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Manfred Schwab
Editor

VOLUME 1

Encyclopedia of Cancer

2nd Edition

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Encyclopedia of Cancer
(2nd edition)

MANFRED SCHWAB (Ed.)

Encyclopedia of Cancer (2nd edition)

With 979 Figures* and 210 Tables

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Preface To The First Edition

Cancer, although a dreadful disease, is at the same time a fascinating biological phenotype. Around 1980, cancer was first attributed to malfunctioning genes and, subsequently, cancer research has become a major area of scientific research supporting the foundations of modern biology to a great extent. To unravel the human genome sequence was one of those extraordinary tasks, which has largely been fuelled by cancer research, and many of the fascinating insights into the genetic circuits that regulate developmental processes have also emerged from research on cancer. Diverse biological disciplines such as cytogenetics, virology, cell biology, classical and molecular genetics, epidemiology, biochemistry, together with the clinical sciences, have closed ranks in their search of how cancer develops and to find remedies to stop the abnormal growth that is characteristic of cancerous cells. In the attempt to establish how, why and when cancer occurs, a plethora of genetic pathways and regulatory circuits have been discovered that are necessary to maintain general cellular functions such as proliferation, differentiation and migration. Alterations of this fine-tuned network of cascades and interactions, due to endogenous failure or to exogenous challenges by environmental factors, may disable any member of such regulatory pathways. This could, for example, induce the death of the affected cell, may mark it for cancerous development or may immediately provide it with a growth advantage within a particular tissue.

Recent developments have seen the merger of basic and clinical science. Of the former, particularly genetics has provided instrumental and analytical tools with which to assess the role of environmental factors in cancer, to refine and enable diagnosis prior to the development of symptoms and to evaluate the prognosis of patients. Hopefully, even better strategies for causal therapy will become available in the future. Merging the basic and clinical science disciplines towards the common goal of fighting cancer, calls for a comprehensive reference source to serve both as a tool to close the language gap between clinical and basic science investigators and as an information platform for the student and the informed layperson alike. Obviously this was an extremely ambitious goal, and the immense progress in the field cannot always be portrayed in line with the latest developments. The aim of the Encyclopedia is to provide the reader with an entrance point to a particular topic. It should be of value to both basic and clinical scientists working in the field of cancer research. Additionally both students and lecturers in the life sciences should benefit highly from this database. I therefore hope that this Encyclopedia will become an essential complement to existing science resources.

The attempts to identify the mechanisms underlying cancer development and progression have produced a wealth of facts, and no single individual is capable of addressing the immense breadth of the field with undisputed authority. Hence, the ‘Encyclopedic Reference of Cancer’ is the work of many authors, all of whom are experts in their fields and reputable members of the international scientific community. Each author contributed a large number of keyword definitions and in-depth essays and in so doing it was possible to cover the broad field of cancer-related topics within a single publication. Obviously this approach entails a form of presentation, in which the author has the freedom to set priorities and to promote an individual point of view. This is most obvious when it comes to nomenclature, particularly that of genes and proteins. Although the editorial intention was to apply the nomenclature of the Human Genome Organisation (HUGO), the more vigorous execution of this attempt has been left to future endeavours.

In the early phase of planning the Encyclopedia, exploratory contacts to potential authors produced an overwhelmingly positive response. The subsequent contact with almost 300 contributory authors was a marvellous experience, and I am extremely grateful for their excellent and constructive cooperation. An important element in the preparation of the Encyclopedia has been the competent secretarial assistance of Hiltrud Wilbertz of the Springer-Verlag and of Ingrid Cederlund and Cornelia Kirchner of the DKFZ. With great attention to detail they helped to keep track of the technical aspects in the preparation of the manuscript. It was a pleasure to work with the Springer crew, including Dr. Rolf Lange as the Editorial Director (Medicine) and Dr. Thomas Mager, Senior Editor for Encyclopedias and Dictionaries. In particular I wish to thank Dr. Walter Reuss, who untiringly has mastered all aspects and problems associated with the management of the numerous manuscripts that were received from authors of the international scientific community. It has been satisfying and at times comforting to see how he made illustration files come alive. Thanks also to Dr. Claudia Lange who, being herself a knowledgeable cell biologist, has worked as the scientific editor. Her commitment and interest have substantially improved this Encyclopedia.

As a final word, I would like to stress that although substantial efforts have been made to compose factually correct and well understandable presentations, there may be places where a definition is incomplete or a phrase in an essay is flawed. All contributors to this Encyclopedia will be extremely happy to receive possible corrections, or revisions, in order for them to be included in any future editions of the 'Encyclopedic Reference of Cancer'.

Heidelberg, September 2001
MANFRED SCHWAB

Preface To The Second Edition

Given the overwhelming success of the First Edition of the Cancer Encyclopedia, which appeared in 2001, and the amazing development in the different fields of cancer research, it has been decided to publish a second fully revised and expanded edition, following the principle concept of the first edition that has proven so successful.

Recent developments are seeing a dynamic merging of basic and clinical science, with translational research increasingly becoming a new paradigm in cancer research. The merging of different basic and clinical science disciplines towards the common goal of fighting against cancer has long ago called for the establishment of a comprehensive reference source both as a tool to close the language gap between clinical and basic science investigators and as a platform of information for advanced students and informed laymen alike. It is intended to be a resource for all interested in information beyond their specific own expertise.

While the First Edition had featured contributions from approximately 300 scientists/clinicians in one Volume, the Second Edition includes more than 1000 contributors in 4 Volumes with an A–Z format of more than 7000 entries. It provides definitions of common acronyms and short definitions of both related terms and processes in the form of keyword entries. A major information source are detailed essays that provide comprehensive information on syndromes, genes and molecules, and processes and methods. Each essay is well-structured, with extensive cross-referencing between entries. Essays represent original contributions by the corresponding authors, all distinguished scientists in their own field, Editorial input has been carefully restricted to formal aspects.

A panel of Field Editors, each an eminent international expert for the corresponding field, has served to ensure the presentation of timely and authoritative Encyclopedia entries. These new traits are likely to meet the expectance that a wide community has towards a cancer reference works.

An important element in the preparation of the Encyclopedia has been the competent support by the Springer crew, Dr. Michaela Bilic, Saskia Ellis and, lately, Jana Simniok. I am extremely grateful for their excellent and pleasant cooperation.

The Cancer Encyclopedia, Second Edition, will be accessible both in print and online versions. Clinicians, research scientists and advanced students will find this an amazing resource and a highly informative reference to cancer.

Heidelberg, March 15, 2008
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2ar

►Osteopontin

17-1A

►EpCAM

A Disintegrin and Metalloprotease

►ADAM Molecules

A-Scan Ultrasonography

Definition

Ophthalmologic ultrasound that provides single-dimension information on the ultrasonic echogenicity of the ocular tissues providing information regarding axial length, size of intraocular structures (such as tumor height), and homogeneity of individual tissues within the eye.

►Uveal Melanoma

AAA^+

Definition

Superfamily of proteins characterized by a segment of ~220 aminoacids (the AAA domain) containing several conserved motifs including those necessary for ATP binding and hydrolysis.

►APAF-1 Signaling

AAMP

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Definition

AAMP stands for angio-associated migratory cell protein and its gene is found on chromosome 2q35. ►HUGO nomenclature symbol is AAMP. AAMP has been conserved in evolution, is distributed intracellularly in many cells and also extracellularly on vascular cells, shares an ►epitope with motility-related proteins (►alpha-actinin and a fast twitch skeletal muscle protein) and contains potential heparin binding and thrombin cleavage sites. Antibody and antisense studies have indicated compartment (intracellular or extracellular) specific roles for AAMP in angiogenesis, cell-cell and cell-matrix interactions, and cell migration.

Characteristics

The cDNA derived from mRNA encoding AAMP was originally cloned from a human melanoma cell library (A2058) in a search for migration-related proteins. AAMP has been found in the cytoplasm of many

nucleated cells, in an extracellular mesh-like network on monolayers of endothelial and vascular-associated smooth muscle cells, and on the apical membranes of endometrial glandular cells. AAMP expression when normalized for tissue source has shown the highest levels of distribution in the esophagus (7.17% of tissue clones) (http://smd.stanford.edu/cgi-bin/source/sourceImage?File = Hs.83347). Local homologies discovered initially to human immunodeficiency viral proteins led to identification of two immunoglobulin-like ►domains in AAMP. In addition to melanoma, expression of AAMP has been observed in a variety of malignant cells, including poorly differentiated colon adenocarcinoma within lymphatics, gastric adenocarcinoma, Jurkat lymphoma, gastrointestinal stromal tumors with mutated ►*c-kit*, breast cancer cell lines and ductal adenocarcinoma *in situ* with necrosis, and brain tumor cells.

Co-culture of astrocytes with endothelial cells (without physical contact) led to increased amounts of extracellular AAMP associated with the endothelial cells. Stimulation of T lymphocytes and monocytes by a ►phorbol ester led to greatly increased AAMP expression, 1.6 kb message and 52 kDa protein. Hypoxia increased expression of the *AAMP* gene in a breast carcinoma cell line.

AAMP has demonstrated compartment-specific effects on endothelial cell migration. Affinity-purified antibodies, that interacted with the extracellular form of AAMP on non-permeabilized endothelial cells, inhibited cell migration and endothelial tube formation. However, anti-sense oligonucleotides, that decreased total AAMP expression, paradoxically increased cell migration, presumably via loss of intracellular AAMP.

The structure of AAMP was initially characterized as having two immunoglobulin-like domains and six ►WD repeats. Now eight WD repeats have been identified in AAMP, UniProt KB/Swiss-Prot Q13685. AAMP has been conserved in evolution. Comparisons of reference sequences for human AAMP (433 aa) with related forms in mouse (434 aa), rat (471 aa), chicken (419 aa), frog (438 aa), and zebrafish (408 aa) have shown 99.5, 98.9, 86.7, 76.5, and 69.0% identity, respectively (UniGene, NCBI, NIH). An acid box (short contiguous run of glutamic or aspartic acid residues) has been identified in the amino terminal regions of several AAMP homologs. They are comprised of seven glutamic acids in human, eight glutamic acids in mouse and rat, and six aspartic acid residues in the zebrafish forms of AAMP.

AAMP contains a strongly immunoreactive ESESES epitope at its ►amino terminal end that has been used to generate an anti-peptide antibody. Under normal reducing conditions, the epitope is immunoreactive for AAMP only in lysates of human brain and activated T lymphocytes. AAMP (52 kDa) shares this epitope with non-skeletal alpha-actinin (100 kDa) and an unidentified fast twitch skeletal muscle fiber protein (23 kDa), as demonstrated with anti-RRLRRMESESES (anti-P189) and related anti-peptide antibodies. The ESESES epitope is linear in AAMP but is discontinuous or conformational (formed by ►secondary structure) in alpha-actinin. The fast twitch skeletal muscle fiber protein with immunoreactivity for anti-P189 was found in the periodic bands (Z discs).

An alternatively spliced, slightly longer form of AAMP (452 aa) includes coding sequence upstream from MESESES. The immediate upstream sequence, RRLRR, potentially functions as a heparin binding site. In addition to an alternative initiating methionine, the upstream human coding sequence differs by only two of seventeen codons when compared to an even longer form of AAMP in rat. The coding sequence of AAMP in rat includes the sequence GRFRRMESESES that corresponds to RRLRRMESESES in the alternative form of human AAMP. In peptide studies, the bipolar RRLRRMESESES sequence was strongly self-aggregating, sensitive to thrombin digestion, and displayed binding to heparin and cells as either an immobilized, single peptide or as an aggregated peptide, without affecting cell viability or adhesion to collagen. Peptide sequencing verified the presence of RLRR in recombinant AAMP translated in *Escherichia coli* following thrombin digestion that cleaved the first R. Although anti-P189 (RRLRRMESESES) did not demonstrate reactivity with the RRLRR epitope in tissue that displayed reactivity with ESESES, the lack of reactivity for RRLRR could have been due to interference by strongly adherent ►glycosaminoglycans.

Thus initial studies of AAMP's distribution and structure are supportive of a role for this protein in cell migration and angiogenesis.

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AAPC

►APC Gene in Familial Adenomatous Polyposis

AAV

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Definition

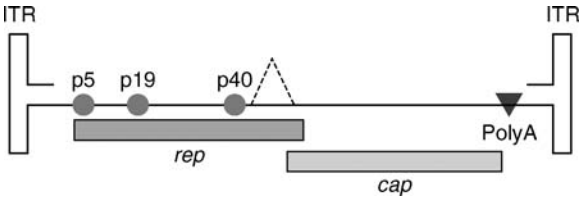
Adeno-associated viruses (AAV) are small DNA-containing viruses that belong to the family of *Parvoviridae*. Thus far, 11 ►serotypes of adeno-associated viruses (AAV-1 to AAV-11) have been cloned from humans and primates, and multiple further isolates were identified in various other species, including birds, bovines, mice, rats and goats. According to current knowledge, none of these naturally occurring viruses are pathogenic in humans. AAV type 2 (AAV-2) has been studied for over 40 years and is the best characterized AAV isolate, hence its frequent referral as the AAV prototype. All AAV serotypes are currently being developed and evaluated as gene transfer ►vectors for the human ►gene therapy of various inherited or acquired diseases, including different types of cancer.

Characteristics

As typical members of the ►Parvovirus family, AAV are characterized by non-enveloped, icosahedral capsids of about 18–24 nm in diameter. These capsids carry linear single-stranded DNA genomes of ~4.6–4.8 kb. The genomes of all known AAV serotypes have been cloned and sequenced. With the exception of AAV-4 and -5, which are distinct (>30%) from the other serotypes at both the nucleotide and amino acid level, all human and primate AAV genomes are related and highly homologous (>80%). Accordingly, their genomic structure and organization are also very similar.

AAV Genome Structure

As an example, the organization of the 4,681 nucleotide AAV-2 prototype genome is described (Fig. 1). The AAV-2 genome consists of two large ►open reading frames (orf), one at the left end encoding the non-structural proteins (replication, *rep* orf), and one at the right end encoding the structural proteins (capsid, *cap* orf). In addition, a single intron sequence is found in the center of the genome, where the *rep* and *cap* orfs overlap. The AAV-2 *rep* gene encodes four closely related proteins (Rep proteins) with partially shared amino acid sequences. On the basis of their molecular weights, these proteins were designated Rep78, Rep68, Rep52 and Rep40. Unspliced and spliced transcripts originating from a ►promoter located at map unit 5 (p5) are translated into the two large Rep proteins, Rep78 and Rep68. Rep52 and Rep40 are expressed from similarly spliced mRNAs that initiate from a second promoter, p19. The third AAV-2 promoter, p40, controls transcription of the *cap* gene. Translation of differentially spliced *cap* mRNAs results in expression of the three proteins that form the AAV-2 capsid, VP1, VP2 and VP3 (in a 1:1:10 ratio). The two viral genes are flanked by short (AAV-2: 145 nucleotides) inverted terminal repeats (ITR), palindromic sequences that are able to fold into T-shaped stem loop structures. The ITRs are necessary and sufficient for replication and encapsidation of the viral genome during a productive infection of cells. Moreover, they are important for integration and rescue of the AAV DNA into, or from, the genome of latently infected cells, respectively. Thereby, the ITRs serve as minimal *cis*-acting sequences during the two different AAV life cycles (see also below).



AAV. Figure 1 Structure of the AAV-2 genome. The 4,681 nucleotide single-stranded genome is depicted as a solid line; by convention, AAV genomes are drawn in 3′-5′ orientation. Shown are the locations of the *rep* and *cap* orfs and the single intron (caret), as well as the position of the three promoters (p5, p19, p40) and the polyA signal, which is used for polyadenylation of all AAV-2 transcripts. Further depicted at the ends of the genome are the palindromic inverted terminal repeat (ITR) sequences in their hairpin configuration.

AAV Life Cycles

AAV serotypes belong to the Parvovirus genus Dependovirus, indicative of their dependence on an unrelated helpervirus to undergo a productive infection of cells. In fact, AAV genomes can only express their genes, replicate and become encapsidated if the cell is simultaneously co-infected by one of these helperviruses. The typical helpervirus for AAV-2 is human ►Adenovirus type 2 or 5, but many other human viruses can also provide full or partial helper functions, including Herpes simplex virus, Vaccinia virus and Cytomegalovirus.

In the case of Adenovirus, one of the major helper functions is to stimulate AAV gene expression, by *trans*-activating the AAV-2 promoters. Additional help for the AAV life cycle is mediated at the post-transcriptional level, where adenoviral proteins and RNAs help to facilitate the cytoplasmic transport of AAV-2 mRNAs. Concurrently, adenoviral functions help to stabilize replicated AAV-2 genomic DNA later in the AAV infection. Notably, once expressed in the infected cell, AAV-2 Rep proteins subsequently further regulate and coordinate gene expression from the AAV promoters. They also play important roles for AAV DNA replication, as well as for packaging of viral genomes into empty new capsids (assembled from AAV-2 VP proteins). To mediate these diverse functions, Rep proteins bind to the AAV-2 ITRs and to sequences located in the AAV-2 promoters. They also interact with various cellular proteins, e.g. the TATA-box binding protein ►(TBP), as well as with each other and the AAV-2 VP proteins. The final step in a productive AAV-2 infection is the helpervirus-mediated lysis of the infected cell. This results in cell death and release of both new AAV-2 and helpervirus particles.

In contrast to this productive (or lytic) phase, AAV-2 can establish latency in the absence of any helpervirus. Rather than replicating, the AAV-2 DNA then integrates into the target cell genome, where it stably persists as a so-called provirus. Important to point out, wildtype AAV-2 integration is not random, as is the case for retroviruses (►Retroviral Insertional Mutagenesis) and other integrating viruses. Instead, it is targeted to a specific region on the long arm of human chromosome 19 (19q13.3-ter). The large Rep proteins (albeit only weakly expressed in the absence of a helpervirus) mediate this site-specific integration through binding to the AAV-2 ITRs, as well as to homologous sequences (AAVS1) located in chromosome 19. However, if a latently AAV-infected cell is later super-infected with a helpervirus, AAV-2 gene expression is induced and the AAV-2 genome is rescued from its integrated state. From this point on, a typical productive AAV-2 infection will occur. Thus, the helpervirus can act as an efficient switch between the two different phases that characterize the AAV-2 life cycle, lytic and latent.

Clinical Relevance

In theory, due to its inherent anti-tumor properties (see below), wildtype AAV-2 (and probably other serotypes alike) could be used as a therapeutic agent for the treatment of human cancers. However, more widely studied and applied are ►recombinant vectors derived from wildtype AAVs. Typically, these vectors are generated by replacing the two viral genes (*rep* and *cap*) with a foreign ►gene expression cassette, encoding RNAs or proteins that mediate an anti-tumor effect (if used for cancer therapy). The general clinical relevance of wildtype and recombinant AAVs is briefly discussed below; for more depth, the reader is referred to recent excellent reviews on the use of AAV for the treatment of human disease (see References below).

Are Wildtype AAVs Pathogenic in Humans?

According to the bulk data available, wildtype AAV serotypes are believed to be non-pathogenic in humans. In fact, despite estimates that up to 80% of adults are ►seropositive for AAV-2, no human disease has ever been causally linked to infection with the wildtype virus. This is even more remarkable considering that AAV-2 can infect a large variety of cells from diverse organs and tissues. Yet, although without gross pathological consequences for the cell, a latent AAV-2 infection can induce subtle changes in the cell ►phenotype. Examples are an increased ability to respond to stress factors, or a perturbation of the cell cycle, resulting in retarded cell growth. Most probably, these various effects are mediated by the large AAV-2 Rep proteins, even at the low expression levels typical for the latent stage.

Is There a Natural Connection Between AAV Infection and Cancer?

One frequently reported observation is that AAV-2-infected cells exhibit an increased resistance to ►oncogene- or tumorvirus-induced transformation. It is moreover known that AAV-2 infection can inhibit the proliferation of cultured cells derived from human cancers, e.g. ►melanomas. Cumulatively, these data strongly suggest that wildtype AAV-2 is not only non-pathogenic, but in fact has oncosuppressive properties. Moreover, certain human cancer cell lines become more sensitive to gamma irradiation (►Ionizing Radiation Therapy) and chemotherapeutic drugs (►Chemotherapy of Cancer, Progress and Perspectives) upon experimental infection with wildtype AAV-2, as compared to non-infected controls. From a clinical point of view, these findings are of particular interest, since a major limitation of cancer chemotherapies is increasing resistance of transformed cells towards the drugs used. The observations of AAV-2-mediated cell sensitization therefore suggest that wildtype AAV might help to improve cancer chemotherapy, when applied in combination with conventional drugs.

What are Recombinant AAV Vectors?

Recombinant AAV (rAAV) vectors are derivatives of wildtype AAV which lack the *rep* and *cap* genes, and instead carry a foreign gene expression cassette inserted between the two viral ITRs. By definition, AAV vectors are thus “gutless” or “gutted” (i.e., devoid of any viral genes). The generation of rAAV vectors is technically feasible and simple, due to the wide availability of molecular clones of the various wildtype viruses. These clones are easily modified using standard molecular

laboratory techniques. Particularly beneficial is that wildtype and recombinant AAV are very small as compared to all other viruses developed as vectors, which aids in their experimental manipulation. Except for the replacement of the wildtype genes with a recombinant DNA, AAV vectors are identical in structure and organization to wildtype viruses and thus also function alike. In fact, AAV vectors will infect the target cell via the same molecular and cellular pathways as the wildtype virus. Ultimately, this will lead to expression of the encapsidated recombinant gene in the cell and thus to the intended therapeutic effect. As gene transfer vehicles, AAV vectors hold enormous promise for therapeutic intervention for a multitude of human acquired or innate genetic diseases, including cancer.

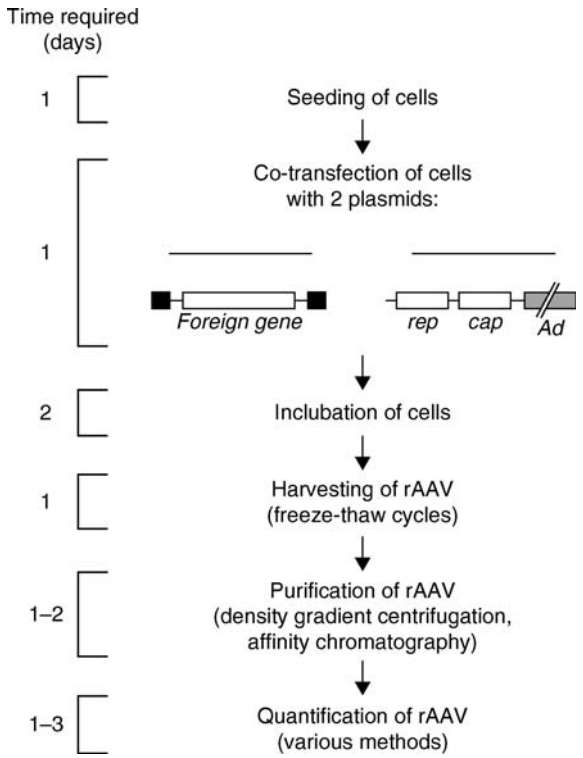
Is AAV Unique as a Human Gene Therapy Vector?

AAV vectors possess a multitude of advantages over all other virus-derived gene transfer vectors currently in (pre-)clinical development. One asset already mentioned is the lack of pathogenicity of the wildtype virus, which is in stark contrast e.g. to Adenovirus, another commonly used virus for gene therapy. Consequently, the production and handling of AAV vectors requires the lowest biosafety levels (S1, i.e., causing minimal risks for humans and the environment). The safety of AAV vectors is further increased by their "gutted" nature, precluding the expression of viral gene products which could cause cellular immune responses in the treated patient (a frequent adverse reaction to adenoviral vectors). A third unique asset, and a further difference to other viral vectors, is the availability of a wide spectrum of human, mammalian and non-mammalian natural serotypes. These isolates typically differ in their ►tropism, i.e., the range of cells and tissues they can infect. Fortunately, it is technically very simple to generate recombinant AAV vectors which carry the same expression cassette, but differ in the viral capsid. This process is called "pseudotyping" and allows for the targeted delivery of a given recombinant DNA to virtually any desired cell or tissue, provided it can be infected by a known wildtype AAV (or a mutant thereof, see below). A plethora of reports have already demonstrated the power of this approach, to use AAV vectors for therapeutic and specific gene transfer to all clinically relevant target organs, including liver, muscle, lung, eye and brain. Last but not least, AAV vectors also differ from all other viral vectors by their capability to mediate persistent and long-term gene expression, both in actively dividing and in quiescent (i.e., non-dividing) cells, and most importantly, without integrating into the host chromosome. Instead, the vector forms stable but extra-chromosomal DNA molecules, which are not capable of perturbing chromosome structures and thus do not pose a mutational risk. This is clinically most pertinent, as many gene therapy applications will require stable gene expression, ideally for the life-span of the patient. The only other viral vectors able to mediate long-term gene expression (and in non-dividing cells) are derived from retroviruses or lentiviruses (HIV). However, these vectors are associated with drastically higher concerns about biosafety, due to the inherent pathogenic nature of the parental wildtype virus, as well as due to their propensity for integration into the human genome. The latter can readily result in insertional mutagenesis, i.e., activation of endogenous oncogenes, or vice versa, inactivation of ►tumor suppressor genes. In both cases, the result is malignant transformation of the infected cell. This potentially serious adverse event from the use of retroviral vectors has indeed been observed in a recent clinical study, where multiple children developed leukemias, and some even died. Likewise, adenoviral vectors and the associated immune response have been blamed for the death of a patient in an early gene therapy trial in 1999. In striking contrast, thus far, none of the over 30 clinical trials using AAV vectors has yielded any evidence for a tumorigenic or lethal potential of this particular vector system.

What are Recent Advances in AAV Vector Technology?

In the early years, AAV vectors have been criticized for their small size (preventing packaging and therapeutic transfer of recombinant DNA >5kb in length), their relatively slow transduction kinetics (resulting from the single-stranded DNA genome and its need for conversion into a transcriptionally active DNA duplex), and their restricted cell and tissue tropism (based on the sole availability of the AAV-2 capsid in the early phase of AAV vector development). Nonetheless, even with those presumed limitations, AAV-2 vectors have been tested successfully in various large animal models and in human patients, addressing diverse diseases such as cystic fibrosis or hemophilia B. Most importantly, all three initial limitations of the AAV vector system have now been overcome, leading to the rapid expansion of AAV-based human gene therapy, especially for cancer treatment. First of all, the issue of limited packaging capacity has been solved with the creation of "split" AAV vectors which exploit the virus' natural propensity for ►concatamerization. In an infected cell, rAAV genomes frequently recombine with each other, resulting in large "head-to-tail" concatamers (i.e., multiple copies of an rAAV genome in the same orientation). This can be exploited experimentally, by splitting a large recombinant DNA (e.g., a gene and its promoter) into two halves, each of which is then delivered by a separate rAAV vector. This strategy effectively doubles the packaging limit of AAV vectors to up to 10 kb, which is sufficient even for large DNAs such as the factor VIII gene (encoding a blood clotting factor missing or defect in hemophilia A patients). Secondly, the inherently slow transduction kinetics of AAV

have been overcome with the development of self-complementary or double-stranded vectors. In these, two copies of a foreign gene expression cassette are cloned and packaged in an inverted format, only separated by a minimal version of an AAV ITR. In the transduced cell, these two inverted copies then rapidly anneal with each other without the need for conversion into a duplex AAV DNA molecule. This results in an extremely rapid onset as well as maximum efficacy of gene expression, both far superior to what is obtained with conventional single-stranded AAV vectors, or most other viral vector systems. Thirdly, the limited host range of AAV-2 was readily overcome with the engineering of the over 100 alternative naturally occurring AAV serotypes as vectors. This approach has not only substantially broadened the range of cells and tissues that can now be infected with AAV vectors, but it has also alleviated concerns over the prevalence of neutralizing antibodies against the AAV-2 prototype in the human population. In fact, a wealth of studies have shown that AAV vectors derived from non-type-2 serotypes are functional in many tissues that are refractory to AAV-2 infection, and most importantly, transduction readily occurred in the (experimentally induced) presence of anti-AAV-2 antibodies, mimicking the situation in most humans. Moreover, very recent work demonstrated the feasibility to create synthetic AAV capsids which are further unique from the AAV-2 prototype, as well as from any of the naturally occurring isolates. Multiple strategies are currently being pursued, including the random mutagenesis of the AAV(-2) *cap* gene, the insertion of peptide pools into exposed regions of the AAV-2 capsid (hoping the peptides will mediate re-targeting to unknown cellular receptors), or the creation of libraries of "shuffled" viruses, in which capsid genes from several parental viruses are mixed and recombined. Most importantly, all of these new approaches and designs remain fully compatible with already established AAV vector technology, allowing for their rapid and straightforward pre-clinical evaluation. In fact, current AAV vector production methodologies are highly advanced and permit the generation of high titer stocks (>1 × 10^{14} recombinant particles per batch) in a very short amount of time (~10 days) (Fig. 2). As a result, AAV vectors have entered clinical evaluation and are currently being studied in about 30 ongoing trials in human patients.



AAV. Figure 2 Streamlined protocol for rAAV production. Cultured cells are transfected with two plasmids: The vector plasmid containing the foreign gene to be packaged into the viral particles, flanked by the AAV-2 ITRs, and the helper plasmid carrying the AAV-2 *rep* and *cap* genes to supply the Rep and VP proteins, respectively. In addition, the helper contains all adenoviral (Ad) genes which encode proteins with supportive function for AAV vector production, but it does not yield Adenovirus after transfection. Helpervirus infection is thus superfluous, and the resulting AAV-2 vectors are free of contaminating Adenovirus. Following a 2-day incubation of the transfected cells, the rAAV particles are harvested, purified and quantified. Note that there are numerous modifications to this basic protocol, e.g., in the number of plasmids (1–3, depending on the arrangement of AAV and adenoviral sequences).

What are Clinically Relevant rAAV Applications in Cancer Treatment?

The sum of assets described above – safety, versatility, efficacy, specificity – makes AAV an ideal vector for multiple and diverse therapeutic applications in humans. With particular respect to cancer, the use of AAV vectors is still in its infancy, but increasing pre-clinical data suggest that this vector system holds enormous potential also for this specific application. Thus far, the approaches can be divided into strategies that either target the tumor cell directly or that modify host mechanisms. In more detail, AAV vectors have been employed in the following major categories: Anti-angiogenesis, ►immunotherapy, tumor suppressors, suicide gene therapy, drug resistance, repair strategies, and, last but not least, purging of tumor cells. For many of those categories, a currently emerging therapeutic modality which is also still in its infancy is RNA interference or RNAi (►RNA interference and Cancer). This term describes the natural phenomenon of gene silencing mediated by short double-stranded RNAs.

The latter can be expressed from AAV vectors and thus be used to effectively and specifically suppress, for instance, expression of cellular or virally-encoded oncogenes. RNAi will likely become a valuable and crucial aspect of AAV-based cancer therapy in the near future, and will complement or perhaps even replace many of the currently existing strategies.

Anti-Angiogenesis

The efficacy of ►angiogenesis inhibitors to undermine tumor ►neovascularization and to block cancer progression as well as formation of metastases (►metastasis) has been established in many animal models. However, this cancer therapy requires that the inhibitors are chronically administered as recombinant proteins, which is usually associated with severe problems. Therefore, AAV vectors with their unique ability to mediate sustained gene expression should prove particularly useful for this type of tumor therapy. Especially promising will be the future combination with synthetic AAV capsids that have been evolved to target the vasculature. Thus far, mostly AAV-2-based vectors have been used to deliver and express various anti-angiogenesis factors in small animals, typically mice. A first important example is angiostatin, which has been expressed from AAV-2 in multiple mouse models of human cancers, including gliomas (►Glioblastoma Multiforme) and liver cancers (►Liver Cancer, Molecular Biology]. In all reported cases, this led to suppression of in vivo tumor growth and to substantial improvements in tumor-free survival rates. Similarly impressive are results with the related anti-angiogenic peptide ►endostatin, whose expression from AAV-2 vectors inhibited the establishment or growth of various human cancers in mice, including liver, ovarian (►Ovarian Cancer), pancreatic (►Pancreas Cancer, Clinical Oncology) and colorectal (►Colon Cancer) tumors. Even better results have been obtained with the co-expression of both angiostatin and endostatin from a single or from two separate AAV vectors, exemplifying the potential for synergistic effects from combinatorial AAV therapies. Other examples for anti-angiogenic AAV therapies already evaluated include the expression of a truncated form of the ►vascular endothelial growth factor receptor (renal tumors), or of tissue inhibitors of ►matrix metalloproteases.

Immunotherapy

Failure of the immune system to recognize cancer antigens can substantially contribute to tumor manifestation and progression. Although tumors can illicit strong immune responses in the early stages, this effect is frequently lost in later phases, eventually allowing for aggressive and metastatic tumor growth. Gene transfer protocols involving AAV (or other viral) vectors have thus been developed which aim to potentiate the patient's antitumor responses, by either targeting the tumor cells directly, or by transducing host-derived immune effector cells. Examples for already reported tumor cell-directed therapies include AAV-mediated delivery of ►interferon genes to ex vivo cultured cancer cells, or via intra-tumoral injection (gliomas). Likewise, AAV-2 has been used to express tumor necrosis factor-related ►apoptosis-inducing ligand (►TRAIL) in colorectal, lung and liver tumor models, resulting in significantly inhibited tumor growth and, in some cases, even in regression. Targeting cells of the host immune system, on the other hand, is a promising alternative approach and could eventually be developed into a vaccination therapy. Already, AAV-2 vectors have been used to deliver dominant tumor epitopes to antigen-presenting cells, such as CD40 ligand which was expressed in B-cells from ►chronic lymphocytic leukemia (CLL) patients, leading to specific proliferation of ►HLA Class I-matched allogeneic T-cells. Another potential vaccine could be AAV vectors expressing an HPV16 (►Human Papillomaviruses) E7 CTL (cytotoxic T-cell) epitope/heat shock fusion protein, based on reports that infected mice became immunized against E7-expressing tumor cells. Last but not least, encouraging studies have identified ►dendritic cells (DC), the most potent antigen-presenting cells, as an attractive target for AAV-based cancer immunotherapies. For instance, DCs transduced with AAV vectors encoding HPV16 E6 or E7 genes caused a stark CTL response against cervical cancer cell lines, while in another study, DCs transduced with CD80-expressing AAVs induced high levels of CD8+ T-cells. Together, these findings suggest that AAV can be used to trigger strong anti-tumor CTL responses, and that AAV-based immunotherapy has substantial clinical potential for cancer treatment.

Tumor Suppressors

Highly attractive targets for AAV-mediated cancer therapy are oncogenes and tumor suppressor genes, respectively, whose expression is frequently dysregulated in malignant human cancers. An important example for a tumor suppressor involved in cellular checkpoint control is p53 (►p53 Protein, Biological and Clinical Aspects), which normally prevents passage of cells with DNA damage through the cell cycle. Consequently, expression of p53 from AAV vectors was consistently found to block the growth of cancer cells in vitro and in vivo, and to mediate apoptosis and cytotoxicity. Similar results were obtained after expression of the fragile histidine triad tumor suppressor (►FHIT), which delayed the growth of human pancreatic tumor ►xenografts and extended long-term animal survival. In a third example, delivery of the gene encoding the monocyte chemoattractant protein MCP-1 from AAV vectors suppressed expression of the HPV E6 and E7 proteins in cervical cancer cell lines, as well as in tumors derived from these cells.

Suicide Gene Therapy

This approach is based on the idea to bio-activate a pro-drug within tumor cells to a toxic species, triggered by the tumor-directed delivery of the activating enzyme from AAV vectors. The best studied example for this category is the Herpes simplex virus-encoded enzyme thymidine kinase (*tk*) in combination with gancyclovir. This system has already been used successfully from AAV vectors to inhibit tumor growth in a variety of human xenograft models, including liver cancer, gliomas and ►oral squamous carcinomas. Notably, the specificity of this approach can be enhanced by the use of tissue- and/or tumor-specific promoters, such as those only active in liver or melanoma cells. Moreover, the overall efficacy of the AAV/*tk* vectors was shown to increase following treatment of transduced cells with irradiation or topoisomerase inhibitors, both known to enhance AAV infection (in addition to their direct effects on cells).

Drug Resistance

Development of multiple drug resistance (MDR) is a major issue with cancer chemotherapies and is often associated with over-expression of the ►P-glycoprotein (an ATPase that pumps chemotherapeutic drugs out of the cancer cell). One recently reported, highly effective approach to reverse the MDR phenotype is to use double-stranded AAV vectors to express anti-P-glycoprotein short hairpin RNAs (effectors of RNAi). In human ►breast cancer and oral cancer cells, this led to a substantial sensitization to chemotherapy, suggesting a high potential to overcome the MDR obstacle with this approach. Another application is expression of the MDR1 gene from AAV vectors in ►hematopoietic progenitors. This should confer myeloprotection in patients undergoing high-dose chemotherapy for advanced tumors, and thus prevent myelosuppresssive effects (►Myelosuppression) from the chemotherapeutic regimen, such as infection or hemorrhaging. However, this strategy has not been fully explored in animal models yet.

Repair Strategies

►Telomerase (the enzyme maintaining and stabilizing the integrity of telomeres, i.e., chromosome ends) is an example for a therapeutically relevant target for repair strategies. Its activity is often elevated in tumor cells, and it was shown that delivery of telomerase antisense molecules [Antisense DNA Therapy] via AAV vectors (in this particular case hybrids with adenoviral vectors) can reduce tumor cell proliferation, as well as induce apoptosis.

Purging of Tumor Cells from Autologous Transplants

Autologous grafts (►Graft Acceptance and Rejection), e.g. peripheral blood progenitor cells, are used for treatment of many solid human cancers. However, they can be contaminated with tumor cells that give rise to relapse after ►myeloablative megatherapy and graft transplantation. There is recent evidence that following infection of such contaminated grafts with recombinant AAV-2, the contaminating tumor cells are preferentially infected, while the hematopoietic progenitors are spared. Indeed, infection of sarcoma cells with AAV/tk vectors (see above) extended the survival of transplanted mice (over non-treated controls), while the same vector was unable to transduce and kill human peripheral blood progenitors. However, it remains to be proven that this strategy can indeed be applied to selectively purge tumor cells from autologous transplants.

RNAi

RNA-mediated silencing of gene expression (RNAi) will clearly become a major part of anti-tumor therapies in the future, as proof-of-concept for the efficacy of this approach is already overwhelming. In combination with AAV, there have only been a few reports thus far, but this field will certainly expand. One described application is to use AAV vectors to deliver short hairpin RNAs against the *hec1* gene, which is highly expressed in mitotic cells where it represents a vital component of the ►kinetochore outer plate. Transduction of glioma cells with anti-*hec1* AAV vectors resulted in selective cell death, while mitotically inactive control cells were unaffected. Likewise, infected xenografts showed lower densities and were highly fibrotic, as a result of AAV treatment. It can generally be predicted that virtually any over-expressed gene that contributes to transformation can be an AAV/RNAi target, including virally-encoded (see above, e.g., HPV E6/7) or cellular oncogenes.

Future Applications

With the current state-of-the-art technology, the AAV vector system is already one of the most powerful and promising toolkits for development as anti-tumor bioreagents. In the future, the versatility of this system will further increase with the discovery and creation of new natural or synthetic capsids, respectively. Likewise, the field will benefit from the engineering of novel tumor- and tissue-specific gene expression cassettes, and from the design of safer and more effective therapeutic sequences, e.g., for the induction of anti-cancer RNAi. A very important approach will be to merge the different strategies into combinatorial therapies, e.g., by mixing immunotherapies with RNAi vectors, or suicide gene expression with repair approaches. Examples for such multimodality cancer therapies with AAV vectors have already been reported, and their numbers will increase in the future. Last but not least, it will also be crucial to combine AAV (or other viral) vectors with further anti-cancer effectors, such as new classes of compounds including proteasome (►Proteasomal Inhibitors) and histone deacetylase (►Histone Deacetylases) inhibitors.

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ABC Drug-Transporters

Definition

Synonym ABC Transporter, ABC (ATP-Binding Cassette) Superfamily. The adenosine-triphosphate (ATP) binding cassette (ABC) transporters form the largest family of transmembrane proteins that use ATP-derived energy to transport various substances over cell membranes. Primary-active transporters, driven by energy released from ATP by inherent ATPase activity, that export substrates from the cell against a chemical gradient, Based on the arrangement of the nucleotide binding domain and the topology of its transmembrane domains, human ABC-transporters are classified into seven distinct families (ABC-A to ABC-G), including ABCB1 (P-glycoprotein), ABCC1 (MRP1), ABCC2 (cMOAT; MRP2), ABCC4 (MRP4), and ABCG2 (ABCP; MXR; BCRP). Structural characteristics based on their ►Walker motif (ATP binding domain) and their nucleotide-binding folds across the membrane are responsible for their classification into this superfamily. Their localization pattern over the body suggests that they have an important role in the prevention of absorption as well as the excretion of potentially toxic metabolites and xenobiotics, both on a systemic and a cellular level. ABC drug-transporters (may) show substrate-overlap. Examples of mammalian ABC transporters include ►P-glycoprotein, MRP (►multidrug resistance protein), ►cystic fibrosis transmembrane conductance regulator (CFTR) and the transporter associated with antigen processing (TAP).

►P-glycoprotein
►Irinotecan
►Fluoxetine
►Glutathione Conjugate Transporter RLIP76
►Major Vault Protein
►Vault Complex

ABC (ATP-Binding Cassette) Superfamily

Definition

ABC (ATP-binding cassette) superfamily contains both uptake and efflux transport systems. These proteins bind ATP and hydrolyze it to energize transport of molecules outside or inside the cells.

ABC Transporter

Definition

ABC transporters are a superfamily of prokaryotic and eukaryotic proteins. They are usually involved in membrane transport and share a homologous nucleotide-binding domain, ABC (ATP-binding cassette). In addition to the ABC domain, ABC transporters contain or interact with hydrophobic domains containing multiple transmembrane segments. Examples of mammalian ABC transporters include P-glycoprotein, MRP (multidrug-resistance protein), cystic fibrosis transmembrane conductance regulator (CFTR) and the transporter associated with antigen processing (TAP).

ABC Transporter Proteins

Definition

ABC transporter proteins are a superfamily of proteins responsible for transporting a broad range compounds across membranes in cells. Structural characteristics based on their Walker domains (ATP binding domain) and their nucleotide-binding folds across the membrane are responsible for their classification into this superfamily.

ABC-Transporters

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Synonyms

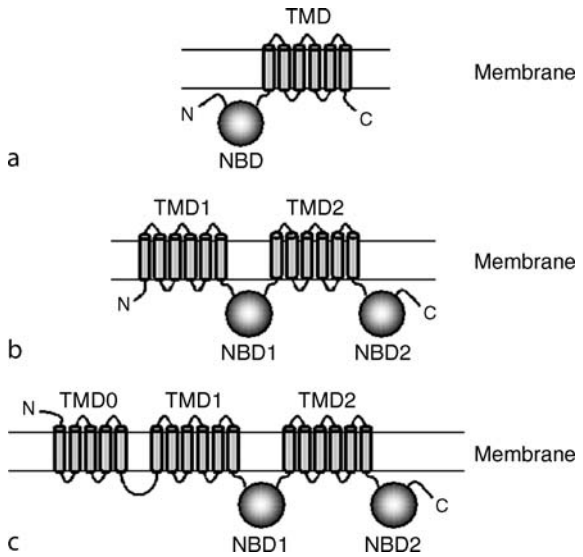
Multidrug resistance transporters; Traffic ATPases; Permeases (for import systems)

Definition

ABC (ATP-binding cassette)-transporters are membrane-embedded proteins with a characteristic ABC domain that utilize the energy from ATP hydrolysis for the transport of their substrates across a cellular membrane.

Characteristics

The superfamily of ABC-transporters comprises one of the most abundant protein families in nature. These transporters are believed to date back in evolutionary time more than 3 billion years and are distributed in all three kingdoms of living organisms, *archaea*, *eubacteria*, and *eukaryotes*. ABC-transporters have to be distinguished from ABC-proteins. Both types of proteins are defined by the presence of a highly conserved ~215 amino acids consensus sequence designated as ABC domain or nucleotide-binding domain (NBD). The domain contains two short peptide motifs, a glycine-rich Walker A and a hydrophobic Walker B motif, both involved in ATP binding and commonly present in all nucleotide-binding proteins. A third consensus sequence is named ABC signature and is unique in ABC domains. ABC-containing proteins couple the phosphate bond energy of ATP hydrolysis to many cellular processes and are not necessarily restricted to transport functions. However, the proper meaning of the term ABC-transporter is satisfied when the ABC-protein is in addition associated with a hydrophobic, integral transmembrane domain (TMD) forming a translocation path. TMDs are usually composed of at least six transmembrane (TM) α-helices. They are believed to determine the specificity for the substrate molecules transported by the ABC-transporter. The minimal structural requirement for a biological active ABC-transporter seems to be two TMDs and two NBDs $[TMD\text{-}NBD]_2$ (Fig. 1). In full-size transporters, this structural arrangement may be formed by a single polypeptide chain and in multi-protein complexes by more than one polypeptide chain. In *prokaryota*, ABC transport systems are often half-size transporters having only one TMD fused to one NBD [TMD-NBD]. Half-size transporters probably dimerize to form a full-size transporter $[TMD\text{-}NBD]_2$ to mediate mainly the influx of essential compounds such as sugars, vitamins, and metal ions into the cell. Eukaryotic ABC-transporters commonly function as exporters mediating the efflux of compounds from the cytosol to the extracellular space or to the inside of intracellular membrane-bound compartments, i.e., endoplasmic reticulum, mitochondria, peroxisomes, or vacuoles. The range of physiologically transported compounds includes lipids and sterols, ions, diverse small molecules, oligo- and polypeptides.



ABC-Transporters. Figure 1 Schematic representation of the predicted domain arrangement of (a) half-size transporters having only one TMD fused to one NBD [TMD-NBD], e.g., ABCG2 (BCRP); and (b,c) full-size transporters $[TMD\text{-}NBD]_2$, whereby (b) shows the predicted structure of ABCB1 (MDR1), and (c) the structure of ABCC1 (MRP1) containing an additional TMD (TMD0) of unknown function. Half-size transporters probably dimerize to form a biological active ABC-transporter. These three ABC-transporters are the most important drug extrusion pumps in multidrug-resistant cancers. TMD, transmembrane domain consisting of six α-helices; NBT, nucleotide-binding domain. It should be noted that the orientation of ABCG2 is reverse to that of ABCB1 and ABCC1.

Human ABC-Transporters

In humans, 48 ABC-transporters distributed to seven subfamilies have been identified (Table 1). Although the number of human ABC-transporters is much smaller than found in bacteria, many of them are of clinical significance. Currently, 18 human genes encoding ABC-transporters have been associated with genetic diseases. Even though the majority of the members of the human ABC-transporter family are active transporters, there are some exceptions in which the energy of ATP hydrolysis is utilized to control alternative biological processes. Thus, ABCC7 (CFTR), well known as mutated in patients suffering on ►cystic

ABC-Transporters. Table 1 Family of human ABC-transporters

Subfamilay	HUGO-nomenclature	Common names	Location	Size [AA]	Function
ABCA	ABCA1	ABC1	9q31.1	2261	Cholesterol-, PS transport
	ABCA2	ABC2	9q34	2436	
	ABCA3	ABC3, ABCC	16p13.3	1704	Surfactant production
	ABCA4	ABCR	1p22.1-p21	2273	N-retinylidene-PE transport
	ABCA5		17q24.3	1642	
	ABCA6		17q24.3	1617	
	ABCA7	ABCX	19p13.3	2146	
	ABCA8		17q24	1581	
	ABCA9		17q24.2	1624	
	ABCA10		17q24	1543	
	ABCA12		2q34	2595	
	ABCA13		7p12.3	5058	
ABCB	ABCB1	MDR1, PGY1	7q21.1	1280	MDR
	ABCB2	TAP1	6p21.3	808	Peptide transport
	ABCB3	TAP2	6p21.3	653	Peptide transport
	ABCB4	MDR3, PGY3	7q21.1	1279	PC transport
	ABCB5		7p15.3		
	ABCB6	MTABC3	2q36	842	Iron transport
	ABCB7	ABC7	Xq12-q13	752	Iron-, Sulfur- cluster transport
	ABCB8	MABC1	7q36	718	
	ABCB9	TABL	12q24	723/766	
	ABCB10	MTABC2	1q42	738	
	ABCB11	BSEP, SPGP	2q24	1321	Bile salt transporter
ABCC	ABCC1	MRP1, MRP	16p13.1	1531	MDR, organic anion transporter
	ABCC2	MRP2, cMOAT	10q24	1545	MDR, organic anion transporter
	ABCC3	MRP3	17q22	1527	Organic anion transporter
	ABCC4	MRP4	13q32	1325	Organic anion transporter
	ABCC5	MRP5	3q27	1437	Organic anion transporter
	ABCC6	MRP6	16p13.1	1503	
	ABCC7	CFTR	7q31.2	1480	Chloride transport
	ABCC8	SUR1	11p15.1	1581	Regulation
	ABCC9	SUR2	12p12.1	1549	Regulation
	ABCC10	MRP7	6p21.1	1464	
	ABCC11	MRP8	16q12.1	1382	
	ABCC12	MRP9	16q12.1	1359	
ABCD	ABCD1	ALD, ALDP	Xq28	745	FA-, FA AcylCoA transport
	ABCD2	ALDL1, ALDR	12q11-q12	740	FA-, FA AcylCoA transport
	ABCD3	PXMP1, PMP70	1p22-p21	659	FA-, FA AcylCoA transport
	ABCD4	PXMP1L, P70R	14q24.3	606	FA-, FA AcylCoA transport
ABCE	ABCE1	RNASELI, OABP	4q31	402	
ABCF	ABCF1	ABC50	6p21.33	807	
	ABCF2		7q36	623	
	ABCF3		3q27.1	709	
ABCG	ABCG1	ABC8, White	21q22.3	638	Cholesterol transport
	ABCG2	BCRP, MXR	4q22	655	MDR
	ABCG4	White2	11q23.3	627	
	ABCG5	White3	2p21	651	Sterol transport

AA, amino acids; FA, fatty acids; MDR, multidrug resistance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

fibrosis, appears as a chloride ion channel; ABCC8 (SUR1) and ABCC9 (SUR2) are both regulatory subunits of the regulatory sulfonylurea receptor (SUR). Other members of the ABC-transporter family couple ATP binding and hydrolysis to the control of translation or ►DNA repair. Although the active transporters have dedicated functions involving the transport of specific substrates, the complex physiological network of ABC-transporters may also have an important role in host detoxification and protection against xenobiotics. This general function is revealed by their tissue distribution. ABC-transporters are highly expressed in important pharmacological barriers, such as the epithelium that contributes to the blood–brain barrier (BBB), the brush border membrane of intestinal cells, the biliary canalicular membrane of hepatocytes, or the lumenal membrane in proximal tubules of the kidney. Anyway, this xenobiotics pump function is the basis for the pivotal role of ABC-transporters in ►multidrug resistance (MDR) of cancer.

ABC-Transporters and Multidrug Resistance of Cancer

MDR is defined as the simultaneous resistance of a tumor against a variety of antineoplastic agents with different chemical structure and mode of action. Thus, MDR is a major obstacle in clinical management of cancer by ►chemotherapy. Although various mechanisms have been identified to mediate a multidrug-resistant phenotype to malignant diseases, the enhanced drug extrusion activity of the ABC-transporter ABCB1 or ►P-glycoprotein (MDR1; PGY1) was the first mechanism that was demonstrated to be the reason for MDR. The substrates of ABCB1 include first and foremost natural product-derived anticancer drugs, such as ►anthracyclines, ►epipodophyllotoxins, ►taxans, and ►vinca alkaloids, but not clinically important drugs like platinum-containing compounds or ►antimetabolites. Besides ABCB1, in particular, ABCC1 (MRP1) and ABCG2 (BCRP) were found to be associated with a multidrug-resistant phenotype, but also alternative ABC-transporters can pump drugs from the inside to the outside of a cancer cell, e.g., ABCC2 (MRP2) is a platinum drug transporter. ABCB1, ABCC1, and ABCG2 have partial overlapping but not identical substrates.

ABC-Transporters as Anticancer Drug Targets

Following the identification of ABCB1 as a pivotal MDR-mediating factor, tremendous efforts were undertaken to identify ABCB1-interacting agents that inhibit its pump activity and, therewith, reverse the MDR phenotype. Such drugs are commonly designated as chemosensitizers or MDR modulators. Although many compounds, e.g., ►verapamil and ►ciclosporin derivatives, were identified as ABCB1 inhibitors or inhibitors of alternative MDR-mediating ABC-transporters, so far all of them failed in clinical trials.

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ABC Transporters (ATP-Binding Cassette Transporters)

Definition

Primary-active transporters, driven by energy released from ATP by inherent ATPase activity, that export substrates from the cell against a chemical gradient, including P-gp, MRP, and BCRP.

ABL

Definition

The ABL gene encodes a nuclear tyrosine kinase that is involved in chromosomal translocations in chronic myeloid leukemia (CML).

►BCR-ABL1
►Chromosomal Translocations

Ablation

Definition

In cancer therapy, the surgical removal or destruction of tumor tissue.

►Photothermal Ablation

ABLES

Definition

Adult Blood Lead Epidemiology and Surveillance.

►Lead Exposure

ABVD

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Definition

Doxorubicin, bleomycin, vinblastine, and dacarbazine combination chemotherapy used for the treatment of patients with Hodgkin lymphoma.

Characteristics

ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) is the most widely used regimen for the treatment of early and advanced stage Hodgkin lymphoma (HL). Treatment of patients with early stage classical HL evolved over the last three decades. Radiation therapy alone as the single treatment modality is no longer practiced. Today, the most widely used approach is combined modality therapy (chemotherapy plus involved field radiation therapy). In general, 2 (for favorable early stage) to 4 (for unfavorable early stage) cycles of ABVD plus 30 Gy of involved field radiation therapy is the most widely used standard of care approach. Using this approach, more than 90% of the patients are expected to be cured of their disease. Patients with bulky stage II disease, (especially with bulky mediastinal mass), or stage II with B-symptoms are usually treated similar to those with advanced stage HL with 6–8 cycles of ABVD followed by involved field radiation therapy to the bulky area.

Use of chemotherapy alone has recently been proposed for a selected group of patients with early stage classical HL. The rationale for this approach is to reduce radiation-induced morbidity and mortality, including second malignancies and cardiac complications. While this approach is appealing, it will need to be further examined after prolonged follow-up. For now, it seems appropriate to treat young female patients with non-bulky early stage classical HL (especially those with mediastinal or axillary adenopathy) with chemotherapy alone to reduce the risk for breast cancer. The risks and benefits of combined modality versus chemotherapy alone should be discussed with patients before making a final treatment recommendation. Based on several randomized studies comparing ABVD with other multi-drug regimens, ABVD became the most widely used combination regimen for the treatment of patients with advanced HL. Chemotherapy alone (6–8 cycles) is usually considered sufficient for treating patients with advanced stage classical HL. However, involved field radiation therapy is frequently added at the end of chemotherapy to areas of bulky disease. This combined modality approach has been recently compared with chemotherapy (MOPP/ABV) alone in a randomized trial in patients with advanced stage classical HL, and showed no survival advantage, especially in those who achieved complete remission after the completion of chemotherapy. Furthermore, meta-analysis review of fourteen clinical trials comparing chemotherapy with combined modality also showed no survival advantage for those receiving the combined modality approach.

Newer treatment programs such as Stanford V and BEACOPP have shown successful results, but remain less widely used compared with ABVD. Although BEACOPP has been shown to be superior to ABVD-like regimens in large-scale randomized trials, the superiority of Stanford V over standard ABVD has not yet been established. Because ABVD may cure only 50–65% of patients with poor risk advanced stage HL, more intensive programs such as BEACOPP may add benefit, despite the increased toxicity. Patients with good risk features have a high cure rate with ABVD, so the use of more intensive and more toxic regimens in this patient population should be used with caution, and preferably within a clinical trial. In fact, a recently published randomized study demonstrated that early intensification with autologous stem cell transplantation after four cycles of ABVD-like chemotherapy did not improve the outcome in patients with advanced stage HL compared with conventional chemotherapy, perhaps because many patients did not have poor risk features as identified by the international prognostic score for HL.

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Accelerated Phase

Definition

Occurs between chronic phase and ►blast crisis. Characterized by 10–19% myeloblasts and >20% basophils in blood or bone marrow and cytogenetic evolution; also increasing splenomegaly, platelet count, or white blood cells if unresponsive to treatment.

►Nilotinib

ACD

Definition

Abbreviation for Appraisal Consultation Document.

►National Institute for Health and Clinical Excellence (NICE)

ACDC

►Adiponectin

Acetaldehyde

Definition

Highly reactive chemical compound (CH_3CHO) that forms following ethanol metabolism. Toxic, mutagenic and carcinogenic.

►Alcohol Consumption
►Hepatic Ethanol Metabolism

Acetaldehydehydrogenase

Definition

The enzyme which oxidizes ►acetaldehyde.

►Alcohol Consumption

Acetylation

Definition

The reversible, covalent attachment of an acetyl group to the lysine residue of proteins. Acetylation and deacetylation of histones play an important role in transcriptional regulation

►Histone Deacetylases

N-Acetylcysteine

Definition

A pharmacological agent used mainly as a mucolytic and in the management of ►paracetamol overdose and is available in different dosage forms for different indications: solution for inhalation – inhaled for mucolytic therapy or ingested for nephroprotective effect; i.v. injection – treatment of paracetamol overdose; or oral solution – various indications.

►Chemoprotectants

Acetylsalicylic Acid

Definition

ASA/aspirin; one of the most successful drugs in combating reactive species overload diseases, and the ►chemoprevention of cancers such as ►colon cancer.

►Inflammation

Acetyltransferase

Definition

An enzymic activity that catalyzes the transfer of an acetyl moiety from acetyl CoA to a specific amino acid on a substrate protein. CBP and p300 are lysine (K)-directed acetyltransferases.

►CBP/p300 Coactivators

N-Acetyltransferase

Definition

Is an acetyl-CoA requiring enzyme that catalyses the acetylation of ►xenobiotics that are aromatic amines or contain a hydrazine group. It participates in the ►detoxification of a plethora of hydrazine arylamine drugs and is also able to bioactivate several known carcinogens. (Vatsis KP, Weber WW, Bell DA et al (1995) Nomenclature for N-Acetyltransferases. Pharmacogenetics 5:1–17)

►Detoxification

ACF

Definition

Aberrant crypt foci; Putative precursors of colon cancer ►Preneoplastic lesions.

►Trefoil Factors
►Colon Cancer
►Conjugated Linolenic Acids

Acharan Sulfate (AS)

Definition

Is isolated from the giant African snail Achatina fulica, responsible for inhibitory effect of tumor growth. AS inhibits tumor growth by binding the ►nucleolin protein on the surface of cancer cells.

Achneiform Rash

Definition

Is a pustular rash with usual distribution over the face, scalp, and upper trunk.

►Erlotinib (Tarceva®)

Acid Rain

Definition

Is formed by absorption of acidic gases as SO_2 or NO_x by water droplets in clouds Precipitation then increases the acidity of the soil, and affects the chemical balance of surface water bodies.

►Xenobiotics

Acidosis

Definition

Is present in a tissue whenever the pH is below 7.0 (or the H^+-concentration is higher than 10–7 mol/L).

►Oxygenation of Tumors

Acinar Cells

Definition

Key cell in the exocrine pancreas. These are arranged around a central lumen and form the bulk of the pancreatic gland. They contain digestive enzymes usually found in the apical region of the cells.

Acites

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Definition

Ascites is derived from the Greek word ασκός (gr. sack, wineskin) and is defined as accumulation of protein rich fluid in the peritoneal cavity. It occurs mainly in ►cirrhosis of the liver, but also in heart failure, tuberculosis and malignancy. Malignant ascites occurs in association with a variety of neoplasms and is defined as the abnormal accumulation of fluid in the peritoneal cavity caused by cancer.

Characteristic

Malignant ascites accounts for around 10% of all cases of ascites and occurs in association with a variety of neoplasms. Malignant effusion is the escape of fluid from the blood or vessels into tissues or cavities, and is a common problem in patients with cancer. All types of cancer can metastasize to any of the body's serous cavities and result in malignant effusion. In the Western World the most common cause of malignant ascites is ►ovarian cancer. Other common primary sites are the pancreas, stomach and uterus, with breast, lung, and lymphoma representing the commonest extra-abdominal sites. Up to 20% of all patients with malignant ascites have cancer of unknown primary origin (►CUP). Except in breast and ovarian cancer, the presence of malignant ascites in patients with neoplastic disease frequently signalizes the terminal phase of cancer. The mean survival time for ovarian cancer is 30–35 weeks and for tumors of lymphatic origin 58–78 weeks, whereas for cancers of the gastrointestinal tract the mean survival is only 12–20 weeks. In patients with CUP the median survival shows a great variability ranging from 1 week to 3 months in different series.

Pathophysiology

Fluid accumulation in the peritoneal cavity is dependent on the amount of fluid generated and the rate at which it leaves the abdominal cavity. When fluid production exceeds its clearance, free transudate will accumulate. Under physiologic conditions, transudation of plasma through capillary membranes of the peritoneal serosa continuously produces free fluid to lubricate the serosal surfaces. This fluid production is under the influence of portal pressure, plasma oncotic pressure, sodium and water retention, hepatic lymph production, and microvascular permeability for macromolecules. Under physiologic conditions, at least two-thirds of the peritoneal fluid reabsorbs into open-ended lymphatic channels of the diaphragm and is propelled cephalad by the negative intrathoracic pressure. This fluid proceeds through mediastinal lymph channels into the right thoracic duct and empties into the right subclavian vein. The ability of the healthy subject to resorb fluid is much greater than the fluid generated, with the result that there is normally only a small volume of approx. 50 mL of fluid in the peritoneal cavity.

Ascites as an abnormal accumulation of fluid in the peritonal cavity can be induced by several causes. In principle, four types of causes can be identified. (i) Ascites due to raised hydrostatic pressure, caused by cirrhosis, congestive heart failure, inferior vena caval obstruction, or hepatic vein occlusion. (ii) Ascites due to decreased osmotic pressure, caused by protein depletion (e.g. nephrotic syndrome), reduced protein intake (malnutrition) or reduced protein production (cirrhosis of the liver). (iii) Ascites due to fluid production exceeding resorptive capacity, caused by infections or malignancies. (iv) Chylous ascites, caused by obstruction and leakage of the lymph channels draining the gut.

The pathophysiology of malignant ascites is multifactorial and is yet incompletely understood. Ascites may result from obstruction of lymphatic drainage by tumor cells that prevent absorption of intraperitoneal fluid and protein as often seen in lymphomas and breast cancer. Since the ascites of many patients with malignant ascites has a high protein content, alteration in vascular permeability has been implicated in the pathogenesis of ascites production. The tumor induces increasing production of peritoneal fluid due to increased microvascular permeability of tumor vasculature and the amount of ascites production correlates with the extent of neovascularization. Aside from mechanical obstruction and cytokines, the pathophysiology of malignant ascites also consists of hormonal mechanisms. Due to decreased removal of fluid as a consequence of obstructed lymphatics, the circulating blood volume is reduced and this activates the renin-angiotensin-aldosterone system, leading to sodium retention. Therefore, reduced sodium intake together with diuretics is often used to treat malignant ascites, but there is no consensus on effectiveness. Available trials considering diuretics often include different patients groups with varying dose regimens and there are no randomized controlled trials to asses the efficacy of diuretics in malignant ascites.

Diagnosis

In most cases, ascites can be diagnosed by careful physical examination and taking a detailed history. The main clinical symptoms of ascites include abdominal

distension, ankle edema, continuous abdominal discomfort or pain, nausea, vomiting, shortness of breath and decreased mobility. Greater quantities of ascites cause abdominal distension, bulging flanks that are dull to percussion, shifting dullness and a fluid wave. Ultrasound is able to detect free peritoneal fluid if its volume is greater than 100 mL. CT and MRI are also able to detect little quantities of ascites. Malignant diagnosis is indistinguishable by physical examination from ascites caused by non-malignant conditions. Ascites detected by ultrasound, CT or MRI in the presence of typical imaging features of a malignant tumor is strongly suggestive of a malignant ascites. Diagnosis is confirmed by positive cytology of malignant cells in the fluid. A positive cytology result has a specificity of nearly 100%, but it is not very sensitive with only about 60% of malignant aspirates being cytologically positive. Compared to ascites caused by cirrhosis, malignant ascites usually contains more white blood cells and a higher level of lactate dehydrogenase. Fibronectin, cholesterol, lactate dehydrogenase, sialic acid, proteases, and antiproteases have been studied with fibronectin performing best in differentiating between malignant and non-malignant acites in most series. However, at present there is no single test available to be used routinely to differentiate between malignant and non-malignant ascites. Tumor markers, especially ►CEA and CA-125 can be useful in diagnosing the primary tumor in malignant ascites, although they lack specificity. In case of doubt abdominal ►paracentesis with chemical and cytologic analysis of the ascitic fluid should be used. The cell count provides immediate information about possible bacterial infection. Samples with a predominance of a least 250 neutrophils per cubic millimeter of ascitic fluid are suggestive of infection. Gram stains and culture for bacterial, fungal, and acid fast organisms are mandatory. ►Spontaneous bacterial peritonitis is characterized by the spontaneous infection of ascitic fluid in the absence of an intra-abdominal source of infection and involves the translocation of bacteria from the intestinal lumen to the lymph nodes, with subsequent bacteremia and infection of ascitic fluid. Third-generation cephalosporins are the treatment of choice. Ascitic fluid amylase content helps to detect pancreatic ascites and gut perforation. 80% of all cases of ascites are caused by cirrhosis of the liver. The chief factor contributing to ascites in liver cirrhosis is ►portal hypertension. Patients with ascites caused by liver disease usually have a ►serum-ascites albumin concentration gradient (calculated by subtracking the albumin concentration of the ascitic fluid from the albumin concentration of a serum specimen obtained on the same day) ≥1.1 g/dL. If serum albumin: ascites albumin gradient is less than 1.1g/dL, portal hypertension can be safely ruled out.

Treatment

In general, practice of managing malignant ascites seems to be influenced by the evidence obtained in the context of non-malignant ascites, especially ascites caused by liver disease. Malignant ascites only accounts for approximately 10% of all cases of ascites whereas over 80% of cases are caused by chronic liver disease. So, most evidence in treatment of ascites is obtained in the context of liver disease. In ascites caused by liver cirrhosis the most important treatments are the restriction of dietary sodium intake and the use of oral diuretics because patients with liver cirrhosis retain sodium and water as a result of the renin-angiotensin-aldosterone pathway. In ascites due to liver disease there is good evidence for the efficacy of a combined therapy with the diuretics spironolactone and furosemide supported by several randomized controlled trials. Treatment options for the minority of patients who are resistant to standard therapy with diuretics are therapeutic paracentesis, ►peritoneovenous shunting, ►transjugular intrahepatic portosystemic shunt (TIPS), extracorporal ultrafiltration of ascetic fluid with reinfusion and liver transplantation. Of these, ►transjugular portosystemic stent shunts, extracorporal ultrafiltration and liver transplantation are specific to liver diseases, whereas abdominal paracentesis and peritoneovenous shunting are often used in managing malignant ascites. In contrast to the treatment of underlying cancer, there is no generally accepted gold standard for the management of malignant ascites so far.

There are two principle approaches in managing malignant ascites. The first attempts to treat the cancer as the underlying cause of the ascites. The main treatments are systemic or intraperitoneal chemotherapies, biological therapies like intraperitoneal α or β ►interferon, tumor necrosis factor TNF or administration of infectious agents in non pathogenic form like corynebacterium parvum or OK-432, a penicillin- and heat-treated powder of Su-strain streptococcus pyogenes A3 in peritoneal cavity. Octreotide, a somatostatin analogue known to decrease the secretion of fluid by the intestinal mucosa and to increase water and electrolyte reabsorption, was used successfully in some case reports of malignant ascites. Novel therapies are radiolabeled monoclonal antibodies and radiocolloids. In tumors associated with increased activity of ►vascular endothelial growth factor (VEGF) like ovarian, gastric, colon, pancreatic carcinomas and omental or hepatic metastatic malignancies, a new concept is to reduce the production of ascites by the inhibition of neovascularization. This is achieved via inhibition of vascular endothelial growth factor (VEGF) or inhibition of ►matrix metalloproteinases, which are a family of enzymes present within the normal healthy individuals, but produced in high

concentrations by a variety of tumors. However, these concepts are comparatively new and are based on experimental results or only partially investigated in Phase I trials and have not yet been evaluated in randomized controlled trials.

The second approach in managing malignant ascites is palliative and relies upon reducing the volume of fluid through a variety of approaches like paracentesis, diuretics, or peritoneovenous shunts. Paracentesis is indicated for those patients who have symptoms of increasing intra-abdominal pressure. Available data show good, although temporary relief of symptoms in most patients. Symptoms seem to be significantly relieved by drainage of up to 5 L of fluid. When removing up to 5 L, intravenous fluids seem to be not routinely required. If the patient is hypotensive, dehydrated or known to have severe renal impairment and paracentesis is still indicated, intravenous hydration should be considered. The only investigated therapy in malignant ascites is infusion of dextrose 5%. There is no evidence of concurrent albumin infusions in patients with malignant ascites. To avoid repeated paracenteses, a peritoneovenous shunting may be considered. Major complications like pulmonary edema, pulmonary emboli, infection or clinically relevant disseminated intravascular coagulation have to be expected in about 6% of patients. There are no randomized trials assessing the efficacy of diuretic therapy. The available data are controversial and there are no clear predictors to identify which patients would benefit. Therefore, the use of diuretics should be considered in all patients with malignant ascites, but has to be evaluated individually. Patients with malignant ascites due to massive hepatic metastasis seem to respond more likely to diuretics than patients with malignant ascites caused by peritoneal carcinomatosis or chylous ascites. Choice of diuretics is also not sufficiently evaluated. As available data suggest that the efficacy of diuretics in malignant ascites depends on plasma renin/aldosterone concentration, aldosterone antagonists like spironolactone should be used, either alone or in combination with a loop diuretic.

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Aclarubicin

Definition

Is an anthracycline anticancer agent; Synonym aclacinomycin A.

►Adriamycin

Acquired Immunodeficiency Syndrome

Definition

AIDS; A life-threatening disease caused by he human immunodeficiency virus (►HIV) and characterized by breakdown of the body's immune defenses.

Acquired Mutation

Definition

A mutation (a genetic change) acquired by a somatic cell after conception.

►Gastrointestinal Stromal Tumor

Acromegaly

Definition

The name “acromegaly” comes from the Greek words for “extremities” (acro) and “great” (megaly), because one of the most common symptoms of this condition is abnormal growth of the hands and feet. A hormonal disorder that most commonly occurs in middle-aged men and women. The prevalence of acromegaly is approximately 4,676 cases per million population, and the incidence is 116.9 new cases per million per year. The symptoms of acromegaly can vary and they

develop gradually over time; therefore, a diagnosis of this condition may be difficult. Early detection is a goal in the management of acromegaly because the pathologic effects of increased growth hormone (GH) production are progressive.

ACRP30

►Adiponectin

ACTH

Definition

Adrencorticotropic Hormone.

►Corticotrophin

Actin

Definition

A protein that is found abundantly in all eukaryotic cells. The protein exists in monomeric (globular or Gactin) and polymeric forms (filamentous or F-actin). Filamentous actin bundles to form long fibers known as Actin Microfilaments or stress fibers. Actin is a globular structural protein that polymerizes in a helix to form an actin filament. Actin filaments determine the cell shape, stabilize the cell mechanically, enable cell movements, and participate in contraction of the cell during ►cytokinesis. Monomers of the protein actin polymerize to form long, thin fibers about 8 nm in a globular protein found in all cells. It is a major component in microfilaments and forms the contractile filaments in muscle cells.

►Huntingtin Interacting Protein 1 (HIP1)
►Micronucleus Assay
►Migration
►Tight Junction

Actin Cytoskeleton

The actin cytoskeleton is a dynamic structure of ►actin bundles and networks in the cytoplasm that provides a framework to maintain cell shape, protects the cell and enables cell locomotion. It also plays an important role in intra-cellular transport.

Actin Filament Severing

Definition

The breakage of noncovalent bonds between ►actin molecules within an actin filament.

►Geloslin

Actinic Keratosis

Definition

Scaly, erythematous patches found on the skin in sun-exposed areas. Radiation induced keratosis (hornification) of the skin. It represents a precancerous lesion also known as solar keratosis or senile keratosis. May undergo malignant progression to form squamous cell carcinoma.

►Epidermoid Carcinoma
►Squamous Cell Carcinoma
►Photodynamic Therapy

Actinomycin D

Definition

Synonym Dactinomycin. An antineoplastic antibiotic used for Nephroblastoma, Rhabdomyosarcoma, and trophoblastic disease in women.

►Placental Site Trophoblastic Tumor (PSTT)

Activated Fibroblasts

►Myofibroblasts

Activated Natural Killer Cells

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Synonyms

K cells; killer cells; K lymphocyte; large granular lymphocyte; lymphokine activated killer; LAK

Definition

White blood cells that kill tumor and virus-infected cells as part of the body's immune system (Unified Medical Language System). A type of white blood cell that contains granules with enzymes that can kill tumor cells or microbial cells (National Cancer Institute). A circulating cellular biosensor, regulating immunity through release of cytokines, maturation of dendritic cells, and recognition and lysis of stressed cells, allowing sampling of cellular contents for delivery to phagocytic cells (our definition).

Characteristics

Biology of NK Cells

►Natural Killer cells comprise 10–15% of circulating lymphocytes in normal adults and are also found in peripheral tissues, including the liver, peritoneal cavity, lymph nodes, and placenta. NK cells were first reported by Wunderlich, Herberman, and Sendo and others in the early 1970s. They were first discovered on the basis of their nonspecific killer activity, disturbing attempts to generate tumor-specific, ►MHC-restricted cytotoxic T lymphocytes (CTLs). NK cell belongs to the innate immune system, bridging ►adaptive immunity in concert with ►dendritic cells. NK cells play a major role in the host defense against tumors and infected cells. NK cells mediate cytolysis of cultured tumor cells, and when lymphokine activated (LAK activity), against freshly acquired tumor cells. "Natural killer" suggests the initial notion that they do not require activation in order to kill target cells. NK cells are large granular lymphocytes (LGL). The targets of NK cells are stressed cells expressing either "nonself" or "the self that changed in quality," prompting their recognition. NK cells, when activated, can recognize cells which fail to express cognate self MHC molecules and simultaneously express (stress-induced) ligands recognized by activating NK receptors. These ligands include MICA/MICB ULPBs, PVR, and Nectin-2 in humans or Rae-1 in mice. NK cytolytic activity is almost nonexistent at birth, increases until 15 years of age, and then gradually reduces through old age. Natural killer cells (NK cells) lack the ability to destroy tumor cells at the time of birth, acquiring cytolytic capacity following recognition. Given their ready acquisition from the peripheral blood, multiple studies have evaluated their activity in various clinical studies; for example, chronic mental stress, fatigue, and physical exertion suppress NK activity. Reduced NK activity may be related to increasing cancer risk. Patients deficient in NK cells prove to be highly susceptible to early phases of herpes virus infection. Many studies indicate that NK activity is reduced in patients with advanced cancer. Tumor infiltrating NK cells of pediatric cancer are significantly less in number than that observed in adult cancers, prompting the notion that this creates a major nosologic difference of adult and pediatric neoplasms.

Role of NK Cells in Human Cancer

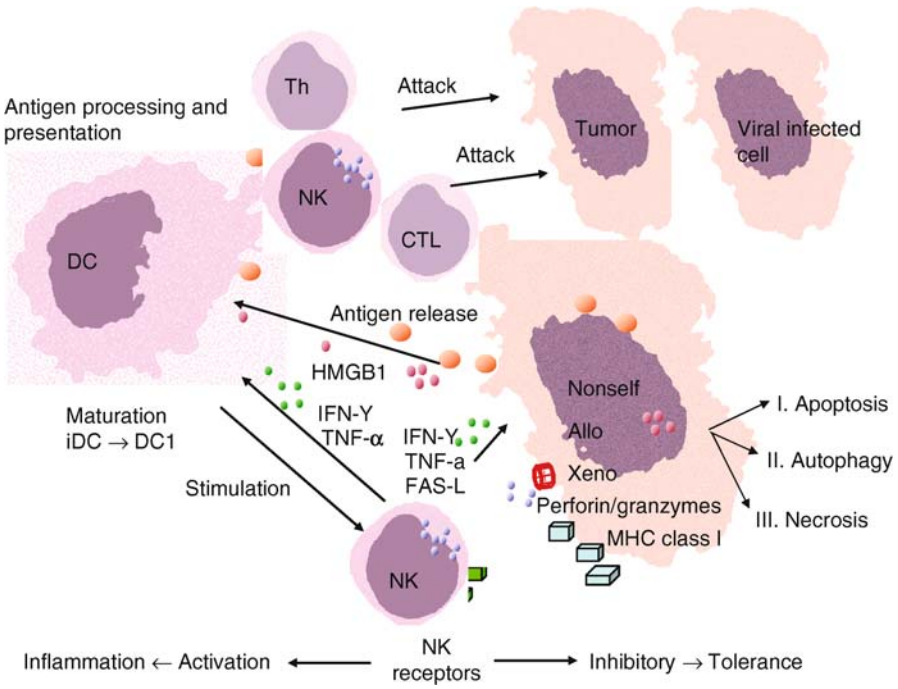
NK cells induce tumor cell death when NK cells recognize tumor cells with NK cell activating receptors. NK cells produce many cytokines including ►IFNs and ►TNF-α and suppress proliferation of tumor and cells and drive type 1 immunity. NK cells help dendritic cells to mature into DC1. NK cells have some suppressive roles against cancer. NK cells have inhibitory receptors. They become tolerant to tumor cells when inhibitory receptors are stimulated with their ligands (Fig. 1).

Markers of NK Cells

NK cells express CD16 (FcγRIII), CD56, ►CD57, CD94, or CD158a. They do not express ►T-cell receptor (TCR) or the pan T cell marker ►CD3 or surface immunoglobulins (Ig) B cell receptor (►CD20). NK cells recognize specific polysaccharide on target cells with NK receptor (CD161; NKR-P1) and expression of MHC class I molecules.

NK Cell Receptors

There are two main types of receptors for MHC class I on NK cells including the KIR (killer cell immunoglobulin-like receptors, one of the immunoglobulin superfamily) and NKG2 receptor (CD94, type C lectin family). In both, there are activating and suppressing forms that accelerate or suppress NK activity. Two explanations for NK-cell self-tolerance have been proposed: first, NK cells from MHC-class-I-deficient hosts have a lower activation potential, owing to decreased activating-receptor expression and/or



Activated Natural Killer Cells. Figure 1 Role of NK cells in tumor immunity. NK cells play multiple roles in tumor immunity. They recognize stressed cells or those failing to express cognate Class I major histocompability molecules, both lysing targets and serving as a source of cytokines important in initiation and perpetuation of the inflammatory response is carried out by them. They serve as helper cells, promoting immune interaction with both T and dendritic cells, critically being required for initiation of the TH1 response. Their absence may also be important in limiting autoimmunity as revealed by their critical absence in the NOD mouse strain, susceptible to autoimmune diabetes. When lysing cells, normal cells capable of undergoing apoptotic or autophagic00128 death, Types I and II death, do so. Many virally infected or transformed cells fail to undergo such death because of block of these pathways, and when lysed, undergo necrotic cell death causing DC maturation and promoting recruitment of additional inflammatory cells. In the absence of viral or bacterial pathogen signals, such chronic necrotic cell death is associated with inhibition of immune effectors and promotion of a wound repair phenotype with angiogenesis and stromagenesis, characteristic of many tumors.

function; or second, NK cells are kept self-tolerant by interactions between non-MHC-dependent receptor–ligand pairs CD94: NKG2, a C-type lectin family receptor, is conserved in both rodents and primates and identifies nonclassical (also nonpolymorphic) MHC I molecules including HLA E. Though indirect, this is a means to survey the levels of classical (polymorphic) HLA molecules. Expression of HLA E at the cell surface is dependent upon the presence of classical MHC class I leader peptides. Ly49 is a relatively ancient, C-type lectin family receptor. Humans have only one pseudogenic Ly49; the receptor for classical MHC I molecules. KIRs belong to a multigene family of more recently evolved Ig-like extracellular domain receptors. They are present in nonrodent primates and are the primary receptors for both classical MHC I (HLA A, HLA B, HLA C) and nonclassical HLA G in primates. KIRs are specific for certain HLA subtypes. ILT or LIR (leucocyte inhibitory receptors) are recently discovered members of the Ig receptor family. ►Carcinoembryonic antigen related cell adhesion molecule 1 (►CEACAM1 Adhesion Molecule) is an inhibitory receptor and its ligands are CEACAM1 itself and CEACAM5, known as CEA. Sialic acid binding immunoglobulin-like lectins (SIGLECs) have a V-set immunoglobulin domain, which binds sialic acid, and varying numbers of C2-set immunoglobulin domains. IRp60, KLRG1, and LAIR1 are other inhibitory receptors recently discovered (Table 1).

NK Cell and Cytokines

NK cells are capable of producing many cytokines including IFN-γ (►Interferon-*f* γ), IFN-α, IFN-β, and TNF-α. They suppress proliferation of tumor and virally infected cells and regulate immune responses. IFN-γ (Interferon-*f* γ) increases NK activity as a positive feedback mechanism. NK cytolytic activity is increased by IFN-α, IFN-β, and IFN-γ (Interferon-*f* γ) (produced by T and NK cells), IL-2\(produced by T cells), IL-10, IL-12, and IL-15 (produced by B cell, monocyte/macrophage, or dendritic cells). NK cytolytic activity is inhibited by IL-4 (►Interleukin-4). IL-15 induces

Activated Natural Killer Cells. Table 1 Inhibitory and Activating NK Cell Receptors and their Ligands Activating NK cell receptors

Receptors	Ligands
2B4	CD48
NKp44	Influenza/unknown
NKp30	
NKp46	Influenza/unknown
CD16	IgG
NKG2D02396	MICA, MICB
NKp80	
DNAM	CD112/CD155
Inhibitory NK cell receptors	
ILT2	MHC01094-A, B, G
KIR3DL2	MHC01094-A
KIR3DL1	MHC01094-B
KIR2DL4	MHC01094-A, B, G
KIR2DL1,2,3	MHC01094-C
CD94	MHC01094-C
CEACAM102441	CEACAM102441, CEACAM5
IRp60	Unknown
KLRG1	Unknown
LAIR1	Unknown
SIGLEC7	Sialic acid
SIGLEC9	Sialic acid

NK cell proliferation. IL-12 induces IFN-γ (Interferon-*f* γ) production by NK cells. IFN-α, IFN-β, IFN-γ (Interferon-*f* γ), and TNF-α produced by NK cells activate monocyte/macrophage, vascular endothelial cells, neutrophils, and induce a local inflammation response.

Cytotoxicity of NK Cells against Tumor or Infected Cells

NK cells release ►perforin from intracellular granules when they bind to target cells, along with granules containing serine proteases known as ►granzymes. Perforin attaches to the membrane inducing an autophagic (►Autophagy) repair process, inducing uptake of vesicles containing granzymes and associated molecules that can target cells for lysis, with perforin allowing escape through pore formation once intracellular. Granzyme induce apoptosis to the target cells utilizing various intracellular pathways. NK cells also induce ►apoptosis to target cells by expressing apoptosis inducing molecules such as FAS ligands or ►TRAIL on the cell surface. The distinction between apoptosis and ►necrosis is important in cancer immunology – necrotic cells release danger/damage associated molecular pattern molecules (DAMPs) such as high-mobility group box 1 (HMGB1) protein, whereas apoptosis leads to retention of HMGB1 within the cells or apoptotic nuclei.

NK Cells and Cancer Immunotherapy

Their rapid cytolytic action and broad target range suggest that NK cells may be promising candidates for cancer cell therapy. The clinical application of ex vivo manipulated cells, including NK cells, is referred to as ►adoptive immunotherapy (AIT). The first clinical AIT trial exploited autologous ex vivo expanded and interleukin 2 (IL-2) stimulated ►lymphokine activated killer (LAK). Although this approach produced nearly 15–20% partial and complete responses in initial trials, subsequent studies showed that a similar antitumor effect could be achieved with administration of high dose IL-2 alone. Purification and enrichment of NK cells on a clinical scale may improve therapeutic outcomes. Alternatively, stimulation of LAK cells with IL-15 or IL-21 instead of IL-2 might increase efficacy.

Myeloid ►dendritic cells (mDCs) support the tumoricidal activity of NK cells, while cytokine-preactivated NK cells activate DCs and induce their maturation and cytokine production. NK–DC interactions promote the subsequent induction of tumor-specific responses of CD4+ and CD8+ T cells, allowing NK cells to act as nominal "helper" cells in the development of the desirable type-1 responses to cancer. NK–DC interaction provides a strong rationale for the combined use of NK cells and DCs in the immunotherapy of patients with cancer. Clinical trials that are being

implemented at present should allow evaluation of the immunological and clinical efficacy of combined NK–DC therapy of melanoma and other cancers.

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Activated Vitamin D

►Calcitriol

Activation Loop

Definition

A 20–25-residue segment within the catalytic domain of protein kinases that functions to regulate their kinase activity.

►B-Raf Signaling

Activator Protein-1 (AP-1)

Definition

A dimeric complex that contains members of the c-jun, c-fos, ATF and MAF protein families. The ►AP-1 transcription factors activate various genes critical in the initiation of DNA synthesis. In ►mesothelioma, it is thought that the persistent induction of these transcription activators following ►asbestos exposure enhances cell division and favors malignant growth.

►Mesothelioma
►Polyphenols
►Retinoid Receptor Cross-talk
►Simian Virus 40
►SV40

Active

►Melanoma Vaccines

Active Cell Death

►Apoptosis

Active Immunity

Definition

Immunity produced by the body in response to stimulation by a disease-causing organism or a vaccine.

Acute Granulocytic Leukemia

►Acute Myeloid Leukemia

Acute Lung Injury (ALI)

Definition

A distinct form of acute respiratory failure characterized by diffuse pulmonary infiltrates, progressive hypoxemia, reduced lung compliance, and normal hydrostatic

pressures. ALI is caused by any stimulus of local or systemic inflammation, principally sepsis.

►Sivelestat

Acute Lymphoblastic Leukemia

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Synonyms

Acute lymphoblastic leukemia; ALL

Definition

Acute lymphoblastic leukemia (ALL) is a malignant disease that arises from several cooperative genetic mutations in a single B or T-lymphoid progenitor, leading to altered blast cell proliferation, survival, and maturation, and eventually to the lethal accumulation of leukemic cells. Although cases can be subclassified further according to the stages of T or B-cell maturation, these distinctions are not therapeutically useful, except for the recognition of a mature B-cell, B-cell precursor, or T-cell stage.

Characteristics

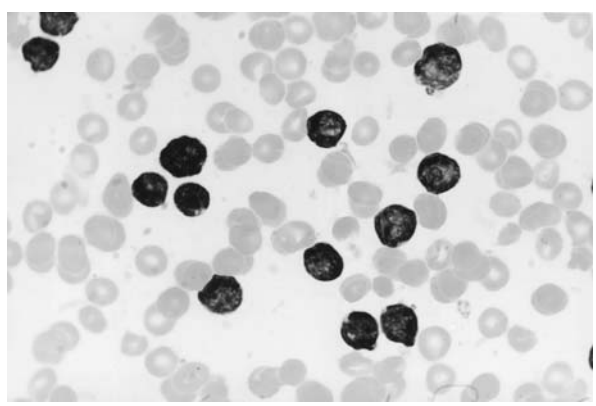
ALL accounts for about 12% of all childhood and adult leukemias diagnosed in developed countries and for 60% of those diagnosed in persons younger than 20 years. It is the most common cancer in children (25% of all cases) and has a peak incidence in patients between the ages of 2 and 5 years, with a second, smaller peak in the elderly.

The factors predisposing children and adults to ALL remain largely unknown. Fewer than 5% cases are associated with inherited genetic syndromes defined by chromosomal instability and defective DNA repair. Ionizing radiation and mutagenic chemicals have been implicated in some cases of ALL, but their contributions appear negligible. Nonetheless, evidence collected over the past two decades has revealed that ALL is essentially a disease of acquired genetic abnormalities. Specific genetic abnormalities are found in the leukemic cells of approximately 75% of patients with ALL to date, and in all likelihood, will be identified in all cases with the improved genetic techniques. These include chromosomal translocations and chromosomal gains or losses, resulting in ►hyperdiploidy or ►hypodiploidy, respectively. Chromosomal translocations often activate transcription factor genes, which in many cases control cell differentiation, are developmentally regulated, and frequently encode proteins at the tops of critical transcriptional cascades. These "master" oncogenic transcription factors, which can exert either positive or negative control over downstream responder genes, are aberrantly expressed in leukemic cells as a single gene product or as a unique fusion protein combining elements from two different transcription factors. Recently, activating mutations of ►*NOTCH1*, a gene encoding a transmembrane receptor that regulates normal T-cell development, and mutations of ►*PAX5*, a gene essential for B-lineage commitment and maintenance, have been identified to be most frequent cooperative mutations in T-cell and B-cell precursor ALL, respectively.

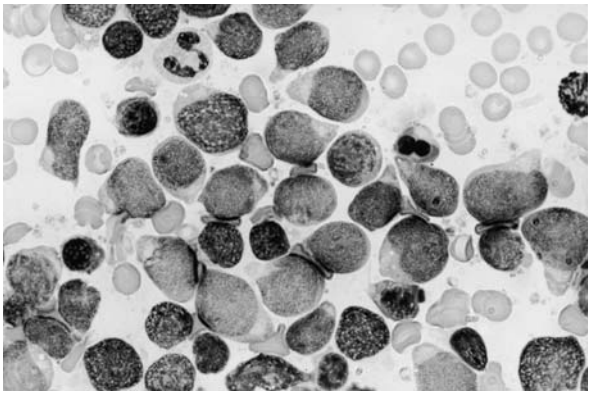
Although most leukemias begin in the bone marrow and spread to other parts of the body, some may arise in an ►extramedullary site, such as the thymus or intestine, and subsequently invade the bone marrow. The presenting features of ALL generally reflect the degree of bone marrow failure and the extent of extramedullary spread. Common signs and symptoms are

- Fever
- Fatigue and lethargy
- Dyspnea, angina, and dizziness (older patients mainly)
- Limp, bone pain, or refusal to walk (young children)
- Pallor and bleeding in the skin or mouth cavity
- Enlarged liver, spleen, and lymph nodes (more pronounced in children)
- Anemia, low neutrophil count, and low platelet count
- Metabolic abnormalities (e.g., high serum uric acid and phosphorus levels)

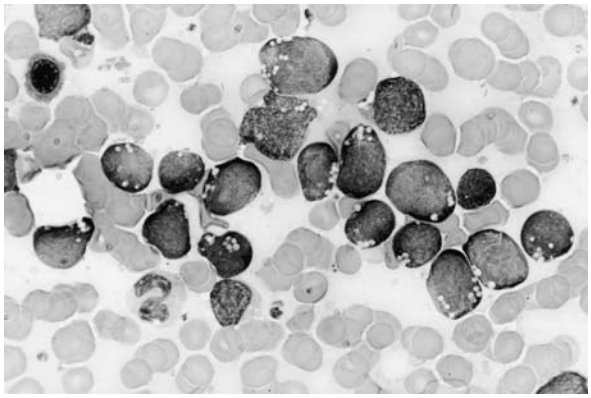
The diagnosis of ALL is based on a morphologic examination of bone marrow cells (Figs. 1–3) and immunophenotype of cells from the same sample.



Acute Lymphoblastic Leukemia. Figure 1 Small regular blasts with scanty cytoplasm, homogeneous nuclear chromatin, and inconspicuous nucleoli.



Acute Lymphoblastic Leukemia. Figure 2 Admixture of large blasts with moderate amounts of cytoplasm and smaller blasts. Such cases may be mistaken for acute myeloid leukemia, emphasizing the importance of immunophenotyping and genotyping to corroborate the differential diagnosis.



Acute Lymphoblastic Leukemia. Figure 3 Mature B-cell ALL blasts characterized by intensely basophilic cytoplasm, regular cellular features, prominent nucleoli, and cytoplasmic vacuolation.

Karyotyping, fluorescence in situ hybridization (FISH), and molecular genetic analysis by RT-PCR (reverse transcriptase-polymerase chain reaction) are now routinely performed by many centers to identify subtypes of ALL with prognostic and therapeutic significance, for example:

- *BCR-ABL* fusion gene due to the t(9;22), or ►Philadelphia chromosome – 25% of adult cases and 3–4% of childhood cases (dismal prognosis)
- ►*TEL-AML1* fusion gene due to a cryptic t(12;21) – 22% of childhood cases (favorable prognosis)
- Hyperdiploidy (more than 50 chromosomes per cell) – 25% of childhood cases (favorable prognosis)
- Hypodiploidy (fewer than 45 chromosomes per cell) – 1% of childhood cases and 2% of adult cases (unfavorable prognosis)

Contemporary risk-directed treatment can cure 80% or more of children and up to 40% of adults with ALL. Cases are generally classified as standard or high risk in adults and as low, standard, and high risk in children. Factors used to determine the relapse hazard include the presenting leukocyte count, age at diagnosis, gender, immunophenotype, ►karyotype, molecular genetic abnormalities, initial response to therapy, and the amount of "minimal residual leukemia" upon achieving a complete ►remission.

Multidrug remission induction regimens almost always include a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and at least a third agent (L-asparaginase or anthracycline), administered for 4–6 weeks. Some treatments rely on additional agents to increase the level of cell kill, thereby reducing the likelihood of the development of drug resistance and subsequent relapse. However, several studies suggest that intensive remission induction therapy may not be necessary for low or standard-risk patients, provided that they receive postinduction intensification therapy. Remission induction rates now range from 97 to 99% in children and from 78 to 93% in adults. Complete clinical remission is traditionally defined as restoration of normal blood cell formation with a blast cell fraction of less than 5% by light microscopic examination of the bone marrow. With this definition, some patients in complete remission may harbor as many as 1×10^{10} leukemic cells in their body. With sensitive and specific methods developed to measure minimal residual disease, it is now recognized that most patients actually have less than 0.01% of residual leukemia after 4–6 weeks of remission induction therapy, and they have excellent treatment outcome. By contrast, patients with 1% or more leukemic cells after remission induction treatment have a poor prognosis and may be candidates for hematopoietic stem cell transplantation. To improve treatment outcome, most protocols specify an intensification (or consolidation) phase in which several effective antileukemic drugs are administered in high doses soon after the patients attain a complete remission. Reinduction treatment, essentially a repetition of the initial induction therapy administered during the first few months of remission, has become an integral component of successful ALL treatment protocol.

Regardless of the intensity of induction, consolidation or reinduction therapy, all children require 2–3 years of continuation treatment, usually methotrexate and mercaptopurine, with pulses of vincristine and dexamethasone for low-risk cases, and multiagent intensive chemotherapy for standard and high-risk cases. The need for continuation therapy in adults is less clear, although in most cases it is discontinued after 2–2½ years of complete remission. The central nervous system can be a ►sanctuary site for leukemic cells, requiring intensive,

intrathecally administered chemotherapy that begins early during the remission induction phase, extending through the consolidation phase and into the continuation phase. Once considered standard treatment, cranial irradiation is now reserved for less than 10% of patients who are at very high risk of relapse in the central nervous system.

For selected high-risk cases, such as patients with Philadelphia chromosome-positive ALL, and those who require extended therapy to attain initial complete remission, hematopoietic stem-cell transplantation is currently the treatment of choice. In light of the development of new therapeutics, the indications for transplantation should be continuously evaluated. For example, therapy with imatinib mesylate (Gleevec; Novartis) or second-generation tyrosine kinase inhibitors has improved the duration of remission of patients with Philadelphia chromosome-positive ALL, but whether this therapy will increase the cure rate remains to be determined. Finally, the optimal clinical management of patients with ALL requires careful attention to methods for the prevention or treatment of metabolic and infectious complications, which may otherwise be fatal.

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Acute Megakaryoblastic Leukemia

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Synonyms

Acute Myeloid Leukemia; Subtype AML-M7 according to the French-American-British (FAB) Classification; Myeloid Leukemia of Down Syndrome (DS-ML); WHO classification: Acute Megakaryoblastic Leukemia (M7)

Definition

Acute Megakaryoblastic Leukemia (AMKL) is defined as a malignant ►clonal proliferation of immature hematopoietic cells of the megakaryocytic lineage. AMKL is a subtype of Acute Myeloid Leukemia (AML). The biologic features of AMKL are heterogeneous and ongoing characterization of the disease pathogenesis is likely to lead to a novel clinically meaningful classification of the disease.

Characteristics

Epidemiology

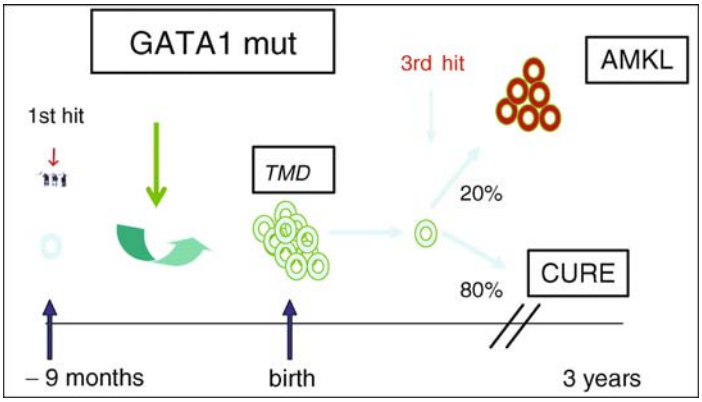
AMKL is diagnosed in 7–10% of infants and children with AML without Down syndrome (DS). In most pediatric cases the disease occurs de novo and subgroups can be identified based on cytogenetic features or biological features as described later. In contrast, AMKL is rare in adults, occurring in 1–2% of all AML cases and is frequently associated with antecedent hematological disorder such as myelodysplastic syndrome.

Children with DS have a markedly increased risk to developing AMKL and represent up to 10 % of children with AML. A large proportion of Children with DS (estimated 10%) are born with a unique transient form of AMKL, often called transient myeloproliferative disorder (TMD) or transient abnormal myelopoiesis (TAM). This congenital leukemia resolves spontaneously in most of the patients. Up to 20% of those patients will relapse with a full blown AMKL by the age of 4 years. Thus the leukemia of DS represents a unique clinical entity of multistep leukemogenesis (Fig. 1).

Clinical and pathologic features

Typical features at diagnosis include hepato-splenomegaly, anemia, thrombocytopenia and myelofibrosis. The fibrosis is probably caused by soluble factors (such as TGF-β) secreted from the malignant megakaryoblasts. Infants with DS may exhibit marked liver failure that sometimes may be life threatening. The liver failure is secondary to liver fibrosis caused by the infiltration of leukemic cells.

►Flow cytometry is the preferred method for ►immunophenotypic characterization of AMKL, although in some cases the diagnosis can only be made from bone marrow or liver biopsies due to extensive myelofibrosis. Typically, the leukemic blasts express at least one megakaryoblastic antigen [CD41(GPIIb)/CD42b(GPIbalpha) or CD61]. Coexpression of the T-lineage marker CD7 is frequently observed, suggesting pathogenic mechanism that could lead to aberrant regulation of lymphoid genes. Expression of erythroid markers (e.g. glycophorin A) and of CD36 (thrombospondine receptor) characterize the AMKL of DS. Because AMKL blasts may display low expression levels of the pan-hematopoietic CD45 antigen, the distinction from metastatic solid tumours may be challenging.



Acute Megakaryoblastic Leukemia. Figure 1 Multistep evolution of AMKL in Down Syndrome. Mutation in GATA1 is acquired during fetal liver hematopoiesis in cells carrying a germline trisomy 21 results in congenital clonal megakaryoblastic proliferation (TMD). In almost all patients TMD resolves spontaneously leading to cure. However in about 20% of the patients additional postnatal acquired mutations in residual cells from the resolved TMD results in the development of full blown acute megakaryocytic leukemia (AMKL) during early childhood.

Cytogenetic and Biological Features

Increasing evidence suggest that distinct subtypes of AMKL can be identified based on genetic and molecular characteristics. Recurrent cytogenetic abnormalities are specifically associated with AMKL and at least in part convey a prognostic significance.

The megakaryoblastic disorders associated with DS (both AMKL and TMD) are characterized by the presence of an acquired mutation in the ►transcription factor GATA1. The mutations occur in exon 2 or in the beginning of exon 3 and uniformly results in the production of a short GATA1 protein (GATA1s) that lacks the amino-terminal of the full length GATA1. GATA1 is a major regulator of normal megakaryopiesis. GATA1s blocks terminal differentiation and enhances proliferation of immature fetal megakaryoblasts. The mutations occur during ►fetal liver hematopoiesis. The initiation of the leukemia during fetal liver hematopoiesis explains the frequent liver dysfunction observed in DS newborns with TMD. Strikingly, GATA1 is located on chromosome X and is mutated only in AMKL with trisomy 21. The precise mechanism by which trisomy 21 promotes the survival of cells with acquired mutation in GATA1 is presently unknown. One hypothesis suggests that genes on chromosome 21 code proteins that enhance fetal megakaryopoiesis. This developmental pressure of megakaryopoiesis coupled with differentiation arresting mutation in GATA1 cause clonal accumulation of megakaryoblasts diagnosed at birth as TMD. GATA1 mutation is necessary and probably sufficient for the development TMD, but additional mutations are required for the occurrence of full blown AMKL in DS patients. Why TMD spontaneously resolves and which mutations cause further evolution to AMKL is largely unknown.

There are several biological subgroups among patients with AMKL that do not have DS. The most frequent recurrent chromosomal aberration detected in non-DS AMKL is the translocation t(1;22), which typically occurs in infants and very young children that present with hepatosplenomegaly and pronounced myelofibrosis. This translocation fuses RBM15/OTT1, an RNA export factor to MKL1/MAL1, a cofactor of the transcription factor SRF (Serum Response Factor). Less commonly fusion translocations between the MLL gene and different partners, often AF10, have been reported in AMKL. Interestingly, a second translocation involving AF10, the translocation t(10;11) which results in the fusion of CALM (clathrin-assembly protein-like lymphoid myeloid) with AF10 was reported in several cases. This translocation was also identified in other AML subtypes and in cases of T-cell ALL. In a mouse model, infection of bone marrow cells with a retroviral vector to express CALM-AF10 results in a transplantable AML, demonstrating that this fusion gene represents a fundamental leukemogenic event.

By ►gene expression profiling, at least two distinct classes of non-DS AMKL could be discriminated based on their molecular phenotype. Approximately one third of the cases display an erythroid expression pattern coupled with expression of CD36 and higher expression levels of the transcription factor GATA1 in absence of detectable mutations. Interestingly this gene expression signature is reminiscent to the increased expression of erythroid markers detected in AMKL from DS patients, which are characterized by increased expression levels of mutated GATA1s. The second subtype of non-DS AMKL samples include all cases with recurrent translocation t(1;22). Interestingly, samples that share similar expression profiles with the samples positive for the translocation t(1;22) are characterized by increased expression levels of another SRF cofactor, HOP,

suggesting that similar regulatory pathways may be involved. This second class is associated with higher levels of expression of the surface antigen CD44, which was associated with worse outcome in other type of malignancies and coexpressed on the leukemia initiating cells from patients with AML. It is currently not possible to determine if the distinction of these two classes by expression profiling has a prognostic significance due to the small numbers of patients that were treated on different therapeutic protocols. A prospective study using selected genes from the AMKL signature will be required to determine if this information could be used as prognostic marker to guide selection of treatment intensity.

Prognosis and Treatment

Treatment results from several international study groups, including the European AML-BFM study group and UK-MRC cooperative groups, and the north american SJCRH and CCG cooperative groups show a marked difference in treatment outcome between DS and non-DS AMKL. Reduction of treatment intensity for patients with DS resulted in a marked decrease in treatment related mortality and an excellent treatment outcome (91% event free survival at 5 years in the AML-BFM 98 study), strongly suggesting a distinct leukemia biology between DS and non-DS AMKL patients. AMKL blasts from patients with DS are extremely sensitive to the chemotherapy drug Cytosine Arabinoside (ARA-C), probably due to a decrease in its cellular degradation caused by an enzyme regulated by GATA1.

The results for patients with AMKL excluding patients with DS are still poor, despite of intensification of AML treatment regimens. The 5–year event free survival (EFS) reported for the most recent treatment regimen correspond to results obtained for other AML subtypes, with EFS of 42% reported for the AML-BFM93/98 trials and of 47% reported by the UK-MRC 10 and 12 trials. Further research is necessary to identify new treatment modalities and biomarkers to guide treatment intensification, including the indication for bone marrow transplantation for patients at highest risk of relapse. Recent data in mouse models suggest that targeted therapy with antibodies directed against the surface marker CD44 may be a future therapeutic.

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Acute Myelogenous Leukemia

►Acute Myeloid Leukemia

Acute Myeloid Leukemia

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Synonyms

Acute myelogenous leukemia; Acute nonlymphocytic leukemia; ANLL; Acute granulocytic leukemia

Definition

Acute myeloid leukemia (AML) is part of a group of hematological malignancies (►hematological malignancies) in the bone marrow involving cells committed to the ►myeloid line of cellular development. It is defined by the malignant transformation of a bone marrow-derived, self-renewing stem cell or progenitor (►stem cells and cancer) which demonstrates a decreased rate of self-destruction and aberrant ►differentiation. Uncontrolled growth of such cells, named blasts, is the result of ►clonal proliferation. Blasts accumulate in the bone marrow and other organs. As a result, mature cells of ►hematopoiesis are suppressed. For the leukemia to be called acute, the bone marrow must include greater than 20% leukemic blasts.

Characteristics

Classification

The first comprehensive morphologic-histochemical classification system for AML was developed by the French-American-British (FAB) Cooperative Group. This classification system categorizes AML into eight major subtypes (M0 to M7) based on morphology and

immunohistochemical detection of lineage markers. This classification of AML was recently revised under the auspices of the World Health Organization (WHO) (see list 1). While elements of the older FAB classification were preserved, the WHO classification incorporates and interrelates morphology, cytogenetics, molecular genetics, immunologic markers, and clinical features in an attempt to define categories that are biologically homogeneous and that have prognostic and therapeutic relevance.

The most significant difference between the WHO and FAB classifications is that the minimum blast percentage for the diagnosis of AML is at least 20% blasts in the blood or bone marrow (the FAB scheme required the blast percentage to be at least 30%). What was known as "refractory anemia with excess blasts in transformation" (RAEB-t) of myelodysplastic syndromes (►MDS), is now included within the broader category of "AML with multilineage dysplasia" as "AML with multilineage dysplasia following a MDS."

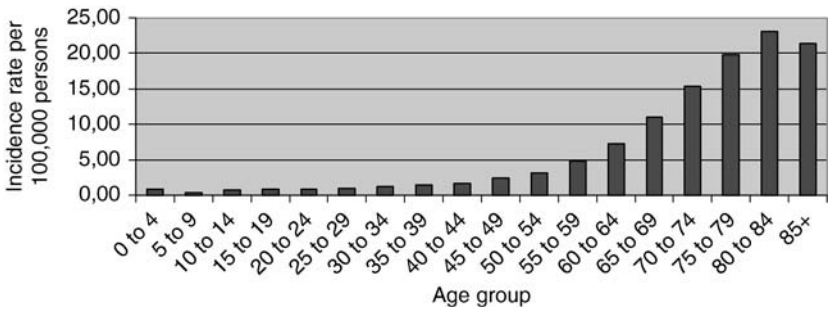
List 1 – WHO classification of AML (the older FAB classifications are given in parentheses where appropriate):

- AML with characteristic genetic abnormalities
  - AML with t(8;21)(q22;q22); (AML/ETO)
  - AML with inv(16)(p13q22) or t(16;16)(p13; q22); (CBFβ/MYH11)
  - Acute promyelocytic leukemia (AML with t(15;17)(q22;q12); (PML/RARα) and variants)
  - AML with 11q23 (MLL) abnormalities
- AML with multilineage dysplasia
  - AML with prior MDS
  - AML without prior MDS
- AML and MDS, therapy-related
  - Alkylating agent-related AML and MDS
  - Topoisomerase II inhibitor-related AML
- AML not otherwise categorized
  - Acute myeloblastic leukemia, minimally differentiated (FAB M0)
  - Acute myeloblastic leukemia without maturation (FAB M1)
  - Acute myeloblastic leukemia with maturation (FAB M2)
  - Acute myelomonocytic leukemia (AMML) (FAB M4)
  - Acute monoblastic leukemia and acute monocytic leukemia (FAB M5a and M5b)
  - Acute erythroid leukemias (FAB M6a and M6b)
  - Acute megakaryoblastic leukemia (FAB M7)
  - AML/transient myeloproliferative disorder in Down syndrome
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
  - Myeloid sarcoma
- Acute leukemias of ambiguous lineage

Epidemiology

AML is infrequent but highly malignant, responsible for a large number of cancer-related deaths. AML accounts for approximately 25% of all leukemias in adults in industrialized countries and, thus, is the most frequent form of leukemia. Worldwide, the incidence of AML is highest in the United States, Australia, and Western Europe.

The age-adjusted incidence rate of AML in the United States in the years 1975–2003 has been relatively stable at approximately 3.4 per 100,000 persons (=2.5 per 100,000 when age-adjusted to the world standard population). The American Cancer Society estimates that 11,930 individuals will be diagnosed with AML in 2006 in the United States. Patients that are newly diagnosed with AML have a median age of 65 years. From 2000 to 2003, the U.S. incidence rate in people under the age of 65 was only 1.8 per 100,000, while the incidence rate in people aged 65 or over was 17 per 100,000 (Fig. 1). AML is thus primarily a disease of later adulthood with an age-dependent mortality of 2.7 to nearly 18 per 100,000. The incidence of AML varies to a small degree depending on gender and race. AML in adults is slightly more prevalent in males in most countries. In the US in 2000, AML was more common in Whites with 3.8 per 100,000 than in Blacks (3.2 per 100,000).



Acute Myeloid Leukemia. Figure 1 Age-specific incidence of AML (USA: 2000–2003) *(Source: SEER)*.

Acute Myeloid Leukemia. Table 1 Risk factors

Genetic disorders	Down syndrome
	Klinefelter syndrome
	Patau syndrome
	Ataxia telangiectasia
	Shwachman syndrome
	Kostman syndrome
	Neurofibromatosis
	Fanconi anemia
	Li–Fraumeni syndrome
Physical and chemical exposure	Benzene
	Drugs as pipobroman
	Pesticides
	Cigarette smoking
	Embalming fluids
	Herbicides
Radiation Exposure	Non-/therapeutic radiation
Chemotherapy	Alkylating agents topoisomerase II inhibitors
	Anthracyclines
	Taxanes

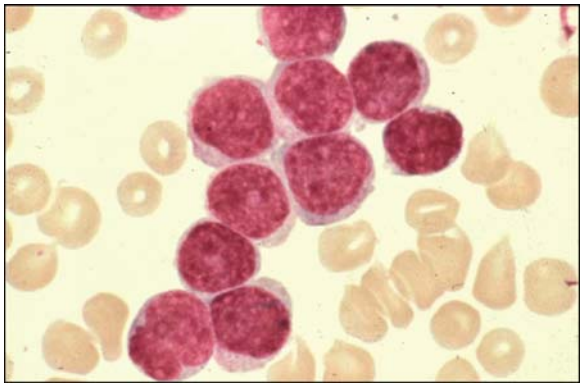
Etiology

The development of AML has been associated with several risk factors summarized in Table 1.

Generally, only a small number of observed cases can be traced back to known risk factors. These include age, antecedent hematological disease, genetic disorders as well as exposures to radiation, chemical or other hazardous substances (e.g., benzene), and previous chemotherapy (e.g., treatment with ►alkylating agents). Leukemogenesis, like ►carcinogenesis is a multistep process that requires the susceptibility of a hematopoietic progenitor cell to inductive agents at multiple stages. The different subtypes of AML may have distinct causal mechanisms, suggesting a functional link between a particular molecular abnormality or mutation and the causal agent. Most cases of AML arise without objectifiable leukemogenic exposure.

Signs and Symptoms of AML

AML can cause different uncharacteristic signs and symptoms such as weight loss, unusual fatigue, and fever. Many patients feel a loss of well-being. Most symptoms can be traced back to bone marrow insufficiency: Anemia, immunodeficiency caused by neutropenia, and thrombocytopenia. Diagnostic procedures and types of specimen necessary to reach the diagnosis of AML are the following:



Acute Myeloid Leukemia. Figure 2 Myeloid blasts in peripheral blood detected by light microscopy.

- Blood cell counts and microscopic blood cell examination (Fig. 2)
- Bone marrow aspiration and biopsy
- Routine microscopic exam of bone marrow
- Flow cytometry
- Immunocytochemistry
- Cytogenetics
- Molecular genetic studies

The peripheral blood count may reveal a decreased white blood cell count (leukopenia) as well as leukocytosis (increased white blood cell count). Leukemia cells do not protect against infection and may cause congestion of blood vessels (leukostasis). Thrombocytopenia, a decrease of platelets, can lead to excessive bruising, ►petechiae, and bleeding. When leukemia cells spread outside the bone marrow, it is called extramedullary manifestation. Small pigmented spots that look like common rashes may indicate skin involvement. A tumor-like collection of AML cells is called chloroma or granulocytic sarcoma. AML sometimes causes enlargement of the liver and spleen.

Prognostic Factors

AML is a curable disease; the chance of cure for a specific patient depends on a number of prognostic factors. Some of the strongest prognostic information can be obtained by ►cytogenetic analysis. Normal cytogenetics indicates average-risk AML. Cytogenetic abnormalities that suggest a good prognosis include translocations t(8;21) and t(15;17), as well as inv(16). Patients with AML that is characterized by deletions of the long arms or monosomies of chromosomes 5 or 7; by translocations or inversions of chromosome 3, t(6;9), t(9;22); or by abnormalities of chromosome 11q23 have particularly poor prognoses.

Further adverse prognostic factors include central nervous system involvement with leukemia, elevated

white blood cell count (>100,000/mm^3), treatment-induced AML, and a history of MDS. Leukemias in which cells express the progenitor cell antigen CD34 and/or the P-glycoprotein (MDR1 gene product) have an inferior outcome. Due to a higher relapse rate, patients with AML associated with an internal tandem duplication of the FLT3 gene (FLT3/ITD mutation) have a poorer outcome.

Beyond these disease-specific factors, patient-specific parameters like comorbidities and frailty have a strong impact on the course of the disease and treatment tolerability, as reflected by the age-dependent surge in mortality.

Therapy

Therapeutic approaches can be differentiated as curative (aimed at long-term cure) or palliative (principally aimed at achieving best quality of life) (►palliative therapy).

Curative intensive chemotherapeutic treatment (►chemotherapy of cancer, progress and perspectives) for AML is considered the standard procedure, usually divided in two phases, induction and consolidation (post-remission) therapy. It is traditionally based on two substances, cytarabine (cytosine arabinoside) and anthracycline. The objective of a curative treatment approach is to rapidly eliminate the cancer cells with induction chemotherapy, called remission. Complete remission occurs in 60–80% of patients. More than 15% of adults with AML (about 25% of those who attain complete remission) can be expected to survive 3 or more years and may be cured. Remission rates in adult AML are inversely related to age, with an expected remission rate of >65% for those younger than 60 years. Duration of remission may be shorter in older patients. Increased morbidity and mortality during induction appear to be directly related to age. This is associated with several factors including the ability to tolerate intensive treatment approaches. Without treatment, the average life expectancy is about 3 months. Complications during treatment include relapse of the disease, severe infections, or life-threatening bleeding. During this time, supportive care consists of patient isolation to prevent infection, antibiotics to treat infections, and transfusion of blood products. After remission is achieved, further treatment is known as consolidation and is necessary in order to achieve a permanent cure. Consolidation may consist of either further chemotherapy or a bone marrow, or stem cell transplantation. The aforementioned treatments are appropriate for all subtypes of AML except for one type of AML known as ►acute promyelocytic leukemia (APL).

Newer treatments, especially for those patients not tolerating intensive chemotherapy, include monoclonal antibodies, demethylating agents, and experimental drugs given in clinical trials. Thus, while the diagnosis of AML in itself does not represent a therapeutic mandate for intensive chemotherapy in all cases, the latter is the only curative approach to treatment. Decisions whether to treat patients with intensive chemotherapy, new agents, or solely best ►supportive care should be based on a sum of patient factors (including age, previous history of MDS, ►comorbidity, frailty, and patients' preferences), in addition to the blast count and the above-described prognostic factors. Careful consideration of these factors is especially relevant in older, multimorbid patients with AML.

►Acute Megakaryoblastic Leukemia
►Nucleoporin

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Acute Myeloid Leukemia 1

►Runx1

Acute Nonlymphocytic Leukemia

►Acute Myeloid Leukemia

Acute Promyelocytic Leukemia

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Definition

Acute promyelocytic leukemia (APL) is a distinct subtype of ►acute myeloid leukemia (AML) characterized by the expansion of leukemic cells blocked at the promyelocytic stage of myelopoiesis. According to the French–American–British (FAB) classification of acute leukemia, APL corresponds to the M3 and M3-variant subtypes, and according to World Health Organization classification (2001) it corresponds to the subtype: AML associated with translocations involving chromosomes 15 and 17 [t(15;17)] and variants. APL accounts for 5–10% of adult AML patients in Caucasian populations and for 20–30% among patients with Latino ancestry. Invariably, APL leukemic cells harbor ►chromosomal translocations involving the *retinoic acid receptor α* (*RARα*) gene on chromosome 17 (Table 1), which may be fused to one of five possible partner genes: *promyelocytic leukemia* (*PML*), *promyelocytic leukemia zinc finger* (*PLZF*), *nucleophosmin* (*NPM*), *nuclear mitotic apparatus* (*NuMA*), and *signal transducer and activator of transcription 5B* (*STAT5b*). This leads to the generation of fusion genes encoding distinct fusion proteins. The sensitivity of APL to the differentiating action of all-trans retinoic acid (ATRA) is differentially mediated by the various fusion proteins (see Molecular Characterization).

Characteristics

Clinical and Laboratorial Presentation

The symptoms of APL are similar to those of other subtypes of AML such as weight loss, fatigue, weakness, pallor, fever, and bleeding. These symptoms manifest acutely and are accompanied by petechiae, bruising, oral bleeding, or epistaxis as well as symptoms and signs related to specific bacterial infections. Patients with APL are particularly susceptible to disseminated intravascular coagulation (DIC) and extensive bleeding is common at onset. The most common sites of clinically overt extramedullary leukemic infiltration include superficial lymphonodes, liver, and spleen. The leukocyte counts are usually lower than those observed in other AML subtypes and the differential counts reveal a variable percentage of blasts in the majority of patients. In most cases, anemia and thrombocytopenia are present at diagnosis. Abnormal promyelocytes constitute more than 20% of marrow-nucleated cells or more than 20% of leukocytes in peripheral blood. Leukemic blasts are morphologically characterized by the presence of distinctive large cytoplasmic granules, frequent multiple Auer rods, and a folded nucleus. The hypogranular variant (M3-variant) is characterized by the expansion of blasts containing large number of small granules that may be difficult to distinguish by light microscopy, and may be wrongly classified as monoblasts. However, both in the classical and variant M3 subtypes the cells are strongly positive for myeloperoxidase staining. A more rare hyperbasophilic variant has been described.

The diagnosis is usually suspected upon the morphological examination of bone marrow and peripheral blood smears. The immunophenotypic profile suggestive of APL is composed by heterogenous intensity of expression of the CD13 surface marker associated with a homogenous expression of CD33; HLA-DR is negative in the majority of cases, and the expression of CD15 and CD34 is mutually exclusive and usually dim. The genetic confirmation of gene rearrangements involving the RARα locus is mandatory and can be done by classical cytogenetics, FISH, or RT-PCR. The

Acute Promyelocytic Leukemia. Table 1 Molecular genetics of acute promyelocytic leukemia

Translocation	Fusion proteins		Response to RA
t(15;17)	PML–RARα	RARα–PML	Good
t(11;17)	PLZF–RARα	RARα–PLZF	Poor
t(5;17)	NPM–RARα	RARα–NPM	Good
t(11;17)	NuMA–RARα	RARα–NuMA?	Good
t(17;17)	STAT5b–RARα	RARα–STAT5b?	Poor?

pattern of immunofluorescence staining using an anti-PML antibody is also useful for a rapid diagnosis of APL. In APL cells a nuclear microspeckled pattern is observed in contrast to other subtypes of AML in which larger and less numerous dots (nuclear bodies) are evident.

DIC occurs in 75% of M3 patients accompanied by secondary fibrinolysis. The cause of coagulopathy is complex, resulting from a combination of tissue factors and cancer procoagulant-induced activation of the coagulation, exaggerated fibrinolysis due predominantly to enhanced expression of annexin II on APL blasts, and blast cell production of cytokines. Laboratory evidence of DIC (prolonged prothrombine time and partial thromboplastin time, decreased fibrinogen and increased fibrin degradation products) should be examined in all APL patients.

Molecular Characterization

APL has been well characterized at the molecular level and has become one of the most compelling examples of aberrant transcriptional regulation in cancer pathogenesis. Due to reciprocal translocations, the *RARα* gene on chromosome 17 is fused to one of five distinct partner genes (for brevity, hereafter referred as *X* genes; Table 1). In the vast majority of cases, *RARα* fuses to the *PML* gene (originally named *myl*) on chromosome 15. In a few cases *RARα* fuses to the *PLZF* gene, to the *NPM* gene, to the *NuMA* gene, or to the *STAT 5B* gene located on chromosomes 11, 5, 11, or 17, respectively. The various translocations result in the generation of *X–RARα* and *RARα–X* fusion genes and the coexpression of their chimeric products in the leukemic blasts. The characterization of the genetic events of APL, and the availability of techniques such as FISH and RT-PCR, render it possible to confirm the diagnosis at the molecular level and to monitor minimal residual disease. RARα is a member of the superfamily of nuclear receptors, which acts as a retinoic acid (RA)-dependent transcriptional activator in its heterodimeric form with retinoid-X-receptors (RXR). In the absence of RA, RAR/RXR heterodimers can repress transcription through histone deacetylation by recruiting nuclear receptor corepressors (SMRT), Sin3A, or Sin3B, which in turn, form complexes with histone deacetylases (HDAC) resulting in nucleosome assembly and transcription repression. PML–RARα represses transcription not only through HDAC, but also via interactions with DNA methyltransferases (DNMTs) leading to hypermethylation at target promoters. The epigenetic changes induced by PML–RARα are stable and maintained throughout cell divisions. ATRA causes the disassociation of the corepressor complex and the recruitment of transcriptional coactivators to the RAR/RXR complex. This is thought to result in terminal differentiation and growth arrest of various types of cells, including normal myeloid hematopoietic cells. The X–RARα fusion proteins function as aberrant transcriptional repressors, at least in part, through their ability to form repressive complexes with corepressors such as NCoR and HDACs. PLZF–RARα can also form, via its PLZF moiety, corepressor complexes that are less sensitive to RA than the PML–RARα corepressor complexes, thus justifying the poorer response to RA-treatment observed in these patients (see also Therapeutics). The X–RARα oncoproteins retain most of the functional domains of their parental proteins and can heterodimerize with X proteins, thus potentially acting as double-dominant-negative oncogenic products on both X and RAR/RXR regulated pathways.

Recently, it has been demonstrated that APL blasts present a marked defect in TGF-β signaling including Smad2/3 phosphorylation and nuclear translocation, which is similar to that in Pml null primary cells. Remarkably, RA-treatment, which induces PML–RARα degradation, resensitizes the cells to TGF-β. It is plausible that PML–RARα may inhibit TGF-β signaling through direct inhibition of the interaction between Smad3 and the cytoplasmic form of PML (cPML).

Modeling APL in Mice

The transgenic approach in mice has been used successfully in modeling APL and in generating faithful mouse models harboring various APL fusion genes. In vivo, transgenic mice (TM) harboring X–RARα oncoproteins develop leukemia after a long latency suggesting that the fusion proteins are necessary, but not sufficient to cause full-blown APL. In the PML–RARα TM model, mice develop a form of leukemia that closely resembles human APL, presenting blasts with promyelocytic features that are sensitive to the differentiating action of RA. A similar phenotype was observed in NuMA–RARα TM, in which leukemia was also preceded by a period of latency, but displayed a higher penetrance. On the contrary, the leukemia developed by the PLZF–RARα TM lacked the distinctive differentiation block at the promyelocytic stage, morphologically resembling more a chronic myeloid leukemia (CML) type of disease, while NPM–RARα TM developed myelomonocytic leukemia. This analysis demonstrated that the X–RARα fusion protein plays a critical role in determining leukemic phenotype as well. Moreover, it is the X moiety of the X–RARα product to determine sensitivity to ATRA, since leukemia in PML–RARα, but not in the PLZF–RARα TM, is responsive to ATRA treatment. Modeling APL in TM contributed to the understanding of the important role of the reciprocal RARα–X fusion proteins. RARα–PML

and RARα–PLZF TM do not develop overt leukemia. However, the coexpression of RARα–PML with PML–RARα increases the penetrance and the onset of leukemia development in double mutants. Strikingly, in the PLZF–RARα TM model, the coexpression of RARα–PLZF with PLZF–RARα metamorphoses the "CML-like" leukemia in PLZF–RARα TM to a leukemia with classical APL features. In addition, RARα–PLZF renders the leukemic blasts even more unresponsive to the differentiating activity of RA. At the transcriptional level, RARα–PLZF acts as an aberrant transcription factor that can interfere with the repressive ability of PLZF. Therefore, RARα–X and X–RARα fusion products act in combination to dictate the distinctive phenotypic characteristics of each APL subtype disease. Modeling of APL in the mouse is thus allowing a better comprehension of the molecular mechanisms underlying the pathogenesis of APL as well as the development of novel therapeutic strategies.

Therapeutics

The exquisite sensitivity of APL blasts to the differentiating action of RA makes APL a paradigm for therapeutic approaches utilizing differentiating agents. This therapeutic approach conceptually differs from the treatments involving drug and/or irradiation therapies, because instead of eradicating the neoplastic cells by killing them, it reprograms these cells to differentiate normally. The utilization of ATRA in APL patient management has reduced early death from DIC-related complications and dramatically improved the prognosis. However, treatment with ATRA alone in APL patients induces disease remission transiently and relapse is inevitable if remission is not consolidated with chemotherapy. Most contemporary therapy protocols incorporate an anthracycline (e.g., dauno or idarubicin) with ATRA during induction, followed by consolidation therapy with ATRA, anthracyclines and cytarabine, followed by maintenance therapy. Leukocyte and platelet counts at diagnosis are frequently used as risk factors for relapse: patients presenting with more than 10,000 leukocytes/μl have high risk in contrast with those with less than 10,000/μl and platelet counts higher than 40,000/μl. In the majority of cases, relapse is accompanied by RA resistance. Unlike t(15;17)/PML–RARα APL, t(11;17)/PLZF–RARα leukemias show a distinctly worse prognosis with poor response to chemotherapy and little or no response to treatment with RA, thus defining a new APL syndrome.

Up to 50% of patients treated with ATRA alone develop an "ATRA syndrome" characterized by a rapid rise in circulating polymorphonuclear leucocytes and associated with weight gain, fever, occasional renal failure, and cardiopulmonary failure, which may be life threatening in some patients. The combination of ATRA and chemotherapy in the induction and consolidation treatment phases has been proven to be an effective strategy to prevent "ATRA syndrome" and achieve long-term disease-free survival.

Arsenic trioxide (As_2O_3), a chemical used in Chinese medicine, is also extremely effective in the treatment of APL. About 90% of APL patients treated with As_2O_3 alone achieve complete remission, especially in relapsed patients who are resistant to RA and/or conventional chemotherapy. RA triggers blast differentiation while As_2O_3 induces both apoptosis and partial differentiation of the leukemic blasts. Utilizing PML–RARα and PLZF–RARα transgenic mouse models of APL, it has been demonstrated that the association of RA and As_2O_3 is effective in the former but not in the latter.

Considering the importance of HDAC-mediated transcriptional repression in APL pathogenesis, the utilization of histone deacetylase inhibitors (HDACIs) such as suberanilohydroxamic acid (SAHA) or sodium phenylbutyrate (SPB) in combination with RA may represent a promising experimental therapeutic approach. Preclinical studies in transgenic mouse models of APL suggest that in fact HDACIs work as growth inhibitors and inducers of apoptosis, and that these effects are potentiated by RA.

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Acute Respiratory Distress Syndrome (ARDS)

Definition

A severe lung disease caused by a variety of direct and indirect insults. It is characterized by ►inflammation of the lung parenchyma leading to impaired gas exchange

with concomitant systemic release of inflammatory mediators causing inflammation, hypoxemia, and frequently resulting in multiple organ failure.

►Sivelestat

Acute Toxicity Studies

►Single Dose Toxicity Studies

Acyclovir

Definition

A deoxyguanosine analog lacking the equivalent of 2υ and 3υ hydroxyl groups; commonly used in the treatment of herpesvirus infections.

►HSV-TK/Ganciclovir Mediated Toxicity
►Human Herpesvirus 6

AD

Definition

Androgen-dependent.

►Cyclin G-Associated Kinase

ADAbp

►CD26/DPPIV in Cancer Progression and Spread

ADA-CP

►CD26/DPPIV in Cancer Progression and Spread

ADAM

Definition

A Disentegrin and Metalloprotease; ADAM Molecules.

ADAM10

Definition

ADAM10, synonym kuzbanian, is a member of a family of zinc-dependent transmembrane metalloproteases, involved in neuronal development in vertebrates and *Drosophila* and expressed in all epithelial tissues. Kuzbanian ADAM10

►Doublecortin
►ADAM Molecules

ADAM17

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Synonyms

TACE; Tumor necrosis factor-alpha converting enzyme; CD156b antigen

Definition

ADAM17 is a zinc-dependent ►metalloprotease belonging to the ►ADAM (A disintegrin and metalloproteinase) family of type I transmembrane proteins. ADAM17 is involved in the ectodomain shedding of a wide variety of membrane-bound ligands and cytokines that are implicated in diverse biological processes including growth and ►inflammation.

Characteristics

Structure

The 50 kb ADAM17 gene, which is located at chromosome 2p25, consists of 19 exons and encodes an 824 amino acid protein. ADAM17 is synthesized as an inactive precursor protein consisting of five domains: the

pro-, metalloprotease, cysteine-rich, transmembrane and cytoplasmic domains. Prior to ADAM17 maturation, a conserved cysteine residue within the pro-domain interacts with the active site zinc atom maintaining the enzyme biologically inert. The active site of the metalloprotease domain contains a histidine consensus sequence (HExxHxxGxxH) that coordinates zinc atoms and water required for the enzymatic processing of ADAM17 substrates. Removal of the pro-domain occurs through a ►furin cleavage site (RVKR), by an unidentified furin or proprotein convertase, enabling the active site zinc to interact with the required histidine residues and to generate the active protease.

While the structural and functional aspects of the pro- and metalloprotease domains have been studied extensively and are well defined, the precise functions of the remaining ADAM17 domains are still somewhat obscure. The cysteine-rich domain consists of two subdomains: the disintegrin and EGF-like domains. A role in cellular ►adhesion has been proposed for the disintegrin domain. In support of this hypothesis, ADAM17 has been shown to interact with at least one ►integrin (α5β1) and modulate cell migration as a result of this interaction. It has also been demonstrated that the cysteine-rich domain is indispensable for the ectodomain shedding of select ADAM17 substrates and thus, might function in substrate recognition through the recruitment of accessory proteins or direct contact with the substrates themselves. The transmembrane domain tethers mature ADAM17 in the cell membrane where it exerts most of its physiological functions. Finally, the cytoplasmic domain comprises several ►Src homology 2 (SH2) and 3 (SH3) domain binding sites as well as phosphorylation sites, and is likely involved in regulatory ►signal transduction pathways.

Expression and Regulation

ADAM17 mRNA is ubiquitously expressed in most adult tissues, albeit at lower levels than those observed in fetal tissues at various stages of development. The ADAM17 zymogen is synthesized in the rough endoplasmic reticulum and is processed in the late Golgi compartment to produce the mature protease lacking the inhibitory pro-domain. This maturation step seems to entail a constitutive process as the majority of cellular ADAM17 exists in its mature form. The greater part of ADAM17 protein is localized in the perinuclear area while the remaining fraction resides at the cell surface, as expected. Notably, it appears that the membrane-bound ADAM17 population is exclusively in the processed form. This surface pool of ADAM17 is relatively stable with a half-life of ~8 h.

The mechanism by which ADAM17 function is regulated is not entirely clear, however, two methods by which the protease can be activated have been described. The first method involves the activation of ADAM17 by growth factors, such as the ►fibroblast growth factor (FGF) and the ►platelet-derived growth factor (PDGF). ADAM17-mediated ligand shedding can also be induced by non-physiological stimuli such as phorbol esters (►Phorbol myristate acetate). Treatment of cells with phorbol esters, such as ►PMA, results in increased ligand shedding without affecting the quantity or localization of endogenous ADAM17 in the cell. There is conflicting evidence with respect to the mechanism by which this stimulation occurs. One study demonstrated that PMA exerts its effects by activating the ►extracellular signal-regulated kinase (ERK) signaling pathway, which results in the phosphorylation of ADAM17 at Thr735 in its cytoplasmic tail, while another group showed that the cytoplasmic tail of ADAM17 is not required for PMA-induced ligand shedding. Although there is no evidence that phorbol esters regulate ADAM17 activity *in vivo*, the ►ERK signaling pathway has also been implicated in growth factor stimulated ADAM17 activation. For this reason, the ERK signaling pathway will likely be the focus of future studies aimed at delineating the mechanisms involved in the positive regulation of ADAM17 activity.

In addition to stimulating ADAM17-mediated ligand cleavage, the treatment of cells with PMA also triggers the establishment of a negative feedback mechanism. Following an increase in ADAM17 activity and ligand shedding, the protease itself is internalized and degraded in response to prolonged treatment with PMA. This negative regulatory mechanism is probably in place to prevent over-stimulation of ligand-activated signaling pathways. In attempt to identify potential regulators of ADAM17 activity, two ADAM17 binding partners were uncovered by yeast two-hybrid screens: synapse associated protein 97 (SAP97) and protein tyrosine phosphatase PTPH1. Overexpression of either molecule results in decreased ligand shedding implicating them in the negative regulation of ADAM17 activity. Whether either of these two proteins regulates ADAM17 activity *in vivo* remains to be seen. The only known endogenous inhibitor of ADAM17 is the tissue metalloprotease inhibitor, TIMP3. The mechanism by which TIMP3 expression results in reduced ADAM17 activity is unknown.

Biological Function

ADAM17 was initially identified as the secretase responsible for the cleavage of tumor necrosis factor-alpha (TNFα), a pro-inflammatory cytokine. The generation of transgenic mice expressing ADAM17 lacking the zinc-binding sequence in its metalloprotease domain ($ADAM17^{\Delta Zn/\Delta Zn}$) allowed for the identification of a multitude of additional ADAM17 substrates. The vast majority of the $ADAM17^{\Delta Zn/\Delta Zn}$ mice die at birth as a result of severe deficiencies in skin, muscle, lung and

neuronal system development that cannot be entirely attributed to loss of TNFα shedding. This indicates the existence of other biologically relevant ADAM17 substrates. Interestingly, the few animals that do survive display a phenotype that is comparable to that of ►transforming growth factor alpha (TGFα) or ►epidermal growth factor receptor (EGFR) knockout mice. This includes the failure of eyelids to fuse as well as defects in skin and hair follicle development. Upon further investigation it was confirmed that ►TGFα, an ►EGFR ligand, is in fact an ADAM17 substrate. Moreover, ADAM17 appears to be the major convertase of several ►EGFR ligands which are involved in a variety of cellular processes including cellular proliferation, survival, migration, and differentiation. The bulk of ADAM17 substrates, including the EGFR ligands, are involved in cell development and differentiation. Other examples include the neurogenic signaling molecule ►Notch, the neurotrophin receptor TrkA, and the EGFR-family receptor HER4. The remaining substrates can be classified as those involved in cellular immunity and regulation of immunogenic responses, like TNFα. These substrates include the TNF receptors (TNF-RI and TNF-RII), the chemokine fractalkine, and the leukocyte adhesion molecule L-selectin to name a few. While many ADAM17 substrates have been identified to date, there is no obvious sequence or structural homology between their cleavage sites. How ADAM17 achieves substrate specificity is a key question that remains to be answered. Nonetheless, it is evident that ADAM17 substrates play an important role in a broad range of fundamental cellular processes.

Clinical Relevance

Due to its involvement in TNFα processing, ADAM17 is considered to be a central mediator in human inflammatory diseases such as rheumatoid arthritis. Direct inhibition of TNFα or ADAM17 in arthritis-affected cartilage has been shown to reduce inflammation. For these reasons ADAM17-based therapies, such as zinc-chelating sulfonamide hydroxamates, are in use for the treatment of such diseases.

In addition to its role in inflammatory diseases, ADAM17 is becoming increasingly implicated in the development and progression of cancer as a result of its role in the processing of EGFR ligands. The upregulation of EGFR expression and signaling is a common feature in human cancer. Unfortunately, ►EGFR inhibitors have rendered disappointing results in ►clinical trials and there is an apparent resistance of several cancer cell lines to these agents. Importantly, ADAM17 is also overexpressed in several neoplastic tissues including ►breast carcinomas, ►colon carcinomas, pancreatic ductal adenocarcinomas, and ►ovarian carcinomas. There is also a positive correlation between ADAM17 expression and the aggressiveness of the malignancy. Thus ADAM17 is most highly expressed in advanced tumors, suggesting that ADAM17 and its substrates play a role in tumor progression.

In accordance with these observations, there is a growing amount of evidence supporting the use of anti-ADAM17 drugs in the treatment of cancer. Several studies have shown that inhibition of ADAM17 activity using a variety of approaches is sufficient to inhibit EGFR ligand release and to prevent the proliferation, migration, and survival of squamous cell, ►kidney cancer, ►bladder cancer and ►breast cancer cell lines *in vitro*. It was recently demonstrated that ►siRNA-mediated silencing of ADAM17 inhibits the release of soluble TGFα in highly malignant ►renal carcinoma cells, thereby abolishing their ability to form tumors in nude mice. This was the first *in vivo* evidence that ADAM17-mediated ligand cleavage is a pivotal step in the establishment of the TGFα/EGFR autocrine ►(Autocrine signaling) growth stimulatory loop and thus in tumorigenesis. Another study revealed that targeting ADAM17, using a ►small molecule inhibitor, prevents ►heregulin cleavage and hence ►HER3 activation in non-small cell lung cancer cells. Not only did this inhibition abolish tumor growth *in vivo* but it also enhanced the sensitivity of the cancer cells to gefitinib, an anti-EGFR based therapy. This result suggests that the concomitant inhibition of ADAM17 and EGFR should improve patient responsiveness to such agents and increase survival. Thus targeting ADAM17 is a promising new alternative to traditional EGFR-based therapies in the treatment of human cancer.

Summary

ADAM17 was originally characterized for its role in TNFα processing and the regulation of inflammatory responses. It has since been demonstrated that ADAM17 is also a physiological convertase of a wide variety of signaling molecules implicated in the development and progression of cancer. The importance of ADAM17 in these oncogenic pathways is highlighted by the finding that silencing of ADAM17 is sufficient to abolish tumor formation *in vivo*. These results validate ADAM17 as a rational therapeutic target and endorse the use of ADAM17 inhibitors in the treatment of human cancer.

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ADAM Molecules

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Synonyms

A disintegrin and metalloprotease; Disintegrin metalloproteases; Metalloprotease disintegrin cysteine-rich; MDC

Definition

A disintegrin and metalloprotease (ADAM) molecules share a common domain structure: a propeptide (prodomain), a metalloproteinase domain, a disintegrin domain, a cysteine-rich region, an epidermal growth factor (EGF)-like domain, a transmembrane region, and a cytoplasmatic domain (Fig. 1). Several ADAMs exist in both membrane-bound and secreted isoforms; the functional significance of this, in most cases, is still unclear. A subset of the presently known ADAM molecules shows catalytic activity. To date, at least 40 ADAMs have been identified in a variety of species. A large proportion (13 ADAMs) is exclusively expressed in the male reproductive system, and only a minority can be found throughout all tissues.

Characteristics

ADAM molecules, with their unique potential to combine ►adhesion, ►proteolysis, and signaling, are involved in a variety of cellular functions. Some have been shown to play an important role in diverse biological processes such as fertilization, myogenesis, cell signaling, inflammatory response, and cell–cell/cell–matrix interactions. However, the respective key function has remained elusive for most ADAMs.

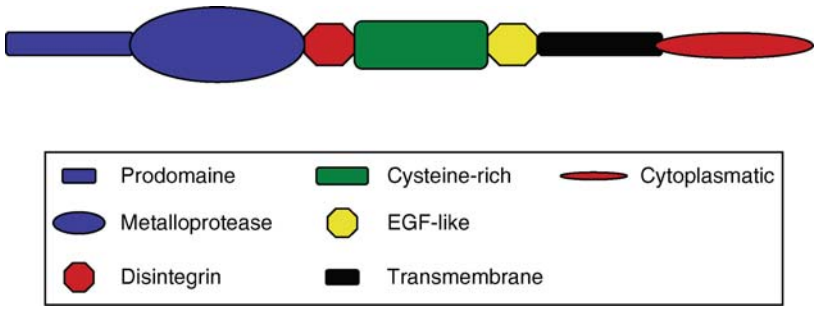
Dysregulation of ADAM molecules has been shown in various diseases. However, there is a growing amount of reports about the role of ADAM molecules in malignant tumors.

Metalloprotease Function

To regulate biological activity, in normal as well as in malignant cells a wide variety of proteins are synthesized as inactive precursors that are subsequently converted to their mature active forms by ADAM molecules.

A well-studied member of the ADAM molecules is ADAM17/►TACE, which was originally described as being responsible for the proteolytic cleavage of the soluble form of ►TNF-α. Subsequent studies have shown that ADAM17/TACE is also involved in the shedding of other biologically active proteins, including growth factors (erbB4/HER-4 and ►transforming growth factor (TGF)-α), surface molecules (L-selectin), and interleukin (IL) receptors (IL-R; IL-1R type II and IL-6R; Fig. 2).

TACE cleavage function in the activation of EGF receptor (EGFR) and EGFR signaling systems, which regulate the proliferation and motility of ►squamous cell carcinoma cells in vitro. The key role of the EGFR/EGFR ligand system for cancer development is well-known. In this context, the transactivation of EGFR via ADAM17/TACE is of special interest. ADAM



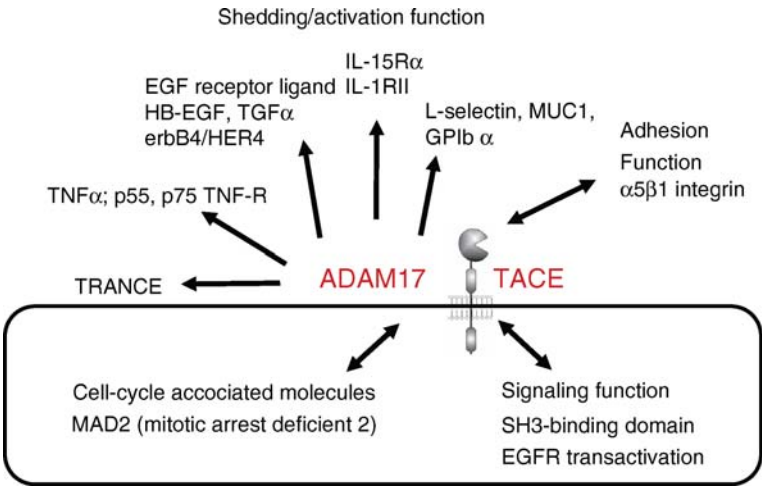
ADAM Molecules. Figure 1 Domain structure of ADAMs. The ADAMs consist of a propeptide domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region, a EGF-like, a transmembrane domain, and a cytoplasmatic domain.

metalloproteases such as ADAM9 and ADAM17/TACE regulate G protein-coupled receptor induced cell proliferation and survival.

Aberrant expression of a proteolytic active ADAM17/TACE has been reported in pancreas ►cancer cells. The increasing prevalence of ADAM17/TACE expression with higher pancreatic intraepithelial neoplasia (PanIN) grade as precursor lesions underlines the role of this molecule in ductal pancreatic adenocarcinoma development. Gene silencing experiments showed a critical role of ADAM17/TACE in the invasion process of pancreatic cancer cells. The aberrant expression of proteolytically active ADAM17/TACE may result in an uncontrolled turnover of activated target molecules, such as TNF-α, TGF-α, and MUC1 (►mucius).

Silencing of ADAM17 in human renal carcinoma cell lines corrects critical features associated with cancer cells, including growth autonomy, tumor inflammation, and tissue invasion. In addition, these cells fail to form in vivo tumors in the absence of ADAM17. It has also been shown that ADAM17/TACE is overexpressed in mammary cancer and other cancer types (Table 1).

ADAM12, which is upregulated, for example, in breast and gastric cancer (Table 1), is expressed in two splice forms, the transmembrane ADAM12-L and the soluble ADAM12-S. In a mouse breast cancer model, ADAM12 decreased tumor cell apoptosis and increased stromal cell apoptosis. The shedding of heparin-binding EGF by ADAM12 was shown to



ADAM Molecules. Figure 2 Schematic overview about the published functions and interactions of ADAM17/TACE.

ADAM Molecules. Table 1 Overview about the aberrant expression of ADAM molecules in different human cancer types as published

ADAM molecule	Human cancer type
ADAM2	Renal
ADAM8	Brain, prostate, lung adenocarcinoma
ADAM9	Prostate, colon, pancreas, liver, gastric, nonsmall cell lung cancer, renal
ADAM10	Breast, colon, prostate, pheochromocytoma, neuroblastoma
ADAM11	Glioma, breast
ADAM12	Breast, gastric, glioblastoma, liver, aggressive fibromatosis, giant cell tumor of the bone, brain
ADAM15	Prostate, breast, lung, ovarian, gastric, brain, bladder
ADAM17/TACE	Pancreas, renal, breast, colon, liver, brain, squamous cell carcinoma cells
ADAM19	Brain
ADAM21	ADAM21-like (ADAM21-L) T-cell leukemia
ADAM22	Brain
ADAM23	Brain, gastric, breast (pancreas)
ADAM28	Nonsmall cell lung carcinoma
ADAM29	Chronic lymphocytic leukemia

promote human ►glioblastoma. In addition, in liver cancers, ADAM12 and ADAM9 expression is associated with tumor aggressiveness and progression. ADAM9 is also described to shed heparin-binding EGF. Overexpression of cytoplasmatic ADAM9 in pancreatic cancer is associated with poor differentiation and shortened survival.

It is of particular interest for cancer development that ADAM molecules reported to shed cell-associated adhesion molecules such as L-selectin, MUC1, and glycoprotein (Gb) 1bα.

In general, the metalloprotease protease function might be involved in various processes of cancer cells and be relevant to promote cell migration and invasion.

Adhesion Function

ADAM molecules are potential ligands for ►integrins due to the presence of binding sites within the disintegrin domain. Only one ADAM (ADAM15) contains the ►RGD integrin-binding motif and it can therefore interact not only with the αvβ3 integrin but also with the αvβ5. Additional ADAM–integrin interactions have been reported: a large number of ADAMs (1, 2, 3, 9, 12, and 15) with α9β1; ADAM9 with α6β1 and αvβ5; and ADAM28 with α4β1. Considering the recently published data on the interaction of ADAM17/TACE with the α5β1 integrin in ►HeLa cells, it is also conceivable that ADAM17/TACE may influence the migration and invasion in other cancer types.

We are beginning to gather insights into ADAM–integrin and ADAM–►extracellular matrix (ECM) interactions. The interplay with integrins and ECM compounds might promote ADAM function in malignant cells. Thus, cell binding to ADAM12 via beta3 integrin results in the formation of focal adhesions. Furthermore, it was shown that the cystein-rich domain of ADAM12 supports tumor cell adhesion through syndecan.

ADAM23 with its inactive metalloprotease domain is exclusively involved in cell-adhesion. It was demonstrated that the interaction between the disintegrin-loop of ADAM23 and the αvβ3 integrin promotes the adhesion of ►neuroblastoma and ►astrocytoma cells. In contrast to the described overexpression or de novo expression in various cancer types, downregulation of ADAMs might also promote cancer development. Thus, ADAM23 gene silencing in breast cancer by promoter ►hypermethylation may result in abnormal cell–cell interactions favoring cell migration.

Signaling Function

Beside the involvement of ADAM molecules in the EGFR transactivation, only few data about the signaling function of ADAM molecules are known. It is intriguing that interactions between integrins and/or ECM- and ADAM-binding domains may induce outside–in signaling. ADAM inside–out signaling pathways might regulate shedding and/or adhesion function of the molecules. However, many ADAM cytoplasmatic domains contain binding motives for the Src homology region 3 (SH3 Domain) of various intracellular proteins. Tyrosine residues could be substrates for tyrosine kinases or could act as ligands for phosphotyrosine-binding domains, when phosphorylated. A number of binding partners have been identified for the cytoplasmatic domains of various ADAM molecules. Interaction of the cytoplasmatic domain of ADAM9 and 15 with endophilin and SH3PX1are reported. ADAM12 and ADAM15 are associated with ►Src protein-tyrosine kinases. However, the shedding of the L1 adhesion molecules in breast cancer cells might involve a Scr protein-tyrosine kinase. Furthermore, mitotic arrest-deficient-2 (►MAD2) was found as binding partner of ADAM17/TACE and ADAM15; MAD2β is linked to ADAM9. To date, the physiological role of this interactions as well as the implication in malignancies is speculative.

Other Functions

Within the ADAM molecules, ADAM11 might play a special role in malignancies. ADAM11 represents a candidate tumor suppressor gene for human breast cancer. This is based on its location within a minimal region of chromosome 17q21 previously defined by tumor deletion mapping.

Taken together, there are rapidly increasing data supporting a critical implication of ADAM molecules in malignancies. But there are still more questions than answers on the function of ADAMs in human cancer and cancer development.

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Adaptive Immunity

Definition

Adaptive immune responses occur when the host comes into contact with immunogenic molecules or organisms. These stimulate the expansion of the antigenspecific lymphocytes, antibody secreting B cells and T cells of the cytotoxic and helper phenotypes, which recognize cells expressing foreign antigens. B cells and T cells are the effector cells of the adaptive immune response. They bear antigen specific receptors of great diversity that are generated by random rearrangement of gene segments and other mechanisms. This results in a vast array of antigen-specific receptors clonally distributed on T and B cells, which clonally expand on contact with antigen. As the immunogen is cleared these clonal populations shrink but leave behind longlived populations of memory cells that are easily recalled on subsequent exposure to the same immunogen. Unlike the innate immune response, adaptive responses are not immediate, requiring 3–5 days for clonal expansion and differentiation of effector lymphocytes. The adaptive immune system allows for a strong immune response as well as immunological memory, where a tumor antigen is "remembered." The adaptive immune response is antigen-specific and requires the recognition of tumor antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to cancer cells and the ability to mount these tailored responses is maintained in the body by "memory cells." Cells of the adaptive immune system are B- and T-lymphocytes. Adaptive humoral responses are mediated by tumor specific antibodies.

►Immunoediting
►Specific Immunity
►Immunoprevention of Cancer
►DNA Vaccination
►Inflammation

Adaptive Response

Definition

The process of adaptation, which allows survival under adverse conditions, is called adaptive response

►Detoxification

Adaptor Proteins

Definition

lack any intrinsic enzymatic activity themselves but instead mediate specific protein-protein interactions leading to formation of protein complexes.

►RAS Activation

ADAR

Definition

A family of "adenosine-deaminase-acting-on-RNA" enzymes that convert adenosines to inosines in double-stranded RNA substrates. This process, known as A → I RNA editing, changes the sequence of the target dsRNA molecule so that it differs from the parent DNA strand. At translation, the inosine is interpreted as a guanosine (G).

►ALU Elements

ADCC

Definition

Antibody-dependent cell-mediated cytotoxicity.

►Immunoprevention of Cancer
►Diabody
►EpCAM

Additive Effects

Definition

Additive effects mean that the effects of ►xenobiotics simply summate.

Adduct

Definition

In biology, adduct is a complex that forms when a chemical binds to a biological molecule, such as DNA or protein.

►Biomonitoring
►Adducts to DNA

Adducts to DNA

Helmut Bartsch
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Synonyms

DNA-bound carcinogens

Definition

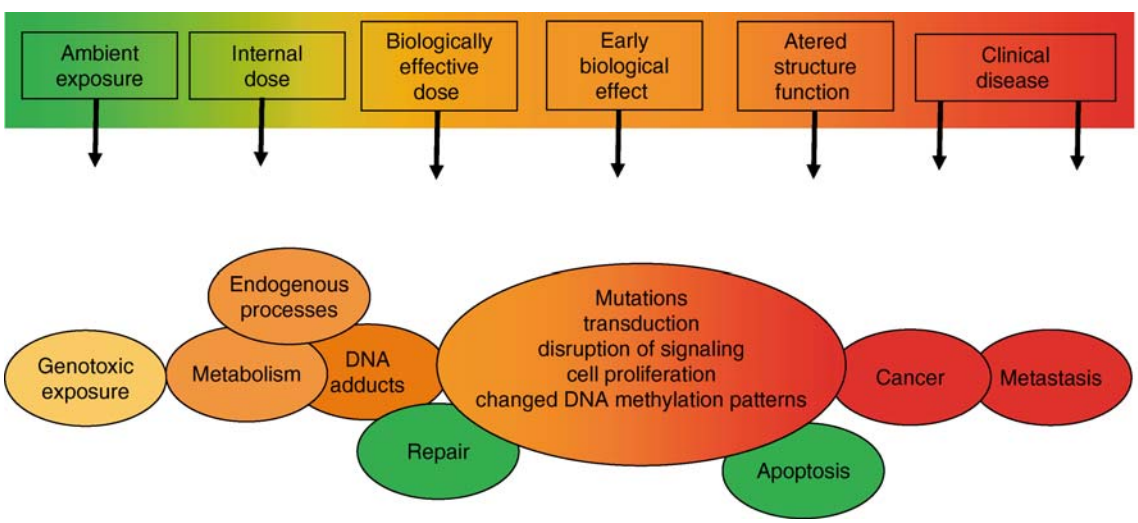
DNA-adducts reflect the amount of a ►xenobiotic that covalently reacts with nucleic acid bases at the target site (biologically effective dose) or in surrogate tissues (►surrogate markers). DNA adducts are mechanistically more relevant to ►carcinogenesis than the internal dose of a carcinogen, since they take into account interindividual differences in metabolism and of DNA repair capacity (Fig. 1). Several hundred DNA adducts, many with miscoding properties, are known to be produced by some 20 classes of carcinogens and through endogenous oxidative processes. DNA adducts are used in human ►biomonitoring as dosimeters of early biological effects and predictors of cancer risk. These ►biomarkers also provide tools for studying disease pathogenesis, etiology and for verifying preventive measures in human cancer.

Characteristics

Rationale for Using DNA Adducts as Biomarkers for Exposure and Adverse Effects

Evidence for the biological significance of DNA adducts in carcinogenesis is supported by the following:

- Over 80% of identified or suspected human carcinogens react often after metabolic activation with nucleic acids and proteins to form macromolecular adducts
- Carcinogen-DNA adducts represent the initiating events leading to mutations in ►oncogenes and ►tumor suppressor genes, and to ►carcinogenesis
- The carcinogenic potency of a large number of carcinogens is proportional to the extent they bind to rodent liver DNA
- Humans with inherited or acquired defects in ►DNA repair have an elevated risk of developing cancer



Adducts to DNA. Figure 1 Paradigm for the multistage process of ►carcinogenesis with DNA adducts as initiating lesions. They are used mostly as biomarkers for the biologically effective dose both of exogenous carcinogens and of DNA-reactive agents produced by endogenous processes, such as chronic oxidative stress. Over the past 40 years emphasis has been placed on the development of accurate and sensitive methods for the detection and quantitation of DNA adducts.

Biological effect markers are defined as indicators of irreversible genetic damage that result from genotoxic interactions at the target site. As DNA adducts do not often cause completely irreversible lesions, because the DNA undergoes repair (which may not be complete), they are not in the strict sense biological effect markers. However, as carcinogen dosage is linked to cancer outcome, and permanent mutations can be caused by DNA adducts, they are associated with cancer risk. This has been shown for many carcinogens and their DNA adducts, when critical toxico-kinetic parameters are taken into account. These include the steady state adduct concentration, the amount of the miscoding adduct compared to others of lesser biological relevance, the adduct half-life after carcinogen exposure has stopped, the organ, cell and gene selectivity of the adduct (Fig. 2).

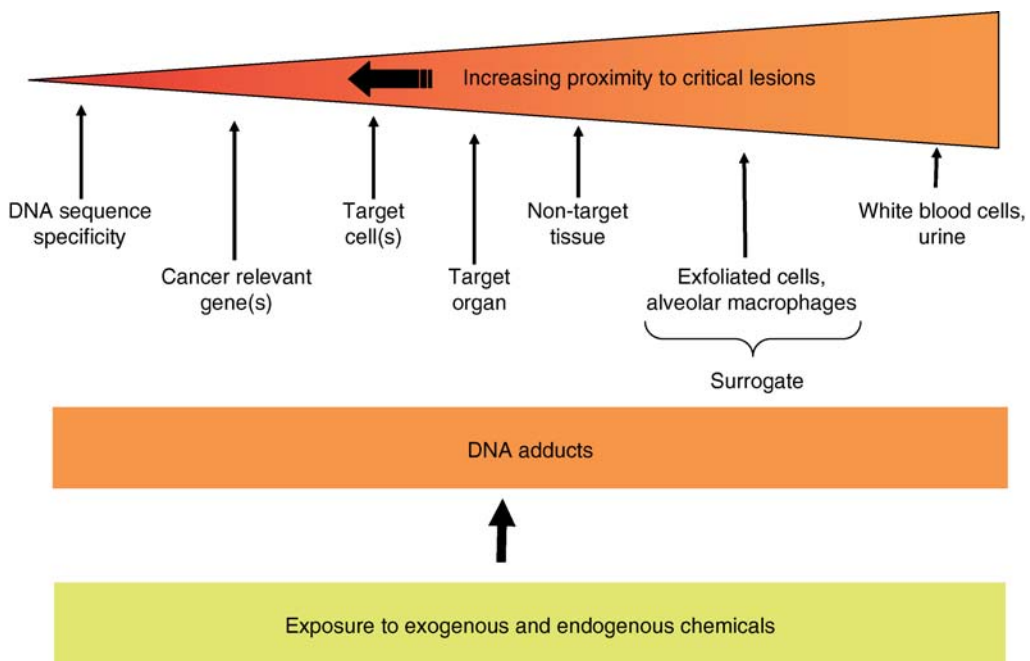
Advantages and Disadvantages of DNA Adducts Compared to Other Biomarkers

For human biomonitoring both DNA and protein adducts can be used for exposure assessment as long as the response in target organs *versus* surrogate tissue is shown to be proportional. The latter has to be determined individually for each carcinogen. The advantage of certain protein adduct measurements is that they often reflect cumulative past exposure (of several months), while the majority of DNA adducts is rapidly repaired or lost after exposure has ceased. However, a small portion of DNA adducts either with slow repair and/or subpopulations of non-dividing cells can survive for several months or even years.

Since somatic genetic or cytogenetic effect markers are neither chemical- nor exposure-specific, only macromolecular adducts allow identification of the structure and thus the determination of the genotoxic exposure sources. Also, cytogenetic markers are more easily affected by lifestyle and environmental components (confounders) that often act as uncontrolled or uncontrollable variables in biomonitoring and molecular epidemiology studies. In addition, at equal levels of carcinogen exposure, DNA adduct levels are a measure for the host's capability of carcinogen metabolism and adduct repair and can be used to determine the overall effect of genetic polymorphisms on DNA damage and cancer susceptibility by a given carcinogen.

Cellular Defense: Repair of DNA Adducts

DNA repair (►repair of DNA) systems such as ►base and ►nucleotide excision repair, O^6-alkylguanine-DNA alkyltransferase and ►mismatch repair operate in human cells to remove adducted and oxidatively damaged DNA bases. Deficiency in nucleotide excision repair genes cause ►Xeroderma pigmentosum (XP) and a high-rate occurrence of skin cancers, as well as a high susceptibility to UV-light and ►polycyclic aromatic hydrocarbon-induced carcinogenesis. A defective mismatch repair system causes hereditary non-polyposis colorectal cancer (HNPCC).



Adducts to DNA. Figure 2 Measurement of carcinogen-DNA adducts in target tissue and cells or in surrogates. The predictive value of DNA adducts for disease risk increases with the proximity of measurements to critical lesions. Accordingly, from right to left, the specificity of this biomarker increases for predicting disease outcome.

Genetic defects in these DNA repair functions or inhibition of repair proteins may have dramatic consequences when DNA adducts, DNA mismatches and DNA loops are not repaired prior to cell replication and when damaged cells are not eliminated by apoptosis. Thus, characterization of germ-line and somatic mutations in DNA-repair genes can identify high-risk subjects who especially in the case of bi-allelic mutations suffer from functional defects of proteins that repair DNA-adducts leading to genetic instability and cancer.

Adduct Measurements in Disease Epidemiology

Cross-sectional and longitudinal studies in cancer epidemiology assess the relationship between carcinogen exposures and biomarker (adduct) levels. Adduct measurement exposed in humans allow the detection, quantification and structural elucidation of specific DNA damage. Findings from such studies include the detection of background exposures manifested in "unexposed" populations and a significant interindividual variation in adduct levels in persons with comparable exposure. The latter is in part due to genetic variation in carcinogen metabolism and DNA-repair processes. Positive correlations between the extent of occupational and environmental exposures, adduct levels and adverse effects, e.g. mutations in oncogenes and tumor suppressor genes have been observed. For example, large-scale studies on geographical variations of ►hepatocellular carcinoma and exposure to ►aflatoxins have used aflatoxin-bound albumin adducts, urinary aflatoxin B1-N7-guanine adducts, and mutational hotspots in the ►TP53 gene as biomarkers. They revealed more than an additive interaction between the hepatocarcinogen and hepatitis B virus (►hepatitis viruses) infection.

►Case-control studies in disease epidemiology allow the evaluation of the role of biomarkers as cancer risk factors and the exploration of underlying mechanisms, but such studies cannot establish causality between biomarker response and cancer causation. This is especially the case when the latency period (between exposure and cancer) is long. Here, adduct measurements are of greater relevance for cancer risk estimation when exposure has been continuous. An optimal study design that can establish causality is a nested case-control study that uses questionnaire data and biological sample collection prior to disease manifestation. Once diagnosis of cancer has been made, cases are matched to appropriate controls and their stored samples analyzed. The predictive value in terms of specificity and sensitivity of a DNA adduct biomarker in biological samples can thus be determined.

Association of DNA Adducts with Cancer Risk

Not all types of DNA adducts are associated with the same cancer risk. Using alkylating agents, aflatoxins and aromatic amines (that induced 50% tumor incidence) DNA adduct levels were compared in animal experiments. A 40- to 100-fold difference in the ability of DNA adducts to induce the same tumor incidence in target tissues was detected. Thus, it is difficult to predict the tumor induction potential of unknown DNA adducts. In the past, assays for DNA adduct determination provided mostly information on the total amount of adducts in bulk genomic DNA. However, new methods are capable of pinpointing adduct profiles in critical target genes (Fig. 2). Because of the multistage and complex nature of human carcinogenesis, carcinogen-DNA adducts *per se* cannot precisely and quantitatively predict an individual's cancer risk. At present risk estimation is limited to a group level.

Background DNA-adduct Levels: Sources, Variations and Cancer Risk Prediction

The major analytical challenge has been to detect levels of DNA adducts at a concentration of 0.1–1 adducts per 10^8 unmodified DNA bases using only low microgram amounts of DNA, and with high specificity and accuracy. Several methods are available including ^{32}P-postlabeling assays often in combination with immunopurification and liquid chromatography coupled to electrospray ionization-mass spectrometry. By using ultrasensitive detection methods background DNA adduct levels have been found in organs of unexposed humans and untreated animals. These are due to physiological lipid peroxidation (LPO) processes, whereby endproducts, such as 4-hydroxynonenal and malondialdehyde when formed in excess in the body, can react with DNA to yield background levels of a variety of exocyclic DNA adducts. These types of adducts generally increase with age, but are significantly increased in human subjects affected by risk cancer factors that induce chronic oxidative stress. These include chronic inflammatory processes and infections, nutritional imbalances, and metal storage disorders. In addition, oxidized DNA bases and LPO-derived DNA adducts occur more frequently in cells with impaired antioxidant defense. Exogenous carcinogens can also induce oxidative stress causing agent-specific DNA adducts and secondary oxidative DNA base damage. The biological relevance of both oxidative and LPO-derived DNA damage is supported by the fact that these adducts are miscoding lesions which are recognized by specific DNA-repair enzymes. There is a growing evidence that both types of DNA lesions, either derived from exogenous and endogenous agents, play a role in the initiation and progression of the multistage carcinogenesis process, as well as other chronic degenerative diseases. Current research addresses some open questions:

- What is the significance of endogenously formed DNA adducts in human cancer, particularly associated with chronic inflammatory conditions and also in relation to spontaneous tumors?

- Has the proportion of cancers that result from environmental agents been overestimated compared to those arising from endogenous DNA damaging processes?
- Can one protect humans against endogenously derived DNA damage and prevent chronic degenerative diseases by administration of chemopreventive (antioxidative) agents, using DNA-adduct measurements to verify their efficacy?
- Will LPO-derived DNA adducts serve as potential prognostic markers for assessing progression of chronic inflammatory cancer-prone diseases?

Contributions of DNA-adduct Measurements to Disease Etiology and Pathogenesis

New insights are gained since

- Adduct analysis permits identification of hitherto unknown exogenous and endogenous DNA-reactive agents and of carcinogenic components in complex exposures, thus increasing the power to establish causal relationships in molecular epidemiology.
- Highly exposed individuals can be more readily identified, and exposure to carcinogenic risk factors can be minimized or even avoided.
- Subgroups in the population (so called pharmacogenetic variants) that are, due to genetic polymorphism of xenobiotic-metabolizing and DNA-repair enzymes, more susceptible to carcinogens, are identifiable by a combination of genotyping and DNA-adduct measurements.
- Repeated applications of dosimetry methods for macromolecular adducts can evaluate the effectiveness of primary and secondary interventions, either by reduction of carcinogen exposure or through (chemo-)preventive strategies.
- Incorporation of DNA-adduct measurements (and of other critical endpoints involved in carcinogenesis) can reduce (i) the enormous uncertainties currently associated with high-to-low dose and species-to-species extrapolation and (ii) yield information on inter-individual risk assessment procedures.
- The role of specific carcinogen exposures may be retrospectively implicated in cancer etiology by analyzing decades after the period of exposure, mutational fingerprints in tumors that arise from exogenous and endogenous agents after their reaction with DNA. Specific mutational signatures, detected in the tumor suppressor gene TP53, were associated with distinct past carcinogen exposures (e.g. tobacco smoke, aflatoxin B_1, vinyl chloride, and UV-light) or inflammatory disease state (such as chronic inflammatory bowel diseases).
- Adducts and derived mutations should allow to study pathogenesis and preventive approaches of chronic degenerative diseases other than cancer (e.g. atherosclerosis, Alzheimer disease).

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Adenine Nucleotides

Definition

Energy-carrying molecules composed of the purine base adenine, the sugar ribose, and one (AMP), two (ADP) or three (ATP) covalently-attached phosphate groups.

►Adenosine and Tumor Microenvironment

Adenocarcinoid

Definition

An appendiceal malignancy that contains both an epithelial neoplasm and a neuroendocrine (carcinoid) neoplasm simultaneously.

►Appendiceal Epithelial Neoplasms
►Carcinoid Tumors

Adenocarcinoma

Definition

A form of carcinoma that originates in glandular tissue. To be classified as adenocarcinoma, the cells do not necessarily need to be part of a gland, as long as they

have secretory properties. This form of carcinoma can occur in some higher mammals, including humans. The term adenocarcinoma is derived from "adeno" meaning "pertaining to a gland" and "carcinoma" which describes a cancer that has developed in the epithelial cells, i.e. cells that line the walls of various organs. This type accounts for about 40% of ►lung cancer. It is usually found in the outer part of the lung.

►Vanadium
►Bile Duct Neoplasms
►Appendiceal Epithelial Neoplasms

Adenomas

►Colorectal Premalignant Lesions

Adenomatous Polyposis Coli

Definition

Familial Adenomatous Polyposis; APC.

Adenomatous Polyps

►Colorectal Premalignant Lesions

Adenomucinosis (DPAM)

Definition

An appendiceal epithelial neoplasm that is noninvasive so that peritoneal surfaces are coated by increasing quantities of a mucinous neoplasm.

►Appendiceal Epithelial Neoplasms

Adenosine and Tumor Microenvironment

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Synonyms

Adenosine; Adenine nucleoside; purine nucleoside; adenine-9-β-D-ribofuranoside

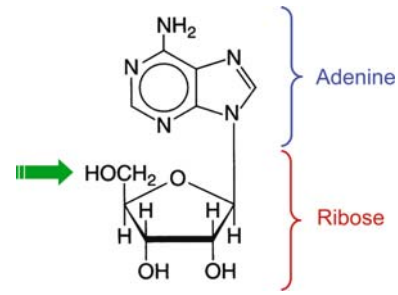
Definition

Adenosine is a small molecule that is released into tissue at high concentrations in response to a deficiency of oxygen, which occurs characteristically in solid tumors. Adenosine has multiple effects within the tumor, including controlling cancer cell growth, locally inhibiting the immune system, and increasing blood vessel formation.

Characteristics

Adenosine (adenine-9-β-D-ribofuranoside, Fig. 1) is a small organic molecule that plays an important part in general cellular biochemistry. Chemically, it is a purine nucleoside. Adenosine is abundant within all cells, predominantly in the form of ►adenine nucleotides (AMP, ADP and ATP) which participate widely in cellular energy metabolism and act as precursor molecules in many processes. However, adenosine itself can exist in a free form both inside and outside of cells, and extracellular adenosine is responsible for the regulation of many processes throughout the body.

Adenosine becomes particularly important when tissues become deprived of oxygen (a state known as



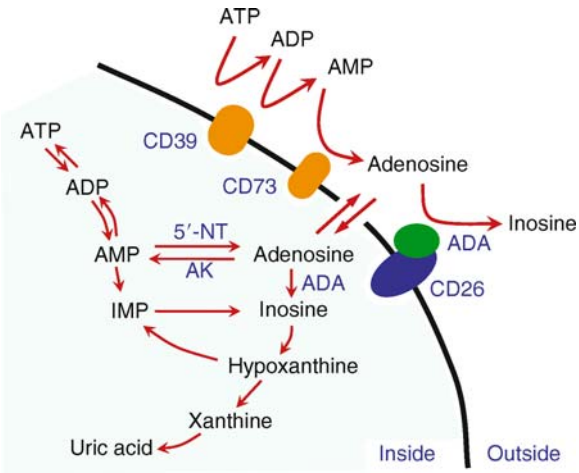
Adenosine and Tumor Microenvironment. Figure 1 The chemical structure of adenosine. Adenosine is composed of a purine base (adenine) linked through a glycosidic bond to a sugar (ribose). Successive phosphate groups may be added at the position indicated by the *arrow* to give AMP (adenosine monophosphate), ADP (adenosine diphosphate) and ATP (adenosine triphosphate).

►hypoxia). This can happen in certain pathological situations, including cancer. It may occur suddenly when blood flow is interrupted, as takes place in a stroke within the brain or during a heart attack. In solid tumors however, hypoxia is a chronic condition because the blood vessels that the cancer forms to nourish itself are not well made and are unable to supply the tissue with sufficient oxygen and other nutrients. For cells to be well oxygenated, they need to be within a distance of about 150 μm of a properly functioning blood vessel. Tumor vessels are typically far apart, are irregular in both size and orientation and can be so poorly regulated that the blood flow may periodically change direction. Cancer cells respond to these harsher conditions by changing their metabolism.

In hypoxic cancer tissues, the balance of energy metabolism in the cells becomes altered. Specific changes in the biochemical pathways of hypoxic cells dramatically change the fate of adenosine. Free adenosine is normally formed principally from adenine nucleotides by the enzyme 5′-nucleotidase inside the cell (some tissues have another pathway that also contributes), and adjacent to the exterior of the cell membrane by a series of proteins including ►CD39 and ►CD73, the latter of which also has 5′-nucleotidase activity (Fig. 2) In hypoxia, the 5′-nucleotidase pathways that lead to adenosine production from adenine nucleotides are activated, while the adenosine kinase enzyme which serves to convert adenosine to AMP is inhibited. These and other changes rapidly increase the concentrations of adenosine within and outside the cell. Since adenosine can pass freely into and out of the cell through various ►nucleoside transporters in the outer membrane, any excess adenosine in the cytoplasm escapes from the cell and further accumulates in the extracellular space. These sources of adenosine contribute to very high extracellular adenosine concentrations in hypoxic tissues.

In tumor tissue the average concentration of adenosine in the extracellular space is approximately 10 μM. Such high concentrations can be found in small tumor nodules of about 2–3 mm in diameter, so are likely to be present in the extracellular fluid of early cancers even before the ►angiogenic switch. Furthermore, because the level of hypoxia varies through the tumor depending upon the proximity of blood capillaries, local levels can be much higher. Finally, adenosine concentrations are highly regulated by ►ecto-enzymes such as adenosine deaminase (ADA) at the cell surface (Fig. 2), so that the ultimate effects of adenosine depend heavily on events at the cell surface.

In normal tissues, where the concentrations of adenosine are low (in the nanomolar range), the principle pathway through which adenosine is metabolized involves phosphorylation to AMP by adenosine kinase. At higher adenosine concentrations, as are present



Adenosine and Tumor Microenvironment. Figure 2 Adenosine production in and around the tumor cell. Adenosine is produced in the cell principally from AMP through the action of 5′-nucleotidase (5′-NT). This pathway is more active under hypoxic conditions, such as exist in solid tumors. Hypoxia also inhibits adenylate kinase (AK), which catalyzes the reverse reaction to convert adenosine to AMP. Outside the cell, adenosine is produced from ATP that is present in the extracellular fluid, by the sequential enzyme activities of CD39 and CD73. The major factor restraining the levels of adenosine that can be reached is the activity of the enzyme adenosine deaminase (ADA), which breaks adenosine down to inosine. This is present both within the cell, and as an enzyme outside of the cell (ecto-enzyme) that is held in place by an anchoring protein, CD26.

inside a tumor, the major route through which disposal of adenosine occurs is by deamination to ►inosine through ADA. ADA is found both within the cell and in the external milieu. The ADA that is present in the extracellular fluid does not remain free, but is largely captured by a 110-kDa binding protein present at the surface of many cells, particularly those of epithelial origin. This ►ADA-binding protein (ADAbp) is found embedded as a dimer in the outer membrane of many cancer cells, where it functions to hold ADA. There is also evidence that some ADA can bind directly to adenosine ►receptors of A_1 and A_{2B} subtypes. ADA held in this way is then able to modify adenosine concentrations immediately next to the cell surface (where the adenosine receptors are located).

One factor that complicates our understanding of how adenosine levels may be regulated within cancer tissue is the fact that adenosine has the capacity to regulate its own levels. This interesting complication arises because ADAbp (also known as CD26 or DPPIV) can be down-regulated at the cell surface by adenosine. That reduces the capacity of the cell to bind ADA at the cell surface and therefore the local rate

of degradation of adenosine. This will extend the half-life of adenosine and increase the persistence of its action. As a result, in the high concentration environment of a tumor, adenosine has the capacity to suppress its own breakdown and enhance its actions still further. (See also ►CD26/DPPIV in Cancer Progression and Spread.)

Although adenosine is a common molecule and has a relatively simple structure, it is able to regulate cellular behavior by interacting with specific receptors. The different types of adenosine receptors are outlined in Table 1 There are four known types, all of which are ►G-protein-coupled receptors with seven transmembrane segments in their structure, embedded in the outer membranes of responsive cells. Adenosine receptors may be found on any of the cell types within a tumor including the cancer cells, the supporting stromal cells, the endothelial cells within blood vessels, or inflammatory cells that are infiltrating the tumor. All four of the adenosine receptor subtypes have been shown to exist on cancer cells; indeed it is possible for a single cancer cell population to express all four forms of the receptor. However, adenosine receptor subtypes A_3 and A_{2B} are the most commonly observed in cancers. The adenosine concentrations that exist in tumors are sufficient to activate all four of the adenosine receptor subtypes.

There are four different types of adenosine receptor Table 1, which differ in their affinity for adenosine and the signalling pathways to which they are linked through G proteins. All of the receptor subtypes are able to act on adenylyl cyclase but may either increase or decrease the production of cAMP as shown. The receptors can also be coupled to phospholipase C (leading to calcium release and activation of protein kinase C), to phospholipase A_2 (causing generation of arachidonic acid and subsequent production of eicosanoid lipid mediators), to phosphatidyl inositol 3-kinase (PI3K, leading to increased activity of the phospholipase D pathway) or in certain cell types can cause the activation of potassium (K) channels.

Adenosine and Tumor Microenvironment. Table 1 The different types of cellular receptors for adenosine

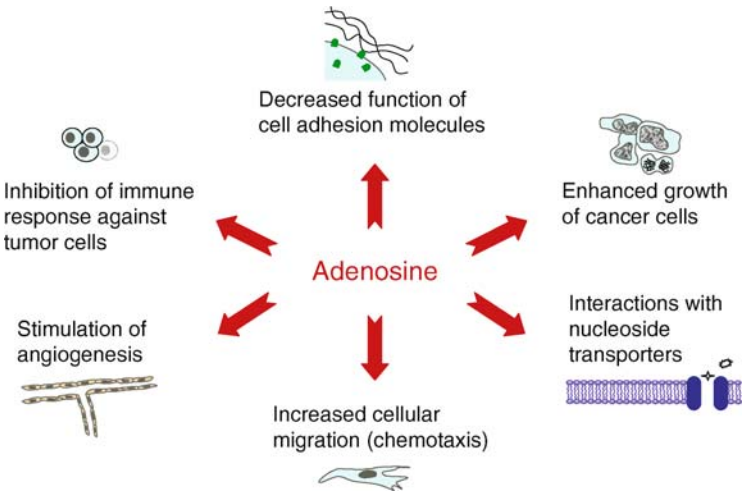
Receptor subtype	Affinity for adenosine	Major G_α protein (s)	Signalling pathways used by receptor
A_1	High	$G_{i/o}$	Adenylyl cyclase (↓ cAMP)
			Phospholipase C
			K^+ channels
A_{2A}	High	G_s	Adenylyl cyclase (↑ cAMP)
			Phospholipase C
A_{2B}	Low	G_s, $G_{q/11}$	Adenylyl cyclase (↑ cAMP)
			Phospholipase C
			Phospholipase A_2
			PI3K
A_3	Low	$G_{i/o}$, $G_{q/11}$	Adenylyl cyclase (↓ cAMP)
			Phospholipase C
			K_{ATP} Channels

The interaction of adenosine with its receptors on the different cell types in a tumor leads to a myriad of different cellular responses. Although it is at times difficult to extrapolate from the experimental approach to the disease itself, these are such as to generally favor the expansion and spread of the cancer (Fig. 3) There is evidence that synthetic agents which target individual receptor subtypes may have different actions to adenosine, sometimes not clearly directed through the adenosine receptor. When adenosine itself is studied at concentrations that are known to be present within the tumor extracellular fluid, it is typically shown to increase the growth of cancer cells. At very high concentrations of adenosine, cells may be triggered to undergo ►apoptosis, although some tumor cells are resistant to this action of adenosine.

In addition to effects on cancer cell growth and survival, adenosine acts on isolated cancer cell populations to increase cell motility, adhesion to the extracellular matrix, the expression of cell attachment proteins and receptors for molecules that can direct cell movement. The patchiness of hypoxia within tumor tissue leads to local areas of high adenosine concentrations that would be capable of influencing tumor cell behavior directionally in this way. While not yet proven, it is possible that within the context of the tumor itself, adenosine may have an influence on the distribution of cells within the tumor and perhaps their dissemination at the later stage of metastasis.

Adenosine receptors are also found on endothelial cells, which are the flattened cells that line blood vessels and which are the major cellular component of the newly-formed vasculature that is formed to supply the expanding cell population with nutrients. Adenosine is able to promote endothelial cell division and motility, and has been shown to enhance the formation of blood vessels (►angiogenesis) in experimental animal models. Adenosine may therefore have an ancillary role alongside other angiogenic factors such as ►VEGF in regulating the formation of the tumor microvascular network.

Probably the greatest potential role for adenosine in the context of cancer however, is as a local immunosuppressant within the tumor. It has long been known that the local tissue environment in cancer is capable of suppressing the immune response, and that this



Adenosine and Tumor Microenvironment. Figure 3 The multiple potential actions of adenosine within a tumor. This diagram summarizes the different ways in which adenosine might act to facilitate the survival and expansion of a malignant tumor. This figure is drawn based upon studies on individual tumor cell populations and other studies *in vivo* in which these responses have been observed.

is one of the factors that limits the capacity of our immune system to eliminate the cancer. Experimental studies have shown that a significant proportion of the immunosuppressive activity is mediated by soluble factors, that it increases in proportion to tissue bulk, and it is seen to decline substantially when the cancer tissue is removed from the animal or patient and dissociated into isolated cells. Adenosine is one of the possible factors responsible for this phenomenon of "metabolic suppression" of the anti-tumor immune response. The capacity for adenosine to act as an immunosuppressant is dramatically illustrated by a rare but well-known genetic disease involving a lack of ADA. In this disorder, levels of adenosine within lymphoid tissues rise and (through a combination of events involving both toxic metabolites and adenosine acting through its receptors) cause a severe immunodeficiency (well known because of the need to protect afflicted children from infection in "biobubble" tents).

Adenosine is capable of interfering with the immune response at different levels and by acting on different cell types. It works through cell-surface adenosine receptors (principally A_{2A} and A_3 subtypes) to suppress various functions of T lymphocytes, natural killer (NK) cells, polymorphonuclear granulocytes, and phagocytic cells such as tissue macrophages that play a key role in recognizing the targets for immunological attack. In the case of T lymphocytes and NK cells, whose infiltration and activity is of key importance to the fate of the tumor and prognosis of the patient, adenosine suppresses successive stages in the evolution and function of the cells. It inhibits proliferation of the cells, the expression of key molecules on the cell surface that are needed to allow full activation, the extent of interaction with the cancer cell, the release of toxic molecules involved in cell killing, and the overall capacity for killing of the cellular targets.

Given the extensive effects of adenosine on nearly all of the cell types present in tumors, it would be appealing to attempt to use drugs that interfere with adenosine pathways as a way of interfering with the growth of the cancer cells, blocking the formation of new blood vessels to nourish the tumor, or relieving the immunosuppression that is due to adenosine. The challenge here lies in the fact that this is a primitive regulatory network in evolutionary terms, and adenosine has a role in the regulation of most organ systems in the mammal. Adenosine receptors of the four subtypes are found on cells throughout the body. Drugs that would a block adenosine's action at its receptors (antagonists) or mimic its actions at a certain receptor subtype (selective agonists) run the risk of interfering with normal processes such as the control of blood flow or the transmission of nerve signals. Nevertheless, there is hope that careful targeting of certain receptors (particularly the A_3 subtype) in cancer may prove to be a useful intervention.

Adenosine Deaminase

Definition

An enzyme involved in the metabolism of deoxyadenosine to deoxyinosine. Congenital deficiency of this enzyme results in the clinical syndrome of severe combined immunodeficiency.

Adenovirus

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Definition

Adenoviruses were originally isolated as etiologic agents for upper respiratory infections. Their name is derived from the initial observation that primary cell explants from human adenoids were found to degenerate secondary to the infection by an, at the time, unknown virus. According to the current official taxonomy, there are four adenovirus genera (Mastadenovirus, Atadenovirus, Aviadenovirus and Siadenovirus), indicating that adenovirus is widely distributed in vertebrates. More than 50 human serotypes have been identified. The individual serotypes are distinguished by different parameters such as immunological properties, tumorigenicity, and DNA sequence. Some serotypes may cause more serious infectious diseases such as epidemic keratoconjunctivitis, gastroenteritis or hemorrhagic cystitis. The adenovirus particle is composed of an outer icosahedric protein capsid with an inner linear double-stranded DNA genome of approximately 36 kilobases (kb) size. There are eleven structural proteins, seven to form the capsid, among them hexon, penton base and fiber being the major constituents of the adenoviral capsid, and four that are packaged in the core. Internalization of the viral particle during infection requires the interaction of the fiber and the penton base with surface proteins (receptors) of the cell. Several virally encoded proteins are associated with the viral DNA.

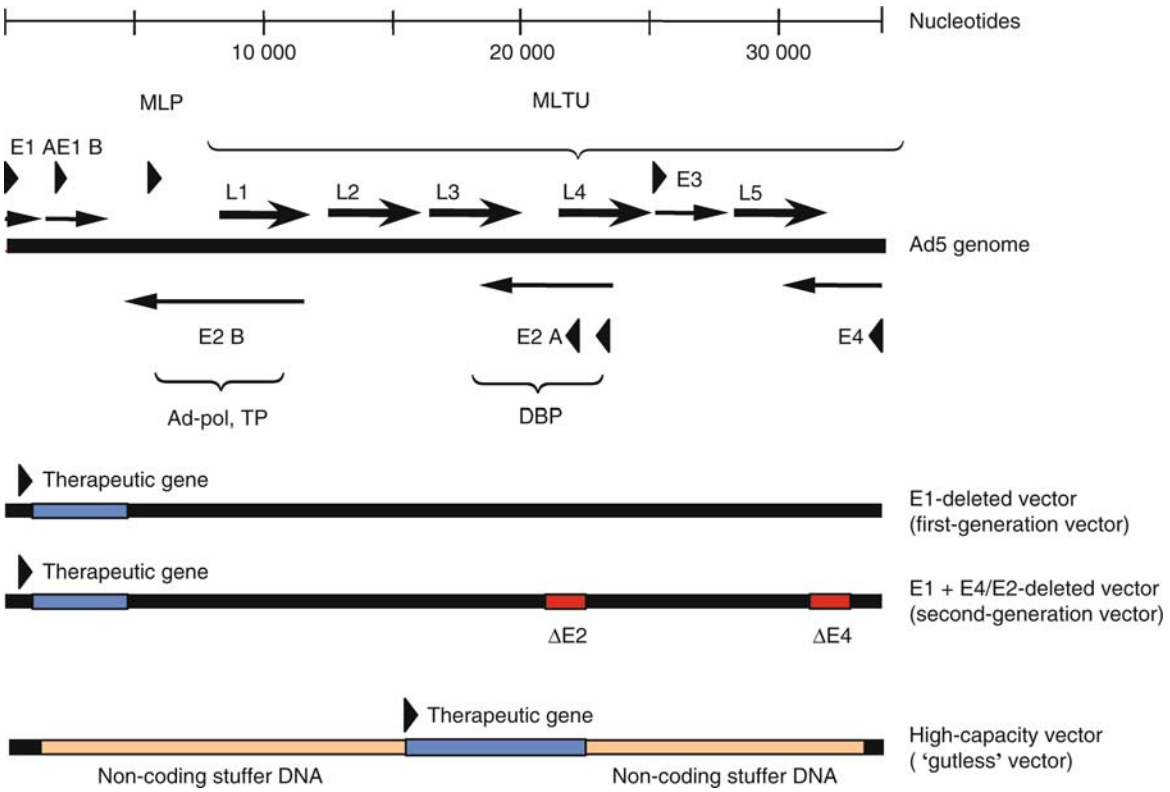
Adenovirus is being used as a gene carrier for ►gene therapy. Most adenoviral vectors (see below) are derived from the serotypes 2 and 5 (Ad2, Ad5) which are frequent causes for mild colds. During childhood most individuals will become immunized against different adenoviral serotypes by natural infection. Ad2 and Ad5 are not oncogenic in humans Adenoviruses have a good safety record based on vaccination studies that have been performed in military recruits two to three decades ago. As detailed below, during natural infection of permissive cells the adenoviral DNA is transcribed, replicated, and packaged into capsids within the nuclei of infected cells. Similar to other DNA viruses, two main phases can be distinguished during infection:

- An early phase that is characterized by the expression of the ►early virus genes E1, E2, E3, and E4
- A late phase after onset of viral replication in which the viral structural proteins are produced

Characteristics

Infection and Viral Transcription

A productive infectious cycle takes approximately 2–3 days and under optimal conditions more than 50,000 particles are produced in every infected cell. In the case of most human adenvirus serotypes the infection begins with the attachment of the virus particle to the cell surface via interaction of the tip of the capsid fiber protein with the membrane protein CAR (Coxsackie–Adenovirus receptor). As it is apparent from the name, CAR is also used by some coxsackie viruses as receptor for entry. Naturally, CAR plays an important role in the interaction of neighboring cells. The adenoviral particle is internalized by receptor-mediated endocytosis into clathrin-coated pits requiring a secondary interaction of the penton base with an αv-integrin. Following endocytosis the viral particle is sequentially disassembled, initally losing the fiber proteins, later most of the other viral structural proteins. Finally, the viral DNA is released as a DNA–protein complex through nuclear pores into the nucleus of the host cell. Shortly thereafter, transcriptional activation of the early genes E1A and E1B initiates a complex transcriptional program designed to first replicate the viral DNA and later to generate new infectious viral particles (Fig. 1). The activation of early and late transcription units follows a relatively well understood transcriptional pattern. The gene products of the E1A and E1B genes are involved in the activation of both viral and cellular genes. Under certain conditions, in particular if infection of a cell does not result in a productive but rather abortive infection (abortive infection = the infectious cycle is blocked at an early stage following infection of the host cell) together with the rare event of integration of the viral DNA into the chromosome, cellular transformation may be a consequence. The E2A and E2B gene products are involved in the replication of the viral genome and include the viral DNA polymerase (Ad-Pol), the terminal protein (TP), and the DNA-binding protein (DBP). The E3 and E4 gene products have diverse functions leading to transcriptional activation of other promoters, preferential export of viral RNAs out of the nucleus of infected cells and suppression of host defenses. With the begin of replication of the viral genome approximately 6 h after infection, late phase transcription units are activated. Most of the late phase proteins are capsid proteins or proteins that are involved in the organization and packaging of the viral genome inside the viral capsid. The most active promoter at this stage is the major late promoter (MLP) that directs the transcription of a large primary RNA transcript that covers more than two thirds of the viral genome. From this transcript five families (L1-L5) of structural proteins are generated by differential splicing and polyadenylation. During the course of an infection the metabolism



Adenovirus. Figure 1 Organization of the adenovirus genome and the different adenoviral vector types employed for gene transfer. Promoters are indicated by arrowheads, transcribed genes by arrows. The genes that are transcribed early during infection are the E1A, E1B, E2, E3, and E4 genes. The main gene products, generated late during infection, are transcribed from the Major Late Promoter (MLP), which directs a very long RNA message (MLTU = major late transcription unit). Different RNA species (L1-L5) that code for structural proteins are generated by alternative splicing and differential polyadenylation (for clarity not all adenoviral genes and gene products are indicated). First-generation adenoviral vectors are characterized by deletion of the E1 genes, second-generation adenoviral vectors by the additional deletion of the E2 and/or E4 genes. High-capacity adenoviral vectors have most of the viral genome removed and retain only the noncoding viral ends. In high-capacity adenoviral vectors, stuffer DNA is included in the vector genome for stability reasons.

of infected cells is redirected to support a predominant production and assembly of viral proteins.

Adenoviral Functions and Oncogenesis

Adenoviruses have played important roles as experimental tools in the discoveries of several fundamental principles in molecular biology, including RNA splicing and oncogenic transformation of cells. In fact, the 1993 Nobel Prize for Physiology or Medicine was awarded to Dr. Phillip Allen Sharp and Dr. Richard John Roberts for the discovery or RNA splicing and was based on their work with adenovirus RNA transcription. The induction of malignant tumors by injection of adenovirus type 12 in newborn hamsters was the first direct demonstration of a human virus causing malignant cellular transformation. This observation greatly stimulated the interest in using viruses as experimental systems in the study of the pathogenesis of cancer. While there is no epidemiological evidence for an involvement of adenoviruses in the pathogenesis of human cancers, several serotypes have been shown to cause tumors in rodents. Some serotypes, such as Ad12 or Ad18 are highly oncogenic in animals, others, for example Ad4 or Ad5 have a low oncogenic potential. Based on several complementing observations cellular transformation is mediated by the viral E1A and E1B genes: In most virus-transformed cells the viral E1 genes are consistently found integrated into the cellular genome where they are expressed. Transfection of cells with the E1A and E1B genes is necessary and sufficient for cell transformation, and viruses with mutations in the E1 genes are defective for transformation. Several RNAs are transcribed from the E1A genes, the main species in Ad5 being the 12S and the 13S RNAs coding for E1A proteins of 243 and 289 amino acids. To a large extent, the E1A proteins exert their transforming activity by interaction with cellular proteins that are involved in cell cycle regulation such as the tumor

suppressor pRB. While E1A alone is capable of immortalizing cells, cooperation with E1B functions is required to achieve a full transformation phenotype. Two main proteins are produced from the E1B gene by alternative splicing. The 21 kD E1B protein that has been shown to inhibit apoptosis, and the 55 kD E1B protein that interacts with the tumor suppressor protein p53. The expression of additional viral functions may contribute to E1 mediated tumorigenesis. For example, a 19 kD protein expressed from the E3 region can decrease MHC class I levels in transformed cells, and certain functions expressed from the E4 region can cooperate with the transforming activity of the E1B 55 kD protein.

Gene Therapy: First- and Second-Generation Adenoviral Vectors

First-generation adenoviral vectors do not replicate in human cells under normal conditions because the E1A and E1B genes are deleted from the vector genome (Fig. 1). These vectors are produced in complementing cell lines that express the E1A and E1B genes. First-generation vectors have been used for gene transfer in cultured cells, animals and even clinical trials in humans to express a large number of genes in different cell types and tissues. So far the results of experiments performed in animals and clinical studies in humans have been relatively disappointing. Several significant disadvantages of first-generation adenoviral vectors have been acknowledged:

- Because first-generation vectors still contain a nearly complete set of viral genes, toxicity and antiviral immune responses are frequently observed resulting in the clearance of transduced cells. Consequently, gene expression is only transient. Contributing factors for short-term gene expression include immune responses directed to the transgenic proteins expressed from the vector, if the organism is not tolerant to that protein.
- The upper DNA packaging limit for adenoviruses is about 38 kb. Because most viral genes are retained on the vector only about 7–8 kb of nonviral DNA can be incorporated into such vectors. However, in many conditions the therapeutic cDNAs are either large, additional elements have to be included to achieve regulated gene expression, or multiple genes need to be expressed to obtain a therapeutic effect. Thus, it is clear that the size constraints in first-generation adenoviral vectors may be a limiting factor for many potential applications. In order to further decrease expression of late viral proteins, adenoviral vectors with inactivation of the E2 and/or E4 functions in addition to the deletion of the E1 region have been generated. These vectors are produced in cell lines that complement both E1 and E2 and/or E4 functions. Currently it is controversial whether these second-generation adenoviral vectors have any significant advantages over first-generation vectors and lead to a longer duration of gene expression.

"Gutless" Adenoviral Vectors

In an attempt to address several of the problems observed with first-generation adenoviral vectors a novel adenoviral vector has been developed that will be useful for the functional analysis of genes in vivo and clinical studies. This vector has been variably called "high-capacity (HC)" adenoviral vector, "gutless", "gutted" or "helper-dependent (HD)" adenoviral vector. Because all viral genes are deleted from this vector the capacity for the uptake of foreign DNA is more than 30 kb. The current production system involves the use of an adenoviral helper virus and takes advantage of the Cre-loxP recombination system. In this production scheme a first-generation adenoviral vector carries two loxP-recognition sequences that flank the adenoviral packaging signal. The vector is produced in E1-complementing cells that express the Cre-recombinase of bacteriophage P1. After infection of these cells both by helper virus and vector the packaging signal of the helper virus is excised. Therefore, vector and only little helper virus is packaged. From several in vivo experiments performed in different animal species it is apparent that these new vectors have clear advantages compared to earlier versions of adenoviral vectors and are considerably improved in safety and expression profiles. Their increased capacity for foreign DNA allows gene transfer of several expression cassettes, large promoters and some genes in their natural genomic context, a significant advantage over first- and second-generation adenoviral vectors.

Replication-Competent Adenoviral Vectors for Cancer Gene Therapy

While the above mentioned adenoviral vectors have been widely used in preclinical and, with the exception of "gutless" adenoviral vectors, also in clinical studies to express a wide variety of transgenes including cytokines, p53 and thymidine kinase (TK), it would be desirable to achieve gene transfer into all or most neoplastic cells within a tumor. This is clearly not possible with current vector technology. Recently, a new concept has been proposed that is based on the use of an adenovirus that is both replication competent and tumor-restricted in its growth. This virus is based on an Ad5 mutant virus that has an inactivating deletion within the E1B gene and does not express the E1B 55 kD protein. Initially, it was thought that replication of the virus was dependent on the p53 status of the host cell and that the virus was able to grow only in cells deficient for function p53 expression. However, more recent results indicate that the growth of this virus is

independent of the p53 status cells and may depend on other cell cycle related factors. Although clinical studies so far have not been or only partially been successful, such a virus has been approved in 2005 in China for cancer therapy and is currently used in combination with chemotherapy and/or radiotherapy.

In addition, replication-competent adenovirus vector are being developed, in which expression of essential viral genes, in particular of E1A, is under control of a tumor-specific promoter. These vectors have been named CRADs (conditionally replicating adenoviruses).

Adenovirus Vectors for Genetic Vaccination

One of the most promising applications of adenovirus vectors is in the area of genetic vaccination. For many common diseases including AIDS or Malaria there are currently no vaccines available. Since adenovirus vectors have been found to induce strong cellular and humoral (antibody) immune responses against expressed genes, many preclinical studies have been performed with the aim of vaccine development. In these studies adenovirus vectors have been found to belong to the strongest inducers of antigen-specific immune responses against different antigens. Therefore, clinical studies have been initiated, in which adenovirus vectors, either alone or in combination with proteins or other vectors, are evaluated for their potential as a vaccine against different infectious diseases.

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Adenylyl Cyclase

Definition

Ubiquitous group of enzymes which catalyze the formation of the second messenger cAMP from ATP. Various mammalian isoforms have been identified which are differentially regulated through G-protein α-subunits and βγ-complexes as well as by Ca^{2+} or protein kinase C.

►G-Proteins
►Signal Transduction

Adherens Junctions

Definition

Adherens junctions are intercellular junctional structures, most prominent in epithelial cells. In the adherens junction, the cell-cell adhesion is mediated by Ca^{2+}-dependent cell adhesion molecules, the cadherins; the cytoplasmic tail of these cadherins is indirectly linked to the ►actin cytoskeleton. Normally are located more basally than ►tight junctions.

►Cell Adhesion Molecules
►E-Cadherin
►Exfoliation of Cells

Adherens Junctions

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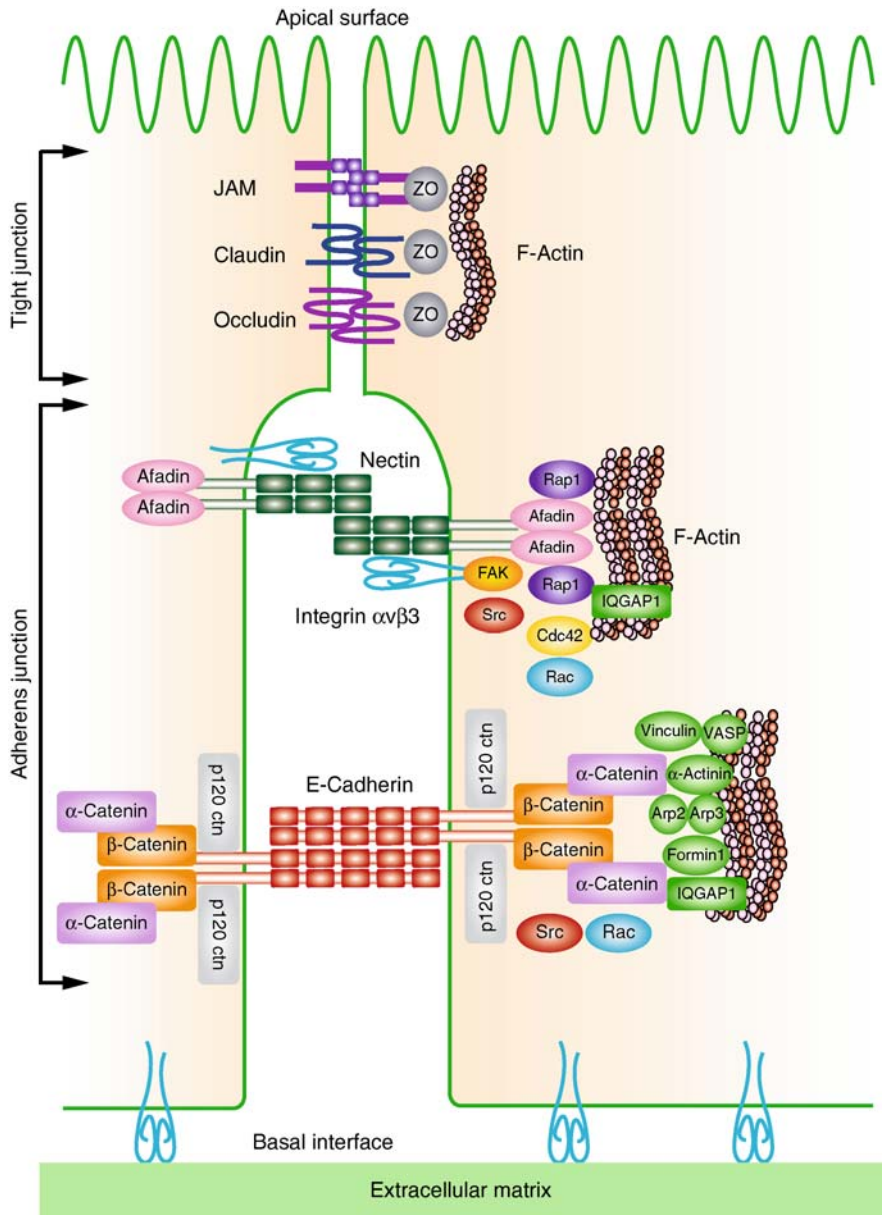
Synonyms

Zonula adherens; intermediate junction

Definition

Adherens junctions are specialized cell–cell attachments composed of transmembrane proteins and cytoplasmic proteins that anchor to the actin cytoskeleton (Fig. 1). Anchoring proteins are clustered with several actin-binding proteins in the cytoplasm adjacent to the junctional membranes. Adherens junctions form punctate or streak-like attachments in nonepithelial tissues, whereas they encircle the apical portion of adjacent epithelial cells below ►tight junctions. Adherens junctions have prototypic roles in stabilizing the epithelium, establishing apical–basal polarity of epithelial cells, and



Adherens Junctions. Figure 1 Epithelial cells joined by the apical adhesion complex. Adherens junctions are located below tight junctions near the apical end of the lateral cell interface in epithelial cells. Nectin and E-cadherin-based cell adhesions are connected via several cytoplasmic proteins into belts of actin filaments that underlie adherens junctions. Nectins are localized to adherens junctions via afadin, and they are associated with integrin αvβ3 in the extracellular space. Afadin binds to the tail of nectin *cis*-dimers as well as F-actin directly, interacting with Rap1. β-catenin binds to the tail of E-cadherin *cis*-dimers directly, and then α-catenin binds to β-catenin. The catenins can mediate interactions to F-actin through binding to several actin-binding proteins such as ZO proteins, afadin, vinculin, α-actinin, VASP, formin-1, and Arp2/3 complex. c-Src, Rac, Cdc42, and FAK play roles in regulating dynamic changes of the actin cytoskelton, facilitated by E-cadherin and nectin clustering.

facilitating cell–cell communication that regulates cell proliferation and movement. Since most human cancers are of epithelial origin, disruption of adherens junctions is one of the hallmarks of cancer cells exhibiting malignant transformation.

Characteristics

Adherens junctions are sites of mechanical attachment regulated by dynamic changes in the actin cytoskeleton, and they also serve as sites of cell–cell communication. Adherens junctions are abundant in many tissues that

are subjected to mechanical stress. In epithelial cells, adherens junctions coalesce into the mature zonula adherens. In cooperation with the zonula occludens (tight junctions), the zonula adherens defines apical–basal polarity by physically separating the membrane into apical and basolateral membrane domains. In addition, adherens junctions mediate nuclear ►signal transduction induced by cell contact. For example, molecules clustered at adherens junctions could mediate contact-dependent inhibition of cell proliferation and movement: the arrest of the cell cycle in G1 phase that occurs when cell density increases to confluence in culture. Thus, the coupling of cell contact and signaling at adherens junctions reflects structural and functional regulations involved in establishing multicellular organisms.

Cadherins and nectins are two major ►cell adhesion molecules in the extracellular space. Cadherins are a superfamily composed of classical cadherins, which are the main components of adherens junctions, and nonclassical cadherins, which include desmosomal cadherins and protocadherins. The classical cadherins share a motif of five cadherin repeats in the extracellular domain, and they are divided into several subtypes including epithelial ►(E) cadherin, placental (P) cadherin, neural (N) cadherin, and vascular endothelial (VE) cadherin. On the other hand, nectins are immunoglobulin-like adhesion molecules composed of four members.

Adherens junctions facilitate cell–cell adhesion through homophilic binding between cadherin molecules, as well as homophilic and heterophilic bindings between nectin molecules on adjacent cells. It remains controversial whether or not the extracellular domain of E-cadherin first binds to form *cis*-dimers on the surface of the same cells, and then promotes cell-cell contacts by forming *trans*-dimers in a Ca^{2+}-dependent manner. On the other hand, each member of nectins forms *cis*-dimers, and then promotes homophilic or heterophilic *trans*-dimer formation in a Ca^{2+}-independent manner. Heterophilic *trans*-interactions have been detected between nectin-2 and nectin-3, between nectin-1 and nectin-3, and between nectin-1 and nectin-4. Importantly, heterophilic *trans*-dimers form stronger cell–cell attachment than homophilic *trans*-dimers, which actually determines the type of cell–cell adhesion. Namely, cadherins exclusively promote adhesion between homotypic cells, whereas nectins have a dual role in promoting adhesion between homotypic cells and between heterotypic cells. Heterophilic engagement of nectins may thus play key roles in cell recognition and sorting *in vivo*.

The intracellular domain of cadherins is associated with a cytoplasmic complex consisting of α-catenin and β-catenin, and forms structural links to the actin cytoskeleton. α-catenin does not act as a stable link to filamentous actin (F-actin) but possibly acts as a molecular switch that regulates actin dynamics at adherens junctions. The catenins could also mediate interactions with F-actin via binding to proteins such as ►ZO protein-1, afadin, vinculin, and α-actinin. The intracellular domain of nectins directly binds to afadin that links nectins to the actin cytoskeleton. Localization of nectins to adherens junctions depends on the presence of afadin. Thus, the catenins and afadin cooperatively contribute to form adherens junctions that are strong yet easily remodeled.

Nectin-based cell–cell adhesions establish adherens junctions, both independently and cooperating with cadherin-based cell–cell adhesions. In Madin–Darby canine kidney (MDCK) cells in culture, nectins first form cell–cell adhesion and then recruit cadherins to the nectin-based cell–cell adhesion sites to establish adherens junctions. Nectins further promote formation of tight junctions in MDCK cells by recruiting JAM (junctional adhesion molecule)-A, claudin-1, and occludin. On the other hand, nectins and integrin αvβ3 are physically associated through their extracellular domains to cooperatively regulate cell movement, proliferation, adhesion, and polarization. Thus, nectins play roles in establishing apical junctional complex, as well as in communication between cell–cell and cell–matrix junctions.

Trans-interacting E-cadherin induces activation of Rac small ►G-protein, which stabilizes non*trans*-interacting E-cadherin on the cell surface by inhibiting endocytosis through the reorganization of the actin cytoskeleton. p120 catenin ($p120^{ctn}$) also plays a role for inhibiting endocytosis of E-cadherin. In contrast, E-cadherin undergoes endocytosis when adherens junctions are disrupted by the action of an extracellular signal, such as hepatocyte growth factor/►scatter factor. Activated c-Src enhances endocytosis of E-cadherin by inducing the tyrosine phosphorylation and ubiquitylation of the E-cadherin complex. On the other hand, *trans*-interaction of nectins activates Cdc42 and Rac, which promotes the formation of adherens junctions mediated by the ►IQGAP1-dependent actin cytoskeleton. In addition, afadin and activated ►Rap1 complex interacts with $p120^{ctn}$ to strengthen the binding between $p120^{ctn}$ and E-cadherin. Furthermore, the cell polarity proteins Par-3, Par-6, and aPKC that form a ternary complex could be implicated in the assembly of adherens junctions. They regulate the association of afadin with nectins in MDCK cells. These cell polarity proteins and afadin could play cooperative roles in the formation of adherens junctions and tight junctions although the mechanism is largely unknown. Thus, E-cadherin and nectin *trans*-interactions induce elaborate interactions between peripheral proteins to establish mature adherens junctions.

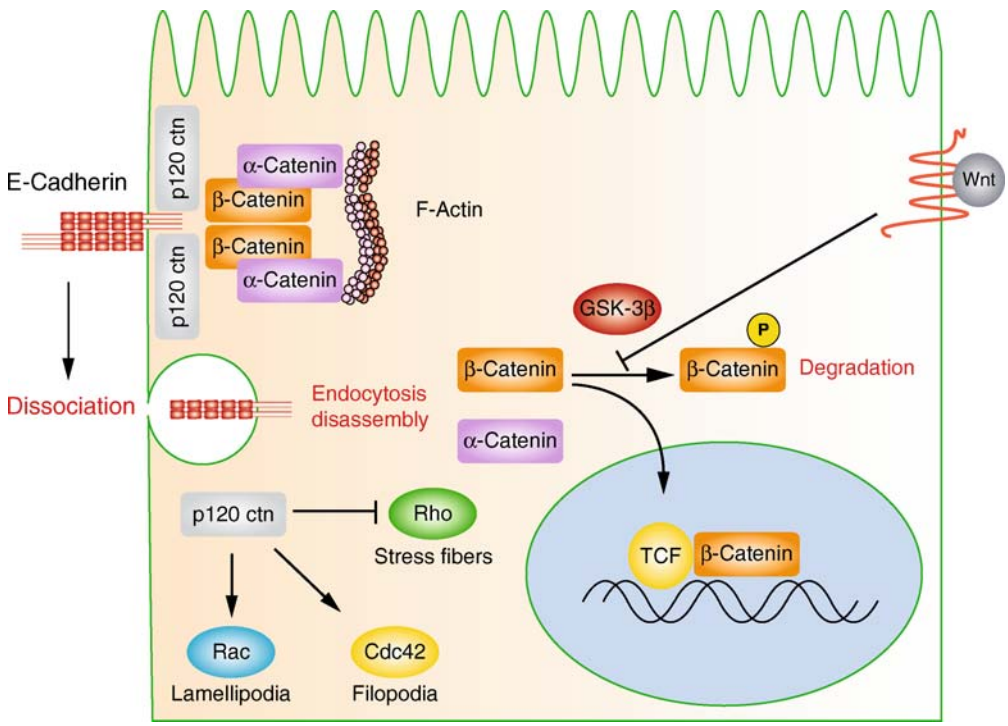
β-catenin is able to translocate to the nucleus, where it binds to lymphoid enhancer factor–T-cell factor (LEF/TCF) that regulates gene transcription. β-catenin is involved in several signaling pathways including the

wingless-type mammary virus integration-site family (►Wnt) ►signaling pathway. When Wnt proteins bind their receptors, they inactivate the serine/threonine kinase GSK3β that phosphorylates β-catenin and targets it for destruction in the proteosome. Mutations involving the serine/threonine residues of β-catenin that are phosphorylated by GSK3β can stabilize the β-catenin protein or increase its nuclear localization. Furthermore, tyrosine phosphorylation of β-catenin also disrupts the association between E-cadherin and β-catenin, allowing β-catenin to transduce signals to the nucleus.

Necl-5, a member of nectin-like cell adhesion molecules (Necls), originally identified as a poliovirus receptor, could mediate growth arrest that has been known as contact inhibition of cell proliferation and movement. Necl-5 is overexpressed in human colon carcinoma, as well as in NIH3T3 cells transformed by ►Ras activation. Necl-5 colocalizes with integrin αvβ3 and growth factor receptors at leading edges of migrating cells and regulates growth factor induced cell migration. When Necl-5 interacts *in trans* with nectin-3 at cell–cell contacts in NIH3T3 cells, Necl-5 undergoes downregulation from the cell surface, resulting in reduction of cell proliferation and movement. Thus, nectins and Necls have roles in mechanical cell–cell adhesion as well as cell–cell communication.

Implications in Cancer

Adherens junctions control epithelial cell polarity while other adhesion apparatus tends to inhibit cell migration, which is crucial for the differentiation and morphogenesis of many tissues. Loss of adherens junctions, as well as aberrant signaling involving the Wnt pathway, could contribute to carcinogenesis and ►metastasis by causing cell depolarization, loss of contact-dependent inhibition of proliferation, and increased ►motility and invasiveness (Fig. 2). Cancer cells that show migratory properties undergo ►epithelial to mesenchymal transition (EMT), with the induction of transcriptional repressor proteins, such as ►snail transcriptional factor, slug, and Twist, that downregulate E-cadherin gene expression. EMT is a basic mechanism that mediates disruption of epithelial polarity and disintegration of cancer cell nests.



Adherens Junctions. Figure 2 Signaling induced by loss of E-cadherin. Disruption of adherens junctions is caused by mutation or transcriptional repression of E-cadherin and growth-factor signaling. Dissociation of homophilic binding of E-cadherin promotes the endocytosis of E-cadherin and the disassembly of the catenins. $p120^{ctn}$ further promotes cell motility by activating Rac and Cdc42 to form lamellipodia and filopodia, and inhibits Rho activity that leads to stress-fiber formation. β-Catenin dissociated from the E-cadherin and catenin complex accumulated in the cytoplasm. Part of β-catenin translocates to the nucleus and binds to TCF to activate transcription of key genes required for survival of detached cells, while the other part of β-catenin is modified by phosphorylation and ubiquitination, leading to proteosome degradation. The Wnt pathway promotes β-catenin signaling by repressing the phosphorylation of β-catenin mediated by GSK-3β.

Reduced E-cadherin levels in cancer cells are accomplished by genetic events such as somatic mutation and reduced gene expression mediated by repressor proteins or by methylation of the promoter region of the E-cadherin gene. The genetic defects of E-cadherin have been found in human lobular breast carcinomas and scirrhus-type ►gastric cancers, both of which have highly metastatic potentials. Mutations of β-catenin also promote migration and ►invasion of cancer cells by the loss of interaction of adherens junctions with the actin cytoskelton.

Distributions of E-cadherin and β-catenin tend to change depending on sites of tumor remodeling. In epithelial structures in the centre of cancer, E-cadherin and β-catenin are mostly present in adherens junctions. However, solitary cells at the invasive front of cancer plates shows no signal for E-cadherin but often produce signals for nuclear β-catenin. Thus, decreased E-cadherin expression promotes the release of solitary cancer cells at the invasive front and increases the survival of cancer cells by stimulating β-catenin signaling.

Strategy for restoring adherens junctions, as well as cell–cell and cell–matrix communication may prevent cancer-cell invasiveness. Therapeutic targets might be molecules involved in pathways affecting the adhesive properties of E-cadherin and the assembly of the adherens-junction complex: c-Src and other tyrosine kinases, tyrosine phosphatases such as PTP-LAR, Rho, Rac, and Rap small G-proteins, transcriptional repressor proteins, and ►merlin and the ►ERM proteins. For example, c-Src regulates both disruption of adherens junctions and focal-adhesion turnover that are required for cancer cell motility. Twist is highly expressed in human cancers with reduced E-cadherin mRNA expression levels. In contrast, podoplanin promotes cancer cell invasion in the absence of EMT, suggesting cancer cells can also migrate as a mass, not necessarily as a single cell. Restoring E-cadherin-mediated cell adhesion could be means of preventing EMT in cancer and metastasis although EMT is not essentially required for cancer-cell invasion.

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Adhesion

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Definition

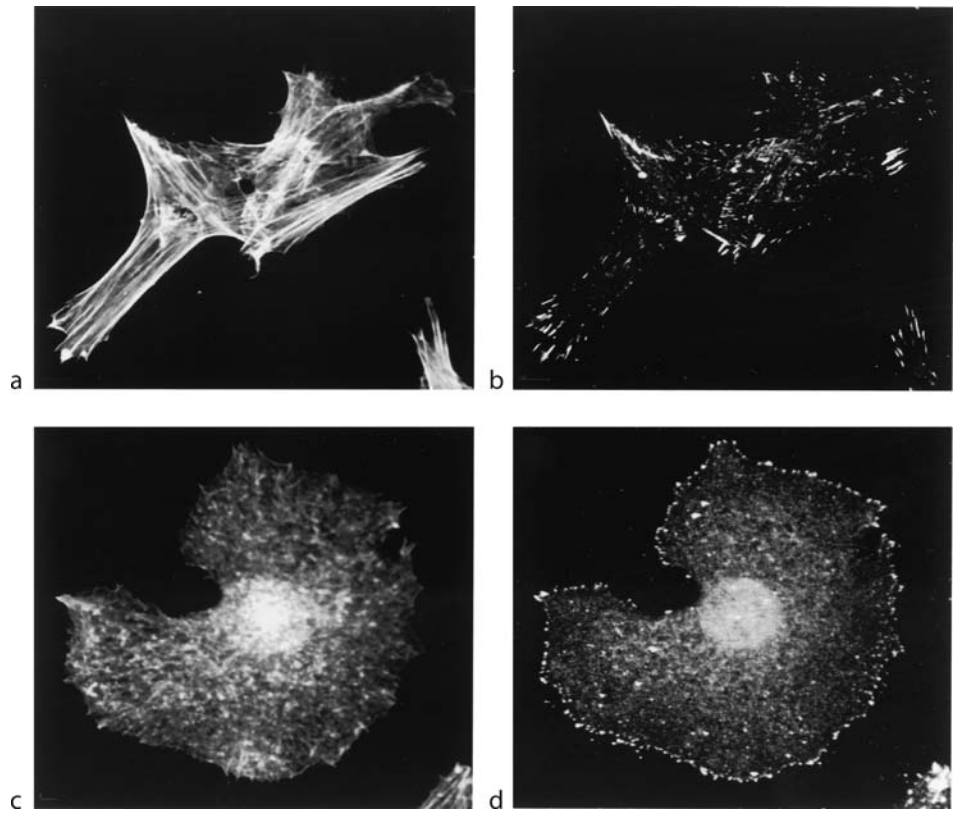
Cell adhesion is a dynamic process that results from specific interactions between cell surface molecules and their appropriate ligands. Adhesion can be found between adjacent cells (cell-cell adhesion) as well as between cells and the ►extracellular matrix (ECM) (cell-matrix adhesion). Besides keeping a multicellular organism together, cell adhesion is also a source of specific signals to adherent cells; their phenotype can thus be regulated by their adhesive interactions. In fact, most of the cell adhesion receptors were found to be involved in ►signal transduction. By interacting with growth factor receptors they are able to modulate their signaling efficiency. Therefore, gene expression, cytoskeletal dynamics and growth regulation all depend, at least partially, on cell adhesive interactions (Fig. 1).

Characteristics

Cell Adhesion Receptors

Cell adhesion molecules were grouped into distinct classes according to structural and/or functional homologies. The following receptors have been directly implicated in the malignant phenotype of tumor cells.

- Integrins represent a family cell surface ►glycoproteins that depend on divalent cations and are important in cell-ECM and cell-cell adhesion. The non-covalent association of an alpha and a beta subunit results in heterodimers that span the plasma membrane, enabling contacts with elements of the ►cytoskeleton and signal transducing intermediates.
- The immunoglobulin superfamily of adhesion receptors is mainly involved in cell-cell adhesion. Named after a 90–100 amino acid domain that is also present in Ig molecules, these kind of receptors can be expressed either as plasma membrane-spanning molecules. However, some of them are alternatively spliced and are anchored to the cell membrane by covalent linkage to phosphatidylinositol.
- ►Selectins represent a class of structurally related monomeric cell surface glycoproteins that bind specific carbohydrate ligands via their ►lectin-like domains. Since the ligands are expressed in a specific way by vascular endothelial cells, selectins are important in lymphocyte trafficking and homing of malignant tumor cells.
- Cell surface ►proteoglycans consist of ►glycosaminoglycans (GAG) attached to core proteins through



Adhesion. **Figure 1** Cell adhesion in normal (a, b) and cancer (c, d) cells. Normal mesenchymal cells show regular actin stress fibers (a: stained with phalloidin) and focal contacts (b: stained with anti-vinculin antibodies). In contrast, cancer cells (a highly motile melanoma cell is shown) often present with a completely disorganized actin cytoskeleton (c) and few focal contacts (d). Vinculin is typically arranged in patches at the periphery of the cell (d) (Confocal micrograph courtesy of Dr. Jörg Hagmann, FMI, Basel).

an O-glycosidic linkage. They can mediate cell-cell and cell-ECM adhesion.

- ►CD44 comprises a large family of proteins generated from one gene by alternative splicing. Variants of CD44 (CD44v) differ from the standard form (CD44s) by their implementation of ten variant exons in various combinations. Some variants have been causally related to the metastatic spread of some tumor cells. Among the ligands for CD44 are hyaluronic acid (HA), fibronectin and collagen, and chondroitin sulfate-modified proteins.
- ►Cadherins are surface glycoproteins involved in cell-cell interactions. They are involved in the formation of adherens-type functions between cells. Through their cytoplasmic tail they interact with catenins, which are important for the signal transducing ability of cadherins.
- ►Connexins are gap junction-forming proteins that oligomerize into specialized intercellular channels, connecting apposing plasma membranes. They allow the exchange of low molecular weight metabolites such as second messengers that are important in signal transduction (Table 1).

Adhesion and Cancer

The selective adhesion of one cell to another or to the surrounding ECM, is of paramount importance during embryonic development as well as for the maintenance of normal adult tissue structure and function. Severe perturbations of these interactions can be, at the same time, cause and consequence of malignant transformation and also play a fundamental role during malignant progression and metastatic dissemination (Fig. 1).

- Adhesion to the ECM through integrin receptors is important for anchorage dependent cell growth and cell survival. Normal cells that are detached from the ECM are locked in the G1 phase of the cell cycle (by loss of activity of the cyclinE/cdk2 complex) and undergo apoptosis (anoikis). Transformed cells, in which integrin signaling is altered, acquire the ability to grow in suspension and do not succumb to anoikis.
- Adhesion to neighboring cells, mediated by cell-cell adhesion molecules (e.g. N-CAM and C-CAM) and by gap-junctions, inhibits growth of normal cells (what is commonly known as "contact growth inhibition"). Loss of these contacts due to the

disrupted function of the relative adhesion molecules may result in uncontrolled proliferation.

- The differentiated state of mature cells (their "identity") is also maintained through specific adhesion to the ECM and adjacent cells: a loss of identity is thus a likely consequence if specific contacts are lost, finally resulting in the ambiguous phenotype of many tumor cells (Fig. 2).

Certain genes that code for cell adhesion molecules may therefore be considered as ►tumor suppressor genes or even ►metastasis suppressor genes since their loss or a functional mutation can strongly contribute to the acquisition of the malignant phenotype.

Adhesion in Metastasis

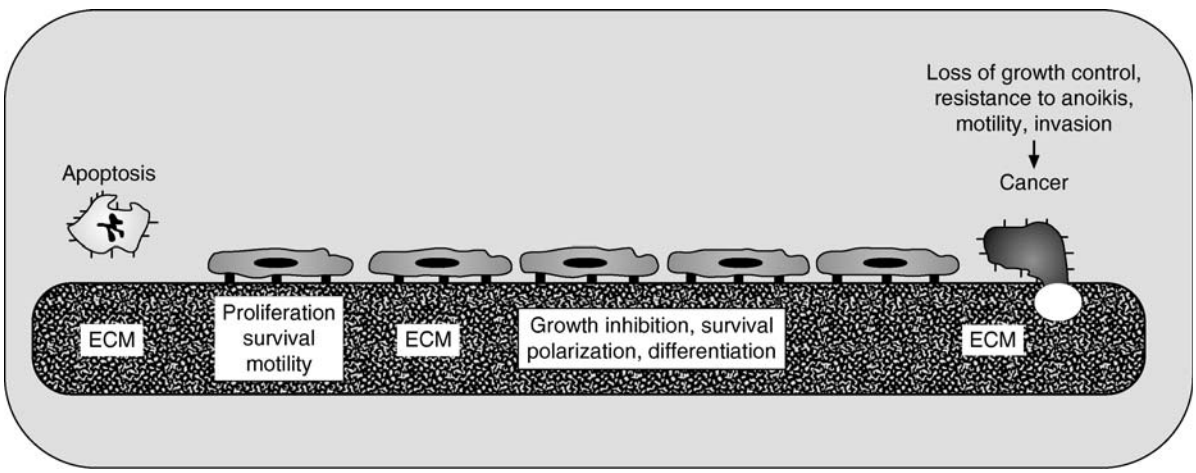
Adhesive interactions play a very critical role in the process of metastatic tumor dissemination, and the abnormal adhesiveness that is generally displayed by tumor cells appears to contribute to their metastatic behavior. Both positive and negative regulation of cell adhesion are required in the metastatic process, since metastatic cells must break away from the primary tumor, travel in the circulation where they can interact with blood cells and then adhere to cellular and extracellular matrix elements at specific secondary sites.

Adhesion within The Tumor Mass

The majority of normal adult cells are restricted by compartment boundaries that are usually conserved during the early stages of development of a tumor. Therefore, the detachment of malignant cells from the primary tumor is an essential step for the initiation of the metastatic cascade. During ►tumor progression changes on the cell surface that lead to a weakening of the cellular constraints, contribute to the release of such mutant cells

Adhesion. Table 1 Adhesion receptors

Family	Main members	Type of adhesion
Integrins	Characterized by the different α- and β-subunits	Cell-ECM cell-cell
IgG superfamily	ICAM-1, V-CAM, N-CAM, CD2 (LFA2), LFA3, CD4, CD8, MHC (class I and II)	Cell-ECM cell-cell
Cadherins	E, P, L	Cell-cell (adherens junction)
Selectins	E, P, N	Cell-cell
Connexins	26 (tumor suppressor) 32 (liver) 43 (glial cells)	Cell-cell (gap junctions)
Cell surface proteoglycans	Syndecan, glypican	Cell-ECM cell-cell
CD44	CD44s, CD44v	Cell-ECM cell-cell



Adhesion. Figure 2 Cell adhesion and maintenance of a normal differentiated phenotype: Detachment of a normal cell from the extracellular matrix (ECM) would normally lead to apoptosis. Normal cells that keep contact with the ECM are protected from apoptosis and may migrate and grow. Normal cells tend to be organized as sheets onto the ECM, which contributes to their polarization and differentiation. Extensive intercellular contacts among cells adhered onto the ECM lead to contact-mediated growth inhibition. Tumor cells do not undergo apoptosis when detached from the ECM and may grow, migrate and invade into the matrix, to enter the circulation and give rise to distant metastases.

from the primary tumor mass. Indeed, it was found that tumor cells separate more easily from solid tumors than normal cells from corresponding tissues.

- Cadherin expression has been shown to influence intercellular cohesion in direct correlation with invasive behavior. An increased cadherin expression in tumor lines, generally causes a tighter association of tumor cells. In vitro experiments have shown that cells which do not express cadherins or in which cadherins are functionally inhibited are more invasive than cells with normal cadherin activity. In cases where E-cadherin was involved, re-introduction of a wild type copy of the gene could revert the invasive phenotype. The loss of cadherin activity however, is not sufficient to make cells invasive. ►Invasion also requires other cellular activities, such as ►motility and protease production. In vivo, tumors expressing low levels of cadherins tend to be less differentiated and to exhibit higher invasive potential, although they are not necessarily more metastatic. In human cancer, a reduction in cadherin activity correlates with the infiltrative ability of tumor cells, a correlation that in many tumors is also retained in distant metastasis.
- A different type of cellular constraint is provided by gap junction communication. Gap junctions play an essential role in the integrated regulation of growth, differentiation and function of tissues and organs. The disruption of gap junction communication can cause irreversible damage to the integrity of the tissue and finally contribute to tumor promotion and malignant progression by favoring local cell isolation. There is experimental evidence that a loss of intercellular junction communication affects the metastatic potential of cell lines. Normal cells use gap junctions to control the growth of tumor cells. Once gap junctional communication is lost, the signaling mechanism responsible for the exertion of such growth control is also lost. Both quantitative and qualitative changes in gap junction protein (connexins) expression were found to be associated with tumor progression during multistage skin carcinogenesis in the mouse model system as well as with tumorigenesis in a rat bladder tumor cell line.

Malignant Tumor Cells in the Blood Stream: Adhesion to Blood Cells and Platelets

Blood-borne tumor cells undergo various homotypic and heterotypic interactions, the effect of which will also influence their metastatic behavior. Some of these interactions may be detrimental to circulating tumor cells such as tumor cell recognition by natural killer (NK) cells, or by tumor infiltrating lymphocytes (TIL). Others may provide, to a certain extent, a protective effect and/or contribute to metastatic spreading, such as interactions with platelets or, in certain cases, with leucocytes.

- De novo expression of the cell adhesion molecule ICAM-1 by melanomas might lead to heterotypic adhesion between melanoma cells and leukocytes bearing the relative receptor (LFA-1). Such interaction might thus enhance tumor cell adhesion to migratory and invasive leukocytes, thereby contributing to further dissemination of malignant tumor cells. In this regard, it has been suggested that site specificity of cancer metastasis might be, at least partially, a consequence of the formation of "multicellular metastatic units" (MSU) consisting of tumor cells, platelets and leukocytes. A subset of leukocytes within the "MSU" would be responsible for site-specific endothelium recognition, adhesion and stable attachment, thus serving as "carrier cells" targeting the metastatic "spheroids" to specific sites of secondary tumor foci formation.
- Several lines of evidence have provided strong support for the concept that tumor cell-platelet interaction significantly contributes to hematogenous metastasis. Two categories of molecules can trigger tumor cell induced platelet aggregation (TCIPA) and activation; soluble mediators and adhesion molecules. The latter are likely to be responsible for the initial contact between tumor cells and platelet cells, and might later stabilize the interaction. P-selectin and αIIbβ3 integrin on the platelet surface may bind Lex carbohydrate determinants and fibrin on the surface of tumor cells, thus triggering platelet activation. Sialylation appears to be a general requirement for TCIPA, and ►sialoglycoconjugates present on both tumor cells and platelets have been involved in tumor cell-platelet interactions. Mechanistically, platelets may contribute to metastasis by stabilizing tumor cell arrest in the vasculature, shielding tumor cells from physical damage, providing additional adhesion mechanisms to endothelial cells and subendothelial matrix, and serving as a potential source of growth factors. If tumor cell interaction with host platelets occurs while tumor cells are circulating, an organ-specific colonization ability of blood-borne tumor cells may be influenced. In fact, the resulting embolus will be more easily arrested in the vasculature of the first organ downstream from the primary tumor site. If this organ represents a favorable milieu for tumor growth, then interaction with platelets will enhance tumor metastasis at that site; if this is not the case, it may prevent tumor cells from reaching their preferred organ and thus cause a reduction of the metastatic potential. It seems, however, that in most cases platelets are involved only after tumor cells have arrested, and platelet activation may then stabilize the initial tumor cell arrest in the microvasculature.

Adhesion in the Target Organ

Circulating tumor cells, either as single cells or most likely as homotypic and/or heterotypic aggregates that have escaped killing by the host immune system and lysis by mechanical shear forces associated with passage in the blood stream, need now to arrest in the microvasculature and extravasate into the organ parenchyma. In fact, the survival time of tumor cells entering the circulation is very short, usually less than 60 min. Therefore those cells that can rapidly arrest and are able to get out the blood stream might have a selective advantage in giving rise to metastatic colonies.

Specific adhesion in the target organ has been proposed as a critical determinant of organ specific metastasis, and experimental data indicates that malignant tumor cells preferentially adhere to organ-specific adhesion molecules. Tumor cells, for instance, adhered more efficiently to disaggregated cells or to histologic sections prepared from their preferred site of metastasis than from other organs. These type of assays, however, do not accurately mimic the physiological situation in vivo, where the first contact of circulating tumor cells happens with the luminal surface of the vascular endothelium, and, after endothelial retraction, with the subendothelial ►basement membrane.

Adhesion to Endothelial Cells (EC)

The arrest of tumor cells in the capillary bed of secondary organs and their subsequent extravasation occur through interactions with the local microvascular endothelium and the subendothelial matrix.

- Biochemical heterogeneity of EC is related to both the heterogeneous microenvironment within tissues and the size of the vessel. Heterogeneity is seen in the differential expression of plasma membrane glycoproteins, cytoskeletal proteins and surface receptors in microvascular endothelium of different organs. Such heterogeneity of endothelium underscores the importance of using organ-specific capillary endothelium in studying the role of organ-specific tumor cell adhesion in metastasis.
- The specificity of the adhesive interactions that depends on the heterogeneity of microvascular EC and tumor cells, may favor, in a selective way, the initial adhesive events in preferred metastatic sites. As a consequence it may also facilitate metastatic dissemination to those organs, in a way that is similar to extravasation of lymphocytes from high endothelial venules of lymphoid tissues. Infact, lymphocyte "homing" represents the paradigm for organ-specific cell adhesion and it has been shown to follow specific interactions between surface "homing" receptors on lymphocytes with vascular "addressins" expressed on the high endothelial venule surface. In a similar way, tumor cells express various combinations of cell surface molecules that may serve as ligands for EC surface receptors, which are typically induced upon stimulation by mediators of inflammation. A local inflammatory response might thus facilitate circulating tumor cells adhesion and arrest. The relevance of this type of interaction in directing tumor metastasis has been recently demonstrated in vivo using strains of transgenic mice that constitutively express cell surface E-selectin either in all tissues or in the liver alone. Metastatic tumor cells that do not express the ligand, colonized mostly the lung. However, following the induction of ligand expression, tumor cell colonization was redirected to the liver with tremendous efficiency.

Adhesion to Extracellular Matrix Components

Mammalian organisms are composed by a series of tissue compartments separated from one another by two types of extracellular matrix (ECM): basement membranes and interstitial stroma. ECM consists of three general classes of macromolecules, including collagens, proteoglycans and non-collagenous glycoproteins (such as fibronectin, laminin, entactin and tenascin among others), which are expressed in a tissue-specific fashion.

Malignant cells arrested in the microcirculation sometimes do not migrate further into the organ parenchyma but grow locally in an expansive fashion until they rupture the vessel wall. In most cases however, the contact between tumor cells and the endothelium results in EC retraction with exposure of the underlying basement membrane, followed by invasion of tumor cells in the tissue.

The presence of specific adhesion receptors on the membrane of metastatic cells, and the peculiar composition of the extracellular matrix at a given site will influence tumor cell retention, motility and invasion, and growth at target organs.

- Electron microscopy observation on the formation of pulmonary metastasis has shown that tumor cells often adhere to regions of exposed basal lamina. The exposed subendothelial matrix is usually a better adhesive substrate for tumor cells than the endothelial cell surface.
- In order to move through the ECM, tumor cells must make firm contacts with matrix molecules, be able to break these adhesive contacts as they move on and respond to chemotactic molecules that direct their movement. Interactions with the ECM may fulfill all these scopes, through the signaling effect of several cytokines (growth factors, motility factors, enzymes and enzyme inhibitors) that are stored bound to ECM molecules, and released upon interaction with tumor cells. Moreover, ECM macromolecules themselves may also function as motility attractants, and have

been shown to stimulate both ►chemotaxis and ►haptotaxis. Haptotactic migration over insoluble matrix components may occur predominantly during the initial stages of metastatic invasion, while at later stages partially degraded matrix proteins, derived from proteolytic processing of the matrix, could be the major determinant of directed motility.

- Finally, it has to be considered that some ECM components may actually impede cell adhesion and thus might influence directional tumor cell motility by promoting the localized detachment of the trailing edge of migrating cells. ECM-associated chondroitin sulfate proteoglycans such as decorin, or the glycoprotein tenascin, have been suggested to modulate tumor cell adhesion and motility in this way.

Adhesion and Drug Resistance

The malignant phenotype of tumor cells depends, at least partially, on the weakening of cell-matrix and cell-cell interactions that occurs during tumor progression. However, late stage tumors maintain some level of intercellular adhesion, or even tend to reactivate certain adhesion mechanisms, indicating that modulation of cell adhesion is a dynamic process. Given the beneficial effect of cell adhesion on apoptosis resistance, an increased level of adhesion may facilitate survival of tumor emboli, and there is evidence that it can help tumor cells to evade the cytotoxic effects of anticancer therapy.

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Adhesion Molecules

Definition

Are transmembrane cell adhesion proteins which extend across the cell surface ►membrane and typically have domains that extend into both the extracellular space and the intracellular space. The extracellular domain of a cell adhesion protein can bind to other molecules that might be either on the surface of an adjacent cell (cell-to-cell adhesion) or part of the ►extracellular matrix (cell-to-ECM adhesion).

►Furin
►Sjögren Syndrome

ADI

►Arginine-Depleting Enzyme Arginine Deiminase

Adipocyte Complement-Related Protein of 30 kDa

►Adiponectin

Adipocyte C1q

►Adiponectin

Adipocytes

Definition

Fat storing cells.

►Signal Transduction
►Adipose Tumors

Adipocytic Tumors

►Adipose Tumors

Adipokine

Definition

refers to a cluster of adipocyte-secreted molecules, which consist of growth factors, metabolic hormones, and cytokines etc. Many adipokines, such as leptin, adiponectin, resistin, visfatin, and adipocyte fatty acid binding protein, play important roles in obesity and its related diseases.

►Adiponectin

Adiponectin

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Synonyms

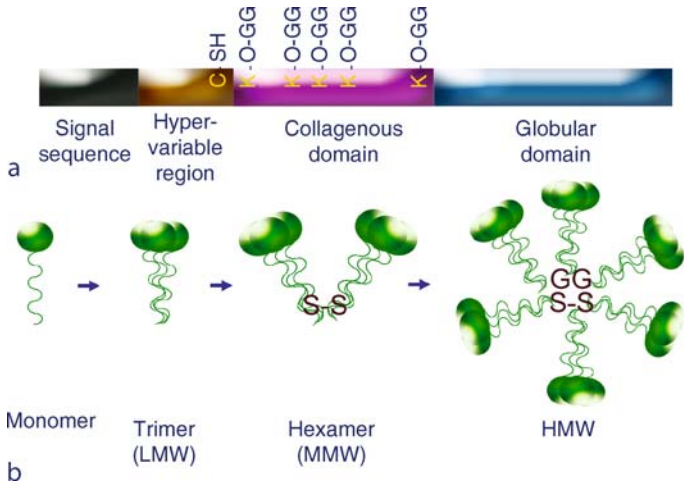
Gelatin-binding protein 28; GBP28; AdipoQ; Adipocyte complement-related protein of 30 kDa; ACRP30; Adipose most abundant gene transcript 1; apM1; Adipocyte C1q and collagen domain containing; ACDC

Definition

Adiponectin is a major ►adipokine secreted exclusively from adipocytes. This adipokine has been demonstrated to possess antidiabetic, antiatherogenic, antiinflammatory and, more recently, antitumorigenic properties.

Characteristics

Adiponectin was originally identified as an adipose-specific gene dysregulated in ►obesity. Human adiponectin gene is located on chromosome 3q27 and encodes a 244 amino acids polypeptide comprising of an NH_2-terminal secretory signal sequence, followed by a hypervariable region, a collagenous domain, and a COOH-terminal globular domain (Fig. 1a). Circulating concentrations of adiponectin range from 3 to 30 μg/ml, which accounts for about 0.05% of total human blood proteins. Endogenous adiponectin is predominantly present as several characteristic oligomeric complexes. The basic building block of the adiponectin complex is a trimer or low molecular weight (LMW) oligomer, which is formed via hydrophobic interactions within its globular domain. Two trimers self-associate to form a disulfide-linked hexamer or middle molecular weight (MMW) oligomer, which further assembles into a bouquet-like high molecular weight (HMW) multimeric complex that consists of 12–18 monomers (Fig. 1b). Posttranslational modifications, including disulfide bond formation at a conserved cysteine residue and glycosylations occurred on several hydroxylated lysine residues within the collagenous domain, are involved in the assembly and stabilization of the



Adiponectin. Figure 1 Schematic representation of the primary structure (a) and the oligomeric complexes of adiponectin (b). Adiponectin contains a NH_2-terminal signal sequence peptide and a hypervariable region, followed by a conserved collagenous domain and a COOH-terminal globular domain. A cysteine residue within the hypervariable region is involved in the disulfide bond formation. Several lysine residues located within the collagenous domain are hydroxylated and glycosylated. (b) Adiponectin exists as three oligomeric species, including the trimer (LMW), hexamer (MMW), and HMW. Disulfide bond formation and glycosylation are involved in its oligomeric formation. GG, glucosylα(1–2)galactosyl group; S−S, disulfide bonds.

oligomeric structures. Different oligomeric complexes of adiponectin activate distinct signaling pathways and possess different biological functions.

Two putative adiponectin receptors, termed AdipoR1 and AdipoR2, have recently been identified. AdipoR1 is highly expressed in skeletal muscle whereas AdipoR2 is most abundantly expressed in liver. Both receptors are integral membrane proteins containing seven transmembrane spanning domains. AdipoR1/R2 mediates the effect of adiponectin on activation of ►AMP-activated protein kinase (AMPK) and stimulation of glucose uptake and fatty acid oxidation. T-cadherin, which is highly expressed in endothelium and smooth muscle, has been identified as an adiponectin coreceptor with preference for hexameric and HMW adiponectin multimers. Both adiponectin analogues and adiponectin receptor agonists represent the potential therapeutic targets for obesity-linked diseases.

Adiponectin and Carcinogenesis

Although adiponectin is secreted exclusively from fat cells, the circulating adiponectin levels are paradoxically reduced in obese individuals and obesity-related pathological conditions, such as ►insulin resistance, type 2 diabetes, and atherosclerosis. Adiponectin has been proved to have insulin-sensitizing, antidiabetic, and antiatherogenic activities. In addition, growing evidence has demonstrated adiponectin to be a potent inhibitor of carcinogenesis. Numerous clinical studies have observed an inverse association between blood adiponectin concentrations and risks of several ►obesity-related cancers, such as ►prostate, ►breast, ►endometrial, ►gastric, and ►colorectal cancers.

Prostate Cancer

Obesity is associated with prostate cancer progression, increased tumor aggressiveness, and poor prognosis. Higher plasma adiponectin is associated with a marked reduction in risk of prostate cancer, independent of other risk factors. Additionally, blood adiponectin levels in those with high-grade prostate cancer are significantly lower than those in the low-grade and intermediate-grade groups, suggesting that plasma adiponectin levels are negatively associated with the histologic grade and disease stage of prostate cancer. Adiponectin has been shown to inhibit leptin- and/or ►insulin-like growth factor-1 (IGF-1)-stimulated DU145 androgen independent prostate cancer cell growth and dihydrotestosterone-stimulated growth of androgen-dependent LNCaP-FGC cells at subphysiological concentrations. In addition, adiponectin enhances the inhibitory effects of the cytotoxic chemotherapy agent, doxorubicin, on prostate cancer cell growth. These data suggest that adiponectin could play an important role in the pathogenesis of prostate cancer, and may be used as a drug target for therapeutic interventions.

Breast Cancer

Excess adiposity over the pre- and postmenopausal years is an independent risk factor for the development of breast cancer, and is associated with late-stage disease and poor prognosis. Clinical studies have shown that low plasma adiponectin levels are significantly associated with an increased risk for breast cancer in both pre- and postmenopausal women, particularly in a low estrogen environment. Moreover, tumors from women with low plasma adiponectin levels are more likely to show a biologically aggressive phenotype. In line with these clinical findings, experimental evidence supports the role of adiponectin as an inhibitory factor for breast cancer development. Adiponectin at physiological concentrations suppresses the proliferation and induces ►apoptosis in the ►estrogen receptor (ER)-negative human breast carcinoma MDA-MB-231 cells and the ER-positive human MCF7 breast cancer cells. It also inhibits insulin- and growth factors-stimulated cell growth in another ER-positive T47D human breast cancer cells. Furthermore, adiponectin replenishment therapy suppresses mammary tumorigenesis of MDA-MB-231 cells in nude mice.

Endometrial Cancer

Adiponectin is decreased in obesity, insulin resistance, type 2 diabetes, and polycystic ovary syndrome, all of which are well-established risk factors for endometrial cancer. Several case-control studies have demonstrated an inverse correlation between plasma levels of adiponectin and the risk of endometrial cancer, independent of other obesity-related risk factors as well as the major components of the IGF system. Moreover, a stronger inverse association is observed among obese women than among nonobese women. Further studies are needed to investigate whether adiponectin deficiency plays a causative role in the pathogenesis of endometrial cancer.

Gastric and Colorectal Cancer

Low plasma levels of adiponectin have been observed in patients with gastric cancer, especially in those with upper gastric cancer. Furthermore, plasma adiponectin levels tend to decrease as the tumor size, depth of invasion, and tumor stage increases. Additionally, the negative correlation is more significant in undifferentiated forms than in differentiated forms of gastric cancers. These data raise the possibility that adiponectin might play a potential role in the progression of gastric cancer, especially in the upper stomach.

Although colorectal carcinogenesis is related to abdominal obesity and insulin resistance, the associations between low adiponectin levels and colorectal cancer are not conclusive. Two independent studies have suggested that men with low plasma adiponectin levels have a higher risk of colorectal cancer,

whereas another report does not support this association. Moreover, adiponectin has been reported to have growth-promoting and proinflammatory actions on HT-29 colonic epithelial cancer cells.

Leukemia

Adiponectin inhibits cell proliferation and induces apoptosis in myelomonocytic cell lines. Decreased levels of plasma adiponectin have been found to be associated with ►childhood acute myeloblastic leukemia (AML), but not with ►acute lymphoblastic leukemia of B (ALL-B) or T (ALL-T) cells. Adiponectin levels are also reported to be inversely associated with ►chronic lymphocytic leukemia and myeloproliferative diseases. However, it is worthy to note that adiponectin concentrations can be modulated by various inflammatory cytokines and interferon therapy in these conditions. Thus, whether low adiponectin level is a causal factor of leukemia, or a secondary response to ►inflammation, needs to be further clarified.

Mechanisms

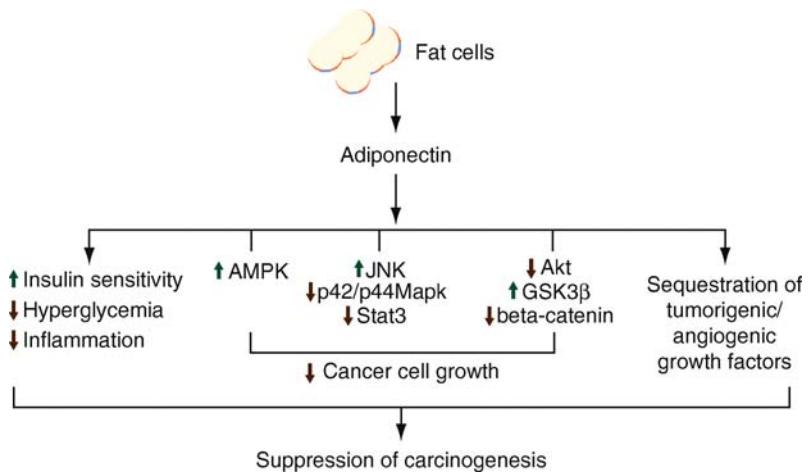
As summarized above, both clinical and experimental evidence support the role of adiponectin as a suppressor of tumorigenesis. Adiponectin has direct antiproliferative effects in a number of cancer cell lines. Although the underlying mechanisms remain poorly understood, adiponectin has been shown to modulate several intracellular signaling cascades involved in regulating cell proliferation and apoptosis (Fig. 2).

AMPK

The phosphorylation-dependent activation of ►AMPK is the major signal transduction pathway evoked by adiponectin. AMPK mediates the insulin-sensitizing effects of adiponectin in liver and muscle. It is also involved in the regulatory activities of adiponectin on endothelial cell functions and cardiac remodeling. AMPK is a negative regulator on cell growth. The upstream kinase LKB1 that activates AMPK is a ►tumor suppressor. ►Tuberous sclerosis complex 2 (TSC2), another tumor suppressor, is downstream of AMPK and a key player in regulation of the ►mammalian target of rapamycin (mTOR) pathway. Through inactivation of ►mTOR, AMPK negatively regulates protein synthesis and de novo fatty acid synthesis, two essential elements for rapid cancer cell growth. In addition, AMPK controls phosphorylation and activation of the ►P53 tumor suppressor and expression of the cell cycle inhibitor ►p21. These molecular events might represent the potential mechanisms through which adiponectin regulate carcinogenesis. Indeed, it has been reported that adiponectin at subphysiological concentrations can induce AMPK phosphorylation and reduce the cell growth in human breast cancer MCF-7 cells.

c-Jun N-Terminal Kinase (JNK) and Signal Transducer and Activator of Transcription 3 (STAT3)

Both ►JNK and STAT3 are the important regulators of cell proliferation, apoptosis, and differentiation in various physiological and pathophysiological conditions. Constitutive activation of STAT3 is crucial in malignant transformation and cancer progression. It has been reported that adiponectin stimulates the phosphorylation of JNK in prostate cancer DU145, PC-3, and LNCaP-FGC cells, as well as in hepatocellular carcinoma HepG2 cells. On the other hand, adiponectin inhibits constitutive activation of STAT3 in DU145 and HepG2 cells, suggesting that activation of JNK and inhibition of STAT3 may contribute to the suppressive effect of adiponectin on carcinogenesis. In addition, the inactivation of p42/p44



Adiponectin. Figure 2 Adiponectin elicits its antitumorigenic activities through multiple mechanisms.

MAP kinase has been implicated in the antiproliferative effects of adiponectin in human beast carcinoma MCF-7 and T47D cells.

Glycogen Synthase Kinase (GSK) 3β/β-Catenin Signaling Pathway

Hyperactivation of the canonical ►Wnt/β-catenin pathway is one of the most frequent signal abnormalities in many types of cancers. The central event in this pathway is the stabilization and nuclear translocation of β-catenin, where it binds to the transcription factor TCF/LEF and consequently activates a cluster of genes that ultimately establish the oncogenic phenotype. β-catenin is phosphorylated by GSK3β and then modified by ►polyubiquitination for ►proteasome-mediated degradation. Adiponectin could modulate the GSK3β/β-catenin pathway in human breast cancer cells. In MDA-MB-231 cells, prolonged treatment with adiponectin markedly reduces serum-induced phosphorylation of GSK3β, decreases intracellular accumulation and nuclear translocation of β-catenin, and suppresses ►cyclin D1 expression. These effects can be inhibited by inhibitors of GSK3β. These data suggest that the cross-talk between adipokines and the Wnt signaling pathway might represent a key mechanism underlying the development of obesity-related cancers.

Other Pathways

In addition to its direct suppressive effect on cancer cell proliferation, as an insulin-sensitizing hormone, adiponectin could ameliorate tumorigenesis indirectly by alleviating ►hyperglycemia and insulin resistance, the two established risk factors for many obesity-related cancers. Furthermore, adiponectin possesses antiinflammatory activity and can inhibit the production of a number of inflammatory factors involved in promoting tumorigenesis, such as IL6, IL8, TNFa, and MCP-1. There is also evidence supporting the anti ►angiogenesis activity of adiponectin. It inhibits tumor neovascularization in mice, through suppression of endothelial cell proliferation, migration, and tubular formation. Moreover, adiponectin can act as a decoy for several proangiogenesis growth factors, including basic fibroblast growth factor (bFGF), platelet-derived growth factor BB (PDGF-BB), and heparin-binding epidermal growth factor (HB-EGF). In this manner, adiponectin prevents these growth factors from activating their respective receptors and effectively impedes their tumor-promoting activities.

In summary, both experimental and clinical evidences support the suppressive role of adiponectin in tumorigenesis. In humans, adiponectin deficiency is closely associated with increased risks of several obesity-related cancers. Therefore, adiponectin and its agonists might represent a novel class of the anticancer agent for the treatment of these malignant tumors. Further studies are warranted to investigate the prospective associations between plasma adiponectin levels and the risk of several obesity-related cancers, and to elucidate the detailed molecular events underlying the antitumor activities of adiponectin.

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AdipoQ

►Adiponectin

Adipose Most Abundant Gene Transcript 1

►Adiponectin

Adipose Tumors

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Synonyms

Lipomatous tumors; Adipocytic tumors; Lipomas; Liposarcomas

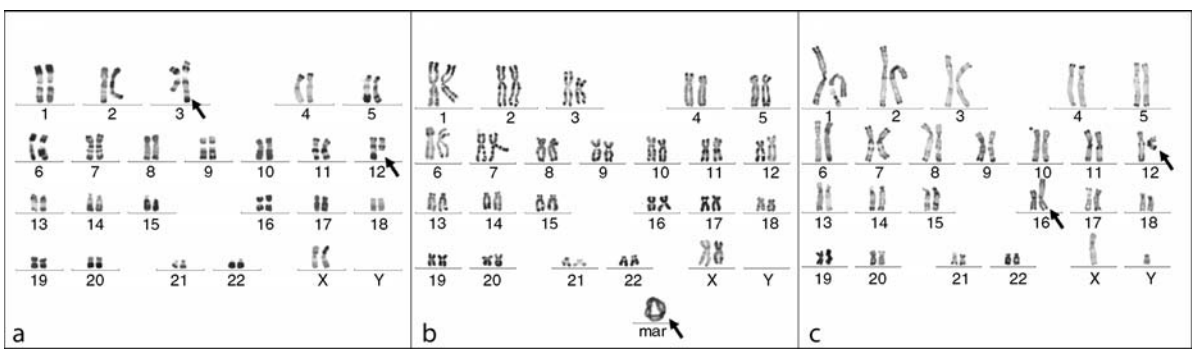
Definition

Adipose tumors (AT) are mesenchymal neoplasms that form the largest group of human tumors. They include benign tumors, such as the very common lipomas, as well as rare malignant tumors with various degrees of clinical aggressiveness. Histologically, AT consist of adipocytic cells showing different levels of differentiation, from mature adipocytes in benign lipomas up to undifferentiated lipoblastic cells in high-grade ►liposarcomas. The 2002 World Health Organization classification distinguishes seven entities of benign AT: lipoma, lipoblastoma/lipoblastomatosis, angiolipoma, myolipoma of soft tissue, chondroid lipoma, spindle cell/pleomorphic lipoma, and hibernoma. Malignant AT, also called liposarcomas, include three types: well differentiated liposarcoma/dedifferentiated liposarcoma, ►myxoid/round cell liposarcoma, and pleomorphic liposarcoma. Except for the ordinary superficial lipomas, differential diagnosis between benign and malignant AT and between AT and other kinds of tumors is sometimes difficult. Studies based on tumor karyotypes have identified chromosomal abnormalities specific to benign and malignant AT and recent advances in molecular cytogenetics improved AT diagnosis. It is now possible to directly detect the genic rearrangements resulting from chromosomal alterations on interphase nuclei such as those in formalin-fixed and paraffin embedded tumor tissue sections using fluorescence in situ hybridization (FISH) (►interphase cytogenetics) or polymerase chain reaction (PCR).

Characteristics

Benign Adipose Tumors

The most common benign AT are the so-called superficial "conventional lipomas." The other types of benign AT are rare and may be the cause of diagnostic difficulties because of their clinical or histological resemblance to malignant soft tissue tumors. In most cases benign AT do not require any treatment. Surgical removal may be necessary in case of functional or cosmetic impairment. ►Conventional lipomas are the most common soft-tissue neoplasm in adults. They occur mainly in the 5–7th decades of life and are generally located superficially in subcutaneous fat. They can also be situated deeply in muscles or on the surface of bones or rarely in visceral and other organ sites. Lipomas usually present as a small (<5 cm), well-circumscribed painless mass under the skin of the neck, shoulders, back, arms or thighs. However, almost all subcutaneous anatomical locations have been reported. Occasionally, lipomas can be painful if they grow large or press nearby nerves. Microscopically they are composed of mature adipocytes, which are usually indistinguishable from those observed in normal adipose tissue. Cytogenetic studies have shown that lipomas are characterized by clonal chromosomal aberrations in approximately two thirds of the cases. A majority of these aberrations involve the 12q13–15 region. Such rearrangements are often balanced ►chromosome translocations or inversions that frequently result in fusion of the *HMGA2* gene (12q15), with a variety of partners. The most frequent aberration is the translocation t(3;12)(q28;q15) that fuses *HMGA2* to ►*LPP* (3q28) (Fig. 1a). In lipomas, the *HMGA2* breakpoints are preferentially clustered in the large third intron of the gene. Extragenic breakpoints located 5′ or 3′ to *HMGA2* also have been described. Of note, *HMGA2* is rearranged not only in lipomas but also in several benign mesenchymal tissue tumors such as uterine leiomyomas, pulmonary chondroid hamartomas and chondromas. It has been proposed that the truncation of *HMGA2* might be the critical step in tumorigenicity by inducing derepression of the gene. The importance of the role of *HMGA2* in adipose proliferation has been shown in studies of *Hmga2*-null and heterozygous mice. These mice are resistant to diet-induced or genetic obesity because of the retarded proliferative capacity of preadipocytes. Moreover, several transgenic mouse



Adipose Tumors. Figure 1 Representative RHG-banded karyotypes from a case of lipoma (a), a case of well-differentiated liposarcoma (b) and a case of myxoïd liposarcoma (c). (a) Balanced translocation t(3;12). (b) Supernumerary ring chromosome. (c) Balanced translocation t(12;16). The arrows indicate the abnormal chromosomes.

models have demonstrated that the misexpression of *HMGA2* is sufficient to induce benign mesenchymal tumors such as lipomas. Among conventional lipomas without involvement of chromosome 12, the most frequent rearrangements are translocations involving 6p21–23 in the region of *HMGA1* (6p21), loss of 13q material with breakpoints in 13q12–14 and/or 13q22 and 8q anomalies. However, a variety of other chromosomal aberrations – most often simple structural rearrangements – can be observed in conventional lipomas. ►Spindle cell or pleomorphic lipomas are usually seen in the subcutaneous tissue of the neck and upper trunk of middle-aged men. They are composed of varying amounts of fat cells and bland CD34+ spindle cells present within a background of wiry collagen and myxoid ground substance. Only a few cases have been investigated cytogenetically. The main characteristic features are complete or partial losses of chromosomes 16 and 13. When located in an anatomical site other than neck and shoulders, spindle cell lipomas may be difficult to diagnose. ►Chondroid lipomas have a significant female predilection. Histologically, they consist of mature fat cells, with myxoid, chondroid, and hyalinized areas. Cells contain vacuoles resembling lipoblasts or chondroblasts and stain positively for S100 protein. The differential diagnosis includes myxoid liposarcomas (see below) or extraskeletal myxoid chondrosarcomas, and distinguishing these can be sometimes difficult. Only four cases of chondroid lipoma have been investigated cytogenetically and all showed translocation t(11;16)(q13;p13). ►Angiolipomas are subcutaneous lesions most commonly affecting male in the late teens or early twenties. The most frequent sites of involvement are the forearm, the trunk and the upper arm. Multiple lesions are seen in the majority of cases. The familial prevalence is estimated at 5%. Angiolipomas consist of mature adipocytes and small capillaries often containing fibrin thrombi. The pathogenesis of such lesions is unknown and no specific cytogenetic aberration has been reported to date. ►Hibernomas consist of brown fat and usually occur in young adults. The thigh is the most common location. Hibernomas are cytogenetically characterized by deletions of the long arm of chromosome 11. ►Lipoblastomas are made up of embryonal white fat and occur in the first 3 years of life more frequently in males. However lipoblastomas may occasionally affect older patients. Lipoblastomas most commonly manifest as asymptomatic, circumscribed masses in the superficial or subcutaneous soft tissue of the extremities although less frequent locations including mediastinum, retroperitoneum, trunk, neck and various organs have been reported. In contrast, the diffuse type (diffuse lipoblastomatosis) tends to infiltrate not only into the subcutis but also into the underlying muscle. These lesions are composed of an admixture of lipoblasts and mature adipocytes, organized into lobules separated by fibrous septa. The stroma is myxoid with a plexiform capillary network. Therefore, confusion with myxoid liposarcoma (see below) may occur. Cytogenetic investigations have shown that lipoblastomas are characterized by rearrangements of chromosome bands 8q11–13, with breakage of the *PLAG1* gene. ►Myolipomas of soft tissue are extremely rare benign lipomatous lesions occurring most often in adult females. These lesions are frequently located deeply in the abdominal cavity, retroperitoneum, and inguinal areas. Histologic analyses demonstrate a variable admixture of smooth muscle and mature adipose tissue.

Malignant Adipose Tumors

Liposarcomas are the most common type of soft-tissue sarcoma. They usually present as painless mass, the size of which can be larger than 20 cm. Once the diagnosis is suspected by clinical examination and imaging, complete staging and treatment performed by a experienced multidisciplinary team are required. Treatment usually involves a combination of surgery with preoperative or postoperative radiotherapy. Chemotherapy represents the mainstay of treatment for patients with metastatic disease and can be useful in some cases of localized-tumors. ►Well-differentiated liposarcomas (WDLPS) are the most common type of liposarcomas. They are defined as tumors of intermediate malignancy given their high rate of local recurrence and low rate of metastatic evolution. WDLPS occur almost exclusively in adults with a peak in the early seventh decade. They are frequently located in the deep soft tissues of the limbs, most often the thigh, or the retroperitoneum. WDLPS usually consists of mature fat with a variable number of spindled cells with large hyperchromatic and pleomorphic nuclei and monovacuolated or multivacuolated lipoblasts (immature fat cell). Histologically, WDLPS may be confused with lipomas, especially when lipomas are deep-seated or infiltrating the muscle, or show secondary changes in the form of fibrosis or liponecrosis (Fig. 1b). WDLPS may also be hard to distinguish from spindle cell/pleomorphic lipomas. ►Dedifferentiated liposarcomas (DDLPS) are biphasic neoplasms occurring in the same age group as WDLPS, with one component being a WDLPS and the other a non lipogenic sarcoma of variable histological grade. Dedifferentiation can be observed in primary or recurrent lesions. Distant metastases are observed in 20% of cases of DDLPS, worsening the prognosis in comparison to WDLPS. In case of heterologous differentiation DDLPS, are likely to be confused with a wide range of spindle cell or pleomorphic undifferentiated tumors including fibrosarcomas, malignant peripheral nerve sheath tumors (MPNST), leiomyosarcomas, rhabdomyosarcomas, chondrosarcomas and osteosarcomas. WDLPS and DDLPS share similar cytogenetic features:

they are both characterized by the presence of supernumerary ring or giant marker chromosomes (Fig. 1b). These supernumerary chromosomes contain amplified sequences from the 12q13–15 chromosomal region, including the *MDM2* and *CDK4* genes. The detection of *MDM2* and *CDK4* overexpression or ►amplification using methods such as immunohistochemistry (IHC), real-time PCR or FISH is now recognized to be reliable and useful for identifying WDLPS and DDLPS among benign or malignant tumors. The comparative genomic hybridization analyses on DNA micro-arrays (►array CGH) have been very useful in demonstrating that a susbset of tumors with 12q amplification that were formerly classified as malignant fibrous histiocytoma (MFH) were indeed DDLPS with an exclusive or highly prominent dedifferentiated component. Another characteristic feature of WDLPS/DDLPS supernumerary chromosomes containing 12q amplification is their absence of ►alpha-satellite centromeric sequences. Alpha-satellite negative chromosomes are generally acentric and unstable and are eliminated with successive mitoses. In contrast, WDLPS supernumerary chromosomes are stable and contain a functional centromere called a "►neocentromere." WDLPS is the only example of a tumor type in which the formation of a neocentromere is a recurrent and consistent pathognomonic feature. ►Myxoid liposarcomas and round cell liposarcomas (MRLPS) are the second most frequent subtype of liposarcomas. Histologically, MRLPS are composed of uniform non lipogenic mesenchymal cells with variable numbers of small signet-ring lipoblasts in a prominent myxoid stroma with a characteristic arborizing capillary network. These lesions occur in adults most frequently in the 4–5th decades of life, and are preferentially located in the deep soft tissues of the extremities, such as the medial thigh and popliteal regions. The prognosis is highly related to the proportion of round cells. Metastases occur in 25% of patients with a round cell component of less than 5% and 55% of patients with a round cell component of more than 25%. Cytogenetically, MRLPS are characterized by a specific t(12;16)(q13;p11) translocation which fuses exons 5, 7 or 8 of *FUS* with exon 2 of *DDIT3* (Fig. 1c). A t(12;22)(q13;q12) translocation which fuses exon 7 or exon 10 of *EWS* with exon 2 of *DDIT3* is present in about 5% of the cases (►see fusion genes). Of note, nine molecular variants of the *DDIT3-FUS* fusion transcript have been described, with no demonstrable prognostic effect. MRLPS shows significantly higher response rate to chemotherapy compared to other liposarcomas. ►Pleomorphic liposarcomas (PL) are the least common subtypes of liposarcomas, accounting for only 5–15% of all liposarcomas. PL are characterized by a relatively high malignant potential with metastases present in 30% of cases. PL affect adults older than 50 years of age with no gender predilection and are most frequently located on the lower extremities. They are composed of a variable number of pleomorphic lipoblasts in a background of a high grade pleomorphic sarcoma. The karyotype of PL is usually complex, showing multiple numerical and structural chromosomal alterations. No specific molecular anomaly has been identified so far.

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Adjuvant

Definition

A substance that is a powerful stimulant of the immune response while not being antigenic itself.

A substance that is used in concert with a therapy whose efficacy it enhances; ►adjuvant therapy.

►Herceptin

Adjuvant Chemoendocrine Therapy

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Synonyms

Adjuvant chemohormonal therapy; Post surgical systemic therapy; Adjuvant cytotoxic and hormonal therapy

Definition

Adjuvant chemoendocrine therapy is a combination of ►chemotherapy and hormonal treatments that may be

administered to patients after surgery for a nonmetastatic tumor with the goal of decreasing the risk of cancer recurrence.

Characteristics

Adjuvant chemoendocrine therapy is potentially useful for tumors which are responsive both to cytotoxic chemotherapy and to hormonal interventions. The growth of these types of tumors is boosted by hormones and usually it may be limited not only by blocking these hormones but also by chemotherapy. Adjuvant chemoendocrine therapy is a standard treatment for early ►breast cancer, while it is experimental in ►prostate cancer, and it is not generally an option in other tumors with possible endocrine responsiveness such as endometrial cancer or ovarian cancer.

Breast Cancer

Surgery generally remains the first step for treating early-stage breast cancer with the goal to remove any visible tumor. Radiotherapy is often offered to patients after surgery, particularly when ►conservative surgery has been performed, to reduce the risk of local recurrence. However, more than half the women with operable disease who receive only surgery, with or without radiotherapy, eventually will die from metastatic disease. This indicates that ►micrometastasis may be present at the time of initial clinical presentation. ►Adjuvant therapy is intended to eliminate potential breast cancer cells lingering in the body: it is an "insurance policy" that may be used even if there is no direct proof that cancer has spread. Together with mammography screening, adjuvant chemoendocrine therapy contributed to the reduction of breast cancer mortality, which has been registered in the past decade in western countries. Adjuvant systemic therapies for breast cancer currently include endocrine therapy, chemotherapy, new ►targeted therapy such as trastuzumab (►Herceptin), which may be differently combined on the basis of the characteristics of the patients and of the tumor.

Adjuvant Hormone Therapy

Breast cancer is generally considered as an endocrine responsive disease. However, sensitivity to hormonal therapy may be quite variable among breast cancer patients. Currently endocrine responsiveness is tested evaluating the presence of ►estrogen receptors (►ER) and progesterone receptors (►PR) with immunohistochemistry on breast tumor sections. Approximately 70% of breast cancers at diagnosis are ER and/or PR positive, with increasing ER positivity according to age. Diseases with strong ER and PR expression may be considered as endocrine responsive, while hormonal treatment will not be useful in tumors with no detectable ER and PR. Endocrine responsiveness may be uncertain for tumors with intermediate or low hormone receptor expression, lack of PR irrespective of ER expression, ►HER-2/neu overexpression, and high number of axillary metastatic lymph nodes.

Hormonal (antiestrogen) therapies either reduce the amount of oestrogen (►Estradiol) in the body or block oestrogen's effects. Different hormonal therapies are currently available and are usually given by pill or, less commonly, by injection: Tamoxifen and other selective estrogen receptor modulators, ►fulvestrant and the ►aromatase inhibitors (anastrozole, letrozole, and exemestane). Besides ►ovarian function suppression may be used for premenopausal patients.

Tamoxifen. Tamoxifen has been the mainstay of hormonal therapy in both early and advanced breast cancer patients for approximately three decades. After binding to the estrogen receptor, it competitively blocks estrogens from binding to tumor cells.

Clinical studies and ►metaanalyses have shown that tamoxifen is effective in all patients with expression of hormone receptors, independently by age or menopausal status and chemotherapy use. Five years of tamoxifen lead to a proportional reduction of the annual recurrence rate of about 40% and of the breast cancer mortality of 31%. Furthermore, local recurrence is decreased and the risk of contralateral breast cancer is reduced by 40–50%. Five years of tamoxifen are more effective than 2 years, while there is no evidence that longer duration would improve results.

The side effects of tamoxifen include a small increased risk of uterine cancer, an increased risk of blood clots, hot flashes, loss of libido, vaginal dryness, vaginal discharge or bleeding, and rarely ocular alterations.

Aromatase Inhibitors (►*AIs*). In contrast with tamoxifen, they act by inhibiting oestrogen synthesis and are effective only in postmenopausal women.

Clinical studies in early breast cancer have shown that AIs are more effective than tamoxifen and improve relapse-free survival when started upfront or after 2–3 years of treatment with tamoxifen rather than continuing tamoxifen. In addition, when used as extended therapy after 5 years of tamoxifen, AIs reduce the risk of recurrence and improve the survival in patients with positive axillary lymph nodes. AIs could be more effective in PR negative tumors and with HER-2/neu overexpression or ►amplification. AIs, in combination with ovarian ablation, are experimental in premenopausal patients.

AIs cause fewer hot flashes, less vaginal discharge, less vaginal bleeding, less endometrial cancer, and venous thromboembolism than tamoxifen; however, they may cause joint and muscle pain, osteoporosis, and an increased risk of bone fracture.

Ovarian Function Suppression. It is the oldest treatment for breast cancer, known from 1889, useful only for premenopausal women. In the past years, it could be induced with surgical oophorectomy or radiotherapy. Surgical oophorectomy causes an immediate and permanent drop in ovarian estrogens production; radiation induced ovarian ablation may be incomplete or delayed in some women, and biochemical verification of ovarian function cessation may be advisable. Injections of Luteinizing hormone-releasing hormone (►LH-RH) agonists are an effective therapeutic alternative with the advantage of reversibility of induced menopause. Chemotherapy may also induce temporary or definitive menopause and this side effect could explain in part the beneficial activity of chemotherapy in women with endocrine responsive disease. Age and type of chemotherapy may influence the likelihood of chemotherapy induced menopause.

In patients with endocrine responsive tumors, ovarian function suppression may be as effective as ►CMF combination chemotherapy; combined hormone therapy with ovarian ablation and tamoxifen may be superior to CMF in the same subset of patients.

Symptoms of menopausal estrogen deprivations are the most common side effects of ovarian function suppression.

Adjuvant Chemotherapy

Chemotherapy is the use of cytotoxic drugs to kill cancer cells. Chemotherapy may be given orally (by mouth) or intravenously (injected into a vein) and is usually given in cycles in an outpatient setting.

The results of different randomized ►clinical trials and metaanalyses have shown that combination adjuvant chemotherapy is more effective than single agent chemotherapy. Chemotherapy including anthracyclines is more effective than others such as CMF. Moreover, adding taxanes (►Paclitaxel or docetaxel) to ►adriamycin or other anthracyclines or the use of ►dose-dense chemotherapy may improve results. Efficacy of adjuvant chemotherapy is greater in younger than older women, but this observation needs to be interpreted on the basis of hormone receptor status of disease.

Adjuvant chemotherapy should start within 12 weeks from surgery; however, different subsets of patients, such as young women with negative hormone receptors could benefit from starting chemotherapy earlier.

Side effects of adjuvant chemotherapy can include fatigue, nausea and vomiting, lowered white blood cell count and a corresponding increased risk of infection, mouth sores, hair loss, and premature menopause. Most of these side effects go away once treatment is stopped and are not long-term. However, long-term effects may occur including heart damage, nerve damage, or secondary cancers.

Planning the Adjuvant Treatment: Adjuvant Chemoendocrine Therapy

If both adjuvant hormone therapy and chemotherapy are active for early breast cancer and have different toxicity profiles, it would seem reasonable to combine them in all patients to improve results. However, the problem is more complex than what it seems. Doctors should take into account many factors in order to tailor the therapy for each patient. Patient's age and general health, menopausal status, risk of breast cancer recurrence, likelihood of tumor response to different therapies and patient's preference are all important items. The main risk factors for the development of metastatic disease after surgery are the involvement of axillary lymph nodes, a poor histological grade, large tumor size, histological evidence of ►lymphovascular invasion, and age. In addition, tumor proliferation rate, the absence of oestrogen and progesterone receptor, and HER-2/neu overexpression or amplification also carries an adverse prognosis. The next important step for better choice of adjuvant therapy should be the evaluation of responsiveness of tumors to different adjuvant therapy. Endocrine responsiveness (see above) is the first target to be determined because it suggests if disease is likely to respond to hormone therapy. However, recent accumulating evidence seems to indicate that adjuvant chemotherapy, in general, and chemotherapy including anthracyclines and/or taxol and docetaxel could be more effective in patients with endocrine unresponsive disease or HER-2/neu overexpression or amplification; on the contrary, the benefit of chemotherapy seems smaller in patients with strong ER and PR expression. Finally, overexpression or amplification of HER-2/neu defines a subset of breast cancer patients who strongly benefit from immunotherapy with the monoclonal antibody trastuzumab. The evaluation of gene-profile expression of tumors could help to improve risk prediction and treatment outcomes, but its successful implementation in clinical practice will depend on the integration with existing clinicopathologic markers and validation on large clinical trials. These evaluations will guide the doctors to choose whether adjuvant treatment is needed and whether it should include only hormone therapy (and what kind of hormone therapy), only chemotherapy, or their combination. Hormone therapy should be offered to all patients with endocrine responsive disease for whom adjuvant treatment is indicated.

For decades, chemotherapy was considered the adjuvant treatment of choice for premenopausal patient, regardless of hormone receptor status. However, metaanalyses showed that, in receptor positive women younger than 50 years, the use of tamoxifen reduces the risk of recurrence and death regardless of the use of chemotherapy. In the same subset of patients, ovarian ablation with or without tamoxifen provides results

similar to CMF and represents an alternative to chemotherapy when the risk of recurrence is low or medium. Women with higher recurrence risk or uncertain endocrine responsiveness should receive a combination of endocrine therapy and chemotherapy. For patients younger than 35 years with endocrine responsive disease receiving chemotherapy, additional endocrine therapies should be considered.

In postmenopausal patients with clear endocrine responsiveness, adjuvant chemotherapy probably adds little benefit to adjuvant hormone therapy. Endocrine treatment should include an AI, either upfront or sequentially after tamoxifen. The concurrent use of AIs and tamoxifen is not useful and should be avoided. Two or three years of tamoxifen followed by 3 or 2 years of an AI, 5 years of an AI upfront, or 5 years of tamoxifen followed by an AI are all possible options. According to St. Gallen Consensus, chemotherapy should be added in patient with high-intermediate risk of recurrence, particularly when endocrine responsiveness is uncertain. We have only limited information from clinical studies about adjuvant treatment for older women. Data from one study suggest that older patients with receptor positive disease benefit even from a short tamoxifen therapy (1 year); in patients with endocrine unresponsive tumors clinical decision about offering them chemotherapy should strongly consider biological age and concurrent diseases; enrollment in clinical trials, if available, should be encouraged. Finally, when both tamoxifen and chemotherapy are to be used, current evidence suggests a sequential treatment: it is preferable to give chemotherapy first and to start tamoxifen after chemotherapy completion. Indeed concurrent tamoxifen seems to decrease effectiveness of chemotherapy and to increase the risk of thrombotic events. At present time it is not known if this modality should be extended to other hormonal treatments such as ovarian ablation and aromatase inhibitors.

Prostate Cancer

Prostate cancer is the most common cancer in European and American men. The growth of prostate cancer cells in most cases is due to ►androgens and these types of tumors may respond to hormonal therapy. The aim of hormonal therapy in prostate cancer is to lower androgen levels or to prevent their action on prostate cancer cells. Hormone interventions for prostate cancer include ►orchiectomy, LH-RH analogs, and antiandrogens and are currently used in advanced prostate cancer. Eventually metastatic prostate cancer develops endocrine refractoriness. Recently, taxotere has been approved for hormone refractory metastatic prostate cancer.

Treatment choices for early prostate cancer are based on clinical and histopathologic factors: clinical stage, tumor grade, and level of ►PSA. For patients undergoing radical surgery, if the resection margins are positive or if there is seminal vesicle or capsular invasion, adjuvant strategy options are radiotherapy or close surveillance until a detectable PSA develops. Adjuvant hormonal treatment is not the standard at this time. Some trials have explored if hormonal therapy after primary treatments (surgery or radiotherapy) is useful, but results are inconclusive and it seems that only patients with positive lymph nodes could have some benefit from these drugs. There are more randomized trials (and one ►metaanalysis) comparing radiotherapy alone versus radiotherapy and hormonal treatment (LH-RH analogs or orchiectomy): the survival seems to be better adding prolonged hormonal therapy to radiotherapy. Adjuvant chemoendocrine therapy is not standard in prostate cancer and is under evaluation in randomized clinical trials.

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Adjuvant Chemohormonal Therapy

►Adjuvant Chemoendocrine Therapy

Adjuvant Cytotoxic and Hormonal Therapy

►Adjuvant Chemoendocrine Therapy

Adjuvant Effect

Definition

Describes the helper-effect of an (in most cases) immunostimulatory substance or compound. Well-known is the use of Freund adjuvant to enhance the effect of antigens in immunization strategies. If containing immunostimulatory mycobacterial subcomponents Freund adjuvant is referred to as complete Freund adjuvant. Enhances cell-mediated immunity and antibody production.

►Bacillus Calmette-Guérin

Adjuvant Therapy

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Definition

Adjuvant therapy is an auxiliary therapy (e.g. ovarian ablation) administered concomitant with another therapy (e.g. surgery or radiation) in the treatment of primary ►breast cancer.

Characteristics

As defined in Webster's Deluxe Unabridged Dictionary adjuvant is:

- "An assistant"
- "In medicine, a substance added to a drug to aid in the operation of the principal ingredient" (e.g. Freund's adjuvant in immunotherapeutic research)

In clinical cancer research and treatment any therapy that in some way helps another modality is considered an "adjuvant." Most of the time cancer therapy is a multidisciplinary endeavor involving specialists from many treatment modalities. These specialists include (but are not limited to) medical oncologists, surgical oncologists and radiation oncologists. As more specific and targeted drug treatments become available, specialists in biologic and immunotherapeutic approaches will need to join the multidisciplinary team.

Cancer treatment for many tumor types involves some combination of surgery, cytotoxic chemotherapy and radiation therapy. In some hormonally-sensitive tumor types such as breast and prostatic cancer, hormonal manipulations may also be utilized. At present "adjuvant" therapies usually imply systemic therapies such as chemotherapy or hormonal therapies, with surgery and/or radiation therapy as the primary treatments. It is possible that as systemic therapies become more effective they may be the primary treatments, with surgery and radiation therapy then being used as "adjuvants."

It has been well established in animal tumor systems, that forms of chemotherapy unable to cure established metastatic cancer could lead to a cure in animals initially rendered disease-free by surgery. These seminal studies have been translated into the clinical setting. The concept of adjuvant therapy is based on the premise that even relatively early cancers may have already disseminated to distant sites by the time of diagnosis. With current diagnostic technologies it is seldom possible to detect systemic metastasis that are less than 1 cm in size. Since this would contain approximately one billion tumor cells, it is likely that many tumors may have already disseminated microscopically via the bloodstream leaving tumor foci of millions of tumor cells undetectable by current diagnostic imaging techniques. This premise underlies current theories attempting to explain why only 70% of women with early stage breast cancer (tumors between 1 and 2 cm with negative axillary lymph nodes) are cured by standard surgical techniques. Much research is being done in an effort to identify those 30% of women destined to relapse and ultimately die of metastatic breast cancer, so that aggressive chemotherapeutic "adjuvant" therapies can be directed toward that high risk subset, and thus sparing the cured 70% the toxic side effects of chemotherapy. Various attempts at prognostication including (but not limited to) the analysis of bone marrow cells for micrometastasis using immunocytochemistry or, more recently, polymerase chain reaction technologies still require validation in large-scale clinical trials.

As examples, reasonably well accepted adjuvant therapies include; the use of chemotherapy, hormonal therapy or both after primary surgery ± radiation therapy for stages I and II breast cancer; radiation therapy to the breast for women undergoing lumpectomy instead of mastectomy for early stage breast cancer; chemotherapy for primary colon cancer that has spread to regional lymph nodes; and radiation therapy after surgery for women with locally advanced cancers of the uterine cervix. In some cancers, such as small cell carcinoma of the lung, chemotherapy is generally considered to be the primary form of therapy thus making surgery and/or radiation therapy, when used, the "adjuvants." Similarly in certain forms of leukemia where chemotherapy is considered the standard form of treatment, the use of whole brain radiation to prevent central nervous system relapse has been used as another example of "adjuvant" therapy.

In summary, as of the year 2000 the term adjuvant therapy in clinical cancer therapy is usually applied to the use of systemic cytotoxic chemotherapy and or hormonal

therapy (for hormonally sensitive tumors). However, in reality the term adjuvant can be applied to any treatment modality applied (generally with curative intent) after whatever initial treatment modality is considered to be the standard primary therapeutic intervention.

►Menopausal Symptoms After Breast Cancer Therapy
►Mucoepidermoid Cancer
►Induction Chemotherapy
►Herceptin
►Taxotere

ADMET

Definition

Is an abbreviation for Absorption, Distribution, Metabolism, Excretion and Toxicity.

►ADMET Screen

ADMET Screen

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Definition

An ►ADMET Screen is the application of a group of experimental assays to measure characteristics of a drug candidate in order to predict the Absorption, ►Distribution, Metabolism, Excretion and ►Toxicity properties of that drug.

Characteristics

ADMET is an abbreviation for Absorption, Distribution, Metabolism, Excretion and Toxicity. The term ADMET is often loosely used to denote those properties required for an orally active drug that are apart from the inherent biological activity of the drug. In a stricter sense the term ADMET denotes the properties denoted by the abbreviation that are the focus of specialized knowledge in the fields of medicinal chemistry, drug metabolism, pharmaceutical sciences and toxicology. The five properties in ADMET can be determined experimentally and to some degree computationally. As of 2007 all these properties can to variable degree be experimentally measured in manual or automated, medium to high capacity experimental assays (screens). The term "ADMET Screen" often refers to a group of screens that measure these properties. The term "screen" is quite appropriate because these ADMET assays are often used in a screening sense to remove chemical compounds with severe ADMET flaws that will never become real orally active drugs. Compounds are screened to remove flawed compounds just like a metal screen (sieve) might be used to remove sand or gravel from a water supply.

Drugs (medicines) are chemicals. They broadly consist of two types. There are those drugs that are of small size, i.e. low molecular weight (MWT). Typically these are below 500 Da (►Daltons) (atomic mass units). Currently the average MWT of an FDA approved drug is 347. About 90% of these low MWT drugs are orally active, i.e. are given to the patient by mouth and swallowed. The term ADMET overwhelmingly refers to these compounds. Another type of drug consists of ►biologicals. Biologicals are large drug compounds e.g. vaccines, antibodies, proteins. These are not given by mouth. The properties of these latter compounds are so different from those of the small orally active compounds that there is little or no overlap in the ADMET properties and screens for the two groups. Broadly, the properties of biologicals tend to be very specific to the individual compound and broadly useful ADMET screens for biologicals mostly do not exist.

What is required for a useful small MWT orally active drug? Broadly, two types of effects are required. The chemical (drug) must have the desired biological effect and the chemical must possess a variety of other properties (the ADMET properties) that are known by historical experience to be required for an orally active drug. There is a misconception prevalent in academia that a medicine requires only the desired biological effect plus an avoidance of toxicity. This is completely wrong. In developing small orally active drugs, solving the ADMET problems is frequently more difficult than obtaining the desired biological activity.

►Intestinal absorption is the entry of the drug from the contents of the gastrointestinal (GI) tract into the tissue lining the GI tract. Little or no absorption occurs in the stomach. Generally absorption starts as soon as soluble drug passes out of the stomach into the duodenum which is the part of the GI tract joining the stomach to the first section of the small intestine. With few exceptions, absorption is finished by the time the drug passes into the large intestine. A generalization says that to be absorbed a drug must be soluble (dissolved) in the contents of the GI tract. Near neutral water at about pH 6.5 is a very simple mimic of the GI tract contents at the point in the duodenum where absorption starts. In actuality, the real duodenal tract contents are more complicated than water and contain biological detergents, i.e. bile acids that help dissolve

►lipophilic (literally fat loving -greasy) drugs. Because of this complexity a small number of lipophilic drugs are in reality more soluble and better absorbed than would be suggested by their solubility in aqueous medium. Nevertheless, drug solubility in water is an excellent predictor for drug absorption. Within limits better aqueous solubility correlates with better absorption. Measuring drug aqueous solubility in water buffered at pH 6.5 or 7 is universally used to assess the likelihood of absorption and highly efficient automated equipment to make this measurement is readily available. Problems of a chemical (potential drug) having low water solubility are today the single most important early discovery problem in the discovery and development of low MWT orally active drugs. Across the board about 30–40% of chemicals in a drug discovery program might be expected to have an aqueous solubility problem.

The vast majority of orally active drugs must move from being dissolved in the aqueous content of the GI tract and must move across the GI tissue wall and then dissolve into the blood on the other side of the GI tract to get to their eventual site of action. The process of passing across the GI wall is termed "►intestinal permeability." Simplifying greatly, the GI wall can be considered as a greasy barrier. Think of a porous material soaked with fat. Chemicals that are very water loving (►hydrophilic) dissolve easily in water but do not dissolve easily in fat. A specific example would be sucrose (table sugar). So to be absorbed a chemical must have an appropriate balance between being water soluble (hydrophilic) and fat soluble (lipophilic). If the chemical is too hydrophilic it will dissolve easily in the GI contents and in blood but will not cross the GI wall (often called the GI barrier). If the chemical is too lipophilic it could cross the GI barrier easily but because of poor solubility in the GI contents the amount that crosses into the blood is very low. Highly efficient automated experimental equipment exists and is widely used to identify compounds likely to have difficulty crossing the GI barrier. A very widely used assay is termed ►PAMPA (Parallel Artificial Membrane Permeability Assay). In PAMPA a measurement is made of the speed with which a chemical crosses a porous material like fritted glass or plastic soaked with a fat. Across the board about 10% of chemicals in a drug discovery program might be expected to have a permeability problem.

When a chemical is absorbed it moves along a concentration gradient from high concentration in the GI tract to low in the blood. The GI barrier contains biological pumps that may literally pump the chemical out of the GI wall back into the GI tract. The best studied of these pumps in humans is called ►p-glycoprotein (PGP) and is the same biological pump that makes cancer cells resistant to chronic chemotherapy. If this pumping occurs to a significant extent, absorption is hindered. To measure this process requires an assay mimicking the live cells of the GI wall. A very common assay to do this is called ►Caco-2. The assay involves growing cells originally obtained from a human colonic cancer cell line on a porous plate. The cells form a continuous sheet on the porous plate substrate. The upper surface resembles the inner (GI tract exposed) surface of the GI wall. This is termed the ►apical surface. The surface growing on the porous substrate is termed the ►basolateral surface. It is possible to measure the speed of the chemical crossing the live cell sheet which gives a rough measure of how fast the chemical will cross the GI wall. It is possible also to measure the rate of chemical crossing the cell layer from either direction; the normal absorption direction termed apical to basolateral (abbreviated A to B) and the reverse pumped out direction termed basolateral to apical (abbreviated B to A). A to B rate divided by B to A rate gives a measure of the probability that the pump will hinder passage of chemical across the GI wall. Ratios of A to B divided by B-A are termed ►efflux ratios. A value greater than 1 suggests the beginnings of a problem. Ratios greater than 10 suggest further studies to see how much of a problem actually exits in a whole animal experiment. High efflux rates can lead to poor absorption and to dose non linearity, i.e. as the chemical dose increases the amount of drug in blood initially low suddenly jumps as the capacity of the efflux pump is exceeded. This is a highly undesirable effect if it occurs in the clinical dose range.

Some chemicals enter into the GI wall but are chemically destroyed either at the inner GI tract surface or within the GI wall. The most common problem is chemical oxidation of the drug by an iron containing enzyme called in humans ►cytochrome P450 CYP-P450 3A4. This enzyme occurs both in the GI wall and especially in the liver. About 60% of drugs might to a variable extent be subject to this process. Accordingly, measuring this oxidation is important both to detect compounds that are rapidly oxidized and to minimize potential interactions with the many other drugs that are oxidized by this same enzyme. Assays for so called drug-drug interactions are very common in ADMET assays. High capacity assays are commonplace to detect interactions with CYP-P450 3A4 as well as four other important CYP's such as ►CYP P-450 2D6 (important in metabolism of CNS drugs), CYP P-450 2C9, CYP P-450 2C19 and CYP P-450 2A1.

Distribution is the term describing how a chemical distributes among various compartments in the body. Fifteen years ago it was thought that distribution was governed by a chemical's physicochemical properties, e.g. size, polarity, lipophilicity acidity or basicity. We now believe that most or all distribution processes are governed by ►biological transporters which move a chemical from one place in the body to another and that

physicochemical properties are simply a crude surrogate measure for whether a chemical interacts with a particular transporter. There are myriads of ►transporters in the body many/maybe most of which are incompletely or entirely uncharacterized. As a result distribution is the property in the ADMET suite that is currently most poorly understood and for which assays are poorest. In general, with few exceptions, it is not possible to predict a priori the tissue distribution or localization of a new chemical.

Metabolism is the term that describes the biological caused change in a compounds chemical structure that occurs to a chemical when it gets into the body. The general pattern is that chemistry occurs to make a chemical more polar and more hydrophilic and more water soluble so that it can be more easily excreted by the body through the kidneys or the liver. Across the board, about 80% of drugs undergo ►oxidative metabolism. Frequently this consists of replacing a carbon hydrogen bond in the chemical by a carbon oxygen bond or the breaking of a carbon nitrogen bond (N-dealkylation). The family of CYP's are the most common cause of this chemistry although there are a handful or more of other less common enzymes that do similar transformations. Assays for these types of oxidations are a part of every ADMET assay suite. Compounds can be incubated with components of a liver which contains many CYP's. Artificially formed cell structures called ►microsomes can be formed from chopped up liver and these ►microsomes can be simplistically thought of as miniature livers. A chemical/drug is warmed up with the microsomes and measurements are made to see if the structure of the starting chemical changes. Very often the results are reported in terms of half life, i.e. does the chemical stay unchanged for less than or more than a certain length of time under some standard set of assay conditions. The interaction of a chemical with CYP's can also be measured by incubating the chemical with cells containing a single CYP enzyme.

Metabolism for about 15% of drugs occurs by a process termed "►phase II metabolism." A very polar small biologically occurring molecule like a special type of acid containing sugar group, e.g. ►glucuronic acid or a ►sulfate group is chemically attached to the starting chemical. This process can occur directly on the starting chemical if it contains something to attach the polar piece or it can occur after the starting chemical is changed by a CYP oxidation. A soluble liver fraction termed ►S-9 is used in an incubation to measure this process.

About 5% of drugs are not metabolized. Ultimately this can be advantageous clinically but in the drug discovery process it is currently a disadvantage since learning how long a chemical survives unchanged in an animal requires animal testing and animal in-vivo testing is always more difficult than testing in a test tube (also termed in-vitro meaning literally in glass).

Metabolism is sometimes termed detoxification, however this is really a misnomer. It is true that metabolism can reduce or abolish biological activity (including toxicity) in a starting chemical but it can just as well lead to an equally active (or even more active) biologically active compound. The biological activity can be the desired effect or something unwanted (toxicity). It is possible to start with a biologically inactive compound and have it biologically converted to a biologically active compound. Such a compound is called a ►prodrug. This process is surprisingly common amounting to about 25% of older drugs. Today this happens much less often because metabolism is scientifically much better understood than even a decade ago and because a directly biological active drug is generally considered advantageous over a chemical that requires metabolism for biological activity.

Excretion is the process by which a chemical is removed from the body. Overwhelmingly this is by excretion through the kidney into the urine (►renal excretion) or by excretion by the liver by way of the gall bladder emptying into the intestine and ultimately into the feces (►hepatic excretion). There has been great progress in understanding both modes of excretion and assays exist for some of the major biological transporters that are important in both processes. The non expert reader will have difficulty understanding the science because the terminology (►transporter nomenclature) used to describe the various transporters is frankly a mess and is changing currently and the same transporter may be described in the literature by multiple names and some of the names themselves unfortunately can be misleading. Very minor methods of excretion exist such as excretion through the lung and sweat. A rough rule of thumb is that MWT governs whether a compound or more often its metabolite or conjugate is excreted through the kidney or liver. A MWT less than 300 suggests excretion through the kidney is more likely. As MWT increases above 400 excretion through the liver becomes more likely. Many drugs show excretion through both liver and kidney. This profile is considered an advantage because damaged excretion through either kidney or liver is common in sick people.

Toxicity is a term denoting unwanted biological activity. Currently, the target and thus the desired mechanism of action (on target activity) is known for over 95% of newer drugs. Knowing the target mechanism is extremely useful when something untoward (toxicity) happens during ►preclinical studies (before ►clinical studies start) or during clinical studies (while the compound is studied in humans). Very commonly the toxicity is off target, i.e. not related to the desired biological activity. This is a good finding because it means that

changing to a different chemical structure but with the same desired mechanism will solve the toxicity problem.

Based on history, about 50% of drugs will cause tumors in one or more tissues in male or female rats in lifetime carcinogenicity studies. These studies may or may not be relevant to cancer risk in human patients. A major relevant factor is whether the drug is inherently ►mutagenic (causing DNA chromosomal damage). It is very common to exclude chemicals from drug development if they contain any chemistry pieces (moieties) previously associated with mutagenic activity and to rigorously test for in-vitro mutagenic activity. Mutagenic assays like the many versions of the ►Ames assay are used. A compound is incubated with genetically crippled but still viable bacteria designed to be very sensitive to chromosomal damage. The dose used is one that is not toxic to normal bacteria. A positive finding of mutagenicity is killing of crippled bacteria at doses where normal bacteria are unaffected. In general it is common that tumor findings in a compound clean with respect to no suspect chemical features and no mutagenicity can be explained as being irrelevant to human risk. For example, long term tissue irritation or stimulation of a growth factor or formation of a metabolite in rodents but not in man or damage to a cell type not found in man can cause tumors in rodents but these causes are likely irrelevant to human risk.

Liver toxicity is extremely common in drug discovery hence a whole variety of assays for liver damage are commonly used. The goal in general is to improve the prediction of liver damage at an earlier time point in the discovery process with less use of resources and with smaller amounts of compound.

►Attrition (loss) of compounds in the entire drug discovery and development process is well understood in the pharmaceutical industry. Generalizing across all types of therapeutic areas: about one in four projects succeeds from first inspiration to choice of a compound to enter the clinic (pre-clinical phase); about in 10–12 projects succeeds in the clinical (human testing phase). This gives an overall success rate of 1 in 40 or 48. Genomically derived projects have particularly poor success rates of 1 in 50 or 100. Cancer and CNS are the two therapeutic areas with poorer clinical success rates of about 1 in 20.

In the current era there are three major causes of roughly similar importance in clinical attrition. These are (i) toxicity; (ii) lack of efficacy and (iii) commercial reasons. These three factors are interrelated and it is very common for there to be multiple causes of failure. For example, Pfizer the largest pharmaceutical company worldwide identified three causes of failure in that organizations attrition analysis of many hundreds of failed clinical candidates over a time period of more than a decade. In this authors opinion toxicity is the most complex attrition cause and therefore the most resistant to a fix. We can expect toxicity to persist as a major attrition cause for multiple decades into the future.

ADMET assays have been summarized so far from an experimental perspective. However, if one tests enough compounds it is, in theory, possible to develop a computer program to predict an assay outcome based just on the structure of the compound. Computational prediction of ADMET is an extremely active area of research and many computational prediction programs of variable quality already exist. For example, the Lipinski "►rule of five" is a very widely used algorithm (with 2,000 literature citations to date) that is used to predict the likelihood of poor oral absorption. The rule states that poor oral absorption is more likely if: the MWT is over 500; the lipophilicity as measured by logP is over 5; there are more than five hydrogen bond donors and there are more than ten hydrogen bond acceptors. There are actually only four rules. The five in the name comes from the number 5 appearing in the parameter cutoff values. The reader is referred to the many review articles on rules and filters and computational prediction of ADMET.

Common sense is needed with regard to predictions. There is a natural importance order in drug discovery and development. Most important is the clinical information. This trumps all. Next in importance is high quality experimental information. Prediction has its best value and impact when neither clinical nor experimental information is available.

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Admixture Population

Definition

Family-based studies of genetic association and linkage play a key role in mapping susceptibility genes of complex human diseases. Recent admixture between

genetically differentiated populations can result in high levels of linkage disequilibrium even at far apart loci. Studies involving admixture population utilize mtDNA.

►Mitochondrial DNA

Adopted Orphan Nuclear Receptors

►Orphan Nuclear Receptors

Adoptive Immunotherapy

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Definition

Adoptive immunotherapy involves the passive transfer of cellular products designed to augment immunity against cancer or infections. To date, clinical trials have evaluated the efficacy of different cell populations including, but not limited to: unmanipulated leukocyte infusions from bone marrow donors, cytokine induced T cells, lymphokine activated killer cells, tumor infiltrating lymphocytes, ►NK cells and antigen specific T cells.

Characteristics

Unmanipulated T Cells

Adoptive immunotherapy with unmanipulated donor lymphocytes (DLI) has effectively treated patients with relapsed or residual disease after hematopoietic stem cell transplant (HSCT). DLI provides the host immune system an additional graft vs. leukemia (GVL) effect and has been most successful in patients with chronic myeloid leukemia who relapse post transplant (70–80% cytogenetic remission rate) or with ►Epstein Barr virus-associated lymphoproliferative disease (EBV-LPD) (up to 90%). Moderate success has been seen when DLI was used after relapse in other malignancies such as: ►acute myeloid leukemia (15–40%), low-grade lymphomas (~60%), and metastatic ►multiple myeloma (40–60%). However, less than 5% of patients with relapsed acute lymphoblastic leukemia (ALL) respond to DLI alone. While the etiology is unclear, this could be due to lack of antigenic expression, down-regulation of T cell recognition molecules, and/or overall tumor burden at the time of treatment.

Although treatment with DLI has led to remission in patients with disease after HSCT, unmanipulated cells also contain alloreactive T cells and can induce graft versus host disease (GVHD). The incidence of GVHD ranges from 55 to 90% and is associated with a 20% treatment-related mortality rate. Different methods to maintain the graft versus tumor (GVT) effect while decreasing the incidence of GVHD have been investigated. One strategy is to transduce donor T cells with a "suicide gene" like herpes simplex virus type 1 thymidine kinase that can be activated by ganciclovir if GVHD develops. An additional method to spare the GVT effect involves selectively depleting alloreactive cells in the T cell product prior to adoptive transfer.

Cytokine Induced Killer Cells

Cytokine induced killer cells (CIK) have been generated from both peripheral blood and cord blood mononuclear cells stimulated with anti-CD3, interferon-γ, and interlekin-2 (IL-2). CIK derived from peripheral blood cells have induced either partial responses or stable disease in patients with lymphoma after autologous HSCT. Pre-clinical studies using cord blood CIK have been promising by demonstrating cytotoxic responses when stimulated with either lymphoma or myeloid leukemia cell lines.

Lymphocyte Activated Killer Cells

Lymphocyte activated killer cells (LAK) were one of the first adoptive immunotherapy approaches used to treat patients with advanced-stage malignancies. These cells were generated by culturing peripheral blood mononuclear cells with IL-2; however, they provided no additional clinical benefit compared to administration of IL-2 alone in patients with renal cell carcinoma or melanoma.

Tumor Infiltrating Lymphocytes

Tumor infiltrating lymphocytes (TIL) are cells harvested from tumor sites and expanded *ex vivo* with IL-2. As the cells are being expanded, increased tumor specificity has been achieved by pulsing the cells with tumor-specific peptides or exposing the lymphocytes to a retrovirus encoding a tumor-specific T cell receptor (TCR). Although a major limitation is that patients must have pre- existing lymphocytes that can both respond to tumor and be expanded *ex-vivo*, one study has reported that transfer of these cells led to tumor regression in 50% of lymphodepleted patients with metastatic melanoma.

Antigen-Specific Cytotoxic T Lymphocytes

Ex vivo generation of cytotoxic T lymphocytes (CTL) provide another avenue for increasing the antitumor or antiviral specificity of adoptively transferred cells. Two major prerequisites for generating antigen-specific CTL are the identification of appropriate viral or tumor target antigens and the availability of suitable antigen-presenting cells (APC). Once identified, CTL lines can be generated by co-culturing T cells with APC that express the target antigen. These lines are then expanded by restimulation with the antigen of choice and the addition of cytokines such as IL-2.

Initial studies with antigen-specific CTL were undertaken in patients diagnosed with viral infections after HSCT. In Phase I studies, the prophylactic use of cytomegalovirus (CMV)-specific CTL after HCST decreased the percentage of CMV reactivation in a small cohort of patients. Our group has successfully used EBV-specific CTL not only for the prophylactic treatment of EBV-LPD, but also for the treatment of patients with EBV-LPD after HSCT and solid organ transplant. Furthermore, EBV-CTL have been used with moderate success to treat patients with type II latency EBV-associated malignancies like ►Hodgkin's lymphoma, ►non-Hodgkin's lymphoma and ►nasopharyngeal carcinoma. Patients with HIV have also been treated with CTL therapy. Autologous gag-specific CTL were reported to accumulate in lymph nodes and transiently reduce the levels of circulating infected $CD4^+$ T cells. More recently we have developed an approach where multivirus specific CTL can be generated *ex-vivo* and provide protective immunity against adenovirus, CMV and EBV in recipients.

These strategies are limited by the time required for CTL generation using current good manufacturing processing techniques. Rapid selection techniques such as tetramer selection and gamma-interferon capture are being evaluated, but the CTL product generated may have a more restricted specificity. An alternative approach is to use banked allogeneic CTL lines, but persistence of a mismatched product may be clinically suboptimal.

NK Cells

►Natural killer (NK) cells are effectors from the innate immune system, which also mediate antiviral and antitumor immunity. Recent studies have shown that haploidentical NK cells infused after lymphodepleting chemotherapy can have antitumor effects.

Enhancing the Function of Adoptively Transferred Cells

To increase treatment efficacy of adoptively transferred cells, investigators are lymphodepleting patients prior to adoptive cell transfer or genetic modifying cells to enhance effector function. Lymphodepletion, removal of the host's lymphocytes, prior to adoptive transfer should allow the infused cells to expand using the body's own homeostatic cytokines like IL-7 and IL-15. Data from the Rosenberg group has shown improved outcomes in patients with metastatic melanoma who were lymphodepleted prior to receiving melanoma specific T cells. Additionally, infused T or NK cells may be genetically modified with artificial receptors targeting tumor antigens or with molecules that may confer resistance to tumor evasion strategies.

►Natural Killer Cell Activation

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Adrenal Cortex

Definition

The most superficial layer of this gland, synthesizes corticosteroid hormones that have multiple functions, including responding to stress and regulating blood pressure, glycemia (cortisol), sexual maturation (androgens), and water and electrolytes metabolism (aldosterone).

►Childhood Adrenocortical Carcinoma

Adrencorticotropic Hormone

Definition

ACTH.

►Corticotrophin

Adrenocortical Cancer

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Synonyms

Malignant adrenocortical tumor; Carcinoma of the adrenal cortex

Definition

Adrenocortical cancer (ACC) is a malignant tumor from the adrenal cortex. It is a rare tumor with a poor prognosis. The consequences of ACC are due to tumor growth and metastasis and also due to steroid oversecretion.

Characteristics

Epidemiology of Adrenocortical Cancer

ACC is a rare disease with an estimated incidence between 1 and 2 per million and per year in adults in North America and Europe.

Pathophysiology of Adrenocortical Cancer

Analysis of the pattern of X-chromosome inactivation in heterozygous female tissue has shown that ACC consists of monoclonal populations of cells. A large number of molecular techniques, such as comparative genomic hybridization (CGH) and microsatellite analysis, have identified alterations affecting various chromosomes and loci in ACC. Most of the changes observed concern losses on chromosomes 2, 11q and 17p, and gains on chromosomes 4 and 5. Studies using microsatellite markers have demonstrated a high percentage of loss of heterozygosity (LOH) or allelic imbalance at 11q13 (≥90%), 17p13 (≥85%), and 2p16 (92%) in ACC.

IGF-II (Insulin-Like Growth Factor II)

The insulin-like growth factors system is involved in the development of the adrenal cortex and its role has been largely documented in adrenocortical tumors. The ►*IGF-II* gene located at 11p15 encodes an important fetal growth factor, is maternally imprinted, and is therefore expressed only from the paternal allele. Genetic or epigenetic changes in the imprinted 11p15 region, resulting in increases in *IGF-II* expression, and mutations of the ►*p57kip2* gene have been implicated in Beckwith-Wiedemann syndrome. This overgrowth disorder is characterized by macrosomia, macroglossia, organomegaly and developmental abnormalities (in particular abdominal wall defects with exomphalos), embryonal tumors – such as Wilms' tumor – and ACC, neuroblastoma, and hepatoblastoma.

Many studies have demonstrated that *IGF-II* is strongly overexpressed in malignant adrenocortical tumors, with such overexpression observed in ~90% of ACC. Transcriptome analysis of adrenocortical tumors has demonstrated that *IGF-II* is the gene most overexpressed in ACC by comparison with benign adrenocortical adenomas or normal adrenal glands. The mechanisms underlying *IGF-II* overexpression are paternal isodisomy (loss of the maternal allele and duplication of the paternal allele) or, less frequently, loss of imprinting.

β-Catenin Activation in Adrenocortical Cancer

Genetic alterations of the Wnt signaling pathway were initially identified in familial *adenomatous polyposis coli* (►*APC*) and have been extended to a variety of cancers. Adrenocortical tumors have been observed in some case reports of patients with familial *APC*. Furthermore, familial *APC* patients, with germline mutations of the *APC* gene that lead to an activation of the Wnt signaling pathway, may develop ACTs. The Wnt signaling pathway is normally activated during embryonic development. β-catenin is a key component of this signaling pathway. Interestingly, gene profiling studies in various types of adrenocortical tumors have shown the frequent activation of Wnt signaling target genes. In both benign and malignant ACT, β-catenin accumulation can be observed. These alterations seem very frequent in ACC, consistent with an abnormal activation of the Wnt signaling pathway. This is explained in a subset of adrenocortical tumors by somatic mutations of the *β-catenin* gene altering the Glycogen synthase kinase 3-β (GSK3-β) phosphorylation site.

TP53

The tumor suppressor gene ►*TP53* is located at 17p13 and involved in the control of cell proliferation. Germline mutations in *TP53* are identified in 70% of families with Li-Fraumeni Syndrome (LFS). This syndrome displays dominant inheritance and confers susceptibility to breast carcinoma, soft tissue sarcoma, brain tumors, osteosarcoma, leukemia, and ACC. Germline mutations in *TP53* have been observed in 50–80% of children with apparently sporadic ACC in North America and Europe. The incidence of pediatric ACC is about ten times higher in Southern Brazil than

in the rest of the world, and a specific germline mutation has been identified in exon 10 of the *TP53* gene (R337H) in almost all cases. In sporadic ACC in adults, somatic mutations of *TP53* are found in only 25–35% of cases. LOH at 17p13 has been consistently demonstrated in ACC but not in adrenocortical adenomas. LOH at 17p13 was recently reported to occur in 85% of ACC.

Diagnosis and Treatment of Adrenocortical Cancer

Clinical and Hormonal Investigations

Symptoms leading to the diagnosis of ACC can be due to hormone hypersecretion and/or tumor mass and metastasis. The majority of ACC are usually secreting tumors when careful hormonal investigations are performed. By contrast with benign adrenocortical tumors (that usually secrete a single class of steroid), ACC can secrete various types of steroids (glucocorticoids, androgens, and mineralocorticoids). Cosecretion of androgens and cortisol is the most frequent and highly suggestive of a malignant adrenocortical tumor. Cortisol oversecretion (classified as "ACTH-independent Cushing's syndrome" in case of ACC) can induce centripetal obesity, protein wasting with skin thinning and striae, muscle atrophy (myopathy), diabetes, hypertension, psychiatric disturbances, gonadal dysfunction, osteoporosis…

Imaging of Adrenocortical Cancer

Imaging is an essential diagnostic step for ACC. It is important for both, not only for the diagnosis of malignancy of an adrenal mass but also for the extension work-up. Adrenal computed tomography scan (CT-scan) is a very informative imaging procedure for adrenocortical tumors. In ACC, it shows a unilateral mass, which is most often large (above 5–6 cm, typically 10 cm and above), lowering the kidney. MRI can also be used in the diagnosis of liver nodules and venous invasions. More recently studies have demonstrated that ACCs almost invariably have a high uptake of 18-fluorodesoxyglucose ((18)-FDG). Thus (18)-FDG PET scan appears to distinguish between benign and malignant adrenal tumors. This simple, nontraumatic imaging procedure also participates in the extension work-up.

Pathology and Molecular Analysis

As often with endocrine tumors, the diagnosis of malignancy of adrenocortical lesions is not always easy for the pathologist. Combinations of various histological parameters allowing the calculation of a "score" for a given tumor have been developed. The most widely used is the Weiss score made of nine different items. It is assumed that a score above three is most likely associated with a malignant tumor. Since the Weiss score has limitations and is dependant on the experience of the pathologist, there is an effort to develop molecular markers of malignancy. IGF-II overexpression and allelic losses at 17p13 have been suggested as useful markers. Immunohistochemistry of Cyclin E or Ki-67 that are higher in malignant adrenocortical tumors has also been suggested in the literature as potential useful tools.

Prognosis of Adrenocortical Cancer

The overall prognosis of ACC is poor with a 5 year survival rate below 35% in most series. Among the various clinical parameters that have been shown to impact on ACC prognosis, tumor staging has been demonstrated as one of the most important. The MacFarlane staging is the most commonly used and relies on surgical finding and extension work-up. Four different stages are differentiated with this score. Stage 1 and Stage 2 tumors are localized to the adrenal cortex and present a maximum diameter below or above 5 cm, respectively. Locally invasive tumors or tumors with regional lymph node metastases are classified as Stage 3, whereas Stage 4 consists of tumors invading adjacent organs or presenting with distant metastases. The prognosis of Stage 1 and 2 tumors is better than that of Stage 3 or 4 tumors. A better survival is usually reported in younger patients. Some pathological features as a high mitotic rate or atypical mitotic figures have been shown to be associated with a poor prognosis.

In the future, it is expected that molecular tools will help to a better prediction of the prognosis of ACC. Gene profiling approach can already differentiate malignant from benign tumors.

Treatment of Adrenocortical Cancer

Surgery of the adrenal tumor is the major treatment of Stage 1 to 3 ACC. It can also be discussed in Stage 4 patients. Only complete tumor removal can lead to long term remission. Radiofrequency thermal ablation of liver and lung metastasis below 4–5 cm of maximal diameter can be an alternative to surgical removal. Chemoembolization has also been used for liver metastasis. Surgery of bone metastasis can be indicated to reduce fracture risk, or, in case of spinal localization, neurological symptoms. Radiation therapy is usually considered as not very effective to control tumor growth. However, it has been recently suggested that tumor bed radiation therapy could help to prevent local recurrence after surgical removal.

When complete tumor removal is not possible, or in case of recurrence, medical treatment with O,p'DDD (ortho, para', dichloro-, diphenyl-, dichloroethane, or Mitotane) is recommended. It has both an anticortisolic action and a cytotoxic effect on the adrenocortical cells. Objective tumor regression could be observed in 25–35% of the patients. A mitotane blood level of

at least 14 mg/l seems to improve the tumor response rate. However, the side effects of mitotane (mainly digestive and neurologics) often limit the ability to reach this suggested optimal level. Since O,p'DDD can induce adrenal insufficiency, substitutive glucocorticoid and mineralocorticoid therapy should be associated. Several cytotoxic chemotherapy regimens have been used in ACC. They are usually considered in patients with tumor progression under mitotane therapy reaching the plasma blood level of 14 mg/l or presenting severe side effects limiting its use. Various drugs have been used and the experience is still limited. It is currently accepted that the combined treatment with Cis-platine, Etoposide, Doxorubicin (EDP regimen) associated with O,p'DDD, and Streptozotocin also given with O,p'DDD are the better regimens. However, there is obviously an important need for prospective controlled studies and for new therapies in patients with advanced ACC.

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Adrenocortical Tumors

Definition

Adrenocortical tumors (ACT) are divided into two histologic groups, depending on several criteria. Large tumors (>200 g) with nuclear atypia, more than five mitoses on 50 high-power fields, vascular and capsular invasion, broad fibrous bands, and extensive necrosis are highly suggestive of carcinoma. Small tumors without the mentioned features are considered to be adenomas. In children, distinguishing between adenoma and carcinoma is difficult because the histologic features of small and large lesions overlap. Only about 20% of childhood ►adrenocortical tumors are classified as adenomas.

►Childhood Adrenocortical Carcinoma
►Adrenocortical Cancer

Adrenomedullin

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Definition

Adrenomedullin (AM) is a member of the ►calcitonin superfamily of peptides. It is produced in virtually every organ by many different cell types and it is secreted into the plasma where it occurs at picomolar concentrations. Over the past several years AM has increasingly received the attention of the scientific community by virtue of its implication in many normal and disease states.

Characteristics

Adrenomedullin is a small peptide (52 amino acids) first isolated from a ►pheocromocytoma in 1993. It was initially described as a hypotensive peptide although after more than a decade of research and about 2,000 published articles published, AM is now recognized as a pluripotent peptide-hormone implicated in many normal and pathological processes ranging from vascular tone and diabetes to ►angiogenesis and embryogenesis/ ►carcinogenesis.

Adrenomedullin: Peptide and Gene Structure

Adrenomedullin is generated as part of a larger precursor molecule named preproadrenomedullin (preproAM) (Fig. 1). PreproAM is 185 amino acids long and contains an N-terminal 21 amino acid signal peptide which is cleaved during the transport of the molecule across the cell membrane to produce the 164 amino acids prohormone proAM. Further processing of proAM by endopetidases generates four peptides termed proadrenomedullin N-terminal 20 peptide (PAMP), mid-regional pro-adrenomedullin (proAM 45–92), adrenomedullin (AM) and adrenotensin (proAM 153–185). From these, PAMP, ProAM 45–92 and AM are present in plasma and PAMP, AM and Adrenotensin are biologically active peptides. Both PAMP and AM peptides are produced as a ►glycine (Gly)-extended inactive peptides which coexists in plasma with the active form generated

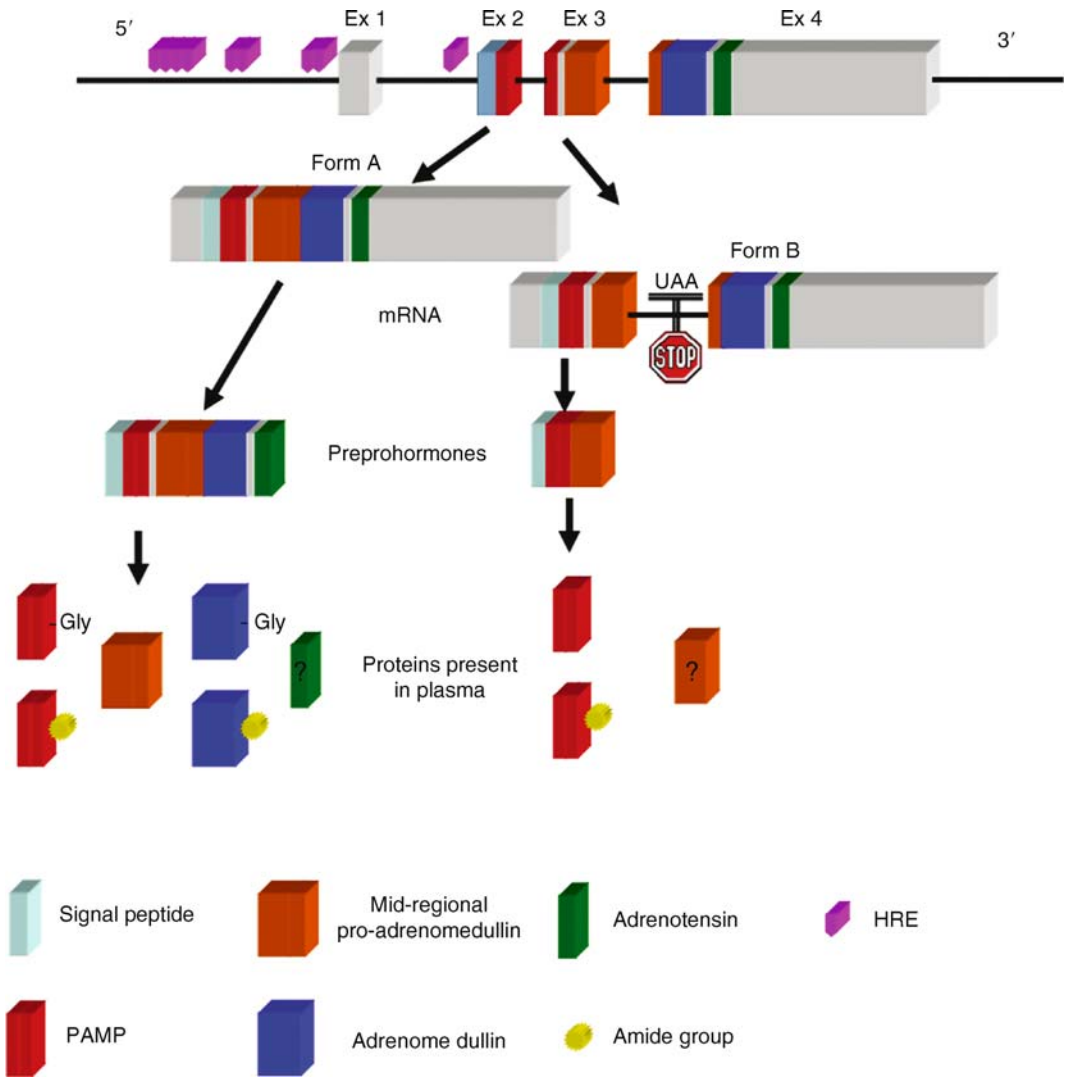
upon enzymatic ►amidation. AM shares homology with several vasoactive peptide members of the calcitonin superfamily including calcitonin, calcitonin gene related peptide (CGRP), amylin and intermedin. Members of this family share the presence of an intramolecular disulfide bond which generates a six-member ring structure and an amidated carboxy terminal, both of which are required for biological activity.

In humans, the single locus of the adrenomedullin gene is located in the short arm of chromosome 11. The complete gene (2,319 bp) contains four exons and three introns which are ►alternatively spliced during the transcription process to generate two different transcripts (Fig. 1). The shortest mRNA form includes exons 1–4 and therefore codes for a complete preprohormone which results in stoichiometric amounts of the four peptides referred above. The longest transcript incorporates the third intron that contains an early termination codon, resulting in a truncated preprohormone which only expresses PAMP.

AM is an ancient gene that, based on our current knowledge, first appeared in the starfish with a potential dual function of neurotransmission and host defense. It shows a remarkable degree of conservation in genomic organization and peptide structure from fish to humans which supports its critical role in species survival.

Signal Transduction

As most soluble peptides, AM transduces its signal upon interaction with a receptor located in the cellular surface. The discovery of the AM receptor in 1998 represented a novel paradigm in the field of ►G-protein couple receptor (GPCR) signaling. A functional receptor for AM requires physical interaction in the cellular



Adrenomedullin. Figure 1 Genomic organization of the AM gene.

membrane of the seven transmembrane domain receptor calcitonin-receptor-like-receptor (CRLR) and either the receptor-activity-modifying protein (RAMP) 2 or RAMP3. CRLR has two alternative pharmacological profiles that are conferred by association to the accessory proteins RAMP1 (producing the CGRP receptor) and RAMP2/3 (producing the AM receptor). Therefore, the expression pattern of functional AM receptors is determined by the presence of these two components. In healthy individuals, RAMP2/3 is equally expressed among most tissues, excluding lung, female reproductive system and adipocytes which show higher levels of expression. CRLR expression although lower, parallels that of RAMP2 which suggests that the majority of CRLR signaling units in the body are complexed with RAMP2 to produce adrenomedullin receptors. Modest but robust changes in the expression of the complex CRLR-RAMP2 have been reported in certain physiological and disease states such as pregnancy, sepsis and ►cancer. The same physiological conditions are related to high levels of AM expression. Other stimuli which result in coordinated regulation of AM, CRLR and RAMP2 include hypoxia, endocrine hormones and inflammatory cytokines.

Upon binding to its receptor AM induces cAMP elevation through an adenylyl cyclase-PKA mediated pathway. While multiple reports including the seminal paper by Kitamura have consistently demonstrated cAMP mediated effects of AM, other more scarce ones have shown cAMP independent actions such as vasodilation via elevation of Ca^{2+} and K^{+}-ATP, and activation of endothelial nitric oxide synthase. AM also activates Akt, ►mitogen-activated protein kinase and focal adhesion kinase in endothelial cells which mediate its angiogenic potential.

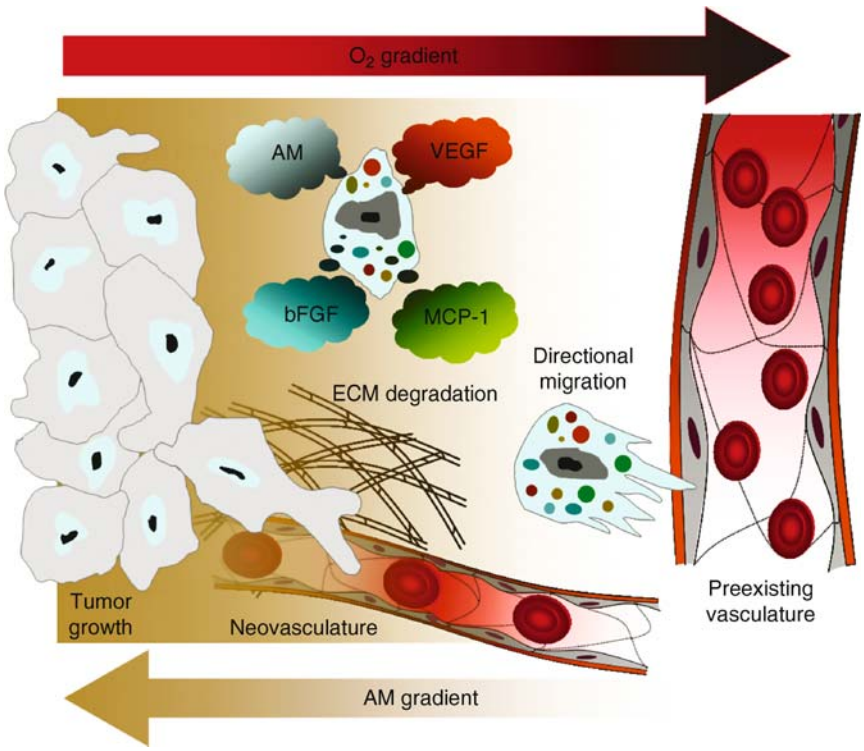
AM Serves as a Common Language Between the Different Cellular Components of the Tumor Microenvironment

Many disease states have been reported to modulate the expression of AM including cancer. As we mentioned before, AM was originally isolated from an adrenal gland tumor. A wealth of subsequent studies have found that AM and its receptor are overexpressed in many human cancers and tumor cell lines establishing an ►autocrine loop mechanism that tumor cells exploit to maintain an autonomous proliferative state. AM is intimately intertwined at several levels in the multistep process of tumor development. At the initial stage of tumor growth, rapid accumulation of malignant cells results in the establishment of an avascular nutrient-depleted ►hypoxic environment. Low oxygen tension within and surrounding the tumor body triggers a number of survival mechanisms which allow neoplastic cells to overcome this inhospitable microenvironment. Many of these encompass the upregulation of AM's expression. In fact one, if not the most important driving force for AM upregulation in tumor cells is hypoxia. Cellular responses to hypoxia are mediated through a well known hypoxia inducible factor (HIF)-dependent mechanism. HIF is a heterodimeric transcription factor which is stabilized under hypoxic conditions and binds to specific DNA sequences denoted hypoxia response elements (HRE) which are present in the ►promoter regulatory region of the AM gene (Fig. 1). Hypoxia also upregulates the expression of the AM receptor gene in many tumor types hence establishing a rational explanation behind the aforementioned autocrine growth mechanism underlying carcinogenesis (Fig. 2).

As tumor derived AM is released into the microenvironment it establishes a peptide gradient which ultimately disseminates to reach a teeming collection of cell types known to be able to respond to this peptide and to be involved in further development of the tumor, including the cancer cell itself. AM not only stimulates tumor cell proliferation via its mitogenic activity but also by involving an antiapoptotic state.

Although the advantageous effects of AM for the tumor cell are apparent, its actions are not restricted to this compartment within the tumor. On the contrary, AM acts as an integrative molecule allowing the crosstalk between all different compartments within the tumor microenvironment. As an example, AM is a ►migratory factor for different inflammatory cells, including ►mast cells. Mast cells migrate towards the tumor mass following the preestablished tumor-derived AM gradient. Hence, as mast cells approach the tumor they are exposed to increasingly higher concentrations of AM. Only when certain concentration of AM is reached in the proximity of the tumor, mast cells degranulate liberating to their immediate milieu numerous inflammatory factors (including AM) which not only enhance the tumor progression but also perpetuate the inflammatory process. AM is also implicated as a potential immune system suppressor, inhibiting macrophage function and acting as a negative regulator of the complement cascade, protective properties which help cancer cells circumvent immune surveillance.

One of the most significant features distinctive of hypoxic tumors is their ability to induce angiogenesis. Tumor-induced angiogenesis is a pathological condition that results in ectopic neovascularization. Of most therapeutic interest is the finding that AM is an essential factor that regulates normal and pathological vascularization. AM was first described as a potent hypotensive peptide although its connection to the normal and pathological biology of the vascular system is much deeper than initially thought. AM is an essential factor for the normal development of vasculature as revealed by mice lacking the AM gene in mice which is embryonically lethal due to abnormal vascularization. AM also induces pathological neovascularization via



Adrenomedullin. Figure 2 Model of the AM/tumor cell/inflammatory cells relationship in human carcinogenesis. The microenvironment around the tumor is hypoxic and stimulates expression of AM by the tumor cells. Tumor-derived AM is released into the microenvironment setting up a concentration gradient of peptide that contributes to angiogenesis and attracts distal MCs to infiltrate the tumor site. Neovasculature makes possible tumor metastasis and it is used as a point of entrance for inflammatory cells (i.e. MC). As MCs migrate up the peptide gradient, higher AM concentrations are reached stimulating MC-derived angiogenic factors (AM, VEGF, bFGF, MCP-1) expression and ultimately release at the tumor site. AM mediates a paracrine tumor survival effect (direct mitogen, angiogenic factor, and anti-apoptosis), and functions as a paracrine recruitment factor drawing additional MCs to the area, thus perpetuating the inflammatory process and enhancing tumor promotion.

CRLR-RAMP2 present in the endothelial cells. Angiogenesis is a multistep process which commences with the growth of endothelial cells which is enhanced by tumor derived AM. AM also prevents hypoxia-triggered apoptosis in endothelial cells enhancing the neovascularization process. Additionally, AM participates in the remodeling of the extracellular matrix and tridimensional rearrangement of endothelial cells in the tissue which results in the establishment of the new intratumoral vasculature by stimulating migration and tube formation of endothelial cells. AM increases the permeability of the endothelial cells in the newly established vasculature which supplies the tumor with the necessary nutrients for expansion additionally it creates an access route for inflammatory cells which are attracted to the tumor site and migrate in following gradients of ►chemoattractant and migratory factors produced by the tumor, such as AM. The same route can simultaneously be utilized by tumor cells as the entrance point to the vascular system facilitating the metastasis process. The invasive capability of tumor cells is thus enhanced by AM.

Concluding Remarks

Conclusions gleaned from the studies carried over the past fourteen years portrait AM as a molecular connector with competence to entangle and allow communication between the different cellular components of the tumor machinery which conspire under the tumor cell direction to promote cancer. It is not only the direct effect that AM has on tumor cells but also its ability to interact with all these cellular elements which makes this peptide an attractive therapeutic target for cancer. The collective research effort is shifting from trying to discern whether AM is a causative agent of cancer to better understanding its central role as a multifaceted exchange currency among the multiple cellular players involved in tumor development. Strategies utilizing blocking agents aimed at disruption of this loop might be proven successful to impede tumor growth.

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Adriamycin

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Synonyms

Doxorubicin; 14-Hydroxyldaunorubicin

Definition

Adriamycin is an antineoplastic ►anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius*. It is widely used in the treatment of various different types of cancers. Proposed mechanisms for its antitumor activity include intercalation into DNA, inhibition of ►topoisomerase II, and promotion of free-radical formation. However, the clinical utility of this drug is seriously limited by the development of cardiomyopathy and ►myelosuppression.

Characteristics

Chemical Properties

Adriamycin is an orange–red compound, soluble in water and aqueous alcohols, moderately soluble in anhydrous methanol, and insoluble in nonpolar organic solvents. It consists of an aglycone (adriamycinone), a tetracyclic ring with adjacent quinone–hydroquinone groups in rings C-B, coupled with an amino sugar (daunosamine). It is generated by C-14 hydroxylation of its immediate precursor, ►daunorubicin (see Fig. 1). Semisystematic derivatives of adriamycin include ►epirubicin, an axial-to-equatorial epimer of the hydroxyl group at C-4′ in daunosamine, and ►pirarubicin, 4′-O-tetrahydropyranyl-adriamycin, etc.

Clinical Aspects

Therapeutic Applications

Adriamycin has a broad antitumor spectrum. It is used to treat hematopoietic malignancies such as leukemias, lymphomas (non-Hodgkin disease, ►Hodgkin disease) and ►multiple myeloma, and different solid tumors (breast, thyroid, gastric, ovarian, bronchogenic, head and neck, prostate, cervical, pancreatic, uterine and hepatic carcinomas, as well as transitional cell bladder carcinomas, ►Wilms tumor, ►neuroblastoma, and soft tissue and bone sarcomas). Adriamycin is applied as a component of combination chemotherapy, rather than a monotherapy. Adriamycin-based combination chemotherapy regimens include ABVD (Adriamycin, Bleomycin, Vinblastine, Dacarbazine) for non-Hodgkin disease, CHOP (Cyclophosphamide, Adriamycin, Vincristine, Prednisone) for ►Hodgkin disease, and M-VAC (Methotrexate, Vinblastine, Adriamycin, Cisplatin) for urothelial carcinoma.

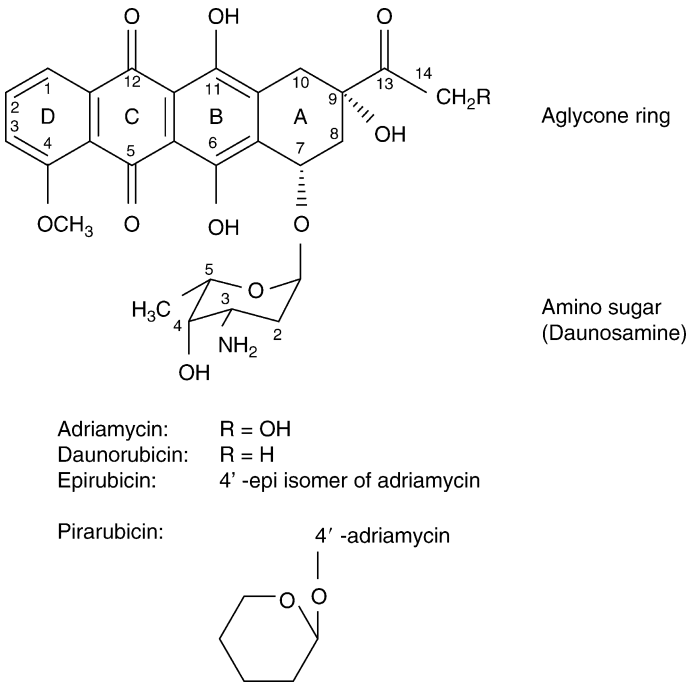
Pharmacokinetics

Adriamycin is rapidly cleared from the plasma, quickly taken up and only slowly eliminated from organs such as the spleen, lungs, kidneys, liver, and heart. It does not cross the blood–brain barrier. Adriamycin is converted to an active metabolite, adriamycinol, through a two-electron reduction of the side chain C-13 carbonyl moiety by NADPH-dependent cytoplasmic aldo/keto reductase or carbonyl reductase. It is converted to inactive metabolites in the liver and other tissues, and predominantly excreted in the bile.

Clinical Toxicities

The usual toxic side effects of adriamycin including stomatitis, nausea, vomiting, alopecia, gastrointestinal disturbance, and dermatological manifestations, are generally reversible. The dose-limiting side effects of the anthracyclines including adriamycin are myelosuppression and cardiotoxicity. Myelosuppression with leukopenia, neutropenia, and occasionally thrombocytopenia is dose-related and potentially life-threatening.

Cardiotoxicity is characteristic of the anthracycline antibiotics, of which adriamycin is the most toxic. Adriamycin-induced cardiotoxicity can be acute, chronic, or delayed. The acute effect is not dose-related, and is characterized by sinus arrhythmias and/or abnormal electrocardiographic (ECG) changes (nonspecific ST-T wave change, prolongation of QT interval). Acute toxicity of this type is transient and rarely a serious problem. Chronic cardiotoxicity is a much more serious problem, being related to cumulative dose. It is irreversible and leads to dilative cardiomyopathy and congestive heart failure (CHF), usually unresponsive to cardiotonic steroids (digitalis) and β-blockers. The risk of developing CHF increases markedly at total cumulative doses in excess of 500 mg/m^2. Moreover, the effects of this chronic cardiotoxicity may manifest precipitously without antecedent ECG changes. The risk of life-threatening cardiac dysfunction can be decreased by regular monitoring of endomyocardial



Adriamycin. Figure 1 Structures of adriamycin and its analogues.

(EM) biopsy histopathological changes and left ventricular ejection fraction (LVEF) as measured by the multigated radionuclide angiography (MUGA) method and/or echocardiography (ECHO). Finally, adriamycin can also cause delayed cardiotoxicity, possibly, related to the dose. This occurs after an asymptomatic interval, mostly in people who were treated as children.

Several approaches have been proposed to overcome adriamycin cardiotoxicity and that of the anthracycline antibiotics generally. Administration by slow continuous intravenous infusion (over 48–96 h) rather than the standard bolus injection decreases the likelihood of chronic cardiotoxicity. ►Dexrazoxane (ICRF-187), an iron chelator that prevents the formation of complexes between adriamycin and iron, and subsequent production of ►reactive oxygen species (ROS), is sometimes used as a cardioprotectant. However, it may decrease antitumor activity. Liposomal encapsulation is designed to increase safety and efficacy by decreasing cardiac and gastrointestinal toxicity through decreased exposure of these tissues to the drug, while effectively delivering it to the tumor. Polyethyleneglycol-coated (Pegylated) liposomal adriamycin (Doxil (USA), Caelyx (UK)) is currently used for treating AIDS-related ►Kaposi sarcoma, refractory ovarian cancer, and some other solid tumors. In order to improve therapeutic efficacy and decrease side effects by promoting drug accumulation inside tumors, the water-soluble N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer, magnetic targeted carriers, and ►immunoliposome conjugates with the specificity of whole monoclonal antibodies (e.g. antibodies against CD19 or MUC-1) or FAB' fragments have been developed as carriers of adriamycin.

In further efforts to decrease the risk of developing cardiotoxicity, several derivatives of adriamycin or daunorubicin, such as epirubicin, pirarubicin, ►idarubicin, and ►aclarubicin have been developed. Although these agents may be less cardiotoxic than adriamycin itself, they do have a decreased antitumor activity.

Pharmacological Mechanisms

Mechanisms of Action

Several mechanisms appear to contribute to the cytotoxic effects of adriamycin, including inhibition of DNA replication and repair; inhibition of RNA and protein synthesis via intercalation of the aglycone portion of the molecule between adjacent DNA base pairs, especially G-C base pairs; promotion of the cleavage of DNA by formation of adriamycin-topoisomerase II-DNA ternary complexes; inhibition of topoisomerase I; and direct binding to the cell membrane. Formation of free radicals is another major mechanism of cytotoxicity. One-electron reduction of the quinone moiety in the C ring of adriamycin by some flavin-containing enzymes (mitochondrial NADH dehydrogenase, microsomal NADPH-cytochrome P450 reductase, and xanthine oxidase) generates adriamycin-semiquinone radicals. These rapidly react with oxygen

to form superoxide anions, which then generate hydrogen peroxide and hydroxyl radicals in the presence of redox-active metals such as iron (III) and copper (II). The final result is DNA damage and lipid peroxidation. The semiquinone radical can be transformed into an aglycone C7-centered radical that also mediates cellular damage by DNA alkylation and lipid peroxidation. Adriamycin can bind to metal ions such as iron, copper, and manganese, by forming adriamycin–metal complexes, which may lead to generation of ROS and damage to cell membranes.

Mechanisms of Resistance

Development of resistance to the drug is a major obstacle in chemotherapy with adriamycin. Drug efflux pumps are important for defending cells against anticancer drugs. The acquisition of adriamycin resistance involves promotion of excretion of the drug by overexpressing the ATP-binding cassette (ABC) transporters ►P-glycoprotein (P-gp)/ABCB1, ►multidrug resistance-associated proteins (MRPs)/ABCC (MRP1, MRP2, and MRP6 etc.), and breast cancer resistance protein (BCRP)/ABCG2. P-gp transports hydrophobic compounds including adriamycin, while MRP1 and BCRP can extrude predominantly these glutathione conjugates. In addition, RalA-binding protein 1 (RALBP1)/Ral-interacting protein of 76 kDa (RLIP76) is a nonclassical ABC transporter involved in drug excretion. RALBP1 catalyzes ATP-dependent efflux of xenobiotics including adriamycin as well as its glutathione conjugates. In fact, the level of expression of these efflux pumps correlates with the clinical efficacy of adriamycin. ►Glutathione-S transferases (GSTs) are a family of enzymes involved in the cellular detoxification of xenotoxins. Adriamycin and its metabolites (adriamycinol) are conjugated with glutathione by GSTs and transported by MRPs and BCRP, etc. Increased expression of GSTs, especially GSTπ, also confers adriamycin resistance by promoting detoxification.

Lung resistance protein (LRP), the 110 kDa major vault protein (MVP), is a main component of vaults, which are multisubunit structures that may be involved in nucleocytoplasmic transport, and is involved in resistance to anticancer drugs including adriamycin. LRP may affect the intracellular distribution of adriamycin, but the detailed mechanisms remain unknown. Furthermore, a relationship between adriamycin resistance and qualitative and quantitative changes in the expression of topoisomerase II, a major target for adriamycin, has been reported.

Mechanisms for Development of Cardiotoxicity

The molecular mechanisms leading to adriamycin-induced cardiotoxicity may include lipid peroxidation by generation of ROS, abnormalities in intracellular calcium homeostasis through inhibition of sarcomeric reticulum Ca^{2+}-ATPase (SERCA2), Na^{+}-K^{+}-ATPase and Na^{+}-Ca^{2+} exchanger of sarcolemma, inhibition of mitochondrial creatine kinase, and interaction with cardiolipin, which is a phospholipid of the inner mitochondrial membrane in the heart. Adriamycin also promotes apoptosis by activation of p38 mitogen-activated kinases (►MAPK) in cardiac muscle cells. Moreover, adriamycin downregulates the expression of genes for sarcomeric proteins (such as α-actin, myosin, troponin I, and myofibrillar creatine kinase) and for proteins involved in calcium homeostasis in the sarcomeric reticulum, such as SERCA2, cardiac muscle ryanodine receptor (RYR2), calsequestrin, and phospholamban, by suppression of transcription factors (e.g. MEF2C, HAND2, and GATA4) and/or activation of a the transcriptional repressor Egr-1.

Adriamycinol (doxorubicinol), a secondary alcohol metabolite, may also be involved in the development of adriamycin-induced cardiotoxicity, via enhancing the inhibitory effects of SERCA2, Na^{+}-K^{+}-ATPase, and Na^{+}-Ca^{2+} exchanger of sarcolemma. Adriamycinol also inhibits the iron-regulatory protein/iron-responsive element (IRP/IRE) system, which plays a crucial role in iron homeostasis, and may lead to cardiotoxicity.

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Adult-Onset Diabetes

Definition

Diabetes Type 2.

Adult Stem Cells

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Synonyms

Somatic stem cells; Tissue stem cells; Postnatal stem cells

Definition

An undifferentiated cell found in a differentiated tissue that can renew itself and (with certain limitations) differentiate to yield all the specialized cell types of the tissue from which it originated.

Characteristics

Adult ►stem cells are defined as undifferentiated tissue-specific stem cells with extensive self-renewal capacity, which can proliferate to generate mature cells of the tissue of origin. The primary roles of adult stem cells are to maintain and/or regenerate the cells of damaged tissues. Adult stem cells were first described in organs and tissues characterized by high cell turnover, such as blood, gut, testis, and skin but have to date also been isolated from many other organs and tissues including brain, bone marrow, liver, heart, lung, retina, and skeletal muscle.

Stem cells differ from ►somatic cells with their different potentials and their proliferation ability. There are three kinds of stem cells: Embryonic, germinal, and adults stem cells that are classified according to their developmental potential ranging from totipotency to unipotency. The fertilized oocyte and the blastomere up to the 8-cell stage are considered as totipotent (►totipotent stem cells) as they can differentiate to generate a complete organism. ►Embryonic stem cells, the cells derived from the inner cell mass of the blastocyste, are pluripotent (►pluripotent stem cells) and have the ability to differentiate into cells and tissues from all three germ layers: the endoderm, the ectoderm, and the mesoderm. ►Germinal stem cells are also pluripotent and are derived from so-called primordial germ cells and give rise to the gametes (sperm and eggs) in adults. In contrast, adult stem cells are generally believed to be multipotent (►multipotent stem cells) or unipotent (►unipotent stem cells) which means that they can only give rise to progeny restricted to the tissue of origin. Hematopoietic stem cells (►HSC), bulge stem cells in the hair follicle, and mesenchymal stem cells (►MSC) are examples of multipotent stem cells, which can differentiate into multiple cell types of a single tissue, whereas epidermal stem cells, myosatellite cells of muscle, and endothelial progenitor cells are examples of unipotent stem cells, which only give rise to one mature cell type. Recently, some studies have shown that many adult tissues may contain cells with pluripotent capacity capable of generating differentiated cells from an unrelated tissue. This process is termed ►stem cell plasticity.

In most tissues/organs renewal is compensated by tissue-specific stem cells. The stem cells normally divide very rarely, but stimuli caused by damaged or injured tissue or a need to generate progeny to maintain the tissue can induce proliferation and produce daughter cells that can differentiate into the specific cell lineages of the respective tissue type. Stem cell division typically leads to the formation of committed progenitor cells with more limited self-renewal capacity as e.g., transit amplifying cells in the epidermis or lymphoid or myeloid progenitors in the bone marrow. Tissue progenitor or transit amplifying cells provides an expanded population of a proliferating tissue that differentiate into more mature and determined cells that eventually no longer proliferates and die. To maintain the balance in the adult tissues/organs, the number of progenitor/stem cells that proliferates must be equal to the number of cells that determinedly differentiate and die. If the number of proliferating cells is higher than the number of cells that maturate and die, it will give the primary feature of a cancer. Studies have shown that many of the pathways that regulate normal stem cell proliferation are dysregulated and cause neoplastic proliferation in cancer cells. Therefore, cancer may be considered a disease of dysregulated cellular self-renewal capacity.

Adult stem cells reside in a special ►microenvironment termed the stem cell niche. Stem cell niches are composed of a group of cells that provide a physical anchorage site and extrinsic factors that control stem cell proliferation and differentiation and enable them to maintain tissue homeostasis. Deregulation of the niche signals has been proposed to lead to cancer. A decrease in proliferation-inhibiting signals, or an increase in proliferation-promoting signals, may lead to excessive stem cell production and thereby development of cancer stem cells (see later). Investigation of the interaction between stem cells and their niche may reveal possible targets for cancer treatments. For example, the blocking of proliferation signals, enhancing of antiproliferative signals or induction of differentiation from the stem cell niche may be used to target the cancer stem cells. It has furthermore been suggested that targeting the stem cell niche may prevent cancer metastasis. Some cancers metastasize to sites that cannot be explained by circulation distribution, lymphatic drainage or anatomic proximity. These sites may, however, provide favorable niches that support the survival of the cancer stem cells.

Much effort is put into the identification of ►stem cell markers to be able to isolate the stem cells of interest. Isolation of stem cells makes it possible to enhance the knowledge of stem cell identity and to use them therapeutically.

Therapeutic Potential

Adult stem cell transplantation has been used in several years for the treatment of ►hematological malignancies and lymphomas. The main purpose of stem cell transplantation in cancer treatment is to make it possible for patients to receive very high doses of chemotherapy and/or radiation. High-dose chemotherapy and radiation can severely damage or destroy the bone marrow while killing cancer cells. Before treatment, bone marrow or peripheral blood stem cells are harvested from the patient itself (for autologues transplantation) or from a donor (for allogen transplantation), frozen down, and then transplanted after the patient has received high doses of chemotherapy, radiation therapy, or both. The transplanted healthy stem cells replace the stem cells destroyed by high-dose cancer treatment and allow the bone marrow to produce healthy cells.

Stem cells in general, due to their high proliferative capacity and long-term survival in comparison to somatic cells, make them very ideal candidates to use for regenerative medicine and cell replacement therapy. Lately, there has been an increasing interest in the potential use of adult stem cells in cell replacement strategies and in tissue engineering, including gene therapy. This current interest rose due to the discovery of adult stem cells with pluripotential capacity and/or transdifferentiating (►transdifferentiation) ability, which means that cells from one tissue can differentiate into mature and functional cells of another tissue. There are reports that HSCs under certain conditions can evolve into cells of neural lineage, liver, muscle, skin, and endothelium; skeletal muscle stem cells can evolve into blood cells and neural cells; and hair follicle stem cells can evolve into neural lineage cells. Other adult stem cells that can be induced to a different cell type include MSCs, cardiac muscle stem cells, neural stem cells, and testis-derived stem cells. These cells have the advantage that they can be used as autologous transplants and have been proposed as an attractive alternative to ►embryonic stem cells in genetic therapy.

An alternative approach for therapeutic use of stem cells is to use them as cellular vehicles. It has been demonstrated that genetically modified MSCs can be used to target delivery of anticancer agents and ►suicide gene therapy vectors to tumor cells. Upon administration, MSCs can target microscopic tumors, proliferate and differentiate, and contribute to the formation of a network of cells surrounding the tumor (tumor stroma). MSCs genetically modified to express interferon beta has, for example, been shown to inhibit the growth of tumor cells by local production of interferon beta. MSCs are not the only stem cells that have been used as shuttle vectors for delivery of gene therapies into growing tumors. It has also been demonstrated that neural stem and progenitor cells migrate selectively to tumor loci in vivo in mice. These studies clearly suggest that a stem cell-directed prodrug therapy approach may have great use for eradicating tumors as well as to treat the residual cancer cells remaining after therapy.

Genetic manipulation of adult stem cells may also be used to increase the functionality and proliferative capacity of these cells. HSCs are one of the most promising candidates for correction of single gene disorders as e.g., ►cystic fibrosis and ►hemoglobinopathies, due to their capability of targeting solid organs and high success rate in their isolation by using a combination of surface markers. Infants with forms of ►severe combined immunodeficiency syndrome have successfully been treated with genetically engineered bone marrow stem cells. The stem cells were harvested from the patients, a functional gene inserted, and the genetically modified cells reintroduced to the same patient. To increase the success of chemotherapeutic treatment, drug resistant HSCs have been produced by introduction of the ►multidrug resistance gene with the aim of limiting the myelosuppressive effects of standard chemotherapeutic agents on the stem cells. However, even though adult stem cells have shown to carry great potential to function as therapeutic agents for targeting human diseases such as cancer, degenerative and chronic diseases, they do have some restrictions such as having limited self-renewal capacity. This limitation can be overcome by the introduction of immortalizing genes that increases the cells proliferative capacity. ►Telomerase has been, in this connection, highlighted among the numerous genes that are capable of immortalizing stem- or progenitor cells. However, suggested oncogenic potential of ►immortalized cells release caution that before the therapeutic use of stem cells in the clinic, a thorough screen for transformation phenotype is required.

Cancer Stem Cells

The theory that cancer stem cells (►cancer stem-like cells and ►stem-like cancer cells) are involved in many types of cancer has recently gained popularity. There are many similarities between adult stem cells and cancer stem cells. Both have the ability to self-renew and differentiate into more mature diversified cells. Cancer stem cells and normal stem cells share many cell surface markers and utilize many of the same signal transduction pathways.

Cancer stem cells have been identified in most types of hematopoietic malignancies, including acute myeloid leukemia, chronic myeloid leukemia, acute

lymphoblastic leukemia, and multiple myeloma. Recently, cancer stem cells have also been isolated from solid tumors such as breast, lung, and brain tumors. The cancer stem cells only represent approximately 1% of the tumor, making them difficult to detect and study. Studies have shown that cancer stem cells may cause tumors when transplanted into a secondary host indicating that the cancer stem cells can initiate and repopulate a tumor. A study of human leukemia shows that the normal hematopoetic stem cell and the neoplastic clone share common molecular mechanisms governing proliferation which is supportive of the normal hematopoetic stem cell being a target for transformation. Due to stem cells are able to divide over the lifespan of the individual they seem to allow accumulation of a number of mutations and perhaps epigenetic changes (►epigenetics) that cause neoplastic development. In addition, it has been shown that adult stem cells can be targets for neoplastic transformation by introducing the telomerase gene into a purified stem cell. The transduced cell line showed characteristic alterations of neoplastic development such as contact inhibition, anchorage independence, and in vivo tumor formation in immunocompromised mice. All these findings give a very large support to the existence of cancer stem cells and the strong links between normal adult stem cells and cancer stem cells suggest that stem cells are targets for neoplastic transformation. Cancer stem cells may also be derived from differentiated cells. Loss of the ►tumor suppressor genes $p16^{Ink4}$ and $p19^{Arf}$ combined with constitutive activation of the EGF receptor (►EGFR) caused loss of differentiation in mature brain astrocytes and the cells regained stem cell properties.

The identification of cancer stem cells strongly suggests that these cells are the key targets for future therapeutic development as they fuel the replicative capacity of the cancer. Therefore, as much as understanding the nature of a cancer cell it is very crucial to understand the neoplastic potential of the stem cells. Analysis of the differences between adult stem cells and cancer stem cells is very important to be able to specifically target the cancer stem cells whilst sparing the normal stem cell population. Several studies indicate that some stem cell markers are expressed differently in normal and cancer stem cells and these may be potential targets in the development of future cancer treatments.

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Adult T-cell Leukemia

Definition

ATL; A leukemia of mature T lymphocytes (►T cells) developing in adults, resulting from infection with the ►human T-cell leukemia virus (HTLV) and characterized by circulating malignant T-lymphocytes, skin lesions, lymphadenopathy (enlarged lymph nodes), hepatosplenomegaly (enlarged liver and spleen), hypercalcemia (high blood calcium), lytic ("punched out") bone lesions, and a tendency to infection. There are four categories of ATL, based on the aggressiveness of the disease – smoldering, chronic, lymphoma, and acute.

Adult Tissue Stem Cells

Definition

These cells are set aside during development in order to provide a source for replenishment of tissue over time in response to damage or simply wear and tear.

►Stem Cell Telomeres

Advanced Breast Cancer

Definition

Tumors of stage III or IV (larger than 5 cm and/or have metastasized).

►Fulvestrant
►Breast Cancer

AEL

Definition
Acute erythro leukemia.

►ETV6

Aerobic Glycolysis

►Warburg Effect

Aesthetic and Reconstructive Surgery of the Breast

Definition
Surgical procedures that reshape the breast to improve their appearance.

►Oncoplastic Surgery

Afaxin

Definition
►Retinol.

Affinity-Matured IgG Response

Definition
Affinity is the strength of binding between a receptor, such as the antigen-binding site on an antibody and a ligand, as for example an epitope on an antigen. High titer, high affinity IgG antibodies are characteristic of an antigen-driven immune response

►Autoantibodies

Aflatoxin B_1

Definition
Aflatoxin B_1 is a potent hepatocarcinogen produced by the mold Aspergillus flavus; ►aflatoxins

►Detoxification
►Carcinogenesis

Aflatoxins

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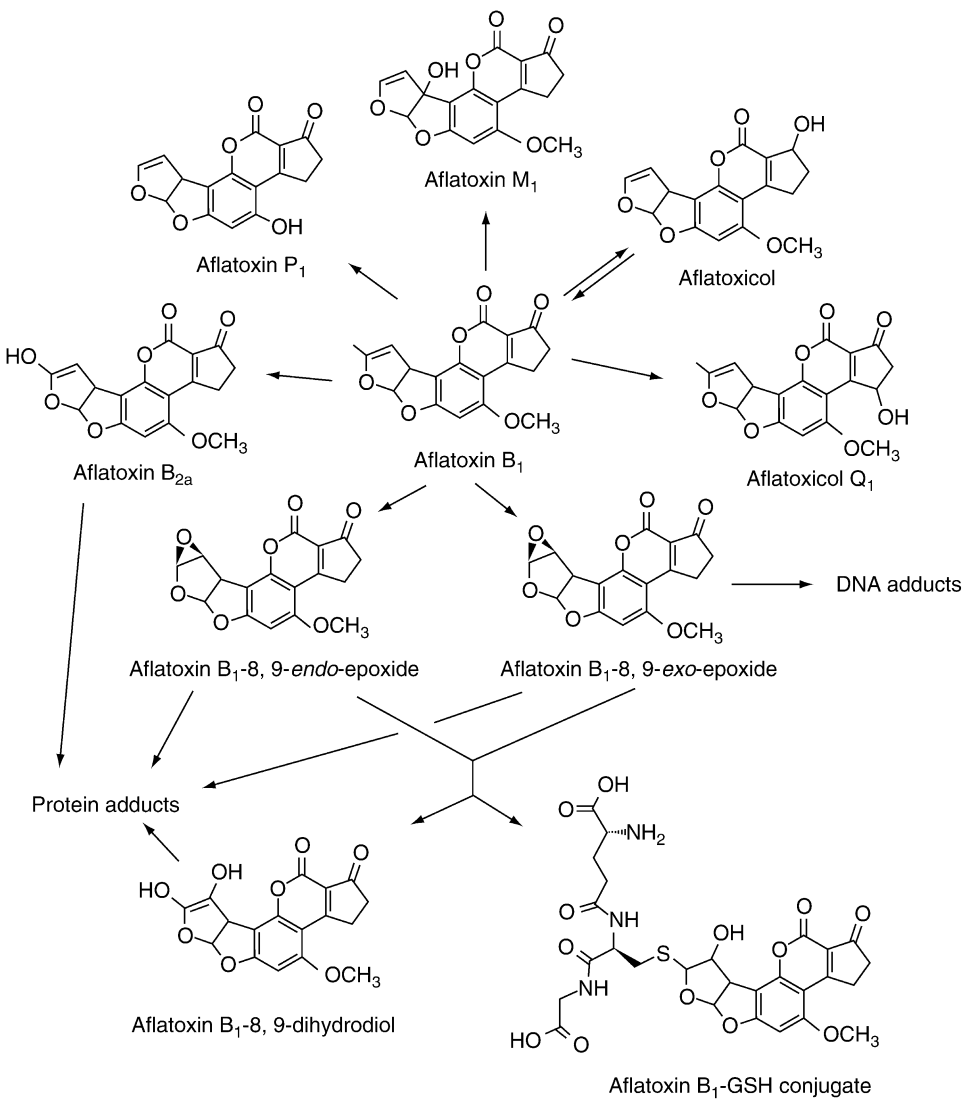
Definition
Mycotoxins are contaminants of a number of agricultural products, including peanuts, corn, and other grains in warm and moist conditions. Human exposure to aflatoxins is primarily through ingestion and results in acute hepatic necrosis, marked bile duct hyperplasia, acute loss of appetite, wing weakness, and lethargy.

Characteristics
In the early 1960s, an outbreak of hepatotoxic disease in turkeys, which became known as turkey "X" disease, gained the attention of many investigators worldwide. This condition was characterized by acute hepatic necrosis, marked bile duct hyperplasia, acute loss of appetite, wing weakness, and lethargy. It was deduced that the condition was caused by consumption of peanut meal contaminated with a mycotoxin, which is a toxin of fungal origin. The culprit fungi in turkey "X" disease turned out to be strains of *Aspergillus flavus, A. parasiticus, and A. nomius,* and thus the term aflatoxins was coined for the toxic metabolites. More specifically, *A. flavus* and *A. parasiticus* can produce aflatoxins B_1, B_2, G_1, G_2, and M_1. These mycotoxins can contaminate a number of agricultural products, including peanuts, corn, and other grains in warm and moist conditions. Human exposure to aflatoxins is primarily through ingestion. In addition to outbreaks of liver failure and gastrointestinal bleeding in Southeast Asia and Africa having been attributed to aflatoxins, ►liver cancer incidence was observed to be elevated in regions with high endemic aflatoxin concentrations. The two major risk factors for human ►hepatocellular carcinoma, the fifth most common cancer worldwide, are hepatitis B infection and ingestion of aflatoxins.

Aflatoxin B_1 (AFB_1), the most prevalent and carcinogenic of the aflatoxins, is classified as a group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer. Although the majority of AFB_1 research has focused on its hepatic effects, AFB_1 also targets other organs, including the lung and the kidney. In the lung, exposure to inhaled AFB_1, particularly from contaminated grain dusts, has been linked to ►respiratory cancers (►Lung cancer). Due to a significant proportion of ingested mycotoxin being excreted via the urine, the renal nephron is exposed to AFB_1 and its metabolites. AFB_1 accordingly alters kidney function, and is a known renal carcinogen.

Biotransformation

AFB_1 is defined as a procarcinogen, as its bioactivation is required for carcinogenicity (Fig. 1). The initial metabolism of AFB_1 involves four types of reactions: *O*-dealkylation, hydroxylation, epoxidation, and ketoreduction. The enzymes responsible for the metabolism include members of the ►cytochrome P450 family (CYPs), prostaglandin H synthase (PHS), lipoxygenase (LOX), and a cytosolic NADPH-dependent reductase. In experimental animals, CYPs involved in AFB_1 bioactivation include members of 1A, 2B, 2C, and 3A subfamilies. In humans, there are multiple p450 isozymes implicated, including CYP1A2, CYP2A3, CYP2B7, CYP3A3, and CYP3A4. CYP3A4 is thought to play a predominant role in the metabolism of AFB_1 in human liver; although CYP1A2 has the highest affinity for AFB_1 at low concentrations, it is expressed at much lower levels than CYP3A4. PHS and LOX are involved in ►xenobiotic bioactivation by catalyzing the oxidation of ►arachidonic acid to produce lipid



Aflatoxins. Figure 1 Biotransformation of AFB_1.

peroxyl radicals, which are known epoxidizing agents. Cooxidation by PHS and LOX may be a significant mechanism of AFB_1 bioactivation in extrahepatic tissues such as lung, which has high PHS and LOX expression, but overall P450 activity is lower than that in the adult liver.

Regardless of the enzyme catalyzing the reaction, epoxidation of AFB_1 results in formation of AFB_1-8,9-epoxide, which can exist in both *endo* and *exo* conformations. The *exo*-epoxide is the isomer implicated in the ►alkylation of DNA, with its reactivity being at least 1,000 fold greater than that of the *endo*-epoxide. Hydroxylated metabolites of AFB_1 include AFM_1, AFQ_1, AFP_1, and AFB_{2a}. The formation of aflatoxicol from AFB_1 is reversible, and therefore aflatoxicol is considered to be a "reservoir" for AFB_1 rather than a bioactivation or ►detoxification product.

The two pathways for AFB_1-epoxide detoxification are glutathione conjugation and epoxide hydrolysis, with glutathione conjugation being quantitatively the most important (Fig. 1). Glutathione conjugation is catalyzed by ►glutathione-S-transferases (GSTs), which can be highly polymorphic. Human GSTM1-1 (hGSTM1-1), which is absent in ~50% of individuals, has the highest activity towards AFB_1 *exo*-epoxide, but the importance of this polymorphism in AFB_1 carcinogenicity has not yet been clearly established. AFQ_1, AFP_1, and AFB_{2a} are not highly mutagenic and therefore are considered to be detoxification products. They can form glucuronide or sulfate conjugates, which are excreted. AFM_1, a metabolite of AFB_1 identified in milk and urine, is less biologically active than AFB_1, but regardless is a potent carcinogen. The AFM_1-epoxide can also bind to DNA, forming AFM_1-N^7-guanine.

Carcinogenesis

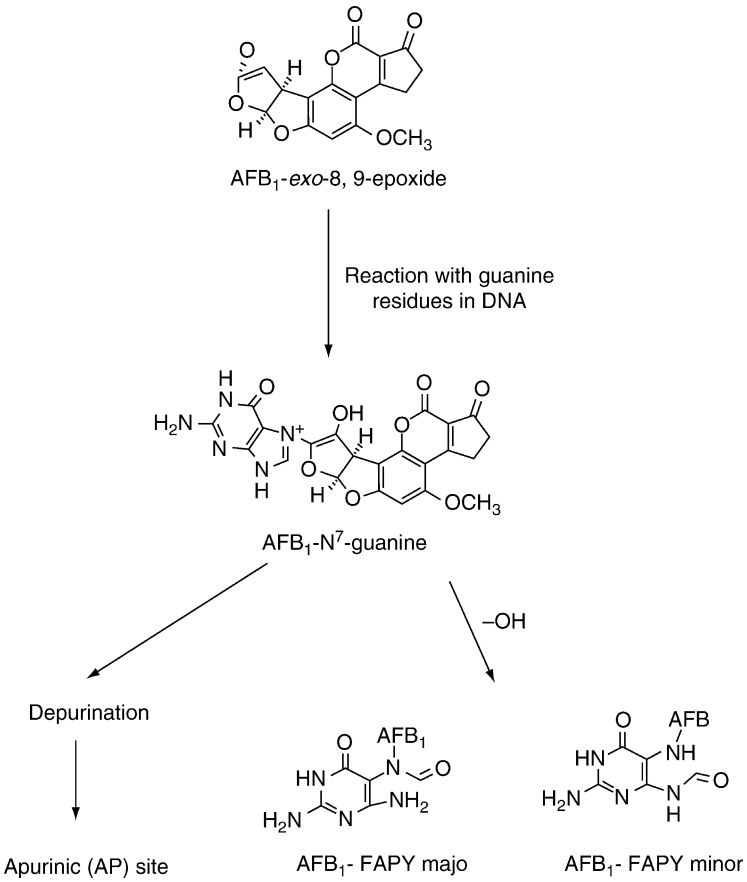
AFB_1 is considered to be a complete carcinogen, possessing activity as both an initiator and a promoter. Initiation occurs by ►DNA damage, as well as cytotoxicity, which stimulates cell division, thus promoting tumor formation. There are many characteristics of AFB_1 that makes it a useful tool for investigating ►chemical carcinogenesis. First, the metabolites of AFB_1 have been extensively investigated and their toxicity elucidated. Second, the toxicity of AFB_1 is determined by a balance between bioactivation and detoxification of the AFB_1-8,9-epoxide. Third, there exists multiple mechanisms of bioactivation that can be compared in terms of carcinogenic metabolites produced. Fourth, not only does the susceptibility of a species/tissue relate to ►DNA repair capabilities (►Repair of DNA), but AFB_1 itself has effects on DNA repair activity. Fifth, the specific AFB_1-DNA adduct formed can be used to predict the mutagenic responses. Finally, the parent compound and several metabolites fluoresce, facilitating detection.

The *exo* epoxide of AFB_1 can alkylate proteins and ►nucleic acids, with the second guanine from the 5′ end in guanine di- and trinucleotide sequences in DNA being the favored target. The major adduct formed by the *exo*-epoxide is 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy AFB_1, also known as AFB_1-N^7-Gua (Fig. 2). AFB_1-N^7-Gua can undergo three reactions: release of AFB_1-8,9-dihydrodiol restoring guanine; depurination resulting in an apurinic site in DNA; and base-catalyzed hydrolysis to form the AFB_1-formamidopyrimidine adduct (AFB_1-FAPY). AFB_1-FAPY, representing a significant proportion of AFB_1 adducts *in vivo*, exists in equilibrium between two rotameric forms, designated AFB_1-FAPY major and AFB_1-FAPY minor. The structure of AFB_1-FAPY has not been completely defined, although the proposed structure is presented in Fig. 2. It has also been shown that metabolism of AFB_1 can lead to formation of 8-hydroxy-2′-deoxyguanosine in rat, duck, and woodchuck liver and in mouse lung. G to T transversion, the most frequently observed mutation induced by AFB_1, results from DNA alkylation and subsequent AFB_1-N^7-Gua formation, and possibly by the ►oxidative DNA damage as well. A proportion of mutations in DNA formed by AFB_1 occurs at the base 5′ to the modified guanine, or even further away, due to helical distortion resulting from the AFB_1 adduct.

►*P53*, a ►tumor suppressor gene considered the "guardian of the genome," has controls on cell cycle, DNA repair, and ►apoptosis. *P53* is the most frequently targeted gene in human carcinogenesis, with a mutation frequency of 50% in most major cancers. In geographical regions with a high dietary exposure to AFB_1, such as China and Sub-Saharan Africa, mutations in *p53* have been implicated AFB_1-induced human liver tumorigenesis. AFB_1 produces mutations at the 3rd base of codon 249 in *p53*, causing a G→T transversion, and an amino acid substitution (arginine to serine), and thus a structural alteration of this tumor suppressor protein. This may result in deregulation of the cell cycle, and thus loss of tumor suppression by *p53*. The K-►*ras* proto-►oncogene, important in ►signal transduction, is often implicated in human and mouse lung tumors. AFB_1-induced point mutations at specific "hot spots" (e.g. codons 12 and 13) of the K-*ras* gene, which cause activation of the protein, occur in AFB_1-induced mouse lung tumorigenesis and rat hepatocarcinogenesis.

Repair

In mammals, ►nucleotide excision repair (NER) is important for protection against AFB_1-induced carcinogenesis. NER is a DNA repair process that deals with a wide array of DNA helix-distorting lesions that affect normal base pairing, thus altering transcription and replication. In *E. coli*, NER is responsible for the repair of both AFB_1-N^7-Gua and AFB_1-FAPY. In yeast, NER



Aflatoxins. Figure 2 AFB_1-*exo*-8,9-epoxide and DNA damage.

is also the main repair pathway, although ▶homologous recombination is also involved in the repair of AFB_1-induced damage. In mammals, NER is important in protection against AFB_1-induced carcinogenesis. NER is the main repair mechanism for the AFB_1-N^7-Gua adduct. AFB_1-FAPY is repaired less efficiently by mammalian NER than is AFB_1-N^7-Gua, an effect that is attributed to AFB_1-FAPY being less distortive of DNA architecture. Apurinic sites generated by AFB_1-DNA adduct formation are repaired by base excision repair (BER), although insertion of an incorrect base is a frequent occurrence.

Species/Tissue Susceptibility

Susceptibility to the toxic and carcinogenic effects of AFB_1 varies between species, as well as between different tissue types. In humans, the liver is the main target for this toxin. In rat, duck, and trout, administration of AFB_1 results in hepatocarcinogenesis, whereas this is not the case in the mouse, monkey, hamster, and mouse. The reason for this has been attributed to differences in AFB_1 biotransformation and DNA repair. For example, the mouse is susceptible to pulmonary carcinogenesis by AFB_1, regardless of the route of administration, but does not develop hepatocarcinogenesis. The mouse liver expresses an alpha-class GST with high specific activity towards the *exo*-epoxide, and higher NER activity as compared to the rat liver. On the other hand, mouse lung has lower DNA repair activity than does liver. AFB_1 is able to alter NER activity (by inhibition or elevation) in different animal species and organs, which may contribute to differential susceptibility to the mycotoxin's carcinogenicity.

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Aflatoxin B1

Definition
A potent liver carcinogen produced by fungi that infest cereal grains.

►Carcinogen Metabolism

AFP

►Alpha-Fetoprotein
►Alpha-Fetoprotein – Historical
►Alpha-Fetoprotein – Modern

AFP-L3

Definition
An AFP fraction, which binds to LCA (lens culinaris agglutinin) lectin because this type of AFP has a core fucose on the *N*-glycan.

►Fucosylation

Agammaglobulinemia

Definition
An almost total lack of immunoglobulins, or antibodies.

AGEs

Definition
►AGEs are advanced glycation end products. The formation and accumulation of these senescent macroprotein derivatives progress under inflammatory or diabetic conditions. AGEs are involved in various types of disorders mainly through the interaction with its receptor RAGE.

►Minodronate
►Inflammation

Aggressive Fibromatosis

►Aggressive Fibromatosis in Children
►Desmoid Tumor

Aggressive Fibromatosis in Children

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Synonyms
Aggressive fibromatosis; Desmoid tumor

Definition
►Aggressive fibromatosis (AF) is a rare soft tissue tumor and rare in childhood with high potential for local invasiveness and recurrence. Primary ►surgery with ►negative margins is the most successful primary treatment modality for ►children with ►AF. Positive resection margins after surgery indicate a high risk for ►relapse. Multicenter prospective (randomized) trials are necessary to clarify the role of and best strategy for ►treatment in pediatric AF after ►incomplete surgery. For this purpose, ►chemotherapy or alternatively ►radiotherapy can be considered, each with its own potential side effects in consequence.

Characteristics
Aggressive fibromatosis (AF) (►Supportive care) is a soft tissue tumor, which arises principally from the connective tissue of muscle and the overlying fascia (aponeurosis). The previously most used synonym is ►desmoid tumor. The histological pattern is characterized by elongated fibroblast-like cells. Although AF is a nonmetastasizing tumor with benign histological features, it has a significant potential for local invasiveness (►Invasion) and ►recurrence. The overall incidence of

AF in children is 2–4 new diagnoses per 1 million a year. Childhood AF has an age distribution peak at approximately 8 years (range 0–19 years) with a slight male predominance.

Clinical Presentation

The typical clinical presentation of AF is a painless, slowly growing, deep-seated mass. Predilection sites are shoulder, chest wall and back, thigh, and head/neck. Children with AF of head/neck have shown to be to be younger at diagnosis than children with AF at other sites. From 1986 until 2004, ten pediatric AF case series reported a total of 206 patients. In 64 of the reviewed patients site of involvement and age at diagnosis were specified. The children with AF of head/neck had a median age of 3.6 years at diagnosis (range 0.2–9.9 years), whereas the children with AF of trunk/limb had a median age of 7.8 years (range 0.0–15.7 years) ($p<0.01$). This difference in age distribution may be influenced by referral and selection bias; however, it may reflect the site distribution in different age groups in children with AF.

Diagnostic Approach

The diagnosis of AF is based on histology. It arises principally from the connective tissue of muscle and the overlying fascia (aponeurosis). The fibromatosis lesion is characteristically poorly circumscribed and infiltrates the surrounding tissue, which is usually striated musculature. The proliferation consists of elongated fibroblast-like cells of uniform appearance surrounded by and separated from one another by abundant collagen, with little or no cell-to-cell contact. The cells lack hyperchromasia or atypia and the mitotic rate is variable. Using immunohistochemistry, the spindle muscle cells stain strongly with vimentin, whereas smooth muscle actin (SMA) and muscle-specific actin stain variable. Rare cases also stain with desmin and S-100.

Pathogenesis

The pathogenesis of AF is suggested to be multifactorial, i.e., genetic predisposition, endocrine factors, and trauma seem to play an important role. Local physical trauma before developing AF was reported in 20% of 108 reported pediatric AF patients from three studies. Apparent chromosome aberrations and nonrandom X-chromosome inactivation in adult and pediatric AF suggests a true neoplastic character (Chromosome translocations). This is supported by a report of eight pediatric AF cases in one study, of which five (63%) had an abnormal karyotype (two at initial diagnosis, and three at relapse) with trisomy 8 (n = 4) and trisomy 20 (n = 1) being the only recurrent features (►Chromosome translocations). Sporadic cases of adult AF contain a somatic mutation in either the adenomatous polyposis coli (APC) gene (21%), identified on chromosome 5q22 and associated with familial adenomatous polyposis (FAP), or β-catenin gene and protein expression (52%) (►APC Gene in Familial Adenomatous Polyposis; ►APC/β-Catenin Pathway).

A high prevalence of ►desmoid tumor has been reported in 126/880 (14.3%) of adult FAP patients with proven APC gene mutation. Insulin-like growth factor binding protein 6 (IGFBP-6) appears directly down-regulated by the β-catenin/TCF complex in adult AF, and implies a role for the IGF axis in the proliferation of AF. In addition, a high prevalence of AF (38%) was reported for patients with Gardner syndrome. In contrast, in a review of all reported pediatric AF studies no patient with a history of familial AF or FAP, and only two patients with Gardner syndrome was seen. This illustrates that routine karyotyping has a relatively limited value, and the significance of the APC and β-catenin genes in the pathogenesis of childhood AF and their value for differentiating fibroblastic tumors has not yet been established. In adults, a correlation between tumor growth rate and the level of endogenous estrogen was suggested in female patients, because of high amounts of estrogen receptors (ER) in their tumor tissue. These are important findings as the presence of antiestrogen binding sites (AEBS) distinct from ER are suggested to play a role in treatment with antiestrogens in adult ►AF02254. So far, in two studies, only four children with AF were tested and did not express ER, indicating that the role of expression of ER and AEBS in pathogenesis of childhood AF may be limited.

Treatment

As these tumors at presentation clinically mimic other more malignant soft tissue tumors like ►rhabdomyosarcoma, non-ossyfying ►Ewing sarcoma sooner or later pediatric AF patients come to the attention of a pediatric oncologist. However, as the tumor is heterogeneous with regards to site and extension, treatment strategy in each individual patient is ideally determined by a multidisciplinary team which consists of pediatric oncologists, surgeons, and radiotherapists, supported by the diagnostic expertise of pediatric radiologists and pathologists. Aggressive fibromatosis still lacks general recommendations for its clinical management. Although spontaneous regression has been observed in sporadic cases, surgery is generally the primary treatment modality in adults and children with AF, unless there is a risk of significant mutilation and/or functional impairment. Seven of ten pediatric AF studies report treatment of the primary tumor, and all generally treated their patients (n = 168) with initial surgery. The other three series report treatment of recurrent tumor, two of them initially treated their patients (n = 15) with chemotherapy [vinblastine (VBL) and methotrexate (MTX)], whereas in the third study (n = 4) radiotherapy 01859 was administered.

Relapse rate in the reviewed children with primary AF was approximately 50%. Most relapses (89%) have been observed within 3 years, and nearly all (97%) by 6 years, although relapse after 10 years has been reported. All relapses are local or regional with a pattern consistent with infiltrative growth. Three deaths are reported, caused by invasive tumor destruction of vital organs (►Progression), all three located in the head/neck region.

In 85 pediatric patients in whom primary surgery was performed, information on resection margins and relapse was available. Remarkably, only 16% of the patients with ►free microscopically margins after surgery relapsed, versus 67% of patients with positive margins ($p<0.01$). In case of positive resection margins, 74% of patients without additional therapy relapsed, versus 40% of patients who received adjuvant treatment ($p = 0.064$). Adjuvant treatment consisted of chemotherapy (n = 8) or radiotherapy (n = 2) (►Adjuvant therapy). Although this is a retrospective analysis, which implies disadvantages like selection biases, the high risk for relapse in case of positive resection margins may indicate that the role of adjuvant treatment in patients with positive margins needs further exploration. In adults, the standard approach for patients with microscopically positive margins after surgery is adjuvant radiotherapy resulting in a high local control rate of approximately 80%, which is therefore considered to be beneficial regardless of surgical margins. In pediatric patients the high doses of radiotherapy (55–60 Gy) necessary for tumor control in AF harbors a large risk for growth problems and development of secondary malignancies (►Radiation Sensitivity; ►Radiation-Induced Sarcomas after Radiotherapy).

One pediatric AF study reported 11 children with partially excised or recurrent lesions who received radiotherapy, and who had at least 3-year follow-up. Four (36%) children relapsed, including two of five who had a dose of ≥50 Gy. In contrast, another pediatric AF study reported 11 of 13 (85%) children with relapse after irradiation, including 6 of 8 who had a dose of ≥50 Gy. The role of radiotherapy in childhood AF as adjuvant treatment in case of SP is not yet established and needs further prospective randomized. studies which will not only evaluate response and survival but also late sequelae.

The use of chemotherapeutic and other systemic agents might be a reasonable alternative to avoid radiotherapy in the growing child. However, also chemotherapy carries the risk for potentially adverse side effects, like second malignancies, fertility problems, and cardiotoxicity. A recent review concerning mainly adult AF reported a median overall response rate of 50% (range 17–100%) with combination chemotherapy [doxorubicin, actinomycin-D, methotrexate (MTX), and vinca alkaloids], in 16 single-arm studies. Reviewing all pediatric AF cases treated with chemotherapy in total, 27 out of 187 pediatric patients were treated with chemotherapy only at initial diagnosis (n = 10) or at relapse (n = 17). A combination of VBL and MTX was the most common reported regimen. Response on chemotherapy only was complete remission (CR) in 26%, partial remission (PR) in 18%, whereas stable disease (SD) was found in 30%, progressive disease (PD) in 11%, and response was not reported in 15% of the reviewed cases. Overall relapse rate (RR) after treatment with chemotherapy only was 26%.

Comparing the relapse rate (respectively 74% versus 50%) of 46 pediatric patients with positive margins after primary surgery may suggest an advantage in outcome of adjuvant treatment with chemotherapy (n = 8), as compared with patients who did not receive adjuvant treatment (n = 38), however, numbers of cases are small and derived from different series. This illustrates that the role of chemotherapy in childhood AF is not yet established and should be further explored. Currently, a collaborative study of MTX/VBL chemotherapy for children with AF is initiated. Based on the reported experiences, the response of pediatric AF to chemotherapy has shown to be slow and it has been suggested that treatment should be continued for prolonged periods from 12 to 18 months. The chronic and prolonged course that many of these children with AF endure as a result of these slow-growing lesions suggests that the use of (combinations of) noncytotoxic drugs, like antiestrogens, ►nonsteroidal anti-inflammatory drugs (NSAIDs), imatinib mesylate, interferon-alpha (IFN-α), and ►retinoic acid for part of their treatment might be reasonable treatment options to explore.

Side Effects in Survivors

So far, information on toxicity of treatment is available from five pediatric AF case series (n = 128) with a median follow-up time of 4 years (range 0–25 years). Two studies reported a limited range of motion of the primary area as the most frequent late complication (42%). Severe short-term toxicity of treatment was reported in three patients, two died of cardiotoxicity after treatment with doxorubicin and one died of severe radiation induced dermatitis with chronic ulcers. During this short median follow-up, one secondary malignancy was reported; a papillary carcinoma of the thyroid gland, which developed 11 years after radiotherapy.

Conclusion

Primary surgery with negative margins is the treatment of choice for children with AF. In case of unresectable tumors, the use of chemotherapy and/or noncytotoxic drugs in children with AF could be a reasonable alternative. Positive margins after surgery indicate a high

risk for relapse. Multicenter prospective (randomized) trials are necessary to clarify the role of and best strategy for adjuvant treatment in pediatric patients with aggressive fibromatosis.

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Aging

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Definition

Aging is defined at many levels, from the mitotic age of cells to the organismal-wide aging of tissues and organs. The appearance of cancer is only one clinical manifestation of the aging process. Age-associated ►epithelial cancers, such as ►breast cancer, ►colon cancer and ►prostate cancer, however, contribute significantly to the morbidity and mortality of the elderly and are the second leading cause of death.

Characteristics

Aging

During an organism's lifespan almost every aspect of its phenotype will undergo modification. The complexity of aging has led to a plethora of ideas about the specific molecular and cellular causes and how these alterations lead to age-associated diseases, such as epithelial cancers. Underlying all of these theories is the assumption that aging occurs from the bottom-up beginning with damage to DNA and proteins and ending with organismal frailty, disability, and disease. There is a vast amount of evidence to support the following aging theories: ►somatic mutation, telomere loss, mitochondrial damage, and altered proteins and waste accumulation. Somatic mutation theory suggests that age-related accumulation of ►DNA damage demonstrates a decline in DNA repair mechanisms, while the telomere loss theory argues that telomere shortening confers a finite lifespan to many human ►somatic tissues. Shortening of telomeres leads not only to a loss of chromosome replicative ability but also to an increased propensity for recombination events, such as chromosomal translocations, that may induce oncogenesis. The mitochondrial theory makes a connection between age-related accumulation of mutations in mitochondrial DNA and impaired ATP production and thus reduced tissue bioenergenesis. Finally, the altered proteins and waste accumulation theory argues that accumulation of damaged proteins, due to either a decline in the function of chaperone proteins or proteosomes, leads to cellular damage, which then contributes to a range of age-related disorders. There is increasing consensus that all of these mechanisms interact to play a role in aging.

Epithelial Cancers

The body defends itself against epithelial cancers by halting replication of damaged cells either through ►apoptosis, in which the cell dies, or by ►senescence, in which the cell replicatively arrests but remains metabolically active. Both of these mechanisms are important in preventing the formation of epithelial tumors in the young. As one ages, the number of senescent cells increases. The accrual of these senescent cells may alter the ►microenvironment of the tissue such that cells harboring preneoplastic damage are permitted to proliferate and eventually undergo transformation. Senescent cells may contribute to this milieu, in part, by secreting paracrine factors that compromise tissue structure and function. Consequently, senescence inhibits cancer formation early on, but with time the build up of senescent cells alters the microenvironment to one that promotes the growth of epithelial cancers.

Cellular Senescence

Most studies on senescence and cancer focus on the role played by senescent fibroblasts in the transformation of epithelial cells. Fibroblasts can undergo senescence as a result of various processes including: ►replicative exhaustion (telomere shortening), ►oxidative stress, DNA damage, ►epigenetic changes to ►chromatin organization, or activation of ►oncogenes such as ►Ras, all of which appear to signal primarily through ►p53-dependent pathways, although some oncogenes trigger senescence via p16. Once a cell has entered senescence, its transcriptome is altered such that genes associated with wound healing (e.g. ►inflammatory cytokines, ►epithelial growth factors, and ►matrix metalloproteinases (MMPs)) are activated.

The alteration in gene expression affects not only the senescent fibroblast itself, but the cells surrounding it as well. Senescent fibroblasts that were co-cultured with breast or prostate epithelial cells increased the proliferation and tumorigenicity of those epithelial cells, both *in vitro* and *in vivo*.

Epithelial cells can also undergo senescence due to oxidative stress, DNA damage, epigenetic changes, or activation of oncogenes. The pathways that trigger epithelial senescence include both p53- and p16/►pRb-dependent as well as independent pathways. While the specific genes triggered by senescence can vary between the two cell types, the pattern of activation is similar: senescent-associated genes exhibit chromosomal clustering. Genes up-regulated in senescent fibroblasts include various cell cycle proteins, interleukins, ►growth factors, integrins, MMPs, and caspases. Those up-regulated in senescent epithelial cells include various cell cycle proteins, epithelial growth factors, transcription factors, integrins, ►laminins, ►fibronectin, MMPs, and ►tissue inhibitors of metalloproteinases (TIMPs). It is important to note that not all of these genes were up-regulated in all samples or studies, only that these genes have been mentioned in various studies on senescence. In addition, it remains to be seen which genes trigger senescence and which are activated during senescence.

Alterations in the Microenvironment

Tissue architecture is important for maintaining proper cellular function and thus serves as a protective mechanism against diseases, including cancer. Accordingly, a defining characteristic of epithelial cancers is ►loss of tissue architecture. The microenvironment, which includes the ►extracellular matrix (ECM) (collagens, laminins, nidogens, proteoglycans) and soluble factors that are released by the cells or transmitted by other organs (hormones, cytokines, growth factors, enzymes), can serve as a powerful tumor suppressor, keeping damaged cells in check. A microenvironment that provides the correct cues can revert cells containing preneoplastic as well as oncogenic mutations back to a normal phenotype. But tissue architecture is not static: it is continually undergoing alterations due to the processes of living. The traditional focus in cancer has been on interactions between cells and various growth factors. However, there is increasing interest in other components of the extracellular space as well as in the bi-directional cross-talk between the ECM and cells. The ECM interacts with cells via cognate receptors on the cell membrane, including integrins and syndecans. These receptors are connected to the cytoskelton of the cell, which is connected to the nuclear matrix and chromatin. Thus signals travel back and forth between the ECM and the cell that regulate gene expression and, in turn, protein expression, which then alters the make-up of the microenvironment. This bidirectional interaction between ECM and cells is termed dynamic reciprocity.

The appearance of cancer cells disrupts the microenvironment and thereby destroys tissue architecture. Moreover, many oncogenic epithelial cells overexpress matrix metalloproteinases. These enzymes degrade various proteins in the basement membrane, including collagens and laminins. The subsequent disruption of the ECM allows the transformed epithelial cells to migrate into the stroma and form tumors. In breast and prostate carcinomas, the microenvironment consists of transformed epithelial cells, ►reactive stroma, recruited blood vessels, and infiltrating immune cells such as macrophages, lymphocytes, and leukocytes. Numerous studies also demonstrate that components of the ECM, such as collagen and laminin, are modified by and contribute to further tumor growth. Alterations in ECM protein are mirrored by changes in cell membrane receptors, such as integrins and ►growth factor receptors.

Tumor Progression in Aging

Whereas aging confers the greatest risk of developing cancer (as discussed above), it is widely accepted that most histologically similar epithelial tumors behave less aggressively in the aged. This longstanding impression arose from clinical studies in humans and was further supported by animal models, in which young and aged mice received identical inocula of tumor cells and were subsequently monitored for tumor growth and aggressiveness. Proposed mechanisms have focused on age-related deficits in immune mediated responses that directly and indirectly promote tumor growth (such as a lack of inflammatory cells and their associated cytokines) and decreased ►angiogenesis. It has been argued that the less permissive milieu of tissues is an adaptive response to the greater risk of cancer conferred by senescence and environmentally induced changes in the epithelial and stromal cells.

Implications for Treatment

A major difficulty with assessment of treatment options in the elderly is that many solid tumor treatment protocols have not been tested and optimized for the elderly. Many ►clinical trial phase-II and -III treatment protocols stop recruitment at age 75 years. This is a problem since bone marrow recovery may be compromised by age and drug dosages may need to be modified due to age-related changes in drug metabolism and clearance. Additionally, standard therapies used for younger individuals may be inappropriate and further contribute to morbidity of the elderly, especially for some cancers, such as prostate, which may have a natural history that extends beyond the patient's expected lifespan. Finally, it is important to understand the cell biology of senescence since many chemotherapeutic agents function by halting cell replication through induction of a senescent phenotype. The

ability to induce cell senescence in a cancer cell should create a new class of therapeutic agents for cancer treatment in the elderly.

►Aging-Associated Inflammation

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Aging-Associated Inflammation

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Synonyms

Senescence-associated chronic inflammation

Definition

Aging: Aging encompasses a set of interconnected processes that contributes to decline in performance, productivity and health ultimately culminating in death with the passage of time.

Inflammation: Inflammation is fundamentally a protective response occurring in the vascularized connective tissue to any insult the ultimate goal of which is to eliminate the organism which was the cause of cell injury (such as microbes, toxins) and the consequences of such injury (such as necrotic cells and tissues).

Characteristics

A unified theory has been sought to explain how the single physiological process of aging may lead to diverse pathological events culminating in diverse aging-associated pathological conditions in different organs, such as Alzheimer's, Parkinson's and other neurodegenerative disorders, rheumatoid arthritis, atherosclerosis, macular degeneration, etc., The free radical theory of aging, as proposed by Harman, is the most plausible and currently acceptable mechanism to explain the aging process. The central premise of this theory proposes that aging and its related disease processes are the net result of free radical-induced damage and the inability to counterbalance these harmful effects by antioxidative defenses. The generation of reactive oxygen and nitrogen species (ROS and RNS) activates redox sensitive transcription factors leading to the generation of pro-inflammatory molecules and a state of chronic inflammation. On the other hand, chronic inflammation itself results in the generation of ROS and RNS thus activating a feedback loop that amplifies the process of damage and deterioration. This oxidative stress and the subsequent chronic inflammation have been implicated as a mitigating factor for almost all of aging-associated maladies.

The hallmarks of chronic inflammation, infiltration of macrophages and circulating levels of pro-inflammatory chemical mediators are observed in aging-associated diseases. Activated macrophages (microglia) are observed in the senile plaques and surrounding tissue in the brain of patients with Alzheimer's disease versus similar regions in control brains. Activated microglia are also detected in affected regions in Parkinson's disease and Amyotropic Lateral Sclerosis (ALS). Similarly, many activated macrophages are found in arterial plaques of atherosclerosis and in infracted heart tissue even years after an acute event. The presence of these activated macrophages/microglia may on one hand be beneficial, and on the other hand be harmful. While, the activated macrophages release toxic materials injurious to viable host tissues, they also have phagocytic potential and an ability to destroy invading pathogens. In a state of persistent inflammation the injurious events overwhelm the protective balance leading to chronic degeneration.

ROS and RNS generated from the activated macrophages induce oxidative stress and free radical-induced injuries are evident in AD cortex, PD substantia nigra and ALS spinal cord in the form of modification of proteins by glycation, the existence of low molecular weight compounds that have been oxidized and nitrated (such as 4-hydroxynonenal, malondialdehyde, 3-nitrotyrosine, 3-nitro-4-hydroxyphenylacetic acid, 5-nitrotocopherol and 8-hydroxy-deoxyguanosin) and peroxidation of lipids.

Additional evidence is the presence of the chemical mediators of inflammation in aging-associated diseases. The tangles and plaques of AD contain activated complement fragments C4d and C3d. The membrane attack complex (MAC) derived from the activation of the complement cascade is evident in dystrophic neuritis in AD brain and in substantia nigra in PD indicating autolytic attack. The mRNAs for complement proteins

are sharply upregulated in affected regions of AD and PD brains and also in infarcted heart tissue.

Cytokines play important roles as pro-inflammatory mediators and studies have documented increased blood level of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and IL-8 in aged individuals as compared to young individuals. Plasma levels of TNF-α were positively correlated with IL-6 and acute phase proteins such as C-reactive proteins (CRP) in 126 centenarians, indicating an interrelated activation of the entire inflammatory cascade. However, the increase in circulating inflammatory parameters is far from levels seen during acute inflammation indicating that aging is associated with a chronic low-grade inflammatory activity. In a large study of 1,727 elderly Americans aged 70 years or older, age was associated with increased circulating plasma levels of IL-6.

Polymorphisms in the promoter and untranslated regions that favor increased expression of pro-inflammatory genes, such as IL-1β, have been observed in patients with AD and PD. Inheritance of the polymorphic allele of apolipoprotein E4 (apoE4) in combination with the high-risk allele of TNF-α significantly increases the risk of AD. Similarly simultaneous inheritance of high-risk alleles for IL-1αα889 and IL-1β+3953 significantly increases the odds ratio for developing AD.

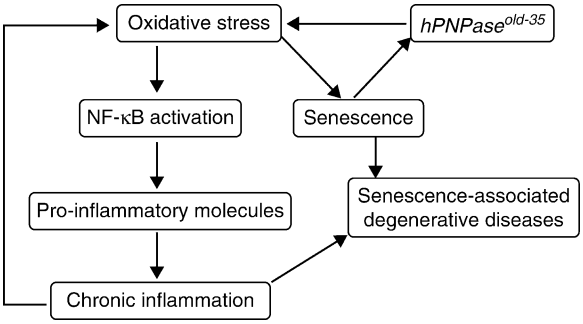
The association between increased plasma levels of TNF-α and atherosclerosis was demonstrated in 130 humans aged 81 years. The individuals with high TNF-α concentrations showed a significant clinical diagnosis of atherosclerosis. Multiple studies have established an association between elevated levels of IL-6 and diseases of old age. IL-6 induces the production of C-reactive protein (CRP), an important risk factor for myocardial infarction. High concentrations of CRP predict the risk of future cardiovascular disease in apparently healthy men. IL-8 plays a crucial role in initiating atherosclerosis by recruiting monocytes/macrophages to the vessel wall, which promotes atherosclerotic lesions and plaque vulnerability. Type 2 diabetes, atherosclerosis and cardiovascular diseases have common antecedents. High plasma TNF-α concentrations were shown to predict insulin insensitivity with advancing age in 70 healthy humans with a large age range. Elevated levels of IL-6 and CRP predicted the development of type 2 diabetes in healthy women. In another study, elevated serum IL-6 levels predicted future disability in older adults especially by inducing muscle atrophy. IL-6 and CRP also play a pathogenic role in several diseases such as osteoporosis, arthritis and congestive heart failure all of which have increasing incidence with age. Moreover, increased serum levels of IL-6 and IL-8 have been detected in patients with chronic obstructive pulmonary diseases and chemokines such as IL-8 and RANTES play important roles in the pathogenesis of these diseases. Various inflammatory mediators, such as IL-1, TNF-α, IL-6, IL-8, RANTES, MMP-3 are responsible for chronic inflammatory rheumatoid diseases, such as osteoarthritis and rheumatoid arthritis both of which occur during aging.

In vitro studies and experiments in animals also establish an intricate relationship between aging and inflammation. Gene expression analysis by microarray in human hepatic stellate cells confirms that replicative senescence in these cells is associated with a pronounced inflammatory phenotype characterized by upregulation of pro-inflammatory cytokines, including IL-6 and IL-8. An aging-induced pro-inflammatory shift in cytokine expression profile has been observed in rat coronary arteries.

How does the pro-inflammatory shift occur during aging? A prominent mechanism by which ROS modulates diverse intracellular molecular processes is by regulating the activity of transcription factors, most notably nuclear factor (NF)-κB. By turning on pro-inflammatory mediators such as TNF-α, IL-1, IL-6, IL-8, IFN-γ, iNOS, ICAM-1, VCAM-1, COX-2 and acute phase proteins, NF-κB functions as a central transcription factor for the development of chronic inflammatory diseases.

Unfortunately very few studies were carried out in aging humans to establish a clear correlation between NF-κB activation and chronic inflammation. Strong NF-κB DNA binding and COX-2 transcription was detected in aging and in sporadic AD superior temporal lobe neocortex. An increase in constitutive NF-κB DNA binding in older animals over young animals has been demonstrated in multiple studies. A gradual rise in ROS was evident in kidneys from Fischer rats from 6 to 24 months of age and this increase correlated with an age-dependent augmentation in binding of NF-κB and elevated expression of cyclogenase-2 (COX-2), an NF-κB-responsive enzyme involved in pro-inflammatory prostanoid synthesis. Vascular smooth muscle cells from 18-month old rats showed considerably higher NF-κB DNA binding than that from new-born rats which correlated with increased expression of inducible nitric oxide synthase and intracellular adhesion molecule-1, two pro-inflammatory molecules, in old smooth muscle cells upon inflammatory stimulation. A similar age-dependent elevation in NF-κB DNA binding has been reported in mouse and rat liver and heart and in rat brain indicating a potential involvement of NF-κB in regulating aging-associated chronic inflammation.

The molecular events leading to the generation of ROS and the development of chronic inflammation during aging are still not deciphered. Recent studies show that human polynucleotide phosphorylase ($hPNPase^{old\text{-}35}$) might be the key element linking aging with the inflammatory process. $hPNPase^{old\text{-}35}$ is a 3′-5′ exoribonuclease involved in mRNA degradation (Fig. 1). Its expression is induced during senescence



Aging-Associated Inflammation. Figure 1 Schematic representation flowchart showing the proposed involvement of *hPNPase*$^{old-35}$ in senescence associated degenerative diseases. See text for details.

and ectopic overexpression of *hPNPase*$^{old-35}$ induces a senescent phenotype in normal and cancer cells. Overexpression of *hPNPase*$^{old-35}$ generates ROS with resultant increase in NF-κB DNA binding and increased production of proinflammatory cytokines such as IL-6, IL-8, RANTES and MMP-3. These effects might be inhibited by an anti-oxidant *N*-acetyl-L-cysteine (NAC).

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Agnogenic Myeloid Metaplasia

►Primary Myelofibrosis

Agranulocytosis

►Neutropenia

AHNP

Definition

Is a small designer molecule with a structure that allows it to bind to p185 ►HER/neu. Is functionally similar to the anti-p185HER2/neu monoclonal antibody ►Herceptin (synonym ►Trastuzumab). The AHNP mimetic specifically binds to p185HER2/neu with high affinity with the result of inhibition of proliferation of p185HER2/neu-overexpressing tumor cells inhibition of both colony formation in vitro and growth of p185HER2/neu-expressing tumors in ►nude mice. In addition, the mimetic sensitizes the tumor cells to ►apoptosis when used in conjunction with ►ionizing radiation therapy or ►chemotherapy. AHNP mimetic has a biological efficiency spectrum that is similar to that of Herceptin. One of the advantages of this antibody-mimic is that it can be synthesized in vitro in large quantities.

►Anti-HER2/Neu Peptide Mimetic

AHR

Definition

Aryl Hydrocarbon Receptor.

AI

Definition

Androgen-Independent.

►Cyclin G-Associated Kinase

AIB1

►Amplified in Breast Cancer 1

AIE2

►Aurora Kinases

AIF

Definition
Apoptosis-inducing factor.

►Photodynamic Therapy

AIK

►Aurora Kinases

AIK2

►Aurora Kinases

AIK3

►Aurora Kinases

AIM1

►Aurora Kinases

AIM-1

►Aurora Kinases

AIPC

Definition
Androgen-independent prostate cancer.

AIs

Definition
►Aromatase inhibitors.

►Adjuvant Chemoendocrine Therapy

AIT

Definition
Adoptive Immunotherapy.

AJCC

Definition
American Joint Committee on Cancer.

Ajuba

Definition
A member of the zyxin family of cytoskeletal adaptor proteins. It localizes in the cytosol, at cell-substratum and cadherin-mediated cell-cell junctions and shuttles to the nucleus under certain conditions. Ajuba contains three several copies of a LIM domain at its carboxyterminus and a proline-rich pre-LIM region. Interaction partners include Grb2, GLT-1, α-catenin, F-actin, Aurora A, p130Cas, PIPKIα, atypical protein kinase C scaffold protein p62, TRAF6, PKCζ, LATS2, and the SNAG domain. Ajuba plays a role in cell proliferation

and cell migration, and is a negative regulator of the Wnt signaling pathway.

►Lipoma Preferred Partner

AKT

Definition

Akt is the mammalian homologue of retroviral oncogene v-akt encoding a serine/threonine protein kinase, also termed protein kinase B (PKB). It is a serine/threonine protein kinase, the cellular homolog of the viral transforming oncogene v-Akt. The c-Akt protein contains a ►pleckstrin homology domain and a catalytic kinase domain. c-Akt is activated by translocation to the cell membrane mediated by its lipid-binding pleckstrin homology domain and enzymatic activation by specific phosphorylation events. These phosphorylations are mediated by protein kinases that are themselves activated by phosphatidylinositol-3′-kinase. Scatter factor-induced activation of c-Akt results in a cell survival signal that renders cells resistant to apoptosis.

►Scatter Factor
►Autophagy
►APAF-1 Signaling
►Major Vault Protein
►p21(Waf1/Cip1/Sdi1)
►Transduction of Oncogenes
►Fragile Histidine Triad

Akt Signal Transduction Pathway

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Synonyms

Protein kinase B

Definition

Akt, also called protein kinase B, represents a serine/threonine protein kinase subfamily. Three members of this family have been cloned to date, namely, AKT1/PKBα, AKT2/PKBβ, and AKT3/PKBγ. The overall homology of between these three isoforms is >85% at amino acid level.

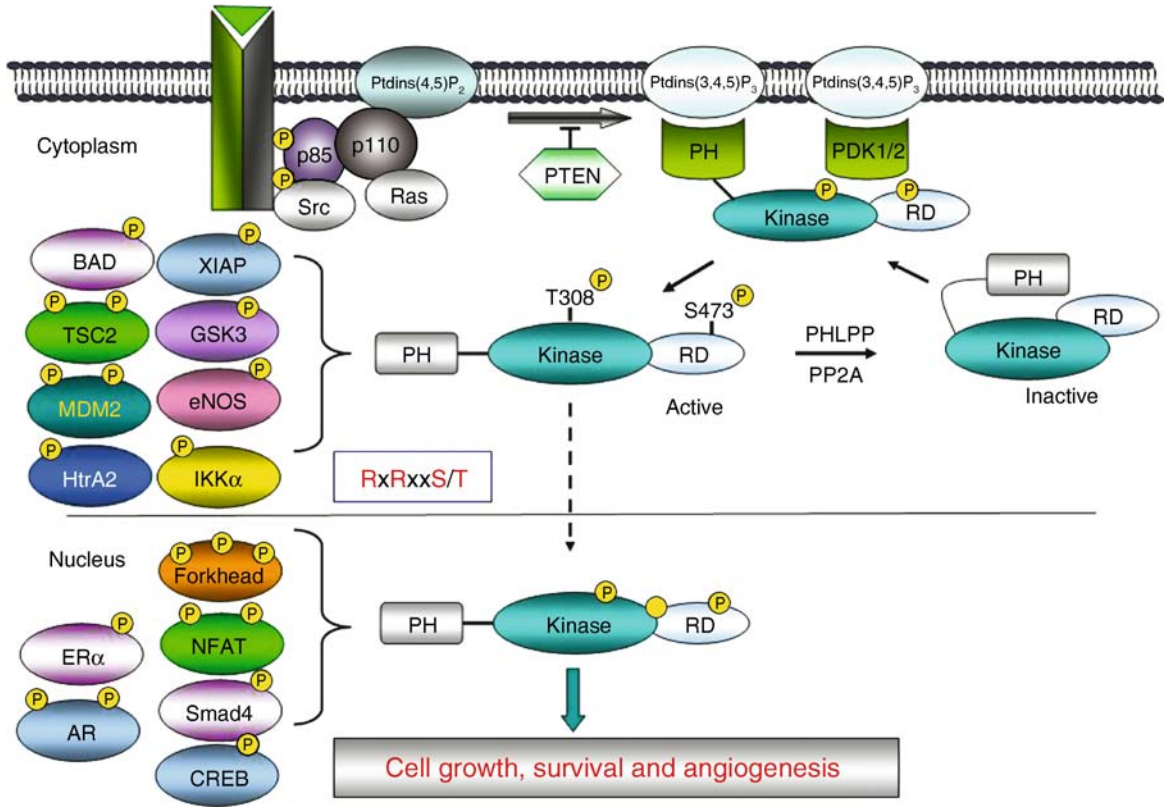
Characteristics

AKT1, *AKT2*, and *AKT3* share a very similar structure, which contains an N-terminal pleckstrin-homology (PH) domain, a central kinase domain, and a serine/threonine-rich C-terminal region (Fig. 1). The PH domain and C-terminal region between these three isoforms are more diverse (homology 73–84% at amino acid level) as compared to the kinase domain (90–95%), suggesting that PH and C-terminal regions may represent functional difference between AKT1, AKT2 and AKT3. All three members of Akt localize to the cytoplasm, however, they could translocate to the nucleus upon activation. In addition, they are located on different human chromosome (AKT1 on 14q32, AKT2 on 19q13.1–13.2, and AKT3 on 1q44).

Akt in Human Malignancy and Different Function of Akt Family Members

Although *AKT1*, *AKT2* and *AKT3* display high sequence homology, there are clear differences between these three members in terms of biological and physiological function: (i) *AKT1* expression is relatively uniform in various normal organs whereas high levels of *AKT2* and *AKT3* mRNA are detected in skeletal muscle, heart, placenta and brain; and (ii) overexpression of wild type AKT2, but not AKT1 and AKT3, transforms NIH 3T3 cells. Amplification of the *AKT2* has been observed in 15% of human ovarian carcinomas and 20% of human pancreatic cancers. Infrequent mutations of the Ak have been also detected in human cancer. However, activation of Akt kinase due to alterations of its upstream molecules occurs up to 50% of all human tumors, and thus Akt is a critical target for anti-cancer drug discovery.

Recently, increasing evidence suggests that AKT members have different cellular functions. Of note, knockout of individual AKT member resulted in distinct phenotypes. *AKT1*-deficient mice exhibited a uniform reduction in organ size, while *AKT2*-null mice develop typical type II diabetes, and *AKT3*-deficient mice displayed a selective impairment of brain development. Moreover, although *AKT1*- and *AKT3*-deficient brains are reduced in size to comparable degree, the absence of AKT1 reduces neuronal cell number, whereas the lack of AKT3 results in smaller and fewer cells. In tumor biology and invasion process, overexpression of only AKT2, not AKT1 or AKT3, recapitulated the invasive effects of PI3K in breast cancer cells. Additionally, only the expression of dominant negative AKT2, not its counterparts, inhibited invasion induced by either activation of PI3K or overexpression of Her2/Neu. These observations suggest that AKT1 and 3 may be acting more in a cellular growth and survival role, while AKT2



Akt Signal Transduction Pathway. Figure 1 Proposed pathways of Akt signaling. Activation of Akt through the PI3-kinase pathway involves recruitment of the Akt to the cell membrane by means of PH domain binding to product of PI3-kinase: PI(3,4,5)P3, promoting a conformational change in Akt which results in phosphorylation of Thr^{308} and Ser^{473} by PDK1 and "PDK2," respectively. Upon its release from the membrane, Akt would become available to phosphorylate a number of molecules to induce cell growth, survival and angiogenesis (KD = Kinase domain, RD = regulatory domain).

may be more involved in regulating cellular metabolism, mobility, invasion and metastasis.

Signal Transduction

Akt is activated by a variety of stimuli, including growth factors, protein phosphatase inhibitors, and cellular stress in a PI3-kinase dependent manner. Activation of Akt depends on the integrity of the pleckstrin homology (PH) domain, which mediates its membrane translocation, and on the phosphorylation of Thr^{308} and Ser^{473}. Phosphoinositides, PtdIns-3,4-P2 and PtdIns-3,4,5-P3, produced by PI3K bind directly to the PH domain of Akt, driving a conformational change in the molecule, which enables the activation loop of Akt to be phosphorylated by PDK1 at Thr^{308}. Full activation of AKT1 is also associated with phosphorylation of Ser^{473} within a C-terminal hydrophobic motif characteristic of kinases in the AGC kinase family. Although the role of PDK1 in Thr^{308} phosphorylation is well established, the mechanism of Ser^{473} phosphorylation is controversial (Fig. 1). A number of candidate enzymes responsible for this modification have been put forward, including ILK, DNA-dependent kinase, and the rictor-mTOR complex. The activity of Akt is negatively regulated by tumor suppressor PTEN, which is frequently mutated in human malignancy. *PTEN* encodes a dual-specificity protein and lipid phosphatase that reduces intracellular levels of PtdIns-3,4,5-P3 by converting them to PtdIns-4,5-P2, thereby inhibiting the PI3K/Akt pathway (Fig. 1). In addition, recent studies have identified PHLPP phosphatase dephosphorylation of the Ser^{473} leading to inactivation of Akt. Akt phosphorylates and/or interacts with a number of molecules to exert its normal cellular functions, which include roles in cell proliferation, survival, angiogenesis and differentiation. A couple dozens of molecules have been identified to be downstream targets of Akt, including TSC2, XIAP, Bad, FOXO, IKKα, ASK and EZH2. The vast majority of Akt substrates contain Akt phosphorylation consensus sequence **RXRXXS/T** (R is arginine; S/T is serine/threonine).

Akt Pathway as a Target for Cancer Intervention

Since Akt functions as a cardinal nodal point for transducing extracellular (growth factor and insulin)

and intracellular (receptor tyrosine kinases, Ras and Src) oncogenic signals, it presents an exciting new target for molecular therapeutics. Lipid-based inhibitors of Akt were the first to be developed, including perifosine, PX-316 and phosphatidylinositol ether lipid analogues, which were designed to interact with the PH domain of Akt. In addition, several Akt antagonists have been identified using high-throughput screening of chemical libraries and rational design. These inhibitors include Akt/PKB signaling inhibitor-2 (API-2), 9-methoxy-2-methylellipticinium acetate, the indazole-pyridine A-443654, and isoform-specific canthine alkaloid analogues. Following its identification in a screen of the NCI diversity set, API-2 was shown to inhibit Akt kinase activity and stimulate apoptosis of xenografts of human cancer cells exhibiting high Akt activity. API-2 is a tricyclic nucleoside that previously showed antitumor activity in phase I and phase II trials conducted, but multiple toxicities, including hepatotoxicity, hyperglycemia, thrombocytopenia, and hypertriglyceridemia, precluded further development. The recent identification of Akt inhibition as a mechanism underlying API-2 activity has provided new interest in studying this drug and raises the possibility that lower doses may inhibit Akt and induce tumor cell apoptosis without the previously associated side effects.

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ALA

Definition

δ-Aminolevulinic Acid

►Photodynamic Therapy

5-ALA

►5-Aminolevulinic Acid
►Hypericin

ALAD

Definition

Delta-Aminolevulinic Acid Dehydratase

►Lead Exposure

Alcohol Consumption

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Definition

Alcohol is a widely used stimulant, toxin and nutrient, depending on doses and drinking pattern. Its chronic abuse damages almost all cells in the human body and results in organ injury, including the development of certain cancers.

Characteristics

Alcohol is responsible for 390,000 cancer cases worldwide, representing 3.6% of all cancers (5.2% in men and 1.7% in women). In February 2007 the International Agency for Research on Cancer (IARC) invited 26 scientists from 15 countries to evaluate the evidence for ethanol and ethanol-containing beverages as a cancer causing agent. The experts reviewed all epidemiological and experimental studies covering this topic and came finally to the following conclusion:

"Regular alcohol consumption is associated with an increased risk for cancer of the oral cavity, pharynx, larynx, esophagus, liver, breast and colorectum. There is substantial mechanistic evidence in humans deficient in aldehyde dehydrogenase that ►acetaldehyde derived from the metabolism of ethanol contributes to causing maligant esophageal tumors.

The studies demonstrate that ethanol and not the type of alcoholic beverage is responsible for the tumor risk."

Epidemiology

Cancer of the Upper Aerodigestive Tract

A large number of prospective and case-control studies have shown that the risk for Upper Aerodigestive Tract (UADT) cancer is significantly dose-dependent, increased two- to threefold at a daily consumption of 50 g of ethanol or more. Smoking has an additionally synergistic effect. A carefully performed French study showed an 18-fold increased risk for esophageal cancer when 80 g of ethanol were consumed daily. Twenty cigarettes per day increased cancer risk by a factor of 5. However, drinking and smoking were associated with 44-fold increased cancer risk. Other factors which increase the ►alcohol mediated cancer risk are oral bacterial overgrowth (poor oral hygiene and dental status) as well as ►gastroesophageal reflux disease (GERD).

Hepatocellular Cancer (HCC)

HCC develops in 1–2% per year of patients with alcoholic liver ►cirrhosis of the liver every year. The risk for HCC is between 4.4- and 7.3-fold at an alcohol dose of 80 g/day. HCC in a non-cirrhotic liver is extremely rare. Chronic alcohol consumption also increases HCC risk in patients with other liver diseases such as chronic hepatitis B and C, hereditary hemochromatosis and non-alcoholic fatty liver disease (NAFLD). Patients with chronic ►hepatitis C have a threefold increased risk when they consume 80 g of ethanol or more as compared to hepatitis C alone. In hepatitis B patients ethanol in doses of 40 g or more shortens the development of a HCC by approximately 10 years.

Breast Cancer

A clear cut dose-dependent association between alcohol intake and breast cancer has been reported in more than 100 publications. The risk starts at a dose of 18 g of alcohol per day. According to a meta analysis of 38 studies, one, two or three drinks increase breast cancer risk by 10, 20 and 40%. Every additional 10 g of alcohol increase breast cancer risk by 7%. At 50 g of alcohol daily, cancer risk is enhanced by 50%. In the United States it has been calculated that 4% of all newly diagnosed breast cancer cases are due to alcohol, resulting in a total of approximately 8,000 cases per year.

Colorectal Cancer

More than 50 prospective- and case-control studies found a positive association between colorectal cancer and alcohol consumption. According to pooled data from eight cohort studies and data from a recent meta analysis, a 1.4-fold increased cancer risk was found in patients with an alcohol intake of more than 50 g as compared to non-drinkers. Excessive alcohol consumption also favors high risk polyp or colorectal cancer occurrence among patients with adenomas. Five out of six studies also showed an increased risk for colorectal polyps following chronic alcohol consumption as compared to abstinence. Epidemiologic studies also underline the importance of the lack of dietary factors such as methionine and folate which modulate the ethanol-associated colorectal cancer risk.

Mechanisms of Alcohol Mediated Carcinogenesis

Acetaldehyde

Acetaldehyde is the first metabolite of ethanol oxidation. Acetaldehyde binds to proteins and DNA; it has been found to be mutagenic and carcinogenic in animal experiments. The most convincing evidence for the role of acetaldehyde as cancer causing agent comes from genetic linkage studies in populations who accumulate acetaldehyde following alcohol consumption. Fifty percent of Japanese have a mutation of the ►acetaldehydehydrogenase (ALDH)2 gene which codes for an ALDH enzyme with low activity. When these individuals drink alcohol, acetaldehyde accumulates in the blood, and they develop a flush syndrome with tachycardia, nausea and vomiting. In addition, acetaldehyde also accumulates in the saliva, rinses the mucosa of the upper aerodigestive tract, and may enter the mucosal cells, resulting in DNA adduct formation. Homocygotes of the polymorphism for ADH1B and ADH1C. ALDH2 2/2 Ten percent of the Japanese population, who have zero ALDH activity, are incapable of consuming alcohol, even in small doses. Despite the unpleasant side effects of flushing, however, heterocygotes of the ALDH2 2/1, 40% of the Japanese population with low ALDH activity, may consume alcohol. These individuals have a significant increased cancer risk for upper aerodigestive tract cancer, in particular esophageal cancer and for colorectal cancer. This gene mutation does not exist in Caucasians. However, Caucasians have a gene polymorphism for the ADH1B and ADH1C gene. While the ADH1B*2 allele encodes for an ADH enzyme with a 40-fold increased acetaldehyde production as compared to the ADH1B*1 allele, the ADH1C*1 allele encodes for an enzyme with a 2.5-fold increased ADH activity as compared to the ADH1C*2 allele. Thus, heavy drinkers who are homozygous for the ADH1C*1 allele not only have an increased concentration of acetaldehyde in their saliva, but also seem to have an increased risk for upper aerodigestive tract cancer.

Considerable amounts of acetaldehyde can also be produced from ethanol by microorganisms in the oral cavity and in the colon. Therefore, poor oral hygiene leading to bacterial overgrowth is a risk factor in the alcoholic for cancer of the oral cavity.

Oxidative Stress

►Reactive oxygene species (ROS) are generated during the oxidation of ethanol via ►cytochrome P-4502E1, and during intramitochondrial reoxidation of NADH generated by ethanol oxidation through ►alcoholdehydrogenase. This is especially relevant in the liver. ROS cause lipiperoxidation, and lipidperoxidation products such as 4-hydroxynonenal can bind to DNA, forming exocyclic DNA-etheno adducts with mutagenic and carcinogenic properties.

Under normal conditions ROS are neutralized by the anti-oxidative defense system, which, however, is severely altered by chronic ethanol consumption.

Altered Methyl Transfer

Chronic ethanol consumption results in a significant reduction of S-adenosyl-methionine (SAMe), the active methyl donor. This is due to multiple effects of ethanol and acetaldehyde on enzymatic reactions leading to the generation of SAMe, including folate deficiency. The lack of SAMe results in a reduction of all methylation processes. With respect to ►carcinogenesis the most important methylation process is the methylation of cytosine bases within the DNA. This DNA hypomethylation results in a dimished silencing of oncogenes and therefore favors carcinogenesis.

Reduced Retinoic Acid

Chronic ethanol consumption results in a decrease of retinol and ►retinoic acid (RA) in the liver, associated with an activation of the AP-1 gene resulting in an increased expression of c-jun and c-fos and finally hepatocellular hyperproliferation associated with increased cancer risk. The decrease of RA is predominantly due to the ethanol-mediated induction of CYP2E1, since CYP2E1 is also responsible for the metabolism of RA and retinol. An enhanced metabolism of RA and retinol induced by CYP2E1, results in the generation of metabolites with apoptotic properties. In this context it is important to note that the concomitant administration of ß-carotin for the prevention of bronchial cancer and the use of alcohol in a dose of more than 12 g/day increases, instead of decreasing, the risk of bronchial carcinomas in smokers.

Specific Mechanisms (Cirrhosis, Gastroesophageal Reflux Disease, Estrogens)

In the liver, cirrhosis caused by chronic ethanol consumption is a prerequisite for the development for a HCC due to mechanisms not clearly understood, but predominantly due to chronic inflammation with inflammation-driven oxidative stress and proliferative changes during the development of cirrhosis. HCC in a non-cirrhotic alcoholic liver is extremely rare.

Gastroesophageal reflux disease (GERD) is an additional factor, which favors carcinogenesis in the esophagus due to acid-mediated chronic inflammation of the esophageal mucosa. GERD is favored by alcohol, since alcohol decreases the tonus of the lower esophageal sphincter which facilitates GERD.

Increased ►estrogens levels due to alcohol consumption, even in small quantities, is most likely an important pathophysiologic factor to explain the increased risk of breast cancer in regular drinkers. The mechanism by which alcohol increases estradiol levels is not known.

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Alcohol Dehydrogenase (ADH)

Definition

Enzyme expressed in hepatocytes capable of metabolizing/oxidizing ethanol to acetaldehyde.

►Hepatic Ethanol Metabolism
►Alcohol Consumption

Alcohol Mediated Cancer

Definition

Chronic alcohol consumption results in an increased cancer risk for the upper aerodigestive tract (oral cavitiy, pharynx, larynx, esophagus) for the liver (hepatocellular cancer (HCC)), for the breast and for the large intestine.

►Alcohol Consumption

Alcoholic Beverages Cancer Epidemiology

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Definition

Drinking beverages that contain ethanol such as wine, beer, or hard liquors. Other alcoholic beverage types are specific to certain geographic regions or countries such as rice wine in East Asia or arrack in India. In some cultures, alcoholic beverages are also made locally or in the home.

Characteristics

A causal link has been established between alcohol drinking and cancers of the oral cavity, pharynx, esophagus, liver and breast. For other cancers, a causal association is suspected. The importance of alcohol as a human carcinogen is often underestimated. There is increasing evidence of an important role of genetic susceptibility to alcohol-related cancer, and knowledge on possible mechanisms of the carcinogenic action of alcohol has evolved in recent years.

The major non-neoplastic diseases caused by alcohol drinking are alcoholic polyneuropathy, alcoholic cardiomyopathy, alcoholic gastritis, depression and other mental disorders, hypertension, hemorragic stroke, liver cirrhosis and fibrosis, as well as acute and chronic pancreatitis. In addition, alcohol drinking is a major cause of several types of injuries, and alcohol consumption during pregnancy is associated with various adverse effects including fetal alcohol syndrome, spontaneous abortion, low birth weight, prematurity, and intrauterine growth retardation. On the other hand, there is strong evidence that moderate consumption of alcohol reduces the risk of ischemic heart disease, ischemic stroke, and colelithiasis.

Epidemiology of Alcohol-Related Cancer

A causal relationship between elevated alcohol drinking and ►oral squamous cell carcinoma, and that of pharynx, larynx and esophagus have been demonstrated. In epidemiological studies of this group of tumors, an effect of heavy alcohol intake and a linear relationship with both duration and amount of drinking have been consistently shown. A synergism between alcohol drinking and tobacco smoking has been demonstrated, and has become since a paradigm of interaction of two environmental factors in human carcinogenesis.

Studies on the association of alcohol drinking and adenocarcinoma of the esophagus have not been consistent. Some studies reported risk estimates for adenocarcinoma of the esophagus and gastric cardia together on the order of 1.5- to 4-fold increases in risk. Many of the studies that have reported risk estimates for adenocarcinoma of the esophagus have tended to be small, while the larger studies have reported no association with ever alcohol consumption and no indication of dose-response relations.

Heavy alcohol intake increases the risk of ►hepatocellular carcinoma. Dose-response relations between the amount of alcohol consumed and the risk of hepatocellular carcinoma have been demonstrated. The most likely mechanism of alcohol-related liver carcinogenicity is through development of liver cirrhosis, although alternative mechanisms such as alteration in the hepatic metabolism of carcinogens may also play a role. Alcoholic liver cirrhosis is probably the most important risk factor for hepatocellular carcinoma in populations with low prevalence of ►HBV and ►HCV infection, such as North America and northern Europe. Synergistic interactions on the risk of liver cancer are also thought to occur between tobacco and alcohol, and between HBV/HCV and alcohol. (Hepatitis Virus-Associated Hepatocellular carcinoma).

The association between alcohol consumption and the risk of breast cancer has been reported fairly consistently in numerous studies, though the risk is thought to be moderate. The association is observed among both premenopausal and postmenopausal women, though it is unclear whether the period of life in which drinking occurs modifies the carcinogenic effect of alcohol. Although the magnitude of the excess risk of breast cancer due to alcohol drinking is not very large, the high incidence of this cancer results in a large number of cases.

Several studies have provided evidence, although not fully consistent, of an association between elevated intake of alcohol, and increased risk of colorectal adenoma and adenocarcinoma. Dietary factors such as low folate intake are thought to increase the risk of colorectal cancer by 2- to 5-fold, and alcohol adversely affects folate metabolism. There may be a synergistic interaction between alcohol consumption and low folate intake, or alcohol may be acting through folate metabolism to increase colorectal cancer risk. Since risk estimates reported suggest a moderate association between alcohol drinking and the risk of colorectal cancer, residual confounding by such dietary factors or other strong risk factors for colorectal cancer is of concern. However, it is doubtful that residual confounding is entirely responsible for the observed increases in colorectal cancer risk due to alcohol consumption. Though the effects may be moderate, there does appear to be a causal relationship between alcohol consumption and colorectal cancer risk.

There is no consistent evidence that alcohol drinking influences the risk of cancers of the stomach, pancreas, lung, endometrium, bladder or prostate. In the

case of ovarian and kidney cancers, the evidence from epidemiological studies is of a possible protective effect, but further investigation is necessary to clarify the relationships. The risk of non-Hodgkin lymphoma was reported to be reduced among alcohol drinkers: this effect, if real, might differ by lymphoma type, which may explain the inconsistencies in results of earlier studies of alcohol and lymphoma.

Mechanisms of Alcohol Carcinogenicity

The mechanisms by which alcoholic beverages exert their carcinogenic effect are not fully understood, and, as in the case of other multi-site carcinogen, they are likely to differ by target organ. Table 1 lists the main mechanistic hypotheses, together with a subjective assessment of the strength of the available supporting evidence. The table is restricted to mechanisms known or suspected to operate in cancers with an established association with alcohol drinking.

Ethanol in its pure form does not act as a carcinogen in experimental models, and one explanation is that alcoholic beverages act as a solvent for penetration of carcinogens through the mucosa of upper aero-digestive organs. Although this mechanism would explain the synergistic effect of tobacco smoking and alcohol drinking, it would not account for the increased risk observed among never smokers.

The primary metabolite of ethanol, acetaldehyde, is a plausible candidate for the carcinogenic effect of alcoholic beverages although direct evidence linking acetaldehyde as a cause of cancer in humans is lacking. Acetaldehyde forms ►adducts to DNA in human cells in vitro, as well as in rats chronically exposed to ethanol. In experimental models, acetaldehyde inhalation has been shown to cause tumors of the respiratory tract, particularly adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters. It also damages hepatocytes, leading to increased proliferation. Autoantibodies against acetaldehyde-modified proteins have been detected in blood and bone marrow of alcohol abusers. Overall, studies strongly suggest that DNA damage occurs in humans following heavy alcohol consumption, and acetaldehyde can be responsible for it. The increasing evidence of a role of polymorphism in enzymes implicated in the oxidation of ethanol and acetaldehyde in modulating alcohol-related cancer risk further supports the hypothesis of a mechanistic role of acetaldehyde.

Production of ►reactive oxygen species and nitrogen species is an additional possible mechanism of alcohol-related carcinogenesis. ►Oxidative stress leads to ►lipid peroxidation, whose products are reactive electrophilic compounds reacting with DNA to form exocyclic DNA adducts, and reactive aldehydes. This mechanism can be particularly relevant to liver carcinogenesis and might explain the synergistic effect of alcohol and viral infection. In the liver, oxidative stress is induced by alcohol via induction of CYP2E1, stimulation of parenchymal cells in response to cytokines and activation of Kupffer cells.

Heavy alcohol intake may lead to nutritional deficiencies by reducing the intake of foods rich in micronutrients, by impairing intestinal absorption, and by altering metabolic pathways. The most relevant effect appears to be on folate metabolism, resulting in alteration in DNA ►methylation and, hence, control of genes potentially involved in carcinogenesis. Intake, absorption and metabolism of vitamin B12 and vitamin B6, may also be affected by alcohol intake, resulting in further alterations of DNA methylation pathways. Vitamin A deficiency has also been proposed as alcohol-mediated carcinogenic mechanism. Alcoholics have a lower level of serum vitamin A and β-carotene, and vitamin A metabolism is altered by chronic alcohol intake.

Alcoholic Beverages Cancer Epidemiology. Table 1 Possible mechanisms of carcinogenicity of alcoholic beverages

Mechanism	Potential Target Organs
Strong evidence[a]	
DNA damage by acetaldehyde	Head and neck, esophagus, liver
Increased estrogen level	Breast
Moderate evidence[a]	
Solvent for other carcinogens	Head and neck, esophagus
Production of reactive oxygen and nitrogen species	Liver, others?
Alteration of folate metabolism	Colon and rectum, breast, others?
Weak evidence[a]	
DNA damage by ethanol	Head and neck, esophagus, liver
Nutritional deficiencies (e.g. vitamin A)	Head and neck, others?
Reduced immune surveillance	Liver, others?
Carcinogenicity of constituents other than ethanol	Head and neck, esophagus, liver, others?

[a]Subjective assessment of strength of supportive evidence.

Alcohol drinking can reduce immune surveillance, thus favoring cancer development as well as metastatic potential. This hypothesis is supported by experimental data showing reduced resistance to metastasis of alcohol-exposed mice.

Components of alcoholic beverages other than ethanol, including impurities and contaminants, have been proposed to increased risk of cancer among drinkers. ►Polycyclic aromatic hydrocarbons have been found in dark hard liquors, and N-nitrosoamines have been detected in beers, but in general information on composition of alcoholic beverages, and in particular hard liquours, is limited. If components in alcoholic beverages represented an important factor contributing to carcinogenicity, one would predict a role of type of beverage in determining the risk.

These mechanisms are mainly relevant to head and neck, liver and colorectal carcinogenesis; in the case of breast cancer, the main hypothesis to explain alcohol carcinogenicity is increased ►estrogen level. The evidence is strongest for postmenopausal women using ►hormone replacement therapy, but the available data suggest an effect also in other groups of women. Additional possible mechanisms include increased susceptibility to endogenous and exogenous carcinogens, and greater invasiveness potential. An effect mediated by folate metabolism, mentioned above for colorectal cancer, would be also relevant to breast carcinogenesis.

Conclusions

Alcohol drinking is one of the most important known causes of human cancer, second only to tobacco smoking, chronic infections, and possibly overweight/obesity (obesity and cancer risk). With the exception of ►aflatoxin, for no single dietary factor there is such a strong and consistent evidence of carcinogenicity. In the case of breast and colorectal cancer, two major human neoplasms, a causal association with alcohol drinking has been established only recently, and the public health implications of these associations have not been not fully elucidated. In many countries, people of lower socioeconomic status or education consume more alcohol, which contributes to social inequalities in cancer burden.

Given the linear dose-response relationship between intake of alcohol drinking and the risk of cancer, control of heavy drinking remains the main target for cancer control. For example, the most recent version of the European Code Against Cancer recommends keeping daily consumption within two drinks (about 20–30 g alcohol) for men and one drink for women. Total avoidance of alcohol, although optimal for cancer control, cannot be recommended from a broader public health perspective, in particular in countries with high incidence of cardiovascular diseases.

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ALD

Definition

Alcoholic liver disease.

►Hepatic Ethanol Metabolism
►Alcohol Consumption

Aldehyde Dehydrogenases

Definition

A group of NAD (P)-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding acids. Seventeen forms exist in humans and they are present in all tissues. Several of these forms are important in detoxification of anticancer drugs.

►Alkylating Agents
►Detoxification
►Hepatic Ethanol Metabolism

Aldehydes

Definition

An organic molecule containing a -COH group; reactive aldehydes originate from lipid peroxidation of cellular membranes.

►Inflammation

Aldo-Keto Reductase

Definition

A superfamily of enzymes that catalyze the NADPH-dependent reduction of numerous carbonyl-containing compounds; substrates include, not only xenobiotics, but also monosaccharides, prostaglandins and steroid hormones. Some isoenzymes may also catalyze the oxidation of alcohols to aldehydes and ketones; detoxication.

►Detoxification
►Reductases

Aldose Reductase

Definition

It is the first and rate-limiting enzyme of polyol pathway of glucose metabolism that converts glucose to sorbitol with the help of cofactor, reduced nicotinamide adenine dinucleotide phosphate (NADPH). It is a monomeric, cytoplasmic enzyme with broad substrate specificity. This enzyme efficiently reduces oxidative stress-induced lipid aldehydes and their glutathione conjugates, steroids, and phospholipids. Newly discovered function of aldose reductase is to work as an excellent nonsteroidal anti-inflammatory agent. Inhibition of this enzyme has been shown to prevent inflammatory response including the progression of human colon cancer cells in nude mice xenografts. Since this enzyme is known to be involved in the pathophysiology of secondary diabetic complications, its inhibitors are being used for these disorders in Japan and are in phase-III clinical trials in the United States.

►Nonsteroidal anti-inflammatory drugs
►Inflammation

Alemtuzumab

Definition

A recombinant DNA-derived humanized monoclonal antibody directed against the cell surface glycoprotein CD52. Alemtuzumab is an IgG1 kappa with human variable framework and constant regions, and complementarity-determining regions derived from a rat monoclonal antibody. This agent selectively binds to CD52, thereby triggering a host immune response that results in lysis of CD52 + cells. CD52 is a glycoprotein expressed on the surface of essentially all normal and malignant B cells and T cells, a majority of monocytes, macrophages and natural killer cells (NK cells).

►Chronic Lymphocytic Leukemia

ALK

Definition

Anaplastic lymphoma kinase; a cell surface receptor protein with enzymatic activity (phosphorylation) originating from the alk gene.

►ALK Protein
►Anaplastic Large Cell Lymphoma

ALK Protein

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Synonyms

ALK; Anaplastic lymphoma kinase; Anaplastic lymphoma kinase; Ki-1; CD246

Definition

Anaplastic lymphoma kinase (ALK) is a ►receptor tyrosine kinase with a potential role in early neural and muscle development. ALK phosphorylates intracellular molecules for the transduction of signals from the exterior of the cell to the nucleus. Aberrant expression of full-length ALK receptor proteins has been reported in ►neuroblastoma and ►glioblastoma while the presence of ALK fusion proteins in ►anaplastic large cell lymphoma has resulted in the identification of the tumor entity ALK-positive anaplastic large cell lymphoma. ALK is a rare example of a receptor tyrosine kinase that is expressed in both hematopoietic and non-hematopoietic tumors.

Characteristics

ALK Protein – Structure, Distribution and Function in Normal Tissues

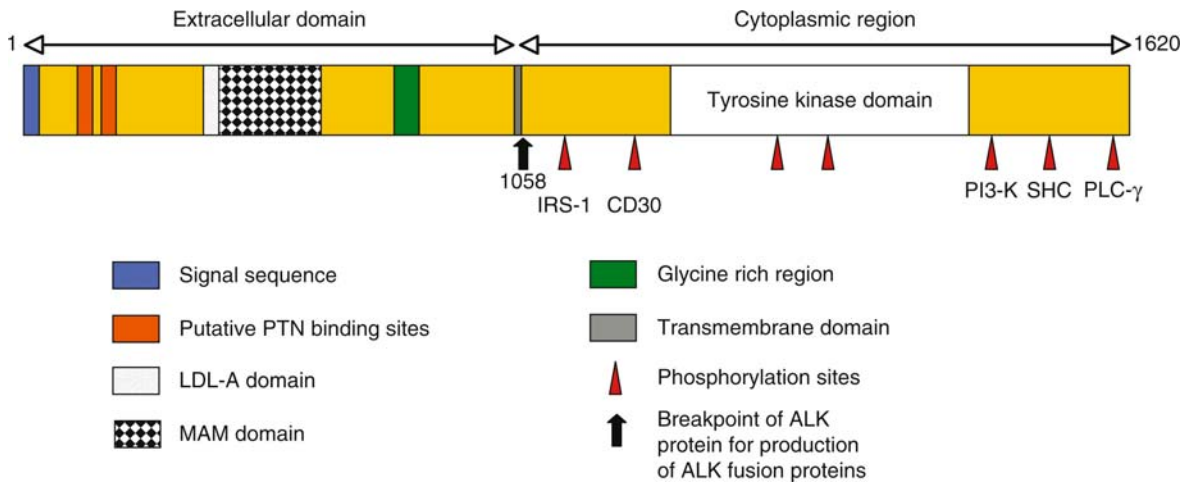
The *anaplastic lymphoma kinase (ALK)* gene (HUGO approved name anaplastic lymphoma kinase (Ki-1)) was originally identified on chromosome 2 at position p23 in the t(2;5)(p23;q35) translocation associated with anaplastic large cell lymphoma. The ALK protein product is a 200 kD ►receptor tyrosine kinase protein and a member of the ►insulin receptor superfamily bearing significant homology to ►leucocyte tyrosine kinase (LTK), Other members of the insulin receptor subfamily include: ►insulin growth-1 receptor (IGF-1R), TRK neurotrophin receptors, MET and cFOS. ALK is a highly conserved single-chain transmembrane protein of 1,620 aminoacids in the human (Fig. 1), 1,621 aminoacids in the mouse and 1,701 aminoacids in Drosophila. The ALK protein was given the designation of CD246 at the VIIth Leucocyte Typing Workshop. Full details on the ALK can be obtained from the following websites: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = gene&cmd = Retrieve&dopt = Graphics&list_ uids = 238 at and http://symatlas.gnf.org/SymAtlas/.

Initial studies described the presence of *ALK* mRNA in human fetal liver, brain, testis, placenta and the enteric innervation. Subsequent studies also identified *Alk* mRNA in the central and peripheral nervous system, as well as in testis, ovary and midgut of fetal rats and mice while *DAlk* was detected in the brain, ventral nerve and gut musculature of Drosophila during embryonic development. The expression of both *Alk* mRNA and Alk protein decreased rapidly in rodent neonates while ALK protein was detected in only rare scattered cells in the brain in humans. Both of these findings are suggestive of a role for ALK in early neural development. The use of DAlk mutants also provided evidence for its role in the development of the ventral mesoderm in Drosophila.

The ligand(s) for full length ALK are at present, still unknown. Possible candidates include a neurotrophic factor and the two heparin-binding growth factors ►pleiotrophin and ►midkine. *In vivo* experiments using flies expressing loss-of-function mutant *DAlk* genes have identified ►Jelly belly protein (Jeb) as a ligand for DAlk in Drosophila. The expression of both Jeb and dAlk proteins were essential for activation of the ►RAS/MAP kinase pathway in visceral gut muscle development. In common with other receptor tyrosine kinases, binding of ligand to the extracellular receptor of ALK results in dimerization of the ALK proteins with permitting the subsequent autophosphorylation and creation of binding sites on the intracellular regions of ALK protein for downstream signaling molecules. Interactions between full length ALK and members of the ►MAP-Kinase pathway, IRS-1 and c-Cbl have been identified in the differentiation of neurites. Other proteins involved in ALK signaling pathways are discussed below with reference to the ALK fusion proteins.

ALK Protein and Cancer

ALK is a receptor tyrosine kinase that has been implicated in the development of both non-hematopoietic as well as hematopoietic tumor development.



ALK Protein. Figure 1 Diagram of the human full length ALK and ALK fusion proteins. The extracellular region contains 16 N-glycosylation sites. The presence of these increases the size of ALK from a predicted 170 kD up to 200 kD. Recognition sites of some of the major intracellular interacting proteins are indicated. The arrow at aminoacid 1,058 indicates the site of cleavage of the ALK protein occurring as a result of the t(2;5)(p23q35) translocation.

Full Length ALK

The expression of full length ALK protein has been reported in a number of mesenchymal tumors such as malignant peripheral nerve sheath tumors and leiomyosarcoma. *ALK* mRNA has also been detected in cell lines arising from ►rhabdomyosarcoma, ►neuroblastoma, ►melanoma, ►glioblastoma and ►breast cancer. The precise role of ALK protein in oncogenesis is uncertain at present although there is evidence that ALK signaling may be a limiting factor in glioblastoma.

ALK Fusion Proteins

In contrast to the situation with full length ALK protein, *in vivo* and *in vitro* studies have led to the conclusion that ALK fusion proteins play a primary role in tumor development. Indeed the aberrant expression of ALK fusion proteins in lymphoid cells is a marker of malignancy.

Structure

Translocations affecting the *ALK* gene result in the production and expression of chimeric ALK fusion proteins. The most common translocation is the t(2;5)(p23;q35), involving the *ALK* gene at 2p23 and the ►*nucleophosmin (NPM)* gene at 5q35, resulting in the expression of the ►NPM-ALK fusion protein. Twelve other variant ALK fusion proteins have been identified (listed below in Table 1).

All of these proteins consist of the N-terminal of the partner proteins and the intracytoplasmic region of ALK containing the tyrosine kinase domain (Fig. 2a). With the exception of ►MSN-ALK and ►MYH9-ALK, all of the fusion proteins contain the final 563 amino acids of ALK while MSN-ALK and MYH9-ALK contain the final 567 and 566 amino acids, respectively.

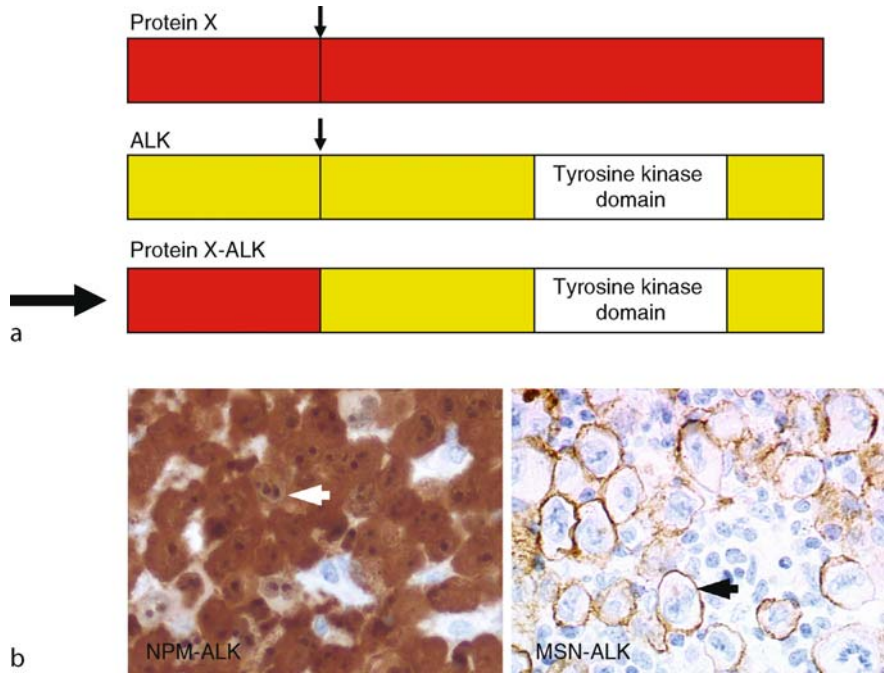
Distribution

Partner proteins of 11 ALK fusion proteins all contain an oligomerization domain in their amino-region. The presence of these domains permits the formation, not only of homodimers of ALK fusion proteins, but also heterodimers of the ALK fusion protein and the normal wild type partner protein. Variations of this mechanism may occur with the MSN-ALK and MYH9-ALK proteins. The ability of the ALK-fusion proteins to dimerise results in each of the ALK fusion proteins having a characteristic subcellular distribution. NPM-ALK, for example, has a nuclear, nucleolar and cytoplasmic localization due to the presence of NPM-ALK homodimers in the cytoplasm of the cell while the presence of a nuclear localization motif present in wild type NPM results in a nuclear and nucleolar distribution

ALK Protein. Table 1 Characteristics and distribution of ALK fusion proteins

Fusion protein	Chromosomal translocation	Subcellular location	Size fusion protein (kD)	Expression in tumors
NPM-ALK	t(2;5)(p23;q35)	Nucleus, nucleolus and cytoplasm	80	ALCL, B cell lymphoma
TPM3-ALK	t(1;2)(p25;p23)	Cytoplasm	104	ALCL, IMT
TFG-ALK_S		Cytoplasm	85	
TFG-ALK_L	t(2;3)(p23;q21)	Cytoplasm	97	ALCL
TFG-ALK_{XL}		Cytoplasm	113	ALCL
ATIC-ALK	inv(2)(p23q35)	Cytoplasm	96	ALCL
CLTC-ALK	t(2;17)(p23;q23)	Granular cytoplasmic	250	ALCL, B cell lymphoma IMT
MSN-ALK	t(2;X)(p23;q11–12)	Cell membrane-associated	125	ALCL
TPM4-ALK	t(2;19)(p23;p13.1)	Cytoplasm	95–105	ALCL, IMT
ALO17-ALK	t(2;17)(p23;q25)	Cytoplasm	ND	ALCL
RANBP2-ALK	t(2;2)(p23;q13) or inv(2)(p23q11–13)	Nuclear periphery	160	IMT
MYH9-ALK	t(2;22)(p23;q11.2)	Cytoplasm	220	ALCL
CARS-ALK	t(2;11;2)(p23;p15;q31)	Cytoplasm	130	IMT

NPM = nucleophosmin; TPM3 = Tropomyosin 3; ►TFG = ►TRK-fused gene; ►ATIC = 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC); also known as PurH; ►CTLC = ►Clathrin heavy chain; ►MSN = ►moesin; ►TPM4 = ►Tropomyosin 4; ►ALO17 = Unknown gene, ALK lymphoma.
oligomerization partner on chromosome 17; ►RANBP2 = ►RAN binding protein also known as Nup358; ►MYH9 = ►Non-muscle myosin heavy chain; ►CARS = Cysteinyl-tRNA synthetase enzyme.
►IMT = inflammatory myofibroblastic tumor.



ALK Protein. Figure 2 Structure and distribution of ALK fusion proteins. (a) The general mechanism of translocations affecting genes encoding ALK and a partner protein (Protein X). As a result of the translocation (shown by the *small arrows* and *dotted lines*), the N-terminus of Protein X is joined to the intracytoplasmic region of ALK to produce a chimeric Protein X-ALK protein. (b) Immunoperoxidase labeling of tissue sections from cases of anaplastic large cell lymphoma to illustrate the different subcellular distribution patterns of ALK fusion proteins. NPM-ALK is present in the nucleus, nucleolus and cytoplasm of the tumor cells (*white arrow*) while MSN-ALK is present at the cell membrane (*black arrow*).

of ►NPM/NPM-ALK heterodimers (Fig. 2b). Other "variant" ALK fusion proteins exhibit a variety of distribution patterns again determined by the identity of the partner protein (Table 1 and Fig. 2b).

Function

Another consequence of an oligomerization domain in the ALK fusion proteins is that it mimics ligand-mediated aggregation of the full length ALK protein with the subsequent constitutive activation of the ALK tyrosine kinase domain. This results in the aberrant activation of multiple downstream signaling pathways involved in mitogenesis and ►apoptosis. Examples of these pathways include the ►AKT signal transduction pathway, Janus kinase and signal transducer and activator of transcription (JAK/STAT), BCL2, GRB2, JNK, ►FOX03A, phospholipase Cγ (PLC-γ), ►phosphatidylinositol 3-kinase (PI3K) and MAP Kinase. NPM itself may also play a role in tumor development through activation of p53. Proteomics-based studies have confirmed the complexity of NPM-ALK signaling pathways in cell proliferation, cellular structure and migration, protein synthesis and the ability of cells to evade apoptosis. Proteins identified in this way include additional adaptor molecules (suppressors of cytokine signaling, ►Rho family proteins and RAB35), kinases (such as MEK kinase 1 and protein kinase C) and phosphatases (meprin, PTPK and protein phosphatase 2 subunit). One potential role of the ALK fusion proteins in oncogenesis is the relocation of interacting proteins away from their normal site of activity within the cell. FOX03A, for example, is redirected to the cytoplasm rather than to the nucleus. Further studies are, however, necessary to understand fully the mechanisms employed by ALK proteins in cell proliferation, differentiation and survival in both normal and disease states.

Tumor Types

Although representing only 5% of ►non-Hodgkin lymphomas (NHL), ALK-positive anaplastic large cell lymphoma constitutes 40% of pediatric large cell tumors. These CD30-positive tumors are of T and null phenotype. NPM-ALK is the most common ALK fusion protein being expressed in 60–80% of the cases, ►TPM3-ALK is detected in about 15% of cases while ►ATIC-ALK, TFG-ALK and ►CLTC-ALK fusion proteins are present in approximately 8% of tumors. The other ALK fusion proteins are present in the remaining 2% of ALK-positive lymphomas. The differential diagnosis

of ALK-positive anaplastic large cell lymphoma is important since these lymphomas are associated with a good prognosis with an overall 5-year survival of 71–80% compared to only 15–46% for ALK-negative anaplastic large cell lymphoma.

ALK fusion proteins may also be implicated in the development of other tumors. ►NPM-ALK and ►CLTC-ALK have been reported in a small subset of CD30-negative B cell lymphoma, while ►TPM3-ALK, ►TPM4-ALK, ►RanBP2-ALK and ►CLTC-ALK fusion proteins have been identified in ►inflammatory myofibroblastic tumors.

Therapeutic Options

Current treatments include the use of various combination chemotherapy protocols originally developed for T-cell lymphoblastic tumors and high-grade B–cell non-Hodgkin lymphomas. Autologous and allogeneic stem cell transplantation techniques have also been utilized. However, 20–30% of patients fail to respond to current treatment regimens and so improved therapeutic options still continue to be sought. One approach is to use ALK as a specific target through the use of ALK specific tyrosine kinase inhibitors (a paradigm is the ABL kinase inhibitor imatinib mesylate or Gleevec used in chronic myeloid leukemia), ALK ribozymes and small interfering RNA probes. Recognition of ALK as an immunogenic tumor-associated antigen has also highlighted its use as a potential target for ►immunotherapy, either for antibody-based therapies for treatment of tumors expressing full length ALK protein or through the use of T-cell mediated immunity in the case of tumors bearing intracellular ALK fusion proteins. Another avenue that has shown promise is the use of small molecule inhibitors affecting proteins involved in the ALK signaling pathways, examples of this include the ansamycin class of natural ►HSP90 inhibitors.

In conclusion, the ALK receptor tyrosine kinase and ALK fusion proteins have been implicated in a diverse range of cellular functions. However, despite major advances, in depth analysis of the signaling pathways is necessary to unravel the full role of this RTK in both normal and neoplastic cells and tissues.

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Alkeran

Definition

►Melphalan.

Alkyl

Definition

Univalent radical containing carbon and hydrogen atoms arranged in chain.

►Bisphosphonates

Alkylating Agents

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Definition

Alkylating agents (*al-ka-LAYT-ing AY-jints*) are a family of anticancer drugs that interfere with cell's DNA and inhibit cancer cell growth. They are so named because of their ability to add alkyl groups to negatively charged groups on biological molecules such as DNA and proteins. Alkylating agents are among the first group of chemicals determined to be useful in ►cancer chemotherapy. They remain to be the most important components of modern chemotherapeutic protocols (individually or in combination with other drugs) because of their proved and significant clinical anticancer activities.

Characteristics

Discovery of alkylating agents as anticancer drugs has its origin in the use of sulfur mustard gas for warfare during World War I. Sulfur mustard gas was not

only fetal but it also showed ►myelosuppression/immunosuppression in its victims as well as in animal models. The latter observation lead to the development of less volatile mustargen (mechlorethamine) with strong antitumor activity against lymphomas and other cancers. Eventually mustargen (nitrogen mustard) was developed for clinical use to treat ►Hodgkin disease. Following the discovery of mustargen, less toxic and more clinically effective nitrogen mustard derivatives, e.g., ►cyclophosphamide, and other alkylating agents in clinical use today were developed (Table 1).

Alkylating agents, as suggested by their names contain reactive alkyl groups. An alkyl is an ►univalent reactive group containing only ►carbon and ►hydrogen atoms arranged in a chain with a general formula of C_nH_{2n+1}, e.g., *methyl*, CH_3 (derived from ►*methane*) and *butyl* C_4H_9 (derived from ►*butane*). Alkylating agents used as anticancer drugs are cable of reacting with biological molecules such as DNA and proteins, and disrupt cellular function by either killing the cell or by prevent its growth. The most common biological functional moiety alkylated by these compounds is guanine, a nucleobase. The anticancer activities of alkylating agents are caused in two ways. (i) Through cross-linking two different DNA strands via the reaction with guanine nucleobases present on the opposing strands of DNA and (ii) Preventing/affecting the activities of critical DNA processing enzymes and there by stimulating apoptosis via the reaction with guanine nucleobases on a single DNA strand. The cross-linking of DNA makes it impossible to uncoil DNA during cell division thus preventing its growth. Based on the reactivity, alkylating agents are of two types; (i) monofunctional (monoalkylating; alkylate nucleobases on one DNA strand) and (ii) bifunctional (dialkylating; alkylate nucleobases on both DNA strands and cross-link them).

Classification

Alkylating agents currently used as anticancer drugs are divided into five major classes. The examples of the clinically used agents (most common) under each of these classes and their clinical utility are shown in Table 1.

Mechanism of Action

Alkylating agents are a diverse group of chemical compounds with a common characteristic of forming positively charged (electrophillic; electron poor) alkyl groups in aqueous solutions under physiological conditions. The positively charged alkyl groups are capable of reacting with basic/negatively charged (nucleophillic; electron rich) groups present in DNA and proteins/peptides. Such reactions lead to adding alkyl groups at oxygen, nitrogen, phosphorous, or sulfur atoms (nucleophillic centers) and thus altering the biological

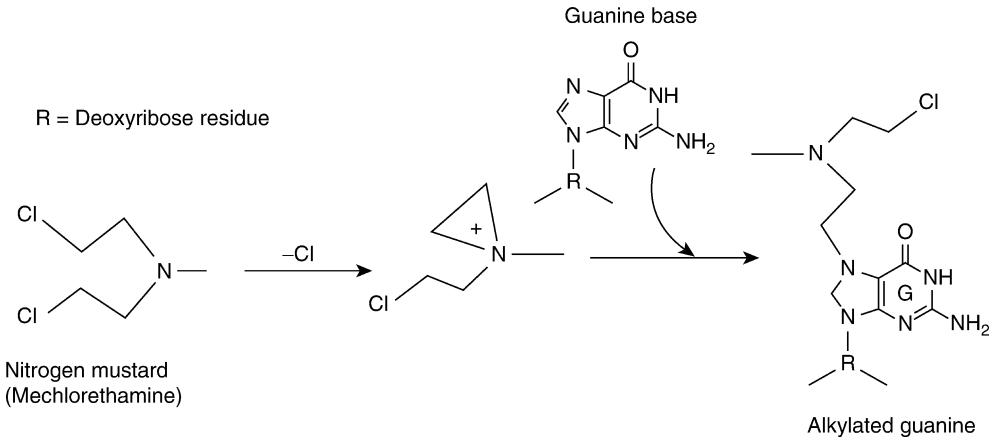
Alkylating Agents. Table 1 Classification of clinically used alkylating agents

Class	Clinically used agents	Cancer/other disease treated
Nitrogen mustards	Cyclophosphamide	►Breast cancers, most lymphomas, and ►childhood cancers
	Ifosfamide	
	4-Hydroxycyclophosphamide	High dose therapies in conjunction with bone marrow transplantation
	Mafosfamide	
	Melphalan	Multiple myeloma, melanoma, and sarcomas
	Chlorambucil	B-cell chronic lymphocytic ►leukemia and immunosuppressive therapy for autoimmune diseases
Aziridines and epoxides	Thiotepa	Breast, ovarian, and ►bladder cancers
	Mitomycin C	►Esophageal, breast, and bladder cancers
	Dianhydrogalactitol	Breast, cervical, and brain cancers
Alkyl Sulfonates	Busulfan	Bone marrow transplantation for Chronic myelogenous leukemia
Nitrosoureas	BCNU [*N*,*N*′-Bis(2-chloroethyl)-*N*-nitrosourea]	►Brain tumors (glioma, ►glioblastoma, medulloblastoma, and astrocytoma), multiple myeloma, and lymphoma
	CCNU [*N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea]	
	MeCCNU [*N*-(2-chloroethyl)-*N*′(4-methylcyclohexyl)-*N*-nitrosourea]	
Hydrazine and triazine derivatives	Procarbazine	Hodgkin lymphoma and certain brain cancers such as glioblastoma multiforme astrocytoma, and ►melanoma
	Dacarbazine	
	Temozolomide	

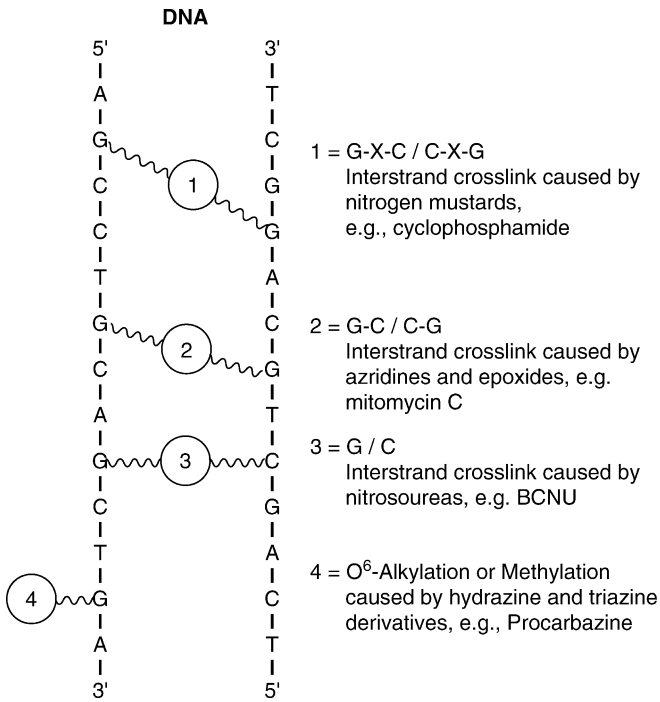
function of DNA and proteins. The most important reaction of alkylating agents with regard to their antitumor activity is their reactions with DNA nucleobases. The most preferred DNA nucleobase for alkylation is guanine and the alkylation preferentially occurs at N7 position on guanine (Fig. 1). Other nucleobases alkylated and the atomic positions at which alkylation occurs in order of preference include N1 and O6 positions of guanine, N1, N3, and N7 positions on adenine, N3 position on cytosine and O4 position of thymidine.

DNA Cross-Links

Various techniques used to elucidate the reactions of alkylating agents with DNA and the possible basis for their anticancer activities has lead to identifying at least four different types of DNA adducts (DNA cross-links) (Fig. 2). Nitrogen mustard (mustargen) and its derivatives, e.g., cyclophosphamide, as well as alkylsulfonates, e.g., busulfan, produce interstrand cross-links in -G-X-C-/-C-X-G- configuration of DNA double helix in greater frequency. The cross-link



Alkylating Agents. Figure 1 Reaction between nitrogen mustard and guanylate residue on DNA at N7 position of guanine.



Alkylating Agents. Figure 2 Schematic representation of alkylation (interstrand cross-links and *O*-alkylation) of DNA by alkylating agents.

involves the N7 atoms of the guanylates in the -G-X-C-/-C-X-G- configuration of the DNA double helix (cross-link 1; Fig. 2). Aziridine and epoxide alkylating agents produce DNA cross-links in -G-C-/-C-G- configuration of DNA. Agents such as thiotepa and dianhydrogalactitol in this class drugs react with N7 position of the guanylate groups. Whereas mitomycin C reacts with the extracyclic N2 atom of the amino group in guanylates (cross-link 2, Fig. 2). Nitrosoureas such as BCNU produce DNA cross-links between a guanine and a cytidine in a -G-/-C- base-pair configuration of the DNA double helix (cross-link 3; Fig. 2). Hydrazine and triazine derivatives such as procarbazine, dacarbazine, and temozolomide decompose to produce a methyl diazonium ion which in turn will methylate guanines on DNA at O6 position (cross-link 4, Fig. 2). Other types of guanylate-alkyl cross-links of type 4, e.g., O6-ethylguanine and O6-benzylguanine, have also been observed.

Molecular Pharmacology, Drug Resistance and Clinical Efficacy

Metabolism:

Alkylating agents are strong electrophiles and react with many biological nucleophiles within the tumor cells. Many of these reactions result in inactivation/detoxification of alkylating agents and thus lead to drug resistance.

The most abundant and principal nucleophile in the cell is ►glutathione (GSH; concentrations in mM levels). The cysteine sulfhydryl (nucleophile) reacts with alkylating agents both in enzyme and no-enzyme catalyzed reactions resulting in glutathione conjugates. The glutathione conjugates of alkylating agents are generally nontoxic. The enzyme catalyzed conjugation of alkylating agents to GSH is catalyzed by ►glutathione S-transferases (GSTs). Tumor cells resistant to alkylating agents commonly have increased levels GSTs. Inhibitors of GSTs such as sulfasalazine and inhibitors of ►gamma-glutamylcysteine synthase (a rate limiting enzyme in the synthesis of GSH) such as buthionine sulfoximine have been shown to reverse the resistance originating due to elevated levels of GSH in both in vitro and in vivo settings.

GSH conjugates of some alkylating agents, e.g., melphalan and chlorambucil, are good substrates for ►ATP-binding cassette membrane proteins (membrane transporter multidrug resistance proteins; MDR; ►P-glycoprotein) and modulation of these enzyme systems is also believed improve clinical efficacy of alkylating agents.

Thiol groups in ►metallothionein enzymes have been shown to sequester alkylating agents such as chlorambucil, melphalan, and phosphoramide mustard (activated cyclophosphamide) and cause resistance. This has been proved by transfection and overexpression, as well as induced expression of genes coding of metallothionines in tumor cells. Modulation of this enzyme system is also expected to increase efficacy of alkylating agents.

Cyclophosphamide and its analogs (nitrogen mustard derivatives) are prodrugs and undergo extensive metabolism. During their metabolism three aldehyde intermediates, viz., aldophosphamide, acrolein, and chloroacetaldehyde are formed. Although all three aldehydes are toxic to cells, the pivotal aldehyde metabolite of the three is aldophosphamide as it gives rise to the DNA alkylating mustard that is finally responsible for the anticancer activity of these agents. ►Aldehyde dehydrogenases catalyze NAD-dependent oxidation of aldehydes in tumor cells. These enzymes have also been shown to oxidize aldophosphamide and cause resistance to cyclophosphamide and its derivatives in various tumor cell models in both in vitro and in vivo settings. Inhibitors of aldehyde dehydrogenases have been shown to reverse resistance to cyclophosphamide and its analogs, as well as increase their efficacy in vitro. Relatively large concentrations of aldehyde dehydrogenases are naturally present in critical normal cells such as bone marrow stem cells, intestinal progenitor cells, and the liver cells. Accordingly, these normal cells are protected from toxicities due to cyclophosphamide and its analogs.

The main mechanism by which alkylating agents present their anticancer properties is via alkylation of DNA. Alkylation further leads to the formation various DNA adducts (Fig. 2) which in turn is responsible for inhibition of tumor cell growth. Removal of such adducts is yet another mechanism by which tumor cells become resistant to alkylating agents.

►O6-Alkylguanine-alkyltransferase has been shown to remove alkyl groups from the O6 position of guanine. This process leads to alkylation of the enzyme alkyltransferase and the alkylated enzyme is rapidly degraded. This mechanism has been shown to be very effective against DNA methylating agents such as procarbazine and temozolomide. The same enzyme has also been shown to remove other alkyl and aryl groups, e.g., dealkylation of O6-ethylguanine and debenzylation of O6-benzyguanine. Inhibitors of O6-alkylguanine-alkyltransferase have been successfully used to prevent resistance to certain clinically used alkylating agents, e.g., BCNU.

DNA cross-links of type 1–3 (Fig. 2) have been shown to be removed via ►nucleotide excision repair and poly(adenosine diphosphate-ribose) polymerase pathways, however the exact mechanism by which this is achieved is not clear.

Toxicology

The most common toxicities associated with administering alkylating agents to treat cancers are as follows.

1. Hematopoietic toxicity – In general, the clinical dose-limiting toxicity for alkylating agents is hematopoietic toxicity, particularly suppression of granulocytes and platelets exhibited for 8–16 days after treatment. The toxicity usually disappears after 20 days and granulocytes and platelets return to their normal levels.
2. Gastrointestinal toxicity (nausea and vomiting) – Damage to the gastrointestinal tract is a toxicity that frequently occurs with high-dose regimens of alkylating agents. These toxicities are characterized by mucositis, stomatitis, esophagitis, and diarrhea. This toxicity can be managed by administering corticosteroids and antiemetics.
3. Gonadal toxicity – Treatments with alkylating agents have been shown to cause testicular lesions leading to depletion of sperm in male patients and decrease in ovarian follicles in female patients.
4. Pulmonary toxicity – Pulmonary toxicities characterized by interstitial pneumonitis and fibrosis leading to dyspnea and nonproductive cough that may lead to cyanosis, pulmonary insufficiency, and death have also been observed in patients treated with alkylating agents.
5. Alopecia – Although the association between an alkylating agents and alopecia was first described with busulfan therapy, this toxicity is predominantly associated with cyclophosphamide and ifosfamide therapy. Alopecia is caused by introduction of nicks in the hair fibers due the temporary stoppage in synthesis of hair in hair follicles by alkylating agents.
6. Teratogenicity – All therapeutically used alkylating agents cause teratogenicity (developmental defects) in animal models. Fetal malformations have been observed in mothers receiving alkylating agents in the first trimester of pregnancy but not second and third trimesters.
7. Carcinogenicity – Reports of the incidence of leukemia and increased frequency of incidence of solid tumors have been reported in patients receiving therapies that include alkylating agents.
8. Immunosuppression – Alkylating agents have been shown to inhibit antibody production. All alkylating agents produce some degree of immunosuppression however severe immunosuppression is caused by cyclophosphamide and its analogs, and chlorambucil. Accordingly, therapies that include high dose cyclophosphamide or chlorambucil without bone marrow transplantation are now being used to treat some autoimmune diseases.

►Acute Myeloid Leukemia
►Adducts to DNA
►Cisplatin
►Mitomycin C
►Toxicological Carcinogenesis

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ALL

Definition

►Acute lymphoblastic leukemia.

►ETV6
►Childhood Cancer

Allele Imbalance

Definition

Alteration of the normal 1:1 ratio of the two alleles at a given genetic locus. The altered ratio can be secondary to increased copy number of one allele (due to amplification or aneuploidy) or decreased copy number of one allele (also known as ►loss of heterozygosity).

►Diethylstilbestrol

Allelic Association

►Linkage Disequilibrium

Allelic Loss

Definition

Is the deletion of one of the two ►alleles from the two homologous chromosomes. If two alleles can be distinguished by molecular genetic means (e.g., microsatellite analysis), allelic loss can be identified as loss-of-heterozygosity (►LOH).

►Microsatellite Instability

Allergen

Definition

An environmental agent capable of initiating an allergic immune response. Common allergens include pollens, molds, and mites.

►Allergy

Allergen-Specific Immunotherapy

Definition

Allergen-Specific Immunotherapy reduces allergen-induced ►inflammation by administering gradually increasing quantities of an allergen extract to an allergic subject in order to ameliorate the symptoms associated with subsequent exposure to the causative allergen (desensitization therapy).

►Immunotherapy

Allergic Asthma

►Allergy

Allergic Disease

Definition

Disease caused by a peculiar or excessive susceptibility especially toward a specific factor (allergen).

►Amine Oxidases

Allergic Rhinitis or Conjunctivitis

►Allergy

Allergy

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Synonyms

Hypersensitivity; Type-1 hypersensitivity; Atopy; IgE-mediated hypersensitivity; Asthma; Allergic asthma; Hay fever; Allergic rhinitis or conjunctivitis; Eczema; Atopic dermatitis

Definition

This field of study refers to the evaluation of the association between allergy and cancer, usually with the hypothesis of allergic status as a protective factor in cancer development.

Characteristics

The presence of allergic disorders has been suggested to be related to cancer development, although this association remains controversial. In particular, the presence of atopic allergic disorders, including allergic asthma and hay fever for example, has been suggested to confer a protective effect against cancer overall and at several sites.

►Atopic disorders are characterized by the inherited predisposition to produce high levels of ►immunoglobulin E (IgE) in response to allergens commonly present in the environment. Upon ►allergen re-exposure, a cascade of events occur leading to a two-phase

(immediate and late) inflammatory immunological response. Atopic disorders arise due to a complex set of genetic and environmental factors and their interactions that remain to be fully elucidated. The prevalence of atopy varies according to a variety of geographic, environmental, and demographic factors and has recently been suggested to be increasing in many western countries. Although atopic disorders have been commonly assumed to offer little benefit to the individual, it has been hypothesized that this atypical, ►Th2-dominated, immune response may in fact represent a state of enhanced or hyperactive immune function.

The immune system has long been recognized as playing a potential role in cancer development (►Immunoprevention of cancer). ►Immune surveillance theory suggests the ongoing search for and eradiation of cancer cells by the immune system as a self-protective mechanism against the development of cancer. Traditionally, it has been the ►Th-1 response however, that has been thought to play a predominant role. The role of the immune system in cancer development can be clearly observed by the fact that individuals who are ►immunosuppressed or immunodeficient tend to display higher rates of certain types malignancies. It is possible that certain groups may exhibit an enhanced ability for immune surveillance, including those with a history of atopic allergy. The hypothesized state of enhanced immune function and immune surveillance among individuals with atopic allergic disorders may be related to cancer development.

Epidemiological Studies

The association between allergy and cancer has been examined in a number of ►analytic epidemiological studies. Over 100 studies conducted throughout the world have evaluated some aspect of the association between a history of allergy and cancer occurrence. Although many of these studies have suggested an association between allergy and cancer, either overall or at a specific cancer site, it is difficult to draw conclusions due to insufficient evidence, inconsistent findings, and limitations of previous studies. The nature of the relationship also likely depends on the cancer site studied.

Previous epidemiological studies have tended to be associated with a variety of limitations associated with exposure assessment, confounding, and certain types of bias. The majority of previous studies have relied on self-reported history of allergy disorders as an indicator of allergic status. Few studies have evaluated ►skin prick testing or serum allergen-specific IgE levels in relation to cancer occurrence and few large prospective studies exist.

It remains unclear if an allergic history is associated with overall cancer occurrence. Although results from a number of early case-control or cohort studies suggested an inverse association, results from more recent larger cohorts although suggestive, in total have provided little clear evidence for an association. Perhaps the strongest evidence for an association between a history of allergy and cancer overall are results from an analysis of a large prospective cohort of nearly 1.2 million US adults followed for 18 years. A statistically significant 12% reduction in the risk of overall cancer mortality among participants who reported a history of both asthma and hay fever at baseline was reported, however, the results among never smokers attenuated slightly.

Among cancer site-specific studies, strong inverse associations have been reported in studies of pancreatic cancer and brain cancer (glioma), although there remain a variety of limitations (►Pancreas cancer, basic and clinical parameters; ►brain tumors). A recent study combining the results of previous published studies (a ►meta-analysis) reported a statistically significant 18% and 29% reduction in risk of pancreatic cancer associated with a history of any allergy or atopy respectively. Results from a number of studies, including a large international case-control study with eight study centres reported a statistically significant inverse association between risk of glioma and a history of any allergic disease, where this study noted a 40% in risk. Numerous other inverse associations have been reported at other cancer sites; however the results findings remain to be confirmed.

Contrasting these findings, lung cancer has tended to be found to be positively associated with a history of asthma, however certain limitations including potential misclassification and differential recall bias in previous studies are still of concern. This positive association, if causal, is thought not to be related to an overall immune response, but rather to local processes of repeated inflammation, cell regeneration, and free radical production due to the allergic lung disease increasing the occurrence of malignancy.

Biological Details

At this point in time, no precise explanation has been put forward to explain an overall inverse association between a history of allergy and cancer risk. Enhanced immune surveillance is most often suggested when inverse associations are reported in the epidemiologic literature. The atopic immune response involves the release of a number of factors including histamine, leukotrienes, and chemotactic factors among others from IgE-mast cell complexes upon allergen exposure. The resulting response initially involves smooth muscle contraction, mucus secretion, vasodilation, and a loss of microvascular integrity which is then followed by the infiltration and activation of eosinophils, neutrophils, Th2-type CD4+ cells, and macrophages that typically lasts for several hours. It remains unclear however, how

this overactive immune response to common environmental agents may be related to the development of cancer. Allergy medications have also been investigated in relation to cancer occurrence; however few associations have been reported.

Overall, although it is plausible that enhanced immune surveillance in individuals with a history of allergy may be associated with reduced cancer risk; specific evidence remains limited and controversial. Further understanding of such a potential association may be relevant for the development of cancer prevention and treatment options.

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Allodynia

Definition

Type of pain that is frequently associated to cancer chemotherapy or cancer itself. Allodynia is caused by a stimulus – such as touch, pressure and warmth – that does not normally provoke pain.

►Cannabinoids and Cancer
►Hyperalgesia

Allogeneic

Definition

Referring to two genetically distinct members of the same species.Describing the relationship between two sets of cells or tissues deriving from different genetic backgrounds. An allogeneic transplant is one in which the donor and recipient are two different individuals but are of the same species.

Allogeneic Bone Marrow Transplantation

►Allogeneic Cell Therapy

Allogeneic Cell Therapy

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Synonyms

Allogeneic cellular immunotherapy; Allogeneic hematopoietic stem cell transplantation; Allogeneic bone marrow transplantation

Definition

Allogeneic cell therapy consists of chemoradiotherapeutic conditioning therapy followed by transplantation of hematopoietic stem cells and lymphocytes isolated from allogeneic healthy donors to generate an effective graft-versus-malignancy immune response in patients with treatment-refractory malignant disorders.

Characteristics

Rationale

Allogeneic hematopoietic stem cell transplantation (HSCT) aims to break autologous immunotolerance towards malignant cells in tumor-bearing patients. The treatment approach is based on the alloreactive graft-versus-malignancy effect that is mainly mediated by T cells of donor origin. These donor T cells are infused together with allogeneic hematopoietic stem cells (HSC) at the time of transplantation or originate from donor HSC in the patient thereafter. Allogeneic HSCT is capable of inducing long-term disease control in patients with chemotherapy-refractory leukemias and other ►hematological malignancies.

Procedure

Allogeneic HSCT requires chemoradiotherapeutic conditioning therapy to allow the engraftment of subsequently infused allogeneic HSC and lymphocytes of donor origin. Preclinical as well as clinical research has demonstrated that the long-term leukemia control following allogeneic HSCT depends on the immunological graft-versus-leukemia (GVL) effect rather than on the intensity of the pre-transplant ►chemoradiotherapy. This important observation led to a change of paradigm shifting the antileukemic effect of the allo-transplantation procedure from the preparative cytostatic drugs to alloreactive immune effector cells. Consequently, reduced-intensity conditioning (RIC) regimens were developed that do not irreversibly destroy recipient hematopoiesis, but are sufficiently immunosuppressive to permit the engraftment of allogeneic HSC. This results in an initial co-existence of donor and recipient hematopoiesis ('mixed hematopoietic ►chimerism') that can be gradually shifted to complete hematopoietic donor chimerism by modulating the post-transplant immune system using immunosuppressive agents or ►donor lymphocyte infusions (DLI). The majority of these non-myeloablative RIC regimens are combinations of 1–3 different chemotherapeutic drugs and low-dose total body irradiation. Compared to conventional myeloablative conditioning protocols based on high-dose radiochemotherapies, RIC regimens carry a much lower treatment-related morbidity and mortality allowing the use of allogeneic cell therapy in patients until 60–70 years of age or in patients with significant co-morbidities.

The allogeneic HSC donors are healthy related and unrelated volunteers who are matched with the patients for ►human leukocyte antigen (HLA) class I and II molecules. Although transplantation of donor HSC with single or multiple HLA allele or antigen disparities is feasible, this increases the risk of immune-mediated graft rejection and ►graft-versus-host disease (GVHD). The HSC can be harvested either by bone marrow aspiration during general anesthesia or by apheresis. The apheresis procedure requires the prior mobilization of HSC into the peripheral circulation by a 3 to 5-day treatment course with recombinant human ►granulocyte-colony stimulating factor (G-CSF). HSC express the hematopoietic ►stem cell marker CD34 that enable their detection in clinical samples by ►flow cytometry.

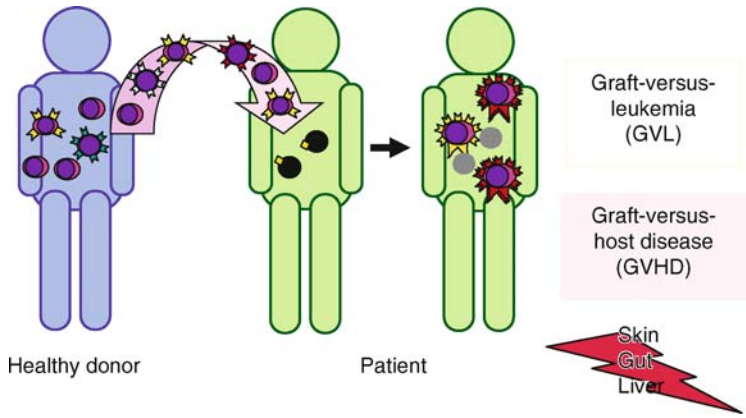
After conditioning therapy and transplantation of the HSC allograft, the patient enters a 1–3 week-long period with a low neutrophil cell count (►neutropenia) in which the patient is susceptible to bacterial and fungal infections. The hematopoietic engraftment is indicated by the re-occurrence of circulating neutrophils. These neutrophils are of donor origin which can be verified by analyzing the proportion of donor and patient derived DNA in chimerism assays. Early graft rejection is prevented by treating the patient with immunosuppressive medication, mainly consisting of the calcineurin inhibitors cyclosporine A and tacrolimus, the antimetabolite methotrexate, and T-cell depletion antibodies. The strong suppression of T cell immunity increases the risk of infections with opportunistic agents of which herpes family viruses (e.g. cytomegalovirus, varizella zoster virus, ►Epstein-Barr virus) and pneumocystis carinii have the greatest clinical significance during the first 1–2 years after transplantation. The drug-induced immunosuppression is also necessary to lower the incidence and severity of GVHD.

GVHD is a life-threatening complication of allogeneic HSCT in which donor T cells attack the tissues of the transplant recipient after perceiving host tissues as antigenically foreign. GVHD is mainly directed against epithelial tissues of the skin, liver, and gastrointestinal tract. Other GVHD target organs include the hematopoietic tissues such as bone marrow and thymus, and the lungs in the form of idiopathic pneumonitis. Clinically, GVHD is divided into acute and chronic forms. The acute form is observed within the first 100 days post-transplant, and the chronic form occurs following day 100 after HSCT. Chronic GVHD damages the above mentioned organs, but also causes changes to the connective tissues including skin and exocrine glands. If the GVHD is severe and requires intense immunosuppressive treatment, the patient may develop serious infections as a result of the immunosuppression and may die of infection. Moderate forms of GVHD are associated with a lower incidence of relapse of the underlying malignant disease, and, therefore, require no escalated immunosuppressive treatment.

If the patient develops disease relapse after allogeneic HSCT, donor lymphocyte infusions (DLI) can be administered to augment the GVL effect. The DLI are collected from the original stem cell donor by apheresis without prior G-CSF treatment. DLI therapy is most efficient in patients with low disease burden. Moreover, DLI carry a superior GVL effect in chronic leukemias compared with acute leukemias. This may rely on disease-inherent factors such as the growth kinetic and immunogenicity of leukemic blasts that favors efficient immune reactions in chronic leukemias over their acute counterparts. The major complication of DLI therapy is an accompanying severe GVHD reaction, particularly if high lymphocyte doses are administered.

Mechanisms

The therapeutic success of allogeneic HSCT relies on the GVL immune effect that is closely linked to GVHD (Fig. 1). However, there are a considerable number of patients who develop efficient GVL reactions in the absence of GVHD. The main effectors that induce the GVL reaction as well as the GVHD are T lymphocytes of donor origin. In allogeneic HLA-identical HSCT, the donor lymphocytes generate a ►T cell response against



Allogeneic Cell Therapy. Figure 1 Donor-derived T lymphocytes infused into the leukemia patient are key mediators of the alloreactive graft-versus-leukemia effect (GVL) and the graft-versus-host disease (GVHD). Main GVHD target organs are skin, gut, and liver.

a group of proteins (called ►minor histocompatibility antigens, minor Hag) that are genetically polymorphic between donor and recipient. The peptide epitopes derived from minor Hag are presented by HLA molecules on recipient cells, and there are well-described examples of HLA class I and II associated minor Hag recognized by $CD8^+$ and $CD4^+$ donor T cells, respectively. It is comprehensible that minor Hag exclusively expressed in the hematopoietic tissue lineage promotes the engraftment of donor hematopoiesis as well as the GVL effect, while minor Hag with an ubiquitous expression pattern including epithelial tissues will facilitate the development of GVHD.

There is also increasing evidence that donor T cells can recognize non-polymorphic antigens that are de novo expressed or over-expressed on leukemic cells of the recipient. Hematopoietic minor Hag and leukemia-associated antigens are ideal candidates to re-direct donor immunity specifically against the hematopoietic recipient cells including leukemia, either by vaccination with ►cancer vaccines or by ►adoptive immunotherapy. A great deal of current research on allogeneic HSCT involves attempts to separate the undesirable GVHD aspects of T cell pathophysiology from the desirable GVL effect.

For many leukemia patients lacking an HLA-matched hematopoietic stem cell donor, transplantation of HLA-incompatible HSC remains the only curative treatment option. In haplo-identical transplantation, the donor shares only one haplotype with the recipient. Because disparate HLA alleles are strongly immunogeneic targets of alloreactive T cells, these regimens require concomitant T cell depletion to prevent graft rejection and severe GVHD. Several research groups have demonstrated that in HLA-mismatch transplantation settings incorporating extensive T cell depletion, the main immunological effector cells are ►natural killer cells of donor origin that recognize recipient hematopoietic (including leukemia) cells lacking the expression of natural killer cell inhibitory receptors.

Clinical Aspects

Allogeneic HSCT is a curative treatment modality for patients with insufficient hematopoietic stem cell function such as aplastic anemia, and for patients with chemotherapy-refractory forms of hematological malignancies including ►chronic myeloid leukemia, ►acute myeloid leukemia, and ►acute lymphoblastic leukemia. Ongoing studies explore the role of allogeneic HSCT in patients with ►Hodgkin disease, non-Hodgkin's lymphoma, and ►chronic lymphocytic leukemia. With the development of less-toxic RIC regimens, many groups are currently trying to establish allogeneic HSCT for the treatment of diseases with a dysfunctional immune system, e.g. autoimmune disorders and solid tumors such as ►renal carcinoma. The general idea is to first generate stable hematopoietic donor chimerism in the patient as a platform allowing in a second step the re-direction of immunity using adoptively transferred donor lymphocytes with beneficial specificity.

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Allogeneic Cellular Immunotherapy

►Allogeneic Cell Therapy

Allogeneic Hematopoietic Stem Cell Transplantation

►Allogeneic Cell Therapy

Allograft

Definition

Allograft refers to cells, or tissue, of an animal of one genetic background grafted into a host animal of another genetic background both from the same species.

►Allogeneic
►Graft Acceptance and Rejection

Allograft Rejection

Definition

Allograft rejection refers to the rejection of a donor tissue graft by the immune system of a recipient because the donor and recipient are genetically different.

Allopurinol

Definition

A xanthine oxidase inhibitor, which is used to inhibit uric acid formation; utilized in the chronic treatment of gout, to retard the rapid metabolic degradation of 6-mercaptopurine, and to prevent tumor lysis syndrome.

►Rituximab

Allylic Structures

Definition

A feature of many anticancer drugs, sphingolipids, and some metabolites that affect cancer cell growth or apoptosis. The oxygen can be an alcohol, a ketone, an ether, or part of an amide or ester; similar groups are found in N compounds. Such drugs act as ►alkylating agents or inhibitors of mitochondrial oxido/reduction processes.

►Sphingolipid Metabolism

Alpha-Actinin

Definition

An F-►actin binding and crosslinking protein. Distinct isoforms are found in skeletal muscle, smooth muscle, and non-muscle cells. Alpha-actinin may function as an actin bundling protein and/or a linking protein attaching actin filaments to a variety of intracellular structures. A homodimer with a subunit molecular weight of 94–103 kDa.

►AAMP

$Alpha_1$-Fetoglobulin

►Alpha-Fetoprotein – Modern

Alpha-Fetoprotein

Definition

AFP is a fetal serum protein that shares sequence homology with albumin. Serum levels of AFP are typically elevated in patients with hepatocellular carcinoma and in men with nonseminomatous germ cell tumors (NSGCT). Although AFP levels >400 ng/ml are highly associated with HCC, not all HCC secrete AFP. It may also be elevated in patients with cirrhosis,

viral hepatitis, drug or alcohol abuse as well as pregnancy, and may be used for screening of fetal spinal cord defects and placental disease.

►Serum Biomarkers
►Hepatocellular Carcinoma

Alpha-Fetoprotein – Historical

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Synonyms

AFP; Tumor markers; Oncodevelopmental proteins; Carcinofetal proteins; Feto-specific proteins; Oncofetal antigens

Definition

AFP or alpha-fetoprotein is a serum protein of mammalian fetuses that is hardly detectable in healthy adults. Its re-occurrence in serum of adults may often attest to specific malignancy especially in high-risk patients, such as those with hepatocellular carcinomas (►hepatocellular carcinoma, ►hepatoblastoma) and chronic hepatitis B or C virus infection (►hepatitis associated hepatocellular carcinoma). It also serves in evaluation (►serum biomarkers, ►surrogate endpoints) of therapy and disease progress in patients with embryonal carcinomas (►germ cell tumors, ►platinum refractory testicular germ cell tumors).

Characteristics

The studies of fetal serum proteins came from different corners: from researchers interested primarily in the development of proteins and from those studying proteins of tumor-bearing laboratory animals. These two groups were at the beginning not very aware of each other's results. The fetal protein history began with the physicochemical and biochemical studies of serum proteins, which depended, as this often happens in the laboratory endeavor, on the development, improvement and refinement of laboratory methods. In the field of serum proteins, the electrophoretic and immunochemical techniques (►proteomics) were of crucial importance, especially in the case of fetal proteins, where usually only minute volumes of sera were available. Studies of electrophoretic patterns of serum proteins in human fetuses showed some considerable differences when compared with the sera of adults. Thus, in 1956 Bergstrand and Czar, using filter paper electrophoresis reported on the special fetal band, (called substance X) which was located between albumin and alpha-1 globulins. Substance X was absent from maternal sera and from sera of healthy adults. Also, Halbrecht and Klibanski reported similar findings in the same year. The first immunochemical studies of the substance X were done by Muralt and by Masopust in 1961 and 1962, respectively. Using antisera to fetal serum proteins (rabbits were immunized with the human fetal sera), an additional precipitin line with alpha globulin mobility was observed on immunoelectrophoresis (IEP) of human fetal serum, however, it was not present in adult sera. This fetal component was called independently "alpha-foeto-proteine" by Muralt and "fetoprotein" by Masopust. These findings resembled older observations in large animals; in 1944 Pedersen studied bovine fetal sera by ultracentrifugation and found a distinct gradient, not present in sera of adult animals. The fraction was named fetuin. Thus, it was believed by some that the human fetoprotein was related to fetuin and the term "human fetuin" was used in some papers on human fetoprotein. Physicochemical properties of fetuin, which was found to be a typical glycoprotein, were studied by a number of workers; its physiological and pathologic properties attracted much less interest. Because fetuin and fetoprotein were present in higher concentrations in fetuses and undetectable in adults, they were sometimes called "feto-specific proteins."

Immunochemical Techniques

For the detection of feto-specific proteins, the immunochemical techniques became the methods of choice in the 1960s. Antisera to these proteins were prepared by the immunization of animals, usually rabbits, with fetal sera. To obtain specific antisera to feto-specific proteins, the antisera were absorbed with the sera of adult men or animals. The absorbed antisera should contain only the antibodies directed to the feto-specific protein(s) of a given species. In some cases, the absorbed antisera showed two to three precipitin lines on IEP of fetal serum. Sometimes, in human fetal sera, two lines with the absorbed antiserum were observed. The line in alpha zone of IEP was that of human fetoprotein, the other line, in beta position, was sometimes incorrectly, without justification, called beta fetoprotein. The lines showed no antigenic relationship each to other. For this reason, the original term "fetoprotein" was changed to "alpha fetoprotein" and consequently the abbreviation of AFP came to life. The term "beta-fetoprotein" ceased to be used since the beta protein was later identified as fetal ferritin.

AFP in Pathology

In 1964, a study of a possible occurrence of AFP in sera of patients was started. The putative presence of AFP was

tested by double radial immunodiffusion (Ouchterlony test). After hundreds of negative results, a patient was identified, who had a definitely detectable serum concentration of AFP. The diagnosis of this patient, confirmed histopathologically at the autopsy, was that of hepatocellular carcinoma. In 1966 and 1967, the occurrence of AFP in four children with a malignant growth of embryonic character was reported. One of them was a 5 year-old boy with embryonal cell carcinoma of the left testicle (►testis cancer, ►childhood cancer, ►germinoma) and another patient was a 14 year-old girl with malignant teratoblastoma of the right ovary (►ovarian cancer, ►ovarian tumors during childhood and adolescens). Also, Abelev published in 1967 the finding of "alpha fetal globulin" in patients with embryonal testicular cancer. Several pediatric patients with non-cancerous liver diseases, such as infectious hepatitis and some unspecified hepathopathies were identified, who had detectable AFP serum levels. A highly sensitive technique, radioactive single radial immunodiffusion (employing the second, 125 Iodine-labeled antibodies to the primary antiAFP immunoglobulin fraction), enabled to quantify previously undetectable levels of AFP in various body fluids. By such means, AFP serum levels of patients with hepatocellular carcinomas were studied in a correlation with their individual histopathologic findings. A further increased sensitivity of AFP quantitation was facilitated by the development of a radioimmunoassay. This technique made the quantitation of AFP in healthy persons such as pregnant women a routine test in clinical laboratories. In the 1970s, a number of reviews on AFP were published along the studies of AFP physicochemical properties. The first studies on serum concentrations of AFP and their changes in the course of diseases were done in those years. Thus, the impact of the therapy could be evaluated and monitored in some malignancies.

Fetuin versus AFP

In the early years, AFP was considered by some investigators to be a protein similar to bovine fetuin and therefore called "human fetuin." Fetuin was isolated from fetal calf serum and the antisera were prepared to fetuin, and to serum proteins of human and bovine fetuses. With the use of absorbed antiserum to calf serum, an additional protein component was detected in alpha zone of bovine fetal serum, which was not detectable in sera of adult animals. This component could be considered as a "bovine fetoprotein." Antiserum to this protein did not react with isolated fetuin and conversely the specific antiserum to fetuin did not react in immunodiffusion experiments with the "bovine fetoprotein." The protein was not detected in adult healthy animals; it was, however, found in sera of two, out of four, adult cows with hepatocellular carcinomas (►comparative oncology). No antigenic relationship was observed in double radial immunodiffusion and the precipitin lines of fetuin and "bovine fetoprotein" crossed each other, showing thus the pattern of antigenic non-identity.

AFP in laboratory Animals

Rat sera were studied electrophoretically already in the 1950s. A "fetal" protein was detected by Beaton (1961) in the macroglobulin fraction of starch gel electrophoresis. This protein migrated as an alpha-2 globulin in electrophoretic media without molecular sieve effect (filter paper) and slowly in starch gel. Therefore, it was called "alpha-2 slow globulin." The protein was found in sera of rat fetuses and newborns, as well as in pregnant rats, but not in healthy, non-pregnant adult rats. It was present, however, in sera of tumor-bearing rats and in animals with various inflammatory processes, e.g. with turpentine abscess. Another alpha globulin was found by Darcy in fetal rat sera; it was also present in sera of pregnant animals and adult rats with tumors and/or with inflammations. Protein was also detectable in much lower concentrations in healthy, non-pregnant rats. Wise in 1963, using two-dimensional electrophoresis (filter paper-starch gel) demonstrated in rat fetal sera special proteins, named "fetal postalbumins" (two electrophoretic bands), which were not present in sera of adult animals. Altogether, at least three fetal components were reported in rats. To address this question, rabbit antiserum directed to rat fetal serum proteins was prepared. The absorbed antiserum (with the serum proteins of adult, healthy, non-pregnant animals) did not react with sera of adult, healthy non-pregnant rats, or with the protein described by Darcy. It did react, however, with three different proteins on IEP of fetal rat sera; two of them located in alpha-2, and one in alpha-1 globulin zone. The antibody to the protein in alpha-2 zone could be absorbed with the serum from an adult rat with turpentine abscess. This protein was also detected immunochemically in extracted proteins from macroglobulin position in starch gel electrophoresis of fetal serum. The protein obviously corresponded to alpha-2 slow globulin of Beaton. The other precipitin line in alpha-2 globulin zone was stainable with lipid stains (Red Oil and Sudan Black B), and represented most probably a lipoprotein-esterase found by Stanislawski–Birencwajg in fetal rat serum. The precipitin line in alpha-1 zone, present in sera of fetuses, absent from sera of adult rats, either healthy or with the acute inflammation, was considered to be a typical feto-specific protein, probably related to human AFP. However, no cross- reaction was seen by immunodiffusion between human AFP and antiserum to rat fetal proteins. To prepare a monospecific antiserum to alpha Ft protein, it was important to remove the antibodies to alpha-2 slow globulin, e.g. by using sera of adult rats with some inflammatory pathology. In 1963, Abelev reported the finding of "embryonal alpha globulin" in serum of adult mouse with transplatable

hepatoma; the globulin was also present in sera of fetal mice (►mouse models).

Much progress has been done since the early modest beginnings of AFP research. Presently, a review of AFP literature shows almost fifteen thousand papers related to the topic (►AFP-modern).

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Alpha-Fetoprotein – Modern

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Synonyms

AFP; Alpha$_1$-fetoglobulin; Embryo-specific alpha-globulin; Embryonal serum alpha-globulin; Foeto-protein; Fetuin; Fetuin-A; α-feto-protein; α_1-fetoglobulin; Embryo-specific α-globulin; Embryonal serum α-globulin

Definition

Alpha-fetoprotein (AFP) is a 68.7 kDa plasma protein synthesized primarily by the fetal liver and embryonic yolk sac that is highly homologous with human albumin. Widely expressed in the fetal liver, AFP mRNA is down-regulated in post-natal hepatocytes. Serum AFP levels are used clinically for detection, confirmation and follow-up of human ►hepatocellular carcinoma (HCC) and non-seminomatous ►germ cell tumors, although lack of sensitivity and specificity complicate its use.

Characteristics

Alpha-fetoprotein (AFP) is a 590 amino-acid plasma protein that shares 40% amino acid and 40–44% nucleotide sequence homology with human serum albumin and is a member of the albumin gene superfamily. The AFP gene covers approximately 22 kB of DNA and has 15 exons and 14 introns. The human albumin gene lies 14.5 kB upstream to its AFP homologue. Regulation of AFP protein production occurs mainly at the transcriptional level. In human cells, the AFP enhancer region contains binding sites for several liver-enriched transcription factors (HNF1-4, C/EBP) which control tissue specific expression. Expression of AFP also appears to be positively regulated by NFκB, by steroids via retinoid X receptors as well as by interactions with extracellular matrix.

AFP is normally expressed by villous trophoblasts in the human placenta during pregnancy and by fetal hepatoblasts. In fetal and newborn rats, AFP mRNA can be detected at low levels in the kidney, pancreas, heart and gastrointestinal tracts as well. In early postnatal life, AFP production is repressed in normal hepatocytes and silenced in non-hepatic parenchymal cells.

The mechanisms for the repression or silencing of AFP expression have largely been characterized. In mice, an unlinked locus called alpha-fetoprotein regulator 1 (Afr1) on chromosome 15 appears to interact with the AFP promoter region; repression of Afr1 appears to be associated with postnatal repression of AFP expression. The AFP promoter may also interact with Ku inducing a hairpin tertiary structure that may abrogate HNF1 binding to the promoter. Postnatal repression of AFP expression in the liver has also been shown to be ►p53- and ►TGFβ1-dependent whereas genetic silencing primarily involves epigenetic mechanisms that concomitantly silence the upstream albumin gene.

In the adult liver, AFP expression is present but repressed. In situ hybridization studies confirm the presence of minute quantities of AFP mRNA, but at levels generally below the sensitivity of immunohisto-chemical detection. In the setting of hepatocyte regeneration, e.g. ischemic injury, surgical resection, and chronic viral hepatitis, in ►hepatoblastoma as well as in a subset of hepatocellular carcinoma (HCC) (and rarely ►cholangiocarcinoma) AFP expression is de-repressed. AFP production also occurs in non-seminomatous germ cell tumors such as choriocarcino-ma, mixed germ cell tumors and teratomas. In fetal and newborn rats, AFP mRNA can be detected at low levels in the kidney, pancreas, heart and gastrointestinal tracts as well. Rarely in adults, non-hepatic/non-germ cell malignancies such as ►gastric cancer, pancreatic cancer, endometrial cancer, colon cancer, and ►ovarian cancer are associated with loss of silencing of AFP expression.

The critical activities of AFP *in vivo* remain poorly defined. Many cell types including vascular endothelium and T-cells express receptors for AFP. AFP administration in human cell lines has been associated with differential expression of FasL and ►TRAIL relative to fas and ►TRAIL receptor, leading to postulation of a role for AFP in escape from tumor immunosurveillance. AFP also appears to inhibit ►TNF

receptor 1-signalling-mediated tumor cell apoptosis. Paradoxically, some studies suggest a pro-apoptotic role for AFP in tumor cells lines via interactions with X-linked inhibitor of apoptosis protein (XIAP). Other studies postulate that AFP may mediate anti-inflammatory effects that suppress autoimmunity and anti-fetal immune responses during pregnancy, possibly via inhibition of CD4 T-cell proliferation.

Serum AFP determinations has two main clinical uses. First, it is used to screen women during pregnancy for fetal developmental abnormalities. Second, AFP is used as a tumor marker for hepatocellular carcinoma (HCC) and non-seminomatous germ cell tumors.

Serum AFP determinations have been used since the late 1960s to detect hepatocellular carcinoma despite limitations in its sensitivity and specificity. While AFP levels greater than 400 ng/ml are considered diagnostic of HCC, such elevations are rarely present. The sensitivity and specificity of AFP determinations also appears to be dependent on the underlying cause of liver disease that results in HCC development. Using a cutoff of 20 ng/ml, sensitivity ranges from 41% to 65% and specificity ranges from 80% to 94%.

The role of serum AFP in screening programs for HCC in patients with cirrhosis remains controversial. It remains unclear if the addition of AFP determinations to routine imaging examinations, e.g. ultrasound every 6 months, provides any incremental benefit.

Current guidelines from the United Network of Organ Sharing (UNOS) in the United States support the use of AFP levels greater than 400 ng/ml to confirm the presence of HCC when a hypervascular lesion on CT or MRI imaging is seen. Exception points may be petitioned from UNOS to provide the rare individual patients with AFP levels greater than 400 ng/ml but no visible tumor to increase the priority of such patients for liver transplantation.

Several glycoforms (AFP-L1, -L2 and -L3) of AFP have been resolved based on differences in glycosylation groups. Lectin-reactive AFP (AFP-L3) in some studies has been associated with intrahepatic cholangiocarcinoma. In other studies a high percentage of total AFP made up of the L3 fraction has been associated with hepatocellular carcinomas. Measurement of specific glycoforms is not in routine clinical use.

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Alpha-Particles

Definition

Helium nuclei produced as radioactive-decay products.

Alpha-SMA

Definition

Alpha-smooth muscle actin.

►Stromagenesis

Alternative Lengthening of Telomeres (ALT)

Definition

Alternative lengthening of telomeres (ALT) is a telomere maintenance mechanism that does not involve telomerase, which probably involves recombination. It is found in a minority of cancers and immortalized cell lines. A minority of immortalized cell lines and cancers have no detectable telomerase activity and maintain their telomeres by an alternative mechanism. Although the details are not yet known, it is likely to be a recombinational mechanism in which one telomere uses another telomere (or itself via looping back) as a template for synthesis of new telomeric DNA. Cells that maintain their telomeres by ALT characteristically have very heterogeneous telomere lengths, ranging from undetectable to extremely long.

►Senescence and Immortalization
►Stem Cell Telomeres

Alternative Reading Frame

►ARF Tumor Suppressor Protein

Alternative RNA Splicing

Definition

Alternative RNA splicing refers to the synthesis of different RNA molecules from a single product by differential usage of splicing junctions, which are the sequences surrounding the exon–intron boundaries.

►Progestin

Alu Elements

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Definition

The most abundant class of dispersed repeat elements in the human genome, and one member of the family of short interspersed repeat elements (SINEs). An estimated one million copies comprise about 10% of DNA in human cells.

Characteristics

Structure

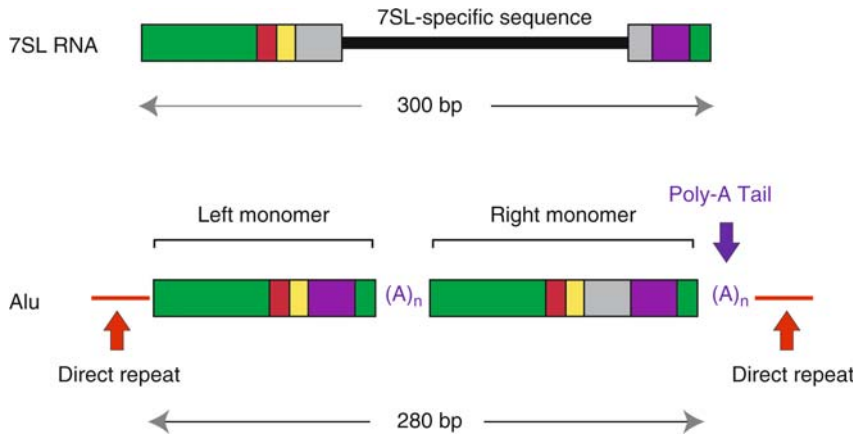
Alu elements are 280 bp in length, and consist of two similar monomers that have homology to, and were originally derived from, the ►7SL RNA gene (Fig. 1). Individual Alu elements are flanked by direct repeats, end in a 3′ A-rich tract, and the left monomer contains an internal RNA polymerase III promoter which directs transcription initiation to the first residue of the element. Alu are ►retrotransposable elements, and several subfamilies, mobilized from different "source" genes at different evolutionary times, can be recognized on the basis of their sequence divergence and diagnostic bases. Some of the more recently integrated subfamilies are polymorphic, occupying regions on some chromosomes that are not occupied at the same locus on others.

Function

The function of Alu elements has been subject to intense investigation, debate and speculation over the past two decades. Proposed roles include modulation of chromosome structure and packaging of DNA around ►nucleosomes, initiation or switch sites for DNA replication, regulation of gene transcription through Alu-specific protein binding domains, RNA editing as preferential templates for adenosine-to inosine (A-to-I) substitution by the ►ADAR family of enzymes, and regulation of translation by RNA transcribed from Alu elements. Although Alu expression increases in cells stressed by chemical agents or viral infection, most human Alu repeats are silent in somatic cells, with only the minor, evolutionarily younger subgroups actively transcribed. Consistent with the latter observations, CpG sites in the majority of Alu sequences are normally fully methylated in most somatic cell types, a state which is considered to suppress expression and therefore transposition. However, Alus located adjacent to CpG islands show sequence features amenable to an unmethylated state, and which may therefore have increased mobility. Alu elements are also differently methylated in male and female germ cells, with at least a subset of the recently integrated Alus being almost completely unmethylated in sperm DNA.

Role in Human Cancer

Alu-mediated gene rearrangement underlies several important constitutional diseases, including familial



Alu Elements. Figure 1 Alu elements have a dimeric structure that originated from 7SL RNA. Colored areas show 7SL sequences present in the Alu repeat consensus.

cancers. Different mechanisms for these rearrangements include recombination between homologous or non-homologous regions of Alu elements at different locations within a gene, or on the same or different chromosomes, expansion of 3′ polynucleotide tracts to form fragile sites, or disruption of coding regions of functional genes by transpositional insertion of actively transcribed Alu elements. Instability of 3′ polynucleotide tracts may also indicate a DNA ►mismatch repair deficiency.

Because of their high density in the human genome, non-random chromosomal distribution and the high degree of homology between individual elements, Alu repeats are also recognized candidates to mediate somatically acquired gene rearrangements with neoplastic potential. Specific underlying mechanisms for involvement of Alu in somatic rearrangements have only recently begun to be explored, with possibilities including promotion of DNA exchange by sequences within Alu that share homology with known recombinogenic ►translin DNA-binding motifs or the ►χ-like Alu core sequence, preferential recombination between DNA regions that are localized within Alu-rich clusters on the same or different chromosomes, or otherwise unknown features of individual Alu elements that predispose to recurrent recombination events associated with some ►breakpoint cluster regions.

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ALVAC-CEA

Definition

A cancer vaccine constructed from canary pox virus (ALVAC) and combined with the human carcinoembryonic antigen (CEA) gene.

►Carcinoembryonic Antigen (CEA)

Alveolar Soft Part Sarcoma

Definition

Rare tumor of young people comprised of large epithelioid acidophilic cells arranged in alveolar structures separated by thin vessels. The cells show light atypia and contain crystalline inclusions. There are not specific markers.

►Uncertain or Unknown Histogenesis Tumors

AMAP1

Definition

A regulator of the small GTPase Arf6, which is involved in actin cytoskeletal remodeling. AMAP1 is overexpressed in invasive carcinomas and functions in invadopodia by binding to paxillin and ►cortactin.

AME Transcription Factor

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Synonyms

RUNX1/MDS1/EVI1; AML1/EVI-1

Definition

AME is an aggressive oncoprotein (chimeric transcription factor) associated with several types of ►acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative disorders (MPD).

Characteristics

The legendary discovery of chromosomal translocations by Janet D. Rowley in 1972 has revolutionized leukemia research and therapy by allowing biological interrogation and classification of these disorders. Several recurring translocations have been identified and the participating genes cloned and characterized at the molecular level. One such recurring abnormality is the balanced translocation between the long arms of chromosomes 3 and 21, t(3;21)(q26;q22), originally discovered in a patient with

therapy-related chronic myelogenous leukemia (CML) which is classified as an MPD.

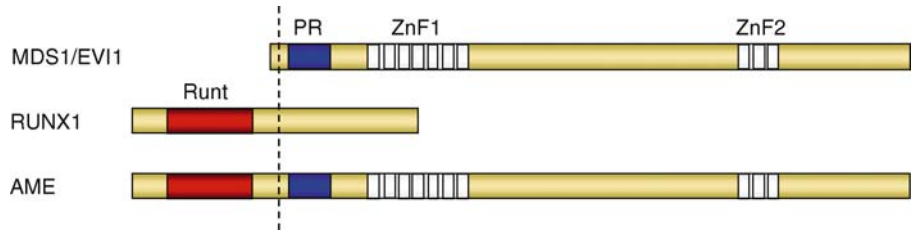
The t(3;21) is a complicated chromosomal rearrangement that employs a mechanism of intergenic splicing to generate several ►fusion genes of which *AME* is perhaps the best characterized and the most important. Among the less frequent translocations involving *RUNX1* (also known as *AML1, CBFA2*, and *PEBP2*) *AME* is the only fusion gene that has been cloned and characterized at the molecular level. *AME,* obtained by in frame fusion of the truncated *RUNX1* and *MDS1/EVI1 (ME)* genes, is controlled by the *RUNX1* promoter which becomes active during the execution of multiple steps of hematopoietic program, especially during the development of myeloid lineage.

The t(3;21) is a relatively rare translocation infrequently seen in *de novo* leukemias. It was observed in ~1% of AML, MDS and MPD cases, and often associated with secondary leukemia that arises in patients previously treated with ►alkylating agents or topoisomerase inhibitors for other malignancies. In particular, the t(3;21) was detected in the patients after administration of cytostatic drugs such as busulphan, teniposide, etoposide, hydroxyurea, ►fludarabine, ►5-fluorouracil and others. There is no unique clinical picture of t(3;21)-associated leukemias such as restriction to a certain FAB (French-American-British classification) category and it has been classified as M1, M2, M4 and M7 subtypes. The common morphologic feature of t(3;21)-positive AML is minimally differentiated blasts with prominent nucleoli and scant cytoplasm. There is no age or gender specificity for t(3;21)-associated diseases but, as for many other ►cancers, older individuals are at higher risk. In contrast to many other translocations, the t(3;21) causes a very aggressive myeloid leukemia/►blast crisis of CML characterized by a low response to the existing therapeutic treatments and a poor prognosis. In the largest clinical investigation of t(3;21) patients published to date, the majority of AML patients died between 1 week and 8.5 months (median 2 months) after presentation whereas MPD patients survived 1–21 months (median 6.5 months) after presentation.

RUNX1 is a DNA-binding subunit of the transcription factor CBF which is essential for hematopoiesis and is involved in several chromosomal abnormalities associated with human leukemias. RUNX1 consists of an N-terminal DNA-binding domain called Runt with homology to the product of the *Drosophila* segmentation gene *Runt* and a C-terminal activation domain. ME is a zinc-finger transcription factor related to the leukemia-associated protein ecotopic viral integration 1 site (EVI1) of unknown function. ME contains a conserved N-terminal region, called PR domain, two sets of DNA-binding zinc finger domains, a proline-rich central domain, and an acidic C-terminal domain. AME consists of the DNA-binding domain Runt of RUNX1 fused to almost the entire ME (Fig. 1).

Forced expression of AME upregulates the cell cycle and blocks granulocytic differentiation of the murine hematopoietic cell line 32Dcl3, and delays the myeloid differentiation of normal murine bone marrow progenitors *in vitro*. The exact mechanisms of AME oncogenic activation are unknown and several possibilities exist. Also, as with many other oncoproteins, most probably AME alone is insufficient to transform a healthy normal cell into leukemic one and additional cooperating genetic abnormalities are necessary. It has been shown that the majority of AME-positive patients have, in addition to t(3;21), several other chromosomal abnormalities readily detected by cytogenetic analysis: translocations, deletions and duplications the most common of which is t(9;22)(q34;q11) found in CML patients.

One of the first investigated properties of AME was its effect on a subset of target promoters regulated by both parent proteins. RUNX1 is generally considered a transcription activator through its C-terminus, which interacts with several transcription coregulators and regulates critical genes in hematopoiesis. ME is also considered a transactivator, and both parent proteins act as antagonists of AME. Therefore, it was suggested that AME could act as a bifunctional transcription factor possessing the ability to bind to and repress/deregulate both the RUNX1- and ME-dependent promoters. In support of this hypothesis, it was shown that AME directly interacts with the corepressors C-terminal binding protein (CtBP) and ►histone deacetylase 1 (HDAC1) which are often a



AME Transcription Factor. Figure 1 Diagram of ME, RUNX1 and the fusion protein AME. The Runt, PR and two zinc finger (ZnF) domains are shown. The vertical dashed line indicates the breakpoint fusion.

part of big repressor complexes transiently formed at the promoter sites. AME has distinct regions for HDAC1 and CtBP binding and, taking in consideration that both corepressors are able to dimerize and interact to each other, one AME molecule can recruit several molecules of the corepressors. AME represses the target promoters by CtBP-dependent and CtBP-independent mechanisms, probably reflecting the dual nature of this protein. *In vitro* CtBP enhances not only AME repression potential but also the ability of AME to upregulate growth and deregulate differentiation in murine hematopoietic cells, suggesting that AME repression is necessary for its oncogenic activity. However, the transcription properties of AME are more complicated because it also interacts with histone acetyltransferases p300/CBP-associated factor (P/CAF) and general control of amino-acid synthesis 5-like (GCN5), which are generally considered as co-activators of transcription. Both P/CAF and GCN5 efficiently acetylate the central region of AME *in vivo*, but the function of this modification and its role in oncogenesis are still unknown.

Similar to many other fusion proteins that are activated by chromosomal translocations in human leukemia, AME is able to oligomerize and displays a complex pattern of self-interaction that involves at least three oligomerization regions, which are the proximal and the distal zinc finger domains and the Runt domain. The distal zinc finger domain is quite important in AME oligomerization because it mediates the interaction with the other two domains and an internal deletion that removes the three zinc finger motifs virtually sufficient to repair (though not completely) the self-renewal and differentiation programs of normal murine bone marrow progenitors *in vitro*. *In vitro*, this domain efficiently co-operates with CtBP in disrupting normal hematopoiesis and the internal deletion and a point mutation that abolishes CtBP binding re-establishes almost completely the hematopoietic differentiation in murine cells. Probably AME belongs to a growing group of chimeric transcription factors which inappropriately maintain high local concentration of corepressors at the specific promoter sites because of their ability to oligomerize, resulting in the deregulation of genes involved in differentiation, ►apoptosis, and proliferation.

It is highly possible that the aggressiveness of AME as an oncoprotein is in part mediated by AME ability to abrogate the growth-inhibitory effect of ►transforming growth factor β (TGF-β) that controls cell expansion and inhibits proliferation of different cell types. The repression of TGF-beta signaling depends on the ability of the proximal zinc finger of AME directly interact with and repress ►Smad3, an intracellular mediator of TGF-β signaling. It should be noted that in contrast to AME, ME co-operates with TGF-β and increases the sensitivity of hematopoietic cells to its stimulus.

AME is also indirectly involved in deregulation of the hematopoietic program. It has been shown that CCAAT/enhancer-binding protein α (C/EBPα), a crucial transcription factor for normal granulopoiesis, is suppressed at translation level by more than 90% in AME-expressing U937 cells. In AML patients harboring t(3;21) the C/EBPα level is reduced even more whereas in AML patients without the t(3;21) C/EBPα is not affected. The mRNA levels remain unchanged in both cases indicating that AME does not affect C/EBPα transcription. Most probably AME acts through an intermediate effector, ►calreticulin, a ubiquitous multifunctional calcium-binding protein, which expression is strongly correlated with both, AME expression and C/EBPα suppression.

It has been shown in reporter gene assays and in Rat1 fibroblasts that AME stimulates activator protein 1 (►AP-1) activity with dependence on the distal zinc finger domain. AP-1 activation may increase cell proliferation potentially contributing to AME oncogenic properties.

A ►mouse model of AME-positive leukemia, generated by bone marrow transplantation of *AME*-expressing cells using BALB/c mice, showed that AME induces acute myeloid leukemia with a latency of 5–13 months indicating that additional genetic abnormalities are necessary for leukemogenesis. The disease was clonal in origin and resembled human acute myelomonocytic leukemia (AML FAB-M4). It has been also shown in this model that AME efficiently co-operates with breakpoint cluster region/abelson tyrosine kinase (►BCR/ABL), a product of t(9;22) frequently seen in CML patients. Both proteins together are able to block myeloid differentiation during the pre-leukemia stage and induce AML within 1–4 months.

The second mouse model for AME utilized bone marrow infection and transplantation using C57BL/6 mice. The animals displayed a variety of clinical features that are observed in essential thrombocythemia (ET) that resulted in their death after 8–16 months. The molecular etiology of ET, which is classified as an MPD, is poorly understood. Recently an activating somatic point mutation (V617F) of Janus kinase 2 (Jak2) was identified in MPD patients. Nonetheless, this mutation was not detected in ~50% of ET patients, indicating that some other molecular mechanisms exist and t(3;21) could be one of them.

The differences between these two mouse models can be explained by taking into consideration that the BALB/c strain of mice is well known to have a higher tumor incidence as compared with C57BL/6 mice (because it has a mutated *inhibitor of Cdk4/alternative reading frame (INK4a/ARF)* locus that at least partially disables $p16^{Ink4a}$, a ►tumor suppressor protein which is frequently mutated in many cancers).

A mouse model of AME knock-in has been also reported. The heterozygous mutant embryos obtained

by breeding of AME chimeric male (ICR strain) and wild type female (C57BL/6 strain) were not viable and died of fetal liver hematopoiesis failure at around day 13.5E. Fetal liver hematopoietic progenitor cells from these mice displayed increased self-renewal capacity and impaired erythropoiesis. In addition, myeloid and megakaryocytic cells appeared dysplastic indicating that AME induces multiple defects in several myeloid lineages. Interestingly, the majority of AME chimeric mice demonstrated sudden death at the age of about 7 months without any significant signs of any disease whereas one of them developed a disease resembling megakaryoblastic leukemia at 5 months of age.

Since 1987, when the t(3;21) was described for the first time, our knowledge about AME has increased vastly, however the prognosis of patients with this abnormality is still extremely poor. Hopefully, the cumulative efforts of different research groups will provide new approaches for the search of a treatment for this selected group of patients.

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Amenorrhea

Definition

Absence or cessation of menstruation.

►Granulosa Cell Tumors
►Prolactin

Ames Assay

Definition

Ames assay comprises a family of widely used bacterial assays to screen for mutagenicity. Bacteria are genetically altered so that they are sensitive to mutagenic damage. Bacterial cell death due to mutagen is the assay readout.

►ADMET Screen

Amidation

Definition

A process in which glycoxylate is removed from a precursor peptide (glycine-extended form) and an α-amide group is added. In cells this process is catalyzed by the amidation enzyme complex peptidyl-glycine α-amidating monooxygenase (PAM).

►Adrenomedullin

Amifostine

Definition

A cytoprotective ►adjuvant used in cancer chemotherapy involving DNA-binding chemotherapeutic agents. It is also used to decrease the cumulative nephrotoxicity associated with cisplatin and cyclophosphamide.

►Chemoprotectants

Amine Oxidases

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Definition

Amine oxidases (AOs) are a class of enzymes which is heterogeneous in terms of structure, catalytic mechanisms, and substrate specificity. ►Biogenic amines, mono, di, and polyamines as well as *N*-acetyl amines, are oxidatively deaminated by AOs in a reaction that consumes O_2 to produce the corresponding aldehydes,

ammonium ions, and ►hydrogen peroxide (H_2O_2) according to the following reaction:

$$E_{ox} + R - CH_2 - NH_3^+ \rightarrow E_{red} - NH_3^+ + RCHO$$

$$E_{red} - NH_3^+ + O_2 \rightarrow E_{ox} + NH_4^+ + H_2O_2$$

(E_{ox} = oxidized enzyme E_{red} = reduced enzyme)

Characteristics

Two classes of AOs can be described, which contain different prosthetic groups: the FAD-dependent AOs (FAD-AOs) containing the flavin adenin dinucleotide (FAD), and the copper-dependent AOs (Cu-AOs) containing copper and an organic cofactor produced by the copper self-catalyzed posttranslational oxidation of a tyrosine residue, i.e., TPQ (trihydroxyphenylalanine quinone).

The FAD-AOs are subdivided in monoamine oxidase A and B (MAO A, MAO B), polyamine oxidase (PAO) and the recently discovered spermine oxidase (SMO). The two latter enzymes are cytosolic, catalyze the oxidation of secondary amino groups, and participate in the interconversion metabolism of polyamines. MAOs are tightly bound to a component of the mitochondrial outer membranes.

Cu-AOs are often also named SSAO (semicarbazide sensitive amine oxidase) because of their inhibition by semicarbazide, which binds the organic cofactor. When strictly necessary, the name of the best substrate is used to characterize the enzymes. For instance, Cu-AOs, which oxidize diamine, histamine, and elastin, are named diamine oxidase (DAO), histaminase, and lysyl oxidase (LXAO), respectively. LXAO contains, instead of TOPA quinone, lysyl tyrosyl quinone. Sometimes, a single enzyme, such as the enzyme purified from pig kidney, may display both DAO and histaminase activities, so that the name may not imply a specific enzyme.

The X-ray structure is available for several Cu-AOs, PAO, and MAO B.

Functions

A plethora of physiological functions, sometimes in contrast with one another, is ascribed to AOs. Although the exact molecular mechanism of their biological activity is not well-defined, a role of these enzymes in various processes through the action of either substrates or reaction products is postulated. Evidences have accumulated on the physiopathological relevance of polyamines, histamine, hydrogen peroxide, and aldehydes in cell death and ►differentiation, ►allergic diseases, and ►postischemic reperfusion damage.

Histamine is considered to be a main factor involved in allergic diseases. A plant Cu-AO, showing high histaminase activity, counteracts acute allergic asthma-like reaction in actively sensitized guinea pigs. The same enzyme modulates cardiac anaphylactic response in guinea pig. Protective effects of the plant enzyme were also observed in myocardial ischemia and reperfusion injury in in vivo rats. Bovine serum Cu-AO was shown to present an antioxidant effect, in vitro, against electrolytically induced ►reactive oxygen species (ROS) (O_2^-, OH, $^1O_2$) . Among other physiopathological functions ascribed to AOs are, for example, the involvement of MAO in psychiatric diseases like schizophrenia, by regulating the dopamine metabolism, and of Cu-AOs in cataract, by the lens damaging effect of amine oxidation products.

A primary involvement of AOs was demonstrated in cancer growth inhibition and progression, especially by means of aldehydes, H_2O_2, and other ROS, the AOs-mediated products of biogenic amines oxidation. Aminoaldehydes were shown to interact with nucleotides or with DNA. Microinjection of Cu-AO into chick embryo fibroblast, rat cells, and glioma cells caused the inhibition of ►DNA damage and protein synthesis. Tumor cells, with higher polyamines content than the normal controls, were more sensitive to the injected AOs. When an immobilized Cu-AO was injected into the peritoneal cavity of Swiss mice, 24 h after viable Ehrlich ascites tumor cells (►ascites) transplantation or into a mouse (►melanoma) model, a strong inhibition of tumor growth was observed. An induction of tumors in rat bowels (►colon cancer) was observed on inhibition of DAO by aminoguanidine. An induction of tumors in rats was observed after carcinogenic treatment combined with PAO inhibition. Both H_2O_2 and aldehydes contribute to cytotoxicity, as demonstrated by incubation of Chinese hamster ovary cells with purified bovine serum AO in the presence of spermine. Catalase, the enzyme involved in H_2O_2 elimination, is absent in many tumor cells and thus ►apoptosis occurs. The direct relationship between AOs, apoptosis, and cancer appears to be related to the regulation of biogenic amines and their metabolic products. H_2O_2 is considered to be a mediator of apoptotic cell death but the mechanism is unclear. H_2O_2 produced by MAO-catalyzed monoamines oxidation seems extremely important for apoptosis induction by considering the fact that MAO inhibitors are able to prevent apoptosis in human melanoma cells and that catalase inhibits the apoptosis induced by polyamines or their analogs and cathecolamines. The catalytic products of active amine oxidation are strong inducers of mitochondrial ►membrane permeability transition (MPT). Taken together, these results indicate that active amines, operating as AO substrates, play a critical role in controlling apoptosis through their effects on MPT and the ►respiratory chain activity by means of fluctuations in their concentrations. The conclusions of the above results may be that apoptosis is induced by polyamines through their oxidation products. Other studies exist demonstrating instead the ability of polyamines to protect

cells from apoptosis. This discrepancy can be explained by taking into account the protective effect of the same polyamines, probably due to a scavenging action of ROS.

A crucial role of AOs in cancer promotion has also to be considered. High levels of DAO activity were occasionally found in rapidly growing tissues, while in some patients, even affected by metastatic tumors, the level of circulating DAO was unaltered. A strong correlation between serum AO activity and the factor responsible for ►angiogenesis was recently found in non-small cell lung cancer patients. DAO activity in the small intestine mucosa was reported to increase in parallel with the degree of cell maturation, being highest in differentiated villus tip cells and lowest in the proliferative compartment. It was also found to increase in regenerating rat liver, with a peak between 16 and 48 h after partial hepatectomy. DAO activity peaks at the outset of growth and falls during the logarithmic growth phase of the cells. An increasing degree of malignancy associated with an increase of MAO A activity and decrease of MAO B and Cu-AOs activities in chemically-induced mammary cancer in the rat has been observed. Elevated activity of AO was found in skeletal metastases of prostatic cancer (►Prostate cancer, clinical oncology). DAO and arginase, an enzyme that catalyses the synthesis of ornithine from arginine, increase in tumor tissues as compared with benign prostatic hyperplasia. A linear correlation between arginase and DAO activities was observed in patients with cancer.

A high concentration of PAO and DAO was found in the cervical intraepithelial neoplasia. The rise from normal conditions seems to produce cytological changes and to play a role in the aethiology of ►cervical cancer. DAO activity is present at high levels both in tumor tissues and in biological fluids of tumor-bearing subjects. A correlation between the degree of tumor malignancy and their levels of AO activity has been observed in astrocytomas, where the activity is proportional to the degree of malignancy. The oxidation products of biogenic amines should also be carcinogenic. Acrolein, produced from the oxidation of spermine and spermidine by AOs, appears to be both carcinogenic and cytotoxic. This compound is considered to be a component of a universal cell growth regulatory system. It may act as mediator of cell transformation under oxidative stress when cells are pretreated with benzopyrene, a major carcinogenic found in cigarette smoke. The oxidation products of spermine, spermidine, and putrescine should be cofactors in the development of cervical cancer.

The balance between the cell content of biogenic amine oxidizing enzymes and antioxidizing enzymes appears to be a crucial point for cancer inhibition or progression. As a general conclusion, the cancer inhibition/promotion effect of AOs might be explained by taking into consideration the full pattern of the enzymes contained in the cells. A long-lasting imbalance of antioxidizing enzymes and AOs activity may be carcinogenic, while AOs are rapidly cytotoxic for cancer cells, because of their higher biogenic amines concentration in comparison with normal cells.

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D-Amino Acid

Definition

Is an optical isomer that is the mirror-image of the corresponding L amino acid. Only L amino acids are constituents of natural proteins.

►Lactoferricin Antiangiogenesis Inhibitor

Amino Terminal End

Definition

The amino terminus (N-terminus) is the end of a polypeptide chain that carries an unreacted amino group. A ribosome synthesizes a polypeptide in the direction from the amino terminal end to the carboxyl terminal end.

►AAMP

Amino-Bisphosphonate

►Minodronate

Aminoflavone

Definition

5-Amino-2,3-fluorophenyl-6,8-difluoro-7-methyl-4*H*-1-benzopyran-4-one. New cytostatic drug entering clinical trials. It requires bioactivation by ►cytochromes P450 and sulfotransferases (SULTs). Its growth-inhibiting activity in 60 human tumor cell lines was primarily determined by the level of expression of SULT1A1.

►Sulfotransferases

5-Aminolevulinic Acid

Definition

Is the precursor of protoporphyrin IX in the heme synthetic pathway. It is used as a photodiagnostic agent.

►Fluorescence Diagnostics

δ-Aminolevulinic Acid

Definition

A member of the heme biosynthesis pathway and a precursor of the photosensitizer protoporphyrin IX (PpIX). PpIX is the last reaction step before heme and its photochemical and fluorescent properties are used in photodynamic therapy/►fluorescence diagnosis for treatment/diagnosis of malignant and nonmalignant diseases.

►Photodynamic Therapy

AML

Definition

►Acute myeloid leukemia.

►ETV6
►Childhood Cancer

AML1

►Runx1

AML1/ETO

►Chromosomal Translocation t(8;21)

AML-1/ETO/CBFβ/TEL in Chromosomal Translocations

Definition

AML-1 has been renamed RUNX1. ETO is also known as MTG8 and is part of the RUNX1-MTG8 translocation, previously known as AML1-ETO. CBFβ binds RUNX1. TEL is also known as ETV6 and occurs in the t(12;21) translocation with RUNX1.

►RUNX1
►Chromosome Translocation
►Acute Myecloid Leukemia

AML1/EVI-1

►AME Transcription Factor

AML1/MTG8

►Chromosomal Translocation t(8;21)

AMN107

►Nilotinib

Amosite

Definition

Is an amphibole form of ►asbestos. The term refers to Asbestos Mines of South Africa. This mineral contains 31% iron; when inhaled, it is highly carcinogenic.

Amph II

►Bin1

Amphibian Gastrin-Releasing Peptide

►Gastrin-Releasing Peptide

Amphipathic

Definition

Amphipathic molecules contain both a hydrophilic and a hydrophobic moiety.

Amphiphysin II

►Bin1

Amphiphysin-like

►Bin1

Amphiregulin

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Synonyms

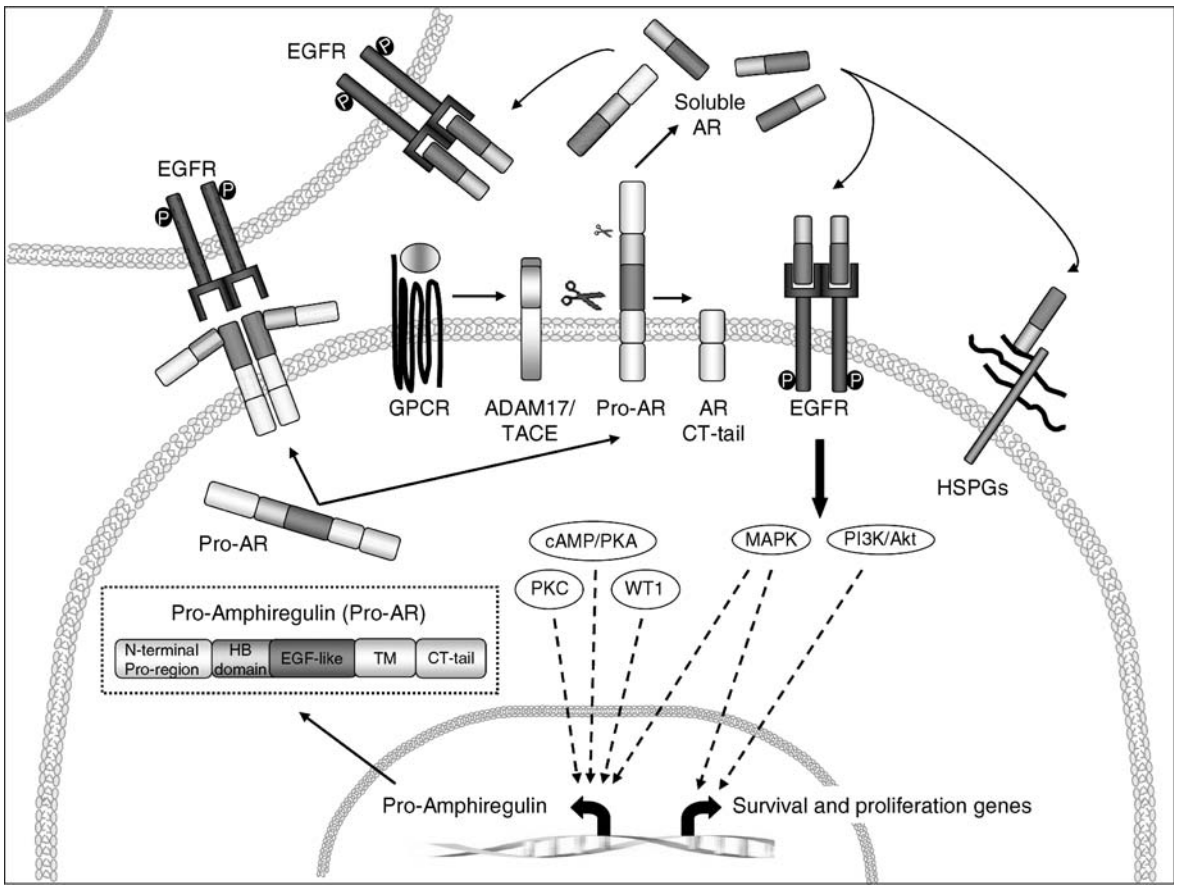
Schwannoma-derived growth factor; SDGF

Definition

Amphiregulin (AR) is a growth factor that belongs to the ►epidermal growth factor receptor (EGFR) family of ligands. AR was originally described as a regulator of cell growth present in the conditioned media of MCF-7 breast tumor cells. AR has been implicated in different physiologic processes including mammary gland and bone development, lung and kidney branching morphogenesis, and trophoblast growth. The expression of AR is upregulated in a variety of cancerous tissues, and signaling triggered by AR is believed to be important in tumorigenesis.

Characteristics

The AR human gene spans 10 kb in the genomic DNA and it is composed of six exons, upon transcription it produces a 1.4 kb mRNA. AR gene shows broad constitutive expression, being more prevalent in human ovary and placenta although it is also expressed in pancreas, cardiac muscle, testis, colon, breast, lung, spleen, and kidney, whereas it is undetectable in liver. Transactivation of AR promoter and AR gene expression can be induced by the ►Wilms' tumor suppressor and through the activation of the ►protein kinase c (PKC), mitogen associated protein kinase (►MAPK), and ►cyclic AMP/protein kinase A (cAMP/PKA) pathways (Fig. 1). AR is synthesized as a 252-amino acid transmembrane glycoprotein, also known as transmembrane precursor or pro-form (Pro-AR) (Fig. 1). Pro-AR consists of a hydrophilic extracellular N-terminus (or ectodomain), a hydrophobic transmembrane domain (TM), and a hydrophilic cytoplasmic C-terminus (CT-tail) (Fig. 1). In the extracellular N-terminus we can distinguish an N-terminal pro-region containing glycosylation sites followed by a heparin-binding domain and an EGF-like region (Fig. 1). The EGF-like region is



Amphiregulin. Figure 1 Transcription of the AR gene can be activated in response to the WT1 protein and the PKC, cAMP/PKA or MAPK signaling pathways. AR is synthesized as a membrane-anchored precursor (Pro-AR) encompassing an EGF-like domain, a heparin-binding domain (HB), a transmembrane region, and a carboxy-terminal cytosolic tail (CT-tail). Upon digestion by the protease TACE/ADAM17, soluble AR forms are shed from the cell surface and can interact with the EGFR in an autocrine or paracrine fashion, or bind to heparan-sulfate proteoglycans (HSPG) in the extracellular millieu. Alternatively, the yuxtacrine interaction of membrane-anchored Pro-AR with the EGFR is also possible. Shedding of AR by TACE/ADAM17 can be enhanced in response to activation of ►G-protein coupled receptors (GPCRs). Binding and activation of the EGFR by AR triggers growth and survival signals essential for the tumor cell.

shared by other members of the ►EGF family of ligands. At the plasma membrane Pro-AR undergoes proteolytic cleavage to release the mature soluble factor in a process known as "ectodomain shedding." Cleavage of Pro-AR at two N-terminal sites gives rise to two major soluble forms of ~19 and ~21 kDa. Alternatively, Pro-AR cleavage can produce a larger 43-kDa soluble protein corresponding to the entire extracellular domain. Cleavage of Pro-AR at the cell surface can be mediated by tumor necrosis factor-α converting enzyme (TACE), a member of the disintegrin and metalloproteinase (ADAM) family also known as ►ADAM17 (Fig. 1). Shedding of AR allows the autocrine or paracrine interaction of the mature ligand with its cognate receptor, the ►EGFR (also known as ErbB1), a transmembrane protein endowed with tyrosine kinase activity, although yuxtacrine interaction between membrane-bound Pro-AR and the EGFR has also been observed (Fig. 1). Binding of AR to EGFR triggers key intracellular signaling pathways, such as the mitogenic MAPK and survival ►PI3K/Akt pathways, which have been demonstrated to participate in the transduction of AR effects (Fig. 1).

Amphiregulin Structure, Expression, and Function

AR was originally identified as a factor capable of inhibiting the growth of certain carcinoma cell lines, while stimulating the proliferation of normal cells, a fact that motivated its denomination. In fact, depending on its concentration and the nature of the target cell AR promotes the growth and survival of most cell types, both normal and transformed. AR gene overexpression

has been frequently demonstrated in cancerous tissues like colon, breast, bladder, prostate, pancreas, lung, ovary, squamous cell carcinomas, hepatocarcinoma, and myeloma cells. Besides changes in AR gene expression, different stimuli can also influence the availability of this growth factor through the stimulation of Pro-AR cleavage at the cell membrane. This is achieved by the activation of TACE/ADAM17 in response to agonists acting through GPCRs in a process termed ►EGFR transactivation (Fig. 1). The existence of EGFR transactivation involving the release of AR has been demonstrated in a variety of cancer cells, suggesting that AR could be an important mediator between diverse stimuli acting on GPCRs and the activation of protumorigenic signals conveyed through the EGFR. Interference with AR production by means of specific antisense RNAs or ►siRNAs, or treatment with AR neutralizing antibodies, has been shown to revert many of the neoplastic phenotypic traits of cancer cells in vitro, even though the expression of other EGFR ligands was preserved in these cells. This suggests that AR plays a nonredundant role in carcinogenesis. Observations performed in vivo also lend support to a role for AR in the initiation and maintenance of the neoplastic properties of tumor cells. For instance, tissue-specific transgenic overexpression of AR in pancreas results in enhanced cell cycle progression, and in mice older than 1 year it induces dysplastic changes and premalignant alterations. Although, so far most of the evidences that support a role for AR in cancer development and progression have been gathered under experimental conditions, there are also clinical studies that point in the same direction. In this regard, a significant correlation has been established between elevated tumor tissue AR mRNA levels and poor survival in bladder carcinoma patients, or elevated serum AR concentrations and increased mortality in non-small cell lung cancer patients.

In summary, the current knowledge on AR in cancer suggests that increased availability of this growth factor can provide transformed cells with a selective advantage. Targeted inhibition of AR expression or action may therefore represent a useful therapeutic strategy for a wide variety of cancers.

►Epidermal Growth Factor (EGF)-Like Ligands

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Amphitropic Proteins

Definition

►Peripheral Membrane Proteins.

AMPK

Definition

Synonym ►5'AMP-activated protein kinase is a fuel-sensing enzyme that plays a central regulatory role in cellular energy metabolism. It stimulates fatty acid oxidation and glucose uptake, inhibits cholesterol and triglyceride synthesis, and modulates cell growth and death.

►Adiponectin
►Autophagy

AMPL

►Bin1

Amplaxin

►Cortactin

A

Amplification

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Definition

Amplification is the selective increase in DNA copy number either intracellularly, as a local genomic change, or experimentally, by polymerase chain reaction (►PCR). Increase in the level of mRNA or protein should not be referred to as amplification.

Characteristics

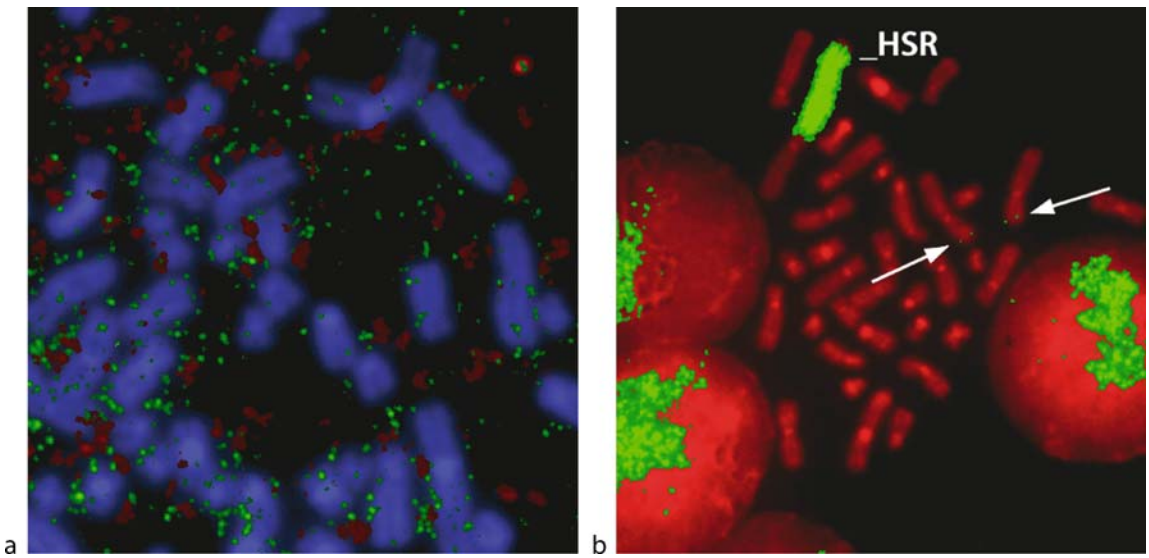
Intracellular amplification results in a selective increase in gene copy number with the consequence of elevated gene expression. Gene amplification has been seen in three different settings

- Scheduled amplification as part of a developmental gene expression program, e.g. chorion genes in ovaries of the fruitfly *Drosophila melanogaster* or actin genes during myogenesis in the chicken
- Unscheduled amplification during acquisition of cellular ►drug resistance. For example; amplification of the gene encoding dihydrofolate reductase (*DHFR*) can result in up to 1,000 gene copies per cell with the consequence of cellular resistance against methotrexate.
- Unscheduled amplification of cellular genes involved in growth control (►oncogenes) during ►tumor progression. Amplification of oncogenes can result in up to several hundred gene copies and enhanced gene expression. Usually large DNA stretches (from 100 Kb up to several Mb) are amplified, and therefore ►syntenic genes in addition to the particular oncogene can be co-amplified due to their close linkage to the oncogene. Alternatively, different ►non-syntenic oncogenes can amplify independently in the same cell. The prototypic human cancer with oncogene amplification is ►neuroblastoma. Here, the amplified gene, *MYCN*, is a ►biomarker for patient management.

Amplified DNA can be visualized cytogenetically as a ►homogeneously staining region within chromosomes (►HSR), as ►double minutes (►DM) or as ►C-bandless chromosomes (CM) (Fig. 1).

Cellular Regulation

Amplification can follow different pathways, the "onion skin model" and "breakage fusion-bridge" (BFB) cycles (Fig. 2) both fit experimental observations.

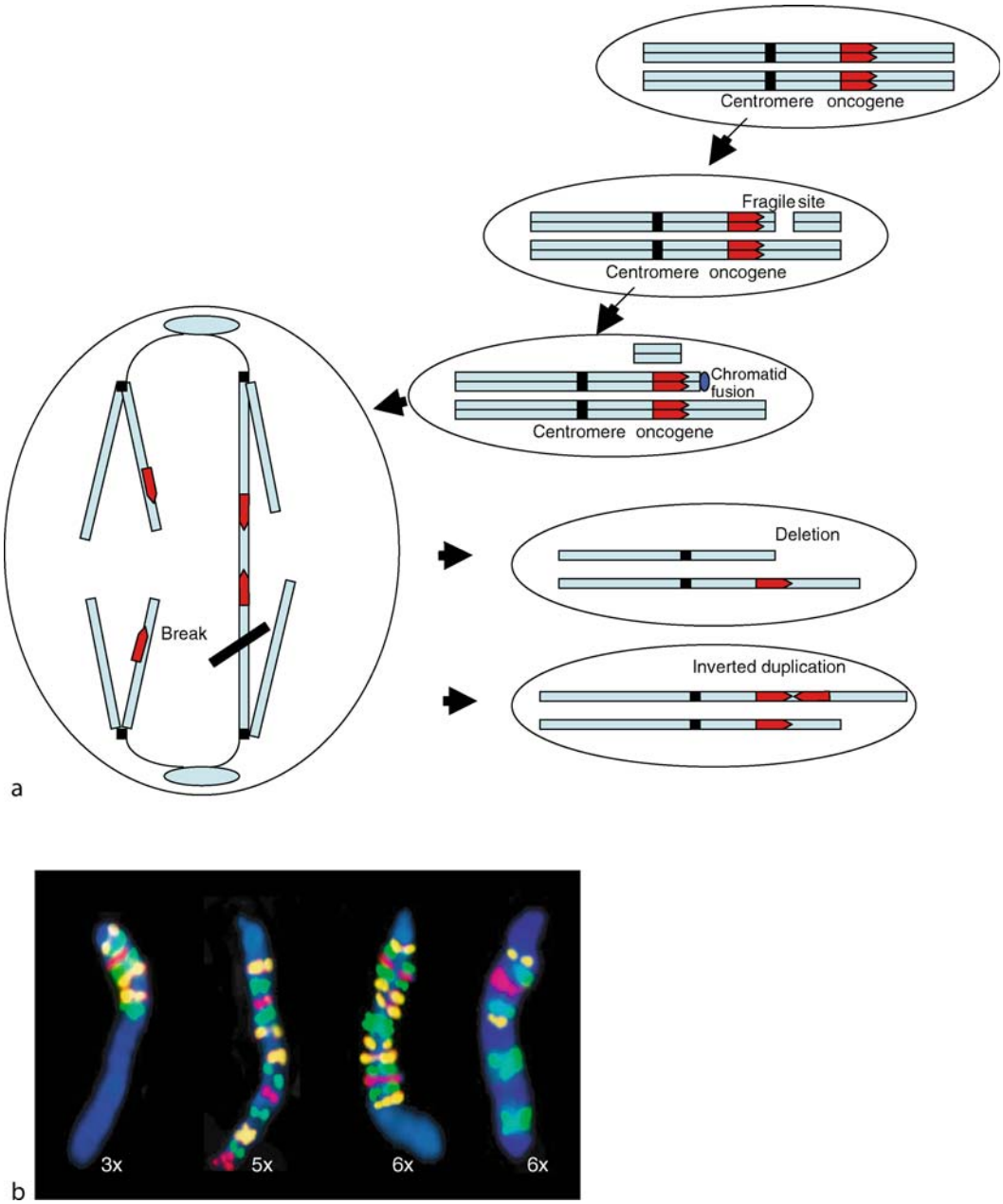


Amplification. Figure 1 Cytogenetics of *MYCN* amplification in neuroblastoma cells. Chromosomal fluorescence in situ hybridization (FISH). High-level *MYCN* amplification appears in human neuroblastoma cells as two alternative cytogenetic manifestations: (a). Double minutes (DMs) (*left*), this tumor cell has in addition to amplified *MYCN* (*red*) amplification of another oncogene *MDM2* (green). The two oncogenes are non-syntenic (2p24, and 12q13–14, respectively), and the amplification is the result of two independent genetic events. (b). Homogeneously staining region (HSR) (*right*), multiple copies are amplified in an HSR on chromosome 12 (with strong signal), while single copy gene is retained on the two parental chromosomes (*arrows*). The retention of *MYCN* at 2p24 indicates that not the original *MYCN* gene but rather a copy, presumably the result of extra-replication, has been amplified. Note also the strong signal in interphase nuclei which allows detection of amplified *MYCN* in tumor biopsies when chromosomes cannot be prepared.

Little is known about genomic or environmental elements involved in amplification. Unscheduled amplification presumably is a sporadic event that can become stabilized under selective pressures, i.e. cytostatic drugs or if cells acquire a growth advantage within a certain tissue architecture.

Clinical Relevance

Resistance against cytostatic drugs poses a big problem in cancer therapy. Amplified oncogenes contribute to tumor progression, many different oncogenes have been found amplified (e.g. ►*RAS*, ►*MYC*, ►*MYCN*, ►*MYCL*, *HER-2* [►*HER-2/neu*], ►*ABL*, etc.), in some



Amplification. Figure 2 Breakage-fusion-bridge (BFB) cycles in early stages of amplification. (a). BFB cycles start from common ►fragile sites, where a DNA break can occur in both ►sister chromatids. DNA repair systems will be recruited to the break and may join the free DNA ends of the two sister chromatids to form a dicentric chromosome, one that has two centromers. At anaphase, where sister chromatids are moved to the daughter cells, the dicentric chromosome at some point will break. Of the two daughter cells, one will carry a deletion, the other an inverted duplication of DNA, which is equivalent to a low-level amplification. By subsequent BFB cycles, the level of amplification can increase. (b). Low level amplification as the result of BFB cycles. FISH image, where each color shows the position and copy-number of a particular DNA sequence.

tumor types the oncogene status provides information about patient prognosis: Amplified *MYCN* indicates poor prognosis for stage 1–3 neuroblastoma; and amplified HER-2 indicates unfavorable outcome in a subgroup of ►breast cancer.

►REL

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Amplified in Breast Cancer 1

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Synonyms

Nuclear receptor coactivator 3; NCoA3; Steroid receptor coactivator-3; SRC-3; Receptor-associated coactivator 3; RAC3; Thyroid hormone receptor activator molecule 1; TRAM-1; Coactivator ACTR; p300/CBP-interacting protein; p/CIP; AIB1

Definition

AIB1 is a 160 kDa intracellular protein that enhances gene expression though interacting with nuclear hormone receptors and some other transcription factors and serving as a transcriptional coactivator. The AIB1 gene is amplified and overexpressed in some human breast tumors.

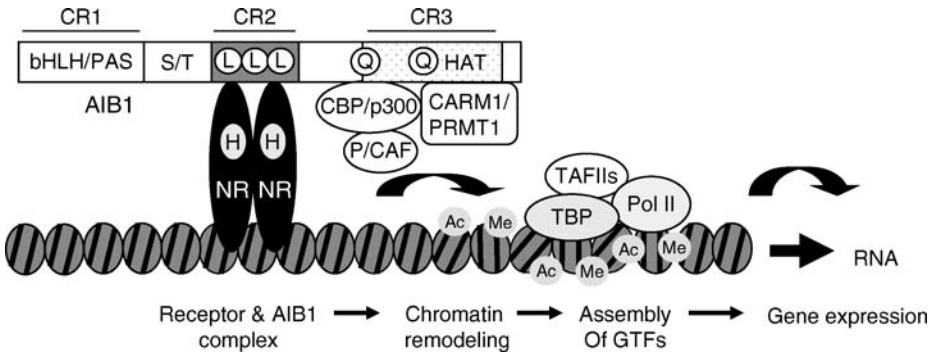
Characteristics

Molecular Structure and Functional Domains

The human AIB1 gene is located in chromosome 20 and it encodes for a 160-kDa intracellular protein with 1402 amino acid residues. AIB1 is a member of the p160 steroid receptor coactivator (SRC) family that also includes SRC-1 and the transcriptional intermediary factor 2 (TIF2). AIB1 contains multiple structural and functional domains (Fig. 1). The N-terminal basic helix-loop-helix/Per-Ah receptor nuclear translocator-Sim (bHLH/PAS) domain is the most conserved region in the molecule with ~70% sequence similarity to the respective regions of SRC-1 and TIF2. The bHLH/PAS domain contains a nuclear localization signal, which is required for AIB1 to get into the cellular nucleus where AIB1-regulated gene transcription takes place and where AIB1 degrades in a proteasome-dependent manner. The bHLH/PAS domain also can interact with certain transcription factors such as myogenin to mediate their transcriptional functions. The serine/threonine (S/T) rich domain contains many serine and threonine residues and some of these residues are targets of serine/threonine kinases. The phosphorylation status of AIB1 is related to its interaction specificity and affinity with transcription factors and other coactivators. A sequence in the S/T domain is also found to interact with transcription factor E2F1. Through interaction and function with ►E2F1, AIB1 can play a role in direct regulation of cell cycle. Following the S/T domain is the second conserved region of AIB1 with ~60% sequence similarity to SRC-1 and TIF2. This region contains three LXXLL (L, leucine, X, any amino acid) α-helix motifs that are responsible for interaction with the ligand-binding domain of nuclear receptors in a hormone binding-dependent manner. The third conserved region located in the C-terminus of AIB1 has ~50% sequence similarity to SRC-1 and TIF2 and contains two poly-glutamine stretches and a weak histone acetyltransferase activity. This domain can steadily interact with CREB (cAMP response element-binding protein) binding protein (CBP) and p300, which are strong histone acetyltransferases. This domain also can interact with the coactivator-associated arginine methyltransferase (CARM1) and the protein arginine methyltransferase 1 (PRMT1), which are histone methyltransferases.

Functional Mechanisms

Two transcriptional activation domains of AIB1 have been identified. The first one is located in the region that interacts with CBP or p300 and the second one is located in the region that interacts with CARM1 or PRMT1 (Fig. 1). The transcriptional activation function of AIB1 is mainly executed through these acetyltransferases and methyltransferases, which are chromatin-remodeling enzymes. In the case of steroid hormone-regulated gene expression, hormone binding triggers a series of events for steroid receptors, including the dissociation of heat shock proteins, change of receptor conformation, receptor dimerization and DNA binding. Importantly, the hormone binding also induces the steroid receptors expose their coactivator-binding motifs in their ligand-binding domains and allows coactivators such as AIB1 to be recruited to the enhancer region of the nuclear receptor target genes. Through the further interaction of AIB1 with CBP, p300, the p300 and CBP-associated factor (p/CAF), CARM1 and PRMT1, a steroid receptor-directed



Amplified in Breast Cancer 1. Figure 1 Schematic presentation of the structure and function of AIB1. CR1, CR2 and CR3, conserved regions 1, 2 and 3 in the p160 SRC family; bHLH/PAS, the basis helix-loop-helix/Per-Ah receptor nuclear translocator-Sim domain; S/T, the serine and threonine-rich domain; L, L and L, the three LXXLL motifs responsible for interaction with nuclear receptors; Q and Q, the two glutamine-rich regions; HAT, the histone acetyltransferase domain; H, hormone; NR, nuclear receptors; CBP, the CREB (cAMP response element-binding protein) binding protein; p300, the 300 kDa protein homologous to CBP; p/CAF, the p300 and CBP-associated factor; CARM1, the coactivator-associated arginine methyltransferase 1; PRMT1, the protein arginine methyltransferase 1; TBP, the TATA binding protein; TAFIIs, TBP-associated general transcription factors (GTFs); Pol II, RNA polymerase II.

transcriptional activation complex is built up on the hormone response elements of their target gene. This protein complex uses its protein acetyltransferase and methyltransferase activities to remodel the chromatin structure, to facilitate the assembly of general transcription factors on the promoter and thereby to promote target gene transcription. In addition to steroid receptors and other nuclear receptors, AIB1 also serves as a coactivator for certain other transcription factors such as E2F1, AP-1 and Ets transcription factors.

Physiological Function

AIB1 mRNA is expressed in many different human tissues and cell lines when examined by Northern blot analysis. Detail analyses with mouse tissues revealed that AIB1 is mainly expressed in the mammary gland epithelial cells, oocytes, vaginal epithelial layer, hepatocytes, smooth muscle cells, endothelial cells, and the hippocampus and olfactory bulbs of the brain. At this time, our knowledge regarding the *in vivo* physiological function of AIB1 is mainly learned from the AIB1 knockout mice. AIB1-deficienct mice have a much lower levels of insulin like growth factor-I and 17β-►estradiol in their circulation. Accordingly, these mice are smaller in size and they exhibit delayed puberty, retarded mammary gland development and reduced female reproductive function. In addition, AIB1 plays a beneficial role in estrogen and ►estrogen receptor-mediated vascular protection after vessel injury by enhancing estrogen receptor function and contributes to the control of acute inflammatory responses by inhibiting the production of pro-inflammatory cytokines.

Role in ►Cancer

The AIB1 gene is amplified (or increased in the number of gene copies) in about 5–10% human breast tumors. The AIB1 mRNA is overexpressed in about 30–60% breast tumors, depending on the resources of reports. However, some study only found about 10% of breast tumors that have elevated AIB1 protein levels. AIB1 overproduction is observed in breast tumors both positive and negative to the estrogen receptor α. In ►tamoxifen-treated patients, high levels of AIB1 are associated with the HER2/Neu expression, the tamoxifen resistance and the lower disease-free survival rates. In the cultured ►breast cancer cells, AIB1, together with the estrogen and estrogen receptor, enhances ►cyclin D1 expression and cell cycle progression. Down regulation of AIB1 in breast cancer cells inhibits cell proliferation, cell motility and anchorage-independent growth in the culture and tumor formation in the immune-deficient mice. Animal experiments further demonstrate that AIB1-deficient mice are resistant to either transgenic ►oncogene- or chemical ►carcinogen-induced mammary gland tumorigenesis. The transgenic v-Ha-ras oncogene can no longer induce mammary gland tumors in the ovariectomized AIB1 knockout mice, suggesting that inhibition of AIB1 function and removal of ovarian ►hormones may be a potential strategy to control breast tumorigenesis. On the other hand, it has been demonstrated that overexpression of AIB1 in the mouse mammary epithelial cells is sufficient to induce a high frequency of mammary gland tumors, indicating that AIB1 is an oncoprotein. Similar to the role of AIB1 in breast cancer, AIB1 is also found to be overexpressed in certain human prostate

tumors and to play a detrimental role in prostate epithelial tumorigenesis in mouse models.

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Amrubicin

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Synonyms

(+)-(*7S,9S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-erythro-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride; SM-5887

Definition

The anthracyclines tested clinically so far have been limited to those produced by fermentation or semisynthetic processes. In contrast, 9-aminoanthracycline, amrubicin is a fully synthetic drug. Amrubicin differs from daunosamine in that it contains a 9-amino group and a simple sugar moiety (Fig. 1).

Characteristics

Amrubicin is converted to its active 13-hydroxy metabolite, amrubicinol, in the liver, kidney, and tumor tissue, through reduction of its C-13 ketone group to a hydroxy group. Despite the similarity of its chemical structure to that of a representative anthracycline, doxorubicin, amrubicin's mode of action differs from that of doxorubicin. Amrubicin and amrubicinol are inhibitors of DNA topoisomerase II, which exert their cytotoxic effects by stabilizing a topoisomerase II-mediated cleavable complex (►Topoisomerase enzymes as drug targets), and are approximately only one-tenth as potent as doxorubicin in producing DNA intercalation.

Preclinical Studies

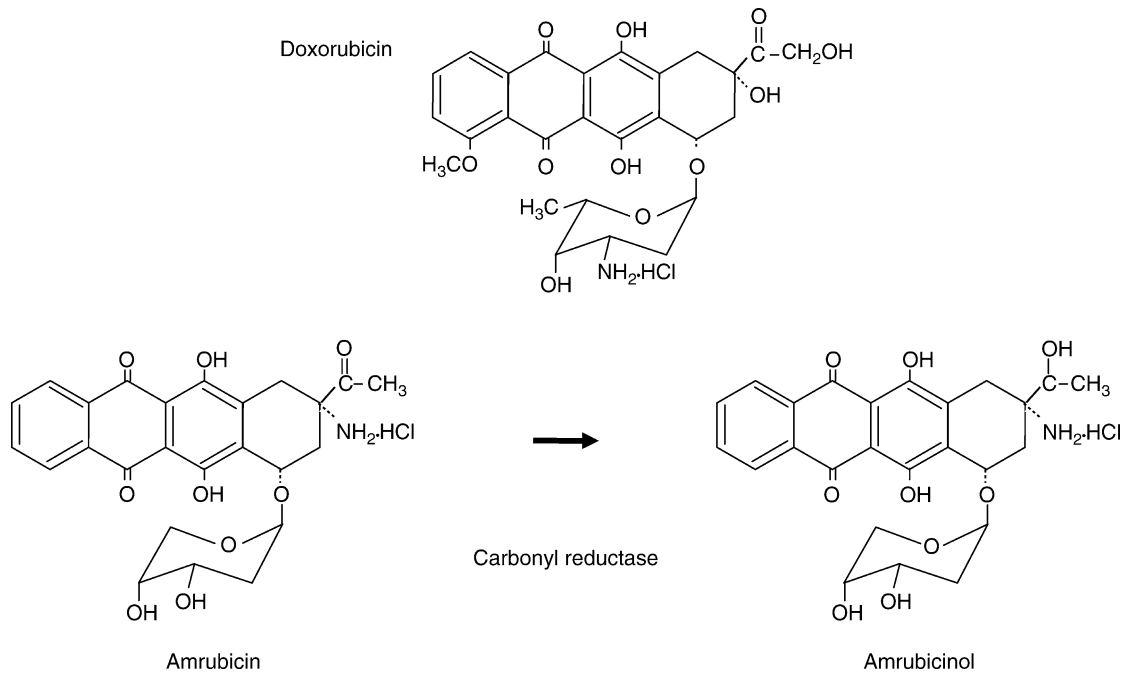
In in vitro experiments, amrubicin and its metabolite amrubicinol have been found to be active against a broad spectrum of human cell lines established from cancers of the lung, prostate, urinary bladder, colon, kidney, pancreas, and uterus. Amrubicinol has been shown to exhibit a 20- to 220-fold more potent antitumor activity in vitro than amrubicin itself, being as potent as doxorubicin. In addition, amrubicin and amrubicinol have also been demonstrated to show some degree of noncross resistance with doxorubicin.

Amrubicin has been shown to be more effective against five human xenografts (one breast cancer, one ►lung cancer, and three gastric cancers), equally effective against two gastric cancers, and less effective against two tumor (one lung and one gastric cancer). Amrubicin caused dose-dependent weight loss, ataxia, myelosuppression, and hair loss in mice after a single intravenous (i.v.) injection. The maximum tolerated (MTD) for such administration was estimated to be 25 mg/kg in four mouse strains. Cardiotoxicity is one of the dose-limiting toxicities of anthracyclines. However, amrubicin showed little delayed-type cardiotoxicity in rabbit and dog experimental models. Furthermore, amrubicin did not aggravate the doxorubicin-induced myocardial injury.

Clinical Studies

Amrubicin Monotherapy

Phase I/II Trial of Amrubicin Given Daily for Three Consecutive Days Every 3 Weeks. Based on the finding that amrubicin exhibited enhanced antitumor efficacy against six of eight cell lines when it was given for five consecutive days instead of on a single day at the MTD dose, a phase I/II trial of amrubicin for three consecutive days was carried out in patients with advanced nonsmall cell lung cancer (NSCLC). In the phase I study, four patients were enrolled at dose level 1 (40 mg/m^2/day) and four at dose level 2 (45 mg/m^2/day). No dose-limiting toxicity (DLT) was observed at these dose levels. At dose level 3 (50 mg/m^2/day), three of five patients experienced DLTs (leukopenia, neutropenia, thrombocytopenia, or gastrointestinal toxicities). The MTD and recommended dose (RD) were determined to be 50 and 45 mg/m^2/day, respectively, from the results of this trial. Seven partial responses (PR) were observed in a total of 28 patients of the phase I/II study, with an overall response rate of 25%.



Amrubicin. Figure 1 Chemical structure of amrubicin and its active metabolite, amrubicinol.

Multicenter Phase II Study of Amrubicin in Patients with Advanced NSCLC. Sixty-one previously untreated patients with stage III or IV NSCLC were entered in this study. Amrubicin was administered by a single i.v. injection daily at the dose of 45 mg/m^2/day for three consecutive days every 3 weeks. One complete response (CR) and 16 PR were observed, with an overall response rate of 27.9%. The median survival time (MST) was 9.8 months. The major toxicity was myelosuppression. The incidences of grade 3 or 4 toxicity were 72.1% for neutropenia, 52.5% for leukopenia, 23.0% for anemia, and 14.8% for thrombocytopenia.

Phase II Study of Amrubicin in Previously Untreated Patients with Extensive-Stage Small Cell Lung Cancer. Thirty-five previously untreated patients with extensive-disease small cell lung cancer (ED-SCLC) were entered in the study. Amrubicin was given by daily i.v. infusion at the dose of 45 mg/m^2/day for three consecutive days every 3 weeks. Of 33 eligible patients, 3 showed CR and 22 showed PR, with an overall response rate of 75.8% (95% confidence intervals (CI), 57.7–88.9%). The MST and 1-year survival were 11.7 months and 48.5%, respectively. The most common toxicity was hematologic toxicity.

Phase II Trial of Amrubicin for the Treatment of Refractory or Relapsed Small Cell Lung Cancer: Thoracic Oncology Research Group Study 0301. SCLC patients with measurable disease who had been treated previously with at least one platinum-based chemotherapeutic regimen were entered into the trial. Amrubicin was administered at a reduced dose of 40 mg/m^2 per day × 3 days every 3 weeks, in view of the prior chemo- and radiotherapy. Sixty patients (16 refractory and 44 sensitive) were enrolled. The grade 3 or 4 hematologic toxicities comprised neutropenia (83%), thrombocytopenia (20%), and anemia (33%). Febrile neutropenia was observed in three patients (5%). The nonhematologic toxicities were mild. The overall response rates were 50% (95% CI, 25–75%) in the refractory group and 52% (95% CI, 37–68%) in the sensitive group. The overall survival and 1-year survival in the refractory group and sensitive group were 10.3 and 11.6 months, and 40 and 46%, respectively. Thus, it was concluded that amrubicin shows significant activity against SCLC, with predictable and manageable toxicities.

Amrubicin Combination Chemotherapy

Phase I-II Study of Amrubicin and Cisplatin for Previously Untreated Patients with Extensive-Stage Small Cell Lung Cancer. This trial was performed to determine the MTDs for combined amrubicin and cisplatin therapy and to assess the efficacy and safety of these drugs at their RD. Patients with histologically or cytologically proven measurable ED-SCLC, no previous chemotherapy, and good prognostic factors were entered into this trial. Amrubicin was administered on days 1–3 and cisplatin on day 1, every 3 weeks. Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2/day and cisplatin 60 mg/m^2) and three patients at dose level

2 (amrubicin 45 mg/m^2/day and cisplatin 60 mg/m^2). The MTD and RD were found to be at level 2 and level 1, respectively. The response rate at the RD was 87.8% (36/41). The MST and 1-year survival rate were encouraging, 13.6 months and 56.1%, respectively.

Phase I and Pharmacologic Study of Irinotecan and Amrubicin for Advanced NSCLC. We conducted a phase I trial of irinotecan (CPT-11), a topoisomerase I inhibitor, combined with amrubicin. The aim was to determine the MTD and DLT of amrubicin combined with a fixed dose of CPT-11 in patients with advanced NSCLC. Eleven patients were treated with amrubicin on days 1–3, combined with 60 mg/m^2 of CPT-11 on days 1 and 8, every 3 weeks. The starting dose of amrubicin was 25 mg/m^2, and the dose was escalated in 5 mg/m^2 increments until the MTD was reached. The 30 mg/m^2 of amrubicin dose was one dose level above the MTD, since three of the five patients experienced DLT, namely, diarrhea and leukopenia. Amrubicin did not affect the pharmacokinetics of CPT-11, SN-38, or SN-38 glucuronide. There were five PRs among the 11 patients, with an overall response rate of 45%. The RD for phase II studies was determined to be 60 mg/m^2 for CPT-11 (days 1 and 8) and 25 mg/m^2 for amrubicin (days 1–3), administered every 21 days.

Conclusion

The preclinical studies described above show that amrubicin has a unique mechanism of action as an anthracycline derivative and a broad spectrum of antitumor activity. The results of clinical studies of amrubicin as a single agent or as one of the drugs in combination regimens for lung cancer have been promising. To determine the exact usefulness of amrubicin in the treatment of SCLC, two randomized trials are now underway in Japan; one of cisplatin + amrubicin versus cisplatin + irinotecan in previously untreated patients of ES-SCLC, and one of amrubicin versus carboplatin + etoposide in elderly patients with ES-SCLC.

Although amrubicin has been investigated over the last decade, many of its characteristics remain unclear. Further studies exploring the usefulness of amrubicin as a single agent or as an agent administered in combination with other cytotoxic agents as well as novel molecule-targeted drugs for the treatment of other malignancies are warranted. Lastly, since all the trials with amrubicin have been conducted in Japan, the results of clinical studies to define the benefits and risk associated with amrubicin for cancer therapy conducted overseas are eagerly awaited.

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Anaerobic

►Hypoxia

Analgesic

Definition

The drugs that prevent or reduce pain.

►Nonsteroidal Anti-inflammatory Drugs

Analytic Epidemiological Study

Definition

A study in a human population designed to evaluate a specific causal relationship.

►Allergy
►Cancer Epidemiology
►Epidemiology of Cancer

Anaphase-Promoting Complex

Definition

The anaphase is a phase of mitosis, during which the paired sister-chromatids (►Sister-chromatids) are separated and drawn to the poles of the cell. This phase is initiated by a ubiquitin-mediated proteolytic pathway (►Ubiquitination) resulting in the degradation of regulatory proteins. The anaphase-promoting complex (APC/C) functions as a protein ubiquitin ligase.

►Securin

Anaphylactic Shock

Definition

A life-threatening allergic reaction characterized by a swelling of body tissues including the throat, difficulty in breathing, and a sudden fall in blood pressure.

Anaplasia

Definition

Refers o lack of cell differentiation in a tumor.

Anaplastic Astrocytoma

Definition

Astrocytic tumor characterized by an intermediate degree of histologic and clinical malignancy

►Brain Tumors

Anaplastic Carcinomas

►Follicular Thyroid Tumors

Anaplastic Large Cell Lymphoma

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Synonyms

Ki1 lymphoma

Definition

Anaplastic large cell lymphoma (ALCL) was originally described in 1985 as a separate entity among the ►non-Hodgkin lymphomas (NHL); it is characterized by the cohesive proliferation of large cells expressing the CD30/Ki1 antigen on their membrane. The World Health Organization more recently stated that the term ALCL should be applied to tumors with a T-cell or null phenotype, thus further restricting the identification of this specific subtype of ►lymphoma.

Characteristics

Systemic ALCL accounts for 2–8% of all ►lymphomas (►Malignant lymphoma, hallmarks and concept) but represents ~10–15% of NHL of childhood. In addition to the primary systemic disease, a form of ALCL limited to the skin is also recognized. Isolated cutaneous ALCL may spontaneously remit, but can also progress to a more aggressive disease.

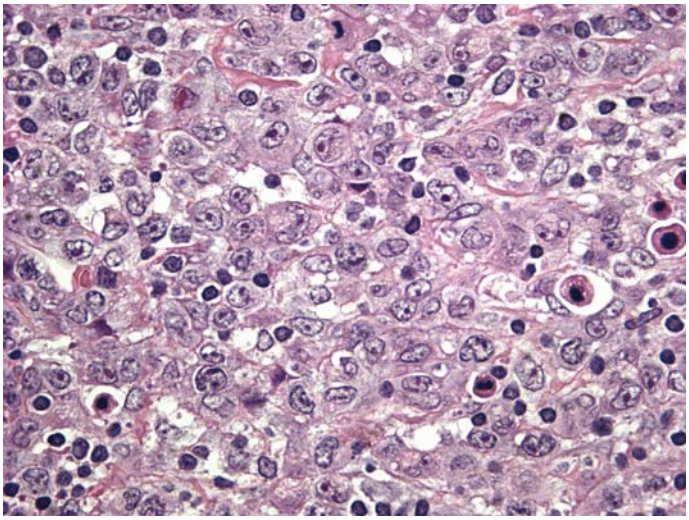
Systemic ALCL has some clinical features that are less common in other NHL subtypes: patients at diagnosis often have B-symptoms (fever, weight loss, sweats) as in Hodgkin lymphoma, mediastinal (►Mediastinum) and extranodal involvement, including skin, bone, and soft tissues. Among lymph nodes, the inguinal nodes are often site of disease, particularly in childhood, and rather frequently lymphadenopathy may be painful. Central nervous system and bone marrow are rare sites of disease, although with more sensitive techniques bone marrow may have submicroscopic infiltration of lymphoma cells more often than previously expected. Clinical differences have been reported between ►ALK (anaplastic lymphoma kinase)-positive and ALK-negative subtypes. Patients with ALK-positive ALCL are significantly younger and have a better prognosis that the negative counterpart, suggesting that ALK-positivity, rather than age, may confer distinct and relevant clinical features to systemic ALCL. Secondary ALCL may arise in the progression of other lymphomas, most commonly during the course of T-cell NHL, mycosis fungoides, Hodgkin lymphoma or lymphomatoid papulosis, and has a poor outcome. Except for the cases of very aggressive disease, ALCL may show multiple recurrences that respond to therapy, although eventually a considerable number of patients die despite intensive treatment.

Diagnosis

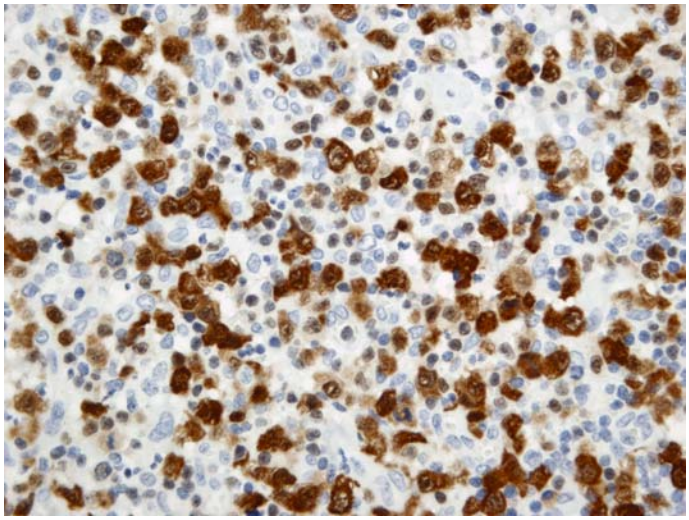
Diagnosis of ALCL relies on histopathology and immunophenotyping, but for a complete characterization of the tumor, chromosomal analysis and molecular genetic studies are warranted. It is now clear that ALCL includes several variants: classical or common type (corresponding to the original description of the disease) that accounts for ~60–70% of the cases, lymphohistiocytic variant, giant cell-rich, small cell type, and mixed. The large cell-rich type is characterized by multinucleated cells, often with Reed-Sternberg-like features that make the differential diagnosis with Hodgkin lymphoma (►Hodgkin disease) sometimes difficult. Irrespective of the histotype, neoplastic ALCL cells are characterized by a distinctive phenotypic profile. They express CD30, a cell membrane glycoprotein, found in activated lymphoid cells, the epithelial membrane antigen (EMA) and T-cell antigens including CD3. Perforin and granzyme B are also expressed in the majority of the cases and, together with absence of CD15 expression, they are useful markers in the differential diagnosis with Hodgkin disease. The development of antibody against the ALCL kinase (ALK) (►ALK protein) has further refined the immunohistochemical analysis of ALCL. Expression of ALK occurs in tumors carrying ►chromosomal translocations that involve the corresponding gene. The most common rearrangement between chromosome 2 and 5 causes strong ALK positivity of neoplastic cells in the nucleus and cytoplasm (Figs. 1 and 2), whereas other chromosomal translocations involving *ALK* produce accumulation of the translocation product at the cytoplasmic level only. ALK reactivity of tumor cells has diagnostic relevance given that ALK is not detected in normal lymphocytes or in Hodgkin lymphoma cells and only few cases of rare forms of lymphoma and nonlymphomatous tumors (inflammatory myofibroblastic tumors, rare neuroblastoma, and rhabdomyosarcoma) show ALK reactivity. Ultimately, combination of morphological, immunophenotypic, and genetic analysis allow the diagnosis of ALCL and the differentiation of this lymphoma from other tumors.

A peculiar aspect to consider is the differentiation of cutaneous ALCL from other CD30-positive lymphoproliferative disorders including lymphomatoid papulosis. In this case, because the great majority of isolated cutaneous ALCL is ALK-negative, clinical observation and evolution of the disease are of foremost importance. In addition, because skin involvement in the context of a systemic ALCL may bear prognostic implications, suspect skin involvement must be documented through biopsy.

Imaging procedures used in the diagnosis and staging of ALCL are similar to other NHL. Special attention should though be given to the examination of soft tissues and skin, given the relatively high frequency of involvement of those sites. Chest x-ray is usually sufficient to detect a mediastinal mass, but a CT scan of the neck, thorax, and abdomen should always be obtained to define the extent of disease. Ultrasound is routinely used for diagnosis and monitoring of lymph nodes, abdominal organ involvement, including liver, spleen, kidney, and soft tissues. This technique is easy to perform and can give accurate information not only for diagnostic purposes, but also to evaluate tumor response to treatment and during follow-up, once treatment is completed. Whole skeletal ►scintigraphy may be useful, especially in cases with bone pain, and should be complemented with x-ray of positive bones.



Anaplastic Large Cell Lymphoma. Figure 1 This panel depicts the classic variant of anaplastic large cell lymphoma with numerous large tumor cells that often contain horseshoe- or kidney-shaped nuclei, distinct nucleoli and abundant cytoplasm.



Anaplastic Large Cell Lymphoma. Figure 2 This panel immunohistochemical staining of anaplastic large cell lymphoma containing the chromosomal translocation t(2;5) originating the NPM–ALK fusion protein. Nuclear and cytoplasmic reactivity to an anti-ALK specific monoclonal antibody is detectable in most lymphoma cells (brownish color) Pictures were a courtesy of Dr. E. S. d'Amore, Institute of Pathology, University of Padua, Italy.

Brain MRI or CT scan are usually performed, but central nervous system involvement at diagnosis is infrequent in ALCL. More recently ►positron emission tomography (►PET) with 18-fluorodeoxyglucose (FDG) has been introduced in the routine evaluation of ALCL patients, mainly for staging. Lymphoma cells characteristically have a higher uptake of FDG compared with normal cells and this gives high activity features to the vital tumor mass. Because a high reactivity may also be seen in other nonmalignant tissues with high glucose metabolism, including reactive lymph nodes and inflammatory tissues, PET findings have to be interpreted with caution at present, until large prospective clinical studies where this recent technology is routinely applied are completed.

To completely define the extent of the disease, as in other NHL, bone marrow biopsy and bone marrow smear should be performed and analyzed for the presence of tumor cells, as well as a lumbar puncture to exclude the presence of lymphoma cells in the central spinal fluid.

Genetics

The ►chromosomal translocation t(2;5)(p23;q35) was originally reported in patients with malignant histiocytosis, which indeed represented ALCL cases diagnosed according to old criteria. Break of chromosome 2 and subsequent fusion to chromosome 5 in a reciprocal chromosome rearrangement is the main genetic feature of ALCL. As a result, the *ALK* gene on chromosome 2 is juxtaposed to the nucleophosmin (►*NPM*) gene on chromosome 5. The ►*NPM–ALK* fusion gene gives rise to a fusion protein composed of ALK and NPM domains that can be detected by ►immunohistochemistry using an anti-ALK monoclonal antibody. While *ALK* is not usually expressed in normal tissues, except in few neuronal cells, *NPM* gene encodes a shuttle protein that undergoes dimerization in the cytoplasm and in this conformation can move to the nucleus. In the cytoplasm of NPM–ALK-positive ALCL cells, heterodimers between NPM and NPM–ALK are formed that, while retaining the ability to move to the nucleus, are reactive against the anti-ALK antibody. This explains the cytoplasmic and nuclear reactivity of ALCL cells harboring the t(2;5) translocation. The wide use of antibodies to ALK protein revealed that about 10% of the ALK-positive ALCLs showed an immunohistochemical reactivity confined to the cytoplasm. Molecular genetic studies demonstrated that those cases were associated to a series of different chromosomal translocations, all involving the *ALK* gene, but with partners other than *NPM*. The deriving fusion genes all cause hybrid protein overexpression that lack the shuttling properties of NPM-containing fusion proteins, thus preventing them from nuclear localization. From the functional point of view, NPM–ALK can dimerize and as such it possesses constitutive tyrosine kinase activity, mimicking the normal functional activity of the ALK receptor in normal cells upon binding and oligomerization by its specific ligand. A number of experimental data suggest that NPM–ALK has a causative role in tumorigenesis of ALK-positive ALCL, although it may need concomitant events. In fact, NPM–ALK displays transforming activity in both hematopoietic and fibroblastic cell lines in vitro.

Overall, ~90% of childhood ALCLs are positive for the ALK-hybrid protein, whereas this percentage is only 50–60% in adult ALCLs. As suggested by clinical studies, ALK-positivity, not only is a relevant tool in the

diagnosis of ALCL, but represents also a prognostic marker in that ALK-positive ALCLs fare better than ALK-negative lymphomas in the adult population.

Therapy

Treatment of ALCL, similarly to other NHL, is almost exclusively based on chemotherapy. The therapeutic approach has witnessed a variety of chemotherapy regimens, ranging from acute lymphoblastic leukemia-type regimens lasting 24 months to shorter chemotherapy more frequently used in aggressive B-cell lymphomas. Differently from most European studies where ALCL is considered as a separate entity, in North America all large cell lymphomas, regardless of the histologic subgroup and ►immunophenotype, are treated according to the same chemotherapy scheme.

Primary systemic ALCL in adults has been treated mostly with CHOP (cyclophosphamide, vincristine, prednisone), CHOP-derived chemotherapy, and MA-COP-B (methotrexate, adriamycin, cyclophosphamide, vincristine, prednisone, and bleomycin) regimens, but occasionally patients have been treated with Hodgkin lymphoma-type chemotherapy (e.g., ABVD regimen).

Collaborative trials have been conducted in childhood and adolescence ALCLs. Overall results were comparable with very different treatment strategies, ranging from leukemia-like treatment, such as modified LSA2-L2 protocol, to chemotherapy regimes derived from B-cell NHL as the BFM (Berlin-Frankfurt-Munster) and the French and British Pediatric Hemato-Oncology Society protocols. The latter are based on the administration of short (usually 5-days) courses of rather high-intensive chemotherapy (based on the rotational use of corticosteroid, anthracyclines, cyclophosphamide or ifosfamide, cytarabine, methotrexate, epipodophyllotoxin) administered at intervals of ~3 weeks. With these treatments, 60–75% of patients obtain cure of their disease and do not experience disease recurrences. Similar results were obtained with the APO regimen in the Children's Oncology Group (USA) that includes higher cumulative doses of anthracyclines without ►alkylating agents (cyclophosphamide/ifosfamide) or epipodophyllotoxins.

Treatment intensity and/or duration have been differentiated based on various risk factors. Although stage of disease has some relevance, in the adult population a high International Prognostic Index (IPI) score, high serum lactate dehydrogenase levels, ALK-negativity and expression of the surface antigen CD56 have been associated to a significant lower outcome. In children and young adults, possibly because of the very high frequency of ALK-positivity, ALK expression does not seem to be a relevant prognostic indicator, whereas high stage of disease, elevated lactate dehydrogenase levels, and specific site of disease, including liver, spleen, lung, and skin involvement, seem to be associated to a less favorable prognosis.

A distinct clinical condition is represented by the isolated cutaneous ALCL. Once other disease localizations have been excluded, given that spontaneous regression of the lesions can occur, a strict monitoring of the patient is often preferred, postponing initiation of chemotherapy when signs of lymph nodes or other organ involvement is demonstrated.

A peculiar aspect of ALCL is that most patients who relapse respond well to salvage therapies. Although early disease recurrences can be extremely aggressive, moderate intensity chemotherapy, including single drug treatment with vinblastine, can achieve long-lasting remission. In case of resistant disease or very aggressive relapse, high-intensive chemotherapy with bone marrow transplant has also been used both in children and adults. Further studies are needed to definitively establish the benefit of bone marrow transplant in ALCL and to identify the subpopulations of patients who may benefit from such a treatment approach.

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Anaplastic Lymphoma Kinase

Definition

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) having an extracellular, a single transmembrane, and an intracellular domain containing the tyrosine kinase activity. ALK belongs to the insulin receptor subfamily of RTKs, most closely related to leukocyte tyrosine kinase receptor. It localizes mostly in neuronal cells and may play a role in the nervous system development and maintenance. ALK and some of the ALK partners or closely related genes are found implicated both in anaplasic large cell lymphoma and in inflammatory myofibroblastic tumors.

►Pleiotrophin
►ALK Protein

Anatomic Pathology

Definition
The study of gross and microscopic tissue features in disease diagnosis.

►Molecular Pathology

Anchorage-Independent

Definition
The ability of cells to survive and multiply in the absence of a protein matrix for adhesion.

►Syk Tyrosine Kinase
►Adhesion Molecules

Anchorage-Independent Cell Growth

Definition
In vitro transformed cells and cancer-derived cells are able to survive and grow in the absence of anchorage to the ►extracellular matrix (ECM) and their neighboring cells, termed anchorage independence of growth, correlates closely with tumorigenicity in animal models. This property of cancer cells presumably reflects the tendency of tumor cells to survive and grow in inappropriate locations in vivo. Such incorrect localization, as occurs in invasion and metastasis, is the characteristic that distinguishes malignant from benign tumors.

►Sprouty

Anchorage-Independent Cell Transformation

Definition
The process by which carcinogenic chemicals, oncogenic viruses, or radiations change the genotype and phenotype of the cell, such that the cells are now able to grow as colonies in three-dimensional suspension in soft agar.

►Chemically Induced Cell Transformation

Androgen

Definition
An agent, usually a hormone (e.g. testosterone) that stimulates the activity of the accessory sex organs of the male.

►Prostate-Specific Membrane Antigen (PSMA)

Androgen Insensitivity Syndrome (AIS)

►Androgen Receptor

Androgen Receptor

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Synonyms
AR; dihydrotestosterone receptor; testicular feminization TFM; spinal and bulber muscular atrophy SBMA; Kennedy disease KD; androgen insensitivity syndrome AIS; NR3C4; SMAX1; HUMARA

Definition
The androgen receptor or AR is a member of the steroid/thyroid receptor superfamily, in which all members share basic structural and functional homology. The AR is an intracellular ligand (androgen)-dependent transcription factor, which regulates the expression of genes that control cell proliferation, ►apoptosis, ►angiogenesis and differentiation in many hormonally regulated tissues including the prostate. It is an important regulator of male sexual differentiation and maturation.

Characteristics

The biological function of androgen is mediated through the androgen receptor. Except for the spleen and bone marrow, the androgen receptor is ubiquitously expressed in human organs. The human androgen receptor gene is more than 90-kb long, and is present as a single allele located at chromosome Xq11.2–12. The AR gene has eight exons and possesses a coding region of 2757 bp. This region encodes a 110 KDa protein (919 amino acids) with four distinct functional domains: a conserved Zn-finger DNA-binding domain (exons 2 and 3), a hinge region (exon 4), a COOH-terminal ligand-binding domain (exons 4–8), and a less conserved and structurally flexible amino (NH2)-terminal transactivation domain (exon 1).

Structure–Function Relationships between Different Domains of Androgen Receptor that are Required for its Transcriptional Activity

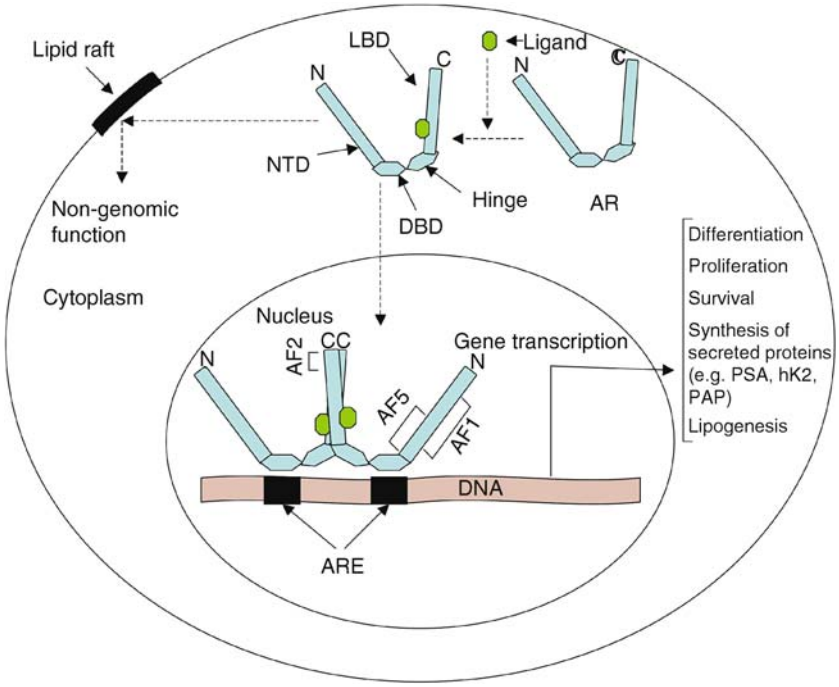
Regulation of Unliganded AR

Unbound AR in the cytoplasm remains in an inactive but androgen responsive state as part of a large dynamic heterocomplex composed of heat shock proteins, co-chaperones, and tetratricopeptide repeat containing proteins. This large complex helps to modulate the ligand-binding domain (LBD) of AR into a relatively stable, partially unfolded, and inactive intermediate which has a high affinity for the potent biologically active androgen, dihydrotestosterone. The androgen-free LBD and the associated chaperone proteins inactivate the function of the transactivation domain of AR. This transactivation domain becomes constitutively active in the mutant AR lacking a ligand-binding domain (LBD). As such, this molecular chaperone complex and the LBD of AR prevent unwanted activation of AR in the absence of androgen

Regulation of Ligand-Bound AR

Because of their lipophilic nature, androgens cross the cell membrane, both passively, and actively via the transport protein, megalin. Once within the cell the androgen binds to the AR, which is stabilized and translocated into the nucleus. Within the nucleus the AR homo-dimerizes and recruits transcriptional cofactors to the promoters and enhancers of AR-target genes, thus facilitating their transcription (Fig. 1). Androgen binding to the LBD of AR induces an overall change in AR structure leading into an active conformation which is characterized by dissociation of the receptor–chaperone complex. However, molecular chaperones also play important roles in the events downstream of AR activation such as translocation to the nucleus, transcriptional activation, transcription complex disassembly and degradation. LBD relieves its inhibitory function upon androgen binding. AR LBD configuration is highly structured and resembles other steroid receptors' ligand-binding domains.

1. AR DNA Binding Domain and Hinge Region: Androgen binding to the LBD initiates a conformational change of AR leading to several secondary effects important for AR transcriptional activity.



Androgen Receptor. Figure 1 A schematic diagram of AR structure and its functions.

One such effect is the unmasking of the bipartite nuclear-localization signal (NLS) that overlaps the DNA-binding domain and hinge regions (amino acid 625–671). Another weak NLS (amino acid 722–805) is present in the LBD of AR, which is also exposed upon androgen binding. The NLS is recognized by an import protein that mediates translocation of the AR through the nuclear-pore complex. AR homodimerization occurs in the nucleus upon treatment with ligand. Both the DNA and ligand-binding domains of AR are involved in subsequent receptor dimerization. The stronger hydrophobic interaction occurs between the ligand-binding domains.The binding of AR dimer to the specific androgen response elements (ARE) of a gene occurs in a co-operative manner. The DNA binding domains of AR, like other nuclear receptor superfamily members, consist of two zinc fingers that provide the structural basis required for ARE recognition in the promoter region of a gene. The consensus ARE is a 6 bp palindromic core sequence (5′-AGEACA-3′) separated by a three nucleotide spacer. Sequences outside the DNA binding domain also play a role in AR-DNA binding.

2. AR AF-2 Domain: A hydrophobic protein-protein interaction surface known as Activation Function-2, or AF2, is present at the carboxy-terminal, and also becomes accessible after androgen binding to AR. AF2 is the potential binding site for AR co-activators such as the p160 family (TIF2, SRC1, and AIB1). These co-activators enhance AR transcriptional activity by modulating AR conformation and by recruiting cofactors to the promoter. Ligand binding also induces interaction of AF2 with a specific motif (FXXLF) (F = phenylalanine, L = leucine and X = any aminoacid) in the NH2-terminal activation domain of AR. The interaction of NTD and LBD or N/C interaction is important for AR transcriptional activity. Although the mechanistic details of how this interaction leads to AR activity are not precisely known, it appears that ligand binding induces intramolecular folding of AR, leading to the interaction between NTD and LBD. This influences receptor dimerization in the nucleus and reduces ligand degradation from LBD, AR protein dissociation, AR chromatin binding ability and receptor transactivation.
3. AR N-Terminal Transactivation Domain: The N-terminal transactivation domain (NTD) of AR is longer and structurally different as compared to other steroid receptors. The highly flexible and disordered domain containing NTD is largely globular in nature. Association with coactivators and transcription factors helps to induce folding in the domains of the NTD to optimize efficient binding. Due to the allosteric nature of binding, the AR NTD is able to bind a broad spectrum of transcriptional coactivators, co-repressors and other factors. Therefore the AR NTD may be responsible for mediating androgen-regulated expression of genes whose functions consist of protein folding, trafficking and secretion, metabolism, cytoskeletal rearrangement, cell cycle regulation and signal transduction. *AF1 and AF5 Domain of NTD*: There are two major overlapping activation functions present in the AR NTD, AF1 (amino acids 142–485) and AF5 (amino acids 351–528). These regions contain microsatellite repeats, protein-protein interaction surfaces, and phosphorylation and sumoylation sites. AF1 is considered the major transactivation domain that binds to basal transcription factors, co-regulators, cell cycle regulatory proteins, heat shock proteins etc. The interaction with molecules like TIFIIF increases folding in the AF1 domain and facilitates further protein-protein interactions for the formation of a transcriptionally competent receptor complex. Unlike AF1, activation of AF5 is ligand independent. An inhibitory domain within AF5 inhibits the DNA-binding domain of AR from binding to AREs. *Glutamine and Glycine Repeats*: AR NTD contains two polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts. The first trinucleotide repeat sequence, CAG, spans amino acids ~58–78, and encodes the amino acid glutamine. The second stretch consists of GGN repeats that encode glycines and span amino acids ~449–472. Shortened trinucleotide repeat stretches result in increased AR activity and are often associated with prostate cancer predisposition. On the other hand, overexpansions of the CAG repeat (more than 40) correlate with a significant decrease in AR activity and are associated with diseases such as X-linked Spinal and Bulbar Muscular Atrophy (SBMA), also known as Kennedy's disease. Both the polyglutamine and polyglycine repeat size appear to regulate the N/C interaction and thereby influence AR activity. *FXXLF and WXXLF Motifs*: The AR NTD also contains two short, highly conserved peptide motifs that mediate the ligand-dependent N/C interaction as discussed previously. These motifs are FXXLF (in humans FQNLF), ranging from amino acids 23–27 and WXXLF (in humans WHTLF), ranging from amino acids 434–438. *AR Co-regulators*: AR functions as a tripartite receptor system involving AR itself, the androgens and AR coregulators. AR interaction with either co-activators or co-repressors enhances or represses its transcriptional activity, respectively, without affecting the basal levels of transcription. Possible mechanisms of co-regulator function include modulation of chromatin structure, promotion of AR post-translational modifications and control of androgen/AR binding affinity, AR expression, AR stability, AR nuclear translocation, and AR recruitment of transcription machinery.

4. Post-translational Modification of AR: Post-translational modification of AR is one of the key mechanisms regulating its function. One of the major post-translational modifications of AR is phosphorylation. These phosphorylation sites may be the sites for possible cross-talk between peptide growth factors and the AR signaling axis, which is important for normal prostate epithelial cell growth and function as well as for the progression of cancer. Most of the phosphorylation sites reside in the NTD at different serine residues. However, recent findings suggest that Src kinase mediates phosphorylation at tyrosine 534 of AR, and therefore acts as a potential regulator of AR transcriptional activity. Another post-translational modification is acetylation of AR at the hinge domain (at lysine residues 630, 632 and 633) by Histone Acetyl Transferases such as p300, p/CAF, and TIP60. This modification regulates recruitment of co-regulators as well as the growth properties of the AR. Acetylation is also required for regulation of the AR by the AKT, PKA and JNK signaling pathways. Sumoylation of AR at two specific domains (NRM1 and NRM2 at NTD) also appears to be important for AR activation, localization and degradation.

Nongenomic Function of AR

Recently, a nongenomic mechanism for AR function independent of its transcriptional activity has been postulated. This nongenomic event is very rapid (2–15 min) and activates signaling events such as the Src/Raf1/ERK, ►PI3K-AKT, and IL-6-STAT3 pathways. Interestingly, membrane lipid rafts are considered to be the privileged site for this AR-mediated nongenomic signaling. Upon androgen binding most of the ARs translocate to the nucleus and act as transcription factors. The few remaining cytosolic ARs may then enter into ►caveolin positive or negative rafts to initiate different signaling pathways.

Androgen Receptor in Human Physiology and Pathology

Androgen and AR are not only necessary for the initiation of prostate development; they are also important factors for the survival, proliferation, secretary function, morphology, angiogenesis and differentiation of the adult prostate gland. AR is also important for Wolffian duct development and spermatogenesis in males. Various clinical disorders due to functional abnormalities of AR have been reported, suggesting that a wide range of physiological responses and developmental processes are mediated by AR.

Androgen Receptor and ►Prostate Cancer: Approximately 80–90% of prostate tumors are dependent on androgen at the initial diagnosis, suggesting the importance of AR signaling in all stages of ►prostate carcinogenesis. A recently developed bioinformatics approach known as Cancer Outlier Profile Analysis (COPA) together with standard genomic techniques has identified recurrent gene fusions of the 5′ untranslated region of the TMPRSS2 gene to ►ETS transcription factors (ERG or ETV1) in human prostate cancer tissues. Interestingly, this gene fusion enables expression of the fused product under the control of AR, as the TMPRSS2 promoter contains an ARE. This ►TMPRSS2-ETS fusion appears to be frequent in prostate cancer and might be an initiating event for prostate cancer, underscoring the importance of AR-mediated signaling in prostate cancer.

Both the tumor epithelia and adjacent stroma express AR. Retardation of tumor growth occurs in response to androgen ablation therapy. Until now, the primary therapy for advanced (locally extensive or metastatic) prostate cancer consists of androgen ablation by pharmaco-therapeutic or surgical means. Eventually, the tumor recurs due to a transition from androgen-dependence to a highly aggressive and androgen-depletion-independent (refractory) phenotype. The detailed molecular mechanism underlying the development of the androgen refractory phenotype of prostate cancer is poorly understood. As such, it has been difficult to develop effective treatments for this stage of the disease. Disruption of androgen receptor function inhibits proliferation of androgen refractory prostate cancer, demonstrating the importance of AR even at subnormal physiological levels of androgen. Mechanisms for ligand-independent AR reactivation include AR mutation, gene amplification, increased stability and nuclear localization, co-regulators and cross talk between different signal transduction pathways. Recent studies also suggest an acquired capacity of recurrent prostate tumors to biosynthesize testicular androgens from adrenal androgens or cholesterol, thereby reactivating the AR.

Targeting AR for Therapy: Androgen ablation therapy, which is achieved by pharmaco-therapeutic (steroidal and non-steroidal antiandrogens) or surgical (subcapsular or subepididymal bilateral orchiectomy) means, is the standard initial systemic therapy for locally advanced or ►metastatic prostate cancer. This therapy is based on inhibiting the synthesis of active androgen or inhibiting the physiological androgen from binding to AR. Novel therapeutic strategies for the androgen-depletion-independent stage of prostate cancer will focus on inhibiting expression of AR, blocking the binding of AR coactivators, and enhancing the binding of AR co-repressors. Using intelligent high-throughput screening and structural and computational chemistry, it may be possible to develop peptide antagonists, ►small molecules or antisense oligonucleotides that target AR-coactivator binding surfaces. Potential drugs targeting the N-terminal domain may also prevent or delay the progression of both hormonal-dependent and -independent prostate cancer.

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Androgen-Independent Prostate Cancer

Definition

AIPC.

►Hormone refractory prostate cancer

Androgens

Definition

Male sexual hormones that are mainly produced in the testicles. Testosterone is the principal androgen hormone.

►Adjuvant Chemoendocrine Therapy

Anemia

Definition

Anemia is abnormally low hemoglobin concentration in the blood (females: <120 g/L, males: <130 g/L).

►Oxygenation of Tumors
►Anoxia
►Erythropoietin
►Gastrointestinal Stromal Tumor

Anergy

Definition

A state of unresponsiveness, induced when the T cell antigen receptor is stimulated, which effectively freezes T cell responses pending a "second signal" from the antigen-resenting cell (►costimulation).

Aneugen

Definition

Any agent that affects cell division and the mitotic spindle apparatus resulting in the loss or gain of whole chromosomes, thereby inducing ►aneuploidy.

►Micronucleus Assay

Aneuploidy

Definition

Aneuploidy is a condition with the abnormal number of chromosomes due to missing or extra chromosomes, as a result, the number of chromosomes by aneuploidy is not a multiple of the haploid set. Normal diploid organisms have 2n chromosomes, where n is the chromosome number in the haploid set. A change in the number of chromosomes can lead to a chromosomal disorder. Aneuploidy is common in tumor cells.

►Micronucleus Assay
►Microtubule Associated Proteins
►Flow Cytometry

Aneuploidy

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Definition

Aneuploidy refers to the presence of either less than or more than the normal diploid number of

chromosomes in a cell. Such losses or gains (aneusomy) of chromosomes may involve segments of chromosomes or complete chromosomes leading to major imbalances in the genetic makeup of the affected cells. Instead of the two copies of structurally intact homologous chromosomes (disomy) present in a normal cell, aneuploidy may result in the loss of one chromosome (monosomy) or both homologous chromosomes (nullisomy) as well as gain of one chromosome or more to each homologous pair of chromosomes (trisomy, tetrasomy etc.) in a cell. Occasionally, gain of one or more copies of the entire haploid set of chromosomes (triploidy, tetrapolidy etc.) or loss of one haploid set of chromosomes (haploidy) have also been reported.

Characteristics

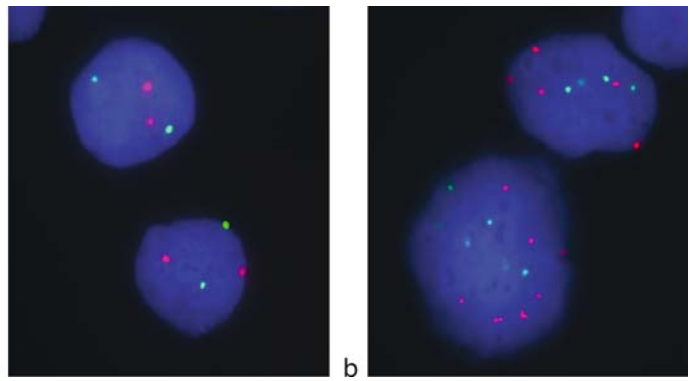
►Chromosomal instability in the form of aneuploidy, is one of the most common genetic anomalies detected in human cancer cells. While von Hansemann had, at the end of the nineteenth century, first discussed the biological consequences of chromosome segregation errors based on observations of abnormal mitoses in human cells, possible contribution of aneuploidy in tumorigenic transformation of cells resulting from multipolar mitosis was later proposed by Theodore Boveri at the beginning of the twentieth century. Germline inheritance of aneuploid condition often leads to developmental defects and somatic origin of similar anomalies are frequently associated with malignant phenotypes. It has been convincingly demonstrated that aneuploidy arises due to underlying genetically determined chromosomal instability in malignant cells. Discovery and functional characterization of genes regulating faithful segregation of chromosomes required for the maintenance of chromsomal stability in somatic cells have helped elucidate the genetic pathways involved in the process. Recent findings of tumor suppressor genes and DNA mismatch repair genes as upstream regulators of these pathways have provided compelling evidence in favor of aneuploidy being a genetically determined phenomenon associated with cancer. Whether aneuploidy is a cause or consequence of cancer has long been debated. Although the physiological relevance of aneuploidy in cancer still remains a subject of investigation, increasing number of observations, over the years, have revealed frequent changes in gene copy number in conjunction with changes in chromosome copy number in cancer cells during cancer initiation and/or progression processes. These observations made in primary tumors with in situ hybridization (ISH) and comparative genomic hybridization (CGH) techniques have revealed that specific chromosome aneusomies sometimes correlate with distinct tumor phenotypes. In addition, aneuploid tumor cell lines and in vitro transformed rodent cells have been reported to display an elevated rate of chromosome instability, thus indicating that aneuploidy is a dynamic chromosome mutation event associated with transformation of cells. Finally and most importantly, a number of genes regulating chromosome segregation have been found aberrantly expressed in human cancer cells and genetically engineered mice expressing abnormal levels of some of these genes have been reported to display higher incidence of aneuploidy associated with tumor development. These findings provide a new direction towards understanding the molecular mechanisms responsible for the origin of aneuploidy in cancer and the knowledge is expected to help design novel cancer therapeutic strategies in the future.

Aneuploidy in Cancer

Aneuploidy involving one or more chromosomes has been commonly reported in human tumors. It is estimated that aneuploidy, is the most prevalent genetic change recorded among over 20,000 solid tumors analyzed thus far. These observations were originally made using classical cytogenetic techniques late in a tumor's evolution and were difficult to correlate with cancer progression. More recent studies have reported an association of specific non-random chromosome aneuploidy with different biological properties such as loss of hormone dependence and metastatic potential.

Classical cytogenetic studies of analyzing metaphase chromosomes from tumor cells had serious limitations in scope since these were applicable only to those cases in which mitotic chromosomes could be obtained. Because of low spontaneous rates of cell division in primary tumors, analyses depended on cells either derived selectively from advanced metastases or those grown in vitro for varying periods of time. In both instances, the metaphase chromosomes analyzed represented only a subset of the tumor cell population. The two major advances in analytical cytogenetic techniques, in-situ hybridization (ISH) and comparative genomic hybridization (CGH), have allowed better resolution of chromosomal aberrations in freshly isolated tumor cells. ISH analyses with chromosome-specific DNA probes can be performed on interphase nuclei and allow assessment of numeric chromosomal anomalies within tumor cell populations in the contexts of whole nuclear architecture and tissue organization (Fig. 1). CGH allows genome-wide screening of chromosomal anomalies without the use of specific probes, even in the absence of knowledge of the chromosomes involved. While both techniques have certain limitations in terms of their resolution power, they nonetheless provide a better approximation of chromosomal changes occurring among tumors of various histology, grade and stage compared with what was possible with the classical cytogenetic techniques.

DNA ploidy measurements, have also been performed with flow cytometry and cytofluorometric methods. Although these assays underestimate chromosome ploidy



Aneuploidy. Figure 1 Fluorescence in-situ hybridization (FISH) analyses of normal ovarian epithelial cells (a) and human ovarian cancer cells (b) performed with chromosome 20 short arm (*labeled with red fluorescent dye*) and long arm probes (*labeled with green dye*). Note the presence of two copies of chromosome 20 in normal epithelial cells in (a) but five to seven copies of chromosome 20 as evidence for aneuploidy in the cancer cells (b).

due to the possibility of a gain occasionally masking a loss in the same cell, several studies employing these methods have concluded that DNA copy number changes or *DNA aneuploidy* correlates with poor prognosis in different cancers. A few published examples of aneuploidy in cancer are mentioned in the following discussion that deal with DNA ploidy measurements as well. Most of these observations are correlative without a direct proof of specific involvement of genes on the respective chromosomes. However, more recent findings on the role of specific genes in regulating chromosome segregation and maintenence of chromosomal stability together with the development of mouse models genetically manipulated to either up regulate or down regulate some of these genes displaying increased incidence of tumors associated with aneuploidy provide strong evidence in favor of aneuploidy playing a critical role in the tumorigenesis process.

In ►renal cancer, either segmental or whole chromosome aneuploidy appears to be uniquely associated with specific histologic subtype. Tumors from patients with hereditary papillary renal carcinomas (HPRC) commonly show trisomy of chromosome 7, when analyzed by CGH. Germline mutations of a putative oncogene MET have been detected among patients with HPRC. It was demonstrated that an extra copy of chromosome 7 results in nonrandom duplication of the mutant ►MET allele in HPRC, thus implicating this trisomy in tumorigenesis. The study suggested that mutation of MET may render the cells more susceptible to errors in chromosome replication, and clonal expansion of cells harboring duplicated chromosome 7 reflects their proliferative advantage. In addition to chromosome 7, trisomy of chromosome 17 in papillary tumors and also of chromosome 8 in mesoblastic nephroma are commonly seen. Association of specific chromosome imbalances with benign and malignant forms of papillary renal tumors not only contributes to understanding of tumor origins and evolution but also implicate aneuploidy of the respective chromosomes in the tumorigenic transformation process.

In ►colorectal cancer, aneuploidy is common occurrence. Molecular allelotyping studies have suggested that the limited karyotyping data available from these tumors actually underestimate the true extent of these changes. Losses of heterozygosity, reflecting loss of the maternal or paternal allele in tumors, are widespread and often accompanied by a gain of the opposite allele. Thus, for example, a tumor could lose a maternal chromosome while duplicating the homologous paternal chromosome leaving the tumor cell with a normal karyotype and ploidy but an aberrant allelotype. It has been estimated that on an average, cancer of the colon, breast, pancreas and prostate may lose 25% of the alleles and it is not unusual for a tumor to have lost over half of all its parental alleles. In clinical settings, DNA ploidy changes indicate high risk of developing premalignant changes among patients with ulcerative colitis and also lymph node metastasis among patients with gastric carcinoma. Similarly, chromosome copy number alterations or aneuploidy has been detected in pre cancerous lesions of colon, cervix, head and neck, esophagus and bone marrow. Between 60% and 80% of colorectal polyps from individuals with adenomatous polyposis syndrome, predisposed to develop colorectal cancer have been reported to show aneuploid changes. Comparative analysis of genomic alterations in AdAPC driven mouse intestinal tumors have identified loci syntenic with human chromosomes 1, 12, 9 and 22 that are frequently gained or lost in familial adenomas and sporadic colorectal cancers suggesting that genetic mechanisms manifested in the form of aneuploidy are conserved across species The molecular karyotype of amplified chromosomal segments (amplotype) generated from colorectal cancer was reported to indicate that over representation of loci on chromosomes 8 and 13 may be critical for metastatic colorectal cancer.

Incidence of chromosome aneuploidy has also been evaluated as a marker of risk assessment and prognosis in several other cancers. Analyzing aneuploidy in non-surgically obtained squamous epithelial cells offers a promising non-invasive tool to identify individuals at high risk of developing head and neck cancer. Interphase FISH studies have revealed extensive aneuploidy in tumors from patients with head and neck squamous cell carcinomas (HNSCC) and also in clinically normal distant oral regions from the same individuals. It has been suggested that a panel of chromosome probes for FISH analyses may serve as an important tool to detect subclinical tumorigenesis and for diagnosis of residual disease. The presence of aneuploid or tetraploid populations are commonly seen in 90–95% of esophageal adenocarcinomas, and when detected in ►Barrett esophagus, a premalignant condition, predicts progression of disease.

Aneuploidy in most solid tumors coexist with structural chromosomal aberrations giving rise to complex karyotypes. Such karyotypic complexities could be reflective of similar underlying mechanisms responsible for the origin of both kinds of chromosomal aberrations as well as their selective value for the evolution of malignant cells during carcinogenesis. These possibilities appear credible in view of the findings that tetraploid p53 null mouse mammary epithelial cells show an increased frequency of whole chromosome missegregation and chromosomal rearrangements together with increased propensity to give rise to malignant mammary epithelial cancers. Despite complex karyotypes, different cancers also show shared minimal regions of gains and losses of specific chromosomes. By analyzing such regions of genomic imbalances in various solid tumors, karyotypic pathways of evolution of cancers involving specific chromosomal aneusomies have been described. For pancreatic cancer, the recurrent early imbalances included loss of chromosomes 1, 5, 7, 8, 15, 17, and 18 while the late recurrent imbalances were identified as gain of chromosomes 2, 6, 7, 11 and loss of chromosome 19.

Besides clinical correlative observations, role of aneuploidy in oncogenesis has also been supported by *in vitro* and *in vivo* transformation experiments performed with human and rodent cells. These studies revealed that aneuploidy is induced at early stages of transformation. Transgenic mouse models with chromosome segment-specific duplications and deletions have been generated to investigate the effect of chromosome ploidy alterations during development. Three duplications for a portion of mouse chromosome 11 syntenic with human chromosome 17 were established in the mouse germline. Mice with duplication of 1 Mb chromosomal DNA developed corneal hyperplasia and thymic tumors. The findings document a direct role of chromosome aneusomy in tumorigenesis. More recent developments of mouse models with targeted upregulation or down regulation of genes regulating chromosome segregation giving rise to increased incidence of aneuploidy and cancer have further strengthened the idea of aneuploidy being a *cause* driving tumorigenesis rather than a *consequence* of cancer.

Aneuploidy as a "Driving Force" and not a "Consequence" in Cancer

The presence of numerical chromosomal alterations in a tumor does not mean that the change arose as a dynamic mutation due to genomic instability. While aneuploidy as a dynamic mutation due to genomic instability in tumor cells would occur at a certain measurable rate per cell generation, a consequential state of aneuploidy is expected to be fixed in similar tumors at an unpredictable random rate possibly decided by differences in environmental factors such as humoral, cell substratum, and cell-cell interaction differences of the tumor and normal cell microenvironments. It could be argued that despite similar rates of spontaneous aneuploidy induction in normal and tumor cells, the latter are selected to proliferate due to altered selective pressure in the tumor cell microenvironment while the normal cells are eliminated through activation of apoptosis. Alternatively, it could be postulated that selective expression or overexpression of anti-apoptotic proteins or inactivation of proapoptotic proteins in tumor cells may counteract default induction of apoptosis in G2/M phase cells undergoing missegregation of chromosomes.

To investigate if aneuploidy is a dynamic mutational event, different human tumor cell lines and transformed rodent cell lines have been analyzed for the rate of aneuploidy induction. When grown under controlled *in vitro* conditions, such conditions ensure that environmental factors do not influence selective proliferation of cells with chromosome instability. In one study, Lengauer and colleagues provided evidence by FISH analyses that losses or gains of multiple chromosomes occurred in excess of 10^{-2} per chromosome per generation in aneuploid colorectal cancer cell lines. The study further concluded that such chromosomal instability appeared to be a dominant trait. Utilizing another *in vitro* model system of Chinese hamster embryo (CHE) cells, Duesberg and colleagues have also obtained similar results. With clonal cultures of CHE cells, transformed with nongenotoxic chemicals and a mitotic inhibitor, these authors demonstrated that the majority of the transformed colonies contained more than 50% aneuploid cells, indicating that aneuploidy would have originated from the same cells that underwent transformation. All the transformed colonies tested were tumorigenic. It was further documented that the ploidy factor, representing the quotient of modal chromosome number divided by the normal diploid number, in each clone correlated directly with the

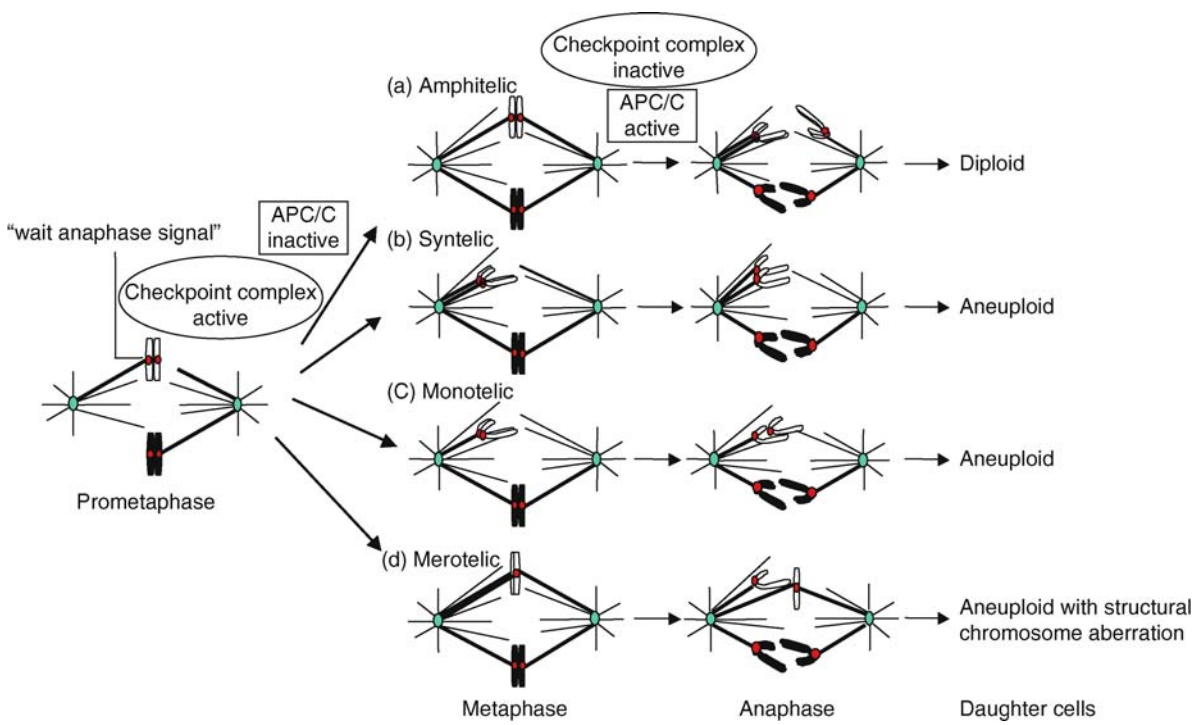
degree of chromosomal instability. Thus chromosomal instability was found proportional to the degree of aneuploidy in the transformed cells, and the authors hypothesized that aneuploidy is an effective mechanism of destabilizing the genome, and changing normal cellular phenotypes.

Genetic Mechanisms of Aneuploidy in Cancer

Numerical chromosomal aberrations giving rise to aneuploidy result when chromosomes are mis-segregated unequally to the daughter cells during mitotic cell division process. Failure to correct mis-attachments of kinetochores with spindle microtubules through mitosis is the major cause of such chromosome mis-segregation. The cell cycle control mechanism that ensures faithful equal segregation of chromosomes during mitosis is referred to as the mitotic checkpoint or the spindle assembly checkpoint (Fig. 2).

The mitotic checkpoint prevents chromosome mis-segregation and aneuploidy by inhibiting metaphase to anaphase transition in cells until the sister kinetochores of all the replicated chromosomes attach appropriately to the spindle microtubules from the two opposing poles in the cell. This form of attachment is known as *amphitelic attachment* and until such time as this attachment is achieved, mitotic checkpoint proteins recruited to the unattached kinetochores generate a diffusible signal (*wait anaphase signal*) that inhibits the anaphase promoting complex/cyclosome (APC/C) from facilitating the degradation of the substrates necessary for transition from metaphase to anaphase and mitotic exit. Thus with an active mitotic checkpoint, inappropriately attached sister kinetochores, such as those with both kinetochores attached to the same pole known as *syntelic attachment* or others with only one kinetochore attached to one pole known as *monotelic attachment* or to the two opposing poles known as *merotelic attachment*, are prevented from proceeding to anaphase with the likely outcome of giving rise to aneuploidy. Aberrant expression of the checkpoint proteins leading to weakening of the mitotic checkpoint, however, allows mis-segregation of inappropriately attached sister chromatids to proceed to anaphase leading to the generation of aneuploid daughter cells.

Chromosome segregation errors may also result in cells with centrosome anomalies giving rise to multipolar spindles. Among the mitotic processes implicated in cancer, defects in centrosome function have been frequently suggested to be involved in a wide variety of malignant human tumors. Centrosomes play a central role in organizing the microtubule network in interphase cells and the mitotic spindle during cell division. Multipolar mitotic spindles have been observed in human cancers *in situ* and abnormalities in the form of supernumerary centrosomes, centrosomes of aberrant size and shape, as well as aberrant phosphorylation of centrosome proteins have been reported in prostate, colon, brain and breast tumors. It is conceivable that cells with abnormal centrosomes may missegregate chromosomes producing aneuploid cells. The molecular genetic mechanism(s)



Aneuploidy. Figure 2 Mitotic checkpoint regulation of chromosome segregation.

regulating centrosome structure/function that are aberrant in cancer cells remain to be elucidated. The presence of supernumerary centrosomes in aneuploid p53-deficient fibroblasts, and over expression of the centrosome associated kinase Aurora-A/STK15 and PLK1 in human cancers have further validated the possibility that aberrant centrosome function is involved in aneuploidy and oncogenesis.

A number of genes involved in the mitotic checkpoint pathway and those regulating chromosome segregation have been found to be aberrantly expressed in human cancer cells raising the possibility that aberrant expression of the respective mitotic checkpoint and chromosome segregation regulatory proteins contribute to the origin of aneuploidy in cancer (Table 1).

In addition to the genes with known functions in mitotic checkpoint and chromosome segregation, mutant alleles of tumor suppressor genes, AdAPC, BRCA1 and BRCA2 have also recently been shown to induce aneuploidy in murine fibroblasts derived form mice expressing mutated forms of these proteins. Similarly, murine fibroblasts lacking the mismatch repair gene Msh2 also reveal widespread aneuploidy indicating that mutations in this gene may be contributing to tumorigenesis by inducing DNA mismatch repair defects and aneuploidy.

Complementing these findings on the likely involvement of aneuploidy inducing genes in the tumorigenesis process, two recent publications on genetically engineered mice aberrantly expressing genes involved in the regulation of chromosome segregation further advance the case for aneuploidy being a *cause* of cancer with some caveats. In one of these studies mice heterozygous for Cenp-E gene, involved in the alignment of chromosomes on mitotic spindle, were reported to develop cancer accompanied by an increase in age dependent whole chromosome aneuploidy although Cenp-E heterozygosity inhibited tumorigenesis in animals lacking the tumor suppressor gene p19/ARF. In the second study mice over expressing the mitotic checkpoint protein Mad2 developed a wide range of tumors with extensive chromosomal rearrangements. However, silencing of Mad2 after tumor formation had no effect on tumor growth, suggesting that Mad2 over expression acts early to promote tumorigenesis. Together, these studies indicate that, like other types of genetic instability, aneuploidy promotes susceptibility to cancer rather than make it obligatory. The concept gains further credence from observations in the human genetic disease *mosaic variegated aneuploidy*, associated with inactivated mitotic checkpoint gene Bub1b, which reveal constitutional aneuploidy and predisposition to develop cancer.

Aneuploidy. Table 1 Genes-Proteins Regulating Chromosome Ploidyin Cancer

Gene name	Function	Mutation/altered expression	Human cancer	Animal models of cancer
Cenp-A	Kinetochore assembly	Upregulated	Yes	
Bub 1	Mitotic checkpoint	Mutated/upregulated/ downregulated	Yes	
Bub R1	Mitotic checkpoint	Mutated/upregulated/ downregulated	Yes	
Bub 3	Mitotic checkpoint	Upregulated/downregulated	Yes	
Mad1	Mitotic checkpoint	Upregulated/downregulated		
Mad 2	Mitotic checkpoint	Mutated/upregulated/ downregulated	Yes	Yes
Cenp E	Motor protein/mitotic checkpoint			Yes
KIF 4	Motor protein			Yes
Aurora-B	Chromosome segregation	Upregulated	Yes	
PTTG (Securin)	Sister chromatid cohesion	Upregulated	Yes	
Survivin	Chromosome segregation	Upregulated	Yes	
Aurora-A	Chromosome segregation	Upregulated	Yes	Yes
PLK 1	Chromosome segregation	Upregulated	Yes	
Nek 2	Chromosome segregation	Upregulated	Yes	
Brca1	Tumor suppressor	Mutated/downregulated	Yes	Yes
Brca2	Tumor suppressor	Mutated	Yes	Yes
AdAPC	Tumor suppressor	Mutated/downregulated	Yes	Yes
Msh2	DNA mismatch repair	Mutated/upregulated/ downregulated	Yes	Yes

Conclusions

The role of aneuploidy as a cancer causing mutation event helps resolve the paradox that with known mutation rate in somatic cells (~10^{-7} per gene per cell generation), tumor cell lineages cannot accumulate enough mutant genes during a human life time. Evidence from human tumor cytogenetic and molecular genetic studies provide compelling evidence in favor of aneuploidy being directly involved in the development of tumor phenotypes. Results from clinical findings support a correlation between origin of aneuploidy and tumorigenic transformation of cells. Molecular genetic analyses of tumor cells suggest that mutations/aberrant expression of genes involved in controlling mitotic checkpoint and chromosome segregation play critical roles in causing chromosome instability leading to aneuploidy in cancer.

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Angiogenesis

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Synonyms

Formation of new blood vessels; Neovascularization

Definition

Angiogenesis is the formation of new capillary vasculature out of pre-existing blood vessels under the regulation of growth factors and inhibitors. It occurs in physiological (e.g. wound healing, ovulation, placental growth) and pathological (e.g. cancer, arthritis, inflammation) conditions.

Characteristics

The formation of new blood vessels out of pre-existing capillaries, the process that is called angiogenesis, is a sequence of events that is of key importance in a broad array of physiologic and pathologic processes. Normal tissue growth such as in embryonic development, wound healing and the menstrual cycle is characterized by dependence on new vessel formation for the supply of oxygen and nutrients as well as for removal of waste products. Also, in a large number of different and non-related diseases, formation of new vasculature is involved in abnormal physiology. Among these pathologies are diseases such as tissue damage after reperfusion of ischemic tissue or cardiac failure, where angiogenesis is low and should be enhanced to improve disease conditions. In a larger number of diseases excessive angiogenesis is part of the pathology. These diseases include cancer (both solid and hematologic tumors), cardiovascular diseases (atherosclerosis), chronic inflammation (rheumatoid arthritis, Crohn's disease), diabetes (diabetic retinopathy), psoriasis, endometriosis and adiposity. These diseases may benefit from therapeutic inhibition of angiogenesis.

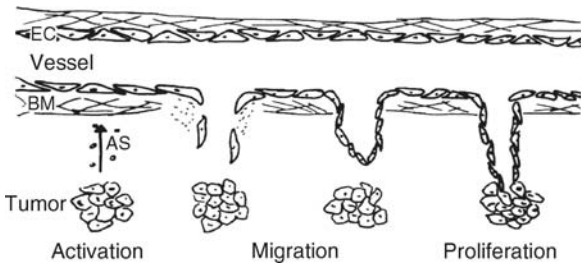
The initial recognition of angiogenesis being a therapeutically interesting process, began in the oncological arena in the early 1970s, when the hypothesis was put forward that tumors are highly vascularized and therefore most vulnerable at the level of their blood supply [1]. It was hypothesized that the process of angiogenesis might be a target for therapy. Since then, it was only after the discovery of the first compounds with specific angiostatic effects in the early 1990s, that the research field of angiogenesis rapidly expanded and provided an increasing body of evidence that inhibition of angiogenesis could attenuate tumor growth [2,3].

The endothelial cells that line the blood vessels play a pivotal regulatory role in the execution of angiogenesis. The sequence of events in endothelial cells that follow the initiation of angiogenesis by exposure to (e.g. tumor derived) angiogenic stimulation consists of:

- Synthesis of proteases that degrade the extracellular matrix
- Migration towards the stimulus
- Proliferation to increase the number of endothelial cells
- Differentiation in order to form a functional vessel (see Fig. 1)

Negative interference in the different steps of the angiogenesis cascade enables different approaches for treatment of cancer:

- Neutralization of angiogenic factors – anti-growth factor antibodies (Avastin), dominant negative growth factor receptors



Angiogenesis. Figure 1 The angiogenesis cascade of endothelial cell activation, degradation of the extracellular matrix and the basement membrane, migration and proliferation. EC, endothelial cell; BM, basement membrane; AS, angiogenic stimulus.

- Inhibition of growth factor receptors – anti-growth factor receptor antibodies
- Desensitization of growth factor mediated intracellular signalling pathways – ►receptor tyrosine kinase inhibitors
- Inhibition of ►matrix metalloproteinases
- Inhibition of endothelial cell ►adhesion
- Inhibition of endothelial cell ►migration
- Inhibition of endothelial cell growth/proliferation

Clinical Aspects

Although the field of angiogenesis research is rather new, the first compounds with angiostatic activity (►Anti-angiogenic drug) have been approved by the US Food and Drug Administration [4]. Most of these compounds are based on interference with growth factors produced by the tumor. Avastin is a monoclonal antibody that blocks ►vascular endothelial growth factor. Other currently approved compounds act through inhibition of signaling (kinase-inhibitor function) of growth factor receptors. Other angiogenesis inhibitors that directly act on endothelial cells are currently in development. One of the advantages of anti-angiogenic therapy is believed to be the lack of induction of resistance to the therapy. This is explained by the fact that endothelial cells are genetically stable cells that are considered not to mutate into drug resistant variants. Although this is a beneficial feature of the anti-angiogenic approach, it is expected that inhibitors of angiogenesis will mainly be used in the future in combination with other anti-cancer modalities such as chemotherapy, irradiation and/or ►immunotherapy.

►Genistein
►Grape Seed Extract
►Prostate-Specific Membrane Antigen (PSMA)
►Tissue Inhibitors Of Metalloproteinases (Timps)

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Angiogenesis Inhibiting Agents

►Vascular Targeting Agents

Angiogenic Factors

Definition

Angiogenic factors are growth factors stimulating proliferation of endothelial cells and new formation of capillaries.

►Sentinel Node
►Vascular Endothelial Growth Factor
►Vascular Disrupting Agent

Angiogenic Switch

Definition

►Angiogenic switch is an alteration in the balance of pro-angiogenic and anti-angiogenic molecules that leads to tumor neovascularization. It is generally thought as a major event in tumor growth and expansion.

►Minodronate
►Macrophages
►B-Raf signaling
►Adenosine and Tumor Microenvironment

Angiogenic/Angiostatic Chemokines

►CXC Chemokines

Angiosarcoma

Definition

Malignant tumor of blood vessels.

►Hepatic Epithelioid Hemangioendothelioma

Angiostatin

Definition

Is a fragment of plasminogen and produced by proteolysis. Kringles 1–4 constitute angiostatin. Kringles domains are folded polypeptides stabilized by three disulfide bonds. A number of blood clotting factors and fibrinolytic components have kringle domains. Angiostatin (kringle 1–4) inhibits endothelial cell proliferation and angiogenesis. In addition, kringle 5 (K5) of plasminogen is also found to inhibit angiogenesis.

►Endostatin
►Kringles Domain

Angiotensin

►Angiotensin II Signaling

Angiotensin II Signaling

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Synonyms

Angiotensin

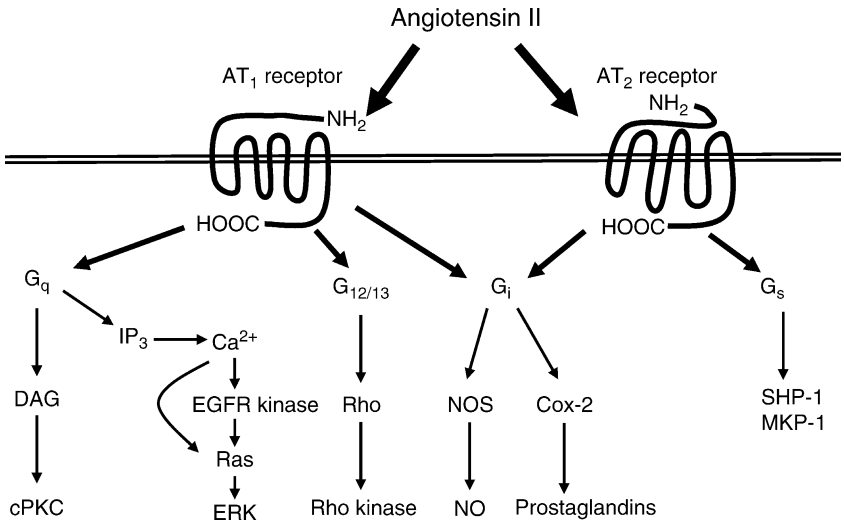
Definition

The angiotensin peptides (angiotensin I, II, III, IV and -(1–7)) are derived from the precursor angiotensinogen by sequential processing proteases such as renin, angiotensin I-converting enzyme (ACE), chymase and other peptidases. Among these peptides, angiotensin II has been well studied and is shown to be the most biologically active peptide. This peptide hormone production system is called the renin-angiotensin system and is one of the phylogenetically oldest hormone systems that has been conserved throughout evolution. The renin-angiotensin system plays a key role in the maintenance of arterial blood pressure and fluid and electrolyte homeostasis. There are two well-defined receptors of angiotensin II (subtype 1 (AT_1) and subtype 2 (AT_2)), both of which are seven trans-membrane, ►G-protein coupled receptors and are encoded by different genes (AT_1 (agtr1), 3q21–25; AT_2 (agtr2), Xq22–23). The major isoform, AT_1 receptor, is expressed in a wide variety of tissues. The AT_2 receptor, the second major isoform, is expressed abundantly in fetal mesenchymal tissues, but its expression decreases significantly immediately after birth. The AT_2 receptor expression level is low in adult tissues but is inducible and functional under pathophysiological conditions. In addition to these angiotensin II receptors, leucyl/cystinyl aminopeptidase and *Mas*-related G-protein coupled receptor member F have been identified as receptors for angiotensin IV and angiotensin-(1–7), respectively.

Characteristics

Angiotensin II Signaling in Carcinogenesis

The renin angiotensin system plays a key role in fluid homeostasis and in blood pressure control. Circulating renin, produced by the juxtaglomerular apparatus of the kidney, and other tissue renin cleaves angiotensinogen to angiotensin I. Angiotensin I-converting enzyme (ACE) catalyzes the subsequent production of the active peptide angiotensin II. Angiotensin II stimulates a variety of biologically important actions, such as vasoconstriction, aldosterone release and cell proliferation. A large portion of these biological actions are executed by locally generated angiotensin II in an autocrine and paracrine manner. The diversity of angiotensin II-induced biological reactions is determined through the expression of two receptors and their coupling with various ►G-proteins. The AT_1 receptor is expressed in a wide variety of tissues and is mainly responsible for most angiotensin II-dependent actions in cardiovascular/renal tissues. The AT_1-mediated angiotensin II signaling stimulates an increase in vasoconstriction (Gq), cardiac hypertrophy (Gq), cell mortality ($G_{12/13}$), nitric oxide (Gi) and ►prostaglandin (Gi) formation (G-proteins in the parenthesis indicate their specific roles, Fig. 1). AT_1-mediated signaling also stimulates production of various growth factors such as ►EGF, basic-FGF, TGF-β and ►VEGF. AT_2 receptor-mediated angiotensin II actions are also diverse, and this diversity is also determined through Gi and Gs protein coupling. Protein tyrosine and serine/threonine



Angiotensin II Signaling. Figure 1 Schematic illustration for diverse angiotensin II signaling.

phosphatase activation (Gs), nitric oxide/cGMP and arachidonic acid/prostaglandin production (Gi) are involved in the mechanism of AT_2 receptor-mediated biological reactions (Fig. 1). The AT_2 receptor can function to counteract AT_1 receptor-mediated angiotensin II bioreactions. However, the AT_1 and AT_2 receptors can also unidirectionally mediate the angiotensin II signal. Angiotensin II also stimulates FGF-2 expression through both the AT_1 and AT_2 receptors. In addition, the AT_2 receptor mediates ►apoptosis in a few types of cells derived from cardiovascular and neuronal tissues *in vitro*.

The stimulation of cell proliferation by angiotensin II-AT_1 signaling has been studied in various cancer cell lines such as ►breast cancer, pancreatic cancer, ►ovarian cancer and prostate cancer. The activation of AT_1 stimulates growth factor pathways such as tyrosine kinase phosphorylation and induces phospholipase C, leading to activation of downstream proteins such as MAPK, JNK and STAT pathways. Furthermore, AT_1 signaling also stimulates ERK1/2 via ►epidermal growth factor receptor (EGFR) transactivation. The AT_1 signaling-induced shedding of heparin-binding EGF by stimulation of metalloproteinases causes the transactivation of EGFR. However, since it is implied that the involvement of transactivation of EGFR by AT_1 signaling is dependent on cell type, pathophysiological significance of angiotensin II-AT_1-dependent EGFR transactivation in carcinogenesis is not yet clear.

Clinical Aspects

Angiotensin II induces the expression of protooncogenes, such as *c-fos* and *c-myc*, and promotes cell proliferation and growth through the AT_1 receptor. AT_1 receptor signaling also stimulates the expression of hypoxia-inducible factor (HIF) 1α and VEGF, which causes resultant neovascularization, a requirement for solid tumor growth. Accordingly, angiotensin II is a mitogenic and pro-angiogenic factor. The AT_1 receptor expression has been shown in the tissues of breast cancer, ovarian cancer, pancreatic cancer, melanoma, prostate cancer and bladder cancer. There is a strong positive relationship between the expression level of the AT_1 receptor and ovarian cancer malignancy and the survival rate of AT_1 positive ovarian cancer patients is significantly lower than the AT_1 negative patients. ACE is also detected in tumor stroma of several types of cancers. These observations suggest that local renin-angiotensin system exists in these various cancer tissues and the AT_1 receptor-mediated angiotensin II signaling may play a significant role in tumor growth. Subcutaneous tumor xenografts in AT_{1a}-KO mice demonstrated that AT_1 signaling in host stromal fibroblasts is also an important regulator of tumor-associated ►angiogenesis. Angiogenesis is an important support mechanism in tumor development. Angiotensin II can directly stimulate capillary network formation by up-regulation of VEGF production in endothelial cells and vascular smooth muscle cells. VEGF is known as a strong angiogenic factor in a variety of cancers. VEGF promotes endothelial cell proliferation, migration and survival. An ACE inhibitor attenuates VEGF-mediated tumor growth, accompanied with the suppression of neovascularization in the tumor and VEGF-induced endothelial cell migration. VEGF expression is up-regulated by AT_1 signaling not only in cancer cells but also in tumor-associated stromal cells including fibroblasts and infiltrated macrophages. Angiotensin II-AT_1 signaling also induces tumor-associated macrophage infiltration. Angiotensin II significantly induced cyclooxygenase-2 (►COX-2) expression in the mouse lung stromal fibroblasts through AT_1 signaling. Prostaglandin E_2, the main product of COX-2 is known to have a

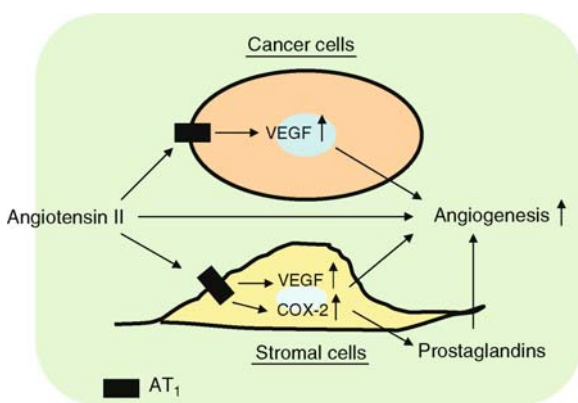
pro-angiogenic effect as well. In fact, the COX-2 inhibitors reduced tumor growth accompanied by an anti-angiogenic effect on tumor tissue. The expression levels of COX-2, and VEGF appears to be tightly associated since VEGF stimulates COX-2 mRNA expression and prostaglandin E_2 increases VEGF mRNA expression in vascular endothelial cells. The COX-2-specific inhibitor suppresses tumor angiogenesis by decreasing VEGF expression in a rat colon cancer model. Furthermore, the selective COX-2 inhibitor Celecoxib and ACE inhibitors or AT_1 receptor antagonists synergistically inhibited colon cancer growth. Accordingly, angiotensin II-AT_1 signaling promotes tumor growth by upregulation of both COX-2 and VEGF expression in cancer cells and stromal cells (Fig. 2).

Attenuation of the AT_1 receptor function by a clinically employed AT_1-specific receptor antagonist has been shown to block lung metastasis of renal cell carcinoma in mice.

A potential mechanism underlying the AT_2 receptor-dependent modification of carcinogen susceptibility appears to be in part due to a modulation of cytochrome P450 expression and stromal fibroblast-dependent support of tumor growth. In addition to angiotensin II receptor blockers, ACE inhibitors retard the growth of cancer cells *in vitro*. ACE inhibitors also inhibit angiogenesis and the growth of tumor xenografts in rats. Therefore, the renin-angiotensin system is an important component in both cancer and cardiovascular diseases.

Epidemiological Study of the Effect of ACE Inhibitors on Cancer Risk

Although the ACE inhibitors (e.g. captopril, lisinopril, enalapril or perindpril) have demonstrated significant anti-tumor effects in *in vitro* studies or animal studies, results of epidemiological studies are not consistent with these studies. In 1998, Lever *et al*. reported that ACE inhibitors decreased the risks of cancer, particularly breast and lung cancer for the first time. However, most of the other epidemiological studies did not find any clear association between ACE inhibitors and risk of cancer. Although it remains unclear the reason for these controversial results, the variety of conditions among studies (the use of different ACE inhibitors, populations, the dose and duration of treatment) might cause these different results. Since angiotensin II is produced not only by ACE but also by other enzymes such as chymase, ACE inhibitors can not completely block the effect of angiotensin II. Therefore, an epidemiological study to determine the association between AT_1 receptor inhibitors and risk of cancer will also be required. Perhaps the most critical issue is that there is no ACE inhibitor or AT_1 receptor blocker case controlled study. There is a strong negative correlation between the expression levels of AT_1 in ovarian cancer tissue and the 5-year survival ratio of patients. Although the sample number is small, this study indicates that angiotensin II signaling has a crucial impact on some types of cancer prognosis. Taken together, angiotensin II signaling is an important component in carcinogenesis and is a potential target for chemoprevention/therapy for various cancers.



Angiotensin II Signaling. Figure 2 The schematic model of the angiogenic effect of angiotensin II in tumorigenesis.

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Aniline

Definition

Is an aromatic amine. It is a colorless, oily liquid, originally obtained from indigo, a blue dyestuff derived from several plants, by distillation. Today it is largely manufactured from coal tar or nitrobenzene as a base from which many brilliant dyes are made.

Aniridia

Definition
Congenital absence of the iris.

►Nephroblastoma

Ankyrin Repeat

Definition
One of the most common protein–protein interaction motifs. Ankyrin repeats are tandemly repeated modules of about 33 amino acids consisting of two alpha helices separated by loops.

►Gankyrin

ANLL

►Acute Myeloid Leukemia

Ann Arbor Staging System

Definition
Is a staging system for lymphomas, both in Non-Hodghia lympsoma and in Hodghin lymphoma.

Local disease on one side of the diaphragm is stage I-II, on both sides of diaphragm is stage III. Stage IV denotes one or more extranodal sites not contiguous with lymph nodes. A and B denote with or without constitutional symptoms (fever, night sweats, weight loss);

►Mantle Cell Lymphoma
►Malignant lymphoma, Hallmarks and Concepts

Anoikis

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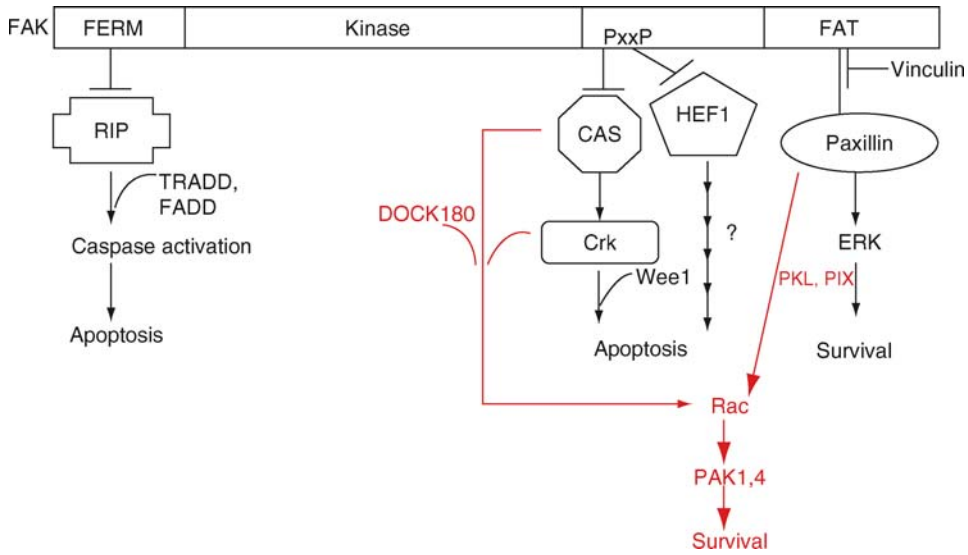
Synonyms
Detachment-induced cell death; Integrin-mediated death

Definition
Apoptosis that is suppressed by extracellular matrix.

Characteristics
Cells that are released from extracellular matrix attachment or cells that are attached to an inappropriate type of matrix are normally programmed to undergo apoptosis. This phenomenon prevents the re-attachment and possible mis-localized colonization of epithelial cells shed during normal turnover, for example, in the gastrointestinal tract. Metastatic tumor cells have undergone genetic or epigenetic changes that invariably render them resistant to anoikis, permitting them to survive during metastasis and underscoring the cancer relevance of this phenomenon. Anoikis is primarily a property of epithelial and endothelial cells, and the epithelial-to-mesenchymal transition (EMT) of tumor cells is accompanied by resistance to anoikis. Accordingly, many activated oncogenes confer anoikis-resistance.

Mechanisms
The prevailing current opinion is that anoikis occurs when survival signaling by ligated integrins is interrupted. An alternative, non-mutually exclusive mechanism is that unligated integrins may also trigger apoptosis by recruiting and activating certain caspases, a phenomenon that has been termed "Integrin-Mediated Death/IMD." However, the data to support this latter view are incomplete at present. This effect, if eventually demonstrated, would represent a novel mechanistic explanation of anoikis.

Survival signaling by integrins is complex. Two pivotal effectors are the ERK sub-family of MAP-kinases and the kinase known as Akt/PKB. ERKs can promote cell survival through several different effects, including: (i) phosphorylation and inactivation of the pro-apoptotic action of the Bcl-2 family member BAD; (ii) down-regulation of the pro-apoptotic Bcl-2 family member Bim and up-regulation of Bcl-xl; (iii) phosphorylation and inactivation of caspase-9. Akt activates several other survival pathways, through other effects: a. inactivation of Glycogen Synthase Kinase-3, which regulates both Wnt/APC/beta-catenin/LEF-1 signaling as well as certain pro-apoptotic transcription factors; b. activation of the pro-survival transcription factor complex NF-kB;



Anoikis. Figure 1 A partial diagram of mechanisms by which FAK promotes cell survival, counteracting anoikis.

c. inactivation of the p73-associated co-factor, YAP; (iv) phosphorylation and inactivation of caspase-9.

Upstream of these kinases, Focal Adhesion Kinase (FAK) contributes substantially to integrin-mediated cell survival. Epithelial cells containing a constitutively active FAK construct are resistant to anoikis, and many human tumors over-express FAK protein. Thus, FAK is considered a potential anti-cancer drug target.

FAK, usually in a complex with c-src, can activate ERKs through several pathways, including: (i) by binding paxillin and augmenting signaling through p21-activated kinase (PAK), the p130cas/crk complex and an exchange factor (PIX) for rac-related GTPases, which are, in turn, important factors in determining anoikis-sensitivity; (ii) by activating the Ras/Raf/MEK/ERK pathway through Grb2/sos1 interaction. FAK can also activate PI3-kinase, activating Akt. FAK can also rescue cells from anoikis by inactivating the pro-apoptotic activity of RIP1, a death receptor adaptor protein. The multiple pathways by which FAK counteracts anoikis are diagrammed in Fig. 1.

Certain Bcl-2 family members now have an established role in anoikis. Although the translocation of Bax to mitochondria occurs in detached cells, a recent report shows that it is the conformational change of Bax rather than its translocation per se that is rate-limiting. Mitochondrial permeabilization by Bax is regulated by several factors, including the "BH3 domain-only" Bcl-2 family members, Bim and Bmf. Both of these latter factors play an important role in anoikis, and they are both regulated transcriptionally as well as post-translationally by their association/dissociation with respect to the actin cytoskeleton.

Several highly cancer relevant genes have been implicated in regulating anoikis recently, two of which are non-integrin proteins involved in cell adhesion and one is a receptor. First, E-cadherin is a major invasion suppressor protein involved in epithelial cell-cell adhesion that is frequently down-regulated in carcinoma cells. Interestingly, mouse genetics data show that cells lacking E-cadherin (in a p53-null background) are resistant to anoikis, indicating that epithelial cells are normally sensitized to anoikis through E-cadherin-mediated cell interactions. This has important implications for the mechanism by which EMT allows tumor cells to resist anoikis.

Second, the neurotrophin receptor protein, trkB, that is over-expressed in pancreatic and prostate tumors, is a potent activator of the PI3-kinase/Akt pathway and thus renders these tumor cells resistant to anoikis, providing an opportunity for trkB-based therapy.

The third is carcinoembryonic antigen (CEA), which is over-expressed on the surface of a variety of tumor cells, appears to program tumor cells to resist anoikis by causing integrin clustering and ensuing survival signaling.

Finally, the Integrin-Linked Kinase (ILK) plays an important role in anoikis because it potently activates Akt as well as promoting EMT, both of which counteract cell death strongly; it also links integrin and growth factor receptor signaling.

Anoikis

Definition

The disruption of interactions between extracellular matrix and specific cognate integrins triggers apoptosis in epithelial cells, in a process termed "anoikis."

►Stem Cells

Anomalous Pancreaticobiliary Ductal Junction

Definition

APBDJ is a congenital malformation at the level of the confluence of the pancreatic and bile ducts. In this disease, the absence of a septum between the ducts results in the formation of a common channel for the bile and pancreatic fluid. APBDJ is classified as three types: a bp type, in which the insertion of the bile ducts is in the pancreatic duct; a pb type, in which the pancreatic duct joins the common bile duct; a Y type, in which there is a long common channel.

►Lysophosphatidylcholine

Anorexia

Definition

Loss of appetite.

►Hepatic Epithelioid Hemangioendothelioma

Anoxia

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Synonyms

Hypoxia; Severe hypoxia; Extreme hypoxia

Definition

Literal definition. Anoxia literally means absence of oxygen (O_2), and has been described as the state where no O_2 (0% O_2) is detected in the tissue.

Conceptual definition. Anoxia has been referred to as extremely low levels of oxygen that trigger secondary responses, additional to the adaptive response of cells to hypoxia, which means low levels of oxygen supply to tissues. Cellular fates in hypoxia can be death, survival, continued proliferation, quiescence, senescence, differentiation, which would depend on the severity of hypoxia and the genetic background of the cell type.

Hypoxia is essentially a functional definition, because there are marked normal differences in the body in oxygen tension, e.g. venous versus arterial circulations in general, but renal medulla arteries have much lower pO_2 than pulmonary veins. Thus a decrease in the normal O_2 for a tissue or organ, sufficient to induce a molecular or physiological response would be an operational definition.

The distinction of terminology between hypoxia and anoxia is important because in hypoxia cells have a much better chance to adapt and survive compared to anoxia. The key biological differences relate to the pathways induced, the much greater death rate, and absence of oxygen in anoxia versus hypoxia.

Characteristics

Oxygen is absolutely essential for life, so the molecular mechanisms underlying responses to low oxygen are central to the cell. The cell has to be able to sense the level of oxygen present so to respond appropriately. For example, when the cell senses hypoxia, such as during intensive exercise, signaling pathways of anaerobic metabolism are switched on, which enables the cell to produce energy and survive under anaerobic conditions, and can be considered as a normal physiological process. This is via the ►hypoxia inducible factor 1 (HIF) pathway.

From a medical point of view, anaerobic metabolism as a consequence of hypoxia is not identical with a pathological situation of anoxia, as exemplified by the oxygen deficit accumulated through extreme exercise. Therefore, it would be important for cells to discriminate and distinguish between hypoxia and anoxia. Whereas the consequences of hypoxia are that of an adaptive response, which ensure cellular survival, the consequences of anoxia could initially be survival, but followed eventually by cell death with time, where cells cannot adapt anymore.

Experiments performed with *Caenorhabtitis elegans* have demonstrated that sensing anoxia is a separate pathway to sensing and adapting to hypoxia. Such distinction between hypoxia and anoxia is also conserved in mammals. It is now well established that tumors also contain areas of hypoxia and anoxia, and the phenomenon of sensing and discriminating between hypoxia and anoxia has recently been emerged and demonstrated in cancer cells and tumors.

In vitro Creation of Hypoxia and Anoxia

Several units have been used to describe the amount of oxygen present. It has been proposed that the partial pressure of oxygen be given in the SI unit kiloPascal (kPa (1,000 N/m^2)) to be in line with international

agreements. 1 kPa equals 10 Bar or 7.5 Torr (or mmHg where 760 mmHg equals 100% O_2). In gas mixtures containing 10,000 ppm (parts per million) of oxygen, the partial pressure is 1 kPa. Most reports have used the unit mmHg or % O_2 to refer to the amount of oxygen used in experiments. The use of ambient air has been referred to the "normal oxygen tension" (normal levels of oxygen) or normoxia as means of control, to which hypoxia is compared to. Typically, experiments testing the effects of hypoxia tend to culture cells in incubators with a gas mix of 5% CO_2 and 95% N_2 until the desired level of hypoxia is reached. The hypoxic cells are then compared to cells cultured in normoxic incubators (normoxia, which is defined as 21% O_2) which consist of ambient air and 5% CO_2.

Anoxia has been achieved in vitro by using incubators with an atmosphere of 5% CO_2, 90% N_2, 5% H_2 and a palladium catalyst to scavenge traces of oxygen. Alternatively, a continuous flow of 95% N_2 and 5% CO_2 has been used. Such conditions have achieved O_2 levels lower than 0.1% and even 0.001% O_2 in tissue cultures of moderate to low cell density, and therefore anoxia has been addressed as O_2 levels <0.1 or 0.001% in several publications.

In vitro normoxia, defined as 21% O_2 (160 mmHg, pO_2), is at least 4 times higher than the physiological in vivo normoxia in most arterial beds. Therefore it has been proposed to make in vivo conditions the standard against which in vitro values should be measured. In venous blood, there is an average 5.3% O_2 (or 40 mmHg O_2), and while some tissues have higher than average oxygen levels, in some tissues (and especially solid tumors) oxygen levels are lower than the average. 21% O_2 is not physiological, especially for tumors where oxygen levels of around 1% (5–10 mmHg) can be a borderline between well and poorly oxygenated tumors. Thus, normally oxygenated (>10 mmHg or 1% O_2) tumors are mostly hypoxic compared to in vitro conditions of 21% O_2, and express HIF1, indicating that many tumors live under hypoxia. The in vitro conditions of hypoxia and anoxia coincide well with oxygen measurements performed with polarographic O_2 electrode needles on patient tumors, which have demonstrated extremely low levels of oxygen such as <2.5 mmHg (<0.3% O_2), including 0 mmHg (0% O_2).

Causes and Consequences of Tumor Anoxia

Areas of low oxygen in tumors may be a consequence of several mechanisms such as abnormal tumor vasculature, limited tissue perfusion, and tumor-associated or therapy-associated ►anemia leading to a reduced oxygen transport capacity of the blood. Over the past decade, clinical studies with oxygen electrodes and molecular markers have shown oxygenation patterns in human tumors to be heterogeneous with respect to the severity and duration of exposure to levels of low oxygen, ranging from 10 mmHg to below 2.5 mmHg including 0 mmHg.

Two types of tumor hypoxia (which if prolonged or severe will lead to anoxia) can be distinguished: perfusion-limited and diffusion-limited hypoxia. Hypoxia will precede anoxia and consequently anoxic tissues will have had induction of hypoxic pathways too. Perfusion-limited or acute hypoxia/anoxia is transient and may be a result of severe structural and functional abnormalities of the tumor microvessels. These abnormalities cause disturbance in the blood supply, leading to temporal shutdown of vessels, gradients of oxygen and nutrients, and even reversal of blood flow. Lack of oxygen can also be caused by an increase in diffusion distances between cells and O_2, resulting in diffusion-limited O_2 supply, leaving cells chronically deprived of oxygen and other nutrients. Over this course of chronic hypoxia and/or anoxia some cells die, but some survive due to mutations in death pathways. This results in areas of ►necrosis that demarcate areas of hypoxia and anoxia, also termed perinecrotic region.

Whereas adaptation to hypoxia is a survival mechanism, anoxia can result in cell death, and has been viewed by some as a protective mechanism to prevent possible cellular transformation associated with anoxic cellular damage. Control of gene expression during anoxia is emerging as an important cellular response, distinct from the response to hypoxia. One feature of cancer is that tumor cells can escape the death pathway of hypoxia and anoxia, attributed to defects in several death pathways, and/or activation of survival pathways. Escaping anoxic cell death could result in selection of cells that have diminished death potential, which results in a more aggressive and therapy resistant tumor type.

The HIF and p53 Interaction Relevant to the Hypoxic and Anoxic Cellular Fate

Under tissue culture normoxia the transcription factor HIF-1α is unstable, degraded and virtually undetectable. As soon as cells experience hypoxia, HIF1α is rapidly stabilized and induced (see hypoxia chapter), regulating majority of the adaptive/survival genes such as glycolytic enzymes and ►VEGF. HIF1α is also induced by anoxia, but several reports have shown that prolonged anoxia results in downregulation of HIF1α in vitro. This in vitro finding has also been observed in some human tumors, which have shown lack of HIF1α expression in perinecrotic anoxic regions. Of some importance to this understanding is the interplay between HIF-1α and the pro-apoptotic transcription factor/tumor suppressor p53.

p53 is expressed at low levels in unstressed cells due to degradation by the ►proteasome. Upon exposure to extremely low levels of oxygen, termed severe hypoxia, close to or equivalent with anoxia, p53 is stabilized,

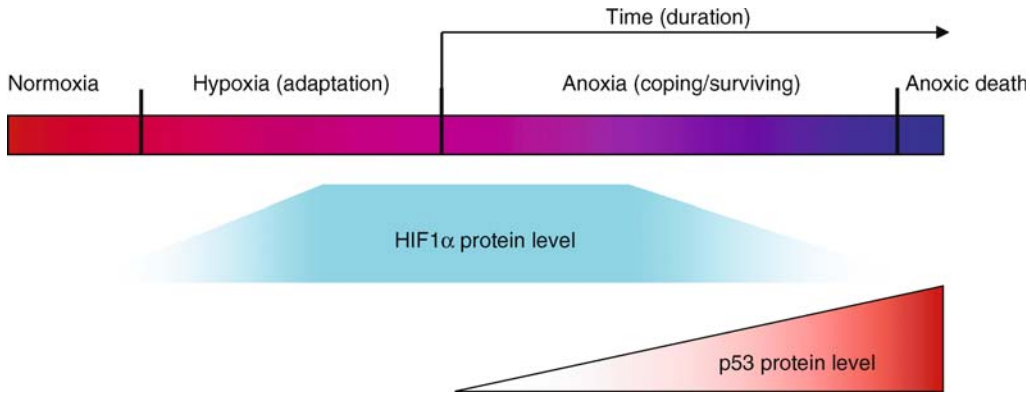
becomes active and facilitates cell death. There has been evidence that HIF1α can interact/bind together with p53, and it has been suggested that p53 accumulation does not overlap with HIF1α accumulation in response to hypoxia but rather to a prolonged period of severe hypoxia or anoxia. Accumulation of p53 in severe hypoxia/anoxia has been shown to both inhibit HIF1α transcriptional activity and reduce the HIF1α protein levels, which might explain why some reports have observed HIF1α levels to decline under prolonged anoxia. This scenario might have implications for prolonged anoxia-induced cell death. In hypoxia, transactivation of HIF1α could serve to protect cells enabling adaptation and survival. In anoxia, inhibition and destruction of HIF1α via p53 may result in a switch from an adaptive hypoxic response into an anoxic death response (Fig. 1). This HIF1α-p53 interactive pathway is one potential mechanism that could determine cellular fate when hypoxia and anoxia need to be discriminated by the cell. This cellular fate of hypoxia versus anoxia can become deregulated in cancer. Tumor cells defective in p53 can escape the anoxic death, and endure longer periods of anoxia, resulting in selection of more aggressive cancer cells.

Oxygen Sensing/Signaling Pathways Relevant to Anoxia

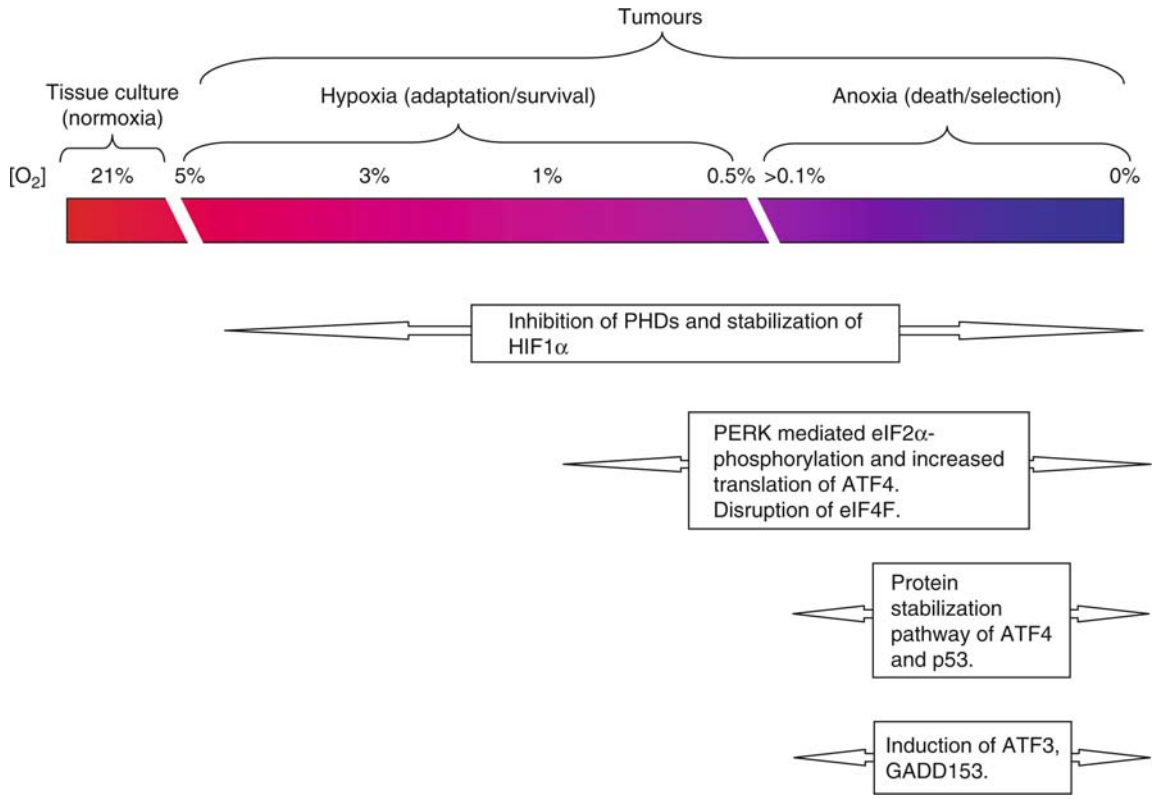
Several distinct oxygen sensing/signaling pathways have been discovered that together determine the cellular response to hypoxia and anoxia. The best characterized of these is a transcriptional response initiated by oxygen-dependent stabilization of HIF1α in hypoxia and anoxia. The stability of HIF1α and its transcriptional activity are regulated by oxygen-dependent hydroxylation of specific amino acid residues. Hydroxylation at two prolyl residues (Pro 402 and Pro 564 in human HIF1α) enables interaction of HIF1α with the von Hippel-Lindau protein (pVHL) that targets HIF1α to proteasomal degradation. These hydroxylations are catalyzed by a series of three closely related HIF prolyl hydroxylases, known as orthologs of C. elegans Egl-9, designated as PH (prolyl hydroxylase) domain containing enzymes (PHD), i.e. prolyl hydroxylases (PHD1, PHD2, PHD3). Transcriptional activity of HIF1α is controlled by hydroxylation of an asparaginyl residue (Asn 803 in human HIF1α), catalyzed by a HIF asparaginyl hydroxylase, also termed factor inhibiting HIF (FIH). Hydroxylation at this site blocks transcriptional activation. The HIF hydroxylases are all iron (II)- and 2 oxoglutarate dependent dioxygenases that have an absolute requirement for molecular oxygen. In hypoxia and anoxia, therefore, hydroxylation is reduced, which allows HIF1α to accumulate by escaping proteasomal degradation. The PHDs could be considered as O_2 sensors of the hypoxia and anoxia HIF1α pathway (Fig. 2).

Anoxia results in induction of several factors including the ATF/CREB (activating transcription factor/cyclic AMP response element binding protein) family of basic region-leucine zipper (bZip) transcription factors such as ATF3 and ATF4, the CCAAT/enhancer binding protein (C/EBP) transcription factor family member ►GADD153, and the transcription factor ►XBP1. Such anoxic response is independent of HIF1α and is mediated by the ►unfolded protein response (UPR), which activates the ►PERK kinase, ►IRE1 and ►ATF6, and takes place after the hypoxic HIF response (Fig. 2). The signaling from downstream effectors of IRE1, PERK and ATF6 merges in the nucleus to activate transcription of UPR target genes.

The regulation of mRNA translation has emerged as an important mediator of the cellular response to hypoxia and anoxia. Distinct mechanisms of translational control have shown to discriminate between initial and prolonged conditions of in vitro generated anoxia. Anoxia results in inhibition of global mRNA



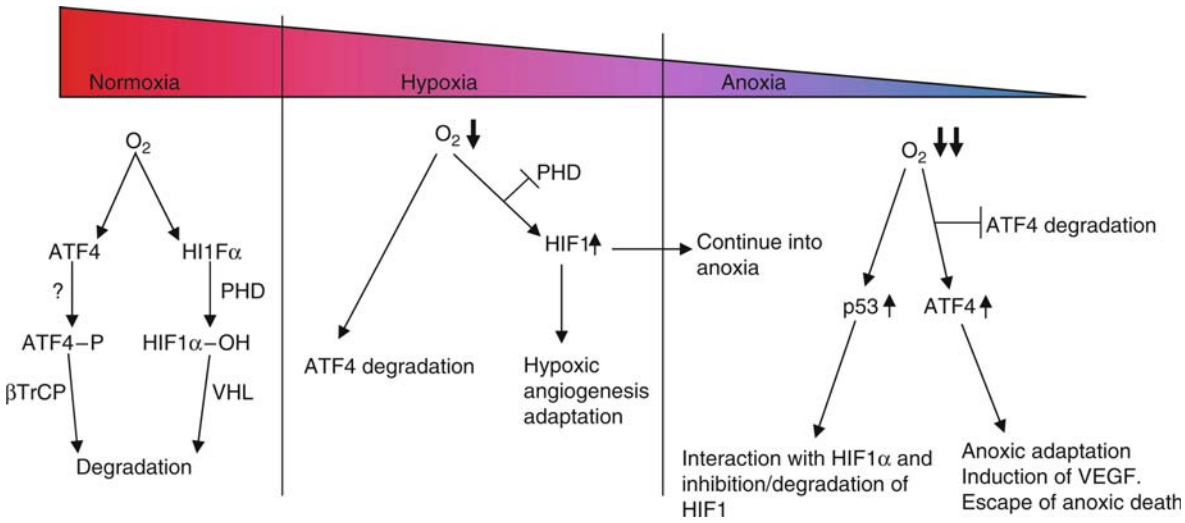
Anoxia. Figure 1 Proposed model (adapted from Schmid T et al [5]) showing the relation of HIF1α and p53 in hypoxia and anoxia. Hypoxic activation of HIF1α is attenuated when p53 starts to accumulate. With progressing time under anoxia p53 accumulates further and promotes HIF1α destruction. Cell survival versus apoptosis is one distinction between extreme end points of hypoxia and anoxia.



Anoxia. Figure 2 Oxygenated tumors are mostly hypoxic compared to in vitro conditions of 21% O_2 and express HIF1α over a broad range of oxygen level, which is sensed primarily by PHDs. Secondary responses, after the induction of HIF1α in hypoxia, include translational control and protein stabilization pathways, which are more specific to sensing anoxia, rather than hypoxia, and can result in induction of factors such as ATF3, ATF4, and GADD153.

translation, which is a biphasic response. A central mediator of the initial translational response to anoxia is phosphorylation of the ►eukaryotic initiation factor 2α (eIF2α) by PERK protein kinase. Phosphorylation of eIF2α in anoxia is extremely rapid, whereas under hypoxia eIF2α is phosphorylated to a smaller degree, and requires prolonged hypoxic exposure. Cells can also distinguish between initial and prolonged anoxia by eliciting a biphasic inhibition of translation. The first phase is due to transient eIF2α phosphorylation, and the second phase of translation inhibition correlates with disruption of the cap binding complex eIF4F. The initial anoxic response of translational inhibition may be important during acute anoxia in tumors, which could be important for enduring anoxia. Although the phosphorylation of eIF2α results in global translational reduction, it specifically induces/increases the translation of ATF4 mRNA, subsequently increasing ATF4 protein levels, which is important for anoxia survival. Indeed, acute anoxic stress (2 h) has shown to be capable of eliciting a cytoprotective pathway, improving the survival of transformed cells following prolonged anoxic stress (24 h), resulting in the clonogenic outgrowth of a population of adapted cells.

Another important mediator of the cellular response to anoxia may be protein stabilization pathways. HIF1α is stabilized and induced rapidly at hypoxic conditions of 5% O_2. Upon reoxygenation it is rapidly degraded. In contrast to HIF1α, the transcription factor ATF4 is not induced by such hypoxic conditions, but is induced by anoxia (Figs. 2 and 3). Like HIF1α, ATF4 protein is unstable and degraded in presence of oxygen. Therefore, modulation of protein degradation pathways seems to be part of a sensing mechanism of both hypoxia and anoxia, but whereas the PHD pathway of HIF1α degradation is blocked in hypoxia, hypoxic degradation of ATF4 is still active. The exact mechanism of ATF4 stabilization in anoxia remains unclear, but under normal oxygen levels of tissue culture ATF4 is degraded by two mechanisms: (i) ATF4 stability modulated by the ►$SCF^{\beta TrCP}$ class of ubiquitin ligase and (ii) ATF4 stability modulated by the histone acetyltransferase p300 (HAT p300). ATF4 contains the βTrCP recognition motif DSGXX(X)S and when the serine of this motif is phosphorylated it results in interaction with βTrCP and subsequent degradation by the proteasome. Histone acetyltransferase p300 induces ATF4 stabilization by inhibiting ATF4/βTrCP



Anoxia. Figure 3 Diagram demonstrating similarities and differences between HIF1α and ATF4 in discriminating between hypoxia and anoxia. Whereas both HIF1α and ATF4 are degraded in normoxia, only HIF1α is induced in hypoxia, whereas ATF4 and p53 are induced in anoxia. In all cases, different protein degradation pathways are involved. Which kinase phosphorylates ATF4 with subsequent recognition by βTrCP targeting ATF4 to proteasomal degradation remains unknown and is shown as?

interaction and subsequent degradation. How this relates to anoxic stabilization of ATF4 remains unknown (Fig. 3). Other mediators of the cellular response to anoxia include mRNA stability pathways and the ►MAPK pathway. The transcription factor ATF3 has been shown to be induced by anoxia. PHDs have been suggested to be potentially involved in regulation of ATF3 induction in anoxia, but a precise role remains unclear. ATF3 mRNA has been shown to be more stable in anoxia compared to normoxia, and translation of ATF3 is also increased in anoxia in a PERK dependent manner. Induction of ATF3 in MKK7 knock-out primary mouse embryonic fibroblasts is fully blocked, which has suggested that the MKK7 pathway might be part of mediating the induction of ATF3 in anoxia. Thus, multiple pathways can converge into regulating ATF3 in anoxia.

Therapeutic Implications of Anoxia

Hypoxia and anoxia are known to directly or indirectly confer resistance to X- and gamma-radiation, and some chemotherapies leading to treatment failures. Many classical radiobiological studies have shown that anoxic cells (O_2 below 0.5 mmHg) are maximally resistant to the lethal effects of irradiation. Therefore, both hypoxia and anoxia are therapeutic problems.

Factors that are induced by anoxia, such as ATF3 and ATF4 have shown to be potentially involved in ►tubulogenesis, induction of VEGF and ►angiogenesis, cell survival, and metastasis. In response to anoxia, protein synthesis is decreased by 60–70% within 1 h, and remains significantly repressed for up to 24 h, but is completely reversible upon reoxygenation. Hence, in anoxia some cells might survive, be selected for and continue to grow upon reoxygenation. Indeed, cells that lack downstream targets of PERK (for example ATF4) and IRE1 (for example XBP1) have shown to be sensitive to anoxia compared to wt cells that contain ATF4 or XBP1. Such anoxic survival is not entirely dependent on HIF1, and cells that are deficient in HIF1 are not more sensitive to anoxia compared to wt cells that contain HIF1. Thus, downstream targets of PERK and IRE1 are important for surviving and adapting to anoxia, as apposed to hypoxia where HIF1 mediates the major survival pathway. Targeting HIF1 alone might select for more aggressive cells with an intact anoxic response pathway.

In addition to hypoxia, anoxia might confer a total separate drug resistance pathway to hypoxia. For example, overexpression of the anoxic factor ATF4 has shown to result in multidrug resistance, and hence, cells under anoxia that induce ATF4 may become selected to a treatment resistance phenotype, if only the hypoxia cascade of HIF1α is targeted.

►Oxygenation of Tumors

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Anoxic

►Hypoxia

Ansamycin Class of Natural Product Hsp90 Inhibitors

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Synonyms

Benzoquinone ansamycin; Geldanamycin; GM

Definition

Ansamycins are a family of antibiotics with antitumor activity. These include geldanamycin (GM), herbimycin A and the macbecins, all of which contain benzoquinone structures. These antibiotics induce tumor cell death via inhibition of the heat shock protein 90 (►Hsp90) chaperone complex and induce the degradation of key client proteins that are required for tumor survival.

Characteristics

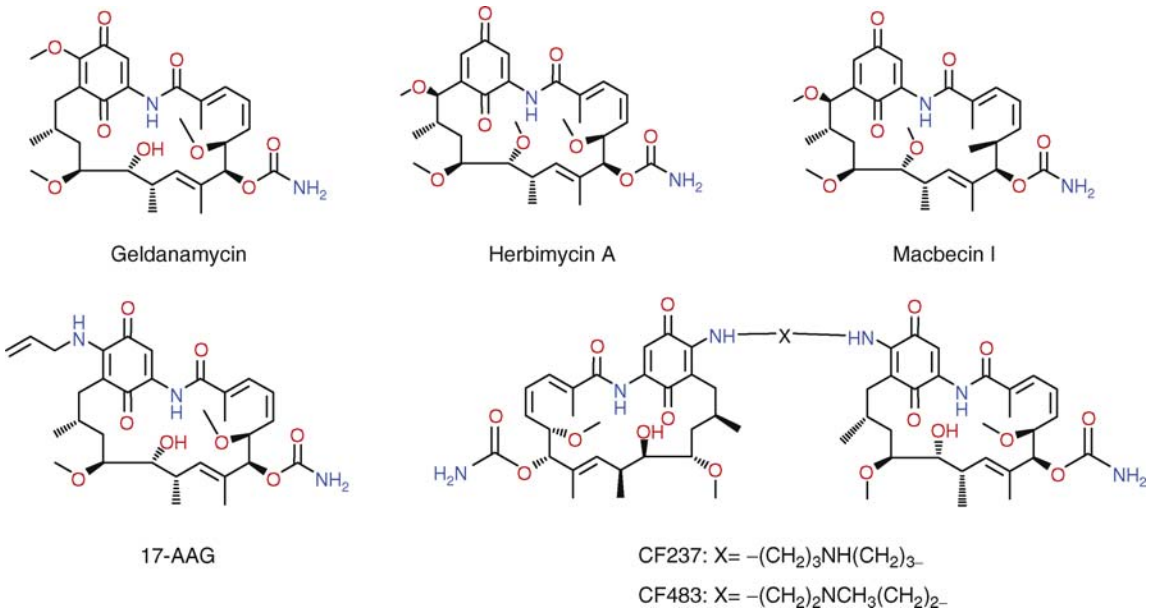
Ansamycins, especially benzoquinone ansamycins, such as herbimycin and geldanamycin, were the first natural product Hsp90 inhibitors identified. Both molecules contain a benzoquinone moiety, which distinguishes them from other ansamycins and confers selectivity for Hsp90 inhibition. Since discovery, these molecules have been widely explored due to their broad-spectrum antitumor activity.

Benzoquinone ansamycins were initially discovered as naturally occurring antibiotics in the fermentation broth of Streptomyces *hygroscopicus* in 1970, with activity against the growth and development of protozoa. Later studies revealed that these molecules possess anti-tumor activity due to their ability to reverse v-Src-induced transformation in cells. However, no direct effect on v-Src was observed *in vitro* experiments, suggesting that they worked by targeting some other factors and indirectly affected v-Src activity. Affinity purification with immobilized GM as bait demonstrated that these molecules exert their function by binding to the Hsp90 chaperone complex and that v-Src activity was blocked because v-Src is an Hsp90 "client" protein that is dependent upon the chaperone for stability and/or function. Co-crystallization of Hsp90 and GM at later time demonstrated that GM bound tightly to the ATP-binding pocket of Hsp90 at the N-terminal of the protein, therefore confirmed that Hsp90 is the true target of benzoquinoid ansamycins.

As the first Hsp90 inhibitor, GM has proved to be of great value in identifying new Hsp90 client proteins and in understanding the biology of Hsp90. Hsp90 is a conserved molecular chaperone that mediates the maturation and stability of a set of cancer-associated proteins which are crucial in oncogenesis. These proteins are collectively referred to as "clients," the members of which are continually growing and include a wide variety of key proteins and kinases that are crucial for carcinogenesis, such as steroid receptors, EGFR family members, IGFR, c-MET, Raf-1 kinase, AKT, Bcr-abl, mutant p53, CDK4 and many other oncogenic molecules. Hsp90 functions as a super-chaperone complex in association with various co-chaperones. Binding of GM or its derivatives to Hsp90 disrupts the complex and sends the dependent client proteins to be degraded in proteasome. This results in simultaneous interruption of many signal transduction pathways and eventually leads to cell death, especially in tumors whose survival and growth depend on Hsp90 client proteins.

Anti-Tumor Activity

Numerous studies with GM demonstrated that the molecule had significant anti-tumor activity *in vitro* and in human xenograft models. However, the molecule also exhibited intolerable hepatotoxicity in animals, rendering it unsuitable for clinical development. Subsequent screening among GM derivatives led to the identification of 17-allyamino-17-demethoxygeldanamycin (17-AAG), that differs from GM at the 17-position (Fig. 1). The co-crystal structure of GM bound to Hsp90 revealed that the quinine ring binds Hsp90 towards the surface of the protein, leaving the 17-position at the entrance of the pocket. This suggested that modification of this position would not affect its anti-tumor activity. Indeed, 17-AAG retained the antiproliferative ability of GM and demonstrated broad-spectrum cytotoxicity against the NCI60 panel. In animal studies, 17-AAG showed reduced hepatotoxicity, but comparable



Ansamycin Class of Natural Product Hsp90 Inhibitors. Figure 1 Benzoquinone ansamycins and its derivatives.

anti-tumor activity. Furthermore, 17-AAG exhibited selectivity for cancerous versus normal tissue where it preferentially bound to the Hsp90 complex in tumors and thus accumulate to high concentration. 17-AAG became the first HSP90 inhibitor to enter clinical trials in cancer patients in NCI-sponsored phase I studies in 2001. The drug was generally well tolerated. Hepatotoxicity was dose-limiting and may be in part related to formulation. Clinical activity has been observed in patients treated with 17-AAG both as a single agent and in combination with trastuzumab, bortezomib or standard chemotherapeutic agents. The responsive tumor types include myeloma, breast cancer, prostate, lung, melanoma, GIST and acute myeloid leukemia. The drug is currently under phase III investigation.

Although 17-AAG is promising in clinical trials, it is poorly soluble, difficult to formulate and has limited bioavailability. Numerous efforts have been made to overcome these problems, including formulation optimization, such as KOS-953, a cremophor-based formulation of 17-AAG, and CNF1010, a nanoemulsion of 17-AAG. Alternatively, more soluble ansamycin analogs, including 17-DMAG and IPI-504 (a quinine reduced form of 17-AAG), are also under phase I evaluation. Although phase I trials are not designed for examining antitumor activities, responses from patients have been observed. Apart from ansamycin derivatives, several companies have also developed small molecule inhibitors with totally novel chemical structures, which are either in early clinical or late discovery stages.

During the development of Hsp90 inhibitors, we also noticed that 17-AAG showed a limited duration of its effects on target proteins and lost antiproliferative activity precipitously under conditions of brief cellular exposure. Since active Hsp90 exists as obligate dimer and the two ATP binding sites are in close apposition in the multichaperone complex, we reasoned that two GM moieties separated by an optimized flexible linker might be able to engage both ATP sites simultaneously and may therefore inhibit Hsp90 more efficiently. GM dimers were originally reported by Zheng FF et al. These early compounds were relatively weak Hsp90 inhibitors that primarily induced the degradation of the most sensitive Hsp90 client, HER-2, but were largely ineffective on other clients. By screening an internally generated library of GM dimers linked at 17-position, we identified some active dimeric ansamycins, represented by CF237 and CF483 (Fig. 1), which readily cause Hsp90 inhibition and client protein degradation. The dimers are more potent under conditions of limited exposure compared to 17-AAG, whose biochemical and cellular effects were relative transient. This presumably results from continuous binding of dimeric compounds to dimeric Hsp90, as dissociation does not occur until both GM moieties dissociate from their binding sites concomitantly. This long-lived binding leads to prolonged biological activity in tumor cells even after the compound was removed, a situation which more closely reflects physiological conditions. Indeed, dimer-treated cells displayed markedly reduced client protein levels for at least 48 h after drug removal; in comparison, 17-AAG induced only transient suppression of the same clients and the pathways they control. The dimers were also retained for longer in tumor xenografts and displayed superior antitumor activity in vivo, especially when the drugs were given at long intervals.

Dimeric drugs with improved biological activity have been reported. Vancomycin dimers show enhanced antibiotic effect against both susceptible and drug-resistant gram-positive bacteria than that of the parent monomer. In a mammalian system, longer-acting bivalent modulators against dimerizing target, G-protein-coupled β2-adrenergic receptor, was also recorded. The binding of high-affinity dimeric Hsp90 inhibitors to their target may be essentially irreversible, but these compounds do not form covalent adducts with the Hsp90. Therefore, they should not inherit the drawbacks of irreversible inhibitors, which can result in increased toxicity. Indeed, the dimeric ansamycin was well tolerated at effective doses in xenograft studies. Moreover, the prolonged acting of dimeric compounds on Hsp90 may increase the range of susceptible tumors due to sustained inhibition of the target. For example, both CF237 and CF483 are active in Rb negative and Bcl-2 overexpressing cells, while 17-AAG is not. It is believed that continuous suppression of client proteins induced by dimers depletes the essential elements for survival and leaves no field for the activated oncoprotein to carry out its function. All these results indicate that monomeric and dimeric ►Hsp90 inhibitors have distinct biological profiles and work differentially toward target inhibition. However, on the other side of the coin, the greater mass of dimeric compounds (~1,200 kDa) would likely retard their permeation through the tightly packed cells and fibrous stroma that characterize solid tumor masses. Similarly, the slow dissociation of dimers from their target would not favor penetration of the drug to distant extravascular sites. Although activity was observed with CF237 and CF483 in solid tumors in preclinical models, the limitations should be considered for future applications.

Since cancer cells normally accumulate multiple mutations, inhibition of single pathway is usually not sufficient to suppress tumor growth. Therefore targeting Hsp90 and hence its chaperoned proteins and pathways becomes increasingly intriguing in cancer therapy. In addition, tumor cells frequently develop resistance by mutating the target protein and escaping apoptotic cell death. It is becoming clear that mutant oncoproteins are more reliant on Hsp90 for maturation and function, thus making Hsp90 inhibitors particularly attractive in resistant tumors and in the scenario where tumor growth is driven by mutated client proteins, such as mutant EGFR in non small cell lung cancer or mutant BRaf in melanoma, even though the wild type counterparts of these two proteins are not Hsp90 clients.

The promising results from 17-AAG and its derivatives in clinical trials are encouraging researchers to develop more potent Hsp90 inhibitors. The beneficial effect of novel drugs with diverse chemical structures has to be determined clinically, and in turn, clinical results will drive the design of superior generations of Hsp90 inhibitors.

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Ansomia

Definition

Is the lack of the sense of smell.

►Nasopharyngeal Carcinoma

Antagonism

Definition

Occurs when two or more agents in combination have an overall effect which is weaker than the sum of their individual effects.

►Xenobiotics

Anterior Pituitary Gland

Definition

The pituitary gland which is a master-regulatory endocrine gland situated in the pituitary fossa of one of the skull bones referred to as the sphenoid, consists of two parts that are called the anterior and posterior pituitary. The anterior pituitary secretes hormones that include, prolactin, thyroid stimulating hormone (TSH),

growth hormone (GH), adrenocorticotrophin (ACTH), luteinising hormone (LH), and follicle stimulating hormone (FSH).

►Multiple Endocrine Neoplasia Type 1

ANTH

Definition

AP180 N-terminal Homology domain. A primary sequence of amino acids that is found in endocytic adaptor proteins and binds to inositol lipids. This binding is thought to be necessary for promoting a number of steps in endocytosis.

►Huntingtin Interacting Protein 1 (HIP1)

Anthocyanins

Definition

A group of natural antioxidants widely distributed in colored fruits and vegetables.

►Chemoprotectants

Anthracycline

Definition

Anthracyclines, consisting of daunosamine and tetrahydro-naphthacene-dione, are a class of chemotherapeutic agents used to treat many cancers. These compounds exhibit cytotoxic activity through intercalation into DNA, inhibition of topoisomerase II, and the production of free radicals. In cancer chemotherapy with anthracyclines, the serious problem is the side effects, cardiotoxicity and bone marrow depression, and the emergence of the drug-resistance.

►Adriamycin
►ABC-Transporters
►Liposomal chemotherapy

Anthropogenic Activity

Definition

Human activities, as opposed to those occurring in nature without human influences

►Lead Exposure

Anti-Angiogenic Drugs

Definition

Therapeutic agents designed to inhibit the growth of new blood vessels into a solid tumor. Anti-angiogenic agents deprive tumors of oxygen and nutrients and inhibit their survival and continued growth. Examples of anti-angiogenic drugs include bevacizumab (Avastin®), a recombinant humanized monoclonal antibody directed against the ►vascular endothelial growth factor (VEGF) and targeted protein-tyrosine kinase inhibitors such as sorafenib (Nexavar®), which inhibits VEGF receptor kinase activity and thereby blocks the propagation of pro-angiogenic signals.

►Anti-Angiogenic Drugs
►Angiogenesis

Anti-HER2/Neu Peptide Mimetic

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Synonyms

AHNP

Definition

AHNP is a rationally designed biologically active ►peptidomimetic that mimics an ►anti-Her2/neu monoclonal antibody (Mab)'s anti-tumor function. AHNP is the smallest antibody fragment, which is derived from the ►complementarity determining region (CDR) heavy chain 3 (H3) of the anti-HER2/neu antibody rhuMab 4D5 (►Herceptin (trastuzumab)).

Receptor Characteristics

The ►epidermal growth factor (EGF) family of ►tyrosine kinase receptors includes four structurally related members: erbB1 (EGFR, HER1), erbB2 (HER2/neu, p185), erbB3 (HER3), and erbB4 (HER4). ErbB receptors are crucial for mediating cell proliferation, differentiation, and survival. They are glycoproteins composed of an ectodomain, a single transemembrane region, and a cytoplasmic tyrosine kinase domain flanked by noncatalytic regulatory regions. The ectodomain contains four subdomains: L1, S1, L2, and S2, where L and S are acronyms for large and small, respectively. These subdomains are also referred to as subdomains I–IV. Subdomains II and IV are Cys-rich subdomains. A family of ligands, EGF-like peptide growth factors, binds to the ectodomain of erbB receptors, leading to the formation of homo- and heterodimers. However, HER2/neu is an unusual member of the erbB family. It dimerizes in a ligand-independent manner. Moreover it is apparently ligandless, since no authentic ligand that directly binds to it has yet been defined. Dimerization consequently stimulates the intrinsic tyrosine kinase activity of the receptors, triggering tyrosine autophosphorylation in the cytoplasmic domain. Phosphorylated tyrosine residues serve as docking sites for intracellular signaling molecules involved in the regulation of signaling cascades. Thus the dimeric species are considered the active form and responsible for signaling. Persistent signaling contributes to tumorigenesis.

Her2 Regulation

Deregulated expression of erbB receptors, in particular, erbB1 and Her2/neu, has been implicated in the development and malignancy of numerous types of human cancers. Hence, various strategies have been developed to target erbB receptors, including antireceptor MAbs, toxin-MAb and toxin-erbB ligand conjugates, synthetic tyrosine kinase inhibitors, and antisense therapy. We have demonstrated that MAbs to the extracellular domain of Her2/neu can downmodulate the receptor from the cell surface, resulting in a reduction in the malignant phenotype and a conversion of the cell phenotype into a more normal one in vitro and in retardation of tumor growth in vivo. This early work has been translated into the creation of humanized anti-receptor antibody (Herceptin or trastuzumab), which has been approved for the treatment of metastatic ►breast cancer. Other anti-erbB receptor antibodies or antibody like molecules are currently under development as therapeutic agents.

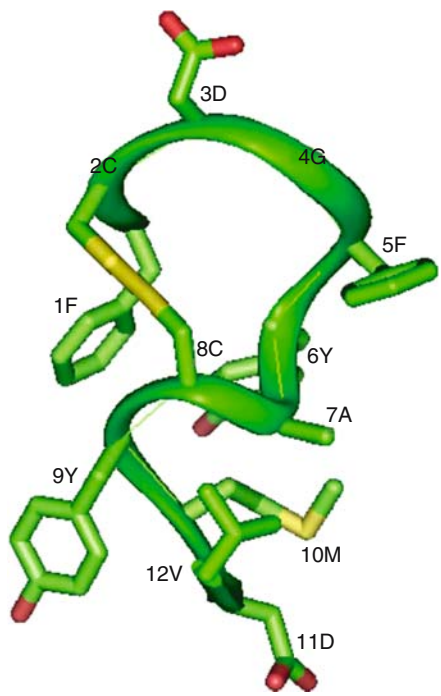
AHNP Characteristics

Rational for Design of AHNP

An antibody is large, difficult to produce and expensive to synthesize and purify. Due to their large size they often are impeded at the periphery of solid tumors and are unable to enter inside the tumor mass. Several studies have reported the creation of smaller antibody fragments such as ►single chain antibody (ScFv). These single chain antibodies have limited antitumor activity to date. A promising alternative approach to overcome the limitations of high-molecular-weight therapeutics is to design mimetic peptides derived from the antigen-binding site of antibodies. The advantages of peptidomimetics over therapeutic proteins include the ease of manufacturing, low immunogenity and potential applicability to a wider range of disease targets, including those inside the cell.

AHNP

A 1.5 kDa anti-HER2 peptide mimetic (AHNP) (Fig.1) derived from the structure of the CDR-H3 loop of the anti-HER2 rhu MAb 4D5 has been designed with demonstrated in vitro and in vivo activities in disabling HER2 tyrosine kinases are comparable to the MAb. AHNP has been shown to bind to the rhu MAb 4D5 epitope on Her2/neu with submicromolar affinity. It inhibited proliferation of Her2/neu-overexpressing tumor cells and colony formation in vitro, as well as growth of Her2/neu-expressing tumors in athymic mice. In addition, AHNP sensitized the tumor cells to ►apoptosis when used in conjunction with ionizing radiation or ►chemotherapeutic agents. AHNP was as effective as Herceptin in reducing tumor size and more effective in its ability to block proliferation.



Anti-HER2/Neu Peptide Mimetic. Figure 1 NMR structure of AHNP.

AHNP Analogs

To further develop AHNP as an anti-tumor agent and as a radiopharmaceutical for tumor imaging, a number of derivatives of AHNP have been designed. Structure–function analysis of AHNP analogs was used to optimize their biophysical and therapeutic properties. Some of the designed AHNP analogs had improved binding properties, solubility, and cytotoxic activity relative to AHNP. Residues in the exocyclic region of AHNP appeared to be essential for high-affinity binding. The study also led to important observations of the analog properties that are essential for their biological activities. Kinetic and equilibrium analysis of peptide-receptor binding for various AHNP analogs revealed a strong correlation between peptide-binding characteristics and their biological activity. For AHNP analogs, dissociation rate constants have been shown to be better indicators of peptide biological activity than receptor-binding affinities. This study has demonstrated that the well-documented biological effects of antibodies, accounting for their applications in tumor therapy, can be mimicked by much smaller antibody-based cyclic peptides with potentially significant therapeutic advantages.

AHNP as a Drug Carrier

Due to high specificity of AHNP to the HER2/neu receptor, it has been used as a ►drug delivery agent against HER2/neu-overexpressing tumors in vitro and in vivo. The breast tumor targeted peptide carrier P3-AHNP has been developed by conjugating AHNP with a modified HIV TAT-derived cell-penetrating peptide. A signal transducers and activators of transcription 3 (STAT3)-inhibiting peptide conjugated to this peptide carrier (P3-AHNP-STAT3BP) was delivered more efficiently into HER2/neu-overexpressing than HER2/neu low-expressing cancer cells in vitro and successfully decreased STAT3 binding to STAT3-interacting DNA sequence. P3-AHNP-STAT3BP inhibited cell growth in vitro, with HER2/neu-overexpressing 435.eB breast cancer cells being more sensitive to the treatment than the HER2/neu low-expressing MDA-MB-435 cells. Compared with HER2/neu low-expressing MDA-MB-435 xenografts, i.p. injected P3-AHNP-STAT3BP preferentially accumulated in 435.eB xenografts, which led to more reduction of proliferation and increased apoptosis and targeted inhibition of tumor growth. This novel peptide delivery system provided a sound basis for the future development of safe and effective new-generation therapeutics to cancer-specific molecular targets.

In another study, the HER2/neu-targeting/neutralizing function of AHNP was exploited by coupling AHNP to a mitochondriatoxic proapoptotic peptide PAP. The engineered chimeric peptide, BHAP, selectively triggered apoptosis in HER2/neu-overexpressing tumor cells and inhibited growth of HER2/neu-overexpressing human mammary xenografts established in SCID mice. BHAP was selectively internalized by human breast cancer cells through HER2/neu-mediated endocytosis and induced apoptosis in vitro and in vivo. The peptide was effective against HER2/neu-overexpressing cells, even those that have been previously described as Herceptin resistant. To increase the avidity of the chimeric peptide for HER2/neu, a tetrameric form has been created through ►streptavidin binding of ►biotin-labeled BHAP. Tetramerization resulted in a significant (19–80 fold) improvement of the efficacy of BHAP against the HER2/neu positive breast cancer cells.

►Taxol (paclitaxel) has been coupled to a bivalent form of AHNP for targeting the chemotherapeutic drug to HER2-overexpressing cells. The prodrug conjugate released Taxol after receptor-mediated internalization resulting in selective toxicity towards HER2/neu positive cells.

The studies where AHNP was used as a tumor-targeting agent have demonstrated a ►context independent nature of its biological activity. Indeed, AHNP could effectively carry drugs to tumor cells when it was included either in the middle of the chimeric construct or at the amino or carboxy termini. This important property facilitates the design of AHNP-drug conjugates and can largely explain the popularity of AHNP as a ►drug carrier agent.

AHNP in Breast Cancer Diagnosis

Since AHNP show antitumor effect in an context-indpendent manner, it was used in a highly sensitive tumor detection technique, termed as “IDAT”. Furthermore, AHNP was engineered as a fusion protein containing AHNP and a ►non-immunoglobulin (Ig) protein scaffold, streptavidin (SA) to improve the receptor-binding and pharmacological properties of AHNP. The recombinant tetrameric protein, AHNP-SA bound to the HER2/neu receptor with high affinity, inhibited proliferation of HER2/neu overexpressing cells and reduced tumor growth induced by HER2/neu-transformed cells. These studies suggest that the tetrameric form of AHNP, as an antibody-surrogate molecule, can be used for tumor diagnosis.

Relevance to Cancer Therapy

►Monoclonal antibody therapy provides high target specificity, but has limitations and challenges in drug development because of the large size of the therapeutic agent resulting in high cost and significant delivery problems. Therapeutic antibodies are efficient against hematologic malignancies. Such targeted antibodies reactive against solid tumors are less efficacious due to barriers that limit their entry into the tumor tissues. The main barriers for antibody transport into tumor mass are dense ►extracellular matrix (ECM) and high interstitial pressure in the tumor. Due to these barriers, therapeutic

molecules must diffuse across the tumor mass. Since the rate of diffusion is inversely proportional to the molecular radius, large antibody molecules diffuse very slowly. Thus, diffusion of antibodies across solid tumors usually takes weeks to months making larger tumor masses especially difficult to treat with monoclonal antibody therapy. To overcome the ►pharmacokinetic limitations of the antibodies, several studies report design of lower molecular weight constructs including ►Fab and Fab′2 fragments, ScFvs, multivalent ScFvs, minibodies, bispecific antibodies, and camel variable functional heavy chain domains. Diffusion of a smaller fragment such as ScFv into tumors is usually faster than that of a full antibody, but could still take up to a month. Tumors are known to be genetically unstable and may acquire resistance if antibody therapies are slowed down by poor penetration. AHNP is the smallest antibody fragment that has been shown to possess anti-tumor effects comparable to an anti-Her2/neu antibody in vivo. Because of their significant advantages over antibodies in terms of molecular weight, tumor penetration and ability to be easily conjugated with cytotoxic drugs, AHNP-like peptide mimetics are expected to be very effective for tumor therapy.

Conclusions

AHNP is the first rationally designed small antibody fragment containing molecule that possesses the ability to reduce tumor growth when used either alone, or in combination with cytotoxic drugs. Its specificity also has been shown to be useful in tumor diagnostics. Due to its small size AHNP is amenable for further modification to create molecular species for clinically useful tumor therapy. AHNP represents a new paradigm in antibody based tumor therapy.

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Anti-human p185neu Receptor Immunoglobulin G1

►Herceptin

Anti-idiotype Vaccination

►Idiotype Vaccination

Anti-Idiotypic Antibodies

Definition

bind specifically to the antigen-binding site (idiotope) of another antibody and are therefore bound specifically by the other antibody. When used for ►immunotherapy, an ►antibody mimicking an epitope of a tumor-associated protein is administered in an effort to stimulate the patient's immune system against the tumor, via the tumor-associated protein. Anti-idiotype monoclonal antibodies structurally resembling tumor-associated antigens can be used as antigen substitutes in cancer patients.

Anti-Inflammatory Drugs

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Synonyms

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Definition

Anti-inflammatory drugs include steroid and nonsteroidal anti-inflammatory drugs (also called ►NSAIDs). Generally, they are used to relieve clinical symptoms such as ►inflammation, swelling, stiffness, and pain.

Characteristics

Steroid Anti-Inflammatory Drugs

Steroid anti-inflammatory drugs such as ►glucocorticoids are hormones naturally produced by the adrenal gland with a variety of important physiological activities within the body. They are prescribed for treatment of diseases such as adrenal insufficiency, arthritis, asthma, ►inflammatory bowel disease, transplant rejection, and the graft-versus-host disease. Glucocorticoids are also a very effective anticancer drug for childhood ►acute lymphoblastic leukemia (ALL), as well as in other lymphoid malignancies. The immune suppressive effect and anticancer therapy of glucocorticoids is mediated by the glucocorticoid receptors to specifically induce ►programmed cell death (also called apoptosis) on lymphocytes in lymphoid tissues. Thus, glucocorticoids are pivotal in the treatment of ►ALL clinically.

Glucocorticoids have been used in huge amounts in the last 40 years. Unfortunately, long-term use of steroids is associated with very severe side effects, including obesity, hyperlipidemia (higher levels of lipid contents in blood), hyperglycemia (higher levels of blood glucose), and hypertension (an increase in blood pressure).

Nonsteroidal Anti-Inflammatory Drugs

►NSAIDs are commonly prescribed medications for the inflammation of arthritis and other inflammatory diseases such as in tendinitis and bursitis. There are two classes of NSAIDs, including a classical NSAIDs (aspirin, sulindac, and various related agents) and ►cyclooxygenase-2 (COX-2) inhibitors (►celecoxib, rofecoxib, and others). All classical NSAIDs act as nonselective inhibitors of the enzyme ►cyclooxygenase and are able to inhibit both COX-1 and COX-2 enzymes with a predominant effect on COX-1, whereas COX-2 inhibitors bind selectively to COX-2. Both COX1 and COX1 are able to catalyze arachidonic acid, resulting in synthesis of ►prostaglandins (PGs) including PGD2, PGE2, prostacyclin, PGF2alpha, and thromboxane. PGs regulate various pathophysiological processes such as inflammatory reaction, gastrointestinal cytoprotection. NSAIDs alleviate pain and inflammation by counteracting the COX activities, thereby inhibiting prostaglandins and thus reducing or eliminating inflammation and pain.

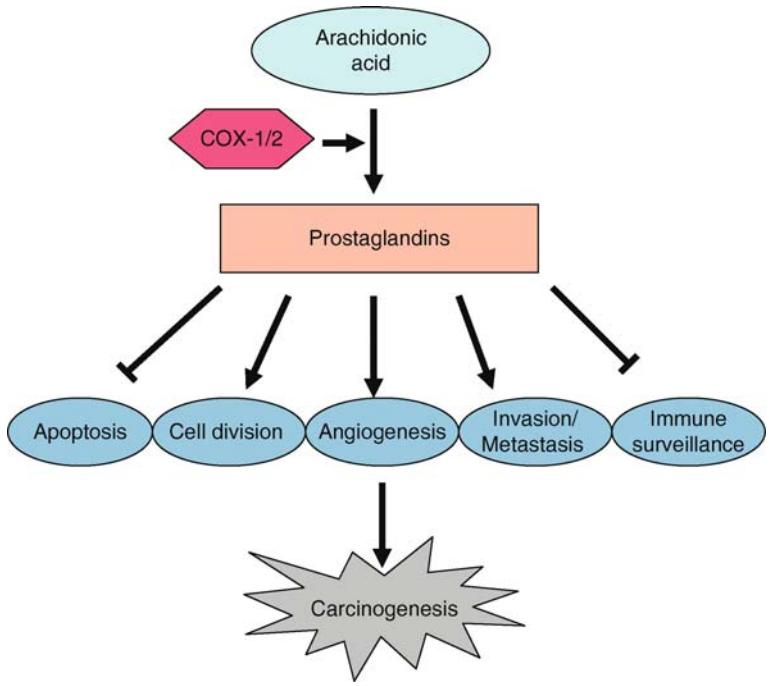
It is becoming increasingly apparent that many cancers are associated with a chronic inflammatory response such as the development of gastric carcinoma in patients with ►*Helicobacter Pylori* infection. Indeed, the tumor microenvironment such as tumor stroma, tumor-associated ►macrophages, cytokines, ►chemokines, and reactive oxygen/nitrogen species contributes significantly to the ►carcinogenesis. Signaling pathways that link inflammation with cancer include the COX-2, NF-kappaB, and phosphatidyl inositol 3-kinase (PI3K)/Akt pathways. Thus, therapies against the inflammatory process also act as either treatment or prevention of cancers.

Increased levels of COX-2 expression have been reported in carcinomas of the colon, stomach, breast, esophagus, cervix, lung, liver, prostate, and pancreas. High levels of COX-2 expression appears to be involved in the development of cancer by promoting ►cell division, inhibiting ►apoptosis, stimulating ►angiogenesis, altering cell adhesion and enhancing ►invasion and metastasis, and influencing ►immune surveillance (Fig. 1). All these actions result in cancer development and progression. The inhibition of COX-2 activity by NSAIDs blocks these activities and thus may account for the anticarcinogenic activity of these drugs.

In addition to mechanisms that involve the inhibition of COX-2, mechanisms independent of COX-2 also participate in the anticarcinogenic activities of NSAIDs. Celecoxib mediates antitumor effects through the inhibition of a signaling pathway called phosphoinositide-dependent kinase-1 (PDK-1)/Akt, which is Cox-2-independent. Thus, blockade of PDK-1/Akt by celecoxib in cancer cells triggers programmed cell death. However, such an observation has not yet been described for rofecoxib. Thus, each traditional NSAID appears to have its own, more or less specific, COX-independent anticancer mechanisms, which requires further investigation.

Side Effects of NSAIDs

Long-term use of NSAIDs, which inhibit both COX-1 and COX-2, is associated with serious side effects. Some 10–50% of patients are unable to tolerate NSAID treatment because of abdominal pain, diarrhea, bloating, heartburn, and upset stomach. Approximately 15% of patients on long-term NSAID treatment develop ulceration (an open wound) of the stomach and duodenum. The worse is that those with unaware of their ulcers are at risk of developing serious ulcer complications such as bleeding or perforation of the stomach. These side effects have been attributed to the inhibition of COX-1, which mediates gastroprotective prostaglandin production. To overcome these side effects associated with COX-1 inhibition, selective COX-2 inhibitors, such as celecoxib and rofecoxib, were developed with fewer gastrointestinal side effects than traditional NSAIDs. Nevertheless, the group of COX-2 inhibitors, including celecoxib, rofecoxib, valdecoxib, etoricoxib, and lumiracoxib, is under critical investigation because increased risk of cardiovascular side effects such as an increase in blood pressure, stroke, and myocardial infarction have been reported after long-term use of rofecoxib and of valdecoxib. Thus, for those taking NSAIDs for more than 1 or 2 months or in large amounts, consultation



Anti-Inflammatory Drugs. Figure 1 Mechanisms of COX inhibitors in anticancer. Both COX1 and COX1 are able to catalyze arachidonic acid and stimulate synthesis of prostaglandins (PGs), which play a role in carcinogenesis by promoting cell division, inhibiting apoptosis, stimulating angiogenesis, enhancing invasion and metastasis, and influencing immune surveillance. Administration of the COX-1/2 inhibitors blocks COX-1/2 activities and thus reduces prostaglandins, resulting in prevention or treatment of cancers.

with the doctor the good that it can do as well as the risks of taking it is highly advised.

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Anti-Ro/SSA and Anti-La/SSB Antibodies

Definition

Anti-Ro/SSA and anti-La/SSB antibodies, which are directed against two extractable nuclear antigens, have been detected with high frequency in patients with ►Sjogren syndrome.

Antiangiogenesis

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Definition

The prevention or inhibition of the process of new blood vessel formation by endothelial cells from pre-existing adjacent vessels (►angiogenesis).

Characteristics

Rationale for Antiangiogenesis for Therapy

Formation of new blood vessels is one of the hallmarks of ►cancer. Therefore, preventing this process – antiangiogenesis – has emerged as a potential therapeutic strategy to halt cancer growth. This was based on the concept promoted by Dr. Judah Folkman (Harvard Medical School, Boston, USA) that new blood vessel formation plays a determinant role in tumor growth and progression to ►metastasis. Given its importance in tumor progression and treatment, angiogenesis has prompted enormous interest in the oncology field over the last few decades. But angiogenesis plays a central

role in other pathological states such as vascular diseases, benign tumors, obesity or atherosclerosis. In cancer and other diseases, the new vessels form due to the chronic overexpression of multiple proangiogenic factors: for example, ►Vascular Endothelial Growth Factor A (VEGF-A or ►VEGF), ►basic Fibroblast Growth Factor (bFGF), and ►Placental-derived Growth Factor (PlGF). As a result, the new vessels are immature and structurally and functionally abnormal. This in sharp contrast to angiogenesis during physiological states (embryonic and postnatal development, wound healing, the menstrual cycle, and pregnancy); in these cases, angiogenesis is tightly regulated spatially and temporally by well-balanced endogenous pro- and antiangiogenic factor expression, and the resulting vessels are fully functional and specialized for organ-specific functions. More recently, Dr. Rakesh K. Jain (Harvard Medical School, Boston, USA) has proposed that antiangiogenic therapy may restore the balance between pro- and antiangiogenic factors, and "normalize" structurally and functionally tumor vasculature. Preclinical and clinical data have validated this hypothesis. The ►vascular normalization concept may be critical for combinations of antiangiogenic therapy with cytotoxic therapies for cancer, but also for a variety of other diseases (see below).

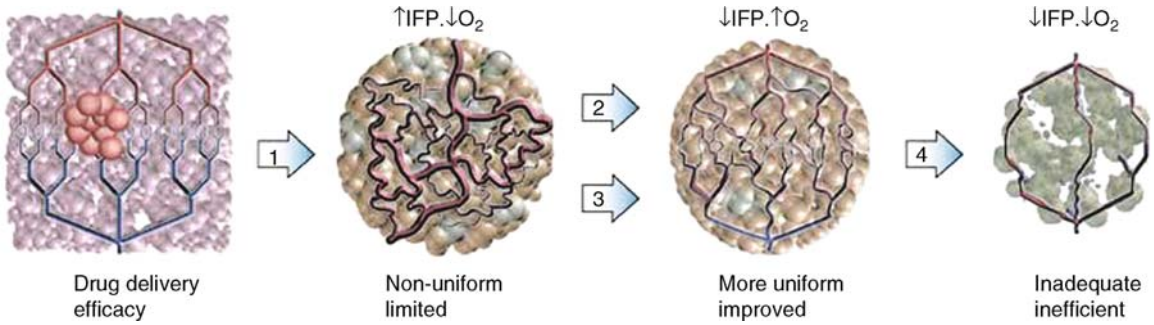
Molecular and Cellular Players in Angiogenesis: Potential Targets for Antiangiogenesis

Specific and efficient targeting of the new vessels that form in pathological states requires an in depth understanding of the cellular and molecular events that lead to angiogenesis in each disease type. For cancer therapy, such specificity and efficiency has not been achieved to date, presumably due to the complexity of the angiogenesis process and the heterogeneity of tumors.

Several strategies have been proposed and are currently under clinical development.

First, antiangiogenesis strategies have been developed to target specific molecules responsible for angiogenesis in cancer. Most of our knowledge in angiogenesis to date derives from studies of VEGF. VEGF is overexpressed by most cancers and is a main player in diseases such as macula degeneration and obesity. However, VEGF is also a key component of physiological angiogenesis. Moreover, VEGF may modulate vasculogenesis (i.e., de novo formation of new blood vessels from endothelial precursor cells), a ►neovascularization process complementary to angiogenesis. Finally, VEGF is a highly pleiotropic cytokine and exerts effects on nonendothelial cells that express VEGF receptors such as hematopoietic cells or cells of the nervous system. A similar complexity emerges from current studies of other proangiogenic factors such as bFGF, PlGF, or ►stromal-derived factor 1 alpha (SDF1α), to name just a few. These factors are also expressed in many tumors and can compensate for VEGF during anti-VEGF therapy. Therefore, molecular and cellular target identification and validation remains of great interest in the antiangiogenesis field (Fig. 1).

A second strategy is to use endogenous factors that are inhibitors of angiogenesis. Upregulation of the expression or exogenous delivery of recombinant protein for factors such as endostatin, tumstatin,



Antiangiogenesis. Figure 1 Potential mechanisms of action of antiangiogenesis on tumor vasculature. Angiogenesis helps tumors make the transition from in situ carcinoma to frank carcinoma (1). At this stage, tumors become hypervascular, but the vessels are leaky and the blood flow is spatially and temporally heterogeneous. This leads to increased interstitial fluid pressure and focal hypoxia, creating barriers to delivery and efficacy of therapeutics. The mechanism of action of the antiangiogenesis may be twofold: inhibition of new vessel formation and killing of immature tumor vessels (2); and normalization of the remaining vasculature by decrease in macromolecular permeability and hypoxia, and improvement of blood perfusion (3). Another effect of antiangiogenesis may be the direct killing of cancer cells in subsets of tumors. Regardless of the mechanisms involved, antiangiogenesis alone is not curative because it cannot kill all cancer cells. In the longer term, it leads to a vasculature that is inefficient for drug delivery (4) and to tumor relapse using alternative pathways for neovascularization. Reproduced with permission, from [5].

canstatin, thrombospondin, and other blood circulating proteins may counteract the overexpression of tumor-derived proangiogenic factors.

Third, proliferating endothelial cells in the angiogenic tissue may be a good target for cytotoxic regimens such as radiotherapy or chemotherapy. Thus, continuous or frequent exposure of the endothelium to cytotoxic treatment may also have an antiangiogenic effect.

Finally, therapies that directly target the cancer cells, either nonspecifically (with chemo and/or radiotherapy) or specifically (with newer molecularly targeted agents), may indirectly affect angiogenesis by killing the main source of the proangiogenic factors – the cancer cells. Increasing evidence has been recently offered to support the concept that tumor associated mesenchymal cells (fibroblasts, myofibroblasts, and perivascular cells) and hematopoietic cells (monocyte/machrophages, neutrophils) play key roles in pathologic angiogenesis. However, to date their roles in antiangiogenesis, or as a potential target for antiangiogenesis, are incompletely understood.

The Antiangiogenesis Strategy in Cancer Patients

While basic researchers continue to explore a multitude of pro- and antiangiogenic pathways as therapeutic targets, so far only the approaches blocking VEGF have proven their utility in the clinic. Many of the early trials for antiangiogenic agents yielded disappointing results. This was in contrast to the efficacy of antiangiogenic agents seen in preclinical models of cancer. But after over three decades of basic research and clinical development, two antiangiogenesis approaches have yielded survival benefit in patients with metastatic cancer in randomized, placebo-controlled phase III trials. In one approach, the addition of bevacizumab (a VEGF-specific antibody, Avastin®, Genentech/Roche) to standard therapy improved overall and/or progression-free survival in colorectal, lung, breast, and renal cancer patients. In the second approach, multitargeted agents that block growth factor pathways in both endothelial and cancer cells (such as sunitinib, Sutent®, Pfizer and sorafenib, Nexavar®, Bayer, and Onyx) demonstrated clinical benefit in gastrointestinal stromal tumor, renal cell carcinoma and hepatocellular carcinoma patients. In contrast, bevacizumab failed to increase survival with chemotherapy in patients with previously treated and refractory metastatic breast cancer and in pancreatic cancer patients. Furthermore, the addition of vatalanib (PTK787, Novartis), a selective VEGF ►receptor tyrosine kinase inhibitor, to conventional cytotoxic therapy did not show a similar benefit in metastatic colorectal cancer patients. These contrasting responses raise critical questions about how these agents work in patients and how to combine them optimally. Specifically, what are the mechanisms of action of antiangiogenic agents? And how can we monitor the effects of antiangiogenesis? Significant research is currently undertaken to answer these questions. The answers will provide invaluable insight on how these agents work, and facilitate an optimized use of antiangiogenesis in the clinic.

Antiangiogenesis for Ocular Diseases

The other application of antiangiogenesis – currently approved by the Food and Drug Administration of the United States for use in patients – is age-related wet macular degeneration. This disease is the result of VEGF-driven angiogenesis and increased permeability and edema into the retina, which causes partial or complete loss of vision. Novel treatments using a VEGF inhibiting aptamer (Macugen®, Eyetech/Pfizer) or antibody fragment (Lucentis®, Genetech) have been demonstrated to efficiently block VEGF and angiogenesis. A number of antiangiogenic agents are currently being tested in other ocular diseases such as diabetic retinopathy.

Mechanisms of Action of Antiangiogenic Agents

Several mechanisms of antitumor action have been proposed for antiangiogenic agents in cancer patients. This is in part due to the fact that proangiogenic growth factor receptors (e.g., VEGF receptors) can be expressed not only on endothelial cells in tumors, but also on subsets of malignant cells. In preclinical models, these agents have demonstrated direct antivascular effects, blockade of angiogenesis, and regression or delay of tumor progression in mice. More recent studies have proposed and demonstrated an anti-"vasculogenic" effect of VEGF blockers, manifested through the blockade of recruitment of blood-borne endothelial precursors in tumors. Based on preclinical observations, our laboratory has proposed in 2001 a new hypothesis that antiVEGF agents can "normalize" tumor vasculature. Since then, this hypothesis has been validated in mouse models of mammary and brain tumors.

Emerging clinical data is beginning to shed light on the potential mechanisms of action of antiangiogenesis in humans. In rectal cancer patients, using multiple functional, cellular, and molecular investigations, we demonstrated that bevacizumab has antivascular effects. In brief, bevacizumab alone reduced the tumor tissue vascular density approximately by half at day 12 after first infusion, reduced significantly the tumor blood flow evaluated by computed tomography (CT), and the number of blood ►circulating endothelial cells (CECs), and ►circulating progenitor cells (CPCs) evaluated by flow cytometry. Clinically, bevacizumab treatment produced a less hyperemic/hemorrhagic appearance of the tumor, but with no significant regression. While the significant pruning of tumor vasculature led to a significant increase in cancer cell death by ►apoptosis, it also led to a more mature (perivascular cell covered)

tumor vasculature, and a stable or increased cancer cell proliferation. Twelve days after bevacizumab therapy alone, the tumor interstitial fluid pressure was consistently decreased. Collectively, these findings suggested that in human tumors, similar to mouse models, the tumor microenvironment was normalized by the reduction of the excessive vascularization and potentially sensitized the tumor to the subsequent cytotoxic therapy. Imaging studies landed more supportive data for the normalization hypothesis: despite the significant reduction in vessel density and blood flow, the 18-Fluorodeoxyglucose-uptake measured by positron emission tomography (a measure of tumor metabolic activity) and the permeability surface area product (proportional to the penetration of tracer in tumor) evaluated on CT scans did not significantly change at day 12. Whether the increase in tumor cell apoptosis was due to a direct or indirect effect of bevacizumab is currently unclear. In recurrent ►glioblastoma patients, a panVEGF receptor tyrosine kinase inhibitor (AZD2171, AstraZeneca) normalized tumor vasculature for at least 28 days, decreased tumor enhancement volume on magnetic resonance imaging (MRI), and significantly reduced peritumor edema throughout the course of treatment. The relative vessel size measured by MRI was reduced during the "normalization window," but increased as tumors relapsed.

These mechanistic insights bring great hope that antiangiogenic agents are not only active antitumor agents, but they may increase survival in many tumor types by acting in synergism with conventional or new molecularly targeted anticancer therapies.

►Biomarkers of Antiangiogenesis

Biomarker identification and validation for this novel type of therapy are currently facing important hurdles. Unlike preclinical models, the phase III bevacizumab experience in metastatic colorectal cancer patients did not identify *p53*, *k-ras*, or *b-raf* status, VEGF or TSP2 expression, or microvascular density at baseline as predictive markers of response. No surrogate marker for antiangiogenesis has been yet validated for bevacizumab, sunitinib, or sorafenib therapy.

Our results in rectal cancer patients demonstrated that bevacizumab decreased tumor microvascular density and the number of viable CECs, consistent with an antivascular effect. Whether these changes have predictive value is currently being investigated in ongoing trials at multiple institutions. Plasma angiogenic proteins have also been investigated in multiple trials of antiangiogenic agents. We reported that the plasma levels of VEGF and PlGF are significantly elevated in cancer patients receiving bevacizumab or AZD2171. Other groups have reported similar observations with a variety of antiVEGF agents and have also found a decrease in soluble VEGF receptor 2 levels in plasma. These data strongly suggest a potential "pharmacodynamic biomarker" value for these markers. Of great interest for the field would be to identify biomarkers that predict disease progression through antiVEGF therapy. Although we were unable to identify significant changes in bFGF in the rectal cancer patients, our data from a recently completed phase II trial of AZD2171 in recurrent glioblastoma patients showed a significant correlation between bFGF and SDF1α and tumor progression. These differences may be due to the excellent clinical response in rectal cancer patients, or to disease or agent specificity. In addition, we discovered that viable CECs correlate with progression of glioblastoma during antiangiogenic therapy, while CPCs predicted relapse after drug interruptions. With the development of improved flow cytometric and protein array analysis techniques and subsequent standardization, circulating cell, and plasma protein measurements hold great promise for identification of valid biomarkers for antiangiogenesis.

Toxicity of Antiangiogenesis

The toxicity of targeted antiangiogenic agents is considered relatively mild, but serious side effects have occurred in rare instances, including treatment-related deaths due to hemorrhage or bowel perforations. Experimental studies have shown that, in 11 of the 17 healthy organs studied, VEGF blockade can significantly decrease the number of normal capillaries. In cancer patients, most antiVEGF agents often induce proteinuria, hypertension, thyroid-stimulating hormone elevation and gastrointestinal toxicity, but agent specific toxicities have also been reported. In addition, the long-term effects of antiangiogenesis in patients with less advanced cancers or other pathologies remain to be established.

Future Directions in Antiangiogenesis

The major directions for the immediate future are further understating of the mechanisms of action and identification of the first biomarkers for antiangiogenesis. Achieving this would allow optimization of current treatment protocols and reduction of the adverse effects.

First, identifying the vascular "normalization window" in patients would allow synergistic combinations with chemotherapeutics or radiation. Second, understanding the mechanisms of vessel pruning and cancer cell apoptosis induced by antiVEGF therapy, and tumor escape from it, may allow further sensitization of tumor cells to cytotoxic therapies. Third, characterization of the effect of antiangiogenesis on host-derived cells contribution to cancer growth and relapse after treatment would allow judicious and more effective approaches to therapy involving these cells. To this end, new biomarkers and improved imaging techniques will play a major role in monitoring the effects and stratifying the patients with the ultimate goal of individualized therapy.

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Antiangiogenic

Definition

Refers to a chemical or biological agent that inhibits formation of new blood vessels (►angiogenesis).

►Methoxyestradiol

Antibodies to Self Antigens

►Autoimmunity and Prognosis in Cancer

Antibody

Definition

Is a protein that binds specifically to a particular substance – its antigen. Each antibody molecule has a unique structure that enables it to bind specifically to its corresponding antigen, but all antibodies have the same overall structure and are known collectively as immunoglobulins or Igs. Antibodies are produced by plasma cells in response to infection or immunization, and bind to and neutralize pathogens or prepare them for uptake and destruction by phagocytes.

►Sjögren Syndrome
►Bispecific Antibodies

Antibody-Dependent Cell Mediated Cytotoxicity

Definition

ADCC is a passive immune response in which the Fc fragment of a (therapeutic) monoclonal antibody binds or ligates activating immunoglobulin Fc receptors, e.g., Fc RI (CD64), Fc RIIa (CD32a), Fc RIIc (CD32c) or Fc RIII (CD16), present on monocytes, macrophages, granulocytes and natural killer (NK) cells, driving cytotoxic effector functions to target membrane-associated antigens.

►Immunotherapy
►Immunoprevention
►Diabody
►Bispecific Antibodies

Antibody Fragments

Definition

Recombinant immunoglobulin fragments produced in bacteria or in yeast. These fragments include Fabs, Fv and engineered Fv (scFv, dsFv), their variants minibodies, CRABs, multifunctional and multi-specific diabodies, triabodies, tetrabodies) and fusion constructions (immunodrugs, immunotoxins, BRM). *Applications:* in vivo radioimmunodetection and in situ radiotherapy, drug, toxin and BRM-targeted delivery, ►detoxification of drugs and toxins, direct or indirect neutralization of viruses and microorganisms, effects on cell receptors and ligand, substitution of whole antibodies in immunoaffinity purification procedures, homogeneous diagnostic assays and catalysis.

►Radioimmunotherapy

Antibody Microarray

Definition

Is a specific form of protein microarray in which a collection of capture antibodies are spotted and fixed on

a solid surface (such as glass, plastic, and silicon chip) for the purpose of detecting antigens. Arraying antibodies with different specificities allows the testing of single or multiple samples for many targets. After antibodies are printed in a defined array, a conventional immunoassay protocol is performed and the completed assay slide is evaluated on a slide reader adapted to the detection method. While consuming only minuscule amounts (<μl scale) of reagents, ultrasensitive assays (zeptomole range) can readily be performed in a highly multiplexed manner. It is often used for detecting protein expressions from cell lysates in general research and specific biomarkers from serum or urine for diagnostic applications.

►Proteinchip
►Microarray (cDNA) Technology

Anticancer Therapeutic Synergy

Definition

It is not uncommon for the therapeutic effect of two anticancer drugs on a particular cancer to be greater than the effect of each drug treatment alone, or the sum of the individual effects. The presence of one drug enhances the effects of the second. This is called a synergistic effect or synergy, and the drugs are sometimes described as showing anticancer synergism.

►Cisplatin

Anticipation

Definition

A phenomenon whereby an increase in severity and/or an earlier age of onset of disease is seen in succeeding generations. In diseases such as fragile-X syndrome this phenomenon is due to continued expansion of the associated trinucleotide repeat.

►Fragile Sites
►Cowaten Syndrome

Antiestrogens

Definition

It is referred to those drugs natural or man-made that are blocking the synthesis of estrogen or that abrogate the interaction of the estrogen with its receptor.

►Estradiol
►Fulvestrant
►Estrogenic Hormones

Antigen

Definition

Molecules recognized by the immune system as non-self (including parts of bacteria, viruses and malignant cells). Is any molecule that can bind specifically to an antibody. Their name arises from their ability to generate ►antibodies. However, some antigens do not, by themselves, elicit antibody production; those antigens that can induce antibody production are called immunogens.

►Sjögren Syndrome
►Cancer-Berlinc (CG) Antigens
►HLA Class I
►Cancer-Germline (CG) Antigens
►Prostate-Specific Membrane Antigen (PSMA)

Antigen of the Cromer blood group

►Decay-Accelerating Factor

Antigen-Presenting Cells (APCs)

Definition

Antigen-presenting cells are highly specialized cells that can process antigens and display their peptide

fragments on the cell surface together with molecules required for T-cell activation. The main antigen-presenting cells for T cells are ►dendritic cells, macrophages, and B cells.

►Sjögren Syndrome
►Autoantibodies
►Peptide Vaccines
►T-cell Response
►DNA Vaccination
►Accessory Cells

Antigen–Antibody Complexes

Definition

Antigen–antibody complexes are noncovalently associated groups of ►antigen and ►antibody molecules that can vary in size from small soluble complexes to large insoluble complexes that precipitate out of solution; they are also known as immune complexes.

►Sjögren Syndrome

Antihormonal Therapy

Definition

►Endocrine Therapy.

Antihormones

Definition

Antihormones are steroid receptor antagonist, either steroidal or nonsteroidal compounds, that compete for binding with the steroid hormone and prevent activation of receptors.

►Progestin

Antilipid Peroxidation

Definition

Halting the process that makes fatty acids/lipids get rancid by oxidation.

►Grape Seed Extract

Antimetabolite

Definition

A chemical with similar structure to a substance required for normal biochemical reactions, yet different enough to interfere with the normal functions of the cell.

Substance class of anticancer drugs that are synthetic derivatives of physiological metabolites, e.g., 5-fluorouracil and methotrexate.

►ABC-Transporters
►Fluorouracil

Antinuclear Antibody

Definition

ANA; An autoantibody directed against a substance in the cell's nucleus.

Antioxidant

Definition

Antioxidant is any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly prevents or delays the oxidation of that substrate. Neutralizes free radicals and prevents cell damage that may lead to cancer. Any nutrient or chemical that reacts with and neutralizes free radicals to prevent oxidative damage to cells (e.g. oxidation of lipid membranes, DNA damage). A good biological

antioxidant is able to accept an unpaired electron to form a free radical intermediate with a relatively long half-life in the normal biologic environment. There is a complex intracellular enzymatic antioxidant system, including superoxide dismutases, catalase, and enzymes of the glutathione peroxidase family. Nonenzymatic antioxidants include arginine, vitamins A, C, E, β-carotene, glutathione, polyphenols, and minerals (selenium and zinc).

►Oxidative Stress
►Lipid peroxidation
►Phytoestrogens
►Coffee Construmption
►Carotenoids
►Oxidative DNA damage
►Reactive Oxygen Species

Antioxidant Capacity

Definition

Is the ability of a substance to inhibit oxidation protecting body cells from detrimental effects due to oxidation.

►Polyphenols
►Oxidative stress
►Autioxidant

Antioxidant Defenses

Definition

Are intrinsic compounds (e.g. albumin, glutathione, uric acid) and enzymes (catalase, superoxide dismutase) that can convert, scavenge or inactivate free radicals (►oxidative DNA damage). Dietary ►antioxidants, such as carotenoids, vitamins E and C, aid this process.

Antioxygen

Definition

Scavenge oxygen free radicals.

►Grape Seed Extract

Antiproliferative

Definition

Cancerous cells go through an exponential growth by doubling. Each generation of cells doubles in numbers compared to the previous generation. It refers to a chemical or biological agent inhibits this cell growth (proliferation).

►Methoxyestradiol

Antipyretic

Definition

The drugs that prevent or reduce body temperature (fever).

►Nonsteroidal anti-inflammatory drugs

Antiradical

Definition

Scavenge free radicals.

►Grape Seed Extract

Antisense DNA Therapy

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Definition

Refers to the introduction of short antisense strands of DNA, which then bind with target mRNA. Many cancers are due to overexpression of the genes that promote cell proliferation, called tumor suppressor

genes. Antisense RNA might be able to inhibit this overexpression. Antisense DNA is single stranded DNA of various length that is complementary to the mRNA of a given gene. The antisense DNA binds to the mRNA and, by mechanisms that are not completely understood, inhibits its natural function, i.e., translation into protein. Antisense nucleic acids are widely used to study the effect of genes in cultured cells. The potential of antisense nucleic acids in gene therapy, for instance to therapeutically downregulate the expression of overexpressed genes, is being evaluated.

Characteristics

It is increasingly clear that the process of tumorigenesis is intimately associated with the accumulation of specific genetic abnormalities. This recognition has led to the design of novel therapeutic strategies based on suppressing the activity of genes involved in tumorigenesis. Gene expression can be disrupted by a variety of methods targeted to the gene itself (e.g. homologous recombination), to the gene's transcriptional product (e.g. antisense strategies), or to the gene's protein product (e.g. expression of proteins with dominant-negative activity). These strategies are usually successful in tissue culture where cells subjected to gene transfer can be identified and expanded; they are, however, of limited value in anti-cancer DNA therapies where it is essential that many tumor cells carry and/or express the exogenous DNA sequences that can disrupt the function of the genes responsible for the growth advantage of neoplastic cells. Among the strategies directed to the suppression of gene expression, the most widely used (at least in preclinical models) involves the so-called "antisense" oligodeoxynucleotides (ODNs). ODNs are short (15–20 nucleotides) single-stranded DNA sequences synthesized as exact reverse complements of the desired mRNA target's nucleotide sequence. Compared to longer DNA molecules, ODNs should exhibit more favorable cell uptake while preserving high specificity of sequence complementarity to the mRNA target. Once the ODNs form a specific DNA-mRNA duplex, translation of the message might be prevented and mRNA degradation promoted by activation of RNase H that cleaves the RNA component of the DNA-RNA duplex. The potential for highly specific targeting of mRNA transcripts of cancer genes contrasts with the mechanism(s) of action of conventional anti-cancer chemotherapeutic agents, which block enzymatic pathways or randomly interact with nucleic acids irrespective of the cell phenotype. Anti-cancer chemotherapeutic agents exploit differences in biochemical or metabolic processes (e.g. growth rate) between normal and cancer cells for the preferential killing of neoplastic cells. In contrast, antisense ODNs have the potential to exploit the presence of genetically defined characteristics that distinguish neoplastic cells and are responsible for their growth advantage over normal cells. In recent years, the antisense strategy for cancer therapy has progressed from in vitro culture studies to investigations in animal models, and now to clinical studies. The principles underlying the in vitro experiments such as choice of target mRNA, oligonucleotide design, assessment of antisense effects apply also to the in vivo studies. We describe here the current state of progress toward gene-directed antisense-based therapies, primarily from studies in animal models and phase I clinical investigations in hematological malignancies.

Target Choice and Oligonucleotide Design

The choice of the target mRNA selected for inhibition by antisense ODNs is dictated by the biology of a particular disease process and by the ability to predict the effects that may be achieved by inhibiting the expression of a particular cancer gene. For example, the bcr/abl (►BCR-ABL1) transcripts of chronic myelogenous leukemia (►CML) cells serve as an ideal target because of the role of the BCR/ABL oncoprotein in hematopoietic cell transformation and in the maintenance of the leukemic phenotype. Since bcr/abl genes are only found in leukemic cells, targeting their mRNA transcripts might also provide the advantage of a specific effect against tumor cells. Targeting ►BCL-2 mRNA in lymphomas with the t(14;18) translocation is appropriate not only for the disease-causing effect of ►BCL-2 expression but also for the importance of interfering with anti-apoptotic pathways in drug response. Thus, ►BCL-2 antisense ODNs, in addition to their direct effects on target cells, may also sensitize these cells to chemotherapeutic agents that promote ►apoptosis. In most published studies, the sequence of the ODN targeting mRNA transcripts of a disease-causing gene is selected empirically with a preference for the mRNA transcription initiation sequence or the nucleotides surrounding the translation initiation codon. However, there are now novel approaches of oligonucleotide design based on the use of the DNA chip (►microarray (cDNA) technology) technology and hybridization with labeled RNA to dissect accessible sites in the mRNA tertiary structure.

Early investigations of ODN-targeting of growth-regulatory mRNA transcripts employed natural DNA; the realization that natural ODNs are rapidly cleaved by endo-and exonucleases led to the development of nuclease-resistant ODNs by modification of the internucleotide linkages. The most common modification is the replacement of the nonbridging oxygen atoms in the phosphate group with a sulfur group. This type

of modification generates the so-called phosphorothioate ODNs extensively used in preclinical studies and in phase I clinical trials. The phosphorothioate modification results in several desirable properties such as nuclease resistance, water solubility, and activation of RNase H. Nevertheless, it presents also certain disadvantages, including impaired uptake caused by the polyanionic nature of phosphorothioate ODNs, and non-sequence-dependent effects attributed to charge interactions between phosphorothioate ODNs and proteins in the extracellular environment, on the cell surface, and intracellularly. A number of strategies have been utilized to minimize the undesirable effects of the phosphorothioate ODNs while preserving their useful properties. Since these modified phosphorothioate ODNs have not been tested sufficiently in vitro and in in vivo models, we will focus on first-generation phosphorothioate ODNs with regard to delivery, subcellular trafficking, pharmacodynamics, and applications in mouse models and in humans.

Delivery, Subcellular Trafficking, and Pharmacodynamics of ODNs

Native and phosphorothioate ODNs are polyanionic molecules that cross cell membranes inefficiently. There is evidence that ODN uptake is time- and concentration-dependent. Below a concentration of 1 mmol/l, uptake of phosphorothioate ODNs is predominantly via a receptor-like mechanism, while fluid-phase endocytosis appears to predominate at higher concentrations. Several receptor-like proteins potentially involved in ODNs uptake have been identified, but evidence that they are responsible for ODNs uptake is still lacking. In culture, ODN uptake may be enhanced by a number of procedures directly or indirectly modifying the permeation properties of the ODNs. The most common methods are ►electroporation and streptolysin treatment which result in physical disruption or enhanced permeabilization of cell membranes. Such procedures are impractical for in vivo studies, which, at present, rely on the administration of naked DNA. Inside the cells, ODNs accumulate in vacuoles, presumably endosomes and lysosomes, and slowly redistribute to the cytoplasm and nucleus where they may interact with their target mRNA molecules. Accordingly, strategies that promote the release of ODNs from endosomal structures may enhance the ODN's antisense effects. Pharmacokinetics and metabolism of antisense ODNs have been investigated in a variety of animal systems and also in few human trials. In most reports, the analyses were carried out after intravenous or intraperitoneal administration. Approximately 30% of the injected dose is excreted in the urine within 24 h and intact material is detected in most tissues up to 48 h, and up to 7 days in liver and kidney, the organs where most ODNs accumulate. Plasma clearance is biphasic with an initial half-life of 15–25 min and a second half-life of 20–40 h. Potential toxic effects of ODNs administration have been reported in rodents and in primates. Mice receiving high doses of phosphorothioate ODNs show decreased platelet counts probably related to the polyanionic charge of the ODNs. Cardiovascular toxicity, rapid peripheral vasodilatation and death have been reported in monkeys. These effects were noted after rapid bolus administration of large doses, while slow infusion of similar doses appeared to be well tolerated.

Clinical Applications of Antisense ODNs in Hematological Malignancies

Early clinical experiences with antisense ODNs have been reported by groups targeting oncogene or apoptosis regulators. These studies were based on encouraging anti-tumor effects of systemically delivered ODNs in mice injected with leukemia or lymphoma cells of human origin. For example, the disease process induced by Philadelphia1 leukemia cells was suppressed by the systemic delivery of antisense ODNs targeting ►BCR-ABL or c-►myb transcripts. In particular, the anti-leukemia effects of the bcr/abl antisense ODNs was markedly enhanced by the combination with low-doses of ►cyclophosphamide. In the context of ►chronic myelogenous leukemia (CML), oligodeoxynucleotides targeting ►BCR-ABL or c-myb mRNA have been used as marrow purging agents in the chronic as well as accelerated phase of the disease. Eight patients with CML in advanced phase were subjected to autologous bone marrow transplantation after bone marrow purging with bcr/abl antisense ODNs.

Infusion of the ODN-treated cells was followed by prompt engraftment and hematologic reconstitution in all patients. Evaluation of anti-leukemia effects by standard cytogenetic analysis and fluorescence in situ hybridization showed a complete karyotypic response in two cases and a minimal or no response in the other six. Survival of transplanted patients exceeded three years in some cases, but it is not clear that the protocol had therapeutic efficacy.

However, lack of toxicity, prompt hematopoietic reconstitution, and karyotypic response in some cases, are all encouraging observations for designing additional clinical trials. In a different study, eight CML patients were subjected to bone marrow transplantation using autologous hematopoietic progenitors (CD34+) cells that were pre-treated with antisense ODNs targeting the mRNA transcripts of the c-myb gene, a key regulator of normal and leukemic hematopoiesis. After transplantation, seven of eight patients engrafted. Of these, four patients showed 80–90% normal metaphases

3 months post autologous bone marrow transplant, suggesting that the antisense ODNs treatment eliminated the majority of Philadelphia1 CML cells. These patients showed hematologic improvement during the period (6–24 months) following the bone marrow transplant.

Eighteen patients with refractory acute myelogenous leukemia were also treated by continuous infusion of c-myb antisense ODNs at dose levels ranging from 0.3 to 2.0 mg/kg/day for 7 days. There was no treatment-related toxicity, but only one patient showed a therapeutic response.

Studies in a mouse model of lymphoma with the t(14;18) associated with BCL-2 overexpression have demonstrated dose-dependent disease eradication in most mice treated with antisense ODNs targeting a segment of the BCL-2 open reading frame.

On the basis of these preclinical data, BCL-2 antisense ODNs were given via a continuous subcutaneous infusion for 2 weeks to lymphoma patients with high BCL-2 expression and resistant to conventional therapies. Therapeutic responses assessed by computed tomography scanning were demonstrated in six out of nine patients. The specificity of the antisense effects was validated by showing a decrease in BCL-2 levels in lymph node aspirates taken at different times after initiating the antisense ODNs therapy.

Prospects for Antisense DNA Therapy

Continuous advances in understanding the genetic basis of tumorigenesis are leading to the identification of an ever-increasing number of gene targets for antisense ODNs-based therapies. Most disease-causing genes identified by molecular genetics belong to the class of cell-cycle and apoptosis regulators. Accordingly, antisense ODNs may be used individually or in combination against these targets; moreover, antisense ODNs might be combined with conventional chemotherapeutic drugs to enhance apoptosis susceptibility of tumor cells. It might therefore be conceivable that various therapeutic strategies involve ODNs that target tumor-causing genes. Considering this, the success of antisense ODNs-based anti-tumor therapies is likely to depend on the development of antisense ODNs as effective therapeutic agents. Delivery of sufficient amounts of ODNs to tumor cells remains an important problem. Administration procedures that may guide ODNs to tumor cells are of great interest; in a recent in vitro study, neuroblastoma cells were targeted on the basis of the expression of the neuroectodermal-specific GD2 disialoganglioside by antibody-coupled neutral liposomes encapsulated with c-myb antisense ODNs. Although it is unknown if such an approach may function in vivo, this is an example of a potentially useful strategy. The delivery of sufficient amounts of ODNs to tumor cells does not guarantee that they will find the mRNA targets once inside the cells. Thus, methods promoting the intracellular trafficking of ODNs, to enhance the access to as many as possible mRNA target molecules, will be invaluable for efficacious ODNs-based therapies. The development of novel classes of ODNs with fewer non-specific interactions to non-target molecules will also improve the efficacy of antisense ODNs therapies.

If the goal of making effective ODN drugs is to be achieved, these and other problems need to be addressed.

While the principles underlying ODNs-based therapies remain highly attractive, the field of DNA therapeutics is now at a crossroad where rigorous validation in clinical trials is necessary.

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Antisense Nucleic Acid

Definition

Are nucleic acids (single-stranded DNA or RNA of various length) that are complementary to the mRNA of a certain gene. The antisense nucleic acid binds to the mRNA and, by mechanisms that are not completely understood, inhibits its natural function, i.e., translation into protein. Antisense nucleic acids are widely used to study the effect of genes in cultured cells. The potential of antisense nucleic acids in gene therapy, for instance to downregulate the expression of overexpressed genes, is being evaluated.

Antiserum

Definition

Serum that contains antibodies.

Antitoxins

Definition

Antibodies that interlock with and inactivate toxins produced by certain bacteria.

Antitumor Vaccines

Definition

Represent relatively new therapeutics for cancer. Such vaccines consist of tumor-associated components (tumor antigens) plus an ►adjuvant (non-specific activator) which may be a cell, such as a ►dendritic cell (DC), or a specially formulated compound capable of activating various immune cells. Most recent therapeutic vaccines for cancer have been prepared with patients' own DC which are cultured and pulsed or loaded with tumor-derived antigens. Antitumor vaccines can be administered to patients as a single modality therapy or in combinations with cytokines, antibodies or chemotherapeutic drugs. Most efficacious antitumor vaccines induce autoimmune sequelae in patients, especially those who achieve clinical responses following vaccination.

►Autoimmunity and Prognosis in Cancer

Antizyme Inhibitor

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Definition

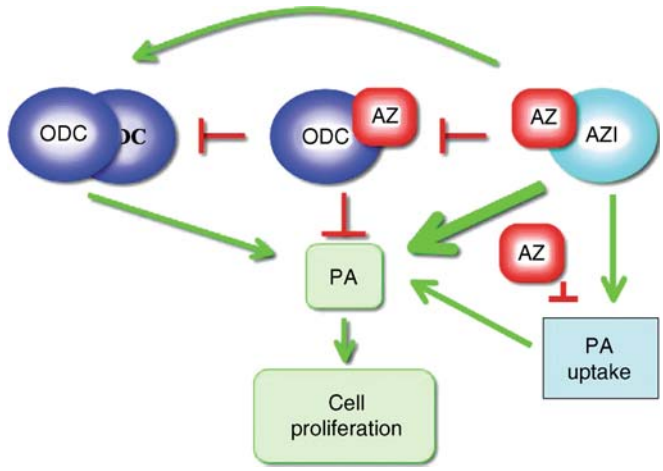
AzI is an ►ornithine decarboxylase (ODC)-related protein that functions as a positive regulator of the ►polyamines biosynthesis pathway thus contributing to enhanced cellular proliferation.

Characteristics

AzI is a cellular protein that shares high homology with ODC, the first rate-limiting enzyme of the polyamine biosynthesis pathway. However, in contrast to ODC, AzI retains no ornithine decarboxylating activity. The Polyamines spermidine, spermine, and their precursor putrescine are organic polycations that are essential for cellular proliferation. Depletion of cellular polyamines results in growth cessation that will resume upon provision of exogenous polyamines. The exact mechanisms by which polyamines affect cellular functions were not yet identified. Optimal cellular polyamine balance is the net result of their biosynthesis, degradation, uptake catabolism, and excretion. Polyamines biosynthesis is executed by the two highly regulated enzymes, ODC and S-adenosylmethionine decarboxylase, followed by the action of the two constitutively active enzymes, spermidine and spermine synthase. ODC is unique in being a short-lived protein whose degradation is performed by the 26S ►proteasome without requiring ►ubiquitination, the standard way of marking proteins for degradation. Instead, its degradation is greatly stimulated by interaction with a polyamine-induced protein known as antizyme (Az). Az expression is regulated at the translational level by a polyamine induced ribosomal frameshifting constituting a cellular polyamine sensing mechanism. Recently, it was demonstrated that Az as a central regulator of the cellular polyamine metabolism is itself subjected to regulation by a molecule termed AzI.

AzI was originally identified as a factor capable of inhibiting Az activity. Following its characterization and cloning, it was revealed that AzI is highly homologous to ODC, exhibits the same homodimeric structure, but lacks ornithine decarboxylating activity. AzI binds Az with higher affinity than ODC. The interaction between AzI and Az neutralizes Az functions leading to stabilization of ODC and to increased polyamine uptake activity, resulting in augmented cellular proliferation (Fig. 1).

Several independent lines of evidence support the notion that AzI is a regulator of cellular proliferation and tumor development. AzI was demonstrated to be upregulated in gastric tumor and in some tumor cell lines including Ras transformed cells. The AzI gene is located in a chromosomal segment whose amplification was connected with the development of prostate and ovarian cancer. Forced overexpression of AzI increased cell proliferation while downregulation of AzI by specific ►siRNA inhibited cell proliferation. Like ODC also AzI is transcriptionally induced by growth promoting stimuli. While it is clear that AzI regulates cell growth by negating Az functions, it was also suggested that AzI might function also in an Az-independent manner by regulating ►cyclin D1 stability.



Antizyme Inhibitor. Figure 1 Regulation of cellular polyamines. Cellular polyamines are provided through their synthesis initiated by the activity of ornithine decarboxylase (ODC) and through their uptake from external sources. Antizyme (Az) regulates both processes, it binds ODC monomers resulting in their inactivation and in their degradation and it reduce polyamine uptake via a yet undefined mechanism. Antizyme inhibitor (AzI), that has higher affinity to Az compared to that of ODC, traps Az into a stable complex leading to ODC stabilization and increased polyamine production. In parallel, Az neutralization increases polyamine uptake. This dual direction increase in the cellular polyamine level results in increased cellular proliferation.

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AP-1

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Definition

Activating protein-1 (AP-1) is a transcription factor usually consisting of a member of the Jun family and a member of the Fos or ATF family of proto-oncogenes. AP-1 is activated in response to cytokines, growth factors, and stress factors during cell differentiation, tumor formation, or mitogenic response.

Characteristics

Much of our present knowledge about transcription factors comes from the discovery and study of the activating protein-1 (AP-1) family. AP-1 (and the transcription factor NFκB) has served to detect one of the decisive DNA binding motifs required for gene regulation by a variety of extracellular signals including growth factors, cytokines, tumor promoters, such as the phorbol ester TPA (12-*O*-tetradecanoyl-phorbol-13-acetate), and carcinogens, for example UV irradiation and other DNA damaging agents. One of its members, the heterodimer Fos-Jun was found in the mid 1980s, as a protein complex containing the viral ►oncogene product Fos without a clue of its function. The term AP-1 was coined for an activity that supports both basal and inducible transcription of several genes containing AP-1 binding sites (5′-TGA$^{G}/_{C}$TCA-3′), also known as TPA-responsive elements (TRE), in their promoter region. AP-1 was purified from cell extracts by TRE-based affinity chromatography and despite multiple rounds of purification, the AP-1 preparations contained several distinct polypeptides. Within a year it became evident that these polypeptides correspond to members of *jun* and *fos* gene families and that the first member of the Jun family, c-Jun, represents the cellular homologue of the transforming oncogene (v-Jun) of the chicken retrovirus ASV-17. At present, the Jun protein family

consists of c-Jun, JunB, and JunD and the Fos protein family consists of c-Fos, FosB, Fra-1, and Fra-2. During the past decade additional proteins, such as members of the ATF family have been identified (mostly by yeast-two-hybrid screening), which share structural homologies and form heterodimeric complexes predominantly with Jun proteins (see below) to bind to TRE-like sequences.

General Structure of the AP-1 Subunits

According to its function in controlling gene expression the prototype of a transcription factor has to comprise at least two properties: a region of the protein that is responsible for binding to a specific DNA recognition sequence (DNA binding domain) and a second region that is required for transcriptional activation (transactivation domain) following DNA binding.

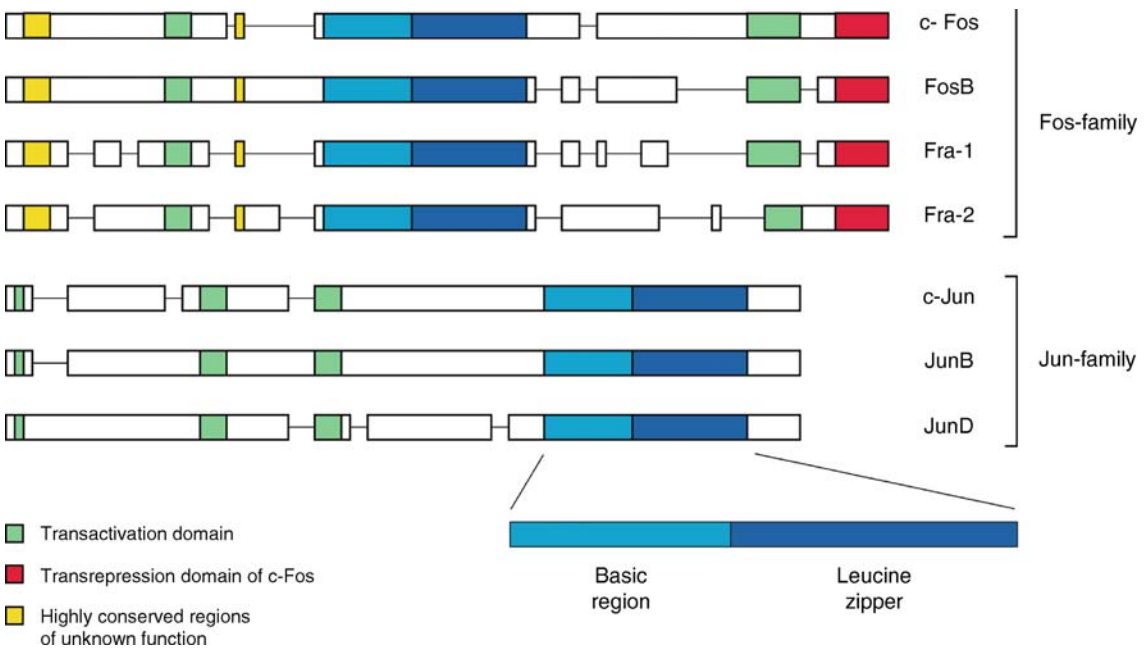
DNA Binding Domain

The DNA binding domain is evolutionarily conserved between the Jun, Fos, and CREB/ATF proteins, thus defining the protein family called "bZip" proteins. bZip stands for the amino acid sequences of the two independently acting sub-regions of the DNA binding domain: the "basic domain", which is rich in basic amino acids and responsible for contacting the DNA, and the "leucine-zipper" region, which is characterized by heptad repeats of leucine being part of the well known "4–3 repeats" forming a coiled-coil structure. The latter domain is responsible for dimerization, which is a prerequisite for DNA binding (Fig. 1). In addition to the leucines other hydrophobic and charged amino acid residues within the leucine zipper region are responsible for specificity and stability of homo- or heterodimer formation between the various Jun, Fos, or CREB/ATF proteins. The Fos proteins do not form stable homodimers but heterodimerize efficiently with the Jun proteins. The Jun proteins can form homodimers, although with reduced stability compared to Jun/Fos or Jun/ATF. Jun–Jun and Jun–Fos dimers preferentially bind to the 7-bp motif 5′-TGA$^{G}/_{C}$TCA-3′ whereas Jun-ATF dimers or ATF homodimers prefer to bind to a related, 8-bp consensus sequence 5′-TTACCTCA-3′. Therefore, individual AP-1 dimers are expected to regulate specific subsets of AP-1 target genes depending on the characteristics of the AP-1 site in their promoter.

In addition to the "classical" AP-1 members (Jun, Fos, ATF) on the basis of DNA sequence specificity and heterodimer formation with Jun and Fos proteins, several new bZip proteins have recently been defined. These include Maf and Maf-related proteins, Smads and Jun-dimerizing partners (JDPs). The exact function of these proteins in AP-1-regulated process is still largely ill-defined. Binding of AP-1 to DNA also supports binding of other transcription factors to adjacent or overlapping binding sites (composite elements) to allow the formation of larger complexes. The interaction of NFAT and Ets proteins with DNA on the IL-2 and collagenase promoters, respectively, serves as paradigms for this type of protein–protein interaction.

Transactivation Domain

In contrast to the well-defined DNA binding domain the structural properties of the domains in the AP-1 proteins



Ap-1. Figure 1 Structural organization of the Fos and Jun proteins.

mediating transcriptional activation of target genes (transactivation domain, TAD) are still poorly understood. The activity of the TAD can be transferred to heterologous DNA binding domains, such as the yeast transcription factor GAL4. By employing such chimeric proteins, which in contrast to the wild-type proteins do not depend on a dimerization partner, critical amino acids in the TADs could be identified. Moreover, it is clear that the various Jun, Fos, and ATF proteins greatly differ in their transactivation potential. Usually, c-Jun, c-Fos, and FosB are strong transactivators, whereas JunB, JunD, Fra-1, and Fra-2 exhibit only weak transactivation potential. Under specific circumstances, they may even act as repressors of AP-1 activity by competitive binding to AP-1 sites, or by forming inactive heterodimers with c-Fos, FosB, or c-Jun. Most importantly, transactivation studies using fusion proteins led to the identification of protein kinases, which bind to and phosphorylate AP-1 proteins in the TAD in response to extracellular signals thereby controlling expression of AP-1 target genes.

Transcriptional and Posttranslational Control of AP-1 Activity

Regulation of AP-1 net activity in a given cell can be achieved through changes in transcription of genes encoding AP-1 subunits, control of the stability of their mRNA, posttranslational processing and turn-over of preexisting or newly synthesized AP-1 subunits, and specific interactions between AP-1 proteins and other transcription factors or co-factors.

The *jun* and *fos* genes are members of a class of cellular genes, termed early response or "immediate-early" genes. They are characterized by a rapid and transient activation of transcription in response to changes of environmental conditions, such as growth factors, cytokines, tumor promoters, carcinogens and expression of certain oncogenes. Since this type of regulation of promoter activity is also observed in the absence of ongoing protein synthesis, it is generally accepted that preexisting factors, whose activity gets altered by changes in posttranslational modification (described in detail in the subsequent section), are responsible for the regulation of promoter activity.

Transcriptional Activation

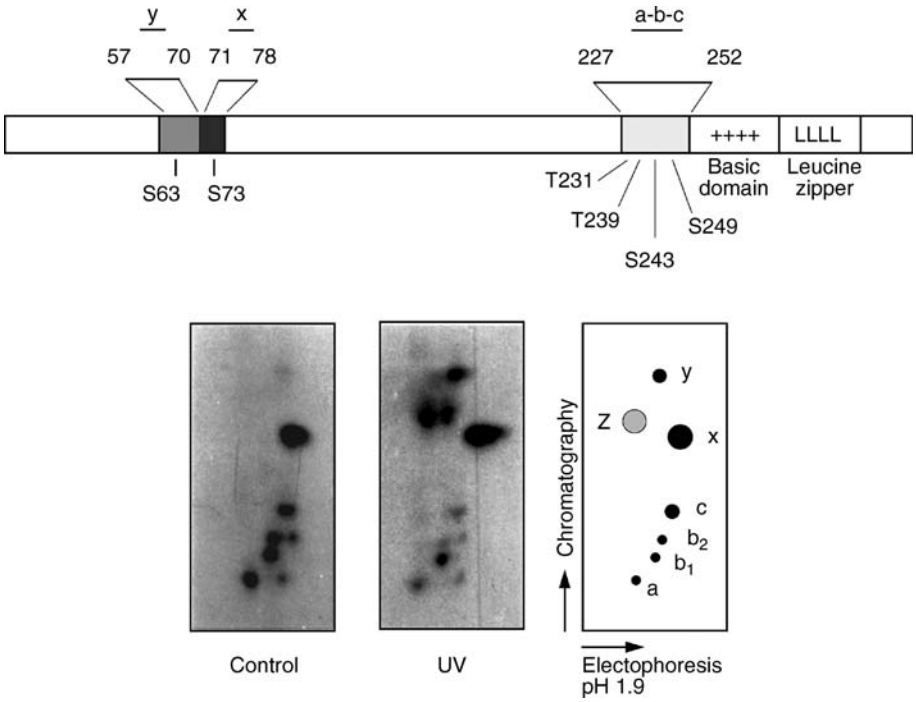
Most of our current knowledge on transcriptional activation of immediate early genes is derived from studies on deletion and point mutations within the *c-fos* and *c-jun* promoters, combined with in vitro and in vivo footprinting analyses. The serum response element (SRE) is required for induced transcription in response to the majority of extracellular stimuli including growth factors and phorbol esters. The ternary complex containing the transcription factor p67-SRF and p62-TCF, which stands for a class of related proteins described as Elk/SAP, specifically bounds to this element. Changes in the phosphorylation pattern of SRF and, predominantly, TCF regulates *c-fos* promoter activity by these stimuli. Other elements include the cAMP response element (CRE) and the Sis-inducible enhancer (SIE), which is recognized by the STAT group of transcription factors. These factors are at the receiving end of the Jak/Stat signaling pathway initiated by specific classes of cytokines. The element responsible for negative autoregulation of the *c-fos* promoter has not yet been identified conclusively.

Analysis of deletion mutants within the *c-jun* promoter identified two AP-1-like binding sites (Jun1, Jun2), which are recognized by Jun/ATF heterodimers or ATF homodimers and are involved in transcriptional regulation in response to the majority of extracellular stimuli affecting *c-jun* transcription. In response to ►G-protein coupled receptor activation (e.g., the muscarinic acetylcholine receptor), or treatment EGF and other growth factors with the AP-1 sites and an additional element in the *c-jun* promoter recognized by MEF2 proteins cooperate in transcriptional control of the *c-jun* gene. Similar to the factors binding to the *c-fos* promoter, the activity of factors binding to the *c-jun* promoter is regulated by their phosphorylation status.

Regulation of AP-1 Activity

The most critical members of the class of protein kinases regulating the activity of AP-1 in response to extracellular stimuli are mitogen-activated protein kinases (►MAPKs). Depending on the type of stimuli, these proline-directed kinases, can be dissected into three subgroups. The extracellular signal-regulated kinases (ERK-1, -2) are robustly activated by growth factors and phorbol esters, but are less efficiently activated by cytokines and cellular stress-inducing stimuli (UV irradiation, chemical carcinogens). In contrast, Jun-N-terminal kinases (►JNK-1, -2, -3), also known as stress-activated kinases (SAPK), and a structurally related class, p38 MAP kinases (p38α, -β, -γ), are strongly activated by cytokines and environmental stress, but are poorly activated by growth factors and phorbol ester. These kinases themselves are under strict control of upstream kinases and phosphatases, which are part of individual signaling pathways initiated by specific classes of extra- and intracellular stimuli (e.g., growth factors, DNA damaging agents, oncoproteins). This network, which exhibits a high degree of evolutionary conservation between yeast, drosophila, and mammals is, however, far too complex to be discussed in greater detail in this review (for in-depth information on this subject see [5]).

ERK1 and ERK2 carry out mitogen-stimulated phosphorylation of JunD and phosphorylation of distinct serine residues at the C-terminus of c-Jun and Fos family members, have also been postulated to depend



Ap-1. Figure 2 *Top*: schematic diagram of the human c-Jun protein. Amino acids are numbered. The numbers on top refer to the trypsin cleavage sites that lead to the appearance of phosphopeptides after in vivo labeling of cells with ^{32}P-orthophosphate. The location of the tryptic peptides "a–c" in the DNA binding domain and peptides "x" and "y" in the transactivation domain are indicated. *Bottom*: Autoradiogram of in vivo labeled c-Jun protein, isolated by immunoprecipitation from untreated and UV-treated cells, digested with trypsin and separated by gel electrophoresis into two dimensions. On the right the positions of the tryptic peptides are schematically illustrated. Peptide "z" most likely represents a peptide-containing residual phosphorylation at threonine-89 and/or threonine-91 of c-Jun.

on the ERK pathway. The JNK/SAPKs were originally identified by their ability to specifically phosphorylate c-Jun at two positive regulatory sites (Ser-63, Ser-73) residing within the TAD (Fig. 2). Hyperphosphorylation of both sites, which was originally identified by 2D-phospho-amino acid-peptide mapping (peptides x, y in Fig. 2), is observed in response to stress stimuli as well as oncoprotein expression and is required for transcriptional activation of numerous c-Jun target genes. The JNKs can also phosphorylate and potentiate the activity of JunD and ATF-2. Notably, the nuclear protein Menin that is encoded by the tumor suppressor gene *MEN1* specifically interacts with JunD and inhibits ERK- and JNK-dependent phosphorylation of JunD, but also of c-Jun. The amino acids that are phosphorylated on ATF2 by JNKs also serve as phospho-acceptor sites for p38, while Ser-63 and -73 of c-Jun are not affected by p38. Most likely, hyperphosphorylation of Jun and ATF proteins results in a conformational change of the TAD allowing more efficient interaction with co-factors, such as CBP, which facilitate and stabilize the connection with the RNA polymerase II/initiation complex to enhance transcription of target genes. In addition to enhanced transactivation, phosphorylation-dependent changes in the half-life of Jun and Fos proteins have been observed. In nonstimulated cells, the DNA binding domain of c-Jun becomes phosphorylated at multiple site (peptide a, b2, and c in Fig. 2) by GSK-3 and/or casein kinase II (CK-II) resulting in reduced DNA binding. In response to extracellular stimuli, such as UV, phosphorylation is reduced leading to enhanced DNA binding. The mechanism (reduced activity of the kinase or enhanced activity of a phosphatase) has not yet been defined conclusively. Recently, GSK3 mediated phosphorylation of c-Jun was also detected at the C-terminus creating a high affinity binding site for the E3 ligase Fbw7, which targets c-Jun for ►poly-ubiquitination and proteosomal degradation.

In addition to phosphorylation, other mechanisms of posttranslational processing have been identified, which regulate AP-1 activity including redox-dependent DNA binding and regulation of nuclear localization.

The mutual interference between AP-1 and steroid hormone receptors, particularly the glucocorticoid receptor (GR) represents another extensively analyzed example of protein–protein interaction-based crosstalk. In this context, there is experimental evidence that the

anti-inflammatory and immunosuppressive activities of glucocorticoids are mediated, at least in part, by GR-mediated repression of AP-1 activity. In addition to GR, numerous transcription factors (e.g., C/EBP, Ets, Gata, MyoD, NFAT, NFκB, Runx, Smad, SP-1, Stat, TCF, and the Lim-only protein YY1), transcriptional co-factors (e.g., alphaNAC, Jab1, p300/CBP, TAF1, TAF4b, TAF 7, Trip6, and WWOX), subunit of the chromatin remodeling complex (e.g., SWI/SNF and HDAC3), as well as other types of cellular proteins (e.g., DexD/H-box RNA helicase RHII/Gu and BAF60a) have been found to physically interact and modulate AP-1 activity. In most cases, the exact mechanism of interaction between AP-1 and these proteins remains to be determined.

AP-1 in Physiology and Pathology

The generation of genetically modified mice harboring genetic disruption and/or transgenic overexpression, as well as the availability of genetically defined mutant cells isolated from these animals represent a major breakthrough in our understanding of the regulatory functions of AP-1 subunits Table 1–2. Distinct and overlapping phenotypes of the individual knockout

Ap-1. Table 1 Knockout and knockin mouse models

Genotype	Phenotype	Affected tissues
c-Jun$^{-/-}$	Embryonic lethality at E12.5	Liver, heart
c-Jun$^{AA/AA}$ for c-Jun	Rescue of embryonic lethality and resistance to epileptic seizures and neuronal apoptosis induced by excitatory amino acid kinate	Liver, heart, CNS
JunB for c-Jun	Rescue of embryonic lethality until birth	Liver, heart
JunD for c-Jun	Rescue of embryonic lethality until birth	Liver, heart
c-Jun$^{\Delta/\Delta}$ Alfp-Cre	Impaired postnatal hepatocyte proliferation and liver regeneration	Liver
c-Jun$^{\Delta/\Delta}$ Bal1-Cre	Malformation of axial skeleton	Skeleton
c-Jun$^{\Delta/\Delta}$ Col2a1-Cre	Increased apoptosis of notochordal cells, fusion of ventral bodies, and scoliosis of axial skeleton	Skeleton
c-Jun$^{\Delta/\Delta}$ K5-Cre	Eye lid closure defect	Skin
c-Jun$^{\Delta/\Delta}$ Nestin-Cre	Impaired axonal regeneration	CNS
JunB$^{-/-}$	Embryonic lethality at day E8.0 to E10	Extraembryonic tissue, placenta
JunB$^{\Delta/\Delta}$Col1a2-Cre	Pronounced epidermal hyperplasia, disturbed differentiation and prolonged inflammation	Skin, immune system
JunB$^{\Delta/\Delta}$LysM-Cre	Osteopetrosis	Skeleton
JunB$^{\Delta/\Delta}$ More-Cre	Osteopenia and myeloproliferative disease	Immune system, Skeleton
JunB$^{-/-}$ Ubi-JunB	Myeloproliferative disease Altered T-helper 2-cell differentiation, impaired allergen-induced airway inflammation, and osteoporosis-like phenotype	Immune system, Skeleton
c-Jun$^{\Delta/\Delta}$JunB$^{\Delta/\Delta}$ K5-Cre-ER	Psoriasis-like phenotype	Skin
JunD$^{-/-}$	Male sterility, growth retardation, cardiomyocyte hypertrophy, and impaired T-helper-cell differentiation	Testis, heart, immune system
c-Fos$^{-/-}$	Osteopetrosis and accelerated light-induced apoptosis of photoreceptor cells	Skeleton, CNS
c-Fos$^{-/-}$ H2Kb-Fra1	Rescue of osteopetrosis and photoreceptor cell apoptosis	Skeleton, CNS
c-Fos$^{\Delta\Delta}$ Nestin-Cre	Impaired long-term memory and synaptic plasticity	CNS
FosB$^{-/-}$	Nurturing defect	CNS, hypothalamus
Fra1$^{-/-}$	Embryonic lethality at E9.5	Extraembryonic tissue, placenta
Fra1$^{\Delta/\Delta}$ More-Cre	Osteopenia	Skeleton
Fra2$^{-/-}$	Postnatal lethality and defective chondrocyte differentiation	Skeleton
Fra2$^{\Delta/\Delta}$ Coll2a1-Cre	Defective chondrocyte differentiation and kyphosis-like phenotype	Skeleton

−/− conventional knockout, Δ/Δ Cre-induced conditional knockout.

Ap-1. Table 2 Transgenic mouse models

Genotype	Phenotype	Affected tissues
H2kb-c-Jun	None	None
UbiC-JunB	Increased bone mass	Skeleton
CD4-JunB	Altered T helper cell differentiation	Immune system
UbiC-JunD	Reduced peripheral T- and B-cells and impaired T-cell activation	Immune system
H2Kb-c-Fos	Osteosarcoma	Skeleton
H2Kb-FosB	None	None
Tcrb-ΔFosB	Impaired T-cell differentiation	Immune system
NSE-ΔFosB	Osteosclerosis and impaired adipogenesis	Bone, fat tissue
H2Kb-Fra1	Osteosclerosis	Bone
CMV-Fra2	Ocular malformation	Anterior eye structure
H2Kb-Fra2	Increased bone mass	Bone

mice induced by defects in cells or tissues in which the subunit was particularly important or where its absence became rate-limiting, support the notion that AP-1 subunits exhibit unique but also common functions in vivo. As a general rule derived from all studies, the AP-1 family members must be present in a complementary and coordinated manner in order to ensure proper development or physiology of the organism.

Conventional knockout approaches demonstrate that expression of JunD, c-Fos, and FosB is dispensable for normal embryogenesis Table 1. However, *junD* null mice develop age-dependent defects in reproduction, hormone imbalance, and impaired spermatogenesis in male and cardiomyocyte hypertrophy that is enhanced by chronic moderate pressure overload. Additionally, *junD* deficiency impacts T helper cell differentiation. An important regulatory role for JunD in lymphocyte maturation and activation is supported by reduced peripheral T- and B-cell populations in transgenic mice with ectopic JunD overexpression. Whereas, adult $fosB^{-/-}$ females nurture insufficiently, tissue-specific overexpression of ΔFosB, a naturally occurring truncated form of FosB that arises from alternative splicing of the *fosB* transcript, causes impaired T-cell differentiation or osteosclerosis, respectively. Lack of c-Fos expression in adult animals causes an accelerated light-induced ▶apoptosis of photoreceptor cells as well as osteopetrosis and further experimental evidence support that c-Fos is a master regulator of osteoclastogenesis. Noteworthy, both phenotypes could be rescued by transgenic Fra-1 overexpression in $c\text{-}fos^{-/-}$ animals in a dose-dependent manner implicating that Fra-1 is an important c-Fos target gene in vivo.

In contrast to the AP-1 subunits discussed so far, c-Jun, JunB, Fra1, and Fra2 expression is indispensable for embryonic development or postnatal survival Table 1. While *c-jun* null embryos die at midgestation (E12.5) due to failure in heart and liver development, lethality of *junB* (E8.0 to E10) and *fra1* (E9.5) deficient embryos is caused by placentation failure due to multiple defects in the extra-embryonic tissue. These data suggest that JunB and Fra1, possibly as heterodimers, address common target genes responsible for the generation of a functional placental labyrinth. Knockin approaches revealed complete restoration of c-Jun dependent defects during embryogenesis by JunB and JunD indicating that spatial and temporal regulation of Jun protein expression may be more important than the coding sequence of the individual family member (Table 1). Finally, Fra-2-deficient mice die shortly after birth, are growth retarded, and show defective chondrocyte differentiation.

Embryonic or postnatal lethality largely prevented functional studies in vivo and therefore, conditional tissue- and cell-type specific ablation has become an important tool to study the regulation and function of AP-1 subunits in physiological and pathological processes Table 1. These approaches confirmed initially seen phenotypes during embryogenesis, but also revealed novel nonoverlapping and common functions of distinct AP-1 family members in adult animals, specifically in skeletal and bone morphogenesis, the immune system, skin homeostasis, and the central nervous system. In adult mice, *c-jun* deficiency results in axial skeleton malformation accompanied by accelerated apoptosis of notochordal cells, fusion of ventral bodies, and scoliosis, while compromised JunB or Fra-2 expression is associated with defective endochondral ossification partially due to impaired chondrocyte differentiation. Postnatal and cell-type specific loss of *junB* also causes osteopenia or osteopetrosis, respectively, due to failure in osteoblasts and osteoclast differentiation and physiology. Again, *junB* or *fra*-1 deficiency in osteoblasts (osteopenia) or overexpression

in transgenic mice (osteosclerosis and increased bone mass) results in comparable phenotypes. Furthermore and similar to JunD, JunB is required for a proper regulation of T-helper-cell-specific cytokine expression and differentiation that is also confirmed by T-cell specific JunB overexpression in transgenic mice (Table 2).

Animal studies further unraveled an important role of c-Jun in skin development and homeostasis as an important regulator of keratinocyte proliferation as well as differentiation through transcriptional regulation of the epidermal growth factor receptor (EGFR). In contrast, JunB can antagonize keratinocyte proliferation and an inducible down-regulation of both, c-Jun and JunB, in epidermal keratinocytes causes a psoriatic-like phenotype with epidermal hyperplasia as well as deregulated cytokine expression. Finally, specific ablation of AP-1 subunits in cells of the central nervous system revealed crucial functions for c-Jun in axonal regeneration upon transection of the facial nerve and for c-Fos in long-term memory and synaptic plasticity.

Importantly, primary and immortalized cells could be isolated from almost all mice lacking individual AP-1 members. Analysis of fibroblasts revealed that c-Jun acts as positive regulator of the cell cycle by suppressing ►p53 and indirectly the p53 target gene ►p21. Moreover, loss of c-Jun results in reduced ►cyclin D1 activity, while its overexpression was found to upregulate cyclin D levels. On the other hand, JunB contributes to both positive and negative regulation of cell-cycle progression by induction of the cyclin-CDK inhibitor p16, down-regulation of c-Jun and cyclin D expression, or transcriptional activation of cyclin A. JunD-deficient fibroblasts exhibit specific alterations in cell proliferation depending of p53 and p19-ARF expression. Moreover, data from fibroblasts lacking both c-Fos and FosB established a critical role of these AP-1 subunits in cyclin D expression, whereas fibroblasts lacking either c-Jun or c-Fos cannot be transformed by oncogenes, such as ►Ras and Src, providing additional evidence for a critical role of AP-1 members in the control of cell proliferation and transformation. In addition to these cell-autonomous effects, critical and antagonistic functions of c-Jun and JunB on cell proliferation in trans were observed using knockout fibroblasts in an in vitro skin equivalent model system with primary human keratinocytes.

As described before, AP-1 activity is also greatly enhanced upon treatment of cells with genotoxic agents, implying that AP-1 target genes are involved in the cellular ►stress response, such as DNA repair, induction of survival, or initiation of the apoptotic program. Detailed studies demonstrate that AP-1 subunits, depending on the cell type and quality of the stimuli, are involved in both anti- and pro-apoptotic responses. As an example, fibroblasts lacking the c-Fos protein are hypersensitive to UV irradiation compared to wildtype cells, which is caused by a higher rate of apoptosis rather than the inability to repair damaged DNA. However, *c-fos*$^{-/-}$ deficiency results in the loss of light-induced apoptosis of photoreceptor cells in retinal degeneration. In contrast to *c-fos*$^{-/-}$ fibroblasts, the ability of *c-jun*-deficient fibroblasts to undergo apoptosis is greatly reduced due to the absence of CD95 (Fas/APO)-ligand induction. Vice versa, c-Jun overexpression induced apoptosis in fibroblasts. Reduced CD95-L induction was also observed in cells from mice expressing a c-Jun mutant protein, which lacks the critical JNK/SAPK phosphorylation sites in its transactivation domain (JunAA). Reduced apoptosis in response to genotoxic agents was also observed in mice lacking members of the JNK/SAPK family of protein kinases, suggesting that c-Jun and ATF proteins are the major substrates of JNK/SAPKs to mediate the cellular stress response. However, primary liver cell cultures and erythroblasts derived from *c-jun*$^{-/-}$ embryos exhibit increased apoptotic rates. Finally, while JunD participates in anti-apoptotic regulation, JunB appears to be part of a pro-apoptotic pathway through negative regulation of anti-apoptotic genes, at least in myeloid cells.

AP-1 Subunits in Cancer

As described previously, AP-1 activity is enhanced in cells that are stimulated by agents promoting cell proliferation. Moreover, oncogenic versions of c-Jun and c-Fos have been isolated from retroviruses, and various membrane-associated or cytoplasmic oncogenes (e.g., Ras, Src, Raf) permanently upregulate AP-1 abundance as part of their transforming capacity, suggesting that AP-1 members play an important role in cell proliferation and transformation. Initial evidence for this assumption has been obtained by blocking AP-1 activity either through expression of a transdominant-negative c-Jun mutant, by expression of antisense sequences, or by microinjection of Jun- and Fos-specific antibodies. Under these conditions cell-cycle progression was disturbed in cultured cells and the efficiency of oncoprotein-mediated ►cell transformation was reduced. However, different lines of evidence suggested that members of the Jun and Fos families play specific roles during these processes or may even antagonize each other.

Genetic analysis of AP-1 function in transgenic mice revealed that overexpression of c-Fos induces ►osteosarcoma formation. More recently, expression of transdominant-negative or phosphorylation-defective mutants and studies using knockout mice confirmed an essential contribution of distinct AP-1 subunits not only in osteosarcoma formation, but also ►skin carcinogenesis, intestinal tumors, liver tumors, lymphomas, and ►rhabdomyosarcomas.

These studies demonstrate that JNK-dependent phosphorylation of c-Jun as well as RSK-2 dependent phosphorylation of c-Fos on Ser-362 are essential

for osteosarcoma formation in mice and may also be important for human osteosarcomas. Additionally, expression and phosphorylation of c-Jun is critically implicated in skin tumor formation, whereas c-Fos function is absolutely required for malignant ►progression in mouse models of skin carcinogenesis. Tissue-specific ablation of c-Jun also reduces tumourigenesis in the APC (Min) mouse model of intestinal cancer and chemically induced ►hepatocellular carcinoma, respectively. During chemically induced liver tumourigenesis c-Jun prevents apoptosis by antagonizing p53 activity and thereby contributes to early-stage hepatocellular cancer development. In contrast to c-Jun, JunB was identified as a potential tumor suppressor gene, at least in hematopoietic cells, since inactivation of JunB in postnatal mice result in a transplantable myeloproliferative disorder eventually progressing to blast crisis and resembling early human chronic myelogenous leukaemia (CML). More recently, JunB has also been shown to inhibit proliferation and transformation of B-lymphoid cells and to function as a gatekeeper for B-lymphoid leukemia. Surprisingly, *p53/c-fos* double knockout mice develop highly proliferative and invasive rhabdomyosarcomas suggesting tumor suppression also by the c-Fos oncogene under specific conditions.

Despite a broad knowledge concerning genes, which harbor AP-1 binding sites in their regulatory elements, only a few directly regulated AP-1 target genes have been identified, which are affected in AP-1 null mice or cells derived thereof and may critically contribute to cellular transformation and tumor formation in vivo. In addition to AP-1 target genes involved in cell proliferation, differentiation, and apoptosis, the most well-characterized AP-1-responsive genes in cancer are those implicated in ►signal transduction (e.g., EGFR), ►chromatin remodeling (e.g., DMNT1, HDAC3), ►invasion (e.g., ►MMPs, ►uPA), ►metastasis (e.g., ►CD44, ►osteopontin), and ►angiogenesis (e.g., VEGF). Some of these target genes support the notion that AP-1 critically contributes to the aggressive spread of malignant tumor cells and metastasis that is a major cause of death in cancer patients (for in-depth information on this subject see [5]).

Despite the fact that AP-1 has been identified two decades ago, it still maintains a lot of its mystery. Further research on tissue-specific inactivation of AP-1 members and the identification of subunit-specific target genes may yield an even more complex picture of function and regulation of AP-1.

Acknowledgments

We like to thank Marina Schorpp-Kistner and Axel Szabowski for help in the preparation of figures.

►Major Vault Protein

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AP2

Definition

AP2 is a heterotetrameric protein constituted of four polypeptides, called adaptins: α and β2 (~ 100 kD each), and σ2 (~ 25 kD) and μ2 (~ 50 kD). α and β2-adaptins both bind to clathrin, whereas μ2 binds to receptor tails; the α subunit further binds to several accessory endocytic proteins in a spatially and temporally organized fashion. AP2 is also often spelled as AP-2.

►Poly(ADP-Ribosyl)ation
►Endocytosis

Apactin (mouse)

►Vomeroglandin (mouse)

APAF-1

Definition

Apoptotic protease activating factor 1 (APAF-1), also known as CED4, binds to caspase 9 (apaf-3) to activate caspase 3, resulting in apoptosis. It is an ubiquitously expressed cytoplasmic protein of 1194 aa and 135 kD. The human gene maps to 12q22.

►Apoptosis
►APAF-1 Signaling
►Photodynamic Therapy

APAF-1 Signaling

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Synonyms

Apoptotic protease activating factor-1; Cytoplasmic scaffolding apoptotic protease activating factor; *C.elegans* cell death 4 homolog; KIAA0413

Definition

APAF-1 was identified in 1997 as a homolog of *C. elegans* cell death 4 (►*CED-4*) gene. APAF-1 is a cytoplasmatic protein of 1194 aminoacids able to bind ►cytochrome-c and contributing to ►caspase-9 activation. APAF-1 protein exists in cells in an inactive monomeric form. Signals that activate the ►intrinsic pathway of apoptosis (such as developmental cues, genomic stress, endoplasmic reticulum stress, cytotoxic damage, ►hypoxia, growth factor deprivation, and cell detachment) lead to ►mitochondria outer membrane permeabilization (MOMP). As a result of MOMP, cytochrome-c, a component of the mitochondrial respiratory chain, present in the intermembrane space, is released from mitochondria into the cytosol, where it binds to APAF-1. Upon binding of cytochrome-c, in the presence of dATP/ATP, APAF-1 undergoes a conformational change. This triggers APAF-1 oligomerization into a heptameric complex named apoptosome. The APAF-1-cytochrome-c apoptosome is a wheel–like multiprotein particle with seven spokes and a central hub that is able to recruit and activate the initiator caspase-9. In turn, caspase-9 activates other effector caspases, such as caspase-3 and caspase-7, which execute the cell death program.

Characteristics

APAF-1 Gene Structure and Regulation

The *APAF1* gene, encoding the APAF-1 protein, spans about 55 kb of genomic region mapping on chromosomal band 12q22, between the polymorphic markers D12S296 and D12S346. Several allelic variants of the *APAF1* gene have been described. Some of these alleles (such as the E777K, N782T, C450W, and Q465R variants) have been shown to segregate with major depression (MDD) in families where a significant linkage had been found previously between MDD and markers at 12q22. The intron–exon structure of the *APAF1* gene comprises 26 introns and 27 exons. The APAF-1 mRNA is ubiquitously expressed in human adult and fetal tissues and yields a 130 kDa cytoplasmatic protein. The *APAF1* gene is one of the transcriptional targets of ►p53 in DNA damage-induced apoptosis. A genomic region upstream of the APAF-1 transcription start site (at -604 to -570 relative to the transcriptional start site) contains two consensus palindromic sequences defined as p53-responsive elements. Expression of the gene is promoted also by ►UVC irradiation which enhances translation of *APAF1* by a cap-independent mechanism facilitated by internal ribosome entry (►IRES) elements located in the 5′-UTR of the gene.

APAF-1 Protein Structure

The APAF-1 protein belongs to the superfamily of AAA^+ (AAA^+) proteins. AAA^+ proteins form large ring-shaped complexes acting as energy-dependent unfoldases of macromolecules. Most AAA^+ proteins have a single ATPase domain containing a canonical phosphate-binding (P-loop) domain. In the APAF-1 protein the AAA^+ ATPase domain is located between an N-terminal caspase recruitment domain (►CARD) and a C-terminal domain containing several ►WD-40 repeats. The overall structure of the APAF-1 protein is as follows: (i) an N-terminal ►CED-3-like domain (aminoacids 1–89) named CARD that binds to the CARD domain of procaspase-9; (ii) a CED-4 homologous domain (aminoacids 94–412) containing a P-loop sequence that binds dATP/ATP and a putative Mg^{2+}-binding site; (iii) a C-terminal regulatory domain (aminoacids 412–1194) containing 12 WD-40 repeats involved in the regulation of APAF-1. APAF-1 has different splice isoforms. These include APAF-1L, APAF-1XL, APAF-1M, and APAF-1XS. These alternative APAF-1 forms differ in the number of WD-40 repeats (12 or 13) and/or for the presence of additional sequences inserted between the CARD and the CED-4 homologous domains.

Assembly and Structure of the Apoptosome

The inactive form of APAF-1 is thought to form a compact monomer containing bound dATP. According to current models, when cytochrome-c binds to the C-terminal regulatory region of APAF-1, it promotes dATP hydrolysis (dATP→dADP). Subsequently, nucleotide exchange (dATP for dADP) takes place leading to an "active monomer" conformation that is poised to apoptosome assembly. Analysis of the apoptosome structure first at 27 Å and more recently at 12.8 Å resolution, by electron cryomicroscopy, has confirmed that the heptameric complex is a wheel-like structure with seven spokes radiating from a central hub. The N-terminal CARD domains of seven APAF-1 molecules contribute to build the central hub of the apoptosome (the "CARD ring"). The CARD ring represents the active center of the apoptosome where interaction with procaspase-9 takes place. At this level, procaspase-9 molecules may bind to the apoptosome, by CARD–CARD interactions with the hub domain.

Activation of procaspase-9 molecules is then thought to occur by an intermediate step requiring the formation of caspase-9 dimers. The C-terminal regulatory regions of each of the seven APAF-1 molecules in the apoptosome contribute an arm and a Y shape domain ending with two lobes. Each lobe, made of six or seven WD-40 repeats (depending on the APAF-1 isoform) folds as a ►β propeller, and a cytochrome-c molecule binds between the two β propellers.

Positive and Negative Regulation of Apoptosome Function

The apoptosome is subjected to positive and negative regulatory interactions with several molecules. In general, activation or inhibition of APAF-1 function may be achieved by mechanisms that interfere with: (i) APAF-1 oligomerization; (ii) APAF-1 interaction with caspase-9 or cytochrome-c; (iii) caspase-9 activation. Examples of positive regulators are: (i) NAC, a CARD-containing protein that associates with APAF-1 and promotes the activation of procaspase-9 by the apoptosome; (ii) Nucling, a protein that binds the apoptosome promoting its translocation to the nucleus and the activation of apoptosome-associated caspase-9; (iii) PHAPI, a protein that promotes caspase-9 association with the apoptosome. Examples of negative regulators of the apoptosome include: (i) IAP proteins, as ►XIAP, that can associate with the apoptosome and inhibit caspase-9; (ii) heat shock proteins (HSP) that can bind to cytochrome-c (as Hsp27) thus preventing its association with APAF-1, or that can bind to APAF-1 (as Hsp70 and ►Hsp90) and prevent caspase-9 activation; (iii) posttranslational modifications of apoptosome components, as exemplified by phosphorylation of caspase-9 at serine 196 (by ►AKT), at threonine 125 (by ERK), or at serine 144 (by PKC) that prevent caspase-9 activation, or recruitment to the apoptosome; (iv) a caspase-9 splice variant (named Casp-9γ), retaining only the CARD domain but lacking the catalytic domain, that may compete with functional caspase-9 for binding to the apoptosome.

APAF-1 Expression and Apoptosome Regulation in Cancer

Most of the chemotherapeutic drugs used in the treatment of cancer promote apoptosis by the mitochondrial pathway that leads to cytochrome-c release and apoptosome assembly. As resistance to apoptosis is one of the hallmark of cancer, alterations in APAF-1 expression and apoptosome function have been shown to be common in both solid tumors and hematological malignancies. Loss of expression of *APAF1* gene was initially described in advanced ►melanoma, by a mechanism involving ►methylation-induced transcriptional silencing and ►allelic imbalance (►loss of heterozygosity). *APAF1* ►promoter methylation and allelic imbalance have been described even in tumors other than melanoma. For example, reduced mRNA levels for *APAF1* has been shown in primary acute myeloblastic leukemia cells, due to ►CpG methylation in a region between +87 and +128 of the *APAF1* gene. Similarly, methylation of *APAF1* gene has been described in ►carcinomas of the bladder and in clear cell ►renal carcinomas. Allelic imbalance for *APAF1* gene, associated with reduced *APAF1* mRNA levels, has been described in ►colorectal carcinomas.

Defects of APAF-1 expression may be a marker of neoplastic transformation and/or tumor ►progression. In human melanoma, expression of APAF-1 protein is lower in neoplastic cells than in melanocytes and decreases with increasing thickness of the primary tumor as well as in the progression from primary lesion to metastatic disease. In ►nonsmall cell lung cancer patients, the subcellular localization of APAF-1 has been shown to represent a significant prognostic factor. In fact, nuclear localization of APAF-1 was associated with 5-year survival rates of 89% compared to 54% in patients with cytoplasmic localization of APAF-1 in the tumor cells. This suggests that nuclear translocation of APAF-1 may be associated with an apoptosis-prone phenotype of the neoplastic cells.

Inactivation of the *APAF1* gene, first shown in human melanoma, provides evidence for a mechanism that may prevent the execution of the apoptotic program in neoplastic cells following cytotoxic stress. The initial evidence indicated that reduced/absent APAF-1 protein expression was associated with chemoresistance of melanoma cells to DNA damaging drugs that mediate apoptosis by the p53 pathway. Reduction of APAF-1 protein can be achieved in neoplastic cells even by sequestration in discrete subcellular domains, not only by reduced protein expression as in melanoma cells. In Burkitt lymphoma cells, APAF-1 has been shown to be associated with discrete domains of the plasma membrane, instead of being free in the cytosol. Such APAF-1 sequestration prevents apoptosome formation in the presence of cytochrome-c and is associated with resistance to etoposide in Burkitt lymphoma.

Altered regulation of apoptosome assembly and function is another antiapoptotic strategy activated in neoplastic cells. For example, the constitutively active tyrosine kinase ►Bcr–Abl of chronic myelogenous leukemia has been shown to inhibit interaction of caspase-9 with APAF-1. Reduced caspase-9 binding to the apoptosome, not explained by reduced levels of caspase-9 or APAF-1, has been proposed as a chemoresistance mechanism even in ►ovarian cancer. In nonsmall cell lung cancer, a defect in apoptosome function has been linked to overexpression of the inhibitor of apoptosis XIAP that binds to the processed form of caspase-9, thus suppressing activation of downstream effector caspases.

Apoptosome-Dependent and -Independent Pathways of Apoptosis in Normal and Neoplastic Cells

The role of the APAF-1 pathway in apoptosis depends on the cell-context and on the specificity of the pro-apoptotic signal. In some experimental models, and in some human tumors, APAF-1 expression and function has been shown to be required for apoptosis in response to different proapoptotic drugs. For example, in mouse embryonic fibroblasts from mice lacking APAF-1 (APAF-$1^{-/-}$ mice), susceptibility to apoptosis induced by the ►proteasome inhibitor bortezomib is inhibited. In human leukemic cells, apoptosis promoted by etoposide requires caspase-10 activation, but small interfering RNA-mediated downregulation of APAF-1 prevents etoposide-mediated caspase-10 activation and inhibits apoptosis.

On the other hand, thymocytes from APAF$1^{-/-}$ mice have normal susceptibility to Fas-mediated cell death, indicating that APAF-1 is dispensable for the execution of apoptosis by the extrinsic pathway (Extrinsic pathway of apoptosis) (i.e., ►death receptor-induced apoptosis). In addition, thymocytes from mice expressing a mutant cytochrome-c unable to bind APAF-1 have been shown to be susceptible to apoptosis regulated by the ►intrinsic pathway (and induced by stimuli as etoposide, γ and ►UV irradiation). In these cells caspase-9 and caspase-3 could be activated after γ-irradiation, in spite of the absence of APAF-1 oligomerization, indicating the existence of apoptosome-independent, caspase activation pathways in response to cytotoxic stress.

Apoptosome-independent pathways of cell death in response to chemotherapeutic drugs exist in neoplastic cells. These pathways may promote apoptosis even when APAF-1 is not expressed, although APAF-1 expression can amplify the cellular response to some drugs. In melanoma cells, APAF-1 expression has been shown to be dispensable for caspase-9 activation and apoptosis promoted by drugs as ►cisplatin, camptothecin, betulinic acid, and etoposide. In agreement with these results, analysis of APAF-1 expression in a panel of 60 cell lines used for drug screening, and including the most frequent solid tumors and leukemias, has not provided evidence for APAF-1 as a major determinant of drug sensitivity.

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AP2-alpha

Definition

Activating enhancer-binding protein 2 alpha, activates gene transcription via GC-rich DNA sequences and its expression is required for normal growth and morphogenesis during mammalian development.

►Insulin Receptor

APC

Definition

Abbrevisation refers to a number of very different definitions.

1. Adenomaous polyposis coli; familial colon cancer ►APC gene in Familial Adenomatous Polyposis
2. ►Antigen-presenting cell
3. ►Anaphase-Promoting Complex

APC/β-Catenin Pathway

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Synonyms

The terms Wnt pathway and APC/β-catenin pathway have been used interchangeably. Because some Wnt proteins have β-catenin-independent effects, and since β-catenin can be affected by upstream pathways other than Wnt, the term APC/β-catenin is used here

Definition

The APC/β-catenin pathway is a signal transduction pathway important in development and tumorigenesis.

Signaling by this pathway is defined as the stabilization of β-catenin and transcriptional activation of several target genes.

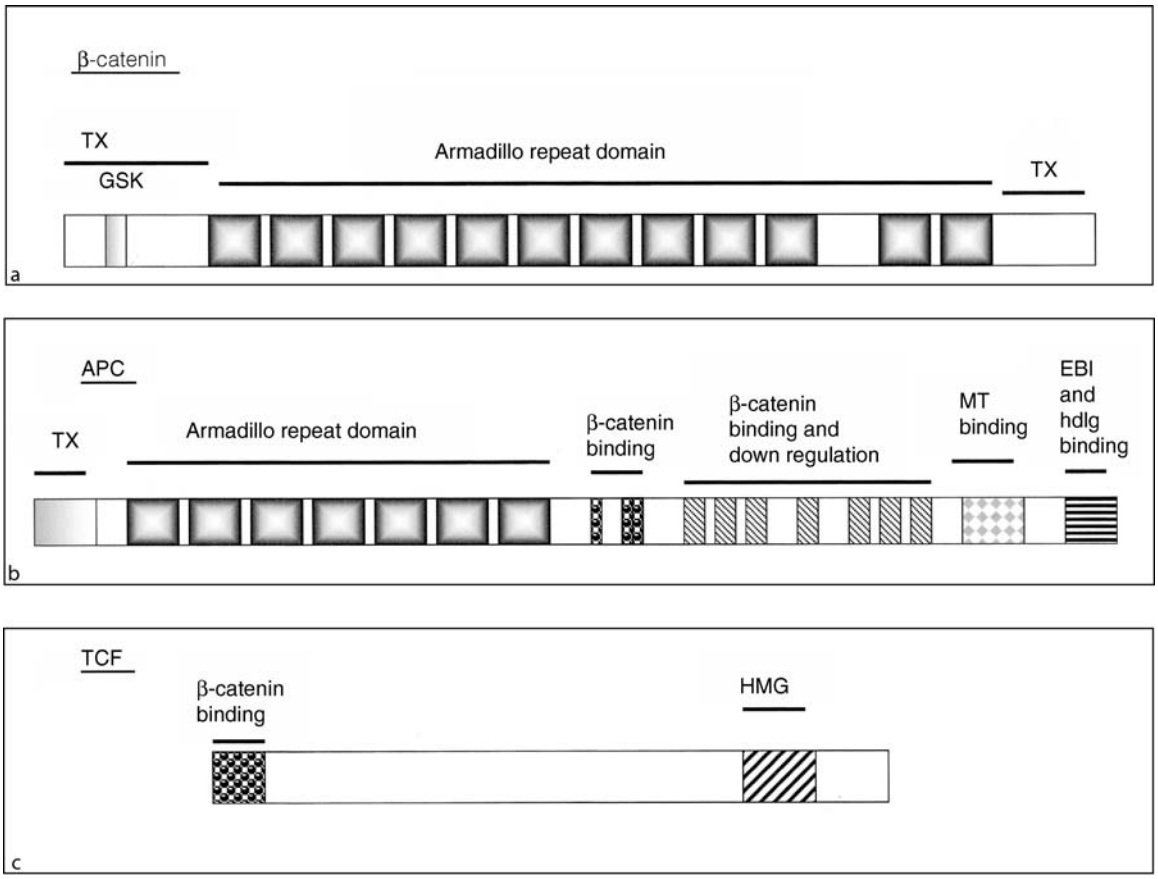
Characteristics

►β-Catenin is a multi-functional protein with roles in adhesion and signal transduction. The adhesion properties of β-catenin reflect its ability to interact with ►E-cadherin at the cellular membrane, and the alteration of this important cellular function has been associated with an increased invasion potential of cancer cells. In addition to this important role, β-catenin is also found in a cytoplasmic/nuclear pool and is believed to act as a signal transduction molecule. Cytoplasmic/nuclear β-catenin (sometimes called free β-catenin) can associate with the T cell factor (TCF) family of transcription factors and activate transcription of specific genes. The TCFs provide the DNA binding domain while β-catenin contains the transcriptional activation domain (Fig. 1). Transcriptional target genes of the pathway include cMYC (►MYC family), ►Cyclin D1 and MMP-7. APC/β-catenin signaling is regulated mainly through degradation of β-catenin at the protein level (see below). This regulation is complex, and inappropriate activation of the pathway can facilitate the development of several malignancies in humans and in animal models.

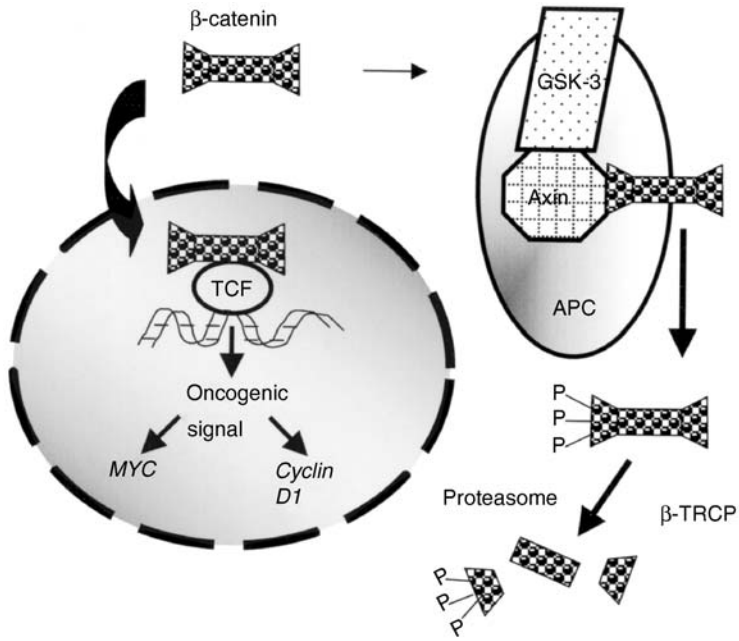
The pathway is highly conserved through evolution and the Drosophila melanogaster homolog of β-catenin, Armadillo, interacts with Drosophila TCF (Pangolin) to activate genes important for cellular fate determination during fruit fly embryonic development. Studies of the wingless pathway in Drosophila and Xenopus have been instrumental in unraveling the regulation of this pathway in normal and cancer cells.

Regulation

Normally, in differentiated cells, β-catenin protein is constantly degraded through the ubiquitin-►proteasome pathway. β-catenin is earmarked for degradation through



APC/β-Catenin Pathway. Figure 1 Schematic representation of the β-catenin, APC, and TCF proteins. (a) β-catenin protein, showing the transcriptional activation domains, Armadillo repeats, and the N-terminal GSK-3-β phosphorylation domain (GSK). (b) APC, showing armadillo repeats, β-catenin binding and downregulation domains and the microtubule binding region. Also shown are the binding domains for EB1 and the human discs large protein. (c) A simplified representation of the TCF family of proteins showing the β-catenin binding site, as well as the DNA-binding HMG domain.



APC/β-Catenin Pathway. Figure 2 APC/β-catenin signaling pathway. In the absence of signaling, β-catenin is targeted by the APC complex for proteasome degradation. When stabilized, β-catenin interacts with TCF to activate transcription of genes such as c-Myc and Cyclin D1. See text for details.

phosphorylation of specific residues at its N-terminus by GSK3β. The ubiquitin ligase β-TRCP can bind phosphorylated β-catenin, which is then polyubiquitinated (►ubiquitination). This effectively targets β-catenin for proteasome degradation. The phosphorylation and ubiquitination of β-catenin is regulated by a large complex of proteins that include GSK3β, Axin and APC (Fig. 2). In addition to its action on β-catenin, GSK3β phosphorylates APC and Axin but the consequences of these phosphorylation events are unclear. It appears that Axin increases the ability of GSK3β to phosphorylate β-catenin, since GSK3β phosphorylation of β-catenin is inefficient in vitro. APC has been suggested to act as a scaffold for these phosphorylation events. APC, a protein believed to be exclusively cytoplasmic, was shown to be present in the nucleus. In addition, APC contains a nuclear export signal (NES) and may be involved in shuttling β-catenin from the nucleus to the cytoplasmic degradation complex. The regulation of the complex and the roles of the different proteins within the complex are incompletely understood. The multifaceted regulation of β-catenin likely reflects the importance of the pathway in development and in tumorigenesis.

Clinical Relevance

The APC/β-catenin pathway is deregulated in several common human cancers. APC mutations are the cause of FAP (►APC gene in Familial Adenomatous Polyposis), a familial colon cancer predisposition syndrome. Genetic testing of FAP families allows the identification of individuals at risk of colon cancer and the establishment of appropriate management options. In addition, APC mutations are found in 80% of all sporadic cases of colon cancer. Mutations of APC lead to a lack of regulation of the β-catenin protein and inappropriate activation of the downstream genes. Interestingly, in sporadic colon tumors without APC mutations, β-catenin itself is frequently mutated. The mutations typically affect GSK3β phosphorylation sites in the N-terminus of β-catenin, leading to a protein resistant to phosphorylation and subsequent degradation. While mutations in APC are not frequently observed in cancers other than colon, mutations in β-catenin have been reported in several human malignancies such as melanoma, colon cancer, prostate cancer and skin cancer. Axin, another member of the pathway is also found mutated in liver cancer. Cellular consequences of the activation of the APC/β-catenin pathway are unclear but several lines of evidence suggest that the pathway is important for the maintenance of stem cell characteristics, including longevity. In any event, it is clear that many human malignancies gain a selective advantage through activation of this pathway. Selective inhibition of β-catenin activation may represent a useful therapeutic strategy for a large number of cancers.

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APC Gene in Familial Adenomatous Polyposis

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Synonyms

Familial polyposis coli; FPC; Adenomatous polyposis coli; APC; Familial adenomatous polyposis; FAP; Gardner syndrome; GS; Attenuated adenomatous polyposis coli; AAPC

Definition

Familial adenomatous polyposis (FAP) is a dominant condition predisposing to the development of multiple colorectal adenomas (polyps) during adolescence. Adenomatous polyps are benign tumors that can degenerate into malignant adenocarcinomas and subsequently into metastases if the affected segment of the bowel is not surgically resected (Fig. 1a and b).

Characteristics

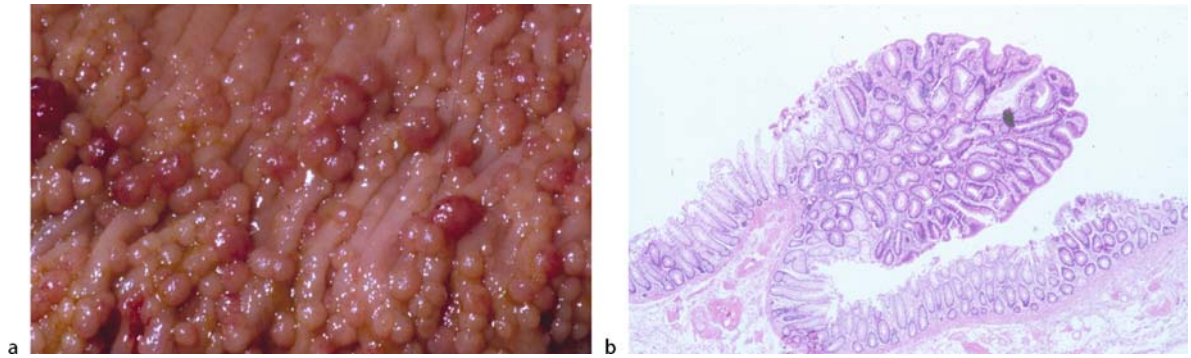
FAP affects on the average 1 in 10,000 individuals and, although the polyps represent the hallmark of the disease, it might be regarded as a condition of the whole body since it is often characterized by a number of extraintestinal manifestations involving all three embryonic lineages:

- Tumors of the stomach, duodenum, and of the biliary tree
- ►Osteomas, desmoids (Fig. 2), liver tumors, and dental abnormalities
- Epidermal cysts of the skin, congenital hypertrophies of the retinal pigment epithelia (►CHRPE), and endocrine tumors

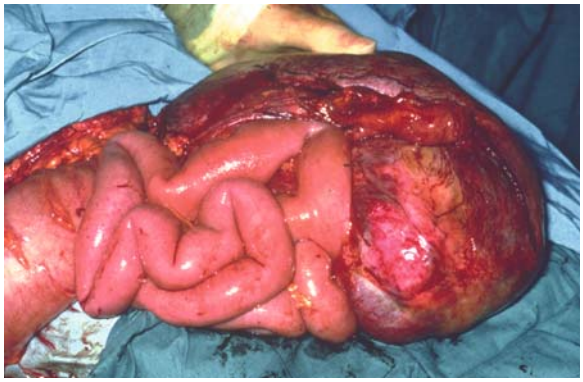
Of these tumors, the duodenal polyps and the abdominal desmoids occur respectively in 44% and 13% of the FAP patients. Next to colorectal cancer (►colon cancer) and eventual metastases, these tumors represent the most clinically relevant complication of the disease.

The most benign manifestation of FAP, the CHRPE in the eye, is found to be consistently associated in about 80% of the cases even before the appearance of the polyps in the large bowel, thus representing a very useful diagnostic biomarker.

FAP is an autosomal dominant condition with very high ►penetrance (close to 100%). Germline mutations in the adenomatous polyposis coli (APC) gene are responsible for FAP. APC encodes for a large (312 kD) and multifunctional protein involved in several biological processes ranging from cell ►adhesion, migration, and signal transduction.



APC Gene in Familial Adenomatous Polyposis. Figure 1 (a) Macroscopic detail of adenomatous polyps from an FAP patient; (b) Section of an adenomatous polyp; HE staining (Courtesy of Dr. Alex Kartheuser, Brussels, Belgium).



APC Gene in Familial Adenomatous Polyposis. Figure 2 Surgical specimen of a large andominal desmoid tumor resected from a patient with familial adenomatous polypoics.

APC is nowadays considered as the gene for colorectal cancer as somatic APC mutations occur early in the adenoma-carcinoma sequence and are found in the vast majority (>85%) of sporadic adenomas and carcinomas. Functional studies have shown that APC plays a critical role in controlling WNT signal transduction (►WNT signaling) by regulating β-catenin levels in the cytoplasm, and this feature is likely to represent APC's tumor suppressive function. Indeed, colorectal tumors with an intact APC contain oncogenic β-catenin mutations that alter phosphorylation sites which make the protein resistant against proteolytic degradation. The ►WNT signal transduction pathway plays a critical role in a broad range of biological processes such as differentiation, cell polarity, and the specification of cell fate (for a schematic representation of the WNT pathway see http://www.ana.ed.ac.uk/rnusse/pathways/pathway.html). In the absence of the WNT stimulus, a multi-protein complex composed of GSK3, Axin, Conductin, and APC, earmarks β-catenin for proteolytic degradation. In the presence of the secreted WNT glycoproteins, these interact with the frizzled receptors thereby inhibiting the formation of the above complex and β-catenin degradation. Accumulation of β-catenin in the cytoplasm results in its translocation to the nucleus where it complexes with TCF transcription factors thereby activating downstream target genes. Hence, loss of APC in mammalian cells or oncogenic activation of β-catenin, leads to constitutive signaling and cell transformation due to uncontrolled activation of downstream target genes.

A large number of disease-causing mutations in individuals affected by FAP have been characterized. The vast majority of APC mutations identified to date are clustered within the 5′ half of the gene (upstream of codon 1600) and are predicted to result in the truncation of the corresponding protein products.

Genotype–phenotype Correlations

The identification of a large number of mutations, together with the availability of the corresponding clinical data, offers a unique opportunity to establish genotype–phenotype correlations at the APC gene. Mutations located close to the 5′ end of the APC gene result in a generally mild and variable FAP phenotype, the so-called attenuated adenomatous polyposis coli (AAPC), characterized by a variable and reduced polyp multiplicity and a delayed age of onset. Mutations beyond APC codon 1600 are rare and are also often associated with attenuated phenotypes. Consistent correlations between germline mutations at the APC gene and FAP extraintestinal manifestations such as ►desmoid tumors, CHRPE's, and osteomas have also been reported.

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APC-Min Mouse

Definition

A mutant mouse, Min (multiple intestinal neoplasia); characterized by a mutated *Apc* gene, similar to that in patients with familial adenomatous polyposis (FAP) and in many sporadic intestinal cancers.

►Arachidonic Acid Pathway
►APC Gene in Familial Adenomatous Polyposis

Apheresis

Definition

Removal of whole blood form a donor or patient.

►Flow Cytometry and Cancer

Aphidicolin

Definition
An antibiotic that specifically inhibits DNA polymerases.

►Fragile Sites

API4

►Survivin

Apical Surface

Definition
As applied to the GI tract wall refers to the wall surface on the inside (the side exposed to swallowed drugs) of the gastrointenstinal tract or in Caco-2 cell culture the cell surface that mimics the inside of the GI tract.

►ADMET Screen

APL

Definition
►Acute Promyelocytic Leukemia.

apM1

►Adiponectin

APO-1

Definition
►FAS

APO2 ligand

►TNF-Related Apoptosis Inducing Ligand

APO2L

►TNF-Related Apoptosis Inducing Ligand

Apoptogenic Death

Definition
Cell death by ►apoptosis.

Apoptosis

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Synonyms
Programmed cell death

Definition
Apoptosis is a T cell death process which occurs during development and aging of animals. It is also induced by cytotoxic lymphocytes (CTL), anti-cancer drugs, γ- or UV-Tradiation, a group of cytokines called death factors and deprivation of survival factors.

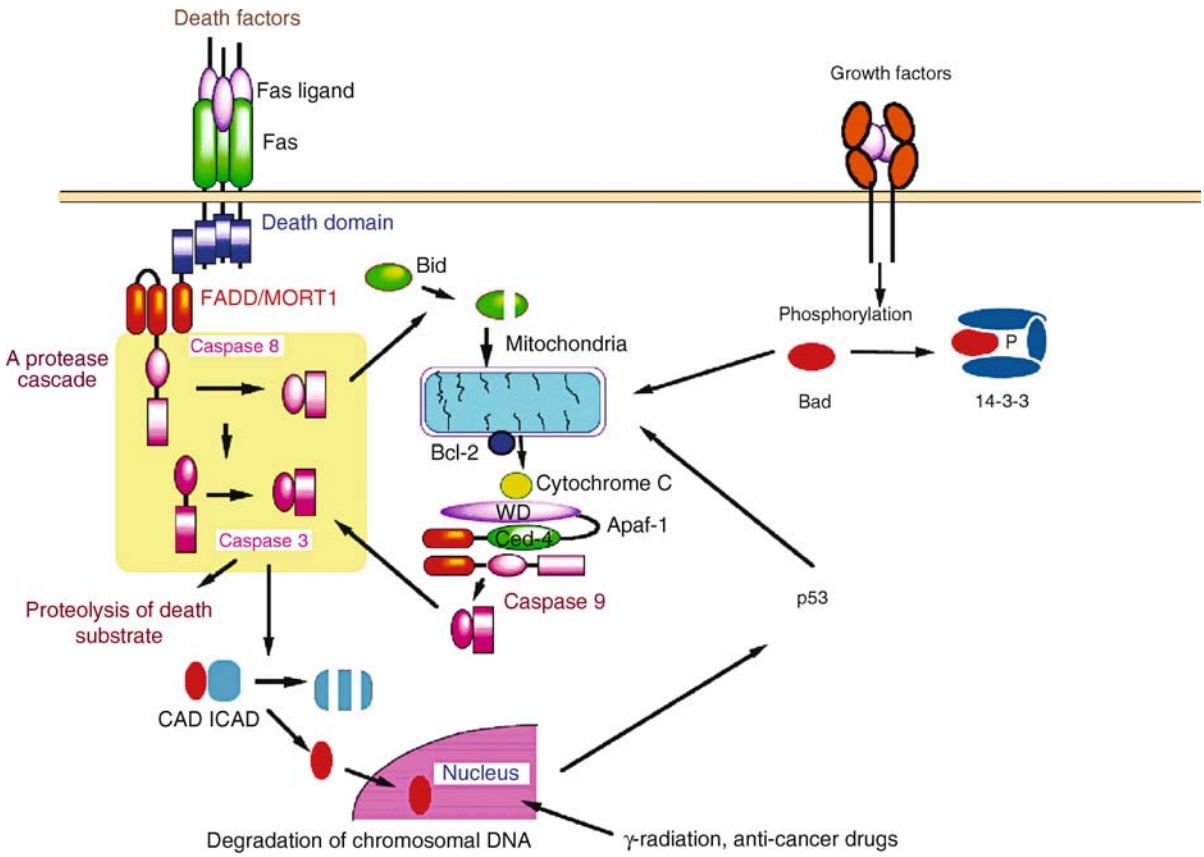
Characteristics
Apoptosis was initially characterized by morphological changes of dying cells. During apoptosis cells shrink, and microvilli on the plasma membrane disappear. The nucleus is also condensed and fragmented. At the final stage of apoptosis the cells themselves are fragmented

with all cellular contents inside. One of the biochemical hallmarks of apoptosis is the fragmentation of chromosomal DNA into ►nucleosome size units (180 bp).

Apoptotic cells can be recognized by staining of the condensed nuclei with fluorescence dyes Hoechst or DAPI. Apoptotic cells expose phosphatidylserine to the cell surface, which can be stained with fluorescently labeled annexin V. The fragmented DNA can be detected by ►TUNEL (terminal deoxynucleotidyltransferase-mediated UTP end labeling) procedure, or by electrophoresis of the isolated DNA on an agarose gel, which yields a ladder of DNA fragments with a unit size of 180 bp.

Cellular Regulation

Apoptosis is mediated by a family of proteases called ►caspases that are activated by processing from its inactive precursor (zymogen). Thirteen members of the human caspase family have been identified. Some of the family members are involved in apoptosis, and these can be divided into two subgroups. The first group consists of caspase 8, caspase 9, and caspase 10,



Apoptosis. Figure 1 Signal transduction for apoptosis. Inducers of apoptosis are categorized into three groups (death factors, genotoxic anti-cancer drugs, and factor deprivation). Fas ligand, a representative of death factors, binds to Fas receptor, and causes its trimerization. The trimerized death domain in the Fas cytoplasmic region recruits pro-caspase 8 through a FADD/MORT1 adaptor, and forms a DISC. The pro-caspase 8 is autoactivated at DISC, and becomes a mature active enzyme. Two routes have been identified to activate caspase 3 by caspase 8. In one route, caspase 8 directly processes pro-caspase 3 in the downstream, and caspase 3 cleaves various cellular proteins including ICAD. CAD is released from ICAD, and degrades chromosomal DNA. In another route, caspase 8 cleaves Bid, a pro-apoptotic member of Bcl-2, which translocates to mitochondria to release cytochrome C into the cytosol. Bcl-2 or Bcl-xL, anti-apoptotic members of the Bcl-2 family, inhibits the release of cytochrome C, the mechanism of which is not well understood. The cytochrome C then activates caspase 9 together with Apaf-1, and caspase 9 in turn activates caspase 3. The genotoxic anti-cancer drugs such as etoposide and γ-radiation generate damage in chromosomal DNA. The signal seems to be transferred to mitochondria in a p53-dependent manner by as yet an identified mechanism. This releases cytochrome C from mitochondria, and activates caspase 9 as described above. The apoptosis induced by factor-deprivation is best studied with IL-3-dependent myeloid cell lines. In the presence of IL-3, the signal from the IL-3 receptor causes phosphorylation of Bad, a pro-apoptotic member of the Bcl-2 family. The phosphorylated Bad is trapped by an adaptor called 14–3–3. In the absence of IL-3, non-phosphorylated Bad is released from 14–3–3, and translocates to mitochondria to release cytochrome C to activate caspase 9.

which contain a long prodomain at the N-terminus and function as initiators of the cell death process. The second group contains caspase 3, caspase 6, and caspase 7, which have a short prodomain and work as effectors, cleaving various death substrates that ultimately cause the morphological and biochemical changes seen in apoptotic cells. The other effector molecule in apoptosis is ►Apaf-1 (apoptotic protease activating factor), which, together with cytochrome C, recruits pro-caspase 9 in an ATP (or dATP)-dependent manner, and stimulates the processing of pro-caspase 9 to the mature enzyme.

The other regulators of apoptosis are the ►Bcl-2 family members. Eighteen members have been identified for the Bcl-2 family, and divided into three subgroups based on their structure. Members of the first subgroup, represented by Bcl-2 and Bcl-xL have an anti-apoptotic function. Members of the second subgroup, represented by ►Bax and Bak (►BAK1), as well as members of the third subgroup such as ►Bid and ►Bad are pro-apoptotic molecules.

The signal transduction pathway for a death factor (►Fas ligand)-induced apoptosis has been well elucidated. Binding of Fas ligand to its receptor results in the formation of a complex (disc, death-inducing signaling complex) consisting of FAS/APO-A/CD95, FADD and pro-caspase 8. Pro-caspase 8 is processed to an active enzyme at the disc. There are two pathways downstream of caspase 8. In some cells, such as thymocytes and fibroblasts, caspase 8 directly activates 3. In type II cells such as hepatocytes, caspase 8 cleaves Bid, a member of the Bcl-2 family. The truncated Bid then translocates to mitochondria and stimulates release of cytochrome c, which activates caspase 9 together with Apaf-1. The activated caspase 9 causes processing of pro-caspase 3 to the mature enzyme. In addition to the death factors, anti-cancer drugs, γ-irradiation or factor-depletion induce apoptotic cell death. Although cytochrome C is released from mitochondria during apoptosis induced by these stimuli, the molecular mechanism that triggers the release of cytochrome C from mitochondria is not known (Fig. 1).

Caspase 3 activated downstream of the caspase cascade activates a specific DNase (CAD, caspase-activated DNase). CAD is complexed with its inhibitor, ICAD (inhibitor of CAD), in proliferating cells. When caspase 3 is activated in apoptotic cells, it cleaves ICAD to release CAD. CAD then causes DNA fragmentation in the nuclei.

Clinical Relevance

Blocking of apoptosis by loss-of-function mutations of apoptosis-inducing molecules such as Fas, Fas ligand and caspases, or overexpression of apoptosis-inhibitory molecule such as Bcl-2, causes cellular hyperplasia. In some cases it leads to tumorigenesis, as evident in B-cell lymphomas, which over-express Bcl-2 due to the translocation of the Bcl-2 gene to the immunoglobulin gene locus. Some multiple myeloma and non-Hodgkin lymphoma carry loss-of-function mutations in the Fas gene. Somatic mutation in the Fas gene can also be found in patients of autoimmune diseases called Canale-Smith syndrome or ►autoimmune lymphoproliferative syndrome (ALPS).

Exaggeration of apoptosis causes tissue damage. For example, administration of Fas ligand, exposure to γ-irradiation, or treatment with a high dose of glucocorticoid kill test animals by causing massive apoptosis in the liver or thymus. Hepatitis, insulitis, graft-versus-host disease, and allergic encephalitis are due to the excessive apoptosis by Fas ligand expressed on CTL. Apoptotic cells are detected in the brain of ischemia

Apoptosis. Table 1 The apoptosis factory

Worker	Synonym	Apoptosis job		Chromosome
		Pro	Anti	
Fas	CD95	+		10q24
	Apo-1			
FADD	MORT-1	+		11q13
Granzyme B	GZMB	+		14q11
Apaf-1	CED4	+		12q23
Casp 2	ICH1	+		7q35
	NEDD2			
Casp 3	CPP32	+		
	Yama			4q33
	Apopain			
Casp 4	TX	+		
	ICH-2			11q22
	ICE-rel-II			
Casp 6	MCH2	+		4q25
Casp 7	MCH3	+		10q25
	ICE-LAP3			
Casp 8	MACH	+		2q33
	MCH5			
	FLICE			
Casp 9	APAF3	+		1p36.3–p36.1
	MCH6			
	ICE-LAP6			
Casp 10	MCH4	+		2q33
CAD	DFF40	+		1p36.3
Bak		+		6p21
Bax		+		19q13
Bcl-2			+	18q21
Bid		+		22q11
Bik		+		22q13.3
XIAP			+	Xq25
UBL1	SUMO-1		+	2q32
	Sentrin			

or Alzheimer patients, suggesting that apoptosis is at least in part responsible for the disease manifestation in these patients.

A proper dose of anti-cancer drugs or γ-irradiation can kill cancer cells by activating the apoptotic death program in the target cells. Some cancer cells are resistant to these drugs by an unknown mechanism. It is hoped that elucidation of the molecular mechanism of apoptosis leads to development of an efficient cancer therapy (Table 1).

►Orphan Nuclear Receptors and Cancer

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Apoptosis-inducing Factor (AIF)

Definition

Is a 57 kDa flavoprotein and is involved in the execution phase of ►apoptosis. Following apoptotic signals, AIF translocates from the intermembrane space of mitochondria to the nucleus contributing to chromatin condensation and fragmentation of the nucleus. It is suggested that it may also induce the translocation of cytochrome c from mitochondria to the cytoplasm.

►Photodynamic Therapy

Apoptosis-Induction for Cancer Therapy

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Definition

►Apoptosis is a highly coordinated ►homeostasis mechanism that ensures the timely and safe removal of superfluous, damaged, or dangerously altered cells without causing collateral damage. Imbalances in apoptosis have been implicated in a variety of pathological conditions, including cancer. Tumor cells typically have an elevated threshold for endogenous pro-apoptotic signals, which can lead to a dangerously extended cellular life span and progressively malignant behavior. Conventional cancer treatment, such as ►chemotherapy and/or ►ionizing radiation therapy, overcomes apoptosis resistance by inducing extensive and indiscriminate damage in all rapidly dividing cell types, including many normal cell types. Consequently, the therapeutic efficacy of conventional cancer therapeutics is usually limited by their severe side-effects. Therefore, cancer researchers have focused on the design of new strategies that more selectively tip the balance of cellular fate of cancer cells towards apoptosis, while sparing normal cells.

Characteristics

In every cell a variety of pro- and anti-apoptotic receptors and signaling molecules are involved in a continuous decision-making process on whether it is safe to live or better to die by apoptosis. The decision to activate apoptosis can originate from within the cell itself and, alternatively, from surrounding tissue or immune effector cells. In the course of ►malignant progression cancer cells evolve mechanisms to evade the activation of apoptosis, most notably by acquiring cancer-specific intracellular aberrancies in the apoptotic machinery. Many of these aberrancies have been identified in recent years and as such have become promising targets for cancer-selective therapy. Indeed, the design of targeted agents that selectively induce apoptosis in cancer cells is a rapidly moving field which has yielded novel promising anti-cancer strategies. Roughly spoken, these strategies can be divided in (i) those aimed at (re)-activation of intracellular pro-apoptotic systems, (ii) those aimed at inhibition of intracellular anti-apoptotic systems, and (iii) those aimed at selective delivery of additional external pro-apoptotic stimuli to cancer cells.

Re-activation of Intracellular Pro-Apoptotic Systems

One of the most commonly found aberrancies in cancer cells is the inactivation of the ►tumor suppressor protein ►p53. P53 is a transcription factor central to many of the cell's anti-cancer mechanisms. When needed, p53 can induce ►growth arrest, ►senescence, and apoptosis in order to maintain genetic integrity. In normal cells, p53 is maintained quiescent by the protein HDM-2, which binds to p53, inhibits its action, and promotes degradation of p53 by the ►proteasome. However, upon intracellular stress, e.g. resulting from UV radiation or drugs that induce ►DNA-damage,

p53 is rapidly activated by ►phosphorylation at sites within the HDM2-binding region. As a result, HDM-2 dissociates and p53 activates the cellular response to stress, which in most cases is the activation of cell death by apoptosis. The importance of p53 is evidenced by the fact that p53 is inactivated in over 50% of human tumors. Furthermore, tumors that do express a functional p53 often overexpress HDM-2.

These notions have led to the rational design of drugs that aim to reactivate the functionality of p53 in cancer cells. Research focused on p53 reactivation is two-fold. One aim is to reactivate mutant p53, a second aim is to release the HDM-2 mediated block on functional p53. An interesting example of the first strategy is the compound called PRIMA-1, which stands for p53-dependent reactivation and induction of massive apoptosis-1. PRIMA-1 selectively restores activity of mutant p53 and thereby potently activates apoptosis. An interesting example of the second strategy is the compound called RITA, which stands for reactivation of p53 and induction of tumor cell apoptosis. RITA inhibits the HDM-2/p53 interaction by binding to p53, which also leads to potent p53-mediated apoptosis induction, without significant toxicity towards normal cells.

Inhibition of Intracellular Anti-Apoptotic Systems

Many cancer cell types are characterized by upregulation of members of the so-called ►Inhibitor of Apoptosis Protein (IAP) family. IAPs represent an integral checkpoint during apoptosis that by inhibition of key proteases, the so-called caspases, can block the execution of apoptosis. Of particular interest is XIAP (X-linked IAP), which has been shown to directly inhibit various pivotal caspases. Intriguingly, this direct caspase-inhibitory activity appears to be a unique feature of XIAP that is not shared by the other IAPs.

A group of polyphenylurea compounds selectively inhibit XIAP. These compounds release the blockade of XIAP on effector caspases, resulting in potent and selective apoptosis induction, while normal cells appear to be resistant. In experimental animal models, these XIAP inhibitors strongly retarded the growth of human tumors, providing an intriguing insight in the apoptosis-prone nature of cancer cells. Apparently, relieving the XIAP anti-apoptotic brake is sufficient to reveal an intrinsically higher sensitivity of cancer cells to apoptosis than normal cells.

Selective Delivery of Additional External Pro-Apoptotic Stimuli

Highly specialized immune effector cells possess a variety of mechanisms to eliminate cancerous cells, including the targeted induction of apoptosis. However, due to the complexity of the immune system, direct therapeutic manipulation for cancer therapy has proven to be difficult. Nevertheless, several of the pro-apoptotic effector molecules of these immune cells have a promising therapeutic potential in their own right.

►TNF-related Apoptosis Inducing Ligand (TRAIL) is normally expressed on immune effector cells as a transmembrane protein. TRAIL can also be cleaved from the cell surface, yielding a functional soluble derivative (sTRAIL) that retains pro-apoptotic activity. Several recombinant forms of sTRAIL have been generated, all displaying promising therapeutic activity towards malignant cell types, with minimal activity towards normal cells.

The tumor-selective binding of sTRAIL, as well as its family member ►sFasL (Fas Ligand), can be strongly enhanced by genetic fusion to a tumor-selective antibody fragment. Binding of such fusion proteins to cell surface-expressed target antigens converts the soluble death ligands into membrane-bound molecules capable of cross-linking agonistic death receptors in an autocrine and paracrine manner. In this way also neighboring tumor cells devoid of target antigen can be effectively eliminated by the so-called bystander effect. This bystander effect solely depends on accretion of fusion proteins to the cell surface of targeted cells and does not require further cellular processing other than intact death receptor signaling pathways. Proof of principle for this approach has been obtained for sTRAIL and sFasL in both solid tumors and leukemia with no or minimal activity towards normal cells.

Perspectives for Selectively Tipping the Apoptotic Balance in Cancer

The future direction in cancer therapy strongly points to the selective induction of apoptosis in cancer cells by targeting cancer-cell specific aberrancies in the apoptotic machinery. However, an important question that remains to be fully addressed is how "selective" cancer-cell selective apoptosis induction is and ultimately can be. In particular, since apoptosis is a pivotal process in both normal and cancer cells, it remains to be determined whether specific induction of apoptosis in cancer cells is feasible without causing harm to normal cells. Single-agent therapy is likely to be not selective and/or effective enough and combinatorial strategies will be required. The most promising combinations of anti-cancer drugs will be those that work along different or complementary apoptotic signaling routes with non-overlapping toxicities towards normal cells.

Such rationally designed combinatorial strategies will only be feasible when specific cancer-related aberrancies can be rapidly diagnoses in individual patients. Therefore, development of reliable, cost-effective and high-throughput diagnostic tools will have to be pursued for each of the cancer-selective strategies to be successful. Taken together, as more and

more cancer-selective strategies designed to target the molecular aberrations in apoptosis regulation in cancer cells are being developed, the application of tumor-selective apoptosis induction in a clinical setting is slowly turning into reality.

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Apoptosis Inhibitor 4

►Survivin

Apoptosis Pathways

Definition

There are two major pathways involving apoptosis, i.e., intrinsic, mitochondrial pathway and extrinsic, receptor pathway; the term "core machinery of apoptosis" refers to the intrinsic, mitochondrial pathway.

►Mcl Family
►Apoptosis

Apoptosis Regulator Bcl2

►BCL2

Apoptosis Signaling

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Synonyms

Type I Programmed Cell Death

Definition

►Apoptosis is an evolutionarily conserved, genetically regulated form of Programmed Cell Death that is driven by ►caspases.

Characteristics

Apoptosis is a major tumor suppression mechanism and mechanism of tumor cell killing by anti-cancer drugs consequently, the study of apoptosis is an important aspect of cancer research. Apoptotic cell death occurs when a family of about twelve cysteine-directed proteases called caspases are activated. Apoptotic caspases fall into two broad categories, initiator caspases, which start the apoptotic cascade and effector caspases, which disassemble the cell. Other members of the caspase family such as caspase-1 play a less important role in apoptosis and are instead involved in processing pro-inflammatory cytokines. Apoptosis is characterized by specific morphological changes in the dying cell including degradation of the cytoskeleton, membrane blebbing, nuclear condensation and fragmentation, DNA degradation and the formation of apoptotic bodies. An important aspect of apoptotic cell death is the translocation of phosphatidyl serine from the inner cell membrane to the outer cell membrane. This serves as a signal for apoptotic cell ►phagocytosis resulting in orderly disposal of the cell corpse with little inflammation. These morphological characteristics are caused by caspase cleavage of particular substrates (amounting to several hundred separate proteins) and provide the basis for methods that are used to assess apoptosis such as the ►TUNEL assay, which detects cleaved DNA after the activation of a caspase-dependent nuclease in apoptotic cells. Other commonly used assays directly measure the presence of caspase-cleaved substrates or the active form of effector caspases such as ►caspase-3. Such methods are often used to assess levels of apoptosis in tumors during development and treatment of cancer. Caspases do not completely degrade theirs substrates, instead cleavage usually occurs at one or two sites in the protein resulting in fragmented proteins with altered activity. The fate of the cell depends on an integration of both pro- and anti-apoptotic signals, which are constantly being received from extracellular and

intracellular sources. The net effect is that either the pro-apoptotic signals will win out and cause a cell to cross its apoptotic threshold and activate the apoptotic machinery sufficiently to lead to cell death or, the cell will fail to cross this threshold and thus remain alive. Therefore a key concept in understanding apoptosis regulation is that each of the various signaling pathways that impinge upon apoptosis are not necessarily more or less important than any other. Although one signal may appear to make all the difference and cause the cell to undergo apoptosis, this signal is not necessarily a stronger apoptotic stimulus than all the others. Instead, it may be just enough to tip the balance over the apoptotic threshold. A consequence of this is that when one tries to manipulate apoptosis, for example, during cancer treatment, it may be difficult to identify any single pathway that is most important for determining the ultimate fate of the cell. Another genetically controlled form of Programmed Cell Death (Type II programmed cell death or autophagic cell death) occurs through ►autophagy. Although there are close connections between apoptosis and autophagy, autophagic cell death does not occur as a result of caspase activation and the morphological changes associated with this form of death (e.g. formation of large number of vesicles in the cell) is quite different from apoptosis.

Death Receptor Signaling

►Death receptors are members of the ►Tumor Necrosis Factor (TNF) superfamily that directly activate apoptotic caspases to stimulate the death receptor or "extrinsic" apoptosis pathway. The best understood death receptors are ►Fas/APo-1/CD95, which signals in response to ►Fas ligand, ►TNFR1, which signals in response to ►TNFα, and two receptors for TNF-Related Apoptosis Inducing Ligand (►TRAIL) called ►TRAIL receptor 1 or DR4, and ►TRAIL receptor 2 or DR5. Death receptor signaling is important in cancer treatment and tumor surveillance. Recombinant TRAIL and agonistic antibodies that recognize TRAIL receptors kill tumor cells while leaving most normal cells unscathed and several clinical trials have been performed to test if these agents may be useful to treat cancer. Fas ligand and TNFα are toxic when administered systemically, which has limited their use as anti-cancer treatments, although TNF is used clinically in isolated limb perfusion treatments. Signaling via the death receptors is also important in mediating the effects of other chemotherapeutic agents. TRAIL signaling is important in mediating tumor cell killing by ►NK cells and T cells, which leads to reduced ►metastasis and inhibition of TRAIL signaling in human tumors has been associated with metastatic disease and poor clinical prognosis. In addition, lymphocyte-induced tumor cell killing by Fas Ligand acts as an anti-tumor mechanism. This mechanism can however be turned on its head by tumor cells, which can sometimes release soluble forms of Fas Ligand to kill lymphocytes that are infiltrating into the tumor.

The various death receptors function as trimers, however the active signaling complex consists of two or more of these trimers and usually involves the formation of large aggregates with many trimer subunits. Apoptosis induction by all the known death receptors relies upon an adaptor protein called ►FADD, which upon ligand binding to the receptor interacts with the intracellular death domains of Fas and the TRAIL receptors, or, in the case of TNFR1 signaling, interacts with a second adaptor protein called ►TRADD rather than the receptor itself. FADD also binds to ►caspase-8 and the receptor/FADD/caspase-8 complex forms the basis of the Death Inducing Signaling Complex, also known as the ►DISC. In the DISC, ►caspase-8 forms catalytically active dimers leading to activation of downstream effector caspases such as caspase-3 that digest cellular proteins to cause apoptotic cell death. ►FLIP, a catalytically inactive homolog of caspase-8, also regulates this step by being recruited to the DISC where it modulates caspase-8 activation. Overexpression of FLIP occurs in various human tumors providing a mechanism by which death receptor signaling can be inhibited during tumor development. In addition to caspase activation, other signaling pathways leading to ►NFκB activation and activation of the ERK, JNK and p38 MAP kinase (►MAP kinase) pathways are induced; other cytoplasmic complexes that form after the DISC generate these signals, which can counteract the pro-apoptotic signaling caused by caspase activation.

Mitochondrial Regulation of Apoptosis

Diverse stimuli including most anti-cancer agents and cell damaging events cause release of mitochondrial proteins to activate the other apoptosis pathway – the mitochondrial apoptosis pathway, also called the "intrinsic" pathway. Members of the ►Bcl-2 family regulate caspase activation and apoptosis induced by stimuli that work through this pathway. There are more than twenty Bcl-2 family proteins that contain a series of conserved protein domains (BH1, 2, 3 and 4) and fall into two broad classes; those that stimulate apoptosis such as ►Bax and ►Bak and those that inhibit it such as ►Bcl-2 and ►Bcl-xL. Broadly speaking the anti-apoptotic Bcl-2 family proteins prevent release of proteins from the mitochondria while the pro-apoptotic proteins promote release of mitochondrial proteins. Bcl-2, Bcl-xL, Bax and Bak possess multiple ►BH3 domains, however, some of the pro-apoptotic family members contain only one of the conserved BH domains. These BH3-only proteins can only kill in cells that possess functional Bax or Bak and serve as sensors of cellular damage/stress and allow control over the initiation of cell death from diverse kinds of stimuli.

They are expressed in particular subcellular locations and monitor particular kinds of death stimuli. For example, two BH3-only proteins called Noxa and ►PUMA are induced by the tumor suppressor ►p53 and are critical for apoptosis following DNA damage (Bax is also a p53 target). Death receptors activate the intrinsic pathway through cleavage of ►Bid, a BH3 protein that is cleaved by caspase-8 and then translocated to mitochondria. This step allows the signal from the death receptor to be amplified by the mitochondrial apoptosis pathway. Other BH3-only proteins regulate cell death after detachment from the extracellular matrix, a process called ►anoikis, alterations in the cytoskeleton, and activation of intracellular signaling transduction pathways regulated by various protein kinases and phosphatases. The mechanisms by which the various pro- and anti-apoptotic proteins work is incompletely understood. However a major aspect of this mechanism is through direct protein-protein interactions where the BH3 domain of the pro-apoptotic BH3-only proteins interacts in a groove on the surface of the anti-apoptotic proteins that is formed by the BH1, 2 and 3 domains. This titrates the anti-apoptotic proteins thus allowing Bax and Bak to promote mitochondrial protein release. In addition conformational changes and intracellular translocations are induced in Bax and Bak by stressors, which activate their pro-death activities. Mitochondrial recruitment of other proteins, including p53 can also have similar effects leading to release of proteins from mitochondria. This mechanism of p53-mediated apoptosis is separate from its ability to regulate expression of pro-apoptotic BH3-only proteins.

Whatever the upstream signals leading to activation of the mitochondrial apoptosis pathway, the end result is release of proteins that reside in the intermembrane space between the inner and outer mitochondrial membranes into the cytoplasm. The most important of these proteins is ►cytochrome c. Released cytochrome c interacts in the cytoplasm with ►Apaf-1, ►caspase-9 and dATP to form a protein complex called the ►apoptosome. This complex activates caspase-9, which then activates effector caspases such as caspase-3 leading to cell death. Other mitochondrial proteins are also released from the intermembrane space. These include: Apoptosis Inducing Factor, ►AIF, an NADH oxidase that is important for efficient oxidative phosphorylation and a protector against oxidative stress. When AIF is released from the mitochondria, it can translocate to the nucleus where it causes caspase-independent chromatin and DNA condensation and degradation. Another protein released from the mitochondria is ►Smac/DIABLO an inhibitor of a class of proteins called Inhibitors of Apoptosis Proteins, ►IAPs. IAPs bind directly to caspases to inhibit their activity and, in some cases, to promote caspase ubiquitylation and degradation via the proteosome. Thus, a concerted mechanism whereby activation of the apoptosome by cytochrome c occurs simultaneously with Smac/Diablo-mediated inhibition of the IAPs promotes efficient activation of the caspase cascade. Other released mitochondrial proteins include Endonuclease G, an endonuclease that digests nuclear DNA and Omi/HtrA2, a serine protease, which can also inhibit IAPs.

Endoplasmic Reticulum Regulation of Apoptosis

A third pathway that regulates apoptosis occurs in response to ►endoplasmic reticulum stress (ER stress). Proper ER function is required for regulation of protein synthesis, trafficking and secretion, vesicle trafficking, lipid and membrane biosynthesis. Dysfunction of the ER leads to a set of adaptive pathways called the ER stress response, which usually allows the cell to restore ER homeostasis, however excessive and/or sustained ER stress triggers apoptosis. These processes are important in the regulation of tumor growth and progression and are, for example, critical for tumor cell survival and growth in hypoxic tumors. ER-specific stressors that lead to apoptosis are also potential therapeutic agents for treating cancer. The signaling pathways that regulate ER stress-induced apoptosis are less well understood than those regulating the death receptor and mitochondrial apoptotic pathways. In addition, extensive crosstalk with these other pathways occurs. For example, several members of the Bcl family that regulate the mitochondrial pathway are also found at the ER. Bax and Bak are located at the ER membrane where they regulate apoptosis induced by ER-specific stressors. This can be achieved through regulation of calcium levels in the ER; upon induction of ER stress, calcium is released from the ER in a Bax and Bak-dependent manner that can be inhibited by ER-targeted Bcl-2. The elevation of cytosolic calcium levels leads to activation of calcium-regulated proteases called ►calpains which can, in turn, activate caspases and stimulate apoptosis. The initiator caspases involved in ER stress-induced apoptosis may differ in different organisms. In mice caspase-12 is associated with the ER membrane and is activated by ER stress-induced apoptosis and required for cell death in response to ER stress. This can lead to caspase-9 activation via a mechanism that is independent of Apaf-1 and the apoptosome. However in most humans the caspase-12 gene is mutated so that it is not an active protease. In human cells, caspase-4 may be an initiator caspase responsible for ER stress-induced apoptosis.

Other mechanisms involved in ER stress-induced apoptosis are activated as a result of the ER stress response itself. Upon accumulation of unfolded proteins, the unfolded protein response (UPR) is induced.

This leads to activation of an ER-localized transcription factor called ATF6, which binds to an ER protein called ►BiP/GRP78, which functions as a chaperone protein. During ER stress BiP is titrated away from ATF6 allowing its translocation to the Golgi where it is cleaved by the same enzymes that regulate sterol-controlled gene expression. This cleavage allows ATF6 to translocate to the nucleus where it activates transcription of various target genes that encode chaperone proteins and other UPR targets that help the cell recover from the ER stress and targets that promote apoptosis. One ATF6 target gene encodes a transcription factor called CHOP, which can inhibit expression of Bcl-2 and increase expression of the TRAIL receptor DR5 providing a direct link to the other apoptotic signaling pathways that promotes apoptosis. A second BiP target is IRE1, which interacts directly with Bax and Bak to provide a physical link between the core apoptotic machinery and a regulator of the UPR. IRE1 can also interact with TRAF2, an adaptor protein that is involved in cytokine signaling including the TNFR signaling pathway. The IRE1/TRAF2 interaction can, in turn, recruit a protein kinase called ASK1, a pro-apoptotic kinase that activates the JNK MAP kinase, which can be either pro- or anti-apoptotic depending upon the cellular context.

Regulation of Apoptosis by Tumor Suppressors and Oncogenes

A complex network of signaling pathways that involve numerous ►tumor suppressors and ►oncogenes regulates apoptosis. As might be expected, oncogenes, which promote tumor growth often inhibit apoptosis while tumor suppressors promote apoptosis. For example, ►Bcl-2 was initially identified as a result of a translocation between chromosomes 14 and 18 that links the immunoglobulin heavy chain locus to the Bcl-2 gene. This leads to overexpression of Bcl-2 and causes follicular B-cell lymphoma. The recognition in the late 1980s that the oncogenic activity of Bcl-2 is achieved through its ability to block cell death was a paradigm shifting event that led to the now widely accepted view that defective apoptosis and not just increased cell growth is a central step in tumor development. Other oncogenes that inhibit apoptosis include ►Akt and ►Mdm2, which inhibits the tumor suppressor p53. Tumor suppressors including p53 and the lipid phosphatase ►PTEN directly promote apoptosis and a major aspect of their tumor suppressor activity is achieved through this mechanism and mutation during tumor development inactivates these mechanisms. p53 activates apoptosis by several mechanisms, the best understood of which is the activation of various target genes including ►BH3 proteins that are components of the apoptotic machinery and inactivation of p53 occurs in many tumors. The primary mechanism by which PTEN promotes apoptosis is through its inhibition of the ►PI3k signaling pathway leading to reduced levels of Akt kinase activity. Akt phosphorylates numerous downstream pathways that directly or indirectly regulate the core apoptosis machinery; examples include the BH3 only protein ►Bad, ►IκB, which is an inhibitor of NFκB, Mdm2 and caspase-9. Activation of the Akt pathway as a result of PTEN inactivation or activation of growth factor receptors by molecules such as the ►Insulin Like Growth factors is associated with tumor development and progression and reduced response to therapy.

Other defects that arise during tumor development can also affect the apoptotic machinery. Examples include epigenetic alterations such as promoter ►methylation of caspase-8, TNFR1 and Apaf1, which reduce expression of key components of the death receptor and mitochondrial apoptotic pathways respectively. Apoptosis inhibitors that are overexpressed in tumors including ►survivin, a member of the IAP family that is one of the most commonly overexpressed genes in human tumors. Other overexpressed proteins are Insulin-like growth factors and their receptors, which are strong activators of the PI3 kinase pathway, FLIP, the negative regulator of death receptor signaling, ►decoy receptors (DcR1 and DcR2), which inhibit TRAIL signaling and FAP1, an inhibitor of Fas signaling. In addition to p53 and PTEN mutations, inactivating mutations in Bax occur in colon cancers while various mechanisms in many tumors activate the transcription factor NFκB. This inhibits apoptosis largely through the activation of various IAP genes and anti-apoptotic Bcl family members. However, the picture is not simply that oncogenes inhibit apoptosis while tumor suppressors promote apoptosis. Instead, and somewhat counter-intuitively, oncogenes often promote apoptosis while bona fide tumor suppressors can inhibit it. For example c-Myc (►Myc) a transcription factor that promotes cell cycle progression, which is activated in very many tumors also sensitizes the tumor cell to apoptosis by diverse stimuli. Similarly, the ►E2F transcription factors that are major drivers of the cell cycle and viral oncogenes that activate E2F such as the ►adenovirus E1a protein or inactivation or loss of the ►retinoblastoma (Rb) tumor suppressor also sensitizes cells to apoptotic stimuli. This sensitization arises in part because core components of the apoptotic machinery such as caspases, BH3 proteins and Apaf1 are expressed by the same E2F driven-promoters that drive the genes required for cell cycle progression. This mechanism means that dividing cells express genes that promote apoptosis at the same time as they express genes required for DNA synthesis and cell division. As a result, tumor cells where the Rb pathway is inactivated

and/or Myc and E2F transcription factors are activated are not necessarily more resistant to apoptosis. Indeed, they may be closer to their apoptotic threshold and easier to kill than normal cells, which are not undergoing aberrant cell cycle progression. The apoptosis sensitization caused by oncogenes such as ►c-Myc oncogene also explains some aspects of the phenomena of oncogene co-operation where a single oncogenic event is not sufficient to cause tumor growth unless it is combined with another defect. For example increased expression of either Myc or Bcl-2 on their own may not be sufficient to cause tumor growth but the two together usually can cause tumor growth. This happens because the growth signals caused by Myc are counteracted by increased apoptosis sensitivity, however when Bcl-2 is also expressed in the cell the anti-apoptotic effects of Bcl-2 counteract the Myc-induced apoptosis sensitization leading to robust growth of the tumor.

Apoptosis Regulation During Cancer Treatment

Much of the interest in apoptosis as it relates to cancer involves understanding the mechanism by which anti-cancer drugs induce tumor cell apoptosis, elucidating the ways that cancer cells subvert the effects of the drug by avoiding these mechanisms and finding new ways to manipulate these pathways to make more effective cancer treatments. Although one of the hallmarks of cancer is the acquisition of defects in apoptosis regulation, most tumor cells are not inherently resistant to apoptosis. In fact, because of the apoptosis sensitization by oncogenes tumor cells may be closer to their apoptotic threshold than normal cells. In a growing tumor, anti-apoptotic mechanisms are keeping the tumor cells alive, however these mechanisms are only just keeping the cell below its apoptotic threshold and the price that the tumor cells pay for their continued growth is that they are perilously close to undergoing apoptosis. This effect explains part of the preferential killing of tumor cells compared with most normal cells (the therapeutic window) by cytotoxic chemotherapy including DNA damaging agents, drugs that interfere with the cytoskeleton and metabolic inhibitors – these treatments provide an extra pro-apoptotic signal that are enough to push the tumor cells over the edge. The sensitization to apoptosis also occurs in other cycling cells and partly explains why side effects caused by cytotoxic chemotherapy usually affect fast growing normal cells such as the epithelia that line the gastrointestinal tract and bone marrow. Like the cycling tumor cells, these normal cells are growing rapidly and are therefore close to their apoptotic threshold, thus upon damage caused by the chemotherapy, they may undergo apoptosis. Most of the current anti-cancer drugs (DNA damaging agents, cytoskeleton-targeted agents and metabolic inhibitors) induce apoptosis primarily by activating the mitochondrial apoptosis pathway through particular Bcl-2 family proteins. For example the BH3-only protein ►Bim is activated by ►Taxol, while ►PUMA is activated by several DNA damaging drugs leading to mitochondrial dysfunction by the mechanisms. Thus, anti-apoptotic mechanisms such as Bcl-2 overexpression that affect the mitochondrial pathway can confer tumor cell resistance to the treatment. However the other pathways of apoptosis regulation can also be involved in therapeutic tumor cell killing. For example, some DNA damaging agents induce expression of death receptors and inhibition of death receptors can, at least in animal models, reduce the efficacy of cytotoxic chemotherapy.

An exciting new approach to cancer treatment that arises from our understanding of apoptosis signaling is to directly target the apoptotic regulators. Several such approaches are in clinical development including direct activation of death receptors using recombinant ►TRAIL and antibodies that activate the TRAIL receptors. Similarly, although systemic delivery of ►TNFα is toxic, recombinant TNFα is used clinically in isolated limb perfusion procedures as a therapy for localized soft tissue ►sarcomas and ►melanomas. Other strategies that can directly target apoptosis regulators include small molecules that disrupt specific protein-protein interactions between Bcl-2 family members, which can be used to selectively inhibit the anti-apoptotic proteins and activate apoptosis. Agents that disrupt the p53-Mdm2 interaction can also promote tumor cell apoptosis. Other "targeted" therapeutic agents target apoptosis regulators more indirectly. For example, the proteasome inhibitor Velcade, which is useful in treating ►multiple myeloma has its therapeutic effect in large part through inhibition of NFκB signaling. This occurs because the inhibition of the proteasome prevents the degradation of the NFκB inhibitor IκB. The reduction in NFκB activity leads to decreased expression of IAP proteins and anti-apoptotic Bcl-2 family members thus pushing the tumor cells over their apoptosis threshold. As we develop a better understanding of apoptosis signaling mechanisms it will hopefully be feasible to refine and extend these kinds of strategies to more effectively treat cancer.

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Apoptosome

Definition

A cytoplasmic macromolecular complex consisting of cytochrome *c*, ►Apaf-1, and caspase-9 whose formation is instrumental in promoting caspase-9 activation.

►TNF-Related Apoptosis Inducing Ligand (TRAIL)

Apoptotic Protease Activating Factor-1

►APAF-1 Signaling

Appendiceal Epithelial Neoplasms

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Synonyms

Pseudomyxoma peritonei; Colloid carcinoma; Cystadenocarcinoma; Mucinous cystadenocarcinoma; Appendiceal mucinous tumor of uncertain malignant potential; Borderline appendiceal mucinous tumor

Definition

►Appendiceal epithelial neoplasms include a broad spectrum of tumors that vary greatly in their biological aggressiveness. Usually, they produce copious mucus as do normal appendiceal epithelial cells. The tumors usually rupture the wall of the appendix prior to diagnosis causing widespread peritoneal dissemination of the disease. New treatment options are directed at definitive treatment of the peritoneal surface component of this disease which will cause death from intestinal dysfunction unless properly managed.

Characteristics

Epithelial Appendiceal Neoplasms

Table 1 summarizes the unique characteristics of these tumors and contrasts them with colorectal cancer. Appendiceal neoplasms show varying amounts of invasiveness. About 75% are non-invasive and grow slowly, allowing patients to survive a decade or longer even without specialized treatments. However, some appendiceal tumors are very invasive, progress rapidly, and can cause death 1–2 years after the initial diagnosis.

Nearly all patients with these tumors have ►peritoneal dissemination at the time of diagnosis, a notable contrast with colorectal cancer, in which only about 15% of patients present with ►carcinomatosis. Progression is usually confined to the peritoneal space, and most patients with minimally invasive tumors die from loss of intestinal function as the mucinous tumors expand within the abdomen and pelvis.

Most patients with appendiceal neoplasms have no lymphatic or hematogenous ►metastases; 2% of patients have ►metastases in the lymph nodes and 2% in the liver; thus extensive ►local-regional treatments can eliminate the disease. Surgical management of the primary tumor is usually appendicectomy or caecectomy, and an appendiceal lymph-node dissection is needed to rule out regional lymph-node metastases.

Appendiceal ►mucinous neoplasms spare the small bowel, which qualifies them for aggressive local-regional treatment. Even though large volumes of mucinous neoplasm are sometimes located within the greater and lesser omentum, the space between the liver and the diaphragm, and within the pelvis, the small bowel is usually free of disease. Carmignani and

Appendiceal Epithelial Neoplasms. Table 1 Contrasting features of appendiceal neoplasms and colorectal cancer

	Appendiceal epithelial neoplasm	Colorectal cancer
Incidence (cases per year in USA)	1500	150,000
Mucinous histology	85%	15%
Aggressiveness pathology	10%	95%
Lymph-node metastases at initial diagnosis	2%	50%
Liver metastases at initial diagnosis	2%	20%
5-year survival with traditional surgical treatment	30%	70%
10-year survival with combined treatment	60%	NA

NA = not available.

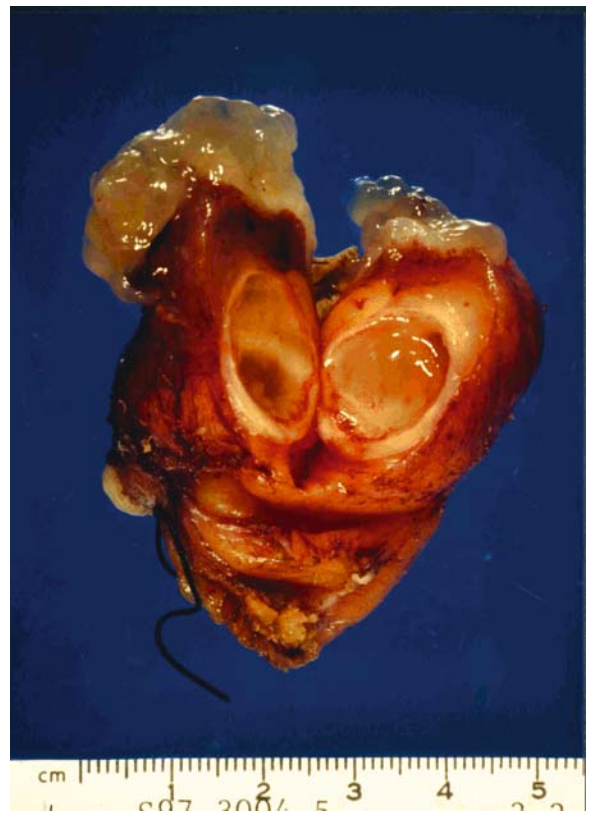
colleagues reported that the constant peristaltic activity of the small bowel prevents neoplastic cells from adhering to its surfaces or to the small-bowel mesentery, except to the part of the jejunum that is adjacent to the ligament of Treitz and the terminal ileum or ileocecal-valve area, which are tethered by a short mesentery to the retroperitoneum.

Before surgery confirms the diagnosis, patients with ►intestinal type adenocarcinoma of the appendix are usually diagnosed with appendicitis, a right lower quadrant abscess, or a tumor mass. In contrast, mucinous appendiceal adenocarcinomas usually have developed a free perforation before diagnosis, causing the tumor to spread to the ovary, or to present as peritoneal carcinomatosis within a hernia sac. An aggressive ►mucinous adenocarcinoma can invade the retroperitoneum and appear as a mucus accumulation in the buttock or thigh. Also, the tumor could invade the abdominal wall with an enterocutaneous fistula or the bladder with an enterovesical fistula. The right ureter can also be invaded by a mucus-containing tumor. If symptoms other than increasing abdominal girth or appendicitis arise, the tumor is probably aggressive.

Pseudomyxoma Peritonei Syndrome

These less aggressive mucinous appendiceal epithelial neoplasms constitute a large proportion of the cases of appendiceal neoplasms. They have a high propensity for spread to peritoneal surfaces, but almost never metastasize through lymphatic channels into lymph nodes, or through venules into the liver. After the tumor ruptures the wall of the appendix (Fig. 1), ►adenomucinosis can progress for months or even years within the abdomen and pelvis without causing any symptoms. As the disease progresses, the peritoneal cavity becomes filled in a characteristic pattern with mucinous neoplasm and mucinous ►ascites. The greater omentum is thickened (omental cake) and infiltrated extensively by the tumor (Fig. 2). All parts of the abdomen that entrap malignant cells also contain tumor, including the undersurface of the right and left hemidiaphragms, the right subhepatic space, the splenic hilus, the right and left abdominal gutters, and the pelvis and cul-de-sac. An important clinical feature of ►pseudomyxoma peritonei is that tumors spare the mobile portions of the small bowel, and the involved parietal and visceral peritoneal surfaces can thus be removed by ►peritonectomy (Fig. 3).

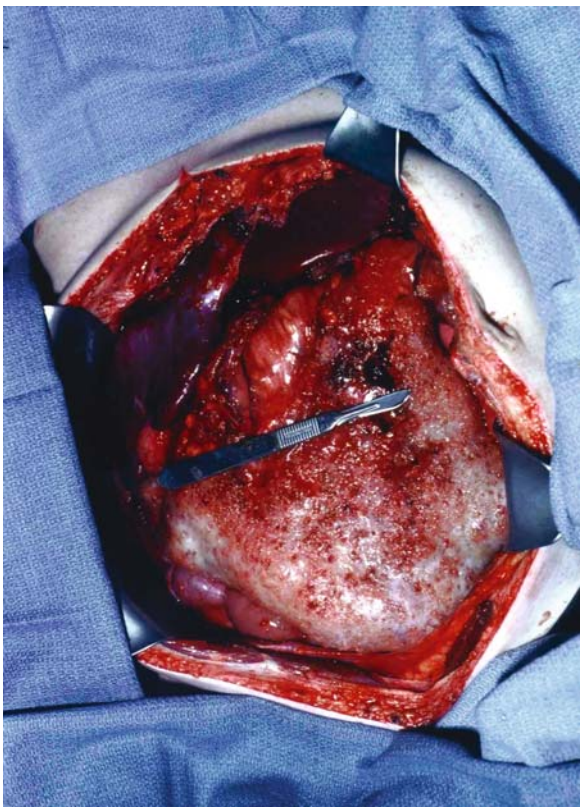
The symptoms and signs of pseudomyxoma peritonei differ greatly from those of appendiceal adenocarcinoma. The most common symptom in both men and women with pseudomyxoma peritonei syndrome is a gradually increasing abdominal girth. Women often develop an ovarian mass, usually on the right side, which is commonly diagnosed at a routine gynecological examination, and men can have new-onset hernia as the next most frequent symptom. The hernia sac is filled by mucin, a mucinous tumor, or both. The third most common presenting feature is appendicitis, a clinical manifestation of a ruptured appendiceal mucocele with local inflammation.



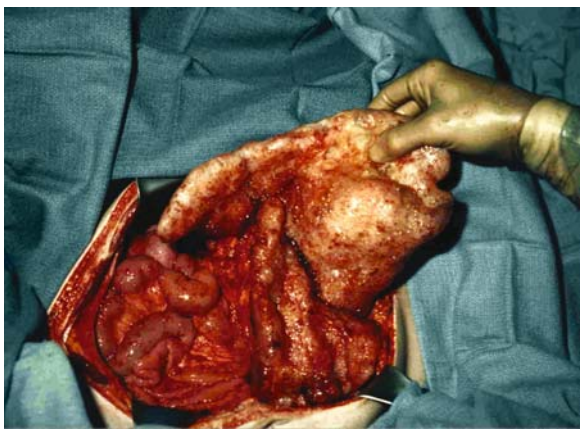
Appendiceal Epithelial Neoplasms. Figure 1 Distal portion of appendix has ruptured from pressure of mucin accumulation within mucocele.

Pseudomyxoma peritonei syndrome can also develop months or even years after planned laparoscopic appendicectomy, if a mucocele is found and ruptures during the procedure. Table 2 shows the symptoms and signs of pseudomyxoma peritonei syndrome as reported by Esquivel and Sugarbaker.

When a patient presents with increasing abdominal girth as a result of presumed malignant ascites, diagnosis is usually established with a paracentesis or laparoscopy and biopsy. In many women with this disease an ovarian tumor will be found. In all instances, paracentesis or laparoscopy with a biopsy should be done directly within the midline and through the linea alba. These sites can be excised as part of a midline abdominal incision. No lateral puncture sites or port sites should be used because they cause the tumor to seed into the abdominal wall causing difficulty with eradication of the disease.



Appendiceal Epithelial Neoplasms. Figure 2 Omental cake characteristic of pseudomyxoma peritonei syndrome.



Appendiceal Epithelial Neoplasms. Figure 3 Mucinous tumors spare the mobile portions of the small bowel.

Treatment

Treatment options for malignant diseases are determined by the anatomical location of the cancer and by its biological aggressiveness. Appendiceal epithelial neoplasms differ greatly from other gastrointestinal cancers in both these categories. Unfortunately, in the past, statistics have been combined for appendiceal neoplasms and colorectal cancer. The international classification of disease designates appendiceal neoplasms together with colorectal cancer. This disease has a unique natural history and requires very different management as compared to colon cancer.

Management in Absence of Peritoneal Dissemination

In patients with invasive non-mucinous adenocarcinoma (►intestinal type) of the appendix, a right hemicolectomy may improve the survival achieved with routine appendicectomy. Patients with invasive intestinal type appendiceal adenocarcinoma with lymph nodes involved should receive a right hemicolectomy either at the appendectomy procedure or at a subsequent time. When the surgeon finds aggressive tumor in the appendix during an appendectomy the mesoappendix should be resected and emergency cryostat sectioning performed. If the bowel is prepared adequately and if adenocarcinoma is identified in lymph nodes, a right hemicolectomy should be done immediately. In some patients, a caecectomy with preservation of the ileocecal valve has been used, and this procedure is recommended if the appendiceal lymph nodes are negative by cryostat sectioning.

Management of Mucinous Neoplasms with Peritoneal Dissemination

Tumor tissue is removed from the abdominal gutters, pelvis, right subhepatic space, and right and left subphrenic spaces by use of a greater omentectomy, lesser omentectomy, splenectomy, and peritonectomy. The probability that a peritonectomy will eradicate the tumor from all surfaces in the abdomen and pelvis is controlled by two factors: the size of the tumor and its invasive capabilities. A small tumor will have negligible or no extension through the serosal layer, so that peritonectomy with electrosurgical dissection (Fig. 4) will result in a small but adequate margin of resection. In a larger tumor, ►invasion through the serosal layer is expected, yet, on structures undamaged by deeper electrosurgical dissection such as the liver, a negative margin is still possible. However, larger tumors on small-bowel surfaces will need resection to eradicate mucinous adenocarcinoma. By contrast, adenomucinous nodules will not invade through the serosal layer and can be resected adequately by peritonectomy from small bowel surfaces.

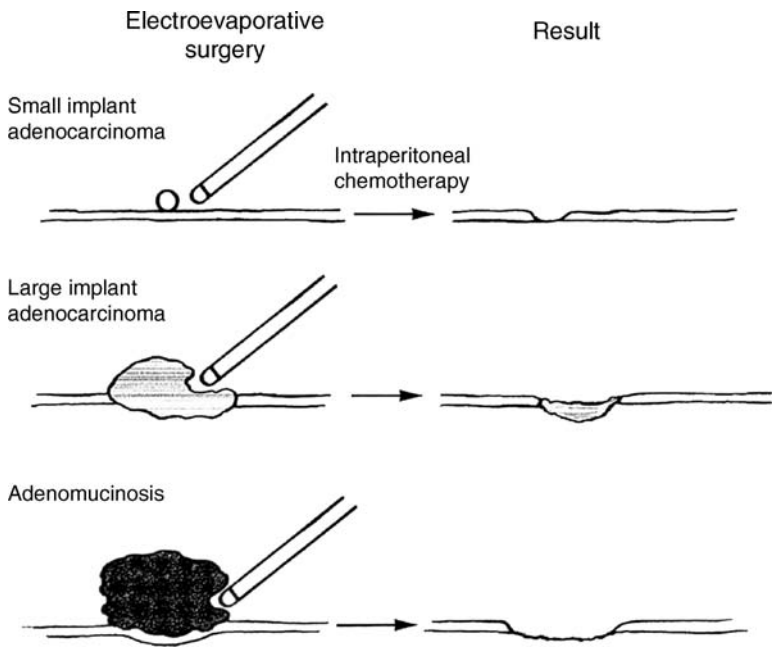
For mucinous appendiceal neoplasms that are perforated at the time of surgery and that result in peritoneal carcinomatosis or pseudomyxoma peritonei, peritonectomy is combined with ►intraperitoneal chemotherapy.

After resection, and with the abdomen open, the peritoneal space is washed thoroughly with warm (41.5°C) chemotherapy solution containing mitomycin C and doxorubicin by the surgeon's hand with gauze debridement of all surfaces. Also, a window of time

Appendiceal Epithelial Neoplasms. Table 2 Frequency of symptoms and signs of pseudomyxoma peritonei syndrome

	Men (*n* = 105)	Women (*n* = 112)
Appendicitis	36 (34%)	22 (20%)
Increased abdominal girth	28 (27%)	21 (19%)
Ovarian mass	NA	44 (39%)
Hernia	26 (25%)	4 (4%)
Ascites	5 (5%)	4 (4%)
Abdominal pain	5 (5%)	3 (3%)
Other	5 (5%)	14 (13%)

NA = not available.



Appendiceal Epithelial Neoplasms. Figure 4 Peritonectomy using electroevaporative surgery for removal of carcinoma implants.

exists in the early postoperative period when all intraperitoneal surfaces are available for treatment with intraperitoneal ►fluorouracil. Consistent exposure of all peritoneal surfaces to intraperitoneal chemotherapy can be achieved if the chemotherapy is used during the first week after surgery. As the postoperative 5-fluorouracil solution remains in the abdominal and pelvic space, the solution can be distributed by turning the patient alternately on to their right and left side and into the prone position.

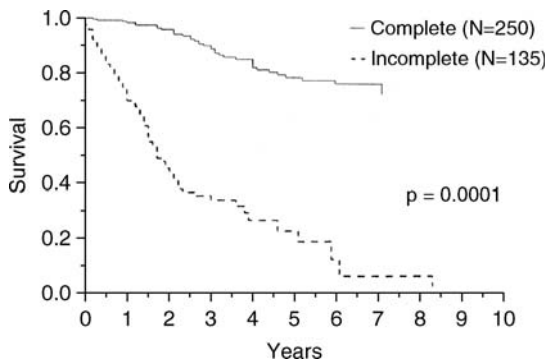
In a prospective investigation of this technique, perioperative intraperitoneal chemotherapy increased the frequency of anastomotic disruptions. Patients who have had previous extensive surgery who need many hours of adhesion lysis, are more likely to develop fistulas after surgery, presumably because of the combined effects of damage to the small bowel from electrosurgical dissection of adhesions (seromuscular damage), and systemic effects of intraperitoneal chemotherapy on the intestine (mucosa and submucosa damage).

►Adjuvant bidirectional chemotherapy (combined intravenous and intraperitoneal) is recommended for patients who have peritoneal dissemination of high-grade appendiceal mucinous neoplasm. Second-look surgery is recommended about 6 months after the ►cytoreduction in some patients, usually those who need ostomy closure. If small tumor foci are found on the peritoneal surface of the abdomen or the pelvis at the staging celiotomy, the nodules are resected and a final treatment with intraperitoneal chemotherapy may be necessary.

Definitive treatment of peritoneal carcinomatosis or pseudomyxoma peritonei should be done in a timely way. Every non-definitive ►debulking surgical intervention makes potentially curative cytoreductive surgery more difficult and because the ►peritoneum acts as the first line of defense against peritoneal dissemination, every effort should be made to keep it intact for optimum results in these procedures. Also, whereas the small bowel is spared early in the natural history of mucinous appendiceal neoplasms and pseudomyxoma peritonei, the fibrous adhesions that inevitably result after several surgical procedures will become infiltrated by tumor, leading to widespread involvement of the small bowel. Eventually, safe cytoreduction becomes impossible, and the effects of the intraperitoneal chemotherapy by itself are not adequate to keep the patient free of disease.

Outcomes

The results of treatments for peritoneal surface dissemination of appendiceal neoplasms have been unexpectedly good. In 385 patients with either peritoneal adenomucinosis or mucinous carcinomatosis who were followed up for an average of 37.6 months, all had documented peritoneal surface disease, and most had large tumors. After cytoreductive surgery, all patients had their abdomen inspected for residual disease and the ►completeness of cytoreduction was scored for all patients on the basis of the size of remaining tumors. Figure 5 shows the survival of patients who had a ►complete cytoreduction compared with those who had an incomplete cytoreduction. In statistical analysis of these data, survival did not differ significantly between patients with near complete cytoreductions and those with grossly incomplete cytoreductions. Furthermore, the only variable that was an independent predictor of survival was the completeness of cytoreduction (complete *vs.* incomplete).



Appendiceal Epithelial Neoplasms. Figure 5 Survival by cytoreduction of 385 patients with peritoneal dissemination of appendiceal epithelial neoplasms treated by cytoreduction and perioperative intraperitoneal chemotherapy.

Survival differed between patients with ►adenomucinosis and those with hybrid or ►mucinous adenocarcinoma ($p < 0.0001$). Patients with non-invasive disease are therefore more likely to benefit from this treatment strategy. No significant differences were noted between patients with hybrid histology and those with mucinous adenocarcinoma. Furthermore, patients with negligible or moderate extent of previous surgery had an improved survival compared with those who had had extensive previous surgery ($p = 0.001$).

This finding shows the importance of the peritoneum as a protective barrier in patients in whom the tumor has spread to this site. Multiple previous dissections had a negative effect on survival when all patients were included in the analysis, but not when patients who had had complete cytoreduction were excluded, suggesting that previous debulking can worsen prognosis by impeding complete removal of the tumor in some patients. Debulking surgery with incomplete cytoreduction allows neoplastic cells to implant on abdominal and pelvic surfaces that have been cleared of peritoneum. Once these neoplastic cells implant deep to the peritoneum, removal with an adequate margin is unlikely.

Analysis of 21 patients with ►adenocarcinoid of the appendix with peritoneal carcinomatosis showed that median survival of patients who had complete cytoreduction and intraoperative and postoperative intraperitoneal chemotherapy was 18.5 months (range 3.2–95.1); 5-year survival was 25%. In some patients, an attempt at complete resection is warranted. If debulking results in gross residual disease, only palliative surgical efforts associated with low morbidity and mortality are indicated because survival is limited.

Extensive cytoreductive surgery combined with early postoperative intraperitoneal chemotherapy is associated with high morbidity. Nevertheless, only 2–10% of patients who receive this treatment die. Anastomotic leaks were more common in these patients than in those who have conventional surgery (5%). Overall morbidity in patients with grade III–IV disease was 20–50%. No morbidity or mortality was associated directly with administration of intraperitoneal chemotherapy. Rather, the frequency of complications depended on the extent of the surgery, number of peritonectomy procedures, and time needed to complete the cytoreduction.

Traditionally, appendiceal epithelial neoplasms have been managed by serial debulking, which removes the bulk of the disease. Although the midabdomen can be cleared by suctioning the mucous neoplasm, washing the intestinal surfaces, and resection of the greater omentum, disease often remains around the liver and deep in the pelvis. Because the tumor recurs after 2–3 years, debulking is often repeated, but is more difficult at a second operation. After three or four debulkings, loops of the small bowel become encased with scar

tissue and mucinous neoplasm, and further surgery is impossible. The function of the gastrointestinal tract is gradually restricted by the accumulation of a large mucinous tumor now embedded within the scar tissue, and the patient dies from long-term starvation. Sometimes, systemic chemotherapy can be of transient benefit.

Approaches to Management

Appendiceal neoplasm with peritoneal dissemination could be an indolent disease process. The assessment of any treatment regimen should allow for a minimum of 10 years' follow-up, and follow-up of 20 years would be ideal. A 20-year follow-up with cytoreductive surgery and perioperative intraperitoneal chemotherapy was reported by Sugarbaker and colleagues, who showed that patients who had complete cytoreduction for less-aggressive disease had a projected survival of 70% at 20 years, a sharp contrast with the results achieved with serial debulking. However, these results should be interpreted with the knowledge that these treatment strategies have not been compared directly. These positive results have led to establishment of treatment centers for appendiceal neoplasm in the USA and in nearly all countries in Europe.

In the UK, combined treatments have become a part of the overall plan for healthcare. Moran and colleagues have established a treatment center dedicated to management of appendiceal malignant diseases and pseudomyxoma peritonei syndrome, to which any patient enrolled in the UK National Health Service, from anywhere in the UK, can be referred. The center, based in Basingstoke, UK, started treatment for this disease in 1994, and about 40 cytoreductions are done there every year. Because of the large number of patients needing treatment for appendiceal malignant diseases with peritoneal surface dissemination, a second treatment center was established in 2002 in Manchester, UK. These centers show that referral to a treatment center is now standard practice for this disease in the UK and allows doctors to use standard treatments, improve treatment regimens, and refine the surgical skills needed for optimum management of these patients. This approach has improved patient care and reduced costs because patients can be referred early before disease progresses to a symptomatic state.

A New Standard of Care

Ideally, new treatments should evolve through the clinical trials process. A phase III trial should be undertaken to compare traditional treatment options with new treatments. However, until such data are available, the issue remains of which treatment option is best for these patients. The available evidence suggests that cytoreductive surgery with perioperative intraperitoneal chemotherapy should replace serial debulking as the standard of care for patients with peritoneal spread of appendiceal epithelial neoplasms.

However, phase III trials are difficult to do in this setting because they would need to compare a potentially curative treatment option with a palliative one. Patients are thus likely to be reluctant to be randomized, no matter how carefully the trial is designed and explained. Follow-up of about 20 years would be needed to assess the best treatment plan. However, such long-term follow-up would mean that meaningful data might never be available within the lifetime of the principal investigator. Furthermore, the disease is uncommon and only a few institutions, especially those designated as pseudomyxoma peritonei treatment centers, can accumulate sufficient numbers of patients to give meaningful results. The only trial that would accumulate sufficient patients for assessment over a reasonable time would be a multinational trial with most of the institutions participating from the USA and Europe, meaning the expense and the coordination would be prohibitive. Acceptance of combined treatment as the standard of care would allow treatment centers to investigate new and possibly more effective local-regional treatment strategies that can improve the overall results and decrease morbidity and mortality by prospective randomized studies. The skills, judgments, and treatments offered vary between the many treatment centers, and results of treatment might not therefore be consistent. With experience, the morbidity and mortality associated with cytoreductive surgery and intraperitoneal chemotherapy should lessen, as should objections to this procedure. Physicians at experienced centers learn that addition of chemotherapy as a planned part of cytoreductive surgery improves patient care rather than results in excessive morbidity and mortality. Correct selection of patients is an important part of successful treatment of this disease.

Summary of Changes in Management of Appendiceal Epithelial Neoplasms

Several distinct changes are needed to the surgical techniques used to treat patients with peritoneal dissemination of an appendiceal mucinous neoplasm. Because chemotherapy has little effect on large tumors, definitive cytoreduction should be attempted to reduce the cancer within the abdomen and pelvis to its smallest volume. Such reduction requires use of peritoneal stripping, now commonly referred to as peritonectomy, in which the patient often needs to spend many hours in the operating theatre. Frequently, the abdomen is left without peritoneal surfaces except for that found on the small bowel. This approach is a change from the previous conservative surgical approach to peritoneal carcinomatosis.

Several changes are also needed in the use of chemotherapy in these patients. Administration of chemotherapy should change from intravenous to intraperitoneal with maximum doses of mitomycin, doxorubicin and 5-fluorouracil. Intraperitoneal chemotherapy should be

done perioperatively to contact all abdominal and pelvic surfaces before wounds start to heal. Once fibrinous deposits become organized, chemotherapy will not be able to reach residual tumors and local recurrence will occur where the surfaces are adherent. Perhaps most important for favorable results, selection of patients for treatment should change. Patients should receive maximum cytoreductive surgical procedure and perioperative chemotherapy as a first time management strategy. The target of these treatments should be directed at minimal residual disease on both the parietal and visceral surfaces. Patients with metastases that cannot be resected or with gross residual disease of the peritoneal surface after cytoreductive surgery has been done should be excluded from a curative approach. With these changes in surgical approach and chemotherapy administration, patients with peritoneal dissemination of appendiceal mucinous tumors could have an improved survival.

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Appendiceal Mucinous Tumor of Uncertain Malignant Potential

►Appendiceal Epithelial Neoplasms

Appendix

Definition

The vermiform appendix is a worm-shaped blind-ending appendage which opens into the caecum of the colon. The commonest tumor arise from this organ is carcinoid, but appendiceal epithelial neoplasia occur and specifically are important in the development of pseudomyxoma peritonei.

►Pseudomyxoma Peritonei
►Carcinoid Tumors

Appraisal Consultation Document

Definition

First draft of guidance on a new health technology produced for consultation purposes by the UK's National Institute for Health and Clinical Excellence.

►National Institute for Health and Clinical Excellence (NICE)

Aptamer

Definition

A single-stranded oligonucleotide that can adopt a particular structure such that it can interact with high specificity and affinity to a particular receptor. Usually identified through a combinatorial selection method such as ►SELEX.

►Combinatorial Selection Methods
►Aptamer Bioconjugates for Cancer Therapy

Aptamer Bioconjugates for Cancer Therapy

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Characteristics

Over the past two decades, a large body of data has been generated that demonstrates the feasibility of antibodies for tissue targeting. The first FDA approval

of a humanized monoclonal antibody for the treatment of cancer came in 1997 when rituximab (Rituxan) entered the market for the treatment of patients with relapsed or refractory low-grade or follicular, CD20 positive, B-cell non-Hodgkin's lymphoma. A wide variety of ligand-drug conjugates are now under clinical development or in clinical practice today. For example, Gemtuzumab (Mylotarg) is an FDA approved chemoimmunoconjugate for the treatment of Acute Myelogenous Leukemia. Mylotarg, and is one of four FDA approved therapeutic conjugates. There are many others that are currently in various stages of clinical and pre-clinical development. In addition to antibodies, there is a growing list of ligand classes under development that are capable of binding to target antigens with high affinity and specificity. One example is nucleic acid ligands referred to as ►aptamers, which are small in size, potentially non-immunogenic, easy to synthesize, characterize, modify, and exhibit high specificity and affinity for their target antigen. In the short time since the groups of Jack Szostak and Larry Gold independently described the methodology for *in vitro* selection of aptamers, known as ►SELEX, these ligands have been explored for a variety of applications, as therapeutics, diagnostics and research enablers. Aptamers have also been exploited as targeting molecules for cell- or tissue-specific delivery of controlled release polymer drug delivery vehicles. Recently we described the first proof-of-concept drug delivery vehicles utilizing aptamers for targeted delivery and have gone on to show efficacy of these vehicles in tumor reduction *in vivo*.

Aptamers are single stranded DNA, RNA or unnatural oligonucleotides that have been selected *in vitro* from a pool of ~10^{14} to 10^{15} random oligonucleotides for their ability to bind to a target molecule. Aptamers have a molecular weight in the 6–25 kDa range and derive their name from the Latin word "aptus" meaning "to fit." Aptamers fold through intramolecular interaction to create tertiary conformations with specific binding pockets which bind to their target molecules with high specificity and affinity. This tertiary conformation is analogous to the globular shape of tRNA.

Unlike antisense compounds, which are single-stranded nucleic acids that affect the synthesis of a targeted protein by hybridizing to the mRNAs that encodes it, aptamers may inhibit a protein's function through directly binding to it. Aptamers typically bind with an equilibrium dissociation constant (Kd) in the range of 10 pM to 10 μM to a wide array of molecular targets including other nucleic acids, proteins, peptides and small molecules. Aptamers can be described by a sequence of approximately 15–80 nucleotides (A, U, T, C, and G). The conformation of the ►aptamer confers specificity for a target molecule through interacting with multiple domains, or a binding pocket. Small changes in the target molecule can foil interactions and thus aptamers can distinguish between closely related but non-identical targets. For example, specific RNAs were identified that have a high affinity for the bronchodilator theophylline (1,3-dimethylxanthine) yet exhibit a >10,000 times weaker binding affinity to caffeine (1,3,7-trimethylxanthine) which differs form theophylline only by the substitution of a methyl group at the nitrogen atom N7 position. Based on their unique molecular recognition properties, aptamers have found great utility for applications in areas such as *in vitro* and *in vivo* diagnostics, analytical techniques, imaging, and therapeutics.

Although aptamers are highly stable and may tolerate a wide range of temperature, pH (~4–9) and organic solvents without loss of activity, these molecules are susceptible to nuclease degradation or renal clearance *in vivo*. Therefore, their pharmacokinetic properties must be enhanced prior to *in vivo* applications. Several approaches have been adopted to optimize the properties of aptamers such as: (i) capping their terminal ends, (ii) substituting naturally occurring nucleotides with unnatural nucleotides that are poor substrates for nuclease degradation (i.e. 2′-F, 2′-OCH3 or 2′-NH2 modified nucleotides), (iii) substituting naturally occurring nucleotides with hydrocarbon linkers, and (iv) use of L-enantiomers of nucleotides to generate mirror image aptamers commonly referred to as spiegelmers. Aptamers can also be stabilized using locked nucleic acid modifications to reduce conformational flexibility. Alternatively, a nuclease resistant aptamer may be selected *de novo* using a pool of oligonucleotides with 2′-F or 2′-OCH3 modified nucleotides. Through combining some of these strategies, an aptamer's half life can be prolonged from several minutes to many hours. To prolong the rate of clearance of aptamers, their size may be increased by conjugation with polymers such as polyethylene glycol (PEG).

►Nanoparticles are referred to structures that are in the 1–100 nm scale in at least one dimension, and may have any form including spherical, cylindrical or pancake like. To put this size range in perspective, a small molecule, a virus, a bacterium, and the cross section of a human hair are around 1, 100, 1,000, and 100,000 nm, respectively. Several classes of materials have been used for the development of nanoparticles including organic and inorganic biomaterials. Biodegradable polymer nanoparticles which are a type of organic nanoparticles, have been extensively investigated for cancer therapy. Polymeric nanoparticles can be designed to have a prolonged systemic circulating half-life by conjugating, or adsorbing sterically amphiphilic polymers such as polyethylenglycol (PEG) to the particle surface. These nanoparticles can be used to release the encapsulated drugs at a controlled rate surface or bulk erosion, diffusion, or swelling followed

by diffusion, in a time or condition dependent manner. The rate of drug release can be controlled by modification of the polymer side chain, development of novel polymers, or synthesis of copolymers. In general, these biodegradable polymer systems can provide drug levels at an optimum range over a longer period of time than other drug delivery methods, thus increasing the efficacy of the drug and maximizing patient compliance, while enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs. Liposomes are another type of nanoparticles made of amphiphilic unilamellar/multilamellar membranes of natural or synthetic lipids. Lipids are characterized by a hydrophilic head group and a hydrophobic tail. Doxorubicin encapsulated liposome (Doxil) was the first liposome to gain FDA approval in 1995 and have potent antineoplastic activity against a wide range of human cancers including Kaposi's sarcoma and ovarian cancer. A variety of other nanoparticles platforms including dendrimers, nucleic acid based nanoparticles, nanoshells to name a few have been, or are currently being, developed for drug delivery applications.

The conjugation of aptamers to nanoparticles can result in the development of therapeutic or diagnostic conjugates. Covalent conjugation of aptamers to nanoparticles can be achieved most commonly through succinimidyl ester – amine chemistry which results in a stable amide linkage or through maleimide – thiol chemistry. Potential non-covalent strategies include affinity interactions (i.e. streptavidan-biotin) and metal coordination (i.e. between polyhistidine tag at the end of the aptamer and Ni^{+2} chelates with immobilized nitrilotriacetic acid on the surface of the polymer particles). When aptamers are conjugated to drug encapsulated nanoparticles, these ►bioconjugates can direct the delivery of therapeutic agents in a targeted manner to specific cells or tissue. The payload of nanoparticles may include small molecule drugs such as chemotherapeutics; protein based therapeutics such as antibodies or hormones; nucleic acid therapeutics such as anti-sense oligonucleotide, RNAi or gene therapy vectors; or agents for neutron capture therapy or photodynamic therapy. Aptamers may also be bound to imaging nanoparticles to facilitate diagnosis and identification of tumor metastases. For example, it may be useful to bind aptamers to optical imaging agents including fluorophores and quantum dots (nanocrystals) or MRI imaging agents such as magnetic nanoparticles for detection of small foci of cancer metastasis. Multiplex systems comprising drug laden nanoparticle aptamer conjugates together with imaging agents represents a prospective avenue to future research.

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Apudomas

Definition

Apudomas referring to tumors capable of amine precursor uptake and decarboxylation; ►neuroendocrine tumors.

►Neuroendocrine Carcinoma

AR

Definition

Androgen Receptor.

►Androgen Receptor
►Cyclin G-Associated Kinase

9-β-D-Arabinosyl-2-fluoroadenine (F-ara-A) Monophosphate

►Fludarabine

Arachidonic Acid

Definition

It is an omega-6 fatty acid with carbon chain length of 20. An unsaturated fatty acid that occurs in most animals fats, which is a precursor of ►prostaglandins. Usually it is esterified in phospholipids (especially phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) of biological membranes. Under oxidative stress, the ►phospholipase A2 (PLA2) enzyme catalyzes the release of arachidonic acid from membranes. It is oxidized by lipoxygenases and cyclooxygenases into three classes of lipid mediators: prostaglandins/thromboxanes, leukotrienes and lipoxins. Its metabolites play important roles in acute/chronic ►inflammation and ►carcinogenesis.

►Nonsteroidal Anti-Inflammatory Drugs
►Celecoxib
►Leukotrienes
►Lipid Mediators
►Arachodinic Acid Pathway

Arachidonic Acid Pathway

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Synonyms

Cyclooxygenase; COX; metabolism; Lipoxygenase; LOX; metabolism; Eicosanoid signaling

Definition

The arachidonic acid pathway describes the biosynthesis of ►eicosanoids from arachidonic acid (AA) including its formation from omega-6 polyunsaturated fatty acids (PUFAs) and the synthesis of eicosanoids from eicosapentaenoic acid (EPA) including its formation from omega-3 PUFAs. Eicosanoids are short-lived biologically potent, autocrine or paracrine acting, lipid signaling molecules.

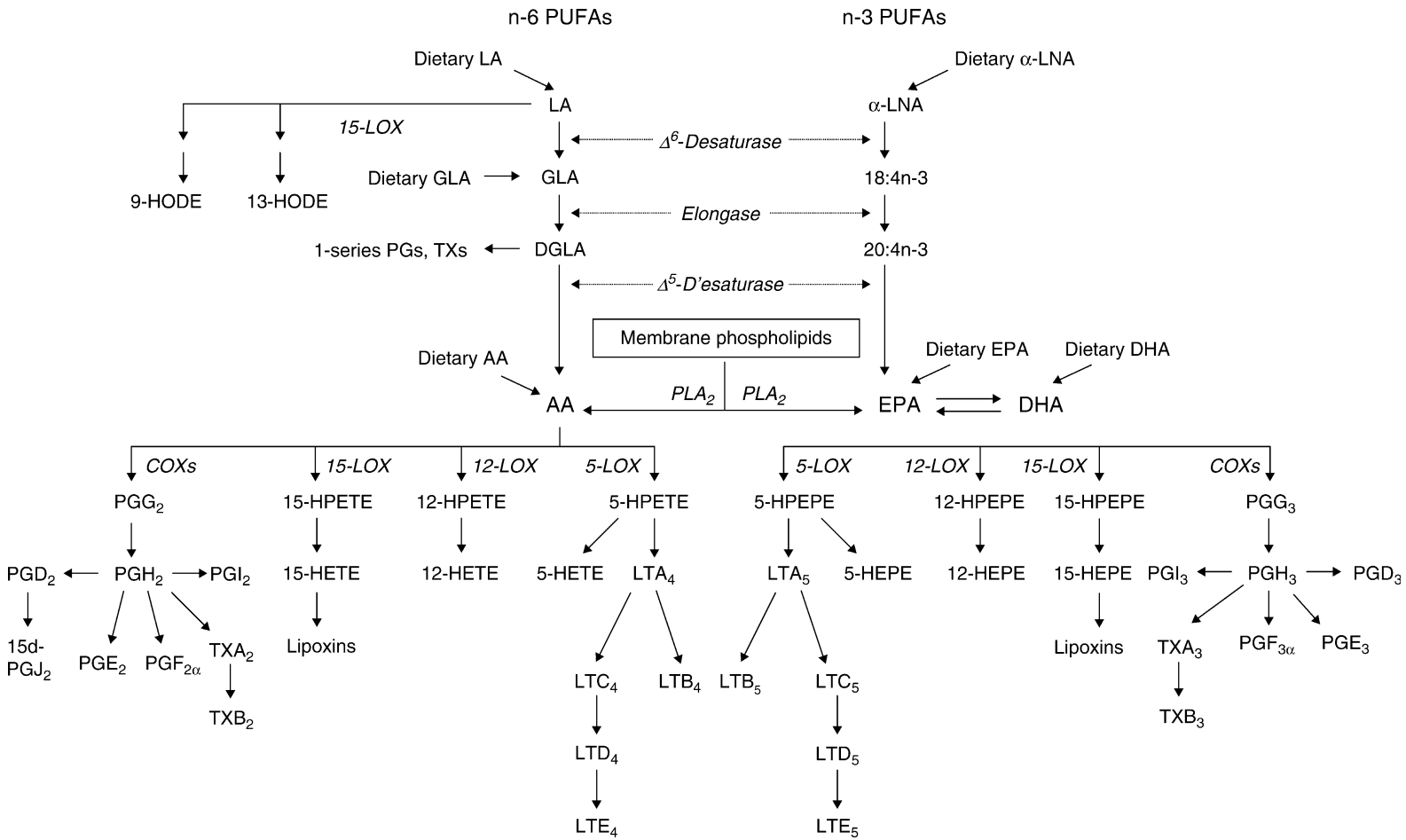
Characteristics

The AA pathway is involved in many physiological processes including ►inflammation and cancer. The pleiotropic effects of modulating this pathway are manifold and are depending on the levels of fatty acids from (dietary) substrates, which in turn modulate the level of eicosanoid precursors (AA and EPA) determining the amounts of eicosanoid lipid mediators actually produced. Initial and still ongoing research on the pharmacological inhibition of inflammatory reactions by ►nonsteroidal antiinflammatory drugs (NSAIDs) is one of the major disciplines/fields of research contributing to our current understanding of the AA pathway, including all its tissue-specific differences.

Fatty Acids as Dietary Precursors

PUFAs that enter the body through our diet are first metabolized via a series of enzymatic changes into the eicosanoid precursors arachidonic acid (AA) and EPA. The main PUFA in our diet is linoleic acid (LA), a member of the omega-6 (n-6) family of PUFAs that have their first double bond at the sixth carbon from the methyl terminus. The omega-3 (n-3) PUFA family, with the first double bond at the third carbon atom, has as its parent compound α-linolenic acid (ALA) (see Fig. 1). Both LA and ALA are converted in several steps by tissue-specific elongases and desaturases into AA and EPA. The latter two can be directly obtained from the diet as well. These eicosanoid precursors are incorporated and stored into cell membranes until they are released by different members of the phospholipase A_2 (PLA_2) family of enzymes, thereby producing free AA and EPA.

The hypothesis that omega-3 PUFAs have a protective effect against (colorectal) cancer originates from observational studies with Greenland Eskimos and a Japanese population. These populations are characterized by a significantly lower incidence of colorectal cancer, and by a fish-enriched diet containing substantially more omega-3 PUFAs compared to Western diets. This protective effect is lost after migration to Western countries like the US, and adoption of the Western lifestyle. The accompanying shift in balance between omega-6 and omega-3 fatty acids is hypothesized to play an important role in cancer promotion and development. Epidemiological prospective studies, however, have not been very consistent and a recent meta analysis even suggested no protective effect at all of omega-3 PUFAs for cancer in general. However, drawing of general conclusions is hampered by the many uncertainties that exist in the way exposure to omega-3 PUFAs is estimated (food frequency questionnaires, analysis of fatty acids in sera or bodyfat biopsies) as well as by tissue- and cancer type specific differences. Furthermore, animal and in vitro studies keep reinforcing a potential protective effect of increased omega-3 PUFA intake on (intestinal) cancer. For example, dietary supplementation with fish oil and/or EPA/DHA decreases tumor number in chemically induced animal models of colorectal tumors as well as in APC-Min mice (►APC-min mouse). Omega-3 PUFAs have also been shown to induce apoptosis and suppress cell growth in vitro.



Arachidonic Acid Pathway. Figure 1 Overview of the arachidonic acid pathway adapted from Larsson et al. [1].

There are several proposed mechanisms by which omega-3 PUFAs can exert their protective effect on tumor formation, as reviewed by Larsson et al. [1]. One of the major mechanisms leads to the suppression of omega-6 PUFA-derived eicosanoids. Higher intake of omega-3 PUFAs, compared to the omega-6 variety, would result in a decrease in available AA for eicosanoid production through the incorporation of the omega-3 PUFAs into membrane phospholipids. This effect is further enhanced by competition between omega-3 and omega-6 PUFAs for the elongases and desaturases that convert these PUFAs, since omega-3 PUFAs have a higher affinity for these enzymes. Omega-3 PUFAs can also directly inhibit cyclooxygenases (COX) (see next section) and compete with omega-6 PUFAs for COX-2 to form ►prostanoids. Moreover, EPA is the preferred substrate for the lipoxygenase (LOX) enzymes that utilize both AA and EPA, resulting in an increase in omega-3 PUFA-derived leukotrienes.

Production of Eicosanoids – from AA and EPA by COX and LOX Pathways

AA and EPA are the central eicosanoid precursors in the majority of mammalian cells and are released by phospholipases from cell-membrane bound phospholipids. The biosynthesis of eicosanoids from AA and EPA is controlled by two major metabolic routes, the COX and LOX pathways. Via these routes, AA is converted into 2-series prostanoids (►Prostaglandins, PGs; prostacyclins, PGIs; and thromboxanes, TXs) and 4-series leukotrienes, and EPA is converted into 3-series prostanoids and 5-series leukotrienes (see Fig. 1). The COX enzymes, also called prostaglandin-endoperoxide synthase (PTGS), catalyze the formation of prostanoids. There are three COX isozymes, the constitutively expressed COX-1, the inducible COX-2, and the more recently identified COX-3 which is actually a splice variant of COX-1, probably producing a truncated inactive protein. The LOX family of enzymes, including 5-LOX, 8-LOX, 12-LOX, and 15-LOX, catalyzes the formation of leukotrienes, "hydroxy fatty acids," and lipoxins. Carcinogenesis in humans and experimental animals is consistently linked to aberrant arachidonic acid metabolism through these COX and LOX pathways.

Regarding COX, tumor development in several different tissues is frequently associated with overexpression of COX-2 in both premalignant and malignant stages, indicating that activation of COX-2 may be an early event in carcinogenesis. This overexpression often starts in tissues adjacent to the transformed epithelium giving rise to "activated" stroma. Expression of COX-2 is induced by numerous growth factors, cytokines and oncogenes, and regulated both transcriptionally and posttranscriptionally, especially through increased mRNA stability. Several pathways are involved and interconnected in the modulation of COX expression and are discussed in more detail in the section on "Interactions with cancer signaling pathways." Both genetic and pharmacologic studies support a causal role of COX in cancer development. Genetic inactivation of COX-2 strongly reduces tumor formation in several animal model systems including the classical two-stage mouse skin cancer model, and in Apc mutant mouse models. These effects are not limited to COX-2 but also apply in part to COX-1. These data are corroborated by pharmacologic intervention studies using both nonselective COX inhibitors, like ►aspirin and other NSAIDS and COX-2 selective inhibitors. The subsequent reduction in tumor formation has been documented in numerous experimental animal model studies, but also in patients with familial adenomatous polyposis (FAP) and supports the outcome of many epidemiological studies suggesting a chemoprotective effect of long-time regular use of these drugs.

With respect to LOX, the situation is less clear and seems more complicated, illustrated by the identification of six LOX genes so far in humans (and seven in mice) and the different profiles of LOX that have been observed in human and rodent tissues. A general but oversimplified picture emerging is to divide the LOX genes/enzymes into "procarcinogenic" and "anticarcinogenic" isoforms. 5-LOX and p12-LOX are considered "procarcinogenic" because they can induce proliferation, antiapoptotic effects, angiogenesis and metastasis, whereas 15-LOX(1 + 2), l + e12-LOX and 8-LOX are considered "anticarcinogenic" because they are associated with differentiation, growth inhibition, and apoptosis.

Signaling by Eicosanoids

There are several mechanisms involved in eicosanoid signaling, which are frequently divided into COX-dependent and COX-independent routes. The COX-dependent tissue-specific production of prostaglandins, leukotrienes, and thromboxanes represents the major route in the formation of lipid mediators. The most prominent example in relation to cancer is prostaglandin E2 (PGE_2)-mediated signal transduction through its G-protein-coupled EP2 receptor, which has been demonstrated to play a pivotal role in (intestinal) carcinogenesis (see also the last section). Another important topic is the type of substrate that is used by the COX and LOX enzymes for the formation of eicosanoids (see also the previous sections). The 3-series prostanoids and 5-series leukotrienes derived from omega-3 PUFAs are generally considered to be less inflammatory and inhibiting tumor formation, whereas the 2-series prostanoids and 4-series leukotrienes derived from omega-6 PUFAs are believed to be proinflammatory and contain several tumor promoting

properties, including increased cell proliferation, inhibition of apoptosis, and stimulating angiogenesis

A variety of other effects, in part COX-independent, have been put forward from research on the effects of (high concentrations) of NSAIDs and its derivatives. An example of a COX-independent process includes the induction of apoptosis directly by AA through the production of ceramide and the mediation of apoptotic signaling by the LOX-15 production of 13-S-HODE. Furthermore, leukotriene production can be reduced due to the inhibition of LOX-5 by nitric oxide-releasing aspirin. Lastly and less clarified, eicosanoids may exert their biological effects in an intracrine way, similar to specific PUFAs, as activating ligands of transcription factors of the ►PPAR family, especially PPARγ and PPARδ.

NSAIDS and Eicosanoid Signaling

NSAIDs, of which aspirin is the best known example, are a class of drugs mainly used for analgesic purposes and to reduce inflammation, and are also used as anticoagulants for individuals at increased risk of cardiovascular disease. From the effects on high risk individuals (Gardner syndrome), it became apparent that NSAIDs also played an active role in the protection against (colorectal) cancer. Numerous epidemiological studies have found associations between regular use of NSAIDs and decreased risk of (colorectal) cancer and adenomas. Animal studies have also given clear indications about the protective effect of NSAIDs. The mechanisms of action of NSAIDs are generally divided into COX-dependent and COX-independent mechanisms. The ability of NSAIDs to inhibit the COX enzymes was originally thought to represent the main underlying mechanism of action, thereby reducing the production of prostanoids and increasing the pool of free AA, resulting in immune modulation, inhibition of tumor angiogenesis, and promotion of apoptosis. One of the first clues that other enzymes might play a role in the mechanism of action of NSAIDs, the COX-independent mechanisms, came from studies on the sulindac metabolites sulindac sulfone and sulindac sulfide. More recently, other enzymes within the pathway have been considered as targets for NSAIDs. Based on in vitro studies 15-LOX1 and PLA2G4A have been implicated as alternative targets of NSAID (aspirin) action. Two subtypes of the PPAR family, which can be activated by the products of the AA pathway, can also act as direct targets of NSAIDs. Sulindac can bind to PPARδ, after which its activity and protein expression is downregulated, inducing apoptosis. NSAIDs can also act as ligands for another receptor subtype, PPARγ. In contrast to PPARδ, this subtype has been shown to be activated by sulindac, which resulted in growth inhibition and apoptosis of cancer cells.

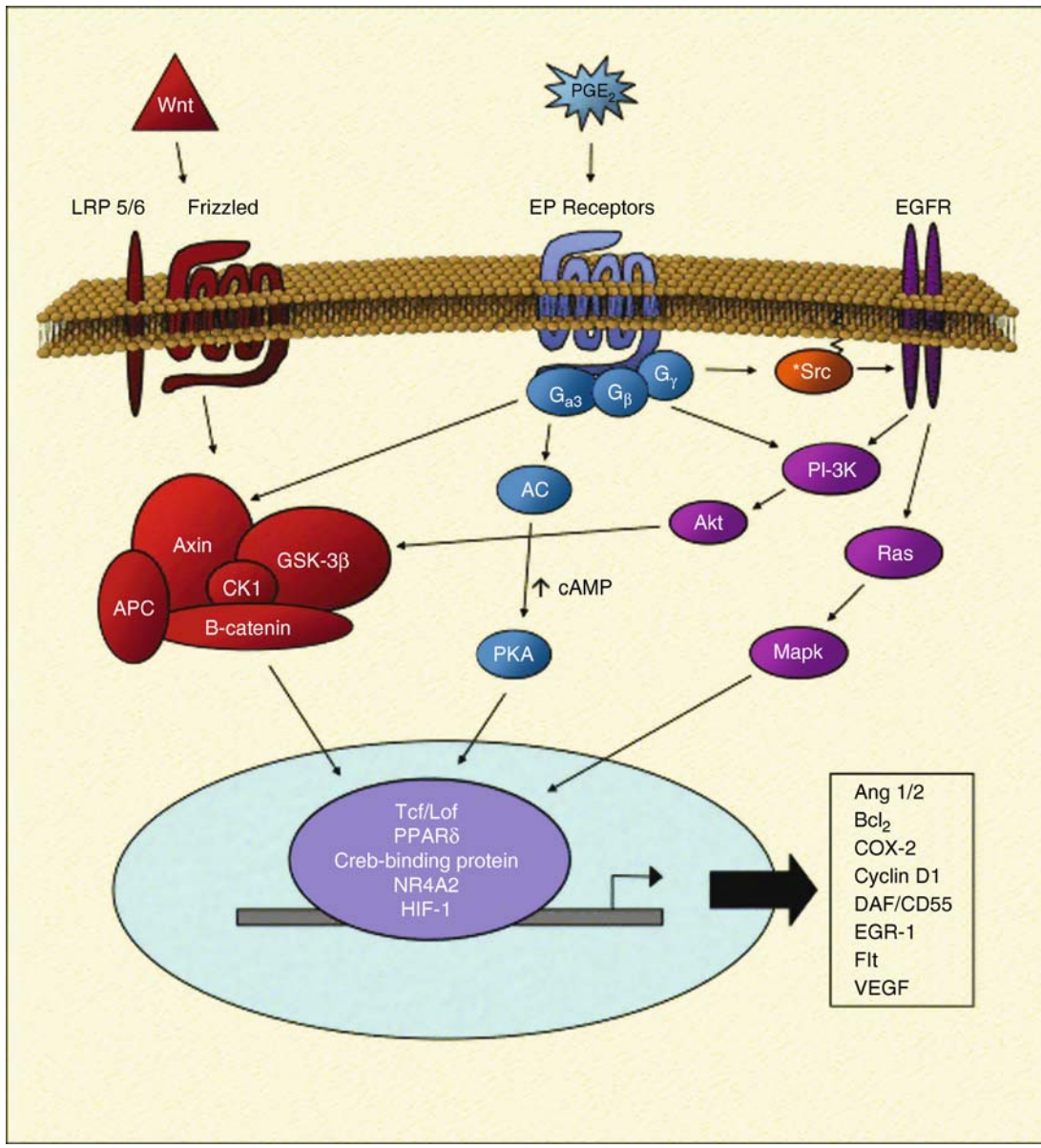
Other recent research in the field of lipid mediators has further linked omega-3 PUFAs and NSAIDs to inflammation. It is now hypothesized that increased omega-3 PUFA intake not only results in decreased production of proinflammatory eicosanoids due to competition with AA, but also produces mediators with potent antiinflammatory effects of their own. Moreover, new evidence suggests that acetylation of COX-2 by aspirin does not only result in complete inhibition of the COX activity of the enzyme, but induces a conformational change resulting in a shift to a LOX function, producing potent lipoxins. This prompted others to study the effect of omega-3 PUFA metabolites derived from acetylated COX-2 on inflammation. They found that these mediators could resolve inflammation, and subsequently called them resolvins. An orphan receptor ChemR23 was identified as a specific receptor of the EPA-derived resolvin RvE1, which inhibits activation of NF-κβ by TNF-α, and is among other tissues also expressed in the gastrointestinal tract. It is likely that these mediators play an important role in reducing inflammation in the GI tract, and that omega-3 PUFAs and aspirin exert their protective effect on colorectal cancer partly through this pathway.

Over time, the serious gastric toxicity of NSAIDS, resulting from the inhibition of COX-1, stimulated the development of specific COX-2 inhibitors, the so-called ►Coxibs. Although highly effective in cancer prevention, their improved risk profile for gastric toxicity seems to occur at the expense of cardiovascular toxicity.

Finally, pharmacokinetics and genetics of NSAID metabolism have been documented to greatly influence the chemopreventive potential of various NSAIDs. Polymorphisms in metabolic activation (CYP2C9) and elimination pathways (UGTs) but also in the AA pathway (COX-2) have been demonstrated to interact with NSAIDs with respect to (colorectal) cancer risk.

Interactions with Cancer Signaling Pathways

At present many molecular signaling pathways linked to cancer have been identified. It is also becoming increasingly clear that the interactions between cellular signaling pathways are key to the (phenotypic) outcome of the molecular signaling circuitry. Two important pathways, the canonical Wnt signaling pathway and one of the RAS effector pathways, the PI3K/AKT pathway have been recently demonstrated to functionally interact with PGE_2-EP2 receptor signaling providing another example of signal modulation by cross-talk between an G-protein-coupled receptor and growth factor receptors. The integrating picture can be summarized by recognizing that the $G_{\alpha s}$ and $G_{\beta\gamma}$ subunits of the EP2 receptor display different downstream signaling properties (Fig. 2). Next to activating adenylatecyclase (AC) and subsequently PKA, it was demonstrated that the $G_{\alpha s}$ subunit is also capable to



Arachidonic Acid Pathway. Figure 2 Prostaglandin induced transactivation of the canonical Wnt and EGFR signaling pathways, adapted from Buchanan and Dubois [5].

associate directly with axin, destabilizing the signaling complex, which leads to an increase in β-catenin and resulting in activation of Wnt signaling. Another way of destabilizing the same complex is to inactivate GSK-3β by phosphorylation. It was also demonstrated that PI3K and AKT could be activated through the Gβγ subunit of the EP2 receptor, leading to inactivation of GSK-3β. In summary, PGE2 seem to exert its G-protein-coupled signaling through axin, GSK-3β and PKA, but how these signals are integrated is still far from clear.

The role of NF-κβ signaling, producing a proinflammatory transcription factor, as an important central player in cancer development and progression is well documented and still intriguing. Activated NF-κβ has been linked to many cancer promoting processes including cellular proliferation, apoptosis suppression, invasion, and angiogenesis, and depending on tissue context also to tumor suppression. Normally, NF-κβ is ►sequestered in the cytoplasm by IkB. A variety of receptors and afferent signals activate IKK, which phosphorylates IkB, tagging it for degradation. The "liberated" NF-κβ can now translocate to the nucleus where it activates ~150 different genes, including COX-2. Well known inflammatory conditions introduced by infectious agents like for example *Helicobacter pylori*

in the stomach and HBV in the liver are established risk factors for respectively gastric and liver cancer. However, the persistent activation of NF-κβ is also documented to result in the loss of the tumor suppressor protein CYLD, leading to the benign human syndrome called ►cylindromatosis. Further details of the interplay between NF-κβ signaling and the AA pathway are eagerly awaited.

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Archaea

Definition

A unique group of microorganisms classified as bacteria (Archaeobacteria) but genetically and metabolically different from all other known bacteria. They appear to be living fossils, the survivors of an ancient group of organisms that bridged the gap in evolution between bacteria and the eukaryotes (multicellular organisms).

ARE

Antioxidant response element, is a *cis*-acting DNA regulatory element which is located in the 5′-flanking region of many cytoprotective phase 2 genes and causes increased transcription of the target gene after binding by the Nrf2 complex.

►Phase 2 Enzymes

ARF

Definition

Is an alternatively spliced product of the INK4a locus. When triggered by E1A, myc or E2F or other illegitimately activated oncogenes, ARF binds MDM2. This leads to a rise of the p53 level, causing growth arrest and/or apoptosis.

►ARF Tumor Suppressor Protein
►Multistep Development
►INK4
►MDM Genes
►Myc Oncogene
►P53 family

ARF Tumor Suppressor Protein

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Synonyms

ARF; alternative reading frame; $p14^{ARF}$; human; $p19^{ARF}$; mouse

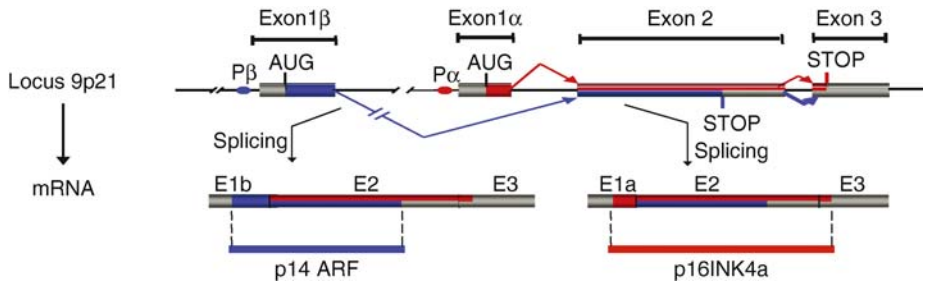
Definition

ARF (alternative reading frame), is a ►tumor suppressor protein that accumulates in the nucleolus in response to aberrant oncogenic/hyperproliferative signals and induces cell cycle arrest in G1/S or G2/M transition and ►apoptosis. Consistent with its tumor suppressor status, ARF inactivation (deletion, promoter methylation) impairs cellular response to oncogenic stress, and frequently occurs in a wide spectrum of cancers (30–40% of cancers).

Characteristics

Genomic alterations of the 9p21 chromosome region occur in various types of human solid tumors and hematological malignancies. Most, if not all, involve the *CDKN2* locus, where the *INK4a* and *ARF* genes are superimposed. Both gene products result from alternative splice transcripts in different reading frames (Fig. 1) and have been functionally characterized as tumor suppressor genes.

1. $p16^{INK4a}$ is the prototypic member of the *INK* (►*INK4*) gene family that encodes inhibitors of



ARF Tumor Suppressor Protein. Figure 1 Schematic representation of the genomic *INK4a/ARF* locus. Exons are depicted by boxes. Coding regions of *ARF* are colored in blue, those of *INK4a* in red, untranslated regions are in grey. ARF and INK4a promoters are indicated respectively by Pβ and Pα. *INK4a*/*ARF* locus utilizes alternative first exons and shares downstream exons to encode two totally unrelated tumor suppressors.

►cyclin-dependent kinases 4 and 6 and induces cell cycle arrest by negatively regulating Rb (►Retinoblastoma protein, biological and clinical functions) phosphorylation.

2. The *ARF* gene encodes a small basic nucleolar protein: $p14^{ARF}$ in human (132 amino acids, 14 KDa) and $p19^{ARF}$ in mouse (169 amino acids, 19 kDa). $p14^{ARF}$ and its murine homologue $p19^{ARF}$ only share 50% sequence identity. The N-terminus region of ARF (exon 1β) is the most conserved among species and retains the main functions of the protein (cell cycle arrest, nucleolar localization, and MDM2 binding). However, the C-terminal domain of ARF also presents functional domains and is needed for efficient nucleolar localization of $p14^{ARF}$ as well as for interaction with specific partners.

ARF is induced in response to aberrant oncogenic or hyperproliferative signals (►ras), ►E2F, E1A, MYC (►Myc oncogene)…, or DNA damage. The growth suppressive function of ARF mainly depends on its ability to regulate ►p53 stability, but recent data indicate that ARF also exerts p53-independent functions.

p53-Dependent Functions of ARF

The ARF–MDM2–p53 Pathway

The major pathway through which ARF exerts its control on cell cycle progression is currently designed as the ARF–MDM2–p53 pathway (Fig. 2.1). Upon mitogenic activation, ARF is expressed in the nucleolus and shuttles into the nucleoplasm where it directly binds to MDM2, which functions as a negative regulator of p53 through its ►E3-ubiquitine ligase activity. The ARF–MDM2 interaction results in the inhibition of p53 ubiquitination and proteasomal degradation. Accordingly, p53 is stabilized and induces upregulation of antiproliferative genes leading to cell cycle arrest or apoptosis. The exact mechanism whereby ARF stabilizes p53 is still not clear. Initial data suggested that the expression of ARF was correlated with MDM2 delocalization from the nucleus to the nucleolus but ARF can also stabilize p53 without relocating MDM2 into nucleoli. Moreover, additional feedback loops regulate this pathway as p53 is able to modulate its own activity by stimulating MDM2 transcription and repressing ARF (Fig. 2.1).

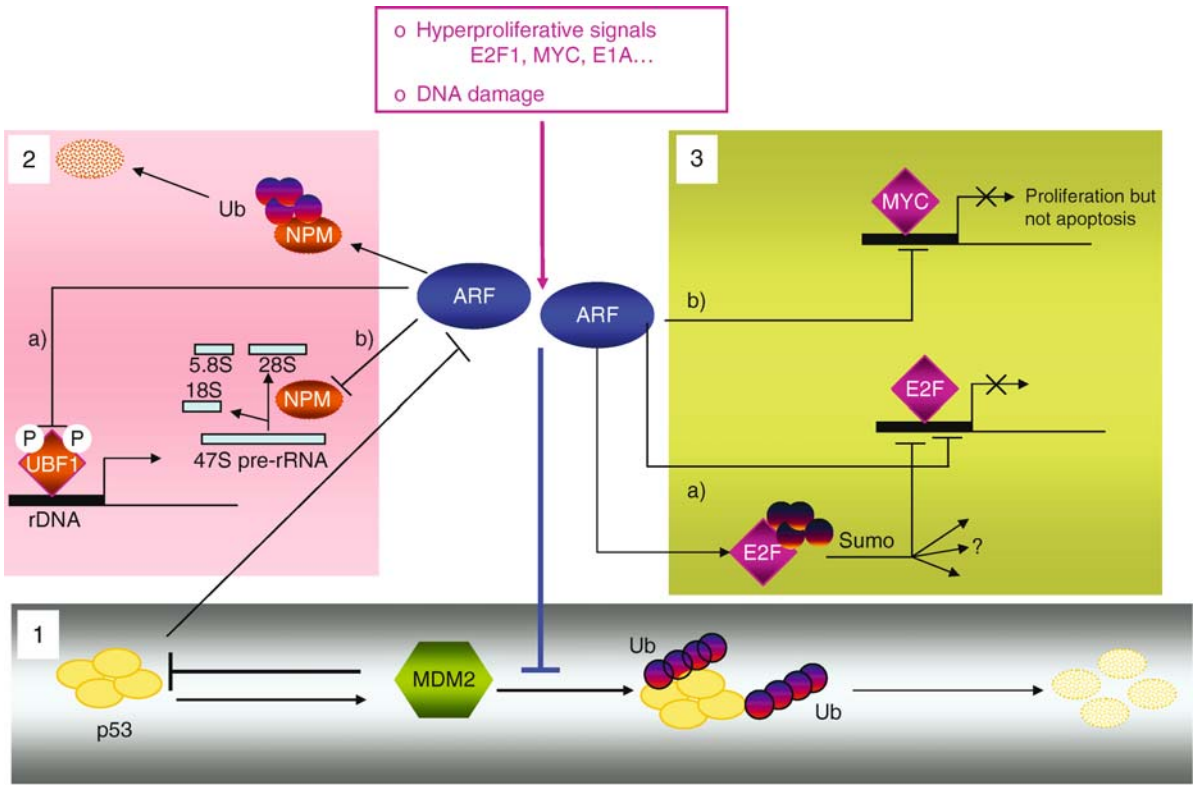
ARF and Replicative Senescence

The induction of $p19^{ARF}$ and p53 by activation of the ARF–MDM2–p53 pathway in mouse embryonic fibroblasts (MEFs) results in premature senescence or apoptosis depending on the cellular context. The alteration of either ARF or p53 function is sufficient to bypass senescence and immortalize MEFs. In contrast, $p14^{ARF}$ expression does not correlate with the onset of senescence in human fibroblasts in which replicative senescence seems to be preferentially mediated by $p16^{INK4a}$.

p53-Independent Functions of ARF

Mice lacking ARF ($ARF^{-/-}$) essentially develop sarcomas and lymphomas and to a lesser extent gliomas. Mice lacking p53 ($p53^{-/-}$) develop with short latency lymphocytic lymphomas but also sarcomas, a phenotype similar to that observed with ARF-null mice. Double ($ARF^{-/-}$ $p53^{-/-}$) or triple knockout (KO) mice ($ARF^{-/-}$, $p53^{-/-}$, $MDM2^{-/-}$) develop with decrease latency a wide spectrum of tumors, suggesting that ARF is involved in alternative pathway(s) to exert its antiproliferative function. Moreover, $p19^{ARF}$ re-expression in triple-KO MEFs is sufficient to induce cell cycle arrest.

The investigation of the p53-independent functions of ARF led to characterize many proteins that physically and/or functionally interact with ARF. The biological relevance of most of these interactions remains unclear, however some of these led to characterize new functions or cellular pathways involving the ARF protein.



ARF Tumor Suppressor Protein. Figure 2 p53-dependent and independent functions of ARF. (1) The ARF–MDM2–p53 pathway (grey frame): ARF binds to MDM2 and exerts a negative control on MDM2-mediated p53 degradation. p53 tightly regulates its own activity by stimulating *MDM2* and repressing *ARF* transcription through a direct binding on their promoters. ARF exerts a negative control on ribosome biogenesis (pink frame). (a) Phosphorylated UBF1 is required for the RNA polymerase I-dependent transcription of the 47S pre-rRNA. Human ARF binds to the transcription factor UBF1 and partially inhibits its phosphorylation, impairing the recruitment of the transcriptional complex on the rDNA promoter. (b) By interfering with the nucleolar protein NPM/B23 within preribosomal particles, ARF induces NPM/B23 degradation and partially reduces 28S rRNA maturation. ARF is a transcriptional regulator (green frame). (a) $p14^{ARF}$ interacts with the transcription factor E2F1. ARF delocalizes E2F1 to the nucleolus and inhibits its transcriptional activity. Recently, ARF has also been shown to enhance the sumoylation of E2F1. The significance of ARF-induced sumoylation is not clear yet, but it could regulate the functions of ARF partners in different ways. (b) When overexpressed, MYC directly associates with the *ARF* promoter and upregulates ARF expression. In turn, ARF associates with MYC and inhibits the transcription of genes required for cell cycle progres.

ARF Negatively Regulates Ribosome Biogenesis

In murine cells, $p19^{ARF}$ has been shown to associate in very high molecular weight complexes with NPM/B23, a multifunctional protein involved in the maturation of the 32S rRNA into 28S rRNA (Fig. 2.2). These complexes are detected in the granular component of nucleolus and correspond to mature preribosomal particles. Given the high amounts of NPM/B23 in the nucleolus when compared with ARF, all ARF molecules are bound to only a fraction of NPM/B23, suggesting that NPM/B23 could sequester ARF into the nucleolus to avoid its binding with some of its nuclear targets. The human homologue $p14^{ARF}$ also colocalizes with NPM/B23 and has been shown to promote its ubiquitination and degradation, resulting in the decrease of mature 28S rRNA levels. The interaction of ARF with NPM/B23 delays rRNA processing and induces a partial cell growth arrest.

Several molecular models connecting the p53-dependent and -independent activities of ARF have recently emerged, based on the consequence of ARF–NPM/B23 interaction on the cellular localization of both partners, and can be summarized as follows: in response to oncogenic insults, ARF is rapidly and highly expressed in nucleoli where it binds to NPM/B23 and interferes with rRNA maturation to inhibit cell growth in a MDM2 and p53-independent manner. Upon DNA damage or other stress signals, ARF shuttles from the nucleolus and is redistributed in the nucleoplasm, where it binds MDM2 to activate the so-called ARF–MDM2–p53 pathway and inhibit cell proliferation.

In addition, recent data indicate that human $p14^{ARF}$ specifically associates with the transcription factor UBF1 and partially inhibits Pol I-dependent transcription of rRNA (Fig. 2.2). UBF1 is hypophosphorylated upon $p14^{ARF}$ expression and unable to recruit the transcriptional complex. These results suggest that ARF could exert a negative regulation of ribosome biogenesis at both transcriptional and posttranscriptional levels through independent pathways. Moreover, human $p14^{ARF}$ has been described to interfere with polysome formation and could in that way complement p53-dependent functions through the negative regulation of protein translation.

ARF and the Control of Transcription

ARF expression can be induced in response to aberrant hyperproliferative signals conveyed by the E2F1 transcription factor. In turn, ARF physically associates with E2F1 and regulates its functions in different ways. Upon ARF expression, E2F1 is delocalized from the nucleus to the nucleolus and its transcription activity is inhibited (Fig. 2.3). Moreover, the presence of ARF is correlated with a dramatic rate of E2F1 proteolysis. Recent data indicate that $p14^{ARF}$, through its interaction with the SUMO-E2 conjugating enzyme can promote the sumoylation of some of its binding partners including E2F1. SUMO is an ubiquitin-like protein that covalently associates to proteins and alters alternatively their stability, subcellular localization, or their function in cell cycle progression. In consequence, $p14^{ARF}$ could diversely regulate its own functions by targeting the sumoylation of its partners.

ARF is also induced upon MYC overexpression, and leads to cell cycle arrest or apoptosis through the stabilization of p53. In turn, ARF has been shown to physically associate with MYC and inhibit some of its functions, characterizing a negative feedback loop, independent of p53 or MDM2 (Fig. 2.3). It is not clear whether this interaction occurs in the nucleolus, suggesting a sequestration of MYC in this compartment, or takes place in the nucleoplasm after ARF exclusion from nucleoli. Anyhow, ARF directly interacts with two functional regions of MYC: the C-terminal domain required for its heterodimerization with MAX, and the amino-terminal transcriptional regulatory (activation or repression) domain.

MYC is a dual transcription factor that regulates genes to either stimulate cell proliferation or induce apoptosis. $p19^{ARF}$ participates in both mechanism and differentially controls MYC transcriptional activity depending on the target genes; the transcription of MYC-responsive genes involved in G1 to S phase progression is dramatically decreased upon $p19^{ARF}$ expression. Chromatin immunoprecipitation experiments suggest that this control of MYC functions could depend on the binding of ARF on selected MYC-responsive promoters. In fact, ARF binding doesn't inhibit the recruitment of MYC–MAX on those promoters, but alters the activation of different cofactors such as TRRAP and TIP 60. These coactivators are responsible for chromatin remodeling through their respective helicase and histone acetyl transferase (HAT) activity. Their association with ARF specifically maintains chromatin in a condensed state and block MYC activation of proliferating genes. In contrast, ARF has not been shown to modify MYC ability to repress transcription or activate apoptosis.

Conclusion

The *INK4a/ARF* locus encodes two unrelated tumor suppressor genes, $p16^{INK4a}$ and ARF, initially considered to regulate cell proliferation through independent pathways:

The first mechanism elucidated, by which ARF inhibits cell proliferation, was its ability to block MDM2-dependent p53 degradation. As the tumor suppressor function of the protein still remained in p53 deficient cells, new partners have been characterized and delineate new modes of action. By interacting with nucleolar proteins like NPM/B23 or UBF1, ARF can limit ribosome biogenesis independently of p53 and MDM2. Moreover, ARF can interact in the nucleoplasm with transcription factors and control the expression of target genes by varied mechanisms such as transcription factor degradation or delocalization, inhibition of promoter binding, inhibition of promoter activity. Interestingly, the control of E2F1 transcriptional activity by ARF characterizes this protein as a regulator of the RB pathway, and delineates a functional connection between the two tumor suppressors encoded by the INK4a/ARF locus.

The emerging notion is that the ability of ARF to control cell proliferation is tightly dependant on its cellular localization. In particular, the pivotal role of NPM/B23 in ARF nucleolar functions should be considered to improve therapeutic strategies restoring a functional p53 pathway.

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Argentaffin Carcinoma

►Carcinoid Tumors

Arginase

Definition

Is a manganese-containing enzyme. The reaction catalyzed by this enzyme is: ►arginine + H_2O → ornithine + urea. It is the final enzyme of the urea cycle.

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

Arginine

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Definition

►Arginine is a "semi-essential" amino acid that is a key component of several metabolic pathways. Excess arginine, however, has been implicated in ►carcinogenesis in animal model systems and also in humans.

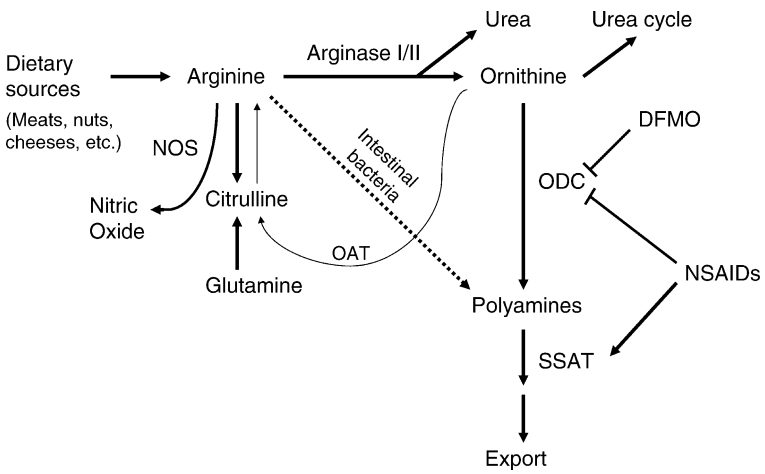
Characteristics

Arginine Metabolism

Arginine is derived from dietary sources and is also synthesized by the kidney. It is considered to be a "semi-essential" amino acid because many conditions affect its synthesis (i.e. sepsis, burns, inborn errors of protein metabolism), resulting in dependence on exogenous arginine supplementation. Dietary sources rich in arginine include all types of meat, nuts, and certain other foods (cheeses, etc.). Arginine is important for protein synthesis, wound healing, spermatogenesis, and it is involved in numerous metabolic pathways. Arginine is well-described as a component of the urea cycle, which is involved in the processing and cellular export of ammonia. L-arginine is a key substrate in the biosynthesis of ►nitric oxide (NO), and the ►polyamines (i.e. putrescine, spermidine, and spermine). These naturally-occurring arginine-derived substances (i.e. nitric oxide and the polyamines) have long been the focus of carcinogenesis research. Thus the majority of investigations on arginine and cancer relate to carcinogenesis research related to these important arginine-derived compounds. Arginine is catabolized primarily by arginase I in the liver for processing of waste via the urea cycle. In the kidney and small bowel, arginase II catabolizes arginine in the mitochondria. A simplified schema for arginine metabolism is provided in (Fig. 1).

Nitric Oxide, Polyamines, Carcinogenesis, and Chemoprevention

Arginine is catabolized by the enzyme nitric oxide synthase (NOS) to form citrulline and nitric oxide (NO).



Arginine. Figure 1 Arginine as the central substrate for polyamine and nitric oxide metabolism. Alternate pathways and inhibitors of these pathways are indicated. NOS, nitric oxide synthase; DFMO, difluoromethylornithine; ODC, ornithine decarboxylase; NSAIDs, non-steroidal anti-inflammatory drugs; SSAT, spermidine/spermine *N*1-acetyltransferase; OAT, ornithine aminotransferase.

Nitric oxide is a signaling molecule with well-defined vasodilatory properties. Three main isoforms of NO synthases exist: NOS1 (neuronal NOS), NOS2 (inducible NOS), and NOS3 (endothelial NOS). Nitric oxide has been implicated in numerous aspects of carcinogenesis. However, nitric oxide has also been observed to inhibit carcinogenesis – thus the biology of nitric oxide is complex, and further research is needed to clarify the role of nitric oxide and NOS in various carcinogenesis models. Nitric oxide has been reported to be involved in cell migration, tumor invasiveness, and angiogenesis in murine mammary adenocarcinoma cell lines. Based on promising experimental data, the field of ►chemoprevention continues to dedicate significant research into inhibitors of inducible-nitric oxide synthase (NOS2). Various NOS2 inhibitors have been developed, and tested in animal carcinogenesis models. For example, selective NOS2 inhibitors have been shown to inhibit colonic aberrant crypt foci (i.e. colorectal adenoma ►preneoplastic lesions) in rat colon carcinoma model systems.

The hepatic arginases convert L-Arginine to ornithine, which is converted directly to putrescine via the rate limiting enzyme of polyamine synthesis: ►ornithine decarboxylase (ODC). In excess, polyamines have been shown to promote tumorigenesis in epithelial tissues in human and murine systems. The field of chemoprevention has long-sought to capture a therapeutic advantage of this finding, through polyamine inhibition via the irreversible ODC-inhibitory agent α-►difluoromethylornithine (DFMO). In experimental studies using murine models, inhibition of polyamines with DFMO suppresses arginine-induced tumorigenesis. In humans, phase IIa clinical biomarker trials of oral DFMO on high-risk populations have demonstrated polyamine inhibition in target tissues (prostate and colorectum). These polyamine-inhibitory effects were sustained during the treatment period (3–12 months), and administration of DFMO resulted in a favorable toxicity profile.

Arginine and Cancer: Experimental Studies

Various cell culture and animal models have demonstrated a carcinogenic role of arginine. In cell culture experiments, viability of malignant cells has been demonstrated to be dependent on arginine supplementation. Normal cells become quiescent in an arginine-deficient medium, whereas malignant cells die. A series of relevant murine and rat studies have been performed demonstrating the effects of dietary arginine intake on model systems of cancer. Rodents treated with L-arginine exhibit increased growth of carcinomas and sarcomas, and treatment with D-arginine inhibits carcinogenesis. Hepatic arginase and arginine deiminase are enzymes that degrade arginine and have been investigated as potential anticancer agents. Experimental results demonstrate that human breast cancer cells have express high levels of arginase. For example, arginase-inhibition results in decreased proliferation of human breast cancer cells. Dietary arginine supplementation has been demonstrated to increase tumor size in familial adenomatous polyposis (FAP) mouse models, and also increase the intestinal tumor number in these mice in a dose-dependent manner. The increase in tumor number was found to be due to an increase in high-grade tumors, in a manner dependent on *Nos2*. This process is inhibited dramatically by treatment with DFMO, various ►non-steroidal anti-inflammatory drugs (NSAIDs, which modulate polyamine metabolism, Fig. 1), or a combination of these agents. Thus an experimental basis for arginine-induced tumorigenesis has been established, and various inhibitory agents and mechanisms are being developed to antagonize this process.

Arginine and Cancer: Epidemiologic Studies

Methods of epidemiologic assessment of arginine intake in cancer research is typically from 24-h food recall or food frequency questionnaires, which collect data on the type and quantity of ingested foods. Further analysis with food composition tables must then be performed to calculate estimated arginine intake for each study participant. Alternatively, serum arginine levels have been tested in epidemiologic studies, and these patient serum levels correlate fairly well with corresponding data from food recall questionnaires.

As mentioned, arginine is primarily derived from meat, and it is estimated that approximately 40% of daily arginine intake comes from meat consumption in Western and European diets. It is a common misunderstanding that high arginine content is restricted to beef. In fact, pork, chicken, various fish, and shellfish have similarly high arginine levels when compared to beef. While a large body of epidemiologic data have associated meat consumption with risk of epithelial cancers (particularly colorectal cancer), the contribution of arginine to this risk is unknown. However, among colorectal cancer patients it has been demonstrated that total dietary arginine intake highly associated with meat consumption quantity, and that meat consumption was associated with poor survival in familial colorectal cancer patients. Such findings implicate potential gene-environment modifying effects between arginine and an unknown genotype(s) among familial colorectal cancer patients – possibly involving the nitric oxide (e.g. *Nos2*) or polyamine synthetic pathway (e.g. *Odc*).

Arginine and Cancer: Clinical Aspects

The putative benefits of arginine restriction on cancer have led to translational applications among cancer patients. However, limited data are available to demonstrate the efficacy and tolerability of such treatments.

Determining the safety of arginine restriction among cancer patients (i.e. as a tertiary cancer prevention

strategy) is of great importance in ►clinical trial development, since these patients often have special nutritional requirements, or cachexia. As noted, many clinical studies have focused on polyamine inhibition through various agents such as DFMO or NSAIDs. Data on the safety and tolerability of arginine restriction among cancer patients is quite limited, however. Interestingly, severe arginine restriction has been prescribed to pediatric patients with gyrate atrophy of the eye (the result of an enzymatic dysfunction leading to excess arginine production and resultant retinal morbidity) without untoward events. Such children are given a prescribed arginine-restricted dietary regimen with amino acid supplementation – which delays disease progression. This dietary regimen is quite stringent and adherence is difficult, but it has demonstrated sustained efficacy in children treated over a period of many years.

Various strategies for arginine restriction have been attempted in the setting of clinical trials, including early-phase clinical trials of pegylated-arginine deiminase (an arginine-degrading enzyme derived from *Mycoplasma*) among hepatocellular carcinoma (HCC) patients. Treatment of HCC patients with arginine deiminase was shown to be well tolerated, despite depletion of plasma arginine levels over a 3-month period, and modest efficacy has been reported. Other investigators have reported responses of various tumors to arginase treatment. Currently, our group at the University of California, Irvine is developing a phase IIa clinical biomarker trial involving arginine restriction among ►colon cancer patients. The goal of this study is to favorably alter surrogate endpoint biomarkers of polyamine metabolism through dietary arginine restriction and oral aspirin therapy. Modest arginine dietary restriction (i.e. ~50% decrease in daily intake) will be prescribed individually to each study participant after a thorough dietary assessment. The customized dietary regimens will be prescribed in a manner that will not restrict total protein intake. Thus the prescribed regimens are not particularly stringent, and are expected to result in good compliance. Safety and tolerability assessments are critical components of this trial, as significant toxicity is not expected.

As more data emerge from clinical trials involving arginine restriction (through various mechanisms) among cancer patients, the optimal delivery methods and management issues will be discovered. However, until then, such approaches remain investigational, and broad-based dietary recommendations directed at cancer patients can not be supported.

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Arginine-Depleting Enzyme Arginine Deiminase

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Synonyms

ADI

Definition

►Arginine deiminase (ADI; EC 3.5.3.6) is an enzyme from prokaryotes that has been used to deplete arginine in the treatment of arginine-requiring ►cancers.

Characteristics

To starve cancer cells through amino acid deprivation can be a strategy in cancer therapy. Specific amino acids are required for the growth of certain tumor cells while normal cells can synthesize sufficient amounts for their own needs. For example, an enzyme called asparaginase has been used to deplete ►asparagine in the treatment of ►acute lymphoblastic leukemia and a few sub-types of non-Hodgkin lymphoma (►Malignant Lymphoma, Hallmarks and Concepts) because these cancers lack asparagine synthetase and are ►auxotrophic for asparagine. Asparaginase degrades both asparagine and glutamine. The antitumor activity of this enzyme is due to its ability to degrade asparagine, and some of its deleterious side effects were due to its degradation of glutamine.

ADI is an enzyme that hydrolyzes arginine to generate energy in many parasitic microorganisms including ►*Mycoplasma arginini* and *Mycoplasma hominis*. This enzyme has potent anticancer activities. Some cancers have an elevated requirement for arginine (Arginine and Cancer), such as metastatic ►melanoma

and ►hepatocellular carcinoma (HCC). These cancer cells are unable to produce arginine and therefore take it from the blood since they need it for their rapid growth. ADI catalyzes the hydrolysis of arginine to citrulline, is currently being used as a chemotherapeutic agent against these arginine-requiring cancers and has gained much attention in recent clinical trials. ADI appears to degrade only arginine and does not appear to metabolize any other amino acids. When administrating ADI to patients with metastatic melanoma or HCC, it destroys the arginine present in the blood, and the cancer cells are thus deprived of their supply, and they cannot grow and eventually die.

Sensitivity of Cancer Cells to ADI-Treatment

Arginine is a ►non-essential amino acid for humans and mice because it can be synthesized from citrulline in two steps via the ►urea cycle enzymes ►argininosuccinate synthetase (ASS) and ►argininosuccinate lyase (ASL). ASS catalyzes the conversion of citrulline and aspartic acid to argininosuccinate. Argininosuccinate is then converted to arginine and fumaric acid by ASL. Some melanomas, HCCs and ►prostate carcinomas (►Prostate Cancer, ►Clinical Oncology) do not express ASS mRNA but do express ASL mRNA. Although it is not known why these cancer cells are unable to express ASS, there is ample evidence that ASS deficiency results in the arginine ►auxotrophy.

The sensitivity of various cell lines to ADI has been reported to be dependent upon the expression of ASS, the rate-limiting enzyme in the conversion of citrulline into arginine. Resistance to ADI-treatment may correlate with cellular ASS activity, allowing cell survival by conversion of the product of the ADI reaction, i.e. citrulline to arginine. Many ASS-positive HCC cell lines are resistant to ADI-treatment, although most require arginine for proliferation. Recombinant human ►arginase has been reported as an arginine-depleting enzyme for killing ASS-positive tumors which expressed ASS, but not ►ornithine transcarbamylase (OTC), the enzyme that converts ornithine, the product of degradation of arginine with recombinant human arginase, to citrulline, which is converted back to arginine via ASS. Recent data suggest that the growth of OTC-deficient HCC tumor cells (ASS-positive and ADI-resistant) in mice is inhibited by treatment with ►pegylated recombinant human arginase, which is in clinical trials and development by Bio-Cancer Treatment International Limited. Arginine deprivation causes many types of tumor cells to die, because they cannot recover or convert urea cycle intermediates into arginine.

Mechanisms of Anti-Tumor Activity of ADI

As a promising enzyme in the treatment of tumors without ASS expression, ADI has shown its anti-proliferative and anti-angiogenic activities in a variety of cancer cells and endothelial cells *in vitro* and *in vivo*. The exact mechanism of its anti-tumor activity remains unclear. To elucidate the mechanisms, many data have been collected. For example, the *Mycoplasma arginini* ADI inhibits the growth of mouse hepatoma cell line MH134 *in vitro*, and its concentration required for 50% growth inhibition (IC_{50}) is about 10 ng/mL. The IC_{50} value of the *Mycoplasma hominis* ADI against the same cell line is about 100 ng/mL. Arginine restores, in a dose-dependent manner, the growth of mouse MH134 hepatoma and Meth A fibrosarcoma cell lines that have been inhibited by ADI, indicating that the tumor cell growth inhibition caused by ADI originates from the depletion of the essential nutrient arginine. ADI is also effective at nanogram quantities per milliliter in Chinese Hamster Ovary (CHO) cells, HeLa cells (human epithelial cells from a fatal cervical carcinoma (►Cervical Cancers)), human T cells and T lymphoblastoid cell lines, but not B-precursor and myeloid cell lines. Renal cell carcinoma (RCC) (►Renal Carcinoma) does not express ASS and is also sensitive to arginine deprivation via ADI. RCC cells treated with ADI showed growth retardation in a dose dependent manner. ADI also exerted *in vivo* anti-proliferative effect on the allografted renal cell carcinoma (RENCA) tumor cells and prolonged the survival of tumor-bearing mice.

ADI may inhibit cell proliferation not only by depletion of arginine, but also by mechanisms involving the cell cycle and death signals. Low concentrations of ADI inhibit proliferation of various cultured cells by arresting the cell cycle in G1- and/or S-phase with higher ADI concentrations leading to subsequent ►apoptosis. For T lymphoblasts, ADI induces apoptotic cell death and cell cycle analysis shows that G1→S transition is blocked in these ADI-treated cells, with increase of apoptotic nuclei in the sub-G1 fraction. Recent data suggest that ADI inhibits proliferation of human leukemia cells more potently than asparaginase by inducing cell cycle arrest and apoptosis. This inhibition of cell proliferation involves cell growth arrest in the G1- and/or S-phase and eventually apoptotic cell death. For human leukemic CEM cells, ADI suppresses expression of c-myc, a potential key regulator of cell proliferation and apoptosis, and increases expression of p27Kip1 cyclin-dependent kinase inhibitor.

Since arginine is involved in several pathways for regulation and maintenance of cellular functions, such as protein synthesis, polyamine synthesis, and ►nitric oxide (NO) production, ADI may modulate these physiological pathways. Human mammary adenocarcinoma (MCF-7) (►Breast Cancer) and human lung carcinoma (A549) (►Metastasis Signaling) cells, express diverse ASS activity which regenerates arginine, have different sensitivity to ADI. In A549 cells, the anti-proliferative activity of ADI might be due to the

inhibition of protein synthesis, but not polyamine synthesis. Recent data suggest that ADI inhibits ►*de novo* synthesis of protein in cells with low ASS activity, but not in cells with high ASS activity, due to the fact that the lack of extracellular arginine in protein synthesis can be replaced by the regenerated arginine via ASS and ASL in the cell. Polyamine synthesis is not affected by ADI, even in cells with no ASS activity. Therefore, inhibitory effect of ADI on the proliferation of ADI-sensitive tumor cells is most likely due to the inhibition of *de novo* protein synthesis.

On the other hand, arginine is the precursor of NO, and the latter modulates ►angiogenesis. ADI is a selective modulator for NO production via inducible (iNOS) and endothelial (eNOS) nitric oxide synthases. Hydrolysis of plasma arginine to citrulline by ADI suppresses lipopolysaccharide-induced NO synthesis. ADI treatment affects tube-like (capillary) formation of human umbilical vein endothelial cells. Inhibition of angiogenesis by ADI is reversed when a surplus of exogenous arginine is provided, indicating that its anti-angiogenic effect (►Antiangiogenesis) is primarily due to arginine depletion. Recent data suggest that acting as an anti-angiogenic agent (►Anti-Angiogenic Drug), ADI inhibits *in vivo* growth of neuroblastomas with unfavorable properties and that these effects are potentiated by simultaneous irradiation. Combination of ADI with irradiation does not increase tumor hypoxia. The anti-proliferative and anti-angiogenic effects of ADI might be a consequence of protein synthesis that involves cell growth and tumorigenesis and polyamine synthesis that involves cell proliferation and differentiation. Due to its two-pronged attack as both an anti-proliferative and an anti-angiogenic agent, ADI may be highly beneficial in cancer therapy.

Clinical Trials

Phoenix Pharmacologics Inc. is developing ADI-PEG-20 (ADI conjugated to polyethylene glycol 20,000 molecular weight), a pegylated ADI for the potential treatment of HCC, for which the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products have granted the drug Orphan Drug status, and melanoma, for which the FDA has also awarded ADI-PEG-20 Orphan Drug status. In addition, ADI-PEG-20 is being investigated for the potential treatment of influenza virus infection and hepatitis C virus infection. The *Mycoplasma hominis* ADI has been mutated and is produced by recombinant technology in E. coli. It is formulated with PEG to decrease its immunogenicity and to increase its circulating half-life *in vivo*.

Reported in 2004, ADI-PEG-20 was used to lower plasma arginine to treat patients with unresectable HCC in phase I/II studies. Pharmacodynamic studies indicated an ADI-PEG-20 dose level of 160 U/m^2 was sufficient to lower plasma arginine from a resting level of ~130 μmol/L to below the level of detection (<2 μmol/L) for more than 7 days. This therapy appeared to be well tolerated, even in patients who had no detectable plasma arginine for three continuous months of therapy. Of the 19 patients enrolled, two had a complete response, seven had a partial response, seven had stable disease, and three had progressive disease. The median survival for the 19 patients enrolled on this study was 410 days.

Reported in 2005, ADI-PEG-20 was also used to treat patients with metastatic melanoma in phase I and II studies. After treatment, plasma arginine levels in individuals with metastatic melanoma were lowered; NO levels also were lowered. There were no grade 3 or 4 toxicities directly attributable to the drug. Six of 24 phase I to II patients responded to treatment (five partial responses and one complete response; 25% response rate) and also had prolonged survival. However, ADI-PEG-20 does have a number of shortcomings. First, ADI is a bacterial enzyme and antigenicity may still be a problem despite ►pegylation. In phase II studies that have been reported, autoantibodies were detected as early as the fifth week and continued to increase with treatment. This may potentially render the drug ineffective on prolonged treatment. Second, ADI converts arginine to citrulline and free ammonia, which could pose problems in patients with cirrhosis liver and hepatic decompensation with further elevation of ammonia levels, leading to prehepatic encephalopathy in man. Third, ADI product citrulline is readily recyclable and rescues cells not only from arginine-free medium but also from arginase-induced deficiency. This has led to the major limitation of ADI: it only kills cancer cells that are ASS deficient. ASS expression has been detected in some tested human tumor biopsy specimens which might cause the tumors to be resistant to ADI therapy.

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Argininosuccinate Lyase

Definition

ASL is an enzyme that starts the reaction in which the amino acid ►arginine is produced from argininosuccinate in the urea cycle.

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

ARK1

►Aurora Kinases

ARK2

►Aurora Kinases

Armadillo Family

Definition

Arm family; a family of proteins that are characterized by a central protein domain that is composed of a series of imperfect 42 amino acid repeats (arm motifs). The family includes the Drosophila protein armadillo and its vertebrate homologues β-catenin and plakoglobin. β-catenin is a structural protein in adherens junctions and has an important signaling function in the APC/β-catenin pathway. Plakoglobin is interchangeable with β-catenin in adherens junctions and is a structural constituent of desmosomes. Plakoglobin may act as a signaling molecule in the ►APC/β-catenin pathway.

►Desmosomes

Armed Viruses

Genetically modified viruses used in ►oncolytic virotherapy that carry therapeutic transgenes such as ►suicide genes or genes encoding immunomodulatory proteins.

►Oncolytic Virotherapy

Aromatase

Definition

Aromatase is a key enzyme converting a precursor into an estrogenic hormone

►Aromatase Inhibitors
►Aromatase and its Inhibitors
►Estrogenic Hormones

Aromatase and its Inhibitors

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Synonyms

Aromatase; Estrogen synthase; CYP 450_{arom}

Definition

Estrogens are involved in numerous physiological processes including the development and maintenance of the female sexual organs, the reproductive cycle, reproduction, and various other neuro-endocrine functions. These ►hormones have crucial roles in certain disease states, particularly in mammary and endometrial hyperplasias and ►breast cancers.

Estrogens are biosynthesized from androgens by the ►cytochrome P450 enzyme complex called "aromatase" first discovered in 1955.

Characteristics

Composition

The aromatase enzyme complex is bound in the endoplasmic reticulum of the cell and is comprised of two major proteins. One protein is cytochrome $P450_{arom}$, a hemoprotein that converts C_{19} steroids (androgens) into C_{18} steroids (estrogens) containing a phenolic A ring. The second protein is NADPH-cytochrome P450 reductase, which transfers reducing equivalents to cytochrome $P450_{arom}$. Three moles of NADPH and three moles of oxygen are used in the conversion of one mole of substrate into one mole of estrogen product.

Distribution and Regulation

In premenopausal women, the enzyme is expressed in the ovarian granulosa cells of large preovulatory follicles and syncytiotrophoblasts of the placenta during pregnancy. Additionally, former theca interna cells of early to mid luteal phase corpus luteum are the major source of aromatase in premenopausal ovaries. Aromatase is not found in pre-pubertal infant ovaries. In addition, aromatase is expressed in a number of other tissues throughout the body. The most important site of non-ovarian estrogen is adipose tissue where production increases with age and is the primary source of circulating estrogen in post-menopausal women. However, aromatase levels in breast tissue have been found to be several fold higher levels that found in plasma. A number of reports indicate that aromatase activity and mRNA is present in normal breast tissue and breast tumors. Approximately, 60% of the ►breast cancers express aromatase activity. Although gonadal aromatase is regulated by follicle stimulating hormone, aromatase in extra-gonadal sites is regulated by other factors such as glucocorticoids, cAMP and prostaglandin E_2. Thus, in postmenopausal women, estrogen synthesis is independent of pituitary-ovary feedback regulation. Furthermore, the expression of aromatase is highest in or near breast tumor sites.

Expression

Human aromatase is encoded by CYP19, a single-copy gene on 15q21.2 and is expressed in the endoplasmic reticulum. CYP19 comprises ten exons, with exons II through X encoding the open reading frame of aromatase. At least nine different first exons are known to encode unique 5′-untranslated regions of aromatase mRNA. Each first exon has its own upstream promoter region. First exons and corresponding promoters are used alternatively in a tissue or cell-specific manner, enabling tissue or cell-specific regulation of aromatase. For example, the most proximal promoters, PII and I.3, are used predominantly for the gonads, whereas promoter 1f drives brain-specific transcription of aromatase, and promoter I.4 is used for adipose tissue and skin. The most distal promoter, I.1, located 100 kb upstream of exon II, is used almost exclusively for the placenta. Each promoter displays different *cis*-elements to which cell specific transcription factors bind. Accordingly, tissue or cell-specific expression and regulation of aromatase is realized by the level of promoter selection and by the cell-specific profiles of transcription factors. The first step toward understanding the regulation of aromatase expression in a specific tissue is to elucidate the associated promoter use.

Aromatization Reaction

Aromatization of androgens to estrogens is last in the series of reactions in steroid biosynthesis is the rate limiting step for estrogens synthesis. Therefore, there are no steroids normally produced downstream to be affected by inhibition of aromatase. Aromatization of androstenedione, the preferred substrate, occurs via three successive oxidation steps, with the first two being hydroxylations of the angular C_{19} methyl group and characteristic of P450 hydroxylations. The final oxidation step involves aromatization of the A ring of the steroid molecule and loss of the C_{19} carbon atom as formic acid. Although the mechanism of the third and last step remains unclear, it is thought to involve nucleophilic attack of the 19-aldehyde by the reduced ferrous peroxy intermediate to produce a peroxo hemiacetal that decays as a consequence of *cis* elimination of the 1β-hydrogen by the proximal oxygen atom and results in aromatization of the A ring of the steroid and formic acid release. Analysis of the reduced ferrous peroxy intermediate indicates that the 1β-hydrogen removal by the proximal oxygen of the peroxo hemiacetal intermediate encounters a high energetic barrier (>60 kcal/mol) that is enzymatically inaccessible. Furthermore, the resulting species do not directly fragment to the experimentally observed formic acid and aromatized steroid products. It has been reported that steroid models that contain the 2,3-enol moiety have a markedly lower barrier for 1β-hydrogen atom abstraction (<7 kcal/mol) due to the ability of the enolized A ring to delocalize the impending radical. Formation of the 2,3-enol appears to be necessary as transition states containing the 2,3-enol moiety and the 19-gem diol decay directly to the aromatized product, formic acid, and the aqua-bound model cytochrome P450 enzyme. Analysis of the reaction vectors indicate that the second hydrogen transfer occurs with a concerted, nonsynchronous mechanism without an energetic barrier. Thus, the final catalytic step of aromatase appears to involve the cytochrome P450 oxene intermediate, 1β-hydrogen atom abstraction, and release of formic acid. Although aromatase shares common features with the other P450

enzymes the unique characteristics of the aromatization reaction, involving loss of the C19 carbon and conversion of steroidal A ring to an aromatic ring provide the opportunity to develop inhibitors selective for $P450_{arom}$. The importance of selective inhibition is that potent inhibitors bind to the target enzyme with high affinity. Because only low concentrations of the drug are required to suppress the enzyme, interactions with other enzymes is unlikely to occur. For example, 11β-hydroxylase mediates the synthesis of the adrenal steroid cortisol and is inhibited along with aromatase and other P450 enzymes by general inhibitors of steroid biosynthesis, such as aminoglutethimide. This compound was used in breast cancer treatment in the 1960s, initially to produce medical adrenalectomies but later was used to inhibit aromatase in conjunction with cortisol replacement. However, because of its lack of selectivity, this compound is a relatively weak aromatase inhibitor and causes a number of toxicities in patients.

Since aromatase has both an iron-containing and a steroid-binding site there are two possible ways inhibitors may interact with the enzyme. Aromatase inhibitors have been traditionally divided into the two classes of Type I and Type II inhibitors.

Type I Inhibitors of Aromatase

Type I or mechanism based inhibitors of aromatase include steroidal structural analogs of the substrate Δ4A. These are competitive inhibitors of the enzyme androstenedione and interact with the substrate binding site of the enzyme. This leads to covalent bond formation with the nucleophilic site of the enzyme leading to irreversible enzyme inhibition. Since, the inhibitor binds irreversibly to the target site, a new enzyme molecule must be produced for estrogen synthesis to occur.

Formestane

First selective aromatase inhibitor: Investigations on the development of aromatase inhibitor began in 1970s and have expanded greatly in the past three decades. The initial approach taken to develop the first selective AI was to design substrate analogs based on the structure of Δ4A. These inhibitors have chemical substituents at various positions on the steroid nucleus. Modifications at C4 have produced several effective inhibitors including 4-OHA (4-hydroxyandrostenedione). Other structural modifications can be made on the B-ring of the steroid nucleus.

Following initial ►clinical trials in the 1980s, 4-OHA was introduced into the market in 1993 as the first selective inhibitor under the name formestane (LENTARON® Ciba-Geigy, now Novartis) with the indication for the treatment of advanced breast cancer in postmenopausal women. In clinical trials, weekly deep intramuscular injections with 500 mg of formestane to unselected breast cancer patients resulted in a 60% suppression of plasma ►estradiol levels and an overall response rate of almost 30%. Similar responses, obtained with a daily oral administration of 500 mg, were however indicative of a lower bioavailability by this route.

Exemestane

Subsequently AIs with improved oral activity were developed. This resulted in exemestane (Aromasin®) which received US FDA approval in 1999 for treatment of advanced breast cancer.

Type II Inhibitors of Aromatase

Non-Steroidal Reversible Inhibitors. Type II inhibitors are reversible inhibitors of aromatase and include nitrogen containing compounds such as letrozole (Femara) and anastrozole (Arimidex). The nitrogen in these compounds reacts and forms a co-ordinate bond with the heme atom of the P450 enzyme complex. These inhibitors are based on known P450 enzyme inhibitors, such as ketoconazole and are more likely to inhibit multiple P450 enzymes in addition to aromatase. Type II inhibitors can have lower specificity than type I inhibitors, for example Type II inhibitors also include aminoglutethimide which inhibits 11β-hydroxylase and cortisol production. However, the newer, third generation AIs such as letrozole and anastrozole exhibit a great degree of specificity for aromatase, are very potent and have good bioavailability. In post-menopausal women, letrozole decreases plasma concentration of estradiol, estrone and estrone sulfate by 75–95% from baseline with maximal suppression achieved within 2–3 days of treatment initiation. At clinically used dosage, letrozole does not impair adrenal synthesis of glucocorticoids or aldosterone.

In 1998, letrozole was approved for the treatment of advanced breast cancer in post-menopausal women with hormone receptor positive or unknown breast cancer who had failed prior ►tamoxifen treatment (second-line). In 2001, FDA approved letrozole for first-line treatment of postmenopausal women with hormone receptor positive locally advanced or metastatic breast cancer.

Applications and Indications

AIs are approved by US-FDA for the treatment of post-menopausal hormone dependent (►ER positive or unknown) as a first-line treatment and after tamoxifen relapse. Recent reports of the MA-17 and IES clinical trials suggest that AIs are effective in early breast cancer following tamoxifen. Based on data from these and other multiple, large randomized trials, it was recommended by the American Association of Clinical Oncology (ASCO) technology assessment panel that optimal ►adjuvant hormonal ►therapy for a postmenopausal woman with receptor-positive breast cancer include an aromatase inhibitor as initial therapy or after treatment with tamoxifen. Clinical data shows advantage of AI over

tamoxifen for as long as 10 years. AIs are well tolerated with less gynecological symptoms than tamoxifen. A low incidence of bone toxicity and muculoskeletal effects are associated with AI compared to tamoxifen.

Some of the newer studies also show AIs may have advantages over clomiphene citrate for ovarian stimulation (ovulation induction).

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Aromatic Amine

Definition

Is an amine (-NH_2, -NH-) with an ►aromatic hydrocarbon substituent, whose structure usually contains one or more benzene rings (►benzene and leukemia). ►Aniline is an example. Acetylation of aromatic amines by ►Arylamine N-Acetyltransferases is an important ►bioactivation pathway of many drugs including those involved in ►carcinogen metabolism.

►Carcinogen Metabolism

Aromatic Hydrocarbon

Definition

A hydrocarbon that contains one or more benzene rings that are characteristic of the benzene series of organic compounds.

►Benzene and Leukemia
►Polycyclic Aromatic Hydorcarbons
►Arsenic

Arp2/3

Definition

A seven-subunit protein involved in the regulation of actin filament assembly; ►E-Cadherin.

►E-Cadherin

Arp2/3 Complex

Definition

Actin-related protein 2/3 complex is a protein complex consisting of seven subunits that is responsible for initiating actin polymerization in cells. It is localized within ►lamellipodia and provides the driving force for lamellipodia extension during cell ►motility.

►Cortactin

Array-based Comparative Genomic Hybridization

►ArrayCGH

ArrayCGH

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Synonyms

Array-based comparative genomic hybridization

Definition

Array-based comparative genomic ►hybridization (array CGH) is a technique to assay the genome for identifying chromosomal segments with copy number alterations. This technique utilizes printed microarrays (►Microarray (cDNA) technology) to perform a simultaneous analysis of selected genomic regions or even

the entire genome. These arrays can be based on printed genomic material (►BACs), cDNAs, or ►oligonucleotide fragments and are analyzed by the same apparatus and software used for cDNA microarray analysis.

Characteristics

DNA sequence copy number changes have been shown to play an important role in the pathogenesis of cancer. Array CGH is a further development of CGH, which was originally used to detect ►chromosomal imbalances. CGH allowed the detection of chromosomal copy number changes in cell and tissue samples but, unfortunately, conventional CGH has a low resolution. This problem has been overcome by the introduction of array-based CGH (array CGH). This technique is based on differential labeling of a test sample and reference DNA that are cohybridized on a slide with several thousand DNA clones representing specific regions of the human genome.

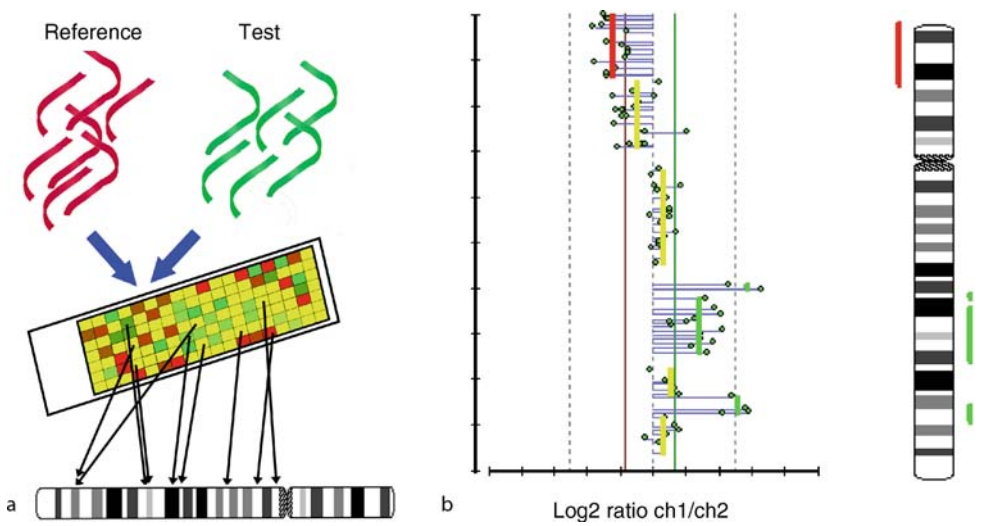
Since chromosomal copy numbers cannot be measured directly, two samples of genomic DNA (reference and test DNAs) are differentially labeled with fluorescent dyes and competitively hybridized to known mapped sequences spotted onto a slide (Fig. 1a). Because the fluorescence intensity is related to the copy number of single clones, the ratio of intensity between test and reference samples will be null in cases of equal amount of fluorescent signal or either negative or positive in cases of low copy number or high copy number of test DNA clones, respectively. The fluorescence intensity ratio for the labeled DNA populations is computed and a fluorescence profile is generated for each chromosome according to the physical position of their corresponding probe on the genome (Fig. 1b). By this method, array CGH can provide improved quantitative accuracy, resolution, and dynamic range compared to conventional CGH, and the measurements can be referenced directly to the positions on the genome sequence.

The difficulty in obtaining the correct result depends on many factors. The types of aberration, for example, strongly influences the array CGH analysis. It is much easier to detect large increases/decreases in copy number due to ►amplifications/►deletions of large genomic regions than single copy gains and losses of affecting small genomic DNA fragments. Heterogeneity of cells in the tumor sample, for example, the presence of normal cells within tissues, and different protocols used for tissue fixation can cause problems. Obviously, another important factor is the type of array CGH technology used providing different results in terms of reproducibility and resolution with different abilities to correctly identify genomic DNA gains or losses.

There are many different types of probes used to generate slides for array CGH analysis. The platforms initially developed used mainly BAC, ►YAC, or ►PAC clones. Recently, synthetic oligonucleotides have been introduced as an alternative substrate for array CGH, and today this approach seems to offer the highest resolution.

Clinical Applications

Chromosomal alterations represent an important feature of tumors that, up to now, have not been investigated exhaustively due to the low resolution of conventional array methods. Compared to microscopic chromosomal cytogenetic techniques, array CGH analyses provide



ArrayCGH. Figure 1 (a) Two DNA samples, isolated from "test" and "reference" cells, are labelled with two different dyes and competitively hybridized to genomic clanes which are spotted onto a slide. (b) The fluorescent signal intensity ratios, measured at each array spot, are normalized and a fluorescent profile is generated for each chromosome.

many advantages because they have the present in tumor cells. For this reason, the role of array-based CGH technology in cancer research is to detect new rearrangements or genomic alterations important for neoplastic transformation and to provide a more accurate diagnosis of cancer. In fact, a more accurate diagnosis of genomic alterations could be of patient's benefit because pathologists and clinicians can better classify and define each single tumor providing a more effective and specific approach to patient care. A wide spectrum analysis could provide important information for a more accurate prognosis as well as helping to predict the outcome to a specific cancer treatment.

Array CGH could also be useful for analyzing not only chromosomal rearrangement but also ►epigenetic alterations, which are another important feature of tumor cells. Hypermethylation of CpG islands in promoter regions is known to be associated with silencing of ►tumor suppressor genes, whilst conversely, ►hypomethylation events are associated with ►oncogene activation. For this reason, a technique that provides an analysis of the vast majority of ►methylation alterations present in tumor cells could be very useful to clarify the mechanism of tumor transformation. Using array CGH methods, it is possible to check the methylation status of genomic DNA combining CpG-island array and differential methylation hybridization techniques (DMH).

Finally, crucial information can be obtained by the ability of array CGH to detect focal homozygous deletions in regions of frequent heterozygous deletions or loss of heterozygosity because these alterations can provide important information to detect specific tumor suppressor genes. Unfortunately, alterations that do not change copy number cannot be detected by this technique. However, the development of array CGH analyses based on ►single nucleotide polymorphism (SNP) profiling could override this problem being an important tool for tumor characterization.

The main limitation for array CGH to be used in clinical environment is the high price of array CGH platforms that do not permit a rapid diffusion in laboratory and clinical practices. However, in the next few years it is possible to predict an increment of this technique and a parallel decrease of cost per sample with a larger diffusion in diagnostic applications and standardization of different protocols and applications.

►Amplification
►Aneuploidy
►Epigenetics
►Hypomethylation
►Methylation
►Microarray (cDNA) Technology
►Oncogene
►Tumor Suppressor Genes

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Arrhenoblastoma

Definition

Arrhenoblastoma is a rare cancer of the ovaries. The cancer cells produce and release high level of a male sex hormone testosterone, which may cause the women to develop male physical characteristics, including facial hair and a deep voice. While the tumor can occur at any age, it develops most often in young adults.

►Sertoli-Leydig Cell Tumor

Arsenic

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Definition

►Arsenic is a "trace element" that is found naturally in the environment. A "trace element" refers to chemical elements present or required in minute quantities. Arsenic (As) is considered to be an important factor in human health. Humans are exposed to arsenic through the air, drinking water, and food. No documented evidence exists that would support a beneficial biological function or documented clinical deficiency in humans. Arsenic has been associated with cancer as far back as 1887, and current evidence suggests a positive correlation between arsenic exposure and increased risk for developing various cancers, especially ►skin cancer.

Characteristics

Arsenic is a metalloid that is found in various oxidation states, depending on the pH and the presence of oxidizing and reducing substances. Arsenite (As^{3+}) and arsenate (As^{5+}) forms are the main forms of arsenic found in drinking water.

Arsenic compounds are classified as inorganic or organic arsenic. Inorganic arsenic is usually combined with oxygen, chlorine, or sulfur, whereas organic arsenic is found in combination with carbon and hydrogen. The inorganic forms of arsenic are more toxic compared to the organic forms. The trivalent forms of arsenic are more toxic and react with thiol groups. On the other hand, the pentavalent forms are less toxic but uncouple cellular oxidative phosphorylation.

The odorless and tasteless properties of most arsenic compounds make them attractive poisons. Arsenic has been called the Poison of Kings and the King of Poisons because of its use by the ruling class to murder one another and its potency and discreetness. One of the most notorious poisoner in the nineteenth century was Goeie Mie ("Good Mary") of Leiden, The Netherlands, who poisoned 102 friends and relatives by giving them As_2O_3 in hot milk after opening life insurance policies in their names. Arsenic acts by disrupting ATP production ultimately leading to death due to multisystem organ failure. Physiologically, both As^{3+} and As^{5+} bind to sulfhydryl groups, but As^{3+} has about a tenfold greater affinity for binding than As^{5+}. Arsenic tends to accumulate in the skin, hair, oral mucosa, and esophagus probably because it can bind with sulfhydryl groups of keratin commonly found in these tissues. In addition, arsenic can be found at high levels in tissues such as hair, nails, skin and lungs, which are rich in cysteine groups.

History of Use

Arsenic compounds were mined by the early Chinese, Greek, and Egyptian civilizations and were used for medicinal purposes, including treatment of syphilis and arthritis. The compound arsenic was probably first identified and isolated by Albertus Magnus (Albert the Great, 1193–1280), a German alchemist, in 1250. Arsenic was mixed with vinegar and chalk and eaten or rubbed on their faces and arms by women during the Victorian era to improve their complexion. In 1878, Dr. Thomas Fowler created "Fowler's solution," a combination of arsenic and potassium bicarbonate. "Fowler's solution" was used to treat leukemia and Hodgkin's disease, but also eczema, psoriasis, and asthma. In 1910, Nobel laureate Paul Ehrlich developed salvarsan (organic arsenical) that was used to treat syphilis and trypanosomiasis (sleeping sickness). Arsenic was used to treat leukemia until the 1970s at which time its use in Western countries was almost totally discontinued due to the advent of more effective chemotherapy and radiotherapy in addition to its perceived toxicity and potential carcinogenicity. However, the clinical use of arsenic has experienced a major resurgence. Arsenic trioxide is now considered to be an effective treatment for relapsed and/or refractory ►acute promyelocytic leukemia (APL) and has been approved by the FDA for general treatment of APL.

Paradox of Arsenic

As^{3+} and its monomethylated and dimethylated derivatives are associated with risk for cancers of the skin, ►lung, ►bladder, kidney, and/or ►liver. Thus arsenic is a well-established human ►carcinogen, but paradoxically, arsenic trioxide is also an extremely valuable therapeutic tool for treating hematological malignancies, including various leukemias and ►multiple myeloma. In particular, arsenic has been very useful in treating relapsed or all-trans-►retinoic acid (ATRA)-resistant APL patients. Arsenic has also been reported as effective against ►neuroblastoma, ►esophageal carcinoma, ►gastric cancer, ►hepatocellular carcinoma, head and neck cancer, ►cervical cancer, ►prostate cancer, ►transitional cell cancer, ►glioblastoma, ►renal cell carcinoma, and ►breast cancer.

This creates a paradox for which no unified agreement has yet been reached regarding the molecular mechanisms that determine whether arsenic will act as a carcinogen or as an effectual chemotherapeutic agent. The suggestion has been made that high doses of arsenic result in toxicity, whereas lower doses induce cellular differentiation. Another key element contributing to the puzzle has been the nonresponsiveness of animal models to tumorigenesis induced by oral administration of arsenic alone. This suggests that arsenic may act to enhance mutagenicity induced by other carcinogens or it may function as a cocarcinogen, acting by inhibiting repair of carcinogen induced DNA damage. Furthermore, a diverse sensitivity to arsenic exists among individuals and this variation may be related to differences in metabolism. The metabolism of arsenic in humans occurs through alternating steps of reduction and methylation. Cellular glutathione (GSH) can reduce arsenate (As^{5+}) nonenzymatically, but evidence also indicates that arsenate reductase may catalyze the GSH reduction of As^{5+} to As^{3+}. As^{3+} is then methylated to form monomethylarsonic acid (MMA), which is further methylated to dimethylarsenic acid (DMA). The relative contributions of these metabolites to the toxicity and ►carcinogenesis of arsenic have not yet been fully elucidated.

Mechanisms of Action – Cancer Causing Effects of Arsenic

The precise mechanisms of arsenic's cancer-causing effects have been elusive most likely because arsenic's

effects are enigmatic. Arsenic has not been shown conclusively to be an initiating or a promoting agent of carcinogenesis in animals, thus making its classification as a carcinogen difficult, and in contrast to classic tumor promoting agents, its effects are not reversible. In addition, the majority of scientific evidence does not suggest that arsenic is a mutagen in vivo, although it has been shown to interact with DNA to cause damage. Arsenic's ability to contribute to carcinogenesis has been suggested to be associated primarily with genotoxicity (chromosome abnormalities), ►oxidative stress, and alteration of cellular ►signal transduction pathways.

Genotoxicity

Exposure to arsenic compounds can result in the induction of chromosomal abnormalities, including micronuclei formation, deletions, sister chromatid exchanges, and aneuploidy in both humans and animals. Arsenic, and especially the methylated trivalent form, seems to have potent clastogenic activity (i.e., causes breaks in chromosomes). Some suggest that whether arsenic acts as a clastogen or an aneugen (i.e., agent that affects cell division and the mitotic spindle apparatus resulting in the loss or gain of whole chromosomes, thereby inducing an aneuploidy) is related to dose (i.e., high dose = clastogen). Arsenite appears to induce gene amplification at the *dhfr* locus in both human and animal cells. Arsenite can also increase telomerase activity to promote cellular proliferation.

Possible Involvement of Reactive Oxygen Species (ROS)

►Reactive oxygen species, such as superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radical, might be involved in both the initiation and promotional stages of carcinogenesis. Low levels of ROS can act as second messengers to mediate gene expression, whereas high levels are suggested to result in cell damage and death. ROS causes damage through lipid peroxidation, modification of DNA including base-pair mutations, rearrangements, deletions, insertions, and sequence amplifications. ROS have been reported to be involved in ►cell transformation signaling. ROS can damage DNA and proteins both directly and indirectly. Exposure to arsenic results in the induction of oxidative stress-associated enzymes such as heme oxygenase and NADPH oxidase in various human and animal cell lines. Induction of these enzymes appears to increase production of ROS, which can cause DNA damage. Elevated levels of characteristic DNA adducts (i.e., 8-hydroxy-2′-deoxyguanosine) associated with oxidative injury have also been observed in various tissues that are common sites of arsenic-induced carcinogenesis. Because arsenic has a high affinity for the thiol groups of proteins, GSH levels and the activity of various antioxidants and ROS scavenging proteins all contribute to a protective effect against arsenic-induced production of ROS. Arsenite has been reported to suppress excision and ligation thereby inhibiting the DNA repair process. It might directly interfere with DNA ligase activity or indirectly interfere through ROS production.

Aberrations in Signal Transduction

The toxic effects of arsenic are very likely related to arsenic's ability to induce or impair various signal transduction pathways. In particular, arsenic may act as a carcinogen by activating pathways associated with proliferation to induce cell transformation or tumor development or it may act as a chemotherapeutic agent by inducing death (►apoptosis) of tumor cells. Arsenic is reported to increase tyrosine phosphorylation of receptor tyrosine kinases (i.e., ►epidermal growth factor receptor) and nonreceptor tyrosine kinases (i.e., Src), which is associated with abnormal cell signaling leading to uncontrolled cell growth and cancer. Arsenic has been reported to stimulate activator protein-1 (►AP-1) and to either activate or suppress nuclear factor kappa B (NFkappaB). AP-1 and NFkappaB are transcription factors that induce the transcription of genes known to have a major role in determining whether cell transformation or apoptosis will occur. Arsenic clearly induces either transformation or apoptosis in many cell types and the carcinogenic and anticarcinogenic actions of arsenic could very well share a common molecular mechanism that is related to length of arsenic exposure (e.g., chronic vs. acute), level of exposure (high dose vs. low dose), and/or species of arsenic exposure (e.g., arsenite, arsenate, MMA, DMA). Based on these assumptions, the possibility exists that the key to arsenic's actions is the extent to which it disrupts the normal control of apoptosis through its influence on signaling pathways, including the ►mitogen-activated protein kinases (MAPKs) that can lead to activation of AP-1 or NFkappaB.

Arsenic as a Therapeutic Agent

APL is a relatively rare malignancy characterized by a chromosomal translocation t(15;17), which fuses the retinoid acid receptor (RAR)α on chromosome 17 to the promyelocytic leukemia (PML) gene on chromosome 15. The PML–RARα fusion gene and the resulting protein product, PML–RARα, are thought to be responsible for APL pathogenesis. The addition of ATRA to chemotherapy has proven to be an effective treatment. Importantly, arsenic trioxide is now considered to be an important and effective treatment for relapsed and/or refractory APL patients. Similar to ATRA, arsenic trioxide appears to act by inducing differentiation and apoptosis in APL cells expressing the PML fusion protein

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Arsenic Trioxide

Definition

As_2O_3 is a secondary material processed from the ore of arsenic bearing rocks. It is a highly toxic chemical substance, which is not only readily absorbed by the digestive system, but also absorbed through inhalation and skin contact. In Chinese medicine, arsenic trioxide is used externally as a caustic to remove dead tissue for the treatment of furuncle, carbuncle, ulcer, scrofula, and hemorrhoid. For internal use, it is used in minute quantities as an antiasthmatic agent. Although arsenic trioxide has been shown to be a human carcinogen, it is also a chemotherapeutic drug with the efficacy to induce ►apoptosis in ►cancer cells. Arsenic trioxide has been approved by the United States to treat ►acute promyelocytic leukemia that is unresponsive to the first line therapy or recurrent after treatment. It is also being studied in the treatment of other types of cancer, such as ►multiple myeloma.

►Chinese versus Western Medicine

Aryl Hydrocarbon Receptor

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Synonyms

Dioxin receptor; Xenobiotic receptor

Definition

The Aryl Hydrocarbon Receptor is a protein that recognizes a variety of usually plane hydrophobic ►xenobiotics, as well as a few endogenous compounds. Upon activation, the AhR triggers a signaling and transcriptional program leading to adaptive cellular processes and toxic effects.

Characteristics

Historical Perspective

Xenobiotic Stress

Exposure of cells and organisms to foreign compounds called ►xenobiotics leads to an adaptive response, which consists in the induction of a variety of xenobiotic metabolizing enzymes (XMEs), ultimately leading to the metabolization, ►detoxification , and elimination of these putative toxic compounds. In analogy with other cellular stresses, this adaptive response could be termed xenobiotic stress. It is best illustrated in the case of hydrophobic and plane compounds such as polyaromatic hydrocarbons (PAH, for example ►benzopyrenes) or halogenated polyaromatic hydrocarbons (►dioxins), which trigger a similar response and induce a subset of xenobiotic metabolizing enzymes as well as specific transporters. Xenobiotic metabolizing enzymes are classified as phase 1 enzymes such as ►cytochromes P450 (CYP), which add a reactive chemical function (hydroxyl, epoxyde) to the xenobiotic and phase 2 enzymes, usually transferases that conjugate an organic hydrophylic group (glucuronate, ►gluthathione, etc.) to the reactive function. This metabolic pathway renders these hydrophobic compounds less toxic, more hydrophilic, and therefore facilitates their elimination. This pathway can be involved in ►carcinogen metabolism. In addition to the metabolic aspects, a critical parameter of xenobiotic stress is the recognition of the xenobiotics and the induction of the genes encoding phase 1 and phase 2 XMEs and transporters. Genetic and biochemical studies using a variety of cell lines that are more or less sensitive to dioxin and PAHs allowed the identification of their receptor, the Aryl Hydrocarbon Receptor. Other similar studies allowed the determination of the other components of this signaling pathway which was initially viewed as a response to the exposure to the toxic PAH and dioxin-like compounds.

Physiological Effects

In addition to its function as a detector of toxic xenobiotics, there have been considerable speculations about possible physiological functions of the AhR that have been supported by a large body of observations. First, several nontoxic plant-derived molecules such as indolic compounds, ►flavonoids, and ►polyphenols were found to be potent ligands of the AhR. Furthermore, endogenous compounds such as tryptophan derivatives, bilirubin, and sterols were also described as ligands of the AhR. A critical argument in favor of a physiological role of this receptor came from the analysis of the phenotype of AhR−/− ►knockout mice. These AhR-deficient mice exhibited a number of defects, among which small liver

size, probably due to a congenital vascular defect, alterations in thymus and cardiovascular development, as well as decreased fertility. While these defects may be due to a deficiency in cellular modulators such as ►retinoic acid or various growth factors and ►cytokines, they point to a function of the AhR in cellular differentiation and development. Such functions are supported by observations made in nonvertebrate organisms. Drosophila, the AhR orthologs are involved in antenna and leg morphogenesis and, in *Caenorhabditis elegans*, the AhR ortholog appears to be required for the differentiation and migration of GABAergic neurons. In these species, there is no evidence for the involvement of AhR orthologs in xenobiotic detection. In conclusion, the control of cell fate, cell proliferation and migration is a likely endogenous function of the AhR and AhR orthologs.

A Link Between Environment and Cancer?

Most of the studies devoted to the AhR have focused on its function as a mediator of its ligands toxicity. The most studied ligand is the prototypical dioxin: 2,3,7,8-tetrachloro-dibenzoparadioxin, TCDD, Dioxin) since it is widely accepted that most, if not all effects of this pollutant, are related to the activation of the AhR. Dioxins are usually present in the environment as a mix of chlorinated congeners. Sevetyfive possible congeners have been identified and their toxicity is systematically expressed in relation to that of Dioxin by means of a "Toxic Equivalent" quantification factor (TEQ). Dioxin's toxic effects are numerous and include teratogenicity, immunosuppression, metabolic and endocrine disruption, skin toxicity such as chloracne and keratosis and cancer. Dioxin is considered by the ►IARC as a human carcinogen. This conclusion is mostly based on its mechanism of action and on animal studies and therefore relies primarily on the ability of dioxin to activate the AhR. Dioxin displays a carcinogenic effect on a wide variety of tissues and organs. In rodents, most studies have focused on liver carcinogenesis in which dioxin behaves as a cancer promoter. In humans, according to epidemiological studies, it is believed that dioxin is a relatively weak nonspecific carcinogen that could mildly increase the risk to develop a variety of tumors such as non-Hodgkin lymphomas, sarcomas, and breast cancer. The difference between the sensitivity of the various species to the carcinogenic effect of Dioxin (high in some rodent species, mild in human) correlates well with the affinity of the AhR for Dioxin in these species. Furthermore, AhR−/− knockout mice are resistant to the carcinogenic effects of Dioxin. In addition, overexpression of a constitutively active AhR in mice leads to the development of stomach and liver cancers. All these observations establish a firm link between the carcinogenicity of dioxin and the activation of the AhR. Other carcinogens also exert their effects at least partially through the AhR. Benzo(a)pyrene, a PAH present in tobacco smoke and Diesel particles also binds and activates the AhR. Although the mechanisms of its carcinogenicity are distinct from those of dioxin, it has been shown that AhR−/− mice are resistant to benzo(a) pyrene toxicity.

Authors have also searched for evidence of the implication of the AhR in cancer independently of its ligand. One such evidence came from mice overexpresssing a constitutively active AhR, which develop stomach and liver cancers, suggesting that AhR activity is sufficient for such a toxic effect. Conversely, ►immortalized mouse embryo fibroblasts carrying deleted AhR genes exhibited less tumorigenicity than wild type fibroblasts in xenograft mouse models. The other evidence was provided by the examination of *AhR* gene polymorphisms in various human cancers leading, in particular, to the finding that one polymorphism adversely affects survival in patients suffering from soft tissue sarcoma.

The Signaling Pathway

The AhR is localized in the cytoplasm of most cells and is associated with a number of ►chaperone proteins such as ►Hsp90, p23, and XAP, in a conformation exhibiting a high affinity for Dioxin. Upon ligand binding, this complex is sequentially dissociated and the AhR is translocated into the nucleus where it forms a heterodimer with a transcription factor called AhR Nuclear Translocator (ARNT). AhR and ARNT have similar structures including a DNA binding and heterodimerization b►HLH domain and a PAS domain that also contributes to heterodimerization and, only in the case of the AhR, harbors the ligand-binding pocket. The heterodimer then binds to specific DNA responsive sequences, interacts with coactivators and other transcription factors, alters the chromatin structure, and regulates transcription of the target genes. The classical target sequence is called XRE (Xenobiotic Responsive Element); however, alternative sequences called XRE II have been identified. In addition to this classical gene regulation pathway, a number of other AhR signaling pathways have been described including the interaction between this receptor and the transcription factor ►NFkB, the hypophosphorylated ►retinoblastoma protein and the corepressor SMRT and the ►estrogen receptor are the ►progesterone receptor. Furthermore, the AhR has been shown to activate, with rapid or long-term kinetics, several protein kinases such as ►Src, p38 ►MAPK, and Jun kinase. These pathways may be relevant for the carcinogenic effects of several AhR ligands.

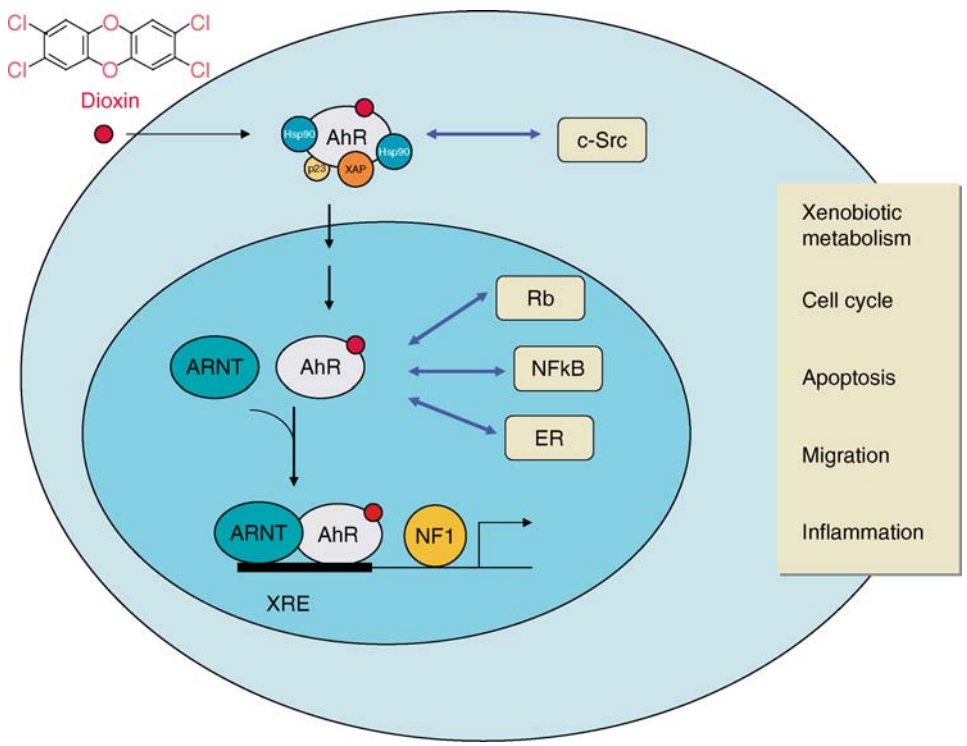
Gene Expression and Biological Pathways

Initial studies on the AhR have focused on its adaptive and putative toxic functions related to the induction of xenobiotic metabolism. The so-called AhR gene battery includes mostly XMEs such CYP1 proteins, glutathione – S transferase (GST) and UDP-glucuronosyl transferase

(UGT). Further biological and large scale genomic studies have shown that the AhR also regulates a number of genes unrelated to xenobiotic stress. Targets of the AhR include proteins involved in cell cycle control such as p27, p21, cJun, SOS1, in cell signaling such as HES1 and IGFBP-1, in cell–cell interaction and migration such as HEF1, ►E-cadherin, and ►MMPs. Some of these regulations have not yet been firmly linked to a biological or toxic effect. These observations suggest that AhR ligands may exert part of their biological or toxic effects independently of the xenobiotic metabolism pathway. Ligands of the AhR (or the AhR alone) could alter cell cycle control, cell fate determination and ►migration. Furthermore, a number of cytokines have been shown to be the target of the activated AhR, which establishes a link between this receptor and inflammation. In the mouse ovary, the AhR is involved in the regulation of aromatase gene expression, which adds another crosstalk between the AhR and hormonal effects. It should be noted that many of these regulations by the AhR could be tissue specific. Repression of some genes has also been reported but was less well characterized. For example, the antagonism between the AhR and estrogen signaling in breast tissue could account for the repression of the pS2 gene.

Mechanisms of Carcinogenicity of AhR Ligands

Different mechanisms could account for the carcinogenicity of the AhR ligands. In the case of PAH such as benzo(a)pyrene, it has been clearly shown that the metabolism of these compounds by the CYP enzymes generates highly reactive metabolites that display ►DNA damage and strong mutagenicity. Phase 2 enzymes detoxify these intermediate metabolites. The balance between phase 1 and phase 2 enzyme activities is maintained through the coordinate regulation of the expression of their genes by the AhR pathway and the ►Nrf2-►oxidative stress signaling pathway. Evidence from both human and animal studies suggests that the xenobiotic metabolizing pathways are protective as long as the fine-tuning between the different enzymes is maintained. ►Genotoxicity could result from an unbalance between the enzymes of the successive phases. The mechanisms of



Aryl Hydrocarbon Receptor. Figure 1 Signaling pathways and functions of the AhR. The AhR is localized in the cytoplasm and interacts with several chaperones such as Hsp90, p23 and XAP. Upon binding to dioxin, the complex dissociates sequentially, the AhR enters the nucleus, interacts with ARNT and the heterodimer binds to target sequences called XREs. The active receptor interacts with transcription factors such as NF1 and activates the transcription of target genes. In addition to this classical pathway, signaling through the AhR could also be mediated through interaction with other regulatory proteins such as c-Src, Rb, NFkB and the ER. Some of the main cellular functions controlled by the AhR are listed to the right. These pathways and functions could contribute to the role of the AhR during the various steps of carcinogenesis.

carcinogenicity of dioxin are distinct from those of PAHs. Indeed, dioxin is not metabolized by the XMEs, which partially accounts for its long half-life (seven years in human). Furthermore, dioxin is not genotoxic. However, the induction of CYP enzymes leads to oxidative stress and therefore to a possible indirect toxicity. Dioxin is considered as a potent cancer promoter, at least in rodents. Such an effect could be mediated by the modification of the gene expression program following AhR activation. The genes that are possibly responsible are those involved in proliferation. However, dioxin also induces several genes implicated in ►apoptosis. To explain this paradoxical effect, it has been suggested that dioxin could promote the proliferation of clones which exhibit resistance to apoptosis and cell cycle arrest. Several other mechanisms could account for the cancer promotion activity of dioxin: increase in ►oxidative stress and ►inflammation, activation of tyrosine kinases such as ►Src, complex interaction with the estrogen signaling pathway. Furthermore, cancer progression could also be stimulated by AhR ligands, since it was shown that the activation of this receptor leads to an increase in cell mobility and plasticity which could be mediated by a number of target gene products.

AhR as a Pharmacological Target

Because of its implication in several pathological processes, AhR was considered as a relevant pharmacological target. Several antagonists, including including plant ►polyphenols or endogenous compounds such as 7-keto-cholesterol, were identified. Other compounds named SahRMs (Selective AhR Modulators) were found to modulate the activity of the AhR in that they prevented the induction of XMEs but did not affect the antagonism between the AhR and the estrogen receptor, a property that is relevant for breast cancer therapy. Several dietary plant constituents have been shown to be activators of both the AhR and the ►Nrf-2 signaling pathways offering potential applications in ►chemoprevention. More generally, pharmacological studies of the AhR have shown that this receptor displays a considerable diversity in its response to different types of ligands, each type selectively activating an overlapping set of pathways and genes. Thus, the signals initiated by pollutants such as dioxin and PAHs and by polyphenols or other dietary plant compounds appears to be distinct. This diversity of the response that has yet to be characterized at a structural and molecular level constitutes the grounds for further pharmacological investigation of this receptor.

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Aryl sulfatase C

►Steroid Sulfatase

Arylamine

Definition

Aromatic amine.

Arylamine *N*-Acetyltransferases

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Synonyms

NAT

Definition

The arylamine *N*-acetyltransferases (NATs; EC 2.3.1.5) are ►phase II enzymes that catalyze the transfer of an acetyl group from acetyl coenzyme A to ►aromatic amine, ►heterocyclic amine or hydrazine substrates. Acetylation catalyzed by NATs is an important biotransformation pathway for many drugs and cancer causing agents that we are exposed to on a daily basis.

Characteristics

There are three human NAT genes. Two encode functional proteins and are designated *NAT1* and *NAT2*, and the third is a pseudogene (*NATP1*) that encodes a truncated nonfunctional protein. All are located on the short arm of chromosome 8 and have been mapped to 8p21.3–23.1, a region commonly deleted in human cancers. Both functional NATs are encoded by single intronless exons and the protein-coding regions share an 87% nucleotide homology and are 870 base pairs in length. NAT1 and NAT2 are cytosolic proteins having an approximate molecular mass of 33 kDa and each consists of 290 amino acids, sharing an 81% homology. NAT1 is ubiquitously expressed and has been identified in both fetal and adult tissues, while NAT2 expression is only evident approximately 12 months after birth and is restricted to the liver and gut. The active site of NAT enzymes contains the catalytic triad cysteine-histidine-aspartate, which is similar to that of the cysteine protease superfamily of proteins.

The importance of exposure to aromatic and heterocyclic amines present in cigarette smoke, car exhaust fumes, and foodstuffs in the etiology of certain toxicities including cancer is well established. Like most chemical carcinogens, arylamines require ►bioactivation in order to exert their oncogenic effects. ►Carcinogen metabolism is a complex process and involves competing reactions that can lead to either ►detoxification or bioactivation. The NATs are versatile enzymes that are able to catalyze both *N*-acetylation and *O*-acetylation reactions. Generally, *N*-acetylation is a detoxification step that produces non-toxic stable *N*-acetates that can be eliminated from the body. An exception is *N*-acetylation of the bladder carcinogen ►benzidine, which is part of the bioactivation process. Bioactivation of arylamines involves an initial *N*-oxidation reaction catalyzed predominantly by ►cytochrome P450 1A2, but also by prostaglandin H synthase and myeloperoxidase. The resultant *N*-hydroxy arylamines and/or their nitroso intermediates are able to react with cellular macromolecules, but subsequent *O*-acetylation by NATs yields highly reactive *N*-acetoxy esters (electrophiles) which can form covalent adducts with nucleophiles such as DNA and protein. ►Adducts to DNA are an essential step in the initiation of ►chemical carcinogenesis.

NATs acetylate a number of important carcinogens. Some compounds, such as the carcinogenic aromatic amines 2-aminofluorene, benzidine, 4-aminobiphenyl, 4,4-dichloroaniline and 2-naphthylamine, and the ►food-derived heterocyclic amines 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,4-dimethyl-imidazo[4,5-*f*]quinoxaline (MeIQx) are *N*-acetylated to varying degrees by both NATs. Generally, *O*-acetylation of the *N*-hydroxy metabolites of carbocyclic arylamines is catalyzed selectively by NAT1, whereas NAT2 *O*-acetylates *N*-hydroxy metabolites of the dietary heterocyclic amine carcinogens.

Acetylation Polymorphism

The acetylation ►polymorphism was one of the first described examples of a ►pharmacogenetic defect affecting ►xenobiotic biotransformation capacity in human populations, and was discovered following the introduction of isoniazid therapy for the treatment of tuberculosis during the 1950s. Since the human *NAT2* locus was established as the site of the acetylation polymorphism, the study of *NAT2* allelic variation has been an area of intense investigation. To date, 36 different *NAT2* alleles have been detected in human populations. Each of the variant alleles is comprised of between 1 and 4 ►SNPs, of which 16 have been identified, located in the protein-coding region of the gene. The correlation between NAT2 ►genotype and ►phenotype is well established. Moreover, there is a gene-dosage effect. Individuals who are homozygous for slow *NAT2* alleles have a slow acetylator phenotype, individuals heterozygous for slow *NAT2* alleles have an intermediate acetylator phenotype, and individuals who lack slow *NAT2* alleles have a rapid acetylator phenotype. Historically, NAT1 was thought to be genetically invariant and referred to as "monomorphic." However, wide inter-individual variability in NAT1 activities was suggestive of a genetic polymorphism. The first reported allelic variation at the *NAT1* locus was in 1993, and marked the beginning of a systematic survey of *NAT1* genotypes. To date, 26 different *NAT1* alleles have been detected in human populations, but only a small number of these alter phenotype. The frequency of slow acetylator alleles for NAT1 is low. The most common NAT1 variant in caucasians is *NAT1*10*, which reportedly results in a rapid acetylator phenotype. Both NAT1 and NAT2 show considerable interethnic variability. In Caucasian and African populations, the frequency of the NAT2 slow acetylation phenotype varies between 40 and 70%, while that of Asian populations, such as Japanese, Chinese, Korean, and Thai, range from 10 to 30%.

Clinical Relevance

Because of the role of acetylation in the metabolic activation and detoxification of arylamine and heterocyclic amine carcinogens, acetylator polymorphisms can modify cancer risk associated with chemical exposures. Unlike the relatively rare but highly penetrant genes involved in familial cancers, those genes responsible for biotransformation polymorphisms have low penetrance and cause only a moderate increase in cancer risk. Nevertheless, their widespread occurrence in the general population suggests they are a significant contributor to individual risk. Many ►cancer epidemiology studies have reported associations between

acetylator status and risk of bladder, colon, breast, head and neck, lung, and ►prostate cancer. However, inconsistent reports have meant that the relationship between phenotype and risk remains unclear. For example, several studies have implicated the rapid phenotype as an increased risk factor for ►colon cancer, whereas others have been unable to confirm this finding. Geographical differences, ethnicity, lack of study power, dietary differences and differences in other risk factors between study groups have been suggested as reasons for variable results from independent studies. Recent reports suggesting that NAT activity may be altered by environmental factors and substrate-dependent down-regulation also may explain why inconsistent associations have been seen. When acetylator phenotype has been linked to carcinogen exposure, more consistent results have been reported. For example, the rapid phenotype has emerged as a strong risk factor for colon cancer in those individuals who have a higher exposure to the food-derived heterocyclic amines. Similarly, the association between slow acetylator status and urinary ►bladder cancer is more consistently observed when exposure is taken into consideration. Individual risks associated with NAT phenotypes are small, but increase when other susceptibility genes and carcinogen exposures are included in the analysis.

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Asbestos

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Definition

Asbestos is a commercial term for a group of crystalline silicates that are composed of long thin fibers. Formerly, the noncombustible mineral was incorporated into a great variety of industrial and building materials. However, asbestos can be easily broken into tiny microscopic fibers and inhaled. In the lungs, the indestructible fibers may, over time, increase in size due to acquisition of ►ferritin/hemosiderin from proximal dying macrophages. After many years, the inhaled fibers can be carcinogenic. Thus commercial use of asbestos has been banned.

Characteristics

Carcinogenic Action of Iron

During the past seventy years, authors of scores of clinical and laboratory studies have reported that one of the many dangers of excessive/misplaced iron is its ability to initiate and to promote neoplastic cell growth. Initiation occurs via iron-catalyzed formation of reactive oxygen radicals (►Reactive oxygen species; ►Oxydative stress) that, when generated in close proximity to DNA, can cause point mutations, cross linking, and DNA strand breaks (►DNA damage). Promotion of neoplastic cell growth by iron occurs via its ability to suppress the tumorcidal action of macrophages and to serve as an essential nutrient for unrestricted cancer cell growth.

Excessive iron enters the body by inhalation, ingestion, or, less often, by injection. Misplaced iron most commonly occurs in conditions of erythrocyte or hepatocyte destruction. A frequently reported example of the association of iron loading with tissue site-specific neoplasia is the development of respiratory tract cancers in humans and animals who have inhaled iron compounds. Items that routinely serve as vehicles for inhaled iron include tobacco smoke (►Tobacco carcinogenesis; Smoking addiction), iron-contaminated coal dust and sand, urban and subway air particulates, iron dross released in mining and industrial processing of ferriferous materials, and, not least, iron-containing asbestos fibers.

Asbestos: A Vehicle for Iron

Asbestos is a commercial term employed for a group of crystalline silicate fibers. The group is divided into amphibole and serpentine configurations. Examples of the former include ►crocidolite, amosite, and ►tremolite; of the latter, ►chrysotile. Crocidolite, amosite, and green tremolite contain high levels of iron and are much more carcinogenic than chrysotile which contains very little iron. Inhalation of carcinogenic forms of asbestos can result, after many years, in carcinoma of lungs (►Lung cancer), esophagus (►Esophageal cancer), and stomach (►Gastric cancer) as well as ►mesothelioma of pleura, peritoneum, and pericardium. In cell cultures and in animal models, strong iron chelators (►Chelators as anticancer drugs) as well as oxygen radical scavengers decrease the toxicity of asbestos.

Following phagocytosis of inhaled iron-containing asbestos by alveolar ►macrophages, iron is leaked (mobilized) over time into low molecular mass pools. Cytotoxicity of asbestos samples is directly proportional

to the amount of mobilized iron. Asbestos fibers not only can release iron but also can acquire the metal as a deposit on their surfaces. The deposit consists of ferritin/hemosiderin derived from proximal decaying macrophages.

Iron coated fibers are termed ►ferruginous bodies. In one study, for example, crocidolite, amosite, or chrysotile injected into rat pleura bound, respectively, 240, 135, or 25 nmol Fe/mg. Accumulation of ferruginous bodies on macrophage cell membranes may kill the defense cells. Moreover, the metal can become catalytically active to result in further oxidant damage.

►Erionite, a ►zeolite silicate of aluminum, calcium, and magnesium, contains no iron but is far more able to cause human mesothelioma than can either crocidolite or amosite. Erionite has an internal cage-like surface area up to fifty-fold greater than crocidolite. Thus when inhaled it acquires a very large quantity of iron. Unlike native erionite, the iron laden fibers catalyze single strand DNA breaks. Destruction of DNA by iron-erionite is prevented by iron chelators.

The iron-dependent mutagenicity of asbestos has been verified by treatment of Chinese hamster cells with crocidolite. A doubling of mutation rate occurred. The increase could be prevented by use of iron-free medium with crocidolite samples from which redox active iron had been removed by the iron chelator, ►deferoxamine (Chelators as anticancer drugs).

Asbestos: Usage and Banishment

For many decades, asbestos was incorporated in such products as brake pads and linings, cement pipes and shingles, cigarette filters, fireproof gloves, flooring and roofing materials, gas masks, hot pipe coverings, and sound proofing material. Although the principal type of asbestos employed was chrysotile, particular samples were contaminated with varying amounts of iron. The elevated risk of carcinomas and mesotheliomas occurred not only in miners and fabricators of iron-contaminated asbestos but also in members of their families as well as in persons who lived downwind from the mines and factories.

Villagers and rural inhabitants who live near asbestos outcroppings likewise are at serious risk of respiratory tract cancers and mesotheliomas. Additional persons at risk include installers of products that contain iron-contaminated asbestos as well as users of those products in which asbestos leaking has developed.

Presently, if building materials are leaking asbestos, the product should be removed by trained biohazard specialists. The latter wear protective masks and clothing. If clothing items are to be reused, they must be laundered carefully in a commercial cleaning establishment rather than in the homes of the biohazard workers.

It has been estimated that, in the U.S., at least eleven million persons have had occupational exposure to asbestos between 1940 and 1979, of whom 2000 die each year of mesothelioma. Since 1979, in the U.S., the commercial use of asbestos in most applications has been banned.

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ASK1

Definition

Apoptosis signal-regulating kinase 1 an upstream kinase of ►Jun N-terminal kinase (JNK). A serine/threonine-specific protein kinase involved in initiating → ►apoptosis. Member of the mitogen-activated protein kinase kinase kinase family (MAPKKK).

►p21(Waf1/Cip1/Sdi1)
►MAP Kinases
►Daxx

Askanazy

Definition

Max Askanazy. German pathologist born 1865-died 1940. He was the first to describe the follicular oncocytic cells of the thyroid in 1898 also known as Hurthle cells.

►Hurthle Cell Adenoma and Carcinoma

Askin Tumor

Definition

Ewing sarcoma or peripheral primitive neuroectodermal tumor of the chest wall.

►Ewing Sarcoma

Asparagine

Definition

Asparagine is one of the 20 most common natural amino acids on Earth. It is considered a non-essential amino acid.

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

Aspiration Cytology

►Fine Needle Aspiration Biopsy

Aspirin

Definition

Trade name from Bayer of acetylsalicylic acid, which belongs to the class of nonsteroidal antiinflammatory drugs (NSAIDS), in some countries used as a generic name.

►Arachidonic Acid Pathway
►Acetylsalicylic Acid

Asrocytoma

Definition

A member of he group of brain tumors. Asrocytomas may arise a any site of the CNS, usually manifest clinically in adults, have a wide range of histopathological features and biological behaviors, show diffuse infiltration of adjacent and distant brain structures and have an inherent tendency to progress to more malignant phenotypes. Oligoastrocytomas.

Assessment of Anaplasia of a Tumor

►Grading of Tumors

Assessment of the Degree of Tumor Differentiation

►Adherens Junctions

Association Study

Definition

A type of case-control study in which the exposure variable is a particular allele of a candidate susceptibility gene. The frequency of the particular allele is measured in a group of patients and compared with the frequency in an ethnically matched control group.

►Cancer Epidemiology
►Epidemiology of Cancer

Aster

Definition

A star-shaped cluster of microtubules radiating from the polar microtubule organizing center at the start of mitosis.

►Paclitaxel

Asthma

►Allergy

Astrocyte

Definition

A cell type found in the central nervous system with radiating star-like processes, the function of which is to surround and support neurons. Astrocyte is the predominant type of glial cell in the brain; it is generally

considered to provide a supportive function to neurons, a structural component to the blood-brain-barrier, contribution to vascular tone, and modulation of synaptic function and intercellular signaling.

►Pannexins
►Oligodendroglioma

Astrocytoma

Definition

Astrocytoma is the collective term for a class of tumors derived from astrocytic precursors.

►Multistep Development
►Brain Tumors
►Oligodendroglioma
►Oligoastrocytomas

Asymmetric Cell Division

Definition

Asymmetric cell division is a mitotic event that produces two daughter cells with different developmental potentials.

►Stem Cell Markers
►Mitosis

Asymmetric Cytokinesis

Definition

►Asymmetric Cell Division.

Ataxia Telangiectasia

Definition

AT is an autosomal recessive human disorder. The gene maps to 11q22. AT is a multisystem disease characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, radiosensitivity, predisposition to lymphoid malignancies and immunodeficiency with defects in both cellular and humoral immunity. Ataxia is the presenting symptom and is usually recognized at the age of 12–14 months, the patient is confined to a wheelchair before adolescence. Telangiectasia has a later onset, usually is observed between 2 and 8 years of age; telangiectasias can affect the eyes, the ears and the butterfly area of the face. The major debilitating features are the progressive neurological abnormalities and immunodeficiency. Chromosome instability with apparently random breaks is a characteristic cytogenetic feature, and it is a common notion that the genomic instability in AT is a major cause of cancer in AT patients, in keeping with the well-established link between cancer and genomic rearrangements.

►TCL1
►ATM Protein

Ataxia-telangiectasia Mutated (ATM)

►ATM Protein

Ataxia-telangiectasia Variant 1 and Variant 2

►Nijmegen Breakage Syndrome

ATF2

Definition

ATF2 is a subfamily of transcription factors that belongs to the transcription factor complex called activator protein 1 (►AP-1).

►JNK Subfamily

ATF6

Definition

Activating transcription factor 6 (ATF6) is an endoplasmic reticulum (ER) transmembrane activating transcription factor. Upon ER stress ATF6α and ATF6β transit to the Golgi compartment where they are cleaved by S1P and S2P proteases to yield a cytosolic fragment. The free ATF6 fragment migrates to the nucleus to activate transcription.

Atheromatous Plaques

Definition

Reduce the interior diameter of the arteries and gradually obstruct them. Atheroma is a fatty deposit (►cholesterol). The combination of cholesterol, cells, and calcium eventually results in the formation of atheromatous plaques on the artery walls. The process of atheroma development within an individual is called atherogenesis, and the overall result of the disease process is termed atherosclerosis.

Atherosclerosis

Definition

Disease condition where plaques are formed by lipids inside the artery.

►Grape Seed Extract

ATL

Definition

►Adult T-Cell Leukemia.

ATM

Definition

ATM Gene.

ATM Protein

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Synonyms

Ataxia-telangiectasia mutated (ATM)

Definition

ATM is a large protein kinase that is the primary activator of the cellular responses to double strand breaks (DSBs) in the DNA – a complex network of signaling pathways. ATM is activated in response to DSB induction and in turn phosphorylates a multitude of substrates, each of which is a key player in a DNA damage response pathway. ATM is missing or inactive in patients with the multisystem genetic disorder ataxia telangiectasia (A-T), characterized by neurodegeneration, immune deficiency, genomic instability, sensitivity to ionizing radiation, and cancer predisposition. The cellular phenotype of cells from A-T patients includes premature senescence, chromosomal breakage, extreme sensitivity to ionizing radiation and radiomimetic chemicals, and defective activation of the extensive array of DSB responses, most notably the activation of the cell-cycle checkpoints, which temporarily halt the cell cycle at specific stages while the DNA damage is assessed. The complex and broad manifestations of ATM deficiency demonstrate the importance of the DNA damage response in maintaining cellular homeostasis.

Characteristics

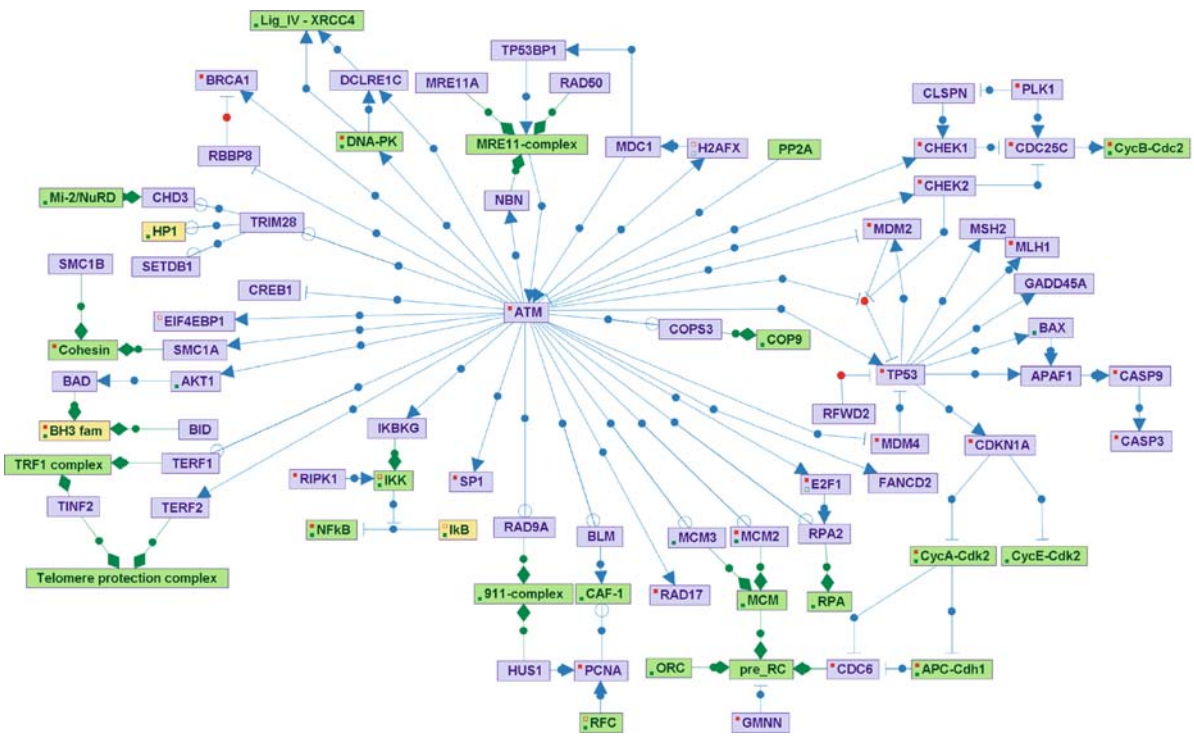
ATM is a large, heavily phosphorylated protein of 370 kDa containing 3,056 amino acids. Its most prominent motif is a carboxy-terminal region of about 350 amino acids that is similar to the catalytic subunit of phosphatidylinositol 3-kinases (PI3-kinases). The large size and PI 3-kinase-related region are common to a family of proteins identified in organisms ranging from yeast to mammals, which are involved in maintaining genomic stability, cell cycle control and responses to genotoxic stress. Most members of this protein family were found to have a serine/threonine protein kinase

activity, i.e., they phosphorylate other proteins on specific serine or threonine residues. A notable member of this group is the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), which is activated by DNA ends. The mammalian PI3-kinase-like protein kinase most closely related to ATM is the ATR protein, which responds primarily to UV damage and stalled replication forks, but in response to DSBs phosphorylates many of ATM substrates, albeit at later time points and slower pace compared to ATM.

Cellular Functions and Molecular Regulation

ATM is primarily a nuclear protein, with a possible minute cytoplasmic fraction. In unprovoked cells, ATM exists as dormant homodimers. Following DSB induction, ATM is rapidly activated in a process that involves its autophosphorylation, followed by release of highly active ATM monomers. ATM activation may be mediated by chromatin reorganization following DNA damage, and requires the activity of several DSB sensors such as the trimolecular complex MRN (Mre11-Rad50-Nbs1). The number of ATM downstream effectors is currently estimated at several hundred. Some of them are shown in Fig. 1. Among them are the tumor suppressor proteins p53 and BRCA1.

Mouse strains lacking the Atm protein were generated by targeted inactivation of their *Atm* gene, the murine *ATM* homolog. These animals recapitulate the human disease, with radiosensitivity and a striking propensity to thymic lymphomas, but they do not show the profound cerebellar degeneration typical of human patients. ATM-deficient mice, like A-T patients, are sterile as a result of extensive chromosomal fragmentation during meiotic recombination. Indeed, in normal mice, the ATM protein is associated with the synapsed chromosomal axes during normal meiosis, pointing to its involvement in meiotic recombination. This indicates that ATM is involved not only in damage responses, but also in responding to DNA breaks that take place during normal physiological processes. Another such process is the maturation of the immune system genes by somatic recombination. The marked immunodeficiency of A-T patients, as well as the appearance of chromosomal translocations involving these genes in



ATM Protein. Figure 1 A partial map of the ATM-mediated network. The map was drawn using a computational software. It contain nodes representing three biological entities: gene/proteins (violet nodes); protein complexes (green nodes, e.g., the MRN complex); and gene families (yellow nodes, e.g., the IκB family of NF-κB inhibitors). The map contains two types of edges: ►Blue edges represent regulations and interactions between genes/proteins. Arrowheads (→) correspond to activation, T-shaped edges (—|) represent inhibition, and open circle edges (—○) denote regulations with yet unknown effect. Blue lines with no head represent physical interactions between proteins. ►Green edges represent containment relations between nodes (e.g., the relationships between a complex and its components). Red and green dots within a node indicate that not all the regulation and containment relations stored in the database of ATM functions for that node are shown on the map.

patient lymphocytes, points to the possible involvement of ATM in this process as well.

Many of the numerous stress responses controlled by ATM end up in activation or down-regulation of gene expression. A global look at gene expression profiles in the cell can be obtained using ►microarray (cDNA) technology, a tool that measures the extent of expression of thousands of genes at the same time. This technique disclosed that ATM is involved in the majority of alterations in gene expression that follow treatments with ionizing radiation or radiomimetic chemicals. This finding further underscores the central role of ATM as a master controller of the DNA damage response.

Clinical Relevance

The clinical manifestation of ATM inactivation is A-T, a genetic disease characterized by a perplexing array of clinical symptoms and cellular defects. The phenotype attests to the centrality of ATM-controlled cellular pathways to the development and proper function of many tissues. The striking predisposition of A-T patients to lymphoid cancers (►lymphoma) clearly shows that ATM controls an important intersection of functions necessary to prevent the development of malignancy in these cells. It is a common notion that the genomic instability in A-T is a major cause of cancer in A-T patients, in keeping with the well-established link between cancer and genomic rearrangements.

It has long been suspected that carriers of A-T mutations bear a certain degree of predisposition to malignancies, primarily breast cancer. This notion stemmed primarily from epidemiological observations and is continuously being re-examined using molecular assays for the detection of carriers of ATM mutations. Evidence is accumulating that some degree of cancer predisposition may be conferred by heterozygosity for specific types of ATM mutations. Since cells from A-T carriers exhibit a moderate degree of radiation sensitivity, heterozygosity for ATM mutations might also lead to adverse side-effects of radiotherapy, such as severe local responses to treatment or radiation-induced secondary cancers.

A further link between ATM sequence alterations and cancer comes from another line of research: In certain hematopoietic malignancies, most notably T-prolymphocytic leukemia, both copies of the ATM gene are inactivated due to somatic mutations and rearrangements. This phenomenon is typical of tumor suppressor genes and points to the role of ATM-dependent processes in guarding mammalian cells from malignant transformation.

Genomic instability is at the heart of cancer development, and inherited genomic instability is directly associated with predisposition to various forms of cancer. On the other hand, because many radiotherapy and chemotherapy agents for cancer induce DNA damage, understanding the DNA damage response also impacts on the refinement of those therapies and may eventually lead to novel treatment modalities for cancer.

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Atopic Dermatitis

►Allergy

Atopic Disorders

Definition

IgE mediated immediate-type hypersensitivity reactions.

►Allergy

Atopy

►Allergy

Atorvastatin

Definition

Member of the ►statins family.

►All-Trans-Retinoic Acid (ATRA)
►Acute Promyelocytic Leukemia (APL)

ATP

Definition

Adenosine triphosphate is produced as an energy source during the processes of photosynthesis and cellular respiration and consumed by many enzymes and a multitude of cellular processes including biosynthetic reactions, motility, and cell division.

ATP-Binding Cassette Transporters

Definition

Are membrane transporters that mediate the ATP-dependent translocation of structurally diverse endogenous and xenobiotic substances and their metabolites across biological membranes. Based on amino acid sequence similarity and phylogeny, the 48 human ABC transporters are grouped into seven subfamilies, termed A–G. Several ABC transporters are important drug efflux pumps overexpressed in drug-resistant cells and contribute to multidrug resistance. Mutations in genes encoding ABC transporters cause a number of human diseases and disorders, including cystic fibrosis, retinal degradation, and cholesterol and bile salt transport defects.

►Membrane Transporters
►ABC Drug Transporters
►Alkylating Agents

ATPase

Definition

ATPase is a protein that catalyzes the conversion of ATP into ADP and free phosphate to yield energy to drive an energetically unfavourable reaction. Most membrane proteins posses this ATPase capability.

►Glutathione Conjugate Transporter RLIP76
►Molecular Chaperones

ATR

Definition

Ataxia telangiectasia and Rad3-related; acronym for a checkpoint kinase (AT- and Rad3-related) that is homologous to the ►ATM protein mutated in Ataxia telangiectasia and the yeast RAD3 kinase. Activation of ATR is a proximal step in the cellular mechanisms that sense and signal DNA damage. ATR is primarily involved in sensing and signaling the presence of stalled replication forks. The gene maps to 3q22-24.

►Fragile Histidine Triad
►Fragile Sites
►Replication Factories and Foci

Atrasentan

Definition

A small molecule antagonist of ET-RA currently in clinical trials for the treatment of hypertension and prostate cancer.

►Endothelins
►Prostate Cancer, Clinical Oncology

Attenuated Adenomatous Polyposis Coli

►APC Gene in Familial Adenomatous Polyposis

Attributable Fraction

Definition

The attributable fraction is the proportion of disease occurrence that can be potentially avoided if exposure was prevented.

►Tobacco-Related Cancers

Attributable Risk

Definition

Proportion of a cancer that can be attributed to a risk factor (to be interpreted with caution).

►Cancer Epidemiology

Attrition

Definition

Attrition is a term referring to loss of compounds in the entire drug discovery and development process. As a rough approximation across all drug targets the attrition is 75% preclinically and 90% clinically.

►ADMET Screen

Atypia

Definition

Atypia is a term used to describe cells that have lost their normal appearance but have not reached the level of abnormality of cancer cells.

►Ovarian Cancer

Atypical Congenital Mesoblastic Nephroma

►Mesoblastic Nephroma

Atypical Mole Syndrome

Definition

Synonym dysplastic nevus syndrome; is a disorder of the skin characterized by the presence of many mole-like tumors (nevi). Most people have 10–20 moles over their bodies. People with atypical mole syndrome often have more than 100 moles, at least some of which are unusual (atypical) in size and structure. These moles vary in size, location, and coloring. They are usually larger than normal moles (5 mm or more in diameter) and have irregular borders. Changes in the appearance of these moles must be taken seriously by patients since such changes may foreshadow the onset of cancerous disease. Individuals with atypical mole syndrome are at greater than others for developing cancer of the skin in the form of malignant ►melanoma. Atypical mole syndrome is thought by some clinicians to be a precursor or forerunner of malignant melanoma. This type of cancer may spread to adjacent parts of the skin or, through the blood and lymph circulation, to other organs.

Atypical Neurocytoma

►Neurocytoma

Atypical Nevi (dysplastic nevi)

Definition

Atypical nevi may be distinguished by their asymmetry, border (indistinct irregular margins), color (presence of unevenness of pigmentation, red-brown color), and diameter ≥5 mm. The presence of multiple nevi of atypical appearance is called the Dysplastic Nevus Syndrome (DNS), the ►Atypical Mole Syndrome (AMS) or, if present with familial clustering of both nevi and melanoma, the Familial Atypical Multiple Mole-Melanoma (FAMMM) Syndrome. Atypical nevi may be found on non-sun-exposed areas, the genitalia, and the iris. Whether familial or not, the presence of multiple atypical nevi constitutes and increased risk for melanoma.

►Melanoma

Atypical Teratoid/Rhabdoid Tumor

Definition

Primitive childhood brain tumor containing highly pleomorphic “rhabdoid” cells

►Brain Tumors

AUC

Definition
Area under the (plasma-)concentration *versus* time curve represents the (systemic) exposure (► pharmacokinetics/pharmacodynamics) to a particular drug.

►Irinotecan
►Pharmacokinetics
►Pharmacodynamics

AURA

►Aurora Kinases

AurB

►Aurora Kinases

AurC

►Aurora Kinases

AURKB

►Aurora Kinases

AURKC

►Aurora Kinases

AURORA2

►Aurora Kinases

Aurora-A

►Aurora Kinases

Aurora-B

Definition
The Aurora B kinase associates with microtubules during chromosome movement and segregation.

►Aurora Kinases
►Forkhead Box M1

Aurora-C

►Aurora Kinases

Aurora Kinases

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Synonyms
Aurora-A; AURA; AIK; ARK1; AURORA2; BTAK; MGC34538; STK15; STK6; STK7; Aurora-B; AURKB; AIK2; AIM-1; AIM1; ARK2; AurB; IPL1; STK12; STK5; Aurora-C; AURKC; AIE2; AIK3; AurC; STK13

Definition
Aurora kinases are mitotic serine/threonine kinases, which regulate mitotic events.

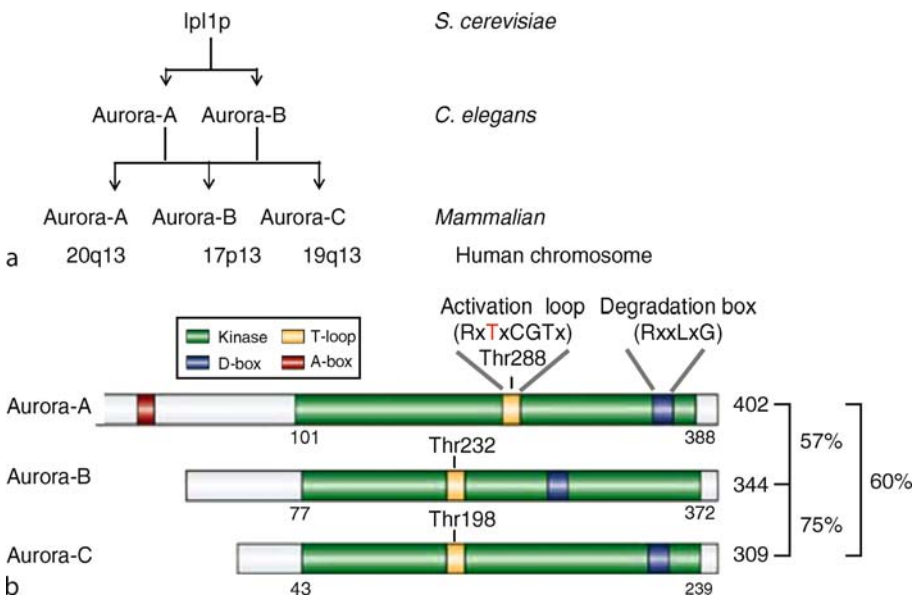
A

Characteristics

Aurora is a subfamily of serine/threonine protein kinase and is conserved from yeast, *Drosophila*, to humans. In mammals, this subfamily of serine/threonine kinases comprises three members: Aurora-A, -B, and -C. *Drosophila* and *C. elegans* also express Aurora-A and -B kinases, whereas *S. cerevisiae* and *S. pombe* have only one Aurora kinase gene, *Ipl1* (Fig. 1a), suggesting that the functions of Auroras have diverged throughout evolution. All Aurora kinases share similar structures, with their catalytic domains flanked by very short C-terminal tails (15–20 residues) and variable lengths of N-terminal domains (39–129 residues). The overall homology between these three members in human is about 60% at amino acid level. The C-terminal domain of human Aurora-B shares 53 and 73% sequence similarity to human Auroras A and C, respectively. The N-terminal domain of Aurora kinases are less conserved, which may determine selectivity of protein–protein interactions (Fig. 1b).

Despite these similarities, the three mammalian Aurora family members differ in their expression patterns, subcellular localization, and timing of activity. Aurora-A is upregulated at the onset of mitosis. It localizes to centrosomes during interphase and to both spindle poles and spindle microtubules during early mitosis. However, it is noted that immunostaining studies show that Aurora-A distributes not only to centrosome but also to cytoplasm and/or nucleus. Aurora-B, whose activity appears to reach maximal levels later in mitosis, displays the dynamic properties of a chromosomal passenger protein. It first associates with centromeres/kinetochores – the sites on chromosomes where microtubules attach – then relocalizes to the midzone of the central spindle, and finally concentrates at the midbody between dividing cells. In line with these distinct localizations, Aurora-A is implicated primarily in centrosome maturation and spindle assembly, whereas Aurora-B is proposed to regulate chromosome condensation and cohesion, kinetochore assembly and bipolar chromosome attachment, the spindle checkpoint and the coordination between chromosome segregation and cytokinesis. Aurora-C has been described only in mammals, where it is expressed in testis and certain tumor cell lines and, like Aurora-B, functions as a chromosomal passenger, which localizes first to centromeres and then to the midzone of mitotic cells. It has been shown that Aurora-C cooperates with Aurora-B to regulate mitotic chromosome segregation and cytokinesis.

Aurora-A is located on chromosome 20q13.2, a region commonly amplified in malignancies, such as melanoma and cancers of the breast, colon, pancreas, ovary, bladder, liver, and stomach. Interest in Aurora has intensified since the discovery that transfection of rodent Rat1 and NIH3T3 fibroblast cell lines with Aurora-A is sufficient to induce colony formation in culture and tumors in nude mice, thus establishing Aurora-A as a bona fide oncogene. Moreover, we and another group have recently shown that overexpression of wild-type Aurora-A induces breast cancer in vivo. *Aurora-B* is located on chromosome 17p13.1, a region not typically amplified in human malignancies. Despite



Aurora Kinases. Figure 1 Aurora kinase family. (a) Aurora kinases are conserved from yeast to human. Their human chromosomal locations are listed. (b) Diagrammatic representation of the domain structure of three Aurora family members. The percentages indicate the degree of the identity between Aurora-A, Aurora-B and Aurora-C.

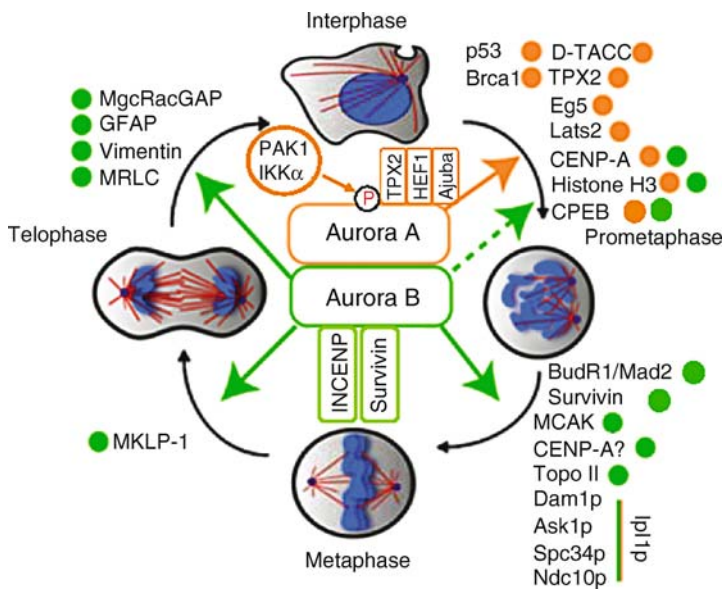
lack of amplification at the gene level, mRNA and protein levels of Aurora-B are frequently increased in human tumors.

Mechanisms

Aurora-A was shown to be activated by autophosphorylation of Thr-288 during G2/M phase upon interacting with TPX, Ajuba, and HEF1 (Fig. 2). However, recent studies have demonstrated that PAK1 and IKKα bind to and phosphorylate Aurora-A on Thr288 and Ser342, which are key sites for kinase activation in mitosis. In vivo PAK activation causes an accumulation of activated Aurora-A. Aurora-A phosphorylates several proteins which are important in mitosis, including histone H3 on Ser10, a key molecule in conversion of the relaxed interphase chromatin to mitotic condensed chromosomes; CPEB (cytoplasmic polyadenylation element-binding protein), best known for its role in promoting polyadenylation of cyclin B and cdc2 mRNA; TACC3, a protein required for stabilization and organization of microtubules; Eg5, a kinesin-like protein involved in both centrosome separation and spindle assembly and stability, TPX2, which is required to generate stable bipolar spindle, and two tumor-suppressor proteins, Lats2 and BRCA1. Further, TPX2, Ajuba, and HEF1 have been shown to interact and activate Aurora-A. The crystal structure of activated Aurora-A in complex with a TPX2 fragment showed that TPX2 binding is sufficient to allow autophosphorylation of the activatory T-loop Thr-288, allowing Aurora-A to adopt a conformation similar to the "active" conformation of other Ser/Thr kinases. With regard to mechanism of Aurora-A regulation of cell survival and proliferation, we have recently demonstrated Aurora-A upregulation of c-Myc to induce telomerase activity. Moreover, we and others have also documented that Auraro-A abrogates p53 DNA-binding activity and induces p53 degradation by direct phosphorylation of p53 at Ser215 and Ser315, respectively. Aurora-B activation is triggered by autophosphorylation after association with its substrates INCENP and survivin, with peak activity in metaphase and telophase (Fig. 2). Key substrates of activated Aurora-B include the centromeric proteins centromere protein A, INCENP, survivin, Borealin; microtubule-destabilizing kinesin mitotic centromere-associated kinesin; the mitotic checkpoint proteins BubR1 and Mad2; the cytoskeletal proteins myosin II regulatory light chain, vimentin, desmin, and glial fibrillary acidic protein; and histone H3. Following mitosis, the D-box region of Aurora-B is recognized by the anaphase-promoting complex/cyclosome, leading to Aurora-B ubiquitination and degradation.

Aurora Kinase as Target for Cancer Intervention

Frequent deregulation of Aurora kinase in human cancer prompted to develop Aurora kinase inhibitors as novel anticancer drugs. A half of dozen of such inhibitors have



Aurora Kinases. Figure 2 Activation, cell cycle execution points and substrates of Aurora-A and Aurora-B kinases. Schematic diagram illustrating (i) the activation of Aurora-A through phosphorylation of Thr288 by PAK1 and IKKα as well as via the interaction with TPX2, HEF1, and Ajuba, (ii) activation of Aurora-B by binding to INCENP and survivin, and (iii) known execution points and substrates of Aurora-A and -B across the cell cycle. The substrates phosphorylated in each phase of the cell cycle are color coded: orange circles indicate Aurora-A substrates and green circles indicate Aurora-B substrates. Ipl1p substrates are indicated as a double green and orange line.

been reported and a few of them are in clinic trails, which include VX-680 (Merck), AZD1152 (AstraZeneca), MLN8054 (Millenium), and PHA-739358 (Nerviano). However, VX-680 and PHA-739358 are pan-Aurora kinase inhibitors. AZD1152 and MLN8054 also inhibit three members of Aurora kinase, however, AZD1152 preferentially inhibits Aurora-B, whereas MLN8054 is more potent toward Aurora-A. Further investigations are required to identify more potent and selective Aurora kinase inhibitor for cancer intervention.

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Autoantibodies

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Definition

Autoantibodies are those antibodies that recognize proteins, nucleic acids, or carbohydrates derived from the cells of the organism in which they were formed as nonself. Cancer-associated autoantibodies are potential reagents for the early diagnosis and prognosis of cancer. Autoantibodies target molecules involved in signal transduction, cell cycle regulation, cell proliferation, and apoptosis, all of them key processes in carcinogenesis. Molecular studies of antigen–antibody systems in cancer can also yield valuable information on the carcinogenic process. The study of autoantibodies in cancer has broad implications for the discovery of molecular targets for drug therapy and for cancer biomarkers in general. Cancer-associated autoantibodies can be invaluable reagents in the selection of naturally immunogenic molecules as key targets for cancer immunotherapy.

Characteristics

Available serologic markers for the diagnosis of cancer exhibit limited specificity and sensitivity. Thus, there is need to develop biomarkers with high accuracy, which alone or in combination with other screening methods such as ►mammography ►prostate specific antigen, might significantly improve the likelihood of diagnosing cancer at an earlier stage. There is ample evidence that malignant tumors can induce T-cell-mediated as well as humoral responses. For decades, autoantibodies reported in cancer sera have been considered to represent epiphenomena and only recently have autoantibodies emerged as promising biomarkers for the diagnosis and prognosis of cancer. Autoimmunity in the systemic autoimmune diseases constitutes a useful model to look at mechanisms by which autoantibodies arise in cancer patients. The autoantibody response observed in both, the systemic autoimmune diseases and in cancer is antigen-driven. It has been determined in ►medullary breast carcinoma that tumor infiltrating B-cells differentiate into plasma cells in situ and that their IgG genes exhibit patterns of mutations that are consistent with antigenic selection and affinity maturation, suggesting a tumor antigen-driven ►humoral immune response. Although it is not known whether these findings apply to solid tumors in general, other data suggest that the autoantibody response in cancer is driven by antigen. The autoantibodies observed in cancer sera are predominantly of the IgG, and the IgM class of immunoglobulins. IgG antibodies may predict the coexistence of helper T-cell activity. In each of the systemic autoimmune diseases, the autoantibody profile has diagnostic and prognostic significance. Likewise, the picture is emerging pointing to a characteristic autoantibody profile in many types of cancer, expanding the opportunities for serologic diagnosis. Several studies have shown that autoantibodies appear in cancer sera before the disease becomes clinically evident, suggesting that they have the potential of detecting early disease, i.e., when the treatment has the best chance to influence tumor behavior and ideally to achieve a cure. In agreement with this possibility, immunopathological studies of premalignant disease have shown molecular alterations that have been associated with autoreactivity to cancer-associated proteins. Also, studies on breast tumor-infiltrating B-cells suggest that antitumor antibodies are commonly produced in response to solid tumors and that ►B-cell responses occur early in tumor development.

As to the origin of autoantibodies, it has been shown that increased proteolytic cleavage of tumor-associated autoantigens in the process of apoptosis may increase their immunogenicity. Apoptosis is a process which

can make cancer-associated antigens or fragments of cytosolic or nuclear proteins accessible to cell surfaces and may expose cryptic peptides for ►MHC I ►MHC II display by ►antigen-presenting cells. The immunogenicity of many or even most tumor-associated antigens is not due to mutations but appears to be the result of overexpression in tumor or of other tumor factors. From the many tumor-associated antigens reported using autoantibodies as reagents for ►immunoscreening cDNA expression libraries or using proteomics, relatively few are membrane-associated or nuclear antigens, while the majority of the autoantigens identified by these methods are cytosolic proteins.

Autontibodies recognizing cancer-associated autoantigens can also be detected in a small proportion of healthy individuals. This is to be expected since antibodies recognizing cancer-associated autoantigens develop months or years before the clinical diagnosis of cancer. It is clear that the larger the pool of "normal" sera, the more likely will be the presence of tumor-associated clones.

Autoantibodies Can Produce Tissue Injury and May Influence Tumor Behavior

Autoantibodies in the ►paraneoplastic syndromes are markers of cancer, and can also result in tissue injury in regions remote from the tumor. Several neurologic syndromes have been described in association with small cell lung cancer and breast cancer. Although neurologic autoimmune syndromes have not been reported in vaccine trials, the presence of cancer-associated autoantibodies cross-reacting with brain antigens and potentially causing neurologic disease should be considered in the design of anticancer vaccines. Although the paraneoplastic syndromes are rare, studies on the specificity of the autoantibodies suggest that they may be able to influence cellular functions.

It has been presumed that spontaneous regressions rarely occurring in cancer patients have an immunological basis. Although there is ample evidence that autoantibodies can function as effectors of the immune system in the systemic autoimmune diseases, whether autoantibodies can in any way influence tumor behavior in cancer patients is unclear.

Studies of the lymphoplasmacytic cell infiltrate observed in medullary carcinoma of the breast have demonstrated its importance to the improved prognosis of this form of breast cancer, suggesting that the host immune response – most likely the autoantibody response to cancer-associated antigens – is involved in restraining tumor growth. Associations with improved prognosis as well as with poor prognosis have been reported for several naturally occurring humoral responses.

The factors underlying the poor effectiveness of naturally occurring antibody responses against cancer are complex. It has been suggested that ►tolerance, an important obstacle to cellular and vaccine-based immunotherapy of cancer, may constitute a similar obstacle to naturally occurring responses i.e., the decreased response to tumor antigens may not be a function of systemic immunodeficiency, but at least to some extent may be due to specific tolerance to tumor-associated antigens. It is also likely, that at least for some antigens, spontaneous autoantibody responses may be subtherapeutic.

Other possibilities exist to explain why naturally occurring autoantibodies are not more effective against cancer. It has been shown that the collaboration of both humoral and cellular immune responses is required for the complete eradication of antigen-expressing tumors. It is likely that this type of cooperation between humoral and cell-mediated response is necessary for an effective spontaneous antitumor immune response. Another possibility that may negate the beneficial effect of a cancer-associated immune response is that autoantibodies against some tumor antigens may actually stimulate uncontrolled cell proliferation and tumor progression. Indeed, some autoantibody responses may be stimulatory of tumor growth in vivo. In addition antibodies against shed tumor cell surface antigens can also promote tumor invasion and metastases through ►FcR-induced release of angiogenic cytokines in the tumor microenvironment. It seems likely that the ►affinity-matured IgG responses observed in draining nodes in breast cancer represents a mixture of protective and harmful autoantibodies. Thus, it is possible that in addition to T-cell reactivity, the balance of the antagonic effect of the autoantibodies produced in a particular patient will contribute to determine whether the immune response may be protective or harmful.

Autoantibodies as Diagnostic and Prognostic Serologic Biomarkers of Cancer

In 1995 Sahin et al. described ►SEREX technology, in which recombinant tumor cDNA expression libraries were screened with cancer sera. Since then hundreds of autoantigens have been identified using SEREX and its modifications in cancer sera. Proteomics technology has also been successfully used for the identification of tumor-associated antigens. Proteomics is a versatile approach which has generated considerable interest in the field of biomarker discovery for its capability of uncovering antigen–antibody systems in serum and other biological fluids. One advantage of this approach over genomics is its ability to assess the contribution of posttranslational modifications of the autoantigens. Once an autoantigen has been identified in cancer sera, it is essential to establish that it is not only patient-associated but also tumor-related, i.e., recognized by multiple sera from patients with cancer and not by control sera. However, these two conditions may not be sufficient for a potential biomarker to become a

reagent useful for the early diagnosis of cancer. Some studies using genomics or proteomics have reported the association of reactivity of individual IgG autoantibodies or of panels of autoantibodies with the diagnosis of solid tumors, while other studies have reported correlations between autoantibody reactivity and patient survival. Despite the abundance of potential biomarkers, efforts to consistently predict malignant disease based on autoimmunity to individual antigens has not resulted thus far in serologic markers with sufficiently high predicting specificity and sensitivity to merit adoption in a clinical setting. However, it has been shown that the use of panels of autoantigens rather than individual antigens in breast, prostate, and ovarian cancers enhances the likelihood of detecting cancer-associated antigens with potential diagnostic value. High-throughput methodology, genomics using phage display and proteomics are being increasingly used in the identification of biomarkers useful for the diagnosis of solid tumors and hematological malignancies. For all the multiple autoantibodies presently being studied as diagnostic instruments, the appropriate validation rout must be followed.

A factor that should be considered when evaluating the predictive value of autoantibodies as biomarkers for the early diagnosis of cancer is the stage of the tumor of patients donating their sera to probe the antigen collection. Although advanced stage cancer patients have normal cellular and humoral responses to recall antigens, they do not generate effective responses against tumor antigens. In addition, a large proportion of patients with early stages of malignancy have serum antibodies reactive with autologous tumor, while a significantly smaller proportion of patients with metastatic disease have tumor-reactive antibodies. These findings suggest that a decrease in antibody response against tumor-associated antigens may be the result of tumor/autoantigen-specific tolerance mechanisms rather than generalized disease-related immune dysfunction. Not only there may be less autoantibody reactivity in advanced stages of cancer, but also there may be a change in the specificity of the autoantibodies. These considerations are relevant to the design of studies proposing to demonstrate the ability of autoantibodies to contribute to the early diagnosis of cancer, since lumping data from all stages of cancer, and particularly the inclusion of patients with advanced stages of the disease in the assessment of sensitivity and specificity, may dilute the predicting ability of a biomarker for the early diagnosis of cancer. Existing data support the view that serum antibodies may be prevalent in premalignant disease, thus supporting the likelihood that autoantibodies may show significant predicting ability for the diagnosis of early disease.

The field of diagnostic biomarkers is still in the discovery phase, but is rapidly approaching testing in the real world. The use of genomics, proteomics and high throughput technology has led to the identification of a large group of cancer-associated autoantigens. These studies strongly suggest that autoantibodies have potential as biomarkers and may facilitate the early diagnosis of malignancies. It has been suggested that proposed markers for classifying or predicting risk in individual subjects must be held to a much higher standard than merely being associated with outcome, and that their sensitivities and specificities must be shown to be adequate through appropriate statistical evaluations. Validation of the most promising of the proposed biomarkers for diagnosis and prognosis of cancer requires the application of these high evaluative standards in prospective studies of large cohorts of patients with cancer against the present diagnostic standards.

The goal of most of these studies is to develop accurate and reliable serologic tests useful to diagnose cancer early. While the biodiscovery and validation phases are supported by the Early Diagnosis Research Network of NIH in the US, the National Institute of Standards and Technology, also a branch of NIH, directs the efforts of the scientific community in bringing metrology to serology. Indeed, after the usefulness of the autoantibody or of a panel of autoantibodies is established, the gap between the discovery phase and the real world should be bridged by developing new standards for autoantibody measurement with participation of industry, academia, physicians, and patients.

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Autoantigens

Definition

Self antigens to which the immune system makes a response are called autoantigens.

►Sjögren Syndrome

Autocrine

Definition

Autocrine is a factor acting on the same cells that produce it. Following interaction with the endogenous receptor(s), an autocrine activation (autocrine loop) is obtained. Self-stimulation by a cell that expresses both the receptor for a particular hormone or growth factor and secretes the ligand. Autocrine one of three fundamental types (with ►paracrine and ►endocrine) of cellular regulation by intercellular diffusible factors, where the diffusible factor exerts a regulatory influence primarily on the same cell that secreted it.

►Scatter Factor
►Receptor Tyrosine Kinases

Autocrine Growth Factors

Definition

Are multifunctional peptides that are extremely potent for the growth of cancer cells and they can also act on the cell in which they are produced. Growth factors that function via an autocrine mechanism in cancer cells include transforming growth factor α (TGF-α), ►transforming growth factor β (TGF-β), ►platelet-derived growth factor (PDGF), bambesine, and interleukins; each with its own membrane receptor on the cells that produce them.

►Sivelestat

Autocrine Signaling

Definition

Is a form of local area signaling in which a cell secretes a chemical messenger (►autocrine agent) that signals the same cell. In contrast, in the ►paracrine signaling, the signal of the chemical messenger (paracrine agent) is limited to other cells also in the local area.

►Chemoattraction

Autocrine Stimulation

Definition

Production of a factor that stimulates the producer cell itself.

►Platelet-Derived Growth Factor

Autografts

Definition

The engraftment of organs, cells or tissues from one individual to themselves.

►Graft Acceptance and Rejection

Autoimmune Diseases

Definition

Diseases in which the pathology is caused by adaptive immune responses to self antigens are called autoimmune diseases.

►Sjögren Syndrome

Autoimmune Hemolytic Anemia

Definition

Anemia that results from an abnormality of the immune system that attacks and destroys red blood cells. Antibodies and associated complement system components adhere to the red blood cell surface.

►Rituximab

Autoimmune Lymphoproliferative Syndrome

Definition

Synonym: Canale-Smith Syndrome, is a childhood syndrome of autoimmunity with a phenotype of hemolytic anemia and thrombocytopenia with massive lymphadenopathy and splenomegaly within first 2 years of life. Lymphadenopathy is associated with mutations of the FAS gene, and the accumulation of lymphocytes is mainly the result of failure of FAS-mediated apoptosis. Autoimmune manifestations, such as hemolytic anemia and thrombocytopenia, which result from the production of autoantibodies against red blood cells and platelets, can persist into adolescence, and the FAS mutations are compatible with long-term survival. Patients have been detected lacking mutation in the FAS gene, and it is thought that in these cases the impaired apoptosis results from other defects of the FAS pathway (Ref.: Vaishnaw AK, Orlinick JR, Chu J-L, Krammer PH, Chao MV, Elkton KB. (1999) The molecular basis for apoptotic defects in patients with CD95 (Fas/Apo-1) mutations. J Clin Invest 103:355–363).

►Apoptosis
►Sjögren syndrome

Autoimmune Response

Definition

An adaptive immune response directed at self antigens is called an autoimmune response; likewise, adaptive immunity specific for self antigens is called autoimmunity.

►Sjögren Syndrome

Autoimmune Thyroid Disease

Definition

A disease that results from an aberrant immune response directed against its own cells and tissues. The most common autoimmune thyroid diseases are Graves disease and Hashimoto disease.

►Graves Disease
►Hashimoto Disease
►Follicular Thyroid Tumors

Autoimmunity

Definition

Refers to the ability of the host immune system to make a response to self, i.e. to components of the host's own tissues or cells. Autoimmunity includes antibody responses (autoantibodies) or cellular responses mediated by T lymphocytes specific for self components (autoantigens). Healthy individuals do not make such responses, because tolerance to self exists and guards against the development of autoimmunity. In disease or when tissue destruction occurs, tolerance to self may be broken and autoimmunity induced. Uncontrolled chronic immune activation may lead to the development of an autoimmune disease, such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma and others.

►Autoimmunity and Prognosis

Autoimmunity and Prognosis in Cancer

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Synonyms

Immune responses to autoantigens; Antibodies to self antigens; T-cells recognizing autoantigens; Immunoregulatory aberrations in cancer

Definition

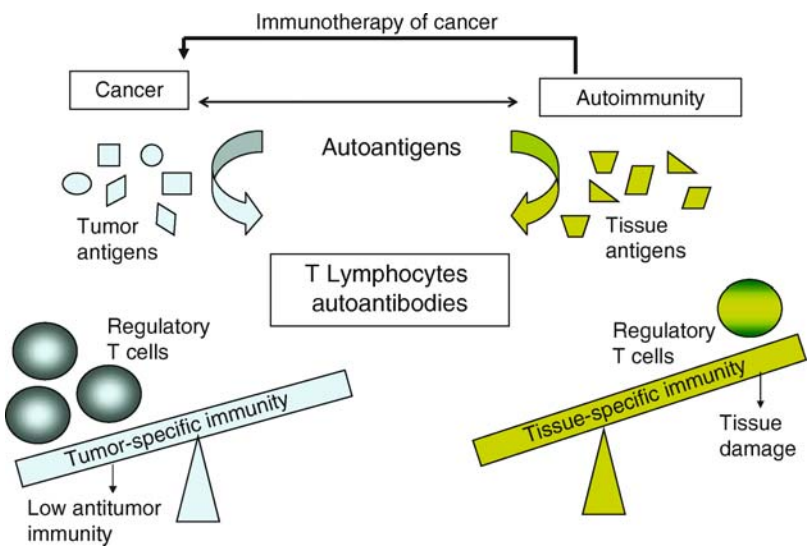
Characteristics

It has often been said that cancer and ►autoimmunity represent "two sides of the same coin." Links between these two disease states have been identified many years ago. However, this relationship has been receiving increased attention recently, largely due to emerging evidence that antitumor immunotherapies, especially ►antitumor vaccines, are often associated with the

manifestation of autoimmunity in cancer patients. Apparently, the most successful vaccinations in terms of their ability to induce immune activation in cancer patients are also most likely to induce autoimmune sequelae. These may be mild or severe and may result in substantial pathology. Immune therapies could also induce autoantibody or self antigen-specific T cell responses without associated clinical symptoms in some patients. It has been well documented that the development of cancer and autoimmune disease is accompanied by aberrations in immune regulation. The crucial role of immune regulation in disease development and progression has been intensively studied in various animal models and, to a lesser extent, in humans with the result that at least some mechanisms responsible for immune dysfunction in autoimmunity and cancer are now defined. The interesting point to consider is that in cancer and autoimmune disease, these mechanisms seem to involve the same immune cell subsets behaving in a diametrically opposite way in response to similar antigenic stimuli. Thus, excessive activation of the immune system to autoantigens is a hallmark of autoimmunity, while in cancer, a down-regulation of autoantigen-specific immune functions allows for cancer development, progression and metastasis.

Evidence for Autoreactivity in Patients with Cancer

In patients with cancer or autoimmune diseases, the presence of circulating antibodies to self antigens is a common finding, and one that has been helpful in diagnosis and management of patients with autoimmune conditions. The development of antibodies to autologous cellular antigens (autoantibodies) in cancer occurs spontaneously, presumably as a result of a tissue damage or trauma in the past, or in response to therapeutic intervention and may or may not be prognostically useful. The types of cellular proteins which induce autoantibody response in malignancy are quite varied and include cellular antigens encoded by mutated normal genes such as p53, cellular proteins that are aberrantly expressed in tumor cells (e.g. alpha-fetoprotein or carcinoembryonic antigen), mucins (e.g. MUC1), inhibitors of apoptosis (e.g. survivin), surface receptors of apoptosis (e.g. CD95) or nuclear antigens such as double-stranded or single-stranded DNA. In recent years the list of the so-called "cancer-related antigens" which initiate humoral immune responses in the host has considerably expanded, and the presence of some of these antibodies has been taken as evidence that the host is not ignorant of the tumor. In patients with melanoma, the appearance of autoantibodies, e.g. anti-nuclear antibodies (ANA), anti-DNA antibodies, anti-thyroid antibodies or of clinical manifestations of autoimmunity or both during immunotherapy with interferon α-2b was associated with good prognosis and significantly improved survival. In many other studies, the presence of autoantibodies against tumor antigens was considered as evidence that tumor-specific immune



Autoimmunity and Prognosis in Cancer. Figure 1 Immunotherapy of cancer can shift the balance of autoantigen-driven immune responses. In cancer, T lymphocytes and /or antibodies specific for tumor-derived antigens, which are self, are generated. However, in the presence of an excess of regulatory Tcells (Treg) these immune responses are suppressed, and tumor-specific immunity is low (*left*). In autoimmuine disease, autoantigens derived from tissues similarly stimulate T-cell and antibody responses. However, because Treg are few or are not functional, tissue-specific immune responses are strongly increased, resulting in immune-mediated tissue damage (*right*). Successful immunotherapy of cancer leads to up-regulation of immune responses to self, similar to what happens in autoimmunity, and to improved antitumor immunity. This may be brought about by a decrease in Treg numbers or function. The ultimate result may be a better prognosis for cancer patients who are predisposed to making immune responses to autoantigens.

responses can be made and could potentially contribute to improved survival of patients with cancer. In reality, these antibodies with specificity for tumor-associated proteins are targeting self antigens and therefore, they represent an autoimmune response. Immune therapies further enhance the host capability to mount these responses, thereby breaking the tolerance to self and contributing to the development of antitumor immunity.

Cellular responses mediated by T cells specific for self antigens are also observed in patients with cancer as well as autoimmune diseases. The self-antigen specific T cells are detectable in the peripheral circulation of patients with cancer and are present at the tumor site. Using MHC tetramers, it has been possible to quantify the frequency of epitope-specific CD8+ T cells in the circulation of normal donors and patients with cancer or autoimmune disease. ►Cancer immunotherapies often increase the frequency and activation levels of such effector T cells, creating an opportunity for autoimmune tissue destruction. There is a valid concern that this type of therapy could potentially result in activation of auto-reactive T cells capable of killing tissue cells, similar to what happens in autoimmune diseases.

Regulation of Autoreactivity

Given that both autoantibodies and self-reactive T cells are present in patients with autoimmune disease and cancer, the question arises as to how immune responses are regulated in these diverse pathologic situations. Presumably, mechanisms allowing for expansion of immune responses to self antigens in autoimmunity and their suppression in cancer are involved. Indeed, recent evidence suggests that a subset of T lymphocytes named ►regulatory T cells (Treg) and able to suppress responses of effector T cells, represents one mechanism that may be relevant to both autoimmunity and cancer. Normally, T reg defined as CD4+CD25+FoxP3+ cells are responsible for maintaining tolerance to self and preventing autoimmunity. Their numbers and functional competence in the local microenvironment determine the nature and magnitude of immune responses. Treg depletion or functional deficiency results in development of autoimmunity. In contrast, Treg accumulations at tumor sites and in the peripheral circulation have been observed in patients with cancer. Further, their presence and suppressive activity in the ►tumor microenvironment have been linked to poor survival in patients with cancer. Therefore, Treg represent a common regulatory mechanism that could account for immune aberrations seen in cancer as well as autoimmunity.

The concept that the same regulatory T-cell subset patrolling responses to self is involved in seemingly opposite functions, i.e. a lack of suppression vs. an excess of suppression, in autoimmunity and cancer, respectively, seems reasonable when it is remembered that the vast majority of non-mutated tumor antigens are seen by the immune system as self. The Janus face presented by Treg in cancer and autoimmunity is a result of biological economy, whereby the same cell subset is differently regulated, depending on the environmental context. Under normal conditions, the immune system maintains tolerance to self by utilizing Treg. In disease, pathologic events accompanied by danger signals trigger inflammatory cascades, which shift the immune balance and activate or down-regulate Treg, depending on the nature of triggering signals. Molecular mechanisms responsible for down- or up-regulation of immunity by Tregs in disease are being intensely investigated at present, and the critical unanswered issues concern their origin, the factors that contribute to Treg generation, the nature of Treg activation stimuli and pathways used for suppression/activation of immune responses. Treg are a heterogenous population encompassing several types of regulatory cells, including natural or nTreg, antigen-dependent Tr1 cells, and perhaps other suppressor cells induced by ►IL-10 or ►TGF-β in the tissue microenvironment. It is unclear whether all or only some of these regulatory cell subsets participate in suppressing antitumor responses and thus, in promoting tumor growth and its escape from immune surveillance. In animals with autoimmune diseases, selective depletion of partially overlapping CD4+ T cell subsets or their adoptive transfers identified the existence of phenotypically distinct Treg which mediate distinct clinical manifestations of disease. The critical role of a cytokine/chemokine milieu, unique cytokine dependency of various Treg subsets and the ability of these subsets to produce distinct cytokines all contribute to tremendous pliability of Treg and suggest organ-restricted regulation of autoimmunity as well as anti-tumor responses. ►Cytokines, especially TNF-α, are implicated in the development of autoimmunity as well as cancer, and their pluripotent activities represent another example of biologic redundancy.

In addition to Treg and cytokines, other links between cancer and autoimmunity have been identified. The role ►dendritic cells (DC) play in regulating T-cell activation is well known. It now appears that DC not only elicit potent effector cell responses but may also cross-talk with Treg. Evidence indicates that DC have the ability to induce and expand subsets of CD4+ Treg cells, with antigen-targeted immature DC promoting induction of Tr1 cells and antigen-loaded mature DC stimulating CD4+CD25+ nTreg. Silencing of cytokine signaling inhibitor SOCS1 in murine DC has been shown to induce unbridled IL-12 signaling and a down-stream cytokine cascade resulting in a breakdown of tolerance and the development of autoimmune responses against normal tissues as well as tumor. Signaling via toll-like receptors (TLR), which are expressed on DC and recognize a set of conserved pathogen-associated molecular structures (PAMPS), may also be involved in regulation of Treg functions.

Thus, molecular signals that DC experience in the tissue microenvironment determine their cross-talk with other immune cells. This cross-talk is most likely driven by cytokines produced by immune and/or tissue cells, and it controls the magnitude of responses mediated by effector cells responsible for antigen-specific or innate immunity. It may well be that the requirements for autoreactive cytolytic T lymphocyte (CTL) responses are defined and regulated at the level of DC-T cell interactions. The outcome could be critical for either cancer progression or development of autoimmunity.

Immunotherapy May Induce Autoimmunity

The mechanisms for breaking tolerance to self and simultaneously up-regulating anti-tumor immunity may operate at the cellular level (e.g. Treg, Treg-DC, DC) or may involve cytokine networks or signaling pathways, such as the NFκB pathway known to regulate pro-inflammatory cytokine responses and recently identified as a potential molecular link between inflammation and cancer. To what extent the same mechanisms are potentially involved in the development of autoimmune disease and cancer is critical to unravel. For many years, reports have been suggesting increased cancer risk among patients with autoimmune diseases. Conversely, the presence of autoimmune disease has been noted in patients with cancer. More recently, the onset of autoimmune diseases among cancer patients immunologically responding to biologic therapies has been observed. In particular, immune therapy of patients with metastatic melanoma using a combination of a peptide vaccine and antibody to cytotoxic T lymphocyte antigen-4 (CTLA-4) was reported to cause durable objective responses, which correlated with the induction of symptomatic autoimmunity. The ability of interferon α-2b to induce autoimmunity in patients with metastatic melanoma and correlations observed by Gogas et al [3], between the development of autoimmunity and the reduced risk of melanoma recurrence convincingly suggest that benefits of immune therapies are restricted to cancer patients in whom evidence of autoimmunity exists or appears in response to therapy. In another interesting report, a patient with melanoma who was treated with anti-CTLA-4 monoclonal antibody in combination with a vaccine containing autologous tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor (GMCSF) developed high titer antibodies against MHC class I chain-related protein A (MICA), while favorably responding to this therapy. These results highlight the therapeutic potential of anti-MICA antibodies, which were shown to be able to opsonize tumor cells for efficient cross-presentation to DC and to lyse tumor cells via complement fixation. In aggregate, clinical and immunologic results of antitumor vaccination trials strongly suggest that cancer patients in whom breaking of tolerance to self can be induced by immunopotentiating therapies are most likely to become clinical responders to the treatment. Seen from this perspective, the reports correlating the presence of autoimmunity to improved prognosis and survival in cancer have important implications for diagnosis, patient selection, immune monitoring and immunotherapy of cancer.

Autoantigens as a Link Between Cancer and Autoimmunity

The above described development of autoimmunity and associated benefits in prognosis or survival of cancer patients treated with immunotherapies, underscores a potential mechanistic link between cancer and autoimmunity. Accumulated data suggest that autoantigens drive the autoimmune response in both instances and are the key factor in pathogenesis. However, it is necessary to distinguish two alternatives. In the first instance, the patient has pre-existing autoimmunity, as indicated by low but detectable titers of pre-existing autoantibodies and/or autoreactive T cells. Such patients with a strong propensity toward autoimmunity might be a group for whom immune therapy should be considered, because memory responses to autoantigens are present. In the second instance, no pre-existing autoimmunity is evident prior to treatment, but immune therapy induces the appearance of autoantibodies and symptoms of autoimmunity that correlate with a favorable clinical response. In such cancer patients autoimmunity develops during effective immunotherapy, perhaps because the tolerance threshold to autoantigens is lowered by therapy, with the result that robust antitumor responses can be generated. The prospective identification of such patients could help clinicians in selecting patients for immunotherapy and lead to improved treatment strategies.

Multiple strategies have been used to optimize immune therapy of cancer and to improve prognosis. It now appears that therapies capable of effectively breaking tolerance in patients who are pre-disposed to autoimmunity are among the most promising. As the cellular and molecular mechanisms of immune regulation become better defined, new opportunities arise for changing the balance in favor of autoimmunity, with a proviso that it can be adequately clinically managed in patients with a malignant disease. The discovery that excess of Treg can be at least transiently decreased with anti-CD25 antibodies, cyclophosphamide or interleukin-2-diphtheria recombinant fusion protein (DAB_{389}IL-2) offers a window of opportunity for delivery of tumor-specific vaccines. It is also now feasible to expand Treg many folds in the presence of

rapamycin (1nM), presenting us with a potentially useful therapeutic tool for ameliorating autoimmune disease. In cancer patients undergoing hematopoietic stem cell transplantation, the post-transplant delivery of expanded, strongly suppressive Treg might help in the control of graft versus host disease (GVHD). These and other strategies targeting regulatory cells or molecular pathways involved in immune regulation are likely to clarify the complex interactions between immune cell subsets mediating autoimmune responses to autoantigens and to provide new insights into more effective approaches to improving prognosis in patients with malignancies.

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Autologous Bone Marrow Transplantation

►Myeloablative Megatherapy

Autologous (hematopoietic) Stem Cell Transplantation

►Myeloablative Megatherapy

Autologous Typing

Definition

A method using normal and cancer cells from the same individual to determine whether patients develop cancer-specific T-cell or antibody responses.

►Cancer-Testis (CT) Antigens
►Cancer-Germline (CG) Antigens

Autonomic Nervous System

Definition

Is the “involuntary” as opposed to the somatic or “voluntary” nervous system. It is responsible for much of the unconscious regulation of body functions such as heart rate, blood pressure and movements of the gut.

►Multiple Endocrine Neoplasia Type 2
►Neuroblastoma

Autophagic Body

Definition

Intralysosomal vesicle containing cytosol or organelles from autophagy.

►Autophagy

Autophagocytosis

►Autophagy

Autophagosome

Definition

Cytoplasmic organelle formed in the process of sequestering cytosol or organelles (mitochondria, peroxisomes) for autophagic degradation.

►Autophagy

Autophagy

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Synonyms

Autophagocytosis; Macroautophagy; Cellular self-digestion

Definition

Autophagy is intracellular uptake of cytoplasm (proteins, nucleic acids, small molecules, whole organelles, etc.) into the ►lysosome and its subsequent degradation. Autophagy is a constitutive as well as a stress-inducible process responsible for the degradation of the majority of cellular proteins.

Characteristics

Lysosomal uptake and degradation of proteins by autophagy can be found in virtually all eukaryotic cells. Autophagy is a way of degrading and recycling long-lived cytosolic proteins and complexes (like ribosomes) and the only way of degrading whole organelles. Autophagy is regarded to be a largely non-selective bulk process, but it also serves more specialized roles in biogenesis of the lysosome (import of lysosomal hydrolases), cellular differentiation and cell death, and in the elimination of mitochondria and ►peroxisomes, organelles which can be degraded with high selectivity by autophagy-related pathways. Autophagy of peroxisomes is referred to as pexophagy. Recently, the autophagic removal of endoplasmic reticulum (ER) has been described.

Autophagy is upregulated as a response to starvation, growth factor withdrawal, during stress response (oxidative stress, chemotherapy, protein aggregation), developmental differentiation, and tumor suppression. An excess of autophagy is involved in diseases like myopathies and neurodegenerative disorders.

Autophagy is highly conserved in evolution. Autophagic degradation is being studied in model organisms like *Dictyostelium discoideum*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Drosophila*, and mice. Most of the autophagy genes (ATG genes) have been discovered in the yeast *Saccharomyces cerevisiae*. Many of them are conserved in higher eukaryotes including mammals.

Cell Biology of Autophagy

There are three morphologically distinct forms of autophagy (Fig. 1): (i) Carrier-mediated uptake involves the recognition of cytosolic proteins by a ►chaperone-related receptor and its subsequent direct translocation through the lysosomal membrane. (ii) In ►microautophagy cytosolic material is sequestered through invaginations of the lysosome. (iii) In macroautophagy, cytosol is engulfed by double membranes thereby forming ►autophagosomes. These autophagosomes fuse with lysosomes, thereby delivering the inner vesicle (►Autophagic body) of the autophagosomes into the lysosome. In subsequent steps, the vesicle membrane and the autophagic cargo are degraded and amino acids and other small molecules are recycled. Macroautophagy is the most prominent form of autophagy, therefore these terms are often used synonymously.

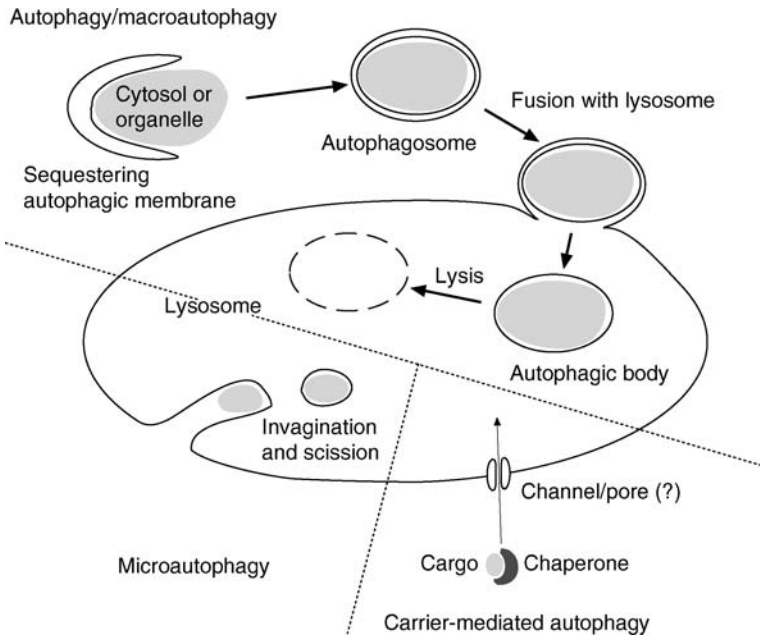
Signal Transduction

When nutrients, amino acids in particular, are abundant, autophagic activity is reduced to basal levels. Concomitantly, active growth factor signaling downregulates autophagy to basal levels. The serine/threonine kinase ►TOR (target of rapamycin) signaling pathway is regarded as a core component in the regulation of autophagy in response to there signals. The TOR pathway transduces extracellular growth factor or hormone signals (e.g. insulin) through receptor tyrosine kinases, phosphatidyl inositol 3-kinase (►PI3K), ►AKT/PKB, TOR, and ►p70S6 kinase, to ultimately regulate cell growth by translational and transcriptional mechanisms (Fig. 2). TOR is thus a nutrient sensor together with the AMP-activated protein kinase (►AMPK) which is responsible to low ATP to AMP ratios.

A number of autophagy protein complexes (mostly Atg proteins) are involved in regulating and executing autophagy: (i) the serine/threonine kinase Atg1 complex that responds to TOR and other upstream signals; (ii) a lipid kinase complex that is involved in vesicle formation in the cytosol; (iii) two ►ubiquitin-like conjugation pathways mediate vesicle expansion; and (iv) a protein recycling pathway is involved in the maturation of autophagosomes.

Autophagy and Programmed Cell Death

Induction of autophagy beyond the constitutive level can be associated with a non-apoptotic form of programmed cell death (PCD), referred to as type II PCD or autophagic



Autophagy. Figure 1 Cell biology of three forms of autophagy. In ►macroautophagy (for short: autophagy) intracellular membranes form in the process of sequestering cytosolic material. The edges of these membranes fuse to form double (or multiple) membrane structures (autophagosomes). The outer membranes of the autophagosomes fuse with the lysosome. Delivery of the inner sequestered material leads to the appearance of autophagic bodies within the lysosome. Phospholipases, proteases and other hydrolases degrade intralysosomal membranes and their content for re-use. Constitutive autophagy replenishes the cellular storages of energy and building blocks. Microautophagy involves the direct uptake of cytosol through membrane invaginations of the lysosome. Microautophagy might be required for membrane homeostasis of the lysosome and might therefore be linked to macroautophagy. In chaperone- or ►carrier-mediated autophagy, the autophagic cargo is recognized in the cytosol by a carrier chaperone and is guided to/though the lysosomal membrane by an unknown mechanism. Drawing Sven Thoms 2007.

cell death. In contrast to classical apoptotic type I cell death, organelle degradation precedes the collapse of cytoskeletal elements in type II PCD. Likewise, ►caspase activation and DNA fragmentation occur at later stages (if at all) in type II PCD. Both, apoptotic and autophagic cell death differ from necrosis by the lack of a tissue inflammatory response.

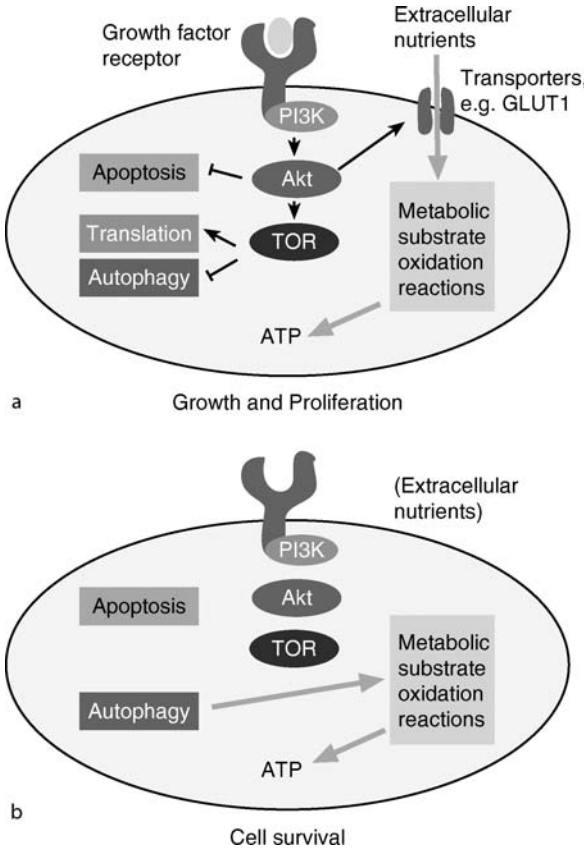
Autophagic cell death is associated with cell reduction in development (e.g. insect metamorphosis or postlactational mammary gland involution). Autophagic cell death is also observed in mammalian tissue treated with chemotherapeutics like ►tamoxifen. The association of cell death with increased autophagy is often only correlative and based on morphological features like the appearance of autophagic vacuoles. Recently it could be shown that in developmental embryonic apoptosis, autophagy is necessary to produce the signals that induce removal of apoptotic cells.

A positive correlation between cell death and autophagy is supported by studies showing that pharmacological inhibition of autophagy can prevent cell death. 3-Methyladenine (3-MA) blocks autophagy by acting on class II PI3K. 3-MA delays or inhibits cell death in various cancer cell lines.

The cell death and survival functions of autophagy do not exclude each other and it is clear from morphological and genetic data that a complex interplay exists between autophagy and apoptosis. Cell death can arise from high levels of autophagy though self-digestion. Low levels of autophagy might contribute to cell death by making the cell more susceptible to environmental stressors that are otherwise eliminated by autophagy.

Autophagy and Cancer

Autophagy is likely to play a role in the development and in treatment of cancer. In the development of cancer, those functions of autophagy that are associated with the restriction of cell growth and proliferation might be more important than the pro-survival functions. It has been speculated that a reduced level of intracellular protein degradation confers a selective advantage to malignant cells over normal cells. Accordingly, the inactivation of autophagy genes promotes tumor development in mice and overexpression of some autophagy genes inhibits formation of breast tumors in mice models. ►Beclin 1, the mammalian homologue of the yeast autophagy protein Atg6/Vps30



Autophagy. Figure 2 Regulation of autophagy in response to growth factor signaling and nutrient availability. (a) In the presence of growth factors and nutrients, TOR kinase is activated through PI3K and AKT and by amino acids. TOR inhibits autophagy and induces translation of household genes. AKT ensures expression of nutrient transporters including ►GLUT1, which in turn contributes to nutrient uptake and ATP production from external sources. Under these conditions, anabolic processes like translation and cell growth are induced, whereas β-oxidation and autophagic turnover are repressed. Not shown: the small GTPase ►Rheb and its GTPase activators, the tuberous sclerosis complex (TSC) proteins are involved upstream in TOR signaling. (b) In the absence of growth factor signaling and under conditions of nutrient deprivation, cell survival is ensured by autophagy. Cell surface nutrient expression is shut down and TOR kinase is inactive. Autophagy provides energy from within the cell by recycling as an alternative to external sources. Excess autophagy can also lead to cell death rather than survival. Redrawn from Nat Rev Mol Cell Biol 6, 442.

is monoallelically deleted in a number of human breast, ovarian, and prostrate cancers. Certain forms of cancer are associated with decreased autophagic activity. In line with these observations, tumor-suppressors like ►p53 or ►PTEN have a positive effect on autophagy, whereas proto-oncogenes like class I PI3K and AKT negatively affect autophagy. Consequently, inhibitors of autophagy have been suggested as anti-cancer drugs.

In cancer treatment, the survival supporting functions of autophagy are in conflict with its role in cell-death. Autophagy might help a cancer cell to survive during nutrient-limitation or to resist chemotherapy or radiation treatment. But autophagy might also support cancer treatment by promoting tumour cell death or the removal of apoptotic cells.

To find out whether the anticancer activity of drugs like tamoxifen, ►rapamycin or ►temozolomide can be (partially) attributed to their autophagy-inducing activity will remain an important area of research.

It is now clear that autophagy and cancer are intimately linked. But it is also becoming evident that autophagy, being a homeostatic as well as a stress-inducible cellular process, is regulated in a complex way in health and disease.

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Autoreactivity

Definition

Describes immune responses directed at self antigens.

►Sjögren Syndrome

Autosomal Dominant

Definition

An inheritance pattern in which the affected individual has one copy of a mutant gene (►allele) and one copy of normal gene on a pair of autosomal (nonsex-determining) chromosomes. The phenotype it gives will be expressed even if the gene is heterozygous. The chances

of an autosomal dominant disorder being inherited are 50% if one parent is heterozygous for the mutant gene and the other is homozygous for the normal gene.

Autotaxin

Definition
Is an enzyme with lysophospholipase D (lysoPLD) activity that hydrolyses lysophosphatidylcholine into lysophospatidic acid (LPA).

►Lipid Mediators

Auxiliary Tissue

Definition
Fatty tissue and lymph nodes in the armpit.

►Oncoplastic Surgery

Auxilin-2

►Cyclin G-Associated Kinase

Auxotrophy

Definition
Is the inability of some cells to synthesize a particular organic compound required for their growth; *auxotrophic* is the corresponding adjective.

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

Avian Erythroblastic Leukemia Viral Oncogene Homolog 2

►HER-2/neu

Awd in Drosophila

Definition
abnormal wing discs.

►NM23 Metastasis Suppressor Gene

Axin

Definition
Wnt/β-catenin pathway scaffold proteins; Axin1 and Axin2 mutated in many tumor types; Axin2 is a direct β-catenin/Tcf target gene. Axis inhibitor, a negative regulator of the oncogenic protein β-catenin.

►Wnt Signaling
►Daxx

Axon Guidance

Definition
The process by which repelling and attractive molecules regulate pathfinding and targeting of axons during development of the nervous system. Process by which axons and growth cones send out signals to identify their correct targets in the developing nervous system.

►Semaphorin
►Slit

Axonal Growth Cone

Definition
The tip of an extending axon. It functions similarly to a migrating cell and senses external factors (guidance cues), but as it moves forward it gradually elongates the axon in the rear.

►Semaphorin

Aza-arenes

Definition
Polycyclic aromatic hydrocarbons containing nitrogen at one of the ring positions.

►Tobacco Carcinogenesis

5-Azacytidine

Definition
5-Azacytidine represents an analogue of the cytosine nucleoside. It is incorporated into the DNA during replication. Inclusion of 5-azacytidine inhibits DNA methylation by the DNA methyl-transferase and results in the demethylation of the 5-Azacytidine containing sequence. DNA methylation controls gene transcription by regulating transcription factor binding.

►5-aza-2″-deoxycytidine
►Melanoma Antigens

5Y-Aza-2Y-Deoxycytidine

Definition
A nucleotide that can substitute for cytosine in DNA during replication but cannot be methylated as cytosine residues can; it is thus used as a drug in cells, animals or humans to cause demethylation of hypermethylated regions of genes.

►Methylation

5-Azadeoxycytidine

►A5-aza-2′ Deoxycytidine

5-aza-2´ Deoxycytidine

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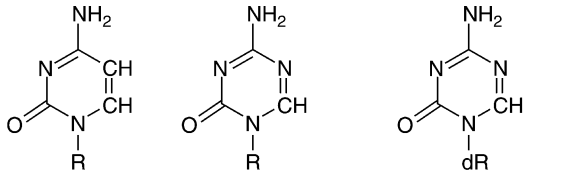
Synonyms
2′-Deoxy-5-azacytidine; Dacogen; Decitabine; 4-Amino-1-(2-deoxy-beta-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1H)-one; 5-Azadeoxycytidine; Deoxyazacytidine; Dezocitidine; DAC

Definition
Sorm and Vesely first described the pyrimidine analogs 5-aza-2′-deoxycytidine and 5-azacytidine in the mid-1960. They were generally considered to be in the antimetabolite class of anticancer agents; however, unique actions of 5-aza-2′-deoxycytidine clearly extend these molecules beyond the realm of most other anti-metabolites. 5-aza-2′-deoxycytidine and 5-azacytidine may be better considered as the prototypical agents of an emergent class of therapeutic agents, epigenetic modifiers (►Epigenetics, epigenetic therapy). The most notable epigenetic effect of these 5-aza-pyrimidines is their ability to demethylate the DNA of proliferating cells, and these drugs are now classified as DNA demethylating agents (►methylation). To attain therapeutic efficacy, enzymatic activation of 5-aza-2′-deoxycytidine to its triphosphate form and incorporation into replicating DNA must occur.

Characteristics
History
Following its synthesis in the early 1960s, 5-aza-2′-deoxycytidine and its close relative 5-azacytidine went through extensive laboratory characterization (Fig. 1). 5-aza-2′-deoxycytidine and 5-azacytidine were shown to be bacterio- and viriostatic, mutagenic, immunosuppressive, antimitotic, and antineoplastic. At the cytotoxic doses examined in the early studies, 5-aza-2′-deoxycytidine and 5-azacytidine altered the activity of multiple enzymes involved in nucleic acid metabolism, and inhibited DNA, RNA, and protein synthesis. 5-aza-2′-deoxycytidine only incorporates into DNA, while



Cytidine 5-azacytidine 5-aza-2′-deoxycytidine

5-aza-2′ Deoxycytidine. Figure 1 Chemical structures of cytidine and two therapeutic analogs, 5-azacytidine and 5-aza-2′deoxycytidine. R: ribose, dR: deoxyribose.

5-azacytidine incorporates into DNA and RNA. 5-aza-2′-deoxycytidine is approximately 10 times more potent than 5-azacytidine, reaffirming that DNA is a critical site of drug action.

Clinical studies to determine the potential role of 5-aza-2′-deoxycytidine and 5-azacytidine as cancer chemotherapeutic agents quickly followed, and their efficacy assessed in various human malignancies. Results from these early clinical studies indicated that these drugs had activity in acute myelogenous leukemia (AML); however, the significant toxicities encountered coupled with the emergence of 1-beta-D-arabinofuranosylcytosine (cytosine arabinoside, araC) for the treatment of AML limited the widespread use of these 5-aza-pyrimidines.

Contemporary Research

Later, investigations found that 5-aza-2′-deoxycytidine and 5-azacytidine had distinctive properties. One example was the striking observation that treatment with 5-azacytidine was able to generate functional striated muscle cells from non-myoblast precursors in a process that resembled a phenotypic conversion. Jones and Taylor shortly thereafter linked this 5-azacytidine-induced phenotypic conversion with inhibition of DNA methylation. Specifically, drug treatment at minimally- or non-cytotoxic doses caused a decrease in genomic levels of 5-methylcytosine. These changes in 5-methylcytosine levels were subsequently shown to induce changes in gene expression. Thus, 5-azacytidine possesses unique properties that produce a transcriptional reprogramming of the cell resulting in significant changes in cell phenotype. This transcriptional reprogramming activity resides in the ability of 5-azacytidine to directly affect the epigenetic landscape of cells. The discovery of these properties has contributed greatly to understanding the therapeutic mechanism of action of these drugs, and has led to a reinvigoration of basic and clinical research on 5-aza-2′-deoxycytidine and 5-azacytidine.

The work by Jones and Taylor also showed that some, but not all, pyrimidine analogs were capable of inhibiting DNA methylation. The few pyrimidine analogs that could inhibit DNA methylation each had modifications at the 5C position of the pyrimidine ring (pseudoisocytidine, 5-fluoro-2′deoxycytidine, and of course, 5-aza-2′-deoxycytidine). Pyrimidine analogs not capable of inhibiting methylation included 6-azacytidine and the clinically active drug, 1-beta-D-arabinofuranosylcytosine. Importantly, the pyrimidine analogs that did not inhibit DNA methylation also did not induce a phenotypic switch. Taken together this work indicated that drug-induced epigenetic changes were not a general effect of pyrimidine analogs, but instead novel mechanisms of action unique to specific compounds.

The primary mechanism by which 5-aza-2′-deoxycytidine and 5-azacytidine decreases 5-methylcytosine levels in mammalian cells is by inhibition of the enzyme responsible for the faithful maintenance and transmission of DNA methylation patterns from one cell generation to the next, DNA methyltransferase I (DNMT1). The DNA methylation maintenance activity of DNMT1 is S-phase specific as is incorporation of 5-aza-2′-deoxycytidine into DNA. Following incorporation into DNA, the 5-aza-pyrimidines act as irreversible inhibitors of DNMT1 and rapidly deplete the cell of this enzymatic activity. The inhibition of this enzyme results in the inability to maintain faithfully DNA methylation levels and patterns in subsequent cell generations. This information indicates that actively proliferating cells are likely to be the most sensitive to the effects of these compounds on epigenetic control systems.

There are additional DNA methyltransferase found in mammalian cells, such as DNMT3a and DNMT3b. These DNMT enzymes appear to be largely responsible for *de novo* methylation of DNA and not maintenance methylation. Although the data is not yet as decisive as it is for DNMT1, it appears that these enzymes are also inhibited by 5-aza-2′-deoxycytidine. Additionally, considering that the enzymatic mechanism of action for DNMT3a and 3b is very similar DNMT1, it is likely that these DNA methyltransferases are also targets of 5-aza-2′-deoxycytidine and 5-azacytidine. What role the inhibition of these enzymes plays in the therapeutic efficacy of the 5-aza-pyrimidines is yet unclear.

5-methylcytosine, in collaboration with other epigenetic marks, serves as a part of an epigenetic switch involved in the control of genome function. The precise control of these epigenetic switches are crucial to normal cellular differentiation and proper organism development, since they play functional roles in X chromosome inactivation, the regulation of tissue-restricted genes, gene imprinting, and chromosomal stability. Disruption of the epigenetic switches, reflected in changes to the normal levels and patterns of 5-methylcytosine, are mechanistically linked to human cancer, and have now been implicated in a number of other complex human diseases including diabetes, cardiovascular disease, autoimmune syndromes, and psychiatric and behavioral disorders.

The normal levels and patterns of 5-methylcytosine is non-random in the mammalian genome. In mammalian cells, 5-methylcytosine occurs only in cytosines found in the nucleotide doublet, 5′-CpG-3′. In general, normal levels and patterns of DNA methylation can be viewed as follows. It is estimated that 60–80% of CpG cytosines are methylated. High copy satellite sequences in pericentromeric heterochromatin are heavily or completely methylated. Other mid to high copy sequences such as ALU sequences, on average, are also heavily methylated. In most single copy genic and intergenic regions of the genome, CpG cytosines are variably methylated. Regions in which CpG cytosines are not methylated in normal genomes are ►CpG islands. CpG islands are areas of the genome that are relatively CpG-rich, having about a threefold overrepresentation of CpG dinucleotides when compared to the genome overall. CpG islands average about 1 kb in size, but can range from 200bp to greater than 10kb. CpG islands are often found in the 5′ end of genes and overlapping the transcription start site, and therefore are coincident with gene regulatory sequences. It has been estimated that 50% of human genes have CpG islands at their 5′ ends.

In cancer, the normal levels and patterns of DNA methylation are disrupted. Overall, there is a generalized DNA ►hypomethylation of the cancer genome when compared to its healthy counterpart. The relative hypomethyaltion is largely driven by a decrease in the levels of DNA methylation seen in high copy elements, such as satellite sequences. While the overall cancer genome becomes hypomethylated, some areas of the genome become aberrantly methylated or hypermethylated. The genomic targets of hypermethylation are frequently CpG islands, and thousands of these CpG islands can become hypermethylated during ►carcinogenesis. The aberrant methylation of these CpG islands is linked to the acquisition of a repressive chromatin state and the inappropriate silencing of the associated gene. Common and important CpG island targets of aberrant DNA methylation are those associated with genes that have tumor suppressor functions, including those involved in cell cycle control, DNA repair, invasion and metastasis, and apoptosis (►tumor suppressor genes). As such, aberrant DNA methylation of CpG islands is an epigenetic mechanism involved in gene inactivation, in much the same way that mutation and deletion are genetic mechanisms involved in gene inactivation in cancer, except that epigenetic inactivation can be a reversible process (►Epigenetic gene silencing).

The therapeutic activity of 5-aza-2′-deoxycytidine and 5-azacytidine is thought to involve their ability to reactivate these critical genes and restore their function. In support of this idea are a variety of studies that show genes associated with aberrantly methylated CpG islands do reactivate following treatment with the 5-aza-pyrimidines, albeit often not to gene expression levels seen in normal tissue counterparts. This gene reactivation is often linked to a demethylation of the associated CpG island, providing further support for an epigenetic mechanism of action of the 5-aza-pyrimidines. Indeed, successful clinical trials that tested 5-aza-2′-deoxycytidine and 5-azacytidine in patients with myelodysplastic syndrome also monitored and detected changes in the DNA methylation state of the CpG island promoter associated with the p15 tumor suppressor gene. Thus, basic epigenetic mechanisms of action operative *in vitro* now extend to the *in vivo* situation, as well.

While 5-aza-2′-deoxycytidine and 5-azacytidine have clear effects on DNA methylation, they may very well have multiple mechanisms of action, and there is experimental evidence to support this possibility. For example, 5-aza-2′-deoxycytidine can induce expression of genes lacking CpG methylation, such as p21WAF1, CDKN2D and APAF-1 and 5-azacytidine has also been shown to activate gene expression in an organism lacking DNA methylation). One alternative mechanism of action is reactivation of the tumor suppressor genes maspin and desmocollin-3 in breast cancer cell lines. In this case, gene reactivation was directly linked to significant decreases in a different repressive epigenetic modification – di-methylation of the K9 residue of histone H3. Indeed, not only did 5-aza-2′-deoxycytidine reduce H3 K9 di-methylation levels in the CpG island promoters of maspin and desmocollin-3, drug treatment also led to global decreases in H3 K9 di-methylation, as well as in the key enzyme responsible for this H3 K9 di-methylation, G9A (EHMT2).

In summary, contemporary research on 5-aza-2′-deoxycytidine and 5-azacytidine demonstrates that the future clinical utility of these compounds likely resides in their ability to alter the epigenetic landscape of diseased cells. This drug-induced change in the epigenetic landscape produces a transcriptional reprogramming of cells, and results in a variety of potential phenotypic outcomes that include, differentiation, loss of malignant properties, or even programmed cell death. Reversal of aberrant DNA methylation is clearly one epigenetic mechanism by which these 5-aza-pyrimidines produce this effect; however, recent work suggests that the reversal of other aberrant epigenetic marks may also participate in the cellular response.

Clinical Research

Renewed clinical interest in 5-aza-2′-deoxycytidine and 5-azacytidine grew out of results from phase I/II, II and III clinical trials in patients with myelodysplastic syndrome (MDS). These studies, conducted in the 1990s and early 2000s, emphasized low dose approaches that would more selectively target the epigenetic mechanisms of actions of 5-aza-2′-deoxycytidine

and 5-azacytidine, and are in stark contrast to the high dose approaches taken in the clinical trials of the 1970s that focused on standard cytotoxic activity. Using the low dose approach, multiple independent clinical groups largely obtained similar positive clinical results.

The common conclusions drawn from these clinical trials were that treatment of MDS patients with low doses of 5-aza-2′-deoxycytidine or 5-azacytidine resulted in significantly higher response rates, an improved quality of life for the patient, a decrease in leukemic transformation, and improved survival compared with the standard intervention, supportive care. Analysis of molecular markers of patients before and after treatment indicated that 5-aza-2′-deoxycytidine and 5-azacytidine could induce DNA demethylation both globally as well as at target loci. Clinical data also suggested that patients with "high risk" chromosomal abnormalities, in particular, might benefit from this treatment strategy.

There has been clear progress in the clinical use of 5-aza-2′-deoxycytidine and 5-azacytidine. Their use in MDS produces hematologic improvement in about 50% of patients and results in an improvement in the quality of life. Unfortunately, complete and partial responses occur in only about 20% of patients and the duration of response remains under two years. Taken together these results indicate the clinical story of 5-aza-2′-deoxycytidine and 5-azacytidine is incomplete. Current efforts to improve their therapeutic efficacy include dose optimization based on their ability to modulate epigenetic markers of disease; identification of patients who may be more likely to benefit due to their molecular profile; and combining 5-aza-2′-deoxycytidine and 5-azacytidine with other epigenetic modifiers, such as histone deacetylase inhibitors (►histone deacetylases). Other strategies considered involve a general debulking of the tumor with cytotoxic agents followed by low dose 5-aza-2′-deoxycytidine or 5-azacytidine in an effort to drive differentiation of the remaining tumor cells to a nonmalignant state. It is likely that an increasingly detailed molecular dissection of clinical responders and non-responders will help investigators better understand the mechanisms governing response, and aid in the further development of therapeutic agents that target the epigenetic landscape.

►Cancer-Germline (CG) Antigens

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Aza-epothilone B

►Epothilone B Analogue

Azathioprine

Definition

Is a potent immunosuppressive drug that is converted to its active form in vivo and then kills rapidly proliferating cells, including lymphocytes responding to grafted tissues.

►Sjögren Syndrome

Aziridine

Definition

Reactive three sided organic molecule (C_2H_5N).

►Mitomycin C

Azoxymethane

Definition

A widely used synthetic carcinogen for inducing colon cancer in laboratory animals.

►Sulforaphane
►Conjugated Linolenic Acids

B Cell

Definition

A B cell, or B lymphocyte, is one of the two major types of lymphocytes. The antigen receptor on B lymphocytes, usually called the B-cell receptor, is a cell-surface immunoglobulin. On activation by antigen, B-cells differentiate into cells producing antibody molecules of the same antigen specificity as this receptor. B Cells play a major role in the body's response to foreign materials (viruses, bacteria, parasites, etc) by generating antibodies to fight these intruders.

►Fluoxetine

B-1 Cells

Definition

B-1 cells, also known as CD5 B cells, are a class of atypical, self-renewing B cells found mainly in the peritoneal and pleural cavities in adults. They have a far less diverse repertoire of receptors than do B-2 cells (also known as conventional B cells), which are generated in the bone marrow throughout life, emerging to populate the blood and lymphoid tissues.

►Sjögren Syndrome
►Omental Immune Aggregates

B7 Molecules

Definition

The major T-cell costimulatory molecules are the B7 molecules, B7.1 (CD80) and B7.2 (CD86). They are closely related members of the immunoglobulin gene superfamily and both bind to the CD28 molecule on T cells. They are expressed differentially on various antigen-presenting cell types.

►Sjögren Syndrome

B Symptoms

Definition

Constitutional symptoms consisting of fever >38°C, night sweats and/or unintentional weight loss of >10% the body weight over a period of up to 6 months. B symptoms are relevant in non-Hodgkin lymphoma and Hodgkin lymphoma staging and are related to tumor burden and prognosis.

►Diffuse Large B-Cell Lymphoma
►Malignant Lymphoma, Hallmarks and Concepts

BAC

Definition

Acronym of bacterial artificial chromosomes. A vector system used to clone large DNA fragments.

►ArrayCGH

BACH1 Helicase

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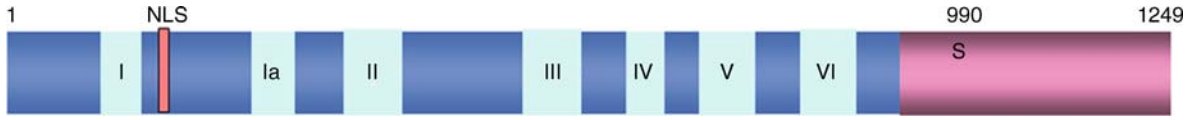
Synonyms

BRIP1; FANCJ



BACH1 Helicase. Figure 1 Schematic representation of BACH1. BACH1 is composed of 7 helicase domains (light blue) which is characteristic of the DEAH helicase, and its interaction with BRCA1 depends on the ►phosphorylation status of a serine residue (S) situated at the carboxy-terminal 990 position.

Definition

The ►BRCA1 associated Carboxy (C)-terminal helicase (BACH1) (Fig. 1) gene encodes a 1249 amino acid nuclear protein that is characteristic of the DEAH family of DNA helicases. BACH1 is an ATP dependent DNA helicase that catalyzes the destabilization of hydrogen bonds between complementary nucleic acids. The C-terminal region of BACH1, including a phosphorylated serine 990 residue, interacts directly with the C-terminal BRCT repeats of BRCA1. In addition, BACH1 also participates in the ►Fanconi anemia (FA) ►DNA damage response pathway as it was identified as the FA gene product, FANCJ.

Characteristics

BACH1 and Hereditary Breast Cancer

Breast cancer afflicts over 200,000 individuals each year, and is the leading cause of cancer death among US women with cumulative lifetime risk of one in nine. Hereditary breast cancer account for approximately 10% of all breast cancer, making it the most commonly inherited cancer in the US. Germline mutations in the Breast Cancer Associated genes *BRCA1* and *BRCA2* account for ~30–40% of these cases. Thus, research has focused on understanding how the BRCA gene products function to support normal cell growth and ultimately suppress tumor formation.

BACH1 was Identified by its Direct Interaction with BRCA1

BRCA1 is a nuclear phosphoprotein with an amino (N)-terminal zinc finger domain and C-terminal BRCT (*BR*CA1 *C*-Terminal) repeats. The integrity of the BRCT repeats is critical for BRCA1-mediated DNA damage response. BRCT region missense and deletion mutations disrupt the BRCA1 DNA damage repair function and are also associated with cancer predisposition. Thus, the integrity of the BRCT region is linked to BRCA1 tumor suppression. Consistent with this role, the BRCA1-BRCT repeats are highly conserved and are also present in other proteins involved in the DNA damage response. The BRCT repeats in BRCA1 and other proteins have been shown to bind phosphopeptides. In fact, the BRCA1-BRCT repeats bind directly to BACH1 when a phosphoserine residue (pSer990) at the C-terminal of BACH1 is phosphorylated.

BACH1 is Mutated in Hereditary Breast Cancer Patients

Due to the direct interaction between BRCA1 and BACH1, it was hypothesized that, like BRCA1, BACH1 may also function to suppress breast cancer development. Consistent with this hypothesis, early genetic screens identified two early-onset breast cancer patients with BACH1 germline mutations. Further analysis of these mutations *in vitro*, revealed that these sequence changes resulted in defective BACH1 protein disrupting the helicase activity. Recently BACH1 truncating mutations were identified in 9 out of 1212 breast cancer patients, whereas, similar mutations were only presented in 2 out of 2018 healthy individuals. Overall, these data suggested that BACH1 mutations conferred a two-fold risk in the development of breast cancer.

BACH1 is Important for Mediating the DNA Damage Response

The BRCA1/BACH1 interaction suggests that BACH1 likely contributes to BRCA1 DNA repair functions. BACH1 similar to BRCA1, following DNA damage is modified by phosphorylation, displays a BRCA1-like nuclear foci pattern and co-localizes with ►gamma (γ)-H2AX. The BACH1/BRCA1 complex is unaltered by DNA damage, but both proteins contribute to the localization of each other to DNA damage foci. In the absence of BRCA1, BACH1 fails to localize to site of DNA damage. While BACH1 is not required for BRCA1 localization to sites of DNA damage, the intensity of BRCA1 foci is diminished in BACH1 deficient cells. Furthermore, this interaction has been shown to be required for activation of the ionizing radiation (IR)–induced ►G2/M checkpoint. Cell cycle analysis revealed a G2/M checkpoint defect in BACH1 siRNA expressing HeLa cells that was corrected by expression of wild-type BACH1, but not with the S990A version of BACH1 disrupted for BRCA1 binding. BACH1 depleted cells also exhibit an elevated S-phase accumulation compared to control cells after treatment with low dose of aphidicolin, which is known to activate the intra ►S-phase checkpoint. BACH1's role in intra S-phase checkpoint was further validated when BACH1-deficient cells showed ►radioresistant DNA synthesis (RDS).

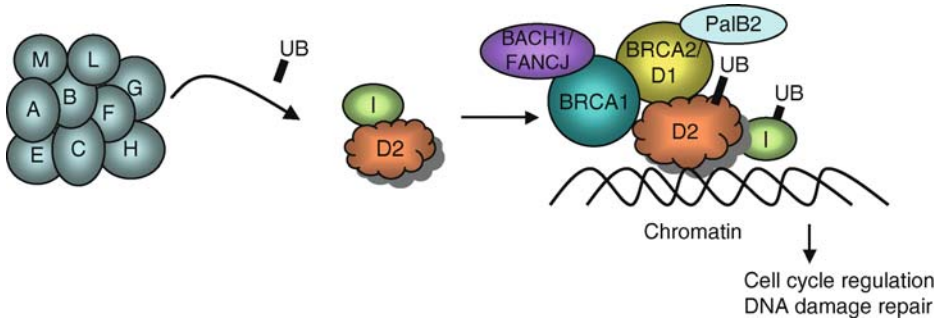
BACH1 is Required for DSBR

As part of the DNA damage response, BACH1 is required for the repair of ►DNA double strand breaks (DSBs). The contribution of BACH1 to double stranded break repair (DSBR) was first apparent through the observation that BRCA1 clinical mutations that disrupted its interaction with BACH1 also resulted in defective DNA damage repair. In addition, overexpression of a helicase inactive version of BACH1 (K52R) displayed a marked delay in repair, as shown by an increase in the level of unrepaired breaks detectable after IR. This repair delay was dependent on BACH1 binding to BRCA1 suggesting that the K52R BACH1 functions as a dominant negative to perturb DSBR in a BRCA1 dependent manner.

Moreover, similar to BRCA1, BACH1 promotes the repair of DSBs by promoting ►Rad51-dependent ►homologous recombination (HR). Specifically, BACH1's role in HR was examined using mammalian cell based homology directed repair assay, where it was found that suppression of either BRCA1 or BACH1 disrupted HR to a similar degree. In support of a role for BACH1 in HR, BACH1 is able to unwind D-loop recombination intermediates in vitro. Since BACH1 does not unwind holiday junction intermediates similar to other DNA repair helicases, such as Bloom syndrome (BLM) and Werner syndrome (WRN), BACH1 most likely has a distinct HR function. The D-loop is the initial structure formed in recombination when the Rad51 nucleoprotein filament invades the duplex DNA in search of homologous repair-template. Thus, Rad51 focal accumulation has been speculated to represent the development of these HR intermediates. Although suppression of either BRCA1 or BACH1 protein results in defective HR, one difference observed between BRCA1 and BACH1 deficient cells was the visualization of Rad51 foci following DNA damage. Rad51 foci formation in BRCA1 suppressed cells is dramatically reduced compares to control cells. However, BACH1 suppressed cells retain robust Rad51 foci. The unaltered presence of Rad51 foci, but reduced HR in BACH1-deficient cells suggests that some form of Rad51 based recombination is active. One possibility is that BRCA1 functions upstream of Rad51 and BACH1 functions downstream of BRCA1. Alternatively, it was proposed that BRCA1 regulates BACH1 helicase activity in a manner that regulates Rad51 foci formation. In this model, it was proposed that in the absence of BRCA1, BACH1 helicase activity would be unregulated and disrupting of Rad51 foci due to perpetual unwinding of recombination intermediates.

BACH1 and Fanconi Anemia

FA is a recessive disorder, in which, FA patients are characterized by congenital abnormalities, bone marrow failure, and predisposition to leukemia and solid tumors. FA is a multigenic disorder and to date, there are 12 FA complementation groups FA-A, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M (Fig. 2). Germline mutations in the BACH1 gene were identified in patients from the FA-J complementation group. FA-J cells, similar to other FA cells, exhibit chromosome abnormality and hypersensitivity to DNA interstrand crosslinking agents such as mitomycin C and cisplatin. Most likely FA proteins are critical for processing crosslinked DNA. In the absence of processing, ►interstrand cross links (ICLs) are extremely deleterious because DNA strands are covalently tethered, which can block DNA replication and compromise cell viability. Consistent with a role for the FA proteins in this process, cellular exposure to ICLs activates the FA core complex (FANCA, -B, -C, -E, -F, -G, -L and -M) and promotes the ►monoubiquitination of the effector protein, FANCD2. Activated FANCD2 translocates into chromatin and forms nuclear foci that co-localizes with BRCA1, BRCA2/FANCD1 and BACH1/FANCJ. Activation of the FA pathway through monoubiquitination of FANCD2 and FA protein relocalization are essential for coordinating the



BACH1 Helicase. Figure 2 The FA pathway is composed of 12 FA complementation groups, with the central feature being the monoubiquitination of FANCD2 in response to DNA damage. Activated FANCD2 translocates to chromatin associated foci where it localizes with BACH1/FANCJ, BRCA1 and BRCA2/FANCD1 to promote cell cycle regulation and DNA damage repair.

ICL repair response. BACH1/FANCJ is considered downstream of the FANCD2, since it is not required for FANCD2 monoubiquitination. The FA proteins, BRCA2/FANCD1 and the newly identified PALB2 proteins are also downstream of FANCD2. While the role of BACH1/FANCJ in the FA pathway is not yet clear, its helicase activity, but not BRCA1 binding activity appears to be essential to correct the ICL-response in chicken and patient cells.

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Bacillus Calmette-Guérin

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Synonyms

Mycobacterium bovis BCG; BCG

Definition

Bacillus Calmette-Guérin (►BCG) is a ►mycobacterium belonging to the Mycobacterium tuberculosis complex. It is a live attenuated organism and generally nonpathogenic for healthy human subjects. Originally developed as a vaccine against tuberculosis, since the mid 1970's it is increasingly used as a successful active immunotherapeutic agent in the treatment of nonmuscle invasive ►bladder cancer.

Characteristics

Microbiological Background on BCG and Tuberculosis Vaccination

BCG is an attenuated derivative of virulent *Mycobacterium bovis* (*M. bovis*), the causative pathogen of bovine tuberculosis. The name of BCG acknowledges the contributions of two French researchers Albert Calmette, a microbiologist, and Jean-Marie Camille Guérin, a veterinarian. Those two, between 1908 and 1921, passaged a virulent strain of *M. bovis* in an attempt to generate a vaccine against tuberculosis in humans. By using a continuous in vitro passage and a special bile-glycero-potato culture medium they succeeded in generating a nonvirulent mycobacterial strain that did no longer cause disease in experimental rodent animals. Shortly after these observations the vaccine was first applied to human subjects as a vaccine against tuberculosis. Although controversial since its first use, the vaccine has now been administered to over 3 billion people world-wide with an excellent safety profile. In spite of BCG's efficacy as a preventive vaccine in general, its highly variable efficacy in preventing pulmonary tuberculosis in adults (with some studies showing no protection at all) remains a major challenge.

The original live BCG vaccine required continuous culture and was spread over various continents for clinical application. By the time lyophilization was available as a method to preserve viable mycobacteria, several substrains of the original strain were created which are collectively known as BCG. Characteristic for BCG is its relatively slow growth in in vitro culture with doubling times between 8 and 36 h depending on the strain and the respective culture conditions. Modern molecular biological analyses are now used to follow the "in vitro evolution" of BCG vaccines. Although the genetic basis for BCGs attenuation has not yet been fully elucidated, several regions of genetic difference between virulent *M. tuberculosis* and BCG have been identified and characterized.

Today, for use in cancer ►immunotherapy, several substrains of BCG mycobacteria are available as freeze-dried lyophilisates with a guaranteed amount of viable bacilli at the end of the shelf-live, which usually exceeds 1 year. Easy clinical use is facilitated by the availability of aliquots pretitrated for one single instillation and delivered together with the respective dilution buffer.

BCG in Cancer Immunotherapy

At the end of the nineteenth century, the American Surgeon W. E. Coley observed occasional remissions of lymphosarcomas following local and/or systemic bacterial infections. Based on these observations, he used bacterial extracts for the adjuvant treatment of cancers of the head and neck which have become known as Coley toxin. Furthermore, it was noted that patients with tuberculosis rarely developed malignant neoplasms. These observations stimulated investigators to further explore the potential use of BCG for various anticancer applications.

In 1976, Morales et al. combined this experience and investigated a new form of application for BCG. They developed a schedule for the effective adjuvant intravesical treatment of nonmuscle invasive bladder tumors following transurethral resection (TUR) with BCG. Since that time, adjuvant BCG-immunotherapy for bladder cancer has remained largely unmodified. Additionally, a large number of prospective controlled phase III trials were published confirming the efficacy of BCG and strengthening its role in uro-oncology. Today, an estimated number of 1 million annual BCG treatments are given to cancer patients.

According to recent metaanalyses of published randomized clinical trials, the following conclusions can be drawn from these trials: (i) patients after TUR in combination with adjuvant BCG will suffer from less recurrences as compared to TUR alone, (ii) BCG-immunotherapy is superior to intravesical chemotherapy for the reduction of bladder cancer recurrences, and (iii) BCG is the only treatment option for preventing and/or delaying bladder cancer progression to muscle invasive disease.

BCG-immunotherapy is first applied at 1–3 weeks following complete TUR of the primary tumors. It consists of a 6-week induction cycle including weekly intravesical instillations of about 81 mg BCG reconstituted in 50 ml saline corresponding to $1–5 \times 10^8$ ►colony-forming unit (►CFU) viable mycobacteria. Retention of the BCG mycobacteria in the urinary bladder for not less than 2 h is recommended to ensure sufficient immunostimulation. While the efficacy of various BCG strains has never been compared in prospective trials, data from a recent metaanalysis suggest that at least the five most commonly used strains Tice, Pasteur, Connaught, RIVM and A. Frappier do not differ in terms of preventing tumor progression. Contraindications against the intravesical use of BCG are given in patients with a compromised immune status, active tuberculosis, acute urinary tract infections, lesions in the lower urinary tract, fever of unknown origin, a history of radiotherapy to the bladder and/or pelvis, and during pregnancy or active nursing.

Recent clinical approaches aimed at improving BCG efficacy by addition of a so-called maintenance therapy schedule. BCG maintenance consists of three intravesical instillations given in weekly intervals at 3, 6, 12, 18, 24, 30, and 36 months after initiation of the induction cycle. Initial data suggest that this modified treatment protocol provides additional benefit for preventing tumor recurrence and progression.

In addition to the current success in bladder cancer, historically, BCG has been used as an anticancer agent in a variety of other cancer types including ►melanoma and lung cancer among many others. However, in most cases these treatment regimens have not become standard clinical practice. With the identification of ►toll-like receptor agonist structures present on the BCG surface, an immunological rationale for the well known ►adjuvant effect of BCG has now emerged. Currently, a variety of approaches are trying to utilize the immunostimulatory effect of BCG and its subcomponents as additives in autologous tumor vaccines.

Mode of Action in Antitumor Activity

When BCG was first used in clinical practice more than 30 years ago, its mode of action has been far from understood. Progress in the fields of infection biology, ►innate immunity, and tumor immunology has set the stage for a better understanding of the mechanism of BCG's antitumor activity. Mechanistic and descriptive studies in vitro, in rodent bladder cancer models and with patient material have provided a clear picture of the processes leading from instillation of mycobacteria into the bladder lumen to antitumor effects.

Based on in vitro studies initial data have suggested a direct antiproliferative, cytotoxic, or proapoptotic effect of BCG on tumor cells. While this effect can certainly be demonstrated in vitro, mechanistic studies in rodent models now provide convinving evidence that a functional immune system of the tumor-bearing host is absolutely essential for most of BCGs antitumor activity in vivo.

BCG mycobacteria have a characteristic outer cell-wall consisting of various complex structural biomolecules. Microbiologists, biochemists, and immunologists have identified and characterized the interaction of a variety of those biomolecules with their respective-receptors on target cells of the host. Collectively, immunologically active parts of those molecules are today referred to as ►pathogen-associated molecular patterns (PAMP). Those PAMPs on the mycobacterial surface are biochemically quite diverse and, for example, include peptidoglycan, mycolic acids, lipomannan, and lipoarabinomannan. Although the role of the mycobacterial surface molecules is better defined in infection biology, it can be assumed that initial contact to primary host target cells in physiologic mycobacterial infection and in instillation immunotherapy is mediated by similar mechanisms.

In BCG-immunotherapy of bladder cancer the vast majority of the original instillation dose of several hundred million of mycobacteria will be washed out with the first postoperative micturition. The remaining mycobacteria will adhere to the bladder wall. This contact will lead to an activation of epithelial cells in the bladder, which can respond with the release of immunologically active mediators such as ►cytokines-like interleukin-(IL-)8, IL-6, and IL-1. Within days after instillation BCG initiates a complex inflammatory cascade in the bladder wall resulting in the enhanced production of a vast array of cytokines and ►chemokines. Most of these cytokines and chemokines

are proinflammatory or promoting a so-called ►T-helper 1 (Th1) response associated with the induction of cell-mediated immunity. Animal experiments have shown that this Th1 response is essential for induction of protective antitumor immunity. The local inflammatory response (►inflammation) in the bladder tissue consists of different phases and changes in the cellular composition of the bladder wall become apparent already a few hours after instillation and can last for up to several month in human patients. A large number of studies have analyzed the cellular immune response (►Th1 response) induced by BCG in immunotherapy of bladder cancer. Collectively, these studies suggest the following scenario: In an immediate and early inflammatory phase, the cellular infiltration of the bladder wall is dominated by ►neutrophil granulocytes (Neutrophils) known to the immunologist as prototypic inflammatory cells. This neutrophilic infiltration is characteristic for the early postinstillation urine and the cellular composition of the urine early after instillation is dominated by more than 90% granulocytes. Polymorphonuclear neutrophil granulocytes (PNG; PMN) then direct in a second phase the subsequent influx of mononuclear cells including macrophages, T cells, and NK cells by mechanisms which are only beginning to emerge. The complex interplay of these immunocompetent cells mediates potent antitumor effects and mechanistic studies have shown that concurrent activation of different effector cell populations is required for full therapeutic efficacy. In a third chronic inflammatory phase, granuloma-like structures of cellular infiltration can persist for up to several month possibly representing a long-lasting immunostimulatory event.

Inspired by the plethora of immune mediators induced during BCG–immunotherapy, researchers have tried to identify a prognostic immunologic marker which could be used to predict a clinical response to this type of immunotherapy. However, until now no definite prognostic immunologic marker has been identified, although certain good candidates (e.g., IL-2) do exist and will be further explored in the future.

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Bacteriophage

Definition

A bacteriophage is a virus that infects bacteria.

►Phage Display

Bacteriophage Display

►Phage Display

Baculoviral IAP-repeat Containing Protein 5

►Survivin

Baculovirus

Definition

Is a pathogen that attacks insects and other arthropods. Like some human viruses, they are usually extremely small (less than a thousandth of a millimeter across), and are composed primarily of double-stranded DNA that codes for genes needed for virus establishment and reproduction.

BAD

Definition

Is a Bcl-2 antagonist of ►apoptosis/death, a pro-apoptotic member of the Bcl-2 family of proteins. It is a 18 kD protein that binds to Bcl-xL and Bcl-2. It competes out the binding of Bcl-xL and Bcl-2 to Bax, thus promoting apoptosis.

BAF47

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

Bak

Definition

Bcl2-antagonist/killer 1, a member of Bcl-2 protein family. Bak functions in a similar manner as Bax to induce ►apoptosis.

►PUMA (p53 Upregulated Modulator of Apoptosis)

BAK1

Definition

synonym: Bak-1, Is a Bcl-2 antagonist/killer-1, a pro-apoptotic member of the Bcl-2 family of proteins. It is a 23 kD protein that binds to and antagonizes Bcl-2. It also forms heterodimers with anti-apoptotic Bcl-xL and binds to adenovirus E1b19k.

►Apoptosis

BALT

Definition

The lymphoid cells and organized lymphoid tissue in the respiratory tract are termed the bronchial-associated lymphoid tissues (BALT). These tissues are very important in the induction of immune responses to inhaled antigens and to respiratory infection.

►Sjögren Syndrome

Bannayan-Riley-Ruvalcaba Syndrome

Definition

Is a rare inherited disorder characterized by excessive growth before and after birth; an abnormally large head (macrocephaly) that is often long and narrow (scaphocephaly); normal intelligence or mild mental retardation; and/or benign tumor-like growths (hamartomas) that, in most cases, occur below the surface of the skin (subcutaneously). The symptoms of this disorder vary greatly from case to case. Additional abnormalities associated with this disorder may include abnormal skin coloration (pigmentation) such as areas of skin that may appear "marbled" (cutis marmorata) and/or the development of freckle-like spots (pigmented macules) on the penis in males or the vulva in females. In some cases, affected individuals may also have skeletal abnormalities and/or abnormalities affecting the muscles (myopathy). Bannayan-Riley-Ruvalcaba syndrome is inherited as an autosomal dominant genetic trait. Bannayan-Riley-Ruvalcaba is the name used to denote the combination of three conditions formerly recognized as separate disorders. These disorders are Bannayan-Zonana syndrome, Riley-Smith syndrome, and Ruvalcaba-Myhre-Smith syndrome.

BAR Domain

Definition

Banana-shaped domain seen in adapter proteins that bind and tubulate cellular membranes during the dynamic processes of endocytosis, vesicle trafficking, organelle fission, and specialized membrane formation events (e.g. muscle T tubules). BAR domains also bind small GTPases and their regulators as well as phosphatidylinositol lipid binding proteins.

►Bin1

BARD1

Irmgard Irminger-Finger
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Synonyms

BRCA1-associated Ring domain (gene/protein) 1

Definition

BARD1 codes for a protein that forms a functional heterodimer with the breast cancer predisposition gene product ►BRCA1. This BRCA1–BARD1 complex has ►ubiquitin ligase activity, but not the respective monomers. The specific targets of the BRCA1–BARD1 ubiquitin ligase relate to the tumor suppressor functions of BARD1 and BRCA1. ►BRCA1-independent functions are attributed to BARD1, based on its role as inducer of ►p53-dependent apoptosis.

Characteristics

The tumor suppressor BARD1 was originally identified as a protein binding to the BRCA1 gene product, BRCA1. BRCA1 depends on binding to BARD1 for most of its tumor suppressor functions mostly through the ubiquitin ligase activity of the BRCA1–BARD1 heterodimer. This is presumably due to the fact that the stability of both proteins depends on their interaction via their N-terminal ►RING finger domains.

BARD1 Structure, Conservation, and Expression

BARD1 is a RING finger protein, like BRCA1, and both have ►BRCT domains at their C-terminus. *BARD1* genes have been found in several species: mouse, rat, *Xenopus*, *C. elegans*, and a database entry is found for the tropical fish *Takifugu rubripes*. While the N-terminal RING finger and the BRCT domains of BARD1 are evolutionary conserved with at least 90% identity of amino acids, the regions between these structures show only little conservation (Fig. 1). In addition to these two conserved domains, BARD1 possesses ►ankyrin (ANK) repeats that are found in proteins of diverse functions. Besides similar structure, BARD1 has several features in common with BRCA1: embryonic lethality of knock out in animal models, and genetic instability in cellular model systems of BARD1 or BRCA1 depletion.

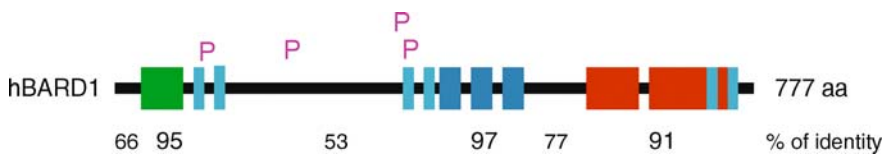
BARD1 is composed of 11 exons. First observation of ►alternate splice variants was BARD1β in pre-leptotene ►spermatocytes, while the wild-type form of BARD1 was expressed in ►spermatogonia. An isoform BARD1δ, derived form ►differential splicing, was found in a rat ovarian cancer cell line and in HeLa cells.

BARD1 mRNA expression was found in most proliferative tissues, mostly parallel with the expression pattern of BRCA1. However, in hormonally regulated tissues BARD1 expression was different from BRCA1. Further evidence for hormonally controlled expression of BARD1 was found in ►spermatogenesis. BARD1 expression is also induced by hypoxia.

Functions of the BARD1–BRCA1 Heterodimer

BARD1 and BRCA1 interact with a number of proteins involved in various functions, most cited are ►homologous repair and ubiquitin ligase activity. Dissection of repair pathways showed that the BRCA1–BARD1 heterodimer has a role in homologous repair before the branch point of HDR (homology derived repair) and SSA (single strand annealing).

The RING finger domains of BARD1 and BRCA1 are required for ubiquitin ligase functions, and missense mutations in the BRCA1 RING finger that abrogate this ubiquitin E3 ligase function, are found in breast and ovarian cancer, suggesting that the ubiquitin ligase activity is linked to tumor suppressor functions. Specifically, BARD1 protein residues 8-142 could enhance ubiquitin ligase activity of BRCA1. The results of mutagenesis studies indicate that the enhancement of BRCA1 E3 ligase activity by BARD1 depends on direct interaction between BARD1 and BRCA1. Across its ubiquitin ligase activity, the BRCA1–BARD1 heterodimer controls cell cycle progression and exerts tumor suppressor functions. BARD1–BRCA1 might also regulate cell cycle progression by interacting with another potential target for ubiquitinations, PolI, or the ►nucleolar protein nucleophosmin/B23 (NMP).



BARD1. Figure 1 Human BARD1 domain structure. RING (green), ANK (blue), and BRCT (red) domains are indicated and location of potential NLS (light blue) and phosphorylation sites (P). Evolutionary conservation is indicated as percentage of identical amino acids between human and mouse BARD1 sequences within distinct regions.

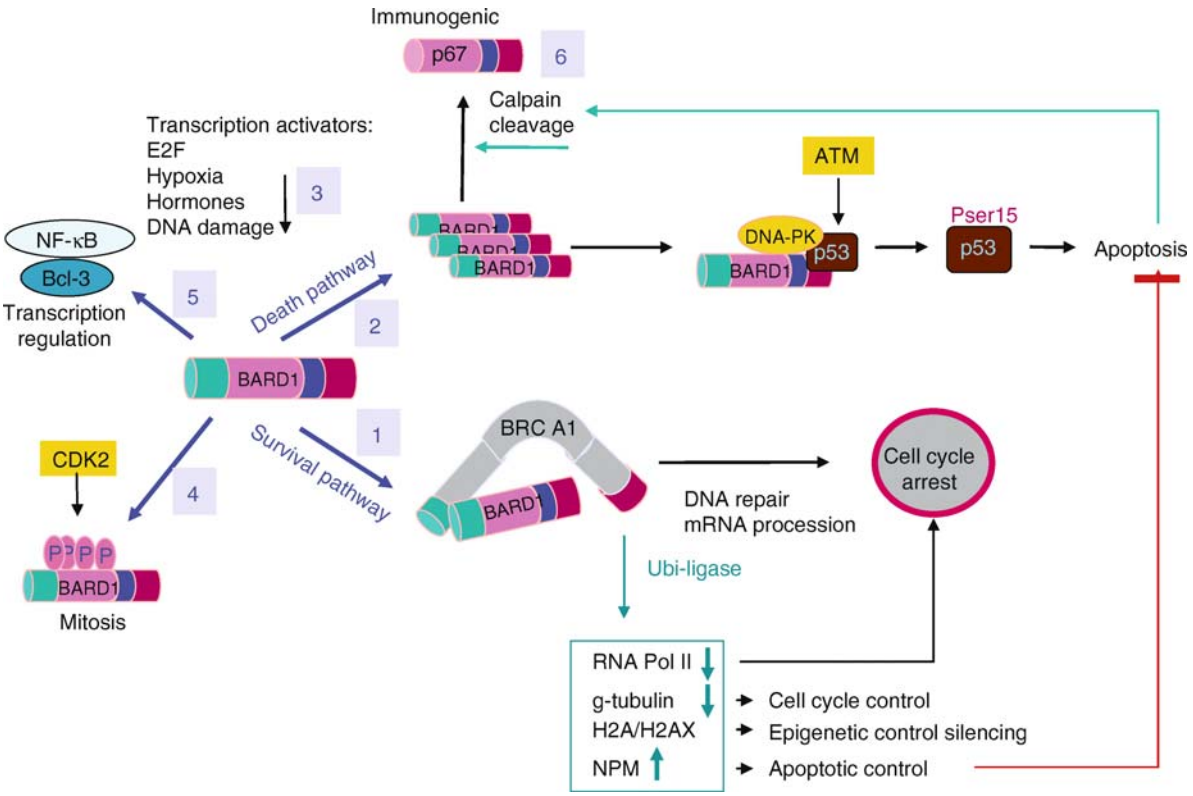
A function in regulation of transcription is exerted via BARD1 binding to the ►Bcl-3 oncoprotein, which acts as a bridging factor between ►NF-κB/Rel and nuclear coregulators. The functional interaction of BARD1 with polyadenylation factor ►CstF-50, provides another possible regulatory mechanism by linking ►mRNA 3′ end formation to DNA damage and tumor suppression.

BRCA1-Indendent Tumor Suppressor Function of BARD1

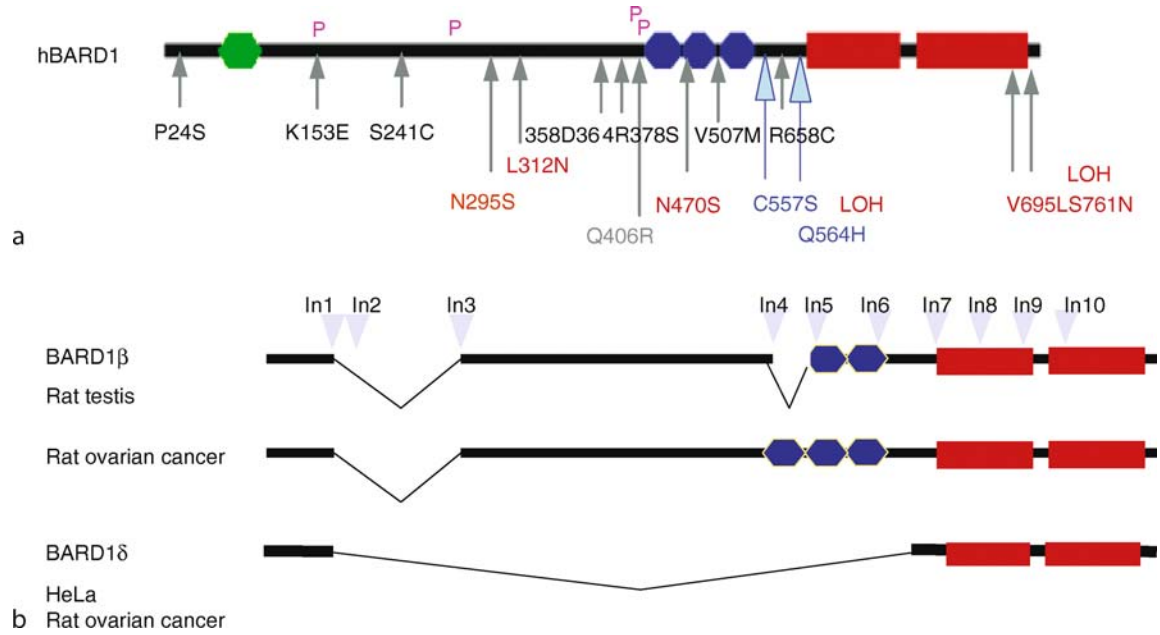
Independently of BRCA1, BARD1 acts in an ►apoptosis pathway by binding and stabilizing ►p53. The mechanism of p53-dependent apoptosis, induced by BARD1, is based on BARD1 binding to the ►Ku-70 subunit of ►DNA-PK and to p53 thus facilitating or catalyzing the phosphorylation of p53 on serine 15. This phosphorylation is required for stabilization of p53 and for initiation of apoptosis. An excess of BARD1 over BRCA1 can induce apoptosis and binding to BRCA1 inhibits BARD1 apoptotic function. Upregulation of BARD1 expression can be induced by various types of cellular stress.

BARD1 deletions that lack the RING finger domain are stable, suggesting that the RING finger is targeting BARD1 for degradation, and proteolytic cleavage of the RING finger leads to the apoptosis pathway. During apoptosis BARD1 (97 kDa) translocates to the cytoplasm where it is cleaved to become the more stable p67, and consistently the apoptotic function of BARD1 was mapped to a region within p67, including ANK, the regions between ANK and BRCT, and part of BRCT.

Therefore BARD1 acts as a cellular switch from repair mode with BRCA1 and moderate BARD1 levels to apoptosis without BRCA1 and excessive BARD1 levels (Fig. 2).



BARD1. Figure 2 Major BARD1 and BRCA1–BARD1 pathways and functions. BARD1 participates in several pathways: (1) survival pathway as BRCA1BARD1 heterodimer and (2) death pathway in a BRCA1-independent function in apoptosis. BRCA1–BARD1 acts in survival pathway. BRCA1–BARD1 ubiquitin ligase activity leads to RNAPolII degradation and cell cycle arrest, to γ-tubulin degradation and regulation of centromere duplication, to H2A/H2AX ubiquitylation and epigenetic control, and to NPB ubiquitylation. Upregulated NPB is a known inhibitor of apoptosis, it causes centromere amplification and genetic instability; hence NPM antagonizes BARD1 functions. (3) BARD1 is transcriptionally upregulated by different conditions, (4) is modified upon apoptosis by phosphorylation, (5) and can modulate NF-kb function. The proteolytic cleavage product p67 is immunogenic and has antitumorigenic properties (6).



BARD1. Figure 3 BARD1 mutations and isoforms. (a) Schematic drawing of BARD1 protein structure with RING (green), ANK (blue), BRCT (red) motifs indicated. Phosphorylation sites are marked with P. Mutations are marked in red and polymorphisms in black underneath. The Q406R mutation was found recently in ovarian cancer. (b) Splice variants found for *BARD1*: BARD1β in preleptotene spermatocytes, BARD1δ in ovarian cancer cell line and in HeLa cells. The N-terminal exons 1–5 were frequently lost in ovarian and ovarian cancer cells cancer, as shown by immunohistochemistry and RT-PCR.

BARD1 in Mitosis

A role of BARD1 in mitosis was suspected, since BARD1 protein levels are increased in mitosis. Mice deficient for BARD1 are embryonic lethal, and embryos die at day 8 of embryonic development due to deficient proliferation. A novel function of BARD1–BRCA1 in mitosis implies interaction with the ►Ran-GTPase and provides an explanation for the genetic instability and lethality observed in BARD1 repressed cells and embryos.

Clinical Relevance

BARD1 Mutations in Cancer

Interestingly, mutations in *BRCA1* occur all over the protein-coding region, leading to truncated presumably unstable proteins. Missense mutations are mostly found in the RING finger and disrupt the BRCA1–BARD1 interaction, suggesting that the BRCA1 function is linked to the function of the BARD1–BRCA1 heterodimer. On the contrary, mutations in BARD1 are found mostly around the ANK repeats, the BRCT domains and the region in between these domains (Fig. 3). This C-terminal region has been shown to be stable in the absence of BRCA1 and to play a role in apoptosis, which suggests that BARD1 has tumor suppressor functions independently of BRCA1.

Considering the multiple functions of *BARD1* as tumor suppressor, it is expected that its functions might be lost or abrogated in cancer cells. Surprisingly, in human breast and ovarian tumors, elevated expression and mislocalization to the cytoplasm of BARD1 was found, as compared to the healthy tissue. Most samples showed 5′ truncations and the upregulation of BARD1 isoform expression in cancer was correlated with poor prognosis.

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Barrett Esophagus

Definition

Barrett esophagus is the metaplastic epithelial lining of the distal esophagus, recognized as a premalignant condition.

►Fluorescence Diagnostics
►Esophageal cancer

Barrett High-grade Dysplasia

Definition

►Barret esophagus.

Basal Cell Carcinoma

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Definition

Non-melanoma skin cancers are the most common malignant neoplasms in the United States, representing one third of all cancers diagnosed every year. Basal cell carcinoma (BCC) represents 75% of non-melanoma skin cancers and has an estimated annual incidence of more than 700,000 cases in the United States outnumbering ►squamous cell carcinoma (SCC) 4–1. Over half a million BCCs are diagnosed in the United States annually, outnumbering squamous cell carcinoma (SCC) 4–1. The US average annual incidence of BCC in whites is currently 191 per 100,000 and is increasing at a rate of 3–7% per year. The peak incidence of BCC occurs in the seventh decade of life and is rare in children.

Characteristics

Basal cell carcinoma has multiple distinctive clinical forms, and these clinical subtypes can often be correlated with histologic subtypes. BCC is protean in its manifestations. For this reason, biopsy for histologic confirmation is necessary. The various clinical forms of BCC include nodular, morpheaform and superficial. Nodular BCC is the most frequent form of BCC. It usually presents as a waxy, pearly or translucent papule/nodule with overlying fine telangiectasias, with frequent ulceration or erosion of the surface. BCCs may occasionally be pigmented to varying degrees. Superficial BCCs most commonly arise on the trunk and extremities, but may be seen anywhere on the body. The tumors are characterized by an erythematous macule or patch, which may be variably pigmented. There may also be an overlying fine scale, a superficial erosion or hemorrhagic scale crust. Superficial BCC is the variant most frequently seen in chronic arsenism and as late sequelae of radiation therapy. Individuals may have broad areas of superficial BCC that are multiple and disconnected. Morpheaform or sclerosing has a scar-like appearance. It consists of a dermal plaque with overlying epidermal atrophy in a sun-exposed distribution. As with infiltrative BCC, subclinical extension is often great and treatment failures frequent.

Risk Factors and Therapy

Location, histologic subtype, clinical characteristics and size are predictive factors for the biologic behavior of BCCs. The typically indolent growth pattern of BCC accounts for the resistance and fusion planes of the central facial zone being a more significant determinant of subclinical extension. Size is also a good predictor of high risk BCCs. Cure rates with ►Mohs micrographic surgery (MMS) decreases as tumor size increases. A cure rate of 99.8% for tumors less than 2 cm in diameter, 98.6% for tumors between 2 and 3 cm and 90.5% for tumors greater than 3 cm has been reported.

Micronodular, infiltrative and ►morpheaform BCCs have a much higher incidence of positive surgical margins after surgical excision (18.6–33.3%) as compared with tumors with a nodular or superficial histologic pattern. Morpheaform BCCs may have significant subclinical extent, with the average subclinical extension being 7.2 mm. Similarly, significant subclinical extension in infiltrative BCC has been noted. BCC with marked squamous differentiation has been determined to be a more virulent tumor (a local recurrence rate of 45.7% and metastatic incidence 8.6% of 35 such tumors as compared to rates of 24.2%/0.09% for BCC). As with SCC, the perineural space can serve as a conduit for significant subclinical tumor extension.

Although BCC is rarely life threatening its capacity for local tissue destruction can result in significant functional or cosmetic morbidity. Untreated or inadequately treated BCCs have an insidious growth pattern and may result in death. ►Metastasis from BCC is a rare event, with estimates of metastatic incidence ranging from 0.0028% to 0.1%. Metastasis is associated with the metatypical (basosquamous) BCC and with duration and size of the lesion. The most frequent site of

metastasis is the lungs, followed by bone, lymph nodes, and liver. For these reasons, great importance is attached to the early diagnosis and treatment of this malignancy.

BCC is related to chronic ultraviolet radiation (►UV radiation) exposure. UVR exposure is partly responsible for both BCC and SCC, as evidenced by the preponderance of these lesions on sun-damaged skin after chronic exposure to sunlight. More than 99% of individuals developing BCC are Caucasians, and 85% of these tumors arise on the head and neck. The nose is most common of all sites, accounting for 25–30% of all tumors. Individuals of Scottish, Celtic, or Scandinavian ancestry are at higher risk. Affected persons usually have a history of significant occupational and/or recreational sun exposure. There is evidence that BCC arising before the age of 40 years corresponds with childhood or recreational sun exposure but does not correlate directly with cumulative sun damage. Thus, in areas of the world where the UV radiation is most intense, such as the sun belt in the United States, childhood sun exposure is at a maximum and younger patients are at a higher risk of developing BCC.

It is debatable whether BCC is more aggressive in children. As total incidence rates of BCC continue to rise, childhood cases may become more common. This increase in pediatric BCC may be especially true in areas of high UV radiation exposure. The percentage of sunny days during the year, higher altitude, and location closer to the equator may place children in these areas at increased risk. There exist other significant risk factors for the development of BCC: Prior injury such as trauma, burns, or vaccinations at the tumor site is frequently noted by persons with BCC. Carcinomas arising as a late sequelae of radiation therapy most frequently takes the form of BCC on the head, neck, and trunk, and SCC on the hands. Prior exposure to inorganic arsenic can also lead to the formation of BCC. In this setting, tumors are often multiple, truncal, and superficial lesions. Immunosuppressed individuals are also prone to the development of BCC, although their risk is greater for SCC than for BCC.

Basal cell ►nevus syndrome (BCNS), ►xeroderma pigmentosum (XP), Baze syndrome and albinism represent inherited genetic disorders that predispose those affects to BCC and SCC. Patients with basal cell nevus syndrome are found to have a germline mutation in ►PCTH, a ►tumor suppressor gene located on 9q22.3. PTCH is the human homolog of Drosophila patched. Approximately 1/3 of cases result from a new germline mutation. Approximately 80% of PTCH mutations result in premature truncation of the patched protein. Inactivation of this gene was found in tumor tissue in 68% of BCCs examined and did not correlate directly with sun exposure or age. Typically, multiple BCCs develop at a young age in ►BCNS. Multiple BCCs, odontogenic keratocysts and palmo-plantar pits constitute the primary features of BCNS. Approximately 5% of infants with BCNS develop ►medulloblastoma. ►Radiation therapy for the medulloblastoma can result in a crop of BCCs in the radiation port.

Xeroderma pigmentosum (XP) is due to a genetic defect in the biochemical pathway to eliminate the carcinogenic potential caused by the damage of ►ultravilet light B (UVB) to DNA. Several genes, those for XP groups B, D, and G and ►Cockayne syndrome groups B code for components of transcription factors, the protein complexes that bind the promoter regions and control gene transcription. BCCs and SCCs occur at a much higher rate and a much earlier age. Keratoacanthomans, fibrosarcomas and melanomas are also common in patients with XP.

BCC can be treated with multiple modalities providing 90% cure rates for primary disease in most cases. Cure rates for ablative surgery and excisional surgery vary with a number of factors including the clinical size of the tumor, the location, the histological subtype and whether or not it is recurrent. Cure rates for ablative surgery are less than 90% for BCC exceeding 0.5 cm in diameter on the face and over 2.0 cm in diameter on the trunk and extremities. In these instances, consideration should be given for excisional surgery with adequate margin control. BCCs exceeding 0.5 mm in diameter of the central facial zone and aggressive growth pattern tumors with sclerosing stromas are best treated with Mohs micrographic surgery. The histologic subtype or growth pattern is a good predictor of cure rate. These tumors do not respond well to superficial or ablative surgery. Nodular and superficial BCC respond well to curettage and electrodesiccation, cryotherapy or shave excision can result in less morbidity than full-thickness excisional surgery. For adequate cure rates morpheaform or sclerosing, micronodular or infiltrative variants of BCC require excisional surgery with histologic margin control.

Squamous Cell Carcinoma

SCC is the second most common skin cancer, representing 20% of cutaneous malignancies. Over 100,000 cases of SCC are diagnosed annually in the United States, accounting for an incidence of 41.4 per 100,000. SCC of the skin is the fifth most common cancer among men and the sixth most common cancer among women in Sweden. SCC in situ or ►Bowen disease is the most common benign/precancerous tumor among men, while among women, it is second only to in situ cervical cancer. It most commonly affects individuals in mid to late life. SCC usually cause local tissue destruction and in advanced cases it may cause cosmetic and functional morbidity.

Clinically, typical SCC is a hyperkeratotic papule, nodule or plaque with variable ►erythema. Associated pain may suggest perineural extension. The central part

of the face is the area at highest risk for recurrence. Tumors in this region tend to grow down or extend at various resistance planes such as the perichondrium of auricular and nasal cartilages. The tarsal plates of the eyelids or embryonic fusion planes at the junction of the nasal and nasolabial folds, and along the nasal columella or in the periauricular region. The size of the tumor also affects risk for recurrence. Tumors less than 1 cm have a 99.5% cure rate by Mohs micrographic surgery, compared with 82.3% for tumors 2–3 cm and 58.9% for tumors greater than 3 cm. Tumors under 2 cm of diameter have a local recurrence rate of 7.4% in contrast to a 15.2% recurrence rate for tumors greater than 2 cm. Therefore, margins of excision are adjusted according to size, with a 4 mm margin recommended for tumors less than 2 cm and 6 mm for tumors of 2 cm or greater.

Deeply invasive tumors have a greater tendency for local recurrence and metastases. Tumors with less than 4 mm in depth have a local recurrence rate of 5.3% compared with a rate of 17.2% for tumors 4 mm or greater. SCCs that penetrate though the dermis to the subcutaneous tissue have a recurrence rate of 19.8%. Tumors greater than 1 cm in diameter or a histologic grade 2 or higher are more likely to extend to subcutaneous tissue. The degree of histologic differentiation has a propensity for aggressive disease. SCC Broder grades 2 or higher usually require larger resections and has greater risk of local recurrence. Well differentiated SCC have a 13.6% recurrence rate in contrast to a 28.6% recurrence rate for poorly differentiated SCC. SCC with neurotropic growth pattern, which invade the perineural space, have a greater risk for local recurrence.

SCC usually have a low metastasis rates ranging from 0.3 to 3.7%. SCC arising in the lip, ear, penis, scrotum and anus have a higher risk for metastases. There is a greater risk of metastases for SCC more than 2 cm in size, with depth of invasion to at least 4 mm, ►Broader histologic classification of 2 or greater and perineural extension. SCC usually metastasis to the regional lymph nodes. The 5 year survival rates for patients with regional lymph node metastases is 26%, and 23% in patients with distant metastases.

Risk Factors and Therapy

The risk factors for SCC include exposure to UV light and ►arsenic compounds, immunosuppression and underlying genetic predisposition. Cellular atypia is often equally high among those with in situ SCC or invasive SCC, and it is difficult to use cytological criteria to define in situ SCC as a benign lesion, in spite of an intact basement membrane in histological specimens. Even when using molecular markers such as the expression of p53 gene, in situ and invasive SCC are indistinguishable. The incidence of invasive SCC is two times higher for men, whereas in situ SCC is more common among women. It is possible that there are close etiological links between in situ and invasive SCC, and in situ appears to be as important a marker for subsequent cancer risk as invasive SCC.

SCC can also be treated satisfactorily with different modalities. Histologic growth pattern is less important in SCC than clinical size and depth of invasion, with the exception of rare histologic subtypes such as adenosquamous cell carcinoma. SCC exceeding 1 cm in diameter and tumors that invade into the mid-dermis or deeper, particularly those involving cartilage and bone, are high-risk tumors. SCC on the lip, ear, temple, genitalia and those associated with preexistent conditions such as radiation or burn scars are all higher risk tumors. In these instances excisional surgery with careful margin control should be the treatment of choice. Postoperative radiation therapy may also be considered for these aggressive high-risk tumors on a case-by-case basis. Superficial or ablative procedures such as curettage and electrodesiccation, cryotherapy and shave excision should be reserved for SCC in situ (►Bowen disease) or SCC that invades only the superficial dermis. The depth of the invasion can be measured with an adequate preoperative biopsy. Indurated tumors with an undermining infiltrative border are often deeply invasive and should be treated with excisional surgery.

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Basal Cell Nevus Syndrome (BCNS)

Definition

Is a heritable autosomal dominant tumor syndrome, characterized by a multitude of developmental abnormalities including basal cell carcinomas of the skin, keratocysts of the jaw, palmar and plantar pits, fibromas of the ovaries and heart, medulloblastomas, and less

commonly polydactyly, syndactyly, and spina bifida. Approximately 2% of patients with BCNS develop medulloblastomas. The disease results from germline mutations in the *PTCH1* gene in chromosome 9q22.3.

►Gorlin Syndrome
►NBCCS
►Naevoid Basal Cell Carcinoma Syndrome
►Medulloblastoma

Basal-like Breast Cancer

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Synonyms

Basal phenotype breast cancer; Basal-subtype breast cancer; Basal-type breast cancer

Definition

Basal-like breast cancer is an aggressive type of breast cancer that expresses genes characteristic of epithelial cells in the outer or basal layer of the normal breast, including the basal ►cytokeratins. These tumors are usually ►hormone receptor negative and have a poor prognosis.

Characteristics

The normal adult breast is a glandular tissue organized into ducts and lobules that are made up of an inner layer of ►luminal epithelial cells, which surround the lumen of these structures and produce milk, and an outer or basal layer of ►myoepithelial cells (basal epithelial cells), which are surrounded by a basement membrane, separating them from the adjacent connective tissue. Luminal and basal epithelial cells can be distinguished by their expression patterns of the cytokeratins (CK), a family of cytoskeletal proteins. To understand the basis for nomenclature, it should be noted that the name "basal cytokeratin" was originally given to those (namely CK5/14/17) expressed in the basal cells of the stratified epithelium of the skin. Cytokeratins 8, 18, and 19 were named as "luminal" according to their strong expression in luminal epithelial cells. The basal or suprabasal layer of the breast also contains a population of multipotent CK5-positive ►progenitor or adult stem cells (►breast stem cells) that are capable of generating both epithelial cell types and likely play an important role in regenerating the gland after pregnancy and lactation.

Intriguingly, human breast tumors have distinctive ►gene expression profiles or "molecular portraits" that likely reflect the contribution of luminal epithelial cells, myoepithelial cells and/or breast stem cells. These gene signatures have recently been used to classify breast cancer into several molecular types, including ER-positive luminal groups, which expresses genes characteristic of luminal epithelial cells (ER, PR, and the luminal cytokeratins 8 and 18), and two major ER-negative groups, the HER2 group, characterized by amplification and overexpression of the proto-oncogene HER2, and the basal-like group, which express genes characteristic of myoepithelial cells (basal cytokeratins 5 and 17 and HER1/EGFR). The vast majority of basal-like breast tumors are negative for ER and PR and lack amplification of HER2. Hence, basal-like tumors are sometimes referred to as ►triple negative breast cancer, although not all basal-like tumors are triple negative and vice versa (see comments below). Other genes expressed in basal-like breast cancer have been implicated in apoptosis-resistance (e.g., α6 and β4 integrins, the molecular chaperone αB-crystallin, and pleiotrophin) and multiple molecular events in metastasis, including epithelial-to-mesenchymal transition (EMT), cell migration, and invasion (e.g., TGFβ2, the chemokine CXCL1, and the matrix metalloproteinase MMP14). These genes may contribute to the aggressive nature of basal-like breast cancer, which has a poor prognosis due in large part to its tendency to metastasize to distant organs. Basal-like tumors also express genes characteristic of breast stem cells, such as c-kit, vimentin and α6 integrin, suggesting that these tumors may have their origin in primitive breast stem/progenitor cells rather than in differentiated myoepithelial cells.

Basal-like breast tumors have also been identified by their expression of basal cytokeratins. In general, all or nearly all sporadic breast carcinomas show strong expression of luminal cytokeratins CK8, CK18 and CK19. The tumors expressing only luminal CK8, CK18 and CK19 are called "luminal" or "non-basal." A small fraction (~10%) of breast cancers also express CK5 together with its major partners CK14 and CK17, which are normally found in the basal layer of the breast, leading to their designation as "basal" or "basal phenotype" tumors. Tumor classification into the basal-like

subtype by cytokeratin immunohistochemistry and gene expression microarrays is very similar, but not completely identical. Some breast tumors lacking immunohistochemically detectable CK5 and CK14 may display a typical basal-like gene expression profile, and vice versa.

Basal cytokeratin expression can be seen in the tumor either uniformly in almost every carcinoma cell or strikingly heterogeneously in a checker-board manner. This feature can be used to further classify basal phenotype tumors as "basal" and "basoluminal" subtypes. All tumors in both subtypes show strong expression for luminal cytokeratins. Thus, in heterogeneously CK5/14-positive tumors, a large number of tumor cells express luminal cytokeratins only. Based on this observation, these tumors were called "basoluminal," in contrast to the uniformly CK5/14-positive "basal" tumors. Basoluminal tumors are also characterized by higher rates of HER2 amplification and a poorer prognosis than "basal" tumors.

Molecular Etiology

Despite the distinctive gene expression profile of basal-like breast cancer, which has been validated in multiple studies, the specific role of individual basal-like genes in the etiology of these tumors is poorly understood at the present time. Nevertheless, the gene signature of basal-like tumors has provided invaluable insights into potential pathogenic mechanisms. Indeed, the observation that the gene expression profiles of breast tumors in women who have inherited a mutation in the *BRCA1* tumor suppressor gene are largely basal-like strongly suggests that defects in BRCA1 function may play an important role in the etiology of non-hereditary basal-like breast cancer. BRCA1 normally functions to protect the genome through its actions in DNA damage repair and cell cycle checkpoint activation. Although mutations in *BRCA1* do not occur in non-hereditary breast cancer, basal-like tumors often have diminished expression of BRCA1 mRNA and protein due to promoter methylation and other mechanisms. Similar to basal-like tumors, hereditary *BRCA1*-related breast tumors are typically triple negative. Moreover, mutations in the *TP53* tumor suppressor gene and overexpression of the cell cycle regulator cyclin E occur commonly in both hereditary *BRCA1*-related breast tumors and non-hereditary basal-like tumors and may contribute to the genomic instability and the rapid proliferation rates of these tumors. Defective X chromosome inactivation is also a hallmark of both tumor types (and BRCA1 dysfunction), although the role of this abnormality in their pathogenesis is unclear. Taken together, these findings suggest that BRCA1 dysfunction and resultant defects in DNA damage repair may play a key role in the etiology of basal-like breast cancer and provide a molecular target for therapy.

Diagnosis

The highly reproducible gene expression profile of basal-like breast cancer remains the definitive method for diagnosing basal-like tumors. However, gene expression profiling is not routinely available in the clinic. Consequently, basal-like tumors can be identified by basal cytokeratin expression by immunohistochemistry or by double negative (ER and HER2 negative) status combined with positive immunostaining for either CK 5/6 or HER1/EGFR. As noted, these methods identify similar, but not identical, subsets of breast tumors given the likely heterogeneity of the basal-like group, an important caveat for clinical studies. In the near future, it may become possible to diagnose basal-like tumors using gene signatures of a subset of basal-like genes (20–50 genes) determined by ►reverse transcription-polymerase chain reaction (RT-PCR) methods on clinical specimens.

Clinical Features

The unfavorable prognosis of basal cytokeratin expressing breast cancer was first described in 1987. Thereafter, it has been shown in many immunohistochemical studies that basal cytokeratin expressing tumors associate with poor survival and early relapse. More recently, the molecular classification of breast cancer by gene expression profiling has been shown to provide important prognostic information for patients. Indeed, patients with basal-like and HER2 tumors have the shortest overall and relapse-free survival of all the molecular types. When basal-like tumors are further subclassified into basoluminal and basal subgroups, as described earlier, the basoluminal tumors show shorter survival estimates than the basal tumors. This difference is not due to more frequent amplification of HER-2 in the basoluminal subgroup.

Consistent with their aggressive behavior, basal-like tumors are poorly differentiated, highly proliferative with high mitotic counts, and are characterized by genomic or chromosomal instability, resulting in numerous chromosome abnormalities, including gains and losses. In addition to these properties, basal-like tumors often show aggressive morphological features like pushing borders, lymphocyte infiltration, tumor necrosis, central scarring, and the presence of spindle cells. Many of these features are typical for medullary and/or metaplastic carcinomas, rare histologic types of breast cancer, which express basal cytokeratins and have a basal-like gene expression profile. Moreover,

basal-like breast cancer tends to metastasize via the blood stream ("hematogenous" route) to distant organs such as the brain and lung, rather than spreading through the lymphatic system to adjacent lymph nodes. These metastases are particularly prevalent during the first five years after diagnosis and are largely responsible for the poor survival of patients with basal-like breast cancer. Intriguingly, hereditary *BRCA1*-related breast tumors are also characterized by early metastases to distant organs that account for their poor prognosis. Approximately 10–20% of all breast cancer cases are basal-like. However, basal-like tumors appear to be more prevalent in younger women, particularly African American women. Hence, the clinical impact of these poor prognosis tumors is compounded by their tendency to strike younger woman.

Treatment

Unlike the other molecular types of breast cancer, there are no currently available, targeted therapies for basal-like breast cancer. For instance, most basal-like breast tumors do not respond to endocrine therapy such as tamoxifen or aromatase inhibitors because they are ER-negative. Similarly, trastuzumab (Herceptin), a therapeutic antibody targeting HER2, is of little benefit against most basal-like tumors because they lack amplification of HER2. Although our understanding of the molecular etiology of basal-like tumors is limited, several candidate drug targets have been identified by gene profiling, including the receptor tyrosine kinases HER1/EGFR and c-Kit, the MEK-ERK MAP kinase pathway (activated by HER1/EGFR and c-Kit), and the cell cycle regulator cyclin E. Importantly, drugs targeting several of these molecules have already been developed and may prove to be useful in treating basal-like tumors. Another potential therapeutic target is the likely BRCA1 dysfunction in basal-like tumors. Recent studies indicate that breast cancer cells expressing mutant *BRCA1* are highly sensitive to chemotherapy drugs which cause DNA double-strand breaks (e.g., topoisomerase II inhibitors) or cross-link DNA (e.g., cisplatin), consistent with the established role of BRCA1 in repairing DNA damage. Hence, these agents may be effective against basal-like tumors, particularly those with reduced *BRCA1* expression. Moreover, inhibition of *BRCA1* expression by RNA interference sensitizes cancer cells to apoptosis by inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), which catalyzes poly(ADP-ribosyl)ation of target proteins. These findings suggest that PARP inhibitors may be of benefit in basal-like tumors. Given the aggressive nature of basal-like tumors and the lack of targeted therapies at present, there is a clearly a pressing need for carefully designed clinical trials to evaluate these and other agents in basal-like breast cancer.

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Basal Phenotype Breast Cancer

►Basal-like Breast Cancer

Basal-subtype Breast Cancer

►Basal-like Breast Cancer

Basal-type Breast Cancer

►Basal-like Breast Cancer

Basalioma

Definition

Refers to a skin cancer with extended growth, but no metastasis; develops in skin areas exposed to sun, such as face, nose, or ears.

Base Excision Repair (BER)

Definition

Is a DNA repair pathway that repairs mainly non-bulky adducts that causes only minor disturbances in the helical structure of DNA, such as oxidized, alkylated, or even absent bases.

►Mutagen Sensitivity
►Nucleotide Excision Repair

Basement Membrane

Definition

The basement membrane (BM) is a thin mat of extracellular matrix that separates epithelial sheets and many types of cells, such as muscle cells and fat cells, from connective tissue. The characteristic components of BMs are laminin, collagen type IV and heparan sulfate proteoglycan.

►Adhesion
►Basal-Like Breast Cancer
►Extracellular Matrix Remodelling
►Extranuclear Matrix Remodelling
►Heparanase
►Metastatic Colonization

Basic FGF

Definition

►Fibroblast Growth Factor 2.

Basic Fibroblast Growth Factor (bFGF)

Is a cytokine that belongs to a homologous family of at least 18 proteins. Basic FGF binds FGF receptors 1 and 2, acts as a potent mitogen for endothelial cells, and is expressed in many benign and malignant tumors.

►Antiangiogenesis

Basic Helix-Loop-Helix Domain

Definition

A phylogenetically conserved domain that defines a class of transcription factors. The basic region, rich in positively charged amino acids, mediates interactions with DNA while two amphipathic alpha helices separated by a flexible, unstructured loop mediate hetero- or homodimeric interactions with other bHLH-containing transcription factors.

►E2A-PBX1
►Myc Oncogene

Basolateral Surface

Definition

As applied to the gastrointestinal tract wall refers to the wall surface on the outside (the side exposed to the bodies blood flow) of the GI tract or in Caco-2 cell culture the cell surface that mimics the outside of the GI tract. In Caco-2 cultures this is the cell surface that grows on the semi-permeable support membrane.

►ADMET Screen

Basophil

Definition

A white blood cell that contributes to inflammatory reactions. Along with mast cells, basophils are responsible for the symptoms of allergy.

BAX

Definition

Bax is a Bcl-2-associated X protein of 192 aa and 21 kD that is membrane-bound and expressed widely in different tissues. It has proapoptotic activity, by binding

and antagonizing the antiapoptotic Bcl-2, thereby accelerating apoptosis. The gene maps to 19q13.

►Apoptosis

bbc3

►PUMA

BCC

Definition

►Basal Cell Carcinoma
►Photodynamic Therapy

BCDF

►Interleukin-6

B-cell CLL/lymphoma 2

►BCL2

B-cell Differentiation Factor

►Interleukin-6

B-cell Interferon

►Interferon-α

B-cell Leukemia/Lymphoma-2 Gene (*Bcl2*)

►BCL2

B-cell Leukemias

►B-cell Tumors

B-cell Lymphoid Neoplasm

►B-cell Tumors

B-cell Lymphoma

Definition

B-cell lymphoma is a tumor of B-cells; ►B-cell Tumors.

►Photodynamic Therapy
►Diffuse Large B-Cell Lymphoma
►B-cell Tumors

B-cell Lymphoma Protein 2

►BCL2

B-cell lymphoproliferative Disorders/ Diseases

►B-cell Tumors

B-cell Malignancy

►B-cell Tumors

B-Cell Response

Definition

Response of B-1 and B-2 cells to antigen stimulation. B-1 cells bear high levels of surface IgM, lower levels of surface IgD, and most express the cell surface antigen CD5, they frequently secrete high levels of polyspecific antibody with relatively low affinity. The majority of B-cells are B-2 cells which express low levels of surface IgM, do not express CD5 and secrete highly specific antibody. While B-1 cells are $CD43^+$ and $CD23^-$, B-2 cells are $CD43^-$ and $CD23^+$.

►Autoantibodies

B-cell Stimulating Factor-2

►Interleukin-6

B-cell Tumors

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Synonyms

B-cell lymphomas; B-cell leukemias; B-cell lymphoid neoplasm; B-cell lymphoproliferative disorders/diseases; Hodgkin and Non-Hodgkin lymphomas; B-cell malignancy; cancer of B-lymphocytes

Definition

B-cell lymphomas are malignant tumors of B-lymphocytes. They arise at all stages of B-cell differentiation, from immature B-lymphocytes in the bone-marrow through to terminally differentiated plasma cells (Fig. 1). It is now possible to use immunogenetic analyses to define more clearly the cell origin and clonal history of B-cell tumors.

Characteristics

What is a B-lymphocyte?

B-lymphocytes are cells of the immune system that are destined to express immunoglobulins (antibody molecules). These immunoglobulins (Igs) play a central role in the recognition of foreign antigens like infectious organisms, which could threaten the integrity of the individual.

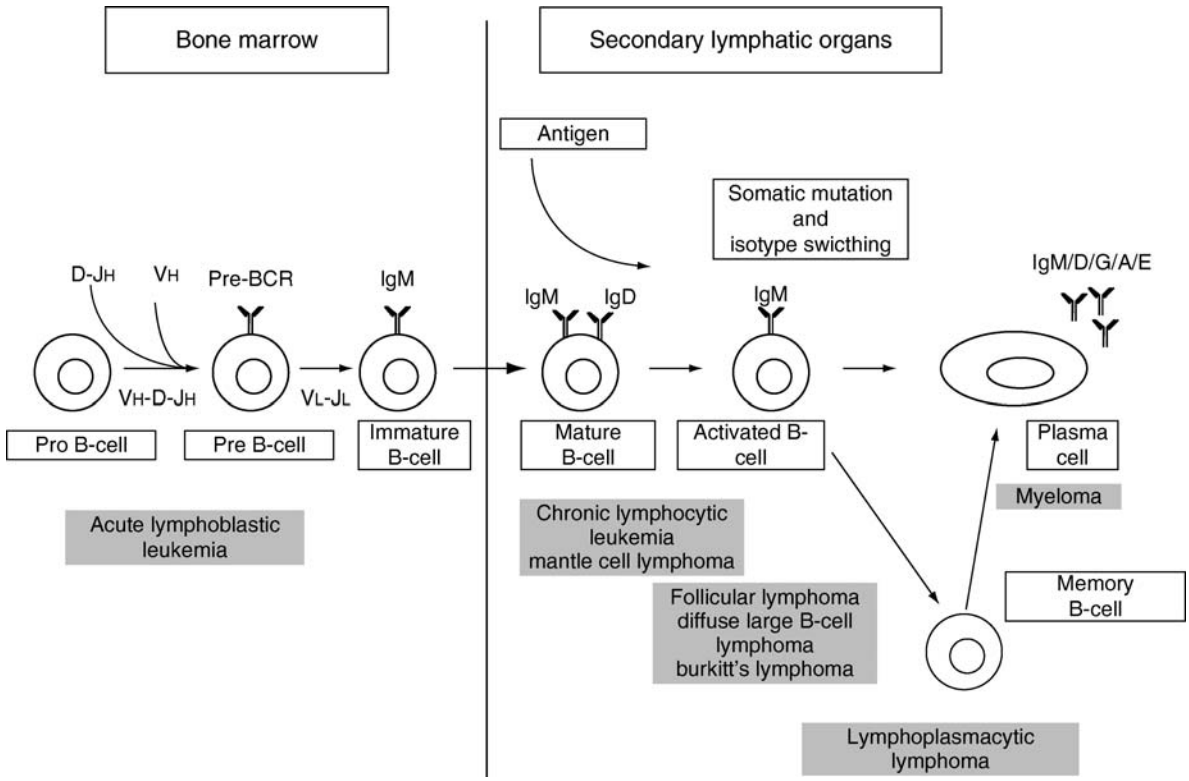
Igs are glycoproteins that can exist either as membrane-bound molecules on the cell surface or as secreted molecules in the serum. There are five classes of Ig with different size, structure and function; IgM, IgD, IgG, IgA and IgE. Each basic Ig molecule contains two identical heavy chains (μ, δ, γ, α or ε) and two identical light chains (κ or λ). A mature B-cell carries about 105–106 identical Igs on its cell surface. Both light chains and heavy chains can be subdivided into distinct regions. The N-terminal variable (V-) regions mediate antigen contact and their amino acid sequence is specific to each B-cell. The C-terminal constant regions are common to all antibodies of the same class.

The sequence variability, which is necessary to recognize the vast number of different antigens present in the environment, is created by two processes. The first is ►immunoglobulin gene rearrangement and the second is somatic mutation. ►Class switching changes Ig effector function.

Immunoglobulin Gene Rearrangement

During this remarkable process, double-stranded DNA breaks are created and repaired in a tightly controlled fashion. Rearrangement brings together one representative from different gene families: variable region (VH) genes, diversity region (D) genes and joining region (JH) genes for the Ig heavy chain, VL and JL for the Ig light chain (Fig. 1). The process of VH-D-JH and VL-JL joining is imprecise; non-templated nucleotides (N-additions) can be inserted and the ends of the joined segments can be trimmed back. Thus the final products of rearrangement, the VH-D-JH and VL-JL, will have a unique nucleotide sequence V(D)J Recombination.

The heavy chain variable (VH) region is about 120 amino acids (aa) long and can be subdivided into discrete structural sections. Three complementarity determining regions form the classical antigen binding site; CDR1, CDR2 and CDR3. While CDR1 and CDR2 are encoded in the germline, CDR3 is created de novo in each B cell by VH-D-JH rearrangement. In the antibody molecule this sequence corresponds to the central part of the antigen recognition site. The CDRs alternate with four framework regions (FR1–4). Light chain variable



B-cell Tumors. Figure 1 In the bone marrow first the D-JH then VH-D-JH recombination takes place. This heavy chain is expressed on the cell surface with the surrogate light chain to form the pre-B-cell receptor (pre-BCR). Next the light chain genes are rearranged. The B-cell now expresses surface Ig and leaves the bone marrow. Mature B-cells encounter antigen, and are stimulated to somatically mutate their V genes. Additionally class switching is initiated. Some B-cells then leave the germinal centre to become plasma cells, some become memory cells. The grey blocks illustrate, at which stage of B-cell differentiation some B-cell tumors are thought to originate.

regions (VL) regions are about ten aa shorter, but contain similar structural motifs.

In the bone marrow the Ig heavy chain genes are rearranged first followed by the light chain genes (Fig. 1). Ultimately a B-cell that successfully completes this process will have a unique immunoglobulin heavy and light chain gene sequence for antigen recognition. The nucleotide sequence in CDR3 can be viewed as its molecular marker or "fingerprint."

Somatic Mutation and Class Switching

Following antigen encounter, further variability is introduced into the rearranged variable region genes by somatic mutation that occurs in secondary lymphatic organs. Somatic mutation can change the amino acid sequence of variable region genes and may therefore impact on the antigen binding of the resulting protein.

The process of class switching changes the effector function of the antibody molecule (complement activation, binding to Fc receptors or uptake by phagocytic cells). During class switching the DNA segment of one constant region (e.g. IgM) is deleted and the variable region of the heavy chain brought into the vicinity of another constant region gene (e.g. IgG or IgA). This process conserves the unique variable region.

What is a B-cell Tumor?

In the broadest sense ►B-cell tumors are malignancies in which tumor cells have undergone rearrangements of their ►immunoglobulin genes. Analysis of the status of these genes provides information that defines origin and clonal history of the tumor cell. Figure 1 shows key steps in normal B-cell development, and gives examples of B-cell malignancies that may arise at a particular stage. Within each cancer, the tumor cells are clonally related, as revealed by the common CDR3 sequence in the tumor cell population.

In some lymphomas the tumor clone has non-functionally rearranged VH genes, as appears to be the case in Hodgkin lymphoma (►Hodgkin disease). This sets these lymphomas apart from normal B-cells, which can only survive if they express immunoglobulins. The antigenic determinants, derived from the variable regions of the immunoglobulin molecule, provide us with a

unique tumor antigen called ►idiotype. This tumor antigen is now being exploited in new immunotherapeutic strategies.

Characteristics of B-cell Tumors

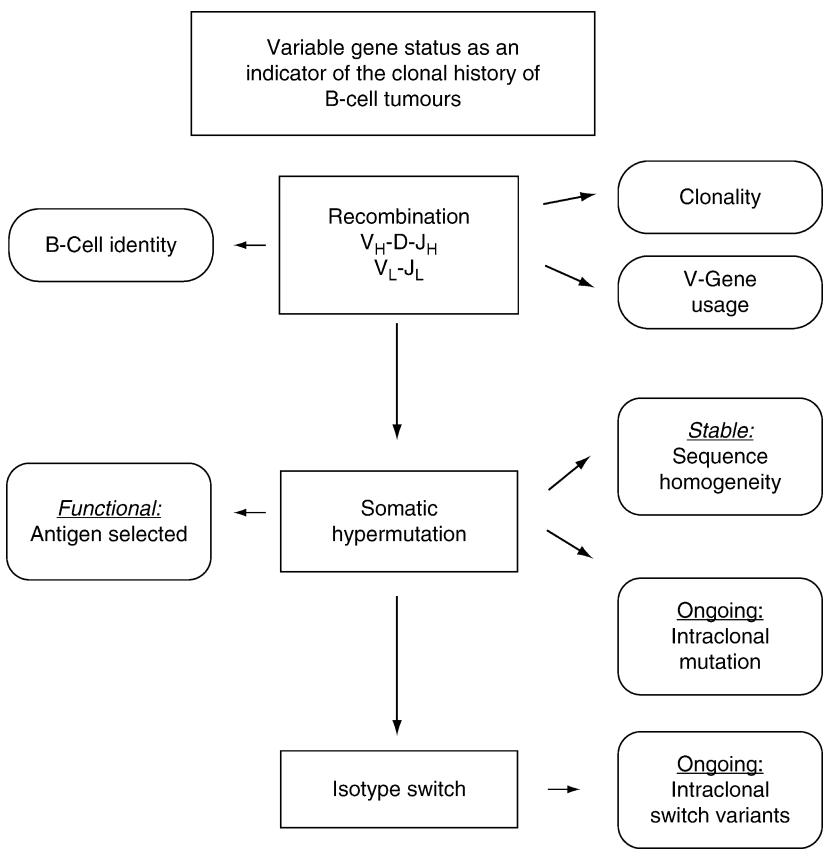
B-cell tumors account for about 3% of all cancers. For unknown reasons their incidence is rising steadily at about 6% per year world-wide. B-cell tumors are the most common malignancies in childhood. In adults, the frequency of B-cell tumors increases steadily with age, with a median of 50–60 years. They occur more frequently in men than women.

The presentation of B-cell tumors at the clinical and morphological level can vary widely. Aggressive malignancies are at the one end of the spectrum, which if untreated will cause death in weeks but are frequently curable with combination chemotherapy. Indolent malignancies are at the other end of the spectrum, which are usually incurable but can remain untreated for decades. The diagnosis of B-cell malignancies relies on the clinical picture, histological analysis and immunophenotype of the tumor. Increasingly, hallmark genetic abnormalities are being defined in individual entities. They frequently involve translocations into the immunoglobulin loci of the heavy chain genes on chromosome 14 or the κ or λ light chain loci on chromosomes 2 and 22.

Different ways of grouping lymphoid malignancies in a logical fashion have been applied. They were based on the need of clinicians to determine a suitable course of treatment as well as the desire of pathologists to distinguish morphological similarities. Although these classifications were used in parallel, they are difficult to compare since similar entities were often attributed to different categories. In 1994, an attempt was made to divide lymphoid malignancies taking into account the combined available information from clinical patterns, morphology, immunophentoype and genetic characteristics. Also, as far as possible, the normal counterparts were attributed to each malignancy. This led to the "Revised European-American Classification of Lymphoid Neoplasms" (REAL). This REAL classification provides the first truly international view of lymphomas and has been developed further in the form of the recently proposed WHO classification.

Immunogenetics of B-cell Lymphomas

Genetic analysis of B-cell lymphomas has aided our understanding of malignant lymphoma. Specific chromosomal rearrangements in many of the lymphoma



B-cell Tumors. Figure 2

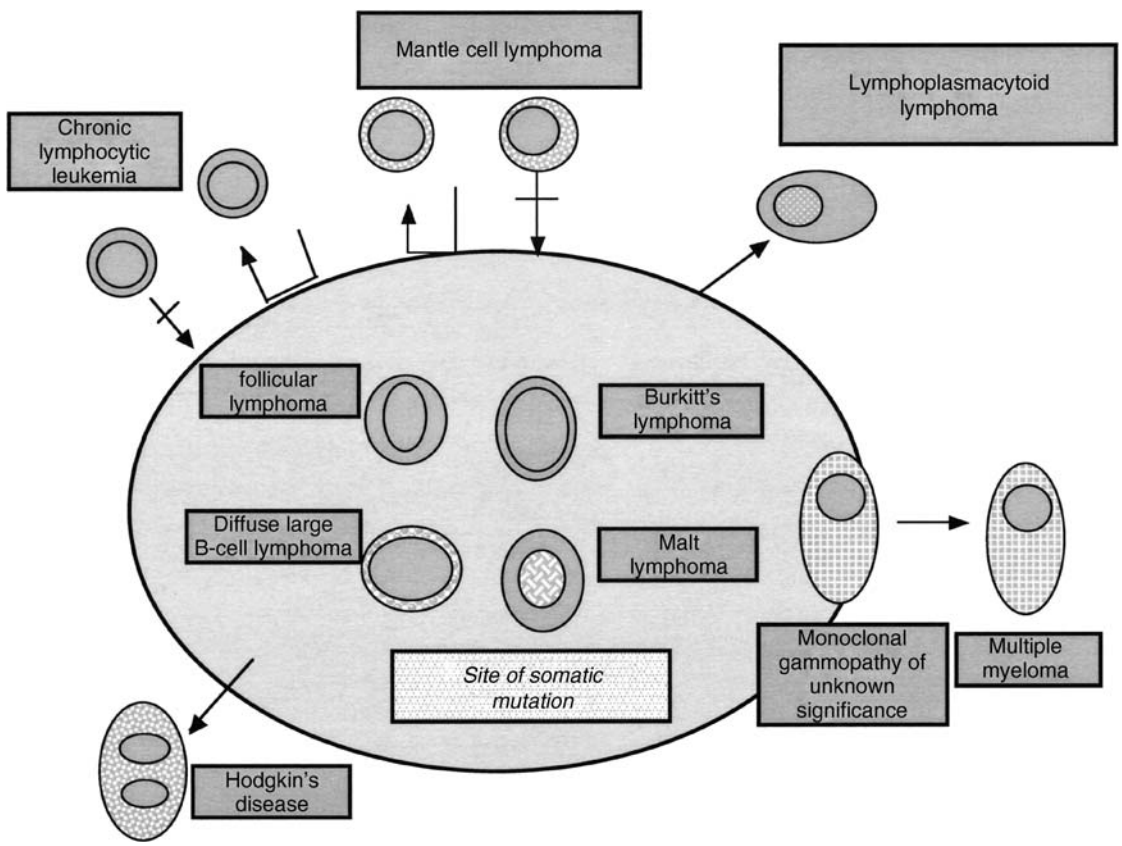
entities indicate that a particular type of genetic damage in the precursor cell is important for the development of the lymphoma. For example t(14;18) translocation is characteristic of ►follicular lymphoma. The isolation of the same translocation in cells from healthy individuals suggests that this genetic change may be a necessary but insufficient condition for the development of follicular lymphoma.

More recently, the analysis of the status of the immunoglobulin genes in B-cell tumors has shed new light on the events which shape the malignant cell. Figure 2 summarizes the information that V-gene analysis of B-cell tumors can reveal. The presence of rearranged immunoglobulin genes defines the cells under investigation as being of B-cell origin (Fig. 2). In this way it could finally be established that in the majority of cases Hodgkin lymphoma is a B-cell tumor.

Sometimes it can be very difficult to assess if an abnormal population of B-cells represents a true malignancy. Examples include low grade ►MALT lymphomas in the stomach or lymphoproliferations after organ transplants. Here the analysis of the Ig genes can help to separate a poly- or oligo-clonal and pre-malignant lesion from a truly clonal and cancerous one (Fig. 2).

In some B-cell lymphomas (follicular lymphoma, diffuse large B-cell lymphomas) the observed VH gene usage is similar to that of normal B-cells. In other tumor types, however, a marked over- or under-representation of certain VH genes has been detected. For example, a member of the VH4 gene family, called V4–34, is used by about 6% of normal cells. In contrast, all known cases of ►Waldenstrom macroglobulinemia with cold agglutinins of anti-I activity use the V4–34 gene. This suggests that B-cell superantigens may play a role in the pathogenesis of cancer in these B-cell lymphomas.

Since gene rearrangement, somatic mutation and class switching all leave their traces in the Ig-genotype of a B-cell, Ig analysis can provide important information about the clonal history of the malignant B-cell. V-gene analysis allows us to determine which processes the



B-cell Tumors. Figure 3 Origin and development of B-cell tumors in relation to the site of somatic mutation in the germinal centre (GC). V-gene mutational patterns can be used to classify tumors as follows: (i) not entering the GC (*blocked arrows*); (ii) passing through the GC (*arrows out*); (iii) remaining in the GC (*no arrows*). Monoclonal gammopathy of unknown significance may in some cases remain in the GC. CLL has subgroups, with different patterns of mutations, which thus possibly arise from different stages of B cell development.

B-cell has been exposed to and also suggests which 'normal' counterpart the tumor cell may be related to.

The majority of B-cells in the periphery will have been exposed to somatic mutation in the germinal centre of the secondary lymphatic organs. Figure 3 relates the origin and development of B-cell tumors relative to the germinal centre. The analysis of the tumor related VH-D-JH genes in tumors can reveal evidence that the tumor cell clone has entered this site, if somatic mutations are found in the VH-D-JH gene. Within the same tumor type some cases may show somatic mutation, while others do not. ►Chronic lymphocytic leukemia (CLL) segregates into two categories; patients with unmutated (pre-germinal centre) CLL have a significantly worse prognosis than those with mutated VH-D-JH genes.

Evidence for ongoing mutation can be identified by detecting micro-heterogeneity in clonally-related sequences from the tumor. While the clonal fingerprint of the tumor is shared between all cells, some cells have acquired additional mutations that are not shared by other cells. This type of pattern is found in follicular lymphoma, ►Burkitt lymphoma and diffuse large B-cell lymphomas (DLBCL) (Fig. 3). The tumor cells of DLBCL and hairy cell leukemias are also able to produce transcripts for more than one Ig isotype. This provides additional evidence that the malignant tumor cells are less frozen in their development than previously thought.

Malignancies like multiple myeloma (MM) have mutated VH-D-JH genes, but all sequences are identical (they are "stable"). This suggests that MM has undergone somatic mutation but that the tumor cells have then left the site of somatic mutation (post germinal centre tumors). ►Monoclonal gammopathy of undetermined significance (MGUS) can show or lack intraclonal heterogeneity.

The available data now allow us a detailed description of human B-cell tumors. Immunogenetics has contributed to the classification by providing information that is independent of the morphology and clarifies the developmental stage at which the final transforming event occurred.

It is likely that in the future new methods like the gene ►microarray technology will help us understand which disease entities should be further subdivided. Additionally, we are likely to predict better within lymphoma entities and tailor treatment according to more accurate prognostic factors.

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BCG

Definition

Attenuated form of Mycobacterium bovis ►Bacillus Calmette-Guérin. Generated by multiple in vitro passages on a special culture medium. Named after two French microbiologists working at the Pasteur Institute in the 1920's.

►Bacillus Calmette-Guérin

Bcl2

►BCL2

BCL2

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Synonyms

B-cell leukemia/lymphoma-2 gene (*Bcl2*); Bcl2; Apoptosis regulator Bcl2; B-cell CLL/lymphoma 2; B-cell lymphoma protein 2

Definition

The Bcl2 family of proteins belong to a peculiar class of proteins regulating ►apoptosis, ►cell cycle, ►differentiation, and ►autophagy; in oncology, the genes

coding for these proteins could not be defined neither as dominant transforming ►oncogenes (such as ►*myc*), nor ►tumor suppressor genes (such as ►*p53*). They could be best defined as apoptosis-related genes, a definition that stresses the importance of apoptosis (and of its dysregulation) in the genesis and development of cancer in humans and other species. Dysregulation of apoptosis is involved also in the development of diseases other that cancer, such as autoimmune diseases, AIDS and various degenerative pathologies.

Characteristics

The Bcl2 family encompasses several members divided in antiapoptotic and proapoptotic proteins; among the antiapoptotic are Bcl2 and BclXL, whereas among the proapoptotic are Bax, Bak, ►Bid, and Bad. These proteins contain conserved Bcl2 homology (BH) domains (termed BH1, BH2, BH3, and BH4), together with a transmembrane domain, all being identified as crucial for the regulation of apoptosis. In addition, based on functional studies and the conservation of BH domains, the Bcl2 family can be further divided into three subgroups. The Bcl2 subgroup includes all antiapoptotic proteins, such as Bcl2 and BclXL. The Bax subgroup consists of proapoptotic members, such as Bax, Bak, and Bad. Both groups contain more than one BH domain. The third subgroup contains BH3-only proteins, such as Bid and Bim, which can interact with either antiapoptotic proteins or proapoptosis members. The observation that inhibitors and inducers of cell death interact with each other by forming homodimers or heterodimers suggests that apoptosis is regulated, at least in part, by protein–protein interaction. By means of two alternative transcripts (a and b), Bcl2 codes for a protein of 205 amino acids (Bcl2b), or 239 amino acids (Bcl2a); both proteins contain BH domains for homo/heterodimerization with members of the Bcl2 family of proteins. The BH4 domain is required for antiapoptotic activity and also for interaction with the serine/threonine kinase encoded by ►Raf-1, an oncogene homologous to ►protein kinase C, that is the target of several tumor promoters. The hydrophobic carboxyl terminus of the protein determines association with cellular membranes; also this tail seems necessary for the antiapoptotic function. Proteins of the *Bcl2* gene family are evolutionarily conserved from the Sponges to man.

Functions

The main biological function of Bcl2 is to inhibit apoptosis or, conversely, to promote cell survival. Other related biological functions concern the control of cell cycle. In fact, Bcl2, as well as the antiapoptotic members of this family of proteins, are antiproliferative by facilitating G0, thus suggesting that cell survival is maintained at the expense of proliferation. In hematopoietic cell lines, these functions are crucial for differentiation, and Bcl2 might also have a direct role in cell fate decision beyond strict cell survival. In addition, Bcl2 family members are involved in the control of autophagy. As far as cell survival is concerned, it appears that the cell fate is dependent on the amount of intracellular Bcl2 protein; an increase (overexpression) of Bcl2 protein is associated with prolonged survival and apoptotic protection, whereas its decrease is associated with apoptosis or enhanced sensitivity to apoptosis-inducing agents. For example, HIV-specific CD8 + T-cells show a significantly reduced expression of Bcl2, potentially priming them to apoptosis. Conversely, in the development of cancer, Bcl2 overexpression inhibits the apoptosis of cancer cells bearing mutations, thus being a key determinant of neoplastic cell expansion and resistance to anticancer treatments. As a consequence, cancer cell death is delayed, and cancer cell accumulation occurs.

At the molecular level, inhibition of apoptosis as well as control of cell cycle, differentiation, and autophagy occur through a complex process of protein–protein interaction. In the inhibition of apoptosis this process involves heterodimerization, especially with the proapoptotic member of the Bcl2 family. In addition to homo/heterodimerization within the Bcl2 family members, the antiapoptotic members of the Bcl2 family also interact with other proteins regulating apoptosis, such as ►caspases and ►APAF1. Formation of complexes with these proteins involved in the actuation of apoptosis prevent them to initiate the protease cascade eventually leading to cell death.

The multiple independent functions of Bcl2 proteins are mediated by the BH domains and the hydrophobic helices. These functions can be grouped in two main categories: (i) A function as membrane channels for ions and proteins and (ii) A function as membrane adaptor/docking proteins. The first hint about Bcl2 function came from studies on the three-dimensional structure of the Bcl2 analog, antiapoptotic Bcl-XL. It showed a surprising similarity to the pore-forming domains of some bacterial toxins that cause the formation of channels for ions, proteins, or both. It was observed that Bcl2 and its homologues are localized to intracellular membranes, in particular, the outer ►mitochondrial membrane, the endoplasmic reticulum and the intracellular membrane of the nuclear envelope. In these areas they have a membrane transport function for calcium ions and proteins. The channels created by Bcl2 insertion into membranes resemble the pores formed by certain bacterial toxins. Thus, the two long hydrophobic helices of the protein core insert deeply through the phospholipid bilayer,

perpendicular to the membrane surface, and the rest of the protein undergoes conformational changes resembling the opening of an umbrella with the five surrounding amphipathic helices resting on the top of the membrane. The ability to form channels, by insertion of the two hydrophobic helices, is essential for Bcl2 antiapoptotic function. However, by analogy with other channel-forming proteins, the Bcl2 channels are formed by two or more proteins of the Bcl2 family. Thus, there is the possibility that anti- and proapoptotic members of the Bcl2 family form homo- or heterodimers. In fact, the proapoptotic members of the family also have channel forming activity, although the channels formed by these proteins might have different transport selectivity or subcellular localization. Heterodimerization of anti- and proapoptotic Bcl2 family proteins might lead to the formation of different channels or, alternatively, the heterodimers might be unable to form channels at all. Schematically, the channels formed by Bcl2 and the other antiapoptotic members prevent apoptosis, possibly transporting back, and thus antagonizing, the proapoptotic factors that outflow through the channels formed by the proapoptotic members of the Bcl2 family. For example, Fas ligand, a well characterized inducer of apoptosis, activates a member of the caspase family (caspase 8) that cleaves proapoptotic Bid. Once truncated, Bid translocates to mitochondria where it might function as a channel protein to release cytochrome *c*, thus activating cytosolic caspases which are the terminal effectors of apoptosis. Bcl2 inhibits the release of cytochrome *c* either by plugging the channels opened by Bid, or by transporting cytochrome *c* back to the mitochondria. Also in this case, the level of expression and the ratio between antiapoptotic and proapoptotic Bcl2 family proteins is critical in deciding cell death or survival.

In addition to the channel forming properties, Bcl2 family proteins interact with a number of signal transducing proteins involved in apoptosis and other crucial cellular processes. These include the protein kinase C homologue Raf-1, the ►G-proteins H-Ras and R-Ras, the p53-binding protein p53-BP2, the proapoptotic protein CED-4, (homologue to APAF1), and the protein phosphatase calcineurin. These interactions are mediated by specific BH domains; for example, the BH4 domain has been reported to bind with calcineurin, Raf-1, and CED-4. The association between Bcl2 and these proteins might be responsible for their translocation to intracellular membranes where Bcl2 is anchored. This may lead to changes of their activity, such that they might be sequestered and inactivated, or targeted for interaction with other membrane-associated proteins. For example, Raf-1 is a serine/threonine kinase which transduces mitogenic signals from membrane receptors to the nucleus. Association between Raf-1 and Bcl2 causes translocation of the protein kinase to the mitochondrial membrane where Bcl2 is located. Once there, Raf-1 phosphorylates and inactivates Bad, one of the proapoptotic members of the Bcl2 family. Phosphorylated Bad is sequestered in the cytosol, encaged by an adaptor protein termed 14-3-3, and thus unable induce apoptosis. In the absence of growth/survival factors (such as in IL-3 deprivation of IL-3-dependent hematopoietic cell lines), Raf-1 is not activated and the unphosphorylated Bad is able to induce apoptosis. Protein–protein interaction is also responsible for Bcl2 biological functions other than control of apoptosis. In fact, interaction between the catalytic domain of Raf-1 and the BH4 domain of Bcl2 in multipotent hematopoietic progenitor cells is critical in determining the erythroid/myeloid fate of differentiating cells. Another protein originally isolated as a Bcl2-interacting protein, is Beclin 1, the first identified mammalian autophagy gene product. Bcl2 negatively regulates Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death, thus raising the possibility that Bcl2 family members also regulate autophagy.

Regulation

The first association between Bcl2 and human cancer was observed in follicular lymphomas bearing the t(14;18) chromosomal translocation by which the gene was cloned. This translocation brings the *Bcl2* gene to chromosomal location 18q21 into juxtaposition with the immunoglobulin heavy-chain locus at 14q32, resulting in transcriptional deregulation of the *Bcl2* gene. This event does not involve alterations of the coding regions of the gene. Subsequently, *Bcl2* overexpression was recognized as a general feature of various types of hematological and solid malignancies. Thus, many members of the Bcl2 family have been found to be differentially expressed in various malignancies, and some are useful prognostic cancer biomarkers. Whether through its function as a channel protein or as an adaptor/docking protein, the final result on cell fate, however, depends upon the level of expression of *Bcl2*. Therefore, the control of Bcl2 expression has been the object of numerous studies of transcriptional, translational, and posttranslational regulation. Overexpression of Bcl2 has been associated with hypomethylation in the promoter region. In normal cells, once apoptosis is initiated, Bcl2 is proteolytically cleaved by caspases. Interestingly, the cleaved protein, lacking the BH4 domain, has proapoptotic activity, and causes the release of cytochrome *c* into the cytosol thus promoting further caspase activity. Bcl2 family proteins are also regulated by phosphorylation that affects their activity and conformation. The structural

analysis of antiapoptotic members of Bcl2 family led to the discovery of an unstructured "loop region" near the N-terminus exposed to the cytoplasm. The antiapoptotic members of Bcl2 family such as Bcl2 and Bcl-XL are phosphorylated on specific serine/threonine residues within this unstructured loop in response to diverse stimuli including treatment with chemotherapeutic or chemopreventive agents. In most instances, such phosphorylation has been associated with the loss of their biological (antiapoptotic) function. The chemoresistant tumors often overexpress Bcl2/Bcl-XL. In these instances, the apoptosis yielding effect due to phosphorylation of antiapoptotic Bcl2 family members is quite interesting because phosphorylation–dephosphorylation pathway of these antiapoptotic proteins could be an ideal molecular target for therapy of subpopulation of cancer in which these cell death repressors are essential prognostic markers. Thus, further gaining the knowledge on the mechanism of inactivation of Bcl2/Bcl-XL by phosphorylation might be of significant importance to therapy for human malignancies in which overexpression of these antiapoptotic proteins is recognized.

Bioactivity

Oncogenes and tumor suppressor genes modulate *Bcl2* expression with profound results on death or survival of cancer cells. The tumor suppressor gene *p53* can induce apoptotic cell death by downregulation of Bcl2 and upregulation of Bax. The p53-dependent negative response element on Bcl2 has the features of a transcriptional silencer, mediating inhibition of transcription in an orientation-dependent manner. In a variety of tumors, p53 expression is associated with apoptosis and with sensitivity to DNA damaging agents (anticancer drugs and ►ionizing radiations), by enhancing the transcription of a gene that favors apoptosis (Bax), at the same time blocking the transcription of a gene that would protect cancer cells from apoptosis (i.e., *Bcl2*). *Bcl2* overexpression is able to hinder p53-induced apoptosis, but it is ineffective against p53-dependent growth arrest. However, when Bcl2 is expressed together with the proto-oncogene *c-myc*, both p53-induced growth arrest and apoptosis are counteracted. In recent years, however, the role of mutations of single genes in the genesis of cancer has been questioned, and it was proposed instead that cancer is a chromosomal disease. According to this hypothesis, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes, ►aneuploidy corrupts teams of proteins that segregate, synthesize, and repair chromosomes. Aneuploidy is thus considered a steady source of karyotypic–phenotypic variations from which selection of further cancer-specific aneuploidies encourages the evolution and subsequent malignant progression of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4–7 orders of magnitude. In this scenario, the role of antiapoptotic genes, such as *Bcl2*, is even more paramount as they provide the opportunity for cancer cells to survive despite gross aneuploidy and to accumulate complex, malignant phenotypes.

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Bcl-2 Binding Component 3

►PUMA

Bcl-2 Family Proteins

Definition

These molecules can regulate apoptosis positively or negatively at the site of mitochondria. The prototype is ►Bcl-2, which is originally isolated at the t(14, 18) breakpoint from the follicular lymphoma. All members share the Bcl-2 homology (BH) domain. Three subgroups have been defined. The anti-death members include Bcl-2, Bcl-x_L, Mcl-1 and Ced-9. They possess the BH1, BH2, BH3 and BH4 domains. The multi-domain pro-death members include ►Bax, ►Bak and Bok, which have the BH1, BH2 and BH3 domains. The BH3-only pro-death members consist of Bad, Bik/Nbk/Blk, Bid, Bim, Bmf, Hrk/DP5, EGL-1, Noxa and ►PUMA, which all contain the BH3 domain only.

►Bid
►Apoptosis

BCL3

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Definition

BCL3 stands for B-cell leukemia/►lymphoma 3. The BCL3 gene is a proto-oncogene mapping to chromosomal band 19q13. It encodes a phosphoprotein of 446 amino acids exhibiting an apparent molecular weight between 47 and 60 kDa. BCL3 is an ►IκB-like protein that primarily functions as a transcriptional cofactor, especially in cooperation with ►NF-κB (nuclear factor κB).

Characteristics

Structure and Molecular Function

As its main structural feature, BCL3 protein exhibits seven so-called ankyrin repeat elements in its central domain. This structure is characteristic of the IκB family of proteins. Ankyrin repeats are tandemly arranged modules of about 33 amino acids. Through these motifs, IκB proteins interact with, and modulate the activity of NF-κB transcription factors. The NF-κB family consists of five members called RelA, RelB, c-Rel, p50 and p52. These subunits form various homo- and heterodimers that regulate the transcription of target genes by binding to specific (κB) sites present in promoter or enhancer elements. Unlike the other NF-κB subunits, p50 and p52 contain a DNA-binding domain but lack a transactivation domain. Thus, DNA-bound p50 and p52 homodimers inhibit gene transcription.

BCL3 differs from classical IκB family members by acting as a transcriptional cofactor. Hence, in many cells BCL3 is primarily located in the nucleus. Its proline-rich amino terminus and proline/serine-rich carboxyl terminus appear to function as transactivation domains. BCL3 preferentially binds to NF-κB p50 and p52 homodimers. These complexes can either activate or repress transcription of target genes. Two mechanisms of transcriptional activation by BCL3 have been described. It can either directly activate transcription by providing its transactivation activity to p50 and p52 homodimers, or cause de-repression by removing these inhibitory subunits from κB sites. Alternatively, BCL3 can also enhance binding of p50 and p52 to DNA, thereby inducing transcriptional repression. The circumstances leading to either effect are not well understood. The dual role of BCL3 in transcriptional regulation is reflected by its interaction with the basal transcription machinery and coactivators such as ►p300/CBP, SRC-1, and the ►histone acetyltransferase Tip60 and with corepressors such as ►histone deacetylases (HDACs). In addition to its role in NF-κB-dependent transcription, BCL3 has been described to function as a coactivator in complex with transcription factors ►AP-1 and ►retinoid X receptor by potentiating their activities.

Additionally, apart from its role in transcriptional regulation, BCL3 seems to exert a function in intracellular signaling. This conclusion results from the observation of BCL3 expression in thrombin-activated platelets. These cells are anuclear and incapable of gene transcription. Here, BCL3 has been found to associate with the ►Src-related protein kinase Fyn. The molecular relevance of this interaction is not known.

Regulation

BCL3 protein is modified by phosphorylation and polyubiquitination (►Ubiquitination). Phosphorylation occurs extensively and constitutively, predominantly at the serine-rich C-terminal domain. BCL3 exhibits several protein forms differing in their phosphorylation state. A major protein kinase shown to act on BCL3 is ►glycogen synthase kinase-3 (GSK3) that constitutively phosphorylates BCL3 at serines 394 and 398. This modification is followed by polyubiquitin linkage on N-terminal lysine residues of BCL3 and its subsequent degradation through the proteasome pathway. Therefore, this mechanism regulates BCL3 turnover. In addition, GSK3-mediated phosphorylation also influences the transcriptional function of BCL3 by modulating its interaction with HDAC transcriptional repressors, and attenuates its oncogenicity. Independent of these findings, the extent of BCL3 phosphorylation has been shown to affect its interaction with both NF-κB p50 and p52. Further information on signaling pathways leading to BCL3 phosphorylation is missing.

In addition, polyubiquitination has also been shown to regulate BCL3 entry into the nucleus. In B and T-cells, BCL3 exhibits a predominantly nuclear localization, while in several other cell types (e.g. erythroblasts, hepatocytes, keratinocytes) BCL3 resides in the cytoplasm and needs activation prior to nuclear translocation. Current data reveal that BCL3 requires a lysine 63-linked polyubiquitin chain in order to enter the nucleus and regulate gene transcription. This polyubiquitin modification acts as a "molecular ticket," probably by facilitating the interaction with nuclear transport receptors (called importins) and mediating transport through the nuclear core complex. Nuclear translocation of BCL3 is prevented by the de-ubiquitinating enzyme ►CYLD, which was identified as a tumor suppressor. Loss of CYLD results in de-ubiquitinylation of BCL3, which in turn facilitates nuclear accumulation of BCL3 and transcription of target genes which are able to promote cellular transformation.

Expression

The BCL3 gene is composed of nine exons, spanning 11.5 kb. Its transcript shows a broad expression pattern in multiple cells types. It is highly expressed in spleen and liver, with no apparent expression in brain. Transcription of BCL3 is regulated through several signaling pathways. In addition to regulatory elements in the promoter, two enhancer regions have been identified within the second intron.

BCL3 itself is an NF-κB target gene whose expression is initiated by a number of classic NF-κB-inducing stimuli (e.g. TNF-α, interleukin-1) but also upon activation of the T cell receptor. The corresponding κB sites have been found in the promoter and first intronic enhancer. BCL3 transcription is further induced by the Jak/Stat pathway (►Signal transducers and activators of transcription in oncogenesis). Stat3-activating cytokines (e.g., ►interleukin-6, -9 and -10) initiate BCL3 transcription primarily via Stat binding sites in the second enhancer. In mice, an AP1-dependent mechanism of BCL3 gene expression was found in T cells upon ►interleukin-4 stimulation. Moreover, BCL3 autoregulates its own transcription in a repressive manner. The negative feedback is mediated via the κB motifs.

In platelets, which lack nuclei and cannot synthesize mRNA, BCL3 expression is regulated on the translational level. In resting platelets, a preformed BCL3 mRNA pool exists whose translation is constitutively repressed. Upon activation, an ►mTOR-dependent rapid increase of BCL3 protein synthesis takes place. This specialized translational control pathway is mediated by a cascade also involving PI3K (►PI3K signaling) and PDK1 protein kinases and culminates in phosphorylation of the translation repressor ►4EBP-1, causing its dissociation from eukaryotic translation initiation factor 4E and allowing translation to proceed.

Physiological Function

Knockout mouse studies provide some information on the physiological function of BCL3. Although BCL3 is widely expressed, it seems to play its primary role in the immune system. BCL3 knockout mice appear developmentally normal, but are susceptible to certain kinds of pathogens. They are severely impaired in producing antigen-specific T and B cell responses. The altered microarchitecture in the spleen and lymph nodes, including the lack of ►germinal center formation, is thought to underlie the immunological defects.

In accordance with its observed role in immune responses, BCL3 functions have been found in immunologically relevant cells. BCL3 is selectively up-regulated in mature dendritic cells, and its absence results in failure of normal follicular dendritic cell differentiation. This finding might be the main reason for the observed defects in the microarchitecture of secondary ►lymphoid organs and T cell responses in BCL3-deficient mice. BCL3 was further shown to be required for the survival of activated T cells as well as for the attenuation of the pro-inflammatory (►inflammation) action of activated macrophages.

Moreover, BCL3 has been found to be transiently upregulated by DNA damage and to suppress p53 activation (see below). The data suggest a physiological role of BCL3 in B cell development. According to this hypothesis, BCL3 expression allows germinal center B cells to tolerate the DNA damage required for immunoglobulin ►class switch recombination and ►somatic hypermutation without mounting an apoptotic (►apoptosis) response.

BCL3 expression is highly upregulated in thrombin-activated platelets. In these activated platelets, BCL3 is required for retraction of fibrin clots, which is an important step in wound healing.

Oncological Relevance

The BCL3 gene was initially identified through its involvement in a t(14;19)(q32;q13) chromosomal translocation found in some patients with chronic lymphocytic leukemia (B-CLL) or other B-cell neoplasms. This translocation leads to juxtaposition of the BCL3 locus at chromosome 19q13 to the enhancer of the immunoglobulin heavy chain gene on chromosome 14q32, resulting in high-level expression of the BCL3 transcript. Recent studies have shown that elevated BCL3 expression is not limited to the rare cases of CLL or lymphomas with this translocation. High BCL3 expression has also been reported in subsets of ►diffuse large B-cell lymphomas, T-cell lymphomas (especially ►anaplastic large cell lymphoma), and (►Hodgkin disease). Furthermore, increased nuclear levels of BCL3 have been demonstrated in a growing number of non-lymphoid tumors such as breast cancer and nasopharyngeal carcinomas. Oncogenically activating mutations within the coding region of BCL3 have not been found so far. Consequently, elevated expression of BCL3 is hypothesized to contribute to oncogenesis by dysregulating target genes involved in cell proliferation, apoptosis and differentiation.

Consistent with a direct oncogenic function, BCL3 overexpression has been shown to lead to transformation of murine fibroblasts and induction of tumor growth *in vivo*. In contrast, transgenic mice expressing BCL3 in both B and T cells develop a lymphoproliferative disorder but no lymphoid neoplasms, indicating that BCL3 overexpression alone is not sufficient for the direct transformation of lymphoid cells.

A few target genes potentially involved in the oncogenic potential of BCL3 have been identified

so far. Transcription of the cyclin D1 (►cyclin D) gene, whose product acts as a key factor in driving cell cycle progression, is activated by BCL3 through its cooperation with p52 homodimers bound to an NF-κB motif in the cyclin D1 promoter. Concerted elevation of BCL3, p52 and cyclin D1 levels have been found in breast cancer cells. In these cells, *in vitro* studies also suggested a BCL3-mediated activation of the anti-apoptotic ►BCL-2 gene.

BCL3 can suppress the activation of tumor suppressor protein p53 (►p53 protein, Biological and Clinical Aspects), which is a crucial guardian of genomic integrity. Normally, p53 is kept at low levels mainly by its interaction with the Mdm2 protein, which mediates the proteosomal degradation of p53. When cells are exposed to genotoxic stress, this interaction is disrupted, and p53 accumulation results in either cell-cycle arrest or apoptosis. One proposed mechanism in this regulatory circuit is the ability of BCL3 to induce the expression of the p53 inhibitor Mdm2 via its recruitment to κB sites in the promoter occupied by p50 or p52. A more complete understanding of the role of BCL3 in human cancers is still lacking.

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BCL-6

Definition

B-cell CLL/lymphoma 6, also known as Bcl-6, BCL5, LAZ3, and zinc finger protein 51 (ZNF51) is a zinc-finger protein of 706 amino acids and 78 kD. The human BCL6 locus maps to 3q27 and the mouse bcl-6 gene locus to chromosome 16 (13.90 cM). BCL6 is a transcriptional regulator that probably plays an important role in lymphomagenesis. It is involved in a form of B-cell non-Hodgkin lymphoma characterized by chromosomal translocation t(3;14)(q27;q32) and t(3;22)(q27;q11) that involves BCL6 and immunoglobulin gene regions, and also in a t(3;4)(q27;p11) chromosomal translocation with Arhh (Ttf).

►BCL6 Translocations in B-Cell Tumor

BCL6 Translocations in B-cell Tumors

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Synonyms

BCL6 translocations in B-cell tumors are chromosomic translocations involving the 3q27 chromosome band

Definition

Mature B-cell tumors are often associated with chromosomal translocations that lead to the juxtaposition of cellular oncogenes with the ►immunoglobulin gene (IG) loci. The 3q27 translocation is unique, fusing the BCL6 gene on 3q27 to either one of the three IGs but also another non-IG partner. Cytogenetic and molecular analyses have demonstrated that alteration of 3q27 and/or BCL6 is one of the most common genetic abnormalities in B-cell tumors.

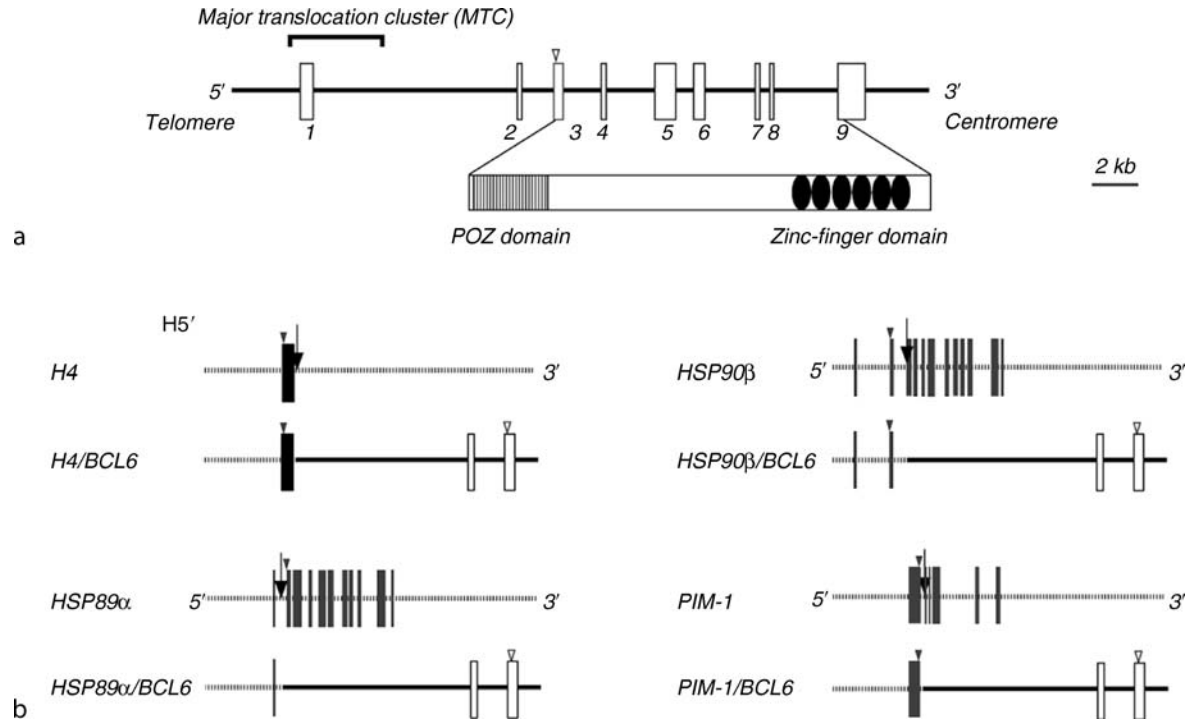
Characteristics

The BCL6 Gene and Gene Product

The BCL6 gene spans 26-kb and contains nine exons (Fig. 1a). At least two types of mRNA are produced by ►alternative splicing that may or may not contain exon 2. The ATG signal for initiation of protein synthesis is within exon 3 and is followed by an open reading frame, which encodes the Bcl-6 protein consisting of 706 amino acids with a calculated molecular weight of 79 kD. The Bcl-6 protein is a sequence-specific transcriptional repressor that contains two identified functional domains (Fig. 1a). The C-terminal region comprises six ►Cys2-His2 zinc finger motifs, each separated by a conserved stretch of seven amino acids. Hence, the Bcl-6 protein was classified as belonging to the Krüppel-like subfamily of ►zinc finger proteins. The N-terminal region contains a conserved protein-protein interaction motif, the POZ domain, which plays a role in homodimerization and heterodimerization. It has been shown that the repressing activity of Bcl-6 protein is exerted by the recruitment of both the ►SMRT co-repressor and a SMRT/mSin3A/HDAC-containing complex. Targeted inactivation of BCL6 in the mouse germline prevents germinal center formation in the lymphoid tissues and alters Th2-mediated immune responses, indicating that BCL6 is an important regulator of lymphoid development and function.

BCL6 Translocation Affecting the IG Loci

The most common type of 3q27/IG translocation is t(3;14)(q27;q32) involving IG heavy chain gene (IGH) on 14q32 [2, 3]. The breakpoints on IGH are within



BCL6 Translocations in B-cell Tumors. Figure 1 (a) Schematic presentation of the BCL6 gene and its protein product. (b) Non-IG/BCL6 translocations involving ►histone H4 gene, HSP89α heat shock protein gene, HSP90β gene [see ►HSP] and ►PIM-1 proto-oncogene. Open (BCL6) and closed (partner genes) boxes indicate the exons, and arrowheads indicate the translation initiation sites of each gene. Arrows indicate breakpoints of each translocation. All the translocations occurred with the same transcriptional orientation.

the switch regions of IGH, and the IGH upstream sequences are juxtaposed to BCL6 in the same transcriptional orientation. The breakpoints on BCL6 are clustered within a 4 kb region spanning the non-coding exon 1 and intron 1 (major translocation cluster; MTC; Fig. 1a); in the majority of cases, breakpoints are localized immediately 3′ of exon 1 (hyper-cluster) [2]. Thus, the coding regions of BCL6 remain intact. On the reciprocal junction, the 5′-BCL6 sequences are fused to downstream sequences of IGH. t(3;14) generates chimeric IGH/BCL6 transcripts that initiate from the IGH germline transcript promoters (Iμ and Iγ), and are followed by the BCL6 coding sequences [3].

Forms of the second, "variant" type of translocation are t(3;22)(q27;q11), involving IGλ light chain (IGLλ) on 22q11 and t(2;3)(p12:q27), involving IGLκ on 2p12 [2]. The last two "variant" translocations lead to juxtaposition of the 3′ sequences of IGLs to the BCL6 in divergent orientation. The breakpoints within the two IGLs are varied; 5′ of V genes, 5′ of Vλ/Jλ complex, at a point between the Vλ2–1, which is the most 3′ V gene of IGLλ, and the Jλ1 segment, and between the Jλ and Cλ segments [2]. Thus, the positions of breakpoints on the IGLs are not related to the regions in which V/J recombination normally occurs. Since IG translocations presumably occur as the result of errors in the recombination process, the positions of breakpoints on IGs can reflect the B cell stage where the translocation develops. While IGH/BCL6 translocations are most likely associated with the isotype class switching process following completion of V/D/J recombination, this "mis-class switch" mechanism may not be applicable to the variant IGL/BCL6 translocations.

BCL6 Translocations Affecting Non-IG Loci

Non-IG partners have been cloned by 5′-rapid amplification of cDNA ends (5′-►RACE) strategy. Another method to obtain a sequence of non-IG partner is long-distance ►inverse PCR. The Table 1 lists non-IG partners that have been identified using these two PCR-based approaches. These non-IG partners are not random but recurrently identified.

TTF is the first identified non-IG partner, which has been renamed RhoH (►Rho family proteins). The RhoH/TTF product possesses many Rho-hallmarks and defines a new group within the Rho subfamily. Other non-IG partners were registered in the GenBank database, and the exon-intron structure as well as the sequence of the regulatory region was studied. Figure 1b shows the representative organization of der(3) chromosome affected by BCL6 translocation.

BCL6 Translocations in B-cell Tumors. Table 1 Diverse partner genes of *BCL6* translocation in B-cell tumors

Genes		Gene product	Chromosomal locus
IG genes	*IGH*	Immunoglobulin heavy chain	14q32
	*IGL*κ	Immunoglobulin κ light chain	2p12
	*IGL*λ	Immunoglobulin λ light chain	22q11
Non-*IG* genes	*RhoH/TTF*	Rho GTP-binding protein	4p13
	BOB1/OBF1	B cell-specific transcriptional coactivator	11q23.1
	H4	H4 histone	6p21.3
	*HSP89*α	Heat shock protein 89α	14q32
	*HSP90*β	Heat shock protein 90β	6p12
	CIITA	MHC class II transactivator	16p13
	PIM-1	Pim-1 proto-oncogene product	6p21.2
	eif4AII	Eukaryotic initiation factor 4AII	18p11.2
	TFRR	Transferrin receptor	3q29
	Ikaros	Ikaros	7p12
	LCP1	L-plastin	13q14
	α-*NAC*	α Chain of the nascent polypeptide-associated complex	12q23–q24.1

The common molecular features of non-IG/BCL6 translocations include:

- The gene fusion occurs in the same transcriptional orientation
- The breakpoint on the partner gene is localized in close proximity to its promoter sequence
- The complete set of the promoter is fused upstream to the coding region of BCL6 on the der(3) chromosome

As the result of the translocation, many types of regulatory sequences on each partner locus substitute for the 5′ untranslated region of the BCL6 gene, and the rearranged BCL6 gene is presumed to be under the control of the replaced promoter activity (promoter substitution). Partner genes are transcriptionally activated by a variety of stimuli, including cell-cycle control, changes in the physical environment and response to cytokines. As ►germinal center (GC) B-cells proliferate rapidly in response to antigen stimulation, it is likely that the BCL6 gene affected by the translocation is inappropriately expressed during B-cell proliferation. A key experiment to establish the role of non-IG/BCL6 translocation would be the creation of a transgenic mouse model, in which the translocation is re-created by placing BCL6 under the control of these diverse promoters.

The 5′ Non-coding Region of BCL6 Undergoes Somatic Hypermutation

Somatic mutations within the 5′ non-coding region of BCL6 have been described in a significant proportion of ►GC/post-GC type B-cell tumors. The majority of the mutations cluster around the 3′ of exon 1, which has been referred to as the Major Mutation Cluster (MMC). These mutations are often multiple, are frequently biallelic and are independent of BCL6 translocation or linkage to IGs. Somatic mutations within the MMC were observed in a large proportion of memory B-cells isolated from normal individuals as well as GC B-cells from a reactive tonsil. The presence of cis-acting elements in BCL6, which are shared with IG and essential for targeting the mutation, has been suggested. Although the MTC and MMC apparently overlap, possible linkage between the two genetic alterations remains to be determined.

Clinical Relevance

Large numbers of B-cell tumors with 3q27 translocation in association with the IG gene loci were first described in 1989. 3q27/IG Translocations were identified in 20 of 318 (6.3%) cases of B-cell ►non-Hodgkin lymphoma (B-NHL) with clonal chromosomal abnormalities. There are nearly 20 cytogenetically identifiable non-IG chromosomal sites, some of which have been reported by several independent laboratories. The overall incidence of 3q27 abnormalities in NHL has been estimated to be 15.9% of all NHL types and 23.1% of ►DLBCL.

An initial study of BCL6 rearrangement, using Southern blot analysis with a probe for MTC, indicated a specific correlation of the rearrangement with diffuse large B-cell lymphoma (DLBCL). However, later studies of panels of many NHL types invariably showed that a significant number of cases with ►follicular lymphoma (FL) carried such rearrangements. The range of BCL6 rearrangements in B-NHL subtypes are 5–15% in FL, 20–40% in DLBCL and its variants, and 20% in ►acquired immunodeficiency syndrome (AIDS)-associated DLBCL.

The 3q27 translocation and/or BCL6 rearrangement sometimes coexist with other IG translocations associated with B-cell tumors, i.e. t(8;14)(q24;q32) and t(14;18)(q32;q21) and their variants. In some cases, alteration of the BCL6 locus was not a primary genetic abnormality but may have occurred at the time of transformation from low- to high-grade disease.

IG translocations sometimes correlate with clinical features of B-cell tumors. For instance, B-NHL associated with t(8;14) and/or its molecular equivalent shows aggressive clinical behavior even though the translocation can occur not only in ►Burkitt lymphoma but also in other types of B-cell tumors. An earlier study showed that BCL6 rearrangement more frequently occurs in extranodal DLBCL than in node-based disease and is correlated with a favorable clinical outcome. However, later studies failed to confirm these observations. Another interesting issue is whether the diverse partner genes can affect the clinical features of NHL carrying a particular BCL6 translocation. It has been observed that the overall survival of DLBCL with non-IG/BCL6 translocation was significantly inferior to that of those with IG/BCL6 translocation. Although the total number of patients analyzed is quite small, additional studies of larger cohorts are warranted.

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BCL-x

Definition

Bcl-x belongs to the ►BCL-2 family of proteins and is associated with cell survival.

►Signal Transducers and Activators of Transcription in Oncogenesis

Bcl-X_L

Definition

Is a member of the pro-survival subfamily ►(Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1 and also Bcl-B in humans) that protects cells exposed to diverse cytotoxic conditions by promoting cell survival. It is a member of an evolutionarily conserved 'stress' pathway, which is triggered by developmental cues and diverse intracellular stresses that activate caspase-9 on a scaffold formed by Apaf-1 in response to cytochrome *c* released from damaged mitochondria. This pathway, also termed 'mitochondrial' or 'intrinsic', is primarily regulated by the Bcl-2 family.

►NUP98-HOXA9 Fusion

BCNS

Definition

Basal Cell Nevus Syndrome; synonym ►Gorlin Syndrome, nevoid basal cell carcinoma syndrome (NBCCS).

BCR-ABL1

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Definition

BCR-ABL1 is a hybrid (fusion or chimaeric) gene that arises when genomic ►DNA of the *BCR* gene on chromosome 22 and of the *ABL1* gene on chromosome 9 breaks and recombines. The *BCR-ABL1* hybrid gene is transcribed to produce a hybrid mRNA that is subsequently translated into a functional BCR-ABL1 protein. The *BCR-ABL1* mutation causes and is diagnostic of human ►chronic myeloid leukemia (CML) and some acute leukemias including particularly ►acute lymphoblastic leukemia (ALL).

Characteristics

A Somatic Mutation of Bone Marrow Progenitor Cells

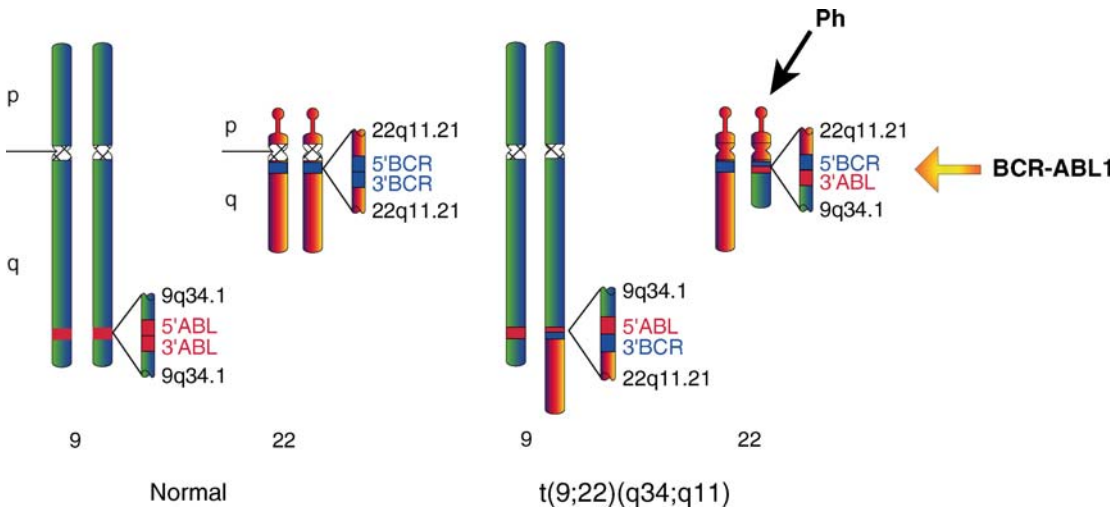
The *BCR-ABL1* mutation is somatically acquired. ►Recombination between the *BCR* and *ABL1* genes

occurs in an early progenitor or stem cell of the bone marrow, and usually results in the microscopically visible ►chromosome translocation t(9;22)(q34;q11) (Fig. 1).

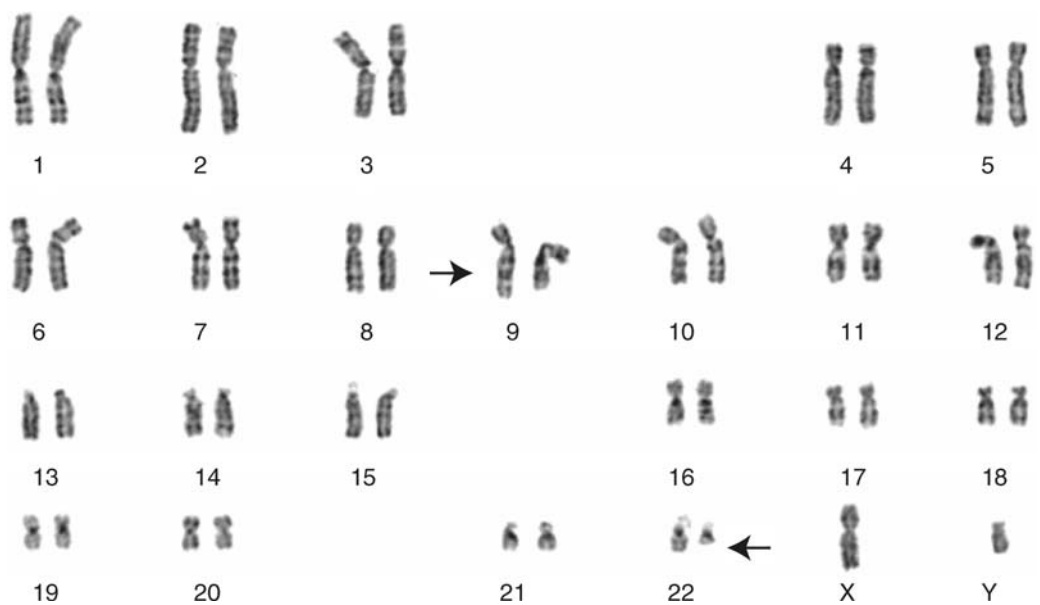
One product of this translocation is the well known Philadelphia (Ph) chromosome (Fig. 2) a shortened chromosome 22 identifiable in leukemic metaphase cells of ~90% of patients with CML. The Ph and associated *BCR-ABL1* hybrid gene are also found recurrently in ALL, although with higher frequency in adult (15–20%) compared with childhood ALL (5%). The discovery of the Ph chromosome in 1960 was a milestone for cancer research, providing the first clear indication that specific cancer types were characterized by recurrent genetic changes with potential proliferative advantage.

Molecular Features of BCR-ABL1 Recombination

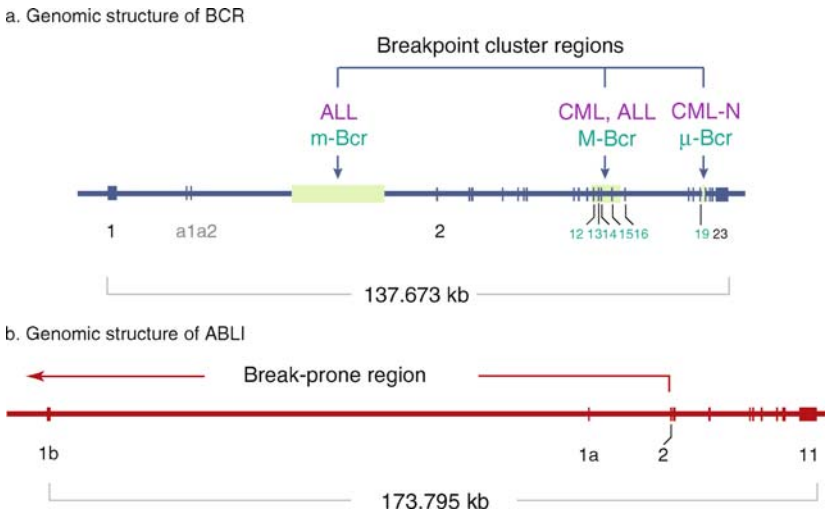
Recombination between the *BCR* and *ABL1* genes usually generates two products: a *5′ABL1–3′BCR* hybrid gene on the derivative 9q + chromosome that is in some cases transcribed but not apparently



BCR-ABL1. Figure 1 Ideogrammatic representation of chromosomes 9 and 22 before (left) and after (right) recombination between the *BCR* and *ABL1* genes to form the leukemia-initiating hybrid *BCR-ABL1* gene.



BCR-ABL1. Figure 2 Karyotype of a leukemic metaphase cell showing the standard Ph translocation, 46,XY,t(9;22)(q34;q11).



BCR-ABL1. Figure 3 Genomic structure and features of the human *BCR* and *ABL1* genes. (a) Exons 1–23 of *BCR* and alternatives (a) are indicated as blue boxes; The minor breakpoint cluster region (m-Bcr), major ▶breakpoint cluster region (M-Bcr) and micro breakpoint cluster region (μ-Bcr) are shaded in green. Disease subtypes associated with the different regions are shown as ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia and CML-N, neutrophilic chronic myeloid leukaemia; (b) Genomic structure of the human *ABL1* gene. Exons 1–11 and alternatives (a) are indicated as red boxed regions.

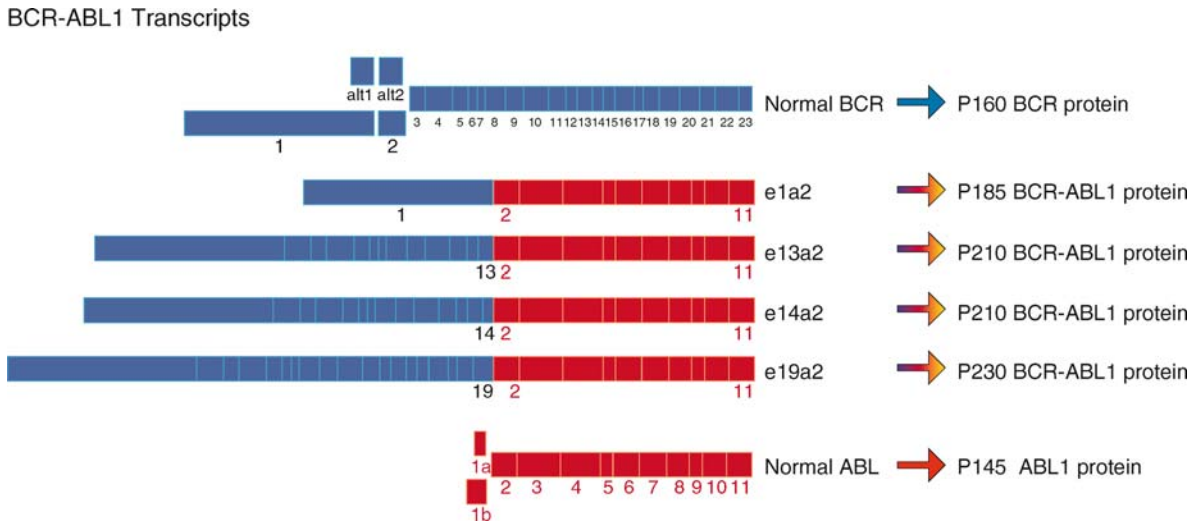
translated, and a *5′BCR-3′ABL1* product on the derivative 22q- or Ph chromosome that is both transcribed and translated. The leukemia-causing properties of the 5′BCR-3′ABL1 protein have been proven in a variety of animal models, and it is to this product that the BCR-ABL1 acronym usually refers.

Both *BCR* and *ABL1* are large genes, at ~138 and 174 kb, respectively (Fig. 3). Several viable in-frame *BCR-ABL1* fusions have been reported or predicted. However, depending on the location of the breakpoint site within *BCR*, those associated with leukemia generally differ according to the number of *BCR* exons that link with the constant *ABL1* exons 2–11 (Fig. 4).

- p210 BCR-ABL1: Breaks occur within the 5-kb major breakpoint cluster region (M-Bcr) of BCR in most cases of CML, and in about 50% of BCR-ABL1 rearrangement-positive cases of ALL. In these cases, the *BCR-ABL1* fusion gene is transcribed as a large chimaeric mRNA that is spliced into an 8-kb mRNA with BCR exon 13:ABL1 exon 2 (e13a2) and/or BCR exon 14:ABL1 exon 2 (e14a2) junctions. This hybrid mRNA is in turn translated to form a 210-kD BCR-ABL1 fusion protein.
- P230 BCR-ABL1: A larger 230-kD protein identifies a subgroup of patients with neutrophilic CML (CML-N) that present with a lower white cell count than usual and for whom progression to blast crisis is slow. In these cases, a breakpoint occurs in a region more 3′ in *BCR* (m-Bcr) to form a BCR exon 19: ABL1 exon 2 (e19a2) mRNA transcript in which almost the entire BCR gene is joined with ABL1.
- P190 BCR-ABL1: For the remaining 50% of BCR-ABL1 positive ALL cases, breakpoints usually occur at different sites across a wider ~35-kb region designated m-Bcr (minor breakpoint cluster region), which maps ~46-kb upstream of M-Bcr. A BCR exon 1:ABL1 exon 2 (e1a2) transcript is expressed in these cases, which is translated into a smaller 185-kD BCR-ABL1 protein. The e1a2 transcript is occasionally found in CML patients when it may be associated with a more aggressive clinical course.
- For all BCR-ABL1 leukemias, although sites of preferential breakage in the *ABL1* gene have been identified, breakpoint locations overall are more variable than those in *BCR*, and may occur at different sites within a >200-kb region extending from a point 9-kb 5′ of the entire gene to exon 2.

Complex BCR-ABL1 Rearrangements

About 10% of CML cases show more complex *BCR-ABL1* rearrangements that involve other chromosomal sites, and which may be camouflaged by a normal karyotype. In all of these cases, the 5′ part of *BCR* is fused with the 3′ part of *ABL1* to form the characteristic *BCR-ABL1* fusion gene essential for the development of CML. However, the 3′ part of *BCR*, which unites with the 5′ *ABL1* remnant in the standard t(9;22)(q34;q11), usually recombines with one of the additional chromosomes in the complex translocations or with a part of chromosome 9 outside of the *ABL1* gene. Although patients present with clinical features typical of BCR-ABL1



BCR-ABL1. Figure 4 Normal BCR and ABL1 transcripts and the most frequently detected variant BCR-ABL1 fusion transcripts. Corresponding protein products are shown to the right. Alternative (alt) exons are marked above the normal transcript for BCR and as 1a or 1b for ABL1.

leukemia, the biological and pathological consequences of complex recombination variants to treatment response and disease course is still unresolved.

Translocation-Associated Genomic Deletions

Another level of complexity in *BCR-ABL1* rearrangement is found in the form of translocation-associated deletions. These genomic deletions, either proximal to the 5′ *ABL1* breakpoint or distal to the 3′ *BCR* breakpoint are associated with the derivative 9q + of the standard t(9;22) or with sites of recombination on additional partner chromosomes in complex variant *BCR-ABL1* rearrangements. The deletions, which were initially identified fortuitously after development of new fluorescent *in situ* hybridization (►FISH) probe systems for detecting ►minimal residual disease in interphase cells of CML patients, are found in ~10–15% of all CML patients, with an increased frequency reportedly associated with complex *BCR-ABL1* rearrangements. The deletions can be large, with variable proximal and distal breakpoints located up to 8 Mb from *ABL1* and 4 Mb from *BCR* on the derivative 9q + or derivative additional partner chromosome. They can occur simultaneously during the *BCR-ABL1* recombination translocation-forming process or occasionally as a subsequent step after the initial translocation. Patients having translocation-associated deletions tend to have a considerably worse prognosis and survival than patients without deletions. The biological basis for the survival disadvantage associated with positive deletion status is presently not known, but may possibly be due to loss of tumor suppressor genes within the deleted region.

Detection of BCR-ABL1

The *BCR-ABL1* fusion gene may be detected in leukemic cells by one or more of the following molecular procedures:

- Amplification of predicted e1a2, e13a2, e14a2, e19a2 or variant splice junctions using polymerase chain reaction (►PCR) after reverse transcription of leukemic cell mRNA (RT-PCR)
- FISH on single cells (metaphase or interphase) using a combination of large insert *BCR* and *ABL1* probes
- ►Southern blotting using leukemic DNA digested with appropriate restriction enzymes and one or more probes from within M-Bcr or other relevant regions of *BCR*
- By immunoprecipitation and Western blot analysis of the chimaeric protein

What Causes BCR-ABL1?

The mechanism that underlies *BCR-ABL1* gene rearrangement in most leukemias is unknown, but new and relevant clues are gradually emerging. For example, there is a clear association, both epidemiologically and in the laboratory, between exposure to ionizing radiation and the development of BCR-ABL1 leukemia. This increased risk is reflected in the increased incidence of CML in atomic bomb survivors compared to the general population, and in the increased occurrence of *BCR-ABL1* mutations in cultured cells subjected to high-dose gamma-irradiation and X-irradiation. Recent findings suggest that ionizing radiation can influence the generation of leukemia-specific fusion genes by juxtaposing genes normally

distanced in the interphase cell nucleus, and that certain cell types with a lineage-specific 3-D chromatin distribution may be more or less susceptible to a particular fusion gene rearrangement than others.

BCR-ABL1 in Healthy Individuals

BCR-ABL1 transcripts have been identified, using RT-PCR, at very low levels in circulating peripheral blood granulocytes of more than two thirds of healthy adults. The identification of other leukemia-associated fusion transcripts in different studies provides good evidence that aberrant recombination occurs ubiquitously at a baseline level in somatic cells of normal individuals. These findings also suggest that additional selective processes, such as immunological tolerance or cell type origin and stage of differentiation, are required to provide BCR-ABL1 cells with a proliferative advantage and produce the leukemic phenotype.

Why Breakpoint Cluster Regions?

The molecular factors that determine preferential breakage sites in *BCR*, and precipitate *BCR-ABL1* recombination are presently unknown, but the ►Alu element is a strong candidate to facilitate this process. Sequence analysis of M-Bcr has identified a single Alu element central within an ~3-kb region where more than 70% of the breakpoints occur. In addition, analysis of reciprocal *BCR-ABL1* and *ABL1-BCR* breakpoint junctions from several cases of CML and ALL has identified sequence homology to Alu elements at, or close to, the sites of recombination. M-Bcr also recombines preferentially with Alu elements at chromosomal sites outside of *ABL1* in complex *BCR-ABL1* rearrangements, and in these cases an association with gene coding domains and ►translin-specific binding motifs was also suggested. Further research is needed to clarify the significance of these findings.

Molecular Consequences of BCR-ABL1

The leukemia-causing properties of the BCR-ABL1 protein have been demonstrated in a range of *in vivo* and *in vitro* laboratory models, including mice made transgenic for different forms of the hybrid oncogene or transplanted with BCR-ABL1 transfected stem cells. BCR-ABL1 cells are more proliferatively active, differentiate abnormally, show an increased resistance to ►apoptosis and have altered adhesion properties compared with their normal counterparts. Much recent work has sought to understand the mechanisms that precipitate these features and precisely how the BCR-ABL1 mutation activates cell transformation in vivo.

Normal BCR proteins are found in the cytoplasm and have at least two enzymatic activities, serine/threonine kinase at the N-terminal, and GAP activity at the C-terminal. The normal ABL1 protein is a non-receptor protein tyrosine kinase that is localized to the cytoplasm, where it is weakly associated with actin filaments, and the nucleus, where it is associated with chromatin. In BCR-ABL1 hybrid proteins, the fused BCR sequences block nuclear translocation and activate the actin binding function that is required for BCR-ABL1 to efficiently transform cells. Because of its heightened ►tyrosine kinase activity, the *BCR-ABL1* protein can phosphorylate a range of different substrates, so activating multiple different cytoplasmic and nuclear signal-transduction pathways relevant to hematopoietic cell growth and differentiation. Examples of signaling cascades activated by BCR-ABL1 include the ►Ras pathway, the Jun-kinase pathway, the phosphatidylinositol-3 kinase pathway, a variety of CRKL-linked signaling processes, the Jak-STAT (►Signal transducers and activators of transcription in oncogenesis) pathway and the Src pathway.

Clinical Relevance

- Chronic myeloid leukemia (CML) is a myeloproliferative disorder that develops after the BCR-ABL1 mutation occurs in a pluripotent bone marrow stem cell. The affected stem cell gains a proliferative advantage and a malignant leukemic clone becomes established. CML, characterized by overproduction of granulocytes in the bone marrow and peripheral blood, accounts for about 25% of all human leukemias, with an incidence of ~1 in 100,000 per year. CML affects both sexes and all age groups, but occurs most commonly at 40–50 years. Patients typically present with symptoms of fatigue, bleeding, moderate weight loss, an enlarged palpable spleen, and a high white blood cell count.
- ►Blast crisis CML: CML is a biphasic disease, and without effective treatment usually progresses within 3–5 years of diagnosis to an aggressive and terminal acute phase or blast crisis. The precise molecular events that determine blast crisis are still unknown, although there is much evidence from cytogenetic and molecular studies that non-random and lineage-specific accumulation of gene mutations may be important.
- BCR-ABL1 is also found in leukemic cells of patients with adult (10–20%) or childhood (5%) acute lymphoblastic leukemia (ALL L1 or L2), and in rare cases (~3%) of acute myeloid leukemia (AML, mostly M1 or M2).

Anti-BCR-ABL1 Therapies

Bone marrow transplantation and/or alpha-interferon therapy have been the treatments of choice for BCR-ABL1 leukemias. But the introduction of ►imatinib mesylate, a synthetic tyrosine kinase inhibitor designed to specifically impede BCR-ABL1 fusion protein activity, has significantly improved the overall outlook and prognosis for the majority of CML patients and

this drug is now considered standard therapy for CML. New anti-BCR-ABL1 therapies are additionally being developed to improve outlook for the proportion of patients who do not respond or to help overcome leukemic cell resistance developed in some cases to imatinib.

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BCSG1

Definition

Breast cancer specific gene 1. Encodes the protein ►synuclein γ.

►Synuclein

BDNF

Definition

Brain-derived Neurotrophic Factor.

Beckwith-Wiedemann Syndrome

Definition

BWS is a rare, congenital overgrowth disorder in which babies are large at birth and may develop low blood sugar. Other common symptoms include a large tongue, large internal organs, and defects of the abdominal wall near the navel. Beckwith-Wiedemann syndrome increases the risk of developing certain cancers, especially ►Wilms tumor.

►BWS
►Beckwith-Wiedemann Syndrome-Associated Childhood Tumors

Beckwith-Wiedemann Syndrome Associated Childhood Tumors

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Definition

Beckwith-Wiedemann syndrome (BWS) is a complex overgrowth disorder caused by a number of genes that are subject to genomic imprinting. A high incidence of solid childhood tumors is seen in patients that present with BWS.

Characteristics

Diagnostic Criteria

Beckwith-Wiedemann syndrome is a disorder first described by Beckwith in 1963 at the 11th annual meeting of the Western Society for Pediatric Research. Later, Wiedemann and Beckwith described the syndrome in more detail [1, 2]. BWS is characterized by a great variety of clinical features, among which are abdominal wall defects, macroglossia, pre- and post-natal gigantism, earlobe pits or creases, facial nevus flammeus, hypoglycemia, renal abnormalities and hemihypertrophy. BWS patients have a 7.5% risk of developing (mostly intra-abdominal) childhood tumors. Tumors most frequently found are ►Wilms tumor (WT), adrenocortical carcinoma (ACC), rhabdomyosarcoma (RMS) and hepatoblastoma (HB). Patients can be classified as having BWS according to the clinical criteria proposed by Elliot or DeBaun, although cases of BWS are known that do not comply with either set of criteria.

(Epi)-Genetics

The syndrome occurs with an estimated incidence of 1:13,700 and most cases are sporadic (85%). The genetic predisposition for BWS lies on chromosome 11p15 (linkage analysis, chromosome abnormalities, loss of imprinting (LOI), gene mutations). The syndrome is

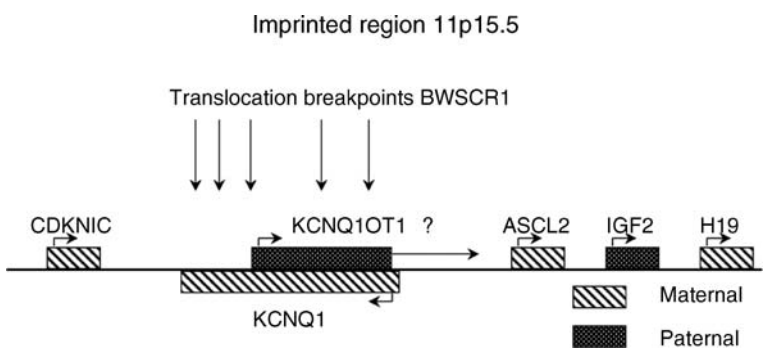
subject to genomic imprinting since maternal transmission seems to be predominant. In addition chromosomal translocations are of maternal origin, duplications and uniparental disomies (UPD) of paternal origin. All hitherto known causative genes are imprinted. The translocation breakpoints on chromosome 11 map to three distinct regions within 11p15.3-pter. Beckwith-Wiedemann syndrome chromosome region 1 (BWSCR1) near INS/IGF2, BWSCR2 5-Mb proximal to BWSCR1 and BWSCR3 2-Mb even more proximal. This already points to genetic heterogeneity, but also at the clinical level there seems to be heterogeneity. Chromosomal translocations in BWSCR1 and BWSCR3 are associated with the classical BWS phenotype, and BWSCR2 with minor BWS features but pronounced hemihypertrophy. BWSCR 1 and BWSCR2 have been cloned, and genes isolated from this region were shown to be involved in the development of this disorder. All genes involved are subject to genomic ►imprinting.

BWSCR1

This region consists of a number of imprinted genes (Fig. 1). All known translocation breakpoints disrupt KCNQ1, a gene coding for a potassium channel involved in the Romano-Ward en Jervell-Lange-Nielsen cardiac arrhythmias syndromes. However, this imprinted gene is most likely not directly involved in BWS, but a gene transcribed in the antisense orientation of KCNQ1 clearly is. This gene, KCNQ1OT1, shows aberrant methylation in 50–80% of BWS cases. It does not code for a protein and functions through its RNA. CDKN1C is an inhibitor of cyclin-dependent kinases. Heterozygous mutations have been identified in about 20% of BWS patients in two studies. Others, however, have not been able to confirm this mutation frequency. The gene is not a major cause of BWS. It is, however, possible that in certain countries the mutation frequency is elevated (e.g. Asia). In addition, it has been reported that this gene is more frequently involved in familial cases of BWS. CDKN1C mouse models revealed some of the clinical BWS features such as omphalocele and renal adrenal cortex anomalies. In humans, CDKN1C also seems to be more frequently associated with abdominal wall defects. Another strong candidate for involvement in the aetiology of BWS is the embryonic growth factor IGF2. Mouse models overexpressing IGF2 displayed a phenotype overlapping with the BWS phenotype. Loss of IGF2 imprinting is often seen in BWS patients. H19, another non-coding gene, lies downstream of IGF2 and the expression of IGF2 and H19 seems to be linked. H19 is important for the maintenance of the imprinting status of IGF2. Mouse studies underline the link between IGF2 and H19 expression and overgrowth phenotypes were found. H19 loss of imprinting (silencing of the gene) is frequently seen in BWS cases although not always in combination with IGF2 loss of imprinting (LOI). Interestingly, overexpression of H19 seems to lead to the Silver Russell Syndrome (SRS), characterized by intrauterine growth retardation, poor postnatal growth, asymmetry a classic facial phenotype and no increased risk for childhood tumors. Finally a gene called ASCL2 is localized to the 11p15-imprinted region. Although no direct involvement in the BWS aetiology is known, this gene might account for the fact that most, if not all, BWS cases with uniparental disomy (UPD) present in a mosaic form. The mouse homologue codes for a transcription factor, which is expressed during early mouse development and is essential for the development of the placenta. Therefore, also in humans, complete lack of expression might be lethal.

BWSCR2

Two patients with balanced chromosomal translocations define this second chromosomal region, one of which developed a Wilms tumor. Both translocations in 11p15.4 disrupt a paternally imprinted zinc-binding finger gene ZNF215. Parts of the 3′ end of this gene are transcribed from the antisense strand of a second zinc-finger gene, ZNF214. Although putative mutations in



Beckwith-Wiedemann Syndrome Associated Childhood Tumors. Figure 1 Imprinted genes on 11p15 involved in BWS. The parental expression (imprinting) of these genes is indicated.

these genes in other sporadic BWS cases were found, their involvement in BWS needs to be further elucidated by functional studies.

Diagnostics

BWS can be diagnosed in the laboratory with cytogenetics (<5%) or DNA-diagnostics. The current major test involves methylation assays or LOI studies at the RNA level. The majority of cases (50–80%) exhibit aberrant methylation of KCNQ1OT1 with or without aberrant methylation of IGF2/H19. These former cases often show UPD for 11p15 (in a mosaic form) which explains this aberrant methylation for multiple genes. However, the majority of cases with KCNQ1OT1 defects and some cases with H19/IGF2 defects have no UPD 11p15. Therefore, an imprinting switch can be assumed, involving an imprinting centre analogous to the Prader-Willi and Angelman syndromes. The current data are most compatible with two distinct imprinting centers for either KCNQ1OT1 or IGF2/H19. CDKN1C mutation analyses might be considered, especially in familial cases of BWS. The increased tumor risk for BWS patients seems to be associated with UPD in general and H19 methylation defects in particular. KCNQOT1 methylation defects only seem to be a reliable prognostic factor since tumors are seldomly associated with this group of patients. Recurrence risks for a second pregnancy can be assessed with UPD studies. In cases of a UPD in a mosaic form, there is no increased recurrence risk for BWS in a second pregnancy since the genetic defect occurred post-fertilization.

BWS Associated Tumors

Although childhood solid tumors associated with BWS share some common genetic features, the spectrum of genetic changes found in these tumors is diverse and complicated with many genetic alterations seen.

Wilms Tumor

The tumor most often found to be associated with BWS is Wilms tumor (WT) or nephroblastoma (59% of the tumors found in BWS patients). Overall it occurs with a frequency of 1 in 10,000 children, mostly in children under the age of 5 years. In patients suffering from BWS the incidence is 800–1000 times increased. A high percentage (38%) show loss of heterozygosity (LOH) of chromosome 11p. This region can be subdivided roughly into two parts: LOH of markers on 11p13 and LOH of markers on 11p15. The region on 11p13 has been shown to be deleted in patients affected by WAGR. WAGR stands for the combined occurrence of sporadic aniridia, WT, genitourinary abnormalities and mental retardation. A gene in the candidate region (WT1) has been cloned. Mutations of this gene occur in only 10–15% of sporadic Wilms tumors, suggesting the existence of additional genes involved in the development of this tumor. The Denys-Drash syndrome, another syndrome associated with Wilms tumor, shows constitutional mutations of the WT1. The region on 11p15 showing LOH in WTs can be subdivided into two regions: An 800 kb region containing the WT2 locus near IGF2 and an additional locus of 336 kb proximal to WT2. WT can also be found in association with other syndromes, like the trisomy 18 syndrome, the Perlman syndrome and the Simpson-Golabi-Behmel, the Sotos syndrome and the Klippel-Trenaunay syndrome. The ►Li-Fraumeni syndrome is a rare familial tumor syndrome and patients suffering from this disease contain germline point mutations in the ►p53 tumor suppressor gene. The tumors that develop in these patients show a deletion of the wild type p53 allele. Although WT is not considered to be part of the Li-Fraumeni syndrome there have been few reports of the occurrence of WT in families affected by this syndrome. Mutations in the tumor suppressor gene p53 have been found in sporadic WTs and seem to be associated with a histological subtype. In a series of 140 WTs, mutations were restricted to tumors of the anaplastic subtype, showing aberrations in 8/11 samples. This subtype is linked to a poor prognosis. In 10–25% of the Wilms tumors, LOH of 16q markers is found. It has been suggested that LOH of 16q is associated with an adverse prognosis. Another genetic abnormality, which seems to confer an adverse outcome, is LOH of 1p. This abnormality was found in 12 and 18% of the cases respectively. Chromosome 7 also seems to be involved in Wilms tumor. According to the literature in 23% of the cases chromosome 7 is rearranged. Another region found to be frequently involved in LOH (14%) is on chromosome 22q. In a study which quantified chromosome 12 allelic imbalance in a series of 28 Wilms tumors, duplications were detected in 18%. An inventory of all quantitative chromosome aberrations occurring in a series of 46 WTs was made using comparative genomic hybridization analysis (CGH). Chromosome regions showing loss of DNA in three or more samples included 1p (11%), 11p (9%), 16q (13%) and 17p (7%). Regions showing gain of DNA in three or more samples included 1q (20%), 7q (9%), 8 (7%), 12q (17%), 17q (7%) and 18 (7%). In 2007, it became clear that a somatic deletion of an X-linked gene (WTX) is found in 1/3 Wilms tumors.

As expected, imprinting seems to play a major role in WT development since 11p15 LOH is always of maternal origin. This resulted in the hypothesis that a paternally imprinted tumor suppressor gene is involved in Wilms tumorigenesis. Alternatively, a maternally imprinted gene involved in stimulation of cell growth could be involved in the cases showing paternal UPD of (part of) chromosome 11. At present there are three

candidate genes on 11p15 that show parent-of-origin-dependent monoallelic expression and belong to one of these two categories: the tumor suppressor genes H19 and CDKN1C which are maternally expressed and the paternally expressed growth-promoting gene IGF2. Evidence for the involvement of these genes has been found i.e. loss of imprinting, or increased expression of IGF2 or reduced expression of CDKN1C or H19.

Adrenocortical Carcinoma

The second most common tumor found in BWS-patients is adrenocortical carcinoma (ACC). It is found in 15% of patients that develop a tumor. In the general population ACC is found to be an extremely rare tumor with an incidence of 1.7 new cases per 1,000,000 per year. As in BWS, IGF2 seems to be involved in sporadic ACC-tumorigenesis. A considerable proportion of the malignant tumors (~60%) display LOH of the 11p15.5 region, presumably all representing uniparental disomies. This is seen in both adult and childhood ACCs. In these cases a good correlation was found with over-expression of the IGF2 gene. These phenomena were found in a much smaller percentage in the benign adenomas. It has been hypothesized that adrenocortical tumorigenesis is a multistep process with sequential progression from the normal to the adenomatous and then to the malignant cell. If this is the case then IGF2 could be involved in the transition from adenoma to carcinoma. ACC is also found in association with other syndromes. One of these is the Li-Fraumeni syndrome, which is associated with mutations of the p53 gene on chromosome 17p. In one study, in which sporadic ACC's were analyzed for the presence of LOH at three different chromosome regions, chromosome 17p (containing the p53 gene) had become homozygous in all informative samples. LOH of 17p was not found in adrenocortical adenoma, the benign counterpart of ACC. Again, if the hypothesis that adrenocortical tumors develop from normal tissue to adenomas to carcinomas is correct, this would mean that LOH of 17p could be a late event in ACC tumorigenesis. Two other groups identified mutations in the p53 gene in ~30% of sporadic ACC's. In addition, CGH analysis showed loss of 17p in 50% of the (sporadic) cases. Another hereditary tumour syndrome associated with adrenocortical tumors is ►multiple endocrine neoplasia type 1 (MEN1). In most cases associated with MEN1 adrenocortical adenomas are found. The disease is caused by mutation of the menin tumor suppressor gene (MEN1), located at 11q13.

Other regions found to be lost in ACC's include chromosome 13q, which was shown to have lost heterozygosity in 50% of informative patients, and chromosome 2. Genetic aberrations that were found in 38% of the tumors in this study were gains of chromosomes 12, 15q, 16q and 19p and losses of chromosomes 3p, 6q, 8p, 9p, 11p, 17q, 18q and 22q. There are numerous differences between the genetic aberrations found in adrenocortical adenomas and adrenocortical carcinomas. These differences may reflect various stages along the carcinogenic pathway.

Evidence for an involvement of imprinting in ACC again comes from LOH 11p15 studies (maternal loss) and LOI and expression studies for IGF2 and H19. It should be noted that LOI of IGF2 was associated with the malignant phenotype, since it was not detected in the adenomas but only in the carcinomas.

Rhabdomyosarcoma

Although rare, rhabdomyosarcoma (RMS) represents the most common soft-tissue sarcoma in children under the age of 15 years. It occurs with a frequency of 1.3–4.5 cases per million children per year. Based on their histology, rhabdomyosarcomas can be subdivided into three major subtypes: embryonal (E-RMS), alveolar (A-RMS) and pleomorphic (P-RMS) rhabdomyosarcoma, of which E-RMS is the subtype associated with BWS. Of all newly diagnosed cases 60% are E-RMS and 20% are A-RMS. Patients with E-RMS have a better prognosis than patients with A-RMS. LOH of chromosome 11p is an abnormality found frequently in RMS. In one study it was found in 72% of primary E-RMS and 20% of primary A-RMS. A gene located in this region, GOK (gene on chromosome 11) or STIM1 (stromal interaction molecule 1) was postulated to be a candidate tumor suppressor gene in RMS. No expression was found in seven RMS cell lines, and transfection of the gene into the RMS cell line RD was followed by growth arrest of the cells. LOH of 16q was also found in both types (in 55% of E-RMS and 40% of A-RMS). In total, LOH of 6p was found in 28% and LOH of 18p in 32% of the cases. Studies of A-RMS have shown that they often (~90%) contain a specific translocation. In most of these cases (68%) a t(2;13)(q35;q14) is found. In a smaller subset of A-RMS (14%) a variant translocation of t(1;13)(p36;q14) has been detected. Both these translocations cause the formation of a chimeric protein. In the case of the t(2;13) a PAX3-FKHR fusion product is expressed and in tumors with the t(1;13) a PAX7-►FOXO1A product is detected. PAX3 and PAX7 are both transcription factors involved in embryonal myogenesis. In the chimeric proteins the DNA binding domains of the PAX genes are retained and fused to the C-terminal region of the FKHR gene containing a strong transactivation domain. It has therefore been proposed that both fusion proteins function as transcription factors that aberrantly regulate transcription of genes, controlled by PAX3 or PAX7 binding sites. The PAX3-FOXO1A fusion protein has been shown to be a strong transcriptional activator. In addition both PAX3-FOXO1A and PAX7-FOXO1A

are over-expressed in A-RMS either by increased transcription (PAX3-FOXO1A) or by gene amplification (PAX7-FOXO1A). Although the presence of either translocation is considered to be a characteristic of A-RMS, some cases with the t(1;13) show mixed histology of both the embryonal and the alveolar type, and a case of E-RMS containing the t(2;13) has been described. In addition the age at diagnosis in patients with the t(1;13) is more consistent with E-RMS. Cytogenetic analysis of RMS showed a high incidence of trisomy 2 (in 9/9 E-RMS samples) and a high incidence of structural rearrangements of chromosomes 1 and 3 (both in 4/5 RMS samples). The alterations on chromosome 3 seem to cluster within 3p14–21. The presence of a der(16)t(1;16)(q21;q13) is also noted in both RMS types and has been categorized as a secondary structural abnormality. RMS was one of the first tumors found to be associated with the Li-Fraumeni syndrome. DNA amplifications have been identified for regions on chromosome 2p and 12q. Both A-RMS and E-RMS have been studied by CGH and the results showed clear differences between the two RMS subtypes. Aberrations found in E-RMS concerned gains and losses of whole chromosomes or large parts of chromosomes: Gains were most frequently found for chromosomes 2, 8, 12, 13 (in 6/10 cases), chromosome 7 (in 5/10 cases), and chromosomes 17, 18 and 19 (in 4/10 cases). Losses were identified most often for chromosome 16 (in 4/10 cases), chromosome 10 (in 3/10 cases) and chromosomes 14 and 15 (in 2/10 cases). One tumor showed an amplification of 12q13-q15. In the A-RMS samples whole (or part of) chromosome gains and losses were found to a much smaller extent. In ten tumors and four cell lines gain of chromosome 17q was found in four cases. However, in a high percentage amplifications were present. Chromosome regions most often involved were 12q13-q15 (in seven cases) and 2p25 (in five cases). The latter region contains the N-MYC gene which is known to be amplified in A-RMS. The regions containing the PAX7 and FKHR genes on 1p36 and 13q14 were found to be amplified in two cases.

As for Wilms tumor, abnormal genomic imprinting of chromosome region 11p15 appears to play a role in the development of RMS (paternal LOH, LOI of IGF2). Increased expression of IGF2 in tumors with monoallelic expression of the gene confirm the important role postulated for IGF2 in the development of this tumor. The imprinting status of H19 has also been examined in RMS and was found to be normal in both subtypes. However, the expression was reduced significantly in 13/15 E-RMS and 2/11 A-RMS. This phenomenon was associated with either loss of the maternal (expressed) allele or LOI of IGF2. In contrast to the situation for Wilms tumor, reduced expression of H19 was not seen in all cases with LOI of IGF2.

Hepatoblastoma

Hepatoblastoma (HB) is a rare malignant epithelial tumor of the liver with an incidence of one case per million children. However, it is the most common malignant hepatic neoplasm of childhood, and occurs with a predominance in males. Although most cases are sporadic, some HBs are associated with either BWS or familial adenomatous polyposis coli (FAP; ►APC gene in Familial Adenomatous Polyposis). Since FAP patients carry mutations in the adenomatous polyposis coli (APC) gene, sporadic HBs have also been analyzed for the presence of mutations in this gene. Indeed, alterations of the APC gene were found in 69% of the sporadic cases. When FAP occurs in combination with extracolonic symptoms it is commonly referred to as Gardner syndrome. Patients suffering from this disease also have an increased risk for the development of HB. The trisomy 18 syndrome can also be associated with HB, as has been found in four patients. One of the phenotypic features of trisomy 18 syndrome is the presence of an omphalocele (also found in BWS patients). It has been suggested that this feature may be one of the factors important in the development of HB in cases in which part of the liver has herniated into the omphalocele. As was found for the other BWS associated tumors, LOH of 11p15 has also been found independently by several researchers for HB (up to 33%). An LOH study of chromosome 1 showed frequent loss of alleles in HBs. In 32 cases 34% had lost heterozygosity for (a part of) chromosome 1, of which 22% were homozygous for markers on the (distal) short arm. There has been a report of the occurrence of HB in the Li-Fraumeni syndrome, and in addition one study showed mutation of the p53 gene in 1/3 sporadic HB samples. Cytogenetic analysis of HB revealed certain consistent chromosome anomalies. Extra copies of chromosomes 2q and 20 are most frequently found. There has also been a report about a recurring translocation: t(1;4)(q12;q34) that results in partial trisomy of most of chromosome arm 1q and partial monosomy of distal 4q. CGH analysis identified mostly gain of DNA. Chromosomes affected in more than 30% of the cases included 1, 2, 7, 8 and 17. When determining the parental origin of 11p alleles lost in HBs it became clear that in this BWS-associated tumor LOH of 11p15.5 was exclusively of maternal origin. When looking directly at the imprinting status of the IGF2 and H19 genes biallelic expression was detected. Two studies showed LOI of IGF2 with normal imprinting of H19 in 1/3 HBs and in 1/5 HBs. A third study showed LOI of both genes in 1/5 cases.

Common Genetic Pathways

When reviewing all genetic and epigenetic data it becomes clear that the most evident abnormality found in all BWS-associated tumors affects chromosome

region 11p15. This is the region to which the syndrome has been linked. All four tumor types show LOH of markers in this region. To date, data has been published for all except ACC showing LOH affecting the maternal allele, with retention of the paternal allele (one ACC with paternal UPD has been described). This suggests the involvement of genomic imprinting. Indeed, abnormal imprinting was found for these tumors, as it was for BWS: They display LOI of the maternally imprinted IGF2 gene. Therefore, this growth factor may play a central role in the development of the overgrowth syndrome and its associated tumors. Increased expression has been noted for WT, ACC and E-RMS, and LOI of IGF2 has been associated with decreased expression of the supposed tumor suppressor gene H19. There is an additional genetic abnormality common between all four types of neoplasms. They all show mutations in the p53 gene. However, this is found in a large proportion of all cancers and therefore is considered not to be specific for the development of tumors associated with the BWS.

Besides genetic evidence there are also pathological data indicating an association between these tumors. Both WT and HB may contain rhabdomyomatous tissue, whereas primary tumors of the liver have been shown to consist of ACC and RMS.

There are also several chromosome aberrations found in a subset of these tumours. When considering abnormalities found in three of the four tumor-types there seems to be a strong connection between WT, E-RMS and HB. They share seven common genetic abnormalities. Besides the abnormalities already mentioned above, they all might contain extra copies of chromosomes 7q, 8 and 17q. Therefore, these chromosome regions may contain genes that play a role in the normal embryonic development of the affected tissues. Since these affected regions are large it would be very difficult to identify the genes involved. More interesting therefore is the abnormality of chromosome 1p that was found in these tumors. This presented either as LOH or structural abnormality of the short arm of chromosome 1. Since these aberrations affect small(er) regions of the chromosome they may be very helpful in the identification of genes. This applies especially to the analysis of translocation breakpoint regions, as has been shown for the regions involved in BWS. Extra copies of chromosome 12 have been identified in the subset consisting of WT, ACC and E-RMS. These tumours are also characterized by increased expression of IGF2.

When analyzing the published data, it becomes clear that WT and E-RMS share most genetic aberrations, with a total of 12. Therefore, the genetic relationship is most evident between these two tumor-types. In addition to the abnormalities already mentioned they have both been shown to contain extra copies of chromosome 18, and in both tumor types decreased expression of H19 has been found. Further elucidation of the common genetic pathways involved in the aetiology of the BWS associated tumors awaits identification of the genes involved.

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Beclin 1

Definition

Mammalian homologue of the yeast autophagy protein Atg6/Vps30. Found in an inhibitory complex with Bcl-2. After release of ►Bcl-2, beclin 1 can associate with class III PI3K and stimulate ►autophagy. Beclin 1 is monoallelically deleted in some human cancers.

Becquerel

Definition

A Becquerel (Bq) is a measure for the disintegration per second. A disintegration of 1 nucleus per second equals 1 Bq.

►Radon

Behcet Disease (BD)

Definition

Synonym Behcet syndrome; was named in 1937 after the Turkish dermatologist Hulusi Behçet, who first

described the triple-symptom complex of recurrent oral aphthous ulcers, genital ulcers, and uveitis. This complex, multisystemic disease includes involvement of the mucocutaneous, ocular, cardiovascular, renal, gastrointestinal, pulmonary, urologic, and central nervous systems and the joints, blood vessels, and lungs. It is characterized by oral aphthae and by at least two of the following: (i) genital aphthae, (ii) synovitis, (iii) posterior uveitis, (iv) cutaneous pustular vasculitis, (v) meningoencephalitis, (vi) recurrent genital ulcers, and (vii) uveitis in the absence of inflammatory bowel disease or collagen vascular disease. The cause of BD is not known; however, immunogenetics, immune regulation, vascular abnormalities, or bacterial and viral infection may have a role in its development.

Benign Tumor

Definition

A tumor that remains confined to its site of origin and neither invades surrounding tissue nor spreads to other organ sites.

Benzene and Leukemia

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Definition

Benzene and Leukemia addresses leukemogenic effect of benzene, representing a complex model of chemical carcinogenesis in humans.

Characteristics

The relationship between benzene, the smallest and most stable ►aromatic hydrocarbon, and leukemia has been reported in the past for workers with high exposures, when benzene in the commercial form (►Benzol) was used largely as a solvent, especially in the shoe industry and in rotogravure printing. Today, occupational exposures are controlled by law and are at most reserved to workers in the petrochemical industry, workers exposed to automobile emissions such as urban officers or gas station attendants, firefighters, and vehicle mechanics. Currently most European countries and the USA have fixed the threshold of acceptable occupational exposure at 1.63–3.25 mg/m^3 (0.5–1 ►ppm). Benzene, even at much lower concentrations, is also a pollutant of the general environment. Among major sources of benzene for the general population (usually below 50 μg/m^3, 15 ►ppb) are traffic exhaust fumes, since benzene is still a typical component of gasoline (1%), and cigarette smoking, which remains a significant source of exposure in both occupationally and nonoccupationally exposed individuals.

Benzene Toxicity and Carcinogenicity

Since the nineteenth century benzene has been recognized as the cause of hematotoxicity of various degrees, up to aplastic anemia, in workers chronically exposed to high concentrations. However, high-dose benzene leukemogenicity was first reported only in 1928 by Delore and Borgomano in a subject showing benzene intoxication. Subsequent studies confirmed an increased risk of leukemia in different occupational settings characterized by high exposure. In Italy, outbreaks of severe benzene poisoning and leukemia have been observed from the thirties to the early sixties, when benzene as a solvent was prohibited by law. Similar findings were reported in the seventies in Turkey and more recently in China. In Italy, most cases of fatal aplastic anemia and leukemia occurred in shoe manufacturing and in rotogravure printing where commercial benzene was used as a solvent of glues and inks respectively. The estimated or measured exposures were in the order of hundreds ppm. Less severe cases of benzene toxicity were observed in subjects exposed to several tens ppm. The acceptable threshold in the 1960s (25 ppm) was later reduced in many countries after the confirmation of benzene leukemogenic activity at lower exposures, claimed on the basis of some epidemiologic studies in chemical and rubber workers, in USA.

Benzene was recognized as a group A carcinogen ("a known carcinogen") by ►EPA in 1979 and as a group 1 human carcinogen ("known to be carcinogenic to humans") by ►IARC in 1982. Based on the general assumption that no threshold might exist for carcinogenic substances and the fact that benzene exposures in certain industries cannot be avoided, the threshold has been lowered to less than 1 ppm, anyway the lowest technically possible threshold.

Conflicting results, however, have been reported in low-level exposure populations such as drivers, police

traffic officers, and gasoline station attendants. Concerns about health effects of benzene at very low doses have been raised recently by results of a study showing a reduction of white blood cells and platelets also in subjects chronically exposed to less than 1 ppm in air, but the values reported were anyway within normal ranges.

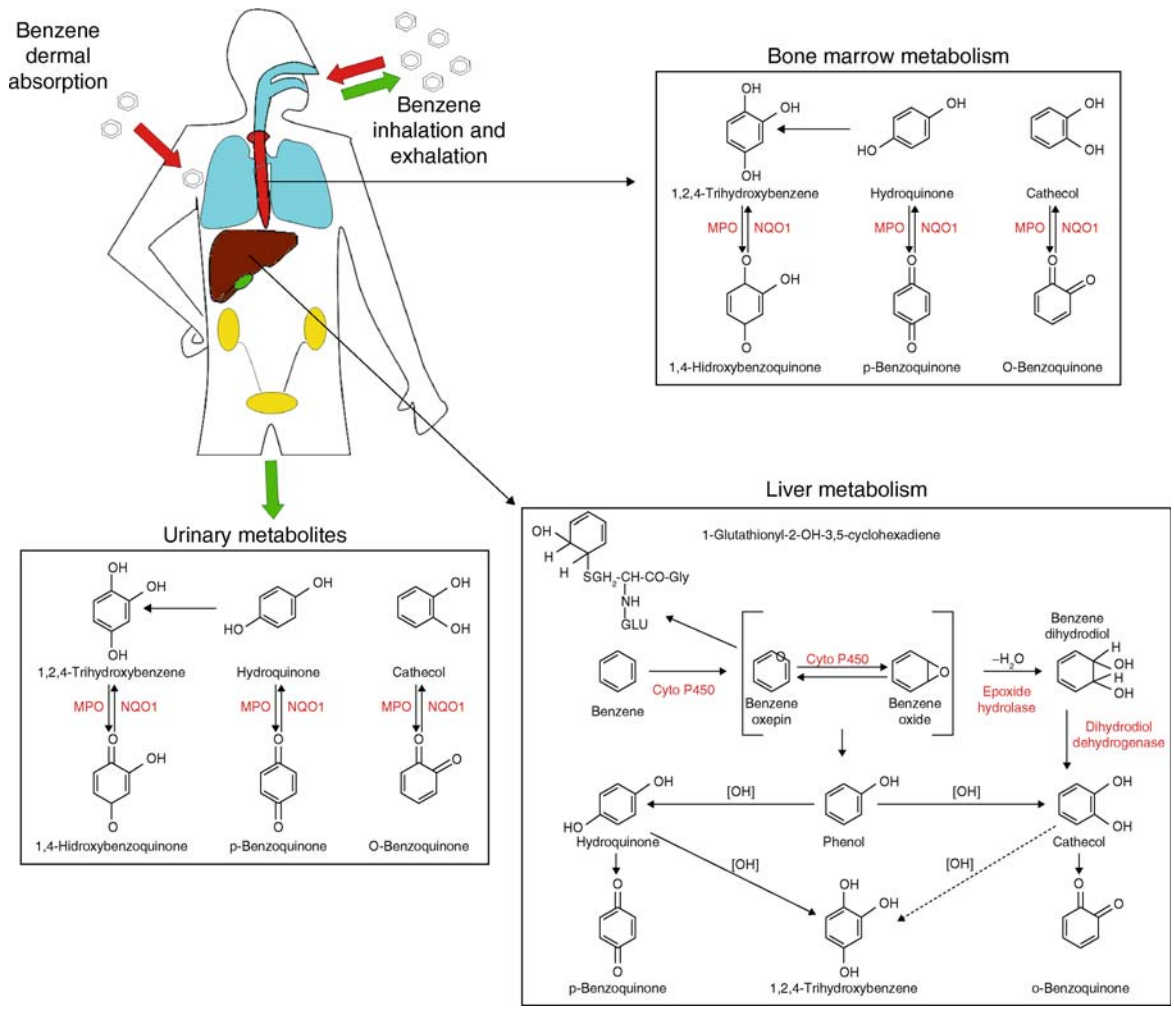
The bone marrow depression of chronic benzene poisoning, resulting in hyporegenerative anemia, leukopenia and thrombocytopenia of varying degree, may slowly recover after removal from exposure, but sometimes persists and evolves into fatal aplastic anemia or into ►acute myeloid leukemia (AML). AML may be preceded by a myelodysplastic syndrome (or preleukemic syndrome), consisting in abnormalities of bone marrow cells surviving ►apoptosis and of blood precursor cells differentiation.

The majority of benzene AMLs are myeloblastic, but other rarer subtypes (e.g., erythroleukemia) have been reported. Many cases of benzene leukemia have low white cell counts, or show only a moderate leukocytosis with a small percentage of immature cells, except in the terminal stage.

Aplastic anemia may occur in subjects while they are still exposed to high concentration of benzene. Leukemia may occur at the same time, or more or less shortly after cessation of exposure. In a few cases a long latency period between the end of work with benzene and occurrence of leukemia has been reported.

Benzene Metabolism and Toxicity

Benzene is not toxic and carcinogenic per se, but rather its toxicity is through its metabolites. Experimental evidences indicate that reactive intermediates are necessary for benzene carcinogenicity and toxicity, but the metabolite(s) responsible are still not fully identified. Inhaled benzene is partly eliminated in the exhaled air. The remaining is rapidly distributed, crosses blood–brain, placental, and gonadal barriers, and is found in



Benzene and Leukemia. Figure 1 Benzene metabolism in humans.

several organs including the bone marrow. Benzene is transformed in the liver to benzene oxide, phenol, cathecol, hydroquinone, and 1,2,4-trihydroxybenzene by the microsomal ►cytochrome P-450 monooxygenase system (►CYP2E1). Cathecol and hydroquinone oxidation results into the reactive intermediates *ortho*-benzoquinone and *para*-hydroquinone. Hydroquinones may also be produced from benzene derived ►quinones via ►NAD(P)H:quinone oxidoreductase (►NQO1) (Fig. 1). Benzene metabolites such as hydroquinone and cathecol, reach the bone marrow and can be further activated by ►myeloperoxidase (►MPO), present at high levels in stromal ►macrophages, resulting in the production of quinones and ►reactive oxygen species, which bind covalently to biological macromolecules. Unmetabolized benzene and several metabolites are eliminated through the kidney and some of them can be measured to assess benzene exposure.

In order to explain the different susceptibility to benzene poisoning in workers with similar levels of exposure, some ►metabolic polymorphisms have been examined in benzene-exposed subjects. A rapid CYP2E1 activity and a loss of NQO1 function polymorphism were found to be associated with increased benzene toxicity in workers exposed to high levels of benzene (>10 ppm) in Shanghai (China). More recent results highlighted the role of MPO and NQO1 polymorphisms even at exposures lower than 1 ppm. CYP2E1 and NQO1 are polymorphically distributed in human populations: in Caucasians, the estimated frequency of CYP2E1 rapid metabolizers is around 10% and a loss-function NQO1 polymorphism has been identified with a 40% frequency.

Benzene, Chromosome Changes and Leukemia

Benzene metabolites are not mutagenic, but are able to generate oxygen reactive species, which might be responsible for DNA damage, both by genetic and ►epigenetic mechanisms.

In the 1960s, the possibility of studying human chromosomes in lymphocytes, stimulated to divide in culture, and in direct preparations of bone marrow cells, raised the interest for cytogenetic studies in benzene-exposed workers with or without signs of benzene toxicity and in cases of benzene leukemia. Exposure to high concentrations of benzene was demonstrated to induce structural (►Chromosome translocations, breaks, deletions) and/or numerical chromosome changes, persisting in lymphocytes also for decades after cessation of exposure and in bone marrow cells at the time of benzene poisoning or during persisting myelodysplastic syndrome.

Structural chromosome changes in benzene-exposed workers were studied more recently with special techniques and resulted to be nonrandom, involving specific chromosomes, both for breaks and for translocations.

In vitro studies of hematopoietic progenitor cells from human bone marrow or umbilical cord blood, cultured in the presence of hydroquinone, showed specific deletions and/or numerical changes in chromosomes more frequently involved in benzene-induced myelodysplastic syndrome and leukemia.

On the basis of some clinical reports, the hypothesis is suggested that myeloid precursor cells with different chromosome changes either die by apoptosis or necrosis, or survive giving rise to atypical cell clones. One clone with selective advantage might proliferate and be responsible for the evolution into leukemia. This mechanism might be enhanced by the bone marrow microenvironment conditions and be favored by benzene-induced immunodepression.

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Benzidine

Definition

Is a carcinogenic ►aromatic amine that has been used in the synthesis of dyes. It has been linked to bladder cancer and ►pancreas cancer. ►Arylamine N-Acetyltransferases.

Benzo(a)pyrene

Definition

An environmental carcinogen belonging to the polycyclic aromatic hydrocarbon family that is primarily

found in sources such as tobacco smoke. Its role in smoke-causing ►lung cancer is extensively studied.

►Sulforaphane
►Polycyclic Aromatic Hydrocarbonal
►Tobacco Carcinogenesis
►Tobacco-Related Cancers

Benzol

Definition

A commercial form of benzene that is a mixture of benzene and its homologues (toluene and xylene).

►Benzene and Leukemia

Benzo[*a*]pyrene Diol Epoxide (BPDE)

Definition

Is a potent mutagenic and carcinogenic metabolic product of ►benzo[*a*]pyrene, one of the most well-known combustion products in cigarette smoke and vehicle exhausts. BPDE induces DNA bulky adducts and is commonly used in epidemiologic studies as a challenge mutagen.

►Mutagen Sensitivity
►DNA Adduct to DNA

Benzoquinone ansamycin

►Ansamycin Class of Natural Product Hsp90 Inhibitors

Benzpyrene

Definition

Member of he group of ►polycyclic aromatic hydrocarbons. Benzpyrenes are present in coal tar at low levels and are considered carcinogenic (cancer-inducing). Traces of benzpyrenes are present in wood smoke, and this has given rise to some concern about the safety of naturally smoked foods.

Berlin Breakage Syndrome

►Nijmegen Breakage Syndrome

Beta-2 Microglobulin (β2 Microglobulin)

Definition

Component of MHC class I molecules present on virtually all cells except red blood cells. β2 microglobulin has no trans-membrane region.

►Plasmacytoma

Beta-glucosidase

A glycoside hydrolase enzyme that cleaves sugar residues from compounds.

►Genistein

Beta Subunit of Human Chorionic Gonadotropin (β-hCG)

Definition

β-hCG is normally produced by the placenta and human fetal tissue. Elevated serum β-hCG is most commonly associated with pregnancy, gestational trophoblastic

disease and germ cell tumors. It can also be found in hypogonadal states and with marijuana use.

►Serum Biomarkers

Betacellulin (BTC)

Definition

Has been isolated from conditioned media from a pancreatic β cell tumor cell line. BTC is also expressed in a variety of mesynchymal and epithelial cell lines and in many tissue including pancreas, liver, kidney and small intestine. Membrane-bound proBTC is processed by ADAM10 to release soluble BTC. Transgenic chicken actin promoter-driven BTC over-expression in mice causes bony deformations of the skull, pulmonary hemorrhage syndrome, and complex eye pathology. Transgenic animals showed decrease in the weight of pancreas and increase weight of the eye, lung and spleen.

►Epidermal Growth Factor (EGF)-Like Ligands
►ADAM Molecules

Betel Quid

Definition

Also known as *pan*, this material consists of four main ingredients: tobacco, areca nuts, and slaked lime wrapped in a betel leaf. Betel quid chewing is widely practiced in South-East Asia, particularly in India.

►Tobacco Carcinogenesis
►Tobacco-Related Cancers

Betulin

Definition

A triterpenol from birch bark.

►Urothelial Carcinoma

Betulinic Acid

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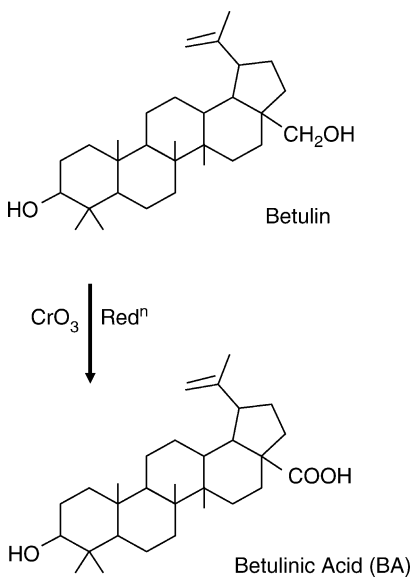
Definition

►Betulinic acid is a naturally occurring triterpenoid acid and a potent anticancer drug.

Characteristics

►Betulin is a pentacyclic triterpenol natural product that is found in tree bark and this compound may constitute up to 30% of the bark from birch trees. Betulinic acid (BA) is a minor bark constituent but is readily synthesized from betulin by oxidation to betulonic acid followed by reduction to betulinic acid (Fig. 1). Birch bark extracts containing betulin and BA have been used in traditional folk medicines; however, in recent years, BA alone or some of its derivatives have been developed as pharmacological agents for treating multiple diseases. For example, these compounds are antiviral agents that inhibit HIV-1 replication and exhibit antimalarial, antihelmintic, and antibacterial activity as well as antiinflammatory and analgesic effects. Many of these responses induced by BA and its



Betulinic Acid. Figure 1 Betulin, a major component of birch bark is readily oxidized by chromic acid to betulonic acid which is reduced with sodium borohydride to betulinic acid.

derivatives are structure-dependent and involve changes in structure of one or more regions in the molecule.

Many of the naturally occurring triterpenoid acids, such as ►ursolic acid, ►glycyrrhetinic acid, ►oleanolic acid and ►betulinic acids, exhibit some cytotoxicity to various cancer cell lines; however, among these natural products, BA is by far the most potent anticancer agent. Initial studies by Pisha et al. showed that BA was a highly potent drug for treatment of melanoma in mouse xenograft model. In this study, athymic mice were injected with melanoma cells (MEL-2 or MEL-1) and treated with BA at doses of 50, 250, or 500 mg/kg every third day and this resulted in significant tumor growth inhibition. Moreover, BA also decreased tumor volume in mice already bearing relatively large tumors. It was also reported that tumor growth inhibition could be observed at doses of BA as low as 5 mg/kg (X 6), whereas at doses as high as 500 mg/kg, minimal toxic side-effects were observed in the animals. It was also reported that BA induced ►apoptosis in melanoma cell lines and the high cytotoxocity of BA was observed in melanoma cells but not in squamous, breast, colon, sarcoma, prostate, lung, neuroblastoma, and glioma cancer cell lines.

Subsequent studies on the cytotoxicity of BA in cancer cell lines demonstrated that comparable effects were observed in cells derived from multiple tumor types. BA alone inhibited proliferation of various cancer cell lines, and several reports show the potential chemotherapeutic advantages of using BA in combination with other anticancer drugs such as vincristine, tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), doxorubicin, taxol, and irradiation. Interactions of these anticancer drugs with BA generally enhance the overall cytotoxicity of the combination compared to the treatments alone; however, these interactions are highly cell context-dependent. Recombinant TRAIL is now being investigated in clinical trials and the protein is a ligand for cell membrane death receptors and activates the extrinsic apoptosis pathway characterized by caspase-8-dependent PARP cleavage. Treatment of neuroblastoma cells with TRAIL plus BA (combination) clearly enhanced apoptosis compared to treatment with the individual agents. TRAIL and BA alone tend to activate the extrinsic and intrinsic apoptosis pathways and the combination of these drugs results in mutual enhancement of both pathways. Since many tumor types are highly resistant to cell death, the combination of BA and other proapoptotic agents may offer many advantages for clinical treatment of some tumors.

Although BA induces apoptosis in most cancer cell lines, there is also evidence that activation of other responses may also contribute to the anticancer activity of this drug. In human melanoma cells, BA induces reactive oxygen species (ROS) and this is accompanied by time-dependent and persistent activation of p38 and c-Jun NH_2-terminal kinase (JNK). ROS acts upstream of these mitogen-activated protein kinases; however, both p38 and JNK can be involved in apoptotic pathways induced by BA in melanoma cells. Interestingly, studies in other melanoma cell lines showed that BA-induced effects on some cell cycle proteins and apoptosis (PARP cleavage and DNA laddering) were dependent on persistent activation of MAPK, since all of these responses were inhibited by the MAPK inhibitor U0126. Thus, BA-induced apoptosis is linked to activation of multiple kinases and differences in their action are highly dependent on cell context. The anticancer activity of BA may also be associated with other effects including the inhibition of topoisomerase 1 and antiangiogenic activity. This latter response was determined in ECV 304 endothelial cells in a Matrigel tube formation assay where BA and three substituted analogs exhibited angiogenic activity.

The mechanism of the anticarcinogenic activity of BA is complex and cell context-dependent and may include contributions from the direct effects of this compound on mitochondria and activation of kinase pathways. Research in our laboratory has focused on studying some of the underlying mechanisms of cancer cell and tumor growth, survival and angiogenesis. Using RNA interference, we have shown that specificity protein (►Sp) transcription factors are responsible, in part, for the growth and survival of cancer cells and their ability to metastasize and grow at distal sites. Sp1, Sp3, and Sp4 are overexpressed in colon and pancreatic cancer cells and tumors, and play a key role in overexpression of the ►angiogenic factors vascular endothelial growth factor (►VEGF), VEGF receptor 1 (VEGFR1) and VEGFR2, the survival gene survivin, and Sp3-dependent suppression of the cyclin-dependent kinase inhibitor p27. These results directly link overexpression of Sp proteins to the enhanced growth and survival and potential for metastasis/angiogenesis of cancer cells and tumors, and also suggest that inhibiting Sp protein-dependent gene expression or by inducing ►Sp protein degradation may be an important strategy for developing effective anticancer drugs. The first example of this approach was the identification and application of ►tolfenamic acid, a nonsteroidal antiinflammatory drug, which inhibited pancreatic cell growth through activation of proteasome-dependent degradation of Sp1, Sp3, and Sp4. Moreover, in an orthotopic model for pancreatic cancer, we also observed that tolfenamic acid inhibited tumor growth and metastasis and this was accompanied by degradation of Sp proteins in pancreatic tumors. Based on the reported proapoptotic/antiangiogenic effects of BA in cancer cell lines, we hypothesized that this compound

may also act, in part, through Sp protein degradation. In LNCaP prostate cancer cells, we have shown that BA induced proteasome-dependent degradation of Sp1, Sp3, and Sp4 and we have observed similar responses in cell lines derived from other tumor types. Moreover, in in vivo studies in athymic nude mice bearing human LNCaP cells as xenografts, BA also inhibited tumor growth and this was accompanied by decreased Sp1, Sp3, and Sp4 expression in the tumors. These results demonstrate that some of the anticancer activities BA in multiple cell lines may be partially due to degradation of Sp proteins and we have observed that this process is due to the activation of both proteasome-dependent and -independent pathways. Thus, betulinic acid and some of its derivatives are part of a new class of anticancer drugs that work through targeting Sp proteins and Sp-dependent genes involved in cancer cell survival, growth, and angiogenesis.

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Bevacizumab

Definition

Monoclonal antibody against the ►vascular endothelial growth factor. Bevacizumab has shown activity in colorectal cancer, non small cell ►lung cancer, and ►breast cancer.

►Erlotinib (Tarceva®)

bFGF

Definition

►Basic fibroblast growth factor is a mitogenic and angiogenic growth factor involved in wound healing and tumor growth.

►Fibroblast Growth Factor 2 (FGF2)
►Basic Fibroblast Growth Factor (FGFB)
►Securin

BGP, Biliary Glycoprotein

►CEACAM1 Adhesion Molecule

BH

Definition

►Bcl-2 homology domain. There are four BH domains, referring as BH1, 2, 3, and 4 domains.

►PUMA

BH3

Definition

►Bcl-2 homology domain-3.

►Mcl Family

BH3 Binding Pocket

Definition

A single α-helix called the ►BH3 region. Activity is proposed to be mediated through the association of the

BH3 region of one protein, including Mcl-1, with a large hydrophobic pocket on the other binding partner, such as Bak. Mcl family

►Mcl Family

BH3-Interacting Death Domain Agonist (Bid)

Definition

Bid is a 195 amino acid, 22 kDa proapoptotic BH3-only protein of the ►Bcl-2 family that is localized to chromosome 22q11. Upon death receptor activation, Bid is cleaved by caspase-8 into a C-terminal 15 kDa and a N-terminal 6 kDa fragment. Truncated C-terminal Bid then translocates to mitochondria to trigger cytochrome *c* release by binding to Bax and/or Bak, causing them to oligomerize.

►Bid
►Caspase-8

BH3-interacting Domain Death Agonist

►Bid

BHD Syndrome

►Birt–Hogg–Dubé Syndrome

bHLH

Definition

Basic helix-loop-helix (bHLH) is a protein motif shared by a group of transcription factors, therefore named bHLH proteins (or E proteins). For DNA binding, mono- or heterodimerization is compulsory, which is mediated by the helix-loop-helix motif. The basic region is composed of basic amino acids and determines DNA sequence-specific binding of the dimer.

►E-box
►Myc Oncogene

bHLH-PAS Proteins

Definition

A family of transcription factors characterized by a basic-helix-loop-helix (bHLH) structural motif and PAS sequence homology. The bHLH motif consists of an amino acid sequence in which the secondary structure has two α helices connected by a loop. The PAS sequence refers to a highly homologous region found in the proteins Per, Arnt, and Sim.

Biallelic Mutations

Definition

Pathogenic sequence alterations, albeit not necessarily identical, are present in both copies of the same gene.

Bias

Definition

A flaw in the study design or method of collecting or interpreting information that leads to an erroneous result

►Coffee Consumption
►Cancer Epidemiology
►Epidemiology of Cancer

Bicalutamide

Definition

Non steroidal antiandrogen.

►Gynecomastia

Bid

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Synonyms

BH3-interacting domain death agonist

Definition

Bid is a pro-death ►Bcl-2 family protein. Structurally, it contains only one Bcl-2 homology domain, the BH3 domain. Thus it belongs to the BH3-only subfamily, which also includes Bad, Bik/Nbk/Blk, Bim, Bmf, Hrk/DP5, EGL-1, Noxa and PUMA.

Characteristics

The Bid Molecule

Bid was first cloned in 1996 from an expression cDNA library screened with recombinant Bcl-2 and Bax. Its ability to interact with both the anti-death and the pro-death Bcl-2 family proteins is a distinguished feature of this molecule among the BH3-only members. Bid was re-cloned in 1998 by two other laboratories. In both cases, Bid was identified as a substrate of ►caspase-8.

Bid is phylogenetically conserved. The mouse Bid gene is located at chromosome 6 (6 F1; 6 54.0 cM), while the human Bid is localized in a syntenic region, chromosome 22q11.2. The major protein product is derived from the originally defined five exons with 195 amino acids (about 22Kd) in both human and mouse. The Bid molecule is widely expressed. At the protein level, the full length Bid is a long lived protein, but caspase-8 cleaved truncated Bid (tBid) is degraded through the ubiquitination proteasome system and has a half-life of less than 1.5 h.

The structure of Bid has been resolved with NMR. It is the only structure resolved for a BH3-only molecule. Bid is composed of eight alpha helices. The central hydrophobic helices (alpha 6 and alpha 7) are surrounded by the amphipathic helices. Such an arrangement is conserved among other Bcl-2 family proteins, such as Bcl-2, Bcl-x_L and Bax. There is a non-structural loop between alpha 2 and alpha 3, which is subjected to regulatory modifications by protease cleavage or phosphorylation. Similar loops are present in Bcl-x_L, Bcl-2 and Bax, which play the same regulatory role.

Bid does not have a transmembrane domain. It shares sequence homology with other Bcl-2 family proteins in the BH3 domain, which is important for its interaction with other family members and for its pro-apoptotic activity. Interaction of Bcl-2 or Bcl-x_L suppresses Bid by preventing it from interacting with the pro-death Bax or Bak.

Bid is a Pro-Death Sensor for Specific Protease Activation

Early studies based on transient transfection or an inducible system demonstrated that over-expression of full length Bid could induce ►apoptosis. However, in most cases it seems that Bid may function in a truncated form. Bid was initially found to be cleaved and activated by caspase-8 following death receptor activation and thus considered to be specific to the death receptor pathway. However, studies in recent years indicate that Bid can be cleaved in a specific and limited way by several other proteases such as Granzyme B, calpains and cathepsins. These proteases are first activated in response to a plethora of stimuli, including death receptor activation, cytotoxic T cell attack, ischemia/reperfusion injury and lysosome damage. These observations indicate that Bid is in general a sentinel to protease activation resulted from various injury stimuli. As such Bid serves a critical role in connecting these stimuli to the ►mitochondria, allowing the death process to be advanced or amplified.

The cellular death receptor pathway is activated when the death receptors, Fas, TNF-R1 or ►TRAIL-R1, are engaged by their ligands or agonistic antibodies. Both in vitro cell lines and in vivo animal models have been used to study the signaling events. In a murine model of anti-Fas antibody-induced liver injury, Bid has been found to play a significant role in Fas-mediated apoptosis. Normal wild type mice are particularly susceptible to the administration of a Fas agnostic monoclonal antibody (clone Jo2), which induces significant hepatocyte apoptosis and severe liver injury. However, *bid*-deficient mice are resistant to such a treatment with minimal hepatocyte apoptosis and liver injury. In these mice, while caspase-8 is appropriately activated, the downstream effector caspase-3 is not. Caspase-3 activation is arrested in *bid*-deficient hepatocytes in a pattern consistent with being suppressed by XIAP, the X-linked inhibitor of apoptosis protein. In the wild type mice, Bid is cleaved by caspase-8 and the truncated Bid is translocated to the mitochondria to induce the release of cytochrome c and Smac. While cytochrome c could activate Apaf-1 and therefore caspase-9, Smac is able to bind to XIAP to release its suppression on caspase-3 activation. In this scenario, Bid connects the death receptor pathway to the ►mitochondria pathway, which is necessary for the prompt activation of effector caspases and subsequent apoptosis in hepatocytes.

Activation of Mitochondria by Bid

The ability of Bid to activate the mitochondria pathway is related to its ability to interact with the mitochondria and to permeabilize the mitochondrial outer membranes. Bid is able to induce the release of multiple mitochondrial inter-membrane space proteins,

including cytochrome c and Smac/DIABLO. Full length Bid is usually much weaker than truncated Bid in this capability. Bid is also able to induce several other prominent mitochondria dysfunctions, including mitochondrial permeability transition, mitochondrial depolarization, mitochondrial cristae reorganization and the generation of mitochondrial ▶reactive oxygen species. While the mechanisms for some of the phenomena are better understood, others are not.

In the case of cytochrome c release, it seems that Bid could activate at least two different mechanisms. One is based on protein interactions and the other is based on lipid interactions. Bid can interact with either Bax or Bak, the multi-domain pro-death Bcl-2 family proteins, via its BH3 domain, to promote their oligomerization on the mitochondrial outer membranes. Indeed, mice deficient in both Bax and Bak are much like *bid*-deficient mice and are resistant to anti-Fas induced hepatocyte apoptosis in vivo. The other important mechanism is based on the interaction of Bid with cardiolipin at the mitochondrial contact site. This interaction promotes mitochondrial cristae reorganization, which contributes to the mobilization of stored cytochrome c and its subsequent release. As the majority of cytochrome c is tightly bound to cardiolipin, a full release of this molecule would require its dissociation from cardiolipin, which is facilitated by Bid-cardiolipin interactions.

The two mechanisms activated by Bid, involving proteins and lipids, respectively, seem to be well coordinated. Bid interaction with Bak or Bax is the primary mechanism, which initiates mitochondrial leakage. This requires the BH3 domain and can be blocked by Bcl-2 or Bcl-x_L. On the other hand, the serial events of Bid-cardiolipin interaction and cristae reorganization do not require the BH3 domain of Bid and could not be suppressed by Bcl-2/Bcl-x_L. The mobilized cytochrome c is released through the mechanism enforced by Bid-Bak or Bid-Bax interactions.

Bid as a Pro-Life Sensor for Cell Cycle Progression and DNA Damage

Although Bid was initially defined as a pro-death molecule, recent studies have shown that Bid can possess functions important to the life of a cell. Bid has a pro-proliferation activity and can also serve as a DNA damage sensor to participate in cell cycle arrest. Other Bcl-2 family proteins, such as Bcl-2, Bcl-x_L, Bax and Bad have also been shown to possess the function of regulating cell cycle progression. From a broad point of view, it seems that Bcl-2 family proteins do not just simply regulate cell death, but also affect other key cellular events.

Bid seems to be able to regulate the G0-G1/S transition, as shown in several types of cells entering cell cycle from the resting stage. As a result, *bid*-deficient cells are often delayed in entering S phase upon mitogen stimulation. How Bid may promote cell proliferation is not clear at the moment. Regulation at the cyclin and cyclin-dependent kinase could be a key mechanism, although this has yet to be determined.

Another function of Bid in cell cycle regulation relates to S/G2 transition in cells with DNA damage or under replication stress. Thus *bid*-deficient cells fail to be arrested at S/G2 boundary in these conditions. Further studies showed that Bid can be a phosphorylation substrate of ATM/ATR. Mutagenesis studies indicate that Bid phosphorylation by ATM/ATR is required for the S phase arrest following DNA damage. However, it is not clear how Bid may then contribute to the S phase arrest. It seems that this function of Bid is beneficial to cells so that they would not have to go into mitosis in the presence of DNA damage. Thus the protective effect of Bid phosphorylation in this case could be largely due to its effect in inducing S phase arrest. Finally, this ability of Bid is not dependent on its BH3 domain.

Role of Bid in Oncogenesis

In general, neoplasia could be resulted from an uncontrolled cell proliferation owing to the activation of oncogenes, or from deregulated cell survival owing to the over-expression of anti-death molecules or the loss of pro-death molecules. For the Bcl-2 family proteins, it is generally assumed that their role in tumorigenesis is related to their ability to regulate cell death. However, other functions of the Bcl-2 family proteins can be equally important in tumorigenesis.

Bid-deficient mice develop spontaneous chronic myelomonocytic leukemia when they become aged. This may be explained by the loss of the pro-death activity of Bid. However, *bid*-deficient mice do not have an enhanced development of liver cancers following the administration of a chemical carcinogen, diethylnitrosamine (DEN). In contrast, they manifest a delayed development of tumors despite that there is a reduced cell death in the affected livers. These observations could be better explained by the role of Bid in promoting cell proliferation. Indeed, Bid was subsequently found to have such a function. Since this ability to regulate cell cycle progression is also possessed by other Bcl-2 family proteins, it is possible that Bcl-2 family proteins can in general affect tumorigenesis via both of their functions in cell death and cell proliferation. The net effects could be specific to the affected tissue or the etiology of the tumor.

In addition, as far as Bid is concerned, one may have to also consider whether the regulation of mitosis checkpoint by Bid following DNA damage can be another key factor in affecting tumorigenesis. There

is a significant presence of genomic instability in *bid*-deficient myeloid cells. It is possible that the myeloid cells are prone to DNA damage, which in the absence of Bid would lead to an accumulation of DNA abnormalities and subsequent leukemogenesis.

Summary

Bid is a versatile multi-function BH3-only molecule. While its function was initially defined to be pro-apoptosis, it is now clear that it can also regulate cell proliferation and genomic stability. These functions could be intimately connected and are overall responsible for the role of Bid in cell death, tissue injury, cell proliferation and oncogenesis. Future studies would be devoted to understand how these functions are integrated and regulated, and what the underlining mechanisms are.

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Bidirectional Differentiated Malignant Tumors

Definition

Possess features of both endothelial cells and mesenchymal cells. ►Melanoma, alveolar rhabdomyosarcomas, mesothelial sarcomas, synovial sarcomas, and epithelioid sarcoma belong to bidirectional differentiated malignant tumors.

►Vasculogenic Mimicry

2,3′-Biindolinylidene-2,3′-diones

►Indirubin and Indirubin Derivatives

BIK Proapoptotic Protein

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Synonyms

BIK (BCL-2 Interacting Killer); Bp4; NBK (Natural Born Killer)

Definition

BIK is a ►BH3-only protein localized exclusively on the cytosolic surface of the endoplasmic reticulum (ER) membrane. Although the amino acid sequence of mouse Blk (BIK-like killer; also known as Biklk for Bik-like) has only 43% identity to that of human BIK, Blk is usually considered mouse ortholog of BIK due to their functional similarities. The official gene symbol of Blk is *bik*. Blk should not be confused with *B lymphoid tyrosine kinase*, a member of the SRC family non-receptor protein tyrosine kinase and whose official gene symbol is *BLK*.

Characteristics

Discovery

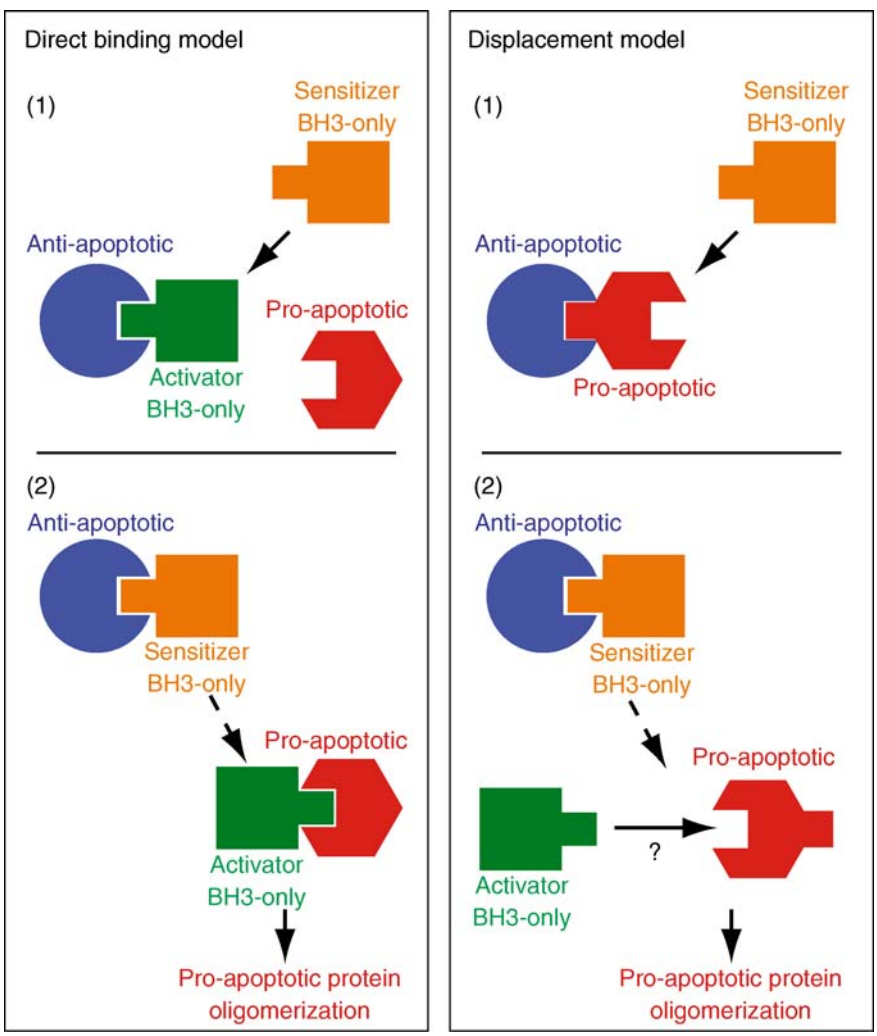
BIK was originally cloned in a two-hybrid screening as a protein that interacts with cellular antiapoptotic proteins ►BCL-2 and BCL-xL as well as viral antiapoptotic proteins ►Epstein-Barr virus BHRF1 and the ►19-kDa adenovirus E1B protein, both of which were viral homologues of human ►BCL-2. In an independently performed two-hybrid screening using the 19-kDa adenovirus E1B protein as bait, BIK was isolated as an E1B-binding protein and described as cDNA clone Bp4, which was later renamed NBK. These early studies characterized BIK/NBK as an apoptosis-inducing protein. Later, a DNA microarray study performed by an independent investigator identified BIK as a protein induced by the ►Adenovirus E1A protein in human KB epithelial cells.

Protein Structure and Molecular Functions

Human BIK consists of 160 amino acids, and its ►BH3 domain spans from amino acid 57(Leu) to 71(Ser). The strongly hydrophobic, leucine-rich C-terminal transmembrane domain (amino acids 136–159) selectively anchors BIK to the ER membrane, thus almost entire BIK protein including its N-terminus is exposed to cytosol. In human cells, BIK is phosphorylated at 33(Thr) and 35(Ser) by an unidentified, casein kinase II-like enzyme. Substitutions of these amino acids with alanines reduce the proapoptotic activity of BIK without significantly affecting its protein stability or ability to heterodimerize with BCL-2. Conversely,

substitutions of these amino acids with aspartic acids enhance the proapoptotic activity.

When expressed in mammalian cells, either endogenously or from exogenous vectors, BIK potently induces apoptosis. This activity involves the release of cytochrome c from mitochondria and is entirely dependent on BAX, a ►proapoptotic member of the BCL-2 family. However, BIK does not directly bind to BAX, nor affect mitochondrial membrane potential or voltage-dependent anion channel activity. Instead, BIK directly binds to BCL-2, BCL-xL, and BCL-w ►antiapoptotic members of the BCL-2 family. Therefore, BIK is a typical ►sensitizer-type BH3-only protein. In contrast, the ►activator-type BH3-only proteins bind directly to BAX or BAK, the proapoptotic members of the BCL-2 family that possess multiple BCL-2 homology (BH) domains and oligomerize on the mitochondrial outer membrane to form a channel that release cytochrome c to the cytosol. The antiapoptotic members of the BCL-2 family bind to the activator-type BH3-only proteins and thus sequester them from interacting with BAX or BAK (Fig. 1). Since each sensitizer and activator BH3-only protein has discrete binding specificity and affinity to the antiapoptotic members of the BCL-2 family, the apoptotic signal is activated only when correct combinations of these three groups of proteins are expressed (Table 1). Reflecting its weak affinity to MCL-1, an antiapoptotic member of the BCL-2 family, BIK cannot kill cells in the presence of sufficient expression of MCL-1. When MCL-1 expression is weak and BCL-2/BCL-xL/BCL-w expression is high, BIK can kill cells in the presence of ►tBID as an activator BH3-only protein, showing specificity similar to that of BMF. However, BIK also has to cooperate with another, weak BH3-only protein such as NOXA to cause rapid release of mobilized cytochrome c and subsequent activation of caspases.



BIK Proapoptotic Protein. Figure 1 Mechanism of the proapoptotic actions of the BH3-only proteins.

BIK Proapoptotic Protein. Table 1 Effects of combinations of anti-apoptotic, sensitizer BH3-only, and activator BH3-only proteins on induction of cell apoptosis

Anti-apoptotic BCL-2 family	Activator BH3-only	Sensitizer/Inactivator BH3-only Proteins				
		None	BAD	NOXA	BMF	BIK
BCL-2 BCL-xL	tBID	n	Y	n	Y	Y
	BIM	n	Y	n	n	n
	PUMA	n	Y	n	n	n
MCL-1	tBID	n	n	Y	n	n
	BIM	n	n	Y	n	n
	PUMA	n	n	Y	n	n

n, no apoptosis; Y, apoptosis. Modified from Kim et al. [2].

The activator BH3-only proteins such as tBID or BIM directly bind to BAK or BAX, the proapoptotic multi-BH domain members of the BCL-2 family. These activator BH3-only proteins are sequestered by the antiapoptotic members of the BCL-2 family proteins BCL-2, BCL-xL, BCL-w, and/or MCL-1. When the sensitizer BH3-only proteins bind to the antiapoptotic members, the activator BH3-only proteins are released from them and instead bind to BAK or BAX, which then oligomerizes to form channels through the outer membrane of mitochondria to release cytochrome c to the cytosol. Thus, the sensitizer and activator BH3-only proteins compete for binding to the antiapoptotic proteins.

At the ER, BIK can initiate early release of Ca^{2+} from the ER lumen to cytosol in response to apoptotic stimuli. This BIK-activated Ca^{2+} release requires BAK recruitment to the ER membrane. The reuptake of cytosolic Ca^{2+} by mitochondria causes recruitment and activation of the fission enzyme ►DRP1 at discrete sites on the mitochondrial tubular network, resulting in mitochondrial fragmentation and cristae opening but minimal release of cytochrome c. Since loss of the GTPase activity of DRP1 results in suppression of mitochondrial fission and cytochrome c release during apoptosis, the BIK-dependent early morphological changes in mitochondria may enhance cytochrome c release, which is later induced by activation of BAX/BAK in the presence of both BIK and NOXA.

Gene Structure, Expression, and Phenotypes

The *BIK* gene is found in human, bovine, rat, and mouse genomes but not in frog or fish. Therefore, among the BH3-only proteins, BIK seems a relatively new, mammalian-specific member. The human *BIK* gene is localized to chromosome 22q13.3 and is comprised of five exons spanning in about a 19-kb region. The minimal BIK promoter is localized to a region between −211 bp and +153 bp relative to its transcription initiation site. Although it was originally reported as a TATA-less promoter, a later study and the EST data suggest possible involvement of a TATA-like sequence in its transcriptional activity. No evidence of alternative promoters or splicing has been found in the EST database or literature.

The BIK mRNA transcripts are strongly expressed in lymphatic tissues and endothelial cells of the venous (but not arterial) lineages. Normal adult mammary and prostate glands also express significant amounts of BIK mRNA. C57BL/6 mice express Bik mRNA in the liver, lung, heart, and kidneys; weaker expression was also detected in spleen, skeletal muscle, and salivary gland. Mechanisms of the BIK tissue-specific distribution are unknown. At least one strain of *bik* gene knockout mice was generated, but no significant phenotype has been observed with them. However, simultaneous knockdown of *bik* and *bim* genes causes male infertility with severely perturbed spermatogenesis. Bik and Bim may share the role of eliminating supernumerary germ cells during the first wave of the permatogenesis, a process critical for normal testicular development.

The A/WySnJ mice, which have 90% fewer peripheral B cells than normal animals and fail to make significant immunoglobulin memory response, overexpress the Bik mRNA transcripts, and their transitional B cells rapidly succumb to apoptosis in vitro. During the transition from naïve B cell to centroblast B cell, expression of the BIK mRNA transcripts increase by about 8.5-fold, and the high level of BIK mRNA expression is maintained in memory B cells. These observations suggest possible roles of BIK during B-cell maturation.

Expression of the BIK mRNA transcripts and protein is inducible in human cells by overexpression of wild type ►p53 protein. Adenovirus E1A protein also induces BIK in a manner dependent on p53 protein. Apoptosis-inducing stimuli that involve p53 protein activation, such as doxorubicin or gamma-irradiation, induce BIK expression as well. Doxorubicin may be able to activate *BIK* gene transcription by a mechanism independent of

p53 protein but involving the E2F transcription factors. In MCF-7 human ►breast cancer cells, antiestrogens such as ►tamoxifen and ►fulvestrant induce BIK expression in a manner dependent on p53 protein, but its mechanism may be independent of the transcription factor activity of p53 protein.

Relevance to Cancer Genetics and Therapy

Significant frequency of missense mutations in the *BIK* gene was observed in peripheral B-cell lymphomas. Chromosomal deletions causing loss of heterozygosity (LOH) of the *BIK* locus has been reported for head and neck tumors, colorectal cancers, glioblastomas, and clear-cell renal cell carcinomas. Epigenetic silencing of BIK mRNA expression was reported for the KAS-6/1 multiple myeloma cell line and in a number of cell lines of renal cell carcinoma. Although these data suggests possible roles of *BIK* in human carcinogenesis, this remains to be established by further studies.

Since the strong proapoptotic activity of BIK potently induces ►apoptosis even in malignant cells, a number of studies reported the possible application of BIK-expressing vectors in the context of ►gene therapy. A chimeric protein consisting of gonadotropin releasing hormone (GnRH) and BIK specifically killed adnocarcinoma cell lines expressing plasma membrane GnRH receptor in vitro. In vivo growth of a human melanoma cell line stably transfected with a doxycycline-inducible expression plasmid for BIK as nude mice xenograft was strongly inhibited by doxycycline administration in drinking water. When administered systematically by intravenous injection, a cationic liposome-based gene delivery system of a BIK expression plasmid effectively suppressed growth of human breast cancer xenografts in nude mice. An adenoviral expression system of BIK induced apoptosis in glioma cell lines, and intratumoral injection of an ►adenovirus vector expressing BIK significantly suppressed growth of prostate (PC-3) and colon (HT-29) tumor xenografts in nude mice. A liposome-based, systematic intravenous delivery of a plasmid that expressed BIK using the pancreatic cancer-specific, cholecystokinin type A receptor promoter completely suppressed growth of human PANC-1 cells as xenograft in nude mice. These results suggest promise for BIK as a cytotoxic protein agent in gene therapy.

Because of the apoptosis sensitizing activity of BIK, low-level expression of exogenously introduced BIK that cannot induce apoptosis by itself may still be able to enhance cellular sensitivity to apoptotic stimuli. Thus, BIK-enhanced sensitivity of the H9 human T-cell leukemia cell line to chemotherapeutic agents increased apoptosis by 10- to 39-fold. Breast cancer cell lines selected for doxorubicin resistance were also effectively sensitized by expression of exogenously introduced BIK. These results suggest possible use of BIK in adjuvant gene therapy in combination with apoptosis-inducing chemotherapy.

The intracellular half-life of BIK protein is very short because of its rapid degradation by a proteasome-dependent mechanism. Therefore, when cells are exposed to proteasome inhibitors such as lactacystin, MG-132, or ►bortezomib, strong intracellular accumulation of BIK protein is often observed. Bortezomib does not cause significant accumulation of other BCL-2 family proteins except for NOXA. The bortezomib effect to resensitize malignant cells resistant to the death receptor ligand ►TRAIL is dependent on BIK protein accumulation. Bortezomib-induced ZR75–1 breast cancer cell apoptosis is also largely dependent on the accumulated BIK protein. Thus, proteasome inhibitors might be useful as adjuvant therapy agents for the purpose of increasing expression of the endogenous BIK protein in malignant cells to enhance their sensitivity to apoptosis-inducing chemotherapy.

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Bilateral Acoustic Neurofibromatosis

►Neurofibromatosis 2

Bile

Definition

Bile is a yellow, green digestive juice produced by the liver and stored in the gallbladder. It is passes through the bile ducts into the duodenum where it aids in digestion and absorption of dietary fat. Bile is

predominately composed of bile salts, cholesterol, phospholipids, bicarbonate and waste products such as bilirubin. The electrolytes present in bile especially bicarbonate which neutralize acid secretions from the stomach as they enter the duodenum.

►Bile Duct Neoplasms
►Gallbladder Cancer

Bile Acids

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Definition

Bile acids are complex physiological molecules that are essential for solubilization, absorption, and transport of dietary lipids in the intestine. On the other hand, bile acids are potentially toxic to cells.

Characteristics

Primary bile acids such as cholic acid and chenodeoxycholic acid are derived from cholesterol in the liver and are secreted and stored in the gallbladder as glycine or taurine conjugates. For example, cholic acid is stored as either glycocholic acid or taurocholic acid, and chenodeoxycholic acid is stored as either glycodeoxycholic acids or taurodeoxycholic acids. Bile acids are then released into the intestinal tract when fat enters the proximal portion of the intestine. About 90–95% of released bile acids are absorbed in the terminal ileum. Bile acids are transported via the portal vein to the liver and extracted for re-use in the so-called "enterohepatic circulation." However, about 5–10% of secreted bile acids reach the colon, where conjugated cholic acid and chenodeoxycholic acid undergo deconjugation and 7α-dehydroxylation by the anaerobic bacterial flora, forming the secondary bile acids deoxycholic acid and lithocholic acid, respectively. The tertiary bile acid ursodeoxycholic acid is subsequently formed by epimerization of chenodeoxycholic acid. In the colon, deoxycholic acid is partly absorbed and enters the enterohepatic circulation. Consequently, about 2–5% of secreted bile acids, consisting mainly of lithocholic acid, are excreted in the stool.

Toxicity of Bile Acids

Bile acids are probably not genotoxic but may be cytotoxic. ►(Toxicological carcinogenesis) Specifically, unconjugated bile acids are known to be toxic. Conjugation reduces the pKa of bile acids, thereby increasing solubility at low pH. Thus, at a pH lower than the physiological value, unconjugated bile acids precipitate easily, whereas conjugated bile acids remain soluble. Taurine conjugates are especially soluble even at more acidic pH. Hence, at physiological pH, bile acids usually remain as ionized forms and may be termed bile salts, which cannot pass through the cell membrane. However, at acidic pH, unconjugated bile acids are nonionized and can accumulate inside mucosal cells and potentially cause damage. An acidic pH thus does not affect the toxicity of conjugated bile acids, but may potentiate the toxicity of unconjugated bile acids. On the other hand, both conjugated and unconjugated bile acids reduce the pH sensitivity of cells. Decreased pH sensitivity results in the induction of cyclooxygenase-2, which is rapidly induced in response to tumor promoters, cytokines, and growth factors. Furthermore, cholic acid and chenodeoxycholic acid are considered to be tumor promoters and increase the incidence of benign adenomas and malignant adenocarcinomas when administered after carcinogens. It is thus likely that pH and bile acids are dual drivers of metaplasia, acting in combination to fuel inflammation and mediate cellular change.

Effects of Bile Acids on Organs

Upper Gastrointestinal Tract

Several studies indicate that duodenogastroesophageal reflux leads to esophagitis or Barrett esophagus and may be related to esophageal adenocarcinoma. Although the pathogenesis of ►esophageal cancer remains to be fully elucidated, bile acids are somehow involved, probably by being cytotoxic rather than genotoxic. As such, bile acids stimulate the development of esophageal squamous cell carcinoma, not to mention esophageal adenocarcinoma or ►gastric cancer, by promoting ►angiogenesis via the cyclooxygenase-2 pathway. ►(Arachidonic acid-pathway and cancer) In contrast, unconjugated bile acids, which are more toxic than conjugated forms, appear more frequently in the bile acid profiles of patients with severe esophagitis. Although reflux of unconjugated bile acids has not been demonstrated in patients with an intact stomach, such reflux has been found in the stomach after partial gastrectomy and in the esophagus after total gastrectomy. However, further studies are required to establish whether bile acids have a role in gastric cancer.

Lower Gastrointestinal Tract

Several studies indicate that ►colorectal cancer is associated with higher fecal levels of secondary bile acids. Deoxycholic acid and lithocholic acid appear to promote carcinogenesis and tumorigenesis by activating multiple oncogenic signaling pathways. ►(Cycloxygenase-2 in colorectal cancer) Furthermore, a high fat diet and cholesterol are implicated in the

pathogenesis of human colorectal cancer, presumably because both of these factors promote the synthesis and secretion of bile acids. Carcinogenesis is also associated with conditions such as ileal resection, cholecystectomy, or ileal inflammation such as that accompanying ►Crohn's disease, which can alter intestinal exposure to bile. These conditions can result in incomplete active reabsorption of bile acids from the distal ileum and interrupt the enterohepatic circulation of bile acids. In addition, increased colonic concentrations of bile acids are also associated with diarrhea, which may respond to bile acid sequestrants.

Liver, Gallbladder, and Bile Ducts

Several etiological studies indicate that bile acids might induce carcinogenesis in the gallbladder. ►(Gallbladder cancer) The gallbladder and bile ducts are exposed to high concentrations of bile acids, most of which are unconjugated. If retained for a long time in the gallbladder and bile ducts, bile acids may induce carcinogenesis in the biliary tree, although the mechanism remains unclear. Furthermore, numerous studies have shown that elevated concentrations of bile acids in the liver induce hepatocyte apoptosis. Some evidence suggests a relation between the hydrophobicity of bile acids and the induction of apoptosis. Thus, the degree of hepatocellular damage may be related to bile acid hydrophobicity, and lithocholic acid, the major constituent of hydrophobic bile acids, is the most hepatotoxic. These findings suggest a possible mechanism for bile-acid-mediated liver injury, but since most hepatic bile acids are in conjugated form, the hydrophobicity of bile acid is reduced, thereby decreasing its entry into cells. Moreover, the hepatotoxicity of chenodeoxycholic acid treatment in patients with cholelithiasis appears to be caused by secondary increases in lithocholic acid production.

Pancreas

Epidemiological studies have demonstrated a positive correlation between the incidence of pancreatic cancer and a high fat diet in association with the secretion of bile acids. ►(Pancreas cancer, clinical oncology) Pancreatic adenocarcinoma tends to develop in the head of the gland, which is more exposed to bile. These findings suggest that bile acids participate in carcinogenesis of the pancreas, although underlying mechanisms remain to be clarified.

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Bile Duct

Definition

Bile ducts are long tube-like structures lined by epithelial cells that carry bile (originating from the bile canaliculus) from the liver to the hepatic duct, which joins with the cystic duct to form the common bile duct opening into the duodenum. Bile ducts within the tissue of the liver are termed intrahepatic, whereas those outside the liver are termed extrahepatic.

►Bile Duct Neoplasms
►Gallbladder Cancer

Bile Duct Adenoma

►Bile Duct Neoplasms

Bile Duct Carcinoma

►Cholangiocarcinoma
►Klatskin Tumors

Bile Duct Hamartoma Biliary Cystadenoma

►Bile Duct Neoplasms

Bile Duct Neoplasms

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Synonyms

Bile duct adenoma; Bile duct hamartoma; Biliary cystadenoma; Carcinoid; Cholangiocarcinoma Cholangiosarcoma; Intraductal papillary mucinous tumors

Definition

Bile duct neoplasms are classified as benign and malignant tumors of the cells comprising the bile ducts of the liver, which include the bile duct epithelial cells and the connective tissues supporting the bile duct structure.

Characteristics

The biliary system is comprised of intra- and extrahepatic ►bile ducts. The function of this system is to transport ►bile from the liver to the duodenum where bile aids in the digestion of dietary fats. Bile ducts have a tube or vessel-like appearance. The interior of the ducts is lined with columnar epithelial cells, which have been termed ►cholangiocytes. Cholangiocytes are surrounded by a subepithelial layer of tough connective tissue, which contains a scant number of smooth muscle cells. Also, within this connective tissue layer, resides a population of mucous cells. The biliary system is surrounded by a network of nerves, blood vessels and lymphatics. Bile duct neoplasms are an extremely rare condition that includes both benign and malignant growth of cholangiocytes and the surrounding supporting tissues of the bile duct.

Benign Bile Duct Neoplasms

Benign tumors of the intrahepatic and extrahepatic biliary system are exceedingly rare as the large majority of bile duct neoplasms are malignant. These benign tumors include adenomas and papillomas and neoplasms of the supporting structure of the bile duct, such as bile duct hamartomas, carcinoids, leiomyomas, and fibromas. Some benign biliary lesions, such as papillomas, adenomas carcinoids and cystadenomas result in biliary obstruction and symptoms of ►cholestasis and ►jaundice. The most commonly diagnosed benign neoplasms are bile duct hamartomas (von Meyenburg complexes), carcinoids, and cystoadenomas.

Bile duct hamartomas are characterized by the growth of many tiny noncancerous nodules in the intrahepatic bile ducts, which are the result of the malformation of the ductal plates of the liver during embryonic development. Pathologically hamartomas are characterized by cysts dilated embedded in a fibrous, collagenous ►stroma. Hamartomas have for the large part been defined to be innocuous. Hamartomas have been associated with increased neoplastic transformation resulting in biliary adenocarcinoma (i.e., ►cholangiocarcinoma). Biliary cystoadenomas arise from von Meyenburg complexes and are also a rare neoplasm of the bile duct that is difficult to diagnose preoperatively. Biliary cystoadenomas occur more often in females. The predominant treatment for cystoadenoma is surgical ablation due to the high potential for malignant transformation.

Benign carcinoid tumors are also extremely uncommon. These neoplasms arise from enterochromaffin cells of the biliary tract. Due to the rarity of this type of tumor, carcinoids have been poorly characterized. Biliary carcinoids have not been associated with the production of functional hormones that has been reported for carcinoids in other areas of the gastrointestinal tract. Patients with carcinoids often present with symptoms mimicking cholangiocarcinoma and/or choledocholithiasis. Biliary carcinoids are slow growing and thus, have a low potential for malignant transformation. The predominant treatment for carcinoids is surgical removal.

Diagnostically, benign bile duct neoplasms are virtually impossible to differentiate from malignant neoplasms. Since these neoplasms are extremely rare, there is a lack of understanding of the potential for these tumors to become malignant. In certain cases, benign neoplasms are thought to contribute to ►inflammation of the liver from damage due to cholestasis. Therefore, surgical resection is the current and predominant treatment course.

Malignant Bile Duct Neoplasms

Over 95% of bile duct neoplasms are malignant. Recent reports indicate that there is an increase in global incidence of malignant bile duct tumors. These malignant tumors include cholangiocarcinoma (an ►adenocarcinoma), cholangiosarcoma, malignant carcinoids, and intraductal papillary mucinous adenocarcinoma.

Cholangiocarcinoma is the predominant cancer of the bile ducts. Cholangiocarcinoma results from the malignant transformation of cholangiocytes, which are epithelial cells that line the biliary system. Cholangiocarcinoma occurs in ~2 per 100,000 people. Approximately 13% of primary ►liver cancers are cholangiocarcinomas. Cholangiocarcinoma is divided

into two types: (i) intrahepatic that occurs in the bile ducts residing within the liver; and (ii) extrahepatic that arises in the right and left hepatic ducts, common hepatic and common bile duct. Risk factors for this cancer share long-standing inflammation of the liver and chronic damage of the biliary epithelium. Increased proliferation of biliary epithelium due to chronic damage of the liver is thought to play a key role in the pathogenesis of malignant bile duct neoplasms. The list of risk factors includes: ►gallstones or gallbladder inflammation, ►chronic ulcerative colitis, or chronic infection of the parasitic worm, ►*Clonorchis sinensis*, and ►primary sclerosing cholangitis (PSC). The prognosis for cholangiocarcinoma is grim due to lack of early diagnostic modalities and effective treatment paradigms. Cholangiocarcinomas are slow growing, metastasize late during the cancer's progression, and present with symptoms of cholestasis due to the blockage of the bile duct by tumor growth. In most cases, the tumors are well advanced at the time of diagnosis, which results in limited treatment options. Many of these tumors are too advanced to be removed surgically and chemotherapy and radiation therapy usually are not effective. In addition to cholangiocarcinoma, cholangiosarcoma is a tumor arising from the cells constituting the connective tissue layer of the bile ducts. Cholangiosarcoma is rarely reported and information is lacking on prevalence.

A subset of cholangiocarcinoma tumors have been defined as papillary cholangiocarcinoma or interductal papillary mucinous neoplasms. These tumors are characterized by frondlike, papillary projects that occasionally produce large amounts of mucous. The excessive mucous secretions may disturb bile flow and cause dilation of the bile ducts, which results in symptoms of obstructive cholestasis or bile duct stones. Interductal papillary tumors have low-grade malignancy penetrating the bile duct wall in the late stages of pathogenesis. Diagnostically, these tumors are often confused with bile duct stones due to the constant sloughing of tumor debris into the bile. The predominant treatment is surgical removal.

Malignant carcinoids are also extremely rare neoplasms. Similar to benign carcinoids, the treatment of choice is surgical removal with chemotherapy used when the tumors are metastatic.

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Biliary Glycoprotein

Definition

A member of the ►CEA gene family first found in bile. The BGP group of molecules consists of seven members formed by alternative splicing. There are differences in the number of Ig domains and there are two types of cytoplasmic domains present. The forms will longer cytoplasmic domains can participate in signaling.

►CEA

Bin1

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Synonyms

Amphiphysin II; Amph II; Amphiphysin-like; AMPL; SH3P9

Definition

Bin1 is a cancer suppression gene that functions in membrane dynamics, ►vesicle trafficking, and nucleocytosolic signaling processes. The Bin1 gene maps to human chromosome 2q14–2q21.

Characteristics

Bin1 encodes a set of BAR adapter proteins that bind and tubulate curved membranes in the cytosol and that can restrict gene expression in the nucleus. Bin1 protein structure is varied by alternate RNA splicing events that determine its cancer suppression activity. All Bin1 proteins include an N-terminal ►BAR domain and a C-terminal ►SH3 domain. Two isoforms found in all

cells localize to the nucleus and cytoplasm and both display cancer suppression activity. Several other tissue-specific isoforms found mainly in neurons include specialized membrane targeting sequences that prevent nuclear entry. These isoforms lack cancer suppression activity. In fact, Bin1 suppression activity is often inactivated in cancer cells by a specific RNA missplicing event that adds a neuron-specific exon (12a) preventing nuclear entry by the aberrant Bin1 protein generated. Studies of RNA splicing patterns in cancer cells indicate that this aberrant event is among the most common missplicing events occurring in human cancer.

Loss of heterozygosity at the Bin1 locus occurs with some frequency in ►prostate cancer, but in general deletions of Bin1 seem to be rare in human cancer. In contrast, Bin1 is often attenuated at the level of missplicing or loss of expression, including in breast (►Breast Cancer), prostate, lung (►Lung Cancer), and ►colon cancer and in ►astrocytoma, ►neuroblastoma, and malignant ►melanoma. In breast cancer, loss of nuclear Bin1 protein may predict poor prognosis. Restoring normal expression in cancer cells can restrict cell proliferation and/or survival, including by eliciting a caspase-independent mechanism of cell suicide. Thus, attenuation of the nuclear function (s) of Bin1 is important during cancer development or progression.

Genetic and cell biological studies in animal model systems indicate that Bin1 acts at several levels to suppress cancer, including by blocking cell proliferation, survival, motility, and ►immune escape. Bin1 was initially identified through its ability to interact with and inhibit the transcriptional and oncogenic activity of the ►Myc oncogene. There is also some evidence that Bin1 may also facilitate Myc-mediated ►apoptosis in certain settings. Furthermore, genetic ablation of Bin1 in the mouse mammary gland drives the progression of lesions initiated by activation of the Ras pathway, which cooperates with Myc in triggering neoplastic cell transformation. Thus, Bin1 may act in part to suppress cancer by restraining the oncogenic activity of Myc. In animals where Bin1 is more widely ablated, inflammation (►Inflammation), premalignant lesions, and tumors occur with a markedly increased incidence during aging. In particular, lung or liver tumors occur within 18 months in most animals where Bin1 is ablated. Mouse model studies further indicate that Bin1 acts to restrict immune escape, an important trait of cancer which is highly relevant to the emergence of clinical disease. At this level, Bin1 acts by restricting expression of ►indoleamine 2,3-dioxygenase, an important modulator of T cell immunity in cancer. Thus, Bin1 loss during tumor development influences the immune ►microenvironment as well as the cancer cell itself.

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BING2

►Daxx

Bioactivation

Definition

Is the transformation of a compound within an organism into a more biochemically active metabolite.

►Arylamine *N*-Acetyltransferases (NAT)

Bioactive Lipids

►Lipid Mediators

Bioavailability

Definition

Bioavailability of nutrients: Refers to the fraction of a mineral nutrient intake that is biologically available to

meet the essential metabolic and/or structural functions associated with that mineral nutrient in the body. Bioavailability incorporates the concepts of absorption, distribution, metabolic transformation (where necessary to meet biological function) and excretion. In some circumstances the main component of bioavailability is absorption across the gastrointestinal wall, so that sometimes the term bioavailability may be used interchangeably with absorption.

Bioavailability of drugs: Refers to the percentage of drug that is detected in the systemic circulation after its administration. Losses can be attributed to an inherent lack of absorption/passage into the systemic circulation and/or to metabolic clearance. Detection of drug can be accomplished ►pharmacodynamically or ►pharmacokinetically. Oral bioavailability is associated with orally administered drugs.

►Personalized Cancer Medicine
►Lead Optimization
►Mineral Nutrients
►Genistein

Biochip

►Proteinchip

Bioconjugate

Definition

A covalent or non-covalent coupling two or more distinct molecules together to confer a specific functionality. For example, the coupling of a aptamer targeting molecules to drug encapsulated nanoparticles can result in a nanoparticle drug delivery bioconjuate that is targeted to specific cells or tissues.

►Aptamer Bioconjugates for Cancer Therapy

Biodribin

►Cladribine

Biogenic Amines

Definition

A group of naturally occurring, biologically active amines, such as monoamines (norepinephrine, histamine, tyramine, dopamine, and serotonin) and polyamines (putrescine, spermidine, spermine).

►Amine Oxidases

Bioinformatics

Definition

Is a discipline covering all aspects of biological information acquisition, processing, storage, visualization, distribution, as well as analysis and interpretation that combines the tools of mathematics, computer science, and biology to advance the scientific understanding of the biological significance of huge amount of data. It involves the creation and advancement of algorithms, computational and statistical techniques, as well as the theories to solve formal and practical problems inspired from the management and analysis of biological data. Genomics and proteomics analyses generate expression information from tremendous amount of genes or proteins, which have to be organized, stored, and analyzed with the aid of bioinformatics.

►Drug Design
►Personalized Cancer Medicine

Biologic Therapy

Definition

Treatments for autoimmune disease or cancer that comprise proteins such as antibodies and cytokines or fragments of proteins or synthetic peptides are called biologic therapy. The term also encompasses the use of cells such as bone marrow-derived cells for treatment.

►Sjögren Syndrome

Biological Clock

Definition
The mechanism within an organism that generates repeated cycles (rhythms, oscillations) in behavioral, biochemical, metabolic and/or physiological activity that can be synchronized by environmental stimuli, primarily light.

►Melatonin

Biological Markers

►Clinical Cancer Biomarkers

Biological Monitoring

►Biomonitoring

Biological Response Modifiers

Definition
Substances, either natural or synthesized, that boost, direct, or restore normal immune defenses, BRMs include interferones, interleukins, thymus hormones, and monoclonal antibodies.

Biological Therapy

►Immunotherapy

Biologically Effective Dose (BED)

Definition
The quantity of a drug that results in a therapeutic benefit. The accurate measurement of the BED is essential for the clinical evaluation of cytostatic molecularly-targeted drugs.

►Drug Design

Biologicals

Definition
Biologicals are large drug compounds with atomic mass units typically in the tens of thousands, e.g. vaccines, monoclonal antibodies, fusion proteins, peglated proteins (proteins chemically modified with long chains of polyethylenglycol).

►ADMET Screen

Bioluminescence Imaging

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Synonyms
BLI

Definition
A non-invasive imaging approach that relies upon the detection of light resulting from the oxidation of a specific substrate by luciferase enzyme expressing cells.

Characteristics
Bioluminescence imaging (BLI) comprises a relatively new preclinical imaging modality that has become popular with researchers across a broad range of biological disciplines. From an oncology perspective, the high sensitivity, versatility and speed afforded by BLI has made it a particularly attractive modality

for measuring many aspects of *in vivo* tumor biology within cohorts of animals.

In general terms and in comparison to the other non-invasive imaging modalities, the hardware and consumable reagents needed for BLI are relatively cheap and safe (i.e. non-radioactive). *In vivo* images of multiple subjects can be acquired quickly (typically ranging between 1 and 180 s) so throughput is high. Moreover, when used in conjunction with small animal models of disease, where tissue depths seldom exceed 1–2 cm, BLI can exhibit sensitivity limits that compare very favorably with ►PET (►Positron emission tomography) (currently deemed the most sensitive non-invasive imaging approach). Unlike ►CT (►Computed tomography) or ►MRI (►Magnetic resonance imaging), BLI does not generate images with a high degree of anatomical detail. The wavelengths of light generated in this approach are prone to scattering and absorption as they pass through tissue, resulting in an imaging resolution of approximately 1 mm. As BLI is reliant on the expression of a luciferase transgene, it is also a highly versatile technique that can be used to non-invasively determine diverse aspects of *in vivo* tumor biology, ranging from the relative quantification of tumor burden to measuring the activation states of various cellular processes or detecting specific protein: protein interactions. In addition, as only viable labeled cells generate bioluminescence in the vast majority of cases, BLI has proven particularly useful for testing the *in vivo* efficacy of experimental cancer treatments using tumor cell lines and small animal models of cancer.

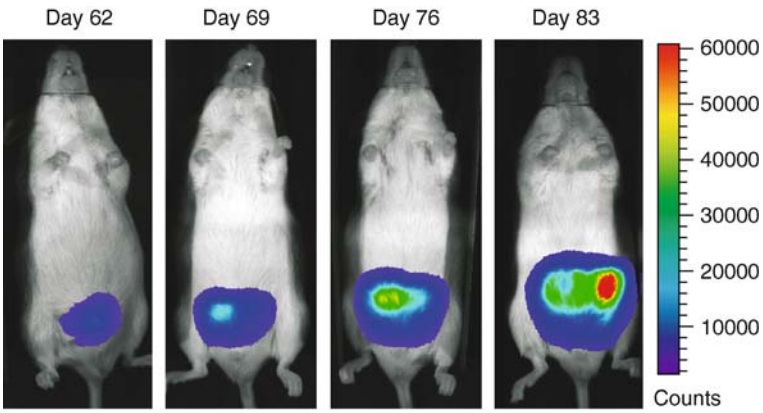
About Luciferases

As stated in the definition, BLI relies upon the external detection of light produced by cells that express a luciferase enzyme. These are not endogenously expressed by mammalian cells, therefore, luciferase transgene expression must first be introduced prior to the direct imaging of tumor cells by this method. The most commonly used luciferase enzymes for such purposes have been the ►codon-optimized (Codon-optimization) forms of firefly luciferase (derived from the North American firefly, *Photinus pyralis*) and renilla luciferase (derived from the sea pansy, *Renilla reniformis*). Also validated for expression in mammalian cells are the click beetle luciferases (green or red; derived from the Jamaican click beetle *Pyrophorus plagiophthalamus*) and gaussia luciferase (derived from a copepod called *Gaussia princeps* and unique amongst the enzymes mentioned in that it is naturally secreted by expressing cells).

These individual luciferase enzymes share little homology and vary in the efficiency that they produce light. To generate bioluminescence, firefly and click beetle luciferases specifically catalyze the oxidation of D-luciferin in the presence of O_2, ATP and Mg^{2+}. Renilla and gaussia luciferases, however, specifically catalyze the oxidation of a different substrate, coelenterazine, in the presence of O_2 and independently of ATP. Neither of these substrates are produced endogenously by mammalian cells and so must be administered either to tissue culture medium or by injection/►osmotic pump to enable *in vitro* or *in vivo* bioluminescence imaging respectively. Both substrates are relatively small molecules that are broadly taken up by cells throughout the body and can be administered repeatedly without eliciting an immune reaction. The *in vivo* use of coelenterazine is slightly more complicated than luciferin, however, as it is less soluble and prone to auto-oxidation (resulting in bioluminescent "noise") and deactivation in serum. It has also been shown that coelenterazine is actively pumped out of cells that express high levels of ►P-glycoprotein. Therefore, renilla or gaussia luciferases are likely not ideal reporters to directly image multi-drug resistant (i.e. P-glycoprotein over-expressing) tumor cells *in vivo*. The emission spectra produced by these luciferases are also characteristically broad (spanning >100 nm) and differ. For example, the peak emission of renilla luciferase is 480 nm, whereas firefly luciferase is 610 nm. Collectively, these factors ensure that bioluminescent signals from selected pairs of luciferase enzyme can be discerned upon the basis of substrate exclusivity as well as their spectral signature. This is highly useful as it enables the employment of powerful dual-labeled BLI studies, where the light generated by different luciferase enzymes can be detected sequentially to measure multiple parameters within the same cell or individual animal (e.g. viable tumor burden measured by renilla luciferase and the activation of a cellular process by firefly luciferase). The development of increasingly sophisticated ►spectral unmixing image analysis techniques should soon make it possible to routinely discern the optical spectra from two different luciferases when both substrates are administered simultaneously.

How is In Vivo Bioluminescence Detected?

The intensity of light generated by luciferase labeled cells in a typical bioluminescence imaging experiment is sufficiently low that a highly sensitive light detector is needed to measure it. Such detectors are commercially available and typically comprise a cryogenically cooled CCD camera (Charge-Coupled Device; cooled to $\leq -90°C$ to reduce thermal noise and increase light sensitivity) that is housed behind a lens within a completely light tight box. The non-visible levels of light associated with bioluminescence imaging can be detected by the pixels of the cold CCD, which results in a fully digitized and quantifiable 2-D map of light intensity across the field of view. An image of this light intensity map is then superimposed over a digital



Bioluminescence Imaging. Figure 1 The above figure shows a series of bioluminescent images of an individual mouse taken at weekly intervals and shows the development of a spontaneous and bioluminescent prostate tumor. Note that the colors associated with these images and accompanying scale bar correspond to light intensity and do not reflect the color of detected light. This figure is reproduced with modification from Fig. 4A (Lyons SK, Lim E, Clermont AO et al (2006) Noninvasive bioluminescence imaging of normal and spontaneously transformed prostate tissue in mice. Cancer Res 66(9):4701–4707) by copyright permission of the AACR.

photograph of the subject (taken in normal light conditions immediately prior to bioluminescence acquisition) to indicate the regions of the subject where labeled cells reside (Fig. 1).

In a manner analogous to conventional photography, the exposure time and aperture settings of the CCD camera can be adjusted to modify the sensitivity of bioluminescence acquisitions. This ensures that CCD pixels do not become saturated when imaging relatively bright subjects and maximizes sensitivity when imaging relatively dim subjects. Computer software can be used to add together (or "bin") the signals detected by adjacent CCD pixels to further increase imaging sensitivity, but this gain is made at a cost to image resolution. Software tools are also used to create "regions of interest" to quantitatively measure light emission from any area of the image in fully calibrated physical units (i.e. photons/s/cm^2/►steradian).

Considerations to Maximize In Vivo Imaging Sensitivity

Currently BLI is considered one of the most sensitive non-invasive preclinical imaging modalities when used in conjunction with small animal models of disease. There are, however, several important factors that will affect the sensitivity of any *in vivo* BLI approach and so influence the minimum number of cells that can be detected or the ability to visualize the activity levels of a cellular process above noise.

One obvious issue relates to the extent of luciferase enzyme expression in the target cell; labeled cells that express relatively low levels of luciferase will be harder to detect than an equivalent number of labeled cells that express greater amounts of luciferase.

Another key issue is the depth of signal, as the wavelengths of light produced by the commonly employed luciferases are prone to scatter and absorption as they pass through mammalian tissue. Red wavelengths of light (>600 nm) pass through tissue with greater efficiency than relatively bluer wavelengths (<500 nm). Therefore, in principal, luciferases that produce light with the highest proportion of red light should be better-suited for *in vivo* imaging. The quantum yield or relative brightness of the different luciferases also varies however, and can compensate for issues relating to the colour of emitted light. For example, even though gaussia luciferase generates predominantly blue/green light, imaging sensitivity has been reported to be roughly comparable to that associated with firefly luciferase as the relative efficiency of light production is sufficiently high. The absorbance of *in vivo* bioluminescence is increased when overlying tissues, skin or fur are darkly pigmented. Indeed, whenever BLI sensitivity issues become prevalent for any given *in vivo* application, the use of albino mouse strain variants or the local removal of pigmented fur is highly recommended.

The fact that different colors of light have inherently different tissue transmission properties has led to the development of algorithms that can predict the depth of firefly luciferase expressing cells in tissue. As red light passes through tissue with greater efficiency than green, the ratio of red to green light on the surface of the animal is proportional to tissue depth (i.e. the presence or absence of green light in relation to the amount of measured red light is indicative of a shallow or deep bioluminescent origin respectively).

Another key factor that influences the sensitivity of BLI is the extent of bioluminescent background. In terms of imaging labeled tumor ►xenograft models, this is not a serious issue as non-labeled host tissue does not emit light at appreciable levels. This issue does become pertinent when working with luciferase labeled transgenic mice (as certain lines may express significant levels of luciferase in a non-specific manner) or when attempting to detect micro-metastases that reside near to the primary tumor. Attempts to image the activation of a molecular pathway in a population of cells can also prove challenging in transgenic mice, if basal levels of luciferase expression are already high.

Oncology Model Applications

A tremendous degree of versatility is afforded by the fact that the direct imaging of mammalian cells by BLI relies upon the expression of a luciferase transgene. A wide range of validated transgenic strategies currently exist to control transgene expression at the transcriptional level or reporter gene functionality following translation. As a consequence, a diverse range of tumor biology related parameters can be imaged *in vivo* using BLI.

One of the most common applications of BLI in cancer research is the detection and repeated measurement of *in vivo* tumor burden within the same subject over time.

For tumor xenograft based studies, this can be achieved by introducing stable constitutive luciferase expression into the cell line of choice prior to implantation. Derivatives of strong viral promoters such as CMV or SV40 have been frequently employed for such purposes, but promoter sequences from eukaryotic house-keeping genes (e.g. β-actin or GAPDH) can also be used and may be preferable for ensuring robust luciferase expression in certain cell types.

It is now well established that when luciferases are constitutively expressed in tumor cells (with the exception of secreted gaussia luciferase), overall light emission is proportional to tumor cell viability. At the early to mid stages of tumor development, this is typically reflected by a strong correlation between measured bioluminescence and tumor volume. This correlation becomes less pronounced when tumors near end-stage, as extensive regions of tumor necrosis (which contribute to tumor volume but not bioluminescence), variable tissue-depth and tumor perfusion/substrate bioavailability (affecting measured bioluminescence but not volume) become more prevalent.

BLI can also measure spontaneously arisen tumor burden in transgenic mice. Imaging such tumors is more complicated than xenograft models as, in order to maintain sensitivity, strategies must be implemented to ensure that luciferase expression is maximized in the tumor, yet kept to a minimum in proximal non-transformed tissues. Tissue specific promoters can be useful when tumorigenesis occurs in a target organ or cell type population that is relatively small (tumors arising spontaneously in the pituitary and prostate glands have been successfully imaged in this way). The growth dynamics of spontaneous tumors arising in other more elaborate conditional (►Cre/loxP dependent) tumor models can also be measured using a generally expressed but conditional (Cre/loxP dependent) luciferase allele.

Constitutive promoter strategies can also be used to detect the appearance of tumor ►metastases *in vivo*. As the primary tumor may be relatively large (and consequently bright) at the time that metastases appear, imaging sensitivity is maximal when metastases develop at sites that are spatially distinct from the primary tumor. Tissue depth will also vary between the metastatic sites within a cohort of subjects. Therefore, longitudinal BLI measurements indicate only the presence and relative growth dynamics of each metastatic lesion as opposed to the absolute quantification of tumor burden at every location. BLI can also be performed on freshly excised organs at necropsy (i.e. *ex vivo* BLI) to quickly validate the presence of metastases at the end of an experiment.

The sensitivity of BLI is such that many other tumor associated processes (and the effects of drug treatment upon them) can also be imaged non-invasively when luciferase expression or functionality is regulated in different ways. For example, the relative levels of tumor cell proliferation can be imaged before and after drug treatment using a luciferase allele that is only expressed at the onset of ►S-phase in replicating cells. Tumor cell ►apoptosis has also been imaged *in vivo* by employing engineered luciferase alleles that have extra peptide domains fused to them that cause either reporter instability or impair function in normal cells. The activation of specific ►caspase enzymes, which mark the onset of apoptosis, specifically cleave this interfering peptide domain from the reporter, resulting in reporter functionality and the generation of bioluminescence. Several approaches have also been devised to image specific protein:protein interactions in tumor cells *in vivo*. One involves the splitting of firefly or renilla luciferase into two separate but complementary domains. When these intrinsically inactive N- and C-terminal reporter fragments are fused to two different proteins, bioluminescent activity is only reconstituted when the proteins that they are fused to bind each other. Again, the ability to garner this type of information non-invasively *in vivo* is incredibly useful for characterizing fundamental aspects of tumor biology or examining the effects of experimental therapeutics.

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Bioluminescent Reporter Gene Assays

►Luciferase Reporter Gene Assays

Biomarkers

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Definition

Biomarkers are parameters that provide information on exposure to xenobiotics and to chemopreventive compounds, or on the effects of that exposure in an individual or in a group.

Characteristics

Biomarkers used for the detection of cancer risk factors and in studies of chemoprevention can reveal an overall body load of genotoxins (which should be avoided) or of chemoprotective components (which should be enhanced).

Background

Most forms of cancer are due to somatic alterations (mutation, amplification, recombination) in proto-oncogenes, in tumor suppressor genes or in DNA repair genes. These are acquired in the tumor target tissues during life time, accumulate and produce a clonal selection of cells with aggressive and invasive growth properties. Only ~1% of all cancers are due to inheritance of these types of genetic alterations. Most other cancers are dietary related, are due to inhalation of tobacco smoke, or may be a consequence of inflammation or viral infections. Therefore the majority of all human tumors are considered to be preventable by avoiding exposure to risk factors. Biomarkers may be used in human trials and in studies of ►molecular cancer epidemiology to study these types of exposures and to identify measures to reduce cancer risks.

Types of Biomarkers

The most straight forward determination of risk is to identify people already carrying the disease on account of having tumor cells in their body. These markers are of diagnostic value. In the context of exposure and health, however, other parameters that can be detected prior to manifestation of tumors are considered more feasible. These include:

- Susceptibility biomarkers (predetermining damage) to identify people at high risk, since they carry cancer prone genetic alterations (mutations, gene amplifications, or recombination) in cancer target genes (e.g. ►APC deletions; hMSH mutations, K-►ras amplification).
- Susceptibility biomarkers (predisposing alterations) to identify people at different degrees of risk because they carry frequent alterations in genes that are more indirectly related to the process of carcinogenesis. These indirect mechanisms include features of carcinogen metabolism (►metabolic polymorphisms) or pharmacological variations (e.g. receptors for micronutrients, sensory dispositions). There is some evidence available that single genetic polymorphisms, or a combination of these, can be associated with cancer risk.
- Biomarkers of early effects in cells and tissues to identify past exposure to risk factors by determining genetic damage (►DNA adducts, DNA breaks, ►oxidative DNA damage, genome instability) in somatic cells. This is based on the assumption that increased DNA damage is the result of a higher load of genotoxic agents that will cause the complex process of carcinogenesis. Additional cellular processes that may serve as biomarkers are cell proliferation or ►apoptosis (intermediate endpoints). These may also be decreased on account of exposure to protective factors. Furthermore, the modulation of gene expression, such as induction of phase II enzymes may render the cell less vulnerable and more resistant to risk factors, and the measurement of these effects are thus novel biomarkers of chemoprevention.
- Biomarkers of exposure (risk and protective factors) to identify current exposure to risk compounds (e.g. carcinogens from tobacco (►tobacco carcinogenesis) or food, reactive oxygen species, products of lipid

peroxidation) or protective compounds (e.g. antioxidants, metabolites of chemopreventive agents, ►fermentation products of the gut flora) by measuring their concentrations in urine or blood. For complex associations such as ►diet and cancer, the shift of these groups of substances relative to each other can then be evaluated as contributing to an increase or to a decrease of risk.

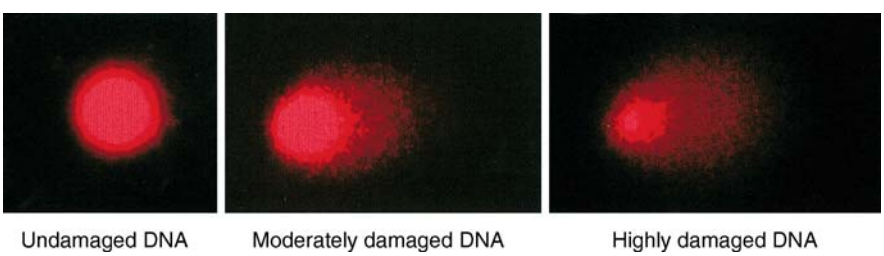
Biomarker Techniques and Fields of Application

Depending on the source of body fluid or cells analyzed, the biomarkers will reveal systemic or tissue specific exposures. Specific endpoints will be more suited for molecular cancer epidemiology studies, whereas non-specific end points are also of value for occupational types of exposure assessment or for dietary intervention studies. Non-invasive methods should be better suited for large scale studies, whereas invasive methods will be employed more selectively. In this context, largely depending on the degree of invasiveness, biomarkers may be categorized as follows:

- Non-invasive methods using body fluids or exfoliated cells include techniques such as the analytical detection of single compounds or of their metabolites. The methods are indicators of exposure. Also, a functional determination of mutagenic or genotoxic effects of body fluids using cultured cells as target organisms (e.g. determination of fecal water genotoxicity) are biomarkers for determining exposure. Other non-invasive methods are directed at analyzing genetic alterations in isolated exfoliated cells from these body fluids. Examples are the analysis of micronuclei e.g. in sputum or urinary and buccal cells.
- Relatively non-invasive methods using cells of the peripheral blood stream are aimed at detecting exposure-related genotoxic damage. The endpoints include DNA-strand breaks, oxidized DNA bases (using the single cell microgel electrophoresis assay, also referred to as the ►comet assay), DNA adducts (detected with ►32P-postlabeling) and cytogenetic endpoints (micronuclei, sister chromatid exchanges, chromosomal aberrations). The development of the techniques for genetic damage has been largely based on their utilization as methods to assess exposure in occupational, environmental settings or subsequent to tobacco smoke inhalation. They have only sporadically been used to study associations of diet and cancer.
- Invasive methods using cells from tumor target tissues make use of cells from biopsies (e.g. colon, breast, kidney) to determine functional parameters in potential tumor target tissues. The parameters indicate cellular responses and genetic alterations (proliferation, K-ras-, p53-mutations, APC-alterations and DNA damage). The end points are indicators of very early response to risk factors and are biomarkers of effect. However, they are invasive, and thus may be limited to studies on special exposures or in specific groups of patients. In any case, their utilization and development will serve as basis for the refinement of non-invasive methods with exfoliated cells as outlined above (Fig. 1).

In conclusion, a variety of biomarkers to assess the impact of risk and of protective factors is available. Already research has provided evidence that biomarkers can measure the efficacy of exposure as well as of exposure reduction. Many of the techniques, however, need to be further validated for their applicability, reliability and predictivity of potential tumor risks in human studies. Another set of techniques is available that can serve as a meaningful basis for the development of potentially new biomarkers. Altogether these methods are of value to serve as indicators of effects and indicators of exposure by risk and protective compounds. Depending on the specificity of the end point or on the technical feasibility, individual methods will be more or less suited for use in dietary intervention studies, in occupational exposure settings and/or in larger scale trials of molecular epidemiology.

►Carcinogen Macromolecular Adducts
►Oncopeptidomics



Biomarkers. Figure 1 Images of undamaged to damaged DNA from single human peripheral lymphocytes in the "comet assay." Cells were embedded into agarose on microscopical slides, lysed, subjected to alkaline electrophoresis and stained with ethidium bromide. Usually the proportion of damaged cells and degree of damage is quantified for 50–100 cells per slide, using an image analyzer.

►Molecular Pathology
►Clinical Cancer Biomarkers
►Biomonitoring
►Tissue Inhibitors of Metalloproteinases
►BORIS
►CCCTC-Binding Factor (CTCF)

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Biomonitoring

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Synonyms

Biological monitoring

Definition

Biological monitoring (i.e., biomonitoring) has conventionally been defined as "the periodic measurement of ►xenobiotic(s) or their ►metabolite(s) in accessible biological media for the comparison with an appropriate reference." At present, a broader definition could be used that included effect ►biomarkers and biologically relevant dose, as well as biomarkers of susceptibility.

Characteristics

Biomonitoring is mainly aimed at (i) defining the existence of an occupational or environmental exposure; (ii) quantifying the ►internal dose; and (iii) verifying that exposure limits are respected. The most commonly used matrices for biomonitoring are blood (and its components, e.g., serum and plasma) and urine.

Biological monitoring is complementary to the two other monitoring programs that are carried out to evaluate the health risk associated with exposure to pollutants, i.e., ambient monitoring and health surveillance. The basis of these monitoring programs is defined by following up the fate of a chemical from the environment to the target molecules in the organism. The main characteristics of biological and ambient monitoring are summarized in Table 1.

Once absorbed and present in the circulation, the chemical may be eliminated unchanged, mainly in urine or in expired air, or distributed to different compartments of the body. Organic chemicals usually undergo a ►biotransformation to more water-soluble compounds that are more easily excreted via urine or bile than the parent compound. If not excreted, the chemical or its ►metabolites may bind to different sites on the target molecules. Binding on critical sites may give rise to adverse health effects at least when the amount bound has reached a certain level and the protective mechanisms are inadequate or insufficient.

Biological monitoring may offer several advantages over environmental monitoring to evaluate the internal dose and hence to estimate the health risk. One of the main advantages is that biological monitoring takes into consideration all routes of absorption (inhalation, skin, ingestion) in both occupational and leisure activities, accounting for individual differences in absorption rate due variations in, e.g., work load or coexposure to additional components of complex mixtures. It also takes into account the variations in individual

Biomonitoring. Table 1 Main characteristics of biomonitoring and ambient monitoring

	Biomonitoring	Ambient monitoring
Quantifies	Dose	External exposure
Routes of absorption	All routes	Inhalation
Measurement	Biomarkers	Direct
Interpretation	Complicated	Easy
Variability	High	Usually low
Confounding	Metabolic ►phenotype	Protection devices
Cost	Usually high	Usually low

►metabolic capability, due to either genetically determined or acquired changes in gene expression and enzyme activity. The greatest advantage of biomonitoring, however, is the fact that the biological parameter of exposure is more directly related to the adverse health effects that one attempts to prevent than any environmental measurement.

On the other hand, biomonitoring is more complex in terms of ►standardization and interpretative efforts as compared with ambient monitoring. Since biomonitoring rely on the use of biomarkers, rational biological monitoring is only possible when sufficient ►toxicological information has been gathered on the mechanism of action and/or the metabolism (absorption, biotransformation, distribution, excretion) of ►xenobiotics to which people may be exposed. When a biomonitoring method is based on the determination of chemical or its metabolite in biological media, it is essential to know how the substance is absorbed via the lung, the gastrointestinal tract, and the skin, and subsequently how it is distributed to the different compartments of the body, ►biotransformed, and finally eliminated. It is also important to know whether the chemical can accumulate to the body.

According to the National Research Council (NRC), biomarkers can be classified as (i) biomarkers of exposure; (ii) biomarkers of effect; and (iii) biomarkers of susceptibility. The use of biomarkers rather than their intrinsic properties may define their classification.

Biomarkers of Exposure

Exposure biomarkers are widely used, e.g., in occupational ►toxicology for a more accurate risk assessment. In workers exposed to similar air concentrations of chemical pollutants, various factors can determine the actual absorbed dose, including physical workload, additional skin absorbance due to bad working practice or, on the contrary, the use of personal protection devices, and differences in individual uptake and metabolism.

The meaning of the marker may depend on the sampling time. Therefore, the choice of the biomarker should rely on a number of considerations, but mainly on ►kinetic parameters and on the knowledge of the mechanistic basis of adverse effects. An ideal biomarker of exposure should be (i) specific for the exposure of interest; (ii) detectable in small quantities; (iii) measurable by noninvasive techniques; (iv) inexpensive; (v) associated with prior exposure; and (vi) able to provide an excellent positive predictive value to a specific health status. Several biomarkers of exposure are often available for the same chemical, e.g., the parent compound itself, a metabolite, or a macromolecular ►adduct (to DNA or protein).

A great majority of the currently available biomonitoring tests are based on determination of the chemical or its metabolite in a biological media. According to their selectivity, these tests can be classified into two subgroups: (i) the selective tests based on the direct measurement of the unchanged chemicals or their metabolites in biological media; (ii) the nonselective tests used as nonspecific indicators of exposure to a group of chemicals.

DNA and protein ►adducts are primary measures of exposure to carcinogenic compounds. DNA adducts are mechanistically linked to cancer formation, as they may cause gene mutations and chromosomal alterations in growth controlling genes. Measurement of DNA adduct levels allows insight into the impact of metabolic variations, the interactions between components of complex mixtures, and coexposure to compounds that enhance the effect of carcinogens. In human studies, DNA adduct levels in the target organ are consistent with the excess risk noted for populations with specific exposures. For instance, significant differences in DNA adduct levels have been detected in persons exposed to passive tobacco smoke in the face of only modest differences in exposures. Moreover, simple interventions have been shown to reduce DNA adduct levels in the target organ of an exposed population. So, while analysis of carcinogen DNA adducts remains primarily a research tool, these research studies have begun to validate its wider use in biological monitoring of exposed human.

Biotransformation is obviously a central issue for any biomarker used in biomonitoring of xenobiotic exposure. Variation in individual metabolism is expected to be an important contributor to variation in biomarker levels. Metabolic differences among individuals can stem from acquired factors, such as enzyme induction or inhibition, or from inherited polymorphisms of xenobiotic-metabolizing enzymes (XMEs).

Most absorbed xenobiotic chemicals undergo biotransformation that eventually aims at disposal of the chemical with as little harm as possible. For indirectly toxic chemicals, phase I reactions mediated by cytochrome P-450 (CYP)-dependent monooxygenases usually comprise metabolic activation, while phase II conjugation reactions are part of detoxification and lead to excretion. In many cases, the metabolism is, however, complicated, and metabolic activation and detoxification does not follow this simple model.

During last decade many of the XME-genes have been shown to be polymorphic, resulting in individual differences in the metabolic capability related to these enzymes. For some enzymes, the polymorphism involves ►genotypes that are associated with no enzyme activity, while in other cases ►phenotypic differences between the genotypes are subtler. The phenotypic consequences of many metabolic polymorphisms are, however, inadequately known. Accordingly, we are just beginning to understand the possible toxicological

impacts of genetic polymorphisms in environmental exposures.

Biomarkers of Effect

Biomarker of effect has been defined as "any measurable biochemical, physiological, or other alteration within an organism that, depending on the magnitude, can be recognized as an established or potential health impairment or disease." Research on biomarkers of effect is rapidly generating a large amount of data measuring intermediate end-points occurring probably after exposure and possibly before illness. Such biomarkers are expected to reflect early modifications preceding progressive structural or functional damage at the molecular, cellular, and tissue level. A wide spectrum of biomarkers may be used for this purpose. Cytogenetic (►Cytogenetics) tests involving scoring of microscopic chromosomal damage are the oldest of biomarkers used and are still applied in a wide variety of exposures.

The main conceptual basis for using cytogenetic assays for biological monitoring is that genetic damage in a nontarget tissue, most often peripheral blood lymphocytes, reflects similar events in cells involved in carcinogenic process. The conventional chromosomal damage assessments include determination of (i) structural chromosomal aberrations (CAs); (ii) sister chromatid exchanges (SCEs); and (iii) micronuclei (MN). Recently, in situ fluorescence techniques (FISH) have been used in order to score specific chromosomes and chromosomal loci. Rigorous study design is necessary in all cytogenetic biomonitoring methods, since many interindividual factors that are not related to the specific chemical exposure(s) of interest may affect the parameters studied. Experimental confirmation of the chromosome damaging potential of the test agent(s) is therefore a prerequisite in performing human cytogenetic studies.

A good example of the potential applicability of chromosomal damage as surrogate for disease comes from recent prospective studies on cytogenetic biomarkers and cancer risk that followed several European cohorts; subjects in the group of highest frequency of CAs were at a more than doubled overall risk for cancer with comparison to the lowest frequency group. The use of MN as a measure of chromosomal damage, on the other hand, has become a widely used assay in both genetic toxicology testing and human biomonitoring studies. Analysis of results from European cohorts indicated that subjects with cancer had a significant increase in MN frequency.

Biomarkers of Susceptibility

The concatenation of environmental exposure, genetic effect, and ►individual susceptibility is a key issue in assessment of risks for populations exposed to environmental pollutants. In view of the interindividual differences in susceptibility to xenobiotics, one might consider the detection of increased susceptibility to a chemical hazard. A biomarker of susceptibility is defined as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance. For instance, the ability to acetylate aromatic amines has been shown to be genetically determined and it has been suggested that carriers of allelic variants of the *N*-acetyltransferase (NAT2) gene that result in decreased *N*-acetylation capacity are at increased risk for colorectal cancer when exposed to carcinogenic aromatic amines.

Genetic polymorphisms in the activity of the aryl hydrocarbon hydroxylase (AHH) has also been suggested as an example of the relationship of metabolic variation to individual susceptibility to develop lung cancer in case of exposure to polycyclic aromatic hydrocarbons (PAHs).

The genetic polymorphisms potentially important for a particular biomarker largely depend on the exposing agent and biological material examined. As ►genotype effects have only occasionally been considered in ►biomarker studies, much basic research is needed, and no general conclusions can yet be drawn on their real importance. It is, however, expected that genotype differences exist in biomarker response to many exposures. In such cases, information on this effect will be very valuable for correct assessment of exposure and effect biomarkers. If genotyping can be shown to markedly improve routine biomarker reliability, the ethical question whether this tool should be utilized only in setting standards or incorporated as part of the analysis must be addressed.

Ethical and Social Implications

Biological monitoring has been a major tool of medical health surveillance in most EU member states already for several decades. Biomonitoring data can also be used to fine tune or even launch environment and health policies; it allows policy makers to identify priorities and provides early warning on potential threats and enables them to assess how effective the strategies are (time trends analysis).

For many pollutants, however, interpretation of health significance is still hampered by the lack of toxicological and medical information. Moreover, ►internal doses cannot be directly linked to the external exposure source. And because human biomonitoring has to do with people, an ethical and communication framework has to be further developed in order to ensure that the biological monitoring surveys respect ethical and privacy considerations.

Much work has therefore still to be done, especially with respect to proper interpretation of human biomonitoring data and its translation into policy actions.

In line with this, developing a coherent approach to human biomonitoring is one of the main priorities of the European Union Environment and Health Action Plan 2004–2010.

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Biopsy

Definition

Sample of tissue obtained for diagnostic examination by a special surgical intervention.

►Exfoliation of Cells

Bioreductive Drug

Definition

Is an agent that is reduced in the state of oxygen deficiency, usually to produce a more active metabolite that can be cytotoxic or that can be used for the detection of O_2-depleted tissue areas.

►Oxygenation of Tumors

Biosensor

Definition

A sensor to detect a biological component or a sensor that provides bioanalytical information by mean of applying biological molecules such as nucleic acids, enzymes, or antibodies which functions as biological recognition element.

►Surface Plasmon Resonance

Biosynthesis

Definition

Is the production (usually enzymatic) of components in living cells

►Estrogenic Hormones

Biotechnology

Definition

The use of living organisms or their products to make or modify a substance. Biotechnology includes recombinant DNA techniques (genetic engineering) and hybridoma technology.

Biotechnology Derived Therapeutic Proteins

►Recombinant Therapeutics

Biotin

Definition

(vitamin H or B7) A water-soluble B-complex vitamin which is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring. Biotin

is important in the catalysis of essential metabolic reactions to synthesize fatty acids, in gluconeogenesis, and to metabolize leucine.

►Anti-HER2/Neu Peptide Mimetic (AHNP)

Biotransformation

Definition

Chemical conversion of substances by living organisms or enzyme preparations.

►Biomonitoring

BiP/GRp78

Definition

BiP (Binding protein for immunoglobulins)/GRp78 (Glucose-regulated protein of 78 kDa) is a molecular chaperone of the Hsp70 family, and expressed in the endoplasmic reticulum. It recognizes non-native folding intermediates through hydrophobic amino acids in proteins.

►Calreticulin

BIR Domain

Definition

►Baculovirus IAP Repeat Domain; Is an approximately 70 amino acid zinc-binding domain, first identified by sequence homology among proteins belonging to the ►Inhibitors of Apoptosis (IAP) family. Present in one to three tandem copies per protein, the BIR domain has been identified in over 80 different proteins in eukaryotic organisms. Most of what is known about BIR domains come from their role in IAP proteins. IAPs bind to and inhibit caspases, a class of cysteine proteases involved in propagating ►apoptosis signals within the cell.

BIRADS

Definition

Acronym for ►Breast Imaging Reporting and Data System.

Birbeck Granule

Definition

Is a Langerhans' cell specific rod-shaped or tennis racquet-shaped organelle detected by electron microscopy, whose function is unknown.

►Langerhans Cell Histiocytosis

BIRC5

►Survivin

Birt–Hogg–Dubé Syndrome

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Synonyms

BHD syndrome; Folliculin (FLCN); Fibrofolliculomas with trichodiscomas and acrochordons; Hornstein–Knickenberg syndrome

Definition

Birt–Hogg–Dubé syndrome is a rare, autosomal dominantly inherited genodermatosis characterized by multiple, benign cutaneous hair follicle tumors (fibrofolliculomas), trichodiscomas, and acrochordons (skin tags), lung cysts, spontaneous pneumothorax (lung wall collapse), colon polyps and colon carcinoma, lipomas, angiolipomas, parathyroid adenomas, parotid oncocytomas, and an increased risk for developing kidney

tumors such as oncocytomas, chromophobe, papillary, and clear renal cell carcinoma (RCC).

Characteristics

BHD syndrome was originally described in 1977 by Birt, Hogg, and Dubé as a rare form of inherited ►autosomal dominant syndrome in large kindred, wherein 15 of 37 members were older than 25 years of age. Originally, it was characterized as a triad of multiple, skin hamartomatous lesions (fibrofolliculomas, trichodiscomas, and acrochordon). The fibrofolliculomas and trichodiscomas appear as multiple, small, dome-shaped, smooth, 2–4 mm, yellowish or skin-colored papules, scattered over the forehead, face, neck, nose, chest, scalp, and upper trunk. The onset of skin lesions typically begins during the third or fourth decade of life. Skin lesions tend to increase in size and number with age. The acrochordons appear as small and soft skin tags (furrowed, 1–2 mm soft papules) composed of loose connective tissues. Histogenesis of skin lesions confirmed that trichodiscomas originated from the mesenchymal component of the pilar complex, acrochordons from epithelial components, and fibrofolliculomas from both epithelial and mesenchymal proliferation. Since its initial description, more than 60 families have been identified with BHD syndrome and a number of other features of BHD have been recognized, including an increased incidence of ►renal carcinoma, most commonly, chromophobe and hybrid oncocytic/chromophobe ►renal cell carcinomas (RCCs), lung cysts, pleural blebs, spontaneous pneumothorax, developing colonic adenomas and carcinoma, neurothekeomas, meningiomas, flecked chorioretinopathy, parathyroid adenomas, multiple lipomas, intraoral papules, parotid oncocytoma, and other cutaneous tumors such as, collagenomas, perivascular fibromas, angiofibromas, and ►melanomas.

Individuals with BHD syndrome were found to have sevenfold higher risk of developing kidney neoplasm, 50-fold higher risk of developing spontaneous pneumothorax, and 80-fold higher risk of developing pulmonary cysts over the general population. The first report of BHD syndrome with renal pathology when examined showed bilateral kidney tumors with a one clear RCC and one chromophobe RCC. Further, in a study of 13 patients with BHD syndrome, seven had renal neoplasms, including renal oncocytomas and papillary RCCs. BHD patients with renal ►neoplasia display multifocal, bilateral tumors of several histopathological variants including chromophobe RCC (34%), oncocytic hybrid (50%) with features of chromophobe RCC and renal oncocytoma, and less frequently, clear cell RCC (9%), renal oncocytoma (5%), and papillary RCC (2%). Oncocytoma and chromophobe RCC originate from the intercalated cells of renal collecting tubules and share overlapping histologic features. A study on 98 patients with BHD syndrome described the occurrence of both oncocytoma and chromophobe RCC with predominancy of chromophobe RCC in renal cancer, found in 7 of 14 histologically examined tumors. Chromophobe RCCs are slowly progressive, locally invasive, and average 7–9 cm in diameter but rarely metastasize. Mean age at diagnosis of kidney tumors is 50.7 years. Recent findings suggest that microscopic oncocytic lesions may be precursors of hybrid oncocytic tumors, chromophobe RCCs, and perhaps clear cell RCCs in patients with BHD syndrome. Strong associations between renal neoplasms and pulmonary cysts and spontaneous pneumothorax have been observed in BHD families. The lung cysts in BHD affected individual are mostly bilateral and multifocal and have a high risk of developing spontaneous pneumothorax. Pneumothorax likely occurs in younger individuals with BHD syndrome. Male gender and older age has been associated with increased risk of renal tumors, whereas the risk of spontaneous pneumothorax is inversely associated with age. Based on these clinical manifestations, penetrance of BHD syndrome is considered to be very high. Thus, the BHD syndrome conferred an increased risk for the development of renal tumors, spontaneous pneumothorax, and lung cysts.

Diagnostic Criteria

The following diagnostic features may be considered in a patient with BHD syndrome: the presence of 10–100 cream to flesh-colored, smooth, firm skin papules on the face, neck, or upper torso, with at least one histologically confirmed fibrofolliculoma with or without family history of BHD or a single renal tumor or history of spontaneous pneumothorax; a patient with multiple and bilateral chromophobe, oncocytic, and/or oncocytic hybrid renal tumors; single oncocytic, chromophobe, or oncocytic-hybrid tumor and a family history of renal cancer with any of above renal cell tumor types; and a family history of autosomal dominant primary spontaneous pneumothorax without a history of chronic obstructive pulmonary disease.

BHD Gene Mutations

The genetic defect responsible for BHD syndrome has been mapped to the pericentromeric region of chromosome 17p11.2 by linkage analysis and the gene in this region has been cloned and is believed to be responsible for the BHD syndrome. This region is interesting because of the presence of low copy number repeat elements, unstable, and associated with a number of diseases. Several heterozygous ►germline mutations have been identified in a novel gene, *BHD*, in BHD families. The human *BHD* gene encodes a tumor-suppressor protein, folliculin (*FLCN*), a cytoplasmic protein with an open reading frame of 579 amino acids,

64-kDa protein. Human *FLCN* consists of 14 exons. Folliculin contains a glutamic acid rich, coiled-coil domain with no significant homology to any known human protein. Folliculin homologs have been identified in many species, including *Drosophila*, *Caenorhabditis elegans*, mouse, dog, and rat, implying a critical biological role for folliculin. Although the function of the *BHD* gene is unknown, germline mutations in *FLCN*, with somatic mutations and loss of heterozygosity in tumor tissue, suggest that loss of function of the folliculin protein is the basis of tumor formation in BHD syndrome. Recently, it has been shown that *FLCN* binds with *FNIP1* (folliculin interacting protein 1) and may be involved in energy and/or nutrient sensing through AMPK and mTOR signaling pathways. Further, a recent study demonstrates that the *Drosophila* homolog of gene BHD regulates male germline stem cell maintenance and functions downstream of the JAK/STAT (janus kinease/signal transducer and activator of transcription) and Dpp (decapentaplegic) signal transduction pathways. This study suggests that the BHD may regulate tumor formation through modulating stem cells in human.

The germline mutations identified in BHD families so far are frameshift or nonsense mutations, predicted to truncate folliculin, including insertions or deletions (44%) of a hypermutable tract of eight cytosines (C8) in exon 11. Initially, the distinct germline mutations on exon 11 of the folliculin gene (c.1733insC and c.1733delC) in three of four families with BHD syndrome, was identified. Later, mutations along the entire length of the coding region of the folliculin gene has been identified, including 16 insertion/deletion, 3 nonsense, and 3 splice site mutations in 51 of 61 families with BHD syndrome. Interestingly, among patients with a mutation in the exon 11 hot spot, significantly fewer renal tumors were observed in patients with the C-deletion than those with the C-insertion mutation. Two unique features of renal tumors in patients with BHD syndrome are the variable expression of the phenotype among members of a given family who carry the same germline mutation, and between families who carry the "hot spot" mutation in exon 11. Mutational hot spot is also reported to be a target of mutation in ►microsatellite instability (MSI) sporadic colorectal cancer. Five of 32 (16%) sporadic colorectal cancers with MSI were found to have insertion/deletion mutations in the poly(C)8 tract of the *BHD* exon 11. In addition, mutations truncating folliculin have been described in patients with 4-bp deletions in *BHD* exon 4, dominantly inherited lung cysts and/or spontaneous pneumothorax without skin lesions or kidney tumors. Moreover, germline mutation in the rat and dog homologs of the *BHD* gene also resulted in inherited kidney tumors, suggesting that the *BHD* gene has a tumor suppressor function. Furthermore, recent evidence of somatic second "hit" mutations in renal tumors from BHD patients in which 53% showed a second somatic mutation and 17% showed loss of ►heterozygosity (LOH) of the wild type allele, strongly supports the Knudson "two-hit" tumor suppressor model for *BHD* and suggesting that BHD is a new ►tumor suppressor gene with roles in both human and animal carcinogenesis.

BHD mRNA is expressed in a wide variety of normal tissues including the differentiated epidermal layers of the skin, the outer and inner root sheath supporting structures of the hair follicle, lung, and kidney and also expressed in a variety of secretory cell types, including acinar cells of the parotid gland and pancreas, brain, lymphocytes and ductal cells of the breast and prostate. Tissues with reduced expression of folliculin mRNA included heart, muscle, and liver. Folliculin immunoreactivity also occurred in the nucleolus of normal cells and was associated with mitosis. In addition, folliculin mRNA was expressed strongly in fibrofolliculomas but loss of folliculin expression was seen in oncocytoma (3.3%), chromophobe RCC (60.7%), papillary RCC (36.4%), and clear cell RCC (21.1%). Abnormal accumulation in the cytoplasm was also observed in oncocytoma (76.7%), chromophobe RCC (3.6%), and clear cell RCC (14.7%). Thus, the protein may have important biological functions in a variety of tissues and organisms. Furthermore, the defective protein in BHD patients may affect the cell's cytoskeleton, disrupting the extracellular matrix and affecting the regulation of cellular proliferation.

Screening and Possible Treatment for BHD

BHD syndrome is inherited in an autosomal dominant manner. A child having a parent with mutation on *BHD* has a 50% chance of inheriting that mutation. No specific screening guidelines for BHD syndrome have been described. However, due to the risk of kidney cancer and other associated abnormalities, it has been suggested that individuals with BHD syndrome or a family history of BHD syndrome should have yearly ultrasounds of their kidneys from the age of 25 and abdominal computerized tomography (CT) scan or magnetic resonance imaging (MRI) every 2 years. Further, BHD syndrome can be identified by skin biopsies to confirm the fibrofolliculomas and X-rays to look for lung cysts and previous spontaneous pneumothorax. Individuals with BHD syndrome should avoid smoking because of increased risk of kidney cancer associated with smoking. No curative medical treatment is currently available for the cutaneous lesions associated with BHD syndrome. However, surgery and electrodesiccation have provided definitive treatment of solitary perifollicular fibromas and multiple lesions, respectively. Treatment of folliculoma/trichodiscoma shows substantial improvement after laser ablation but

can be reverted. Renal tumors can be treated with nephron sparing surgical approaches, depending on the size and location of the tumors. Individuals with spontaneous pneumothorax may avoid high ambient pressures, which can precipitate spontaneous pneumothorax. Consider colonoscopy for colonic polyps and colonic adenocarcinoma. Genetic testing for BHD syndrome is also available. Use of molecular genetic testing for early identification of at-risk family members before disease causing mutations are manifested, may improve diagnostic certainty and reduce costly screening procedures. Methods of using BHD encoding sequence also allow for a differential genetic diagnosis of spontaneous pneumothorax, or collapsed lung.

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Bispecific Antibodies

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Definition

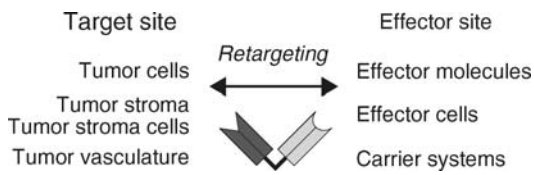
Bispecific antibodies are ►antibodies possessing antigen-binding sites with specificity for two different structures (dual specificity).

Characteristics

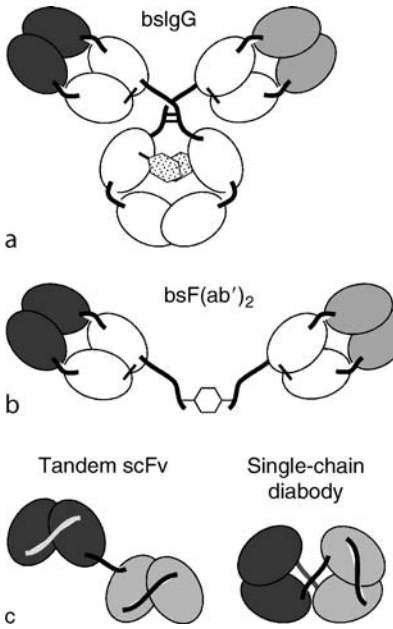
Bispecific antibodies are molecules able to simultaneously bind to two different ►epitopes on the same or different antigens. Bispecific antibodies act as mediators or adaptors bringing two different structures into close contact. In cancer therapy, possible applications include the retargeting of effector molecules (e.g. radionuclides, drugs, enzymes, ►cytokines), effector cells (e.g. cytotoxic T lymphocytes, ►natural killer cells), or carrier systems (e.g. drug-loaded liposomes, genetic vehicles) to tumor-associated target sites, such as tumor cells, tumor stroma cells and extracellular components as well as cells and structures associated with the tumor vasculature. Thus, potential applications of bispecific antibodies cover the areas of immunotherapy, ►chemotherapy, radiotherapy (►radioimmunotherapy) and ►gene therapy. Bispecific antibodies can lead to increased selectivity and improved efficacy of natural effector functions and are able to expand therapeutic effects to those not exerted by normal immunoglobulins used in the clinic (e.g. IgG molecules) (Fig. 1).

Generation of Bispecific Antibodies

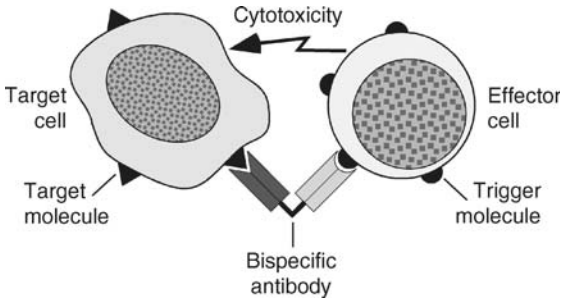
Bispecific antibodies are not found in nature and hence have to be generated in vitro. Various methods have been established including somatic hybridization of two antigen-producing cells, chemical cross-linking of two Fab′ fragments derived from different antibodies, and genetic approaches leading to recombinant antibody molecules. Somatic hybridization, e.g. of two ►hybridoma cells, leads to hybrid-hybridomas or quadromas. These quadromas produce light and heavy chains of both antibodies within one cell, which assemble into bispecific antibodies. However, also nonfunctional or monospecific antibodies are produced due to random association of light and heavy chains. Thus, this approach results in a heterogeneous population of antibodies. Alternatively, Fab′ fragments produced by proteolytic cleavage of two antibody molecules with different specificity can be chemically conjugated to form bispecific $F(ab')_2$ fragments. More recent approaches utilize genetic engineering to combine two different antigen-binding sites within one molecule. A large variety of different formats have been developed. Currently, the most widely used formats are



Bispecific Antibodies. Figure 1 Possible applications of bispecific antibodies in cancer therapy. Bispecific antibodies can act as mediators to retarget effector molecules, effector cells or carrier systems to tumor-associated target sites.



Bispecific Antibodies. Figure 2 Various forms of bispecific antibodies. Bispecific antibodies can be generated by (a) somatic hybridization of two antibody-producing cells, (b) by chemical cross-linking of two Fab′ fragments, or (c) by genetically combining two different antigen-binding sites, e.g. as tandem scFv or as single-chain diabody format.



Bispecific Antibodies. Figure 3 Bispecific antibodies for the retargeting of effector cells. Bispecific antibodies are able to retarget effector cells such as cytotoxic T lymphocytes or natural killer cells to target cells by simultaneous binding to a target molecule on the target cell and a trigger molecule on the effector cell leading to killing of the target cell.

Bispecific Antibodies. Table 1 Effector cells and trigger molecules

Effector cell	Main trigger molecule	Costimulus	Activating cytokine
Cytotoxic T lymphocytes (CTL)	TCR/CD3	B7, anti-CD28	IL-2
Natural killer cells (NK)	CD16	–	IL-2
Macrophages	CD64, CD16, CD89	–	GM-CSF, IFN-γ
Neutrophils	CD89, CD64	–	G-CSF, GM-CSF, IFN-γ

tandem scFv molecules linking two ►single-chain Fv fragments (scFv) by a flexible linker and diabodies or single-chain diabodies with a more rigid structure. Compared to whole antibodies or $F(ab')_2$ fragments these recombinant formats are much smaller with a molecular weight of 50–60 kDa (Fig. 2).

Effector Cell Retargeting

Preclinical and clinical developments of bispecific antibodies for cancer therapy have a strong focus on the retargeting of effector cells of the immune system to tumor cells. Suitable effector cells of the immune system include cytotoxic T lymphocytes (CTL); Natural killer cells (NK), macrophages and neutrophils, which can efficiently kill target cells by antibody-independent or ►antibody-dependent cellular cytotoxicity (ADCC). Retargeting of these effector cells to target cells requires binding of the bispecific antibody to one or more trigger molecules on the effector cell (Fig. 3).

Cytotoxic T cells are among the most potent effector cells of the immune system. Trigger molecules on CTLs are molecules associated with the T cell receptor (TCR) such as CD3. Bispecific antibodies thus bypass normal ►MHC-mediated T cell activation. This is of special interest since many tumor cells escape from a T cell response by down-regulation or loss of MHC expression during tumorigenesis. T cell activation, however, depends on a costimulatory signal, e.g. through binding of B7 to CD28 on CTLs. In a therapeutic setting this costimulatory signal can be provided by anti-CD28 monoclonal antibodies or by bispecific or bifunctional antibodies (Table 1). Interestingly, some anti-CD3 antibodies are able to activate T cells without the need for costimulation. One recombinant bispecific antibody (MT103) directed against CD19 and CD3 is based on such a costimulation independent anti-CD3 antibody. This antibody is currently in a phase I trial for the treatment of non-Hodgkin lymphoma (NHL).

Fc receptors are the trigger molecules employed for retargeting of NK cells, macrophages, and neutrophils. Fcγ receptor III (CD16) represents the main trigger

molecule on NK cells, while Fcγ receptor I (CD64) or the Fcα receptor (CD89) have been utilized for retargeting of macrophages and neutrophils, respectively. The expression of these trigger molecules on effector cells can be increased by activating cytokines such as interleukin-2 (IL-2), granulocyte/macrophage-colony stimulating factor (GM-CSF), or ►interferon-γ (INF-γ) (Table 1).

Clinical Experience with Bispecific Antibodies

Various bispecific antibodies have entered clinical trials. However, as to yet none has been approved for therapeutic applications. Initial problems were associated with the use of whole bispecific immunoglobulins derived from murine hybridomas, especially Fc-mediated toxicity due to the release of inflammatory cytokines (cytokine storm) and a neutralizing immune response against the murine antibodies (human–anti-mouse antibodies, HAMA). Further studies using bispecific $F(ab')_2$ fragments in combination with activating cytokines such as GM-CSF could demonstrate some biological effects, however, clinical responses remained vague. Current research focuses on the development of novel antibody formats including costimulation-independent bispecific tandem scFv molecules for the retargeting of CTLs, the retargeting and activation of CTLs as well as Fcγ receptor expressing effector cells by combination therapy with two bispecific antibody molecules or trispecific antibodies, but also on bispecific antibody molecules with improved pharmacokinetic properties.

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Bisphosphoglycerate

Definition

BPG is a molecule able to inversely modulate the affinity of hemoglobin for oxygen.

►Polycythemia

Bisphosphonates

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Definition

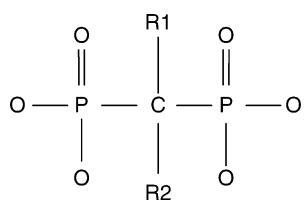
Bisphosphonates are potent inhibitors of ►osteoclast mediated ►bone resorption. These compounds are stable analogues of the inorganic pyrophosphate (PPi), which is an endogenous regulator of bone mineralization.

Characteristics

Bisphosphonates were developed in the nineteenth century for industrial use, in particular as "water softeners." The first clinical use of bisphosphonates in humans was in the 1960s for the treatment of ►Paget disease, a focal disorder of bone remodeling due to abnormally increased osteoclast-mediated bone resorption. So far, bisphosphonates have been successfully studied in several clinical disorders characterized by an alteration in bone resorption, such as metastatic and osteolytic bone diseases, hypercalcemia of malignancy and ►osteoporosis.

All bisphosphonates share a common structure which consists of two phosphate groups attached to a single carbon atom (P-C-P) (See Fig. 1).

The P-C-P group is responsible for the affinity of these drugs for the bone, since it is essential for binding to ►hydroxyapatite or hydroxylapatite. The substitution in the R1 and R2 side chains give rise to a variety of compounds with different potency and biological effects. For instance, the presence of a ►hydroxyl group in the R1 side chain confers a higher affinity for the bone mineral. The R2 side chains directly influence the potency of bisphosphonates for inhibiting osteoclast-mediated bone resorption. In particular, the bisphosphonates containing a basic primary nitrogen atom in an ►alkyl chain (such as pamidronate and alendronate) are 10–100 times more potent than non



Bisphosphonates. Figure 1 Generic structure of bisphosphonates.

nitrogen bisphosphonates. Indeed, the higher antiresorptive potency is obtained when the R2 side chain contains a nitrogen atom within a heterocyclic ring (as in risendronate and zoledronate).

Thus, according to the chemical structure of the R2 side chain, bisphosphonates are generally classified as follows:

- Non nitrogen containing bisphosphonates:
  - Etidronate (Didronel®)
  - Clodronate (Bonefos®, Loron®)
  - Tiludronate (Skelid®)
- Nitrogen containing bisphosphonates
  - Pamidronate (Aredia®)
  - Neridronate
  - Olpandronate
  - Alendronate (Fosamax®)
  - Ibandronate (Bondronat®)
  - Risendronate (Actonel®)
  - Zoledronate (Zometa®)

Mechanism of Action

Bisphosphonates bind to the bone mineral in particular at sites of active bone metabolism, where they achieve therapeutic concentration. During the process of bone resorption, since the acid environment osteoclasts causes dissolution of the hydoxyapatite bone mineral, bisphosphonates are released in this sub-cellular space, and are internalized by osteoclasts. At this point, osteoclasts lose the ruffled border and show cytoskeleton alterations, and eventually become apoptotic.

The toxic effect of bisphosphonates on osteoclasts can be explained in at least two different ways, according to their chemical structure.

First-generation, non nitrogen containing bisphosphonates, such as clodronate and etidronate, are metabolized by osteoclasts to nonhydrolyzable adenosine triphosphate (ATP) analogues, with subsequent inhibition of ATP-dependent intracellular enzymes. The intracellular accumulation of these metabolites inhibits osteoclast function and can induce apoptosis. On the other hand, aminobisphosphonates, following internalization in the osteoclasts, inhibit the farnesyl diphosphonate (FPP) synthase, affecting the biosynthetic mevalonate pathway. This pathway is involved in the production of sterols such as cholesterol, and isoprenoid lipids such as isopentenyldiphosphate, farnesyldiphosphate (FPP) and geranylgeranyldiphosphate (GGPP). FPP and GGPP are essential for the post-translational modification of small ►GTPases (Ras, Rab, Rho and Rac). These signaling proteins are involved in the regulation of cell proliferation, cytoskeletal organization, membrane ruffling, intracellular vesicle transport, and apoptosis. In addition, aminobisphosphonates can also induce the formation of intracellular ATP analogues which may directly induce osteoclast apoptosis.

More recently, a growing number of preclinical data have consistently demonstrated the direct antitumor effect of bisphosphonates. The mechanisms responsible for this effect are not still fully elucidated. However, it has been shown that bisphosphonates can inhibit angiogenesis, cell proliferation and adhesion. Moreover, the effects of bisphosphonates on osteoclasts result in an inhibited release of growth factors in the bone microenvironment, thus rendering the bone less hospitable to cancer cell homing.

Pharmacokinetic

Bisphosphonates are characterized by very low absorption from the gastrointestinal tract (less than 6% for clodronate and etidronate). The plasma half-life ranges between 20 min and 2–3 h, depending on the type of bisphosphonates and the individual rate of clearance. However, because of the high affinity for the bone matrix, half-life in bone is very long, ranging from months to years.

Clinical Use

Hypercalcemia of Malignancy (HCM)

HCM is a severe clinical condition that can occur in up to 20% of patients with advanced cancer, in the presence or absence of bone metastases. HCM is a consequence of osteoclast activation due to the presence of cancer cells in the bone (metastatic bone disease), or the production by cancer cells of parathyroid hormone-related protein (humoral hypercalcemia). Tumors more frequently inducing episodes of HCM include non Hodgkin lymphomas, myeloma, lung cancer, breast cancer, ►renal cancer and ►prostate cancer.

HCM occurs when total serum calcium is above 10.2 mg/dl (2.55 mmol/l), and causes a variety of symptoms including gastrointestinal manifestations (anorexia, vomiting, constipation), renal function deterioration, alteration of cardiac rhythm (EKG abnormalities and arrhythmias), and neurological disorders (from asthenia to lethargy and coma).

Treatment of HCM includes iv hydration and diuretics to facilitate renal calcium excretion, and bisphosphonates to inhibit calcium resorption from the bone. A single bisphosphonate iv infusion can obtain a sustained serum calcium normalization in about 80% of the patients.

Treatment of Bone Metastases

Bone metastases represent a major health problem and can occur in a significant proportion of patients with solid tumors. Bone metastases are frequent (up to 70–80% of the patients) in common tumor types such as prostate cancer, breast cancer and lung cancer. Bone metastases develop when circulating tumor cells home in the bone marrow and stimulate the activation of osteoclasts that eventually initiate bone matrix resorption. Bone metastases can be lytic, sclerotic, or mixed,

depending on the balance between bone resorption induced by osteoclasts and new bone formation by ►osteoblasts.

Metastatic bone disease causes considerable morbidity, leading to several complications including pain, pathologic fractures, spinal cord compression, ineffective hematopoiesis, and HCM. In addition to the specific anticancer therapy (e.g. chemotherapy, hormonal therapy, or biologic agents), the current options to treat bone metastases are radiation therapy, orthopedic surgery, radiopharmaceuticals and bisphosphonates. Currently, bisphosphonates are considered the mainstay of the treatment for metastatic bone disease from myeloma, breast cancer, prostate cancer and other solid tumors including lung and renal cancer.

In clinical trials, the efficacy of bisphosphonates has been measured on the basis of their capacity to reduce or delay the skeletal related events (SRE). An SRE is defined as the occurrence of pathologic fractures, radiation therapy for bone pain or to treat/prevent a fracture, surgery to stabilize bone fractures, hypercalcemia of malignancy, or spinal-cord compression.

The following sections will briefly summarize the clinical experience with different bisphosphonates according to tumor types.

Multiple Myeloma

►Multiple myeloma (MM) is associated with relevant skeleton morbidity, since lytic lesions are present in more than 90% of the patients. The lytic process in MM is different from bone metastases from other cancers, where bone destruction is generally followed by new bone formation. Several bisphosphonates including clodronate, pamidronate and ►zoledronic acid, are effective in preventing or delaying skeletal complications. Oral clodronate have shown a significant reduction in non-vertebral and vertebral fracture rates over a placebo. Again compared to placebo, intravenous pamidronate significantly reduce the proportion of patients with any SRE. It was also associated with significant decrease of bone pain. More recently, zoledronic acid was shown not only to be as effective as iv pamidronate, but to also produce an additional 16% risk reduction of skeletal complication as measured by multiple event analysis.

Bone Metastases from Breast Cancer

Several bisphosphonates have been approved in the United States and Europe for the treatment of skeletal metastases from breast cancer.

The efficacy of pamidronate has been known since the early 1990s. In two pivotal, phase III randomized trials, pamidronate significantly reduced the incidence and delayed the onset of SREs as compared to placebo. It was also effective in the reduction of pain scores.

The more potent bisphosphonate zoledronic acid has been directly compared to pamidronate. The pivotal trial, including breast cancer and multiple myeloma patients, was designed as a non inferiority trial, the primary end point being the percentage of patients with at least 1 SRE at 25 months. Zoledronic acid was at least as effective as pamidronate according to the primary end point. Furthermore, the multiple events analysis demonstrated that zoledronic acid was significantly more effective in reducing the risk of SREs in the subset of breast cancer patients.

Zoledronic acid was also compared to placebo in a trial conducted in Japan, where pamidronate is not approved for the treatment of bone metastases from breast cancer, showing a clear superiority in reducing the SRE rate ratio, the percentage of patients with at least 1 SRE, and in delaying the time to first SRE. The multiple event analysis showed a 44% reduction in the risk of developing an SRE, and significantly reduced mean pain scores from baseline over 12 months.

Ibandronate is a single-nitrogen bisphosphonate available in both intravenous and oral formulations. In terms of reduction of SREs and pain control, the efficacy of iv and oral ibandronate has been confirmed in three placebo-controlled phase III randomized trials. Ibandronate is currently approved in more than 40 non-US countries for the treatment of patients with breast cancer and bone metastases. The approval of the US Food and Drug Administration is still pending. A direct comparison between ibandronate and zoledronic acid is ongoing. Recently, in a review of 21 randomized controlled trials of bisphosphonates in breast cancer by the Cochrane Collaboration, it has been shown that zoledronic acid reduces the risk of SRE by 41%, compared with 33% by pamidronate, 18% by iv ibandronate, 14% by oral ibandronate, and 16% by oral clodronate.

Bone Metastases from Prostate Cancer

Bisphosphonates have also been studied in patients with prostate cancer and bone metastases. In patients with symptomatic bone disease, pamidronate failed to show any advantage over placebo in pain scores, analgesic use, and SREs. On the contrary, as compared to placebo, zoledronic acid significantly reduced the proportion of patients with an SRE over 2 years and delayed the time to first SRE by approximately 6 months. Therefore, zoledronic acid was approved for the treatment of patients with prostate cancer metastatic to bone and progression of disease despite first line hormonal therapy.

More recently, ibandronate has shown some palliative benefit in a small open-label study, but its efficacy over placebo in a randomized trial is still to be demonstrated.

Bone Metastases from Lung Cancer and Other Solid Tumors

The first bisphosphonate with proven efficacy in the treatment of bone metastases from lung cancer and solid tumors is zoledronic acid. In a placebo-controlled randomized trial in more than 700 patients, zoledronic acid significantly reduced the proportion of patients experiencing at least one SRE, and delayed the median time to first SRE as well. Currently, zoledronic acid is the only bisphosphonate approved for the treatment of metastatic bone disease from solid tumors other than breast cancer.

Prevention of Bone Metastases

Because of their mechanism of action, bisphosphonates have the potential to prevent cancer cells homing in the bone, thus changing the entire process of metastatic spread. The data from the earlier, weaker generation of bisphosphonates are conflicting, and to date bisphosphonates are not recommended for the prevention of bone metastases. However, ongoing studies with the newer, more potent intravenous bisphosphonates will establish the role of these compounds in the prevention of bone metastases in several tumor types such as breast, prostate and lung cancer.

Osteoporosis

In both men and women, bone mass decreases with age. In men, this process is constant over time while women usually experience a significant increase in the rate of bone loss after menopause. The most standardized method to evaluate the bone mineral density (BMD) is the dual energy X-ray absorptiometry (DXA) at the recommended site of the proximal femur. Patients are classified as osteoporotic when the BMD (as expressed as T-score) is 2.5 standard deviation (SD) or more below the average value for premenopausal women. When the T-score is between −1 and −2.5 SD patients are classified as osteopenic. For each SD reduction in BMD there is a doubling in the risk of fracture.

Bisphosphonates have been successfully used to treat osteoporosis. In particular, alendronate and etidronate can increase the BMD and almost halve the fracture rates in postmenopausal women, representing the most frequent agents used worldwide in this setting.

Besides the risk of bone metastatization, the bone health of cancer patients can be further affected by cancer therapies. This particular condition, known as cancer-treatment-induced bone loss (CTIBL), reflects the effects of cancer therapy (both chemotherapy and endocrine therapy) on bone mineralization. In brief, all cancer therapies that directly or indirectly antagonize the effect of estrogen or androgen significantly enhance the loss in bone mineral density, thus dramatically increasing the risk of fractures. Because of the higher severity of CTIBL, the common strategies to treat benign osteoporosis, such as oral bisphosphonates, calcium/vitamin D supplements, and calcitonin, might not be sufficient. In early breast cancer, both daily oral clodronate and intermittent oral risendronate have shown superiority over placebo, although clodronate was unable to completely prevent bone loss in patients with chemotherapy-induced ovarian dysfunction. The use of more potent intravenous bisphosphonates is under investigation in three large trials. In prostate cancer patients receiving androgen deprivation therapy, zoledronic acid was able to reverse CTIBL, and even increase bone density at multiple sites. Several trials in prostate cancer are under way to confirm these results. With these potent agents, a less frequent schedule (every 3 or 6 months) seems to be effective, while the monthly dose is used for metastatic bone disease.

Side Effects

The safety profile of bisphosphonates varies depending on the route of administration. Treatment with intravenous bisphosphonates is usually well tolerated, with transient side effects such as mild to moderate flu-like symptoms following initial infusions, generally self-limited. However, iv bisphosphonates have the potential to adversely affect renal function, and sporadic episodes of both acute and chronic renal failure have been described. The risk of renal failure is directly related to dose and to the drug infusion time: when bisphosphonates are administered at the recommended doses and infusion rates, the incidence of elevated serum creatinine is generally low (<10%), and severe adverse renal events are rare. Nevertheless, accurate renal-function monitoring is recommended in the use of iv pamidronate and zoledronic acid. In breast cancer patients, iv ibandronate has shown a renal safety profile similar to a placebo, and because no case of renal failure has been described at the time of writing this, the monitoring of serum creatinine prior to each ibandronate administration is not mandatory.

Oral administration of bisphosphonates can cause esophagitis and other gastrointestinal side effects such as mucositis, nausea, vomiting, and diarrhea.

In the past few years, a growing number of cases of ►jaw osteonecrosis have been associated with the use of aminobisphosphonates, prompting labeling changes for pamidonate and zoledronic acid. Several reports have described a frequency of jaw osteonecrosis ranging from 0.6 to 4.3% for patients with breast cancer and from 3 to 9.9% for those with multiple myeloma. The exact mechanism underlying jaw osteonecrosis has not yet been fully elucidated. Dental disease, dental surgery, periodontal disease, trauma and poor oral hygiene are the most often reported precipitating factors. Several reports

have also identified a relationship between dose and duration of treatment and the development of this complication. Because jaw osteonecrosis is not reversible in the majority of the cases, physicians should focus on the prevention of this complication. It is therefore recommended to assess the dental status of patients before starting bisphosphonates, and avoid invasive dental procedures while on bisphosphonate therapy.

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BL

Definition

Burkitt lymphoma.

►Childhood Cancer

Bladder Cancer

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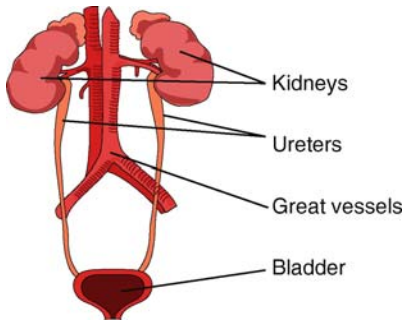
Definition

Bladder cancer is a malignant neoplasm which arises from the epithelial lining of the bladder (Fig. 1). Several histological forms have been identified. Cancers with urothelial histology (UC) comprises more than 90% of the neoplasms, while ►squamous cell carcinoma (SCC) and adenocarcinoma account for 5% and 2%, respectively. In areas with endemic schistosomiasis, SCC is the predominant histological form. It is also not uncommon for urothelial cell malignancies to have minor elements of adenomatous or squamous cell histology. However, from the clinical management standpoint, urinary neoplasms with minor components of these two histologic types are treated for their primary component. The clinical relevance of these minor components or the percentage at which a minor component becomes significant is unclear. An important prognostic criteria in urothelial cell carcinoma is tumor grade. Tumor grading most commonly follows World Health Organization (WHO) guidelines in which malignant tumors are classified as papillary urothelial neoplasm of low malignant potential (PUNLMP), low grade or high grade, regardless of invasion status.



Bladder Cancer. Figure 1 Anatomy of the urinary tract.

Characteristics

Clinical Epidemiology and Risk Factors

Carcinoma of the urinary bladder is the second most common urologic malignancy. The worldwide incidence of bladder cancer is approximately 200,000 patients per year with 120,000 annual deaths, accounting for 3.2% of all malignancies. It affects more males than females by a 3:1 ratio. In the United States, the incidence is higher in Whites than Blacks, although survival is longer in Whites and men than in Blacks and women. The disease can affect all ages (even children) but the median age at presentation is 70 years. It is rarely found as an incidental finding at autopsy suggesting that these cancers do not have a long latent or subclinical course. Bladder cancer incidence has increased 50% between 1985 and 2005; however the mortality rate has decreased by 33% in the past four decades.

Environmental risk factors for ►urothelial cell carcinoma include cigarette smoking, aniline dyes, pelvic radiation, benzidine, 2-naphthylamine, and other aromatic amines. Acrolein, a metabolic product of cyclophosphamide, can increase the risk of bladder cancer ninefold. Smoking increases the risk of bladder cancer fourfold, and at least one quarter of cases

can be attributed to smoking. Chronic cystitis and long-term bladder catheters increase the risk of squamous cell carcinoma. *Schistosoma haematobium* infection not only increases the risk of SCC significantly, but also increases the risk of urothelial cell carcinoma. Epidemiologic evidence does not exist for a hereditary etiology for bladder cancer.

Tumor Biology and Genetics

Urothelial cell carcinoma is a field change disease rendering the entire urothelium susceptible to malignant transformation. Polychronotopicity refers to the propensity of tumors to arise at different times and sites in the urothelium. Both the ►TP53 and ►RAS genes are known targets of chemical carcinogens. The most frequent genetic alterations in urothelial cell carcinoma are monosomies of chromosome 9 (57%), and losses on chromosome arms 11p (32%), 17p (32%), 8p (23%), 4p (22%), and 13q (15%). Deletions specifically associated with higher grades and stages of cancer, indicative of tumor progression to muscle invasive disease, have been identified at 3p, 4q, 8p, 10, 15, 17p, 18q among many others. Other studies utilizing immunohistochemical techniques have suggested that overexpression of p21Ras protein, mutated TP53, and the epidermal growth factor receptor (EGFR) in bladder tumors are related to bladder tumor progression. In addition, loss of RB1, DCC, and ►E-cadherin (CDH1) expression has also been related to this transition. Tumors with p53 mutations tend to exhibit more aggressive behavior when present in both noninvasive and invasive disease, while chromosome 9 alterations and ►fibroblast growth factor receptor 3 (FGFR3) mutations are associated with low grade noninvasive disease. In fact, FGFR3 mutations are found in up to 90% of non-muscle invasive cancers while only found in 10% of muscle invasive or metastatic cancers.

Characteristics of Nonurothelial Cell Carcinomas

SCC comprises only 1–3% of bladder tumors in the U.S. and Britain but represents 75% of tumors in Egypt. Most of the SCCs found in Egypt are due to *S. haematobium* ("bilharzial" bladder cancer) and are well differentiated with lower risk of metastases than urothelial cell carcinoma. Non-bilharzial squamous cell tumors are caused by chronic inflammation from infection, stones, indwelling catheters, or bladder diverticuli. Although these tumors' prognosis is similar to urothelial cell carcinoma by stage, non-bilharzial tumors tend to present with late-stage disease. Primary bladder adenocarcinomas represent approximately two percent of bladder tumors and are more common in extrophic bladders, urachi and intestinal conduits or augmentations. They may produce mucin and can be associated with cystitis glandularis. Most are poorly differentiated and present with advanced disease.

Clinical Presentation

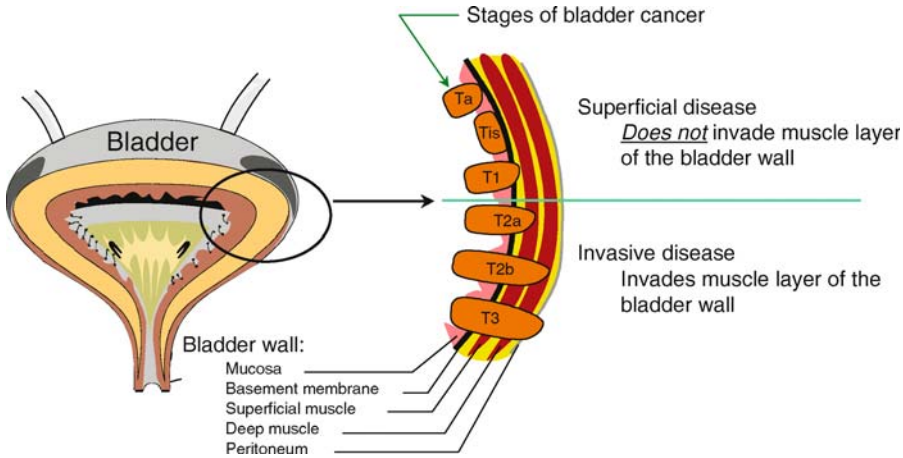
Bladder cancer frequently presents with painless hematuria, although urinary frequency, urgency, and dysuria can occur as well. Gross hematuria is common, and bladder cancer is rarely diagnosed in the absence of at least microscopic hematuria although this can be intermittent. Bladder cancer can also present with flank pain and hydronephrosis if the tumor obstructs the ureteral orifice.

Diagnosis and Staging

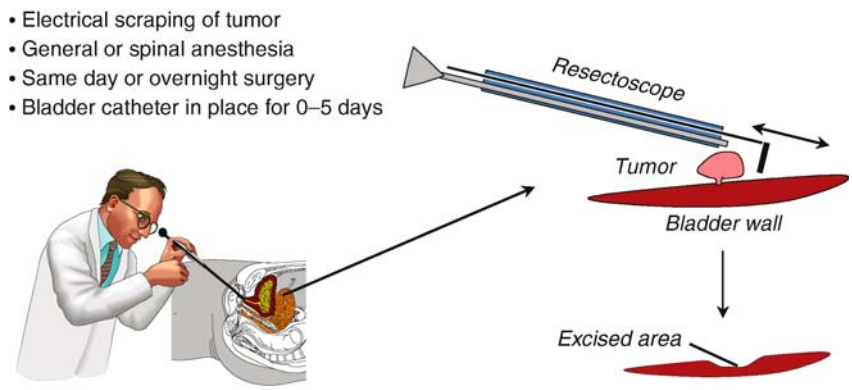
The diagnostic evaluation of bladder cancer begins with a history and physical examination including bimanual pelvic exam, urinalysis, cytology, and cystoscopy. In the past, intravenous urography (IVU) was indicated in all patients with bladder tumors to evaluate the upper urinary tracts. Retrograde ureteropyelograms (IVP) can also be performed at the time of cystoscopy if IVU does not provide an adequate view of the upper tracts. Currently, IVP has been replaced by CT scanning of the abdomen and pelvis with 3-D reconstructions allowing for both the assessments of extravesical spread (contiguous and metastatic) and urography (CT urogram). This is discussed in more detail later. Cytologic examination of bladder cells that slough off into urine is useful in the diagnosis of carcinoma in situ (CIS) or high grade tumors, but low grade tumors are more difficult to detect by cytology. Cytology is primarily used in the diagnosis and follow up of patients at risk for recurrent disease. Novel ►biomarkers such as the nuclear matrix protein (NMP-22) assay, ►survivin, BLCA4, and FISH analysis (UroVysion) offer promise for enhanced detection of symptomatic patients, screening of high risk populations, and treatment follow up and monitoring.

At presentation, 85% of patients with urothelial cell carcinoma of the bladder have disease limited to the organ, while 10% have regional disease and 5% have metastatic disease. Of the 85% with localized disease, 80% have non-muscle invasive disease (stages Tis/CIS, Ta, T1) and 20% have muscle invasive disease (stages T2–T4) (Fig. 2). These stages are according to the UICC/AJCC system, the most common staging system in use today. Urothelial cell carcinoma may grow in papillary, sessile, nodular, or flat (Tis) forms. Papillary tumors with orderly cellular arrangement and minimal nuclear atypia are designated PUNLMP. Such tumors rarely progress to invasive disease and are considered benign. Tis/CIS is sessile, poorly differentiated urothelial cell carcinoma involving only the urothelium. Although CIS can cause irritative voiding, it is often asymptomatic. Cystoscopy may be normal or exhibit erythematous patches, and urine cytology is 80–90% sensitive.

Transurethral resection (TUR) of bladder tumors not only provides tissue for pathologic diagnosis, but can



Bladder Cancer. Figure 2 Stages of bladder cancer.



Bladder Cancer. Figure 3 The trans urethral resection (TUR).

represent definitive therapy in the most clinical stage Ta and T1 tumors as long as the whole tumor is resected (Fig. 3). After the intraluminal portions of the tumor are resected, the tumor base is frequently resected as a separate pathologic specimen to ensure complete resection and accurate staging. T1 tumors undergo a second resection 3–6 weeks later to reduce the risk of understaging. It is critical that muscularis propria is included in the specimen to exclude the presence of muscle invasion by the tumor. Routine performance of random biopsies of the bladder or prosatic fossa mucosa remain controversial. However, these may be indicated to evaluate for CIS in patients with positive cytology or in patients that are candidates for orthotopic neobladder or partial cystectomy. If the tumor appears invasive (i.e., sessile, solid configuration), the resection is tailored so as to accurately determine clinical stage and to optimize subsequent definitive therapy. For example, if the patient is likely to choose radical cystectomy as the treatment of choice then complete TUR is not necessary. Conversely, if the patient is likely to select bladder sparing therapy with radiation and chemotherapy, then resection of as much tumor as safely possible should be carried out.

As alluded to earlier, the staging of bladder cancer is based primarily on the specimen generated by the TUR and is classified according to the 1997 UICC/AJCC system (Table 1) and revised by the World Health Organization/International Society of Urological Pathology WHO/ISUP Consensus Classification (Table 2). In 1998, the WHO/ISUP Consensus Classification of urothelial neoplasms of the urinary bladder was developed to unify the numerous diverse grading schemes for noninvasive bladder cancer and provide detailed histological criteria for papillary urothelial lesions. In addition, the new classification system allows for designation of a lesion papillary urothelial neoplasm of low malignant potential, which biologically has a very low risk of progression, but is not entirely benign. Therefore an intermediate classification enables these patients to avoid the label of having cancer with its psychosocial and financial implications and prevent them

Bladder Cancer. Table 1 UICC/AJCC consensus classification

UICC/AJCC 1997	Description
Ta[1]	Papillary tumor, epithelium confined
Tis	Carcinoma in situ: "flat tumor"
T1	Lamina propria invasion
T2a	Tumor invades non-muscle invasive muscle (inner half)
T2b	Tumor invades deep muscle (outer half)
T3a	Tumor invades perivesical fat microscopically
T3b	Tumor invades perivesical fat macroscopically (extravesical mass)
T4a	Tumor invades prostate or uterus or vagina
T4b	Tumor invades pelvic wall or abdominal wall
N1[2]	Single node <2 cm
N2	Single node >2–5 cm or multiple nodes <5 cm
N3	Any node >5 cm
M1	Nodal meets above bifurcation of common iliac vessels
M1	Metastasis

[1]Suffix "*m*" after T stage to indicate multiple tumors.
[2]Nodes of "true pelvis" (i.e., below bifurcation of common iliac vessels).

Bladder Cancer. Table 2 WHO/ISUP Consensus Classification

World Health Organization/International Society of Urological Pathology Classification
Hyperplasia
Flat hyperplasia
Papillary hyperplasia
Flat lesions with atypia
Reactive (inflammatory) atypia
Dysplasia
Carcinoma in situ (CIS)
Papillary neoplasms
Papilloma
Papillary neoplasm of low malignant potential (PUNLMP)
Papillary carcinoma, low grade
Apillary carcinoma, high grade

from being diagnosed as having a benign lesion, whereby they might not be followed as closely.

The pathologic exam of bladder specimens may be complicated by difficulty in differentiating muscularis propria from the more non-muscle invasive and thin muscularis mucosa, the latter which does not represent "true muscle" invasion and thus direct communication between urologist and pathologist is essential. If pathologic examination reveals tumor invasion into muscularis propria, CT or MRI of the abdomen pelvis is used to evaluate for gross extravesical spread, lymphadenopathy, or hepatic metastases. In general, these methods fail to detect lymph node spread in as many as 30% of patients. A radionucleotide bone scan can be obtained to evaluate for bony metastases, but the yield of a bone scan in the face of a normal alkaline phosphatase is low. Chest X-ray or CT scan is obtained to rule out pulmonary metastases.

Management of Noninvasive Disease

The therapy of noninvasive (Ta/T1) bladder cancer consists of TUR and fulguration. Because approximately 30% of these tumors tend to recur and 10% may progress to muscle invasion, follow up cystoscopy at regular intervals is mandatory. Tumors that invade the lamina propria (T1) should be considered potentially more aggressive, particularly if high grade. Argon and Nd:Yag lasers have also been used successfully for ablation of noninvasive bladder tumors especially those that are multifocal or difficult to access via the resectoscope used for TUR. The disadvantage of these techniques is the lack of tumor specimen to be analyzed by pathology and thus only lesions with a high likelihood of being noninvasive should be treated in this way.

Patients with recurrent, high grade Ta, T1 tumors or CIS may benefit from intravesical therapy with ►Bacillus Calmette–Guerin (BCG) or ►mitomycin C. These treatments can be given in two clinical contexts. They can be given for the treatment of residual disease which could not to be removed at TUR. Alternatively, they can be used to reduce the incidence of recurrence and progression in patients that have completely resected tumors. Furthermore, Mitomycin C delivered perioperatively in one dose has successfully reduced the incidence of tumor recurrence following TUR. BCG

is a live attenuated strain of *Mycobacterium bovis*, which stimulates a local and possibly systemic immune response. BCG can often delay recurrence and progression of high grade noninvasive disease and CIS. Side effects of BCG include bladder irritability, granulomatous prostatitis, systemic disseminated infection requiring antitubercular agents, and rarely death. Contraindications for intravesical delivery include: active tuberculosis, immunosuppression, traumatic catheterization, gross hematuria, and prior severe reaction to BCG. Mitomycin C is an alkylating chemotherapeutic agent that inhibits DNA synthesis. BCG is superior to Mitomycin C in reducing the risk of progression in high grade tumors in some but not all studies. BCG can also be used for maintenance therapy where it has been shown to reduce recurrence even further. Other intravesical compounds include interferon, keyhole-limpet hemocyanin, bropirimine, mycobacterial cell wall DNA extract, doxorubicin and its derivatives, thiotepa, and ►gemcitabine. The effectiveness of these agents compared with the two mentioned earlier in delaying progression and recurrence in initially treated patients is generally less and thus their use has been reserved for the salvage setting. Similarly, the addition of interferon gamma to BCG has been used to treat patients who have recurred after initial BCG therapy. Among patients with Ta, T1, or CIS, radical cystectomy is reserved for diffuse, symptomatic, recurrent high grade or unresectable papillary tumors unresponsive to intravesical therapy.

Recurrence polychronotropism (multiple reoccurrences in space and time) in noninvasive bladder tumors is uniquely elevated when compared to other organ sites. 20–70% of patients suffer disease recurrence. While in the absence of progression recurrence per se is not life threatening, this phenomenon nonetheless constitutes a cause of significant morbidity and treatment expense. While less common, the progression of noninvasive tumors to muscle invasion is associated with a marked decrease in 5-year disease-specific survival. Progression risks vary widely by stage and grade, ranging from less than 5% for low grade papillary tumors and greater than 50% for T1 lesions with associated CIS.

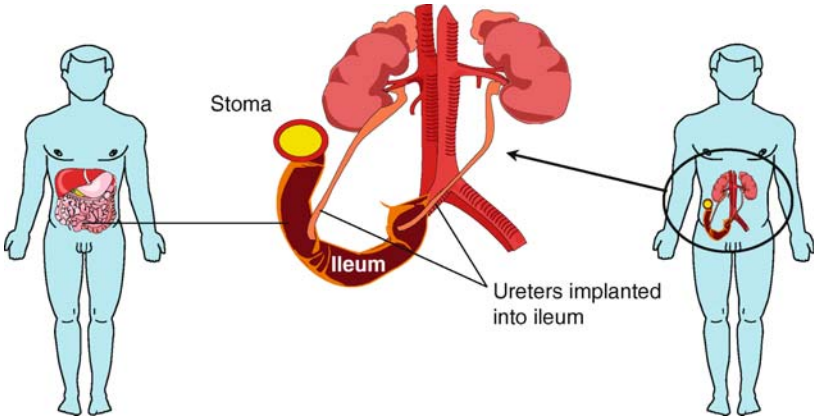
Management of Invasive and Metastatic Disease

Radical cystectomy with urinary diversion or bladder sparing protocols, using a combination of radiation and chemotherapy, are the treatments of choice for patients who have resectable muscle invasive bladder cancer. Radical cystectomy includes wide excision of bladder and prostate in male patients and typically bladder, uterus, ovaries and anterior vaginal wall in females. Perioperative mortality from cystectomy is approximately 1% in most centers. The 5-year disease-free survival is 65–80% for pT2 tumors and 37–61% for pT3 tumors. Microscopic involvement of local lymph nodes decreases 5-year survival to approximately 5–20% depending on the number and extent of nodal involvement. Pelvic recurrence rates after cystectomy range from 2 to10% and depends on the stage of the primary tumor as well as the presence of pelvic nodal involvement. In addition, an interval longer than 12 weeks between the diagnosis of muscle invasive bladder cancer and radical cystectomy is associated with decreased survival. Recently, the use of ►neoadjuvant therapy consisting of a four drug regimen MVAC (methotrexate, vinblastine, doxorubicin, and ►cisplatin) has demonstrated a survival advantage for patients with localized bladder cancer undergoing cystectomy. The use of ►adjuvant therapy has been suggested by some authors however there is limited evidence of benefit of this approach. Ongoing clinical trials are addressing this question.

Recurrence or persistence rates after bladder sparing protocols approach 50%. By careful patient selection these latter protocols can achieve comparable disease specific survival rates to those obtained by radical cystectomy. Large tumors that are only minimally resectable by TUR and those causing hydronephrosis have a significantly worse response rate with such bladder sparing protocols. Complications of radiotherapy include dysuria, frequency, or diarrhea in up to 70% of patients.

Following cystectomy, multiple options in urinary diversion exist, most of which utilize intestinal segments. An ileal conduit using a short portion of the terminal ileum to carry urine from the ureters to the anterior abdominal wall is the simplest most commonly performed diversion and the one associated with the least number of complications. Patients wear an external appliance on the stoma. Possible complications include parastomal hernia, stomal stenosis, or stricture at the ureteroileal anastomosis. A cutaneous continent urinary diversion such as the indiana (ileocecum) pouch forms an internal reservoir which can then be intermittently catheterized via a small cutaneous stoma (Fig. 4). In selected patients, such continent reservoirs can be anastomosed to the native urethra and in this setting are called “orthotopic neobladders” Such continent diversions are technically more difficult and require motivated patients to manage the postoperative care required. Ureterosigmoidostomies are now rarely performed because of difficulties with reflux, urolithiasis, electrolyte imbalance, and increased risk of adenocarcinoma of the colon.

Recent advances in laparoscopic and robotic surgery have enabled minimally invasive techniques to be applied for treatment of various benign and malignant conditions of the urinary bladder. Multiple centers worldwide are reporting their initial experience with



Bladder Cancer. Figure 4 The ileal conduit urinary diversion.

laparoscopic radical cystectomy and urinary diversion. The majority of centers perform an intracorporeal laparoscopic cystoprostatectomy and complete the urinary diversion extracorporeally through a mini-laparotomy incision.

Metastatic urothelial cell carcinoma has traditionally been treated with MVAC with a response rate of 15–35%. Complete remission is seen in approximately 13% of patients and mean survival can be improved from 8 to 12 months. However, MVAC is associated with significant toxicity, as 20% experience neutropenic fever and sepsis associated mortality approaches 3–4%. Newer agents have recently been used with a significantly lower morbidity and mortality than MVAC. Gemcitabine is an antimetabolite chemotherapeutic agent. The combination of gemcitabine and cisplatin has demonstrated similar effectiveness to that of MVAC with a better safety profile and tolerability. However, larger randomized trials are needed to conclusively prove this point.

►Urothelial Carcinoma

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Blast Crisis

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Definition

Blast crisis is the aggressive and rapidly fatal terminal phase of ►BCR-ABL1 positive ►chronic myeloid leukemia (CML). This phase of the disease is characterized by accumulation of immature myeloblasts or lymphoblasts similar to those found in patients with acute leukemia.

Characteristics

Clinical Features

When left untreated, CML is a biphasic disease. Patients typically present in a relatively benign chronic phase which is characterized by symptoms of fatigue and lethargy, bleeding, moderate weight loss, an enlarged palpable spleen, and a high white blood cell (WBC) count. The increased WBC population in large part constitutes cells of the myeloid compartment, with over-representation of the granulocyte series. Within a

period of 3–5 years, the natural course of the disease is to accelerate, then to transform to an aggressive and rapidly terminal acute phase or blast crisis of 4–6 months duration. Features associated with this transformation include an increasing number of leukocytes, particularly immature blasts, in the blood and bone marrow, progressive anemia, thrombocytopenia and lack of response to therapy. In a small proportion of patients, the blast transformation may occur outside the bone marrow (extramedullary) in sites such as the lymph nodes, spleen, skin or meninges. There is no known cure for blast crisis CML. However, transition to accelerated disease may be postponed for several years or prevented by treatment early during chronic phase CML with the BCR-ABL1 tyrosine kinase inhibitor ►imatinib mesylate or allogeneic bone marrow transplant.

Blast crisis can be divided into two general forms: lymphoid and myeloid. Lymphoid blast crisis develops in about 30% of patients, and the blast cells are phenotypically similar to the common form of ►acute lymphoblastic leukemia (ALL). In rare cases, T-cell morphology has been described. Myeloid transformation is heterogeneous, where myeloblasts are the usual blast cell type, but megakaryoblasts or erythroblasts have been frequently identified. Occasional patients show blasts with myelomonocytic, monocytic or very rarely, basophilic blast differentiation. It is important to differentiate between myeloid and lymphoid blast cells, as patients in lymphoid blast crisis respond better to treatment.

Biological Basis

The precise molecular events that determine blast crisis are still poorly understood. However, the destabilized proliferation status that is imposed by expression of the BCR-ABL1 fusion gene in a self-renewing leukemic stem cell is a likely mitigating influence. Much evidence has shown that there is lineage-specific selection for, and accumulation of, cytogenetic and molecular gene rearrangements in the affected myeloid or lymphoid cell compartments. Cytogenetic evolution of the BCR-ABL1 fusion gene positive clone occurs in ~80% of cases with CML that transform to blast crisis, and a change in karyotype is considered to be a poor prognostic sign, heralding or accompanying the acute transition. Diverse karyotype abnormalities are observed, both structural and numerical, either singly or in combination, and there is marked non-random involvement of certain chromosomes. Duplication of the Ph chromosome (and therefore the BCR-ABL1 fusion gene), i(17q), +8 or +19 are observed alone or in various combinations in 60–80% of cases having additional abnormalities. Recurring molecular changes have also been identified at transformation in some cases, and include mutation of the ►TP53 and ►retinoblastoma 1 (RB1) genes, activation of ►RAS, and, in lymphoid blast crisis cells, homozygous loss of the tumor suppressor gene ►CDKN2A (p16). These and other studies suggest that BCR-ABL1 cells are genetically unstable, and preferentially accumulate non-random genomic mutations that are compatible with the BCR-ABL1 oncogene product and provide a proliferative advantage. Similar non-random accumulation of genomic aberrations is also observed in advanced leukemias of BCR-ABL1 transgenic mice. New evidence suggests that the biological characteristics of blast crisis CML will alter in the post-imatinib mesylate era.

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Blast Phase

Definition

Last phase of CML; similar to an acute leukemia (poor prognosis). Characterized by >20% myeloblasts or lymphoblasts in blood or bone marrow.

►Nilotinib

Blastocyst

Definition

A mammalian embryo before implantation. Blastocysts consist of outer trophectoderm cells, which allow the embryo to implant, surrounding the inner cell mass, within which are found the pluripotent epiblast cells.

Bleomycin

Definition

A chemotherapeutic drug categorized as a cytotoxic/antitumor antibiotic. As an anti-cancer drug, it is typically used in the treatment of cervical cancer, head and neck cancer, Hodgkin disease, non-Hodgkin lymphomas, and testicular cancer. Bleomycin interferes with cell growth by damaging DNA and preventing DNA repair. An anticancer antibiotic that can induce DNA strand breaks. It is the first mutagen applied to ►mutagen sensitivity assay.

►Hyperthermia
►Malignant Lymphoma, Hallmarks and Concepts
►Mutagen Sensitivity

BLI

►Bioluminescence Imaging

BLL

Definition

Blood lead level.

►Lead Exposure

Blood–Brain Barrier

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Synonyms

Blood–brain barrier; Brain microvascular endothelial cells; Brain capillaries

Definition

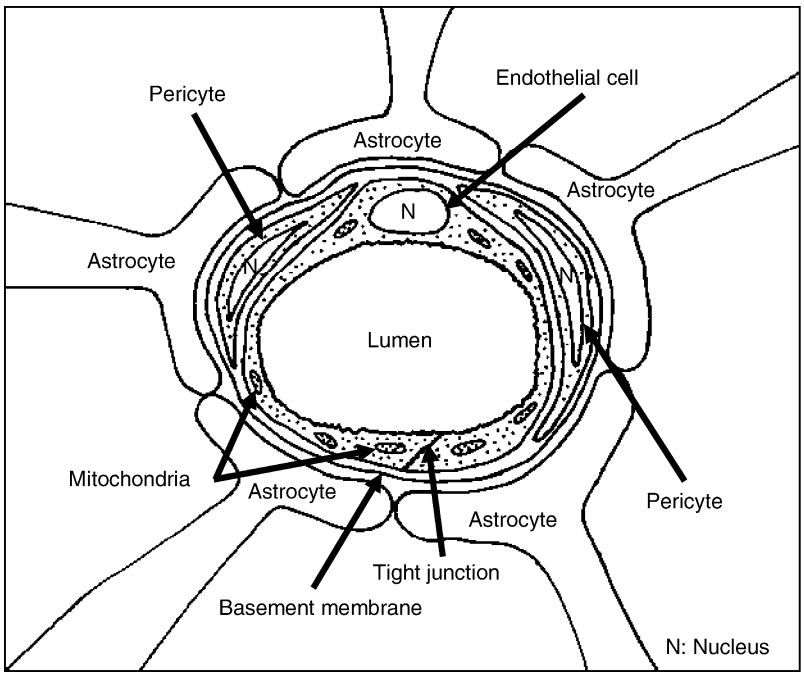
The blood–brain barrier (BBB) is formed by brain capillary endothelial cells. The BBB is composed of cerebral endothelial cells, astrocyte end-feet, and pericytes and regulates the homeostasis of the central nervous system (CNS).

Characteristics

The BBB was identified by Paul Ehrlich in 1885 [1–2]. However, the biology of the BBB, its importance in health and disease, and its anatomical definition were mostly revealed over the last 30 years. The BBB is a well-differentiated network of brain microvessels that maintains the homeostasis of the brain microenvironment. The BBB regulates the interface between the peripheral circulation and the CNS. It restricts the nonspecific flux of ions, proteins, and other substances into the CNS environment, thereby protecting neurons from harmful components of the blood, and also allows the uptake of essential molecules from the blood to the CNS. The BBB is a selective diffusion barrier at the level of the cerebral microvascular endothelium. The anatomy of the brain microvascular endothelial cells (BMEC) of the BBB, which are a major component of the BBB, are distinguished from other types of endothelial cells in the periphery by increased mitochondrial content, a lack of fenestration, minimal pinocytotic activity, and the presence of ►tight junctions (TJs). The tight junctions create a barrier in the BBB that helps to maintain brain homeostasis and provide high transendothelial electrical resistance (100–2000 ohm/cm^2), resulting in decreased paracellular permeability. BMEC are surrounded with pericytes, often divided into granular and filamentous subtypes, and astrocytic end-feet, which play an essential role in maintaining the structure of the BBB (Fig. 1). Astrocytes confer protection to the BBB against hypoxia and aglycemia.

The development of the BBB involves brain angiogenesis and BBB differentiation. First, brain endothelial cells derived from permeable vessels invade the vascular neuroectoderm and form intraneural vessels. Next, during the late embryonic and early postnatal periods, brain capillaries, in concert with astrocytes, differentiate, gradually mature, and are remodeled into the BBB, with impermeable properties.

Failure to maintain BBB integrity can have profound effects on the CNS. The disruption of the BBB may result in many brain disorders, including brain tumors. Changes in BBB function are associated with several neurological disorders, including stroke, multiple sclerosis, and Alzheimer's disease as well as inflammatory diseases such as chronic relapsing multiple sclerosis. Many of these changes have been linked to alterations in the tight junctions of the BBB.



Blood–Brain Barrier. Figure 1 Anatomical view of major components of the blood–brain barrier (BBB). The BBB is formed by endothelial cells, pericytes, basement membrane, and astrocytes. The BBB forms a highly restricted barrier that controls the exchange of materials between brain tissue and the circulatory system to maintain brain homeostasis. Tight junctions are more abundant than in other vessel systems and play a major role in regulating the permeability changes of the BBB. N: nucleus.

The BBB Junctional Complexes

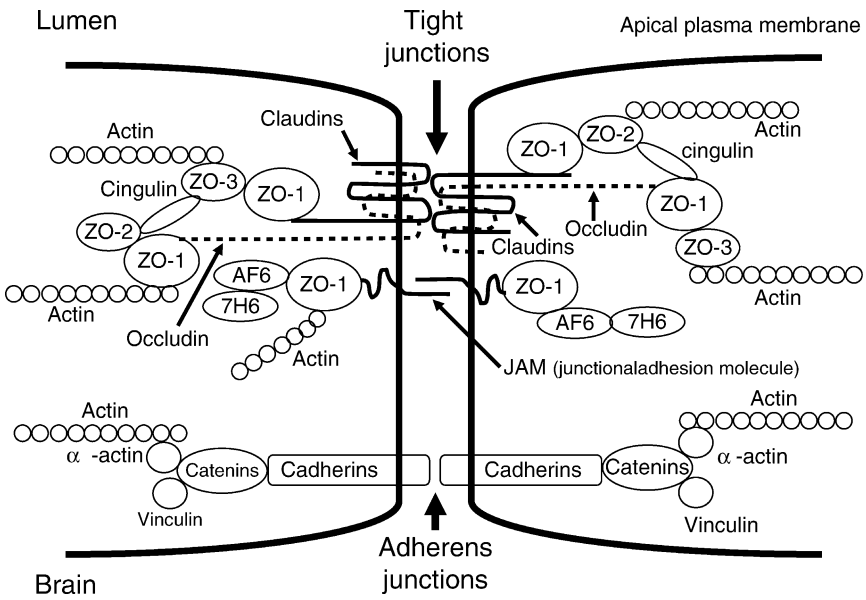
The interendothelial space of the cerebral microvasculature is characterized by the presence of a junctional complex that includes adherens junctions (AJs), tight junctions (TJs), and ►gap junctions (Fig. 2) [3]. While the gap junctions mediate intercellular communications, both AJs and TJs act to restrict permeability across the endothelium.

The TJs are dynamic structures. The physiological and pathological conditions of the BBB affect TJ organization and function in the BBB. Disruption of the TJs by disease or drugs can lead to impaired BBB function and thus compromise the CNS microenvironment. Changes in TJ expression, subcellular localization, and/or posttranslational modification or changes in the protein–protein interactions of TJs can lead to alterations in BBB permeability and integrity.

AJs are ubiquitous in the vasculature and mediate the following functions: (i) adhesion of endothelial cells to each other; (ii) contact inhibition during vascular growth and remodeling; (iii) initiation of cell polarity; and (iv) partial regulation of paracellular permeability. The components of AJs include VE-cadherin, alpha-actinin, and vinculin which all link to the actin cytoskeleton, thus stabilizing the AJ complex.

The TJs form a continuous network of parallel, interconnected, intramembrane strands of protein arranged as a series of multiple barriers. It is the TJs that confer the low paracellular permeability and the high electrical resistance. The TJs are composed of transmembrane proteins that form a primary seal linked via accessory proteins to the actin cytoskeleton. The proteins of the tight junctions include: junctional adhesion molecule (JAM-1), occludin, and the claudins.

1. JAM-1 is a 40-kDa member of the IgG superfamily and is believed to mediate the early attachment of adjacent cell membranes via homophilic interactions. JAM-1 is composed of a single membrane-spanning chain with a large extracellular domain.
2. Occludin is a 60–65-kDa protein that has four transmembrane domains with the carboxyl and amino terminals oriented to the cytoplasm and two extracellular loops which span the intercellular cleft. It is highly expressed along the cell margins in the cerebral endothelium. Occludin increases electrical resistance in TJ-containing tissues, and has multiple sites for phosphorylation on serine and threonine residues. In addition, the cytoplasmic C-terminal domain is likely involved in the association of occludin with the cytoskeleton via accessory proteins, such as the ►zonula occludens ZO-1 and ZO-2.
3. Claudins are a family of 20–24-kDa membrane proteins that includes 24 members. The assumption



Blood–Brain Barrier. Figure 2 Major tight junction and adherens junction proteins in the blood–brain barrier (BBB). Three transmembrane proteins, claudin, occludin, and junctional ►adhesion molecule (JAM), form integral tight junctions between adjacent endothelial cells. They provide the primary seal and regulate the paracellular permeability of the BBB. Other accessory proteins, such as zonula occludens (ZO-1, ZO-2, and ZO-3), AF6, 7H6, and cingulin, are involved in structure support, regulation, location recognition, and signal transduction for the tight junctions. Adherens junctions consist of one transmembrane protein, cadherin, and three structure support proteins, catenin, α-actinin, and vinculin, that link to the major cytoskeletal protein, actin.

is that claudins form the primary seal of the TJs and that occludin acts as an additional support structure. In the brain endothelium, claudin-5 is the most critical for BBB permeability.

4. In addition to the transmembrane components of the TJs, there are several accessory proteins that associate with them in the cytoplasm. These include members of the membrane-associated guanylate kinase-like (MAGUK) homolog family. MAGUK proteins are involved in the coordination and clustering of protein complexes to the cell membrane and in the establishment of specialized domains within the membrane. Three MAGUK proteins have been identified at the TJs: ZO-1, ZO-2, and ZO-3. ZO-1, which is abundantly expressed in BMEC, is a 220-kDa protein that links transmembrane proteins of the TJs to the actin cytoskeleton. This interaction is critical to the stability and function of the TJs and is important for the integrity and permeability of the BBB.
5. Additional accessory proteins of the BBB include cingulin, AF6, and 7H6.

Permeability Properties of the BBB

The BBB significantly impedes entry from the blood into the brain of virtually all molecules, except those that are small and lipophilic. However, there are sets of small and large hydrophilic molecules that can enter the brain, and they do so by active transport. One of the important transporters is P-glycoprotein (Pgp), which is present in relatively high concentrations in brain capillaries and is also part of the barrier. Pgp is associated with multidrug resistance (MDR) in numerous tumors. The discovery of Pgp on the BBB has contributed to an understanding of the penetration of various drugs into the brain.

Net fluid influx and brain extracellular fluid homeostasis are regulated by hormones produced in the CNS that affect blood–brain transport. Transcytosis of insulin and transferrin has been well-defined and these pathways have been utilized for targeted delivery to the brain and brain tumors. The presence of active efflux transporters in the BBB prevents many systemically administered drugs from entering the brain and is a major obstacle in designing drugs to treat neurological disorders.

In Vitro BBB Models

Research on BBB functionality has been facilitated by the availability of in vitro BBB culture systems [4]. Culturing of the in vitro BBB involves the isolation of capillaries and culture of BMEC alone or in combination with astrocytes or astrocyte-conditioned medium.

BBB in Disease

The BBB is sensitive to the pharmacodynamic effects of compounds and disease mediators that may result in

changes in BBB integrity and function [5]. Alterations of the barrier tight junctions are a hallmark of many CNS pathologies, including tumor, stroke, HIV, encephalitis, and bacterial meningitis. BBB breakdown or TJ protein rearrangement seems to be involved in both the direct and indirect effects of stress responses and inflammatory mediators. Traumatic brain injury leads to an upregulation of ►vascular endothelial growth factor (VEGF) and the VEGF receptors, VEGFR-1 and VEGFR-2. Although a compromised BBB has been reported under some pathologic conditions, the precise role of a disrupted BBB in the pathogenesis of neurological diseases is not well-defined.

In addition, the BBB presents a major obstacle to the treatment of malignant brain tumors and other CNS diseases. Delivery of therapeutics to the CNS is critical for the successful treatment of brain tumors and other neurological diseases. In this context, the current view is that the BBB, BMEC along with glia cells, pericytes, and neurons, should be viewed as a neurovascular unit for drug delivery.

Future studies should be aimed at understanding BBB dysfunction and the factors that regulate its recovery as well as at designing new approaches for the prevention and treatment of neurological diseases including brain tumors.

►Pharmacogenomics in Multidrug Resistance

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Blood Stasis due to Vital Energy Stagnancy

Definition

Is a pathogenesis and syndrome in Chinese medicine. Various pathogenic factors such as emotional depression, unhealthy diet, infection, and injury obstruct the circulation of vital energy and result in vital energy stagnancy. Chronic or severe stagnation of vital energy may lead to blood stasis, with syndrome characterized by distention, pain, ecchymosis, and even mass formation. The principle of its treatment is to supplement vital energy, promote blood circulation, and resolve blood stasis.

►Chinese versus Western Medicine

Bloom Syndrome

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Synonyms

Bloom-Torre-Mackacek syndrome; Congenital telangiectatic erythema

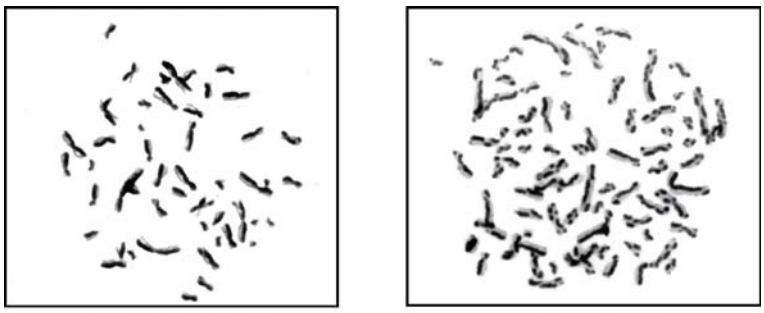
Definition

Bloom syndrome (BS) is a rare human autosomal recessive disorder that belongs to the group of "►chromosomal breakage syndromes," and is characterized by marked ►chromosomal instability associated with a greatly increased predisposition to a wide range of ►cancers commonly affecting the general population. BS was first described by David Bloom in 1954 as "congenital telangiectatic erythema resembling lupus erythematosus in dwarfs." The predominant and constant clinical feature of BS is proportionate pre- and postnatal growth retardation. Additional clinical features are described below. The hallmark of BS cells is an approximately tenfold increase in the rate of ►sister chromatid exchanges (SCEs) compared to normal cells. This increased level of SCE is the only objective criteria for BS diagnosis (Fig. 1). SCEs frequency averages 0.24 per chromosome in normal cells and 2.12 per chromosome in BS cells.

Characteristics

Clinical Description

A surveillance program, the Bloom Syndrome Registry, was established in 1960 by James German and Eberhard Passarge, in which the follow-up of 168 BS patients (93 males, 75 females) was reported until 1991. From the data in this Registry, it appears that the two constant clinical features associated with BS are growth retardation starting *in utero* and persisting throughout life with normal proportioning and accompanied by



Bloom Syndrome. Figure 1 Increased sister chromatid exchange in Bloom Syndrome cells. The sister chromatids in the images are differentially labelled so that regions of chromatid exchange can be seen as regions of light and dark staining. Little chromatid exchange is seen in normal cell metaphase (left panel), whereas most of the chromosomes in a Bloom Syndrome cell metaphase (right panel) show chromatid exchange.

dolichocephaly, and predisposition to all types of cancers. The mean adult height for men is 147.5 cm (range 130–162), and for women is 138.6 cm (range 122–151). Eleven additional clinical features that are not constant and that vary in severity among BS patients were also reported by James German: (i) a "bird-like" facies with a narrow face and prominent nose, and malar and mandibular hypoplasia, (ii) sun-sensitive erythema affecting the butterfly area of the face (similar to that caused by lupus erythematosis), and sometimes affecting the dorsa of the hands and forearms, (iii) spots of hyper- and hypopigmentation of the skin ("café au lait" spots), (iv) a high-pitched voice (Mickey Mouse voice), (v) a variable degree of "vomiting and diarrhea" during infancy, (vi) diabetes mellitus (diagnosed at a mean age of 24.9 years in 20 of the 168 BS patients in the Registry), (vii) small testes accompanied by a total failure of spermatogenesis in men and early cessation of menstruation accompanied by reduced fertility in women, (viii) immunodeficiency manifested by recurrent respiratory tract infections complicated by otitis media and pneumonia (life-threatening ear and lung infections are common), and manifested by the gastrointestinal problems mentioned in (v), (ix) some minor anatomic abnormalities such as obstructing anomalies of the urethra, which were of major clinical importance in several cases, (x) average intelligence (sometimes mental deficiency), (xi) clinical features that occurred in only one or a few BS patients and that are not to be considered part of BS itself, such as congenital thrombocytopenia, mild anemia, asthma, or psoriatic arthritis.

The 100 cancers that arose in 71 of the 168 BS patients recorded in the Bloom Syndrome Registry have been reported, and the distribution of the sites and types of these cancers is similar to that found in the general population. The main conclusions of this report are that nearly half of the registered BS (71/168) patients have had at least one cancer by the mean age of 24.7, and of those patients, 40% have had more than one primary cancer (29/71). ►Acute leukemias (21% of cancers), lymphomas (23%) and rare tumors (5% including ►medulloblastoma, ►Wilms tumor, osteogenic sarcoma) predominate in the first two decades of life, whereas carcinomas (51%) start to appear late in the second decade of life.

BLM-Deficient Cells

BS cells display an increase in chromosome breaks, a spontaneous ►mutation rate ten times higher than that in normal cells, an increased frequency of spontaneous ►symmetric quadriradial interchanges and sister chromatid exchanges, and increased ►loss of heterozygosity (LOH). BS cells also display replication abnormalities, including retarded replication-fork elongation and abnormal replication intermediates, and a general delay in the timing of replication associated with an increased level of constitutive ►DNA damage in mid- to late-S-phase. BS cells bud out large number of micronuclei during S phase and have constitutively high levels of RAD51-containing nuclear foci. Chronic overproduction of the superoxide-free radical O_2^- (►Reactive oxygen species) has also been reported in BS cells.

BLM Gene

BS arises through mutations in both copies of the BLM gene, which is located on chromosome 15 at 15q26.1. Nonsense or frameshift mutations leading to a premature termination codon, and missense mutations have been found in BLM gene from BS patients. One particular BLM gene mutation corresponding to a 6-bp deletion and a 7-bp insertion at nucleotide position 2207, referred to as the blm^{Ash} mutation, is homozygous in nearly all BS patients with Ashkenazi Jewish ancestry and is due to a founder effect. Screening for

BLM gene mutations can be done by analyzing the 21 coding exons (4,437 bp total length).

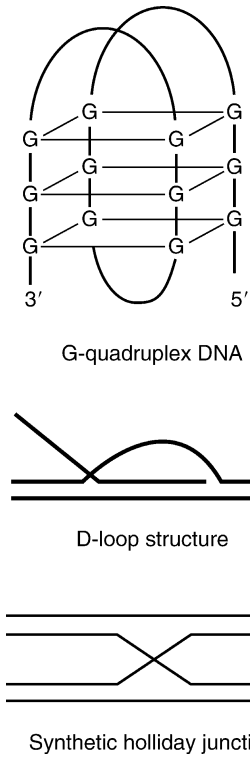
Frequency

BS affects all human populations, and its reported frequency is 1 in 10,836,000 in Japan, 1 in 3,331,000 in the United States, 1 in 5,590,000 in West Germany, and 1 in 2,395,000 in the Netherlands. In the Ashkenazi Jewish population, the frequency of BS is ~1 in 48,000. This is due to a founder effect, and ~1% of the Ashkenazi Jewish population are heterozygous carriers for the blm^{Ash} mutation.

BLM Protein

The BLM gene codes for the BLM protein, which is 1417 amino acids in length with a predicted molecular mass of 159 kDa, and it belongs to the DExH box-containing ►RecQ helicase subfamily. BLM displays an ATP- and Mg^{2+} dependent 3′-5′-DNA helicase activity that separates the complementary strands of DNA in a 3′-5′ direction. However, the exact function of BLM is still unclear. BLM protein accumulates in S and ►G2/M phases of the cell cycle, and localizes in two distinct nuclear structures, PML nuclear bodies (also called ND10) and the nucleolus. The preferred substrates for BLM are ►G-quadruplex DNA (G4 DNA), ►D-loop structures and ►X-junctions (Fig. 2). BLM promotes branch migration of RecA-generated Holliday junctions and effects, with topoisomerase IIIα, the resolution of a recombination intermediate containing a double Holliday junction with no flanking sequence exchanges (►Reverse branch migration activity). BLM also catalyzes the annealing of complementary single-stranded DNA molecules (DNA strand annealing activity). BLM interacts with several proteins involved in the maintenance of genome integrity. It participates in a super complex of ►BRCA1-associated proteins called BASC (BRCA1-associated genome surveillance complex) which includes BRCA1 (mutated in some familial breast cancers) ►ATM defective in Ataxia Telangectasia, AT, NBS1 (defective in ►Nijmegen Breakage syndrome) and MRE11 (defective in ataxia-telangiectasia-like disorder), MLH1, MSH2 and MSH6 (involved in Human Non-Polyposis Colorectal Cancer, HNPCC syndrome or ►Lynch syndrome), and several other proteins known to be involved in replicational and/or post-replicational ►repair processes. BLM also participates in a complex called BRAFT (BLM, RPA, FA, Topoisomerase IIIα), which contains five of the ►Fanconi anemia (FA) complementation group proteins (FANCA, FANCG, FANCC, FANCE and FANCF), RPA and topoisomerase IIIα (which are also known to interact independently with BLM), and a newly identified factor called BLAP75. Among the other proteins known to co-localize and/or to interact physically and/or functionally with BLM in undamaged cells and/or in cells submitted to ►genotoxic stresses, are the tumor suppressor protein ►p53, WRN protein (a RecQ helicase defective in the Werner syndrome), RAD51 (a key protein in ►homologous recombination), RAD51L3 (a RAD51 paralog), ATR (ataxia telangiectasia and rad^{3+} related kinase), TRF2 (a double-stranded telomeric DNA binding protein), Mus81 (a DNA-structure specific endonuclease), γ-H2AX (►GammaH2AX; histone H2AX phosphorylated on Ser 139 in response to DNA double-strand breaks), hp150 (the largest subunit of chromatin assembly factor 1, CAF1), FEN1, (flap endonuclease 1, involved in the removal of RNA primers of Okazaki fragments), FANCD2 (Fanconi anemia complementation group D2 protein), and the Chk1 kinase (a serine/threonine protein kinase that is a key mediator in DNA damage-induced cell cycle checkpoints).

Altogether, these data support a major role for BLM in maintaining genomic stability during DNA replication, homologous recombination and repair. Several models for the role of BLM have been proposed and suggest that BLM acts as a "roadblock" remover during DNA replication by disrupting complex structures such



Bloom Syndrome. Figure 2 Preferred substrates of the BLM helicase. The recombinant BLM protein efficiently unwinds DNA structures such as G-quadruplex, D-loop and synthetic holiday junctions.

as G-quadruplexes or DNA hairpins. BLM may also restart replication after the fork stalls and/or resolve recombination intermediates during DNA double-strand break repair through its ►reverse branch migration and DNA strand annealing activities.

Mouse Models

Among the five BS knockout alleles that have been generated, four led to embryonic lethality when the targeted allele was homozygous, and only one resulted in viable "BS" mice through a complex rearrangement of the targeted region. By 20 months of age, 29% of these Blm-deficient mice had developed a wide spectrum of cancer, similar to human BS patients.

Genetic Counseling

Due to the autosomal recessive transmission of BS, sibs of two heterozygous carriers are at 25% risk of having BS and at 50% risk of being carriers. When the risk of BS transmission has been well evaluated, prenatal diagnosis can be proposed (SCE analysis of fetal cells or detection of a specific *BLM* gene mutation when causative mutations are identified).

Therapy

There is no curative treatment for BS. However, a physician should carefully follow BS patients to ensure early cancer diagnosis.

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Bloom-Torre-Mackacek Syndrome

►Bloom Syndrome

Blue Dye

Definition

A blue substance that visualizes lymph vessels and (sentinel) lymph nodes, which helps identifying tumor sites.

►Sentinel Lymph Nodes

BM-40

►Secreted Protein Acidic and Rich in Cysteine

BMP

Definition

Bone morphogenetic protein; Group of growth factors able to induce the formation of bone and cartilage.

►Dental Pulp Neoplasms

BMS-247550

►Epothilone B Analogue

Body Mass Index

Definition

The body mass index (BMI) is a ratio between weight and height, a mathematical formula that correlates with body fat. BMI is determined by calculating your weight in kilograms divided by your height in meters squared (BMI = kg/m^2).

Bombesin (BBS)

Definition

Neuropeptide hormone from the fire-bellied toad *Bombina bombina* analogous to the mammalian gastrin-releasing peptide (GRP).

►Gut Peptides

Bone Loss, Cancer Mediated

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Synonyms

Osteolysis; Osteolytic bone disease; Osteoblastic bone disease; Cancer-mediated bone loss; Osteolytic lesions of bone; Osteoblastic lesions of bone; Skeletal complications (skeletal-related events)

Definition

Metastasis describes the spread of cancer from its site of origin (the "primary site") to another location in the body (the "secondary site"). Bone is the most frequent site affected by metastatic cancer. While any type of cancer can spread to bone, metastatic bone disease is most commonly associated with cancers whose primary origin is the breast, prostate or lung. Although less common, bone metastases are also associated with cancers arising in the thyroid, kidney stomach, uterus, colon, bladder and rectum (Table 1). Metastasis of cancer to bone often results in significant skeletal morbidity which manifests as severe bone pain, pathologic fractures, spinal cord compression and life threatening ►hypercalcemia.

Bone Loss, Cancer Mediated. Table 1 Incidence of primary tumors which metastasis to bone as a percentage of all bone metastases

Primary tumor	As a percentage of all bone metastases
Breast	35
Prostate	30
Lung	10
Kidney	5
Thyroid	2
Others	18

Characteristics

Types of Bone Metastasis

Bone metastases are classified as osteolytic, osteoblastic, or mixed, based on their radiographic appearance. Patients can present with either osteolytic or ►osteoblastic metastasis, or mixed lesions containing both elements. Bone metastases from prostate cancer are predominantly osteoblastic, whereas bone lesions from breast cancer can be osteoblastic, osteolytic, or mixed. Only in multiple myeloma do purely lytic bone lesions develop. Regardless of the mechanisms involved in the formation of osteolytic or osteoblastic metastases, the end result is a change to the bone architecture which predisposes the patient to a variety of skeletal co-morbidities.

Bone Physiology: Control of Normal Bone Remodeling

Healthy bone is a dynamic organ which is constantly rejuvenated by the coordinated activity of two cells types; namely the bone resorbing ►osteoclasts and the bone forming ►osteoblasts. Osteoblasts are responsible for the synthesis of collagen and non-collagenous bone proteins which are involved in the mineralization of the bone matrix. Normal bone remodeling (or turnover) is initiated by osteoclasts which degrade the bone matrix, creating cavities or lacunae. Once resorption is complete, osteoblasts synthesize a new osteoid matrix which is then mineralized. Under normal physiological conditions, the amount of newly formed calcified matrix is equal to the amount of bone resorbed by the osteoclast, thereby maintaining bone mass and skeletal integrity.

The bone resorbing osteoclasts are derived from hematopoietic stem cells. Osteoclast formation and activity is regulated by systemic hormones and growth factors which are synthesized in the bone microenvironment, including macrophage colony stimulating factor (M-CSF) and *r*eceptor *a*ctivator of *n*uclear *f*actor κB (RANK) ligand (RANKL). RANKL, a member of the tumor necrosis factor family, is expressed on the surface of stromal cells and osteoblasts. RANKL binds the RANK receptor on the osteoclast precursor cell surface and induces the formation of osteoclasts by signaling through the nuclear factor κB and Jun N-terminal kinase pathways. A number of osteotropic factors, such as parathyroid hormone, 1,25-dihydroxyvitamin D3, and prostaglandins, induce the formation of osteoclasts by increasing the expression of RANKL on marrow stromal cells and osteoblasts rather than by acting directly on osteoclast precursors. The activities of RANKL are further regulated by the decoy receptor, ►osteoprotegerin (OPG) which is synthesized by numerous cells including

marrow stromal cells and osteoblasts. The binding of OPG to RANKL precludes RANKL from binding to RANK, thereby inhibiting the differentiation and activity of osteoclasts. The ratio of RANKL to OPG will ultimately determine whether osteoclast formation will occur. Osteoclasts resorb bone by forming a tight seal between the ruffled border of the plasma membrane and the bone surface and secreting proteases and acid which dissolves the bone matrix.

Bone-forming osteoblasts are derived from mesenchymal stem cells. The transcription factor, Runx-2, (also termed core-binding factor α 1; CBFA1), is critical for the differentiation of osteoblasts by activating the expression of numerous genes which "drive" osteoblast differentiation. Many factors can enhance the growth and differentiation of osteoblasts, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF)-β and the bone morphogenetic proteins (BMP).

Bone is a Unique Environment for Metastasis

In 1889, Stephan Paget proffered the "seed-and-soil hypothesis" to explain why primary cancer cells selectively colonize distant organs. Paget suggested that cancer metastasis is reliant upon multiple interactions ("cross-talk") between cancer cells (the "seeds") and certain organ environments ("the soil") which provide a favorable environment for tumor localization and growth. To this day, this hypothesis still holds true. Metastasis to bone is a complex multi-step event which involves a bidirectional interaction of the tumor cells with cellular elements in three different microenvironments: (i) the site of primary tumor, (ii) the circulation and (iii) the bone microenvironment. The metastatic tumor cells must first break away from the primary tumor and move into the circulation and reach various skeletal sites, where they colonize, proliferate, and induce metastatic lesions. The preferential skeletal localization of tumor cells is attributed to the biologic and molecular characteristics of tumor cells as well as that of the bone microenvironment. For example, while unable to confer a bone metastatic potential individually, over-expression of an array of proteins, including the chemokine receptor, (CXCR)-4, interleukin (IL)-11, connective tissue growth factor (CTGF), and matrix metalloproteinase, (MMP)-1, along with the osteopontin, enhance the metastatic potential of breast cancer cells to bone. These proteins participate in one or more of the steps involved in the homing, invasion, angiogenesis, and proliferation of tumor cells in the bone microenvironment.

The multi-focal nature and predilection of tumor cells for the hematopoietic marrow sites in the proximal long bones and axial skeleton (vertebrae, pelvis, ribs, and cranium) can be attributed to the continuous and dynamic turnover of the bone matrix and bone marrow which provides a fertile ground for tumor cells to utilize resources (cells, growth factors, cytokines, and receptors) for their homing and growth. The anatomical and molecular characteristics of bone also make it a favorable site for metastasis, with the slow-moving but copious metaphyseal blood flow assisting intimate interactions between endothelium and tumor cells, a process necessary for the initial colonization of tumor cells within the bone marrow. In addition, various growth factors and cytokines in the bone marrow such as endothelin (ET)-1, basic fibroblast growth factor (bFGF), TGF-β, IL-6 and IL-8 serve as paracrine regulators of the initial growth of metastatic tumor cells. The interaction of chemotactic extracellular matrix and stromal cell expressed proteins (stromal cell derived factor-1, vascular cell adhesion molecule-1, fibronectin, type I collagen, type IV collagen, vitronectin, osteopontin, osteocalcin, bone sialoprotein and osteonectin) with ligands that are over-expressed on tumor cells (integrins α4β1 and α5β1, CTGF and CXCR4) promote tumor colonization within the bone marrow. Moreover, the bone matrix is a large repository of latent growth factors such as insulin-like growth factor (IGF), TGF-β, BMPs, PDGF and vascular endothelial growth factor (VEGF), which are released during the formation of both osteolytic and osteoblastic lesions and serve to stimulate the "vicious cycle" of tumor growth and progression of bone lesions.

The Vicious Cycle of Osteolytic Metastasis

The most common manifestation of bone metastasis is osteolysis (osteolytic metastasis). Osteolytic bone metastases are common in solid tumour metastases of lung, renal, breast cancer and the hematological malignancy, multiple myeloma. In osteolytic metastases, skeletal destruction is mediated primarily by osteoclasts rather than the tumor cells. However, the factors responsible for the activation of osteoclasts vary depending on the tumor type. The pathogenesis and progression of osteolytic metastases are often the result of a complex "vicious cycle" which involves the interactions between tumor cells, bone cells (osteoclasts and osteoblasts), and the bone matrix. The tumor cells secrete various soluble factors including IL-6, IL-8, IL-11, TNF-α, M-CSF, prostaglandin E2 (PGE2) and parathyroid related protein (PTHrP) that directly or indirectly promote osteoclast differentiation, proliferation, and activation leading to increased osteolysis. Furthermore, the process of bone resorption itself results in the release of growth factors, including TGF-β, IGF, bFGF and BMP, from the bone matrix which support the growth and survival of the tumor cells. In turn, the growing tumor secretes more pro-osteolytic factors, which results in further osteolysis and perpetuation of the vicious cycle.

The Vicious Cycle of Osteoblastic Metastases

Osteoblastic bone metastases are characterized by an increase in woven bone formation which radiographically appear as sclerotic lesions, and are most commonly seen in patients with prostate cancer. Tumor cells forming osteoblastic metastases secrete numerous pro-osteoblastic factors that drive bone remodeling toward a predominant bone-forming state. Furthermore, activated osteoblasts secrete numerous growth factors during woven bone formation, including TGF-β, BMP and VEGF, which further stimulate tumor survival and growth and perpetuate the vicious cycle. In addition, the Wnt (wingless int) pathway, the ET axis, and the BMP pathway have emerged as key regulators in the establishment of osteoblastic skeletal metastasis. Wnt proteins are soluble glycoproteins that promote embryonic and postnatal bone formation by binding to a membrane receptor complex comprised of frizzled (FZD) G-protein-coupled receptor and a low-density lipoprotein receptor-related protein. The formation of this ligand-receptor complex initiates a number of intracellular signaling cascades that modulate differentiation, survival, and activity of the osteoblasts. ET-1 promotes osteogenic differentiation, stimulates bone matrix formation, and inhibits osteoclast formation and motility. BMPs (BMP-2, BMP-6 and BMP-7) play a central role in skeletal development and postnatal bone repair, and are implicated in metastatic bone formation due to their osteoinductive properties.

Osteolytic Multiple Myeloma

Multiple myeloma is a hematological malignancy characterized by the "homing" of a clonal population of neoplastic plasma cells from secondary lymphoid organs to sites within the bone marrow, close to the bone surface. Here, multiple myeloma plasma cells recruit and activate the bone resorbing activities of osteclasts. Lytic lesions are observed in more than 80% of patients afflicted with multiple myeloma. Several osteoclastogenic factors have been implicated in the increased activity of osteoclasts in myeloma, including macrophage inflammatory protein (MIP)-1α, SDF-1 and RANKL. RANKL is produced by both the myeloma cells and marrow stromal cells in response to factors secreted by the myeloma cells themselves. In the bone microenvironment of myeloma patients, RANKL production is increased and ►osteoprotegerin is markedly decreased. MIP-1α is a potent inducer of osteoclast formation and enhances both RANKL- and IL-6–stimulated osteoclast formation. MIP-1α also stimulates integrin-mediated adhesion of myeloma cells to stromal cells resulting in an increased production of IL-6, RANKL and MIP-1α, which in turn further stimulates bone destruction. MIP-1α levels in myeloma patient serum correlate strongly with the presence of osteolytic lesions. Similarly, levels of the CXC chemokine, SDF-1, are elevated in myeloma patients which exhibit radiographically detectable bone lesions. While incapable of stimulating osteoclast formation in the absence of RANKL, myeloma cell-derived SDF-1 serves to hyper-activate the resorptive activity of osteoclasts leading to bone loss.

Bone lesions in myeloma are purely lytic and are not accompanied by an osteoblastic response. While the basis for the lack of an osteoblastic response in myeloma remains to be determined, recent studies suggest roles for the soluble Wnt-signaling antagonists, dickkopf 1 (DKK1) and soluble frizzled related protein, sFRP-2 (Fig. 1). High serum levels of soluble DKK1 and sFRP2 have been correlated with myeloma-induced focal bone lesions.

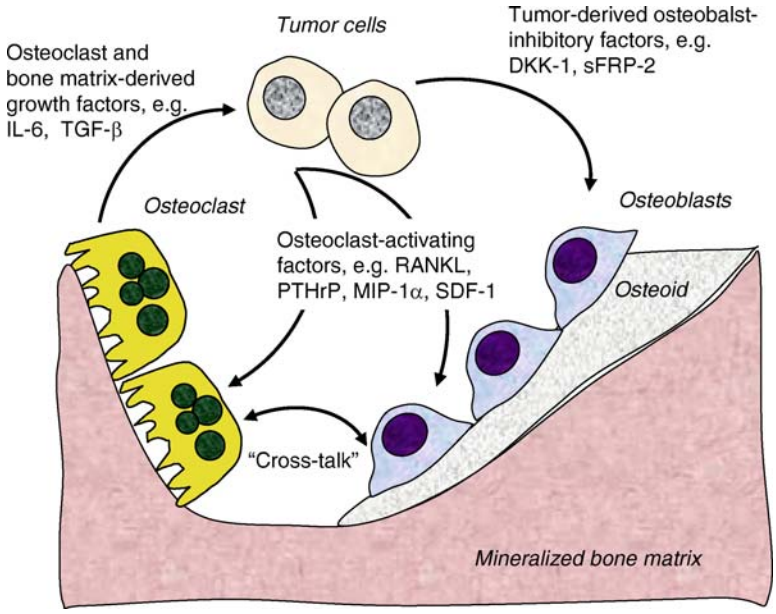
Myeloma tumor cells secrete numerous factors (RANKL, MIP-1a, SDF-1 and PTHrP) which directly or indirectly (via the osteoblast) stimulate osteoclast formation and activity. Myeloma tumor cells also express factors which suppress normal osteoblast function, including the Wnt signaling pathway antagonists DKK-1 and sFRP-1.

Osteolytic Metastasis from Breast Cancer

Breast cancer commonly metastasizes to and destroys bone, causing pain and fracture, and the median survival of patients with bone metastases is only 19–25 months. Almost one third of patients present with radiographically detectable mixed osteoblastic and osteolytic lesions. Tumor cells in breast cancer produce factors that directly or indirectly induce the formation of osteoclasts, including parathyroid hormone-related protein (PTHrP), interleukin (IL)-6, IL-8, IL-11, M-CSF, PGE-2, TNF-α, and RANKL. The resultant osteoclastic bone resorption releases growth factors from the bone matrix which further stimulate tumor growth and bone destruction. In particular, PTHrP functions by inducing the expression of RANKL on marrow stromal cells which further stimulates osteoclast formation and activity. The resultant osteoclastic resorption releases growth factors such as TGF-β from the bone matrix, with TGF-β in turn stimulating PTHrP production by tumor cells, and perpetuating the vicious cycle of breast-cancer metastases.

Osteoblastic Metastasis in Prostate Cancer

Prostate cancer is the most prevalent non-dermatologic cancer in males, and the clinical course of patients with metastatic prostate cancer can be relatively long with a median survival measured in years not months. At presentation, 10% of patients have bone metastases, and almost all patients who die of prostate cancer have skeletal involvement. Based on radiographic



Bone Loss, Cancer Mediated. Figure 1 The formation of lytic bone disease in myeloma.

appearance, prostate metastases are classified as osteoblastic, however studies have show that both bone resorption and bone formation are dysregulated in this disease. Although the mechanisms and factors involved in osteoblastic metastasis are unknown, a number of factors have been implicated, including ET-1, PDGF, urokinase-type plasminogen activator (u-PA), and prostate specific antigen (PSA). ET-1 stimulates the formation of bone and the proliferation of osteoblasts in bone organ cultures, and serum endothelin-1 levels are increased in patients with osteoblastic prostate metastases. Overproduction of u-PA by prostate-cancer cells imparts an enhanced capacity to initiate osteoblastic metastases. Prostate-cancer cells also release PSA, a kallikrein serine protease, which can cleave and inactivate parathyroid hormone–related peptide which can block tumor induced bone resorption. It may also activate osteoblastic growth factors released in the bone microenvironment during the development of bone metastases, such as IGF-I and -II or TGF-β. These data suggest that a vicious cycle may also be responsible for osteoblastic metastasis. While markers of bone resorption are also increased in prostate cancer patients with metastasis, there is usually no histologic evidence of increased numbers of osteoclasts. Studies show that blocking osteoclastic bone resorption in patients with prostate cancer decreases the number of skeletal-related events suggesting that bone resorption may precede bone formation in the development of osteoblastic metastases.

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Bone Marrow

Definition

Is the site of hematopoiesis, the generation of the cellular elements of blood, including red blood cells, monocytes, polymorphonuclear leukocytes, and platelets. The bone marrow is also the site of B-cell development in mammals and the source of stem cells that give rise to T cells upon migration to the thymus. Thus, bone marrow transplantation can restore all the cellular elements of the blood, including the cells required for adaptive immunity.

Bone Marrow-Derived Mesenchymal Stem Cells (MSC)

Definition

Synonym marrow stromal cells; these bone marrow fibroblasts act as a supportive framework within bone marrow and support hematopoiesis (i.e., blood cell development). MSC are also an adult stem cell population. The have the ability to self-renew, and they can give rise to osteoblasts (bone), adipocytes (fat), and cartilage.

►Desmoplasia

Bone Morphogenetic Protein

Definition

►BMP.

Bone Neoplasms

►Bone Tumors

Bone Resorption

Definition

Is a complex biological mechanism leading to the degradation of bone organic and mineral extracellular matrix and is closely related to osteoclast activity. Bone resorption corresponds to an extracellular degradation mechanism associated with the release of protons and proteases by osteoclasts.

►Zoledronic Acid
►Bisphosphonates

Bone Sarcomas

►Bone Tumors

Bone-seeking Malignant Phenotypes

►Bone Tropism

Bone Sialoprotein

►Osteopontin

Bone Tropism

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Synonyms

Skeletal secondary tumors; Bone-seeking malignant phenotypes

Definition

Bone tropism is the propensity of certain tumors to spread from the organ(s) in which they initially originated and target preferentially the skeleton, in which they may eventually grow into secondary tumors or ►metastasis.

Characteristics

Solid tumors at their initial stage present unique challenges for the selection of the appropriate treatment. Several considerations come into play, including the feasibility of their surgical ablation, the age of the patient and the histological grade of the neoplasia, among others. However, the risk of metastatic spread is indeed one of the most critical factors in deciding the therapeutic strategy to adopt. It is also widely recognized that the detection of secondary tumors at

the time of the initial diagnosis poses additional and most often prohibitive hurdles to a positive clinical outcome. This is because after secondary tumors become clinically evident there is no turning back and, despite slight individual differences in disease progression, both the prognosis and the quality of life dramatically worsen.

Although primitive tumors may be rapidly lethal or are defeated before a significant metastatic disease delineates itself, a defined group of solid tumors including prostate and breast adenocarcinomas consistently spread to the skeleton. Major complications caused by the growth of cancer cells in the skeleton include pathological fractures, spinal cord compression and an overall organ impairment affecting both mielogenic and immunological properties of the bone marrow. For several forms of neoplasia, skeletal metastases are the sole site of spread in ≥80% of patients; their distribution overlaps that of bone marrow in the adult and they represent the major cause of death. In fact, patients may succumb to the metastatic disease years after their primitive tumors were surgically removed.

Bone secondary tumors are extremely difficult to treat; since surgical procedures are commonly limited to mere ►palliative therapy, the treatments of choice are either ►chemoradiotherapy or immunotherapy. However, cancer cells located in the bone marrow benefit from the "sanctuary" characteristics of this tissue. Small foci of cancer cells can grow almost undisturbed because of the restricted access to the site combined to the high availability of nutrients and growth factors, which are constantly produced locally or delivered by the blood circulation.

There are several postulated mechanisms that could explain the bone tropism of cancer cells:

Blood Circulation Patterns

The anatomical description of the Paravertebral Venous Plexus, made by Batson in 1940, led to postulate that retrograde blood flow would deliver cells from tumors such as prostate and colon carcinomas preferentially to the spine, thereby determining the occurrence of secondary tumors at the vertebral level. Based on this paradigm, migrating cancer cells would not specifically arrest to the vertebrae but rather be passively forced by blood-flow patterns to settle in the first capillary bed they encounter. Their lodging into the bone would then occur by size restriction arrest, with capillaries progressively smaller in diameter trapping the traveling cancer cells.

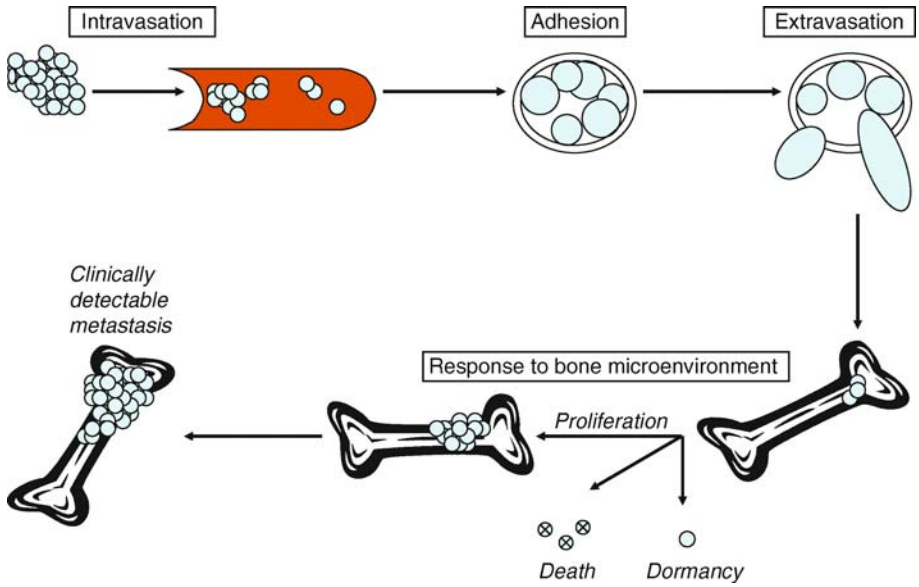
The role of blood flow and circulatory patterns in hematogenous skeletal metastases is obviously fundamental; however, a large body of evidence discounts mechanical entrapment as the exclusive factor and points toward additional mechanisms underlining the bone tropism of tumors. For example, certain tumors, such as prostate cancer, affect the skeleton both at the pelvic and the upper torso regions, an unlikely scenario for mechanical trapping by simple blood-flow distribution; in addition, several studies have shown that cancer cells can bypass more than one capillary bed and lodge in selected distant organs.

Cellular Adhesive Interactions

When cancer cells reach the marrow of different bones through blood circulation, they encounter the vascular wall of the bone sinusoids and interact with the endothelial cells which are lining it. The times required for adhesion of cancer cells to the endothelium by simple vicinity of cellular surfaces are in fact considerably in excess of their luminal transit times, suggesting the formation of adhesive bridges. Therefore, unique characteristics of bone marrow endothelial cells could promote the specific adhesion of cancer cells (Fig. 1).

This scenario is compatible with the trafficking of immuno-competent cells and their adhesion to different types of endothelia, including that of bone marrow sinusoids. For instance, the recruitment of leukocytes into sites of inflammation requires the sequential execution of events such as *capture* and subsequent firm *adhesion* to endothelial cells. The capture of leukocytes from the lumen of blood vessels involves the intervention of specific ►cell adhesion molecules (CAMs) exposed on the surface of leukocytes and endothelial cells. These molecules – including ►integrins and ►selectins among others – need to establish firm interactions with their respective substrates, such as ►fibronectin, to resist the shear force exerted by the blood flow while arresting the cells traveling through the lumen of the vessel. Similar mechanisms are in place to ensure the trafficking of hematopoietic progenitor cells from the bone marrow to the peripheral blood and vice versa.

It has been proposed that adhesive mechanisms involving CAMs could be usurped by cancer cells, allowing them to colonize the skeleton, among other organs. The activation of selected CAMs can be induced by molecules involved in the inflammatory response, named chemotactic cytokines or ►chemokines, through their interaction with specific receptors located on the surface of leukocytes and other cell types. During the last few years, a large number of studies have shown that different types of cancer cells express receptors for chemokines that are produced in organs which are common sites of metastasis. For example, breast cancer cells express the ►CXCR4 receptor for the chemokine CXCL12/SDF-1, which is detected in the bone marrow. Similarly, prostate cancer cells express the ►CX3CR1 receptor for the chemokine ►CX3CL1/fractalkine, which is constitutively anchored to the surface of the bone marrow endothelium, whereas



Bone Tropism. Figure 1 Bone tropism. Schematic representation of the sequential steps of skeletal metastasis that can be determinant in conferring bone tropism to cancer cells (Artwork by Whitney Jamieson).

other endothelia need inflammatory conditions to express it. Comparable chemokine/receptor interactions have been reported for different types of cancer targeting the skeleton, suggesting that chemokine-induced adhesive interactions might promote the preferential, albeit not exclusive, arrest of cancer cells into a specific tissue and indeed represent a crucial determinant of bone tropism.

Chemoattraction of Cancer Cells

Cancer cells can proliferate and grow within the blood vessels at their primary site of attachment to the endothelium, as shown by studies conducted by Dr. Muschel and co-workers, among others. However, particularly for skeletal metastases, simply adhering to the bone marrow endothelial wall may not suffice to invade the bone. Similarly to leukocytes, cancer cells need to migrate from the luminal side of the endothelial cells into the surrounding bone marrow stroma. This process, named ►extravasation, is comparable to the diapedesis observed for leukocytes and can be similarly regulated by chemokines. Thus, the mutual interactions between chemokines present in the bone tissue and their receptors expressed by bone-seeking cancer cells could exert an attractive force and induce migratory events involved in the skeletal secondary location of specific tumors. In support of this hypothesis, numerous studies have found CXCL12/SDF-1, CX3CL1/fractalkine and other chemokines being produced at high levels by the cells of the bone marrow.

However, bone marrow-produced chemokines are also abundant in other organs – for example the optic nerve and cardiac muscle for CXCL12/SDF-1 – which are seldom sites of metastasis from breast cancer cells, which express the compatible receptor CXCR4. Therefore, bone tropism likely depends on multiple factors; the idea of favorable conditions for cancer cell growth offered only by selected organs is epitomized by the ►seed and soil hypothesis.

Tissue Conditions Supporting the Growth of Cancer Cells

Once cancer cells have migrated into a foreign tissue, they will need to find favorable conditions to survive and proliferate. The seed and soil hypothesis – originally conceived by the English surgeon Stephen Paget in 1889 – emphasizes the importance of appropriate local trophic factors (the soil) in determining the growth of disseminated cancer cells (the seeds) into secondary tumors. Thus, cancer cells targeting distinct organs would differ in their responsiveness to molecules produced by different tissues; this idea could also explain the discrepancy between the relatively high blood supply of certain organs and their relatively low frequency of metastatic growth.

The seed and soil hypothesis might imply that tumor cells leave the blood and lymphatic circulation to the same degree at all organs, but survive and proliferate only in those organs producing congenial growth factors. However, it is highly plausible that selective adhesion/extravasation as well as trophic interactions between cancer cells and skeletal tissue are not mutually exclusive phenomena and they all play a crucial role in determining the bone tropism of tumors.

The bone is composed of different cell types and is characterized by sustained levels of metabolic activity due to bone remodeling or repair, inflammation and reactive hematopoiesis, throughout the entire life of the individual. Thus, bone tissue homeostasis is orchestrated by a plethora of trophic molecules to which cancer cells might also be sensitive. In fact, an important role for bone turnover in the preferential location of prostate cancer cells to the skeleton has been recently reported.

The quest to identify factors produced by the bone microenvironment that could support the growth of cancer cells has led to implicate several molecules, including ►transforming growth factor, ►osteopontin, ►platelet derived growth factor, among others.

The disruption of the trophic interactions between cancer cells and bone, for example by using inhibitors of growth factor receptors such as ►imatinib, has been recently attempted. In general, the identification of crucial molecules supporting the survival of cancer cells in the bone will provide novel targets for the treatment or prevention of skeletal tumors by pharmacological, immunological or gene therapy approaches.

Cancer cells may leave the primitive tumors and enter the blood circulation in large numbers, a process called ►intravasation. However, the metastatic process is highly inefficient and it has been shown that the vast majority of disseminated cancer cells fail to produce secondary tumors. Using animal models, only a very small fraction of cells delivered into the blood stream were seen reaching peripheral organs; of those that located to secondary organs some would proliferate while others would remain dormant. Thus, growth factors produced by the bone tissue can be determinant for supporting the small foci of cancer cells immediately after their arrival and their successful lodging in the skeleton.

Alteration of Bone Microenvironment by Cancer Cells

The small number of cancer cells reaching the skeleton are probably unable to immediately affect the surrounding cells and stroma and will depend on growth factors already present in the microenvironment to sustain their survival and growth. However, it is conceivable that once the initially small foci of malignant cells have reached significant size, functional cross-talking with the bone resident cells is established. This phenomenon has been shown to regulate phenotype and genotype of tumor as well as bone stromal cells and ultimately modify the microenvironment in a fashion that can potentially benefit metastatic growth.

An intriguing hypothesis is that cancer cells, in order to survive in the bone, need to acquire bone-like properties. This phenomenon – defined ►osteomimicry – is characterized by the expression of bone cell-related genes and is regulated by the interaction with bone resident cells, which can occur through either physical contacts or released factors. Some bone cell-related genes are those for osteopontin, osteocalcin and bone sialoprotein. A soluble factor, ►osteoprotegerin, a member of the ►tumor necrosis factor superfamily, can be detected in high levels in the serum of patients affected by advanced prostate cancer, which is almost invariably associated with skeletal metastasis. Osteoprotegerin acts as a ►decoy receptor and blocks ►TRAIL/Apo 2L, a cytokine that can trigger cancer cell death by interacting with death-receptors. Interestingly, osteoprotegerin is overexpressed by cancer cells as a consequence of osteomimicry. However, as bone marrow stromal cells also produce this protein during bone remodeling, the bone microenvironment could independently protect cancer cells from the TRAIL-induced death. Thus, the production of osteoprotegerin by cancer cells upon osteomimicry would essentially amplify this phenomenon.

Because of the obvious problems posed by bone secondary tumors for prognosis and quality of life, therapies to successfully counteract malignant cells growing at the skeletal level are of utmost importance for the management of cancer.

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Bone Tumors

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Synonyms

Bone neoplasms; Bone sarcomas

Definition

Bone tumors are space-occupying lesions arising in bone that are usually derived from primitive connective tissue elements.

They constitute a diverse group of neoplasms that are collectively less common than those of almost all other body sites. Malignant primary bone tumors have an approximate incidence one new malignant bone tumor per 100,000 individuals per year. Since the incidence of benign bone tumors is about half that of malignant tumors, the average aggregate incidence of all bone tumors is about one in 67,000 persons per year.

With a few notable exceptions, bone tumors have a predilection for individuals in the second and third decade; there is a second, smaller peak of incidence in the sixth and seventh decade. In most large series of bone tumors there is a slight male predominance. Each tumor type has a characteristic range age, skeletal distribution, and sometimes sex and racial predilection.

Characteristics

Etiology

While the link between bone tumors and a predisposing cause is not demonstrable in most cases, there are conditions in which the incidence of bone tumors is increased. The presence of pre-existing ►Paget disease of bone, ►idiopathic bone infarctions, and ►fibrous dysplasia are all associated with increased frequency of bone sarcomas. Ionizing radiation, whether given for therapeutic purposes or accidentally acquired through external exposure or ingestion also predisposes to the development of bone sarcomas. Ollier's disease, a congenital but non-hereditary disorder characterized by multiple ►enchondromas predisposes to malignant cartilage tumors in 10–20% of cases. About half of all patients with Maffucci syndrome, which is characterized by multiple soft tissue ►hemangiomas in patients with Ollier's disease, develop malignant cartilage tumors. Multiple hereditary exostoses, an autosomal dominant disorder, is associated with a specific genetic abnormality, usually at the EXT-1 or EXT-2 chromosomal loci. In this disorder, which is characterized by the development of multiple ►osteochondromas, resulting in bone modeling abnormalities, the incidence of malignant cartilage tumors is in about 10% of patients.

Other familial disorders having an increased incidence of other malignant neoplasms also have an increased predisposition for ►osteosarcomas. Chief amongst these are the Li–Fraumeni Syndrome, in which mutations in the p53 gene causes an increased incidence of many tumors (►p53 gene family). Patients with familial retinoblastoma gene mutations (Rb) have a high risk for the development of osteosarcoma. Two other autosomally recessive disorders that usually present with skin abnormalities, the ►Rothmund–Thomson syndrome and ►Bloom syndrome, have associated specific chromosomal mutations that result in an increased association with osteosarcomas. A specific and reproducible chromosomal abnormality, namely, a fixed reciprocal translocation between chromosomes 11 and 22 and, less frequently, between chromosomes 7 or 21 and 22 results in a chimeric gene product that encodes for the proliferation protein promoter found in the cells of more that 90% of patients with ►Ewing sarcoma.

Diagnosis

Bone tumors present with fairly non-specific symptoms, the most common of which is pain that is noticed at rest or is severe enough to wake a patient from sleep. Interference with normal limb function or the presence of a mass may also be present, but these usually occur later in disease evolution. A specific diagnosis is made by biopsy, which may be as simple as a fine needle aspiration or as extensive as a complete excision. Since the overlying soft tissues and skin cover the bones, bone tumors are essentially invisible without clinical imaging studies. The most important of these are routine radiographic studies done using multiple views. Imaging studies should always be reviewed in tandem with the histology prior to rendering a diagnosis, because not only do they provide information regarding the location, extent and aggressiveness of the disease, but also correlative information on the likelihood that the biopsy is representative of the process.

Primary bone tumors are classified into their various histologic subtypes on the basis of their histologic grade, their anatomic location, and especially by the kinds of extracellular connective tissue (e.g. bone, cartilage, fibrous tissue) produced by each or whether there is differentiation into any other types of soft tissue elements (Table 1). For example, osteosarcoma is a malignant tumor in which the cells have the capacity to produce bone. While the diagnosis is usually made from the histologic features, some bone tumors that look deceptively benign histologically may actually be diagnosed as malignant tumors when the imaging and histologic features are correlated. In addition, a very few varieties of tumor are characteristically unpredictable in biologic potential. A few primary bone tumors may behave in a locally destructive fashion but have an unpredictable propensity for metastatic behavior. The most important examples include ►giant cell tumors of bone and ►hemangioendotheliomas.

Because they often produce so large a quantity of extracellular matrix, the volume of primary bone tumors tend to be comprised more of extracellular connective tissue elements rather than by tumor cells. This relationship is the exact opposite of what is observed in carcinomas. With the notable exception of chordoma, adamantinoma, and neural tumors, it is

Bone Tumors. Table 1 Bone tumor classification

Matrix	Benign	Malignant
Bone	Osteoma	Osteosarcoma surface
	Osteoid osteoma	
Osteoblastoma	Osteoblastoma	
Cartilage	Enchondroma	Central chondrosarcoma
	Periosteal chondroma	Peripheral chondrosarcoma
	Osteochondroma	
Chondroid	Chondroblastoma	
	Chondromyxoid fibroma	
Fibrous	Non-ossifying fibroma	Fibrosarcoma
	Desmoplastic fibroma	Malignant fibrous histiocytoma
	Benign fibrous histiocytoma	
Fat	Lipoma	Liposarcoma
Muscle	Leiomyoma	Leiomyosarcoma
		Rhabdomyosarcoma
Notochord		Chordoma
Neural	Schwannoma	Ewing's sarcoma/primitive neuroectodermal
Tumor	Neurofibroma	
Vascular	Hemangioma	Angiosarcoma
	Hemangioendothelioma	
Epithelial		Adamantinoma
Lymphoid		Lymphoma
Myeloid		Myeloma
Histiocytic	Giant cell tumor	

believed that almost all primary bone tumors are derived from embryonic ►mesoderm. This is again in contrast to adenomas and carcinomas, which are derived from ►ectodermal or ►endodermal precursors.

Staging and Prognosis

Benign tumors of bone are almost always of a limited growth potential and their morbidity is confined to interference with local anatomy or function. They are usually controlled or cured by limited surgery such as curettage or simple excision. Locally aggressive bone tumors may require more aggressive surgery depending upon their size, location, and whether there has been previous treatment. Most malignant bone tumors are high-grade lesions and are usually treated as systemic diseases even if they seem localized at the time of diagnosis. With the exception of ►chondrosarcoma, malignant tumors are now treated with systemic chemotherapy (►neoadjuvant Therapy). A few tumors, notably lymphoma and myeloma, may also be treated with local radiation therapy, but most other bone tumors are no longer irradiated. Most malignant bone tumors spread systemically to the lungs though other bones and sometimes other parenchymal organs may be affected. In general, regional lymph nodes are not affected, so the most meaningful staging schemas of bone tumors does not necessarily include the presence of nodal disease. The musculoskeletal tumor society staging system first proposed by Enneking takes into account only whether a malignant tumor is high grade or low grade, confined to one or more compartments, or is metastatic. This system both summarizes tumor biology and helps to clarify the treatment regimen required for a particular patient.

In general, patients with low-grade malignant bone tumors have a good prognosis so long as their initial surgery is adequate. If surgery is not adequate, malignant tumors recur locally. The longer a low-grade tumor persists locally or the more times it recurs, the greater is the likelihood for systemic spread by the tumor. Certain low-grade tumors such as central chondrosarcoma and low-grade osteosarcomas are notorious for undergoing ►dedifferentiation, with greatly increased likelihood for metastasis. The prognosis of patients with high-grade bone sarcomas has improved with the advent of present-day chemotherapy. For example, the 5-year survival for patients with osteosarcomas was in the range of 20% but is now better than 70% in some series.

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Borderline Appendiceal Mucinous Tumor

►Appendiceal Epithelial Neoplasms

BORIS

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Synonyms

Brother of the regulator of imprinted sites; CTCFL (stands for CTCF-like); CTCF-T (stands for CTCF-testis specific)

Definition

BORIS (acronym for *B*rother of the *R*egulator of *I*mprinted *S*ites) is a member of Cancer–Testis Antigen (CTA) family and a mammalian paralogue of ►CTCF, with the suggested role in the regulation of epigenetic reprogramming.

Characteristics

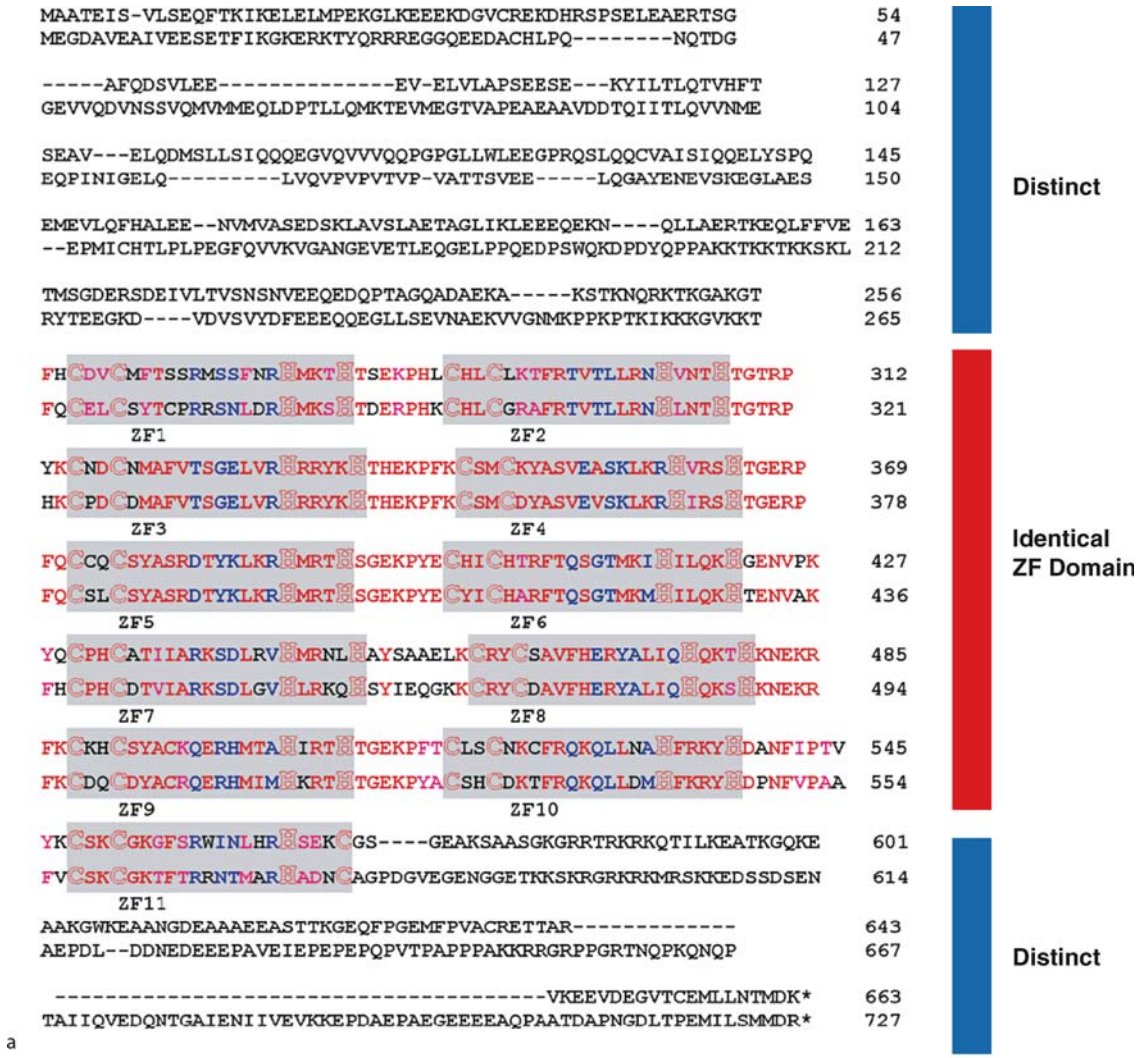
Studies on a transcription factor CTCF, a candidate ►tumor suppressor gene (TSG) involved in transcriptional regulation, control of imprinted genes, and insulators, led to a discovery of its paralogue, termed BORIS. The rationale behind the discovery of BORIS was to explain the process of resetting of ►imprinting marks in testis, in other words, how methylation-sensitive CTCF binding sites can be established in germ cells de novo. The existence of a protein with certain characteristics in these cells was therefore postulated. On one hand, such a hypothetic protein needed to recognize the same DNA sequences as CTCF to modify CTCF binding sites. On the other hand, this protein should be associated with a different biochemical machinery to erase and/or establish the methylation marks in germ cells that will later be read by CTCF in somatic cells. This hypothesis of the existence of germ cell specific molecule was later confirmed by the identification of BORIS, which was the ideal candidate for the role.

Firstly, in contrast to the ubiquitous CTCF, BORIS was only expressed in germ cells (spermatocytes) in testis; other testicular cells (Sertoli, Leydig, and the somatic cells) were BORIS negative. It is worth noting that, contrary to BORIS, CTCF was significantly down-regulated in the spermatocytes. Secondly, BORIS had the DNA binding domain identical to CTCF (74% identity), whereas the flanking N- and C-terminal domains were dissimilar (Fig. 1a). These structural features indicated that BORIS could recognize the same set of DNA targets as CTCF, while the dissimilar flanking domains could bring different interacting partners to the sequences to perform epigenetic modifications. The ability of BORIS to bind to a variety of DNA targets of CTCF was tested experimentally. In this study, all CTCF DNA targets inspected formed complexes with BORIS. This implies that evolutionary pressure maintained the same specificity of the DNA binding domain in CTCF and BORIS, thus suggesting important functions for both proteins; these functions are discussed in this and CTCF essays in the Encyclopedia.

BORIS is a 85 kDa protein; it can be detected both in the nucleus and in the cytoplasm, which is not common for transcription factors. This is in contrast to CTCF, which has strictly nuclear localization. The cytoplasmic form of BORIS may represent the inactive, sequestered protein, although the precise function(s) of the cytoplasmic BORIS remains to be investigated.

The human BORIS gene is mapped to chromosome 20q13 (Fig. 1b), whereas its mouse counterpart is mapped to chromosome 2, bands H3-4. Of note, the mouse chromosome 2 has significant homology to the human chromosome 20q. Surprisingly, BORIS not only possesses the DNA binding domain similar to CTCF, but its gene also preserved similar exon/intron structure, with identical splice sites for each exon–exon junctions in the ZF domain as the mammalian CTCF (Fig. 1c). It is worth noting that the exon/intron structure of

B



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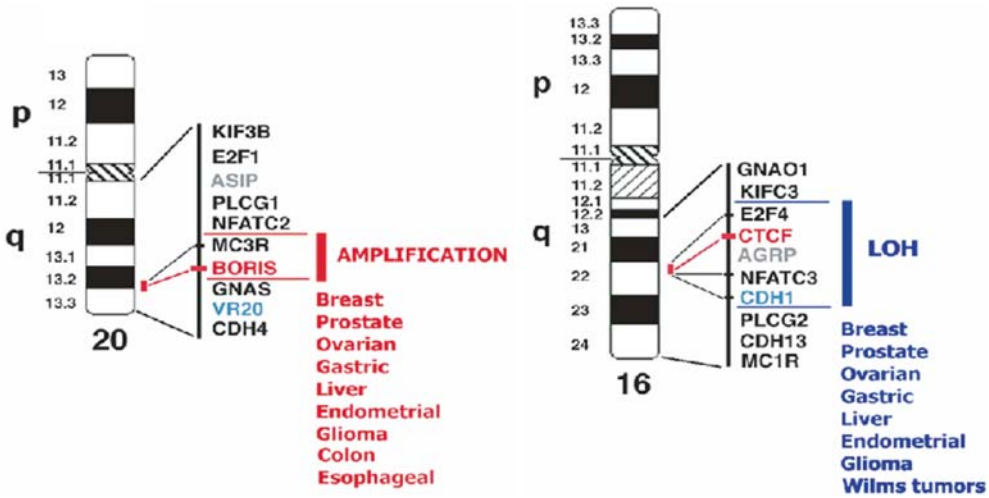

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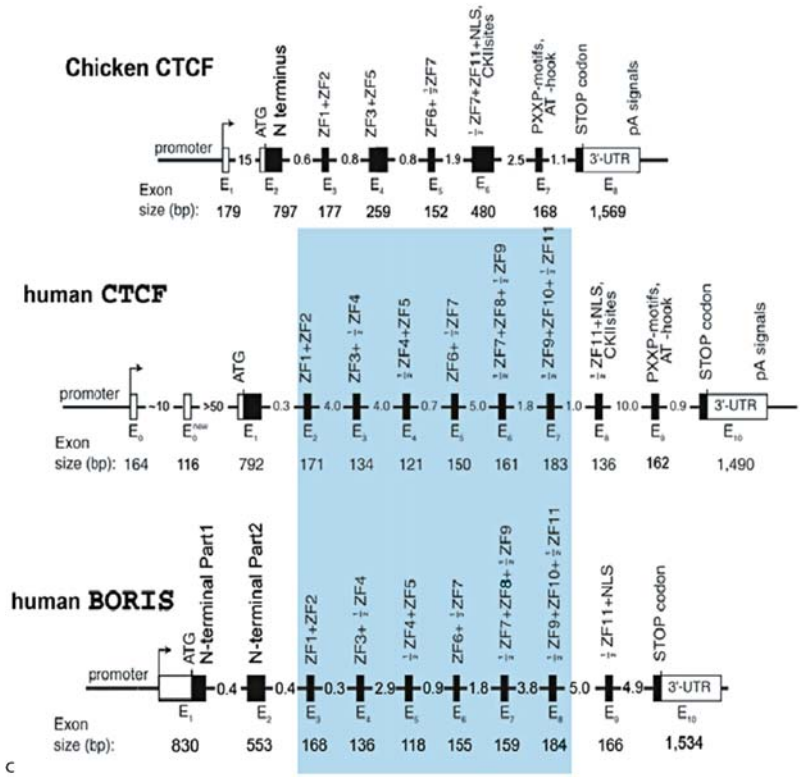
BORIS. Figure 1 Continued



BORIS. Figure 1 Comparative amino acid alignment of human CTCF and BORIS. Identical and homologous residues in the ZF domain are shown in red, homologous in magenta, nonhomologous in black. Residues responsible to form DNA contacts in Zn-fingers are shown in blue. Residues chelating Zn ion are shown in larger font sizes. (b) Both BORIS and CTCF map to cancer-associated "hot spots": on 20q13 and 16q22, respectively. According to the modified "two hit" hypothesis, two hits occur on different chromosomes – first in the region of frequent LOH at 16q22 where CTCF is localized and second in a region of 20q13 frequently amplified in multiple human cancers, where BORIS is localized. BORIS in this context can be regarded as a dominant-negative mutant of CTCF. (c) Comparison of the overall exon/intron structures of CTCF and BORIS genes. The region of homology over the exons encoding the ZF domain is highlighted. BORIS contains duplicated ZF-coding exons of mammalian, but not chicken, CTCF.

chicken CTCF is very different from that of the mammalian CTCF suggesting that BORIS gene most likely appeared in the evolution after the separation of mammals and birds possibly due to the duplication and translocation of the CTCF DNA-binding domain (Fig. 1c).

So far, the human and mouse BORIS genes have been cloned, while the rat, dog, and chimpanzee homologues of BORIS can be easily found using bioinformatics approaches, such as a homology search of the Genbank.

BORIS Functions

Although indirect evidence indicates that BORIS plays important functional roles in the cells where it is expressed, there are only a few published reports investigating BORIS functions. The fact that CTCF and BORIS are expressed in a mutually exclusive manner during male germline development led to the hypothesis that BORIS may be important for epigenetic reprogramming occurring in these cells during development. Indeed, BORIS has been implicated into the initiation of a series of methylation events at the imprinting control regions (ICR), in the vicinity of the CTCF/BORIS binding sites. In this model, BORIS interaction with a protein arginine methyltransferase PRMT7 leads to methylation of histones, followed by methylation of DNA by the de novo methyltransferase Dnmt3b and the establishment of a heterochromatic (silent) configuration of chromatin in this locus. However, the exact role that BORIS plays during spermatogenesis needs to be further investigated using knock-out and/or knock-in models.

When BORIS is introduced into cells that do not normally express this molecule, for example, into somatic cells such as normal human dermal fibroblasts, it activates a group of members of CTA family (MAGE-A1, MAGE-A2, MAGE-B1, MAGE-B4,

GAGE-3-8, RAGE-2, NY-ESO-1 (CTAG1B), LAGE-1 (CTAG2)) and also transcription factors playing a role in maintaining germ/stem cells phenotype (Oct-3/4, or POU5F1). The molecular events underlying this function of BORIS involve demethylation of the promoters of the genes in question, followed by activation of these genes. This implies the involvement of different biochemical machinery recruited by BORIS to the DNA targets in these cells. Interaction between BORIS and a transcription factor, SP1, facilitates derepression of *NY-ESO-1* gene in lung cancer cells. These findings further signify the importance of protein partners interacting with BORIS; the identification of such proteins will be instrumental in the understanding of the exact molecular mechanisms of BORIS functions in the processes of DNA methylation and demethylation.

Some of the CTAs (for instance MAGE-A1 and NY-ESO) are considered to be potential clinical targets for cancer immunotherapy. Therefore the investigations into how these genes may be regulated by BORIS are very important as they may provide the means to increase the expression from the relevant genes and enhance the response from the patients' immune system.

BORIS is capable to compete with CTCF on different targets both in vitro and in vivo. Taking into account the same DNA binding specificity, it is conceivable that the aberrantly expressed BORIS can act as an interfering mutation to CTCF. As CTCF regulates several genes implicated in cancer development (►*c-Myc*, *hTERT*, ►*BRCA1*, *IGF2*, ►*p53*, ►*p27*, ►*p21*, ►*ARF* etc. – see the essay on CTCF in this Encyclopedia), aberrantly expressed BORIS can compete with CTCF for binding thus ultimately leading to deregulation of those genes.

BORIS in Cancers

Some of BORIS features indicate that BORIS can be classified as an ►oncogene. It is located at the 20q13, together with Aurora kinase; this small 20q amplicon is a hotspot for chromosomal amplification in many human cancers (Fig. 1b). BORIS proximity to Aurora and their frequent coamplification raises an interesting possibility of potential cooperation of those two potential oncogenes in the process of cell transformation.

Normal BORIS expression is restricted to adult testis while abnormal expression is detected in a wide variety of cancers including breast, prostate, colon, melanomas, testicular, endometrial, and others. This expression pattern and the ability to induce immune response in patients, both antibody and cellular, place BORIS into a category of CTAs. The CTAs include around 14 families of tumor antigens. The function of the majority of the CTAs is still unknown, although some CTAs are though to be implicated in the regulation of gene expression and others may control gametogenesis. As a member of the CTA family, BORIS is now seen as an attractive target for both diagnostics and therapy of many human tumors (see below).

Although the mechanisms of BORIS activation in cancers are not yet known, the consequences of such an event can be dramatic. For example, BORIS can reverse the function of CTCF as a TSG by binding to CTCF targets and deregulating them. The possibility of such a "two chromosome hit" scenario, when a TSG is inactivated by the events occurring on two different chromosomes, creates a necessity to revise Knudsen's "two hit" theory, suggesting that while one copy of a TSG can be eliminated by loss of heterozygosity (LOH), the other copy can be either inactivated by epigenetic means or by somatic mutations. For the CTCF/BORIS pair, the second hit can occur at a different chromosome (20q13) by activation and subsequent chromosomal amplification of a gene with the same DNA binding specificity but different regulatory domains. This activation of a different gene on a different chromosome but capable of interfering with a tumor suppressor can be considered as analogous to the action of a dominant negative mutant (Fig. 1b). The aberrantly expressed BORIS is likely to interfere with the CTCF regulatory pathways that include a number of cancer-related genes, thus leading to deregulation of these genes and contributing to transformed phenotype.

BORIS interaction with the protein arginine methyl transferase PRMT7 that can result in DNA methylation and formation of heterochromatin may be responsible for silencing of some tumor suppressor genes that are inactivated in cancers due to aberrant methylation of the promoter regions. Finally, BORIS appears to be capable of the reversing the epigenetically silenced multiple CTAs, which results in activation of these genes and may contribute to tumor development.

Clinical Aspects

BORIS as a Cancer Biomarker

The identification of new markers to discriminate tumorigenic from normal cells, as well as the different stages of tumor pathology, has now become of critical importance for cancer diagnosis, prognosis, and monitoring. All currently available tumor markers are not ideal as in most cases they lack of sensitivity for early cancer and specificity for malignancy. Therefore, the quest to identify additional "cancer genes" implicated in breast tumorigenesis, along with delineation of prognostic biomarkers, has now become the most important step towards developing better diagnostic tools and a possibility of curing the disease. The finding that, similar to other CTAs, BORIS is aberrantly expressed in a wide variety of cancers points to important practical applications, namely, using BORIS as a molecular ►biomarker of cancer, especially for early diagnostics of the disease.

BORIS has the potential to be an early circulating marker, the detection of which may indicate the existence of a cancerous condition in the patient or of a predisposition of the patient to such a condition. In these investigations, BORIS was found to be present in the white blood cells (or leukocytes) in patients with breast cancer, but not in healthy donors. The type of the leukocytes was determined as the neutrophil polymorphonuclear granulocytes (PMNs). These findings place BORIS in a new category of cancer biomarkers, different from those currently used in medical practice.

The molecular mechanisms of BORIS activation and the functions in PMNs of breast cancer patients remain to be established. However, it is acknowledged that this is a tumor-related occurrence because BORIS was not detected in PMNs in donors with injuries, immune, and inflammatory diseases. This opens the perspective to utilize BORIS as a valuable blood marker for early detection of breast cancer (and may be other types of tumors), as well as the attractive target for early intervention and prevention of the disease.

This discovery is of great importance in the context of an ongoing quest to identify accurate circulating markers as far there are no established ►circulating tumor markers available for clinical use in the determination of cancer susceptibility, screening, diagnosis, and prognosis. The presence in such markers in blood makes them particularly useful since clinical analysis will involve relatively noninvasive procedures.

BORIS as a Target for Cancer Immunotherapy

Multiple CTAs are promising candidates for immunotherapeutic approach to treat cancer, although they have limitations. One of the main disadvantages for using these targets is their relatively narrow expression patterns. Another problem lies in the fact that sometimes during the treatment of the cancerous condition, the expression of the target antigen ceases without affecting the tumor growth, thus allowing tumor cells to escape from the immune response directed against them.

The fact that BORIS belongs to cancer–testis gene family as well as its wide expression in multiple cancers suggests that BORIS can be a good candidate for cancer immunotherapy. Using a mouse model, it was recently demonstrated that immune response to BORIS can be developed in the organism and, furthermore, such immune response has protective effects against several mouse tumors of different origin. Importantly, this immune reaction seems to be of a MHC class I-restricted response of cytotoxic T lymphocytes (CTLs) against histologically diverse tumor cells expressing only endogenous BORIS.

As BORIS seems to reverse tumor suppressor functions of CTCF in cancer, it is likely that BORIS is important to sustain the transformed phenotype. Therefore, if some tumor cells escape the anti-BORIS immune cells following the cessation of BORIS production in these cells, such BORIS-negative cells are expected to have much weaker growth/tumorigenic potential than BORIS-positive cells. This means that even if complete elimination of tumor can not be achieved, the patient still might benefit from anti-BORIS therapy.

In summary, BORIS has an excellent potential for immunotherapy and even preventive vaccination against cancers, although more research is required to advance BORIS to clinical trials.

►CCCTC-Binding Factor (CTCF)

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Bortezomib

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Synonyms

PS 341; Velcade®

Definition

An antineoplastic agent targeting the proteasome.

Characteristics

Mechanism of Action

Bortezomib is a proteasome inhibitor and the first drug to emerge from a new class of therapeutic agents

targeting the ubiquitin–proteasome pathway. This pathway mediates the degradation of polyubiquitinated proteins and accounts for 80% of the protein degradation in eukaryotic cells. Proteins that are marked for degradation undergo conjugation of polyubiquitin chains to lysine residues in a process called ubiquitination. The proteasome is an enzymatic complex that recognizes ubiquitin-tagged proteins and catalyzes their proteolytic breakdown in an ATP-dependent fashion. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells. It has been approved in the United States and Europe for the treatment of ►multiple myeloma and has recently been approved in the US for the treatment of relapsed ►mantle cell lymphoma. In addition, it has also shown significant antitumor activity in many other types of cancer. Due to the wide range of proteins that are subject to ubiquitination and subsequent proteasomal degradation, bortezomib interferes with multiple pathways ultimately leading to ►apoptosis. Proposed mechanism of action includes NF-κB inhibition trough reduced Iκα degradation, leading to reduced NF-κB-dependent synthesis of antiapoptotic factors such as c-Flip. Other mechanisms include inhibition of apoptosis (IAP)1/2 and ►BCL-2, stabilization of ►p53, deregulation of cyclin turnover, and subsequently ►cyclin-dependent kinases activity as well as effects on stability of cdc25 family proteins, ►KIP1 and WAF1 during cell cycle. In addition, it has been shown to influence the balance between pro- and antiapoptotic Bcl-2-family proteins, stabilizes JNK, as well as increases c-Jun phosphorylation and AP-1 DNA-binding activity with subsequent Fas upregulation. Other pathways include deregulated proapoptotic signaling via ►tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and disruption of the unfolded protein response with endoplasmic reticulum (ER) stress induction. Increased intracellular reactive oxygen species and oxidative stress may also contribute to its antitumor activity. Preclinical data suggests that bortezomib reverses the antiapoptotic effects of ►interleukin (IL)-6 and ►insulin growth factor (IGF)-1. It also reduces tumor cell migration in multiple myeloma cells and squamous cell cancer cells due to its effects on ►VEGF and dysregulation of focal ►adhesion assembly. Furthermore, it may affect the tumor ►microenvironment thus exerting an anti-angiogenic effect. As a result, bortezomib ultimately triggers an apoptotic cascade mediated by ►caspases. The number of proposed mechanisms is likely to grow in the future as none of these pathways can fully explain the clinical effectiveness of bortezomib in malignancy, and research is under way to fill these gaps. Depending on the tumor cell type, one or a combination of several pathways may be responsible for the clinical effects and different cell types may therefore be more or less sensitive to the effects of bortezomib. For example, terminally differentiated immumoglobulin-secreting plasma cells have impaired proteasome activity, which could explain bortezomib's activity against malignant plasma cells in multiple myeloma.

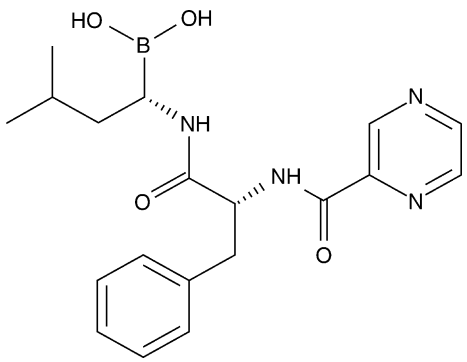
Pharmacology

The chemical formula for bortezomib is [3-methyl-1-(3-phenyl-2-pyrazin-2-ylcarbonylamino-propanoyl)amino-butyl] boronic acid. The chemical structure is shown in Fig. 1. Bortezomib is administered intravenously and weekly once and twice dosing schedules have been tested. The mean elimination half-life of bortezomib after first dose ranges from 9 to 15 h at doses ranging from 1.45 to 2.00 mg/m^2 in patients with advanced malignancies. In vitro studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is oxidatively metabolized primarily via cytochrome P450 enzymes 3A4, 2C19, and 1A2, while bortezomib metabolism by CYP 2D6 and 2C9 enzymes is minor. The major metabolic pathway is deboronation to form two deboronated metabolites that subsequently undergo hydroxylation to several metabolites, which are inactive as 26S proteasome inhibitors. The most common side effects include asthenia, nausea, diarrhea, anorrhexia, constipation, ►thrombocytopenia, peripheral neuropathy, and pyrexia.

Clinical Aspects

In Cancer

The effectiveness of bortezomib is based on response rates which led to the accelerated approval of bortezomib by the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). The approval of bortezomib was based on an open-label, single-arm, multicenter study of 202 subjects with multiple myeloma who had received at least two prior therapies. An IV bolus



Bortezomib. Figure 1 Chemical structure of bortezomib.

injection of bortezomib (1.3 mg/m2/dose) was administered twice a week for 2 weeks, followed by a 10-day rest period (21-day treatment cycle) for a maximum of eight treatment cycles. Subjects who experienced a response to bortezomib treatment were allowed to continue treatment in an extension study. Results showed 52 (27.7%) subjects achieved an overall response rate, 5 (2.7%) achieved a complete response, 47 (25%) achieved a partial response, and 33 (17.6%) demonstrated a clinical remission. The ►Kaplan–Meier estimated median duration of response was 1 year. In another trial, bortezomib was compared with high-dose ►dexamethasone in 669 patients with relapsed multiple myeloma, which confirmed superior activity of bortezomib over dexamethasone resulting in a higher response rate, a longer time to progression (the primary end point), and a longer survival than patients treated with dexamethasone. The combined complete and partial response rates were significantly longer for the group receiving bortezomib (38% vs. 18%), and the complete response rates were 6% and less than 1%, respectively. Median times of progression in the bortezomib and dexamethasone groups were 6.22 months (189 days) and 3.49 months (106 days), respectively. The 1-year survival rate was 80% among patients treated with bortezomib and 66% among patients treated with dexamethasone, and the hazard ratio for overall survival with bortezomib was 0.57. Since then, more data from phase 2 and 3 trials have emerged and have confirmed the activity of bortezomib in relapsed and newly diagnosed multiple myeloma. Trials evaluating the combination of bortezomib and other drugs such as ►thalidomide and its analog lenalidomide are ongoing and the results will be available in the near future. Bortezomib has recently been approved for the treatment of relapsed and refractory mantle cell lymphoma. The approval was based on data from an open-label, single-group, multicenter phase 2 clinical trial of 155 patients with relapsed or refractory mantle cell lymphoma who had received at least one prior therapy. Participants received single-agent bortezomib (1.3 mg/m^2 twice a week for 2 weeks every 21 days) for up to a year. Results showed that 31% of patients achieved overall response to bortezomib, with 8% demonstrating a complete response, and 23% a partial response, as determined by computed tomography scan reviews. The median number of cycles in responding patients was eight, and the median time to response was 40 days. The median duration of response was 9.3 months overall and longer for those achieving complete response compared with those with partial response to bortezomib. The median time to progression of disease was 6.2 months.

Trials are also investigating thalidomide in combination with bortezomib in patients with ►myelodysplastic syndromes. Phase 2 studies on bortezomib in other types of lymphoma are ongoing and interesting results have been presented as abstracts which indicate that bortezomib may be useful in the treatment of these diseases with high response rates and substantial number of complete remissions in pretreated patients.

Early data is also emerging showing that bortezomib has activity in various solid tumors such as lung, head and neck, prostate cancer. Preclinical and early clinical data suggests that bortezomib may enhance the activity of EGF-R inhibitors through the upregulation of the EGF-receptor. As trials are under way to evaluate the use of bortezomib alone and in combination with other agents, other indications for bortezomib in solid tumors are likely to emerge.

In Graft Versus Host Disease and Immune Disorders

The use of bortezomib in graft versus host disease (GVHD) is based on bortezomib's effect on antigen procesing, apoptosis, cell-cycle onco-stimulation, and chemotaxis. The ability of proteasome inhibitors to prevent NF-κB activation has made proteasome inhibitors attractive candidates for the treatment of immune-mediated disorders. Preclinical and early clinical data suggests that proteasome inhibitors may be effective in the treatment of osteoarthritis, psoriasis, and a spectrum of other autoimmune conditions. In preclinical models, GVHD was effectively prevented without affecting the beneficial graft versus tumor effects. At this point it is unknown whether this will be reproducible in humans. It appears that bortezomib has direct proapoptotic effects on human T-lymphocytes which has been shown in vitro which may explain the clinical observation of lymphopenia in some patients undergoing treatment with bortezomib.

Other Uses

Preclinical models suggest that bortezomib may also be useful in the treatment of cardiovascular disease and ischemic strokes. It is unclear whether these effects may be related to its effects on the NF-κB inactivation; cytokine secretion and modulation of cell adhesion may also play an important role.

Future Directions

Other compounds that target the proteasome are in preclinical and clinical development. NPI-0052, a compound derived from the marine actinomycete *Salinospora tropica*, an inhibitor of the 20S subunit of the proteasome, is currently undergoing early phase clinical testing. It has shown significant activity in multiple myeloma.

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Bovine Papillomavirus

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Definition

Oncogenic DNA viruses causing both benign and malignant epithelial and mesenchymal tumors in cows and equids.

Characteristics

Bovine papillomaviruses (BPV) belong to the Papillomaviruses (PV) family. BPV are small oncogenic DNA viruses strictly specie-specific and, even in experimental conditions, do not infect any other host than the natural one. The only known case of cross-species infection is the infection of horses and other equids by BPV type 1 (BPV-1) or BPV type-2 (BPV-2). Papillomavirus infections usually regress, but occasionally they develop to cancer.

Ten BPV types (BPV 1–10) have been characterized associated with different histopathological lesions. The different genotypes have been classified into three genera. Two noval BPV were characterised further and the phylogenetic analysis showed that both viruses were new BPV types: one was designated as BPV-7 and classified as a member of a new PV genus, and the other was designated as BPV-8 and classified as a member of the *Epsilonpapillomavirus* genus.

Recently, two new BPV types belonging to the genus *Xipapillomavirus* have been characterised and designed as BPV-9 and BPV-10.

Xi-papillomaviruses encompassing the pure epitheliotropic BPV-3; BPV-4 and BPV-6; Delta-papillomaviruses encompassing BPV-1 and BPV-2 associated to fibropapillomas (i.e., benign tumors of both epithelium and underlying derma) and Epsilon papillomavirus comprising the BPV-5 whose genome seems to share similarities with the former two BPV groups.

The BPV virion is a nonenveloped structure of 55–60 nm diameter containing a double-stranded covalently closed circular DNA. Three different regions compose the genome: the long control region (LCR) and two regions encoding for early and late genes.

The LCR is the genome region containing signals for both viral DNA replication and transcription. E2 regulates BPV transcription at LCR level; the LCR of BPV-4 contains different E2 binding sites; depending on the sites involved the transcription may be repressed or activated. The E2 sites are also bound by different cellular transcription factors and the E2 can also bind to mitotic chromosomes resulting in efficient distribution of the BPV genome into daughter cells.

BPV Gene Products

E5

The papillomavirus ►E5 proteins are short hydrophobic polypeptides [from 83 amino acid residues in ►human papillomavirus type 16 (HPV-16) to 42 residues in BPV-4], many of which have transforming activity. *BPV-1 E5* oncogene encodes for a 44 amino acid protein that is the major BPV transforming oncoprotein. It is a type II transmembrane protein which is expressed in the deep layers of the infected epithelia and is largely localized to the membranes of the endoplasmic reticulum (ER) and Golgi apparatus (GA) of the host cells. BPV E5 is expressed in the cytoplasm of both basal and suprabasal transformed epithelial cells with a typical juxtanuclear pattern due to its localization in the GA. It may also be expressed in neoplastic cells of mesenchymal origin such those of endothelial origin.

Due to its relative small size BPV E5 has no intrinsic enzymatic activity and its transformation activity is related to the activation of several kinases, from growth factor receptor to cdk cyclins. E5 interacts with the 16-K subunit c protein, a component of the vacuolar H^+-ATPase pump. This proton pump acidify the lumen of intracellular compartments, (endosomes, lysosomes, and GA) that process growth factors so that E5 binding may result in alteration of this processing. Another consequence of E5-mediated impaired acidification is the downregulation (both in vivo and in vitro) of the major histocompatibility complex class I (►MHC-I) expression, representing one of the mechanisms by which the BPV evade the immunoresponse by the host.

The mechanism by which BPV-1 E5 induces cell transformation is its binding to and activation of the cellular β receptor for the ►platelet-derived growth factor (PDGFβ). The activation of endogenous PDGFβ receptors is characterized by the formation of stable E5-receptor complexes, persistent tyrosine phosphorilation of the receptor, its dimerization and cellular transformation. This interaction takes place also in naturally occurring BPV-2 associated bovine urinary bladder cancer.

E6

The *BPV-1 E6* gene of Xi BPV encodes an oncoprotein of 137 amino acids. It binds to paxillin blocking its

interaction with vinculin and the focal adhesion kinase. It also binds to several others cellular proteins such as ERC-55, the E3 ubiquitin ligase E6AP, and with the AP-1. Finally, it has been demonstrated that E6 interacts with the CBP/p300 inhibiting the p53.

E7

The *BPV E7* gene encodes a 127 amino acids zinc binding protein which cooperates with E5 and E6 in inducing cell transformation. Once ►E7 is coexpressed with E5 and E6 its transformation capacity increases many folds, and such coexpression may also occur in tumors of mesenchymal origin. BPV-1 E7 transformation function correlates with its binding to a cellular target p-600, which is a shared transformation pathway of HPV-16 E7.

The BPV-4 E7 can also cooperate with E8 in inducing cellular transformation and the activation of the ►ras oncogene is responsible for morphological changes of primary bovine fibroblasts (PalF). Like other E7 PV, BPV-4 E7 has a p105Rb-binding domain whose mutation may reduce or abolish its transforming activity,

BPV E7 localizes in the cytoplasm and nucleoli of basal and lower spinous epithelial cells. It may also be found in mesenchymal neoplastic cells.

L1 and L2

The BPV late proteins L1 and L2 are expressed into the more differentiated epithelial cells. The former mediates virus interaction with cellular receptor, the latter induces virion assembly by binding to viral DNA.

Infection by delta-PVs leads to transformation of subepithelial fibroblasts followed by epithelial acanthosis and then papillomatosis, while infection by Xi-PVs induces transformation only of the epithelial component. Virus replication can take place only in keratinocytes undergoing terminal differentiation to squamous epithelium, so it is seen only in the epithelial component of the tumors and only at certain stages of its development. Virus replication has never found in fibroblasts where the BPV genome is present in a nonintegrated episomal form, although *BPV* viral gene expression has been recently found in tumors of mesenchymal origin such those arising from blood vessels (hemangioma and ►hemangiosarcoma) suggesting a role of the virus even in neoplastic transformation other than epithelial.

BPV and Papillomas

►Papillomas and fibropapillomas may occur in different organs in cattle and different BPV genotypes are found. BPV-1, BPV-5, and BPV-6 are associated to papillomas of the teats and udders in cows. This can become a great economic problem once the papillomas spread around the primary tumors and the cows cannot be milked, veals are unable to suckle properly and the site may become infected inducing mastitis. Occasionally, the herds should be culled if the papillomatosis progress.

Epithelia of both prepuce and penis may be infected by BPV-1 resulting in fibropapillomas. The tumors can spread along the perineum and even up toward the back; they can become necrotic and cause loss of reproductive functions.

BPV-4 induces fibropapillomas of the upper gastrointestinal (GI) tract. All sites form the tongue to stomach can be affected. Healthy cattles normally recover from papillomatosis in approximately one year time, but if the animals are not able to reject the tumors they are at high risk to develop cancer such as squamous cell carcinoma.

Normally, these benign lesions (papillomas and fibropapillomas) regress but some animals may even die due to widespread cutaneous or mucosal papillomatosis if they are not able to reject the infection. Progression of benign persistent lesions to cancer occur once cofactors synergize with the virus.

BPV and Cancer

One of the major environmental cofactor involved in BPV-associated carcinogenesis is the ►bracken fern (genus *Pteridium*), the only higher plant proven to cause cancer naturally in animals.

Bracken eating animals may develop cancer since the plant contains immunosuppressants as well as a number of mutagens and oncogenic principles such as ►ptaquiloside. Bracken-fed cows become chronically immunosuppressed and the latent BPV is activated. Full malignant transformation depends on others mutagens that are believed to trigger *BPV* gene expression leading to initiation and development of cancer.

Additionally, bracken eating animals develop a clinical syndrome known as chronic enzootic hematuria and chromosomal abnormalities.

Field cases of urinary bladder and GI cancers in cattle occur wherever the plant is spread. The disease is known to occur in continental Europe, Azores Islands, in some regions of Kenya, Brasil, New Zealand, India, and in China. Human exposure to bracken fern directly or indirectly through milk from bracken eating cattle has been linked to human GI cancer.

Cows affected by BPV-4-associated papillomas of the upper GI tract and naturally exposed to bracken fern are at high risk of developing carcinoma. The fern induces immunosuppression and the fibropapillomas spread, additionally the mutagens from the plant such as ►quercetin and ptaquiloside act synergistically with

the virus in the carcinogenic process. The BPV-4 E7 oncoprotein cooperates with quercetin for neoplastic transformation, in so doing the ras oncogene is activated, the p53 is mutated and the number of the cellular receptors for epidermal growth factors is increased. From a comparative point of view it is worth noting that some human GI cancer may have the same etiology: papillomavirus and bracken suggesting that similar molecular mechanisms underlying bovine cancer may even occur in humans.

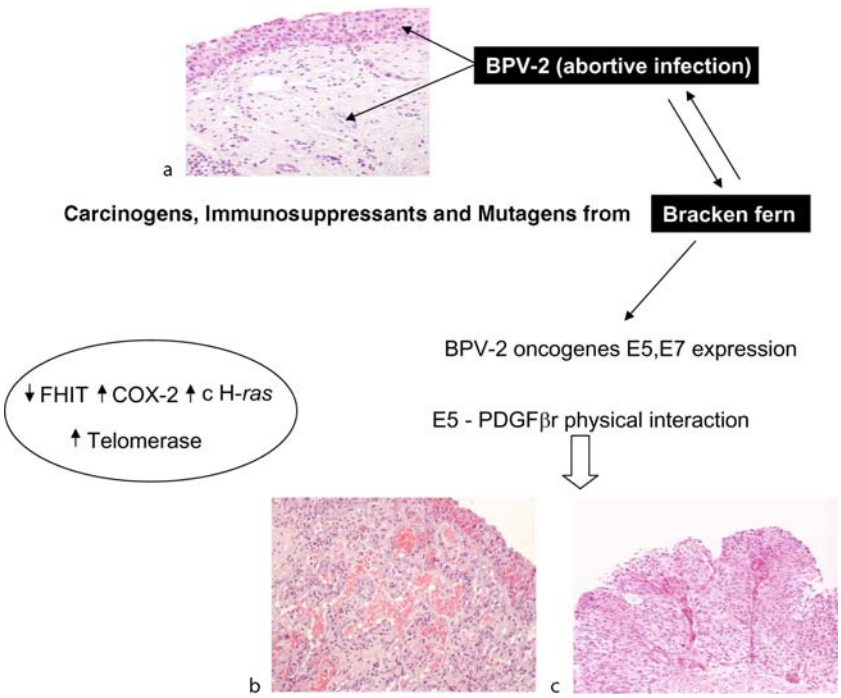
At the same time cows suffering from chronic enzootic haematuria may develop urinary ►bladder cancer. The cancer are of both epithelial and mesenchymal origin with ►hemangiosarcomas being the most frequent histotype. In both cases the BPV-2 is involved testifying that the virus is not a pure epitheliotropic agent in its natural host. The BPV-2 infects the urinary bladder mucosa inducing an abortive and latent infection with no production of virions. The exposure to immunosuppressants, mutagenic and carcinogenic principles from bracken triggers viral gene expression leading to cell transformation. In both cancers of epithelial and mesenchymal origin the BPV-2 E5 oncoprotein is expressed and is in complex with the activated form of the PDGFβ receptor. Additionally, in ►urothelial cancers the ►telomerase activity is upregulated, expression of ras and ►cyclooxygenase-2 (COX-2) is increased and, as already observed in HPV-associated ►cervical cancer, the ►fragile sites are disrupted and the expression of the tumor suppressor fragile histidine tetrads (►FHIT) is downregulated (Fig. 1).

BPV and Equine Sarcoids

The ►sarcoids are benign tumors of fibroblastic skin origin affecting horses, mules, and donkeys. They are locally invasive often occurring at sites of previous injury or scarring. Tumors can exist as single or multiple lesions in different forms. Clinically, five different types of sarcoids can be distinguished: Occult sarcoid: is an hairless circular area of the skin; Verrucous: tumors with wart-like appearance; Fibroblastic sarcoids present as a fleshy mass; Nodular sarcoids consist of firm masses lying under the skin and mixed sarcoid show a combination of features of verrucous, fibroblastic, and nodular types.

It is the most common dermatological neoplasm reported in horses. The most common sites of appearance is the skin of the head, ventral abdomen, legs, and the paragenital region.

Despite the failure to isolate any papillomavirus from the sarcoids, a large body of evidence strongly support the hypothesis that BPV is the etiological agent of this tumor.



Bovine Papillomavirus. Figure 1 Schematic representation of the multi-step carcinogenesis of bovine urinary bladder tumors. Histological sections of (a) normal bladder mucosa; (b) hemangiosarcoma; (c) papillary urothelial carcinoma.

Both BPV-1 and BPV-2 have been detected in sarcoid tumors with the BPV-1 being the predominant type. The BPV exsists as episomally and its major oncoprotein E5 is expressed, thus suggesting the viral genes are expressed.

Equine sarcoids is a biologically attractive tumor since it is the only known case of natural cross-species PV infection. Moreover, while BPV infection in cattle produce benign lesions that may regress, the sarcoids are nonpermissive for virus production, locally aggressive and nonregressing.

Cell cycle regulatory proteins are involved in the pathogenesis of equine sarcoids. P53 is stabilized in sarcoid cells being expressed in the nuclei as well as in perinuclear region, however its transactivation function is abrogated. Low levels of cell proliferation are characteristic of sarcoids with no overexpression neither of cyclin A, $p27^{kip1}$ nor of CDK-2.

The loss of p53 function and the low levels of cell proliferation indicate that sarcoid cellular and molecular pathology may not be associated with abnormal cell cycle control mechanisms.

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Bowen Disease

Definition

A red patch on the mucosa that is not attributable to any obvious cause. Generally, these lesions have a well-defined border and a soft, velvet-like appearance. Their atrophic nature contributes to the red coloration, as underlying vasculature is more prominent. Around 90% show signs of severe dysplasia or carcinoma-in-situ and may progress to invasive squamous cell carcinoma.

►Squamous Cell Carcinoma

Bowman-Birk Inhibitor (BBI)

Definition

Serine protease inhibitor consisting of a single chain of 71 amino acid residues cross-linked by seven pairs of disulfide bonds and a well characterized ability to inhibit trypsin and chymotrypsin. It has been shown to be capable of preventing or suppressing carcinogenic processes in a wide variety of *in vitro* and *in vivo* animal model systems.

►Lunasin

Boyden Chambers

Definition

A hollow plastic chamber sealed at one end with a porous membrane and suspended in a well containing chemoattractants. Cells are placed inside the chamber and allowed to migrate through the pores to the other side of the membrane.

53BP

Definition

p53 Binding Protein 1, a checkpoint molecule that acts as a mediator for transducing DNA damage signals, especially following detection of DNA double-stranded breaks

►BRIT1 Gene
►p53 family

BR 27-29

►Serum Biomarkers

Brachytherapy

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Synonyms

Endocurietherapy; Radioactive seed therapy

Definition

Brachytherapy treatments deliver radiation dose using radioactive isotopes placed via applicator devices or catheters directly into tumors or into cavities in close approximation to the tumor.

Characteristics

Radiation therapy is the treatment of cancer with radiation. Radiation targets the DNA in cells and causes DNA strand breaks. Normal cells have the ability to repair the DNA damage, whereas cancer cells lack such repair mechanisms.

Brachytherapy is one method of delivering radiation. The word "brachy" is derived from Greek meaning "short." The radiation from the radioactive isotopes penetrates a short distance, allowing for conformity to a target volume or tumor while sparing the normal structures in the vicinity. The ►dose fall-off for brachytherapy sources follows the inverse square law, in that the distance traveled by the radiation is inversely proportional to the square of the radius of distance ($d = 1/r^2$).

Historically, the isotopes used for brachytherapy were radium, radon and its derivatives, and gold. In modern times, isotopes must be non-gaseous, have effective energies for treatments, be able to be encapsulated in a size that is clinically useful, and have a ►half-life either suitable for permanent implants or temporary implants (Table 1).

Dose Rate

Brachytherapy treatments can utilize different ►dose rates to treat cancer. The definitions for dose rates were defined by the International Commission on Radiation Units and Measurements Report #38. Low dose rate (LDR) is defined as a range of 0.4–2 ►Gray (Gy)/h. Medium dose rate (MDR) is a range of 2–12 Gy/h, and high dose rate (HDR) is defined as >12 Gy/h. VLDR (very low dose rate) radiation is used in permanent radioactive seed implants, at a dose rate of less than 40 cGy/h. Temporary implants are placed into the tumor/adjacent tissues in order to deliver LDR, MDR, or HDR treatments. VLDR implants typically reside permanently in the tissue implanted, but decay over the course of a few months. In the delivery of LDR or MDR radiation, the temporary implant stays in place over several hours, whereas HDR treatments usually last only a few minutes.

LDR techniques involve the static placement of radiation isotopes within the applicators for a period of time. ^{137}Cesium (^{137}Cs) for gynecologic brachytherapy or ^{192}Iridium (^{192}Ir) for ►gynecologic cancers or sarcomas are most commonly used. The radiation is either manually afterloaded by a physician or can be remotely afterloaded if a cesium selectron afterloader (for gynecologic brachytherapy) is available. HDR treatments involve a single ^{192}Ir source fixed to a wire that is guided remotely by a computer. The HDR afterloader attaches to individual applicators by transfer tubes. Computer programming determines the position of the radiation isotope within the applicator, and calculates a radiation ►isodose curve that may be manipulated by altering the dwell times. ►Dwell positions are defined along the applicators every

Brachytherapy. Table 1 Characteristics of some commonly used radioisotopes in the United States

Isotope	Half-life	Energy (MeV)
^{137}Cesium	30 years	0.66
^{192}Iridium	74 days	0.29–0.6
^{125}Iodine	60 days	0.028
^{103}Paladium	17 days	0.023

2.5–10 mm, and the isotope remains at designated dwell positions for a preset time as determined by the optimized plan. LDR radiation may have a ►radiobiological advantage over HDR radiation, as the normal tissue is more likely to be able to repair sublethal damage. Additionally, the continuous dose may prevent repopulation of the tumor cells, and the longer period of time that the cells are exposed to radiation allows the cell cycle to move through radio-resistant and radiosensitive phases. HDR radiation may lead to an increase in normal tissue toxicity if the total dose delivered compared to LDR is not decreased. It is important to fractionate the HDR radiation sufficiently and deliver as small a fraction size as feasible depending on the tissue treated, the indications for treatments and the amount of normal tissue in proximity to the source. In ►cervical cancer brachytherapy, packing the vagina can reduce the amount of normal tissue exposed to radiation.

Pulsed dose rate (PDR) brachytherapy uses an HDR afterloader and source but attempts to mimic the radiobiologic effect of LDR by giving a large number of very small fractions over a longer period of time than HDR.

Dose Calculations

Historically, the dose delivered to a treatment volume was hand-calculated, based on one of three methods of implantation. The Paris and Quimby systems place parallel sources with uniform spacing and source activity, to give a higher central dose compared with the periphery. The Paterson–Parker method utilizes higher peripheral radioactivity compared with the centers resulting in increased ►dose homogeneity throughout the implant. These methods have been replaced in several radiation oncology clinics by computer programs that utilize information gathered from imaging techniques such as CT scans to define the target volume and identify the implant geometry within that volume to calculate the dose.

Implantation Techniques

The placement of the radiation source in relation to the treatment volume is the most important determinant in the effectiveness of brachytherapy. Therefore the techniques used depend on the location of the tissue being targeted.

Surface applicators involve sculpting a radiotherapy delivery system on or around the target surface area. A superficial dose may be delivered to lesions of the skin or intraoperatively to exposed tumor beds.

Intracavitary radiation utilizes orifices within the human body to introduce applicators in close proximity to the tumor. Common examples of intracavitary radiation include gynecologic malignancies, in which the vagina, cervical os, and uterine cavity allow for the relatively easy placement of applicators. Other intracavitary treatments include the bronchus, esophagus, and rectum.

Interstitial radiation entails passing catheters through normal tissue to reach the target volume or placing tubes within a surgical bed at the time of operation.

Clinical Applications of Brachytherapy

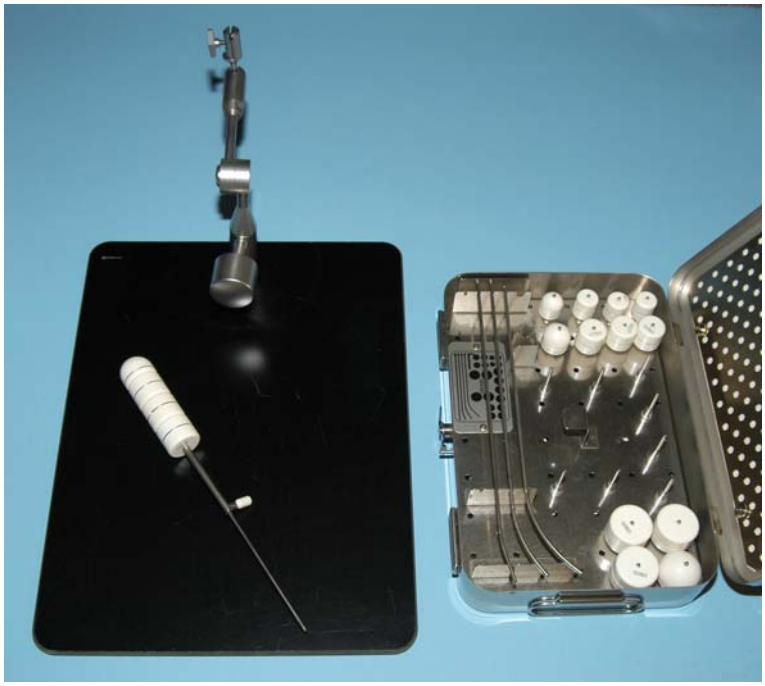
Brachytherapy may be administered alone or in combination with external beam radiation, chemotherapy, or surgery to provide either cure or palliation for the patient. The most common uses of brachytherapy are discussed below.

Gynecologic Malignancies

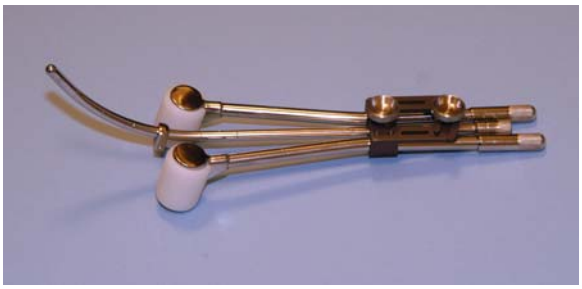
The most common gynecologic malignancy treated with brachytherapy in the United States is ►endometrial cancer. Intracavitary radiation targets the vaginal vault in women thought to be at high risk of local recurrence to the vagina following definitive surgery. This treatment involves the insertion of a cylinder into the vagina (Fig. 1). LDR or HDR radiation may be used. The dose and fractionation of the radiation depend on both the dose rate and the patient's history of prior external beam radiation therapy. The dose may be prescribed at either the surface of the applicator or at a depth, typically 5 mm, from the applicator.

Cervical cancer is treated using a combination of external beam radiation with or without chemotherapy and brachytherapy, commonly referred to as a tandem and ovoid application. A central uterine tandem is placed through the cervical os into the uterine cavity. Vaginal ovoids or a vaginal ring or cylinder are secured to the central tandem (Fig. 2). Historically, cervical cancer brachytherapy was administered using LDR radiation, most commonly using tandem and ovoids placed twice with one week between treatments. In most centers, plain films assess the location of normal tissue structures; however, several radiation oncology clinics have acquired CT imaging capability, allowing for 3D imaging of the normal tissues and more accurate dose calculation. In more recent times, several centers have incorporated HDR radiation into the management of cervical cancer. HDR tandem and ovoid dose is delivered in minutes, and most commonly requires four or five separate insertions, with each treatment lasting several minutes. The HDR isodose curve approximates a standard LDR loading (Fig. 3).

Vulvar and vaginal cancers are rare, but their treatment may involve interstitial or intracavitary radiation after external beam radiation.



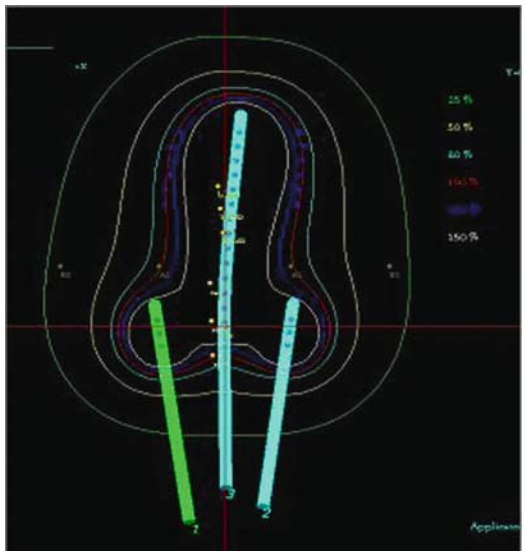
Brachytherapy. Figure 1 A high dose rate vaginal cylinder is inserted into the vagina to treat the vaginal surface for patients who have had a hysterectomy for uterine or cervical cancer. The applicator is attached to a brachytherapy board for stabilization.



Brachytherapy. Figure 2 Low dose rate Fletcher–Suit–Delclos tandem and ovoid applicator will be loaded with ^{137}Cs. The central tandem is inserted into the uterus. The ovoids may have plastic caps placed over them in order to fill the vaginal fornices. A flange rests outside the external os of the cervix. The apparatus is held in place by vaginal packing.

►Prostate Cancer

Brachytherapy in prostate cancer may be the sole treatment for low-risk disease or in combination with external beam radiation as a form of dose escalation. VLDR brachytherapy places permanent radioactive seeds of either ^{125}I or ^{103}Pd into the prostate through the perineal skin, under image guidance and using catheters. The seeds remain permanently within the prostate and deliver a low dose of radiation continuously until they have decayed. HDR brachytherapy for prostate cancer is currently being investigated in research protocols.



Brachytherapy. Figure 3 High dose rate tandem and ovoid isodose curve demonstrates the 100% isodose line optimized to point A, a point 2 cm above and lateral to the cervical os.

Other

Other cancers that can be treated with brachytherapy as part of combined care include head and neck cancers, including nasopharynx and tongue, breast cancers, sarcomas, thoracics and some gastrointestinal cancers.

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Bracken Fern

Definition

A worldwide diffuse plant belonging to *Pteridium* genus known to cause cancer naturally in animals. Bracken fern eating is also related to human cancer.

Bradykinin

Definition

Bradykinin is an active peptide of the kinin protein group. It consists of nine amino acid residues and is a potent vasodilator.

►Kallikreins

B-*raf*-1

►B-Raf Signaling

BRAF1

►B-Raf Signaling

B-Raf Signaling

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Synonyms

v-*raf* murine sarcoma viral oncogene homolog B1; B-*raf*-1; BRAF1; EC 2.7.11.1; MGC126806; MGC138284; *RAFB*1; p94; c-R*mil*

Definition

B-Raf signaling comprises the activation of the proto-oncogene product B-Raf and its downstream effectors and represents a key regulatory step in the activation of the canonical ►MAP kinase pathway by various extracellular stimuli and oncogene products such as ►RAS and activated receptor tyrosine kinases like ►NTRK and ►RET. Aberrant B-Raf activity as a result of somatic mutations is observed in 8% of human cancers.

Characteristics

Physiological Aspects of B-Raf Signaling

B-Raf is a member of the ►Raf kinase family and represents an important component of the Ras/Raf/MEK/ERK MAP kinase signal transduction pathway, which plays a pivotal role in growth control and differentiation. Dysregulation of this pathway is observed in about 30% of human tumors and represents an established mechanism for tumorigenesis. In their role as gatekeepers of this pathway, Raf-kinases appear as attractive targets for therapeutic intervention. The Raf-family contains three genes in vertebrates, A-Raf, B-Raf and Raf-1 as well as D-Raf and LIN-45 in *Drosophila* und *Caenorhabditis*, respectively. While the *RAF1* gene displays a ubiquitous and prominent expression pattern, B-Raf is predominantly expressed in neuro-ectoderm derived tissues, placenta, the hematopoietic system and the testis. However, gene targeting experiments in mice and ►DT40 B cells revealed that B-Raf represents the major ERK activator, even if it is expressed at barely detectable levels, whereas Raf-1 serves as an accessory ERK activator. Among the three mammalian isoforms, B-Raf displays the highest affinity towards its substrate MEK and has the highest activities in biological and ►*in vitro* kinase assays. In many cell types, B-Raf plays a non-redundant role in the maintenance of ERK signaling induced by various extracellular signals and thereby regulates directly, or in concert with other signaling pathways, the expression of important target gene products such as growth factors and cytokines. The importance of B-Raf for the efficient expression of ERK-regulated target gene products is most likely

explained by the fact that ERK activation is not only required for the induction of ►immediate early genes transcription, but also for the stabilization of the resulting proteins by phosphorylation through sustained ERK signaling. The correlation between B-Raf expression and sustained ERK signaling has been implicated in various physiological processes such as lymphocyte activation, myelopoiesis, angiogenesis, development of extra-embryonic tissues as well as for the growth-factor-mediated survival of neurons and their effector functions. The discovery of germ-line mutations with mostly slight to moderate gain-of-function character in the *SOS*, *KRAS*, *HRAS*, *SHP2/PTPN11*, *BRAF* and *MEK1/2* genes in patients suffering from the various ►neuro-cardio-facial-cutaneous syndromes illustrates that tight control of this pathway upstream or at the level of the B-Raf/MEK interface is key to the normal development and homeostasis of many organs.

B-Raf Signaling and Tumor Development

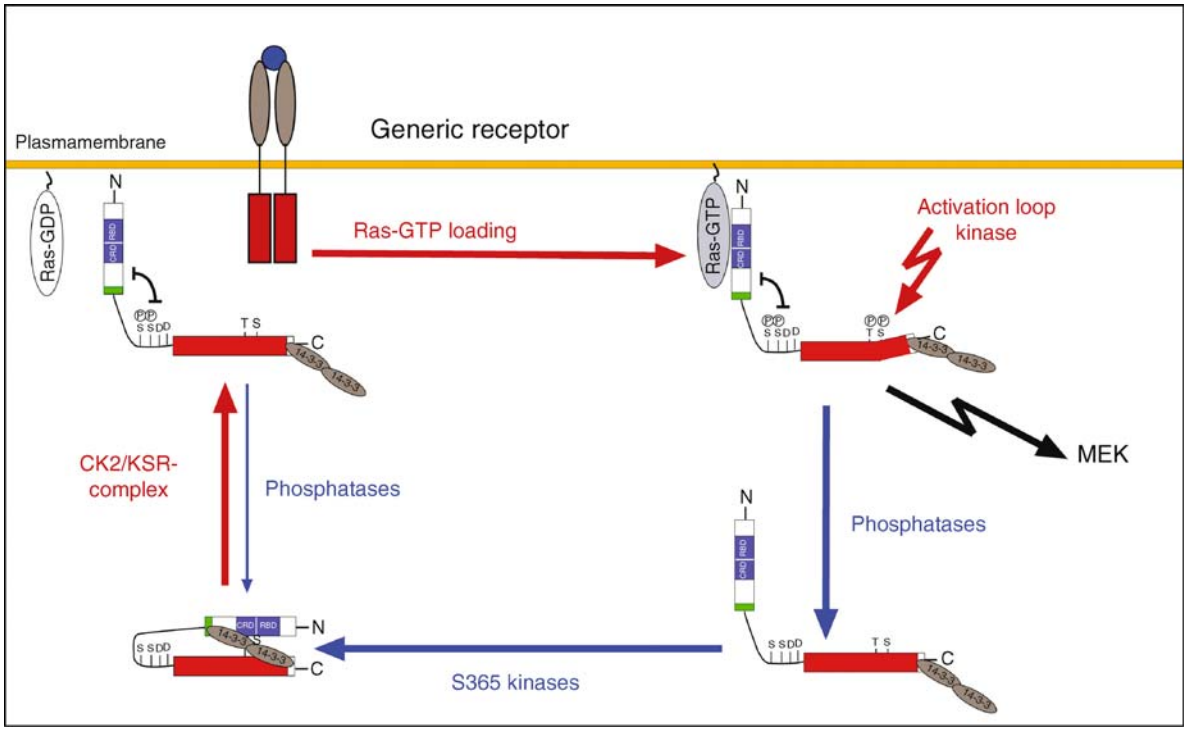
The high biological relevance of B-Raf is also reflected in the discovery that ►somatic alterations of the *BRAF* gene occur in about 8% of all human tumors with particular high frequencies in ►melanoma (70%), ovarian (30%), thyroid (27%), colorectal and biliary tract carcinoma (both 15%). Many of the resulting mutant B-Raf proteins cause chronic ERK activation and transform a variety of cell types *in vitro*. Furthermore, the B-Raf^{V600E} oncoprotein, which is the most frequently found mutant and occurs in 7% of human tumors, induces neoplasms in transgenic mice and zebrafish. Apart from their established role as ERK activators, B-Raf^{V600E} and other oncogenic mutants have been shown to activate the ►NF-κB pathway, although the exact mechanism for this oncologically relevant aspect of B-Raf sigaling remains elusive.

Dysregulated B-Raf signaling in the absence of any *BRAF* mutations has been also implicated in various neoplastic diseases. For example, hyper-activation of wild type B-Raf has been observed in ►Polycystic Kidney Disease. Similarly, over-expression and de-regulation of B-Raf have been implicated in ►Kaposi Sarcoma. Likewise, amplification and/or overexpression of the *BRAF* gene were described as alternative events to *BRAF* mutations in melanoma. Furthermore, B-Raf serves as an important signal transducer of upstream oncogene products such as RAS or activated receptor tyrosine kinases (RTKs) such as ►RET, ►NTRK, ►Epidermal Growth Factor Receptor family members or the ►Kit/Stem cell factor receptor. In many cell types where the chronic activation of the RAF/MEK/ERK effector arm by these oncoproteins represents a major mechanism of cellular transformation, a mutual exclusivity is observed between mutations in *BRAF* or genes encoding its upstream activators. For example, gain-of-function mutations in either the ►*RET*, ►NTRK, ►*RAS* or *BRAF* proto-oncogenes account for 70% of papillary thyroid carcinoma and provoke similar transformed phenotypes indicating that the activation of B-Raf effectors such as ERK and NF-κB is a major driving force in thyrocyte transformation. Similar constellations have been described for *RAS* and *BRAF* in melanoma, colorectal and ovarian carcinoma. However, Ras and B-Raf transformed cells differ in their responsiveness to MEK-inhibitors showing that both oncoproteins, while having a large group of effectors in common, also trigger ►oncogene addiction through distinct mechanisms. Oncogenic B-Raf not only mimics growth factor signaling, but also induces a variety of auto- and paracrine acting growth factors itself, e.g. ►Heparin-Binding Epidermal Growth factor (EGF)-Like Growth Factor, chemokines and pro-inflammatory and angiogenic cytokines like ►Vascular Endothelial Growth Factor A. Apart from tumor initiation, tissue culture experiments suggest that oncogenic B-Raf also contributes to tumor progression by inducing two additional key events in metastasis: the ►Epithelial to Mesenchymal Transition of the oncogene-bearing cell and the ►angiogenic switch in its environment through the aforementioned growth factors and cytokines.

Aberrant B-Raf activity does not necessarily result in tumorigenesis unless profound changes in the regulatory network underlying cell cycle control have occurred. Through the ERK and NF-κB pathways, oncogenic B-Raf stimulates not only the production of positive cell cycle regulators such as Cyclin D1, but also induces negative regulators such as cyclin-dependent kinase inhibitors like p16^{INK4A}. Consequently, chronic B-Raf/ERK signaling ultimately results in cell cycle arrest and cellular ►senescence. For example, melanocytes with an intact cell cycle control program become growth arrested by chronic B-Raf signaling and develop only into benign nevi. However, if important negative cell cycle regulators and tumor suppressor genes like ►INK4A or ►p53 are lost, oncogenic B-Raf signaling will trigger cell cycle progression and drive tumor development.

B-Raf Structure and Regulation

Like many other protein kinases, B-Raf is part of a large multi-protein complex or ►signalosome in which the individual components regulate B-Raf conformation and activity through various protein-protein interactions in a dynamic spatio-temporal manner. Key to the understanding of the (dys-)regulation of B-Raf is the knowledge of its modular structure. B-Raf shares three highly conserved regions (CR) with the other members of the Raf-family (Fig. 1): the N-terminal CR1 contains the Ras-GTP binding domain, which initiates the interaction with activated Ras, and the Cystein-rich domain involved in the stabilization of



B-Raf Signaling. Figure 1 Model of the B-Raf activation cycle. B-Raf contains three conserved regions: CR1 (blue) consisting of the Ras-binding domain (RBD) and the Cystein-rich domain (CRD), CR2 (green) and the kinase domain CR3 (blue). Inactive B-Raf resides in the cytoplasm in a closed, inactive conformation stabilized by 14-3-3. Interaction of B-Raf with a complex consisting of CK2 and the scaffold protein KSR results in phosphorylation of S446 (and perhaps S447) in the N-region thereby transferring B-Raf into a more open conformation. The constitutive basal phosphorylation of B-Raf at S446 suggests that a large fraction of B-Raf resides in this primed state. Interaction with activated Ras (Ras-GTP) leads to phosphorylation of T599 and S602 within the activation loop, which induces a conformational change within the CR3 and renders B-Raf active. B-Raf is supposedly inactivated by phosphatases, re-phosphorylation of the inhibitory residue S365 and transition into the closed conformation.

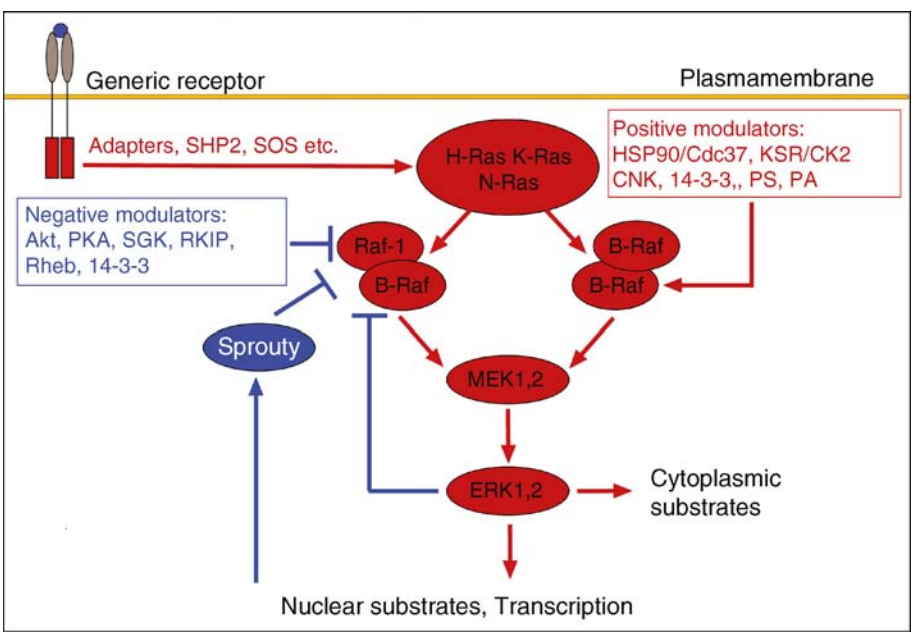
Ras/Raf interaction. The CR2 contains a negative regulatory serine residue (S365) that serves as a binding site for ►14-3-3 proteins upon phosphorylation by ►Akt and other kinases. The catalytic domain (CR3) harbors phosphorylation sites for Raf-regulating enzymes within two segments, the N-region and the ►activation loop. B-Raf carries a second 14-3-3 binding motif around S729 at the C-terminal end of the CR3 domain, which is essential to couple B-Raf to its downstream effector MEK.

Similar to the better-characterized Raf-1 isoform, B-Raf is activated by its interaction with small GTPases of the RAS family. Although no crystal structure for any of the full-length Raf-proteins is available, various experimental approaches imply that Raf activation is accompanied by a transition from a closed, auto-inhibited into an open, active conformation in which the N-terminal lobe consisting of the CR1 and CR2 domains is displaced from the C-terminal lobe encompassing the CR3 (Fig. 1). The degree of auto-inhibition of B-Raf is influenced by the inclusion/exclusion of amino acid sequences within the linker region between N- and C-terminal lobe, which are encoded by alternatively spliced, tissue-specific exons and various phosphorylation events. Among the latter, two phosphorylation sites within the CR3, the N-region and the activation loop, are of particular importance (Fig. 1). The introduction of negative charges into the N-region, which is located at the N-terminal end of the CR3 domain, plays a critical, multi-faceted role in Raf activation. While the N-region of Raf-1 is charged through phosphorylation of its $S^{338}SYY^{341}$-sequence in a RAS-dependent manner by Ser/Thr- and Tyr-kinases, the equivalent serine residues within the N-region of B-Raf ($S^{446}SDD^{449}$-motif) are phosphorylated in a constitutive and RAS-independent manner (Fig. 1). Although structural data are still missing, several lines of evidence propose that N-region phosphorylation primes B-Raf for activation at the membrane by reducing the affinity between N-terminal and C-terminal lobe. The significance of the aspartate residues, which are the functional equivalents of the phosphotyrosine residues in the SSYY-sequence of Raf-1, is

twofold: firstly the negative charge of the aspartate residues are supposed to prime B-Raf for N-region phosphorylation by Casein Kinase 2 (CK2). Secondly, the D448 residue stabilizes the conformation of activated B-Raf through the formation of a salt-bridge with R506 within the αC-helix of the CR3. The important role of the SSDD-sequence is highlighted by the fact that mutation of the serine and/or aspartate residues results in drastic reduction of the basal *in vitro* kinase and biological activities. Furthermore, it has been suggested that the different mechanisms that supply the N-region of B-Raf and Raf-1 with negative charges, account not only for the aforementioned isoform-specific differences in the enzymatic, biological and transforming activities, but also predispose the *BRAF* gene for oncogenic hits. However, while tissue culture experiments demonstrated that the rare B-Raf^{E586K} mutant indeed requires an intact SSDD-sequence to induce MEK/ERK activation and oncogenic transformation, the biological activity of the most frequently found mutant, B-Raf^{V600E}, is not affected by N-region neutralization, at least not in experimental approaches involving the ectopic expression of this oncoprotein.

The interaction with Ras recruits B-Raf to the plasma membrane followed by the phosphorylation of the activation loop residues T599 and S602 (Fig. 2). This phosphorylation event presumably leads to the dislocation of the activation loop relative to the overall catalytic domain thereby resulting in full B-Raf activity. The importance of the activation segment phosphorylation is established by the fact that mutation of these phosphorylation sites to alanine residues renders B-Raf resistant to extracellular signals and even to strong activators like oncogenic Ras^{G12V}. Conversely, mutations that mimic the phosphorylation-induced dislocation of the activation segment, such as *BRAF*^{V600E}, lock B-Raf in an active conformation and confer high constitutive enzymatic and transforming activities to B-Raf independent of RAS. Consequently, these activation loop mutations are frequently found as somatic alterations of the *BRAF* gene in human tumors.

Intracellular B-Raf activity is also regulated by the phosphorylation-dependent recruitment of ▶14-3-3 proteins in an opposing manner (Fig. 1). Binding of 14-3-3 proteins to phospho-S729 at the C-Terminus of B-Raf is essential to couple B-Raf to the MEK/ERK pathway. In contrast, phosphorylation of S365 within the CR2 by Protein kinases A, Akt or Serum-and-Glucocorticoid-induced kinase (SGK) generates a second binding site for 14-3-3 proteins, which negatively regulates B-Raf activity, most likely through the



B-Raf Signaling. Figure 2 Modulation of B-Raf signaling. Extracellular signals received by various receptor classes trigger the activation of Ras-GTPases by stimulating their loading with GTP. Activated Ras not only recruits B-Raf and promotes its phosphorylation by unknown activation loop kinases, but also stimulates its homo- and hetero-dimerisation. The activity of B-Raf (and Raf-1) is fine tuned by a multitude of positive and negative modulators. The longevity of B-Raf/Raf-1 heterodimers is determined by a rapid negative feedback loop from ERK. In a delayed negative feedback loop, sustained B-Raf/ERK signaling also induces the transcription of Sprouty-2, a negative regulator of B-Raf.

stabilization of the auto-inhibited conformation through the simultaneous binding of the 14-3-3 dimer to S365 and S729 (Figs. 1 and 2). 14-3-3 proteins are also involved in the RAS-stimulated formation of homo-dimers of B-Raf and its hetero-dimerisation with Raf-1 (Fig. 2). Indeed, B-Raf/Raf-1 hetero-dimers represent the most potent form of Raf-activity within the cell. Activated ERK limits the longevity of these dimers by targeting an evolutionary conserved phosphorylation motif at the C-terminus of B-Raf (Fig. 2). In addition, B-Raf activity is modulated by other components of the signalosome such as the ►HSP90/Cdc37 chaperone complex and ►scaffold proteins like Kinase-suppressor-of-Ras (KSR) and Connector-and-enhancer-of-KSR (CNK). Membrane phospholipids such as phosphatidylserine (PS) and phosphatidic acid (PA) are also discussed as important regulators of Raf activation. B-Raf is also negatively regulated by Sprouty-2 and Raf-kinase-inhibitory protein (RKIP), two proteins, which are both often down-regulated in human cancer raising the possibility that their epigenetic silencing represents an alternative mechanism to gain-of-function mutations in genes linked to the Ras/Raf/MEK/ERK pathway in human cancer. Similarly, B-Raf^{V600E} and other activation loop mutants are incapable of interacting with Sprouty demonstrating that the V600E mutation not only uncouples B-Raf from positive (interaction with Ras, N-region and activation loop phosphorylation) but also negative regulatory mechanisms.

B-Raf as a Therapeutic Target

The growing importance of B-Raf in tumor biology has fostered the development of therapeutic strategies aiming at either reducing the expression or activity of B-Raf or its downstream effector MEK. Various MEK inhibitors are currently in clinical trials and experiments in tissue culture and xenograft models indicate that tumor cells harboring the *BRAF*^{V600E} mutation, but not those with RAS mutations, are highly "addicted" to ERK activity and are consequently particularly sensitive towards MEK inhibition. Similar results have been obtained in experiments in which the expression of B-Raf^{V600E} but not of wild type B-Raf was specifically abolished by allele-specific ►RNA interference illustrating the importance of this oncoprotein for the maintenance of the tumor phenotype. Recent strategies also target B-Raf directly. The orally available multi-kinase inhibitor BAY 43-9006 (also known as Sorafenib or Nexavar), which was originally designed to block Raf-1, inhibits B-Raf as well as several receptor tyrosine kinases (RTKs) involved in neo-angiogenesis and tumor progression. However, it is assumed that the inhibition of the latter kinase class or the simultaneous inhibition of several kinases, rather than the inhibition of Raf itself, is responsible for the anti-tumor activity of BAY 43-9006, in particular in renal cell carcinoma. A third approach employs the requirement of the HSP90/Cdc37 chaperone complex for the stability of B-Raf. In this regard, the HSP90 inhibitor ►Geldanamycin was shown to trigger the degradation of B-Raf by disrupting its association with the HSP90/Cdc37 chaperone complex. The stability of most activated B-Raf mutants, including B-Raf^{V600E}, appears to be more reliant on the chaperone complex than those of wild type B-Raf suggesting that tumor cells driven by *BRAF* mutations will be particularly sensitive to Geldanamycin.

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B-Raf Somatic Alterations

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Definition

Somatic alterations of the *BRAF* gene in cancer, either caused by point mutation or genomic rearrangement of the *BRAF* proto-oncogene.

Characteristics

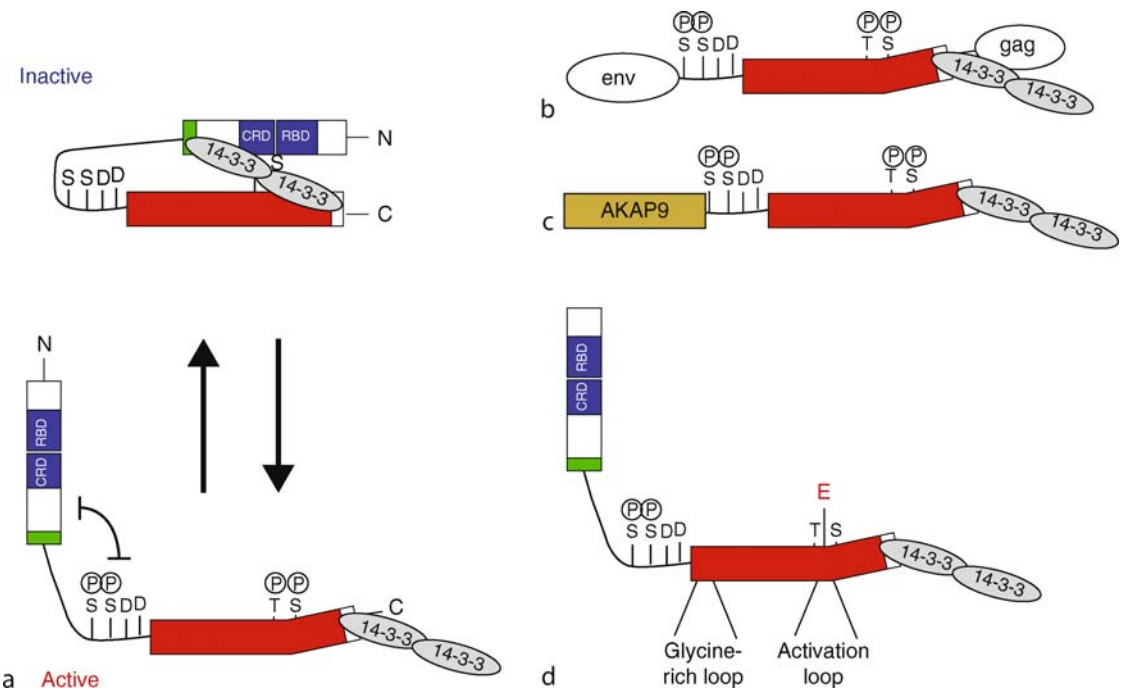
The Ser/Thr-kinase B-Raf, a product of the human *BRAF* proto-oncogene, plays a pivotal role in the activation of the classical ►ERK/MAP kinase pathway that is involved in the control of proliferation and differentiation of various tissues. Consequently, alterations of the expression level or the activity of B-Raf are associated with malignancies like ►polycystic kidney disease and various cancers. Proto-oncogenes can be converted into oncogenes by point mutations, amplifications, genomic rearrangement, e.g. translocation or inversion, or by retroviral transduction. Interestingly, all four mechanisms of oncogene activation have

been documented for the *BRAF* genes in human and/or animal tumors.

History of the *BRAF* Proto-Oncogene

The discovery of the *raf*-oncogenes originates back to the isolation of the chicken Mill Hill 2 (MH2) retrovirus by Begg in 1927. Genetic studies in the 1980s demonstrated that MH2 contains two unrelated retroviral oncogenes that were designated as v-*myc* and v-*mil*. Subsequent analysis of v-*mil* revealed a high sequence homology to the v-*raf* oncogene of the murine sarcoma retrovirus 3611. Further analyses showed that both v-*mil* and v-*raf* arose independently by retroviral transduction from the chicken c-*mil* and mammalian *raf*-1 genes, respectively. In 1988, a v-*mil* related oncogene was discovered in ►transforming retroviruses that were generated by passaging the non-oncogenic Rous-associated virus type 1 (RAV-1) on embryonic chicken neuroretina cells. Due to its origin in retinal cultures, this relative of v-*mil* was designated as v-R*mil*. Subsequent studies showed that v-R*mil* was generated by retroviral transduction from the proto-oncogene c-R*mil*, which is related but distinct to the c-*mil*/*raf*-1 proto-oncogenes and represents the avian ►orthologue of *BRAF*. Similar to the avian c-R*mil*/B-*raf* gene, the *BRAF* genes of other vertebrates display a conserved exon/intron structure with 18–20 coding exons, in which the first eight exons encode the N-terminal autoinhibitory region (Fig. 1a).

At the same time as v-R*mil* was discovered, the human *BRAF* oncogene was identified in a ►NIH3T3 transformation assay using ►Ewing sarcoma DNA. Importantly and in striking analogy to v-Mil and v-Raf, both the v-Rmil and the B-Raf oncoprotein from the Ewing sarcoma isolate represent N-terminally truncated B-Raf proteins (Fig. 1b), which have lost the N-terminal regulatory lobe and consequently the ability for auto-inhibition (►B-Raf signaling). Therefore, all these Raf-oncoproteins display constitutive activity and induce chronic activation of the ERK pathway. Thus, loss of exons encoding for the auto-inhibitory N-terminal moiety is a common mechanism of oncogenic activation of *raf* proto-oncogenes. This notion is further supported by recent experiments showing that the



B-Raf Somatic Alterations. Figure 1 B-Raf oncoproteins. (a) Situation for wildtype B-Raf. In its inactive state, the *BRAF* proto-oncogene product resides in a closed conformation stabilized by 14-3-3 proteins. Activation of B-Raf by activated RAS results in a displacement of the N-terminal auto-inhibitory region (Conserved region (CR) 1 in blue, CR2 in green) from the CR3 or kinase domain (red) allowing access of the activation loop kinase to the the TVKS-motif. Phosphorylation of T599 and S602 within this motif renders B-Raf active. (►B-Raf signaling). (b) Schematic representation of v-Rmil. Due to the retroviral transduction event, the genome of RAV encodes for a fusion protein flanking the B-Raf (CR3) kinase domain with an N-terminal portion encoded by the *env* gene and a C-terminal moiety encoded by a portion of the *gag* gene. Both the *env* and *gag* genes are integral components of retroviral genomes. (c) Schematic representation of AKAP9-B-Raf. (d) Schematic representation of B-Raf proteins with point mutations as exemplified for the activation loop mutant B-Raf^{V600E}.

murine *Braf* gene represents a frequent integration point for the *Sleeping Beauty* transposon. All transposon integrations were observed between exons 9 and 10 resulting in a disruption of the coding sequence of full-length B-Raf and expression of an N-terminally truncated B-Raf protein with an intact kinase domain and structural similarity to v-Rmil and v-Raf. However, it should be mentioned that neither retroviral B-Raf oncogenes nor transposon-mediated oncogenic activation of the *BRAF* gene have been observed in human beings. Likewise N-terminal truncations of B-Raf like those found in the original publication on the human B-Raf gene, which most likely represents a transfection artifact, have not been found in human tumors until recently. Nevertheless, the human *BRAF* proto-oncogene is affected by somatic alteration in about 7% of human tumors. The following alterations are observed in human tumors:

Chromosomal Aberrations

A recent study has identified an oncogenic BRAF allele in about 11% of papillary thyroid carcinomas (PTC) in children and adolescents that had been exposed to radiation following the Chernobyl nuclear power plant station accident in 1986. This oncogene was generated *via* a paracentric inversion of the *BRAF* locus on chromosome 7q34 resulting in an in-frame fusion with exons 1–8 of the *A-kinase anchor protein* 9 (*AKAP*9) gene on 7q21-22. The resulting AKAP9-B-Raf fusion protein is made up by exons 1–8 of *AKAP*9 and exons 9–18 of *BRAF*. Thus, this AKAP9-B-Raf protein contains an intact kinase domain, but the auto-inhibitory N-terminal regulatory domain of B-Raf is replaced by the AKAP9 moiety, which cannot confer autoinhibition (Fig. 1c). Consequently, the activity of this fusion protein is, similar to the situation in v-Rmil, unrestrained and able to transform NIH3T3 cells. Interestingly these mutations were only found in tumors that had developed within a short latency period suggesting that this chromosomal aberration is a driver of radiation induced PTC rather than being a secondary event.

Another recent study has reported the occurrence of chromosomal translocations involving the human *BRAF* gene in two cases of large congenital melanocytic nevi, which can progress into malignant melanoma. In both cases and similar to the situation of the AKAP9-B-Raf fusion protein, these translocations give rise to fusion proteins, which lack the exons encoding the auto-inhibitory N-terminal regulatory domain, but again contain an intact B-Raf kinase domain.

Somatic and Germ-Line Point Mutations

Although Raf proteins were implicated early on as important effectors of human oncoproteins, e.g. Ras, they were not considered as frequent mutational targets in cancer. In 2002, however, the ►cancer genome project (CGP) reported a high frequency of somatic point mutations in the human *BRAF* gene in malignant melanoma (27–70%). Subsequent studies also revealed high point mutation frequencies in thyroid (36–53%), ovarian (30%), biliary (14%) and colorectal cancer (522%) and lower frequencies in a wide range of other human tumors. It is estimated that the human *BRAF* gene bears somatic mutations in about 7% of all human cancers. In contrast to the aforementioned alterations of the *BRAF* gene, these point mutations do not affect the overall primary structure of B-Raf (Fig. 1d), but mostly bypass critical regulatory events required for the activation of wildtype B-Raf (►B-Raf signaling).

While mutations in the human *CRAF* gene are still considered as a very rare event, over 40 different somatic mutations, involving 24 different codons, have been identified in *BRAF* since 2002. Most alterations represent point mutations, however, codon deletions or in-frame insertions have been occasionally identified as well. A detailed overview on these mutations can be found on the CGP homepage (http://www.sanger.ac.uk/perl/genetics/CGP/cgp_viewer?action =gene&ln=BRAF). Most mutations cluster within the activation loop codons and, to a lesser extent, within the nucleotide sequence encoding the glycine-rich loop (also known as P-loop; Fig. 1d). Among the activation segment mutations, the thymidine to adenine transversion at nucleotide 1799, which results in the substitution of valine 600 within the $T^{599}V^{600}KS^{602}$-motif in the activation segment by glutamate, represents the most common mutation and is found in 6% of human cancers. Structural analysis of the B-Raf kinase domain suggests that the inactive conformation of B-Raf is stabilized by a hydrophobic interaction between the activation loop residues with the glycine rich loop, with V600 and F467 playing key roles in this process. Upon activation of wildtype B-Raf by activated Ras, T599 and S602 in the activation loop become phosphorylated by an unknown kinase resulting in the disruption of the inhibitory hydrophobic interaction between the activation and glycine rich loop and consequently full activation of B-Raf (Fig. 1a). In a similar way, any mutation in either the activation or glycine-rich loop mutation that disrupts this hydrophobic interaction, e.g. replacement of V600 by bulky and/or charged amino acids like glutamate, mimics the activated state and confers constitutive activity to B-Raf. As described in ►B-Raf signaling, the current model of B-Raf activation proposes a sequence of positive regulatory events leading to a relief of auto-inhibition by the N-terminal lobe followed by activation loop phosphorylation and full B-Raf activation. According to this sequential model of B-Raf activation, the V600E mutation not only bypasses these events, but is also

able to counteract auto-inhibition, which would explain why this mutation is so frequently found in tumors driven by chronic ►B-Raf signaling. However, why the V600E mutation occurs more frequently than any other activation loop or glycine-rich loop mutations that would also disrupt the inactive conformation, remains controversial. The V600E codon might represent a mutational "hotspot" or, due to still unknown details of B-Raf activation, might be an extremely efficient oncogene that subjects B-Raf^{V600E} expressing cells to a particularly strong positive selection. It should be also mentioned that the occurrence of the *BRAF*^{V600E} allele in colorectal cancer is correlated with ►microsatellite instability (MSI) and widespread methylation of CpG islands in a highly statistically significant manner. However, it remains to be clarified as to whether the MSI phenotype that is caused by absence or hypo-activity of DNA mismatch repair genes and is characterized by a widespread methylation of CpG islands, reflects a cause or a consequence of dysregulated B-Raf signaling.

In 2006, germ-line mutations in the human *BRAF* gene were found in patients suffering from the ►cardio-facial-cutaneous (CFC) syndrome. Some of these mostly gain-of-function mutations in CFC patients are also found in cancer, however, mutations conferring high activity to B-Raf such as *BRAF*^{V600E} have not been found. Indeed, *knock-in* experiments in mice have shown that ubiquitous expression of B-Raf^{V600E} confers early embryonic lethality suggesting that high levels of chronic B-Raf activity would not be tolerated during human development as well.

However, it should be noted that not all of the point mutations found in cancer or CFC patients represent obvious gain-of-function mutations as some of them actually display impaired *in vitro* kinase activity. Nevertheless, these impaired activity mutants still appear to activate the ERK pathway within the cell, either through stimulating the activity of Raf-1 in Raf-1/B-Raf heterodimers or, potentially, by acting as a buffer against negative regulators, e.g. RKIP, or negative feedback loops controlling ►B-Raf signaling.

Amplification

Amplification of the *BRAF* locus is another mechanism contributing to elevated B-Raf protein expression and activity. Studies in malignant melanoma have described the amplification of *BRAF* alleles with point mutations such as V600E at the expense of the wildtype *BRAF* allele.

Genetic experiments have identified B-Raf as an important factor for ERK activation under basal and steady state conditions (►B-Raf signaling). Experiments in various cell types have shown that increasing levels of wildtype B-Raf enhanced basal and steady state ERK signaling suggesting that over-expression of endogenous wildtype B-Raf might contribute to tumorigenesis. Indeed, amplification of the *BRAF* locus in the absence of any mutations in exon11 (Gly-rich loop) and exon 15 (activation loop) was described as an important contributor to the proliferation of malignant melanoma cell lines.

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Bragg (Curve) Peak

Definition

A characteristic dose distribution of a single-energy charged particle beam (e.g. protons) with a sharp peak close to the end of the range. The range is a distance that particles travel inside the medium.

►Radiation Oncology

Brain Capillaries

►Blood–Brain Barrier

Brain Microvascular Endothelial Cells

►Blood–Brain Barrier

Brain Tumors

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Definition

Primary ►brain tumors present most commonly as ►meningioma or various grades of ►astrocytoma. ►Gliomas constitute 78% of all malignant brain and central nervous system tumors. It is estimated that ~20,500 individuals will be diagnosed with cancer of the brain and nervous system in 2007, or about 1.4% of all newly occurring malignancies. Of those diagnosed, there will be 10% more men than women. Primary brain and nervous system cancers will account for 2.3% of the estimated 560,000 cancer deaths in 2007. Based on the most recent report of the Central Brain Tumor Registry of the United States, benign tumors of the CNS arise in numbers comparable to malignant brain tumors. In children and young adults, brain tumors are responsible for 25% of all cancer-related deaths, second only to leukemia in this age group. The estimated 5-year relative survival rate for malignant brain tumors is 29%, but there is much variation in survival, depending on tumor histology. The 5-year survival rate exceeds 91% for pilocytic astrocytomas, but is less than 4% for glioblastomas. Generally, survival decreases with increasing age at diagnosis.

Characteristics

Classification and Pathology

The cell of origin of commonly occurring brain tumors is not known, although recent evidence suggests that these tumors arise either from ►neural stem cells or from other cells that take on many characteristics of neural stem cells as a result of malignant transformation caused by the activation of oncogenes and the inactivation of ►tumor suppressor genes within the cells. Pathologically, these tumors are classified according to the World Health Organization (WHO) nomenclature and grading criteria. Tumors that share cytologic and histologic evidence of astrocytic differentiation are known as ►astrocytoma and are the most frequent primary intracranial neoplasms. Their neuropathological appearance is highly variable. Tumors with evidence of oligodendroglial differentiation are known as ►oligodendroglioma. Some tumors that have cells reminiscent of both lineages are known as ►mixed oligo-astrocytomas. Each of these tumor types can be graded histologically according to a four-tiered system of increasing malignancy from Grades I through IV. Grade I, for example, has an excellent prognosis following surgical excision, and Grade IV, ►glioblastoma multiforme, has multiple features of clinical aggressiveness and is typically incurable. Hypercellularity with evidence of high mitotic activity, nuclear and cytoplasmic atypia, endothelial proliferation, and necrosis correspond closely to tumor virulence and are most characteristically present in Grade IV tumors. The overwhelming majority of gliomas arising in adults are high-grade and arise in a supratentorial location. High-grade tumors do not have a clear margin separating neoplastic and normal tissue. This finding is consistent with the observation that tumor cells usually have infiltrated adjacent normal brain by the time of diagnosis, when complete resection is oftentimes not possible. Tumor cells capable of initiating new tumor foci can now be recognized as tumor stem cells.

Cytogenetic examination of chromosomes within the cells of a ►brain tumor has revealed characteristic regions that tend to be altered in specific tumor types (Table 1). Frequent sites for chromosomal DNA loss in astrocytic tumors include chromosomes 17p, 13q, and 9. In oligodendroglioma, DNA from 1p and 19q is frequently lost, and in ►meningiomas, 22q is often lost. Molecular genetic analysis can also reveal evidence of tumor-specific genetic alterations at sites where chromosomes appear normal upon cytogenetic

Brain Tumors. Table 1 Cytogenetic and genetic alterations in brain tumors

Tumor type	Chromosomal alteration	Genetic changes	
		Oncogene	Tumor suppressor gene
Astrocytoma	$1p^{-}$, 7^{+}, $9p^{-}$, de110, $11p^{-}$, $12q^{-}$, $12q^{+}$, $13q^{-}$, $13q^{+}$, $18p^{+}$, $17p^{-}$, $17q^{-}$, $19p^{-}$, $19q^{-}$, $22q^{-}$, DMs[a]	*EGFR, PDGFRA, KIT, CROS, MET, CDK4, NEU, RAS, MDM2, GLI, CMYC*	*P53, RB1, NF1, PTEN, DMBT, CDKN2A, CDKN2D, RASSF1A*
Oligodendroglioma	$1p^{-}$, $19q^{-}$, 7^{+}, 10^{-}	*EGFR*	*TP53, PTEN, CDKN2A, CDKN2D, PIK3CA*
Medulloblastoma	$5p^{+}$, $5p^{-}$, $5q^{-}$, del6, $8p^{-}$, $8q^{+}$, $9q^{-}$,$10q^{-}$, $17p^{-}$, $17q^{-}$, $17q^{+}$, $21q^{+}$	*GLI, CMYC, CTNNB1*	*TP53, PTCH, SUFU, APC, RASSF1A*

[a]DMs, double minute chromosomes.

analysis. Using a variety of molecular technologies, it has been possible to document the alteration of many different genes in brain tumors, particularly astrocytic tumors (Table 1).

While the particular constellation of genetic alterations that activate oncogenes and inactivate tumor suppressor genes varies among individual brain tumors that appear to be histologically indistinguishable, an accumulation of mutations is typically associated with increasingly aggressive malignant behavior. Glioblastoma multiforme (►GBM) typically presents without evidence of a precursor lesion, referred to as de novo or primary GBM. These tumors typically have evidence for chromosome 10 deletions in the region where the tumor suppressor ►*PTEN* is known to be located, and activation of the ►epidermal growth factor receptor (►*EGFR*) gene either by amplification or deletion of ~275 amino acids from the extracellular domain of the receptor. This and closely related mutations occur in ~60% of GBM. *EGFR* is the gene most frequently activated in malignant astrocytomas. *EGFR* amplification and activation by mutation occurs in ~5% of low-grade astrocytomas and about 30% of GBM, indicating that this molecular change is principally associated with the progression from low- or intermediate-grade neoplasia to high-grade astrocytic neoplasia. In fewer than 20% of cases, GBM arises in association with progressive genetic alterations after the diagnosis of a lower-grade astrocytoma. These tumors are referred to as secondary GBMs. The most widely described alterations are amplification or overexpression of the ►*PDGF* receptor, mutations of ►*p53* or ►*MDM2* and ►*INK4a*, and loss of *PTEN* (Table 1). Deletion of the ►*CDKN2* gene, which encodes the cyclin-dependent kinase inhibitor *p16*, has been reported to occur in ~40–70% of glioblastoma. The ►*RB1* tumor suppressor gene is homozygously deleted or mutated in about 30% of high-grade gliomas.

The protein products of tumor suppressor genes are proteins that act to regulate or suppress cell growth or promote cell death. These genes are inactivated during tumorigenesis, and several such genes have been implicated in the development of astrocytoma. Occasionally, inactivation of one of these alleles in the germline can occur without disturbing development, and patients who carry germline mutations of some tumor suppressor genes can be predisposed to the development of cancer. Several inherited cancer-predisposition syndromes are known to be associated with the development of different brain tumors. Patients with ►Li-Fraumeni syndrome, caused by an inherited constitutional *p53* mutation, have a predisposition for the development of brain tumors. The *p53* gene, located on chromosome *17p*, has been found to influence multiple cellular functions thought to be important in tumorigenesis. *p53* mutations have been reported in sporadically arising astrocytic tumors of all grades, occurring in ~40% of astrocytomas, in 30% of ►anaplastic astrocytomas, and in a slightly smaller fraction of GBM. Other brain tumor predisposition syndromes associated with the inactivation of one copy of a particular gene in the germline include ►neurofibromatosis type 1 (*NF1* gene), which is associated with meningioma and optic glioma; ►neurofibromatosis type 2 (*NF2* gene), which is associated with acoustic neuroma and glioma; familial ►retinoblastoma (►*Rb* gene), which is associated with retinoblastoma and pinealoblastoma; ►von Hippel-Lindau syndrome (*VHL* gene), which is associated with cerebellar hemangioblastoma; ►tuberous sclerosis (*TSC1* and *TSC2* genes), which is associated with subependymal giant cell astrocytoma; ►Turcot syndrome (►*APC* gene), which is associated with astrocytoma and ►medulloblastoma; and Gorlin's syndrome (*PTCH* gene), which is associated with desmoplastic medulloblastoma.

The second most common primary brain tumor is oligodendroglioma, which has a more benign course than astrocytoma. Many ►gliomas have mixtures of cells with astrocytic and oligodendroglial features. If this mixed histology is prominent, the tumor is termed a mixed glioma or an ►oligoastrocytoma. Many investigators believe that the greater the oligodendroglial component, the more benign the clinical course. The presence of such histologic characteristics as mitosis, necrosis, and nuclear atypia generally is associated with a more aggressive clinical course. If these features are prominent, the tumor is termed a malignant oligodendroglioma. The highest grade oligodendroglioma are indistinguishable from glioblastoma multiforme.

Other malignant primary brain tumors include ►primitive neuroectodermal tumors (►PNET) such as ►medulloblastoma, ►ependymoma, and ►atypical teratoid/►rhabdoid tumors; ►germinomas; and CNS ►lymphoma. Cerebral PNETs and medulloblastoma, a PNET that arises in the posterior fossa, are highly cellular malignant tumors thought to arise in neural precursor cells. These tumors are difficult to distinguish from one another and typically appear histologically as sheets of small round malignant cells. Germline mutation of *PTCH* and *SUFU* in rare patients has called attention to the importance of sonic hedgehog signaling in medulloblastoma. Similarly, *APC* germline mutations in rare patients implicate *WNT* signaling as well. These tumors most commonly occur in children. Ependymomas are rare tumors, and when these occur in children, they typically are within the fourth ventricle, where they are thought to arise from cells lining the fourth ventricle. In adults, they arise more frequently in the spinal cord. Patients with neurofibromatosis type 2 are at increased risk of developing ependymoma, and 30% of sporadically occurring tumors exhibit deletion of Ch22q where the *NF2* gene is located. Histologically, these tumors exhibit diagnostic ependymal rosettes.

Atypical teratoid/rhabdoid tumors histologically appear as fields of undifferentiated malignant neuroectodermal cells that are indistinguishable from PNET, except for infrequent cells that exhibit evidence of rhabdoid differentiation and the presence of mesenchymal and epithelial elements. Germinomas arise most commonly during the second decade of life at midline locations. Both malignant and benign variants occur frequently. These tumors present with hypothalamic–pituitary dysfunction and visual field deficits.

Primary CNS lymphomas are most commonly seen in immunocompromised patients, and have a clinical presentation similar to other primary brain tumors with signs and symptoms referable to cerebral and cranial nerve involvement. Imaging studies typically demonstrate a uniformly enhancing mass lesion. Secondary CNS lymphoma almost always occurs in association with the progression of systemic disease. Several kinds of tumors that are most often benign also occur in the nervous system. ►Meningiomas are derived from cells of the arachnoid membranes. They are more frequent in women than in men, with a peak incidence in middle age. Meningiomas rarely have histological evidence of malignancy. Other tumors that have a benign clinical course include giant cell astrocytomas, pleomorphic xanthroastrocytomas, neurocytomas, and gangliogliomas. Colloid cysts, dermoid cysts, and epidermoid cysts also occur in the brain.

Clinical Presentation of Brain Tumor Patients

The most common symptoms that bring patients with a tumor arising in the brain to their physician include a slow progressive focal neurological disability, or a nonfocal neurological syndrome such as headache, dementia, gait disorder, or seizure. Other systemic symptoms suggest a tumor from some other location that may have metastasized to the brain, since patients with primary brain tumors typically do not exhibit systemic symptoms. Patients with primary brain tumors rarely have any biochemical abnormalities; thus CT (Computerized Tomography) and MR (Magnetic Resonance) imaging are key diagnostic modalities for the identification of brain tumors. The characteristic imaging features of brain tumors are mass effect, edema, and contrast media enhancement. Positron emission tomography (PET) scanning and single photon emission computed tomography (SPECT) have ancillary roles in the imaging of brain tumors. Meningiomas and other slow-growing tumors may be found incidentally on a CT or MRI scan or they may present with a focal seizure, a slow progressive focal deficit, or symptoms of increased intracranial pressure. As described above, brain tumors are also recognizable in many inherited syndromes including von Recklinghausen syndrome (neurofibromatosis type 1), neurofibromatosis type 2, Li-Fraumeni syndrome, ►Multiple endocrine neoplasia type 1, tuberous sclerosis, Turcot syndrome, and Gorlin syndrome.

Clinical Management of Brain Tumor Patients and Prognosis

Stereotaxic needle biopsy may establish the histological diagnosis of primary brain tumor, although open biopsy is also often utilized to establish the diagnosis. The primary modality of treatment for most primary brain tumors is surgery. The goals of surgery are to obtain tissue for pathological examination, to remove tumor, and to control mass effect. In the case of low-grade and benign tumors, the removal of tumor tissue can be curative or contribute substantially to extending the time to symptomatic progression. In higher-grade tumors, the role of surgery in contributing to curative therapy is less clearly defined, but in younger patients most surgeons aggressively pursue the removal of as much tumor as possible. Following total excision of an ependymoma, the prognosis is excellent. However, many ependymomas cannot be totally excised. Following surgery, ►radiation therapy has been shown to prolong survival and improve the quality of life of patients with high-grade glioma, PNET, ependymoma, or meningioma when malignant histologic elements can be pathologically identified within the tumor.

The medical management of most brain tumors is symptomatic, although a role for chemotherapy is clearly defined in oligodendroglioma and medulloblastoma. In patients with oligodendroglioma, a combination of procarbazine, lomustine, and vincristine has been shown to be most effective in patients with a deletion of Ch1p. Various combination therapies have been shown to contribute to the treatment of medulloblastoma, which has a propensity to spread throughout the neuroaxis. If medulloblastoma is limited to the posterior fossa and completely resected, this tumor has a good prognosis. Temozolomide given during radiation therapy for glioblastoma has been shown to contribute to longer overall survival time. Chemotherapy and radiation typically play a central role in the treatment of germinomas, although there is a role for surgery as well. Patients whose brain tumors are associated with surrounding edema benefit symptomatically from the administration of high doses of glucocorticoids. Anticonvulsants are useful in the control of seizures. Some glioma patients receive anticoagulation therapy to avoid complications of venous thrombosis that occurs in these patients.

The prognosis for patients with primary brain tumors varies greatly as a function of the histology and location of the tumor. Benign tumors are often cured by surgery alone. ►Germinomas and medulloblastomas are more sensitive to cytotoxic therapies than are other brain tumors, and the prognosis for patients with these tumors is generally better than for patients with high-grade

glioma. In modern studies, the median survival of patients with high-grade glioma is ~1–2 years.

Complications of Therapy

Neurological damage associated with surgical intervention presents a key challenge in the management of brain tumors. Furthermore, the nervous system is vulnerable to injury by therapeutic radiation, and this is frequently manifested by neuropsychological compromise and disability, particularly in very young children who have been treated with high doses of radiation. Pathologically, there is demyelination, hyaline degeneration of small arterioles, and eventually brain infarction and necrosis. Endocrine dysfunction is also commonly seen when the hypothalamus or pituitary gland has been exposed to therapeutic radiation. Depending on the radiated field, secondary tumors such as glioma, meningioma, sarcoma, and thyroid cancer occur following radiation therapy. Toxicities associated with chemotherapy can be significant, but they are not usually different from the toxicities associated with comparable treatments for tumors arising elsewhere in the body.

►Neuro-Oncology: Primary CNS Tumors

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Brain-Derived Neurotrophic Factor

Definition

BDNF; Is a neurotrophin in the central nervous system (CNS), predominantly in the brain and the periphery. This is in contrast to ►NGF acting predominantly in the peripheral nervous system. It acts on certain neurons of the CNS and the peripheral nervous system that helps to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. In the brain, it is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory, and higher thinking. BDNF was the second neurotrophic factor to be characterized after NGF.

Branching Morphogenesis

Definition

Branching morphogenesis refers to the formation of tree-like networks of epithelial tubes through reiterated cycles of branch initiation, branch outgrowth and branch arrest. This process relies on the precise spatio-temporal control of gene expression, cell proliferation and migration, and is essential for the physiological function of many organs including the lung, the vascular system and the kidney.

►Sprouty

BRCA1-associated Ring Domain (Gene/Protein) 1

►BARD1

BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk

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Definition

Mutations in the ►breast cancer genes BRCA1 and BRCA2 cause elevated risks to breast and ovarian cancer. BRCA1 maps to chromosome 17 (band q21), BRCA2 maps to chromosome 13 (band q12).

At the genetic level there are interesting analogies between the two genes, even though they are not detectably related by sequence. Both genes are large (coding regions of 5.6 and 10.2 kb, respectively),

complex (22 and 26 coding exons, respectively), and span about 80 kb of genomic DNA. Both have extremely large central exons encoding >50% of the protein. The majority of the mutations in both genes detected to date lead to premature termination of protein translation, presumably resulting in an inactive truncated protein. Gene changes are distributed nearly ubiquitously over the coding exons and immediate flanking introns. Even though more than half of all mutations are found only once, many mutations have been detected repeatedly in certain populations. For most of these, this has been shown to be the result of a founder effect: these mutations arose a long time ago, and have since spread in the population. Typical founder mutations are the 1185delAG and 15382insC in BRCA1 and 26174delT in BRCA2 that have a joint frequency of about 2.5% among individuals of Ashkenazi Jewish descent.

Characteristics

Clinical Characteristics

Female carriers of a deleterious BRCA1 mutation were estimated by the Breast Cancer Linkage Consortium (BCLC) to have an 87% cumulative risk to develop breast cancer before the age of 70, and 40–63% risk to develop ovarian cancer before that age (Fig. 1). The gene frequency of BRCA1 was estimated at 1 in 833 women, implying that 1.7% of all breast cancer patients diagnosed between the ages of 20 and 70 are carrier of such a mutation. The estimated cumulative risk of breast cancer conferred by BRCA2 reached 84% by age 70 years. The corresponding ovarian cancer risk was 27% (Fig. 1). These estimates imply that BRCA2 mutations are about as prevalent as BRCA1 mutations. It has been suggested that the ovarian cancer risks are dependent on the position of the mutation in the gene, for BRCA1 as well as BRCA2 mutations. There is also some evidence that cancer risks can be modified by other factors. For example, a strong variability in phenotype can be seen among families segregating the same mutation. This can range from early-onset breast cancer and ovarian cancer, to late-onset breast cancer without ovarian cancer. Even within a single pedigree, ages of onset of cancer can vary substantially. It seems likely that environmental and hormonally related factors (smoking, oral contraceptives) importantly co-determine disease outcome in carriers.

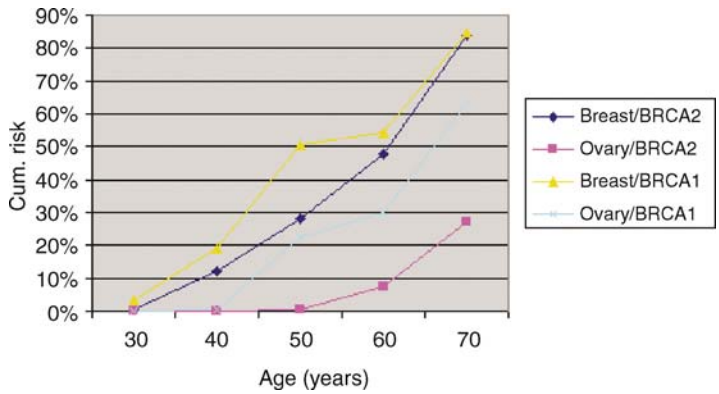
Molecular and Cellular Characteristics

Tumor Suppressor Genes

The first clues to the roles of BRCA1 and BRCA2 in tumorigenesis were genetic. The fact that most germline mutations are predicted to inactivate the protein, and the observed loss of the wild type allele in almost all breast and ovarian cancers arising in mutation carriers, are strong indicators that BRCA1 and BRCA2 proteins act as tumor suppressors. This is supported by the finding that induced overexpression of wild type but not mutant BRCA1 in MCF-7 breast cancer cells leads to growth inhibition and inhibited tumor growth in nude mice.

Expression of BRCA1 and BRCA2

In normal cells, BRCA1 and BRCA2 encode nuclear proteins, preferentially expressed during the late-G1/early-S phase of the cell cycle, but down-regulated in quiescent cells. While apparently at odds with the



BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Figure 1 Overall penetrances of BRCA1 and BRCA2 for breast and ovarian cancer. Estimates were obtained by maximizing the LOD score with respect to all the different penetrance functions in those families with strong evidence of the breast and ovarian cancers being caused by the gene (done by linkage analysis). This is equivalent to maximizing the likelihood of the marker data, which is determined only by disease phenotype data. This will give an unbiased estimation of the penetrance irrespective of ascertainment of families on the basis of multiple affected individuals. Data were compiled from Ford et al. (1994) Lancet 343:692–695 and Ford et al (1998) Am J Hum Genet 62:676–689. The graphs can be read in such a way that, for example, an unaffected carrier of a BRCA1 mutation has a 50% risk to develop breast cancer before age 50.

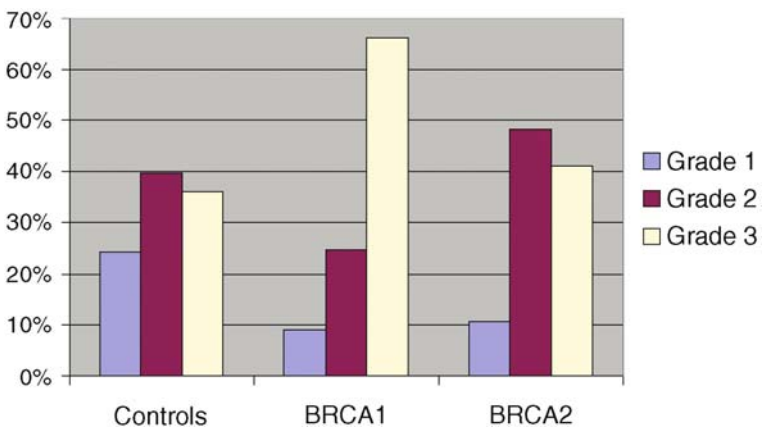
above-mentioned observations that BRCA1 expression inhibits cellular proliferation, the proliferation-induced expression could represent a negative feedback loop tending to decrease breast cancer risk. However, BRCA1 expression can also be up-regulated in a proliferation-independent way in mammary epithelial cells induced to differentiate into lactating cells by glucocorticoids. Hence, BRCA1 might also play a role in controlling mammary gland development. In mice, expression of BRCA1 and BRCA2 is coordinately up-regulated with proliferation of breast epithelial cells during puberty, pregnancy and lactation. Intriguingly, BRCA1 might suppress estrogen-dependent mammary epithelial proliferation by inhibiting ER-α mediated transcriptional pathways related to cell proliferation. Whatever the cellular function of BRCA1, it appears to be regulated by phosphorylation: it becomes hyperphosphorylated at G1/S with dephosphorylation occurring at M phase. BRCA1 might regulate the G1/S checkpoint by binding hypophosphorylated retinoblastoma protein. BRCA1 and BRCA2 have also been suggested to regulate the G2/M checkpoint by controlling the assembly of mitotic spindles and the appropriate segregation of chromosomes to daughter cells.

BRCA1- and BRCA2-Related Breast Cancer

A close examination of the pathology of BRCA1- and BRCA2-related breast cancers has defined a typical pathology for each category, differing from that in sporadic cases. In general, cancers in carriers are of higher grade than age-matched controls (Fig. 2), and the BRCA1-cancers more frequently display a "medullary"-like appearance. This is due to a higher mitotic count and lymphocytic infiltrate. BRCA2-related breast cancers generally show fewer mitoses and less tubule formation. For both BRCA1- and BRCA2-related cancers, greater proportions of the tumor show continuous pushing margins. Although a role for BRCA1 and BRCA2 in non-inherited sporadic breast cancer is unclear, protein expression of BRCA1 was found to be reduced in most sporadic advanced (grade III) ductal breast carcinomas.

BRCA1 and BRCA2 as Caretakers of the Genome

To date, several biological roles for BRCA1 and BRCA2 have been demonstrated, and a number of observations indicate that they function in a similar pathway. Both maintain genomic stability through their involvement in homologous recombination, transcription-coupled repair of oxidative DNA damage and double-strand break repair. These roles are suggested by interactions of the Brca1 and/or Brca2 proteins with proteins known to be involved in DNA damage repair, most notably RAD50 and RAD51. Murine embryonic stem cells and mice in which both copies of BRCA1 or BRCA2 have been mutated show a repair deficiency and defects in cell-cycle checkpoints. BRCA1 and BRCA2 play a role in transcriptional regulation, through interactions or complex formation with RNA polymerase II and various transcriptional regulators, although this is presently more firmly established for BRCA1 than for BRCA2. A transcriptional response to DNA damage is well-documented,



BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Figure 2 BRCA1- and BRCA2-related breast cancers are generally of higher grade than age-matched controls. Histological sections from 118 breast tumors attributable to BRCA1, and 78 attributable to BRCA2, were evaluated by five histopathologists, all experts in breast disease. Every slide was seen by two pathologists. An age-matched group of 547 apparently sporadic female breast cancer cases served as control. The overall grade of both BRCA1 and BRCA2 breast cancers was significantly higher than that of controls ($p < 0.0001$ and $p < 0.04$, respectively). For BRCA1 breast cancers this was due to higher scores for all three grade indices, whereas for BRCA2 breast cancers the grade was only significantly higher for tubule formation. Data taken from The Breast Cancer Linkage Consortium (1997) Lancet 349:1505–1510.

and identification of downstream targets of BRCA1/2-mediated transcription regulation might help to further understand how BRCA1 and BRCA2 suppress tumor formation. Microarray-based screening of genes regulated by BRCA1 were recently found to fall into two categories, cell-cycle control genes and DNA damage response genes.

Clinical Relevance

When to Take the DNA-test?

Diagnosis of gene defects became possible after the identification of BRCA1 and BRCA2 in 1994 and 1995, respectively. In many countries, testing for mutations is being offered to women with a high priori familial risk in Clinical Genetic Centres or multidisciplinary Cancer Family Clinics. A few studies have presented models to determine the prior probability that the counselee is a BRCA mutation carrier, by combining breast and ovarian cancer family history data with results from comprehensive mutation-testing. These models enable the genetic counselor to decide when a DNA-test is indicated.

Why Take the DNA-test?

A clear positive result of the DNA-test, i.e. the presence of a deleterious mutation, is being used to enter these women into early-detection cancer screening programs or in the decision for or against prophylactic surgery. A woman in which breast cancer has just been diagnosed can benefit from knowledge about gene carrier status, since the risks to the contralateral breast and ovaria must be considered. The treatment of such cancer by lumpectomy will not reduce recurrence risks dramatically, as opposed to complete mastectomy. Healthy women who test positive can take action to prevent cancer developing, although the efficacy of the preventive options currently offered to a woman remains without formal supporting evidence. Chemoprevention is still controversial, and good prospective data on BRCA carriers will probably never become available, given the ethical and clinical difficulties surrounding randomization. Prophylactic surgery, intuitively the most secure way to reduce breast cancer risk to below population levels, is socially ill-accepted in many parts of the world, and formal proof of its preventive effect in BRCA carriers is also lacking. Clearly, this area is fraught with clinical dilemmas.

Interpreting a Negative Test Result

Paradoxically, a negative test result (the absence of a deleterious mutation) presently still has limited power in excluding the presence of a strong susceptibility allele. A negative test result is presently being found in 70–80% of all probands tested in most non-Ashkenazi Jewish populations. Among probands with a family history for ovarian cancer, a negative test result is found less frequently (although still in 40–60% of the cases). There are several levels of uncertainty.

- The first is technical: no single mutation-detection method is 100% sensitive, and therefore only exhaustive testing, using a range of different methodologies sensitive to various types of mutation-mechanisms,

BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk. Table 1 Mutation types in BRCA1 and BRCA2 and their predicted effects

	BRCA1		BRCA2	
	% of Total	% of Distinct	% of Total	% of Distinct
Mutation type				
Frameshifting	47.1	38.7	33.7	36.5
Nonsense	11.3	11.1	11.5	10.2
Splice-site	4.4	7.9	2.2	3.6
In-frame del/ins	0.6	1.8	0.4	1.0
Missense	28.4	28.4	44.3	35.4
Neutral	3.5	3.9	3.1	5.5
Intronic change	4.7	8.3	4.9	7.8
Mutation effect				
Protein truncating	62.6	56.9	41.4	47.9
Missense	2.2	1.5	0.7	1.9
Neutral polymorphism	11.0	7.2	14.4	13.7
Unclassified variant	24.2	34.4	43.4	36.4

The entire Breast Cancer Information Core (BIC) database was down-loaded on March 1, 2000 from http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic. There were 3,086 *BRCA1* mutations and 1,892 *BRCA2* mutations. The total numbers of distinct changes were 724 and 670, respectively.

and investigating the entire coding regions and regulatory domains, can detect any changes. This is obviously very cost- and labor-intensive.

- The second level of uncertainty relates to the interpretation of sequence changes that do not predict a truncated protein. Of the almost 5,000 BRCA1 and BRCA2 mutations submitted to the Breast Cancer Information Core (BIC) database, about one-third are either missense, in-frame deletions or insertions, base-substitutions not leading to an amino acid change (neutral changes) or intronic changes with unknown effect on mRNA-processing (Table 1). Only a small proportion of these have been unmasked as polymorphisms unrelated to disease outcome. They include missense changes and intronic variants, but, intriguingly, also a nonsense mutation in BRCA2. The K3326X mutation was found in 2.2% of over 400 controls tested. Only a few missense changes (e.g., BRCA1C61G) have been called a deleterious disease-related mutation, mainly because they reside in a validated functional domain of the protein or affect an evolutionary conserved residue. As a result, about 35% of all the distinct gene changes detected to date are lumped into the "unclassified variant" category, meaning that their relevance to disease outcome is uncertain. Almost certainly, a substantial proportion of these represent rare polymorphisms but equally certainly, a number of them will turn out to be true deleterious mutations.
- A third reason for a negative test result is that the familial clustering of breast cancer in a family is due to an unknown gene or in fact is a non-genetic chance event. The proportion of truly missed, deleterious mutations is therefore difficult to gauge. A study by the BCLC has suggested that a combination of incomplete testing and missed or misinterpreted gene changes, causes false-negative test results in over 30% of all family types with some evidence of being linked to BRCA1. This proportion was independent of the mutation-screening methodology used.

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BRCT Domain

Definition

Named after BRCA1 c-terminal repeat. The domain consists of a 90–100 amino acid unit that occurs as a single element or as multiple repeats in several proteins involved in the DNA-damage response. Heterodimerization between BRCT repeats promotes protein-protein interactions. A subset of tandem BRCT repeats adopt a conserved head-to-tail structure. Such tandem repeats can function as a phospho-peptide binding module that binds proteins with specific phosphorylation motifs.

►Fanconi Anemia
►BRIT1 Gene
►BRCA1/BRCA2 Germline orientation and Breast Cancer Risk

Breakpoint

Definition

Point of separation on a chromosome involved in translocation or other structural rearrangement.

►E2A-PBX1
►Chromosomal Translocations

Breakpoint Cluster Region

Definition

A localized site of recurrent DNA breakage.

►ALU Elements
►BCR-ABL1

Breast Cancer

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Definition

Breast cancer may originate from more than one cell type in the breast as a result of different subsets of molecular changes. It is therefore a collection of diseases with different characteristics, different risks and different treatments. It occurs predominantly in women but it may also occur in men (0.5% of cases). In addition to invasive disease, several benign pre-malignant and non-invasive forms exist. Although the broad pathological categories are generally accepted there are several alternative systems of sub-categorization.

- Benign conditions: include sclerosing conditions and obliterative mastitis; mild moderate and severe hyperplasias and atypical hyperplasias; fibrocystic conditions, fibroadenomas and related conditions (note that the Oxford Textbook of Pathology presents a "simplified nomenclature" of 50 subtypes of benign breast disease).
- Non-invasive carcinoma: generally divided into Lobular Carcinoma in situ (LCIS) and Ductal Carcinoma in situ (DCIS). DCIS was originally sub-divided into comedo, cribriform, papillary, solid and clinging but has more recently been categorized as well, moderately and poorly differentiated.
- Invasive carcinoma: broadly defined as of "special type" (30% of cases) and "no special type" (70% of cases).

Characteristics

Epidemiology

Breast cancer is the most common fatal malignancy in women in the Western world representing about 10% of all cancer deaths. It is however much less common in other countries probably as a result of environmental rather than genetic factors. Behavioral risk factors have been identified. Early pregnancy to term and multiple pregnancy are protective for breast cancer incidence probably due to a reduced exposure to estrogens. There are several reports of weak associations with diet and use of oral contraceptives.

Screening

In some countries mammographic screening is available to women to detect early disease, as it is likely that earlier treatment is beneficial for survival. Educational programs are also active, for instance in promoting self-examination as a method of early detection. The value of these approaches for improving patient survival is not yet fully established.

Genetics

About 5–10% of breast cancer cases are associated with a genetic predisposition to the disease. Recently three genes have been identified where the inheritance of variants is associated with a very high incidence (penetrance) of the disease. The ►BRCA1 gene was the first to be identified, and gene carriers are thought to have as much as a 70–80% chance of developing the disease, generally at an earlier age than women with "sporadic" (not genetically predisposed) disease. Subsequently a second gene called ►BRCA2 was also found in other families that predisposes to breast and ovarian cancers. The particular risk in an individual gene carrier may be determined by the nature of the particular gene defect and by environmental and hormonal factors and by their background genetic makeup (►breast cancer genes BRCA1 and BRCA2). A further inherited condition, called ►Li-Fraumeni syndrome is associated with increased risk to breast and many other types of cancers. This is due to the inheritance of a rare mutant copy of the ►p53 gene.

Molecular Biology

Breast cancer is the most studied form of human cancer, due to its common occurrence and to the availability of many immortal breast cancer derived cell lines that can be grown in tissue culture (in contrast to prostate cancer for instance). About 60% of breast cancers at diagnosis express the estrogen receptor. Several genes have been found to be altered by mutation or amplification in invasive cancer and in DCIS. The ►HER-2 or ERBB2 gene (also known as c-erbB-2 and neu) is amplified in about 25% of invasive breast cancers leading to overexpression of the growth factor receptor which it encodes. The c-myc gene is similarly found to be amplified in about 20% of breast cancer also resulting in overexpression of the c-myc protein. The ►cyclin D gene, which specifies a protein important in regulating the cell cycle, is also amplified in a proportion of breast cancers. The p53 gene has been found to be point mutated in ~20% of invasive breast cancers and to be overexpressed in about 50% of cases. Other point mutations have been found in E-cadherin gene, which encodes a cell adhesion molecule. In this case the mutations are most common in lobular cancers. More subtle changes occur in the expression of apparently normal proteins including growth factors such as those in the epidermal growth factor (EGF) family and the FGF family of proteins, the receptor tyrosine kinase c-erbB-3 and the Src tyrosine kinase. Some of these altered proteins represent targets for new forms of treatments.

Treatment

Three methods of treatment are available, surgery, radiotherapy and chemotherapy/hormonal therapy. Benign disease and lobular carcinoma in situ are rarely treated but are observed, as they are associated with an increased risk of developing breast cancer. DCIS of the breast has often been treated by mastectomy as it is frequently quite widespread within the breast but may also be treated by local surgery. It is possible that the different pathologically defined forms of DCIS may be associated with different relative risks of recurrence and recurrence as invasive disease. Invasive disease is generally first treated by surgery, and lymph nodes are sampled to determine, by pathological diagnosis, if there is evidence of tumor spread. This procedure may be limited to a single node (called the sentinel node) or may involve a greater degree of surgery. Patients with invasive breast cancer are often treated with radiotherapy to the breast to reduce the chances of local recurrence. Even if the cancer has not apparently spread, patients are sometimes offered preventative or "adjuvant" therapy using drugs, as this helps to prevent recurrence of the disease. Chemotherapy or hormonal therapy are generally offered to patients where metastatic spread of the disease has occurred. Hormonal therapy is frequently offered to women whose tumors express the estrogen receptor. Specific methods of treatment still vary depending on the patient and the institution where it is given, although more generally agreed protocols are becoming accepted.

New Treatments

Breast cancer is frequently a hormonally dependent disease. Thus treatments with drugs such as tamoxifen, which binds to the estrogen receptor and reduces its activity, or other drugs that suppress the production of estrogen, such as aromatase inhibitors, are frequently employed. However, new drugs directed to known molecular changes in the cancer cells are under development. These include signal transduction inhibitors directed to molecules such as the epidermal growth factor receptor, monoclonal antibodies to the c-erbB-2 receptor and drugs which inhibit proteolytic enzymes thought to be involved in the process of metastasis. Several of these are now in clinical trials that will determine their usefulness for the treatment of the disease.

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Breast Cancer Genes BRCA1 and BRCA2

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Definition

►*BRCA1* and ►*BRCA2* are cancer-predisposition genes, germline mutations in which are associated with a high risk of developing breast, ovarian and other cancers. Much information has been accumulated on the function of their large, nuclear-localized protein products, which implicates them in the cellular response to DNA damage, the control of mitotic cell division, and the regulation of gene transcription. BRCA1 and BRCA2 are very distinct genes, despite the similarity in their acronyms.

Characteristics

Roughly one-tenth of all ►breast cancer cases exhibit a familial pattern of inheritance. Of these familial cases, germline mutations in either one of two genes, *BRCA1* or *BRCA2*, occur in 20–60% (that is, in 2–6% of all cases). Somatic mutations in *BRCA1* or *BRCA2* do not appear to be a feature of non-familial (that is, sporadic) breast cancer, but there is evidence that epigenetic suppression of *BRCA* gene expression, or genetic alterations affecting the biological pathways in which they participate, can occur in sporadic breast cancer.

BRCA1 and *BRCA2* were first identified in 1994–1995 through the analysis of families exhibiting a predisposition to early-onset breast cancer. Founder mutations affecting these genes occur in Iceland and amongst the Ashkenazim, where they confer a highly penetrant risk of breast, ovarian and other cancers (including cancers of the male breast, pancreas and prostate). In other populations, germline *BRCA1* or *BRCA2* mutations are found in the great majority (up to 80%) of families that suffer from multiple occurrences of breast plus ovarian cancer. Germline *BRCA2*

mutations affecting both alleles also occur in the rare D1 complementation group of Fanconi anaemia.

The *BRCA1* and *BRCA2* genes have been assigned to human chromosomes 17q and 13q, respectively. In both genes, exon 11 (~3.4 kb in *BRCA1*, or 5 kb in *BRCA2*) encodes a large portion of the protein. Overall, the murine and human genes are no more than 60% identical at the amino acid level, although small regions exhibit a much higher degree of conservation. Proteins encoded in alternative splice products, such as the IRIS protein encoded by the *BRCA1* gene, also exist.

Protein

BRCA1 and *BRCA2* encode large proteins (human BRCA1 is 1,863 amino acids long; and human BRCA2 is 3,418 amino acids) that localise to the nucleus in mitotic and meiotic cells (Fig. 1). They bear little resemblance to proteins of known function. At its N-terminus BRCA1 protein contains a RING-domain known to mediate hetero-dimerization with the RING domain of BARD1, forming an active E3 ubiquitin ligase. At its C-terminus, BRCA1 includes two copies of a ~95 amino acid motif (the BRCT domain, for BRCA1 C-terminal) later detected in a number of different proteins implicated in DNA repair and cell cycle checkpoint control. This domain, whose atomic structure has been elucidated, mediates a number of protein-protein interactions with phosphorylated targets by serving as a phosphopeptide-binding module.

Eight repeated sequences (the BRC repeats), each of about 30 amino acids, are encoded in *BRCA2* exon 11. The BRC repeats, but not their intervening sequences, are conserved between several mammalian species suggestive of a conserved function. Indeed, the interaction of BRCA2 protein with RAD51, a mammalian homologue of bacterial RecA essential for genetic recombination, occurs through the BRC repeats. There is good evidence from genetic, structural and biochemical studies that the BRC repeats regulate the activity of RAD51 in reactions that lead to DNA repair by recombination. Two other regions of BRCA2 have been implicated in the control of recombination. A domain carboxyl-terminal to the BRC repeats interacts with the small protein Dss1 to form a structure capable of binding junctions between single-stranded and double-stranded DNA, which can displace the ssDNA-binding protein RPA from recombination substrates, whereas an additional RAD51-binding region of uncertain function is located near the extreme C-terminus of BRCA2.

Cellular and Molecular Regulation

The transcripts and protein products encoded by BRCA1 and BRCA2 are expressed in dividing cells of many types. Expression is also high in meiotic cells. These expression patterns speak to the possible functions of BRCA1 and BRCA2 proteins.

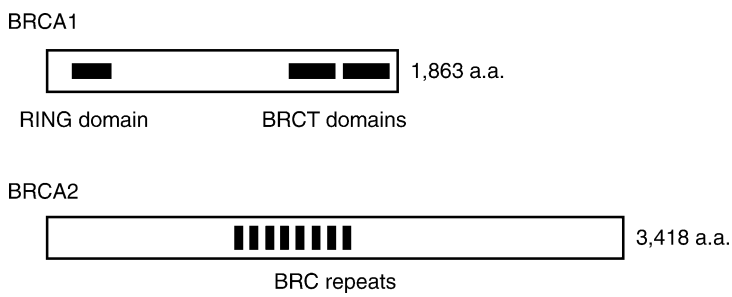
Role in the Cellular Response to DNA Damage

Both BRCA1 and BRCA2 proteins localize to the nucleus. In meiotic cells, co-localisation has been reported to the synaptonemal complexes of developing axial elements. This is suggestive of a role in meiotic recombination, a process that is initiated by DNA double-strand DNA breakage. Similarly, there is good evidence that BRCA1 and BRCA2 are essential in mitotic cells for the repair of DNA double-strand breaks by homologous recombination.

Several lines of evidence are indicative of such a role:

- Cells in which BRCA1 or BRCA2 or their homologues in other species have been inactivated exhibit genotoxin hypersensitivity and chromosomal instability suggestive of defects in DNA double-strand break repair.
- Second, homology-directed repair of double-strand DNA breaks introduced into chromosomal substrates is impaired by the disruption of BRCA1 or BRCA2, although pathways for non-homologous end joining remain largely unaffected.
- Finally, BRCA1 and BRCA2 localize after DNA damage to nuclear foci where they interact with molecules implicated in DNA recombination, including RAD51, and the Fanconi anemia proteins.

The precise mechanisms that may underlie such a function remain to be determined. BRCA2 interacts directly, and at a relatively high stoichiometry, with



Breast Cancer Genes BRCA1 and BRCA2. Figure 1 Structural features of the BRCA1 and BRCA2 proteins (not drawn to scale).

RAD51, a protein essential for DNA repair by recombination, to modulate RAD51 activity or availability. The interaction of BRCA1 with RAD51 is less well defined, although both proteins co-localize – along with BRCA2 – to discrete nuclear foci following DNA damage. BRCA1 may participate in the cellular mechanisms that sense and signal DNA damage, culminating in the activation of cell-cycle checkpoints and the machinery for DNA repair. The protein kinases ATM (encoded by the gene mutated in Ataxia telangiectasia), ATR, chk1 and chk2 (mutated in ►Li-Fraumeni syndrome) are proximal components of these sensing/signaling mechanisms. ATM, chk1 and probably the other checkpoint kinases, phosphorylate BRCA1 following DNA damage, a modification essential for its proper function. These observations are important because they place BRCA1 – and by extension, possibly BRCA2 – in the same pathway as genes such as ATM (►ATM protein), germline mutations in which are also associated with an increased risk of breast and other cancers. Thus, a common DNA damage response pathway may be defective in a significant fraction of breast cancers.

BRCA1 and BRCA2 have also been implicated in the enforcement of cell cycle checkpoints during the G2 and M phases, and in the regulation of centrosome number. Additional functions have recently been described in the control of mitosis. BRCA1 regulates proteins such as MAD2 that act in the mitotic spindle assembly checkpoint, and has an essential function in directing the correct formation and function of the mitotic spindle, whereas BRCA2-deficient cells exhibit defects in the completion of cell division by cytokinesis. Thus, BRCA1 and BRCA2 appear to work in multiple processes responsible for maintaining the integrity of chromosome number as well as structure in dividing cells, which may help to explain why they are potent tumor suppressors.

Other Functions

It is difficult to reconcile the disparate nature and severity of the cellular and developmental defects induced by the disruption of murine homologues of BRCA1 and BRCA2, with functions exclusively in the response to DNA damage. Indeed, evidence is accumulating that BRCA1, in particular, can control gene transcription. Several proteins that interact with BRCA1 are known to regulate transcription or mRNA processing. Moreover, at least a fraction of the total intracellular pool of BRCA1 is linked to the general transcription machinery – the RNA polymerase II holoenzyme – through its RNA helicase subunit.

Intriguingly, BRCA1 has been implicated in the control of X-inactivation in female cells, a process whose dysregulation is associated with breast cancer predisposition. How this function may be exerted is not clear, but it may work through the control of localization of the Xist product.

In addition, roles for BRCA1 and possibly BRCA2 have been reported in the control of oestrogen receptor expression and signaling.

Tumor Suppression by BRCA1 and BRCA2

Inheritance of a single defective copy of *BRCA1* or *BRCA2* confers cancer predisposition in humans. However, the second allele is almost invariably lost in the cancers that arise in predisposed individuals, indicating that *BRCA1* and *BRCA2* behave in some respects as ►tumor suppressor genes.

Abnormalities in growth or in DNA repair have not yet been reliably detected in murine or human cells heterozygous for *BRCA1* or *BRCA2* mutations. Thus, there is currently little to suggest that cancer predisposition arises solely from haplo-insufficiency, or a trans-dominant deleterious effect induced by a single mutant *BRCA1* or *BRCA2* allele. Rather, as has been proposed for other tumor suppressor genes, germline mutations in one allele may simply increase the likelihood that the gene is wholly inactivated by loss of the second allele through somatic mutation. However, aneuploidy as a consequence of abnormal cytokinesis has been reported in cells and tissues heterozygous for *BRCA2* mutations, and the possibility that this effect of haplo-insufficiency contributes to carcinogenesis remains to be determined.

Whatever the events that lead to loss-of-heterozygosity, inactivation of BRCA1 or BRCA2 would initiate genetic instability by destabilizing chromosome structure and number, allowing the rapid evolution of tumors due to increased somatic alterations in genes that control cell division, death or lifespan. Thus, *BRCA* genes are proposed to work as "caretakers" of genetic stability. This "caretaker" role is most likely to arise through the function of the BRCA proteins in DNA repair and mitosis. Cells that harbor disruptions in BRCA1 or BRCA2 accumulate aberrations in chromosome structure, reminiscent of diseases like Bloom syndrome or ►Fanconi anemia, where chromosomal instability is associated with cancer predisposition. They also exhibit aneuploidy and defects in cell division. These defects could together elevate the rate of genomic instability, leading to somatic mutations or alterations in gene copy number that promote carcinogenesis.

It is unclear why carcinogenesis accompanied by loss of the second *BRCA* gene allele in individuals who inherit one mutant allele should occur preferentially in tissues such as the breast or ovaries. Both BRCA1 and BRCA2 are widely expressed and appear to perform functions essential to all tissues. Currently there is little evidence to help distinguish between the several possible explanations that can be advanced.

The chronology of the molecular events during carcinogenesis in *BRCA* gene mutation carriers is not known. Loss of the second allele is clearly very frequent, but it is unclear at what stage in tumor

evolution this may occur. However, the catastrophic cellular consequences of homozygous inactivation of *BRCA1* or *BRCA2*, which quickly lead to cell death, does emphasize that other genetic alterations will be necessary. Current evidence favors the notion that the inactivation of cell-cycle checkpoint genes, particularly those that enforce mitotic checkpoints, is an important additional step during carcinogenesis in *BRCA* gene mutation carriers.

Viewed in this way, it is conceivable that the tissue specificity of carcinogenesis represents differences in the ability of cells which have lost both alleles of *BRCA1* or *BRCA2* to survive for long enough to acquire these additional genetic alterations. For instance, BRCA-deficient cells in epithelial tissues such as the breast and ovary may take advantage of hormonal or local inter-cellular interactions to support survival until the accumulation of additional genetic alterations allows outgrowth. By contrast, BRCA-deficient cells in non-target tissues may quickly be eliminated.

Clinical Relevance

Germline mutations in *BRCA1* or *BRCA2* are frequently associated with familial, early-onset, breast and ovarian cancer, particularly in those families that suffer from multiple cases of cancer in both sites. This has obvious important implications for genetic testing and counselling in the clinic. The mutations have been estimated to carry a cumulative life-time cancer risk of between 40–70%.

There is some evidence that the pathological features of breast and ovarian cancers associated with *BRCA1* or *BRCA2* mutations differ from those of sporadic tumors. So far, these differences seem to be insufficiently well-marked to be of diagnostic significance. One notable association is that BRCA1-deficient cancers often exhibit a "basal-like" phenotype usually characterized by the expression of specific markers, and negativity for oestrogen receptor expression.

It is also unclear if the prognosis of breast and ovarian cancers associated with *BRCA1* or *BRCA2* mutations will differ significantly from that of sporadic cases. Conflicting results have been reported in the literature, their interpretation made difficult by the varied study designs and by the relatively small numbers of cases that have been compared. Similarly, the value of prophylactic interventions, whether surgical or drug-based, in *BRCA* gene mutation carriers awaits evaluation.

Emerging evidence suggests that the DNA repair defect inherent in *BRCA*-mutant tumors can be exploited in cancer therapy. Thus, both BRCA1 and BRCA2-deficient cancers appear to be hypersensitive to the effect of DNA cross-linking agents such as carboplatin, and also to novel chemical inhibitors of the PARP1 enzyme.

Breast Cancer Resistance Protein

Definition

The breast cancer resistance protein is a plasma membrane transporter and member 2 of the subfamily G of ATP-binding cassette transporters. It is predominantly localized in the epithelium of small and large intestine, in hepatocyte canalicular membranes, in ducts and lobules of the breast, on the placental syncytiotrophoblast, and in the plasma membrane of stem cells. Physiological substrates include sulfated steroids. Transported xenobiotics are e.g., several dietary carcinogens, the chlorophyll breakdown product pheophorbide, and the receptor tyrosine kinase inhibitor imatinib. The breast cancer resistance protein is involved in conferring multidrug resistance.

►Membrane Transporters

Breast Conservation

Definition

Surgical removal of cancer from the breast while preserving the remainder of the breast tissue.

►Oncoplastic Surgery

Breast Imaging Reporting and Data System

Definition

BIRADS; http://www.birads.at/index.html

Breast Implant

Definition

Device placed into the body to enlarge or reconstruct the breast after removal.

►Oncoplastic Surgery

Breast Reconstruction

Definition
Surgical procedure to simulate the appearance of the breast after it has been removed for the treatment of cancer.

►Oncoplastic Surgery

Breast Reduction

Definition
Surgery to reduce the size of the breast.

►Oncoplastic Surgery

Breast Regressing Protein 39 Kd

►Serum Biomarkers

Breast Stem Cells

Definition
Self-renewing cells in the breast that are capable of producing all of the breast epithelial cell types and likely play an important role in breast cancer.

►Basal-like Breast Cancer

Breast Surgery

Definition
Surgical procedures on the breast used to treat congenital deformities or to remove cancerous tissue.

►Oncoplastic Surgery

Breast Transillumination

►Optical Mammography

Breslow Depth

Definition
Referring to ►cutaneous desmoplastic melanoma tumor thickness (according to *Breslow*). The single most important factor in predicting survival for patients with stage I/II melanoma. Is measured from the top of the granular layer (for non-ulcerated lesion) or from the ulcer base overlying the deepest point of invasion (for ulcerated lesions) to the deepest extension of the tumor using an ocular micrometer. According to the AJCC-criteria for malignant melanoma, tumor thickness and the presence or absence of ulceration are the primary criteria for the tumor classification.

►Desmoplasia
►Desmoplastic Melanoma

BRG- and BRM-associated Factor, 47 kDa

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

Brilliant Yellow S

►Curcumin

BRIP1

►BACH1 Helicase

BRIT1 Gene

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Synonyms

Microcephalin; MCPH1

Definition

BRCT-repeat inhibitor of hTERT expression.

Characteristics

Significance of BRIT1 in the Development of Cancer

►BRIT1 was first described through a genetic screen for transcriptional repressors of the catalytic subunit of ►human telomerase, (hTERT). This catalytic subunit, hTERT, is the rate-limiting determinant of and is necessary for telomerase activity and thus is highly significant for cellular immortalization by preventing natural cellular senescence. Therefore, molecules that negatively control hTERT activity, such as BRIT1, directly influence the development of pre-cancerous and cancerous cells.

The BRIT1 amino acid sequence matched a previously identified gene, ►Microcephalin (►MCPH1) that is implicated as one of the contributing factors in the autosomal recessive neuro-develomental disorder, primary microcephaly. Additional biological roles for BRIT1 in the DNA damage response pathway were suggested by the protein structure. The presence of three ►BRCA1 carboxy-terminal (BRCT) domains within its structure connected BRIT1 with a group of proteins involved in DNA damage repair and checkpoint control such as ►53BP1, ►MDC1 and BRCA1. Proper cellular response to DNA damage is essential for the maintenance of genomic stability and is consequently crucial in prevention of neoplastic transformation. Depletion of BRIT1 by experimental manipulation abolishes normal DNA damage response and introduces chromosomal and centrosomal abnormalities. The reduction of BRIT1 expression in normal human mammary epithelial cells by experimental RNA interference generated chromosomal breaks, dicentric chromosomes and telomeric fusions. Additional chromosomal aberrations were introduced when BRIT-deficient cells were submitted to genotoxic insult. The resultant genomic instability generated from the loss of an appropriate BRIT1-mediated checkpoint and DNA repair mechanism may contribute to tumor formation. Functional impairment or loss of proteins, such as BRIT1, may significantly contribute to tumorigenic development by allowing the perpetuation of damaged or mutated genes within a cell, resulting in the inappropriate expression and control of the affected genes.

In addition to the influence on hTERT expression, BRIT1 appears to control the expression of two vital checkpoint-regulating proteins; BRCA1 and Chk1. BRCA1 and Chk1 protein levels are dramatically reduced in cells where BRIT1 has been experimentally reduced by RNA interference. A concurrent reduction in mRNA levels of BRCA1 and Chk1 were observed in BRIT1 knockdown cells suggesting that BRIT1 exercises an influence on the transcription of these genes. The significance of BRCA1 in breast cancer has been previously established. Whether BRIT1 functions directly as a specific transcription factor or as a chromatin modifying factor is unclear at this time, however, as a controller of these key players in the DNA damage checkpoint control network, BRIT1 is extremely important to the maintenance of normal cell function and thus in the prevention of tumorigenesis.

Aberrations of BRIT1 have been identified in various different human cancers. BRIT1 mRNA and protein expression is aberrantly reduced in several breast cancer cell lines and is reduced in some human ovarian and prostate epithelial tumors as compared to the corresponding normal tissue. Reduction of BRIT1 gene copy number significantly correlated with genomic instability found in the specimens. Additionally, reduced BRIT1 expression correlated with the duration of the relapse-free intervals and with the occurrence of metastases in some breast cancer patients suggesting that BRIT1 deficiency may contribute to the aggressive nature of breast tumors. A mutant form of BRIT1, isolated from one human breast tumor specimen, lacked two C-terminal BRCT-domains of the protein. This shorter form of BRIT1 resulted in a loss-of-function with respect to DNA damage response when tested experimentally. Therefore, significant evidence exists to directly link defective or reduced BRIT1 protein expression to several forms of cancer and to implicate BRIT1 as a novel tumor suppressor gene.

BRIT1 Function in DNA Damage Response

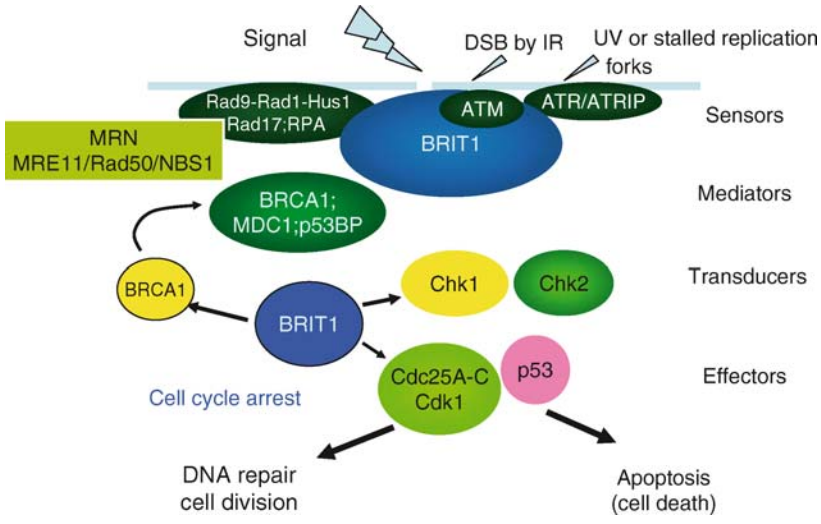
In the normal course of events, cellular DNA is subjected to a variety of endogenous and environmental factors that induce damage within its structure. In response to these insults, normal cells activate complex mechanisms to detect, signal the presence of and subsequently repair DNA damage when possible. Propagation of the DNA damage alarm progresses

through a complex signal transduction network that includes the BRIT1 protein. Initially, sensor proteins recognize the location of damaged or altered DNA structure and transmit a signal through mediator molecules to transducer proteins. The transducer proteins transmit the signal to numerous downstream effectors involved in specific pathways (Fig. 1). Two distinct DNA damage repair networks, both requiring BRIT1 activity, have been described. The ►ATM (ataxia telangiectasia mutated) pathway is activated by double-stranded breaks in the DNA observed after exposure to ionizing radiation while the ►ATR (ATM and Rad3-related) pathway is activated by prolonged presence of single-stranded DNA induced by either ultraviolet radiation or stalled DNA replication. ATM and ATR are essential kinases that are responsible for phosphorylating numerous transducer and effector proteins in the DNA damage network. BRIT1 co-localizes with numerous molecules associated with these two signaling networks including ►γH2AX, MDC1, 53BP, ►NBS1, p-ATM, ATR, p-RAD17 and p-RPA34 after DNA damage is induced. In the absence of functional BRIT1, all of these molecules with the exception of γH2AX, fail to localize to sites of DNA damage strongly suggesting that the BRIT1 molecule is an essential mediator for the subsequent repair processes. However, BRIT1 expression does not influence the chromatin binding of proteins unrelated to DNA damage such as Orc-2 indicating that the BRIT1 function is highly specific. The depletion of BRIT1 inhibits the recruitment of phosphorylated ATM to double-stranded DNA broken ends and subsequently blocks the phosphorylation of multiple down-stream members of the ATM repair pathway including Chk2 and NBS1. Depletion of BRIT1 also abolishes the UV-induced phosphorylation of RPA34 and reduces the levels of phosphorylated RAD17 indicating the importance of BRIT1 in the ATR signaling pathway. Based on these observations, it has been determined that BRIT1 is a pivotal protein in both of the DNA damage signaling networks and for this reason has great significance in the prevention neoplastic transformations of cells.

Figure 1 illustrates a simple model for the function of BRIT1 based on current experimental evidence. After exposure to ionizing radiation, double-stranded breaks in DNA occur resulting in the recruitment the MRE11/RAD50/NBS1 (MRN) complex and MDC1 to the damaged site thus facilitating the recruitment and kinase activity of ATM. Activated p-ATM phosphorylates NBS1, H2AX and BRCA1 which localize to sites of DNA damage. Increased single-stranded DNA by exposure to ultraviolet radiation induces the coating of RPA on DNA leading to the recruitment of ATRIP and ATR to the sites of DNA damage. Activated ATR then phosphorylates critical downstream molecules such as Rad17 and Chk1 further propagating the DNA damage signal in the cell. BRIT1 appears to regulate the recruitment of NBS1, MDC1 and thus the MRN complex in the ATM pathway. BRIT1 also regulates the recruitment of RPA, which in turn recruits ATRIP/ATR complex initiating the ATR signaling cascade.

BRIT1 Function in Cell Cycle Control

Normally, the progression of a cell through the cell division cycle is stalled to allow for its DNA repair and if the damage cannot be repaired, the cell enters programmed cell death (apoptosis). This retardation or cell cycle checkpoint is essential to maintain the



BRIT1 Gene. Figure 1 BRIT1 Function in DNA Damage and Cell Cycle Control.

integrity of cellular DNA that insures normalcy in consecutive descendant cells. Key molecules involved in cell cycle arrest, (p53, chk1 and chk2), are all activated when phosphorylated by ATM or ATR. BRIT1 clearly impacts the activity of both ATM and ATR by affecting the association of these molecules to damaged DNA. Activated p53 induces cell cycle arrest at G1 while p-Chk1 and p-Chk2 negatively regulate Cdc25 phosphatases that promote transition through the cell cycle thereby inducing the execution of G1/S, G2/M and intra-S checkpoints of the cell cycle. BRIT1 is required for the activation of intra-S and G2/M cell cycle checkpoints after cellular exposure to ionizing radiation. The influence of BRIT1 on control of these cell cycle checkpoints may result from BRIT1's regulation of three checkpoint regulator proteins, Chk1, BRCA1 and NBS1. In the absence of BRIT1, BRCA1 and Chk1 expression is significantly reduced and NBS1 fails to be phosphorylated. BRCA1 plays a significant role in homologous recombination DNA repair and possibly serves as a scaffold for ATM and ATR thus affecting phosphorylation of many downstream effectors proteins. Therefore the regulation of BRCA1 by BRIT1 dramatically affects multiple aspects of cell cycle control and DNA damage repair.

The normal cellular response to ionizing radiation is to arrest the cell cycle also at G2, allowing for the initiation of DNA repair, however, BRIT1-depleted cells continue to progress through G2 indicating that BRIT1 is essential in the activation of this important cell cycle checkpoint. Additionally, BRIT1-depleted cells continue to synthesize DNA and proceed through mitosis unlike normal cells exposed to ionizing radiation. Replication of DNA damaged by ionizing radiation could easily result in the propagation of mutated or disrupted genes and contribute to tumorigenesis.

BRIT1 also controls the cell's entry into mitosis by affecting the stabilization of the cdc25A, a key phosphatase in cell cycle control. Cells derived from a microcephaly patient (BRIT1 defective) maintained a persistent level of the phosphatase, cdc25A following UV treatment. Cdc25A is targeted for degradation when phosphorylated by Chk1 kinase during normal cell division and its degradation is usually amplified by UV exposure. Degradation of cdc25A abolishes the activation of the Cdk2-cyclin complex inhibiting DNA synthesis. Conversely, inappropriate persistence of cdc25A allows for the continued DNA synthesis despite aberrations or damage in the structure. These BRIT1 mutant cells also harbor reduced levels of phosphorylated Cdk1-cyclin B complex that is essential for mitotic entry. It was proposed that the regulation of mitosis by BRIT1 is both ATR dependent through regulation of cdc25A stability and ATR independent through regulation of Cdk1-cyclin B phosphorylation.

The affect of BRIT1 on cell cycle control is therefore multi-faceted (Fig. 1). Complete loss of the BRIT protein results in reduced protein levels of BRCA1 and Chk1 and impairs the activity of a multitude of vital proteins through their diminished phosphorylation. Presence of a mutated BRIT1 protein allows for expression of BRCA1 and Chk1 but still blocks proper signal transduction by inhibiting the activities of both ATM and ATR kinases.

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BRM

Definition

Biological response modifier.

BRMS1

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Definition

BRMS1 (Breast Metastasis Suppressor 1) is a human ►metastasis suppressor gene that, when re-expressed, suppresses metastasis of human breast carcinoma, ovarian, and melanoma cell lines in immunocompromised mouse models.

Characteristics

The *BRMS1* gene is located on chromosome 11q13.1-q13.2. It spans over 8.5 kb and is comprised of ten exons, the first exon being untranslated. *BRMS1* cDNA is 1485 base pairs and encodes a 246 amino acid protein (MW ~28.5 kD, although it runs more slowly (~35 kDa) by SDS-PAGE). It is highly conserved with the mouse ortholog (*Brms1*) having 95% homology at the amino acid level. Like human *BRMS1*, the mouse ortholog has suppresses metastasis in murine models of breast cancer.

BRMS1 protein contains two nuclear localization sequences, two coiled-coil motifs and imperfect leucine zippers. It also contains a glutamic acid rich N-terminus and a potential endoplasmic retention signal. *BRMS1* shows almost ubiquitous expression in human tissues; highest expression is in kidney, placenta, peripheral blood lymphocytes and testis and lowest expression in brain and lung. Subcellular fractionation and immunofluorescence studies have determined that *BRMS1* protein is predominantly (>90%) nuclear. *BRMS1* protein is stabilized by interaction with the chaperone protein, Hsp90, and is further regulated by proteasomal degradation.

Cellular and Functional Characteristics

BRMS1 re-establishes gap junctional cell-cell communication in human breast cancer cell lines. Studies using MDA-MB-435, MDA-MB-231, and the ovarian cancer cell line HO-8910PM show an inverse effect of *BRMS1* expression on cell motility. Further, over-expression of *BRMS1* in H1299 human lung carcinoma cells and MDA-MB-435 cells results in suppressed growth in soft agar. Additionally, *BRMS1* transfection increases apoptosis in suspended non-small cell lung carcinoma.

The exact mechanism by which *BRMS1* affects these phenotypes is as yet unknown. However, it is known that *BRMS1* interacts with several different proteins and large (megadalton) protein complexes, most notably with class I and class II histone deacetylases (HDACs) and the transcription factor NFκB. *BRMS1* is specifically a core member of mSin3-HDAC chromatin remodeling/transcriptional repression complexes but its involvement is implicated in other HDAC complexes. Additionally, *BRMS1* and HDAC1 function as NFκB co-repressors; chromatin bound *BRMS1* facilitates HDAC-1-mediated deacetylation and inactivation of NFκB. Studies also suggest that direct interaction of *BRMS1* and the RelA/p65 subunit of NFκB represses the transactivation potential of NFκB. Further, *BRMS1* leads to a reduction in NFκB translocation by inhibiting the phosphorylation and degradation of the NFκB inhibitor, IκB.

Studies show reduced phosphoinositide signaling in *BRMS1* transfected cells. Decreased phosphoinositide signaling results in decreased mobilization of intracellular calcium, a known regulator of metastasis. *BRMS1* expression also downregulates fascin, an actin-bundling protein associated with cell motility. Further, repression of NFκB results in decreased expression of anti-apoptotic genes and two tumor-metastasis activators, osteopontin and urokinase-type plasminogen activator.

Clinical Relevance

BRMS1 is regulated at both the RNA and protein levels. To date, only a single study has examined levels of *BRMS1* protein in patient samples to find a loss of *BRMS1* in nearly 25% of 238 breast cancer cases. Further, the study showed loss of *BRMS1* correlated with disease-free survival when stratified by loss of estrogen receptor, progesterone receptor, or Her2 over-expression. Other clinical studies have studied *BRMS1* mRNA expression in human cancers compared to adjacent non-cancerous tissues or regional lymph nodes. Since *BRMS1* is regulated at the protein level, looking exclusively at mRNA may be misleading. Nonetheless, the majority of studies show high levels of *BRMS1* correlates with increased disease-free survival and diminished progression.

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BRN-5547136

►Temozolomide

Broder Histological Classification

Definition

Refers to histological classification of differentiation in ►squamous cell carcinoma. Devised by Broder. Grades 1, 2 and 3 denoted ratios of differentiated to undifferentiated cells of 3:1, 1:1 and 1:3, respectively. Grade 4 denoted tumor cells having no tendency towards differentiation.

Bromodeoxyuridine (BrdU)

Definition

A compound that, due to its chemical structure, can substitute for thymidine in DNA.

►Fragile Sites

Bromodomain

Definition

Conserved domain that specifically recognizes and binds to acetylated lysine residues that occur within a protein.

►Histone Modifications

Bronchogenic Carcinoma

►Lung Cancer

Brother of the Regulator of Imprinted Sites

►BORIS

Brown Adipose Tissue

Definition

BAT is present in many newborn or hibernating mammals, and its primary function is to generate heat.

►Cachexia

brp-39

►Serum Biomarkers

Brush Border

Definition

Is formed by the densely packed microvilli of the surface of columnar epithelial cells, e.g., in the intestine and in the proximal tubules of the kidney. Microvilli are small projections of the plasma membrane which greatly enlarge the surface area of the cell. Individual microvilli can only be distinguished using an electron microscope; in a light microscope, the microvilli are observed collectively as a fuzzy fringe at the surface of the epithelium which has, therefore, been termed brush border.

►Membrane Transporters

Bryostatin-1

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Definition

Bryostatins are a class of macrocyclic lactones. Bryostatins are potent modulators of ►Protein Kinase C (PKC). Bryostatin-1 was isolated from the marine invertebrate *Bugula neritina*. Bryostatin-1 is currently not available for commercial use.

Characteristics

Rationale for Targeting the PKC

PKC is a family of homologous serine/threonine protein ►kinases that transduce signals linked to diverse cellular processes including proliferation, differentiation, angiogenesis, and ►apoptosis. The PKC family includes 12 isoforms subdivided into three major classes based on their co-factor requirements for activation. Aberrant regulation of the PKC enzymes activity has been demonstrated in a number of malignancies including: breast, colorectal, pancreatic and non-small cell lung cancer.

Preclinical Activity of Bryostatin-1

Treatment of cancer cell lines with bryostatin-1 results in the activation of PKC. However, prolonged exposure to bryostatin-1 induces PKC inhibition most probably through ubiquitin-mediated degradation. Inhibition of PKC activity results in cell cycle arrest, apoptosis, cell differentiation and modulation of chemoresistance.

Bryostatin-1 has been shown to potentiate the effects of several classes of cytotoxic agents including: vincristine in diffuse large cell lymphoma, melphalan in Waldenstrom's macroglobulinemia, gemcitabine in pancreatic and breast cancer, paclitaxel and mitomycin C in gastric cancer cell lines. The synergism between bryostatin and cytotoxic agents is sequence dependent.

Single Agent Activity of Bryostatin-1

Phase I trials of bryostatin-1 used two different schedules. The maximal tolerated doses were 25 μg/m^2 when infused over 24 h and 120 μg/m^2 when infused over 72 h. The most common side effects included ►myalgia. Other observed toxicities included headache, phlebitis, and transient ►thrombocytopenia.

Single agent bryostatin-1 has been studied in phase II trials for lymphoma, renal, colorectal, head and neck, sarcoma, and melanoma. Bryostatin-1 did not demonstrate single agent activity in any of these diseases.

Bryostatin-Based Combinations

Since PKC activation contributes to chemoresistance the combinations of bryostatin-1 and cytotoxic agents were tested. In Chronic Lymphocytic Leukemia (CLL) and indolent Non-Hodgkin Lymphoma, bryostatin-1 was evaluated in combination with fludarabine. Patients received fludarabine daily for 5 days and bryostatin-1 over 24 h infusion either before or after fludarabine. The combination was well tolerated. Partial and complete responses were observed in 6 and 2 patients (total number 27), respectively. Bryostatin-1 and vincristine was evaluated in patients with refractory B-cell lymphoma. Twenty four patients were enrolled on the study. The bryostatin-1 was well tolerated at a dose of 50 μg/m^2 over 24 h infusion. The regimen had activity with five patients having objective response and five having stable disease.

Bryostatin-1 was also evaluated in combination with cisplatin in two phase I trials. In the first trial, bryostatin-1 (30 μg/m^2 over 24 h infusion), had no significant activity. In the second trial bryostatin-1 was administered at a dose of 15–55 μg/m^2 over 72 h. In this study, three responses were reported. A phase I trial of gemcitabine and bryostatin-1 (25–35 μg/m^2 over 24 h) revealed that the regimen was well tolerated and resulted in stable disease in 8 out of 36 patients. Bryostatin-1 (15–50 μg/m^2 infused over 24 h) was evaluated in combination with paclitaxel. Partial responses were observed in patients with pancreatic and gastroesophageal cancer. The common finding in these trials is that bryostatin-1 can be combined safely with cytotoxic chemotherapy agents.

Phase II trials evaluating the activity of bryostatin-1 with cisplatin in cervical cancer had disappointing results. Fourteen patients were enrolled on the trial and there were no treatment responses. Ajani et al. reported on thirty seven patients with gastroesophageal and gastric cancer treated with bryostatin-1 (40 μg/m^2 infused over 24 h) and weekly paclitaxel (80 mg/m^2). The response rate was 29% which is higher than the previously reported response rates with paclitaxel. In a phase II trial, bryostatin-1 (50 μg/m^2 infused over 24 h) and weekly paclitaxel (90 mg/m^2) were evaluated in patients with non-small cell lung cancer. Of eleven response evaluable patients, stable disease was seen in five patients. Therefore, the bryostatin-1 and paclitaxel combination did not demonstrate significant activity in lung cancer.

Future Directions

Mixed results have been observed in the trials evaluating bryostatin-1 and cytotoxic chemotherapy agents. The future challenges in the development of bryostatin-1 include, the identification of biomarkers that can predict activity and the development of combination therapy with other targeted agents. Another approach for the development of bryostatins is through the modification of the chemical structure in order to identify analogues with better safety or efficacy profiles than bryostatin-1.

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BSA

Definition

Body surface area; The current practice of using body-surface area (calculated using a formula derived from height and weight) in dosing anticancer agents has been implemented in clinical oncology about half a century ago. By correcting for BSA, it was generally assumed that the interindividual variation in the pharmacokinetics of the drug administered would be reduced which would lower the risk of serious adverse effects without reducing the agent's therapeutic effect. Recently, doubt has arisen to this hypothesis, and for many anticancer drugs the rationale for individualization of dosage based on BSA is lacking.

►Irinotecan
►Pharmacokinetics/Pharmacodynamics

B-Scan Ultrasonography

Definition

Ophthalmologic ultrasound the provides a two-dimensional ultrasound image of the echogencity of the ocular structures providing a cross-sectional view allowing the for the diagnosis and characterization of multiple disorders including retinal and choroidal detachments, vitreous hemorrhages, vitritis, and intraocular tumors.

►Uveal Melanoma

BSF-2

►Interleukin-6

BTAK

►Aurora Kinases

BUB1

Definition

budding uninhibited by benzimidazoles 1 is a protein that is required for the spindle assembly checkpoint. BUB1 is a protein kinase; it phosphorylates CDC20 and inhibits ubiquitin ligase activity of APC/C. In mammals, BUB1 depletion causes embryonic lethality in mice.

►Mitotic Arrest-Deficient Protein 1 (MAD1)
►Synucleius

Bulk Minerals

Definition

Are mineral nutrients that are typically required to be ingested by humans in amounts of hundreds of milligrams to a few grams per day. This category includes calcium, phosphorus, and magnesium and, along with electrolytes, are sometimes referred to as macrominerals.

►Mineral Nutrients

Bulky

Definition

A lymphoma is bulky if a nodal lymphoma mass with largest dimension of 10 cm or greater is present.

►Diffuse Large B-Cell Lymphoma

Burkitt Lymphoma

Definition

Burkitt lymphoma is caused by ►Epstein-Barr virus (EBV) and occurs mainly in sub-Saharan Africa.

Burkitt Lymphoma Cell Lines

Definition

Burkitt lymphoma cell lines are EBV-infected B cell lines established from Burkitt lymphoma biopsies; these cells are tumorigenic in nude mice.

►BCL6 Translocations in B-cell Tumors
►Epstein-Barr Virus

Bystander Effect

Definition

When cells are killed indirectly by virtue of neighboring cells that transfer toxic products to them.

►HSV-TK/Ganciclovir Mediated Toxicity

C33

►Metastasis Suppressor KAI1/CD82

C75

Definition

Derivative of cerulenin inhibits fatty acid synthase activity by interfering with the binding of malonyl-CoA to the β-ketoacyl synthase domain of fatty acid synthase.

►Fatty Acid Synthase

c-Abl

Definition

A protein tyrosine kinase which activation leads to cell cycle arrest and apoptosis. A chromosomal translocation of c-Abl to the Bcr locus results in a fusion gene. Constitutively active tyrosine kinase Bcr/Abl expression associates with chronic myelogenic leukemia.

►Protein Kinase C Family
►BCR-ABL1

C.elegans cell death 4 homolog

►APAF-1 Signaling

CA19-9

Definition

Carbohydrate antigen 19-9 is used as a tumor-marker when measured in serum. It is thought to be a sialylated Lewis blood group antigen. CA19-9 levels are elevated in many gastrointestinal malignancies including cholangiocarcinoma and pancreatic cancer, as well as some non-malignant conditions such as cholangitis and peritoneal ►inflammation/infection. Patients who have a genetic deficiency in a fucosyltransferase specified by the Le gene are Lewis^{a-b-} and are unable to make this antigen; thus CA19-9 testing in Lewis^{a-b-} patients can be falsely negative.

►Cholangiocarcinoma
►Fucosylation

CA125

Definition

CA125 (cancer antigen 125) is a mucin-like protein of high molecular mass estimated at 200–20,000 kDA. CA125 cell surface expression is upregulated when cells undergo metaplastic differentiation into a Müllerian-type epithelium. CA125 is the most extensively studied biomarker for possible use in ovarian carcinoma early detection. It is elevated in some cases of ►endometriosis.

►Mesothelin
►Ovarian Cancer
►Serum Biomarkers

Ca^{2+}-activated Phospholipid Dependent Protein Kinase

►Protein Kinase C Family

Ca^{2+} ATPase

Definition

ATPases are a class of enzymes that catalyze the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP). The Ca^{2+} ATPase uses this process to transport Ca^{2+} against a concentration gradient (e.g., Ca^{2+} transported from the cytoplasm into the endoplasmic reticulum).

►Celecoxib

Ca^{2+}-Release Channels

Definition

Membrane receptors localized in the sarcoplasmic/endoplasmic reticulum that once activated mediates the release of Ca^{2+} from the ER to the cytosol.

►Endoplasmic Reticulum Stress

CaBP3

►Calreticulin

Cachexia

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Definition

Cachexia came from the Greek "kakos" and "hexis" meaning "bad conditions." Cachexia is a complex metabolic syndrome characterized by progressive weight loss with extensive loss of skeletal muscle and adipose tissue, which is secondary to the growing malignancy.

Characteristics

Most cancer patients develop cachexia at some point during the course of their disease, and nearly one-half of all cancer patients have weight loss at diagnosis. Cachexia prevents effective treatments for cancer and predicts a poor prognosis because the severity of wasting inversely correlates with survival. The consequences of cachexia are detrimental and cachexia is considered to be the direct cause of about 20% of cancer deaths. The pathogenesis of cancer cachexia remains to be fully understood, but it is evidently multifactorial.

Weight Loss

Clinically, cachexia should be suspected if involuntary weight loss of more than five percent of premorbid weight occurs within a six-month period. Weight loss is not simply caused by competition for nutrients between tumor and host as the tumor burden may be only 1–2% of total body weight. The frequency of weight loss varies with the type of malignancy, being more common and severe in patients with cancers of gastrointestinal tract (►gastrointestinal tumors) and lung (►lung cancer). Gastric and pancreatic cancer patients may lose large amounts of weight, up to 25% of initial body weight. Over 15% of weight loss in patients is likely to cause significant impairment of respiratory muscle function, which probably contributes to premature death. Weight loss can arise from several metabolic changes that take place during malignancy, for example, reduced food intake, increased energy expenditure, and tissue breakdown.

Poor Appetite

Loss of the desire to eat or lack of hunger is common in cancer patients. It can be related to the mechanical effect of the tumor such as obstructions (especially of the upper gastrointestinal tract), side-effects of chemotherapy or radiotherapy (►chemoradiotherapy), and emotional distress. Some tumors may secrete products which act on the brain to inhibit appetite. Regulation of food intake involves the integration of the peripheral and neural signals in the hypothalamus and other brain regions. In the hypothalamus, the orexigenic signals such as ►neuropeptide Y (NPY), the most potent appetite stimulant, increase food intake, and the anorexigenic signals including the pro-opiomelanocortin/cocaine and amphetamine regulated transcript (POMC/CART) inhibit appetite. Dysregulation of NPY in the hypothalamic pathway can lead to decreased energy intake but higher metabolic demand for nutrients. It has been demonstrated that NPY-immunoreactive neurons in the hypothalamus are decreased in experimental model of cancer anorexia. In contrast, reduced food consumption can be restored to normal levels by blocking the POMC/CART pathway in tumor-bearing animals.

High level of leptin, a hormone primarily secreted by adipocytes, inhibits the release of hypothalamic NPY. In cancer cachexia the leptin feedback loop appears to be deranged, altering the signaling pathway of NPY. Cytokines such as interleukin 1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) are implicated to be involved in cancer anorexia, possibly by stimulating corticotrophin-releasing factor, a neurotransmitter which suppresses food intake at least in rodents, and/or by inhibiting neurons that produce NPY in the hypothalamus.

Increased Metabolism and Energy Expenditure

Maintaining normal body weight requires energy intake to equal energy expenditure. In some patients with cancer cachexia, energy balance becomes negative as reduced food intake is not accompanied by a parallel decrease in energy expenditure. For example, patients with lung and pancreatic cancers generally have higher ►resting energy expenditure (REE) compared with normal control subjects; however, REE is usually normal in patients with colorectal cancer. The mechanisms of increased energy expenditure are not clear although studies suggest that it might be through the upregulation of uncoupling proteins, a family of mitochondrial membrane proteins, which are proposed to be involved in the control of energy metabolism. ►Uncoupling protein-1 (UCP-1), which decreases the coupling of respiration to ADP phosphorylation thereby generating heat instead of ATP, is only expressed in ►brown adipose tissue (BAT). UCP-1 mRNA levels in BAT are increased in mice bearing the MAC16 colon adenocarcinoma. Although BAT is uncommon in adults, the prevalence of BAT has been found to be higher in cancer cachectic patients than the age-matched control subjects. mRNA levels of UCP-2 (expressed ubiquitously) and UCP-3 (expressed in skeletal muscle and BAT) in skeletal muscle are upregulated in rodent models of cancer cachexia. In humans, skeletal muscle UCP-3 mRNA levels are over fivefold higher in cachectic cancer patients compared with patients without weight loss and health controls. Elevated expression of UCP-2 and -3 has been suggested to contribute to lipid utilization rather than whole-body energy expenditure. Cytokines such as TNFα and/or other tumor products may be responsible for the changes in UCP expression at least in rodents. Additional energy consumption could arise from the metabolism of tumor-derived lactate via "futile cycles" between the tumor and the host. The main energy source for many solid tumors is glucose, which is converted to lactate and transferred to the liver to convert back into glucose. This "futile cycle" requires large amount of ATP, resulting in an extra loss of energy in cancer patients.

Loss of Adipose Tissue

Fat constitutes 90% of normal adult fuel reserves and depletion of adipose tissue together with ►hyperlipidemia becomes a hallmark of cancer cachexia. Computed tomography (CT) scanning has revealed that cachectic cancer patients with gastrointestinal carcinoma had significantly smaller visceral adipose tissue area than control subjects. Increased lipolysis is implicated in cancer-associated adipose atrophy. The activity of hormone-sensitive lipase, a rate-limiting enzyme of the lipolytic pathway, is increased in cancer cachectic patients, which causes elevated plasma levels of free fatty acids and triglycerides. Meanwhile, there is a fall in lipoprotein lipase (LPL) activity in white adipose tissue, thus inhibiting cleavage of triglycerides from plasma lipoproteins into glycerol and free fatty acids for storage, causing a net flux of lipid into the circulation. Finally, glucose transport and de novo ►lipogenesis in the tissue are reduced in tumor-bearing state, leading to a decrease in lipid deposition. There is also evidence that loss of adipose tissue in cancer cachexia could be the result of impairment in the formation and development of adipose tissue. The expression of several key adipogenic transcription factors including CCAAT/enhancer binding protein alpha, CCAAT/enhancer binding protein beta, peroxisome proliferator-activated receptor gamma, and sterol regulatory element binding protein-1c are markedly reduced in adipose tissue of cancer cachectic mice.

Various factors produced by tumors or the host's immune cells, responding to the tumor can disturb lipid metabolism. TNFα has been shown to affect adipose tissue formation by inhibiting the differentiation of new adipocytes, causing dedifferentiation of mature fat cells and suppressing the expression of genes encoding key lipogenic enzymes. TNFα has also been associated with increased lipolysis probably through suppression of LPL activity in adipocytes. In addition, both TNFα and IL-1 are able to inhibit glucose transport in adipocytes and consequently decrease the availability of substrates for lipogenesis. Certain prostate, gut and pancreatic tumors secrete a lipid-mobilizing factor (LMF), also produced by a mouse adenocarcinoma model. LMF has been shown to be identical to the plasma protein zinc-α2-glycoprotein (ZAG). It is recently found to be secreted by human adipocytes and upregulated in adipose tissue of mice with cancer cachexia. ZAG causes rapid lipolysis in vitro and in vivo, possibly through activation of intracellular cyclic AMP. ZAG also stimulates expression of UCPs in brown fat of mice, which may contribute to increased energy expenditure as well as lipid catabolism during cachexia. Moreover, ZAG has been shown to reduce glucose metabolic rate in adipose tissue, consistent with a decrease in glucose transportor-4 transcript in white fat of mice bearing a ZAG-producing tumor.

Loss of Muscle Protein

Weakness, commonly seen in cancer cachectic patients, is directly related to wasting of muscle that accounts for almost half the body's total protein and bears the brunt of enhanced protein destruction. Reduced protein synthesis together with enhanced proteolysis have been observed in experimental animal models and in muscle biopsies from cancer patients with cachexia, and whole-body protein turnover can be markedly increased in cachectic cancer patients.

Some mediators and pathways of excessive protein breakdown have been incriminated in cancer cachexia. TNFα appears to be involved, as treatment with recombinant TNFα enhances proteolysis in rat skeletal muscle and activates the ubiquitin–proteasome system. Ubiquitin, an 8.6 kD peptide, is crucially involved in targeting of proteins undergoing cytosolic ATP-dependent proteolysis. There is an increase in ubiquitin gene expression in rat skeletal muscle after incubation with TNFα in vitro. Tumors also produce cachectic factors such as proteolysis-inducing factor (PIF), a 24 kD glycoprotein initially isolated from a cachexia-inducing tumor (MAC16) and the urine of cachectic cancer patients. PIF induces muscle protein breakdown by stimulation of the ubiquitin–proteasome proteolytic pathway.

There is increasing evidence that both cytokines and PIF cause protein degradation by activation of ►nuclear factor kappa B (NFκB), a transcription factor that regulates the expression of a number of proinflammatory cytokines. TNFα and PIF can upregulate components of the ubiquitin–proteasome pathway in an NFκB-dependent manner. Activation of NFκB by TNFα in murine muscle cells suppresses mRNA of the transcription factor ►MyoD, inhibiting skeletal muscle cell differentiation as well as preventing the repair of damaged skeletal muscle fibers.

Treatment

Current treatment designed to ameliorate cancer cachexia has limited benefit. Nutritional supplementation (oral or parenteral) alone has little effect and, critically, does not restore muscle mass, improve quality of life or prognosis in cancer patients. Appetite stimulants such as megestrol acetate and medroxyprogesterone acetate are commonly used at present in the treatment of anorexia and cachexia. These agents are believed to stimulate orexigenic peptide NPY in the hypothalamus, and inhibit the synthesis and release of proinflammatory cytokines. Their effects on appetite and well being are short-termed and they do not influence lean body mass and survival. Cannabinoids (►cannabinoids and cancer) have also been studied as potential appetite stimulants. However, dronabinol has failed to prevent progressive weight loss in patients with advanced cancer.

Therapeutic interventions include anticytokines such as thalidomide (►thalidomide and its analogs) with multiple immunomodulatory properties. It suppresses the production of TNFα, IL-1β, IL-12, and cyclooxygenase-2, which is probably through inhibiting NFκB activity. Thalidomide has been shown to attenuate total weight loss and loss of lean body mass in cachectic patients with advanced pancreatic cancer.

►Eicosapentaenoic acid (EPA), a polyunsaturated fatty acid from fish oil, has attracted attention as a potential anticachectic agent. EPA has been shown to attenuate the increased expression of the components of the ubiquitin–proteasome proteolytic pathway in skeletal muscle of mice with cancer cachexia, and EPA can block PIF-induced protein degradation in vitro. In randomized clinical trials, cachectic patients with unresectable pancreatic cancer receiving EPA have shown a stabilization in the rate of weight loss, fat and muscle mass as well as the REE. Recent data from animal studies suggest that EPA combined with the leucine metabolite beta-hydroxy-betamethylbutyrate seems to be more effective in the reverse of muscle-protein wasting.

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Cachexia-inducing Agent

►Leukemia Inhibitory Factor

Caco-2

Definition

Caco-2 is an immortalized cell line originally derived from a human ►colon cancer. It can be grown in-vitro in

such a way as to mimic the gastrointestinal tract wall and is used in cell culture models to measure drug intestinal permeability.

►ADMET Screen

Cadherin 1

Definition

CDH1; synonyms Epithelial cadherin, Uvomorulin.

►E-Cadherin

Cadherins

Definition

Cadherins are a family of cell adhesion receptor glycoproteins that are involved in the calcium-dependent cell-to-cell adhesion. E-, V-, and N-cadherins are distinct in immunological specificity and tissue distribution. They promote cell adhesion via their homophilic binding interactions. Class of type-1 transmembrane proteins important in cell adhesion. They are dependent on calcium (Ca^{2+}) ions to function, hence their name. Cadherins play an important role in regulation of morphogenesis. Cadherins inhibit invasiveness of tumor cells.

►Doublecortin
►E-Cadherin
►EpCAM
►Tight Junction
►Adhesion
►Cell Adhesion Molecules

Cafe Au Lait Spots

Definition

Synonym Cafe-au-lait Macule; Coffee-with-milk-colored spots on the skin that are seen characteristically in the neurofibromatosis type 1 (NF1) syndrome.

Cajal Bodies

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Synonyms

Coiled bodies

Definition

Small nuclear organelles (0.1–2.0 μM in diameter), present in all eukaryotic cells, involved in a number of different nuclear functions.

Characteristics

The nucleus of eukaryotic cells contains a number of different highly specialized organelles. Unlike cytoplasmic organelles these nuclear structures are not delimited by a membrane but are by all means compartments that contain a number of specific proteins. Most of the organelles can be clearly identified trough immuno-staining using antibodies directed against specific marker proteins; however, it should be kept in mind that these organelles are highly dynamic structures that often exchange components and therefore many proteins can be found in more than one organelle.

Among these organelles are Cajal Bodies (CBs), described over a century ago by Ramon y Cajal. CBs were originally described in neuronal cells but have since been described in a variety of cell types, both in animals and in plants, suggesting that they are involved in some fundamental cellular process. Due to their characteristic ultra-structural appearance as a tangle of coiled fibrillar strands they have also been called coiled bodies. They usually vary in size from 0.2 μM to 2 μM, but can be occasionally larger. The number of CBs is usually between 0 and4 in normal diploid cells; however, many more can be found in some cancer cells. The number of CBs per cell is regulated during the cell cycle. Indeed, CBs disappear in prophase nuclei, to reappear in G1 at the same time of the nucleolus. Their number is then doubled, usually reaching the number of four, in the S phase. It has been suggested that in these cells the number of CBs depends on the ploidy of the cells or more specifically on the number of chromosomes 1 and 6. CBs can be found associated with specific gene loci such as snoRNA, snRNA and histone gene clusters. In addition, CBs can also be found in association with other nuclear bodies such as cleavage bodies and ►PML bodies, suggesting that there is an exchange of components between the different nuclear organelles.

CBs have a heterogeneous composition, containing different Small Nuclear Ribonucleoproteins (snRNPs), Small Nucleolar Ribonucleoproteins (snoRNPs), cell cycle regulating proteins, and transcription factors, as well as other proteins, whose function still needs to be determined.

The generally recognized marker of CBs is p80 coilin. The function of this protein is still unknown, its deletion in mice results in reduced coilin –/– animal litters, suggesting a developmental defect; however surviving animals appear normal. Deletion of coilin results in residual bodies that still contain some components such as fibrillarin, Nopp140 and FLASH, but not others like splicing snRNPs.

While their function is still in part elusive, recent work suggests that they are involved in several nuclear functions. CBs are supposed to be the site of assembly of the three eukaryotic RNA polymerases (pol I, pol II and pol III) with their respective transcription and processing factors that are then transported as multiprotein complexes to the sites of transcription.They are also involved in the modification of Small Nuclear RNAs (snRNAs) and Small Nuclear Ribonucleoproteins (snRNPs), which are important for spliceosome formation. Indeed CBs contain newly assembled snRNPs and snoRNPs that later accumulate in speckles and nucleoli and it has recently been suggested that CBs are sites of modification for snRNPs and particularly sites where 2′-O-methylation and pseudouridine formation occur. This process requires a novel class of CB specific small RNAs (scaRNAs) that pair with the snRNAs and function as guides for 2′-O-methylation. The reaction is probably mediated by the fibrillarin, a CB and nucleolar associated protein with methyl transferase activity. CBs have also been implicated in ►replication dependent histone gene transcription, and a subset of CBs is physically associated with histone gene clusters on chromosomes 1 and 6. Phosphorylation of a CB component p220/NPAT by cyclinE/Cdk2 is required for activation of histone transcription, exit from G1 and progression through S phase of the cell cycle. Moreover it has been shown that another CBs component FLASH is essential for this function. Downregulation of FLASH results in structural alteration of CBs, reduction of replication dependent histone gene transcription, and block of cells in the S phase (►S-phase damage-sensing checkpoints) of the cell cycle. In addition, CBs are involved in U7 snRNA dependent cleavage of the 3′ end of histone pre-mRNA before the mature mRNA can be exported to the cytoplasm.

Finally, a role for CBs in regulating ►telomerase function has been proposed. Based on the presence of the RNA component of telomerase (hRT) in CBs of cancer cells, it has been suggested that CBs play a role in the maturation of hRT or in the assembly of the telomerase complex. However, CBs might represent only a site of accumulation of hRT; alternatively, this could be an altered localization only present in cancer cells; therefore further studies are required to clarify this potential CB function.

Alteration of CB structure, as well as other nuclear structure alterations, has been observed in various diseases; however, in most cases it is not clear if these defects are a consequence of altered nuclear functions or play a role in the disease pathogenesis.

CBs have been found associated with the aggregates formed in CAG triplet expansion diseases and ataxin-1; mutated in ►Spinocerebellar ataxia type 1 (SCA1) it has been shown to interact with coilin. The role of these findings in the disease pathogenesis is yet to be established.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disease characterized by motor neuron degeneration associated with muscular atrophy and paralysis; it is usually caused by mutations of the surviving motor neurons 1 gene (SMN1). SMN is a 294 amino acid protein, ubiquitously expressed; it bears no homology to other known proteins and its function is still unknown. It is localized both in the cytoplasm and in the nucleus where it is found in two different nuclear organelles: Cajal bodies (CBs) and Gems (for Gemini of CBs). Pathogenesis of SMA is not clearly understood but reduction of SMN levels results in an alteration of CB structure.

Alteration of CBs in cancer has not been thoroughly studied yet and a role for these organelles in cancer has not been clearly established. However cancer cell lines often show an increased number of CBs and some alteration of CBs can be found in specific cancers. In ►MLL-ELL leukemia (►Acute myeloid leukemia) the presence of the MLL-ELL fusion protein results in alteration of CBs structure and altered localization of coilin. The TLS/CHOP fusion protein generated by the t(12;16) translocation (►Chromosome Translocations), found in liposarcomas shows high transforming capacity and is in part localized in CBs.

In conclusion, while recent studies have started to shed light on the function of CBs and on the interrelationship between these organelles and other nuclear structures, more work is required to clearly understand the molecular mechanisms involved in their formation and clarify their different roles in nuclear function. This in turn will provide information on their potential role in the pathogenesis of a range of human diseases.

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CAK1 Antigen

►Mesothelin

Cal

Definition

Is a member of the ►zyxin family of proteins. Also known as migfilin.

►Lipoma Preferred Partner

Calcineurin

Definition

Is a highly conserved, Ca^{2+}/calmodulin-dependent serine/threonine phosphatase, also called protein phosphatase 2B (PP2B). Calcineurin is best known for its role in the Ca^{2+}-dependent regulation of the nuclear factor of activated T-cells (NF-AT) pathway which is involved in T-cell activation.

►Calreticulin

Calcitonin

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Definition

Calcitonin (CT) is a 32-amino acid peptide synthesized in mammals by the C cells of the thyroid gland. Several extrathyroidal sites including the prostate gland, gastrointestinal tract, thymus, bladder, lung, pituitary gland, and central nervous system (CNS) also produce this peptide molecule.

Characteristics

Almost all cells of human body synthesize and secrete procalcitonin (proCT), a precursor of the CT peptide, in response to infection/►inflammation. Only cells of the thyroid and ►neuroendocrine organs can process proCT to produce mature CT molecule. CT sequence among various species shows remarkable divergence. However, all sequences contain 32 amino acids, a carboxy-terminal proline amide, and a disulfide bridge between cysteine residues at positions 1 and 7. In addition to CT, other biologically and chemically diverse molecules such as CT gene-related peptide (CGRP), ►adrenomedullin (►ADM), and ►amylin (AMY) are considered as CT family of peptides because of their ability to interact with CT receptor (CTR) and induce biological response. Each of these peptides displays selective tissue distribution and distinct physiological effects. For example, CGRP is predominantly present in central and peripheral nervous system, and is important for neurotransmission and neuromodulation. ADM is relatively abundant in vascular space, and plays an important role in the regulation of cardiovascular and respiratory functions, and CT is essential for calcium balance. However, CT does not regulate calcium in extrathyroidal tissues but is implicated to play an important role in cell growth, cell differentiation, and other regulatory functions.

Biosynthesis

Four CT genes, CALC-I, CALC-II, CALC-III, and CALC-IV with significant nucleotide homologies have been identified. However, CT is encoded by only CALC-I gene. CALC-I and CALC-II encode two different forms of CGRP, CGRP-I and CGRP-2. CALC-III is thought to be a pseudo gene, and CALC-IV produces AMY. Human (h) CT (CALC-I) gene is located in the p14qter region of chromosome 11. CT gene encodes two distinct peptides CT and CGRP, which arise by tissue-specific alternative splicing of the same primary mRNA transcript. The primary mRNA transcript is spliced almost exclusively to CT mRNA in thyroid, and to CGRP in the nervous system.

CT is synthesized as part of a larger precursor protein of 136 amino acids. The DNA sequence of the hCT gene predicts that the hormone is flanked in the precursor by N- and C-terminal peptides. Both N-terminal and C-terminal flanking peptides are detected in the plasma and thyroidal tissues of both normal and ►medullary thyroid carcinoma (MTC) patients. However, no biological function for either of these two peptides has been conclusively determined. Cyclic adenosine monophosphate (cAMP), pentagastrin, and progesterone are potent stimulators of CT gene expression. In contrast, testosterone and estrogens have inhibitory effect.

There is evidence for ►polymorphisms in CALC-I gene that leads to increased risk for ovarian ►cancer in carrier women (T-C 624 bp upstream of translation initiation codon); and 16 bp microdeletion polymorphism has been reported in a family multiple cases of unipolar and bipolar depressive disorders.

Biological Actions

Actions on Bone

CT is the only hormone that inhibits bone resorption by direct action on osteoclasts in the bone. It is characterized by the rapid loss of osteoclast ruffled borders, reduced cytoplasmic spreading, decreased release of lysosomal enzymes, and inhibition of collagen breakdown. This role is physiologically more relevant at times of stress on skeletal calcium conservation such as pregnancy, lactation, and growth, when bone remodeling by osteoclasts and the consequent release of calcium stores in the bone need to be tightly regulated to prevent unnecessary bone loss. In normal adult humans, even large dose of CT has little effect on serum calcium. However, in pathologies created by increased bone turnover such as thyrotoxicosis, ►metastatic bone disease, or Paget's disease, CT treatment effectively inhibits bone resorption and lowers serum calcium.

Renal Actions

CT increases urinary excretion rate of sodium, potassium, phosphorus, and magnesium. CT also enhances 1-hydroxylation of 25-hydroxy vitamin D in the proximal straight tubule by stimulating the expression 25-hydroxy vitamin D 1-hydroxylase.

Central Actions

Central administration of CT produces analgesia, affects sleep cycles producing insomnia, major reduction in slow wave sleep and long period of alteration of rapid eye movement (REM) sleep and wakening. The centrally mediated actions of CT correlate well with the location of CT binding sites. CT also demonstrates multiple hypothalamic actions such as modulation of hormone release, decreased appetite, gastric acid secretion, and intestinal motility. Administration of CT in clinical situations of bone pain is very effective in ameliorating the pain symptoms.

Other Actions

CT and its receptors have been identified in a large number of other cell types and tissue sites suggesting multiple roles for CT–CTR axis. CTR binding sites have been identified in the kidneys, brain, pituitary, testis, prostate, spermatozoa, lung, and lymphocytes. There is evidence to suggest the involvement of CT in cell growth and differentiation, tissue development and tissue remodeling. CT appears to be important for blastocyst implantation and development of the early blastocyst.

CT in Cancer

Overexpression of CT has been reported in cancer-derived cells from ►thyroid, ►lung, ►breast, ►prostate, ►pancreas, ►pituitary, ►bone (osteoclastoma, osteogenic sarcoma), and embryonal carcinoma, suggesting the deregulation of CT expression is an important event in several malignancies. The results from our laboratory have shown that CT and CTR are present in undifferentiated basal cells, but absent in differentiated secretory cells of normal human prostate gland. However, CT and CTR become detectable in malignant secretory epithelium suggesting malignancy-associated deregulation of CT/CTR expression. CT and CTR transcripts in malignant human prostate become detectable as early as high-grade ►PIN, and progressively increase with increase in tumor grade. In human pancreas, CTR is present in benign as well as malignant regions but CT is exclusively detected in malignant sections of multiple pancreatic carcinomas, including ductal adenocarcinomas.

Mechanism of CT Action

Receptors

CT acts by binding to receptors on the plasma membrane of responding cells. CTR cDNA has been cloned in multiple mammalian species. Analysis of the protein translated from CTR cDNA sequence reveals the size of approximately 500 amino acids, and the receptor belongs to the class B family of G protein-coupled receptors (►GPCRs), which also includes numerous potentially important drug targets. The human CTR gene is located on chromosome 7 at 7q21.3. The CTR gene exceeds 70 Kb in length, comprises of at least 14 exons, separated by introns ranging in size from 70 nucleotides to >20,000 nucleotides.

Multiple polymorphic sites in CTR gene have been identified, and several of them lead to lower bone mineral density in postmenopausal women.

Receptor Isoforms

Human CTR (hCTR) is known to exist in mutiple isoforms that arise from alternative splicing of the same primary transcript. The two most common hCTR variants arise by alternative splicing of intracellular domain 1. The most common variant (Type 1 hCTR) leads to the addition of a 16 amino acid insert in the first intracellular loop. Alternative splicing of this small exon leads to the expression of type 2 hCTR, which differs from type 1 hCTR (abundant in the brain and the kidneys) by the absence of a 16-amino acid insert in the first intracellular loop. Type 2 CTR is predominantly expressed in malignant prostate and pancreatic cells. It has been shown that the lack of 16-amino acid insert in

first intracellular loop enables type 2 hCTR to coactivate both adenylyl cyclase and phospholipase C. In addition, another receptor referred as calcitonin receptor-like receptor (►CRLR) has been reported.

Modulation of CTR Specificity

CTR displays high affinity for CT, but low affinities for other CT family peptides. However, the ligand specificity of CTR is significantly altered when it binds to a ►RAMP protein. Several ►RAMPs have been identified but three (RAMP1, RAMP2, RAMP3) are investigated. hCTR displays low affinity for AMY, but association with RAMPs enables hCTR to bind AMY with high affinity. Similarly, ligand specificity of CRLR depends on the complexed RAMP. For example, CRLR-RAMP1 serves as CGRP receptor whereas CRLR-RAMP2 acts as ADM receptor. CRLR-RAMP3 displays high affinity for ADM as well as CT. This phenomenon opens up a possibility that ligand specificity of CTRs can be regulated by modulation of RAMPs expression.

CTR Signaling

G protein-Mediated Signaling

The intracellular mechanisms by which CTR produces biological effects are still being elucidated. However, the signaling pathways appear to vary with cell type as well as animal species. As with most other GPCRs, the CTRs show coupling with multiple G proteins, which also depends on the isoform of CTR. For example, type I CTR preferentially couples to ►Gαs, leading to the activation of adenylyl cyclase and elevation in the intracellular levels of cAMP. The inhibitory action of CT on osteoclasts is accompanied by increase in cAMP levels. Forskolin, a direct activator of adenylyl cyclase, as well as dibutyryl cAMP, which elevates intracellular cAMP levels independent of adenylyl cyclase, mimic CT actions on bone resorption. Similarly, CTR is known to activate adenylyl cyclase in kidney as well as in cancers of lung, breast, and bone.

Unlike type 1 CTR, type 2 CTR simultaneously couples with Gαs and ►Gαq, leading to the coactivation of adenylyl cyclase and phospholipase C. This results in the elevation of intracellular levels of cAMP, as well as inositol triphosphates, and thence increased cytosolic calcium levels. This, together with coliberated diacyl glycerols, activates protein kinase C. In brain tissue, CT couples to G proteins other than Gαs as indicated by limited activation of adenylyl cyclase in neural tissues. In hepatocytes, CT increases cytosolic calcium levels without activating adenylyl cyclase. In LLC-PK1 kidney cells, CT increases either intracellular cAMP levels or cytosolic calcium levels in a cell cycle-dependent manner; and in pituitary lactotrophs, CT inhibits TRH-induced increases in cytosolic levels and activation of protein kinase C. In ►prostate cancer cells, CT coactivates protein kinases A and C, and pathways activated by these enzymes play an important role in CT-stimulated growth, invasiveness and tumorigenicity of prostate cancer cells.

G Protein-Independent Signaling

Recent evidence suggests that GPCRs also activate G protein-independent signaling by interacting with proteins referred as GPCR interacting proteins (GIPs). GPCRs activate this signaling by binding to GIPs through one or more of structural interacting domains such as ►Src homology 2 (SH2) and SH3, plackstrin homology, ►PDZ and Eva/WASP homology (EVH) domains.

Examination of CTR sequence reveals that the last four amino acids at the extreme C-terminus of the ►C-tail form E-S-S-A tetramer (amino acids 447–50), which conforms to the canonical type I ►PDZ ligand. A single serine-to-alanine substitution in the PDZ ligand of prostate CTR almost abolished CT-elicited increase in invasiveness and tumorigenicity of PC-3 prostate cancer cell line, raising a strong possibility that metastasizing ability of CTR is dependent upon its ability to interact with intracellular proteins containing ►PDZ domain(s). CTR seems to induce ►metastasis by disassembly of tight junctions on prostate cancer cells, which leads to the loss of cell polarity, activation of proteases such as urokinase type plasminogen activator, matrix metalloproteinases 2 and 9. These results raise a possibility that the prevention of interaction between CTR and its intracellular partner through the PDZ ligand can be an effective strategy to prevent CT-mediated metastasis. With current advances in medicinal chemistry and peptide mimetics, it should be possible to design a small peptide of 4–6 amino acids or a small molecule to prevent this interaction.

CT also activates ►phosphoinositol-3-kinase (PI3K)–Akt–Survivin pathway and induces chemoresistance and ►apoptosis in multiple prostate cancer cell lines through as yet uncharacterized mechanism. CT activated protein kinase A plays a key role in multiple actions of CT on prostate cancer cell lines, suggesting that both, G protein-dependent and G protein-independent actions of CTR may act in concert to increase oncogenicity of prostate cancer cell lines.

Significance of CT–CTR axis in Cancer: Clinical Aspects

CT is "Oncogene" for Prostate Cancer but "Tumor Suppressor" for Breast Cancer

Although growing body of evidence suggests elevated expression of CT and CTR in multiple cancers, extensive studies on CT actions have been conducted only in ►prostate cancer and ►breast cancer (►tumor suppressor) cell lines. Interestingly, CT displays sexual dimorphism in these two cancers, raising a possibility

of the modulatory role of sex hormones on CT actions in these two organs. For example, CT is a potent ►oncogene for prostate cancer as indicated by the progressive increase in CT and CTR expression in primary prostate cancers with tumor progression, and potent stimulatory actions of CT on tumorigenicity of prostate cancer cell lines. In contrast, CT and CTR is constantly expressed in normal mammary ductal epithelium, the loss of CTR expression is associated with the progression of breast cancer to ►metastatic phenotype, and CT inhibits growth of some breast cancer cell lines. Although opposing actions of CT on prostate and breast cancer cell lines remain to be thoroughly investigated, initial studies in the author's laboratory suggest that CT protects junctional complexes in breast cancer cell lines, and estradiol attenuates the actions of CT in estradiol receptor-positive breast cancer cell lines. These results emphasize the importance of CTR actions on junctional complexes in cancer, and its significance in cancer cell growth and metastasis.

CT is an Angiogenic Factor

►Angiogenesis, the process of new vessel formation or neovascularization, has aroused increasing interest over last 25 years. Expansion of the tumor cell mass is dependent on both the degree of tumor vascularization and the rate of angiogenesis. Our recent results have demonstrated the presence of CTR in ►HMEC-1 cell line, and that CT stimulates in vivo angiogenesis in nude mice, and directly stimulates all major phases of in vitro angiogenesis including endothelial cell migration, invasion, proliferation, and tube morphogenesis. The stimulatory actions of CT on in vitro angiogenesis are comparable to the actions of ►vascular endothelial growth factor (VEGF). Importantly, silencing of CTR in HMEC-1 cells completely abolishes CT-induced tube morphogenesis. Furthermore, ►prostate and thyroid cancer cell lines expressing high levels of CT form large, highly vascular tumors. In contrast, the silencing of CT expression in these cell lines markedly reduces tumor growth and vascularity. These results may also explain the findings that malignancies displaying high levels of CT expression (such as MTCs and ►multiple endocrine neoplasias) also produce highly vascular tumors. Considering that therapeutic use of CT for pain relief is fairly widespread in cancers as well as other diseases, it will be important to consider oncogenic and angiogenic effects while determining CT therapy in these patients.

In summary, CT and CTR expression has been well-investigated in ►breast and prostate carcinomas. CT is a potent stimulator of tumor growth, angiogenesis, and metastasis in prostate cancer cell lines. In contrast, CTR expression is lost with breast cancer progression, and CTR attenuates growth of breast cancer cell lines. Significant expression of CT and CTR has also been reported in MTCs, multiple endocrine neoplasias, and ►carcinomas of lung, ►pancreas, ►gastrointestinal tract, ►thymus, and ►bladder. However, the significance of CT–CTR axis in these carcinomas remains to be investigated.

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Calcitriol

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Synonyms

CALCITRIOL; Activated vitamin D; 1α,25-Dihydroxy-vitamin D_3

Definition

Calcitriol, the hormonally active form of vitamin D, is the major regulator of calcium homeostasis in the body and is critically important for normal mineralization of bone. Calcitriol is produced by sequential hydroxylations of vitamin D in the liver (25-hydroxylation) and the kidney (1α-hydroxylation) to produce the active hormone. Like other steroid hormones, calcitriol, working through the vitamin D receptor (VDR), functions by a genomic mechanism similar to

the classical steroid hormones to regulate target gene transcription. In other words, vitamin D is converted into a hormone that acts similarly to other hormones (steroids, thyroid hormone, retinoids, etc.) whose mechanism of action is via nuclear receptors. The traditional actions of calcitriol are to enhance calcium and phosphate absorption from the intestine in order to maintain normal concentrations in the circulation and to provide adequate amounts of these minerals to the bone-forming site to allow mineralization of bone to proceed normally. This action is critical to prevent rickets in children and osteomalacia in adults. However, in the past two decades, it has become increasing clear that calcitriol has many additional functions that implicate the hormone in a wide array of actions relating to bone formation as well as to other areas unrelated to bone or mineral metabolism including antiproliferative, prodifferentiating and immunosuppressive activities. Pharmaceutical companies and academic centers have actively studied analogs of calcitriol in an attempt to design a drug with increased potency to treat osteoporosis, cancer or autoimmune diseases while being less likely to cause hypercalemia and renal stones, the predictable side-effects of high doses of calcitriol, Several recent reviews of the mechanism of action and function of calcitriol have been published as well as a comprehensive book addressing all areas of vitamin D.

Characteristics

Vitamin D exists in two forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). When written without a subscript the designation vitamin D denotes either D_2 or D_3. Sunlight, in the form of UV-B rays, cleaves the B ring between carbon-9 and 10 to open the ring and create a secosteroid structure. By this process the precursor (provitamin) molecules, 7-dehydrocholesterol in animals and ergosterol in plants, are converted to the secosteroids, vitamin D_3 and vitamin D_2, respectively. The two secosteroids differ only in the presence of a methyl group at carbon 28 and a double bond between carbon 22 and 23 on the side chain of vitamin D_2. Vitamin D_2 and vitamin D_3 are handled identically in the body and converted, via two hydroxylation steps, first in the liver and then in the kidney to the active hormones, $1,25(OH)_2D_2$ or $1,25(OH)_2D_3$ (calcitriol). Calcitriol then acts in multiple target tissues throughout the body by binding to its nuclear receptor, the vitamin D receptor (VDR) to regulate gene expression. Since few dietary sources contain high levels of vitamin D, sunlight exposure or ingestion of supplements or vitamin D-supplemented food is essential to maintain adequate vitamin D levels. In recent years it is being increasingly recognized that vitamin D deficiency in many people is related to inadequate sunlight exposure, dark skin, living in northern latitudes or other life-styles that limit vitamin D production.

Calcitriol and Cancer

A number of epidemiologic studies have found a protective relationship between vitamin D status and decreased risk of cancer. Higher rates of cancer mortality have been observed in regions with less sunlight UV-B radiation, among African-Americans and among overweight people, each associated with lower levels of circulating 25(OH)D, the precursor of calcitriol. These data suggest that there is a beneficial effect of vitamin D on cancer development and mortality. The preponderance of observational studies of vitamin D status in relation to the risk of colon, breast, prostate and ovarian cancers has found that vitamin D sufficiency lowers cancer risk. Several studies have demonstrated an inverse relationship between sunlight exposure and the incidence of colon and prostate cancers. Studies correlating the measured plasma levels of vitamin D metabolites with cancer incidence have shown an inverse relationship between plasma 25(OH)D levels and colorectal cancer whereas in the case of prostate cancer, the results have been variable. Several studies have also examined the association between polymorphisms in the VDR gene and the risk for colon and prostate cancers and the results have also been variable but suggestive that some forms of VDR alter the risk of developing cancer.

Mechanisms

VDR, the receptor through which calcitriol exerts its actions, is expressed in many normal and malignant cell types indicating a wide array of previously unrecognized potential targets for calcitriol action. In many of these normal and malignant cells, calcitriol and its analogs exert pleiotropic actions to inhibit cell proliferation and promote differentiation. A number of important mechanisms have been implicated in calcitriol-mediated growth inhibition. A primary mechanism appears to be the induction of cell cycle arrest in the G_1/G_0 phase, due to an increase in the expression of cyclin-dependent kinase inhibitors such as $p21^{Waf/Cip1}$ and $p27^{Kip}$, inhibition of cyclin-dependent kinase activity and regulation of the phosphorylation status of the retinoblastoma protein (pRb). As the loss of the expression of cell cycle regulators has been associated with a more aggressive cancer phenotype and decreased prognosis and poorer survival, these observations suggest that calcitriol may be a suitable therapy to inhibit cancer progression. In addition, calcitriol induces apoptosis in some cancer cells and down-regulates anti-apoptotic genes like *bcl-2*. Other mechanisms include the stimulation of differentiation, modulation of growth factor actions and regulation of the expression and function of oncogenes and tumor

suppressor genes. The inhibition of invasion and metastasis of tumor cells as well as the suppression of angiogenesis have also been shown to contribute to the anti-tumor effects of calcitriol. Recent studies in prostate cancer have revealed anti-inflammatory effects of calcitriol through the inhibition of prostaglandin synthesis and actions as well as the inactivation of stress-induced kinase signaling and down-stream production of inflammatory cytokines. Since inflammation and prostaglandins are associated with carcinogenesis and cancer progression, these anti-inflammatory actions suggest yet another role for vitamin D in cancer chemoprevention and treatment.

Role of Vitamin D or Calcitriol in Cancer Prevention or Therapy

Because of its actions to inhibit cell proliferation and promote differentiation, calcitriol has been considered a good candidate for possible "chemoprevention" or "differentiation" therapy in a number of malignant cell types that possess VDR.

Colon Cancer

VDR are present in the colon, in colon cancer cell lines as well as in surgically removed colon cancers. The possibility that calcium and/or vitamin D may be active in decreasing colon cancer has been examined by several groups and an adequate intake of calcium (in the range of 1,800 mg/day) and vitamin D (800–1,000 IU/day) has been found in some studies to have a protective effect against the development of colon cancer. Studies in a number of colon cancer models have demonstrated the tumor inhibitory and pro-differentiation effects of calcitriol or its analogs both *in vitro* and *in vivo*. A recent study in the APC(min) mouse model has demonstrated that both vitamin D and calcium individually exert inhibitory effects on the development of precancerous polyps and exhibit a synergistic effect when used together. VDR expression correlates with colon cancer prognosis: high VDR levels are associated with favorable prognosis and VDR expression is down-regulated in high grade tumors.

Breast Cancer

VDRs are present in normal breast and breast cancer cell lines and in many human cancer specimens. Adequate calcium and vitamin D intake has been shown to enhance survival rates among breast cancer patients in some studies. Calcitriol suppresses the growth of human breast cancer cell lines in culture and also *in vivo* in xenografts of human breast cancer cells in nude mice and carcinogen-induced breast cancer in rats. A number of investigators have shown that calcitriol or its analogs exhibit antiproliferative effects in cultured breast cancer cells through a number of different mechanisms. Calcitriol has also been shown to decrease estrogen receptor-alpha levels in breast cancer cells and inhibit estrogen stimulation of breast cancer cell growth. In addition to its antiproliferative effects, calcitriol stimulates apoptosis in some breast cancer cells and may enhance the responsiveness of breast cancer cells to conventional cytotoxic agents. Studies in VDR null mice (mice in which the VDR was genetically deleted) reveal that calcitriol participates in the negative growth control of normal mammary gland. Disruption of VDR signaling results in abnormal morphology of the mammary ducts, an increase in preneoplastic lesions and accelerated mammary tumor development suggesting that vitamin D compounds may play a beneficial role in the chemoprevention of breast cancer.

Prostate Cancer

In a prediagnostic study with stored sera, calcitriol blood levels were found to be an important predictor for palpable and anaplastic tumors in men over 57 years of age but not for incidentally discovered or well differentiated tumors. VDR are present in prostate cancer cell lines and in normal prostate. Calcitriol inhibits the growth of all these cell types in culture. Calcitriol and vitamin D analogs exert antiproliferative effects in multiple prostate cancer models and several mechanisms mediate these effects. The induction of apoptosis may also play some role in the growth inhibitory activity of calcitriol in some prostate cancer cells. One of the recently discovered molecular mechanisms mediating calcitriol effects in prostate cells is the inhibition of the synthesis and actions of growth-stimulatory prostaglandins, through multiple calcitriol actions including a decrease in the expression of the pro-inflammatory molecule, cyclooxygenase-2 (COX-2). Moreover calcitriol has been shown to cause synergistic inhibition of prostate cell growth when combined with non-steroidal anti-inflammatory drugs (NSAIDs), suggesting that a combination of vitamin D or its analogs with NSAIDs may be useful in prostate cancer therapy. Calcitriol also induces the expression of MAP kinase phosphatase-5 in primary prostate cells leading to the inactivation of the stress kinase p38 and inhibition of interleukin-6 production. These new mechanisms of action support an anti-inflammatory role for calcitriol in prostate cancer and suggest that it may have beneficial prostate cancer chemopreventive effects. The efficacy of calcitriol as a chemopreventive agent has recently been evaluated using mutant mice, that recapitulate stages of prostate carcinogenesis from the pre-cancerous lesion known as prostate intraepithelial neoplasia (PIN), to high-grade PIN, to adenocarcinoma. The findings reveal that calcitriol is beneficial at the early-stage preventing the development of high-grade PIN, providing support for its use in the chemoprevention of prostate cancer.

Several vitamin D analogs exhibit greater antiproliferative potency than calcitriol, raising the possibility of the therapeutic potential of these drugs in the treatment of prostate cancer. Clinical trials have begun to address the utility of calcitriol or its analogs in treating prostate cancer patients. Recent studies have demonstrated that intermittent administration of very high doses of calcitriol are well tolerated by prostate cancer patients without significant toxicity or renal stones. In combination with the chemotherapy drug docetaxel, calcitriol given at extremely high doses once weekly, produced favorable effects on the time to disease progression and survival. An unanticipated benefit of the combination therapy was decreased side-effects of docetaxel. A phase III placebo-controlled randomized trial is currently under way testing the safety and efficacy of this combination in prostate cancer patients.

Other Malignancies

Calcitriol or other related vitamin D compounds have been shown to exhibit anti-cancer effects in multiple other malignancies as well. The growth inhibitory action of calcitriol on tumor cells was first demonstrated in human melanoma cells. Since then a large body of evidence has accumulated indicating the antiproliferative and pro-differentiation effects of calcitriol in melanocytes as well as malignant melanoma cells and melanoma xenografts. Evidence for a potential beneficial role of vitamin D compounds in hematologic, ovarian, pancreatic, and lung cancers has also been developed. Clinical trials employing calcitriol or vitamin D analogs are currently under way to evaluate the benefits of vitamin D therapy in chemoprevention or therapy of a number of cancer types.

Note Added in Proof

After this article was written the phase III trial in advanced prostate cancer patients to compare docetaxel plus calcitriol with docetaxel plus placebo was halted because of safety concerns in the clacitriol arm of the study. The details are not yet available to explain the nature of the problem.

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Calcium-Binding Proteins

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Definition

Calcium-binding proteins are ►proteins that participate in calcium signaling pathways by binding to Ca^{2+}. The most ubiquitous Ca^{2+}-binding protein, found in all eukaryotic organisms including yeasts, is calmodulin. With their role in signal transduction, Ca^{2+}-binding proteins contribute to all aspects of the cell's functioning, from homeostasis to ►cancer.

Characteristics

Normal cell cycle division is a highly coordinated progression of molecular events that is subject to control mechanisms from both outside and inside the cell. Commitment to cell cycle initiation is made from outside and occurs as a response to extracellular signals such as growth factors. Inside the cell, control mechanisms exist to determine the timing of intracellular events such as nuclear and cytoplasmic cleavage. Under normal conditions, growth-regulating mechanisms endeavor to maintain homeostasis. Homeostasis within a cell is regulated by the balance between proliferation, growth arrest, and ►apoptosis. Intracellular Ca^{2+} is an important modulator of a variety of biochemical processes associated with cell cycle progression. With few exceptions, the controls exerted by intracellular Ca^{2+} are transduced through site-specific interactions with specialized Ca^{2+}-binding proteins. There exist at least three main families of Ca^{2+}-binding proteins. The first of these is represented by proteins that possess one or more EF-hand helix–loop–helix structural motifs predicting Ca^{2+}-binding domains as typically found within calmodulin. The second class of Ca^{2+}-binding proteins is known generically as annexins. A possible third family of Ca^{2+}-binding proteins is the "calreticulin-like" group of proteins that include ►calreticulin, Grp78, endoplasmin, and protein disulfide isomerase. Ca^{2+} has also been implicated in cell growth under pathological states. An

altered cellular response to extracellular calcium ion concentration is one of the earliest changes induced in mouse epidermal cells by chemical carcinogens. However, whereas some human breast cancer cell lines and leukemia cell lines exhibit Ca^{2+}-induced cell proliferation, other carcinoma cell lines exhibit retarded growth in the presence of Ca^{2+} or no sensitivity to Ca^{2+} at all. In a human breast cancer cell line, which is sensitive to Ca^{2+}, the administration of calcium channel antagonists lowered intracellular (Ca^{2+}) and inhibited cell proliferation. A sustained physiological elevation of intracellular calcium ion concentration (Ca^{2+}i) may be responsible for a loss of proliferative potential in neoplasmic keratinocytes. It appears from preliminary evidence that Ca^{2+} is not only important to cell cycling and growth in normal cells, but the abnormal regulation of Ca^{2+} may also contribute to changes in these processes in disease conditions like cancer.

EF-Hand Motif Calcium-Binding Proteins

The ►EF-hand protein structural motif was first discovered in the crystal structure of parvalbumin. It consists of two alpha helices positioned roughly perpendicular to one another and linked by a short loop region (usually about 12 amino acids) that often binds calcium ions. A consensus amino acid sequence for this motif has aided the identification of new members of this family that now has over 200 members. A few of these proteins are present in all cells, whereas the vast majorities are expressed in a tissue-specific fashion. Some members, like S100 family, calcineurin, calmodulin, etc. have proved to be useful therapeutic markers for a variety of cancers.

S100 family: The S100 ("Soluble in 100% saturated solution with ammonium sulfate") family, the largest family within the EF-hand protein, comprises at least 26 members, 19 of which (S100A1–16, profillagrin, trychohyalin, and repetin) are located in the epidermal differentiation complex situated at 1q21, while S100B, S100G, S100P, S100Z, and S100A7L2–S100A7L4 are present at other genomic locations (21q22, xp22, 4p16, 5q14, and 1q22, respectively). Their gene structure is highly conserved, in general comprising three exons and two introns, of which the first exon is noncoding. The S100 family is a remarkable group of proteins that acquired highly specialized functions during their evolution, even though they are small proteins (9–13 kDa acidic proteins) with a single functional domain. S100 proteins, which exhibit dramatic changes in the expression, are involved in tumor progression and include S100A1, S100A4, S100A6, S100A7, and S100B (11–14), whereas S100A2 has been postulated to be a tumor suppressor (Table 1). Such changes might be caused by rearrangements and deletions in chromosomal region, which are frequently observed in tumor cells. Although in most cases, the function of S100 proteins in cancer cells is still unknown, the specific expression patterns of these proteins can be used as a valuable prognostic tool.

The other members of EF-hand motif Ca^{2+}-binding family involved in cancer are calcineurin, recoverin, calretinin, oncomodulin, etc. (Table 2).

Annexins and Other non-EF-Hand Motif Proteins

Annexin (AnxA1) is a Ca^{2+}-binding and acidic phospholipid binding protein with antiinflammatory properties. AnxA1 has been found in leukocytes, tissue

Calcium-Binding Proteins. Table 1 S100 proteins involved in cancer with characteristic features

S100 protein	Previous name	Cancer type	Characteristic features
S100A2	CAN19, S100L	Esophageal SCC	Function in carcinogenesis is dependent upon the context of tissue and associated tumor type as well as stage of malignancy
		Thyroid	
		Oral SCC	
		Laryngeal SCC	
		Melanoma	
		Skin tumors (other)	
		NSCLC	
		Lung SCC	Marker of patient prognosis
		Gastric	
		Lymphoma	
		Prostate	
		Ovarian	
		Breast	
S100A3	S100E	Astrocytomas	Level of S100A3 protein expression identified pilocytic astrocytomas

Calcium-Binding Proteins. Table 1 S100 proteins involved in cancer with characteristic features (Continued)

S100 protein	Previous name	Cancer type	Characteristic features
S100A4	CAPL, Calvasculin, MTS1, Metastasin, p9Ka, FSP1	Thyroid carcinoma	Stimulate angiogenesis
		NSCLC	Overexpression is associated with many different cancer types
		Colorectal	
		Gastric	
		Prostate	Poor patient prognosis in breast, colorectal, NSCLC and bladder cancers
		Breast	
		Gallbladder	
		Bladder	
		Pancreas	
		Esophageal SCC	
		Melanoma	
		Oral SCC	
S100A5	S100D	Meningioma	Expression can be associated with prognostic value in recurrence of meningiomas
S100A6	CABP, CACY, Calcyclin, MLN 4, PRA, Prolactin receptor-associated protein	Thyroid	Upregulation of S100A6 appears to be an early event in progression towards pancreatic cancer
		Pancreas	
		Breast	
		Lung	
		Melanoma	Potentially act as a predictor of clinical outcome
		Colorectal	
S100A7	PSOR1, Psoriasin	Breast	Expression is restricted to keratinocytes and breast epithelial cells
		Esophageal SCC	
		Bladder SCC	
		Melanoma	Overexpression has a role in early breast tumor progression
		Skin SCC	
		Gastric	
S100A8	Calgranulin A, MRP-8, MIF, NIF, P8, CFAG, CGLA, CP-10, Calprotectin	Prostate	Upregulated in PIN and in prostatic adenocarcinomas
		Breast	
		Esophageal SCC	
		HNSCC	
		Gastric	
		Pancreas	Early involvement of the proteins in prostate cancer
		Bladder TCC	
		Endometrial	
		Ovarian	
		Colorectal	
S100A9	Calgranulin B, MRP-14, P14, Calprotectin	Prostate	Upregulated in PIN and in prostatic adenocarcinomas
		Breast	
		HNSCC	
		Esophageal SCC	
		Liver (hepatocellular)	
		Gastric	
		Lung	
		Ovarian	

Calcium-Binding Proteins. Table 1 S100 proteins involved in cancer with characteristic features (Continued)

S100 protein	Previous name	Cancer type	Characteristic features
S100A10	CAL1L, CLP11, GP11, p10, ANX2LG	NSCLC	Annexin 2 protein ligand
		Gastric	Overexpressed in human renal cell carcinoma
		Renal cell carcinoma	
		Lymphoma	
S100A11	Calgizzarin, MLN70, S100C	Breast	Downregulated at transcriptional level in malignant bladder cells
		Bladder	
		Prostate	
		Thyroid	Expression may be involved in tumor suppression and better prognosis
		Lymphoma	
		Gastric	
		Colon	Cytoplasmic staining pattern in Papillary carcinomas
S100A12	Calgranulin C, MRP6, p6, CAAF1, ENRAGE	Esophageal SCC	Downregulated
S100A14	BCMP84, S100A15, S114	Esophageal SCC	Used for CTC monitoring in peripheral blood
		Circulating tumor cells	
S100A16	S100F, DT1P1A7	Circulating tumor cells	Used for CTC monitoring in peripheral blood
S100B	S100, S100 protein (beta chain), NEF	Melanoma	Putative cancer biomarker
S100P	S100E, MIG9	NSCLC	Isolated from placenta
		Pancreas	
		Prostate	Upregulation is early event in pancreatic cancer valuable marker for the prediction of clinically relevant early pancreatic lesions
		Breast	
		Colon	

SCC, squamous cell carcinoma; HNSCC, head and neck SCC; NSCLC, nonsmall cell lung carcinoma; TCC, transitional cell carcinoma; CTCC, circulating tumor cells.

Calcium-Binding Proteins. Table 2 Other EF-hand motif family members

EF-hand motif protein	Cancer type	Characteristic features
Recoverin	Cancer-associated retinopathy	Autoimmune response
Calretinin	Colon adenocarcinoma and mesothelioma of epithelial type	Belongs to the calbindin subgroup, autoantigen in a paraneoplastic disease
Sorcin	Ovarian carcinoma	Overexpression leads to paclitaxel resistance
Calcineurin B	Squamous cell carcinoma of cervix, pancreatic cancer	Calcineurin B subunit appears to be a significant biological response modifier due to its anticancer effects
Oncomodulin/ Parvalbumin	Carcinoma cell lines characterized by translocative activity	Related to motile behavior of carcinoma cells, can be a possible candidate for tumor marker
Calmodulin	Osteoclast apoptosis	Calcium ion receptor in neoplastic cells

macrophages, T-lymphocytes, and epithelial cells of the respiratory and urinary systems. Cellular functions of AnxA1 include regulation of membrane trafficking, cellular adhesion, cell signaling, and membrane fusion in exocytosis and endocytosis. The AnxA1 protein is involved in maintaining normal breast biology. The *AnxA1* gene expression may provide data about the future therapeutic plan of breast carcinomas. The

decreased expression of *ANXA1* gene in normal histological sections of breast may warn the clinician that a malignant version of the cancer is about to form from the benign gland. This observation carries an important prognostic clinical value on microscopic reading of the surgical specimen, especially if these normal glands are adjacent to surgical margins. Similar result was reported as a prognostic factor with down-regulation of AnxA1 and other Anxs in the development of the lethal prostatic carcinoma phenotype.

The other major protein that belongs to this category is clusterin (CLU). CLU is a disulfide-linked heterodimeric protein associated with the clearance of cellular debris and apoptosis. In prostate, breast, and colorectal cancers, the CLU was found to have anti- or proapoptotic activity regulated by calcium homeostasis. Reports so far suggest "two faces" of CLU activity: the calcium-dependent cytoplasmic localization of CLU positively correlates with cell survival, whereas nuclear translocation of this protein promotes cell death in calcium-deprived cells. The cytoplasmic retention and high level of the 50-kDa CLU protect tumor cells from apoptotic stimuli induced by chemotherapeutic drugs or natural ligands, such as FasL, whereas its nuclear localization (nCLU) enhances cell apoptosis. The 50-kDa CLU isoform is mainly overexpressed in cancer cells and retained in the cytoplasm, promoting cancer progression and aggressiveness. Cytoplasmic CLU could easily translocate into the nucleus in the presence of various inducers, such as IR, chemotherapy, hormones, or cytokines, or depletion of cellular calcium. These findings support CLU as a valid therapeutic target in strategies employing novel multimodality therapy for advanced prostate cancer.

Calreticulin-like Proteins

Calreticulin is a 46-kDa Ca^{2+}-binding chaperone protein found across a diverse range of species. The human gene for calreticulin is located on chromosome 19 at locus p13.3–p13.2 and the homologous gene in the mouse maps to chromosome 8. Calreticulin at the cell surface may play a role in cell adhesion, cell–cell communication, and apoptosis. Calreticulin has also been implicated in the pathology of some cancers. The protein also plays an important role in autoimmunity and cancer. For example, it appears that calreticulin might be an excellent molecular marker for prostate cancer. The expression of calreticulin is downregulated in metastatic melanoma and ►squamous cell carcinoma, whereas significantly upregulated in colon cancer. Further, the N-domain of the protein has been reported to have inhibitory effects on tumors and to inhibit ►angiogenesis on endothelial cells. This observation is of great interest because the development of angiogenesis inhibitors is currently a highly promising approach in anticancer therapy.

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Calcium-binding Reticuloplasmin of Molecular Weight 55 kDa

►Calreticulin

CALI

►Chromophore-Assisted Laser Inactivation

Calnexin

Definition

CNX is an 88-kDa type I membrane protein in the Endoplasmic Reticulum. CNX and ►calreticulin (CRT) are ►paralogs and both function as molecular chaperones for glycoproteins by binding through monoglucosylated N-linked oligosaccharides (Glc1Man5–9GlcNAc2).

►Calreticulin
►Endoplasmic Reticulum Stress

Calpain

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Definition

The calpains represent a unique class of intracellular protein degrading enzymes. This class of ►proteases was named "calpains" to reflect their dependency upon calcium ions for proteolytic activity, and homology to the ►papain family of cysteine proteases. In mammalian species, the calpain protein family is comprised of 15 members, of which nine are ubiquitously expressed in all tissues and the remainder are expressed in a tissue-specific manner. The ubiquitously expressed calpain 1 and calpain 2 are the most well characterized isoforms. Calpain 1 and calpain 2 function as heterodimeric enzymes composed of a large catalytic subunit (calpain 1 and calpain 2) bound to a small regulatory subunit (calpain 4). Calpain activity *in vivo* is tightly regulated by the ubiquitously expressed endogenous inhibitor ►calpastatin. Calpains result in the proteolysis of a broad spectrum of cellular proteins. No unique consensus amino-acid sequence has been identified as a calpain-binding or -cleavage site, rather, it appears that calpains target substrates for cleavage by recognition of unidentified tertiary structure motifs. Another distinguishing feature of calpain proteases is their ability to confer limited cleavage of protein substrates into stable fragments, rather than complete proteolytic digestion. Thus, the calpain-calpastatin proteolytic system represents a major pathway of post-translational modification of proteins, that influences various aspects of cellular physiology. The recent application of pharmacological and molecular intervention strategies against calpain activity demonstrates a broad role for this class of proteases in the control of proliferation, ►migration and ►apoptosis in most cell types.

Characteristics

Cell proliferation, migration and apoptosis are key processes that have to be tightly regulated in order to maintain optimal tissue homeostasis, required for development and viability of multicellular organisms. Deregulation of any of these cellular processes will ultimately result in pathological outcomes, such as cancer. A number of studies have identified a correlative link between modulation of calpain gene expression and/or activity with cancer development and progression *in vivo*. For example, in human renal cell carcinomas, significantly higher levels of calpain 1 expression are found in tumors that metastasized to peripheral lymph nodes relative to tumors that had not metastasized. In addition, elevated calpain activity was detected in breast cancer tissues relative to normal breast tissues and was determined to be greater in estrogen receptor (►ER)-positive tumors than ER negative tumors. Calpain-mediated proteolysis of the tumor suppressor protein neurofibromatosis type 2 (NF2 or ►Merlin) is associated with the development of schwannomas and menigiomas. Experimental studies performed *in vitro* demonstrate that total cellular calpain activity is elevated upon transformation induced by the *v-src*, *v-jun*, *v-myc*, *k-ras* and *v-fos* oncogenes. Furthermore, calpain activity is necessary for full cellular transformation induced by such oncogenes. A number of intervention studies utilizing small molecule inhibitors or oligonucleotides that impair calpain activity have demonstrated a role for calpain during tumor cell progression *in vitro* and *in vivo*. Cell proliferation, migration and apoptosis are controlled by a plethora of regulatory proteins that participate in complex biochemical signaling cascades, of which calpain is a pivotal regulator. Thus, targeting calpain activity may represent an effective strategy for cancer prevention and/or treatment.

Calpain and Cell Proliferation

Studies using pharmacological inhibitors of calpain activity, overexpression of calpastatin and cells expressing depleted levels of calpain activity have all implicated calpain in the promotion of cell proliferation. Sequential progression through G1, S, G2 and M phases of the cell-cycle is required for mitosis and cell proliferation. Several studies indicate that calpain can cleave a number of cell-cycle control proteins such as ►cyclin D, cyclin E and $p27^{kip1}$ all of which regulate progression through G1 and S phase. Calpain has also been demonstrated to cleave upstream regulators of cell-cycle control proteins such as p53 and p107. Consequently, elevated calpain levels and activity in tumors may contribute to cancer cell proliferation through cleavage of cell-cycle control proteins and deregulation of normal cell cycle control.

More detailed mechanistic studies also demonstrate that calpain 2 is an important downstream component of many growth factor receptor and non-receptor tyrosine kinase signaling pathways that include signaling kinases such as, epidermal growth factor receptor (►EGFr), platelet derived growth factor receptor (►PDGFr), Src and ►focal adhesion kinase (►FAK). Such signaling molecules play an important role in transmitting extracellular signals to intracellular mediators that control cell proliferation, and are often constitutively activated in cancer cells. Activation of receptor and non-receptor kinases subsequently leads to activation of the Ras/►MAPK pathway, which results in

►ERK-mediated phosphorylation of calpain 2 on a serine residue (Ser 50). Phosphorylation of calpain 2 on Ser 50 initiates a conformational switch culminating in enhanced proteolytic activity. This evidence, together with pharmacological and molecular intervention studies targeting calpain activity, suggests that activation of calpain, in part, mediates growth factor receptor and non-receptor induced cell proliferation and migration of cancer cells.

Calpain and Cell Migration

The interaction between cell surface adhesion receptors known as ►integrins and their ►extracellular matrix substrates controls the migration of all cells. Integrin-linked ►focal adhesions are large complexes of structural and signaling proteins that provide both a structural and biochemical link between the extracellular environment and intracellular proteins. Dynamic spatial and temporal regulation of focal adhesion assembly and disassembly is required for optimal cell motility. Several studies indicate that calpains localize to integrin-associated adhesions. Furthermore, many of the protein components of focal adhesions are known substrates of calpain. Calpain-mediated cleavage of the focal adhesion components, FAK, paxillin, talin and possibly others promotes the disassembly of these complexes, contributing to reduced cell adhesion and increased migration. In fact calpain-mediated cleavage of talin has been reported to represent the rate-limiting step in adhesion turnover. In addition to mediating focal adhesion turnover, emerging evidence suggests a role for calpain in regulating components of the actin cytoskeleton involved in cell spreading and membrane protrusion, mechanisms that are also essential for persistent and directed cell migration. It is likely that calpain cleavage of actin-binding and -regulatory proteins such as, ezrin, rhoA and cortactin influences the dynamic formation and retraction of membrane structures known as ►filipodia and ►lamellipodia thereby influencing cancer cell migration and ►invasion. Pharmacological and molecular inhibition of calpain activity has been shown to impair cancer cell migration across experimental two-dimensional substrates and invasion into three-dimensional extracellular matrix substrates *in vitro*. Furthermore, a recent intervention study demonstrates that antisense-mediated suppression of calpain 2 gene expression reduced the invasion of prostate cancer cells both *in vitro* and in a mouse model *in vivo*. Thus, evidence strongly indicates that calpain activity contributes to the invasion and ►metastasis of cancer cells.

Calpain and Apoptosis

Apoptosis is defined as the process of programmed cell death. Apoptosis often follows activation of the caspase family of cysteine proteases, which degrade numerous proteins that are essential for cell viability. Regulated apoptosis is critical for the development of multicellular organisms and also restricts the growth and spread of malignant cancer cells. Conflicting roles for calpain activity in the promotion and suppression of cell apoptosis have been proposed. Calpain activity has previously been shown to play a pro-apoptotic role through the activation of caspase 3 and caspase 12 and cleavage of Bax and ►Bid proteins to their pro-apoptotic forms. Enhanced calpain activity has also been implicated as the major proteolytic pathway resulting in breakdown of essential proteins during caspase-independent mechanisms of apoptosis. Conversely, calpain-mediated cleavage of caspase 7 and caspase 9 has been found to suppress their activity and subsequent apoptosis. In addition, calpain-mediated cleavage of IκBα can lead to activation of the NFκB transcription factor resulting in subsequent expression of anti-apoptotic survival proteins. Many chemotherapeutic agents such as cisplatin induce their tumoricidal effect *via* inducing apoptosis of cancer cells. Tumor cell resistance to cisplatin-induced apoptosis is a common feature frequently encountered during chemotherapy of cancer patients. Inhibition of calpain activity has been shown to sensitize resistant tumor cells to cisplatin-induced death, whereas other studies suggest that calpain potentiates cisplatin induced cell death. Thus, the role of calpain during cell apoptosis is context dependent and determined by cell type, the apoptotic stimuli and status of intrinsic regulators of cell apoptosis.

In contrast to the aforementioned studies suggesting a pro-tumorigenic role for calpain activity, an anti-tumorigenic role is also supported by studies indicating that calpain degrades a number of oncogene-generated protein products such as PDGFr, EGFr, c-Jun, c-Fos, c-Src, and c-Mos. Also, calpain-mediated cleavage of protein kinase C (►PKC), a downstream effecter for tumor promoting phorbal esters, inhibits malignant transformation. Furthermore, specifically calpain 9 (nCL-4) activity contributes to the suppression of cell transformation in vitro and gastric tumors in vivo. Although the calpain 9 substrates that mediate this anti-tumor effect remain to be determined.

A substantial body of evidence has accumulated demonstrating that activity of the calpain family of proteases plays a broad and important role in the physiology of both normal and cancer cells. Further investigation into the complex and multifaceted role of calpain in cancer may lead to the discovery of novel therapeutic approaches targeting calpain activity that may impact on the development, progression and prevention of cancer.

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Calpastatin

Definition

Is an endogenous protease inhibitor that acts specifically on calpain. It consists of four repetitive sequences of 120–140 amino acid residues (domains I, II, III and IV), and an N-terminal non-homologous sequence (L).

►Calpains

Calreticulin

Definition

Is the molecular chaperone that binds initially to ►MHC class I, MHC class II, and other proteins that contain immunoglobulin-like domains, such as the T-cell and B-cell antigen receptors.

►Sjögren Syndrome
►Molecular Chaperones

Calreticulin

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Synonyms

CRP55; calcium-binding reticuloplasmin of molecular weight 55 kDa; Calsequestrin-like protein; CaBP3; ERp60; HACBP; high affinity Ca^{2+} -binding protein; Reticulin; CRT

Definition

Calreticulin (CRT) is a Ca^{2+} -binding multifunctional ►molecular chaperone in the endoplasmic reticulum (ER). CRT is a 46-kDa soluble protein with a cleavable N-terminal amino acid signal sequence and the C-terminal sequence Lys-Asp-Glu-Leu (KDEL), a retrieval signal in the ER. ►Calnexin (CNX), a membrane-binding paralog of CRT, shares the ►chaperone function in the ER. CRT is expressed in a variety of tissues and organs, but its levels are particularly high in the pancreas, liver, and testis. It is also a highly conserved protein with over 90% amino acid identity in mammals including humans, rabbits, rats and mice. The CRT gene has been mapped to human chromosome 19 at p13.2, and its expression is upregulated by ER stress such as unfolded protein responses and deprivation of Ca^{2+} in the ER.

Characteristics

Structure of CRT

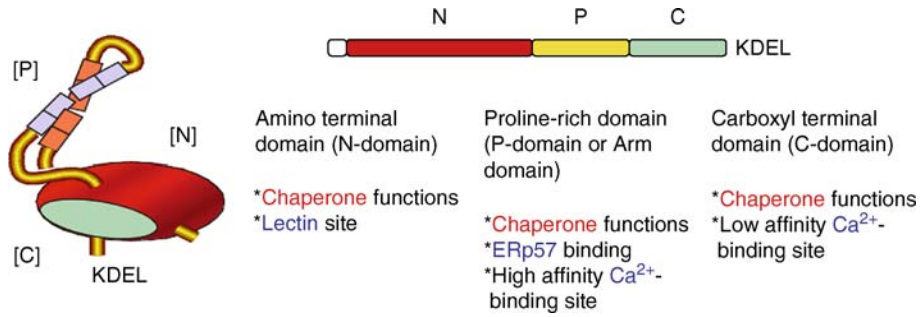
Based on structural and functional studies, CRT can be divided into three distinct domains; N-terminal [N], proline-rich [P], and C-terminal [C]. The proline-rich P-domain shows a characteristic structure with an extended and curved arm connected to a globular N-domain. The N-terminal region encompassing the N and P-domains of CRT interacts with misfolded proteins and glycoproteins, binds ATP, Zn^{2+}, and Ca^{2+} with high affinity and low capacity, and is likely to be involved in the chaperone function of the protein. The C-domain binds Ca^{2+} with high capacity and plays a role in the storage of Ca^{2+} in the ER in vivo, though no structural information is available at present (Fig. 1).

Functions of CRT in the Cell

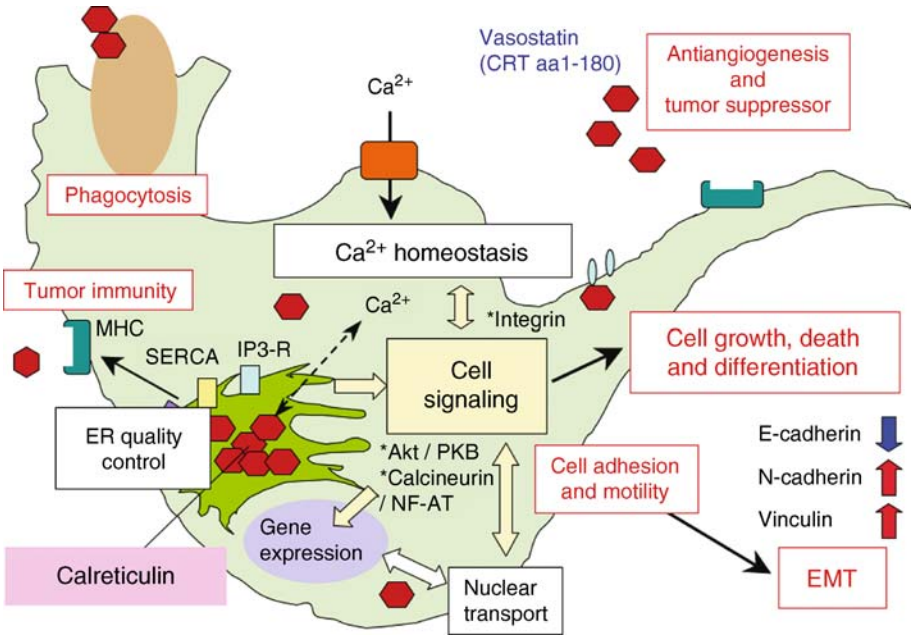
CRT is involved in a number of biological processes including the regulation of glycoprotein folding, Ca^{2+} homeostasis and intracellular signaling, cell adhesion, gene expression, and nuclear transport (Fig. 2).

CRT, a Lectin-like Molecular Chaperone in the ER

A molecular chaperone function of CRT has been reported for several protein substrates. In the biosynthesis of glycoproteins bearing ►N-linked glycans in the ER, the oligosaccharide Glc3Man9GlcNAc2 (Glc, glucose; Man, mannose; GlcNAc, N-acetylglucosamine) is attached to the Asn residue contained in the consensus sequence Asn-X-Ser/Thr, of newly synthesized polypeptides. CRT or CNX binds the Glc1Man5–9GlcNAc in glycoproteins after the processing of sugar chains. The N-domain of CRT and CNX is speculated to be the oligosaccharide-binding site (►lectin site). If the glycoprotein is completely folded in the ER, the terminal glucose is removed by glucosidase-II and the glycoprotein is released from the CNX/CRT chaperone cycle. However, if the glycoprotein is not properly folded, the terminal glucose is once again attached by



Calreticulin. Figure 1 Schematic structure of calreticulin.



Calreticulin. Figure 2 Functions of calreticulin in the cell. CRT is involved in a variety of cellular processes including the quality control of glycoprotein synthesis in the ER, Ca^{2+} homeostasis, intracellular signaling, gene expression, and nuclear transport. In cancer cells, the altered expression of CRT may lead to alterations in cellular characteristics, such as growth, adhesion, motility, immune responses, and susceptibility to apoptosis. Furthermore, extracellular CRT fragments (i.e., vasostatin) elicit antiangiogenic or tumor-suppressing activities.

the action of UDP-Glc: glycoprotein glucosyltransferase, which discriminates between folded and unfolded substrates. Together, CRT and CNX form a specific chaperone cycle for the biosynthesis of glycoproteins in the ER. Because of the preference of CNX/CRT for oligosaccharides as substrates, CNX and CRT are called "lectin-like chaperones." CRT and CNX function with the help of other chaperones such as ►ERp57 and ►BiP/GRp78. The binding site for ERp57 has been identified in the P-domain of CRT or CNX. As a chaperone, CRT plays an important role in the formation of major histo-compatibility complex (►MHC) class I to aid in antigen presentation.

CRT, a Regulator of Ca^{2+} Homeostasis in the ER

The ER is the main reservoir of intracellular Ca^{2+} and plays an important role in Ca^{2+} homeostasis. CRT has two Ca^{2+} -binding sites and this characteristic contributes to the function of the ER as a Ca^{2+} reservoir. Ca^{2+} is released from the ER by receptors for inositol-1,4,5-trisphosphate (IP3) and ryanodine, and taken up into the ER by sarcoplasmic and endoplasmic reticulum Ca^{2+} -ATPase (SERCA). With respect to the regulation of the Ca^{2+} level, the involvement of CRT and SERCA2b or the IP3-receptor has been reported. Furthermore, the store-operated release of Ca^{2+} from the ER was shown to be suppressed by overexpression of CRT protein. These

findings indicate that CRT is not only a reservoir of Ca^{2+} but also a regulator of Ca^{2+} -homeostasis in the ER.

Other Miscellaneous Functions of CRT In and Out of the ER

CRT is involved in cell ►adhesion by affecting integrin-related cell signaling. In CRT-deficient embryonic stem cells, integrin-mediated Ca^{2+} influx was impaired leading to a decrease in cell adhesion to fibronectin and laminin. It is still not clear whether CRT affects integrin directly or indirectly to regulate cell adhesion signaling.

Cell surface expression of CRT has also been reported in various cell types, and may be related with cell adhesion and ►migration. The cell surface CRT may modulate cell adhesion by binding with extracellular matrix proteins, such as ►fibrinogen, ►laminin, and ►thrombospondin. Furthermore, extracellular CRT is implicated in the pathological processes of autoimmune diseases. ►Autoantibodies against CRT were found in ~40% of patients with systemic lupus erythematosus, patients with secondary Sjogrens syndrome, rheumatoid arthritis, celiac disease, complete congenital heart block, and halothane hepatitis. CRT is known to bind to complement, C1q, and compete with antibodies for binding to C1q and inhibition of C1q-dependent hemolysis. In autoimmune diseases, impairment of the classical pathway of compliment causes a failure to clear immune complex, resulting in progression of the disease. Therefore, extracellular CRT may contribute to the progression of autoimmune diseases by preventing the clearance of immune complex. Furthermore, it has been reported that cell-surface CRT is involved in the mechanism for clearance of viable or apoptotic cells through the trans-activation of LDL-receptor-related protein (LRP) on phagocytes. However, it is still controversial whether CRT is exported from necrotic cells or apoptotic cells under pathologic conditions.

Cytosolic CRT functions as an export factor for multiple nuclear hormone receptors, such as steroid hormone, non-steroid hormone, and orphan receptors. This function is consistent with previous findings that CRT suppresses the transactivation of nuclear hormone receptors including ►androgen receptor and vitamin D. However, the mechanisms by which CRT molecules are transported into, and retained in, the cytosol/nucleus are not fully defined.

CRT and Development

CRT is essential for cardiac and neural development in mice. CRT-deficient embryonic cells showed an impaired nuclear import of nuclear factor of activated T cell (NF-AT3), a transcription factor, indicating that CRT functions in cardiac development as a component of the Ca^{2+}/►calcineurin/NF-AT/GATA-4 transcription pathway. Actually, cardiac-specific expression of calcineurin reversed the embryonic lethality of CRT-deficient mouse.

CRT transgenic mice suffer a complete heart block and sudden death, and CRT-dependent cardiac block involves an impairment of both the L-type Ca^{2+} channel and gap junction ►connexins (Cx40 and Cx43). Phosphorylated Cx43 was also decreased in CRT transgenic heart, suggesting that the functions of protein kinases are altered via the regulation of Ca^{2+} homeostasis. Collectively, CRT plays a vital role in cardiac differentiation and function, though how has not been fully clarified.

CRT and Cancer

Expression of CRT in Cancer

In terms of the relationship between CRT and cancer, proteomic analysis has revealed a new functional role of CRT in the early diagnosis of cancers. CRT is proposed to be a new tumor marker of bladder cancer. In addition, it was reported that the expression of CRT is up-regulated in a variety of malignant cells or tissues including progressive fibrosarcoma cells, colorectal cancer cells, and pituitary adenomas. Furthermore, autoantibodies to CRT isoforms have utility for the early diagnosis of pancreatic cancer. These reactions are not indicative of malignant properties of CRT, but rather are markers of immunogenicity and anticancer responses. On the other hand, another report demonstrated that CRT is over-expressed in the nuclear matrix in ►hepatocellular carcinoma, compared with normal liver tissue, suggesting a relationship between over-expressed CRT and malignant transformation. In contrast, it was also reported that CRT expression correlates with the differentiation of ►neuroblastomas to predict favorable patient survival.

Pathophysiological Relevance of CRT in Malignant Disease

Susceptibility to ►apoptosis is important in terms of cancer treatments including the use of antibiotics and irradiation. In embryonic fibroblasts from CRT knock-out mice, susceptibility to apoptosis was significantly suppressed, indicating that CRT functions in the regulation of apoptosis. Furthermore, it was found that overexpression of CRT modulates the ►radiation sensitivity of human glioma U251MG cells by suppressing ►Akt/protein kinase B signaling for cell survival via alterations of cellular Ca^{2+} homeostasis. These findings suggest that the expression level of CRT is well correlated with the susceptibility to apoptosis. In contrast, overexpression of CRT provides resistance to oxidant-induced cells death in renal epithelial LLC-PK1 cells. The function of CRT in the regulation of apoptosis may differ in specific cell types, and is still controversial.

As for cell adhesion, it was reported that CRT expression modulates cell adhesion by coordinating up-regulation of N-cadherin and vinculin. Recently, it has been reported that overexpression of CRT induces ►epithelial-mesenchymal transition (EMT)-like morphological changes and enhances cellular invasiveness in renal epithelial MDCK cells. The enhanced invasiveness mediated through ►E-cadherin gene repression was regulated by the gene repressor, ►Slug, via altered Ca^{2+} homeostasis caused by overexpression of CRT in MDCK cells. This study suggests that expression of CRT may play some causative role in the gain of invasiveness during the process of malignant transformation. In addition, it has been reported that cellular migration and binding to collagen type V are apparently suppressed in embryonic fibroblasts from CRT knock-out mice, indicating that the cellular level of CRT is important for the regulation of cell motility.

Furthermore, CRT protein binds to GCN repeats in mRNA of the myeloid transcription factor ►CCAAT/enhancer-binding protein α (CEBPA), and thereby impedes translation of the CEBPA mRNA, suggesting that CRT plays a functional role in the differentiation block in ►acute myeloid leukemia through suppression of CEBPA by the leukemic ►fusion gene *AML1-MDS1-EVI1*. Together, these findings suggest that CRT is involved in the regulation of cancer characteristics, although the overall mechanisms are still not clear.

CRT as a Tool for Cancer Therapy

CRT can form complexes with peptides in vitro to elicit peptide-specific CD8+ ►T cell responses. In addition, peptide-bound CRT purified from tumor extracts elicits an antitumor effect specific to the source tumor. Antigen-specific cancer immunotherapy is an attractive approach to the eradication of systemic tumors at multiple sites in the body. It has been reported that vaccination with DNA encoding chimera for CRT and a tumor antigen, ►human papilloma virus type-16 (HPV-16) E7 [CRT/E7], resulted in a significant reduction in the number of lung tumor nodules in immunocompromised mice. All together, the use of CRT represents a feasible approach for enhancing tumor-specific T cell-mediated immune responses.

Therapeutic agents that target the tumor vasculature may prevent or delay tumor growth and even promote tumor regression or dormancy. As another approach to cancer therapy, CRT or a fragment thereof (amino acids 1–180) (i.e., ►vasostatin) inhibits ►angiogenesis and suppresses tumor growth. The combination of vasostatin and IL-12 as well as vasostatin and interferon-inducible protein-10 had a suppressing effect on the cell growth of Burkitt lymphoma and colon carcinoma in mouse metastasis models. Although this suggests some potential for use in cancer therapy, the molecular mechanism of CRT actions at the cell surface is not fully understood.

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Calsequestrin-like Protein

►Calreticulin

CAM

Definition

Complementary alternative medicines are popular all over the world. The general concept that natural products are harmless by definition should be changed into a more realistic and responsible attitude.

cAMP

Definition

Cyclic adenosine monophosphate, second messenger induced in cells treated with various peptide hormones.

►Suppressors of Cytokine Signaling

cAMP Response Element Binding Protein (CREB)

Definition

A transcription factor that is activated by serine phosphorylation triggered by increased intracellular levels of cAMP or calcium.

►Signal Transduction

Campath-1H

Definition

A chimeric anti-CD52 monoclonal antibody regimen that has been successfully used for the treatment of refractory ►Chronic Lymphocytic Leukemia.

►Mcl Family

Camptothecin

Definition

Is a plant alkaloid isolated from *Camptotheca acuminata* (family Nyssaceae) with cytotoxic potential. Various (semi-)synthetic and water-soluble anticancer drugs, including 9-aminocamptothecin and 9-nitrocamptothecin, diflomotecan, topotecan, lurtotecan, and the prodrug ►irinotecan have been derived from camptothecin.

►Irinotecan
►Membrane Transporters
►Topoisomerases

CAMs

►Cell Adhesion Molecules

CAMTA1

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Definition

CAMTA1 is a candidate ►tumor suppressor gene encoding a member of a protein family designated as calmodulin-binding transcription activators (CAMTAs). It resides within a distal portion of chromosomal arm 1p that is frequently deleted in a wide range of human malignancies.

Characteristics

CAMTA1 maps to 1p36.31-p36.23 and its 23 exons are spread over 982.5 kb. The 6,582 bp cDNA encodes a protein of 1,673 amino acids. The protein's primary structure contains a nuclear localization signal, two DNA-binding domains (CG-1 and TIG), a transcription activation domain, calmodulin binding motifs (IQ motifs), and ankyrin domains. Although the expression of *CAMTA1* is seen in various organs, highest levels are found in neuronal tissues. Information on the physiologic roles of CAMTAs is scarce and most data derive from plant and drosophila studies.

CAMTAs are transcription factors that typically bind to CGCG boxes via their CG-1 domain. An alternative mechanism of transcriptional activation has been described for CAMTA2, the second human CAMTA homolog. It acts as a coactivator of another transcription factor, Nkx2-5, to stimulate gene expression. This function is inhibited by binding of class II ►histone deacetylases to the ankyrin-repeat region of CAMTA2. Upstream signaling components can activate CAMTA2 by promoting the export of class II histone deacetylases to the cytoplasm, relieving their repressive influence on CAMTA2.

The sole fly homolog of CAMTA1 induces the expression of an ►F-box gene, the product of which inhibits a Ca^{2+}-stimulating ►G-protein-coupled receptor (GPCR). The controlled deactivation of Ca^{2+}-stimulating GPCRs is needed to tune Ca^{2+}-mediated signaling and prevent abnormal cell proliferation. As CAMTA activity is increased by the Ca^{2+}-sensor calmodulin, the Ca^{2+}/calmodulin/CAMTA/F-box protein pathway may mediate a negative feedback loop controlling the activity of Ca^{2+}-stimulating GPCRs. This regulatory loop is of special interest taking into account the fundamental links between GPCR-mediated pathways and cancer biology.

Clinical Relevance

Deletions within 1p occur in various types of human malignancies, ranging from virtually all types of

solid cancers to leukemias and myeloproliferative disorders. Functional evidence for a role of 1p in tumor suppression derives from experiments in which the introduction of 1p chromosomal material into ►neuroblastoma cells resulted in reduced tumorigenicity. In neuroblastoma and other cancers, deletion of 1p36 is a predictor of poor patient outcome. Therefore, it is widely assumed that distal 1p harbors a gene (or genes) with tumor suppressive properties. To define the DNA, deleted from 1p, more precisely in pursuit of identifying the gene(s) of interest, substantial mapping efforts have been undertaken with the most detailed picture being worked out for neuroblastoma. In this tumor entity, the combination of ►loss of heterozygosity (LOH) fine mapping studies allowed to considerably narrow down a smallest region of consistent deletion spanning only 261 kb at 1p36.3 and pinpointing the *CAMTA1* locus. Sequence analysis revealed no evidence for somatic mutations in the remaining *CAMTA1* copy of neuroblastomas with 1p deletion. However, a rare sequence variant leading to amino acid substitution within the ankyrin domain was seen in a subgroup of neuroblastomas. More importantly, low *CAMTA1* expression is significantly associated with markers of unfavorable tumor biology and is itself a marker of poor neuroblastoma patient outcome. Moreover, *CAMTA1* expression is a neuroblastoma predictor variable that is independent of the established molecular markers including 1p deletion. Thus, the measurement of this variable should allow an additional biological stratification of neuroblastomas and help to assign patients to the appropriate therapy.

Additional evidence for a role of CAMTA1 in tumor development comes from ►glioma and ►colon cancer in which 1p is frequently deleted. In glioma, a 1p minimal deleted region spans 150 kb and resides entirely within *CAMTA1*. In colorectal cancer, a genome-wide analysis of genomic alterations revealed that loss of a 2 Mb recurrently deleted genomic region encompassing *CAMTA1* has the strongest impact on survival when compared with other genomic changes. Furthermore, as in neuroblastoma, low expression of *CAMTA1* is an independent marker of poor patient outcome. The high prevalence of *CAMTA1* deletion in neuroblastoma, glioma, and colorectal cancer together with the independent predictive power of low *CAMTA1* expression for neuroblastoma and colorectal cancer outcome are consistent with the idea that low CAMTA1 levels mediate a selective advantage for developing tumor cells.

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Canale-Smith Syndrome

Definition

►Autoimmune Lymphoproliferative Syndrome

Canals of Hering

Definition

Hepatocytes secrete bile into bile canaliculi which in turn drain into the canals of Hering – small ductules lined in part by cholangiocytes and in part by hepatocytes.

►Cholangiocarcinoma

Cancer

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Definition

Cancer is a deregulated multiplication of cells with the consequence of an abnormal increase of the cell number in particular organs. Initial stages of the developing

cancer are usually confined to the organ of origin whereas advanced cancers grow beyond the tissue of origin. Advanced cancers invade the surrounding tissues that are initially connected to the primary cancer. At a later stage, they are distributed via the hematopoetic and lymphatic systems throughout the body where they can colonize in distant tissues and form ►metastasis. The development of cancers is thought to result from the damage of the cellular genome, either due to random endogenous mechanisms or caused by environmental influences.

The origin of cancers can be traced back to alterations of cellular genes. Genetic damage can be of different sorts:

- Recessive mutations in ►tumor suppressor genes
- Dominant mutations of ►oncogenes
- Loss-of-function mutations in genes, involved in maintaining genomic stability and ►repair of DNA (resulting in ►genomic instability)

History

Human cancer is probably as old as the human race. It is obvious that cancer did not suddenly start appearing after modernization or industrial revolution. The world's oldest documented case of cancer comes from ancient Egypt, in 1500 BC. The details were recorded on a papyrus, documenting eight cases of tumors occurring on the breast. It was treated by cauterization, a method to destroy tissue with a hot instrument called "the fire drill." It was also recorded that there was no treatment for the disease, only palliative treatment. The word cancer came from the father of medicine, Hippocrates, a Greek physician (460–370 BC). Hippocrates used the Greek words, carcinos and carcinoma to describe tumors, thus calling cancer "karkinos." The Greek terms actually were words to describe a crab, which Hippocrates thought a tumor resembled. Hippocrates believed that the body was composed of four fluids: blood, phlegm, yellow bile and black bile. He believed that an excess of black bile in any given site in the body caused cancer. This was the general thought of the cause of cancer for the next 1,400 years. Autopsies done by Harvey in 1628 paved the way to learning more about human anatomy and physiology. By about the same time period, Gaspare Aselli discovered the lymphatic system, and this led to the end of the old theory of black bile as the cause of cancer. The new theory suggested that abnormalities in the lymph and lymphatic system as the primary cause of cancer. The lymph theory replaced Hippocrates' black bile theory on the cause of cancer. The discovery of the lymph system gave new insight to what may cause cancer, it was believed that abnormalities in the lymphatic system was the cause. Other theories surfaced, such as cancer being caused by trauma, or by parasites, and it was thought that cancer may spread "like a liquid" (Bentekoe, 1687; Heinrich Vierling, personal communication). The belief that cancer was composed of fermenting and degenerating lymph fluid was predominant.

The discovery of the microscope by Leeuwenhoek in the late seventeenth century added momentum to the quest for the cause of cancer. By late nineteenth century, with the development of better microscopes to study cancer tissues, scientists gained more knowledge about the cancer process. It wasn't until the late nineteenth century that Rudolph Virchow, the founder of cellular pathology, recognized that cells, even cancerous cells, derived from other cells. The early twentieth century saw great progress in our understanding of microscopic structure and functioning of the living cells. Researchers pursued different theories to the origin of cancer, subjecting their hypotheses to systematic research and experimentation. John Hill first recognized an environmental cause from the dangers of tobacco use in 1761 and published a book "Cautions Against the Immoderate Use of Snuff." Percivall Pott of London in 1775 described an occupational cancer of the scrotum in chimney sweeps caused by soot collecting under their scrotum. This led to identification of a number of occupational carcinogenic exposures and public health measures to reduce cancer risk. This was the beginning of understanding that there may be an environmental cause to certain cancers.

A virus causing cancer in chickens was identified in 1911 (Rous sarcoma virus). Existence of many chemical and physical carcinogens were conclusively identified during later part of the twentieth century. The later part of the twentieth century showed tremendous improvement in our understanding of the cellular mechanisms related to cell growth and division. The identification of ►transduction of oncogenes with the discovery of the ►*SRC* gene, the transforming gene of Rous sarcoma virus, led to formulating the oncogene concept of tumorigenesis and can be viewed as the birth of modern molecular understanding of cancer development. Subsequently, tumor suppressor genes were identified. Many genes that suppress or activate the cell growth and division are known to date, their number is ever growing. It is conceivable that in the end the confusing situation may arise to recognize that all genes of the human genome, in one way or another, take part in signaling normal or cancerous cellular growth.

Characteristics

A large proportion of genetic changes appears to arise by mechanisms endogenous to the cell, such as by errors occurring during the replication of the ~3×10^9 base pairs present in the human genome. Environmental factors have a major role as well, predominantly as:

- Chemical carcinogens (e.g. aflatoxin B1 in liver cancer (►liver cancer, molecular biology), tobacco smoke in lung cancer; ►tobacco carcinogenesis)
- Radiation
- Viruses (such as ►hepatitis B virus (►Hepatitis viruses) in liver cancer, or ►human papillomavirus in cervical cancer)

Types of Genetic Damage

Damage to oncogenes and tumor suppressor genes can be of different sorts:

- Point mutations resulting in the activation of a latent oncogenic potential of a cellular gene (e.g. ►RAS) or in the functional inactivation of a tumor suppressor gene by generating an intragenic stop codon that leads to premature translation termination with the consequence of an incomplete truncated protein (e.g. ►p53) or the failure for maintaining genomic stability (►mismatch repair genes in ►HNPCC)
- ►Amplification leading to an increase of the gene copy number beyond the two alleles normally present in the cell (copy number can reach 500 and more; example: ►*MYCN* in human ►neuroblastoma)
- Translocation, which is defined as an illegitimate recombination between non-homologous chromosomes, the result being either a fusion protein (where recombination occurs between two different genes such as *BCR-ABL* in Chronic Myclogenous Leukemia) or in the disruption of normal gene regulation (where the regulatory region of a cellular gene is perturbed by the introduction of the distant genetic material such as ►*MYC* in Burkitt lymphoma (►Epstein-Barr virus))
- Viral insertion by the integration of viral DNA into the regulatory region of a cellular gene. This integration can occur after a virus has infected a cell. Viral insertion is well documented in animal tumors (HBV integration in the vicinity of ►*MYCN* in liver cancer in experimental animals; liver cancer, molecular biology)

Cellular Aspects

Cancer in solid tissues (solid cancer) usually develops over long periods (often 20–30 years latency period) of time. An exception are solid cancers (such as neuroblastoma) in children, which often are diagnosed shortly after birth. Malignant cancers are characterized by their ability to develop metastasis (i.e. secondary cancers at distance from the primary tumor), often they also show multidrug resistance, which means that they hardly react to conventional chemotherapy. It is thought that the development of a normal cell to a metastatic cell is a continuous process driven by genetic damage and genomic instability, with the progressive selection of cells that have acquired a selective advantage within the particular tissue environment (►multistep development). Studies of colorectal cancers have identified 6–7 genetic events required for the conversion of a normal cell to a cell with metastatic ability. This is in contrast to leukemias, which usually require one genetic event, most often a translocation, for disease development.

Sporadic Versus Familial Cancer

The vast majority of cancers are "sporadic," which simply means that they develop in an individual. Descendants of this individual do not have an increased risk because the cellular changes that have resulted in cancer development are confined to this individual. In contrast, ~10% of cancer cases have a hereditary background, they show familial clustering (prominent examples include retinoblastoma (►Retinoblastoma, ►cancer genetics), ►breast cancer, FAP (►APC gene in Familial Adenomatous Polyposis) and HNPCC as familial forms of colorectal cancer (►colon cancer), ►melanoma). Familial cancers have been identified to result from germline mutation of genes. These germ line mutations do not always directly dictate cancer development, although they are considered "strong" hereditary determinants. They represent susceptibility genes that confer a high risk for cancer development to the gene carrier. The relative risk of the individual carrying the mutant gene can vary considerably. For instance, the risk of carriers of one of the breast cancer susceptibility genes ►*BRCA1* or ►*BRCA2* for breast cancer development can vary between approximately 60 and 90%. In reality this means that the risk for cancer development is difficult to predict, and individuals may not develop cancer at all in spite of the presence of a mutated gene in their germ line. The molecular basis for the differences in risk are unknown. Formally the activity of modifying factors, either environmental or genetic, has been suggested. Such modifying factors appear to be less important for some other familial cancers, such as retinoblastoma, where the risk is constant between 90 and 95% for gene carriers.

Polygenic Determinants of Risk

The relative risk of the individual for cancer development can also be determined by so called "weak" genetic factors. Normal cells contain a number of genes involved in ►detoxification reactions. Different allelic variants of these genes exist in the human population that encode proteins with slightly different enzymatic activities. Although the exact contribution of individual allelic variants to cancer development is difficult to assess, it is reasonable to assume that individuals that have inherited "weak" enzymatic activities in different detoxification systems are likely to have a higher risk. It is likely, therefore, that the risk for such cancers is "polygenic."

►Toxicological Carcinogenesis

Cancer and Cadmium

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Definition

Cadmium is a metal that has the symbol Cd and atomic number 48 in the periodic table. Cadmium has high toxic effects, an elimination half-life of 10–30 years, and accumulates in the human body, particularly the kidney. Roughly 15,000 tons of cadmium is produced worldwide each year for nickel-cadmium batteries, pigments, chemical stabilizers, metal coatings, and alloys.

Characteristics

Urinary excretion of cadmium over 24 h is a biomarker of lifetime exposure. Exposure to cadmium occurs through intake of contaminated food or water, or by inhalation of tobacco smoke or polluted air. Occupational exposures can be found in industries such as electroplating, welding, smelting, pigment production, and battery manufacturing. Other exposures to cadmium can occur through inhalation of cigarette smoker. Gastrointestinal absorption of cadmium is estimated to be around 5–8%. Inhalation absorption is generally higher, ranging from 15 to 30%. Absorption after inhalation of cadmium fume, such as cigarette smoke, can be as high as 50%. Once absorbed, cadmium is highly bound to the metal-binding protein, metallothionein. Cadmium is stored mainly in the kidneys and also the liver and testes, with a half-life in the body of 10–30 years. In general, nonsmokers have urinary cadmium concentrations of 0.02–0.7 μg/g creatinine, which increase with age in parallel with the accumulation of cadmium in the kidney. Cadmium is a global environmental contaminant. Populations worldwide have a low-level intake through their food, causing an age-related cumulative increase in the body burden of this toxic metal. Environmental exposure levels to cadmium, that are substantially above the background, occur in areas with current or historical industrial contamination for instance in regions of Belgium, Sweden, UK, Japan, and China.

As an environmental carcinogen, cadmium could have substantial health implications. Three lines of evidence explain why the International Agency for the Research on Cancer classified cadmium as a human carcinogen. First, as reviewed by Verougstaete and colleagues, several, albeit not all studies in workers showed a positive association between the risk of lung cancer and occupational exposure to cadmium; discrepancies between these studies should not be ascribed to the better design of the more recent studies. Verougstraete and colleagues suggested that such inconsistencies might be attributed to the high relative risk of cancer in the presence of coexposure to ►arsenic, nickel, or toxic fumes, and that the increasingly stringent regulations with regard to levels of exposure permissible at work might be a confounding factor (►Lead exposure, nickel carcinogenesis). Second, data from rats showed that the pulmonary system is a target site for carcinogenesis after cadmium inhalation. However, exposure to toxic metals in animal studies have usually been much higher than those reported in environmentally exposed humans to toxic metals. Third, several studies done in vitro have shown plausible pathways, such as increased oxidative stress, modified activity of transcription factors, and inhibition of DNA repair. Most errors that arise during DNA replication can be corrected by DNA polymerase proof reading or by postreplication mismatch repair. In fact, inactivation of the DNA repair machinery is an important primary effect, because repair systems are required to deal with the constant DNA damage associated with normal cell functions. The latter mechanism might indeed be relevant for environmental exposure because Jin et al. found that chronic exposure of yeast to environmentally relevant concentrations of cadmium can result in extreme hypermutability. In this study the DNA-mismatch repair system is already inhibited by 28% at cadmium concentrations as low as 5 μM. For example, the prostate of healthy unexposed humans accumulates cadmium to concentrations of 12–28 μM and human lungs of nonsmokers accumulate cadmium to concentrations of 0.9–6 μM. Further, in vitro studies provide evidence that cadmium may act like an estrogen, forming high-affinity complexes with estrogen receptors, suggesting a positive role in breast cancer carcinogenesis.

Along with this experimental evidence, two epidemiological studies in 2006 gave important positive input into the discussion on the role of exposure to environmental cadmium in the development of cancer in human beings. First, the results of a population-based case-control study noticed a significant twofold increased risk of breast cancer in women in the highest quartile of cadmium exposure compared with those in the lowest quartile. Second, we conducted a population-based prospective cohort study with a median follow-up of 17.2 years in an area close to three zinc smelters. Cadmium concentration in soil ranged from 0.8 to 17.0 mg/kg. At baseline, geometric mean urinary cadmium excretion was 12.3 nmol/day for people in the high-exposure area, compared with 7.7 nmol/day for those in the reference (i.e, low exposure) area. The risk of lung cancer was 3.58 higher than in a reference population from an area with low exposure. 24-h urinary

excretion is a biomarker of lifetime exposure to cadmium. The risk for lung cancer was increased by 70% for a doubling of 24-h urinary cadmium excretion. Confounding by coexposure by arsenic could not explain the observed association. Epidemiological studies did not convincingly imply cadmium as a cause of prostate cancer. Of 11 cohort studies, only 3 (33%) found a positive association.

In conclusion, recent experimental and epidemiological studies strongly suggest environmental exposure to cadmium as a causal factor in the development of cancer of the lung and breast.

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Cancer Antigen

Definition

Cell surface proteins specific for cancer cells.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Cancer Antigen 15-3 (CA 15-3)

Definition

Carbohydrate antigen 15-3 is a tumor marker associated with breast cancer, and has much less specificity and sensitivity in patients with ovarian, lung, or prostate cancers. Increased levels may be associated with pregnancy and lactation, benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis.

►Serum Biomarkers

Cancer Associated Antigen 19-9 (CA 19-9)

Definition

Serum levels of CA 19-9, an intercellular adhesion molecule, was initially found in patients with colorectal cancer, and subsequently also identified in patients with pancreatic and biliary tract cancers, and less often in gastric, ovarian, lung, breast and uterine cancer. Non-cancerous conditions that may elevate CA 19-9 include gallstones, cholecystitis, pancreatitis and cirrhosis of the liver.

►Serum Biomarkers

Cancer Associated Antigen 27-29 (CA 27-29)

Definition

Cancer antigen 27-29 (synonym: BR 27-29) is a normal epithelial cell mucin-1 (MUC1) apical surface glycoprotein. Elevated serum levels are highly associated with breast cancer. However they can also be found in cancers of colon, stomach, kidney, lung, ovary, pancreas, uterus and liver and in a number of noncancerous conditions, including first trimester pregnancy, ►endometriosis, ovarian cyst, benign kidney, liver and breast disease.

►Serum Biomarkers

Cancer of B-lymphocytes

►B-cell Tumors

Cancer Cachexia Syndrome (CCS)

Definition

Loss of weight in the form of lean body mass and fat which results from a complex interaction between cytokines and tumor factors.

►Nutrition Status

Cancer Causes and Control

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Synonyms

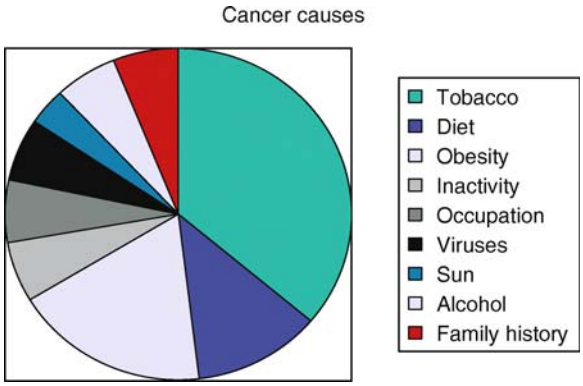
Etiology; Prevention

Definition

The process of identifying causes of cancer and developing strategies to change cancer risk through health care providers, regulations that reduce risk, or individual and community level changes.

Characteristics

Over 6 million people around the world die from cancer each year. There is overwhelming evidence that lifestyle factors impact cancer risk and that positive, population-wide changes can significantly reduce the cancer burden. Current epidemiologic evidence links behavioral factors to a variety of diseases, including the most common cancers diagnosed in the developed world – ►lung cancer, ►colorectal cancer, ►prostate cancer and ►breast cancer. These four cancers account for over 50% of all cancers diagnosed on western countries. As summarized in Fig. 1, ►tobacco causes some 30% of cancer, lack of physical activity 5%, obesity 15%, diet 10%, ►alcohol 5%, ►viral infections 5%, and ►UV light by excess sun exposure 3%. Because of the tremendous impact of modifiable factors on cancer risk, especially for the most common cancers, it has been estimated that at least 50% of cancer is preventable. Currently in the US not all risk factors are equally distributed across race and social class. Trends in risk factors should also be considered when assessing potential for prevention. To bring about dramatic reductions in cancer incidence, widespread lifestyle changes will be necessary.



Cancer Causes and Control. Figure 1 Causes of cancer.

Rose advocates the need for population approaches for prevention of chronic disease. He emphasizes that when the relation between a lifestyle factor or biological predictor of risk is continuous, the majority of cases attributable to the exposure will likely arise in those who are not classified as being at high risk. He illustrates this with examples of blood pressure and rates of coronary heart disease. Specifically, even small changes in blood pressure at the population level can translate into large reduction in the rates of coronary disease and stroke. To reduce the risk of disease in the population-substantial benefits can be achieved by a small reduction for all members of the society rather than just focusing on the high-risk groups. Because population wide trends in cardiovascular risk factors show continuing improvement, the rate of coronary heart disease incidence and mortality continues to decrease.

When we consider population approaches to cancer prevention, we must address the etiologic process, which covers a different time course and sequence from coronary heart disease. Although cardiovascular disease is the end point of the chronic process of atherosclerosis, treatment focuses on the reversal and subsequent prevention of the acute thrombotic process of myocardial infarction. Cancer, on the other hand, is the result of a long process of accumulating DNA damage (►multistep development), leading ultimately to clinically detectable lesions such as in situ and invasive cancer. For example, studies of the progression in ►colon cancer from first mutation to invading malignancy suggest that DNA changes accumulate over a period of as long as 40 years. The goal of cancer prevention is to arrest this progression; different interventions interrupt carcinogenesis at different points in the process. Further, most cancers do not have a late "acute" event, analogous to thrombosis, which can be prevented with medical interventions.

The benefits of cancer prevention and control programs take time to be observed. The fact that different

interventions will impact at different points along the pathway to cancer, that can stretch over nearly half a century, has implications for when we can expect to see pay-off in terms of lower cancer rates. Research has demonstrated that those who initiate smoking during early adolescence greatly increase risk of lung cancer even when one takes into account both the dose and duration of smoking. If we could delay the age at which most adolescents first start to smoke, we would probably substantially reduce lung cancer rates, but this benefit will not be observable for 20–40 years after the intervention. Adult cessation, on the other hand, reduces risk more rapidly, but fails to address the continuing recruitment of the next generation of smokers. Recent declines in the incidence of lung cancer among younger men and women in the United States reflect reductions in the rate of smoking among younger adults. Other lifestyle interventions may act as preventive early in the DNA pathway to cancer. For example, ►aspirin and ►folate appear to act early in the pathway inhibiting colon cancer.

Population-wide prevention strategies for cancer do work. For example, reductions in lung cancer rates in the United States mirror changes in cigarette smoking patters, with marked decreases seen first in young men, then older men, and finally in women. Introduction of the Papaniculou test for cervical cancer in the 1950s was followed by a dramatic decline in cervical cancer in those countries that made wide-spread ►screening available. The decline in Australian ►melanoma mortality for those born after 1950 is an additional example of effective intervention at the population level. Behavior change is possible and offers great potential for cancer prevention. The recommendations for cancer risk reduction include reducing tobacco use, increasing physical activity, maintaining a healthy weight, improving diet, limiting alcohol, avoiding excess sun exposure, utilizing safer sex practices, and obtaining routine cancer screening tests.

Age is the dominant factor that drives cancer risk; for all major malignancies, risk rises markedly with age. The importance of age is exemplified by the fact that the aging U.S. population together with projected population growth will result in a doubling of the total number of cancer cases diagnosed each year by the year 2050, assuming that incidence rates remain constant. With this estimated growth in cancer from 1.3 million to 2.6 million cases per year, it is expected that that both the number and proportion of older persons with cancer will also rise dramatically.

Tobacco

Tobacco is the major cause of premature death around the world accounting for some 5M deaths each year. In the United States, adult smokers lose an average of 13 years of life because of smoking, and approximately half of all smokers die of tobacco-related disease. Smoking is well known to cause over 90% of lung cancers in addition to a range of other malignancies (►tobacco carcinogenesis). It causes about 30% of all the cancer in the developed world, including lung cancer, ►mouth cancer, larynx cancer, ►esophagus cancer, ►pancreas cancer, ►cervix cancer, ►kidney cancer, and ►bladder cancer. Smoking also increased risk of cancers of the colon, stomach, cervix, liver, and prostate, as well as to leukemia. In addition, smoking leads to many other health problems, including heart disease, stroke, lung infections, emphysema, and pregnancy complications.

Tobacco may act on multiple stages of carcinogenesis; it delivers a variety of carcinogens, causes irritation and inflammation, and interferes with the body's natural protective barriers. The health risks of tobacco use are not limited to cigarette smoking. Cigar and pipe use increase the risk of disease, as does exposure to second-hand smoke and smokeless tobacco use.

Avoiding initiation of tobacco use clearly offers the greatest potential for disease prevention. However, for those who use tobacco products, there are substantial health benefits that come with quitting. There are numerous effective cessation methods, and in the past 25 years, 50% of all living Americans who have ever smoked, have successfully quit.

Quitting smoking has immediate and significant health benefits for men and women of all ages. For example, former smokers live longer than individuals who continue smoking. Those who quit before age 50 have approximately half the risk of dying in the next 15 years. This decline in mortality risk is measurable shortly after cessation and continues for at least 10–15 years.

Strategies to assist smoking cessation and decreasing youth initiation from both a population and clinical perspective are essential steps to reducing the burden of cancer.

Trends

Current smoking among US adults has remained steady over the past decade.

Once quite pronounced, gender disparity in smoking rates is now relatively small and has been stable since 1990. In 2002, 25.7% of men were current smokers compared to 20.8% of women. Given the profound impact of smoking on cancer, disparities in smoking rates and in access to effective cessation methods will continue to translate directly into differences in the burden of smoking-related cancers.

Physical Activity

Lack of physical activity causes over 2M deaths each year around the world. People in the US and in other developed nations are extremely inactive – over 60% of

the US adult population does not participate in regular physical activity, which includes 25% of adults who are almost entirely sedentary. Fortunately, the negative effects of a sedentary lifestyle are reversible: increasing one's level of physical activity, even after years of inactivity, can reduce mortality risk.

Lack of physical activity increases the risk of colon and breast cancer and likely endometrial cancer, as well as diabetes, osteoporosis, stroke, and coronary heart disease. Overall, sedentary lifestyles have been linked to 5% of deaths from cancer. Among both men and women, high levels of physical activity may decrease the risk of colon cancer by as much as half. Using a variety of measures of activity, studies have consistently shown higher physical activity lowers risk of colon cancer. Physical activity also appears to lower the risk of large adenomatous polyps, precursor lesions for colon cancer, suggesting that it may influence the early stages of the adenoma-carcinoma sequence. In addition, the relationship between physical activity and breast and colon cancer are seen across levels of obesity, indicating that physical activity and obesity have separate or independent effects on cancer incidence. Growing evidence suggests that physical activity may also be protective against lung and prostate cancer.

Several mechanisms have been proposed to explain these associations. Physical activity reduces circulating levels of insulin, a growth factor for colonic epithelial cells. Additionally, it is postulated that cancer risk is reduced through alterations in prostaglandin levels, improvement in immune function, and modification of bile acid metabolism.

Potential mechanisms for the reduction of breast cancer risk include physical activity's lowering of the cumulative lifetime exposure to circulating estrogens and improving immune pathways.

The benefits of physical activity include the prevention of cancer and a large number of other chronic diseases. Increasing levels of physical activity, even after years of inactivity, reduces mortality risk. As little as 30 min of moderate physical activity (such as brisk walking) per day significantly reduces disease risk.

Trends

One major determinant of activity level that has changed over time is the amount of activity required for work and daily living. With advances in technology and the development of labor-saving devices, there is now a greatly reduced need for physical activity for transportation, household tasks, and occupational requirements. Overall, the prevalence of physical inactivity in the United States is remarkably high; in 1996, about 28% of Americans reported absolutely no participation in leisure-time physical activity. In addition, physical activity in schools has declined, and almost half of young Americans between the ages of 12–21 are not vigorously active on a routine basis.

Given the trends in our society, it is unlikely that this decreasing energy expenditure will reverse rapidly. Accordingly, the burden of cancer due to lack of physical activity will increase in the years ahead unless new strategies to promote activity are rapidly implemented.

Weight Control and Obesity Prevention

Overweight and obesity is increasing at epidemic rates in the United States, around the world, and is estimated to account for 2.6M deaths each year. Currently almost 65% of American adults are overweight (body mass index (BMI) $\geq$25 kg/m^2), and over 30% are considered obese (BMI $\geq$ 25 kg/m^2).

Overweight and obesity cause a variety of cancers; colon, postmenopausal breast, endometrial, renal, and esophageal. The proportion of cancer caused by obesity ranges from 9% for postmenopausal breast cancer to 39% for endometrial cancer. One large US study suggested that obesity influences an even broader range of cancers, increasing the risk of death from cancers of the colon and rectum, prostate, breast, esophagus, liver, gallbladder, pancreas, kidney, stomach, uterus, and cervix in addition to non-Hodgkin's lymphoma and multiple myeloma. Overall, obesity causes 14% of cancer deaths among men and 20% of cancer deaths among women. Excess body fat may act by altering levels of hormones and tumor growth factors. It is clear that excess weight has severe health consequences.

In addition to raising the risk of cancer, overweight and obesity also increase the risk of a multitude of other diseases and chronic conditions, such as stroke, cardiovascular disease, type 2 diabetes, osteoarthritis, and pregnancy complications.

The International Agency for Research on Cancer has proposed a comprehensive set of recommendations to address the issue of weight control at multiple levels, including steps by health care providers, regulatory approaches to create adequate access to safe places for exercise (including school, worksite, and community), and family and community level actions.

Trends

In the U.S., the prevalence of overweight and obesity has increased so dramatically and so rapidly, it is frequently referred to as an obesity epidemic. The trend is also being seen among children and adolescents.

This epidemic has affected people of all ages, races, ethnicities, socioeconomic levels, and geographic regions. Given limited long-term success in weight reduction programs, the cancer burden due to obesity will likely continue to follow the rising prevalence of this risk factor in the coming years.

Dietary Improvements

Fruit and vegetable intake has been most consistently evaluated as a cancer prevention strategy. The global burden of inadequate intake is estimated to account for over 2M deaths each year. While evidence for cardiovascular benefits and reduced risk of diabetes are clear, evidence for cancer risk reduction has become less convincing with the results of numerous prospective cohort studies showing weaker associations with cancer risk. Low intake of fruits and vegetables are probably related to increased risk of pancreas, bladder, lung, colon, mouth, pharynx, larynx, esophagus and stomach cancer.

Although the effect of fruit and vegetable consumption on the risk of prostate cancer has been examined in nearly twenty studies, data remain inconsistent. The majority of studies suggest that overall fruit and vegetable intake has little effect if any on the risk of prostate cancer. However, individual fruits and vegetables may offer the potential for greater risk reduction, with tomatoes being the most promising, with a 40–50% reduction in risk among men who consumed large amounts of tomatoes and tomato products. The carotenoid ►lycopene is hypothesized to be responsible for the protective effect.

A number of mechanisms have been suggested to explain the protective effect of fruits and vegetables, but it is not known if specific agents, such as ►carotenoids, folic acid, and vitamin C, or a special combination of factors create anticarcinogenic effects. It is also possible that diet in childhood and adolescence is more important than later in life in driving risk of cancer.

A number of studies have found that as folate intake increases, the risk of colorectal cancer (as well as polyps) decreases. The Nurses' Health Study found that a high intake of folate from fruits and vegetables was sufficient to lower risk but that supplementation with a multivitamin that contained folate offered even greater reductions. The underlying biologic role of folate and its interaction with the MTHFR gene add support to the causal relation between low folate and colon cancer.

In addition to the reduction in risk of colon cancer, growing evidence points to folate also reducing the adverse effect of alcohol on breast cancer. Based on this evidence and the benefits for prevention of neural tube defect and cardiovascular disease, use of a daily vitamin supplement containing folate is recommended.

Dietary Fat

Variations in international cancer rates have often been attributed to differences in total fat intake, yet evaluation has shown no clear link between dietary fat and breast, colon or prostate cancer. Although dietary fat overall does not appear to impact cancer risk, there is some evidence to suggest that certain types of fat, such as animal fat, may increase risk.

Fiber has been shown to reduce the risk of heart disease and diabetes, but it does not appear to offer protection against cancer. Long believed to help prevent colon cancer, the data do not support this hypothesis.

Red Meat

High intake of red meat, including beef, pork, veal and lamb is associated with an elevated risk of colorectal cancer. The mechanism of this increased risk is not well understood, but it may be related to the high concentrations of animal fat or to carcinogens such as heterocyclic amines produced when the meat is cooked at high temperatures.

Calcium

Higher calcium intake has been linked to a reduced risk of colorectal adenomas and colorectal cancer. However, increased dietary calcium is also associated with an increased risk of prostate cancer. Research indicates that there may be a moderate intake of calcium that provides protection against colorectal cancer risk without causing a large increase in prostate cancer risk.

Excess Caloric Intake

One consistent dietary finding is that excess calories from any source result in weight gain and increased cancer risk. As the obesity epidemic continues to spread, the importance of balancing caloric intake with caloric expenditure becomes even more evident for the prevention of cancer and other chronic diseases.

Whole Grains

Although grain products in general have not been shown to affect cancer risk, whole-grain foods may provide some protection against stomach cancer. Grains such as wheat, rice, and corn form the basis for most diets worldwide. Some grain products, such as whole-wheat bread and brown rice, are consumed in the "whole-grain" form, while others, like white bread and white rice, are more refined. During the process of refining grain, most of the fiber, vitamins, and minerals are removed, thus whole-grain foods tend to be more nutrient-rich than refined foods and may offer more in terms of disease prevention. The benefits of whole-grain foods in reducing cardiovascular disease and ischemic stroke are well established.

Vitamin A and Carotenoids

Isolated ►vitamin A and carotenoids are not likely to play a large role in cancer prevention. Some observational data support a probable inverse relation with lung cancer risk, but randomized trials of beta-carotene intake found either no effect or an increased risk of lung cancer. It has also been suggested that beta-carotene impacts breast cancer risk, however, it seems that at

best, there is only a small decrease in breast cancer risk associated with a high intake of carotenoids.

Selenium

Ecological studies have suggested that increased ►selenium intake is associated with decrease risk of colon and breast cancer. A randomized control trial of selenium for skin cancer prevention showed no effect of selenium on skin cancer incidence, however it did show a reduction in incidence of lung, colon and prostate cancer. Despite these promising results, the impact of selenium remains unclear. Fortification of the soil in Finland in the mid-1980s led to higher blood selenium levels, but no decline in incidence or mortality has been noted for prostate or colon cancer.

Vitamin D

Growing evidence relates lower levels of ►vitamin D to increased risk of cancer and to poor survival after diagnosis.

Trends

The proportion of adults consuming the recommended five servings of fruits and vegetables a day varies between 8 and 32%. While these estimates are clearly low for the entire population, certain groups, as defined by gender, race/ethnicity, education, and income, are of particular concern.

Given constraints due to both financial resources and physical access to markets that provide fresh fruit and vegetables, it remains likely that SES gradients in diet will continue. Interventions are needed to overcome these existing barriers and make healthy foods readily available to all.

Limitation of Alcohol Use

Globally, alcohol intake in excess is responsible for 1.8M deaths each year. Clear benefits of moderate alcohol intake have been shown in terms of reducing cardiac and diabetes risk, but alcohol remains a risk factor for cancer mortality.

Alcohol is a known carcinogen that may raise cancer risk in several ways. For example, it may act as an irritant, directly causing increased cell turnover, or it may allow for improved transport and penetration of other carcinogens into cells. Alcohol use is a primary cause of esophageal and oral cancer, and it is associated with an increased risk of breast, liver, and colorectal cancer. Multiple other risks are also associated with alcohol use, including the risk of hypertension, addiction, suicide, accident, and pregnancy complication.

To balance the cardiovascular benefits with the risks of cancer and other negative consequences, it is recommended that those who drink alcohol should do so only in moderation. Intake should be limited to less than one drink per day for women and less than two drinks per day for men.

Safer Sex and Decreased Viral Transmission

►Unsafe sex is responsible for 2.9M deaths each year, primarily due to the transmission of ►HIV. However, unprotected sexual contact also results in the spread of multiple other sexually transmitted infections including oncogenic viruses. Some of these viruses may also be spread through exposure to blood and blood products.

►Human papillomavirus causes cervical cancer, vulvar, penile and anal cancer; ►hepatitis B virus and ►hepatitis C virus cause ►hepatocellular cancer; human lymphotropic virus-type 1 is associated with adult T cell leukemia (►human T-cell leukemia virus); human immunodeficiency virus-type 1 causes ►Kaposi sarcoma and non-Hodgkin lymphoma; and human herpes virus causes Kaposi sarcoma and body cavity lymphoma.

Prevention strategies to contain the spread of these viruses should include behavioral and educational interventions to modify sexual behavior, and structural and regulatory changes to promote safer sex and make condoms readily available. Biomedical interventions to administer vaccines are also needed. For example, it is estimated that vaccination programs could reduce the global burden of liver cancer by 60%. Additional strategies to prevent viral spread include needle exchange programs for intravenous drug users; regulation of tattooing and acupuncture; screening of blood donors, and the development of artificial blood products.

Trends and Disparities

Current U.S. data on the prevalence of these different viruses is not adequate to predict trends in cancer incidence. In addition, the recent development of new technologies such as vaccines against HPV, suggest a new era in prevention of cervical cancer. However, for success, such vaccines must be available and accessible to the entire population. Assuring access remains a policy priority to maximize the potential benefit of this cancer prevention strategy.

Sun Protection

The American Cancer Society estimates over 50,000 melanoma diagnoses each year in the U.S. The incidence of melanoma is rising more rapidly than that of any cancer in this country. Exposure to the sun (►UV radiation) is the major modifiable cause of melanoma and other skin cancers. For most people, the majority of lifetime sun exposure occurs during childhood and adolescence, and migrant studies clearly show that age at migration to high-risk countries has a strong impact on risk of this malignancy. For this reason, early intervention has the greatest potential for prevention.

The risk of melanoma and other less aggressive forms of skin cancer exists for all racial and ethnic groups, but skin cancers occur predominantly in the non-Hispanic white population. Constitutional characteristics including hair color, mole count, and family history contribute to risk of melanoma. However, studies show that established risk factors alone do not identify a sufficient proportion of cases to focus prevention efforts on only a subset of the population. Because identifying high-risk individuals will miss the majority of cases, population-based efforts provide greater protection. There is tremendous potential to substantially reduce the burden of this common malignancy through effective prevention efforts.

Screening

Screening for cancer can provide protection in several ways. In the case of colorectal and cervical cancers, screening can detect premalignant changes that can be treated to prevent cancer from developing. This primary prevention has the potential to substantially reduce the burden of cancer. With colorectal screening the mortality from colon cancer is reduced by a half or more.

If cancer is already present, screening can act as a secondary prevention (►early detection), such as mammography for breast cancer, facilitating early diagnosis and treatment, thereby decreasing morbidity and mortality. This type of prevention is an added benefit of colorectal and cervical screening, and is the main goal of breast cancer and prostate cancer screening.

Trends and Disparities

Trends in cervical cancer screening have been impacted by the breast cancer cervical cancer screening act which provided resources to states, via the Centers for Disease Control and Prevention, to bring screening services to low income women. Despite these efforts, national data suggest that low income and Hispanic women are less likely to be current with screening recommendations. Lack of access to care, defined as not having a usual source of health care, was associated with significantly lower compliance with cervical screening. Evidence from the 1998 Health Interview Survey indicates that all US born women have comparable and high compliance with screening for cervical cancer. Foreign born women, however, appear to be under-screened, accounting for the disparity among Hispanic women and suggesting a priority area for prevention as the U.S. continues to have a large immigrant population at risk of cancer. Surveys of colorectal screening suggest that the rates of screening have been rising and that Caucasians are more likely to be up to date with screening than other racial or ethnic groups.

Conclusion

Lifestyle changes offer tremendous potential for prevention of cancer and multiple other chronic conditions. This potential is often underestimated. To achieve the maximal benefit through behavioral change, interventions are necessary at multiple levels. Societal changes are needed to support and encourage the behavior modification of individuals. Approaches are needed to target individuals, communities, and systems, and create an environment less inductive to high-risk lifestyles. Social systems and regulatory efforts must complement individual behavior changes if these changes are to be sustained and the benefits of reduced disease burden realized.

Overall, the major lifestyle factors considered here account for the majority of cancer and could be modified to prevent at least half of all cancers. However, the burden of cancer is not limited to just the major lifestyle factors considered here. For example, occupational and environmental exposures also account for a relatively small number of cancer cases compared to the lifestyle factors considered above. Yet the burden of exposure to these harmful agents may be disproportionately high among low-income populations, accentuating their cancer risk. In large part, these exposures can be prevented through adequate enforcement of regulatory changes, and this should remain a high priority.

Small individual changes can result in large population benefits, but efforts to create prevention programs for only certain members of our society limits the potential for prevention. We must largely reframe our approach to the issue. Identifying risk factors and setting goals for reduction is only the beginning. Research and policy must now focus on bringing about population-wide lifestyle change, addressing the issues of disparities, and leaving no group or community behind.

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Cancer Cell-platelet Microemboli

►Tumor Cell-Induced Platelet Aggregation

Cancer Epidemiology

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Synonyms

Population-based cancer research

Definition

Knowledge about causes and preventive strategies for malignant neoplasms has greatly advanced during the last decades. This is largely attributable to the development of cancer epidemiology. In parallel to the identification of the causes of cancer, primary and secondary preventive strategies have been developed. A careful consideration of the achievements of cancer research, however, suggests that the advancements in knowledge about causes and mechanisms have not been followed by an equally important reduction in the burden of cancer. Part of this paradox is explained by the long latency occurring between exposure to carcinogens and development of the clinical disease. In addition, the most important risk factors of cancer are linked to lifestyle, and their modification entails cultural, societal and economic consequences. The failure to identify valid biomarkers of cancer risk is another reason of the limited success in cancer control.

Cancer epidemiology investigates the distribution and determinants of cancer in human populations. Although the main tool in cancer epidemiology is the ►observational study, ►the intervention study, of experimental nature, is conducted to evaluate the efficacy of prevention strategies, such as screening programs and chemoprevention trials (clinical trials are usually considered outside the scope of epidemiology). Intervention studies follow the randomized trial design. Observational epidemiology can be broadly divided in ►descriptive epidemiology and analytical studies. Analytical studies can be based on data collected at the individual or population level. The former consist of ►cohort study, ►case-control study and ►cross-sectional study (and a few variations on these themes), the latter of so-called ►ecological study. Family-based studies are used in ►genetic epidemiology to identify hereditary factors. An additional useful distinction among etiological studies concerns the nature of the information on exposure: while some studies use information routinely collected for other purposes, such as censuses and medical records, in other circumstances exposure data are collected ad-hoc following a variety of approaches including questionnaires, pedigrees, environmental measurements, measurement of biological markers. A method-oriented (rather than subject- or design-oriented) approach has lead to the identification of specific sub-disciplines such as ►molecular epidemiology.

Characteristics

A distinctive feature of cancer epidemiology is the availability in many countries of a population-based ►cancer registry, which allow the calculation of valid and reliable estimate of the occurrence of cancer (incidence, mortality, prevalence, survival). Typically, registries collect routinely demographic data of patients, which are used to generate statistics according to period of diagnosis, age, sex, and other characteristics. These studies of descriptive epidemiology have been critical in developing etiological hypotheses. One particular type of descriptive studies concerns migrants from low- to high-risk areas, or vice-versa: the repeated demonstration of rapid changes in the risk of many cancers among migrants (from that prevalent in the area of origin towards that of the host area) provided very strong evidence of a predominant role of modifiable factors in the etiology of human cancer. While cancer registries were initially established in high-resource countries, a growing number of population s in middle- and low-resource countries are now covered by good-quality registries, thus providing a solid infrastructure for most ambitious research projects.

Other routinely collected data are used in epidemiology. Mortality statistics are available in many countries of the world, and provide a good approximation of the incidence of the most fatal cancers. In a growing number of populations, automatic linkage is possible between incidence or mortality data and other population-based registries (e.g., hospital discharges, use of medications). ►Record-linkage study may represent an efficient alternative to investigations based on ad-hoc collection of data.

The number of new cases of cancer which occurred worldwide in 2002 has been estimated at about 10,800,000. Of them, 5,800,000 occurred in men and 5,000,000 in women. About 5,000,000 cases occurred in developed countries (North America, Japan, Europe including Russia, Australia and New Zealand) and 5,800,000 in developing countries. Among men, lung, prostate, stomach, colorectal, and liver cancers are the most common malignant neoplasms, while breast, cervical, colorectal, lung and stomach cancers are the

most common neoplasms among women. The number of deaths from cancer in 2002 was estimated at about 6,700,000 and that of 5-year prevalent cases at about 24,600,000.

Epidemiology has been instrumental to identify the causes of human cancer. In several cases, the epidemiological results preceded the elucidation of the underlying mechanisms. In other areas, however, epidemiological techniques are not sufficiently sensitive and specific to lead to conclusive evidence on the presence or absence of an increased risk. As for other branches of the discipline, the observational nature of epidemiology represents an opportunity for bias, including that generated by confounding, to generate spurious results. Techniques have been developed to prevent, control and assessment the presence and extent of bias in epidemiological studies.

Cancer epidemiology has lead to the identification of tobacco smoking and use of smokeless tobacco products, chronic infections, overweight, alcohol drinking and reproductive factors as major causes of human cancer. Other important causes include medical conditions, some drugs, perinatal factors, physical activity, occupational exposures and ultraviolet and ionizing radiation. A role of diet in cancer risk has been suggested, but for very few dietary factors there is conclusive evidence of an effect on cancer risk. With a few exception of little relevance in most populations, the role of pollutants on cancer is not established. Tobacco smoking is the main single cause of human cancer worldwide. It is a cause of cancers of the oral cavity, pharynx, esophagus, stomach, liver, pancreas, nasal cavity, larynx, lung, cervix, kidney and bladder, and of myeloid leukemia. The proportion of cancers in a population attributable to tobacco smoking depends on the distribution of the habit a few decades earlier. Therefore, in populations in which the tobacco epidemic has not fully matured (e.g., men in many low-income countries and women in most European countries), the full effect of tobacco smoking on cancer burden is not yet observed.

The notion that genetic susceptibility plays an important role in human cancer is old, and genetic epidemiology studies have characterized familial conditions entailing a very high risk of cancer have been identified, such as the Li-Fraumeni syndrome and the familial polyposis of the colon, and have identified high-risk cancer genes responsible for these syndromes. However, such high-risk conditions explain only a small fraction of the role of inherited susceptibility to cancer. The remaining fraction of genetic predisposition is likely explained by the combination of common variants in genes involved in one or more steps in the carcinogenic process, such as preservation of genomic integrity, repair of DNA damage. The identification of such low-penetrance susceptibility genes and of their interactions with exogenous factors (so-called ▶gene-environment interactions) represents a challenge to genetic epidemiology.

Advances in molecular biology and genetics offer new tools for epidemiological investigations, and have lead to the development of new methodological approaches, broadly defined as molecular epidemiology. The application of biomarkers to epidemiology has lead to advances in the identification of human carcinogens (e.g., the role of aflatoxin in liver cancer) and in the elucidation of mechanisms of carcinogenesis (e.g., *TP53* mutations in tobacco-related carcinogenesis).

Exposure to most known carcinogens – at least in theory – be avoided or reduced. This is true in particular for tobacco smoking and chronic infections, the two major known causes of cancer. Tobacco control measures have been implemented in most countries, and effective vaccination is today available against two of the main carcinogenic viruses, Hepatits B and Human Papilloma. Control of workplace exposure to known and suspected carcinogens in high-resource countries is another example of successful primary prevention of cancer. In many instances, however, primary prevention of cancer would require major changes in lifestyle, which are difficult to achieve.

Detection of preclinical neoplastic lesions before they have developed the full malignant phenotype, and notably the ability to metastasize is a highly appealing approach to control cancer. The effectiveness of screening has been demonstrated via epidemiological studies for cervical cancer (cytological smear), breast cancer (mammography) and colorectal cancer (colonoscopy). The development of effective strategies for the early detection of other neoplasms is an active area of research.

Cancer epidemiology exemplifies the strengths and the weaknesses of the discipline at large. Cancer epidemiology has the privilege of using complete and good quality disease registries available in many populations and covering a broad spectrum of rates and exposures. In many occasions, cancer epidemiology has been the key tool to demonstrate the causal role of important cancer risk factors. The best example is the association between tobacco smoking and lung cancer, which lead in the early 1960s to the establishment of criteria for causality in observational research. These findings have brought important regulatory and public health initiatives as well as lifestyle changes in many countries of the world. These epidemiological "discoveries" share two important characteristics: they involve potent carcinogens and methods are available to reduce misclassification of exposure to the risk factor of interest and to major possible confounders. It has been therefore possible to demonstrate consistently an association in different human populations. Note that it is not necessary for the prevalence of exposure to

be high (although this obviously has an impact on the population ►attributable risk): examples are the many occupational exposures and medical treatments for which conclusive evidence of carcinogenicity has been established on the basis of epidemiological studies conducted in small populations of individuals with well characterized exposure.

When these conditions are not met, however, the evidence accumulated from epidemiological studies is typically inconsistent and difficult to interpret. The history of cancer epidemiology presents many examples of premature conclusions, which have not been confirmed by subsequent investigations and have damaged the reputation of the discipline. Exposure misclassification, uncontrolled confounding, emphasis of positive findings generated by chance, and inadequate statistical power are the most common limitations encountered in epidemiological studies. Several solutions have been proposed to overcome these problems. First, epidemiological studies should be very large in size. This is achieved either by conducting multicentric studies including thousands of cases of cancer, or by performing pooled and meta-analyses of independently investigations. Second, as mentioned above, the use of biological markers of exposure and early effect might contribute to reduce exposure misclassification, increase the prevalence of the relevant outcomes and shed light on the underlying mechanisms. Finally, guidelines have been developed to improve and standardize the conduct are report of observational epidemiological studies.

Although relatively young, epidemiology has become a key component in cancer research. Most cancer centers have an epidemiological research group, and cancer is a major subject of research in most academic departments of epidemiology. Epidemiologists are more and more often invited to meetings of clinicians and basic researchers not only to provide an introduction to the distribution and the risk factors of a given cancer, but to participate in interdisciplinary discussions on clinical, preventive or mechanistic aspects of the disease. The strongest cancer epidemiology groups in the world are those combining different lines of expertise, from biostatistics to molecular biology and genetics to medical oncology. Despite its limitations, cancer epidemiology remains one of the most powerful tools at the disposal of the research community to combat cancer at all levels.

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Cancer Epigenetics

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Definition

►Epigenetics is defined as chromatin modifications that can alter gene expression, are heritable during cell division, but do not involve a change in DNA coding sequence.

Characteristics

In the context of normal biological processes, epigenetic mechanisms establish regions within the genome containing transcriptionally active (termed euchromatin) and silent (termed heterochromatin) DNA. Further, epigenetic mechanisms are responsible for stably inherited patterns of gene expression such as X chromosome inactivation and genomic imprinting (i.e., selective expression of maternal or paternal alleles). Chromatin modifications that alter gene expression are both changes to the methylation state of DNA and post-translational modifications to histone complexes.

It is well recognized that genetic mutations occur in cancer cells and that these events can exert profound and disease-associated changes in gene expression and/or function. However, it is becoming widely accepted that cancer cells also exhibit aberrant epigenetic alterations and that these changes can play a prominent role in disease initiation and progression. Epigenetic changes are potentially as important as genetic mutations in causing cancer since chromatin alterations can exert an influence regional gene expression, thereby changing the transcriptional profile of multiple genes. In this chapter we summarize the principal epigenetic alterations that occur in cancer cells: regional DNA hypermethylation and ►histone modifications, and global DNA hypomethylation.

DNA Hypermethylation

Chromatin structure is influenced by cytosine ►methylation, the only known naturally occurring base

modification in DNA. Cytosine methylation occurs at 5′-CG-3′ dinucleotides (referred to as CpGs) and is catalyzed by a class of enzymes termed ►DNA methyltransferases (DNMTs). Several DNMTs have been characterized in mammalian cells including DNMT1, DNMT3a and DNMT3b. These enzymes catalyze the transfer of a methyl group from S-adenosylmethionine (SAM) to the 5-carbon position of cytosine, forming 5-methycytosine. DNMT3a and DNMT3b appear to be principally involved in methylating previously unmodified cytosines (termed de novo methylation). In contrast, DNMT1 preferentially methylates hemimethylated DNA and is thus viewed as the DNA methyltransferase principally responsible for continuation of DNA methylation patterns in daughter cells (termed *maintenance* methylation).

From a statistical standpoint, the human genome is depleted in CpG dinucleotides; however, ~60% of genes in our genome are associated with regions ranging from 200 to 4,000 bases in length containing high density of CpG dinucleotides relative to the bulk genome. These regions are referred to as ►CpG islands and are usually located within upstream promoter regions or gene transcriptional start sites. In normal somatic cells, gene-associated CpG islands are usually unmethylated and associated with genes in a transcriptionally active euchromatic state. In cancer cells, hypermethylation of such CpG islands is strongly correlated with the transcriptional silencing of genes. Thus, through this epigenetic mechanism, tumor cells can dramatically down regulate expression of numerous genes, including ►tumor suppressor genes (TSGs). At present, numerous TSGs have been characterized as targets for epigenetic silencing through hypermethylation of associated CpG islands. Table 1 is a partial listing of characterized TSG whose promoter regions have been shown to be hypermethylated in various tumor types. Given the wide spectrum of tumor types that display epigenetic silencing of TSGs, mounting evidence clearly supports the assertion that epigenetic silencing is a prominent mechanism driving the process of tumorigenesis.

Cancer cells often over express DNMTs. Compared to normal tissues, the expression of DNMT1 is almost always increased in tumors. However, since DNMT1 expression is normally regulated during the cell cycle with increased abundance paralleling entry into S-phase, much of this increased expression may simply reflect increased cell proliferation within the tumor. Although demonstrable in model experiments, it remains unresolved if increased expression of DNMT1 is responsible for aberrant methylation in cancer cells. In contrast, increased expression of DNMT3a and DNMT3b observed in some tumors is likely significant, since these enzymes are normally expressed at low levels in somatic cells. However, it is still unclear to what extent over expression of these enzymes is responsible for cancer-associated DNA hypermethylation especially when one considers that cancer cells exhibit overall genome hypomethylation (discussed below). Thus, it remains unclear how CpG islands associated with specific TSG are targeted for hypermethylation during the process of tumorigenesis.

One mechanism by which DNA methylation can negatively impact gene expression is by simply blocking the binding of essential transcription factors to gene promoter sequences. While several examples of this are documented, it is also apparent that CpG methylation is also capable of directing transcriptional repression through promoting additional layers of chromatin alteration. Specifically, several proteins have been characterized that bind to methylated CpG dinucleotides and are capable of promoting further chromatin condensation and consequential transcriptional repression through recruitment of chromatin-modifying activities.

Cancer Epigenetics. Table 1 Genes subject to epigenetic silencing in cancer

Gene	Function	Tumor types
APC	Regulation of β-catenin, cell adhesion	Colorectal, gastrointestinal
BRCA1	DNA repair	Breast, ovarian
CDH1 (E-cadherin)	Homotypic epithelial cell–cell adhesion	Bladder, breast, colon, liver
MGMT	DNA repair	Brain, colorectal, lung, head and neck
MLH1	DNA repair	Colorectal, endometrial, ovarian
CDKN2A (p16)	Cell cycle control	Lung, brain, breast, colon, bladder, melanoma prostate
PTEN	Regulation of cell growth and apoptosis	Prostate, brain, endometrial, melanoma
VHL	Inhibits angiogenesis, regulates transcription	Renal cell carcinoma
ATM	DNA damage response	Breast, colorectal, head and neck

Histone Modification

Owing to technical considerations, DNA methylation is the most widely analyzed type of epigenetic alteration in human tumors. However, another extremely important epigenetic modification capable of altering gene expression during carcinogenesis involves various types of histone modifications.

The fundamental packing unit of chromatin within the nucleus is termed the ►nucleosome. A single nucleosome unit contains 146 base pairs of DNA wrapped around eight histone subunits (histone octamer). The histone octamer contains two copies each of histones H2A, H2B, H3 and H4. Structural studies have determined that each histone possesses an amino-terminal tail rich in the amino acid lysine. These lysine residues can undergo a variety of post-translational modifications including acetylation, methylation, phosphorylation, and ubiquitination. Such modifications are recognized by various proteins and protein complexes and combinations of histone modifications constitute a proposed histone code important in establishing a given gene's transcriptional profile.

Perhaps the best-studied histone modifications is acetylation of the ε-amino group of lysine residues within the amino-terminal tail of H3 and H4 although acetylation of both H2A and H2B occurs as well. This is a reversible modification that is carefully controlled by two large enzyme families: histone ►acetyltransferases (HATs) and ►histone deacetylases (HDACs). The net positive charges carried by these lysine residues are proposed to contribute to the high affinity of histones for negatively charged DNA. Acetylation of lysine residues by HATs neutralizes this positive charge thus decreasing histone/DNA interaction. This raises molecular access to DNA and promotes gene transcription. Conversely, HDACs promote transcriptional repression by supporting chromatin condensation into a heterochromatic conformation.

Several proteins that bind specifically to methylated CpG through a conserved methyl-binding domain (MBD) motifs have been discovered. The first such protein to be characterized, termed MeCP2, is capable of recruiting the co-repressor molecule mSin3 to the sites of methylated DNA. In turn, mSin3 binds to HDAC1 and HDAC2, thus promoting localized histone deacetylation. The importance of HDAC activity in transcriptional repression is underscored by the observation that repression of several gene promoters that can be partially relieved by HDAC inhibitors. A functionally similar complex termed MeCP1 binds to methylated DNA via an associated protein termed MBD2. The MeCP1 complex contains multiple subunits besides MBD2, including components of the NuRD complex, a characterized repressor complex containing both chromatin remodeling and HDAC activities.

In addition to acetylation, lysine residues within histone tails can be methylated and exist in either mono-, di-, or trimethylated states. Similar to the effects of histone acetylation/deacetylation, methylated lysine 4 of H3 (H3K4) is associated with transcriptionally active chromatin, while transcriptionally silent chromatin generally contains methylated lysine 9 of H3 (H3K9). H3K9 is methylated by a number of histone methyltransferases including ESET, Eu-HMTase, G9a, and the closely related methyltransferases SUV39-H1 and SUV39-H2. Histone methylation is likely a dynamic process since a histone demethylase, termed LSD1, was recently characterized. Methylated H3K9 binds to the chromodomain protein heterochromatin protein 1 (HP1) which promotes heterochromatin formation and gene silencing. Moreover, since H3K9 methylation cannot occur when this position is acetylated, it is clear that H3K9 acetylation and methylation represent opposing forces in determining chromatin conformation.

In a broad view, it is reasonable to propose that CpG methylation and histone acetylation/deacetylation act synergistically in the progressive silencing of genes. One model that accounts for tumor-suppressor gene silencing by epigenetic mechanisms invoke abnormal hypermethylation of the promoter CpG island followed by recruitment of MBD proteins, including complexes such as MeCP2 and MeCP1 that recruit HDACs to the area of hypermethylation and promote further transcriptional repression through histone modification. An alternate model proposes that transcriptionally repressive histone modifications are the first event in gene silencing and subsequently promote CpG methylation resulting in further transcriptional repression. Experimental evidence supports both of these models and may be reflective of the variety of gene promoters and model systems used for study. Less equivocal is the fact that DNA methylation appears the dominant silencing mechanism since inhibition of DNA methylation generally restores gene expression while HDAC inhibitors generally exert more modest effects on gene silencing.

DNA Hypomethylation

While CpG islands associated with gene promoters are generally unmethylated in normal adult somatic cells, the majority of CpG dinucleotides elsewhere in the genome are generally methylated. Moreover, despite the fact that many CpG islands are subject to hypermethylation in cancer cells, it is equally well documented that tumor cells display an overall loss of methylated cytosines compared to normal tissue. This tumor-associated global DNA hypomethylation predominantly occurs in repetitive DNA sequences within the human genome although the molecular mechanisms responsible for this loss of DNA methylation are poorly understood.

Recent work on a human genetic disorder has underscored an important role for DNA methylation in maintenance of genome stability. The immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome is a rare autosomal recessive disease characterized by germline mutation of the DNMT3B gene. Loss of DNMT3B activity in ICF leads to hypomethylation of repetitive satellite DNA sequences within heterochromatin adjacent to the centromeric region of chromosomes. The loss of methylation is most prominent within the pericentric regions of human chromosomes 1 and 16 and leads to multiple chromosomal abnormalities including chromatin decondensation, chromosomal translocations and deletion, and multiradial chromosomal structures. These observations, as well as those made on cells with engineered disruption of DNMTs, clearly support the view that DNA methylation is critical in maintaining normal chromosome structure. Since cancer cells often show chromosomal rearrangements, it is likely that cancer-associated DNA hypomethylation allows for heightened rates of chromosomal instability.

Retrotransposon sequences of the LINE (long interspersed nuclear element) and SINE (short interspersed nuclear element) classes as well as human endogenous retroviruses (HERVs) are major targets of tumor-associated DNA hypomethylation. Mobility of these DNA elements is kept in check in normal tissues owing, in part, to dense methylation of CpG dinucleotides within their genomic structure. It follows that increased mobility of these dormant mobile elements occurs as a result of cancer-associated DNA hypomethylation and there have been reports of retrotransposition-like insertions involving LINE-1 sequences in tumors although retrotransposition of endogenous elements seemingly occurs more often in rodents than humans.

In addition to the hypomethylation of CpG dinucleotides present within repetitive DNA elements, cancer-associated hypomethylation also occurs in regions of the genome encoding single-copy genes. Dysregulation of allele-specific methylation will result in the loss of imprinting (LOI) and allow for both maternal and paternal gene expression. Perhaps the best-studied example of this is the insulin-like growth factor 2 (IGF2) gene where LOI occurs in primary tumors and in patients with the inherited, cancer-prone ►Beck-with-Wiedemann. While not as well-studied as gene silencing due to DNA hypermethylation, it is likely that additional examples of cancer-promoting increased gene expression stemming from DNA hypomethylation will be uncovered in the future.

Epigenetic Alterations as Targets for Diagnosis and Therapeutic Intervention

Sequencing data obtained from the human genome project are currently undergoing analysis to construct a human epigenetic map based on CpG content. This knowledge coupled with cross-species comparisons of the epigenome will be invaluable in deciphering the epigenetic elements involved in gene regulation. Epigenetic alterations typically occur early during the oncogenic process, and detection of such early abnormalities may aid in early diagnosis and/or preventing cancer progression through dietary alterations or pharmacological intervention. With increasing awareness of the importance of epigenetics in tumorigenesis, and the advent of sensitive laboratory approaches to analyze epigenetic alterations, it is likely that epigenetic profiles will ultimately be used in the clinical setting to provide information useful in predicting an individual's predisposition to cancer, assisting in tumor staging, and guiding optimal therapeutic approaches.

A promising feature of alterations in DNA methylation patterns and chromatin structure in cancer cells is their potential for reversibility, because these modifications occur without changing the primary nucleotide sequence. At present, two major pharmacological targets associated with these epigenetic changes are DNMTs and HDACs. The DNMT inhibitor 5-aza-deoxycytidine (5-azadC) and related compounds cause transcriptional reactivation of endogenous genes with hypermethylated promoters. This drug, also termed Decitabine, is currently used to treat certain types of hematological malignancies, especially advanced ►myelodysplastic syndromes (MDS). HDAC inhibitors, such as trichostatin A and sodium butyrate, have been shown to increase the level of histone acetylation in cultured cells, and to cause growth arrest, differentiation and apoptosis. Based on these observations, the potent HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) is in clinical trials.

Cancer Genome Project

Definition

The Cancer Genome Project (CGP) was founded at the Wellcome Trust Sanger Institute in 2000 and aims to establish an unbiased catalogue of mutations involved in human tumorigenesis by complete sequencing of candidate genes. By sequencing the human *BRAF* gene, the CGP discovered a hitherto unknown mechanism of oncogenic activation of B-Raf that is not only found in 7% of human cancers, but also provided more insight into the complex process of B-Raf activation.

►B-Raf Somatic Alterations

Cancer Germline Antigens

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Synonyms

Cancer-testis (CT) antigens; CG antigens

Definition

CG ►antigens (Cancer-testis (CT) antigens) are a class of immunogenic tumor antigens encoded by genes expressed in ►gametogenic cells of the testis and/or ovary and in human cancer.

Characteristics

Identification

The main criterion for classification of a gene as a CG antigen pertains to its expression pattern in gametogenic, somatic, and tumor tissues. A gene is generally considered to be a CG antigen if it is expressed in the gametogenic cells of the testis or ovary (including fetal ovary) and in some proportion of human cancers, but is expressed in two or fewer normal somatic tissues. CG antigen genes are also commonly expressed in ►trophoblast tissue. CG antigens were originally identified from searches for auto-antigens expressed in human cancer. The original method for antigen screening used ►autologous typing, in which ►T-cells (T-lymphocyte) from a ►melanoma patient were screened for reactivity with tumor cells from the same patient; this method led to the identification of MAGE-A (named for ►melanoma antigen)/CT1 genes. In later studies, another immunological assay was developed to identify tumor antigens and this method, ►SEREX (Serological analysis of recombinant cDNA expression libraries), was used to successfully identify a variety of important CG antigen genes, including NY-ESO-1/CT6 and SSX/CT5. Recognition of the unique expression pattern of CG antigen genes has led to the use of gene expression analyses (including EST or SAGE database searching) to identify CG antigen genes. This method has led to the identification of additional CG genes, including XAGE-1/CT12 and SCP-1/CT8. Although formally classified as CG antigen genes, genes identified by the latter non-immunological method may not be antigenic in cancer patients.

Nomenclature

A nomenclature system for CG antigens has been devised, which is based on their chronology of discovery, and also accounts for the numerous family members that exist for certain CG antigens. In this system, CG antigen genes are referred to by their original given names and also are assigned a separate CT identifier or CT#. Currently, over 40 CG antigen gene families are recognized, comprising more than 89 distinct mRNA transcripts. CG antigen genes have been assigned into two groups on the basis of chromosomal localization. ►CG-X antigens: These genes reside on the X-chromosome where, interestingly, close to 10% of the total number of genes encode CG antigens. CG-X genes are typically members of large multigene families, e.g. MAGE-A/CT1, MAGE-B/CT3, and MAGE-C/CT7. In normal tissues, CG-X genes are often expressed in pre-meiotic spermatocytes in the testis. All of the current important targets of CG antigen ►cancer vaccines are members of this group, including MAGE-A1/CT1.1, MAGE-A3/CT1.3, and NY-ESO-1/CT6.1. ►Non-X CG antigens: A number of CG antigen genes are located on autosomal chromosomes. Unlike CG-X genes, these genes are highly dispersed in the genome and do not exist in multigene families. In normal tissues, non-X CG genes are often expressed during meiosis, where some members play roles in DNA recombination, including SCP-1/CT8 and SPO11/CT35. The members of this gene group do not include any currently validated cancer antigens, although certain members are expressed at high levels in cancer.

Regulation of Expression

Certain cancer types appear to expresses CG antigen genes frequently, while others rarely express them. Tumor types that frequently express CG antigens include melanoma, lung, ovary, and ►bladder cancer; tumors that rarely express CG antigens include ►colon cancer, renal cancer, and leukemia/lymphoma. CG antigen genes show coordinate expression in human cancer. That is, the great majority of tumors either do not express CG antigen genes or express two or more CG antigen genes simultaneously, while relatively few tumors express only one CG antigen gene. Another characteristic of CG antigen gene expression in cancer (revealed by immunohistochemical staining) is that tumors that express CG antigens show heterogeneous expression within the tumor: often only focal staining is observed. The coordinate but heterogeneous expression of CG antigens in cancer has led to the intriguing hypothesis that CG antigen expression is indicative of the activation of a normally dormant gametogenic program in tumor cells (possibly corresponding to tumor stem cells).

The observation of coordinate expression of CG antigen genes suggests that CG antigen gene activation may be controlled by a common molecular mechanism. Supporting this idea, a number of studies

have suggested a key role for ►DNA methylation in regulating CG antigen gene expression. Promoter DNA hypermethylation has been observed to correlate with CG antigen gene repression in normal tissues and non-expressing tumors, while treatment of tumor cell lines *in vitro* with DNA methyltransferase inhibitors such as ►5-aza-2′-deoxycytidine (DAC) leads to CG antigen gene activation, coincident with promoter DNA ►hypomethylation. Conversely, tumor cell lines and tissues that endogenously express CG antigen genes often display promoter DNA hypomethylation. Many CG antigen genes have CpG-rich promoter regions that serve as targets for regulation by DNA methylation. Other studies have shown that ►histone deacetylase (HDAC) inhibitors can either augment DAC-mediated CG antigen gene activation or can activate CG antigen genes on their own. As DNA ►methylation and ►chromatin structure (in the form of histone modification status) are intimately linked, it is not surprising that both of these ►epigenetic mechanisms serve as important regulators of CG antigen gene expression. Consistent with the model that epigenetic mechanisms regulate CG antigen gene expression is the observation that DNA hypomethylation occurs during gametogenesis, which is the normal setting for CG antigen gene expression.

Function

CG antigens are a rare group of genes in that clinical studies designed to target these antigens for ►immunotherapy of cancer are more advanced than is our basic knowledge of the function of the gene products. However, some information about CG antigen gene function has recently come to light. As mentioned earlier, many non-X CG antigens have roles in germ cell maturation, including mediating the structure of synaptonemal complexes (SCP1/CT8), facilitating DNA recombination during meiosis (SPO11/CT35), and contributing to spermatid function (ADAM2/CT15, OY-TES-1/CT23). In tumors, the function of CG antigen genes is less clear, but recent studies of the MAGE-type antigens, which share a region referred to as the MAGE homology domain (MHD), indicate that these proteins might serve as transcriptional repressors via interactions with other transcriptional regulatory proteins that themselves recruit co-repressors such as HDACs. CG antigen genes have also been reported to play a role in the evolution of ►chemotherapy resistance in cancer cell lines, suggesting that the CG antigen gene products could serve as viable targets for anticancer therapy. An recent report appears to link these two observations by showing that MAGE-A2/CT1.2 disrupts ►p53 function by recruiting HDAC3 to p53, leading to chemotherapy resistance in cancer cells.

Clinical Studies

The identification of CG antigens as tumor-specific antigens has led to a great deal of interest in treating cancer by targeting CG antigens via vaccine-based immunotherapy. In particular, MAGE-A1/CT1.1, MAGE-A3/CT1.3, and NY-ESO-1/CT6.1 have been developed as targets for this approach. In early studies, the antigenic peptides from CG antigens that elicited ►T-cell dependent responses were mapped, and these peptides were utilized for vaccination. Because responses to peptide-based vaccine formulations are limited by patient ►HLA type, more recent vaccination approaches targeting CG antigens have utilized full-length ►recombinant proteins. These recombinant proteins can be introduced using viral vectors, including vaccinia and fowlpox viruses. Alternatively, recombinant CG antigen proteins can be assembled with ►adjuvants such as ISOMATRIX, which further enhances immune responses. A common finding in CG antigen vaccine clinical studies is that the treatment is safe and elicits both ►antibody and T-cell mediated immune responses *in vivo*. In particular, NY-ESO-1/CT6.1 vaccine trials have shown encouraging results, with durable and multifaceted immune responses, as well as suggestive data indicating clinical benefit, in terms of disease stabilization and prolonged time to recurrence. Many of the patients targeted in these clinical trials have had malignant melanoma, and a proportion of these patients displayed evidence of immune recognition to the target antigen prior to vaccine therapy. In virtually all cases, patients have been selected for inclusion in CG antigen vaccine trials based on the expression of the antigenic target in tumor biopsies. To expand the patient population that would benefit from this immunotherapy approach, a number of investigators have proposed using DNA methyltransferase and/or HDAC inhibitors (which are FDA approved and known to augment CG antigen gene expression) in combination with CG antigen directed vaccines. The potential benefit of this multi-modality approach awaits clinical testing.

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Cancer of the Large Intestine

►Colon Cancer

Cancer-mediated Bone Loss

►Bone Loss, Cancer Mediated

Cancer Networks

Definition

Local management teams, responsible for population areas of about 500,000, whose role is to co-ordinate cancer service within the National Health Service in England and Wales.

►National Institute for Health and Clinical Excellence (NICE)

Cancer-Prone Genetically Modified Mouse Models

Definition

Transgenic or knockout mice prone to cancer development as a consequence of ►oncogene activation or ►tumor suppressor gene inactivation.

►Oncomouse
►Immunoprevention of Cancer

Cancer Registry

Definition

System of on-going registration of all newly diagnosed cases of cancer in a given population. The files of cancer registries typically contain demographic and clinical characteristics.

►Cancer Epidemiology

Cancer Stem Cells

Definition

A minor population of cells within a tumor that is thought to be necessary for tumor growth and propagation. Like all stem cells, cancer stem cells are characterized by their capacity for self renewal and unlimited replication.

►Stem Cells and Cancer

Cancer Stem-Like Cells

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Synonyms

Tumor initiating cells

Definition

Cancer stem(-like) cells are those cells that possess the capacity for self-renewal and for causing the heterogeneous lineages of cancer cells that comprise the tumor.

Characteristics

The definition follows a consensus at a workshop on cancer stem(-like) cells (CSC) organized by the American Association for Cancer Research (AACR). There is considerable debate and some controversy on the CSC concept, so that a consensus definition is required. The importance of the debate is proportional to its relevance to the change in our perception of cancer, intirinsic to the CSC paradigm, implying that not all cancer cells are equal but that only a small fraction of them is endowed with the properties of perpetuating the disease. This hierarchical model has not only important biological consequences but also relevant therapeutic implications, as we discuss in this essay.

The CSC paradigm fits in a model of cancer as a caricature of an organ that is already present in the literature as suggested by data published 30 to 40 years ago. In particular, Hamburger and Salmon established growth conditions for cancer cells in soft agar medium and found that tumor stem cell colonies, arising from different types of cancer with 0.001–0.1% efficiency, had differing growth characteristics and colony morphology. Studies by Dick and co-workers in the 1990s showed that in several forms of acute myeloid leukemia (AML) cells that could engraft in immunodeficient mice are restricted to a minority subpopulation defined as [$CD34^{+}/CD38^{neg}$]: these cells, therefore, shared a cell surface phenotype with normal human primitive hematopoietic progenitors, suggesting that they may have originated from normal stem cells rather than from committed progenitors. Also of interest was the observation that leukemic cells engrafted in NOD-SCID mice (non obese diabetic-severe combined immunodeficiency: an immunodeficient mouse strain characterized by lack of B, T and NK lymphocytes) showed similar phenotypic heterogeneity to the original donor: thus, [$CD34^{+}$, $CD38^{neg}$] retain the differentiating capacity necessary to give rise to $CD38^{+}$ and Lin^{+} cells (lineage positive).

The presence of CSC has also been demonstrated in chronic myeloid leukemia (CML). This disease has a chronic phase and a terminal stage; the blast crisis and molecular events underlying this evolution are not completely understood. In the chronic phase, the chromosomal translocation t(9:22)BCR-ABL, a diagnostic marker of CML, can be detected in most circulating mature lineages. In the blast crisis, however, highly undifferentiated BCR-ABL^{+} cells accumulate in the blood. In particular, an expansion of granulocyte-macrophage progenitors (GMP) is present in blast cells, showing aberrant acquisition of self-renewal properties and nuclear expression (i.e. activation) of beta-catenin, a key, positive regulator of stem cell self-renewal. These observations imply that during progression of CML the GMP subfraction of leukemic progenitors acquire stem cell characteristics. Thus, the functional hierarchy of CSC can be modified during the natural history of this tumor as a result of its progression.

The requirement for a periodical renovation is not only present in blood but also in the skin and epithelia of the respiratory, gastrointestinal, reproductive and genito-urinary systems. Other tissues like brain, previously considered as exclusively post-mitotic, contain stem cells that can be mobilized and activated under conditions of stress, such as hypoxia. Thus the CSC model could also be applied to solid tumors, and a series of recent papers report data supporting the identification of a stem cell population in different cancers (see Table 1). Initial data were gained in breast cancer where a small population of cells with a $CD44^{+}$ /$CD24^{neg\text{-}low}$ phenotype appears exclusively capable of tumor initiation.

The most malignant of brain tumors, glioblastoma multiforme (GBM), was also found to contain a fraction of neoplastic cells identified and selected on the basis of CD133 expression. Not only could $CD133^{+}$ cells self-renew and differentiate into different neural lineages but also, in vivo, only the $CD133^{+}$ cells were able to re-initiate malignant gliomas with phenotype similar to the original tumor.

The CSC paradigm may also help to explain intra-tumor heterogeneity, a frequent finding in most cancers: heterogeneity could be consequent to functional diversity of cells at different states of differentiation. On the other hand, the patterns of tumor heterogeneity and gene expression profiles can be highly similar in the original tumor and in distant metastasis.

It is rather obvious that the existence of cancer stem cells may provide novel therapeutic targets of increased effectiveness in contrasting or even eliminating cancer. Brain tumors have provided a highly fertile ground to start verifying this hypothesis, as outlined in Table 2. Data are piling up indicating that $CD133^{+}$ GBM CSC are highly pro-angiogenic, because of the high levels of VEGF expression, and have greater resistance to chemotherapy and radiotherapy. As a consequence,

Cancer Stem-Like Cells. Table 1

Tumor	Markers	Reference
Acute Myeloid Leukemia	$CD34^{+}$/CD38neg	Bonnet and Dick 1997
Breast cancer	$CD44^{+}$/CD24-/neg	Al-Hajj et al 2003
Glioblastoma	$CD133^{+}$	Singh et al 2003
Myeloma	CD138neg	Matsui et al 2004
Prostate cancer	$CD44^{+}$/alpha2beta1 integrin high/ $CD133^{+}$	Collins et al 2005
Melanoma	CD20+	Fang et al 2005
Lung cancer	Sca-1^{+}/ CD45neg/ Pecam neg/CD34pos	Kim et al 2005
Colon cancer	$CD133^{+}$	O'Brien et al 2007, Ricci-Vitiani et al 2007
Pancreatic cancer	$CD44^{+}$/$CD24^{+}$/ESA (epithelial specific antigen)$^{+}$	Li et al 2007

Cancer Stem-Like Cells. Table 2

Pathway/mechanism in CSC	Potential treatment	Reference
Angiogenesis-Increased production of VEGF	Bevacizumab	Bao et al Cancer Res 2006
Increased resistance to radiation	Chk1 and Chk2 inhibitor	Bao et al Nature 2006
Specific patterns of expression	Dendritic cell targeting	Pellegatta et al 2006
Cell cycle deregulation	Bone Morphogenetic Protein 4	Piccirillo et al 2006
Resistance to chemotherapy	BCRP1/MGMT inhibition (?)	Liu et al 2006

specific therapeutic strategies can be attempted and combined to overcome CSC. Upon radiotherapy $CD133^{+}$ GBM CSC activate checkpoint kinases 1 and 2 and repair mechanisms more effectively than $CD133^{neg}$ cells. Resistance to chemotherapy can be linked to an intriguing aspect of the CSC phenotype, the side population (SP) phenotype. SP cells have the ability to extrude the DNA binding dye Hoechst 33342 via the drug transporter BCRP1/ABCG2. Interestingly, the BCRP1/ABCG2 pump can also effectively extrude chemotherapeutic drugs such as mitoxantrone.

Also related, although of less immediate relevance in the clinical setting, are the observations reported by Pellegatta et al. using glioma neurospheres as a target for dendritic cell (DC, the most potent of antigen presenting cells) immunotherapy. Normal neural stem cells may grow as neurospheres (NS) in the absence of serum and in the presence of two critical growth factors, EGF and bFGF. NS are enriched in neural stem cells but also contain partially committed progenitors as well as a differentiated progeny. Oncospheres with similar characteristics were obtained from GBM but also from other solid tumors like breast or colon carcinomas. Pellegatta et al set up a murine model showing that DC loaded with GBM NS are much more effective in protecting mice against the GBM challenge than DC loaded with GBM cells where CSC are poorly represented. Thus, CSC targeting by immunotherapy is feasible and highly effective, opening new scenarios for clinical immunotherapy and supporting the idea that CSC are at the heart of malignant growth. Also of interest is the observation by Piccirillo et al. that treating GBM CSC with the differentiating factor BMP4 can block growth in vitro and avoid tumor formation in the majority of mice in vivo.

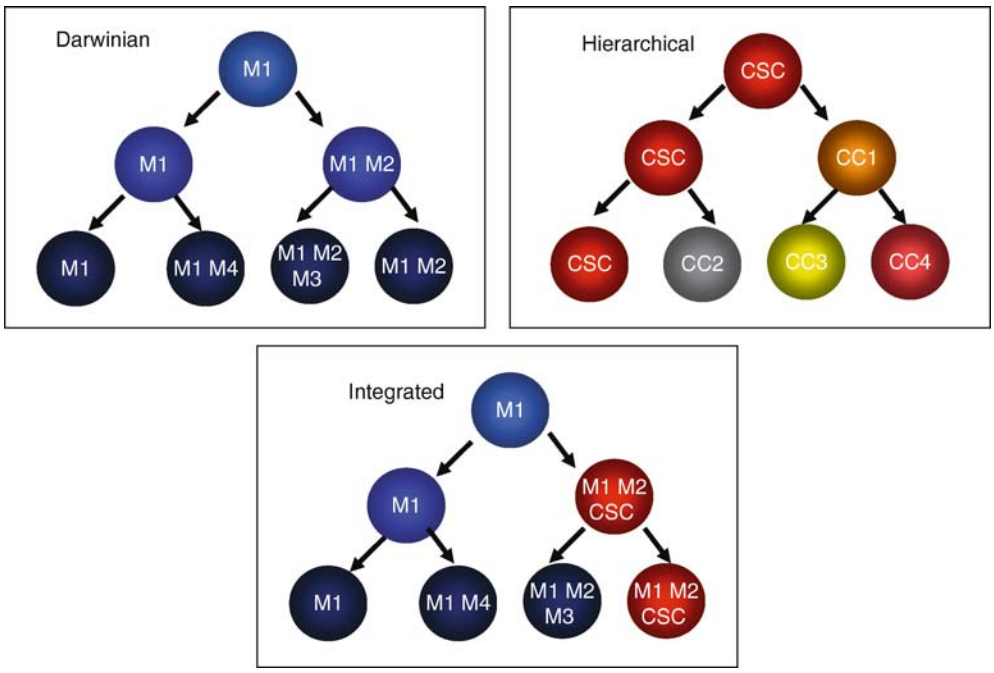
Given the increasing number of observations supporting the CSC paradigm in different tumors, it is expected that more therapeutically relevant observations will be proposed in the near future.

Together with therapeutic and clinical implications the CSC concept seems to have important consequences for our understanding of tumor biology. Modern genetics and molecular biology have given a definition of cancer as a genetic disease in which a growing burden of mutations leads to a progressively more aggressive and ultimately lethal phenotype (Fig. 1). A Darwinian selection for these mutations, privileging those that can confer resistance to different challenges, like hypoxic stress or immune attack, appears to be the most plausible rationale for making sense of this evolutionary catastrophe. The hierarchical, CSC model seems to introduce an element of rigidity in this highly flexible scenario, implying that only cells endowed with stem cell properties can afford tumor perpetuation (Fig. 1). Are these two models different or are they compatible? A convincing answer to this tough question will undoubtedly require a lot of robust science in the time to come but comments can be given on the basis of data that are already available. One important issue that the CSC model addresses is that of the cell of origin for cancer(s): stem cells, because they are long-lived and self-renewing, are excellent candidates to play the "cell of origin" role. A stem cell hosting a critical mutation could be quiescent for years and then be engaged in a repair response requiring mobilization and proliferation. For example, hypoxic stress may activate the CXCR4 pathway that not only attracts stem cells but may also favour their proliferation, thus being the spark initiating the cancer fire. However, an initiating mutation could also arise in a more committed progenitor (see the integrated model in Fig. 1): acquisition of a stem-like phenotype could in this context be the consequence of environmental challenges; in vivo, for instance, hypoxia could play an important role in de-differentiation; in vitro, the modification of growth factors could have similar consequences. Epigenetic changes could play important roles in mediating rapid and genome-wide changes that can substitute for genetic mutations and lead to de-differentiation.

In the *Darwinian model* different mutations (M1 through M4) accumulate during evolution and confer heterogeneity.

In the *Hierachical model* tumor arises in a stem cell, thus becoming a cancer stem cell (CSC): heterogeneity is conferred by asymmetrical divisions creating different types of cancer cells (CC1 through 4).

In the *Integrated model* a first mutation (M1) can arise in a progenitor or even a committed cell. During progression, though, external stimuli may give rise



Cancer Stem-Like Cells. Figure 1 Biological models for tumor evolution.

to a cancer stem cell that through asymmetric division will create other CSC as well as more differentiated tumor cells.

►Stem-Like Cancer Cells

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Cancer Testis Antigens

Definition

Cancer Testis Antigens are genes that can be defined by predominant expression in various types of cancer and undetectable expression in normal tissues except germ cell.

►BORIS

Cancer (or Tumor) Stroma

►Tumor Microenvironment

Cancer Vaccines

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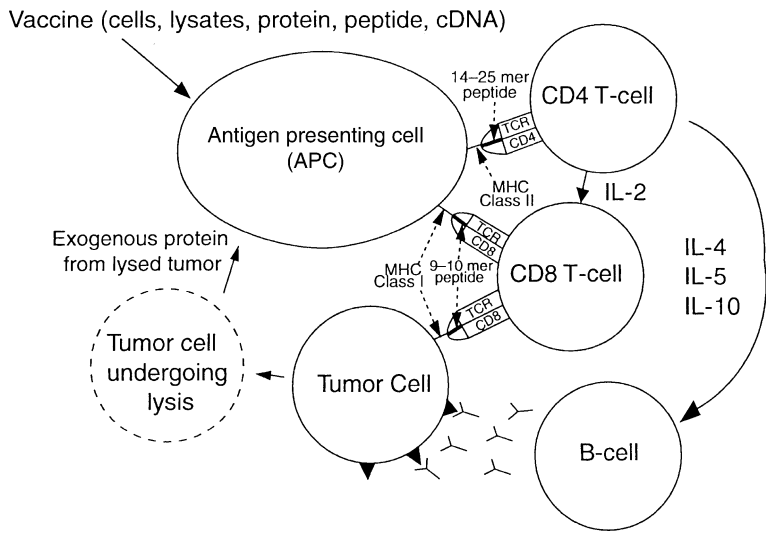
Definition

A vaccine should activate a unique lymphocyte (B and/or T cell) response, which has an immediate anti-tumor



Cancer Vaccines. Figure 1 T-cell activation. T-cells recognize antigens as fragments of proteins (peptides) presented with major histocompatibility complex (MHC) molecules on the surface of cells. The antigen presenting cell processes exogenous protein from the vaccine or from the lysed tumor cell in to a peptide, and presents the 14/25 mer peptide to CD4 helper-T-cells on a class II molecule. There is also data that suggests that exogenous proteins can be processed into 9/10 mer peptides that may be presented on MHC class I molecules to CD8 cytotoxic T-cells. Activated Th1 CD4 helper T-cells secrete Th1 cytokines such as IL-2 that upregulate CD8 cytotoxic T-cells. Activated Th2 CD4 helper T-cells secrete Th2 cytokines such as IL-4, IL-5 and IL-10 that activate B cells.

effect as well as memory response against future tumor challenge (Fig. 1). The primary role of a cancer vaccine is the treatment of cancer or in prevention of recurrence in a patient with surgically resected cancer, rather than "prevention" of cancer in a person who has never had cancer. Therefore, cancer vaccines are not thought of in the traditional sense of vaccines that are used for infectious diseases. If the current cancer vaccines prove to be useful in the above respects, then they may have a future role in preventing cancer in persons who have never had cancer but are at high-risk for a particular type of cancer.

Characteristics

The first and most obvious types of vaccines are prepared from autologous or allogeneic tumor cells. Alternatively, membrane preparations from tumor cells may be used. In some instances, tumor cell vaccines have been combined with cytokines such as granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-2 (IL-2). More recently, with advances in molecular biological approaches, gene modified-tumor cells expressing antigens designed to increase the immune response, or gene modified to secrete cytokines have been an additional tool used in vaccination. In addition, increase in our knowledge of tumor associated antigens (TAA) have led to the use of purified TAAs, DNA-encoding protein antigens, and/or protein derived peptides. All of these approaches are currently being tested in the clinic.

Mechanistically, the ultimate aim of a vaccine is to activate a component of the immune system such as B lymphocytes, which produce antibodies or T lymphocytes, which directly kill tumor cells. Antibodies must recognize antigens in the native protein state on the cell's surface. Once bound, these molecules can mediate antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity, both mechanisms which are capable of destroying tumor cells. T lymphocytes, on the other hand, recognize proteins as fragments or peptides that vary in size, presented in the context of major histocompatibility (MHC) antigens on the surface of the cells recognized (Fig. 1). The proteins from which the peptides are derived may be cell surface or cytoplasmic proteins. MHC antigens are highly polymorphic, and different alleles have distinct peptide binding capabilities. The sequencing of peptides derived from MHC molecules have led to the discovery of allele-specific motifs that correspond to anchor residues that fit into specific pockets on MHC class I or II molecules.

T Lymphocytes

There are two types of T lymphocytes, helper T lymphocytes and cytotoxic T lymphocytes (CTLs) that recognize antigens through a specific T cell receptor (TCR) in close conjunction to the CD3 molecules, which is responsible for signaling. CD4 helper T cells recognize antigens in association with class II MHC gene products, and CD8 positive CTLs recognize

antigens in association with class I MHC gene products. CD4 helper T cells are activated by binding via their TCR to class II molecules that contain 14–25 amino acid peptides in their antigen-binding cleft. Specialized antigen presenting cells (APCs), such as dendritic cells (DCs), macrophages, and B lymphocytes, capture extracellular protein antigens, internalize and process them, and display class II-associated peptides to CD4 helper T cells. The CD8 positive CTLs are activated by binding via their TCR to class I molecules that contain 9–10 amino acid peptides in their antigen-binding cleft. All nucleated cells can present class I-associated peptides, derived from cytosolic proteins such as viral and tumor antigens, to CD8 positive T cells.

There are two types of CD4 helper T cells capable of generating either antibody or cell-mediated immune responses, based on the type of signaling they receive. Th1 CD4 helper T cells stimulate cell-mediated immunity by activating CTLs through the release of cytokines such as IL-2. Th2 CD4 helper T cells mediate an antibody response through the release of cytokines such as IL-4 and IL-10.

Tumor Cells

The most straightforward means of immunization is the use of whole tumor cell preparations (either autologous or allogeneic tumor cells). The advantage of this approach is that the potential TAAs are presented to the immune system for processing and presentation to the appropriate T cell precursors. The difficulty with this approach lies in the availability of fresh autologous tumor material and the sparcity of well-characterized long-term tumor cell lines. Regardless, whole tumor cell vaccines have been an area of intense interest. A variety of trials using autologous tumors for colon cancer and malignant melanoma have been reported. In one trial, freshly thawed autologous colon cancer cells were inactivated with radiation, mixed with ►BCG (bacille Calmette-Guerin) and injected into patients who had their primary colon cancer resected but were at risk for recurrence. This study did reveal disease-free survival and overall survival trends in favor of the vaccine arm. In a melanoma study, autologous tumor cells were mixed with dinitrophenyl (DNP) and mixed with BCG. Promising results were reported for patients with metastatic disease and for patients with locally resected melanoma.

The weakness of autologous cell vaccines can be overcome with the allogeneic approach: First an allogeneic vaccine is generic and developed from cell lines selected to provide multiple TAAs and a broad range of HLA expression. Second, allogeneic cells are more immunogeneic than autologous cells. Third there is no requirement to obtain tumor tissue by surgical resection for a prolonged course of immunotherapy.

A polyvalent melanoma cell vaccine called CancerVax developed for allogeneic viable melanoma cell lines has demonstrated promising results for patients with resected metastatic disease and for resected local disease. Randomized phase III studies are ongoing in the United States comparing CancerVax plus BCG versus BCG for patients with stage III melanoma.

Another variation of cell vaccines is using "shed" antigen vaccines. These are vaccines that are prepared from the material shed by viable tumor cells into culture medium. The potential advantage is that it contains a broad range of antigens expressed on the surface of melanoma cells and the shed antigens are partially purified. Trials of such vaccines in melanoma patients have demonstrated specific humoral and cellular immune responses in patients and promising early clinical results.

Another approach to tumor cell vaccines is the introduction of foreign genes encoding cytokines such as IL-2 and GM-CSF into tumor cells. Alternatively, molecules designed to increase the immunogenicity of the tumor cell such as CD80 and CD86. Gene transfer can be accomplished by transfection of plasmid constructs (electroporation) or transduction using a viral vehicle such as a retrovirus or an adenovirus. Another option tested for gene transfer is physical gene delivery in which a plasmid or "naked" DNA is delivered directly into tumor cells. There are a number of mechanisms to carry this out including liposomes as gene carriers, use of a "gene gun," electroporation and calcium phosphate-mediated gene transfer. In one phase I trial, 21 patients with metastatic melanoma were vaccinated with irradiated autologous melanoma cells engineered to secrete human GM-CSF. Metastatic lesions resected after vaccination were densely infiltrated with T lymphocytes and plasma cells and showed extensive tumor destruction.

Peptides and Carbohydrates

An advantage to peptide vaccines is that they can be synthetically generated in a reproducible fashion. The major disadvantage is that they are restricted to a single HLA molecule and are not of themselves very immunogenic. To increase their immunogenicity, peptides may be injected with adjuvants, cytokines or liposomes or presented on DCs. Whole proteins have the advantage over peptides in that they can be processed for a wider range of MHC class I and II antigens.

Mucins such as MUC I are heavily glycosylated high molecular weight proteins abundantly expressed on human cancers of epithelial origin. The MUC I gene is over-expressed and aberrantly glycosylated in a variety of cancers including colorectal cancer. MUC 1 is being widely used as a focus for vaccine development.

Using expression-cloning techniques, several groups have cloned the genes encoding melanoma antigens

recognized by T cells and have identified the immunogenic epitopes presented on HLA molecules. Ten different melanoma antigens have been identified. Direct immunization using the immunodominant peptides from the tumor antigens or recombinant viruses such as adenovirus, fowlpox and vaccinia virus encoding the relevant genes have been pursued to immunize patients with advanced melanoma. Initial results have demonstrated increased anti-tumor T cell reactivity in patients receiving peptide immunization. Immunization in melanoma patients with melanoma antigens have been reported. One study showed that immunization of melanoma patients with MAGE-1 peptide pulsed on DCs induced melanoma-reactive and peptide specific CTL responses at the vaccination sites and at distant tumor deposits. Administration of the gp-100 molecule in conjunction with high-dose bolus IL-2 to 31 patients with metastatic melanoma revealed an objective response of 42%. This is compared with the typical response of high-dose systemic IL-2 without peptide of only 15%. Based on these data, a randomized trial was initiated to compare the peptide vaccine plus IL-2 versus IL-2 alone in metastatic melanoma patients.

Immunization against tumor-associated carbohydrate antigens has also been attempted. Carbohydrate antigens typically bypass T cell help for B cell activation. Investigators demonstrated that some carbohydrates may activate an alternative T cell pathway. Vaccine studies have been reported using the GM-2 ganglioside vaccine. Patients were pretreated with low dose cyclophosphamide. After a minimum follow up of 72 months, there was a 23% increase in disease-free interval and a 17% increase in overall survival in patients who produced antibody against GM-2. This suggested a benefit to the GM-2 ganglioside vaccine which has led to a current phase III trial.

Recombinant Vaccines Expressing Tumor Antigens

The carcinoembryonic antigen (CEA) is highly expressed on colorectal cancer and on a variety of other epithelial tumors, and is thought to be involved in cell-cell interactions. A recombinant vaccinia virus expressing human CEA (rV-CEA) stimulates specific T cell responses in patients. This was the first vaccine to demonstrate human CTL responses to specific CEA epitopes and class I HLA-2 restricted T cell mediated lysis, and demonstrated the ability of human tumor cells to endogenously process CEA to present a specific CEA peptide in the context of a MHC for T-cell mediated lysis.

Anti-idiotype Vaccines

The idiotype network offers an elegant approach to transforming epitope structures into idiotypic determinants expressed on the surface of antibodies. According to the network concept, immunization with a given TAA will generate production of antibodies against these TAA, which are termed Ab1; the Ab1 is then used to generate a series of anti-idiotype antibodies against the Ab1, termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structure of the TAA identified by the Ab1. These Ab2 can induce specific immune responses similar to those induced by the original TAA and therefore can be used as surrogate TAAs. Immunization with Ab2 can lead to the generation of anti-anti-idiotypic antibodies (Ab3) that recognize the corresponding original tumor-associated antigen identified by Ab1. The anti-idiotype antibody represents an exogenous protein that should be endocytosed by APCs and degraded to 14–25 mer peptides to be presented by class II antigens to activate CD4-helper T cells. Activated Th2 CD4-helper T cells secrete cytokines such as IL-4 that stimulate B cells that have been directly activated by Ab2 to produce antibody that binds to the original antigen identified by Ab1. In addition, activation of Th1 CD4-helper T cells secrete cytokines that activate T cells, macrophages and natural killer cells that directly lyse tumor cells, and in addition, contribute to ADCC. Th1 cytokines such IL-2 also contribute to the activation of a CD8-CTL response. This represents a putative pathway of endocytosed anti-idiotype antibody. The anti-idiotype antibody may be degraded to 9/10 mer peptides to present in the context of class I antigens to activate CD8-cytotoxic T cells, which are also stimulated by IL-2 from Th1 CD4-helper T cells.

Anti-idiotype antibodies that mimic distinct TAAs expressed by cancer cells of different histology have been used to implement active specific immunotherapy in patients with malignant diseases including colorectal carcinoma, malignant melanoma, breast cancer, B cell lymphoma and leukemia, ovarian cancer, or lung cancer. A murine monoclonal anti-idiotype antibody, 3H1 or CeaVac, which mimics CEA was developed by the authors and was used in a phase I clinical trial. Among 23 patients with advanced colorectal cancer, 17 patients generated anti-anti-idiotypic Ab3 responses, and 13 of these responses were proven to be true anti-CEA responses. The median survival of 23 evaluable patients was 11.3 months, with 44% 1-year survival. Toxicity was limited to local swelling and minimal pain. In another clinical trial, 32 patients with resected colorectal cancer were randomized to treatment with CeaVac. All 32 patients entered into this trial generated high-titer IgG anti-CEA antibodies, and ~75% generated CEA specific T cell responses. These data demonstrated that 5-fluorouracil based chemotherapy regimens did not have any adverse effect on the immune response developed by CeaVac. TriGem, an anti-idiotype monoclonal antibody that mimics the disialoganglioside GD2 was used as a vaccine in

clinical trial consisting of 47 patients with stage IV melanoma. Forty of 47 patients developed high-titer IgG anti-GD2 antibodies. Seventeen patients were stable on the study from 8 to 34 months. Disease progression occurred in 27 patients on the study from 1 to 9 months. For the 26 patients with soft tissue disease, the median overall survival has not been reached. For 18 patients with visceral metastasis, the median overall survival was 15 months. These results exceed historical controls with stage IV melanoma. Another anti-idiotype monoclonal antibody, TriAb, which mimics the human milk fat globule (HMFG) membrane antigen, is highly overexpressed on breast cancer cells and a variety of other cancer cells, including ovarian cancer, non small-cell lung cancer, and colon cancer. Immunizations with this anti-idiotype antibody elicited both anti-HMFG antibodies and idiotype specific T cell responses in patients with breast cancer in the adjuvant setting as well as in patients with advanced disease following autologous bone marrow transplantation. Although these initial clinical data are promising, active specific immunotherapy with anti-idiotype antibodies need to be tested in combination with other conventional and experimental therapies to overcome the multiple mechanisms by which tumor cells escape immune recognition and destruction. The anti-idiotype vaccine therapy for patients with minimal residual disease might be curative in the adjuvant setting and may improve the quality of patients' life.

Dendritic Cell-based Vaccines

DCs are the professional APCs of the immune system and are present in peripheral tissues, where they capture antigens. These antigens are subsequently processed into small peptides as the DCs mature and move towards the draining secondary lymphoid organs. There the DCs present the peptides to naïve T cells, thereby inducing a cellular immune response that involves both CD4 T helper 1 (Th1) cells and cytotoxic CD8 T cells. DCs are also important at inducing humoral immune response through their capacity to activate naïve and memory B cells. DCs can also activate natural killer (NK) cells and natural killer T (NKT) cells. Therefore, DCs can conduct all of the elements of the immune orchestra, and they are therefore a fundamental target and tool for vaccination.

The development of ex vivo techniques for generating large numbers of DCs in vitro from mouse bone marrow cells supplemented with either GM-CSF alone or GM-CSF plus IL-4 allowed the approach of DC-based tumor vaccination to be fully exploited. Numerous studies in mouse tumor models have shown that DCs pulsed with tumor antigens can induce protective and therapeutic anti-tumor immunity. In 1996, Hsu et al reported the first DC-based clinical trial of follicular B cell lymphoma patients who were treated with peripheral blood-derived DCs pulsed with a tumor-specific idiotype (Id) protein. Of these ten patients, eight developed a proliferative cellular response to Id and one patient developed an Id-specific CTL response. However, tumor regression was not reported in these DC-vaccinated patients. In several other trials a correlation between immunological and clinical outcome has been demonstrated. However, the efficacy of therapeutic DC-based vaccination has been modest and these trials have had similar clinical outcome: mainly, immunized patients often demonstrate significant activation of adaptive immunity to the targeted tumor antigen(s) as shown by various methods such as tetramer analysis, IFN-γ ELISPOT, and ^{51}Cr-release assay; but only a limited number of immunized patients demonstrate significant tumor regression.

The complexity of the DC system requires rational manipulation of DCs to achieve protective or therapeutic immunity. Further research is needed to analyze the immune responses induced in patients by distinct ex vivo generated DC subsets that are activated through different pathways. These ex vivo strategies should help to identify the parameters for in vivo targeting of DCs. Overall, we remain optimistic that improved cancer vaccines will ultimately yield favorable clinical results, particularly after these approaches have been modified in a manner that integrates recent progress related to the physiology of DCs and our improved understanding of how tumors and the host immune system interact with each other.

Conclusion

There exist several promising immunologic approaches to vaccine therapy of cancer. The challenge of immunotherapy research is to determine which combination of approaches leads to a favorable clinical response and outcome. Several studies have shown enhanced survival of patients receiving vaccines; however, a randomized phase III clinical trial has yet to show a statistically significant improvement in the survival of such patients.

►Cancer-Germline (CG) Antigens
►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy
►T-cell Response

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Cancer Without Disease

►Dormancy

Cancers of Hormone-responsive Organs or Tissues

►Endocrine-Related Cancers

Cancer-Testis-Antigens

Definition

Tumor antigens which are only expressed by malignant tumor cells as well as by germ line cells in the testes. The MAGE1 protein was one of the first cancer-testis antigen originally identified in melanoma cells but also expressed by various other tumor types. It belongs to a large family of "MAGE-related" proteins whose biological function is still unclear. It is thought that common epigenetic alterations in tumors lead to re-expression of genes which are normally only expressed in the germ line.

►Melanoma Vaccines
►Cancer Germline (GC) Antigens
►GAGE Proteins

Candidat of Metastasis 1

►p8 Protein

Cannabinoids

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Synonyms

Phyto-cannabinoids; Endocannabinoids; Synthetic cannabinoids; Marijuana

Definition

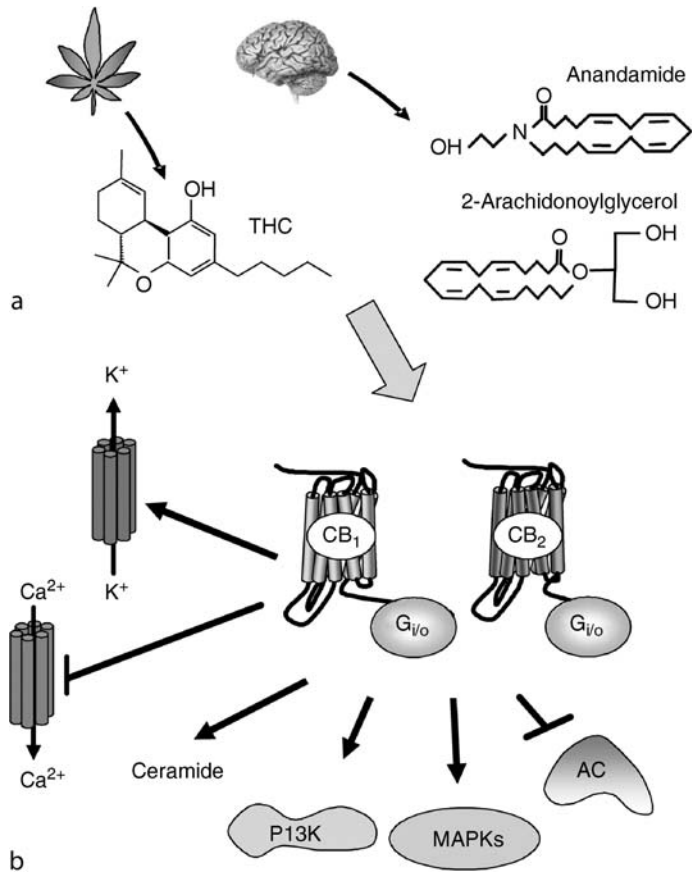
Cannabinoids are a family of lipid molecules that comprises a series of metabolites produced by the hemp plant *Cannabis sativa* (the phyto-cannabinoids); several fatty-acid derivatives endogenously produced by most animals (the endogenous ligands for cannabinoid receptors), and different synthetic compounds structurally or functionally related with the natural cannabinoids. Activation of cannabinoid receptors by some of these molecules reduce the symptoms associated to cancer chemotherapy and inhibit the growth of tumor cells in culture and in animal models of ►tumor xenografts.

Characteristics

The hemp plant *Cannabis sativa* produces approximately 70 unique compounds known as cannabinoids, of which Δ^9-tetrahydrocannabinol (THC) is the most important owing to its high potency and abundance in cannabis. THC exerts a wide variety of biological effects by mimicking endogenous substances – the endocannabinoids anandamide and 2-arachidonoylglycerol – that bind to and activate specific cannabinoid receptors (Fig. 1a and b). So far, two cannabinoid-specific ►G-protein-coupled receptors have been cloned and characterized from mammalian tissues: The CB_1 receptor is particularly abundant in discrete areas of the brain, but is also expressed in peripheral nerve terminals and various extra-neural sites. In contrast, the CB_2 receptor was initially described to be present in the immune system, although recently it has been shown that expression of this receptor also occurs in cells from other origins including many types of tumor cells.

Signaling Pathways Modulated by Cannabinoid Receptors

Most of the physiological, therapeutic and psychotropic actions of cannabinoids rely on the activation of CB_1 and CB_2 receptors (Fig. 1a and b). Extensive molecular and pharmacological studies have demonstrated that cannabinoids inhibit adenylyl cyclase through CB_1 and CB_2 receptors. The CB_1 receptor also modulates ion channels, inducing, for example, inhibition of N- and



Cannabinoids. Figure 1 Cannabinoids, cannabinoid receptors and its mechanisms of action. (a) Δ^9-tetrahydrocannabinol (THC), the main active component of marijuana, and the endocannabinoids anandamide and 2-Arachidonoylglycerol are ligands of cannabinoid receptors. (b) Both CB_1 and CB_2 receptors belong to the family of G-protein-coupled receptors. Binding of cannabinoids to cannabinoid receptors leads, among other actions and depending on the cell context, to: inhibition of adenylyl cyclase, modulation of the activity of several ion channels, modulation of phosphatidylionsoitol-3 kinase (PI3K) and of mitogen activated protein kinase cascades, or stimulation of ceramide generation.

P/Q-type voltage-sensitive Ca^{2+} channels and activation of G protein-activated inwardly rectifying K^+ channels. Besides these well-established signaling events that mediate – among others – the neuromodulatory actions of the endocannabinoids, cannabinoid receptors also modulate several pathways that are more directly involved in the control of cell proliferation and survival, including extracellular signal-regulated kinase, c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, ►phosphatidylinositol 3-kinase/Akt and focal adhesion kinase. In addition, cannabinoids stimulate the generation of the bioactive lipid second messenger ceramide via two different pathways: sphingomyelin hydrolysis and ►ceramide synthesis *de novo*.

Palliative Effects of Cannabinoids in Cancer

Cannabinoids have been known for several decades to exert palliative effects in cancer patients, and nowadays capsules of THC (Marinol-TM) and its synthetic analogue nabilone (Cesamet-TM) are approved to treat nausea and emesis associated with cancer chemotherapy. In addition, several clinical trials are testing other potential palliative properties of cannabinoids in oncology such as appetite stimulation and analgesia.

Mechanism Involved in the Antiemetic Effect of Cannabinoids

One of the most important physiological functions of the cannabinoid system is to modulate synaptic transmission. Thus, activation of cannabinoid receptors at presynaptic locations leads to reduced neurotransmitter release. As the CB_1 receptor is present in cholinergic nerve terminals of the myenteric and submucosal plexus of the stomach, duodenum and colon, it is likely that cannabinoid-induced inhibition of digestive tract motility is due to blockade of acetylcholine release in

these areas. There is also evidence that cannabinoids act on CB_1 receptors localized in the dorsal vagal complex of the brainstem – the region of the brain that controls the vomiting reflex. In addition, endocannabinoids and their inactivating enzymes are present in the gastrointestinal tract and may play a physiological role in the control of emesis.

Mechanism Involved in Appetite Stimulation by Cannabinoids

The endogenous cannabinoid system may serve as a physiological regulator of feeding behavior. For example, endocannabinoids and CB_1 receptors are present in the hypothalamus, the area of the brain that controls food intake; hypothalamic endocannabinoid levels are reduced by leptin, one of the most prominent anorexic hormones; and blockade of tonic endocannabinoid signaling with the CB_1 antagonist rimonabant – inhibits appetite and induces weight loss. CB_1 receptors present in nerve terminals and adipocytes also participate in the regulation of feeding behavior.

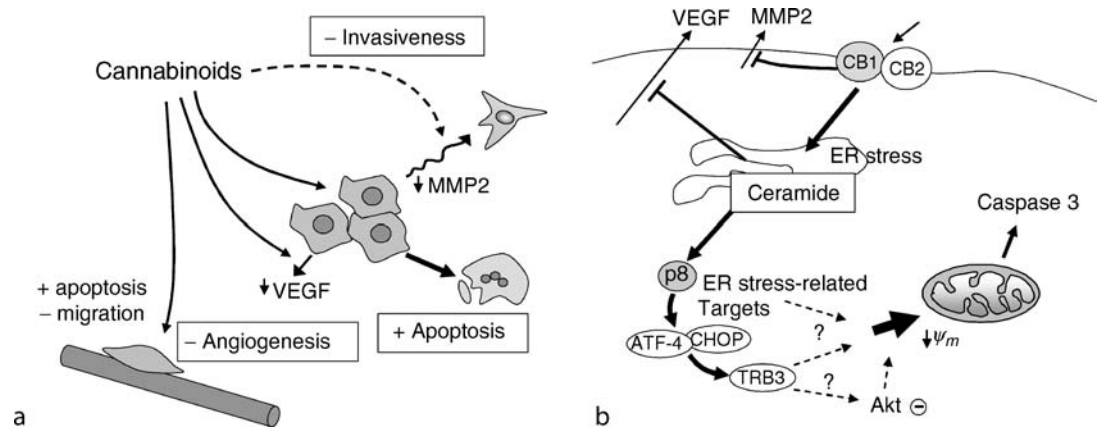
Mechanism Involved in the Analgesic Effect of Cannabinoids

Cannabinoids inhibit pain in animal models of acute and chronic ►hyperalgesia, ►allodynia and spontaneous pain. Cannabinoids produce antinociception by activating CB_1 receptors in the brain (thalamus, periaqueductal grey matter, rostral ventromedial medulla), the spinal cord (dorsal horn) and nerve terminals (dorsal root ganglia, peripheral terminals of primary afferent neurons). Endocannabinoids serve naturally to suppress pain by inhibiting nociceptive neurotransmission. In addition, peripheral CB_2 receptors might mediate local analgesia, possibly by inhibiting the release of various mediators of pain and inflammation, which could be important in the management of cancer pain.

Antitumoral Effects of Cannabinoids

Cannabinoids have been proposed as potential antitumoral agents on the basis of experiments performed both in cultured cells and in animal models of cancer. A number of plant-derived, synthetic and endogenous cannabinoids are now known to exert antiproliferative actions on a wide spectrum of tumor cells in culture. More importantly, cannabinoid administration to nude mice curbs the growth of various types of tumor xenografts, including lung carcinoma, glioma, thyroid epithelioma, lymphoma, skin carcinoma, pancreatic carcinoma and melanoma. The requirement of cannabinoid receptors for this antitumoral activity has been revealed by various biochemical and pharmacological approaches, in particular by determining cannabinoid receptor expression in the tumors and by using selective cannabinoid receptor agonists and antagonists.

Although the downstream events by which cannabinoids exert their antitumoral action have not been completely unraveled, there is substantial evidence for the implication of at least two mechanisms: induction of ►apoptosis of tumor cells and inhibition of tumor ►angiogenesis (Fig. 2a).



Cannabinoids. Figure 2 Mechanism of cannabinoid antitumoral action. (a) Cannabinoid administration decreases the growth of tumors by several mechanisms, including at least: (i) reduction of tumor angiogenesis, (ii) induction of tumor cell apoptosis, and perhaps (iii) inhibition of tumor cell migration and invasiveness. (b) Cannabinoid treatment induces apoptosis of several types of tumor cells via ceramide accumulation and activation of an ER stress-related pathway. The stress-regulated protein p8 plays a key role in this effect by controlling the expression of ATF-4, CHOP and TRB3. This cascade of events triggers the activation of the mitochondrial intrinsic apoptotic pathway through mechanisms that have not been unraveled as yet. Cannabinoids also decrease the expression of various tumor-progression molecules such as VEGF and MMP2.

Induction of Apoptosis

Different studies have shown that the pro-apoptotic effect of cannabinoids on tumor cells relies on the stimulation of cannabinoid receptors and a subsequent activation of the proapoptotic ►mitochondrial intrinsic pathway. In glioma and pancreatic tumor cells, treatment with cannabinoids leads to accumulation of the pro-apoptotic sphingolipid ceramide which in turn leads to up-regulation of the stress-regulated protein ►p8, which belongs to the family of ►HMG-I/Y transcription factors. The acute increase of p8 levels after cannabinoid treatment triggers a cascade of events that involves the up-regulation of several genes involved in the ►endoplasmic reticulum (ER) stress response including the activating transcription factor 4 (ATF-4) and the C/EBP-homologous protein (CHOP). These two transcription factors cooperate in the induction of the ►tribbles homologue 3 (TRB3), a ►pseudokinase that is involved in the induction of apoptosis (Fig. 2b).

The processes downstream of ER stress activation involved in the execution of cannabinoid-induced apoptosis of tumor cells are not completely understood yet but include inhibition of the anti-apoptotic kinase Akt and activation of the mitochondrial intrinsic pathway.

Of interest the pro-apoptotic effect of cannabinoids is selective of tumor cells. For instance, treatment of primary cultured astrocytes with these compounds does not trigger ceramide accumulation, induction of the aforementioned ER stress-related genes or apoptosis. Furthermore, cannabinoids promote the survival of astrocytes, oligodendrocytes and neurons in different models of injury, supporting the notion that cannabinoids activate opposite responses in transformed and non-transformed cells.

Inhibition of Tumor Angiogenesis

To grow beyond minimal size, tumors must generate a new vascular supply (angiogenesis) for purposes of cell nutrition, gas exchange and waste disposal, and therefore blocking the angiogenic process constitutes one of the most promising antitumoral approaches currently available. Immunohistochemical analyses in mouse models of glioma, skin carcinoma and melanoma have shown that cannabinoid administration turns the vascular hyperplasia characteristic of actively growing tumors to a pattern of blood vessels characterized by small, differentiated and impermeable capillaries. This is associated with a reduced expression of ►vascular endothelial growth factor (VEGF) and other proangiogenic cytokines such as angiopoietin-2 and placental growth factor, as well as of type 1 and type 2 VEGF receptors, in cannabinoid-treated tumors. Pharmacological inhibition of ceramide synthesis *de novo* abrogates the antitumoral and antiangiogenic effect of cannabinoids *in vivo* and decreases VEGF production by glioma cells *in vitro* and by gliomas *in vivo*, indicating that ceramide plays a general role in cannabinoid antitumoral action.

Other reported effects of cannabinoids might be related with the inhibition of tumor angiogenesis and invasiveness by these compounds (Fig. 2a and b). Thus, activation of cannabinoid receptors on vascular endothelial cells in culture inhibits cell migration and survival. In addition, cannabinoid administration to glioma-bearing mice decreases the activity and expression of ►matrix metalloproteinase-2, a proteolytic enzyme that allows tissue breakdown and remodeling during angiogenesis and metastasis. In line with this notion, cannabinoid intraperitoneal injection reduces the number of metastatic nodes produced from paw injection in lung, breast and melanoma cancer cells in mice.

Therapeutic Potential of Cannabinoids as Antitumoral Agents

On the basis of these preclinical findings, a pilot clinical study of THC in patients with recurrent ►glioblastoma multiforme has been recently run. Cannabinoid delivery was safe and could be achieved without significant psychoactive effects. Also, although the limited number of patients involved in the trial did not permit the extraction of statistical conclusions, median survival of the cohort was similar to other studies performed in recurrent glioblastoma multiforme with temozolomide and carmustine, the drugs of reference for the treatment of these tumors. In addition THC administration correlated with decreased tumor cell proliferation and increased tumor cell apoptosis.

The significant antiproliferative action of cannabinoids, together with their low toxicity compared with other chemotherapeutic agents and their ability to reduce symptoms associated to standard chemotherapies, might make these compounds promising new antitumoral agents.

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Cap

Definition
Structure added to the 5′-end of nascent eukaryotic mRNA molecules. It aligns eukaryotic mRNAs on the ribosome during translation.

►Funnel Factors

Cap-Binding Complex

Definition
A protein complex that recruits messenger RNA to the ribosome.

►MCT-1 Oncogene

Cap-Dependent Translation

Definition
In eukaryotes, translation can usually be initiated at the 5′ end "cap" of the mRNA since cap-recognition is required for the assembly of the initiation complex. Most of the transcripts are translated by cap-dependent translation. Alternatively, some transcripts might be translated from an internal ribosome entry site.

►Rapamycin
►Funnel Factors

Capecitabine

Definition
Capecitabine is a 5-fluorouracil (Fu) oral ►prodrug.

►Erlotinib (Tarceva®)

CapG

Definition
Is, a 45-kDa protein composed of 348 amino acids, is a member of the gelsolin/villin actin-modulating protein family. The subcellular localization of CapG protein is largely nuclear, though other ►gelsolin family members localize only in cytoplasm, suggesting that CapG may have a function in addition to cytoskeleton modulation. The human *CapG* gene, mapped at 2p11.2, is ubiquitously expressed in normal tissues, but down-regulation of the expression was observed in roughly one third of melanomas, stomach, and lung cancers. Furthermore, CapG has been shown to possess tumor suppressor activity when transfected into certain cancer cell lines.

►Gelsolin family

Capillary Hemangioblastoma

Definition
Low-grade tumor comprised of stromal cells and abundant capillaries, preferentially occurring in the cerebellum. Multiple hemangioblastomas are associated with the Von-Hippel Lindau disease.

►Uncertain or Unknown Histogenesis Tumors
►Von Hippel-Lindau Tumor Suppressor Gene

CAR

►Chimeric Antigen Receptor on T Cells

Carbonyl Reductases

►Reductases

Carboplatin

Definition
Second-generation platinum compound with a broad spectrum of antineoplastic properties. Carboplatin contains a platinum atom complexed with two ammonia groups and a cyclobutane-dicarboxyl residue. This agent is activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups

such as GC-rich sites in DNA, thereby inducing intrastrand and interstrand DNA ►cross-links, as well as DNA-protein cross-links. These carboplatin-induced DNA and protein effects result in ►apoptosis and cell growth inhibition. This agent possesses tumoricidal activity similar to that of its parent compound, ►cisplatin, but is more stable and less toxic.

Carboxylesterase

Definition

Carboxylesterases, present in serum, in the epithelial lining of the intestines, in tumor tissue, and in high content in the liver, are enzymes responsible for metabolizing (hydrolysis) a wide variety of drugs and ►xenobiotics. The carboxylesterase genes are located on chromosome 16q13-q22 and are supposed to be highly conserved during evolution.

►Irinotecan

Carboxypeptidase

Definition

A type of protease or hydrolase that removes the amino acid at the free carboxyl end of a polypeptide chain.

►Prostate-Specific Membrane Antigen

Carcinoembryonic Antigen

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Synonyms

CEA; CD66e; CEACAM5

Definition

CEA is a glycoprotein of approximately 150–180 kDa. It's measurement in serum is used clinically as a ►biomarker for a number of cancers (pancreas, breast, stomach, ovary, lung and medullary carcinoma of the thyroid) but its primary use is in monitoring cancers of the colon and rectum.

Characteristics

Protein Structure

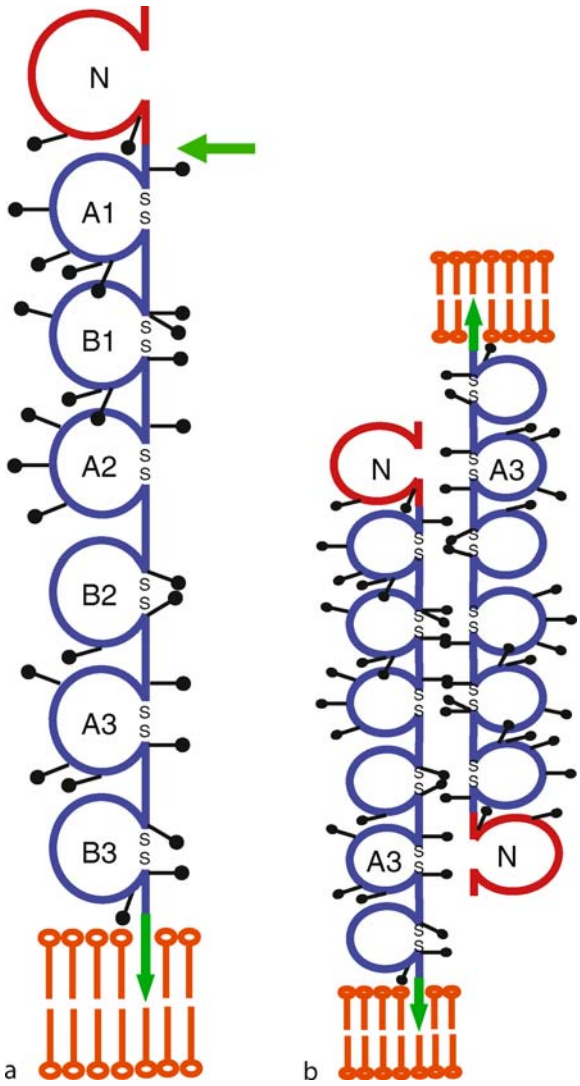
CEA was discovered in 1965 in ►colon cancer and fetal tissue extracts and was described as an ►oncofetal antigen. Many of the advances in tumor marker research lead directly back to the discovery of CEA The protein component of CEA is 79 kDa in size and the balance of 70–100 kDa is made from up to 28 complex N-linked multi-antennary carbohydrate structures containing *N*-acetyl-glucosamine, mannose, galactose, fucose and sialic acid. Low resolution X-ray studies have shown an elongated monomeric structure that could be described as a bottle brush. The molecule is composed of a series of six disulphide linked immunoglobulin like domains (IgC2-like) of either 93 (type A) or 85 (type B) amino acids and a seventh N-domain of 108 amino acids which is an IgV (variable antigen recognition domain) structure without the stabilizing disulphide bridge. CEA can attach to the cell membrane and this is achieved by post translational modification of a small (26 amino acids) hydrophobic C-terminal domain to a ►glycosyl phosphatidyl inositol linkage (see Fig. 1A). Cleavage of this linkage by ►phospholipases releases CEA into the lumen of the intestine or other extra-cellular compartments.

The CEA Gene Family

The complete gene for CEA has been cloned and it includes a promoter region that confers cell type specific expression. The ►CEA gene family comprises of 29 genes or pseudogenes located between the q13.1 and q13.3 regions of chromosome 19. The family can be divided into three groups: The CEA group of 12 genes, the pregnancy specific glycoprotein group (PSG) of 11 genes and a third group composed of 6 pseudogenes. Only 16 of the 29 genes are expressed. Sequence data has shown that the CEA family is a subset of the Immunoglobulin supergene family. Comparative sequence studies of the CEA gene family from various species suggest that the CEA family have a common ancestry and arose relatively recently in evolution.

Function of CEA in Normal and Cancerous Tissue

In general members of the CEA family subgroup have a ubiquitous distribution in adult tissues. However CEA itself has a more restricted expression being found only in colon, pyloric mucus cells, epithelial cells of the prostate, in sweat glands and in squamous cells in the tongue, cervix and esophagus. In the colon CEA is located at the apical surface of colonic enterocytes and is associated with the ►glycocalyx or fuzzy coat. In the normal colon CEA is maximally expressed on columnar cells at the level of the free luminal surface. CEA is also found in goblet cells in association with mucins. The function of CEA in the normal individual is not well understood and has been the subject of much



Carcinoembryonic Antigen. Figure 1 CEA Structure (a) Showing insertion of CEA into the plasma membrane (down arrow) the Ig domain structure and the position of the N-linked sugar chains. An arrow marks the position of the PELPK receptor recognition sequence. (b). Shows homotypic binding between two CEA molecules with attachment between the N and A3 domains. (Structures are modified from the CEA Homepage http://cea.klinikum.uni-meunchen.de)

speculation. It has been estimated that the normal person can produce 70 mg or more of CEA a day and excrete it in the feces. CEA has been shown to bind to various fimbriated gut pathogens and therefore it has been suggested that it has a function in protecting the gut epithelia. In cancer cells CEA may perform a number of functions. Unlike the normal colonocyte where CEA expression is highly polarized in cancers this polarity is lost and its expression occurs through the whole of the cell surface. It has been shown that CEA can act as a Ca^{2+} independent homotypic adhesion molecule binding with itself through an interaction between the N and A3 domains (Fig. 1B) and causing aggregation of tumor cells. This allows the malignant epithelium to adopt a multi-layered structure and may disrupt the normal pattern of differentiation. CEA can also bind heterotypically to other members of the gene family including the ►nonspecific cross reacting antigen (NCA, CD66c, CEACAM6) and the ►biliary glycoprotein (BGP, CD66a, ►CEACAM1) of which seven different forms have been identified. It is unlikely that CEA functions as a ►cell adhesion molecule in the normal colon because of its apical expression. CEA is cleared from the circulation by the hepatic ►macrophages (►Kupffer cells). A cell surface receptor identical to the heterogeneous nuclear RNA binding protein ►hnRNP M4 recognizes a penta-peptide (Pro–Glu–Leu–Pro–Lys (PELPK)) located at the hinge region between the N and first immunoglobulin loop domain (A1) of CEA. Patients with a mutation in the region coding for this peptide have extremely high circulating CEA levels presumably due to the inability of Kupffer cells to clear the protein from the blood. CEA has also been implicated in the development of hepatic ►metastasis from colorectal cancers by the induction of a localized inflammatory response that affects retention and implantation in the liver. Cytokines produced also protect the tumor cells against the toxic effects of hypoxia. CEA producing cells therefore have a selective advantage for growth in the liver. Recent studies have also shown that CEA can protect cancer cells from a form of programmed cell death called ►anoikis and this also seems to involve the PELPK motif and inhibition of Trail-R2 (►DR5) signaling. CEA is also protective against other forms of ►apoptosis including drug and UV light induced programmed cell death. The related protein CEACAM-1, however, is a pro-apoptotic protein.

Clinical Aspects

The main clinical use for CEA is as a tumor marker especially for cancers in the colon and rectum and approximately 90% of these cancers produce CEA. CEA has also been used as a marker for breast and small cell cancer of the lung. Approximately 50% of breast and 70% of small cell cancers express CEA. Accurate immuno-assays are commercially available for its measurement in body fluids. Immunohistochemistry on biopsy or resection specimens is also often carried out, for example the intensity of CEA staining has been associated with a worse prognosis for breast cancer. Normal serum levels are <2.5 ng/ml unless the subject is a heavy smoker when the normal cutoff becomes <5 ng/ml. CEA levels can be elevated in a number of non-malignant conditions such as pancreatitis, inflammatory bowel disease including ►Crohn disease, hereditary polyposis including ►Gardener

syndrome, polycystic disease of the liver and a variety of other liver diseases including ►cirrhosis, hepatitis and benign biliary duct obstruction. Rarely do these diseases result in a CEA elevation over 10 ng/ml. CEA levels above 20 ng/ml almost always indicate the presence of a malignant tumor. Patients with colorectal cancer who present with a CEA level of over 5 ng/ml have a poorer prognosis and are at higher risk for developing metastasis to the liver. However, because the CEA assay lacks sensitivity for early stage colorectal cancer it cannot be used as a population screen. CEA is most useful for the early detection of liver metastasis in colorectal cancer patients. It is not as effective in detecting loco-regional or pulmonary metastases. Elevated CEA levels that fall to normal following tumor resection are an indication of a successful surgery; however, a rising CEA level post operatively indicates a progression or recurrence of the tumor. There is no clear agreement on how often CEA measurements should be taken following curative surgery. The guidelines put out by the American Society of Clinical Oncologists (ASCO) recommend measurements every 2–3 months for a minimum of 2 years. CEA measurements can give a lead time of up to a year before the onset of clinical symptoms of recurrence. Serial serum CEA measurements have been shown to be useful in the follow up of patients with breast cancer and small cell cancer of the lung. The reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to detect CEA producing circulating cancer cells. More recently a real time PCR method has been developed for the quantitative detection of CEA mRNA transcripts in blood, peritoneal washings and lymph nodes. These methods can be used for a more exact staging and prognosis in cancer patients. CEA has been used as a target antigen for both ►radio-immunodetection and ►radio-immunotherapy of cancers. Imaging after administration of radiolabeled anti-CEA antibodies provides information on the location and extent of disease. Radio-labeled anti CEA antibodies have shown therapeutic effects in reducing tumor size in metastatic disease. Radio-labeled anti-CEA antibodies are also used to guide second look surgery and can detect occult disease. More recently CEA has been used as the antigen of choice for cancer vaccines against colorectal cancers. Clinical trials have been conducted using recombinant ►CEA-vaccine virus and recombinant ►ALVAC-CEA vaccines. The vaccines were well tolerated and elicited CEA specific T-cell responses. This promises to be a useful addition to standard therapies.

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Carcinoembryonic Antigen (CEA)

Definition

Is a normal mucosal cell oncofetal glycoprotein involved in cell adhesion. It is overexpressed in gastric, pulmonary, breast, pancreatic and predominantly colorectal adenocarcinomas. It may also be elevated in the serum of heavy smokers and patients with ulcerative colitis, pancreatitis and cirrhosis. Its role as a screening tool of colorectal carcinoma remains uncertain because of poor sensitivity and specificity. In patients with established disease, the absolute serum level of CEA correlates with disease burden and has prognostic value.

►Serum Biomarkers
►CEA

Carcinofetal Proteins

►Alpha-Fetoprotein – Historical

Carcinogen

Definition

The causal agent which induces tumors. They include external factors (chemicals, physical agents, viruses)

and internal factors such as hormones. Chemical carcinogens are structurally diverse and include naturally-occurring substances as well as synthetic compounds. An important distinction can be drawn between 'genotoxic' carcinogens and 'non-genotoxic' carcinogens or between 'direct-acting' carcinogens and 'indirect-acting' carcinogens.

►Toxicological Carcinogenesis
►Carcinogen Metabolism
►Fragile Histidine Triad
►Chemically Induced Cell Transformation
►Chemical Carcinogenesis

Carcinogen Dosimetry

Definition

Measures of internal dose (DNA adducts) or surrogate internal dose (protein adducts), occurring as a result of carcinogen dosing by inhalation, topical exposure or oral exposure, in target tissues for tumor formation (mostly epithelial tissues), in their surrogates (typically leukocytes), or excreted in urine and/or feces.

►Carcinogen Macromolecular Adducts
►Adducts to DNA

Carcinogen Macromolecular Adducts

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Definition

Carcinogen macromolecular adducts are chemical modifications ("addition products") of nucleic acids and proteins that form in tissues and cells exposed to reactive chemical species.

Characteristics

Chemical carcinogens that induce macromolecular damage may be exogenous or endogenous (Table 1). Exogenous chemicals, including environmental pollutants or drugs most often require activation, and before they are activated they may be referred to as ►procarcinogens. Following the generation of a chemically reactive species, spontaneously or more commonly through metabolism, covalent binding to nucleic acids and proteins can occur resulting in adduct formation. In the case of reactive chemical species forming adducts with DNA, a mutation can result if the DNA is not properly repaired. When reactive chemical species bind covalently to a protein, the resulting adducts persist for the lifetime of the protein. Formation of DNA adducts is considered necessary for carcinogenesis, while protein modification is considered an indicator of exposure and a surrogate for DNA adduct formation.

Sometimes, normal physiological (endogenous) processes require chemical modification of nucleic acids. For example, selective 5-methylation of cytosine in DNA regulates normal gene expression, and 7-methylation of guanosine in 5′ cap structures of mRNA is necessary for efficient protein synthesis in eukaryotes. Normal endogenous metabolic processes, including ►redox cycling, lipid peroxidation, nitric oxide metabolism and endogenous nitrosation can produce oxygen free radicals, oxidative-DNA adducts, etheno-adducts and nitrosamine adducts.

Exogenous carcinogenic agents that form macromolecular adducts can be direct-acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide and ozone. However, most are inert, like the polycyclic aromatic hydrocarbons (PAHs), and require biotransformation through ►metabolic activation. Examples of some chemical carcinogens responsible for macromolecular adduct formation, their origins and how humans become exposed are presented in Table 1.

Carcinogen Metabolism Leading to Macromolecular Adduct Formation

In order to form macromolecular adducts, exogenous agents that can be inhaled, ingested or absorbed through the skin, are altered metabolically by families of enzymes. These enzymes convert a small fraction of the initial dose to highly reactive intermediate metabolites that become bound covalently to specific bases in DNA or amino acids in protein.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants composed of variable numbers of fused benzene rings that are chemically unreactive and insoluble in water. The prototype PAH, benzo[*a*]pyrene (BP), forms macromolecular adducts in the body by metabolism to simple epoxides through the action of ►cytochrome P450 enzymes, hydration through the action of epoxide hydrolase and epoxidation (cytochrome P450s) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to a positively charged, highly reactive free radical called a carbocation (the ►ultimate carcinogen), which binds

Carcinogen Macromolecular Adducts. Table 1 Selected chemical carcinogens involved in DNA and protein adduct formation, their origins and routes of exposure

Chemical class	Examples	Occurrence
Polycyclic aromatic hydrocarbons (PAHs)	Benzo[a]pyrene, dibenz[a,h]anthracene, 5-methylchrysene	Charbroiled foods[a], water[b], coke oven emmissions[c], urban air[b], soil[b], tobacco smoke[d], coal tar ointment[e]
Nitro-PAHs	1-Nitropyrene, 1,8-dinitropyrene	Diesel exhaust[b,c], coal smoke[b]
	3-Nitrobenzanthrone	
Aromatic amines	4-Aminobiphenyl, benzidine	Tobacco smoke[d], dyes[c], urban air[b]
	2-Acetylaminofluorine	Candidate pesticide
Heterocyclic amines	2-Amino-3-methyl-imidazo-[4,5-f]quinoline (IQ)	Fried or broiled meats and fish[a,g]
	3-Amino-1-methyl-*5H*-pyrido[4,3-b]indole (Trp-P1)	
	2-Amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP)	
Nitrosamines	*N*-nitroso-*N*-methylethylamine	Tobacco smoke[d], alcoholic beverages[d]
	N-nitrosodimethylamine	Various foods[a], urban air[b], soil[b]
	4-(methylnitrosamino)-4-(3-pyridyl)-1-butanone (NNK)	Tobacco specific nitrosamine (see below)
Nitrosoureas	*N*-nitroso-*N*-methylurea	Cancer chemotherapeutic agents[e]
Naturally occurring molecules	Aflatoxins, ochratoxin, free radicals (O_2, OH, NO) ozone, tobacco specific nitrosamines (e.g., NNK)	Molds/fungi[a] (*Aspergilius, Penicillium*)
		Immune response/microbes[f] (*H. Pylori*)
		Urban air[b]
		Plant: tobacco[e], tobacco smoke[e]
Miscellaneous	Vinyl chloride, ethylene oxide, benzene	Manufacture of polyvinylchloride[c]
		Sterilization of medical equipment[c]
		Petrochemicals/glues/solvents[c]

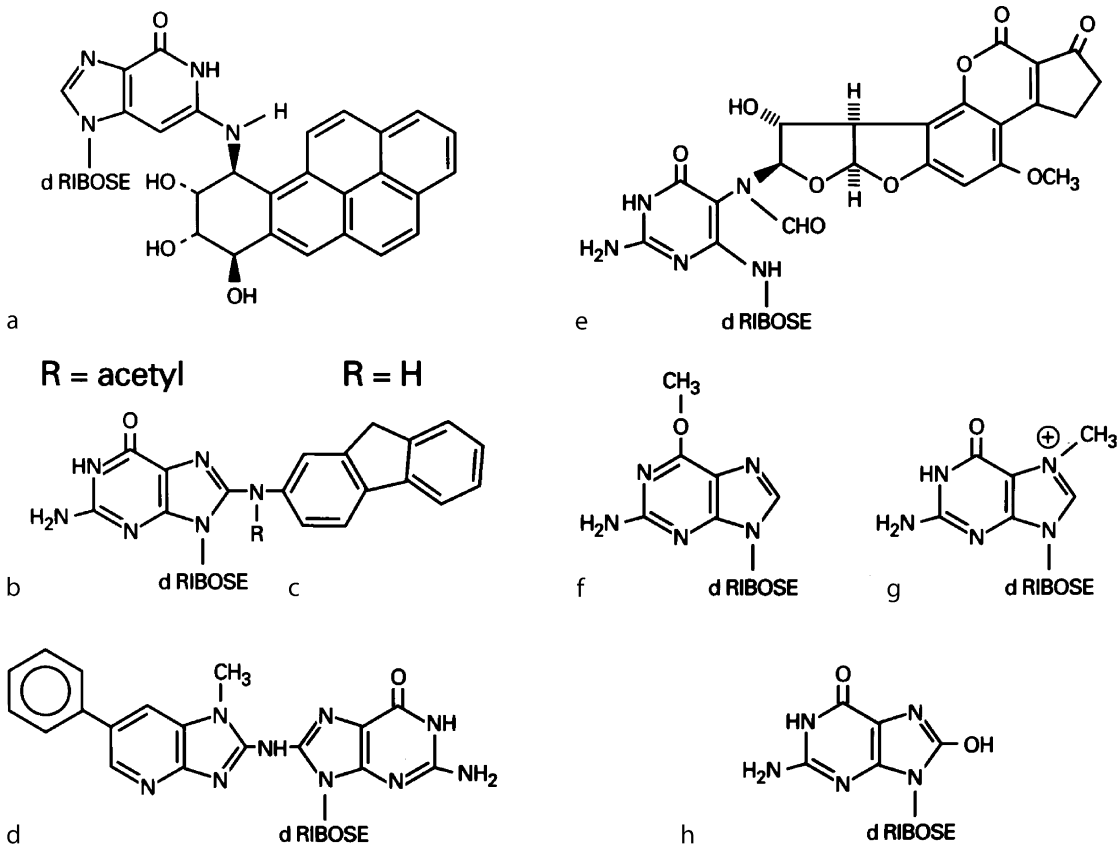
[a]Diet, [b]environmental, [c]occupational, [d]life-style, [e]medicinal, [f]infectious agents, [g]formed by pyrolysis (150°C) of amino acids in the presence of creatinine and glucose.

covalently to DNA and protein. The structure of the major DNA adduct formed by BP with deoxyguanosine is shown in Fig. 1a. BP also forms adducts with protein (Fig. 2).

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), is implicated in human bladder cancer. An additional class of environmental contaminants, nitrated polycyclic aromatic hydrocarbons are related to aromatic amines by nitroreduction. The presence of the amino-group, which can be either acetylated or non-acetylated, bestows a complex variety of metabolic options for aromatic amine metabolism. Activation of aromatic amines proceeds by *N*-oxidation with sulfotransferase catalysis resulting in the formation of acetylated and non-acetylated guanine adducts as shown in Fig. 1(b and c) for the carcinogen *N*-2-acetylaminofluorene. Aromatic amine adducts with protein were among the first protein adducts to be identified and characterized.

Heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), are a class of aromatic amines that are formed during high temperature cooking of meat and fish (Table 1). These compounds constitute the so-called "food ►mutagens." To form DNA adducts these compounds undergo *N*-hydroxylation (cytochrome P450) and enzymic *O*-esterification. The major guanine adduct of PhIP is shown in Fig. 1d.

Fungal mycotoxins contaminate cereals, grains and nuts, and ingestion of the prototype aflatoxin B_1, is correlated with a high incidence of liver cancer. Aflatoxins are hetercyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P450) across the olefinic double bond at the 8,9-position giving rise to a carbocation. Some addition products with DNA are unstable and lead to purine ring-opening followed by non-mutagenic depurination. The major aflatoxin-guanine adduct is shown in Fig. 1e. Serum and albumin



Carcinogen Macromolecular Adducts. Figure 1 (a) (7R)-N2-{10-[r-7, t-8, t-9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene]-yl}-deoxyguanosine; (b) *N*-deoxyguanosin-(8-yl)-2-acetylaminofluorene; (c) *N*-deoxyguanosin-(8-yl)-2-aminofluorene; (d) *N*-deoxyguanosin-(8-yl)-2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine; (e) Aflatoxin B1-N7-deoxyguanosine; (f) O6-Methyl-deoxyguanosine; (g) N7-Methyl-deoxyguanosine; (h) 8-Hydroxy-deoxyguanosine.

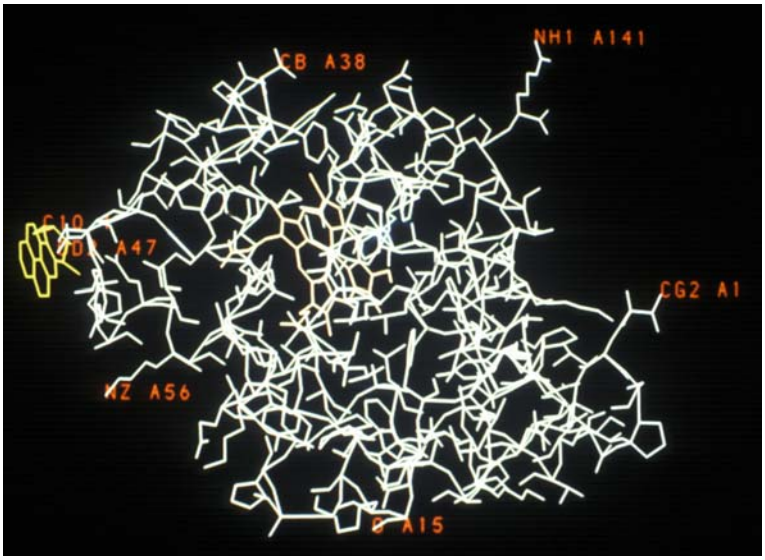
adducts of aflatoxin have been characterized and used extensively as human ►biomarkers.

Carcinogenic *N*-nitrosamines can be found in many substances including food, alcoholic beverages and tobacco. *N*-nitrosodimethylamine is activated (cytochrome P450) through α-hydroxylation to produce an unstable α-hydroxy-nitrosamine, which forms formaldehyde and methyl diazohydroxide. The methyl diazohydroxide becomes a free radical and a powerful methylating agent, which leads to the production of multiple DNA modifications (e.g., Fig. 1f and g). Some nitrosamines such as the tobacco-specific nitrosamine, 4-[methylnitrosoamino]-1-[3-pyridyl]-1-butanone (NNK), are asymmetrical and also give rise to the formation of bulky DNA adducts. Nitrosamines are also known to form protein adducts.

Oxygen radical damage produced by both endogenous and exogenous events can result in the formation of macromolecular adducts. Pathways that lead to the formation of oxygen radicals include degradation of organic peroxides (catechol, hydroquinone, and 4-nitroquinoline-*N*-oxide), hydrogen peroxide, lipid peroxidation and the catalytic cycling of some enzymes (e.g., ►redox cycling). Stomach cancer has been associated with *Helicobacter pylori* infection, and consequent oxidative damage linked with the inflammatory process is thought to contribute to carcinogenesis. Treatment with certain drugs or exposure to plasticizers can stimulate peroxisome proliferation, also giving rise to oxyradicals. Exposure to tumor promoters indirectly increases oxyradical formation; examples include the action of phorbol esters, mediated by protein kinase C and chronic inflammation, mediated by nitric oxide. Oxygen free radicals produce multiple DNA adducts including 8-hydroxydeoxyguanosine (Fig. 1h).

Structural Modification of DNA to Form Carcinogen-DNA Adducts

The structure of DNA bases can be modified through various mechanisms. These include oxidation, alkylation, dimerization, deamination and reaction with large, bulky aromatic-type carbocations. Endogenous



Carcinogen Macromolecular Adducts. Figure 2 Hemoglobin α-chain modified with benzo(a)pyrene-7, 8-diol 9,10-epoxide (BPDE) (yellow structure) on aspartate residue 47 (see Chem Res Toxicol (1991) 4:359–363). (The model shown here was provided by Billy Day and Steven R. Tannenbaum, Massachusetts Institute of Technology, Cambridge, MA).

and exogenous pathways lead to the formation of oxygen free radicals and the formation of oxidative DNA damage. Examples of oxidative DNA adducts include thymine glycol, 8-hydroxydeoxyguanosine, uracil glycol, 5-hydroxyuracil, 5-hydroxy-methyluracil and 6-hydroxy-5,6-dihydrocytidine.

Alkyl-radicals form during the ►metabolic activation of certain *N*-nitrosamines or spontaneously in the case of *N*-alkylureas (*N*-methyl-*N*-nitrosourea) or *N*-nitrosoguanidines. Protonated alkyl-functional groups, which become available to modify DNA, attack nucleophilic centers. There are ten of these: N1, N3, and N7 of adenine; N3 of cytosine; N2, O6 and N7 of guanosine; O2, N3, and O4 of thymidine. Repair of some of these lesions is correlated with mutagenicity. For example, O6-methyl-deoxyguanosine (Fig. 1f) can be repaired and is a promutagenic lesion, whereas N7-methyl-deoxyguanosine (Fig. 1g) is neither repaired nor mutagenic.

Larger, bulky aromatic-type adducts bind to DNA producing three-dimensional structures that reside either in the minor or the major groove of the DNA helix. Activated BP binds preferentially to the exocyclic (N2) amino group of deoxyguanosine (Fig. 1a). While guanine is a preferred site for PAH modification, covalent binding to deoxyadenosine and deoxycytosine are also possible. Aromatic amines form adducts at the C8, N2, and O6 positions of deoxyguanosine and deoxyadenosine but the major aromatic amine adducts form at the C8 position of deoxyguanosine (Fig. 1b and c). Evidence suggests that activation of aflatoxin B_1 produces adduction primarily at the N7-position of deoxyguanosine (Fig. 1e).

Methods to Measure Carcinogen-DNA Adducts

Single methods currently in use for carcinogen-DNA adduct detection include radiolabeling, immunoassays, immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, mass spectrometry, atomic absorbance spectrometry and electrochemical conductance. Single methods are typically not able to chemically characterize specific adducts in human tissues, although they work well for animal models exposed to a single chemical agent. Using human samples, greater success in DNA adduct characterization has typically been obtained by combining preparative methods (immunoaffinity chromatography, high performance liquid chromatography or gas chromatography) with immunoassays, ^{32}P-postlabeling, synchronous fluorescence spectrometry or mass spectrometry. These assays are typically able to detect as little as 1 adduct in 10^9 nucleotides using ~5–100 μg of DNA. Accelerator mass spectrometry, can detect 1 adduct in 10^{12} nucleotides but requires administration of exceedingly low levels of carcinogens labeled with a distinguishing isotope (e.g., ^{13}C or ^{2}H).

Importance of DNA Adducts in Chemical Carcinogenesis

The presence of a DNA adduct in a critical gene provides the potential for occurrence of a mutagenic event, resulting in subsequent alterations in gene expression and a loss of growth control. A substantial

period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis since other events, such as mutagenesis and cell proliferation, must also take place. DNA adduct levels, measured at any point in time, reflect tissue-specific rates of adduct formation and removal, which depend upon carcinogen activation, ►DNA repair, adduct instability and tissue turnover. In experimental models dose-response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis induced by chemicals, while reductions in DNA adduct levels have been associated with chemoprevention. However, some adducts are highly-mutagenic and associated with carcinogenesis, while others are not. Studies in animal models have demonstrated an association between mutation "hot-spots" in proto-oncogenes or tumor suppressor genes, and specific adducts. Mutations considered potentially carcinogen-specific have been observed in *p53*, *ras*, and other reporter genes in humans. An important example is the *p53* codon 249 G to T mutation associated with aflatoxin B_1-N^7-deoxyguanosine formation and liver cancer in Qidong PRC. Molecular epidemiologic studies involving DNA adduct measurements have the potential to elucidate the role of DNA adduct formation in human cancer risk.

Significance of Carcinogen-Protein Adducts

In the same way that DNA can be modified by reactive chemical species, endogenous or exogenous carcinogens can become bound to proteins after direct interaction or metabolic activation. Typically, a reactive cation binds covalently at a nucleophilic amino acid. Alkylating agents most commonly attack the amino acid cysteine, however, aspartate, histidine, valine, tryptophan, glutamate, and lysine residues are also targets.

Because protein adducts are not repaired, protein adduct measurements are considered to reflect ►carcinogen dosimetry. Chemically-stable adducts are thought to provide a measure of dose integrated over the life time of a given protein. The blood proteins hemoglobin and serum albumin have been most studied as human ►biomarkers because they are readily accessible and have known rates of turnover. However, histone and collagen adducts have been explored as indicators of longer-term exposures.

Methods to Measure Carcinogen-Protein Adducts

The earliest animal model studies that examined hemoglobin or serum albumin adducts involved the use of a radiolabeled carcinogen. More recent approaches have included immunoassays, HPLC with fluorescence detection and various mass spectrometry approaches. These methods are particularly powerful because of the ability to determine the specific chemical structure of the purified protein adduct. In addition, sensitivity can be typically as low as ~0.1 fmole of adduct in mg quantities of protein. The strengths of this approach are the specificity of the methods and the availability of large quantities of sample material.

Significance of Protein-Adducts

The utility of proteins for human dosimetry in environmental and occupational chemical exposures was first demonstrated by the kinetic relationship between protein adduct persistence and protein lifetime. This important principle was established for hemoglobin modified by ethylene oxide or alkylating agents. It provided the basis for subsequent studies that investigated associations between carcinogen-protein adduct levels and carcinogen exposures. Protein adduct formation is a valuable surrogate for DNA adduct formation since many chemical carcinogens bind to both DNA and protein in blood with similar dose-response kinetics. In addition, blood proteins are available in large quantities, enhancing the feasibility of measuring carcinogen-protein adducts in human biomonitoring studies.

Many protein adduct studies have considered human exposures to different chemicals including ethylene, methylmethane sulfonate, BP, aflatoxin B_1, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), dimethylnitrosamine, ethylene and propylene oxide, NNK and styrene. Hemoglobin adducts formed through the metabolic activation of aromatic amines have proven to be excellent indicators of tobacco smoking. Tobacco smokers are readily distinguished from non-smokers, and a dose response has been observed between smokers of black tobacco containing high levels of 4-ABP and blonde tobacco containing low levels of 4-ABP. Protein adducts have been measured for twenty aromatic amines contained in cigarette smoke. The 3-aminobiphenyl-hemoglobin is a unique marker for passive smoking because 3-aminobiphenyl is present in side-stream but not main stream tobacco smoke. Hydroxyethylvaline in hemoglobin is also a dosimeter of tobacco smoking, but it is less specific because ethylene oxide has other environmental origins in addition to tobacco smoke. Although questions remain concerning the relationship between protein adduct levels and disease risk, measurement of protein adducts has been, and will continue to be, a valuable tool in ►molecular epidemiology studies.

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C

Carcinogen Metabolism

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Definition

The transformation of chemicals is important in carcinogenesis both in terms of bioactivation as well as detoxication. Most chemical carcinogens need to be activated within the body. Such reactive forms can then cause biological damage (Fig. 1). As an example for competing processes aflatoxin B_1 was chosen (Fig. 2) (►Adducts to DNA). Exactly what proportion in human cancers is the result of chemical exposure is not clear. However, in most countries at least one third of cancer cases are due to tobacco carcinogens (►Tobacco carcinogenesis, ►tobacco-related cancers). A significant number of cancer cases may be related to diet, although it is unknown exactly which chemicals in food cause or influence cancer. As a result of precautions adapted in the course of the last century, the number of cases due to industrial exposure seems to be very low.

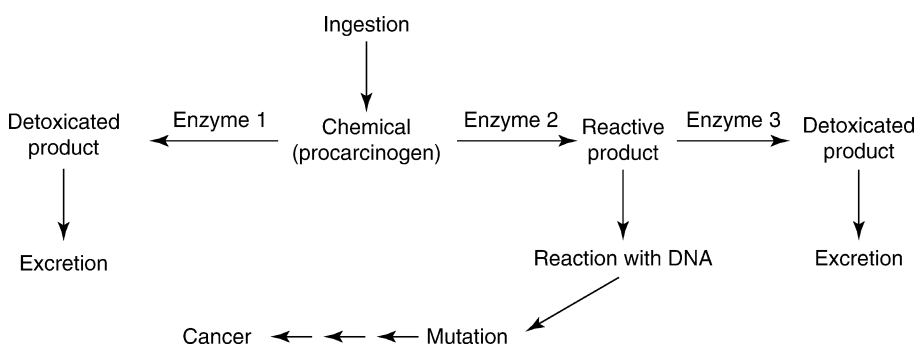
Characteristics

History

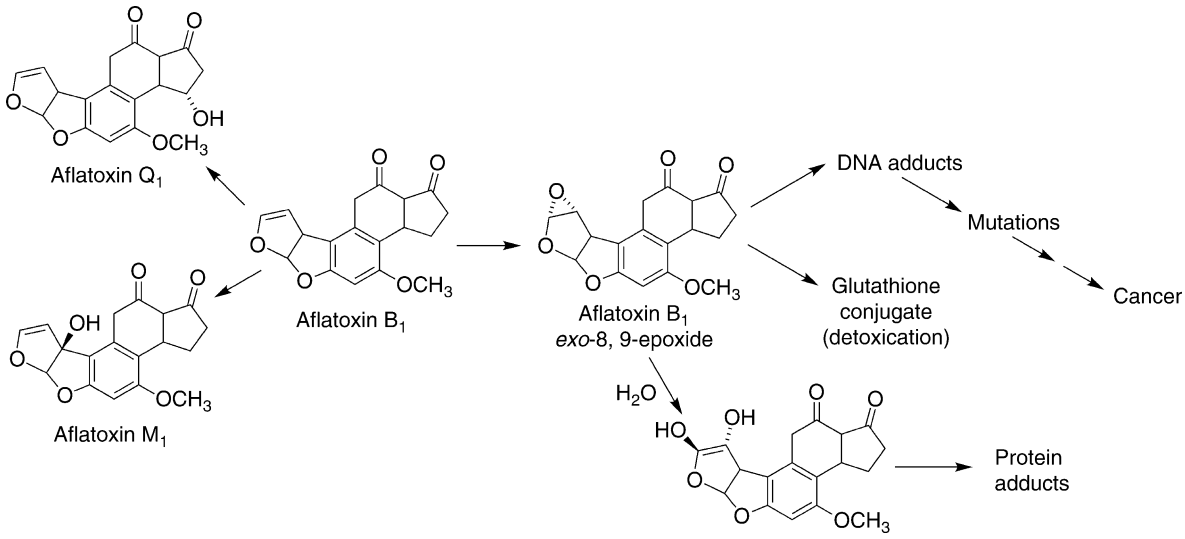
In 1761, the London physician J. Hill associated the use of snuff with nasal cancers (tobacco carcinogenesis, tobacco-related cancers). More than one hundred years later in 1895, Rehn and others reported a link of large-scale arylamine exposure of workers in the aniline dye industry in Germany and Switzerland to bladder cancer (►Aromatic amines). In Japan, Yamagiwa and Ichikawa were in 1915 the first to demonstrate the formation of tumors in rabbits exposed to coal tar, a mixture of polycyclic hydrocarbons (►Polycyclic aromatic hydrocarbons). The concept that metabolic processes are a necessity for the bioactivation of chemical carcinogens was primarily developed by J. A. and E. C. Miller at the University of Wisconsin in the early 1940s (►DNA damage). Over the next few decades, they and others provided further insight, defining metabolically derived carcinogenic products that react with DNA ("ultimate carcinogens") (Adducts to DNA). However, although the relationship between carcinogens and mutagenesis had been considered, it was not clearly defined. It was only after B. N. Ames developed a (still widely used) bacterial mutation system in which rat liver extracts are able to transform carcinogens into mutagens that the correlation between carcinogenesis and mutagenesis became obvious (►Genetic toxicology). Advances in enzymology and recombinant DNA technology made it possible to discern the role of individual human enzymes in various steps in carcinogen metabolism. Using inbred mouse strains and knockout mice it was possible to demonstrate the critical role of mouse orthologues in carcinogen activation.

Metabolism

Metabolism of carcinogens occurs in many tissues throughout the body (►ADMET screen). Many in vitro studies utilize liver tissue samples because many enzymes of interest are concentrated there. However, for tumors that originate elsewhere, extrahepatic sites are of greater interest. The question of which kind of tissue is most important is related to the site of entry of a carcinogen, as well as how much the activated form(s) of the carcinogen are able to circulate within the body before reacting with the target tissue. Examples of



Carcinogen Metabolism. Figure 1 General paradigm for carcinogen metabolism, including both bioactivation and detoxication reactions.



Carcinogen Metabolism. Figure 2 Major events in the metabolism of the hepatocarcinogen aflatoxin B_1.

important carcinogens and their metabolism are given below (►Xenobiotics).

- Polycyclic aromatic hydrocarbons are systems of fused benzene rings that are found in carcinogenic soots, tars, and tobacco smoke (Polycyclic aromatic hydrocarbons). A widely studied member of this class of compounds is benzo[*a*]pyrene. It is widely believed that the main metabolic pathway involves the oxidation of benzo[*a*]pyrene by cytochrome P450 (P450) to an epoxide. The hydrolysis of this epoxide to a dihydrodiol is followed by another oxidation by P450 that generates highly reactive diol epoxides (►Cytochrome P450). The latter can either react with DNA or are detoxicated by glutathione transferase.
- Aflatoxin B_1 is a mycotoxin and a prominent contributor to human liver cancer (►Hepatocellular carcinoma). A critical feature of its metabolism is the formation of an epoxide by P450 enzymes Fig. 2. The epoxide (with a half life in water of $t_{1/2}$ = 1 s) is able to react with DNA or can be conjugated with glutathione. P450 enzymes can also detoxicate aflatoxin B_1 by catalyzing several other oxidation steps (e.g., the oxidation to 3α- and 9α-hydroxylated products).
- Olefins (alkenes) can be oxidized to epoxides (►Alkylating agents). A member of this group is vinyl chloride, a carcinogenic substance that was shown to cause a rare liver hemangiosarcoma in people working in the rubber industry.
- Another problematic group of substances are *N*-nitrosamines. They can result from some industrial settings but that are also produced endogenously from amines and nitrites in the acidic environment of the stomach. Sources are the so-called tobacco-specific nitrosamines as well as sodium nitrite that are used to preserve processed meats (Tobacco carcinogenesis, tobacco-related cancers). As in the examples stated above, P450 activates *N*-nitrosamines by oxidation. The formation of an alcohol on the adjacent carbon atom yields an unstable product that decomposes and alkylates DNA.
- Another group of chemicals of concern presents in food and tobacco is heterocyclic amines, substances that derive from creatinine and amino acids following pyrolysis (Aromatic amines). Amine activation involves its oxidation by a P450 enzyme to a hydroxylamine (–NHOH). An unstable compound (–NHOAc) is the result of the enzymatic transfer of an acetyl group (►Arylamine *N*-acetyltransferases (NAT) and cancer, ►biomarkers). It ultimately breaks down to a nitrenium ion ($-NH^+$) that can react with DNA. Detoxication involves other P450 enzymes, glutathione transferases, and UDP glucuronosyl transferases.

Mechanisms

Conjugation reactions (including those catalyzed by the enzyme *N*-acetyltransferase) (Arylamine *N*-acetyltransferases (NAT) and cancer) are usually involved in detoxication reactions; they can, however, also be part of bioactivation schemes (►Sulphotransferases). An example is the pesticide ethylene dibromide ($BrCH_2Cl_2Br$) and related compounds where the enzymatic conjugation of ethylene dibromide with endogenous tripeptide glutathione yields a molecule (in this case

glutathione-CH_2CH_2Br) that can react with DNA (►Glutathione-S transferase).

Cancers

Numerous studies support the important role of carcinogen metabolism in human cancers.

First, substances, such as aflatoxin B_1, whose metabolic products can cause cancers Fig. 2, have been identified in foods (Xenobiotics). Second, it has been shown in animal models that either the absence or the induction of certain enzymes that are involved in carcinogen metabolism can have a dramatic effect on chemical-caused cancers. Third, humans are known to show great phenotypic variation in many enzymes involved in carcinogen metabolism. Dramatic effects on the metabolism of drugs have been demonstrated with these enzymes. Large international and other inter-individual differences in cancer incidence, as well as the documented effects of diet on cancer, justify the considerable interest to study carcinogen metabolism, particularly in humans. Research in carcinogen metabolism and its applications can be divided into several areas. Investigating cancer cause and cancer etiology depends upon the understanding of basic chemistry, enzymology, and physiology of metabolic processes as well as how the chemicals react with DNA once they are activated (►Carcinogen macromolecular adducts). Molecular epidemiology utilizes information about carcinogen metabolism in order to establish their relevance in human cancer (►Biomarkers). A related topic is risk assessment, which uses the knowledge of carcinogen metabolism derived from animal bioassay studies and sometimes epidemiology, to determine critical exposure levels of environmental carcinogens in humans (►Cancer epidemiology).

Metabolism mechanisms play an important role in cancer safety assessment studies of prospective new drugs, including those used to treat cancer. Another important area is chemoprevention where beneficial effects of certain chemicals are investigated, e.g., their ability to change the metabolism of carcinogens.

►Detoxification

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Carcinogenesis

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C

Definition

Carcinogenesis is the process by which cancer develops in various tissues in the body.

Characteristics

In most cases carcinogenesis occurs via a stepwise process that can encompass a major fraction of the lifespan (►multistep development). These progressive stages often include hyperplasia, dysplasia, metaplasia, benign tumors and then, eventually, malignant tumors. Malignant tumors can also undergo further progression to become more invasive and metastatic, autonomous of hormones and growth factors and resistant to chemotherapy or radiotherapy.

Causes

Known causes of carcinogenesis include various chemicals or mixture of chemicals present in several sources. This includes cigarette smoke, the diet, the workplace or the general environment, ultraviolet and ionizing radiation, specific viruses, bacteria and parasites and endogenous factors (►oxidative DNA damage), DNA depurination, deamination). According to the International Agency for Research on Cancer (IARC) 69 agents, mixtures, and exposure circumstances are known to be carcinogenic to humans (group 1), 57 are probably carcinogenic (group 2A) and 215 are possibly carcinogenic to humans. Some of these agents, or their metabolites, form covalent ►adducts to DNA and are ►mutagenic. Others act at the epigenetic level by altering pathways of signal transduction and gene expression. These include tumor promoters, growth factors and specific hormones. Dietary factors also play an important role. Fruits and vegetables often have a protective effect. Excessive fat and/or calories may enhance carcinogenesis in certain organs. Hereditary factors can also play an important role in cancer causation. Indeed, human cancers are often caused by complex interactions between these multiple factors. An example is the interaction between the naturally occurring carcinogen ►aflatoxin and the chronic infection with hepatitis B virus in the causation of liver cancer in regions of China and Africa.

Molecular Genetics

Recent studies indicate that the stepwise process of carcinogenesis reflects the progressive acquisition of

activating mutations in dominant acting ►oncogenes and inactivating recessive mutations in ►tumor suppressor genes. It is also apparent that epigenetic abnormalities in the expression of these genes also play an important role in carcinogenesis. Thus far over 100 oncogenes and at least 12 tumor suppressor genes have been identified. Tumor progression is enhanced by genomic instability due to defects in DNA repair and other factors. The heterogeneous nature of human cancers appears to reflect heterogeneity in the genes that are mutated and/or abnormally expressed. Individual variations in susceptibility to carcinogenesis are influenced by hereditary variations in enzymes that either activate or inactivate potential carcinogens, variations in the efficiency of DNA repair and other factors yet to be determined. Age, gender and nutritional factors also influence individual susceptibility.

Clinical Relevance

Prevention

Cancer is a major cause of death throughout the world. Therefore, the prevention of carcinogenesis is a major goal of medicine and public health. The carcinogenic process can be prevented by avoidance of exposure to various carcinogenic factors (i.e. cigarette smoking, excessive sunlight, etc.), dietary changes, early detection of precursor lesions and chemoprevention.

►Toxicological Carcinogenesis
►Nucleoporin

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Carcinogenicity Studies

Definition

Are conventionally conducted in rats and mice where several groups of animals are administered drug for a period typically of 2 years which is a large part of the lifetime of these species. The route chosen is that of the intended clinical dosage route where practicable. The high dose group is usually at or near the maximum tolerated dose when physically achievable. Group sizes are larger (50 per sex per group) so that any effect on tumor development can be detected. Old rodents develop tumors naturally so that it requires a sufficient numbers of animals for an effect of the drug to be seen against this background. These animals are simply housed and treated without any other intervention for the period of the experiment, killed humanely either when sick or at 2 years and subject to a detailed autopsy. From each animal at least 30 tissues and all tumors are examined and diagnosed by a pathologist. The numbers and types of tumors would be evaluated and statistical analysis conducted to detect any increases in the incidence of tumors in drug-treated animals. These studies are not normally performed with anticancer drugs that are strongly mutagenic in short term tests. This would be considered evidence of carcinogenic activity and the clinical use of such drugs would be limited to life-threatening conditions.

►Preclinical Testing
►Carcinogenesis

Carcinoid

►Bile Duct Neoplasms

Carcinoid Syndrome

Definition

Carcinoid Syndrome is a clinical syndrome characterized by a variety of symptoms such as diarrhea, flushing, bronchial constriction, and right heart failure. It is caused by the release to the systemic circulation by ►carcinoid tumors cells of one or several of the following hormones: serotonin, histamine, tachykinins, bradykinins, and prostaglandins.

►Neuroendocrine Carcinoma

Carcinoid Tumors

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Synonyms

Neuroectodermal tumor; NET; neuroendocrine carcinoma; Argentaffin carcinoma

Definition

Carcinoid tumors represent a family of diseases derived from ►neuroendocrine cells. These tumors were first described by Langhans in 1867 but were not described in detail until Lubarsch described them in 1888. The name *karzinoide* was not used until 1907 by Oberndorfer, and was chosen to reflect his idea that these were benign growths. However, these tumors have a wide range of clinical presentations and outcomes from benign to malignant. Clinicians must recognize the nature of carcinoid disease, because these often have a significantly different clinical course than typical carcinomas occurring within the body. Additionally, with few exceptions, neuroendocrine tumors (NETs) comprise a tiny fraction of tumors within any specific organ. These neoplasms cause <1% of all malignancies in the United States, currently occurring at a rate of 2.5–4.5/100,000 people.

NETs can arise in almost any tissue within the body, but most are derived from the embryonic ►foregut, ►midgut, and ►hindgut with over two-thirds of these occurring in the gastro-entero-pancreatic axis, with approximately 25% occurring in the foregut. NETs of the ►midgut (true carcinoids) are the only ones which secrete ►serotonin and only these tissues give rise to tumors causing carcinoid syndrome. Carcinoid syndrome is the most recognized complication of carcinoid tumors, originally described in 1890 by Ransom. This manifestation was not recognized as an endocrine or paraneoplastic syndrome until 1914 by Gosset. Carcinoid syndrome is caused by carcinoid in the midgut which secretes serotonin. In its localized state, venous drainage of tumor secretions are metabolized by the liver and serotonin is deactivated. However, upon metastasis to the liver, secretions are not as readily processed, serotonin not deactivated, and so is released into systemic circulation, causing the syndrome. This is often characterized by flush, diarrhea, and cramping.

Characteristics

Within the GI system, the small intestine is the most frequent site of NETs, followed by the rectum. Overall, 32–45% of NETs will spread, but the propensity for these tumors to metastasize also varies individually by the organ in which they develop. As with most neoplasms, the set of symptoms that each tumor will cause the patient is dependent on where the tumor begins, and thus NETs will probably present in different stages depending on site. For instance, >70% of NETs starting in the cecum and pancreas spread regionally or distantly, compared to NETs of the rectum and stomach, which spread less than 18 and 33% of the time, respectively. Overall 5-year survival rates for NETs are 67%, with survival rates after spread to localized or distant sites exceeding 75% and below 40% respectively. Accordingly, 5-year survival rates vary by site, with the highest and lowest rates being in the rectum (88%) and liver (18%), regardless of stage.

Risk for developing NETs can also be part of inherited familial cancer syndromes. Although rare, this most frequently occurs in ►Type I multiple endocrine neoplasia (►Multiple endocrine neoplasia Type 2) and ►Type I neurofibromatosis, with isolated case reports in other inherited cancer syndromes. However, the site of NETs in these syndromes is most often the duodenum or pancreas, and not the midgut, which is the site of most sporadic carcinoids. The molecular genetics of *MEN1* (the gene which is mutated MEN I) in carcinoids is complex, but includes point mutation as well as loss of one allele. In lung NETs, for example, 4/11 tumors showed both point mutation as well as deletion of one allele. Additionally, *MEN1* is located on chromosome 11q13, which is frequently lost from NETs and will be discussd later. *MEN1* which encodes the protein menin, is frequently altered in sporadic carcinoid, in addition to Type I MEN. Menin behaves as a nuclear protein when consisting of the wild-type sequence, but does not localize to the nucleus in many mutated forms. As with almost all genes which are implicated in familial cancer syndromes, Menin predictably behaves as a tumor supressor gene. It has been shown to dampen transcriptional transactivational activity of such oncogenes as NF-κβ and JunD. However, menin is also known to behave as an oncogene, and in fact is required for mixed lineage leukemogenesis in relevant models of the disease. Menin interacts with the Mixed-Lineage Leukemia (MLL) protein, which is also known to regulate transcription. Indeed, deletion of the Menin-interaction domain from MLL results in failed leukemogenesis in a validated model for this disease. MLL behaves as a ►histone methyltransferase for histone H3-K4, and H3-K4 methylation is known to be strongly associated with transcriptional activation. Therefore, it appears Menin retains transcriptional activating as well as repressing activity, and behaves in oncogenic as well as tumor suppressive signaling pathways. Only

a handful of other genes are known to be definitively mutated in NET and carcinoid, including succinate-ubiquinone oxidoreductase subunit D (*SDHD*) and ►β-catenin (►APC/►β-Catenin Pathway). Notably, mutation of "classical" oncogenes and tumor supressors like ►*RAS* and ►*TP53* are not present in NETs.

Much more work has been done to identify chromosomal losses in NETs or carcinoid tumors. Identification of regions of DNA which are consistently lost in carcinoid tumors implies that carcinoid- or NET-specific tumor suppressors may reside in these deleted regions. These regions most frequently include 11q, which contains *MEN1* as previously discussed, but this region may contain other tumor suppressors as well. Another chromosomal region frequently lost in these malignancies is18q. This occurs in 33–88% of NETs and varies by NET site. For instance, lung NETs, like carcinoids, often have loss of 11q, but rarely have loss of 18q. Several known tumor suppressors reside on 18q which include two transcription factors in the ►TGF-β signaling pathway (►Smad proteins in TGF-β signaling) (*SMAD2* and *SMAD4*) and *DCC*, all of which are known to be altered in pancreatic and colorectal cancer. At least one study has delimited the region of lost genomic DNA in midgut carcinoids to the sequence between 18q22 and the 18q ►telomere. Interestingly, *SMAD2*, *SMAD4*, and *DCC* do not reside in the region of recurrent loss in carcinoid, strongly suggesting that other genes in 18q22-qter are carcinoid tumor suppressors. Less evidence is available regarding the loss of 9p21 (which contains *p16/ARF*), 3p (which contains ►peroxisome proliferator-activated receptor and cancer, *RASSF1A*, ►von Hippel-Lindau tumor suppressor gene, ►*FHIT*, and ►retinoic acid) and 16q21.

The ability to measure chromosomal abnormalities and point mutation of specific genes in NETs combined with the fact that NETs are often multifocal has led to the question of whether each tumor focus originated as an independent event or if multifocality is secondary to local invasion and metastasis. This question can be answered in a variety of ways, but currently the most definitive method is based on molecular analysis of the genomic content of each individual focus. By analyzing regions which are frequently lost and/or genes which are commonly mutated in each tumor individually, inferences regarding the clonality of multiple tumor foci can be drawn. If multiple tumors form independently, then one would expect that genetic alterations at specific loci would occur randomly. However, If the same genetic alterations are seen in a majority of tumors from the same patient, then it is likely that the tumors do not form independently. This was shown by Katona et al using microdissected tissues from patients with multiple pancreatic NETs or carcinoids. Seventy-two tumors from 24 patients were analyzed using LOH analysis. The results of this study showed that indeed at least some tumors show identical patterns of allelic loss, indicating that carcinogenesis in these patients probably occurred once and that each tumor focus represents a locally invasive metastasis. However, the largest fraction of patients had tumors with non-identical LOH pattern in each tumor, indicating that these tumors actually formed independently. Other groups have shown a similar pattern of evidence with different genetic markers implicating multiple independent foci in carcinoid carcinogenesis.

The implications of identifying the mechanism of multiple tumor formation are directly related to therapy, especially with regard to surgical intervention. This is directly related to the pathogenesis of NET formation. One would surmise that multiple tumors arise independently in an organ for one of a few reasons: cells were exposed to an endogenous mitogen or trophic factor; cells were exposed to a genotoxin, causing genetic damage to accumulate in many cells which independently acquire further mutations; or intrinsic germline ►polymorphisms provide a propensity for tumors to form in multiple independent cells. In all three cases, the idea of a ►field defect, first described by Slaughter in 1953 plays a significant role. A field defect may be thought of as normal-looking tissue which, at a gene-by-gene level, is not normal. In other words, it is a pre-benign lesion. If the entire organ at the site of NET formation harbored pre-malignant genomic changes, then more aggressive surgical intervention would predictably result in better outcomes for patients, because the procedure would leave behind tissue with a propensity to continue to undergo carcinogenesis. However, if multiple NETs arise in an organ due to local spread, then wider surgical margins would probably not result in higher survival rates, assuming the procedure was able to remove all of the malignant tissue.

Much has been learned about carcinoid since its original description over 100 years ago. Much is left to be learned about this disease as well, including exactly which signaling pathways are altered in the disease and identification of genes which are in chromosomal regions of frequent alteration. As yet, there are very few reports of experimental carcinoid models which to study, both at the cell line or animal level. Detailed epidemiological studies become difficult to perform due to the rare nature of this disease, which also complicates prospective studies. Overcoming or averting these obstacles will significantly speed the rate of progress in carcinoid research.

►Neuroendocrine Carcinoma

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Carcinoma

Definition

Malignant neoplasm derived from epithelial tissue.

►Salivary Gland Malignancies
►EpCAM
►Pathology

Carcinoma In Situ

Definition

CIS is a lesion that exhibits the cytologic changes of invasive ►carcinoma but that is limited to the epithelium with no invasion of the basement membrane. Transitional cell CIS carries a high risk of eventual progression to muscle invasion.

Carcinoma of the Adrenal Cortex

►Adrenocortical Cancer

Carcinoma with Amine Precursor Uptake Decarboxylation Cell Differentiation

►Extrapulmonary Small Cell Cancer

Carcinomatosis

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Synonyms

Peritoneal carcinomatosis; Peritoneal malignancy; Peritoneal tumor

Definition

Until 1980s, "Carcinomatosis" was a condition typically characterized by widespread dissemination of malignant metastases throughout the body. It was then used to describe conditions with more limited spread as in "►leptomeningeal carcinomatosis," "►lymphangitic carcinomatosis," and "peritoneal carcinomatosis." Since 2000, due to the worldwide diffusion of cytoreductive surgery combined with intra abdominal chemotherapy, "carcinomatosis" is a word now describing tumoral spreading within the peritoneal cavity.

Peritoneum is the mesothelial tissue (serosa) that covers most of the organs in the abdominal cavity as well as the interior part of the abdominal wall (parietal peritoneum covers the abdominal walls and visceral peritoneum covers intra abdominal organs like stomach, colon, gallbladder, spleen, liver, etc). Peritoneum, which is now regarded as an organ by itself, could be the site for "peritoneal carcinomatosis" arising from primary peritoneal tumors or from metastatic nonperitoneal tumors.

Characteristics

Etiology of Peritoneal Carcinomatosis

Primary peritoneal carcinomatosis could arise (i) from pseudomyxoma: a rare border line or malignant mucinous tumor, generally originating from the appendix, incidence of which is estimated 1/million/year and sometimes called the "jelly-like fluid disease" – only the histopathologic analysis is able to determine the grade 1(peritoneal adenomucinosis, 84% 5-year survival), the grade 3 (peritoneal mucinous adenocarcinoma,

7% 5-year survival), and the grade 2 (intermediate, 37% 5-year survival), (ii) from mesothelioma: peritoneal location of mesothelioma is not so frequent as pleural location, and about 250 new cases are diagnosed in USA each year; contrary to pleural mesothelioma, its asbestosis exposure relation remains controversial mainly in women, and other carcinogenic agents were reported such as virus, abdominal irradiation, chronic peritonitis, mica or thorium dioxide exposure, (iii) from primary serous carcinoma (a very rare disease mostly diagnosed in women and sometimes related to a chromosome deletion), (iv) from desmoplastic tumors, or (v) from psammocarcinoma (the estimated incidence for these three last diseases remains unknown and is probably <1/10 million/year).

Metastatic peritoneal carcinomatosis is common; it mainly arise from colorectal cancer (detected in about 10% of patients at the time of primary cancer resection), ovarian cancer, and gastric or pancreatic cancers. The mechanisms causing carcinomatosis are multifactoral and include peritoneal dissemination of free cancer cells as a result of serosal involvement of the primary tumor, implantation of free cancer cells caused by the presence of adhesion molecules, and presence of cancer cells in lymph fluid or venous blood retained within the peritoneal cavity (the role of laparoscopic approach in malignant cell diffusion, as well as the role of surgeon during the tumor handling is still controversial). Metastatic peritoneal carcinomatosis could also arise from extra peritoneal cancer (lymph and/or blood dissemination) such as breast cancer, uterus cancer, thyroid cancer, etc.

Natural history of metastatic peritoneal carcinomatosis is well known from three international prospective series; without curative treatment the overall median survival is 3–7 months, according to the stage of the peritoneal carcinomatosis.

Diagnosis

Primary and metastatic peritoneal carcinomatosis have no specific symptoms; due to the absence of specific symptoms, clinical diagnosis could be a rather difficult one. Peritoneal carcinomatosis is often diagnosed during surgical exploration of a known primary tumor; if not, symptoms can include abdominal or pelvic pain, changes in bowel functions (up to intestinal obstruction), increase of abdominal volume (caused by ascitis or by tumoral volume itself), abdominal swelling and bloating, infertility (mainly in pseudomyxoma), loss of weight, anorexia, asthenia, etc. Clinical examination of the patient could reveal ascitis or malignant nodules detectable through the abdominal wall.

Abdominal ultrasonography could reveal ascitis or primary ovarian cancer but remains unuseful for detection of peritoneal lesion <1 cm in diameter. While CT scan and MRI help the diagnosis when peritoneal carcinomatosis is made of >5 mm in diameter lesions, PET scan is still under evaluation. Laparoscopic exploration is useful to perform large biopsies and to stage the peritoneal carcinomatosis using Gilly staging system (Fig. 1) and Sugarbaker peritoneal cancer index (Fig. 2); combination of these two scoring systems was demonstrated as an independent survival predictive factor.

Stages carcinomatosis	
Stage 1	< 5mm, one part
Stage 2	< 5mm, diffuse
Stage 3	5mm to 2 cm
Stage 4	Large malignant cakes

Carcinomatosis. Figure 1 Gilly staging system for peritoneal carcinomatosis.

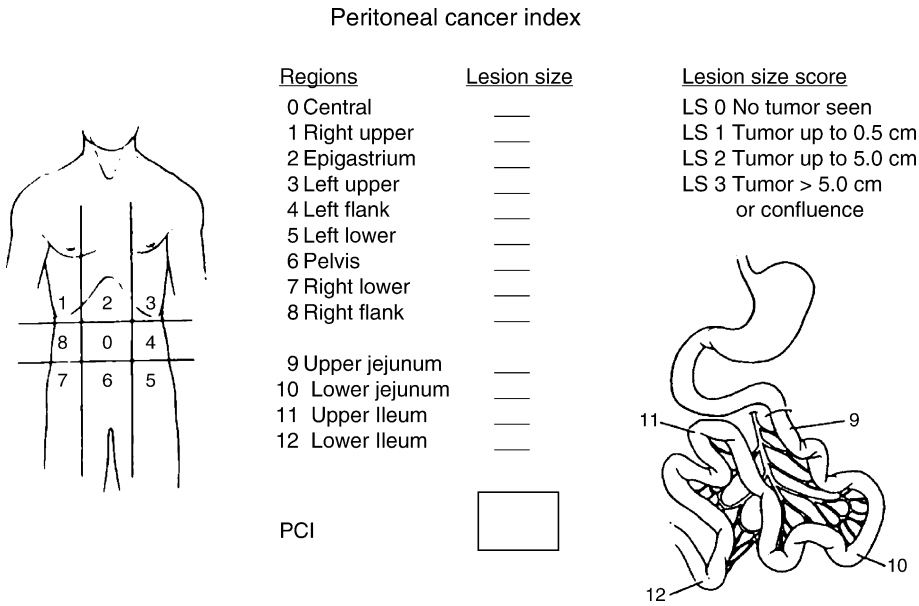
There are no biologic specificity; tumoral markers (CEA, CA 19-9, CA 125, etc) could be increased in relation to or not with the primary tumor and molecular markers are still being evaluated.

Microscopic examination of biopsies (or surgically removed tumor) is the key for the diagnosis and could need immune analysis using calretinine, B72.3, Ber EP4, estrogen, and progesterone receptors (mainly to differentiate mesothelioma and primary serous carcinoma).

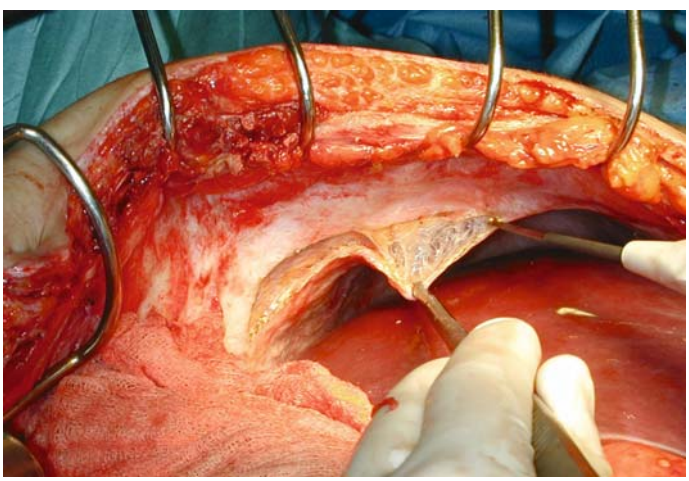
Treatment

While peritoneal carcinomatosis was thought to be a terminal disease for a long time, most oncologists regarded it a condition only to be palliated. Systemic chemotherapy (mainly 5-Fluoro-uracil-, oxaliplatinum-, and irinotecan-based) combined or not with antiangiogenic drugs achieve a 15-month median survival (most of the available trials are related to liver and/or pulmonary metastases with only few information regarding peritoneal metastases). Since the 1980s, a renewed interest in peritoneal surface malignant diseases developed through new multimodal therapeutic approaches, mainly with cytoreductive surgery combined with intraperitoneal chemotherapy (using mitomycin C, cisplatinum, oxaliplatinum, doxorubicin) with or without hyperthermia (42–43°C). Despite these aggressive and multidisciplinary approaches are reserved to experienced teams, many phase II and phase III studies revealed a strong advantage for selected patients with colorectal carcinomatosis, peritoneal pseudomyxoma, and mesothelioma.

Cytoreductive surgery (also called peritonectomy procedure) aims to remove as much tumor as possible within the abdominal cavity. The objective is to clear the entire peritoneal cavity of all macroscopic



Carcinomatosis. Figure 2 Sugarbaker peritoneal cancer index (PCI) for peritoneal carcinomatosis.



Carcinomatosis. Figure 3 Operative view of right diaphragmatic cupula peritonectomy (the parietal peritoneum is stripped to remove all the macroscopic lesions).

detectable disease. Procedures for cytoreductive surgery (Fig. 3) have been described extensively by Sugarbaker: parietal peritonectomy (which is a stripping of the parietal peritoneum) combined with organ resections (where visceral peritoneum is involved) followed by immediate intra peritoneal chemotherapy with or without hyperthermia (using closed or opened technique) aims to clear all potential microscopic residual disease. Whatever the used technique is, intraperitoneal chemohyperthermia is defined as a heated fluid circulation with cytotoxic drugs for 30–90 minutes within the abdominal cavity under a nonstop control of core temperature as well as cardiac flow rate. At least, combination of optimal cytoreductive surgery and intraperitoneal chemohyperthermia is a long surgical, oncologic, and anesthetic procedure (5–10-h long) which requires an experienced multidisciplinary team.

These therapeutic strategies need a strict patient selection (younger than 70 years who have not had cardiorespiratory or renal failure) and need to be done in experienced centers involved in the management of peritoneal surface malignancies. Combination of cytoreductive surgery and intra peritoneal chemotherapy leads to a 1–5% mortality rate and 30% morbidity rate (related to the extent of the carcinomatosis, the duration of surgery and the number of digestive anastomoses performed).

Postoperatively, the patients also received systemic chemotherapy, according to the primary tumor location.

Results are currently encouraging ones. For colorectal carcinomatosis, the Dutch randomized trial showed that 2-year survival was 43% using cytoreductive surgery and intraperitoneal chemohyperthermia versus 16% in the control group; the international registration (including more than 500 patients) showed that 5-year survival was 33% for patients treated by optimal cytoreductive surgery combined with intraperitoneal chemotherapy. Concerning pseudomyxoma (the natural history of this disease is not extensively documented but the prognosis is better than that for colorectal carcinomatosis), the 5-year survival is 80% for patients with complete cytoreductive surgery (whatever the pathologic grade is). Concerning peritoneal mesothelioma (the median survival in the past was approximately 12 months), optimal cytoreductive surgery and intraperitoneal chemohyperthermia achieve a 5-year median survival. Peritoneal carcinomatosis arising from gastric or ovarian cancer, as well as the use of intraperitoneal chemohyperthermia in a prophylactic way for high locoregional recurrence risk tumors are still under evaluation.

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CARD

Definition

Caspase recruitment domain is a protein-binding motif that interacts with caspases through CARD–CARD interaction.

►APAF-1 Signaling

Cardiac Dysrhythmia

Definition

Any abnormality in the rate, regularity, or sequence of cardiac activation.

►Rituximab

Cardiac Myxoma

Definition

Is a benign tumor of the heart and the most common type of heart tumor (►cardiac tumors) in adults. Cardiac myxomas can appear in an isolated case or in families, sometimes as part of an hereditary syndrome called the ►Carney complex.

Cardiac Tumors

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Definition

Cardiac tumors, like all other tumors, may be classified as primary and secondary and were previously considered as incidental curiosities seen at autopsy. In comparison to the incidence and range of neoplastic proliferations seen in other organs, tumors of the heart are uncommon and have a fairly limited morphologic spectrum. With the advent of innovative diagnostic techniques such as echocardiography, computed tomography (CT) scan and the magnetic resonance imaging (MRI) and better delineation, this has changed and most tumors are now diagnosed antemortem. With new therapeutic techniques, both surgical and pharmacologic, patients with cancers live longer and show more evidence of cardiac involvement.

Characteristics

The heart was considered the "Royal Organ" and hence immune to damage, including tumors. We know now that the heart is as prone to disease as most other organs and that includes tumors. The prevalence of immunodeficiency states (especially ►human immune deficiency virus infection) has also lead to an increase in some cancers, involving the heart.

Incidence

Secondary tumors or metastases to the heart and pericardium are 100–1,000 times more common than the primary cardiac tumors with an incidence of about 1.23%. In contrast, the frequency of primary neoplasms ranges from 0.001% to 0.030%, and three quarters of these are benign. The types of benign tumors vary with age. The myxoma and papillary fibroelastoma are more common in adults, while in children, the common ones are cardiac rhabdomyoma and fibroma. Primary cardiac cancers comprise the remaining quarter, and most of these are ►sarcomas.

Clinical Features

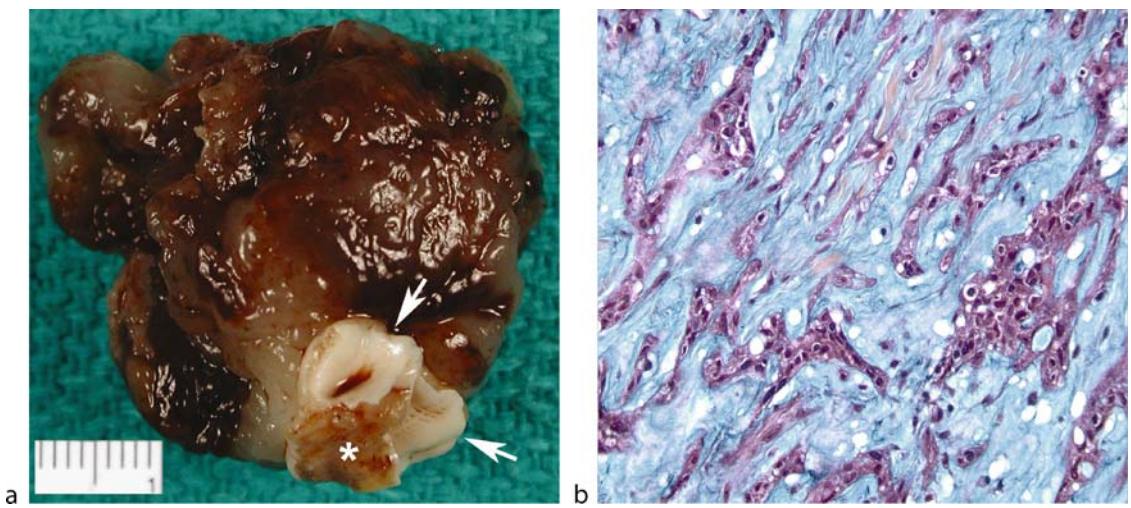
Cardiac tumors, in general, can have a varied clinical presentation, and the pattern depends on the location of the tumor. Most primary tumors, benign and malignant, usually produce intracavitary masses. Such lesions produce one or more patterns of the classic triad of constitutional symptoms, obstruction to the inflow and outflow of the blood within the cardiac chambers and/or complications related to tumor ►embolization. Tumors with significant myocardial involvement may lead to arrhythmias or features related to coronary artery disease due to narrowing or obliteration of small intramyocardial coronary arteries. If the tumor is large or multifocal, it can by itself produce frank, symptomatic cardiac failure. Pericardial tumors are often associated with varying degrees of serous or hemorrhagic effusion with or without ►tamponade. This is also the presentation with ►metastatic tumors, which more commonly involve the pericardium. It is noteworthy that 12% of primary tumors and almost 90% of metastases to the heart are clinically silent and detected only at routine assessment or are a surprise at necropsy.

Primary Tumors

Benign

Cardiac myxoma is the commonest tumor encountered in adults and accounts for nearly half the cases. It is more common in females than in males. While no age is immune, it is more common in the 30–60 years old age group. A familial predisposition has been noted in some of these patients. In the familial form, the tumors appear at younger age, involve the right side of the heart, are often multicentric and have a high rate of recurrence. There is a germline mutation in PRKARIA gene, and other associations such as pigmented adrenal micronodular hyperplasia, cutaneous melanocytic or neurogenic tumors are inherited as autosomal dominant disease. These are collectively known as the ►Carney complex. A majority of myxomas are solitary, arising as smooth surfaced, firm, grey-white sessile polypoid masses on either side of the interatrial septum (Fig. 1a and b). Approximately 75% of myxomas are located in the left atrium, where they produce features related to the classical triad. Patients with this tumor often present with a typical complaint of "hearing a plop" as they bend forward, associated with severe shortness of breath, and that they hear a second "plop" and improvement of symptoms, as they bend backwards. This is due to the tumor "plopping" into the mitral valve orifice and obstructing it. Systemic embolization is often related to the softer, gelatinous, blunt papillary fronds.

Superimposed thrombus or even infection can occur and these can embolize. The cut surface characteristically appears "wet," yellowish white, gelatinous and translucent with foci of fibrosis, hemorrhage, calcification and rarely ossification. Histologically, nestling amidst the mucopolysaccharide (jelly like), are the



Cardiac Tumors. Figure 1 (a) Polypoidal glistening hemorrhagic myxoma in the left atrium, resected with underlying interatrial septal endocardium (*arrows*) and myocardium (*). (b) Myxoma cells in cords within a mucopolysaccharide (greenish blue) rich stroma (b). stain Movat pentachrome, original magnification, ×10.0).

myxoma cells or lipidic cells arranged in the form of cords or form vessel-like structures. Foci of hematopoiesis and intestinal glandular metaplasia may be seen occasionally.

The next common tumor is the papillary fibroelastoma, representing about 8% of cardiac tumors, with no gender predilection. The tumor arises from the endocardium, especially over the cardiac valves, particularly the aortic. The true incidence is not known as they are often small, escaping gross detection; besides the surgically excised native valves are not always subjected to rigorous histological examination. The morphology of this tumor is best appreciated by holding the mass under water, when a short central stalk with multiple papillary fronds (up to 1 cm or more in length) are seen (Fig. 2a and b). The whole tumor resembles a sea anemone. These papillae are delicate and often break off and embolize. Additionally, thrombi can also occur over the surfaces of the papillae with subsequent embolization. In fact, one of the presentations of this tumor is the sudden development of blindness of one eye or the sudden development of chest pain. Sudden death has also been reported. The fronds have a soft myxoid core surrounded by collagen and elastic tissue fibers, lined by endothelial cells.

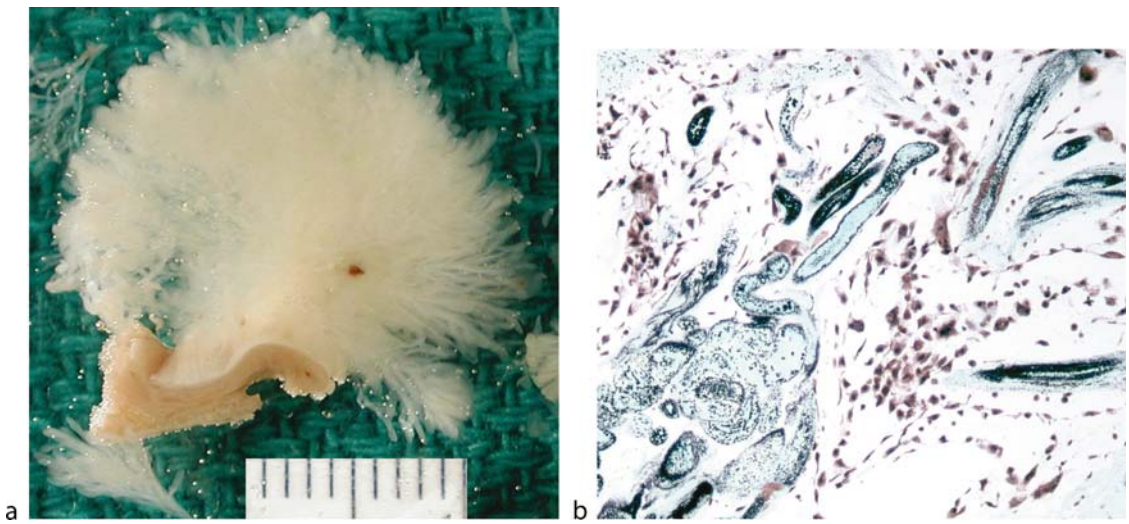
Rhabdomyoma, considered a hamartoma, is the most common tumor found in children, with an overall incidence of about 5%. Sporadic rhabdomyomas are mostly seen as solitary, small or large (0.1 cm or more), firm, opaque white, and well-circumscribed endocardial nodules. They are usually found in the left ventricle and the interventricular septum and produce obstructive symptoms. In contrast to the sporadic variants, about 50% of patients with tuberous sclerosis have multiple rhabdomyomas, leading to intra-uterine hydrops fetalis and stillbirths. It is important to rule out the presence of tuberous sclerosis in not only multiple tumors, but also in patients with solitary lesions. The rhabdomyomas are composed of ballooned out cardiac myocytes with clear vacuolated pink-staining cytoplasm, radiating from the centrally located nucleus to the periphery, and responsible for the "spider-cell" appearance.

Cardiac fibroma, the second common tumor in the pediatric population; is seen as a solitary, circumscribed, firm to hard, grey-white fasciculated tumor occurring in the ventricular chambers with a predilection to affect the interventricular septum. The mean age of presentation is around 13 years and these are the commonly resected tumors at that age. There is a proliferation of innocuous appearing fibroblasts, which characteristically entrap islands of myofibers (pseudo-invasion). With increasing age, the tumors are rendered paucicellular, with multifocal areas of calcification.

Both rhabdomyomas and fibromas are considered as congenital lesions that undergo gradual spontaneous regression or cease to be progressive. Other benign lesions include the hemangioma (vascular) and lipoma (fat-cell tumor). Brief mention must be made of the cystic tumor of the atrioventricular node, the smallest known tumor, associated with sudden death. It is composed of small fluid-filled endoderm-derived spaces in a connective tissue stroma. The spaces may be lined a variety of epithelial and epitheliod cells.

Malignant

Sarcomas are the most common malignant primary tumors of the heart, forming ~10% of the surgically



Cardiac Tumors. Figure 2 (a) "Sea anemone"-like appearance of papillary fibroelastoma, photographed under water. (b) Delicate papillae with a core of collagen and elastic, lined by plump endothelial cells (b) Stain Hematoxylin and eosin, original magnification, ×10.0).

resected cardiac neoplasms. Angiosarcoma is the commonest with an incidence of 35–37%, slight male predominance and occurs in the third to fifth decade of life. Most often, it is seen as an irregular, soft, friable, hemorrhagic mass in the right atrium, projecting into the cavity as well as infiltrating the wall and adjacent structures. Clinical presentation is often with recurrent, hemorrhagic pericardial effusion and/or symptoms related to metastases (seen in more than half the patients), frequently pulmonary or rarely even distant. At times, the angiosarcomas may be purely pericardial, forming a sheet-like mass. Depending on the degree of differentiation, angiosarcomas show irregular, anastomosing vascular channels lined by plump, atypical endothelial cells with papillary projections or sheets of epithelioid or spindled cells. If areas of spindle-shaped cells predominate, presence of red blood cells in the stroma and presence of intracellular vacuoles offer important clues to the diagnosis. Areas of necroses and brisk mitotic activity are evident. Some of the tumors are also associated with chromosomal abnormalities as seen by cytogenetic analysis.

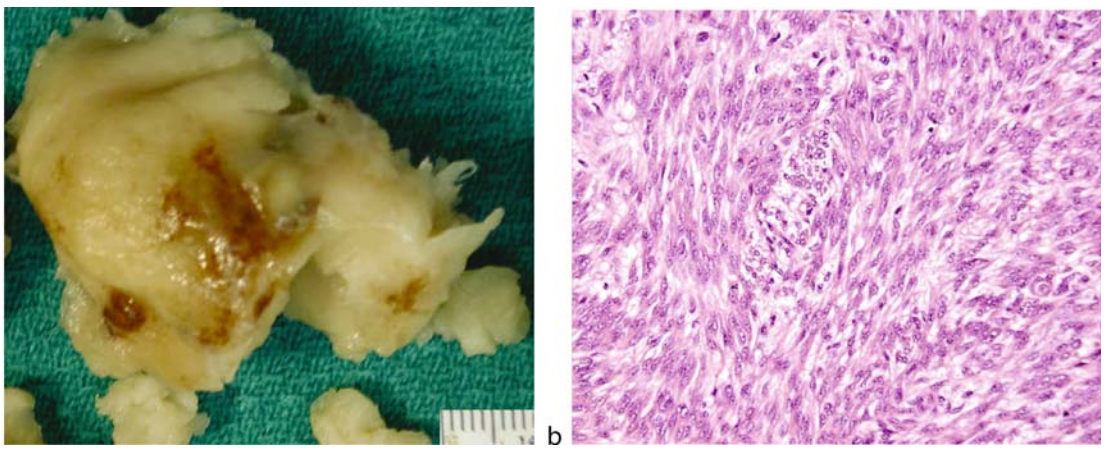
The next common sarcomas are a group of sarcomatous proliferation, which share many morphological and clinical features. These are designated as myofibroblastic sarcomas and include malignant fibrous histiocytoma, fibrosarcoma, fibromyxosarcoma and myxosarcoma (Fig. 3a and b). Many of them in addition can show focal osteosarcomatous or chondrosarcomatous differentiation. The mean age of presentation is around 40 years of age with no gender predilection. They form bulky, lobulated polypoidal grey-white masses projecting into the left atrium with the result that many of these patients present with the left-sided inflow tract obstruction. Most of them resemble their soft-tissue counterparts, but often these tumors in their cardiac location show a prominent myxoid change. Hence at times, many such tumors are misdiagnosed as myxomas.

Undifferentiated sarcomas constitute about 10–24% of these malignant cardiac tumors. They are designated undifferentiated, as they are not associated with specific, classifiable morphological, immunohistochemical or ultrastructural features. These are present as large, lobulated, polypoidal masses, chiefly in the left-sided chambers in middle-aged adults. They can affect either gender, with a wide age range of occurrence. Patients are often symptomatic at an early stage. The tumors are composed of proliferating spindle cells or epithelioid looking cells and pleomorphic cells in varying pattern.

Rhabdomyosarcomas, which constitute about 5% of the tumors, are common in younger patients, especially children. They too form very bulky and infiltrative masses in either of the ventricular chambers. Majority are embryonal rhabdomyosarcomas. The other sarcomas include leiomyosarcoma and synovial sarcoma, though virtually any type of soft tissue sarcoma may occur.

Primary lymphomas of the heart are extremely rare. However, their incidence has increased, in patients who are HIV positive as well as those with other causes of immunosuppression. An important criterion in these patients is the demonstrable absence of lymphomatous proliferation or any other sarcoma at any other site, before they can be categorized as a primary cardiac tumor. These patients therefore require thorough imaging investigations. In this type of lymphoma, there is a slight male predominance, and multiple, soft to firm, creamy white nodules are seen, especially in the right atrium. They mostly exhibit the full range of the neoplastic B cell proliferation, though large cell lymphomas are common.

Sarcomas can also affect the great vessels. Leiomyosarcomas involve chiefly the veins especially the



Cardiac Tumors. Figure 3 (a) Left atrial sarcoma resected in pieces. (b) Malignant spindle-shaped cells arranged in intersecting bundles, suggestive of leiomyosarcoma (b) Stain- Hematoxylin and eosin, original magnification, ×20.0).

inferior vena, and less commonly the superior vena cava and azygous vein. Women with mean age of 49 years are affected, with clinical features of pain and venous obstructions. The growth may be intraluminal or extraluminal. Extraluminal tumors, despite their extension into the surrounding tissues, appear circumscribed and lobulated. Metastases usually involve the lungs, though other organs like liver or kidney can also be affected, especially with inferior ven caval tumors. Sarcomas of the great arteries, pulmonary trunk and the aorta arise from the multipotential mesenchymal cells of the intima, and designated as intimal sarcomas. Despite the intimal origin, the tumor can have an intraluminal or mural growth patterns. The patients are usually elderly and the symptoms depend on the growth pattern. Aortic involvement is more common than pulmonary. The luminal tumors often produce sheet-like or plaque-like growths, which in due course of time can form an intraluminal polyp. There can be superimposed thrombi, which may form the major chunk over the tumor, leading to misleading diagnosis. The patients present with effects of obstruction or embolization. The mural growth pattern is uncommon and there is medial and adventitial infiltration with resultant local invasion. Most of them are of the undifferentiated type, which have 50% shorter survival as compared with the differentiated types. The latter includes myxofibrosarcoma, angiosarcoma, malignant fibrous histiocytoma, leiomyosarcoma or myxoid chondrosarcoma.

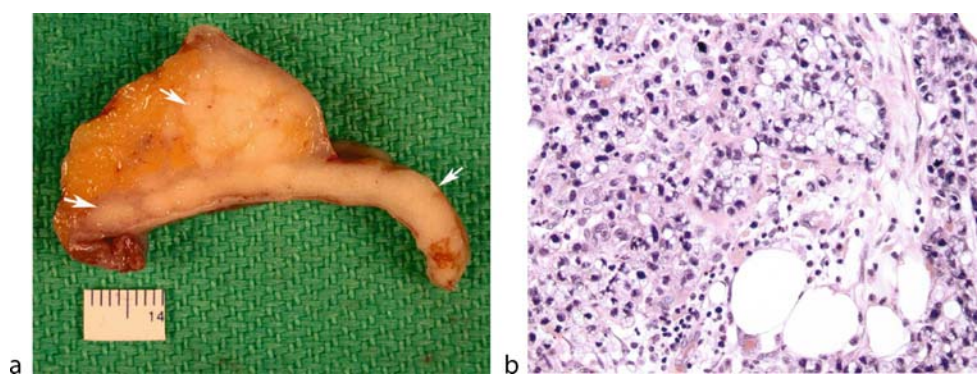
Secondary or Metastatic Tumors

The commonest tumor seen in the heart is the metastatic or secondary tumor, and is seen in at least 3% of patients with cancer, that is at least 3% of patients with a malignancy(usually advanced ones) have cardiac metastases (Fig. 4a and b). Primary cancers can spread to the heart by direct extension from adjoining structures, hematogenous or lymphatic spread and sometimes as extensions through the inferior vena cava and even the pulmonary veins. The non-cardiac solid organ primary cancers may be placed in three groups, depending on their propensity to produce metastases: Uncommon malignant tumors with a high incidence of cardiac metastases (malignant melanoma and malignant germ-cell tumors); common cancers with an intermediate frequency of cardiac involvement (carcinoma of lung in males and breast cancer in females); and, common cancers with rare metastases (cervical carcinoma).

Of all malignancies, leukemias have the highest incidence of cardiac involvement, however this infiltrative process, usually does not produce symptoms. There is a diffuse or patchy interstitial infiltrate of neoplastic cells. On the other hand, the solid tumor metastases produce multiple or single nodules over the epicardial surface. Occasionally, nodules may also be found in the myocardium or on the endocardial surface. The mode of presentation therefore depends on the location of the tumor. The tumors do not pose a diagnostic problem, as the histologic appearance is usually similar to that of the primary site.

Therapy and Prognosis

Benign tumors, which are symptomatic or located at sites that might lead to catastrophic complications, are resected. Primary cancers with limited growth and no evidence of metastases are treated by surgical resection with adjuvant chemotherapy and radiotherapy. The latter may be the only treatment option available in very large tumors which are not amenable to even palliative debulking. Auto-transplantation or orthotopic transplantation may be an option in some cases. Primary lymphomas, on the other hand, are usually best treated by combined chemotherapy and radiotherapy. The prognosis in benign tumors after surgical excision is usually excellent, except for a rare case of recurrences,



Cardiac Tumors. Figure 4 (a) Cut surface(viewed en-face) of an excised piece of pericardium showing metastatic tumor, with diffuse grey-white thickening and a large nodule,with some surrounding yellow adipose (fatty) tissue. (b) Histology shows a metastatic adenocarcinoma composed of clusters of mucin-secreting cells (Stain Hematoxylin and eosin original magnification, ×20.0).

likely due to incomplete resection. The prognosis for cardiac sarcoma is extremely dismal with a mean survival of 3 months to a year.

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Cardio-Facial-Cutaneous (CFC) Syndrome

Definition

Is a rare genetic disorder characterized by integumental defects (sparse, brittle and curly hair, skin defects), skull and skeletal abnormalities (macrocephaly; bi-temporal constriction of the head, short stature), congenital heart defects, mental retardation and failure to thrive. CFC syndrome is a autosomal dominant genetic disorder caused by a germ-line mutations in the human BRAF or the MEK1 or MEK2 genes. However, not all affected individuals carry a mutation in one of these genes suggesting that other genes are also associated with CFC. CFC belongs to the neuro-cardio-facial-cutaneous syndrome complex some of which are discussed as pre-disposition for neoplastic diseases.

►B-Raf Somatic Alterations

Cardiomyopathy

Definition

A deterioration of function of the myocardium and a disease or disorder of the heart muscle

►Chemoprotectants

Cardiotoxicity

Definition

Death of heart muscle tissue.

►Herceptin

Caretaker Genes

Definition

Caretaker genes have evolved to tackle the surveillance and maintenance of DNA integrity, the loss of which gives rise to genomic instability and defects in mismatch repair, which is a major cause or effect of ►carcinogenesis. As major barriers to the initiation and ►progression of ►cancer, caretaker genes represent the third major class of genes subject to epimutations in ►cancer, alongside the ►oncogenes and ►tumor suppressor genes.

►Chinese versus Western Medicine
►Mismatch Repair in Genome Stability

Caries

Definition

Chronic destructive tooth demineralization caused by microorganisms.

►Dental Pulp Neoplasms

Carney Complex

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Synonyms

LAMB; lentigineses, atrial myxoma, mucocutaneous myxoma, blue nevi; NAME; nevi, atrial myxoma, myxoid neurofibroma, ephelide; CNC

Definition

The Carney complex (CNC) is a dominant autosomal hereditary multiple neoplasia syndrome characterized mainly by cardiac myxomas, spotty skin pigmentation, and endocrine tumors. It was first described in 1985 by J. Aidan Carney, a pathologist at the Mayo Clinic.

Characteristics

The manifestations of CNC can be numerous and vary between patients. Even in the same kindred, phenotypic variability can be observed. The estimated frequencies of these manifestations are listed in Table 1. Endocrine, dermatologic, and cardiac anomalies are the main manifestations of the disease. The ►lentiginosis is observed in most patients and is so characteristic that can makes the diagnosis. It appears as small brown to black macules typically located around the upper and lower lips, on the eyelids, ears, and the genital area. Multiple blue nevi and junctional or compound nevi may also be observed in CNC, as well as cutaneous myxomas. The skin myxomas present as nonpigmented subcutaneous nodules. Myxomas can also be located in the ear canal.

Table 1 lists the most frequent features of CNC and their estimated frequency. The incidence of each manifestation depends on its presentation and might not reflect true prevalence. For instance, according to autopsy studies, ►primary pigmented nodular adrenocortical disease (PPNAD) is a constant feature in CNC patients, however, reports of ►Cushing syndrome in the literature indicate that only 25–45% of CNC patients have PPNAD.

►Cardiac myxoma is an important manifestation of CNC. It may be the cause of the high rate (16%) of sudden death historically reported in CNC families, thus underlying the importance of its early diagnosis. In the past, underdiagnosis of cardiac myxomas may have accounted for the majority of deaths due to CNC. In contrast with sporadic myxoma, they can develop in any cardiac chamber and may be multiple. Cardiac myxoma can be the cause of stroke due to embolism and cardiac deficiency. It is therefore important to screen regularly (by ultrasound) patients with CNC for the presence of cardiac myxoma. In difficult cases, transesophageal ultrasound and cardiac magnetic resonance imaging (MRI) can be very helpful.

Carney Complex. Table 1 Main manifestations of Carney complex

Main features of Carney complex	Frequency (%)
Primary pigmented nodular adrenocortical disease (PPNAD)	25–60
Cardiac myxoma	30–60
Skin myxoma	20–63
Lentiginosis	60–70
Multiple blue nevus	
Breast ductal adenoma	25
Testicular tumors (LCCSCT: large cell calcifying Sertoli cell tumor) (in male)	33–56
Ovarian cyst (in female)	20–67
Acromegaly	10
Thyroid tumor	10–25
Melanotic schwannoma	8–18
Osteochondromyxoma	<10

Endocrine tumors are also a major manifestation of the disease. Most characteristic is the adrenocorticotropic hormone (ACTH)-independent ►Cushing syndrome due to PPNAD observed in 30–60% of patients with CNC. The disease was named after the macroscopic appearance of the adrenals that is characterized by the small pigmented micronodules observed in the cortex. The disease is usually bilateral with primary involvement of both adrenals. Cushing syndrome due to PPNAD is most often observed in children and young adults, with a peak during the second decade of life. Diagnosis of Cushing syndrome due to PPNAD is often difficult because hypercortisolism can develop progressively over years. In contrast, a large and rapid burst of cortisol excess can be observed in some patients who might spontaneously regress. In some cases of PPNAD, clearly cyclic forms of hypercortisolism have been documented. PPNAD can also be diagnosed by systematic screening in patients with CNC, investigated for other clinical manifestations of the complex, or after familial screening. Despite the unusual time course of Cushing syndrome observed in some patients with PPNAD, clinical signs are quite similar to those observed in patients having other causes of hypercortisolism. Urinary cortisol is increased in most patients at the time of diagnosis of PPNAD, but its level can be highly variable. The circadian rhythm of cortisol secretion is usually completely abolished. As with ACTH-independent Cushing syndrome due to other causes, patients with PPNAD have low plasma levels of ACTH and show no stimulation of cortisol or ACTH secretion after ►corticotropin-releasing hormone (CRH) injection. In addition, dexamethasone fails to suppress cortisol secretion, even after high dose administration. Pathological investigation reveals that adrenal glands from patients with PPNAD are usually normal in size and weight (between 4 and 17 g). In keeping with this finding, adrenals appear normal on computed tomography (CT) scan in one out of three patients. In the other patients, micronodules can be visible and, more rarely, macronodules (>1 cm diameter) in one or both glands. Iodocholestrol scintigraphy, when performed, usually shows a bilateral uptake despite ACTH suppression by endogenous hypercortisolism.

►Acromegaly due to a pituitary ►GH-secreting tumor is not very frequent, but most patients with CNC

present with a mild increase in GH, and sometimes in ►prolactin (PRL) secretion.

Alterations in the rhythm of GH secretion are frequently observed. Thyroid tumors are most often benign, nontoxic adenomas, mostly of follicular type. Some patients present with papillary carcinoma that can be multiple and sometimes quite aggressive. Testicular tumors (►Large cell calcifying Sertoli cell tumors (LCCST)) are easily detected by ultrasound investigation as bilateral microcalcifications. They can be diagnosed by ultrasound. Ovarian cysts and cystadenoma have been observed in CNC patients.

Various other tumors, some of them quite specific for CNC, can be observed. ►Melanotic schwannoma is a rare tumor and occurs mainly in CNC. It is a pigmented tumor that can be misdiagnosed as a melanoma. This tumor can be observed in any peripheral nerve and can be, in rare cases, malignant. Breast ductal adenomas, breast myxomas, and ►osteochondromyxoma are among the tumors also observed in CNC.

CNC is an autosomal dominant hereditary disease and at least two loci have been postulated: 2p16 and 17q22-24. The *CNC1* gene, located on 17q22-24, has been identified as the regulatory subunit (R1A) of the ►protein kinase A (*PRKAR1A*). PRKAR1A is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis. Heterozygous inactivating mutations of *PRKAR1A* have been detected in about 65% of CNC families. In CNC patients with Cushing syndrome, the frequency of *PRKAR1A* mutations is about 80%, suggesting that families with PPNAD are more likely to carry a 17q22-24 defect. Interestingly, patients with isolated PPNAD and no familial history of CNC may also carry a germline de novo mutation in *PRKAR1A*. In the tumors of CNC patients, loss of heterozygosity (LOH) at 17q22-24 may be observed, suggesting that *PRKAR1A* is a tumor suppressor gene. Somatic mutation of *PRKAR1A* in a patient with PPNAD already carrying a germline mutation may lead to inactivation of the wild-type allele. However, inactivation of the remaining wild-type allele by genetic alteration does not appear to be a constant step in PPNAD and CNC tumor development. In a mice transgenic model with heterozygous inactivation of *PRKAR1A*, tumors may develop without allelic loss. This suggests that the classic model of tumor suppressor gene with a germline inactivating first allelic alteration, followed by a second genetic hit leading to inactivation of the remaining wild-type allele, might to some extent be applicable to PRKAR1A. It is also possible that in PPNAD, a general polyclonal expansion might be stimulated by haploinsufficiency due to the first germline defect; a second genetic hit would then lead to inactivation of the wild-type allele and further stimulate tumorigenesis and the development of adrenocortical nodules. PRKAR1A inactivation in transgenic models is associated with an increased PKA activity. Stimulation of the MAP Kinase pathway as well as mTOR phosphorylation has been observed in experimental models of PRKAR1A inactivation and might be a mechanism for oncogenesis in CNC.

Considering the genetics of isolated PPNAD, the clinical manifestations in a subgroup of very young PPNAD patients may differ from those in older patients with CNC. In these patients the classical pathological finding of pigmented nodules may be absent although micronodules are present. In this subgroup of very young PPNAD patients, Cushing syndrome may occur between birth and the age of 5-years. The main reason for differentiating this group of PPNAD or PPNAD-like patients is the lower rate of germline inactivating mutation. This observation lead to the identification by genome wide screen of a gene responsible for isolated PPNAD: the phosphodiesterase PDE11A4. The affected patient present with a germ line heterozygous mutation of *PDE11A4* gene located at 2q31-35. An allelic loss at 2q31-35 is observed in adrenal tissue from these patients, suggesting also that PDE114 might be a tumor suppressor gene. Inactivating mutations lead to increased cAMP and cGMP levels in keeping with the observation that PDE11A4 is a dual phosphodiesterase.

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Carotene 15,15′-Oxygenase

Definition

Formerly called β-carotene 15,15′-dioxygenase is a non-heme iron oxygenase enzyme which catalyzes the symmetric cleavage of carotenoids at their central 15,15′ double bond.

►Carotenoids

β-Carotene

Definition

A fat-soluble, isomeric form of carotene that is widely distributed in nature and most efficiently converted to vitamin A by the body.

►Chemoprotectants

Carotenes

Definition

Carotenes are un-oxidized (oxygen-free) carotenoids such as α-carotene, β-carotene, lycopene.

►Carotenoids

Carotenoids

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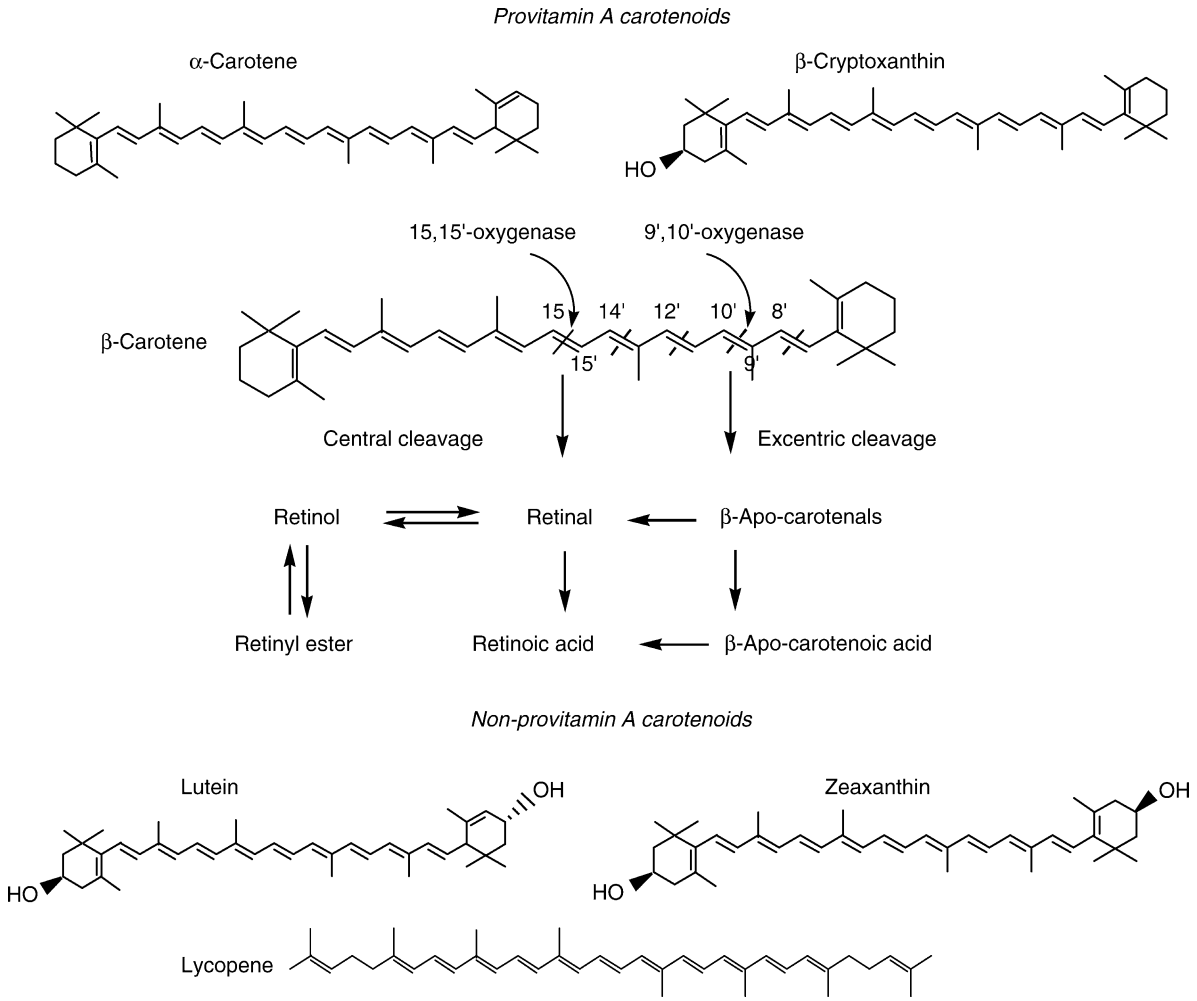
Definition

Carotenoids are lipophilic plant pigments with polyisoprenoid structures that occur naturally in plants and other photosynthetic organisms. There are over 600 known carotenoids with chemical structures characterized by a large (35–40 carbon atoms) conjugated polyene chain, sometimes terminated by ring structures. Carotenoids are divided into to major groups: ►xanthophylls, oxygenated carotenoids including lutein, zeaxanthin, and astaxanthin, and ►carotenes, hydrocarbon carotenoids that are either cyclized, such as α-carotene and β-carotene, or linear like lycopene. The most abundant carotenoids in human plasma include lutein, lycopene, β-carotene, zeaxanthin, β-cryptoxanthin, α-carotene, and α-cryptoxanthin. The two main mechanisms by which carotenoids may influence cancer risk are by exerting ►antioxidant effects and through interaction with ligand-dependent ►nuclear hormone receptors and their signaling pathways. The capacity of carotenoids to act as lipid-soluble antioxidants serves a functional and protective role in plants during photosynthesis, and may protect animals against free radical damage to lipid membranes and DNA. In addition, carotenoids may enhance cell-cell gap junctional communication and induce phase II detoxifying enzymes (see ►carcinogen metabolism). Provitamin A carotenoids (e.g. β-carotene, α-carotene, and β-cryptoxanthin) can be cleaved to generate vitamin A and other metabolites that interact with signaling pathways controlling gene expression. Non-provitamin A carotenoids (e.g. lutein, zeaxanthin, and lycopene) not only have significant antioxidant activity, but their metabolites may also exert effects on gene expression (Fig. 1).

Characteristics

►DNA damage by ►free radicals (including ►reactive oxygen species and reactive nitrogen species) is thought to be a major contributor to ►carcinogenesis, and carotenoids contain an extended system of ►conjugated double bonds that make them efficient scavengers of free radicals. While antioxidant supplements and diets high in antioxidant nutrients, including carotenoids (lycopene, β-carotene, lutein, β-cryptoxanthin), have been shown to reduce DNA strand breaks and other ►biomarkers of ►oxidative DNA damage, it is still unclear whether these changes are sufficient to lower cancer risk in humans with chronic exposure to low levels of carcinogenic compounds over a lifetime. Epidemiological studies suggest that a higher dietary intake of carotenoids and high levels of certain carotenoids in the plasma may offer protection against the development of certain cancers (e.g., lung, prostate, stomach, colon, breast), as well as other health conditions linked to oxidative damage (e.g. heart disease, macular degeneration, cataracts). However, two intervention trials, the Beta-Carotene and Retinol Efficacy Trial (CARET) and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), have shown that supplementation with high dose β-carotene, alone or in combination with vitamin A, does not reduce the risk of lung cancer and may even increase that risk in smokers and ►asbestos workers. These findings have led to an increased effort to better understand the role of carotenoids and ►retinoids (vitamin A and its derivatives) in the process of carcinogenesis, with special attention to dose and the oxidative environment at the tissue level. Based on the accumulated evidence, it appears that low-dose carotenoids (similar to the amounts consumed in a diet high in fruits and vegetables) may act as antioxidants and protect against cancer, whereas at high doses carotenoids may lose their effectiveness as antioxidants, function as pro-oxidants, and/or interfere with retinoid signaling pathways, increasing cancer risk.

The series of conjugated double bonds in the central chain of carotenoids make them susceptible to oxidative cleavage and isomerization from *trans* to *cis* forms.



Carotenoids. Figure 1 Metabolic pathway of β-carotene, and chemical structures of provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) and non-provitamin A carotenoids (lutein, zeaxanthin, and lycopene).

Cleavage can result in the formation of potentially bioactive metabolites, such as retinoids and other biological compounds. For provitamin A carotenoids, such as β-carotene, α-carotene, and β-cryptoxanthin, ►central cleavage by ►carotene 15,15′-oxygenase (formerly called β-carotene 15,15′-dioxygenase), a non-heme iron oxygenase enzyme which can cleave carotenoids at their central 15,15′ double bond, is a major pathway leading to vitamin A formation. An alternative pathway for carotenoid metabolism into vitamin A in mammals is ►excentric cleavage or asymmetric cleavage. Recent characterization and study of ►carotene 9′,10′-oxygenase has demonstrated that this enzyme can catalyze the excentric cleavage of both provitamin A carotenoids and non-provitamin A carotenoids. Since disruption in retinoid metabolism and signaling may play a key role in the process of carcinogenesis, understanding the molecular details behind the actions of these carotenoid oxidative metabolites may yield insights into both physiological and pathophysiological processes in human health and disease, particularly the potential for beneficial effects of small quantities of carotenoids and harmful effects of large quantities of carotenoid metabolites.

Disruption of carotenoid and retinoid metabolism and signaling due to diet and lifestyle factors has been associated with increased risk of cancers at multiple sites (see ►nutritional status and cancer, ►hepatic alcohol metabolism and ►smoking addiction). Cigarette smoking is associated with substantially decreased plasma levels of carotenoids, despite only slightly lower intakes of carotenoids in smokers compared to non-smokers. Several hypotheses have been proposed to explain this increased metabolism of carotenoids in the tissues of smokers, including increased induction of metabolic enzymes, excentric cleavage of β-carotene into harmful oxidative products, and oxidative degradation of cellular antioxidants (e.g. ascorbic acid,

α-tocopherol) that normally serve to stabilize the reduced form of β-carotene. While initial cleavage of provitamin A carotenoids can lead to the generation of ►retinoic acid, a bioactive form of vitamin A, additional oxidation can lead to degradation into polar metabolites. With respect to lung cancer, laboratory studies have demonstrated that the oxidative cleavage products of β-carotene, when formed in large quantities in the cell after supplementation with high-dose β-carotene in the highly oxidative environment of the smoke-exposed lung, enhance catabolism of retinoic acid by their induction of ►cytochrome P_{450} enzymes (CYP enzymes) and facilitate the binding of carcinogen ►adducts to DNA. Lower retinoic acid levels then combine with smoke-induced changes to alter ►signal transduction pathways and promote lung carcinogenesis. On the other hand, low dose β-carotene supplementation, particularly when combined with other antioxidants, inhibits tobacco smoke-induced changes in retinoic acid levels and signaling in the lung tissue, preventing the formation of smoke-induced ►preneoplastic lesions (see ►tobacco carcinogenesis).

Therefore, the effects of provitamin A carotenoids can be mediated by conversion to retinoic acid and transcriptional activation of a series of genes with distinct antiproliferative or proapoptotic activity or by induction of ►apoptosis, eliminating cells with unrepairable alterations in the genome or killing neoplastic cells. Certain carotenoids may also be able to interact directly or indirectly with transcription factors, such as peroxisome proliferator activated receptors (PPARs), nuclear factor E_2-related factor 2 (Nrf2), or orphan receptors, or may indirectly influence transcriptional activity of redox-sensitive transcription systems, such as activator protein-1 (►AP-1), nuclear factor-κB (►NF-κB), and the antioxidant response element (ARE). Greater understanding of the biological functions of carotenoids mediated via their oxidative metabolites through their effects on these important cellular signaling pathways and molecular targets, as well as their significance to cancer prevention, is needed. In considering the efficacy and complex biological functions of carotenoids in human cancer prevention, it seems that provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) combined with other antioxidants (ascorbic acid, α-tocopherol) would be an effective chemopreventive strategy against cancer development.

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Cas

Definition

Crk-associated substrate is a tyrosine-phosphorylated docking protein that is indispensable for the actin cytoskeletal organization and cell migration. Cas binds directly to ►Src, phosphorylated and localized to focal contacts.

►Focal Adhesion Kinase

Case Control Association Study

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Synonyms

Case–Control association analysis; Population candidate gene association study; Genetic association study

Definition

Case–control association study aims to detect association between one or more genetic marker (usually a ►polymorphism but also may be a ►microsatellite) and a trait, which might be a disease (e.g., lung cancer), a quantitative characteristic (e.g., serum level of a ►cytokine) or a discrete attribute.

Characteristics

Several genetic methods are used for detecting genes responsible for the development of ►complex human diseases; these are nonparametric ►linkage analysis, case–control association analysis, and ►DNA microarray.

Case–control association analysis involves selecting genes that are likely to be associated with the pathogenesis of disease based on our understanding of its pathophysiology. Then ►genetic polymorphisms in these candidate genes are investigated in a large number of unrelated patients and healthy ethnically matched controls. Significant differences in ►genotype or ►allele frequencies between the two groups suggest either that (i) the polymorphism predisposes one to the disease, (ii) the polymorphism is in ►linkage disequilibrium with a disease susceptibility gene, or (iii) there is a confounding factor such as poor ethnic matching between the cases and controls. When several markers are being examined for association with the same trait, it is advisable to check them for linkage disequilibrium and disease-associated ►genetic haplotypes.

Association studies have greater power than linkage analysis. They can detect genes with a relative risk of 1.5 at nearly 80% probability if several hundred samples are collected. However, since association studies examine much smaller regions than linkage analyses, many more markers would need to be typed to conduct a ►genome-wide association study. This is not possible with current technology. At present, association studies are limited to the investigation of candidate genes and regions identified in linkage analysis. As association studies are not comprehensive, the possibility that the most important genes have been overlooked cannot be excluded. Choosing candidate genes from areas spotted by linkage analysis might be the most fruitful practice.

When performing case–control association studies factors such as study design, methods for recruitment of case and controls, selection of candidate genes, functional significance of polymorphisms chosen for study, and statistical analysis require close attention to ensure that only genuine associations are detected.

Potential Problems in Association Studies

Marked inconsistency can be observed between association studies in different or even the same ethnic groups. The association between tumor necrosis factor alpha promoter polymorphism and gastric cancer is an example for inconsistency while association of head and neck cancers with M1 polymorphism of glutathione S-transferase gene is an example for the reproducible associations. This situation has led some commentators to question the value of genetic association studies, suggesting that association studies should be restricted to polymorphisms that have been shown to have a direct effect on gene function. Possible explanations for these inconsistent results include:

1. Different populations might have different genetic components for the same disease phenotype. Furthermore, the degree of association between the specific gene and disease is different between populations. It is also possible that the different environments to which every population is exposed will interact differently with the genetic components responsible for the development of the disease.
2. Most complex human diseases are heterogeneous disorders, e.g., leukemia. It may happen that patients diagnosed with leukemia in different ethnic groups have distinct types of polymorphisms causing a specific type of leukemia.
3. When the population under study consists of a mixture of two or more subpopulations that have different allele frequencies, associations between genotype and outcome could be confounded by population stratification.
4. Ascertainment errors include undiagnosed affected individuals in the control group or including patients with heterogeneous etiologies for the complex disease in the patient group.
5. Failure to check for ►Hardy–Weinberg equilibrium. The presence of disequilibrium in the control group can result from genotyping errors, inbreeding, small sample size, or mutation.
6. A possible reason for failure to replicate positive findings is that subsequent studies are underpowered.
7. Failure to exclude chance is the most likely explanation for difficulty in replication of reports of genetic associations with complex diseases. Applying a significance level of $p = 0.05$ leads to 1 false positive in 20 results. In order to avoid type I error, p values calculated from association studies must be corrected for the number of loci analyzed (x) and the number of alleles at each loci (y). In the Bonferroni correction, the required significance level should be divided by x ($y-1$). However, this method is too conservative because closely located loci are not usually independent. The appropriate correction for multiple comparisons in association studies remains unclear.
8. Publication bias should be considered. Negative results in association studies may not be submitted for publication.

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Case–Control Association Analysis

►Case Control Association Study

Case–Control Study

Definition

A study in which a group of persons with a particular disease or condition (cases) and a group of persons without the disease or condition (controls) are compared with respect to exposure to possible risk factors. Also called a retrospective study.

►Obesity and Cancer Risk
►Stress
►Epidemiology of Cancer
►Cancer Epidemiology
►Coffee Consumption

CASH

►FLICE Inhibitory Protein

CASP-8

Definition

►Caspase-8

Caspase

Definition

Are protein degrading enzymes (proteases) that act as mediators of programmed cell death (►apoptosis). Proteins within the large family of these cell-death proteases are all similar to each other. Caspases are highly conserved during evolution and can be found in humans as well as in insects and worms and are even found in lower multicellular organisms. More than a dozen caspases have been identified in humans. Usually caspases selectively cleave a restricted set of target proteins in the primary sequence at one position, or at a few positions at most. Cleavage always occurs behind an aspartate amino acid. The caspase-mediated cleavage of specific substrates supplies an explanation for several characteristic features of apoptosis. Cleavage of the nuclear lamins, for instance, is required for nuclear shrinking. Cleavage of cytoskeletal proteins causes the overall loss of cell shape. In healthy cells, caspases normally lie dormant. In response to diverse stimuli they become activated when cell death is required. Dormant caspases exist as precursor polypeptides or "proenzymes" that are largely activated by proteolytic processing. This involves cleaving of proenzymes at specific points to generate the large and small subunits that associate to the active caspase enzyme. The proenzymes have low protease activity themselves and can therefore process each other when brought into vicinity. This process starts, when an external stimulus, a "death ligand" binds to a receptor (such as CD95/FAS/APO-1) on the cell surface. Ligand binding results in the aggregation of procaspase-8. The high density of caspase-8 proenzymes has the result that they mutually activate each other. Caspase-8 is an initiator caspase that can activate downstream procaspases, in particular procaspase-3, either by direct cleaving or indirectly by cleaving BID and inducing cytochrome C release from mitochondria.

An alternative mechanism of caspase activation in response to death stimuli involves procaspase-9. In this case, the adaptor molecule APAF-1 sequesters several procaspase-9 molecules that, within this complex (often referred to as apoptosome), are activated by a change in conformation, not by proteolysis. In response to that change, they can activate downstream caspases.

In short, initiator caspases become primarily activated by regulated protein-protein interaction, whereas downstream effector caspases are activated proteolytically. Besides caspase pathways, other death-inducing pathways must exist, since developmental apoptosis is

functional in mice that are defective in regard to the ►caspase-8 and caspase-9 pathways.

►APAF-1 Signaling
►PUMA
►Autophagy

Caspase-3

Definition

►Caspase-3 belongs to the family of cystein-dependent aspartate-directed proteases. It is responsible for the proteolytic cleavage of cellular proteins leading to the characteristic apoptotic features, e.g. cleavage of caspase-activated DNase resulting in internucleosomal DNA fragmentation.

►Minodronate
►Caspases

Caspase-8

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Synonyms

FLICE; FADD-like ICE; Mach; Mch5

Definition

Caspase-8 belongs to the family of cysteine proteases called caspases that act as mediators of programmed cell death (►apoptosis). It is a protein of 480 amino acids and 55 kDa that is widely expressed in various tissues. Caspase-8 displays 20% identity to the *ced-3*-encoded protein of *Caenorhabditis elegans*. The gene maps to 2q33.

Characteristics

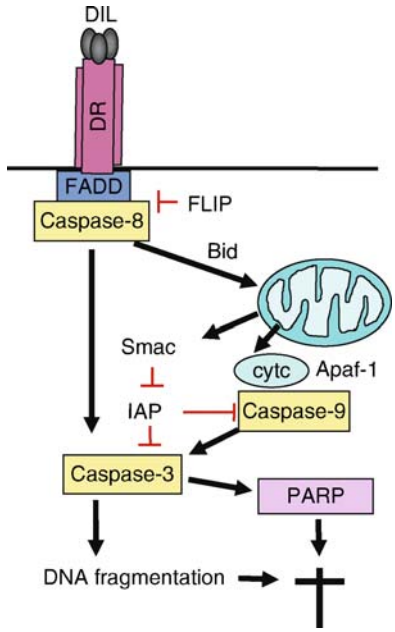
Structure and Physiological Functions of Caspase-8

Caspase-8 contains two ►death-effector-domains (DED) in the N-terminal prodomain that serve as protein–protein interaction sites and a catalytic protease domain at the C-terminus consisting of a large and small subunit. The active caspase-8 molecule is composed of a hetero-tetramer of two of each of the large and small subunits (Fig. 1). The preferred substrate specificity for caspase-8 is I/L/V/E X D (where X is any amino acid).

Caspase-8. Figure 1 Caspase-8 structure. Caspase-8 is a 480 amino acid protein that consists of two death-effector-domains (DED) and a catalytic protease domain with a large subunit (p20) and small subunit (p10).

Caspase-8 exists in different splice variants of which caspase-8a and -8b are expressed in most cell lines and catalytically active. Caspase-8L is generated by ►alternative splicing of intron 8 of the human *caspase-8* gene generating a 136 bp insertion between exon 8 and exon 9 of full-length caspase-8 mRNA. This produces a premature stop codon and a truncated protein that contains only the two N-terminal DED domains, but lacks the C-terminal proteolytic domain.

Caspase-8 is an ►initiator caspase that is expressed as proenzyme (zymogen) in an inactive state and becomes activated during apoptosis through oligomerization in a multimeric complex. Crosslinking of ►death receptors such as CD95 or the agonistic ►TRAIL receptors TRAIL-R1 and TRAIL-R2 by their corresponding ligands CD95 ligand or TRAIL or by agonistic antibodies initiates receptor trimerization, clustering of the receptors ►death domains, and recruitment of adaptor molecules such as ►Fas associated with a death domain (►FADD) through homophilic protein–protein interactions mediated by the death domains (Fig. 2). FADD in turn recruits caspase-8 to activated death receptors through interaction via the DED domains to form the ►death-inducing signaling complex (DISC). Oligomerization of caspase-8 upon DISC formation drives its activation through autoproteolysis. Once activated, caspase-8 cleaves downstream effector caspases such as caspase-3. For the CD95 signaling pathway two distinct prototypic cell types have been identified. In type I cells, caspase-8 is activated upon CD95 ligation at the DISC in quantities sufficient to directly activate downstream effector caspases such as caspase-3. In type II cells, however, the amount of active caspase-8 generated at the DISC is insufficient to fully activate caspase-3. In these cells a mitochondrial amplification loop is required for complete activation of the caspase cascade involving caspase-8-mediated cleavage of ►BH3-interacting death domain agonist (Bid), which translocates to mitochondria to trigger the release of apoptogenic proteins such as cytochrome *c* from mitochondria into the cytosol. Also, a similar cell type dependent organization (type I and type II) of the TRAIL



Caspase-8. Figure 2 Apoptosis signaling pathways. Apoptosis pathways can be initiated by crosslinking of death receptors (DR), e.g., CD95 or TRAIL receptors, by death-inducing ligands (DILs) such as CD95 ligand or TRAIL followed by recruitment of the adaptor molecule FADD and caspase-8, which drives caspase-8 activation through autoproteolysis (receptor/extrinsinc pathway). In type I cells, caspase-8 is activated at the receptor level in quantities sufficient to directly activate effector caspase-3. In type II cells, caspase-8 initiates a mitochondrial amplification loop to activate effector caspases by cleaving Bid, which translocates to mitochondria to trigger the release of cytochrome *c* (cytc) and Smac. The mitochondrial (intrinsic) pathway is initiated by the release of cytochrome *c* or Smac from mitochondria into the cytosol. Cytochrome *c* triggers caspase-3 activation via formation of the cytochrome *c*/Apaf-1/caspase-9-containing apoptosome complex, while Smac neutralizes the inhibitor of apoptosis proteins (IAP)-mediated inhibition of caspase-3 and -9. cellular ►FLICE-inhibitory protein (c-FLIP) inhibits apoptosis by blocking caspase-8 activation.

signaling pathway has been described. Besides its activation at the DISC caspase-8 can also be activated downstream of mitochondria upon initiation of the intrinsic apoptosis pathway, e.g., through cleavage by caspase-6.

In addition to its established role in ►apoptosis signaling, recent evidence indicates that caspase-8 can also exert several nonapoptotic functions. For example, caspase-8 is required to maintain homeostasis of peripheral T cells by controlling T cell proliferation via regulation of IL-2 production. In addition, caspase-8 is involved in the regulation of differentiation and proliferation of B cells, NK cells and hematopoietic progenitors. Also, caspase-8 has been reported to be important for NF-κB activation through the T cell receptor, for CD95 clustering and internalization upon CD95 stimulation as well as for the survival of endothelial cells. Furthermore, it has been shown that caspase-8 can promote cell motility by regulating activation of ►calpains, Rac, and lamellipodial assembly and regulates cell spreading by cleaving the cytolinker plectrin, a component of hemidesmosomes and focal adhesion complexes. Loss-of-function mutation in caspase-8 is lethal to the mouse embryo around day 12.5, an indication of its critical role during normal development. Caspase-8 knockout mice die in utero as a result of defective development of heart muscle and display abdominal hemorrhage and fewer than normal hematopoietic progenitor cells. Together, these findings indicate that constitutive caspase-8 activity is relevant to normal physiology.

Caspase-8 and Cancer

It is well established that the evasion of apoptosis is one of the hallmarks of ►cancer. It is therefore not surprising that some cancers have used the inactivation of caspase-8 to avoid apoptotic signals suggesting that caspase-8 may act as ►tumor suppressor. In principle, caspase-8 expression and/or function can be altered through genetic or ►epigenetic mechanisms or alternatively, caspase-8 function can be compromised in cancers. For example, caspase-8 expression can be impaired by mutations. Such mutant variants of caspase-8 may act in a dominant-negative manner, e.g., by blocking the recruitment of wild-type caspase-8 to activated death receptors, thereby inhibiting apoptosis. Despite the key role of caspase-8 for cell death execution, caspase-8 mutations in human tumors have, however, only been identified at low frequency in some tumors, e.g., in colorectal, head and neck, or vulvar carcinoma. In addition, homo- or heterozygous genomic deletions were found in some neuroblastoma. In contrast to these rare genetic alterations, caspase-8 expression is frequently impaired by epigenetic mechanisms in cancer cells. To this end, caspase-8 expression was found to be inactivated by hypermethylation of a regulatory sequence of the *caspase-8* gene, which maps to the boundary between exon 3 and intron 3. Silencing of caspase-8 was detected in a variety of cancers, e.g., in ►neuroblastoma, ►medulloblastoma, ►malignant glioma, ►rhabdomyosarcoma, ►Ewing sarcoma, ►retinoblastoma, and small lung cell carcinoma both in cell lines and in primary tumor samples. Although this regulatory region of caspase-8 does not meet the criteria of a classical ►CpG island and shows no promotor activity, the ►methylation status of this domain correlated with caspase-8 expression in several human tumors. In

addition, treatment with the demethylating agent 5-aza-2′ deoxycytidine (5-AZA) resulted in demethylation of this regulatory sequence, which in turn led to increased caspase-8 promotor activity and re-expression of caspase-8. This suggests that demethylation of a *trans*-acting factor may be involved in controlling activity of the caspase-8 promotor. Another level of transcriptional regulation of caspase-8 in cancers is alternative splicing, for example in leukemia or neuroblastoma cells. Alternative splicing of intron 8 of the *caspase-8* gene generates caspase-8L that misses the catalytic site but retains the two N-terminal DED repeats. Thus, caspase-8L is recruited to activated death receptors where it acts as a dominant-negative inhibitor of apoptosis in cancer cells by interfering with the recruitment of wild-type caspase-8.

In addition to genetic and epigenetic mechanisms caspase-8 signaling can also be functionally impaired in cancer cells, e.g., by overexpression of antiapoptotic proteins that interfere with caspase-8 activation at the death receptor level. Examples are cellular ►FLICE-inhibitory protein (►c-FLIP) or ►phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15kDa (►PED/PEA-15) that exert their antiapoptotic function by blocking the recruitment of caspase-8 to activated death receptors. The adenoviral E1B19K early protein has similar properties.

The biological relevance of caspase-8 inactivation in cancers follows from its key role in the apoptotic machinery. Tumor cells with loss of caspase-8 were found to be resistant to death receptor-triggered apoptosis. Similarly, embryonic fibroblasts derived from caspase-8 knockout mice were completely resistant to apoptosis induced by death receptors including CD95, TRAIL receptors, or TNF receptor-1, whereas they retained sensitivity to other apoptotic stimuli such as UV irradiation, ►ceramide, or several anticancer drugs. These findings indicate that caspase-8 plays a necessary and nonredundant role in transducing the death signal from activated death receptor to intracellular effector caspases. In addition, chemotherapeutic drugs can initiate caspase-8 activation in a receptor-dependent and also in a receptor-independent manner. Of note, loss of caspase-8 expression has been reported to significantly correlate with unfavorable survival outcome in medulloblastoma patients, while no correlation with survival or established parameters of poor prognosis was found in neuroblastoma. Restoration of caspase-8 expression by gene transfer or by demethylation treatment in cancer cells where caspase-8 is epigenetically silenced also sensitized resistant tumor cells for death-receptor- or drug-induced apoptosis. In addition, treatment with the cytokine IFNγ caused transcriptional activation of caspase-8 in cancer cells lacking caspase-8 and enhanced expression of caspase-8 through interferon-sensitive response elements within the caspase-8 promotor and ►STAT-1.

Loss of caspase-8 fosters cancer ►metastasis and ►invasion by rendering cancer cells resistant to integrin-mediated cell death. Integrin receptors that are unable to find appropriate ligands can form a large molecular complex containing caspase-8, thereby initiating an apoptosis cascade. In this context, caspase-8 may function as metastasis suppressor gene that, together with integrins, regulates cell death of cancer cells that ►migrate from the primary tumor. Thus, loss of caspase-8 may promote metastasis of cancers by providing a survival advantage in foreign ►microenvironments.

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Caspase-eight-related Protein

►FLICE Inhibitory Protein

Caspase-9

Definition

A member of caspase family of cysteine proteases implicated in apoptosis and cytokine processing. Caspase-9 is synthesized as 46 kDa precursor protein. It consists of three domains: an N-terminal prodomain, a large subunit, and a small subunit.

►APAF-1 Signaling
►Caspases

Caspase Homologue

►FLICE Inhibitory Protein

Caspase-like Apoptosis-regulatory Protein

►FLICE Inhibitory Protein

CASPER

►FLICE Inhibitory Protein

CASTing

Definition
Cyclic amplification and selection of targets (CASTing) is a combinatorial selection method used primarily to identify consensus protein-binding sites on duplex DNA.

►Combinatorial Selection Methods

Catalytic Domain

Definition
Catalytic domain, the region that exhibits the enzyme's active site. Active site is normally a small pocket at the surface of the ►enzyme that contains residues responsible for the substrate specificity and for the catalysis.

►Furin

Catalytic Pocket

Definition
Structural part of an enzyme that forms a pocket where a biochemical reaction is catalyzed.

►Histone Deacetylases

Catastrophe Promoter

Definition
Agents that facilitate the switching of microtubules from growth to shrinking phase.

►Microtubule Associated Proteins

Catechin

►Epigallocatechin

Catechins

Definition
►Green Tea Catechins

Catechol Estrogen

Definition
Is the hydroxylated form of ►estradiol and is of special importance because it can be easily autooxidated to semiquinones and subsequently quinones, both of which are electrophiles capable of covalently binding to nucleophilic groups on DNA via a Michael addition and, thus, serve as the ultimate carcinogenic reactive intermediates in the peroxidatic activation of catechol estrogens.

Catenate

Definition
To link in a series. Decatenation is the process of separating linked DNA molecules. Trypanosome

kinetoplast DNA represents a catenated network of interlinked DNA circles and is used to assay for decatenatory activity by ►topoisomerase II.

►Decatenation G2 Checkpoint

β-Catenin

Definition

Multifunctional cytoplasmic protein that is involved in cadherin-mediated cell–cell adhesion, linking cadherins to the actin cytoskeleton. Can also act independently as a gene regulatory protein. Has an important role in animal development as part of a Wnt signaling pathway.

►Nucleoporin
►Wnt Signaling
►Carcinoid Tumors

β-Catenin Stabilization

Definition

A core event in the canonical *wnt/Wingless* signaling pathway that directs cell fate in many cell types and plays a central role in development and in tumor progression.

►Protease Activated Receptors

Cathepsin-D

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Definition

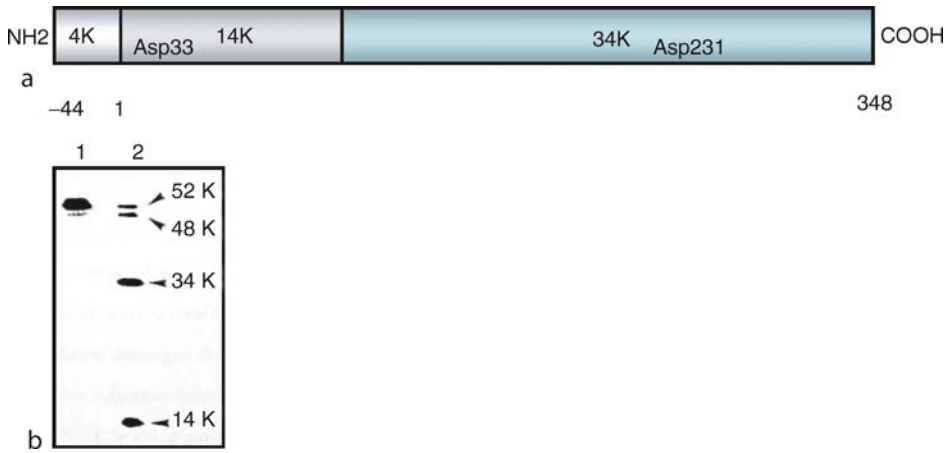
►Cathepsin-D (E.C. 3.4.23.5) is a ubiquitous lysosomal aspartic endo-►proteinase cleaving preferentially -Phe-Phe-, -Leu-Tyr-, -Tyr-Leu-, and -Phe-Tyr- bonds in peptide chains containing at least five amino acids at an acidic pH.

Characteristics

Cathepsin-D is ubiquitously distributed in ►lysosomes. It was considered for a long time that the main function of cathepsin-D was to degrade proteins in lysosomes at an acidic pH. Apart from its function in general protein turnover, cathepsin-D can also activate precursors of biologically active proteins in pre-lysosomal compartments of specialized cells. ►Knock-out of cathepsin-D gene induces death shortly after birth with severe apoptotic and necrotic phenotypes. Its pH optimum depends on the enzyme source and on the substrate used for the determination of the activity and ranges between 2.8 and 5. No endogenous cathepsin-D tissue inhibitor is known in mammals. Pepstatin, a natural inhibitor of aspartic proteases isolated from various species of actinomycetes, inhibits its catalytic activity. Cathepsin-D, like other aspartic proteases such as renin, chymosin, pepsinogen, has a bilobed organization. Crystal structures of native and pepstatin-inhibited forms of mature human cathepsin-D, revealed a high degree of tertiary structural similarity with other members of the aspartic proteinase family (*e.g.* pepsinogen and human immunodeficiency virus protease). The human cathepsin-D gene containing nine exons is located in chromosome 11p15 and expresses a single transcript of 2.2 kb. Cathepsin-D is synthesized as a 52 kDa catalytically-inactive precursor (Fig. 1). During its transport to lysosomes, cathepsin-D can be found in the endosomes where it is present as partially active 48 kDa single-chain intermediate (Fig. 1). This intermediate is subsequently transported to the lysosomes where it is converted into the fully active mature protease that is composed of a 34 kDa heavy and a 14 kDa light chain (Fig. 1). The human cathepsin-D catalytic site includes two critical aspartic residues (amino acids 33 and 231) located on the 34 and 14 kDa chains (Fig. 1a). Mannose-6-phosphate (M6P) receptors are involved in lysosomal routing of cathepsin-D and in the cellular uptake of the secreted pro-cathepsin-D. In ►breast cancer cell lines, over-expressed cathepsin-D is hyper-secreted in the extracellular environment and can be ►endocytosed (Endocytosis) by both ►cancer cells and fibroblasts *via* M6P receptors and other as yet unidentified receptor(s) (Fig. 1b). Endocytosed pro-cathepsin-D also undergoes successive maturations leading to the 48 kDa and 34 + 14 kDa forms. In addition, secreted pro-cathepsin-D, like pepsinogen, is capable of acid-dependent auto-activation *in vitro*, resulting in a catalytically active pseudo-cathepsin-D, an enzyme species that retains 18 residues (27–44) of the pro-segment.

Apoptosis

Cathepsin-D is a key mediator of ►apoptosis induced by many apoptotic agents, such as IFN-gamma, FAS/APO, TNF-alpha, ►oxidative stress, ►adriamycin, etoposide,



Cathepsin-D. Figure 1 Cathepsin-D structure and expression in breast cancer cells. (a) Schematic representation of the human 52 kDa pro-cathepsin-D sequence. Location of 4 kDa cathepsin-D pro-fragment, 14 kDa light and 34 kDa heavy mature chains are indicated. Intermediate 48 kDa form (not shown) corresponds to non-cleaved 14 + 34 kDa chains. Number 1 corresponds to the first amino acid of the mature cathepsin-D. Position of the 2 aspartic acids of the catalytic site is shown. Molecular mass is shown in K (kDa). (b) Expression of Human cathepsin-D in MCF-7 breast cancer cell line. MCF-7 cells were metabolically labeled with [^{35}S]Methionine and human cathepsin-D immunoprecipitated from cell extract (lane 2) and medium (lane 1) was analyzed by SDS-PAGE.

cisplatin and 5-fluorouracil as well as staurosporine. The role of cathepsin-D in apoptosis has been linked to the lysosomal release of mature 34 kDa cathepsin-D into the ►cytosol, leading in turn to the mitochondrial release of cytochrome c into the cytosol and the activation of pro-caspases-9 and -3.

Regulation

Studies on ►estrogen receptor positive breast cancer cell lines revealed that this housekeeping enzyme is highly up-regulated by ►estrogens (Estradiol) and growth factors (*i.e.* IGF1, EGF). In estrogen receptor positive breast cancer cell lines, both estrogens and growth factors stimulate cathepsin-D protein and mRNA accumulation levels. The regulation of cathepsin-D mRNA accumulation by estrogens is mainly due to increased initiation of transcription. Estrogen-responsive elements have been defined in the proximal ►promoter region of the gene, and in conjunction with other regulatory sequences (*e.g.* SP1, AP1), they may be responsible for the stimulation of cathepsin-D gene expression. Studies in estrogen receptor negative breast cancer cell lines that are the more aggressive, invasive and metastatic indicated a constitutive over-expression of cathepsin-D. The mechanism of this over-expression is still unknown but does not seem to involve ►gene amplification or major ►chromosomal rearrangements (►Chromosome translocation).

Cancer

Cathepsin-D over-expressed by cancer cells stimulates tumorigenicity and ►metastasis in nude mice. The direct role of cathepsin-D in cancer metastasis was first demonstrated in rat tumor cells in which transfection-induced cathepsin-D over-expression increased their metastatic potential *in vivo*. In this rat tumor model, the cathepsin-D mechanism responsible for metastasis stimulation seemed to be a positive effect on cell proliferation, favoring the growth of micrometastases. Using an RNA antisense strategy, cathepsin-D was then shown to be a rate limiting factor for the outgrowth, tumorigenicity and lung colonization of MDA-MB-231 breast cancer cells. Several reports have indicated that cathepsin-D stimulates cancer cell proliferation. Purified pro-cathepsin-D from MCF-7 breast cancer cells stimulated MCF-7 cell growth. Moreover, 3Y1-Ad12 rat cancer cells transfected with human cathepsin-D cDNA grew more rapidly both at low or high cell densities *in vitro* and showed an increased experimental metastatic potential *in vivo*. In addition, pro-cathepsin-D was also mitogenic for breast and prostate cancer cells.

Clinical Aspects

Different approaches, such as cytosolic immunoassay, ►immunohistochemistry, *in situ* hybridization, and Northern and Western blot analyses, have indicated that in most breast cancer tumors, cathepsin-D is over-expressed from 2- to 50-fold compared to its concentration in other cell types such as fibroblasts or normal mammary glands. Several independent clinical studies have shown that the cathepsin-D level in primary breast cancer cytosols is an independent prognostic parameter correlated with the incidence of clinical metastasis

and shorter survival times. The major cathepsin-D producing cells appear to be ►epithelial cancer cells ►(Epithelial tumors) and stromal ►macrophages. Cathepsin-D production by fibroblasts appears variable according to various publications. Certain studies have indicated that cathepsin-D production is low relative to cancer cells as shown by immunohistochemistry and *in situ* hybridization with antisense RNA. Other studies have indicated a prognostic role for cathepsin-D over-expression by reactive stromal cells. Pro-cathepsin-D is also increased in the plasma of patients with metastatic breast cancer, indicating that part of the pro-cathepsin-D secreted by tumors can be released into the circulation.

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Cathepsins

Definition

Are mainly lysosomal cysteine proteases (human cathepsins B, C, F, H, K, L, O, S, V, X, W), other cathepsins belong to the serine (cathepsin G) and the aspartic (cathepsins D, E) proteases. Cathepsins were long believed to be involved in intracellular protein degradation, recently it has become evident that they are involved in a number of specific cellular processes and that their irregular function is associated with pathological conditions, including cancer. Cathepsins were originally defined as a group of digestive proteases present in lysosomes and involved in lysosomal protein breakdown. From a genetic, biochemical and catalytic point of view, cathepsins constitute an extremely heterogeneous group of proteases. This diversity assures in most tissues complete degradation of ingested proteins. With the identification of select cathepsins in other vesicular compartments of the secretory and endosomal system, however, the definition of cathepsins has evolved to also take into account their capacity to act by limited proteolysis on certain proteins

►Cystatins
►Stefins

Catheters

Definition

A hollow tube that can be inserted into a body cavity, duct, vessel, or tissue. Catheters allow drainage or injection of fluids, as well as access for surgical instruments or thermometry devices.

►Hyperthermia

Caveolae

Definition

Are small caveolin-coated invaginations of the cell plasma membrane and function as transport and signal transduction compartments.

►DLC1 (Deleted in Liver Cancer 1)

Caveolins

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Definition

Caveolins are integral membrane proteins responsible for the formation of caveolae, small vesicular invaginations of the plasma cell membrane. They play a key role in membrane trafficking and ►signal transduction.

Characteristics

Caveolae ("little caves") are flask-shaped, "smooth," vesicular invaginations of the plasma membrane

(50–100 nm in diameter) distinct from the larger electron-dense clathrin-coated pits. As a subset of detergent-resistant liquid-ordered lipid rafts, which are clustered protein microdomains within a "sea of homogeneously distributed lipids," they are uniquely enriched in cholesterol, sphingolipids, and phosphatidylethanolamine, and additionally contain essential structural marker proteins termed caveolins. Specifically, caveolins are highly conserved hairpin loop-shaped (both the C-terminus and the N-terminus face the cytoplasmic side of the membrane), oligomeric, integral membrane proteins of 22–24 kDa with a typical short stretch of eight amino acids (FEDVIAEP), the "caveolin signature sequence." Three distinct caveolin genes have been identified: caveolin-1 or VIP-21 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3). Cav-1 exists in two isoforms Cav-1α (containing residues 1–178) and Cav-1β (containing residues 32–178); Cav-2 exists in three isoforms, the full-length Cav-2α, and two truncated variants, Cav-2β and Cav-2γ. Cav-1 and Cav-2, proposed to function as an accessory protein to Cav-1, are co-expressed in most differentiated cells, including adipocytes, endothelial cells, pneumocytes, Schwann cells and fibroblasts; whereas Cav-3 is found specifically in skelatal muscle, the diaphragm, and the heart. Apart from the plasma cell membrane, caveolins are also present in other cellular localizations including endocytic vesicles called caveosomes, mitochondria, the endoplasmatic reticulum (ER), the Golgi/trans-Golgi network (TGN) and secretory vesicles. In addition Cav-1 is secreted by some cells into the extracellular space. Functionally, caveolae and caveolins have been implicated in vesicular transport (transcytosis, pinocytosis, and clathrin-independent ►endocytosis) and cholesterol homeostasis. Moreover, caveolins in general and Cav-1 in particular, interact through the caveolin scaffolding domain (CSD) with a vast variety of proteins thereby sequestering and organizing protein complexes and regulating multiple intracellular signaling pathways. Such molecules include ►Src family tyrosine kinases, ►G protein α subunits, G protein-coupled receptors and ►receptor tyrosine kinases (i.e. receptors for ►epidermal growth factor (EGFR), ►insulin-like growth factor (IGFR), ►placenta-derived growth factor (PDGFR), ►interleukin-6 (IL-6), ►vascular endothelial growth factor (VEGFR)), Ca^{2+} pumps, endothelial ►nitric-oxide synthetase (eNOS), ►integrins, ►Protein Kinase C α, as well as components of the tumor growth factor β (TGFβ/SMAD, ►Wnt/β-catenin/Lef-1 and ►MAP Kinase (e.g. H-Ras, ►Raf kinase, p38) pathway. In addition to the CSD, SH2 domain-containing molecules (i.e. Grb7) interact with Cav-1 *via* the growth-factor/cytokine-triggered phosphorylation of the tyrosine residue 14. Dysregulation of caveolins is associated with the pathogenesis of several human diseases including type II diabetes; Alzheimer's disease; atherosclerosis; muscular dystrophy; and ►cancer.

Clinical Aspects

The ability of Cav-1 to interact with and regulate the activity of proteins involved in cell transformation, growth, invasion, and cytoskeletal rearrangement, renders Cav-1 a key role in tumorigenesis. While initial studies have demonstrated that Cav-1 negatively regulates signaling molecules thereby mediating cell growth inhibition, several recent reports clearly show a positive correlation between high Cav-1 expression, ►tumor grade, ►progression/►metastasis and chemoresistance. One hypothesis for this dual role may be that by Cav-1 downregulation in early tumors stages growth-inhibitory signals pivotal for clonal expansion are avoided, and conversely, by Cav-1 upregulation in progressed ►tumor stages (characterized by increased genomic instability and accumulation of genetic changes) survival benefits are conferred through inhibition of ►apoptosis and acquisition of multidrug resistance (MDR). For example, increased Cav-1 expression has been linked to the progression of tumors including human ►prostate cancer; primary and metastatic human ►breast cancer; progression of ►thyroid cancer; high-grade ►bladder cancer; metastasis of ►lung and ►pancreatic cancer; lymph node metastasis in ►esophageal squamous cell carcinoma; and ►multiple myeloma. Based on these proposed roles of Cav-1 in tumor progression, ongoing studies are now exploring caveolins as novel therapeutic targets in cancer therapies. High levels of Cav-1 expression in vascular endothelial cells additionally provide the rationale for using Cav-1 targeted therapy to inhibit tumor ►angiogenesis. Approaches to target caveolins in general and Cav-1 in particular include the use of Cav-1 antisense and Cav-1 ►siRNA, as well as the use of synthetic CSD, which competitively inhibits protein interactions with Cav-1. Further therapeutic strategies include attempts to inhibit or disrupt caveolae formation using either statins (3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors), which block the production of the cholesterol intermediate mevalonate; or the cholesterol-binding agent methyl-β-cyclodextrin (MβCD). Alternatively, caveolae might be used as a drug and gene delivery transport system to specifically target anticancer therapies to tumor cells thereby reducing required dosages and overall toxicity.

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C-Bandless Chromosome

Definition

Is a centric chromosome lacking the typical banding pattern after C-banding; chromosome arms show features of a homogeneously Staining region.

►Amplification

CBFA2

►Runx1

CBP/p300

Definition

Cyclic AMP response element- binding protein (CBP) and its homologue p300 are transcriptional co-activators of various sequence-specific transcription factors that are involved in a wide array of cellular activities, such as DNA repair, cell growth, differentiation and apoptosis. The ability of CBP/p300 to activate transcription resides in its capacity to acetylate the core histone proteins associated with enhanced promoter regions of the genes it activates. This induces conformation changes in chromatin and allows the recruitment of auxiliary proteins to activated promoters. CBP/p300 has the capacity to acetylate and regulate the activity of a variety of transcription factors including p53, NF-B and c-Myc. Acetylation in this context affects both the ability of transcription factors to bind DNA, and also to recruit other binding proteins.

►NUP98-HOXA9 Fusion
►HCBP/p300 Coactivators

CBP/p300 Coactivators

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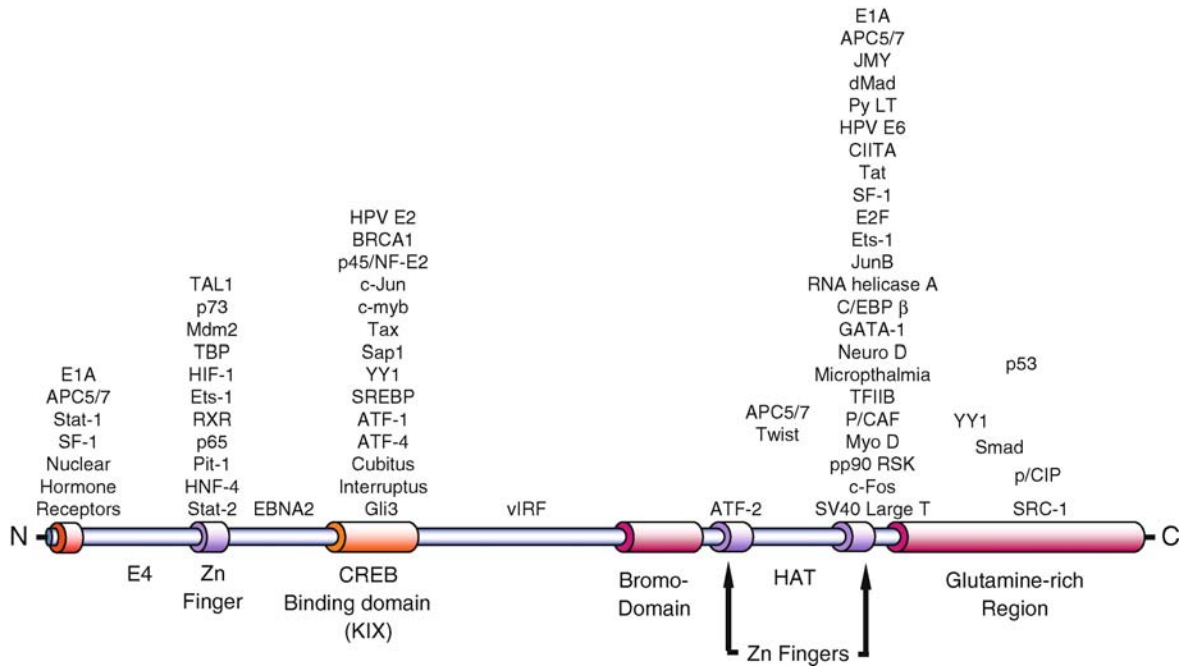
Definition

CBP is an acronym for cAMP-regulated-enhancer (CRE)-binding protein (CREB)-binding protein. p300 is a protein that is highly homologous to CBP, and has been named according to its approximate molecular weight. Coactivators are a group of cellular proteins that enhance transcription factor-dependent transcriptional activation.

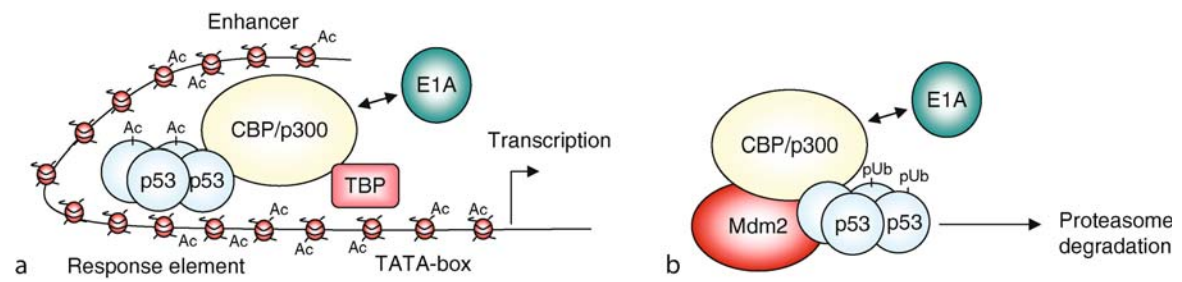
Characteristics

CBP was initially identified as an auxiliary cofactor required for the CREB-mediated activation of cAMP-stimulated gene ►transcription. CBP binds specifically, at CREs, to an activated CREB species which has been suitably modified through phosphorylation by the cAMP-responsive protein kinase, PKA. p300 was subsequently characterized, independently, upon the basis of its interaction with the protein product of the adenoviral transforming *E1A* gene, and like CBP can function as a coactivator in CREB-mediated transcriptional activation. CBP, akin to p300, also binds to E1A. CBP and p300 are highly-related at the amino acid sequence level, sharing approximately 60% identity and both proteins have predicted molecular weights of 265 kDa [1]. Although CBP and p300 bind to a similar set of cellular proteins, share identical enzymic activities (Fig. 1), and overlap functionally in regulating ►cell-cycle and ►differentiation pathways, it is important to note that they also possess distinct biological functions. For example, discrete roles for CBP and p300 during retinoic-acid-induced differentiation, cell-cycle exit and ►apoptosis of embryonal carcinoma F9 cells have been identified. p300, but not CBP, was found to be required for both retinoic-acid induced differentiation, and transcriptional upregulation of the cell-cycle inhibitor $p21^{CIP1/WAF1}$. In contrast, CBP, but not p300, was required for transcriptional induction of $p27^{KIP1}$. Interestingly, both CBP and p300 were required for retinoic acid induced apoptosis.

CBP and p300 function primarily as transcriptional coactivators for many sequence-specific transcription factors. In this capacity both CBP and p300 function as lysine (K)-directed ►acetyltransferases [ATs; (Fig. 2a)]. They modify ►chromatin structure and function through acetylation of the core histones H2A, H2B, H3 and H4 at numerous sites within their N-terminal tail regions. Specific p300-directed acetylation sites within



CBP/p300 Coactivators. Figure 1 Schematic depiction of CBP/p300 primary sequence displaying conserved domains. The diagram shows the binding sites for a number of proteins including the APC/C subunits APC5 and APC7, p53 as well as the adenoviral E1A protein: E4, ubiquitin E4 ligase activity; HAT, histone-directed AT activity.



CBP/p300 Coactivators. Figure 2 Role of CBP and p300 in acetylation and ubiquitylation. (a) CBP and p300 bind to enhancer and promoter regions and promote the acetylation of the core histones in order to promote the recruitment of transcription factors and auxiliary factors to sites of transcription. Acetylation of the transcription factor p53, promotes its binding to p53-response elements, Ac: acetylation (b) CBP and p300 accelerate Mdm2-mediated polyubiquitylation (pUb) of p53 promoting its degradation by the proteasome. The adenoviral E1A protein binds to CBP/p300 to regulate both acetylation and ubiquitylation activities.

►nucleosome-associated histones have been identified. p300 acetylates H2A upon K5, H2B upon K5, K12, K15 and K20, H3 upon K14 and K18, and H4 upon K5, K8 and K12. Histone acetylation by CBP and p300 facilitates further epigenetic histone modifications and the recruitment of other proteins involved in transcriptional activation to promoter/enhancer regions, potentially through reducing the affinity of histone tails for DNA. Interestingly, p300 AT activity itself is enhanced by auto-acetylation of critical lysine residues in an activation loop motif found within its AT domain. Specifically, auto-acetylation of critical residues K1499, K1549, K1554, K1558, and K1560 enhances AT activity.

CBP and p300 also enhance transcription through their ability to interact with, and acetylate non-histone proteins and regulate their cellular activities. Indeed, CBP and p300 acetylate a variety of transcription factors directly, including ►p53, E2F-1, NF-κB and c-Myc. For example, p300 has been shown to enhance p53 transcriptional activity by promoting p53 sequence-specific binding to DNA through the acetylation of multiple residues in p53's C-terminal region.

Lysine residues K370, K372, K373, K381 and K382 have all been found to be substrates for p300-directed acetylation *in vitro*. Consistent with these observations K373 is acetylated *in vivo* in circumstances when p53 transcriptional activity is stimulated by UV- and ionizing-radiation. Interestingly, Mdm2 the ►E3 ubiquitin ligase that targets p53 for degradation, inhibits p300-mediated acetylation of p53. CBP and p300 can also function as transactivators independently of AT activity. Thus CBP and p300 mutants that lack the AT domain can still stimulate transcription. CBP and p300 function in this regard through specific binding to transcription factors such as nuclear receptors, or p53. p300 also possesses an N-terminal ►E4 ubiquitin ligase domain. It has been shown that this domain catalytically enhances the Mdm2-directed poly-ubiquitylation of p53, promoting degradation (Fig. 2b). E1A inhibits p300 function in this regard.

A role for CBP and p300 in cell-cycle and cellular ►transformation was first established during early studies with E1A. E1A mutants incapable of binding to CBP and p300 were found to be defective in their ability to promote S-phase and initiate DNA synthesis in Baby Rat Kidney (BRK) cells; E1A was also shown to induce S-phase by a redundant pathway through its interaction with the protein product of the *Retinoblastoma* gene, pRb. Interestingly, E1A's capacity to induce mitosis in BRKs requires its interaction with both pRb and CBP/p300. Moreover, the ability of E1A to transform primary rodent cells in tissue culture was found to be wholly-dependent upon its interaction with CBP and p300, suggesting that both CBP and p300 might function as ►tumor suppressors. *In vitro* models suggest that E1A inhibits CBP/p300-directed AT activity and represses CBP/p300-dependent transcription programmes. Alternatively, E1A could utilize CBP/p300 acetyltransferases during ►tumorigenesis to promote an altered programme of gene expression. A role for the E3 ubiquitin ligase, the ►APC/C, in CBP/p300 function has recently been determined. E1A and APC/C subunits APC5 and APC7 share evolutionarily conserved CBP/p300-binding domains within their primary sequence. Studies have suggested that E1A de-regulates CBP/p300 during tumorigenesis by disrupting CBP/p300-APC/C cell cycle function. Interestingly, E1A residue K239 is acetylated by CBP/p300 *in vivo* and E1A associates with CBP/p300 AT activity from adenovirus infected and transformed cells. Acetylation of E1A has been proposed to affect its interaction with the corepressor CtBP, and alter its nuclear localization by disrupting E1A association with importin-α. Whether acetylation of E1A is required for transformation with either Ras or E1B is not known. The requirement for the CBP/p300 E4 ligase in E1A-mediated transformation is similarly not known.

There is increasing evidence to suggest that CBP and p300 might be functionally de-regulated in ►cancer. In support of this notion, studies have indicated that both CBP and p300 genes are functionally de-regulated in ►acute myeloid leukemia (AML). Specifically, chromosomal translocations occur during AML tumorigenesis where a significant portion of the gene encoding the monocytic leukemia zinc-finger AT (MOZ) fuses with a large part of the CBP or p300 gene to form MOZ-CBP or MOZ-p300 chimeras. It is proposed that these chimeric proteins possess aberrant AT activity which is important in promoting tumorigenesis. Chromosomal rearrangements are more common for CBP than p300 in this regard. Mixed lineage leukemia (MLL), MLL-CBP and MLL-p300 translocations have also been described. Studies have also indicated that somatic mutations in one p300 allele, accompanied by ►loss of heterozygosity (LOH) of the second wild-type allele also occur in isolated cases of human colorectal and breast tumors. Similarly, biallelic somatic inactivation of CBP has been observed in ovarian tumors, esophageal squamous cell carcinomas and some lung cancers, suggesting that both CBP and p300 might function as classical tumor suppressors in epithelial cancers.

In support of these findings, germ-line monoallelic inactivation of CBP is the genetic basis for Rubinstein Taybi Syndrome (RTS), a disease characterized by pleiotropic developmental abnormalities and an increased incidence of malignancies, usually childhood tumors of neural crest origin. Whether these tumors are characterized by LOH is however, not known. Interestingly, mice displaying monoallelic inactivation of CBP also display characteristics of RTS, whilst mice engineered heterozygous for CBP display hematological developmental abnormalities, and with increased age develop a number of hematological malignancies, that in some instances are characterized by LOH. Germ-line monoallelic mutations in p300 also result in RTS. It is not known at present however, whether these RTS patients also have an increased risk of developing tumors. However, mice heterozygous for p300 do not develop malignancies at a higher frequency. The ability of CBP and/or p300 to function as ►tumor suppressor genes may reside in their capacity to directly interact with tumor suppressor gene products and ►oncogene products, or through regulating, indirectly, multiple signaling pathways that coordinate cell cycle progression and/or differentiation programmes.

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CBs

Definition

►Cajal Bodies

CCAAT/Enhancer-Binding Protein (CEBPA)

Definition

Is a myeloid transcription factor crucial for normal granulopoiesis. The expression of CEBPA is specifically suppressed in ►acute myeloid leukemia patients carrying the *AML1-MDS1-EVI1* (*AME*) translocation in their chromosomes.

►Calreticulin

CCCTC-Binding Factor

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Synonyms

CTCF

Definition

CTCF (acronym for a "CCCTC-binding factor") is a highly conserved and ubiquitous protein with multiple functions, which include regulation of transcription, chromatin insulation and genomic imprinting.

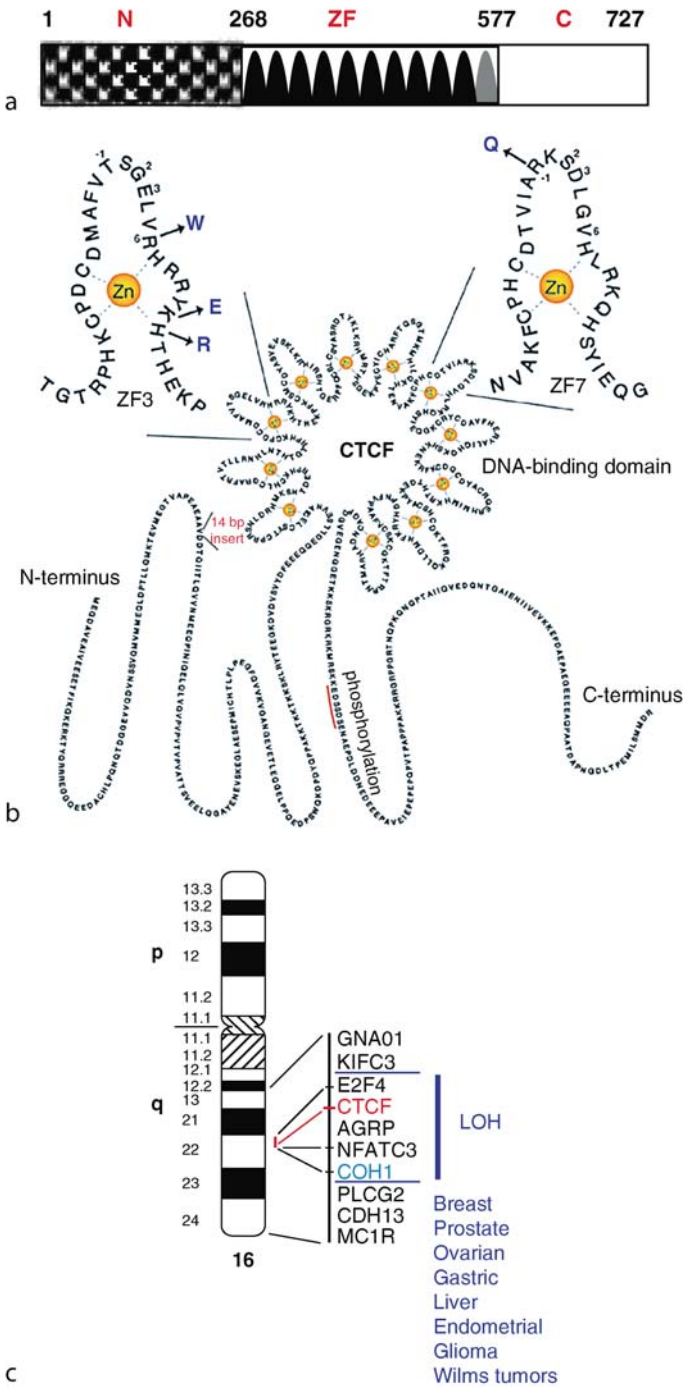
Characteristics

The CTCF protein was originally identified for its ability to bind to a promoter element of the chicken c-myc gene. The sequence recognized by CTCF contained the CCCTC repeats and therefore the protein was defined as CTCF (the CCCTC binding factor). However it was later discovered that other CTCF target sequences (or CTSs) were remarkably dissimilar, and the term "multivalent transcription factor" was coined for CTCF. Another unusual feature of the CTSs is their length: the analysis of binding patterns of CTCF to multiple sites demonstrated that CTCF requires about 50–60-bp-long sequence to form a complex with DNA.

The ability of CTCF to bind such diverse targets has been attributed to its DNA binding domain, which is composed of eleven Zinc Fingers (ZFs), ten of them of the C_2H_2 class and one ZF of C_2HC-class (Fig. 1a and b). According to this model, the combinatorial utilization of different ZFs results in binding to diverse DNA targets. In addition, CTCF-DNA complex formation can be regulated by DNA ►methylation, if symmetrically methylated CpG dinucleotides present on both DNA-strands within any given CTS coincide with the DNA-bases required for the CTS recognition by a particular subset of CTCF fingers. Not all CTCF-target sequences contain CpG bp that can be modified by methylation, nevertheless the capability of CTCF to distinguish differentially methylated DNA targets is one of the major features of CTCF with a broad spectrum of functional implications.

The CTSs have been identified in many genomic elements. It is estimated there may be well over 30,000 of CTSs in the human genome, with ~14,000 localized in potential insulators. Many of these sites are methylation sensitive and map to promoter, intergenic and intragenic regions, and both exons and introns. Examples of CTCF-target promoters include 5′-non-coding regions of the c-*Myc* oncogene, chicken lysozyme, *IRAK2*, ►*BRCA1*, the amyloid precursor protein (*APP*), the exon regions of *hTERT* and the intron regions of the serotonin transporter gene, *SLC6A4*. Other CTCF-driven regulatory elements include vertebrate enhancer-blocking elements (insulators), classic examples of which are chicken *β*-globin insulators that flank *β*-lobin gene cluster. Such intergenic insulators seem to have a consensus binding motif for CTCF. CTCF sites are universally present in all mammalian differentially methylated domains/regions (DMD/DMR) or imprinting control regions (ICR), as exemplified by CTSs in ICRs of such imprinted gene-clusters as *IGF2/H19*, *Rasgfr*, *KvDMR* and other loci, deregulation of which through aberrant (biallelic) CTS-methylation or CTS-demethylation contributes to cancer.

CTCF has now been cloned from various organisms which include insects, fish, amphibians, birds, rodents, and primates. The comparison between the proteins



CCCTC-Binding Factor. Figure 1 (a) Schematic drawing of the CTCF protein. The three domains of CTCF are depicted as follows: N – N terminal domain (Patterned box); ZF – ZF domain (box with half ovals designating 11 Zinc Fingers; the black half ovals refer to the C_2H_2 class and the grey half oval refers to the C_2HC-class); C – C-terminal domain (open box). The amino acid numbers for the start and the end of each domain are indicated above the diagram. (b) The cartoon illustration of the wild-type human CTCF protein represents the N-terminal and C-terminal domains of CTCF and the DNA binding domain of CTCF composed of 10 ZF of C_2H_2-class and one ZF of C_2HC-class. (c) The locations of the tumor specific mutations in the CTCF protein are shown. The mutations CTCFHR, KE, RW are located in ZF3 and the mutation CTCFRQ is located in ZF7. The position of the 14 bp insertion is indicated.

revealed a high degree of homology between the CTCF from different organisms, especially in the ZF DNA binding domain. Thus this domain is 100% identical at the protein level among mouse, man and chicken, whereas the full-length protein is 93% identical in those three species; the *Drosophila* CTCF protein has a 46% identity within the zinc-finger regions and 27% overall identity.

Typically for a transcriptional factor, CTCF is localized to the nucleus. It is ubiquitously expressed in various tissues and cells, in different organisms. Such conservation in the protein composition and also wide representation in cells/tissues signifies the important and general cellular functions mediated by CTCF.

The size of the CTCF protein varies depending on the organism. For example, the human CTCF protein is composed of 727 amino acids, chicken CTCF of 728 and *Drosophila* CTCF of 818 amino acids. The structure of the human CTCF is shown in Fig. 1 (panels a and b). The ZF-DNA binding domain is positioned in the centre of CTCF and accounts for about one third of the protein's size.

The N-terminal domain of human CTCF is composed of 268 amino acids and is rich in proline residues. The C-terminal domain is the smallest part of the molecule (150 amino acids) and is highly negatively charged. These CTCF domains play an important role in the modulation of CTCF functions in the regulation of transcription. In some cases this regulation relies on post-translational modifications. For example, the C-terminal domain contains the sites of phosphorylation by the protein kinase CK2 (former casein kinase II), whereas the N-terminal domain contains the sites for ►poly(ADP-ribosyl)ation by the PARP-1 (poly(ADP-ribose) polymerase-1).

The post-translational modifications and interactions with protein partners have been demonstrated to modulate important functions of CTCF. For example, specific phosphorylation of CTCF by CK2 affects the CTCF functions in transcriptional regulation. Poly (ADP-ribosyl)ation was found to be important for insulator function of CTCF and CTCF-dependant nucleolar transcription. Post-translational modifications of CTCF have also been implicated in human myeloid cell differentiation.

Regulation of CTCF-dependent molecular processes also involves CTCF associations with other proteins. Thus, CTCF interactions with sin3 and YB-1 are shown to modulate CTCF function as a transcriptional repressor. Cooperation of CTCF with nucleophosmin, Kaiso and helicase protein CHD8 has been linked to the control of insulator function of CTCF and epigenetic regulation. Interaction of CTCF with another transcription factor, YY-1, is required to control the X chromosome inactivation and cooperation of CTCF with RNA Polymerase II may be important for regulation of transcription.

A testis-specific paralogue of CTCF has been reported. This protein was termed ►*BORIS* (the acronym for Brother of the Regulator of Imprinted Sites). BORIS possesses the eleven ZF domain homologous to that of CTCF; the flanking N-and C-terminal domain, on the other hand, are dissimilar. These structural features indicate that BORIS could recognize the same set of DNA targets as CTCF, while different flanking domains could be important for regulation of BORIS-specific functions.

CTCF Functions

The diverse nature of the CTSs harmonizes with the multitude of CTCF functions. They include gene activation, repression and silencing; CTCF is also involved in the control of insulator function and ►imprinting. All vertebrate enhancer-blocking elements tested so far contain CTCF binding sites. The importance of the insulator function of CTCF was further demonstrated in the regulation of CTG/CAG repeats in the *DM1* locus and in the X-chromosome inactivation. It is now generally accepted that the molecular basis for the insulator function of CTCF lies in the ability of CTCF to influence chromatin architecture by mediating long-range chromatin looping and modification of histones. Such alterations then settle the balance between active and repressive chromatin.

CTCF binding to many of its targets can be regulated by DNA methylation; the ability of CTCF to read such epigenetic marks contributes significantly to the versatility of CTCF functions. Several findings support the concept of CTCF being a ►tumor suppressor gene (TSG). Firstly, CTCF suppresses cell growth and proliferation, and, further, in some cell systems (for example, myeloid cells) induces cell differentiation. Secondly, the CTCF gene maps within the smallest region of overlap for loss of heterozygosity (LOH) that has been observed at chromosome 16q22.1 in breast, prostate, and Wilms tumors (Fig. 1c). Finally, functionally significant, tumor-specific CTCF mutations in the ZF domain of CTCF were identified in various sporadic cancers including breast, prostate and Wilm's tumors in the remaining allele (Fig. 1b). All four reported tumor specific point mutations in the CTCF Zn finger domain result in a missense codon at a position predicted to be critical for ZF formation or DNA base recognition. Another reported tumor-specific mutation constituted of a 14 bp insertion in the N-terminal domain of CTCF (Fig. 1b). In familial non-*BRCA1*►*/BRCA2* breast cancers, two sequence variants, G240A in the 5′ untranslated region and C1455T (S388S) in exon 4, were also identified.

The CTCF function as a negative regulator of cell growth has been well documented on various cellular models. Thus, over-expression of CTCF leads to inhibition of cell growth and proliferation. Normal embryonic rat cells, made haploinsufficient for CTCF by the retroviral insertion into the intron upstream of the first coding exon, manifest all major features of cancerous transformation in vitro. The mechanism of this function of CTCF, at least in part, lies in the ability of CTCF to control genes responsible for regulation of cell growth and proliferation, negatively ►oncogenes and positively TSG. Examples of such CTCF-target genes include oncogenes ►*MYC*, *PIM-1, PLK, E2F1, TERT, IGF2* and TSGs ►*p19ARF(p16/INK4a)*, BRCA1, ►p53, ►p21, and ►*p27*. Based on these findings, CTCF emerges as a key versatile element linking genetics, epigenetics, development, and disease.

The ability of CTCF to interact with the repeated sequences and read epigenetic marks (DNA methylation) may provide a causal link not only to some forms of neoplasia, but also to degenerative and neurological conditions. Epigenetic disturbances in these diseases are frequently associated with the instability of repeats, which is considered to be the hallmark of this pathology.

Clinical Aspects

A link between CTCF and the disease development has been generally recognized. Various genetic and epigenetic mechanisms that result in CTCF malfunction can lead to pathogenesis.

The tumor specific mutations in CTCF can dramatically change the normal biological functions of the wild type CTCF protein. The sets of the genomic targets of the mutant CTCF variants may alter due to the loss of binding to the usual CTCF targets and/or binding of the mutants to the new targets, especially if the wild type allele is lost. Each ZF mutation abrogate CTCF binding to a subset of target sites within the promoters and/or insulators of certain genes involved in regulating cell proliferation, but do not alter binding to the regulatory sequences of other genes. These observations suggest that CTCF may represent a novel tumor suppressor gene that displays tumor-specific "change of function" rather than complete "loss of function."

The 14 bp insertion in the N-terminal domain, on the other hand, most likely leads to the loss of function of CTCF as it creates a premature stop codon, thus generating a truncated CTCF protein. The significance of the sequence variants in the familial breast cancers, however, is not yet clear.

The genetic alterations in CTCF are rare events therefore considerable efforts are being currently made to identify epigenetic mechanisms responsible for inactivation of CTCF. The rationale behind these studies is that the binding of CTCF to its DNA targets is methylation sensitive, with the current view that the bound CTCF can protect the CpG islands of DNA against methylation. Indeed, it has been reported that de-repression of the maternal *IGF2* allele is linked to abnormal methylation of the CTCF target sites within the *ICR H19* in a wide range of cancer types (breast, prostate, colorectal, Wilm's tumor). This has been explained by the inability of CTCF to bind to the methylated *ICR H19*, and therefore its failure to establish the chromatin insulator function on the maternal allele thus leading to activation of *IGF2*.

There is a growing body of evidence to suggest that even mutations of a single CTCF site leads to dramatic biological consequences. For instance, mutations of the CTCF site in the Xist promoter that alter CTCF binding result in the skewed X chromosome inactivation in affected families. Furthermore, deletions of CTCF sites in human *ICR H19* lead to predisposition to Wilm's tumors in families with Beckwith-Wiedemann Syndrome (BWS). Finally, a mutation of the single CTCF site in the homologous *ICR H19* predisposes the mice carrying such a mutation to colorectal cancer.

Epigenetic inactivation of a number of cancer genes due to aberrant methylation of the CpG islands within their promoters has also been established. Interestingly, many of these genes are regulated by CTCF. As in the case with the *ICR H19*, CTCF may be necessary to protect the promoters of the TSGs from unwanted DNA methylation. According to another, yet to be proven model, CTCF may demarcate the boundary between methylated and unmethylated genomic domains, as may be the case for the BRCA1 promoter.

The utility of CTCF as a cancer ►biomarker is yet to be established, although there are indications that CTCF may be an interesting target for therapy in breast tumors where levels of CTCF were found elevated compared with breast cell lines with finite life span and normal breast tissues. Such up-regulation of CTCF in breast cancer cells has been linked to resistance of these cells to apoptosis. The results of the experiments in breast cancer cell lines point to a possible link between CTCF expression and sensitivity to apoptosis; that is, higher levels of CTCF may be necessary to protect the more sensitive cancer cells from apoptotic stimuli. These findings may be relevant to the potential use of CTCF as a therapeutic target in breast cancers: reducing the levels of CTCF would then result in apoptotic cell death of cancer cells hopefully without affecting normal breast tissue; the effect of CTCF down-regulation may be more dramatic in high grade breast tumors. On the other hand, elevated levels of CTCF in breast tumors may correlate with several clinical and/or pathological parameters, which make CTCF a potential

prognostic marker. More research is needed to clarify the full potential of CTCF as a clinical target and a cancer biomarker.

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CCI-779

►Temsirolimus

CCL5/RANTES

Definition

CCL5 (chemokine (C-C motif) ligand 5)/RANTES (regulated upon activation, normal T-cell expressed, and secreted) is a small cytokine belonging to the CC ►chemokine family, binds and activates CCR5, and is chemotactic for T cells, eosinophils and basophils.

►Langerhans Cell Histiocytosis

CCL20/MIP-3α

Definition

CCL20 (chemokine (C-C motif) ligand 20)/MIP-3α (macrophage inflammatory proteins-3α) is a small cytokine belonging to the CC ►chemokine family, binds and activates CCR6, and is strongly chemotactic for lymphocytes.

►Langerhans Cell Histiocytosis

CCND1

Definition

Synonyms PRAD1; Is an oncogene localized to chromosomal band 11q13. This gene encodes the protein Cyclin D1 that functions as a cell cycle regulator. Amplification of CCND1 has recently been reported in several human tumors, including breast and head neck carcinomas.

►Cyclic D

CCR4

Definition

Chemokine (C–C motif) receptor 4; chemokine receptor, expressed selectively on Th2 lymphocytes and its ligand thymus and activation-regulated ►chemokine (►TARC).

CCR6

Definition

CCR6 is ►chemokine receptor for CCL20, and is expressed on unactivated memory T-cells and immature ►dendritic cells.

►Langerhans Cell Histiocytosis

CCRG-81045

►Temozolomide

CCV

Definition
Clatherincoated vesicles.

►Cyclin G-Associated Kinase

CD1a

Definition
Is expressed on the surface of Langerhans cells. It is related to class I MHC molecules and involved in the presentation of lipid antigens to CD1-specific T cells.

►Langerhans Cell Histiocytosis
►CD Antigens

CD2AP

Definition
CD2 associated protein is an adaptor protein that couples endocytic proteins to the actin cytoskeleton.

►Cortactin
►CD Antigens

CD3 Complex

Definition
Is the complex of α:β or γ:δ T-cell receptor chains with the invariant subunits CD3γ, δ, and ε, and the dimeric ζ chains.

►Sjögren Syndrome
►CD Antigens

CD4

Definition
CD4, the cell-surface protein, is important for recognition by the T-cell receptor of antigenic peptides bound to ►MHC class II molecules. It acts as a coreceptor by binding to the lateral face of the MHC class II molecules.

►Sjögren Syndrome
►CD Antigens

$CD4^+$T-Cells

Definition
$CD4^+$ helper T-cells are a sub-group of lymphocytes that plays an important role in establishing and maximizing the capabilities of the immune system. These cells are unusual in that they have no cytotoxic or phagocytic activity; they cannot kill cancer cells. $CD4^+$ helper T-cells are involved in activating and directing anti-tumor immune responses. They are essential in activation and growth of cytotoxic T cells, and in maximizing anti-tumor humoral immune responses.

►DNA Vaccination
►CD Antigens

CD5 B Cells

Definition
Are a class of atypical, self-renewing B cells found mainly in the peritoneal and pleural cavities in adults. They have a far less diverse receptor repertoire than conventional B cells, and since they are the first B cells to be produced they are also known as B-1 cells.

►Sjögren Syndrome
►CD Antigens

CD8

Definition
A the cell-surface protein, is important for recognition by the T-cell receptor of antigenic peptides bound to ►MHC class I molecules. It acts as a coreceptor by binding to the lateral face of MHC class I molecules.

►Sjögren Syndrome

CD8+ Cytotoxic T-Cells

Definition

Synonym TC, CTL, T-Killer cell or killer T cell; belong to a sub-group of T-cells capable of inducing the death of cancer cells. Most cytotoxic T cells express T-cell receptors (TcRs) that can recognize a specific antigenic peptide bound to Class I major histocompatibility complex (MHC) molecules. The binding of CD8 to the MHC molecule keeps the $CD8^+$ cytotoxic T-cells and the target cell bound closely together. Cell death is induced by the release of perforin and granzyme as well as by expression of FAS ligand which induces ►apoptosis of the cancer cell.

►DNA Vaccination

CD8 T Cells

Definition

CD8 T cells are T cells that carry the coreceptor ►CD8. They recognize antigens, for example, viral antigens, that are synthesized in the cytoplasm of a cell. Peptides derived from these antigens are transported by TAP, assembled with ►MHC class I molecules in the endoplasmic reticulum, and displayed as peptides: MHC class I complexes on the cell surface. CD8 T cells differentiate into cytotoxic CD8 T cells.

►Sjögren Syndrome

CD14

Definition

Is a membrane-associated glycosylphosphatidylinositol-linked protein expressed at the surface of cells, especially ►macrophages. CD14 acts as a co-receptor (along with the Toll-like receptor TLR 4) in the cellular response to bacterial endotoxins.

►Kupffer Cells
►CD Antigens

CD15

Definition

Carbohydrate antigen called the X hapten, found in several glycolipids and glycoproteins. The antigen is present on normal myeloid cells and a wide variety of epithelial cells, as well as their corresponding tumors.

►Hodgkin Disease, Clinical Oncology
►CD Antigens

CD20

Definition

Is a non-phosphorylated glycoprotein expressed on all mature B cells and in less density on pre-B cells. CD20 functions as regulator of transmembrane Ca^{2+} -conductance and is thought to play a role in B cell-activation and -proliferation.

►Monoclonal Antibody Therapy
►CD Antigens
►Hodgkin Disease, Clinical Oncology

CD24

Definition

Cell surface glycoprotein that has been shown to regulate E-cadherin and TGF-beta3 expression.

►Pancreatic Cancer Stem Cells
►CD Antigens

CD26

►CD26/DPPIV in Cancer Progression and Spread

CD26/DPPIV in Cancer Progression and Spread

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Synonyms

CD26; Dipeptidyl peptidase IV; DPPIV; ADAbp; ADA-CP

Definition

CD26/DPPIV is a multifunctional protein in the outer membrane of normal and cancer cells, that can (i) remove an amino-terminal dipeptide from many regulatory peptides, terminating their activity, (ii) bind the enzyme adenosine deaminase (ADA) from the extracellular fluid, and (iii) associate directly with proteins of the ►extracellular matrix. Levels of CD26/DPPIV are variable but typically decline as cancer develops, and this has been linked to disease progression and the shift to metastasis.

Characteristics

CD26/DPPIV is a molecule that has been known in different forms since the 1960s, but whose key role in cancer has only been appreciated since the early 1990s when it was shown that the absence or presence of CD26/DPPIV in ►melanocytes determined whether or not those cells showed behavior that was characteristic of a cancer (►melanoma). Our understanding of CD26/DPPIV has an interesting history, as it reflects the collective findings of four different areas of research – in fact directly reflecting the multifunctional nature of the protein itself. The different aspects of the function of this molecule are illustrated in Fig. 1.

Some of the earliest data on this molecule were obtained in studies of the major binding protein for the enzyme adenosine deaminase (ADA) in gastrointestinal epithelia. When ADA was isolated from tissue it was found to exist in both high-molecular-weight and low-molecular-weight forms. The high-molecular-weight form was found to be a complex of ADA itself with a larger, 110-kilodalton protein, subsequently referred to as ADA-complexing protein (ADA-CP) or ADA-binding protein (ADAbp). This anchoring protein for ADA was later shown to be identical to CD26/DPPIV, the extracellular part of which has a region that acts to bind ADA from outside of the cell.

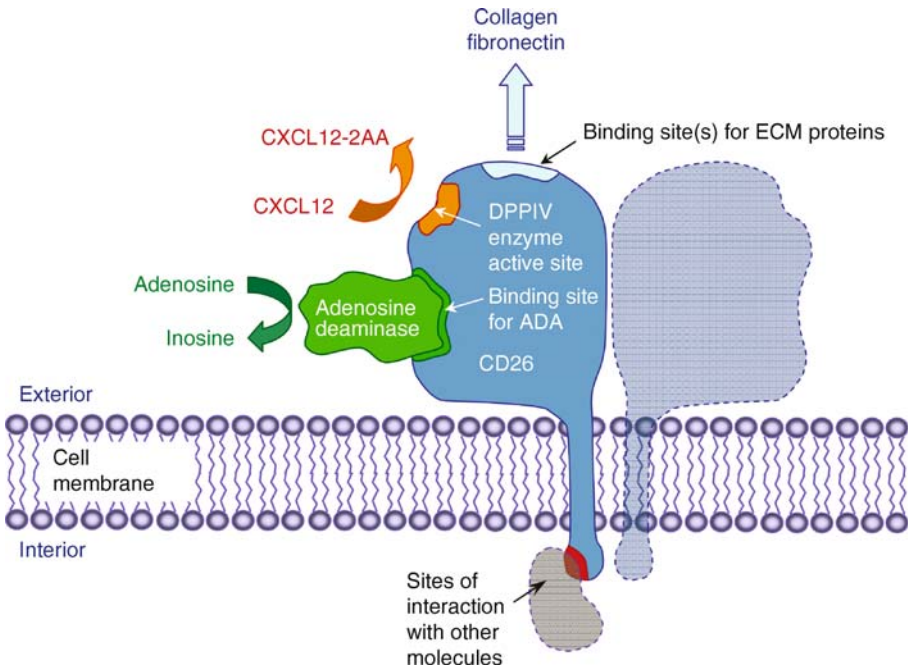
Some of the major substrates for this activity are listed in Table 1. Early studies on CD26/DPPIV also addressed its enzyme activity. The dipeptidyl peptidase IV (DPPIV) activity is an intrinsic part of the molecule itself, and was initially studied mostly at a biochemical level. This very selective form of enzyme activity removes just two amino acids from the N- (amino-) terminus of a peptide, which is why is called a dipeptidase. The characteristic activity of DPPIV requires that the penultimate N-terminal amino acid has a particular identity; usually ►proline and less commonly ►alanine. This is a part of the peptide that often has effects on its stability within the body – the existence of a proline in that position typically confers greater stability. So the removal of this dipeptide by DPPIV is a means of regulating the persistence and bioactivity of important regulatory peptides.

The relative susceptibilities to cleavage of the substrates are given on an arbitrary scale based upon their specificity constants (k_{cat}/K_m). A high number indicates that the peptide is a good substrate for the dipeptidyl peptidase-IV activity of CD26/DPPIV.

The third area of research that led to our present knowledge of CD26/DPPIV involved the way in which lymphocytes become activated. Lymphocytes normally reside in the body within particular tissue structures – specialized structures called lymph nodes or at specific sites within the gut mucosa, for example – in numbers that are necessary to be able to respond to almost all of the threats that may be encountered. In the event of such a challenge, however, the cells that are most able to deal with the threat are mobilized, divide so as to make a larger population of specialized defenders, and become armed to respond in the appropriate way. As these cells become activated, various important proteins are produced at the cell surface. These "activation proteins" are given "CD" numbers as unique identifiers ("CD" refers to "cluster of differentiation" markers, or antigens). The differentiation antigen designated CD26 has proven to be identical to the molecules ADAbp and DPPIV.

The last of the roles for CD26/DPPIV follows from its ability to bind to extracellular matrix molecules, primarily ►collagen and ►fibronectin. These are embedded within the molecular scaffold that surrounds all cells and which provides particular cues for cellular behavior in three dimensions. For the CD26/DPPIV that is present on cancer cells, this opens up the possibility that it may act as an additional anchor to tether cells to the extracellular matrix, along with dedicated cell adhesion molecules such as the ►integrins. The reverse situation may also be important during the process of metastasis. It has been shown that the CD26/DPPIV that is present at the surface of endothelial cells lining blood vessels can interact with a form of fibronectin that is deposited on the surface of cancer cells. This may cause arrest of circulating cancer cells that have become detached from the main tumor, and help to seed the cancer at secondary sites like the lung.

The same molecule therefore has four different functions, and has four different names that have been used



CD26/DPPIV in Cancer Progression and Spread. Figure 1 The different domains and functions of CD26/DPPIV. The CD26 protein is anchored in the plasma membrane of the cell, with the bulk of its molecular structure on the outer face. The enzyme domain that underlies its dipeptidyl peptidase-IV activity, removing pairs of amino acids (AA) from substrates such as the chemokine CXCL12, comprises one of three functional sites in contact with the external environment. A separate domain acts as the major cellular binding site for another enzyme, adenosine deaminase (ADA), which is present in the extracellular fluid. There are also at least two potential sites for the binding of the extracellular matrix proteins collagen and fibronectin. CD26/DPPIV usually exists as a dimer; the second molecule is shown in outline. The intracellular portion of CD26/DPPIV is small and no functional domains have been identified. CD26/DPPIV must signal intracellularly by coupling with other cellular components.

CD26/DPPIV in Cancer Progression and Spread. Table 1 Some of the major substrates for the dipeptidyl peptidase-IV activity of CD26/DPPIV

Molecule	Full name and main function(s) in normal tissues	DPPIV sensitivity (k_{cat}/K_m)
CXCL12	SDF-1α (stromal cell-derived factor-1α): Involved in development of the nervous system, bone marrow and intestine, and in the homing of stem cells.	100
CCL22	Macrophage-derived chemokine: Is an attractant for various types of white cells and functions in immune and inflammatory responses	80
GRP	Gastrin releasing peptide: Released by nerves in the stomach to cause the production of gastrin from G cells in the mucosa.	40
NPY	Neuropeptide Y: Peptide neurotransmitter found in the brain that has a role in regulating normal physiological processes.	20
GLP-1	Glucagon-like peptide-1: Gut hormone secreted by L cells in the intestine, has a role in control of insulin levels.	4
CCL11	The chemokine eotaxin-1: Causes the recruitment of eosinophils into tissues and plays a role in allergic responses.	1.6
CCL5	The chemokine RANTES ("Regulated on Activation, Normal T Expressed and Secreted"): Selective attractant for memory T lymphocytes and monocytes.	0.8
VIP	Vasoactive intestinal peptide: Peptide hormone produced by various tissues, with effects on blood vessels and secretory processes.	0.2

over the years with greater or lesser frequencies. The designation CD26 is probably the most neutral, because although CD proteins have been studied primarily in white cells, they also exist in other tissues, and the nomenclature has no link to function. The abbreviation "DPPIV" refers to its enzyme activity and – given the other activities this talented component incorporates, is not a valid name for the overall molecule. However, as so as much research on this protein has focused upon its enzymatic role, and this facet of its action is of significance in certain diseases such as cancer and diabetes, the term "CD26/DPPIV" serves as a compromise.

CD26/DPPIV is found at the surface of the cells that form the functional barrier (epithelium) in most of the major sites that give rise to cancer in adults (e.g. intestine, lung, breast and prostate). The levels detected in cancer (the "expression") vary from those of the corresponding normal tissue, but the pattern is not consistent across all cancers and within a single cancer type there may be variable findings. So, for example, while the prevailing change in adult solid cancers (lung and prostate cancer, for example) is for CD26/DPPIV to decline, in certain less common cancers such as those of the thyroid and kidney, CD26/DPPIV levels actually increase. This suggests that the absence or presence of CD26/DPPIV does not universally favor or disfavor cancer progression, but that its role depends very much on the tissue type – meaning that changes in CD26/DPPIV as a tissue becomes cancerous will depend very much on its normal role. Additionally, in some cancers (such as colorectal cancer) the expression of CD26/DPPIV is very variable, not just between different tumors but in different regions of the same cancer. This points to a likelihood that CD26/DPPIV levels can be regulated by factors that are generated within the developing cancer tissue.

The ability of CD26/DPPIV to bind the enzyme ADA seems to be part of a fundamental mechanism whereby cells can resist the actions of the purine nucleoside adenosine in certain disease situations. This helps them to resist a threat to their survival by high concentrations of adenosine, or the risk of responding excessively to adenosine when it persists in the environment for an extended period. High concentrations of adenosine can occur persistently in the disorganized environment of a solid cancer (►Adenosine and tumor microenvironment). By retaining ADA close to the cell surface, the cell has a greater chance of scavenging adenosine near to the cell and preventing excessive action through adenosine receptors that are embedded in the cell membrane.

This dynamic situation involving extracellular adenosine production (from ATP breakdown and through cellular export) and breakdown (ADA bound to CD26/DPPIV) next to the cell surface provides substantial opportunity for the cell to modulate other signals that might be acting on it from other sources. Adenosine modulates many of the signals that are produced to act on leukocytes in inflammation and cancer, leaving CD26/DPPIV – as the docking site for ADA – in a unique position to act as one of the central determinants of the overall cellular response. In leukocytes, this seems to allow cells to resist somewhat the immunomodulatory effects of adenosine that may be produced during inflammation. Indeed levels of CD26/DPPIV, either on the surface of leukocytes or in a soluble form (sCD26) that is shed from cells and can be recovered from blood plasma, have been used to indicate levels of inflammation.

In cancer, the status quo is altered by two things. Firstly, as indicated above, adenosine levels in solid cancers are persistently high. Secondly, cellular levels of CD26/DPPIV are altered from normal and (with the exception of a few specific cancers) are typically low. These factors will combine to leave cells within a cancer (the tumor cells, the supporting fibroblastic cells, and infiltrating leukocytes) more susceptible to the effects of adenosine.

The two factors may be linked, as it has been shown that persistently high adenosine levels can cause the amounts of CD26/DPPIV at the surface of cancer cells to decline precipitiously. Adenosine, which is produced regionally within cancers, is likely a major factor responsible for the spatial variations in CD26/DPPIV expression within certain cancers.

Changes in CD26/DPPIV levels in cancer will also have an impact as a result of alterations in the DPPIV enzyme activity available. The substrates of this enzyme are typically hormones and other peptide regulators that are important in controlling the functions of epithelial and nervous cells, as well as cells involved in the body's defenses (Table 1). Amongst the most sensitive of the various mediators that are substrates for this enzyme is a chemokine molecule called CXCL12. (Chemokines are small peptide mediators that play an important role in controlling cellular arrangement in developing tissues and directing cell movement in the immune and inflammatory systems of our body's defenses.) CXCL12 is important in cancer because it seems to be one of the major factors that provides the "right environment" for cancer cells that have left the original tumor to settle into new locations in the process of metastasis. It provides a signal that activates a receptor on cancer cells called CXCR4 to facilitate their seeding and growth in such metastatic sites as the lungs, liver and bone marrow (►CXCR4 chemokine receptor).

Changes in CD26/DPPIV levels in cancer likely help cancers to grow by affecting the activities of these mediators that are substrates for the DPPIV enzyme activity. The result of excising the N-terminal two amino acids in most cases is to inactivate the mediator or cause it to be more rapidly degraded. In the common

cancers in which CD26/DPPIV tend to have declined, there will therefore be a shift to higher levels of the active mediator(s). As mediators such as CXCL12 are strongly linked to cancer progression, this will be one of the many different ways in which cancers can act to encourage their own expansion.

CD30

Definition

Member of the tumor necrosis factor/nerve growth factor receptor superfamily. It is an activation-associated antigen that is most often expressed on lymphoid cells but also found on embryonal carcinoma cells.

►Hodgkin Disease, Clinical Oncology
►CD Antigens

CD33

Definition

Is a glycoprotein expressed on myeloid and myelomonocytic progenitors, but not on pluripotent progenitor cells in normal hematopoesis. In 85–90% of cases of adult and pediatric acute myeloid leukemia CD33 can be detected on leukemia blasts. Functionally, CD33 is involved in cell-cell interactions and signaling in the hematopoietic and immune system.

►Monoclonal Antibody Therapy
►CD Antigens

CD34

Definition

A surface antigen that is present on human hematopoietic stem cells and progenitors but not on more differentiated cells. The presence of CD34 and absence of CD38 characterizes both normal and leukemic hematopoietic stem cells.

►NUP98-HOXA9 Fusion
►CD Antigens

CD39

Definition

A cell-surface protein that removes diphosphates from nucleotides such as ATP (to form AMP); also known as ecto-nucleoside triphosphate diphosphohydrolase (NTPDase-1).

►Adenosine and Tumor Microenvironment
►CD Antigens

CD44

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Synonyms

Cluster of differentiation 44; Hyaluronan receptor; Homing receptor; ECMRIII; Phagocytic glycoprotein-1; pgp-1; H-CAM; $gp90^{Hermes}$

Definition

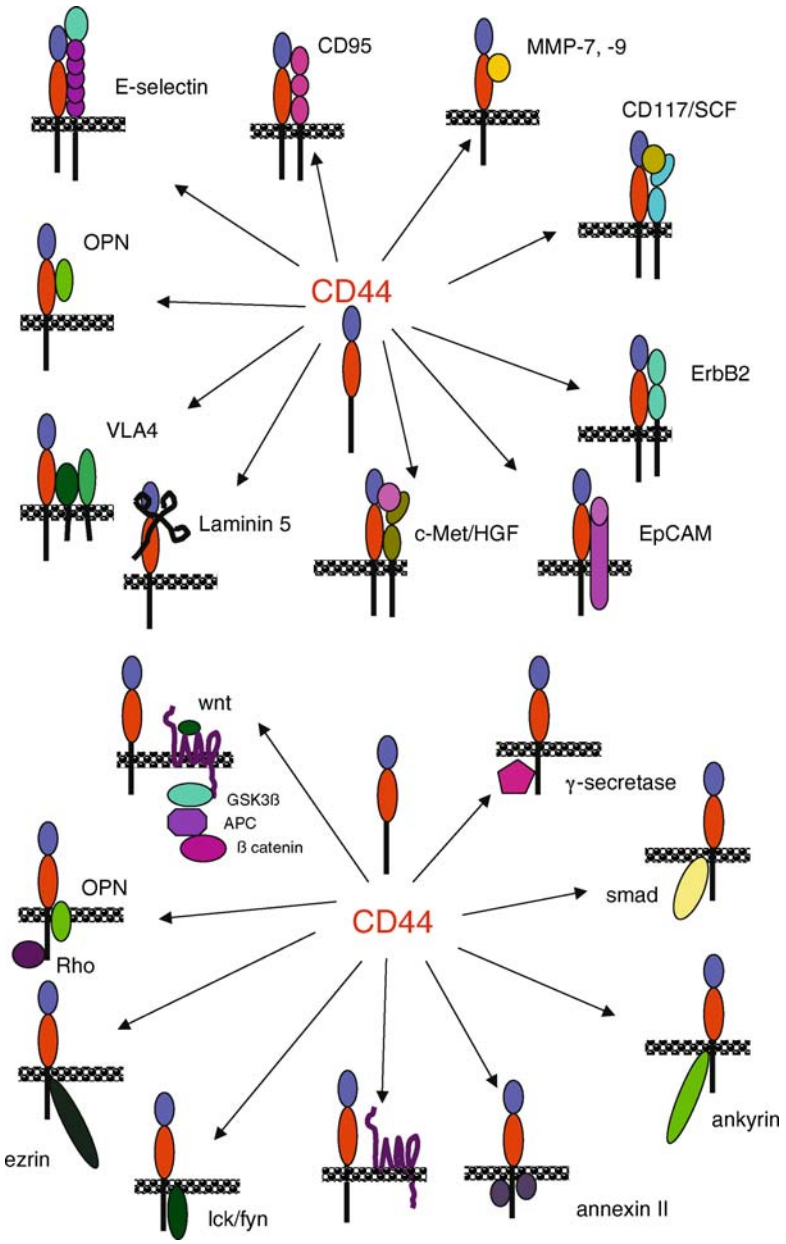
CD44 is a type I transmembrane glycoprotein, which exists in a large number of isoforms. The gene contains 20 exons within a region of ~60 kb on chromosome 11p13 in humans and on chromosome 2 at 56 cM in mice. CD44 is in close proximity to the recombination-activating-genes Rag-1 and -2.

Characteristics

CD44 is the major receptor for ►hyaluronic acid and other ►extracellular matrix molecules (►fibronectin, ►laminin 5, collagen type IV, serglycin). The standard molecule is heavily ►glycosylated by N- and O-linked residues and chondroitin sulfate side chains, while some of the ►variant isoforms carry in addition ►heparan sulfate moieties, which can present various ►growth factors and ►chemokines (for local concentration and activation). The number of extracellular molecules that can associate with CD44 is ever growing, among them ►matrix metalloproteinase-7, -9 (►MMP-7, MMP-9) inducing activation of latent transforming growth factor β (►TGF-β) and hence promote ►invasion and ►angiogenesis. Further associating molecules are ErbB2 (►HER-2/neu), EpCAM, ►E-selectin, ►CD74, and ►VLA-4 (Integrin α4β1). While ►c-met/►scatter factor receptor, c-kit/►stem cell factor receptor, ►osteopontin

(OPN), and ►CD95 have specifically been shown to associate with CD44 variant isoforms, association with the other molecules has not been specified to a CD44 isoform. The association between VLA-4 (integrin α4β1) and CD44 directs cells into ►inflammatory regions, while the c-met/CD44v6 interaction is required for c-met/scatter factor receptor signaling leading to ►Ras activation; and when CD44v6 associates with CD95, trimerization of the death receptor is prevented and hence ►apoptosis signaling is blocked (see Fig. 1).

Upon cellular activation, CD44 localizes to ►plasmamembrane microdomains and associates (see Fig. 1) with nonreceptor ►tyrosine kinases lck and fyn, smad-1, membrane bound OPN, and ►Rho. Via ►ezrin (►ERM protein), ankyrin or annexin II the cytoplasmic region of CD44 is linked to the cytoskeleton. CD44 is involved in the ►wnt signaling pathway. ►P-glycoprotein, the product of the multidrug resistance (MDR) gene has also been demonstrated to interact physically and functionally with CD44, thus promoting cell



CD44. Figure 1 Multiprotein complexes can be formed between CD44 and various cell surface, membrane-linked and intracellular molecules.

►migration and invasion and possibly enforcing resistance to ►chemotherapy. The p-glycoprotein – CD44 interaction is the first hint of a functional association between MDR and ►metastasis formation, involving CD44. Further it is of importance that the presenilin-dependent ►γ-secretase cleaves off the intracellular domain (ICD) of CD44, which then translocates to the nucleus and acts as a transcription factor for genes containing TPA (12-O-tetradecanoyl phorbol 13-acetate) response elements in their promoter. The ICD of CD44 promotes the fusion of ►macrophages, is localized in the nucleus of macrophages, and promotes the activation of ►nuclear factor kappa (NF-κ) B.

Cellular and Molecular Regulation

The standard form of CD44 (CD44s) is expressed in almost all tissues and leukocytes and is encoded by exons s1–s10, yielding a product of 90 kDa. The variant isoforms (CD44v) are generated by ►alternative splicing of the nuclear RNA between exons s5 and s6, and are encoded by exons v2–v10 (exon v1 is silent in humans, but not in mice and rats). Combinations of different variant exons with the standard backbone result in numerous variant isoforms, with masses of 100–250 kDa. All the variant regions are located extracellularly and are highly hydrophilic. In contrast to the ubiquitous expression of CD44s, CD44v isoforms are expressed in a highly restricted manner in nonmalignant tissues: in early embryogenesis, ►stem cells of epithelia and hemopoiesis, activated leukocytes and memory cells. However, in malignant tissues, CD44v isoforms are often upregulated, e.g., in ►carcinoma, various ►hematological malignancies, and in ►autoimmune lesions.

A positive feedback loop was identified which couples Ras activation with alternative splicing of the CD44 variant isoforms. The presence of CD44v6 then sustains ►Ras signaling, which is in turn important for cell cycle progression.

CD44 is implicated in various aspects of ►tumor progression: invasion, migration, and ►apoptosis blockade.

Clinical Relevance

Originally identified by its metastasizing potential in rats, CD44v isoform expression was identified in various human tumors and correlated with clinical relevance. *Upregulation* of CD44v correlates with poor prognosis in ►gastric and colorectal carcinoma, ►nonsmall cell lung tumors, ►hepatocellular carcinoma, ►pancreatic cancer, ►B-cell chronic lymphocytic leukemia, ►multiple myeloma, ►non-Hodgkin lymphoma, and ►acute myeloblastic leukemia. *Downregulation* of CD44v correlates with poor prognosis in ►esophageal squamous cell carcinoma, ►bronchial carcinoid tumors, ►ovarian neoplasms, ►uterine cervical tumors, ►transitional cell bladder tumors, and ►prostate cancers, while downregulation of CD44s correlates with amplification of ►MYCN and is indicative for an unfavorable outcome in ►neuroblastoma patients. In ►breast carcinoma, controversial data between CD44v expression and survival were established and need further evaluation.

Elevated serum levels of CD44v have prognostic value for ►gastric and ►colon carcinoma, non-Hodgkin lymphoma, which are indicative for a poor prognosis.

An emerging new field (although hypothesized some 150 years ago) is the area of cancer-initiating cells, also termed ►cancer stem-like cells. They exist as a small population in every tumor and determine the capability of the ►tumor to grow and propagate. In tumors of the ►breast, the ►pancreas, the ►prostate, ►head and ►neck, the ►brain (►glioblastoma), and in the blood system (►leukemia), the cancer-initiating cells are $CD44^+$. A major goal currently is to identify specific markers (►stem cell markers) that enable to distinguish between normal, benign tissue stem cells and those that are cancer-initiating.

CD44 is also strongly upregulated in ►inflammatory lesions of patients with ►autoimmune diseases(►inflammatory bowel disease (Crohn's disease), multiple sclerosis, rheumatoid arthritis).

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CD45

Definition

Family of protein tyrosine phosphatases expressed exclusively on the surface of almost all hematolymphoid cells and their progenitors; synonym leukocyte common antigen.

►Hodgkin Disease, Clinical Oncology
►CD Antigens

CD52

Definition

Is a glycoprotein expressed on B- and T-lymphocytes and in less density on monocytes, macrophages, eosinophils and on parts of the male reproductive tract. There is little information about the function of CD52.

►Monoclonal Antibody Therapy
►CD Antigens

CD55

►Decay-Accelerating Factor

CD57

Definition

Antigen present on lymphocytes with natural killer and killer cell activity. The antigen is also found in subpopulations of T lymphocytes, some neural and neuroendocrine cells, and a wide variety of neoplasms. Hodgkin disease

►Hodgkin Disease, Clinical Oncology
►CD Antigens

CD62E

►E-Selectin-Mediated Adhesion in Cancer

CD66a

Definition

Cluster of Differentiation Antigen 66 a.

►CEACAM1 Adhesion Molecule

CD73

Definition

A cell-surface enzyme that removes a phosphate group from AMP to form adenosine; also known as ecto-5′-nucleotidase.

►Adenosine and Tumor Microenvironment
►CD Antigens

CD82

Definition

►Metastasis Suppressor KAI1/CD82

CD95

Definition

The CD95 gene (synonyms: FAS, APO1, APT1, TNFRSF6) encodes a cell-surface receptor that mediates ►apoptosis signaling.

CD95

Definition

Fas/APO-1/CD95.

CD99

Definition

Cluster definition for a transmembrane cell surface sialoglycoprotein previously named MIC2 or E2 or HBA71 antigen; present on almost every human tissue but most highly expressed on early hematopoietic precursor cells, some lymphomas and all Ewing sarcoma family tumors. Ewing family tumors express at high levels an antigen determined by the *MIC2* gene.

The product of *MIC2* is a glycoprotein (also designated CD99 or p30/32 MIC2) with a molecular weight of approximately 30,000 daltons located on the cell surface and probably involved in cell adhesion. Although immunohistochemical detection of membrane-localized *MIC2* expression is a sensitive diagnostic marker for Ewing family tumors, it lacks specificity in that many other tumors are also immunoreactive with anti-MIC2 antibodies in some cases.

►EWS-FLI (ets) Fusion Transcripts
►Ewing Sarcoma
►Adhesion

CD156b Antigen

►ADAM17

CD184

►Chemokine Receptor CXCR4

CD246

►ALK Protein

CD314

►NKG2D Receptor

CD Antibody Microarray

Definition

A device able to identify large numbers of surface molecules on a suspension of cells in a single analysis.

►CD Antigens

CD Antigens

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Synonyms

Surface molecules; Cellular antigens; Immunophenotypic determinants

Definition

The human cluster of differentiation (CD) antigens are surface molecules originally detected on white blood cells (►leukocytes) from peripheral blood. The first Human Leukocyte Differentiation Antigen (HLDA) workshop was held in Paris in 1982 where 15 surface molecules were assigned based upon the "►clustering" of submitted antibodies whose reactivities were screened against a panel of cell lines. Different antibodies that showed similar or identical patterns of reactivity against the panel of cell types were considered to be reacting with the same surface molecule. This clustering of antibody reactivity enabled designation of a specific CD number for a particular surface molecule. The identification of CD antigens was facilitated by the prior development by Kohler and Milstein of a procedure for generation of ►monoclonal antibodies against a particular antigen. Meetings of the HLDA group were held approximately every 4 years, culminating in HLDA8 that was held at Flinders University in 2004. At that workshop, further CD antigens were added to the list to give a total of 339 CD antigens, and more have been added since that time. The CD antigen organization has now been renamed Human Cell Differentiation Molecules (HCDM) in recognition that CD antigens are not found uniquely on leukocytes, and a number of additional CD antigens have been designated. Indeed CD antigens are found on all types of human cells in different repertoires controlled by the genetic program of the tissue.

Characteristics

The CD antigens are a diverse group of ►surface glycoproteins with a multitude of functions, providing the interface between a cell and the external environment that includes other cells. The CD antigens may be cell-cell or cell-matrix adhesion molecules, cytokine receptors, ion pores, or nutrient transporters. The CD antigens perform a variety of roles in immune system function. CD1 for example, presents lipids to T-cells and is essential for immunity against the mycobacterial infections that cause tuberculosis and leprosy. CD4 is a

co-receptor in antigen-induced T-cell activation and is a receptor for HIV, CD35 is a complement receptor, CD40 is a member of the TNF receptor family with the ligand CD154, and CD54 is an intercellular adhesion molecule.

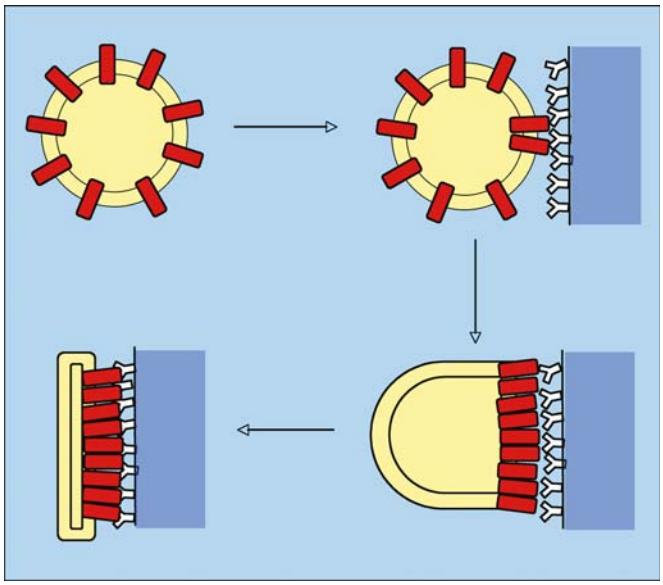
The method of discovery of CD antigens has classically involved testing monoclonal antibodies submitted to a workshop against a panel of 75 cell types using fluorescently-tagged antibodies and ►flow cytometry. Hierarchical cluster analysis is then performed and a dendrogram plotted. Monoclonal antibodies that cluster show similar patterns of interaction with the panel of cells. With recent development of sophisticated procedures for membrane proteomics, this clustering procedure is becoming out-dated and CD antigens may in the future be designated using different criteria. There are certainly several thousand cell surface proteins that could, in principle, be detected and characterized using methods of higher sensitivity. The discovery of further CD antigens will continue to involve raising monoclonal antibodies against antigens on intact cells in the traditional manner, but will certainly utilize modern ►proteomic techniques such as two-dimensional gel electrophoresis and multi-dimensional chromatography with detection and identification of proteins using mass spectroscopy and extensive protein databases.

CD Antigens Provide Immunophenotypes of Leukocytes

The repertoires of surface CD antigens found on different types of leukocytes reflect the genetic programs that operate in particular cell types. Thus, cells may be classified according to their cell surface profile (►immunophenotype). This concept is illustrated in Fig. 1 as a Venn diagram for T-cells, B-cells and myeloid cells. T-cells (yellow) express certain antigens uniquely such as CD2, CD3 and CD4, B-cells (blue) express CD19, CD20, CD21 and CD22, and myeloid cells (red) express CD13, CD14, CD15 and CD33. Certain CD antigens are shared between two lineages of leukocytes, for example, CD5 and CD38 (green), are shared between T-cells and B-cells. The so-called pan leukocyte markers are shared between all 3 categories of leukocytes and include well-known antigens such as CD44 and CD45. All leukocytes originate from stem cells via proliferation and differentiation of cells down lineages to form the many types of mature leukocytes. The stem cell antigen CD34 (black) is a marker of undifferentiated cells.

Classification of Leukemias Using CD Antigens

The principles described above for normal cells can also be applied to cancers such as leukemias. Most leukemias arise as mutations in precursors of leukocytes in the lineages of differentiation found in the bone marrow. A mutation will stop further differentiation of a precursor cell, and there is proliferation rather than differentiation. The resultant identical (monoclonal) cells accumulate in the circulation and the patient is eventually diagnosed with leukemia. Most leukemias are monoclonal and the leukemic cells usually have a similar or



CD Antigens. Figure 1 Venn diagram showing the differential expression of CD antigens on different categories of leukocytes.

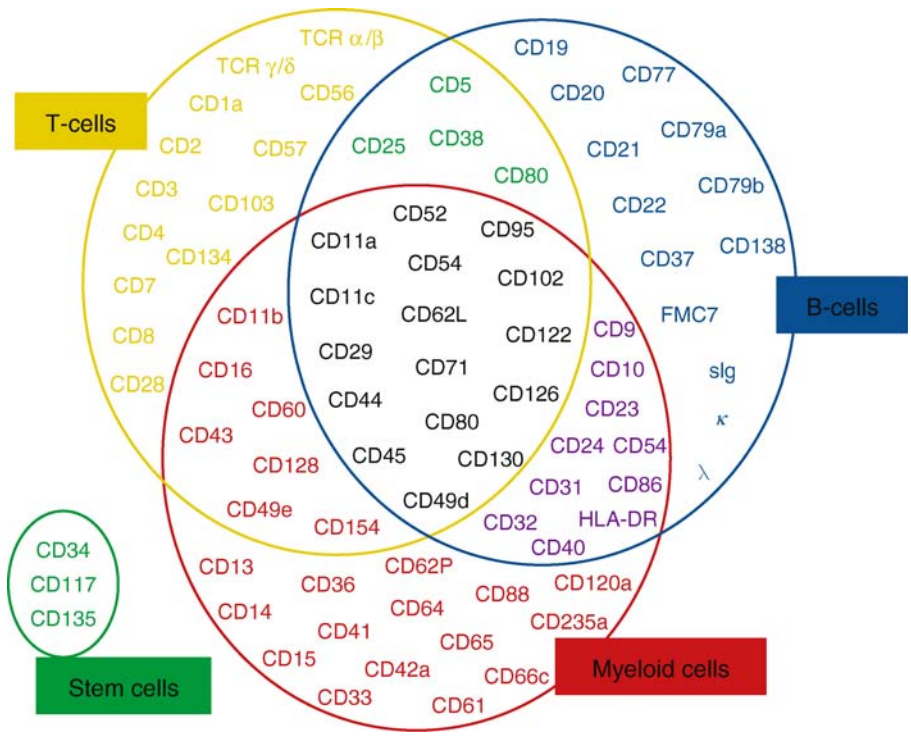
identical surface expression profile (immunophenotype) to that of the precursor cell from which the leukemia arose. Thus, identification of a large number of CD antigens using flow cytometry ►or antibody microarrays may be sufficient to diagnose a leukemia.

CD Antigens as Targets for Therapeutic Antibodies

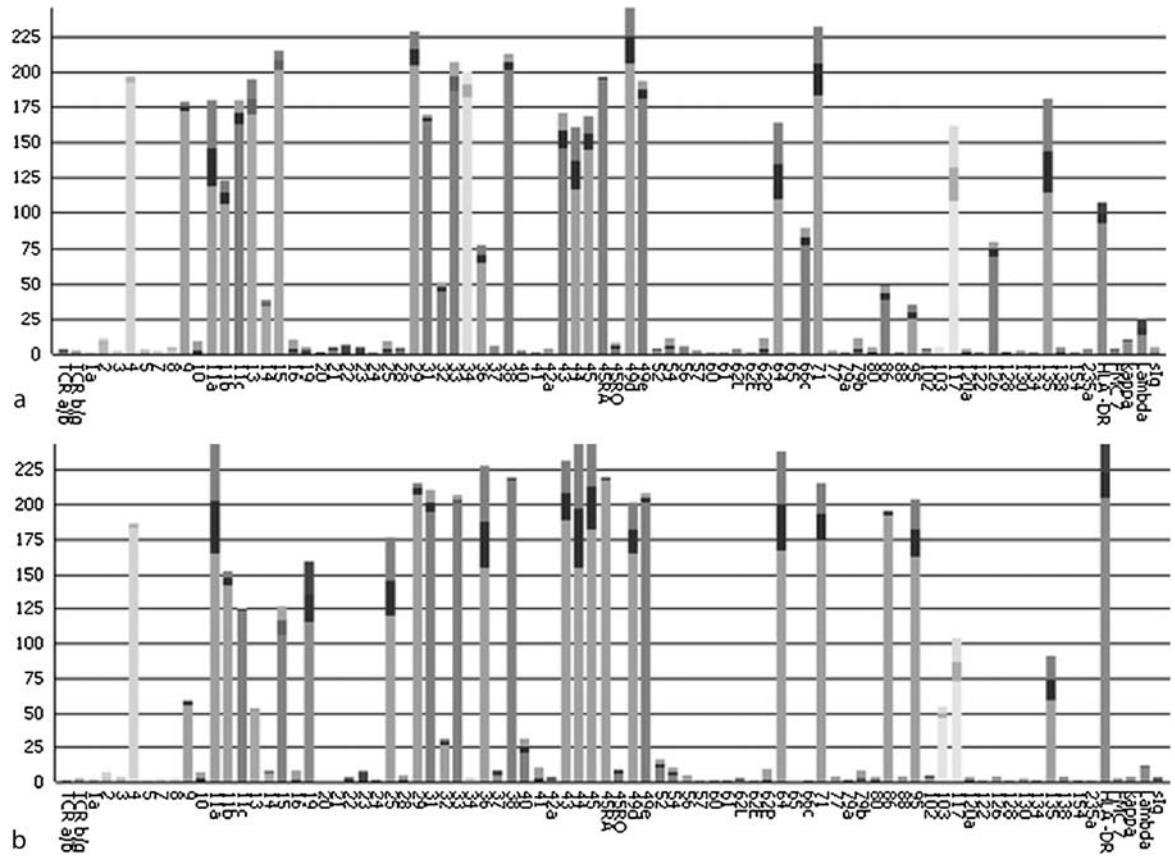
These cell surface proteins are potential targets for therapeutic antibodies. Such antibodies may block the function of a receptor, selectively activate leukocyte sub-populations, carry a toxin or radioisotope, or act as a site for antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) where the target cell is eliminated by cytotoxic cells such as neutrophils, monocytes and natural killer cells. There are a number of therapeutic antibodies in clinical use for treatment of a variety of leukemias and lymphomas. For example, Rituximab is specific for CD20 and is used to treat chronic lymphocytic leukemia (CLL) and non-Hodgkins lymphoma (NHL). Both are B-cell cancers that express CD20 (Fig. 1) and are killed by this antibody. Mylotarg is specific for CD33, contains a toxin and is used to treat certain types of acute myeloid leukemia (AML). Campath-1H (Alemtuzimab) binds to CD52 and is used to treat NHL. There are many more therapeutic antibodies in development, one of the most rapidly growing area of pharmaceuticals, where monoclonal antibodies are first made against the desired CD antigen and the characteristics of the antibody are then "engineered" to make it suitable for use in patients.

Methods for Identification of CD Antigens

Flow cytometry has been the "gold standard" for identification of a limited number of CD antigens on the surface of leukocytes. In this method, the leukocytes in suspension are mixed with a fluorescently-labeled antibody that is specific for the extracellular portion (epitope) of a surface molecule thought be expressed on the cells. The fluorescently-labeled sample is aspirated into the flow cytometer, and the cells pass singly through a narrow aperture where a laser beam individually excites fluorescent antibodies bound to single cells. The emitted fluorescence is detected and data accumulates for a large number (e.g., 10,000 cells). Flow cytometry can detect three different fluorescent antibodies simultaneously; more sophisticated systems can detect eight and up to 17 CD antigens. To diagnose leukemias, 10–15 CD antigens are usually identified using several cycles of flow



CD Antigens. Figure 2 Capture of live leukocytes on the CD antibody microarray. The *red bars* across the cell membrane represent a CD antigen (e.g., CD20) that forms an initial interaction with antibodies against CD20 that are immobilized on a solid support as a dot in the microarray. Cell capture occurs progressively as CD20 moves in the membrane of the cell and becomes progressively captured by the antibodies on one side of the cell.



CD Antigens. Figure 3 Cell surface expression profiles from an antibody microarray. (a) Acute myeloid leukemia (AML) cells from peripheral blood; (b) AML cells from bone marrow. Numbers on the x-axis refer to antibodies against the corresponding CD antigens. Values on the y-axis are average dot intensities.

cytometry, and the information is combined with other criteria such as cell morphology, cell staining, an image of the chromosomes, and sometimes analysis of the DNA in the cells.

More recently, a ►CD antibody microarray has been developed that detects the presence of 147 different CD antigens on leukocytes in a single assay. This microarray called DotScan (Medsaic Pty Ltd, Eveleigh, NSW, Australia), consists of CD antibodies immobilized on a microscope slide. Live cells (3 million) are placed on the microarray that is ~0.5 cm square and contains more than 300 antibody dots. Cells are captured by an immobilized antibody if the cell has the corresponding CD antigen on its surface (Fig. 2). After one hour, unbound cells are gently washed off and the resultant dot pattern is the immunophenotype (surface expression profile, disease signature) for the leukemia. The dot pattern for a leukemia is stored as a digital image and may be analyzed with a variety of software to provide an expression profile (Fig. 3) that in many cases enables diagnosis of the type of leukemia.

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CdA

►Cladribine

2-CdA

►Cladribine

CDC

Definition

Centers for Disease Control and Prevention

►Lead Exposure

CDC7

Definition

Cell division cycle 7; is a protein kinase that promotes the initiation of replication forks at licensed replication origins.

►Replication Licensing System

CDC20

Definition

Cell division cycle 20; is a component of the spindle assembly checkpoint. Phosphorylated CDC20 (by BUB1) interacts directly to APC/C and represses its ubiquitin ligase activity.

►Mitotic Arrest-Deficient Protein 1 (MAD1)

CDC25

Definition

Cell division cycle 25; Dual specific phosphatases targeting phosphorylated proteins at serine and threonine residues.

►Trefoil Factors

CDC37

Definition

Cell division ccle 37; A co-chaperone of the Hsp90 chaperone complex that mediates the stabilizing interactions of the chaperone with a wide variety of protein kinases.

CDC42

Definition

Cell division cycle 42; is a small GTPase which cycles between an active GTP-bound and an inactive GDP-bound state. It plays an important role in actin cytoskeleton remodeling, and in epithelial cell polarization processes.

►Mitogen-Inducible Gene 6 (MIG-6) in Cancer

CDDP

►Cisplatin

CD66e

►Carcinoembryonic Antigen

CDH1

Definition

Cadherin 1.

►Epithelial Cadherin
►Uvomorulin
►E-Cadherin

CDK

►Cyclin Dependent Kinases

CDK1 Kinase

►Cyclin Dependent Kinases

CDK2/cyclin A-associated protein p45

►Ubiquitin Ligase SCF-Skp2

CDK4I

►CDKN2A

CDK Inhibitors

Definition

Usually negatively modulate cell cycle progression by binding to and inhibiting the activity of cyclin/Cdk complexes.

►Forkhead Box M1
►Cyclin Dependent Kinases

CDKN2

►CDKN2A

CDKN2A

Definition

A ►tumor suppressor gene. Germline mutations in the CDKN2A have been shown to predispose to ►cutaneous malignant melanoma. Synonyms: INK4A or INK4A/ARF

►Neurofibromatosis 1
►INK4

CDKN2A

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Synonyms

CDKN2; CDK4I; CMM2; INK4A; MTS1; p16; $p16^{INK4}$; $p16^{INK4A}$; $p16^{INK4a}$; Cyclin-dependent kinase inhibitor 2A

Definition

Cyclin-dependent kinase inhibitor 2A gene (CDKN2A), the first identified ►melanoma predisposition gene, encodes the tumor suppressor proteins p16 and ARF.

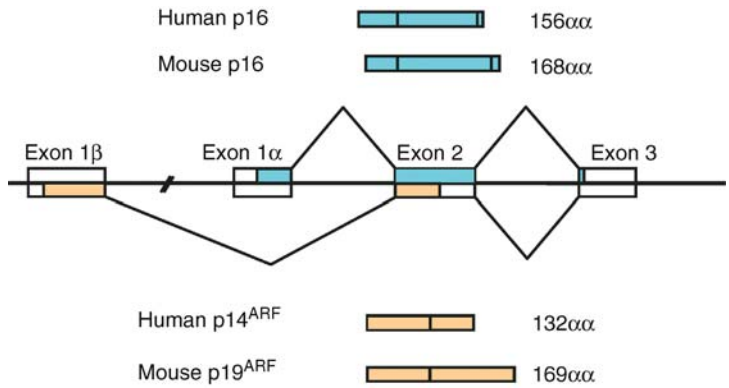
Characteristics

Identification of CDKN2A

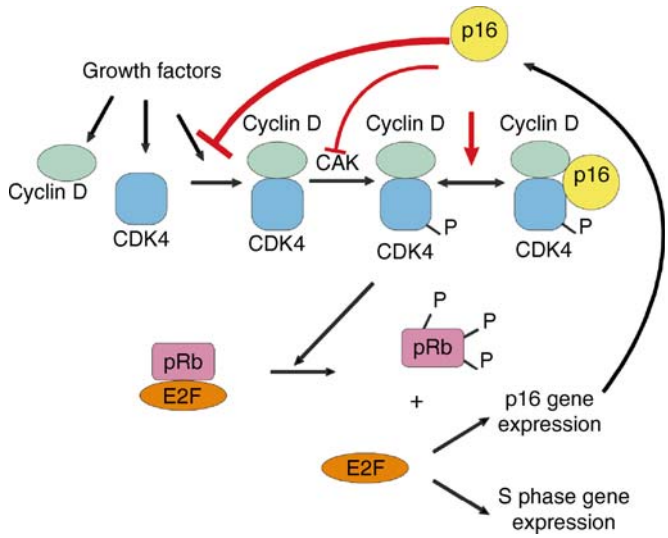
The 9p21-22 chromosomal region was originally implicated in the development of melanomas through a combination of cytogenetic and loss of heterozygosity (LOH) studies. Subsequent linkage analysis in melanoma families indicated that this region harbored a melanoma predisposition locus. Homozygous deletions in cell lines derived from several different tumor types narrowed down the region significantly. This led to the isolation, by two independent groups, of the cell cycle regulatory gene encoding the cyclin-dependent kinase (CDK) inhibitor, p16, which had been previously identified in a yeast two-hybrid screen to identify proteins that bound to CDK4 (Fig. 1).

Gene Structure of CDKN2A

In the original description of human p16 the initiating methionine was incorrectly identified. It was later found that the protein included eight additional amino acids at its amino terminus, although these residues



CDKN2A. Figure 1 Alternative transcripts and products encoded by the CDKN2A locus. The exons of CDKN2A are shown as boxes and identified as exons 1β, 1α, 2 and 3. Alternative splicing occurs as indicated to give rise to two transcripts, exons that splice to encode p16 are shown above and those that encode p14ARF are shown below. The sizes and composition of the respective mouse and human proteins are indicated.



CDKN2A. Figure 2 Schematic representation of the protein interactions in the cyclin D/CDK4/p16/pRb pathway. Through a complex system of signal transduction, growth factors lead to the assembly of cyclin D and CDK4. This complex is then activated through phosphorylation by the CDK-activating kinase (CAK), and cyclin D/CDK4 in turn phosphorylates pRb, leading to the release of transcription factors of the E2F family. These are then capable of transactivating the genes necessary for entry into S phase and p16 has been shown to inhibit this process in several ways; by binding to the complex and inhibiting the kinase activity of CDK4, inhibiting CAK dependent phosphorylation of CDK4, or inhibiting the assembly of the cyclin D/CDK4 complex, with the latter being the principal mechanism of inhibition in vivo. The scheme provided is necessarily simplistic, however, it appears that p16 may also inhibit the phosphorylation of pRb by indirectly inactivating other CDKs, e.g. CDK2, as a consequence of the redistribution of other CDK inhibitors, e.g. p27 and p21. There is also a feedback loop whereby the release of the E2F transcription factor results in the activation of p16 expression, although the absence of E2F binding sites in the CDKN2A promoter preclude direct transactivation by E2F. Aberration of this pathway through either; deletion or mutation of pRb, the binding of viral ►oncogenes to pRb, overexpression or activation of CDK4 or cyclin D, or deletion or mutation of CDKN2A all can result in constitutive transactivation of S phase genes by E2F transcription factors.

are not present in murine p16. Three exons, spread over approximately 7.2 kb of genomic DNA, encode the 156 amino acid protein with predicted molecular weight of 16,533 Da, designated p16. The primary structural feature of p16 is the four tandem ankyrin-like repeats that comprise approximately 85% of the protein. This domain is believed to facilitate protein-protein interactions (Fig. 2).

The sizes of the translated regions encoded by exon 1α, exon 2 and exon 3 are 150, 307, and 11 bp, respectively. The CDKN2A-locus also has the capacity to encode two distinct transcripts from two different promoters. This is achieved by alternative splicing and the use of different reading frames. Each transcript has a specific 5′ exon; exon 1α (E1α) or exon 1β (E1β), which is spliced onto common second (E2) and third (E3) exons. The E1α-containing transcript encodes p16, and the E1β-containing transcript encodes a protein translated into an alternate reading frame initiated in E1β, designated p19ARF in mice and p14ARF in humans. In contrast to p16, where the murine and human genes share 85% amino acid homology, the alternative reading frame (ARF) proteins share only 59% amino acid homology. The different sizes of the encoded proteins are brought about by the earlier truncation of the ARF transcript in exon 2 in humans. Two different translation start sites have been reported for the ARF protein, which has lead to some confusion in the numbering of the ARF protein amino acids in publications.

Tumor Suppressor

CDKN2A is a tumor suppressor gene for multiple tumor types. The frequency of mutations at this locus in various cancers is rivaled only by mutations in TP53. As with other classical tumor suppressor genes, both alleles need to be abrogated for tumorigenesis to occur. A wide variety of mechanisms of inactivation of CDKN2A have been documented, including intragenic mutation, homozygous deletion and transcriptional silencing through methylation of the promoter. Notably in melanomas, many of the intragenic mutations are C > T or tandem CC > TT transitions, implicating ►ultra-violet radiation (UVR) as the causal somatic mutagen. Although CDKN2A is inactivated in the majority of melanoma cell lines examined, deletions and interstitial mutations of CDKN2A are much less common in uncultured melanoma tumors. Present studies indicate that only 5–10% of uncultured melanomas demonstrate mutations in CDKN2A, a surprisingly low figure given the obvious importance of CDKN2A in familial melanoma and the frequency of LOH seen at chromosome 9p21 in melanomas.

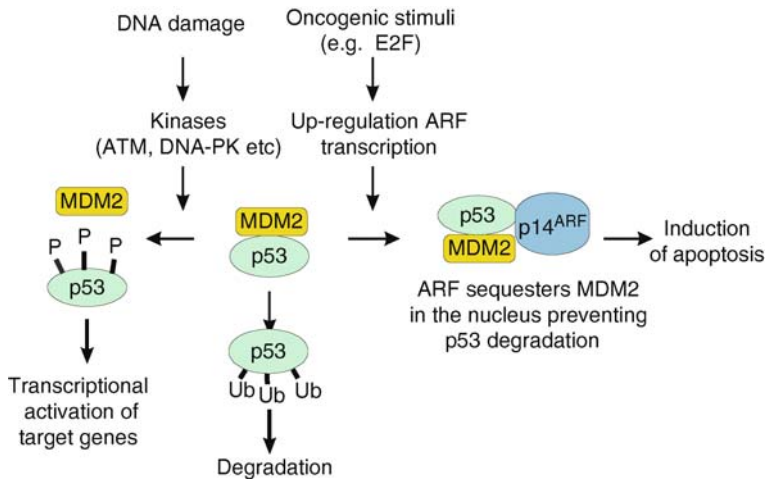
P16 is a CDK Inhibitor

P16 is the archetype member of the ►INK4 (inhibitor of CDK4) family of CDK inhibitors, which is comprised of p16INK4A, p15INK4B, p18INK4C and p19INK4D; encoded by CDKN2A, CDKN2B, CDKN2C and CDKN2D, respectively. Each of the proteins inhibits CDK4- or CDK6-mediated phosphorylation of the ►retinoblastoma susceptibility gene product, pRb, thereby providing a powerful negative signal, or "brake," to progression through the cell cycle.

The ►cyclin D1/CDK4/p16/pRb signaling pathway is the major growth control pathway for entry into the cell cycle. For cells to progress through G1 into S phase they must pass the late G1 restriction point, which controls entry into S phase. For progression past this restriction point, cyclin D/CDK4 must phosphorylate the ►retinoblastoma protein pRb. During G0/G1 the Rb protein exists in a DNA-bound protein complex, where it is bound to the transactivation domain of E2F transcription factors, preventing transactivation of E2F target genes. The phosphorylation of pRb results in the disassociation of this protein complex and the release of E2F such that it can transactivate genes required for entry into S phase. Over-expression of p16, inhibits progression of cells through the G1 phase of the cell cycle by binding to CDK4/cyclin D complexes (or CDK6/cyclin D) and blocking the kinase activity of the holoenzyme. Given that p16 normally functions to inhibit CDK4, it is easy to understand how inactivation of this gene could result in uncontrolled cellular growth leading to cancer. In many tumor types, an inverse correlation between mutations of p16 and pRb has been observed. Since p16 lies upstream of pRb, inactivation of both proteins would be redundant.

Role of the Alternative Reading Frame (ARF) Product

The ARF protein also regulates the G1/S phase transition via a distinct pathway involving the ►TP53 ►tumor suppressor gene product p53, and MDM2, which function upstream of p21 (a cyclin-dependent kinase inhibitor closely related to p16) and the CDK2/cyclin E complex (Fig. 3). p53 is a transcription factor that plays a major role in monitoring the integrity of the genome, and can be activated to inhibit cell cycle progression or initiate apoptosis through two distinct pathways: (i) in response to a variety of cellular stresses including ►DNA damage and ►hypoxia, (ii) via overexpression of viral or cellular oncoproteins such as E1A and c-myc. In this way, cells prevent the repair of mutations in successive generations by inducing apoptosis in incipient cancer cells. ARF plays a crucial role in p53-induced apoptosis. Murine p19ARF is capable of inducing a p53-dependent G1 cell cycle arrest that is not mediated through the direct inhibition of known CDKs. Ectopic expression of ARF leads to stabilization of p53 in multiple cell types, but unlike other known upstream effectors of p53 this activation is not through phosphorylation. Instead, ARF binds to MDM2 and blocks both MDM2-mediated p53 degradation and the transactivational silencing of p53. MDM2 continuously shuttles between the nucleus and the cytoplasm. This shuttling is essential for its ability to promote p53 degradation, indicating that MDM2 must export p53 from the nucleus to the cytoplasm to target p53 to the cytoplasmic proteosome. ARF activates p53 by binding to MDM2 in the nucleus and blocking the



CDKN2A. Figure 3 Schematic representation of the role of ARF in p53 activation by DNA damage and oncogenic stimuli. ARF functions to sequester MDM2 in the nucleus preventing nucleo-cytoplasmic shuttling of the MDM2/p53 complex, however, the details have not yet been fully elucidated and results suggest the mechanism may differ between humans and mice.

transport of the MDM2/p53 complex out of this organelle. Results obtained with murine and human ARF are somewhat different. In murine cells results indicate that p19ARF sequesters MDM2 away from p53 into the nucleolus. In human cells p14ARF moves out from the nucleolus to form discrete nuclear bodies in conjunction with MDM2 and p53, thereby blocking their nuclear export and leading to p53 stabilization. The discovery that ARF transcription is induced by the over-expression of a variety of cellular and viral oncoproteins including c-myc, E1A and E2F has provided the link by which hyperproliferative signals result in p53-dependent apoptosis.

To determine whether mutations in CDKN2A contribute to tumorigenesis via p19ARF in addition to p16, cDNAs carrying a variety of exon 2 mutations have been transfected into cell lines and cell cycle arrest monitored. These mutations have included several that are silent in p16 but caused missense mutations in p19ARF, as well as several deletion mutants that removed either exon 1β or various portions of exon 2. Results indicate that the majority of p19ARF activity is encoded by the exon 1β sequences, as all missense mutations in exon 2 of p19ARF remained fully active in blocking cell cycle progression, and removal of exon 2 sequences only marginally reduced the ability to induce arrest. In contrast, deletion of exon 1β resulted in a transcript that was incapable of inhibiting cell cycle progression. Missense mutations in exon 2 of the human p14ARF transcript similarly did not reduce the growth suppressive function of p14ARF.

Senescence

p16 is not normally expressed at detectable levels in most cycling cells, however, CDKN2A mRNA and p16 protein accumulate in late-passage non-immortalized cells, implicating a role for p16 in cellular ►senescence. This is supported by studies revealing that loss of p16 expression is a critical event in ►immortalization (the flip side to senescence) of a range of cell types. This conclusion was initially alluded to by finding that the frequency of deletions and intragenic mutations of CDKN2A in uncultured tumors was considerably lower than in immortalized cell lines. Growth and survival experiments using cells with impaired CDKN2A function suggest that a p16/pRb-dependant form of senescence may be particularly important in melanocytes. Individuals with defective p16INK4a have been found to have increased numbers of naevi, and it has been speculated that naevi are senescent clones of melanocytes.

Mouse Models

The generation of a CDKN2A "knockout" mouse, carrying a germline homozygous deletion encompassing exons 2 and 3 of the gene, revealed that p16 and p19ARF (since both proteins are eliminated by deletion of exon 2) were not essential for viability or organomorphogenesis. However, the mice did demonstrate abnormal extramedullary hematopoiesis, suggesting that p16 or p19ARF may regulate the proliferation of some hematopoietic lineages. In addition, the mice developed spontaneous tumors at an early age, specifically fibrosarcomas and B cell lymphomas, and were highly sensitive to carcinogens. In contrast to wild-type mouse embryonic fibroblasts (MEFs), cultured MEFs from Cdkn2a nullizygous mice (Cdkn2a−/−) failed to undergo senescence crisis, and could be transformed by oncogenic ras alleles. Although Cdkn2a−/− mice did not develop melanomas, transformation of

Cdkn2a−/− MEFs by activated ras prompted experiments to cross the Cdkn2a−/− mice with a previously generated transgenic mouse in which an activated ras allele was targeted exclusively to melanocytes under the control of the tyrosinase promoter. These mice spontaneous developed melanomas at high frequency and with short latency.

To determine whether p16 or p19ARF was the principal mediator of the above effects, knock-out mice strains with targeted deletions of p16 and p19ARF were generated. In general, p19ARF null animals were observed to develop a tumour spectrum more closely related to p53 null rather than p16 null mice. Tumours observed in p19ARF null mice included lymphomas and an increased incidence of soft tissue sarcomas, carcinomas and osteosarcomas. Mice lacking p16 were found to develop soft tissue sarcomas, osteosarcomas and melanomas. Mouse strains with specific inactivation of either p16 or p19ARF were tumour prone, but neither was as severely affected as animals lacking both p16 and p19ARF, suggesting cooperation between p16 and p19ARF loss in tumourgenesis.

Clinical Aspects

CDKN2A Mutations and Melanoma

Germline CDKN2A mutations have been observed in approximately 20–40% of melanoma families worldwide. However, melanoma appears to segregate with chromosome 9p markers in a far greater proportion of families than have been shown to carry mutations of CDKN2A. This suggests that melanoma predisposition in some of these families is caused by: (i) another gene in the vicinity of CDKN2A, (ii) mutations outside of the p16 coding region, (iii) another gene somewhere else in the genome, with linkage to this region occurring simply by chance. The most parsimonious explanation is that a combination of all these possibilities is likely.

Overall, approximately 40% of pedigrees with 3 or more cases of melanoma have been found to harbor mutations in the CDKN2A gene. This figure varies with location and is lowest in regions of high ►UV radiation (UVR), e.g. Australia (20%) and higher in regions with low incident UVR, e.g. Europe (57%).

There is a significant increase in the yield of CDKN2A mutations with increasing number of affected cases in families with melanoma. In addition, an early age of diagnosis, the presence of family members with multiple primary melanomas or with ►pancreatic cancer have also been shown to be significantly associated with an increased likelihood of finding a CDKN2A mutation.

The population based frequency of CDKN2A mutations in melanoma cases is of the order of 1–2%, even in those individuals that had developed multiple primary tumors, much lower than observed in families selected for multiple cases of melanoma.

Disease associated mutations are distributed along the entire length of the p16 coding region. At least one mutation has been described in the promoter of the gene, and several putative mutations have been identified in the intronic sequences. The most frequent CDKN2A mutations identified to date are c.255_243del19 (also known as p16 Leiden), p.M53I, p.G101W, c.331_332insGTC (p.R112_L113insR), (all in exon 2), c.-34G>T (promotor) and c.IVS2-105A>G (intron). There are considerable differences in the frequencies and distribution of CDKN2A mutations across the world. Many mutations have been shown to arise from a common founder, and are more frequent in particular geographic locations. For example Sweden and the Netherlands have single predominant founder mutations (p.R112_L113insR, and p16 Leiden respectively) involving over 90% of families tested. The G101W mutation, common in Italy, France and Spain, has been calculated to arise from a single genetic event approximately 93 generations ago. Many additional mutations have been repeatedly reported, and where analysis has been performed these have invariably been shown to be due to common founders. The only exception to this appears to be a 24 bp insertion in exon 1a, that has arisen multiple times, presumably because of DNA slippage over a 24 bp repeat region.

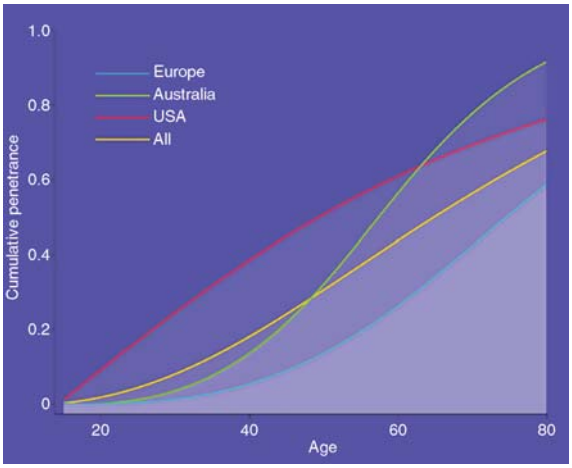
Mutation of ARF

Germline mutations affecting ARF but not p16INK4a have been reported in a small number (~3%) of melanoma families. Whereas the distribution of p16 mutation types (approximately 70% missense or nonsense, 23% insertion or deletion, 5% splicing and 2% regulatory) is consistent with that observed in the Human Genome Mutation Database, the reported ARF specific mutations are almost all either splicing mutations (affecting the 3′ splice site of exon 1b) or large deletions.

Penetrance

The pattern of susceptibility in melanoma pedigrees is consistent with the inheritance of autosomal dominant genes with incomplete penetrance. The overall penetrance of CDKN2A mutations in melanoma families has been estimated to be 0.30 by age 50 years and 0.67 by age 80 years. There is significant variation in the penetrance of CDKN2A mutations with geographical location. By age 50 years penetrance was estimated to be; 0.13 in Europe, 0.5 in the United States, and 0.32 in Australia, by age 80 years; 0.58 in Europe, 0.76 in the United States, and 0.91 in Australia (Fig. 4).

This indicates that the CDKN2A mutation penetrance varies with melanoma population incidence rates, thus the same factors that effect population incidence of melanoma may also mediate CDKN2A penetrance.



CDKN2A. Figure 4 Age specific penetrance estimates for CDKN2A mutations. Penetrance is shown for melanoma pedigrees from Australia, Europe, America (US) and all geographic locations combined.

Multiple Primary Melanoma

General characteristics of inherited susceptibility to many types of cancer are early age of onset and the development of multiple primary tumors. Hence the presence of multiple primary melanomas (MPM) in an individual may be a sign of them being a CDKN2A mutation carrier. This is the case for a small proportion (13/133, 10%) of MPM cases without a family history of the disease. In contrast, analysis of MPM cases with a family history of disease yields CDKN2A mutations in 55/139 (40%) of samples tested. The proportion of CDKN2A mutations in sporadic MPM cases increases with increasing number of melanomas (10/119 (8.5%) of cases with two primary melanomas, compared to 11/83 (33%) cases with 3 or more primary tumors).

CDKN2A Mutations and Non-Melanoma Cancers

Since CDKN2A is a tumor suppressor found to be inactivated in a wide range of different tumors, one might expect individuals carrying germline mutations of CDKN2A to be prone to cancers other than melanoma. ►Breast, ►prostate, ►colon, and ►lung cancers, have been suggested to be associated with CDKN2A mutations, however, these common cancers may occur in CDKN2A positive pedigrees by chance. Convincing evidence for susceptibility to another tumor type has been shown only for pancreatic cancer, which has been shown to be significantly associated with CDKN2A mutations in all regions except Australia, the reason for this is not yet understood.

There appears to be no evidence of an association between neural system tumors (NSTs) and CDKN2A mutations involving p16. However, there is marginal evidence for the association of NSTs with ARF specific mutations.

Modifiers of Penetrance of CDKN2A Mutations

The MC1R gene (16q24) which encodes for the melanocyte-stimulating hormone has been shown to be a risk factor in families with segregating CDKN2A mutations. MC1R variants have been shown to act as modifier alleles, increasing the penetrance of CDKN2A mutations and reducing the age of onset of melanoma.

CDKN2a Polymorphisms as Low Risk Factors

The A148T variant, located in exon 2 of the CDKN2A gene has no observed effect on p16 function, and does not segregate with disease in melanoma pedigrees. The contribution of this polymorphism to melanoma risk remains unclear, an association with increase in risk has been seen in some populations, but not in others.

The 500 C > G and the 540 C > T polymorphisms in the 3′ untranslated region of the CDKN2A gene have been shown to be associated with melanoma risk. The frequencies of the rare alleles at these loci have been shown to be higher in melanoma cases than in controls. It is possible that these variants might alter the stability of the CDKN2A transcript or the level of transcription, or that they may be in linkage disequilibrium with an unidentified variant which is directly responsible for melanoma predisposition. The contribution of these polymorphisms to melanoma risk is likely to be small in comparison to that of CDKN2A inactivating mutations.

CDKN2A and the Atypical Mole Syndrome

Since the description of the "B-K mole syndrome" much debate has ensued regarding the association between melanoma and the ►atypical mole syndrome (AMS). Several authors have concluded that atypical moles segregate independently of CDKN2A mutations, although individuals with high numbers of naevi in melanoma-prone families are three times more likely to be CDKN2A mutation carriers than those with a low number of naevi. Support for the notion that CDKN2A is naevogenic comes from a study of a large series of 12-year-old twins in which total naevus count was found to be tightly linked to CDKN2A. This finding has recently been corroborated by two independent genome wide association studies that have mapped loci responsible for naevi in twin cohorts. Both studies showed peaks of high linkage scores at 9p21 directly over the CDKN2A gene.

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cDNA chips

►Microarray (cDNA) Technology

CEA

►Carcinoembryonic Antigen

CEA Gene Family

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Synonyms

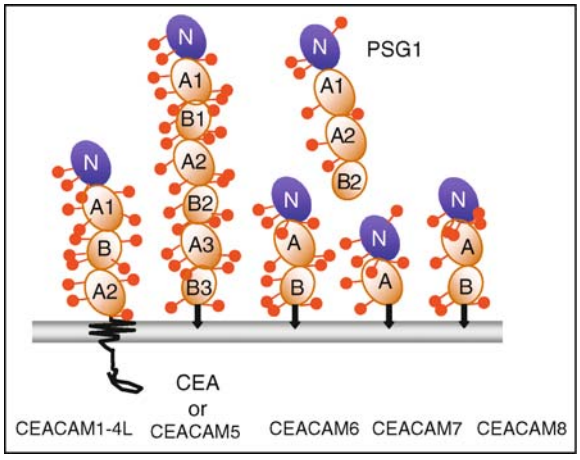
CEACAM1=BGP, C-CAM, CD66a; CEACAM5=CEA, CD66e; CEACAM6=NCA, CD66c; CEACAM7=CGM2; CEACAM8=CGM6, CD66b

Definition

The Carcinoembryonic Antigen (►CEA) gene family comprises 33 genes, 22 of which are expressed. All family members share similar structural features encompassing immunoglobulin (Ig) variable and/or constant domains and therefore constitute members of the large immunoglobulin superfamily. These proteins are either secreted or membrane-bound. Several CEACAMs function as homophilic or heterophilic intercellular ►cell adhesion molecules. CEA, ►CEACAM1, ►CEACAM6 and ►CEACAM7 also play a significant role as regulators of tumor cell proliferation and differentiation and their overexpression (CEA and CEACAM6) or their down-regulation (CEACAM1 and CEACAM7) contributes to progression of many ►epithelial cancers and immune dysfunctions.

Characteristics

The *CEA* gene family encodes a set of 22 genes and 11 pseudogenes clustered in a 1.8 Mb region on human chromosome 19q13.2 between the *CY2A* and *D19S15* marker genes. The *CEA* genes encompass an N-terminal Ig variable domain followed by one to six Ig constant-like domains. A striking characteristic of these proteins is their extensive ►glycosylation on asparagine residues with multi-antennary carbohydrate chains. CEA and CEACAM1 are further modified by addition of ►Lewis^x and sialyl-Lewis^x high-mannose residues. The proteins differ however, in their C-terminal regions producing either secreted entities such as the pregnancy-specific glycoproteins (►PSG1–11) or others, tethered to the cell surface by either a glycosyl phosphatidylinositol linkage (CEA, CEACAM6–8) or a *bona fide* transmembrane domain (CEACAM1, CEACAM3, CEACAM4, CEACAM18–21) (Fig. 1). The *CEACAM1* gene is unique in this family in that it produces twelve different splicing variants. More information on the structural features of the *CEA* gene family members is available at http://cea.klinikum.uni-muenchen.de. CEA is a monomeric protein adopting a



CEA Gene Family. Figure 1 Schematic representation of some members of the CEA family. Most CEA family members, except the pregnancy-specific glycoproteins (PSG) that are secreted proteins, are associated with the cell membrane (depicted in grey). The immunoglobulin variable-like domains (the N domain) are shown in blue and the immunoglobulin constant-like domains are represented in orange. The N-linked glycosylation sites are indicated by sticks and balls, colored in dark orange. The glycosylphosphatidylinositol membrane anchors are represented by arrows. The *CEACAM1* gene expresses many splice variants. However, only the CEACAM-4L isoform containing four Ig domains and the longer cytoplasmic tail is shown here.

β-barrel cylindrical shape resembling a "bottle brush," whereas CEACAM1 is present as both a monomeric and dimeric protein.

Expression and Functions of CEA Family Members in Normal and Tumor Tissues

Although not ubiquitous, CEA family members exhibit a wide tissue distribution. CEA and CEACAM6 are found mainly in columnar epithelial and goblet cells of the colon in the early fetal period and are maintained in adult life. In the colonic brush border, CEA, CEACAM1, 6 and 7 demonstrate maximal expression at the free luminal surface, although CEACAM1 and 7 are also found at the lateral membrane. In addition to its expression in epithelia, CEACAM1 is located on granulocytes, lymphocytes and on endothelial cells, whereas CEACAM6 is also expressed on granulocytes and monocytes. CEACAM3 and 8 are found exclusively on granulocytes.

CEA, CEACAM1 and CEACAM6 are recognized as cell adhesion molecules contacting each other by antiparallel self-binding (homophilic). Some associations are exclusive, such as ►CEACAM8-CEACAM6. The first Ig domain is crucial in these interactions. Various CEA family members also act as heterophilic partners for ►E-selectin and ►galectin-3. Another striking feature of CEA family members is their ability to act as pathogen receptors binding to outer membrane proteins of *Neisseria* gonococci and *Haemophilus influenzae* as well as fimbriae of *Salmonella typhimurium* and *Escherichia coli*. In addition, CEACAM1 is the receptor for the mouse hepatitis viruses. The bacterial and viral adhesin functions of the CEA family members confer strong immunosuppressive activity in T and B lymphocytes, whereas they enhance integrin-dependent cell adhesion in epithelial cells with concomitant increase of the TGF-β1 receptor CD105. Other functions for CEA and CEACAM6 include inhibition of cellular differentiation as demonstrated in a number of cellular systems and inhibition of the ►apoptotic process of ►anoikis by activation of β1 integrins.

PSG1–11 are mainly expressed in syncytiotrophoblast during the first trimester of pregnancy where they act as immunomodulators and inhibit cell-matrix interactions.

CEA is abundantly expressed in tumors of epithelial origin such as colorectal, lung, mucinous ovarian and ►endometrial adenocarcinomas. For these reasons, CEA has a long history as a marker of colonic, intestinal, ovarian and breast tumor progression and its high expression is associated with poor prognostic and recurrence of disease post-surgically. High pre-operative CEA levels are indicative of a poor prognosis whereas low levels are associated with increased survival of the patients. The tumorigenic potential of CEA and CEACAM6 was recently clarified by transgenic overexpression of a bacterial artificial chromosome fragment of 187 kb encoding the full *CEA*, *CEACAM6* and *CEACAM7* genes. When the *CEABAC* transgenic mice were treated with the azoxymethane ►carcinogen to induce colon cancers, expression of CEA and CEACAM6 was increased by 2–20 fold, a situation reminiscent to that observed in the human cancer. Information on CEACAM7 expression in tumors is more limited. It is down-regulated in colorectal cancers, but increased in gastric tumors. CEACAM6 however, exhibits a broader distribution than in the cancers described above, as it is additionally found in gastric and breast carcinomas, and ►acute lymphoblastic leukemias. In fact, overexpression of CEACAM6 in ►pancreatic cancer confers increased resistance to anoikis and increased metastasis. It also modulates chemoresistance to the ►gemcitabine agent, thereby suggesting that CEACAM6 determines cellular susceptibility to apoptosis.

Expression and Functions of CEACAM1

CEACAM1 expression is more complex. It is down-regulated in colon, prostate, hepatocellular, bladder, endometrial, renal cell and 30% of breast carcinomas, but overexpressed in gastric and squamous lung cell carcinomas and ►melanomas. In thyroid carcinomas, CEACAM1 was shown to restrict tumor cell growth. However, it increases the thyroid cancer metastatic potential. Manipulation of CEACAM1 expression levels in colonic, prostatic and bladder tumor cell lines, negative for CEACAM1, has indeed confirmed that expression of the longer variant, CEACAM1-4L, produces reduction of tumorigenic potential *in vitro* and inhibition of tumor growth in xenograft mouse models. The importance of cell surface CEACAM1 expression for maintenance of normal epithelial cellular behavior has recently been confirmed *in vivo*; a *Ceacam1*-null mouse exhibits a significantly increased colon tumor load compared to the wild-type littermates upon carcinogenic induction of colorectal cancer.

CEACAM1's role as a modulator of tumor progression depends on the involvement of its cytoplasmic domain in signaling via its tyrosine and serine phosphorylation. Two Tyr residues are positioned within Immunoreceptor Tyrosine-based Inhibition Motifs (►ITIM). The membrane-proximal Tyr488 is a phosphorylation substrate of Src-like kinases as well as of the Insulin and Epidermal growth factor receptors. Upon Tyr phosphorylation, CEACAM1-L associates with the tyrosine phosphatases SHP-1 and SHP-2. The SHP-1-CEACAM1-L protein complex regulates its function in various tissues such as inhibition of epithelial cell growth, $CD4^+$ T cell activation and insulin clearance from hepatocytes. CEACAM1-L ►tyrosine phosphorylation also stimulates its association with the cytoskeletal proteins G-actin, tropomyosin and paxillin, thereby

influencing cell adhesion, and with the ►β3 integrin, hypothesized to influence cell motility. The CEACAM1-L cytoplasmic domain also carries seventeen serine residues most of which lie in consensus sequences recognized by serine kinases. However, little is know about their functional implications apart from the CEACAM1-S Thr/Ser452 and Ser456, shown to modulate direct binding to G- and F-actin, tropomyosin and calmodulin, and CEACAM1-L's Ser503 whose mutation to an Ala residue enhances colonic or prostatic tumor development in xenograph models. Additionally, Ser503 renders permissive Tyr488 phosphorylation by the insulin receptor. Transgenic mice overexpressing a Ser503Ala CEACAM1-L mutant in the liver developed hyperinsulinemia, secondary insulin resistance and defective insulin clearance. As a consequence of the decreased insulin receptor endocytosis and altered insulin signaling, the transgenic mice became obese demonstrating increased visceral adiposity, elevated serum free fatty acids and plasma and hepatic triglyceride levels.

CEACAM1-L also contributes to important functions in the immune system. It functions as an inhibitory co-receptor in T lymphocytes. Its conditional deletion in these cells amplified TCR-CD3 signaling, whereas overexpression in T cells was responsible for decreased proliferation, ►allogeneic reactivity and cytokine production *in vitro*, with delayed type hypersensitivity and inflammatory bowel disease *in vivo*. Regulation of this function involves the ITIM motifs and the SHP-1 tyrosine phosphatase. A similar function and mechanism have been described in B lymphocytes and natural killer cells. Indeed, CEACAM1-mediated intercellular adhesion between melanomas with increased CEACAM1 expression and NK cells allows inhibition of NK-cell-elicited killing, thereby conferring upon CEACAM1 a role in tumor immunosurveillance. Similarly, heterophilic engagement of CEACAM1 with CEA, overexpressed in many tumors, also inhibits lymphocyte-mediated and NK-cell-mediated killing having therefore detrimental effects on immune surveillance. In addition, increased expression of CEACAM1 on endothelial cells present in tumors in response to ►VEGF activation and/or hypoxia provokes a pro-angiogenic switch with increased endothelial tube formation and invasion. Therefore CEACAM1's contribution to cancer progression most likely depends on its positive or negative expression and signaling in epithelial tumor cells, on its systemic effects on metabolism and adiposity, on its role in immunosurveillance and most probably on endothelial proliferation and invasion.

Transcriptional Regulation

The upstream promoters of the *CEA* and *CEACAM1* genes have been dissected to identify important binding sites responsible for their transcriptional regulation. These two genes do not encompass classical TATA and CAAT boxes and are considered members of the housekeeping gene family. Their distal promoter regions (> −500 bp) contain highly repetitive elements, whereas their proximal promoter regions are rich in GC boxes and SP1 binding sites. Five footprinted regions have been identified in the *CEA* promoter, the first three binding respectively, to the Upstream Stimulatory Factor (USF), and SP1 and SP1-like factors. Similarly, the human *CEACAM6* promoter is regulated by the USF1 and USF2 as well as SP1 and SP3 transcription factors. A silencer element has also been located in its first intron. In contrast, the human *CEACAM1* promoter does not bind the SP1 factors, but associates with an AP-2-like factor and the USF and HFN-4 transcription factors. The gene is additionally controlled by the hormonal changes (estrogens and androgens) and can be induced by cAMP, retinoids, glucocorticoids and insulin. Moreover, many genes of this large family are triggered by inflammation via interferons, tumor necrosis factors and interleukins. It has been reported more recently that expression of the *CEACAM1* gene is influenced by TPA and calcium ionophore in endometrial cancers, the expression of ►BCR/ABL in leukemias, the expression of the β3 integrin in melanomas and VEGF and hypoxia in angiogenic situations. In prostate cancer, there is an inverse correlation between the down-regulation of CEACAM1 and the increased expression of the transcriptional repressor Sp2 that acts to recruit histone deacetylase to the *CEACAM1* promoter.

The Next Frontier

The diversity of functions of the members of the CEA gene family and their dynamic expression patterns in normal and tumor tissues has slowed the development of effective targeted therapies. More recently, effective strategies have been devised using vaccination with CEA peptide-loaded mature dendritic cells that induced potent CEA-specific T cell responses in advanced colorectal cancer patients. Effective protection from tumor development have also been seen with delivery of adenoviral vectors encoding CEA fused to immunoenhancing agents such as tetanus toxin or the Fc portion of IgG1. Likewise, targeting of CEACAM6 in pancreatic cancer may result in decreased tumor load. The therapeutic and selective targeting of CEACAM1 in melanomas, gastric and lung carcinomas as well as its location in tumor endothelia may prove to be a favorable avenue of future interventions.

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CEA-related Cell Adhesion Molecule 1

►CEACAM1 Adhesion Molecule

CEA-Vaccine Virus

Definition

A vaccine constructed from a recombinant vaccine virus containing the human carcinoembryonic antigen gene.

►Carcinoembryonic Antigen (CEA)

Ceacam1, Ceacam6, Ceacam7, Ceacam8

Definition

Carcinoembryonic antigen-related cell adhesion molecules. Members of the CEA family.

►CEA Gene Family

CEACAM1=BGP, C-CAM, CD66a

►CEA Gene Family

CEACAM5

►Carcinoembryonic Antigen

CEACAM5=CEA,CD66e

►CEA Gene Family

CEACAM6=NCA, CD66c

►CEA Gene Family

CEACAM7=CGM2

►CEA Gene Family

CEACAM8=CGM6, CD66b

►CEA Gene Family

CEACAM1 Adhesion Molecule

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Synonyms

NCA-160, nonspecific cross-reacting antigen with a Mw of 160kD; BGP, biliary glycoprotein; CD66a, cluster of differentiation antigen 66 a; CEACAM1 CEA-related cell adhesion molecule 1

Definition

CEACAM1 (CEA-related cell adhesion molecule 1) belongs to the CEA (►Carcinoembryonic antigen, ►CEA gene family) family of cell surface glycoproteins, a subfamily of the immunoglobulin gene superfamily. The CEA family comprises two major groups, the CEA-related molecules and the PSG (pregnancy-specific glycoprotein)-related molecules. Additionally, a number of pseudogenes have been identified. To date, 29 genes are known, which are clustered on human chromosome 19 (19q13.1-19q13.2). The CEA-related members of the CEA family display a complex expression pattern on human healthy and malignant tissues. They are linked to the cell membrane via GPI anchors, or they are transmembrane proteins with a cytoplasmatic tail. The PSG-related molecules are soluble glycoproteins; their expression is restricted to the placenta, more specifically, to the syncytiotrophoblast, which is the outermost fetal component of the placenta. CEACAM1 has been structurally and functionally conserved in humans and rodents.

Characteristics

Properties of CEACAM1

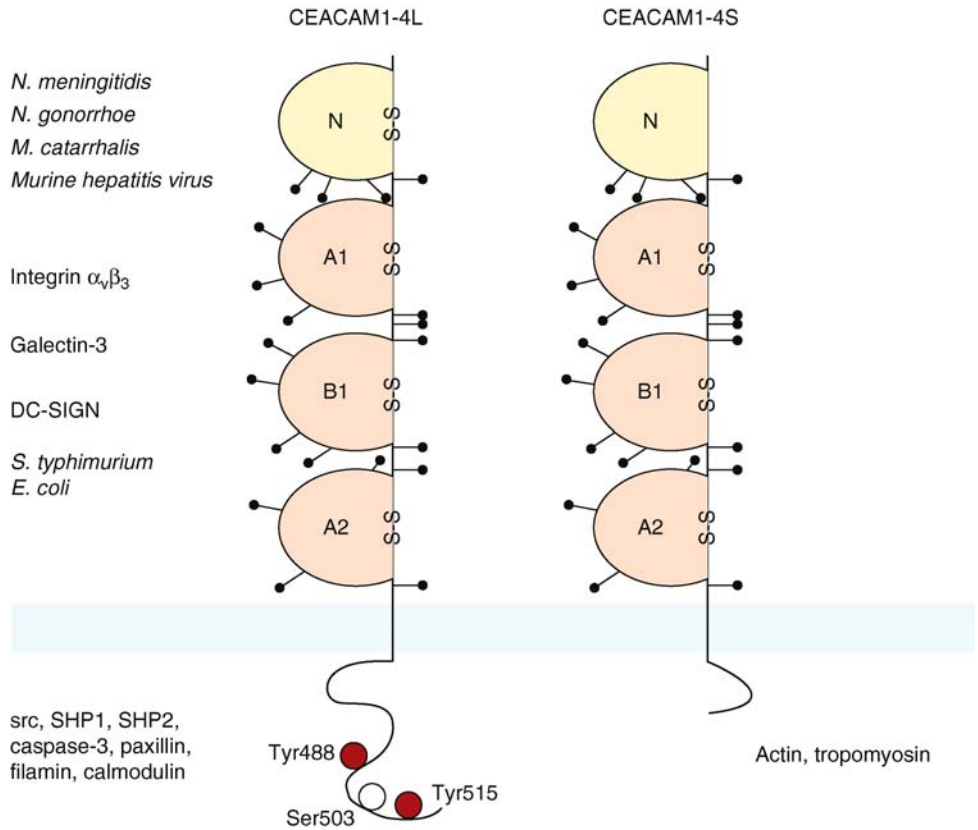
Human CEACAM1 has been originally identified in human bile due to its crossreactivity with CEA-antisera. It was therefore named biliary glycoprotein I or nonspecific cross-reacting antigen at first. Amongst the cluster of differentiation antigens on human leukocytes, CEACAM1 used to be referred as CD66a. However, with the latest revision of the nomenclature for the CEA family, CD66a, BGP, or NCA-160 became CEACAM1. Its structural similarities to CEA and the immunoglobulin superfamily proteins became apparent, once the cDNA sequence for CEACAM1 became available.

CEACAM1 displays the broadest expression pattern amongst CEA family members; it has first been described as a cell–cell ►adhesion molecule on rat hepatocytes. CEACAM1 is expressed on epithelia, endothelia, and on leukocytes.

CEACAM1 is a heavily glycosylated molecule that exists in 11 known isoforms emerging from differential splicing and proteolytic processing. The two major isoforms of CEACAM1 consist of four extracellular Ig-like domains, a transmembrane domain, and either a long or a short cytoplasmic tail, referred to as the long (CEACAM1-4L) and the short isoform (CEACAM1-4S), respectively. In addition to these transmembrane isoforms, soluble CEACAM1 isoforms are found in body fluids, for example, in saliva, serum, seminal fluid, and bile. Glycans on the extracellular domains of CEACAM1 are linked to the protein backbone via N-glycosidic linkages. It is presently unknown whether all of the 19 motifs that may render N-linked ►glycosylation actually harbor sugar moieties. On human granulocytes, CEACAM1 is a major carrier of Lewis^x glycans that are implicated in cellular adhesion to cognate lectins on blood vessels, within the extracellular matrix, or antigen presenting cells. CEACAM1 also elicits cell–cell adhesion via self-association in a homomeric fashion or via formation of heteromers with other CEA-family members and different adhesion molecules that are either located on the same cell or on neighboring cells. The resulting adhesive properties are modulated by differential expression ratios between the long and short CEACAM1 isoform, respectively. Through its long and short cytoplasmic tail, CEACAM1 mediates molecular interactions with cytoskeletal components or adapter proteins, which are integral parts of various key signal transduction pathways (►Signal transduction, cell biology). These interactions are in part dependent on differential phosphorylation of the CEACAM1-4L cytoplasmic domain on tyrosine and serine residues. The overall phosphorylation status of the CEACAM1-4L cytoplasmic domain relays signals, which contribute to cellular motility and differentiation, and thus determine cell fate by promoting proliferation or cell death. Phosphorylation of CEACAM1-4L cytoplasmic tyrosines that are part of an imperfect ITIM (immune receptor tyrosine-based inhibition motif) and serine residues regulate the interaction with kinases, phosphatases, cellular receptors for insulin (►Insulin receptor), the epidermal growth factor (►Epidermal growth factor receptor ligand, epidermal growth factor receptor inhibitor), and other cellular adhesion molecules, for example, integrin $\alpha_v\beta_3$ (►Integrin signaling and cancer). These qualities make CEACAM1 an important tool for cellular communication and they illustrate why so many different biological functions have been attributed to CEACAM1 in different biological contexts (Fig. 1).

CEACAM1 in Cancer

The first report on CEACAM1, in the context of human pathological conditions, was on elevated serum levels of a biliary glycoprotein in patients with liver or biliary tract disease. Later, aberrant CEACAM1 expression in a broad variety of human malignancies has been reported. In the progression of malignant diseases, two general patterns in the changes of CEACAM1 expression levels have emerged. In the first group of tumors, CEACAM1 expression is downregulated in the course of progressing disease. In the second group of tumors, CEACAM1 expression appears to be upregulated; often, this upregulation of CEACAM1 expression is observed in the context with increased invasiveness (►Invasion) of the primary tumor or is found on microvessels in progressing (►Progression) tumor areas (Fig. 2).

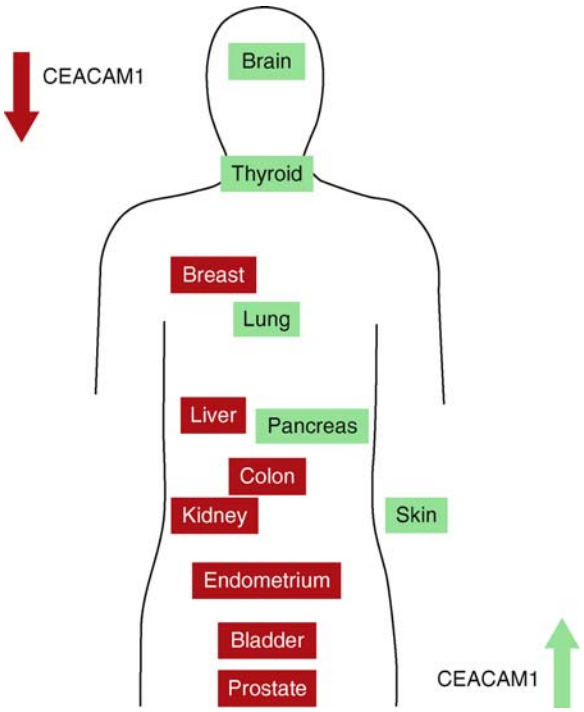


CEACAM1 Adhesion Molecule. Figure 1 Schematic representation of CEACAM1-4L and CEACAM1–4S and their participation in extracellular and intracellular communication. The two major CEACAM1 isoforms consist of four extracellular immunoglobulin-like domains, a transmembrane domain and either a long or a short cytoplasmic tail. The N-terminal domain (N) resembles a variable-like Ig domain but lacks the cystin bond usually found in Ig members. The A1, B1, and A2 domain resemble constant I-type-like Ig domains. Motifs for N-linked glycosylation are represented by lollipops. With its extracellular domains, CEACAM1 mediates recognition of various pathogens, such as *Escherichia coli, Salmonella typhimurium, Moraxella catarrhalis, Neisseria gonorrhoeae, Neisseria meningitidis*. The murine homologue of CEACAM1 is the receptor for the murine hepatitis virus: Additionally, CEACAM1 binds to galectin-3, DC-SIGN (dendritic cell ICAM3-grabbing nonintegrin), and integrin $\alpha_v\beta_3$. Tyrosine and serine residues involved in relaying CEACAM1-4L-mediated signal transduction are indicated by red and grey circles, respectively. Through its long cytoplasmic tail, CEACAM1-4L interacts with intracellular kinases of the SRC-family ►(*SRC*), the tyrosine phosphatases SHP-1 and SHP-2, caspase-3 as well as with paxillin, filamin, and calmodulin. Differential phosphorylation of the CEACAM1-4L cytoplasmic domain is required for its interaction with the insulin receptor, regulating insulin receptor internalization and recycling, and for modulating immune responses elicited by lymphocytes, for example. The short cytoplasmic domain of CEACAM1–4S binds to actin and tropomyosin.

Loss of CEACAM1 Expression in Tumorigenesis and Tumor Progression

Human cancers that show downregulation of CEACAM1 expression in the course of tumor progression are carcinomas of the liver (►Hepatocellular carcinoma), colon (►Colon cancer, colorectal premalignant lesions), kidney (►Renal cell carcinoma, renal carcinoma), urinary bladder (►Bladder cancer, bladder tumors), prostate (►Prostate cancer, clinical oncology), mammary gland (►Breast cancer), and the endometrium (►Endometrial cancer). In general, downregulation and subsequent loss of CEACAM1 expression is more frequent in high-grade tumors that are poorly differentiated, and often associated with a larger tumor size.

On epithelia, especially those that form a lumen, CEACAM1 exhibits a pronounced apical expression, like in the entire gastrointestinal tract, breast, liver, prostate, bladder, and kidney. CEACAM1 expression has been implicated in morphogenesis of lumen formation. In the process of building an asymmetrical epithelium, lateral CEACAM1 expression on neighboring cells is lost and often becomes entirely apical once a lumen or a duct has been formed. The loss of CEACAM1 expression in the context of tumorigenesis



CEACAM1 Adhesion Molecule. Figure 2 Dysregulation of CEACAM1 expression in human cancers. Changes of epithelial CEACAM1 expression in the course tumor progressison: In mammary carcinomas and carcinomas of the liver, colon, endometrium, kidney, bladder, and prostate, CEACAM1 expression is downregulated on tumor epithelium (►Epithelial cancers). Downregulation of CEACAM1 levels often correlates with dedifferentiation of the tumor and loss of tissue architecture. In carcinomas of the thyroid, ►non-small cell lung cancer (Lung cancer), pancreatic tumors (►Pancreas cancer, clinical oncology), and malignant melanomas (►Melanoma), CEACAM1 is induced or upregulated in the course of tumor growth. Here, CEACAM1 expression is found on the invasive front of the tumors and is related to development of metastatic disease (►Metastasis) and poor prognosis. In pancreatic cancer, CEACAM1 has been identified as a novel biomarker (►Biomarker, clinical cancer biomarker) that indicates presence of malignant disease.

has been studied most extensively in the context of breast, colonic, and prostate carcinomas.

A hallmark of carcinomatous lesions is the loss of polarity of their epithelial structures. In colonic epithelium, for example, loss of polarity is accompanied by the loss of apical CEACAM1 expression that occurs in early adenomas and carcinomas. In these tumors, presence and absence of CEACAM1 correlate with normal and reduced apoptosis (►Apoptosis, apoptosis signals), respectively. Furthermore, the naturally occurring process of ►anoikis, once cells lose contact to their substratum, is compromised. This observation and the fact that the *CEACAM1* gene is silenced in the course of aberrant cell growth prompted the hypothesis that CEACAM1 acts as a ►tumor suppressor. In intestinal cells, presence of the long CEACAM1 isoform is required to suppress tumor growth, and lack of CEACAM1-4L expression is accompanied by a decrease in proteins that inhibit cell cycle progression.

In human mammary epithelial cells, CEACAM1 expression is causally related to lumen formation and differentiation. In mammary glands, CEACAM1-4S is the predominating isoform, and only the short cytoplasmic tail induces apoptosis of the central cells and subsequently leads to lumen formation in mammary morphogenesis. During tumor progression, CEACAM1-4S expression is lost and acinar polarity no longer can be observed.

However, since particular mutations or allelic loss of the *CEACAM1* gene in human cancers has not been described so far, it is likely that dysregulation of CEACAM1 expression rather than irreversible loss of the *CEACAM1* gene are linked to tumorigenesis and tumor progression in vivo. Hence, gene silencing may attribute to the loss of the tumor suppressive qualities of CEACAM1. Though there are no changes in promoter ►methylation of the *CEACAM1* gene linked to tumor progression, CEACAM1 promoter activity appears to be regulated by binding of the transcription factor Sp2. In high-grade prostate carcinomas, Sp2 is highly abundant, whereas CEACAM1 expression is lost. Sp2 localizes to the CEACAM1 promoter and imposes repression of gene transcription by recruiting ►histone deacetylase.

Upregulation of CEACAM1 Expression in Malignant Diseases

Opposed to its tumor suppressive functions, certain tumors gain CEACAM1 expression in the course of cancer development. In the case of malignant melanomas and thyroid carcinomas, expression of CEACAM1 correlates with an increase of tumor invasiveness and development of metastatic disease. In primary cutaneous malignant melanomas, for example, CEACAM1 expression is found at the invasive front of the tumors, and its coexpression with integrin $\alpha_v\beta_3$ indicates that CEACAM1 may directly promote on cellular invasion. In a follow-up study, CEACAM1 was identified as an independent prognostic marker, predicting the development of metastatic disease and poor survival. In this context, it is noteworthy that CEACAM1 on melanoma cells forms homophilic cell–cell contacts with CEACAM1 molecules on tumor-infiltrating lymphocytes, and leads to inhibition of their cytolytic function. Similarly, in human non-small cell lung cancer, CEACAM1 expression correlates with advanced disease, whereas it is not expressed on the normal bronchiolar epithelium; this CEACAM1 neoexpression

was identified as an independent prognostic marker, indicating lower incidence of relapse-free survival.

In pancreatic carcinomas, CEACAM1 has been identified as a novel serum biomarker, with an increased CEACAM1 expression on neoplastic cells of pancreatic adenocarcinomas and elevation of serum levels at the same time. Additionally, significant differences in CEACAM1 serum levels were found in patients with either pancreatic cancer or chronic pancreatitis. Opposed to the classical pancreatic tumor marker CA19-9, CEACAM1 was confirmed as an independent marker to distinguish between the presence of malignant disease and pancreatitis.

CEACAM1 and Tumor Angiogenesis

CEACAM1 expression on human blood vessels is restricted to newly formed vessels, and usually, no CEACAM1 is found on mature, large vessels. The first indication that CEACAM1 is related to ►angiogenesis was the description of CEACAM1 neoexpression on newly formed vessels in the human placenta. Furthermore, CEACAM1 is expressed on vessels in wound healing tissues and on tumor vessels of human bladder carcinomas, the prostate, hemangiomas, and ►neuroblastomas. CEACAM1 expression in endothelia is induced by VEGF (►Vascular endothelial growth factor)-dependent pathways and appears to favor vessel maturation.

In human prostate carcinomas, CEACAM1 shows divergent expression on tumoral blood vessels and the tumor epithelium. The presence of epithelial CEACAM1 is observed in the context of poor tumoral blood vessel growth and loss of epithelial CEACAM1 expression parallels enhanced tumor angiogenesis. Especially in high-grade prostate carcinomas, tumor proximal vessels are expressing CEACAM1. Contrary to prostate carcinomas, microvessels in human neuroblastomas are CEACAM1-positive only during tumor maturation, but absent in undifferentiated, high-grade tumors. In ►Kaposi sarcomas, CEACAM1 upregulation is observed, indicating that CEACAM1 might be related to lymphatic reprogramming of the vasculature in these tumors.

Studying CEACAM1 in Cancer: Animal Models

In animal models investigating CEACAM1 function in tumorigenesis in vivo, the observations from human diseases could be confirmed. The focus of the mouse and rat models (►Mouse model) studied to date was set largely on the tumor-suppressive effects or enhancement of metastatic disease of CEACAM1-4L on the progression of colonic cancer, prostate cancer, hepatocellular carcinomas, and malignant melanomas. In CEACAM1-knockout mice, chemically induced colonic tumor growth was significantly increased in terms of tumor numbers and size opposed to CEACAM1-expressing wild type littermates. In syngeneic and xenotypic transplantation of tumor cells of the colon, prostate, and hepatocellular carcinomas, the tumor-suppressive effects of CEACAM1-4L expression could also be validated. After xenotransplantation of human CEACAM1-expressing melanoma cell lines into immune-deficient mice, enhanced metastasis was observed when compared to transplantation of CEACAM1-negative cell lines.

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C/EBPα

Definition

The transcription factor CCAAT enhancer binding protein-α is a tumor suppressor gene and a crucial regulator of granulopoiesis through inhibition of c-JUN. Disruption of C/EBPα, including dominant negative mutations of C/EBPα, are found in acetyl myeloid leukemia.

►NUP98-HOXA9 Fusion
►Tumor Suppressor Gene
►Chromosomal Translocation t(8;21)

C/EBP Homologous Protein

Definition

CHOP; synonym growth arrest and DNA damage-inducible gene 153; Is a C/EBP family transcription factor which is involved in ►endoplasmic reticulum stress-mediated ►apoptosis.

CED

►Convection Enhanced Delivery

CED-3

Definition

One of three genes (Ced-3, -4, and -9) that control the process of programmed cell death in *Caenorhabditis elegans*. Ced-9 is homologous of mammalian ►BCL-2 family members and acts upstream of Ced-3 and Ced-4. Ced-4 is homologous of APAF-1 and Ced-3 is homologous of the proapoptotic cysteine proteases known as caspases.

►APAF-1 Signaling
►Apoptosis

CED-4

Definition

Homologous of APAF-1 in *C. elegans*. CED-4 is one of three genes (Ced-3, -4 and -9) that control the process of programmed cell death in *C. elegans*.

►APAF-1 Signaling
►CED-3

Celastrol

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Synonyms

Tripterine; Quione methide friedelane tripterene (2R,4aS,6aS,12bR,14aS,14bR)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxylic acid

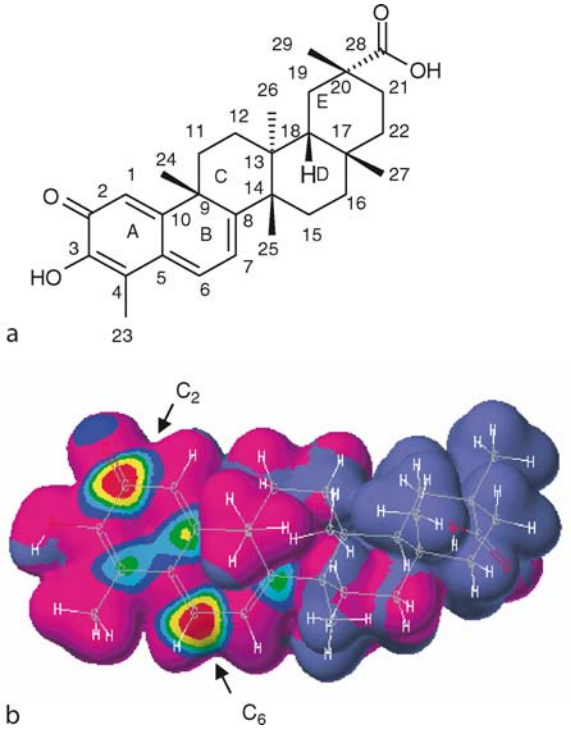
Definition

Celastrol is a natural quione methide friedelane tripterene, widely found in the plant genuses of *celastrus, maytenus* and *tripterygium*, all of which are present in China. For example, celastrol is one of the active components extracted from *tripterygium wilfordii Hook F,* an ivy-like vine also known as "Thunder of God Vine," which belongs to the family of *celastraceae* and has been used as a natural medicine in China for hundreds of years (Fig. 1).

Characteristics

Biological Properties

Celastrol has strong antifungal, anti-inflammatory and antioxidant effects. It has been shown that celastrol isolated from the roots of *Celastrus hypoleucus* (Oliv) Warb f argutior Loes exhibited inhibitory effects against diverse phytopathogenic fungi. Celastrol was also found to inhibit the mycelial growth of *Rhizoctonia solani*



Celastrol. Figure 1 The chemical structure and nucleophilic susceptibility of celastrol. (a) The chemical structure of celastrol is shown. (b) Nucleophilic susceptibility of celastrol analyzed using CAChe software. Higher susceptibility was shown at the C_2 and C_6 positions of celastrol.

Kuhn and *Glomerella cingulata* (Stonem) Spauld and Schrenk *in vitro*. Furthermore, celastrol has good preventive effect and curative effect against wheat powdery mildew *in vivo*.

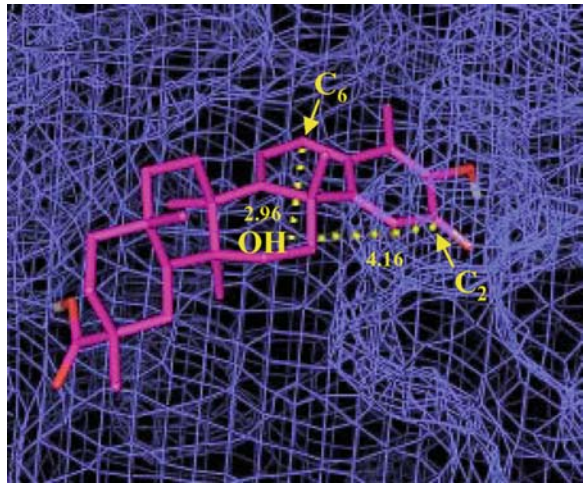
Celastrol in low nanomolar concentrations suppresses the production of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) by human monocytes and macrophages. Celastrol also decreases the induction of class II major histocompatibility complex (►MHC) expression by microglia. In macrophage lineage cells and endothelial cells, celastrol decreases induction of nitric oxide (NO) production. Celastrol also suppresses adjuvant arthritis in the rat, demonstrating *in vivo* anti-inflammatory activity. Low doses of celastrol administered to rats could significantly improve the performance of these animals in memory, learning and psychomotor activity.

In an isolated rat liver assay of lipid peroxidation, the antioxidant potency of celastrol (IC_{50} 7 μM) is 15 times stronger than that of α-tocopherol or vitamine E. Under *in vitro* conditions, celastrol was found to inhibit ►cancer cell proliferation and induce programmed cell death (or ►apoptosis) in a broad range of tumor cell lines, including 60 National Cancer Institute (NCI) human cancer cell lines. As a ►topoisomerase II inhibitor, celastrol was 5-fold more potent than the well-known topoisomerase inhibitor etoposide to induce apoptosis in HL-60 leukemia cells. Celastrol was also found to be a tumor ►angiogenesis inhibitor. In a sharp comparison, celastrol can block neuronal cell death in cultured cells and in animal models. These unique features of celastrol suggest potential use for treatment of cancer and neurodegenerative diseases accompanied by inflammation, such as Alzheimer's disease.

Potential Molecular Targets

Celastrol is a naturally occurring potent inhibitor of the ►proteasome and nuclear factor kappa B (NFκB). Proteasome, or 26S proteasome, is a multicatalytic protease complex consisted of a 20S catalytic particle capped by two 19S regulatory particles. The ubiquitin-proteasome pathway is responsible for the degradation of most endogenous proteins involved in gene transcription, cell cycle progression, differentiation, senescence and apoptosis. Inhibition of the proteasomal chymotrypsin-like, but not trypsin-like activity is associated with induction of apoptosis in tumor cells.

Both computational and experimental data support the hypothesis that celastrol is a natural proteasome inhibitor. Atomic orbital energy analysis demonstrates high susceptibility of C_2 on A-ring and C_6 on B-ring of celastrol toward a nucleophilic attack. Computational modeling shows that celastrol binds to the proteasomal chymotrypsin site (β5 subunit) in an orientation and conformation that is suitable for a nucleophilic attack



Celastrol. Figure 2 Docking solution of celastrol. Celastrol was docked to S_1 pocket of β5 subunit of 20S proteasome. Celastrol was shown in pink while β5 subunit was shown in purple. The selected conformation with 92% possibility showed the distances to the OH group of N-Thr from C_6 and C_2 were 2.96 Å and 4.16 Å, respectively.

by the hydroxyl (OH) group of N-terminal threonine of β5 subunit. The distances to the OH of N-terminal threonine of β5 from the electrophilic C_6 and C_2 of celastrol are measured as 2.96 Å and 4.16 Å, respectively. Both carbons, more probably C_6, of celastrol potentially interact with N-terminal threonine of β5 subunit and inhibit the proteasomal chymotrypsin-like activity (Fig. 2).

Celastrol potently and preferentially inhibits the chymotrypsin-like activity of a purified 20S proteasome with an IC_{50} value 2.5 μM. Celastrol at 1–5 μM inhibits the proteasomal activity in intact human prostate cancer cells. The inhibition of the cellular proteasome activity by celastrol results in accumulation of ubiquitinated proteins and three natural proteasome substrates, ►IκB-α, Bax and p27, leading to induction of apoptosis in ►androgen receptor (AR)-negative PC-3 cells. In AR-positive LNCaP cells, celastrol-mediated proteasome inhibition was accompanied by suppression of AR protein, probably by inhibiting ATP-binding activity of heat shock protein 90 (Hsp90) that is responsible for AR folding. Treatment of PC-3 tumor-bearing nude mice with celastrol (1–3 mg/kg/day, *i.p.*, for 1–31 days) resulted in significant inhibition (65–93%) of the tumor growth. Multiple assays using the animal tumor tissue samples from both early and end time-points demonstrated *in vivo* inhibition of the proteasomal activity and induction of apoptosis after celastrol treatment.

Antitumor activity of celastrol was also observed in a breast cancer mouse model. Celastrol inhibited ~60% tumor growth in breast cancer xenograft through

NFκB inhibition. NFκB inhibition by celastrol includes inhibition of its DNA-binding activity and inhibition of IκBα degradation induced by TNF-α or phorbol myristyl acetate. Further investigation showed that the cysteine-179 in the IκBα kinase was a potential target of celastrol-suppressed IκBα degradation. Since the proteasome is required for the activation of NFκB by degrading IκBα, the proteasome inhibition may also contribute to the NFκB inhibition by celastrol.

TNF could send both anti-apoptotic and pro-apoptotic signals. The effects of celastrol on cellular responses activated by the potent proinflammatory cytokine TNF have also been investigated. Celastrol was able to potentiate the apoptosis induced by TNF and chemotherapeutic agents and inhibited invasion, both regulated by NFκB activation. TNF induced the expression of gene products involved in anti-apoptosis (IAP1, IAP2, ►Bcl-2, Bcl-X_L, c-FLIP, and survivin), proliferation (cyclin D1 and COX-2), invasion (MMP-9), and angiogenesis (VEGF), and celastrol treatment suppressed the expression of these genes. Celastrol also suppressed both inducible and constitutive NFκB activation. Furthermore, celastrol was found to inhibit the TNF-induced activation of IκBα kinase, IκBα phosphorylation, IκBα degradation, p65 nuclear translocation and phosphorylation, and NFκB-mediated reporter gene expression. Therefore, celastrol potentiates TNF-induced apoptosis and inhibits invasion through suppression of the NFκB pathway.

Clinical Relevance

Due to its antioxidant or anti-inflammatory effects, celastrol has been effectively used in the treatment of autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus), asthma, chronic inflammation, and neurodegenerative diseases. As a bioactive component in Chinese traditional medicinal products from the extract of the roots of *Tripterygium wilfordii Hook F,* celastrol has been used since 1960s in China for autoimmune diseases, but has showed some side effects such as nausea, vomit, *etc*. Celastrol has not been used solely as a medication product. Celastrol has anti-tumor activities *via* inhibition of the proteasome and NFκB activation, indicating that celastrol has a great potential to be used for cancer prevention and treatment. This finding can be applied to various human cancers and diseases in which the proteasome is involved and on which celastrol has an effect.

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Celebra

►Celecoxib

Celebrex

►Celecoxib

Celecoxib

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Synonyms

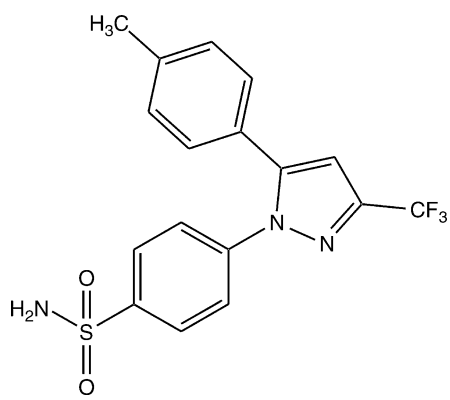
Celebrex; Celebra; 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide

Characteristics

Celecoxib, a diaryl-substituted pyrazole drug, was developed by G. D. Searle & Company, and is currently

marketed by Pfizer Incorporated under the brand names Celebrex and Celebra. Celecoxib is a member of the class of agents known as ►non-steroidal anti-inflammatory drugs (►NSAIDs). NSAIDs are the most commonly used therapeutic agents for the treatment of acute pain, fever, menstrual symptoms, osteoarthritis, and rheumatoid arthritis. Because of their ability to reduce tissue ►inflammation, which is often associated with ►tumorigenesis at various sites in the body (e.g., gastrointestinal tract and lung), celecoxib and certain other NSAIDs are also considered to have a potential in ►cancer chemoprevention as exemplified by their ability to prevent the formation and decrease the size of polyps in ►familial adenomatous polyposis (FAP) patients. Orally administered celecoxib exhibits good systemic bioavailability and tissue distribution with an estimated plasma half-life of approximately 11 h. Celecoxib binds to plasma albumin, and is metabolized primarily by hepatic enzymes prior to excretion. In humans, long-term exposures to celecoxib taken for arthritis pain relief at 100 mg twice daily caused no biologically significant adverse reactions. However, higher doses of 400 mg twice daily recommended for patients with FAP resulted in threefold increased risk of cardiovascular events (Fig. 1).

►*Cyclooxygenase Dependent Mechanisms for Cancer Chemoprevention by Celecoxib*. Cyclooxygenases are enzymes that are indispensable for the synthesis of ►prostaglandins. Prostaglandins are ►hormones generated from ►arachidonic acid, and they are found in virtually all tissues and organs. Prostaglandins typically act as short-lived local cell signaling intermediates that regulate processes associated with inflammation. In the early 1990s, cyclooxygenases were demonstrated to exist as two isoforms, cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2). COX-1 is characterized as a constitutively expressed housekeeping enzyme that mediates physiological responses like platelet aggregation, gastric cytoprotection, and the regulation of renal blood flow. In contrast, COX-2 is recognized as the inducible cyclooxygenase isoform that is primarily responsible for the synthesis of the prostaglandins that are involved in pathological processes (e.g., chronic inflammation) in cells that mediate inflammation (e.g., macrophages and monocytes). COX-2 is inducible by ►oncogenes (e.g., ►RAS and ►SRC), interleukin-1, ►hypoxia, benzo[*a*]pyrene, ultraviolet light, epidermal growth factor, ►transforming growth factor β, tumor necrosis factor α. Many of these inducers activate nuclear factor kappa B (NF-κB), which controls COX-2 expression and has been associated with tumorigenesis in various cell types.



Celecoxib. Figure 1 The chemical structure of celecoxib.

The COX-2 isoenzyme is frequently unregulated in cancer cells, as well as cells that constitute premalignant lesions, which are important targets for ►cancer chemoprevention. The expression of the inducible COX-2 is enhanced in 50% of colon adenomas and in the majority of human ►colorectal cancers, as opposed to COX-1, which typically remains unchanged. Thus, the increase in COX-2 expression, which is an early event in colon carcinogenesis, is believed to be necessary for tumor promotion. Aberrant COX-2 expression has also been implicated in tumorigenesis in the lung, ►prostate, esophagus, ►breast, ►liver, ►pancreas, and ►skin. The activity of COX-2 to produce arachidonic acid metabolites appears to enhance the proliferation of transformed cells and/or increases their survival through the suppression of ►apoptosis. Furthermore, COX-2 expression by tumor cells can stimulate ►angiogenesis at the tumor site and alter tumor cell adhesion to promote ►metastasis.

Celecoxib is a highly selective inhibitor of COX-2. Traditional NSAIDs (e.g., aspirin) inhibit both COX-1 and COX-2 isozymes. In contrast, celecoxib is approximately 20 times more selective for COX-2 inhibition compared to its inhibition of COX-1. This specificity allows celecoxib, and other selective COX-2 inhibitors, to reduce inflammation while minimizing adverse drug reactions (e.g., stomach ulcers and reduced platelet aggregation) that are common with non-selective NSAIDs. This selectivity for COX-2 is also intimately associated with the putative cancer chemopreventive activity of celecoxib, which has been demonstrated in ►colorectal cancer prevention. Epidemiological studies have shown that persons who regularly take aspirin have about a 50% lower risk of developing colorectal cancer. Celecoxib was the most effective ►NSAID in reducing the incidence and multiplicity of colon tumors in a rat colon carcinogenesis model. Moreover, in a clinical setting celecoxib has been used effectively to suppress the development and/or reduce the number of colorectal polyps in patients with FAP. This inflammatory disease often predisposes individuals to the development of ►colorectal cancers. The anti-inflammatory mediated anticancer effects of

celecoxib may be tissue-specific considering that celecoxib reduced lung inflammation in mice, but failed to inhibit the formation of chemically induced lung tumors in these animals.

Cyclooxygenase Independent Mechanisms for Cancer Chemoprevention by Celecoxib. The results of several *in vitro* and animal studies suggest the celecoxib may suppress tumorigenesis through several COX-2-independent mechanisms, which may account, at least in part, for celecoxib's anti-cancer effects in humans. For example, celecoxib inhibited the proliferation of various cancer cell types *in vitro* irrespective of their expression of COX-2, including transformed haematopoietic cells and immortalized and transformed human bronchial epithelial cells that were deficient in COX-2 expression. Celecoxib also inhibited the growth of human COX-2-deficient ►colon cancer cells that were transplanted as xenografts in nude mice. Thus, the chemopreventive effect of COX-2-specific inhibitors like celecoxib may be due to their effect on COX-2 as well as targets other than COX-2.

One putative COX-2 independent target for celecoxib is the ►phosphatidylinositol 3-kinase (PI3K) pathway, which is often deregulated in tumor cells. Celecoxib appears to directly inhibit the phosphoinositide-dependent kinase-1 (PDK1), and its downstream substrate protein kinase B/AKT, in the ►PI3K pathway. Protein kinase B/AKT inhibits apoptosis through the ►phosphorylation, and thus inactivation, of the proapoptotic ►BCL-2 family protein BAD. During apoptotic stimuli, BAD antagonizes BCL-2 and BCL-X_L activity, which can promote ►mitochondrial membrane permeabilization and cell death. The inhibition of the PI3K pathway by celecoxib is believed to be specific in its ability to promote apoptosis in transformed cells. For example, rofecoxib, another specific COX-2 inhibitor, had only marginal protein kinase B/AKT inhibitory activity in tumor cells during apoptosis induction.

Another presumed COX-2 independent target of celecoxib in tumor cells is ►sphingolipid metabolism. Celecoxib treatment increases the level of the ►sphingolipid ceramide in murine mammary tumor cells irrespective of COX-2 expression. This increase in ►ceramide was considered essential to apoptosis induction in these cells. Ceramide has been shown to mediate apoptosis in response to inflammatory cytokines like Fas and tumor necrosis factor α, and/or conditions associated with ►oxidative stress. During conditions of cell stress, the deregulation of ceramide generating and/or utilizing processes are believed to cause a net increase in cellular ceramide that is sufficient to trigger apoptosis induction via a mitochondrial membrane permeabilization mechanism.

Celecoxib treatment has also been shown to suppress the activity of the ►$Ca^{2\pm}$ ATPase located in the endoplasmic reticulum of human ►prostate cancer cells. The inhibition of the $Ca^{2\pm}$ ATPase by celecoxib disrupted Ca^{2+} homeostasis in the prostate cancer cells. This activity was highly specific for celecoxib, and was not associated with the exposure to other COX-2 inhibitors, including rofecoxib. Microsome and plasma membrane preparations from the human prostate cancer cells showed that only the $Ca^{2\pm}$ ATPases located in the endoplasmic reticulum were the direct targets of celecoxib. The disruption of Ca^{2+} homeostasis played a central role in apoptosis induction in the prostate cancer cells because it was required for the activation of Ca^{2+}-dependet hydrolyses that carried out cellular degradation. Moreover, mitochondrial membrane permeabilization, which releases cytochrome *c* to activate cell death, is sensitive to elevations in intracellular free Ca^{2+}. Consequently, the celecoxib-induced inhibition $Ca^{2\pm}$ ATPases located in the endoplasmic reticulum may provide a link to mitochondrial membrane permeabilization for apoptosis induction much in the same way that Celecoxib inhibition of the PI3K pathway can regulate BAD phosphorylation to trigger mitochondrial-mediated cell death.

It is apparent that the central hypothesis of a dominant role for COX-2 inhibition in cancer prevention by celecoxib may need re-examination. Furthermore, the COX-2 dependent and independent action of celecoxib in cancer prevention may be tissue specific. Since the aberrant expression of COX-2 is implicated in the pathogenesis of various types of human cancers, perhaps this inducible enzyme may be a useful surrogate ►biomarker of the anticancer activity of celecoxib when evaluating the chemoprevention of cancer at various sites in the body. Although the precise molecular mechanism for its chemopreventive effects are still fairly unknown, celecoxib may be still useful as a chemopreventive agent for a variety of malignancies, especially since it triggers less toxicity and adverse side effects during long-tern use when compared to traditional NSAIDs. Celecoxib may be useful when combined with other cancer chemopreventive/therapeutic agents to control the process of tumorigenesis.

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Cell Adhesion Molecules

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Synonyms

Cell adhesion receptors; Adhesion molecules; CAMs

Definition

Cell ►adhesion molecules are transmembrane or membrane-linked glycoproteins that mediate the connections between cells or the attachment of cells to substrate (such as stroma or basement membrane). Dynamic cell-cell and cell-substrate adhesion is a major morphogenetic factor in developing multicellular organisms. In adult animals, adhesive mechanisms underlie the maintenance of tissue architecture, allow the generation of force and movement, and guarantee the functionality of the organs (e.g. to create barriers in secreting organs, intestines and blood vessels) as well as the generation and maintenance of neuronal connections. Cell adhesion is also an integrated component of the immune system and wound healing. At the cellular level, cell adhesion molecules do not function just as molecular glue. Several signaling functions have been attributed to adhesion molecules, and cell adhesion is involved in processes such as ►contact inhibition, growth and ►apoptosis. Deficiencies in the function of cell adhesion molecules underlie a wide variety of human diseases including cancer. By their adhesive activities and their dialogue with the ►cytoskeleton, adhesion molecules directly influence the invasive and metastatic behavior of tumor cells, and by their signaling function they can be involved in the initiation of tumorigenesis.

Characteristics

At the molecular level, cell adhesion is mediated by molecules that are exposed on the external surface of the cell and are somehow physically linked to the cell membrane. In essence, there are three possible mechanisms by which such membrane-attached adhesion molecules link cells to each other (Fig. 1a). First, molecules on one cell bind directly to similar molecules on the other cell (►homophilic adhesion). Secondly, adhesion molecules on one cell bind to other adhesion receptors on the other cell (►heterophilic adhesion). Finally, two different adhesion molecules on two cells may both bind to a shared secreted multivalent ligand in the extracellular space. Also, cell-cell adhesion between two identical cells is called ►homotypic (cell) adhesion, while ►heterotypic (cell) adhesion takes place between two different cell types. In the case of cell-substrate adhesion the adhesion molecules bind to the ►extracellular matrix (ECM).

Cell Adhesion Molecules and the Cytoskeleton

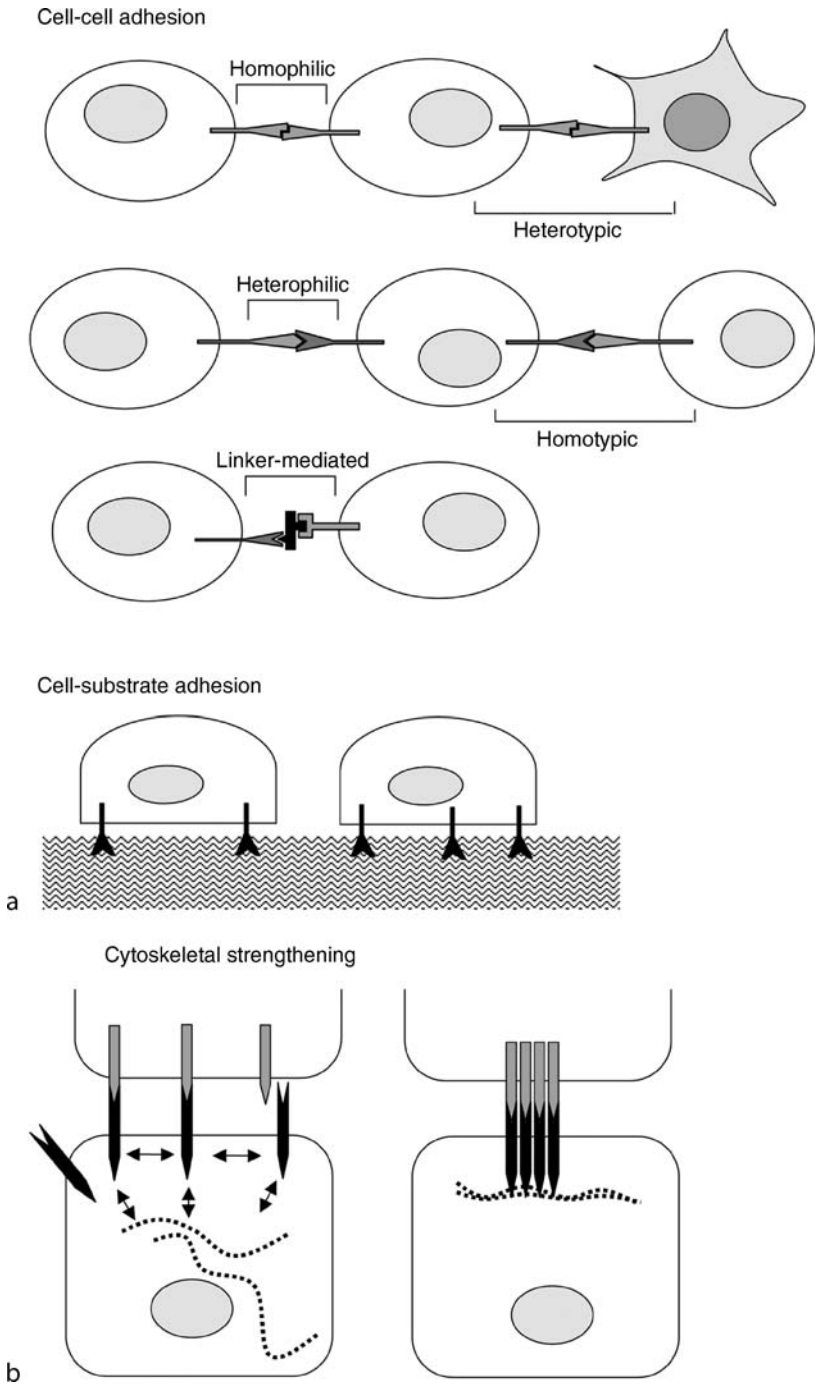
Adhesion molecules can be associated with the cell membrane either by a glycosylphosphatidyl-inositol (GPI) anchor or by a membrane-spanning region. In the latter case the cytoplasmic part of the molecule often associates indirectly with components of the cytoskeleton (e.g. actin, intermediate filaments or submembranous cortex). This implies that adhesion molecules, which by themselves establish extracellular contacts, can be structurally integrated with the intracellular cytoskeleton, and they are often clustered in specific restricted areas in the membrane, the so-called ►junctional complex (Fig. 1b). This combined behavior of linkage to the cytoskeleton and clustering, considerably strengthens the adhesive force of the adhesion molecules. In some cases, exposed adhesion molecules can be in a conformational configuration that does not support binding to its adhesion receptor. A signal within the cell can induce a conformational change that activates the adhesion molecule. Dynamic adhesion can also be mediated via regulated endocytosis of the adhesion molecules. These mechanisms of regulation allow for a dynamic process of cell adhesion that, amongst others, is required for morphogenesis during development and for efficient immunological defense.

Classification of Cell Adhesion Molecules

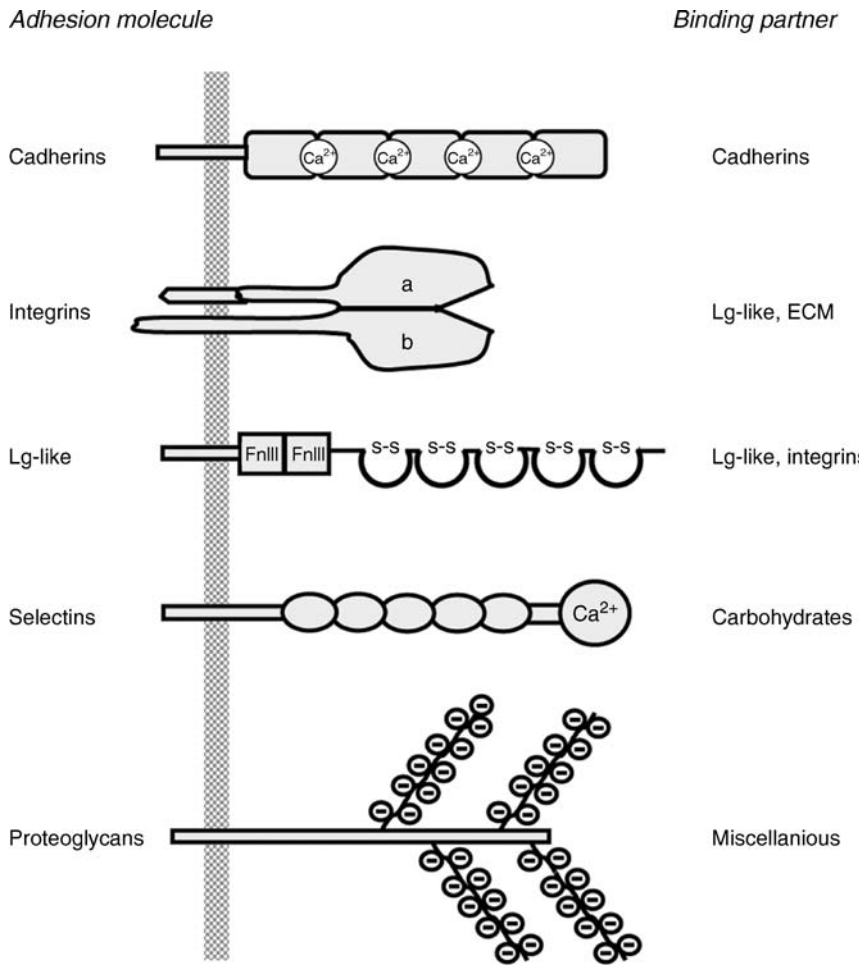
Based on their molecular structure and mode of interaction, five classes of adhesion molecules are generally distinguished; the ►cadherins, ►integrins, immunoglobulin (Ig) superfamily, selectins and ►proteoglycans (Fig. 2).

Cadherins

Cadherins and proto-cadherins form a large and diverse group of adhesion receptors. They are Ca2+-dependent adhesion molecules, involved in a variety of adhesive interactions both in the embryo and the adult. Cadherins play a fundamental role in metazoan embryos, from the earliest gross morphogenetic events (e.g. separation of germ layers during gastrulation) to the most delicate



Cell Adhesion Molecules. Figure 1 Different modes of cell-cell and cell-substrate adhesion and the mechanism of cytoskeletal strengthening. (a) Three possible mechanisms by which cell adhesion molecules mediate intercellular adhesion. A cell surface molecule can bind to an identical molecule (homophilic adhesion) on the opposing cell or can interact with another adhesion receptor (heterophilic adhesion). Alternatively, cell adhesion receptors on two neighboring cells can bind to the same multivalent, secreted ligand (linkermediated adhesion). Intercellular adhesion can take place between identical cell types (►homotypic adhesion) or between cells of different origin (►heterotypic adhesion), independently of the involved adhesion molecules. Cell-substrate adhesion molecules attach cells to specific compounds of the extracellular matrix. Cell-cell and cell-substrate adhesion can occur simultaneously. (b) Intercellular and cell-substrate adhesion can be strengthened by indirect intracellular linkage of the cytoplasmic tail of the adhesion molecules to the cytoskeleton and by lateral clustering in the membrane.



Cell Adhesion Molecules. Figure 2 The five major classes of cell adhesion molecules and their binding partners. Cadherins are Ca2+-dependent adhesion molecules that consist of a varying number of cadherin repeats (five in case of the classical cadherins). The conformation and activity of cadherins is highly dependent on the presence of Ca2+-ions. In general, cadherin binding is homophilic. Integrins are functional as heterodimers and consist of an a- and b-subunit. They interact with members of the immunoglobulin superfamily or with compounds of the extracellular matrix (e.g. fibronectin, laminin). Members of the immunoglobulin superfamily (Ig-like proteins) are characterized by a various number of immunoglobulin-like domains (open circles). Membrane-proximal, fibronectin type III repeats are often observed (gray boxes). They can either bind to other members of the Ig-family (homophilic) or to integrins. Selectins contain an N-terminal Ca2+-dependent lectin domain (circle) that binds carbohydrates, a single EGF-like repeat (gray box) and a number of repeats that are related to those present in complement-binding proteins (ovals). Proteoglycans are huge molecules that consist of a relatively small protein core to which long side chains of negatively charged glycosaminoglycans are covalently attached. They bind various molecules, including components of the extracellular matrix.

tunings later in development (e.g. molecular wiring of the neural network). The extracellular part of vertebrate classical cadherins consists of a number of cadherin repeats whose conformation is highly dependent on the presence or absence of calcium ions. Homophilic interactions can only be realized in the presence of calcium, usually by the most distal cadherin repeat. Classical cadherins are generally exposed as homodimers, and their cytoplasmic domain can be structurally or functionally associated with the actin cytoskeleton. Cadherins are the major adhesion molecules in tissues that are subject to high mechanical stress such as epithelia (►E-cadherin) and endothelia (VE-cadherin). However, finer and more elegant intercellular interactions, such as synaptic contacts, also involve cadherins.

Integrins

Integrins are another group of major players in the field of cell adhesion. They are involved in various processes

such as morphogenesis and tissue integrity, homeostasis, immune response and inflammation. Integrins are a special class of adhesion molecules, not only because they mediate both cell-cell and cell-substrate interactions (with components in the ECM such as laminin, fibronectin and collagen) but also because they function as heterodimers consisting of an α- and β-subunit. To date, at least 16 α-subunits and 8 β-subunits have been indentified. Of the theoretical 128 heterodimeric pairings, at least 21 are known to exist. While most integrin heterodimers bind to ECM components, some of them, more particularly those expressed on leukocytes, are heterophilic adhesion molecules binding to members of the Ig superfamily. The α-subunit mostly contains a ligand-binding domain and requires the binding of divalent cations (Mg2+, Ca2+ and Mn2+, depending on the integrin) for its function. Interestingly, integrins may be present on the cell-surface in a non-functional and a functional configuration. The cytoplasmic domain appears to be responsible for the conformational change that activates the integrin.

The Ig Superfamily

Among the classes of adhesion molecules discussed here, the Ig superfamily is probably the most diverse. The main representatives are the neural cell adhesion molecules (NCAMs) and V(ascular)CAMs. As the name suggests, the members of this family all contain an extracellular domain consisting of different immunoglobulin-like domains. NCAMs sustain homophilic and heterophilic interactions that play a central role in regulation and organization of neural networks, specifically in neuron-target interactions and fasciculation. The basic extracellular structure consists of a number of Ig domains, which are responsible for homophilic interaction, followed by a discrete number of fibronectin type III repeats. This structure is linked to the membrane either by a GPI anchor or a transmembrane domain. The VCAM subgroup, including I(ntercellular) CAMs and the mucosal vascular addressin adhesion molecule (MAdCAM), are involved in leukocyte trafficking (or homing) and extravasation. They consist of membrane-linked Ig domains that make heterophilic contacts with integrins. Other members of this family that are associated with cancer are carcinoembryonic antigen (►CEA), "deleted in colon cancer" (DCC) and platelet endothelial (PE)CAM-1.

Selectins

These types of adhesion molecules depend on carbohydrate structures for their adhesive interactions. Selectins have a C-type ►lectin domain, that specifically binds to discrete carbohydrate structures present on cell-surface proteins. Intercellular interactions mediated by selectins are of particular interest in the immune system, where they play a fundamental role in trafficking and homing of leukocytes.

Proteoglycans

Proteoglycans are large extracellular proteins consisting of a relatively small protein core to which long chains of glycosaminoglycans are attached. Although poorly documented, proteoglycans may bind to each other or may be the attachment site for other adhesion molecules.

Role of Adhesion Molecules in Cancer

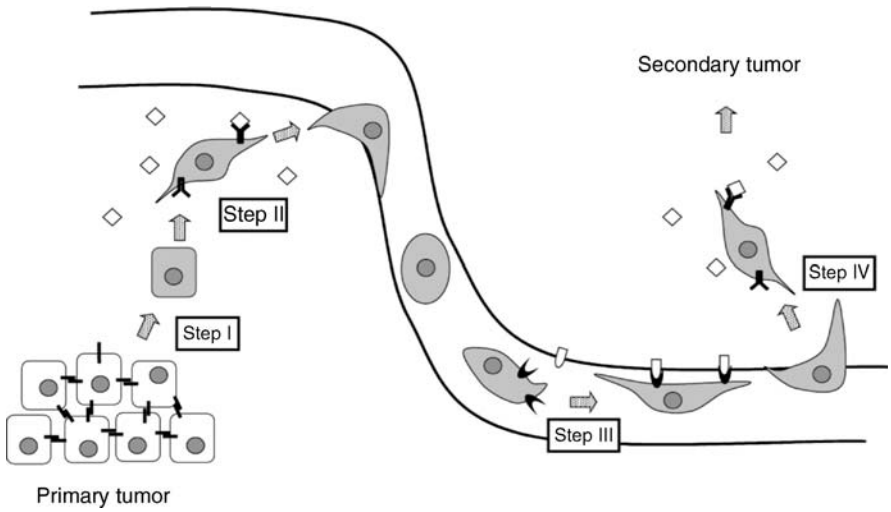
The Metastatic Cascade

Cell adhesion molecules play an important role during the progression of tumors, more particularly in the metastatic cascade (Fig. 3). When a benign tumor becomes malignant, cells at the periphery of the tumor will lose cell-cell contact (step I) and invade the surrounding stroma (step II) (see also ►invasion). Cells then extravasate and enter the vasculature or lymphatic system, where they are further transported. A fraction of the circulating tumor cells survives and is arrested at a distant site, attaches to the endothelium (step III) and extravasates through the blood vessel wall and into the surrounding tissue (step IV). Here the tumor cells grow, attract blood vessels and develop to a secondary tumor (►metastasis).

Adhesive Events in Metastasis

All the classes of cell adhesion molecules play a role in the metastatic cascade. During the first step, tumor cells need to disrupt intercellular junctions in order to detach from the primary tumor. This step often involves suppression of cadherin function. The second step of ►migration through the stroma and into the blood or lymphatic vessels requires dynamic cell-substrate adhesion, mostly mediated by integrins. In the third step, where cells arrest in the circulation by aggregation with each other or attachment to platelets, leukocytes and endothelial cells, critical roles have been attributed to cell adhesion molecules of the Ig superfamily, selectins, integrins and specific membrane-associated carbohydrates. The fourth step is similar to step II and mostly involves integrins. Details on the adhesive events associated with metastasis are outlined below.

- In benign epithelial tumors, cells maintain firm intercellular adhesive contacts, mostly by formation of a junctional complex (including tight junctions, ►adherens junctions and desmosomes). Establishment and maintenance of such a strong junctional complex requires expression and function of cadherins (more particularly E-cadherin). Loss of E-cadherin expression or function appears to be a hallmark of progression of a benign epithelial tumor (adenoma)



Cell Adhesion Molecules. Figure 3 Cell adhesion processes involved in the metastatic cascade. A subset of cells (gray) growing in a primary tumor will reduce cell-cell contacts (Step I) and migrate in the surrounding stroma by increasing specific cell-substrate adhesion (Step II). These invasive tumor cells can extravasate into the circulation and, at distant sites, attach to the endothelial blood vessel wall through specific cell-cell interactions (Step III). Once these cells have extravasated through the vessel wall they use cell-substrate adhesion molecules to invade the surrounding stroma (Step IV). See text for details.

to a malignant one (carcinoma). Epithelial tumor cells often acquire invasive properties by mutational inactivation of E-cadherin or one of its cytoplasmic binding partners (catenins). It is important to keep in mind that cadherin-mediated adhesion is a dynamic process and that E-cadherin can be temporarily inactivated at the functional level, for example by phosphorylation or other posttranslational modifications. E-cadherin and other molecules of the junctional complex are very often suppressed or functionally modulated in the epithelial-mesenchymal transitions (EMT), a hallmark of malignant tumor progression. EMT can be a tumor-intrinsic feature or can be induced by their microenvironment. Paracrine factors such as scatter factor or juxtacrine signaling via Ephrin/Eph receptor or via Semaphorins/plexins can affect adhesion via direct activity on the cell adhesion molecules or via regulation of the cytoskeleton.

- Dynamic cell-substrate adhesion is a critical factor in the migration of invasive tumor cells into the surrounding stroma. Integrins are instrumental in this process. Several studies have correlated the migratory behavior of tumor cells either with an increased or decreased expression of particular integrins. This apparent paradox may be explained by the fact that firm but temporary cell-substrate contacts are required for cells to migrate on a substrate. In order to crawl directionally through the stroma, a cell needs to "grab" the ECM, release after pulling itself forward and then has to establish the next contact. Both inhibiting adhesion and preventing release of the substrate contacts "locks" the cell in its position and prevents migration. It should be remembered that integrins may exist in two functional states and that signals passed through the cytoplasm determine whether membrane-exposed integrins are functional or not.
- In the third step of the metastatic cascade, cell-cell interactions are again the most determining. Homotypic interactions between circulating tumor cells promote formation of aggregates that are preferentially retained in the capillary network. PECAM-1 is a cell adhesion molecule potentially involved in this process. It should be pointed out that (re)expression of the invasion-suppressor molecule E-cadherin would actually promote metastasis formation. Besides these homotypic interactions, heterotypic interactions are also of major importance in the metastatic process. Tumor cells can attach to the blood-vessel wall either directly or indirectly through platelets and leukocytes. The adhesion molecules involved in this process are similar to those involved in the "multistep adhesion cascade" observed during homing and extravasation of leukocytes or trafficking of lymphocytes. Cell adhesion events include interactions of tumor-associated lectins with selectins expressed on platelets, leukocytes and endothelium (P-, L- and ►E-selectins, respectively). These adhesion molecules are also involved in the initial transient low-affinity interactions (rolling) of circulating leukocytes (and probably tumor cells) with the endothelium. Other and

more stringent heterotypic heterophilic interactions in this metastatic stage include the binding of integrins on tumor cells to ICAMs expressed on the surface of the endothelial cells.
- The fourth step in the metastatic cascade is extravasation and invasion at a distant site. This process is very similar to step 2 and the same adhesion molecules are likely to be involved. Specific interactions of the tumor cells with molecules present on the endothelial cells (e.g. N-cadherin) will facilitate the extravasation process.

Other Cancer-Related Functions of Cell Adhesion Molecules

Recently, it has become clear that some cell adhesion molecules are involved in signaling processes that are relevant to cancer. Germline mutations in E-cadherin predispose patients to the development of diffuse gastric carcinomas, and in lobular breast carcinoma E-cadherin seems to act as a tumor suppressor. Interestingly, β-catenin, a protein cytoplasmically linked to cadherins, has a central role in ►Wnt signaling and has oncogenic properties that are counteracted by the adenomatous polyposis coli (►APC) gene product. Signaling by integrins can also be an important factor that prevents cells from undergoing apoptosis (apoptosis upon loss of cell adhesion is called ►anoikis), which might be critical when tumor cells are traveling in the circulation. Interdisciplinary research has revealed new unexpected functions for known cell adhesion molecules. The suspected tumor suppressor DCC, a member of the Ig superfamily of adhesion molecules, turned out to be the receptor for netrin-1, an axonal chemoattractant crucial in neuronal development. Other molecules known to have adhesive or repulsive activities in the axonal growth cone or in migrating neural crest cells, turn out to have similar activities in tumor cells (see also the chapters on ►EPH receptors, ►Ephrin signaling in cancer, ►Semaphorins and ►Plexins).

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Cell-Adhesion Molecules (CAM)

Definition

Are cell-surface proteins that are involved in binding cells together in tissues and also in less permanent cell–cell interactions.

►Adhesion

Cell Adhesion Receptors

►Cell Adhesion Molecules

Cell Biology

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Definition

Cell biology deals with all aspects of the normal and of the tumor cell, their normal and abnormal multiplication, their differentiation, their stem origins, and their regulated cell death.

Characteristics

The Cell

The intracellular environment is separated from the external environment by a lipid bilayer called plasma membrane. The plasma membrane controls the movement of substances in and out of the cell and it is important for the cell to sense the surrounding environment. Within the cell the nucleus occupies most of the space. The cell nucleus contains genes, which drive all cellular activities and processes. Genes are organized in chromosomes (i.e., genome) and are made of DNA. The genetic information is used to produce proteins, which are the critical effectors required for all cellular processes. The nucleus is separated from the rest of the cellular content by the nuclear membrane,

which remains in contact with the cytoplasm as well as the nucleoplasm. In the cytoplasm, proteins are organized into specific functional structures and also connected with the structural network referred to as cytoskeleton network, which physically sustains the cell. Moreover several intracellular organelles are located in the cytoplasm (e.g., mitochondria, Golgi apparatus) and allow the cells to self sustain. To continuously adjust the intracellular processes and to promptly respond to the demands of the extracellular environment, cells need to exchange matter, energy, and information with the external milieu.

Cell Division and Reproduction

One of the unique features of cell is its ability to divide and produce two daughter cells that are an exact copy of their parental cell, by a process called "mitosis." However, some differentiated cells undergo the process of meiosis. For simplicity, meiotic division can be considered as the sum of two successive mitotic divisions, which result in four daughter cells with half the number of chromosomes and rearranged genes. These specialized cells (i.e., gametes) serve as reproductive cells. The fusion of the female and male gametes (eggs and spermatozoa, respectively) results in a new cell called zygote. The zygote, by definition, is a stem cell. Following mitotic division, it becomes an embryo and, at the end of the embryonic development, results in a new organism.

Cell Proliferation

The physiological functions of an organ require maintenance of homeostasis, a process of regulated balance between cell proliferation and cell death (also known as ►apoptosis), in the differentiated tissue. Indeed, a variety of extracellular stimuli activate specific ►signal transduction pathways that affect the expression and activity of molecules involved in the control of cell proliferation or cell death. Thus, the balance between ►cell cycle progression and apoptosis defines the cell fate, and this process depends on genetic factors as well as the kinetics of signal transduction pathways in exponentially growing cells.

Cell Cycle

In mammalian cells, one cell cycle takes about 24 h in most cell types and can be schematically divided into two stages: mitosis and interphase. Mitosis (M phase) consists of a series of molecular processes that result in cell division. On the other hand, the interphase can be subdivided into three major gaps (G1, S, and G2 phase). The G1 phase of the cell cycle separates the M and S phases. In G1 phase, cells express a specific pattern of gene products required for the DNA synthesis; the G2 phase of the cell cycle resides in between the S and M phases and is important for the completion of processes that are necessary for mitosis. The G0 phase of the cell cycle is entered by the cells from the G1. In the G0 phase, cells are out of the cell cycle and into a quiescent state where they do not proliferate.

Regulation of Cell Cycle Progression

Cell cycle progression is achieved through a series of coordinated molecular events that allow the cells to transit across the restriction points, also known as cell cycle checkpoints. There are three main restriction points in the cell cycle (G2/M, M/G1, and G1/S, respectively). Broadly, these checkpoints are defined as points after which the cell is committed to progress to the next phase in a nonreversible manner. Therefore, the transition between the phases of the cell cycle is strictly regulated by a specific set of proteins. ►Cyclin-dependent kinases (CDK) act in various phases of the cell cycle by binding to its activating proteins called cyclins. For example, both ►cyclin D/CDK4 and cyclin E/CDK2 complexes regulate transition of the cells through G1/S phase whereas cyclin A/CDK1, cyclin A/CDK2, and cyclin B/CDK1 complexes are active during the rest of the cell cycle. On the other hand, another class of regulatory proteins, the cyclin-dependent kinase inhibitors (CKI) (e.g., $p21^{Cip/Kip}$; $p19^{Ink4d}$) antagonizes the activation of CDK activity, thus impeding the progression of the cell cycle.

Programmed Cell Death

Programmed cell death (PCD) is a physiological process of eliminating a living cell. The PCD involves activation of specific intracellular programs that commit cells to a "suicidal route." The process of PCD plays an important role in a variety of biological events, including morphogenesis, maintenance of tissue homeostasis, and elimination of harmful cells. To date, different forms of PCD have been described among which apoptosis, necrosis, and ►autophagy are the most common.

Apoptosis

One of the critical events in apoptosis is the activation of cystein proteases, called caspases, upon a given signal. The initiator caspases (►Caspase 8 and 9) are the first enzymes involved in the activation of the apoptotic cascade. Caspase 8 and 9 activate the downstream effector caspases (caspase 3, 6, and 7) by proteolytic cleavage which in turn results in the hydrolysis and inactivation of the enzymes involved in the processes of DNA repair such as by poly-ADP-ribose polymerase (PARP). Upon stimulation of apoptotic cascade, cells display a specific set of characters, which constitute the hallmark of apoptosis (DNA fragmentation, cell shrinkage, cytoplasmic budding, and fragmentation). The activation of caspases

is achieved through two principle pathways – an extrinsic pathway that transduces signals from the plasma membrane directly to the caspases, and an intrinsic pathway that involves activation of caspases through a series of biochemical events leading to permeabilization of the mitochondrial membrane and release of cytochrome c (►Cytochrome P450) in the cytoplasm. Apoptotic cells are eventually eliminated by the immune system without the activation of inflammatory reactions (►Inflammation).

Necrosis

Necrosis results from a severe physical, mechanical, or metabolic cellular damage. The necrotic phenotype is very different from those of an apoptotic cells. Overall, the cell switches off its metabolic pathways and the DNA condenses at the margins of the nucleus and the cellular constituents start to degrade. In general, necrosis consists in a general swelling of the cell before it disintegrates. Furthermore, upon leakage of the intracellular content, necrotic cells stimulate an inflammatory response that usually damages the surrounding tissue.

Autophagy

Autophagy, i.e., autophagic cell death, occurs by sequestration of intracellular organelles in a double membrane structure termed autophagosome. Subsequently, the autophagosomes are delivered to the lysosomes and degraded. Autophagy is responsible for the turnover of dysfunctional organelles and cytoplasmic proteins and thus, contributes to cytosolic homeostasis. Autophagy can occur either in the absence of detectable signs of apoptosis or concomitantly with apoptosis. Indeed, autophagy is activated by signaling pathways that also control apoptosis.

Signal Transduction

Extracellular signals are transduced by the activation of a series of phosphorylation-dependent intracellular pathways initiated by cell surface receptors. Eventually, such signals feed into the nucleus, stimulate transcription factors, and regulate gene transcription.

Signaling Targets

Signaling pathways regulate gene transcription by triggering the promoter activity of the target gene. For example, regulation of cyclin D is critical for cell cycle progression. The extracellular signal-mediated activation of specific signal transduction pathways stimulates the activity of transcription factors such as AP-1, SP-1, and NF-κB, which coordinate the activation of the cyclin D1 promoter and thus lead to cyclin D1 expression. On the other hand, signaling molecules can also change the activity of a preexisting protein. For example, activation of p21-activated kinase (PAK) induces the phosphorylation of phosphoglucomutase (PGM) that stimulates its enzyme activity and the phosphorylation of ►estrogen receptor alpha (ERα) thus inducing its transcriptional activity. One of the most studied signaling pathways is the extracellular-regulated kinase (ERK) (►MAP kinase) cascade. It consists of three steps of sequential phosphorylations that impact on diverse cellular effectors. The ERK cascade is activated by mitogenic stimuli (e.g., growth factors (►Fibroblast growth factors)) and plays a critical role both in cell proliferation and cell survival. Indeed, activation of ERK induces the activation of AP-1 transcription factor, which, in turn, regulates cyclin D1 expression in addition to many of other proliferative molecules. Further, ERK activity leads to an increased expression of the antiapoptotic protein ►Bcl-2 and inactivation of the proapoptotic protein ►Bad. Conversely, the JNK/SAPK (►JNK subfamily and cancer) and the p38/MAPK (MAP kinase) pathways mediate stress and apoptotic stimuli (e.g., UV, ischemic-reperfusion damage). Activation of JNK/SAPK and p38/MAPK often results in an increased expression of proapoptotic proteins (e.g., Bax), and in the activation of the caspase cascade and cytochrome c release from the mitochondria.

Systems Biology

Systems biology represents a new analytical tool that has begun to emerge for balanced comprehensive analyses of cellular pathways at the level of genes and proteins. Signal transduction pathways often cross-talk and influence each other, and the functionality of the effector molecule is influenced by the overall outcome of a set of signaling pathways. Thus, cells form a web of intracellular interactions that are critical for a timely and dynamic response. The intracellular signaling network is considered a complex system rapidly adapting to extracellular challenges. Therefore, an additional level of complication is the evaluation of the network as a whole, rather than the individual pathway.

Cell Motility and Migration

►Motility and ►migration are important components for the functionality of a variety of cell types, and are involved in physiologic processes such as embryonic development, immune response, as well as in pathologic processes such as ►invasion and ►metastasis. Cell motility and migration are coordinated physiological processes that allow the cells to move or to invade the surrounding tissues, respectively. They occur as a result of a complex interplay between the focal ►adhesion sites (cell-to-substrate contacts) and the ►extracellular matrix (ECM) (substrate). Phenotypically, migratory cells develop motile structures such as pseudopodia, lamellipodia, and filopodia. An ordered sequence of events (protrusion of motile structures, formation and disruption of focal contacts) generate the traction forces

that drive the cell movement. Moreover, when migration is required, cells secrete specific proteolytic enzymes (matrix metalloproteinases, MMPs) that digest the ECM, thus opening a passage across the substrate. Cytoskeleton is critical for the correct occurrence of cell motility and migration.

Cytoskeleton

Cytoskeleton is a network of cytoplasmic proteins, which define the cell "bones." Many different protein filaments are important for cytoskeleton functions. In particular, microtubules, built from different types of tubulin, originate from specific intracellular structures called microtubules organizing centers (MTOC). Dynamic changes in the polymerization and depolymerization of tubulin maintain microtubule integrity and resulting functions. Furthermore, actin microfilaments form a network of cytoskeleton-associated proteins and connect the focal adhesion with the intracellular cytoskeleton. The dynamic remodeling of microtubules and microfilaments has an impact on cell motility, migration and cell–cell adhesion, ►endocytosis, intracellular trafficking, organelle function, cell survival, gene expression, and cell division.

Signaling Regulation

At the focal adhesion sites, cells accumulate receptors (e.g., growth factor receptors), adaptors (e.g., vinculin), and signaling molecules, as well as structural and motor proteins (e.g., actin, myosin). Migration-specific stimuli (e.g., integrins engagement of ECM, growth factor stimulation, and mechanical stimuli) activate specific biochemical pathways. ►Focal adhesion kinase (FAK), integrin-linked kinase (ILK), PAK, and ►Src play key roles in modulating cell migration and invasion. The FAK/Src complex regulates the assembly and disassembly of focal contacts, F-actin cytoskeleton remodeling, and the formation of lamellipodia and filopodia through the activation of specific downstream cytoskeleton-associated signaling pathways. Further, ILK is also implicated in cell motility and migration by linking integrins with cytoskeleton dynamics through the ►PI3K signaling pathway. Also, PAK1 dynamically regulates cytoskeletal changes by coordinating upstream signaling with multiple effectors. By acting on actin reorganization, PAK1 drives directional cell motility and migration.

Tumor Biology

Cancer is a progressive disease that arises from the clonal expansion of a single transformed cell into a mass of uncontrolled proliferating cells. Tumorigenesis is a multistep process and involves progressive conversion of a normal cell into a malignant cell, which subsequently invades the surrounding tissues. The process of tumorigenesis consists of major steps (initiation, promotion, and progression), each involving specific molecular mechanisms, often interlaced with each other, that drive tumor development.

Initiation and Promotion

In general, initiation of tumorigenesis is referred to as the first oncogenic stimulus. However, such as initial event is not sufficient for tumor induction. In most cases, a second oncogenic stimulus must occur in a restricted time frame, thus promoting an irreversible effect. Chemical (e.g., aromatic compounds (►Polycyclic aromatic hydrocarbons)), physical (e.g., ►UV radiation), as well as biological (e.g., viruses as ►human papillomavirus) stress have impact on the cells and can induce DNA mutations (e.g., point mutations). In addition, gene deletion or duplication also alters gene function and contributes to the process of tumorigenesis. These genomic changes result in the production of proteins with altered functions or in the overexpression or downregulation of specific proteins, which affects the associated cellular functions.

Protooncogenes or oncogenes are genes that encode for proteins involved in the induction of cell proliferation (e.g., *cyclin D1*, *CDK*, *EGFR*, *Src*, *Ras*, etc.) and whose overexpression or hyperactivation leads to an uncontrolled cell proliferation. On the other hand, tumor suppressor genes are genes encoding for proteins that negatively regulate cell proliferation (e.g., *p53*, *PARP*, *CKI*, etc.). Inactivating mutations or downregulation of tumor suppressor genes are also critical for enhanced cell proliferation. In addition to DNA damage, oncogenes and tumor suppressor genes, abnormal changes in the epigenetic cellular information (e.g., DNA► methylation) can also participate in clonal evolution of human cancers.

Progression

The modified balance between the growth-inhibitory programs and proliferative networks allow the cell to escape the physiological growth restrains. These selective growth advantages produce a population of more aggressive or transformed cells that resist clearance by the immune system (i.e., immune defense escape), and in turn, contributes to the accumulation of additional mutations and eventually, in tumor growth. In this context, an in situ tumor develops, that is the uncontrolled mass of transformed cells stays within the limit of the tissue in which the first cell resided. During this phase, tumor volume increases in parallel with an increased dedifferentiation of the cells that also secrete angiogenic factors (►Angiogenesis) to promote blood vessels formation in the tumor.

Metastasis

Metastasis is the process by which highly vascularized tumor cells acquire the ability to invade the

blood-stream and seed in distant organs. Deregulation of cytoskeleton-associated proteins and secretion of protein factors play a critical role in the functionality of the metastatic cells.

Stem Cell Biology

In 1998, the group of Prof. James Thomson reported the isolation of a human embryonic stem cell line from the blastocyst stage of a human embryo. This cell line showed stability in a specifically developed culture medium and, upon transplantation in the nude mice, had the ability to form tumor-like structures made up of all the major human tissue types. This pioneer study opened the field of stem cell biology. Since then, enormous research efforts have been focused on the understanding of stem cell biology as well as their potential medical and therapeutic implications. Nonetheless, although the last 10 years witnessed an enormous progress, the field of stem cell research is in its infancy. The first controversy is the definition of stem cell itself. For simplicity, a stem cell is a clonal self-renewing entity that is multipotent and can generate several different cell types. This definition introduces three major characteristic of the stem cells: self-renewal, clonality, and potency.

Self-Renewal and Clonality

Self-renewal is the process by which a stem cell undergoes an asymmetric mitotic division that produces, rather than two identical daughter cells, one cell that is completely identical to the parental stem cell and another cell that is already committed to a more restricted developmental path and more specialized abilities. Thus, stem cells have both the ability to self-maintain their clonal cell population and to produce a population of clones with more differentiated characteristics. In this way, stem cells form a hierarchy of potency.

Potency

Stem cells have the ability to give rise to a population of daughter stem cells with a reduced differentiation. The totipotent cells are the first embryonic cells that can become any kind of cell type (e.g., zygote). These cells become pluripotent cells, which can differentiate in most but not all cell types (e.g., embryonic stem cells). Next, cells that are committed to produce only a certain lineage of cell types (e.g., ►adult stem cells) are the multipotent cells. Some multipotent cells can only generate one specific kind of terminally differentiated cell type and thus, such cells are called unipotent cells.

Environmental Regulation

The molecular mechanism by which regulatory processes occur in stem cells are not clear but are believed to be tightly regulated to avoid imbalance in stem cell population or mutation that can lead to tumorigenesis. One possibility is that the asymmetric division produces two daughter cells and, because of intrinsic factors, such cells follow different fates in spite of residing in the same ►microenvironment. Alternatively, the two daughter cells become functionally different because they are exposed to different extrinsic factors. Most likely, both intrinsic and extrinsic factors are integrated in the milieu of the surrounding microenvironment, also known as the stem cell niche. Signals from the niche determine the type of gene regulation that allows the asymmetric division to take place. In this model, one daughter cell stays in the niche and the other one moves out. Indeed, the importance of the microenvironment in stem cell biology is highlighted by the ability of a particular stem cell to transdifferentiate or to dedifferentiate when put in a different niche. Although the concept of plasticity is debated in the literature, it is part of the "stemness" of a cell, which is the hallmark for a cell to be defined as a stem cell.

Social Implications

The ability to scientifically manipulate the human embryo or human adult stem cells has opened new perspectives for treatment of several human diseases. However, it has also initiated intense philosophical and political debates on the ethical issues associated with the use of such potential tools in medical practice.

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Cell Block

Definition

Consists of a paraffin block made from the cellular material of cytologic specimens (most commonly fine needle aspiration biopsies and body fluids) and is processed similar to histology. Can be a useful adjunct in cytology because it gives a better idea of tissue

architecture and allows for multiple sections for ancillary stains.

►Fine Needle Aspiration

Cell Cycle

Definition

The sequence of cellular transformations that accompany transition from one mitotic cell division to another. The cell cycle is composed of four phases known as G1, S, G2 and M. S is the period of DNA synthesis, M is mitosis when sister chromatids are condensed and segregated to two daughter cells. G1 lies between M and S and is a phase of preparation for DNA synthesis, G2 is between S and M and is a phase of preparation for mitosis. G_0 refers to a quiescent state into which some cells in multicellular organisms enter from G_1. Cells typically achieve full differentiation in the G_0 phase.

►Decatenation G2 Checkpoint
►Cell-Cycle Targets for Cancer Therapy
►Cyclin Dependent Kinases
►Chelators as Anticancer Drugs

Cell Cycle Arrest

Definition

The halt of the ►cell cycle, often as a result of cellular stress with physical or chemical treatment as a mechanism of cellular defense.

►Sulforaphane

Cell-Cycle Checkpoint

Definition

The cell-cycle checkpoint is a mechanism for stopping progression through the cell cycle when a key event, such as DNA replication, is not completed or when the genome is damaged. It is a restriction point during the cell cycle in which a cell monitors if preceding events required for cell division have been correctly completed. It is a regulatory mechanisms that monitors the progression of the cell cycle, so that one phase is not started before another has finished. The activation of checkpoints, for example by damaged DNA, arrests cell cycle progression.

►Hypoxia
►HSP90
►Decatenation G2 checkpoint

Cell-Cycle Targets for Cancer Therapy

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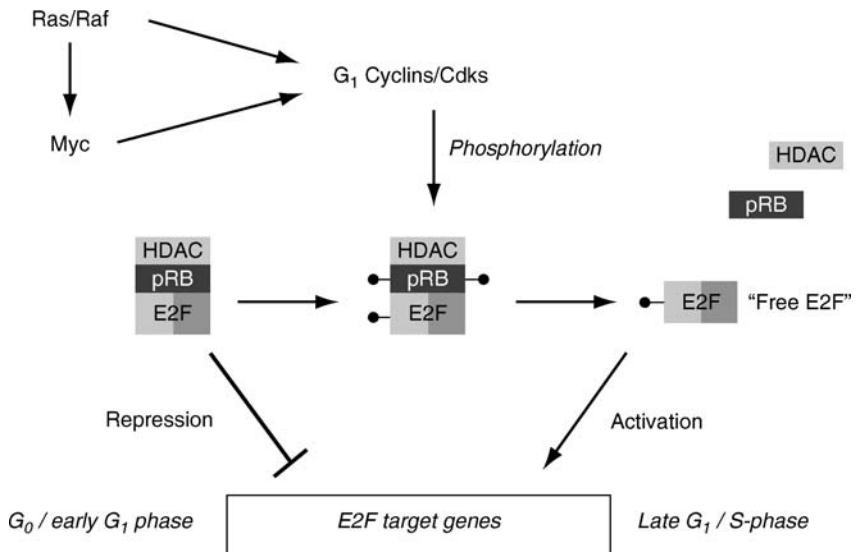
Definition

Knowledge of the molecular mechanisms governing the mammalian ►cell cycle and their dysfunction in cancer cells has grown considerably in the last decade. It is now clear that the cell utilizes two distinct kinds of regulatory mechanisms to control cell-cycle progression: while progression past the ►restriction point in late G1 is solely governed by extracellular signals, ►checkpoints sense cellular damage or dysfunctions that are not compatible with a proper cell division, such as DNA damage. The detailed knowledge of the underlying molecular mechanisms, pathways and molecules provides the basis for a new approach to cancer therapy.

Characteristics

Cell-cycle progression in mammalian cells is controlled through fundamentally different regulatory pathways. Progression through G1 across the restriction point (R-point) is controlled by external signals that are transmitted, for example, by mitogens or through cell adhesion processes. Beyond this point, cell-cycle progression is governed by a genetic program that is largely independent of extracellular signals but regulated by internally controlled checkpoints. These checkpoints ensure proper DNA replication, DNA integrity, progression through G2 and mitosis. A central role in cell-cycle progression is exerted by the ►cyclin-dependent kinases (CDKs), which are composed of a regulatory cyclin subunit (e.g., cyclin A, B, D or E) and a catalytic kinase subunit (e.g., CDK1, 2, 4 or 6). The activity of CDKs is controlled by phosphorylation, phase-specific expression and proteolysis as well as the

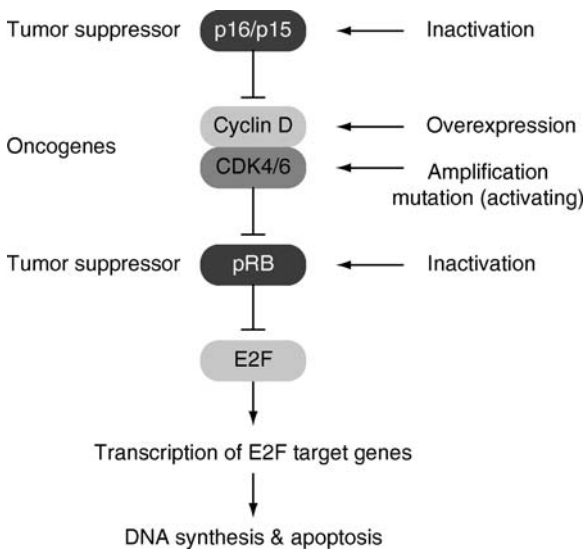


Cell-Cycle Targets for Cancer Therapy. Figure 1 The E2F pathway and its regulation by Rb and G1 CDKs.

association with CDK inhibitors (CDIs) belonging to the INK4 (p15, ►p16, p18, p19) or KIP (p27, p57)/CIP (p21) families.

Restriction Point Control

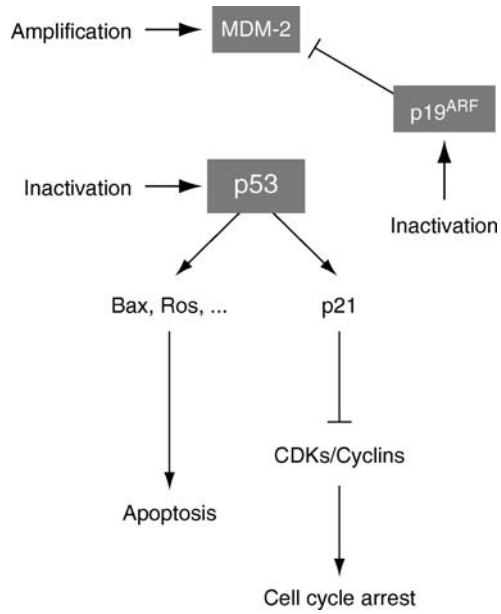
The G1 CDK-cyclin complexes regulate progression across the restriction point through phosphorylation of the ►retinoblastoma protein Rb and its kins p107 and p130. In early-mid G1 the transcription factor ►E2F is found in complexes with Rb and ►histone deacetylase (HDAC). These complexes actively repress transcription via E2F binding sites in the respective target genes. The phosphorylation of the E2F-Rb-HDAC complexes by ►cyclin D-CDK4/6 and cyclin E-CDK in mid-late G1 leads to the disruption of these complexes and the generation of transcriptionally active "free" E2F, which results in the induction of numerous E2F target genes (Fig. 1). The relevance of R-point control for tumorigenesis is emphasized by the fact that the ►INK4-cyclin D-CDK4-Rb pathway is defective in the vast majority of human tumors due to genetic alteration of its components (Fig. 2). Therefore, this pathway is of major interest with respect to therapeutic intervention.



Cell-Cycle Targets for Cancer Therapy. Figure 2 Deregulation of E2F activity in cancer cells through impairment of the INK4-cyclin D-CDK 4–8211; Rb pathway.

Checkpoint Control

A major role in checkpoint control is exerted by the ►p53 tumor suppressor pathway. In response to DNA damage (or other insults to the cell) p53 induces a number of genes that either invoke cell-cycle arrest (such as the CDI p21/CIP) or trigger apoptosis (Fig. 3). The activity and the steady-state level of p53 is regulated by ►MDM-2, a oncoprotein that associates with p53, inhibits its transcriptional activity and targets p53 for degradation by the proteasome. MDM-2 itself is targeted for proteolysis by the tumor suppressor ►p14ARF (or p19ARF in mice). The importance of this pathway is demonstrated by the fact that each of its components can be a target for genetic alterations in human tumors, and that a defective p53 pathway is found in more than 50% of all human malignancies. This emphasizes the relevance of p53 for the development of new anti-cancer therapies.



Cell-Cycle Targets for Cancer Therapy. Figure 3 Loss of p53 function in human cancer cells.

Another checkpoint activated in G2 in response to DNA damage is governed by the checkpoint kinase-1 (►CHK1; Fig. 4). CHK1 phosphorylates the CDC2 (CDK1) phosphatase CDC25C, which results in the association of CDC25C with a p53-induced specific isoform of 14–3–3. This renders CDC25C inactive, so that the cyclin B-CDC2 complex remains in its phosphorylated inactive form. As a consequence, progression into mitosis is prevented and DNA repair can occur. Since many anti-cancer drugs exert their function through DNA damage, this checkpoint may have a negative impact on their efficacy. An analogous checkpoint operating in G1 has recently been identified. This checkpoint is activated when the CDK2 phosphatase CDC25B is targeted for degradation in response to DNA-damage that will leave the cyclin E kinase in an inactive (phosphorylated) state. As a consequence, cell-cycle progression into S-phase is prevented.

Clinical Relevance

Cancer is clearly a proliferative disease resulting from deregulated cell-cycle progression. The inhibition of specific proteins driving the cell cycle is therefore an obvious strategy for the rational discovery of new anti-cancer drugs. In this context it is of particular interest that the interference with coordinated cell-cycle progression can result in apoptosis of tumor cells. This is exemplified by the observation that the deregulated expression of proteins, such as ►Myc or E2F-1, in conjunction with a non-physiological cell-cycle block is incompatible with cell survival. It has also been shown that the direct inhibition of CDKs, for example by CDIs or through ►antisense nucleic acid, can trigger programmed cell death in tumor cells. These and other findings have laid the foundation for the definition of a new class of anti-tumor agents that function through a direct inhibition of proteins driving the cell cycle.

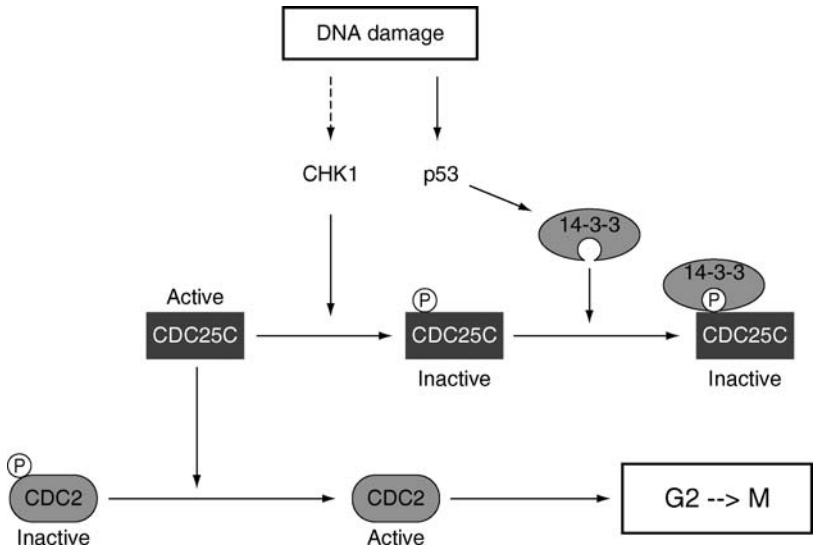
One of the prototypes of this class of compounds is the synthetic flavone ►Flavopiridol. Flavopiridol is a general inhibitor of CDKs, induces cell-cycle arrest and apoptosis, and is not influenced by many of the genetic alterations conferring resistance on human tumor cells. Accordingly, Flavopiridol has shown promising tumor responses in preclinical models and is currently undergoing clinical trials. Numerous other chemical CDK inhibitors have recently been identified and are currently being evaluated for their anti-tumor properties. It can be anticipated that CDK-inhibiting drugs will constitute a new class of powerful chemotherapeutics.

Other interesting targets for therapeutic intervention are the proteins governing checkpoint control, for instance in response to DNA damage. Checkpoint control can invoke a transient cell cycle block, but can also trigger apoptosis. Both types of checkpoints are of relevance to tumor therapy. While the functionality of an apoptosis-inducing mechanism in response to drug- or radiation-induced cellular damage is desirable, checkpoint control leading to cell-cycle arrest is counterproductive for any therapy that relies on cell proliferation, such as radiation or conventional therapy.

The p53 checkpoint is lost in many tumor cells, and thus the ability to undergo apoptosis in response to chemo- or radiotherapy. The restoration of this checkpoint could therefore sensitize many tumor cells to conventional therapies. Strategies along these lines involve the development of compounds that can reactivate mutant p53 or inhibit MDM-2, or the use of gene therapeutic approaches for the reintroduction of functional p53 genes.

Other drug-based strategies aim to improve the efficacy of existing therapies that rely on DNA-damage, such as radiation or DNA-damaging chemotherapy. A prime candidate in this context is the kinase ►CHK1 that regulates the G2 checkpoint (Fig. 4). First results obtained with an inhibitor of the G2 checkpoint, UCN-01, suggest that this may indeed be the case.

Numerous other mechanisms controlling cell-cycle progression have been discovered, and approaches for therapeutic intervention are being developed, pointing to the great potential of targeting the cell cycle for the development of new anti-cancer drugs. It can be anticipated that this new class of anti-cancer drugs will lead to a clear advance in clinical oncology.



Cell-Cycle Targets for Cancer Therapy. Figure 4 Regulation of the G2 checkpoint.

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Cell Differentiation

Definition

This is a concept from developmental biology describing the process by which cells acquire a "type". The morphology of a cell may change dramatically during differentiation, but the genetic material remains the same, with few exceptions.

A cell that is able to differentiate into many cell types is known as pluripotent. These cells are called stem cells in animals and meristematic cells in higher plants. A cell that is able to differentiate into all cell types is known as totipotent. In mammals, only the zygote and early embryonic cells are totipotent, while in plants, many differentiated cells can become totipotent with simple laboratory techniques.

►Orphan Nuclear Receptors and Cancer

Cell Division

Definition

Synonym cell proliferation; Is the process of cell doubling by which a cell, called the parent cell, divides into two cells, called *daughter cells*. Cell division is a physiological process that occurs in almost all tissues. However, a process of pathological cell division can be seen in cancers.

►Cell Cycle

Cell Fate

Definition

The ultimate differentiated state to which a cell has become committed.

►Polycomb Group

Cell-free Circulating Nucleic Acids

►Circulating Nucleic Acids

Cell Lines

Definition

Are cell populations with the feature of dividing indefinitely when growing in culture. There are tumor and non-tumor cell lines from different organisms including humans.

Cell Locomotion

►Migration

Cell-Mediated Immunity

Definition

Synonym Cell-mediated immune response describes any adaptive immune response in which antigen-specific T cells have the main role. It is defined operationally as adaptive immunity that cannot be transferred to a naïve recipient with serum antibody.

►Sjögren Syndrome

Cell Membrane

Definition

►Plasma membrane

Cell Migration

A highly complex process, regulated by multiple gene pathways enabling the motility of cells through the adhesion and invasion of extracellular matrices.

►Tissue Inhibitors Of Metalloproteinases (Timps)

Cell Motility

►Migration
►Motility

Cell Movement

►Motility

Cell Polarity

Definition

Cell direction or orientation to maintain the property of having two opposite poles, apical and basolateral domains.

►Tight Junction

Cell Scattering

Definition

A common tissue culture assay used to monitor ►Met receptor activation. When non-transformed dog kidney epithelial (MDKC) cells are grown in tissue culture, the cells spontaneously arrange themselves into tightly connected epithelial sheets. Following Met activation, these cell sheets breakdown and the individual cells migrate away from each other.

Cell Signaling

Definition

Synonym ►signal transduction, refers to the process by which a cell converts one type of stimulus into another using ordered sequences of biochemical reactions.

Cell-Surface Receptors

Definition

Are cell surface receptor is a cellular proteins embedded within the cell membrane that receive and respond to extracellular soluble ligands such as neurotransmitters, hormones, growth factors, or chemokines.

►CXC Chemokines

β-Cell Tumor of the Islets

►Insulinoma

Cellular Antigens

►CD Antigens

Cellular Atypia

Definition

Histological features associated with epithelial dysplasia, the degree of which is determined by the number of atypia present in the dysplastic lesion. Atypia include densely stained nuclei, pleomorphic nuclei, altered nucleus:cytoplasm ratio, aberrant mitosis, frequent mitosis, supra-basal mitosis, disorganized tissue architecture, de-differentiation, loss of cellular adhesion, loss of cellular polarity, keratinization within the deeper areas of the epithelium.

►Squamous Cell Carcinoma

Cellular Immortalization

Definition

The process by which cells cultured in vitro, or in the organism, escape from cellular senescence and grow forever. This can happen spontaneously, or can be caused by chemical carcinogens, oncogenic viruses, or radiations.

►Chemically Induced Cell Transformation
►Senescence and Immortalization

Cellular Immunity

Definition

Immune protection provided by the direct action of immune cells (as distinct from soluble molecules such as antibodies).

Cellular Self-Cannibalism

►Autophagy

Cellular Senescence

Definition

The process of programmed cell aging, by which cells die after a specific number of population doublings, usually 60 population doublings.

►Chemically Induced Cell Transformation
►Senescence and Immortalization

Cellular Transformation Assay

Definition

Cell biological test to demonstrate oncogenic activity of a candidate gene. Typically the gene in question is cloned into a mammalian expression vector, transfected into appropriate recipient cells and expressed. The transforming activity is detected on the basis of morphological alterations such as the loss of the typical fibroblastic or epithelial cell shape, anchorage independent proliferation, as determined in semi-solid agar medium, or tumor formation following injection of transfected cells into nude mice. The classical cellular transformation assays were done with donor DNA prepared from tumors and pre-neoplastic mouse NIH/3T3 cells as recipients.

►RAS Transformation Targets

CENP-E

Definition

Centromeric protein E; Is a kinetochore-associated kinesin-like motor protein that is responsible for chromosome movement and alignment in mitosis. In animal model, CENP-E deletion in mice causes early embryonic lethality, with embryos unable to implant or develop past implantation.

►Mitotic Arrest-Deficient Protein 1 (MAD1)

Censoring

Definition

Censoring, particularly in survival studies, occurs when the outcome of interest is not measured fully as, for example, when a trial is ended after a specified period of time so that failure times are not precisely measured.

►Kaplan–Meier Survival Analysis

Central Cleavage

Definition

Central cleavage refers to the symmetric cleavage of ►carotenoids at their central 15,15′ double bond by carotene 15,15′-oxygenase, a main pathway for vitamin A formation from provitamin A carotenoids.

Central Nervous System

Definition

The brain and spinal cord.

►Brain Tumors

Central Neurocytoma

►Neurocytoma

Central Neurofibromatosis

►Neurofibromatosis 2

Centrocytic (Mantle Cell) Lymphoma

►Mantle Cell Lymphoma

Centromere

Definition

Constricted portion of the chromosome. The centromere divides the chromosome into a short “p” and a

long "q" arm. The centromere is a region of chromosomes with a special DNA sequence and structure. The centromere plays a role in cellular division and it is the region where sister chromatids join after doubling the chromosomes during prophase and metaphase of mitosis.

►Micronucleus Assay

Centrosome

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Synonyms

Major microtubule organizing center; MTOC; Spindle pole body; SPB, in yeast

Definition

The centrosome is a nonmembranous organelle (1–2 μm in diameter) normally localized at the periphery of nucleus, and its primary function is to nucleate and anchor microtubules.

Characteristics

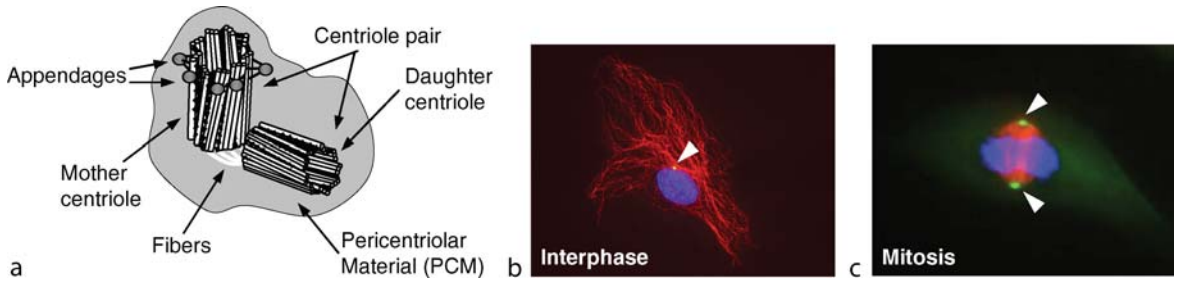
Structure and Function

The centrosome in mammalian cells consists of a pair of centrioles and the surrounding protein aggregates consisting of a number of different proteins (known as pericentriolar material; PCM). The centrioles in the pair structurally differ from each other; one with a set of appendages at the distal ends (mother centriole) and another without appendages (daughter centriole). These appendages are believed to be important for nucleating and anchoring microtubules. The daughter centriole acquires the appendages in late G2-phase of the cell cycle. As the primary function of the centrosome is to nucleate and anchor microtubules, centrosomes organize the cytoplasmic microtubule network during interphase, which is involved in vesicle transport, proper distribution of small organelles, and establishment of cell shape and polarity. In mitosis, centrosomes become the core structures of spindle poles and direct the formation of mitotic spindles. (Fig. 1).

Centrosome Duplication

Upon cytokinesis, each daughter cell receives only one centrosome. Thus, the centrosome, like DNA, must duplicate once prior to the next mitosis. In other words, cells have either one unduplicated or two duplicated centrosomes at any given time point during the cell cycle. Since DNA and centrosome are the only two organelles that undergo semiconservative duplication once in a single cell cycle, cells are equipped with a mechanism that coordinates these two events, likely to ensure these two organelles to duplicate once, and only once. In late G1/early S-phase, the centrosome initiates duplication by physical separation of the paired centrioles, which is followed by the formation of a procentriole in the proximity of each preexisting centriole. During S and G2, the procentrioles elongate and two centrosomes continue to mature by recruiting PCM. By late G2, two mature centrosomes are generated.

The coupling of the initiation of DNA and centrosome duplication is in part achieved by late G1-specific activation of cyclin-dependent kinase 2 (CDK2)/cyclin E. CDK2/cyclin E triggers initiation of both DNA synthesis and centrosome duplication. The activation of CDK2/cyclin E is controlled by the late G1-specific expression of cyclin E as well as the basal level expression of p53 and its transactivation target $p21^{Waf1/Cip1}$ (p21), a potent CDK inhibitor. Several



Centrosome. Figure 1 Structure and function of centrosomes. (a) The centrosome consists of a pair of centrioles and surrounding protein aggregates (PCM). (b and c) Mouse embryonic fibroblasts were immunostained for γ-tubulin (one of major centrosomal proteins, green – appearing in yellow) and α- and β-tubulin (primary constituents of microtubules, red). Cells were also counterstained for DNA with DAPI. Panel b: interphase cell, panel c: mitotic cell.

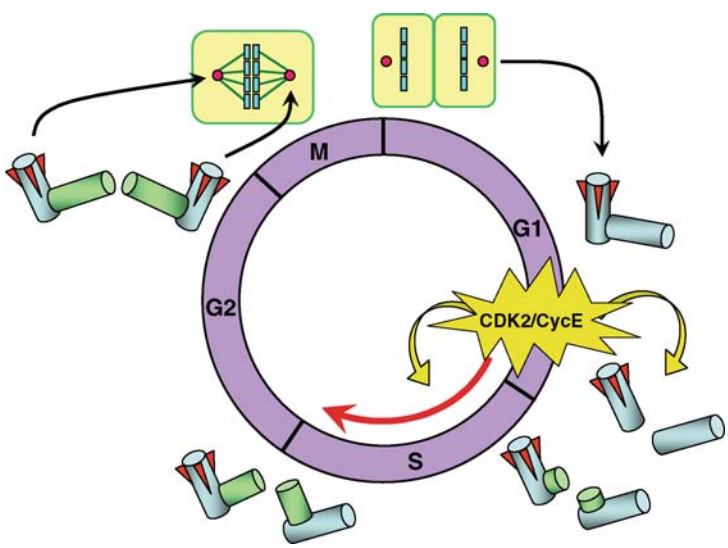
potential targets of CDK2/cyclin E for centrosome duplication have been identified, including nucleophosmin, Mps1 kinase, and CP110. For instance, nucleophosmin localizes between the paired centrioles, likely functioning in the pairing of the centrioles. CDK2/cyclin E-mediated phosphorylation promotes dissociation of nucleophosmin from the centriole pairs, leading to physical separation of the paired centrioles. (Fig. 2).

Abnormal Amplification of Centrosomes and Chromosome Instability in Cancer

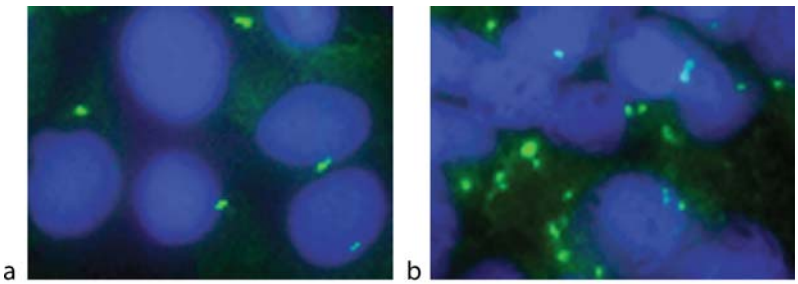
The presence of two centrosomes at mitosis ensures the formation of bipolar mitotic spindles. Since chromosomes are pulled toward each spindle pole, the bipolarity of mitotic spindles is essential for the accurate chromosome segregation into two daughter cells during cytokinesis. Abrogation of the regulation underlying the numeral homeostasis of centrosomes (i.e., regulation of centrosome duplication) results in abnormal amplification of centrosomes (presence of >2 centrosomes), which in turn increases the frequency of mitotic defects (i.e., formation of >2 spindle poles) and chromosome segregation errors/chromosome instability (see [1] for the full description of the mechanisms for generation of centrosome amplification). Chromosome instability has been recognized as a hallmark of cancer, and contributes to multistep carcinogenesis by facilitating the accumulation of genetic lesions required for acquisition of various malignant phenotypes. To date, a number of studies have shown that centrosome amplification is a frequent event in almost all types of solid tumors, including breast, bladder, brain, bone, liver, lung, colon, prostate, pancreas, ovary, testicle, cervix, gallbladder, bile duct, adrenal cortex, and head and neck squamous cell, to name a few. Centrosome amplification has also been observed in certain cases of leukemia and lymphoma. Many studies have also shown the strong association between the occurrence of centrosome amplification and a high degree of aneuploidy. Thus, centrosome amplification can be reasonably considered as a major contributing factor for chromosome instability in cancer. (Fig. 3).

Loss of Tumor Suppressor Proteins and Centrosome Amplification

In view of carcinogenesis, it is important to mention that loss or inactivating mutation of certain tumor suppressor proteins, most notably p53 and BRCA1, results in centrosome amplification. For both p53 and BRCA1, they were initially implicated in the control of centrosome duplication and numeral homeostasis of centrosomes by the observations that centrosome amplification and consequential mitotic aberrations were frequent in the embryonic fibroblasts (as well as various tissues) of p53-null mice as well as mice harboring BRCA1 mutation, which implies that destabilization of chromosomes due to centrosome amplification contributes to the cancer susceptibility



Centrosome. Figure 2 The centrosome/centriole duplication cycle. Late G1-specific activation of CDK2/cyclin E triggers initiation of both DNA and centrosome duplication. Centrosome duplication begins with the physical separation of the paired centrioles, which is followed by formation of procentrioles. During S- and G2-phases, procentrioles elongate, and two centrosomes progressively recruit PCM. In late G2, the daughter centriole of the parental pair acquires appendages (shown as red wedges), and two identical centrosomes are generated. During mitosis, two duplicated centrosomes form spindle poles, and direct the formation of bipolar mitotic spindles. Upon cytokinesis, each daughter cell receives one centrosome.



Centrosome. Figure 3 Representative immunostaining images of centrosome amplification in human cancer. The touch preparations of G3 tumor grade bladder cancer specimens and the adjacent normal bladder epithelium samples were subjected to immunostaining for γ-tubulin (centrosome, green) and counterstained for DNA with DAPI (blue). No centrosome amplification can be seen in normal bladder epithelium (a), while a high frequency of centrosome amplification in the G3 tumors (b).

phenotype associated with loss or mutational inactivation of p53 as well as BRCA1.

Centrosome Amplification and Cancer Chemotherapy

In cells inhibited for DNA synthesis (i.e., by exposure to DNA synthesis inhibitors such as aphidicolin (Aph) or hydroxyurea (HU)), centrosomes undergo multiple rounds of duplication in the absence of DNA synthesis, resulting in abnormal amplification of centrosomes. However, this phenomenon preferentially occurs when p53 is either mutated or lost. In the presence of wild-type p53, centrosome duplication is also blocked by exposure to DNA synthesis inhibitors; p53 is upregulated upon prolonged exposure to Aph or HU, leading to transactivation of p21, which in turn blocks the initiation of centrosome duplication via continuous inhibition of CDK2/cyclin E. In contrast, in cells lacking p53, p21 fails to be upregulated in response to the cellular stress imposed by DNA synthesis inhibitors, allowing "accidental" activation of CDK2/cyclin E, which triggers initiation of centrosome duplication. Considering the high frequency of p53 mutation in human cancer, it is important to address the effect of commonly used anticancer drugs targeting S-phase (DNA replication) on centrosomes. When p53-null cells were exposed to subtoxic concentrations of the S-phase targeting chemotherapeutic agents (i.e., 5′-fluorouracil, arabinoside-C), centrosome amplification was efficiently induced. Moreover, after removal of drugs, these cells resumed cell cycling, and suffered dramatic destabilization of chromosomes. This finding may be significant in the context of cancer chemotherapy using the S-phase targeting drugs. During chemotherapy, not all cells in tumors receive a maximal dose of drugs – such cells may not be killed, but only arrested for cell cycling. If these cells harbor p53 mutations, centrosome amplification occurs during the drug-induced cell cycle-arrest. Upon cessation of chemotherapy, these cells resume cell cycling in the presence of amplified centrosomes, and suffer significant mitotic aberrations and chromosome instability, which increases the risk of acquiring further malignant phenotypes. This may in part explain why the recurrent tumors after chemotherapy are often found to be more malignant than the original tumors. Many S-phase targeting anticancer drugs have been found to be effective, and there is no doubt that DNA duplication should be one of the major targets for future development of more effective anticancer drugs. However, the possibility that the S-phase targeting drugs may exacerbate a chromosome instability phenotype by inducing centrosome amplification should be taken into consideration.

Another important issue to be addressed is the concept of centrosome duplication as a target of cancer chemotherapy. Like DNA replication, centrosome duplication occurs only in proliferating cells. Inhibition of centrosome duplication will not only suppress centrosome amplification and chromosome instability, but also block cell division and possibly induce cell death – cells with one centrosome fail to form bipolar mitotic spindles, and are often undergo cell death. Moreover, in contrast to genotoxic drugs which impose an increased rate of secondary mutations through interfering with DNA metabolisms, such side effects will likely be minimal in the protocol designed to block centrosome duplication.

►Genomic Imbalance
►Microtubule-Associated Proteins

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Ceramide

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Definition

Ceramide belongs to the group of sphingolipids and is constituted by the amide ester of the sphingoid base D-erythro-sphingosine and a fatty acid of C_{16} through C_{32} chain length. At present, the differential biological function of different ceramide species is unknown and, thus, the term ceramide is used collectively to represent all long chain ceramide molecules.

Characteristics

Formation of Ceramide

Ceramide molecules are very hydrophobic and exclusively present in membranes. Sphingomyelin, the choline-ester of ceramide is hydrolyzed by acid, neutral and alkaline sphingomyelinases to release ceramide. Ceramide is also de novo synthesized via a pathway involving the serine-palmitoyl-CoA transferase. Under some circumstances ceramide can be also formed from sphingosine by a reverse activity of the acid ceramidase.

Ceramide-Induced Changes of Biological Membranes

The formation of ceramide within biological membranes results in a dramatic change of the biophysical properties of the lipid bilayer. Ceramide molecules have the tendency to self-associate and to form small ceramide-enriched membrane microdomains. These membrane microdomains spontaneously fuse to large ceramide-enriched membrane macrodomains that constitute a very hydrophobic and stable membrane domain. Furthermore, ceramide molecules seem to compete with and displace cholesterol from membrane domains. Ceramide-enriched membrane platforms serve to re-organize and cluster/aggregate receptor molecules in the membrane resulting in a very high density of receptors within a small area of the cell membrane. At least for some receptors the transmembranous domain of the receptor determines its preferential partitioning in ceramide-enriched membrane platforms.

Ceramide-enriched membrane macrodomains are also involved in the recruitment or exclusion, respectively, of intracellular signaling molecules that mediate transmission of signals into the cell via a particular receptor. In general, clustering of receptors in ceramide-enriched membrane domains serves to amplify a weak primary signal. For instance, it was shown that ceramide-enriched membrane platforms amplify CD95 signaling ~100-fold.

Ceramide in Receptor-Mediated Signaling

Death receptors, in particular CD95 or DR5, activate the acid sphingomyelinase and trigger the translocation of the enzyme onto the extracellular leaflet of the cell membrane. Translocation of the acid sphingomyelinase onto the extracellular leaflet of the cell membrane may occur by fusion of intracellular vesicles that are mobilized upon receptor stimulation with the cell membrane. Surface exposure and stimulation of the acid sphingomyelinase results in very rapid release of ceramide in the cell membrane. Ceramide forms membrane platforms and mediates clustering of the death receptors, which is required for the induction of cell death via these receptors (Fig. 1).

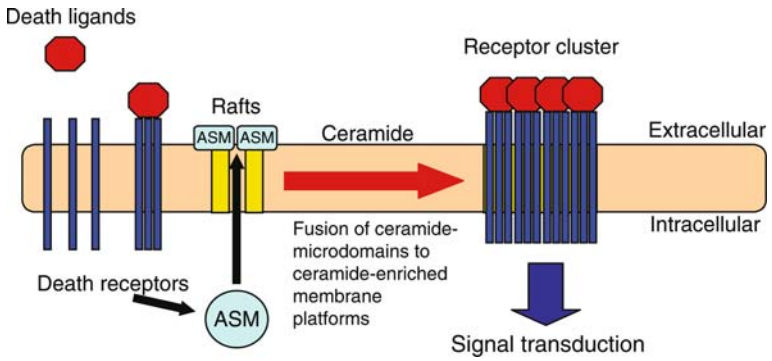
However, ceramide is not only involved in the mediation of apoptotic stimuli, but also many other stimuli trigger the release of ceramide including CD40, CD20, FcγRII, CD5, LFA-1, CD28, TNFα, Interleukin-1 receptor, PAF-receptor, infection with *P. aeruginosa*, *S. aureus*, *N. gonorrhoeae,* Sindbis-Virus, Rhinovirus, γ-irradiation, UV-light, doxorubicin, cisplatin, gemcitabine, disruption of integrin-signaling and some conditions of developmental death.

Signaling Molecules Regulated by Ceramide

Ceramide interacts with and activates phospholipase A_2, kinase suppressor of Ras (KSR; identical to ceramide-activated protein kinase), ceramide-activated protein serine-threonine phosphatases, protein kinase C isoforms and c-Raf-1. Furthermore, ceramide inhibits the potassium channel Kv1.3 and calcium release activated calcium (CRAC) channels. Lysosomal ceramide specifically binds to and activates cathepsin D resulting in translocation of cathepsin D into the cytoplasm and induction of cell death via the pro-apoptotic proteins Bid, Bax and Bak.

Ceramide in Mitochondria and Cell Death

Besides a function of ceramide in the plasma membrane and lysosomes for the induction of cell death, ceramide is also generated in mitochondria via the de novo synthesis pathway, a reverse activity of the ceramidase and/or activity of the acid sphingomyelinase. Although at present the function of ceramide in the mediation of mitochondrial pro-apoptotic events is poorly defined,



Ceramide. Figure 1 Receptors cluster in ceramide-enriched membrane domain to transmit signals into cells. The interaction of a ligand with its receptor results in translocation of the acid sphingomyelinase onto the extracellular leaflet and a concomitant release of ceramide. Ceramide spontaneously forms ceramide-enriched microdomains that fuse to large ceramide-enriched macrodomains. These domains trap activated receptor molecules finally resulting in clustering of many receptor molecules within a small area of the cell membrane. The high density of receptor molecules and associated intracellular molecules amplifies the primarily weak signal, permits the generation of a strong signal and, thus, efficient transmission of the signal into the cell. Modified from A. Carpinteiro et al. Cancer Letters.

it was suggested that C_{16}-ceramide molecules form large channels in mitochondrial membranes that may permit the exit of cytochrome c from mitochondria to execute death.

Genetic Evidence for a Function of Ceramide in Apoptosis

The role of the acid sphingomyelinase and ceramide for CD95 and DR5-triggered apoptosis was evidenced by studies on acid sphingomyelinase-deficient cells or mice, respectively, that revealed a resistance of these cells to CD95- and DR5-triggered apoptosis, but also γ-irradiation- and UV-light- or *P. aeruginosa*-triggered cell death.

Ceramide in γ-Irradiation- and UV-A Light-Induced Apoptosis

The acid sphingomyelinase and ceramide are critically involved in the response of cells to γ-irradiation. Animals or cells lacking the acid sphingomyelinase are resistant to γ-irradiation-induced cell death. In particular, endothelial cells in acid sphingomyelinase-deficient mice are resistant to γ-irradiation.

Ceramide also plays a critical role for UV-light induced apoptosis. UV-A and UV-C light activate the acid sphingomyelinase, trigger the release of ceramide and the formation of large ceramide-enriched membrane domains in the cell membrane to initiate.

Ceramide and Chemotherapy

In addition to a central role of ceramide in γ-irradiation-induced cell death, ceramide is also critically involved in the induction of cell death by at least some chemotherapeutic drugs. Thus, doxorubicin-, cisplatin- und gemcitabine-induced cell death of malignant and non-malignant cells requires expression of the acid sphingomyelinase, release of ceramide and/or the formation of ceramide-enriched membrane platforms to trigger death. Rituximab, an anti-CD20 antibody, requires expression of the acid sphingomyelinase and the generation of ceramide to kill leukemic cells.

Short Chain Ceramide

Short chain ceramide molecules composed of a fatty acid chain with C_2 through C_{12} length are water-soluble and, thus, very much differ from endogenous long ceramide molecules (C_{16}-C_{32}). However, they are very efficient reagents to kill tumor cells in vitro. Cationic pyridinium-ceramides seem to accumulate in mitochondria of tumor cells and may, thus, serve as a new class of anti-tumor reagents, although at present no convincing concepts are available to selectively target tumor cells in vivo by and to avoid effects of short chain ceramide on normal cells.

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Ceramide Kinase (CerK)

Definition

Ceramide kinase (CerK) has important roles in leukocyte functions, including the role in degranulation of mast cells and the phagocytosis of polymorphonuclear leukocytes.

►Ceramide

Ceramide-1-Phosphate (C1P)

Definition

Ceramide-1-phosphate (C1P) is a phosphorylated form of ►ceramide and possesses antitumor properties.

►Lipid Mediators

C-erb-B2

►HER-2/neu

Cerebral Edema

Definition

Is the accumulation of fluid in the brain, often as a result of a pathological condition.

►Convection Enhanced Delivery (CED)

Cerulenin

Definition

An antifungal antibiotic isolated from several species, including Cephalosporium, Acrocylindrum, and Helicoceras. It inhibits the biosynthesis of fatty acid by irreversibly binding to the active site cysteine thiol in the β-ketoacyl-synthase domain of fatty acid synthase.

►Fatty Acid Synthase

Cervical

Definition

Pertaining to the neck.

Cervical Cancers

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Definition

The regions of the uterus are the corpus and the cervix. Cancer originating from the cervix is defined as cancer of the cervix. When cancers are simultaneously detected in the cervix and corpus, squamous cell carcinoma (SCC) is designated as a cancer of the cervix and adenocarcinoma is designated as a cancer of the corpus. When cancer occupies both the cervix and vagina without the junctional area (the fornix), the cancer extending to the exocervix is recognized as a cancer of the cervix. Thus, cervical cancer is defined apart from cancer of the uterine corpus (cancer of the uterine endometrium) and cancer of the vagina.

Characteristics

The main gynecological cancers originate from the cervix, endometrium, and ovary. Among them, cervical cancer is the most common malignancy in women.

Main risk factors are

- Young age at first intercourse, especially shortly after the menarche
- High number of sexual partners
- High number of sexual partners of the partner
- High number of children
- Excessive douching

Smoking appears to increase the incidence of SCC, but not of adenocarcinoma or adenosquamous carcinoma. Immunosuppression by smoke-derived nicotine and its metabolite cotinine in the cervical mucus may enhance

the effects of sexually transmitted disease (STD) including human papillomavirus (HPV) infection. Most epidemiological risk factors for cervical cancer are associated with STDs. HPV induces an STD, human venereal condyloma, which is associated with cervical, vaginal and vulvar dysplasia, and invasive carcinomas. HPV particles and DNA, especially HPV-16, HPV-18, and HPV-33, are detected in cervical and vulvar dysplasia and in invasive carcinomas. Additionally, it has been demonstrated that HPV transforms human cell lines. HPV infection of the cervix is a main etiology of cervical cancer.

Symptoms

Main symptoms of cervical cancer are

- Vaginal bleeding, which may be recognized as postmenopausal bleeding, irregular menses, or postcoital bleeding
- Abnormal vaginal (watery, purulent or mucoid) discharge

In advanced cases, corresponding local symptoms occur. A Pap smear even in unsymptomatic cases is useful for the early detection of cervical dysplasia and cancers. Among women over the age of 18 who have had sexual intercourse, high-risk women should be screened at least yearly.

Pathology

Histopathological types in cervical cancers are mainly SCC and adenocarcinoma, which account for about 90% of all cervical cancers (adenosquamous carcinoma, glassy cell carcinoma, adenoid cystic carcinoma, adenoid basal carcinoma, carcinoid, small cell carcinoma, and undifferentiated carcinoma also occur). SCCs are keratinizing or nonkeratinizing in most cases and may be verrucous, condylomatous, papillary, or lymphoepithelioma-like carcinomas in a few cases. Adenocarcinomas are classified into mucinous, endometrioid, clear cell, serous, and mesonephric adenocarcinomas; mucinous adenocarcinomas are subclassified with endocervical type into adenoma malignum and villoglandular papillary adenocarcinoma, and intestinal type adenocarcinoma.

Staging

Clinical staging represents the degree of advancement of the tumor, and is defined by the FIGO classification established in 1994 and by the TNM classification of malignant tumors set by the UICC in 1997 as follows (classified by FIGO [TNM]):

- Stage 0 (Tis): carcinoma in situ (preinvasive carcinoma).
- Stage I (T1): cervical carcinoma confined to the uterus.
- Stage II (T2): tumor invades beyond the uterus but not to the pelvic wall or to the lower third of the vagina.
- Stage III (T3): tumor extends to the pelvic wall and/or involves the lower third of the vagina and/or causes hydronephrosis or nonfunctioning kidney.
- Stage IVA (T4): tumor invades the mucosa of the bladder or rectum and/or extends beyond the true pelvis.
- Stage IVA (Ml): distant metastasis.

Stage IA (TIa) has been further classified by microinvasive depth and width into stage IA1 (Tlal) (depth of stromal invasion ≤3 mm, horizontal spread ≤7 mm) and stage IA2 (Tla2) (depth of stromal invasion >3 mm, ≤5 mm; horizontal spread ≤7 mm). Stage IB (Tlb) has been further classified by tumor size into stage IB1 (Tlbl) (greatest dimension ≤4 cm) and stage IB2 (Tlb2) (greatest dimension >4 cm). In cases staged IA2 (Tla2) or less advanced, colposcopically directed biopsy in the transformation zone of the cervix, endocervical curettage or cervical conization are required.

Prognosis

Unfavorable prognostic factors include younger age, advanced clinical stage, certain histopathological types, vessel permeation, large tumor volume, parametrium involvement, and lymph node metastasis. Nodal metastasis is an especially critical prognostic factor after curative resection. Vascular endothelial growth factor (VEGF)-C and osteopontin contribute to the aggressive lymphangitic metastasis in uterine cervical cancers. Platelet-derived endothelial cell growth factor (PD-ECGF) contributes to the advancement of metastatic lesions as an agiogenic factors. PD-ECGF, VEGF-C, and osteopontin levels in metastatic lesions are prognostic indicators. Furthermore, serum PD-ECGF level reflects the status of advancement of cervical cancers and is recognized as a novel tumor marker for both SCC and adenocarcinoma of the cervix, while the tumor marker SCC is well known only as an indicator for SCC of the cervix. VEGF-C and osteopontin contribute to the aggressive lymphangitic metastasis in uterine cervical cancers.

Therapy

The treatment for cervical cancer consists mainly of surgery and radiation. Chemotherapy is performed in combination with surgery and/or radiation for advanced cases, and immunotherapy is an adjuvant treatment for surgery, radiation, and chemotherapy. The standard treatment for carcinoma in situ is cervical conization or total hysterectomy. The standard treatment for microinvasive carcinoma stage IA (Tla) is modified radical hysterectomy regardless of regional lymphadenectomy. The standard surgical treatment for

invasive carcinoma is radical hysterectomy with regional lymphadenectomy. Although oophorectomy can be avoided in some cases during the reproductive period, ovarian metastasis must be considered especially in adenocarcinoma of the cervix. When ooporectomy is avoided, the ovary is better shifted out of radiation area. For patients who undergo oophorectomy, hormone replacement therapy can be useful. In more advanced cases, extended radical hysterectomy or pelvic exenteration is appropriate. After surgery external irradiation is followed in some cases. The standard radiotherapy without surgery for invasive carcinoma is intra-cavitary and/or external irradiation. Recently, neoadjuvant therapy (chemotherapy) has been tried in order to make surgery more successful, and concurrent radio-chemotherapy has been tested for the purpose of enhancing the effect of radiation.

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Cetuximab

Definition

Is a chimeric IgG1κ monoclonal antibody specifically binding to epidermal growth factor receptor (EGFR).

Epidermal growth factor receptor (EGFR) (EGFR; ErbB-1; HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. Ligands which induce activation of EGFR are epidermal growth factor and transforming growth factor α, for example. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer. EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity resulting in activation of several signal transduction cascades which lead to DNA-synthesis and cell proliferation. EGFR mutations can lead to EGFR overexpression or overactivity and consequently result in uncontrolled cell division. Mutations of EGFR have been identified in several types of cancer, such as lung cancer and colorectal cancer.

Cetuximab is approved for treatment of EGFR-positive, irinotecan-refractory ►metastatic colon cancer.

►Monoclonal Antibody Therapy
►Epidermal Growth Factor Receptor Inhibitors

c-FLICE-like Inhibitory Protein

Definition

c-FLIP, also known as FLAME-1/I-FLICE/CASPER/CASH/MRIT/CLARP/Usurpin, is a death-effector-domain (DED)-containing protein that exists in three different isoforms, $FLIP_L$, $FLIP_S$, and $FLIP_R$. All c-FLIP isoforms contain two N-terminal DEDs, whereas only $FLIP_L$ also harbors a C-terminal part of catalytically inactive caspase-like domains homologous to caspase-8. $FLIP_L$, $FLIP_S$, and $FLIP_R$ function as inhibitors of apoptosis by blocking caspase-8 activation at the death-inducing signaling complex (DISC). In addition, $FLIP_L$ may promote ►apoptosis at low expression levels by facilitating autocatalytic activation of procaspase-8 and is also involved in nonapoptotic pathways, e.g., NF-κB or MAPK signaling.

►c-FLIP
►Usurpin
►Caspase-8

c-FLIP

Definition

►c-FLICE-like Inhibitory Protein

CG

►Cancer Germline Antigens

cGMP

Definition
Cyclic guanosine monophosphate.

►Nitric Oxide

CGP57148

►STI-571
►Imatinib

ζ Chain

Definition
A transmembrane protein associated with key functional receptors of the immune system, the T cell antigen receptor (TCR) and NK killing receptors (NKP30, NKP46 and CD16). The ζ chain has a key role in receptor assembly, expression and signaling function. Down-regulation of ζ chain expression associated with immunosuppression has been shown in various chronic pathologies characterized by chronic inflammation, including cancer, autoimmune, and infectious diseases.

►Inflammation

Channels

Definition
Channels are pores in biological membranes that have a rather limited specificity. Substance flow through channels is controlled by the channels' open probability so that high transport rates of 10^7–10^9 molecules per second can be achieved.

►Membrane Transporters

Chaperone

Definition
Molecular chaperones are proteins that aid the proper folding and assembly of proteins incompletely folded under conditions of cellular stress or protein synthesis. Molecular chaperones include small Hsp, Hsp40, Hsp60, Hsp70, Hsp90, Hsp100, the calnexin/calreticulin families, and so forth.

►Calreticulin
►Dioxin
►Methylation-Controlled J Protein (MCJ)
►Autophagy

Chaperonins

Definition
A family of conserved ►chaperone proteins which have a characteristic multi-subunit ring structure. They function by enclosing a nascent protein and preventing its non-specific aggregation during assembly.

►Molecular Chaperones

Charged Particle Therapy

►Proton Beam Therapy

Checkpoint

Definition
Checkpoints represent intrinsic mechanisms that are activated when cell-cycle progression would be detrimental to the cell, as in case of DNA-damage, incomplete DNA synthesis, metabolic dysfunctions or mitotic spindle damage. In such cases, cell-cycle progression is transiently halted until the respective problem is fixed, for instance by the DNA repair

machinery. Major checkpoints are governed by the tumor suppressor gene product p53 or the ►check point kinases CHK1 and CHK2. Control mechanism that ensures that the next step in the cell cycle does not proceed until a series of preconditions have been fulfilled including the completion of all previous steps; this is particularly true for all pathways associated with DNA replication and chromosome formation; impaired chromosome checkpoints can result in chromosomal.

►Cell-Cycle Targets for Cancer Therapy
►Fragile Histidine Triad
►Chromosomal instability
►Aneuploidy

Checkpoint Kinases

Definition

Checkpoint kinases are a group of at least four protein kinases (ATM, ATR, CHK1 and CHK2) and their relatives, which play an important role in the mechanisms that sense and signal DNA damage, culminating in the activation of cell-cycle checkpoints and DNA repair.

►Checkpoint
►Cell-Cycle Checkpoint

Chelation Therapy

►Chelators as Anti-Cancer Drugs

Chelator

Definition

A chemical or drug capable of binding metal ions.

►Chelators as Anticancer Drugs

Chelators as Anti-Cancer Drugs

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Synonyms

Chelation therapy

Definition

Iron is an element fundamental for life. Many vital cellular processes such as energy metabolism and DNA synthesis consist of reactions that require catalysis by iron-containing proteins. These proteins include ►cytochromes, and ►ribonucleotide reductase (RR). The latter is more significant in the context of cellular proliferation due to its role in catalyzing the rate-limiting step of DNA synthesis. Ultimately, the importance of iron is highlighted by the fact that iron-deprivation leads to G_1/S ►cell cycle arrest and ►apoptosis. Cancer cells in particular, have a higher iron requirement because of their rapid rate of proliferation. In order to satisfy their iron requirement, some cancer cells have altered iron metabolism. In addition, iron ►chelators also demonstrate the ability to inhibit growth of aggressive tumors such as ►neuroblastoma. For these reasons, iron-deprivation through iron chelation is seen as an exploitable therapeutic strategy for the treatment of cancer.

Characteristics

Iron Metabolism in Cancer Cells

In order to attain more iron, cancer cells have higher numbers of the transferrin receptor-1 molecule (TfR1) on their cell surface. The TfR1 binds the serum iron transport protein, transferrin (Tf). Hence, cancer cells are able to bind more Tf, and thus, take up iron at a greater rate than their normal counterparts. This is reflected by the ability of tumors to be radiolocalized using a radioisotope of gallium, ^{67}Ga, which binds to the iron-binding site on Tf for delivery *via* TfR1. ^{67}Ga can bind to iron-binding sites of Tf due to the similar atomic properties between gallium(III) and iron (III). Additionally, gene therapy by administration of anti-sense TfR1 targeted to the sequences of *TfR1* mRNA also showed selective anti-cancer activity, further demonstrating the importance of TfR1 in mediating cancer cell growth.

Apart from TfR1 up-regulation, the expression of the iron-storage protein ferritin is also often altered in neoplastic cells, especially neuroblastoma (NB) and breast carcinoma. In childhood NB, serum ferritin levels are elevated at stages III and IV of the disease.

In a longitudinal study, it was found that the elevated level was associated with a markedly poorer prognosis of the disease. In addition, serum ferritin levels also exceeded the normal limit in ►hepatocellular carcinoma and were found to be directly related to axillary lymph node status, presence of metastatic disease (►metastasis) and clinical stages of breast cancer.

Desferrioxamine, an Iron Chelator with Some Anti-Cancer Activity

Desferrioxamine (DFO) is a natural ligand secreted by the bacterium *Streptomyces pilosus* to selectively sequester iron for biological use (Fig. 1). DFO is used clinically for the treatment of iron overload disorders such as the transfusion-related iron overload in β-thalassemia.

DFO is active against aggressive tumors including NB and leukemia in cell culture and clinical trials. The cytotoxicity of DFO *in vitro* was prevented by co-incubation of the cells with iron or iron saturated DFO, indicating that its anti-proliferative activity was due to depletion of cellular iron. Furthermore, DFO induces a block in cell cycle progression. Therefore, it was proposed that the mechanism of action of DFO involved the depletion of cellular iron, leading to the inhibition of ribonucleotide reductase for DNA synthesis and cell cycle arrest. In human NB cells, 5 days of exposure to DFO resulted in approximately 90% cell death. In contrast, the effect of DFO was minimal on non-NB cells, suggesting that it had selective anti-NB activity. A clinical trial showed that seven of nine NB patients had up to 50% reduction in bone marrow infiltration after a course of DFO administered for 5 days. Other clinical trials using DFO as a single agent and in combination with other chemotherapeutic drugs confirmed the anti-cancer potential of this chelator. However, in some animal studies and clinical trials, DFO was found to exhibit limited or no activity.

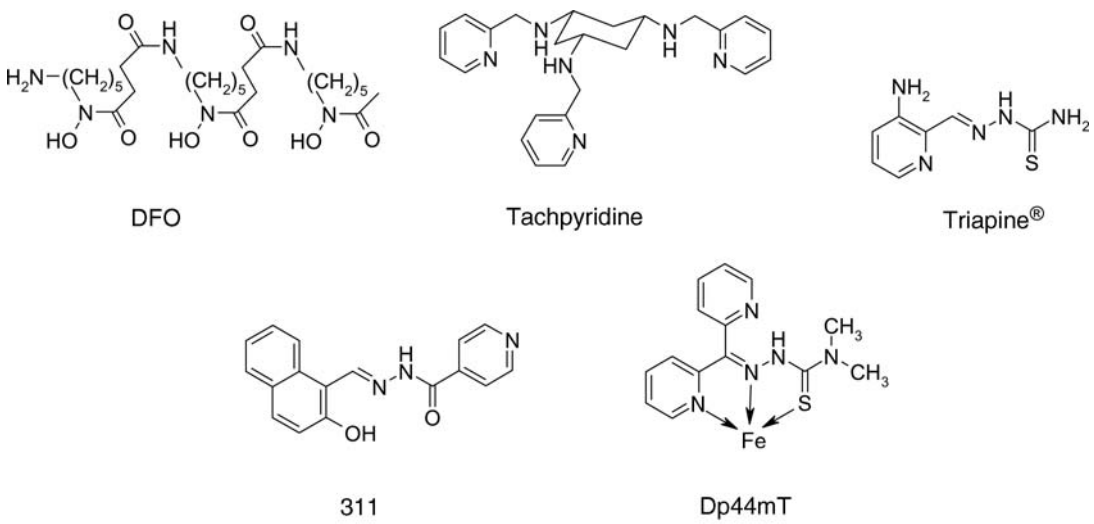
DFO also suffers a number of limitations as a result of its highly hydrophilic nature. It has poor gastrointestinal absorption and a short plasma half-life of about 12 min due to rapid metabolism. As a result, DFO is not orally active and needs to be administered via subcutaneous infusion for prolonged periods ranging from 8 to 12 h for five to seven times per week. The prolonged infusion results in pain and swelling, which consequently leads to poor patient compliance. DFO is also expensive to produce.

Despite these limitations and mixed results in clinical trials, DFO nonetheless provides "proof of principle" that iron chelation therapy may be specific and useful for cancer treatment.

Other Chelators with Anti-Cancer Potential

The limitations of DFO as an anti-cancer agent have encouraged the search for other active iron-chelating drugs against cancer.

Other experimental iron chelators include Triapine® (3-AP; Fig. 1), an iron-binding thiosemicarbazone-based drug currently in clinical trials for cancer therapy. Triapine® is a chelator that binds iron via a sulfur and two nitrogen donor atoms, and is suggested to be one



Chelators as Anti-Cancer Drugs. Figure 1 Chemical structures of the iron chelators desferrioxamine (DFO), *N,N′,N″*-tris(2-pyridylmethyl)-*cis,cis*-1,3,5-triaminocyclohexane (tachpyridine or tachpyr), 3-aminopyridine-2-carboxaldehyde-thiosemicarbazone (3-AP or Triapine®), 2-hydroxy-1-napthaldehyde isonicotinoyl hydrazone (311) and di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT) showing coordination to iron (Fe) through pyridyl nitrogen, aldimine nitrogen and thionyl sulfur donor atoms.

of the most potent inhibitors of RR yet identified. In clinical trials, high doses of Triapine® (160 mg/m^2/day) resulted in dose-limiting toxicities, including reduction in white blood cells, jaundice, nausea and vomiting. Lower doses of Triapine® administered as a 96-h iv infusion at 120 mg/m^2/day every 2 weeks was found to be well tolerated. In clinical trials with patients with advanced cancer, Triapine® was combined with the cytotoxic cancer drug gemcitabine, which also targets DNA synthesis. Of the 22 patients examined after treatment with gemcitabine and Triapine®, three were observed to have an objective response, and one patient had evidence of tumor reduction. In this trial, Triapine® was suggested to cause oxidation of hemoglobin to methemoglobin. This may have led to or contributed to the hypoxia, acute hypotension, and electrocardiogram changes in patients receiving this chelator. An asymptomatic myocardial infarction was also observed in one individual administered Triapine® and this may also be related to its oxidative effects. Triapine® continues to be examined in clinical trials, particularly in combination with standard chemotherapy drugs. However, these deleterious effects must be considered when designing future studies with compounds of this class.

Tachpyridine, (or tachpyr; Fig. 1) is a novel chelator based upon the framework of the triamine *cis,cis*-1,3,5-triaminocyclohexane. Tachpyridine is cytotoxic to cultured bladder cancer cells with an activity approximately fifteen times greater than that of DFO. Although tachpyridine has the potential to chelate a number of metals, including calcium(II), magnesium (II), manganese(II), copper(II) and zinc(II), toxicity studies on tachypyridine complexes suggest that iron and zinc depletion mediates its cytotoxic effects.

Similar to Triapine®, Tachpyridine induces apoptotic cell death independent of functional p53 (see Iron Chelation and Cell Cycle Control Molecules, below) (►p53 gene family). In addition, tachpyridine-iron complexes produce toxic free-radicals (►reactive oxygen species), which was also thought to contribute to its anti-tumor activity.

Tachpyridine arrests cells at the G_2 phase, whereas the majority of iron chelators arrest cells at the G_1/S phase due to inhibition of ribonucleotide reductase. The G_2 phase stage of the cell cycle is particularly sensitive to the effects of radiation. Ionizing radiation increases the sensitivity of tumor cells to the action of tachpyridine. Currently, tachpyridine is in preclinical development with the National Cancer Institute, USA.

PIH Chelators

The most comprehensively assessed alternate chelators for cancer treatment are the pyridoxal isonicotinoyl hydrazone (PIH) analogues. This class of chelators bind iron through the carbonyl oxygen, imine nitrogen, and phenolic oxygen (Fig. 1).

Originally conceived for the treatment of iron overload disorders, several chelators of the PIH class were found to inhibit the growth of cancer cells. In fact, the chelator 311 (Fig. 1) was found to be highly active against a range of cancer cells. These compounds also showed marked ability to remove Fe from cells and prevent cellular Fe uptake from transferrin. The marked anti-cancer activity of chelator 311 was attributed to its relatively high ►lipophilicity, which facilitates entry into the cell. Indeed, a general trend observed with the PIH analogues was that anti-cancer activity increased as the chelator became more lipophilic. Mechanistically, PIH analogues have multiple modes of anti-cancer activity, aside from chelation of iron and inhibition of ribonucleotide reductase. Some members of the PIH class of chelators (e.g. DpT chelators, see below) increase the generation of toxic free-radicals (reactive oxygen species) in cancer cells and affect the expression of cell-cycle control molecules (see Iron Chelation and Cell Cycle Control Molecules, below). Additional studies with 311 have also shown that it can markedly induce the expression of the metastasis suppressor protein, ►Drg-1 in tumor cells. The Drg-1 protein is known to play a critical role in suppressing tumor growth and metastasis. Hence, induction of Drg-1 by potent iron chelators such as 311 may significantly contribute to the anti-cancer activity of these analogues.

The DpT Chelators: Dp44mT

The DpT class of chelators are structurally-related to PIH analogues, but feature a sulfur donor atom instead of the hydrazone oxygen donor atom (Fig. 1). The chelator Dp44mT has recently been shown to be the most effective of the DpT series of ligands in terms of anti-cancer activity. It acts with selectivity against tumor cells and has much less effect on the growth of normal cells. Dp44mT also showed high iron chelation efficacy and prevented cellular uptake of iron from iron-labeled Tf. Another mechanism of its action involves the generation of toxic free-radicals (reactive oxygen species) when Dp44mT interacts with cellular iron pools.

Initially, *in vivo* studies of Dp44mT in mice bearing chemotherapy-resistant M109 lung carcinoma showed a reduction in the size of the tumor by 53% after 5-days of treatment. A later investigation also found marked inhibition of the growth of human lung, neuroepithelioma and melanoma xenografts growing in mice. In fact, a 7-week administration of Dp44mT in mice bearing human melanoma xenografts resulted in the decrease of tumor growth to 8% of that in untreated control mice. At the dose given, no hematological abnormalities were detected, although at a higher dose, myocardial fibrosis was identified. This side effect at a high dose may be due to the marked redox activity of the Dp44mT-iron complex. However, at a lower dose

Dp44mT was well tolerated with no hematological abnormalities and less cardiotoxicity. Other studies with Dp44mT showed that it also markedly increased the expression of the metastasis suppressor protein, Drg-1 in tumor cells. Induction of Drg-1 could potentially be a very significant component of the anti-cancer mechanism of Dp44mT. Further development of DpT series chelators is currently underway.

Iron Chelation and Cell Cycle Control Molecules

Iron-deprivation generally leads to G_1/S phase cell cycle arrest as a result of inhibition of ribonucleotide reductase. This has prompted many studies assessing the effect of iron chelation by DFO and chelator 311 on the expression of many cell cycle control molecules, namely, cyclins, ►cyclin dependent kinases (cdks), cdk inhibitors and p53 (p53 gene family). Consistently, these studies found that iron chelation markedly decreased the expression of ►cyclin D (D1, D2 and D3), and to a lesser extent cyclin A and B. The expression of cdk2 and cdk1, but not cdk4, were also decreased upon iron chelation. These effects were dependent on iron-deprivation, as iron-chelator complexes were unable to induce such effects.

Cyclins D, E, A and cdks 2, 4 and 6 are involved in progression through the G_1 phase, although cyclin E, A and cdk2 are also involved in S phase progression. The formation of the cyclin A-cdk2 complex is essential for G_1/S progression. Cyclin B and cdk1 on the other hand, are important for mitosis. During the G_1 phase, cyclin D and E bind to cdk4 and cdk2 respectively to phosphorylate (►phosphorylation) the ►retinoblastoma protein (pRb) (►Biological and Clinical Functions). This results in the release of molecules such as the ►E2F transcription factor from pRb that promotes the expression of genes for S phase. The decrease in the expression of these cyclins upon iron chelation causes hypophosphorylation of pRb, which in turn leads to the G_1/S phase arrest.

In addition to cyclins and cdks, iron chelation also affects the expression of cell cycle modulatory molecules. In particular, iron chelators caused a marked increase in the expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ (►p21(WAF1/CIP1/SDI1)) at the mRNA level. $p21^{WAF1/CIP1}$ mediates G_1/S phase arrest by directly binding the cyclin-cdk complexes. It was speculated that the increased level of $p21^{WAF1/CIP1}$ upon iron chelation was consistent with its potential role in the G_1/S phase arrest. However, an increase of $p21^{WAF1/CIP1}$ expression only occurred at the mRNA level, with either no change or a decrease in $p21^{WAF1/CIP1}$ protein expression being observed. This was unexpected and it was subsequently demonstrated that $p21^{WAF1/CIP1}$ protein level could be controlled by proteasomal (►proteasome) degradation after iron chelation.

In contrast, investigations examining p53 showed that its protein expression and DNA-binding activity were increased after chelation. p53 is a tumor suppressor and acts as a transcription factor that is involved in the transcription of a variety of genes involved in cell cycle arrest, differentiation, apoptosis and DNA repair. An increase in p53 after iron chelation may be the result of a decrease in deoxyribonucleotide levels due to inhibition of RR activity or changes in intracellular redox status. Despite the fact that $p21^{WAF1/CIP1}$ is a down-stream effector of p53, elevated expression of $p21^{WAF1/CIP1}$ upon iron chelation occurs through a p53-independent pathway. The ability of chelators to potentially inhibit tumor cell growth by a p53-independent pathway is significant, since p53 is the most frequently mutated gene in cancer. This also explains why cells with wild-type or mutant p53 are similarly sensitive to the growth inhibitory effects of iron chelators. However, the function of increased p53 expression after chelation remains a subject for further investigation.

Conclusions

The demonstration that some iron chelators may be clinically useful for cancer treatment followed on from initial observations that rapid cancer cell proliferation requires iron. Currently, the iron chelator, Triapine®, is being examined in a variety of clinical trials, with focus on a potential role in combination chemotherapy. The search for more effective anti-cancer Fe chelators than DFO has also led to the development of other potent Fe chelators, including Dp44mT and tachpyridine, and significant progress has been made towards understanding their molecular targets. However, further *in vivo* experiments and pre-clinical studies will be necessary to build upon the promise of these agents.

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Chemical Biology

Definition

The application of chemical tools and ideas to biological problems.

►Small Molecule Screens

Chemical Biology Screen

►Small Molecule Screens

Chemical Carcinogenesis

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Definition

►Chemical carcinogenesis (►carcinogenesis) is the process of the genesis of a ►tumor (carcinoma), and the series of sequential steps that occur when lower animals or humans are treated with ►chemical carcinogens that leads to tumor development. After all these steps are accomplished, the physiological mechanisms regulating control of growth in the normal cells are degraded, and the normal cells are degraded and converted into tumor cells. The tumor cells then grow in an unregulated fashion and evade the host immune system, leading to development of visible tumors.

Characteristics

Normal Cell Types in Animals and the Tumors They Give Rise to

During embryogenesis in mammals (warm-blooded animals), there are three primary germ layers of the early embryo which develop into all the basic cell types, tissues, and organs in the body. These are the ectoderm, the endoderm, and the mesoderm. The ectoderm and endoderm are epithelial layers. Most of the epithelial organs in the body are derived from the endodermal and the ectodermal germ layers. The epidermis of the skin, the corneal epithelium, and mammary glands develop from the ectoderm. The endoderm layer develops into the liver, pancreas, stomach, and intestines. The mesoderm develops into the kidney and linings of male and female reproductive tracts. Three types of cells are important in chemical carcinogenesis. These cell types are (i) ►epithelial cells, which form the coverings and internal parts of organs, (ii) ►fibroblasts, which are connective tissue cells derived from primitive mesenchymal cells, and (iii) cells of the hemato-lymphopoietic series, which are derived from the blood-forming elements. These cell types all have special, specific characteristics.

In humans, 92% of the tumors that arise are derived from epithelial cells (►Epithelial cell tumors). These tumors are called carcinomas. The remaining 8% of the tumors are derived from a combination of tumors derived from fibroblasts, called sarcomas, and tumors derived from white blood cells, called leukemias (►Leukemia diagnostics) and lymphomas.

Carcinogens

There are a group of molecules and radiations referred to as "carcinogens." A ►carcinogen (►Carcinogen macromolecular adducts) is any molecule, or group of molecules, such as viruses (►Virology), or radiation (►Radiation carcinogenesis; ►radiation oncology) that can cause tumors in lower animals and humans, when they are exposed to this agent. This happens when carcinogens cause normal cells to transform, or convert into transformed cells and tumor cells during experiments in vitro, called chemical transformation experiments.

Chemicals referred to as chemical carcinogens (chemical carcinogenesis) can cause tumors in lower animals in humans exposed to them. Examples of chemical carcinogens are vinyl chloride, aflatoxin B1 (a metabolite and biocide of the fungus, *Aspergillus flavus*) (►Aflatoxins), benzo(a)pyrene (a polycyclic aromatic hydrocarbon formed when organic matter is pyrolyzed in the absence of oxygen) (►Polycyclic aromatic hydrocarbons), and beta-naphthylamine (an aromatic amine used to manufacture dyestuffs that causes bladder cancer in animals and humans) (►Aromatic amines). Nitrosamines are another class of chemical carcinogens. An example is dimethylnitrosamine (DMN). Many nitrosamines are synthetic compounds. Some are believed to form in the stomach of humans when amines (derived from fish in the diet) contact nitrous acid (formed from the nitrate from fertilizer that is used to grow foodstuffs) in the acidic conditions (acid pH) of the stomach. Chemicals in all these classes of carcinogens can cause tumors in humans and in lower mammals.

There are also a number of radiations that cause tumors in humans and lower animals. These include ionizing radiations, such as alpha particles (charged helium nuclei), beta particles (naked electrons), and gamma particles. There are also tumor viruses, consisting of RNA (RNA tumor viruses) and DNA (DNA tumor viruses). When animals are treated with these viruses, tumors are formed. Examples of RNA tumor viruses are the Rous sarcoma virus, the Abelson leukemia virus, and the Kirsten Ras virus. Examples of DNA tumor viruses are the polyoma virus, the SV40 (simian virus 40) (►SV40) virus, the ►Epstein Barr virus, and the human papilloma viruses 16 and 18 (►Human papilloma viruses).

Mechanisms of Chemical Carcinogenesis

There are two broad mechanisms of chemical carcinogenesis. In the first type, which we refer to here as "►complete carcinogenesis," a mammal is treated with a large dose of a chemical carcinogen, such as 7,12-dimethylbenz(a)anthracene, and the animals treated eventually develop tumors. Carcinogenesis with complete carcinogens is usually dose-dependent, such that the higher doses of carcinogens that the animals are treated with, the high the yield of tumors per animal and in the percentage of animals with tumors.

The second mechanism of chemical carcinogenesis, discovered by Dr. Isaac Berenblum of the Weizmann Institute in Israel, is referred to as ►"two-step carcinogenesis," or "initiation and promotion". In initiation and promotion experiments, Berenblum treated mice on the skin of their shaved backs with chemical carcinogens at low doses and also with ►tumor promoters. Berenblum was testing the hypothesis that carcinogenesis was due to irritation and inflammation. Hence, he used croton oil, a product of the plant, Euphorbia lathyris, which the plant uses as a biocide against insects. Croton oil is a very irritating substance, which is important in the plant's use of it as a biocide against insects. When mice were treated with low doses of 7,12-dimethyl-benz(a)anthracene (DMBA, a carcinogenic PAH), one time, they exhibited no tumors. A second group of animals was treated with the tumor promoter, croton oil, once per week, and the animals also exhibited no tumors. When the mice were treated with a low dose of DMBA, and then once weekly with croton oil, they developed many tumors. If the latter treatment was reversed, i.e. the animals were treated first with croton oil once per week, and then later treated with a low dose of DMBA, the animals showed no tumors. If the animals were treated with a low dose of DMBA, then no treatment was performed for a significant amount of time, then the animals were treated with croton oil once per week, the animals also developed a high yield of tumors. In this system, treatment of the animals with the low dose of DMBA is referred to as the "initiation step," and later treatment with croton oil is called the "promotion" step. Initiation is believed to be a ►genotoxic event, likely a ►mutation, and is an irreversible step. Initiated cells can be promoted to tumors cells if they are treated with croton oil long enough. The promotion step is believed to be due to the binding of tetradecanoyl-phorbol acetate (TPA, the most active constituent of the mixture of phorbol esters in croton oil), to protein kinase C, triggering signal transduction and cell division in cells bearing mutations in ►proto-oncogenes. If promotion is interrupted, then tumorigenesis is reversible, i.e. the cellular death rate will equal the cellular growth rate, and the tumor will regress. If promotion is continued long enough, the tumor becomes fixed and will not regress. Eric Hecker of the German Cancer Research Center (Deutsch Krebs Forschung Zentrum) in Heidelberg, Germany, fractionated croton oil used by Berenblum, by high pressure liquid chromatography, and found that TPA was the most active tumor promoter in it.

From experiments with high doses of chemical carcinogens, and experiments with initiation and promotion, we now have evidence that chemical carcinogens such as DMBA cause mutations in proto-oncogenes, such as ras genes, converting them into activated ►oncogenes. In complete carcinogenesis experiments, further mutations in other proto-oncogenes can also occur, leading to activation of additional oncogenes. In addition, activated metabolites of the carcinogens (formed in the animals/mammals by cytochrome P450 or other enzymes of metabolic activation), also cause mutational inactivation of ►tumor suppressor genes, or breakage of chromosomes bearing them, leading to loss of these tumor suppressor genes. Together, activation of oncogenes and inactivation of tumor suppressor genes is believed to lead to the genesis of tumors in mammals.

Insights into Mechanisms of Chemical Carcinogenesis from Studies of Chemically Induced Neoplastic Transformation

Studies of the abilities of chemical carcinogens to convert normal cells into tumor cells in cell culture dishes have given us substantial insight into the molecular mechanisms of chemical carcinogenesis. In cell culture, normal fibroblasts and normal epithelial cells grow if they are fed properly, until they eventually fill the culture dish, and touch each other. Growth then ceases. This process is called contact inhibition of cell division. Cells can then be removed from the cell culture dish with a protease called trypsin, diluted, and replated into new cell culture dishes. This process can be repeated many times, until the population of total cells has undergone sixty population doublings. At this point, the cells senesce (►Senescence and immortalization), or die. This is due to progressive shortening of telomeres (►Telomerase), structures at the end of chromosomes, with each successive DNA replication

and cell division. Telomere shortening acts as a cellular and molecular "clock," to mark the lifetime of the cell. This process aids in the control of the normal physiology of the organism, by removing old cells which accumulated many mutations, which could eventually lead to cancer.

►Chemically induced cell transformation is the process by which normal cells are treated with chemical carcinogens in vitro in a cell culture dish or flask, and then their growth control mechanisms degrade, converting or transforming them into transformed cells. There are two mechanisms by which cells can be converted by chemical carcinogens into transformed cells. Firstly, cells can be treated with genotoxic (DNA-damaging) (►Genetic toxicology) chemical carcinogens. Many of these genotoxic carcinogens are ►mutagens (►Mutation rate). These carcinogens either already are direct mutagens (rare), or more commonly they are pre-carcinogens, and can be converted into mutagenic proximate carcinogens by ►cytochrome P450 enzymes or other enzyme systems that activate the pre-carcinogens into mutagens. The carinogens (benzo(a)pyrene, aflatoxin B1, and nitrosamines are all examples of pre-carcinogens that are metabolically activated into mutagens by various types of cytochrome P450 enzymes. Most pre-carcinogens are hydrophobic (fat-loving) compounds that would bioaccumulate in the body, and cause alterations in the properties of enzymes and membranes in cells. Mammals must therefore derive strategies to eliminate hydrophobic pre-carcinogens. The cytochrome P450 enzyme system, and other enzyme systems, have evolved in order to metabolize these pre-carcinogens, to make them water-soluble, so they can be excreted in the urine and removed from the body. Since these compounds are inherently chemically inert, a necessary first chemical reaction step has evolved, in which cytochrome P450 enzymes attack pre-carcinogens like benzo(a)pyrene (BaP) with molecular oxygen and reducing equivalents (NADPH and NADH) to generate epoxides and diol epoxides from it. These metabolites are mutagens, and this step results in "metabolic activation." In a second step, which is closely coupled to the first step, these active metabolites are reacted with and conjugated to, molecules of water by the enzyme, epoxide hydrolase, converting them to trans-dihydrodiols and tetraols, which are highly water-soluble, so they are excreted in the urine. The small amount of epoxides and diol epoxides derived from BaP then bind covalently to DNA bases, resulting in mutations in proto-oncogenes, activating them into oncogenes, and mutations in tumor suppressor genes, inactivating them.

In a second mechanism of ►carcinogenesis, chemicals called "non-genotoxic carcinogens," transform normal cells into tumor cells in a different way, by non-mutagenic mechanisms. One example is the chemical, 5-azacytidine, a chemical analog of a normal base. 5-azacytidine binds to DNA methyltransferases (►Methylation), inhibiting them. This results in a loss of methylation of the cytidine in DNA. If this occurs in quiescent proto-oncogenes, then these can become transcriptionally activated, leading to cell transformation. Other examples of non-genotoxic carcinogens include hormones, such as testosterone and estrogen. Higher steady-state levels of testosterone and estrogen are believed to lead to aberrantly high numbers of cell divisions in prostate and breast tissue. The resultant spontaneous mutations that occur are believed to lead to prostate cancer and breast cancer, respectively.

The process by which a normal cell is converted into a tumor cells, or chemically induced ►neoplastic transformation (►Neoplastic cell transformation), occurs in four steps. In the first step, when cells are treated with mutagenic chemical carcinogens, there occur mutations in proto-oncogenes, activating them to oncogenes, and mutations in tumor suppressor genes, inactivating them. The cells then develop the ability to grow in multi-layers, and form foci. This is particularly true for fibroblastic cells, less so for epithelial cells. This first step in cell transformation is called morphological cell transformation, or focus formation. Further genetic changes occur in the transformed cells. The second step that occurs is that the cells become immortal, and do not die or senesce. Some activated oncogenes (v-myc) can cause cells to become immortal. This step would be called transformation to cellular immortality. In the third step, cells develop the ability to grow in soft agar, in three dimensional suspension. This step is called anchorage-independent cell transformation, or transformation to anchorage independence. A final step that develops after further genetic change is that cells develop the ability to form tumors when injected into athymic (nude) mice. This step is called neoplastic transformation, or the ability of cells to be transformed so that they form neoplasms, or new growths, which we call tumors. Often, a number of activated oncogenes, two or more, may cooperate together to perturb normal cellular physiology, to cause neoplastic transformation of normal rodent or human cells in culture. It is now believed by scientists that activation of proto-oncogenes into oncogenes, and inactivation of tumor suppressor genes, such that approximately eight total genes are genetically altered, leads to the aberrant expression of approximately 150 genes or more in the tumor cells. This then leads to neoplastic transformation of cells in culture, hence to chemical cacinogenesis in the animal. We believe that chemically induced neoplastic transformation is a good model for how cells in the animal become converted (transformed) into tumor cells when the animal is treated with chemical carcinogens.

Significance of Chemical Carcinogenesis

The significance of the process of chemical carcinogenesis is two-fold. Firstly, the assay for chemical carcinogenesis in lower animals, usually mice and rats, can be used to test chemicals to determine whether they are carcinogens by virtue of their ability to induce tumors in mice and rats. Those chemicals that are able to cause a reproducible, dose-dependent induction of tumors in mice and/or rats, are presumed to be human carcinogens. This presumption is due first to the relationship that rodents and humans are both warm-blooded animals, or mammals. As such, their biochemistry and physiology is similar. In addition, many chemical carcinogens were first found to be carcinogenic in rodent carcinogenesis bioassays, and later found to be carcinogens in humans. Almost all carcinogens that have been shown to be carcinogenic in humans are also carcinogenic in rodents (aflatoxin B1, vinyl chloride, asbestos, cigarette smoke, asbestos, polycyclic aromatic hydrocarbons).

Secondly, the process of chemical carcinogenesis as studied in rodents has led to unique insights into the mechanisms of carcinogenesis. Investigators frequently use whole animal carcinogenesis bioassays to study how proto-oncogenes are activated into oncogenes, how tumor suppressor genes are inactivated by chemical carcinogens, and how oncogene activation and tumor suppressor gene inactivation leads to induction of tumors in mammals. Studying the mechanisms of carcinogenesis in rodents has also led to the identification of agents that interfere with this process, and may eventually be used to prevent the induction of cancer in humans.

►Toxicological Carcinogenesis
►Genetic Toxicology

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Chemical Castration

Definition

Removal of the gonads (ovary and testis) is required to ablate serum levels of sex steroids (progesterone, estrogen, and testosterone). As continuous administration of GnRH analogs removes the influence of the pituitary to regulate gonadal function, this inhibitory effect became known as "Chemical castration."

►Gonadotropin-Releasing Hormone

Chemical Genetic Screen

►Small Molecule Screens

Chemical Mutagenesis

►Genetic Toxicology

Chemical Tool

Definition

A chemical tool is a small, drug-like molecule that can be used to identify new targets in a ►signal transduction pathway. The chemical tool is usually identified through the screening of a cell-based or *in vivo* assay, and then used as an affinity probe to identify its molecular target. The chemical tool provides the link between the target and the desired phenotype in the assay.

►Luciferase Reporter Gene Assays

Chemically Induced Cell Transformation

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Definition

►Chemically induced cell transformation is the series of sequential steps that occur when mammalian cells are treated with ►chemical carcinogens, and converted into tumor cells.

The intermediate cell phenotypes (cell properties) are acquired one at a time, including first morphological ►transformation (change in cell shape, leading to criss-crossing of cells in abnormal patterns), then anchorage independence (growth of cells as colonies or balls of cells in three dimensional suspension of agar, without attachment to the plastic dishes cells are usually grown on), and finally ►neoplastic transformation (►Neoplastic cell transformation), or the ability of cells to form tumors when injected into nude (athymic) mice.

Characteristics

Normal Growth of Normal Cells

In the mammalian organism (warm-blooded animals), there are many types of cells. In general, these cell types are divided into (i) ►epithelial cells, which form the coverings of organs, (ii) ►fibroblasts, which are connective tissue cells, and (iii) cells of the hemato-lymphopoietic series, which are derived from the blood-forming elements. These cell types all have special, specific characteristics.

These three general cell types can be grown outside the body in an artificial situation, in cell culture medium in plastic cell culture dishes. This constitutes a model system in which the physiology of cells can be studied outside of the complicated conditions of the body. When grown in cell culture, epithelial cells and fibroblastic cells attach to the cell culture dish, by virtue of the surface charge of the cell relative to that of the plastic of the cell culture dish. These normal fibroblastic and epithelial cells must anchor to the bottom inside of the cell culture dish in order to be able to replicate their DNA and divide. This is called anchorage dependence of cell growth. These cells continue to grow if fed properly with cell culture medium, containing 5–10% fetal calf serum and cell culture medium. Cell culture medium consists of sugars, amino acids, salts, and buffers, along with an indicator to detect the acidity of the culture medium (pH indicator), all dissolved in water. In cell culture, the normal fibroblasts and normal epithelial cells continue to grow if they are fed properly, until they eventually fill the culture dish, and touch each other. Growth then ceases. This process is called ►contact inhibition of cell division. These cells can then be removed from the cell culture dish with a protease called trypsin, diluted, and replated into new cell culture dishes. This process can be repeated many times, until the population of total cells has undergone approximately 60 population doublings. This is called the "Hayflick Limit," after Dr. Leonard Hayflick, who discovered it. At this point, the cells undergo ►cellular senescence (►Senescence and cellular immortalization), or die. This is due to progressive shortening of telomeres (►Telomerase), structures at the end of chromosomes that are progressively shortened with each successive DNA replication and cell division. Hence, telomere shortening acts as a cellular and molecular "clock," to mark the lifetime of the cell. This process is believed to aid in the control of the normal physiology of the organism, and to rid it of old cells which have many ►mutations, which could eventually lead to cancer. If these normal cells are injected into mice lacking an immune system (athymic or "nude" mice), they will not grow and will not form tumors.

In contrast, cells of the hemato-lymphopoietic series grow in three-dimensional suspension (the blood) in vivo. Hence, when grown in vitro (outside the body), these cells must also be grown in three-dimensional suspension. A common practice is to grow the cells in varying concentrations of agar. When injected into athymic or "nude" mice, these normal cells, whether cells of the hematopoietic (red blood cell) or lymphoid (white blood cell) lineages, will not form tumors.

Carcinogens

There are a group of molecules and radiations referred to as "carcinogens." A ►carcinogen (►Carcinogen macromolecular adducts) is any molecule or group of molecules, such as viruses (►Virology) or radiation (►Radiation carcinogenesis; ►radiation oncology) that can cause tumors in lower animals when they are treated with this agent. These agents can also cause normal cells to transform (convert) into transformed cells and tumor cells.

There are a group of chemicals referred to as chemical carcinogens (►Chemical carcinogenesis). These are specific chemicals that can cause tumors in animals treated with them. Examples of these are vinyl chloride, aflatoxin B1 (a metabolite and biocide of the fungus, *Aspergillus flavus*) (►Aflatoxins), benzo(a)-pyrene (a polycyclic aromatic hydrocarbon formed when organic matter is burned in the absence of

oxygen) (►Polycyclic aromatic hydrocarbons), and beta-naphthylamine (an aromatic amine used to manufacture dyestuffs that causes bladder cancer in animals and humans) (►Aromatic amines). Another class of chemical carcinogens is called nitrosamines. An example is dimethylnitrosamine (DMN). Many nitrosamines are synthetic compounds. Some are believed to form in the stomach of humans when amines (derived from fish in the diet) contact nitrous acid (formed from the nitrate from fertilizer that is used to grow foodstuffs) in the acidic conditions (acid pH) of the stomach. Chemicals in all these classes of carcinogens can cause tumors in humans and in lower mammals.

There are also a number of radiations (Radiation carcinogenesis) that can cause tumors in humans and lower animals. These include ionizing radiations, such as alpha particles (charged helium nuclei), beta particles (naked electrons), and gamma particles.

In addition, there are also tumor viruses, consisting of RNA (RNA tumor viruses) and DNA (DNA tumor viruses). When animals are treated with these viruses, tumors are formed. Examples of RNA tumor viruses are the Rous sarcoma virus, the Abelson leukemia virus, and the Kirsten Ras virus. Examples of DNA tumor viruses are the polyoma virus, the SV40 (simian virus 40) (►SV40) virus, the ►Epstein Barr virus, and the human papilloma viruses 16 and 18 (►Human papilloma viruses).

Chemically Induced Cell Transformation – Description and Mechanisms

Chemically induced cell transformation is the process by which normal cells are treated with chemical carcinogens in vitro in a cell culture dish or flask, and they then convert or transform into transformed cells. There are two mechanisms by which cells can be converted by chemical carcinogens into transformed cells. Firstly, cells can be treated with ►genotoxic (DNA-damaging) (►Genetic toxicology) chemical carcinogens. Many of these genotoxic carcinogens are ►mutagens (►Mutation rate). These carcinogens either already are direct mutagens (rare), or more commonly they are pre-carcinogens, and can be converted into mutagenic proximate carcinogens by ►cytochrome P450 enzymes or other enzyme systems that activate the pre-carcinogens into mutagens. The Pre-carinogens benzo(a)pyrene, aflatoxin B1, and nitrosamines are all examples of pre-carcinogens that are metabolically activated into mutagens by various types of cytochrome P450 enzymes. The perspective for this process is that most pre-carcinogens are hydrophobic (fat-loving) compounds that would bioaccumulate in the body, and cause alterations in the properties of enzymes and membranes in cells. Hence, the organism must derive a strategy to eliminate these hydrophobic pre-carcinogens. Therefore, the cytochrome P450 enzyme system, and other enzyme systems, have evolved in order to metabolize these pre-carcinogens, to make them water-soluble, so they can be excreted in the urine and removed from the body. Since these compounds are inherently chemically inert, a necessary first chemical reaction step has evolved, in which cytochrome P450 enzymes first attack pre-carcinogens like benzo(a)pyrene (BaP) with molecular oxygen and reducing equivalents (NADPH and NADH) to generate epoxides and diol epoxides from it. These metabolites are mutagens, and this step results in "metabolic activation." In a second step, which is closely coupled to the first step, these active metabolites are reacted with and conjugated to, molecules of water by the enzyme, epoxide hydrolase, converting them to trans-dihydrodiols and tetraols, which are highly water-soluble, so they are excreted in the urine. The small amount of epoxides and diol epoxides derived from BaP then go on to bind covalently to DNA bases, resulting in mutations in proto-oncogenes, activating them into ►oncogenes, and mutations in ►tumor suppressor genes, inactivating them.

In a second mechanism of ►carcinogenesis, chemicals called "non-genotoxic carcinogens," transform normal cells into tumor cells in a different way, by non-mutagenic mechanisms. One example is the chemical, 5-azacytidine, a chemical analog of a normal base. 5-azacytidine binds to DNA methyltransferases (►Methylation), inhibiting them. This results in a loss of methylation of the cytidine in DNA. If this occurs in quiescent proto-oncogenes, then these can become transcriptionally activated, leading to cell transformation. Other examples of non-genotoxic carcinogens include hormones, such as testosterone and estrogen. Higher steady-state levels of testosterone and estrogen are believed to lead to aberrantly high numbers of cell divisions in prostate and breast tissue. The resultant spontaneous mutations that occur are believed to lead to prostate cancer and breast cancer, respectively.

The process of chemically induced neoplastic transformation, or the process of generating a tumor cell, falls into at least four steps. In the first step, when cells are treated with mutagenic chemical carcinogens, there occur mutations in proto-oncogenes, activating them to oncogenes, and mutations in tumor suppressor genes, inactivating them. The cells then develop the ability to grow in multi-layers, and form foci. This is particularly true for fibroblastic cells, less so for epithelial cells. This first step in cell transformation is called ►morphological cell transformation, or focus formation. Further genetic changes occur in the transformed cells. The second step that occurs is that the cells become immortal, and do not die or senesce. Some activated oncogenes (v-myc) can cause cells to be come immortal. This step would be called transformation to cellular immortality. A third step that occurs is that the cells develop the ability to grow in soft agar,

in three-dimensional suspension. This step is called ►anchorage-independent cell transformation, or transformation to anchorage independence. A final step that develops after further genetic change is that the cells develop the ability to form tumors when injected into athymic (nude) mice. This step is called neoplastic transformation, or the ability of the cell to be transformed so that it forms neoplasms, or new growths, which we call tumors. Often, a number of activated oncogenes, two or more, may cooperate together to perturb normal cellular physiology, to cause neoplastic transformation of normal rodent or human cells in culture.

Significance of Chemically Induced Neoplastic Transformation

The significance of the process of chemically induced neoplastic transformation is twofold. Firstly, the assay for chemically induced morphological cell transformation can be used an assay to detect chemical carcinogens. Those chemicals that have the ability to induce foci of morphologically transformed cells are highly likely to be able to induce tumors in animals. Hence, this assay can detect chemical carcinogens by virtue of their ability to induce foci of morphologically transformed cells.

Secondly, the study of chemically induced morphological, anchorage-independent, and neoplastic transformation in vitro is frequently used as a model system to study the process of chemical carcinogenesis. Investigators frequently use these assays to study how proto-oncogenes are activated into oncogenes, and how tumor suppressor genes are inactivated by chemical carcinogens, and how oncogene activation and tumor suppressor gene inactivation leads to induction of morphological transformation, cellular immortality, anchorage-independent transformation, and neoplastic transformation.

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Chemoattractant

Definition

A molecule that is capable of promoting cell movement by inducing ►chemotaxis.

►Chemokine Receptor CXCR4

Chemoattractant Cytokine

►Chemokine

Chemoattraction

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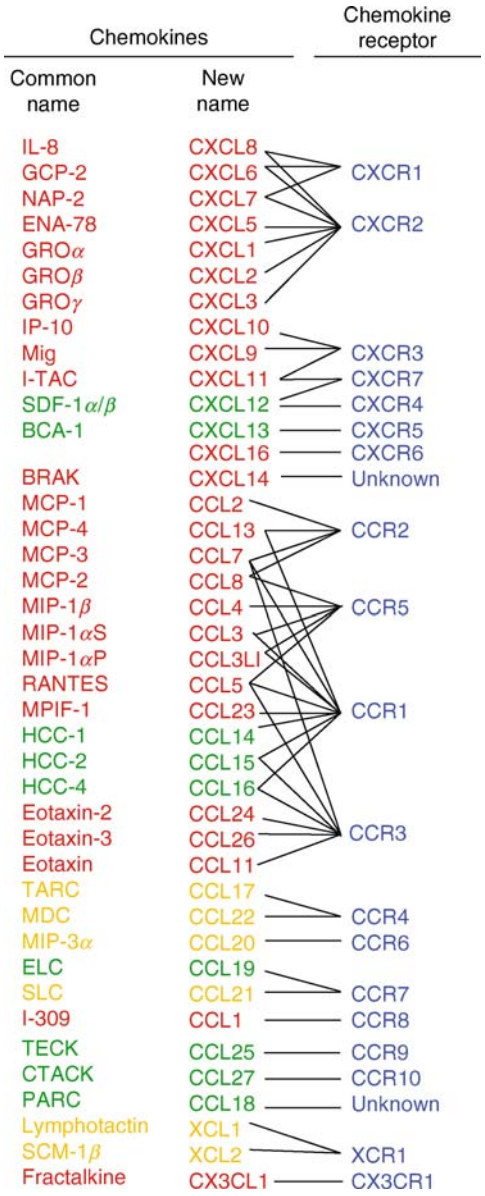
Synonyms

Directed migration; Directed motility

Definition

Chemoattraction is the process whereby a cell detects a chemical gradient of a ligand called chemoattractant and, as a consequence, gets oriented and subsequently moves in the direction from a low to a high concentration of the chemoattractant. Chemoattraction is controlled by specific chemoattractant receptors that are able to detect selectively these ligands. Chemoattraction is called ►*chemotaxis* or ►*haptotaxis* when the chemical gradient of the chemoattractant is presented to the cell either in a soluble or bound to a substrate form,



Chemoattraction. Figure 1 Classical and new names of chemokines are included. Red identifies "inducible" or "inflammatory" chemokines, green "homeostatic" agonists and yellow ligands belonging to both realms. BCA, B cell activating chemokine; BRAK, breast and kidney chemokine; CTACK, cutaneous T-cell attracting chemokine; ELC, Epstein–Barr virus-induced receptor ligand chemokine; ENA-78, epithelial cell-derived neutrophil-activating factor (78 amino acids); GCP, granulocyte chemoattractant protein; GRO, growth-related oncogene; HCC, human CC chemokine; IP, IFN-inducible protein; I-TAC, IFN-inducible T-cell α chemoattractant; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; Mig, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NAP, neutrophil-activating protein; PARC, pulmonary and activation-regulated chemokine; RANTES, regulated upon activation normal T cell expressed and secreted; SCM, single C motif; SDF, stromal cell-derived factor; SLC, secondary lymphoid tissue chemokine; TARC, thymus and activation-related chemokine; TECK, thymus expressed chemokine.

respectively. As it is not clear which one of these two types of motile processes takes place *in vivo*, it is more appropriate to refer to these directional motile processes with the more general term of chemoattraction.

Characteristics

Chemoattractants use specific chemoattractant receptors to guide different migratory cell types towards specific sites in the organism. These receptors, upon binding to the chemoattractant, transform the information of this ligand in intracellular signals that result in the movement of the migratory cell towards the positions where chemoattractant is present at high concentration. Therefore, the analysis, in a specific context, in one hand, of the type of chemoattractant receptors expressed by a certain migratory cell and, on the other hand, the position in the organism of the chemoattractants recognized by these receptors, allow to make predictions on the potential tissues where this cell can be attracted. Upon arrival to the position where the chemoattractant is at a high concentration, adhesive receptors may contribute to slow down (function largely performed by selectin adhesive receptors for cells in blood vessels) and eventually attach (cells use ►integrin receptors for this function in most cell types) the cells to these sites.

Chemoattractants can be conveniently classified according to the type of receptor that they bind. In this regard, the first and the largest group include chemoattractants that bind members of the ►G-protein coupled receptor (GPCR) superfamily. In this first group is included the family of ►chemokines. A second group is formed by chemoattractants that bind tyrosine kinase receptors (e.g. Epidermal Growth Factor (EGF), Platelet Derived Growth Factor (PDGF)). A third group includes ligands that bind receptors different of the two aforementioned families (e.g. laminin and fibronectin, which bind integrin receptors). This article deals mainly with the chemokines because they have been the chemoattractant family most studied in relation to ►cancer and ►metastasis.

Chemokines

Chemokines (chemotactic chemokines) are a family of peptides (60–100 amino acid (aa)) that includes some 50 members (Fig. 1). Based on the number and spacing

of the conserved Cystein (C) residue in the N-terminus of the protein, chemokines are subdivided in four families (C, CC, ►CXC, CX_3C), where X is any intervening amino acid between the cysteins. Chemokines receptors transmit intracellular signals that can control either chemoattraction or other functions (Fig. 1). The chemokine receptors (some 20 members) are included in the G-protein coupled receptor (GPCR) superfamily. They are classified based on the class of chemokines that they bind, i.e. receptors that bind C, CC, CXC, CX_3C chemokines are called respectively to CR, CCR, CXCR and CX_3CR receptors. Based largely on studies performed in the immune system, chemokines have been classified in three functional groups, homeostatic, inducible and dual function (Fig. 1). The first group, which includes chemokines constitutively produced by "resting cells" in specific organs or in tissues inside these organs, controls homeostatic migratory processes that determinate the correct location of different cell types in the organism under normal conditions. Inducible or inflammatory chemokines are secreted in different tissues in emergency situations and serve to attract to these places specialized cell types that contribute to the resolution of the emergency situation. A third group is formed by dual function chemokines, which can be either homeostatic or inducible depending on the context (Fig. 1). Although chemoattraction is the function most commonly regulated by chemokines, however, studies performed mainly on leukocytes have demonstrated that these peptides, acting through specific chemokine receptors, may control additional cellular functions, including proliferation, ►adhesion, motility, survival or protease secretion, among other functions. By controlling these activities, chemokines may contribute to modulate the functions of leukocytes and other cell types.

Chemokines and Cancer

Cancer is a disease where cells have disrupted the mechanisms that regulate their normal growth and, consequently, proliferate without control. This affliction becomes life threatening when cancer cells become metastatic, that is, they acquire the ability to leave their original sites of growth (primary tumor) and invade other tissues or organs where the uncontrolled growing cells can form new colonies (►metastases) that can interfere with vital functions. The process leading to metastasis formation has been divided into several steps. In a first step, the cancer cells detach from the substrate and from the neighboring cells and escape from the primary tumors. A second step involves the penetration of the cancer cells into the blood or lymphatic vessels and their ►migration through these vessels. In the case of cells that migrate through the afferent lymphatics, they migrate first to the lymph nodes from where they can exit through the efferent lymphatics, eventually ending up in the blood vessels. In a third stage, cancer cells extravasate from blood vessels and home into new sites in the organism where new metastatic colonies can be formed. During these migratory processes the cells undergo changes in their adhesive properties that are regulated by modulation of the activities and/or levels of integrin receptors. Moreover, cancer cells and/or associated stromal cells secrete proteases which, by degrading extracellular matrix (ECM) proteins of connective tissues facilitate the moving of the cells and the ►invasion of other tissues. Finally, at the metastatic sites, the cancer cells attach and grow as secondary colonies. In addition, they may secrete chemokines and other soluble factors that induce new vascular vessel formation (►angiogenesis) and contribute to maintain the growth of the metastatic cells. Although millions of cells may be shed into the blood from primary tumors, however only a reduced percentage of these cells are able form metastases, suggesting that metastatic cells develop mechanisms that increase their survival in the face of a hostile environment.

Chemoattraction: A Key Process to Attract Cancer Cells to New Biological Niches

Since the work of Stephen Paget in the second half of the nineteenth century, it is known that metastatic cells do not move randomly, displaying in contrast a marked tropism toward specific organs (Table 1). A variety of experimental data indicates that chemokines may play an important role in determining this bias of the metastatic cells. Analysis of the phenotype of multiple metastatic cell types shows that these cells express specific sets of chemokine receptors (Table 1). Furthermore, a clear correlation has been observed between the expression of a specific chemokine receptor by a metastatic cell and the presence of its respective ligands in the metastatic sites, suggesting the involvement of these receptors in the homing processes (Table 1). Finally, a direct role for chemokines and their receptors in the control of the tropism of metastatic cells is corroborated in studies that show that interference with the binding to the chemokine receptors impairs the ability to metastasis to specific organs. For instance, antibody neutralization of ►CXCR4 in breast cancer cells reduced the ability of these cells to form metastases in the lung, both upon intravenous injection and after ►orthotopic implantation of the cells. Conversely, over-expression of CCR7 in B16 ►melanoma resulted in a dramatic enhancement in the ability of these cells to form metastases in the draining lymph nodes upon intravenous injection of the cells in mice. From these studies it has also emerged that CCR7 and

Chemoattraction. Table 1 Chemokine receptors involved in cancer metastases

Chemokine/s receptor/s/ligand/s	Site/s of metastases	Cancer cell types	Function/s regulated by chemokine receptor
CXCR3/CXCL9, -10, -11	Lung, bone, lymph node	Acute lymphoblastic leukemia, Chronic myelogenous leukemia, colon, melanoma	Chemoattraction
CXCR4/CXCL12	Lung, bone, lymph node	Breast, ovarian, prostate, glioma, pancreas, melanoma, esophageal, lung (small cell lung cancer), head and neck, bladder, colorectal, renal, stomach, astrocytoma, cervical cancer, squamous cell cancer, osteosarcoma, multiple myeloma, intraocular lymphoma, follicular center lymphoma, rhabdomyosarcoma, neuroblastoma, B-lineage acute lymphocytic leukemia, B-chronic lymphocytic leukemia, non-Hodgkin lymphoma, acute myeloid leukemia, thyroid cancer, acute lymphoblastic leukemia, chronic myelogenous leukemia	Chemoattraction, angiogenesis, survival, growth
CXCR5/CXCL13	Lymph node	Head and neck, chronic myelogenous leukemia	Chemoattraction
CXCR7/CXCL11, -12	Lymph node	Breast, cervical carcinoma, glioma, lymphoma, lung carcinoma	Adhesion, survival, growth
CCR4/CCL17, -22	Skin	Cutaneous T-cell lymphoma	Chemoattraction
CCR7/CCL19, -21	Lymph node	Breast, Melanoma, lung (non-small cell lung cancer), head and neck, colorectal, stomach, chronic lymphocytic leukemia	Chemoattraction
CCR9/CCL25	Small intestine	Melanoma, prostate	Chemoattraction
CCR10/CCL27	Skin	Melanoma, cutaneous T-cell lymphoma	Chemoattraction, growth, survival

CXCR4 are the chemokine receptors most commonly expressed by metastatic cells. This finding contributes to explain the ability of multiple metastatic cell types that express these receptors to colonize the lymph node and other organs where CXCL12 (ligand for CXCR4 and CXCR7) and CCL19 and CCL21 (both ligands of CCR7) are expressed (Table 1).

Premetastatic niche is the name given to the specific regions, whose formation is induced by soluble factors released by primary tumor cells, which eventually become colonized by distant metastatic cells from the primary tumors. It has been shown that chemokines expression may confer premetastatic niches the ability to attract metastatic cells from the distant primary tumor. In this regard, it has been shown that chemokines S100A8 and S100A9, expressed by myeloid and endothelial in premetastatic niches in the lung, are responsible of attracting incoming Lewis Lung carcinoma metastatic cells to these niches because neutralization of the chemokines with antibodies reduced the metastases in these areas. In sum, chemokine/chemokine receptor pairs are important factors that control the colonization of cancer cells to specific sites in the organism.

Other Biological Effects of Chemokines on Cancer Cells Apart from Chemoattraction

Chemokines may affect cancer not only by regulating chemoattraction, but also by regulating other functions that control cancer progression.

Chemokines can Contribute to Regulate the Growth of Cancer Cells

Uncontrolled growth is a hallmark of cancer cells. Considering that chemokines may control cell growth in different cell types, the effect of chemokines on the proliferation of cancer cells is not unexpected. The growth of tumor cells may be affected by chemokines that can be either released in an ►autocrine signaling fashion by the cancer cells or secreted by the stromal tissues associated to the cancer cells. As an example of the first case, it is known that CXCL1, -2, -3, and -8, secreted as autocrine growth factors by melanoma, pancreatic and liver cancer cells, regulate the proliferation of all these cell types. As an example of the second case, it has been reported that CXCL12, which is secreted in lung and lymph nodes, leads to the increase in the growth of ►glioma, ovarian, small cell lung, basal cell carcinoma and renal cancer, all cancer cells

types that colonize the aforementioned organs. The effects of chemokines on growth can be complex because, for instance interference with CCR5 seems to increase the proliferation of ►xenografts of human breast cancer, suggesting that CCR5 inhibits the growth of this cancer cells.

Chemokines can Contribute to Regulate the Survival of Cancer Cells

A reduced susceptibility to ►apoptosis, leading to a concomitant extended survival, is also an important factor to explain the uncontrolled growth and the ability of cancer cells to form metastases. Chemokines have been involved in regulating survival in leukocytes and other cells, therefore these ligands may potentially contribute to regulate the carcinogenic phenotype by modulating this function. Stimulation of melanoma B16 cells expressing CCR10 with its ligand CCL27 enhances the resistance of these cells to the apoptosis induced by stimulation of the death receptor CD95. These *in vitro* results are consistent with *in vivo* experiments that show that the neutralization of CCL27 ligand with antibodies results in the blocking of tumor cell formation. Also, stimulation of glioma cells with CXCL12 protects these cells from the apoptosis induced by serum deprivation. Recently, it has been shown that, CXCR7, a novel second receptor for CXCL12, is expressed in a variety of cancer cells. It has been indicated that CXCR7 may regulate survival, growth and adhesion. Thus, it is possible that CXCR7 may also contribute to control all these functions in cancer cells.

Chemokines can Contribute to Regulate the Adhesion to New Sites in Cancer Cells

Migratory cancer cells experience changes in adhesion, including processes of attachment and detachment, as they move through the organism. Enhanced adhesion is particularly crucial at the final stages of cancer progression where these cells require attaching to the new metastatic sites. Stimulation of cancer cells with chemokines may change the adhesion of these cells either by increasing the activity of ►integrins or by inducing changes in the expression levels on the membrane of these receptors. As an example of the first case, it has been observed that stimulation of B16 melanoma cells with CXCL12 leads to an increase in the affinity of the β1 integrin by the ligand VCAM-1 both in *in vitro* and in *in vivo* experiments. As an example of the second case, stimulation of prostate tumor cells with CXCL12 induces enhanced expression of the integrins α3 and β5.

Chemokines can Contribute to Control Protease Secretion in Cancer Cells

Metalloproteins are largely responsible for ECM remodeling and play key roles in solid tumor cell invasion. In this regard, it has been shown that chemokines enhance in protease secretion in some cancer cell types. For instance, stimulation of myeloma cells with CXCL12 induces metalloproteinase secretion.

Chemokines can Contribute to Control Angiogenesis in Cancer Cells

At metastatic sites cancer cells induce formation of new vessels (angiogenesis), which allow the nourishment of the metastatic colonies. Angiogenesis is a finely orchestrated process where endothelial cells proliferate, secrete proteases, change their adhesive properties, migrate and, finally, differentiate into new vessels. Chemokines can act as positive or negative regulators of the angiogenesis in the tumor microenvironment. In this regard, the members of the ►CXC chemokine family play an important role during this process. The CXC family has been divided into two groups. A first group that includes members that present the triplet Glutamic-Leucine-Arginine (ELR) before the first Cys (ELR^+ CXC chemokines), and a second group that include the members that lack this three amino acids (ELR^- CXC chemokines). Although there are exceptions, by and large, ELR^+ CXC chemokines (including CXCL1, -2, -3, -5, -6, -7 and -8) play proangiogenic roles, promoting vessel formation through the stimulation of the CXCR2 receptor. For instance, in human ovarian carcinoma CXCL8 induces both angiogenesis and tumorigenesis. Furthermore, treatment of mice that bear CXCL8-producing non-small cell ►lung cancer cells with anti-CXCL8 antibodies blunted the growth of these tumors in the mice. Exceptions to the rule ELR^+ CXC=angiogenic chemokines are the ELR^+ CXC members CXCL1 and 2, which are angiostatic i.e. they inhibit angiogenesis.

ELR^- CXC chemokines, including CXCL9, -10, -11, are generally angiostatic. For instance, CXCL9 and CXCL10 inhibit Burkitt's lymphoma tumor formation probably by blocking blood vessel formation. An exception to the rule ELR^- CXC = angiostatic chemokine is CXCL12 that is angiogenic, as suggested by CXCL12 and CXCR4 KO mice that display cardiovascular development defects. It is believed that the angiogenic effects of CXCL12 are mediated by the vascular endothelial growth factor (VEGF) that is secreted by endothelial cells upon stimulation with CXCL12. The latter chemokine can be secreted in the tumor microenvironment by both the cancer cells and associated stromal cells. Finally, apart from CXC chemokines, other chemokines families may also regulate angiogenesis. In this regard, the CC chemokine CCL21 is angiostatic. In contrast, three CC family members (CCL1, -2, -11) and one CX3C family member (CX3CL1) can induce angiogenesis. All these chemokines, secreted inside the tumor, may potentially regulate the growth of the metastatic cells.

Therapeutical Aspects

The multiple points at which chemokines may regulate cancer progression make them attractive targets to develop anti-cancer drugs. Several strategies have been adopted to harness the power of chemokines against cancer, including the use of antibodies against the overexpressed chemokine receptors in the target cancer cells to induce apoptosis of these cells. One common strategy has been the development of inhibitors to block the binding of the chemokines to the receptors and consequently the function of these receptors. The fact that chemokine receptors are on the membrane and that much information is available on the sequences, both on the ligands and on the receptors, necessary for receptor-ligand binding have enabled the development of numerous peptide or small molecule inhibitors that interfere with chemokine function. Some of these inhibitors have been developed against CCR1, CCR5, CXCR7 and CXCR4. Most of these inhibitors relay on their ability to inhibit survival or angiogenesis in the target cells. As CXCR4 is one of the most broadly expressed chemokine receptor in cancer cells, at least six peptides or small molecule inhibitors of the function of CXCR4 have been developed and used in preclinical cancer models. CXCR4 is particularly interesting due to its pro-angiogenic functions. A variety of data indicate that the growth and persistence of tumors and their metastases depend on an active angiogenesis at the tumor sites. In this regard, interference with this process is a powerful strategy to inhibit tumor growth. Interference with CXCR4 has been used in several cancer models, including many of the cancers indicated in Table 1. Although peptide inhibitors of chemokine receptors may not have by itself ►tumoricidal affects, however, along with other strategies may be a powerful therapy against tumors.

Summary and Final Conclusions

Upon becoming carcinogenic and metastatic, a variety of cancer cells up-regulate the expression of chemokine receptors. In this regard, the microenvironment conditions inside the tumors are also known to induce chemokine receptor expression in some cases. For instance, the low oxygen concentration (►hypoxia) inside a tumor induces CXCR4 expression which concomitantly leads to a more aggressive metastatic phenotype in cancer cells. Chemokine receptors endow cancer cells with "postal codes" that determine their migration to tissues where the ligands of these receptors are expressed and therefore are important for the metastatic ability of these cells. In addition, these receptors may confer or modulate cancer cells functions that, by regulating different steps in cancer progression, may contribute to the carcinogenic and metastatic phenotype of these cells. The case of the Kaposi's sarcoma herpesvirus (KSHV), which induces cancer lesions similar to that of Kaposi sarcoma, is a dramatic example that shows the important role that chemokines and their receptors may play in cancer. Interestingly, this virus encodes a constitutively active receptor that displays a high degree of sequence similarity to chemokine receptors CXCR1 and CXCR2 and which can even be further activated by the CXCR2 ligands CXCL1 and/or CXCL8. KSHV is also pro-angiogenic and induces survival effects in the cancer cells where is expressed. Further supporting a causative role of CXCR2 in cancer, a constitutive form of CXCR2 can induce cell transformation in susceptible cell types.

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Chemoembolization

Definition

Chemoembolization is a procedure in which the blood supply to a tumor is interrupted through mechanical or surgical interventions (embolization) and cytotoxic drugs are administered directly into the tumor. This technique is used in hepatocellular carcinoma and neuroendocrine carcinomas, among other cancers.

►Neuroendocrine Carcinoma

Chemokine

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Synonyms

Chemotactic cytokine; Chemoattractant cytokine

Definition

Chemokines are a large group of small proteins that play multiple biological roles, including stimulating directional migration (►chemotaxis) of leukocytes and tumor cells via their membrane-bound receptors.

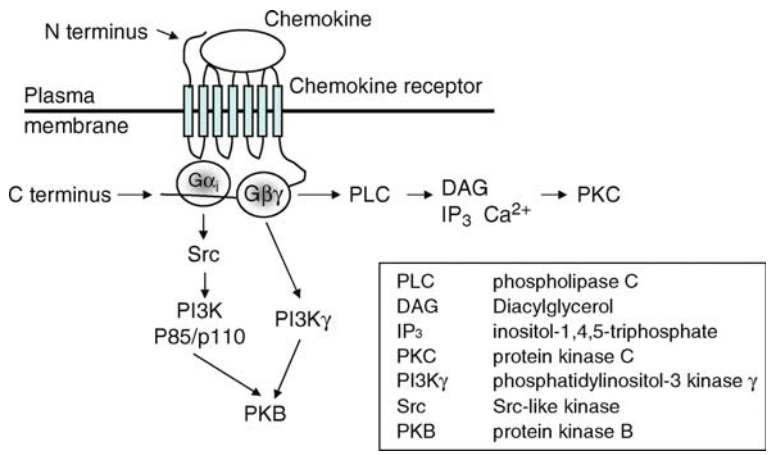
Characteristics

Chemokines are divided into four subgroups (C, CC, CXC, and CX_3C) based on the spacing of the key cysteine residues near the N terminus of these proteins. The CC and CXC families represent the majority of known chemokines. Chemokines signal through seven-transmembrane-domain receptors, which are coupled to heterotrimeric G_i-proteins. Activation of phospholipase C (PLC) and ►phosphatidylinositol-3-kinase γ (PI3Kγ) by βγ subunits of ►G-proteins is well established.

So far, approximately 50 chemokines and 18 chemokine receptors have been identified. Some chemokine receptors bind to multiple chemokines and vice versa, suggesting possible redundancies in chemokine functions. Chemokine receptors permit diverse cells to sense small changes in the gradient of soluble and extracellular matrix-bound chemokines, thus facilitating the directional migration of these cells toward higher relative concentrations of chemokines. While soluble chemoattractants can induce directional migration, chemokines (due to their net positive charges) will often be bound to and presented by negatively charged macromolecules such as endothelial cell-derived proteoglycans *in vivo*. Chemokine gradients bound to solid surfaces are capable of mediating ►haptotaxis of leukocytes and other cells. Chemokine receptor activation can also trigger conformational changes in membrane ►integrins, permitting strong cell–cell adhesion in the presence of appropriate integrin receptors. This signaling pathway is particularly relevant in triggering cellular integrins found on leukocytes and cancer cells to bind to their respective receptors (e.g. ICAM-1) on vascular endothelial cells, facilitating stable binding and spreading of cells to endothelium. The stable binding of metastatic tumor cells to vascular endothelial cells at distant sites of metastasis is likely to be a crucial early step in the process of ►metastasis.

Circumstantial evidence supports the idea that tumor cells use chemokines to promote their own survival and metastasis through multiple mechanisms. For example, certain chemokines secreted by tumor cells contribute to tumor growth and ►angiogenesis. Members of chemokines that contain an ELR motif (Glu–Leu–Arg) act as angiogenic factors, which are chemotatic for endothelial cells *in vitro* and can stimulate *in vivo*. In contrast, members without an ELR motif inhibit angiogenesis. Chemokine-mediated tumor cell activation through cellular kinases such as PI3K, ►Akt kinase, and other downstream mediators (Fig. 1) influences tumor cell resistance to apoptotic death. For example, activation of the chemokine receptor CCR10 prevents ►Fas-mediated tumor cell death induced by cytolytic antigen-specific T cells.

Selected chemokine receptors are upregulated in a large numbers of common human cancers, including breast, lung, prostate, colon, and melanoma. Chemokine receptors expressed on tumor cells coupled with chemokines preferentially expressed in a variety of organs are believed to play critical roles in cancer metastasis to vital organs as well as draining lymph nodes. CXCR4 is by far the most common chemokine receptor expressed on most cancers. In addition, CXCL12, the ligand for CXCR4, is highly expressed in lung, liver, bone marrow, and lymph nodes, which represent the common sites of metastasis of many



Chemokine. Figure 1 ►Chemokine receptor signaling. Upon stimulation by chemokine, βγ subunits of G-protein are dissociated from $G\alpha_i$ subunit. βγ subunits activate phospholipase C (PLC) and phosphatidylinositol 3 kinase γ (PI3Kγ), Whereas $G\alpha_i$ subunit directly activates ►Src-like kinase.

cancers. Chemokine receptor expression on cancer cells may influence the conversion of small, clinically insignificant foci of cancer cells at metastatic sites to rapidly growing, clinically serious secondary tumors. Cancers that upregulate CCR7 expression also facilitate their entry into lymphatic vessels, which strongly express the CCR7 ligand (CCL21), and subsequent retention within CCL21-rich secondary lymphoid organs. Upregulation of chemokine receptors such as CCR7 may be a major reason for efficient lymph node metastasis observed in many epithelial cancers.

Chemokines released by tumor cells have been shown to attract ►regulatory T cells, thus suppressing host responses to invasive tumors. Moreover, chemokine and their receptors are involved in ►dendritic cell maturation, B and T cell development, and ►T_h1 and ►T_h2 polarization of the T cell response. These actions suggest the possibility that chemokines may play a role in altering the magnitude and polarity of host immune responses to cancer cells.

Although individual chemokine and chemokine receptor appear to affect many aspects of cancer cell survival, migration, angiogenesis, and the host response to cancer cells, it is still unclear which of these functions predominate in the multistep establishment of primary tumors and secondary metastases.

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Chemokine Receptor CXCR4

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Synonyms

Receptor for CXCL12; Receptor for stromal cell-derived factor-1 alpha (SDF-1α); CD184; Fusin

Definition

CXCR4 is a cell-surface protein that acts as a receptor for the molecule CXCL12 (stromal cell-derived factor-1 alpha, SDF-1α). CXCL12 is one of a class of signaling molecules called chemokines that regulate the movement and other activities of cells throughout the body. Although CXCL12 and CXCR4 play major roles in regulating stem cells and cells of the immune system, CXCR4 is also found on many cancer cells and plays a part in metastasis, spread of the cancer cells being influenced by tissue levels of CXCL12.

Characteristics

Chemokines are a class of peptide mediators that play important roles in controlling cellular homing and migration both in embryonic development and in the regulation of cell populations in the adult. There are at least forty different chemokines that fall into four classes depending upon their peptide structure. The different classes are "C", "CC", "CXC" and "CX_3C" chemokines, for which characteristic sequence motifs involve residues of the amino acid cysteine (C) either in sequence or separated by one or three other amino acids (X or X_3). The chemokines themselves are peptides that can exist freely in solution in biological fluids and act by binding to corresponding ►receptors. In the language of molecular interactions a chemokine is therefore known as a ►ligand. Chemokines are denoted by the letter L within their name. CXCL12 is thus a ligand, and a chemokine of the CXC class of chemokine mediators.

The chemokine receptors are named according to the chemokine class of their binding partner (or ligand), with the letter "R" to designate their receptor status. CXCR4 is therefore a receptor. As for chemokines, the numbers serve to distinguish individual members of the overall family. The partnership between chemokine receptors and the chemokines is not monogamous, and some chemokine receptors may bind as many as ten different chemokines. However, most receptors have between one and three distinct partners. With very few exceptions, these partnerships are within a particular chemokine class (e.g., CXCL chemokines bind selectively to certain CXCR receptors). At this point, the only chemokine factor known to bind to CXCR4 is CXCL12, although CXCL12 itself is able to bind to an alternate receptor (CXCR7, previously known as RDC-1) as well as to CXCR4.

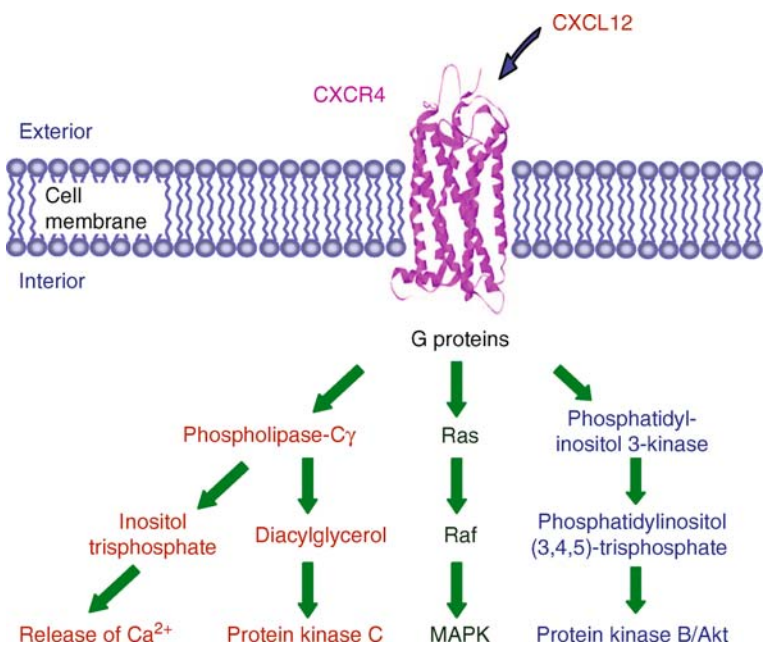
Chemokine receptors such as CXCR4 are seven-transmembrane, ►G-protein-coupled receptors. The protein chain of CXCR4 therefore winds back and forth across the outer membrane of the cell so that it crosses the membrane a total of seven times. One end of the protein chain (the amino terminus) protrudes from the outside of the cell. This region of the protein,

together with certain parts of the three extracellular loops, forms the binding domain for CXCL12. The part of the receptor that protrudes from the inner face of the membrane (composed of the carboxy terminus and three intracellular loops) contains the characteristics that allow it to provoke a cascade of events within the cell (Fig. 1). These steps are initiated firstly by a linkage to one or more of a small family of proteins that interact directly with the receptor, called ►G proteins (in this case primarily $G_{\alpha i}$ and $G_{\alpha q}$). G protein involvement leads to the activation of three major signaling pathways: (i) the phospholipase C-diacylglycerol/IP_3 pathway, (ii) the ras-raf-MAP kinase pathway, and (iii) the PI 3-kinase pathway.

CXCR4 is a crucially important member of the chemokine receptor family. If CXCR4 or CXCL12 is absent during embryonic development, the organism is unable to survive. The key dependence on CXCL12 and CXCR4 reflects the importance of this signal/receptor pair in marshalling the correct formation of cells as tissues are formed from their more rudimentary cellular precursors in the embryo. The CXCL12:CXCR4 axis, as it is often called, is a central part of the normal development of the central nervous system (the brain itself) and the exquisitely organized tissue that replenishes the different cells of the blood through adult life (the ►hematopoietic system). In addition, CXCR4 and CXCL12 seem to play a particular role in the development of the gut, and their participation is important for the proper development of the blood vessel system that is required for efficient intestinal function in the adult. In adult organisms, CXCR4 and CXCL12 partly reprise their developmental role during tissue damage by participating in repair processes.

Once the organism is fully formed, the most evident role for CXCR4 and CXCL12 in a normal individual is that of continued regulation of the hematopoietic system. This takes place mainly in the bone marrow, which acts as a reservoir for the ancestral cells (stem cells and other progenitor cells) that are needed for the continued production of various white cells (leukocytes) and other progeny that are required to ensure a proper defense against infection or injury, or to deal with replacement and remodeling of damaged tissues. These stem cells – which need to be maintained safely by the body until required to respond – are located within the protected environment of the bone marrow and are supported and nourished by a specialized grouping of cells that together are referred to as the "►microenvironmental niche." These supporting cells or "►stromal cells" secrete a number of factors that serve to nourish the stem cells and to keep them within a safe environment in their primitive and "resting" state.

Notable amongst these factors is CXCL12 (the "stromal cell-derived factor"), which can bind to CXCR4 on the stem cells. The binding of CXCL12



Chemokine Receptor CXCR4. Figure 1 The cellular signaling pathways of CXCR4. When the chemokine ligand CXCL12 binds to its receptor CXCR4, one or more of several pathways can be activated through initial links involving G proteins that associate with the receptor. These pathways, which are shown only in outline, involve a further network of interactions that eventually lead to a cellular response that may ensure cell growth, migration or survival.

to its receptor has several effects on cell behavior, but the principal outcome is to attract cells toward the source of CXCL12. In the case of stem cells in the bone marrow, this results in retention within the microenvironmental niche, or directs migrant stem cells back to this location. This ability of the CXCL12:CXCR4 axis to direct cell movement is what underlies its key role in orchestrating tissue development and repair. The phenomenon can be demonstrated in experiments using isolated cells, such that cells that have the CXCR4 receptor can be induced to migrate through pores in an artificial filter in response to an upward concentration gradient of CXCL12 in the fluid. This is a cellular response known as ►chemotaxis, and CXCL12 is referred to as a ►chemoattractant.

Unfortunately, this normal and very important process by which CXCL12 and CXCR4 assist directed cell movement has been subverted by cancer cells to assist the spread of a cancer, or metastasis. Normal tissues that are not subject to inflammation or repair processes typically have very low levels of CXCR4. However, when cancers are formed the affected cells frequently experience a dramatic increase ("upregulation") of CXCR4. This has been shown for the common adult cancers (carcinomas of the breast, colon, lung, prostate, cervix etc), which arise in the membranous linings (epithelia) of certain organs; but CXCR4 levels are also elevated in cancers arising in bone (e.g., osteosarcoma), muscle (e.g., rhabdomyosarcoma), nervous tissue (e.g., glioblastoma) or white cells (various leukemias).

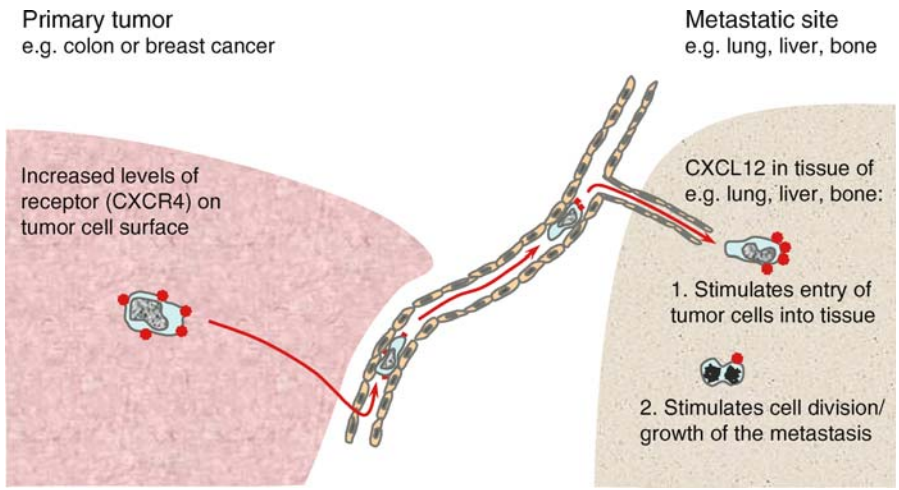
This is such a consistent finding that in many cancers the level, or "expression," of CXCR4 can be used as cancer ►biomarker. The levels of CXCR4 that are present on the cells give an indication of how the cancer is likely to behave in the future, and what therapeutic steps might need to be considered. Levels are assessed using a technique called immunohistochemistry. In this approach very thin slices or "sections" – no more than 0.005 mm thick – are taken from the suspect tissue onto glass slides. Special protein reagents called ►antibodies are used that recognize any molecules of CXCR4 in the tissue, and additional steps in the process generate color wherever the antibody has bound. The resulting picture under a microscope tells the pathologist not only about the architecture of the tissue and the characteristics of the cells, but whether or not they have high levels of CXCR4. High levels (expression) of CXCR4 are associated with cancer aggressiveness, a likelihood that the cancer will spread or metastasize, and means that the outlook for the patient is likely to be poorer.

The link between cancer aggressiveness/metastasis exists because the CXCL12:CXCR4 axis has a similar role of "directing traffic" in cancer as it does in normal circumstances. In this situation it is the cancer cells that possess the receptor – CXCR4 – and have levels at the cell surface that are much greater than are found on their normal counterparts. The exact reasons for these elevated levels of the chemokine receptor are not fully understood. Undoubtably the genetic changes that are characteristic of cancer cells lead to alterations in ►transcription of the CXCR4 gene that may provide certain subpopulations with greater amounts of the CXCR4 protein, and these cells have a selective advantage. However, there are also indications that factors within the environment of the tumor can make the situation worse by stimulating the cell to make even more CXCR4. The hypoxic nature of tumor tissue causes an increase in CXCR4 gene transcription through a pathway involving ►hypoxia-inducible factor-1 alpha (HIF-1α). Various small-molecular-weight and polypeptide mediators have also been shown to enhance the cellular expression of this chemokine receptor.

The cancer cells are therefore equipped to be attracted toward sources of CXCL12 and to be captured within environments that are high in concentrations of CXCL12. Thus, it is no coincidence that the tissues that are high in CXCL12 are also those in which cancers form secondary tumors or metastases. Such tissues include the lymph nodes – central filters in the system that drains fluid from all tissues – as well as the liver, lung and bone marrow. CXCL12 is believed to be one of the major factors driving metastasis (Fig. 2). As a colorectal cancer develops in the large intestine, for example, and small groups of tumor cells are shed into the blood circulation and the lymphatic drainage, circulating cells will find an attractive home as they encounter lymph nodes in the mesenteric fat around the intestinal wall, when they are delivered to the liver through the portal circulation, or as they lodge in the capillary beds of the lung after traversing the systemic circulation. Conversely, they have a much reduced probability of taking up residence in sites such as the heart or skeletal (voluntary) muscle, which are low in CXCL12.

In addition to being attracted and retained in tissues that have high concentrations of CXCL12, the CXCR4-bearing cancer cells may respond in other ways. Although this may not be the case for all cancers, in some types (carcinomas of the colon and prostate, for example) there is evidence that once the cells have settled in to their new location, the presence of CXCL12 acting through CXCR4 also enhances their ability to grow and colonize the tissue. In this way, CXCL12 can also be regarded as a growth factor, alongside other polypeptide growth stimulators that participate in tumor expansion.

One additional factor that makes CXCR4 of interest for many different clinicians and researchers is that it is one of the two major coreceptors by which the AIDS virus infects human cells. One of the proteins that is



Chemokine Receptor CXCR4. Figure 2 How CXCR4 and CXCL12 work together to facilitate metastasis. Tumor cells have increased levels of the receptor at their cell surface. When the tumor grows sufficiently for the cancer cells to find their way into the bloodstream, some cells lodge in tissues (e.g., lungs, liver and bone marrow) that have high concentrations of CXCL12, the molecule for which CXCR4 is the receptor. CXCL12 both encourages the entry of cells into the tissue and promotes growth of the cell population, facilitating metastatic spread. Tissues that have low levels of CXCL12 are much less likely to accept metastases.

present within the outer surface of the HIV-1 virus, called gp120, binds to CXCR4, although at a slightly different site to CXCL12. When the virus binds to its major target (the CD4 protein) on susceptible cells, it requires a coreceptor in order to complete its cellular attack. This allows it to complete the molecular changes that allow it to infect the cell. Depending on the exact cell and viral type, the coreceptor may be CXCR4 or another chemokine receptor, CCR5. While the link with AIDS has limited direct relevance to most cancers, the two fields of research have synergized to extend our present understanding of CXCR4.

Chemokines

Definition

The name comes from "Chemotactic cytokines," these small cytokines induce migration of diverse immune cells. The family of the chemokines is quite numerous, as are the chemokine receptors, and often there is "promiscuity," in that a single chemokine can active multiple receptors and multiple chemokines can activate a single receptor. These molecules direct trafficking of leucocytes. Two chemokine receptors are also the principal co-receptors for HIV involved in viral entry: CCR5, expressed on monocytes and macrophages as well as other cells, and the more widely expressed CXCR4. The tropism of specific chemokine receptors is associated with HIV clinical effects, with CCR5 linked to infection and CXCR4 tropism linked to progression to AIDS.

►TAT Protein of HIV

Chemokinesis

►Motility

Chemoprevention

Definition

Chemoprevention involves the use, in healthy people, of natural or laboratory made substances to prevent cancer or reduce cancer risk both in high-risk individuals as well as in the general population. The

aim is to reduce the cancer burden in humans. Most work is being done to reduce the risk for ►oral cancer, ►prostate cancer, ►cervical cancer, ►lung cancer, ►colorectal cancer, and ►breast cancer. The first chemopreventive agent to reach the clinic – and possibly the best known – was ►tamoxifen, which has been shown to cut breast cancer incidence in high-risk women by 50%. It was followed by ►finasteride, found to reduce ►prostate cancer incidence by 25% in men at high risk for the disease. However, the large-scale trials that confirmed these benefits brought to light a troublesome issue: the drugs caused serious side effects in some patients. This is an issue of particular concern when considering long-term administration of a drug to healthy people who may or may not develop cancer. Obviously, this is raising a number of ethical issues. An effective chemopreventive agent should not significantly alter quality of life, and should be ideally inexpensive, safe, well tolerated, and effective in preventing more than one cancer.

Experience with ►celecoxib (Celebrex) and other ►COX-2 inhibitors illustrates the importance of an assessment of the risk/benefit ratio for patients. COX-2 inhibitors have shown impressive efficacy in the prevention of colon cancer and several other forms of cancer, but they also increase the risk of serious cardiovascular side effects.

Attention has focused on ►nutraceuticals and ►phytochemicals as chemopreventive agents. ►Curcumin (found in the curry spice turmeric), has shown dramatic anticancer results in preclinical studies owing to its significant anti-►inflammation properties. Curcumin has been used for thousands of years in the diets of people in the Middle and Far East and therefore is believed to have a low probability of serious side effects. Under investigation for their potential in breast cancer chemoprevention are ►aromatase inhibitors, a class of ►estrogen blockers, which are approved to treat metastatic breast cancer in post-menopausal women. While the idea of cancer chemoprevention is extremely attractive, much research remains to be done to make this a generally applicable option for reducing the human cancer burden. An important element will be to identify informative ►biomarkers to assess individual cancer risk and to possibly provide information of patients tolerance towards individual chemopreventive agents.

►Celecoxib
►Chemoprotectants
►COX-2
►Cyclooxygenase 2
►Detoxification
►Photochemoprevention
►Phytochemicals and Cancer Prevention

Chemoprotectants

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C

Synonyms

Chemoprotection; Chemoprevention

Definition

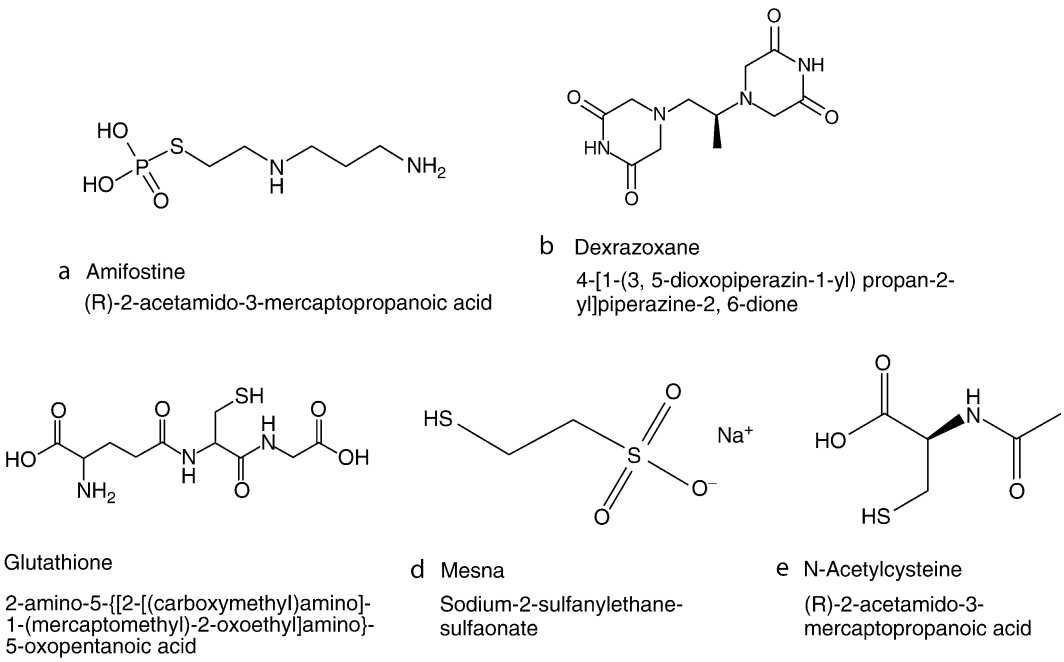
►Chemoprotectants are natural or synthetic chemical compounds which exhibit the ability to ameliorate, mimic, or inhibit the toxic or adverse effects of structurally different chemotherapeutic agents, ►radiation therapy, cytotoxic drugs, or naturally occurring toxins, without compromising the anticancer or antitumor potential of the chemotherapeutic drugs. Chemoprotectants shouldn't affect the ►therapeutic efficacy of the chemotherapeutic agents, radiation or drugs, disrupt the serum enzyme levels, or induce significant injury to the tissues/organs. These chemoprotectants include anticancer, antitumor, anti►angiogenic, and antioxidant compounds and used as an adjuvant in cancer ►chemotherapy.

Characteristics

According to the World Health Organization (WHO), cancer accounts for 7.6 million (or 13%) of all deaths in 2005, and the incidence of cancer is expected to rise with an estimated 9 and 11.4 million deaths from cancer in 2015 and 2030, respectively. Cancer chemotherapy and radiation therapy are the most promising choice available for the cancer patients. The global outlook of cancer therapy has made dramatic improvement since the discovery of various ►synthetic and ►natural chemoprotectants which slows down the progress of this deadly disease and enhances the life span of the cancer patients. Chemoprotectants may exert toxic effects. Thus, it is very important to determine the right dosage and exposure scenario for each chemoprotectant prior to the exposure to demonstrate adequate safety.

Synthetic Chemoprotectants

Amifostine. A white powder, water-soluble organic thiophosphate compound, chemically known as 2-[(3-aminopropyl)amino]-ethanethiol dihydrogen phosphate (ester) or 2-(3-aminopropylamino)ethylsulfanyl phosphonic acid or aminopropylaminoethyl thiophosphate (Fig. 1a), and used as a ►cytoprotective adjuvant in cancer chemotherapy to reduce the incidence of ►neutropenia-related fever and infection caused by



Chemoprotectants. Figure 1 Structures and IUPAC nomenclature of (a) Amifostine, (b) Dexazoxane, (c) Glutathione, (d) Mesna, and (e) *N*-acetylcysteine.

DNA-binding chemotherapeutic agents including cyclophosphamide and cisplatin. ►Amifostine (empirical formula $C_5H_{15}N_2O_3PS$; molecular weight 214.22; trade name Ethyol, synonyms: Ethiofos, Ethanethiol, Gammaphos, WR2721, NSC-296961) is used to decrease the cumulative nephrotoxicity caused by cisplatin in patients with ovarian or lung cancer, as well as to reduce the incidence of moderate to severe xerostomia (dry mouth) in patients undergoing radiotherapy for head and neck cancer. Amifostine is dephosphorylated by alkaline phosphatase in tissues to a pharmacologically active free thiol metabolite, which readily scavenge noxious reactive oxygen species (ROS) generated by exposure to either cisplatin or radiation, as well as detoxify reactive metabolites of platinum and other alkylating agents. Pharmacokinetic studies show that amifostine is rapidly cleared from the plasma with a distribution half-life of <1 min and an elimination half-life of approximately 8 min.

Ethyol is supplied in 500 mg vials and administered intravenously (i.v.). Amifostine induced adverse side effects include nausea, vomiting, flushing, chills, dizziness, shortness of breath, fainting, seizures, cardiovascular problems, skin rash, hives, and swelling of the throat.

Dexrazoxane. A whitish crystalline powder, sparingly soluble in water, and chemically known as (S)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione or 4-[1-(3,5-dioxopiperazin-1-yl) propan-2-yl]piperazine-2,6-dione (Fig. 1b). ►Dexrazoxane (empirical formula $C_{11}H_{16}N_4O_4$; molecular weight 268.28; trade names: Zinecard®, ICRF-187, ADR-529, or NSC 169780, synonym: 2,6-piperazinedione) is a cyclic derivative of EDTA that readily penetrates cell membranes and a potent intracellular chelating agent. Dexrazoxane is used to protect the heart against the cardiotoxic side effects of anthracycline chemotherapy, and to reduce the incidence and severity of ►cardiomyopathy associated with doxorubicin administration in women with breast cancer. Dexrazoxane is hydrolyzed by the enzyme dihydropyrimidine amidohydrolase in the liver and kidney to active metabolites, which have been shown to chelate both free and bound intracellular iron, thereby preventing the formation of cardiotoxic ROS and prevent anthracycline-mediated cardiomyopathy. However, dexrazoxane may potentiate hematological toxicity induced by chemotherapy or radiation. Dexrazoxane is rapidly distributed into body's tissues and fluids, while the highest concentration is found in the hepatic and renal tissues. Urinary excretion plays an important role in the elimination of dexrazoxane (half-life, $t_{1/2}$ 2–4 h). Forty-two percent of the 500 mg/m^2 dose of dexrazoxane is excreted in the urine.

Dexrazoxane is available in 250 and 500 mg for i.v. administration. Adverse effects include alopecia, nausea, vomiting, fever, fatigue, anorexia, urticaria, leucopenia, hematologic thombocytopenia, and ►neurotoxicity.

Glutathione A tripeptide, made of the amino acids γ-glutamic acid, cysteine, and glycine, is the predominant nonprotein thiol and functions as a redox buffer

and exhibit diverse antioxidant activities and protects cells from oxidative stress. ►Glutathione (synonyms: reduced glutathione, monomeric glutathione, GSH; empirical formula $C_{10}H_{17}N_3O_6S$; and molecular weight 307.33) is chemically known as *N*-(*N*-L-γ-glutamyl-L-cysteinyl)glycine (Fig. 1c), while its dimer is known as oxidized glutathione, glutathione disulfide, diglutathione, and GSSG, chemically known as L-γ-glutamyl-L-cysteinyl-glycine disulfide (empirical formula $C_{20}H_{32}N_6O_{12}S_2$). The primary function of GSH is to act as a nonenzymatic reducing agent to help keep cysteine thiol side chains in a reduced state on the surface of proteins. GSH levels in intracellular fluids decline dramatically with advancing age, and thus the ability to detoxify ROS diminishes. GSH is available as a single ingredient ►dietary supplement or in combination products. Daily dosage ranges from 50 to 600 mg daily. No adverse effects were reported.

Mesna. A synthetic sulfhydryl compound, chemically known as sodium-2-mercaptoethane sulfonate or sodium-2-sulfanylethane-sulfonate, and forms a clear and colorless aqueous solution. ►Mesna (HS-CH_2–CH_2SO_3–Na^+; empirical formula $C_2H_5O_3S_2Na$; molecular weight 164.18; trade names: Uromitexan, Mesnex) (Fig. 1d) is a thiol uroprotective chemoprotectant used as an adjuvant in cancer chemotherapy to protect the bladder and kidneys from the urotoxic side effects of the chemotherapy drugs ifosfamide (Mitoxana, Ifex, and Holoxan), trofosfamide (Ixoten), and cyclophosphamide (Endoxan). It was developed as a prophylactic agent to reduce or detoxify the risk of hemorrhagic cystitis and ►hematuria (excretion of blood in urine) induced by ifosfamide or cyclophosphamide, and to decrease the incidence of ifosfamide-associated urothelial toxicity. Hematuria can also happen with higher doses of cyclophosphamide chemotherapy, but is less common. Higher doses of mesna are recommended if blood is detected in the urine. Ifosfamide or cyclophosphamide is converted to urotoxic metabolites such as acrolein and oxazaphosphosphorine metabolites, while mesna neutralizes these metabolites by binding through its sulfhydryl moieties, and increases urinary cysteine excretion. Analogous to the physiological cysteine–cystine system, mesna is rapidly oxidized to its biologically inert disulfide metabolite, mesna disulfide or ►dimesna. Both mesna and dimesna are very hydrophilic and, therefore, remain in the intravascular compartment, where they are rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic ifosfamide metabolites including acrolein and 4-hydroxy-ifosfamide, resulting in their detoxification. After oral administration, mesna has a bioavailability of 50–75% and urinary mesna concentrations are approximately one half of those observed after i.v. infusion. The mean terminal half-life of mesna is 0.4 h, and the half-life of dimesna is 1.2 h.

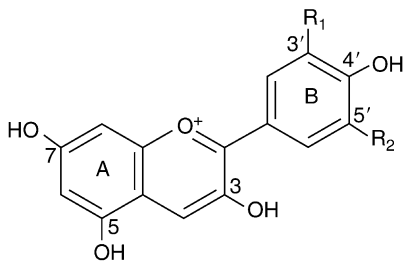
Mesnex Injection contains 100 mg/ml mesna and is recommended for both oral and/or i.v. Adverse effects include nausea, vomiting, taste changes, headache, diarrhoea, weakness, pain, skin rash, itching, irritation, and mood swings.

N-*acetylcysteine*. It is a precursor of intracellular glutathione and cysteine, has an impressive array of mechanisms and protective effects toward DNA damage, carcinogenesis, and other mutation-related diseases. ►*N*-acetylcysteine (empirical formula $C_5H_9NO_3S$; molecular weight 163.19; synonyms: LNAC, NAC, *N*-acetyl-L-cysteine; trade names: ACC, Mucomyst, Acetadote, Fluimucil, Parvolex) (Fig. 1e) is chemically known as (R)-2-acetamido-3-mercaptopropanoic acid and used mainly as a mucolytic (mucus dissolving) in a variety of respiratory conditions or in the management of paracetamol overdose. However, novel applications of NAC, alone and in combination with other anticancer compounds have been shown to be successful in treatment of tumor cell growth. First, it scavenges noxious ROS, and later, NAC is deacetylated in many tissues and cells to form L-cysteine, supporting glutathione biosynthesis that serves directly as an antioxidant or as a substrate in the glutathione redox cycle. Efficacy of different doses of NAC on potent carcinogens such as benzo(a)pyrene, 2-aminofluorene, and alfatoxin B_1 have been reported. NAC, a precursor of intracellular glutathione, is also capable of stimulating phase II enzymes in the glutathione cycle (GSH peroxidase, GSSG, reductase, GSH S-transferase). Repair of DNA damage has also been found to be stimulated by thiols like NAC and glutathione. In a rat hepatocarcinogenesis model, NAC administered by gavage, inhibited the formation of carcinogen–DNA adducts. NAC (250–1500 mg/day) is well-tolerated while mild gastrointestinal upset reported at very high doses.

ORG 2766. A neuroprotective chemoprotectant which slows down the neurotoxic effect or ►neuropathy of the cancer chemotherapy drug cisplatin, while leaving the antitumor activity of cisplatin unaffected. ►ORG 2766, a hexapeptide analog of ACTH-(4-9) [synonym: adrenocorticotropic hormone-(4-9)], prevents taxol-induced neuropathy in rats, and cisplatin-induced ►ototoxicity (ear poisoning). ORG 2766 is given subcutaneously in a dose of 0.25 mg/m^2 (low dose) or 1 mg/m^2 (high dose). No adverse effects were reported.

Natural Chemoprotectants

Berry Anthocyanin. Natural ►anthocyanins (synonyms: anthocyanins, anthocyanidins) including petunidin, malvidin, pelargonidin, peonidin, delphinidin, and cyanidin (Fig. 2), provide pigmentation (color) to fruits (especially berries), vegetables, and red wine, and



R_1 = H	R_2 = H	:	Pelargonidin
R_1 = OH	R_2 = H	:	Cyanidin
R_1 = OH	R_2 = OH	:	Delphinidin
R_1 = OCH_3	R_2 = H	:	Peonidin
R_1 = OCH_3	R_2 = OH	:	Petunidin
R_1 = OCH_3	R_2 = OCH_3	:	Malvidin

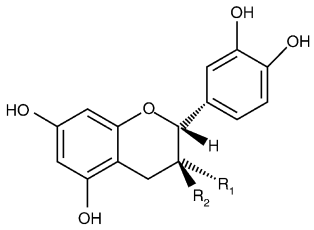
Chemoprotectants. Figure 2 Structures of berry anthocyanins.

demonstrate novel chemotherapeutic, anticancer, anti-inflammatory, and antimutagenic properties. Blueberry, bilberry, cranberry, strawberry, lingonberry, tart cherry, black raspberry, and red raspberry as such, and their extracts, have exhibited potential cancer chemopreventive properties. Extensive studies were conducted on six edible berry extracts including wild blueberry, wild bilberry, cranberry, elderberry, raspberry, seed and strawberry, and accordingly a novel synergistic combination of these six berry extracts known as "►OptiBerry" was developed. The six berry extracts and OptiBerry demonstrated excellent antiangiogenic properties. OptiBerry was also shown to eradicate ►*Helicobacter pylori*, a causative factor for diverse gastrointestinal diseases including gastric cancer. Anthocyanins can be identified in human blood plasma and serum after consumption of berries.

Grape Seed Proanthocyanidins (GSP). OPC is the acronym for "oligomeric proanthocyanidins" [synonyms: procyanidins, grape seed extract, ►grape seed proanthocyanidins (GSP)], a class of polyphenolic bioflavonoids especially found in grape seeds and the bark of maritime pine trees. Catechin, epicatechin, and OPC dimers, trimers, and tetramers are shown in Fig. 3. GSP exhibited excellent free radical scavenging ability and provided significantly better protection as compared to ►vitamin C, ►vitamin E, and ►β-carotene in both in vitro and in vivo models. GSP exhibited significant protection against acetaminophen-induced hepato- and nephrotoxicity, amiodarone-induced pulmonary toxicity, dimethylnitrosamine (DMN)-induced splenotoxicity, cadmium chloride-induced nephrotoxicity, doxorubicin-induced cardiotoxicity, and *O*-ethyl-S, S-dipropyl phosphorodithioate (MOCAP)-induced neurotoxicity in mice. GSP was shown to induce selective cytotoxicity toward cultured human MCF-7 breast cancer, A-427 lung cancer and CRL-1739 gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells and murine macrophage J774A.1 cells. The protective ability of GSP was assessed against chemotherapeutic drug-induced cytotoxicity towards normal human liver cells. Chang liver cells were treated with idarubicin (Ida) (30 nM) or 4-hydroxyperoxycyclophosphamide (4-HC) with or without GSP. GSP dramatically reduced the growth inhibitory effects of Ida and 4-HC on liver cells. Thus, GSP can serve as a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents used in the treatment of cancer. Another study demonstrated that long-term exposure to GSP may serve as a potent barrier to all three stages of DMN-induced liver carcinogenesis and tumorigenesis by selectively altering oxidative stress, genomic integrity, and cell death patterns in vivo. No adverse effect is known.

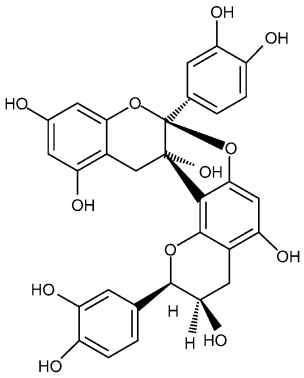
Green Tea Catechins and Polyphenols. The history of tea as a beverage is traced by the Chinese to about 2700 BC. The green tea polyphenols (synonym: green tea extract) are composed of seven different kinds of catechin derivatives including (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate, (−)-gallocatechin-3-gallate and (−)-epigallocatechin-3-gallate, (Fig. 3). ►Green tea catechins exhibit powerful antioxidant, antitumor, and anticancer properties. These help DNA molecules against oxidative damage, as well as eradicate *H. pylori*. High consumption of green tea and a low incidence of prostate and breast cancers have been reported in epidemiological studies. Case-control studies have demonstrated that high consumption of green tea, especially more than 10 cups a day, is associated with cancer chemoprevention, while consumption of five cups lower the risk of esophageal, stomach, and gastric cancer. Green tea polyphenols increase the activity of both glutathione peroxidase and catalase in the intestines, liver, and lungs of mice, and suppress spontaneous mutagenesis mediated by peroxide in the microenvironment of DNA following a substantial reduction of activated carcinogens. EGCG inhibits the growth and causes regression of human prostate and breast tumors. The advantages of cancer chemoprevention with green tea components are safety, economical, and early to mass-produce.

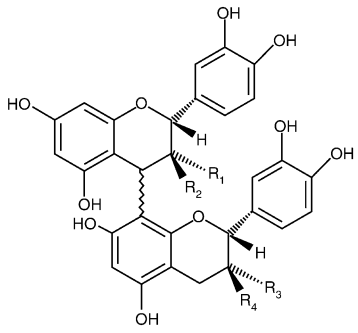
Lycopene. A bright red carotenoid pigment, chemically a terpene assembled from eight isoprene units, is a natural pigment biosynthesized by and accumulated in various fruits mostly in deep-red color of ripe tomatoes, vegetables, plants, and algae. ►Lycopene (empirical formula $C_{40}H_{56}$; molecular weight 536.87; synonyms: all *trans* lycopene) (Fig. 4a) is one of



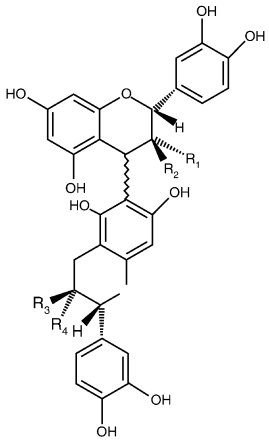
(+)-Catechin: R_1=H; R_2=OH
(–)-Epicatechin: R_1=OH; R_2=H
(–)-Epicatechin-3-O-gallate: R_1=OG; R_2=H
(+)-Catechin-3-O-glucose: R_1=OGlu; R_2=H

Dimer A_2

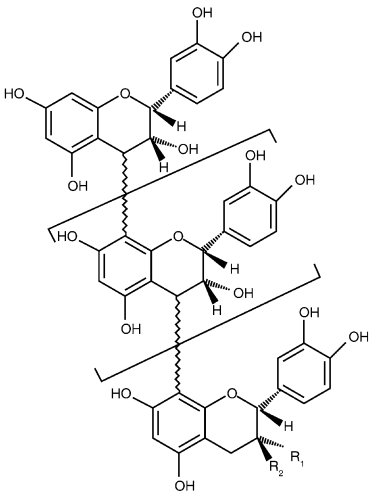




Dimer B_1: R_1=OH; R_2=H; R_3=H;R_4=OH
Dimer B_2: R_1=OH; R_2=H; R_3=OH;R_4=H
Dimer B_3: R_1=H; R_2=OH; R_3=H; R_4=OH
Dimer B_4: R_1=H; R_2=OH; R_3=OH; R_4=H

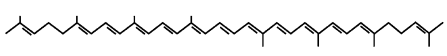


Dimer B_5: R_1=OH; R_2=H; R_3=OH;R_4=H
Dimer B_7: R_1=OH; R_2=H; R_3=OH;R_4=H



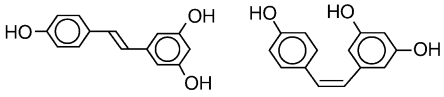
Trimer C_1: R_1=OH; R_2=H; n=1
Trimer EEC: R_1=H; R_2=OH; R_2=OH; n=1
Tetramer EEEC: R_1=H; R_2=OH; n=2

Chemoprotectants. Figure 3 Structures of grape seed proanthocyanidins.



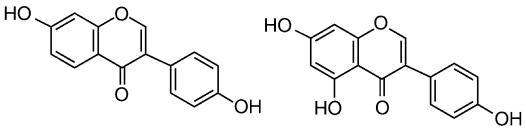
a Lycopene

(6E, 8E, 10E, 12E, 14E, 16E, 18E, 20E, 22E, 24E, 26E)-2, 6, 10, 14, 19, 23, 27, 31-Octamethyldotriaconta-2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30-tridecaene



b *trans*- Resveratrol *cis*- Resveratrol

Trans-3, 4′, 5-trihydroxystilebene; 3, 4′, 5-stilbenetriol; trans-resveratrol; (E)-5-(p-hydroxystyryl)resorcinol; 5-[(E)-2-(4-hydroxyphenyl)-ethenyl]benzene-1,3-doil



c Diadzein
4′, 7-Trihydroxyisoflavone

Genistein
4′, 5, 7-Trihydroxyisoflavone

Chemoprotectants. Figure 4 Structure and IUPAC nomenclature of (a) Lycopene, (b) *trans*- and *cis*-Resveratrol, and (c) Diadzein and genistein.

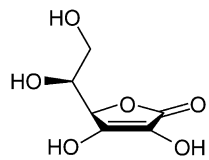
the most potent carotenoid antioxidant in the human body, as well as a potent chemoprotectant. Lycopene exhibits anticancer properties by regulating cancer cell growth by interfering with cell cycle progression thereby inhibiting proliferation. An inverse association exists between intake of tomatoes or plasma levels of lycopene and a lower risk for cancer was strongest for cancers of the lung, stomach, and prostate gland and was suggestive for cancers of the cervix, breast, oral cavity, pancreas, colorectum, and esophagus. Lycopene exhibited a cancer risk reduction of 30–40%. The red color of lycopene is due to many conjugated C = C double bonds, which absorbs most of the visible spectrum. The antioxidant properties are responsible for the anticancer properties of lycopene. Lycopene in combination with vitamin D or ►vitamin E has been reported to inhibit cancer cell growth. It was shown that lycopene, with a half-maximal inhibitory concentration of 1–2 μM, more effectively impaired growth of select cancer cell types as compared with α-carotene or β-carotene. No adverse effects were reported.

Resveratrol. A ►phytoalexin, a natural antibiotic, conferring disease resistance in the plant kingdom and is produced under the conditions of UV radiation, fungal infection, and pathogenic attack. ►Resveratrol (3,4′,5-trihydroxystilbene, 5-[(E)-2-(4-hydroxyphenyl) ethenyl]benzene-1,3-diol; empirical formula $C_{14}H_{12}O_3$; molecular weight 228.25) (Fig. 4b), is mostly found in grapes, berries, nuts, red wine, and Japanese knotweed, and produced with the help of the enzyme stilbene synthase. The *trans*- configuration of resveratrol is the only naturally occurring isomer. Resveratrol was demonstrated to function as a potent antimutagen including the induction of phase II drug-metabolizing enzymes (antiinitiation activity), inhibition of cycloxygenase and hydroperoxidase functions (antipromotion activity), and induction of human promyelocytic leukemic cell differentiation (antiprogression activity). Resveratrol was also found to possess chemopreventive activity by inhibiting ribonucleotide reductase and cellular events associated with cell proliferation, tumor initiation, promotion, and progression. Rapid absorbtion of resveratrol occurs at the intestinal level in both animals and humans, and reaches the highest concentrations in the blood plasma approximately 1 h after administration. No adverse effects were reported.

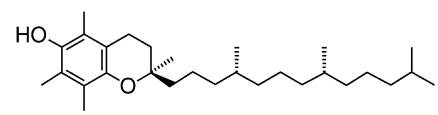
Soy Isoflavonoids. Isoflavonoids, natural plant estrogens (►phytoestrogen), exhibit novel antioxidant and anticancer properties. Soybeans contain beneficial isoflavones such as ►diadzein (empirical formula $C_{15}H_{10}O_4$; molecular weight 254.25; chemical name 4′,7-dihydroxyisoflavone; synonyms: 7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 4′,7-dihydroxyisoflavone) and ►genistein (empirical formula $C_{15}H_{10}O_5$; molecular weight 270.24; chemical name 4′,5,7-trihydroxyisoflavone; synonyms: 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 4′,5, 7-trihydroxyisoflavone) (Fig. 4c). Being a weak form of estrogen, isoflavones can positively interact at estrogen receptor sites and maintain the requisite amount of estrogen in the blood level to reduce the risk factor for breast cancer and menopausal symptoms. Genistein reduces the risk factor for breast and prostate cancer, and slows down the prostate cancer growth and render prostate cancer cells to die. No adverse effects were reported.

Vitamins C, E, and β-carotene. These antioxidants are associated with decreased risk of cancer.

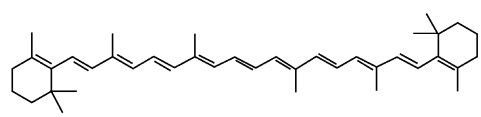
Vitamin C. It is a water-soluble, highly bioavailable antioxidant and chemically known as 2-oxo-L-threo-hexono-1,4- lactone-2,3-enediol *or* (*R*)-3,4-dihydroxy-5-((*S*)-1,2-dihydroxyethyl)furan-2(5*H*)-one (Fig. 5a).



a Vitamin C
2-oxo-L-threo-hexono-1, 4-lactone-2, 3-enediol; (R)-3, 4-dihydroxy-5-((S)-1, 2-dihydroxyethyl)furan-2(5H)-one



b Vitamin E
(R)-2, 5, 7, 8-tetramethyl-2((4R,8R)-4, 8, 12-trimethyltridecyl)chroman-6-ol



c β-Carotene
3, 7, 12, 16-tetramethyl-1, 18-bis(2, 6, 6-trimethyl-1-cyclohexenyl)-octadec; a-1, 3, ,5, 7, 9, 11, 13, 15, 17-nonaene

Chemoprotectants. Figure 5 Structure and IUPAC nomenclature of (a) Vitamin C, (b) Vitamin E, and (c) β-Carotene.

►Vitamin C (synonyms: L-ascorbate, L-ascorbic acid; L-xylo-ascorbic acid; empirical formula $C_6H_8O_6$; molecular weight 176.13) induces antioxidant efficacy in the biological systems. The low one-electron reduction potentials of ascorbate and the ascorbyl radical enable them to react with and reduce ROS and reactive nitrogen species. The ascorbyl radical may scavenge another radical, or rapidly dismutates to form ascorbate and dehydroascorbic acid. Vitamin C acts as a coantioxidant by regenerating α-tocopherol from the α-tocopheroxyl radical. Recommended dietary allowance (RDA) for vitamin C is from 75 to 90 mg/day.

Vitamin E. A fat soluble antioxidant present in cell membranes and lipoproteins, and chemically known as (*2R*)-2,5,7,8-Tetramethyl-2-[(*4R,8R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-chromen-6-ol (Fig. 5b). The term vitamin E (synonyms: α-tocopherol, tocopherol, (±)-α-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol; empirical formula $C_{29}H_{50}O_2$; molecular weight 430.69) describes a family of eight plant-derived antioxidants, α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Only the *RRR*-stereoisomer occurs naturally. Vitamin E inhibits lipid peroxidation by reacting with lipid peroxyl radicals much faster than these radicals can react with polyunsaturated fatty acids to propagate the chain reaction of lipid peroxidation. The α-tocopheroxyl radical is relatively stable and can be reduced back by a coantioxidant such as vitamin C. RDA for vitamin E is 15 mg/day.

β-Carotene. Another fat soluble vitamin present in cell membranes and chemically known as 3,7,12,16-tetramethyl-1,18-bis(2,6,6-trimethyl-1-cyclohexenyl)-octadeca-1,3,5,7,9,11,13,15,17-nonaene (synonyms: β,β-Carotene, Provitamin A, β-cryptoxanthin, all *trans* β-carotene; empirical formula $C_{40}H_{56}$; molecular weight 536.85) (Fig. 5c). Carotenoids, a class of carotenes, are a group of more than 600 naturally occurring pigments, of which only about 50 can be bioconverted to vitamin A. Carotenoids circulate in the blood with lipids in lipoproteins, while liver and adipose tissue are the major tissues where intact carotenoids accumulate. β-Carotene is the primary provitamin A carotenoid in the human diet. Vitamin A plays essential roles in visual function, immune system function, and cell growth and differentiation. The Institute of Medicine recommends 3–6 mg of β-carotene/day.

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C

Chemoprotection

►Chemoprotectants

Chemoradiation

►Chemoradiotherapy

Chemoradiotherapy

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Synonyms

Chemoradiation; Radiochemotherapy; Combined modality treatment

Definition

►Chemoradiotherapy refers to a combination of cytostatic drugs and external beam irradiation, and can be applied sequentially or concurrently. There are several arguments to combine both modalities. While radiotherapy is aimed at controlling the primary tumor, chemotherapy is used to eradicate distant (micro-) metastases ("spatial cooperation"). Both modalities may be active against different tumor cell populations ("independent cell-killing effect"). In addition, chemotherapy may synchronize cells in a vulnerable phase for radiotherapy, decrease repopulation after radiotherapy, and enhance reoxygenation, by shrinking a tumor, which is advantageous for radiotherapy. However, this concept has failed in most clinical trials, probably due to

fast repopulation of tumor cells after cytoreduction with chemotherapy before the start of radiotherapy. In contrast to sequential regimens, concurrent chemoradiotherapy results in a reduction of the overall treatment time, minimizing the risk of clonogenic repopulation. Moreover, it exploits the ability of chemotherapeutic agents to sensitize radio-resistant tumors to the lethal effect of ionizing irradiation.

Characteristics

In this review we will mainly focus on chemoradiotherapy, the combined use of chemotherapy and ►ionizing radiation therapy, in a number of solid tumors. Several clinical trials carried out during the last decades clearly show that concurrent delivery of both treatment modalities significantly improves local control in a variety of advanced solid tumors. In most of these trials, ►cisplatin alone or in combination with other drugs has been used. This has led to improved survival rates in head and neck, lung, and cervical cancer. An additional important advantage of this combined treatment is the possibility to obtain a higher organ-preservation rate, such as in patients with advanced head and neck or anal cancer. Finally, in the preoperative and ►adjuvant therapy setting, concurrent ►chemoradiation has contributed to a better outcome in terms of tumor downsizing/-staging (esophageal and rectal cancer) and survival (►gastric cancer). Major further improvement can be expected from the combination with biological agents that specifically target deregulated pathways in tumor cells. Examples include ►epidermal growth factor receptor (EGFR) inhibitors and ►antiangiogenic drugs.

Chemoradiotherapy for Non-Small Cell Lung Cancer

For many years radiotherapy has been the standard of care for inoperable stage III non-small cell lung cancer (►NSCLC). These patients, however, show a poor outcome with long-term survival rates of 5–10%. Therefore, many groups have explored the possibility to improve these results by adding chemotherapy to the radiation treatment. In 1992, the EORTC reported the results of a randomized phase III study of concomitant cisplatin (weekly or daily) and radiotherapy versus radiotherapy alone in patients with inoperable NSCLC. The results indicated that the combination of cisplatin with radiotherapy was associated with improved survival and control of local disease. The largest and significant benefit was seen in the radiotherapy/daily cisplatin arm. Two recent meta-analyses confirmed the benefit of concurrent cisplatin-based chemoradiotherapy compared with radiation alone and consolidated this regimen as standard treatment for stage III NSCLC. Whether chemoradiotherapy should be given sequentially or concurrently, has also been the topic of several studies. Four randomized trials have demonstrated that concurrent is better than sequential chemoradiotherapy, but is also associated with more acute toxicity, mainly esophageal. So far, no significant increase in late toxicity has been reported.

Chemoradiotherapy for Small Cell Lung Cancer

In the treatment of limited stage SCLC, the central role of chemotherapy has been widely recognized. To define an additional role of thoracic irradiation, several large phase III studies have been performed. These, together with subsequent meta-analyses established the positive impact of thoracic irradiation in combination with chemotherapy in terms of local tumor control and survival. Regarding the timing of both treatment modalities, it has been shown that early thoracic irradiation during chemotherapy is superior to its late scheduling.

Chemoradiotherapy for Cervical Cancer

The introduction of chemoradiotherapy in the treatment of cervical cancer shows many similarities with that in NSCLC: until the 1980s radiotherapy was the standard therapy for patients with locally advanced tumors. Despite modifications of the total radiation dose and overall treatment time, more than 70% of these patients developed a local regional recurrence. Therefore, improvements of these results were sought into the addition of chemotherapy to radiotherapy. In 1999, three articles were published reporting on studies comparing chemoradiotherapy with conventional radiotherapy for locally advanced cervical cancer. In all three studies, the combination of radiotherapy with cisplatin was significantly better than the control arms. An interesting observation came from Rose and colleagues, demonstrating that the single use of cisplatin was as effective as a combination of three drugs, the latter scheme being much more toxic. In the meantime, three additional trials on the concomitant use of cisplatin in cervical cancer have been published, demonstrating now in five out of six trials a significant improvement of local control and survival when concomitant cisplatin and irradiation was used. This is in contrast with eight out of nine phase III studies of ►neoadjuvant chemotherapy prior to radiotherapy, showing no benefit. Based on these results, concurrent chemoradiotherapy is nowadays the standard of care for cervical cancer. Several open questions persist, however. For example, it is unclear whether patients with very large tumors benefit as much from chemoradiotherapy as those with smaller tumors. Also, it remains to be established what the optimal chemotherapy or combination of cytostatic drug is for combined use with radiation.

Chemoradiotherapy for Head and Neck Cancer

Several chemoradiation trials have been conducted in patients with previously untreated head and neck cancer using cisplatin alone; cisplatin and ►5-fluouracil (5-FU); and other combinations. In eight single institutional studies, the average complete response to concomitant therapy was 67.5%. A recent meta-analysis performed by the MACH-NC group concerning the updated results of 63 randomized trials including 10,717 patients demonstrated a clear benefit of 8% ($p = 0.0001$) improved disease-free survival for the concomitant chemotherapy treatment. In the same analysis adjuvant and neoadjuvant chemotherapy showed no improvement. Subsequent trials confirmed that the concomitant use of cisplatin or carboplatin and irradiation leads to improved local cure and survival when compared with radiotherapy alone, including in the postoperative setting.

Despite these encouraging results, cisplatin-based chemoradiation protocols for advanced head and neck cancer are still associated with too many locoregional recurrences. Besides dose-escalation strategies, molecular targeted drugs represent a new and promising approach to further improve treatment results. One of these is the humanized monoclonal antibody directed against the EGFR which is frequently overexpressed in head and neck cancer and associated with chemo-/radioresistance and poor outcome. In 2006 a large multicenter randomized phase III study was published comparing radiotherapy alone with radiotherapy plus cetuximab in patients with locally advanced head and neck cancer. The results were very encouraging and demonstrated that the addition of cetuximab to radiotherapy significantly improved locoregional control and survival. Whether cetuximab (or other EGFR-blocking strategies) can replace chemotherapy or further improve the results when added to chemoradiotherapy, is subject of ongoing trials.

Chemoradiotherapy for Esophageal Cancer

Surgical resection is currently the preferred treatment for ►esophageal cancer. Neoadjuvant chemotherapy may improve the results of surgery and may prevent patients from recurrent disease. However, a recent Cochrane meta-analysis based on seven phase III randomized trials with neoadjuvant chemotherapy failed to demonstrate such a beneficial effect. In a number of studies sequential chemoradiotherapy or concurrent chemoradiotherapy was compared with radiotherapy alone. The RTOG 85-01 phase III study comparing radiotherapy alone with 5-FU/cisplatin-based chemoradiotherapy showed a statistically significant survival difference in favor of the chemoradiotherapy arm. Treatment-related toxicity was increased in the chemoradiotherapy arm, 44% severe and 20% life-threatening side effects versus 25 and 3% in the radiotherapy alone arm. Late toxicity was not increased as has been reported in other studies with concomitant chemoradiotherapy. Al-Sarraf reported on an additional group of patients treated with the same chemoradiotherapy regime. The 5-year survival was 26% versus 0% in the chemoradiotherapy arm and radiotherapy alone arm, respectively. These studies show that concurrent chemoradiation is recommended compared with radiotherapy alone. In most concurrent chemoradiotherapy studies the classic 5-FU/cisplatin regimen has been used. More recently, studies with taxanes as concurrently administered cytotoxic drugs showed promising results. Preoperative chemoradiotherapy with a weekly schedule of paclitaxel and carboplatin appears highly effective in tumor downstaging and obtaining a radical resection.

Chemoradiotherapy for Gastric Cancer

Surgical resection remains the cornerstone of curative treatment of gastric cancer. However, the long-term prognosis remains poor for patients with locally advanced disease. Therefore, different (neo-) adjuvant strategies have been evaluated in the past decades to improve these results. Adjuvant chemotherapy only resulted in a small survival benefit of 3–5% as shown in multiple meta-analyses. Preoperative radiotherapy also showed a small, but significant improvement in survival. Recently, MacDonald et al. performed a randomized phase III study comparing surgery alone with surgery and postoperative adjuvant therapy, combining radiotherapy with 5FU-leucovorin. In this study of 556 patients a statistically and clinically significant reduced risk of relapse and improved survival were observed. Median overall survival in the surgery alone group was 27 months, compared with 36 months in the chemoradiation group. An update of these results provided at the ASCO GI 2004 meeting showed a persistent positive effect with a 3-year survival rate of 41% versus 50% ($p = 0.005$). In 2005 final results of the MAGIC-study on perioperative chemotherapy have been presented. In this large multicenter study patients were randomized between surgery only and three cycles of preoperative ECF (epirubicin, cisplatin, 5-FU) followed by surgery and then another three cycles of ECF chemotherapy. This regimen resulted in a 10% higher resectability rate and a significant survival benefit of 13% (23% vs. 36% at 5 years). Which of both strategies – postoperative chemoradiotherapy or perioperative chemotherapy – is superior, remains to be determined. Since preoperative-combined chemoradiotherapy has shown a beneficial impact on surgical outcome in esophageal and rectal cancer, this is an attractive approach to explore in operable gastric cancer as well. Because of tumor downsizing by neoadjuvant chemoradiotherapy in gastric cancer the rate of tumor negative surgical resection margins will improve.

Chemoradiotherapy for Rectal Cancer

Surgical resection is the only curative treatment for colorectal cancer. However, following resection, local recurrence rate varies between 5 and 40%. Total mesorectal excision (TME), the standard surgical technique for primary resectable rectal cancer, has significantly improved the outcome of this disease, in particular through the realization of free circumferential margins. The Dutch TME trial demonstrated that short-term preoperative radiotherapy is effective in preventing local recurrences, but not in patients with a positive resection margin. Although positive margins can be partly due to poor surgical techniques, they occur more often in locally advanced tumors. For these stages, a more aggressive (neo-) adjuvant approach is required. Postoperative chemoradiation has been mainly evaluated in the United States. The Gastrointestinal Tumor Study Group conducted a four arms study: surgery only, postoperative chemotherapy, postoperative radiotherapy, and postoperative chemoradiotherapy (GITSG 71-75). Pairwise comparisons showed superior survival and local recurrence rates in the chemoradiation arm versus the surgery-only arm. The North Central Cancer Treatment Group compared radiotherapy with postoperative chemoradiation and demonstrated lower local and distant recurrence rates in the combined treatment arm. Survival was significantly increased (NCCTG 794751). The evidence that the addition of chemotherapy to preoperative radiotherapy improves local control rates has recently been provided by two separate trials. The EORTC 22921 trial has a two by two factorial design and randomized between preoperative radiotherapy versus preoperative 5-FU-based chemoradiotherapy. A second randomization took place for postoperative chemotherapy versus no adjuvant treatment. The results demonstrated an increased local control rate for the chemoradiation arm: 92% versus 87%. A similar result was found in the French FFCD 9203 study, which randomized between preoperative radiotherapy and preoperative 5-FU-based chemoradiotherapy, with local recurrence rates of 16.5 and 8%, respectively. All studies that compare preoperative radiotherapy with preoperative chemoradiotherapy demonstrate an increase in toxicity in the combined modality arm.

Recently, it became clear that apart from cytotoxic agents, biological agents may play a role in the achievement of tumor response. In a recent experimental study, ►VEGF blockade enhanced radiotherapeutic activity, probably due to reduction of tumor vascular permeability and tumor interstitial pressure, thereby increasing the delivery of large therapeutic compounds to the tumor. In an early report on a small number of patients treated with the combination of an anti-VEGF monoclonal antibody, bevacizumab, 5-FU and radiotherapy, significant downstaging occurred in all six patients.

Chemoradiotherapy for Anal Cancer

Over the past decades, the treatment of anal cancer has shifted from a surgical approach toward organ-sparing radiotherapy with or without concurrent chemotherapy. It was shown in two randomized studies that concomitant radiotherapy and 5-FU and ►mitomycin C (MMC) is superior to radiotherapy alone and significantly reduced the number of local recurrences. These anal cancer trials also clearly demonstrated the advantage of organ preservation by combined modality treatment as it results in an improved colostomy-free survival. The enhanced acute toxicity which was observed during these combined regimens did not translate in a significant increase in late side effects. MMC has contributed significantly to these results. In a RTOG study patients were randomized to radiotherapy and 5-FU, or radiotherapy, 5-FU, and MMC. The colostomy-free survival rate at 4 years was significantly better in patients who received both 5-FU and MMC compared with those who received 5-FU only (71 and 59%, respectively). In addition, others found that by deleting MMC from a comparable combined treatment protocol the local tumor control rate at 2 years dropped from 87 to 58%. In order to minimize treatment-related toxicity cisplatin has been evaluated as a replacement for MMC, with good results in nonrandomized studies. Randomized trials are now underway to confirm at least equal efficacy of cisplatin and MMC. At the annual ASCO meeting 2006 Ajani et al. reported the first results of the RTOG 98-11 study. This phase III randomized trial compared 5-FU plus MMC and radiation to 5-FU plus cisplatin and radiation in 632 anal carcinoma patients. Preliminary 5-year estimated disease-free, colostomy-free, and overall survival was not significantly different. Hematological toxicity grade 3/4 was significantly more severe in the MMC-containing arm. Based on these results, it was concluded that MMC, 5-FU, and radiotherapy remain the standard of care for patients with anal canal carcinoma. Further improvements in treatment results are expected from the application of novel biological agents.

Concluding Remarks

The combination of radiotherapy and chemotherapy has resulted in a major step forward in the treatment of patients with advanced solid tumors. The recognition that concurrent chemoradiotherapy is superior to sequential regimens may be viewed as one of the major achievements in clinical oncology of the past decades. In general, the interaction between radiation and cytostatic agents is time-, dose-, and sequence-dependent as shown for cisplatin, the most widely used ►radiosensitizer. In the near future, a number of new drugs will

become available for testing in concomitant chemotherapy and radiotherapy approaches. These agents should be selected based upon their mechanisms of action. Given the results of many randomized clinical studies, it is quite likely that chemoradiotherapy will be the standard of care for an increasing number of advanced squamous cell cancers, but until the best regimen of each disease has been determined, there is now more than ever an urgent need to encourage treatment of patients within the framework of carefully controlled clinical trials.

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Chemorepulsive Cues

Definition

Growth factors and ►chemokines that repell cells.

►Plexins

Chemoresistance

Definition

The lack of typical cellular response to a chemical. In the context of cancer, chemoresistance is the property which allows a cancer to progress during chemotherapy which would otherwise cause similar cancers to regress.

►Drug Design

Chemosensibilization

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Synonyms

Resistance modulation; Resistance reversion

Definition

The sensitization to chemotherapeutic agents of resistant tumor cells is known as chemosensibilization.

Characteristics

Chemosensibilization is used when tumor cells no longer respond to chemotherapeutic drugs. This resistance can be inherent to tumor cells or can be acquired during ►chemotherapy treatment, leading to the inefficiency of a wide range of antineoplastic agents. This phenomenon is named multidrug resistance (MDR). Once MDR appears, chemotherapy is not efficient anymore even when using high doses of drugs, which stimulates the resistance mechanism and brings toxic side effects. To overcome this problem, several strategies aiming at restore drug sensitivity are used. The main approach to achieve chemosensibilization is the use of substances capable of bypassing the resistance mechanism, named chemosensitizers or MDR modulators. A typical chemosensitizer should not really have inherent antitumor properties; however, most chemosensitizers exhibit antitumor activity and act synergistically with antineoplastic drugs to kill tumor cells. After the discovery of the mechanisms leading to chemoresistance, much effort has been devoted to discover such candidate molecules. Although numerous chemosensitizers have been developed, few of them have reached clinical trials. MDR is a multifactorial phenomenon and the best characterized mechanisms are resistance to apoptosis and enhanced drug efflux due to the overexpression of ATP-binding cassette transporters (►ABC transporters) such as ►P-glycoprotein (P-gp), MRP, or BCRP in tumor cells.

Chemosensibilization of Tumors by Targeting P-gp

Inhibitors of P-gp Activity

The development of chemosensitizers to overcome chemoresistance mediated by ABC transporters mainly focus on the inhibition of P-gp that is overexpressed in numbers of malignancies (Table 1). Many chemosensitizers are administered simultaneously with anticancer drugs. They overcome drug resistance by functioning as competitive or non competitive inhibitors for P-gp and by binding either to drug modulation sites or to

Chemosensibilization. Table 1 Compounds used to chemosensitize P-gp-mediated MDR tumor cells

Antiarrhythmics	Quinidine, amiodarone, propafenone
Antibiotics	Cephalosporins
Antihistaminics	Terfenadine, azelastine
Antihypertensive	Reserpine
Antimalarials	Quinine, quinacrine, mefloquine
Calcium channel blockers	Verapamil, dexverapamil, nicardipine, azidopine
Calmodulin antagonists	Trifluorperazine, chlorpromazine
Immunosuppressants	Cyclosporin A, SDZ PSC 833, staurosporine, rapamycin
Neuroleptics	Phenothiazine, fluoxetine, haloperidol
P-gp-specific chemosensitizers	MS 209, GF 120918, XR 9576, VX-710, LY 335979
Steroid hormones and synthetic derivatives	Progesterone, tamoxifen
Alkaloids	Cyclopamine, tetrandrine, fangchinoline
Flavonoids and dietary compounds	Quercetin, genistein, curcumin, green tea polyphenols, ginsenoside Rg, indole-3-carbinol, diallyl sulfide
Anti-P-gp antibodies	Monoclonal antibodies, immunization-induced antibodies
siRNA, antisense oligonucleotides	
Anti-MDR1 ribozymes	

other modulator binding sites. Other reversing agents act by interfering with ATP hydrolysis required to P-pg activity.

The first chemosensitizers used to inhibit P-gp-mediated MDR were drugs that possess unrelated pharmacological functions. Among them, verapamil was the first substance that showed chemosensitizing activity. It was originally used as a calcium channel blocker in the treatment of heart disease. Verapamil is a substrate of P-gp and inhibits the transport of chemotherapeutic drugs in a competitive manner without interfering with its catalytic cycle. Cyclosporin A, a commonly used immunosuppressant for organ transplantation, can also sensitize MDR tumor cells by interfering with both substrate recognition and ATP hydrolysis. Unfortunately, the use of these first generation chemosensitizers in clinical studies has been limited. These sensitizers reverse MDR at high concentrations, which brings toxic side effects due to their innate pharmacological function.

The search for nontoxic second generation chemosensitizers resulted in newer analogs of the first generation modulators that were more potent and considerably less toxic. Structural analogs of verapamil show increased reversal activity when used at lower doses and are less cardiotoxic than verapamil. PSC 833, a potent and non immunosuppressive analog of cyclosporine, efficiently reverses MDR and has been used in combination with anticancer drugs in clinical studies. It restores sensitivity to chemotherapy by direct interaction with P-gp. Although these modulators are more efficient than the first generation chemosensitizers, they influence the pharmacokinetics of anticancer drugs, elevating plasma concentration beyond acceptable toxicity.

Third generation chemosensitizers were designed using structure-activity relationships specifically for high transporter affinity and low pharmacokinetic interaction. The latest synthetic compounds, including VX-710, LY 335979, GF-120918, and XR 9576, are currently used in clinical trials in association with anticancer drugs to sensitize tumor cells.

Several other compounds with different pharmacological functions possess chemosensitizing effects in several models of resistant tumor cells expressing P-gp (Table 1).

Other Strategies to Inhibit P-gp

Alternative strategies that include the use of chemicals as chemosensitizers can be applied to restore sensitivity of tumors:

1. *Anti-P-gp Antibodies*. Antibodies specific to P-gp have been developed and are capable of potent reversal of MDR by disrupting P-gp drug efflux activity. These antibodies can be generated by several ways. ►Monoclonal antibodies generated from hybridomas have been developed to target P-gp. The monoclonal antibody UIC2 recognizes a conformational epitope that involves several peptide fragments of the human P-pg. The binding of UIC2 to P-gp induces the blockade of conformational changes required to the activity of the efflux pump, leading to an increase in intracellular accumulation of drugs. Recombinant antibody fragments targeted

to extracellular loops of P-gp are also used in vitro to sensitize MDR cells to chemotherapy, as well as antibodies induced by immunization with P-gp-derived peptides that allow the *in vivo* sensitization of tumor cells to anticancer drugs.

2. *Altered Levels of MDR1 mRNA*. Downregulation of the *MDR1* gene coding for P-gp is another way to overcome chemoresistance. It is based on the use of molecules such as antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and ribozymes whose activity leads to altered level of a specific mRNA (►Antisense DNA therapy, ►RNA interference and cancer). They can specifically modulate the transfer of the genetic information from DNA to proteins. ASOs are short single strand DNAs that hybridize to a unique mRNA sequence. Hybridization of ASOs to mRNA leads to the formation of mRNA/DNA hybrid duplexes that become the target of RNase H, an enzyme that catalyzes the cleavage of RNA in RNA/DNA duplexes. siRNAs are small RNAs duplexes that assemble into a RNA-induced silencing complex (RISC). These complexes target a specific mRNA that is cleaved and degraded. The targeting of a unique mRNA can also be achieved by using catalytic RNAs called ribozymes. These small RNAs hybridize to a complementary sequence of mRNA and catalyze site-specific cleavage of the substrate. Specific ASOs, siRNAs, and ribozymes targeted to the MDR1 mRNA are used to prevent P-gp expression in tumor cells and can improve sensitivity toward chemotherapeutic drugs in resistant tumor cells.

Chemosensibilization of Tumors by Triggering Apoptosis

Tumor cells can become resistant to chemotherapy due to a reduced susceptibility to die by ►apoptosis, by the overexpression of antiapoptotic proteins and activation of prosurvival signaling pathways, or by the downregulation of proapoptotic proteins. Cancer treatment by chemotherapy kills cells principally by inducing apoptosis. Therefore, modulation of the key elements of apoptotic signaling directly influences therapy-induced tumor cell death and represents another way to sensitize tumor cells to apoptosis-inducing drugs. Given that antiapoptotic Bcl-2 family members are overexpressed in many types of cancers, specific ASOs directed against these proteins can be useful to improve drug sensitivity. The administration of Bcl-2 and Bcl-x_L antisenses in combination to anticancer drugs results in a decreased level of these antiapoptotic molecules and the subsequent improved efficiency of drugs. In many cases, complete cure of mice bearing Bcl-2 or Bcl-x_L-overexpressing tumors occurs. Downregulation of other proteins inhibiting apoptosis including survivine and XIAP and silencing of prosurvival signaling mediated by the PI-3kinase/Akt pathway and NFκB can also sensitize tumor cells to programmed cell death

A growing interest has been placed upon the use of dietary polyphenols, which induce apoptosis in cancer cells while they protect normal cells. These compounds not only have the capacity to trigger cell death when used as single agents, but also enhance apoptosis triggered by numerous anticancer drugs in several tumor cell lines by interfering with multiple pathways leading to chemoresistance (►Polyphenols and cancer).

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Chemotactic

Definition

Refers to ►Chemotaxis.

Chemotactic Cytokine

►Chemokine

Chemotactic Factors

Definition

Substances that can either attract or repulse cells. The cells may move towards the source of chemotactic substances (towards an increasing concentration gradient) or away from these factors.

►Inflammation

Chemotaxis

Definition

The characteristic movement or orientation of an organism or cell along a chemical or protein concentration gradient. Attraction indicates on a positive chemotaxis whereas repellence indicates on negative chemotaxis.

►Chemokine
►Chemoattraction
►Chemokine Receptor CXCR4
►Motility

Chemotherapy

Definition

Treatment of disease by chemicals that kill cells.

►Gastrointestinal Stromal Tumor

Chemotherapy of Cancer

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Definition

Chemotherapy is defined as the use of chemical agents for treatment. Chemotherapy as used for cancer generally refers to small molecules that damage proliferating cells. It represents systemic treatment in contrast to radiotherapy and surgery that represent local treatment. Classes of systemic agents may also include hormones, cytokines and vaccines.

Characteristics

The Challenge

Cancer is the most feared, morbid and mortal of diseases. In the United States five million people contract cancer per year, of whom one third, or almost half a million citizens will die of their disease. Most cancers start in a specific location (e.g. breast, lung) and spread to regional lymph nodes; in breast cancer spread is to the armpit and subsequent dissemination by the bloodstream to distant organs. For cancers that are diagnosed before such dissemination, local treatment with surgery and/or radiotherapy may be curative. Most patients who die of cancer die because of disseminated metastatic tumor. These are either clinically present at the time of diagnosis or occur months to years after diagnosis because of microscopic clinically undetectable cancer that only becomes clinically evident following local treatment. Cancer chemotherapy along with hormone therapy and immunotherapy is designed to treat and ideally eradicate metastatic cancer.

The Agents

Most of the currently effective chemotherapeutic agents were discovered by serendipity and/or empiricism (by trial and error). For example, the first effective agent, nitrogen mustard, was a derivative of chemical warfare studies conducted in World War I. Among the side effects of mustard gas was the suppression of normal bone marrow. Because of this, it was given to mice bearing a tumor derived from the bone marrow, i.e. leukemia, and found to be effective. Subsequent clinical trials affirmed this effectiveness. Analogs were synthesized and mustard-like compounds, termed alkylating agents, are effective in many forms of cancer.

The antimetabolites are compounds that are similar to normal metabolites, such that they enter the same metabolic system but because of slight differences, inhibit or antagonize that system. For example, white cells consume high quantities of the vitamin folic acid, and this is particularly true of cancerous white cells, that is leukemic cells. Slight chemical modifications of folic acid have lead to the antifol class of compounds, and these have been found to be active in patients with leukemias, as well as in many solid tumor patients.

Two important classes of compounds are

- The anthracyclines
- The platinum analogs both of which were discovered by serendipity, and developed largely through screening methods. All of the above compounds target DNA and therefore cell proliferation. Another class of compounds target the cytoskeleton of the tumor cell. These are derived from fungi and plants, and include the vinca alkaloids and the recently discovered ►taxol.

These examples of currently used agents, while varyingly effective against human cancers (see below), have a significant limitation in the area of specificity. They attack not only the tumor but also certain rapidly growing normal tissues such as the bone marrow and bowel, and hence produce dose-limiting toxicity relating to depression of the marrow (infection and bleeding), nausea and vomiting and ulceration of the gastrointestinal tract.

The use of high dose combination chemotherapy with stem cell rescue for patients with breast cancer was the subject of considerable enthusiasm during the 1980s and early 1990s. However, in the late 1990s and particularly since the American Society of clinical Oncology (ASCO) reports in 1999 have been considered to be largely ineffective. This paper considers some of the reasons for this change and particularly on the basis of preclinical and clinical models, considers current and future directions. The intensification regimen may produce resistance that could compromise the important intensification component. Microenvironmental and clinical trials of adjuvant chemotherapy strongly indicate that one cycle of intensification is not enough and that two and perhaps three will be required. The components of the intensification regimen are reviewed with respect to dose response and with respect to mechanisms of resistance, cross resistance and potential additive or synergistic effects.

To reiterate, the major limitation of classical cancer chemotherapy is the lack of specificity for the tumor as compared to normal tissue. This limitation is being addressed by basic science particularly relating to molecular biology, a summary of which follows:

Cancer is a genetic disease of somatic cells, following a series of mutations or genetic events incident to lifestyles such as smoking.

►Tobacco carcinogenesis and genetic susceptibility, a sufficient number of events occurs such that a cell becomes transformed into a cancer cell. The vast majority of cancers therefore derive from a single cell. However, the process that produces cancer also results in a marked increase in genetic instability such that daughter cells are variable. This variation permits selection of those daughter variants that have a survival advantage, such as resistance to certain drugs, a higher proliferative thrust or a greater capacity to invade and metastasize. This clonal evolution to heterogeneity is adverse. However, it does lead to events that are unique to the cancer cell, that is, they are not present in normal cells in the same person. For example, chronic myelogenous leukemia is due to white cells being driven to cancer behavior by a product of the fusion of two genes. By advanced pharmacologic techniques including designer drug synthesis and high throughput screening, an agent termed STI571 was developed that inhibits the action of the fusion gene protein product. STI571 has been found to be capable of producing complete regression of leukemia in the majority of patients, and in contrast to essentially all chemotherapy, is non-toxic. There are a number of molecular targets in other tumors that have been identified, and academia and the pharmaceutical industry have given major priority to the development of agents capable of selectively attacking and inhibiting such molecular targets. It is this process more than any other that leads to optimism on the part of cancer investigators concerning the future of cancer treatment.

Clinical Strategies

Although there are numerous different types of cancer, they often share many biological and molecular processes, an important feature to keep in mind.

In the late 1950s a series of integrated clinical trials were conducted. As a result, the cure rate for childhood leukemia increased from 0% to 70% and many scientific principles of cancer therapy were established. These were (in chronological order):

- The application of quantitative clinical trials, involving comparisons and randomizations.
- The use of agents in an appropriate combination can increase the complete remission rate from zero to more than 90%.
- The generation of complete remission as the most powerful discriminant for survival.
- The identification of active agents in an experimental model with the duration of complete remission (DCR) being central parameter of response.
- The use of the DCR model to develop and evaluate optimal doses, schedules, and combinations.
- It was observed that meningeal leukemia occurred with increasing frequency in patients with prolonged complete remission. Pharmacological and clinical trial studies found this be due to the failure of standard anti-leukemia agents to cross the blood/brain barrier. The introduction of intrathecal chemotherapy and radiotherapy to the brain, markedly reduced this type of complication.
- The importance of supportive and symptomatic care; for example, platelet transfusions, antibiotics and anti-emitics, markedly reduced the morbidity and mortality of chemotherapy.

The result of these advances was an increase in the cure rate for childhood leukemia from 0% in 1955, to 35% by 1970, up to 80% within the last 15 years. This experience with childhood leukemia had a profound effect on the field of cancer chemotherapy in general. Most importantly, it established the position that it can be done, that is, systemic cancer could be cured by systemic therapy.

Most patients who die of cancer die because of disseminated metastatic tumor. Today, in major centers and cooperative groups the cure rate of childhood leukemia is close to 80%. It was hoped that the solid tumors would follow on closely behind the leukemias, but they have in the main proven more difficult to treat. This is unfortunate as adult solid tumors such as breast, bowel and lung cancer constitute 80% of all cancer. A major strategy has been to use agents in combination. This is because solid tumor cells are heterogeneous and thus they have multiple targets.

The second rationale for combination chemotherapy is that it works, with essentially all highly effective and certainly curative cancer chemotherapy involving combinations. The best of combinations produce partial responses in 30–50% of patients with, for example, metastatic cancer of bowel and lung. Complete tumor regression rarely occurs.

It has long been known that in experimental tumors, for example in mice, tumor burden is critical to chemotherapeutic effect. Thus, chemotherapy that has a minor effect on palpable tumor, is often curative of the same tumor in microscopic form. This led to the strategy known as adjuvant chemotherapy. Here patients known to be of high risk of having micrometastatic cancer at the time of initial treatment, are given chemotherapy immediately following surgery and/or radiotherapy. This increases the cure rate some 20% for breast and large bowel cancers. In a more recent strategy (termed neoadjuvant chemotherapy), chemotherapy is given prior to surgery. This moves chemotherapy still further forward in the disease. It provides shrinkage of the primary tumor and thus facilitating the use and effectiveness of local treatment. It may decrease the need for radical surgery in certain tumors such as head, neck and bladder cancer. Finally, combination chemotherapy can be given concurrently with radiotherapy as initial treatment. In addition to the above advantages, local control may be superior with many chemotherapeutic agents since some of them, particularly the platinum analogs and fluoriuracil, are highly radiosensitizing.

In addition to combination chemotherapy, dose is a significant factor in cancer chemotherapy. The dose of certain chemotherapeutic agents, particularly the alkylating agents, can be substantially increased if one protects the bone marrow. Protection is provided by harvesting marrow stem cells before the high dose chemotherapy and returning the marrow to the patient following chemotherapy. Such peripheral blood stem cell rescue has been effective in the leukemias and lymphomas, and is under study often in combination with some of the above-mentioned strategies in selected solid tumors.

Supportive Care

Bone marrow or peripheral blood stem cell transplantation is one form of supportive care. The first major advance in supportive care involved platelet transfusions for the treatment and prevention of thrombocytopenic hemorrhage, starting in the late 1950s and early 1960s. The nausea and vomiting associated with cancer, and some forms of cancer treatment, has been markedly reduced by the development of anti-emetics. Pain control has markedly improved and radical surgery has been reduced by neoadjuvant approaches.

Long Term Effects of Cancer Treatment

Perhaps the most worrisome long-term effect has been the development of second cancers, particularly leukemia and leukemia-like illnesses. There is a latent period of 5–10 years for most of these secondary cancers, and for solid tumors it is even longer. Clinical treatment, environmental and genetic factors, and in vitro and in vivo laboratory models are being developed to study these events. The alkylating agents and X-ray are the chief offenders. ►Hodgkin disease was found to be curable by strategies similar to that of acute lymphocytic leukemia, but there was a cumulative long-term risk of secondary cancer. With this knowledge and the development of newer active agents for Hodgkin's disease, the combination regimen that included alkylating agents has been modified without loss of effectiveness, but with major diminution in secondary cancers.

Conclusions

With a marked increase in support for cancer research including both basic and clinical and the extraordinary increase in molecular sophistication of such research, it is expected that major progress in the curative treatment of most, if not all cancers, will be achieved in the next decade. It is the clinical scientist who must translate this progress in basic research to the clinic. This ultimate challenge would require the most sophisticated of treatment methodology and must always be conducted in a setting where the primary beneficiary of such research is the patient.

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Chest Tube

Definition

A plastic tube placed into the chest cavity between the chest wall and the lung, to remove trapped air or fluid. The tube is connected to suction and allows the lungs to reinflate.

►Pleural Effusion

χ-(Chi)-Like Sequence

Definition

In *Escherichia coli*, the recBC dependent recombination system is stimulated near recombinational hotspots called "χ," which consist of the sequence 5Y-GCTGGTGG-3Y and its complement.

►ALU Elements

CHI3L1

►Serum Biomarkers

Child-Pugh Score

Definition

►Hepatocellular Carcinoma

Childhood Adrenocortical Carcinoma

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Definition

Adrenocortical carcinoma (ACC) is a cancer of the cortex of the adrenal gland ►(Endocrine-Related Cancers). There are two types. In one type, the tumor continues to secrete the hormones normally produced by the cortex, including ►glucocorticoids, ►mineralocorticoids and adrenal ►sex hormones. However, these steroids may be produced in excessive amounts, with negative effects on the body. In the other type, the tumor does not produce these hormones and may go undiscovered until it metastasizes.

Characteristics

Incidence

Cancer of the ►adrenal cortex is exceedingly rare: only about 300 cases are diagnosed in the United States each year. ►Adrenocortical tumors (ACT) represent only about 0.2% of all malignancies in children. The frequency of ACT is 0.4 per million during the first 4 years of life, 0.1 per million during the subsequent 10 years, and 0.2 per million during the late teens. These tumors also occur in adults, usually during the fourth to fifth decades of life. The incidence of ACT differs across geographic regions, ranging from 0.1 per million in Hong Kong and Bombay to 0.4 in Los Angeles and 3.4 in southern Brazil.

Causes

Predisposing genetic factors have been found in the majority of children and adolescents with ACT (Table 1).

The most common genetic abnormalities in young children with ACT are germline mutations in various exons of the ►*TP53* tumor suppressor (►Li-Fraumeni Syndrome, or LFS). However, low-penetrant mutant alleles can also reduce, rather than abrogate TP53 tumor suppressor activity and contribute to ACT without being associated with LFS. Strong evidence suggests that one such inherited *TP53* mutation (Arg337His) explains the extraordinarily high incidence of pediatric adrenocortical carcinoma in southern Brazil.

Adrenocortical tumors may also occur in the context of Beckwith–Wiedemann Syndrome (BWS) (►Beckwith–Wiedemann Syndrome Associated Childhood Tumors), which is characterized by a loss of heterozygosity at chromosome 11p15, resulting in the overproduction of ►insulin-like growth factor II (IGF-II) (Insulin-Like Growth Factors) and diminished levels of the cyclin kinase inhibitor $p57^{Kip2}$. ►Carney complex, hemihypertrophy, congenital adrenal hyperplasia, and multiple endocrine neoplasia type I (inherited mutations in the *MENIN* ►tumor suppressor gene) also give rise to pediatric ACT.

Normal Physiology and Tumor Biology

The human fetal adrenal cortex rapidly develops into two morphologically distinct zones (fetal and definitive) and is essentially adult size by midgestation. The outer, definitive zone exhibits high proliferative activity and is thought to be the germinal cell compartment from which the lipid-dense fetal zone cells migrate. Cortisol is also synthesized primarily by the definitive zone.

The inner, fetal zone produces large amounts of dehydroepiandrosterone sulfate (DHEA-S), which maintains placental function and integrity. Soon after birth, the fetal zone atrophies by undergoing massive cell death through an apoptotic mechanism. Remodeling of the cortex in the neonate results in three functional

Childhood Adrenocortical Carcinoma. Table 1 Constitutional syndromes associated with adrenocortical tumors

Condition	Tumor types	Observations
Germline *TP53* mutations, including Li-Fraumeni Syndrome (►IARC TP53 Database) (►p53 Family) (►p53 Protein, Biological and Clinical Aspects)	Adenomas, sarcomas, carcinomas	Penetrance of ACT is about 10% or less
Beckwith–Wiedemann syndrome (Beckwith–Wiedemann Syndrome Associated Childhood Tumors)	Adenomas, carcinomas	ACT is the second most common tumor (~15% of children with this syndrome)
Hemihypertrophy	Adenomas, carcinomas	20% of these tumors are ACT
Congenital adrenal hyperplasia	Adenoma, carcinoma	Very rare occurrence of ACT
Carney complex	Primary pigmented nodular adrenocortical disease	ACT occurs in ~25% of patients; common in children
Multiple endocrine neoplasia I	Nodules, adenomas, carcinomas	ACT very rare in children

regions: (i) zona glomerulosa, (ii) zona fasciculata, and (iii) zona reticularis. The outer zona glomerulosa primarily produces aldosterone, and the zonae fasciculata and reticularis synthesize corticosteroids and androgens, respectively.

Adrenocortical tumorigenesis in children, in contrast to adults, often results in the hyperproduction of steroids, which is readily apparent by marked physical changes (see below and Fig. 1).

The extensive growth of the adrenal cortex during gestation and its postnatal resolution are likely important in the sensitivity of this gland to small losses in TP53 tumor suppressor activity in those carriers of germline *TP53* mutations. An adrenocortical cell that was destined to cease proliferation during the expansion phase or was targeted to die after birth could continue to survive and divide. The timing of tumor development by 3 or 4 years of age is consistent with this hypothesis. The fetal zone or the zona reticularis have been implicated as the source of cells that contribute to ACT formation, although definitive proof is lacking.

Pediatric adrenocortical tumorigenesis relies on the acquisition of multiple genetic hits. In addition to frequent germline *TP53* mutations, *Steroidogenic factor-1* (*SF1*) which encodes a transcription factor required for normal adrenal gland development, is amplified on chromosome 9q34 and overexpressed in ~90% of patients with ACT. Inactivation of the TGFβ-related *Inhibinα (INHA)* on chromosome 2q33 by mutation of one allele and deletion of the remaining wild-type allele is also common in ACT. The gross overexpression of IGF-II in pediatric ACT, regardless of the genetic predisposing factors (LFS or BWS), has been convincingly established. Adult ACT shares this biochemical alteration and clinical trials of drugs to inactivate IGF-II signaling in both childhood and adult ACT are being designed.

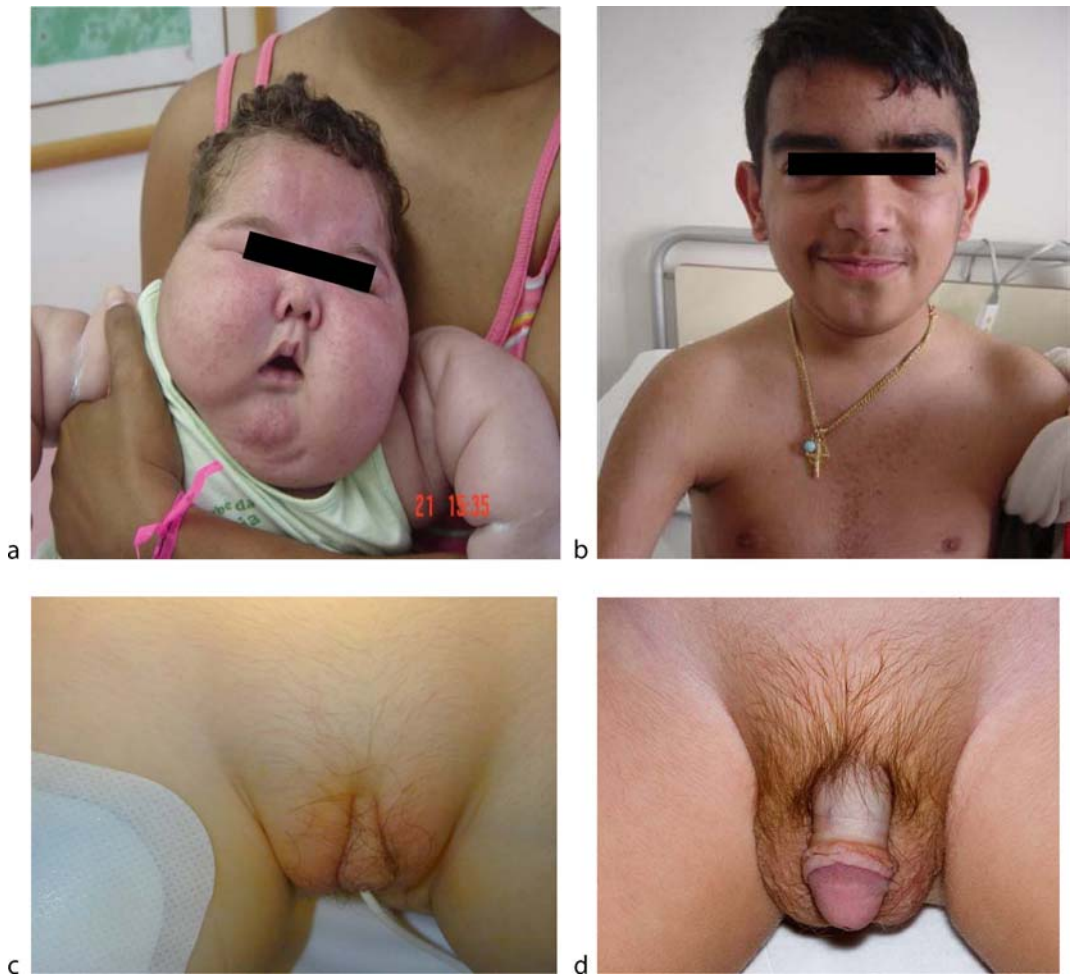
Clinical Manifestations

Features of ►virilization, including pubic hair, facial acne, clitorimegaly, voice change, facial hair, hirsutism, muscle hypertrophy, growth acceleration, and increased penis size are the most common clinical manifestations of ACC. Virilization can be observed either alone (virilizing tumors, 40% of patients) or with clinical manifestations resulting from the overproduction of other adrenal cortical hormones, including glucocorticoids, androgens, aldosterone, or estrogens (mixed type, 45%; Fig. 1). About 10% of patients show no clinical evidence of an endocrine syndrome at presentation (non-functional tumors). Finally, overproduction of glucocorticoids alone (►Cushing Syndrome) is evident in about 3% of patients. Primary hyperaldosteronism (►Conn Syndrome) and pure ►feminization can also occur. In some circumstances, clinical manifestations of ACT can be present at birth.

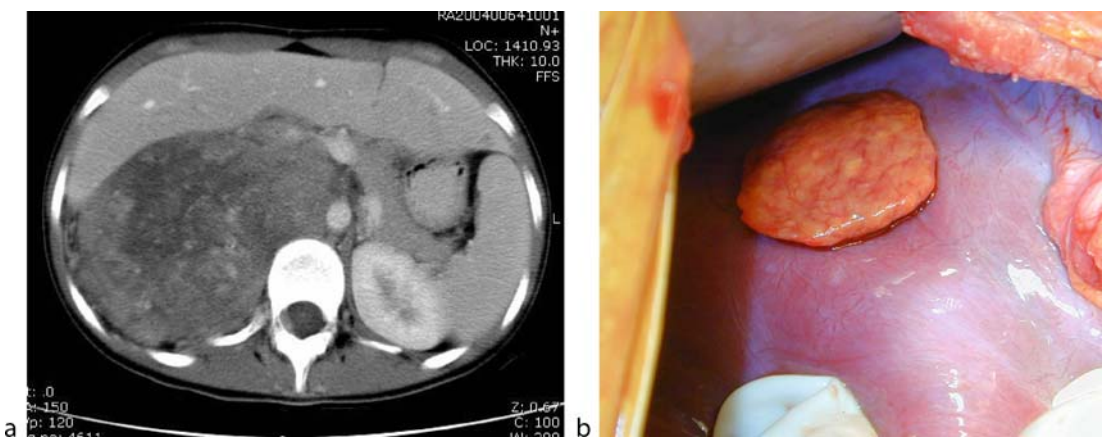
Testing

Routine laboratory evaluation for suspected ACT includes measuring urinary 17-ketosteroids (17-KS), 17-hydroxycorticosteroid (17-OH), and free cortisol, as well as plasma cortisol, DHEA-S, testosterone, androstenedione, 17-hydroxyprogesterone, aldosterone, renin activity, deoxycorticosterone, and other 17-deoxysteroid precursors. Most patients with ACT who are tested have elevated levels of 17-KS. Plasma DHEA-S levels are abnormal in ~90% of cases. Elevated glucocorticoid and androgen levels are strong indications of an adrenal tumor.

Several different imaging modalities are used to diagnose ACT. Computed tomography (CT; Fig. 2), sonography, magnetic resonance imaging (MRI), and



Childhood Adrenocortical Carcinoma. Figure 1 Clinical signs of adrenocortical tumors. (a) Typical facies of a patient with hypercortisolism. (b) Patient with hirsutism. (c) Precocious pseudopuberty (clitorimegaly) in a girl with adrenocortical carcinoma. (d) Precocious pseudopuberty in a boy with adrenocortical carcinoma.



Childhood Adrenocortical Carcinoma. Figure 2 (a) Axial computed tomographic image of a large adrenocortical tumor showing a central area of stellate appearance caused by hemorrhage, necrosis, and fibrosis. Small calcifications are also seen. (b) Regional relapse is apparent adjacent to the liver surface. This patient's initial surgery was complicated by tumor rupture and spillage.

►positron emission tomography (PET) are the most commonly used.

Ultrasound is useful for evaluating tumor extension into the inferior vena cava and right atrium. On CT imaging, ACT is usually well demarcated, with an enhancing peripheral capsule. Large tumors usually have a central area of stellate appearance that is caused by hemorrhage, necrosis, and fibrosis. Calcifications are common. Because ACT is metabolically active, ►fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging is frequently used in patients with ACT. PET imaging can also detect tumor recurrence in areas that routine follow-up CT imaging may miss.

The definitive diagnosis of ACT is made on the basis of the gross and histologic appearance of tissue obtained surgically. Tumors are classified as adenoma or carcinoma, although even an experienced pathologist can find it difficult to differentiate between benign and malignant tumors.

Treatment

Surgery is the mainstay of treatment for ACT. A curative, complete resection may be attempted in patients with local or regional disease (70–75% of cases). *En bloc* resection, including the adjacent structures invaded by the tumor, is required for good local control. Nephrectomy and resection of liver segments and portions of the pancreas may be included. Because of tumor friability, rupture of the capsule with resultant tumor spillage is common (Fig. 2). When ACT is suspected, laparotomy and a curative procedure are recommended rather than fine-needle aspiration, to avoid the risk of tumor rupture.

Infiltration of the vena cava by tumor thrombus occurs in 20% of patients and may make radical surgery difficult; a combined thoracic and abdominal approach may be required in those cases. The pattern of recurrence is locoregional (15–25%), combined local and distant (25–30%), or distant alone (50%). Chemotherapy with ►mitotane is indicated for unresectable and recurrent disease, although it has a small impact on overall outcome. At low doses, mitotane suppresses the secretion of adrenal steroids, providing symptomatic improvement and partial regression of endocrine dysfunction in most patients with functional tumors. Higher doses (>3 g/day) are required for an adrenolytic effect.

Although responses to mitotane alone may occur in 20–30% of cases, most responses are transient, and the prospect for long-term survival is uncertain. The antitumor effect of mitotane is influenced by its pharmacokinetics and by the duration of its therapeutic exposure. Serum concentration plateaus after 8–12 weeks of treatment, and antitumor responses occur only when a serum concentration of at least 14 μg/mL is maintained for a prolonged period. The severe gastrointestinal (nausea, vomiting, diarrhea, and abdominal pain) and neurologic (somnolence, lethargy, ataxia, depression, and vertigo) toxic effects of mitotane reduce patient adherence. Because mitotane is adrenolytic, all patients receiving this agent should be considered to have severe adrenal insufficiency and treated accordingly. ►Cisplatin-based regimens, usually including etoposide and doxorubicin, are used in combination with mitotane, although less than 40% of patients respond.

The use of radiotherapy in pediatric ACT has not been consistently investigated, although ACT is generally considered to be radioresistant. Furthermore, because many children with ACT carry germline *TP53* mutations that predispose them to cancer, radiation may increase the incidence of secondary tumors. For most patients with metastatic or recurrent disease that is unresponsive to mitotane and chemotherapy, repeated surgical resection is the only alternative. However, given the infiltrative nature of the disease, complete resection is difficult. Image-guided tumor ablation with radiofrequency currently offers a valid alternative for these patients.

Prognosis

Complete tumor resection is the single most important prognostic indicator. Patients who have distant or local with gross or microscopic residual disease after surgery have a dismal prognosis. Long-term survival (5 years or more after the diagnosis) is about 75% for children after complete tumor resection. Among those who undergo complete tumor resection, tumor size has prognostic value. The estimated event-free survival is 40% for those with tumors weighing more than 200 g and 80% for those with smaller tumors. Children whose tumors produce excess glucocorticoid appear to have a worse prognosis than children who have pure virilizing manifestations. Classification schemes or disease staging systems (Table 2) are still evolving. Prognosis will likely be further refined by adding other predictive factors, including those from gene expression studies.

Concluding Remarks

Adrenocortical tumors remain difficult to treat, and little progress has been made in developing effective chemotherapeutic regimens. The rarity of ACT hinders the opportunity to conduct adequately powered clinical trials, including biological studies. Therefore, efforts must be coordinated and resources must be consolidated to advance our understanding and treatment of ACT. In this regard, a long-standing international ACT registry and tissue bank has been established [http://www.stjude.org/international-outreach/0,2564,455_2265,00.html]. Short-term goals are to establish tissue culture, xenograft transplants, and genetically engineered mouse models to explore novel therapies. Clinical investigators,

Childhood Adrenocortical Carcinoma. Table 2 Staging criteria for childhood adrenocortical tumor

Stage	Description
I	Tumor totally excised, tumor size <100 g or <200 cm^3, absence of ►metastasis, and normal hormone levels after surgery
II	Tumor totally excised, tumor size ≥100 g or ≥200 cm^3, absence of metastasis, and normal hormone levels after surgery
III	Unresectable tumor, gross or microscopic residual tumor, tumor spillage during surgery, persistence of abnormal hormone levels after surgery, or retroperitoneal lymph node involvement
IV	Distant tumor metastasis

physicians, and basic scientists are encouraged to participate in these studies.

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Childhood Cancer

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Definition

Childhood cancer is defined as cancer occurring in children below 20 years of age.

Characteristics

Incidence

The annual incidence of childhood cancer is 150/million/year. Worldwide, cancer is very rare among children compared to adults. There are variations among different populations in the incidence of certain cancers that can provide some insight into etiology. In the United States of America, it is estimated that about 12,400 children (<20 years) were diagnosed with cancer in 1998, and 2,500 died of cancer in the same year. The probability of developing cancer by age 20 in a newborn male is 0.30% (1:300) and for a newborn female 0.32% (1:333). Cancer related deaths ranked forth in children 1–19 years of age after unintentional injuries, homicides and suicides. Reports from the ►SEER (Surveillance Epidemiology and End Results) program on childhood cancer since the 1970s have shown a gradual increase in incidence (although it appears to have leveled off in the past decade) with a decrease in the mortality rate from cancer (Childhood cancer; Fig. 1).

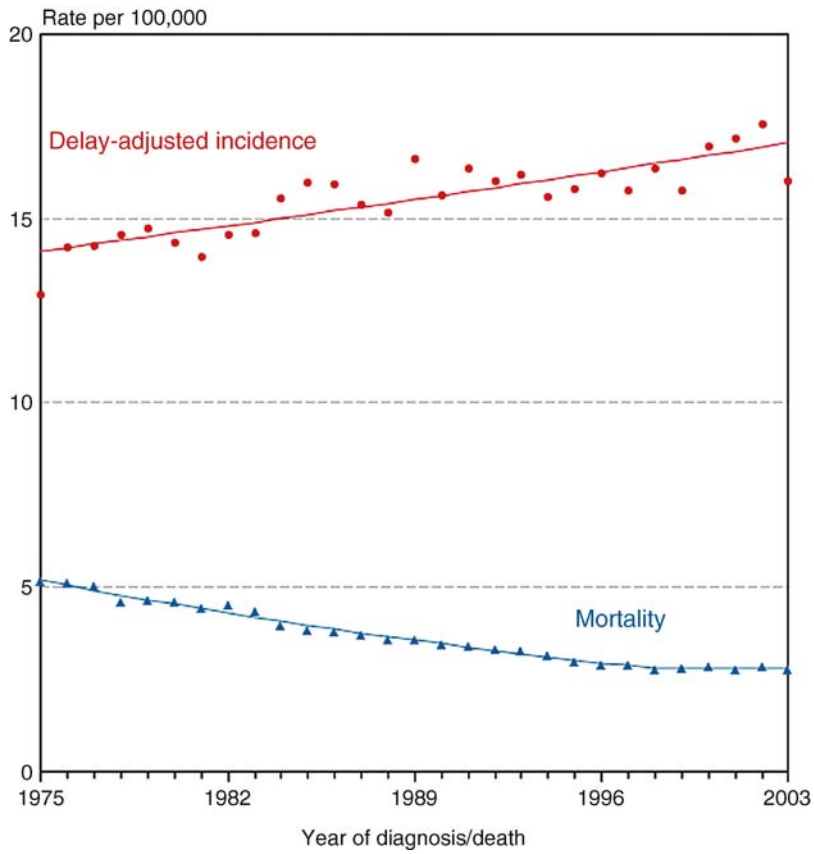
The most common childhood cancer diagnoses are ►leukemia followed by brain tumor and ►lymphoma (Childhood cancer; Fig. 2a and b). The incidence and type of cancers varies with age with certain cancers occurring mostly in early childhood (►retinoblastoma, neuroblastoma, ►Wilms tumor) and others at later ages (lymphoma, bone tumors, germ cell tumors) (Childhood cancer; Fig. 3).

Survival following childhood cancer has improved over the last three decades mainly due to increased survival of childhood acute lymphoblastic leukemia (►ALL) with introduction of multi-agent chemotherapy drugs, central nervous system prophylaxis and most recently development of risk adapted therapy. Additionally, the improvement in supportive care has contributed to an improved survival in all types of childhood cancer.

Causes and Risk Factors

Identifying the causes of cancers in children remains a great challenge for physicians and researchers. However, studying epidemiologic patterns can shed some light over possible causes occurring alone or as part of multiple risk factors that work together and lead to cancer. Some of these factors are discussed below:

1. *Socioeconomic Conditions*: Geographical variations in childhood cancers reflect environmental factors that may play a crucial role in the development of



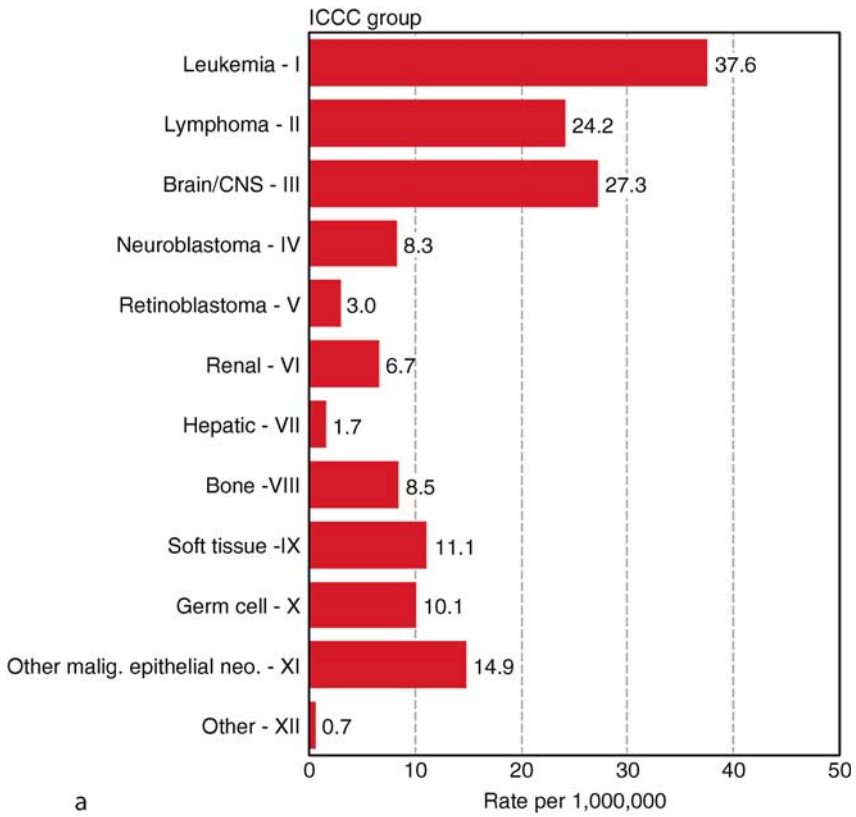
Childhood Cancer. Figure 1 SEER delay-adjusted incidence and US mortality all childhood cancers, under 20 years of age, both sexes, all races, 1975–2003.

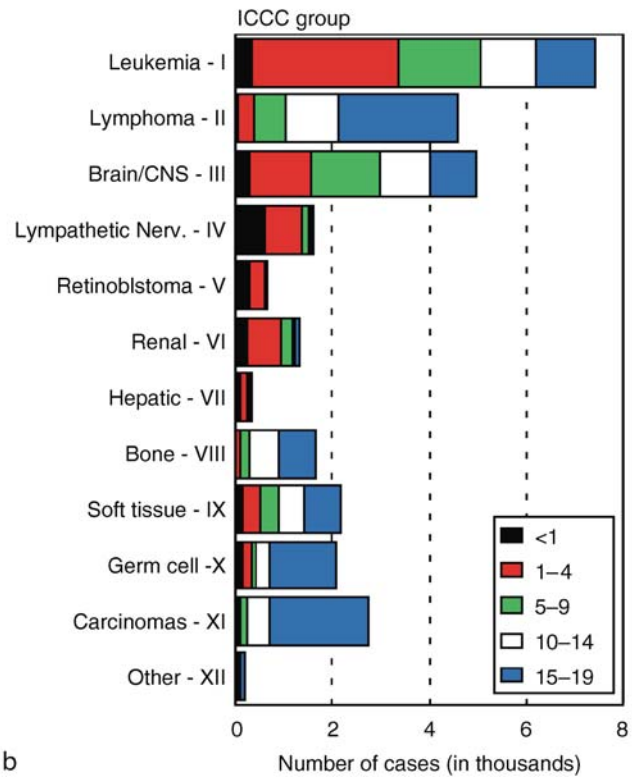
such cancers. The predominant childhood cancers among whites in Europe, the Americas, Oceania, and in East Asia are leukemias accounting for 35–50/million. Lower incidence is seen in South Asia, Middle East, American Blacks and sub-Saharan Africa (<30/million). This variation is mostly due to acute lymphoblastic leukemia (ALL) which accounts for 80% of all childhood leukemias. Higher incidence occurs in higher levels of socioeconomic development. The commonest subtype of ALL (►pre-B ALL) seen in ~70% within the high incidence populations occurs in only a third of cases in the lower-incidence populations where ►T-cell ALL has a higher frequency. With improving socioeconomic conditions, a similar trend is seen as with increasing early childhood peak of common ALL suggesting a strong association between environmental factors and the etiology of ALL.

2. *Infections*: Epidemiological studies of the incidence of Hodgkin's lymphoma (►HD) worldwide have shown the strong association of poor socioeconomic conditions and Epstein-Barr virus (►EBV) infection. EBV is implicated in more than 50% of cases of HD worldwide and EBV positivity in malignant cells is commonly seen in mixed cellularity HD and in cases from developing countries. The development of HD may be a response to both environmental factors and EBV infection.

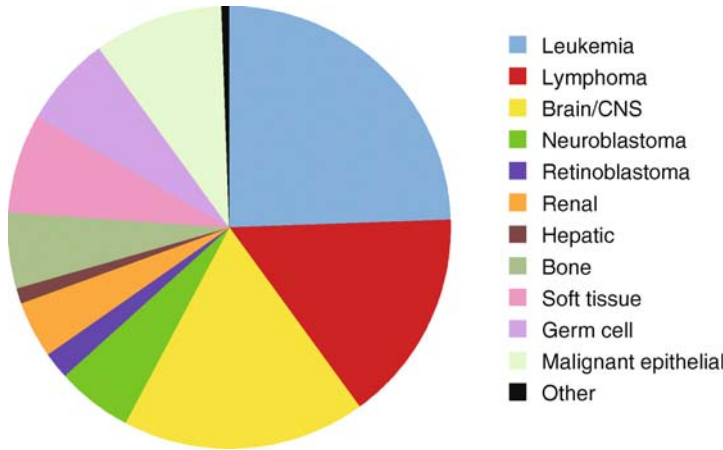
Burkitt Lymphoma (►BL) has also been strongly linked with EBV infection and malaria as a cofactor as seen in all BL cases in tropical Africa and in Papua New Guinea. In other areas of the world, EBV is associated with 50–70% of BL in North Africa and South America and 20% in Europe and North America. In addition, BL can be identified by breakages and translocations involving chromosome 8. Nasopharyngeal carcinoma has the highest incidence in North Africa (3/million) and is also associated with EBV infection. Other unidentified factors may play a role.

Kaposi Sarcoma is the most common soft tissue sarcoma among children in sub-Saharan countries. The incidence with endemic ►HIV (human immunodeficiency virus) infection was 2–2.5/million and this increased to 10–50/million due to the AIDS (acquired immunodeficiency syndrome) epidemic and heightened infection with ►HHV8 (human herpesvirus 8) virus.





Childhood Cancer. Figure 2 (a, b) Childhood cancer SEER incidence rates by ICCC group, 1975–2003, under 20 years of age, both sexes, all races.



Childhood Cancer. Figure 3 Number of cases of all childhood cancers by ICCC and age group, all races, both sexes, 1975–1995.

Hepatocellular Carcinoma (►HCC) is a rare hepatic tumor in Europe and North America, but is still the most common childhood liver tumor in Saharan Africa, East and South East Asia and Melanesia and is associated with chronic hepatitis B infection. Mass immunization against Hepatitis B in Taiwan resulted in a decreased incidence of HCC.

3. *Radiation Exposure*: Following the Chernobyl explosion, areas in Belarus, Russia and the Ukraine contaminated with radiation fallout, reported a significant increase of more aggressive forms of thyroid carcinoma. The incidence fell to pre-Chernobyl levels in children conceived after the explosion.
4. *UV Light Exposure*: Malignant Melanoma has an incidence of 1/million in children. The highest rate is seen in Oceania and is attributable to heavy sunlight exposure in this region.
5. *Ethnic Factors*: Wilms tumor occurs at the lowest rates in East Asia. This low incidence continues in ethnic East Asian groups in California and Hawaii (<5/million) compared to blacks from US and Africa (9–12/million) and Whites (6–10/million) suggesting the role of genetic factors in the incidence of certain cancers. Ewing's Sarcoma is another example of a disease that has markedly different incidences based on race; it is extremely rare in Blacks and East Asians compared to Whites.

Genetic and Chromosomal Syndromes

Genetic factors play a crucial role in the development of cancer. There are several genetic diseases that are associated with increased risk of cancers. Familial neoplastic syndromes, inherited immunodeficiency and bone marrow failure syndromes, miscellaneous genetic syndromes and numerical chromosome abnormalities will be discussed:

1. *Familial Neoplastic Syndromes*: Heritable Retinoblastoma is the classic example where two mutations involving the *RB1* gene locus result in retinoblastoma. Heritable disease is bilateral and is seen often with a family history of retinoblastoma. The first *RB1* mutation is pre-zygotic and is usually inherited, however, sometimes a rare germ-cell mutations can occur. The inheritance is autosomal dominant with 90% penetrance. Additionally, patients with mutant *RB1* are at an increased risk for osteosarcomas in the first three decades of life as well as other types of cancers with advancing age as melanomas and carcinomas of the lung and bladder. Familial Wilms tumor is rare compared to retinoblastoma, occurring in 1.5% of children diagnosed with Wilms tumor in the US. Two genes are associated with familial Wilms tumor; *FWT1* on chromosome 17q12–21 and *FWT2* on chromosome 19q13, but clear patterns of causation are not established. The Li-Fraumeni cancer family syndrome (►LFS) increases the risk of childhood cancers (relative risk of 20) and results in soft tissue sarcoma, adrenocortical carcinomas, premenopausal breast carcinoma, central nervous system tumors and osteosarcomas. Germline mutations of the *TP53* tumor suppressor gene are seen in 77% of LFS families. Other familial neoplastic diseases include Neurofibromatosis type 1(►NF1) that increases the risk for brain tumors (relative risk of >40) and similarly for soft-tissue sarcomas. NF1 patients also have increased risk for juvenile myelomonocytic leukemia (relative risk of 200) and ALL and non Hodgkin's Lymphoma (►NHL) (relative risk of 5). Neurofibromatosis type 2 patients have an increased incidence of meningiomas but less frequently than NF1.

2. *Inherited Immunodeficiency Syndromes*: There is an increased risk for leukemias and lymphomas in patients with inherited immunodeficiency syndromes accounting for a very small fraction (<0.1%) of childhood cancers. Children with ataxia telangiectasia (AT) have a 10% chance of developing lymphoma or leukemia in their first 15 years of life. Similarly, patients with Wiskott-Aldrich syndrome have an increased incidence of NHL. Other immunodeficiency diseases as Bloom syndrome, common variable immunodeficiency, X-linked agammaglobulinemia, IgA deficiency, severe combined immunodeficiency syndrome (►SCID), Duncan's disease and Nijmegen breakage syndrome are associated with a high risk of ALL and NHL. Blooms syndrome is also associated with increased Wilms tumor and osteosarcoma.
3. *Bone Marrow Failure Syndromes*: The bone marrow failure syndromes that demonstrate an increased risk for childhood cancer include: Fanconi anemia (►AML, hepatocellular carcinoma), Diamond-Blackfan anemia (AML, Osteosarcoma), and Shwachman-Diamond syndrome (myelodysplasia).
4. *Non Neoplastic Genetic Syndromes*: Many other syndromic diseases cause patients to have an increased risk of childhood cancer including: Xeroderma Pigmentosa (skin carcinoma and melanoma), WAGR syndrome (Wilms tumor), Denys Drash syndrome (Wilms tumor), Beckwith-Widemann syndrome (Wilms, hepatoblastoma, neuroblastoma, pancreatoblastoma).
5. *Numerical Chromosomal Abnormalities*: Down syndrome (trisomy 21) accounts for the largest number of cancers including leukemias with a 50-fold risk in the first 5 years of life and tenfold risk in the next 10 years. Less commonly, Down syndrome patients are diagnosed at a higher frequency with germ cell tumors, lymphomas and retinoblastoma. Patients with trisomy 18 have an increased risk of Wilms tumor. Turner's syndrome patients are at increased risk for Neuroblastoma and Wilms and patients with Klinefelters have increased risk of germ cell tumors.

Clinical Presentation and Diagnosis

The presenting symptom of childhood cancer can mimic many of the common childhood diseases and may be difficult to diagnose. Different cancers have different symptoms related to the site of disease and type of cancer. The most commonly diagnosed childhood cancer is acute lymphoblastic leukemia (ALL). ALL usually presents with symptoms related to bone marrow infiltration as anemia (fatigue and pallor), neutropenia (fever and infection), and thrombocytopenia (bleeding and petechial rash). A relatively common misdiagnosis occurs when patients present with generalized aches and pains, specifically bone pains secondary to leukemic infiltration, but lack peripheral blood abnormalities. Constitutional symptoms with weight loss, fever and anorexia are frequent symptoms of metastatic disease. Extensive evaluation for an underlying etiology should occur when unexplained symptoms in children last longer than 10–14 days.

Solid tumors present with signs and symptoms related to the organ involved. Brain tumors, the commonest solid tumors of childhood and second common childhood cancer after ALL, usually present with symptoms related to increased intracranial pressure as headaches, vomiting, seizures and change in the level of consciousness, or other neurological signs. The presence of headaches along with other neurological symptoms, such as facial nerve palsy or ataxia warrants more urgent further evaluation.

Young children can present with abdominal masses that may be noticed by the parents, or an adult who gives the child a bath, as a protuberant abdomen. Abdominal masses may cause symptoms such as pain, vomiting, constipation or intestinal obstruction. Abdominal masses can be diagnosed as Wilms tumor, Neuroblastoma, lymphoma or sarcoma, and can sometimes be benign. This heterogeneity of etiology makes surgical biopsy critical when an abdominal mass is discovered. Other symptoms as hypertension and hematuria may develop with renal tumors and neuroblastoma.

Children may present with enlarged lymph nodes in the neck, axilla or groin. The nodes may be asymptomatic, painful or compress local structures as blood vessels and nerves. Lymph node enlargement within the chest or the presence of a mediastinal mass may result in cough and dyspnea. These presentations are common with lymphomas that may also be associated with constitutional symptoms as fever, weight loss and night sweats.

Tumors of the extremities may present with a localized swelling with or without pain, and often unrelated to trauma. Sometimes, a pathological fracture draws attention to a bone tumor.

Primary care physicians are often the first to suspect such a diagnosis. Although many of these symptoms can be seen in common childhood illnesses, it is the duration, unusual associations, course and quality of such symptoms that alerts the physician to the possibility of an underlying oncologic disorder. Once this is determined, then the child needs to be referred to a Pediatric Hematology and Oncology Center urgently for further evaluation to establish the diagnosis. Such an evaluation would include the following:

1. Complete blood counts
2. Liver and kidney function tests
3. Radiological evaluation: X-rays, CT scans, MRI, PET scan, bone scan and MIBG scans as determined by symptoms and location

4. Bone marrow examination or surgical excision or biopsy of the suspected lesions

Treatment

Children with cancer are best cared for at a pediatric comprehensive cancer care center that includes a team of pediatric oncologists, nurses, surgeons, radiation oncologists, pathologists, and supportive care services for the patients and their families. Treatment includes a combination of chemotherapeutic agents with or without local radiation therapy and surgery. Chemotherapy is usually given intravenously, intraspinally, intramuscularly and by mouth. The specific agents depend on the disease being treated based upon 40 years of clinical trial experiences. Side effects include hematological toxicities (anemia, neutropenia and thrombocytopenia), nausea and vomiting, alopecia, infections, cardiac, renal and liver toxicities depending on the chemotherapeutic agents used. Response to treatment is assessed during therapy and treatment is adjusted based upon tumor response and patient side effects.

Outcome and Late Effects

Improved childhood cancer care including the development of combination of chemotherapeutic regimens, supportive care guidelines to minimize infection and bleeding resulted in a dramatic improvement in survival and cure of most childhood cancers and a corresponding drop in the mortality rate (childhood cancer; Fig. 1). However, physicians have become aware of late side effects to therapy that became apparent as early as weeks following completion of treatment up to several decades later. Some of these side effects were related to the cumulative dose of chemotherapy agents and resulted in dose modification or replacement in newer regimens to minimize immediate and late toxicity without affecting efficacy. Similar modifications have been made with radiation therapy by instituting techniques to improve delivery of radiation to the tumor and minimizing damage to neighboring healthy tissues (involved field radiotherapy) as well as reducing the total dose of radiation delivered.

Currently, several pediatric oncology centers have established clinics for childhood cancer survivors where they are followed for life. Patients are given advice about their risks of developing secondary cancers, long term organ toxicities (cardiac, pulmonary, neurological, hepatic and renal), fertility and psychosocial late effects.

Newer clinical trials are underway and will provide more information about childhood cancer survivors and their late effects.

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Chimeric

Definition

A mouse that carries a transgene in some tissues but not others; a mosaic animal.

►Mouse Models

Chimeric Antibodies

Definition

Hybrid immunoglobulins in which the original murine variable regions are preserved and the constant regions are switched for those of a human antibody to try to gain human effector functions. *Applications:* Antibody therapy when effector and other Fc-associated functions and properties are needed.

►Immunotherapy
►Diabody

Chimeric Antigen Receptor on T Cells

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Synonyms

CAR

Definition

A chimeric antigen receptor (CAR) consists of an extracellular antigen-binding exodomain, typically derived from a single-chain antibody fragment (scFv) of a monoclonal antibody (mAb), a spacer (such as an antibody Fc region), a transmembrane region, and one or more intracellular signaling endodomains, which can be genetically introduced into hematopoietic cells, such as T cells, to redirect specificity for a desired cell-surface antigen.

Characteristics

Background on Manipulating T-Cell Responses to Cancer

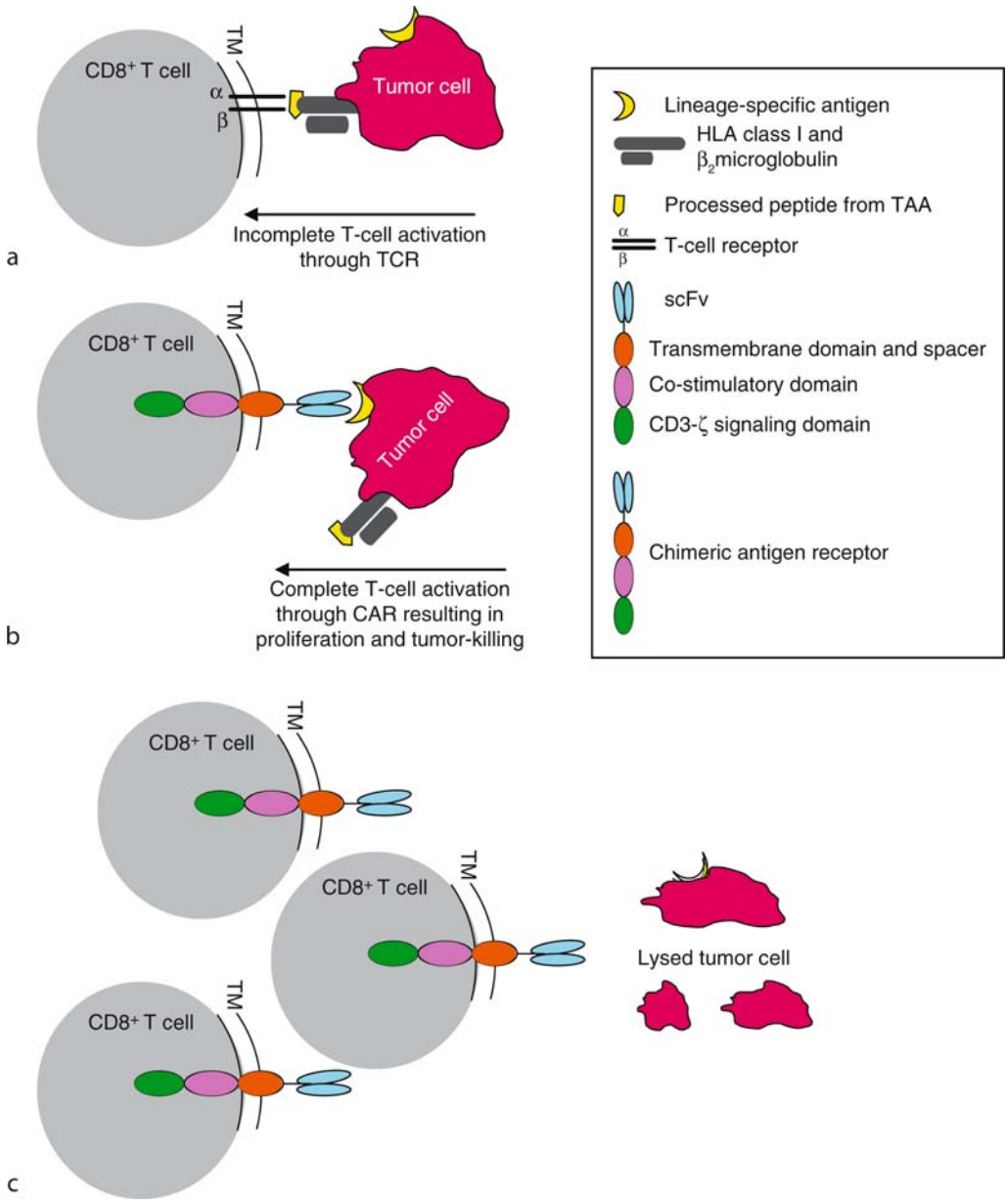
Adoptive transfer of tumor-specific T cells in mouse models can function as potent anti-cancer biological agents leading to elimination of established malignancies. Continued advances in tumor immunology support the premise and promise for ►adoptive immunotherapy as a treatment for human malignancies. Yet, infusion of tumor-specific T cells has only been partially successful in clinical oncology trials. Indeed, most of these trials demonstrate the safety and feasibility of infusing T cells, but with the exception of treating melanoma and chronic myelogenous leukemia (CML), only occasionally show a sustained anti-tumor effect. In contrast, infusing viral-specific T cells has successfully treated and protected patients from opportunistic diseases associated with adenovirus, CMV, and EBV. Why is it that augmenting an immune response against neoplasms by infusing tumor-specific T cells has proven more challenging than engendering an effective anti-viral response? The answer is partly due to the relative inability of T cells to recognize, via an endogenous T-cell receptor (TCR), poorly-immunogenic tumor-associated antigens (TAAs) compared with the highly-immunogenic/stimulatory viral antigens presented in the context of human leukocyte antigen (HLA). While TAAs generally have little or no expression in normal post-natal tissues outside of sanctuary sites, naturally-arising T cells are typically not reactive to tumors expressing a TAA due to immunologic tolerance. However, investigators have been able to manipulate T cells into recognizing TAA in the context of HLA molecules. This has been exploited by injection/infusion of (i) vaccines presenting TAA to overcome tolerance and stimulate T-cell immunity to tumors, (ii) tumor-specific T cells which have been culled from the patient and massively expanded in the laboratory, and (iii) T cells that have redirected specificity for tumor by genetically introducing pre-defined tumor-specific immunoreceptor genes. Clinical trials are currently evaluating all of three approaches.

While vaccines will likely have application for cancer prevention (►cancer vaccines), vaccination to eradicate most established tumors is a difficult challenge at this time due in part to iatrogenic damage of the underlying immune system from chemotherapy and accumulation of large tumor masses in sanctuaries which are functionally protected from immune recognition and response. Rather than vaccinate, or addition to vaccination, investigators have chosen to augment T-cell response through infusion of effector T cells (adoptive immunotherapy). Indeed, isolation, *ex vivo*-expansion of autologous tumor-infiltrating cytotoxic T-lymphocytes (CTLs), and subsequent transfer of these CTLs in lymphodepleted patients can mediate regression of metastatic melanoma. The widespread therapeutic success of adoptive immunotherapy using T cells that have not been genetically manipulated is, however, limited because of (i) difficulties in obtaining sufficient number of tumor-reactive CTLs from patients, (ii) TAAs are typically poor immunogens, (iii) existence of immune-regulatory mechanisms that prevent T-cell dependent reactivity against TAA (such emergence of tumor escape variants with loss of HLA), and (iv) the requirement that patients have pre-existing tumor-reactive cells that can be expanded *ex vivo*.

To overcome these hurdles investigators have combined the endogenous effector function of CTLs with re-directed antigen specificity that results from genetic introduction of an immunoreceptor. This introduced immunoreceptor transgene can be engineered *ex vivo* to provide non-physiologic signaling via a chimeric antigen receptor (CAR) that co-opts the ability of recombinant antibody to bind to tumor targets leading to T-cell activation. The development of CAR^+ T cells can overcome the relative inability of antibodies to localize to and penetrate into tumor masses and provides a self-renewing source of chimeric antibody linked to T-cell effector function.

Tumor-Specific CAR Development

Generally, a CAR consists of an extracellular domain composed of a single chain variable fragment (scFv) derived from a monoclonal antibody (mAb) against a cell-surface tumor-antigen which is typically a lineage specific molecule, such as CD19 on B cells. The scFv is typically suspended from the cell surface by a spacer (e.g. mAb Fc region) and uses a transmembrane (TM) region (e.g. from CD4 or CD28) to affix the scFv–Fc to the cell surface. This TM region is in turn fused in frame to one or more signaling modules that are normally present in an endogenous TCR signaling complex, such as the CD3-ζ chain (Fig. 1). The CAR can confer scFv-mediated antigen-recognition to T cells that is independent of HLA on tumor cells and endogenous TCR. T cells genetically modified to express a CAR can be propagated *in vitro* and demonstrated to exert robust CAR-dependent effector function. Upon antigen-mediated cross-linking of the CAR, the intracellular



Chimeric Antigen Receptor on T Cells. Figure 1 (a) Incomplete activation of T cells recognizing TAA through αβ TCR in context of HLA class I. (b) Fully-competent activation signal by T cells recognizing lineage-specific antigen (e.g. CD19) independent of HLA by introduced CAR. (c) Complete CAR-mediated activation results in T-cell proliferation and tumor lysis.

signaling domain or domains initiate cellular activation which can result in proliferation, cytokine secretion, and specific cytolysis of the antigen-expressing target cells. Several requirements need to be met to enable T cells expressing CAR to exert a pronounced therapeutic effect. These include, (i) expression of the CAR at sufficient density to activate effector function upon binding antigen, (ii) generation of clinically-meaningful numbers of T cells suitable for infusion, (iii) traffic T cells to and within tumor stroma, and (iv) conditional activation upon antigen binding, manifesting appropriate T-cell effector mechanisms such as cytokine secretion, proliferation, and cytolysis leading to tumor destruction. We and others have generated a panel of CARs and demonstrated that T cells (and NK cells) equipped with CAR mediate a highly efficient anti-tumor immune response against antigen-defined tumor target cells *in vitro* as well as *in vivo*. These CAR-grafted T cells, thus are appealing candidates for adoptive immunotherapy.

Clinical Application of CAR-Specific T Cells

Adoptive transfer of T cells for treatment of tumors is an attractive therapeutic option as it has the potential to cure disease refractory to conventional therapies. Successful allogeneic hematopoietic stem-cell transplantation (HSCT) with engraftment of donor derived tumor-specific T cells and adoptive transfer of T cells genetically rendered specific for melanoma antigens currently provide the two cornerstones for the rational application of adoptive transfer of T cells genetically modified to express CAR. Along with other investigators, we have chosen to develop CD19-specific CAR, since the CD19 molecule is widely expressed on most cancers arising from B cells. B-cell tumors are as a class poorly immunogenic with few described TAA, thus isolating and expanding endogenous T cells with specificity for malignant B cells has proven difficult in the context of allogeneic HSCT and near-impossible in the autologous setting. Thus, to target B-lineage neoplasms, an initial CD19-specific CAR (designated CD19R) was generated from the variable regions of a mouse mAb specific for CD19, and T-cell activation was achieved through chimeric CD3-ζ endodomain. The CD19 molecule is a 95-kDa membrane glycoprotein, found on human B lymphocytes at all stages of maturation, although it typically disappears upon differentiation to terminally differentiated plasma cells. It is expressed on B-lineage acute leukemias and lymphomas as well as chronic lymphocytic leukemia and is rarely lost during the process of neoplastic transformation. It is not expressed on hematopoietic progenitor cells or on normal tissues outside the B-lineage and is not thought to be shed into the circulation. An advantage of CD19-directed therapy for lymphomas over targeting CD20, another B-lineage antigen, is that unbound rituximab (CD20-specific therapeutic mAb) will not interfere with binding of CD19-specific T cells. A clinical-grade DNA plasmid vector coding for CD19R and bifunctional hygromycin (Hy) phosphotransferase selection gene fused to thymidine kinase (TK) suicide/imaging fusion gene (HyTK) was developed and produced by the National Gene Vector Laboratory. Using this vector, a gene therapy trial was opened (BB-IND 11411, ClinicalTrials.gov Identifier: NCT00182650) to determine the feasibility and safety of infusing autologous T cells co-expressing CD19R and HyTK transgenes, along with exogenous low dose recombinant human IL-2 (as a surrogate T_h-response) in patients with refractory lymphoma.

Improved Therapeutic Potential of CAR$^+$ T Cells

Ongoing projects in our laboratory to improve therapeutic efficacy have focused on prolonging the survival of the infused CAR$^+$ T cells mainly *via* three approaches.

1. *To produce T cells those are capable of endogenous IL-2 production.* To generate an effective anti-tumor response for CD19-redirected effector T cells in tumor microenvironment we introduced a chimeric CD28 T cell co-stimulatory molecule into the CD19R CAR. This is based on the rationale that T-cell binding of CD28 to B7 molecules on target cells generates critical regulatory signals necessary for full T-cell activation and preventing T-cell apoptosis after CAR engagement. However, massively *ex vivo*-expanded genetically modified T cells may lose endogenous CD28 cell-surface expression. Therefore, to provide genetically modified CD28^{neg} T cells with tandem activation and co-stimulation upon engagement with B7^{neg}CD19$^+$ B-lineage tumors, the CD19R CAR was modified to include CD28-signalling domain. This second generation CAR, designated CD19RCD28, has been expressed in primary T cells and shown to activate genetically modified T cells for killing, IFN-γ, and IL-2 cytokine production, and improve *in vivo* survival of adoptively transferred CD19RCD28$^+$ T cells resulting in a greater anti-tumor effect, compared with first-generation CD19R$^+$ T cells.
2. *To target IL-2 to the tumor micro-environment.* In addition to engineering CAR for T-cell production of IL-2, a cytokine that *ex vivo*-expanded T cells typically depend on for continued *in vivo* persistence, we have directed exogenous IL-2 cytokine to the B-cell tumor microenvironment using a CD20-mAb fused to IL-2 (immunocytokine), and this combination immunotherapy enhanced the anti-tumor effect of infused T cells.
3. *To produce T cells with improved biologic potential.* Shortening the *ex vivo* manufacturing time may improve therapeutic efficacy, as extensively propagated T cells differentiate *in vitro* into cytolytic effectors lacking desired homing receptors and a tendency to undergo replicative senescence. Therefore, we have developed a new rapid propagation technology using a CD19$^+$ immortalized artificial antigen presenting cell (aAPC) that can be lethally-irradiated and used in co-culture to numerically expand cytolytic CD19-specific T cells. To develop non-invasive biomarkers and to evaluate adoptively transferred T-cell distribution and function, we have used radionuclides that are metabolized by TK which acts as a reporter gene for detection of T cells by positron emission tomography (PET).

Future Challenges

There has been a paucity of published data on the safety and feasibility of infusing T cells expressing CAR and at this time there are no reports describing sustained clinical response of adoptive transfer of CAR$^+$ T cells. This will soon change as multiple clinical trials are currently

underway world-wide using adoptive cellular immunotherapy with T cells expressing CAR. To maximize therapeutic efficacy, future trials will likely infuse CAR^+ T cells combined vaccine to deliver a T-cell activation signal though endogenous αβ TCR, or CAR^+ T cells combined with cytokine, such as immunocytokine, to deliver co-stimulatory signal through endogenous cytokine receptors. One of the major advantages of the strategy infusing CAR^+ T cells lies in the modular composition of the CAR molecule that combines an antigen-binding domain with signaling domains for effector-cell activation. This allows investigators to swap the CAR exodomain with a scFv or ligand that recognizes or binds to a desired cell-surface antigen. Future experiments will build on the catalog of antigen-targeting receptors to evaluate expression level, density, and stability of the CAR expression on the T-cell surface, as well as affinity of the binding domain for antigen in the context of tumor targets with varying antigen density. These studies will impact CAR-mediated immunotherapy since low-density antigen-positive tumor cells or low affinity CAR may lead to emergence of tumor escape. Just as genetic engineering can be used to alter the CAR exodomain, so the transmembrane, or endodomain may be altered to provide a fully-competent antigen-dependent T-cell activation signal. The majority of the CARs generated harbor CD3-ζ signaling chain which is currently considered more efficient in activating T cells for cytolysis, compared to chimeric FcεRI-γ. Other CARs have been generated activating T cells through syk, lck, but the full-effect of these receptors with respect to cellular activation and stability of receptor expression on the cell surface has yet to be determined. Similarly the CAR endodomain has been altered to express a co-stimulatory signaling molecule, such as chimeric CD28, to provide a coordinated signal with CD3-ζ to provide a more complete T-cell activation signal than achieved by signaling through CD3-ζ alone. Because different types of co-stimulation may result in different patterns of cellular activation, it will likely be beneficial to explore alternative co-stimulatory pathways in CAR-mediated T-cell activation. Increasingly, investigators are developing the tools to genetically modify T cells using techniques that cause minimal manipulation of the product leaving intact the full range of T-cell homing and proliferation potentials. Finally, genetic modification is being used to accomplish more than redirect T-cell specificity. For example, experiments are underway to render the T cells resistant to the anti-inflammatory and deleterious effects of iatrogenic glucocorticoids and TGF-β secreted by tumor cell.

Conclusion

Although limitations remain, genetic modifications enable investigators to engineer T cells with augmented therapeutic potential. It is expected that infusion of genetically modified T cells will be a center-piece of personalized medicine rivaling the influence of therapeutic mAbs as a treatment for malignancies.

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Chimeric Genes

►Fusion Genes

Chimeric Oncogenes

►Fusion Genes

Chimeric Oncoproteins

Definition

Chimeric proteins generated by ►chromosome translocations of two cellular genes in which the functional domains of two separate genes are fused together and/or alter regulation of gene expression. Many chimeric oncoproteins characterized to date appear to act as aberrant transcription factors, likely functioning in

transformation by dysregulating the expression of key target genes.

►Epigenetic Therapy
►Fusion Genes
►BCR-ABL1

Chimeric T Cell Receptors

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Synonyms

Recombinant T cell receptor; Immunoreceptor; T-body

Definition

Chimeric T cell receptors are recombinant transmembrane receptor molecules, which are derived from the ►T cell receptor (TCR) complex and consist of an extracellular binding domain, a transmembrane domain, and an intracellular domain to initiate T cell activation upon engagement of the specific receptor ligand.

Characteristics

Recombinant TCRs provide the basis to engineer T cells with predefined specificity in order to redirect the ►T cell response toward defined target cells and to break tolerance for use in ►adoptive immunotherapy. Moreover, chimeric TCRs are valuable tools for the analysis of receptor driven activation of immune cells including T and NK cells. Several formats of recombinant TCRs have been designed during the last years, most of them are chimeric molecules composed of an extracellular binding and an intracellular signaling domain.

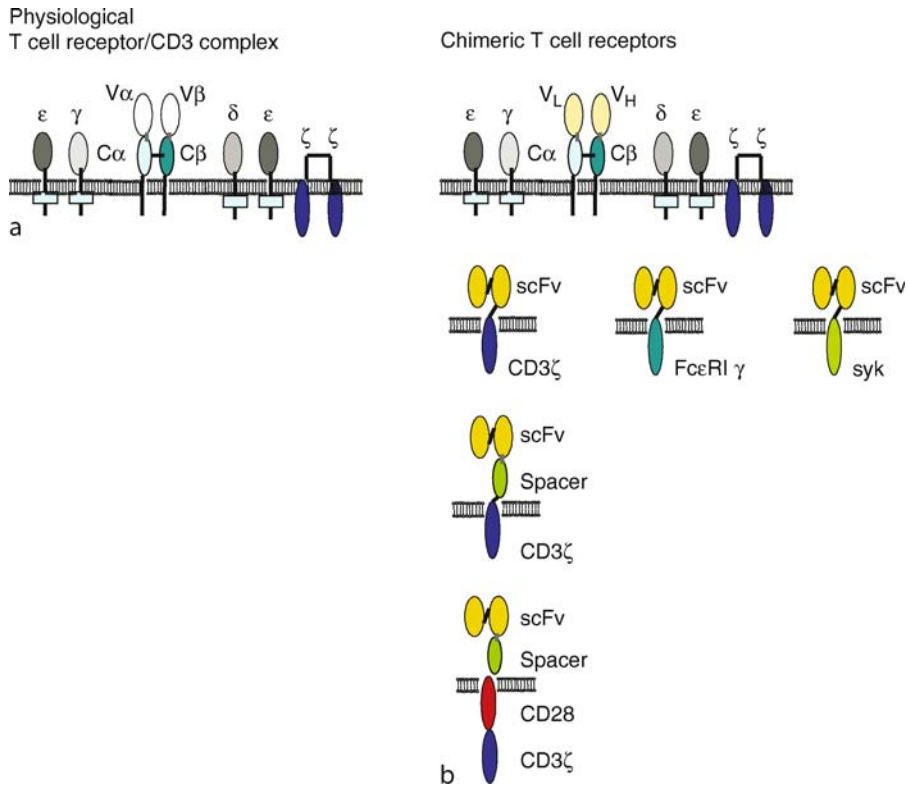
T cells exhibit their binding specificity via the TCR complex. For a number of applications in ►molecular therapy, T cells with predefined specificity would be desirable. By ►viral vector-mediated gene transfer, T cells can be grafted with a recombinant TCR of predefined specificity, which allows to redirect engineered T cells toward defined target cells. The approach, however, is hampered by the fact that the TCR of wanted specificity is frequently not available. In this situation, various formats of recombinant chimeric TCRs with predefined specificity were designed.

The TCR Format

Based on the similarity of the primary structure and the spatial conformation of the variable regions of immunoglobulin (Ig) and TCR α and β chain molecules, antibody derived binding regions V_H and V_L for antigen were grafted on to the constant domain of the TCR α and β chains, respectively (Fig. 1). Thereby, the TCR Vα and Vβ domains are replaced resulting in chimeric TCR molecules with antibody directed T cell recognition. The strategy, however, requires simultaneous expression of two modified TCR chains in engineered T cells. Moreover, the transfected TCR α and β chains frequently heterodimerize with the corresponding chains of the endogenous TCR resulting in chain mispairing and reduced expression of the recombinant TCR.

The Single Chain Format

The alternative format of a chimeric TCR (immunoreceptor, T-body) consists of one polypeptide chain which is composed of an extracellular binding and an intracellular signaling domain (Fig. 1). The single chain format avoids a number of difficulties that rise with the TCR format including simultaneous expression of the two recombinant TCR chains and mispairing with the endogenous TCR. The antigen binding domain is derived from a single chain fragment of variable regions (►scFv) antibody, the signaling moiety is preferentially derived from the CD3ζ chain of the TCR/CD3 complex or, alternatively, from the γ chain of the high affinity IgE Fc receptor (FcεRI). The scFv moiety used for binding is generated from an antibody molecule by genetically joining the V_H and V_L immunoglobulin regions via a flexible peptide linker, e.g., $(Gly_4Ser)_3$, resulting in a continuous polypeptide chain of the V_H-linker-V_L or V_L-linker-V_H type. ►Phage display techniques are applied to isolate scFv antibodies with high binding affinity. Due to the antibody derived binding domain, chimeric immunoreceptors can be generated that bind antigen of any chemical composition or conformation including classical and nonclassical T cell targets like carbohydrates, as far as an antibody exists. Thereby, a broad variety of chimeric TCRs were generated against tumor associated antigens like ►HER-2/neu, or ►cancer germline antigens. Instead of an scFv domain, any polypeptide with specific binding properties, e.g., receptor ligands, may be suitable as targeting domain as well. The majority of chimeric TCRs of the immunoreceptor type harbor antibody derived binding domains that mediate T cell activation independently of MHC. However, antibodies are available that recognize the processed peptide in the context of MHC. MHC/peptide recognizing antibodies when integrated into a chimeric TCR mediate T cell recognition in a MHC dependent fashion. It is so far not elucidated whether MHC dependent or independent, chimeric TCRs are



Chimeric T Cell Receptors. Figure 1 Modular composition of chimeric T cell receptors (TCRs). The physiological TCR complex consists of the α and β chain for antigen recognition and the CD3 complex to initiate intracellular signaling. Fusion of the antibody derived binding domains V_H and V_L to the α and β chains of the TCR creates a chimeric TCR. In an alternative format the chimeric TCR consists of one polypeptide chain with the single chain fragment of variable region (scFv) antibody as antigen binding domain which is fused via spacer and transmembrane (TM) domain to the intracellular signaling domain which is preferentially derived from the CD3ζ chain or the FcεRI γ chain, or alternatively from lck, syk, or CD3ε. Chimeric TCRs of the second generation harbor in addition to the CD3ζ chain a costimulatory domain, e.g., the CD28 domain.

superior in redirecting T cells in vivo. Some advantages of MHC independent chimeric receptors, however, are obvious, including targeting of unconventional T cell antigens like carbohydrates and targeting of cells even when their target antigen is not properly processed or presented in the MHC. Both $CD8^+$ and $CD4^+$ T cells can be redirected by the same immunoreceptor. The latter is of clinical relevance since redirected T cells of both $CD4^+$ and $CD8^+$ subpopulations execute granule-dependent cytolysis of target cells when stimulated via a chimeric TCR that circumvents MHC restriction.

The intracellular signaling domain of the chimeric TCR is most frequently derived from the CD3ζ or the FcεRI γ chain. Both signaling chains contain immunoreceptor tyrosine activation motifs (►ITAMs), the CD3ζ chain three, the FcεRI γ chain one ITAM, which become phosphorylated upon receptor crosslinking. The signaling domains thereby serve as specific adaptors for downstream signaling proteins of the TCR complex. Alternatively, signaling moieties of downstream kinases like lck can be used as activation domains in chimeric TCRs.

Expressed in cytotoxic T lymphocytes (►CTLs), crosslinking of the chimeric receptor by antigen engagement initiates T cell activation resulting in proliferation, cytokine secretion, and specific cytolysis of antigen expressing target cells. Chimeric TCRs define a new specificity of the grafted effector cell. This is indicated by the fact that (i) T cells with immunoreceptor are activated upon coincubation with target cells that express the defined antigen on the cell surface whereas antigen-negative target cells do not initiate receptor signaling in receptor grafted T cells, (ii) T cells without immunoreceptor or with immunoreceptor of other specificity are not activated upon coincubation with antigen expressing target cells, and (iii) receptor triggered activation can be blocked by antibodies directed toward the scFv domain of the immunoreceptor. The efficacy of T cell activation depends on various parameters including the expression level of the

receptor on the effector cell surface, the density of antigen on the target cell, the targeted epitope and the position of the epitope within the antigen.

Chimeric T Cell Receptors with Primary and Costimulatory Signal

Naive T cells, in contrast to preactivated T cells, are not fully activated when stimulated via CD3ζ but require ►costimulation via CD28. When T cells encounter antigen but lack costimulatory signals antigen-specific tolerance or anergy is induced. Current chimeric TCR strategies therefore aim to provide appropriate costimulatory signals on effector T cells in order to avoid induction of anergy. This is done by simultaneous expression of two recombinant immunoreceptors, one with the CD3ζ and the other with the CD28 signaling domain or, technically more convenient, by combining the intracellular CD3ζ chain together with the CD28 signaling domain into one polypeptide chain receptor molecule (Fig. 1). Different costimulatory domains result in different activation profiles. T cells triggered by the combined CD28-CD3ζ signaling chimeric receptor exhibit increased IFN-γ secretion and secretion of substantial amounts of IL-2 whereas T cells equipped with the CD3ζ chimeric receptor secrete lower amounts of IFN-γ and do not secrete IL-2. The specific cytolytic capacity, noteworthy, is not dramatically altered when preactivated T cells are stimulated via the CD28-CD3ζ compared to the CD3ζ signaling receptor.

Clinical Aspects

The major application of chimeric TCR strategy is to redirect cytotoxic T cells (CTLs) toward defined tumor cells or virus infected cells. T cells equipped with a tumor specific, chimeric TCR break tolerance toward autologous tumor cells as demonstrated in mouse models in vivo. Moreover, patient's T cells from the peripheral blood can be redirected in vitro toward the autologous tumor cells isolated from a tumor biopsy of the same patient. The approach is universal since by inserting the appropriate binding domain into the chimeric TCR, T cells can be redirected toward a broad variety of tumor associated antigens expressed on tumor cells of different entities, including ►gastrointestinal tumors and ►pancreas carcinoma (CEA, CA19-9, B72-4), ►melanoma (HMW-MAA, p97, GD3, Mage-1), breast carcinoma and ►epithelial tumors (►Her2/neu ErbB2, Muc1), renal cell carcinoma (G250), ►prostate carcinoma (PSMA), ►ovarian cancer (FBP), ►hematological malignancies including ►multiple myeloma and ►Hodgkin's lymphoma (CD19, CD20, CD30, CD33), and, moreover, toward virus infected cells, including hepatitis B virus infected ►hepatocellular carcinoma cells that express virus encoded proteins on the cell surface.

The strategy of redirecting T cells by chimeric TCRs has advantages over antibody-based therapies. In particular, the strategy makes use of the autologous cellular defense system represented by immunological effector cells that actively penetrate tissues. In contrast to antibodies, the engineered effector cells persist and circulate over long periods of time, i.e., until up to 1 year or even longer, amplify by proliferation upon antigen encounter and interact with numerous target cells by executing their cytolytic attack. Thereby, the chimeric TCR strategy is anticipated to be most powerful in eliminating (micro-) ►metastasis and ►circulating tumor cells. Since T cells in patients with advanced stages of the disease are frequently defective in TCR signaling, the chimeric receptor approach will require repeated administration of "freshly" grafted T cells to substitute burn-out or anergized CTLs. Once initiated the immune reaction is thought to be specifically sustained by chimeric receptor signaling upon repetitive stimulation with antigen. Having target cells eliminated, the immune reaction is supposed to be self-limiting since T cells that do not furthermore interact with their antigen enter apoptosis. It is furthermore anticipated that some engineered T cells convert into memory cells that will be active in ►immunoprevention of tumor relapse.

Clinical trials have been and are currently initiated using engineered T cells equipped with chimeric TCRs in order to eliminate tumor cells, e.g., of ►ovarian carcinoma, renal cell carcinoma, lymphoma, and melanoma. The safety of the immunotherapeutic strategies to redirect T cells by chimeric TCRs is currently explored. One of the major risks of redirecting T cells with chimeric TCRs, however, is the risk of autonomous amplification of engineered T cells independently of antigen. Secondly, there is the risk of an unwanted autoimmune reaction toward healthy tissues that express the same antigen as the target cell, however at lower levels. Costimulatory chimeric TCRs may harbor a higher risk of autoaggression compared to CD3ζ signaling receptors. Crucial questions in the treatment of malignant diseases, however, are whether effector cells with chimeric TCRs have retained their homing capabilities after genetic manipulation in order to accumulate at the tumor site, whether they execute their effector functions at the tumor site and are resistant toward suppression by ►T regulatory cells, and whether they remain silent upon contact with healthy tissues with physiological expression of target antigen.

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Chimeric Transcripts

►Fusion Genes

Chinese Medicine, Traditional Chinese Medicine, Oriental Medicine

►Chinese *versus* Western Medicine

Chinese *versus* Western Medicine

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Synonyms

Chinese medicine, Traditional Chinese medicine, Oriental medicine; Western medicine, Modern medicine, Conventional medicine, Mainstream medicine, Orthodox medicine, Biomedicine, Allopathic medicine

Definition

Chinese medicine is a patient-oriented medical system that treats the ►cancer patients instead of the malignant diseases. It is believed that ►qi (the Chinese term for vital energy) supports the functional activities and blood supplies nutriments for the whole body. There exists a system of channels within the human body, through which the vital energy and blood circulate, and by which the internal organs are connected with superficial organs and tissues, and the body is made an organic whole. Using these holistic and harmonic approaches, Chinese medicine emphasizes to strengthen the body resistance. It attaches importance to the self-healing ability of human body to remove pathogenic factors and recover health. Many of its cancer therapies, such as Chinese medication (including medicinal decoction, patent medicine, and proprietary medicine), medicated diet, acupuncture, and moxibustion, as well as ►qigong and massage, are employed for enhancing this power.

Western medicine is an evidence-based medical system, in which authorized medical and healthcare professionals struggle to avert the destruction of cancer patients by the unrelenting displacement of normal homeostasis accompanying neoplastic growth. The clinical problems faced by oncologists include overcoming the inherent or acquired resistance of the malignant cell to therapy, ameliorating the toxicities of aggressively applied therapies, as well as exploiting the synergistic potency of surgery, radiotherapy, and chemotherapy. In the last few decades, the limited clinical skills demand the use of destructive agent for cancer therapy. In the post-genomic era, ►targeted therapy and novel therapeutic strategy are applied to complement the conventional treatment for an achievement of optimal anticancer results.

Characteristics

Developing History

The Yellow Emperor's Canon of Internal Medicine is believed to be the earliest medical monograph in China, which appeared during the Warring States period (475–221 BC), first defined the etiology of tumor. Since the ancient times, Chinese medicine has made a great contribution to the health of the Asian people. Based on empirical and clinical experience, Chinese medicine has been systematized and theorized in complex practice. Many safe and effective methods have been developed to diagnose and treat cancer over the past thousands of years.

Hippocrates (460–375 BC), the father of Western medicine, first attributed the origin of cancer to natural causes. Improved microscopes, stimulated cancer researches, and important discoveries in human and animal studies have resulted in a better understanding of neoplasia. The last few decades has witnessed spectacular progress in describing the fundamental molecular basis of cancer following the advent of molecular biology and genetics, which allows the device of advanced or targeted therapy for cancer.

Theories and Principles

The philosophical theories and fundamental principles of Chinese medicine include the theory of ►yin and yang, the five elements concept, the physiological functions of viscera and bowels, the conception of vital energy and blood, as well as the theory of the channels and collaterals. According to the basic theories, the physical structure and physiological phenomena of human body as well as the pathological changes are in adaptative conformity with the variations of the natural environment. Hence considerations of ►personalized cancer medicine are based on the patient's constitution, geographical localities, climatic, and seasonal conditions. The therapeutic principle of cancer is to treat the disease by looking into both its root cause and symptoms. Sometimes different treatments are applied to the same kind of cancer in the light of different physical reactions and clinical manifestations, whereas the same therapy can be used to treat different cancers if they are alike in clinical manifestations and pathogenesis.

According to the theories of Western medicine, cancer is not one illness but a variety of disorders with different pathophysiology that can arise from and spread to almost every organ and tissue in the body. Mechanism of the cancer development varies according to the site of the malignant disease and the precipitating cause. Each cancer has its unique pattern of presentation and approach to diagnosis and treatment. Therapy must be directed not only toward cure of the cancer and control of potential ►metastasis, but also to optimize the quality of life.

Etiology and Pathogenesis

There are two categories of etiological factors for cancer, based on the knowledge of Chinese medicine, which includes exogenous and endogenous factors. The exogenous factors refer to the six excessive and untimely atmospheric influences (wind, cold, summer-heat, dampness, dryness, and fire), as well as unhealthy diet. The endogenous factors refer to the excessive emotional changes (joy, anger, melancholy, anxiety, sorrow, fear, and fright) and the deficiency of functional organ. In general, the pathogenesis of cancer can be summarized as accumulation of ►phlegm dampness, internal noxious heat due to accumulation of pathogenic heat, ►blood stasis due to vital energy stagnancy, dysfunction of internal organs, vital energy, and blood deficiency, as well as yin and yang imbalance.

Extensive epidemiological and prospective studies have allowed Western medicine to identify two categories of etiological factors for cancer, which include environmental and genetic factors. The environmental factors refer to smoking, alcohol, unhealthy diet, ultraviolet light, ionizing radiation, carcinogens, certain viruses, and infections. The genetic factors refer to monogenic and polygenic disorders. In general, cancer is a clonal disease arising by the ►multistep development of genetic or epigenetic changes in ►oncogenes, ►tumor suppressor genes, and ►caretaker genes that favor expansion of the new clone over the old. These changes allow a normal cell to achieve the hallmark features of cancer, which include the capacity to proliferate irrespective of exogenous mitogen, the refractoriness to growth inhibitory signal, the resistance to ►apoptosis, the potential to reactivate ►telomerase resulting in unrestricted proliferation, the capacity to recruit a vasculature, as well as the ability to invade surrounding tissue and eventually metastasize.

Diagnostic Methods

Chinese medicine views the human body as a unity, and a malignant disease reflects both the interior and exterior of the body. The diagnosis of cancer is based on an overall analysis and differentiation of the patient's signs and symptoms, which include observation of the patient's mental state and inspection of the tongue, auscultation and olfaction, interrogation, as well as pulse taking and palpation.

Western medicine diagnoses cancer based on tissue diagnosis, which include tissue biopsy, diagnostic medicine, cytology, histopathology, and immunocytochemistry. The tumor-node-metastases classification is applied to establish the anatomical staging for most cancers. In addition, there are a number of specific ►tumor markers which are useful in diagnosis, such as α-fetoprotein for hepatocellular carcinoma, β-human chorionic gonadotrophin for choriocarcinoma, and prostate-specific antigen for prostate cancer.

Treatment Modalities

The principal methods of cancer treatment by Chinese medicine involve Chinese medication (derived from plant, animal, and mineral substances), medicated diet, acupuncture, and moxibustion, as well as ►qigong and massage. In most cases, the patients are treated with medications to strengthen their resistance and dispel the invading pathogenic factors of cancer. Sometimes the malignancy is treated with poisonous medication to combat poison with poison, such as the application of ►arsenic trioxide. The cancer remedy should be made up in accordance with the physique of an individual, pathologic changes occur in the course of cancer, as well as the geographical and seasonal conditions. The prescription for cancer usually consists of various medicinal ingredients with the purpose to produce the desired therapeutic effect in unison and reduction of toxicity or side effects. The principal ingredient provides the principal curative action, the adjuvant ingredient helps to strengthen the principal action, the auxiliary ingredient relieves the secondary symptom or tempers the strong action of the principal ingredient, and the

conductant directs the action to the affected channel or site. More than 100 species of Chinese pharmaceuticals have been used to treat cancer. As there are up to thousands of compounds in a medication, multitargets are exhibited to the malignant disease and some of the compounds may exert a synergistic anticancer effect.

Using the combined modality approach, the principal methods of cancer treatment by Western medicine involve the combined application of surgery, radiotherapy, combination chemotherapy, hormonal therapy, biological therapy, palliative care, and symptom control. Surgery alone is curative in many early stage neoplastic tumors. Radiotherapy is often used after surgery to reduce the chance of recurrence. It can also be used on its own with curative intent or as a palliative treatment. Chemotherapy is a systemic treatment that can reach any part of the body with an adequate blood supply, and therefore it is normally used to treat disseminated cancer. It can also be used as an ►adjuvant therapy to reduce the volume of advanced cancer, with intent to prolong life and relieve symptom. The development of new less toxic chemotherapeutic drugs and more effective antiemetics have reduced many adverse effects of chemotherapy. Hormonal therapy is performed to manipulate the hormone level, which can result in the regression of a number of cancers, particularly for the breast cancer, endometrial cancer, and prostate cancer. Biological therapy exploits insight into the nature of tumor antigen, the molecular and cellular requirement for immune activation, the role of cytokine in amplifying the immune response, as well as the evolution of recombinant DNA approach to introduce the genetic material into eukaryotic cell. Palliative care aims to achieve the best possible quality of life for patient and their family by controlling the physical symptom, as well as recognizing the psychological, social, and spiritual problems.

Western medicine is currently used as the primary therapy for cancer and Chinese medicine is employed as a supplementary therapy in some Asian nations. There is considerable evidence for the promising benefits of Chinese medicine in alleviating the toxic effect of radiotherapy and chemotherapy, strengthening the anticancer activity, as well as enhancing the immune function. Chinese and Western medicines are obviously two distinct medical systems with different diagnostic and therapeutic methods for cancer. However, with a common goal to eradicate this systemic disease, it may be feasible for a certain degree of complementation and integration of these two medical systems in the realm of clinical practice.

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Chitinase-3-like-1

►Serum Biomarkers

CHK1

Definition

Checkpoint kinase 1, synonym CHEK1, is a protein kinase that controls a ►checkpoint in G2. CHK1 is activated by DNA damage and phosphorylates the protein phosphatase CDC25C. This prevents the CDC25C-mediated activation of the CDC2-cyclin B complex and thus M-phase entry.

►Cell-Cycle Targets for Cancer Therapy
►UCN-01 Anticancer drug
►Fragile Histidine Triad

CHK2

Definition

►Checkpoint kinase 2.

►CHEK2

2-chlorodeoxyadenosine

►Cladribine

2-chloro-2′-deoxyadenosine

►Cladribine

CHO Cells

Definition

Chinese hamster ovary cells; an vin vitro established cell line often used to study cellular processes.

Cholangiocarcinoma

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Synonyms

Bile duct carcinoma; Klatskin tumor; Cholangiocellular carcinoma

Definition

Cholangiocarcinoma refers to malignancy within the biliary duct system and is distinct from ►gallbladder cancer. Cholangiocarcinomas generally have features of biliary tract epithelium, such as a glandular appearance, small regular nuclei, and scant cytoplasm. Tumor cells often express cytokeratins, ►CA19-9 and mucin.

- 95% of bile duct tumors are adenocarcinomas
- The remainder is comprised of squamous, carcinoid, sarcomatous, mixed cholangiocellular/hepatocellular tumors, Kaposi's sarcoma, and lymphoma

The characteristics described here refer to the adenocarcinoma cell type of either intrahepatic or extrahepatic bile duct origin.

Characteristics

Etiology

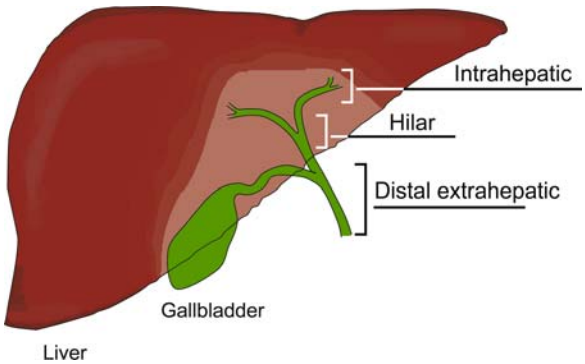
Cholangiocarcinomas are the second most common primary liver cancer, representing 10% of all primary liver tumors. Cholangiocarcinomas occur with an approximate 1:100,000 incidence in the Western world but are more common in Southeast Asia and Japan. There is a slight male preponderance, with tumors occurring most frequently in the seventh decade of life. More than 90% occur sporadically in an otherwise normal liver. Risk factors for the development of cholangiocarcinoma include ►primary sclerosing cholangitis (PSC), congenital dilatation of the biliary tree (i.e. Caroli's disease), ►hepatolithiasis and chronic infestation of the biliary tree by the parasitic ►liver flukes *Opisthorchis viverrini* and ►*Clonorchis sinensis*. Infection with these parasites is endemic in some parts of Southeast Asia, and in part may contribute to the higher incidence of cholangiocarcinoma in this region. These conditions all have in common chronic inflammation of the biliary tree, biliary stasis, or both. The inflammatory milieu likely contributes to two of the cardinal features of cancer, resistance to ►apoptosis, and continuous proliferation.

The cellular origin of malignant cells is not certain. Biliary epithelial cells are long lived under normal circumstances and thus survive long enough to accumulate carcinogenic mutations. Alternatively, multipotent progenitor cells that reside in the ►canals of Hering are not terminally differentiated and have the proliferative capacity that may facilitate malignant transformation. Finally, gland-like structures along the bile ducts can show hyperplasia and dysplasia adjacent to malignant cholangiocarcinoma. It is possible that each of these cellular compartments contributes, depending on tumor focus and carcinogenic insult.

Biology

Grossly, cholangiocarcinomas usually develop at the hilum (60%) and less frequently develop as distal extrahepatic or as intrahepatic tumors (Fig. 1). Lesions can be categorized based on growth pattern as mass-forming, periductal-infiltrating, and intraductal. Microscopically, tumors have relatively few cancer cells in a desmoplastic stroma. This fibrosis leads to a low diagnostic yield of random sampling and the sensitivity of routine cytology from biliary brushings is usually reported in the range of 4–26%.

The mechanistic link between inflammation and the development and progression of cholangiocarcinoma is of interest. Several inflammatory mediators have been shown to stimulate cholangiocyte proliferation, inhibit DNA repair or block apoptosis – all factors important in carcinogenesis. Indeed, interleukin-6 (IL-6) is an effective cholangiocyte mitogen and drives proliferation of this cell type via the MAP-kinase pathway. Additionally, IL-6-driven activation of Signal-Transducer and Activator of Transcription 3 (STAT3) increases expression of ►Mcl-1, a potent anti-apoptotic protein. Mcl-1 acts to protect cancerous cells and may contribute to the refractory nature of cholangiocarcinoma to chemotherapy. Pro-inflammatory cytokines induce cholangiocyte DNA damage and inhibit DNA repair by a nitric oxide-dependent mechanism. In addition, immunohistochemical studies of human cholangiocarcinoma specimens



Cholangiocarcinoma. Figure 1 Diagram of the liver and biliary tree. Cholangiocarcinoma can arise anywhere along the biliary tree, including intrahepatic bile ducts (peripheral cholangiocarcinoma), the hilum (Klatskin tumors), and more distally (distal extrahepatic cholangiocarcinoma). Hilar cholangiocarcinoma can be further subdivided using the Bismuth-Corlette classification depending on the involvement of only the hepatic duct (type I), the common hepatic duct and the confluence of the left and right hepatic ducts (type II), the common hepatic duct and either the left or right hepatic duct (type III), or the common, left, and right hepatic ducts or multifocal tumors (type IV).

have shown the ubiquitous presence of inducible nitric oxide synthase (iNOS). Thus, IL-6 and iNOS with nitric oxide generation in chronically inflamed tissues likely contribute to the initiation and progression of cholangiocarcinoma.

The epidermal growth factor receptor (EGFR) has been implicated in numerous cancers, and activating mutations of EGFR have been described in cholangiocarcinoma. Further, bile acids stimulate signaling through the EGFR, including increasing Mcl-1 protein levels. EGFR activation leads to activation of the survival kinase Akt which is inhibited by phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Experimental PTEN deficiency in mice combined with loss of SMAD4 (a mediator of TGF-beta signaling) leads to cholangiocarcinoma by 4–5 months of age. Thus, dysregulation of EGFR signaling may play a prominent role in cholangiocarcinoma.

Diagnosis

Most patients with cholangiocarcinoma present with signs and symptoms of biliary obstruction, including ►jaundice, pruritis, chalk-colored stools, and dark-colored urine. This is the result of partial or complete blockage of the biliary tract leading to cholestasis, and applies to Klatskin tumors and distal extrahepatic tumors. Intrahepatic tumors do not cause clinically significant biliary stasis.

Because the majority of tumors are not mass-forming, diagnosis by imaging can be difficult. Still, MRI with magnetic resonance cholangiopancreatography (MRCP) can be used to define the location and extent of a lesion. MR angiography is useful for assessment of vascular involvement. CT and CT angiography can also be used to assess tumor location and vascular involvement, as well as lymph node enlargement. Ultrasound is often the initial study used in evaluation of obstructive jaundice. While non-specific, ultrasound can visualize biliary duct dilatation proximal to the obstruction and potentially can visualize an intrahepatic mass.

Endoscopic retrograde cholangiography (ERC) in the case of obstruction is necessary and can be diagnostically and therapeutically useful. ERC can demonstrate the site and extent of a stricture, intraluminal brushings taken within the stricture can provide cells for cytologic and advanced cytologic evaluation, and stenting relieves symptoms due to obstruction. Transluminal or percutaneous biopsy of hilar lesions or lymph nodes is not recommended due to the risk of tumor seeding.

Histologic diagnosis is the gold standard, however often diagnosis is based on the overall clinical picture. Cytologic evaluation has high specificity when positive for malignancy, but due to the desmoplastic nature of the tumor has low sensitivity. Advanced methods of cytologic analysis (digital image analysis and fluorescence in situ hybridization) have recently been used to improve sensitivity without compromising specificity. Serum CA19-9 level can aid in the diagnosis.

Patients with PSC represent a significant diagnostic challenge, as non-cancerous stricture formation is the norm in this disease. Still, with a lifetime incidence of cholangiocarcinoma in PSC patients of 10–20%, a high index of suspicion must be maintained. Stable asymptomatic patients can be surveyed by non-invasive techniques such as MRCP and CA19-9 serum testing.

Treatment and Prognosis

Surgical resection for intrahepatic cholangiocarcinoma is curative for a minority of patients. For extrahepatic tumors (including Klatskin tumors) resectability depends on the extent of biliary and vascular involvement. Involvement of the left and right hepatic lobar structures (bilateral portal vein, main portal vein, or bilateral hepatic ducts) precludes resection, as does underlying liver disease or PSC. In resectable cases, partial hepatectomy improves outcomes. Selected patients with unresectable extrahepatic tumors benefit from liver transplantation; liver transplantation for intrahepatic cholangiocarcinoma is contraindicated due to disease recurrence.

Only a minority of patients present with disease amenable to surgical resection or transplantation. For the remaining patients, palliative treatment improves quality of life. The most important intervention

involves relief of biliary obstruction generally by endoscopic approach or percutaneously. Drainage of one functional lobe is sufficient to relieve obstructive symptoms. Photodynamic therapy in conjunction with stent placement can improve drainage and increase survival by ~1 year. Palliative chemotherapy with gemcitabine leads to only limited responses.

Overall, the prognosis for cholangiocarcinoma remains poor. Five year survival ranges from 10 to 45%, while peri-ampullary tumors have slightly higher 5-year survival (50–60%). Future improvements may come from rationally designed therapy based on the tumor biology, for instance targeting IL-6, EGFR, or Mcl-1.

►Klatskin Tumors

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Cholangiocarcinoma

►Bile Duct Neoplasms

Cholangiocellular Carcinoma

►Cholangiocarcinoma
►Klatskin Tumors

Cholangiocytes

Definition

Are the epithelial cells that line the intrahepatic and extrahepatic bile ducts. Cholangiocytes participate in the modification of bile originating from the canalicular domain of hepatocytes via a series of hormonally regulated processes that lead to the secretion of bicarbonate into ductal bile.

►Bile Duct Neoplasms

Cholangiosarcoma

►Bile Duct Neoplasms

Cholecystectomy

Definition

Is the surgery for removal of the gallbladder. This kind of surgery can be performed with the traditional open incision or can be removed through abdominal incision using a laparoscope (laparoscopic cholecystectomy).

►Gallbladder Cancer

Cholecystitis

Definition

Acute or chronic ►inflammation of the gallbladder. In 90% of cases cholecystitis is associated with the presence of gallstones in the cystic duct (i.e., calculous cholecystitis), while the other 10% represents acalculous cholecystitis.

►Gallbladder Cancer

Cholecystokinin (CCK)

Definition

Gut peptide hormone secreted in response to food, that stimulates gallbladder contraction and pancreatic

enzyme secretion; CCK receptors are shared with another gut peptide, gastrin

►Gut Peptides

Cholera Toxin

Definition

Exotoxin from Vibrio cholerae which ADP-ribosylates the α-subunit of G_s at Arg201 leading to the constitutive activation of the α-subunit by blockade of its GTPase activity. Modification of $G\alpha_s$ in epithelial cells of the gut leads to an increase in cAMP levels which causes watery diarrhea.

►G-Proteins

Cholestasis

Definition

Is a condition caused by interruption in the excretion of bile. Cholestasis is caused by obstruction of bile ducts within the liver (intrahepatic) and/or outside the liver (extrahepatic). Obstruction of bile flow causes bile salts, the bile pigment, bilirubin, and fats (lipids) to accumulate in the blood instead of being eliminated normally.

►Bile Duct Neoplasms

Cholesterol

Definition

Is a white waxy substance that can stick to the inside of blood vessels, resulting in clogged arteries, heart disease, and strokes. Formation of cholesterol can be inhibited by ►statins.

Chondrex

►Serum Biomarkers

Chondroitin Sulfate (CS)

Definition

Synonym dermatan sulfate (DS) is a sulphated glycosaminoglycan, usually found attached to proteins as part of a proteoglycan. CS chains are unbranched polysaccharides of variable length containing two alternating monosaccharides: d -glucuronic acid (GlcA) and *N*-acetyl-d-galactosamine (GalNac), each of which can be sulfated in variable positions and quantities. CS is a major component of extracellular matrix, and is important in maintaining the structural integrity of the tissue. It also readily interacts with proteins in the extracellular matrix due to its negative charges and regulates a diverse array of cellular activities.

►Pleiotrophin

Chondrosarcoma

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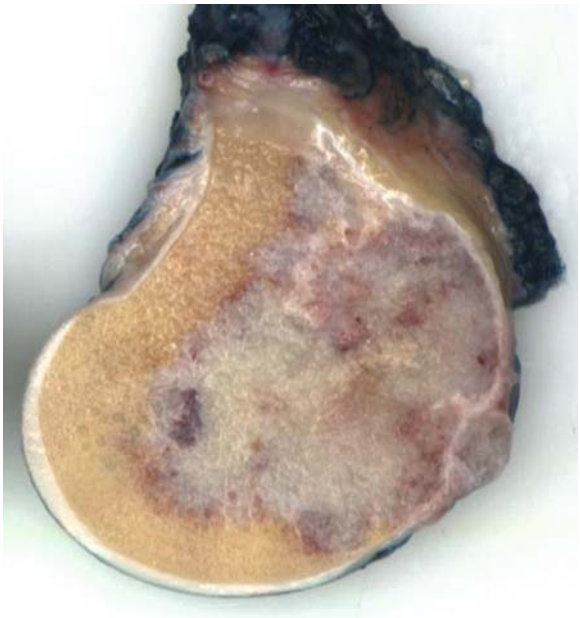
Definition

Chondrosarcoma of bone is a malignant hyaline cartilage forming tumor (Fig. 1). The term chondrosarcoma describes a heterogeneous group of lesions with diverse morphologic features and clinical behavior. Apart from conventional central and peripheral chondrosarcoma constituting the largest subgroup (~85%) this encompasses rare subtypes such as clear cell chondrosarcoma (~1%), mesenchymal chondrosarcoma (~2%), juxtacortical chondrosarcoma (~2%) and dedifferentiated chondrosarcoma (~10%) as well.

Characteristics

The incidence of conventional chondrosarcoma is about 1:50,000. The incidence in males and females is almost equal, and the mean age of diagnosis is 30–60 years. Chondrosarcomas are mostly found in bones that elongate by ►endochondral ossification with the most common sites being the pelvis followed by the proximal femur, proximal humerus, distal femur and ribs. When comparing histologically the different cartilaginous tumors to the growth plate, parallels between normal and neoplastic chondrocyte growth and differentiation



Chondrosarcoma. Figure 1 Macroscopic photograph of gross specimen of chondrosarcoma of distal femur.

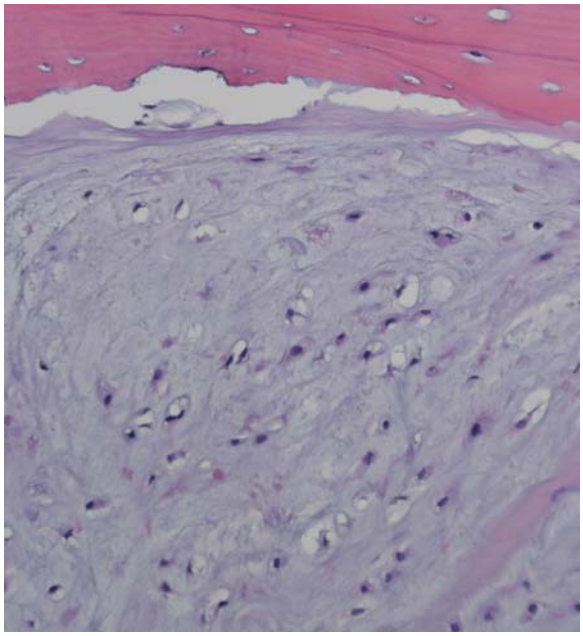
become evident. Resting (primitive, mesenchymal stem-cell like) chondrocytes are found in mesenchymal chondrosarcoma, while clear cell chondrosarcoma consists mainly of hypertrophic chondrocytes. ►Osteochondroma, a benign cartilaginous tumor at the surface of bone, recapitulates all differentiation levels of the growth plate. In contrast, ►enchondroma, a benign cartilaginous tumor in the medullar cavity of bone, and conventional peripheral and central chondrosarcoma mostly contain proliferating chondrocytes, lying in small lacunae. The more rarely occurring dedifferentiated chondrosarcoma is thought to arise from conventional chondrosarcoma in which tumor cells transdifferentiate towards a more spindle-cell phenotype. In addition, the rare subtype juxtacortical chondrosarcoma is recognized, which also contains proliferating chondrocytes. This specific diagnostic term is used as a result of its typical clinicoradiological presentation and its in general relatively favorable prognosis as compared to conventional chondrosarcoma.

There is a clinical as well as a morphological spectrum of cartilaginous tumors. Central chondrosarcoma is the most common subtype (>85%) of conventional chondrosarcoma. Malignant transformation of an enchondroma to a central chondrosarcoma is estimated to be <1%. However, since in 40% of central chondrosarcomas remnants of a pre-existing enchondroma are found, there is considerable debate whether these tumors are secondary to enchondroma or arise mostly primary. The frequency of malignant transformation is significantly higher (15–30%) in patients with multiple enchondromas in the context of the extremely rare non-hereditary disorder ►Ollier disease. Conventional chondrosarcoma at the surface of bone (secondary peripheral chondrosarcoma) per definition develops within a pre-existing osteochondroma. Secondary peripheral chondrosarcomas constitute up to 15% of conventional chondrosarcomas in referral centers. ►Multiple osteochondromas (MO), previously known as hereditary multiple exostoses (HME), is an autosomal dominant disorder and malignant transformation occurs in 1–3% of the cases of MO. In addition, chondrosarcomas may biologically progress: up to 13% of recurrent chondrosarcomas exhibit a higher grade of malignancy than the original neoplasm, with an adverse prognosis.

Diagnosis

Benign cartilaginous tumors are asymptomatic, and are often found by incidence at radiology made for other reasons. In contrast, malignant tumors almost always produce symptoms such as local swelling and pain. The distinction between enchondroma or osteochondroma and low-grade conventional chondrosarcoma is difficult, both at the radiological level (in case of central chondrosarcoma) and the histological level (for both subtypes). Diagnosis should be made in a multidisciplinary setting, based on clinical, radiological and histological aspects. Dynamic MRI has been proven to be informative in distinguishing benign from malignant cartilaginous tumors. Histologically, the distinction between enchondroma and low grade conventional central chondrosarcoma is mainly based on growth patterns and cytomorphological features. Encasement (new shells of reactive bone, formed at the periphery of cartilage nodules), is a feature of benign tumors, while entrapment (permeation of tumor around pre-existing lamellar bone), points to a faster growing process and thus malignancy.

Histologically, chondrosarcomas are divided in three grades of malignancy based primarily on cellularity, nuclear size and chromasia, mitoses and the composition of the matrix. Grade I tumors are moderately cellular and nuclei are uniformly sized and hyperchromatic. Grade II tumors are more cellular and nuclei are atypically shaped, hyperchromatic and larger, and mitoses can be found (Fig. 2). At the end of the spectrum, grade III tumors are hypercellular, with nuclear pleomorphism are found, and mitoses can be frequent. In addition, the extracellular matrix of grade III tumors becomes more mucoid/myxoid compared to the abundant chondroid matrix seen in grade I tumors and their vascularity is increased. Differences in 5 year survival and the occurrence of metastases show the clinical importance of histological grading. While grade I and II tumors rarely metastasize (respectively 0 and 10%), grade III tumors do so in 71% of the cases. 5 year survival is



Chondrosarcoma. Figure 2 Chondrosarcoma of histology.

lowest in patients with grade III tumors (29%) compared to 64% in grade II tumors and 83% in grade I chondrosarcomas.

Therapy

A correct diagnosis is essential for therapeutic decision making. Surgery is the only option for curative treatment since chondrosarcomas are highly resistant to conventional ►chemotherapy and ►radiotherapy. Studies regarding the mechanism underlying resistance are sparse. Therefore, development of targeted therapy for chondrosarcoma would mean a major advance in chondrosarcoma therapy. While for benign lesions a wait and see policy is justified, malignant tumors require more aggressive treatment. Grade I chondrosarcomas are prone to local recurrence but almost never ►metastasize. Therefore, there is a trend in sarcoma centers to treat them by curettage with margin improvement by phenol or ►cryosurgery. In contrast, high grade tumors are usually treated by often mutilating wide en bloc resection or even amputation, since these often metastasize, being lethal in the majority of patients.

Genetics

Although histologically similar, central and peripheral chondrosarcoma have been shown to be genetically, and thereby molecularly, different entities. In Multiple Osteochondromas germline mutations have been identified in the EXT tumor suppressor genes, located on chromosomes 8q24 (EXT1) and 11p11–12 (EXT2), respectively. These ►EXT genes encode glycosyltranferases involved in heparan sulphate biosynthesis. In MO germline mutations in EXT1 or EXT2 with loss of the remaining wild-type allele is found. Recently, in solitary osteochondromas somatic homozygous deletions of EXT1 have been demonstrated. In both hereditary and solitary osteochondromas mRNA expression of EXT1 or EXT2 is decreased. This probably results in intracellular accumulation of heparan sulphate proteoglycans (HSPGs), since the Syndecan2 and the CD44v3 core proteins were shown to aberrantly localize in the Golgi apparatus in solitary and hereditary osteochondroma and peripheral chondrosarcoma. The EXT1 homologue in *Drosophila* (tout velu, ttv) is required for IHH diffusion to its receptor that signals to ►PTHLH and thereby controls chondrocyte proliferation. In contrast to the growth plate, in osteochondroma IHH signaling has become cell autonomous, probably overcoming the diffusion problems caused by defective HSPGs due to EXT inactivation.

Additional genetic alterations are thought to be required for malignant transformation of osteochondroma towards low grade secondary peripheral chondrosarcoma. These additional alterations presumably cause ►chromosomal instability, since peripheral chondrosarcomas are shown to be ►aneuploid with DNA indices ranging from 0.56 to 2.01. At the protein level progression from osteochondroma towards low-grade peripheral chondrosarcoma is characterized by a re-activation of PTHLH signaling. Its downstream target, ►BCL-2, can be used as a diagnostic marker in those cases in which it is hard to distinguish between benign and malignant cases, with osteochondromas being negative in 95% (specificity) and chondrosarcomas scoring positive in 57% (sensitivity). This re-activation of PTHLH is hypothesized to be caused by increased ►TGF-beta signaling, since IHH signaling has been shown to be downregulated in peripheral chondrosarcoma.

Despite the increasing number of genetical studies including peripheral and central chondrosarcomas as separate subgroups, no specific genetic aberrations for the more common central chondrosarcoma have been identified as yet. Mutations in EXT1 and EXT2 have not been reported, and reports on IHH signaling on proliferation in central chondrosarcoma are still inconclusive. A positive relation between histological grade and the degree of karyotypic complexity and aneuploidy was found. Near-diploidy and limited loss of heterozygosity are typical of low-grade central chondrosarcomas rather than of peripheral chondrosarcomas pointing to an oncogenic mechanism with few alterations, sufficient for oncogenesis. Multiple studies report alterations at chromosomal bands 9p21 and 12q13–15. Genetic loss at the 9p21 locus as found by cytogenetics, loss

of heterozygosity analysis and ►comparative genomic hybridization suggest an important role for the CDKN2A/INK4a locus. Loss of protein expression of the tumor suppressor gene p16, encoded by this locus, was found to be associated with increased histological grade in central chondrosarcoma, and thereby to be important for tumor progression.

Rearrangements in the 12q13–14 region have been frequently reported in sarcomas. Several genes in this region have been indicated to be of importance for tumorigenesis, such as SAS (sarcoma amplified sequence), CDK4 (►cyclin dependent kinase 4) and GLI (glioma associated oncogene homologue). Also two other often implicated genes in sarcomas, HMGA2 (high mobility group AT-hook 2) and ►MDM2, are located just outside the 12q13–14 region. Moreover, the progression from low-grade to high-grade central chondrosarcoma is characterized by ►P53 alterations. Despite the large number of studies involving central chondrosarcomas, the exact underlying molecular mechanism is still largely unknown.

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CHOP

Definition

►C/EBP homologous protein

Choroidal Melanoma

►Uveal Melanoma

Christmas Factor

Definition

►Factor IX

Chromate

►Hexavalent Chromium

Chromatid

Definition

One-half of a replicated chromosome that are joined by a single centromere and separate during cell division to become individual chromosomes.

►Mutagen Sensitivity

Chromatin

Definition

Complex of DNA, histones, and non-histone proteins found in the nucleus of a eukaryotic cell; the material of which chromosomes are made.

►Replication Factories and Foci
►Histone Deacetylases
►Histone Modification

Chromatin Modification

►Chromatin Remodeling

Chromatin Remodeling

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Synonyms

Nucleosome remodeling; Chromatin modification

Definition

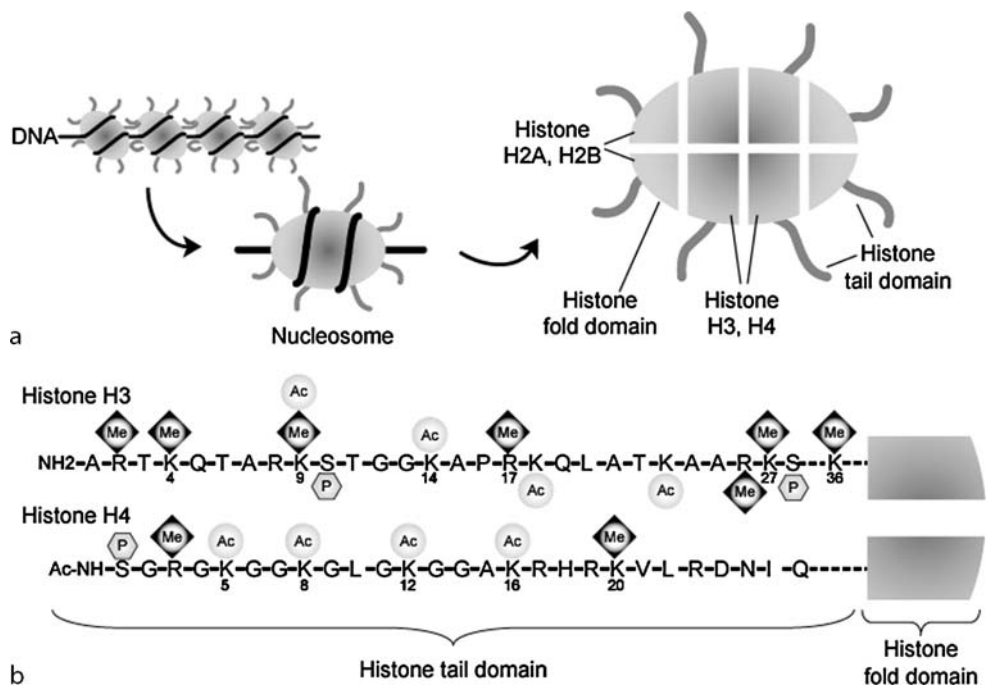
Chromatin remodeling is regulated by reorganization of nucleosome position by ATP-dependent nucleosome remodeling factors (ADNR) and covalent modifications of histone proteins. Because chromatin structure affects the binding of proteins including transcription factors to DNA, it is involved in many essential cellular processes.

Characteristics

Nucleosome (►Nucleosomes) consists of 147 bp DNA wrapped around the histone octamer comprising histone proteins, H2A, H2B, H3, and H4. Since the position of nucleosomes on DNA and the chromatin structure can affect the binding of proteins to DNA, chromatin remodeling is required for all the key processes such as gene expression (►Epigenetic gene silencing), DNA replication, repair, chromosomal recombination, and mitosis. ADNR factors, SWI/SNF type factors, which are the part of multiprotein complexes, shuffle the nucleosomes and change chromatin structure and organization (nucleosome mobility), leading to either activation or repression of gene expression.

The N-terminal domains of all core histones are subjected to chemical modifications, such as acetylation, methylation, and phosphorylation at certain residues (Fig. 1). Histone modifying enzymes (►Histone deacetylases; ►HDACs) bring complexity of posttranslational modifications that can either activate or repress transcription, depending on the type of chemical modification and its location in the histone protein. The modification pattern of histone has been functionally linked to transcription and acts as a "histone code," which alters the structure of higher-order chromatin and helps recruit effector molecules. Various observations have suggested a connection between nucleosome remodeling and covalent histone modifications.

Recent study suggested that histones at transcriptionally active loci can be selectively replaced in a manner



Chromatin Remodeling. Figure 1 (a) DNA is compacted in the nucleus through a hierarchy of histone-dependent interactions. The fundamental repeating unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around an octamer of core histone proteins, H2A, H2B, H3, and H4. (b) Core histone proteins consist of less structured amino-terminal tails (histone tail domain) protruding from the nucleosome and globular carboxy-terminal domains making up the nucleosome scaffold (histone fold domain). Histone tail domain could be a target of a variety of posttranslational modifications, including acetylation (Ac), methylation (Me) and ►ubiquitination of lysine (K) residues, phosphorylation (P) of serine (S) and threonine (T) residues, and methylation of arginine (R) residues.

that is independent of DNA replication; that is, replacement of canonical histone H3 with variant histone H3.3, which is a highly conserved histone variant.

Mutation or dysfunction of these "epigenetic mechanisms" has been implicated in human cancers (►Epigenetics).

Mechanisms and Clinical Aspects

Chromatin Remodeling Complex

In order to obtain the access of proteins to the DNA inside nucleosomes, chromatin remodeling ATPases are required to unwrap the nucleosomal DNA or to slide the nucleosome along the DNA to expose the buried sequences. (Fig. 2) SWI/SNF type chromatin remodeling factors have been shown to be required to this process. These factors are multiprotein complexes containing a central nucleic acid substrate stimulated ATPase belonging to the SWI2/SNF2 family. SWI2/SNF2 family is thought to be involved in gene expression, although their regulation and functions are not fully understood. The mammalian homologues, *Brahma* gene (*BRM*) and *Brahma*-related gene 1 (*BRG1*) are major components with ATPase enzymatic activities in the nucleosome remodeling SWI/SNF complex. BRM and BRG1 have a high degree of homology and either one of them might be contained in each SWI/SNF complex. Mutations or lack of expression of BRG1 have been identified in pancreatic, breast, lung, and prostate cancer cell lines. Germline and somatic mutations in *SNF5* (also called *INI1*), which is another mammalian SWI/SNF complex component but itself does not possess a chromatin remodeling function, cause malignant rhabdoid tumor. Although BRM and BRG1 make a chromatin remodeling complex with SNF5, mutation in SNF5 resulted in more sever phenotype than the tumor harboring either BRM or BRG1 mutations. There could be a degree of functional redundancy between BRM and BRG1, though the functions of SWI/SNF complexes containing BRG1 or BRM might not be interchangeable in some cases. BRG1 is involved in preventing cell cycle progression through its interaction with RB that has been shown to function as a brake on the cell cycle at least in part by establishing stable epigenetic silencing of the target genes. The SWI2/SNF2 family also contains generally one or more domains in addition to helicase-like and ATPase domains. A number of these domains have been shown to interact with surfaces on the nucleosome,

a

b

SNF2 family proteins in mammals

Subfamily	Protein	Characteristc domain	Function
SWI2/SNF2	BRG1/SMARCA4	Bromo	Activation and repression
	BRM/SMARCA2	Bromo	Activation and repression
ISW1	SNF2H/SMARCA5	Sant-like	Chromosome structure
	SNF2L/SMARCA1	Sant-like	Chromosome structure
CHD1	CHD1	Chromo	Activation
	CHD3	Chromo	Repression
	CHD4	Chromo	Repression
INO80	INOB0	DBINO	DNA repair and gene expression
RAD54	ATRX		Regulation of transcription, DNA methylation
DDM1	HELLS/PASG/Lsh		Repression

Chromatin Remodeling. Figure 2 (a) The nucleosome is a substrate of ATP-dependent nucleosome remodeling factor (ADNR). Nucleosome mobility is regulated by ADNR. (b) The SWI2/SNF2 family of ATP-dependent nucleosome remodeling proteins in mammals is classified into different subfamilies. SWI2/SNF2 family contains one or more domains in addition to helicase-like and ATPase domains. SANT (SWI3, ADA2, N-CoR, TFIIIB), DBINO (DNA-binding domain of INO80), Bromo (bromodomain), and Chromo (chromodomain) might interact with surfaces on the nucleosomes.

which are often the targets of posttranslational modifications (see below). For example, bromodomain and chromodomain could bind acetylated lysine and methylated lysine, respectively.

Histone Modifications

Histone modifications are important in transcriptional regulation and are stably maintained during cell division. The less structured N-terminal domains of all core histones protrude from the nucleosomes and are subjected to chemical modifications, such as acetylation, ►methylation, and phosphorylation at certain residues. The modification patterns of histone have been functionally linked to transcription and act as a "histone code," which implies that transcription states can be predicted simply by deciphering this code. Generally, acetylation of lysine (K) residues on histone H3 and H4 leads to the formation of an open chromatin structure, with transcription factors accessible to promoters. Phosphorylation on serine 10 and acetylation on K14 on histone H3 work antagonistically to K9 methylation on H3 leading to the gene activation. Methylation at lysine is considered as a stable modification. There are the extra complications that histone lysine methylation can be either activating (e.g., H3K4 and H3K36) or repressing (e.g., H3K9, H3K27, and H4K20), and the respective enzymes vary in their potential to induce mono-, di-, or trimethylation. Trimethylation at K9 on histone H3 or K20 on histone H4 has been shown to be a marker of heterochromatin from yeast to human. Dimethylation at K9 is associated with inactivation of gene expression. Trimethylation at histone H3K27 is a distinct histone modification involved in the regulation of homeotic (Hox) genes (►Homeobox genes and cancer) expression and in early steps of X-chromosome inactivation in women. Di- or trimethylation on K4 on histone H3 localizes to sites of active transcription and this modification may be stimulatory for transcription. These different combinations of histone tail modifications influence transcription by affecting chromatin structure. Modifications on the lateral surface of core histone could also affect the histone–DNA interactions as well. Control of nucleosome mobility could be regulated by the valance of modifications of acetylation, methylation, and phosphorylation on the lateral surface amino acid residues.

DNA methylation is a crucial epigenetic mechanism for silencing tumor suppressor genes in human cancers, which also affect the chromatin structures. The link between DNA methylation and the histone modifications is mediated by a group of proteins with methyl DNA binding activity, including MeCP2, MBD1, and Kaizo, these proteins localize to DNA methylated promoters and recruit a protein complex that contains HDACs and histone methyltransferases. The DNA methyltransferases may also play a role in direct repression of transcription through cooperation with HDACs in late S-phase. While evidences for interactions between the DNA methylation and histone modifications are accumulating, the critical initiating events in silencing remain to be defined. In fungi, mutations of a histone H3K9 methyltransferase reduced DNA methylation indicate a simple linear model in which H3K9 methylation acts as an upstream epigenetic mark which signals to DNA methylation. However, in mammalian cells, DNA methylation inhibition could also rapidly changes histone methylation, and in plants histone and DNA methylation play distinct roles depending on the locus studied. The interactions between DNA methylation and histone H3K9 methylation currently best fit a model whereby these two changes form a reinforcing silencing loop, and this may explain why silencing is less stable in organisms that lack DNA methylation.

Histone Variant

At transcriptionally active loci, histone H3.3 variant substitutes for the canonical H3 histones. This replacement is independent of DNA replication. Histone modifications that may change histone–DNA or histone–histone contacts also affect catalyzing histone variant exchange. This Replacement process is also catalyzed by ATP-dependent nucleosome remodeling complexes. Histone replacement, which presumably is associated with activating transcription factors on the promoter region, offers an explanation for gene reactivation that were previously silenced via histone methylation. Whether histone replacement might be perturbed in cancer cells remains an open question.

►Epigenetic Therapy

The most promising aspects of this field are that the restoring gene function silenced by epigenetic changes including chromatin remodeling in cancer. "Epigenetic therapy," has the potential of "normalizing" cancer cells, which may lead to differentiation, senescence, or apoptosis. This could have a novel impact on prevention and treatment of human cancers. HDAC inhibitors (►Valproic acid) lead to the accumulation of acetylation in histones resulted in changes of chromatin status and of transcriptional activity to a normal state. P21 is a good example that is induced by HDAC inhibitors. However, exact mechanism through which the HDAC inhibitors mediate antitumor activity remains to be unclear, although these agents have been known to induce apoptosis and to inhibit angiogenesis and metastasis.

In a mouse model of colonic tumorigenesis, reducing DNA methylation genetically and/or pharmacologically has been shown to have tumor-preventive effects, a finding which was recently confirmed. The cytosine analogues, 5-azacytidine, and 5-aza-2′-deoxycytidine

are powerful inhibitors of DNA methylation, which are incorporated into DNA during cell division and trap DNA methyltransferases and lead to cell differentiation and growth repression. Indeed these demethylating agents have been widely studied in hematological diseases and received FDA approval for the treatment of myelodysplastic syndrome. Further, the synergistic effects between DNA demethylating agent and HDAC inhibitors suggest clinical trials of this approach to restore gene function silenced by aberrant chromatin changes in cancers.

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene
►Histone Modification

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Chromium(VI)

►Hexavalent Chromium

Chromium Carcinogenesis

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Synonyms

Chromium-induced carcinogenesis; Hexavalent chromium-induced carcinogenesis; Chromium-induced cell transformation; Chromium tumorigenesis

Definition

►Chromium carcinogenesis (►carcinogenesis) is the process of the genesis of tumors (carcinomas) by specific carcinogenic chromium compounds, containing ►hexavalent chromium (Cr^{+6}). It includes the series of sequential steps that occur when lower animals or humans are exposed to specific hexavalent chromium-containing compounds that leads to tumor development. After all these steps are accomplished, the physiological mechanisms regulating control of growth in the normal cells are degraded. Hence, the normal cells are degraded and converted into tumor cells. The tumor cells then grow autonomously in an unregulated fashion and evade the host immune system, leading to development of visible tumors. Chromium carcinogenesis encompasses carcinogenesis by both insoluble and soluble hexavalent chromium-containing compounds.

Characteristics

Chromium, The Chemical Element, and its Ionic Species and Chemical Compounds

Pure chromium, the element, is a white, hard, lustrous, brittle metal with a high melting point (1,903°C). Pure chromium metal is resistant to mild corrosive agents. Therefore, pure chromium metal is used as a protective coating that can be electroplated onto other metals to protect them from corrosive. Chromium can be found in chemicals, in the +6, +5, +4, +3, +2, and 0 oxidation states. Cr(V) is usually a transient state, which can be best detected by ESR spectroscopy during the reduction of Cr(VI).

Chromium is most commonly found in nature in the ore called chromite, or ferrous chromite ($FeCr_2O_4$). In chromite, chromium is in the +3 valence state, a very reduced state. The mineral, chromite, has Cr(III) at octahedral sties and Fe(II) at tetrahedral sites. Chromite can be reduced with carbon in a furnace. This reduction yields iron, chromium, and carbon monoxide in a carbon-containing alloy called ferrochromium.

High purity chromium metal can be obtained by treating ferrous chromite ore with molten alkali and oxygen. This oxidizes Cr(III) to chromate, containing Cr(VI). The chromate can then be dissolved in water and precipitated as sodium dichromate. The sodium dichromate can then be reduced with carbon to Cr(III) oxide (Cr_2O_3). The chromium (III) oxide can then be reduced with aluminum metal to Cr (0) metal and aluminum oxide.

Important Commercial Uses of Nickel and Nickel Compounds

Chromium is also a very important and useful metal commercially. Chromium is used in large quantities in many important alloys, such as stainless steel (consisting of iron, nickel, and chromium) and ferrochromium

(consisting of iron and chromium). Chromium is also used in the manufacture of paints and pigments, where various chromium compounds impart yellow and orange colors to the paints and pigments. Such compounds as ►lead chromate ($PbCrO_4$), strontium chromate, and barium chromate have been used in paints to paint aircraft due to the anti-corrosive properties of these compounds. Lead chromate is very toxic, and hence has been replaced by strontium and barium chromates. Because chromium metal is resistant to corrosive agents, it is extensively used as a protective coating that is delivered onto other metals by an electroplating process.

Biological Aspects of the Essentiality of Chromium (III) in Mammalian Cells

In mammals, Cr(III) is considered an essential trace element. The required daily uptake of Cr(III) is from 50–200 μg/day. Cr(III) is usually ingested by humans from the diet. Cr(III) is considered an essential trace element because it is a component of the complex called glucose tolerance factor, which aids in glucose and lipid metabolism. Glucose tolerance factor, or "low–molecular–weight Cr binding substance" is an oligopeptide. It is thought that a tetranuclear Cr(III) carboxylate complex may be present in glucose tolerance factor. Glucose tolerance factor is believed to aid insulin in mediating the uptake of glucose into mammalian cells. This area of nutrition requires further investigation.

Exposure of Humans to Chromium Compounds

Cr(VI)-containing compounds are very toxic to humans, both insoluble and soluble Cr(VI)-containing compounds. In terms of toxicology, humans are commonly exposed to chromium as Cr(VI)-containing compounds, primarily during the refining of chromium from chromite ore, and also from the manufacture of chromium compounds for use in pigments and paints and during chromium electroplating. In the past, exposures of workers manufacturing chromate compounds to Cr(VI) compounds resulted in higher incidences of nasal and respiratory cancers. In workers who conducted chromium electroplating, there were also increased incidences of nasal and respiratory cancer. There were also increased incidences of various cancers in workers who were employed at tanneries, where they tanned cow hides, where chromate was also used to enhance the tanning process.

When inhaled, insoluble Cr(VI)-containing compounds can enter the airways, and be ►phagocytosed by macrophages and by normal airway epithelial cells. This leads to deposition of a bolus of Cr(VI) inside the cells. When inhaled, soluble Cr(VI) compounds, in the form of chromate $(CrO_4)^{-2}$, bind to and enter mammalian cells on the sulfate-phosphate anion transport carrier. This anion transport carrier is somewhat nonspecific, and chromate can bind to it in place of phosphate and sulfate. Hence, inhalation of both soluble and insoluble Cr(VI)-containing compounds can cause cancer of the respiratory tract, insoluble Cr(VI) compounds via ►phagocytosis and soluble Cr(VI) compounds by entering cells on the anion transport carrier.

To date, we also know that ingestion of soluble Cr(VI)-containing compounds, or drinking them when they are added to or contaminate drinking water, can lead to these compounds being taken up into cells of the alimentary tract, and entering cells on the sulfate-phosphate anion carrier. This can pose a risk for stomach cancer and cancer of the intestines, as well as a cancer risk to many other internal organs, in both lower animals (rodents) and humans.

Genotoxicity of Chromium Compounds

Chromium (VI) compounds are found in both the soluble and insoluble forms. Soluble and insoluble chromium compounds are both taken up by mammalian cells. Insoluble chromium (VI) compounds are taken up into mammalian cells by phagocytosis, and soluble Cr(VI) compounds are taken up into mammalian cells by the phosphate-sulfate transport carrier. Lead chromate, strontium chromate, and barium chromate are examples of Cr(VI)-containing compounds that are only sparingly soluble in water. Calcium chromate and potassium dichromate, are examples of Cr(VI) compounds that are highly water soluble.

There are many insoluble chromium (VI) compounds, such as lead chromate, barium chromate, and strontium chromate. If these insoluble Cr(VI)-containing chromium compounds are found in particle sizes of <10 μm, they are phagocytosed by mammalian cells. Phagocytosis involves an invagination of the plasma membrane around the particles, to form a phagocytic vesicle. The phagocytic vesicle then is internalized into the cell with its entrapped particle of insoluble Cr(VI)-containing compound. Hence, by the process of phagocytosis, a bolus of insoluble Cr(VI)-containing compound can be taken up into mammalian cells. The phagocytosed insoluble Cr(VI) compounds then enter the lysosomal network. The resultant chromium compounds then dissolve into soluble chromate ions and counterions. The chromate ions then migrate through the cell in an attempt to establish chemical equilibrium. Eventually, some chromate ions will travel into the nucleus. Eventually, the Cr(VI) in the chromate ions will be reduced to Cr(V), Cr(IV), and then to Cr(III). In addition, it is thought that Cr(VI) can act as a pseudo-Fenton reagent, that can generate hydroxyl radical from ►superoxide radical (formed during normal metabolism) plus ►hydrogen peroxide (also formed during normal cellular metabolism).

Soluble Cr(VI)-containing compounds can enter mammalian cells on the sulfate-phosphate anion transport carrier, because they are competitive substrates and

therefore inhibitors of sulfate and phosphate uptake into mammalian cells. Following phagocytosis of insoluble Cr(VI) compounds, or uptake of soluble Cr(VI) compounds on the phosphate-sulfate anion transport carrier, the resultant intracellular Cr(VI) ions cause cytotoxicity, DNA strand breaks, DNA-protein cross-links, and ►chromosomal aberrations, in the forms of gaps, breaks, fragments, dicentrics, and satellite associations, to mammalian cells.

Among the soluble Cr(VI) compounds, calcium chromate, sodium chromate, chromium trioxide, and potassium dichromate all induce mutation to 6-thioguanine resistance in diploid human fibroblasts. Lead chromate, an insoluble Cr(VI) compound, induced a weak yield of mutation to 6-thioguanine resistance in cultured diploid human fibroblasts. Induction of mutation to 6-thioguanine resistance by Cr(VI) compounds, both soluble and insoluble, occurred over the range of 0.05 μM – 1.00 μM. For Cr(III) compounds, weak mutation was induced by soluble chromium chloride and insoluble chromium chloride and Cr(III) oxide, but only at the very high concentrations of 50–1,000 μM, which were 1,000-fold higher than those used to induce mutation by Cr(VI) compounds. Hence, Cr(VI) compounds are 1,000-fold more mutagenic, and cytotoxic, than Cr(III) compounds. Cr(III) compounds are not considered significantly toxic at low concentrations, particularly since Cr(III) is considered an essential nutrient and a component of "glucose tolerance factor."

Once inside mammalian cells, Cr(VI) ions are reduced to Cr(V), Cr(IV), and Cr(III) ions. Cr(III) ion binds to DNA and thereby induce mutation to mammalian cells. There is also some evidence that intracellular Cr(VI) ions and more reduced Cr species, can generate ►reactive oxygen species (ROS) intracellularly in mammalian cells. In mammalian cells, superoxide radicals arise from normal cellular oxidative metabolism. Following the dismutation of two superoxide radicals by superoxide dismutase, hydrogen peroxide is formed, particularly in mitochondria (mitochondrial DNA and cancer). There is some evidence that intracellular Cr(VI) ions can catalyze pseudo-Fenton reactions, in which superoxide radical, hydrogen peroxide, and Cr(VI) ions can catalyze the reaction of superoxide radical and hydrogen peroxide to generate hydroxyl radicals and hydroxyl ions. The resultant hydroxyl radicals are able to cause formation of 8-hydroxy-deoxyguanosine and therefore mutations in DNA. However, this latter pathway has not been demonstrated conclusively with rigorous experimentation.

Chromium-Induced Cell Transformation

As noted above, insoluble Cr(VI)-containing compounds can be phagocytosed into mammalian cells, leading to generation of intracellular Cr(VI)-containing ions. Soluble chromium compounds generate soluble Cr(VI)-containing ions, which are taken up on the sulfate-phosphate anion transport carrier and enter mammalian cells. These intracellular Cr(VI) ions can be reduced by intracellular reductants, such as glutathione, which will then generate Cr(V), Cr(IV), and Cr(III) containing ions. These Cr ions can then induce DNA-DNA cross links, DNA-protein cross-links, DNA strand breaks, mutations, and chromosomal aberrations, which are genotoxic events. Many Cr(VI)-containing compounds, including insoluble lead chromate, and soluble Cr(VI) compounds, can induce morphological and neoplastic transformation of mammalian cells, and in particular in rodent cells, in culture. Lead chromate has been shown to induce morphological, anchorage-independent, and neoplastic transformation of C3H/10T1/2 mouse embryo fibroblastic cells.

It is thought that the mechanism of Cr(VI)-induced morphological transformation is due to genotoxic events, including the mutations and chromosomal aberrations that Cr(VI) causes in mammalian cells. These chromosomal aberrations are thought to lead to loss of regions of chromosomes bearing ►tumor suppressor genes, or disrupt areas of chromosomes bearing tumor suppressor genes, which can lead to loss of tumor suppressor genes from cells, contributing to morphological, anchorage-independent, and neoplastic cell transformation. Similarly, intracellular Cr(VI) species can also generate Cr(III) and reactive oxygen species, which can cause mutations in ►proto-oncogenes, leading to activation of oncogenes, which is a part of the mechanism of Cr(VI)-induced morphological and neoplastic cell transformation. In addition, generation of intracellular Cr(III) ions due to reduction of intracellular Cr(VI) by intracellular reductants, such as glutathione and other reductants, is thought to lead to mutations in proto-onocogenes, leading to activation of oncogenes, which also contributes to cell transformation. It is likely a combination of all these events that leads to Cr(VI)-induced morphological, anchorage-independent, and neoplastic cell transformation.

Chromium Carcinogenesis

Specific insoluble Cr(VI)-containing compounds, and also soluble Cr(VI) compounds, are carcinogens in lower animals such as rodents by the inhalation route. Similarly, both insoluble and soluble Cr(VI) compounds are also carcinogenic in humans when humans are exposed to them by the inhalation route. This is particularly true in the context of chrome electroplating and during the manufacture of chromates, where chromates are contacted by inhalation.

Recently, increasing attention has been paid to the risk of cancer when Cr(VI) compounds are ingested or taken in with the drinking water. This is because Cr(VI) compounds have been used to paint aircraft in order to utilize the anti-corrosive properties of the

Cr(VI) compounds. This has led to contamination of drinking water sources in various states in the United States, such as California. In addition, Cr(VI) compounds have been used in the water of cooling towers. Discharge of this water into the drinking water sources has led to contamination of the drinking water sources with Cr(VI) compounds. When Cr(VI) enters humans or lower animals though the drinking water route, a significant amount of the Cr(VI) is reduced to Cr(III) extracellulary, reducing its toxicity, mutagenicity, and carcinogenicitiy. However, while this extracellular reduction is going on, there is a simultaneous, competitive uptake of Cr(VI) by the anion transport carrier into cells. This leads to a significant fraction of Cr(VI) getting into cells in a competitive mechanistic scheme d (i.e. A →B (cells) or C (enzymes of reduction). In rodents, administration of Cr(VI) by the oral route leads to stomach and intestinal tumors. There is weaker epidemiological data which also indicates that when humans drink water containing Cr(VI), the incidence of various internal tumors is increased. This includes a small increased incidence of stomach tumors, tumors of the bones, leukemias, kidney tumors, and liver tumors. Hence, while the cancer risk due to inhalation of Cr(VI) compounds is large, the cancer risk due to ingestion or drinking water containing Cr(VI) is smaller than the inhalation risk but is still of appreciable significance from the standpoint of induction of human cancer.

Mechanisms of Chromium Carcinogenesis

At present, the molecular mechanisms of Cr(VI) carcinogenesis appear to be due largely to genotoxic effects. These genotoxic effects include the ability of intracellular Cr(VI) ions to be reduced to Cr(V), Cr(IV), and Cr(III) ions, and the ability of Cr(III) ions to bind to DNA and cause mutations. There is also some thinking that Cr(VI) ions can act as redox catalysts to cause pseudo-Fenton reactions. The reduction of Cr(VI) ions is believed to lead to generation of reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radicals, which can cause 8-hydroxyguanosine in DNA, leading to mutations. Generation of 8-hydroxy-deoxyguanosine and the binding of Cr(III) to DNA, would be expected to lead to mutations in many genes, including proto-oncogenes and tumor suppressor genes. This would be expected to result in the activation of proto-oncogenes into oncogenes and the mutational inactivation of tumor suppressor genes, leading to neoplastic cell transformation and eventually carcinogenesis.

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Chromium-induced Carcinogenesis

►Chromium Carcinogenesis

Chromium-induced Cell Transformation

►Chromium Carcinogenesis

Chromium Tumorigenesis

►Chromium Carcinogenesis

Chromocenter

Definition

A punctate condensed collection of chromatin encountered in the interphase cell nuclei of certain cell types with unknown biological significance.

►Oligodendroglioma

Chromodomain

Definition
Conserved domain that specifically recognizes and binds to methylated lysine residues that occur within a protein.

►Histone Modifications

Chromogranin A

Definition
Is a glycoprotein secreted by nearly all types of neuroendocrine tumors used as a diagnostic/monitoring tool. It is found in the wall of secretory vesicles in neuroendocrine cells.

►Neuroendocrine Carcinoma

Chromophore

Definition
A molecule that can selectively absorb photon energy at certain wavelengths.

►UV Radiation

Chromophore-Assisted Laser Inactivation

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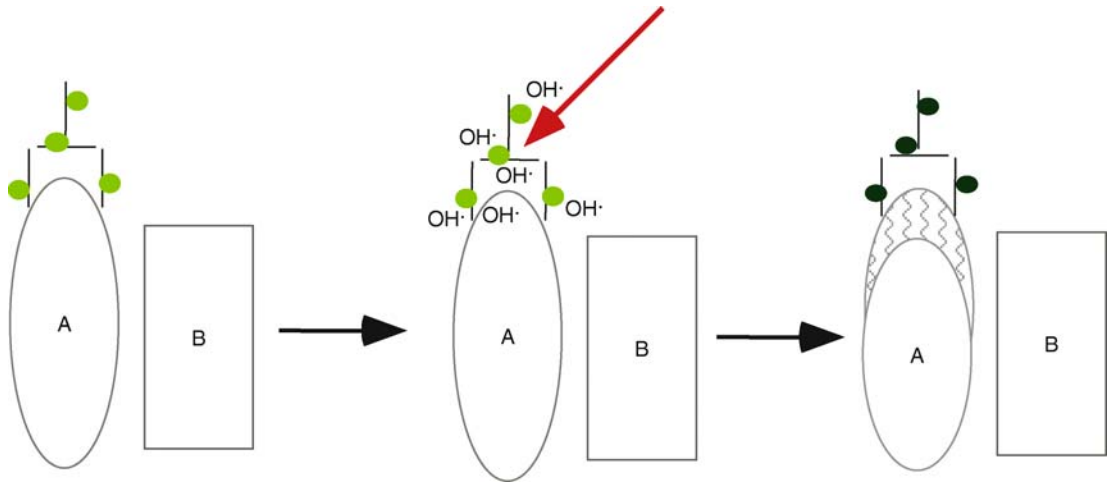
Synonyms
CALI

Definition
Technology to address protein function in situ. CALI uses ►**laser** light of 620 nm, targeted via specific Malachite Green-labeled non-function-blocking antibodies, which generate short-lived protein-damaging free radicals (Fig. 1). This wavelength is not absorbed by cells, such that nonspecific light damage does not occur. The short lifetime of the free radicals generated restricts the damage largely to the bound antigen (~15 Å) such that even neighboring proteins are not significantly affected. Micro-CALI focuses the laser light through microscope optics such that proteins within a 10 μ spot may be inactivated.

Characteristics
The advent of complete genomic information will herald a new revolution in molecular biology to develop a mechanistic understanding of how proteins function together in the living cell. This increased understanding will provide insight into cancer (as well as other diseases) and potentially will help define protein targets for drug discovery. Target validation of proteins of disease relevance (the site of most drugs) is the limiting first step in obtaining drugs of clinical value. In particular, identifying proteins that have essential roles in cancer-relevant cellular processes remains a major challenge. There is a current lack of technology that addresses protein function directly and rapidly, as most functional inactivation approaches target genes or mRNAs. One useful tool to address this is Chromophore-Assisted Laser Inactivation (CALI). CALI uses targeted laser light to inactivate proteins of interest via a dye-labeled antibody that by itself does not block function. CALI provides a high degree of temporal and spatial resolution to acutely perturb protein function in situ.

Advantages
The major advantages of CALI compared to other functional inactivation approaches are its unprecedented temporal and spatial resolution. The range of area for CALI-based inactivation is localized to regions within the beam, between microns to millimeters depending on the focus of the laser. Inactivation occurs acutely upon initiating laser irradiation. Because the loss of protein function is acute and transient, CALI does not appear to be subject to genetic compensation that is occasionally observed in chronic deletion strategies such as gene knockouts in mice. CALI is particularly useful for systems lacking genetic methods. For example, CALI may be used for human tissue culture cells that are disease-relevant. As such, it is not necessary to extrapolate target validation from model systems. Many proteins of interest to cancer research have essential roles in early development and are thus difficult to address by gene knockout. CALI may be used to study the roles of these essential gene products in cellular processes after development is completed. The coupling of micro-CALI with real time imaging has been used for studying dynamic cellular processes. The application of micro-CALI on part of a single



Chromophore-Assisted Laser Inactivation. Figure 1 The principle of CALI. Specific proteins (a) in cells are bound by an antibody and labeled with the chromophore Malachite Green (MG). Irradiation with pulsed laser light of 620 nm (*red arrow*) generates short-lived hydroxyl radicals. These radicals selectively inactivate the bound protein by oxidative damage because their half-maximal radius is about 15 Å due to their short life time in cells. Neighboring proteins (b) are not significantly affected.

cell generates a transient asymmetry of function across a cell, and this has been particularly useful in addressing proteins required for cell motility and migration. The capacity and relative ease of multiplex approaches for CALI (compared to gene knockouts) may lend itself well to whole ►proteome approaches and ►high throughput screens.

Limitations

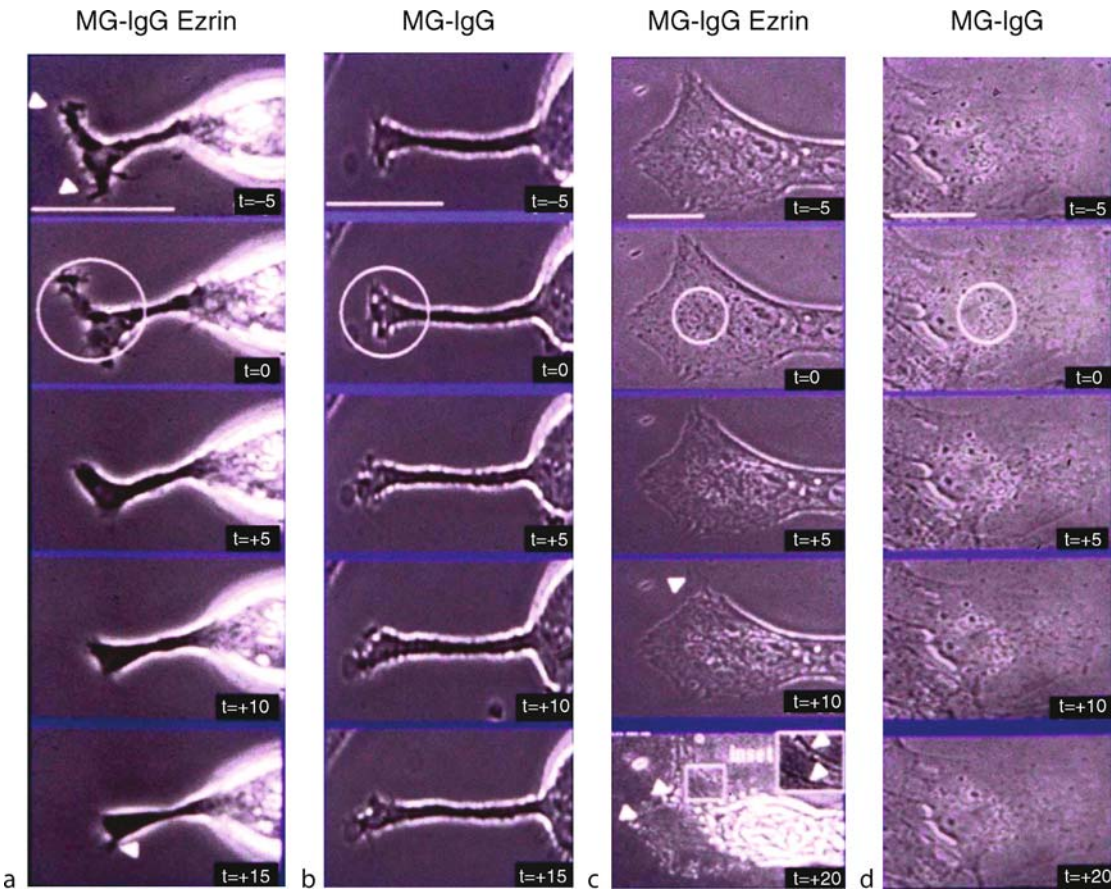
As with any technology, CALI has its limitations, and a clear understanding of these is required for its judicious application. Inactivation is dependent on quality, specificity and site of antibody binding and also on the susceptibility of the targeted protein to free radical damage. It should be noted that only the protein (not the gene) is inactivated and hence recovery is dependent on de novo synthesis. This usually allows a loss of function of hours to perhaps a day. The loss of function may not be complete so that activity is "knocked down" as opposed to "knocked out." As such, residual activity may obscure a potential phenotype. In general, a negative result is difficult to interpret for CALI as with most other inactivation approaches.

Application

Current Applications

CALI has been used for over 50 proteins and been successful in ~90% of these cases. CALI has precisely mimicked Drosophila genetic loss of function mutations in several direct comparisons. The proteins studied in many cell and animal systems span a diverse array that include membrane receptors, cytoskeletal proteins, signal transduction molecules and transcription factors. The understanding of how proteins function in the nerve growth cone has been a major area of study that has utilized CALI. The functional roles of proteins such as NCAM, L1, calcineurin, talin, vinculin, myosin V and Ib, radixin and tau have been recently addressed. For these studies, methods for introducing antibodies into living cells have been optimized including electroporation, trituration and microinjection.

Of particular relevance to cancer research is the application of CALI to proteins that have roles in cancer cell migration. One example of the application of CALI to a protein of cancer relevance is the prototypic ERM-family (►ERM proteins) member, ezrin. Ezrin is an actin-associated protein that shows increased expression and phosphorylation upon fos-mediated transformation of fibroblasts that is correlated with a change in cell shape (from flat to rounded) and motility (from lamellipodial to pseudopodial). CALI of ezrin in transformed fibroblasts causes a decrease in membrane ruffling and pseudopodial retraction. CALI of ezrin in normal fibroblasts causes a marked collapse of the leading edge lamellipodia (Fig. 2). These studies implicate ezrin in cell shape and motility and suggest that ezrin has a critical role in the shape and motility changes associated with oncogenic transformation. A second protein of interest is the tumor supressor ►hamartin. Hamartin binds to ERM proteins and its function is regulated by the small GTPase, Rho. CALI was used to show a role for hamartin in cell adhesion and suggests it might be involved in a rate-limiting step in tumor formation.



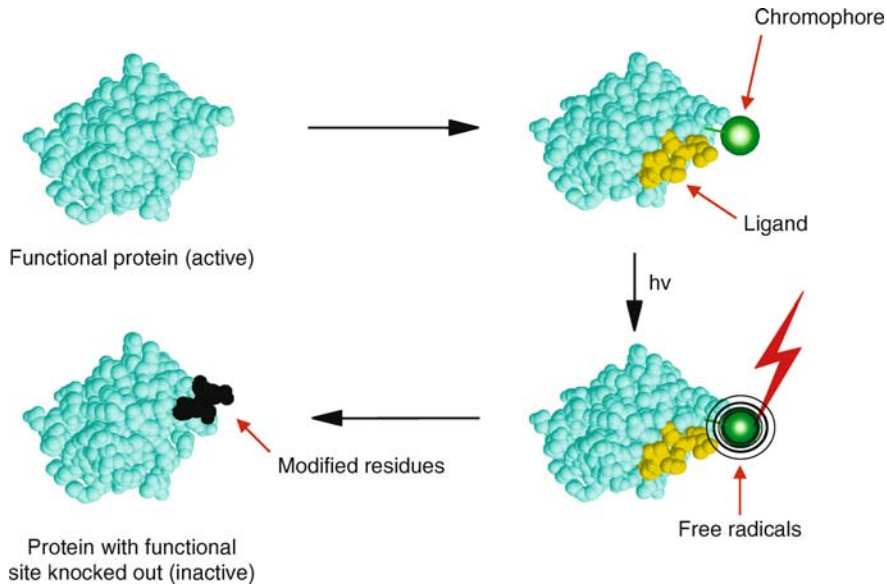
Chromophore-Assisted Laser Inactivation. Figure 2 Micro-CALI of Ezrin affects fibroblast shape and motility. Fibroblasts transformed with v-fos, change their shape and motile behavior and show an increase in the expression and phosphorylation of the actin-associated ERM protein, Ezrin. We applied micro-CALI of Ezrin within the circled areas to v-fos-transformed fibroblasts (A and B) and normal fibroblasts (C and D). Micro-CALI of Ezrin in v-fos transformed cells caused a loss of membrane ruffling (arrowheads) and pseudopodial retraction (a) while laser irradiation of cells injected with Malachite Green-labeled non-immune IgG had no effect on motility (b). Micro-CALI of Ezrin in normal fibroblasts caused a marked collapse of the leading edge (c) with filaments remaining attached to the substratum (arrowheads in inset of panel labeled t = +20). Irradiation of cells, injected with Malachite Green-labeled non-immune IgG had no effect on cell shape (d). Scale bars = 10μm; time is in minutes.

Future Applications

CALI is currently being combined with advances in dynamic imaging to visualize subcellular changes in response to the loss of function of specific proteins. We view that a major application of CALI for cancer research will be in target validation. As CALI lends itself well to combinatorial approaches and high throughput methods, it may be a powerful tool in addressing function in a proteome wide manner. A new use for CALI is in refining drug discovery screens to direct them against binders of a single domain on the target protein. CALI causes localized oxidative damage to modify residues of the protein near the antibody-binding site. By combining CALI with high resolution mass spectrometry to map those sites of damage, it may be possible to correlate loss of function with particular domains on a protein (Fig. 3).

Conclusions

CALI is a means for the inactivation of specific proteins in situ with a high degree of spatial and temporal resolution. CALI converts a binding reagent (such as an antibody) into an functional inhibitor. A large number of studies has demonstrated the potential of CALI in addressing cellular processes. It has recently been employed to address cellular mechanisms of cancer, and we believe that this technology is poised to contribute significantly to target validation and drug discovery for cancer-relevant processes.



Chromophore-Assisted Laser Inactivation. Figure 3 Principle of Xplore. Xplore uses CALI and high resolution mass spectroscopy to map regions of functional importance on proteins. After CALI inactivation, sites of oxidative damage are mapped by high resolution mass spectroscopy, providing a correlation of a protein function with specific domains of the targeted protein.

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Chromosomal Aberrations

Definition

Damage to the chromosomes of a cell by a chemical carcinogen or other genotoxic agent, such that the shape of the chromosome is changed. Examples include gaps in chromosomes, breaks in chromosomes, fragmentation of chromosomes, dicentric chromosome formation, chromosome translocations, and satellite associations.

►Nickel Carcinogenesis
►Chromium Carcinogenesis

Chromosomal Imbalance

Definition

Loss or gain of chromosome copy number.

►ArrayCGH

Chromosomal Instability

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Synonyms

Genetic instability; CIN

Definition

Chromosomal instability is the gain and/or loss of whole chromosomes or chromosomal segments at a higher rate in a population of cells, such as cancer cells, compared to their normal counterparts (normal cells). In some cancers, each cell within the tumor has a different chromosomal constitution (karyotype) due to chromosomal instability, which may be defined in practical terms as numerical and/or structural chromosomal alterations that vary from cell to cell. Although the terms chromosomal instability and genomic instability have been used interchangeably, this is technically incorrect, as they refer to different forms of genetic instability.

Characteristics

Chromosomal instability is a characteristic of cancer cells, especially solid tumors (rather than most hematologic (blood cell) malignancies). Several cellular mechanisms lead to numerical and structural chromosomal instability in cancer cells, including defects in (i) chromosomal distribution to the daughter cells (chromosome segregation), (ii) cell cycle checkpoints that protect against proliferation of abnormal cells, (iii) telomere (specialized structures that cap the ends of chromosomes) stability, and (iv) the DNA damage response. Although in the past, these mechanisms were thought to be unrelated, it has become clear that they are intimately intertwined, connecting the complex network of cellular pathways. Human papillomavirus and other oncogenic viruses interfere with these processes, causing chromosomal instability and tumor formation in the cells that they infect. Chromosomal instability plays an important role in cancer by creating large-scale genetic changes in as little as one cell generation, leading to rapid cancer cell evolution. The rate of discoveries about the mechanisms leading to chromosomal instability in cancer cells is accelerating, improving our understanding of how cells become cancer cells and how cancer cells become more dangerous to the patient by progressing and/or metastasizing.

Both clonal numerical and structural chromosomal alterations and chromosomal instability are common features of human cancers. Aneuploidy is the condition in which the chromosome number in a cell, population of cells, or person is not an exact multiple of the usual haploid chromosome number ($N = 23$ for humans). Aneuploidy results from numerical chromosomal alterations. Cancers with chromosomal instability are characterized by aneusomy, a condition in which a population of cells contain different numbers of chromosomes. In tumor cells, gains and losses of chromosomal segments arise as a result of structural chromosomal alterations, including reciprocal and non-reciprocal chromosomal translocations, homogeneously staining regions (in which a cassette of contiguous genes, including at least one oncogene or growth-related gene, is tandemly repeated (amplified) at least five times on a diploid background), other forms of gene amplification (e.g., double minute chromosomes), insertions, and deletions. Structural alterations may result in a further imbalance in gene expression, resulting in chromosomal instability. In some tumors, each cell within the tumor has a different karyotype due to chromosomal instability.

Historical Background

Chromosomal instability is thought to be the means by which cells develop the features that enable them to become cancer cells. In spite of the presence of cell-to-cell chromosomal instability, the tumor karyotype is thought to be quite stable over time, probably because advanced tumors have evolved a genetic makeup (genotype) optimized for growth, making it less likely that additional genetic alterations will confer an additional growth advantage. Chromosomal alterations and karyotypic instability in human tumor cells have been investigated for nearly a century. David von Hansemann first identified abnormal dividing cells is tissue sections of tumors, including cell divisions that appeared to have asymmetric spindles or multiple spindle poles (multipolar spindles) that would lead to unequal distribution of the chromosomes to the daughter cells, and chromosomes stretched between the two spindle poles late in cell division (anaphase bridges). Theodor Boveri [2], while studying chromosomal segregation in *Ascaris* worms and *Paracentrotus* sea urchins in the early 1900s suggested that malignant tumors arise from a single cell with an abnormal genetic constitution acquired as a result of defects in the mitotic spindle apparatus. Today we know that numerical chromosomal instability arises as a result of chromosome segregational defects, most frequently resulting from multipolar spindles. Structural chromosomal instability results from chromosome breakage and rearrangement due to defects in cell cycle checkpoints, the DNA damage response, and/or loss of telomere integrity. Structural chromosomal instability frequently results from breakage-fusion-bridge (BFB) cycles, first described in maize by geneticist Barbara McClintock in 1938. In this process, a chromatid break occurs, exposing an unprotected chromosomal end which, after replication, is thought to fuse with either another broken chromatid or its sister chromatid to produce a dicentric chromosome. During the anaphase stage of mitosis, the two centromeres are pulled to opposite poles, forming a bridge which breaks, resulting in more unprotected chromosomal ends, and thus the cycle continues. Our studies of cancer cells suggest that structural chromosomal instability, including gene amplification, occurs by BFB cycles. The basis for these BFB cycles is not entirely clear, although recent studies of chromosomal

fragile site breakage, some of which occurs as a result of cigarette smoking and leads to induction of BFB cycles, telomere dynamics, and the DNA damage response, suggest that these critical cellular processes play major roles in the development of structural chromosomal instability. In this contribution, defects in chromosomal segregation, cell cycle checkpoints, telomere function, and the DNA damage response and their role in mechanisms leading to chromosomal instability are introduced and literature citations (References) are provided for the interested reader.

Chromosome Segregational Defects Lead to Chromosomal Instability

One of the fundamental processes required in the life of a cell, whether from a unicellular or multicellular organism, is chromosome segregation. Fidelity of chromosome segregation, whether in meiosis or mitosis, is necessary for genomic stability and the continuation of life as we know it. Abnormal chromosome segregation results in aneuploidy, abnormal numbers of chromosomes being distributed to daughter cells, such that the daughter cells don't match each other or their mother cell. This is the essence of chromosomal instability. Recent studies have shown that several factors can result in segregation defects, including abnormal chromosome-spindle interactions, premature chromatid separation, centrosome amplification, multipolar spindles, and abnormal cytokinesis (cell division). Chromosomal segregational defects (multipolar spindles, lagging chromosomes at metaphase and anaphase, and anaphase bridges) in cancer cell lines are an intrinsic, heritable trait in the general tumor cell population. Tumor cells expressing chromosomal instability cannot be "cloned," as they continue to express numerical and structural chromosomal instability generation after generation. In some cancers ongoing chromosomal instability is a feature of both primary tumors in the patient and cell lines cultured in the laboratory from biopsies removed from those tumors. Many studies of proteins involved in the process of chromosome segregation, spindle function, and cytokinesis are in progress in numerous laboratories. The role of these proteins in chromosomal instability and implications in the diagnosis, prognosis, and therapy of human tumors will be revealed in the next few years.

A Defective Response to DNA Damage Leads to Chromosomal Instability

For many years, cytogeneticists (scientists who examine chromosomes) have known that patients with "chromosome breakage" syndromes express chromosomal instability. Yet, until recently, features of these syndromes have not been utilized to define defects in the DNA damage response in cancer cells. Causes of DNA damage include attack by ultraviolet light, ionizing radiation (X-rays), or environmental chemicals, and cellular errors, such as "spelling errors" (base pair mismatch) during DNA replication, replication fork collapse, or defects caused by naturally occurring reactive oxygen species. One type of DNA damage is the double strand break, which leads to a cascade of cellular events (the DNA damage response) that usually results in repair of the damage or cell death. Failure in the DNA damage response and double strand break repair can lead to genetic alteration or chromosomal instability, which can result in transformation from a normal cell to a cancer cell.

The DNA damage response involves the sensing of DNA damage followed by transduction of the damage signal to a network of cellular pathways, from those involved in the cellular survival response, including cell cycle checkpoints, DNA repair, and stress responses to telomere maintenance, and the apoptotic pathway. To make a simple analogy in an effort to describe the complex DNA damage response to double strand breaks, we can say that our cellular instruction book for all of the activities that go on in our cells and in our bodies is made up of 23 chapters, the chromosomes, and for safety's sake, we have two copies of the book, one from our mother and one from our father, although they aren't exact copies (e.g., the set of eye color genes from your mother may code for blue eyes and the one from your father, brown). The genes are like sentences in a chapter, made up of three letter words composed of the four letters of DNA, A, T, C, and G. The 23 different chromosomes in the cells, composed of many genes, are equivalent to the 23 chapters in the book, made up of many sentences. The total genome is equivalent to the whole instruction book for the cells, and the instructions code for proteins, the molecules that do the work in our bodies. So in total, we have 46 chromosomes, two copies of each one. Sometimes, this very long set of DNA instructions becomes damaged (like the pages in the book can become torn or fall out) from smoking, chemicals, X-rays, oxidants that occur naturally in our bodies (why some of us take "antioxidant" vitamins), or other insults. Although our DNA is a code of letters like words in a book, it really looks like a ladder or even like railroad tracks. To more easily think about DNA repair, we need to visualize it as railroad tracks. Like the railroad company, which has special vehicles that check the integrity of the tracks, we have proteins that check our DNA (sensor proteins and checkpoint proteins). If the sensor protein spots a double strand break or another defect that might derail the train or cause a defect in the cellular instructions (mutation), she then tells the communications officer (signal transducer) to call headquarters which in turn, calls the repair team. This happens in our cells, in which case the repair team is a series of proteins that carry out sequential multistep assessment and repair of the damage (the DNA damage response).

If they find that a cargo train has already been instructed by the defect to race out of control, analogous to a cell proliferating in an uncontrolled fashion, making more and more copies of itself, on the way to making a cancer, they kill that cell. But, what if the protein that has the job of pushing the kill switch is sick that day, the cell cannot be killed and a cancer ensues. In our cells, this DNA damage response pathway is carried out by about 50 proteins in a carefully choreographed process. With the advances in the human genome project, we are learning more about the proteins in this pathway and how defects (mutations) in them can cause predisposition to cancer.

Loss, mutation, or altered function of the genes that code for some of the DNA damage response proteins cause familial cancer syndromes and in some cases, chromosomal breakage syndromes, which may affect heterozygous gene carriers or affected (homozygous) individuals. Although not clear at this time, the role of these critical DNA damage response genes in chromosomal instability merits further investigation. The DNA damage response genes involved in known familial cancer syndromes include ►*ATM*, *TP53*, *BRCA1*, *BRCA2*, *FANC*, *CHEK2*, *BLM*, and *MRE11A*. The involvement of the DNA damage response genes, *BRCA1* and *BRCA2* in familial breast and ovarian cancer is well known. Both genes also appear to be associated with an increased risk of prostate cancer, and *BRCA2* is involved in familial pancreatic cancer. Germline *TP53* mutation carriers have Li-Fraumeni syndrome which is associated with a high risk of breast and brain tumors, sarcomas (muscle tumors), leukemia (blood cell tumors), laryngeal (voice box) and lung cancer, and other tumors. Germline *CHEK2* mutation carriers may present with a Li-Fraumeni-like syndrome and may have an increased risk for a wide range of tumors including breast, prostate, and colorectal (intestinal) cancer. Patients with ataxia telangiectasia, the autosomal recessive genetic disorder characterized by a defective *ATM* gene, manifest progressive cerebellar ataxia (staggering gait), telangiectases ("blood shot" eyes and skin), immune dysfunction, chromosomal instability, increased sensitivity to ionizing radiation (X-rays), and predisposition to cancer, especially leukemia. Heterozygous *ATM* carriers (both human and mouse) of dominant-negative (interfering) missense mutations are at increased risk for solid tumors, including breast cancer. Fanconi anemia (FA) is a rare genetic cancer susceptibility syndrome characterized by skeletal abnormalities, skin pigmentation abnormalities, bone marrow failure, chromosomal instability in the form of rearrangements between non-homologous chromosomes, and sensitivity to DNA crosslinking agents. FA patients are predisposed to developing cancer, primarily leukemia and epithelial tumors, especially squamous cell carcinoma of the mouth and throat (called head and neck cancer) or cervical cancer. The risk of solid tumors in FA patients is ~50-fold higher for all solid tumors compared to the general population, but about 700-fold higher for head and neck cancers. Bloom syndrome is an autosomal recessive disorder characterized by growth deficiency, sun-sensitive facial redness, hypo- and hyper-pigmented skin, sterility in males, reduced fertility in females, predisposition to a variety of malignancies, and chromosomal instability. Thus, patients with cancer predisposition and "chromosomal breakage" syndromes will continue to educate us about the cellular processes that lead to chromosomal instability and cancer.

Telomere Dysfunction May Lead to Chromosomal Instability

Telomere loss or dysfunction is a cause of chromosomal instability in the laboratory mouse. Telomere loss can result from DNA damage or occur spontaneously in cancer cells which often have a high rate of telomere loss due to telomere shortening with each cell division. Telomere alterations in certain genetically engineered mice mirror those in human epithelial tumors, lending support to the hypothesis that telomere defects drive chromosomal instability in cancer cells and age-related epithelial carcinogenesis. Thus, in mouse and man, telomere dysfunction leads to chromosomal instability, as shown by studies of telomere dysfunction in the mouse, chromosomal breakage patterns in human tumors, and the observation that cancer predisposition syndromes can lead to both telomere dysfunction and chromosomal instability. Consistent with this hypothesis, both telomere shortening and cancer incidence increase with age. Telomeres play an important role in chromosomal instability, but the exact details remain under active investigation.

Cell Cycle Disturbances Result in Chromosomal Instability

Oncogenic (cancer causing) viruses, such as human papillomavirus (a sexually transmitted disease which causes cervical cancer in women, penile cancer in men, and oral and anal cancer in both men and women), recapitulate the abnormalities, including defects in chromosome segregation, centrosome dynamics, telomere mechanics, the DNA damage response, cell cycle regulation, and cell cycle checkpoints, that appear to play important roles in the development and maintenance of chromosomal instability. The primary impact of chromosomal instability is cancer. In addition, chromosomal instability is a major cause of tumor evasion of or resistance to therapy. Therefore, a complete understanding of the biological basis of chromosomal instability is essential for developing therapies targeted against the defects in cancer cells.

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Chromosomal Rearrangement

Definition

A chromosome mutation involving new juxtapositions of chromosome parts.

►Chromosome Translocations

Chromosomal Translocation t(9;22)

Definition

Chromosomal abnormality resulting from the rearrangement of sections between two nonhomologous chromosomes. This refers to the Philadelphia chromosome characteristic of ►chronic myeloid leukemia (CML) and some forms of ►acute lymphoblastic leukemia (ALL).

►Nilotinib

Chromosomal Translocation t(8;21)

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Synonyms

t(8;21); t(8;21)(q22;q22); AML1/MTG8; AML1/ETO; RUNX1/CBFA2T1; RUNX1/RUNX1T1

Definition

The ►chromosomal translocation t(8;21) is associated with ►acute myeloid leukemia. The resultant fusion gene AML1/MTG8 (AML1/ETO, RUNX1/CBFA2T1, RUNX1/RUNX1T1) is a repressor of gene transcription. In this chapter, the fusion gene is named *AML1/MTG8*, and the corresponding fusion protein AML1/MTG8.

Characteristics

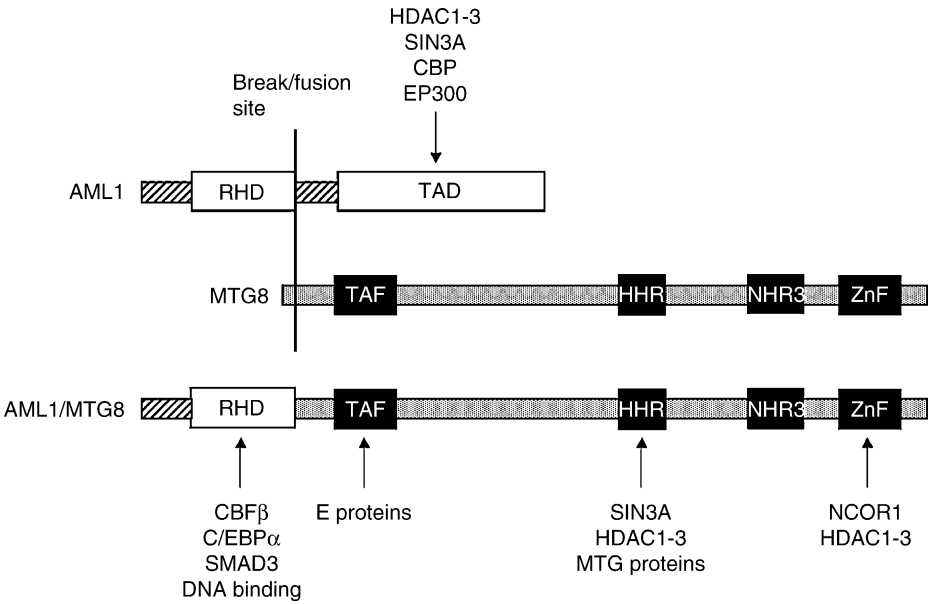
Cytogenetics and Morphology

Almost 50% of all cases of acute leukemia are associated with recurrent chromosomal changes such as inversions or translocations of material from one chromosome to the other. t(8;21)(q22;q22) marks a chromosomal translocation, where the chromosomes 8 and 21 exchanged their long arms (the q arms) from band 22 till the telomere. This translocation is exclusively associated with acute myeloid leukemia (AML). Most commonly, standard cytogenetic analysis is used to detect the t(8;21). In addition, molecular techniques such as ►FISH (fluorescence in situ hybridization) or reverse transcriptase polymerase chain reaction (RT-PCR) are increasingly used for the identification of t(8;21) positive patients. Several studies comparing the sensitivity of PCR techniques and standard cytogenetics for the detection of t(8;21) have found AML1/MTG8 transcripts also in patients with no cytogenetic evidence of this aberration. These findings indicate, that the sensitivity for the detection of a t(8;21) can be increased by molecular screening of all AML patients.

The leukemic blasts of t(8;21)-positive AML patients are often large and display characteristic morphological features such as abundant cytoplasm, numerous granules, and single needle-like Auer rods. In most cases, the leukemic cells express the ►stem cell marker antigen CD34 on their surface. In contrast to most solid tumors, the amount of additional chromosomal changes is rather limited in t(8;21)-positive leukemia. The t(8;21) is significantly associated with the loss of a sex chromosome. Other additional chromosomal changes include a trisomy of chromosome 8 and a deletion of chromosome 9q.

AML1/MTG8

The translocation t(8;21) affects two genes. The *AML1* (►*RUNX*1) gene located on chromosome 21 codes for a transcription factor which is essential for hematopoiesis. The *MTG8* gene on chromosome 8 encodes a corepressor able to interact with several histone deacetylases (HDACs). Because of its reciprocal



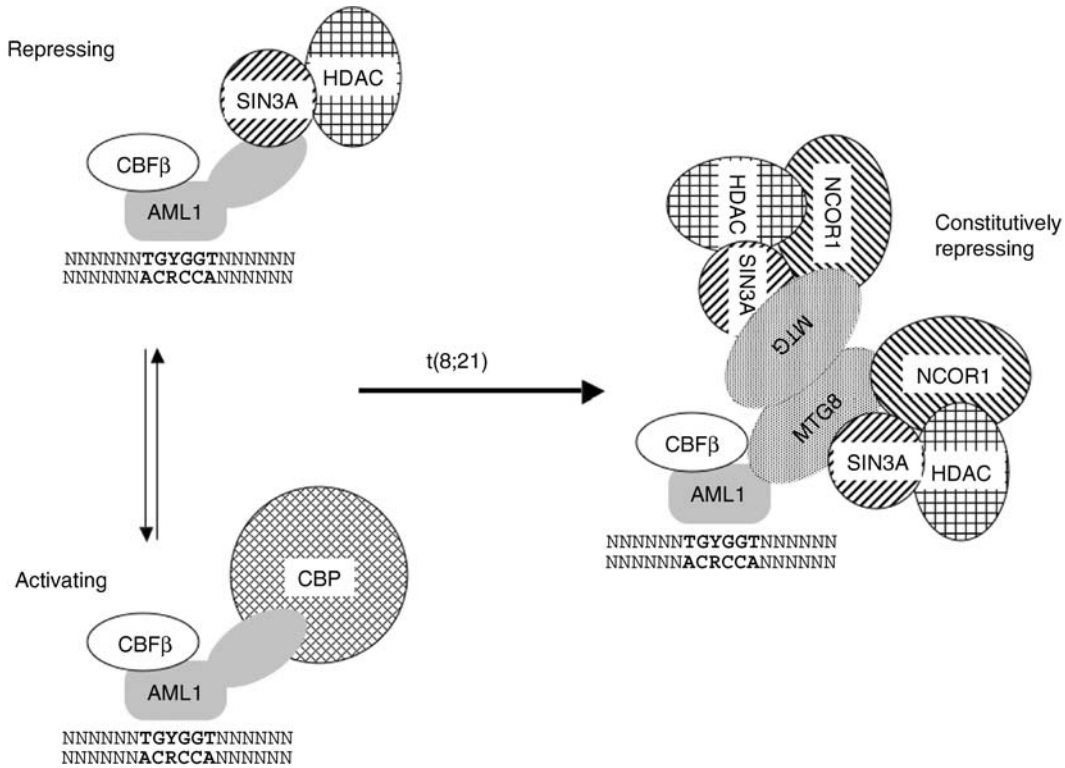
Chromosomal Translocation t(8;21). Figure 1 Primary structure of AML1/MTG8, AML1b, and MTG8. The translocation t(8;21) fuses the N-terminal part of AML1 to the almost complete MTG8 protein. Functions and interacting proteins for the different domains are indicated. A line marks the fusion site. RHD, runt homology domain; TAD, transactivation domain; TAF, TATA box binding protein-associated factor homology domain; HHR, hydrophobic heptad repeat; NHR3, nervy homology region 3; ZnF, zinc-finger region.

nature, the translocation t(8;21) generates two fusion genes, the derivative 8, *MTG8/AML1*, and the derivative 21, *AML1/MTG8*. However, leukemic cells express only *AML1/MTG8*; MTG8/AML1 protein has not been identified yet. In the case of the AML1/MTG8 fusion protein, the DNA-binding domain of AML1 (the runt homology domain, RHD) is linked to the almost complete MTG8 (Fig. 1). As a consequence, the transcriptional modulator AML1 is converted into a constitutive repressor. However, since only one of the two copies of chromosome 8 and 21 are affected by the translocation, each t(8;21)-positive cell still contains one intact copy of these chromosomes and, thus, expresses nonfused wild-type AML1 in addition to AML1/MTG8.

AML1/MTG8 acts as a transcriptional repressor. Via the ►SIN3A and ►NCOR1 (N-CoR) bridging proteins, AML1/MTG8 recruits HDACs to genes, which contain AML1-binding sites in their promoters, thus leading to the deacetylation of histones and, consequently, silencing of the target gene (Fig. 2). Established target genes include cytokine and growth factor receptors such as the gene for M-CSF receptor (*CSF1R)* or cell cycle control genes such as $p14^{ARF}$ (*CDKN2A*). Moreover, AML1/MTG8 interferes with hemopoietic differentiation by sequestering factors essential for these processes such as ►C/EBPα (CEBPA), ►SMADs, or Vitamin D receptor (VDR).

AML1/MTG8 in Leukemogenesis

The translocation t(8;21) is most likely an initiating event in the development of leukemia. AML1/MTG8 expression has been found in blood samples of newborn children at a much higher incidence than the probability to develop leukemia. Furthermore, some of the cured AML patients remain positive for AML1/MTG8. Moreover, AML1/MTG8 supports the expansion of hemopoietic stem cells both in cell culture and in animal models. In conclusion, AML1/MTG8 generates and maintains a pool of preleukemic cells, but is not sufficient to induce leukemia. Additional genetic changes such as mutations in growth factor receptors (e.g., c-kit) or in ►p53 are required for full leukemic transformation. Nevertheless, leukemic persistence requires the continuous expression of AML1/MTG8 as shown by RNA interference experiments. Notably, a C-terminally truncated version lacking a binding domain for NCOR-HDAC complexes has a much higher transforming capacity in a leukemia mouse model than the full-length AML1/MTG8 protein. Interestingly, similar splice variants of *AML1/MTG8* have been identified in patients suffering of t(8;21)-positive leukemia. Because of



Chromosomal Translocation t(8;21). Figure 2 AML1 and AML1/MTG8. Dependent on cellular signaling events, AML1 can switch from a repressive mode (complexed with SIN3A and HDACs) to an activating mode (complexed with the transcriptional activators CBP or EP300). Replacement of the transactivation domain by MTG8 results in oligomerisation with other MTG proteins, recruitment of histone deacetylases and, consequently, in a constitutive repression of AML1 target genes. The DNA binding site is indicated in bold. CBFβ, Core binding factor β (Cofactor of AML1).

its essential role in maintaining leukemia, and due to its exclusive expression in preleukemic and leukemic cells, *AML1/MTG8* might provide a promising target for leukemia-specific therapeutic approaches.

Clinical Relevance and Therapy

The translocation t(8;21) is found in about 10% of adult acute myeloid leukemia (AML) patients. Patients with t(8;21) are generally younger than 60 years. Most cases of t(8;21) positive AML show a ►FAB M2 or, less often, a M1 subtype, that is with (M2) or with minimal (M1) signs of maturation. This translocation marks a subgroup of patients, which responds well to standard chemotherapy and, thus, has a rather good prognosis. Standard induction chemotherapy consisting of ►cytarabine (5-azacytidine) and an anthracycline achieves a very high complete remission (CR) rate of approximately 90% in patients with t(8;21). Moreover, an intensive consolidation with high dose cytarabine or autologous stem cell transplantation yields an overall survival of approximately 50–70%. A low white blood cell count and high platelets at diagnosis are favorable prognostic factors, whereas the loss of the Y-chromosome in male patients has an adverse prognostic effect. In t(8;21)-positive patients in CR, minimal residual disease can be detected by RT-PCR for AML/MTG8 fusion transcripts. As mentioned earlier, some of the patients remain positive for AML1/MTG8 even in long-term CR or after ►allogeneic stem cell transplantation most probably due to the persistence of nonleukemic t(8;21)-positive multipotent progenitors. However, it has been shown that serial quantification of *AML1/MTG8* transcript levels by quantitative RT-PCR might identify patients at high risk for relapse.

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Chromosomal Translocations

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Definition

A chromosomal translocation is a type of chromosomal rearrangement in which two non-homologous chromosomes exchange genetic material. The exchange process involves breakage of each chromosome at a specific point called ►breakpoint, followed by fusion of the fragments generated by these breaks. A causative role has been demonstrated for some chromosomal translocations in various cancer types.

Characteristics

Instability of the genome, ►chromosomal instability in particular, is one of the hallmarks of cancer. Therefore, chromosomal rearrangements are very common in cancer cells. A frequent type of rearrangement is the translocation of genomic fragments between different chromosomal regions. The simplest case is a reciprocal translocation between two chromosomes, but translocations can also involve three or more chromosomes. If no genetic material is lost in the process, translocations are said to be "balanced." A well-known example of a reciprocal chromosomal translocation in cancer is the t(9;22) implicating the *ABL1* gene on chromosome 9 and the *BCR* gene on chromosome 22. This BCR-ABL1 translocation is found in most patients with ►chronic myeloid leukemia.

Chromosome translocations are found both in solid and hematological malignancies. Solid tumors usually display complex ►karyotypes with many different translocations and other types of chromosomal rearrangements such as deletions, amplifications, inversions, etc. In contrast, a frequent feature of most types of leukemias and lymphomas is the presence of a single or a few translocations, many of which are recurrent (i.e., found in different patients with the same type of cancer, or even in different tumor types). For this reason, chromosomal translocations have been best characterized in hematological cancers.

Biology

The mechanism underlying the presence of chromosomal translocations in cancer cells is the subject of active research. Various lines of evidence over the past years have identified several requirements for the generation of chromosome translocations. First of all, two breaks must be created in different chromosomes at the same time. Also, the free ends must be close to each other within the cell nucleus. Finally, some repair pathway must join the broken ends together, and the resulting molecule must provide some proliferative advantage to the cell.

With respect to the initial step, it is now generally accepted that chromosomal translocations are the result of DNA ►double-strand breaks, a type of ►DNA damage in which both strands of the double-helix are broken. Double-strand breaks are created throughout the genome by ►oxidative damage, radiation, replication over a single-strand break, genotoxic chemicals or physiological processes such as the assembly of active immunoglobulin and T-cell receptor genes during lymphocyte development through ►V(D)J recombination. There are three main pathways that repair double-strand breaks in mammalian somatic cells: i) ►homologous recombination repair, which relies on the presence of an intact homologous template in order to repair the DNA lesion; ii) single-strand annealing, which requires some homology at both sides of the break, usually in the form of ►direct repeats; iii) ►non-homologous end-joining, which results in the religation of the ends without the requirement for a template. The general consensus is that, in cancer cells, chromosomal translocations are the result of the repair of double-strand breaks via non-homologous end-joining.

Recent work in the field of chromosome localization has shown that chromosomes occupy specific ►chromosomal territories inside the cell nucleus, and that a substantial amount of intermingling takes place between chromatin loops from neighboring territories. For example, loops from different territories can co-localize if genes present in those loops are transcribed at the same time and utilize the same ►transcription factory. Thus, two chromatin loops (from different chromosomes) that sustain a double-strand break simultaneously and are localized in close proximity are more likely to be involved in a translocation event. This could explain the recurrence of certain chromosomal translocations in specific cancer types.

However, it is possible that many of the translocations generated in a cell never lead to development of cancer. Chromosomal translocations are associated with cancer only when the resulting fusion products possess some oncogenic property that favors the clonal expansion of those cells. In this regard, there are two main mechanisms by which chromosomal translocations disrupt normal cellular processes. In one type of translocations, a gene is separated from its regulatory elements (promoter, enhancers) and juxtaposed to the regulatory elements of a different gene. As a result, the pattern of expression of the gene is altered and this leads to the acquisition of growth or survival advantage to those cells. Translocations involving immunoglobulin genes are the best example of this mechanism. For example, the t(8;14) found in Burkitt lymphoma fuses the ►*MYC oncogene* to the regulatory elements of the gene coding

for immunoglobulin heavy chains, resulting in deregulated and constitutive expression of *MYC* in lymphoid cells. The other type of translocations lead to the acquisition of an oncogenic phenotype because they create a ►fusion gene that is translated into a ►chimeric oncoproteins. This hybrid fusion protein brings together functional domains that were present in both original proteins, and this results in some gain of function which helps the cell to escape normal control mechanisms. For example, the t(15;17) found in patients with ►acute promyelocytic leukemia fuses part of the *PML* gene on chromosome 15 to part of ►retinoic acid receptor A (*RARA*) gene on chromosome 17. The chimeric fusion protein lacks RARA's responsiveness to retinoic acid, a consequence of which is that some bone marrow progenitor cells cannot undergo the normal process of differentiation.

Breakpoints are sometimes clustered non-randomly in specific regions of the genes involved in a translocation. This has prompted an active search for structural DNA elements or sequence motifs that could be associated with chromosomal translocations in cancer. Although some chromatin features have been proposed to promote the appearance of a double-strand break in their vicinity, it is unclear at present whether the localization of a breakpoint is determined primarily by the presence of such elements. Alternatively, double-strand breaks might be generated randomly throughout the genome and the clustering of translocation breakpoints seen in some tumors would result from functional selective pressures, as only those fusion products that confer a proliferative advantage to the cell will eventually initiate or promote tumor progression and be found in cancer cells. These two mechanisms are not mutually exclusive, and it is likely that the non-random clustering of translocation breakpoints that is seen in some cancers is the result of both processes.

Clinical Relevance

The fact that some chromosomal translocations are associated with specific malignancies is also important from the clinical point of view. A complete collection of published chromosomal translocations and the cancer types in which they were detected can be found in the Mitelman Database of Chromosome Aberrations in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

In some cases, especially in ►hematological malignancies, the diagnosis of the disease relies on the detection of a particular chromosomal translocation.The laboratory tools most frequently used in the diagnostic setting are conventional karyotyping (G-banding), ►fluorescence in situ hybridization (FISH) and ►PCR-based molecular techniques. Analysis of cancer patients has also shown that the clinical course of the disease sometimes depends on the presence of specific translocations. Therefore, detection of chromosomal translocations is also important to estimate the probability of response to therapy or the risk that the cancer will recur after treatment. For this reason, specific translocations are part of the international classification system proposed by the World Health Organization for various types of malignancies. Importantly, the detection of specific chromosomal translocations is also used to assess the efficacy of treatment, since it provides a rational way to follow the evolution of the tumor clone and to confirm (or rule out) the presence of ►minimal residual disease.

Finally, the identification of chromosomal translocations has been instrumental in designing new effective therapies against some types of cancer. The best example of this is the new generation of drugs, like ►ST1-SH against ►chronic myeloid leukemia, and other malignancies characterized by the presence of chromosomal translocations involving ►tyrosine kinase genes. The finding that these tumors are the result of deregulated tyrosine kinase activity has led to the development of specific inhibitors and a dramatic increase in response to therapy and in survival rates in those patients.

►Nucleoporin
►Signal Transduction
►Orphan Nuclear Receptors and Cancer

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Chromosome

Definition

Structures in the nucleus, classified according to size, the location of the centromere, and the banding pattern along each arm. The autosomes are numbered from 1 to 22 in descending order of length; the sex chromosomes are referred to as X and Y. Both the long and short chromosome arms consist of one or more regions.

Chromosome Abnormality

Definition

Changes can be either structural, implying that the banding pattern or size of a chromosome is altered, or numerical, which means additional or missing whole chromosomes. Loss or gain of whole chromosomes are indicated by a plus " + " or minus "*" sign before a chromosome number.

Chromosome Band

Definition

Chromosomal area distinguishable from adjacent segments by its lighter or darker staining intensity. Bands are numbered consecutively from the centromere outward along each chromosome arm. Each band can be individually designated by first listing the chromosome number, then the chromosome arm, the region, and band number with the region. For example, 12q13 means chromosome 12, the long arm, region 1, band 3.

Chromosome Condensation

Definition

Chromosome changes occur throughout the cell cycle, however, chromosome condensation is first visible at the G2/M transition. Human naked DNA must be compacted ~10,000 fold to allow it to resolve into pairs of sister chromatids that can be separated from one another during the middle stage of mitosis. The binding of multisubunit phosphoprotein complexes, known as condensins, tightly regulates chromosome condensation. Topoisomerase II, an enzyme that regulates DNA topology, by breaking one DNA duplex and passing another through the gap, is required for complete chromosome condensation. Inhibitors of this enzyme stop complete chromosome condensation.

►G_2/M Transition
►Topoisomerases

Chronic Bronchitis

Definition

A daily, productive cough for at least 3 months in two successive years with no other cause for cough elucidated.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

Chronic Granulomatous Disease (CGD)

Definition

Is a genetically heterogeneous immunodeficiency disorder resulting from the inability of phagocytes to kill microbes they have ingested. This impairment in killing is caused by any of several defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex, which generates the microbicidal respiratory burst. In CGD, phagocytes ingest bacteria normally, but they cannot kill them. It is a primary immunodeficiency that affects phagocytes of the innate immune system and leads to recurrent or persistent intracellular bacterial and fungal infections and to granuloma formation. CGD is a syndrome that typically manifests as pneumonia, infectious dermatitis, and recurrent or severe subcutaneous abscess formation. In addition to increased susceptibility to infections, patients have a higher prevalence of mucosal inflammatory disorders such as colitis, enteritis, and gastric outlet obstruction. Cutaneous disease occurs in 60–70% of patients.

►Reactive Oxygen Species

Chronic Idiopathic Myelofibrosis

►Primary Myelofibrosis

Chronic Liver Disease

Definition

CLD, is the process that involves the immunologically mediated damage or destruction of various cell types

within the liver. CLD encompasses hepatitis (►inflammation of the liver), fibrosis (scarring, which involves the accumulation of connective tissue in the place of liver tissue) and cirrhosis (extensive scarring in which connective tissues completely surrounds islands of remaining liver tissue)

►Hepatitis Virus Associated Hepatocellular Carcinoma

Chronic Lymphocytic Leukemia

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Synonyms

CLL

Definition

CLL is a chronic form of leukemia with accumulation of small mature B-lymphocytes that express the surface membrane proteins CD5, CD19 and CD23.

Characteristics

Diagnosis

A diagnosis of CLL requires persistent absolute lymphocytosis of 5×10^9/l or more, with a characteristic immunephenotype and cytomorphology. Usually the diagnosis can be made based on a blood sample. In the identical lymphoma disorder, small lymphocytic lymphoma, the level of circulating tumor cells in the blood and bone marrow may be very low or absent. The diagnosis of SLL therefore may require a lymph node biopsy.

Epidemiology

CLL is the most common leukemia in the western world, with an incidence of ~5 new cases per 100,000 persons per year. The median age at diagnosis is 70 years, and the incidence increases with age. CLL may be seen in younger adults, but never in children.

Etiology

The causes of CLL are largely unknown. Unlike other leukemias, there is no relation to exposure to chemotherapy or ionizing radiation.

Pathogenesis

The forces that drive the relentless expansion of the CLL clone are unknown. Models of the pathogenesis of CLL have focused on the B-cell receptor. Like other mature B-cells, CLL cells express immunoglobulin in the cell membrane, structurally ordered inside the B-cell receptor (BCR) complex. The immunoglobulin molecules in the CLL BCR-complex are unique in several ways: (i) The repertoire of Ig-genes used by CLL cells is skewed, compared to normal B-cells (ii) The genes encoding the heavy chain variable segments only show signs of somatic hypermutation in about half of cases. (iii) The BCR-complex is expressed at much lower densities, than on normal or other malignant B-cells. (iv) The three-dimensional structure of the immunoglobulin molecules encoded by B-cells are remarkably stereotypic. Taken together, these observations suggest that the BCRs of CLL cells may be activated and transduce signals, by a limited and restricted set of (auto)-antigens, that drive the expansion and survival of the CLL clone. Abnormal expression of certain molecules involved in signal transduction from the BCR, for example overexpression of ZAP-70 or CD38, or low expression of $p72^{syk}$, may further modify the signaling capacity of the BCR in CLL cells. The result of this altered signaling is extended survival of B-CLL cells, and perhaps even increased proliferation. Furthermore, the most common cytogenetic lesion in CLL cells, result in deletion of a segment on chromosome 13q14 encoding the two ►micro-RNA's miR-15 and miR-16. These miR's can target and destroy Bcl-2 mRNA transcripts, leading to abandoned expression of Bcl-2 protein. When miR-15 and miR-16 are lost, due to the 13q14 deletion, the absence of the negative regulation of Bcl-2 may extend the longevity of CLL cells. Given the extended life cycle of a CLL cell, the risk of acquiring additional chromosomal aberrations is increased, and may for example result in losses at 11q22 (the ►*ATM* gene) or 17p (the *p53* gene). Both of these aberrations will further destabilize the negative regulation of Bcl-2, and furthermore, decrease the DNA damage response. In particular, the ability of p53 to induce cell cycle arrest upon DNA damage. In this way, the loss of control over apoptosis, induced by Bcl-2 overexpression, becomes linked to loss of control the G1-restriction point of the cell cycle, resulting in increased proliferation and transformation to the truly malignant and aggressive form of leukemia, seen in the end stages of advanced chemotherapy refractory CLL.

Thus, the highly variable clinical course observed for many years in CLL, is reflected by an equally variable spectrum of molecular aberrations detected in CLL cells. About half the cases of CLL have few molecular aberrations, and are characterized by very slow expansion of the clone, resulting in an indolent form of leukemia that may not affect the mortality or morbidity of the patient. The other half of the patients, show molecular features of aggressive disease, and follows a clinical course that sooner or later develops into aggressive, refractory and lethal leukemia.

Risk Prediction in CLL

Traditionally, the estimation of prognosis in CLL patients has relied on clinical staging systems, the two most widely used systems being those of Rai and Binet. Both systems use practical measurements of tumor size and bone marrow failure for prognostic estimation in CLL. Patients presenting with lymphocytosis alone generally have a favorable prognosis. Patients with lymphocytosis and enlarged lymph nodes, liver or spleen have an intermediate prognosis. Patients presenting with signs of bone failure, i.e. anemia or thrombocytopenia not caused by autoimmunity, have a poor prognosis. However, the clinical staging systems are static, and can only describe the patient status at presentation. The increased usage of standard blood tests in the clinic, results in identification of CLL patients at earlier stages of the disease, thereby eroding the informativeness of clinical staging systems. Today, more than 75% of patients are diagnosed by chance, usually because of examination for a non-CLL related condition, at a stage where lymphocytosis is the only manifestation of the disease.

The identification of biological risk predictors now allows definition of low-risk and high-risk cases, based on molecular features at the time of diagnosis. The major risk predictors are (Table 1).

Patients with no high-risk features have an expected median survival of more than 15 years. Patients with some high-risk features have an expected median survival around 10 years. Patients with many high-risk features have an expected median survival of 5 years or less.

Symptoms and Signs of Active CLL

There is no chemotherapy treatment that can cure CLL at present. ►Non-myeloablative allogeneic hematopoietic cell transplantation may do so, but the considerable morbidity and mortality associated with this treatment, makes it an option only for patients in advanced FC refractory stages. Therefore the treatment strategy is to await signs of active disease, before treatment is initiated. The signs of active CLL was defined by the NCI working group on CLL, also known as the Cheson criteria:

- Progressive bone marrow failure:
  - Development or worsening of non-immune mediated anemia
  - Development or worsening of non-immune mediated ►thrombocytopenia
- Massive or progressive lymph node enlargement
- Massive or progressive enlargement of the spleen
- Progressive lymphocytosis:
  - More than 50% increase in lymphocyte count in less than 2 months
  - Lymphocyte doubling time of less than 6 months
- Disease symptoms:
  - Weightloss (>10%) in less than 6 months
  - Fever of unknown origin for more than 2 weeks
  - Extreme fatigue
  - Night sweats
- Steroid resistant autoimmune cytopenia

Treatment Strategies in CLL

Once the patient has developed active CLL, it is necessary to prepare a long-term treatment strategy, ensuring that the relevant options are available for the patient at the inevitable subsequent relapses. The goal of the treatment must be accessed, i.e. tumor reduction, tumor control or tumor eradication, and developed in the context of patient age, co-morbidity and biological risk prediction. Elderly patients, or patients with significant co-morbidity, may not benefit from aggressive treatment, not at least due to toxicity. Tumor control, using single agent alkylating regimens, such as chlorambucil, may be sufficient. Standard treatment, aiming at tumor control, for patients younger

Chronic Lymphocytic Leukemia. Table 1 Risk prediction in CLL

Predictor	Low-risk CLL	High-risk CLL
►Immunoglobulin heavy chain gene mutations or usage	Mutated. (Less than 98% homology to the germ-line sequence)	Unmutated. (More than 98% homology to the germ-line sequence). H3-21 gene, regardless of mutational status
►Cytogenetics by FISH	Del13q14 as sole abnormality	Del17p and/or Del11q22 and/or Trisomy 12
►Clinical stage	Lymphocytosis only	Bone marrow failure (non-immune mediated anemia/ thrombocytopenia)
►ZAP-70 protein expression	<20% positive cells by flowcytometry	>20% positive cells by flowcytometry
►CD38 protein expression	<30% positive cells by flowcytometry	>30% positive cells by flowcytometry
►CLLU1 mRNA expression	<40-fold upregulation by quantitative RT-PCR	>40-fold upregulation by quantitative RT-PCR

than 70 years of age is the combination of (oral) ►fludarabine and ►cyclophosfamide (FC). The initiation of FC therapy in younger patients is also the evaluation of an ultimative risk predictor. FC non-responders, or early (<1 year) relapses, have a particularly unfavorable clinical course, with a median overall survival of less than 2 years. Therefore, patients younger than 70 years of age, started on treatment with a ►fludarabine containing regimen, should have their options for allogeneic hematopoietic cell transplantation (allo-HCT ►allogenic cell therapy) accessed at start of the treatment, primarily by tissue typing and identification of potential sibling allo-HCT donors. The expected median event free survival, is 1-year for alkylating agents, 2 years for fludarabine monotherapy and 3 years or more for fludarabine combination regimens. None of these strategies will cure CLL, and relapses are inevitable. If the recurrence of active CLL occurs later than the expected time point, the initial treatment may successfully be repeated. If not, it may be considered to escalate to a more aggressive regimen, again considering the age and co-morbidity of the patient.

Given the incurable nature of the disease, the described treatment strategy will eventually select for patients who are resistant to fludarabine containing regimens. These patients constitute a growing and very significant challenge in CLL centers. First of all, their disease at this points have every sign of aggressive leukemia, with bone marrow failure and constitutional symptoms being the most important signs of disease. Secondly, fludarabine refractory patients more often than not, present with a very severe immunodeficiency. It is opportunistic infections, more than the leukemia, that is threatening in advanced stages of CLL. Treatment options at this point, for example monoclonal anti-►CD52 antibodies (►alemtuzumab), may reduce and control the tumor, but will inevitably worsen the immunodeficiency. The only way out of this situation, with aggressive leukemia, and disabled immune function, is allo-HCT.

Allo-HCT in CLL

The effectiveness of allogeneic-HCT relies on two principles, with the aim to eradicate the tumor: (i) To deliver disease effective high dose chemo- or radiotherapy that can eradicate the tumor, and will eradicate normal bone marrow function, which however can be restored by reinfusion of normal bone marrow precursors. (ii) To develop allo-reactivity, that will target and destroy the leukemia, without targeting the patient. Standard transplantation evokes both principles. Non-myeloablative, or reduced intensity transplantation, using modern immunosupression to allow the new immune system to develop, is focused on the second principle. This second form of allo-HCT appears to be particularly effective in CLL. However, at a certain cost. The introduction of a new immune system, will create the risk that the new graft (immune system) will not only target the leukemia, but several tissues in the engrafted recipient. The risk is development of ►graft-versus-host disease (GVHD). The GVH-disease follows an acute and a chronic phase. The acute phase is responsible for a treatment related mortality of ~10%. The deaths caused by chronic GVH occurs at the same frequency, and living with chronic GVH-disease causes severe reduction in life quality. Therefore, allo-HCT cannot be considered an option for the general CLL-population. However, in younger patients, with FC-refractory disease, or deletions at 17p, allo-HCT may be the only way to survive the disease.

In summary, the development of biological risk predictors, new effetive chemo- immunotherapy combinations and the possibility for allo-HCT for at least some patients, has changed the management of CLL considerably since, and will continue to do so over the next years.

Chronic Lymphocytic Thyroiditis

Definition

Hashimoto Thyroiditis.

Chronic Lymphoproliferative Disorders

Definition

Malignancies of the lymphocytes and lymphoid tissue in the body, chronic.

►Mastocytosis

Chronic Myeloid Leukemia (CML)

Definition

Synonym myelocytic leukemia, also chronic myelogenous leukemia a myeloproliferative disorder associated with clonal proliferation of cells of bone marrow stem cell origin with excessive production of mature-appearing granulocytes. It is associated with a reciprocal translocation (t(9;22)(q34;911) leading to the

appearance of the Philadelphia (Ph1) chromosome and the ►BCR-ABL1 fusion protein. Therapy using tyrosine kinase inhibitors targeting ABL has proved very effective in achieving remission.

►ST1-571

Chronic Obstructive Pulmonary Disease and Lung Cancer

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Definition

►Chronic obstructive pulmonary disease (COPD) and ►lung cancer have rising prevalence worldwide with estimates of significant increases in mortality over the next few decades. Both diseases are directly linked to cigarette smoking, environmental exposures and old age, and both cluster in families, suggesting genetic links (►genetic disorders associated with cancer predisposition and chromosomal instability) to disease susceptibility. But irrespective of tobacco history (►tobacco carcinogenesis; tobacco-related cancers) and environment, there is growing evidence supporting an increased incidence of lung cancer in individuals with COPD.

Primary lung cancer is usually divided into two broad classes, small cell lung cancer and non-small cell lung cancer (tobacco carcinogenesis; ►cancer causes and control; ►tobacco-related cancer), which includes adenocarcinoma, squamous cell carcinoma and large cell carcinoma.

Characteristics

COPD

COPD is a chronic disorder characterized by airflow limitation that may be accompanied by hyperreactivity but is not fully reversible and is usually progressive. The disease is primarily caused by cigarette smoking, and to a lesser extent, by other noxious particles and gases. Despite smoking cessation, once COPD is established, the disorder continues to progress, albeit at a slower rate. Although COPD is a lung disorder, it is also associated with significant systemic abnormalities.

COPD is a general category that includes ►chronic bronchitis, an inflammatory airway disorder characterized by a daily, productive cough for at least 3 months in two successive years, and ►emphysema, an abnormal, permanent airspace dilation with destruction of the alveolar walls without evidence of fibrosis. Most patients with COPD have components of both chronic bronchitis and emphysema, and some have superimposed asthma, with airway hyperactivity and reversible limitation to expiratory airflow.

Most cases of COPD start with abnormalities in the small airways, with epithelial changes, ►inflammation in the airway walls, and narrowing of the airway lumen, limiting airflow. As the disease progresses, the airflow limitation is manifest by abnormalities in ►spirometry, a physiologic test that measures inhaled and exhaled volumes of air independently and as a function of time. The most commonly used parameters relevant to COPD are the forced vital capacity (►FVC, the volume of air that can be forcibly exhaled following maximal inspiration) and the forced expiratory volume in one second (►FEV1, the volume of air that is exhaled in the first second of that same maneuver). A reduction in FEV1 directly correlates with the degree of airways disease from inflammation, fibrosis or intraluminal exudates that characterize COPD. Following the use of inhaled bronchodilators, if the FEV1/FVC is <70% and the FEV1 is <80% of expected values, airflow limitation is present. The airflow limitation that characterizes COPD results from the intrinsic airway disease *per se*, as well as the loss of airway structural support from the destruction of the alveolar walls. The alveolar wall destruction also results in a reduction in the diffusing capacity for carbon monoxide, and can be visualized by computerized tomography of the chest.

Lung Cancer

Lung cancer is the leading cause of cancer related deaths worldwide. Like COPD, most (85–90%) of all lung cancers are a result of exposure to smoking. The risk of developing lung cancer, even after smoking cessation, persists for years. In the United States, the rate of lung cancer in former smokers is now equal to the rate in current smokers and is expected to increase even more in the next decades.

Link to Decline in FEV1

The incidence of both lung cancer and COPD increases with >20 pack-year smoking exposure. Smoking one pack of cigarettes per day can increase the normal decline in FEV1 from an expected 30 to 50–60 ml/year. Overall increases in mortality associated with COPD are directly associated with declining FEV1. The decrease in FEV1 associated with COPD is also directly linked to lung cancer, as well as to an increased risk of cardiovascular disease, including coronary heart disease and stroke.

COPD and Lung Cancer Associations

While COPD and lung cancer are both related to smoking, there is data from as early as the mid-1960s to support that the two diseases are even more closely related. Van Der Wal et al were the first to demonstrate a high incidence of lung cancer in patients with "chronic nonspecific lung disease," now recognized as COPD. Goldstein et al in 1968 reported a 32 times higher rate of lung cancer in patients with radiographic evidence of bullous emphysema in hospitalized patients with cancer as compared to other hospitalized patients who were used as controls. These data were supported by several subsequent studies in the 1970s and 1980s.

In 1976, Davis et al suggested that a reduced FEV1 itself might be an independent risk factor for the development of lung cancer, demonstrating a 4–5-fold increase in lung cancer in their patients with COPD as compared to lung cancer rates in previously reported series of smokers without COPD. A prospective study by Skillrud et al comparing 113 people with COPD to 113 matched controls without COPD followed over a 10 year period, found that all cause mortality increased with COPD, a decreased FEV1 was directly linked to a decreased time to death from any cause, and, that despite shorter survival data for patients with COPD, there was a clear, increased risk for the development of lung cancer in the COPD population. Overall, the risk of developing lung cancer in patients with airway obstruction is 4.4 times greater than in those without obstruction to expiratory airflow.

In 1997, a retrospective review of data collected from two large prospective study groups, The Intermittent Positive Pressure Breathing Trial sponsored by the National Heart, Lung and Blood Institute and the Johns Hopkins Lung Project, was conducted by the National Cancer Institute's Cooperative Early Lung Cancer Detection Program. The goal was to evaluate the association between the degree of airway obstruction and the development of lung cancer. This study demonstrated that the frequency of lung cancer was proportional to the degree of airflow obstruction, and that the risk of lung cancer was more closely linked to a decline in FEV1 than to older age or degree of tobacco use.

The 1994 study by Islam et al reviewed prospectively data collected over a 28 year period in a community-based study in Tecumseh, Michigan in 3,900 subjects. They determined that the initial FEV1 and the rate of decline in FEV1 were each independent predictors of lung cancer development. When loss of FEV1 reached 100 ml/year, the risk of lung cancer reached as high as 30 times the rate seen in matched controls.

A study by Mannino, published in 1993, reviewed patients in the First National Health and Nutrition Examination Survey database who had at least a 22 year follow up. They demonstrated that moderate to severe obstructive disease was associated with a higher incidence of lung cancer and that there was no difference in rates of lung cancer when comparing current to former smokers.

Possible Mechanisms of the COPD - Lung Cancer Risk

The relationship between reduced FEV1 and the development of primary lung cancer is not clear, although many theories have been advanced. It is generally accepted that both disorders result from a combination of genetic and environmental factors (cancer causes and control; ►cancer epidemiology). However, while the link to smoking is clear, the commonalities of the smoke components that cause COPD and lung cancer are not clear (genetic disorders associated with cancer predisposition and chromosomal instability), nor are the genetic differences linking a susceptibility to both diseases.

One gene of common interest is ►vascular endothelial growth factor (VEGF). While COPD has been theorized to be associated with decreased availability of VEGF leading to capillary apoptosis in the lung, lung cancer, as with other cancers, is associated with an increased expression of VEGF supporting ingrowth of capillaries into the developing tumor. Linkage studies in both humans and mice have suggested that allelic loss in some regions of chromosome 6q and 12q are associated with lung cancers as well as with COPD. Finally, excess amounts of matrix metalloproteinases (MMPs), enzymes that degrade extracellular membranes are associated with a decline in lung function, as well as an increased risk of lung cancer.

Chronic inflammation (►inflammation in cancer) has long been linked to cancer in many organs, as evidenced by the development of esophageal adenocarcinoma (►esophageal cancer) following chronic gastric reflux. This concept has led to the theory that COPD and lung cancer risks increase in the face of chronic inflammation. Consistent with that concept, cigarette smoking (tobacco carcinogenesis; tobacco-related cancer) has been shown to cause sustained changes in gene expression of respiratory epithelial cells. Some of these changes have been noted to persist, regardless of smoking cessation, for decades. Some of the inflammatory changes are likely associated with the development of both COPD and lung cancer. One example is the loss of the tumor suppressor gene, p53 (►p53 protein, biological and clinical aspects) which generally inhibits inflammation. Allelic loss of p53 is known to lead to inflammatory responses including increases in nuclear factor-κB, a transcription factor linked to both COPD and cancer, via inflammatory pathways. Other genes, including antioxidants, oncogenes and other ►tumor suppressor genes have also been suggested as key risk factors to the development of COPD and lung cancer.

Summary
The reasons why only a minority of smokers develop smoking related lung disease are still not clear and many challenging questions remain unanswered regarding COPD, lung cancer and the relationships between them. More information on their associations with environmental exposures (including but not limited to tobacco), genetic susceptibility, and inflammatory processes is still needed. It is evident that these connections are not simply based on tobacco use and that there is a genetic predisposition to the development of each. While smoking cessation will clearly reduce the incidence of COPD and lung cancer, it will not eliminate either disease for many decades to come. Further well-formulated studies are required to continue to evaluate the connections between environmental exposures, inflammation, genetic expression and the development of COPD and lung cancer, which remain two pulmonary diseases with the highest morbidity and mortality in the world.

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Chronic Obstructive Pulmonary Disease (COPD)

Definition
Chronic airflow limitation that may be accompanied by hyperreactivity but is not fully reversibly and is usually progressive. It is characterized by destruction of alveolar walls without evidence of fibrosis.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

Chronic Phase

Definition
Early phase of chronic myeloid leukemia (CML) characterized by variable duration. Patients often lack symptoms, or are mildly symptomatic; if left untreated will progress to an accelerated phase.

►Nilotinib
►ST1-571

Chronic Ulcerative Colitis

Definition
Causes ►inflammation of the membrane lining the colon (large intestine). The condition causes inflammation and small sores, called ulcers, in the top layers of the lining of the colon.

►Bile Duct Neoplasms

Chronotherapeutics

Definition
Treatment delivery based on the rhythmic organization of relevant biological functions. Cancer chronotherapeutics is mostly based on the control of drug pharmacology and cell cycle by circadian clocks. Through the delivery of anticancer drugs at specific times of the circadian cycle, one aims at improving host tolerability and/or antitumor efficacy.

►Circadian Clock Induction

CHRPE

Definition
Congenital hypertrophy of the retinal pigment epithelia (CHRPE) are pigmented lesions of the retinal epithelium. These patches result from an increased content of

melanin granules within enlarged retinal pigment epithelial cells. The majority of FAP patients (75–80%) have CHRPEs.

►APC Gene in Familial Adenomatous Polyposis

Chrysotile

Definition

Is a serpentine (lizard-like) form of ►asbestos. It is a magnesium silicate but, in some deposits, varying amounts of iron have replaced magnesium. In the absence of iron, it is non-carcinogenic.

Chuvash Polycythemia (CP)

Definition

Is a form of erythrocytosis endemic in Chuvashia (a republic of the Russian Federation) where approximately several hundred cases are recognized among a population of about 1.5 million people. An additional cluster has been subsequently identified in Ischia island (Italy) and a number of cases in other region of world.

►Polycythemia

Chylothorax

Definition

Accumulation of high fat containing fluid (chyle) in the abdomen as a result of obstruction of or abnormal development of lymphatic vasculature.

►Lymphangiogenesis

C.I. 75300

►Curcumin

Ciclosporin

Definition

Cyclic peptide consisting of 11 amino acids residues with immunosuppressive activity.

►ABC-Transporters

Cilia

Definition

Eucaryotic cell organelles (hair-like projections). Apical parts of bronchial epithelial cells have multiple mobile *cilia* moving mucus and microscopic foreign particles out of the lung.

►Exfoliation of Cells

Ciliary Body Melanoma

►Uveal Melanoma

CIN

►Chromosomal Instability

Cip-Interacting Zinc Finger Protein 1 Ciz1

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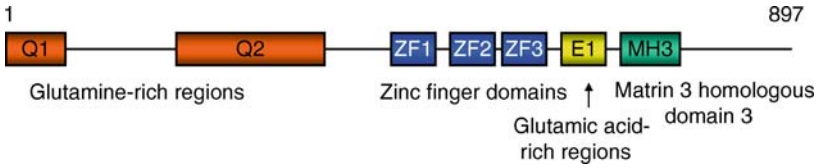
Definition

Ciz1 is a member of the Matrin 3 protein family and also called NP94 (Nuclear Protein 94). At the N-terminal



Cip-Interacting Zinc Finger Protein 1 Ciz1. Figure 1 Schematic representation of protein domains in Ciz1.

region, Ciz1 contains poly-glutamine repeats and glutamine rich regions. Additionally, the C-terminus contains three zinc-finger motifs, an acidic region and a matrin 3-homologous domain 3 (MH3 domain), which shows 40.4 and 37.7% identity (55.8 and 64.2% similarity) to NP220 and matrin 3 (two other members of the Matrin 3 family), respectively (Fig. 1). Ciz1 is expressed in a wide variety of tissues, with highest expression in pancreas, testis, kidney and brain, with the highest expression in cerebellum, and is mainly localized in the nucleus. Ciz1 is expressed in a number of cell lines from multiple organ systems. None of the genes of Yeast, *C. elegans* or *Drosophila* showed any overall structural similarities to Ciz1, suggesting that Ciz1 is a unique protein only found in vertebrates.

Characteristics

Ciz1 directly interacts with ►p21^{WAF1} and is predominantly located in the nucleus. However, upon co-overexpression of Ciz1 and p21^{WAF1}, an enhanced cytoplasmic localization of both proteins was detected. Ciz1 is a DNA binding protein, and its DNA consensus sequence was determined by a modified selected and amplified binding (SAAB) sequence method (ARYSR (0–2)YYAC). Ciz1 is a binding partner of Dynein Light Chain 1 (DLC1), shown by using a modified ►proteomics technique. Ciz1 influences the ►cell cycle progression at the G_1-S transition by affecting the kinase activity of Cdk2. A reduced localization of p21^{WAF1} in nuclei of DLC1 overexpressing cells strengthened the hypothesis that Ciz1 (together with DLC1) in important for the sequestration of p21^{WAF1} in the cytoplasm, which will then release the repression of the Cdk2 kinase complex and induce the G_1-S transition. Also, cell-free experiments have demonstrated that Ciz1 has a role in mammalian DNA replication. The addition of Ciz1 protein increases the number of nuclei that initiate DNA replication, and when mutating the potential ►cyclin-dependent kinase (Cdk) phosphorylation sites, Ciz1 functions were compromised in vitro. Ciz1 co-localizes with PCNA in foci in the nucleus, and Ciz1-depleted cells are unable to replicate their DNA.

Besides significant role in the cell cycle progression, Ciz1 is responsible for potentiating the transactivation activity of the ►Estrogen Receptor alpha (ER). Ciz1 is a coregulator of ER by enhancing ER transactivation activity and recruitment to target gene chromatin. Ciz1 induces the hypersensitivity of ►breast cancer cells to ►estradiol and induces the expression of ER target gene ►cyclin D1 at a femto-molar dose of estrogen, with likely downstream effects on G1 progression and DNA replication. In addition, overexpression of Ciz1 promotes the growth-rate, anchorage-independence and tumorigenesis of breast cancer cells in a xenograft model. Interestingly, Ciz1 is also an estrogen-inducible gene, suggesting that overexpression of Ciz1 in breast cancer cells may support hormone hypersensitivity.

Ciz1 has a functional MH3 domain since its C-terminal region essential for the anchoring of Ciz1 to the nuclear matrix, and this immobilization is cell-cycle dependent and occurs probably during late G1 or early S phase. Although the C-terminus is sufficient for the immobilization of the Ciz1 protein to the nuclear matrix, the N-terminal region is required for the focal localization of Ciz1 in the nucleus. Using biotinylated-dUTP and GFP-tagged Ciz1, Ciz1 was shown to be co-localized with the newly synthesized DNA. Thus, Ciz1 has multiple functions in cell cycle progression and cell proliferation control and has a potential role in tumorigenesis.

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C

Circadian

Definition

Rhythms with a period (cycle duration) of about 24 h (circa, about; dies, day) characterize many biological functions in unicellular organisms, plants, as well as all kinds of animals, ranging from insects to rodents, wildlife, and humanisms. In mammals, rhythms in locomotor activity, body temperature, hormonal secretions, etc. persist in constant environmental conditions. Yet the period of these endogenous rhythms slightly differs from precisely 24 h, being close to 24.8 h on the average in humans.

►Circadian Clock Induction

Circadian Clock

Definition

Most cells in the brain and peripheral tissues contain a molecular clock consisting of at least 12 specific clock genes in mammals. Interacting transcriptional and post (transcriptional loops constitute this molecular clock which rhythmically control many metabolic and proliferation functions in most living cells.

►Circadian Clock Induction

Circadian Clock Induction

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Definition

The ►circadian timing system efficiently orchestrates the physiology of living organisms to match environmental or imposed 24-h cycles. Most cells in the brain and peripheral tissues contain a molecular clock consisting of at least 12 specific clock genes in mammals. This molecular clock rhythmically controls the transcriptional activity of nearly 10% of the genome, ~10% of which are proliferation-related genes, over the 24 h. Among the 12 genes which constitute the molecular clock, *Per2*, *Bmal1*, and *Rev-erbα* play a central role. Thus, a null mutation in these genes results in profound alterations of the circadian phenotype. The intracellular clock mechanisms involve interacting positive and negative transcriptional feedback loops that drive recurrent rhythms in the RNA levels of these key components. High levels of Bmal1 mRNA and protein promote the formation of BMAL1:CLOCK heterodimers that bind to the ►E-box sequences in the promoter of clock genes *Per2* and *Rev-erbα* and activate their transcription. In turn, Rev-erbα negatively regulates Bmal1 transcription, while PER2:CRY1 complexes inhibit the transcription of their own genes by interfering with CLOCK:BMAL1. Phosphorylation of PER and CRY proteins through casein kinase (CK) Iδ/ε activity plays a key role in the regulation of clock proteins degradation and length of precise circadian period.

The circadian organization of drug metabolism pathways as well as ►cell cycle, ►DNA repair, and ►apoptosis is responsible for dosing time dependencies in drug ►pharmacokinetics and pharmacodynamics. As a result, about 24-h changes in anticancer drug tolerability and efficacy call for ►chronotherapeutics, i.e., the delivery of anticancer treatments according to circadian (and other) rhythms. Dedicated ►drug delivery systems can actually administer chemotherapeutic drugs at specific optimal times and/or according to an optimal circadian pattern to cancer patients.

Conversely, destruction of the ►circadian pacemaker in the brain, iterative alterations of the environmental cycles, or clock gene mutations usually produce severe modifications within the ►circadian timing system that result in the uncoupling of coordinated biological functions. Such ►circadian disruptions can influence cancer processes.

Characteristics

Relevance of Circadian Disruption for Cancer Processes

The relative risk of developing breast, colorectal, or prostate cancer is enhanced 50–300% in populations exposed to prolonged shift work, frequent transmeridian flights, or chronic light exposure at night. These latter conditions profoundly alter the circadian timing system and the downstream control it exerts on cellular proliferation, DNA repair, apoptosis, and metabolism. These circadian alterations can consist of a decrease in rhythm amplitude, a phase shift and/or a modification, or a suppression of the circadian period. Circadian

physiology monitoring reveals relevant rhythm alterations in nearly one third of patients with metastatic cancer. In patients with advanced or metastatic cancer, disrupted circadian rhythms in rest-activity or cortisol secretion are associated with poor quality of life and increased risk of an earlier death. In experimental models, the growth rate of transplanted tumors is accelerated with the disruption of the circadian timing system through ablation of the hypothalamic circadian pacemaker or chronic jet lag produced by 8-h advances of light onset every 2 days.

Circadian Clock Control of Cell Cycle

At least three molecular mechanisms link molecular ►circadian clock with the cycling of cell division. The molecular clock controls Wee1 transcription through an E-box-mediated mechanism. WEE1 negatively controls the activity of CDK1/Cyclin B1, which facilitates the ►G2/M transition (Fig. 1). In addition, the BMAL1: CLOCK heterodimers repress ►c-Myc transcription through E-box-mediated reactions in the *c-Myc* gene P1 promoter and PER 2 can suppress c-Myc expression indirectly. In general, knocking down the molecular clock modifies transcription patterns of genes involved in the cell cycle regulation. This can translate into genomic instability, thus favor malignant ►progression or growth. Both *Per1* and *Per2* as well as possibly other clock genes also control DNA repair through interactions with ►*ATM* and *mdm2*.

Circadian Disruption in Cancer Tissues

The circadian expression pattern of core clock genes *Per2*, *Bmal1,* and *Rev-erbα* can be severely altered in experimental tumors in mice. In experimental Glasgow osteosarcoma, a transplantable mouse tumor with a doubling time of 2–3 days, the transcriptional rhythms in these three clock genes displayed altered amplitudes and phase at an early stage of growth that subsequently evolved toward arhythmicity (Fig. 2).

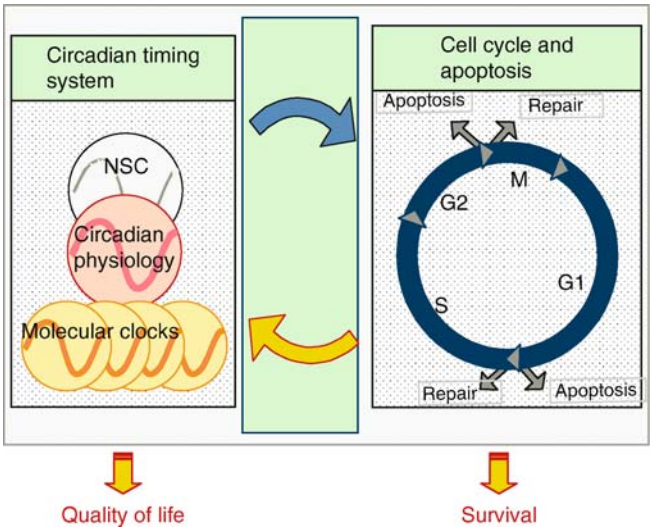
Physiologic Clock

The adjustment of a cosine curve with a 24-h period to mRNA expression of Rev-erbα (blue line), Per2 (red line), and Bmal1 (green line) allows to build a model of the circadian clock in healthy liver of $B6D2F_1$ mice. This model establishes physiological dynamic relations with maxima occurring near Circadian time 6 (CT6) for Rev-erbα, CT15 for Per2, and CT23 for Bmal1.

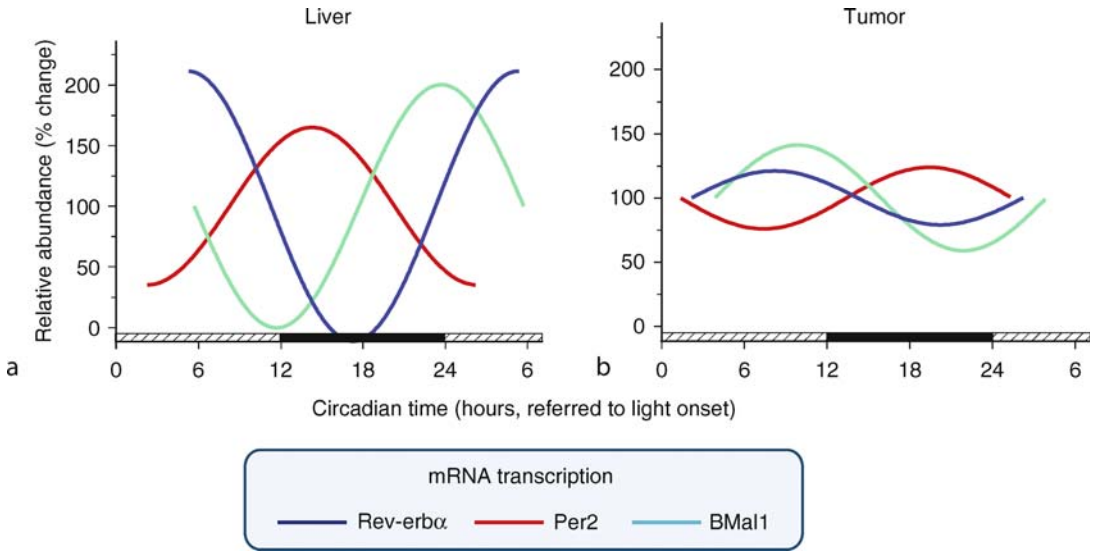
Tumor Clock

The same method is applied to clock genes data for control tumors. No clock gene transcription rhythm is validated.

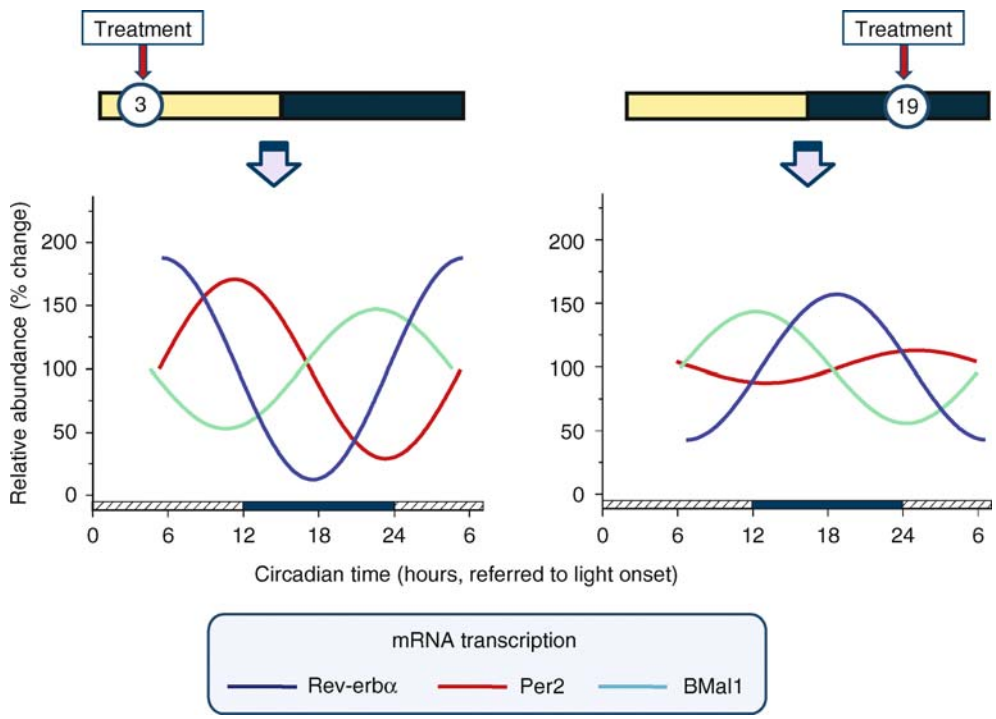
The ablated rhythms in clock gene expression in tumor tissue could result from an altered synchronization of the molecular clocks in each malignant cell or from an impairment of the molecular clock within each individual cancer cell. The first possibility would happen, for instance, if the internal rhythm in each cell beats with a period that differs by minutes or hours from those of its neighbors. Alternatively, clock genes defects could result in nonfunctional molecular clocks through deletions, mutations, or ►methylation of promoter region. In several human cancers, decreased expressions of the Per1, Per2, or Per3 genes were found at a single time-point, in comparison with reference tissues.



Circadian Clock Induction. Figure 1 Schematic representation of the interactions between the circadian timing system and the cell division cycle. SCN: suprachiasmatic nuclei, the main circadian pacemaker in the hypothalamus.



Circadian Clock Induction. Figure 2 ►Cosinor-based model of molecular clock in mouse healthy liver (a) or tumor Glasgow osteosarcoma (b).



Circadian Clock Induction. Figure 3 Example of tumor circadian clock induction with CDKI seliciclib as illustrated with cosinor-based model of molecular clock. Physiologic phase relations between expression patterns of the three core clock genes, similar to those in healthy liver, are induced with seliciclib treatment in the early rest span (at Zeitgeber Time 3, ZT3), but not if the drug is given during darkness (at ZT19).

Circadian Clock Induction

Peripheral ►synchronizers, such as feeding schedules, and drugs, such as ►cyclin-dependent kinase inhibitor (CDKI) seliciclib, can induce rhythmic clock gene expression patterns in malignant tumors with otherwise disrupted or uncoordinated molecular clocks. Indeed, exposure of tumor-bearing mice to chronic jet lag severely disrupts the already altered temporal patterns

of core clock gene expressions in tumor. Programming food availability to 6 h daily induces near normal molecular clock in the tumor and slows down malignant growth. The administration of seliciclib also induces near normal molecular clock in mice dosed in the early light span, but has no such effect if treatment is applied during darkness (Fig. 3). Feeding or pharmacologic induction of the tumor clock is associated with antitumor effect. Both clock induction and antitumor effects depend upon the circadian time of application.

Circadian clock induction improves control of ►G2/M gating through an enhancement of Wee1 transcription, a gene that is unidirectionally controlled by CLOCK-BMAL1. In the case of seliciclib, the induction of the molecular clock involves inhibition of CK1δ/ε, a key determinant of circadian period. In turn, inhibition of CK1δ/ε impairs PER2 degradation and nuclear translocation and results in increased *Bmal1* transcription. Since highly coordinated sequential transcription is a major mechanism of circadian rhythms, programmed food availability or daily seliciclib act as strong resetters of tumor cells that have lost synchrony in functional clocks, through transient inhibition of CK1δ/ε or other pathways within permissive time windows.

In conclusion, while malignant tumors tend to display disrupted molecular clocks and to lose circadian coordination, feeding schedules and drugs like CDKI seliciclib can slow down tumor growth through circadian clock induction. The mechanisms underlying these favorable effects involve the cross talk between the circadian clock and the cell cycle, two biological oscillators whose interactions represent a new relevant dynamic target for cancer therapeutics. Furthermore, the extent of circadian clock induction and that of antitumor activity depend upon the circadian time of drug administration, revealing the relevance of chronotherapeutics of CDKI for improving cancer control.

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Circadian Disruption

Definition

Modifications within the circadian timing system that result in the uncoupling of coordinated biological functions.

►Circadian Clock Induction

Circadian Pacemaker

Definition

The circadian clocks that reside in nearly all mammalian cells are coordinated mainly by the suprachiasmatic nuclei (SCN), a pair of neuronal nuclei located at the floor of the third ventricle in the hypothalamus. This coordination is exerted through known neuroanatomic circuitry and diffusible signals. The SCN also receives direct input pathways from the retina and other brain areas so that it adjusts the phasing of the circadian timing system to the light–dark and social synchronizers.

►Circadian Clock Induction

Circadian Rhythm

Definition

A self-sustained biological rhythm or oscillation of behavior, metabolism, biochemistry and/or physiology that repeats with a cycle of approximately 24 h; literally means a rhythm of about (circa) a day (dian).

►Melatonin

Circular Dorsal Ruffles

Definition

Transient regions of cell membrane that extend from the surface of cells as they initially respond to growth factor

stimulation. They are thought to supply membrane and protein components required for sustained lamellipodia formation, as well as regulating growth factor receptor internalization.

►Cortactin

Circulating Endothelial Cells (CECs)

Definition

Are cell populations circulating in the peripheral blood that exhibit an endothelial cell-specific surface marker phenotype (e.g., positive for CD31, but not for CD45, a pan-hematopoietic marker).

►Antiangiogenesis

Circulating Nucleic Acids

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Synonyms

Cell-free circulating nucleic acids; Extracellular nucleic acids; CNAs

Definition

Circulating nucleic acids (CNAs) are nucleic acids, i.e., DNA, and RNA, isolated from cell-free plasma/serum and other "circulating" body fluids like lymphatic fluid. For nucleic acids obtained from body fluids like liquor, ascites, milk, bronchial lavage fluids, urine, stool, or isolated from cell-free supernatants of in vitro cultivated cells, the terms extracellular or cell-free nucleic acids are better suited. CNAs are found in the animal kingdom, in plants, and in the microbial world as well. In humans and in animals, most of these nucleic acids seem to derive from dead cells (apoptosis, necrosis), but there are probably also mechanisms leading to an active release. The majority of cell-free circulating DNA molecules are of low molecular weight (up to ~300–500 bp). The amount of CNAs in healthy subjects is very low (~1–20 ng DNA/ml plasma), while in patients with a tumor (but with a benign disease as well) a much higher quantity can be found (up to several orders of magnitude).

Characteristics

Biological Aspects

The presence of CNAs in plasma samples of humans was reported by Mandel and Metais in 1948. Leon et al were the first to describe a relationship between CNAs in cancer patients and their clinical parameters, and the group of Drs. Stroun and Anker clearly demonstrated that at least part of CNAs in cancer patients is derived from the tumor itself [1].

Healthy subjects contain a few ng DNA/ml plasma, while patients with a malignant or benign disease may contain an increased quantity of CNAs (up to 2–3 orders of magnitude more), but there is a wide range. The increase in the amount of CNAs in diseased patients is very unspecific, not related to any malignancy in particular, and can be observed in trauma patients as well as in patients with inflammatory conditions (like chronic inflammations in patients with ulcerative colitis).

CNAs are not naked but are released in complexes that make them resistant against attacks of nucleolytic enzymes. So far all known cellular nucleotide sequences analyzed in CNAs (i.e., DNA, and RNA) were also found in plasma/serum and other body fluids. Therefore CNAs are probably a mirror of all cellular nucleic acids, but whether the quantitative ratios are the same in both compartments is unknown so far. The half-life of CNAs seems to be very short (in the range of minutes). The clearance mechanisms in animals, including the human species, are probably an excretion in urine and stool or an uptake by the liver. In plants the traffic of nucleic acids, i.e., DNA, and RNA, plays an important role in cell–cell communication (like spreading of a primary crown gall, which is considered a plant tumor, to "metastatic sites"), and in the animal kingdom CNAs might be involved in ►horizontal gene transfer (HGT).

Clinical Aspects

CNAs isolated from cancer patients may contain all the genetic alterations found in the tumor cells themselves. These include DNA ►amplifications, inversions, point mutations (►Oncogenes), deletion of a ►tumor suppressor gene, ►microsatellite alterations, hypermethylation (►Methylation) of ►CpG islands in promoter regions, mutations in ►mitochondrial DNA, the presence of viral nucleic acids, and the possibility to detect a larger quantity of mRNA for genes that are overexpressed in tumors. While there does not seem to be a well-defined relationship between an increased quantity of CNAs in plasma/serum and the presence of a malignancy or any other clinical parameters (►Tumor staging, ►tumor grading, size of the tumor, presence/absence of ►metastases, etc.), the amount of CNAs in plasma/serum is frequently higher in tumor patients

than in the control populations. The detection of mutations, frequently found in tumor cells (such as in ►*p53* gene family, ►*K-ras*, ►*N-ras*, ►*APC*, ►*BRAF*), has also been possible in CNAs. The detection of microsatellite alterations (i.e., the ►loss of heterozygosity (LOH) or band shifts) in CNAs from tumor patients is one of the most frequent genetic changes found in DNA from plasma/serum. Almost all solid tumors and many ►hematological malignancies harbor microsatellite alterations detectable in CNAs. One of the main problems with assays detecting these alterations is their low sensitivity. Since CNAs are a mix of nucleic acids derived from normal, i.e., healthy, and tumor cells and most often the fraction of the tumor-derived DNA is much smaller than the one derived from normal cells, it can be very difficult or impossible to detect these changes (the sensitivity for methods detecting an LOH is ~1:200). In contrast, the detection of point mutations or hypermethylated sequences as a positive detectable marker is more straightforward and yields higher assay sensitivity. The CpG dinucleotides are found in small stretches of DNA termed CpG islands that are frequently located around the transcription start sites of human genes (promoter regions). Such a promoter hypermethylation is commonly found in so-called ►tumor suppressor genes, leading to a shutdown of these genes (►Epigenetic gene silencing). Recently developed methods (mostly based on PCR (►Real-time PCR)) yield a high test sensitivity (one single hypermethylated allele can be detected against a background of up to 10,000 unmethylated alleles) and also allow a ►real-time quantification of methylated alleles. The presence of cell-free viral nucleic acids had been described before CNAs became popular as new tumor markers, and the strong association of a virus infection and the development of certain types of cancer makes the detection of viral nucleic acids an interesting field. There are strong correlations between the development of a nonnasopharyngeal head and neck carcinoma (NNHNC) and an infection with the Epstein-Barr virus (EBV); between cervical cancer and an infection with the ►human papilloma virus (HPV), especially types 16 and 18; between Hodgkin's disease and other lymphoproliferative diseases and EBV. While DNA is a rather stable molecule, RNA is much more fragile and prone to degradation by the ubiquitous presence of RNA degrading enzymes. Since it is known that the serum RNAse concentration is elevated in cancer patients, it was quite a surprise when EBV-associated RNA was detected in the plasma of nasopharyngeal carcinoma patients and tyrosinase *mRNA*, a gene overexpressed in melanoma cells, was found in the serum of melanoma patients.

The reasons for the observation that frequently there is no complete concordance between alterations found in the primary tumor and in CNAs are based on the facts that extracellular nucleic acids are a mix of normal and tumor-derived nucleic acids, that most solid tumors are heterogeneous and contain subclones that are genetically heterogeneous, and that primary tumors, their subclones, and metastases may differ in the ability to shed nucleic acids into their environment. This makes it even more difficult to establish a marker panel that can be useful in a clinical setting. There are quite a few reports in which a correlation between the presence of genetic alterations in CNAs and clinical parameters was found. It has been shown that the determination of the quantity of circulating viral nucleic acids before and/or after therapy might be a good marker to evaluate the response to a therapy or an overall and disease-free survival. Similar observations were made for the detection of methylated sequences in CNAs (APC, RASSF1A, p15, and p16INK4A) and some clinical parameters. In addition, there might be a correlation between the presence of *K-ras* and *p53* mutations in CNAs and the survival of the affected patients. In any case (and this holds true for all methods and genetic alterations mentioned above) a thorough validation of the potential markers is a conditio sine qua non, since some of the tumor-associated genetic alterations are not only found in the tumor itself, but have also been detected in histologically normal tissue of cancer patients, in people having a certain risk for the development of a tumor, or even in cells obtained from obviously healthy subjects. This was demonstrated for the *p53* tumor suppressor gene whose alterations are found in progenitor lesions of the lung, esophagus, head, and neck, and colon. Some of the tumor-associated changes seen in cellular DNA were also detected in plasma DNA of nontumor patients, like microsatellite alterations in plasma DNA from patients with benign respiratory diseases and a tumor-associated gene promoter methylation in plasma from women who have never smoked. In addition, patients with long-standing ulcerative colitis are frequently tested positive for mutations of the *Ki-ras* and *p53* genes even if there are no signs of a tumor. Finally a couple of important technical issues need to be resolved (like determination of the influence of preanalytical factors on the result, standardization of the different methods, choice of an optimal marker panel, etc.) before the analysis of CNAs can be a useful and clinically meaningful tool.

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Circulating Progenitor Cells (CPCs)

Definition

Are cell populations circulating in the peripheral blood that express progenitor/stem cell-specific surface markers (e.g., CD34, CD133).

►Antiangiogenesis

Circulating Tumor Cells

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Definition

Circulating tumor cells (CTC) are tumor cells spread in blood and/or lymphatic vessels from solid tumors, thus including all types of tumor cells except those derived from leukemia and lymphoma. CTC may circulate as aggregated tumor cells, which are defined as ►circulating tumor microemboli or "►collective tumor cells migration (CTM)."

Characteristics

Tumor cells may circulate in blood spontaneously, i.e., because of their invasive capabilities, or for other causes of cell spreading. Spontaneous circulation of tumor cells represents the early hallmark of the invasive behavior of a proportion of cancer cells and the first step of the process leading to the formation of ►metastases. Nonspontaneous circulation of tumor cells may derive from iatrogenic invasive procedures (biopsy, surgical intervention, etc.), tumor compression and tumor inflammation.

The process by which tumor cells spreading from solid tumors gives rise to metastases includes the following steps: tumor growth, ►angiogenesis, tumor cell detachment, ►epithelial to mesenchymal transition (EMT), motility, ►intravasation, survival in vessels and embolization, collective tumor cells migration (CTM), possible ►extravasation, mesenchymal to epithelial transition (MET), formation of ►micrometastases and growth of ►macrometastases (Figs. 1 and 2).

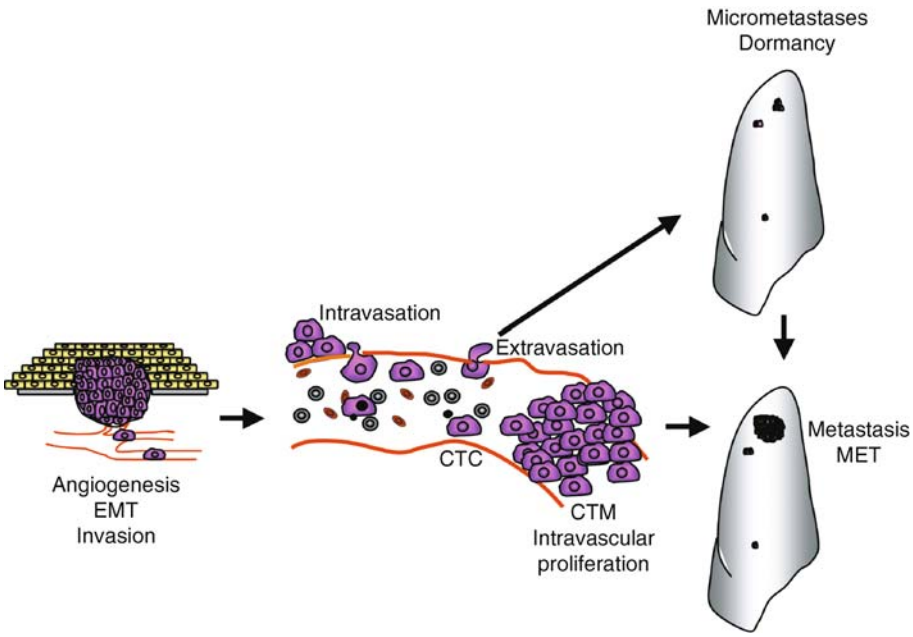
Growing cells rapidly outstrip the supply of nutrients and oxygen and suffer from hypoxia. Hypoxia inducing factor (HIF), which mediates the transcriptional response to hypoxia, is a strong promoter of tumor growth and ►invasion and controls angiogenesis via two key angiogenic factors (VEGF-A and angiopoietin-2). Hypoxia determines cell necrosis and release of inflammatory mediators such as cytokines and ►chemokines which recruit, among other cells, leukocytes and ►macrophages. These, in turn, stimulate angiogenesis, extracellular matrix breakdown, and tumor cells motility. Local production of basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and transforming growth factor beta (TGF-beta) mediate the control of tumor cell survival/►apoptosis balance and of E-cadherin down-regulation leading to reduced cell adhesion and increased tumor cell invasiveness.

Furthermore, hypoxia, acting through LOX induction and Snail activation, leads to E-cadherin repression, a crucial feature of the EMT. During EMT, Twist may need to activate antiapoptotic programs in order to allow epithelial cells to convert to a mesenchymal fate while avoiding ►anoikis.

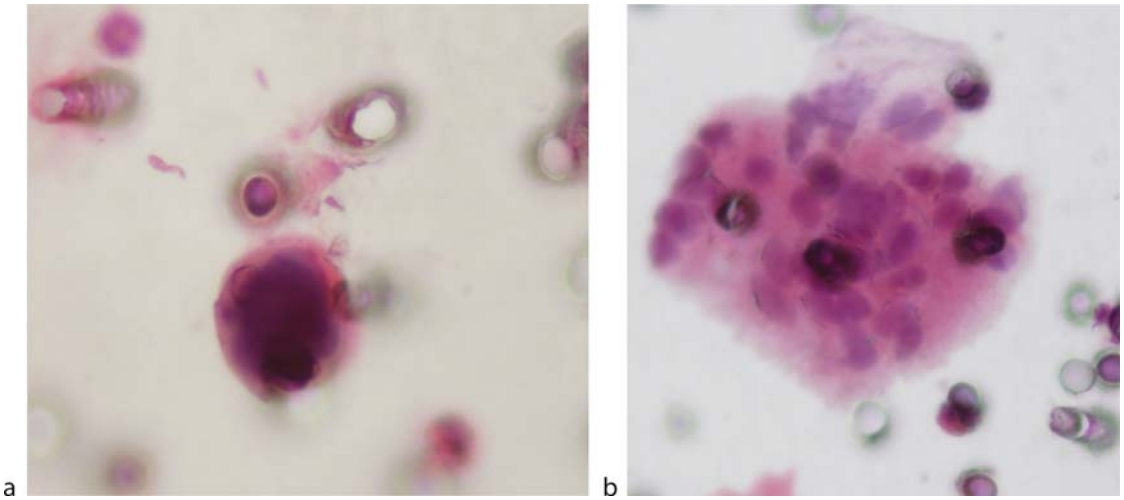
Tumor cells can also invade as multicellular aggregates or clusters (a process known as "collective tumor cells migration." Multicellular aggregates of tumor cells, also called circulating tumor microemboli (CTM), are thought to have potential advantages for survival, proliferation and establishment of micrometastatic lesions in distant organs. Actually, it has been shown that CTM may give rise to metastasis without extravasation, by attaching to vessel walls of arterioles and capillaries, and proceeding to cell proliferation within the vasculature, rupture of capillary walls, and formation of metastases (Fig. 2). Thus, it is generally accepted that the presence of CTM in blood is a marker of highly metastatic potential.

Once the target organ is reached, mesenchymal-like CTC may need to reverse to epithelial-like tumor cells via MET in order to regain the ability to proliferate.

The mechanisms involved in the preferential choice of a target organ for metastatic tumor cell proliferation (►"seed and soil" theory) are still not completely understood. Organ-specific attractant molecules (chemokines) can stimulate migrating tumor cells to invade the walls of blood vessels and enter specific organs.



Circulating Tumor Cells. Figure 1 Main steps leading to development of metastases. Growing tumor cells outstrip oxygen supply and activate angiogenesis. Invading tumor cells undergo the phenotype switch "epithelial to mesenchymal transition (EMT)": they progressively lose epithelial antigens, acquire mesenchymal antigens, and motile propensities (like fibroblasts). After entering blood vessels (intravasation), circulating tumor cells (CTC) undergo apoptosis or circulate as isolated CTC. After extravasation to distant organs, CTC remain as dormant solitary cells or undergo limited proliferation (micrometastases). Unrestrained CTC proliferation gives rise to metastases, via phenotype reversion "mesenchymal to epithelial transition (MET)" and angiogenesis. Circulating tumor microemboli (CTM) represent "collective tumor cell migration" of tumor cells. They cannot extravasate, but arrest in capillaries and proliferate, rupturing the capillary walls and giving rise to metastases.



Circulating Tumor Cells. Figure 2 CTC and CTM enriched by ISET. A CTC (a) from a patient with prostate cancer (hematoxylin and eosin staining, 100×), and a CTM (b) from a patient with kidney cancer (hematoxylin and eosin staining, 83×), enriched by ISET and detected by cytopathological analysis.

Tumor–endothelial interaction, appropriate adhesion molecules expressed by endothelial cells in distant organs, and local growth factors can drive matastatic tumor cell proliferation.

Convergent recent results have led to the present knowledge that invasion can be early and sometimes clinically dormant. Tumor cell dissemination may precede evident primary tumor outgrowth by many

years. The capacity to metastasize may be preordained by the spectrum of mutations acquired early in tumorigenesis, which means that some cancers start out "on the wrong foot." In fact, it has been demonstrated that cancer cells in the primary tumor may harbor a gene-expression signature matching that observed in the metastatic colony and that this signature can be used to predict, with high accuracy, whether the tumor will remain localized or whether the patient will experience metastases and disease relapse.

Epithelial cancer cells have very low survival rates in circulation. The fate of intravasated tumor cells includes a rapid phase of intravascular cancer cell disappearance. This process has been related to "anoikis." Many cancer cell types with increased metastatic potential are resistant to anoikis compared with the parental cells, a tumor cell behavior related to the expression of apoptosis inhibitors.

Animal studies, in which tumor cells are directly introduced into the systemic circulation, have established that around 1/40 CTC give rise to micrometastases and only approximately 0.01% proliferate into macrometastasis. This metastatic inefficiency is principally determined by CTC susceptibility to apoptosis, failure of solitary cells extravasated in distant organs to initiate growth, and failure of early micrometastases in distant organs to stimulate angiogenesis and continue growth into macromatastases. Both solitary cells and micrometastases may remain in "►dormancy" for years. The immune system and angiogenesis have been shown to play a role in tumor cell dormancy, although the mechanisms may be variable in different tumors and are not completely understood. Finally, it has been suggested that any factor that tips the balance between proliferation and apoptosis may result in tumor progression or regression.

CTC Detection and Characterization

The challenge of CTC/CTM detection is related to the requirement of high sensitivity combined with high specificity. Since invasion can start very early during tumor development, identification and counting of CTC when they are very rare (few CTC/CTM per 10 ml of blood, which means few CTC/CTM mixed with approximately 100 million leukocytes and 50 billion erythrocytes) could alert the oncologist about a developing tumor invasion process.

Specificity is also an absolute requirement in this field. In fact, a wrong identification of "nontumor cells" (like epithelial non tumor cells, for instance) as "tumor cells," could generate poor clinical and therapeutical choices having a negative impact on the quality and/or expectancy of life in patients with cancer.

Indirect methods to detect CTC do not provide a diagnostic identification of CTC. They target epithelial cells and/or use organ-specific markers which identify cells from organs but do not demonstrate their tumorous nature. These include immuno-mediated methods and RT-PCR (reverse transcriptase-polymerase chain reaction) methods. Since specific antigens or transcripts characterizing CTC are not known at present (antigens or transcripts which are expressed by all the tumor cells from a solid tumor type and not expressed by leukocytes or by other circulating nontumor cells), authors have used antibodies or transcripts specific to epithelial antigens or organ-specific antigens to identify CTC [for instance, EpCAM, BerEP4, Cytokeratins (CK)]. Epithelial-specific antibodies and transcripts can specifically detect nontumor circulating epithelial cells, or they can nonspecifically detect nontumor, nonepithelial circulating cells, thus giving false positive results. Invasive tumor cells tend to loose their epithelial antigens due to EMT process, and nontumor epithelial cells can also be present in blood. Finally, CTM cannot be reliably detected by immuno-mediated and RT-PCR approaches (as multiple cell labeling tend to dissociate tumor cell aggregates and RT-PCR methods destroy cell membranes). Thus, it appears that a reliable diagnostic identification of CTC and CTM cannot be based on the expression of epithelial-specific transcripts or antigens.

Direct methods, in particular density-gradient isolation and ISET (isolation by size of epithalial tumor cells), followed by cytopathological analysis, are meant to provide a diagnostic identification of CTC [1].

Given the important limitations of immune-labeling and RT-PCR assays, direct diagnosis of CTC/CTM can only be obtained, in a routine manner, by cytopathological analysis of the isolated cells. CTC characterization showing genetic abnormalities can provide clues to the tumorous nature of the cells detected by direct or by indirect methods. However, this approach is not feasible in a routine manner. Genotyping of CTC can be performed by FISH (fluorescence in situ hybridization) or by CGH (comparative genomic hybridization) directed to single tumor cells or pools of tumor cells. Analyses of oncogene amplifications (ex HER2) can be performed by FISH and/or by quantitative PCR after laser microdissection of CTC. Oncogene mutations can be recognized in cytopathologically validated CTC after laser microdissection. Immunolabeling is an interesting approach to characterize the invasive potential of CTC by assessing the expression of tumor markers (for instance HER-2, metalloproteinases, EGF-R, uPAR, alpha-fetoprotein) on enriched cells. However, FISH and immunological staining may have a certain rate of nonspecific labeling.

Detection of apoptotic cells [for instance by TUNEL (TdT-uridine nick end labeling) analysis] may be relevant before and after anticancer therapy, in order to assess the proapoptotic effect of therapeutic

programs. However, the method used to prepare the cells for analysis may induce apoptotic cell death in cells made fragile by blood storage, multiple manipulations, and magnetic particles.

Clinical Impact of CTC Detection

Several studies have shown the potential of CTC/CTM detection and counting in cancer prognosis and follow up. However, the clinical impact of CTC detection is not completely established because a substantial number of studies do not meet essential criteria for quality assurance, stressing the need for a gold standard assay. The definition of a standardized, uniform, cytopathologic method to specifically and sensitively detect CTC/CTM is crucial to perform large clinical trials focused on patients with different types of solid cancers at different clinical stage. These trials are expected to generate reliable results and provide guidelines to use the new marker in clinical oncology.

These assays are also expected to expand the knowledge of the invasion process and to generate new CTC data aimed at improving the patient's quality and expectancy of life.

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Circulating Tumor Microemboli (CTM)

Definition

Synonym "collective tumor cells migration"; are multicellular aggregates or clusters of tumor cells. They have potential advantages for survival, proliferation, and establishment of micrometastatic lesions in distant organs.

►Circulating Tumor Cells
►Micrometastasis

Cirrhosis

Definition

Cirrhosis is a consequence of chronic liver disease characterized by replacement of liver parenchyma by fibrotic tissue and regenerative nodules, leading to progressive loss of liver function. Cirrhosis is most commonly caused by excessive consumption of alcohol and viral infections but has many other possible causes. Cirrhosis has a high mortality due to various complications.

►Alcohol Consumption

CIS

Definition

►Carcinoma in situ

Cis_2His_2 Zinc Finger

Definition

A common protein domain that binds DNA. It contains two cysteine and two histidine residues, which coordinate a zinc ion (crucial for its stability).

►Intrinsically Unstructured Proteins

Cis-Diamminedichloroplatinum

►Cisplatin

Cis-Dichlorodiammineplatinum(II)

►Cisplatin

Cisplatin

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Synonyms

CDDP; cis-Diamminedichloroplatinum; cis-dichlorodiammineplatinum(II); cis-Platinum II; DDP

Definition

Cisplatin is classified as a platinum compound and an alkylating cytotoxic agent. Much of our current understanding of the unique properties of ►platinum drugs has come from studies of cisplatin, especially its antitumor activity. The antitumor activity of platinum (II) complexes requires several unique chemical properties including the presence of chloride, bromide, oxalate, or malonate as leaving group and the neutral complex with inert carrier ligands such as NH_3 groups. Minor variations in the structure of these ligands may have a profound effect on the antitumor activity and toxicity of platinum compounds. The cis conformation is required for a complex to be a biologically effective agent and has significant cytotoxic properties while the trans isomer does not.

Characteristics

Since the discovery of the antitumor potential of cisplatin by Rosenberg and coworkers, therapeutic efficacy of cisplatin as an anticancer agent has been established in a variety of preclinical animal tumor models and in clinical human cancers. Cisplatin has now been one of the most widely used chemotherapeutic agents for treatment of many human cancers. The success of cisplatin in cancer treatment has been due to its many unique properties: a wide spectrum of antitumor activity against drug-sensitive as well as drug-resistant human tumors; a potent inhibition against tumors with varied proliferation and growth characters; effectiveness on both solid and disseminated tumors; and broad cytotoxic activity against viral-induced, chemical-induced, and transplantable tumors with no strain or species specificity.

Mechanisms of Action

The biochemical and biological properties of cisplatin rely on the relative ease of substitution of the chlorine ligands with nucleophilic species such as nucleic acid bases of a DNA strand. It is now widely accepted that cisplatin is similar to the bifunctional alkylating agents and its primary target is DNA. After cisplatin enters the cells, the chloride ligands are replaced by water molecules. This reaction results in the formation of positively charged platinum complexes that form covalent bounds with nucleopholic sites on guanine bases in a DNA strand using intrastrand and interstrand cross-links and create cisplatin–DNA adducts. The most prevalent and unique form of cisplatin–DNA adducts is the 1,2-intrastrand cross-link that cannot form with the inactive isomer of ciaplatin, trans-DDP, suggesting that such an adduct might be responsible for the biological activity of cisplatin. Other platinum–DNA adducts form a distinct structural element that interacts with DNA differently. The formation of these DNA adducts disrupts DNA function and prevents DNA, RNA, and protein synthesis. Regulatory mechanisms that detect the abnormal DNA activate a chain of cellular response to correct or repair the faulty DNA and this ultimately leads to programmed cell death (►apoptosis). Cisplatin-mediated cell killing is believed to be cell cycle phase nonspecific, although there is now much evidence that it may be most effective in G1 phase. Cisplatin also has immunosuppressive, radiosensitizing, and antimicrobial properties.

Cisplatin and Cancer Treatment

Cisplatin is an effective chemotherapeutic drug against a wide spectrum of human cancers. It has been primarily used in the treatment of epidermoid carcinomas of the head and neck, lymphoma, nonHodgkin and Hodgkin disease sarcoma, mesothelioma, osteosarcoma, and adrenal carcinoma and of bladder, brain, and cervical, esophageal, gastric, lung, nasopharyngeal, ovarian, prostate, and testicular cancers. It has also proved to be of benefit in the treatment of other cancers of anal, kidney, liver, breast, penile, and thyroid and of choriocarcinoma, lymphomas, and melanoma. The effectiveness of ciaplatin is however mostly due to the inclusion of other antineoplastic agents into the chemotherapy regimens. For example, such combination therapy of cisplatin along with vinblastine and bleomycin produces complete remission in more than 70% of patients with testicular cancers and substantially improves the survival rate of patients with ovarian cancer. This high success rate is mostly due to synergistic effects, where multidrug combination prevents the drug-induced resistance in tumor cells and, in addition, to the reduced toxic effects of the combination therapy with respect to the total toxicity of each equivalent single

agent. A marked therapeutic synergy has been shown in combination of cisplatin with a wide variety of other chemotherapeutic agents, such as 5-fluorouracil and cytarabine.

Cisplatin is supplied for clinical use as a lyophilized powder in vials that contain 10 mg of the drug, a diuretic, usually mannitol, and salt, or as a 1 mg mL^{-1} aqueous solution. The powder is reconstituted with sterile water to a concentration of 1 mg mL^{-1} and followed by further dilution with saline for intravenous (i.v.) administration. The standard method of administration of cisplatin is as a single slow i.v. injection or infusion every 3–4 weeks. Recently, cisplatin has been shown to be more effective when given locally to the site of the tumors. The most common method is intraperitoneal (i.p.) administration and this type of therapy is most effective for ovarian cancers. The specific dose of cisplatin will vary from patient to patient and depends on a number of criteria.

Cisplatin-Induced Resistance

Even though cisplatin has proven to be a highly effective chemotherapeutic agent for treating various types of cancers, one of the significant limitations toward the successful treatment of malignant cancers with cisplatin and other platinum-based drugs is the emergency of drug resistance. Drug resistance has significant clinical implications and accounts for the failure of a single platinum agent-mediated chemotherapy in curing the majority of cancer patients. When cells become resistant to cisplatin, a large dose escalation has to be applied, which can lead to severe multiorgan toxicities such as failures of the kidneys and bone marrow, intractable vomiting, and deafness. Cellular resistance to these drugs consists of complex mechanisms involving multiple biological pathways. The acquisition or intrinsic presence of resistance significantly undermines the curative potential of these drugs against many human malignant cancers. Although the precise mechanisms by which cells develop resistance to cisplatin are still not well known, several cellular processes have been identified or suggested attributing to invulnerability to cisplatin-induced cytotoxicicty.

Inhibition of Drug Uptake and Decreased Intracellular Accumulation

In order for cisplatin to exercise its cytotoxic effect on tumor cell, it must be taken and accumulated inside of cancer cells to reach and bind to the DNA and cause cell death. The cancer cell, however, has to develop mechanisms either to keep cisplatin out of the cell or to remove cisplatin from the cell to survive. Alterations in cellular pharmacology, including inhibition of cisplatin uptake and reduced cisplatin accumulation by cancer cells, have been observed in numerous model systems and appear to be a major form of acquired resistance. An increase in the production of cellular thiols such as metallothione and glutathione has been shown to block the formation of cisplatin–DNA adducts and sequester cisplatin and remove it from the cell.

Increased DNA Repair

Cancer cells can also become resistant to cisplatin by an enhanced ability to remove cisplatin-DNA adducts and to repair cisplatin-induced ►DNA damages through an upregulated expression and activity of certain DNA repair proteins. For example, a nuclear protein called XPE-BF (xeroderma pigmentosum group E binding factor) has been shown to be upregulated early in the development of cisplatin resistance and be able to repair cisplatin damaging. Another example of a DNA repair protein that may be involved in the recognition of cisplatin damage is ERCC1, one of the essential components of the mammalian nucleotide excision repair (NER) pathway. A higher level of ERCC1gene expression is observed in cisplatin-resistant cells than in cells that are sensitive to cisplatin and in tumor tissues from patients who were clinically resistant to cisplatin therapy than those who responded favorably to the treatment. In addition, an increased level of ERCC1 expression was also found in patients who developed resistance after initial cisplatin treatment. More recently, it has been shown that an enhanced capacity to tolerate cisplatin-induced damage may also contribute to cisplatin resistance. Alterations in proteins that recognize cisplatin–DNA damage (mismatch repair and high-mobility group (HMG) family proteins) and in pathways that determine sensitivity to apoptosis may contribute to damage tolerance. Furthermore, tumor suppressor genes have also been linked to the ability of DNA repair to confer cisplatin sensitivity. Interruption of p53 ►tumor suppressor gene by dysfunctional mutations found in breast, ovarian, and lung cancer cells may increase tumor cells' sensitivity to cisplatin, possibly by a decrease in p53-mediated DNA repair. Recently, NPRL2, a novel tumor suppressor gene identified in human chromosome 3p21.3 region, has been suggested to be involved in DNA mismatch repair, cell cycle checkpoint signaling, and regulation of the apoptotic pathway. The loss of NPRL2 protein expression was significantly correlated to cisplatin resistance in human nonsmall cell lung cancer cells. However, it remains to be determined whether any of these mechanisms contribute significantly to resistance in the clinical setting. Ongoing biochemical modulation and translational correlative trials should clarify which specific mechanisms are most relevant to clinical cisplatin resistance. Such investigations have the potential to improve the ability to predict likelihood of response and should identify potential targets for pharmacological or molecular intervention.

Development of Cisplatin Analogs

Besides a remarkable therapeutic efficacy in a series of solid tumors and outstanding activity of cisplatin the platinum-based therapy is in part accompanied by a set of severe toxic side effects. Analogs or second-generation platinum drugs have being designed and developed to exhibit an exclusive tumor selectivity, enhance the efficacy, improve the toxicity profile, overcome resistance of the original drug, and to be able to be taken orally. Many second-generation analogs of cisplatin have been made. Some have been found to produce the same therapeutic effects as cisplatin but with lower required doses and reduced side effects. Three of these analogs are carboplatin, spiroplatin, and iproplatin. Carboplatin has proven to be the most useful of these three analogs and was approved by the FDA for the treatment of ovarian cancers and for first line lung cancer treatment. Carboplatin and cisplatin have been shown to form an identical type of adduct with DNA and have similar activities against ovarian and lung tumors but is less toxic to the peripheral nervous system and the kidneys. Carboplatin works in some cases when cisplatin has failed. The decreased toxicity of carboplatin and the activity of carboplatin against cisplatin-resistant tumors have led to greater use of carboplatin which has resulted in carboplatin becoming the greater moneymaker of the two drugs. In addition to carboplatin and other second-generation cisplatin analogs, several third-generation drugs have been synthesized and tested, such as platinum (IV) dicarboxylates. These analogs can be taken orally, a significant improvement over cisplatin which can only be administered intravenously. These new platinum complexes and their promising therapeutic strategies in terms improved accumulation and activation at the tumor site are demonstrating a stepwise approach toward the "magic bullet" to human cancer therapy.

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Cisplatin-refractory Germ Cell Tumors

►Platinum-Refractory Testicular Germ Cell Tumors

Cisplatin Resistant Germ Cell Tumors

►Platinum-Refractory Testicular Germ Cell Tumors

Cis-Platinum II

►Cisplatin

c-Jun N-terminal Kinase (JNK)

Definition

JNK, also known as stress-activated protein kinase (SAPK), belongs to the mitogen-activated protein kinase (MAPK) superfamily, which transmits extracellular signals into the nucleus. In response to various environmental stresses, JNK is activated by dual phosphorylation on Thr183 and Tyr185 and in turn phosphorylates Ser63 and Ser73 in the amino terminal activation domain of c-Jun protein. The resultant phosphorylation enhances the transcriptional activity of c-Jun and its heterodimer ►AP-1.

►Doublecortin
►JNK Subfamily
►MAP-Kinases

CK I

Definition

Casein kinase I; serine/threonine protein kinase; CKIα associates with Axin in the β-catenin destruction

complex and phosphorylates β-catenin; CKIγ phosphorylates LRP5/6, facilitating recruitment of Axin.

►Wnt Signaling

CK II

Definition

Casein kinase II (CKII) is a serine/threonine kinase which is involved in many cellular processes, such as DNA replication or transcription. It phosphorylates certain transcription factors such as ►SRF or Pu.1. It is upregulated in many cancers and promotes tumorigenesis

►ETS Transcription Factors

CKI

Definition

Cyclin-dependent kinase (CDK) inhibitor.

►Cyclin D

c-Kit

►Kit/Stem Cell Factor Receptor in Oncogenesis

Cladribine

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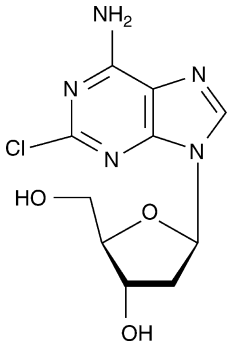
Synonyms

2-chlorodeoxyadenosine; 2-chloro-2′-deoxyadenosine; NSC-10514-F; 2-CdA; CdA; Leustatin; Biodribin



Cladribine. Figure 1 Chemical structure of cladribine.

Definition

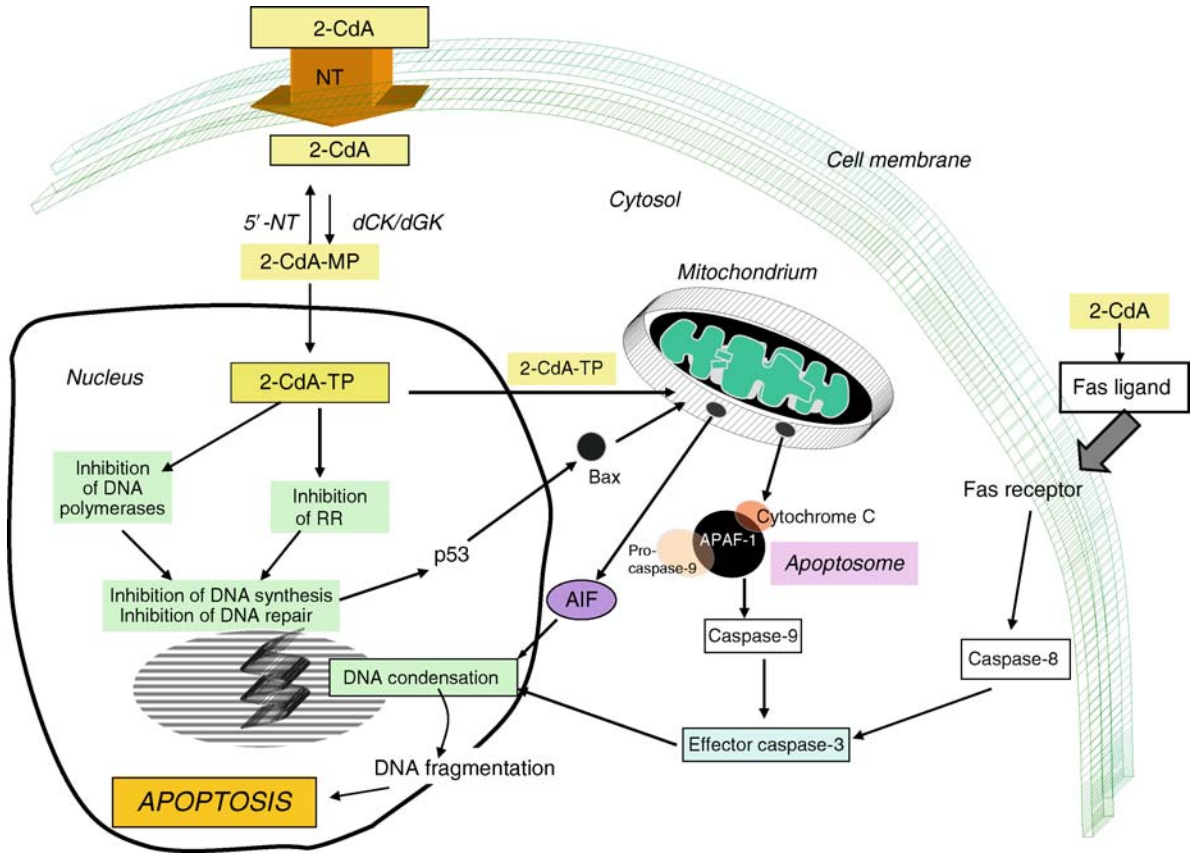
Cladribine is a purine nucleoside analog (PNA) synthesized by a simple substitution of a chlorine atom with a hydrogen atom at the position 2 of the purine ring of deoxyadenosine and resistant to deamination by adenosine deaminase (ADA) (Fig. 1).

Characteristics

2-CdA is a prodrug and its intracellular phosphorylation is necessary for cytotoxic effect to occur. It is phosphorylated by deoxycytidine kinase (dCK) and accumulates as 2-chlorodeoxyadenosine triphopsphate (2-CdATP). High activity of this enzyme in lymphocytes along with their low 5 nucleotidase (5′-NT) activity explains its relatively high selectivity for lymphoid cells. The nucleoside that is formed does not readily exit from the cells through the cell membrane and therefore is accumulated inside the cell. This metabolite disrupts cell metabolism by incorporating into the DNA of the actively dividing cells and freezes cell cycles at S phase. In contrast to other antineoplastic drugs, 2-CdA is cytotoxic to both proliferating and quiescent cells. In quiescent cells 2-CdATP interferes with proper repair of DNA and leads to a total disruption of cellular metabolism via accumulation of breaks in DNA strand, which in turn lead to p53 expression and consequently to induction of apoptosis. Apoptosis induced by 2-CdA can be mediated either via DNA damage and p53 protein expression or directly via mitochondrial permeability transition pore. Inhibition of DNA repair and accumulation of DNA breaks lead to p53 expression, which plays a key role in control of apoptosis and cell cycle and influences the bcl-2 protein family with antineoplastic properties ,as well as bcl-2 like proteins such as bax, bcl-xs and bak, which have proapoptotic action (Fig. 2).

Administration and pharmacokinetics

Clinical pharmacokinetics of 2-CdA have been evaluated in patients with lymphoproliferative diseases and acute leukemia. The drug is usually administrated



Cladribine. Figure 2 -Schematic presentation of cladribine (2-CdA) pathways. (2-CdA-MP-cladribine monophosphate; 2-CdA-DP-cladribine diphosphate; 2-CdA-TP cladribine triphosphate; dCK- deoxycytidine kinase; dGk-deoxyguanine kinase; 5′-NT - 5′- nucleotidase.

i.v. in a dose of 0.12–0.14 mg/kg/day for 5–7 days in continuous infusion or 2-hour infusion. Oral and subcutaneous method of administration can be also used. This routes result in substantial improvement of the quality of life in disorders that require repeated courses of treatment.

After administration of 2-CdA at a dose 0.14 mg/kg as a 2-hour i.v. infusion the mean maximum plasma concentration of the drug is 198 mol/L (range 70–381 mol/L). The steady-stage drug concentration during 24-h continuous infusion of 2-CdA at a dose of 0.14 mg/kg is 23 mol/L. The areas under the concentration time curves (AUC) are similar for both the 2-h (588 mol/L) and 24-h (552 mol/L) infusion. Following administration of 2-CdA at a dose of 0.12 mg/kg as a 2-h i.v. infusion or continous 2-hour infusion the mean cellular concentration of 2-CdA nucleotides are 12.2 mol/L and 10.8 mol/L, respectively. Cellular concentration of the drug exceeded plasma concentration 128 to 373 times. There is a linear dose relationship for 2-CdA between 0.2 and 2.5 mg/m^2/h and elimination followed by two compartment model. The two compartment model showed a half life (T1/2α) of 35 ±12 minutes and T1/2β of 6.7±2.5 h. The mean apparent volume of distribution (Vdr) is 9.2 ±5.4 L/kg.

Clinical activity

2-CdA was approved by the FDA for the treatment of hairy cell leukemia (HCL) and in some European countries for the treatment of refractory/relapsed chronic lymphocytic leukemia (CLL). Moreover several clinical trials continued the value of this agent in low-grade non-Hodgkin lymphoma (LG-NHL), Waldenström macroglobulinemia (WM), cutaneous T-cell lymphoma (CTCL), Langerhans cell histiocytosis (LCH) and systemic mastocytosis. 2-CdA has also some activity in acute myeloid leukemia (AML) and idiopathic myelofibrosis (IM).

1. *Hairy cell leukemia*. 2-CdA induces durable and unmaintained complete response (CR) in about 80% of patients with HCL after a single course

of therapy. However, patients in an apparent clinical and hematological remission following a single course of 2-CdA may have residual disease and 20–30% of them relapse at 10 years follow-up. However, 2-CdA may be equally effective in the reinduction therapy. Moreover, this agent may be also effective in patients with HCL who did not enter remission after splenectomy, interferon-α or even pentostatin.

2. *Chronic lymphocytic leukemia.* 2-CdA used alone or in combination with other cytotoxic drugs showed good efficacy and acceptable toxicity profile in CLL. The drug is more effective in the previously untreated patients than in the patients refractory to or relapsed after conventional therapy with alkylating agents. The overall response (OR) rate ranges from 75 to 85% and CR from 10 to 47% when 2-CdA is used in first line therapy. In the pretreated patients OR rate ranges first 30 to 70% and CR from 0 to 30%. The combination of 2-CdA with cyclophosphamide (CC), cyclophosphamide and mitoxntrone (CMC) or rituximab (RC) can be more effective than 2-CdA alone. ►Randomized studies indicate that 2-CdA alone and CC used as the first-line therapy give similar OR and CR and are of comparable toxicity as fludarabine alone or fludarabine combined with cyclophosphamide, respectively.
3. *Waldenström's macroglobulinemia.* 2-CdA is a reasonable choice for the first line treatment of WM patients. In this disease, 2-CdA has been shown to be active in 64% to 100% of the the previously untreated patients and 14% to 78% of the refractory or relapsed patients. The median time of response to this agent in the previously untreated patients varied between 13 and 28 months. The response rate is higher and the duration of response is longer when 2-CdA is given to the patients with primary refractory disease or to the patients relapsing of therapy rather than to the patients with the disease in resistant relapse.
4. *Cutaneous T-cell lymphoma.* 2-CdA showed some activity in advanced CTCL patients, including Sezary syndrome, and *mycosis fungoides*. This drug produces 25% OR in CTCL. However, high incidence of septic complications and significant treatment related mortality was observed.
5. *Other lymphoid malignancies.* 2-CdA showed remarkable activity in both previously treated and untreated patients with low grade non Hodgkin's lymphoma (LG-NHL). In relapsed/refractory LG-NHL the drug induced durable response with OR rates ranging from 36% to 56% and CR rates between 10% and 20%. 2-CdA is also effective in combination with alkylating agents and/or mitoxantrone in the treatment of refractory or relapsed advanced stage LG-NHL. High activity and low toxicity was reported in the patients with LG-NHL and mantle cell lymphoma treated with 2-CdA combined with rituximab (RC regimen) or rituximab and cyclophosphamide (RCC).
6. *Langerhans cell histiocytosis.* 2-CdA has a major clinical activity in both pediatric and adult patients with LCH. Clinical response was observed in 60–100% patients with a median disease free survival of 33–50 months. Combination with cyclophosphamide may be even more effective in the treatment of LCH.
7. *Systemic mastocytosis.* 2-CdA exerts cytotoxic and anti-apoptotic effect on mast cell leukemia derived cell line HMC-1 cells. Efficacy of 2-CdA in pretreated systemic mastocytosis patients has been reported, with partial response achieved in up to 75% of patients. This agent has a role in the treatment of symptomatic mastocytosis which is unresponsive to conventional therapy with interferon-α.
8. *Acute myeloid leukemia.* 2-CdA as a single agent is more active in pediatric AML than in adults. 2-CdA increases cell concentration of Ara-CTP which is active metabolite of cytarabine (Ara-C). The combined use of both agents is active regimen in the patients with AML. The addition of G-CSF may further improve the effects of 2-CdA and Ara-C (CLAG regimen). Even better results (50% CR) have been achieved in the refractory/relapsed patients when CLAG was combined with mitoxantrone (CLAG-M). Encouraging results with combination of 2-CdA, Ara-C and idarubicin have been also observed in previously untreated elderly AML patients (62% CR).
9. *Idiopathic myelofibrosis*. 2-CdA used alone was investigated in idiopathic myelofibrosis. Clinical and hematological response was seen in about 50% of patients with median response duration of 6 months.

Toxicity and adverse effects

The tolerability profile of 2-CdA is distinguishable from that of other cytotoxic agents. However, bone marrow suppression with prolonged thrombocytopenia, neutropenia and anemia is a common complication of this drug. Moreover the treatment with 2-CdA leads to a decrease in the CD4+/CD8+ ratio for an extensive period of time exceeding even 24 months. In consequence, infections, including opportunistic ones, are frequent events and infections with fatal outcome are reported. The most common infection complications arising from 2-CdA toxicity are respiratory tract infections with bacterial pathogenes and unexplained fever. Oportunistic infections caused by *Pneumocystic carini*, cytomegalovirus, herpes simplex virus, zoster

virus and mycobacteria are also observed. Some reports suggest that 2-CdA may induce autoimmune hemolytic anemia, especially in the patients with CLL. Prolonged immunosuppression related to 2-CdA treatment may increase the risk of the second malignancies.

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Clark Level

Definition

Referring to the level of melanoma invasion, describing the maximal depth of melanoma cell penetration using the anatomical structures of the skin.

Clark Level I: melanoma cells are confined to the epidermis (and appendages); equivalent to melanoma in situ

Clark Level II: melanoma cells extending to the papillary dermis

Clark Level III: extension of the tumor cells filling and expanding the papillary dermis

Clark Level IV: invasion of the reticular dermis

Clark Level V: invasion of the subcutaneous fat

Although level of invasion has historically been shown to correlate with prognosis, its utility in staging primary cutaneous melanoma has been supplanted by Breslow depth, except for thin primary melanomas (i.e. up to 1 mm Breslow depth; AJCC/UICC T1) where both Clark level and Breslow depth are included.

►Cutaneous Desmoplastic Melanoma

CLARP

►FLICE Inhibitory Protein

Class II Tumor Suppressor Genes

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Definition

Class II tumor suppressor genes encode proteins that function in the negative regulation of cell growth. The genes are downregulated in cancer without mutations or deletions in their coding regions. Downregulation is reversible indicating that gene and protein function can be reconstituted upon appropriate treatment.

Characteristics

The term "class II tumor suppressor gene" was invented in 1997 by Ruth Sager, who was the first to realize during the upcoming age of ►gene expression profiling that in human cancers many genes show reduced expression in tumors without being deleted or mutated. Accordingly, two classes of tumor suppressor genes were suggested: Class I tumor suppressor genes (►tumor suppressor genes, TGS) that are lost in cancer due to mutation or deletion and class II tumor suppressor genes that are not altered at the ►DNA level, but rather exhibit strongly reduced expression in tumors as compared with normal tissue.

Examples for classical, bona fide class I tumor suppressor genes are *Rb, p53,* or *WT1*. These genes are found frequently deleted or mutated in the large majority of human cancers. A list of class II tumor suppressor genes that have been identified today are summarized in Table 1 and harbor well-defined genes such as *Thrombospondin* (*THBS*), *H-REV107-1*, and ►*maspin*.

Evidence for a Class II Tumor Suppressive Activity

Due to the reversible nature of class II tumor suppressor gene regulation, their expression levels may vary during tumor development. This is in sharp contrast to class I tumor suppressors and renders the functional characterization of class II genes challenging. The suppressive impact of a given class II tumor suppressor gene might depend on the tumor type and even more on tumor stage and on the underlying genetic alterations. *H-REV107-1* and *maspin* are two class II tumor suppressors, which have been characterized extensively. *H-REV107-1* is downregulated in ovarian cancer and acts as a growth suppressor in vitro and in vivo by inducing ►apoptosis in ►ovarian carcinoma cells. No mutations with the *H-REV107-1* gene have been detected in ovarian tumor samples and overexpression or induction of the gene by interferon γ stimulates apoptosis. Also the mechanism

Class II Tumor Suppressor Genes. Table 1 List of characterized class II tumor supppresser genes involved in negative growth regulation in human tumors

Tumor suppressor gene	Gene function	Cancer type	Mechanism of inactivation
Maspin	Serine protease inhibitor	Breast	Transcriptional repression P53 loss
ING1-4	HDAC/HAT cofactor	NSCLC, breast	Unknown
RNASet2	Secreted glycoprotein	Ovary	Unknown
RARRES3 (TIG3)	Signaling regulator	Colon, ovary	Unknown
H-REV107-1 (HRSL3)	Signaling regulator	ovary	Loss of IRF1
THBS1	Angiogenesis inhibitor	Prostate, ovary	Transcriptional repression by ATF1 or Id1
Tropomyosin	Microfilament compo-nent	Breast	Methylation
Gelsolin	Actin binding	Ovary, breast	Chromatin modification lack of ATF1 binding
CAV1	scaffold protein	Ovary	Unknown
RASSF1	Negative RAS effector	Various	methylation
LOX	Extracelullar cross-linker	Skin, breast	Loss of IRF1, methylation
RARRES1 (TIG1)	Unknown	Prostate, lung	Methylation
PRSS11 (HtrA)	serine protease	Melanoma, ovary	Unknown
KLK10	Secreted serine protease	Breast, testis, ovary	Methylation

of H-REV107-1 action as a signaling regulator indicates that its downregulation is necessary to enable antiapoptotic signaling in ovarian carcinoma. Thus, for the *H-REV107-1* gene, evidence for its class II tumor suppressive nature comes from several studies investigating expression and regulation in vitro an in vivo, as well as mutational and functional analysis. In a similar way, the ►serine protease inhibitor (serpin) *maspin,* originally identified as being downregulated in human breast carcinomas, was characterized. Maspin exerts a number of different functions inside and outside the cell and downregulation of the gene is achieved by various mechanisms in human carcinomas. Maspin inhibits invasion and ►angiogenesis probably by interfering with cytoskeletal signaling thereby altering components of the cytoskeleton. Maspin was also shown to hamper the migration of cultured endothelial cells upon VEGF chemoattraction and to sensitize both tumor and endothelial cells for drug-induced apoptosis.

Class II Tumor Suppressor Identification

Different paths of identification have been used for class I and class II tumor suppressor genes. Class I tumor suppressors are usually localized in critical chromosomal regions often found deleted in cancer. In contrast, most class II tumor suppressors were recognized during large-scale expression profiling as being downregulated in tumor cells and tissues. They light up in approaches such as ►differential display, ►subtractive hybridization, and ►DNA microarray analysis. In addition, some class II tumor suppressors where identified as being encoded in mutational hotspots without directly comprising a target for deletions and mutations in a given tumor. Further proof that an individual gene identified during such an approach is a true class II tumor suppressor requires careful analysis of the genomic sequence and functional analysis of the mechanisms of suppression and function of the protein. Interestingly, high-throughput screening also revealed that the transition between class I and class II tumor suppressors is a smooth one. Canonical tumor suppressor genes such as *BRCA1* and *WT1*, frequently inactivated by mutation in hereditary breast cancer and Wilms' tumor, respectively, can be suppressed by nonmutational mechanisms in sporadic carcinomas and thus turn into class II tumor suppressors in these cancer types. Distinct class II tumor genes, e.g., ING-family members, are inactivated by missense mutations in one cancer type, but lost by downregulation in another cancer type. Therefore, it will be more precise in the future to define a class I mechanism (mutation, deletion) or a class II mechanism (transcriptional or functional inactivation) for an individual tumor suppressor gene in a defined tumor type.

Mechanisms of Inactivation

For the majority of class II tumor suppressors the precise mechanism of downregulation has not been elucidated. However, it has become clear that often alterations in upstream ►signaling cascades and transcriptional regulatory complexes can finally result in the loss of downstream gene expression. Oncogenic signaling pathways emerging from overexpressed ►receptor tyrosine kinases like ►*HER2* and from cytoplasmic oncoproteins such as ►RAS have been shown to suppress class II tumor suppressors in a

reversible manner. Differentiation signals emerging from hormone and vitamin receptors can normally stimulate the expression of class II tumor suppressors such as *RARRES3* and *TIG1* but are lost in cancer cells. Also, deregulation of ►microRNAs might be one mechanism for class II tumor suppressor inactivation. Several class II tumor suppressors, e.g., *CAV1, maspin, THBS1*, have been identified as p53 target genes. *P53*, a bona fide class I tumor suppressor, acts as a transcriptional regulator and belongs to the most frequently lost suppressor genes in human carcinomas. It is evident that loss of p53 entails a loss of targets genes, some of which act themselves as tumor suppressors. For the *maspin* gene, active suppression through a ►hormone-responsive element and lack of transactivation have also been detected. Likewise inactivation of the interferon-responsive transcription factor 1 (►IRF1) was found to determine loss of the *H-REV107-1* class II tumor suppressor involved in the induction of apoptosis in human ovarian carcinomas. In addition, aberrant localization of the maspin protein was found to account for altered function. In ovarian carcinoma, only cytoplasmic maspin localization is associated with poor prognosis, while nuclear maspin was found in less aggressive carcinomas, suggesting a tumor suppressive role of only nuclear maspin. A frequent class II mechanism for gene inactivation is chromatin modification such as ►histone methylation, ►histone acetylation, and DNA ►methylation and a number of class II tumor suppressors, e.g., *RASSF1*, *tropomyosin,* or *TIG1* are suppressed via DNA methylation.

Clinical Relevance

Class II tumor suppressors offer novel therapeutic opportunities because they are present as wild-type alleles in cancer cells. Like class I tumor suppressors, class II tumor suppressor genes are involved in the regulation of apoptosis, cell signaling, differentiation, invasion, and metastasis. As one example, the serine protease inhibitor maspin could be induced by the breast cancer drug Tamoxifen, thereby contributing to the metastasis suppressing effects of the drug. Due to the variety of different mechanisms involved in class II tumor suppressor gene inactivation, therapeutic importance is currently under investigation for most of the class II tumor suppressors. However, the reconstitution of proapoptotic or immune-modulatory properties through interference with chromatin modification and DNA methylation in tumors has already entered clinical trials and will be improved in the near future.

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Class III Histone Deacetylases

►Sirtuins

Class Switching

Definition

Class switching is a process by which the rearranged variable region from an immunoglobulin heavy chain gene is brought into the vicinity of a constant region gene other than IgM or IgD. Typically the intervening DNA between the variable region and the downstream constant region is lost during this recombination.

►B-cell Tumors

Classifier

Definition

Artificial predictive model utilized to assign samples to different categories (classes).

►Supervised Classification

Clastogen

Definition

Is a material which is capable of causing breaks in chromosomes leading to deletions or rearrangements of the chromosomes. This is one of the forms of mutagenesis and can lead to tumorigenesis.

►Micronucleus Assay

Clastogenesis

Definition

Any process that leads to breaks in chromosomal material, or rearrangement, gain or loss of pieces of chromosome.

►Genetic Toxicology

Clathrin

Definition

The major structural component of coated pits constituted by a larger, ~190 KDa protein, the clathrin heavy chain, which is complex to a smaller (~25 KDa) subunit, the clathrin light chain. Heavy–light chain dimers can further assemble into complexes called triskelions, which represent the building blocks of the polygonal array that constitutes the organizing scaffold of a pit.

►Endocytosis
►Huntingtin Interacting Protein 1 (HIP1)

Clathrin-mediated Endocytosis

►Endocytosis

Clear Cell Sarcoma of Soft Tissue

Definition

Sarcoma occurring in young adults, showing melanocytic differentiation and typically affecting the tendons and aponeuroses. It has a nested to fascicular growth pattern, with epithelioid or spindle cells positive for melanoma markers.

►Uncertain or Unknown Histogenesis Tumors

Clear Cell Sarcoma of the Kidney (CCSK)

Definition

Highly malignant childhood renal tumor, often with a polymorphic and challenging histology. Untreated CCSK metastasizes to the bones and lung, recently brain metastases have been reported in patients treated with chemotherapy. Since the introduction of new drugs in the standard treatment, such as cyclophosphamide, doxorubicin, etoposide, and carboplatin, prognosis is improving.

►Mesoblastic Nephroma

Clearance

Definition

The volume of a specific compartment (such as plasma) completely cleared of a specific compound per unit time. Renal clearance refers to the volume of plasma cleared via the kidneys and is measured as a test of kidney function. After intravenous infusion, the clearance of a drug is calculated.

►Irinotecan

Cleavable Complex

Definition

Is the covalent interaction between DNA and the nuclear enzyme topoisomerase I.

►Irinotecan
►Topoisomerases

Clinical Cancer Biomarkers

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Synonyms

Tumor markers; Biological markers; Surrogate endpoints

Definition

A biological analyte that serves as a tool to answer clinically relevant management issues regarding a specific cancer disease.

Characteristics

The analytes must be measurable qualitatively or quantitatively and they may be a biological substance or a process that is dynamically based on a specific tumorigenic pathway. They may occur at the molecular, cellular, or somatic level and should have the ability to detect and thereby reveal sentinel events impacting health outcome with respect to carcinogenesis. They may emanate from the cancerous process itself or the host reaction to the various processes involved in the cancer pathway. The analytes can be measured in a variety of bodily fluids such as blood, saliva, urine, breast fluid, colonic effluent (stool or washings), and sputum or other fluids relevant to the specific cancer disease. There is a current attempt worldwide at standardization of objective assays by criteria such as levels of evidence that attempt validation for innumerable marker candidates.

Diagnostic Tumor Markers

These markers can be applied over the continuum of carcinogenesis from premalignancy to metastatic cancer. While risk markers such as carcinogen-►adducts to DNA are classical risk biomarkers, for example, in ►smokers at risk for ►lung cancer, none have been conclusively validated for utility in individuals at risk while others such as ►adenomatous colonic polyps have been well established in ►hereditary cancer syndromes as well as in sporadic ►colon cancer carcinogenesis. Risk markers and early detection screening markers share the same endpoint for cancer detection but the expectation for accuracy (see formula) differs in that risk markers are not expected to be as accurate as screening markers.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{false negative}} \times 100$$

However both are dependent on the prevalence of the disease in the population being tested. Only two markers are approved for clinical screening in asymptomatic individuals, having been validated using criteria for sensitivity, specificity, accuracy, positive and negative predictive value (see formula) in a prospective manner in multiple clinical trials internationally; the stool-based ►fecal occult blood test (FOBT*)* and the serum-based ►prostate specific antigen (PSA).

$$\text{PPV} = \frac{\text{Prevalence} \times \text{sensitivity}}{(\text{Prevalence} \times \text{sensitivity}) + ([1 - \text{prevalence}] \times [1 - \text{specificity}])}$$

Though neither fulfills the ultimate optimal screening test criteria for cost-effective reduction of overall mortality, FOBT has been shown to reduce disease-specific mortality significantly. FOBT is a qualitative test performed on guaiac-impregnated paper that utilizes the peroxidase activity of hemoglobin to effect a resultant blue color change when an appropriate reaction solution is applied. The hemoglobin in the stool is derived from tumors where a tendency to bleed raises the baseline gastrointestinal blood loss tenfold but clearly cannot differentiate from other endogenous or exogenous dietary sources of hemoglobin. The chance of cancer being discovered on a follow-up ►colonoscopy in the individual patient with any one positive result from two smears each from three consecutive stools is 2–5%. PSA is a serine protease ►kallikrein and is measured by a quantitative immunoassay performed on serum from the blood circulation containing PSA that is secreted by prostatic ductal and acinar cells. PSA functions as a liquefying agent in ►prostate cancer tissue. It is mostly complexed to ►serum proteases or as a minority free PSA form (5–35%). Although the prostate is the major source, other tissues may also secrete PSA such as endometrial, breast, adrenal, or renal tissues. In the individual patient with a test result of >4 ng/ml the chance of cancer as detected by prostatic transrectal, ►ultrasound-guided biopsy is 25–30%. A variety of diagnostic cancer markers are commonly used to complement other clinical diagnostic modalities and some of these may also have prognostic utility, and are available for different cancer types. The ultimate goal of screening is to detect early disease that is amenable to effective treatment ideally demonstrated by efficacy in prospective, randomized trials.

Prognostic/Predictive/Markers

►Prognostic markers are expected to discriminate between patients in predicting a variety of better or worse prognostic outcomes (overall or disease-specific mortality, time to recurrence of disease) independent of treatment and are usually arrived at because of an association with the tumorigenic process under consideration. Predictive markers relate directly to the outcomes of specific therapeutic interventions but many markers may share prognostic and predictive qualities. The optimal marker is one that influences the disease management to improve the clinical outcome although other outcomes such as cost benefit or quality of life may also be evaluated. Prognostic markers are usually compared to traditional clinical prognosticators such as pathologic stage of the disease and ►grade

of tumor. In order to broaden the application of a marker the measurement assay should be simple and reproducible. ►Multivariate analysis statistical analysis of data from retrospective or prospectively trials is the standard for identifying markers as independent prognostic/predictive indicators. The best studied examples of such markers are in the field of ►breast cancer.

Examples of prognostic makers in this clinical scenario are ►estrogen receptors (ER +), ►progesterone receptors (PgR +), and the two components of the ►urokinase-type activation system, the activator (uPA) and activator inhibitor type-1 (PAI-1). The presence of the former two are associated with a better prognosis while patients with low levels of the two latter markers have significantly better survival than the patients with the converse pattern. All these markers are currently in clinical use but the models used to stratify risk differ in that some professional bodies such as the International Consensus Panel on the Treatment of Primary Breast Cancer as of 2003 were using ER + and PgR + status as part of their strategy to designate low risk patients as opposed to the Eastern Cooperative Oncology Group which uses ER + but not PgR +. These models contrast with the expert panel of the American Society of Clinical Oncology which uses neither in their model. The widely used ►Nottingham prognostic index, a model based mainly on pathological parameters, has been validated. Much effort has been invested to complement this system with other markers such as ►*Her-2* and markers of ►angiogenesis. With respect to predictive markers, those that display a treatment by marker interaction quality in predicting response to a specific treatment are likely to be adopted into clinical practice. The ►c-erbB2 marker may be predictive in selection of specific ►adjuvant therapy strategies. Statistical analyses used to evaluate any model also have limitations as significance may not necessarily translate into clinical utility. Lactate dehydrogenase serves as one of the important prognostic marker for ►lymphomas and is a surrogate of tumor burden. Cytogenetic analysis and/or fluorescent in situ hybridization is prognostic of outcome in acute leukemias and other ►hematologic malignancies.

Surrogate/Monitoring Markers

►Surrogate markers are technically surrogate endpoint markers and confer a reasonable likelihood of predicting clinical benefit for a particular therapeutic intervention. The validation measures are accuracy and reproducibility within a controlled study design. Classically, these markers are used to provisionally evaluate a new intervention. A successful outcome in the measured marker favoring the said intervention confers an expectation that future supporting evidence for positive clinical risk:benefit ratio will evolve. Often, a single marker may not account for all observed treatment effects and a battery of markers directed at various components in the tumorigenic pathway may be required. The major advantage of this category of markers is that measuring the effect of an interventional agent with a final conventional endpoint may involve a very large population and many years of follow-up. An example is the use of the chemopreventive effect of acetyl salicylic acid (►aspirin) on ►colorectal cancer. Most studies employ a surrogate endpoint marker represented by a reduction of colorectal adenomatous polyps at the end of a designated follow-up period. In this setting, the adenomas substitute for the ultimate hard endpoint of invasive cancer. Monitoring markers gauge the response to a specific therapy by a parallel change in marker levels with measurable tumor volumes. Alternatively, these monitoring markers may detect occurrence and extent of recurrent disease after primary treatment that may allow for timely institution of secondary treatment modalities but may also be used as surrogate endpoint markers with the same therapeutic intent. Examples are alpha-►fetoprotein and human chorionic ►gonadotropin-beta in ►germ cell tumors (pretreatment levels of which also serve as prognostic markers); ►carcinoembryonic antigen (CEA) and ►CA19-9 in gastrointestinal cancers; and CA125 in ovarian tumors. However, despite their popular use, little actual objective survival benefit has been demonstrated.

Miscellaneous Markers

Emerging markers of tumor metabolism have emerged that utilize ►stable epitope-based dynamic metabolic profiles (SIDMAP). These strategies take advantage of unique anabolic changes induced by protein kinase activation resulting in the preferential nonoxidative utilization of glucose in the pentose cycle for nucleic acid synthesis. This can be used in the development of drugs that impact on protein kinases and effector targets. The most practical expression in clinical practice is the use of the ►positron emission tomography (PET) scan for the monitoring of response to cancer chemotherapy. Assays of tumor ►hypoxia have shown an adverse effect on response to radiotherapy. Oxygenation can be measured directly or by endogenous markers such as ►hypoxia-inducible factor 1 alpha (HIF-1alpha) where overexpression has been shown to confer an adverse outcome suggesting a role for specific hypoxia related treatment.

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Clinical Pathology

Definition

The study by laboratory techniques of body fluids or tissue extracts in disease diagnosis.

►Molecular Pathology

Clinical Studies

Definition

Describe the three phases of clinical study in humans: phase I safety/toleration; phase II early efficacy and phase II definitive efficacy studies.

►ADMET Screen

Clinical Trial

Definition

Refers to the administration of a prospective new drug or therapy to human subjects in a controlled fashion to determine whether the drug or therapy is sufficiently safe and effective to warrant its approval for general medical practice. Typically, clinical drug trials are conducted in three successive phases with increasingly larger test populations: a phase I trial to determine the safe limits of a new drug, a phase II trial to determine whether a new drug is therapeutically effective and to establish its effective dose, and a phase III trial to determine how the effectiveness of the new therapy compares to the existing standard of care and to assess any unexpected adverse effects in the general population.

►Drug design

CLL

►Chronic Lymphocytic Leukemia

CLN

►Conjugated Linolenic Acids

Clonal

Definition

Clonal is an adjective used to describe a group of cells, or an organism that is descended from and genetically identical to a single common ancestor. Malignancies or cancers are usually clonal diseases.

Clonal Proliferation

Definition

Clonal proliferation describes the selection and reproduction of only one type of cell.

►Acute Myeloid Leukemia

Clone

Definition

Cell population derived from a single progenitor. It is common practice in tumor cytogenetics to infer a clonal

origin when a number of cells have the same or closely related karyotypic characteristics. Since subclones may evolve during the development of a neoplasm, clones are not necessarily completely homogeneous.

►Laryngeal Carcinoma

Clonogenic Survival Assay (CSA)

Definition

An assay used to determine the number of cancer cells that retain the ability to divide a sufficient number of times to form a macroscopic colony (typically >50 cells) after treatment or experimental manipulation. Cells are plated in culture dishes and stained colonies are counted ~1–2 weeks later. Clonogenic capacity is usually expressed as the surviving fraction which is the plating efficiency of treated cells divided by the plating efficiency of untreated control cells subjected to the same assay. Plating efficiency is defined as the number of cells counted/number of cells originally plated. The clonogenic assay is particularly useful in cancer research as it integrates many forms of cell death (necrosis, mitotic catastrophe, apoptosis) into one end point critical for cancer therapy – that of continued reproductive capacity.

►Three-Dimensional Tissue Cultures

Clonorchis Sinensis

Definition

Is a human liver fluke in the class Trematoda, Phylum Platyhelminthes. This parasite is found mainly in the common bile duct and gall bladder and feeds on bile. The parasite is endemic to Japan, China, Taiwan, and Southeast Asia.

►Bile Duct Neoplasms

Cluster of Differentiation 44

►Adipose Tumors

Cluster of Differentiation (CD) Molecules

Definition

Cluster of differentiation molecules are cell surface targets on leukocytes (and other cells) recognized by specific sets of antibodies (designated CD-plus a number).

►CD Antigens

Clustering

Definition

A statistical method for identification of patterns that may be similar.

►CD Antigens

CMC

Definition

Complement-mediated cytotoxicity.

►Immunoprevention of Cancer

CMF

Definition

Combination of three cytotoxic drugs: Cyclophosphamide, methotrexate, and fluorouracil.

►Adjuvant Chemoendocrine Therapy

CML

Definition

►Chronic Myeloid Leukemia

CMM2

►CDKN2A

CMMoL

Definition
Chronic myelomonocytic leukemia.

►ETV6

c-Myc

Definition
Transcription factor that is activated by many mitogenic signals. Regulates the expression of various genes involved in cell growth and differentiation and apoptosis. A proto-oncogene.

►Myc Oncogene

CNAs

►Circulating Nucleic Acids

CNC

►Carney Complex

CNDF

►Leukemia Inhibitory Factor

cNOS

Definition
Constitutive nitric oxide synthase.

►Nitric Oxide

Coactivator ACTR

►Amplified in Breast Cancer 1

Coactivators

Definition
Proteins that interact with activated transcription factors and cooperate with this factor to enhance gene transcription.

►Estrogen Receptor

Coagulation Factor II Receptor

►Proteinase-Activated Receptor-1

Coagulation Factor II Receptor-like 1

►Proteinase-Activated Receptor 2

Coagulation Factor II Receptor-like 2

►Proteinase-Activated Receptor-3

Coagulation Factor II Receptor-like 3

►Proteinase-Activated Receptor-4

Coagulation Pathway

Definition

(i) Extrinsic pathway. During coagulation, tissue factor (TF) binds factor VIIa (FVIIa), and the TF-FVIIa complex interacts with factor X (FX) to form active factor Xa (FXa). FXa then interacts with factor Va (FVa), a cofactor for FXa-mediated conversion of prothrombin to thrombin. Thrombin in turn is able to cleave fibrinogen, resulting in fibrin clot formation. (ii) Intrinsic pathway. The intrinsic pathway is triggered by exposure of blood to collagen underlying damaged vessels, and begins with the conversion of FXII to FXIIa, which is catalyzed by kallikrein and kininogen. This conversion then initiates a cascade of events involving activation of FXI, FX and converging with the extrinsic pathway and the FXa-dependent conversion of prothrombin to thrombin.

►Proteinase-Activated Receptors

Coagulopathy

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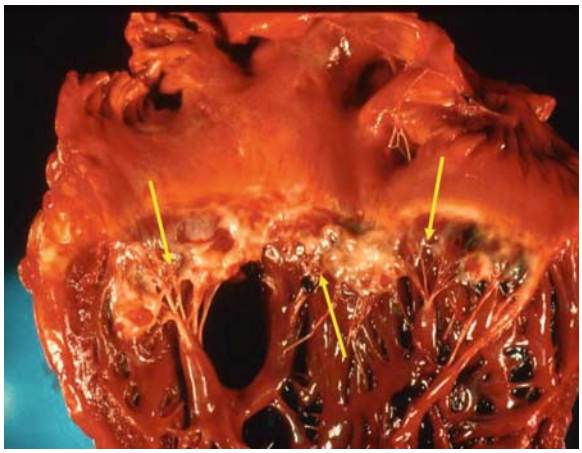
Definition

The changes in pathways of clot formation and lysis, and their interactions with cells and tissues that precede or accompany cancer.

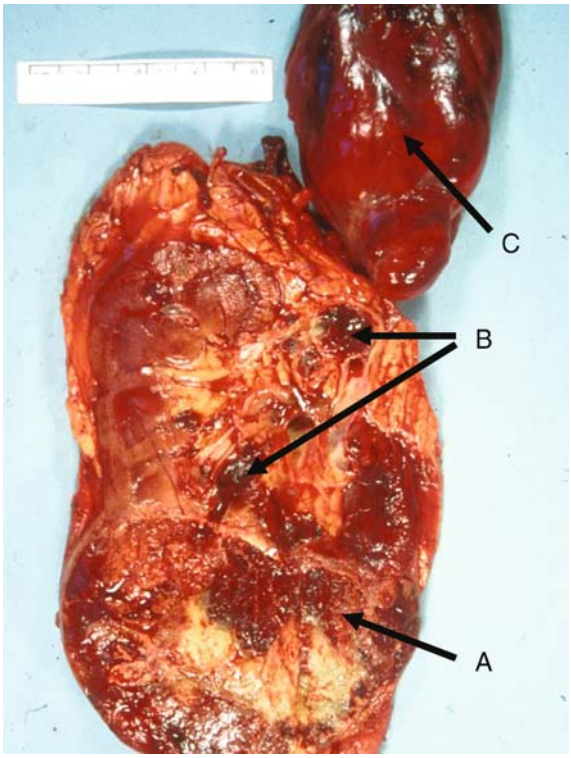
Characteristics

Blood coagulation dyscrasias have been linked to cancer for centuries. Tumor regression with leeching, and bleeding and thrombosis complicating malignancy were among the early cues to an elegant but ruinous cybernetics. In 1867, Trousseau described "painful edema" (deep vein thrombosis, DVT) with both established and as yet undiscovered malignancies. The common occurrence of venous thromboembolism (VTE) with cancer signals poor survival and is a common cause of death in cancer patients. Compression of veins and organ damage by tumor masses, superimposed infection, surgery, radiation therapy, chemotherapy, hormonal therapy, growth factor/cytokine administration and anti-angiogenic therapy predispose to cancer-related thrombosis. Cancer-related thrombotic syndromes besides VTE include non-bacterial thrombotic (marantic; pertaining to wasting disease) endocarditis (Fig. 1), microangiopathy, migratory superficial phlebitis, arterial thromboembolism and Raynaud's phenomenon. Renal cell carcinoma is famous for thriving in a clot in the renal vein and inferior vena cava (Fig. 2). Tumor cells having procoagulant properties may activate coagulation in the adjacent pericellular matrix (►Fibrinogen). More often, heightened cytokine and growth factor expression that mediates malignant growth also triggers host macrophage- or endothelial cell-initiated coagulation activation locally and systemically. In contrast to the physiologic hemostatic response to injury, coagulation activation with malignancy is inexorable (in the absence of intervention) and incapable of self-attenuation; Dvorak's "wound that does not heal."

Laboratory tests of coagulation activation are a sensitive indicator of future cancer risk; abnormalities are virtually universal in established malignancy and signal poor prognosis. Systemic coagulation activation is typically triggered extravascularly by the ►tissue factor-initiated or "extrinsic" (the inciting agent resides on cells and tissues apart from plasma protein coagulation factors) pathway. The more sensitive the test in an up-stream direction in the coagulation pathway, the more likely the test will be abnormal.



Coagulopathy. Figure 1 Non-bacterial thrombotic (marantic) endocarditis of the mitral valve in a patient with gastric carcinoma. Arrows indicate pearly white fibrin vegetations on valve edges.



Coagulopathy. Figure 2 Resected kidney showing: (a) renal cell carcinoma originating in the lower pole; (b) growth of the tumor into the venous drainage to become (c) a tumor thrombus extracted from the inferior vena cava.

Triggering of systemic coagulation activation leads to increased turnover of platelets and fibrinogen (disseminated intravascular coagulation, DIC) in malignancy (Fibrinogen, ►plasminogen-activating system). Early subliminal DIC may be "compensated" when production equals destruction leading to normal levels of both. Worsening DIC becomes "over compensated" and elevated levels of platelets and fibrinogen are common with cancer. The physiologic attempt to resolve injury is subverted resulting in paradoxical promotion of the "wound." Severe DIC may "decompensate" when production no longer meets demand resulting in low platelet and fibrinogen levels (the defibrination syndrome). Bleeding and/or multi-organ failure may follow. Symptomatic DIC may be the first manifestation of a small, as yet undiscovered tumor because of systemic effects of thrombogenic cytokines (eg, IL-1, TNF alpha).

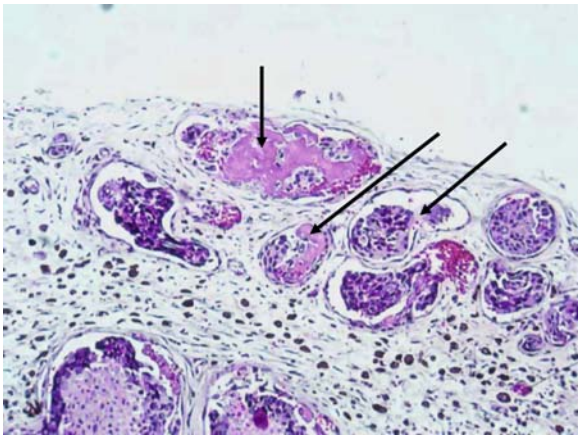
Cancer-related bleeding can occur because of consumption of coagulation factors and platelets due to DIC, but also because of hyperviscosity syndrome, inhibitors of specific clotting factors, and ITP-like thrombocytopenia. Notable changes in coagulation tests include thrombocytosis, the "lupus anticoagulant," heparin-like anticoagulants, and the presence of ►cryofibrinogen. Levels of ►d-dimer predict more advanced disease and reduced cancer survival (Plasminogen-activating system). The ability of low molecular weight heparin (LMWH) treatment to suppress d-dimer levels in advanced malignancy may mark the anti-tumor activity of this class of drugs (►Heparanase, ►heparanase inhibitors).

The inciting force for coagulation activation can exist long before cancer is evident (►Cancer causes and control). Evaluation of patients with DVT for occult malignancy is indicated when no obvious hereditary or acquired (for example, recent surgery, trauma, pregnancy, etc) cause exists. The probability of malignancy within two years of an episode of unexplained (idiopathic) DVT approaches 10% and is higher when DVT is bilateral or recurrent. The history, physical examination (including pelvic examination) and routine laboratory testing with chest X-ray are crucial for evaluating DVT patients at risk for occult malignancy, and re-examination at 6-month intervals for at least two years is appropriate. Tumor markers, abdomino-pelvic scanning, and sometimes cytologic and endoscopic procedures aid discovery of occult disease. Chances of detecting cancer increase with patient age (►Aging and cancer), and a variety of tumor types may be discovered. The old dictum that malignancy discovered on evaluation of a sentinel DVT is untreatable or inoperable is false.

Heparin followed by maintenance warfarin is standard of care for cancer-associated VTE but thrombosis may recur despite "adequate" warfarin anticoagulation. Warfarin control may be precarious due to drug interactions, poor nutrition, compromised liver function due to metastatic disease and other factors. LMWHs that can be self-injected at home allow dose adjustment and more stable anticoagulation in difficult patients, and reduce the risk of DVT recurrence. Use of LMWHs is expected to increase as more clinical trials show they improve cancer survival.

The coagulation mechanism and cancer share metabolic traffic patterns that regulate not only clot formation and dissolution (Plasminogen-activating system) but also tumor growth. Many molecules that participate in coagulation reactions also support tumor cell proliferation, angiogenesis, invasion of extracellular matrix, and metastatic dissemination (Fig. 3) (►Metastasis). A half-century of studies in animal models of malignancy have shown that virtually any manipulation of the coagulation mechanism will alter tumor behavior. However, perplexing variability in response to such interventions signals important differences in mechanisms between tumor models.

Viewing malignancy as a "solid phase coagulopathy" provides insights into regulation of malignant transformation and the aberrant behavior of transformed



Coagulopathy. Figure 3 Tumor cell thrombi in the meningeal microvasculature (arrows) of a patient with metastatic carcinoma.

cells. Platelets are traveling packets of growth factors that "deliver the goods" to sites of vascular invasion by tumor and that enhance metastasis by linking tumor cells to endothelium. Fibrinogen and fibrin in the tumor matrix impart cohesion and induration to tumor masses and provide a scaffolding for tumor cell growth and angiogenesis (Fibrinogen). Thrombin and activated factors VII, X and XII are tumor cell mitogens. Numerous proteins and protein fragments from coagulation and fibrinolytic pathways regulate angiogenesis. Pathologic production of ►urokinase-type plasminogen activator contributes to expression of many features of the transformed phenotype (Plasminogen-activating system).

Molecular participants in coagulation pathways have been mapped in tumor tissue *in situ* and two dominant patterns found. Type I tumors express coagulation factor intermediates with generation of enzymatically active factor X and thrombin on the tumor cells with conversion of fibrinogen to fibrin adjacent to the cells (Fibrinogen). Examples include small cell carcinoma of the lung (SCCL), renal cell carcinoma and melanoma. Type II tumors express a pathway of proteolysis initiated by urokinase-type plasminogen activator but lack a tumor cell coagulation pathway (that may be present on activated macrophages in the tumor matrix). This mechanism has been observed in breast, colon, prostate and non-small cell lung cancers. Other tumor types may have neither of these or an alternative mechanism (lymphomas, mesothelioma). Such information provides a framework for developing hypotheses about how these heterogeneous pathways contribute to tumor growth and dissemination. For example, warfarin and heparins have been shown in randomized trials to improve response rates and survival in SCCL. Aprotinin, an inhibitor of the plasminogen activator-plasmin pathway, has a similar effect in colon cancer. The heparins have attracted attention for cancer clinical trials because they limit coagulation activation and block tumor growth factors, angiogenesis and metastasis (Heparanase, heparanase inhibitors). They might be effective in certain type I and type II tumors (for example, SCCL and colon cancer).

Current research seeks more precise prediction of thrombosis risk, definition of upstream initiators of thrombosis, the identity of common denominators of coagulation activation and carcinogenesis (►Oxidative DNA damage, ►oxidative stress), and clarification of how the coagulation mechanism regulates tumor growth. Cause-and-effect relationships can be defined based on current knowledge of molecular heterogeneity among human tumor types and the availability of drugs that target corresponding components of coagulation and fibrinolysis pathways. For example, evidence from randomized clinical trials showing improvement of cancer outcome upon treatment with anticoagulant heparins should point the way to trials of heparins engineered to block tumor growth but not coagulation that can be tested in increasing doses and combined with interventions targeting other pathways.

Coagulation biology has broadened and deepened our understanding of cancer biology, and suggested testable new and relatively non-toxic strategies for the prevention and treatment of neoplasia. Viewing the coagulation-cancer interaction as an inappropriate "response to injury" invites enquiry into common denominators of coagulation activation and carcinogenesis that stand to disclose the cause(s) of both (Oxidative DNA damage, oxidative stress).

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Co-chaperone

Definition
A protein which interacts with a chaperone and modulates its activity.

►Methylation-Controlled J Protein (MCJ)
►Molecular Chaperones

Cockayne Syndrome

Definition
CS; Have a defect in transcription-coupled repair, which is type of ►nucleotide excision repair. Clinical phenotype include growth failure, progressive neurological degeneration, retinal degeneration, photosensitive skin and deafness. Most CS patients die at an early age.

►Xeroderma pigmentosum

Coding Region

Definition
Region of DNA that encodes the amino acid sequence of a polypeptide (or occasionally a functional mature RNA that does not specify a polypeptide).

Codon

Definition
A nucleotide triplet that specifies an amino acid or a translation stop signal.

Codon-Optimization

Definition
Based upon biases in codon usage between species, a DNA mutagenesis technique that is used to maximize the expression of a transgene in the cells of a foreign species relative to the species that the gene originated.

►Bioluminescence Imaging

COE

►Early B-cell Factors

Cofactor

Definition
A cofactor can be considered a helper or assistant in protein-regulated processes. In general it indicates enzymes, such as kinases or acetylases that enforce transcription factor-mediated transcription.

►Isoflavones

Coffee Consumption

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Definition
Coffee is a beverage made from coffee beans, which have been cleaned, dried, roasted, ground, and brewed with hot water to extract their flavor.

Characteristics
Coffee, along with tea and water, is one of the most frequently consumed beverages in the world. The popularity of coffee is likely related not only to its taste but also to its content of caffeine, which stimulates the central nervous system. Associations between coffee consumption and risk of cancer and other chronic diseases have been studied extensively. Concerns about potential health risks of coffee drinking raised by ►epidemiologic studies in the past were likely

exaggerated by associations between high coffee consumption and unhealthy behaviors, such as smoking and excessive alcohol consumption. More recent knowledge has put coffee in a more optimistic light, and to date there is evidence that coffee consumption may reduce the risk of some chronic diseases, including liver cancer, type 2 diabetes mellitus, and Parkinson disease.

Coffee Constituents

Roasted coffee is a complex mixture of more than a thousand different substances, including lipids, carbohydrates, nitrogenous compounds, alkaloids, phenolic compounds, vitamins, and minerals. Coffee is a major source of caffeine, an alkaloid that occurs naturally in coffee beans, tea leaves, cocoa beans, and other plants. The caffeine content of coffee can be quite variable. A cup of coffee is usually assumed to provide 100 mg of caffeine, but the amount of caffeine in a standard cup (150 ml) of brewed coffee can range from 40–220 mg. Caffeine has been shown to be ►mutagenic. Conversely, caffeine has also been reported to inhibit chemical ►carcinogenesis and UVB light-induced carcinogenesis in animal models.

Two diterpenes, cafestol and kahweol, are found at significant levels in unfiltered coffee. These diterpenes are released from roast and ground coffee beans by hot water, but are largely removed from brewed coffee by paper filters. The amount of cafestol and kahweol in coffee depends on the brewing method. Scandinavian boiled coffee, Turkish coffee, and French press coffee contain relatively high levels of cafestol and kahweol (6–12 mg/cup), whereas filtered coffee, espresso coffee, and instant coffee contain low levels of cafestol and kahweol (0.2–0.6 mg/cup). The coffee diterpenes have been reported to possess anti-inflammatory and anti-carcinogenic properties.

Coffee contains several different chlorogenic acids, and it has been estimated that chlorogenic acid intake is several times higher for persons who regularly drink coffee as compared with non- drinkers. The chlorogenic acid content of a cup of brewed coffee (150 ml) has been reported to range from 15–325 mg. Data obtained from *in vitro* and *in vivo* studies indicate that chlorogenic acid mostly presents ►antioxidant and anti-carcinogenic activities.

Coffee contains significant amounts of ►lignans, which are biphenolic compounds present in plant foods. Lignans can be converted by intestinal bacteria into enterolignans (enterodiol and enterolactone) that possess antioxidant and weak estrogen-like activities. Coffee also contains several micronutrients, in particular magnesium, potassium, niacin, and vitamin E.

Coffee Association with Cancer

Numerous epidemiologic studies have examined the association between coffee consumption and risk of cancer at various sites, particularly the bladder, pancreas, colorectum, stomach, breast, and ovary. Associations between coffee consumption and cancer risk have been reviewed at regular intervals. The literature was extensively reviewed in 1990 by a Working Group of the International Agency for Research on Cancer (IARC). The Working Group concluded that, in humans, there is limited evidence that coffee drinking is carcinogenic in the urinary bladder, lack of evidence of carcinogenicity in the breast and large bowel, and inadequate evidence of carcinogenicity in the pancreas, ovary, and other sites. In 1997, the World Cancer Research Fund in association with the American Institute for Cancer Research concluded that "Most evidence on coffee suggests that coffee drinking has no relationship with cancer risk." The authors of a 2000 review of the epidemiologic literature from 1990 through 1999 wrote that "This updated and comprehensive overview of coffee and cancer epidemiology provides further reassuring information on the absence of any appreciable association between coffee intake and most common cancers, including cancer of the genital tract, digestive tract, and of the breast." Taken together, there is no scientific evidence that moderate consumption of coffee increases the risk of developing cancer. In contrast, emerging evidence suggests that coffee drinking may lower the risk of liver cancer.

Bladder and Lower Urinary Tract Cancer. The association between coffee consumption and risk of ►bladder cancer remains controversial, despite a large amount of epidemiologic data. In general, coffee consumption has been associated with an increased risk of bladder cancer, but the excess risk is generally modest and not dose-related. A 2001 review and ►meta-analysis identified 34 ►case-control studies and three prospective ►cohort studies. The authors found that coffee consumption might increase the risk of lower urinary tract cancer by approximately 20%. In a large ►cohort study in the Netherlands (based on 569 bladder cancer cases) published after that review, coffee consumption was associated with a small increase in risk for bladder cancer in men but was inversely associated with risk in women. A combined analysis of ten European case-control studies published in 2000 attempted to eliminate potential ►confounding by smoking by considering nonsmokers only. In this study, the risk of bladder cancer was not found to be higher in coffee drinkers than in non-coffee drinkers, unless consumption was ten or more cups per day. Hence, overall evidence indicates that coffee drinking is unlikely to have any major influence on bladder cancer risk. The possibility that the relation between coffee consumption and bladder cancer observed in some studies is due to ►bias or confounding is tenable.

Pancreatic Cancer. A possible association between coffee consumption and pancreatic cancer was raised in

the early 1980s when a ►case-control study suggested an almost threefold increased risk of pancreatic cancer associated with drinking three or more cups of coffee per day. However, most subsequent studies have not confirmed a significant relation between coffee consumption and risk of pancreatic cancer. Based on the existing literature in 1990, a Working Group of the International Agency for Research on Cancer concluded that there was little evidence to support a causal relationship between coffee consumption and pancreatic cancer risk. Out of nine cohort studies of coffee consumption and pancreatic cancer conducted since then, two showed increased risks associated with higher coffee consumption and seven observed no association. Overall, the evidence indicates that coffee consumption is unlikely to have any major impact on pancreatic cancer risk.

Colorectal Cancer. ►Case-control and cohort studies have provided different messages about the relation between coffee consumption and risk of colorectal cancer. While most case-control studies have reported an inverse association between coffee drinking and risk of ►colon cancer or colorectal cancer, no association has been found in large prospective cohort studies. However, results of two large cohort studies in the United States showed that men and women who drank two or more cups of decaffeinated coffee per day had approximately half the risk of rectal cancer than those who did not drink decaffeinated coffee. Overall, despite convincing findings in ►case-control studies, it remains unclear whether coffee consumption reduces the risk of colon or rectal cancer.

Gastric Cancer. In the literature to date, there are at least sixteen case-control studies on coffee consumption and risk of ►gastric cancer; a significant association (inverse) was reported in only two of these studies. Of seven prospective cohort studies, a significant increase in risk of gastric cancer associated with higher coffee consumption was found in two studies, including a cohort of Hawaiian-Japanese and a cohort of Swedish women. The remaining five cohort studies observed no substantial association. Thus, it appears unlikely that coffee plays a major role in gastric carcinogenesis.

Breast Cancer. Out of fourteen case-control studies on coffee consumption and risk of breast cancer, three reported an inverse association and eleven observed no association. A Finish case-control study found that coffee consumption was inversely associated with breast cancer risk in postmenopausal (but not in premenopausal) women. A large case-control study in the United States also reported a decreased risk with higher coffee consumption, but the association was limited to premenopausal breast cancer. In a multicenter case-control study of women at high risk for breast cancer due to BRCA gene mutations, breast cancer risk was significantly lower among women who habitually drank six or more cups of coffee per day than among non-coffee drinkers. None of at least eight prospective cohort studies have found any significant association between coffee consumption and risk of breast cancer. Taken as a whole, there is no consistent evidence for a link between coffee drinking and breast cancer risk.

Ovarian Cancer. Of thirteen case-control studies on coffee consumption and risk of ►ovarian cancer, a significant association was found in five studies, with four reporting an increased risk and one a decreased risk with higher coffee consumption. No significant relation between coffee consumption and ovarian cancer was observed in three prospective cohort studies, including a cohort of Seventh-day Adventists based on 51 fatal cases, a Norwegian cohort based on 93 incident cases, and a Swedish cohort based on 301 incident cases. Overall, it appears unlikely that coffee consumption has a major impact on the risk of ovarian cancer.

Prostate Cancer. Of five ►case-control and six prospective cohort studies, none has reported any significant relation between coffee consumption and the risk of ►prostate cancer. Thus, it can be concluded that coffee consumption probably has no relationship with prostate cancer risk.

Kidney Cancer. Seven of eight case-control studies have found no association between coffee consumption and risk of kidney cancer (specifically, renal cell carcinoma). A case-control study in Los Angeles reported a significant increase in risk among women (but not men) who drank more than five cups of coffee per day. A Norwegian cohort study observed no significant association. Likewise, coffee consumption was not associated with risk of renal cell carcinoma in a study consisting of two large prospective cohorts of about 90,000 women and 48,000 men in the United States. Thus, it can be concluded that coffee consumption probably has no relationship with the risk of renal cell carcinoma.

Liver Cancer. To date, there are nine published epidemiologic studies on coffee consumption and risk of primary liver cancer or ►hepatocellular carcinoma, the major type of primary liver cancer. In a prospective cohort study of more than 90,000 Japanese men and women, those who consumed coffee on a daily or almost daily basis had half the risk of developing hepatocellular carcinoma than those who almost never drank coffee. The risk decreased with increasing amounts of coffee consumed; compared with non-coffee drinkers, those who consumed five or more cups per day had a 76% lower risk of hepatocellular carcinoma. Likewise, in a cohort study consisting of approximately 111,000 Japanese men and women, the risk of death due to hepatocellular carcinoma was 50% lower among drinkers of one or more cups of coffee per day than among non-coffee drinkers. Two smaller cohort studies in Japan and five case-control studies (two in Japan and three in

Europe) have confirmed an inverse association between coffee consumption and risk of primary liver cancer or hepatocellular carcinoma. Furthermore, coffee consumption has been associated with a decreased risk of chronic liver disease and hepatic cirrhosis. Compounds in coffee, including caffeine and chlorogenic acid, have been found to inhibit chemically induced hepatic carcinogenesis in animal models. Overall, the evidence suggests that coffee drinking may reduce the risk of liver cancer.

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Cohesins

Definition

Evolutionarily conserved, ring-shaped protein complex responsible for binding the sister chromatids during S phase and to maintain it during G2 and M phases.

►Genomic Imbalance

Cohort Study

Definition

In a cohort study, a defined population with known information on exposure is followed-up. Information on diseases or causes of death are collected. Finally, the correlation of these outcomes with the exposure is analyzed.

►Cancer Epidemiology
►Coffee Consumption
►Obesity and Cancer Risk
►Uranium Miners

Coiled Bodies

►Cajal Bodies

Coiled-Coil Domain

Definition

Is an α-helix protein structure coiled together like the strands of a rope. The most common types are dimers and trimers. Many proteins with coiled-coil domains are involved in important biological functions such as the regulation of gene expression, for example, transcription factors.

►Doublecortin

Colchicine

Definition

Inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to ►mitosis, and therefore colchicine effectively functions as a “mitotic poison.” Since one of the defining characteristics of cancer cells is a significantly increased rate of mitosis, this means that cancer cells are significantly more vulnerable to colchicine poisoning than are normal cells. The therapeutic value of colchicine against cancer is (as is typical with chemotherapy agents) limited by its toxicity against normal cells.

►Vascular Disrupting Agents

Cold Surgery

►Cryosurgery in Bone Tumors

Coley Toxin

Definition

Is a bacterial mixture consisting of the killed species *Borrelia burgdorferi*, *Streptococcus pyogenes* and *Serratia marcescens*, which was used historically as a vaccine for tumor therapy. William Coley noted spontaneous tumor regression in some patients after an episode of septicemia. He showed that cancer can be controlled by injections of bacterial products. The immunological basis of protection reflects properties of the bacterial oligonucleotides.

►Immunotherapy

Collagen

Definition

A fibrous structural protein found in large quantities in the connective tissues. Twenty eight different types of collagen have been characterized. A number of ►angiogenesis inhibitors (e.g. Tumstatin, Canstatin and ►Endostatin) are generated by the proteolytic fragmentation of the carboxyterminal non-collagenous domain (NC1) of collagen.

Collapsin

►Semaphorin

Collective Cell Invasion

Definition

Cell-cell adherence is maintained and the cells migrate as a sheet. This invasion pattern is usually seen in the absence of epithelial-mesenchymal-transition and requires the expression of β1-integrin and surface proteases. Collective cell invasion can be induced by podoplanin.

►Podoplanin
►Epithelial to Mesenchymal Transition

Collier-Olf-EBF

►Early B-cell Factors

Colloid Carcinoma

►Appendiceal Epithelial Neoplasms

Colon Cancer

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Synonyms

Cancer of the large intestine; Malignant neoplastic changes of the colon

Definition

Colon cancer refers to malignant neoplasia of the large intestine. The demarcation line to the more distal rectal cancer is defined as being proximal to 16 cm of the anocutaneous line.

Characteristics

Due to the slow development of precursor lesions in the form of adenomatous polyps or dysplastic lesions, no other tumor offers as many possibilities and as much time for preventive measures. Diagnosis of colon cancer in early stages highly increases the probability of curative resection. A 5-year survival rate of patients diagnosed with stage one colon cancer (limited to the bowel wall) is 90%, which is decreased to 35–60%

in patients with a positive nodal status (stage III) and drops to less than 10% in the metastatic disease (stage IV).

Screening Strategies for the Average Risk Population

Early colon cancer detection programs have been suggested to asymptomatic population of a certain age. The World Health Organisation (WHO), the American Cancer Society and the Agency for Health Care Policy and Research (AHCPR) recommended an annual ►fecal occult blood test (FOBT) and a 5-yearly sigmoidoscopy for the asymptomatic population older than 50. In Germany this proposal has been extended to annual FOBT and rectal-digital examination beginning at the age of 45. In 1994 however, this was followed by only 44.1% of women and 14.4% of men. To date it has not been demonstrated that the rectal digital examination by itself is an efficient means for the early detection of rectal cancer. It therefore seems unreasonable to replace the sigmoidoscopic examination by the rectal digital examination, although it is an essential part of every physical examination in patients older than 50.

FOBT (Fecal Occult Blood Test)

Three large randomized studies carried out in USA, Denmark and Great Britain in a period of 8–13 years, have demonstrated the benefits of FOBT in early colon cancer detection and in the reduction of mortality by 15–33%. Although FOBT is more specific without rehydration, best results were achieved when the test was carried out once a year and included rehydration. FOBT is an adequate screening modality for early cancer detection, reducing mortality rates as well as treatment costs. It is in itself, however, not the appropriate means in cancer prevention since it implies the removal of neoplastic changes even before the event of malignant transformation.

Sigmoidoscopy

Periodic sigmoidoscopy from age 50 onwards, reduces the mortality of rectosigmoidal cancers by 60% and usually a control interval of 5 years is sufficient. The risk of developing colon cancer proximal to the splenic flexure, however, remains unaffected. Compared to FOBT alone, the combination of the two procedures increases the cancer-preventive effect by a factor of 2.2.

Colonoscopy

In the age group of 55- to 64-year-old asymptomatic persons, the combination of FOBT and sigmoidoscopy result will, in the case of positive test results, lead to the recommendation of performing colonoscopy. The question may therefore be raised, if a base-line colonoscopy is a suitable alternative for this age-group. In approximately one third of all patients, polyps will be detected (and removed) such that this strategy would imply a true cancer-prevention. On the other hand, 70% of persons with a negative colonoscopy would not require any further screening modalities for a period of 5 years.

Double Contrast Barium Enema

This radiologic examination cannot replace colonoscopy since the sensitivity is significantly lower (83% versus 95%). The probability of overlooking a small cancer is increased fourfold compared to the endoscopic procedure. Small polyps, however, are also frequently not recognized during the endoscopic examination. In a prospective study, every fourth adenoma under the size of 5 mm was overlooked. The detection of adenomas larger than 1 cm in diameter was reproducible in 94% of the cases. The reliability of colonoscopy depends much on the experience of the person performing the examination.

Preoperative Diagnosis of Colon Cancer

Required examinations:

- History (including family history).
- Physical examination (including rectal-digital examination).
- Colonoscopy with biopsy or double-contrast barium enema with subsequent biopsy of a pathological alteration. If the barium enema does not show a pathological lesion, it may entirely replace endoscopy.
- If a stenosis cannot be surpassed preoperatively, colonoscopic examination in the first 3 months after operation is warranted.
- Ultrasound sonography of the abdomen.
- Radiologic thorax examination.
- Tumormarker ►CEA (carcino embryonal antigen).
- MRI (as an alternative or extended examination).
- CT scan of the thorax if in doubt about lung metastases.
- In case of sigmoid cancers: urine sedimentation, CT scan. If ultrasound examination suggests infiltration of the urinary tract or if red blood cells are demonstrated in the urine sedimentation, cystoscopy is recommended to investigate bladder infiltration. Gynecological examination, if infiltration of the uterus or the ovaries is suspected.

Preoperative (Neoadjuvant) Therapy

To date no benefits have been shown in using ►neodajuvant therapy in colon cancer.

Surgical Therapy (with the Aim to Cure)

Surgery aims at the curative resection of the tumor-bearing segment of the colon together with the regional lymph nodes. In addition, the (partial) resection of

adjacent organs, if these are infiltrated by tumor (multivisceral resection) may be necessary. Colon cancers usually have a circular growth pattern. In order to remove the intramural tumor cell spread, a minimal margin of 2 cm suffices. A regional lymphnode involvement is more widespread. Lymphnodes show a tangential metastatic involvement (up to 10 cm away from the macroscopic tumor), their preferred distribution being towards the center.

Cancers of the Cecum and Ascending Colon

Generally, a right-sided hemicolectomy is the treatment of choice in such patients, including the radicular removal of the lymphnodes of the right colic artery and the ileocolic vessels. The large omentum of the colon is removed together with the colon segment. If the dissection of the gastrocolic ligament is considered, one might be confronted with contrasting opinions regarding the right gastroepiploic artery. Some authors recommend the preservation of the vessel, others do not.

Cancers of the Right Flexure and the Proximal Transverse Colon

As a rule of thumb, extended right-sided hemicolectomy is warranted if the right colic artery is dissected at its origin, out of the superior mesenteric artery. The distal resection lies close to the splenic flexure, allowing circulation. If the blood supply of the distal transverse segment appears insufficient, the additional resection of this segment becomes necessary. The large omentum is completely removed, together with the gastroepiploic ligament and the right gastroepiploic vessels (resection of potentially involved lymphnodes above the pancreas).

Cancers of the Transverse Colon

Cancers in the mid-transverse colon are treated with an entire resection of the segment, including the flexures. The omentum as well as the gastroepiploic ligament and arcade are removed together with the colonic specimen. If the cancers are close to the flexures, hemicolectomy extended to the right and the left is the procedure of choice.

Tumors of the Left Colonic Flexure

Suggested is an extended left-sided hemicolectomy, together with the removal of the lymphnodes of the medial colic vessel and the inferior mesenteric vessels. Equally radical is the central ligature of the left colic artery at its origin, leaving the central part of the inferior mesenteric vessels intact. Under these circumstances the superior rectal vessels remain unaffected, such that the circulation in the remaining sigmoid colon is not impaired. Depending on the exact tumor localization and blood supply, the right colonic flexure may be preserved. Lymphnodes along the central portion of the superior mesenteric vessels should always be removed for diagnostic evaluation.

Tumors of the Descending Colon and Proximal Sigmoid Colon

Generally, left-sided hemicolectomy is recommended, with radicular ligation of the inferior mesenteric vessel. The distal margin of resection lies in the upper part of the rectum and usually the left flexure has to be removed. In order to obtain a tension-free anastomosis, sometimes the medial colic artery has to be sacrificed.

Tumors of the Middle and Distant Sigmoid Colon

In this case, radicular segmental sigmoid resection is the preferred option. The inferior mesenteric artery is ligated either centrally or distal, relative to the origin of the left colic artery. The inferior mesenteric vein should be ligated at the lower edge of the pancreas.

Further Constellations Influencing Surgical Strategy

Multivisceral resections: If adjacent structures are inherent to the tumor, these should, in addition to the lymphnode resection, be resected “en bloc.” In contrast, biopsies to confirm tumor infiltration of adjacent organs are to be avoided since cell dissemination might be initiated. Distant metastases: The resection of synchronous or metachronous metastases of the liver, lung etc. is indicated only, if this resection has curative intent and complies with the oncological principles. If the metastases are unresectable, “palliative measures” apply. Multiple colonic primaries: The extent of resectional surgery depends on additional lymphnode dissections recommended for each tumor. As a result colectomy with ileorectal anastomosis may be indicated. Synchronous occurrence of colonic polyps: Adenomas that are not removable endoscopically should be resected during colon cancer surgery. In this case a margin of 2 cm applies. Extended resection to the segmental lymphnode is not necessary. Cancer diagnosis in endoscopically removed polyps: If, unexpectedly, the histological examination reveals malignancy, oncological resection of the colonic segment is indicated. This may be neglected only in the case of a polyp with tumor-free stem which is confined to the submucosa with “low risk” (pT1, G1–2, no lymph vessel involvement). Segmental resection: In patients with metastatic disease, radical resection of the colonic segment with lymph node removal may not be indicated. Very poor physical condition or the high age of some patients may justify colonic surgery which does not follow oncological principles. Emergency operation: Nevertheless, high-urgency surgery, unavoidable due to bowel obstruction, tumor or colon perforation should comply with oncological principles. Laparoscopic surgery: To date, no data are available

that document the operative outcome in patients that underwent laparoscopic colon cancer surgery. Future consideration of ongoing studies with long follow-up periods is therefore necessary for the optimal treatment of colon cancer. Nevertheless, there are no objections to carry out laparoscopic colon cancer surgery in a palliative setting. Ulcerative colitis and familial adenomatous polyposis (FAP (►APC gene in Familial Adenomatous Polyposis)): Cancers of this type require proctocolectomy, if possible continence-preserving. Especially in an early stage, cancer in the proximal two thirds of the rectum is not a contraindication for ileoanal pouch surgery. ►HNPCC (hereditary nonpolyposis colorectal surgery): In the event of this autosomal-dominant syndrome, many authors suggest extended cancer surgery in the form of prophylactic bowel removal (colectomy and ileorectal anastomosis or restorative proctocolectomy). Occurrence of metachronous colorectal cancer and the observation of so-called interval cancers is significant. However, the benefit of prophylactic colon removal (without the evidence of neoplasia) remains uncertain, especially if considering a reduced penetrance of ~80%.

Intra- and Postoperative Histopathological Diagnosis

Due to technical complications the immediate pathological classification of a tumor/polyp in a frozen section is not an option. Pathological evaluation after oncological surgery is, however, of prognostic significance in the locoregional resection (R-classification), the depth of invasion (pT classification) and the grading and lymphnodal status (pN classification) and forms the basis in the decision process concerning ►adjuvant therapy. The total number of resected lymph nodes and metastatic lymphnode occurrence is therefore of essential relevance. Perforation of the tumor during surgery is of prognostic significance and must be documented.

►Microsatellite tumor instability is of special relevance in the setting of HNPCC, and of increasing interest since response rates to adjuvant therapy have shown that stable and instable tumors differ in their biological response to chemotherapeutic agents. Although the natural course of unstable tumors is more benign, than the natural course of stable tumors, the biological response to conventional chemotherapy in stable tumors is better.

Classification of Colorectal Cancers

There are two classifications that are used separately, the Dukes and the TNM (tumor, lymphnodes, metastases) classification.

The Dukes-Classification (Table 1) is preferred in the US and UK and describes the following stages:

The TNM-staging (suggested by the Union internationale contre le cancer, UICC) (Table 2) is preferred in European countries and distinguishes between the stages listed below. T stands for the expansion of the primary tumor; N for the lack or the presence of metastases of the lymphnodes; M for the lack or the presence of distant metastases. Numbers indicate the extent of malignant processes; p, post-operative.

Colon Cancer. Table 1 Dukes-classification

Dukes A	Growth limited to wall, nodes negative
Dukes B	Growth beyond muscularis propia, nodes negative
Dukes C1	Nodes positive and apical negative
Dukes C2	Apical node positive
Dukes D	Growth beyond originating organ

Colon Cancer. Table 2 TNM-staging

pT1	Local invasion of submucosa
pT2	Local invasion of the muscularis propia
pT3	Local invasion beyond the muscularis propia
pT4	Tumor cells have reached peritoneal surface or invaded adjacent organs
pN0	No lymphnodes affected by metastases
pN1	One to three lymphnodes affected by metastases
pN2	Four or more lymphnodes affected by metastases
pM0	No distant metastasis
pM1	Distant metastasis

Adjuvant Therapy

- In order to recommend adjuvant therapy, complete removal of all regional and metastatic lesions (R0 resection) in addition to the tumor removal is necessary. Recommending adjuvant therapy is based on the pathohistological classification of the tumor, specifically of the pN status. In order to define the lymphnode status, a minimum of twelve regional lymphnodes should be examined. Immunocytological studies of isolated tumor cells in either bone marrow aspiration biopsies or in the peritoneal fluid should not, at least at this point, be referred to in the decision for or against adjuvant therapy since the impact of "minimal residual disease" remains to be established.
- Patients with early-stage colorectal cancer (stage I or II) and patients after R0 resection of distant metastases should receive adjuvant therapy in the setting of controlled studies only.
- The benefits of adjuvant therapy in UICC stage III cancers (all pT stages, pN1-2, M0) remain to be established. A quality-controlled surgical treatment with and without adjuvant therapy is currently under evaluation. Outside these studies, adjuvant therapy is recommended for stage III cancers.
- Adjuvant chemotherapy for stage III colon cancers: One year administration of 5-FU (fluouracil) and levamisol proved to be as effective as a 6-monthly administration of 5-FU and folinic acid. Although there is variation between different adjuvant protocols, general contraindications for adjuvant therapy are listed below:
  - General physical condition under the score of 2 (WHO)
  - Uncontrolled infection
  - Liver cirrhosis
  - Severe coronary heart disease, cardiac insufficiency (NYHA III and IV)
  - Preterminal and terminal renal insufficiency
  - Limited bone marrow function
  - Unavailability for regular control check-ups

To date there appears to be no benefit in the administration of monoclonal antibody treatment (17 1A) in addition to conventional chemotherapy.

Follow-Up

Due to the low rate recurrence rate, no major prognostic advantage from follow-ups is expected for patients with early cancer (UICC I) and R0 resection. The advice to perform two colonoscopies, 2 and 5 years post colon cancer surgery, is aimed towards an early identification of second primaries. An intensified surveillance of individual cases is justified in circumstances that lead to suspect a higher recurrence rate, i.e. tumor perforation, G3 and G4 tumors or histologically verified pericolic vessel infiltration. After palliative tumor resection (R2) a symptomatic follow-up is recommended.

Following R0 resections of tumor stages II and III, the main benefits of follow-up strategies can be expected, if the general physical condition of the patient does not object to recurrent surgical intervention. Two specific follow-ups are recommended within 5 years of primary surgery (year 2 and year 5) and include physical examination, CEA-level, abdominal ultrasound, X-ray of the thorax and colonoscopy. Intensified follow-up is recommended for patients with an increased hereditary risk.

Cellular and Molecular Features

Colorectal tumors provide an excellent system in which to search for and study genetic alterations involved in the development of neoplasia. It appears that most if not all malignant colorectal tumors arise from preexisting benign adenomas. These precursor lesions can be removed and studied at various stages of development. Colorectal tumors develop as a result of oncogene mutations in combination with the mutated tumor suppressor genes, the latter being predominant.

Human colorectal tumors, including very small adenomas, have a monoclonal composition. Adenomas therefore arise from a single or a small number of cells which initiate the process of neoplasia by clonal expansion. Genetic alterations within the majority of neoplastic cells studied so far, suggest an impaired regulation of cell growth that enables those cells to become the predominant cell type, eventually constituting the neoplasm.

The development process in patients with sporadic cancer (as opposed to ►familial cancer) occurs over a period of decades. The series of genetic alterations involves oncogenes such as ►RAS as well as tumor suppressor genes (particularly those on chromosome 5q, 17p and 18q). In general, the three stages are represented by increasing tumor size, dysplasia and villous content. The mutation of the Ras gene (usually K-Ras), appears to occur within a single cell of a preexisting small adenoma followed by clonal expansion which produces a larger and more dysplastic tumor. Deletions of chromosome 17p and 18q generally arise at a later stage of tumorigenesis, than deletions of chromosome 5q or Ras gene mutations. In this ►multistep development the total number of genetic alterations rather than their order of occurrence, determines the biological properties of neoplasia.

Tumors continue to progress once cancers have formed and the cumulative loss of tumor suppressor genes on different chromosomes correlates with the ability of the tumor to metastasize and to cause death.

Recent investigations have shown that ~25% of randomly selected colorectal cancers are unstable, a phenomenon used as an independent prognostic factor in colorectal cancer. In addition, the loss of heterozygosity (►LOH) at a chromosome 8p marker (termed ►allelic imbalance) has, as recently reported, been related with a poor patient outcome. It may therefore be expected, that the molecular characterization of colorectal tumors will increasingly affect the individual risk assessment and the suitable intervention strategies.

The identification and characterization of molecular mechanisms underlying tumor development and tumor growth offer new opportunities in cancer treatment. An intricate genetic scenario is responsible for a complex human neoplastic condition. The relevance of individual steps within, might open doors for therapeuticals that specifically target essential, although malfunctioning, check-points.

Perspective

In developing countries, especially Asia, incidences of colon cancer are rapidly rising. In the United States and in Germany, ~130,000 and 50,000 patients, respectively, are diagnosed with colorectal cancer every year. Colorectal cancer comes second in the group of tumor-related deaths. The life-time risk of developing colorectal cancer in Germany is 4–6%; with the majority of these cancers occurring in people aged 50 and above. Gaining new insight into the molecular pathogenesis of colorectal cancer will allow progress in many facets of disease control. They include the identification of genetically predisposed groups for targeted surveillance and/or chemo-prevention, prognosis for patients with established cancer, predictions of treatment efficacy and the development of novel treatment strategies.

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Colonoscopy

Definition

Is an examination of the rectum and *entire* colon using a lighted instrument called a colonoscope. Colonoscopy can find precancerous or cancerous growths throughout the colon, including the upper part of the colon, where they would be missed by sigmoidoscopy.

Colony-Forming Unit (CFU)

Definition

In microbiology defines the capacity of a given pathogen to form a single clonal culture on an appropriate solid culture medium. Used to define the number of pathogens under laboratory conditions.

►Bacillus Calmette-Guérin

Colony Stimulating Factor-1

Definition

The major growth factor regulating the viability, proliferation and differentiation of cells of the mononuclear phagocytic lineage. It is also a powerful macrophage chemoattractant.

►Macrophages

Colorectal Premalignant Lesions

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Synonyms

Adenomas; Adenomatous polyps; Microadenomas

Definition

Colorectal premalignant lesions are focal lesions which precede cancer development. They include different entities: adenoma is the only one for which the available scientific evidence of its premalignant nature is more convincing.

Colorectal adenomas are polypoid, flat or depressed lumps of epithelial origin, which can be found throughout the large bowel, whose main histological feature is ►dysplasia. They may be single or multiple, rarely hundreds or even thousands, as in patients with ►familial adenomatous polyposis (FAP). Adenomas, if left untreated, grow and finally may become malignant. However, only a fraction of colorectal adenomas acquire malignant features, and sometimes may also regress. Polypoid adenomas are the most frequent and can be sessile (with a large base) or pedunculated (with a stalk attached by a narrow base on the colorectal mucosal surface).

Other kinds of colorectal lesions may be premalignant; they are hyperplastic polyps, hamartomatous polyps, aberrant crypt foci, and dysplasia in inflammatory bowel diseases (IBD).

Hyperplastic polyps are round, sessile, and pale lesions usually with a diameter of less than 1 cm. Histology shows elongated, dilated, and typically saw-like crypts, lined by a single layer of colonic epithelium, and usually no feature of dysplasia. Thus, hyperplastic polyps are not considered premalignant. However, a variant of hyperplastic polyps showing areas of both dysplasia and hyperplasia in the same lesion (referred to as mixed polyps) or others where, in a hyperplastic architecture, cytological signs of dysplasia are present (referred to as serrated adenomas), can be considered premalignant.

Hamartomatous polyps are rare colorectal polypoid lesions whose main histological feature is the presence in the same lesion of different normal colorectal tissues (mainly epithelial glands, connective tissue, and smooth muscle) showing marked alteration of the whole architecture.

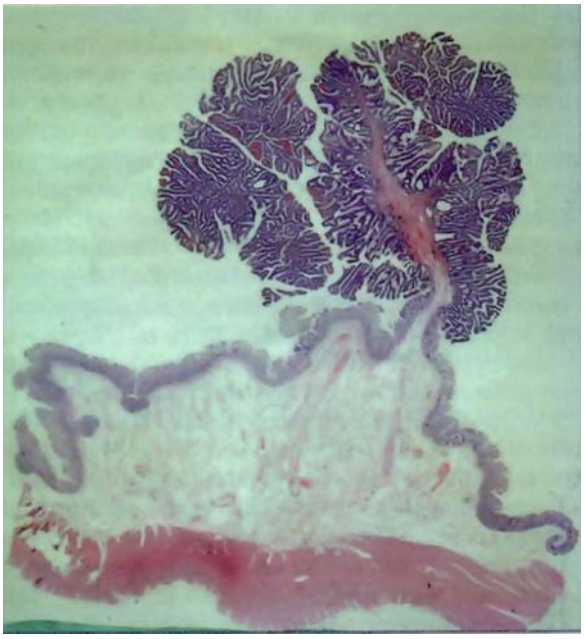
Aberrant crypt foci are microscopic focal lesions which can be observed under a light or a dissecting microscope at 30–40× magnification, or even during magnifying colonoscopy, on the colorectal mucosal surface after staining with a vital dye, methylene blue, in patients with FAP, cancer, or benign diseases of the large bowel. They were originally described in mice exposed to colonic carcinogens and then identified also in humans. They appear as clusters of altered colonic crypts, enlarged, and deeply stained than normal at topological view, often showing abnormal-shaped lumens and slightly bulging on the level of the normal mucosal surface.

In patients with IBD (ulcerative colitis and Crohn's disease) dyplasia may be observed in biopsies taken during colonoscopy as flat lesions or polypoid masses. Dysplasia in IBD is a premalignant feature, which deserves further clinical management.

Characteristics

Adenomas

Adenomas are rare in Africa and in most Asian countries, while they are very frequent in many developed countries, where 30–40% of individuals over 50 years harbor an adenoma of any size in the colon. The widespread use of colonoscopy contributed to the increased prevalence of adenomas in these populations. The geographical distribution of adenomas lends support to the contention that dietary and lifestyle factors in western countries play some role in their etiology. Among them, a high intake of meat (expecially red meat), a low intake of vegetables and fiber, a low physical activity, and insulin resistance are the most consistent. It is well-established that their prevalence increases with age. Adenomas are more frequent in the left colon (distal to the splenic flexure) and in the rectum, reflecting the anatomical distribution of carcinoma, though recently an increased prevalence in the right colon due to the use of pancolonoscopy has been reported. Adenomas are usually less than 1 cm in diameter, sometimes less than or equal to 0.5 cm (diminutive polyps). When larger, more often they show a villous architecture (see later) and proclivity to malignant transformation. Adenomas are single in most cases. In about half of the individuals, two or more lesions can be observed at endoscopy. The number of adenomas is strictly related to the risk of developing cancer; in ►FAP cancer incidence approaches 100%. According to the main architecture of the lesion, adenomas may be divided into three histological types: tubular, tubulovillous, and villous, the latter having the highest probability of becoming malignant. Tubular adenomas show a prevalent glandular architecture in more than 80% of the whole lesion. Villous adenomas have more than 80% of villous architecture, characterized by long fronds of papillary epithelium arising from the mucosal surface of the colon (Fig. 1). Tubulovillous adenomas show both components, each less than 80%. In all colorectal adenomas, dysplasia is graded into three different levels of severity, i.e., mild, moderate, and severe. The current grouping of grades into two categories: "low-grade" (mild and moderate) and "high-grade" (severe) is justified by the poor reproducibility in the separation between mild and moderate grades. This uncertainty stems from the definition of dysplasia, term with a high level of subjectivity. More advanced is the overall grade, higher is the risk of cancer development, which is evident when malignant cells pass through the muscularis mucosae and invade the submucosal layer of the bowel wall, identifying a malignant polyp. This is the earliest form of carcinoma with metastatic



Colorectal Premalignant Lesions. Figure 1
Histological section of a villous adenoma with typical papillary epithelium.

potential; the risk of lymph node metastasis has been estimated around 10% overall.

Flat adenomas are short lumps of mucosa with a reddish surface, sometimes with a central area of depression. At histology these adenomas show the same three types of architecture described for polypoid adenomas, but they progress to cancer with a higher frequency, providing further evidence for the adenoma–carcinoma sequence.

Sometimes adenomas bleed, but most remain asymptomatic, and are discovered by chance during colonoscopy. Flat and depressed adenomas are not easily seen during endoscopy, but it is important to identify and remove them, due to their higher malignant potential. Indeed, the optimal treatment for adenomas (and for colorectal polyps in general) is its removal, usually during colonoscopy, or seldom by surgical operation, expecially large adenomas of the rectum. The pathologic diagnosis is then mandatory for planning the future endoscopic follow-up of the patient. Estimating the risk of developing other lesions after removal of adenomas is not an easy task. The current evidence suggests that larger, multiple, and villous adenomas do recur more frequently and require a closer endoscopic follow-up.

Adenoma–Carcinoma Sequence

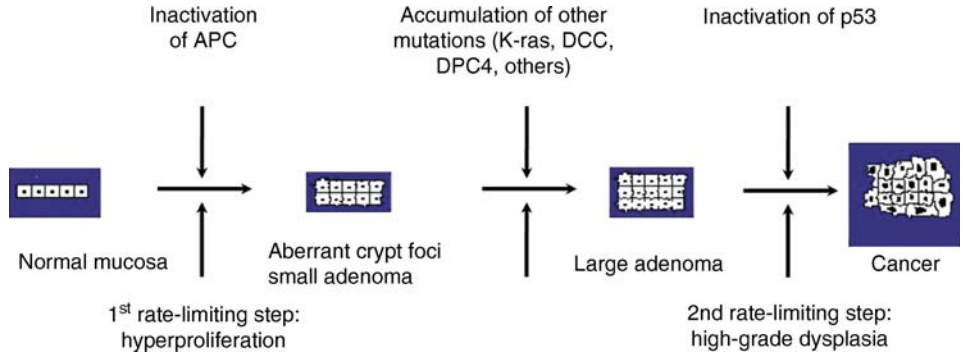
Overwhelming evidence suggests that colorectal carcinomas develop from preexisting adenomas, which is referred to as the so-called adenoma–carcinoma sequence hypothesis based upon the following observations:

1. Focal adenomatous areas have been frequently observed in colorectal carcinomas; on the other hand, focal infiltration by carcinomatous tissue (beyond the muscularis mucosae) can be observed in adenomas;
2. If adenomas are not removed, cancer may develop at the polyp site, and accurate surveillance with removal of polyps leads to a reduction of the expected number of invasive carcinomas;
3. Adenomas and carcinomas share a similar distribution along the large bowel, being more frequent in the descending and sigmoid colon, and in the rectum;
4. Age-specific incidence rate of colorectal adenomas shows a peak that precedes that of cancer by about 5 years, in keeping with the estimated time lag required for malignant transformation;
5. Adenomas have an hyperproliferative epithelium, as carcinomas;
6. Similar patterns of genetic and epigenetic alterations have been reported for adenomas and carcinomas, though usually they are less frequent and severe in adenomas than in carcinomas.

The adenoma–carcinoma sequence has been revisited by a genetic point of view, and definite steps of activation of oncogenes and inactivation by allelic loss of tumor suppressor genes, which underlies colorectal carcinogenesis, have been identified (Fig. 2). In particular APC inactivation seems one of the earliest events causing hyperproliferation of epithelial cells and microadenoma formation, then accumulation of mutations in other genes [K-*ras* (►ras), DCC, and others] are responsible of adenoma growing. Finally, later inactivation of the tumor suppressor gene *p53* [*p53* gene family] seems the key event for malignant transformation. Moreover, in some cases ►microsatellite instability (MSI) (expecially in tumors from patients with ►Lynch syndrome) and hypermethylation of ►CpG islands in the promoter region of several genes, causing gene silencing, has been demonstrated in adenomas.

Hyperplastic Polyps

The premalignant potential of hyperplastic polyps is not supported by most evidence. However, some observations seem to point to the contrary. Polyps with mixed but distinct features of hyperplasia and dysplasia may become malignant. Their nature is not yet understood. It is possible that adenomatous tissue develops within a hyperplastic polyp. Also serrated adenomas have premalignant potential because of some cytological features of epithelial cells lining the crypts; evident



Colorectal Premalignant Lesions. Figure 2 Genetic model of colorectal carcinogenesis, including the main molecular alterations underlying the development of colorectal lesions.

signs of dysplasia have been identified in 30–40% of serrated adenomas. Moreover, it should be mentioned the rare hyperplastic polyposis, a syndrome which seems to have malignant potential, acquired through mutator pathways. Some biomolecular alterations similar to those found in adenomas, have been reported in hyperplastic polyps, including K-*ras*, and p53 mutations, and MSI.

Hamartomatous Polyps

Hamartomatous polyps are observed in two diseases: ►Peutz–Jeghers syndrome and juvenile polyposis. They are not considered precancerous lesions, although some authors have reported the development of adenocarcinoma in hamartomatous polyps, and others the coexistence of adenomatous and carcinomatous tissues in these polyps. Finally, in Peutz–Jeghers syndrome a frequent occurrence of gastrointestinal as well as extradigestive cancers has been observed.

Aberrant Crypt Foci

Aberrant crypt foci are smaller than adenomas, and usually not visible to the naked eye. Luminal openings of aberrant crypts show various shapes, each corresponding to definite histological alterations. Indeed, histologically ACF are rather heterogeneous. They may show various alterations, from hypertrophy (only dilated crypt with no cell alteration), to hyperplasia, to severe dysplasia. Only a minor fraction (10–30%) of the ACF examined is defined as dysplastic (referred to as microadenomas). Dysplasia may be focal in an aberrant crypt focus, and even in a single aberrant crypt, suggesting that a transition from hyperplasia to dysplasia in aberrant crypts is possible. Dysplasia seems more frequent in larger and in proximal colonic ACF. ACF with dysplasia can be considered true premalignant lesions, whereas for ACF with other histological features the matter is questionable, and the mechanisms and steps of the progression remain unclear. The main points in favor of their premalignat potential are as follows. The density of ACF is higher in patients with FAP. In these patients the ACF examined show definite dysplasia at histology in 75–100% of cases. In patients with cancer and benign diseases of the large bowel the density of ACF is lower, but higher in the distal colon and rectum. Older individuals harbor a higher number of ACF in their colon. Epidemiological evidence suggests that density of ACF in patients with colon cancer is related to environmental factors as it happens for adenomas and carcinomas. ACF have a hyperproliferative epithelium. In particular an extension of the proliferative compartment to the upper portion of aberrant crypts is evident in dysplastic ACF. The premalignant potential of ACF is further supported by the finding of several genetic and epigenetic alterations (►epigenetics), similar to those reported in adenomas and sometimes in hyperplastic polyps, including DNA MSI and DNA hypermethylation.

Inflammatory Bowel Diseases

Patients with ulcerative colitis or Crohn's disease may have an increased risk of developing colorectal cancer, depending on the duration of the disease and the extent of the involved mucosa. The risk is related to dysplasia which may be documented in the colorectal mucosa of these patients as flat areas or dysplasia-associated lesions or masses which resembles an adenoma. Dysplasia is the consequence of chronic inflammation typical of these diseases. The lesions are at high-risk of becoming malignant and their presence requires close follow-up and should guide surgical treatment.

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Combination Therapy

Definition

An approach to the treatment of cancer and other diseases in which several different drugs, each with different mechanisms of action, are simultaneously administered to the patient so as to improve the probability of clinical success.

►Drug Design

Combinatorial Chemistry

Definition

The synthesis of a large number of new chemical compounds by combining various sets of compound "building blocks."

►Small Molecule Screens

Combinatorial Libraries

Definition

A collection of candidate drugs which has been systematically expanded by incorporating a range of substituents at various sites on a molecular scaffold, then reiteratively expanded by further modifications of those substituents. Combinatorial libraries are typically created by automated synthetic schemes and analyzed by robotic high-throughput screening assays.

►Drug Design

Combinatorial Selection Methods

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C

Synonyms

CASTing; *In vitro* genetics; REPSA; SAAB; SELEX; TDA

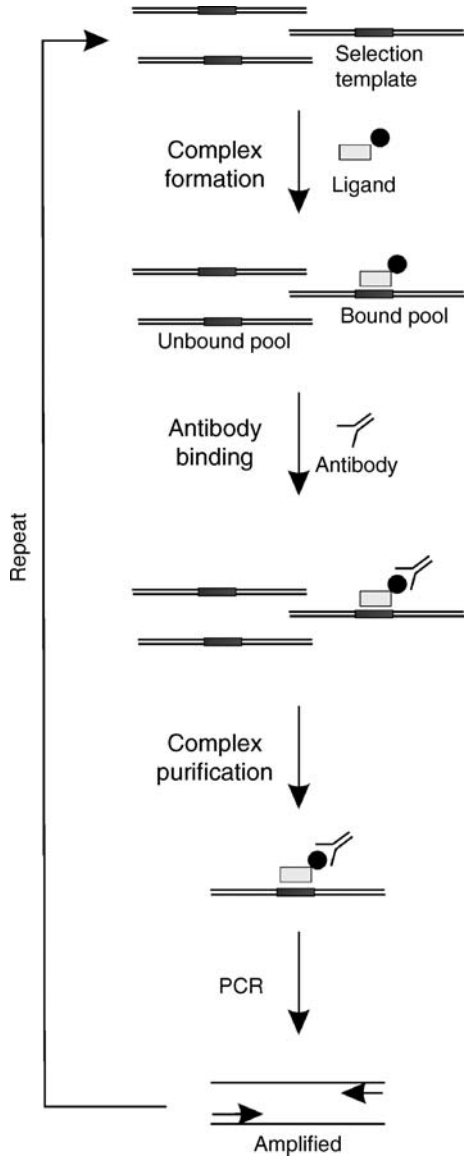
Definition

Combinatorial selection methods refer to a series of reiterative approaches involving large pools of randomized oligonucleotides, a selection process, and PCR amplification for identifying preferred ligand-binding sites on nucleic acid receptors.

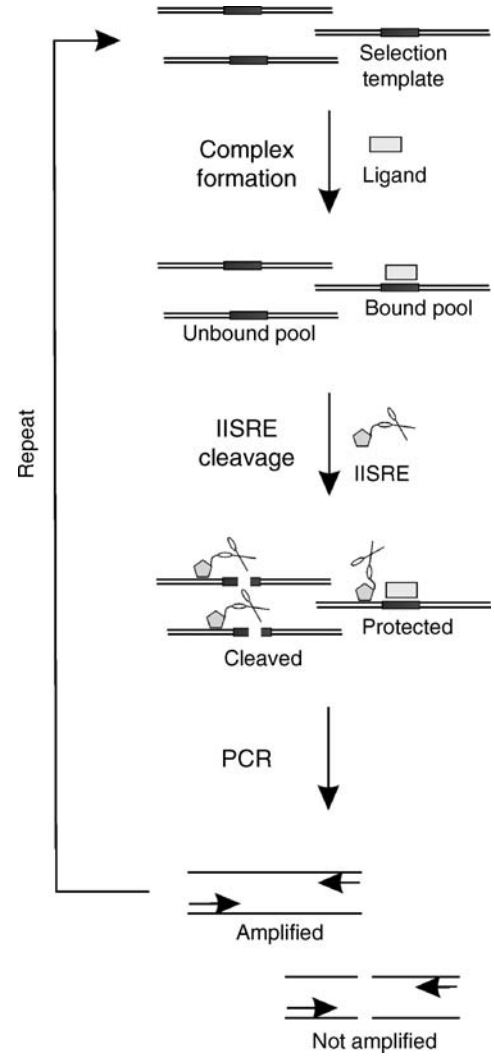
Characteristics

Combinatorial selection methods are reiterative *in vitro* methods used to find the preferred nucleic acid–binding sequences of many ligand types. Examples of combinatorial selection methods include cyclic amplification and selection of targets (►CASTing), ►*in vitro* genetics, restriction endonuclease protection, selection, and amplification (►REPSA), and systematic evolution of ligands by exponential enrichment (►SELEX). Typically these combinatorial methods involve large populations of nucleic acids containing a region of randomized sequence, a selection process, a means of amplifying the selected subpopulation, and the ability to cyclically repeat selection and amplification steps to obtain sequences that bind with high affinity to the selecting ligand. Figures 1–3 show the basics of these combinatorial selection methods. Note: ►phage display, which uses randomized sequences within a bacteriophage genome to allow the expression of a variety of viral coat fusion proteins for use in the selection of peptides that interact with particular ligands, may also be formally considered a combinatorial selection method.

Ligands investigated by combinatorial selection methods include proteins, peptides, nucleic acids, and various small molecules (molecular mass <1000 Da). Receptors are usually DNA or RNA oligonucleotides. Selections are typically performed *in vitro* and require the physical separation of ligand-bound from unbound nucleic acids. Under optimal selection conditions, the selected subpopulation constitutes only a tiny fraction of the input nucleic acid. Thus, amplification of the selected nucleic acid, typically by a polymerase chain reaction (PCR) method, is necessary to acquire workable quantities of material. Amplifications can

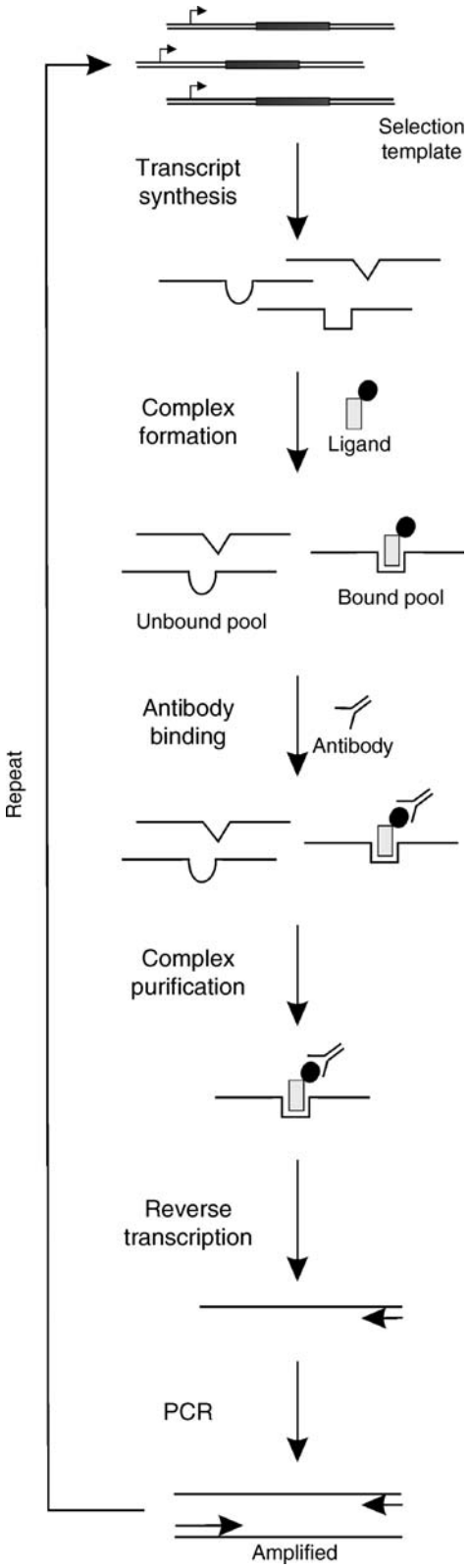


Combinatorial Selection Methods. Figure 1 Steps within a cycle of CASTing. (1) Primary complex formation between ligand and selection template mixture, (2) Secondary complex formation following antibody binding, (3) Secondary complex purification by immunoprecipitation, and (4) PCR amplification of selected DNAs. Steps 1–4 are repeated until a population of selection templates with desired properties is isolated. Open and filled regions on selection templates refer to defined and randomized sequences, respectively.



Combinatorial Selection Methods. Figure 2 Steps within a cycle of REPSA. (1) Primary complex formation between ligand and selection template mixture, (2) IISRE binding and selection template cleavage, and (3) PCR amplification of selected DNAs. Steps 1–3 are repeated until a population of cleavage-resistant selection templates emerges. Open and filled regions on selection templates refer to defined and randomized sequences, respectively.

be performed under highly stringent conditions, to maintain nucleic acid sequence integrity. Alternatively, amplifications may be performed under less stringent conditions (e.g. through the use of a low-fidelity reverse transcriptase), thereby allowing the introduction of mutations that could provide even higher affinity in subsequent selection rounds. Finally, because of limitations in the selection process, often a single round of selection does not yield the highest possible affinity species. Thus, multiple cycles of selection and amplification are often used to obtain the desired results. Progress toward isolating the highest affinity species can be determined after each cycle. Alternatively, many investigators proceed *sola fide*, with the hope that after a certain numbers of cycles, useful material will be obtained.



Combinatorial Selection Methods. Figure 3 Steps within a cycle of SELEX. (1) Preparation of RNA transcript mixture from template DNA, (2) Primary complex formation between ligand and selection transcript mixture, (3) Secondary complex formation following antibody binding, (4) Secondary complex purification by immunoprecipitation, (5) Reverse transcription of selected RNAs, and (6) PCR amplification of selected cDNAs. Steps 1–6 are repeated until a population of selection templates with desired properties is isolated. Open and filled regions on selection templates refer to defined and randomized sequences, respectively. Raised, rightward pointing arrows on SELEX selection templates indicate transcription start sites.

Combinatorial selection methods have been used to identify consensus DNA-binding sequences for specific transcription factors, isolate RNA ▶aptamers for high-throughput proteomic applications, and identify preferred DNA-binding sequences for antineoplastic agents. They have become standard tools in biochemical and molecular biology research, as well as in the development of new drugs and diagnostics, especially in the cancer field. Descriptions of some of the more commonly used combinatorial selection methods and their applications in cancer research and treatment are provided below.

CASTing

CASTing is an archetype for several related combinatorial selection methods, including selected and amplification binding (▶SAAB) and target detection assay (▶TDA). The steps involved in CASTing are shown in Fig. 1. Typically, these methods use randomized double-stranded DNA oligonucleotides as the receptor or ▶selection template and proteins as ligands, although other ligands including small molecules and nucleic acids have also been used successfully. Selection templates include a central element (often 6–20 bp in length) containing a degenerate or randomized sequence flanked by two defined sequences of suitable length and base composition to allow for efficient amplification by PCR. Binding reactions are performed *in vitro* between a ligand and a population of selection templates sufficient for a good representation of all possible sequences. Ligand-bound oligonucleotides are then physically separated from unbound oligonucleotides by various methods, either through capitalizing on the different physical properties of ligand-DNA complexes compared with free oligonucleotides and standard biochemical techniques (e.g. reduced electrophoretic mobility and electrophoretic mobility shift assays (EMSA), increased hydrophobicity and nitrocellulose filter binding) or through affinity methods (e.g. immunoprecipitation, affinity chromatography). Of course, application of these separation methods requires *a priori* knowledge of the ligand and its DNA complex and/or

physical modification of the ligand (e.g. epitope-tagged proteins, biotinylated small molecules). Amplification of the selected oligonucleotides is achieved through direct PCR. Cycles of binding, separation, and amplification are often repeated four to eight times, depending on the length of the randomized region within the selection template and the efficiency of the selection process. The resulting selected oligonucleotides can then be individually subcloned and sequenced or the entire pool directly sequenced, and the resulting information can be used to derive a consensus.

Combinatorial selection methods such as CASTing have been used primarily to determine the specific DNA-binding sites of proteins such as eukaryotic transcription factors. Many of these proteins play important roles in cancer as ►oncogenes or as ►tumor suppressors (e.g. ►c-myc, ►p53).

REPSA

REPSA is a combinatorial selection method similar to CASTing in that it identifies preferred ligand-binding sites on double-stranded DNAs. The steps involved in REPSA are shown in Fig. 2. As in CASTing, a REPSA selection template also contains an element of randomized sequence flanked by two defined sequences. However, in REPSA selection templates, the defined flanking sequences also contain type IIS restriction endonuclease (►IISRE) binding sites oriented so that the IISREs cleave sites within the randomized cassette. Type IISREs differ from conventional type II restriction endonucleases in that they do not cleave DNA directly at their binding site but rather at a fixed distance from their binding site. They also cleave duplex DNA without regard to sequence specificity; thus, they are powerful probes of ligand binding in the randomized cassette. After binding reactions are performed, the mixture of ligand, selection templates, and their complexes is subjected to cleavage by an IISRE. Unbound selection templates are preferentially cleaved, rendering them incapable of serving as templates in subsequent PCR amplifications. Intact templates are amplified, however, and serve as the input of reiterative rounds of selection, cleavage, and amplification. Subcloning, sequencing, and analysis are identical to those in CASTing.

Because REPSA does not require physical separation between ligand-bound and unbound selection templates, it is more versatile than many other combinatorial selection methods. Almost any ligand that can inhibit IISRE cleavage is suitable for use with REPSA. Ligands including proteins, nucleic acids, and both noncovalent- and covalent-binding small molecules have had their preferred duplex DNA-binding sequences successfully determined by REPSA. REPSA can also be used with mixtures of ligands, with unknown and uncharacterized ligands, and with native, unmodified ligands. Given the limitations of alternative methods, REPSA has been proven most effective in determining small molecule binding specificity, especially for those modular DNA-binding small molecules used to target specific genes (e.g. hairpin polyamides). In addition, REPSA has been proven highly effective in determining the binding specificity of several ►small molecule drugs, including ►alkylating agents and ►topoisomerase poisons (e.g. ►adriamycin and ►irinotecan). REPSA can also be used to identify the preferred sites of antineoplastic agent (e.g. ►cisplatin and other ►platinum drugs) and ►carcinogen-macromolecular ►adducts to DNA. It is envisioned that REPSA should be useful in the development of new anticancer drugs that target specific DNA sequences and genes and in better understanding chemical carcinogenesis.

SELEX

SELEX differs from CASTing and REPSA in that it primarily uses single-stranded RNA and DNA oligonucleotides as the receptor for various ligands. These single-stranded oligonucleotides adopt sequence-dependent three-dimensional structures in solution on the basis of base pairing (both through Watson-Crick and noncanonical hydrogen bonding schemes), base stacking, and other interactions. These structures then have the potential to interact with a variety of ligands, including proteins and small molecules. Thus, SELEX affords the possibility of identifying potential receptors within a larger conformational space than usually explored by other combinatorial approaches.

The steps involved in SELEX are shown in Fig. 3. The nucleic acids used in SELEX binding reactions are usually derivatives of double-stranded DNA templates (e.g. single-stranded RNA transcripts). Thus, whereas the flanking regions of the selecting transcripts need to be of sufficient length to allow PCR amplification, the complete templates are often longer to provide information for transcript generation (e.g. a bacteriophage promoter region). In addition, since the single-stranded nucleic acids need to be of sufficient length to adopt a stable three-dimensional structure, the randomized region of SELEX templates tends to be considerably longer (20–100 nucleotides) than that of other combinatorial selection methods. An initial step in SELEX involves the production of selection transcripts from the parent template. The binding and separation steps are comparable to those in CASTing. After selection, RNA transcripts need to be converted into complementary DNA strands by reverse transcription before amplification by PCR. As with other combinatorial selection methods, cycles of transcription, binding, separation, reverse transcription, and amplification can be repeated until desired results are obtained. Note that the long length of some SELEX

randomized regions makes it highly unlikely that all possible sequences are well represented in the initial selection. Thus, either the reverse transcription or PCR amplification steps are performed with enzymes having relatively low fidelity. This allows the introduction of mutations into the selection templates, which provides the opportunity to identify even higher-affinity oligonucleotides than were present in the initial selection. Subcloning, sequencing, and analysis are comparable to those in other combinatorial selection methods.

SELEX, being a combinatorial method that uses single-stranded nucleic acids, has the unique ability to present a large variety of different conformational shapes for selection, rather than just different duplex DNA sequences. This allows SELEX to identify linear nucleic acid sequences that adopt structures capable of interacting with a variety of ligands, including those not normally believed to interact with natural nucleic acids. Oligonucleotides containing these SELEX-selected sequences are known as aptamers, and aptamers have been identified that bind to a variety of proteins and small molecule ligands with specificities and affinities rivaling those of antibodies. Thus, a considerable number of uses for aptamers have been found in microarray-based proteomic analyses, especially in medical diagnostics. In addition, although unmodified nucleic acids have a relatively short half-life *in vivo*, chemically modified oligonucleotides can persist for several days. Thus, modified aptamers (e.g. ►aptamer bioconjugates) are being developed as targeted therapeutic agents for both acute and chronic diseases, including cancer.

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Combined Modality Treatment

►Chemoradiotherapy

Comet Assay

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C

Synonyms

Single cell gel electrophoresis assay (SCGE); Single cell microgel electrophoresis assay

Definition

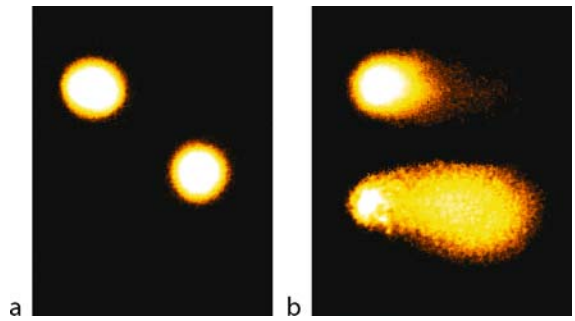
The comet assay is a sensitive electrophoresis technique for studying ►DNA damage and ►repair of DNA in individual cells. It has become one of the standard methods for testing of ►genotoxic stress and it is also frequently utilized in environmental and human ►biomonitoring, molecular ►cancer epidemiology as well as in fundamental cancer research.

Characteristics

The assay can be applied to all cell types including human, animal or plant cells, whether in culture or isolated from organs or tissues. In general, single cell suspensions are prepared, and cells are subsequently embedded in a thin agarose gel on microscope slides. Consecutive cell lysis with detergent and high salt removes cellular and nuclear membranes and proteins, and liberates DNA in the form of a compact, nucleus-like structure which is also called nucleoid. The gel-embedded DNA is then subjected to electrophoresis. DNA migrates toward the anode in a way that is dependent on its size. Thereby, DNA migration corresponds to the number of DNA lesions, i.e. increased electrophoretic migration is correlated with an increased amount of DNA damage present in the cell. DNA is stained by a fluorescent DNA-binding dye and so visualized by fluorescence microscopy as to show DNA migration. Several imaging software programs are commercially available to analyze the microscopic pictures and to quantify DNA migration (Fig. 1).

Modifications

Different modifications of the assay have been developed. The most common version applies alkaline electrophoretic conditions in concert with an alkaline pre-treatment of DNA. This leads to the conversion of so-called alkaline labile sites to strand breaks, and increases the spectrum of DNA lesions that can be detected. Before electrophoresis, additional incubation of DNA with damage-specific DNA repair enzymes,



Comet Assay. Figure 1 Comet assay micrographs of (a) undamaged cells, and (b) cells with DNA damage; DNA stained with SYBR® Green; observation at 250× magnification using a fluorescence microscope; the term comet assay refers to the comet-like structure present in damaged cells after lysis and gel electrophoresis.

such as DNA glycosylases or endonucleases, can enhance both sensitivity and specificity of the assay. These enzymes recognize DNA lesions with high specificity and convert them to strand breaks which increase DNA migration. Examples are endonuclease III to detect oxidized pyrimidines, formamidopyrimidine DNA glycosylase to detect 8-oxoguanine and other altered purines, and T4 endonuclease V to detect ►UV radiation-induced pyrimidine dimers. Neutral electrophoresis conditions facilitate the detection of DNA double strand breaks.

Areas of Application

Genotoxicity Testing

International guidelines have been published for the application of the comet assay in genotoxicity testing and biomonitoring. The assay is widely used in this research area due to several advantages such as (i) sensitivity to detect low levels of DNA damage, (ii) requirement for relatively small amounts of cells and test substances, and (iii) possibility to perform high-throughput analyses implying automated imaging. The technique is able to detect a broad spectrum of DNA damaging agents including both ionizing and UV radiation, ►alkylating agents, chemicals that form free radicals or ►adducts to DNA, and various metal compounds. Modified versions of the assay have been developed to measure the genotoxic effects of DNA–DNA or DNA–protein cross-linking agents such as ►cisplatin. Agents that directly induce DNA strand breaks are readily detectable. Other DNA lesions such as bulky DNA adducts, e.g. formed by ►polycyclic aromatic hydrocarbons do not increase DNA migration by itself. Here, DNA breaks occur only as intermediates during their repair process when these adducts are eliminated from cellular DNA by ►nucleotide excision repair. These intermediate breaks are normally short-lived, especially in dividing cells. They are however effectively detectable by the comet assay when the breaks are accumulated by delaying DNA synthesis with specific inhibitors.

DNA Repair

The comet assay is increasingly used to measure DNA repair. Here, the removal of afore induced DNA lesions is monitored over time, and both clearance of alkaline labile sites and repair-mediated rejoining of strand breaks can be observed as a decrease in DNA migration. The repair activity of intact cells as well as of cell extracts has been successfully analyzed. The repair of specific DNA lesions can be followed (i) by utilizing agents that induce a well characterized type of DNA damage, and (ii) by pre-treatment of the gel-embedded nucleoids with lesion-specific enzymes. An important and prominent example is the detection of 8-oxoguanine, a mutagenic base byproduct which occurs as a result of exposure to reactive oxygen. Studying repair of oxidized bases by pre-teatment of nucleoids with the enzyme Ogg1 (8-oxoguanine DNA N-glycosylase 1) or its bacterial counterpart Fpg (formamidopyrimidine DNA glycosylase) has revealed considerable variation among subjects. Furthermore, the application of the comet assay in human intervention trials showed that the level of oxidative DNA damage can be modulated, e.g. the supplementation of diet with antioxidant-rich fruit increased the antioxidant status of lymphocytes and enhanced DNA repair activity. The comet assay as described provides evidence of DNA damage and repair in the whole genome of the analyzed cell. These measurements can also be focused on specific genomic regions when the analysis is combined with fluorescence in situ hybridization (FISH).

Biomonitoring

The induction of DNA lesions is considered to be a crucial event in ►carcinogenesis and, in the case of absent or imperfect DNA repair, might lead to mutations, a key driving force in cancer development. The comet assay is applied in human biomonitoring studies, e.g. to detect genotoxic environmental or occupational exposures in human white blood cells, and it can be used as a tool to characterize hazards in risk assessment studies. The potential of the assay to detect DNA lesions in peripheral blood lymphocytes has also been exploited in cancer patients receiving antineoplastic chemo- or radiotherapy. It is however important to note that the level of DNA damage that is detected in these studies was shown to be – at least in some studies – influenced by age, gender and a variety of additional environmental or lifestyle factors such as

exposure of subjects to air pollution, sunlight, dietary components, smoking or excessive physical exercise.

Cancer Susceptibility

In addition to its use as a ►biomarker of exposure to genotoxic carcinogens, it can also serve as a biomarker for cancer susceptibility. By analyzing human cell samples, e.g. peripheral blood lymphocytes, it allows estimation of inter-individual differences in response to genotoxic carcinogens and facilitates the identification of susceptible subjects. When applied in molecular epidemiological studies, individual differences both in the extent of induced DNA damage (mutagen sensitivity) and in the ability to repair DNA lesions (DNA repair capacity) can be monitored. The assay was successfully utilized in such studies, e.g. to demonstrate that cells from lung cancer patients showed significantly increased mutagen sensitivity and reduced DNA repair capacity as compared to cells from control subjects. These data, and further results from studies using comparable assays, emphasize the importance of mutagen sensitivity and DNA repair capacity as host factors which are strongly associated with the risk of developing cancer and other diseases. Furthermore, family studies and studies in monozygotic and dizygotic twins provide strong and direct evidence that mutagen sensitivity is highly heritable. Overall, epidemiological studies revealed a positive and consistent association between these at risk phenotypes and cancer occurrence with an increased risk ranging from 2 to 10. Application of the comet assay, e.g. in prospective cohort studies and multi-laboratory trials will further contribute to its validation and, if successful, will offer new possibilities to improve cancer screening programs, to prevent tumor initiation, and to intervene in tumor progression in a patient-tailored manner.

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Committed

Definition

Used to describe cells whose fate is already determined along a particular path of differentiation – at least within the bounds of the experimental assay.

Common Bile Duct

Definition

The duct formed by the junction of the gallbladder duct (cystic duct) and the common hepatic duct from the liver which carries bile to the gut (duodenum).

►Hepatic Epithelioid Hemangioendothelioma

Common Fragile Sites

Definition

Specific chromosomal loci that are especially prone to forming gaps and break as seen on metaphase chromosomes under conditions of replication stress. Found in all individuals, common fragile sites are considered a normal component of human chromosomes.

►Fragile Site

Common Melanocytic Nevi

Definition

Moles composed of ►nevus cells (melanocytes) grouped in collections within the first and/or second layers of the skin. The tendency to develop nevi is influenced by familial factors and sun exposure. Most nevi have a very low malignant potential.

►Protease Activated Recept Family
►Melanocytic Tumors

Comorbidity

Definition

Comorbidity describes any distinct additional clinical entity that has existed or may occur during the clinical course of a patient with a primary (index) disease. There is currently no consensus on how to quantify comorbidities but several scales and indices are available.

►Acute Myeloid Leukemia

Comparative Genomic Hybridization (CGH)

Definition

Is a molecular-cytogenetic method for the analysis of copy number changes (gains /losses) in the DNA content of tumor cells.

►Array (CGH)

Comparative Genomics

Definition

The analysis and comparison of genomes from different species.

Comparative Oncology

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Definition

Comparative oncology is the discipline that includes spontaneous, naturally occurring cancers seen in companion (pet) animals into studies of cancer biology and therapy.

Characteristics

An under-utilized group of animal models in the study of cancer biology and therapy include companion animals, primarily dogs, which naturally develop cancer. In the United States, there are ~60–70 million pet dogs. Based on crude incidence rates, it is estimated that over one million new cases of cancer are diagnosed in pet dogs each year. Due to increasing emphasis on the human-animal bond, the pet owning public is motivated to seek out new and effective treatment options for their pet animals with cancer. This population provides a platform to study cancer biology and therapy in a natural system that can serve as an intermediary step between ►mouse models and human patients. Naturally occurring cancers in pet dogs and humans share many features, including histological appearance, tumor genetics, biological behavior and response to conventional therapies. Tumor initiation and progression are influenced by the same factors for both human and canine cancer, including age, nutrition, sex, reproductive status, and environmental exposures. Several genetic alterations and molecular signaling pathways known to be important in human cancers have been defined and shown to be relevant in cancers of pet dogs.

Some malignant histologies of comparative interest include:

- Canine ►Osteosarcoma
- Canine NH Lymphoma
- Canine Prostate Carcinoma
- Canine Mammary Carcinoma
- Canine Melanoma
- Canine Lung Carcinoma
- Canine Head and Neck Carcinoma
- Canine Soft tissue Sarcoma
- Canine Bladder Carcinoma
- Canine Renal Cystadenocarcinoma

History

The value of naturally occurring cancers seen in companion animals, as models of human cancer has been recognized for over 30 years. Early studies in the field of bone marrow transplantation utilized dogs with non-Hodgkin's lymphoma (►Malignant Lymphoma, Hallmarks and Concepts) to define optimal preparatory regimens for bone marrow transplant. Since then, the activity and optimal use of a wide variety of anticancer agents have benefited from information derived from studies in these large animal naturally occurring cancer models. These have included:

- Limb sparing techniques for osteosarcoma
- Cytotoxic chemotherapy
- Inhalation therapy for pulmonary malignancies
- Immunotherapy

- Peptide vaccine (►Peptide Vaccines for Cancer)
- DNA vaccine
- Cell based immunotherapy

- ►Anti-angiogenic therapy
- Isolated perfusion techniques
- Small molecule inhibitors (►Small Molecule Drugs)

Scientific Advancements

A recognized and long standing weakness of comparative models was a limited opportunity to investigate the biological basis of an anticancer agent's activity or lack of activity. However with the public release of a high-quality draft sequence covering 99% of the canine genome (2.5 billion base pairs) it is now possible to apply many of the same methodologies used to interrogate human cancer, in the dog. The genome sequencing suggests that all ~19,000 genes in the dog have a similar gene in the human genome. The dog and human lineages are more similar than the rodent lineage both in terms of nucleotide divergence and rearrangements. Also the single nucleotide polymorphism (SNP) frequency in dogs is similar to SNP frequency in the human population, even with the diversity of breed phenotypes. Thus, the genomes of dog and human are similar enough to suggest that genomic information learnt about one species can be easily transferred to and be applicable for the other.

Coupled with this more available genomic data, reductions in the cost of generating biological reagents have contributed to the development of novel investigative platforms for the study of canine tissues. Evidence of this includes the availability of commercially available canine oligonucleotide microarray, optimized conditions for proteomic studies, validated canine specific antibodies and characterization of human antibodies that cross react with canine epitopes. Collectively, the opportunity now exists to conduct detailed and biologically intensive studies in dogs that have cancer, that can evaluate cancer associated target genes/proteins and pathways important in cancer biology and therapy.

Need for New Models of Drug Development

Cancer drug development (►Drug Design) is costly, linear, and inefficient. Costs associated with development incrementally rise as the path proceeds. The two most common causes of drug failure are toxicity or lack of efficacy. These failures are most costly once a drug enters phase I human trials but are even more costly if they occur after phase II trials, and beyond (►Clinical Trial). It is therefore essential that "go-no-go" decisions focus on the issue of toxicity and efficacy as early in the development path as possible. An information gap has historically existed between preclinical studies and phase I human trials; however, with the development of novel non-cytotoxic anti-cancer agents, this gap is now equally evident later in the drug development path. After successful completion of phase I human clinical trials, the design of phase II trials often has to take place without sufficient information including biological dose, schedule, and regimen for many of these novel agents. For both cytotoxic and, more importantly novel non-cytotoxic agents, additional model systems are needed. The "model" advantages of companion animal cancers provide an opportunity to integrate studies that include companion animals into the development paths of new cancer drugs. The outcome will include earlier assessment of agent activity and toxicity and the validation of biological endpoints and surrogate markers critical to the design of more informed phase I and phase II human clinical trials.

Comparative oncology models are well-suited for integration into preclinical cancer drug development efforts for several reasons. By their nature, companion animal cancers are characterized by inter-patient and intra-tumoral heterogeneity, the development of recurrent or resistant disease, and metastasis to relevant distant sites. In these ways, companion animal cancers capture the "essence" of the problem of cancer in ways not seen in other animal model systems. The lack of gold standard treatments for canine cancer patients allow for the early and humane testing of novel therapies. The shortened life span of companion animal patients and their early metastatic failure allow rapid completion of clinical trials of novel agents. A further rationale for the use of these models in non-clinical efficacy studies is the immune competence of the host, relevant and species-concordant tumor-microenvironment interactions, spontaneous development of tumors, and more importantly spontaneous development of resistance patterns to standard therapies within an individual animal. Additional attributes of comparative oncology include the opportunity to gather serial biopsies from target and non-target lesions and repeated body fluid collection (serum, whole blood, urine) from the same animal during exposure to an investigational agent. This serial sampling allows for the identification of tumoral and surrogate pharmacodynamics endpoints that can be uniquely correlated to response in ways that are often deemed unacceptable in human trails (►Preclinical Testing).

Clinical Applications

Efforts to perform co-ordinated, multi-center preclinical cancer trails in companion (pet) dogs exist in a number of different formats allowing for the rapid evaluation of cancer drugs in biologically intensive trials. Hallmarks of comparative oncology trials include good clinical practice (GCP) trial conduct, computer-based data management and reporting and multiple study endpoints. The ability to capture data contemporaneously in preclinical dog trials allows for the reporting of

toxicities, if they are to occur, in a rapid and systemic fashion.

Mechanisms and procedures for integration of data from preclinical trials in tumor bearing dogs into the regulatory pathway are currently being defined. However, the use of clinical trials using pet dogs will not only provide information important to the initiation of early human clinical trials (Phase I) but also will inform the appropriate design of later development trials (Phase II/Phase III). It is expected, through the integration of comparative oncology modeling, that the cancer drug development path will become more informed, efficient, and less costly.

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Complement

Definition

The complement system is part of the immune system's defense mechanism against microbes and foreign cells. When initiated, the complement cascade activates a series of complement components, which interact to form a membrane attack complex. The formation of this complex at the surface of the foreign cell/microbe leads to its lysis and killing

►Cystatins

Complement Cascade

Definition

A precise sequence of events usually triggered by an antigen-antibody complex, in which each component of the complement system is activated in turn.

Complement-Dependent Cytotoxicity

Definition

(CDC) refers to the lysis of a target cell in the presence of ►complement system proteins. The complement activation pathway is initiated by the binding and fixation of the first component of the complement system (CIq) to the fragment crystalline (Fc) region of a (therapeutic) antibody complexed with a cognate antigen. The end result is a membrane attack complex that generates a hole in the cell membrane, ultimately causing cell lysis and death.

►Immunotherapy
►Diabody
►Interferon-α

Complement-Mediated Cell Death

Definition

A process used by the immune system whereby members of the complement factor family (comprised of at least 20 distinct proteins) recognize antibody-coated cells and facilitate cell lysis resulting in cell death.

►Osteopontin
►Immunoprevention of Cancer

Complementarity Determining Region

Definition

CDR; Is a short amino acid sequence found in the variable domains of immunoglobulin and T cell receptor proteins that complements an antigen and therefore provides the receptor with its specificity for that particular antigen.

►Anti-HER2/Neu Peptide Mimetic (AHNP)
►Hypervariable Region

Complementation Groups

Definition

Complementation groups are subgroups of DNA repair-deficiency syndromes, each representing a separate (defective) DNA repair gene; xeroderma pigmentosum.

►Xeroderma Pigmentosum
►Fanconi Anemia

Complete Cytoreduction

Definition

Visceral resections and peritonectomy procedures whose goal is to remove malignancy to the extent that subsequent intraperitoneal chemotherapy will result in a complete eradication of the abdominal and pelvic disease.

►Appendiceal Epithelial Neoplasms

Complete Hematologic Remissions

Definition

Achievement of a normal white blood cell (WBC) and platelet count, normal differential, and no signs and symptoms of leukemia cells.

►Nilotinib

Complex Karyotypic Changes

Definition

more than three numerical and/or structural aberrations in a clone.

Compound Screen

►Small Molecule Screens

Compounds from Organisms

►Natural Products

Computed Tomography

Definition

Computed tomography (CT), also known as computerized tomography or computed axial tomography (CAT), is medical technology that uses special X-ray equipment to obtain image data from different angles around the body, and computer processing of the information to produce three-dimensional images. While conventional X-rays provide flat two-dimensional images, CT images show a detailed views of cross-sections of body tissues and organs.

►Radiation Oncology

Concatamerization

Definition

Formation of large molecules consisting of tandem copies of a nucleic acid.

Concomitant

Definition

Given at the same time, e.g. radiotherapy combined with chemotherapy.

►Ionizing Radiation Therapy

Conditionally Replicating Adenovirus

►Oncolytic Adenovirus

Conditionally Replicative Adenovirus

►Oncolytic Adenovirus

Conduit

Definition

A channel, pipe or pore that allows liquids or substances to pass.

►Connexins

Confidence Interval

Definition

CI defines the uncertainty of the risk estimate and is confounded by sample size. The probability that the unknown true risk lies within a 95% confidence interval (before drawing the sample) is 95%. After drawing the sample and calculating the risk estimate, there is considerable assurance that the confidence interval covers the true estimate.

►Radon
►Cancer Epidemiology

Confocal Laser-Scanning Microscopy In Vivo

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Definition

Confocal laser-scanning microscopy (CLSM) in vivo represents a novel imaging tool that allows the noninvasive examination of skin cancer morphology in real time at a resolution for viewing microanatomic structures and individual cells.

Characteristics

In recent decades, enormous strides have been made in noninvasive imaging of cancer tissues with the development and refinement of computerized axial tomography, magnetic resonance imaging, and positron emission tomography, to name a few. Progress in noninvasive skin cancer imaging, however, has been slower than in other specialities due in part to the ease with which skin is visually examined and biopsied. Early detection of malignant skin tumors is essential, and still one of the most challenging problems in clinical oncology. Although surgical excision in early stages of tumor development is almost always curative, delayed recognition of skin malignancies puts the patient at risk for destructive growth and death from disease once the tumor has progressed to competence for metastasis. The early diagnosis of malignant skin tumors by naked-eye examination, however, is still rather poor. Technological advancements have led to the development and investigation of imaging tools to provide information to the clinician that can improve the diagnostic performance for early diagnosis and assist in the management of cutaneous malignancies. Among novel noninvasive imaging techniques, CLSM stands out because of its high resolution. CLSM provides for the first time in vivo imaging of individual cancer cells and offers windows on living tissue. Investigations of CLSM for in vivo examination of human skin was first published in 1995, 2 years later the first commercially available in vivo confocal reflectance microscope was introduced to the research community. Since then, valuable experience has been gained from research labs and hospitals around the world. Imaging is based on the detection of back scattered light with contrast due to naturally occurring refractive index variations of tissue microstructures. A confocal digital imager consists of a point source of light that illuminates a small spot within the biological specimen. The illuminated spot is then imaged onto a detector through a pinhole aperture. This aperture acts as a spatial filter, rejecting light that is reflected from the out-of-focus portions of the object, so the resultant image has the high contrast of a thin-section image. The light source, illuminated spot, and detector have the same foci, or are placed in conjugate focal planes, and are therefore confocal to each other. The diameter of the detector aperture is matched to the illuminated spot through the intermediate optics. Because a small spot is illuminated and then detected through a small aperture, only the plane in focus within the specimen is imaged. Light originating from out-of-focus planes is prevented from entering the detector. A confocal digital imager thus allows imaging of thin

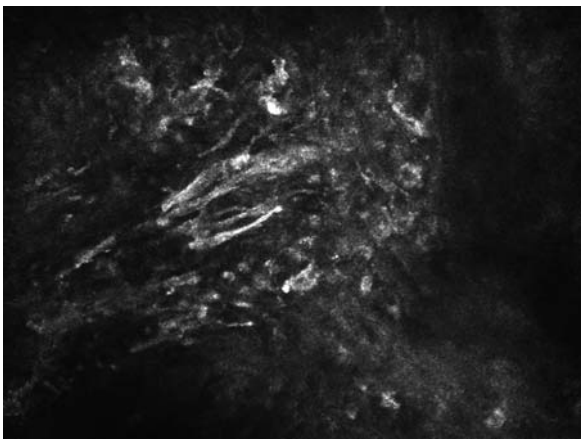
slices of tissue, or optical sectioning, with high axial resolution and contrast. The confocal digital imager illuminates and images only a small spot at a time. To view the whole specimen, the illumination spot is scanned over the desired field of view. The illumination spot is raster-scanned optomechanically to sweep the entire area. The specimen is illuminated point by point, and then the image is created in the corresponding manner. Real-time confocal imaging of human skin involves laser scanning rather than white-light tandem scanning. Laser scanning has the benefits of bright, higher contrast imaging, higher magnifications, and deeper penetration. Infrared lasers coupled to a fast scanner allow video rate imaging of skin to maximum depths of 300 μm. The thickness of the in vivo optical slice obtained is 2–5 μm. To minimize blurring, a skin-to-microscope contact device stabilizes the skin to within ±2 cells. Live images are displayed on a video monitor. With in vivo imaging, the virtual sectioning occurs in the horizontal plane, which correlates to en face sections as opposed to the vertical sections of routine histology. Contrast in the image correlates to naturally occurring variations in refractive index of organelles and microstructures within the skin. Epidermal keratin, for example, varies in refractive index depending on the state of differentiation of the keratinocyte. As the keratinocytes mature within the epidermis and the molecular weight of the keratins within an epidermal keratinocyte increases, the keratinocytes become more refractile, thus causing an increase in refractive index. As a result, the confocal images become brighter and the keratinocytes within the epidermis are well defined. The pigment melanin within the epidermis also has a high refractive index, in fact higher than keratin. Visually, melanin has a characteristic brown-black appearance because of the absorption of visible light. When illuminated with infrared light, however, the absorption is greatly reduced. This reduced absorption, combined with the intrinsic high refractive index, causes enhanced back scattering of reflected light that is collected by the confocal microscope. Higher concentrations of melanin cause an increase of back scattering to occur. Consequently, what appears as brown-black to the naked eye will appear white or bright in a confocal image.

The main advantage of CLSM is the unique opportunity to image thin sections of living tissue at a resolution equal to that of conventional microscopes used to view histology slides. Cellular and architectural details can be examined without having to excise and process the tissue as in standard histology. When the objective lens is placed onto an adapter ring, which is fixed on the tumor, real-time images can be obtained in seconds at the bedside. As a limitation in the current state of technological confocal microscopy development it has to be addressed that assessment of microanatomic structures can only be done to a depth of 300 μm, which corresponds to the papillary dermis. Thus, processes in the reticular dermis and tumor invasion depth cannot be reliable evaluated at the present state of confocal imaging technology.

Clinical Relevance

Initial research has concentrated on the most clinically relevant cutaneous malignancies. Tumors that have been imaged and characterized include ►melanocytic skin tumors and nonmelanoma skin cancer. Confocal images have been qualitatively and quantitatively correlated to corresponding horizontal histology sections. The primary goal was to define and understand skin cancer morphology as seen with a confocal microscope in vivo. These preliminary studies were helping to develop an ability to understand and interpret confocal images of skin cancer. In the present state of investigations, the focus lies on the diagnostic accuracy of the method and consequently the integration into clinically routine procedures. Of all the cancers, ►melanoma of the skin represents one of the greatest challenges in early or preventative detection. Using CLSM, distinct morphologic features can be described for the differentiation of benign common nevi and malignant melanoma (Fig. 1). For example, in general, progression from monomorphic features in benign common nevi to increasing pleomorphism and architectural disarray in dysplastic nevi and melanomas was found.

Melanocyte cytology shows round to oval, bright and monomorphic cells in benign nevi whereas melanomas tend to present polymorphic and irregularly shaped cells. Nevus cell nests can be clearly seen in benign common nevi, but are less defined in dysplastic nevi. Disarray of architecture can be found in melanoma.



Confocal Laser-Scanning Microscopy In Vivo. Figure 1 Confocal in vivo image of malignant melanoma.

Keratinocyte cell borders can be readily detected in benign common nevi, show focal absence in dysplastic nevi and are poorly defined or absent in melanoma. Dendrite-like structures with a complex branching pattern are frequently seen in melanoma, but less frequently in benign nevi, where they are smaller and more delicate. In a recently published study, excellent sensitivity and specificity achieved for the diagnosis of melanoma using confocal microscopy in vivo, based on distinct morphologic features, has been described. Of note, the independent observer received only a 30 min presentation that instructed them in the confocal morphologic features of melanocytic skin tumors. Moreover, statistical analysis showed excellent to perfect inter and intraobserver agreement for the confocal morphologic attributed studied. Nonmelanoma skin cancers are the most common malignancies among the Caucasian population. The most frequent of these are ►basal cell carcinomas and squamous cell carcinomas. Based on several studies, confocal images of both basal cell carcinomas and squamous cell carcinomas show relevant cellular and architectural features comparable to standard pathology. Moreover, a high diagnostic accuracy, prior to naked-eye and other noninvasive imaging techniques, could be achieved by the confocal microscope. Another potential use of CLSM in vivo is presurgical margin detection for skin cancer surgery. Surgical management of amelanotic melanomas as well as melanomas, basal cell carcinomas, and squamous cell carcinomas with ill-defined borders present a significant clinical challenge currently addressed by serial excisions. In these settings, CLSM provides a much improved first approximation of the lateral borders between the tumor and normal skin. The cumulative experience with CLSM by different investigators clearly holds promise for this technology in the future. The results of several studies indicate that in vivo examination of skin tumors by CLSM can provide useful diagnostic information. CLSM represents an opportunity for clinicians to add useful and reliable information in their diagnostic decisions and therefore may spare some patients a biopsy or excision procedure and save time and costs.

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Conformational Diseases

Definition

A group of heterologous disorders, that include Alzheimer, Parkinson and Creutzfeldt–Jakob diseases, which arise from the dysfunctional aggregation of proteins in non-native conformations.

►Endoplasmic Reticulum Stress

Confounding

Definition

A situation in which a measure of the effect of an exposure is distorted because of the relation between the exposure and another factor that influences the disease under study

►Coffee Consumption
►Obesity and Cancer Risk

Congenic Animal

Definition

Mice that carry a small amount of DNA from one strain of mouse and the remainder of DNA from another strain of mice.

►Mouse Models

Congenics

►Mouse Models

Congenital

Definition
A condition or disorder present at birth.

Congenital Hypertrophy of the Retinal Pigment Epithelia

Definition
►CHRPE.

Congenital Lymphedema

Definition
Is characterized by a chronic and disfiguring swelling of the extremities, and associated with heterozygous inactivating missense mutations of the gene encoding ►vascular endothelial growth factor receptor-3.

►Lymphatic Vessels

Congenital Mesoblastic Nephroma

►Mesoblastic Nephroma

Congenital Telangiectatic Erythema

►Bloom Syndrome

Conjugated Double Bonds

Definition
Are double bonds that are separated from each other by one single bond (–C=C–C=C–); conjugated systems with alternating single and double bonds result in a general delocalization of electrons across adjacent atoms allowing for resonance-stabilized structures that impart both capacity to act as biological antioxidants and the ability to absorb and give off certain wavelengths of light that cause a compound to appear colored (e.g. β-carotene is responsible for the orange color of carrots, lycopene for the red color of tomatoes).

►Carotenoids

Conjugated Linoleic Acid (CLA)

Definition
Conjugated linoleic acid (CLA) refers to a family of at least 13 geometric isomers of linoleic acid, which is found preferentially in dairy products and meat of ruminants.

►Lipid Mediators

Conjugated Linolenic Acids

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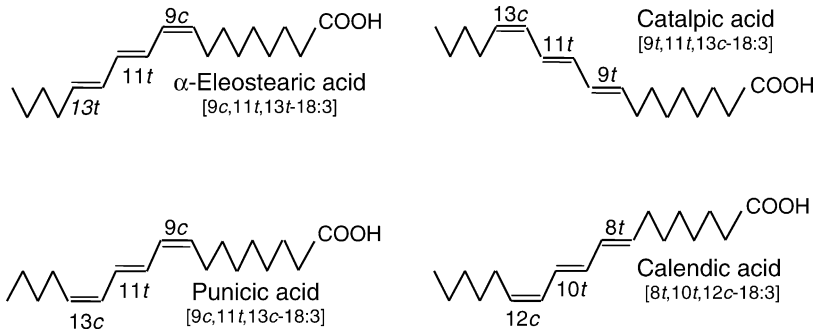
Synonyms
CLN

Definition
Conjugated linolenic acid (CLN) is a general term for the geometrical and positional isomers of octadecatrienoic (18:3) acid with three conjugated double bonds. Conjugated linolenic acids occur in several terrestrial plants (mainly seed oils). They include α-eleostearic acid (9*cis*(c),11*trans*(t),13t-18:3), catalpic acid (9t,11t,13c-18:3), punicic acid (9c,11t,13c-18:3),



Conjugated Linolenic Acids. Figure 1 Structure of conjugated linolenic acids (CLN).

calendic acid (8t,10t,12c-18:3) and jacaric acid (8c,10t,12c-18:3) (Fig. 1). High contents of calendic acid, punicic acid and α-eleostearic acid are found in seed oils of pot marigold, pomegranate and tung/bitter gourd respectively.

Characteristics

In-Vitro Studies

Conjugated linolenic acid (CLN) shows cytotoxic effect on mouse tumor cell (SV-T2). However, there is a difference in the toxicity between CLN isomers. Fatty acid from pot marigold (8t,10t,12c-18:3; 33.4%) has no effect on the cell line up to 250 μM, but other kinds of fatty acids from seed oils are cytotoxic to SV-T2 cells below 20 μM. The same effect is observed in the case of human monocytic leukemia cell (U-937). Generally, 9,11,13-CLN and all trans-CLN are more cytotoxic than 8,10,12-CLN and CLN containing cis configuration, respectively. The higher cytotoxicity of 9,11,13-CLN or all trans CLN isomers is partly due to the different susceptibilities of these CLN isomers to lipid peroxidation. On the other hand, the inhibitory effect of CLN on the growth of colon cancel cells is related to the regulation of peroxisome proliferator-activated receptor (PPAR)γ. ►PPARγ ligands such as troglitazone and 15-d-prostaglandin (PG) J_2 cause growth inhibition and induce ►apoptosis in cancer cells. CLN shows a higher ligand activity on PPARγ than troglitazone. ►Bcl-2, ►GADD45, and ►p53 are known as an important molecular target in apoptosis-inducing pathways. In Caco-2 cell treated with 9c,11t,13t-CLN, Bcl-2 expression is down-regulated, while GADD45 and p53 expressions are up-regulated. Therefore, two possible mechanisms of the anticarcinogenic activity of CLN can be hypothesized *viz.*, induction of apoptosis *via* lipid peroxidation and regulation of target gene and protein.

In-Vivo Studies

CLN from bitter gourd seed oil (BGO) significantly reduces the frequency of colonic aberrant crypt foci (►ACF) in rat as a precursor of colon carcinogenesis. In this case, the proliferating cell nuclear antigen (►PCNA)-labeling indices in ACF and normal-appearing crypts also decreases by dietary feeding of CLN. Furthermore, feeding of CLN enhances apoptotic cells in ACF without affecting the surrounding normal-appearing crypts. ►Chemopreventive ability of BGO on rat colon cancer can be found in a long-term *in vivo* assay. Dietary administration of BGO rich in CLN (9c,11t,13t-18:3) significantly inhibits the development of colonic adenocarcinoma induced by ►azoxymethane (AOM) in male F344 rats without causing any adverse effects. In addition, BGO intake significantly reduces the multiplicities of colorectal carcinoma (number of carcinomas/rats) in rats. Other CLN isomer (9c,11t,13c-18:3) from pomegranate seed oil (PGO) also shows the chemopreventive effect on rat colon cancer induced by AOM. Dietary feeding of PGO suppresses progression of adenoma to malignant neoplasm in post-initiation phase of colon cancer. Dietary feeding of BGO and PGO enhances PPARγ expression in non-lesional colonic mucosa. Synthetic ligands for PPARα and PPARγ effectively inhibit AOM-induced ACF in rats. Therefore, it may be possible that BGO and PGO suppress colon carcinogenesis by means of altering PPARγ expression in colonic mucosa.

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Conjugation

Definition

The joining together of two chemicals (by an enzyme).

►Carcinogen Metabolism

Conn Syndrome

Definition

Conn syndrome, first reported by Jerome W. Conn, is a result of an increased production of aldosterone, a hormone produced by the zona glomerulosa of the adrenal cortex. This hormone causes the retention of water and sodium and excretion of potassium. The clinical manifestations include high blood pressure, headaches, and muscle cramps. A small proportion of children with adrenocortical cancer overproduce aldosterone.

►Childhood Adrenocortical Carcinoma

Connexins

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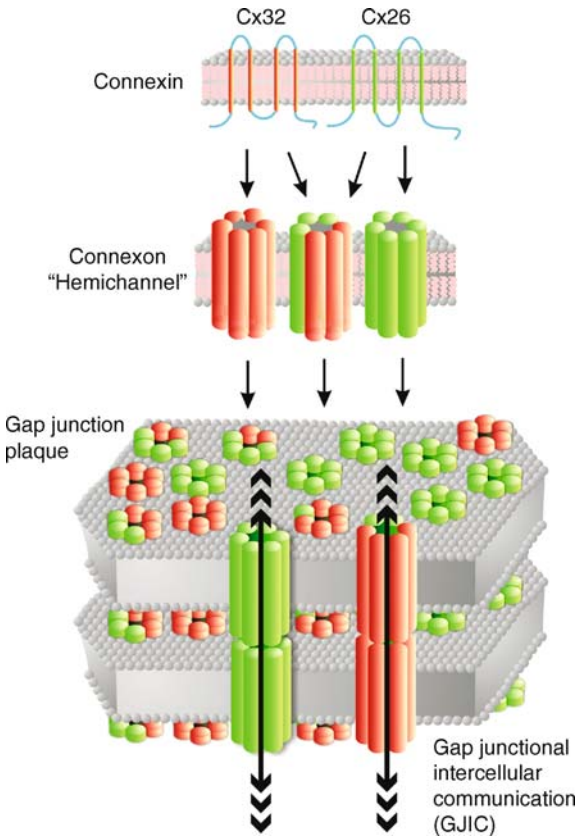
Definition

Connexins are proteins which assemble into channels that allow for small molecules to pass directly from one cell to another.

Characteristics

The family of connexin (Cx) proteins is composed of 21 members in humans. All connexins (►Cx32 and ►Cx26) share common features of assembling into ►connexons also called "hemichannels" consisting of six subunits of the same or different connexins (Fig. 1). Hemichannels from apposing cells dock and the resulting channels cluster into a junctional complex known as a ►gap junction or often referred to as a gap junction plaque. Gap junctions allow for the direct intercellular exchange of secondary messengers and other small molecules, a process termed gap junctional intercellular communication (GJIC) (Fig. 1). Gap junctions have a ubiquitous distribution in human tissues, and these specialized intercellular channels are essential for normal cell function, proper cell differentiation, tissue development, metabolic transport, ion transfer and cell growth control. Importantly, each connexin is assembled into a channel with unique



Connexins. Figure 1 Connexins (e.g. Cx32 or Cx26) are gap junction proteins that thread through the lipid bilayer of cell membranes four times. Connexins of the same type (orange or green rods) or different types (mixtures of orange and green rods) assemble into hexameric arrangements with a central pore known as connexons or "hemichannels." Connexons from opposing cells dock and tightly cluster into gap junction plaques allowing for bidirectional exchange of small molecules a process known as gap junctional intercellular communication (GJIC).

properties that are thought to reflect distinct physiological roles for the different gap junction channel types. In numerous diseases connexins are either not produced or mislocalized (e.g. many cancers) or contain mutations that inhibit the normal function of the resulting channels. Mutations of connexin genes are linked with human diseases including neurodegeneration, skeletal abnormalities, ►keratodermas, and hereditary ►sensorineural deafness. For example, mutations in the gene encoding Cx26 are the most common cause of ►congenital hearing loss.

Connexins as Tumor Suppressors

The evidence that connexins play a role in cell growth control and early tumorigenesis ranges from circumstantial to direct linkages. First, most soft tissue tumors typically have reduced gap junctions due to either decreased connexin expression or an inability to efficiently assemble connexins into gap junctions. Second, tumor promoters, ►mitogens and ►oncogenes are known to reduce GJIC. Third, re-expression of connexins in tumor cells frequently revert cancer cells to a less aggressive cell type and slows cell growth, highlighting the tumor suppressive behavior of connexins. Fourth, mice lacking one member of the connexin family (Cx32) are 10–25 times more at risk of developing chemically or radiation-induced liver or lung tumors. The evidence that mutations in the genes encoding connexins lead to increased susceptibility to cancer is sparse and thus connexins are more commonly thought of as ►Class II tumor suppressors reflecting the consequence of reduced expression or ability to make gap junctions. Collectively, convincing data suggest that connexins play a role in ►carcinogenesis particularly at disease onset, progression and early events associated with ►metastasis. The role of connexins in cancer cells that enter the blood stream and proceed to break through the endothelial wall in later stage metastasis is less clear. In fact, considerable evidence would support the position that connexin expression in later stage disease favors the ability of cancer cells to enter and propagate at new tissue sites. Consequently, a working paradigm is that connexins protect cells from becoming cancerous, act to suppress the growth of primary tumors and play an inhibitory role in cells breaking away from the primary tumor. However, in advanced disease where tumor cells were successful in escaping the primary tumor, connexin re-expression may facilitate the cancer cells exiting the blood system to enter and populate a second tissue site.

Connexins as a Therapeutic Target

The likelihood of connexins being a good target for ►combination therapy for primary tumors remains promising. Considerable evidence supports the notion that upregulation of connexins alone with only minimal increases in GJIC may be sufficient to suppress the growth and expansion of the primary tumor. However, it is likely that such a putative treatment would need to be combined with drugs designed to induce cell death. Unfortunately, there are no drugs in clinical cancer trials that specifically target the regulation of connexins. The reason for this is primarily due to the lack of a non-toxic drug that will specifically up-regulate connexins within the tumor. The need for a tumor specific drug is critical as many studies indicate that a system wide up-regulation of connexins and gap junctions in non-diseased organs would likely lead to pathological side effects. Consequently, any drug or gene therapy development would need to target the cancer cells only to avoid detrimental side effects. In addition to increasing connexin content in tumor cells, it is almost a certainty that any curative strategy would require combinational therapy where connexin up-regulation and increases in GJIC would be accompanied with a second therapeutic strategy. Evidence has suggested that gap junctions could act as ►conduits for delivery of ►pro-drugs deep into the tumor allowing for a more effective cell kill throughout the tumor. Again, such treatment strategies would necessitate good gene targeting or specific drug treatments that restrict their effects to the primary tumor. At a minimum, the increase presence of connexins and gap junctions would be expected to provide a decrease in tumor expansion while a patient is exposed to repeated treatment protocols designed to kill the tumor cells. The importance of connexins as a possible target in the treatment of metastatic disease is relatively unknown. Based on findings in animal models, additional precautions must be considered as connexins have been reported to enhance the movement of tumor cells from the blood to vital organ tissues. Additional research is also necessary to determine what role connexins play in facilitating or inhibiting the interaction of the tumor cell with the surrounding ►milieu of cells and the extracellular matrix that become the "soil" for metastatic tumor cell growth.

In summary, the role of connexins in carcinogenesis and metastatic disease may in fact be two fold. First, the bulk of the evidence would support members of the connexin family as acting as inhibitors of cancer onset, primary tumor growth and early stage events associated with metastasis. As such, this highlights connexins as a viable target for cancer prevention and treatment of primary disease. Second, a paradigm is developing where connexin expression may favor later stage metastatic properties of at least some tumor cell types. Consequently, interventions where connexins are targeted and downregulated only in tumor cells circulating in the blood may serve as an advantage

in treatment strategies of more advanced disease. Clearly more information involving better experimental models is necessary to resolve the full function of connexins in carcinogenesis and disease progression.

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Connexon

Definition

A hexameric arrangement of six connexin family members resulting in a structure with a distinct central pore.

►Connexins

Conservative Surgery

Definition

Surgery that completely removes cancer and leaves as much healthy tissue as possible.

►Adjuvant Chemoendocrine Therapy

Conserved Region

Definition

A conserved region is a region within a gene or protein that remains identical or near-identical between species and through evolution.

►*Myc* Oncogene

Constant Region

Definition

That part of an ►antibody structure that is characteristic for each antibody class.

Constitutive

Definition

Describing a state of activity that occurs at a constant level and is therefore not responsive to modulation by physiologic regulators, or a type of control that yields such a constant output.

Contact Inhibition

Definition

When grown in monolayer, cells arrest growth when they contact each other and reach confluency. Under these circumstances, cancer cells usually continue growth and pile up on top of one another.

►Cell Adhesion Molecules
►Ether à-go-go Potassium Channels

Contact Inhibition of Cell Division

Definition

The process by which cells touch, and then send biochemical signals from the membranes through the microtubules and microfilaments to the nucleus, signaling that they have touched, leading to negative signals which stop further cell growth.

►Chemically Induced Cell Transformation

Contact Normalization

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Synonyms

Heterologous growth control

Definition

The process by which nontransformed cells force tumor cells to assume a normal morphology and phenotype.

Characteristics

Transformed cells often survive medical treatment and lay dormant for many years before they emerge to cause relapse in a patient. Nontransformed cells can force tumor cells to assume a normal morphology and phenotype by a process called "Contact Normalization" (Fig. 1).

Contact Normalization is a powerful global phenomenon. Cells transformed by a variety of chemicals, viral agents, and oncogenes can be normalized by contact with nontransformed cells. This process is dramatically exemplified by malignant tumor cells that form normal adult organs when injected into mouse blastocysts.

Contact Normalization is an important process in vivo. Genetically transformed cells can assume a normal morphology and reside in many organs including skin, breast, and intestine. Moreover, since these "occult tumor" cells are phenotypically normal, they tend to resist chemotherapy. As stated above, Contact Normalization is a powerful process; transformed keratinocytes that comprise up to 4% of epidermal volume can be controlled in human skin for decades.

In at least in some cell systems, Contact Normalization requires direct contact between transformed cells and nontransformed cells. Thus, intercellular junctional proteins such as ►connexins and ►cadherins may augment the ability of nontransformed cells to normalize the growth of adjacent tumor cells. Since these junctions are often disrupted in malignant and metastatic tumor cells, they should be stabilized in tumor cells undergoing Contact Normalization in the microenvironment.

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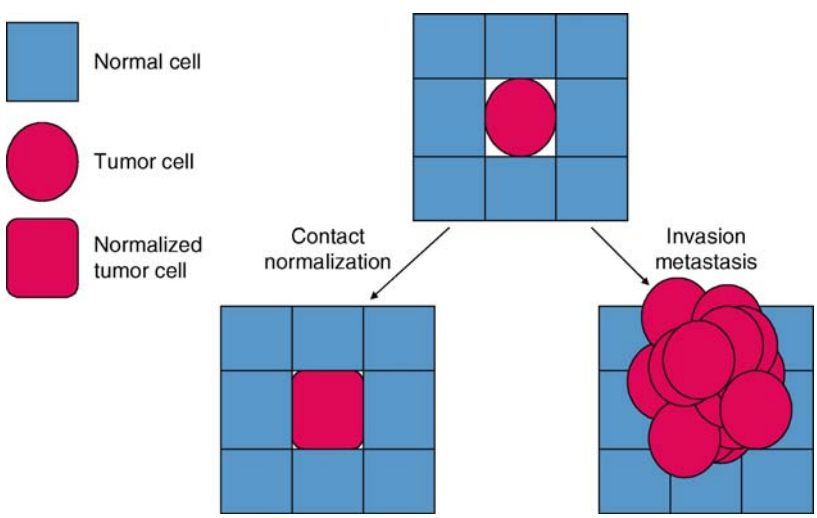

Contact Normalization. Figure 1 Contact normalization. Tumor cells are normalized by contact with nontransformed cells. Cancer arises when this process is unsuccessful. Intercellular junctions are disrupted in invasive and metastatic tumor cells, but should be stabilized during Contact Normalization.

Contralateral

Definition

Contralateral referring to the opposite side.

Contralateral Breast Cancer

Bilateral primary breast cancer; increasing breast cancer incidence rates, improved prognosis, and growing life expectancy have resulted in an increasing number of women at risk of developing bilateral primary breast cancer. In the US alone, there are ~2.2 million living diagnosed at sometime with breast cancer.

Contra-lateral breast cancers are divided in those that are synchronous, that is when breast cancers are diagnosed in both breasts simultaneously, and metachronous, normally defined as diagnosed more than 6 months from the first cancer. The age specific incidence of synchronous breast cancer mimics that of unilateral breast cancer. In contrast, the risk of being diagnosed with a metachronous bilateral cancer is higher among women diagnosed before the age of 45 compared to those older at time of diagnoses of the first breast cancer. The risk of metachronous bilateral breast cancer is elevated throughout the entire life of a woman, and is ~0.5–1% annually.

The incidence of synchronous bilateral breast cancer has shown a steady increase in the last 30 years, although somewhat leveling off the last years. This is in sharp contrast to the metachronous contra-lateral breast cancers where the incidence has decreased by ~30% over the last 30 years. This is most likely a function of the increasing use of postoperative adjuvant therapy.

A woman with a synchronous bilateral breast cancer has a higher mortality rate compared to women with unilateral cancer, this is particularly evident before the age of 50 where a synchronous cancer entails about a two time higher mortality rate. Women who develop a metachronous bilateral cancer within 5 years of the first cancer and before the age of 50 are at a four times higher risk of dying from breast cancer compared to an age-matched woman with unilateral cancer. Time since diagnoses of first cancer also influences the prognosis of the second cancer. Young women with an early metachronous cancer have a particularly bad prognosis, while women who were diagnosed more than 10 years after the first cancer has a prognosis similar of that of a unilateral breast cancer.

Contrast Agents

Definition

Compounds or small particles that are injected into circulation to enhance the ability of a medical image system to detect blood vessels or display differences in blood flow to different tissues. The formulation of a contrast agent depends on the physics of the imaging technology with which the agent will be used.

►Ultrasound Micro-Imaging

Convection Enhanced Delivery

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Synonyms

High-flow microinfusion; Interstitual microinfusion; Intracerebral microinfusion; Intracerebral clysis; CED.

Definition

Convection-enhanced delivery (CED) is a novel delivery method that allows direct drug infusion into the brain in a ►locoregional manner. The delivery is accomplished through surgically implanted catheters in the brain that are connected to external drug infusion pumps that generate a positive infusion pressure. This positive pressure begins the process of convection, which is the augmentation and maintenance of the brain's normal physiologic bulk flow of interstitial fluid. The enhanced bulk flow through the ►interstitial space acts as the carrier of the desired agent.

Characteristics

High-grade primary ►brain tumors, such as ►glioblastoma multiforme, remain one of the most challenging diagnoses to treat effectively. Despite a range of therapeutic options and their combinations, including surgical resection, external beam radiation therapy, and ►chemotherapy, the median survival remains an astounding 12–14 months. Due to the compartmentalized distribution of functional areas within the brain and the consequence to the patient's independence with compromise of these functions, surgical resection must

be tempered. Radiation therapy has proven benefit; however, there is a finite limit to the brain's tolerance to radiation effects. Therefore, focus has shifted toward maximizing the role of chemotherapeutic agents in the treatment of primary brain tumors.

Several factors have contributed to the failure to substantially improve survival among patients with primary brain tumors. While some are attributable to the biology of the disease, others are due to the limited activity of many agents, and the obstacles to effective delivery of therapeutic agents within the brain. Recent advances in the understanding of the pathogenesis of primary brain tumors have also resulted in the development of novel therapeutic ►drug designs, many with high specificity, due to the incorporation of structures such as ►monoclonal antibodies to their structures. Although highly specific in their targeting and activity, these new agents also possess characteristics such as high molecular weight and polarity, and therefore may not be suitable for traditional routes of drug delivery.

One physiological barrier that must be overcome is the ►blood-brain barrier (BBB). The BBB is both a physical and a metabolic barrier that allows entry of selected substance from the circulation into the brain. Specifically, substances with lipid solubility cross the cell membrane, as well as those with specific transport systems. Substances with a molecular weight >500 Da cannot cross through the BBB. This limitation may exclude the use of otherwise highly bioactive chemotherapeutic agents currently in development; in other words, anti-tumor agents must be small is size and lipophilic in nature in order to reach the brain following enteral or parenteral administration.

Free concentration gradients of substances are the driving force for ►diffusion as a passive transport mechanism. Diffusion of substances in the interstitial space of the brain is largely dependent on the molecular weight of the compound, with higher molecular weight compounds resulting in less diffusion than of smaller molecular weight compounds. Diffusion is also a very slow process and the desired agent is subject to many forces that may limit its diffusive capacity, such as capillary uptake and metabolism. Due to these physical limitations, in order to achieve a therapeutically meaningful concentration of a drug, very high concentrations, often supratherapeutic, must be delivered to ensure passage of meaningful concentrations of drug beyond the immediate delivery site. Realization that diffusion results in only millimeter distances of drug penetration through the interstitial space, when the biology of primary brain tumors dictates that regions an order of magnitude greater needs to be covered with the desired agent, led to interest in utilizing the brain's physiology, interstitial bulk flow, as a mechanism for drug delivery to the brain.

Although the brain itself lacks a lymphatic system, the interstitial space is a dynamic compartment where bulk flow of fluid occurs under normal physiologic conditions. Augmenting this bulk flow, by the initiation of a point-source of positive iatrogenic pressure generated by a pump connected to a surgically implanted catheter in the brain, results in fluid convection. When desired agents are dissolved in the diluents and delivered through the catheter, the positive hydrostatic pressure results in distribution of the agents through the interstitial space in a radial, thus spherical, direction. CED has consistently resulted in centimeter-radius volumes of distribution and is able to distribute these agents, independent of their molecular weight, polarity, and concentration. In fact, CED results in a less than one-log decrease in concentration of the delivered agent at the "leading edge" compared to its concentration at the catheter site, unlike the distribution achieved by using diffusion. With the direct infusion of the desired agent into the interstitial space, the BBB is circumvented and theoretically acts as a barrier to keep agents from entering the circulation, which decreases systemic toxicity. To that end, small, lipophilic agents are not deemed favorable agents for CED application.

Clinical Application

CED is a novel technique that allows locoregional drug distribution for the treatment of a locoregional disease such as primary brain tumors; however, CED is not yet considered a clinical standard of care and is practiced only in research settings. However, ►preclinical, Phase I, Phase II, and now Phase III clinical trials have demonstrated CED to be well-tolerated and safe for drug delivery into the brain. Adverse effects, seen in ~30% of patients undergoing CED, appear to be related to the increased ►cerebral edema following drug infusion. Neurological deficits often respond to medical techniques aimed at reducing cerebral edema and thus are transient.

While the volume of distribution achieved by CED increases linearly with the volume of infusion, several technical factors have also been found to influence the efficacy of CED. As the delivery device situated within the brain, the catheter has received particular attention. One of the difficulties encountered with CED is the phenomenon of reflux, or leak back, of the agent along the catheter. This is encountered with high infusion rates, and large catheter diameters. To combat reflux, research has focused on catheter design in an effort to create a reflux-proof catheter.

With regard to infusion rates, one of the disadvantages of CED is the need to infuse the desired agent over a protracted period of time, often lasting several days in duration. This necessity stems from the reflux problem mentioned above which, should it occur, limits the volume of distribution of the agent. Therefore, a rate of infusion is chosen which exceeds the rate at which the

brain can remove fluid from the interstitial space but is less than the rate at which leak back may occur.

The anatomic complexity of the brain and of the region afflicted by a primary brain tumor also affects the efficacy of CED. Due to the presence of tumor tissue, white matter, and gray matter, the intersititial space is not uniform. Furthermore, the pattern of ►gyri and ►sulci, and therefore the ►subarachnoid space, the proximity of the ►ependymal layer that lines the ventricle space, and the presence of tumor necrosis complicate catheter positioning, which must be accomplished after thorough presurgical planning. The subarachnoid space, ependymal layer, and tumor necrosis all represent low-resistance areas that would lead to the potential loss of convection. Radiographic correlate of effective convection is detected on T2-weighted magnetic resonance imaging sequence as an increase in the fluid signal within the targeted region.

Currently, there are several targeted therapeutic agents in advanced clinical development. The most advanced along its development is the agent IL13-PE38QQR, which is a chimeric protein based on the fusion of IL-13 as a ligand and the *Pseudomonas aeruginosa* exotoxin as the cytotoxic agent. The IL-13 receptor is known to be over-expressed in high-grade primary brain tumors, while the exotoxin is a potent inducer of cell death by arresting cellular protein synthesis.

IL13-PE38QQR has undergone rigorous testing thus far, and the results of three Phase I/II trials were recently presented. In aggregate, 74 patients were enrolled in these trials, which determined the maximally tolerated dose of the agent. In addition, patient outcomes were compared when CED was conducted with the delivery catheters located in the peritumoral region compared to the intratumoral space. Improved survival was seen among patients undergoing peritumoral infusions; within this group of patients receiving peritumoral infusions, those who had more than two "optimally" placed catheters had a significantly improved median survival.

A Phase III trial was initiated in 2004 which compared IL13-PE38QQR, delivered by CED, to another local drug delivery technique that relied on diffusion for drug dispersion. Three hundred patients were randomized to this trial and the final results remain to be reported.

Future Directions

While CED represents a novel drug delivery technique, it also remains a field in evolution. Several lines of research are currently focusing on areas for continued improvement. Questions remain regarding the optimal placement and number of CED catheters. In Phase I/II trials of IL13-PE38QQR, patients with "optimally" placed catheters had better outcomes. Whether this will be confirmed in the Phase III trial will be of great interest.

With regard to ►pharmacokinetic parameters, efforts are underway to increase the half-life of therapeutic agents, allowing the agents to remain available long after CED is halted. Investigators have demonstrated that encapsulation of their agents in ►liposomes is one such strategy. Another consideration is that, depending on the pharmacokinetic characteristics of the therapeutic agent, prolonged infusions may yield greater clinical efficacy. In that situation, alternative delivery methods of CED, such as implanted pumps housed entirely under the skin, may provide protection from infectious complications yet retain the drug delivery advantage.

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Convection-Enhanced Delivery

Definition

CED; The delivery approach of small and large molecules in sold tissues, utilizing a pressure gradient to distribute macromolecules to clinically significant volumes of tissue by bulk flow.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Convergent Extension

Definition

A process in which tissues simultaneously lengthen and narrow to facilitate body axis extension, neural tube closure and tissue morphogenesis.

►Wnt Signaling

COP1 (Constitutively Photomorphogenic 1)

Definition
A RING finger E3 ubiquitin ligase with substrates including c-Jun and p53.

►Major Vault Protein

Core Binding Factor A2

►Runx1

Core Fucose

Definition
A fucose attached on the innermost *N*-acetylglucosamine in *N*-glycans

►Fucosylation

Corepressor

Definition
Factor that interacts with different transcription factors to confer a repressor activity. Usually found in multimeric complexes containing associated chromatin remodeling activities (like ►histone deacetylases, methylases and ATPases).

►Snail Transcription Factors

Corin

Definition
Is a ►serine protease (type II) spanning the plasma membrane, highly expressed in cardiac muscle cells. The exact physiological function is not known. Corin might also be involved in hypertensive disease in humans.

►TMPRSS10

Coronary Artery Disease

Definition
Clinical syndrome resulting in obstruction of coronary arteries by atheromatous plaques.

►Statins

Cortactin

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Synonyms
Src8; Amplaxin; EMS1

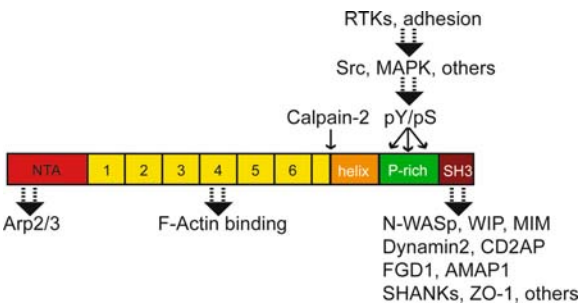
Definition
Cortactin is a protein that is a component of the cortical actin cytoskeleton, where it participates in regulating the assembly and organization of filamentous actin in protrusive structures generated during cellular movement. Cortactin gene ►amplification and overexpression is found in several cancer types, where it contributes to enhanced tumor cell ►motility, ►invasion and ►metastasis.

Characteristics
Cortactin is an actin-binding protein and kinase substrate that is intimately associated with the microfilament network underlying the plasma membrane in most cells. It plays an important role in ►signal transduction pathways that mediate chemotactic cues from the extracellular environment that initiate and maintain cell ►migration. Activation of growth factor receptors or ►adhesion molecules results in the phosphorylation of cortactin at several tyrosine and serine residues. Cortactin phosphorylation is coincident with

changes in plasma membrane architecture that occur during the initial phases of cellular movement, including the formation of ►lamellipodia and ►circular dorsal ruffles that are required for the extension of a cell's leading edge. Cortactin is also enriched in ►invadopodia, ventral protrusive structures that contain membrane-bound proteases and enhance cellular invasion by facilitating the focal degradation of extracellular matrix. In addition to its role in cell motility, cortactin is also associated with various intracellular membrane compartments, including endosomal vesicles and the Golgi apparatus, and plays an important role in the early events of ►endocytosis and in vesicle trafficking.

Structure and Binding Partners

Cortactin is expressed in all tissues except cells of myeloid lineage, where it is functionally replaced by the related protein ►HS1. Based on primary sequence analysis cortactin is subdivided into several distinct domains (Fig. 1). The amino-terminal domain (NTA) contains a series of acidic residues and a binding motif that interacts with the ►Arp2/3 complex. The NTA domain is followed by a series of 37 amino acid tandem repeats, six complete and one incomplete in the predominant isoform. The repeat region interacts with F-actin, with binding activity centered around the fourth repeat. Alternative splicing in some cells is responsible for two additional isoforms that lack either the sixth complete or fifth and sixth complete repeat segments. These forms bind F-actin at reduced affinities. Following the repeats region is an alpha helical domain that is the site of cleavage by the protease ►calpain 2. This is followed by a proline-rich region that harbors serine, threonine and tyrosine residues that serve as the primary sites of phosphorylation. An ►SH3 domain is found at the extreme carboxyl terminus that binds to proline-rich sequences on a variety of proteins including the actin regulatory proteins ►N-WASp, WASp interacting protein and the missing in metastasis protein, the endocytic proteins ►dynamin 2 and ►CD2AP, the small ►GTPase regulatory proteins ►FGD1 and ►AMAP1, scaffolding proteins of the ►SHANK family and the ►tight junction protein ►ZO-1. These structural parameters and binding partners allow cortactin to function as a molecular scaffold by linking a wide variety of diverse regulatory molecules to sites of Arp2/3-mediated actin assembly.



Cortactin. Figure 1 Domain structure of cortactin and associated binding proteins. This is a simplified representation showing domain organization, binding proteins and regulatory signaling pathways. See text for details.

Function

The function of cortactin has been best defined in regards to cell motility. Downregulation of cortactin protein expression reduces cellular movement while overexpression of cortactin enhances this process. Biochemical studies have determined that cortactin activates Arp2/3 complex actin nucleation activity through the NTA domain, and its localization within lamellipodia indicates that cortactin contributes to the formation of the dendritic cortical actin network responsible for lamellipodia protrusion. Important in this aspect is the ability of cortactin to stabilize Arp2/3-produced actin networks, a feature unique among Arp2/3 activating proteins that serves to prolong the half-life of branched F-actin filaments at the cell periphery. Accordingly, cortactin depletion reduces the ability of extended lamellipodia to persist and inhibits efficient leading edge dynamics. Cortactin can effect Arp2/3 mediated actin polymerization by additional alternative mechanisms, most notably by activation of the Arp2/3 regulatory protein N-WASp through association with the cortactin SH3 domain. Cortactin fragments lacking the NTA but containing the SH3 domain are capable of stimulating motility, suggesting that the NTA and SH3 domains can function independently with regards to promoting actin-based cell movement. The interaction of cortactin with dynamin 2 is also noteworthy in that cortactin is recruited to subpopulations of clathrin-coated pits by dynamin 2 and is important for driving the scission of invaginating pits to produce intracellular endocytic vesicles. The cortactin-dynamin complex is also important in regulating cell morphology, invadopodia function and the genesis of vesicles from the trans-Golgi network.

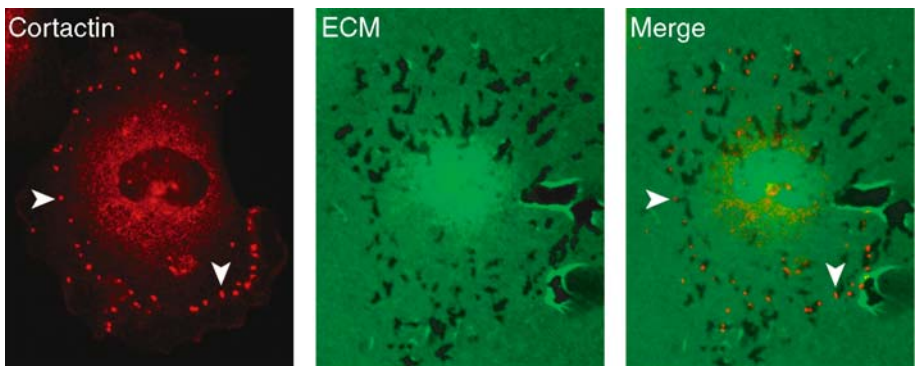
Regulation

Evidence to date indicates that phosphorylation on tyrosine and serine resides are the main factors involved in regulating cortactin function, although the precise mechanisms are unclear. Activation of ►receptor tyrosine kinases or adhesion molecules leads to phosphorylation of three tyrosine sites in the proline-rich domain that are required for efficient cell migration. These sites are direct targets of ►Src and related

non-receptor tyrosine kinases, and are hyperphosphorylated by oncogenic variants (i.e, v-Src). Tyrosine phosphorylated cortactin is enriched within lamellipodia and invadopodia, indicating a potential role in regulating cortical actin dynamics and has been shown to influence F-actin architecture. Cortactin is also phosphorylated on two serine residues by ►MAP kinase in the proline-rich domain, and dual phosphorylation of cortactin by Src and MAP kinase has opposing effects on the ability of the cortactin SH3 domain to interact with and activate N-WASp. This has led to the proposal of a regulatory phosphorylation switch mechanism predicated by cortactin initially existing in an autoinhibited closed conformation, with the SH3 domain binding back and interacting with motifs in the proline-rich domain. Phosphorylation of cortactin by MAP kinase induces a conformation change that renders the SH3 domain accessible for binding and activating N-WASp, whereas phosphorylation of cortactin by Src causes disassociation of N-WASp from the SH3 domain and subsequent downregulation of N-WASp activity. This proposal remains theoretical in part since it is derived primarily from biochemical analysis and evidence for an intramolecular cortactin interaction is lacking. In addition to phosphorylation indirectly regulating N-WASp activity, the serine/threonine kinase ►PAK1 phosphorylates cortactin within the first tandem repeat, resulting in reduced F-actin binding. Subsequent work has identified over 17 additional phosphorylation sites in every domain except the SH3, but the responsible signaling pathways and functional significance of these modifications are currently unknown. Besides phosphorylation, cortactin is also regulated by the calcium dependent protease calpain 2, which cleaves cortactin between the repeats and alpha helical domain and is important in limiting the extent of lamellipodia protrusion.

Role in Cancer

The cortactin gene (*CTTN*, formerly *EMS1*) maps to chromosome 11q13.3, a region that is frequently amplified in a number of cancers with inherently high invasive and metastatic potential, including ►breast, ►head and ►neck, ►ovarian, ►bladder and ►hepatocellular carcinomas. 11q13 and *CTTN* amplification is associated with poor pathological outcome parameters including increased tumor recurrence, advanced disease stage, poor histological differentiation, increased lymph node metastasis and reduced disease specific survival. Mechanistically, cortactin overexpression as a result of *CTTN* amplification increases tumor cell motility and invasion as well as preventing the internalization and ubiquitylation-mediated degradation of ►EGF receptor, a receptor tyrosine kinase often overexpressed in carcinomas that is a potent activator of Src and MAPK. Sustained EGF receptor activity as a result of *CTTN* amplification and cortactin overexpression promotes increased cortactin tyrosine phosphorylation, which has been shown to enhance distant metastasis of breast carcinoma cells in ►mouse models. ►EGF receptor inhibitors suppress tumor cell invasion and cortactin tyrosine phosphorylation, providing further support for the clinical relevance of cortactin phosphorylation in human cancer. Specific functions for cortactin in tumor cell invasion have been identified, most notable being its absolute role in the signaling and structural requirements governing the formation and function of invadopodia (Fig. 2). Cortactin is



Cortactin. Figure 2 Cortactin localization in invadopodia corresponds to sites of extracellular matrix degradation. Shown is a cell from a head and neck squamous cell carcinoma tumor containing invadopodia, visualized by immunofluorescent staining for cortactin in red as focal dots within the cell cytoplasm. The cell was grown on a green fluorescent extracellular matrix (ECM), and sites of matrix degradation are visualized as cleared dark regions against the green background. When merged, these areas correspond with cortactin-labeled invadopodia and are highlighted with arrowheads.

required to recruit and sequester the main invadopodial ►matrix metalloproteinase MT1-MMP into sites of newly initiated invadopodia. Cortactin tyrosine phosphorylation levels within invadopodia correlate to the degree of extracellular matrix degradation activity, but the functional significance of cortactin phosphorylation in invadopodia is currently undefined. Cortactin in invadopodia forms a complex with the focal adhesion protein ►paxillin and other signaling proteins. Often present in invasive carcinomas is amplification and overexpression of AMAP1, which physically links paxillin and cortactin together in promoting tumor invasion. Targeting of the trimeric paxillin-AMAP1-cortactin complex with competitive peptides mimicking the AMAP1 binding site for the cortactin SH3 domain suppresses carcinoma invasion and may show potential value in ►anti-metastatic therapy. In addition to its role in promoting tumor cell invasion and metastasis, cortactin has been shown to be a prominent tumor antigen and is present at high levels in the sera of subsets of breast cancer patients. Recent work has determined that cortactin is an extracellular ligand for ►TEM7, a transmembrane receptor expressed primarily on the surface of tumor endothelial cells. While the function of TEM7 is currently unknown, related TEM proteins promote endothelial cell growth and survival, raising the possibility that cortactin released into the circulation from necrotic or damaged tumor cells, especially tumors with *CTTN* amplification, may serve an unexpected role by promoting or maintaining tumor ►angiogenesis.

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Cortical Bone

Definition

Dense type of bone tissue that forms the surface of bones.

►Lead Exposure

Cortical Neurons

Definition

Nerve cells found in the cortex of the brain.

Corticosteroids

Definition

Are a family of drugs related to steroids that are naturally produced in the adrenal cortex, such as cortisone. Corticosteroids can kill lymphocytes, especially developing thymocytes, inducing apoptotic cell death. They are useful antiinflammatory, antilymphoid tumor, and immunosuppressive agents. Natural and synthetic analogs of the hormones secreted by the pituitary gland. These analogs include glucocorticoids, mineralocorticoids, and corticotropins.

►Rituximab
►Sjögren Syndrome

Corticotrophin

Definition

A hormone produced by the anterior lobe of the pituitary gland that stimulates the secretion of cortisone and other hormones by the adrenal cortex.

►Adrencorticotropic Hormone
►ACTH

Corticotrophin-Releasing Hormone

Definition

CRH; A hormone made by the hypothalamus that stimulates the release of ►corticotrophin by the anterior pituitary gland.

Cortisol

Definition

Cortisol is an important hormone in the body, secreted by the adrenal glands and involved in the following functions and more: Proper glucose metabolism, Regulation of blood pressure Inflammatory response, Insulin release for blood sugar maintenance, and Immune function.

Corynebacterium Diphtheriae

Definition

A bacteria causing Diphtheria that is a contagious disease of the throat.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Cosinor

Definition

Statistical method used to compute rhythm parameters and their 95% confidence limits through the adjustment of the best fitting cosine function with an a priori chosen period (usually, τ = 24 h for circadian rhythms). The main parameters are the amplitude, half the difference between maximum and minimum of cosine function, and acrophase, location of maximum, usually referred to light onset.

►Circadian Clock Induction

Costello Syndrome

Definition

A developmental defect characterized by a complex disorder involving characteristic craniofacial features, failure to thrive, developmental delay, cardiac and skeletal anomalies and a predisposition to develop neoplasia.

Costimulation

Definition

A process that is necessary for full activation of T cells via antigen-specific signaling pathways. Signals during costimulation are provided by the interaction of molecules other than T cell antigen receptor.

►T-Cell Response

Costimulatory Signal

Definition

The proliferation of lymphocytes, requires both antigen binding and the receipt of a costimulatory signal. Costimulatory signals are delivered to T cells by the costimulatory molecules B7.1 and B7.2, related molecules that are expressed on the surface of the cell presenting antigen, and which bind the T-cell surface molecule CD28. B cells may receive costimulatory signals from common pathogen components such as LPS, from complement fragments, or from CD40 ligand expressed on the surface of an activated antigen-specific helper T cell.

►Sjögren Syndrome

Coumestans

Definition

A class of phytoestrogens.

►Phytoestrogens

γ-Counter

Definition

Detection unit for ►γ-irradiation.

Cowden Disease

►Cowden Syndrome

Cowden Syndrome

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Synonyms

Cowden disease; Multiple hamartoma syndrome; PTEN hamartoma tumor syndrome

Definition

Cowden syndrome (CS, OMIM#158350), along with Bannayan-Riley-Ruvalcaba syndrome (BRR, OMIM# 153480), Peutz-Jeghers syndrome (PJS, OMIM#175200) and juvenile polyposis syndrome (JPS, OMIM#174900), is a member of a group of rare autosomally dominant inherited conditions classified as the ►hamartoma syndromes. Proteus syndrome (PS, OMIM#176920), although most frequently of sporadic presentation, has also been classified as part of this hamartoma-tumor syndrome spectrum. CS takes its name from the proposita of the first family described. It is characterized by an increased risk of developing breast, thyroid and endometrial cancer along with the presence of hamartomas in multiple organ systems. The susceptibility gene for both CS and BRR is the tumor suppressor ►*PTEN* (alternatively named ►*MMAC1* or ►*TEP1*).

Characteristics

CS displays variable expressivity within families, however usually presents in the third decade. CS hamartomas are present in tissues derived from all three ►germ cell layers, specifically the breast, thyroid, skin, central nervous system and gastrointestinal tract. The incidence of CS has been estimated to be 1 in 200,000 individuals. Ninety-nine percent of CS patients display the hallmark CS hamartoma known as ►trichilemmomas in addition to mucocutaneous papules. Seventy percent of female CS patients develop breast ►fibroadenomas, 40–60% have thyroid adenomas, whilst gastrointestinal polyps occur in 35–40% of CS patients. Breast cancer develops in 25–50% of female CS patients and on occasion also in males, whilst thyroid cancer develops in 3–10% of all affected individuals. Disease of the central nervous system, most often benign but in some cases malignant, occurs in 40% of cases. Lhermitte-Duclos disease, a condition of dysplastic gangliocytoma of the cerebellum manifesting as seizures, tremors and poor co-ordination, has been reported in conjunction with CS. Megencephaly or macrocephaly occur in approximately 38% of CS patients. Other abnormalities including those of the genitourinary tract may also be present. Given the subtle and poorly recognized physical findings in individuals with CS, it is thought that this condition may often be underdiagnosed. The International CS Consortium has developed and recently revised diagnostic criteria to aid in the identification of this syndrome.

CS shows partial clinical overlap with BRR as patients with either syndrome may develop intestinal hamartomatous polyps, macrocephaly (in nearly 100% of cases of BRR) and ►lipomas (occurring frequently in BRR but in a minority of patients with CS). Other features of BRR include very early age of onset, pigmented macules of the glans penis ("speckled penis") in males, ►hemangiomas, mild mental retardation and developmental delay. Both the unique and overlapping clinical features of CS and BRR are described in the Table 1. A number of anecdotal cases of malignancy affecting the thyroid and brain have been reported in BRR, however whilst malignancy is well described in CS, it is not part of the classic BRR phenotype. Nevertheless, because of the apparent increased risk of developing cancer in BRR recommendations for cancer screening have been made. In addition, a number of families have been reported in which both CS and BRR are present. In these families, CS is generally present in the parental generation, whilst BRR appears in the younger generation, suggesting a form of ►anticipation (Fig. 1 and Fig. 2).

Molecular Features

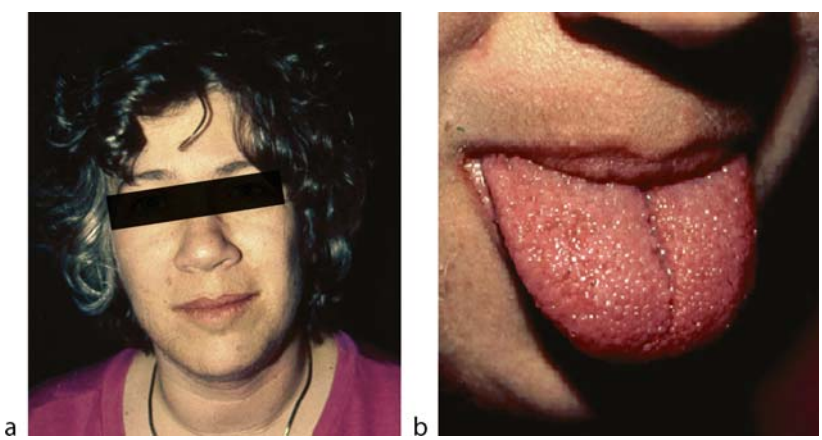
PTEN, the first protein tyrosine phosphatase shown to function as a tumor suppressor, is the susceptibility gene for both CS and BRR. A processed ►pseudogene is located on chromosome band 9p21, missing the initiating methionine present in PTEN but sharing greater than 98% homology with the *PTEN* coding region.

PTEN contains nine exons encoding a dual-specificity ►phosphatase mapped to 10q23.3, with

Cowden Syndrome. Table 1 Clinical features seen in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome

	Cowden syndrome	Bannayan-Riley-Ruvalcaba
CNS	Lhermitte-Duclos disease	Developmental delay[a], seizures, myopathy
Endocrine	Multinodular goitre, adenoma, thyroid anomalies, thyroiditis, hypothyroidism	Hashimoto's thyroiditis[a], diabetes mellitus
►Growth disturbances:		
Generalized	Macrocephaly	Macrosomia at birth, enlarged penis and testes, localized overgrowth, macrocephaly
Skin	Facial trichilemmoma[b], acral keratoses, hemangioma, mucosal lesions, papillomatous papules[b], hypertrichchosis, vitiligo, pseudo-acanthosis nigricans, skin cancers (basal cell, squamous cell and melanoma)	Penile lentigines, acanthosis nigricans, verruca vulgaris type facial changes, tongue polyps, Café au lait spots, angiokeratoma, lipoma/ lipomatosis[a]
Gastrointestinal	Polyps in entire gastrointestinal tract, gastrointestinal cancers	Polyps in distal gastrointestinal tract
Breast	Fibrocystic breast disease, adenocarcinoma	–
Other benign tumor	Uterine leiomyoma, ovarian cysts, fibroma, meningioma, glioma, neuroma	Meningioma, angiolipoma, hemangioma[a] (especially intracerebral and bony) lymphangioma
Other malignant tumors	Thyroid (non-medullary)[b], cervix, uterus, bladder, liver, renal, acute myelogenous leukemia, non-Hodgkin lymphoma, liposarcoma, trichilemmomal carcinoma, renal cell carcinoma	–

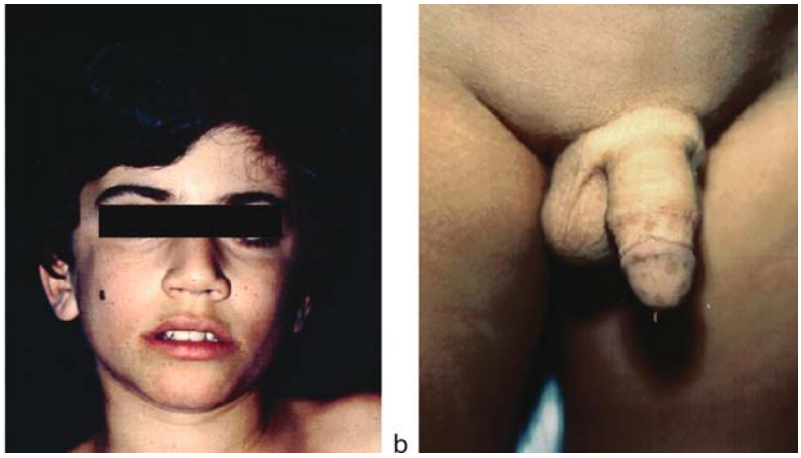
[a]Common in BRR, occasionally seen in CS.
[b]Reported in CS, occasionally seen in BRR.



Cowden Syndrome. Figure 1 Female with Cowden syndrome. (a) Macrocephaly (head circumference of 59½ cm, greater than the 97th percentile). (b) Multiple small papules of the tongue and mouth.

homology to the cytoskeletal proteins tensin and auxillin. Residues 122–132 located in exon 5 encode the classic phosphatase core motif (I/V)HCXXGXXR (S/T)G. The COOH-terminus contains three potential tyrosine phosphorylation sites at residues 240, 315 and 336, as well as two potential serine phosphorylation sites at residues 335 and 338. It also contains a potential ►PDZ binding domain encoded by the last four amino acids (ITKV) that may have a role in its subcellular localization and/or substrate interactions.

PTEN has been shown to reduce tyrosine phosphorylation of focal adhesion kinase (FAK) *in vitro* suggesting a role in cell migration and invasion. However, the major endogenous substrate of PTEN would seem to be phosphatidylinositol 3,4,5-trisphosphate (Ptd-Ins $(3,4,5)P_3$), a phospholipid in the phosphatidylinositol 3-kinase (PI-3 kinase) pathway and an important second messenger in cell growth regulation. In this pathway growth factors such as insulin, platelet derived growth factor and fibroblast growth factor stimulate the enzyme



Cowden Syndrome. Figure 2 Male with Bannayan-Riley-Ruvalcaba Syndrome (son of female in Fig.1). (a) Macrocephaly (head circumference of 59 cm, greater than the 97th percentile). (b) Multiple hyperpigmented macules of the penis.

PI3-kinase to phosphorylate Ptd-Ins(4,5)P_2 to produce Ptd-Ins(3,4,5)P_3. PTEN acts as a 3-phosphatase to dephosphorylate Ptd-Ins(3,4,5)P_3 to Ptd-Ins(4,5)P_2. When PTEN is mutant, Ptd-Ins(3,4,5)P_3 accumulates and activates protein kinase B (PKB)/►AKT to function as an oncogene, thus causing the tumorigenic state. AKT is a ►serine-threonine kinase and a known cell survival (anti-apoptotic) factor. Thus, apoptosis is a likely mechanism for PTEN-induced growth suppression. However, PTEN is also able to cause cell cycle arrest in cells in the G_1 phase, possibly via modulation of levels of RB phosphorylation.

Elucidation of the crystal structure of PTEN has revealed a wider and deeper phosphatase active site than is usually described in other dual-specificity phosphatases that allows the accommodation of Ptd-Ins (3,4,5)P_3. Further, the make up of the residues in this pocket cause it to have a positive charge consistent with the negative charge of Ptd-Ins (3,4,5)P_3 and with the preference displayed by PTEN for highly acidic polypeptide substrates. One particular germline mutant found only in CS, G129E, has been shown to have normal phosphatase activity against non-phospholipid substrates *in vitro* and in cell lines but has no phosphatase activity against Ptd-Ins(3,4,5)P_3. It is believed that mutation of this residue reduces the size of the active pocket so that it can no longer accommodate the phospholipid substrate Ptd-Ins(3,4,5)P_3. However, catalysis of the smaller substrates of phospho-tyrosine, serine and threonine are not disrupted. Furthermore, a C2 domain is present in the C-terminal domain and associates over an extensive interface with the phosphatase domain creating interdomain hydrogen bonds between conserved residues. This interphase region provides strong evidence that the C2 domain not only functions to recruit substrate, but also optimally positions it available to the phosphatase catalytic domain. Germline mutations of conserved residues involved in the creation of this interphase, including serine at position 170, have been reported in BRR. Thus, there is strong evidence that the lipid phosphatase activity of PTEN is essential for its tumor suppressor activity.

Clinical Aspects

PTEN is mutated in the germline of up to 80% of patients with CS and up to 60% of patients with BRR. Mutations are scattered largely along the entire gene with the exception of exon 1, including point mutations, insertions, deletions, deletion-insertions and splice site mutations. Germline mutations and deletions have also been reported in the promoter region of *PTEN* in patients with CS and BRR. In BRR alone, gross ►hemizygous deletions and also a balanced translocation likely affecting the PTEN gene have been reported. Further, loss of the wild type allele has been identified in hamartomas from a subset of CS individuals with *PTEN* mutation, providing additional evidence that PTEN is functioning as a classic tumor suppressor according to Knudson's two-mutation model. However, there are many cases where loss of the wild-type allele is not observed in affected CS tissue. As is suggested by one of the $Pten^{+/-}$ mouse models, PTEN haploinsufficiency may be all that is required for the presence of the characteristic developmental defects and tumor formation seen in CS and BRR. It was first thought that none of the 3 $Pten^{+/-}$ mouse models described developed the classic benign and malignant tumors of CS and BRR, although the presence of colonic microscopic hamartomatous polyps not dissimilar to what is seen in CS and BRR was reported. However, a

study of Pten$^{+/-}$ mice older than 6 months reported the development of a range of tumours more similar to the spectrum of tumors observed in CS patients, specifically breast tumours in 50% of females, 100% of females with endometrial hyperplasia and a high incidence of endometrial cancer, prostate and adrenal neoplasia and tumors of the gastrointestinal tract.

PTEN germline mutations in CS and BRR have been found to cluster in exons 5, 7 and 8, with the great majority occurring in exon 5. This may be a function of the fact that exon 5 is the largest exon of this gene, constituting 20% of the coding region, but this exon also contains the protein tyrosine phosphatase (PTPase) core motif. Of note in CS, most mutations that occur in the core motif are non-truncating, suggesting the importance of this functional domain.

Identical mutations, including Q110X, R130X, R233X and R335X have been reported in both CS and BRR, making the presence of other genetic and/or epigenetic factors such as modifier loci highly likely in the determination of phenotype. Furthermore, a number of families have been reported with CS diagnosed in the older generation and BRR present in the younger generation suggesting some form of anticipation that is currently not well understood. From this, it could be concluded that BRR and CS are different presentations of a single syndrome with broad clinical expression. In fact, it has been suggested that *PTEN* mutation positive CS and BRR patients should be clinically grouped as a single entity and classified as the "PTEN hamartoma-tumor syndrome" (PHTS).

DNA based predictive testing programs can now be incorporated as part of the clinical management of CS and BRR individuals. At the level of clinical management, cancer surveillance coupled with genetic counseling becomes important for CS and BRR patients as well as their first degree relatives.

Preliminary genotype-phenotype correlations have been reported for both CS and BRR and a number of trends observed. Firstly in a study of BRR and CS/BRR overlap families, the correlation of a germline *PTEN* mutation with the presence of lipomas and also with any cancer or breast fibroadenoma was determined. In CS families a number of correlations were observed including an association between the presence of a *PTEN* mutation and breast involvement, as well as the presence of a missense mutation and the involvement of all five organ systems (ie. breast, thyroid, gastrointestinal tract, central nervous system and skin). It is possible that this latter trend may in fact be a positional effect given that the majority of missense mutations occur in the PTPase core motif. One study states that the presence of a *PTEN* mutation in either CS alone, BRR alone or CS/BRR overlap families predisposes individuals to the presence of tumors, whether they be benign such as the lipomas seen predominantly in BRR, or malignant such as the breast, thyroid and uterine carcinomas seen in CS or CS/BRR overlap families. Confirmation of these preliminary findings requires analysis of a larger number of families before they can be directly transferred to the clinic.

Clinical cancer surveillance of patients with CS is recommended. All patients with a *PTEN* mutation should undergo careful annual physical examinations with special attention to the skin and thyroid from the teens. For females, breast self examination from 18 years, annual clinical breast examination from age 25 and annual mammography/MRI at age 30–35 years is recommended. Thyroid ultrasound is recommended at 18 years with annual thyroid ultrasounds thereafter. Annual surveillance of the endometrium, with biopsies of the endometrium from the thirties and annual transvaginal ultrasound examination with biopsy of suspicious areas after menopause should be performed. Urine should be checked annually for blood. All specific cancer screening should be started at least 5–10 years earlier than the earliest appearance of the specific cancer in the family.

In addition to being mutated in the germline of patients with CS and BRR, PTEN has been described as "…the most highly mutated tumor-suppressor gene in the post-p53 era… ." It is mutated in a spectrum of human malignancies including glioblastoma (where *PTEN* mutation would seem to be a late event in tumor progression), endometrial hyperplasias (likely an early event) and carcinomas, prostate cancer and malignant melanoma and less commonly in thyroid neoplasias, breast and colon cancer. Thus, it is likely that syndromic hamartomas and cancers in CS and BRR develop on a background created by loss of the tumor suppressor function of PTEN. Furthermore, *PTEN* is a highly significant gene in the development of a wide range of sporadic human cancers.

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COX

►Arachidonic Acid Pathway

COX-2

►Cyclooxygenase-2 in Colorectal Cancer

Cox-2 inhibitors

►Nonsteroidal Anti-inflammatory Drugs

Cox Proportional Hazards Model

Definition

Cox proportional hazards model is a semi-parametric method of survival analysis that uses an approach similar to regression analysis to estimate relative hazard rates using either continuous or categorical predictor variables.

►Kaplan–Meier Survival Analysis

Coxibs

Definition

Coxibs are cyclooxygenase-2 inhibitors which cause fewer gastrointestinal bleeds than convential ►nonsteroidal anti-inflammatory drugs, but may cause cardiovascular damage.

►Lipid Mediators
►Archidonic Acid Pathway

CPD

Definition

Cyclobutane pyrimidine dimers.

►UV Radiation

CpG

Definition

Bacterially derived DNA triggers innate immune defense mechanisms, the activation of dendritic cells, and the production of TH1 cytokines. This is due to recognition of certain CpG dinucleotide sequences which are immunostimulatory. CpG stimulatory (CpG-S) sequences are hypomethylated. The optimal immunostimulatory sequence is an unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′ pyrimidines. Additionally, flanking regions outside this immunostimulatory hexamer must be guanine-rich to ensure binding and uptake into target cells. CpG-S sequences induce polyclonal B-cell activation and the upregulation of cytokine expression and secretion mediated by Toll-like receptor 9. Stimulated macrophages secrete IL-12, IL-18, TNF-α, IFN-α, IFN-β and IFN-γ, while stimulated B-cells secrete IL-6 and some IL-12 and drive the immune response toward a Th1 phenotype.

►DNA Vaccination
►Toll-Like Receptors

CpG Islands

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Synonyms

*Hpa*II tiny fragments (HTF) islands

Definition

CpG islands are short stretches of DNA sequences with an unusually high GC content and a higher frequency

of CpG dinucleotides as compared to the rest of the genome. Together CpG islands account for about 1–2% of the genome and their location is mainly in the 5′ regulatory regions of all housekeeping genes as well as up to 40% tissue specifically expressed genes.

Characteristics

With the rapid accumulation of sequencing data it became obvious that the distribution of the four bases, adenine (A), cytosine (C), guanine (G), and thymine (T), in the genomic sequence is not even. Normal DNA has an average GC content of 40% and an AT content of 60%. Early work by Bird et al [1] identified stretches of genomic sequence characterized by an unusual high number of HpaII restriction sites (restriction site: C^CGG). These sequences were initially called "HpaII tiny fragments (HTF) islands." Careful inspection of those sequences indicated that the ratio of CpG dinucleotides is higher than in the rest of the genome. Normal DNA sequence contains only 25% of the CpG dinucleotides expected from the base composition. These stretches of DNA sequence, with a high GC content and a frequency of CpG dinucleotides that is close to the expected value, are now called CpG islands.

The following three criteria, established by Gardiner-Garden and Frommer [2], are commonly used to define CpG islands: First, the sequence is longer than 200 bp but can be up to several kilo basepairs in size. Second, the GC content is above 50% while the rest of the genome is at about 40%. Third, the CpG ratio (observed/expected) is above 0.6 while the rest of the genome is 0.2. Changes to this definition have been proposed using slightly modified criteria.

The human genome contains about 29,000 CpG islands and the estimated number in the mouse genome is slightly less. The majority of these sequences are located in the 5′ region (promoter and or exon 1) of all housekeeping genes and a large number of tissue specifically regulated genes. However, CpG islands in the 3′ end of genes or in intronic sequences have been found. The preferential location of CpG islands in 5′ regions of genes can be used for the identification of novel genes. Rare cutting restriction enzymes with GC rich recognition sequences such as NotI (GC^GGCCGC), AscI (GG^CGCGCC), BssHII (G^CGCGC) and EagI (C^GGCCG) can be used for the restriction mapping of large genomic clones. Clusters of those restriction enzyme cutting sites would indicate the presence of a CpG island. It is unknown why the mouse genome has fewer CpG islands than the human genome, when the estimated number of genes in both genomes is expected to be very similar. One possible explanation is that the rate of CpG dinucleotide loss due to deamination (see below) is higher in the mouse than it is in the human genome. The location of CpG islands is in the early replicating, less condensed and GC-rich R-bands of chromosomes.

Preservation of CpG Islands

The origin of CpG islands in the vertebrate genomes is closely associated with DNA ►methylation and a process called deamination. DNA methylation in the vertebrate genomes is found mainly in CpG dinucleotides. Those CpG dinucleotides that are located within CpG islands are usually unmethylated. However, CpG dinucleotides located outside of CpG islands are methylated at the 5′ position of the cytosine. Methylation of CpG dinucleotides makes these sites vulnerable to spontaneous deamination leading to a transition of the 5-methylcytosine to thymine, a process that is believed to be the cause for depletion of CpG dinucleotides from the genome.

Clinical Relevance

Although CpG islands are usually unmethylated there are a few important exceptions. Methylation in CpG islands has been correlated with the transcriptional silencing of the adjacent genes. The detailed molecular process controlling this inactivation, however, is not known. Protein complexes, containing the methyl CpG-binding protein and co-repressor enzymes modifying histone tails as major components, are able to bind to methylated promoters. These protein complexes induce histone deacetylation, which mediates the formation of transcription-repressing chromatin. In *in vitro* experiments, re-expression could be achieved by adding trichostatin A (TSA), a specific inhibitor of histone deacetylases. Two general methylation events in CpG islands can be distinguished: First, the developmentally regulated process of CpG island methylation found in the inactive X-chromosomes, in promoter regions of genes that are regulated in a tissue specific manner and in imprinted genes. Second, the aberrant CpG island methylation in cancer.

Normal, Developmentally Regulated, CpG Island Methylation

- Most CpG islands in the inactive X-chromosome of females are densely methylated. This process of X-chromosome inactivation is linked to the transcriptional silencing of genes on the inactive X-chromosome (phosphoglycerate kinase 1 (PGK1), glucose 6-phosphate dehydrogenase (G6PD) or androgen receptor (AR). Exceptions are found in a few number of CpG islands in genes that escape X inactivation (e.g. STS, ZFX or UBE1).
- Some CpG islands become methylated in other normal developmental processes including cell differentiation and aging. The result of this methylation is the selective inactivation of genes in specific

tissues or at certain developmental stages (e.g. estrogen receptor).
- Genes that are expressed from either the paternal or the maternal allele are called imprinted genes. These genes are found to have CpG island methylation of one allele. While methylation usually occurs in the inactive allele, CpG island methylation was found in some instances in the active allele. This feature of allele specific methylation in a CpG island was used as a tag for the identification of novel imprinted genes in the mouse using the ►restriction landmark genomic scanning (RLGS) technique for a genome wide scan for patterns of allele specific methylation.

Aberrant CpG Island Methylation in Cancer

Hypermethylation of CpG islands in various cancers has been observed and is correlated with the transcriptional inactivation of tumor suppressor genes and other cancer related genes. It was shown that methylation in a CpG island can serve as one of the two "hits" needed for the inactivation of a tumor suppressor gene. While CpG island methylation in some tumors is restricted to a small number of CpG islands, other tumors show a methylation phenotype with up to 10% methylated CpG islands. A subset of CpG islands is methylated in a tumor type specific matter, while other CpG islands can be methylated in different tumor types. It was also shown that many of the genes associated with methylated CpG islands could be reactivated in cell lines by experimental demethylation using 5′-aza-2′deoxycytidine.

►Epigenetic Gene Silencing

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CPT-11

►Irinotecan

Cr(VI)

►Hexavalent Chromium

Cr^{6+}

►Hexavalent Chromium

c-R*mil*

►B-Raf Signaling

cRaf

►Raf Kinase

C-Raf

►Raf Kinase

Cre/loxP

Definition

A site-specific recombinase system derived from bacteriophage P1. Commonly used in mouse models of cancer to specifically and irreversibly switch on or off the expression of oncogenes or tumor suppressor genes.

►Bioluminescence Imaging

C-Reactive Protein

Definition

CRP, is an acute-phase protein that binds to phosphorylcholine, which is a constituent of the C-polysaccharide of the bacterium *Streptococcus pheumoniae*, hence its name. Many other bacteria also have surface phosphorylcholine that is accessible to C-reactive protein, so the protein can bind many different bacteria and opsonize them for uptake by phagocytes. C-reactive protein does not bind to mammalian tissues.

►Sjögren Syndrome

CREB-Binding Protein (CBP)/p300

Definition

CBP and its homologue p300 are transcription co-activators of various GSTFs and are engaged in a wide array of cellular activities, such as DNA repair, cell growth, differentiation and apoptosis. These proteins might be considered as tumor suppressors, albeit their prominent role is the cross-coupling of distinct gene expression patterns in response to various cues.

►Retinoid Receptor Cross-talk
►p300/CBP Co-Activators

CREB/cAMP Signaling

Definition

CREB transcription factors regulate the expression of target genes with a broad functional role including the metabolic regulation of tissues.

►Mucoepidermoid Cancer

Cre-Lox Recombination

Definition

The Cre/lox system is used as a genetic tool to control site specific recombination events in genomic DNA. The system begins with the Cre protein, a site-specific DNA recombinase. Cre can catalyze the recombination of DNA between specific sites in a DNA molecule. These sites, known as *loxP* sequences, contain specific binding sites for Cre that surround a directional core sequence where recombination can occur.

When cells that have *loxP* sites in their genome express Cre, a reciprocal recombination event will occur between the *loxP* sites. The double stranded DNA is cut at both *loxP* sites by the Cre protein and then ligated (glued) back together. It is a quick and efficient process. The effect of recombination depends on the orientation of the *loxP* sites. For two lox sites on the same chromosome arm, inverted *loxP* sites will cause an inversion, while a direct repeat of *loxP* sites will cause a deletion event. If *loxP* sites are on different chromosomes it is possible for translocation events to be catalyzed by Cre induced recombination.

►Orphan Nuclear Receptors

Cremophor EL

Definition

A polyoxyethylated castor oil vehicle, and dehydrated ethanol (1:1, v/v), used to solublize some drugs that are poorly soluble in water.

►Paclitaxel

Cripto-1

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Synonyms

Teratocarcinoma-derived growth factor-1; TDGF-1

Definition

Human Cripto-1 is a cell membrane associated protein important for embryonic development, stem cell renewal and tumorigenesis.

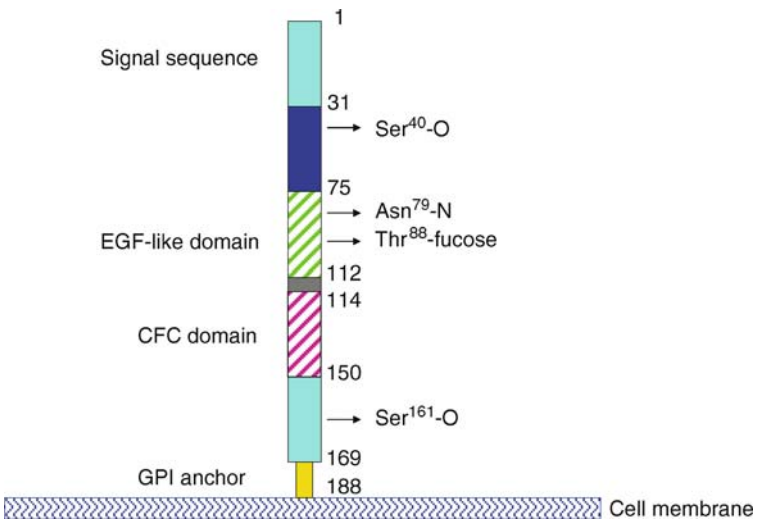
Characteristics

Structure of Cripto-1, a Member of the EGF-CFC Protein Family

Human Cripto-1 (CR-1), originally identified from a human embryonal carcinoma cDNA library, is the founding member of the Epidermal Growth Factor ►(EGF)-CFC (Cripto in humans, FRL1 in *Xenopus* and Cryptic in mice) family of proteins identified only in vertebrates. The ►EGF-CFC protein family includes monkey Cripto-1, mouse Cripto-1 (Cr-1), chicken Cripto-1, zebrafish *one-eyed pinhead* (*oep*), *Xenopus* FRL1 and mouse and human Cryptic. EGF-CFC proteins contain multiple domains consisting of an amino-terminal signal peptide, a modified EGF-like domain, a cystein-rich CFC motif and a short hydrophobic carboxy-terminus containing, in some cases, consensus sequences for a ►glycosylphosphatidylinositol (GPI) anchorage site that serves to attach the protein to the cell membrane (Fig. 1). EGF-CFC proteins are mostly found to be cell membrane-associated. However, human CR-1 can also be detected in the conditioned medium of several cancer cell lines and in the plasma of colon and breast cancer patients, probably by cleavage of the ►glycosylphosphatidylinositol (GPI) linkage by GPI-specific enzymes. An overall sequence identity of approximately 30% exists between the EGF-CFC members across different species. Within the EGF-like domain there is a 60–70% sequence similarity, whereas in the CFC-motif the similarity ranges from 35 to 48%. The modified EGF-like domain corresponds to a region of approximately 40 amino acids containing six cysteine residues. Whereas the canonical EGF-like domain that is present in the EGF family of growth factors (►Epidermal Growth Factor Receptor Ligands) such as EGF, transforming growth factor α (TGFα) and heregulins, contains three loops (A, B and C) due to the presence of three intramolecular disulfide bonds, the variant EGF-like domain in the EGF-CFC proteins lacks the A loop, has a truncated B loop and possess a complete C loop. The presence of this unusual EGF-like domain explains the observation that CR-1 does not directly bind to any of the known *erb*B type I tyrosine kinase receptors including the EGF receptor, *erb*B2, *erb*B3 and *erb*B4. EGF-CFC proteins are glycoproteins that range from 171 to 202 amino acids with an unmodified core protein of 18–21 kDa in size. The native mouse and human Cripto-1 proteins are 24, 28 and 36 kDa in size, although proteins ranging in size from 14 to 60 kDa have been identified in mouse and human normal tissues. This variation in size could be due to the removal of the hydrophobic signal peptide and to posttranslational modifications of the core protein. In fact, all the members of the EGF-CFC family, except for *oep*, are glycoproteins that contain a single N-►glycosylation site and potential O-glycosylation sites (Fig. 1). A single O-linked ►fucosylation site (Fig. 1) has been identified within the EGF-like domain of human CR-1 and a single point mutation in the fucosylation consensus sequence results in the loss of Cripto-1-dependent Nodal signaling (see below).

Cripto-1 During Embryonic Development and in Embryonic Stem (ES) Cells

Cripto-1 functions as a co-receptor for the TGFβ family ligands, Nodal and Vg1/growth and differentiation factor 1 and 3 (GDF1 and 3), during early vertebrate embryogenesis. Genetic studies in zebrafish and mice have defined an essential role for Nodal that functions through Cripto-1 in the formation of the primitive streak,



Cripto-1. Figure 1 Schematic diagram of the human Cripto-1 protein domains. Sites of glycosylations are indicated by *arrows*.

patterning of the Anterior/Posterior axis, specification of the mesoderm and endoderm during gastrulation and establishment of Left/Right asymmetry of developing organs. Cripto-1-dependent Nodal signaling depends upon the Activin type II (Act RII) and type I (Alk4) serine/threonine kinase receptors that activate the Smad-2/Smad-3 intracellular signaling pathway (►Smad Proteins in TGFβ Signaling). Evidence from several studies suggests that Cripto-1 recruits Nodal to the Act RII/Alk4 receptor complex by interacting with Nodal through the EGF-like domain and with Alk4 through the CFC domain. Cr-1 null mice die at day 7.5 due to their inability to gastrulate and form appropriate germ layers. Disruption of Cr-1 in Cr-1$^{-/-}$ embryos results in the formation of embryos that possess a head without a trunk, demonstrating that there is a severe deficiency in mesoderm and endoderm without a loss of anterior neuroectoderm formation. Homozygous knock out of the Cr-1 gene in pluripotential ►embryonic stem cells (ES cells) impairs their ability to differentiate *in vitro* into cardiomyocytes without affecting the ability of ES cells to differentiate in other cell types. In fact, Cr-1$^{-/-}$ ES cells show extensive neuronal differentiation *in vitro* and *in vivo*, suggesting that Cripto-1 could represent a key molecule required for both induction of cardiomyocyte differentiation and repression of neural differentiation. In this regard, Cr-1$^{-/-}$ ES cells, when transplanted *in vivo* at low doses, generate a pool of dopaminergic cells that are able to induce behavioral and anatomical recovery in animal models of Parkinson's disease. It has recently been established that Cripto-1 is a ►stem cell marker in mouse and human ES cells and in conjunction with Nanog, Nodal, Oct3/4 and GDF3 is involved in maintaining self-renewal and pluripotentiality of ES cells. Since malignant ES cells are probably the most appropriate targets for therapy in cancer, stem cell markers could be used as a signature to identify adult tissue cancer stem cells (►Stem Cells and Cancer).

Cripto-1 in Mammary Gland Development

Ovarian hormones and several growth factors, such as TGFβ, TGFα, EGF and insulin ►like growth factor, have been shown to play a crucial role in the regulation of the development and maturation of the mammary gland. In the mouse mammary gland, Cr-1 is detected during different stages of postnatal mammary gland development. In fact, Cr-1 protein has been detected in 4–12-week-old virgin, midpregnant and lactating mouse mammary gland. In the virgin pubescent mammary gland Cr-1 expression is observed in the cap stem cells of the growing terminal end buds and in the ductal epithelial cells from pregnant and lactating mice. Expression of Cr-1 in ductal epithelial cells is enhanced by approximately 3- to 5-fold during pregnancy and lactation. Further support for CR-1 regulation of mammary epithelial cells derives from data showing that CR-1 can modulate milk protein expression in ►HC-11 mouse mammary epithelial cells. HC-11 mouse mammary epithelial cells express the milk protein β-casein after exposure to the lactogenic hormones dexamethasone, insulin and prolactin (DIP). Prior treatment of ►HC-11 cells with exogenous CR-1 during logarithmic growth induces a competency response to DIP with respect to the induction of the milk protein β-casein. In contrast, simultaneous treatment of HC-11 cells with CR-1 in the presence of DIP inhibits β-casein expression. This inhibitory effect of CR-1 on milk protein expression may be biologically significant since soluble CR-1 protein can be found in human milk.

Cripto-1 in Transformation, Tumorigenesis and Angiogenesis

A first clue to the biological activity of Cripto-1 derives from studies demonstrating the ability of human CR-1 to transform mouse NIH-3T3 fibroblasts, mouse NOG-8 and mouse CID-9 mammary epithelial cells *in vitro*. However, NOG-8 and CID-9 cells transformed cells are unable to form tumors in nude mice, suggesting that additional genetic alterations are necessary to complete the tumorigenic phenotype *in vivo*. Further support for the transforming potential of Cripto-1 derives from studies showing increased expression of Cripto-1 in cells transformed by different oncogenes. In this regard, Ha-*ras* (►Ras) has been shown to upregulate Cr-1 expression in rat CREF embryo fibroblasts or rat FRLT-5 thyroid epithelial cells. Also, v-*ras*/Smad-7 transformed keratinocytes develop skin tumors that overexpress CR-1 and TGFα, suggesting that Smad-7 induces tumor formation through upregulation of CR-1 and other EGF-related peptides. Overexpression of Cr-1 in EpH4 mouse mammary epithelial cells increases cell proliferation, anchorage-independent growth in soft agar and the formation of branching structures when the cells are cultured in a three-dimensional type I collagen gel matrix. Furthermore, EpH4 Cr-1 cells show an increase in their migratory behavior in ►Boyden chamber studies and in ►wound healing assay. Exogenous CR-1 protein is also able to stimulate chemotaxis of wild-type EpH4 cells and can induce scattering of NOG-8 mouse mammary epithelial cells grown at low density as colonies on plastic. The scattering effect is characterized by a change in morphology of the epithelial cells to a more fibroblastic-like phenotype and by a decrease in cell-cell adhesion due to reduction in ►E-cadherin expression. These findings suggest that Cripto-1 may play a role in inducing ►epithelial to mesenchymal transition (EMT) of mammary epithelial cells. In fact, MCF7 breast cancer cells overexpressing CR-1 show increased invasion through matrix-coated membranes and mammary hyperplasias and tumors from MMTV-CR-1 ►transgenic mice (see below) show a

dramatic reduction in the levels of expression of the adhesion molecule E-chaderin, whereas the mesenchyme cell cytoskeleton component, vimentin, is significantly increased. Regulation of cell proliferation, cell motility and survival by CR-1 is dependent upon activation of two major intracellular signaling pathways, the *ras*/*raf*/mitogen-activated protein kinase (MAPK) (►Map Kinase) and phopshatidylinositol 3′ kinase (PI3K)/Akt (►AKT Signal Transduction Pathway in Oncogenesis) signaling pathways. Activation of these two intracellular signaling pathways is independent of Nodal and Alk4, since CR-1 can activate MAPK and Akt in EpH4 mammary epithelial cells and MC3T3-E1 osteoblast cells that lack Nodal and Alk4 expression, respectively. Activation of these two signaling pathways is mediated by binding of CR-1 to the GPI-linked heparan sulfate proteoglycan Glypican-1, which can then activate the cytoplasmic tyrosine kinase c-src triggering activation of MAPK and Akt. Finally, an intact c-src kinase is required by CR-1 to induce *in vitro* transformation and enhance migration in mammary epithelial cells.

In addition to regulating cell proliferation and transformation, CR-1 plays an essential role in tumor ►angiogenesis. In fact, CR-1 has a strong angiogenic activity *in vitro* in cultured ►human umbilical vein endothelial cells (HUVECs), stimulating proliferation, migration, invasion and differentiation of HUVECs into vascular-like structures when the cells are grown in ►Matrigel. Furthermore, recombinant CR-1 protein stimulates new blood vessel formation in silicone cylinders filled with Matrigel implanted under the skin of nude mice and microvessel formation in response to CR-1 is significantly inhibited *in vivo* by an anti-CR-1 blocking mouse monoclonal antibody. Finally, tumor xenografts that develop from CR-1 overexpressing MCF-7 breast cancer cells in the cleared mammary fat pad of nude mice have a significantly higher microvessel density than tumor xenografts that form from control MCF7 cells.

Transgenic Mouse Models Overexpressing CR-1 in the Mammary Gland

Transgenic mouse models have shown that overexpression of a human CR-1 transgene in the mouse mammary gland under the control of the ►mouse mammary tumor virus (MMTV) or ►whey acidic protein (WAP) promoter results in mammary hyperplasias and adenocarcinomas. Virgin MMTV-CR-1 transgenic mice exhibit enhanced ductal branching, intraductal hyperplasias and hyperplastic alveolar nodules. Approximately 30–40% of multiparous female mice develop papillary adenocarcinomas. The relatively long latency period suggests that additional genetic or regulatory alterations are required to facilitate mammary tumor formation in conjunction with CR-1. Unlike the MMTV promoter that starts to be active in the virgin mammary gland, the WAP promoter is maximally expressed at mid-pregnancy and lactation. Approximately 50% of old nulliparous WAP-CR-1 mice develop multifocal intraductal hyperplasias and more than half of multiparous WAP-CR-1 female mice develop multifocal mammary tumors of mixed histological subtypes. These tumors are a mixture of regions containing glandular, papillary and undifferentiated carcinoma, as well as myoepithelioma and adenosquamous carcinoma. Mammary tumors of mixed histology are normally phenotypes that are associated with transgenic mice that have alterations in the canonical Wnt/β-catenin pathway (►Wnt Signaling). In fact, increased expression of an activated β-catenin has been found in the mammary tumors of WAP-CR-1 transgenic mice, suggesting that a canonical Wnt pathway may be activated in these tumors.

Expression of CR-1 in Human Carcinomas and Premalignant Lesions

CR-1 is overexpressed, relative to noninvolved adjacent tissue, in ∼50–90% of carcinomas that arise in the colon, breast, stomach, pancreas, lung, gall bladder, testis, bladder, ovary, endometrium and cervix. Furthermore, enhanced expression of CR-1 has also been detected in premalignant lesions, such as colon adenomas, intestinal metaplasia of the gastric mucosa and ductal carcinoma *in situ* of the breast. In this respect, the frequency and level of CR-1 expression in colon adenomas and intestinal metaplasia in the stomach are directly correlated with the size, histological subtype and degree of dysplasia in these lesions, suggesting that CR-1 might be an early marker for malignant transformation in these tissues. CR-1 expression has also been detected in approximately 60% of normal colon mucosa specimens from individuals with a high incidence of colon carcinomas, but only in 20% of colon mucosa from low-risk individuals. In addition, expression of CR-1 in the adjacent noninvolved colon epithelium surrounding colon tumors is significantly correlated with increased lymph node involvement and with a higher rate of recurrence of colorectal tumors. Although no significant correlations have been found between CR-1 expression and prognosis, a recent study suggests that CR-1 is an independent prognostic factor in breast cancer. In fact, in more than 100 invasive breast cancers, overexpression of CR-1 has been found more often in high grade and poor prognosis tumors compared to low grade and good prognosis breast cancers and is significantly associated with decreased patient survival. Another study has also demonstrated a significant increase in the plasma levels of CR-1 protein in patients affected by colon and breast carcinomas, suggesting that CR-1 might represent a novel serological marker for breast and colon cancer.

►Serum Biomarkers

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Cripto-1 (Cr-1)

Definition

Is a glycoprotein member of 188 aa. Cr-l has been implicated in development. Expression of Cr-l was reported in trophoblast and embryoblast of 4-day old mouse blastocysts, and the myocardium of developing heart tubes in 8.5 old mice embryos. Transgenic murine embryos lacking Cr-l are devoid of cardiac specific gene expression (α and β- myosin heavy chain, myosin light chains 2A and 2V). Antisense RNA silencing studies showed that inhibition of Cr-1 expression inhibits the growth of human colon carcinoma cell lines expressing this factor.

►Epidermal Growth Factor (EGF)-Like Ligands

Crk

Definition

Adaptor protein with one SH2-domain and two SH3-domains. It is homolog to v-Crk oncogene. Crk is involved in regulation of cell growth, cell motility, and apoptosis.

►Focal Adhesion Kinase

CRLR

Definition

Calcitonin receptor-like receptor, distinct from CTR. Although it has affinity for calcitonin, it generally binds to other CT family of peptides. It requires association with RAMPs for translocation at the membrane, for ligand binding and generation of biological response. Its ligand specificity can be altered by changing the complexed RAMP molecule

►Calcitonin

Crocidolite

Definition

Is an amphibole form of ►asbestos. Iron predominates over magnesium in its composition. Following inhalation, it is strongly carcinogenic.

Crohn Disease

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Synonyms

Inflammatory bowel disease (also includes ulcerative colitis)-related cancer; Nonprimary APC mutation colorectal cancer

Definition

Crohn disease (also: regional enteritis) is a chronic, inflammatory condition of the gastrointestinal tract, usually episodic in nature. Like ulcerative colitis, the other main entity of ►inflammatory bowel disease (IBD), it is linked to the development of colorectal cancer (CRC), patients suffering from Crohn disease having an approximately six times higher change of contracting the disease.

Characteristics

IBD patients, which include the two related conditions of Crohn disease and Ulcerative Colitis, have an increased risk of developing CRC. The risk depends on disease duration, extent of inflammation, presence of primary sclerosing cholangitis, a positive family history of CRC, age of onset, and the degree of endoscopic and histologic activity (►Inflammation in cancer). Furthermore, CRC accounts for about 15% of deaths related to IBD, however, IBD-related colorectal

carcinoma accounts for only 1–2% of all cases of CRC. Although, the number of IBD-related CRC of the total cases of CRC is low, the mortality rate in patients with a diagnosis of CRCs in the setting of IBD is higher than for those afflicted with sporadic cases of CRC (►Cancer epidemiology). Additionally, the risk for CRC is not related to disease activity, patients who are clinically quiescent do not have a lower risk for developing CRC compared to patients who suffer from a more active disease history. Moreover, during the last decades the incidence of IBD has continued to rise worldwide, reaching incidence rates of 16.6/100,000 in North America and 9.8/100,000 in Europe. IBD, the hereditary syndromes of familial adenomatous polyposis (FAP), and hereditary nonpolyposis colorectal cancer (HNPCC) are together the top three of high-risk conditions for CRC (►Colon cancer; ►Gastrointestinal tumors; ►Peutz-Jeghers-syndrome).

Chemoprevention of IBD-Related CRC

Cancer chemoprevention is based on the arrest of one or several steps in the multistep carcinogenesis process, which attempts to block, reverse, or delay carcinogenesis before the development of invasive disease (►Chemoprotectants). Mesalazine (5-aminosalicylic acid (5-ASA)), for which there is long-term clinical experience in the treatment of patients with IBD, is well tolerated, has limited systemic side effects, and has no gastrointestinal toxicity (►Antiinflammatory drugs). In a rodent model of CRC, mesalazine inhibits tumor growth and reduces the number of aberrant crypt foci colorectal ►premalignant lesions, whereas in patients with sporadic polyps or cancer of the large bowel, mesalazine induces apoptosis and decreases proliferation in the colorectal mucosa. Epidemiological data strongly support a chemopreventive role for mesalazine in ulcerative colitis-associated CRC and especially the data published by Eaden et al. show compelling evidence for a protective effect of long-term mesalazine use in patients with ulcerative colitis [1]. Together, these data suggest a chemopreventive role for mesalazine in CRC development.

Wnt/β-Catenin Pathway (Wnt Signaling)

The most important pathway in CRC is the Wnt/β-catenin pathway. Aberrant activation of this pathway by *Apc* or *β-catenin* mutation occurs in FAP (►*APC* gene in familial adenomatous polyposis) and most sporadic CRCs already early in carcinogenesis. In this pathway, Wnt binds to the transmembrane Frizzled receptor, which leads to activation of the cytoplasmic Disheveled (Dsh) protein. Dsh forms a complex with the β-catenin degradation complex, which consists of the adenomatous polyposis coli gene product (*APC*), glycogen synthase kinase-3β (GSK-3β), axin, and β-catenin. In the absence of ►Wnt signaling, β-catenin within this complex is phosphorylated by GSK-3β, and this leads to its rapid degradation via the ubiquitin pathway (►Ubiquitination). In response to Wnt signals, β-catenin is no longer targeted for degradation and accumulates to high levels in the cytoplasm. This stabilized β-catenin translocates to the nucleus where it binds with members of the T-cell factor (Tcf)/lymphoid enhancer factor (Lef) family of transcription factors, and activates the transcription of Wnt target gene expression (►APC/β-catenin pathway). Constitutive activation of this pathway is seen in almost all CRCs, mostly due to a mutation in the *Apc* gene. However, IBD-associated CRC is usually not associated with *Apc* mutations. Low prevalence of truncating mutations in the *Apc* gene is also seen in esophageal cancers, which is as IBD-associated carcinoma also an inflammatory-related carcinogenesis. Nonetheless, the function of the *Apc* gene is commonly repressed in esophageal carcinomas because the majority of the primary tumors have hypermethylated *Apc* promoter regions. Data on methylation status of *Apc* promoter regions of IBD-associated tumors are not published yet (►Methylation).

This opens a window for drug intervention. We have shown that that using concentrations of mesalazine identical to concentrations seen in patients with IBD, mesalazine inhibits the Wnt/β-catenin pathway via inhibition of protein phosphatase 2A. However, little is known about aminosalicylic acids and Wnt/β-catenin pathway. Hence, much more research should be conducted investigating the effect of NSAIDs on this important pathway in colon cancer.

Conclusions

Chronic inflammation of the Colon, as observed in Crohn disease and ulcerative colitis is closely linked to the development of CRC. The pathogenesis of this disease is different from the classical pathway, involving loss of APC function. Chemoprevention of CRC-development, using mesalazine is essential in this patient group.

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Cross-links

Definition

Are chemical bonds that link one polymer molecule to another. They can be covalent bonds or ionic bonds. Drugs used in anticancer therapy often act by inducing intrastrand and interstrand DNA cross-links, as well as DNA-protein cross-links.

Cross Match

Definition

Testing of the compatibility between donor and recipient antibodies with recipient and donor cells in order to determine the success of transplant or transfusion.

►Flow Cytometry

Cross Priming

Definition

Synonym cross-presentation; denotes the ability of antigen-presenting cells to take up, process and present extracellular antigens with ►MHC class I molecules to CD8 T cells (cytotoxic T cells). This process is necessary for immunity against tumors and against viruses that do not infect antigen-presenting cells directly.

►DNA Vaccination

Cross-Sectional Study

Definition

A study that examines the relationship between cancer and other factors of interest at one particular time.

►Cancer Epidemiology

Cross Talk

Definition

Coupling of pathways, such that one molecule affects the function of another molecule, e.g. a tyrosine kinase receptor may phosphorylate another receptor and affect its function.

Crow-Fukase Syndrome

Definition

►POEMS Syndrome

CRP55

►Calreticulin

CRP-ductin (mouse)

►Deleted in Malignant Brain Tumours 1

CRT

►Calreticulin

Cryofibrinogen

Definition

Fibrin monomer or soluble fibrin, the intermediate between fibrinogen and fibrin, that precipitates at

refrigerator temperatures and that indicates the presence of disseminated intravascular coagulation with malignancy.

►Coagulopathy

Cryosurgery

Definition

Operative cutting of tissue or the targeted destruction of pathological tissue by induced cold necrosis at temperatures down to –196°C.

►Cryosurgery in Bone Tumors

Cryosurgery in Bone Tumors

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Synonyms

Cold surgery; Freeze surgery

Definition

Operative cutting of tissue or the targeted destruction of pathological tissue by induced cold necrosis at temperatures down to –196°C.

Characteristics

Cryobiology

Cryobiology deals with the physical effects of low temperatures and the changing of temperatures in living tissues. The state or phase (vapor, liquid or solid) of water depends on temperature, pressure and volume. The liquid and solid phase of pure water are in equilibrium at atmospheric pressure and 0°C. By increasing the pressure this temperature (0°C) or freezing point can be lowered. This phenomenon is known as ►supercooling.

When its temperature is lowered, water will show ►vitrification or ►crystallization. Very rapid cooling of pure water will induce vitrification that entails the formation of amorphous, transparent, glasslike structures rather than crystals. Crystallization requires initiating nuclei, for instance an insoluble crystalline impurity). Slow cooling rates of water (<1°C/min) will induce large crystals around a few nuclei. During fast cooling rates many small crystals are formed which are thermodynamically unstable and tend to join each other by recrystallization to minimize their surface energies.

During freezing of solutions, ice crystals remove more and more pure water from the solution, elevating the dissolved solute concentration and lowering the vapor pressure of water to that of ice at the same temperature. In this situation solid and liquid phase coexist and this ►supercooled phase ends with a sudden rise of the temperature due to dissipation of latent heat generated by the recrystallization of the thermodynamically unstable small crystals. This phenomenon takes place at a ►eutectic temperature.

The nature of the tissue responding to low temperatures varies with the intensity of the induced cold. A minor cryogenic injury produces only an inflammatory response; a greater injury will produce tissue destruction. The effects of every physical state on living tissue can be divided in immediate and delayed effects. Immediate destructive properties of ►cryosurgery are the result of mechanical damage due to the formation of ice, whereas the delayed effects are due to progressive failure of the microcirculation (vascular stasis), tissue ischemia and ultimately cell death. When tissue temperature is lowered without reaching subzero temperatures cell metabolism is reduced. This is a reversible process and used to its benefit in cardiac surgery. However, if living tissue is continuously subjected to low, but non-freezing temperatures, cell death will occur.

The freezing of tissue is more complicated since its solvent (water) is divided by cell membranes into extracellular and intracellular compartments. Cell membranes in general easily allow the passage of water, but far less readily allow passage of other solutes. When tissue is subjected to a constant slow lowering of temperature it first enters a supercooled phase. Temperatures of 10–15°C below zero will initiate ice formation in the extracellular compartment. The intracellular compartment remains unfrozen because it contains substances with high and low molecular weight, which lower freezing temperatures. Due to the freezing of water in the extracellular compartment concentration of solutes will rise, creating an osmotic pressure induced transport of water from the intra- to the extracellular compartment. This loss of water will lead to shrinkage of the cell, accompanied by higher concentrations of the solutes, which further prevent the formation of ice in the intracellular compartment.

The shrinkage and high concentration of solutes, especially of salts, may be responsible for cell injury. This phenomenon seems especially of importance

during slow freezing rates. Very rapid cooling induces intracellular ice formation, because there is insufficient time for water leaving the cell to maintain osmotic equilibrium across the cell membrane. Intracellular ice formation is believed to be lethal to the cell. Based on histological investigations it has been shown that intracellular ice causes mechanical damage to the membrane, and disturbs the function of mitochondria and other cell organelles and membranes.

Furthermore masses of frozen cells, closely packed will be subjected to shearing forces of ice formation that will injure the tissue structure. Propagating ice will induce cell damage, regardless of the fact that ice is intra- or extracellular. Intracellular ice has to been shown to propagate from one cell to another via intercellular channels.

During thawing the "behavior" of the ice crystals is dependent on the rate of thawing. In contrast to rapid thawing, slow thawing is accompanied by recrystallization and the crystals can grow to damaging sizes. The damaging effect of these intracellular ice crystals, only formed during rapid freezing can therefore be exploited a second time, if slow thawing is allowed, thereby enhancing recrystallization. On the other hand, if tissues have been cooled slowly, causing shrinkage and intracellular dehydration, rapid thawing may be damaging because the cells are exposed to high electrolyte concentrations.

After thawing there is typically a brief period of vasodilation. Additionally the endothelium of blood vessels is particularly sensitive to freeze-thawing, leading to increased permeability of vascular walls, interstitial oedema, slowing of circulation and platelet aggregation. Capillary obstruction and vascular stasis ensues resulting in tissue ischemia and cell death.

Tumors, Suitable for Cryosurgical Treatment

A number of benign and malignant ►bone tumors can be treated by cryosurgery. These include aneurysmal bone cyst, symptomatic enchondroma, borderline ►chondrosarcoma, low-grade chondrosarcoma, chondroblastoma, chordoma and giant cell tumor of bone. In addition radio- and chemotherapy resistant bone metastases may also be effectively treated. The same goes for benign aggressive soft tissue tumors.

Indications for Cryosurgery

Active, or aggressive benign and low-grade malignant bone tumors are ideally treated by ►extralesional excision (►Marginal excision or ►wide excision). For tumors located in expendable bones, like ribs this is the treatment of choice. However, since most benign and low-grade malignant bone tumors tend to occur in the ►metaphysis and/or ►epiphysis of long bones marginal or wide excision would imply segmental loss of bone, compromising normal growth in children and loss of articular surface. Therefore ►intralesional excision (curettage), combined with a powerful local adjuvant is advocated in tumors in which this combination is equivalent to at least marginal a excision.

Cryosurgery is a powerful adjuvant therapy and the main advantage is that the surgeon is in charge of the local extent beyond the surgical margin (7–12 mm) and is able to customize the treatment for a specific benign or low-grade malignant bone tumor.

Cryosurgical Technique

After sufficient exposure of the tumor, thorough curettage of the tumor is performed. To monitor the intralesional temperature and the local extent of the freeze thermocouples are positioned in and around the lesion. Liquid nitrogen is sprayed in the cavity in every direction, until the whole cavity is wetted and becomes frosted. The duration of the freeze is based on the temperature readings and visual observation. Intralesional temperatures of at least minus 50°C are pursued. After spontaneous thawing, two more cycles of freezing and thawing is done, to destroy tumor cells, which may have survived the previous cycle. Finally the cavity is filled with allograft bone chips and when feasible the defect is reinforced with ►osteosynthesis to prevent pathological fracture of the bone.

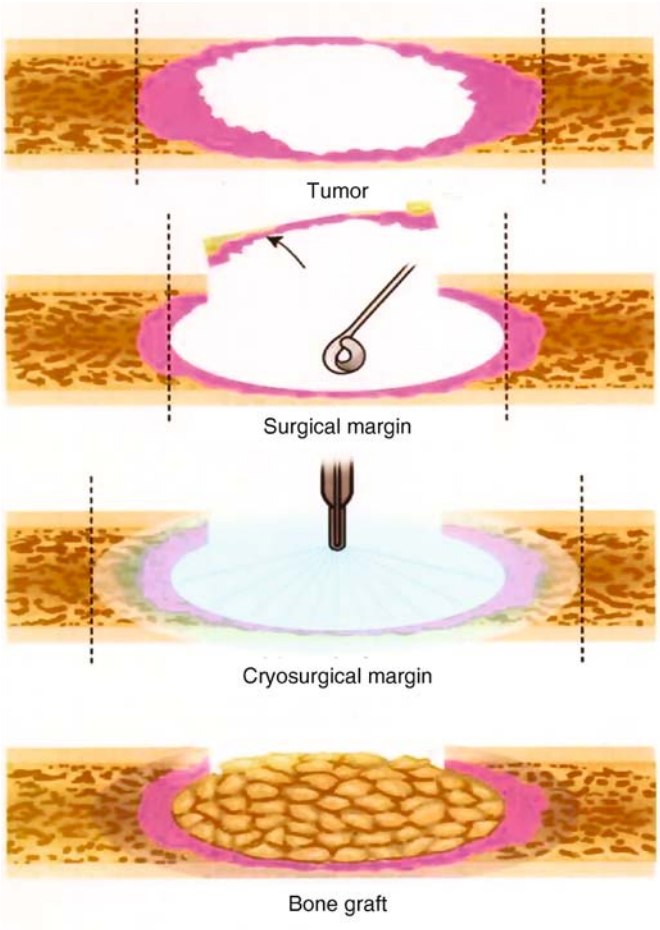
Figure 1 shows this type of cryosurgical therapy for bone tumors.

Results of Treatment

All results will be presented according to the functional evaluation system of the Musculo-Skeletal Tumor Society. Veth et al. reported on 302 patients who had been treated by cryosurgery for a variety of bone and soft tissue tumors. At follow-up 298 of these patients showed NED (no evidence of disease) or CDF (continuously free of disease), whereas 5 were AWD (alive with disease) and 2 DOD (dead of disease). The minimal follow-up period for this review was 2 years.

Of these cryosurgically treated patients 43 had been diagnosed with giant cell tumor of bone, 15 with chondroblastoma, 73 with borderline chondrosarcoma and 44 with chondrosarcoma grade1. Chordoma was diagnosed in seven cases.

Most studies on giant cell tumor report on a rate of local recurrences varying from 0 to 47%. The risk for local recurrence for this tumor is greatly influenced by the method of first surgery. Primary surgery of a giant cell tumor in a non-bone tumor hospital is most likely to induce several local recurrences. The local recurrence rates in chondroblastoma and low-grade chondrosarcoma are respectively 7 and 3%. Figures for chordoma are small and rates of local recurrence vary to a great deal with the extent of the tumor. The functional results according to the MSTS system



Cryosurgery in Bone Tumors. Figure 1 Drawing of cryosurgical technique for bone tumors.

(►MSTS functional evaluation system) for the first 3 tumors are good to excellent in 80% of cases.

Comparing the results it appears, that the risk for local recurrence is small after cryosurgery, compared to different types of treatment and the functional results are at least similar. However an import feature of cryosurgery is that tumor excision is never followed by prosthetic implants, thus the patient keeps a biological reconstruction.

Complications

Possible complications of cryosurgery are:

Wound Infection

Cryosurgery appears to be accompanied by a deep infection rate of about 4%, but this differs between institutions. Sacral lesions are prone for developing an infection.

The following items are of importance in order to avoid infection:

intraoperative broad spectrum antibiotics, adequate drainage of wound fluids, avoidance of accidental freezing of the skin and wound closure with sufficient soft tissue coverage.

Venous Gas Embolism

During cryosurgery liquid nitrogen is either sprayed or poured into the bony cavity and since its boiling point is −195°C nitrogen gasbubbles are rapidly produced at room temperature. In general, whenever a gas is introduced into a body cavity there is the hazard of intravascular introduction of gas bubbles especially when pressure is allowed to develop. Gas emboli in the vascular circulation can cause serious hemodynamic complications.

The risk is increased when the site of the tumor is located in a richly vascularized area such as the metaphysis of the long bones. Unfortunately, this is the location of preference for many bony tumors suitable for cryosurgical treatment.

Fracture

The tumor itself as well as the surgical exposure and resection jeopardize the structural integrity of the bone. Cryosurgery is said to further diminish bone strength

by inducing necrosis of the local bone stock often leading to postoperative fractures.

In the late sixties the pioneers, who used cryosurgery for bone tumors reported rather high fracture rates (up to 10%).

Fractures are most likely to occur 4-8 weeks after the cryosurgical treatment, but they can occur even after 8 months. Diaphyseal lesions are most prone for fracture. Therefore prophylactic internal fixation is advised. Plate and screws are often used, which protects the bone especially from rotating forces. Intramedullary instrumentation is ill advised, because it has the risk of contaminating the entire intramedullary compartment with tumor cells. Titanium alloys are preferred because these implants induce little interference on MRI making tumor follow-up less difficult. Partial weight bearing is usually necessary until three months after the operation.

Experience and improvements in technique have reduced the fracture rate to an acceptable level of 1–2%.

Epiphyseal Damage

Benign bone tumors, especially simple- and aneurysmal bone cysts tend to occur in patients of immature skeletal age. Furthermore these tumors are commonly observed in the metaphysis, often adjacent or very close to the epiphysis. Damage of the epiphysis either by the tumor itself or the use of cryosurgery occurs and may result in arrest or disturbance of normal growth.

Whether an epiphysis is damaged by the bone tumor or by the treatment will not always become clear and in many cases may be the result of both.

Degenerative Osteoarthritis

Some bone tumors like giant cell tumor and chondroblastoma occur almost always extremely close to major joints. Damage of the articular surface either by the tumor itself (intra-articular fracture) or treatment (cryosurgery) may be the result, thus resulting in osteoarthritis.

Damage to Nerves

Nerve palsy is a complication of cryosurgery, which was recognized at the very early beginning of the introduction of cryosurgery for bone tumors.

If nerves are frozen their function is only temporary impaired. Most neuropraxias resulting from freezing will resolve in 6 weeks to 6 months. Very likely regenerating nerve fibers can grow down the nerve sheaths since they are left intact. Furthermore the vital nerve cell nucleus is located away in the dorsal root ganglion. Tourniquets should not be used, in order to keep nerves and skin vascularized and thereby protect them from a freeze injury. Veth et al saw in 302 cryosurgical procedures 10 nerve palsies; only one peroneal nerve failed to regain its function, however not the cryosurgery but surgical traction was very likely the cause of this persistent palsy.

Prospects to the Future

Starting in the mid 1960's, cryosurgery has evolved from a medical tool with limited usefulness for treatment of all kind of tumors into a reliable technique, even for bone tumor patients. A rather recent study showed that 2 instead of 3 freeze-thaw cycles would be sufficient for tumor control. A study by Baust identified apoptosis as a cryosurgery related mechanism of cell death, additive with ice-related cell damage and post-treatment coagulative necrosis. This may provide a possible route to molecular-based optimization of cryosurgical procedures and better results.

The use of cryosurgery in multi recurrent schwannoma of peripheral nerves, chordoma or other sacral tumors as well as its use in bone tumor areas where a peripheral nerve is often involved (proximal fibula) has shown that these nerves may dysfunction after cryosurgery for a period up to 6 months, but in the end mostly recover completely. Additional study on the behavior of nerve tissue during cryosurgery is warranted in order to optimize the temperature for tumor cell kill and reduce the period of nerve dysfunction.

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CSA

Definition

Clonogenic Survival Assay.

CSC

►Stem-like Cancer Cells

CSF

Definition
Colony-stimulating Factor.

Csk

Definition
C-terminal ►Src kinase; Epidermal growth factor receptor (EGFR) and Src tyrosine kinase cooperate in regulating EGFR-mediated cell signaling and promoting cell transformation and tumorigenesis in pathological conditions. Activation of Src is tightly regulated by the C-terminal Src kinase (Csk).

►Fibroelastoma
►Cardiac Tumors
►Epidermal Growth Factor Receptor Ligands

Csk-Binding Protein (Cbp)

Definition
Is a ubiquitously expressed transmembrane protein. Its functions include suppression of T-cell receptor activation through recruiting ►Csk and inhibiting ►Src family kinase (SFK). Cbp functions as a negative regulator of cell transformation and tumor cell growth through downregulation of Src activation, suggesting that Cbp might be broadly involved in ►receptors tyrosine kinases-activated signaling pathways and tumorigenesis.

c-Src

►Src

CT

Definition
Also known as CAT (Computed axial tomography). A non-invasive imaging approach, reliant upon the detection of x-rays, capable of producing high resolution (50–200 μm) 2-D tomographic or 3-D volumetric images of anatomical structures in the body. As x-rays are involved, image resolution is maximized when imaging bone or lung.

►Bioluminescence Imaging
►Computed Tomography

CT4

►GAGE Proteins

CT Scan

Definition
Computed tomography, in which X-rays are passed through the body or body region in a spiral fashion and detected by sensors. A computer then reconstructs a cross-sectional or 3 dimensional image in any desired plane.

C-Tail

Definition
C-terminal tail portion of G protein-coupled receptors, which remains inside the cytoplasm of cells and is shown to interact with intracellular proteins to form signaling scaffolds

►Calcitonin

CTCAE

Definition
Common Terminology Criteria for Adverse Events (National Cancer Institute (NCI)) version 3.0 is a descriptive terminology which can be utilized for Adverse Event reporting. A grading (severity) scale is provided for each Adverse Event term (available at: http://ctep.cancer.gov/forms/CTCAEv3.pdf).

►Irinotecan

CTCF

Definition
"CCCTC-binding factor" is a highly conserved and ubiquitous protein with multiple functions, which include regulation of transcription, chromatin insulation and genomic imprinting.

►BORIS
►CCCTC-Binding Factor

CTCFL

Definition
CTCF-like

►BORIS

CTCF-T

Definition
CTCF-testis specific

►BORIS

CTL

Definition
Cytotoxic T lymphocyte, CD8 + T cell that mediates a cytolytic T cell response upon antigen encounter and appropriate activation. A lymphocyte that is able to kill foreign cells marked for destruction by the cellular immune system.

►Chimeric T Cell Receptors
►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

C-type Lectins

Definition
Lectins, such as selectins, that need Ca^{2+} ions for their glycan binding.

►Glycobiology

CUB Domain

Definition
a protein domain originally identified in ►complement factor, urchin EGF-like protein and BMP-1.

►Platelet-Derived Growth Factor

CUL4A (Cullin4A)

Definition
Through a three-enzyme (E1–E2–E3) cascade, the attachment of ubiquitin to proteins is catalyzed by E3 ubiquitin ligase. The superfamily of the cullin-RING complexes is conserved from yeast to human and the DDB1–CUL4–ROC1 complex is a cullin-RING

ubiquitin ligase which regulates DNA repair, DNA replication and transcription. CUL4A is implicated in ubiquitination of p27, ►JUN and HOX proteins.

►NUP98-HOXA9 Fusion

CUP

Definition

Cancer of Unknown Primary; Presence of lymph node or organ metastases without clinical verification of the primary tumor manifestation site.

►Positron Emission Tomography
►Ascites

Curative

Definition

Treatment given with the aim of destroying the disease (cancer).

►Ionizing Radiation Therapy

Curcumin

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Synonyms

1,7-bis [4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione; Diferuloylmethane; $C_{21}H_{20}0_6$; $[HOC_6H_3(OCH_3)CH{:}CHCO]_2CH_2$; E100; C.I. 75300; Turmeric yellow; Brilliant Yellow S; Natural Yellow 3

Definition

Curcumin (MW 368,3862) (Fig. 1a) is the active ingredient of the natural Indian spice curcuma obtained from the root of the plant *Curcuma Longa L* also called Turmeric, which is a member of the ginger family, *Zingiberaceae* (Fig. 1b). In Ayurvedic medicine, turmeric is thought to have many healthful properties.

Curcumin is known to exhibit anti-oxidant, anti-inflammatory, anti-sceptic, anti-cancer and wound healing properties with low cytotoxicity [1]. Furthermore, it modulates molecular targets including cytokines and growth factors such as tumor necrosis factor (TNF) or interleukins, transcription factors including ►nuclear factor kappa B (NF-κB), ►signal transducer and ►activator of transcription (STAT) 3 or ►AP-1 (activating protein-1) [2]. Pro-inflammatory enzymes like COX-2 (cyclooxygenase 2), LOX (lipoxygenase) and iNOS (inducible nitric oxide synthase), as well as the ►protein kinases c-Jun N-terminal kinase (JNK), protein kinase A (PKA), Janus kinase (Jak) and inhibitor of κB (IκB) alpha were also shown to be inhibited by curcumin [3].

Characteristics

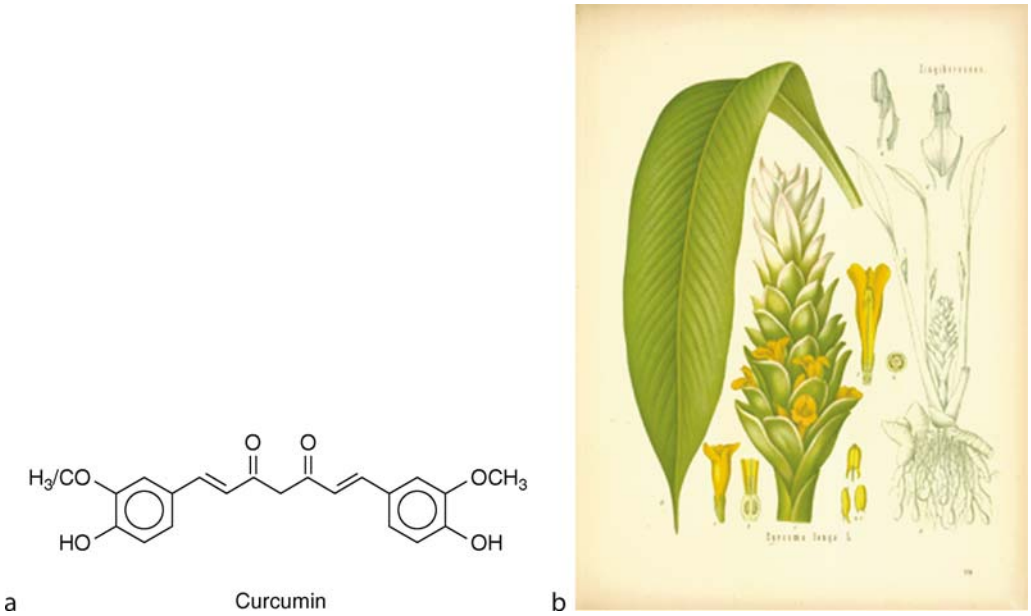
Anti-Carcinogenic Activities

Curcumin inhibits tumor initiation, promotion and progression. It has anti-angiogenesis activities by inhibiting vascular endothelial growth factor (VEGF), angiopoietin 1 and 2 as well as tyrosine kinase Flk-1/KDR (VEGF receptor-2) in various cellular models.

Curcumin leads to reduced expression levels of oncogenes including c-jun, c-fos, c-myc, NF-κB inducing kinase (NIK), mitogen activated protein kinases (MAPK; ►MAP kinase)s, extracellular regulated kinase (ERK), ELK-1, phosphoinosititde-3 kinase (PI3K), protein kinase B (PKB/►Akt) and cyclin dependent kinases (CDK)s. Curcumin can inactivate 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-induced activation of protein kinase C (PKC). β-catenin-mediated transactivation is also inhibited and leads to growth inhibition of colon ►cancer cells.

Effect on Cell Signaling Pathways

Curcumin induces ►apoptosis signaling in cancer cells including HL-60, K562, MCF-7 and HeLa.without cytotoxic effects on normal cells. In human kidney carcinoma cells, apoptosis is initiated by Akt dephosphorylation, B-cell leukemia/lymphoma (Bcl) -2, Bcl-XL and inhibitor of apoptosis (IAP) protein inhibition, as well as cytochrome c release and ►caspase 3 activation. Curcumin induces caspase 8 and 9 whereas p53 levels remain unchanged. Curcumin is also able to inhibit chemotherapeutic effects by reducing camptothecin-, mechlorethamine- or doxorubicin-induced apoptosis in breast cancer cells. Curcumin inhibits the activation of NF-κB and AP-1. IκB kinase (IKK) inhibition by curcumin blocks both IκBα phosphorylation and NF-κB p65 translocation and leads to NF-κB inhibition. Curcumin inhibits interleukin (IL) 1α-, TNFα-, TPA-, lipopolysaccharide (LPS)- and thrombin-induced NF-κB activation. Curcumin was described to impair the proteasome-ubiquitin degradation pathways and thus leads to apoptosis.



Curcumin. Figure 1 (a) Molecular structure of curcumin (diferuloylmethane). (Figure modified from ScienceSlides, VisiScience Corp.). (b) *Curcuma longa L.* (© 1995–2004 Missouri Botanical Garden, http://ridgwaydb.mobot.org/mobot/rarebooks).

Curcumin inhibits the signal transduction pathway leading to ►JNK activation at mitogen-activated protein kinase (►MAPKKK). Furthermore, the signaling pathway leading to MAPK p38 activation is attenuated by curcumin in inflammatory bowel disease cells, whereas Akt kinase is completely inhibited in prostate cancer cells. Curcumin was described to act on the Janus Kinase (Jak)-STAT pathway blocking JAK 2 mRNA expression in Bcr-Abl+ human K562 leukemia cells.

Clinical Trials

Clinical trials are aiming to evaluate if curcumin improves the efficacy of gemcitabine in patients with advanced pancreatic cancer. They also evaluate the combination of curcumine with gemcitabine and celecoxib for patients with colon cancer, to assess whether curcumin can regress colorectal adenomatous polyps in patients with familial adenomatous polyposis and to determine the effectiveness of curcumin in reducing the number of aberrant crypt foci in the colon. Curcumin is also under trial to determine the clinical effects in improving the cytopenias of patients with myelodysplastic syndromes.

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Curie

Definition

Ci; ►Radioactivity. 1 Ci corresponds to 3.7×10^{10} decays per second.

Cushing Syndrome

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Definition

Cushing syndrome is a rare disease entity. The overall incidence of Cushing syndrome is ~2–5 new cases per million people per year. Approximately 10% of the new

cases each year occur in children. As in adult patients, in children with Cushing syndrome, too, there is a female to male predominance, which decreases with younger age; there might even be a male to female predominance in infants and young toddlers with Cushing syndrome. The most common cause of endogenous Cushing syndrome is overproduction of adrenocorticotropin (ACTH) from the pituitary; this is called Cushing disease. It is usually caused by an ACTH-secreting pituitary microadenoma and, rarely, a macroadenoma. ACTH secretion occurs in a semiautonomous manner, maintaining some of the feedback of the HPA axis. Cushing's disease accounts for ~75% of all cases of Cushing syndrome in children over 7 years. In children under 7 years, Cushing disease is less frequent; adrenal causes of Cushing syndrome (adenoma, carcinoma or bilateral hyperplasia) are the most common causes of the condition in infants and young toddlers. Ectopic ACTH production is almost unheard of in young children; it also accounts for less than 1% of the cases of Cushing syndrome in adolescents. Sources of ectopic ACTH include small cell carcinoma of the lungs, carcinoid tumors in the bronchus, pancreas or thymus, medullary carcinomas of the thyroid, pheochromocytomas and other neuroendocrine tumors.

Rarely, ACTH overproduction by the pituitary may be the result of oversecretion of corticotropin-releasing-hormone (CRH) by the hypothalamus or by an ectopic CRH source. However, this cause of Cushing syndrome has only been described in a small number of cases, and never in young children. Its significance lies in the fact that diagnostic tests that are usually used for the exclusion of ectopic sources of Cushing syndrome have frequently misleading results in the case of CRH-induced ACTH oversecretion.

Autonomous secretion of cortisol from the adrenal glands, or ACTH-independent Cushing syndrome, accounts for ~10–15% of all the cases of Cushing syndrome. However, although adrenocortical tumors are rare in older children, in younger children they are more frequent. Adrenocortical neoplasms account for 0.6% of all childhood tumors.

Cushing syndrome is a manifestation of approximately one third of all adrenal tumors. A number of adrenal tumors presenting with Cushing syndrome can be malignant: the majority of patients present under age 5, contributing thus to the first peak of the known bimodal distribution of adrenal cancer across the life span. As in adults, there is a female to male predominance. The tumors usually occur unilaterally; however, in 2–10% of patients they occur bilaterally.

Bilateral nodular adrenal disease has been appreciated more recently as a rare cause of Cushing syndrome. Primary pigmented nodular adrenocortical disease (PPNAD) is a genetic disorder with the majority of cases associated with Carney complex, a syndrome of multiple endocrine gland abnormalities in addition to lentigines and myxomas. The adrenal glands in PPNAD are most commonly normal or even small in size with multiple pigmented nodules surrounded by an atrophic cortex. The nodules are autonomously functioning resulting in the surrounding atrophy of the cortex. Patients with PPNAD frequently have periodic Cushing syndrome.

Massive macronodular adrenal hyperplasia (MMAD) is another rare disease, which leads to Cushing syndrome. The adrenal glands are massively enlarged with multiple, huge nodules that are typical, yellow-to-brown cortisol-producing adenomas. Most cases of MMAD are sporadic, although few familial cases have been described; in those, the disease appears in children. In some patients with MMAD, cortisol levels appear to increase with food ingestion (food-dependent Cushing syndrome). These patients have an aberrant expression of the GIP receptor (GIPR) in the adrenal glands. In the majority of patients with MMAD, however, the disease does not appear to be GIPR-dependent; aberrant expression of other receptors might be responsible.

Bilateral macronodular adrenal hyperplasia can also be seen in McCune Albright syndrome (MAS). In this syndrome there is a somatic mutation of the *GNAS* gene leading to constitutive activation of the Gsα protein and continuous, non-ACTH-dependent stimulation of the adrenal cortex. Cushing syndrome in MAS is rare and usually presents in the infantile period (before 6 months of age); interestingly, a few children have had spontaneous resolution of their Cushing syndrome.

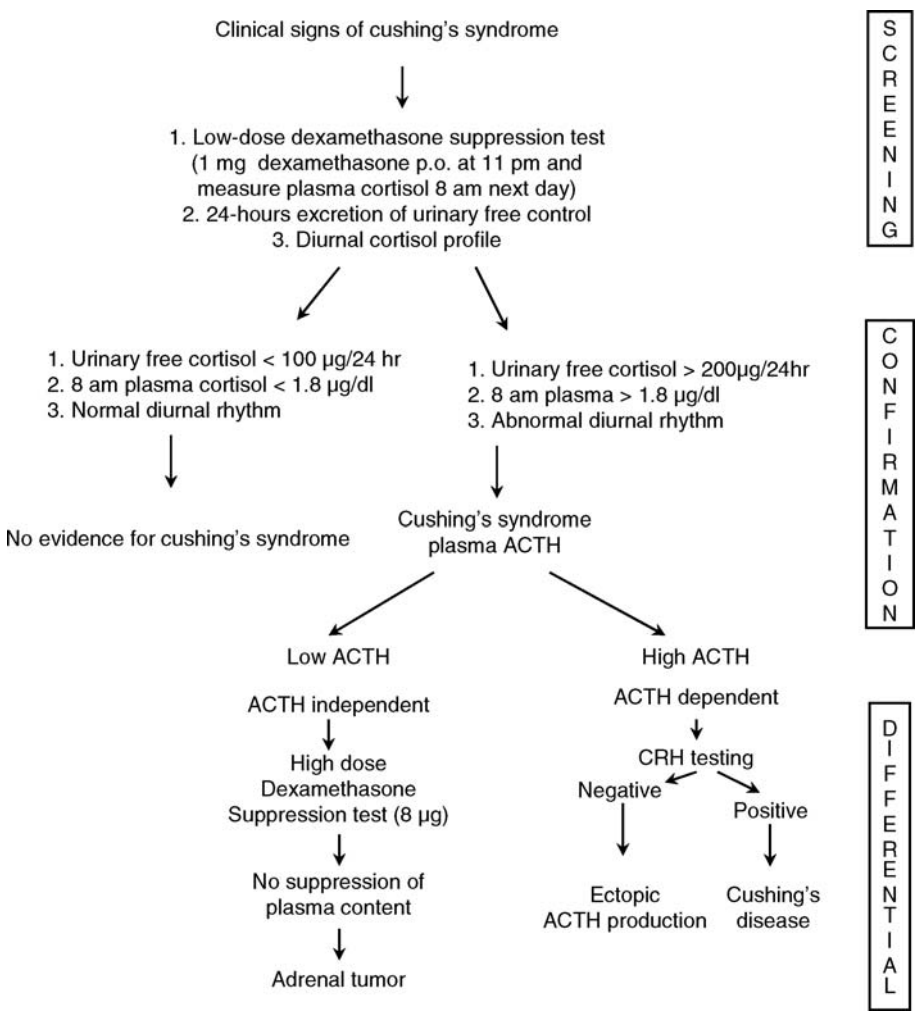
Characteristics

In most patients the onset of Cushing syndrome is rather insidious. The most common presenting symptom of the syndrome is weight gain, although it is not universally present. Almost pathognomonic for Cushing syndrome in childhood is weight gain associated with growth retardation; the combination of the two is among the most consistent and frequently encountered signs. Other common problems reported in patients include facial plethora, headaches, hypertension, hirsutism, amenorrhea, and delayed sexual development. Other patients may present with virilization. Skin manifestations, including acne, violaceous striae and bruising and acanthosis nigricans are also common. In comparison to adult patients with Cushing syndrome, symptoms that are less commonly seen include sleep disruption, weakness and mental changes.

Diagnostic Guidelines

The appropriate therapeutic interventions in Cushing syndrome depend on accurate diagnosis and classification of the disease. The history and clinical evaluation, including growth charts in children, are important to make the initial diagnosis of Cushing syndrome. Upon suspicion of the syndrome, laboratory and imaging

confirmations are necessary. An algorithm of the diagnostic process is presented in the attached diagram (Fig. 1). The first step in the diagnosis of Cushing syndrome is to document hypercortisolism. This step is usually done in the outpatient setting. Because of the circadian nature of cortisol and ACTH, isolated cortisol and ACTH measurements are not of great value in diagnosis. One excellent screening test for hypercortisolism is a 24-h urinary free cortisol (UFC) excretion corrected for body surface area. A normal 24-h UFC value is <70 μg/m^2/day. A 24-h urine collection is often difficult for parents to do in children and may be done incorrectly, especially in the outpatient setting. Falsely high UFC may be obtained because of physical and emotional stress, chronic and severe obesity, pregnancy, chronic exercise, depression, alcoholism, anorexia, narcotic withdrawal, anxiety, malnutrition and excessive water intake (more than 5 l/day). These conditions may lead to sufficiently high UFCs to cause what is known as pseudo-Cushing syndrome. On the other hand, falsely low UFC may be obtained mostly with inadequate collection. Another baseline test for the establishment of the diagnosis of Cushing syndrome is a low dose dexamethasone suppression test. This test involves giving a 1 mg of dexamethasone at 11 P.M. (adjusted for weight for children <70 kg by dividing the dose by 70 and multiplying by the weight of the child) and measuring a serum cortisol level the following morning at 8 A.M. The problem with this test is that it has not been evaluated extensively in children; for adult patients, the cortisol cut-off level should be <1.8 μg/dl (50 nmol/l). If it is greater than 1.8 μg/dl, further evaluation is necessary. This test has a low percentage of false normal suppression; however, at our institution, very rarely we obtain the 1-mg test for screening for Cushing syndrome in children. It should also be noted



Cushing Syndrome. Figure 1 Algorithm of the diagnostic process.

that the 1-mg overnight test (like the 24-h UFCs), does not distinguish between hypercortisolism from Cushing syndrome and other hypercortisolemic states.

If the response to both the 1-mg dexamethasone overnight suppression test and the 24-h urinary free cortisol are both normal, a diagnosis of Cushing syndrome may be excluded with the following caveat: 5–10% of patients may have intermittent or periodic cortisol hypersecretion and may not manifest abnormal results to either test. If periodic or intermittent Cushing syndrome is suspected, continuous follow up of the patients is recommended. Diurnal plasma cortisol variation, including midnight cortisol values, is a fairly good test for the establishment of the diagnosis of Cushing syndrome. In our institution, it has become the test of choice for the confirmation of endogenous hypercortisolemia and is routinely done in patients with confirmed elevated urinary cortisol levels on the outside. There are several caveats for the interpretation of the test of which the most important ones are: (i) The venous catheter has to be placed at least two hours before the test; and (ii) if the patient comes from another time zone, a 1 h per day adjustment should be taken into account prior to obtaining the test. In general, serum cortisol levels are drawn at 11:30 P.M. and 12:00 MN and at 7:30 A.M. and 8:00 A.M., while the patient is lying in bed and asleep; mid-night cortisol levels above 5 μg/dl are abnormal and confirm the diagnosis of Cushing syndrome, whereas an inverted diurnal rhythm is seen in PPNAD and some other adrenal tumors.

If one of the tests is suggesting Cushing syndrome or, if there is any question about the diagnosis, tests that distinguish between pseudo-Cushing states and Cushing syndrome may be obtained. One such test is the combined dexamethasone-CRH test. In this test the patient is treated with low dose dexamethasone (0.5 mg adjusted for weight for children <70 kg by dividing the dose by 70 and multiplying by the weight of the child) every 6 h for eight doses prior to the administration of CRH (ovine CRH – oCRH) the following morning. ACTH and cortisol levels are measured at baseline and every 15 min for one hour after the administration of oCRH. The patient with a pseudo-Cushing state will exhibit low or undetectable basal plasma cortisol and ACTH, and have a diminished or no response to oCRH stimulation. Patients with Cushing syndrome will have higher basal cortisol and ACTH levels and will also have a greater peak value with oCRH stimulation. The criterion used for the diagnosis of Cushing syndrome is a cortisol level of greater than 38 nmol/l (~1.4 μg/dl) 15 min after oCRH administration; all other patients (<1.4 μg/dl) may suffer from a pseudo-Cushing state.

Once the diagnosis of Cushing syndrome is confirmed there are several tests to distinguish ACTH-dependent disease from the ACTH-independent syndrome. A spot plasma ACTH may be measured; if this measurement is <5 pmol/l it is indicative of ACTH-independent Cushing syndrome, although the sensitivity and specificity of a single ACTH measurement are not high because of the great variability in plasma ACTH levels and the instability of the molecule after the sample's collection. Even if one assumes that the sample was collected and processed properly (collected on ice and spun down immediately in a refrigerated centrifuge for plasma separation; the sample should then be immediately processed or frozen at −20°C), ACTH levels that are between 5 and 20 pmol/l are not informative in this era of high sensitivity assays; levels above 20 pmol/l are more suggestive of an ACTH-dependent condition, but again that is not a certainty until single ACTH levels are repeatedly over 70 pmol/l.

The standard six-day low- and high dose dexamethasone suppression test (Liddle's test) is used to differentiate Cushing disease from ectopic ACTH secretion and adrenal causes of Cushing syndrome. In the classic form of this test, after 2 days of baseline urine collection, 0.5 mg of dexamethasone (adjusted per weight for children <70 kg by dividing the dose by 70 and multiplying by the weight of the child) every 6 h are given *per os* starting at 6.00 A.M. on day 3 ("low dose" phase of the test) for a total of eight doses (2 days); this is continued with a 2-mg dose of dexamethasone *per os* (adjusted per weight for children <70 kg by dividing the dose by 70 and multiplying by the weight of the child) on day 5 ("high dose" phase of the test) given every 6 h for another 8 doses (final 2 days) (13–15). Urinary free cortisol and 17-hydroxysteroid excretion are measured at baseline, during, and 1 day after the end of the dexamethasone administration. Approximately 90% of patients with Cushing disease will have suppression of cortisol and 17-hydroxysteroid values, whereas less than 10% of patients with ectopic ACTH secretion will have suppression. Urinary free cortisol values should suppress to 90% of baseline value and 17-hydroxysteroid excretion should suppress to less than 50% of baseline value. The criteria are similar if one uses serum cortisol values obtained at 8 A.M. of the morning after the last dose of dexamethasone, e.g. serum cortisol on day 7 should be 90% of baseline serum cortisol values (obtained at 8 A.M. the day before dexamethasone administration). An increase of urinary free cortisol values of 50% or more over baseline during Liddle test has been used in the differential diagnosis of PPNAD and other micro-nodular adrenocortical disease versus other causes of adrenal causes of Cushing syndrome.

The Liddle test has been modified to (i) giving 2 mg every 6 h (without the preceding low-dose phase);

(ii) administering dexamethasone intravenously over 5 h at a rate of 1 mg/h; or (iii) giving a single high dose of dexamethasone (8 mg, in children adjusted for weight <70 kg) at 11 P.M. and measuring the plasma cortisol level the following morning. This overnight, high dose dexamethasone test has sensitivity and specificity values similar to those of the classic Liddle test. A 50% suppression of serum cortisol levels from baseline is what differentiates Cushing disease (more than 50% suppression) from other causes of Cushing syndrome (adrenal or ectopic ACTH production) (less than 50% suppression).

An oCRH stimulation test may also be obtained for the differentiation of Cushing disease from ectopic ACTH secretion. In this test, 85% of patients with Cushing disease respond to oCRH with increased plasma ACTH and cortisol production. 95% of patients with ectopic ACTH production do not respond to administration of oCRH. The criterion for diagnosis of Cushing disease is a mean increase of 20% above baseline for cortisol values at 30 and 45 min and an increase in the mean corticotropin concentrations of at least 35% over basal value at 15 and 30 min after oCRH administration. When the oCRH and high dose dexamethasone (Liddle or overnight) tests are used together, diagnostic accuracy improves to 98%.

Another important tool in the localization and characterization of Cushing syndrome is diagnostic imaging. The most important initial imaging when Cushing disease is suspected is pituitary magnetic resonance imaging (MRI). The MRI should be done in thin sections with high resolution and always with contrast (gadolinium). The latter is important since only macroadenomas will be detectable without contrast; after contrast, an otherwise normal-looking pituitary MRI might show a hypoenhancing lesion, usually a microadenoma. More than 90% of ACTH-producing tumors are hypoenhancing, whereas only about 5% are hyperenhancing after contrast infusion. However, even with the use of contrast material, pituitary MRI may detect only up to ~30% of ACTH-producing pituitary tumors, although with the use of new modalities (e.g. SPGR-MRI) this percentage may be as high as 60%. Computed tomography (CT) (more preferable than MRI) of the adrenal glands is useful in the distinction between Cushing disease and adrenal causes of Cushing syndrome, mainly unilateral adrenal tumors. The distinction is harder in the presence of bilateral hyperplasia (MMAD or PPNAD) or bilateral adrenal carcinoma (conditions, however, that are rare). Most patients with Cushing disease have ACTH-driven bilateral hyperplasia, and both adrenal glands will appear enlarged and nodular on CT or MRI. Most adrenocortical carcinomas are unilateral and quite large by the time they are detected. Adrenocortical adenomas are usually small, less than 5 cm in diameter and, like most carcinomas, they involve one adrenal gland. MMAD presents with massive enlargement of both adrenal glands, whereas PPNAD is more difficult to diagnose radiologically because it is usually associated with normal or small-sized adrenal glands, despite the histologic presence of hyperplasia.

Ultrasound may not be used to image the adrenal glands for the diagnostic work up of Cushing syndrome, because its sensitivity and accuracy is much less than CT or MRI. A CT or MRI scan of the neck, chest, abdomen and pelvis may be used for the detection of an ectopic source of ACTH production. Labeled octreotide scanning and venous sampling may also help in the localization of an ectopic ACTH source. Since up to 50% of pituitary ACTH-secreting tumors and many of ectopic ACTH tumors can not be detected on routine imaging, and often laboratory diagnosis is not completely clear, catheterization studies must be used to confirm the source of ACTH secretion in ACTH-dependent Cushing syndrome (1–3). Bilateral inferior petrosal sinus sampling (IPSS) may also be used for the localization of a pituitary microadenoma (although not with great accuracy or sensitivity). IPSS is an excellent test for the differential diagnosis between ACTH-dependent forms of Cushing syndrome with a diagnostic accuracy that approximates 100%, as long as it is performed in an experienced clinical center. IPSS, however, may not lead to the correct diagnosis, if it is obtained when the patient is not sufficiently hypercortisolemic or, if venous drainage of the pituitary gland does not follow the expected, normal anatomy. In brief, sampling from each inferior petrosal sinuses is taken for measurement of ACTH concentration simultaneously with peripheral venous sampling. ACTH is measured at baseline and at 3, 5, and 10 min after oCRH administration. Patients with ectopic ACTH secretion have no gradient between either one of the two sinuses and the peripheral sample. On the other hand, patients with an ACTH-secreting pituitary adenoma have at least a 2-to-1 at baseline, and 3-to-1 central-to-peripheral gradient after stimulation with oCRH.

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Cutaneous Desmoplastic Melanoma

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Synonyms

Desmoplastic melanoma

Definition

Desmoplastic (Desmoplasia) ►melanoma (DM) is a rare variant of invasive melanoma of the skin composed of spindle cells surrounded by various degrees of sclerotic stroma. DM has a variable clinical appearance and may mimic several lesions.

Characteristics

Epidemiology

Desmoplastic melanoma (DM) is an uncommon variant of melanoma representing 2–4% of all melanoma cases. Like many melanomas, DM tends to occur in sun-exposed regions of the skin with highest incidence in the head and neck region (>40%), thus suggesting a predilection for chronically sun-damaged skin. Nevertheless, DM may occur anywhere, including acral and mucosal sites. DM predominantly affects elderly individuals; the mean age of onset is consistently 10 years later (~60 years) than it is for patients with conventional (i.e. non-DM) melanoma. DM has a male predominance ratio of ~2:1.

Microscopic Features

Microscopically, DM presents as a poorly circumscribed neoplasm of variable size with dermal and frequently subcutaneous infiltration. There is often an accompanying atypical or frankly malignant melanocytic proliferation in the epidermis. The tumor is present in a sarcoma-like pattern with elongated and usually amelanotic, hyperchromatic fibroblast-like spindle cells arranged singly or in thin fascicles separated from each other by fibrotic stroma. Epitheloid cells may be recognized in the superficial areas of the lesion while in deeper areas the cells are predominantly spindle cell with fibroblast-like features. The spindle cell population varies greatly in appearance: it may be hypocellular and bland with minimal mitotic figures (differential diagnosis: scar, fibromatosis, dermatofibroma) or associated with nuclear atypia and high mitotic activity (differential diagnosis: high-grade sarcoma or sarcomatoid carcinoma). If these latter areas are more than focal, such lesions are not classified as DM but rather as spindle cell melanoma (see below). Focal lymphocytic aggregates are very frequently observed and may aid in diagnosis. By differentiating along Schwannian lines, DM may also mimic neurofibroma, neurotized melanocytic nevus or nerve sheath myxoma. Variants of DM include the "neurotropic" melanoma showing a "neuroma-like" growth pattern with invasion of cutaneous nerves, usually in a spindle cell vertical component with fibrosis. The myxoid (or myxofibrous) variant displays abundant mucinous stroma. DM has been defined as either "pure" if the overwhelming majority of the invasive tumor is desmoplastic or as "combined" or "mixed" DM if the desmoplastic areas constitute less than 90% of the tumor.

The immunohistological profile of DM is different from that of epitheloid melanoma. While S100 is typically positive in 80–94% of cases, other markers often helpful in diagnosing melanoma (►Melanoma antigens) such as HMB45 antigen, tyrosinase, and Melan-A/MART-1, are typically only focally positive or completely negative in DM. Nevertheless, absence of such immunoreactivity does not exclude the diagnosis when the clinical picture and/or histology are characteristic. Inconsistent results regarding the labeling pattern as well as the staining intensity have been noted for antigens such as CD68, NSE (neuron-specific enolase), CD34, and smooth muscle alpha-actin (SMA); as such these markers are usually not helpful in the diagnosis of DM.

Clinical Features

In most cases, DM presents as a slow-growing, painless, and usually amelanotic palpable plaque or nodule that may be associated with a lentigo maligna lesion. The clinical appearance, however, can be variable. Due to its sometimes innocuous appearance and the common lack of pigmentation, it is uncommonly diagnosed at an early stage and thus may be confused with a variety of benign (scars, dermatofibroma, melanocytic nevus) and malignant (carcinoma, sarcoma) lesions. DM commonly presents as a deeply invasive tumor at the time of diagnosis.

Recent studies reported a median tumor thickness between 2.5 and 6.5 mm, and the majority of lesions showed invasion of the reticular dermis or subcutis (►Clark level IV/V). This observation, in conjunction with a commonly described invasion along neural structures (neurotropism) were considered the main reasons for the seemingly locally aggressive biologic behavior and high incidence of local recurrence of 11–55% reported in the literature. Nevertheless, data involving large patient datasets suggest that this may be due to frequent positive, unknown, or relatively narrow

resection margins, particularly in the head and neck region. Excision of DM with a 2 cm margin (e.g. for melanomas over 1 mm in depth) appears to be associated with a reduced rate of local recurrence. This concept has been further substantiated by a recent study involving 65 patients with DM demonstrating no local recurrences after wide local excisions with 2 cm margins.

There is solid evidence to indicate that the incidence of lymph node metastasis in patients with DM is lower than in patients with conventional melanoma of similar thickness, ranging from 0 to 18.8%. With the widespread use of sentinel node biopsy for melanoma, regional lymph node status in patients with DM has often been assessed at the time of diagnosis. So far, all studies assessing SLN status in patients with pure DM showed a lower rate of lymph node involvement as compared to conventional melanoma. Specifically, synchronous microscopic stage III disease among patients with DM of the "pure" histologic subtype is extremely uncommon – i.e. below 3% – and suggests that the behavior of "pure" DM may be more like soft tissue sarcoma, which in general is also associated with a low rate of regional lymph node metastasis. In contrast, DM of the "mixed" phenotype showed a similar rate (~15%) of positive SLN nodes as is generally observed for conventional (i.e. non-DM) melanoma.

Although patients with DM usually present with thicker tumors, survival of patients with DM has repeatedly reported to be equal or better than for other forms of melanoma. The largest study at present reported similar survival rates among patients with desmoplastic and conventional melanoma, with a 5-year survival rate of 75%.

Management

Like any invasive melanoma, wide local excision – with margins appropriate for tumor thickness – represents the mainstay of treatment of the primary DM tumor location. As nerve involvement may be observed, adequate wide excision is in particular advisable in patients with DM whenever possible; such an approach likely enhances local control. The low incidence of regional lymph node metastases in patients with DM suggests that elective (prophylactic) lymph node dissection is not indicated in these patients. While sentinel node biopsy is regarded as the standard of care for patients with intermediate- and high-risk melanoma, only a subset of patients with pure DM may benefit from this procedure due to the expected low yield of positive regional lymph nodes.

In view of studies demonstrating significant local disease control by performing wide local excision with adequate margins, adjuvant postoperative radiation with the aim to reduce the rate of local recurrence may be dispensable in many patients with DM who have had an appropriate wide local excision.

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Cutaneous T-Cell Lymphoma (CTCL)

Definition

A group of lymphoproliferative disorders characterized by localization of neoplastic T lymphocytes to the skin, including mycosis fungoides/Sézary syndrome.

►Mycosis Fungoides
►Sézary Syndrome
►Suberoylanilide hydroxamic acid (SAHA)
►Zolinza
►Vorinostat

CX3CL1/Fractalkine

Definition

A ►chemokine with both cell-adhesive and chemoattractant properties; can be expressed in a transmembrane or soluble form.

►Bone Tropism

CX3CR1

Definition

A receptor expressed on the plasma membrane of normal and malignant cells. CX3CL1/fractalkine is the only know ►chemokine that binds and activates CX3CR1.

►Bone Tropism

Cx26

Definition

Member of the connexin family of gap junction proteins.

►Connexins

Cx32

Definition

Member of the connexin family of gap junction proteins.

►Connexins

CXC Chemokines

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Synonyms

Angiogenic/angiostatic chemokines; ELR+ /ELR- chemokines

Definition

►Chemokines are a large family of small proteins that have been classified into four distinct groups based on the specific pattern of two conserved cysteine residues found within the ►amino terminus. If these two conserved cysteine residues are separated by one aliphatic amino acid, these proteins are classified into the "CXC chemokine" group. CXC chemokines are well known for their role in the immune system. These chemokines mediate ►leukocyte homing to sites of wounding or ►inflammation, thus instigating the immune response. With respect to cancer progression, CXC chemokines have been found to play critical roles in tumor ►angiogenesis, tumor growth, tumor cell invasion, and metastasis.

Characteristics

CXC chemokines are small, soluble proteins that specifically bind and activate their cognate ►cell-surface receptors. CXC chemokine receptors are present on the surface of many different cell types, including endothelial cells, ►epithelial cells, tumor cells, and specific subsets of immune cells. These receptors pass seven times through the plasma membrane and are coupled to heterotrimeric ►G-proteins, through which they mediate ►cell signaling and functional response. Once activated by chemokine ligand, they mediate cellular processes associated with cell motility, cell proliferation, and cell survival.

Chemokines derive their name from the phrase "chemotactic ►cytokines", and function primarily to mediate the process of cellular ►chemotaxis. Chemotaxis refers to the migration of a cell toward an increasing concentration (gradient) of a particular substance (chemoattractant). Therefore, cells expressing CXC chemokine receptors respond to chemokine ligand gradients by migrating in the direction of the increasing concentration of CXC chemokine. Physiological sources of CXC chemokine gradients can include sites of inflammation or sites of active ►wound healing. Interestingly, engagement of CXC chemokine with its cognate receptor not only results in cell movement, but also results in cell proliferation and protection from apoptosis. Therefore, CXC chemokine gradients can act physiologically to recruit immune cells and endothelial cells to stimulate immune response and tissue regeneration. However, dysregulated chemokine expression can either lead to neoplasia and cancer progression, or can result from it. Because of the essential roles that CXC chemokines play in cell survival, proliferation, and chemotaxis, dysregulated chemokine expression has been linked to tumor angiogenesis, tumor growth, tumor cell invasion, and metastatic spread.

Angiogenesis and Tumor Growth

CXC chemokines are intricately involved in the regulation of both physiological and pathological

angiogenesis. Angiogenesis is defined as the growth of new blood vessels from pre-existing vasculature, and is necessary for certain physiological processes such as wound healing, development, or ischemic repair. However, aberrant angiogenesis is not only present within the tumor microenvironment, but is also a requirement of tumor progression and metastatic spread. The more blood vessels that are found within a tumor, the bigger and more invasive that tumor can become. CXC chemokines play a critical role in the regulation of angiogenesis, but can cause dysregulated blood vessel proliferation under tumorigenic conditions.

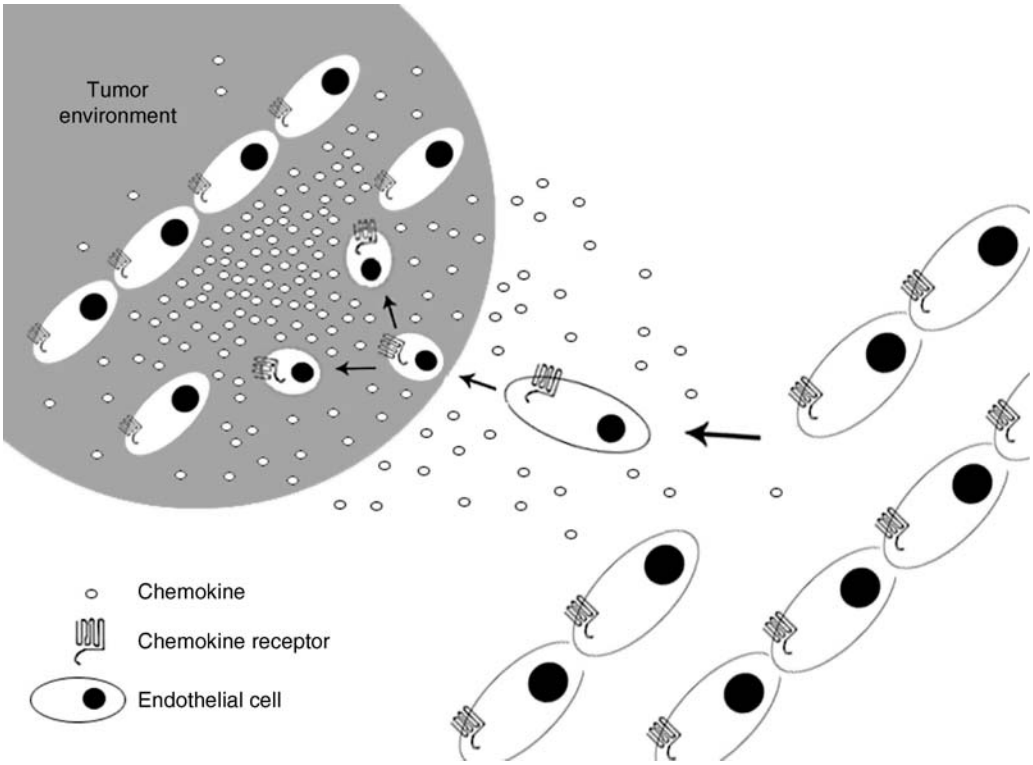
On a cellular level, angiogenesis involves the migration of endothelial cells away from pre-existing blood vessels, toward an angiogenic stimulus. Once these migrating cells reach the stimulus, they proliferate to form new vessel lumen. Because endothelial cells express CXC chemokine receptors, they will migrate toward CXC chemokine gradients produced by various physiological environments, such as areas of active wound repair. Once CXC-receptor expressing endothelial cells have reached the CXC chemokine source, they will begin to proliferate and result in new vascular networks throughout the environment. However, physiological angiogenesis requires that endothelial cell proliferation and migration signals be shut off in a timely manner, to avoid pathological consequences. Interestingly, the stimulus responsible for this inhibition, or ►angiostasis, is actually a subgroup of CXC chemokines, whose expression is tightly regulated. Therefore, CXC chemokines are broken up into two subgroups: the angiogenic CXC chemokines and the angiostatic CXC chemokines (Table 1). Regulated angiogenesis therefore becomes a delicate balance of timing the activation of the angiogenic CXC chemokines with timing the activation of the angiostatic CXC chemokines. Unfortunately, disruption of this balance leads to pathological consequences. For example, many tumor types express elevated levels of the angiogenic CXC chemokines and their cognate receptors. The secretion of these chemokines activates surrounding endothelial cells to migrate into the tumor, proliferate, and form new vasculature within their new environment (Fig. 1). This activation overcomes angiostatic chemokine activity, and leads to increased vessel growth and increased tumor vasculature. Early evidence showed that those CXC chemokines regulating pro-angiogenic activity contained a motif in the amino terminus of the protein consisting of the amino acids glutamic acid, leucine, arginine (ELR). Furthermore, it was noted that those chemokines regulating anti-angiogenic activity did not contain this motif. Therefore, the angiogenic CXC chemokines became known as ELR+, while the angiostatic chemokines were designated as non-ELR+ chemokines (Table 1). However, the one exception to this rule is CXC chemokine CXCL12. CXCL12 is a non-ELR+ chemokine, but it appears to exhibit angiogenic effects via its cognate receptor CXCR4. CXCR4 is found endogenously on the surface of endothelial cells, and its activation by CXCL12 results in cell migration, proliferation, and cell survival. Interestingly, though, the mechanism by which CXCL12/CXCR4 stimulates angiogenesis may be different than the mechanism by which the ELR+ chemokines stimulate a similar process. Instead of the tumor cells themselves secreting the angiogenic chemokine stimulus, it appears that stromal cells within the tumor microenvironment secrete significant amounts of CXCL12. However, the result is the same. Endothelial cells are activated and recruited by CXCL12 chemokine gradient secreted by the tumor, resulting in an increase of vasculature.

CXC Chemokines. Table 1 ELR+/angiogenic chemokines, non-ELR+/angiostatic chemokines, and non-ELR+/angiogenic chemokine CXCL12

Chemokine	Putative chemokine receptor
ELR+/angiogenic chemokines	
CXCL1 (GRO-α)	CXCR2
CXCL2 (GRO-β)	CXCR2
CXCL3 (GRO-γ)	CXCR2
CXCL5 (ENA-78)	CXCR2
CXCL6 (GCP-2)	CXCR2
CXCL7 (NAP-2)	CXCR2
CXCL8 (IL-8)	CXCR2
Non-ELR+/angiostatic chemokines	
CXCL4 and CXCL4L1 (PF-4)	CXCR3
CXCL14	?
CXCL9 (MIG)	CXCR3
CXCL10 (IP-10)	CXCR3
CXCL11 (ITAC)	CXCR3
Non-ELR+/angiogenic chemokine	
CXCL12 (SDF-1)	CXCR4

Adapted from Strieter et al. (2006).

CXC chemokines can not only promote tumor growth through their contribution to angiogenesis, but they can also directly stimulate survival and growth of neoplastic cells in a ►paracrine/autocrine fashion. For example, certain tumor cells can gain expression or overexpression of specific CXC chemokine receptors, and so enhance their response to chemokine stimulation. This stimulation can result in cell proliferation and survival, increasing the size and magnitude of the tumor. In some instances, the tumor cells themselves produce the chemokine stimulus. This production of chemokine and subsequent release into the extracellular space binds and activates chemokine receptor on the tumor cell surface. Activation of chemokine receptor can then signal not only for proliferation and tumor



CXC Chemokines. Figure 1 Endothelial cells are recruited by chemokine gradients produced by tumor cells to induce angiogenesis in the tumor environment.

growth, but also for the production of more chemokine, resulting in an autocrine signaling loop. In other cases, chemokine can be produced by other cells within the tumor microenvironment, such as ►fibroblasts or endothelial cells. This chemokine can then bind to and activate receptor on the surface of the tumor cell, resulting in a paracrine loop that signals for tumor cell proliferation and subsequent tumor growth.

Tumor Cell Invasion and Metastasis

Angiogenesis is not only essential for tumor growth, but is also necessary for the metastatic spread of tumor cells. In order to metastasize, tumor cells must enter the blood stream and travel to distant sites within the body. The more vasculature present within a tumor, the more chance there is for invasive cells to escape the primary mass and enter the blood stream. Furthermore, tumor cell motility is a requirement of metastasis. Tumor cells must actively migrate away from the primary tumor to enter the blood stream and establish distant metastatic sites. Because CXC chemokines are important mediators of cell motility, they have been implicated in tumor cell migration during metastatic progression. Therefore, the angiogenic CXC chemokines contribute to the regulation of metastasis as well as angiogenesis.

Importantly, it has long been unclear why particular cancers preferentially metastasize to certain sites in predictable patterns. For example, breast cancer generally metastasizes to lymph node, bone marrow, liver, or brain. It was hypothesized that tumor cells actually migrate away from their primary tumor toward a chemotractant produced at distant sites within the body. Many types of tumor cells have been found to overexpress CXC chemokine receptor CXCR4, and therefore CXC chemokines were implicated in metastatic spread. Interestingly, high levels of CXCR4 ligand are found in organs such as the liver, bone marrow, brain, and lymph nodes, where cancers typically metastasize. Therefore, it is thought that cancer cells "home" to specific metastatic sites by migrating up the CXCL12 gradient produced by specific organs. Once these tumor cells have reached the point in the gradient that saturates all receptors, they stop migrating and proliferate, resulting in a metastatic tumor.

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C-X-C Motif Chemokines

Definition

Also α-chemokines; Refers to a subgroup of ►chemokines. The two N-terminal cysteines of CXC chemokines are separated by one amino acid, represented in this definition with an "X."

►CXC Chemokines
►CXCL11/I-TAC

CXCL11/I-TAC

Definition

CXCL11 (chemokine (C-X-C motif) ligand 11)/I-TAC (interferon-inducible T-cell alpha chemoattractant) is a small cytokine belonging to the CXC chemokine family, binds and activates CXCR3, and is chemotactic for activated T cells.

►Langerhans Cell Histiocytosis

CXCL12

Definition

Chemokine (C-X-C motif) ligand 12 or stromal-derived-factor-1 (SDF-1) is a small cytokine strongly chemotactic for lymphocytes. The receptor of CXCL12 on leukocytes is CXCR4.

►Stromelysin-1

Cyclin B

Definition

Is a major mitotic cyclin, which binds to Cdk1, active during cell cycle and is polyubiquitinated in mitosis.

►Forkhead Box M1

Cyclin D

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Definition

D type cyclins belong to a family of related proteins that bind to and activate several protein kinases named ►cyclin-dependent kinases (CDKs), which are involved in regulation of the cell division cycle.

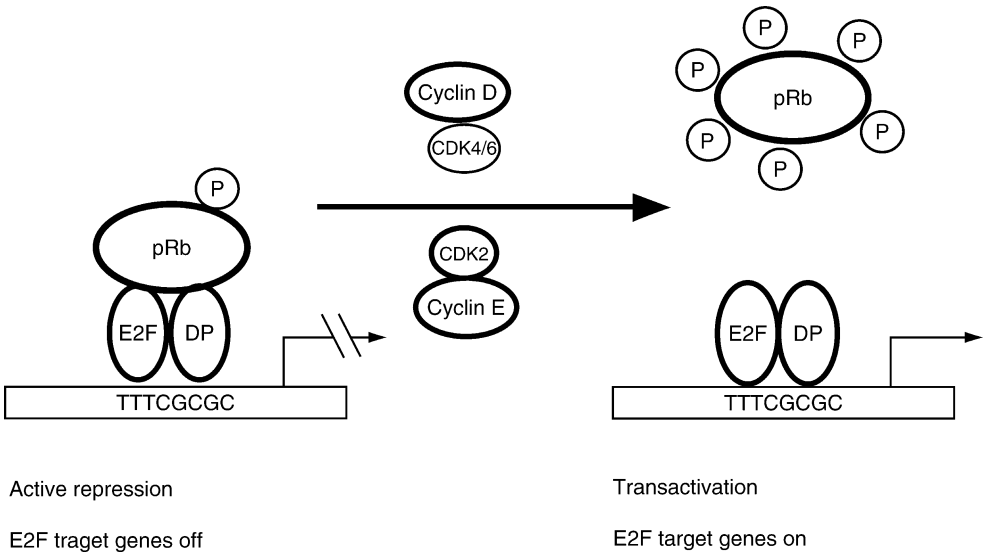
Characteristics

D-type cyclins are encoded by three closely related genes (cyclins D1, D2 and D3) that are expressed in a tissue-specific fashion. Biochemically, D type cyclins act as regulatory subunits of a group of related protein kinases (CDKs), primarily the CDKs 4 and 6. Cyclin D/CDK4/6 complexes, together with cyclin E/CDK2, cause phosphorylation of the family of retinoblastoma proteins (►pRb, p107 and p130) in the G1 phase of the cell cycle, resulting in abrogation of their growth inhibitory activity. Phosphorylation of the retinoblastoma proteins leads to release of ►E2F transcription factors from the retinoblastoma proteins and to progression to the S phase of the cell cycle (Fig. 1).

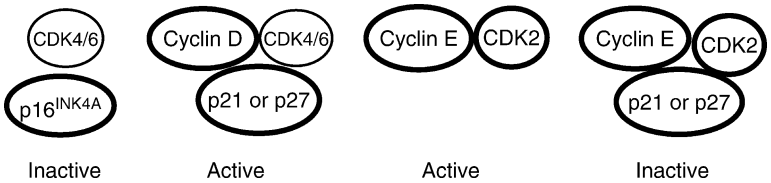
Regulation of D Cyclins

D type cyclins are major downstream targets of extracellular signaling pathways, which act to transduce mitogenic signals to the cell cycle machinery. Transcriptional induction of D type cyclins occurs in response to a wide variety of mitogenic stimuli, including the ►Ras signaling cascade and the ►APC-β-catenin-Tcf/Lef pathway. In addition, cyclin D1 protein turnover and subcellular localization is highly regulated during the cell cycle. Phosphorylation of cyclin D1 by GSK-3β in resting cells renders the protein a target for rapid destruction by the ►proteasome. In contrast, mitogenic stimulation of cells leads to inhibition of GSK-3β and stabilization of cyclin D1 protein. In response to DNA damage, cells initiate an immediate G1 arrest, which is caused by rapid proteolysis of cyclin D1. Together with activation of the p53 tumor suppressor protein, cyclin D1 destruction causes a fast withdrawal from the cell cycle to allow repair of the damaged DNA before DNA synthesis resumes.

Binding of D type cyclins to their CDK partner is antagonized by the ►INK4 family of CDK inhibitors (►CKI). ►INK4 proteins bind to CDK4 and 6 and thereby prevent association of D type cyclins to these CDKs (Fig. 2). The most prominent member of this family is p16INK4A. Mutations in p16INK4A (also



Cyclin D. Figure 1 Regulation of E2F activity through pRb phosphorylation. In the G1 phase of the cell cycle the retinoblastoma protein pRb is hypophosphorylated, allowing it to bind E2F transcription factors. E2F/pRb complexes are able to bind DNA but are inactive in transcription activation. Phosphorylation of pRb by cyclin D/CDK4 and cyclin E/CDK2 complexes causes the release of E2F from pRb. Free E2F is then able to activate transcription of E2F target genes (genes with TTTCGCGC-like E2F sites in their promoters), allowing cells to enter the DNA synthesis phase (S phase) of the cell cycle.

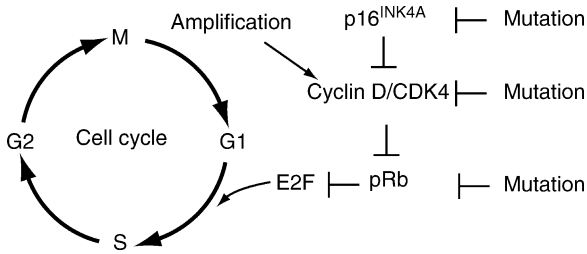


Cyclin D. Figure 2 Effect of CDK inhibitors on cyclin/CDK complexes. CDKs 4 and 6 are activated by binding of D type cyclins. Association of cyclin D to CDKs 4 and 6 is prevented by p16INK4A that binds with high affinity to these CDKs. Thereby, binding of cyclin D to these CDKs is prevented. The CDK inhibitors p21cip1 and p27kip1 bind both to cyclin E/CDK2 and to cyclin D/CDK4 complexes, although with different consequences. Even though these inhibitors antagonize cyclin E/CDK2 activity, they are required for proper assemblage and activity of cyclin D/CDK4/6 complexes.

known as ►CDKN2A) are found in a variety of spontaneous tumors, and heterozygosity for p16INK4A in the germ line predisposes to melanoma. A second family of CKIs consists of three related proteins that bind to cyclin/CDK complexes. Members of this family include p21cip1 and p27kip1. This class of CKIs has quite divergent effects on the different cyclin/CDK complexes. Whereas cyclin E/CDK2 is inhibited by both p21cip1 and p27kip1, cyclin D/CDK4/6 complexes are active when complexed with this class of inhibitors (Fig. 2). In fact, formation of active cyclin D/CDK4/6 complexes requires the presence of p21cip1 or p27kip1 to act as 'assembly factors' of cyclin D/CDK complexes. These opposing effects of p21cip1 and p27kip1 on cyclin E/CDK2 and cyclin D/CDK4 complexes endows cyclin D/CDK4 complexes with an important second, non-catalytic function during the G1 phase of the cell cycle. Synthesis of cyclin D1 by mitogenic stimulation leads to absorption of p21cip1 or p27kip1 into active ternary complexes, thereby facilitating activation of cyclin E/CDK2 by removal of inhibitors.

CDK-Independent Activities of D Type Cyclins

Apart from their role in activation of CDKs, D type cyclins can have several profound effects on cellular physiology independent of their CDK partners. In ►breast cancer, cyclin D1 can bind directly to the estrogen receptor, thereby causing hormone-independent activation of the estrogen receptor. This activity of cyclin D1 may contribute to resistance to anti-hormonal therapy that is often seen in the clinic. In addition, D type cyclins can modulate the activity of Myb transcription factors.



Cyclin D. Figure 3 The p16-cyclin D-pRb pathway: a frequent target in human cancer. E2F transcription factors contribute to G1-S phase progression through the activation of specific target genes. E2F activity is negatively regulated by its binding to the retinoblastoma tumor suppressor gene product, pRb. The ability of pRb to bind E2F is regulated by cyclin D/CDK complexes. The activity of cyclin D/CDK complexes in turn is negatively regulated by p16INK4A that is encoded by the CDKN2A tumor suppressor gene.

In this respect is the ►Myb-like transcription factor DMP1, which has anti-proliferative activity. Expression of cyclin D inhibits this effect on cell proliferation of DMP1 through direct binding to DMP1, which prevents DNA binding by DMP1.

Clinical Relevance

Because of their critical role in linking cytoplasmic signals to nuclear responses it is perhaps not surprising that D type cyclins are frequently deregulated in several types of cancer. Cyclin D1 ►amplification or overexpression is found in a number of human malignancies, the most prominent being breast cancer, in which up to 50% of all cases have elevated levels of cyclin D1 protein. Chromosomal translocations involving cyclin D1 are found in parathyroid adenoma and in mantle cell lymphoma.

Not only is cyclin D1 itself often directly mutated in human cancer, its upstream regulators such as p16►INK4A and its downstream target pRb are frequent targets in human carcinogenesis as well. It is generally believed that this p16INK4A-cyclin D1-pRb pathway is deregulated in virtually all human cancers (Fig. 3).

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Cyclin-dependent Kinase

►Cyclin Dependent Kinases

Cyclin-dependent Kinase Inhibitor 2A

►CDKN2A

Cyclin Dependent Kinases

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Synonyms

CDK; Cyclin-dependent kinase; Cdk1 kinase; Maturation promoting factor; MPF

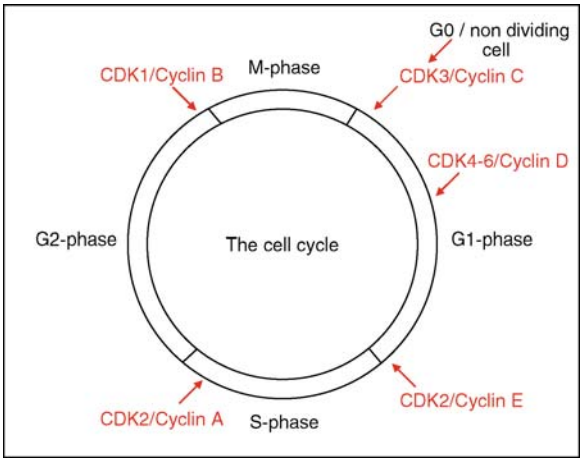
Definition

A class of enzymes that add phosphates to other proteins.

Characteristics

Cyclin-dependent kinases (CDKs) catalyze one of the most important biological events in eukaryotic cells–cell proliferation. When cells proliferate, they follow the four phases of the ►cell cycle that enables them to duplicate their DNA and deliver exact copies into two daughter cells. Many enzymes orchestrate these steps, but the key enzymes that control the phases of the cell cycle are CDKs. (Fig. 1)

CDKs are enzymes in the serine/threonine protein kinase family. They phosphorylate (add phosphate) to substrate proteins on hydroxy amino acids, serine and threonine, but not on tyrosine, which distinguishes CDKs from the tyrosine protein kinase family. Once a substrate is phosphorylated by a protein kinase, the substrate often has different biological properties relative to its nonphosphorylated counterpart. For example, a phosphorylated protein may increase or reduce its catalytic activity, change its cellular localization, bind to or dissociate from other proteins, and increase or decrease its biochemical half-life. The



Cyclin Dependent Kinases. Figure 1 CDKs and the cell cycle. The role of the major CDKs (cyclin-dependent kinases) is shown relative to the four phases of the cell cycle. Each transition from one phase to another during the cell cycle, including entry into the cell cycle from G0 or nondividing cells, requires the formation and activation of a CDK.

prototype CDK, Cdk1, phosphorylates many proteins during the G2/M-phase transition of the cell cycle. The change in phosphorylation state of these proteins causes cells to organize their DNA into chromosomes and begin the process of mitosis and cytokinesis.

CDKs are composed of two protein subunits: a protein kinase catalytic domain and a regulatory cyclin subunit. The catalytic domains of protein kinases can be divided into 11 subdomains, each with a specific sequence that permits the classification of protein kinases into families. Among the 518 protein kinases encoded in the human genome, 13 are members of the CDK family. The signature sequences for cyclin subunits are not as well defined as those of the catalytic subunit, however, it is estimated that there are at least 25 different human cyclin subunits. The one-to-one pairing of a catalytic domain to a cyclin posits that one catalytic subunit can bind to more than one cyclin subunit, although not at the same time. The exact composition of all possible CDKs is currently not known, but it is an important parameter because the pairing combination defines its substrates, its cellular location, and the timing of activation.

Cdk1 is the best-known member of the CDK family. It is composed of the 34 kD Cdk1 catalytic domain that binds to A- or B-type cyclin subunits of 50–55 kD. The Cdk1 catalytic domain is inactive as a monomer and its protein levels do not change during the cell cycle. As cells progress towards mitosis, the cyclin B protein levels increase and bind to the catalytic subunit to form the CDK complex. This complex is now competent to become active, but its activity is constrained by a coordinated network of three other enzymes: Wee1, Cdc25C, and CAK. Wee1 phosphorylates the Cdk1 catalytic subunit on threonine 14 and tyrosine 15, two amino acids that are within the ATP binding site and therefore directly block the interaction between the catalytic subunit and its ATP substrate. Cdc25C, a member of a protein ▶phosphatase family, can dephosphorylate, or remove the phosphates from the ATP binding site. In addition, access to the ATP binding and protein substrate binding sites are hindered by a peptide within the catalytic domain known as the T-loop. Upon phosphorylation of this peptide on threonine by CAK (another member of the CDK family), the domain is displaced thus permitting access of ATP and protein substrates to the catalytic center of Cdk1. The sequential formation of Cdk1 from its subunits followed by phosphorylation by Wee1 enables cells to accumulate a form of Cdk1 that can be rapidly activated. If a number of other cellular events are completed, such as DNA synthesis, then Cdc25C dephosphorylates Cdk1 and the T-loop is phosphorylated by CAK. This system of regulation enables a cell to achieve maximum Cdk1 catalytic activity in a very short time and engages a cell to enter mitosis.

The catalytic activity of Cdk1 is maintained until the cyclin subunit is selectively degraded by a protease. Once cyclin B1 is degraded, the Cdk1 catalytic subunit returns to its inactive state, the cell exits from mitosis and forms two daughter cells. Cdk1 participates in its activation and inactivation by phosphorylating the enzymes that regulate it. This creates an autoactivation loop that results in an all-or-none activity of Cdk1, which is coherent with the all-or-not entry into mitosis during the cell cycle.

The Discovery of CDKs

Cell-division-cycle 2 (Cdc2) kinase was the original name of the prototype Cdk1, based upon the discovery of the role of Cdc2 in mitosis. A convention for naming this family of protein kinases was established by consensus at the Cold Spring Harbor Symposium on the Cell Cycle at Cold Spring Harbor, NY, USA in 1991. The role of Cdk1 in the cell cycle was originally identified by experiments with cells of lower eukaryotic species. The catalytic subunit was first identified by genetic studies in the fission yeast, *Schizosaccharomyces pombe*, as the Cdc2 gene product. Cyclin proteins were first identified in sea urchin eggs. After fertilization, sea urchin eggs enter mitosis in a synchronous manner. This natural synchrony and the experimental methods used to detect new protein synthesis permitted the discovery of B-cyclins, whose levels cycle relative to mitosis. Evidence that cyclins and Cdc2 family members form a complex came from biochemical studies of Xenopus (toad) oocytes and starfish oocytes. Copurification of MPF with the Cdc2 subunit and cyclin B

demonstrated that Cdc2/Cyclin B were the major components of an essential complex for mitosis. The confirmation that Cdc2 was part of a pathway conserved in all eukaryotic cells was made by genetic complementation studies in which yeast cells could proliferate after replacement of the yeast Cdc2 gene with the human CDC2 gene. Human and yeast genomes are separated by 1 billion years of genetic isolation, yet their functional activity has been conserved. Leland Hartwell, Tim Hunt, and Paul Nurse were awarded the Nobel Prize in physiology or medicine in 2001 for their role in the discovery and the characterization of Cdc2 and cyclins in the cell cycle.

Members of the CDK Family

Other CDKs, besides Cdk1, have important roles in progression through the cell cycle. CDK3 with its putative partner cyclin C is activated as cells start the cell cycle from a resting state (G_0). CDK4 and CDK6, which are associated with ►D-type cyclins, respond to extracellular growth signals and permit cells to continue the G1-phase of the cell cycle. CDK4 and CDK6 complexes phosphorylate the ►retinoblastoma protein, which controls the expression of genes required for the G1/S-phase transition and S-phase progression. The CDK2/cyclin E complex also participates in the G1/S-phase transition by phosphorylating replication factors. During S-phase, CDK2/cyclin A phosphorylates different substrates allowing DNA replication and participates in the inactivation of G1 transcription factors. In mouse models, using gene knock-out technology, it has been shown that CDK2 is not essential. By contrast CDK1 and CDK5 are essential genes. In the early phases of the cell cycle, the activity of CDKs is regulated by members of a small protein family, such as ►p21/WAF1 and ►p16/INK4. These proteins bind to the CDK complex and inhibit it. In the final phases of the cell cycle, after DNA synthesis is completed, the Cdk1 complex is activated and triggers the G2/M-phase transition. The cell ends M-phase and enters G1 by phosphorylation of the anaphase promoting complex (APC) by Cdk1. These successive waves of CDK/cyclin activation and inactivation drive the major phases of the cell cycle.

The role of CDK enzymes is not limited to the cell cycle. Some CDKs regulate DNA transcription, including the transcription of genes that are essential for cell cycle progression, therefore, these CDKs have a role that lies at the interface of cell cycle control and basal cell activity. CDK7, which binds to cyclin H, is a component of the transcription factor TFIIH. CDK7 has a dual role because it can phosphorylate RNA polymerase II, which is required for RNA elongation, and it can phosphorylate the Cdk1 and Cdk2 T-loop, which is required for CDK1 and CDK2 activation. The role of CDK8 overlaps with that of CDK7 in transcriptional regulation. CDK9/cyclin T is a component of a transcription elongation factor.

CDK activity can be detected in cells that undergo ►apoptosis (programmed cell death). In some cases, this activity is related to DNA damage, resulting in a type of mitosis that is known as ►mitotic catastrophe, which eventually leads to cell death. Activation of CDKs can occur in nondividing neuronal tissue after chemical shock and may be required for apoptosis. The role of CDKs in apoptosis has implications in chronic inflammation. Neutrophils, a specialized cell that participates in inflammatory response, may be made dependent upon CDKs to engage apoptosis under experimental conditions.

Relative to other CDKs, CDK5 has a specialized role in nondividing neural tissue. Its activity is important for neurite outgrowth and neuronal development, myogenesis and somite organization in embryos. The cyclin subunit, p35, is one of the smallest members of the cyclin family (35 kD) and it can be processed to exist in a smaller form of 25 kD. The three dimensional structure of p35 reveals that it has similar protein folds to other cyclins, which provides a rationale for its capacity to activate CDK5.

The crystal structure of several CDKs has been resolved, which gives insight to the organization of the substrate binding site and the role of the regulatory cyclin subunit. In the case of CDK2, which is composed of Cdk2 catalytic subunit and a cyclin A subunit, it was revealed the kinase complex consists of an amino-terminal lobe rich in β-sheets and a carboxyl-terminal lobe that is larger and mostly α-helical. Between these two lobes is a deep pocket that harbors the ATP binding site, which contains the conserved amino acids that participate in catalysis. The T-loop, which blocks substrate access, moves away from the catalytic cleft after cyclin binding and is then accessible for phosphorylation by CAK. This highlights the role of cyclin and CAK in CDK activation. Cyclin binding also reorientates amino acids within the ATP pocket to permit phosphate transfer from ATP to the protein substrate. The resolution of CDK5 and CDK6 revealed structures that are similar to that of CDK2, suggesting that CDK structures of the entire family are similar.

CDKs in Cancer and Other Human Diseases

CDKs are implicated in a broad range of human disease. Their essential role in cell proliferation, especially in the case of Cdk1, has led to the proposal that these proteins play an important role in human cancers. Although it is known that cancer cells require Cdk1 to proliferate, it remains to be demonstrated that the function of Cdk1 in cancer cells is different from its role in normal, proliferating cells. The overexpression of cyclin E is frequently found in both precancerous and cancerous lesions in human tissue. It is believed that

CDK activation by cyclin E may cause genomic instability. There is much evidence to link CDK5 activity to cytoskeletal abnormalities that can lead to neuronal cell death. This may be, in part, caused by the conversion of its cyclin partner p35 to p25, which leads to activation of CDK5 and alteration of its cellular localization. Uncontrolled activation of CDK5 causes the phosphorylation of neuronal proteins, such as tau, and may be linked to Alzheimer's disease. Chronic inflammatory diseases such as gout, arthritis, Crohn's disease may be partly due to misregulation of CDKs. CDKs also participate in virus replication in infected cells. In the example of HIV, which is linked to the cause of AIDS, CDK9 is recruited by the ►HIV tat protein which enhances transcription of viral genes.

The involvement of CDKs in disease and the detailed knowledge of CDK atomic structure have led to the identification of potent chemical inhibitors that have the potential to become ►small molecule drugs. Many of these have been cocrystallized with CDKs, which gives insight to the molecular docking of inhibitors in the ATP pocket. Pharmacological inhibitors of CDKs are being evaluated for therapeutic use in major human disease such as cancer, neurodegenerative disorders, cardiovascular disorders, viral infections, and parasitic infections.

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Cyclin G-Associated Kinase

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Synonyms

GAK; Auxilin-2

Definition

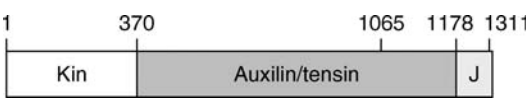
GAK/auxilin-2 is the ubiquitously expressed form of the neuronal-specific protein Auxilin-1. GAK is a member of the Ark/Prk serine/threonine protein kinases family and has an important role in ►endocytosis, uncoating clathrin-coated vesicles (CCVs) in non-neuronal cells, and clathrin-dependent trafficking from the *trans* Golgi network. GAK has been implicated as a transcriptional co-activator of the ►androgen receptor (AR) and may have a role in prostate cancer progression to androgen-independent disease. Expression patterns during androgen-withdrawal therapy suggest a prognostic role for GAK in advanced prostate cancer.

Characteristics

GAK is a large, 140 kDa protein that has three functional domains: (i) an NH_2-terminal Ser/Thr kinase domain, (ii) a central Auxilin/Tensin homology domain, and (iii) a COOH-terminal J-domain (Fig. 1). Although GAK was initially identified by its association with cyclin G, which is a downstream transcriptional target and a negative regulator of the ►p53 tumor suppressor protein, subsequent studies suggest that GAK has an important role in endocytosis and un-coating CCVs in non-neuronal cells.

Kinase assays demonstrated that GAK is one of two kinases present in clathrin-coated vesicles and that the Ser/Thr kinase activity of GAK was directed towards the μ2 component of CCVs. The protein homologue that is responsible for un-coating CCVs in neuronal cells is Auxilin. Consequently, the central Auxilin/Tensin homology domain of GAK is essential for its role in vesicle un-coating and has clathrin-binding motifs that allow for association with CCVs. GAK differs from Auxilin by its kinase domain, and recently GAK was classified as an Ark kinase family member due to its homology with actin regulating kinase (ARK)-1.

The COOH-terminal J-domain of GAK is known to interact with the molecular chaperone Hsc70, which is the constitutively expressed form of Hsp70. Both GAK and Auxilin are DnaJ homologues that possess J-domains. The J-domain recruits ATP-bound Hsc70 to CCVs. The mechanism of clathrin dissociation was first elucidated for Auxilin and then shown to be comparable for GAK. Recruitment of ATP-bound Hsc70 activates dormant ATPase activity of the



Cyclin G-Associated Kinase. Figure 1 Human GAK (hGAK) is a 144 kDa protein that has three functional domains: an NH_2-terminal Ser/Thr kinase domain, a central Auxilin/Tensin homology domain, and a COOH-terminal J domain.

chaperone and destabilizes clathrin-clathrin interactions in CCVs. Hsc70, now in its ADT state, remains tightly bound to clathrin while Auxilin is recycled for another round of un-coating. GAK activity in non-neuronal cells also involves Hsc-70 recruitment and ATPase-dependent destabilization of clathrin interactions. The kinase domain of GAK is indeed functional and, as described above, phosphorylates components of CCVs.

GAK and the Androgen Receptor

The impact of androgens, which are male steroid hormones, on prostate growth is a major consideration when treating advanced prostate cancer. Androgens carry out their function through the androgen receptor (AR), which is a ligand-dependent transcription factor. Activated AR regulates genes that are involved with growth and differentiation of the prostate gland. Dihydrotestosterone (DHT) has up to fivefold greater affinity for AR than testosterone (T) and, consequently, is up to 2.5-times more active as a hormone. For this reason, 5α-reductase inhibitors that prevent conversion of T to DHT were developed for use in prevention and treatment of prostate diseases. Another commonly used therapeutic strategy is removal of androgens by surgical and/or chemical methods to prevent activation of AR. Hormone therapy reduces testosterone levels significantly, down to ~10% of the normal level; low-levels of circulating androgens resulting from adrenal secretions are still present. Removal of hormone by androgen ablation therapy prevents the growth promoting effects of androgens, leads to ►apoptosis of cancer cells, and ultimately results in tumor regression.

The decrease in tumor burden following androgen ablation occurs during the androgen-dependent (AD) stage of prostate cancer when the tumor still requires androgens for survival and growth. Unfortunately, this form of therapy offers limited aid, and the average range of overall survival is only 23–37 months. For reasons that are not fully understood, prostate cancer cells switch from an AD state to one that is androgen-independent (AI), in which cells are able to bypass requirement for the androgenic growth signal, and grow in an uncontrolled fashion. As a result, tumor burden and prognostic disease markers such as ►prostate specific antigen (PSA) increase dramatically.

Transcriptional co-activators of AR interact directly with the receptor to modify AR-mediated transactivation and gene expression. Originally, this class of proteins carried out their role at the level of the promoter, either by altering DNA accessibility to transcription machinery or by bridging AR to the basal transcription machinery. Several of these *classical*, or *Type I*, co-activators have indeed been associated with AR. However, a class of *non-classical*, or *Type II,* co-activators was formed to differentiate their mechanism of action. Non-classical co-activators enhance AR activity by altering other facets of the AR transactivation process. These include: (i) stability of inactive and active AR, (ii) nuclear translocation, and (iii) DNA binding. In addition, non-classical co-activator activity may involve post-translation modification of AR by phosphorylation, acetylation or sumoylation. Unfortunately, the role of co-activators in directing AR-activity is far from clear.

One possible mechanism for AI activation of AR in advanced prostate cancer is related to aberrant activity or expression of transcriptional co-activators. The majority of studies on AR co-activators have been carried out *in vitro* and the relevance of these accessory proteins has yet to be determined *in vivo*. However, transactivation assays with many co-activators have demonstrated that ectopic over-expression of these proteins results in enhanced AR transactivation and increased expression of AR-regulated genes.

GAK was identified as a putative AR interacting protein by using the AR's NH_2-terminal transactivation domain as molecular bait in a modified yeast two-hybrid system. Subsequent studies, including immunoprecipitation and GST-pull down assays, not only confirmed the interaction between AR and GAK in prostate carcinoma cell lines, but also detailed the regions of interaction between the two protein molecules. GAK interacted with all three AR domains (NH_2-terminal transactivation, central DNA-binding, and COOH-terminal ligand-binding domains), whereas only the auxilin/tensin homology domain of GAK is crucial for its interaction with AR.

Transactivation assays, which assess the impact of GAK on AR-mediated transcription, demonstrated that GAK could enhance AR activity from three- to fivefold in the presence of androgens. Importantly, however, over-expression of GAK increased AR activity by up to eightfold in low-androgen conditions, suggesting that increased GAK expression can result in increased AR sensitivity to low concentrations of androgens and may serve as a mechanism for prostate cancer progression to androgen independence.

The exact mechanism by which GAK serves as a transcriptional co-activator of AR is unknown. Also, its role as a Ser/Thr kinase is still being investigated. Upon activation by its ligand, AR is phosphorylated. Additionally, activated AR is sensitive to phosphorylation by signal transduction pathways. Whether these phosphorylation events are integral to the transcriptional activity of AR and whether phosphorylation of AR by GAK or any other kinase has a role in androgen-independent activation of AR remains to be determined.

GAK and Prostate Cancer Progression

The growing incidence of prostate cancer over the past two decades is due, in part, to increased detection through the introduction of PSA screening. PSA is a

useful molecular marker for assessment of disease progression, particularly for emergence of AI disease in patients undergoing androgen ablation therapy, and is used in conjunction with digital rectal exam (DRE) and transrectal ultrasound for diagnosis. In addition, histological grading of prostate biopsies by Gleason score and ►TMN staging are important prognostic tools. Early detection is the most powerful weapon against prostate cancer since the disease is often curable in its early stages. However, patients with advanced disease have poor prognosis and an average life expectancy of 30 months with androgen ablation therapy.

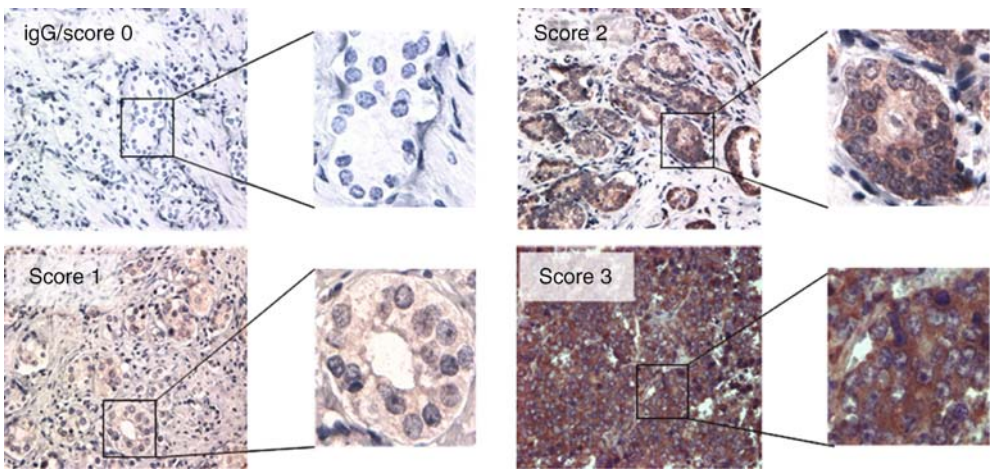
Significant research has been carried out to identify diagnostic and prognostic molecular markers for disease progression as well as molecular targets for therapeutic strategies. Since GAK was identified as an AR-interacting protein with co-activator properties, it may have a role in inappropriate activation of AR in advanced disease. High through-put immunohistochemical analysis of Neo-adjuvant Hormone Therapy (NHT) Tissue Microarray was used to assess GAK's potential as a prognostic molecular marker of prostate cancer.

For the NHT arrays, a total of 112 samples were obtained and sampled in triplicate. Most tissues were radical prostatectomy specimens, while AI tissues were obtained either from transurethral resections from patients with hormone refractory disease or from warm autopsy samples of metastatic tissues. Specimens were chosen so as to represent various treatment durations of androgen withdrawal therapy prior to radical prostatectomy ranging from no treatment (n = 21), ≤6 months (n = 49), and more than 6 months (n = 28). AI tumors were also identified (n = 14).

GAK expression was assessed by immunohistochemistry and staining intensity was scored visually by a pathologist on a scale from 0 to 3, ranging from no staining (score 0) to very intense staining (score 3) (Fig. 2). While GAK expression decreases slightly in response to androgen withdrawal, suggesting that GAK itself may be an AR-regulated gene, the proportion of samples with elevated GAK expression (score 2–3) increases during continued NHT treatment. In AI disease samples, elevated levels of GAK expression are considerably higher than during the AD phase. Since over 95% of AI tumor biopsies exhibit very high levels of GAK (visual score ≥2) the use of GAK as a prognostic marker for disease progression merits further investigation.

The mechanism by which GAK may encourage AI growth of prostate cancer is still unknown. GAK acts as a co-activator of AR and renders AR more responsive to lower androgen levels. Androgen ablation therapy, including NHT, reduces the level of circulating androgens. However, low levels of circulating androgens from adrenal secretions are still present. In prostate cancer cells, it is possible that increased levels of GAK are able to sensitize AR so that the receptor is responsive to these circulating low-level adrenal androgens, as observed *in vitro*. This would allow for AR activation and expression of AR-regulated growth and survival genes.

In summary, recent findings have identified GAK as a putative co-activator of AR and a possible molecular marker for prostate cancer progression. GAK expression may be a useful prognostic tool for assessing prostate cancer progression in patients undergoing androgen ablation therapy. An increase in GAK expression during the therapeutic course would suggest that the cancer has adapted to androgen-withdrawn conditions and are



Cyclin G-Associated Kinase. Figure 2 A NHT tissue microarray was stained with an antibody that recognizes GAK (Santa Cruz Biotechnologies, Inc.). Staining intensity was scored from 0–3 by a pathologist. Examples of IgG control and GAK staining with score = 1, score = 2 and score = 3 are shown. Slides were visualized under × 40 magnification and further magnification of delineated areas is shown.

progressing to an AI stage. These early warnings of disease progression are crucial in allowing physicians the opportunity to re-assess appropriate strategies for disease treatment.

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Cyclins

Definition

Are a family of proteins involved in the cell cycle progression, cooperating with its catalytic partner ►cyclin-dependent kinase (Cdk), which activates the protein kinase function. The most important substrate of cyclin/Cdk complex is ►retinoblastoma protein (Rb).

►INK4a
►Early B-cell factors

Cyclobutane Pyrimidine Dimer

Definition

A ►DNA photoproduct that is generated upon saturation of the 5,6 double bonds of adjacent pyrimidines and formation of a four-membered cyclobutyl ring.

►Solar Ultraviolet Light

Cyclooxygenase

►Arachidonic Acid Pathway

Cyclooxygenases (COX)

Definition

Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids (including prostaglandins, prostacyclin, and thromboxane). There are three COX isoenzymes: COX-1, COX-2, and COX-3. COX-3 is a splice variant of COX-1, while COX-1 is considered a constitutive enzyme expressed in most mammalian cells and upregulated in various carcinomas involving in tumorigenesis. Whereas, *COX-2* is undetectable in most normal tissues, but is inducible with abundant in cells at sites of inflammation and cancers.

►Anti-Inflammatory Drugs
►Inflammation
►Celecoxib

Cyclooxygenase-2 (COX-2)

Definition

Is an enzyme that is responsible for the formation of a number of important biological mediators. To date, three different COX-enzymes are known (COX-1, COX-2, COX-3) that have different biological functions. Pharmacological inhibition of COX-2 can provide relief from the symptoms of ►inflammation. The main COX-2 inhibitors are the ►non-steroidal anti-inflammatory drugs (NSAIDs), such as ►Celecoxib. However, to date none of the different COX-inhibitors is specific against COX-2, they also inhibit the other COX-enzymes, causing serious side-effects. For instance, inhibition of COX-1 results in inhibition of prostaglandin synthesis, with the result of serious health consequences such as gastrointestinal (stomach) bleeding, acute renal failure, or worse. This is due to the acidity of the stomach, where the cells of the stomach are replaced very quickly, within a few days. One of the major roles of PG's is to keep the lining of the stomach intact, and when the prostaglandin system is disrupted (say by taking COX-1 drugs like many NSAIDS) stomach irritation, digestive tract problems and even intestinal or stomach bleeding and death can occur. Such problems can also arise with alleged COX-2 inhibitors, when they are not specific for COX-2 but inhibit also the other COX-enzymes. Even modern drugs, like ►Celecoxib, are simply not selective

enough, not to mention some of the potentially horrible side effects of COX-inhibitors and the associated lawsuits that have been filed due to side effects such as cardiovascular problems, even heart attacks, stroke and blood clots. Major research efforts are directed towards developing specific inhibitors against COX-2, which is more involved in inflammation.

►Cyclooxygenase-2 in Colorectal Cancer

Cyclooxygenase-2 in Colorectal Cancer

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Synonyms

COX-2; Cyclooxygenase (prostaglandin endoperoxide synthase)

Characteristics

Colorectal cancer (►colon cancer) remains a significant health concern for much of the industrialized world, even though mortality rates are beginning to decline in the USA. Diagnosis often occurs at a late stage in the progression of this disease, which reduces the likelihood of treatment being effective. Current treatment strategies often include a combination of surgical resection and adjuvant chemotherapy (►adjuvant therapy). Because of the unsatisfactory outcome of existing treatment methods, much emphasis has been placed on developing new treatment, prevention and screening strategies. Numerous population based studies indicate that use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk for colorectal cancer and decrease the incidence of adenomatous polyps. NSAIDs have been shown to induce polyp regression in familial adenomatous polyposis [FAP (►APC gene in Familial Adenomatous Polyposis)] patients and reduce tumor burden in animal models of colorectal cancer.

Cyclooxygenase-2 and Colorectal Cancer Prevention

COX-2 mRNA and protein levels are increased in intestinal tumors that develop in rodents following carcinogen treatment and in adenomas taken from multiple intestinal neoplasia (►Min) mice. When intestinal epithelial cells are forced to express COX-2 constitutively, they develop phenotypic changes that include increased adhesion to ►extracellular matrix (ECM) and resistance to butyrate-induced ►apoptosis. Both of these phenotypic changes are consistent with an increased tumorigenic potential. COX-2 expression has been detected in 80–90% of colorectal adenocarcinomas but in only 40–50% of premalignant adenomas. These data suggest that elevation of COX-2 expression is secondary to other initiating events such as dysregulation of the APC signaling pathway and/or dysfunction of other genes affected during the adenoma to carcinoma sequence (►multistep development).

The observation of elevated COX-2 expression in three different models of colorectal carcinogenesis has led to consideration of the possibility that COX-2 expression may be related to colorectal tumorigenesis in a causal way. Recent studies have demonstrated a significant reduction in premalignant and malignant lesions in carcinogen-treated rats that were given a selective COX-2 inhibitor.

Tumor growth requires the maintenance and expansion of a vascular network. It has been demonstrated using in vitro assays that COX-2 can influence ►angiogenesis, and treatment with selective COX-2 inhibitors blocks angiogenesis. COX-2 appears to contribute to tumor vascularization and there seems to be a link between COX-2 and regulation of ►VEGF expression.

Summary

Both preclinical and clinical data indicate that selective COX-2 inhibitors have anti-neoplastic activity. The precise role of COX-2 and the ►prostaglandins produced by this enzymatic pathway in carcinogenesis remains to be clearly delineated. Overexpression of COX-2 in epithelial cells leads to inhibition of apoptosis and increased adhesiveness to extracellular matrix. Inhibition of COX-2 activity leads to a marked reduction of tumor growth in a number of experimental models. Treatment with selective COX-2 inhibitors have been clearly shown to inhibit tumor-induced angiogenesis. The most effective role for selective COX-2 inhibitors for prevention and treatment of human cancers is currently under investigation.

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Cyclooxygenase-prostaglandin Endoperoxide Synthase

►Cyclooxygenase-2 in Colorectal Cancer

Cyclopamine

Definition

A naturally occurring teratogenic steroidal alkaloid isolated from the corn lily *Veratrum californicum.* Cyclopamine inhibits Hedgehog signaling at the level of Smoothened. The effect of cyclopamine was uncovered when lambs born to ewes grazing on *Veratrum californicum* were born with cyclopia (single eye). A similar phenotype results from mutations in Sonic hedgehog.

►Hedgehog Signaling

Cyclophosphamide

Definition

Is a bifunctional nitrogen mustard that is most commonly used drug is combination chemotherapy is a DNA alkylating agent that is used as an immunosuppressive drug. It acts by killing rapidly dividing cells, including lymphocytes proliferating in response to antigen.

►Alkylating Agents

Cyclosporin A

Definition

Is a powerful immunosuppressive drug that inhibits signaling from the T-cell receptors, preventing T-cell activation and effector function. It binds to cyclophilin, and this complex binds to and inactivates the serine/threonine phosphatase calcineurin. Cyclosporine A is used to inhibit the immune response in patients who receive donor organs.

►Fluoxetine
►Sjögren Syndrome

CYFRA 21-1

►Serum Biomarkers

CYLD

Definition

Is a tumor suppressor gene that encodes for an ubiquitin-carboxyterminal hydrolase mutated in patients with familial ►cylindromatosis.

►BCL3

Cylindromatosis

Definition

Synonym turban tumor syndrome, is a condition where mutations in the *CYLD* tumor suppressor gene predispose to benign tumors arising in hair follicles and in cells of sweat and scent glands, collectively called epitheliomas.

►Arachidonic Acid Pathway

CYP

Definition

Enzymes located in the smooth endoplasmic reticulum of cells. CYP catalyses a variety of reactions including

epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation. There are numerous ►isoforms of ►cytochrome P450.

►Vanadium

CYP2E1

Definition

Cytochrome P-450 monooxygenase system, member of the cytochrome P450 mixed-function oxidase system, is involved in the metabolism of xenobiotics in the body.

►Benzene and Leukemia
►Cytochrome P450

CYP450

►Cytochrome P450

CYP 450_{arom}

►Aromatase and its Inhibitors

CYP P-450 2D6

Definition

CYP P-450 2D6 is an iron containing enzyme responsible for chemical oxidation (metabolism) of almost all CNS drugs.

►ADMET Screen

Cystadenocarcinoma

►Appendiceal Epithelial Neoplasms

C

Cystatins

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Synonyms

Thiol-protease inhibitors (cytoplasmic α- and β-TPIs; plasma α_1- and α_2-TPIs); Acid, neutral and basic cysteine-protease inhibitors (ACPI, NCPI and BCPI); Thiostatins

Definition

Cystatins were originally defined as endogenous ►inhibitors of thiol- or cysteine-►proteases. Later, the discoveries of several proteins whose primary sequence revealed substantial homology to the typical cystatin domain led to the definition of a cystatin super-family. With the identification of other types of intracellular cysteine-proteases, however, it became clear that cystatins inhibit mainly cysteine-proteases present in ►endosomes and ►lysosomes. Additionally, many of the newer members of the cystatin super-family have less than 30% homology to classical cystatins and do not inhibit lysosomal cysteine-proteases. Moreover, several proteins with little apparent amino acid sequence homology fold into typical three-dimensional structures attributed to cystatins. Thus, the term "cystatins" now refers to a heterogeneous group of proteins still lacking both a uniform identity and a cohesive definition.

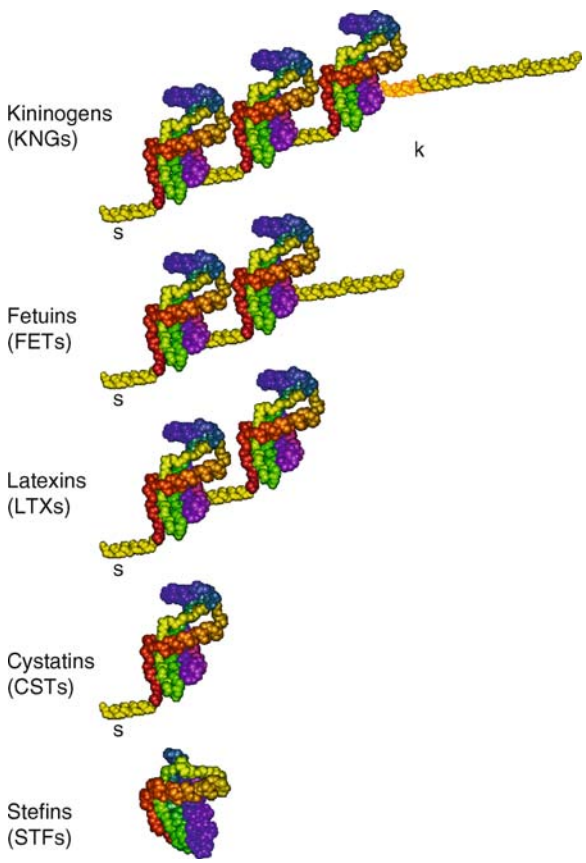
Characteristics

Domain Structure

The primary structure of the archetypal cystatin domain consists of a polypeptide 100–120 amino acids in length. This polypeptide folds into a five-stranded β-sheet, which partly wraps around an inner α-helix, resulting in a three-dimensional structure commonly referred to as the "hot-dog fold." Members of the cystatin super-family have been categorized into several distinct families according to the following criteria: (i) amino acid sequence homology; (ii) location of disulfide bonds; (iii) presence of a ►signal peptide in

the primary translation product; and (iv) number of cystatin-like domains. Based on these criteria, roughly five different families can be distinguished in humans today: stefins, cystatins, latexins, fetuins, and kininogens (Fig. 1). Whether or not these diverse families and all of their respective members are phylogenetically linked remains to be clearly determined. Some members (*i.e.*, fetuins and latexins) may indeed have acquired "cystatin"-like properties through other mechanisms such as convergent evolution.

The stefins (STFs) are 11-kDa cytoplasmic cystatins each containing a single cystatin-like domain and lacking typical features of secreted proteins such as a signal peptide, disulfide bonds and glycosylation. The cystatins (CSTs) are secreted proteins 14- to 24-kDa in mass, which are distinguishable by a single cystatin domain, a typical signal peptide, and disulfide bonds. Some CSTs are ►*N*- or *O*-glycosylated, or exhibit ►Ser/Thr-phosphorylation. The secreted latexins (LTXs) contain two typical cystatin domains, but share little sequence homology to other cystatins, and seem to lack cysteine protease inhibitory activity. Plasma fetuins (FETs) also contain two cystatin-like domains, but share <30% sequence homology to other cystatins, and also lack cysteine protease inhibitory activity. Finally, the kininogens (KNGs) are plasma proteins with three cystatin-like domains, domain 1 being inactive and domains 2 and 3 being active as cysteine protease inhibitors.



Cystatins. Figure 1 Composition of the cystatin super-family. Schematic ball and stick representation of members of the cystatin super-family with one, two or three cystatin-like domains. k, kinin moiety; s, signal peptide.

Gene Structure & Evolution

At the gene level, a typical cystatin domain is generally the product of three coding exons. Consequently, the genes of most members of the cystatin super-family are composed of 3+ (STFs and CSTs), 6+ (LTXs and FETs), and 9+ (KNGs) coding exons. Although the existence of a common evolutionary origin of the individual cystatin genes is still uncertain, a hypothetical model for the evolution of the super-family has been proposed. According to this model, the various members arose from an ancestral STF-like cytoplasmic inhibitor. Upon evolution, the archetype gene acquired additional DNA elements such as, for example, the coding sequence for a signal peptide. This may have resulted in an archetypal gene for a secreted cystatin, which produced members with two and three cystatin domains upon successive gene duplication events. It is also possible that alternative evolutionary mechanisms have contributed to the level of diversity amongst the present members of the cystatin super-family.

Prokaryotes do not seem to harbor any genes even remotely similar to cystatins. Cystatins, instead, seem to have emerged with the development of more complex forms of life in eukaryotes. Typical cystatin-like genes have indeed been identified in various plants, some yeast strains, various unicellular parasites, as well as in worms, insects, fishes, frogs, birds, snakes and mammals.

Function

The primary function of cytoplasmic cystatins, such as STFA (stefin A/CSTA/ACPI/α-TPI) and STFB (stefin B/CSTB/NCPI/β-TPI), is generally assumed to be part of a safeguard mechanism protecting against the transient disruption of the integrity of lysosomes. Lysosomes are intracellular digestive factories filled with hazardous hydrolytic enzymes, including proteases generally referred to as ►cathepsins. Complexes between stefins and lysosomal cysteine-proteases form instantly, and can be readily detected when the integrity of cells and lysosomes is compromised. Shear stress, fever, stress-induced oxidation or glycation of proteins, infection by pathogens, and other stress conditions all contribute to repeated episodes of lysosomal leakage, which must be concealed in space and time for cell survival. In proliferating tissues, these events bear less consequence than in post-mitotic tissues such as cardiac and nervous

tissue. Unsurprisingly, STFB deficiencies have been linked to hereditary forms of monoclonus epilepsies, in which cerebellar granule cells undergo cell death mediated by leakage of lysosomal cysteine-proteases.

Secreted cystatins such as CST3 (cystatin C/BCPI/γ-trace/post-γ-globulin) were long believed to function exclusively as inhibitors of lysosomal cysteine proteases, despite early studies proposing additional or alternate functions. Before CST3 was coined a cystatin, it was believed to be a neuroendocrine peptide hormone. Over the years, five observations have greatly contributed to the need to reconsider the accepted model of CST function: (i) The number of CSTs, which seem to lack cysteine protease inhibitory activity, represents more than 50% of all members of the CST family in mammals. (ii) Several CST functions, such as the modulation of DNA synthesis, cell proliferation, and immune function, do not require cysteine protease inhibitory activity. (iii) Despite the fact that some CSTs form extremely tight molecular complexes with purified proteases *in vitro*, there still is little evidence for the occurrence of such complexes *in vivo*. (iv) Finally, under physiological and healthy conditions, most CSTs would be useless under the current model, as they are secreted proteins and their target enzymes are confined intracellularly.

Based on recent analyses of gene structures, a new model was proposed in which CSTs and ►chemokines evolved from a common ancestral gene and may have conserved similar integrated functions. Comparison of the crystal structures of CSTs and chemokines further underscores this relationship. Besides their similar gene structure (both are generally composed of three exons and two introns), CSTs share similarities in their basic three-dimensional fold: disordered *N*- and/or *C*-terminus, β-pleated sheet comprised of three to five anti-parallel β-strands, one β-strand with a bulge, a central α-helix, and two or three disulfide bonds to stabilize the core domain. CSTs and chemokines also share other intriguing features: Both types of proteins are secreted proteins, of low molecular mass (<24 kDa), which undergo ►*N*- or *C*-terminal processing by serine-proteases, have a tendency to dimerize, and exhibit multiple biological activities. To act like chemokines, however, CSTs would have to bind to specific ►G-protein coupled receptors (GPCRs). Such an interaction was proposed for the cystatin-like protein monellin from African berries, which, upon binding to a GPCR involved in taste perception, elicits an extremely sweet sensation in humans. Further direct evidence is required to establish an unambiguous relationship between CSTs and chemokines.

CSTs may play important roles in the modulation of the humoral and cellular immune responses. As inhibitors of lysosomal cysteine proteases, CSTs can block degradation of ►perforins and ►complement component C3, thus, preventing cytotoxic T cell- and complement-mediated cell lysis, respectively. In antigen presenting cells, lysosomal cysteine proteases play critical roles in major histocompatibility complex class II-mediated antigen processing and presentation. It has, however, not been conclusively determined whether CSTs are able to penetrate inside endosomes and/or lysosomes of antigen presenting cells to modify either process. As potential chemokines, CSTs have been shown to modulate the production of cytokines by various cell types such as macrophages and lymphocytes, as well as the release of nitric oxide, oxidative burst, and ►phagocytosis in neutrophils.

One of the functions of LTXs, as well as of other related members of the cystatin super-family with two tandem cystatin domains, seems to be the inhibition of some serine- and/or metallo- but not cysteine-proteases. LTX is the only known mammalian protein inhibitor of zinc-dependent carboxypeptidases and, thus, could potentially perform an important role in the regulation of peptide hormone activity during tissue growth and differentiation. The FETs regroups major plasma glycoproteins such as FETA (fetuin A/AHSG), FETB (fetuin B), and HRG (histidine- or histidine-and proline-rich glycoprotein). FETA is synthesized in the liver and constitutes a major plasma protein that accumulates in the matrix of bone and teeth. The protein also blocks calcium phosphate precipitation in the blood, thus preventing the spontaneous generation of systemic apatite crystals. This property is shared by HRG but not by FETB. A role for FETA in physiological and pathological mineralization is also evident from FETA knockout mice.

KNGs represent major plasma glycoproteins with three cystatin-like domains, and, like FETs, are also produced in the liver. One important function of KNGs is to serve as precursors of vasoactive kinins, such as bradykinin. Because bradykinin is extremely short-lived, this small peptide needs to be proteolytically released from the precursor molecule in the immediate vicinity of the GPCRs for bradykinin (BKRs). The third cystatin domain in KNGs is assumed to tether KNGs to the cell surface and thus bring the prokinin moiety close to the BKRs. With analogy to the interaction of chemokines with their respective receptors, one could postulate that KNGs perhaps also bind to BKRs in a two-step mode: an initial step in which there is a loose binding of the prokinin moiety, followed by proteolytic excision of the kinin moiety, tight binding, and activation of the receptor. Another important function of KNGs is to scavenge excess lysosomal cysteine-proteases that are locally released from damaged tissue during various injuries, infections, and inflammatory reactions. This buffering capacity of KNGs may limit further tissue damage and, hence, favor tissue remodeling and repair.

Role in Cancer

Lysosomal cysteine proteases have been implicated in multiple steps of tumor progression and recurrence, including early steps of ►immortalization and ►transformation, intermediate steps of tumor invasion and ►angiogenesis, and late steps of ►metastasis and drug resistance. During all these steps, tumor cells must actively ►escape immune surveillance. This may be accomplished, in part, through complete antigen degradation, which would leave no identifiable immunogenic peptides. Alternatively, exocytosis and cell surface binding of lysosomal cysteine proteases could lead to the degradation of the third component of complement C3 or the pore-forming protein perforin and, thus, interfere with immune cell-mediated lysis and the killing of target tumor cells. The importance of lysosomal cysteine proteases in the development of tumors from benign growths to aggressive lesions suggests that cystatins, *i.e.*, STFs and CSTs, may in many ways safeguard against tumor progression. In spite of this, the scenario is not always so simplistic as some CSTs appear to have both tumor promoting and tumor suppressing activities, while others promote metastasis. Thus, STFs and CSTs play important but distinct roles in our current understanding of tumor formation and progression. There is little or no information on LTXs and, the putative roles of FETs and KNGs in tumor ►neovascularization have been recently reviewed elsewhere.

In prostate and breast tissue, STFA is produced in basal and myoepithelial cells, respectively. STFA expression is thus lost with the loss of these cells during progression of most prostate and breast cancers. STFA expression is also lost during skin tumorigenesis, metastasis of oral/pharyngeal squamous cell carcinoma, and lung cancer progression. There is increasing evidence suggesting that STFs regulate initiation or propagation of the lysosomal cell death pathway. This cell death pathway is triggered by many different stimuli, including ►cytokines, ►p53 activation, and ►retinoids. In many tumor cells, the lysosomal cell death pathway involves cathepsin B as a major downstream executioner. Overexpression of STFA in various cancer cell lines indeed reduces their susceptibility to cell death-inducing agents. In addition, administration of STFA to mice bearing myeloid leukemias prolongs their mean survival. Exogenous treatments with STFA also reduce tumor cell ►motility. STFB is a far more ubiquitous and abundant protein than STFA, but like STFA, it also inhibits motility of tumor cells when exogenously administered.

CST3, unlike most other secreted cystatins, is considered a housekeeping-type gene. The mean concentration of this 15-kDa protein in normal human serum is about 77 nM (or 1.16 μg/ml). However, there are considerable differences in the levels of CST3 in other body fluids, suggesting that different tissues exhibit different accumulation rates of the protein or renewal rates of the extracellular fluid. The picture is further complicated by the fact that glomerular filtration is often impaired in patients with advanced cancer. CST3 has been extensively studied as a potential tumor marker, yet clinical studies tend to dismiss this cystatin as a useful diagnostic/prognostic marker. In spite of these clinical data, CST3 displays several noteworthy effects on tumor cells. As a potent inhibitor of lysosomal cysteine proteases, CST3 very efficiently inhibits *in vitro* tumor cell-mediated degradation and invasion of an ►extracellular matrix. In addition, overexpression of CST3 in human glioblastoma cells resulted in little apparent intracerebral tumor take compared to parental and mock controls. CST3, however, can also promote *in vitro* DNA synthesis and long-term proliferation of various cell types including ►stem cells. This may explain the fact that most established cell lines express and secrete this protein. ►Lung colony formation assays, in agreement with a mitogenic function of CST3, reveal that tail vein injection of highly metastatic melanoma cells results in a seven-fold reduction of lung colonies in CST3-null mice, compared to wild-type littermates.

CST6 (cystatin E/M) is expressed in a variety of normal human tissues, but its expression is lost in most established cancer cell lines and tumor tissues. In cancers of the breast, cervix, lung and brain, the CST6 gene is ►epigenetically silenced by promoter hypermethylation rather than deleted or mutated. Overexpression of CST6 in breast and lung cancer cells results in a reduction of colony formation and cell proliferation, suggesting that this cystatin may hold some tumor-suppressing capabilities. This has been confirmed *in vivo*, after inoculation of tumor cell clones into mammary fat pads of ►severe combined immunodeficient mice. CST6 expression in breast cancer cells strongly reduces tumor growth during the first six to seven weeks after inoculation, but has only a minor effect on incidence of metastasis and number of lesions in the lungs. The overall metastatic burden in the lungs and the liver, however, is significantly smaller in the CST6 when compared to the control group. Further studies using the experimental lung colonization assay demonstrated that CST6 expression has no effect on the initial seeding and survival of tumor cells in the lungs, but does reduce the expansion of established lung colonies. Thus, CST6 is a *bona fide* ►tumor suppressor gene for breast cancer.

CST7 (cystatin F/leukocystatin/CMAP) is a highly tissue-specific cystatin, which explains its initial cloning and annotation as leukocystatin. The gene is expressed predominantly in cells of the hematopoietic lineage and not in fetal tissues. CST7 is highly expressed in peripheral blood cells, such as T-cells and monocytes, as well as in stem cell-derived dendritic cells. Most T- and B-cell-derived cell lines, and the promyelo-monocytic

cell line U-937, secrete fair amounts of CST3, but little to no CST7. A significant amount of the CST7 produced by U-937 cells is in fact either retained intracellularly, or is reabsorbed by the cells immediately following secretion. CST7 has also been cloned by RNA differential display as CMAP, a cystatin-like metastasis-associated protein. This gene is preferentially expressed in murine tumor cell lines that metastasize to the liver. ►Transfection of highly metastatic cells with CST7 antisense cDNA led to a reduction of experimental colonization of the liver and spleen by several transfection clones, and improved survival of such tumor-bearing mice more than two-fold. Additionally, CST7 expression was detected in several human tumor cell lines, particularly in those with high propensity to metastasize to the liver (*i.e.* colon, pancreas, and lung cancer cell lines). Other tumor cell lines, which demonstrated high levels of CST7 expression, included cells from melanomas, glioblastomas, and osteosarcomas. Based upon a multivariate analysis of 79 patients with colorectal cancer (including 17 cases with liver metastases), high expression levels of CST7 were identified as the strongest independent factor for liver metastasis. In addition, the 5-year survival rate was significantly lower in patients with high CST7 expression than it was for those who displayed low expression levels.

Conclusion

From the above considerations and hypotheses it is clear that a much better understanding of the dual nature of cystatins could lead to the advancement of novel anti-cancer strategies. Further research could reveal that their roles as protease inhibitors and potential chemokines could be combined to signal anti-cancer immune responses, while also inhibiting tumor growth, invasion, and neovascularization. The literature on CST3 and CST7 suggests that chemokine-like function may need to be disrupted for efficient protease inhibitor-mediated tumor and metastasis suppression. In contrast, the example of CST6 shows that the two functions greatly contribute to tumor suppression.

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Cystatins A, B

►Stefins

Cystectomy

Definition

Surgical removal of the urinary bladder.

►Urothelial Carcinoma

Cysteine Proteases

Definition

A group of proteolytic enzymes that use the sulphydryl group of a cysteine residue for the nucleophylic attack of the scissile peptide bond. 148 human genes code for cysteine proteases.

►Stefins

Cystic Fibrosis

Definition

A hereditary disease caused by mutations within the ABC-transporter encoding gene ABCC7 (CFTR).

►ABC-Transporters

Cystic Nephroma

Definition

Benign multicystic renal tumor. Contrary to the cystic partially differentiated ►nephroblastoma it posses no

blastemal component in histology and can be found in adults too.

►Mesoblastic Nephroma

Cytarabine (1-arabinofuranosylcytosine)

Definition

Nucleoside analog used for lymphoma and acute myeloid leukemia chemotherapy, blocks DNA polymerization.

►Chromosomal Translocation t(8;21)

Cytochalasin B

Definition

The alkaloid cytochalasin B is a cell-permeable mycotoxin which inhibits cytoplasmic division by blocking the formation of contractile actin filaments. Cytochalasin B shortens actin filaments by blocking monomer addition at the fast-growing end of actin polymers. It blocks ►cytokinesis thus preventing the separation of daughter cells after mitosis that leads to the formation of binucleated cells.

►Micronucleus Assay
►Microcell-Mediated Chromosome Transfer

Cytochrome c

Definition

Is a protein of the mitochondrial electron transport chain with heme as a prosthetic group. Furthermore, it is involved in the apoptotic process; that is, following initiation of the mitochondrial pathway of ►apoptosis, it is translocated from the intermembrane space of the mitochondria to the cytosol, where it contributes to the activation of the procaspase 9 in the apoptosome.

►Photodynamic Therapy

Cytochrome c Oxidase

Definition

Is a large transformation protein complex (complex IV) found in mitochondria. It is the last protein in the electron transport chain and is the terminal enzyme responsible for 90% of cellular oxygen consumption in mammals

►Nitric Oxide

Cytochrome P450

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Synonyms

CYP450

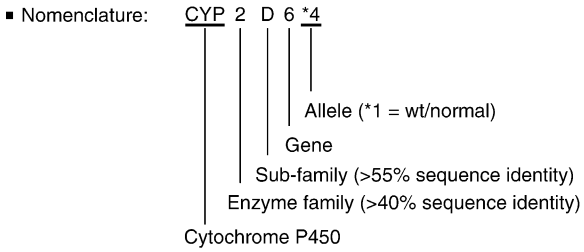
Definition

Cytochrome P450 stands for a superfamily of closely related proteins that protect the individual against potentially harmful substances by modifying these substances by oxidation, hydroxylation, dealkylation or dehalogenation, thereby increasing polarity and solubility and thus facilitating excretion from the body.

Characteristics

The body of an organism has a powerful system to facilitate the excretion of potentially harmful substances: the cytochrome P450 system. Cytochrome P450 stands for a superfamily of more than 50 closely related heme-containing enzymes, which can be divided into families (with at least 40% sequence homology on the amino acid level) and subfamilies (with at least 55% sequence homology), each with their own substrate specificity. The designation P450 is derived from the specific spectral absorbance at 450 nm of these proteins. The nomenclature is "CYP," followed by a Arabic number to indicate the family, then a capital letter indicating the subfamily if more members exist, and finally an Arabic number to indicate the specific enzyme (i.e., CYP2C9 is a member of the CYP2 family, belongs to the CYP2C subfamily) (Fig. 1). Family members include CYP1, CYP2, CYP3, CYP4, CYP11, CYP17, CYP19, CYP21, CYP26, and CYP51. These phase I monooxidases may carry out hydroxylation,

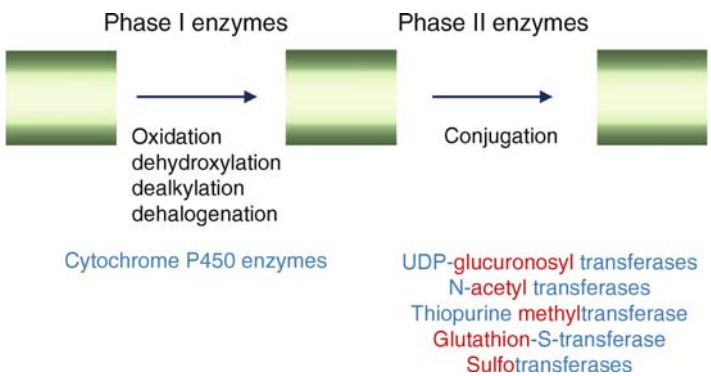
dealkylation, oxidation, or dehalogenation reactions on a variety of lipophilic compounds of exogenous or endogenous origin, which are often followed by conjugation reactions (Fig. 2). Purpose is to increase aqueous solubility by increasing polarity of these molecules, and thus facilitating their elimination from the body. Most of these enzymes are expressed in the liver, but also extrahepatic expression occurs (i.e., intestine, kidney). Within the cell, the enzymes are located in the endoplasmatic reticulum. Besides the systemic action of the CYP450 system, also tumor cells may express CYP450 enzymes, especially those of the CYP1 family, thereby potentially affecting effective treatment with anticancer drugs. Antibodies against the highly expressed CYP1B1 in many tumor types have been developed, which potentially can be used as specific cancer detection. The high CYP1B1 expression in many tumors metabolically inactivates drugs like ►paclitaxel, ►docetaxel, doxorubicin, mitoxantrone, and ►tamoxifen, and as a consequence, mediates anticancer drug resistance. Besides detoxification reactions, part of the CYP450 family is involved in synthesis of endogenous compounds like steroids. The CYP11A1 enzyme catalyzes the conversion of cholesterol to pregnenolone, while CYP21A2 (21-decarboxylase) is involved in the formation of 11-deoxycortisol from 15α-hydroxyprgesterone. If this latter enzyme is deficient, it causes congenital adrenal hyperplasia. The enzyme CYP19 is involved in estradiol formation from androstenedione, making local inhibition of this enzyme in tumors an interesting target in breast cancer therapy.



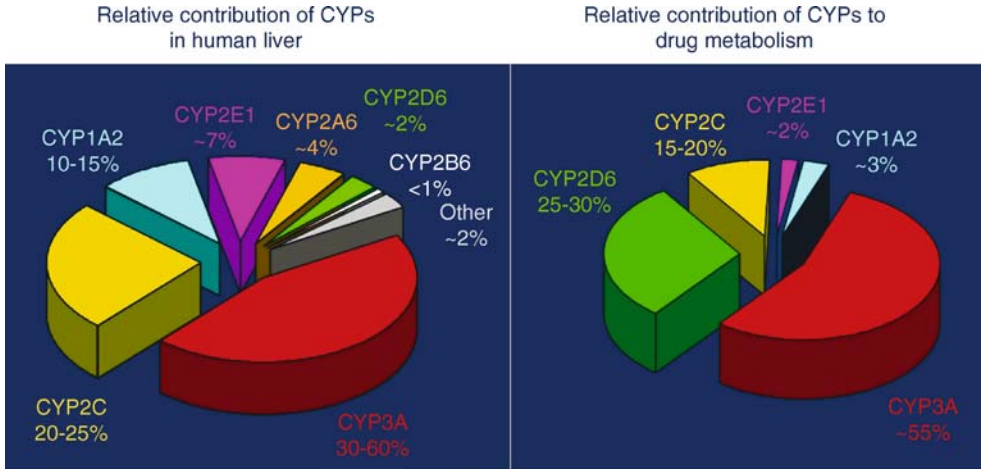
Cytochrome P450. Figure 1 Nomenclature for cytochrome P450 enzymes.

Detoxification

Other important exogenous substrates of the CYP450 enzymes are environmental chemicals, plant toxins, and medical drugs. Metabolism by CYP450 enzymes mostly deactivates these compounds and facilitates urinary or faecal excretion. The best studied members of these detoxifying enzymes are the CYP2D6 enzyme, the CYP3A subfamily, and the CYP2C subfamily. The CYP3A subfamily (CYP3A4, CYP3A5, CYP3A7, CYP3A43), with CYP3A4 being the most important member, accounts for 50% of the total CYP450 protein in the liver in adults. Expression increases during the first year of life, replacing the foetal CYP3A7 expression. CYP3A5 expression is only found in 20% of Caucasians, and the enzyme has considerable substrate overlap with the CYP3A4 enzyme. The role of CYP3A43 is thought to be minimal. The enzyme CYP3A4 is involved in the metabolism of >50% of prescribed drugs, while CYP2D6, accounting only for 2% of total protein, is involved in the metabolism of 25% of these compounds (Fig. 3). Together with the CYP2C family (CYP2C8, CYP2C9, CYP2C19), which metabolizes 5–10% of drugs, these three subfamilies take credit for the majority of phase I reactions of prescribed drugs. Most anticancer agents are substrates for CYP3A4, like docetaxel, vinca alkaloids, paclitaxel, cyclophosphamide, ifosfamide, irinotecan, imatinib, gefitinib, etoposide, teniposide. Important contributions of other CYP450 enzymes are, for instance, CYP2D6, which activates tamoxifen, CYP2B6 for activation of cyclophosphamide, and the prominent role of CYP2C8 in the metabolism of paclitaxel. Furthermore, thalidomide (used in the treatment of chronic myeloid leukemia and prostate cancer) is metabolized by CYP2C19, while the prodrug of 5-fluorouracil, tegafur, needs activation by CYP2A6. All cytochrome P450 enzymes, with the exception of



Cytochrome P450. Figure 2 Reactions catalyzed by cytochrome P450 enzymes.



Cytochrome P450. Figure 3 Percentage contribution of cytochrome P450 subfamilies to total protein in human liver (left panel) or to drug metabolism (right panel).

CYP2D6, are highly susceptible to induction or inhibition by numerous compounds, including drugs and environmental factors. Grapefruit juice, for instance, decreases CYP3A4 activity dramatically, the herb St. John's Wort increases CYP3A4 activity, and smoking increases the transcription and activity of CYP1A2. These inducing and inhibiting events may affect the ability to metabolize drugs, and thus interfere with the expected relationship between dose, blood concentration and effect.

Using CYPs to Improve Therapy

The enzyme CYP1B1 was identified as the main CYP450 present in a wide range of human cancers of different histological types, among which are prostate, lung, and renal cell tumors. It is expressed in primary tumors as well as in metastases and is regarded as a biomarker of the neoplastic phenotype. It thus is an interesting target in the development of novel anticancer prodrugs that need specific activation by CYP1B1. In this way, locally high concentrations of anticancer agents are generated without the high exposure of the total individual, although special precautions are necessary to prevent the premature elimination of those tumor cells expressing the CYP1B1 enzyme. Several patents on this approach have been filed in the last 5 years. Another, novel CYP450 enzyme was recently discovered: CYP2W1, which is expressed during fetal life but can also be found in transformed tissues. This high expression in tumors and the low expression found in normal cells also make CYP2W1 an interesting target for developing new anticancer therapies.

Genetic Polymorphisms

The enzymes of the CYP450 superfamily involved in detoxification reactions are highly polymorphic: genetic variants, usually present as ►single nucleotide polymorphisms (►SNPs), may encode enzymes with reduced activity. Because these ►variant alleles can be determined prior to starting therapy, they can be used to predicting aberrant pharmacokinetics. This discipline of analyzing DNA to predict or explain drug metabolism is called ►pharmacogenetics. These variant alleles are depicted using an asterisk, in which *1 stands for the most common (►Wild-type) active allele, and subsequent variant receive an increasing number (e.g. *CYP2C9*2*, *CYP2C9*3*) (Fig. 1). The power of these predictions depends on the pharmacokinetics of the drug (how much is it dependent on just one CYP450, or is there redundancy?), comedication, kidney and liver function, and environmental factors. For clinical use, these predictions are most valuable for drugs having a narrow therapeutic window, in which the consequences of subtherapeutic treatment on one hand, and toxic side effects on the other hand, are high. Cancer therapy is therefore an important field to explore the potential contribution of pharmacogenetics, in which prediction of metabolism in increasing effectivity and avoiding extreme toxicity are of vital importance.

CYP2B6

Enzymatic activity of CYP2B6 shows a considerable interindividual variation, but in addition, also a difference between males and females exists, the latter having a 1.7-fold higher activity. The *CYP2B6* gene has recently shown to be quite polymorphic, with various variant alleles in the population, encoding CYP2B6 with altered activities. The correlation between specific variations in the DNA and assignment to specific variant alleles has been quite confusing because increasing knowledge showed that certain SNPs proved not as unique as anticipated for a specific variant allele.

The most investigated SNPs at this moment are the 415A > G (Lys139Gln), 516G > T (Gln172His), 785A > G (Lys262Arg), and 1459C > T (Arg487Cys), which can be present in several allelic variants. The prominent role of CYP2B6 in the activation of cyclophosphamide was confirmed in some studies in which the 516G > T polymorphism correlated with a 1.5–2.0-fold increased clearance. Remarkably, this same SNP causes a reduced rather than increased enzymatic activity with respect to another CYP2B6 substrate, efavirenz, stressing the caution for extrapolating conclusions from one substrate to another. The frequency of the variant alleles containing this SNP is 23% for Caucasians and 16% for Japanese individuals, demonstrating that this may affect a substantial part of the patients treated with cyclophosphamide.

CYP2C8

The *CYP2C8*2* (805A > T, Ile269Phe) and *CYP2C8*3* (416G > A, Arg139Lys; 1196A > G, Lys399Arg) genetic variants, occurring with frequencies of 0% (*2) and 13% (*3) in Caucasians, and 18% (*2) and 2% (*3) in African Americans, were tested for their enzymatic activity on paclitaxel. This anticancer drug depends for 85% of its metabolism on CYP2C8 and for 15% on CYP3A4. Both CYP2C8 variant proteins showed a decreased activity on paclitaxel, implying a possible use in paclitaxel metabolism. However, further studies are needed to demonstrate the clinical use of genotyping for paclitaxel therapy.

CYP2C9

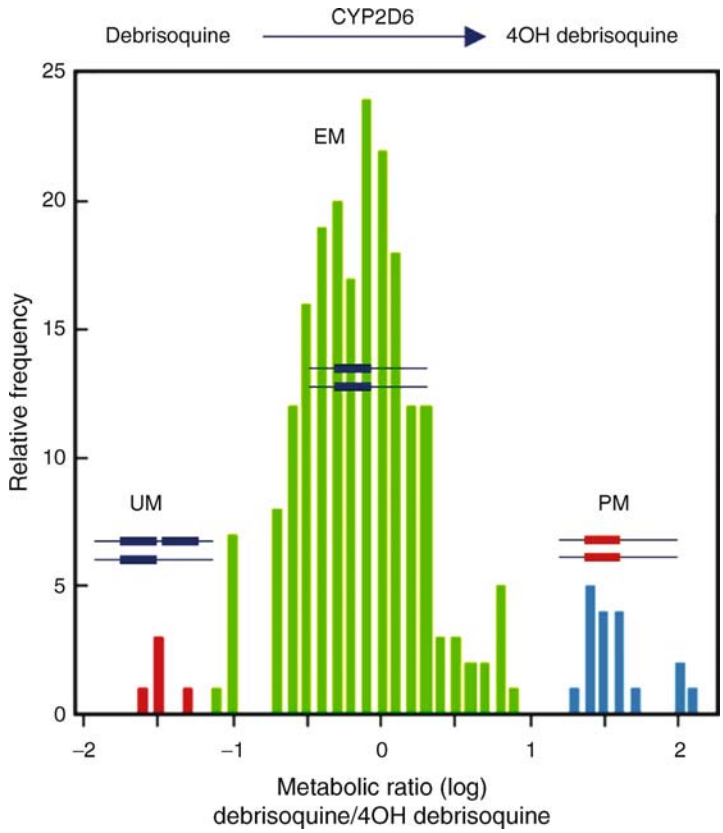
CYP2C9 is the major enzyme of the CYP2C family. The *CYP2C9* gene has numerous variant alleles, yet the *CYP2C9*2* (430C > T, R144C) and *CYP2C9*3* (1075A > C, I359L) are regarded as the most important variant alleles encoding decreased activity. The involvement of CYP2C9 in the metabolism of the anticoagulation drugs warfarin and acenocoumarol, affecting anticoagulation therapy, has resulted in major attention for this enzyme. Allele frequencies are quite high, with 11% (*2) and 7% (*3) in Caucasians while the frequencies of these alleles in Asians (0% for *2 and 3% for *3) and Africans (4% for *2, 2% for *3) are much lower. Although implicated in the metabolism of cyclophosphamide, no correlations between CYP2C9 variant alleles and cyclophosphamide pharmacokinetics are apparent, suggesting a very modest role for this enzyme.

CYP2C19

Population differences in CYP2C19 activity were first described around 1980 in an experiment using mephenytoin. One subject experienced such extreme sedation after taking mephenytoin for several days that he terminated his participation to the study. Based on the amounts of R- and S-mephenytoin in his urine, this individual was shown to be defective in S-mephenytoin 4-hydroxylation. Hereditary defective S-mephenytoin 4-hydroxylation is caused by the inheritance of two defective *CYP2C19* alleles. Approximately 2–3% of Caucasians is a CYP2C19 poor metabolizer, 3–5% of blacks and 10–24% of Asians. The most common defective alleles are CYP2C19*2 (681G > A; splicing defect) and CYP2C19*3 (636G > A; W212X, incomplete protein). Known substrates for CYP2C19 are the proton pump inhibitors (omeprazole, pantoprazole), in which the treatment of individuals for Helicobacter Pylori was found to be less effective in patients with two active alleles compared to patients having one or two deficient alleles. In cancer treatment, a correlation between CYP2C19 genotype and the activation of cyclophosphamide was demonstrated, thereby affecting survival, implicating potential use in cyclophosphamide therapy. Because of the involvement of CYP2C19 in the metabolism of thalidomide, pharmacogenetic analyses for this enzyme in the treatment of chronic myeloid leukemia or prostate cancer might be an interesting option.

CYP2D6

CYP2D6 activity displays a trimodal distribution in Caucasians, differentiating between poor metabolizers (PMs), extensive (normal) metabolizers (EM) and ultrarapid metabolizers (UMs) (Fig. 4). Also an intermediate metabolizer group can be distinguished, although phenotypically this group displays substantial overlap with the extensive metabolizers, and is usually characterized on the genetic level. For CYP2D6, 5–10% of the Caucasian population is practically deficient, which was discovered using the probe drugs sparteine and debrisoquine. This deficiency is due to inheritance of two defective *CYP2D6* alleles, the most common polymorphism being a G to A conversion on position 1846 of the *CYP2D6* gene, leading to a RNA splicing defect, characteristic for the *CYP2D6*4* variant allele. The second most prominent CYP2D6 allele is the *5 allele, in which the whole *CYP2D6* gene is deleted. Over 50 variant alleles have been described until now for CYP2D6, which can be divided in those encoding no activity (null alleles), decreased activity (decreased function alleles), or normal activity (functional alleles). The frequency of variants in the population depends very much on ethnicity. The *CYP2D6*17*, for instance, is mainly found in Africans, while in Asians the decreased activity allele *CYP2D6*10* is found much more frequent than in Caucasians. Patients with a CYP2D6 deficiency who are treated with tamoxifen for breast cancer showed decreased effectiveness of therapy, due to decreased activation. In addition to genetic deficient alleles, *CYP2D6* can also be present as gene duplications in certain individuals, bringing the

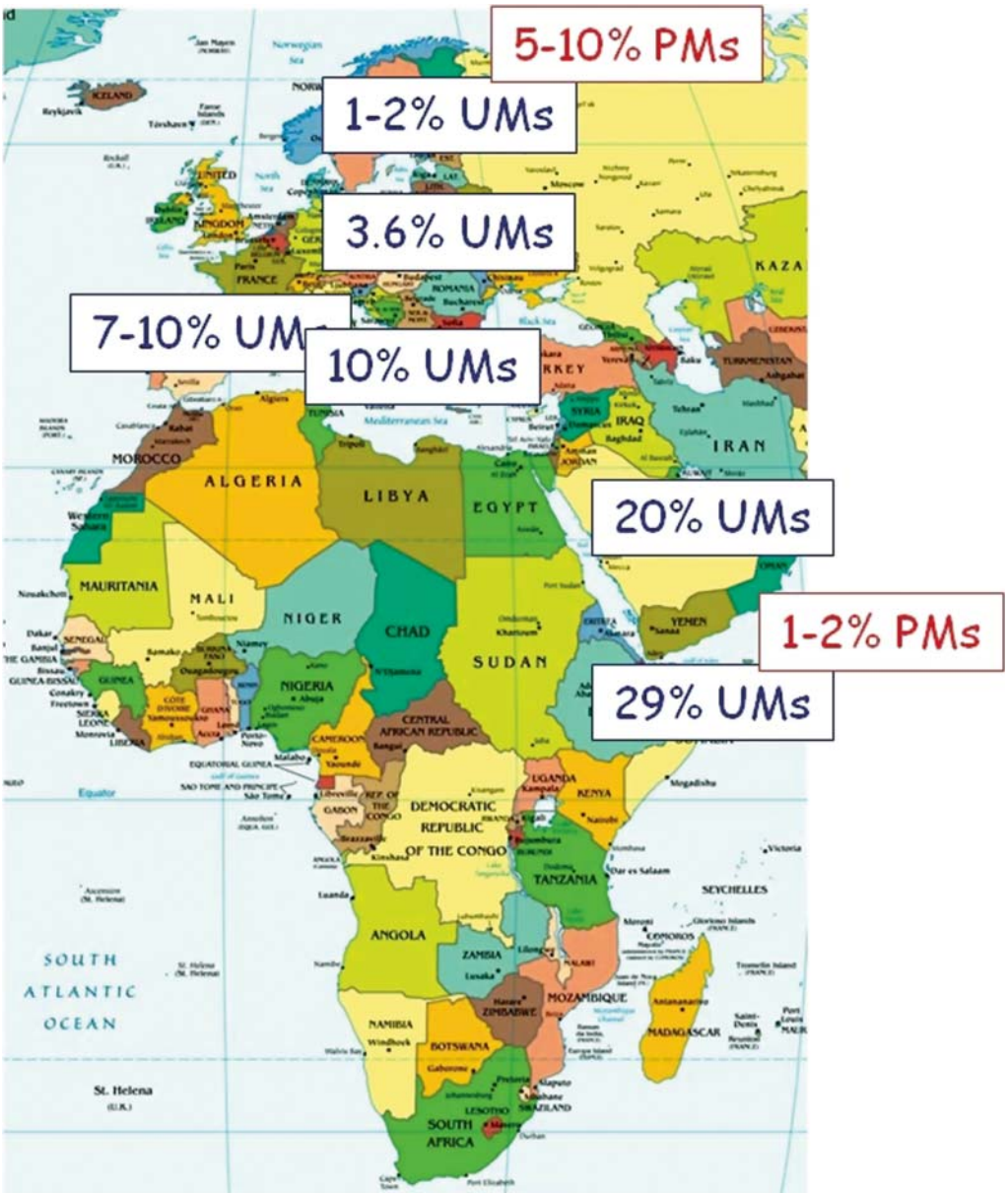


Cytochrome P450. Figure 4 Distribution of CYP2D6 activity in the Caucasian population, showing ultrarapid (UM), extensive (normal) (EM) and poor metabolizers (PM). (Adapted from Guen Guerich 2003, Mol. Intern. 3,194–204).

total number of CYP2D6 alleles to 3, or even higher. In Sweden, a family was identified that had 13 copies of the *CYP2D6* gene. This gene duplication is thought to be a compensating mechanism invented by evolution to circumvent the poor inducibility of CYP2D6. The frequency of this gene duplication displays an interesting north–south gradient, with duplications being present in 1–2% of individuals in Sweden, 3.6% in Germany, 7–10% in Spain, 10% in Italy, 20% in Saudi Arabia, and 29% in Ethiopia. The frequency of poor metabolizers shows the reverse, with 1–2% of poor metabolizers in Ethiopia to 5–10% in Northern Europe (Fig. 5). This gene duplication is accompanied by an increased activity, and leads an ultrarapid metabolizer phenotype. Individuals having these gene duplications may experience severe toxic effects with codeine, which is converted in the liver to morphine by CYP2D6; fatalities have been documented where the breast milk of a mother having codeine contained extreme high levels of morphine, leading to fatal morphine exposure in the child. This high morphine concentration in the milk was a result of the mother being a CYP2D6 ultrarapid metabolizer. In the treatment of cancer, CYP2D6 also plays a minor role in the metabolism of the new anticancer agent imatinib, but thus far the clinical implications of being a poor metabolizer for this kind of therapy are not known.

CYP3A4

This cytochrome is regarded as the most important enzyme, and is involved in the metabolism of over 50% of commonly described drugs. It is highly susceptible to induction and inhibition, with a resulting large interindividual variability in activity. Although extensive research has been done identifying genetic polymorphisms in this enzyme, most SNPs and the 20 variant alleles described until now proved to have low frequencies (<1%) in the different populations. An exception is the promoter variant *CYP3A4*1B* (−392A > G), which has an allele frequency of 2–9% in Caucasians, 35–67% in blacks, but was rarely found in Asians. In vitro experiments demonstrated a slightly higher transcription, and thus CYP3A4 activity, but the clinical consequences of this are not clear yet. Interestingly, this *CYP3A4* polymorphism was shown to be linked genetically to a polymorphism in the *CYP3A5* gene. It was suggested that effects, attributes to the *CYP3A4* polymorhism, were, in fact, due to the



Cytochrome P450. Figure 5 Geographical variation in frequency of CYP2D6 ultrarapid metabolizers (UMs) and poor metabolizers (PMs).

►*CYP3A5* polymorphism. However, several studies have shown correlations with the *CYP3A4*1B* allele without any correlation with CYP3A5 variant alleles. In recent study on cyclophosphamide, a decreased metabolism and a decreased median survival in breast cancer patients were demonstrated having the *CYP3A4*1B* allele. Recently, another variant, the *CYP3A4*16B* (554C > G; Thr185Ser) allele, was indirectly shown by the use of tagging SNPs to be correlated with a 20% decrease in paclitaxel metabolism in Japanese patients, a population in which the allele frequency of this variant was found to be 1.7%.

CYP3A5

For a long time, the interindividual expression of CYP3A5 was poorly understood. However, its expression in only 20% of Caucasians appeared to be caused by a highly frequent genetic polymorphism, 6986A > G, which is characteristic of the *CYP3A5*3* allele, and causes aberrant splicing. About 80% of Caucasians, but only 30% of the African American population, are deficient for this enzyme, most of them having the *CYP3A5*3/*3* genotype. Because of the substantial substrate overlap with CYP3A4, it is not always clear to what extent CYP3A5 contributes to the metabolism of certain drugs.

Cytochrome P450. Table 1 Correlations described for cytochrome P450 enzymes and the incidence of various cancers, either directly or in combination with environmental exposure

	1A1	1A2	2A6	1B1	2C9	2C19	2D6	2E1	3A4	3A5	17	19
Bladder						•						
Breast	•										•	•
Colorectal	•	•		•		•		•				
Dermatologic	•											
Gastric	•		•					•				
Gynecological				•							•	
Head and neck				•				•				
Hematologic												
Hepatocellular	•		•	•			•	•				
Lung	•		•			•		•		•		
Esophageal				•				•		•		
Prostate	•			•			•		•	•	•	•
Renal				•								

This polymorphism does have an impact in the metabolism of the immunosuppressive drug tacrolimus, which was slower in *CYP3A5*3/*3* individuals.

Cancer Incidence

A number of the detoxifying CYP450 enzymes have been associated with cancer incidence, mostly because their involvement in the conversion of procarcinogens to carcinogenic metabolites (Table 1). The xenobiotic benzo(a)pyrene, for instance, is a common environmental pollutant produced from the burning of coal, from the combustion of tobacco products, from food barbeque on charcoal briquettes, and from industrial processing. It is a weak carcinogen, but is converted to a potent carcinogen by CYP1A1, CYP1A2, and CYP1B1, making that variable activities in these enzymes correlate with cancer incidence. CYP1A1 alleles have been implicated in lung, gastric, colorectal, hepatocellular, breast, prostate, and dermatologic cancer, while CYP1B1 variant alleles correlated with colorectal, hepatocellular, prostate, head and neck, renal, and gynecological cancers. For CYP3A4, the *CYP3A4*1B* allele was discovered because it correlated with a more aggressive form of prostate cancer. In nonaggressive bladder cancer, a lower frequency of CYP2C19 poor metabolizers is found while, in contrast, for squamous cell carcinoma a higher frequency of CYP2C19 poor metabolizers was apparent. Also correlations with lung cancer and colorectal cancer have been reported, while CYP2C9 variant alleles showed correlation with colorectal cancer. *CYP2E1* polymorphisms were associated with an increased risk of lung, esophageal, head and neck, gastric, colorectal, and hepatocellular cancer, while CYP2D6 poor metabolizers appeared to be relatively protected for hepatocellular carcinoma but were at some increased risk for prostate cancer in patients who were using tobacco. Although not all mechanisms behind the correlation between variant CYP alleles and cancer incidence are known, it is obvious from the described observations that CYP450 enzymes not only play a role in the treatment of cancer, but may also be involved in the development of cancer.

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Cytochrome P450 (CYP)

Definition

Member of a superfamily of heme-containing monooxygenases involved in xenobiotic metabolism, cholesterol biosynthesis, and steroidogenesis, in eukaryotic

organisms found mainly in the endoplasmic reticulum and inner mitochondrial membrane of cells.

►Toxicological Carcinogenesis

Cytochrome p450 2 E1 (CYP2E1)

Definition

Inducible enzyme forming part of the cytochrome p450 family of enzymes capable of metabolizing ethanol to acetaldehyde

►Hepatic Ethanol Metabolism

Cytochrome P-4502E1

Definition

A specific cytochrome from the huge cytochrome family which is involved in the oxidation of ethanol but also of other ►xenobiotics (procarcinogens and drugs) and which is induced by chronic alcohol consumption.

►Alcohol Consumption

Cytochrome P450 CYP-P450 3A4

Definition

Cytochrome P450 CYP-P450 3A4 is an iron containing enzyme responsible for chemical oxidation (metabolism) of 60% of all drugs.

►ADMET Screen

Cytochrome P_{450} Enzymes

Definition

(CYP) enzymes are a large group of monooxygenase enzymes responsible for the metabolism (both synthesis/bioactivation and breakdown) of thousands of endogenous and exogenous compounds including drugs (Phase I metabolism), toxins, hormones, cholesterol, and vitamins.

►Carotenoids

C

Cytogenetic

Definition

Is the modifier of ►cytogenetics, the study of heredity by examining chromosome structure, learning and describing the relationships between chromosome structure and phenotype, and seeking out the causes of chromosomal abnormalities.

Cytogenetic Alteration

Definition

Chromosomes changes such as deletions, duplications, translocations, and amplifications that are associated with disease states.

►Gastrointestinal Stromal Tumor

Cytogenetic Analysis

Definition

Provides a technique for the evaluation of damage to chromosomes on the basis of direct observation and classification of chromosomal aberrations. Cells arrested in metaphase are examined microscopically for both numerical and structural chromosome aberrations.

Cytogenetic Karyotype

Is the characterization of the chromosomal complement of an individual or a species, including number, form and size of the chromosomes, by conventional banding techniques.

Cytogenetics

Definition

Laboratory analysis of chromosome structure. This analysis requires first growing cells, then "freezing" them in metaphase just prior to the completion of cell division in order to get a good viewing of condensed, paired chromosomes.

►Minimal Residual Disease
►Biomonitoring
►Leukemia Diagnostics

Cytokeratin 19 Fragments (CYFRA 21-1)

Definition

Is a soluble fragment of cytokeratin 19 expressed in normal squamous cells. Elevated serum concentrations are found in tumors of squamous origin, including lung and nasopharyngeal cancer.

►Serum Biomarkers

Cytokeratins

Definition

A large group of proteins expressed in a cell type-specific manner constituting intermediate filaments. They can be used for determining the histological subtype of cancers. A family of intermediate filament proteins that provides structural support to epithelial cells.

►Urothelial Carcinoma

Cytokine

Definition

Any of a variety of secreted polypeptides that control the development, differentiation, and proliferation of hematopoietic cells. The effects of cytokines on lymphocytes are usually mediated through membranebound cytokine receptors and are especially critical during immune responses. A group of proteins mainly functioning as soluble signal transmitters in the immune system. Are primarily released by immune cells but many other cell types can also release cytokines. Direct and mediate various functions of the adaptive and innate immunity and modulate functions of immune cells and other responsive cell types. Their local of systemic effects, elicited by binding to specific cell surface receptors, contribute mainly to innate and adaptive immune responses, so that their production is involved in a range of infectious, immunological, and inflammatory diseases. To this class belong interleukins, interferons, TNF-like molecules, etc. These molecular signals are similar to hormones and neurotransmitters and are used to allow one cell to communicate with another.

►Signal Transducers and Activators of Transcription in Oncogenesis
►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Cytokine Gene Transfer

Definition

Tumor cells can be genetically modified using various gene transfer strategies to transiently or stably express immunostimulatory cytokines in order to enhance their immunogenicity, i.e. their ability to stimulate an anti-tumor immune response. Granulocyte-macrophage colony stimulating factor (GM-CSF) turned out to be one of the most potent cytokines in this regard because it supports growth and differentiation of dendritic antigen-presenting cells which are of key importance for the induction of cellular immunity.

►Melanoma Vaccines
►Granulocyte-Macrophage colony stimulating factor (GM-CSF)

Cytokine Receptor

Definition

Cell surface protein that binds to specific cytokines and transduces intracellular signaling.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

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Synonyms

Cytokine receptor; Immunotherapy; Cancer vaccines; Immunotoxins

Definition

Cancer ►immunotherapy is the treatment of cancer by improving the ability of a tumor-bearing individual to reject the tumor immunologically. In the case of immunotherapy targeting ►cytokine receptors, delivery of tumor antigen proteins induces immune response against cancer cells bearing such tumor antigen to eliminate them. Cytokine receptor-targeting immunotoxins are proteins containing a bacterial toxin or chemical compound along with an antibody or a ligand that binds specifically to its target receptor. Immunotoxins then internalize through the receptors and achieve cytotoxic effect derived from the toxin moiety.

Characteristics

When cancer cells are generated in a body, a variety of mechanisms cooperate in order to kill the cancer cells. Among these mechanisms, the immune system has an important role to eliminate tumor tissues. First, immunocytes like ►macrophages and ►natural killer (NK) cells are activated to attack the newly formed foreign body. If they were unable to completely remove the cancer cells, T-cells and B-cells are the next players to fight against cancer. Many scientists have tried to find how the natural immune system works in order to control and to beat cancer. As cancer cells are known to express specific antigens or cytokine receptors on their cell surface, these molecules may be utilized as a target for tumor immunotherapy and immunotoxin therapy.

Cytokine Receptor as the Target for Immunotherapy

Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. For example, administration of interferon can activate the systemic immunity. Therapeutic cancer vaccine is also included in this category, which is designed to activate the host immune system against tumor cells. Cancer vaccines take advantage of the fact that certain molecules on the surface of cancer cells are either unique or more abundant than those found on normal or noncancerous cells. These molecules act as antigens, stimulating the immune system to evoke a specific immune response. There are few licensed therapeutic vaccines to date. However, several cancer vaccines are in large-scale ►clinical trials.

The Her2/neu (c-erbB2), the target protein of ►Herceptin®, which is the world's first therapeutic antibody to treat ►breast cancer, has long been studied as a target of cancer immunotherapy. The immunization methodology is variable, including administration of plasmid DNA, recombinant protein, or intracellular domain (ICD) peptide; virus vector delivery; dendritic cell pulse therapy; and combination therapy with adjuvant like granulocyte-macrophage colony stimulating factor (GM-CSF) or with anticancer reagents. Her2/neu immunotherapy has been tested in Phase II clinical trials to treat breast cancer, and in Phase I for various cancer types including ►ovarian cancer, prostate cancer, and ►non-small-cell lung carcinoma. In the Phase II trial contributed by Washington University, the ICD of HER-2 vaccine immunizes breast cancer patients with CD4 + helper T epitopes derived from the HER-2 protein. During ►preclinical testing, the rat neu peptide vaccine is effective because it circumvents tolerance to rat neu protein and generates rat neu-specific immunity. In Phase I study, patients underwent intradermal immunization once a month for a total of six immunizations with GM-CSF as an adjuvant. The endpoint of the study was to evaluate the toxicity and the both cellular and humoral HER-2/neu-specific immunity when the vaccinations were completed. The majority of patients (24 of 27 patients, 89%) developed HER-2/neu ICD-specific T-cell immunity. Out of 27 patients 22 patients (82%) also developed HER-2/neu-specific IgG antibody immunity, and over half of the assessable patients retained HER-2/neu-specific T-cell immunity 9–12 months after the completion of immunizations.

►Vascular endothelial growth factor Receptor-2 (VEGFR2) is highly expressed in neovascular endothelial cells in a tumor tissue. The epitope peptides of VEGFR2 were identified, and stimulation using these peptides induces ►CTLs with potent cytotoxicity in the ►HLA class I-restricted fashion against not only peptide-pulsed target cells but also endothelial cells endogenously expressing VEGFR2. In A2/Kb transgenic mice expressing α1 and α2 domains of human HLA-A*0201, vaccination using these epitope peptides in vivo was associated with significant suppression of the tumor growth and prolongation of the animal survival. A clinical trial has initiated to verify its effectiveness on breast and ►gastric cancer at University of Tokyo, Japan.

Another approach attempts to avoid ►immunologic tolerance to cancer vaccines. Cancer immunotherapy utilizes the host immune system, and tolerance is one of the major causes weakening vaccine efficiency.

If immunologic tolerance could be controlled, it is expected to enhance the effect of a cancer vaccine. There are a variety of other strategies which have shown antitumor activity in mouse model of cancer, e.g., dendritic cells pulsed with EphA2 (►ephrin-A2, an angiogenic factor) epitope peptide, KLH-bound EGF receptor variant III peptide, VEGFR1 epitope peptide, and plasmid DNA of IL-13 receptor α2.

Cytokine Receptor as the Target for Immunotoxin Therapy

Immunotoxins are protein toxins connected to a cell binding ligand or antibody. Classically, immunotoxins were created by chemically conjugating an antibody to a whole protein toxin, or, for more selective activity, by using a protein toxin devoid of its natural binding domain. Immunologic proteins that are smaller than monoclonal antibodies (MAbs), like growth factors and ►cytokines, have also been chemically conjugated and genetically fused to protein toxins. Targeted cancer therapy such as immunotoxin therapy which targets tumor-specific cell surface receptors is one of the most effective strategies against cancer. The targeted agents require a threshold level of receptor expression on the cancer cells to achieve their antitumor activity.

At present, only one agent targeting cytokine receptor, which contains human interleukin (IL)-2 and truncated ►diphtheria toxin (ONTAK), is approved for use in cutaneous T-cell lymphoma (CTCL). ONTAK, or Denileukin diftitox, is a fusion protein designed to direct the cytocidal action of diphtheria toxin to cells which express the IL-2 receptor (IL-2R). Among three forms of IL-2R, the high affinity form consisting of CD25/CD122/CD132 subunits is usually found only on activated hemocytes as T lymphocytes, B lymphocytes, and macrophages. A Phase III randomized, double-blind clinical trial was conducted in 71 patients with recurrent or persistent, Stage Ib to IVa CTCL whose malignant cells express the CD25 component of the IL-2R. Administered with 9 or 18 μg/kg/day of ONTAK as an intravenous infusion daily for 5 days every 3 weeks (median: 6 courses), seven patients (10%) achieved a complete response and 14 patients (20%) achieved a partial response. In 1999, the US FDA approved ONTAK indicated for the treatment of patients with persistent or recurrent CTCL whose malignant cells express CD25 component of the IL-2R.

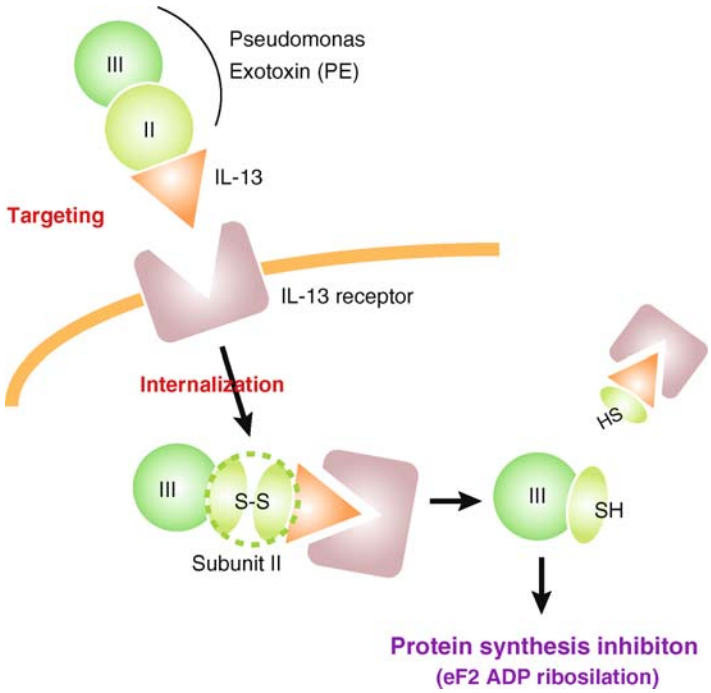
In the case of the IL-13 receptor (IL-13R) system, these receptors are constitutively overexpressed on a variety of human solid cancer cells including ►renal cell carcinoma, glioma, AIDS-associated ►Kaposi sarcoma, head and neck cancer, ovarian cancer, and prostate cancer. To target IL-13R, a recombinant fusion IL-13 cytotoxin termed IL13-PE38QQR, or Cintredekin besudotox, has been developed that is composed of IL-13 and a mutated form of ►*Pseudomonas* exotoxin. In early-phase studies, Cintredekin besudotox was administered via intraparenchymal convection-enhanced delivery (►CED) after resection of supratentorial recurrent malignant glioma. CED is a novel approach for the delivery of small and large molecules in solid tissues, utilizing a pressure gradient to distribute macromolecules to clinically significant volumes of tissue by bulk flow. The CED of Cintredekin besudotox was fairly well tolerated, with a reasonable benefit/risk profile for treatment of patients with glioma. It has received orphan drug designation in Europe and the US as well as fast-track drug development program status from the FDA (Fig. 1).

The ►interleukin-4 receptor (IL-4R), which is related to the IL-13R, is also expressed by a variety of solid tumors and ►hematologic malignancies. The IL-4 cytotoxin, IL4(38–37)-PE38KDEL, which is composed of circular permuted IL-4 and a mutated form of *Pseudomonas* exotoxin, is highly active in killing IL-4R-expressing tumor cells in vitro and in vivo. In a Phase I/II trial of 31 glioma patients, tumor necrosis was observed in 71% of patients, and one patient experienced long-term survival. The Phase II intratumoral study had been completed, but further development has stalled because of severe adverse events as infusion concentration of drug was possibly too high.

TransMID™, a modified diphtheria toxin conjugated to transferrin, is currently in Phase III clinical trials indicated for the treatment of ►Glioblastoma multiforme. Transferrin receptors are particularly prevalent on rapidly dividing cells, and the high level of transferrin receptor expression on glioma cells makes it an ideal target for brain cancer. Phase I and Phase II clinical trials for TransMID™ have been successfully completed in patients suffering from inoperable, recurrent high grade gliomas who have failed to all other forms of treatment. In Phase II study, a reduction in tumor size of 50% or more was noted in 35% of evaluable patients, with a corresponding increase in life expectancy in those patients that did respond. In this study, median survival for patients receiving TransMID™ was ~37 weeks. TransMID™ received fast-track status from the FDA, and orphan drug designation in the US, Europe, and Japan.

TP-38 is a recombinant chimeric targeted toxin composed of the EGFR binding ligand TGF-α and a genetically engineered form of the *Pseudomonas* exotoxin, PE38. A Phase I trial was conducted to define the maximum tolerated dose (MTD) and dose limiting toxicity of TP-38 delivered by CED in patients with recurrent malignant brain tumors. The Phase II studies, in which TP-38 was administered to patients with recurrent glioblastoma using CED, have shown initial encouraging results.

DT388GMCSF has been developed for the treatment of ►acute myeloid leukemia (AML). This molecule



Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy. Figure 1 Model for cytokine receptor-targeted cancer therapy. In this model, IL-13R expressing cancer cells are treated with IL-13 immunotoxin. When IL-13 moiety of this immunotoxin binds to IL-13 receptor, the receptor–immunotoxin complex immediately internalizes into cytosol. Then domain II of *Pseudomonas* exotoxin (PE) is degraded in endosome and domain III of PE shows cytotoxic effect through its irreversible inhibition of eF2.

is composed of amino acids 1–388 of diphtheria toxin (DT), a histidine–methionine linker, and amino acids 1–124 of human GM-CSF. Phase I clinical trial has been completed in patients with AML, and 4 of 37 patients showed clinical remission of disease.

Another unique DT-based approach is VEGF121-DT385 and VEGF165-DT385 immunotoxins, which is a chemical conjugate containing VEGF165, VEGF121, and truncated DT. ►Vascular endothelial growth factor (VEGF) is the most critical inducer of blood vessel formation. In vivo animal studies have shown that these molecules are able to inhibit angiogenesis and tumor growth.

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Cytokinesis

Definition

The last stage of cell division whereby the cytoplasm shared between the mother and daughter cells are split, and cellular content and organelles are redistributed. It usually starts during the late stages of mitosis, splitting a binucleate cell into two cells.

►Micronucleus Assay
►Mitosis

Cytologist

Definition

A professional who works in ►cytology. This can be either a cytopathologist or a cytotechnologist.

►Aspiration Cytology
►Fine Needle Aspiration

Cytology

Definition

Synonym cytopathology; The study of the origin, form, and function and pathology of cells. The medical specialty dealing with the microscopic examination of individual cells and small clusters of cells for the diagnosis of diseases, including cancers.

►Fine Needle Aspiration
►Molecular Pathology

Cytolytic Synapse

Definition

Forms as a cell-cell junction at the contact site between a cytolytic effector (cytotoxic T cell or natural killer cells (NK) cell) and a target cell and serves as a signaling platform; for example cytotoxic granule contents are released into the synapse before entering the target cell; it is also referred to as immunological synapse (IS).

►Natural Killer Cell Activation

Cytometry

Definition

Fluorescence antibodies oustains are applied to cells, and the cell expression of proteins nucleic acids are analyzed to determine the cell type. Cancer cells often show a different pattern of cell surface proteins or DNA content than their normal counterparts.

►Minimal Residual Disease

Cytomorphology

Definition

Microscopic examination of peripheral blood and bone marrow smears using panoptic stains.

►Leukemia Diagnostics

Cytopathologist

Definition

A board certified pathologist who sub-specializes in ►cytopathology. In many countries, an additional year of graduate training in an accredited cytopathology program, followed by successful completion of subspecialty board exams is required.

►Fine Needle Aspiration
►Cytology

Cytopenia

Definition

Refers to a deficiency of cellular element of the blood pancytopenia – an abnormal deficiency in all blood cells (red blood cells and white blood cells and platelets); usually associated with bone marrow tumor or with aplastic anemia haematocytopenia, hematocytopenia – an abnormally low number of red blood cells ►thrombocytopenia – A decrease in the number of platelets.

Cytoplasmic Scaffolding Apoptotic Protease Activating Factor

►APAF-1 Signaling

Cytoplasmic 7SL RNA

Definition

One of the components of the signal recognition particle (SRP) that functions in protein translocation across the endoplasmic reticulum.

►ALU Elements

Cytoprotective Adjuvant

Definition

A drug, chemical or therapy used in conjunction with chemotherapy to reduce or eliminate the adverse effects of chemotherapeutic drugs or radiation.

►Chemoprotectants
►Chemothrapy of Cancer

Cytoreduction

Definition

A surgical procedure that involves visceral resections and peritonectomy procedures so that the peritoneal dissemination is resected to the extent that only cells remain behind as residual disease.

►Appendiceal Epithelial Neoplasms

Cytoreductive Surgery

Definition

Refers to cytoreduction; removal of the major portion of the material composing a lesion. Removal of excess bulk of tissue from a lesion either to assist in healing or as an adjunct to chemotherapy. For instance, cancers of the ovary and fallopian tube tend to spread to the abdominal and pelvic areas. There may be cells found under the diaphragm, on the outsides of digestive organs, or in the omentum, an apron of fatty tissue roughly in front of the small intestine. During cytoreductive surgery, the surgeon carefully looks for all signs of cancer in the abdomen, and removes as much of the tumor as possible. Usually the omentum is removed as well. This makes it more likely that chemotherapy and/or radiation can kill the remaining cancer cells.

►Debulking Surgery
►Surgical Debulking

Cytoreductive Surgery

Definition

►Peritoneal Debulking Surgery

Cytoskeleton

Definition

Is a network of polymeric proteins organized in filaments (microtubules, microfilaments and intermediate filaments) that gives shape to the cell and contributes to transport of molecules inside the cell. It is an intracellular network of structural protein filaments (including actin filaments, microtubules and intermediate filaments like keratins, vimentin, desmin and neurofilaments). The cytoskeleton directs cell shape, mediates the anchoring and movement of cell organelles and is necessary for cell division. It is linked to cell-cell and cell-substrate cell adhesion molecules and increases the structural integrity of tissues and organs.

►Adhesion
►Cell Adhesion Molecules

Cytosol

Definition

The fluid portion of the cytoplasm, outside the organelles.

Cytosolic Transglutaminase

►Transglutaminase-2

Cytostasis

Definition

Arrest of cell growth and proliferation.

Cytostatic

Definition

Resulting in stopping cell division, but not necessarily cell death.

Cytotactin

►Tenascin-C and Cancer

Cytotechnologist

Definition

A medical laboratory specialist who works in the field of ►cytopathology. In many countries, a cytotechnologist must be a graduate of an accredited school of cytotechnology and must have successfully completed board certifying exams. Most cytotechnologists also hold a bachelor's degree.

►Fine Needle Aspiration

Cytotoxic Carcinogen

Definition

A carcinogen causing damage to cell structure or function.

►Toxicological Carcinogenesis

Cytotoxic Chemotherapy

Definition

Involves treatment with chemicals having selective toxicity for cancer cells.

►Chemotherapy
►Estrogenic Hormones

Cytotoxic T Cells (CTLs)

Definition

Synonym Cytotoxic T Lmphocytes; CTLs bear a heterodimeric T cell receptor (TCR) composed of alpha (α) and beta (β) chains that recognize fragments of antigenic proteins, which are associated with Major Histocompatibility Complex (MHC) class I molecules, on the surface of a cell. CTLs bear the CD8 receptor that also associates with MHC class I. This interaction can result in CTL activation and expansion. Activated CTLs secrete cytokines and can kill target cells expressing the same MHC/antigen complex. Since, with a few exceptions, all nucleated cells express ►MHC class I, the role of CTL is to monitor all the cells of the body, ready to destroy any that express foreign antigen fragments in their class I molecules. Thus, they are very important in killing infected cells and clearing viral and intracellular pathogen infections. They are also competent to kill tumor cells that express tumor-associated antigens.

►Immunoediting
►Peptide Vaccines for Cancer
►GAGE Proteins
►Interferon-α

Cytotoxic T Lymphocytes

Definition

Cytotoxic T lymphocytes, or killer T cells, are cells of the immune system that are responsible for the destruction of tumors, elimination of cells infected by virus, organ transplant rejection and autoimmunity.

►BORIS

Cytotoxins

Definition

Cytotoxins are proteins made by cytotoxic T cells that participated in the destruction of target cells. Perforins, granzymes, and granulysins are the major defined cytotoxins.

►Sjögren Syndrome

Cytovilin

►ERM Proteins

DAC

►A5-aza-2′ Deoxycytidine

Dacogen

►A5-aza-2′ Deoxycytidine

DAF

►Decay-Accelerating Factor

DAG

Definition
Diacylglycerol.

Diadzein

Definition
One of several known ►isoflavones, diazein is found in a number of plants, with soybeans and soy products like tofu and textured vegetable protein being the primary food source.

►Chemoprotectants
►Genistein

Daltons

Definition
Are atomic mass units. The simplest atom, hydrogen exists as hydrogen gas (H2) and has an atomic mass of two (2 atoms of mass 1). A single atom of oxygen has 16 mass units (16 Da).

►ADMET Screen

Damage Response

►Stress Response

DAMP

Definition
Acronym for Damage-associated molecular pattern molecules; ►Activated Natural Killer Cells.

Danazol

Definition
A drug used for the treatment of ►endometriosis and other diseases. It combines androgenic activity with anti-estrogenic and anti-progestogenic activity.

DAP6

►Daxx

DAP-10

Definition
DNAX Activation Protein of 10kDa, a signaling adaptor associated with NKG2D. Synonyms are Hcst: Hematopoietic cell signal transducer; KAP10: Killer activating protein 10.

►NKG2D Receptor

DAP-12

Definition
DNAX activation protein 12. A signaling adaptor associated with NKG2D in the mouse. Synonyms are KARAP: Killer-activating receptor-associated protein; TYROBP: TYRO protein tyrosine kinase-binding protein

►NKG2D Receptor

DARC

►Duffy Antigen Receptor for Chemokines

Darier Sign

Definition
Skin lesions that urticate when rubbed or irritated.

►Mastocytosis

Dasatinib

Definition
An oral medication approved as therapy for patients with ►chronic myeloid leukemia and ►Philadelphia chromosome-positive ►acute lymphoblastic leukemia. It is a tyrosine kinase inhibitor, effective against several tyrosine kinases. Is capable of inhibiting ►BCR-ABL, c-KIT, EPHA2, PDGFRβ and ►SRC family kinases (►SRC, LCK, YES, FYN). Dasatinib was originally developed to treat patients with chronic myelogenous leukemia who failed to respond to ►imatinib due to imatinib-resistant mutations in the ►BCR-ABL1 drug target.

►Mastocytosis
►Tyrosine Kinase Inhibitors

Data Over-Fitting

Definition
As in all research with high dimensional data, two practical realities constrain the analysis of mass spectra in ►proteomics. The first is that the data are typically characterized by thousands of features. The second is that the number of samples is limited. Relations that appear statistically significant are just noise. It occurs when the complexity of the statistical model is too great for the present amount of data.

►Oncopeptidomics

Daughter Cells

Definition
Cells that arise from the division of another cell (the parent cell).

►Mitosis

Daunorubicin

Definition
Is an ►anthracycline antineoplastic antibiotic isolated from cultures of *Streptococcus peucetius* var. *caesius*.

►Adriamycin

Daxx

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Synonyms

Death-associated protein 6; DAP6; BING2; MGC126245; MGC126246

Definition

Daxx was originally identified as a protein factor that binds to a transmembrane receptor called Fas (►Fas/Apo-1/CD95), one member of the ►tumor necrosis factor receptor superfamily. The extracellular region of Fas is where its ligand, FasL, binds. The intracellular tail shares sequence similarity with another member of the TNF receptor family, TNF receptor I (TNFRI). The shared sequence is termed death domain for its critical role in signaling cell death upon ligand binding.

Characteristics

Since its identification in 1997, Daxx has been intensively investigated for its biological functions. However, up to date the three-dimensional crystal structure of Daxx has not been resolved. Human Daxx protein is a polypeptide of 740 amino acid residues in length. As depicted in the schematic diagram (Fig. 1), Daxx contains several putative domains and many binding sites for interacting with a wide spectrum of proteins including transcription factors, kinases, tumor suppressors, and chromatin remodeling factors. It is worth to note that for the influence from its acidic region, Daxx displays an aberrant molecular weight during electrophoretic migration on gels, being 120 kDa instead of the calculated 81.3 kDa.

Basic Information

Human *Daxx* gene is mapped to 6p21.3 in the major histocompatibility complex (MHC) region, and transcribes a single 2.4 Kb RNA. It is ubiquitously expressed in human tissues, and is highly conserved in mammals with 69% identity between the human and mouse proteins. Currently at least 35 molecules have been known to interact with Daxx, which were identified by various methods including yeast two-hybrid screening, coimmunoprecipitation, and immunofluorescent costaining in the cell. Most notably, Daxx interacts with the ►tumor suppressor ►p53, ►Axin (Axis inhibitor), ►ubiquitin ligase ►Mdm2 (mouse double minute 2), the Ser/Thr kinase ►HIPK2 (homeodomain-interacting protein kinase 2), and the product of the promyelocytic leukemia (►*PML*) gene.

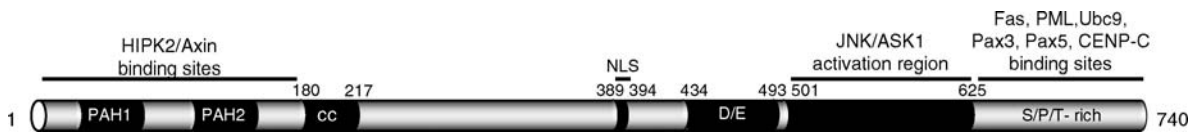
Subcellular Distribution of Daxx Protein

Daxx is predominantly localized in the nucleus. Daxx and PML interact with each other and are colocalized in nuclear punctuate structures known as nuclear bodies (NBs) that have been involved in various processes such as transcriptional regulation, apoptosis, genome stability, and tumor suppression. PML protein is an important tumor suppressor that is essential for the formation of the nuclear bodies, as deletion of the *PML* gene by genetic knockout can fully abrogate the formation of these structures. In $pml^{-/-}$ cells that lack PML-NBs structures, the ability of Daxx to induce cell death is abolished, underscoring the importance of PML for cell death induction by Daxx-mediated signaling.

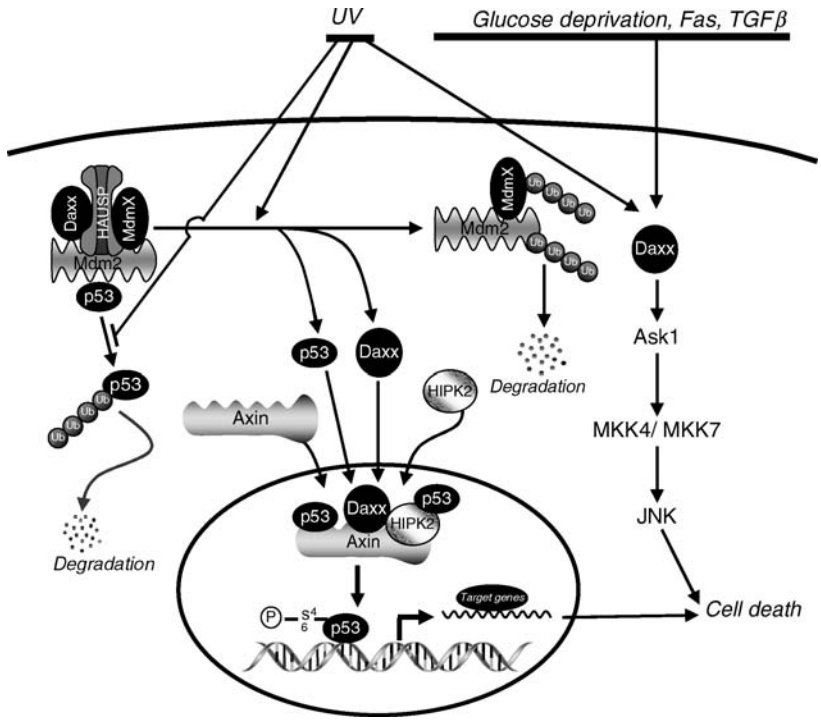
Daxx Mediates Stress-Induced Cell Death

Daxx has been implicated in the modulation of apoptosis induced by a wide range of stimuli, including ►ultraviolet radiation, hydrogen peroxide, ►arsenic trioxide, Fas ligand, transforming growth factor beta (►TGF-β), and interferon-γ. As summarized in Fig. 2, two main apoptotic pathways are thought to be mediated by Daxx.

- The first reported mechanism for Daxx to induce apoptosis is through association and subsequent activation of apoptotic signal-regulating kinase 1 (►ASK1), an upstream kinase of JNK MAP kinase that leads to activation of c-jun N-terminal kinase (►JNK). Recently, glucose deprivation as a stress



Daxx. Figure 1 Schematic diagram showing various domains of Daxx. Human Daxx is a protein with 740 amino acid residues in length that contains several putative domains: two N-terminal paired amphipathic helices (PAH1 and PAH2), a coiled-coil region, an acidic domain (D/E), and a Ser/Pro/Thr-rich domain (S/P/T). Axin and HIPK2 associate with Daxx through binding sites on the N-terminal portion. A region between amino acid 501 and 625 is required for ASK1 binding and the subsequent activation of JNK. The Ser/Pro/Thr-rich domain contains sites for interaction with a series of protein, such as Fas, PML, Ubc9, and CENP-C.



Daxx. Figure 2 Schematic representation of two possible pathways through which Daxx mediates cell death. Upon stimulation by stress signals such as UV and nutrition deprivation, Daxx becomes associated with ASK1, leading to activation of JNK MAP kinase that is required for cell death induction. In parallel, UV irradiation causes dissociation of p53-destabilizing complex to release p53 and Daxx which then assemble into a p53-activating complex consisting of p53, Axin, Daxx, and HIPK2. Upon complex formation, HIPK2 is activated and activates p53 by phosphorylating it at Ser46. As a result, p53 target genes related to apoptosis are activated.

can also stimulate the association of Daxx to ASK1 and activate Daxx-dependent ASK1 activation. TGF-β is a multifunctional growth factor that has principal roles in growth control. In a yeast two-hybrid screening using the cytoplasmic domain of the type II TGF-β receptor as bait, Daxx was found to interact with this receptor and mediate TGF-β-induced apoptosis via facilitating JNK activation. Interference of Daxx function by truncated Daxx mutants or by knockdown of Daxx expression can inhibit TGF-β-, UV-, or oxidative stress-induced apoptosis.

- Another means adopted by Daxx to induce cell death is through Axin/HIPK2/p53 complex. Currently, the best characterized role of Daxx is its ability to modulate the function of the tumor suppressor ►p53, which is mutated in over 50% of all tumor tissues. p53 exerts its tumor suppressional roles by acting as a transcription factor. Upon DNA damage, p53 is activated, which results in cell cycle arrest or apoptosis by inducing expression of a series of proteins such as p21, ►GADD45 (growth arrest and DNA damage), and ►PUMA (p53 upregulated modulator of apoptosis). HIPK2, an upstream Serine/Threonine kinase of p53, can specifically phosphorylate p53 at serine 46 upon UV irradiation and plays an important role in UV-induced p53-mediated apoptosis. ►Axin is a negative regulator of Axis formation in the development of mouse embryos, its deficiency can lead to axis duplication. In recent years, accumulating evidence demonstrates that this protein functions as a tumor suppressor. It has been shown that UV irradiation can enhance the association of Daxx and Axin. Further investigation demonstrates that Axin tethers Daxx to p53, and cooperates with Daxx to stimulate HIPK2-mediated Ser46 phosphorylation and transcriptional activity of p53. Axin is required for Daxx-induced p53 activation and apoptosis, as in $Axin^{-/-}$ cells the ability of Daxx to induce cell death is dramatically attenuated. Downregulation of HIPK2 by specific siRNA remarkably suppresses Daxx-induced apoptosis, suggesting that HIPK2 plays crucial role in Daxx-induced cell death. All above evidence indicates that formation of a new complex containing Daxx/Axin/p53/HIPK2 is important for Daxx-induced cell death. Knock-down of Axin and Daxx by ►siRNA can strongly attenuate UV-induced cell

death, demonstrating that Daxx and Axin complex plays a critical role in UV-triggered cell death.

In Unstressed Cells, Daxx Seems to Play an Antiapoptotic Role

Daxx may also destabilize p53 by promoting the function of Mdm2, a ►ubiquitin ligase E3 that facilitates p53 ►ubiquitination and degradation. In unstressed cells, Daxx simultaneously interacts with Mdm2 (heterodimerized with ►MdmX) and the deubiquitinase ►HAUSP (herpes-associated ubiquitin-specific protease). In this complex, Daxx can stimulate the stabilizing effect of Hausp on Mdm2, which results in accumulation of Mdm2 in the cell. Moreover, Daxx enhances the intrinsic E3 activity of Mdm2 toward p53 leading to the inhibition of the antiproliferative effects of p53. Upon DNA damage, Daxx, HAUSP, and p53 dissociate from Mdm2, which allows Mdm2–MdmX complex to undergo autoubiquitination and subsequent degradation. The dissociated Daxx and p53 may then form an apoptotic complex with HIPK2 and Axin in the nucleus, in which Daxx and Axin cooperatively promote the HIPK2 kinase activity toward p53 at Serine 46. Therefore, in response to stress signals, Daxx enhances the death-inducing function of p53, suggesting that Daxx in fact may exert opposing roles in controlling p53 activity depending on cellular states. However, the detailed molecular mechanisms as to how Daxx dissociates from the Daxx/Mdm2/HAUSP/Mdmx complex and recycles to form an apoptotic complex upon DNA damage remain to be clarified in the future.

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DBCCR1

Definition

A locus at 9q33 that may encode a tumor suppressor in ►urothelial carcinoma and other cancers.

DCC

Definition

Deleted in colorectal carcinoma is implicated as a ►tumor suppressor gene. The gene maps to 18q21.3 and encodes a membrane protein preferentially found in axons of the neurons of the central and peripheral nervous system and in differentiated cell types of the intestine. ►Colorectal tumors that have lost their capacity to differentiate into mucus producing cells, uniformly lack DCC protein expression. Inactivation of DCC due to allelic deletion and/or point mutations may also be involved in both lymphatic and hematogenous metastasis of esophageal squamous cell carcinomas.

►Multistep Development
►Esophageal Cancer

DCIS

Definition

►Ductal Carcinoma In Situ.

DcR3

►Decoy Receptor 3

DCX

Definition

►Doublecortin

D-Dimer

Definition

A plasmin breakdown product of cross-linked fibrin that provides a convenient measure of systemic coagulation activation.

►Coagulopathy

DDP

►Cisplatin

DDS

►Drug Delivery Systems for Cancer Treatment

De novo

Definition

De novo Synthesis refers to the synthesis of complex molecules from simple molecules such as sugars or amino acids, as opposed to their being recycled after partial degradation.

►Arginine-Depleting Enzyme Arginine Deiminase

Deacetylation

Definition

Removal of acetyl from histones, opposite to ►aceylation.

Death-associated protein 6

►Daxx

Death Domain

Definition

DD; A conserved cytoplasmic domain of death receptors (►TRAIL-R2 and ►TNF-R1). Via this domain, death receptors can bind other death domain-containing proteins that act as adaptor proteins for binding ►caspase-8 and/or caspase-10. The death domain is a characteristic region of 80 amino acids in the cytoplasmic tail of death receptors of the TNF receptor superfamily that mediates protein–protein interaction with DD-containing adaptor molecules such as FADD or TRADD.

►Caspase-8
►TNF-Related Apoptosis Inducing Ligand (TRAIL)

Death-Effector-Domain

Definition

DED; Is a protein interaction domain comprised of six α-helical structure. DEDs are found in seven pro- or antiapoptotic proteins involved in the regulation of ►apoptosis and proliferation, i.e. ►caspase-8, caspase-10, ►FADD, c-FLIP, PEA-15/PED, DEDD, and DEDD2.

►TNF-Related Apoptosis Inducing Ligand

Death-Inducing Signaling Complex

Definition

DISC; Is a multiprotein complex that is formed upon crosslinking of death receptors such as ►CD95, ►TRAIL-R1, and TRAIL-R2 by their respective ligands, i.e., CD95 ligand or TRAIL, or agonistic antibodies. It consists of oligomerized death receptors, ►FADD, ►caspase-8 and -10 and is essential for transmitting the apoptotic signal from ►death receptors at the cell surface to the cytoplasmic caspase cascade.

►Apoptosis

Death Receptor-3

Definition

DR3; Is a member of the ►tumor necrosis factor receptor superfamily. These receptors are characterized by the presence of a 70–80 amino-acid homologous

region in the cytoplasmic tail called the death domain. The signaling pathways induced by these receptors rely on oligomerization by ligand binding, recruitment of death domain proteins like TRADD, FAD or RIP1 and subsequent activation of the caspases apoptotic cascade or the transcription factor NF-kappa-B. DR3 is preferentially expressed in peripheral blood leukocytes, and lymphocytes-rich tissues and to a lesser extent in small intestine and colon. The precise role of DR3 is unclear. However, its ectopic expression in mammalian cells induces ►apoptosis and activates the transcription factor NF-kappa-B, depending on the cytoplasmic effectors engaged in the signaling complexes downstream of the death domain. DR3 is also expressed in colon carcinoma cells and is involved in binding to E-selectin and in providing a metastatic advantage to the cancer cells.

►E-selectin-Mediated Adhesion
►Nuclear Factor Kappa B

Death Receptor Apoptosis Pathway

Definition

Also known as the extrinsic death pathway, this pathway is initiated by the death receptor at the cell surface. The receptors include Fas (►CD95), TNF-R1 (CD120a), ►TRAIL-receptor I (DR4) and TRAIL-receptor II (DR5). Upon engagement by their ligands, FasL, TNFα or TRAIL, respectively, the receptors oligomerize and internalize, which subsequently recruit adaptor molecules, such as ►FADD and TRADD. The initiator caspases, ►caspase-8 or caspase-10 are recruited to this ►death inducing signaling complex (DISC) and activated. Subsequently, the effector caspases are activated and ►apoptosis occurs. The initiate caspases could also activate Bid, which then activates the ►mitochondria apoptosis pathway. The latter is particularly important for caspase activation in the so-called Type II cells, which include hepatocytes and certainly lymphoid cell lines.

►Bid

Death Receptors

Definition

Belong to the ►tumor necrosis factor (TNF) receptor gene superfamily, which is defined by similar, cysteine-rich extracellular domains. The death receptors also contain a homologous cytoplasmic sequence termed the "►death domain". Death domains typically enable death receptors to engage the cell's apoptotic machinery, but in some instances they mediate functions that are distinct from or even counteract apoptosis. Some molecules that transmit signals from death receptors contain death domains themselves.

►FLICE Inhibitory Protein

Debulking Surgery

Definition

►Cytoreductive Surgery.

►Surgical Debulking

Decatenation G2 Checkpoint

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Definition

A surveillance system that ensures that ►mitosis does not begin before ►sister chromatids have been sufficiently decatenated.

Characteristics

During DNA replication, daughter DNA duplexes become catenated or entangled through knots and links. These knots and links must be removed before sister chromatids can be properly segregated during mitosis. Type II DNA ►topoisomerases are enzymes that remove knots and links through a concerted set of reactions in which a protein-associated DNA double-strand break is generated, another segment of DNA is passed through the break, and the protein-associated double-strand break is reversed. The decatenation G2 ►checkpoint is an active signal transduction pathway that monitors topoisomerase II-dependent DNA decatenation and controls the location and/or

activity of ►mitosis-promoting factor to delay the onset of mitosis.

Molecular Biology

Drugs that inhibit topoisomerase II fall into two groups known as poisons and catalytic inhibitors. Topoisomerase II poisons stabilize the enzyme when in a covalent complex with cleaved DNA strands. Proteolysis of the protein produces frank DNA double-strand breaks which are potentially lethal lesions. Topoisomerase poisons such as doxorubicin and etoposide are among the most widely used and successful of cancer chemotherapeutic drugs. The catalytic inhibitors of topoisomerase II prevent the enzyme from forming protein-associated DNA double-strand breaks; by preventing formation of protein-associated DNA double-strand breaks, catalytic inhibitors are able to block the toxicity of the poisons.

There are two type II topoisomerases in mammalian cells. Topoisomerase IIβ is a non-essential enzyme that is constitutively expressed in quiescent and proliferating cells. Topoisomerase IIα is an essential enzyme whose level of expression is tightly regulated in the cell division cycle. Maximal expression occurs in G2 and M when ►topoisomerase II is required to separate linked sister chromatids and condense mitotic chromosomes. Catalytic inhibitors of topoisomerase II include ICRF-193 and ICRF-187. ICRF-193 causes mammalian cells to delay progression from G2 to mitosis, and impedes separation of sister chromatids at anaphase in mitosis. This behavior suggests that cells monitor the state of chromatid catenation in G2 and delay entry to mitosis until ►sister chromatids are sufficiently decatenated. Because chromatids retain some catenations into metaphase of mitosis, the decatenation G2 checkpoint monitors the sufficiency of decatenation, not its completion.

Both ICRF drugs cause cells to delay progression from G2 to mitosis. The G2 delay was established as an active checkpoint when various loss-of-function mutations or genetic alterations were shown to reverse the delay. The decatenation G2 checkpoint requires signaling by ►ATR, ►CHK1, ►BRCA1 and WRN to block cellular entry to ►mitosis. Override of decatenation G2 checkpoint function using caffeine induces chromosome aberrations (breaks and exchanges) as mitotic cells attempt to condense and segregate knotted and linked chromatids.

The transition from G2 to mitosis (►G2/M transition) is controlled by mitosis-promoting factor, a ►cyclin-dependent kinase made up of a catalytic subunit, CDK1, and a regulatory subunit, cyclin B1. Cyclin B1/CDK1 complexes can initiate all steps of mitosis including nuclear envelop breakdown, chromosome condensation and formation of the mitotic spindle apparatus. Cyclin B1 is co-regulated with topoisomerase IIα, with peak levels of expression in G2 and mitosis. The activity of mitosis-promoting factor is regulated by phosphorylation/dephosphorylation. Inhibitory phosphates in the enzyme catalytic site are removed at the onset of mitosis by CDC25B/C. Cyclin B1/CDK1 complexes also are actively exported from the nucleus during G2 but at the ►G2/M transition this exportation is inhibited, intra-nuclear transport is enhanced and mitosis-promoting factor accumulates in the nucleus. The decatenation G2 checkpoint blocks cellular progression from G2 to mitosis by preventing the accumulation of mitosis-promoting factor in the nucleus. The decatenation G2 checkpoint appears to function by inhibiting the activity of the Polo-like kinase PLK1 that stimulates nuclear accumulation of mitosis-promoting factor.

Clinical Implications

The decatenation G2 checkpoint is a dependency checkpoint that ensures that an essential event in the ►cell cycle (chromatid decatenation) is achieved before a dependent downstream event (chromatid condensation and separation in mitosis) is initiated. Failure of decatenation G2 checkpoint function is expected to cause instability of chromosome numbers and structure (►Chromosomal instability). Insufficient decatenation can cause non-dysjunction errors when the mitotic spindle breaks, producing ►aneuploidy; alternatively, linked chromatids may break under the force of chromatid condensation or segregation producing deletions, amplifications and rearrangements. Cells with defective decatenation G2 checkpoint function due to defects in ATR, CHK1, BRCA1 and WRN display severe chromosomal instability.

Some bladder, breast and lung cancer lines display defects in decatenation G2 checkpoint function. The breast cancer susceptibility gene BRCA1 is not only required for decatenation checkpoint function, it also regulates the decatenatory activity of DNA topoisomerase IIα (►Breast cancer genes BRCA1 and BRCA2). BRCA1 contains an ubiquitin-ligase activity that is expressed in the presence of the cofactor BARD1. BRCA1 regulates ►ubiquitination of topoisomerase IIα and ubiquitination stimulates decatenatory activity of topoisomerase IIα. Thus, breast cancers with inactivation of ►BRCA1 may display less chromatid decatenation by topoisomerase IIα. BRCA1 also serves as a mediator in DNA damage and decatenation G2 checkpoints, enhancing phosphorylation by checkpoint kinases of their downstream targets. Breast cancers with defects in BRCA1 are less able to decatenate daughter chromatids and less able to delay mitosis when chromatids are catenated.

►Lung cancer lines with defects in decatenation G2 checkpoint function display hypersensitivity to

killing by ICRF-187. This suggests that topoisomerase IIα catalytic inhibitors may be clinically useful for selected cancers.

►Cancer stem cells represent immortal clones that drive malignant progression (►Stem cells and cancer). Mouse embryonic stem cells and human hematopoietic stem cells when grown in cell culture display a defect in the decatenation G2 checkpoint. Because decatenation G2 checkpoint function preserves chromosomal stability during cell division, a checkpoint defect may promote ►chromosomal instability in cancer stem cells.

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Decay-Accelerating Factor

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Synonyms

CD55; Decay-accelerating factor for complement; Antigen of the Cromer blood group; DAF

Definition

Decay-accelerating factor (DAF) participates in the regulation of complement system activity by accelerating the decay of the C3/C5-convertase of the classic as well as of the alternative pathway. The highly polymorphic 50–100 kDa protein facilitates complement regulation by cystein-rich complement control protein repeats (CCP). Most of the DAF isoforms are linked to the cell membrane by a glycosyl phosphatidylinositol (GPI) anchor following the CCPs.

Characteristics

DAF has been detected in all mammalians. Physiologically it is expressed in all cells contacting the complement system enclosing cells within the peripheral blood and epithelial as well as endothelial cells. Soluble DAF is detectable in plasma, tears, saliva, and urine, as well as in synovial and cerebrospinal fluids. Besides its function as a regulator of the complement system DAF inhibits ►natural killer cells, is a ligand of the CD97 receptor, and a receptor for viruses and microorganisms. DAF is also involved in the development of spermatozoa and their survival within the female genital tract.

DAF is a glycoprotein appearing as different isoforms depending partially on different posttranslational glycosylation patterns and on alternative splicing. The DAF protein possesses four units for the control of complement activation following each other and that are designated as CCP 1–4. Therefore, the molecular weight of the mature protein varies between 50 and 100 kDa in different cell types.

DAF has been detected in different malignancies, e.g., ►CLL, ►CML, ►ALL, ►AML, ►colorectal cancer, ►gastric cancer, ►thyroid cancer, ►medullary thyroid carcinoma, malignant ►glioma, ►breast cancer, ►renal cancer, ►non-small cell lung cancer, ►ovarian cancer, ►cervical cancer, and also partially in metastases of colorectal carcinomas. Furthermore, DAF is frequently overexpressed within the stroma of colorectal tumors suggesting that DAF comes from the tumor cells and is either cleaved from the cell membrane into the environment or is secreted by the tumor cells as a soluble form. Expression of more than one DAF isoform originated from different glycosylation patterns (colon cancer) or alternative splicing (breast cancer) has been detected.

Actions of DAF in Cancer (Tab.1)

DAF expression in cancer cells is upregulated by ►interleukins (►IL-4, IL-1α, IL-1β), cytokines (►TNF-α, ►IFN-γ), growth factors (►EGF, ►bFGF), ►prostaglandins (E2), and complement regulatory protein protectin (CD59).

DAF decreases complement deposition on tumor cell membranes as well as complement-mediated lysis in melanomas, renal tumors, thyroid, lung, squamous cell, and cervical carcinoma as well as in some hematological malignancies. DAF decreases cell adhesion of T-lymphocytes to leukemic cells as well as inhibiting effect on ►NK cells which could impair immune surveillance of cancer cells.

The GPI-anchored and membrane linked form of DAF is part of a signal transduction cascade. In consequence, tyrosine phosphorylation in malignant tumors functioning within signal transduction goes far beyond immune modulating effects. For example, the tyrosine

kinase p56lck participates in a signal cascade conveying active motility of breast cancer cells.

DAF has been identified as ligand of the surface receptor CD97 (EGF-TM7), which belongs to the family of class B seven-span transmembrane (TM7) receptors. Predominantly expressed in hematopoietic cells, CD 97 is ectopic expressed in human thyroid, colorectal, gastric, pancreatic, esophageal, and oral squamous cell carcinomas. CD97 promotes invasive growth, ►migration, and ►angiogenesis. DAF, being the ligand of CD97, is synthesized and secreted by colorectal carcinoma cells leading to an autocrine stimulation of invasive growth, migration, and ►metastasis of these cells. A similar mechanism takes place in breast cancer cells as ►HER2-positive mammary carcinoma cells showing increased transendothelial invasiveness selectively overexpress and secrete a 45 kDa splice variant of DAF (Table 1).

Perspectives in Cancer Therapy (Table 2)

Removal of DAF from the cell membrane or neutralization of the protein increases the intensity of a potential

Decay-Accelerating Factor. Table 1 Versatile actions of Decay-accelerating factor in cancer

Action	Mechanisms
►Carcinogenesis	Upregulation of DAF in cancer and precancerous lesions (factors regulating DAF expression: IL-4, IL-1α, EGF, PGE2, TNFα, IFNγ, thrombin, βFGF, VEGF, CD59)
Complement inhibition	Decay-acceleration of the C3/C5-convertase leading to decrease in complement deposition and in complement-mediated lysis of cancer cells
Inhibition of natural killer (NK) cells	Downregulation of NK cell-mediated cell lysis
Oncogenic tyrosine kinase pathway activation	Signal transduction through GPI-anchored (tyrosine phosphorylation of src-kinases, TCR-zeta chain and ZAP-70)
Angiogenesis	Increase of DAF synthesis by VEGF
Invasive growth	Increase in DAF expression, ligand binding of DAF to CD97 (autocrine stimulation by tumor-specific DAF isoforms?)
Cell migration	Ligand binding of DAF to CD97, tyrosine phosphorylation of p56lck
Metastasis	Increase in DAF expression, ligand binding of DAF to CD97 (autocrine stimulation by tumor-specific DAF isoforms?)
Survival and apoptosis	Induction of apoptosis through monoclonal SC-1-antibody against tumor-specific DAF isoform (upregulation of c-myc, activation of caspase-6 and -8, cleavage of cytokeratin 18)

Decay-Accelerating Factor. Table 2 DAF targeted therapies

Targeted Cancer	Therapeutic investigational design and benefit
Stomach adenocarcinoma of diffuse-type	Monoclonal antibody SC-1 against a gastric cancer-specific 82 kD isoform of DAF; induced apoptosis in primary tumors as compared to pretreatment biopsy material in up to 90% of the cases, regression of tumor mass up to 50%
Metastasizing gastric cancer in nude mice	Monoclonal antibody SC-1 against a gastric cancer-specific 82 kD isoform of DAF; reduce the number of disseminated tumor cells in the bone marrow
Non-Hodgkin's lymphoma	Rituximab (chimeric anti-CD20 monoclonal antibody); enhancement of complement-dependent killing activity of Rituximab, by additional application of monoclonal anti-DAF-antibody
Cervical cancer	Monoclonal antibody against DAF showed the widest range of specific reactivity
Renal cancer	Bispecific antibodies binding DAF as well as renal tumor-associated antigen G250; decrease of unwanted side effects
Osteosarcoma of children	DAF as a cancer vaccine additionally applied to myelosuppressive chemotherapy; induction of T-cell proliferation 71% and antigen-specific gammaIFN secretion in 59% of the cases; vaccination was well tolerated
Melanoma xenografts in immunodeficient mice	Coxsackievirus A21 infection; rapid viral oncolysis

local inflammatory reaction as well as complement-mediated cell lysis and could also possibly improve response to special therapeutic strategies. In consequence, DAF was used as a target for molecular cancer therapy. Monoclonal antibody SC-1 binding an isoform of DAF expressed in gastric carcinoma induced ►apoptosis of these cells. In first clinical trials, patients with poorly differentiated stomach adenocarcinoma of diffuse-type have been treated primarily with the SC-1 antibody followed by gastrectomy and lymphadenectomy. A significant induction of apoptotic activity in primary tumors as compared to pretreatment biopsy material in up to 90% of the cases and a significant regression of tumor mass in up to 50% was observed. Application of SC-1 antibody therapy in nude mice with metastasizing gastric cancer reduced the number of disseminated tumor cells in bone marrow. Other studies could show that the complement-dependent killing activity of ►Rituximab, a chimeric anti-CD20 monoclonal antibody, in the treatment of ►non-Hodgkin lymphoma cells is enhanced by additional application of monoclonal anti-DAF antibody. The DAF is also expressed in many physiological human cells. Therefore, use of bispecific antibodies binding DAF as well as another tumor-specific antigen like the renal tumor-associated antigen G250 might be a solution to decrease unbeneficial side effects. Additional application of DAF as a cancer vaccine for ►osteosarcoma under myelosuppressive chemotherapy induced T-cell proliferation gammaIFN secretion. DAF as a receptor for viruses enhanced the systemic effect on metastatic melanoma cells by an infection with coxsackie-A21-viruses (Table 2).

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Decay-accelerating Factor For Complement

►Decay-Accelerating Factor

D

Decitabine

►A5-aza-2′ Deoxycytidine

Decoy Receptor 3

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Synonyms

DcR3

Definition

DcR3 is a member of the ►TNF receptor superfamily (TNFRSF6B), and the DcR3 gene is mapped to chromosome 20q13.3. DcR3 is a glycosylated protein of 300 amino acids and 33 kD. The receptor lacks a transmembrane domain and exists as soluble protein.

Characteristics

Tissue Expression and Decoy Function of DcR3

DcR3 is generally undetectable in normal tissues, but is highly expressed in several human malignant tissues, such as adenocarcinomas of the esophagus, stomach, colon, rectum, pancreas and lung, glioblastomas and lymphomas. In addition, high serum levels of DcR3 have been detected in many cancer patients. Clinical data indicate the significance of detecting serum DcR3 as a novel parameter for the diagnosis, treatment, and prognosis of malignancies. Several lines of evidence suggest a significant role for DcR3 in immune suppression and tumor progression. DcR3 has been regarded

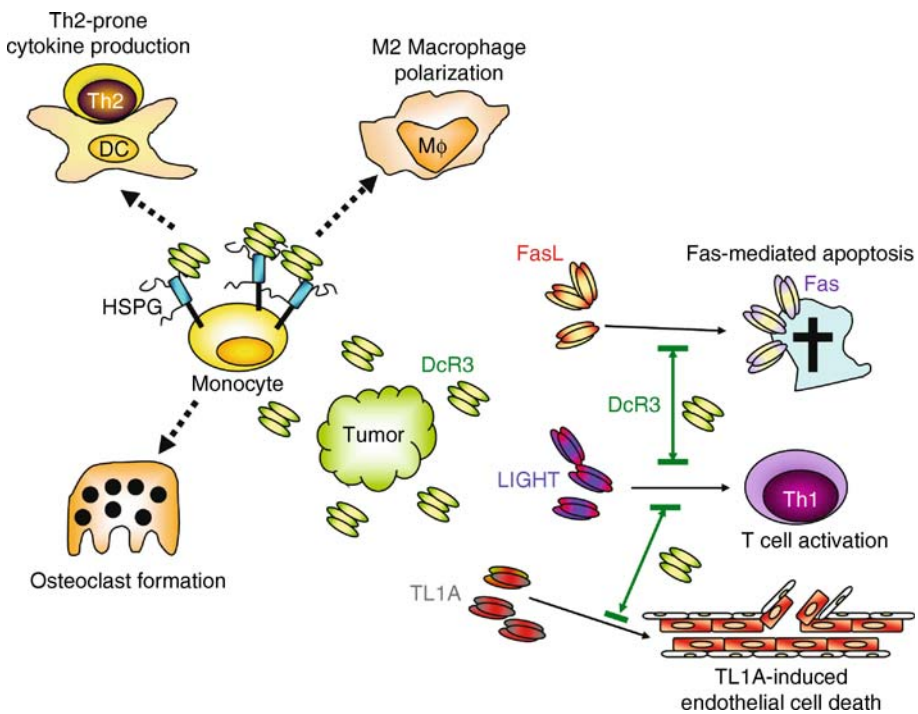
to function as decoy receptor for FasL, LIGHT (homologous to lymphotoxins, shows inducible expression and competes with herpes simplex virus glycoprotein D for herpesvirus entry mediator, a receptor expressed by T lymphocytes) and TL1A, which are three cytokine members of the TNF family. FasL and LIGHT are expressed by activated T cells, and can induce tumor cell death through signaling pathways mediated by Fas and lymphotoxin β receptor (LTβR), respectively. LIGHT is also a T cell co-stimulator, and this action is mediated by receptor herpesvirus entry mediator (►HVEM). Therefore, Fas and LIGHT are two cytokines contributing to the host immune surveillance. TL1A is an angiostatic cytokine releasing from endothelial cells. The cytokine neutralizing actions confer DcR3 function as a tumor molecule to decrease T-cell-mediated immunity and stimulate ►angiogenesis. Tumor cells engineered to release a higher amount of DcR3 may escape from FasL-induced apoptotic cell death.

Decoy Unrelated Novel Actions of DcR3 (Figure 1)

In addition to neutralizing bioactive cytokines, DcR3, which is also an effector molecule, directly modulates the activities of many cell types. DcR3 can regulate ►dendritic cells (DCs) differentiation and down-regulate several co-stimulatory molecules, leading to Th2 polarization. Moreover, DcR3 induces actin reorganization and adhesion of monocytes as well as reducing phagocytic activity and proinflammatory cytokine production in ►macrophages. Osteoclast formation is promoted by the addition of DcR3 to monocyte/macrophage lineage precursor cells. Finally, DcR3 increases monocyte adhesion to endothelial cells via ►NF-κB activation, leading to the transcriptional up-regulation of adhesion molecules and IL-8 in endothelial cells. Thus DcR3 has pleiotropic effects to modulate inflammation and ►osteoporosis. As chronic ►inflammation has long been associated with tumorigenesis, DcR3-induced inflammation might provide a beneficial microenvironment for tumor growth. Increased ►osteoclast activity and decreased bone density are observed in DcR3 transgenic mice; therefore DcR3 might also play an important role in bone erosion and destruction in cancer patients.

New Action Mechanisms of DcR3 (Figure 1)

It is a mystery why DcR3 has such diverse immunomodulatory functions independent of the neutralizing FasL, LIGHT and TL1A. A glycosaminoglycan-binding domain of DcR3 has been identified as binding and cross-linking heparan sulfate proteoglycans (HSPG), such as syndecan 2 and CD44v3, to induce monocyte adhesion via activating ►PKC. Recombinant protein, comprising the HSPG-binding domain (HBD) of DcR3 and Fc portion of human IgG1 (HBD.Fc) has a



Decoy Receptor 3. Figure 1 This figure shows the biological activities of DcR3. Tumor-secreted DcR3 can neutralize FasL, LIGHT and TL1A, and also bind to HSPG of monocytes to trigger signal cascades, resulting in differentiation of M2 macrophages, osteoclasts and Th2- dominant immune response.

similar effect as DcR3.Fc to modulate the activation and differentiation of DCs, macrophages, and induce osteoclast differentiation. Even though Fc stabilizes dimeric DcR3 and enhances cross-linking activity, transgenic mice overexpressing DcR3 also attenuates Th1 differentiation and enhances osteoclast formation. This indicates that the Fc portion is dispensable, and the biological effects of DcR3 in vivo are not restricted to its neutralizing effects on FasL, LIGHT, and TL1A.

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DED

Definition

Stands for death effector domain and is required for the recruitment of initiator caspases containing DEDs to the death-inducing signaling complex.

►FLICE Inhibitory Protein

Dedifferentiation

Definition

The development of a high-grade malignancy within a well-differentiated malignant tumor.

►Bone Tumors

Deeply Infiltrating Endometriosis

►Endometriosis

Definitive Therapy

Definition

Therapy considered potentially curative by itself. Surgery is perhaps the most common form of definitive therapy. However, regimens that combine ►radiation therapy and ►chemotherapy (without surgery) are also sometimes curative by themselves, and therefore considered definitive.

►Induction Chemotherapy

Delayed Type Hypersensitivity Reaction

Definition

DTH; reaction is a type of cellular-mediated immune response mainly mediated by T cells for a given antigen. The DTH test is used to detect prior exposure to the antigen. Typically, when small amounts of antigen are injected intradermally, a discrete reaction is elicited which includes redness, swelling, induration and infiltration of immune cells into the site of the lesion within 24–72 h.

Deleted in Malignant Brain Tumours 1

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Synonyms

DMBT1; Glycoprotein-340 (gp-340; human); Salivary agglutinin (SAG, human); H3 (rhesus monkey); CRP-ductin (mouse); Muclin (mouse); Apactin (mouse); Vomeroglandin (mouse); Ebnerin (rat); Hensin (rabbit)

Definition

DMBT1 located at chromosome 10q26.13 2 was initially identified as a gene that shows frequent homozygous deletions in malignant ►brain tumours. It codes for an extracellular glycoprotein of the evolutionary highly conserved group of scavenger receptor cysteine-rich (SRCR) proteins and it is widely expressed in human tissues with strongest expression in epithelia and associated glands. Based on genomic alterations and loss of expression in several cancer types *DMBT1* has been proposed to play a role in tumorigenesis. The currently available data suggests two physiological functions for DMBT1: a function in innate immunity/mucosal protection and a function in epithelial/stem cell differentiation and regenerative processes.

Characteristics

The human DMBT1-locus is built up by tandem-arrayed repeats with extensive homologies in the coding and non-coding region. The locus spans a region of about 103 kb with 55 exons with the last exon coding for a putative transmembrane domain (TMD). In contrast to the DMBT1 homologues in mouse and rat, the TMD exon has not yet been found to be contained in human transcripts. DMBT1 contains a signal peptide at the N-terminus and ►immunohistochemistry demonstrated that it is expressed and secreted mainly by epithelial cells and glands. The mode of secretion is thought to determine its functions: lumenally secreted ►DMBT1 is part of the mucus (the protective layer of epithelial surfaces) and is assumed to play a role in pathogen defence, while DMBT1 secreted to the extracellular matrix (ECM) may trigger epithelial and stem cell differentiation.

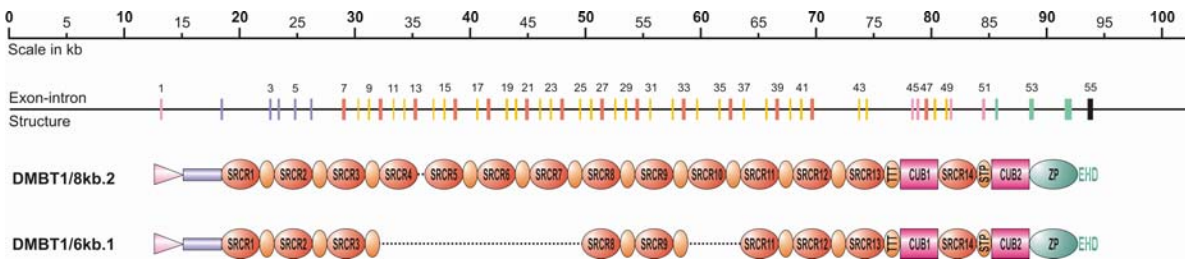
The largest (wildtype) variant of DMBT1 contains 13 highly homologous (87–100% identical) SRCR domains. The SRCR-domains are separated by so called SRCR-interspersed domains (SIDs) harbouring potential sites for *O*-►glycosylation (Fig. 1). The SRCR domains have been shown to function in pathogen binding and in mediating interactions with endogenous proteins involved in pathogen defence.

Functions or ligands for the two C1r/C1s Uegf Bmp1 (CUB) domains and the 14th SRCR domain, which shares less homology to the 13 C-terminal ones, have not been identified so far. The second CUB domain is followed by a so called zona pellucida (ZP) domain (Fig. 1). This type of domain mediates the formation of protein-oligomers in other molecules, which is likely the case for DMBT1 as well. Genetic polymorphisms give rise to DMBT1 alleles with copy number variations resulting in a reduced number of SRCR domains and SIDs. Several DMBT1-alleles were identified coding for transcripts with sizes ranging from 6 to 8 kb (Fig. 1). A hemizygous deletion of SRCR4-SRCR7 was found in approximately 25% of the normal population.

Functional Characteristics

►*DMBT1* and Innate Immunity/Mucosal Protection

There is increasing evidence that DMBT1 is part of the innate immune system against bacteria and viruses. The protein is present at most mucosal surfaces and binds to a broad range of bacteria including ►*Helicobacter pylori*, a human pathogen, which is linked to the development of peptic ulcer and ►gastric cancer. Evidence that the SRCR-domains could mediate the binding to bacteria has been provided by the identification of a small peptide sequence present in these domains, which exerts a broad bacteria-binding and -agglutinating activity. However, several hints have also suggested an involvement of DMBT1-attached



Deleted in Malignant Brain Tumours 1. Figure 1 Genomic organization and domain structure of DMBT1. Topline represents scale in kilobases followed by the exon–intron structure of the DMBT1 gene consisting of 55 exons. The exons are coloured according to the domain that they code for (see below). The presence of a further exon (black box) with coding potential for a transmembrane domain and a short cytoplasmic tail is predicted by homology searches with the cDNA sequences of the rodent homologues. The bottom two lines depict the domain organisation of the wildtype protein (DMBT1/8 kb.2) isolated from human adult trachea and one of the 6 kb transcripts isolated from human fetal lung (DMBT1/6 kb.1). The two variants could represent alternative splice products, but simultaneously also correspond to the largest and the smallest allelic variants identified so far. Pink triangle, signal peptide; blue box, unknown motif; red circles, SRCR domains; orange circles, SIDs, threonine- and threonine-serine-proline-rich domain, respectively; purple boxes, CUB domains; green circle, ZP domain; green letters, Ebnerin homologous domain.

glycosyl-residues in pathogen interactions. Furthermore, DMBT1 binds to viruses like HIV-1 and influenza A and is able to inhibit viral infection *in vitro*. Beside its interactions with microorganisms, DMBT1 is known to interact with mucosal and circulating defence factors among these the surfactant proteins A and D, (s)IgA, ►trefoil factor 2, lactoferrin and complement component C1q. This suggests a potential cooperative action of ►DMBT1 and its endogenous ligands in host defence. Further ►DMBT1 may activate the complement cascade and ►macrophage chemokinesis and regulate neutrophil respiratory burst response *in vitro*. Upregulation of ►DMBT1 has been reported in inflammatory processes for example following tissue injury or infection. Except for a role in innate mucosal protection, ►DMBT1 expression in lymphoid organs may point to a participation in the regulation of the acquired immune system, as has been reported for other proteins of the ►SRCR superfamily.

DMBT1 and Epithelial/Stem Cell Differentiation and Regenerative Processes

Functional studies *in vitro* and *in vivo* suggest a role for ►DMBT1 and its rodent homologues in epithelial/stem cell differentiation. The rabbit homologue of DMBT1 (hensin) was found to be responsible for the switch of cell polarity and the induction of terminal differentiation of intercalated epithelial cells in the kidney. These processes are triggered by the deposition and polymerization of hensin in the extracellular matrix. Localization to the ECM of in human adult multilayered and fetal developing epithelia, and expression along the proliferation-differentiation axis in the intestine may support such function. Rabbit DMBT1 has further been reported to trigger *in vitro* the differentiation of mouse embryonic stem cells towards columnar epithelial cells. DMBT1-deficient mice (termed hensin−/− mice) have been reported to display early embryonic lethality. Based on upregulation of rat DMBT1 in liver stem cells during liver regeneration, a participation in stem cell-mediated regenerative processes has been proposed.

DMBT1 and Tumorigenesis

Genomic alterations in *DMBT1* and a loss or a reduction of its expression in several cancer types have led to the proposal that *DMBT1* might represent a ►tumor suppressor gene. Inactivating point mutations have not been found within *DMBT1*. Homozygous deletions (deletions concerning both copies of the gene) of/or within *DMBT1* initially have been observed in a substantial number of malignant ►brain tumours, ►lung cancer and ►esophageal cancer. Due to the finding that *DMBT1* is a highly polymorphic gene with frequent intragenic deletions within the repetitive SRCR/SID exon containing region, however, it has been proposed that the majority of the previously identified deletions may represent preexisting polymorphisms unmasked by a loss of heterozygosity of the wildtype allele. Hence, inactivation of *DMBT1* at the transcriptional/post-transcriptional level rather than genomic alterations may represent the predominant mechanism. Evidence has been gained that ►epigenetic silencing by DNA ►methylation may account for DMBT1 silencing in a smaller subset of tumours.

Contrary to other cancer types, ►prostate cancer and ►pancreas cancer display highly elevated DMBT1 expression levels, so that ►DMBT1 has been discussed as potential biomarker for these cancer types. A 29 amino acid C-terminal peptide of ►DMBT1 has further been found at high levels in pancreatic cancer and has been suggested as tumor ►biomarker.

These data have led to the suggestion of a complex involvement of DMBT1 in tumorigenesis. Through protection from carcinogenic agents and/or pathogens and/or via regulation of the inflammatory response, DMBT1 may counteract tumour initiation and/or progression. Alternatively or additionally, loss of ►DMBT1 function may interfere with processes of differentiation and promote tumorigenesis.

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Deleted in Pancreatic Carcinoma Locus 4

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Synonyms

SMA- and MAD-related protein 4; SMAD4; DPC4; Mother against decapentaplegic, drosophila, homolog of 4; MADH4

Definition

Deleted in Pancreatic Carcinoma Locus 4 (DPC4), belongs to the class of tumor suppressor genes (►tumor suppressor genes). It was identified within chromosomal band 18q21.1 which is frequently deleted in pancreatic carcinoma. DPC4 is a component of the transcription complex (►transcriptional complex) that mediates cell surface signals to the nucleus which are initiated by transforming growth factor β [►TGF-β]-related growth and differentiation factors.

Characteristics

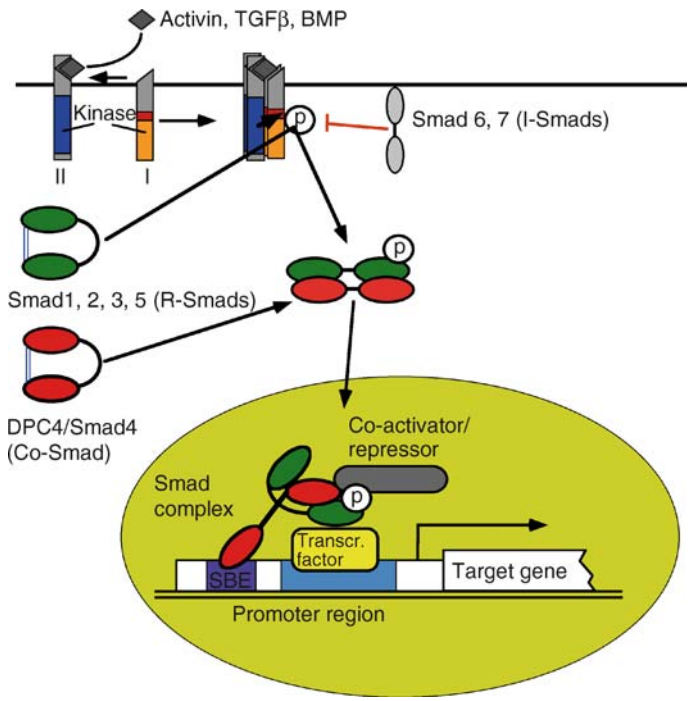
The open reading frame of DPC4 spans 1656 nucleotides and comprises 11 exons that code for a 60 kD protein (552 amino acids). DPC4 belongs to the highly conserved family of Smad genes that has been identified by protein sequence homology studies. The founding member Mad (Mother against decapentaplegic), was identified in ►*Drosophila melanogaster*.

Mad and its homologs mediate signals from cell surface receptors to the cell nucleus (►Smad proteins in TGFβ signaling). Involved in this signaling cascade are serine/threonine kinase receptors of the TGFβ family that become activated upon binding of polypeptides of the TGFβ cytokine family (Fig. 1). At least 25 different cytokines from various species are currently known. They include TGF-β, activin, ►inhibin, ►bone morphogenic protein (BMP) and Müllerian-inhibiting substance and control, among others, important biological functions such as embryonic development, cell growth and cell differentiation, modulation of immune responses and bone formation.

The number of Smad genes identified in humans has grown to a total number of eight (Table 1).

Common to all of them is a characteristic three-domain structure (Fig. 2): a highly conserved region, the Mad homology domain 1 (MH1), is located at the amino (N) -terminal end. Next to it is a poorly conserved, proline-rich linker region which is followed by a second highly conserved domain (MH2) located at the carboxy (C) -terminal end of the protein.

A link between the DPC4 protein and TGFβ signaling cascade was initially established by sequence homology studies of the proteins DPC4 and Mad. At the time of DPC4 discovery its potential tumor suppressor function was hypothesized; it was believed that the loss of DPC4 function in tumors is the reason for the



Deleted in Pancreatic Carcinoma Locus 4. Figure 1 A model for DPC4 signaling. In response to ligand binding to the TGFβ receptor complex, receptor regulated Smads (also called R-Smads) become C-terminally phosphorylated through the receptor kinase. The phosphorylated Smads change their folding pattern and form a heterodimeric or trimeric complex with DPC4. The newly formed ►Smad complex is then translocated into the nucleus. In the nucleus the Smad complex will make contact to transcription factors as well as bind directly to DNA through the ►Smad binding element (SBE), thus stabilizing the higher order DNA binding complex. In addition, transcriptional ►co-activators or ►co-repressors may be recruited into the complex ultimately leading to either activation or repression of target gene expression.

observed resistance towards TGFβ mediated growth inhibition of many tumor types. Although this hypothesis has only been partly proved, a wealth of information regarding the signaling pathway has been collected in recent years. This gave rise to a model of DPC4 protein function within the TGFβ signaling pathway.

TGF-Smad Signaling Cascade

The Smad signaling cascade involves three different classes of Smad proteins: the receptor regulated Smads (R-Smads), the common mediator Smad (Co-Smad) and the inhibitory Smads (I-Smads).

Upon ligand induced TGFβ receptor stimulation, R-Smads can transiently interact with the type I receptor. They become C-terminally phosphorylated by the receptor kinase and, once phosphorylated, are able to form a heterodimeric or trimeric complex with the "common-mediator" DPC4/Smad4 which then translocates into the nucleus. Here it can either up- or down-regulate the transcription levels of target genes by interacting with other nuclear factors and by recruiting transcriptional co-activators or co-repressors.

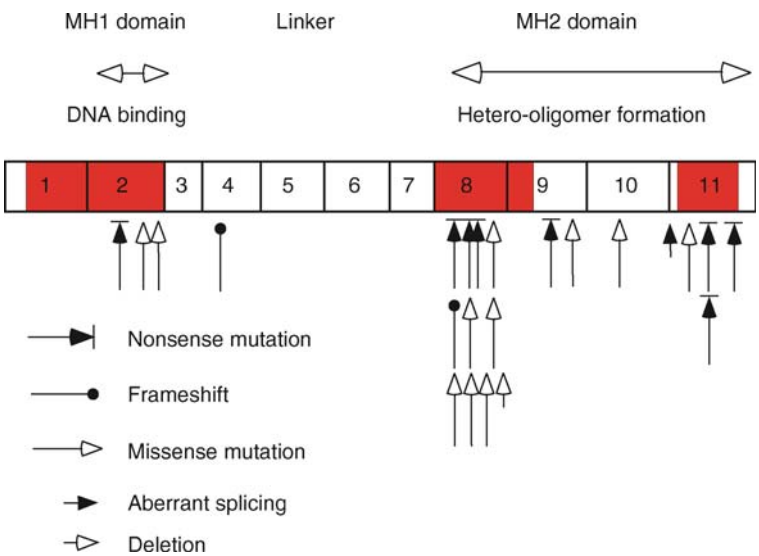
This signaling cascade can be negatively regulated by the I-Smads (Smad6 and Smad7). Whereas Smad7 acts as a more general inhibitor of TGFβ family signaling Smad6 seems to preferentially block BMP signaling. I-Smads can compete with R-Smads for type I receptor binding and can therefore prevent the phosphorylation-dependent activation of R-Smads. Furthermore, Smad7 can interact with the E3 ubiquitin ligases Smurf 1 and 2. Once the Smad7/Smurf complex is bound to the TGFβ receptor it induces TGFβ receptor degradation. Direct binding of I-Smads to R-Smads has also been shown, yielding R-Smads inactive. The expression of I-Smads appears to be regulated by TGFβ and BMP via an autoregulatory feed back loop. Furthermore, it has been shown that ►interferon-γ (interferon-γ) (IFN-γ) via the Jak1/STAT1 pathway, and tumor necrosis factor alpha (TNFα) and interleukin 1 through NFκB (►nuclear factor κB) /RelA can induce the expression of I-Smads to antagonize TGFβ signaling.

Deleted in Pancreatic Carcinoma Locus 4. Table 1
Summary of functional classes of human Smads

Classes of Smad Proteins		
Receptor-regulated	**Common**	**Inhibitory**
Smad 1, Smad 5, Smad 8 } BMP	DPC 4/Smad 4	Smad 6, Smad 7
Smad 2, Smad 3 } TGFβ, Activin		

Transcriptional Regulation through Smads

Since Smad proteins have no intrinsic enzymatic activity, they exert their effector function as transcriptional



Deleted in Pancreatic Carcinoma Locus 4. Figure 2 Functional domains and sites of identified DPC4 mutations. In addition to the mad homology domains MH1 and MH2, DPC4 carries a ►nuclear localization signal (NLS) domain and a ►nuclear export signal (NES) domain responsible for constant shuttling of DPC4 between nucleus and cytoplasm, thus helping the cell to constantly sense the TGFβ receptor activation state. A number of candidate phosphorylation target sites (P) for kinase pathways such as the MAPK (►MAP-Kinase) pathway have been described within the linker region which for example may modify nuclear accumulation rates of DPC4. The numbered squares forming the schematic DPC4 molecule also depict the 11 known exons within the DPC4 transcript.

regulators either directly by binding to specific promoter consensus sequences termed ►Smad-binding elements (SBE) and/or indirectly by associating with transcription factors already bound to the promoter. Therefore, many but not all Smad responsive promoters have two adjacent DNA sequences. One provides the binding site for transcription factors that are cooperating with the ►Smad complex, the other allows direct binding of the Smad complex to the DNA. While R-Smads provide the interface for the binding to ►transcription factors, DPC4 makes the contact to SBE elements. DPC4 thereby stabilizes the formation of a higher order DNA binding complex which is able to recruit transcriptional co-activators or co-repressors.

Many of the factors that cooperate with the Smad complex are regulated independently by other signaling cascades. The function of an active Smad complex can therefore be described as a co-modulator of transcription. It can modulate gene expression positively as well as negatively by integrating various incoming signals -including those mediated by the TGFβ ligand family. Therefore, it is not surprising that currently more than 1,000 genes are described to be either directly or indirectly regulated by DPC4. In addition, many of these DPC4 target genes can only be found in a certain cell type and growth state, again illustrating how much the cellular differentiation and signaling state determines the net gene expression regulation pattern of a DPC4 containing transcription complex.

What makes DPC4/Smad4 Unique among the Other Smad Family Members?

- DPC4 is the only human Co-Smad that is currently known.
- It seems particular to DPC4 that it is, almost without exception, essential for the establishment of a functional active Smad complex, a fact that emphasizes its role as a "master switch" in the regulation of TGF-β-like signals.
- Most somatic and all germ line mutations in human Smad genes identified to date target DPC4. Only very few somatic mutations were found in the human Smad2 gene, none were found in the other members of the human Smad gene family.

Which Human Tumors Show Alterations of the DPC4 Gene?

Changes, resulting in the inactivation of the DPC4 gene were found in approximately 50% of pancreatic carcinomas (►pancreas cancer). Research carried out in a variety of other cancer types suggested that DPC4 may contribute primarily to the formation of pancreatic neoplasia, and to a lesser extend to ►colon cancer ►cervical cancer and biliary cancer (►bile duct neoplasia) as well as the induction of non-producing ►neuroendocrine tumors. However, such changes appear to play only a minor role in the development of other tumor types such as head and neck, ►lung cancer ►ovarian cancer ►breast cancer and ►bladder cancer. The frequency of DPC4 mutations is markedly increased in metastatic colorectal carcinoma (35%) compared to non-metastatic colorectal carcinomas (7%). Furthermore, during pancreatic carcinoma development, a high incidence of biallelic DPC4-inactivation is generally not present before the carcinoma-in-situ stage, suggesting the loss of DPC4 function is critical for the tumor cell to develop characteristics such as the ability to invade into the surrounding tissue and to form metastasis.

In addition, germline mutations of the DPC4 gene have been identified in patients with familial juvenile polyposis, an autosomal dominant disorder that is characterized by a predisposition to hamartomatous polyps as well as an increased risk for gastrointestinal carcinomas.

How are Naturally Occurring DPC4 Mutations Interfering with the Smad Signaling Cascade

Most DPC4 mutations identified to date are located within the C-terminal MH2 domain. Functional studies identified the MH2 domain as providing the binding properties to R-Smads, the latter being important for a functionally active Smad complex. It is therefore likely that compromising mutations of the MH2 domain structure restrict the formation of a functional Smad complex, thus preventing signal transduction to downstream components. In addition, a few mutations have been identified within the N-terminal MH1 domain which was shown to mediate the direct binding of DPC4 to DNA promoter sequences. Such mutations might interfere with Smad signaling by rendering the formation of the higher order Smad-DNA complex unstable. Furthermore, some DPC4 ►missense mutations targeting the MH1 domain result in an instable protein due to a mutation-induced poly-ubiquitination of DPC4 and its subsequent proteasomal degradation.

Does DPC4 Contribute to the Familial Risk for Pancreatic Cancer?

Although the DPC4 gene is most frequently altered in ►sporadic pancreatic carcinoma, to date no germline mutations were found in families with an increased risk of this type of carcinoma. DPC4 is therefore unlikely to play an important role as a heritable genetic risk factor in pancreatic carcinoma.

How does DPC4 Contribute to Tumor Formation?

Although Smad signaling (including DPC4/Smad4) is regarded as central to the TGFβ pathway, there are now numerous examples illustrating that DPC4 inactivation is not simply abolishing TGFβ responsiveness and thus providing the cell a growth advantage. This

can partly be explained by the ability of TGFβ to modulate also Smad-independent pathways such as the Ras (►RAS) -ERK, PI3K (►PI3KSignaling) –AKT (►AKTSignal Transduction Pathway in Oncogenesis) and Rac/Rho pathways. Thus, loss of DPC4 function is not able to completely abrogate TGFβ signaling rather than shifting the balance between DPC4/Smad-dependent and DPC4/Smad-independent TGFβ signaling pathways towards the DPC4/Smad-independent pathways. The output of the latter is dependent on the successful activation of the latent form of TGFβ ligands and intactness of the TGFβ receptors. The cellular context will further modulate the signaling state of the DPC4/Smad-independent pathways through regulating the activity status of their pathway target genes by integrating signals from other signaling pathways. Thus, loss of DPC4 function has been shown cell type dependent to be involved in altering a number of different cell behaviors relevant to tumor formation such as, cell growth rate by modulating the cell cycle and/or the rate of ►apoptosis, altering the extracellular matrix components (►extracellular matrix remodeling), the cell adhesion (►adhesion) properties, and supporting ►epithelial to mesenchymal transition, thereby facilitating tumor ►invasion and ►metastasis, Furthermore, other experiments provided evidence that loss of DPC4 function might promote tumor ►angiogenesis by causing an increase in the concentration of angiogenic factors and/or a decrease its corresponding inhibitors.

Additional insight of DPC4 function was provided by targeted mutagenesis in mice. Mice with two mutated alleles for DPC4 die at embryonic day 7.5, a result that underlines the importance of DPC4 in early embryonic development. DPC4 heterozygous mice develop gastric and duodenal polyps which resemble human juvenile polyps. Furthermore, knockout mice experiments have demonstrated a functional cooperation between the DPC4 and the APC (APC) (adenomatous polyposis coli) gene. In mice that were carrying defect copies of both genes, compared to mice carrying only the mutated APC gene, the induced colonic tumors displayed a much more aggressive phenotype. Lastly, data from primary human tumors and from mice experiments provided evidence that haploinsufficiency of the DPC4 locus may also contribute to progression of cancer. These data clearly support the importance of DPC4 in the suppression of tumorigenesis.

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Deletion

Definition

Is the loss of a chromosomal segment or gene. A chromosomal deletion can be terminal, i.e., involve the end of a chromosome, or it can be interstitial, in which case a segment from within the chromosome is lost. A decrease in specific DNA fragment copy numbers.

►ArrayCGH

Dendrimer

Definition

A type of ►nanoparticle that is a highly branched polymeric molecule synthesized from monomers in a reproducible fashion that may have applications for drug delivery and imaging.

►Nanotechnology

Dendritic Cell-Based Tumor Vaccines

Definition

The system of dendritic antigen-presenting cells derive from hematopoetic precursors and reside as sentinels of the immune system in all tissues, particularly in skin and mucous membranes. They are able specifically equipped with pathogen recognition receptors which enable them to recognize harmful microbial infections and take up foreign antigens. Activation of ►dendritic cells triggers (i) their ►migration to the regional lymph

nodes, (ii) antigen processing and (iii) functional maturation for optimal antigen presentation and stimulation of T cell proliferation. ►Dendritic cells are the key regulators of cellular immunity against both infected as well as malignant cells. They are therefore targets of many ►cancer vaccine strategies both ex vivo (using cultured dendritic cells) as well as in vivo.

►Melanoma Vaccines

Dendritic cells

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Definition

Dendritic cells are a special subset of ►leukocytes that form a complex network of ►antigen-presenting cells (APC) throughout the body. They play a principal role in the initiation of immune responses to invading micro-organisms (bacteria, fungi and viruses), malignant cells and allografts, by activating naïve lymphocytes, by interaction with innate cells and by the secretion of cytokines. At certain developmental stages they grow branched projections, the dendrites, hence the cell's name.

Characteristics

Origin and Function

Dendritic cells (DC) were characterised for the first time by Steinman in 1973 based on their distinct morphology with different cytoplasmic extensions, such as dendrites, pseudopodia and ►lamellipodia, which give the cell its star-shaped feature. Due to their pronounced morphology, DC have a large surface, ensuring close contact with neighbouring cells.

Variations among the tissue distribution of DC and differences in their phenotype and function, indicate the existence of heterogenous populations of DC. DC originate from different hematopoietic lineages in the bone marrow (Table 1). A myeloid progenitor cell can differentiate *in vivo* to different DC populations: ►Langerhans cells that migrate to the skin epidermis and interstitial DC that migrate to the skin dermis and various other tissues (airways, liver and intestine). Circulating or migrating DC are found in the blood and in the afferent lymphatics, respectively (the latter called veiled cells). Interdigitating DC are found in the paracortex of lymph nodes in close proximity with T cells. In addition, monocytes represent an abundant source of DC precursors during physiological stress. Another subset of DC, plasmacytoid DC (pDC) originate from a lymphoid progenitor cell in lymphoid organs. By contrast, follicular DC (FDC) are probably not of hematopoietic origin, despite similar morphology and function to the above mentioned subsets of DC. FDC are APC of the B cell follicles in lymph nodes and central players in humoral immunity.

DC express several different types of membrane molecules that determine their phenotypic and functional characteristics:

1. DC display a high surface density of antigen-presenting molecules, such as CD1a, ►major histocompatibility complex (MHC) class I and class II molecules. The level of expression of these molecules is 10- to 100-fold higher compared to other APC (e.g. B cells).
2. In addition, mature DC have high expression levels of costimulatory and ►adhesion molecules: CD40, ICAM-1/CD54, ICAM-3/CD50, LFA-3/CD58, B7-1/CD80 and B7-2/CD86. Binding of these molecules with their respective receptors on T cells results in T cell activation and subsequently stimulates the expression of cytokines, cytokine-receptors and genes for cell survival.
3. Several members of the integrin family are expressed by DC. ►Cadherins contribute to the generation of cell contacts and selectins are important for the motility of DC.
4. DC also express pathogen-recognition receptors, e.g. DEC-205, a macrophage-mannose receptor capable of binding bacterial carbohydrates and ►toll-like receptors (TLR), recognising a variety of pathogen-associated molecular patterns (PAMP), such as carbohydrates, nucleic acids, peptidoglycans and lipoteichoic acids.
5. Cytokine and chemokine receptors are also important for DC function, since growth, differentiation and migration of DC as well as antigen processing and presentation is tightly regulated by cytokines and/or chemokines.

The widespread distribution of DC and their expression of a variety of membrane molecules underline their sentinel function: they patrol the body to capture invading pathogens and certain malignant cells in order to induce efficient anti-microbial or anti-tumour ►T-cell responses. In their *in vivo* steady state condition, immature DC are specialised in capturing antigens, i.e. they efficiently take up pathogens, apoptotic cells and antigens from the environment by phagocytosis, macropinocytosis or ►endocytosis. However, immature DC remain tissue-resident, expressing only small amounts of (MHC) class II and of

Dendritic cells. Table 1 Different subsets of dendritic cells

CD34+ hematopoietic stem cell				
	Myeloid progenitor cell			Lymphoid progenitor cell
	Monocyte-derived DC	Langerhans cells	Interstitial DC	Plasmacytoid DC
Phenotype				
CD11c	+	+	+	–
CD1a	±	+	–	–
CD123	–	–	–	+
Birbeck granules	–	+	–	–
Factor XIIIa	±	–	+	–
Function				
Endocytosis	+	+	+	+
IL-10*	+	+	+	+
IL-12*	+	–	+	+
IFN-α*	–	–	–	+

*After application of danger signals.

costimulatory molecules, which leads to T cell unresponsiveness. After encounter of a "danger" signal (e.g. TLR ligand) immature DC mature and migrate to the secondary lymphoid organs. Mature DC are considered to be immunogenic, mainly due to the marked upregulation of MHC class II and costimulatory molecules. This maturation step is believed to be a crucial event to regulate DC function and makes DC potent inducers of T cell immunity.

Dendritic Cell-Based Immunotherapy

Despite our immune system's function to protect us from malignant cells, tumour cells grow undisturbed and, unless treated, are fatal to the host. The reasons for the failure to eliminate tumour burden in a majority of patients can be the consequence of different tumour escape mechanisms. For example, tumour-derived inhibitory factors (e.g. IL-10 and/or TGF-β) or tumour cell-induced T regulatory cells (►Treg) might be involved in downregulating or altering immune function. The goal of cancer ►immunotherapy is to resolve or circumvent these problems and generate tumour-specific immune responses. It is important to realise that immunotherapies will likely only be successful after reducing tumour mass via primary therapies: surgery, radio- and/or chemotherapy i.e. in a ►minimal residual disease (MRD) setting.

Because of their pivotal immune-stimulating capacity and their ability to activate naïve tumour-specific T cells, DC-based ►cancer vaccines could have important applications in the future treatment of cancer. For this, it was necessary to cultivate DC with high yields. Several cultivation protocols were developed for *in vitro* generation of DC. First, DC can be differentiated from CD34+ hematopoietic progenitor cells using granulocyte-monocyte colony stimulating factor (GM-CSF), tumour necrosis factor (TNF-α), stem cell factor (SCF), interleukin (IL)-3 and ►Interleukin-6. Second, DC can be generated starting from monocytes using GM-CSF and ►Interleukin-4. Finally, DC can be directly harvested from the peripheral blood of a patient, where they reside at low percentages (0,1%).

Next, cultivated DC can be loaded with the tumour antigen of importance in different ways:

1. DC can be grown *in vitro* in the presence of ►tumor-associated antigens (TAA). This technique is called peptide pulsing and results in direct binding of the immunodominant epitope on an empty MHC class I molecule on the DC membrane. This circumvents the need for antigen uptake and processing and ensures the stimulation of tumour specific cell-mediated cytotoxicity. However, the number of known TAA is still restricted and highly dependent on the human leukocyte antigen (HLA) haplotype of the patient.
2. DC can also be fused with the patient's tumour cells *in vitro* or pulsed with tumour cell lysates. The former method combines sustained tumour antigen expression with the antigen-presenting and immunostimulatory capacities of DC. DC-tumour cell hybrids will also stimulate an active anti-tumoural immune response.
3. Tumour antigen can also be loaded on DC using plasmid DNA transfection or ►viral vector mediated gene transfer. The former method results in only low transfection efficiencies. On the other hand, viral transduction, for example by using adenoviral or lentiviral, vectors is very effective with regard to transfection efficiency. However, the immunogenic character of the viral vector itself is a serious

disadvantage. In both cases, DC will transcribe and process the tumour antigen. This will result in a cytotoxic immune response, necessary for immunological defence against cancer cells.

4. It is also possible to transfect DC using *in vitro* transcribed mRNA coding for tumour antigens or total tumour RNA. It has been shown that electroporation of RNA is the most effective non-viral transfection method for DC (►non-viral vectors for cancer therapy). mRNA is brought directly into the cytoplasm and the cell's metabolism will translate mRNA into proteins, which can be presented onto MHC class I molecules after processing. This will guarantee a specific cell-mediated anti-tumoural immune response.

In a clinical context, *in vitro* cultured and activated DC loaded with appropriate tumour antigens could be administered to cancer patients in a therapeutic setting (active specific immunotherapy). The aimed generation of anti-tumour immunity, mediated by DC, could be of importance for both treatment (as adjuvant to conventional therapies) as well as to prevent relapse in a MRD setting. On the other hand, tumour antigen-loaded DC can also be used for the *ex vivo* generation of tumour-specific cytotoxic T lymphocytes (CTL) in an autologous system. These tumour-specific CTL can, in their turn, be administered, to the patient to exert a direct cytotoxic effect on the patient's cancer cells (passive or adoptive immunotherapy).

The impact of a DC-based cancer vaccine is clear: an antigen-specific anti-tumour vaccine would influence both morbidity and mortality of various cancers. Currently, several phase I-II or III ►clinical trials using TAA-loaded DC are ongoing worldwide in order to stimulate the patient's immune system against tumour antigens. A number of these trials demonstrated some clinical and immunological responses (as evidenced by T cell proliferation, IFN-γ ►ELISPOT and ►delayed type hypersensitivity [DTH reaction] reaction) without any significant toxicity. However, despite the presence of expanded antigen-specific T cells in patients after vaccination, only a minor population of these patients showed a beneficial biologically relevant clinical response, i.e. tumour regression and increased disease-free survival. Until now, clinical trials using DC have only shown moderate, if any, success. Since DC possess the exceptional capacity to stimulate the patient's own immune system against cancer, the reasons for the failure to eliminate tumour burden in a majority of patients needs to be carefully examined in ongoing and future trials.

Dendritic Cells in Cancers

DC can also infiltrate human tumours where they are involved in the induction of anti-tumour immune responses. It is likely that the establishment of tumour-specific immune responses depends on the migratory capacity of DC from the tumour microenvironment to the draining lymph nodes, where tumour antigen presentation to T cells takes place. Moreover, by their expression of costimulatory molecules and several cytokines, such as IFN-α and IL-12, DC also mediate T cell survival by preventing T cell ►apoptosis. In addition, mature DC have been reported to cause direct lysis, apoptosis as well as cell cycle arrest of cancer cells through the secretion of soluble factors. As a consequence, the presence of a high number of DC in the tumoural or peritumoural area, as well as in the draining lymph nodes of various human tumours has been shown to correlate with patients' survival and a better prognosis. Decreased numbers or dysfunction (e.g. decreased expression of costimulatory molecules) of DC are reported in poor-prognosis tumours. Furthermore, tumour cells can secrete certain factors (e.g. IL-10 and TGF-β) that counteract DC maturation and migration and thus actively contribute to DC dysfunction.

Occasionally, neoplasms of accessory immune cells (antigen-presenting cells, dendritic cells) can occur. These are primarily found in lymph nodes and extranodal lymphoid tissues (lymph node interdigitating cell sarcoma), but are also reported from other sites such as the skin (►Langerhans cell histiocytosis). The incidence of dendritic cell tumours is very rare: until now, only a few dozens of cases have been reported in literature.

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Densitometric

Definition

Pertaining to measurement of optical density in a material (e.g. amount of stain).

►Malignancy-Associated Changes

Dental Pulp

Definition

Forms a functional and interdependent unit together with ist adjacent tissue, the dentin. Physiologic or pathologic reactions in one compartment will affect the other compartment as well. Dentin and the dental pulp are of mesectodermal origin. They are also called "pulp-dentin complex"or "pulp-dentin organ".

►Dental Pulp Neoplasms

Dental Pulp Neoplasms

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Definition

Are tumors that are located in the dental pulp.

Characteristics

Dental pulp neoplasms (DPNs) are rare tumors of the dental pulp tissue which is not exposed to the oral cavity. Two types of DPNs can be distinguished: Type 1 originates from the dental pulp itself (primary DPN) and type 2 originates from tissue outside of the tooth (secondary DPN). Most DPNs are somewhat incidental findings in patients with a known tumor anamnesis. Therefore the number of histologic examples of DPNs is rather limited, and one also has to take into account articles from old literature in order to draw a complete clinical picture.

History

In the late nineteenth century, when systematic dental care and oral hygiene in general were considerably more deficient than today, dentists encountered numerous teeth with deep ►caries and sometimes massive exposed pulp tissue. This phenomenon was called "pulpitis chronica sarcomatosa", a chronically inflamed dental pulp supposedly caused by a sarcoma. Later it was found out that this pulpal alteration was in fact nothing to do with a sarcoma but rather was the result of colonization of the exposed dental pulp by free epithelium cells of the gums. This entity is a so-called "dental pulp polyp". However, the first true description of a type 1 DPN was made in 1904 by V. A. Latham from Rogers Park, Illinois. He presented the case of a 56-year-old woman presenting with an upper right canine with a greenish-white tinge. The tooth was vital, symptomless and caries-free, i.e. the dental pulp was not exposed to the oral environment. After tooth extraction (for prosthodontic reasons) and histological processing, this canine proved to have an epithelioma of the pulp. The extraction socket was curetted and subsequently cleaned with iodine and carbolic acid. According to his report, Latham thus seems to have cured the patient from a tumor by simply extracting the tooth. Until today, descriptions of type 1 DPNs are very rare.

First descriptions of type 2 DPNs also date back to the early 20th century where reports of involvement of dental pulps in patients with ►breast cancer, ►lymphoma or neuroma have been given. Three to thirty percent of tumors of the head and neck region (HNR) are associated with involvement of the dental pulp. Carcinomas are more likely to be associated with DPNs than sarcomas or any other type of tumors of the HNR. The maximum incidence of DPNs lies between the fifth and sixth decade of life.

Inflammatory Pulp Reactions

A DPN causes inflammatory reactions (►inflammation) in the dental pulp. Chronic inflammation of the pulp may either lead to calcification of parts of the dental pulp tissue or to resorption of the surrounding hard tissue, i.e. ►dentin. Calcifications – as regularly observed histological findings in dental pulps with a neoplasm – can be explained by the behavior of primary and secondary ►odontoblasts. These cells are determined to secrete dental hard substance. If a bacterial impact is directed towards the pulp (as is the case with dental caries), the primary odontoblasts immediately start to produce tertiary dentin in the targeted area. Thus increasing the distance between the bacteria and the pulp, an early opening of the pulp chamber in the course of the carious process is evaded. Meanwhile, the chronic inflammation of the dental pulp in slowly progressing caries may lead to the calcification of parts of the pulpal tissue via secondary odontoblasts. These particular cells are differentiations of former pulpoblasts. Pulpoblast differentiation can be modified by ►bone morphogenetic protein (BMP) 2, 4, and 11, ►GDF, ►TGF-β, or high calcium concentrations, all of which are present in dentin. It can also be modified by certain medicaments, which originally were only used in periodontal regenerative therapy but have now also been introduced in endodontic therapy as well as dental traumatology as a means of direct pulp capping.

Radiotherapy

As an additional point of discussion the possibility of therapeutically induced DPNs by radiotherapy has to

be mentioned. It is a given fact that one of the risks of radiotherapy of the HNR consists in radiation-induced tumors (►radiation-induced sarcomas after radiotherapy). Establishing a causal connection is often difficult due to a latency period of several years. However, teeth after radiotherapy sometimes show calcifications of the dental pulps, which can be detected in postirradiation radiographs. Since there has not been a study distinguishing between bacterial and abacterial calcifications as signs of chronic inflammations of the dental pulp in postirradiated cases, no predication can be given about a higher risk of DPN after radiotherapy.

Animal Investigations

As to DPN-cases, the pulp tissue reaction with respect to calcifications seems to be the same as in cases of dentin caries. At this point, observations made in experimental animal models become of interest: After several days calcification of the dental pulp tissue (with a simultaneous breakdown of the odontoblast layer) can be detected when inoculating the dental pulps of rodents with virulent sarcoma cells. Regular findings in these studies consist in massive development of intrapulpal dental hard substance like denticles, osteoids, or pulp stones. Also destruction of pulpal cells, particularly of the odontoblasts, by tumor tissue has been described in an animal study. In none of these investigations do the dental pulps survive longer than three weeks. Nevertheless, it is a matter of speculation whether this effect is really due to the sarcoma cells or rather to the increased extravasal pressure of the inflamed pulpal tissue. In these animal models, the sarcomata are able to infiltrate the dental pulps and to proliferate to adjacent tissue like ►periodontium, mandibular bone and masseteric muscle. In later stages, ►metastasis in the regional lymph nodes as well as in the sublingual, submandibulary and parotid glands can be found.

The fact that rodent teeth are substantially different from human teeth must not be neglected. While rodent teeth are growing lifelong and have a largely open apex, human tooth formation literally comes to an endpoint in a constriction at the tip of the root(s).

Clinical Relevance

Since systematic autopsies of the jaws are no longer common, the entity of DPNs have somewhat moved out of the focus of scientific attention.

Type 1 DPNs are certainly of small clinical relevance. In the dental pulp, fibroblasts, subodontoblastic progenitor cells, pericytes, stem cells, and, occasionally, Malassez epithelium remainders of the Hertwig root sheath are cells with mitotic competence and thus are able to undergo neoplastic alteration. A relatively high grade of differentiation of the pulpal tissue limits further differentiation of purported neoplasms.

Due to the restricted anatomical macroenvironment of a tooth and possibly further due to ►microenvironmental interactions a type 1 DPN is more or less self-limited. Concerning the formation of a DPN, the capability of the dental pulp to regularly form calcifications under certain circumstances as well as the fact that one encounters a terminal blood supply in the pulp play a crucial role. Growth of a neoplasm will increase extravasal pressure within the dental pulp and thus stimulate secondary odontoblasts to secrete irritation dentin. A large amount of irritation dentin might influence the blood supply of the dental pulp and thus will probably lead to a hemorrhagic infarct. A growing tumor in the root canal system will contribute to this effect. While becoming necrotic in such a way, the dental pulp does not necessarily have to show clinical symptoms (such as tooth ache). Teeth with necrotic pulps will normally receive endodontic treatment (i.e. root canal therapy) or they will be "cured" by tooth extraction. It can be acclaimed that the specialty about a type 1 DPN lies in its possibility to be removed successfully and in a relatively easy way. The anatomic prerequisite of the root canal system presents the unique fact that while growing atumor is already limiting its further existence.

The risk of metastasis of a DPN is not given in normal-sized teeth. The volume of the dental pulp chamber and the root canal system do not provide sufficient space for a tumor to gain a critical cell mass in order to disseminate clonal cells. Only teeth with incomplete root formation (as in children or adolescents) or ►taurodonts, i.e. teeth with an abnormally large crown and roots, might provide enough space allowing a tumor to gain a critical cell mass. Large animal teeth, whose pulp chambers can surely provide enough space for a tumor (for instance in large mammalians), are not systematically screened for dental pulp diseases.

Type 2 DPNs seem to be mere incidental findings in patients with tumors mainly of the HNR. This leads to the assumption that DPNs are normally symptomless and of relatively small clinical relevance. Nevertheless, type 2 DPNs may also lead to tooth-related symptoms (pain) as has been described in single case reports.

Far more often (and clinically more important) is the opposite case when seemingly healthy teeth with no sign of caries, filling or a positive trauma history mimic toothache. The projected toothache is thus drawing off the attention of a true HNR tumor, which often leads to unnecessary root canal treatment or tooth extraction. Therefore, apart from regular or ofacial neuropathic or nociceptive pain conditions, differential diagnosis therefore should always consider a neoplasm in the HNR.

In common classifications of dental pulp diseases, inflammation of the dental pulp due to neoplasms are neglected. However, animal tumor models (►mouse models) should be reinvestigated for changes within the dental pulp.

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Dentin

Definition

Dental hard substance located between ►dental pulp and enamel/cementum.

►Dental Pulp Neoplasms

Denys-Drash Syndrome

Definition

DDS; Is a rare disorder consisting of the triad of congenital nephropathy, ►Wilms tumor, and disorders resulting from mutations in the Wilms tumor suppressor (*WT1*) gene. Nephropathy is a constant feature.

►Nephroblastoma

Deoxyazacytidine

►A5-aza-2′ Deoxycytidine

2´-Deoxy-5-azacytidine

►A5-aza-2′ Deoxycytidine

Deoxycytidine Kinase

The rate-limiting enzyme in cytarabine metabolism, converting ara-C to ara-CMP. Also catalyzes the conversion of F-ara-A to F-ara-AMP.

2-Deoxy-D-Glucose

Definition

A glucose analog that inhibits glycolysis.

►Jasmonates in Cancer Therapy

Dermoid Cyst

Definition

►Ovarian Teratoma.

DES

►Diethylstilbestrol

DES Daughters

Definition

Women exposed *in utero* to ►diethylstilbestrol.

DES Mothers

Definition

Women administered ►diethylstilbestrol during pregnancy.

DES Sons

Definition
Men exposed *in utero* to ►diethylstilbestrol.

Descriptive Epidemiology

Definition
The branch of cancer epidemiology that deals with the collection and analysis of data on the incidence, mortality, and survival of cancer in populations.

►Cancer Epidemiology
►Epidemiology of Cancer

Desert

Definition
Gene in hedgehog signaling.

►Hedgehog Signaling

Des-Gamma-Carboxy Prothrombin

Definition
(DCP); Has been reported to be useful in the diagnosis of ►Hepatocellular Carcinoma (HCC). This marker is also known as a protein induced by vitamin K absence or antagonist-II (PIVKA-II), or abnormal prothrombin. DCP was originally found in the blood of patients who were deficient in vitamin K or who were receiving a vitamin K antagonist. In 1984, Liebman et al. reported for the first time that serum DCP was elevated in patients with HCC.

Designer Foods

►Nutraceuticals

Desmocollin

Definition
Dsc; a protein belonging to the desmosomal ►cadherin family of cell ►adhesion molecules. In humans three desmocollins (Dsc1–3) are known. Each is encoded by a distinct gene that is located in the desmosomal cadherin gene cluster on chromosome 18q21. Each desmocollin gene encodes two closely related proteins (the larger Dsc "a" protein and the smaller "b" protein) that differ only in the length of their C-terminal tails. The desmocollins are membrane spanning constituents of ►desmosomes and are essential for desmosomal adhesion.

Desmoglein

Definition
Dsg; a protein belonging to the desmosomal ►cadherin family of cell ►adhesion molecules. In humans four desmogleins (Dsg1–4) are known. Each is encoded by a distinct gene that is located in the desmosomal cadherin gene cluster on chromosome 18q21. The desmogleins are membrane spanning constituents of ►desmosomes and are essential for desmosomal adhesion.

Desmoglein-2

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Synonyms
Dsg2

Definition
Dsg2 is one of the calcium-binding transmembrane glycoprotein components of the cell-cell ►adhesion molecules of the ►desmosomes. Dsg2 is one of the ►cadherin cell adhesion molecule superfamily in vertebrate epithelial cells.

D

Characteristics

Cell Junctions

Epithelial cell-cell junctions consists of four junctions, ►tight junctions, ►adherens junctions, ►desmosomes, and ►gap junctions (Fig. 1). Two adhering-type junctions, the adherens junctions and the desmosomes, are responsible for strong cell-cell adhesion. Each of these junctions consists of a transmembrane cadherin and a complex cytoplasmic plaque that serve to link cadherin to actin microfilaments or the intermediate filament cytoskeleton.

Desmosome

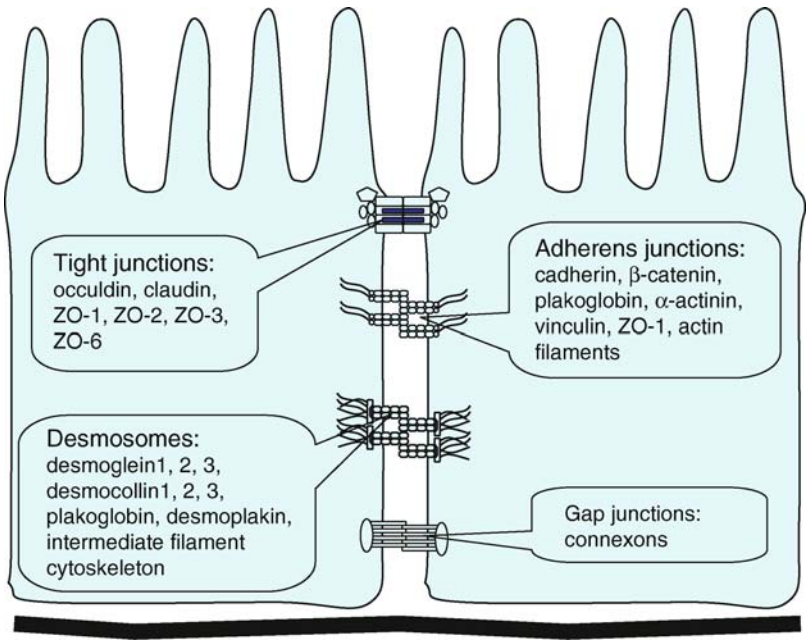
Intercellular junctions known as desmosomes are multimolecular membrane domains that provide intercellular adhesion and membrane anchors for the intermediate filament cytoskeleton. Desmosomes are essential adhesion structures in most epithelia that link the intermediate filament network of one cell to its neighbor, thereby forming a strong bond. Desmosomes contain the desmosomal cadherins, desmoglein (Dsg) and ►desmocollin (Dsc) that are linked to the intermediate filament cytoskeleton through interactions with ►plakoglobin and ►desmoplakin (Fig. 2).

Desmoglein and Cancer

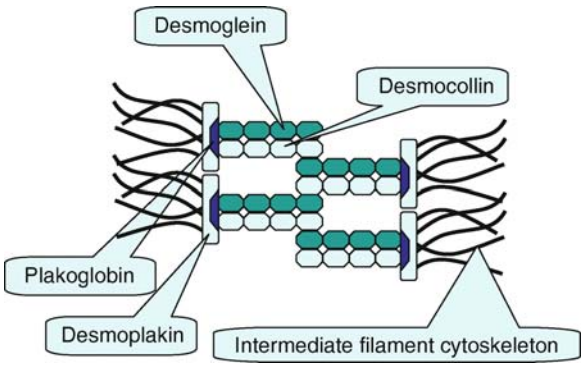
Epithelial cell-cell adhesion is important in tumor development. Dsgs are transmembrane glycoproteins of the desmosome, a cell-cell adhesive structure prominent in epithelial tissues, which have been reported to be associated with tumor development. cDNA and protein studies have revealed that there are subfamilies of Dsg (types 1, 2 and 3) and Dsc (types 1, 2 and 3) [3]. Dsg2 and Dsc2 are widely expressed and are found together in desmosomes of the basal layer of stratified epithelia, simple epithelia, and nonepithelial cells such as in the myocardium of the heart and lymph node follicles, whereas Dsg3/Dsc3 and Dsg1/Dsc1 are more restricted to complex epithelial tissues. Although considerable overlap is exhibited in the distribution of these isoforms in stratified tissues, their expression is clearly differentiation-dependent. Dsg2, but not Dsg1 or Dsg3, is expressed in stomach epithelia. ►Gastric cancers have been classified into two histological types: intestinal-type and diffuse-type. Diffuse-type gastric cancers show decreased cell-cell adhesion, which is associated with metastatic potential. These histological features indicate that a decrease in adhesive junctions may be involved in the emergence of diffuse-type gastric cancers. A decrease in E-cadherin has been reported to be one cause of the decrease in adhesive junctions, but not all diffuse-type gastric cancers show such a decrease. Decreased expression of Dsg2 is associated with diffuse-type gastric cancers and poor ►prognosis in gastric carcinoma.

Adherens Junctions

The adherens junction is composed of a classic cadherin (e.g. E-, P- or N-cadherin) linked to ►β-catenin or plakoglobin [5]. Thus, plakoglobin is found in both adherens junctions and desmosomes, while β-catenin is restricted to the adherens junction. Alpha-catenin links the cadherin/catenin complex to the actin cytoskeleton



Desmoglein-2. Figure 1 Cell junctions. Epithelial cell-cell junctions consist of four junctions, tight junctions, adherens junctions, desmosomes, and gap junctions.



Desmoglein-2. Figure 2 Desmosomes. Desmosomes contain the desmosomal cadherins, desmoglein and desmocollin that are linked to the intermediate filament cytoskeleton through interactions with plakoglobin and desmoplakin.

through interactions with ►α-actinin, vinculin, ZO-1 and actin filaments. Lost or reduced plakoglobin expression has been observed in tumor tissues and metastatic lesions, and has been linked to poor prognosis in a variety of tumors.

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Desmoid Tumor

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Synonyms

Aggressive fibromatosis; Mesenteric fibromatosis; Gardner syndrome

Definition

Desmoid (meaning tendon-like) tumors are a heterogeneous group of rare connective tissue neoplasms, which can occur at almost any anatomical location.

Desmoids have been classified as fibromatoses, along with pathologies such as palmar fasciitis, which are due to proliferation of well differentiated fibroblasts and are locally infiltrative and tend to recur after excision, but do not metastasize.

Characteristics

Desmoids are rare, accounting for less than 0.1% of all tumors, and have an annual incidence of 2–4 per million. While most occur sporadically, 2% are associated with ►familial adenomatous polyposis (FAP), an autosomal dominantly inherited cancer predisposition syndrome due to mutation of the tumor suppressor gene *APC* (►APC gene in familial adenomatous polyposis). Desmoids are over 1,000 times more common individuals with FAP than in the population in general, occurring in about 10–20% of them, and are an important cause of death in this group.

It is useful to classify desmoid tumors as being either sporadic or FAP-associated, and by their location into intra-abdominal, abdominal wall or extra-abdominal.

Pathology

Both sporadic and FAP-associated desmoids have been shown to be clonal proliferations of myofibroblasts. Those associated with FAP result from acquired mutations in the ►wild-type copy of *APC*. Somatic loss of the *β-catenin* gene has been described in sporadic desmoids, and *APC* mutation has also been identified in some cases ►APC/β-catenin pathway. Thus abnormal activation of the Wnt pathway seems to have an important role in desmoid tumorigenesis ►Wnt Signaling. A variety of complex chromosomal abnormalities, including trisomy 8 and gain of 1q21, has also been found in some tumors.

There is no true capsule, and the desmoid compresses and infiltrates surrounding tissues as it grows. Desmoids range in size from a few centimetres to large masses weighing several kilograms. A photograph of a mesenteric desmoid tumor taken at surgery can be found in ►APC gene in familial adenomatous polyposis. Growth rates are very variable. There have been reports of spontaneous resolution, and some desmoids grow relentlessly. The majority, however, either display cycles of growth and resolution or stabilize.

The cut surface is usually pale and whorled. There may be central hemorrhage, necrosis or cystic degeneration. Histologically desmoids consist of mature, highly differentiated spindle shaped fibroblasts in an abundant collagen matrix. The histological appearances are not

necessarily diagnostic, and need to be interpreted in the light of the macroscopic findings.

Aetiology

Trauma, sex hormones and genetics have all been implicated in their aetiology. Many sporadic abdominal wall desmoids seem to arise in women in pregnancy, perhaps as a result of low-grade trauma of stretching, coupled with high levels of ►female sex hormones. There have been numerous reports of desmoids arising at sites of surgical wounds, although many, particularly at extra-abdominal sites, seem to occur in the absence of any previous trauma.

The higher incidence of desmoids in females, association with pregnancy, presence of estrogen receptors, and results of some experimental studies on desmoid cell lines all suggest that estrogens may have a role in stimulating desmoid development.

Desmoids are very much more common in individuals with FAP. Within this group some familial clustering has been observed, in part explained by a ►genotype-phenotype correlation in which families with an *APC* mutation 3′ of codon 1444 have an attenuated colorectal phenotype but a high risk of desmoid development. There is also evidence of the influence of as yet unidentified modifier genes.

Clinical Features

Desmoids most commonly occur in young adults (mean age of onset around 30 years), but have been described in children and even babies. Sporadic desmoids are more frequent in women than men (reported gender ratio 2–5:1), but in FAP there is a less marked gender difference.

Sporadic desmoids are found predominantly in the abdominal wall (50%) and at extra-abdominal sites (40%), whereas about 80% of desmoids associated with FAP are within the abdomen, most in the ►small bowel mesentery. It is not uncommon for an individual to develop desmoids at multiple sites.

Intra-abdominal desmoids characteristically arise in the small bowel mesentery. Potential "desmoid precursor lesions," consisting of small plaques of peritoneal thickening, have been observed in patients with FAP. It is thought that these enlarge, causing a diffuse thickening and puckering of the mesentery which can be seen on ►CT scans. In some cases a frank desmoid mass develops.

Most extra-abdominal desmoids cause symptoms because of their bulk and resulting mechanical effects. At some sites, for example in the neck, they can compress nerves and blood vessels. The overlying skin may ulcerate and abdominal wall desmoids occasionally adhere to and erode abdominal organs. Intra-abdominal desmoids can cause major morbidity and even death, usually due to ureteric obstruction, bowel obstruction or perforation, either due to direct erosion or to compromise of the vascular supply.

►CT and ►MRI are the most useful imaging modalities, showing both tumor size and relationship to neighboring structures. Signal intensity on T2 weighted MRI may reflect cellularity and is correlated with tumor growth.

Treatment

The treatment of desmoids is difficult and controversial. There are numerous case reports and small uncontrolled series in the literature, but these are difficult to interpret, particularly as the natural history of these tumors is so variable.

The drugs most widely used are ►non-steroidal anti-inflammatory drugs (NSAIDs) (particularly sulindac), and anti-estrogens (►tamoxifen or toremifene). Overall the response rates to a variety of drugs in these classes is claimed to be in the region of 50%, but in reality is likely to be considerably less than this. There have been a handful of reports of acute desmoid necrosis, with abscess formation or bowel perforation in some, occurring in the weeks after initiation of drug treatment.

As NSAIDs have little in the way of adverse effects they are often used as first-line treatment. The mechanism of action in this setting is not clear, but there is some evidence that Cox-2 inhibition my inhibit desmoid growth ►celecoxib, ►cyclooxygenase-2 in colorectal cancer. There have been no trials of Cox-2 inhibitors used therapeutically. Anti-estrogens can be used alone or in combination with NSAIDs.

Surgery is widely accepted as the first line treatment for extra-abdominal and abdominal wall tumors. Recurrence rates are high (20–80%), but unaffected by use of prosthetic mesh in reconstruction. Serious morbidity and mortality rates are generally very low, although some sites, such as the neck, pose particular challenges. There are some reports suggesting that radiotherapy given postoperatively might reduce recurrence rates.

Excision of intra-abdominal desmoids is also associated with frequent recurrence, but carries a substantial risk of perioperative mortality or major morbidity. The commonest reason for this is that the tumors lie close to or encase the superior mesenteric blood vessels, so that the blood supply of a large part of the intestine may be damaged or deliberately sacrificed during surgery. This may result in the need for lifelong parenteral nutrition, and in a handful of cases small bowel transplantation has been performed in these circumstances. Careful case selection, using CT angiography and multiplanar reconstruction, together with accumulation of expertise in a specialized institution has been shown to produce better surgical results in the last 10 years. Generally, however, major resection of intra-abdominal desmoids should be avoided. Ureteric obstruction can be successfully overcome by ►stenting, and intestinal obstruction

or fistulation may be managed in many cases, at least acutely, by defunctioning.

Cytotoxic chemotherapy has been used to treat life-threatening desmoids. Response rates of 50% have been obtained using doxorubicin and dacarbazine in combination, and also with a less toxic regimen of methotrexate and vinblastine. In view of the potential toxicity of this type of treatment, it should probably be reserved for progressive, inoperable desmoid tumors in which other treatments have failed.

►Aggressive Fibromatosis in Children

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Desmoplakin

Definition

DP; a protein belonging to the plakin family of cytolinkers. Desmoplakin is found in ►desmosomes and is essential for desmosomal adhesion. The desmoplakin gene encodes two closely related proteins (the larger DPI and the smaller DPII) that differ only in the length of their central rod domain.

►Desmosomes
►Maculae Adherents

Desmoplasia

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Synonyms

Stroma; Stromal cell response; Schirrous (*archaic*)

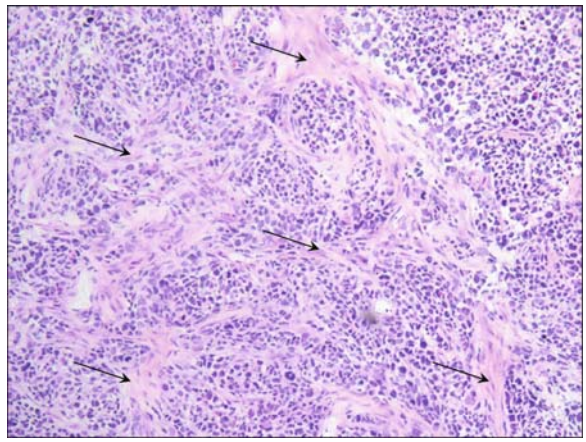
Definition

Desmoplasia is the formation of fibrous connective tissue by proliferation of ►fibroblasts. Desmoplasia is a key component of solid tumor stroma (Fig. 1).

Characteristics

Tumors have many parallels to wounds, including similar inflammatory and desmoplastic responses, and fibroblasts are the key cellular component in development of desmoplasia. Fibroblasts are recruited into the wound or tumor, secrete and remodel ►extracellular matrix (ECM) (►Extracellular Matrix Remodeling), and serve as scaffolding for other cell types in connective tissue. As fibroblasts incorporate into a tumor environment, they undergo a phenotypic change and acquire an "activated fibroblast" appearance, which are also known as a ►myofibroblasts or tumor-associated fibroblasts. Myofibroblasts have similar markers to fibroblasts, but myofibroblasts upregulate proteins such as α-smooth muscle actin (α-SMA), fibroblast activation protein (FAP-1), and ►fibronectin fibrils. During wound repair, the number of myofibroblasts return to a normal level upon wound resolution. In contrast to wound repair, ►tumor microenvironments simulate a chronic wound in many ways. Thus, local fibroblasts and those that were recruited into the expanding stroma are continuously exposed to activation signals. Activated fibroblasts expand and contribute to an increased stromal response known as desmoplasia. Desmoplasia can be associated with increased tumor stage and poor prognosis in ►breast cancer patients, but it is unclear whether fibroblasts are *active* inducers or *passive* participants in cancer progression. It is clear, however, that activated fibroblasts play a large role in the expanding tumor stroma (►Stromagenesis).

Fibroblastic stromal cells and desmoplasia have been linked to several activities that promote cancer growth



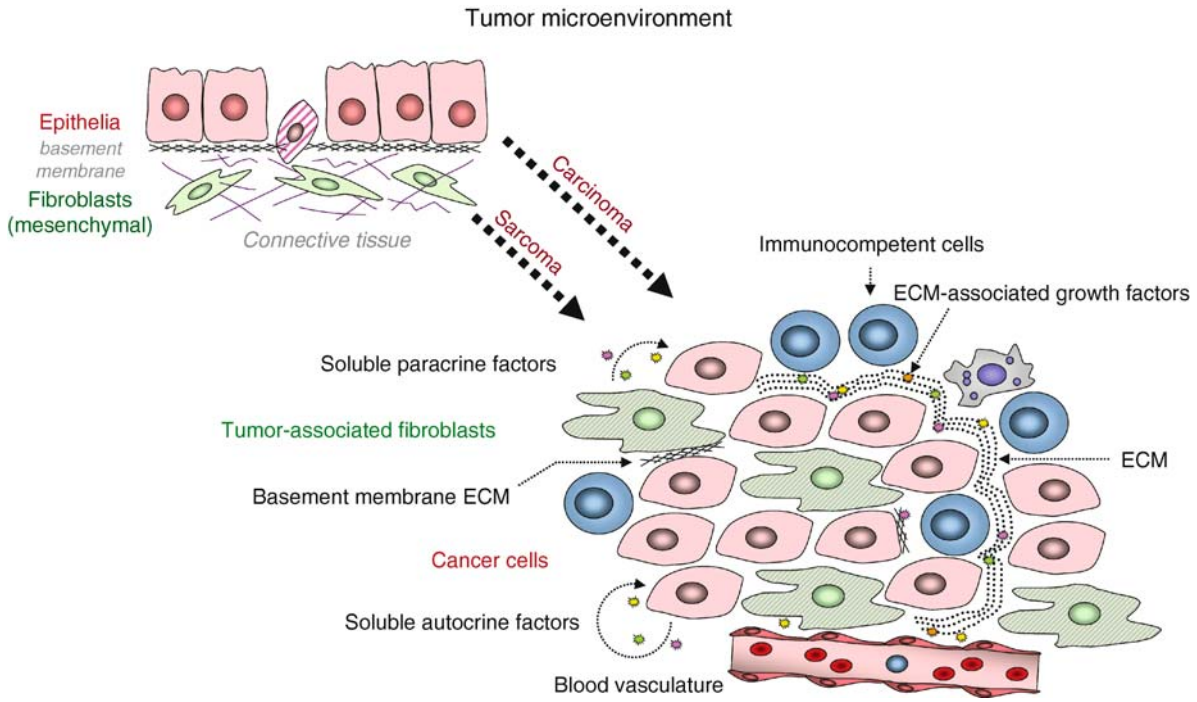
Desmoplasia. Figure 1 Hematoxolin and Eosin (H&E) stain. Tumor fibroblasts (i.e., desmoplasia) appear pink.

and ►metastasis (►Seed and Soil) including ►angiogenesis, ►epithelial to mesenchymal transition (EMT), and progressive ►genetic instability. Additionally, fibroblastic stromal cells can dysregulate anti-tumor immune responses, as exemplified by experiments demonstrating that ►allogeneic murine tumor cells, when co-injected with fibroblastic stromal cells, can engraft across immunologic barriers. Together, these studies suggest that tissue-specific fibroblasts are influential players in progression of metastatic cancer. However, with the exception of promoting epithelial to mesenchymal transition, the direct biological impact on cancer cells themselves has been difficult to distinguish from indirect mechanisms such as enhanced support for angiogenesis or recruitment of inflammatory cells.

The origins of desmoplastic fibroblasts are not fully understood. Some studies have suggested that stromal cell fibroblasts are recruited to the expanding tumor mass from local tissue fibroblasts. However, other experimental evidence supports that additional tumor-associated fibroblasts can be recruited from peripheral fibroblast pools, such as ►bone marrow-derived mesenchymal stem cells (MSC) or fibrocytes. It has been shown that once fibroblasts are recruited into the expanding stroma they change their phenotype and may also undergo selective genetic alterations, which may drive additional tumor growth. Desmoplastic tumor fibroblasts have also been shown to carry unique genetic lesions when compared to those found in expanding tumor cells. These observations offer an additional insight into potential mechanisms for how genetic lesions can induce tumor cell expansion.

The mechanism for recruitment of desmoplastic fibroblasts into a developing tumor remains poorly defined. Yet, several groups have shown that ►platelet-derived growth factor (PDGF) can contribute to the formation of desmoplasia. In a ►xenograft model using the human breast carcinoma cell line MCF-7 expressing the cellular oncogene, c-ras, investigators demonstrated that blocking tumor PDGF inhibited the formation of desmoplasia. Others have shown that blocking TGF-α, TGF-β, IGF-I, and IGF-II had no effect on the desmoplastic response. Since these models used murine xenografts it remains unclear whether PDGF is as critical for development of desmoplasia in human carcinomas (►Epithelial Tumorigenesis).

One important way that desmoplastic fibroblasts can contribute to tumor growth and metastasis is through the production of multiple growth factors (►Fibroblast Growth Factors). ►Paracrine growth factors such as the stroma derived factor 1 (SDF-1/CXCL12) (►angiogenesis), vascular endothelial growth factor (►VEGF) (angiogenesis), ►fibroblast growth factor (FGF) family, ►hepatocyte growth factor (HGF), ►transforming growth factor beta (TGF-β) family, ►interleukin-6 (IL-6), and epidermal growth factor (EGF) have all been linked to increased tumor growth. Desmoplastic fibroblasts also contribute to tumor stroma through the



Desmoplasia. Figure 2 The tumor microenvironment is composed of many cell types that support tumor cell growth and survival.

production of fibrous connective tissues and extracellular matrix proteins (►Fibronectin) (►Focal Adhesion Kinase (FAK)). ►Collagen production is a hallmark feature of desmoplasia. As fibroblasts convert to myofibroblasts or tumor-associated fibroblasts, parallel increases in production of collagen are observed. A pathologist can readily visualize increased levels of tumor collagen using standard histology procedures (►Pathology), and collagen types I and IV are the most prevalent forms of collagen found within most desmoplastic reactions. Collagen bundles interact with extracellular matrix and cell surface proteins such as ►integrins (►Cell Adhesion Molecules) (Focal Adhesion Kinase (FAK)) to influence the stiffness of a given tumor ►microenvironment.

Desmoplasia varies extensively between tumors and even within the same tumor. Some studies have suggested that desmoplasia is a defensive mechanism used to wall off the expanding tumor, but other data demonstrate that desmoplasia is associated with increased tumor growth, invasion, and metastasis. It is unclear, however, which underlying mechanisms determine the extent to which desmoplasia may promote tumor progression. As investigators continue to recognize the importance of the tumor microenvironment (Fig. 2), more detailed studies will allow clarification of the biological impact of desmoplasia in tumor development, survival and metastasis.

►Cutaneous Desmoplastic Melanoma
►Stromagenesis
►Stem Cell Plasticity

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Desmoplastic

Definition

A growth of fibrous or connective tissue around the tumor.

►Desmoplastic Small Round Cell Tumor

Desmoplastic Medulloblastoma

Definition

Histological subtype of ►medulloblastoma characterized by a network of reticulin fibers leaving pale islands of typical medulloblastoma cells. Predominant histological medulloblastoma variant in ►BCNS or ►Gorlin syndrome.

Desmoplastic Melanoma

►Cutaneous Desmoplastic Melanoma

Desmoplastic Small Round Cell Tumor

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Synonyms

Small round-cell tumor; Malignancy of small round blue cell type

Definition

DSRCT; Is a rare and highly aggressive tumor occurring mostly in the abdominal peritoneal cavity of adolescents and young adults. In rare cases, the tumors can also be found in other sites such as pleural cavity, pelvis, bone, and head and neck region. DSRCT belongs to a group of undifferentiated small round cell tumors, which include ►Ewing sarcoma/primitive peripheral neuroectodermal tumor (PNET)/Askin's tumor and ►rhabdomyosarcoma. DSRCT is invariably defined by a ►chromosomal translocation involving chromosomes 11 and 22, t(11;22)(p13;q12), leading to a fusion of two unrelated genes, ►*EWS* and ►*WT1*, into a single ►chimeric gene.

Characteristics

Clinical and Pathological Features

DSRCT was first described in 1989 and is a poorly understood cancer that primarily affects young adults in their second and third decades of life. DSRCT occurs

predominantly in males than females but the reason for this is unknown. Symptoms of DSRCT are usually associated with abdominal pain or pain in the primary site of tumor involvement, distention, and palpable mass. Local invasion or metastasis to liver, lungs, and bone is commonly found at diagnosis. DSRCT displays distinct histological and immunological features. Most of DSRCT cases are presented as tumors in the serosal surface of abdominal cavity, displaying nests of tumor cells surrounded by dense stromal components (hence the term ►desmoplastic) containing spindle-shaped fibroblasts and hyperplastic blood vessels. Though rare, the primary tumors in sites other than abdominal region have been documented. The tumors are positive for various cell lineage markers, such as epithelial membrane antigen, keratin (epithelial), desmin (muscle), and neuron-specific enolase (neural). Thus, the tumor cell origin of DSRCT is not known.

DSRCT is a clinically aggressive tumor with a high risk of recurrence and an overall poor prognosis. The most recent report on the comparison of different treatments of DSRCT patients suggests that compared to patients who received conventional treatments, a multimodal therapy, which include high-dose multiagent chemotherapy, aggressive debulking surgery, and radiotherapy, can prolong overall survival at 3 years (55%) and may provide a possibility of achieving a long-term survival, albeit at a low rate. The two key elements of the multimodal approach are the use of high-dose polychemotherapy, so-called ►P6 protocol, and greater than 90% removal of tumor by surgery. P6 protocol consists of seven courses of high-dose alkylating agents ►cyclophosphamide, ►doxorubicin, ►vincristine, ifosfamide, and ►etoposide. This is followed by aggressive ►debulking surgery, which was shown to be the major determinant in patient survival. Postoperative radiotherapy also contributed to improved survival. Although the multimodal therapy can improve survival at 3 and 5 years, the prognosis of DSRCT still remains extremely low (median survival of 2.5 years).

Molecular Diagnosis

Although clinical, histologic, and immunologic features of DSRCT are distinct, a definitive diagnosis of DSRCT can be provided by genetic techniques. FISH technique, using fluorescently labeled genomic DNA probes derived from *EWS* and *WT1*, can be used to identify the specific t(11;22)(p13;q12) translocation of DSRCT. Alternatively, a definitive DSRCT diagnosis can be made with the use of reverse transcriptase-polymerase chain reaction (►RT-PCR) technique to amplify and detect the DSRCT-specific EWS/WT1 hybrid mRNA transcripts using DNA primers specific for *EWS* and *WT1* genes. This is an extremely sensitive detection method that can provide accurate diagnosis with limiting tumor materials.

Molecular Genetics

Molecular genetic studies revealed that all cases of DSRCT harbor a balanced reciprocal chromosomal translocation, t(11;22)(p13;q12) (►reciprocal translocation) (Fig.1). The breakpoint in chromosome 22 has been mapped to the intron 7 of Ewing sarcoma gene, *EWS*, (breakpoints in other sites of *EWS*, such as in introns 8 and 10, have also been observed in rare cases), while the other breakpoint in chromosome 11 has been invariably mapped to the intron 7 of ►Wilms tumor gene, *WT1*. This DSRCT-specific chromosomal translocation between *EWS* and *WT1* results in a fusion of the N-terminal domain (NTD) of *EWS* to the C-terminal DNA-binding domain of *WT1*.

EWS gene was first isolated from the Ewing sarcoma chromosomal breakpoint, where the translocation generates a fusion between *EWS* and an ETS-family transcription factor gene, *FLI-1*. *EWS* encodes a putative RNA-binding protein with presumptive roles in transcription and splicing. The NTD of EWS mediates potent transcriptional activation when fused to a heterologous DNA-binding domain, while its C-terminal domain, which is lost in the translocation gene product, is involved in RNA recognition. *WT1* encodes a transcription factor which is mutated in a subset of Wilms' tumor, a childhood kidney cancer. *WT1* encodes four Cys_2-His_2 zinc-fingers in the C terminus that mediate sequence-specific DNA binding and the NTD containing both transcriptional activation and repression domains. *WT1* is subjected to two ►alternative RNA splicing events, one of which involves the usage of two alternative splice donor sites at the end of exon 9, leading to inclusion or exclusion of three amino acids, lysine, threonine, and serine (termed KTS), between the zinc-fingers 3 and 4 (Fig.1). The KTS insertion leads to a markedly decreased DNA-binding affinity of WT1. In all *EWS/WT1* translocations examined, only the last 3 exons of *WT1* (exons 8–10) encoding the last three zinc-fingers are fused to the NTD of EWS (Fig. 1), while the first zinc-finger of WT1 is invariably lost. The alternative KTS splicing of *WT1*, however, is preserved. As a result, *EWS/WT1* produces two isoforms: EWS/WT1(−KTS) and (+KTS) that differs in the DNA binding affinity and specificity (Fig. 1). In vitro study has shown that only the EWS/WT1(−KTS) isoform, but not the EWS/WT1(+KTS), possesses the oncogenic activity in ►NIH3T3 ►transformation assay.

DSRCT is a rare disease and has been recognized only recently as a distinct cancer type. Therefore, not much is known about the mechanisms of DSRCT but molecular details are starting to emerge. The novel fusion protein EWS/WT1(±KTS) acts as an aberrant transcription factor to presumably initiate the oncogenic

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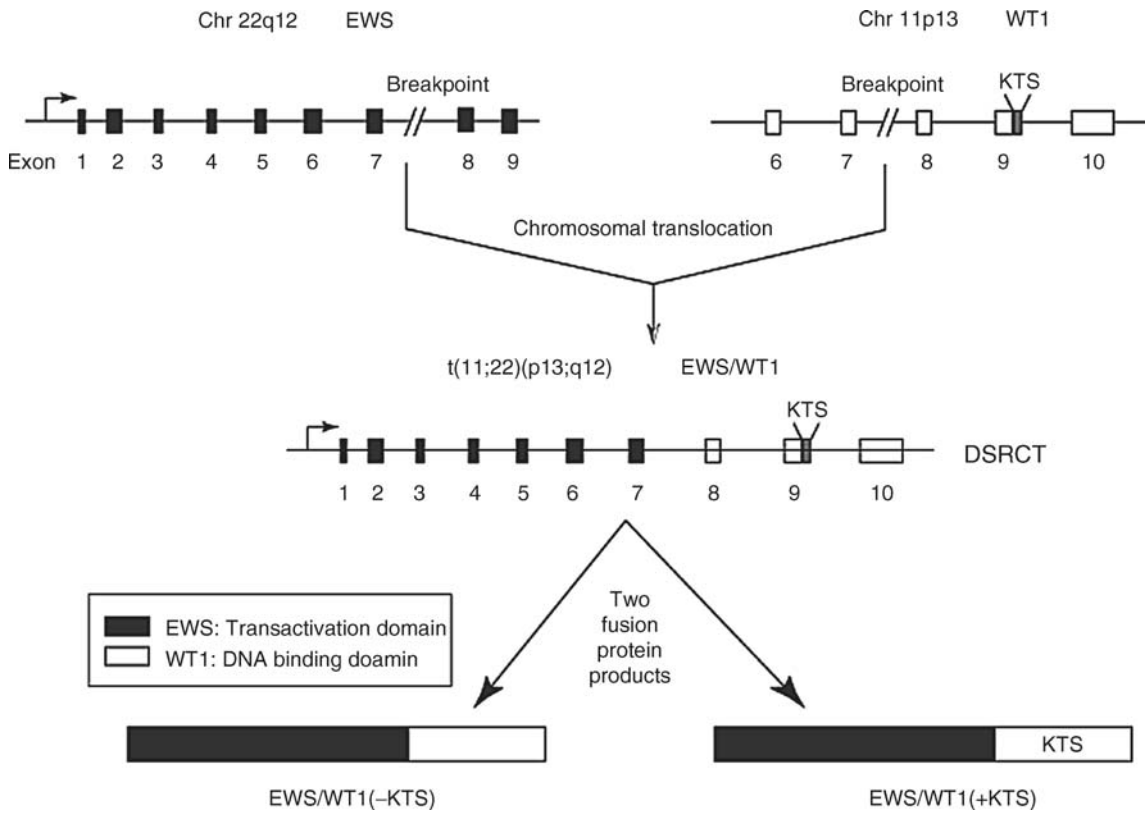

Desmoplastic Small Round Cell Tumor. Figure 1 Schematic representation of DSRCT-specific chromosomal translocation. A reciprocal balanced chromosomal translocation that results in the fusion of *EWS* gene to *WT1* gene is shown. The arrow indicates the promoter of *EWS* which drives the transcription of the fusion gene and the boxes mark the exons. Alternative KTS splicing (grey box, KTS) within the exon 9 of *WT1* is shown. Two isoforms of the fusion product are shown separately. See text for details.

process. To date, a number of direct transcriptional targets of EWS/WT1(−KTS) have been identified, which include *PDGF-A* (platelet-derived growth factor A), *IGFR1* (insulin-like growth-factor receptor 1), *IL2RB* (interleukin 2 receptor beta), *BAIAP3* (BAI1-associated protein 3), a potential regulator of growth-factor release, and *TALLA*-1 (T-cell acute lymphoblastic leukemia-associated antigen 1), a gene encoding a tetraspanin-family protein. There is only one target gene identified for EWS/WT1(+KTS), which is *LRRC15* (leucine-rich repeat containing 15), a gene implicated in cell invasion. All of these target genes are not transcribed by the native WT1 and thus represent EWS/WT1-specific transcripts. Identification of the EWS/WT1 target genes may provide clues to the molecular and cellular pathways that are central to DSRCT. For example, expression of IGFR1 and IL2RB can promote proliferation and survival of the tumor cells, while expression of PDGF-A and BAIAP3 by the tumor cells can enhance recruitment and proliferation of surrounding fibroblasts and stromal tissues, which may further enhance the growth of the tumor cells and may explain the dense stroma (desmoplastic feature) associated with DSRCT. Some of these target genes may also have diagnostic and therapeutic values, but it will require further evaluation.

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Desmosomal Cadherins

Definition

A sub-family of the cadherin super-family of cell ►adhesion molecules. In humans, the desmosomal cadherin family comprises four ►desmogleins and three ►desmocollins. The desmosomal cadherins are constituents of desmosomes and are essential for desmosomal adhesion.

►Desmosomes

Desmosomes

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Synonyms

Maculae adherents

Definition

Desmosomes are intercellular junctions that mediate cellular ►adhesion and maintain tissue integrity. They are found in ►epithelial cells, myocardial and Purkinje fiber cells of the heart, arachnoid cells of brain meninges and follicular dendritic cells of lymph nodes.

Characteristics

Desmosomes are localized at sites of close cell-cell contact (Fig. 1a). They are less than 1 μm in diameter, have a highly organized structure at the ultrastructural level and act as anchoring points for intermediate filaments of the cell cytoskeleton (Fig. 1b). By linking intermediate filaments of adjacent cells desmosomes confer structural continuity and mechanical strength on tissues. Desmosomes are particularly prevalent in tissues, such as the epidermis and heart, that experience mechanical stress. The proteins that form desmosomes belong to three genes families, the ►desmosomal cadherins, the ►armadillo family and the ►plakin family of cytolinkers.

Desmosomal Cadherins

The desmosomal cadherins are the membrane spanning ►cell adhesion molecules of desmosomes. In humans there are seven, four ►desmogleins (Dsg1–4) and three ►desmocollins (Dsc1–3). Each desmoglein and desmocollin is encoded by a distinct gene that is located in the desmosomal cadherin gene cluster on chromosome 18q21. All three desmocollin genes encode a pair of proteins, a larger "a" protein and a smaller "b" protein, that are generated by alternative splicing; the role of the smaller protein in desmosomal adhesion is not yet clear. All desmosomes contain at least one desmoglein and one desmocollin and both are required for adhesion. The desmosomal cadherins show tissue-specific patterns of expression with Dsg2 (►desmoglein-2 adhesion molecule) and Dsc2 ubiquitously expressed in tissues that produce desmosomes and the others largely restricted to stratified epithelial tissues. The extracellular domains of desmosomal cadherins produced by adjacent cells interact in the intercellular space. Within the cell desmosomal cadherin cytoplasmic domains associate with armadillo proteins (Fig. 1c).

Armadillo Family

Armadillo proteins that are found in desmosomes include ►plakoglobin (γ-Catenin) and ►plakophilins. Plakoglobin is indispensable for desmosome function and interacts with desmosomal cadherins, plakophilins and ►desmoplakin. Plakoglobin is also found in ►adherens junctions where it is interchangeable with a closely related armadillo protein, β-catenin. In addition to its structural role in adherens junctions, β-catenin acts as a signaling molecule in the ►APC/β-catenin pathway. There is a strong possibility that plakoglobin also has a signaling function in this pathway although its role has yet to be fully defined. There are three plakophilins (PKP1–3) that exhibit complex tissue-specific patterns of expression. All three plakophilins show dual localization in desmosomes and in the nucleus. The plakophilins have an important structural role in desmosomes and, because of their nuclear localization and similarity to other armadillo proteins, it is possible that they act as signaling molecules.

Plakin Family

►Plakin family proteins bind intermediate filaments and several, including desmoplakin, plectin, envoplakin and periplakin, localize to desmosomes. Of these only the presence of desmoplakin is obligatory for normal desmosomal adhesion. It is a dumbbell shaped molecule with two globular domains separated by a coiled-coil rod domain and is thought to exist as a homodimer. The desmoplakin gene encodes two proteins (DPI and DPII) that are generated by alternative splicing and differ only in the length of their central rod domain; the role of DPII, the smaller of these proteins, is unclear. The N-terminal end of desmoplakin binds to plakoglobin and plakophilins whereas its C-terminal end binds to intermediate filaments. In epithelial tissues desmoplakin anchors keratin intermediate filaments to the membrane, but in myocardial and

Purkinje fiber cells it interacts with desmin intermediate filaments and in arachnoid and follicular dendritic cells it associates with vimentin intermediate filaments.

Null Mutations in Mice

►Genetic ablation studies in mice have shown the importance of desmosomes for embryonic development and normal tissue biology. ►Knock-out mice of either Dsg2, Dsc3 or desmoplakin display early embryonic lethality at around implantation or before. Mice without either PKP2 or plakoglobin survive longer but die during mid-gestation as a result of heart defects. Embryonic survival is not affected by absence of either Dsg3, Dsg4 or Dsc1 but loss of these molecules does result in defects in keratinocyte adhesion and skin and hair abnormalities.

Clinical Relevance

Loss of desmosomal adhesion can result in skin blistering diseases. Pemphigus is an autoimmune blistering disease that is caused by pathogenic ►autoantibodies against desmogleins. Staphylococcal scalded-skin syndrome is caused by toxins with serine protease activity that are released by the bacterium *Staphylococcus aureus* and specifically cleave Dsg1. Mutations in DNA encoding desmosomal constituents result in a variety of diseases that can affect the skin, hair and heart, and sometimes all three.

No mutations in desmosomal cadherins, plakophilins or desmoplakin have been found so far in cancer. However, many reports have documented altered expression of desmosomal constituents in tumorigenesis. For example, loss of Dsg2, Dsc2 and Dsc3 occurs in ►gastric ►colorectal and ►breast cancer respectively. By contrast Dsg2 is overexpressed in ►skin cancer and Dsg3 is overexpressed in head and neck cancer. PKP3 levels are elevated in ►lung cancer and loss of desmoplakin has been correlated with progression in a variety of epithelial tumors. A causal relationship between these changes and cancer has yet to be established.

Mutations in plakoglobin, concomitant with strong nuclear accumulation, have been linked to the pathogenesis of prostate cancer. Nuclear accumulation and improper activation of transcriptional targets as a result of a failure to degrade cytoplasmic β-catenin has been implicated in FAP (►APC gene in Familial Adenomatous Polyposis), a familial syndrome that predisposes to ►colon cancer, and sporadic colon cancer. It remains to be seen whether plakoglobin has, in common with β-catenin, pro-proliferative effects in cancer. In many cancers loss of expression of plakoglobin has been observed and it may be that plakoglobin is anti-proliferative in some cell types. There is little doubt that plakoglobin plays a role in cancer but whether this is related to its participation in desmosomes remains unclear.

Overall it appears that the importance of desmosomes in cancer is twofold. Firstly, as mediators of cell-cell adhesion reduced expression of desmosomal constituents could lead to loss of cell-cell adhesion, ►epithelial-mesenchymal transition, increased invasiveness and metastasis. Secondly, desmosomes may act as signaling centers and variations in expression levels of desmosomal proteins could trigger intracellular signaling cascades that contribute to cancer pathogenesis.

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Destruction Box

Definition

DB; Amino acid sequence that when present in a protein in appropriate location confers the ability to be recognized by ►E3-ubiquitin ligases and the subsequent degradation by the ►proteasome. Usually, DB sequences can act in heterologous contexts.

►Snail Transcription Factors
►Ubiquitination

Detachment-induced Cell Death

►Anoikis

Determination of Tumor Extent and Spread

►Staging of Tumors

Detoxification

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Synonyms

Detoxication; Drug metabolism; Xenobiotic metabolism; Carcinogen metabolism; Xenobiotic biotransformation

Definition

Metabolic and transport processes used to chemically inactivate noxious compounds and eliminate them from cells for subsequent excretion from the body.

Characteristics

Humans are continuously exposed to foreign chemicals (xenobiotics (►Xenobiotic)) through administration of medicines, the consumption of food and drink, and air breathed. Protection against the detrimental effects of xenobiotics is achieved by the concerted actions of a battery of proteins that metabolize, transport and ultimately pump out of cells modified forms of the compounds originally encountered. This process is called detoxification, or detoxication (in instances where no toxicity occurs). Although detoxication occurs primarily in the liver, all cells possess some capacity to metabolize and eliminate unwanted chemicals. The xenobiotics subject to this process are numerous and include mycotoxins, phytoallexins, pesticides, herbicides, environmental pollutants, cytotoxic anti-cancer agents and many pharmacologically-active drugs. Detoxication processes also confer protection against harmful compounds of endogenous origin, many of which arise as a consequence of interaction with reactive oxygen species, such as the superoxide anion, produced normally in the body.

Detoxication is achieved in two distinct stages, the first involving metabolism of the xenobiotic, and the second involving energy-dependent efflux of the xenobiotic from the cell. Historically, description of xenobiotic biotransformation has been divided into phase 1 and phase 2 metabolism, and consequently efflux of xenobiotics is referred to as phase 3 of detoxication.

- Phase 1 drug metabolism involves an initial chemical modification of the xenobiotic that results in the introduction, or exposure, of a functional chemical group (e.g. –OH, –NH_2, –SH, –COOH) into the compound. This usually entails enzyme-catalyzed oxidation reactions by ►cytochrome P450 (CYP) or flavin monooxygenase.
- Phase 2 drug metabolism often involves a second chemical alteration of the xenobiotic, usually at the same region of the molecule where the functional group was introduced. This is performed by enzymes catalyzing conjugation reactions (such as ►glutathione S-transferase (GST), ►N-acetyltransferase (NAT), ►sulfotransferase (SULT) and ►UDP-glucuronosyl transferase (UGT). It should be noted that use of the terms phase 1 and phase 2 to define the detoxication enzymes is somewhat arbitrary and does not necessarily reflect the pathway of biotransformation of all chemicals. Thus, a number of xenobiotics are subject to several modifications by the phase 1 CYP isoenzymes before serving as substrates for the phase 2 enzymes. Alternatively, some xenobiotics do not require modification by phase 1 enzymes before metabolism by phase 2 enzymes, and others are subject to modification by more than one phase 2 drug-metabolizing enzyme. As a result of differences in drug metabolism, the group of enzymes catalyzing reduction of hydrolysis reactions (e.g. ►aldehyde dehydrogenase (ADH), ►aldo-keto reductase (AKR), ►epoxide hydrolase (EPHX) and ►NAD(P)H-quinone oxidoreductase (NQO)) are variously referred to as phase 1 or phase 2 detoxication, depending on the individual xenobiotic being considered and the preferences of research workers. Clearly, these enzymes provide a highly flexible metabolic defense that has evolved to protect against a diverse spectrum of chemicals.
- Finally, phase 3 of detoxication involves ATP-dependent elimination of the parent compound or modified xenobiotic by proteins that are drug efflux pumps (e.g. ►multidrug resistance protein (MDR) and ►multidrug resistance-associated protein (►Multidrug resistance protein) (►MRP)). As a consequence of the combined actions of phase 1 and phase 2 enzymes, a diverse spectrum of xenobiotics acquires a limited number of molecular "tags" (i.e. acetate, glutathione, glucuronide or sulfate moieties) that are recognized by the MRP trans-membrane pumps. Furthermore, the xenobiotic metabolites produced by phase 1 and phase 2 are usually more soluble, and easily excreted, than the parent compound.

Whilst the ability of CYP to oxidize xenobiotics is generally desirable, as it facilitates further metabolism and elimination of harmful chemicals, it can sometimes result in the generation of highly reactive products that may not be readily detoxified. In such instances, modification of intracellular macromolecules will occur resulting in necrosis, ►apoptosis or malignant ►transformation. As an example of the interplay between toxification and detoxification reactions, a scheme depicting metabolism of ►aflatoxin B1 (AFB1), modification of macromolecules by AFB1 metabolites, and efflux of the AFB1-glutathione conjugate from a cell is shown in the illustration (Fig. 1).

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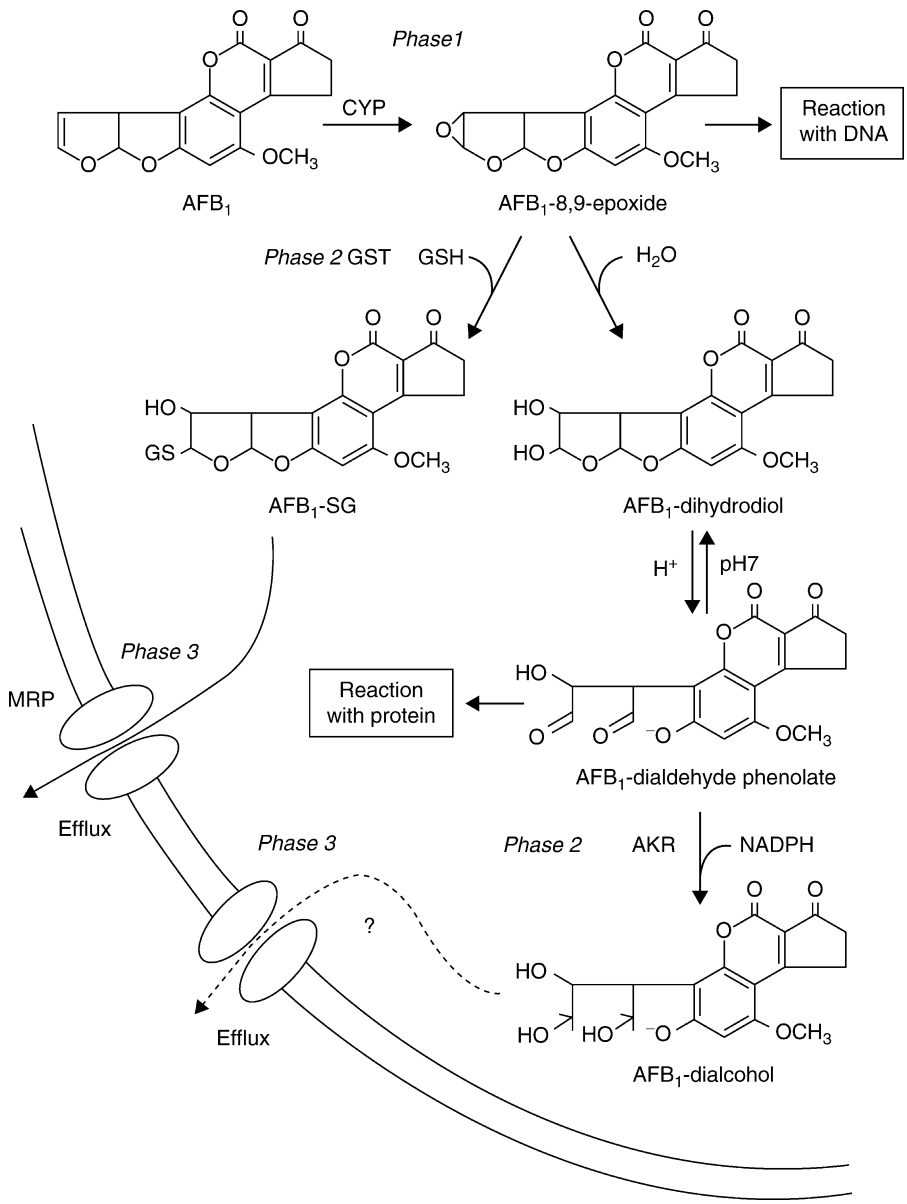

Detoxification. Figure 1 Detoxification pathways for aflatoxin B1. The mycotoxin is converted to the ultimate carcinogen AFB1-8,9-epoxide, by the actions of the hepatic phase 1 CYP enzyme system. The epoxidated AFB1 is highly reactive, and if it is not detoxified it will form DNA adducts that may cause hepatocarcinogenesis. The phase 2 GST enzymes can achieve detoxification of this unstable intermediate, and the resulting AFB1-glutathione conjugate is eliminated from the liver cell by MRP. In addition, AFB1-8,9-epoxide can rearrange to form a dialdehyde-containing metabolite which will covalently modify proteins by forming Schiff's bases. The dialdehyde can be reduced by phase 2 AKR to yield a dialcohol that may be a substrate for SULT or UGT before being transported out of the cell, presumably by MRP.

Genetic Variation

Numerous proteins have evolved that detoxify drugs, and certain of the families listed above comprise over twenty genes. In total, the human probably possesses between 100 and 150 genes encoding detoxication proteins. Substantial variation can occur in the levels of these proteins in tissues from different individuals, and this can result in increased sensitivity of cells to chemical insult. In part, this inter-individual variation is due to ►genetic polymorphisms. By definition, such differences must be present in at least 1% of the population in order to be considered a ►genetic polymorphism. In some instances the variation involves deletion of detoxication genes with complete loss of

specific functions, whereas in other instances point mutations result in alteration of protein structure causing only a modest attenuation of activity. In other cases mutations alter the regulatory regions of genes causing altered expression of normal protein. Detoxication genes that are polymorphic in the human include those for the enzymes CYP3A4, CYP2C9, CYP2C19, CYP2D6, CYP2E1, AKR1C4, GSTM1, GSTP1, GSTT1, NAT2, SULT1A1, SULT1E1, SULT2A1, UGT1A1, UGT1A4, UGT1A6 and UGT2B7, EPHX and NQO1, as well as the MRP2 efflux pump. It is clear additional polymorphisms remain to be identified.

Cellular Regulation

In addition to genetic polymorphisms, induction of detoxication proteins by xenobiotics and environmental agents is a further mechanism that can cause inter-individual differences in detoxification capacity. Induction of detoxication proteins represents an ►adaptive response to chemical and ►oxidative stress, which can be brought about by synthetic drugs or by naturally occurring compounds such as coumarins, indoles and isothiocyanates that are found in edible plants. Increased expression provides short-term resistance to toxic xenobiotics. Enzyme induction also results in increased metabolism of therapeutic drugs. Many of the enzymes and pumps such as CYP, GST, ADH, AKR, NQO and MRP are inducible, often by transcriptional activation of genes encoding the proteins. The promoters of these genes contain enhancers that enable a transcriptional response to a diverse spectrum of chemical agents. The enhancers that are involved in induction of detoxication proteins include ►AP-1 binding sites, the antioxidant responsive element, the xenobiotic responsive element, the phenobarbital responsive enhancer module, progesterone X receptor and peroxisome proliferator-activated receptor enhancer.

Clinical Relevance

It is apparent from studies into the mechanisms of selective toxicity between species that variation in the activity of detoxication proteins influences sensitivity to chemical insult. Increasing evidence suggests that genetic polymorphisms in detoxication enzymes can confer an inherited predisposition to a number of malignant diseases that are influenced by environmental factors (e.g. lung and colorectal cancer). They may also confer a predisposition to adverse drug reactions.

Induction of some phase 2 detoxication systems is believed to represent a major mechanism of cancer ►chemoprevention, and is thought to explain in part the epidemiological data suggesting that consumption of diets rich in fruit and vegetables protect against certain malignant diseases.

Acquired ►drug resistance to chemotherapy is a major problem in the treatment of many cancers. There is overwhelming evidence that the overexpression of several detoxication proteins, particularly GST, MDR and MRP, contributes to the drug-resistant phenotype.

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Detoxication

Definition

Synonym Detoxification; Process, or processes, of chemical modification which make a toxic molecule less toxic.

►Toxicological Carcinogenesis

De-ubiquitinase

Definition

DUB; An enzyme that can specifically remove ►ubiquitin proteins from substrates through an enzymatic cascade that cleaves an isopeptide bond.

►Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) De-Ubiquitinase
►Ubiquitination

De-ubiquitinating Enzymes

Definition

Are a large family of enzymes that cleave chemical bonds formed at the C-terminus of ►ubiquitin. By virtue of their action, the ►ubiquitination of a given protein is reversible. Ubiquitin-protein conjugates are not always degraded by the proteasome, an alternative fate is the protein is spared from degradation through the activity of any of a large family of de-ubiquitinating enzymes. De-ubiquitinating enzymes can remove ubiquitin from ubiquitin-protein conjugates. These enzymes break down abundant multiubiquitin chains that are nor attached to any substrate and produce mature ubiquitin from the precursor forms in which it is synthesized. A second alternative fate for ubiquitin-protein conjugates is that ubiquitinated cell surface proteins may be targeted for endocytosis and eventual degradation via the lysosome rather than the proteasome.

Development of New Lymphatic Vessels

►Lymphangiogenesis

Dexrazoxane

Definition

Is an iron chelator, is a bisdioxopiperazine with cardioprotective and antineoplastic activities.

►Adriamycin

Dexrazoxane

Definition

A cyclic derivative of ►EDTA used to protect the heart against the cardiotoxic side effects of anthracycline chemotherapy.

►Chemoprotectants

Dezocitidine

►A5-aza-2′; Deoxycytidine

dFdC

►Gemcitabine

DHT

Definition

Dihydrotestosterone.

►Cyclin G-Associated Kinase
►Dihydrotestosterone Receptor

DIA

►Leukemia Inhibitory Factor

Diabetes

Definition

Diabetes mellitus; People who are Type 1 Diabetes mellitus must use manufactured ►insulin, usually in an injectable form, to replace the natural insulin that is no longer produced by their body (for instance as the result of beta-cell degeneration). People with Type 2 Diabetes sometimes need to use insulin when their cells become too resistant to the insulin that they produce naturally and when oral medications are no longer working.

Diabetes Type 2

Definition

Type 2 Diabetes, is a metabolic disorder characterized by insulin resistance, relative insulin deficiency, and hyperglycemia. A disorder of glucose and insulin metabolism that is characterized by inappropriately increased blood glucose levels and resistance of tissues to the action of insulin. Insulin levels are elevated during early stages of the disease. Previously referred to as adult-onset diabetes or non-insulin-dependent diabetes.

►Obesity and Cancer Risk
►Adiponectin

Diabody

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Synonyms

Engineered antibody; Single-chain Fv dimer; Multimeric antibody fragments

Definition

Diabody is a noncovalent dimer of single-chain Fv (scFv) fragment that consists of the heavy chain variable (V_H) and light chain variable (V_L) regions connected by a small peptide linker. Another form of diabody is single-chain $(Fv)_2$ in which two scFv fragments are covalently linked to each other.

Characteristics

Advances in antibody technology are enabling the design of antibody-based reagents for specific purposes in cancer diagnosis and ►monoclonal antibody therapy. First, to minimize the immunogenicity and enhance the efficacy in human use, mouse monoclonal antibodies are engineered to ►chimeric antibodies or ►humanized antibodies by grafting to the human constant region or framework. Moreover, fully human antibodies are developed by the use of ►transgenic mice or phage display technology. Second, monoclonal antibodies are designed as immunoconjugates to deliver the cytotoxic agents such as chemotherapeutic drugs, toxins, enzymes, and radioisotopes. These therapeutic antibodies have emerged as potent agents and are used worldwide for cancer therapy. More recently, engineered antibody fragments have been investigated as alternative reagents because of their unique properties resulting from the structure.

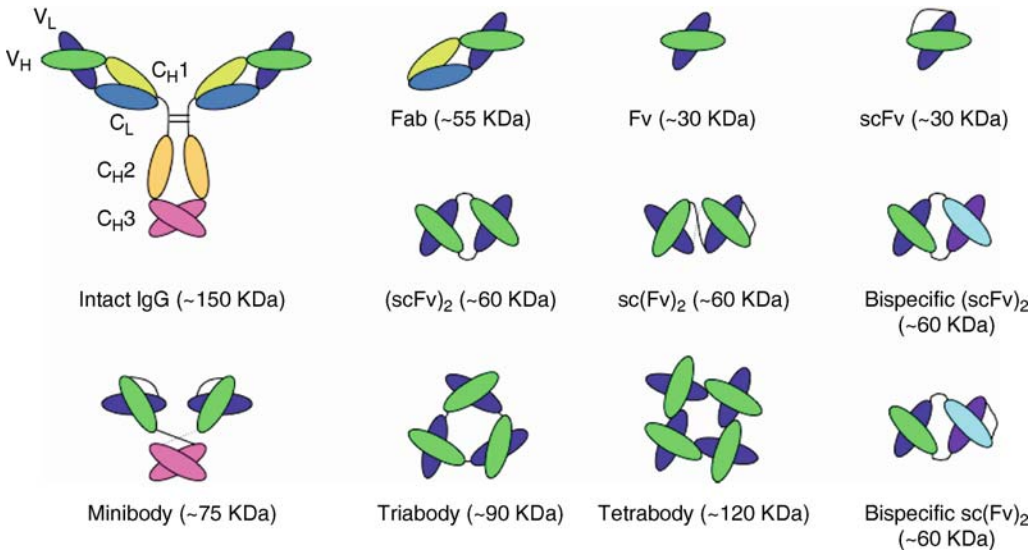
Structure

A variety of antibody fragments are developed including single V_H domain, Fab, scFv, and multimeric formats such as multivalent scFvs (diabody, triabody, and tetrabody), bispecific scFv, and minibody (scFv–CH3 dimer) (Fig. 1). In scFv fragments, the V_H domain binds to its attached V_L domain when the linker is flexible and long enough (a length of at least 12 amino acids). For example, the linker sequence of $(Gly_4Ser)_3$ provides sufficient flexibility for the V_H and V_L domain to form Fv comparable to the parent antibody. In contrast, when the linker is shortened to less than 12 residues (e.g., five amino acids of (Gly_4Ser)), the V_H and V_L domains are unable to bind each other and instead the scFv fragment form a noncovalent dimer by another scFv molecule [$(scFv)_2$ diabody]. Shortening of the linker length between the V_H and V_L domains (less than three residues) promotes the assembly of trimeric or tetrameric structures (triabody or tetrabody). However, this multimer formation also depends on the V-domain orientation either V_H–V_L or reverse V_L–V_H orientation in the scFv constructs. The bivalent Fv fragment can also be designed by linking two scFv domains covalently as a single chain version [$sc(Fv)_2$ diabody]. The $sc(Fv)_2$ version is more stable than $(scFv)_2$, and this structure may form a noncovalent dimer $[sc(Fv)_2]_2$.

The capacity of multivalent binding of these fragments offers a significant opportunity to design multifunctional antibody reagents. The diabody structure is used to form ►bispecific antibodies by linking different V_H and V_L domains of two antibodies (e.g., V_HA–V_LB and V_HB–V_LA). However, when two different polypeptides are produced within a single cell, purification steps are necessary to obtain the active heterodimeric antibody among the inactive homodimers. Therefore, bispecific $sc(Fv)_2$ version is developed by connecting two different scFv domains with the middle-length linker (e.g., V_HA–V_LB–V_HB–V_LA or V_HA–V_LA–V_HB–V_LB).

Pharmacokinetics and Distribution

The ►pharmacokinetics of these antibody fragments is markedly different from intact IgG antibodies that exhibit prolonged circulation ($t_{1/2}$ of up to 3 weeks). The lower molecular weight constructs (below than 60 kDa) are subject to be excreted by renal clearance, resulting in a shorter serum half-life than intact IgG. In most cases, the $t_{1/2}$ values of scFv and diabody are extremely short such as 2 and 6 h, respectively.



Diabody. Figure 1 Schematic structure of intact IgG antibody and engineered antibody fragments. The variable regions of heavy (V_H) and light chains (V_L) contribute to the antigen binding. The V_H and V_L domains can be connected by a peptide linker to form single-chain Fv (scFv). The scFv fragments can form multimers such as $(scFv)_2$ diabody, triabody, and tetrabody depending on the linker length and the V-domain orientation. The bivalent $sc(Fv)_2$ can be generated by connecting two scFvs covalently. Bispecific diabodies can also be engineered by using two different Fv domains.

This rapid pharmacokinetics is the most favorable for imaging applications and ►radioimmunotherapy because of the lower background levels in normal tissues. ►Drug biodistribution studies of radiolabeled scFv and $sc(Fv)_2$ have shown high tumor-to-blood ratios in xenograft models compared with intact IgG antibodies. The fast blood clearance of antibody fragments contributes to avoid undesired toxicity, and these fragments have the remarkable advantage for ►targeted drug delivery of toxins or radioisotopes.

In addition, antibody fragments show better penetration into the tumor mass, but these smaller constructs have shorter retention to tumor cells at the same time. Thus, the valance of penetration and retention of antibodies is an important factor for therapeutic use, especially in solid tumors. The Fab and scFv fragments are monovalent and exhibit poor retention on target cells, but multivalent forms of these fragments such as diabody, triabody, and tetrabody exhibit dramatically increased affinity and high tumor retention compared with the parent scFv. The ideal tumor-targeting reagents are intermediate-sized multivalent antibodies such as bivalent diabodies that show a longer half-life as well. In another approach, the Fc portion is fused to antibody fragments to control the serum levels of the antibody. The scFv–Fc or scFv–CH3 fusion antibodies (minibodies) are expected to have a more prolonged half-life and increased tumor accumulation in vivo. The serum half-life of antibody fragments can also be extended by modification such as linkage to polyethylene glycol (►PEG).

Agonistic Activity

In terms of mechanism of action, intact IgG antibodies kill tumor cells mainly by Fc-mediated effector functions such as ►antibody-dependent cell-mediated cytotoxicity (ADCC) and ►complement-dependent cytotoxicity. In contrast, antibody fragments have the compact structures without the Fc portion, and have unique characteristics for using cancer treatment. The two binding sites of diabodies are located at a distance of about 70Å less than half for those of intact IgG antibodies. Therefore, diabodies can place the antigens more closely to each other than by the parent IgG antibodies, which efficiently induces the ligation of target molecules on the cell surface. When the targets are functional receptors, the diabody can mediate a direct effect or signal transduction in tumor cells, including the stimulation of ►apoptosis or cell death. For example, we and our collaborators have generated $(scFv)_2$ and $sc(Fv)_2$ diabodies that recognize CD47 or ►HLA class I molecules. These diabodies can cross-link the target antigens and show the enhanced cytotoxic activities against hematological malignancies such as leukemia, lymphoma, and myeloma cells when compared with the original IgG antibodies. Thus, enhancement of the cross-linking potential is one of the important bioactivity of antibody fragments.

Application of Diabodies

A variety of target antigens have been evaluated for therapeutic purposes including CD19, CD20, CD22,

epithelial cell adhesion molecule (Ep-CAM), epidermal growth factor receptor (EGFR), HER2, MUC1, and carcinoembryonic antigen (►CEA). Several types of diabodies and minibodies are engineered for targeting these candidate antigens on tumor cells. Immunotoxins are also constructed to deliver the cytotoxic agents, radioisotopes, enzymes, cytokines, and liposomes by using antibody fragments. Previous studies have shown the effectiveness of these reagents in preclinical and clinical trials.

►Bispecific antibodies that comprise two different binding specificities have been studied extensively in cancer diagnosis and therapy. Most of the bispecific reagents are designed for the retargeting of effector cells such as cytotoxic T lymphocytes and NK cells. Recombinant bispecific diabodies such as anti-CD19 x anti-CD3, anti-Ep-CAM x anti-CD3, and anti-HER2 x anti-CD3 have been used in the immunotherapy of ►B-cell lymphoma ►breast ►ovarian and ►colorectal cancer. Another strategy of bispecific antibodies is the recruitment of effector molecules including toxins, drugs, ►prodrugs, ►cytokines, and radionuclides in vivo. First, the tumor cells are targeted by the tumor-specific binding site of the diabody. After the unbound diabody is cleared from the serum, cytotoxic drugs or radiolabeled hapten are administered to be captured by another binding site of the bound diabody.

Future Directions

In principle, selection of target molecules and modification of antibody constructs are key issues of antibody-based strategies in clinical utility. Based on the properties of pharmacokinetics, biodistribution, and manufacturing production, engineered antibody fragments have been investigated as alternative reagents to target cancer cells. Although the efficacy of these antibody fragments needs to be evaluated in clinical settings, the drastic potential of agonistic activity or multivalent activity of these reagents will provide new promises for development of the next generation of antibody drugs in cancer diagnosis and treatment.

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Diacylglycerol

Definition

DAG; Is a lipid with two acyl chains esterified to sn-1 and sn-2 position of glycerol backbone; it is produced by phospholipase C and is involved in selective activation of isoforms of ►protein kinase C (PKC).

►Lipid Mediators
►Protein Kinase C Family

Diagnostic Biomarkers

Definition

Markers to assess the presence or absence of cancer.

Diagnostic Pathology

►Pathology

Dibasic Processing Enzyme

►Furin

Dicer

Definition

An RNaseIII type enzyme that cleaves perfect or partially double-stranded RNA molecules. The hallmark of Dicer cleavage is the production of double-stranded short RNA molecules consisting of two 21 nucleotide RNA strands annealing to each other through 19 base pairs and have a two nucleotide overhang at the 3′-ends. Dicer produces ►siRNAs from long double-stranded RNAs in lower

animals and plants and generates ►microRNA duplexes from stem-loop structure pre-miRNAs.

DIE

►Endometriosis

Diet

Definition

Dietary factors may contribute to enhancing risks for the various cancers. These factors are heterogeneous. In many cases individual compounds have been suggested to be involved but little definitive evidence is available. The individual risk factors are specific for different tissues, and the state of current knowledge has recently been published. In general diets high in total fat or animal fat are considered causative for several common tumors including those arising from tissues of the gastrointestinal tract. In contrast, vegetables and fruits are considered to be protective for many tissues and for all cancers discussed in the review listed below. Phytoprotectants are implicated as contributing to risk reduction by different mechanisms, but it has not been possible to pinpoint individual compounds as the responsible factors. Tissues that seem to be most protected are esophagus, stomach, colon, lung, pancreas and bladder. The least clear cut protection is achievable in the hormone dependent tissues, prostate and breast, although a dietary component can not be excluded for these tumors. Altogether the estimates indicate that at least 35% of all human tumors are dietary related, which means a large proportion of tumors could be prevented by adequate dietary regimens; biomarkers

See also: World Cancer Research Fund and American Institute for Cancer Research; Food, Nutrition and the Prevention of Cancer: a global perspective. Washington DC: American Institute for Cancer Research, 1997

►Biomarkers

Dietary Essential Minerals

►Mineral Nutrients

Dietary Micronutrient

Definition

A trace element, present in the diet that is required for maintenance of normal health.

►Ultra trace Minerals

Dietary Supplement

Definition

An agent intended to supply nutrients, vitamins, minerals, and essential elements.

►Chemoprotectants

Diethylstilbestrol

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Synonyms

DES

Definition

Diethylstilbestrol is a synthetic non-steroidal estrogen with biological properties similar to endogenous estrogens such as estradiol-17-beta and estrone (►Estradiol).

Characteristics

Pharmacology

Diethylstilbestrol is administered orally, is lipid-soluble, and readily absorbed from the proximal gastrointestinal tract. It is metabolized via the hepatic microsomal system to dienestrol, and quinone and epoxide intermediates. It crosses the placenta and is thought to be metabolized by the fetus.

Initial Use and Early Epidemiologic Studies

Diethylstilbestrol (DES), first manufactured by Dodds and associates in London in 1938, was used to treat several gynecologic conditions. In particular, it was

prescribed for the treatment of frequent or threatened miscarriages. As early as 1953, Dieckmann and colleagues demonstrated that DES did not improve pregnancy outcomes. (In fact, in a later re-analysis of these data in 1978, Brackbill and Berendes showed that women exposed to DES had higher risks of premature births, perinatal death, and miscarriages than women who were given placebo.) Other studies also found DES to be ineffective in preventing adverse pregnancy outcomes, but physicians continued to prescribe the drug to try to maintain high-risk pregnancies because its use seemed logical and it was well-established. It was administered to approximately 5–10 million pregnant women in the United States between 1940 and 1971. It remained in use in Europe until the early 1980s.

In 1971, Herbst and colleagues established a strong connection between DES exposure *in utero* and subsequent development of clear-cell adenocarcinoma of the vagina and cervix in young women, aged 14–21 years. The incidence of this cancer in women whose mothers had been administered DES during pregnancy (►DES daughters) is estimated to range from 1.4 cases per 1,000 exposed to one case per 10,000 exposed persons (►Cervical cancer). Previously, clear-cell adenocarcinoma of the vagina and cervix had been observed only rarely, primarily in post-menopausal women (over age 50) not exposed to DES. Consequently, the U.S. Food and Drug Administration issued a drug bulletin recognizing DES as a ►transplacental carcinogen, and banned its use during pregnancy (►Carcinogen). Since then, DES exposure has been observed to cause a range of teratogenic and neoplastic changes in humans and animals. It is used now mainly for treatment of a small subset of hormonally responsive refractory cancers. However, DES exposure can serve as a model for evaluating the potential effects of xenoestrogens (►Hormonal carcinogenesis; ►estrogenic hormones). Therefore, its investigation remains important and should not be limited to the study of the consequences of a specific, unintentionally deleterious administration.

Animal Studies

DES has been studied extensively in animal models. In the Syrian hamster model, exposure to DES induces neoplasms of the liver and kidney, as well as aneuploidy (particularly chromosome gains) in the renal neoplasms (►Aneuploidy, ►chromosome instability). DES exposure in rats elicits tumors of the reproductive tract, pituitary and mammary glands. In addition, Green and colleagues have observed that DES metabolites produce DNA adducts (►Adducts to DNA) and, ultimately, cancer in the breast of female ACI rats. Tumors of the reproductive tract and mammary glands are seen in the murine model as well, along with alterations in the genetic pathways governing uterine differentiation. Data from Newbold and colleagues suggest that an increased susceptibility to tumor formation is transmitted along the maternal lineage to subsequent generations, both male and female. Thus, not only is the developing organism sensitive to the endocrine-disrupting chemical, but transgenerational effects are plausible as well.

Neoplastic Effects in Humans

The only increased risk of hormone-dependent cancers observed in women who took DES during pregnancy (►DES mothers) is ►breast cancer (Table 1). Hatch and colleagues found that DES mothers had a 30% increased rate of breast cancer compared to the general population. In contrast, DES daughters have an increased risk of two types of hormone-dependent cancers, clear cell adenocarcinoma of the vagina and cervix (mentioned above) and breast cancer (►Breast cancer). Clear cell adenocarcinoma of the vagina and cervix generally presents in these women when they are in their teens and twenties; however, because some women have been diagnosed in their thirties and forties, concerns have arisen about whether

Diethylstilbestrol. Table 1 Effects of diethylstilbestrol exposure

	Non-neoplastic effects	Neoplastic effects
DES Mothers	Increased risk of • Adverse pregnancy outcomes	Increased risk of • Breast cancer
DES Daughters	Increased risk of • Adverse pregnancy outcomes • Infertility • Reproductive tract structural abnormalities • Vaginal adenosis	Increased risk of • Clear-cell adenocarcinoma of vagina and cervix • Breast cancer over age 40
DES Sons	Increased risk of • Epididymal cysts • Cryptorchidism • Testicular hypoplasia • Semen and sperm abnormalities	No increased risk of hormone-related cancers

another increase in risk will occur as DES daughters approach the age at which this type of cancer is seen in the general population (the postmenopausal period).

Until recently, there was a paucity of information on breast effects in DES daughters. However, the National Cancer Institute's Continuation of Follow-Up of DES-Exposed Cohorts has proven a rich source of information to study the long-term effects of *in utero* estrogen exposure in humans. These cohorts include over 4,000 women who had documented *in utero* exposure to DES and more than 2,000 unexposed women from the same record sources; all have been followed from 1994 or earlier. As women exposed *in utero* to DES have begun to reach the ages at which breast cancer is more common, it appears that these women have an increased risk. In the most recent data from the National Cancer Institute collaborative follow-up study of DES health effects (Continuation of Follow-Up of DES-Exposed Cohorts), Palmer and colleagues found that at ages 40 and older, DES daughters had double the risk of breast cancer of unexposed women; no association was seen prior to age 40.

To date, males exposed *in utero* to DES (►DES sons) do not exhibit an increase risk of developing hormone-related cancers. However, DES sons do display non-neoplastic abnormalities (see below) that place then at increased risk of developing testicular cancer regardless of DES exposure (►Testicular cancer).

Non-Neoplastic Effects in Humans

In utero exposure to DES has been shown to elicit a variety of non-neoplastic reproductive tract abnormalities, including structural cervical, vaginal, or uterine abnormalities, in addition to changes in the vaginal epithelium such as adenosis (see Table 1). DES dosage and the stage of pregnancy during which the drug was administered appears to be directly linked to the severity of the adenosis, with the most severe manifestations seen in DES daughters whose mothers took the drug during their first trimesters. It is unclear whether these areas of adenosis progress to vaginal clear cell adenocarcinoma. DES daughters have an increased risk of poor pregnancy outcomes, such as ectopic pregnancy or miscarriage, and also have a higher incidence of infertility than the general population.

DES sons are more likely than unexposed men to exhibit genital abnormalities such as epididymal cysts, cryptorchidism, and testicular hypoplasia. Although DES sons show an increased incidence of semen and sperm abnormalities, they have not demonstrated an increased risk of infertility, but this is still under investigation.

Mechanism of Toxicity

The mechanism by which estrogens in general, and DES in particular, exert their toxic and carcinogenic effects is not fully understood. Both proliferative and genotoxic mechanisms have been postulated. Classically, estrogen exerts its effects through interaction with the estrogen receptor α (ERα), which stimulates cell proliferation and inhibits apoptosis (►Estrogen receptor). ERα may also interact with other receptors (such as ERβ, insulin-like growth factor 1 receptor and epidermal growth factor receptor) to influence proliferation, or may act through non-genomic pathways, since it is found in non-nuclear subcellular fractions such as the plasma membrane and the mitochondria. An alternative or additional mechanism that may mediate estrogen's and perhaps DES' toxic effects is through the metabolites' genotoxic capacity. ►Estrogens, and specifically DES, can be oxidatively metabolized into potentially genotoxic intermediates. Estrogen and its metabolites have been reported to induce DNA damage (►DNA damage), manifesting as ►allele imbalance and ►DNA amplification in human breast epithelial cells *in vitro* (►Amplification). DES metabolites have been reported to produce DNA adducts and cancer in mammary glands of female rats. DES is a strong mitotic inhibitor in cell lines, blocking equatorial plate formation, tubulin polymerization, and spindle assembly. This may, in turn, induce ►aneuploidy. Tumors associated with DES exposure exhibit ►genetic instability, such as whole and partial chromosome gains *in vitro* and *in vivo*, in the Syrian hamster model. ►Microsatellite instability has been reported in human vaginal clear cell adenocarcinomas associated with *in utero* DES exposure, as well as in murine endometrial carcinomas after DES treatment.

In contrast to the substantial microsatellite instability seen in human vaginal clear cell adenocarcinomas associated with *in utero* DES exposure, breast neoplasms in DES daughters do not exhibit an increased amount of microsatellite instability. Breast tumors of DES mothers have not been investigated. In fact, little microsatellite instability was observed in breast tumors in both exposed and unexposed women, which is consistent with previous results from unselected human breast cancers, confirming that microsatellite instability is unusual in human breast cancers and suggesting that prenatal DES exposure does not affect ►DNA mismatch repair mechanisms in the breast. Similarly, equivalent amounts of allele imbalance have been observed in breast tissue regardless of exposure, which differs from findings in animal models and *in vitro* systems. Therefore, the effect of *in utero* DES exposure may be tissue, timing and/or species specific, as is the case with other hormonal agents such as ►tamoxifen, which has variable effects on human endometrium and mammary tissue. It remains under investigation as to whether the potential effects of *in utero* DES exposure on human breast carcinogenesis

are mediated by enhanced proliferation, by alternative genotoxic effects, or by other pathways entirely.

Summary

The unfortunate consequences of DES administration to pregnant women have yielded clinical and scientific insights into estrogen's effects on developing and mature tissues. Its continued investigation should provide additional clinical and mechanistic information about these effects, and have relevance to understanding the effects of exposure to xenoestrogens.

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Diferuloylmethane

►Curcumin

Differentiation

Definition

A process whereby a cell undergoes morphological transition from a cell which is capable of undergoing cellular division to a state where the cell becomes post-replicative. Often accompanied by changes in cellular function. The process during which young, immature (unspecialized) cells take on individual characteristics and reach their mature (specialized) form and function. Describes the process by which cells acquire a "type or assignment." The morphology of a cell may change dramatically during differentiation, but the genetic material remains the same, with few exceptions.

Diffuse, Small Cleaved Cell Lymphoma

►Mantle Cell Lymphoma

Diffuse Large B-Cell Lymphoma

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Synonyms

KIEL classification: Centroblastic, B-immunoblastic, B-large cell anaplastic; Working Formulation: Diffuse large cell, Large cell immunoblastic, Diffuse mixed small and large

Definition

Diffuse large B-cell lymphoma is a ►non-Hodgkin lymphoma entity composed of malignant large lymphoid cells with blastic morphologic features, expression of B-cell markers, and with a diffuse growth pattern. The postulated cells of origin are germinal or post germinal centre B-cells. This lymphoma entity is morphologically, clinically and genetically heterogeneous.

Characteristics

Diffuse large B-cell lymphoma is the most common type of lymphoma comprising 30–40% of adult non-Hodgkin lymphomas and approximately 20% of non-Hodgkin lymphomas in childhood and adolescence. Diffuse large B-cell lymphoma can be seen in all age groups, but the incidence increases with age. The median age at diagnosis is approximately 65 years. There is a slight male preponderance. In most patients the tumor resides in lymph nodes, but 40% of patients have predominant extranodal disease. Virtually any extranodal site may be involved, but the most frequently involved organs include the gastrointestinal tract, soft tissue, thyroid, skin, central nervous system, liver, bone, gonads, breast, kidney, lung and salivary glands. So-called transformed diffuse large B-cell

lymphomas arise from indolent lymphomas such as small lymphocytic lymphoma/►chronic lymphocytic leukaemia, ►marginal zone B-cell lymphoma and ►follicular lymphoma. The aetiology of most diffuse large B-cell lymphoma cases is unclear. However, patients with immunodeficiency such as human immunodeficiency virus-infected patients or patients receiving immunosuppressive therapy are at increased risk of developing lymphoma. Diffuse large B-cell lymphoma is the most frequent lymphoma arising in this setting, and these lymphomas are often ►EBV associated.

Diagnosis

The typical clinical presentation of diffuse large B-cell lymphoma patients with nodal disease is rapidly enlarging ►lymphadenopathy. Patients with extranodal presentation of the disease often have symptoms related to dysfunction of the involved organ(s). One third of the patients have ►B symptoms. Approximately half of the patients have localized lymphoma, i.e. Ann Arbor stage I or II, and the remainder have disseminated disease.

The morphological diagnosis is based on the World Health Organization Classification. Diffuse large B-cell lymphoma typically consists of a diffuse proliferation of medium-sized to large transformed B-lymphoid cells with a nucleus at least twice the size a normal lymphocyte. These large cells are a mixture of cells that resemble either the centroblasts or the immunoblasts that normally reside in reactive germinal centres. The diffuse large B-cell lymphoma entity is morphologically quite heterogeneous with several morphologic variants. The two most common variants are the centroblastic variant which is dominated by centroblasts and the immunoblastic variant with >90% immunoblasts. In the T-cell/histiocyte rich variant the majority of cells are small T-cells and histiocytes and less than 10% of the cells are neoplastic B-cells. An anaplastic variant of diffuse large B-cell lymphoma is recognized which has a similar morphology and ►CD30 expression as the T-cell lymphoma anaplastic large cell lymphoma. However, anaplastic diffuse large B-cell lymphoma is clinically and genetically unrelated to anaplastic large cell lymphoma. Other rare variants include plasmablastic diffuse large B-cell lymphoma and diffuse large B-cell lymphoma with expression of full-length *ALK*.

Diffuse large B-cell lymphoma cells usually express CD45 and B-lymphoid markers such as CD19, CD20, CD22, CD79a and PAX5. The proliferation rate is high with most cases expressing the proliferation associated marker ►Ki-67 in >40% of the tumor cells. In some tumors >95% of the malignant cells express Ki-67.

The prognosis is variable with a 5-year overall survival rate for all patients of 45–50%. The International Prognostic Index is widely used for prognostication of diffuse large B-cell lymphoma. It consists of five clinical factors (age, stage, performance score, serum lactate dehydrogenase, number of extranodal sites involved) each with independent prognostic value regarding overall survival. The index allocates 35–40% of the patients to the low risk group with a 5-year overall survival rate of >70%, while 15–20% of the patients have high risk lymphoma and a 5-year overall survival rate of <30%. By adding the anti-►CD20 antibody ►rituximab to the treatment regimens the survival rates cited above may increase with up to 10% or more.

Therapy

Multidrug chemotherapy and radiotherapy represent the mainstay of diffuse large B-cell lymphoma treatment. For several decades CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or CHOP-like regimens have been the gold standard chemotherapy regimens with the achievement of complete remission rates of 70–80% and cure rates of 40–50%. Recent trials have documented that the addition of rituximab to CHOP or CHOP-like regimens significantly increases event-free and overall survival for most diffuse large B-cell lymphoma patients. The treatment strategy is influenced by the age and the International Prognostic Index risk group of the patient, as well as the location and stage of the disease. Patients with non-►bulky stage I lymphoma may be treated with 3 series of CHOP (-like) chemotherapy and radiotherapy of the involved field and possibly with the addition of rituximab. Patients with higher stage disease are typically treated with 6–8 series of CHOP(-like) chemotherapy with the addition of rituximab. The role of autologous stem cell transplantation is not well defined, but may be beneficial to younger patients with high risk disease who are unlikely to do well with standard therapy.

Approximately half of the diffuse large B-cell lymphoma patients either do not attain complete remission or develop a relapse after remission and most of these patients require second-line therapy. A variety of second-line chemotherapy regimens are used, and these regimens typically have a response rate of 50–70%. For patients with chemotherapy-sensitive relapse autologous stem cell transplantation is considered the treatment of choice.

Genetics

The cellular origin of diffuse large B-cell lymphoma is germinal or post germinal centre B-cells. Two major pathogenic mechanisms related to normal germinal centre function contribute to the lymphomagenesis of diffuse large B-cell lymphoma: aberrant ►somatic hypermutation and ►chromosomal translocation often involving the ►immunoglobulin genes. Virtually all diffuse large B-cell lymphoma cases have rearranged

immunoglobulin genes, and most cases are somatically hypermutated in the immunoglobulin variable region genes.

Somatic hypermutation is a physiologic process in the ►germinal center that target immunoglobulin genes and thereby creates antibody diversity. However, aberrant somatic hypermutation is considered an important transformation mechanism in diffuse large B-cell lymphoma. In most diffuse large B-cell lymphoma cases aberrant somatic hypermutation targeting non-immunoglobulin genes results in hypermutation of multiple proto-oncogenes such as ►*MYC*. This process is thought to contribute to the clinical and biological heterogeneity of diffuse large B-cell lymphoma.

Another important mechanism involved in the pathogenesis of diffuse large B-cell lymphoma is chromosomal translocations bringing a proto-oncogene under the influence of an active locus such as the immunoglobulin gene, and thereby causing deregulated overexpression of the oncogene. These translocations may occur as (accidental) by-products of the immunoglobulin remodeling processes, i.e. ►V(D)J recombination, somatic hypermutation and ►class switching, which take place in the germinal centres and require DNA strand breaks. The t(14;18) brings the anti-apoptotic ►*BCL2* gene in juxtaposition with the joining segment of the immunoglobulin heavy chain gene and is a hallmark of follicular lymphoma. Twenty to thirty percent of diffuse large B-cell lymphomas also carry this translocation, and these cases probably evolved from clinical or subclinical follicular lymphoma. The presence of the *BCL2* rearrangement is associated with disseminated and nodal disease but not with prognosis or expression of the bcl-2 protein. In 10% of the cases bcl-2 overexpression is achieved by way of gene ►amplification. Approximately one third of diffuse large B-cell lymphoma cases have a rearrangement of the proto-oncogene ►*BCL6*. Deregulation of bcl-6 expression in germinal centre cells may contribute to lymphomagenesis by functional inactivation of p53 and thereby suppressing p53-mediated ►apoptosis. *MYC* is rearranged, often as a translocation to an immunoglobulin gene, in 10–15% of diffuse large B-cell lymphoma. Cases with rearrangement of both *MYC* and *BCL2* appear to have a very poor prognosis.

Other frequent genetic lesions include ►*TP53* mutations in 20–25% and ►*CD95* mutations in 20% of the tumors. ►*TP53* mutations are associated with poor response to treatment and poor prognosis, whereas ►*CD95* mutations correlate with extranodal disease and autoimmune phenomena.

Gene expression profiling studies with ►microarray technology have identified two major subgroups of diffuse large B-cell lymphoma. One type has an expression profile that resembles normal germinal centre B-cells (GCB-like), and the profile of the other group is similar to activated B-cells (ABC-like). In addition to having expression profiles with hallmarks related to B-cells of different stages of differentiation, these two subgroups of diffuse large B-cell lymphoma also utilize distinct oncogenic mechanisms. For example, *BCL2* is dysregulated by way of the t(14;18) in up to half of the GCB-like cases, but this translocation is not found ABC-like cases. In stead, as an alternative mechanism of *BCL2* activation, amplification of the *BCL2*-containing chromosome region 18q21 is present in 20% of ABC-like tumors, but it is rare in GCB-like cases. Furthermore, 20–25% of ABC-like cases have gain on chromosome 3q and/or trisomy 3, whereas these lesions are not identified in GCB-like lymphomas. Moreover, a major difference is that ABC-like diffuse large B-cell lymphoma has a constitutively active ►NF-κB pathway which is a potentially important therapeutic target. Finally, these molecular differences translate into clinical differences, as GCB-like diffuse large B-cell lymphoma has a better prognosis than ABC-like.

Subtypes of Diffuse large B-cell Lymphoma

From the heterogeneous group of diffuse large B-cell lymphoma several subtypes have sufficiently distinct clinical, morphological and/or genetic features to be recognized as entities.

Mediastinal (thymic) Large B-Cell Lymphoma

Mediastinal large B-cell lymphoma presumably arises from thymic B-cells. These cases comprise up to 10% of all diffuse large B-cell lymphomas. It is a disease with a female predominance. The patients are younger, in general in the third to fifth decade. Typically the lymphoma presents at diagnosis as a bulky and locally invasive anterior mediastinal mass which often causes airway compression and vena cava superior syndrome. In contrast to other diffuse large B-cell lymphoma types, mediastinal large B-cell lymphomas seldom harbor rearrangements of *BCL2*, *BCL6* or *MYC*. Mediastinal large B-cell lymphoma has a gene expression profile distinct from ABC-like and GCB-like diffuse large B-cell lymphoma. However, in common with ABC-like diffuse large B-cell lymphomas mediastinal large B-cell lymphomas have a constitutively active ►NF-κB pathway.

Intravascular Large B-Cell Lymphoma

Intravascular large B-cell lymphoma is a rare neoplasia characterized by proliferation of large CD20-positive lymphoid cells primarily in the lumina of small vessels. The disease is often widespread at diagnosis causing a highly variable clinical picture. The symptoms are mainly caused by occlusion of capillaries in the involved organs. The prognosis is very poor.

Primary Effusion Lymphoma

►Primary effusion lymphomas occur most often in immunocompromised patients and the outcome is extremely poor. Patients usually present with serous effusions without overt tumor masses. Characteristically the lymphomas cells are pleomorphic and lack B-cell-associated antigens such as CD19 and CD20. Instead they often express CD30 and the plasma cell-associated marker CD138. Reflecting the aetiology the lymphoma cells contain ►Kaposi sarcoma herpes virus/human herpes virus 8.

Lymphomatoid Granulomatosis

Lymphomatoid granulomatosis is a rare ►Epstein-Barr virus-associated angiocentric lymphoproliferative disease involving extranodal tissues, most commonly the lung. The lesions are composed of large EBV-positive B-cells in a background dominated by small reactive T-cells. Based on the proportion of EBV-positive B-cells lymphomatoid granulomatosis is graded from grade I (few cells) to grade III (numerous cells). Grade III lymphomatoid granulomatosis fulfil the morphological criteria of a diffuse large B-cell lymphoma subtype and should be treated as such.

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Diffuse Neuroendocrine System

Definition

Consists of a network of specialized cells (neuroendocrine cells), distributed in a variety of organs that release hormones in response to stimulus from the nervous system to control different body functions.

►Neuroendocrine Carcinoma
►Neuroendocrine Tumors

Diffusion

Definition

Is a passive transport mechanism that results in the dispersion of an agent, relying largely on a concentration gradient.

►Convection Enhanced Delivery (CED)

Diffusional Flux

Definition

Is the amount of a substance or gas diffusing from one location to another.

►Oxygenation of Tumors

2′,2′-Difluoro-2′-deoxycytidine

►Gemcitabine

Difluoromethylornithine

Definition

DFMO; An oral agent that irreversibly binds to ►ornithine decarboxylase and inhibits its function, resulting in polyamine inhibition and decreased tumorigenesis in model systems. As such, DFMO has been widely used in ►chemoprevention research as an active cancer preventive agent, with tolerable safety profile.

►Arginine

Dihydrogen Dioxide

►Hydrogen Peroxide

Dihydrotestosterone Receptor

►Androgen Receptor

1,25-Dihydroxyvitamin D_3

►Calcitriol

2-Dimensional Gel Replicon Mapping

Definition

A physical method in which two different concentrations of agarose are used to separate origins of replication from replication forks. ►Origins of replication have a lower mass than replication forks and therefore migrate faster in low concentrations of agarose. However, in higher concentrations of agarose replication origins migrate at a slower rate than replication forks because of their complex structure. Thus, the 2-dimensional gel mapping technique is capable of distinguishing replication origins from ►replication forks.

►S-Phase Damage-Sensing Checkpoints

Dimer Formation

Definition

In biochemistry and molecular biology, dimers of macromolecules like proteins are frequently observed. The dimerization of identical protein subunits is called homodimerization, the dimerization of different subunits or unrelated monomers is called heterodimerization. Most dimers in biochemistry are not connected by covalent bonds with the exception of disulfide bridges. An example of this would be the enzyme ►reverse transcriptase, which is made of two different amino acid chains.

Dimesna

Definition

►Mesna is rapidly oxidized to its biologically inert disulfide metabolite, mesna disulfide or dimesna.

►Chemoprotectants

Dimethyl Benz(a)anthracene

Definition

DMBA; Is a strong ►carcinogen used only in laboratory settings that has not been found in the environment. It induces primarily mammary gland tumors in animal models.

►Sulforaphane

7, 12 Dimethylbenz[a]anthracene

Definition

DMBA; A polycyclic aromatic hydrocarbon chemical that acts to initiate mutations in codons 12, 13, 59, and 61 of the v-Ha-*ras* oncogene in skin.

►Skin Carcinogenesis

Dimethylfumarate

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Definition

Fumaric acid is one of two isomeric unsaturated dicarboxylic acids with the formula $HO_2CCH{=}CHCO_2H$

(the other being maleic acid with carboxylic acid groups in cis). Reactions with alcohol create fumaric acid esters (FAE) and water.

Chemistry and Pharmacodynamics

Dimethylfumarate (DMF) is a lipophilic ester of fumaric acid (Fumaric acid is a dicarbonic acid and a component of the intracellular citric acid cycle). DMF is rapidly hydrolyzed to methylhydrogenfumarate (►MHF) in aqueous solutions at physiological pH.

In both, DMF and MHF, nucleophiles can be added to the double bonds in the center of the molecule by a so called ►Michaelis-type addition. Thus, they interact with ►thiols, as given in the tripeptide ►glutathione (►GSH) or with cystein residues in larger proteins. This reactivity of DMF is higher than that of MHF (Fig. 1).

Characteristics

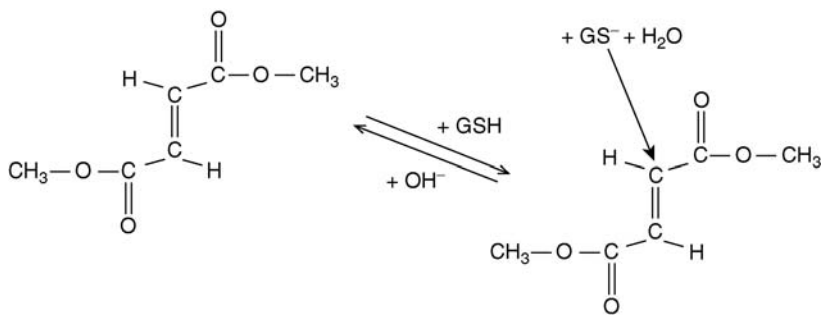
Biological Effects of FAEs In vitro

Due to the chemical structure of ►FAEs as low-molecular weight electrophiles, much work has focused on their antioxidant effects. FAEs directly react with GSH. They initially deplete intracellular GSH levels, which return to normal or even supra-normal levels 6–18 h thereafter. Possibly as a consequence of this GSH-FAE interaction, FAEs induce transcription and activity of so-called phase 2 enzymes, which are important in the intermediate cellular metabolism for ►detoxification. Induction of Phase 2 enzymes, as e.g., DT-diaphorase (NQO1 = NAD(P)H:quinone oxidoreductase I) and cytochrome b5 reductase, enhance cellular metabolism and decrease sensitivity towards environmental toxins. Phase 2 enzymes generally share similar antioxidant or electrophilic response elements (ARE/EpREs) within their promotor sequences. Interestingly, the induction of phase 2 enzymes by DMF is unevenly distributed within different tissues. In astrocytes, DMF leads to the induction of genes containing ARE/EpREs within their promotor sequences. Also in the gut and to a lesser degree in the liver, DMF increases activity of phase 2 enzymes, whereas in prostate tissue, no phase 2 enzymes are induced by DMF. Recently it has been shown that DMF induces heme oxygenase 1 (HO-1) in human peripheral blood mononuclear cells. The induction of HO-1 expression by DMF was paralleled by the DMF-induced depletion of intracellular GSH, but a direct proof for a causal connection is pending.

A second approach towards identification of FAE effects was based on the clinical observation that FAEs reduce tumor necrosis factor (TNF)-induced cell activation. This effect of FAEs was identified in various different cells such as endothelial cells, fibroblasts or ►melanoma cells. FAEs exert these effects by interacting with the ►nuclear factor-κB (NF-κB) pathway downstream the Iκ-kinases (IKK), they retain activated ►NF-κB proteins in the cytosol and prevent their ►nuclear translocation. As a result, NF-κB cannot bind to DNA thereby preventing NF-κB-mediated gene transcription. In these experiments, DMF proved superior to MHF. Although the mode of this inhibition is not yet completely elucidated, it has been speculated that this is also mediated through interaction of FAEs with the redox status of the cell.

Drug Formulations of Currently Used Fumaric Acid Esters (FAEs)

1. Fumaderm®, a mixture of FAEs (120 mg DMF, 87 mg Ca^{2+} Ethylhydrogenfumarate, 5 mg Mg^{2+} Ethylhydrogenfumarate, 3 mg Zn^{2+} Etyhlhydrogenfumarate) was registered in Germany in 1994. It is used for oral treatment of psoriasis, a chronic inflammatory skin condition. Off-label use revealed anecdotic efficacy in other inflammatory skin conditions such as granuloma anulare, cutaneous sarcoidosis or necrobiosis lipoidica. Moreover, Fumapharm has initiated a phase IIa clinical trial



Dimethylfumarate. Figure 1 Reaction between DMF and thiols (modified from Schmidt et al. [5]).

with Fumaderm® for the treatment of multiple sclerosis. This study showed a significant reduction of gadolinium-enhancing lesions on T1-weighted magnetic resonance imaging (MRI) brain scans. This study could also show that exacerbations of the disease could be reduced by low-dose FAE therapy.
2. DMF as a monosubstance, named BG12®, was used for a European placebo-controlled multicenter phase III trial in psoriasis patients. In this study, treatment with BG12 for 16 weeks led to clinical response and improvement in 68% of the BG12 treated patients, whereas placebo treated patients improved only for 10%. Biogen has now initiated a phase III study in patients with multiple sclerosis using BG12.

In dendritic cells and keratinocytes, FAEs possess anti-proliferative and pro-apoptotic properties. It is yet not clear whether this is based on the ability of FAEs to alter the redox status of the cell and/or by a direct interaction with the NF-kB pathway.

In malignancies *in vitro*, effects of DMF have been evaluated in ►lung cancer, ►breast cancer, ►neuroblastoma and ►glioblastoma. DMF has been shown to increase cytotoxicity of chemotherapeutics when given in combination with for example ►mitomycin C or streptonigrin. This was shown for glioblastoma, lung cancer and mammary cancer cell lines. DMF also sensitizes fibroblasts, neuroblastoma cells as well as human ►bladder cancer cell lines to irradiation (and therefore increases their ►radiation sensitivity.

Biological Effects of FAEs In vivo

Anti-inflammatory effects of oral treatment with Fumaderm® and BG12 in psoriasis are well documented in human patients. Although DMF is more active than MHF *in vitro*, it is still unclear, whether DMF or ►MHF mediate biological effects *in vivo*. This is because (i) DMF hydrolyses into MHF and (ii) pharmacokinetic studies failed to detect DMF in human plasma, whereas MHF was measurable. However, these studies did not rule out that DMF was bound to cystein-containing plasma proteins or – due to its lipophilia – has rapidly entered cells and thus escaped detection by HPLC techniques.

For malignant disease, only preclinical data in animal models exist. DMF has been tested in a model of chemically induced ►colon cancer, where DMF reduced cancer ►progression and ►invasion. In humanized ►SCID mouse models for ►melanoma, oral treatment with DMF has reduced tumor growth and ►lymphogenic metastatic spread. In this model, beneficial effects of DMF were based on its anti-proliferative and pro-apoptotic properties on melanoma cells.

Clinical Aspects and Future Perspectives

In many human malignancies the NF-κB pathway is constitutive active. This causes increased expression of many ►tumor progression genes (regulating cell cycle and apoptosis of tumor cells and the composition of the ►tumor matrix). Indeed, animal models have provided a direct link between activation of NF-κB and tumor progression. Moreover, NF-κB-dependent genes are clearly involved in the development of ►chemo-resistance; inhibition of NF-κB has been shown to reduce chemoresistance.

DMF fulfills several criteria to justify clinical trials in human malignancies; first, it has been shown to be beneficial in animal models for malignant diseases; second, it inhibits NF-κB-dependent gene expression, which would reduce tumor growth and ►metastasis and also may reduce the development of chemo-resistance; third DMF has been clearly shown to be safe even in long term treatment in human psoriasis patients.

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Dioxin

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Synonyms

TCDD; 2,3,7,8-TCDD; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Definition

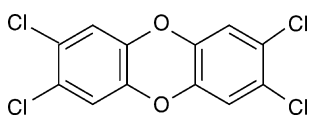
Dioxin is an unwanted by-product of a number of chemical and industrial processes. It was first observed as a contaminant formed in the synthesis of trichlorophenols used for the production of certain herbicides. Later it was recognized that small amounts of dioxin (as well as other dioxins) can be produced during different types of combustion processes including the burning of chlorine-containing materials such as chemical and hospital wastes, and sewage sludge. Dioxin may also be formed during manufacturing processes utilizing chlorine. These include the bleaching of pulp and paper, and chlorine-dependent regeneration of metal catalysts. Dioxin and numerous dioxin-like chemicals are ubiquitously present in trace amounts in the environment (Fig. 1).

Characteristics

Based on studies in experimental animals, dioxin is considered to be one of the most potent tumorigenic agents known. The results of epidemiologic studies are more equivocal in terms of carcinogenic potency in humans. These and other responses elicited by dioxin occur through a unique mechanism involving binding to a transcription factor of otherwise unknown function, and subsequent modulation of gene expression. The exact relationships between particular gene alterations and ultimate carcinogenic responses to dioxin remain obscure. However, several putative functions of the transcription factor and endogenous ►ligands, whose action dioxin likely impedes, offer novel targets for future drug discovery and therapeutic intervention.

Carcinogenic Activity

Dioxin is considered to be one of the most toxic synthetic compounds known with acute lethal doses in several animal species being in the range of μg/kg body weight. In addition, numerous experimental studies have consistently shown dioxin to be a potent carcinogen. Although there are differences in sensitivity among species, some rodents exhibit significantly increased and dose-dependent tumor incidence with average daily exposures of 100 ng/kg body weight and higher. A variety of tumor types in several tissues including liver, lung, and thyroid have been reported. The findings that in most experimental systems dioxin fails to induce mutations, dioxin-derived ►adducts to DNA have not been detected, and dioxin enhances the incidence of various tumor types following initiation with known ►carcinogens, are consistent with dioxin acting primarily, if not exclusively, as a tumor promoter. However, although there is little or no evidence for dioxin to be directly genotoxic, it may appear to be a complete carcinogen due to its exceptionally high potency as a tumor promoter.

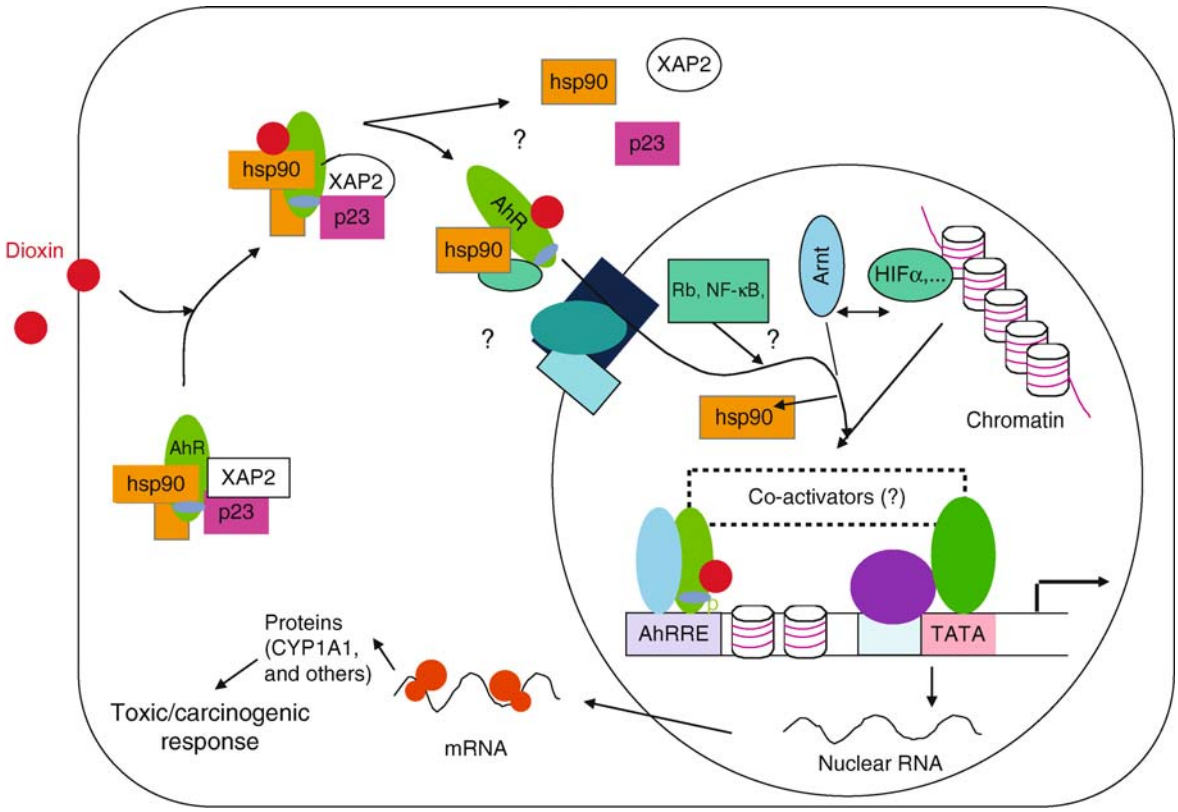


Dioxin. Figure 1 Chemical structure of dioxin.

Mechanisms

The toxic and carcinogenic effects of dioxin are mediated by its binding to and activation of a transcription factor termed the ►aryl hydrocarbon receptor (AhR). This belongs to a family of proteins containing a basic-helix-loop-helix (bHLH) – PAS (Per-Arnt-Sim) ►domain structure. The known ►bHLH-PAS proteins are involved in regulating responses to signals in the tissue environment such as oxygen tension and circadian rhythms, and serve regulatory roles in development and cellular differentiation. Like many of these proteins, the AhR has been conserved through evolution. However, unlike most other proteins in this family, the AhR is ligand-activated and its exact normal function in tissue processes is not clearly resolved. Evidence that the AhR mediates most, if not all, biological responses to dioxin is based in part on studies examining structure-activity relationships of other dioxin-like chemicals, and genetic studies in mice that have a mutant AhR protein. In particular, mice in which expression of a functional AhR has been "knocked out" (►knock-out mice) are insensitive to the toxic effects of dioxin. In the absence of ligand, the AhR appears to be localized primarily in the cellular cytosol complexed with several proteins including the ►chaperone protein ►hsp90, co-chaperone ►p23 protein, and an ►immunophilin-like protein XAP2 (Fig. 2). Upon ligand binding, the AhR is translocated to the cell nucleus, dimerizes with another bHLH-PAS protein, Arnt (also known as ►hypoxia inducible factor (HIF) β) and binds to specific ►response elements (AhR response elements; AhREs) (also called xenobiotic response elements (XREs) or dioxin response elements (DREs)) within regulatory domains of responsive genes to modulate the expression of these genes. The AhR has been shown to interact with several proteins involved in other signaling pathways. These include the ►estrogen receptor and ►NF-κB. As such, some responses elicited by dioxin may not be dependent on the ability of the AhR-Arnt complex to bind AhREs. Nevertheless, the toxic and carcinogenic actions of dioxin are believed to result from its interference with unknown endogenous AhR ligands and/or the inappropriate and prolonged alteration of AhR-responsive genes and signaling pathways.

There are several postulated mechanisms whereby dioxin may act at stages involved in cancer initiation and progression to facilitate the development of malignancies. Of the identified AhR-responsive genes,



Dioxin. Figure 2 AhR signaling pathway. Dioxin binds to the AhR to initiate a series of events leading to modulation of gene expression.

many of these encode enzymes (e.g. ►cytochrome P450 (CYP) 1A1 and 1B1) are known to metabolize and activate carcinogens to intermediates that cause DNA mutations. Although dioxin is resistant to metabolism, the induction of these pathways enhances the likelihood of bioactivation of other potential carcinogens that are in the environment. It has also been proposed that induction of the CYPs may generate ►reactive oxygen species that can directly damage DNA. Several studies have observed dioxin exposure to result in the expression of genes such as ►*p53* and ►*p21Waf1* indicative of DNA damage or cellular stress. Other studies have linked high CYP1A1 enzyme activity with increased cancer risk, and several carcinogens, including benzo[a]pyrene, induce tumors in normal mice but not AhR knock out mice. Increased expression of these genes by dioxin is also known to affect tissue levels of several hormones and growth factors, including estrogen and thyroxine, as well as their receptors. The progression of several tumors is known to be hormone dependent. Dioxin has been shown to alter several signaling pathways regulating cellular differentiation, proliferation and cell death (►apoptosis). Furthermore the modulation of these cellular processes, intrinsic to metastatic progression, are affected in a variety of cell types by dioxin. In particular, many studies demonstrate a role, although as yet not clearly defined, of the AhR in regulating the cell cycle. This may occur through several complex pathways involving AhR-dependent activation of cellular kinases, e.g. ERK, or direct AhR interaction with cell cycle regulatory proteins including the tumor suppressor ►retinoblastoma protein (Rb). The latter interaction has been suggested to regulate key cell cycle components mediated by an alteration of ►E2F transcription factors.

Immune surveillance mechanisms are important in the control of malignant cell growth. In experimental animals, dioxin has consistently been shown to be a potent immunosuppressive agent affecting both humoral and cell-mediated immune pathways. Furthermore, dioxin exposure has been shown to increase tumor cell survival following engraftment. Prolonged inflammation is also associated with increased cancer development, and the AhR is known to interact with NF-kB proteins that are involved in regulating ►inflammatory responses. In addition, several pro-inflammatory genes (e.g. ►cyclooxygenase-2) are AhR-responsive. There is also recent evidence that the AhR may be involved in the regulation of ►angiogenesis important for tumor growth; AhR

knockout animals exhibit defects in tissue vascularization. This has been postulated to be related to cross-talk between the AhR and HIF signaling pathways, since Arnt is also a dimeric partner for HIFα. Ultimately, the exact mechanisms underlying the carcinogenic activity of dioxin are poorly understood. As indicated above, there are likely many complex, and possibly tissue-specific, mechanisms whereby dioxin exhibits potent tumor promoter activity. Clearly, however, the multi-faceted nature of the responses to dioxin reflect an importance of the AhR signaling pathway in fundamental processes, deregulation of which may contribute to altered cellular phenotype and uncontrolled growth.

Clinical Relevance

Several epidemiologic studies link dioxin exposure with cancer in human populations. Among the increased cancers reported were lung cancer, ►lymphomas, and ►leukemias. However, in all of these studies the body burden exposure levels were very high, being often greater than several hundred μg/kg body weight. In many studies the increased cancer incidence was relatively small and the association, although significant, was weak. Based on limited human evidence and sufficient evidence in animals, the International Agency for Research on Cancer (IARC) and the National Toxicology Program classified dioxin as a human carcinogen. Human exposures to dioxin and dioxin-like chemicals occur mainly through food consumption. Average current background body burden levels for most human populations are approximately 10 ng dioxin-eqivalents/kg body weight, and many fold lower than that observed in animal investigations or human epidemiologic studies in which a positive association with increased cancer incidence was observed. As such, the actual risk for cancer development at a low level exposure remains uncertain. Several studies indicate that the human AhR has lower affinity for dioxin than the AhR in most animal species. Identified human AhR ►genetic polymorphisms have not yet been clearly associated with increased or decreased cancer risk. Nevertheless, the ►half-life of dioxin, a molecule resistant to metabolism, appears to be considerably longer in humans, ranging from 7 to 10 years. Furthermore, a variety of human cells and tissues respond to dioxin exposure in a manner similar to those of animals, at least in terms of some acute biochemical and cellular responses (e.g. *CYP1A1* induction and altered cellular differentiation). The full clinical significance of dioxin exposure may not be realized until we have a greater understanding of the physiological role of the AhR. This role and any putative endogenous ligands regulating AhR activity have yet to be clearly defined. The findings that dioxin exposure and/or altered activity of the AhR are associated with direct modulation of particular ►gene batteries resulting in altered immune responses, modulated angiogenesis, as well as deregulated cell cycle and altered cellular proliferation and differentiation, may offer new and unique targets for drug discovery and therapeutic intervention for the treatment of various cancers.

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Dioxin Receptor

►Aryl Hydrocarbon Receptor

Dipeptidyl Peptidase IV

►CD26/DPPIV in Cancer Progression and Spread

Diphtheria Toxin

Definition

Toxin produced by Corynebacterium diphtheriae that inactivates eEF-2 and inhibit protein translocation during eukaryotic protein synthesis.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Diploid

Definition
The full set of chromosomes consisting of one chromosome from each parental set, in a somatic cell; in humans, 46 chromosomes (22 pairs of autosomes and two sex chromosomes).

►Genomic Imbalance

Diplopia

Definition
Objective double vision.

Dipropyl-acetic Acid

►Valproic Acid

Direct Repeats

Definition
A sequence of DNA that is repeated in the same orientation.

►Repetitive DNA
►Chromosomal Translocations

Direct-Acting Carcinogen

Definition
A chemical that acts as the ultimate carcinogen without metabolic activation.

►Toxicological Carcinogenesis

Directed Migration

►Chemoattraction

Directed Motility

►Chemoattraction

DISC

Definition
►Death-inducing signaling complex, which is formed upon activation of death receptors through ligation with their ligands. The DISC consists of death receptors, an adaptor protein, initiator caspases and other proteins and is essential for death receptor-induced ►apoptosis.

►FLICE Inhibitory Protein
►Death-Inducing Signaling Complex (DISC)

Disintegrin Metalloproteases

►ADAM Molecules

Disordered Domain

►Intrinsically Unstructured Proteins

Disposition

Definition
Refers to both the distribution of a drug over the body and its elimination.

Distamycin-A

Definition
An oligopeptide antibiotic that binds to AT-rich regions of DNA.

►Fragile Sites

Distribution

Definition
Is the term describing how a chemical distributes among various compartments in the body.

►ADMET Screen

Disulfide

Definition
A bond between two sulfur atoms. In proteins, such bonds are formed by the oxidation of a ►dithiol motif.

►Thioredoxin System

Dithiol

Definition
A motif with two thiol (-SH) groups. In ►oxidoreductases, dithiols are usually formed from the side chains of two cysteine residues positioned close to each other in the three-dimensional structure.

►Thioredoxin System

dJ889N15.2

►Gankyrin

DKFZ

Definition
Deutsches Krebsforschungszentrum; German Cancer Research Center Prolific Cancer Research Institution in Germany. http://www.dkfz.de/index.html.

DKK

Definition
Dickkopf; family of secreted proteins that normally downregulate Wnt/β-catenin signaling by binding to LRP5/6 Wnt receptors; in most cases downregulated in cancer.

►Wnt Signaling

DLBCL

Definition
►Diffuse large B-cell lymphoma.

►BCL6 Translocations in B-cell Tumors

DLC1

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Definition
DLC1 is a ►tumor suppressor gene frequently underexpressed in various types of cancers. Genomic deletion and ►promoter hypermethylation may account for the inactivation of *DLC1* in human cancers. Functionally, overexpression of *DLC1* can result in the ►tumor suppression in cancer cells and regulation of cytoskeleton organization.

Characteristics

Deleted in liver cancer 1 (*DLC1*), also known as *Rho-*►*GTPase activating protein 7* (*ARHGAP7*) and *START domain-containing protein* (*STARD12*), is a putative tumor suppressor gene identified from primary ►hepatocellular carcinoma (HCC) in 1998. Human *DLC1* gene was mapped to chromosome region 8p21.3–22. The full-length *DLC1* mRNA is 6 kb long and consists of 14 exons which encode a protein of 1,091 amino acids. In-silico analysis indicated that *DLC1* shares 86% sequence homology with rat *p122 RhoGAP* and is likely to be its human homolog. DLC1 protein contains three major functional domains, namely, SAM (sterile alpha motif), RhoGAP (GTPase-activating protein for Rho-like GTPases), and START (steroidogenic acute regulatory (STAR)-related lipid transfer) (Fig. 1). The RhoGAP domain is a characteristic of RhoGAP family proteins, functions to catalyze the intrinsic GTPase activity of Rho proteins.

Functions of DLC1

RhoGAP protein converts the active GTP-bound Rho to the inactive GDP-bound form. On the other hand, guanine nucleotide exchange factor (GEF) acts to activate Rho proteins. Rho proteins are important regulators in the remodeling of ►actin cytoskeleton, regulation of transcription, cell proliferation, metastasis, and tumorigenesis (Fig. 2). The best characterized members of Rho proteins are RhoA, Rac1, and Cdc42. RhoGAP serves tumor suppressor function by downregulating Rho GTPase activity. Therefore, negative regulation of Rho proteins by RhoGAP activity contributes to the functional role of DLC1.

Negative Regulation of Cytoskeleton

The in vitro GAP activities of DLC1 specific for RhoA and Cdc42 have previously been demonstrated. In cell model, expression of DLC1 resulted in inhibition of stress fiber formation and extensive cell rounding. These morphological changes were similar to the effects of Rho inhibitor, C3 exoenzyme, implicating that DLC1 negatively regulates cytoskeletal organization via inhibition of Rho proteins. The importance of RhoGAP of DLC1 was further supported by failure of DLC1 RhoGAP mutant to induce morphological changes. ►Tensin2, a focal adhesion protein has been identified as the first and novel binding partner of DLC1. DLC1–tensin2 protein complex localizes in ►caveolae where Rho GTPases are concentrated. It is postulated that DLC1–tensin2 complex brings DLC1 in close proximity to Rho GTPases which are enriched in caveolae and facilitates the inactivation of Rho proteins by DLC1.

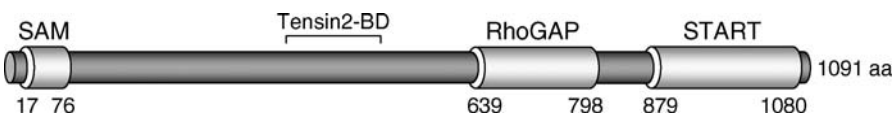
Mouse model of DLC1 revealed that $DLC1^{+/-}$ heterozygous deletion mutant did not display any phenotypic abnormalities, while $DLC1^{-/-}$ homozygous deletion mutant (►knock-out mice) was embryonic lethal. $DLC1^{-/-}$ embryos showed defects in several organs, including neural tube, brain, heart and placenta. Fibroblasts isolated from $DLC1^{-/-}$ ►homozygous deletion embryos displayed aberrant cytoskeletal organization with diminished actin stress fiber formation and focal adhesions.

Tumor Suppressive Effect

Besides the role in the regulation of cytoskeletal reorganization, DLC1 also possesses growth inhibitory activity in cancer cell lines. The growth inhibitory effect of DLC1 was first demonstrated in HCC cell lines. Loss of growth suppressive effect with the DLC1 RhoGAP mutant demonstrated that the RhoGAP activity of DLC1 was associated with its growth suppressive effect in tumor cell lines. Expression of *DLC1* in HCC cells resulted in significant inhibition in cell proliferation, anchorage independent growth, and in vivo tumorigenicity in ►nude mice. Apart from HCC cells, the tumor suppressive effect of DLC1 has also been demonstrated in breast cancer and non-small cell lung carcinoma cells. Moreover, stable expression of *DLC1* resulted in inhibition of migratory and invasive abilities of HCC and breast cancer cells. Furthermore, expression of *DLC1* in HCC cells induced caspase 3-mediated apoptosis. The tumor suppressive role of *DLC1* has also been implicated in genome-wide expression profiling approach. Transcriptional profiling analysis of breast cancer cells that exhibit different metastatic efficiencies using microarrays identified *DLC1* as one of the differentially expressed genes. Functional analysis has further demonstrated that *DLC1* acts as a metastasis suppressor in breast cancer cells.

Clinical Relevance

DLC1 was first discovered from a subtractive hybridization screening study in primary HCC. Recent studies have indicated that *DLC1* possesses tumor suppressing



DLC1. Figure 1 Schematic representation of DLC1 protein. Structural domains of DLC1, namely, sterile alpha motif (SAM), Rho GTPase activating protein (RhoGAP), steroidogenic acute regulatory (StAR)-related lipid transfer (START) and the tensin2 binding domain (*Tensin2-BD*) are shown.

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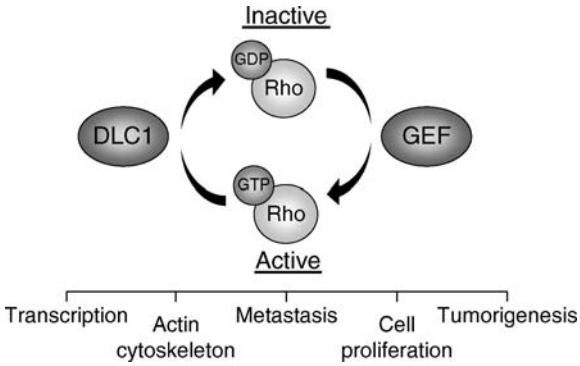

DLC1. Figure 2 Functional role of DLC1 protein. Rho proteins exist as active GTP-bound form and inactive GDP-bound form. RhoGAP functions to convert the active Rho to the inactive state. On the other hand, guanine nucleotide exchange factor (GEF) acts to activate Rho proteins. Rho proteins are important regulators in the remodeling of actin cytoskeleton, regulation of transcription, cell proliferation, metastasis, and tumorigenesis. Negative regulation of Rho proteins by RhoGAP activity may contribute to the functional role of DLC1.

function and is implicated in human ►carcinogenesis. *DLC1* mRNA is frequently underexpressed in various human cancers, particularly in non-small cell lung carcinoma and nasopharyngeal carcinoma, and down-regulation of *DLC1* mRNA expression has been observed in more than 90% of primary tumor samples. The inactivation of *DLC1* in human cancers could be due to both genetic and epigenetic alterations.

Genetic Alteration

DLC1 is mapped at chromosome 8p21.3–22, which is one of the most commonly deleted regions in human cancers. As indicated by its name, *DLC1* gene is frequently deleted in human HCC. ►Loss of heterozygosity and ►single nucleotide polymorphism (SNP) analyses have revealed that hemizygous deletion of *DLC1* gene is present in approximately half of primary HCC and almost 90% of head and neck squamous cell carcinomas. Although not at a high frequency, homozygous deletion has also been reported in human HCC and ►medulloblastoma. Somatic mutation is rarely found in *DLC1* gene and SNP on *DLC1* also shows no association with human cancers.

Epigenetic Silencing

In established cancer cell lines, frequent loss of *DLC1* gene expression, even in the absence of gene deletion, has been observed. In these cancer cell lines, *DLC1* expression can be significantly restored by administrating demethylating drugs or ►histone deacetylase inhibitors to the cells. This indicates that in addition to gene deletion, epigenetic alterations including ►DNA methylation and ►histone deacetylation also play important roles in *DLC1* inactivation. In all human cancer types tested, recurrent DNA ►methylation on the promoter region of *DLC1* gene has been detected in the primary cancer samples, and *DLC1* ►promoter methylation is closely associated with loss of gene expression. In ►cervical cancer, ►nasopharyngeal carcinoma and ►multiple myeloma, DNA methylation accounts for more than 80% of *DLC1* gene inactivation in these diseases. Interestingly, *DLC1* promoter methylation has also been found in 71% of benign prostatic hyperplasia and was closely associated with the serum prostate-specific antigen level. These findings suggest that *DLC1* promoter methylation may occur in early stage of carcinogenesis and has a potential value for early diagnosis of ►prostate cancer.

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DLT

Definition

In clinical drug trials, ►dose-limiting toxicity.

DM

Definition

Double minute; dmin.

►Amplification

D-MAPP

Definition
D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol, a ►ceramide analog that inhibits alkaline/neutral ceramidase.

►Sphingolipid Metabolism

DMBA

Definition
Dimethylbenz(a)anthracene, a chemical ►carcinogen, extensively used in animal models of carcinogenesis. Polycyclic aromatic hydrocarbon with carcinogenic, tumorigenic and teratogenic effects.

►Polycyclic Aromatic Hydrocarbons

DMBA/TPA Mouse Skin Carcinogenesis Model

Definition
The model shows tumor progression from normal skin to benign papillomas and invasive tumors. ►DBMA (7,12-dimethylbenzanthracene) acts as a tumor initiator and ►TPA (12-O-tetradecanoylphorbol-13-acetate) as the promotor.

DMBT1

Definition
Deleted in Malignant Brain tumors 1 (DMBT1) is a polymorph gene located on chromosome 10q26-13 that belongs to the scavenger receptor cysteine-rich (SRCR) superfamily and potentially represents a molecular link between infection, ►inflammation, regeneration and cancer.

DNA

Definition
Deoxyribonucleic acid (DNA) is the nucleic acid that is located in the cell nucleus, encodes developmental and functional information and which represents the hereditary genetic make-up of an individual.

DNA Adducts

Definition
►Adducts to DNA are covalent binding products formed between a chemical and DNA.

►Biomarkers
►DNA Damage
►Tobacco Carcinogenesis
►Tobacco-Related Cancers
►Toxicological Carcinogenesis

DNA Amplification

Definition
An increase in the copy number of DNA. Copy number can be increased over a relatively limited area (in which case the areas are called amplicons).

►Amplification
►Neuroblastoma

DNA Aptamers

Definition
DNA molecule, typically 20-30 nucleotides long, which after folding obtains a 3D structure and binds to other molecules with high affinity.

►Aptamer

DNA-Binding Domain

Definition

DBDs; Are regions of proteins that interact with DNA.

►p53 Family

DNA-bound Carcinogens

►Adducts to DNA

DNA Damage

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Synonyms

DNA lesion or adduct

Definition

DNA damage refers to the myriad of chemical or structural perturbations that can affect the function of genes encoded along the DNA macromolecule of the cell. The continuous exposure of all cells to many types of DNA damaging agents can alter the backbone of the DNA double helix or the ►nucleotide base components that connect the two strands together and make up the four-lettered genetic code – guanine (G), cytosine (C), thymine (T) and adenine (A). Un-repaired or inappropriately repaired DNA damage can therefore alter genetic information controlling cell function, resulting in mutation, ►chromosomal instability or ►aneuploidy – the hallmarks of all cancers.

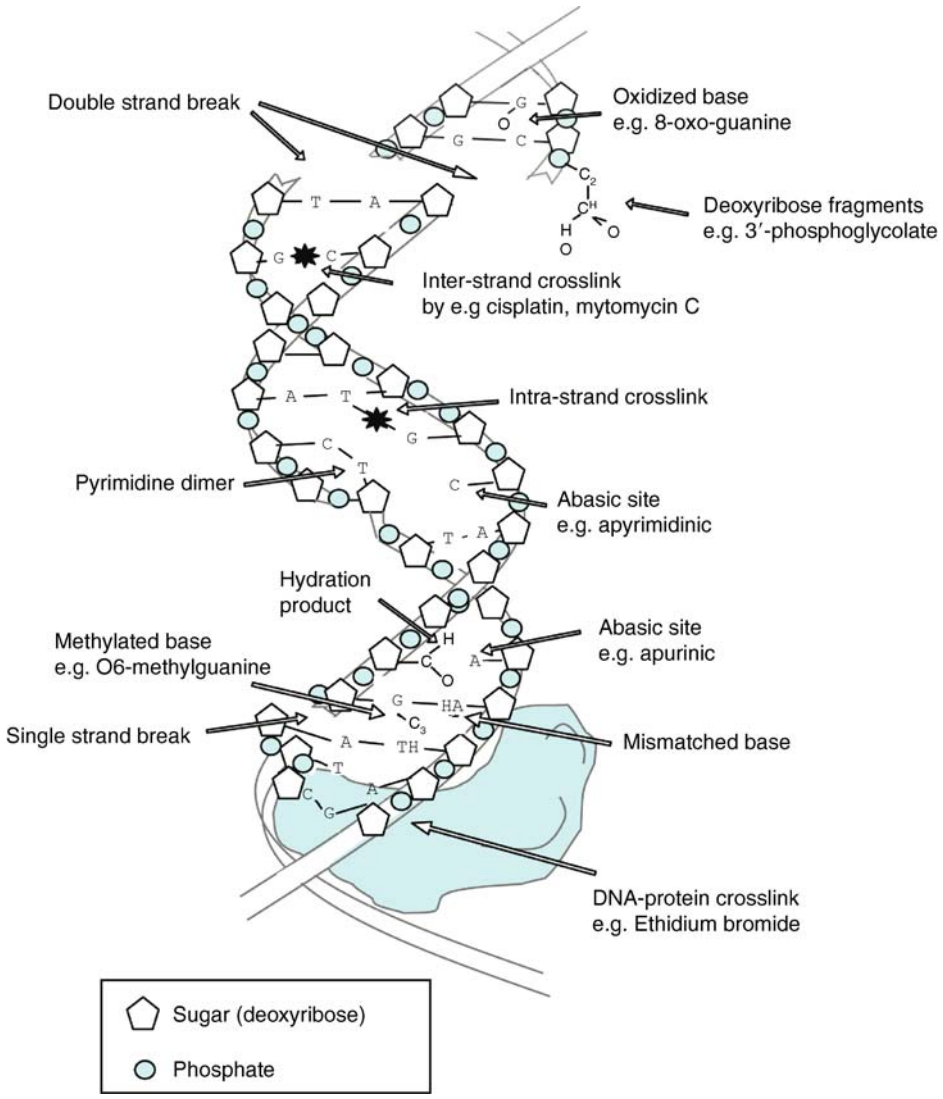
Characteristics

DNA resides in both the nucleus and mitochondria of human cells and both cellular regions contain significant levels of potentially damaging agents, derived either from the environment (exogenous) or from metabolic processes continually occurring inside the cell (endogenous). Although DNA is heavily protected by a complex packaging arrangement of supercoiled strands, wrapped around ►histone proteins within protective chromosomal structures, it is estimated that anything from 1,000 to 1,000,000 DNA lesions occur in any given cell per day. Because genomic DNA inside the nucleus contains the genetic code for expressing most of the proteins required for normal cell function, un-repaired or incorrectly repaired damage to nuclear DNA threatens the integrity of the genome and can result in disruption of cellular processes that control cellular growth and differentiation and therefore the development of cancer. DNA damage and subsequent mutagenesis in somatic cells also drives the processes of ageing, degenerative diseases and evolution.

Constant exposures to a barrage of environmental and cellular metabolic DNA damaging agents creates enormous selective pressures on all prokaryotic and eukaryotic cells on Earth, from bacteria to human, to evolve a complex protective array of DNA damage sensing, signaling and repair proteins. Subsequently, most of these proteins are conserved from single cell organisms to humans. These enzymes and molecular scaffolds constantly interact to scan, recognize, alert and repair many forms of DNA damage. They represent crucial cellular defense pathways that not only protect the integrity of the genome of a single cell but coupled to abortive programmed cell death (or ►apoptosis) pathways, allow whole organisms to clear themselves of problematic and potentially malignant tissue. This is triggered when levels of DNA damage pass a certain threshold and this defines the repair capacity of a cell. This can vary from cell to cell and tissue to tissue and some DNA repair pathways are more error-prone and less efficient than others. Some repair pathways may become dysfunctional or deregulated and indirectly promote ►carcinogenesis, due largely to the activities of inappropriate cellular responses rather than the damage itself. Not surprisingly, individuals who inherit defects or mutations in specific DNA damage sensing and/or repair proteins are predisposed to and significantly more likely to develop early onset cancers, premature ageing and degenerative disorders. There are many types of DNA damage and these are dealt with by several pathways of DNA repair that specialize in recognizing and repairing particular types of lesion.

Agents Causing Bulky, Structurally Distorting DNA Adducts

1. ►UV radiation – predominantly UV-B and UV-C, with UV-B representing the wavelength from ►solar ultraviolet light that can penetrate the Earth's ozone layer. This form of radiation reacts with DNA to form 6,4-photoproducts and cyclobutane pyrimidine dimers (CPDs) (Fig. 1). Chronic exposure of skin cells to solar radiation dramatically increases the cellular load of these adducts and increase the risk of ►skin cancers such as ►malignant melanoma.



DNA Damage. Figure 1 Examples of DNA damage.

An illustration showing examples of structural and chemical alterations to the deoxyribose backbone and the nucleotide bases in between the two strands of DNA. Chemical cross-links are indicated by bold asterisk and a large protein-DNA interaction is shown as a blue globular protein distorting the nucleotide structure.

2. ►Polycyclic aromatic hydrocarbons (PAHs) – atmospheric pollutants found in smoke, soot and tar. The adducts formed by PAH predominantly result in the conversion of G to T (called transversions) (Fig. 1). This results in the mutagenic transformation of affected genes and is responsible for the majority of smoke-related ►lung cancer. ►Tobacco cigarette smoke contains significant amounts of PAHs but also contain extremely high concentrations of acrolein that is one of many ►carcinogens also stimulating G to T transversions. Cigarette smoke is also known to oxidize bases (see Base Damage in next section).
3. Antibiotics such as ►Mytomycin C and ►Adriamycin – These can induce DNA-protein and DNA–DNA inter/intrastrand cross-links (Fig. 1) that covalently join together the nucleotide bases on one/both strands of DNA, respectively.
4. Genotoxic chemotherapeutic drugs such as ►Cisplatin and ►Camptothecin. These are also classed as DNA crosslinking agents and their cytotoxic properties are exploited by oncologists to kill tumor cells such as testicular carcinomas. ►Cisplatin can also induce intrastrand crosslinks (Fig. 1).

Bulky adducts are typically removed by the ►nucleotide excision repair (NER) system. This removes a stretch of DNA harboring the lesion (typically about 30 bases long) and replaces it with the correct sequence of nucleotide bases. Examples of NER enzymes are XPA, XPG and TFIIH. Individuals with genetic defects that affect the function of these components are at a higher risk of developing cancers associated with ►xeroderma pigmentosum.

Nucleotide Base Damage

Many environmental as well as endogenous factors can induce smaller chemical alterations to the DNA sequence-encoding bases – G, C, T and A. Although they are small alterations in comparison, they can have dramatic effects upon the integrity of the genetic sequence encoded along the particular stretch of DNA that harbors such damage. Examples include:

1. ►*Oxidative DNA damage*. The most common form of base damage that can stimulate the conversion of e.g. guanine to ►8-oxoguanine (Fig. 1). Oxidizing agents such as ►reactive oxygen species (ROS) are frequently produced during normal cellular metabolic processes and these are responsible for a significant proportion of base oxidation. ROS are present in the nucleus and cytoplasm and especially inside the mitochondria where energy production from ATP hydrolysis produces high levels. Subsequently, mitochondrial DNA is particularly vulnerable to such damage. Chemicals such as ►hydrogen peroxide (H_2O_2) can also oxidize nucleotide bases and represent powerful DNA damaging agents. Vinyl chloride and chromium (►chromium carcinogenesis) represent industrial chemicals that damage DNA by inducing, among others, etheno-►DNA adducts and base oxidization, respectively. Occupational health hazards such as these are well documented to cause mutations and cancer among exposed workers. Anti-oxidants such as circulating uric acid, the enzyme superoxide dismutase, and those provided in many types of food e.g. ►vitamin C (ascorbic acid) and ►curcumin, work to absorb the free radicals that can oxidize DNA and are generally considered to be important agents that protect cells from cancer-causing oxidants.
2. ►*DNA alkylating agents and* ►*DNA methylation agents*. These can stimulate the conversion of e.g. cytosine to O_6-methylcytosine by adding a methyl group ($–CH_3$) (Fig. 1). DNA alkylating agents like MNNG and 6-TG have been developed as chemotherapeutic agents to kill proliferating tumor cells by damaging their DNA. Unrepaired methylated bases are therefore very toxic to cells.
3. *Mismatched bases*. Unrepaired methylated lesions are also subject to secondary repair by the ►mismatch repair system that normally functions to replace the occasional mismatched base (Fig. 1) with its correct partner (A with T and C with G) (defects associated with ►microsatellite instability, ►colorectal cancer and ►Lynch Syndrome). In doing so, the repair system can become "fooled" by the structural change exerted by ►methylation and, if not sent round a futile cycle of toxic repair, can promote the fixation of potentially carcinogenic mutations.

The ►base excision repair (BER) system is responsible for recognizing and replacing many types of altered bases and there are several components involved. Examples include the APE-1 endonuclease and Nth1 glycosylase enzymes that excise apurinic and apyrimidinic bases and Ogg1, MUTHY and MTH1 that recognize and replace oxidized bases such as 8-oxoguanine.

Single and Double Strand Breaks

DNA damaging agents such as ionizing radiation (X-rays, α and β particles) and free radicals can physically break the covalent bonds between the phosphates, ribose and bases and result in single and double strand DNA breaks (SSB and DSB) (Fig. 1). SSBs are recognized and processed efficiently by dedicated SSB repair proteins such as PARP-1 (►poly (ADP-ribose) polymerase-1), and XRCC1 (which also functions in BER). DSBs are by far the most toxic form of DNA damage and one unrepaired DSB inside a cell is capable of killing that cell. Almost immediately after a DSB forms i.e. after exposure to ionizing radiation, a particular variant of the histones, ►H2AX, which coats the DNA along the entire genome becomes phosphorylated up- (5′-) and down-stream (3′-) of a DSB, some million base pair distances away, thus amplifying the signal and initiating a global cellular response. Other histone modifications are now being linked to DNA damage responses in what seems to be a histone code of responses.

DSBs represent highly reactive substrates for DNA end repair proteins. These are components of the non-homologous end-joining and (NHEJ) and ►homologous recombination ►repair systems. Defects in the protein components of these DSB repair pathways predispose individuals to a range of neurodegenerative disorders and many forms of cancer, e.g. ►ATM (ataxia telangiectasia), ►BLM (►Bloom Syndrome) Ligase IV, MRE11, NBS (lymphoma, ►Nijmegen Breakage Syndrome, ►multiple myeloma), ►BRCA1, ►BRCA2, Fanc D (►Fanconi anemia, ►breast cancer). DSBs are therefore potentially extremely mutagenic lesions, capable of translocating and rearranging gene sequences that can give rise to cancer. A classic

example of malignancy resulting from ►chromosomal translocation and rearrangement is ►Burkitt lymphoma.

The deoxyribose sugars at the ends of SSB and DSBs (3′ or 5′, depending on the direction of the gene transcript) often exist in fragmented forms, particularly if the breaks result from free-radical attack. Examples of these are phosphoglycans. These have to be removed by specific repair enzymes before SSB and DSB repair can proceed. This extensive end-processing can result in loss of base sequence information, loss of heterozygocity (LOH) and cancer-causing mutations if not tightly regulated.

SSBs and DSBs are also actively induced during normal cellular processes such as ►V(D)J recombination and cell division but the way in which these DSBs are discriminated from *bona fide* DNA damage is not fully understood. DSBs can also result from the collapse of regions undergoing DNA ►replication called replication forks Collapse can result from pre-existing DNA lesions that interrupt the DNA replication process.

Furthermore, existing DSBs in the genome represent easy target sites where foreign DNA, such as invading viral DNA, can insert, integrate and rearrange genetic sequences. These random, sequence-independent events are called insertional mutagenic events and can be extremely carcinogenic. Indeed, this process was responsible for the unfortunate development of leukemia in a significant proportion of X-SCID patients who underwent ►gene therapy clinical trials to correct their immunodeficiency. The corrective gene was incorrectly inserted into a region close to the ►oncogene LMO-2 and resulted in uncontrolled proliferation. Metnase is a protein recently identified which promotes NHEJ and the random integration of foreign DNA into human cells.

Cancers Associated with Failure to Signal DNA Damage

A cell requires intricate signaling proteins that alert the presence of DNA damage and activate the appropriate responses. For example, if a cell does not repair DNA damage before it is replicated during cell division, daughter cells will inherit DNA with mutations around that region and the next generation or progeny of cells will be defective. Therefore, appropriate responses such as DNA replication ►checkpoints, or ►cell cycle arrest pathways must be activated to prevent permanent alterations from being passed on. If this is not activated then damaged cells can lose control of proliferation and lead to cancer. Key regulators of this response are the ►p53, ►STRAP, ►MDM2 and Rb (►retinoblastoma protein) that lie at the interface between cell cycle arrest and cell death responses.

Mutations or expression defects in ►p53 are associated with most human cancers, demonstrating the importance of this key responsive network in suppressing cancer development. Prolonged suppression of this response has long been known to contribute to ►tumor progression. It has also been confirmed that, in mice engineered to have p53 expression under inducible control, restoring its function dramatically reduces the size of sarcomas and lymphomas. Furthermore, a whole range of p53 post-translational modifications and down-stream cofactors now seem to govern cell fate after exposure to various DNA damaging agents.

Therapeutic Approaches

The DNA of cancer cells remains one of the most effective targets to damage and destroy cancerous tissue in patients. Traditional DNA damaging ►chemotherapy and ►radiotherapy approaches have served cancer patients very well over the past four to five decades. However, long-term survival rates under such management across the cancer spectrum have not improved that much over the same period of time, clearly defining the need for more advanced techniques.

As we learn more about how cells respond to such damage, it is not surprising that genes such as p53 have been singled out as potentially powerful targets to treat human cancers. Restoring the functions of tumor suppressors such as p53 and suppressing oncogenes and DNA repair proteins that can promote carcinogenesis and cancer cell survival, respectively, are all validated approaches to kill tumor cells and are under current investigation.

►Gene therapy holds great promise for treating all genetically tractable diseases including many cancers. Identifying individuals with DNA repair polymorphisms or mutations and replacing or enhancing the expression of wild-type protein may help restore appropriate responses to DNA damage and protect against cancer development. Furthermore, randomly positioned DNA DSB sites in the genome represent preferential regions where viruses insert their genomes and integrate. Inhibiting DNA repair pathways that promote this random integration may suppress viral infection and reduce the chances of insertional mutagenesis that has so far plagued the promising use of ►viral vector-mediated gene transfer for gene therapy in clinical trials.

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DNA Damage Responses

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Definition

Damage to DNA invokes several cellular responses that enable cells either to eliminate the damage or activate programmed cell death. These responses include activation of ►checkpoints to arrest progression through the ►cell cycle; removal or repair of damaged DNA to prevent the transmission of damaged chromosomes to daughter cells; induction of genes required for DNA repair, cell cycle arrest, and ►apoptosis; and apoptosis to eliminate cells too seriously damaged or deregulated to repair.

Characteristics

Sources of Damage

Endogenous attack by ►reactive oxygen species can induce several types of damage to DNA bases, including oxidation and the generation of DNA strand interruptions; alkylation, depurination, and depyrimidination; and wrongly mismatched bases coupled to a new DNA strand. Exogenous damage is caused by a variety of external agents. ►Ultraviolet light from the sun causes crosslinking between adjacent thymine bases, creating pyrimidine dimers. Ionizing radiation from X-rays and gamma rays results in breaks in the DNA double strands. DNA intercalating compounds create a huge variety of DNA adducts.

Checkpoint Pathway

►Checkpoint responses trigger cell cycle arrest, providing time to repair damaged chromosomes before they enter ►mitosis, thus maintaining genomic integrity. There are five major components of this system. First are checkpoint sensors which include the Rad9–Hus1–Rad1 (9-1-1) complex, the Rad17–RFC clamp loading complex, and the ►Mre11–►Rad50–►Nbs1 (MRN) complex. Second, checkpoint mediators include ►BRCA1, MDC1, 53BP1 and Claspin. Third are the apical signal transducing kinases including ►ATM and ►ATR kinases. Fourth, distal signal transducing kinases are represented by ►Chk1 and ►Chk2. Finally, there are checkpoint effectors encompassing cell cycle regulators such as the ►cdc25 phosphatases, various DNA repair proteins, transcription factors ►p53 or ►E2F1, and chromatin components such as histone ►H2AX (Fig. 1).

G1 Checkpoint

To prevent a cell with damaged DNA from entering the S phase, cells activate checkpoint transducing kinases ATM/ATR and Chk1/Chk2, which target Cdc25A and p53 at the G1 checkpoint. When DNA is damaged, p53 is phosphorylated at Ser15 and Ser20 in its transactivation domain. This stabilizes p53 by inhibiting its interaction with ►Mdm2, thus stabilizing p53. The key transcriptional target of p53 is p21 which inhibits cyclin E-cdk2 activity and prevents the phosphorylation of Rb by cyclin D-cdk4. This sequence of events results in suppression of the Rb/E2F pathway, prolonging transition from G1 to S. The phosphorylation of cdc25A by Chk kinases creates binding sites on cdc25A with ►14-3-3 proteins and excludes them from the nucleus. Lack of active cdc25A in the nucleus results in the accumulation of inactive ►cdk4. The inactive molecule is incapable of loading cdc45 onto chromatin, a step required for initiation of replication origin firing.

Intra-S-phase Checkpoint

When a double-strand break or DNA nick occurs during the S phase, the intra-S checkpoint, involving ATM, the ►MRN complex, and BRCA1, is activated. The ATM-Chk2-Cdc25A-Cdk2 pathway is responsible for the checkpoint response. In contrast, when DNA is damaged by ►uv radiation or chemicals that create bulky base lesions, the main damage sensor is the ATR-ATRIP complex. Activated ATR phosphorylates Chk1, which in turn phosphorylates and down-regulates cdc25A, and thus inhibiting replication origin firing.

G2/M Checkpoint

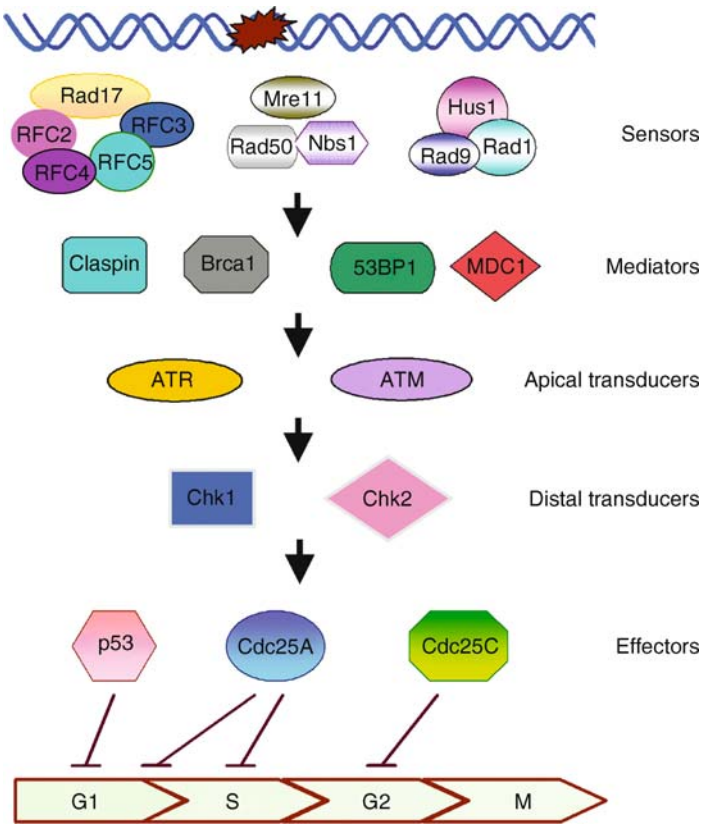
The key downstream target of the ►G2/M checkpoint is the mitosis-promoting activity of the cyclin B/Cdk1 kinase. After DNA damage, ATM/ATR and Chk1/Chk2 kinases downregulate cdc25A and cdc25C. Upregulated Wee1 and downregulated cdc25 collaboratively inhibit the activity of cyclin B/Cdk1 kinase at the G2/M boundary. Again, the phosphorylated cdc25A binds 14-3-3 proteins and is sequestered in the cytoplasm, promoting degradation by the ►ubiquitin-proteasome pathway. Y15-phosphorylated cdk1 accumulates, and ►mitosis is arrested.

DNA Repair Responses

DNA damage alters the spatial structure of the helix, and such alterations can be detected by the cell. Once damage is located, specific ►DNA repair molecules are recruited to the site, inducing other molecules to bind and form a complex that enables actual repair to take place.

Excision Repair

►Base excision repair corrects damage to a single nucleotide. In this process, DNA N-glycosylases



DNA Damage Responses. Figure 1 Signal transduction of the DNA checkpoint responses in human cells. The DNA damage is detected by sensors that, with the aid of mediators, transmit the signal to transducers. The transducers activate or inactivate effectors that directly participate in inhibiting the G1/S, S, or the G2/M transition.

recognize oxidized, alkylated, or deaminated bases and catalyze the hydrolytic removal of the mutated bases. Lesions are then repaired by DNA Pol β/δ/ε, DNA ligase III, ►PCNA, and FEN1. Nucleotide excision repair amends longer strands of 2–30 damaged bases. This process, involving RPA, XPA, and XPC, recognizes bulky, helix-distorting changes such as thymidine dimers as well as single-strand breaks. TFIIH, XPG, and XPF-ERCC1 are recruited to the site to form an incision complex. The gap is filled by DNA Pol δ/ε with the aid of replication accessory proteins PCNA and RFC. ►Mismatch repair fixes errors of DNA replication and recombination that result in mispaired nucleotides.

Double Strand Break Repair

Double strand breaks are produced by ►reactive oxygen species, ►ionizing radiation, and certain antineoplastic chemicals. Two mechanisms exist to repair this damage. The nonhomologous end-joining pathway directly joins the two ends of the broken DNA strands without a template. The Ku heterodimer binds to the two ends of a double-strand break and recruits DNA-PKcs and the ligaseIV-XRCC4 heterodimer to ligate the two duplex termini. The second mechanism, ►homologous recombination repair, requires an identical or a nearly identical sequence as a template. This pathway allows a damaged chromosome to be repaired using the newly created ►sister chromatid as a template. A key intermediate in this pathway is the Holliday junction in which the two recombining duplexes are joined covalently by single-strand crossovers.

Translesion Synthesis Repair

►Translesion synthesis is an error-prone method of DNA repair. This pathway is mediated by DNA polymerases that insert extra bases at the site of damage and thus allow replication to bypass the damaged base.

Interstrand DNA Crosslink Repair

Patients with ►Fanconi anemia have a predisposition to cancer. Their cells are hypersensitive to agents that cause interstrand cross-links. Repair of this type of damage is poorly understood but may involve excision repair and recombination.

Arrest, Repair, or Die?

p53-Dependent Mechanisms

A key determinant as to whether cells with DNA damage live or die is ►p53. ATM phosphorylates p53 at Ser15 and ►MDM2 at Ser395, while ►Chk1 and ►Chk2 phosphorylate p53 at Ser20. These modifications prevent the p53-MDM2 association that targets p53 for proteolysis, thereby allowing increments in cellular levels of p53. The consequences of p53 activation include transcriptional activation of DNA repair activities, cell cycle inhibitors (p21 and 14-3-3σ), and pro-apoptotic factors (►PUMA, ►FAS/APO-1/CD95, and ►Apaf-1).

p53-Independent Mechanisms

One possible mechanism for p53-independent apoptosis involves E2F1, under the control of Chk1- and Chk2-mediated activation. The transcriptional targets of E2F1 include proteins associated with DNA repair (BRCA1, Msh2, Msh6, RFC, and PCNA), cell cycle checkpoints (ARF and Chk1), and apoptosis (Caspase 7, Apaf1). Thus, like p53, E2F1 may determine whether cells live or die.

Cancer Susceptibility

Loss of ►ATM strongly predisposes humans to ►lymphoma and, to a lesser degree, to other malignancies. Patients with mutations in ►NBS1 or ►MRE11, for example, are predisposed to develop cancer. ►Seckel syndrome, in which there is a low level of ATR expression, involves ►chromosome instability after ►mitomycin C exposure. Inherited mutations in one allele of p53 and Chk2 are found in families with the extremely cancer-prone ►Li-Fraumeni syndrome. The inheritance of a single mutated allele of either ►BRCA1 or ►BRCA2 markedly increases the incidence of breast and ovarian cancers in women.

Nucleotide excision repair, homologous recombination, and translesion synthesis are all thought to be defective in ►Fanconi anemia cells. Nucleotide excision repair is defective in ►xeroderma pigmentosum and ►Cockayne syndrome, while ►mismatch repair malfunctions in children with ►Turcot syndrome and in tumor cells derived from ►hereditary nonpolyposis colorectal cancers (HNPCC). Tranlesion synthesis repair is defective in patients with a variant of xeroderma pigmentosum.

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DNA-Damage Tolerance

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Synonyms

DNA lesion bypass; Replicative DNA lesion bypass; Postreplication repair

Definition

DNA-damage tolerance is a biological mechanism in response to DNA damage which overcomes arrested DNA replication, as a result of unrepaired DNA damage, leading to elimination of its potential lethal effects.

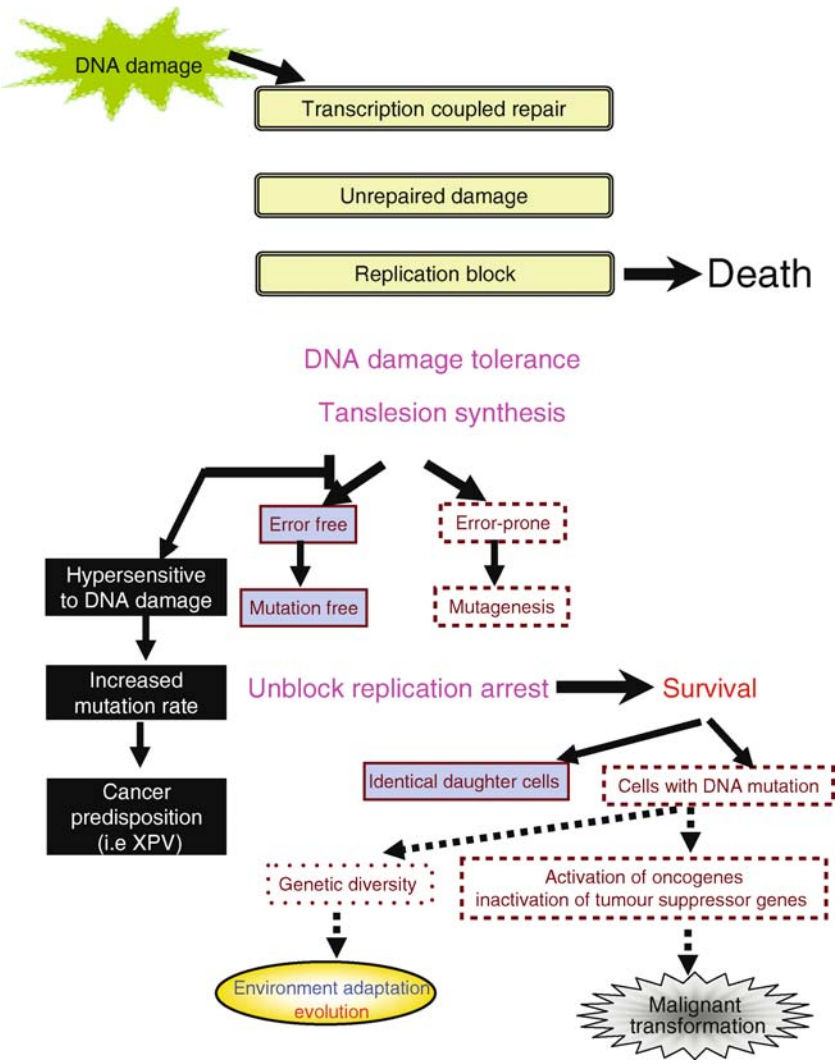
Characteristics

DNA is frequently damaged by endogenous and environmental factors. Base damage in DNA template strands blocks transcription to allow time to activate ►transcription-coupled repairpathways and eliminate DNA base damage. However, some lesions are persistent during replication therefore causing replication blockage and cell death. In order to overcome this problem, cells have evolved a damage tolerance system to allow complete replication in the presence of DNA damage. This process bypasses, rather removes, DNA damage, therefore, it is also named replicative bypass. It enables the cell to tolerate DNA damage and promote cell survival at the expense of high mutation rate. In fact, this process is responsible for most of the damage-induced point mutations and particularly important for oncogenesis. On the other hand, mutations are essential for evolution and adaptation which help a species to survive in a changing environment. The schematic summary of consequences of DNA damage is shown in Fig. 1.

Mechanism

Nonreplicative DNA Polymerases

A number of DNA polymerases have been identified specifically responsible for overcoming damage-induced replication arrest in human cells, which are also called specialized DNA polymerase (or bypass



DNA-Damage Tolerance. Figure 1 Schematic summary of consequences of DNA damage in mammalian cells. Note that DNA-damage tolerance mechanism promotes cell survival, and defects in error-free translesion DNA synthesis result in predisposition to cancer.

polymerase). So far ten of those polymerases have been identified in human including Rev1, Polyη, κ, ι, λ, μ, β, θ, υ, and ζ. Unlike ►replicative DNA polymerases, which can only use the opposite strand as template to initiate DNA synthesis, the specialized polymerases are able to promote stable incorporation of nucleotides opposite the lesion when replication is blocked by a damage. In addition to the conventional domains commonly found in all DNA polymerases such as "fingers," "thumbs," "palm" domains, many of the specialized DNA polymerases have a "little finger" domain and catalytic sites which provide flexible active site and allow the replicative bypass of various types of template structure. During an arrested replication, these polymerases take over temporarily from the replicative DNA polymerases and use either the damage DNA strand or the newly synthesized DNA strand as a template to proceed replication. This event is referred as "polymerase switching." This step is essential for the specialized polymerases to transiently occupy the primer template for initiating synthesis to bypass the damaged site. Unlike the high fidelity replicative DNA polymerases, these specialized polymerases have low fidelity of DNA synthesis and are responsible for mutagenesis in the genome. It is speculated that the reason that these polymerases are excluded from normal DNA replication is to maintain genomic stability in normal cells.

Translesion DNA Synthesis

The predominant and most well-studied DNA tolerance mechanism is known as ►translesion ►synthesis

(TLS). This process allows tolerance of DNA damage by employing specialized DNA polymerases to synthesize DNA directly in order to bypass template DNA damage. The outcome of this process can be both error-free and error-prone. When normal DNA synthesis is blocked by a lesion on one of the template strands, the specialized DNA polymerase(s) use the newly synthesized daughter strand as template to proceed DNA synthesis. Therefore, copying of the damaged site of DNA template is avoided and the DNA replication continues. Because the newly synthesized daughter strand, instead of the damaged strand, is used as template, this process is also named "template switching." In this process, the correct nucleotide is incorporated opposite the damage site; therefore, it is error-free. In contrast, when specialized polymerase(s) use the damaged template to proceed with DNA synthesis, errors may occur. This process is called lesion bypass and usually error-prone because of lack of correct template. However, if the correct nucleotide is incorporated opposite to the damage site, it can be error-free. When an incorrect nucleotide is incorporated opposite the damage site and subsequently extended, a base mutation occurs. Therefore, the error-free lesion bypass is mutation-avoiding mechanism, and error-prone lesion bypass is a mutation-generating mechanism. In human, the poly η is demonstrated to be one of the error-free specialized polymerases while Pol ι, κ, ζ, and Rev1 are found to be mutagenic specialized polymerases. The error-prone translesion synthesis contributes a major mechanism of DNA damage-induced mutagenesis in human.

Association with Cancer

Although the majority of polymerase errors are corrected by ►mismatch repair mechanism, the repair system may not function with 100% efficiency; therefore, errors are likely to escape correction and extended during subsequent replication. In addition, some unrepaired DNA damage, spontaneously formed or induced by environmental agents, will be processed by error-prone lesion bypass mechanism leading to mutagenesis. Therefore, mutations are generated every time the cell replicates itself. Accumulation of mutations in DNA results in activation of ►proto-oncogenes and inactivation of ►tumor suppressor genes resulting in malignant transformation. The significance of translesion synthesis in the development of human cancer comes from the identification of germ line mutations of the *Poly η* gene in a form of hereditary disease named ►xeroderma pigmentosum variant (XPV). Poly η is a highly specific bypass polymerase in repairing UV-induced thymidine dimers in an error-free manner. Loss of its function leads to increased UV-induced mutagenesis and hypersensitivity to sunlight. These XPV patients are hypermutable by ►UV radiation and develop cancer on sun-exposed skin at very young age. Skin abnormalities are caused by defects in bypassing UV-induced DNA damage leading to cell death or malignancy.

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DNA Damage Triggered Death Signaling Pathways

►DNA Damage-Induced Apoptosis

DNA Damage-Induced Apoptosis

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Synonyms

DNA damage triggered death signaling pathways; DNA repair and damage processing

Definition

►DNA damage-induced cell death is executed by apoptosis, necrosis, mitotic catastrophe and autophagy. From these different forms of cell inactivation ►apoptosis is the main route of death following DNA damage. Cells undergo apoptosis upon genotoxic stress via the death receptor and/or the intrinsic mitochondrial pathway. DNA damage-induced apoptosis is thought to be a mechanism protecting against cancer because it eliminates genetically damaged cells. This is most obvious in sun burned skin in which ►p53 upregulation initiates

the apoptotic process in response to light-induced DNA damage.

Characteristics

Summary

Not every type of DNA damage induces apoptosis. Many DNA lesions are tolerated by the cell, some are mutagenic without being toxic and some are more toxic than mutagenic. Apoptosis-inducing lesions are O^6-methylguanine, O^6-chloroethylguanine, base N-alkylations, cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts, benzo[a]pyrene guanine adducts, cisplatin and mustard-induced crosslinks and ►DNA double-strand breaks (DSBs). It is reasonable to suppose that any ►transcription and DNA ►replication blocking lesion bears apoptotic potential, and that transcription blockade and DNA replication fork stalling are apoptosis initiating events. ►Apoptosis signaling competes with DNA repair processes that either remove apoptosis-inducing lesions from DNA or tolerate them by replication bypass. Extended replication inhibition may lead to collapse of replication forks and the generation of DSBs, which are supposed to be the ultimate critical downstream apoptotic lesions for many, if not all, genotoxins. Primary DSBs induced by ionizing radiation and radiomimetic drugs and secondary DSBs derived from chemical DNA adducts during DNA replication are sensed by ►ATM (ataxia telangiectasia mutated) or ►ATR (ataxia telangiectasia related) protein. These ►PI3 kinases phosphorylate a large number of proteins including p53 that regulates apoptosis by upregulating the death receptor ►FAS/Apo-1/CD95, ►PUMA, Bak or translocating Bax to the mitochondrial membrane. Another mechanism implicated in apoptosis signaling rests on sustained upregulation of the transcription factor ►AP-1 by c-Fos and c-Jun activation, thus triggering the Fas ligand expression. The apoptotic pathway that finally becomes activated is cell type- and genotoxin-specific, depending on the p53 status, ►FAS/Apo-1/CD95 responsiveness and DNA repair capacity. DNA damage-induced apoptosis is executed by caspases, with caspase-8 activated by death receptors and caspase-9 by mitochondrial damage. The main executing ►caspases are caspase-3 and -7. These caspases cleave the inhibitor of caspase-activated DNAse (CAD) that in turn cleaves DNA in the typical 180–200 bp nucleosomal fragments.

Physiological Role of Apoptosis Induced by DNA Damage

DNA is subject to spontaneous and induced damage by environmental genotoxins. These include ►alkylating agents, ►polycyclic aromatic hydrocarbons, biphenyls, ►heterocyclic amines, ►ultraviolet light and ►ionizing radiation. DNA is also the main target for most anticancer drugs that react directly with DNA or interfere with DNA metabolism. Two cellular strategies have evolved for coping with DNA damage: (i) the damage is repaired or tolerated and (ii) cells harboring DNA damage are removed from the population by death. DNA damage has harmful consequences, which manifests as chromosomal changes, mutations, gene ►amplification and misregulation, cell death and malignant transformation. Cells respond to genotoxins in a complex way by evoking numerous cellular responses that may ultimately lead to damage repair, damage fixation as mutations, or damage elimination by various routs of cell death (►necrosis, apoptosis, reproductive cell death, interphase death, ►autophagy, ►mitotic catastrophe). Programmed cell death (apoptosis) following DNA damage is based on a complex enzymatic machinery. Apoptosis occurs continuously in the body, notably in changing tissues, and it appears that DNA damage-triggered cell death utilizes the normal cellular suicide program as a strategy to eliminate genetically damaged cells.

DNA damaging agents. There are three lines of evidence that apoptosis induced by genotoxic agents is related to DNA damage: (i) Inability of cells to repair DNA lesions results in hypersensitivity to the killing effect (an exception to the rule is DNA mismatch repair, see below). This has been shown for mutants defective in O^6-methylguanine-DNA methyltransferase (MGMT), base excision repair (BER), nucleotide excision repair (NER), DSB repair and DNA crosslinks repair. (ii) Modified nucleotides such as 6-thioguanine or gancyclovir incorporated in DNA induce apoptosis. (iii) DSBs induced by restriction enzymes in the cellular genome induce a strong apoptotic response. They hardly induce necrosis. The apoptotic pathways activated by a single DNA lesion, O^6methylguanine, as well as its interplay with DNA repair pathways has been studied in great detail. O^6MeG does not block DNA replication. It mispairs with thymine, giving rise to GC to AT point mutations. In the presence of ►mismatch repair (MMR) the O^6MeG/thymine mismatch is recognized by MutSα (consisting of MSH2 and MSH6) and MutLα (heterodimer of MLH1 and PMS2) that provoke a futile mismatch repair cycle leading to the formation of DSBs and apoptosis signaling. MMR deficient cells are highly resistant to simple alkylating agents because they do not undergo apoptosis. They tolerate O^6MeG adducts at the expense of mutations. Therefore, MMR driven apoptosis eliminates pre-mutated cells from the population, reducing mutation load (►Mismatch repair in genome stability).

Apoptotic pathways. The apoptotic pathways employed by cells following the exposure to O^6-methylating agents and other genotoxins differ depending on their p53 status. In p53 mutated cells the mitochondrial apoptotic pathway becomes activated, characterized by a decline in Bcl-2 that increases the Bax/Bcl-2 ratio and allows for the

release of ►cytochrome c from the mitochondria. This release of cytochrome c leads to the activation of the apoptosome consisting of Apaf-1, ATP, cytochrome c and procaspase-9. In turn, ►caspase-3 becomes activated that cleaves the inhibitor of caspase-activated DNAse (ICAD) that degrades DNA, resulting in the characteristic apoptotic DNA fragmentation. In p53 wild-type cells, e.g. human lymphocytes, DNA damage activates the death receptor pathway (Fas/CD95/Apo-1). There are cell types, such as glioma cells, wild-type for p53 in which both the Fas receptor and the mitochondrial damage pathway becomes activated in response to DNA damage. However, a higher level of DNA damage is required in order to induce apoptosis by activating the mitochondrial compared to the death receptor pathway.

Another role for p53 has been ascribed to the regulation of PUMA. DNA damage localizes p53 to the nucleus and transcribes PUMA. PUMA in the cytoplasm liberates cytoplasmic p53 from Bcl-xL, thereby freeing p53 to activate BAX and facilitate mitochondrial apoptosis. Another player in DNA damage-induced apoptosis is caspase-2, the only nuclear localized caspase that seems to act upstream of the mitochondria (►Apoptosis signaling).

DNA damage-triggered signaling: sustained JNK/p38 kinase activation. Some DNA damaging agents provoke the activation of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) and p38 kinase, which results in an increase in c-►Jun level and ►AP-1 activity. This sustained activation of AP-1 is accompanied by transcriptional activation the Fas-L gene. NER repair defective mutants display a higher level of sustained JNK/p38 kinase activation, indicating DNA damage is responsible for the response. Together with DNA damage-induced p53 upregulation that triggers transcription of the Fas receptor, the upregulation of the Fas ligand via AP-1 is effective in driving apoptosis upon DNA damage.

The ATM/ATR/p53 connection. ►DSBs are the most lethal DNA lesions and, therefore, cells have to be equipped with sensors that recognize DSBs immediately upon formation. These sensors signal repair or, if this fails, cell death. DSBs are recognized by proteins containing both signaling (kinase) and repair activity. The most important players are ►ataxia telangiectasia mutated (ATM) protein and A-T and Rad3-related (►ATR) protein. Both are activated by DSBs. Once activated, ATM phosphorylates various downstream substrates such as ►Nbs1. Phosphorylated Nbs1 acts as an adapter molecule for ATM dependent phosphorylation of Chk2 which activates Cdc25 responsible for S phase checkpoint control. Other substrates of ATM are p53, MDM2, ►CHK1 and ►CHK2, ►H2AX and ►BRCA1. While ATM is activated by ionizing radiation-induced DSBs, ATR is activated in response to UV light and presumably all chemical agents that give rise to stalled DNA replication forks. ATM/ATR is implicated in three crucial functions: regulation and stimulation of DSB repair (homologous recombination), signaling cell cycle checkpoints, and signaling apoptosis via p53. The phosphorylation of p53 by ATM leads to its stabilization, nuclear translocation and upregulation of p21 that triggers G1/S arrest. Low levels of DSBs activate only a minor fraction of p53 that is sufficient to drive the p21 gene, causing cell cycle arrest. With a higher level of DSBs, p53 becomes activated strongly and thus drives pro-apoptotic genes such as *bax* and the *fas receptor*. Therefore, the ATM/p53 activation level is very important in DNA damage-triggered apoptosis. Although AT cells (cells in which the ATM gene is mutated) are more sensitive to ionizing radiation, they exhibit less apoptosis after ionizing radiation than normal cells. ATM ►knockout mice also showed a lower apoptotic response after ionizing radiation than the corresponding wild-type mice, indicating the importance of the ATM/p53 pathway in triggering DNA damage-induced apoptosis. However, in a p53 mutated background, ATM knockout fibroblasts are more sensitive to apoptosis induced by alkylating agents than corresponding lines expressing ATM. This suggests that ATM is not indispensable for triggering apoptosis in response of DNA damage. It thus appears that DSBs, notably those induced by chemical genotoxins, are able to trigger apoptosis very efficiently also by another mechanism not involving ATM/p53 signaling. The high level of resistance of ATM wild-type cells to ►MNNG is explained by the role of ATM in DSB repair.

p53 is considered a major player in the apoptotic response, which is in line with the finding that p53 knockout mouse are resistant to the toxic effect of ionizing radiation, which is largely due to impaired death of thymocytes. While p53 deficient thymocytes are more resistant to ►ionizing radiation, p53 deficient mouse fibroblasts are more sensitive. This indicates that in fibroblasts, p53 is not required for inducing apoptosis in response to DSBs. It rather exerts a protective effect, which is most likely due to its involvement in DNA repair and inhibition of DNA synthesis by blocking cells in ►G1/S, transition. The decision of whether p53 exerts a pro- or anti-apoptotic effect appears to be cell type-specific, and the conditions determining whether p53 stimulates or protects against apoptosis have yet to be explored.

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DNA Demethylation

►Hypomethylation of DNA

DNA Double Strand Breaks

Definition

DNA DSBs; Sever of both strands of the double helix, which can lead to genome rearrangements.

►BACH1 Helicase
►Fragile Site

DNA Double-Strand Break Repair

Definition

DNA double-strand break repair in mammalian cells are the pathways for the repair of ►DNA double-strand breaks that include simple rejoining by the non-homologous end-joining reaction, or homology-directed processes including single-strand annealing or homologous recombination.

►Homologous Recombination Repair

DNA Flexibility

Definition

The fluctuation in the twist angle of DNA. Flexibility for any given sequence can be measured by using the FlexStab program (http://leonardo.ls.huji.ac.il/departments/genesite/faculty/bkerem.htm).

►Fragile Sites

DNA Glycosylase

Definition

An enzyme in a cell that removes a damaged or atypical base from DNA.

►Fluorouracil

DNA-Interstrand Crosslinks

Definition

Both DNA strands are covalently linked by a chemical. The binding is highly stable so that it prevents separation of the strands required during DNA replication and transcription. Such type of damage is lethal and must be removed or bypassed for cells to survive. This process is defective in (►Fanconi Anemia) cells.

DNA Lesion Bypass

►DNA-Damage Tolerance

DNA-Methyl-Transferases

Definition

Are a group of enzymes catalyzing the transfer of a methyl group to the DNA. DNA ►methylation serves a wide variety of biological functions.

►DNA Methylation

D

DNA Methylation

Definition

A modification to the 5-position of a cytosine ring in a ►CpG dinucleotides in DNA. Studies have established the involvement of DNA methylation in ►imprinting, gene regulation, chromatin structure, genome stability and disease, especially cancer. In eukaryotes, refers to the modification of cytosine at the 5-position by the addition of a methyl group. Methylated cytosine plays important roles in gene regulation, genomic stability, X-chromosome inactivation, and ►genomic imprinting.

►Cancer-Germline Antigens
►Epigenetic Gene Silencing
►Methylation

DNA Microarray

Definition

Technical device or platform suitable for massive parallel analysis of expressed genes or genomic sequences.

►Microarray (cDNA) Technology

DNA Mismatch Repair Mechanism

Definition

A type of DNA repair pathway that recognizes and corrects particular classes of errors - certain mispaired bases, small deletions and insertions - that arise during DNA replication. These errors may arise from slippage or mispairing of the DNA strands. Normal function of the mismatch repair pathway (as well as other repair mechanisms) preserves genomic integrity.

►Mismatch Repair is Genome Stability

DNA Photoproduct

Definition

Type of DNA damage formed after excitation of the DNA molecule by solar ►ultraviolet light and kovalent binding between adjacent pyrimidines. The most common types are ►cyclobutane pyrimidine dimers and ►6,4-photoproducts.

►Solar Ultraviolet Light

DNA-PK

Definition

Multi-protein complex essential to the repair of ►DNA double strand breaks and is thus a critical determinant of cellular ►radiosensitivity.

►Hsp90

DNA Repair

Definition

Maintenance and/or restoration of the original DNA sequence by removal of a ►carcinogen ►DNA adduct or other carcinogen-induced ►DNA damage is performed by repair mechanisms that include: direct repair of the adduct, ►base-excision repair, ►nucleotide excision repair, ►homologous recombination repair (postreplication repair), DNA ►mismatch repair, and ►non-homologous end joining. DNA-repair enzymes (>150) accomplish the common functions of recognition, incision, excision, degradation, polymerization and ligation by associating in different combinations and acting to remove the damage during a period of cell cycle arrest.

►Carcinogen Macromolecular Adducts
►Mismatch Repair in Genome Stability
►Toxicological Carcinogenesis

DNA Repair and Damage Processing

►DNA Damage-Induced Apoptosis

DNA Repair Capacity

Definition

DRC; All living organisms have a ►DNA repair system to protect the integrity of genomic DNA against constant assault from a plethora of endogenous and exogenous sources. DNA repair capacity (DRC) has often been used in epidemiologic studies as an indicator of the functionality of an individual's DNA repair system. Reduced DRC is associated with increased cancer risk.

►Mutagen Sensitivity

DNA Replication

Definition

Duplication of chromosomes by synthesis of DNA restricted to the S-phase of the ►cell cycle.

►Mitosis

DNA Topoisomerases II

Definition

Are the essential enzymes that play a role in virtually every cellular DNA process catalyzing the transient breaking and rejoining of DNA strands. They are able to cleave both DNA strands at the same time, allowing one DNA duplex to pass through another.

►Nutraceuticals
►Topoisomerases
►Topoisomerases II

DNA Undermethylation

►Hypomethylation of DNA

DNA Vaccination

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D

Synonyms

Genetic immunization

Definition

Vaccination with deoxyribonucleic acid (DNA) against cancer is the most basic type of vaccination that, rather than consisting of the tumor-associated antigen itself, provides genes encoding for the antigen. Once produced in vivo following DNA delivery, the antigen is presented to the immune system inducing an antigen specific immune response. This response is augmented by the immunological properties of the DNA itself, mediated by unmethylated ►CpG sequences. This essay reviews accomplishments and challenges in this area.

Characteristics

DNA vaccination represents a young field in cancer immunotherapy. It started with the observation that injection of plasmid DNA into a mammal resulted in the synthesis of the encoded protein. The unformulated or "naked" plasmid DNA containing a simple expression cassette, consisting of a promoter functioning in mammalian cells and of a gene encoding for a protein antigen, was injected into the muscle of mice. The subsequent induction of antigen specific ►CD8+ cytotoxic T-cells and antibodies was effective in protecting mice from challenges with the pathogenic agent expressing the antigen. This observation was surprising, given the low amount of antigen produced, the apparent lack of transfection of professional antigen presenting cells (APC) and the absence of any replicative step. The robustness of the technology was demonstrated for a variety of disease models.

Mechanisms of Action

The method of DNA delivery critically affects the mechanisms involved in the induction of an immune response. Intramuscular injection of plasmid DNA leads to in vivo transfection of myocytes. Mechanistic studies revealed that antigen specific immune responses following intramuscular injection of DNA is a result from ►cross priming. This mechanism describes production of the antigen by the myocyte and subsequent uptake and presentation by professional APCs. This was clearly demonstrated in bone marrow chimeric mice and in experiments with transfected myoblasts. In both systems,

the induction of an antigen specific immune response depended on the antigen presentation by APCs, and not by the myocyte. The transfer of the antigen from myocyte to APC follows different routes, ranging from uptake of secreted protein, processed peptide alone or with heat-shock proteins or apoptotic bodies by APCs. The transfer of DNA into the myocyte and subsequent induction of an antigen specific immune response can be largely improved by in vivo ►electroporation. The technique involves the application of an electric field around the DNA injection site. There are also needle free systems available, injecting DNA in solution using high pressure liquid jets. Clinical devices were developed by the pharmaceutical industry for application in humans, which are well tolerated.

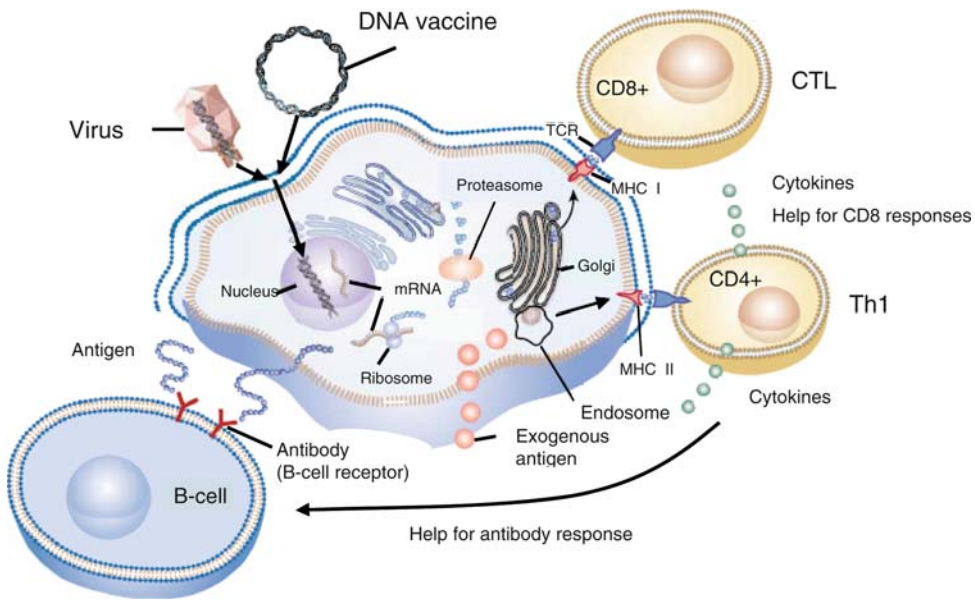
Bombardment of the epidermis with plasmid loaded onto gold particles using the ►gene gun directly transfers DNA into APCs of the skin called ►Langerhans cells. Once the protein antigen is expressed, professional antigen presentation is mediated by this cell type. Efficient induction of an immune response occurs after migration of these APCs from the skin into regional lymph nodes.

Gene transfer of plasmid DNA into APCs is also accomplished by the use of life attenuated bacteria such as salmonella typhimurium or listeria monocytogenes. In both cases, these micro organisms are infectious, but not pathogenic, and therefore serve as in vivo carrier systems for plasmid DNA vaccines. After in vivo application, ►Peyer patches and the spleen become infected. Subsequently, the carrier microbes die due to distinct mutations in their genome and liberate multiple copies of the plasmid DNA vaccines in these ►secondary lymphoid organs. There, the DNA vaccines are expressed by APCs leading to the induction of an antigen specific immune response.

A central role for induction of an immune response by DNA vaccines is antigen expression by APCs and subsequent presentation to $CD8^+$T-cells, ►$CD4^+$ T-cells and B-cells (Fig. 1).

Adjuvant Activity of DNA

Plasmid DNA derived from bacterial expression systems naturally contain unmethylated DNA sequences called CpG motifs. These sequences bind to Toll-like receptor 9 and are strong activators of ►innate immunity. This



DNA Vaccination. Figure 1 Mechanisms involved in the generation of antigen specific humoral and cellular immune responses upon DNA vaccination. Antigen specific activation of cytolytic T lymphocytes ($CD8^+$T-cells) occurs after proteasome dependent antigen processing of intracytoplasmic proteins into peptides associated with newly synthesized MHC class I molecules. MHC class I/peptide complexes are presented on the surface of APCs in conjunction with costimulatory molecules to $CD8^+$T cells. The activation of ►$CD4^+$T-cells is primarily achieved by exogenous protein antigens taken up by the endolysosomal compartment. After degradation, peptides associate with MHC class II molecules which are then translocated to the cell surface. Specific $CD4^+$ helper T cells recognize these MHC class II/peptide complexes and are activated to produce cytokines. These cytokines have multivarious activities helping B-cells to mature into antibody producing plasma cells and $CD8^+$T-cells to transform into cytolytic effector cells. For antibody responses, B-cells recognize and respond to antigens that are either present extracellularly or exposed extracellularly by being transmembrane proteins.

receptor is also expressed on APCs leading to improved antigen processing and presentation as well as the release of pro-inflammatory cytokines and chemokines that help to shift ►adaptive immunity responses from ►Th2 immune response to ►Th1 immune response. Th1 responses are required for most effective anti-tumor immunity. Therefore, CpG motifs in the DNA vaccine backbone can be considered endogenous adjuvants linking innate immunity with ►adaptive immunity, which provides for robust and long lasting antigen specific immune responses.

Tailoring Immune Responses by DNA Vaccine Design

In order to improve antigen specific immune responses, the versatility of DNA vaccine design allows for the simultaneous expression of antigen, co-stimulatory molecules and chemoattractants. These include cytokines, chemokines, molecules of the B7 family and CD40 ligand. The design of the protein antigen itself can be altered to be secreted for induction of B-cell responses or to be targeted into the endopasmatic reticulum or the proteasomal degradation pathway for the generation of T-cell epitopes. Protein antigens can be redesigned as mini genes only encoding for immunodominant peptide antigens. In summary the versatility of DNA vaccines allows for specific tailoring of an optimized immune response following a rational vaccine design.

Formulation

The formulation of DNA vaccines to improve antigen specific immune responses includes transfection-facilitating lipid complexes, nanoparitcles and classical adjuvants. Lipid complexes are varying combinations of DNA with cationic lipids. Microparticles are generated with DNA entrapped in biodegradable poly-lactide-co-glykolactide or complexed with non-ionic block copolymers or polycations. Among the classical adjuvants, aluminium phosphate is noteworthy for its effectiveness and simplicity of preparation. Microparticles appear to improve the trafficking of DNA to APCs by facilitating the transfer of DNA into regional lymphnodes.

Mixed Modality Vaccines

A very promising strategy that is entering clinical trials is to combine DNA vaccines with other gene delivery systems. This is based on observations that if DNA encoding an antigen is given as a prime followed by another gene-based vector system as a boost such as recombinant viruses encoding the same antigen, most optimal immune responses and protection are achieved. The responses are significantly greater than using DNA or the virus for both the prime and the boost or if the order of the administration is reversed.

Results from Clinical Trials

First generation DNA vaccines have been evaluated clinically as a therapeutic vaccine approach for cancer. These vaccines encoded for viral epitopes from transforming viruses, self-antigens expressed on tumors, and tumor specific antigens. In most trials so far, the plasmid DNA was injected intramuscularly, intradermaly or intranodaly. Antigen specific humoral and cellular responses were observed in human cancer trials. However, this did not translate into clinical responses in the trial patient populations characterized by large tumor burden and progressive disease. The clinical trials so far have proved the principle that immune responses can be generated in humans. They also highlight the need to apply strategies to increase the potency of the technology as outlined above and to generate second generation DNA vaccines for future application in cancer patients.

In summary, DNA vaccines hold great potential as immunotherapeutic tools to prevent and treat human cancer. Their advantages include cost effectiveness, versatility, safety, stability, ease of construction and mass production and most importantly ability to induce robust humoral and cellular immune responses. Lack of success in early clinical trials so far is similar to early clinical results with treatments based on monoclonal antibodies which are now established cancer therapeutics. To push for success, the next generation of DNA vaccines will have to incorporate multiple strategies to enhance plasmid DNA immunogenicity. Additional avenues may involve exploring the possibilities of combining adoptive cell therapies with DNA vaccines such as ex vivo gene transfer into autologous dendritic cells.

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DnaJ (Hsp40) Homolog Subfamily C Member 15

►Methylation-Controlled J Protein

DNAJC15

►Methylation-Controlled J Protein

DNAJD1

►Methylation-Controlled J Protein

DNF15S2

►Macrophage-Stimulating Protein

DNMTs

Definition

DNA methyltransferase enzymes; Responsible for maintenance of ►methylation as well as de novo methylation.

►Epigenetic Gene Silencing

Docetaxel

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Synonyms

►Taxotere

Definition

Docetaxel is a new class of anticancer agent that exerts the cytotoxic effects on microtubules. It is a semisynthetic drug with significant activity in a broad range of tumor types that are generally refractory to conventional therapies, including chemotherapy-resistant epithelial ►ovarian cancer, ►breast cancer, ►non-small cell lung cancer, head and neck cancer, ►bladder cancer, and ►gastric cancer.

Characteristics

Docetaxel is derived semisynthetically from 10-deacetyl-baccatin III, is more water soluble than ►paclitaxel and is more potent antimicrotubule agent in vitro.

Mechanism of Action: Docetaxel induces polymerization of ►tubulin, ►microtubule bundling in cells, formation of numerous abnormal mitotic asters. The cytotoxic effects of docetaxel are also severalfold greater than paclitaxel in vitro and in tumor xenografts. Docetaxel inhibit proliferation of cells by inducing a sustained mitotis block at the metaphase–anaphase boundary at much lower concentrations than those required to increase microtubule polymer mass and microtubule bundle formation. These inhibitory effects at low drug concentrations are associated with the formation of an incomplete metaphase plate of chromosomes and an arrangement of spindle microtubules resembling the abnormal organization that occurs at low concentrations of the ►vinca alkaloids.

Docetaxel primarily block cell-cycle traverse in the mitotic phases and prevents the transition from Go to S phase. The inhibitory effects in the nonmitotic cell-cycle phases include the disruption of tubulin in the cell membrane and direct inhibitory effects on the disassembly of the interphase cytoskeleton. These effects may result in the disruption of many vital cell functions such as locomotion, intracellular transport, and transmission of proliferative transmembrane signals.

After disruption of microtubules and other processes by docetaxel, the precise means by which cell death occurs is not clear. Morphologic features and a DNA fragmentation pattern (►nucleosomal DNA fragments) that are characteristic of programmed cell death, or apoptosis, in docetaxel-treated cells indicate that this taxane trigger apoptosis as do many other chemotherapeutic agents. Whether docetaxel-induced ►apoptosis requires a functional ►p53 pathway is unclear and probably depends on the cell line under study. The consensus seems to be that in most cell lines, disruption of p53 has little effect on drug sensitivity.

Mechanism of Resistance: Selection of taxane-resistant cells in vitro is associated with changes in β-tubulin isotype expression. Six different isotypes of β-tubulin are expressed in nonmalignant tissues, with the class I isotype comprising 80–99% of cellular β-tubulin. The β III isotype increase the dynamic instability of microtubules, impairs rates of microtubule assembly, and increases resistance to taxanes.

A second mechanism of acquired taxane resistance fits the general pattern of ►MDR. The particular species of Pgp found in taxane-resistant murine ►macrophages is similar, but not identical, to that found in ►vinblastine- and ►colchicine-resistant cells derived from the same parental line. These cells are cross-resistant with many other natural products, and resistance to docetaxel conferred by ►mdr-1 can be reversed by many classes of drugs, including ►tamoxifen, ►cyclosporine A, antiarrhytmic agent.

Other changes in tumor cells selected for drug resistance have included upregulation of ►caveolin-1, a principal component of membrane-derived vesicles involved in transmembrane transport of small molecules and in intracellular signaling.

Pharmacokinetics

The single 1-hour infusion every 3 weeks is the most common administration of docetaxel. The ►pharmacokinetics behavior on 1 or 2 h schedules is linear at doses of 115 mg/m2 or less and optimally fits a three-compartment model. Docetaxel binds rapidly and avidly to plasma proteins (>90%), especially to albumin, α1-acid glycoprotein, and lipoproteins. In addition, peak plasma concentrations generally exceed levels required to induce relevant biologic effects in vitro. Limited information is available about the distribution of docetaxel in humans. Immediately after treatment, tissue uptake of radioactivity is highest in the liver, bile, and intestines, a finding that is consistent with substantial hepatobiliary extraction and excretion. High levels of radioactivity are also found in the stomach, which indicates the possibility of gastric excretion, as well as in the spleen, bone marrow, myocardium, and pancreas. Docetaxel has hepatic metabolism and biliary excretion and urinary excretion accounts only 2%. Approximately 80% of the administered dose of total radioactivity is excreted in the feces within 7 days after treatment, with the majority of excretion occurring in the first 48 h. In the hepatic ►cytochrome P450-mixed function, oxidases are responsible for the bulk of drug metabolism, and CYP3A, CYP2B, and CYP1A isoforms may play major roles in biotransformation. The main metabolic pathway consists of oxidation of the tertiary butyl group on the side chain at the C-13 position of the taxane ring as well as cyclization of the side chain.

Toxicity

►Neutropenia is the principal toxicity of docetaxel. At dose of 100mg/m2, neutrophil count nadirs are <500/mcl in 50–80% of courses in previously untreated patients. The onset of neutropenia is early, with nadirs usually on day 9 and complete recovery by days 15–21. Neutropenia is not cumulative.

Hypersensitivity reaction (HSR) with premedication with dexamethasone is present in less of 10% of the cycles. Another possible toxicity is fluid retention that is cumulative doses, characterized by edema, weight gain, ►pleural effusion, and ►ascites. Neither hypoalbuminemia nor cardiac, renal, or hepatic dysfunction is found, and capillary filtration studies suggest that docetaxel causes a capillary permeability abnormality. Fluid retention is not usually significant at cumulative doses of <400 mg/m^2.

The incidence of this effect may be reduced by premedication with dexamethasone 8 mg orally twice daily for 5 days, beginning from day 1.

Between 50 and 75% of patients receiving docetaxel develop skin toxicity, typically characterized by an erythematous pruritic maculopapular rash that affects the forearms and hands. Other cutaneous effects include onychodystrophy, onycholysis, soreness and brittleness of the fingernails. Docetaxel also induces palmar-plantar erythrodysthesia and ►alopecia. With respect to neurotoxicity has been reported in 40% of previously untreated patients and may be higher in patients who were previously treated with ►platinum antitumor compounds. The peripheral neuropathy predominately affects large fiber sensory function.

Asthenia has been observed in as many as 60–70% of patients and usually is moderate in severity, and when it is severe, it warrants dosage reduction or discontinuation of treatment. Nausea, vomiting, and severe diarrhea are rare.

Dosage

Docetaxel is indicated at a dose range of 60–100 mg/m^2 over 1 h. Although untreated or minimally pretreated patients generally tolerate docetaxel at a dose of 100 mg/m^2 without severe toxicity, emerging data indicate poorer tolerance in more heavily pretreated patients, in whom 75 mg/m^2 may be a more reasonable dose from a toxicologic perspective. Although weekly drug administration has no clear benefits in terms of antitumor activity, hematologic toxicity is much less than conventional schedules and an unacceptable degree of asthenia and neurotoxicity is evident at doses that exceed 36 mg/m^2 per week.

Indications

Docetaxel has demonstrated antitumor activity in patients with metastatic breast cancer as first line or salvage treatment, recurrent ovarian cancer, non-small cell lung cancer, adjuvant breast cancer, squamous cell head and neck cancer, and gastric cancer. In addition to these indications, docetaxel has demonstrated activity in previously treated patients with carcinomas of ►endometrial cancer, ►esophageal cancer, ►bladder cancer, ►prostate cancer, ►small cell lung cancer, as well as ►lymphomas and other neoplasms.

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DOCK180/ELMO

Definition

Is a 180 kDa protein, which binds to Crk and is associated in a DOCK180/ELMO complex, which serves as a functional GEF (guanosine exchange factor) for RAC. This complex is involved in phagocytosis of apoptotic cells and cell migration through Rac activation.

►Focal Adhesion Kinase (FAK)

Docking Proteins

Definition

►Scaffold Proteins.

Domain

Definition

A portion of a polypeptide that folds to form a compact unit that remains distinct within the tertiary structure of the whole protein. Domains are usually compact globular units within proteins that are the basic units of their tertiary structures. Immunoglobulin-like domains appear to resist proteolysis and are therefore a feature of a large family of mostly extracellular proteins.

Domain Structure

Definition

Refers to an element, motif, or particular amino acid sequence, within the overall protein that often folds and functions independently of the rest of the protein.

Dominant

Definition

Describing an ►allele of a gene that determines the phenotype in spite of the presence of the second allele (►recessive) that specifies a different phenotype. Referring to one of several alternative traits (phenotypes) that can be specified by a genetic locus; when the locus is ►heterozygous and carries information specifying two distinct traits, the dominant trait will be the one actually exhibited.

Dominant Negative

A mutation that occurs in only one ►allele but is able to effect the cellular or organismal phenotype by virtue of inactivating the normal allele at the protein level. Implies that an altered gene product acts antagonistically to the wild-type allele; this usually results in an altered molecular function, which is often inactive.

►Retroviral Insertional Mutagenesis

Donor Lymphocyte Infusion

Definition

DLI; Is used to treat leukemia relapse after allogeneic hematopoietic stem-cell transplantation. Donor lymphocytes mediate the beneficial graft-versus-leukemia effect as well as the undesired ►graft-versus-host disease in the allotransplant recipient.

►Allogeneic Cell Therapy
►Graft Acceptance and Rejection

Dopamine

Definition

A neurotransmitter substance produced in the hypothalamus and elsewhere in the nervous system. It negatively regulates prolactin secretion.

►Prolactin

Doppler Ultrasound

Definition

Doppler effect is a shift in the frequency of a wave produced by motion of a source or reflector. The change in pitch of a train whistle or emergency siren as the vehicle passes the listener is a familiar example of the Doppler effect. In vascular ultrasound imaging, red blood cells are the primary moving reflectors. The Doppler frequency shift observed when an ultrasound pulse reflects from moving red blood cells depends on the velocity of blood flow, the original frequency of the transmitted ultrasound pulse, the angle between the ultrasound beam and the direction of flow, and the speed of sound. Measurements of Doppler frequency shift can therefore be used to estimate blood flow velocity.

►Ultrasound Micro-Imaging

Dormancy

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Synonyms

In situ cancer or carcinoma; Occult cancer; Cancer without disease; In situ carcinoma

Definition

Tumor dormancy describes human tumors with three characteristics: (i) Visible only under a microscope, and therefore, cannot be detected by conventional diagnostic imaging methods. May have an average diameter the size of a pinhead, but range from 0.1 mm to ~2 or 3 mm. (ii) Usually do not expand or spread to other organs. (iii) Usually asymptomatic and harmless, but have the potential to resume growth, and eventually to be fatal to their host.

Characteristics

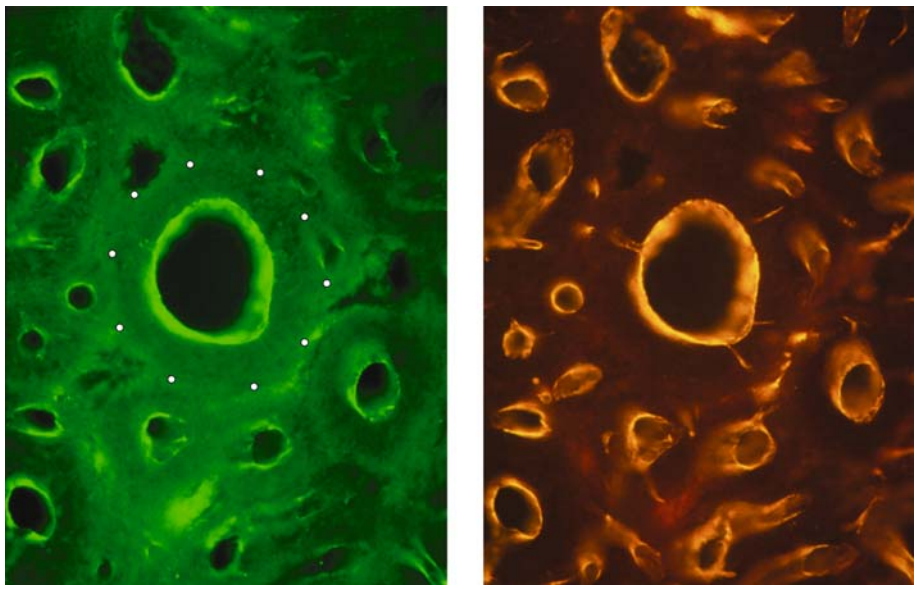
Virtually all adult humans have dormant cancers, as determined from autopsies of individuals who died of trauma (e.g. auto accidents), but who did not have a diagnosis of cancer during their lifetime. From these autopsies, pathologists report microscopic-sized cancers in different organs, often called carcinoma *in situ*. In women 40–50 years old, 39% have dormant *in situ* carcinomas in their breasts (►Preneoplastic lesions), but only 1 out of 100 ever develop ►breast cancer during a normal lifetime (►Ductal carcinoma in situ). Forty-six percent of men from 60 to 70, have carcinoma *in situ* of the prostate, but only 1 out 100 in this age range are ever diagnosed with prostate cancer. In contrast, estimated 98% of individuals age 50–70, harbor carcinoma *in situ* of the thyroid gland, but only 1 out of 1,000 develop thyroid cancer. Dormant carcinoma *in situ* can be found nestled among established capillary blood vessels, but such dormant tumors do not recruit new blood vessels. In other words, most dormant cancers *in situ* are non-angiogenic.

Blocked Angiogenesis

Therefore, one mechanism of tumor dormancy is blocked ►angiogenesis, i.e. the inability of emerging early tumors to recruit new blood vessels. Cancer arises from a single cell, for example, a liver cell. Normal liver cells rarely divide. A cancerous liver cell however, can continue to divide without restraint until it has accumulated offspring of up to ~1 million tumor cells. Nevertheless, such a microscopic tumor becomes dormant when its further expansion is arrested by the limits of oxygen diffusion from the nearest open capillary blood vessel. This oxygen diffusion limit is ~180–200 μ (about 0.2 mm) for tumor cells, and significantly less for normal cells. Virtually every normal cell lives either directly adjacent to a capillary blood vessel, or at least not more than two cell widths from a capillary. However, tumor cells can surround a capillary vessel with multiple cell (Fig. 1). Once a microscopic tumor becomes oxygen depleted, tumor cell death (►apoptosis) increases to match tumor cell proliferation. This "balance" between proliferating and dying tumor cells is one of the hallmarks of the dormant, microscopic, *in situ* cancer which virtually all humans harbor (►Progression).

Maintenance of Tumor Dormancy by Endogenous Angiogenesis Inhibitors

The majority of new tumors, and metastases remain dormant for prolonged periods of ►time (sometimes



Dormancy. Figure 1 *Left panel*: Blood vessels in a human breast cancer (MCa-IV). Tumors were transplanted into mice and subsequently the tumors were perfused with fixative so that the microvessels would not be compressed. The white dots show the outer layer of multiple layers of tumor cells surrounding a new capillary blood vessel (lectin-binding). *Right panel*: Endothelial cells are labeled by antibody to CD31 antigen. This section also shows new capillary sprouts in the breast cancer. (Courtesy of Donald McDonald, University of California, San Francisco.)

for years), in part because of endogenous angiogenesis inhibitors. At this writing, 29 angiogenesis inhibitors have been discovered in the body; none were known before 1980 (►Antiangiogenesis). Most of these angiogenesis inhibitors are proteins, such as ►thrombospondin-1, platelet factor 4, ►maspin, ►angiostatin, ►endostatin, tumstatin, canstatin, ►interleukin-12, ►SPARC, and others. Endostatin and tumstatin are under the control of ►p53. While some inhibitors circulate at low concentrations in the plasma, others are stored in platelets, white blood cells, bone marrow cells, fibroblasts of tissues throughout the body, or in the collagen basement membranes in the stroma underlying most tissue cells. Endothelial cells produce collagen type XVIII ►basement membrane. Tumor cells themselves, also express certain angiogenesis inhibitor proteins directly, such as thrombospondin-1, or express enzymes that mobilize antiangiogenic peptide fragments from larger proteins. Examples of the latter are angiostatin from plasminogen, and endostatin from collagen XVIII. The gene for collagen XVIII is on chromsome 21. Individuals with ►Down syndrome have three copies of this chromosome (trisomy). As a result, elevated levels of endostatin are found in these individuals. They are protected against abnormal angiogenesis. Down syndrome individuals with diabetes are protected against neovascularization in the retina and also from neovascularization in atherosclerotic plaques. They are also protected against cancer. In fact, of the ~200 types of cancer, these individuals only develop ►testicular cancer and a rare, but mild form of ►leukemia. For the other types of cancer, individuals with Down syndrome have <0.1 the expected incidence even though they live into middle age and beyond. In mice genetically engineered to overexpress collagen XVIII so that their endostatin level is increased by ~1.6-fold to mimic individuals with Down syndrome, implanted tumors are poorly neovascularized and grow 300–400% slower. Conversely, tumors grow threefold more rapidly in mice that lack endostatin. The genes for mental retardation are unrelated to endostatin. Furthermore, a second putative antiangiogenic gene, called DSCR1 (Down syndrome critical region), has recently been identified on chromosome 21. Although this phenomenon is correlative and not yet proved to be causal, it provides a thought provoking clinical clue that suggests the question – do individuals with Down syndrome harbor equivalent numbers of microscopic dormant cancers as the rest of the population, but a lower incidence of tumors that become angiogenic?

Escape from Tumor Dormancy by a Switch to the Angiogenic Phenotype

Miles of capillary blood vessels, thinner than a hair, supply every tissue in the body. A pound of fat contains ~1 mile of capillary blood vessels. Vascular endothelial cells which line the inside of these blood vessels normally proliferate only infrequently, to replace lost

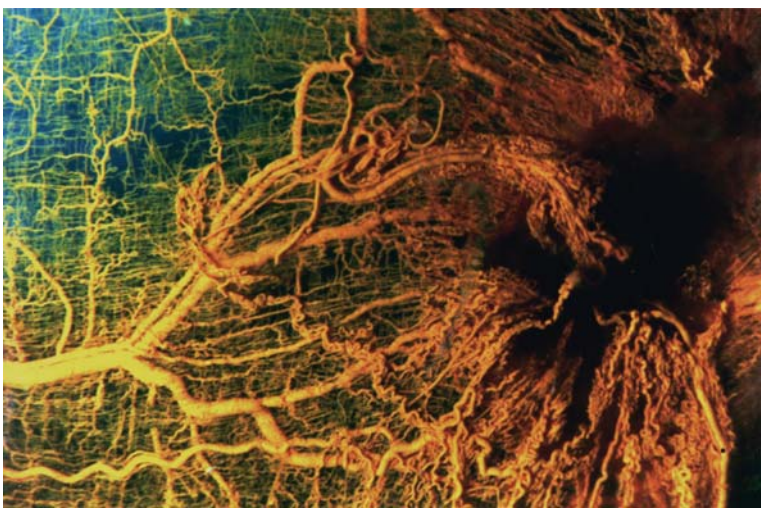
endothelial cells. The entire endothelial lining is replaced or "turned over" in ~3 years, in contrast to the turnover of intestinal epithelial cells that is measured in days. During physiological angiogenesis, such as reproduction, development, or wound repair, endothelial cells can proliferate rapidly, i.e. with a turnover measured in days or weeks. Physiologic angiogenesis is however, self-limited. Angiogenesis during ovulation is turned off after a few days, and in wounds after ~2 weeks. In contrast, once a tumor has become angiogenic, endothelial cells in the tumor bed proliferate continuously, the beginning of the switch to the angiogenic phenotype. As new capillary blood vessels grow toward the dormant tumor, tumor cells grow around them, and the tumor mass now expands rapidly (Fig. 2). Angiogenic tumors become detectable by conventional imaging methods, cause symptoms, and metastasize to other organs. Angiogenic tumors are potentially fatal (►Progression).

Experimental Analysis of the Angiogenic Switch in Human Dormant Cancers

The escape of human dormant tumors to the angiogenic phenotype has been studied in immunodeficient mice (►SCID or ►Nude mice), by cloning single tumor cells from tumor specimens discarded from the operating room, or obtained as tumor cell lines from the American Type Culture Collection. When these clones are expanded *in vitro*, and re-implanted in immunodeficient mice, ~3–5% form *non-angiogenic* tumors of ~1 mm diameter and remain dormant with a high proliferation rate of tumor cells balanced by a high apoptotic rate. The microscopic dormant tumors can be visualized in mice if tumors are stably infected either with luciferase, or green fluorescent proteins (e.g., green flourescent protein). For each tumor type there is a predictable percentage of non-angiogenic tumors that will undergo a spontaneous switch to the angiogenic state (called phenotype), at a predictable time. For example, ~80% of the non-angiogenic tumors from a given type of human breast cancer become angiogenic at ~4 months. For ►liposarcoma (a cancer of fat tissue), the angiogenic switch occurs at 4 months in 95% of non-angiogenic tumors. A human brain tumor (►glioblastoma) becomes angiogenic at 8 months in 60% of tumors. In human bone tumors (►osteosarcoma), the angiogenic switch does not occur until after 1 year, and in only 5–15% of mice. After tumors become angiogenic, they escape tumor dormancy and form lethal tumors in 100% of mice regardless of cancer type. Thus, human tumors studied so far contain a subpopulation of non-angiogenic tumor cells that can form dormant tumors. For some tumor types, the majority of non-angiogenic tumors become angiogenic and escape from dormancy (►Progression) (e.g. liposarcoma). For other tumor types, only a minority of non-angiogenic tumors become angiogenic and the rest remain non-angiogenic and dormant indefinitely.

Molecular Mechanisms of Angiogenic Switching in Dormant Cancers

If human tumors could be restricted to the non-angiogenic dormant phenotype, or if angiogenic tumors could be reversed to the non-angiogenic dormant



Dormancy. Figure 2 Angiogenesis in rat sarcoma. In this micrograph, blood vessels grow toward a sarcoma (dark area at right) in rat muscle. This contrasts with the normal grid-like pattern of blood vessels that appears at the upper left. Tumor cells that have begun to surround the capillary vessels are not shown here. (Courtesy of L. Heuser and R. Ackland, University of Louisville, USA.)

phenotype, a novel anti-cancer therapy could be possible (►Antiangiogenesis). Therefore, molecular mechanisms are being studied. For example, ►transfection of a non-angiogenic human osteosarcoma with the ►*RAS* oncogene causes dormant tumors to become angiogenic and escape from dormancy within weeks to 1 month, in contrast to the spontaneous angiogenic switch which can take up to 1 year. Furthermore, after ►*RAS* transfection, 100% of dormant non-angiogenic tumors become angiogenic and grow rapidly, whereas spontaneous escape from dormancy occurred in only 5–15% of tumors. *RAS* transfection is followed by a 30% increased expression of vascular endothelial growth factor (i.e. ►Adrenomedullin) (►VEGF, a potent pro-angiogenic protein), and is accompanied by a 50% decrease in expression of thrombospondin-1, a potent angiogenesis inhibitor. Many oncogenes induce increased expression of a pro-angiogenic proteins and suppression of an antiangiogenic protein (►Antiangiogenesis). This common pattern could lead to a general molecular mechanism of escape from tumor dormancy by activation of the switch to the angiogenic phenotype.

Other Forms of Tumor Dormancy

Immune Surveillance as a Cause of Tumor Dormancy

Many experimental models reveal that the immune system can maintain a reduced load of cancer cells based on manipulation of ►cytotoxic T lymphocytes (CD8+) which can kill tumor cells expressing specific antigens. Furthermore, escape from tumor dormancy in some models is based on tumor evasion of the immune system. In other experimental systems, growth of mouse lymphoma cells can be suppressed by a T-cell mediated mechanism. It remains to be determined if therapeutic blockade of angiogenesis, by restricting expansion of a dormant tumor population will synergize immune suppression.

Hormonal Depletion as a Cause of Tumor Dormancy

Certain hormonally dependent tumors, such as prostate cancer, become dormant when the hormone, (e.g. androgen), is blocked or depleted. It has been shown that depletion of testosterone from a prostate cancer, decreases VEGF expression, and thus reduces tumor angiogenesis. However, in clinical practice, this therapy is temporary, and within 1 year or more, androgen-independent prostate cancer cells often emerge.

Dormancy of Single Metastatic Tumor Cells

It has been shown in experimental animals that single metastatic tumor cells can exit the circulation at a future metastatic site, for example liver, or lungs, and survive for long periods near a capillary blood vessel without proliferating, (the G_0 state).

Future Directions

Biomarkers to Detect Dormant Tumors

Many laboratories are developing a variety of molecular ►biomarkers in the blood to detect the presence of cancer. Some of these may be useful for detecting the presence of microscopic sized tumors that cannot be located anatomically by conventional imaging methods. This would include non-angiogenic dormant tumors, or those just beginning to switch to the angiogenic phenotype. If a biomarker of high sensitivity could be validated in the clinic, then it could eventually be used to guide non-toxic antiangiogenic therapy, or anti-►telomerase therapy, or ►immunotherapy, to prevent recurrence of cancer years before symptoms, or before anatomical location was possible.

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Dose-Dense Chemotherapy

Definition

A different way of administering ►chemotherapy in which standard medications are given more frequently than in the past.

►Adjuvant Chemoendocrine Therapy

Dose-Density

Definition

Describes the concept of administering optimal dose levels of drug therapy at intervals as close together as

safely permissible so as to limit the degree of tumor regrowth between doses and thereby optimize cell-kill. This is based on the Norton-Simon hypothesis, which describes the positive relationship between small tumor volume and relative rapidity of growth.

►Log-Kill Hypothesis
►Norton-Simon Hypothesis

Dose Fall-off

Definition
The distance between the targeted high-dose region of radiation and the treatment field.

►Brachytherapy

Dose Homogeneity

Definition
Distribution of the dose evenly throughout a target volume.

►Brachytherapy

Dose-Limiting Toxicity

Definition
DLT; A specified quantity of a therapeutic agent, such as a drug or medicine, prescribed to be taken at one time or at stated intervals.

Dose Rate

Definition
The radiation dose delivered per unit time.

►Brachytherapy
►Radiation Oncology

Dose-Response Curve

Definition
A graph that shows the relationship between the dose of a substance and the degree of response it produces.

►Small Molecule Screens

Double Minute

Definition
DM; Synonym dmin, are extrachromosomal small paired chromatin bodies. In tumor cells they indicate oncogene ►amplification and are also seen in cells, where genes involved in drug metabolism are amplified (e.g. dehydrofolate reductase gene in ►methotrexate resistance).

►Neuroblastoma

Double Strand Break

Definition
DSB; Is most notably caused by ►ionizing radiation, is considered the most lethal DNA damage in cells since both DNA strands are profoundly affected.

►Mutagen Sensitivity
►Repair of DNA

Doublecortex

►Doublecortin

Doublecortin

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Synonyms

Doublecortin; Doublecortex; DCX

Definition

Doublecortex (*DCX*) is a brain-specific gene, deficient in ►glioma tumor cells and suppresses tumor in brain. DCX is a substrate of ►Jun N-terminal Kinase (JNK) and highly phosphorylated by JNK. The phosphorylated DCX interacts with a potent tumor suppressor, ►spinophilin ►neurabin II and then associate with the protein serine/threonine phosphatase, protein phosphatase-1 (PP1). This association of protein complex of DCX, neurabin II, and PP1 leads to inhibit anchorage independent growth of glioma tumor cells and eventually suppresses tumor development.

Characteristics

DCX is a ►microtubule-associated protein (MAP), which directly interacts with ►microtubules (MTs) without any additional mediators. This interaction results in stabilization and bundling of MTs both in vivo and in vitro. DCX is a basic protein with an isoelectric point of 10, typical of other MT-binding proteins. DCX is evolutionarily conserved MT binding protein that maintains almost identical amino acid as well as nucleic acid sequences from mouse to human. It is located on chromosome Xq22.3-q23. From Affymetrix gene chip analysis, *DCX* is one of the three dominant genes found in relation to glioma patient survival. Other two genes are ►osteonectin and ►semaphorin. All of them regulate cellular motility. De novo expression of *DCX*, a gene which is absent in wild-type glioma cells, leads to an arrest in the G2 phase of the cell cycle. During this process, the neoplastic cells cease to proliferate and lose their ability to form large colonies in semisolid medium and to induce tumor xenograft in immunocompromised hosts. This growth suppression can be reversibly abrogated by blocking *DCX* transcription via its sequence-specific inhibitor DCX ►siRNA. The absence of the *DCX* gene may increase propensity to develop tumors; thus, boosting endogenous *DCX* gene expression may be therapeutically beneficial and, alternatively, pharmacologic delivery of recombinant DCX may have beneficial therapeutic effects in the treatment of glioma.

Regulation

Regulation of many cellular functions depends on protein phosphorylation and dephosphorylation of multiple substrates by protein kinases and phosphatases. DCX, a substrate of JNK interacts with both ►JNK and ►JNK-interacting protein and is highly phosphorylated by JNK in glioma cells. Aside from ►epidermal growth factor receptor (EGFR), JNK is the only other kinase that is almost exclusively expressed and basally active in primary human glial tumors. The phosphorylated DCX interacts with spinophilin/neurabin II, a tumor suppressor. Neurabin II inhibits anchorage-independent growth of human and mouse cancer cell lines, regardless of ►p53 and ►p14ARF (ARF) status. Ectopic expression of ARF is ineffective for suppression of colony formation in osteosarcoma cancer cells, Saos-2 whereas, neurabin II and ARF coexpression and interaction synergistically inhibits anchorage-independent growth. DCX and neurabin II interaction inhibits proliferation and anchorage-independent growth in glioma cells. In contrast, DCX-mediated growth suppression is lost in neurabin II null HEK 293T cells and is reversed by knocking down neurabin II with siRNA (small interfering RNA). DCX and neurabin II interaction may contribute to strong antitumorigenicity to glioma.

Phosphorylation and dephosphorylation lead to association and dissociation of many proteins such as a phosphoprotein encoded by mouse a4 and the catalytic subunit of protein phosphatase 2A that regulates their activation. Neurabin II belongs to this class of regulators because it negatively regulates the PP1 catalytic subunit activity. Native neurabin II associates with PP1. DCX, neurabin II, and PP1 are found in the same protein complex when PP1 is pulled down with PP1-specific microcystin-agarose beads and also when DCX is immunoprecipitated from mouse brain extracts. ►JNK inhibitors and JNK ►site-directed mutagenesis (T331, S334) in DCX reduced the interaction between DCX and neurabin II in glioma cells and between DCX. JNK activation by MKK7–JNK1 increases DCX phosphorylation and its interaction with neurabin II in glioma cells. Phosphatase inhibitors reduce the interaction between DCX and neurabin II and also inhibit DCX–neurabin II–PP1 complex formation. Thus, phosphorylation and dephosphorylation both may be required for interaction between DCX and neurabin II and DCX–neurabin II–PP1 complex formation. Interaction between phosphorylated DCX and neurabin II induces association of DCX, neurabin II, and PP1 in vivo. PP1, one of the key eukaryotic serine/threonine protein phosphatases, is involved in the mitotic dephosphorylation of the ►retinoblastoma gene (pRb), as well as in the dephosphorylation of specific residues of p53, and regulates the control of cell cycle progression. PP1 dephosphorylates DCX specifically on amino acid residues S331 and T334 both in vitro and in vivo. Microinjection of PP1-neutralizing antibodies and PP1 inhibitors such as ►okadaic acid blocks ►mitosis and alters the progression of the ►cell cycle by accumulating at the nucleus to

associate with chromatin during G2 and M phases. DCX overexpression blocks the G2-M phase of the cell cycle in glioma cells. DCX-mediated growth arrest in the G2-M phase of the cell cycle in glioma cells may be through inactivation of PP1 by neurabin II–DCX interaction. The expression of DCX and neurabin II is dynamic, and they are coexpressed in migrating neurons. Overexpressing the ►coiled-coil domain of neurabin II leads to interaction with DCX, recruits the endogenous neurabin II with PP1, and induces dephosphorylation of DCX on one of the JNK phosphorylated sites. In vitro, DCX is site-specifically dephosphorylated by PP1 without the presence of neurabin II. Overexpression of phosphorylated DCX, therefore, itself may competitively inhibit PP1 and block G2-M phase of cell cycle progression.

►Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) gene, also known as mutated in multiple advanced cancers (MMAC1), and transforming growth factor-β-regulated and epithelial cell-enriched phosphatase (TEP-1), is a tumor suppressor gene located at chromosome 10q23.3. Deletions of all or part of chromosome 10 are the most common genetic alterations in high-grade gliomas. ►Loss of heterozygosity on the long arm of chromosome 10 is found in 75–90% of high grade gliomas. PTEN maps to chromosome region 10q23 and is mutated by the most common genetic alteration of loss of heterozygosity in high-grade gliomas. Transfection of wild-type PTEN into PTEN-deficient glioma cells causes in vitro and in vivo growth suppression. PTEN induces DCX expression in mouse brain subventricular zone precursor cells and even in PTEN-deficient wild-type glioma cells. In addition, DCX siRNA treatment significantly reduces the growth suppression effect in PTEN-overexpressing glioma cells. These data indicate that involvement of DCX in PTEN-mediated tumor suppression is a novel mechanism.

DCX expression induces cell adhesion proteins such as E-, V-, and N-►cadherin in glioma U87 cells at mRNA and protein level. One cell adhesion protein, E-cadherin is widely used as a negative marker of tumor invasion. E-cadherin is typically reduced in levels or absent in invasive tumors, as a result of mutation, transcriptional silencing, or protease-mediated ectodomain shedding. Such loss of E-cadherin function by proteolysis has been demonstrated for several types of protease, including matrix metalloproteinases ►MMPs, ►ADAM10, and ►plasmin. Glioma cells highly express MMPs, particularly MMP-2 and MMP-9. Their high expressions create an environment in favor of ►angiogenesis, cell growth, motility, survival, and eventually proinvasive functions that enable the general spreading of glioma cells into brain. MMPs are proteolytic enzymes that degrade cell–cell adhesion proteins and remodel the ►extracellular matrix (ECM). Induction of E-cadherin by DCX could therefore reverse the effect of invasion of glioma cells. Glioblastomas are the most malignant brain cancers, with a median survival of 10–12 months. Gliomas are the most common type of intracranial tumors and these rapidly invade the brain. Even drastic treatments for glioma such as surgery, radiotherapy, and chemotherapy fail. These treatments have only minimally altered the median survival time of patients with glioma, who eventually die within a year. The identification of the mechanisms of glioma cells invasion into the brain will possibly point toward the therapeutic approach to target the invading cells more specifically and reduce further spreading.

Clinical Relevance

Fundamental questions are how this microtubule-associated protein DCX modifies the growth rate and tumorigenic potential of transformed cells, and whether this growth suppression is also operational in normal cells. These MAPs including DCX are either absent or mutated in tumor cells. The MTs play a critical role during mitosis. The polymerization of MTs into α/β ►tubulin heterodimers produces a complex structure known as the "►mitotic spindle." The process of chromosome segregation is mediated by these mitotic spindles. The depolymerization of MTs is required to segregate sister chromatids on the mitotic spindle. The dynamic instability of MTs is a characteristic property of MTs, which allows them to switch abruptly between state of elongation (polymerization) and rapid shortening (depolymerization). The transition from a state of growth to a state of shrinkage is called "catastrophe" and the transition from a state of shrinkage to a state of growth is called "rescue." The polymerization and depolymerization of MTs depends on activities of two major classes of proteins, the MT-stabilizing and -destabilizing proteins and synchronizes spatially and temporally separation of sister chromatids. The MAPs stabilize the assembled MTs by suppressing catastrophe. The MT-destabilizing proteins are members of the ►kinesin superfamily and the MT-severing proteins. All these proteins can destabilize the assembled MTs by increasing the catastrophe rate of the polymers. These two classes of cellular polymerizers and depolymerizers of MTs play a critical role in the control of cell division. Mutation or absence of either of them in tumor cells disrupts the dynamic instability of microtubules, arrest cell growth in G2-M phase in cell cycle, inhibit cell division, and prevent or delay tumor progression. Similarly, induction of either of them in tumor cells interrupts the dynamic instability of microtubules, and causes accumulation of cells in the G2/M phases of the cell cycle and inhibition of growth and tumor progression. Either an increase or decrease in the level of expression of polymerizers and depolymerizers causes mitotic arrest in the cell cycle.

To complete mitosis in the cell cycle, the level of expression of polymerizers and depolymerizers are therefore required to be balanced. Overexpression of DCX as well as MAP2 leads to microtubule stabilization, cell cycle arrest in G2-M phase, and growth inhibition. Induction of MAPs including DCX in the primary tumor disrupts MT dynamics and spindle check point. Disruption of dynamic instability of MTs can lead to mitotic block, cell cycle arrest. Agent that target MTs such as DCX is, therefore, ideal for treatment of cancer.

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Double-Strand Break DNA Repair

Definition

DNA double-strand breaks are caused by ►ionizing radiation, x-rays or chemicals and can be repaired by two cellular systems, the homology-directed repair (HDR) and the ►nonhomologous end joining (NHEJ).

►Repair of DNA

Doxorubicin

Definition

Synonym ►adriamycin; Is an ►anthracycline drug that interacts with DNA, and induces ►apoptosis or other forms of cell death. It is used in the treatment of a wide range of cancers.

►Rapamycin

DPC4

►Deleted in Pancreatic Carcinoma Locus 4

DPPIV

►CD26/DPPIV in Cancer Progression and Spread

DR4 Antibodies

►TRAIL Receptor Antibodies

DR5

Definition

Is a death receptor involved in ►anoikis and ►apoptosis in cancer cells. It is also known as TNF-related apoptosis-inducing ligand (►TRAIL-R2).

►Carcinoembryonic Antigen

DR5 Antibodies

►TRAIL Receptor Antibodies

Draining Lymph Node

►Sentinel Node

DRF

►Intrinsically Unstructured Proteins

Drg-1

Definition

Differentiation related gene-1 has been shown to be a major inhibitor of tumor growth and ►metastasis. It is a promising new target in cancer chemotherapy that is up-regulated by potent iron chelators.

►Chelators as Anticancer Drugs

Drosophila Melanogaster

Definition

Fruit fly, one of the most widely used model system in developmental biology.

Drug Biodistribution

Definition

The study of the fate of a drug in the body in terms of localization in the tissues.

►Liposomal Chemotherapy

Drug Carrier

Definition

A substance that serves as a mechanism to improve the delivery and the effectiveness of drugs.

►Drug Delivery Systemic-Cancer

Drug Delivery Systems for Cancer Treatment

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Synonyms

DDS

Definition

Drug delivery systems (DDS) are defined as systems that deliver optimal amounts of drugs to a target site, enhancing drug efficacy and reducing adverse effects.

Characteristics

Unconjugated drugs administered in vivo are absorbed, distributed, metabolized, and excreted during the process of delivery to tissues. Therefore, amounts of drugs properly targeting the desired tissues are low and as such difficult to control. Drugs must be administered in large amounts to exert their beneficial effects; therefore adverse effects can occur.

DDS are designed to enhance drug efficacy and reduce adverse effects by controlling drug concentration to a minimum needed at the target tissues. Regulation of the rate of drug release and targeting to specific tissues where the drugs are needed are major objectives of DDS.

If drugs can successfully be administered by a ►sustained release, controlling the release speed and amount of the drug to the targeted site becomes possible and pharmacological therapy can be optimized.

DDS are being actively investigated to develop novel therapies and to optimize therapeutic effects. Drug deliveries using macromolecules, ►liposomes, microcapsules, and ►nanoparticles have been developed. DDS are also finding applications in ►gene therapy.

Advances in developments of DDS would be enhanced with interdisciplinary cooperation among pharmaceutical, medical and dental sciences, biopolymer and material sciences, nanomedicine, tissue engineering, and the science of clinical practices.

Characteristics of Vascular Walls of Cancer

Cancer tissues grow rapidly. To support cancerous growth, ►neovascularization occurs constantly. Newly developing vascular walls are incomplete, and there are multiple slits with widths of several hundred nanometers. Although anticancer drugs adsorbed to micelles or nanoparticles do not penetrate into normal vascular walls, they do penetrate vascular walls in cancer tissues (Fig. 1). Thus, drug carriers of less than several hundred nanometer sizes that are customized for DDS are likely to penetrate to cancer foci through leaky vasculature. As well, ►lymphatic vessels that excrete drugs are poorly developed in cancers, resulting in the retention of administered drugs for longer periods. This phenomenon is called the enhanced permeability and retention effect (EPR effect).

Drug Carriers

Drugs are administered either through the digestive system, intradermal transport system, or by infusion. To achieve targeted delivery of drugs, multiple carriers such as nanoparticles and ►monoclonal antibodies have been developed.

Nanoparticles between 1 and 100 nm have novel electric, optical, and structural properties. Nanoparticles can be used to conjugate various drugs and biomaterials and the resultant conjugated drugs can be customized as ►targeted drug delivery vehicles. This makes it possible to deliver sufficient amounts of therapeutic agents into cancer cells while protecting normal cells from exposure to these drugs.

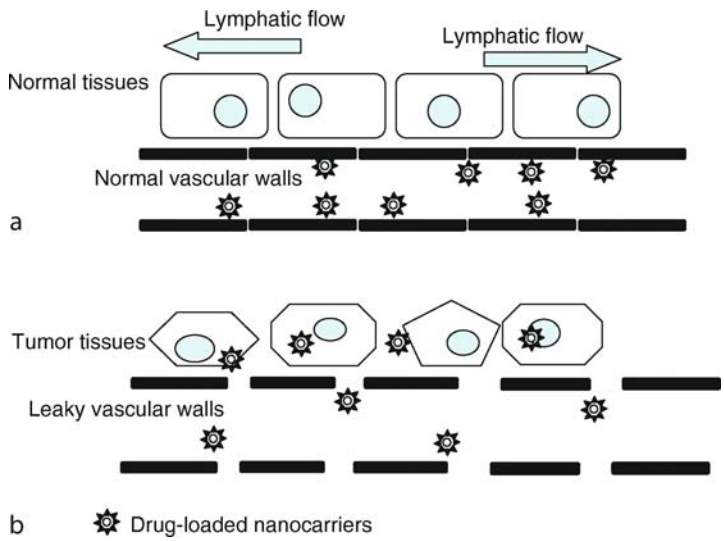
Antibodies are also used as carriers for cytotoxic substances such as drugs, toxins, and radioisotopes. Conjugation of drugs to an antibody changes toxicity and pharmacodynamics in vivo. Positron-emission tomography (PET) is particular useful as an imaging system of cancer cells through the labeling of tumor-associating antibodies using radioisotopes.

Liposomes, micelles, microspheres, and microcapsules also serve as suitable carriers of drugs and have been extensively studied.

Targeted Drug Delivery

Passive targeting takes advantage of the enhanced permeability of tumor vascular walls. Tumor-activated ►prodrug therapy is based on the tumor-specific microenvironment that activates prodrugs in tumor cells.

Active targeting is achieved by conjugating carriers such as nanoparticles with a targeting moiety such as membrane receptors, antigens, and carbohydrates of cancer cells; thus allowing preferential accumulation of the drug at the cancer loci. Vascular cells in cancer and lymphatic vessels in cancer ►metastasis are also good as drug targets.



Drug Delivery Systems for Cancer Treatment. Figure 1 Drug delivery systems for cancer treatment. Enhanced permeability and retention (EPR) effect. (a) Vasculature in normal tissues is tight, preventing drug carriers from penetrating and extravasating. (b) Vascular walls in tumor tissue have multiple narrow slits and are leaky and thus hyperpermeable to drugs. Lymphatic vessels are poorly developed in tumors, causing drug accumulation and retention.

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Drug Design

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Synonyms

Targeted drug design; Drug development

Definition

The process of discovering, inventing, improving and/or testing a new therapeutic agent for the treatment of a specific disease.

Characteristics

Most of the drugs used in classical cancer chemotherapy were discovered by serendipity or by trial and error (►Chemotherapy of Cancer, progress and perspectives). A problem with such drugs arises because, in addition to having a desirable cytotoxic effect on cancer cells, many of these drugs can adversely affect rapidly dividing normal cells, such as gastric mucosal cells lining the stomach or hematopoietic stem cells in the bone marrow. This lack of specificity is a major contributing factor for the severity of side effects of classical cancer chemotherapy, which can include immunosuppression, nausea, vomiting and other gastrointestinal toxicities, and damage to the brain (neurotoxicity), heart (cardiotoxicity), liver (hepatotoxicity), kidney (renal toxicity) and lungs (pulmonary toxicity). The recent trend in anticancer drug development emphasizes the rational design of targeted chemotherapy (►Personalized Cancer Medicine), in which drugs are designed to inhibit a critical molecular target that is essential for the growth and survival of the tumor cell but which, ideally, does not exist in the normal cell.

The prototypical targeted chemotherapeutic agent is ►imatinib, a low molecular weight inhibitor of the protein-►tyrosine kinase BCR/ABL. BCR/ABL is an oncoprotein produced from the fusion of the *bcr* and *abl* genes resulting from a t(9;22)(q34;q11) ►chromosomal translocation. This chromosomal translocation is observed in approximately 95% of adult patients with ►chronic myelogenous leukemia (CML); this subset of patients can be identified by the presence of the ►Philadelphia Chromosome. While imatinib selectively inhibits BCR/ABL, it is not truly specific for this enzyme. In this instance the lack of specificity benefits patients with gastrointestinal stromal tumor (►GIST), because they can be successfully treated with imatinib by virtue of the fact that imatinib also inhibits the tyrosine kinase c-kit (►Kit/Stem Cell Factor Receptor in Oncogenesis). The inappropriate expression of *c-kit* contributes to the development of GIST and therefore provides another target for imatinib. In comparison to classical cancer chemotherapy agents such as ►cisplatin or ►adriamycin, imatinib has a relatively low toxicity profile which can include periorbital edema, dermatitis, or nausea. Following the introduction of imatinib, other protein ►tyrosine kinase inhibitors have been approved for the treatment of cancers. These include the use of epidermal growth factor receptor (EGF Receptor; ►EGFR) tyrosine kinase inhibitors, ►gefitinib and ►erlotinib, for the treatment of ►non-small cell lung cancer. ►Sorafenib, a dual inhibitor of both protein kinase ►Raf and ►VEGFR protein-tyrosine kinase used in the treatment of advanced ►renal cell carcinoma, is another example of successful targeted drug design.

Identification and Validation of Molecular Targets

In principle, drug design entails a progression of distinct stages. The first stage is to identify and validate one or more molecular targets for disease therapy. A molecular target for chemotherapy typically has a causal role in the etiology of the cancer, although some targets have supportive roles in the survival or progression of cancers. Just as ►Koch's Postulates are applied to validate a particular biological agent as a pathogen, so too must analogous postulates be applied in the validation of molecular pathogens. One popular approach to the identification and validation of molecular pathogens is to compare the complex genetic (Genomics), proteinaceous (►Proteomics) and metabolic (Metabolomics) differences between normal and diseased cells in order to find a pathogenic "needle" in a "haystack" of biological information (►Bioinformatics). Interestingly, these studies rarely yield a single unambiguous molecular target. Bioinformatics have typically provided a more complex molecular disease signature involving clusters of biomolecules, and these signatures have identified hitherto unrecognized distinctions amongst cancers which were not distinguishable through conventional diagnostic methods. The recognition of distinct subtypes of diffuse large ►B-cell lymphoma is an excellent example. Bioinformatics theory and its attendant technology and computational methodology are merging in

the evolution of a new scientific discipline called ►systems biology. Systems biology examines not only how cellular components interact to result in cellular structure and function, but also the larger question of how cells interact to result in higher level organization and function. The great potential of these new computational and analytical tools is that they can not only yield new diagnostic and prognostic biomarkers (►Biological Markers), but that they can also be used to validate a molecular pathogen and predict the physiological repercussions of targeting that molecule with a rationally designed drug. The major pitfall is, of course, is that the quality of the results will be strictly dependent on the quality of the data and the validity of the assumptions used to construct the predictive and analytical models. In reality, several of the signal transduction pathways which are the subject of contemporary targeted drug design are imperfectly understood, and this ignorance, far from being synonymous with bliss, has been a root cause for the failure of certain "targeted" drug candidates. In retrospect, it is clear that the success of imatinib against BCR/ABL-dependent CML (Chronic Myeloid Leukemia) occurred because BCR/ABL was thoroughly validated as a molecular pathogen in this disease.

Characterization of Molecular Targets

The second stage of drug design is the detailed characterization of the molecular target. The goal of this effort is to determine whether the target is a "druggable" target. Designers must not only determine whether a target *can* be affected by a drug (in other words, "is it possible?"), but also whether a target *should* be affected by a drug (in other words, "is it prudent?"). Failure to do so will result in the development of a drug that will be ineffective at treating cancer, likely to exhibit unacceptable toxicities, or at worst, both. The molecular pathogens that are best suited for targeted drug design are those that are biochemically distinct from their normal counterparts. Point mutations, chimeric fusions due to chromosomal translocations, post-translational modifications or other unique structural modifications are most likely to yield a good candidate target. The ideal characterization of a molecular target will include the determination of its three-dimensional structure by such techniques as nuclear magnetic resonance (NMR) spectroscopy and x-ray Crystallography, ►Structural Biology which will provide useful insights into the physical interaction between the drug and its target. Designers must also determine whether the candidate target has a functionally distinct role in the cancer cell in comparison to its role in the normal cell (►Pathway Addiction), which further elaborates the information gleaned from the identification and validation phase. By thoroughly characterizing the role of the target in both normal and pathological cellular biology, designers can anticipate potential toxicities that would prove costly if discovered in later development stages. And finally, although it might seem quite obvious, it is important to characterize the biochemical behavior of the target. Most targets are either receptors or enzymes, and one must first understand their kinetic properties before one can compare the efficacy of candidate drugs on the target.

Compound Screening and Optimization

The third and central stage of drug design is that of ►compound screening and ►lead optimization. Before entering this stage, the designer should consider which type of therapeutic agent would be most likely to succeed. The vast majority of drugs are low-molecular weight compounds; once approved for market use, these drugs typically are typically cheaper to produce. On the other hand, low-molecular weight compounds tend to interact with several other non-targeted biomolecules, which commonly causes undesirable adverse effects and toxicities. New therapeutic agents include ►recombinant therapeutic proteins and recombinant ►humanized monoclonal antibodies (see also ►Monoclonal Antibody Therapy). The complex structures of these agents provides the basis for their target specificity, such that if the biology of the target is clearly understood, then these agents are least likely to produce unanticipated adverse effects and toxicities and are most likely to succeed in late stage ►clinical trials. Their primary disadvantage is that, at present, they are exorbitantly expensive. In theory, ►gene therapy holds the promise of correcting the root cause of cancers and other diseases. In reality, the utility of gene therapy is severely hampered by the fact that current methods of gene delivery are inefficient, non-specific, poorly understood at the mechanistic level, and have consistently resulted in unanticipated adverse effects in human subjects.

The goal of compound screening and lead optimization is to rationally determine which structural attributes of a molecule are most important for its usefulness with respect to the target, and to modify the molecule until the best possible drug candidate has been generated. Many targeted anticancer drugs serve as inhibitors of crucial enzymes (►Tyrosine Kinase Inhibitors, ►Proteasome Inhibitors), so it is simplest to discuss compound screening and lead optimization in the context of inhibitor design. Compound screening, also referred to as the discovery phase, involves the rapid systematic evaluation of candidates contained in natural products libraries or combinatorial libraries. Given the large numbers of compounds present in such libraries, robotic processing is required for high-throughput screening (HTS). High-throughput enzyme assays, interaction assays and

cell-based assays must be designed with careful attention to accuracy, precision, sensitivity, specificity and reliability. Methodologies such as ►homogeneous time-resolved fluorescence (HTRF), ►surface plasmon resonance, and ►luciferase reporter assays are commonly incorporated into high-throughput screening assays because of such considerations. Ultimately, the quality of the data depends on the quality of the assay design, and miniaturized design can result in maximized errors. Statistical parameters, such as the ►Z-factor, have been developed to evaluate the quality and statistical reliability of high-throughput assays. In addition to considering the accuracy and sensitivity of cell-based HTS, one must ensure that the HTS strategy can distinguish between generalized toxicity versus target-selective effects before identifying active compounds (or "hits") in a drug library. Once a set of "hits" has been identified, the process of ►lead optimization begins. Lead compound optimization and pre-clinical evaluation form a reiterative loop (which is a formal acknowledgement of the need to "go back to the drawing board"). The chemical structures of the "hits" are ranked according to their HTS-ascribed functional profiles in an effort to define one or more appropriate ►pharmacophores, which provides a general molecular scaffold containing core functional domains that determine the drug's physicochemical and biochemical properties. When the structure of the target is known, computational algorithms such as DOCK can be applied to predict the conformation and orientation of a drug (or pharmacophore) bound to its target, although these predictions should be confirmed by biophysical methods such as Nuclear Magnetic Resonance (NMR), x-ray Crystallography. Computational ►Quantitative Structure Activity Relationship (QSAR) analysis can then be applied to predict which structural modifications might improve the pharmacophore's properties. While a great deal of emphasis is placed on improving the potency of a drug, other properties can have a greater impact on the success of a drug. These properties include aqueous solubility, lipophilicity, pK_a and metabolic stability, which will determine its ►pharmacokinetics/►pharmacodynamics (PK/PD) profile. They also include the drug's specificity or selectivity for a given target, as well as its safety profile. There is a great interest in predicting the ADMET profile (Absorption, Distribution, Metabolism, Excretion, Toxicity, ►ADMET screen) of a drug before engaging in expensive and time-consuming clinical evaluations. It has been estimated that half of all drug development failures are attributable to unsatisfactory pharmacokinetic/pharmacodynamic properties and unacceptable animal toxicities, so the development of cost-effective HTS assays and computational methods to accurately predict ADMET profiles are of the utmost importance to the pharmaceutical industry. Ultimately, the efficacy of a drug is more important than its potency, and the overall drug development process will be most efficient if well-designed lead compounds emerge from this stage.

Pre-Clinical Optimization

The fourth stage of targeted drug design, pre-clinical evaluation, should be considered separately from the screening and optimization stage, although the results of initial pre-clinical evaluations often require a re-optimization of the lead compound. This is especially true when unsatisfactory pharmacokinetic/pharmacodynamic properties or unacceptable toxicities (►Pharmacokinetics/►Pharmacodynamics), ►ADMET Screen are encountered during animal testing studies. Pre-clinical evaluation of a lead compound is the assessment of whether the compound can be safely administered to an animal to induce a therapeutic effect on the disease of interest. In rare instances, animals can be pre-disposed to develop a disease through genetic engineering, or the disease can be chemically induced. If the disease is communicable, it can be induced in the animal by exposure to the appropriate pathogen, as is sometimes the case with cancers caused by viruses. The most commonly used animal in contemporary pre-clinical evaluations of anti-cancer drugs are immunosuppressed, ►SCID, or ►nude mice containing a transplanted tumor of human origin, also known as a xenografted mouse (►Xenograft). This allows one to determine whether the drug can be administered to the animal in a fashion that will lead to regression or eradication of the tumor without causing unacceptable adverse or toxic effects. However, given the compromised immune status of xenografted mice, which forces them to live in an unrealistic sterile environment (not to mention the fact that mice have whiskers and a tail not normally found in humans), such pre-clinical studies do not provide a sufficient evidence to judge whether the lead compound should be safe and efficacious in humans. If a lead compound still appears promising after pre-clinical evaluation in xenografted mice, then it should be administered to other animals to improve the ADMET characterization profile. Other animals commonly used in pre-clinical evaluations of drugs include rat, rabbit, dog and monkey. Theoretical and practical considerations are taken into consideration in designing such experiments; the cost per animal and the physiological similarity of a given animal to humans are two of the most important considerations. Internationally accepted ►Good Laboratory Practices, (GLP) have improved the consistency of pre-clinical toxicology data, and improve the level of confidence in data generated by external laboratories. Such pre-clinical evidence will be carefully scrutinized before regulatory agencies will approve the administration of the first dose of a new drug in humans.

Clinical Evaluation

The fifth and final stage of drug design is the clinical evaluation of the drug. Once the drug has been demonstrated to be safe and efficacious in the treatment of humans, the manufacturer can petition the appropriate government authority for approval to market the drug. The design of clinical trials and the analysis of data from these trials must be impeccable, because clinical trial evidence is of paramount importance in the decision to approve or disapprove the marketing of a drug. Furthermore, sloppy design and analysis of clinical trials can be used in litigation against a manufacturer if a drug is shown to cause harm to patients after it has been approved for market use. Several issues must be considered before clinical trials can begin. First, it is essential to manufacture the drug under current ►Good Manufacturing Practices (GMP) to ensure that it is of consistent quality and purity before administering it to humans. Second, proposed protocols must be independently reviewed to ensure the protection of human subjects, and all investigational personnel must be properly trained in and committed to ethical conduct. Finally, if it has not already been addressed, biomarkers for patient inclusion or exclusion should be incorporated into the clinical trial design. By requiring the detection of ►HER-2/neu in breast cancer biopsies, rather than merely the diagnosis of breast cancer, as an inclusion criterion for human test subjects, investigators were able to demonstrate that ►Herceptin was efficacious in the treatment of certain breast cancers. Retrospective analysis showed that such a demonstration would not have been possible with less stringent inclusion criteria. Similarly, the presence of the Philadelphia Chromosome provided a crucial biomarker for patient selection in the evaluation of imatinib for the treatment of CML (Chronic Myeloid Leukemia). Clinical trials are conducted in three phases. Phase I clinical trials are the initial tests of a new drug in humans. Phase I trials are primarily designed to establish the dose-limiting toxicities of a new drug and to determine its ►maximum tolerable dose (MTD) as a gauge of the dose to be used in the next study phase. Phase II trials are designed to determine whether a new drug is effective against the disease in question, and to establish its effective dose. There is a growing recognition that the ►biologically effective dose (BED) is a more relevant parameter than the MTD for the evaluation of molecularly targeted drugs, and of the need for useful biomarkers to assess the BED. Phase III trials are designed to compare the efficacy of the new drug relative to the best established therapy for a given disease and to examine whether there are any unexpected adverse effects or toxicities in the general patient population. Phase III studies typically involve multiple study arms and are the volunteers are divided into "control" and "experimental" groups. In each of the above, the decision to progress to an advanced phase or to discontinue the evaluation process is predicated on the success or failure in an earlier study phase, and in each case the pool of participating human volunteers will increase in size to accommodate the increasingly rigorous statistical requirements of advanced phase trials.

Ideally, success in each of the preceding five stages of drug design will facilitate the approval of a new anticancer drug for use in the medical market. The work of the drug designer is not complete at this stage, however. Seemingly inevitable problems of drug resistance (►Chemoresistance) will emerge, and if the cause of resistance is known, this problem can be overcome by returning to the stage of lead optimization. Such was the case with the development of ►dasatinib for the treatment of patients with CML (Chronic Myeloid Leukemia) resistant to imatinib. Also, as new drugs are administered to larger and more genetically diverse patient populations, subtle population-based differences in drug efficacy and toxicities begin to emerge. It is hoped that these differences will be positively exploited to achieve the ideal goal of ►personalized medicine, which will require the application of ►pharmacogenomics to the process of drug design. On a final note, it should be remembered that while the process of drug design has been described as a logical strategy, serendipity still plays a major role both in the discovery of new drugs and in the discovery of new applications for old drugs. As Louis Pasteur once said, "Dans les champs de l'observation le hasard ne favorise que les esprits prepares" ("In the fields of observation chance favors only the prepared mind").

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Drug Development

►Drug Design

Drug Latency to Withdrawal

Definition
The time expired between the last use of a drug and the onset of withdrawal symptoms.

►Nicotine Addiction

Drug Metabolism

►Detoxification

Drug Resistance

Definition
Is a major problem in the treatment of many cancers. There is overwhelming evidence that the overexpression of several detoxication proteins, particularly ►GST, ►MDR and ►MRP, contribute to the drug resistant phenotype. It is the biochemical mechanism whereby cancer cells fail to respond to ►chemotherapy, such that there is growth of cancer despite therapy. There are many mechanisms, but the final endpoint is that cancer cells are unresponsive to therapy.

►Amplification
►Detoxification

Drug Resistant

Definition
A characteristic of many cancer cells that are insensitive to one or more anticancer drugs.

►Drug Resistance

Drug Targeting

►Targeted Drug Delivery

Drug Tolerance

Definition
Diminishing impact over time of repeated doses of a drug, or the requirement to use large doses to obtain the same effect.

►Drug Resistance

Dry Eyes Syndrome

►Sjögren Syndrome

Dry Mouth Syndrome

►Sjögren Syndrome

Dsg2

►Desmoglein-2

DSS1

Definition
Deleted in split-hand/split-foot malformation protein 1 (DSS1) is the protein proposed to be aberrant in the ►autosomal dominant form of the split-hand/split-foot malformation and directly interact with BRCA2.

►Breast Cancer Genes BRCA1 and BRCA2
►Gankyrin

DT40

Definition

Due to its extraordinary high rate of homologous recombination and gene targeting activities, the chicken ►B-cell lymphoma line DT40 has become an important experimental system to address various areas in cell biology by combining cellular and biochemical read-outs with gene targeting approaches. Research areas using DT40 cells as an important model system include DNA repair, somatic hypermutation, signal transduction, mitosis, cell cycle regulation, chromatin dynamics, RNA processing etc. DT40 cells are regarded as immortalized immature B cells (bursal stem cells), which are prevented from further differentiation. The transformation event, which gave rise to this cell line, was caused by insertion of the Avian Leukosis Virus into the ►MYC locus thereby causing overexpression of the c-Myc protein, which in turn mediates the differentiation block of DT40. The exact molecular basis for the high rate of homologous recombination between exogenous DNA and the genome is still elusive, but is probably linked to the retained capability of DT40 lymphoma cells to continue ►immunoglobulin gene diversification by gene conversion. DT40 cells are easy to transfect, which suits them for transient and stable expressions of transgenes in order to perform rapid complementation analyses of ►loss-of-function mutation.

►B-Raf Signaling
►Diffuse Large B-Cell Lymphoma

Ductal Carcinoma In Situ

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Synonyms

Intraductal carcinoma; Pre-invasive breast cancer; Non-invasive breast cancer; In situ breast cancer; DCIS

Definition

Ductal Carcinoma *in situ* (DCIS) is a pre-invasive ►breast cancer. The malignant cells remain confined behind an intact basement membrane. The tumor can spread locally along the breast ducts (up to 20 or so) that form the breast, but the DCIS does not possess the ability to invade into surrounding structures or spread to distant sites. If left, over time, a proportion of cases will develop into an invasive breast cancer, which does have the ability to spread and metastasize.

Characteristics

Diagnosis

Over 90% of DCIS that is diagnosed cannot be felt on breast examination and is completely asymptomatic; it is often only detected at screening mammograms. Approximately 70% of these mammographically detected cases present as microcalcifications. Atypical mammographic features include circumscribed nodules, ill defined masses, duct asymmetry and architectural distortion. These screening-detected cases are frequently small (<4 cm) and localized, and are able to be treated by breast-conserving surgery. The remaining 10% of cases are symptomatic, presenting with either a palpable breast lump, nipple discharge or ►Paget disease of the nipple. If these symptoms are present, the underlying disease is usually extensive and will require a ►mastectomy.

Diagnosis can only be confirmed by core biopsy, which takes a cylindrical whole core of tissue through a biopsy-gun. It is performed under local anesthetic and can be done at an out-patients clinic. This is preferred to fine needle aspiration cytology, where a smear of cells are obtained through a finer needle, as this gives no information on whether the basement membrane is intact or if there is evidence of invasion (indicating an invasive breast cancer not DCIS). X-ray or ultrasound image guidance, to ensure the accuracy of sampling of these mainly impalpable lesions, is crucial. If the area of DCIS is extensive (>4 cm in size), multiple areas of the lesion need to be biopsied pre-operatively to ensure that there is no invasive component to the disease and that all the microcalcifications are truly DCIS.

If the definitive diagnosis cannot be made with core biopsy then a surgical ►open biopsy will be necessary, usually under a general anesthetic. The area of concern is localized with fine wires placed pre-operatively under X-ray or ultrasound guidance, to direct the surgeon to the area requiring excision. The excised specimen is X-rayed immediately, after careful orientation with radio-opaque clips, to confirm that all the microcalcifications have been excised. These wire-guided localization procedures are often therapeutic rather than diagnostic and no further procedure is necessary.

Risk Factors

Risk factors for developing DCIS include a family history of breast cancer, older age at first childbirth and never being pregnant. There is no conclusive evidence to date that either the oral contraceptive pill or

►hormone replacement therapy (HRT) increases the risk of DCIS.

As DCIS itself has no ability to metastasize, the main reason to treat DCIS is that a significant proportion will progress onto become an invasive cancer. It is a subsequent invasive cancer that has the ability to spread into local tissue, the lymphatic system, the blood and distant organs, unlike pure DCIS. Screening mammography may detect some cases of DCIS that would not progress to an invasive cancer, but this number is small compared to the potential benefit of detecting more aggressive disease that would in time become an invasive breast cancer and develop the potential to spread.

Classification

DCIS is classified into two major subtypes according to the presence or absence of comedo necrosis. A case of DCIS is termed to be comedo necrotic when necrotic material is seen to fill at least one duct when the specimen is looked at under a microscope. The necrotic material often calcifies and is subsequently visible at mammography.

Non-comedo tumors encompass all the other subtypes of DCIS:

- Solid – where tumor fills extended duct spaces
- Micro-papillary – where tufts of cells project into the duct lumen perpendicular to the basement membrane
- Papillary – where the projecting tufts are larger than in micro-papillary and contain a fibro-vascular core
- Cribriform – where the tumor takes on a fenestrated/sieve-like appearance
- Clinging (flat) DCIS – where there are variable columnar cell alterations along the duct margins

Rarer subtypes also exist including neuro-endocrine, encysted papillary, apocrine and signet cell. In addition, the UK and EU-funded breast-screening programs use the system of low, intermediate and high nuclear grade to classify DCIS. This definition is based on the characteristics of the lesion as seen with a high power microscope lens (×40) and uses a comparison of tumor nuclear size with normal epithelial and red blood cell size. If a lesion contains areas of varying grade it is awarded the highest grade present.

Poorly differentiated high-grade comedo DCIS has low ►estrogen receptor (ER) expression, high rates of cell proliferation, high rates of ►apoptosis (programmed cell death), and over-expresses c-erbB-2 (►HER-2/neu) and ►EGFR. Low-grade lesions have high ER expression, with lower rates of cell proliferation and apoptosis than high-grade lesions and they rarely express c-cerbB-2. ►Progesterone receptor (PR) expression correlates with ER expression in both low and high-grade tumors. In comparison, normal breast epithelium has a low expression of ER, PR, and a very low rate of apoptosis and c-erbB-2 expression.

Treatment

Breast-conserving surgery is now the treatment of choice for small, localized areas of DCIS, <4 cm in diameter; where only the segment of breast containing the tumor is excised. When the specimen is examined under a microscope if the DCIS extends close (<1 mm) to the edges of the specimen the patient should undergo cavity re-excision, as clear margin status is a key factor in predicting a good prognosis.

The multi-disciplinary consensus conference on the treatment of DCIS (1999) recommends mastectomy for patients with large areas of DCIS (>4 cm), for multicentric disease and for patients where ►radiotherapy is contraindicated. Women should also be offered mastectomy if the excision margins are persistently involved following breast-conserving surgery and cavity re-excision. The recurrence rate following mastectomy for DCIS is <1%.

The incidence of macroscopic lymph node metastasis in DCIS is <1%, and formal axillary staging (removal of lymph nodes from the armpit) in women with DCIS should be avoided.

In the USA all patients who have undergone breast-conserving surgery for DCIS are recommended to receive a course of radiotherapy. In Europe, ►adjuvant radiotherapy is recommended for all high-grade DCIS. Intermediate and low-grade DCIS is selected for adjuvant radiotherapy on an individual patient basis.

The use of drug therapy following treatment for DCIS is not yet standardized. The anti-estrogen ►tamoxifen can be used in ER positive women who have undergone breast-conserving surgery; its use is decided upon on an individual patient basis. There is little point in using tamoxifen in ER negative cases. ►Chemotherapy is not offered to women with pure DCIS regardless of type.

Following primary treatment for DCIS and radiological and pathological confirmation that there has been complete excision of all suspicious microcalcifications with clear margins, patients should be given the opportunity to participate in clinical trials. Follow-up in out patient clinics, after the initial post-operative reviews, should be by annual bilateral mammography to detect recurrence. Although most DCIS recurrence is impalpable, clinical examination is still important to detect invasive recurrences.

DCIS in Men

DCIS accounts for up to 15% of breast cancers in men. It mainly presents clinically with symptoms of a cystic-type mass behind the areola or bloody nipple discharge. The standard treatment for DCIS of the male breast is total mastectomy with excision of the nipple areola

D

complex. The percentage of men with DCIS that go onto develop an invasive cancer is not known.

Recurrence

Patients with a recurrence of DCIS, where the primary was treated with breast-conserving surgery alone, can be offered re-excision (ensuring clear margins) followed by post-operative radiotherapy. Patients who have already received radiotherapy following their primary excision should be advised to have completion mastectomy. A skin-sparing mastectomy with Latissimus Dorsi myocutaneous flap ►breast reconstruction gives excellent results. The management of invasive recurrence is again dependent on the initial therapy for DCIS. If the patient did not receive radiotherapy after initial DCIS excision then wide local excision and radiotherapy may still be an option depending on the size and location of the invasive tumor. If wide local excision is not an option then mastectomy and axillary staging is the treatment of choice, with adjuvant therapy dictated by standard protocol as for primary invasive cancers.

The overall recurrence for breast-conserving surgery alone is ~25% at 8 years follow-up, with up to 50% of recurrences (i.e. 12.5% of all cases) being invasive disease. The women who develop invasive disease are at risk of metastatic spread. The remaining 50% of women develop further *in situ* tumors, which by definition, does not metastasize.

A fundamental risk factor for recurrence is inadequate excision following breast-conserving surgery. This is judged as either close (<1 mm), or involved margins and by failure to remove all suspicious microcalcifications.

High-grade tumors and tumors showing comedo necrosis are independent risk factors for recurrence. The degree of tumor differentiation is also predictive of local recurrence. A further risk factor for recurrence irrespective of tumor grade or type is a young age (<40 years) at diagnosis. None of the major trials have found any statistical significance between recurrence and tumor size.

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Ductal Carcinomas

Definition

A cancerous lesion delimited to the intraepithelial compartment of the ductal structure of the breast.

►Estradiol
►Ductal Carcinoma in situ

Duffy Antigen Receptor for Chemokines

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Synonyms

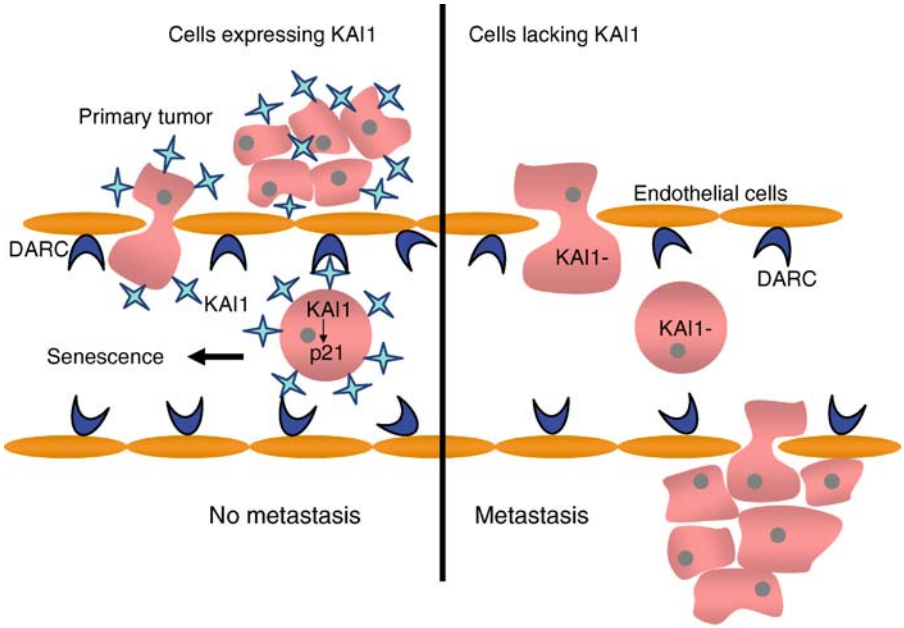
DARC; gp-Fy

Definition

DARC or Duffy antigen receptor for ►chemokines is a seven-transmembrane protein, which has a molecular weight of ~45k Da. DARC is expressed on vascular endothelium (plasma membrane and caveolae) as well as on red blood cells. It binds to chemokines of both C–C and ►C–X–C motif chemokines but signal transduction through the G-protein-coupled receptor or Ca^{2+} flux is not observed. The genomic organization of the *DARC* gene consists of two alleles Fya and Fyb that are associated with a polymorphism at the 44-amino acid residue.

Characteristics

DARC is a receptor for malaria parasite *Plasmodium vivax*, and 70% of the West African population that lack DARC expression on their erythrocytes are resistant to malaria infection. A novel function of DARC has been identified where it has been found to interact with the ►metastasis suppressor *KAI1* CD82. When cancer cells expressing KAI1 intravasate and come in contact with the endothelial lining of small blood vessels during dissemination, KAI1 binds to the DARC expressed on the endothelial cells. The physiologic outcome of this interaction is associated with the inhibition of cell proliferation and induction of cellular ►senescence. DARC constitutes two alleles, Fya and Fyb, and the Fyb allele of DARC is found to be associated with the



Duffy Antigen Receptor for Chemokines. Figure 1 Mechanism of DARC–KAI1 interaction.

KAI1–DARC interaction (Fig. 1). This KAI1–DARC interaction led to the cellular senescence by modulation of the expression of *TBX2* and ►*p21*(WAF1/CIP1/SDI1) genes. Furthermore, the metastasis suppression of KAI1 was significantly compromised in DARC knockout mice, which show that DARC is essential for the function of KAI1 as a metastasis suppressor gene. The majority of West African population, which lacks DARC expression and are resistant to malaria, showed a significantly higher incidence of both ►prostate cancer and ►breast cancer as well as higher rate of metastatic disease than their white counterparts. DARC also serves as a promiscuous receptor for chemokines and is believed to function as "decoy" of excess chemokines. Therefore, DARC is also proposed to have an antitumorigenic role by clearing the angiogenic CXC chemokines. More evidence of DARC's antitumorigenic property has been observed in recent times, where DARC-►knock-out mice were shown to have a significant increase in incidence of prostate tumor in comparison to the wild-type mice. Overexpression of DARC in breast cancer cells significantly suppressed spontaneous lung metastasis. These lines of evidence indicate that DARC functions as an antitumorigenic as well as an antimetastatic protein, and development of drugs that can mimic its function may have a potential utility for the prevention and treatment of cancer.

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DUG

Definition

Rat orthology is Pdcd4.

►Programmed Cell Death 4

Dukes Classification

Definition

Classification of ►colon cancer.

dUTP

Definition

2′-deoxyuridine 5′-triphosphate.

►Replication Factories and Replication Foci

DVL

Definition

Disheveled (Dsh); cytoplasmic protein family activated by Wnt signals; Dvl3 is overexpressed in ►non-small cell lung cancer.

►Wnt Signaling

Dwell Positions

Definition

In ►brachytherapy, the specified locations along a high dose rate (HDR) applicator that the source travels past in a stepwise manner. Conventional dwell positions are defined every 2.5–10 mm along the applicator.

Dynamic Contrast-Enhanced Magnetic Resonance Imaging

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Definition

Dynamic contrast-enhanced ►magnetic resonance imaging (DCE-MRI) is a non-invasive quantitative method of evaluating microvascular structure and function in tumors.

Characteristics

Solid tumors develop a circulatory blood supply of their own by ►angiogenesis to enable growth and survival. Since the vascular networks produce are structurally and functionally abnormal, they offer a potential target for novel anti-cancer therapy. This has led to worldwide interest in identifying and validating ►biomarkers of tumor vascular function that assist diagnosis, inform prognosis or enable the monitoring of treatment effects for both conventional ►chemoradiotherapy and ►anti-angiogenic agents.

T_1 and T_2* DCE-MRI Techniques

Imaging techniques allow repeated measurements of the tumor vasculature to be made in a non-invasive manner. Magnetic resonance imaging, unlike ►positron emission tomography and x-ray computed tomography, does not produce ionizing radiation and is thus considered a safe technique for evaluating functional changes in tumor blood vessels. Two main types of examination can be performed in DCE-MRI. In ►T_1-weighted techniques, repeated images are acquired while an intravenous bolus of ►gadolinium contrast agent traverses the tumor microvasculature. Gadolinium ions are paramagnetic and therefore interact with nearby ►hydrogen nuclei to shorten T_1 ►relaxation times in local tissue water, increasing signal intensity on T_1-weighted images. Signal enhancement within each imaging ►voxel is dependent on tissue perfusion, capillary surface area, capillary permeability and the volume of the extracellular extravascular leakage space (EES), as well as the contrast agent dose, concentration of contrast agent in the artery supplying the tumor vascular bed and native tissue T_1 times. T_1-weighted DCE-MRI is therefore used to estimate the flow, volume and permeability of blood vessels in tumors.

►T_2*-weighted techniques (also known as dynamic susceptibility-contrast MRI) offer an alternative method of investigating the microvasculature. Here, rapid loss of signal intensity observed on T_2*-weighted images is used to calculate the change in concentration of contrast agent for each individual voxel. This method is particularly sensitive to changes in blood flow and volume and assumes that the gadolinium contrast agent remains within the tumor vessels throughout the examination, ignoring the effect of vessel permeability. For this reason, T_2*-weighted methods have generally been limited to studies of the brain where it is assumed that the intact blood-brain barrier limits permeability. While this assumption is untrue in high grade malignancy, numerous DCE-MRI studies of brain tumors employ T_2*-weighted analysis techniques.

Data Acquisition

DCE-MRI protocols are complex. They require considerable technical expertise and interaction between basic

scientists and clinicians. Most examinations are performed on conventional clinical scanners at a magnetic field strength of 1.5 Tesla.

In general, three types of imaging data are acquired in T_1-weighted DCE-MRI. Initial anatomical images are followed by sequences that allow calculation of baseline tissue T_1-values. Finally, dynamic T_1-weighted images are acquired every few seconds over a period of approximately 5–10 min. Most investigators use gradient echo sequences for the dynamic series, as they allow good contrast medium sensitivity, high signal-to-noise ratio, adequate anatomical coverage and rapid data acquisition. Policies for quality control are mandatory to achieve reliable, reproducible data, since DCE-MRI suffers from errors in T_1 calculation, significant motion artefact and difficulties in accurate definition of the tumor region of interest.

Gradient echo pulse sequences are also used in dynamic susceptibility-contrast MRI. Images are ideally acquired every 1–2 s to allow calculation of blood flow and volume. T_2*-weighted techniques are limited by measurement errors and unlike Positron Emission Tomography, usually produce measurements that are relative to large cerebral vessels, rather than absolute values.

Data Analysis

DCE-MRI data analysis may be performed using one or more methods of varying complexity. Most examinations limit the area scanned to around 20 cm or less. Selection criteria for target lesions include size (tumors greater than 2 cm), adequate contrast with background tissue, and organ site free of excessive motion. Initially, a region of interest is defined to cover the entire tumor (for example in three dimensional volume imaging) or part of the tumor (for example one or more two-dimensional slices).

Measures of tumor function are extracted based upon one or more of:

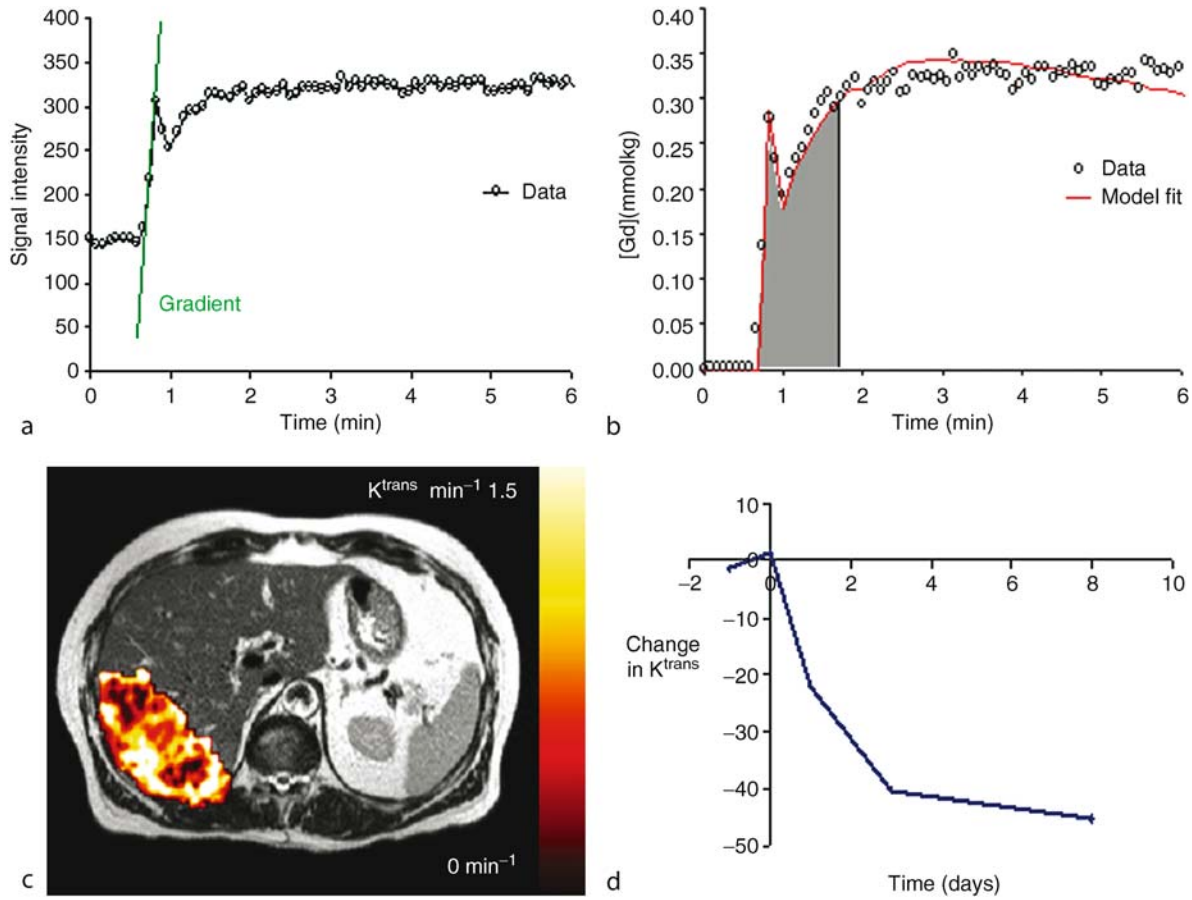
1. Simple features of the signal intensity–time curve (such as fraction of enhancing voxels, gradient or time to 90% peak enhancement).
2. Conversion of signal intensity data into more robust parameters that describe the shape of the contrast agent concentration–time curve. These represent a combination of flow, blood volume, vessel permeability and EES volume (such as ►the initial area under the gadolinium contrast agent concentration–time curve – IAUGC).
3. Fitting data from the contrast agent concentration–time curve to a pharmacokinetic model. This enables parameters that are independent of the data acquisition sequence to be derived.

Modeled parameters enable estimates of physiological characteristics such as flow and capillary endothelial permeability within a tumor; they are more "physiologically meaningful" than either signal intensity or IAUGC measurements. Many related parameters have been described in the literature making comparison between studies difficult. Current expert consensus recommends that the volume transfer coefficient of contrast agent between the blood plasma and the EES (►K^{trans}) and the size of the EES should be preferred in ►clinical trials of Angiogenesis inhibitors using T_1-weighted DCE-MRI, along with IAUGC. ►Cerebral blood volume (CBV) and flow (CBF) are typically derived in brain tumor studies that incorporate T_2*-weighted techniques. Many of the aforementioned quantities have been investigated as potential Biomarkers of tumor biology and drug effect, but are yet to be validated as ►surrogate endpoints.

Finally, a choice of parameter analysis must be made. Median values are most commonly quoted, since both signal intensity and contrast agent concentration within the tumor voxels assume non-normal distributions. Single values may adequately quantify characteristics of tumor tissues, for example median CBV can accurately predict histological ►tumor grading in ►glioblastoma multiforme and discriminate between high grade (higher CBV) and low grade (lower CBV) and identify areas of de-differentiation. However, imaging drug effects in clinical trials of anti-angiogenic agents requires changes in K^{trans} and IAUGC to be calculated from baseline values. Here, percentage change from baseline is frequently quoted. Other forms of data analysis, such as parametric (or calculated) maps demonstrate the spatial heterogeneity of DCE-MRI parameters. Examples of DCE-MRI analyses are illustrated in Fig. 1.

Clinical Findings

DCE-MRI has been used to evaluate tumor microvasculature in numerous clinical studies. Comparison between study methodology and conclusions are difficult since most clinical studies have employed measures of signal intensity, rather than modeled parameters to quantify the microvasculature. Simple signal intensity measures, such as the initial gradient, shape of the enhancement curve and maximum enhancement at 60 s have been shown to reliably distinguish malignant ►breast cancer from benign breast lesions. High and/or increasing relative signal intensity of contrast enhancement in patients with ►cervical cancer before ►radiotherapy has predicted a low incidence of local recurrence. Some centers use DCE-MRI to distinguish between benign bladder wall thickening and ►bladder cancer, to characterize breast cancer and ►hepatocellular carcinoma and to target areas of high grade malignancy in ►glioblastoma multiforme. Despite these encouraging results, DCE-MRI is has few clinical indications at present.



Dynamic Contrast-Enhanced Magnetic Resonance Imaging. Figure 1 Panels a–c illustrate T_1-weighted DCE-MRI data analysis in a patient with a single colorectal liver ►metastasis prior to treatment with an ►anti-angiogenic agent – analysis options include (a) measures from the signal intensity-time curve such as initial gradient, (b) calculated gadolinium contrast agent concentration-time curve with the integrated area under the curve at 60 s after injection (IAUGC), (c) parametric map of median K^{trans} values for each voxel superimposed on an anatomical image. The effect of an anti-angiogenic agent is demonstrated by (d) change in tumor median K^{trans}.

Evaluation of Angiogenesis Inhibitors

One major area of interest in imaging in recent years has been evaluating magnetic resonance based ►biomarkers of changes in tumor microvasculature. An increasing number of clinical trials of ►angiogenesis inhibitors have incorporated DCE-MRI and other forms of imaging in their study design. DCE-MRI is particularly attractive in this setting since anti-angiogenic agents do not typically induce large changes in tumor size, as assessed by traditional imaging endpoints such as ►radiological response criteria.

Significant dose-dependent reductions in K^{trans}, IAUGC and similar parameters have been demonstrated in a number of studies with anti-►vascular endothelial growth factor antibodies and inhibitors of tyrosine kinase ►signal transduction. For example, dose-dependent reduction in a parameter similar to K^{trans} was demonstrated in study of patients with liver metastases from ►colon cancer, following administration of the ►VEGF receptor tyrosine kinase inhibitor vatalanib (PTK787/ZK222584; Novartis, Basel, Switzerland), which correlated with response rate and disease progression. Similar findings have been demonstrated in other studies, where DCE-MRI has been utilized alongside other functional imaging, histology and ►serum biomarkers of tumor pathophysiology.

This study highlights the potential use of DCE-MRI, namely that change in tumor vasculature can be followed at regular intervals in a non-invasive manner. However, it also identifies the shortfalls of the technique – the precise mechanism of action cannot be determined by a technique that measures changes in composite processes at the millimeter scale. Furthermore, while changes in DCE-MRI parameters may be necessary to identify successful therapeutic compounds, they are not sufficient to identify success in a phase III

randomized controlled trial – vatalanib has not produced an improvement in overall survival in patients with metastatic colon cancer when combined with cytotoxic chemotherapy.

Conclusion

The parameters obtained in DCE-MRI analysis undoubtedly provide valuable insight into how the tumor ►microenvironment responds to chemoradiotherapy and novel therapies, but the quantities produced are estimates of composite processes, rather than measurements of physiological mechanisms. Further work is required to validate their role in diagnosis, prognosis and follow-up of solid tumors and as Biomarkers of drug efficacy and mechanism in Clinical Trials.

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Dynamic Instability

Definition

During ►microtubule assembly, hydrolysis of ►tubulin-bound ►GTP destabilizes the internal structure of a microtubule leading to a transition to shortening. This switching between a growing and shortening state of microtubule is referred as dynamic instability.

►Microtubule Associated Proteins

Dynamin

Definition

Is a guanosine triphosphatase (►GTPase) capable of self-assembling into oligomeric "collars" around the necks of coated pits. Dynamin is responsible for the pinching off of a vesicle from the ►plasma membrane.

►Endocytosis

Dysgerminoma

Definition

Most frequent subtype of ovarian malignant germ cell tumors. Dysgerminomas correspond to the testicular seminoma and consist of sheets of immature germ cells with intermixed infiltrating lymphocytes.

►Germ Cell Tumors
►Ovarian teratoma
►Ovarian Tumors During Childhood and Adolescence

Dyskeratosis Congenita

Definition

It is a rare inherited bone marrow failure syndrome associated with abnormalities of the skin, fingernails, and tongue. Other clinical manifestations may include epiphora, lung fibrosis, liver cirrhosis, osteoporosis, and a predisposition to develop a variety of malignancies. The gene responsible for the X-linked form of the disease encodes a protein involved in ribosome biogenesis and in stabilizing the ►telomerase complex, while the ►autosomal dominant form is caused by mutations in the core RNA component of telomerase.

►Stem Cell Telomeres

Dysmenorrhea

Definition
Painful menstruation, usually consisting of lower abdominal cramps.

►Endometriosis

Dysmyelopoietic Syndrome

►Myelodysplastic Syndromes

Dysphagia

Definition
A difficulty in swallowing.

►Nasopharyngeal Carcinoma

Dysphonia

Definition
Altered vocal sounds.

►Nasopharyngeal Carcinoma

Dysplasia

Definition
Is an alteration found principally in epithelial tissues, characterized by a number of changes including loss of single cells uniformity and architectural orientation. Dysplastic cells are highly pleomorphic (variation in size and shape) and often exhibit hyperchromatic nuclei and increase in the nucleus/cytoplasm ratio. Mild-to-moderate dysplasia is often reversible following the removal of causative stimuli. However, severe dysplasia becomes irreversible and may be considered a premalignant lesion due to its ability to progress to carcinoma. Dysplastic epithelium may be precursor of carcinoma and may per se be malignant when associated with direct invasion beyond the muscularis mucosae in the submucosa of the bowel wall. Dysplasia is identified on the basis of a series of microscopic features including architectural alteration of the tissue and cytologic abnormalities, mainly nuclear pleomorphism and hyperchromatism, loss of nuclear polarity, and marked stratification of nuclei. On the skin, dysplasia may appear as a red patch on the mucosa that is not attributable to any obvious cause. Generally, these lesions have a well defined border and a soft, velvet-like appearance. Their atrophic nature contributes to the red coloration, as underlying vasculature is more prominent. Around 90% show signs of severe dysplasia or ►carcinoma in-situ and may progress to invasive squamous cell carcinoma.

►Preneoplastic lesions
►Colorectal Premalignant Lesions
►Squamous Cell Carcinoma

Dyspnea

Definition
Shortness of breath, or difficult or labored breathing.

►Taxotere

Dystroglycan

Definition
Is a membranal protein involved in the ►adhesion of certain cells to the ►extracellular matrix. It is involved in the development of the nervous system and cell adhesion.

►Laminin Signaling
►Extracellular Matrix Remodeling

E1-Activating Enzyme

Definition

An enzyme responsible for activating ubiquitin for the ►ubiquitination enzymatic cascade through the formation of a thiol ester bond.

►Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) De-Ubiquitinase

E2F

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Definition

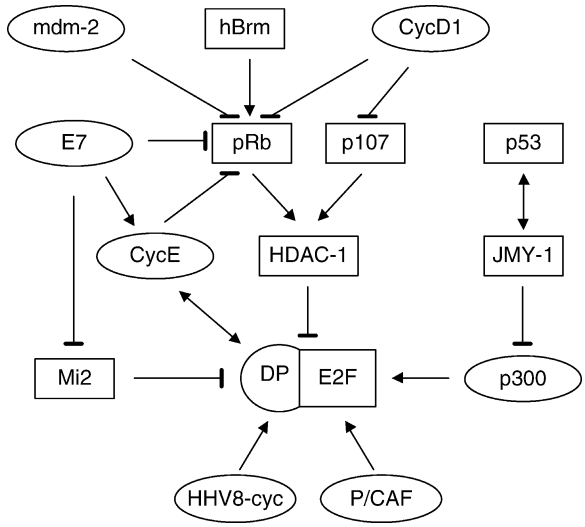
Family of transcription factors involved in cell-cycle regulated transcription; they control the expression of genes coding for growth regulatory proteins.

Characteristics

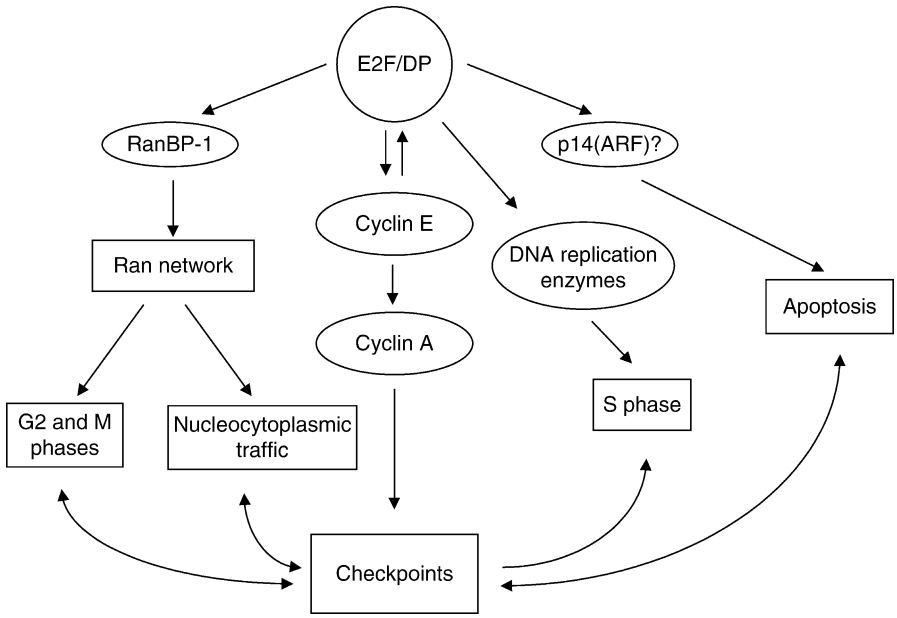
The E2F family of transcription factors has the five members, E2F-1–5, which are capable to either activate or repress transcription, depending on the promoter context and the composition of E2F complexes (see later). A sixth member of the E2F family, referred to as EMA or E2F-6, also binds E2F recognition sites but only acts as a repressor of transcription. Members of the E2F family form heterodimers with members of the DP family. The latter consists of two genes, DP1 and DP2, which encode several isoforms. Heterodimerization is required for DNA binding. E2F/DP heterodimers function as transcriptional regulators of a variety of cellular genes, some of which are involved in cell-cycle checkpoint control.

E2F target genes can be grouped as follows:

- Genes that code for cell-cycle regulators (Fig. 1), such as cyclin E, cyclin A, the ►retinoblastoma protein, p107, and Ran BP1. Expression of these genes is driven by E2F. E2F thereby directly controls the progression from one ►cell cycle phase into the other, particularly at the G1/S and the ►G2/M



E2F. Figure 1 Control of E2F activity in cancer cells. Signals and regulatory pathways that induce or repress the activity of the E2F/DP transcription factors are indicated. The picture contains only proteins with a known function in human cancer; although other proteins that can modulate E2F/DP activity have been identified their role in cancer is not yet clear. The activity of E2F/DP transcription factors is controlled by chromatin-modifying enzymes such as HDAC-1 and Mi2, which block the transcription of E2F-dependent genes by deacetylating histones. In contrast, histone acetyl transferases such as p300 and P/CAF, act as potent coactivators of E2F. These proteins acetylate histones and thereby release E2F-driven genes from chromatin repression. p300 and P/CAF also upregulate the activity of E2F-driven genes by direct acetylation of the E2F partner in the E2F/DP heterodimer. p53 and E2F compete for the adapter protein p300. This interaction is mediated by the currently identified protein JMY1. Finally, human tumor viruses such as HPV-16 or HHV-8, encode genes that directly modulate E2F activity, leading to aberrant activation of E2F target genes.



E2F. Figure 2 E2F regulation of cell-cycle checkpoints. The role of E2F/DP genes in coordination of cell-cycle checkpoint control is schematically depicted. Apart from its role in S-phase entry, E2F acts at the G2/M checkpoint in mammalian cells, which is mediated by Ran-dependent modulation of nucleocytoplasmic traffic. Induction of apoptosis by certain E2F family members is believed to depend on transcriptional activation of the gene encoding p14 (ARF). The major groups of E2F target genes are shown and the interplay of their gene products is indicated by arrows.

transition. E2F also activates expression of the p14 (ARF) gene, an upstream regulator of ►p53.

- Genes that code for enzymes involved in DNA replication, such as thymidilate synthetase, thymidine kinase, dihydrofolate reductase, etc. It is believed that upregulation of these genes prepares cells for DNA synthesis which are in the G1 phase of the cell cycle.

Cellular and Molecular Regulation

- E2F proteins are regulated by cellular tumor suppressor proteins (Fig. 2). Members of the retinoblastoma protein (►Retinoblastoma protein, biological and clinical functions) family (pRB, p107, and p130) associate with the E2F/DP heterodimers, block their transactivation capacity and, in some cases, convert these activators of transcription into transcriptional repressors. The latter conversion appears to involve the tethering of histone deacetylases to E2F/pRB complexes.
- E2F regulates the activation of the tumor suppressor p53 via p14 (►ARF). In turn, E2F function is regulated by p53 via a competition for the p300 coactivator protein (p300/cBP co-activators).
- The function of E2F is positively regulated by cyclin-dependent kinases. This, at least in part, relies on the phosphorylation and the resulting inactivation of members of the retinoblastoma protein family. However, cdk-dependent phosphorylation of the E2F subunit also contributes to E2F activation.
- E2F activity is upregulated by cellular and viral oncogenes, such as ►MDM2, adenovirus E1A, papillomavirus E7, and SV40 T antigen. Activation of E2F by these oncogenes is thought to be mediated by a displacement of the retinoblastoma protein or its relatives from E2F/DP complexes.

Clinical Relevance

E2F-1 acts as a tumor suppressor and at the same time as an oncogene in the mouse, as was exemplified by the complex phenotype of E2F-1 ►knock out mice. It appears that tumor suppression by E2F depends on its interaction with the retinoblastoma protein by enhanced transcriptional silencing. The oncogenic activity of E2F-1 is mediated by free E2F-1/DP complexes which enforce the transcription of E2F-dependent target genes, some of which are required for cell proliferation.

►Amplification of the E2F-1 gene was described in human erythroleukemia cells; the *E2F-5* gene is amplified in some human breast cancers.

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E2F Transcription Factor

Definition
A family of transcription factors that help mediate the G_1/S transition in the mammalian cell cycle. E2F transcriptional targets include ►cyclins, cdks, checkpoints regulators, DNA repair and replication proteins.

►Chelators as Anticancer Drugs
►Cell Cycle Checkpoints
►Repair of DNA

E3 Ubiquitin Ligase

Definition
The last enzyme in the ubiquitination enzymatic cascade responsible for mediating the transfer of the activated ubiquitin from the ►E2-conjugating enzyme to the substrate. This protein also provides substrate specificity for the reaction.

►Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) De-Ubiquitinase
►Ubiquitination

E4 Ubiquitin Ligase

Definition
A protein that kinetically enhances the E3 ubiquitin ligase mediated transfer of ubiquitin to a protein substrate. CBP and p300 possess ►E4 ligase activity. Results in poly-ubiquitin chain assembly.

►CBP/p300 Coactivators

E5

Definition
Short hydrophobic papillomavirus proteins, many of which have transforming activity.

►Bovine Papillomavirus
►Human Papillomavirus

E7

Definition
Oncoprotein required for the full transformation activity of human and bovine papillomaviruses.

►Bovine Papillomavirus
►Human Papillomavirus

E11 Antigen, RTI40

►Podoplanin

E100

►Curcumin

E-Cadherin

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Synonyms
Epithelial cadherin; Uvomorulin; Cadherin-1

Definition

E-cadherin, a 120 kDa molecule, is a prototypical member of the classical cadherin family of single-pass transmembrane proteins that mediate calcium-dependent cell-cell adhesion. Normally, ►epithelial cells are tightly interconnected through several junctional structures, including ►adherens junctions, ►tight junctions, and ►desmosomes. E-cadherin is the main adhesion molecule of the adherens junctions of epithelial cells, and via catenins it is linked to the underlying actin cytoskeleton. Two other members of the cadherin family, desmoglein and desmocollin, mediate adhesion in desmosomes. Via plakoglobin and desmoplakin they are connected to intermediate cytoskeletal filaments. Thus, E-cadherin and other members of the cadherin family play a crucial role in establishing and maintaining the integrity of epithelial tissues.

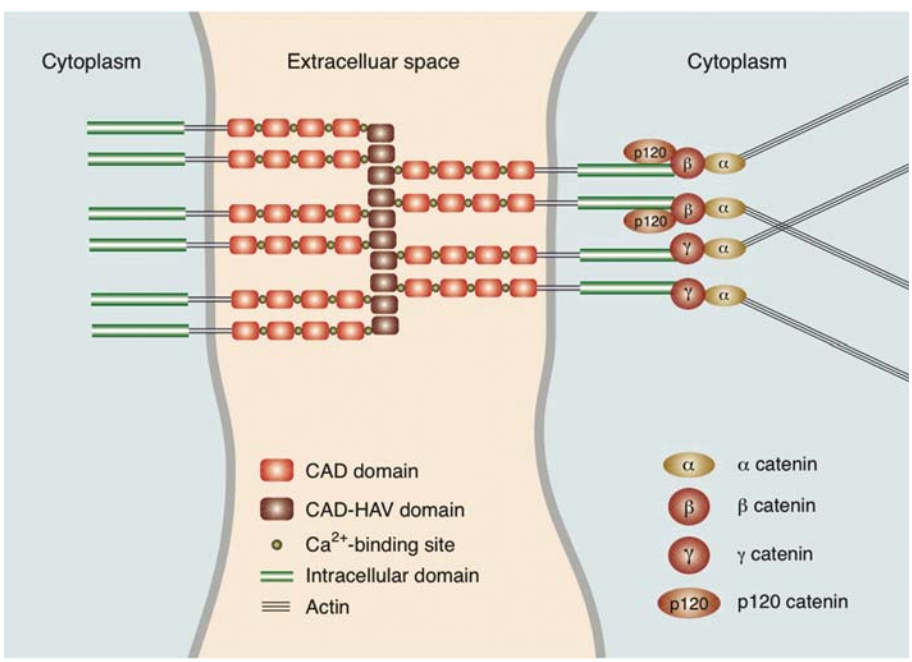
The majority of human cancers (80–90%) are of epithelial origin. Loss of E-cadherin expression correlates with late stage tumorigenesis characterized by tumor cell de-differentiation, invasive tumor growth and metastasis. Moreover, studies in a transgenic mouse model of carcinogenesis have demonstrated that the loss of E-cadherin is a rate-limiting step in the transition from ►benign tumors to ►malignant tumors and the subsequent formation of metastases. In addition, germline mutations of E-cadherin predispose to diffuse gastric cancer.

Characteristics

The extracellular region of E-cadherin consists of five highly conserved cadherin (CAD) domains that combine with calcium ions to form rod-like structures (Fig. 1). The outermost CAD domain contains a conserved His-Ala-Val (HAV) motif, which is thought to mediate homotypic binding *in trans* to an E-cadherin molecule on the surface of an adjacent cell. Dimerization of E-cadherin molecules *in cis* appears to be mediated by their transmembrane domains. Depletion of calcium ions results in the disassembly of E-cadherin mediated adhesive structures and the loss of cell-cell adhesion. Critical for E-cadherin-mediated cell-cell adhesion is the interaction of its cytoplasmic domain with catenins. β-catenin and γ-catenin/plakoglobin associate directly with the cytoplasmic domain of E-cadherin. They bind to α-catenin, which in turn connects the cadherin cytoplasmic complex to the actin cytoskeleton (Fig. 1). An additional catenin, p120 catenin, has been originally identified as a substrate of the non-receptor tyrosine kinase pp60$^{c\text{-}src}$ and binds to the juxtamembrane region in the cytoplasmic tail of classical cadherins. Depending on the cell type, p120 catenin has been found to positively or negatively modulate the strength of cell-cell adhesion.

E-cadherin and the Formation of Adhesion Junctions

Upon contact of the cell membrane with adjacent cells, pre-existing E-cadherin is recruited to the site of the



E-Cadherin. Figure 1 E-cadherin-mediated cell-cell adhesion. E-cadherin homodimers on the plasma membranes of adjacent cells interact in a zipper-like fashion. The most N-terminal CAD domain on each E-cadherin molecule contains a HAV motif thought to interact with an E-cadherin molecule on an adjacent cell. The cytoplasmic cadherin complex, which consists of α-catenin, β-catenin, γ-catenin and p120 catenin, mediates the interaction between E-cadherin and the actin cytoskeleton.

interaction and the junctional complex is formed. Once in place, E-cadherin-mediated cell-cell adhesion can be rapidly dismantled by growth factor-mediated signals. p120 catenin and to a lesser extent β-catenin and γ-catenin are phosphorylated upon treatment of cells with the growth factors hepatocyte growth factor (HGF), epidermal growth factor (EGF) (►Growth factor), or ►platelet-derived growth factor – either directly by the respective receptor tyrosine kinases or indirectly by downstream effector kinases, such as $pp60^{c\text{-}src}$. Phosphorylation of the catenins causes their release from the cell adhesion complex resulting in the disruption of cell adhesion and eventually cell scattering. Similarly, treatment of cells with potent inhibitors of tyrosine phosphatases induces dissociation of the cytoplasmic cell adhesion complex. Deletion of the cytoplasmic catenin-binding domain or any disruption of the intracellular E-cadherin-catenin complex results in loss of cell-cell adhesion. Hence, mutations in genes other than E-cadherin itself might also affect E-cadherin function.

The mechanism through which β-catenin and α-catenin increase the adhesive strength of E-cadherin is not clear. Recent studies indicate that α-catenin may not simultaneously bind E-cadherin/β-catenin and actin, but rather influence the structure of the actin cytoskeleton via ►Arp2/3. The formation of adhesive junctions is also regulated by Rho-GTPases (►GTPase). Forced expression of constitutive-active forms of Rac or Cdc42 inhibits HGF-induced scattering of epithelial cells. Similarly, overexpression of Tiam-1, a guanine nucleotide exchange factor (GEF) for Rac, enhances E-cadherin-mediated adhesion.

Signals Elicited by the Loss of Cell Adhesion

Besides being a major component of the cytoplasmic cadherin complex, β-catenin also takes part in ►Wnt signaling. In the absence of a Wnt-signal, free β-catenin is rapidly phosphorylated by glycogen synthetase kinase 3β (GSK3β) in the adenomatous polyposis coli (APC)-GSK3β complex and subsequently degraded by the ►ubiquitin-proteasome pathway. If the tumor suppressor APC is non-functional, or if GSK3β activity is blocked by the activated Wnt signaling pathway, β-catenin accumulates at high levels in the cytoplasm. Subsequently, it translocates to the nucleus, where it binds to a member of the TCF/LEF-1 family of transcription factors and modulates the expression of TCF/LEF-1 target genes. Loss of function mutations of APC are a hallmark of ►colon cancers and stabilizing mutations of β-catenin are frequently found in a number of cancer types. However, in several human cancer types and experimental tumor models, tumor progression induced by the loss of E-cadherin was shown to be independent of β-catenin. Therefore, β-catenin that is released from the E-cadherin adhesion complex does not necessarily feed directly in the Wnt signaling pathway, unless β-catenin degradation is inhibited.

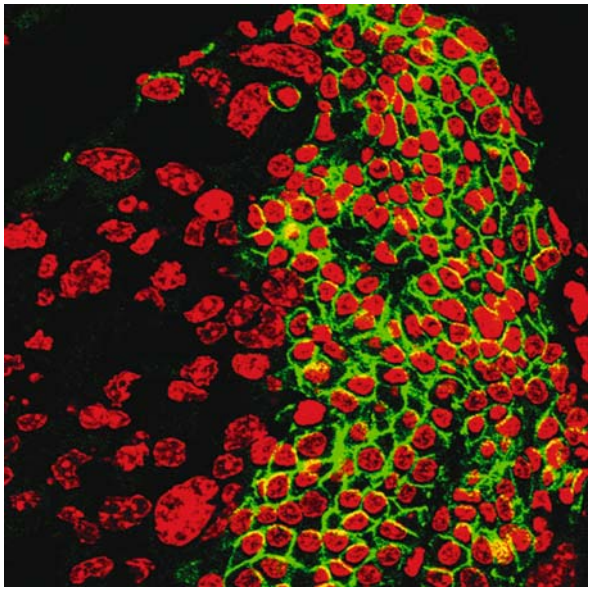
α-Catenin is not only connecting adherens junctions with the actin cytoskeleton but also interacts with several actin binding proteins, including vinculin, ZO-1 (►Zonula occludens (ZO) protein-1) and α-actinin. It is also involved in the communication between cell adhesion and changes in cellular phenotype. As outlined above, E-cadherin and the junctional complex can also interact with Rho-GTPases. Experiments suggest a direct influence of Rac and Cdc42 on the cadherin-catenin complex via the protein IQGAP1 (►IQGAP1 protein). IQGAP1 accumulates at contact sites of cells expressing E-cadherin, where it directly interacts with the N-terminus of β-catenin and the cytoplasmic tail of E-cadherin. Active Rac and Cdc42 prevent the interaction of IQGAP1 with β-catenin by sequestration of IQGAP1. Upon release of IQGAP1 from GDP-bound, inactive Rac and Cdc42, however, IQGAP1 displaces α-catenin from β-catenin, resulting in the dissociation of α-catenin from the cytoplasmic cadherin complex and concomitant loss of E-cadherin-mediated cell adhesion. Thus, Rac and Cdc42 seem to positively regulate cell adhesion by suppression of IQGAP1 activity. Finally, p120 catenin was shown to translocate to the nucleus, interact with Kaiso (a transcriptional repressor), and thereby modulate gene expression. Furthermore, recent evidence suggests that Frodo, a functional regulator of Dishevelled, may link p120 catenin to the Wnt pathway as well.

Apart from signaling through the catenins, the intracytoplasmatic tail of E-cadherin can be cleaved by presenilin1, a γ-secretase, and participate in the regulation of cellular transcription and lysosomal degradation of amyloidogenic proteins.

E-Cadherin in Animal Models of Cancer

The effect of loss of E-cadherin on tumor progression was studied in two animal models of human cancer. In the Rip1Tag2 mouse model of insulinoma, the expression of a dominant-negative E-cadherin (resulting in a complete ablation of E-cadherin-mediated adhesion) promoted the formation of carcinomas and induced lymph node metastasis (Fig. 2). Forced expression of E-cadherin in the same model arrested tumor progression at the stage of an adenoma. Furthermore, the transgenic expression of ►podoplanin, a small mucin-like glycoprotein, led to the formation of carcinomas in the presence of E-cadherin and the other components of the zonula adherens.

In the p53 knock-out ($K14Cre;Trp53^{F/F}$) mouse model of breast cancer, somatic inactivation of E-cadherin shifted the invasion pattern of carcinomas from an expansive (ductal-like) to an infiltrative (lobular-like) invasion pattern and induced the formation of distant metastasis. Interestingly, isolated knock-out of E-cadherin in the mammary epithelium was not sufficient to induce breast cancer formation.



E-Cadherin. Figure 2 Loss of E-cadherin expression during tumor progression. Right hand: E-cadherin (green) is expressed at the plasma membranes of tumor cells with benign, epithelial phenotype and normal nuclei (red). Left hand: In contrast, E-cadherin expression is focally downregulated within the portion of the tumor that exhibits invasive growth and atypic nuclei (red).

Taken together, loss of E-cadherin increases the formation of carcinomas, shifts the invasion pattern from collective to single cell invasion, and thereby promotes metastasis. However, in certain animal models the formation of carcinomas was possible in the presence of E-cadherin, and those carcinomas which expressed E-cadherin tended to invade by adopting an expansive/collective invasion pattern.

E-Cadherin in Human Cancer

E-cadherin's adhesive function can be lost during the development of human epithelial cancers, including carcinomas of the breast, colon, prostate, stomach, liver, esophagus, skin, kidney and lung. In general, decreased E-cadherin function correlates with de-differentiation of tumor cells, infiltrative tumor growth, metastasis and poor prognosis. Several different mechanisms appear to cause the loss of E-cadherin function in human tumors. Hereditary mutations in the E-cadherin gene are evident in cases of familial gastric cancers. Patients with a germline mutation of E-cadherin have a 70–80% lifetime risk to develop diffuse gastric cancer, indicating that the penetrance of the E-cadherin mutation is similar to that of ►BRCA1 carriers in breast cancer.

Considering the penetrance and the aforementioned animal models of cancer, mutation of E-cadherin predisposes to neoplasia, but the mutation alone is not sufficient to induce the disease.

Apart from mutations of E-cadherin, expression of truncated α-catenin or truncated β-catenin abrogate E-cadherin function. Whereas mutations in the α-catenin gene or reduced α-catenin protein levels thus far have only been found in cultured tumor cell lines, mutations in the β-catenin gene are evident in many primary human tumors, including melanoma, colon cancer, gastric cancer and prostate cancer.

Epigenetic mechanisms such as chromatin rearrangement and loss of transcription factor binding also coincide with suppression of E-cadherin promoter activity in invasive carcinoma cells. In many tumor types, ►hypermethylation of the regulatory region of the E-cadherin gene and thus transcriptional silencing of the gene appears to be a major mechanism underlying E-cadherin loss of function. In several cellular systems, the epigenetic silencing of E-cadherin is mediated by the activation of transcriptional repressors. One of these repressors, Snail (►Snail transcriptional factor), has been shown to downregulate E-cadherin expression along with claudin-3, a protein involved in the formation of tight junctions. Finally, proteases that are upregulated during tumor progression are able to degrade the extracellular portion of the E-cadherin molecule resulting in the disruption of cell-cell adhesion and the cytoplasmic cadherin complex.

Other family members of classical cadherins are expressed in epithelia. Recently, it was observed that in several cancer types expression of N-cadherin was upregulated during tumor progression concomitant with the loss of E-cadherin function. This phenomenon was termed "cadherin switch," and is related to ►epithelial to mesenchymal transition (EMT), a process implicated in the transition from benign neoplasia to malignant cancer. In these cases, N-cadherin appeared to induce tumor cell invasion and metastasis.

Clinical Relevance

E-cadherin is used as a diagnostic and prognostic marker for several human cancers. In particular, expression of E-cadherin is lost in most lobular breast cancers, making the lack of E-cadherin a diagnostic criterium for the disease. Moreover, detection of mutations in the E-cadherin gene is diagnostic for hereditary diffuse gastric cancer. Therapeutic approaches that are based on the loss of E-cadherin function will have to await the identification of downstream effector genes that may be more amenable to therapeutic intervention.

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E2-Conjugating Enzyme

Definition

An enzyme for the second step of the ►ubiquitination enzymatic cascade responsible for receiving the activated ubiquitin protein via a thiol group.

►Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) De-Ubiquitinase

E Motif

►E-Box

E-Selectin

Definition

Is a cell ►adhesion protein that is involved in the binding of neutrophils and monocytes to cytokine activated ►endothelial cells. It recognizes carbohydrate antigens that are related to the sialylated lewis x antigens. These antigens are also expressed on the surfaces of cancer cells and can be responsible for cancer cell endothelial cell interactions.

►Kupffer Cells
►CEA Gene Family and Cancer
►E-Selectin-Mediated Adhesion in Cancer

E-Selectin-Mediated Adhesion in Cancer

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Synonyms

E-Selectin; CD62E; Endothelial leukocyte adhesion molecule-1; ELAM-1; Leukocyte endothelial cell adhesion molecule-2; LECAM-2

Definition

►Adhesion of circulating cancer cells to the ►endothelium is a prerequisite for ►metastasis. The specificity of the cancer cell-endothelial cell interactions constitutes the basis of the organ selectivity or homing of metastatic colonization. Subsequently to adhesion, cancer cells form metastases either by growing locally in the capillaries or by invading the surrounding tissue following ►extravasation. This latter process is associated with the induction of a bidirectional signaling that contributes to increase the motile potential of the cancer cells and the permeability of the endothelium.

Characteristics

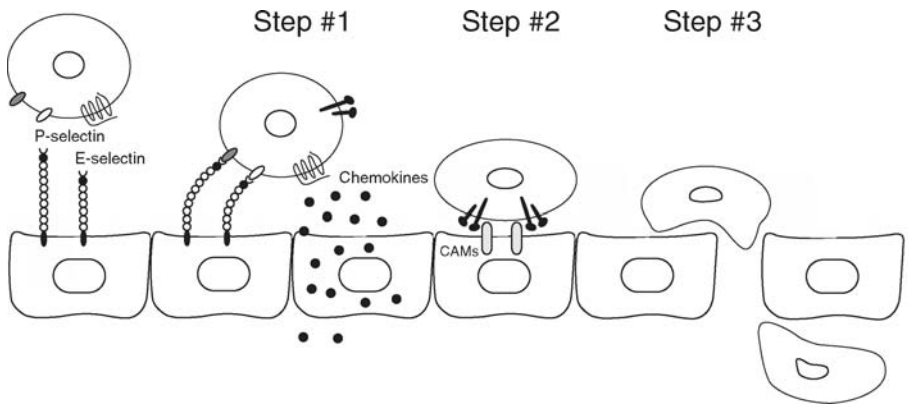
E-selectin is a calcium-dependent transmembrane receptor of the selectin family that also contains L- and P-selectin. L-selectin is expressed constitutively by lymphocytes. P-selectin is found in platelets and endothelial cells where it is stored in α-granules and Weibel-Palade bodies before being translocated at the cell surface by stimulation. E-selectin is expressed exclusively by endothelial cells, following a challenge by pro-inflammatory stimuli such as TNFα and interleukin-1β. The selectins are characterized by an extracellular domain containing a C-type lectin domain of 120 amino acids in the N-terminal followed by an epidermal growth factor (EGF) domain of 30–40 amino acids and 2–9 short repeats of ~60 amino acids found in complement regulatory proteins. The selectins are anchored in the membrane through a single helicoidal transmembrane domain that is completed by a short cytoplasmic tail. The usual role of selectins is to mediate the adhesion and rolling of leukocytes to the endothelium allowing their extravasation into inflamed tissues. Several lines of evidence indicate that cancer cells "parasite" the inflammatory system and interact with selectins to extravasate and form metastases. For example, several studies showed that breast carcinoma, bladder cancer, gastric carcinoma, pancreatic carcinoma, leukaemia and lymphoma form metastases in an

E-selectin-dependent fashion in organs as various as liver, bone marrow, skin and lung. Interestingly, immunohistochemical studies on colon cancer tissues have shown that the small blood vessels surrounding cancer cell nests frequently express E-selectin and that its expression is inversely correlated with the distance of blood vessels from the cancer nests. These results suggest the possibility that E-selectin expression may be involved in ►angiogenesis and that cancer cells have the ability to induce the expression of vascular E-selectin. This hypothesis was confirmed by *in vivo* experiments. For example, Lewis lung carcinoma cells trigger the expression of E-selectin by liver sinusoidal endothelium, increasing their metastatic potential and suggesting that E-selectin contributes to the organ-specificity of metastatic colonization. Along these lines, it is well documented that ►colon carcinoma metastasizes specifically to the liver in an E-selectin-dependent manner. Most interestingly, highly metastatic human colorectal and mouse lung carcinoma cells, on their entry into the hepatic microcirculation, trigger a rapid host pro-inflammatory response by inducing TNF-α production in resident Kupffer cells. In turn, this triggers E-selectin expression by endothelial cells, presumably enhancing the binding and extravasation of the cancer cells. As discussed in the next section, the binding of cancer cells to E-selectin involves a counter-receptor for E-selectin that is composed of ►sialyl Lewis-a/x determinants that are borne by a carrier protein or lipids on cancer cells.

Mechanisms

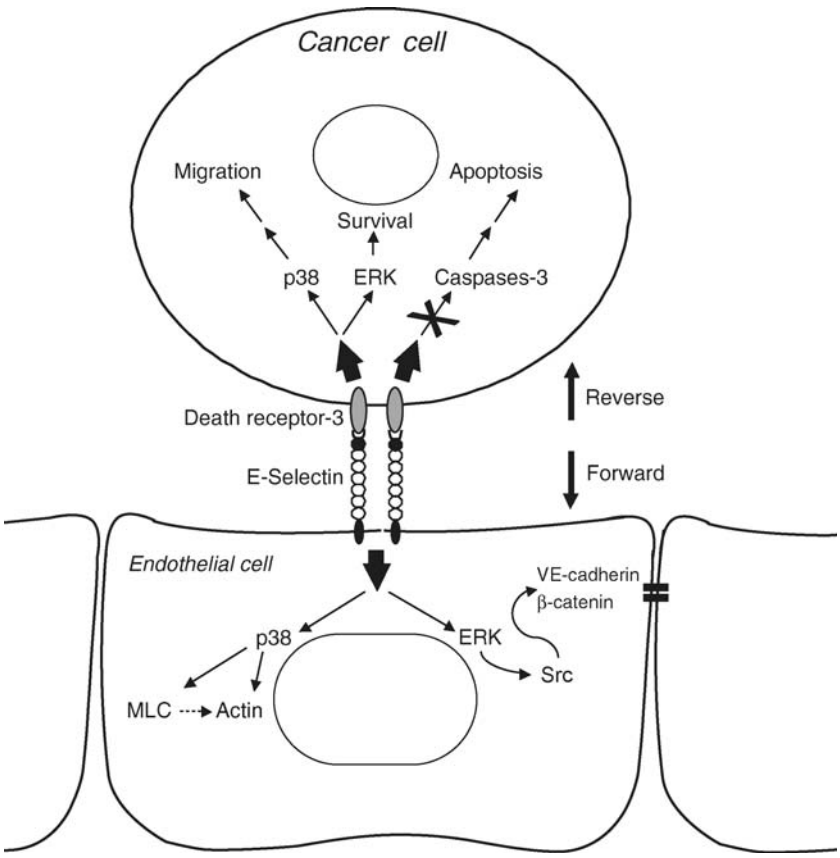
The binding of E-selectin to sialyl Lewis-a/x determinants borne by binding cancer cells is Ca^{2+}-dependent, and it is mediated through the N-terminus lectin domain of E-selectin. This primary adhesion is transient and unstable under shear stress and it allows the rolling of cancer cells on endothelial cells. Moreover, it induces signaling in the cancer cells that may confer them a metastatic advantage, as reported below. The E-selectin-dependent adhering and activated cancer cells are released into the circulation unless secondary adhesion mechanisms are brought into play. In particular, many chemoattractants are produced in response to E-selectin-mediated attachment, and these ►chemokines contribute to activate adhesion receptors in order to mediate firm attachment. Both β_1 and β_2 integrins present on cancer cells are able to modulate the binding to ►cell adhesion molecules like ICAM-1, ICAM-2 and VCAM-1 on endothelial cells. In each step, soluble factors and activated receptors trigger signaling pathways that regulate the endothelial barrier integrity. The destruction of endothelial cell-cell junctions and endothelial ►cell-extracellular matrix junctions combined with retraction of endothelial cells enable extravasation of the cancer cells (Fig. 1). In capillary beds submitted to lower blood pressure, the firmer adhesion that follows the primary adhesion to E-selectin may be associated with the local proliferation of the adhering cancer cells that will form metastatic foci in the blood vessel without extravasating.

Sialyl Lewis-a borne by carrier proteins plays major roles in the binding to E-selectin of cancer cells derived from the lower digestive organs, such as the colon and rectum, as well as from the pancreas and biliary tract. Sialyl Lewis-x is the representative carbohydrate involved in the E-selectin binding of breast, ovarian and pulmonary cancer cells. In contrast, little is known



E-Selectin-Mediated Adhesion in Cancer. Figure 1 Extravasation of cancer cells is a multi-step process. The first step consists in the transient adhesion of cancer cells to the endothelium. It involves endothelial adhesion molecules such as E-selectin and P-selectin and their counter-receptors present on cancer cells. This step is associated with the rolling of the cancer cells on the endothelium. The second step consists in a firmer adhesion of cancer cells to endothelial cells. It is mediated through chemoattractants and adhesion molecules on the endothelium and integrins on the cancer cells. The third step is characterized by the extravasation of cancer cells through endothelial cell-cell junctions.

about the proteins that bear these carbohydrates and that serve as E-selectin counter-receptors on cancer cells. A retrospective study suggests that recombinant P- and E-selectins and sialyl Lewis-x antibodies transmit activation signals through tyrosine phosphorylation of several proteins in Hodgkin's lymphoma-derived cell lines. In turn, this results in changes in cell morphology. On cancer cells from solid tumors, LAMP-1, LAMP-2, ►CD44 and ►death receptor-3 (DR3) were identified as E-selectin ligands. Among them, only DR3 has been described as a functional and signaling sialylated ligand that binds E-selectin on colon cancer cells. The subsequent DR3 activation increases the motile and survival potentials of the cancer cells through activation of the p38 and ERK ►MAP kinase pathways, respectively. On the other hand, the activation of E-selectin induces a forward signaling in endothelial cells that initiates its endocytosis and degradation through lysosomal compartments. In addition, the activation of E-selectin by adhering colon cancer cells or cross-linking antibodies stimulates both ERK and p38 MAP kinase pathways in the endothelial cells. In turn, this contributes to increase endothelial permeability enabling transendothelial ►migration of cancer cells by regulating the opening of the inter-endothelial spaces (ERK) and the contractility of endothelial cells (p38). Overall, these findings suggest that E-selectin-mediated adhesion of cancer cells regulates their metastatic potential by inducing a bidirectional signaling that contributes to increase their intrinsic motile and survival potential as well as the permeability of the endothelium. This mechanism is illustrated in Fig. 2.



E-Selectin-Mediated Adhesion in Cancer. Figure 2 Reverse and forward signaling induced by E-selectin in cancer cells and endothelial cells. Adhesion of colon cancer cells to endothelial cells expressing E-selectin induces a reverse signaling in the cancer cells that increases their motile potential, and a forward signaling in the endothelial cells that increases interendothelial permeability and enables extravasation. For example, we found that adhesion of colon carcinoma cells to endothelial cells involves the binding of E-selectin on endothelial cells to Death Receptor-3 (DR3) on cancer cells. This interaction induces the reverse activation of p38 and ERK MAP kinases in cancer cells, which increases their motile and survival potentials. Reciprocally, the interaction between DR3 and E-selectin triggers the forward activation of the same MAP kinase pathways in endothelial cells. This results in myosin-light chain (MLC)-mediated cell retraction and in dissociation of the VE-cadherin-β-Catenin complex and thereby destruction of *adherens* junctions leading to increased endothelial permeability and extravasation of cancer cells.

Clinical Relevance of E-selectin and its Ligands in Cancer

The finding that cancer cell binding to E-selectin-expressing endothelial cells is associated with metastasis is of great clinical relevance and opens several therapeutic avenues. Since this adhesion process requires expression of endothelial E-selectin as well as sialyl Lewis-a/x determinants on cancer cells, various efficient strategies targeting these molecules are promising to suppress E-selectin-mediated cancer cell adhesion. The approaches include: antibodies directed against E-selectin or sialyl Lewis-a/x antigens, compounds that mimic the sialyl Lewis-a/x determinants, and antisense-cDNA of the genes encoding for the enzymes that synthesize these carbohydrates. Controlled clinical trials are still waiting but their reliability is strongly supported by solid experimental findings. For example, interfering with the binding of colon carcinoma cells to lung endothelium, by using an anti-E-selectin-antibody, impairs lung metastasis. Similarly, antibodies directed against sialyl Lewis-a/x determinants inhibit the formation of metastasis by human pancreatic and gastric cancers in nude mice. Moreover, sialyl-Lewis-a mimetic compounds inhibit the metastatic potential of tumor cells expressing sialyl Lewis-a, whereas antisense-cDNA for fucosyltransferase genes (FUT III/VI) suppresses metastatic colonization by colon cancer cells. Along the same lines, encouraging results are obtained in colon cancer by using cimetidine to inhibit E-selectin expression or celecoxib, an inhibitor of ►cyclooxygenase-2, to impair the expression of sialyl Lewis-a.

The expression of E-selectin as well as sialyl Lewis-a/x determinants may reveal to be efficient markers of metastasis. In cancer patients, an elevated level of serum E-selectin is generally recognized as a marker of endothelial cell activation and is associated with clinical metastasis. For example, in patients with breast cancer, high concentrations of circulating soluble E-selectin are associated with liver metastasis. In contrast, low levels of circulating sE-selectin are associated with a strong prognostic value for an overall and disease-free survival in patients with node-negative breast cancer. In patients with non-small cell lung cancers, a high serum concentration of E-selectin correlates with a worse prognosis when the cancer cells express sialyl Lewis-a/x. Similarly, elevated levels of serum E-selectin is indicative of a poor prognosis in patients with colon cancer, when the cancer cells express sialyl Lewis-a. Hence, the determination of blood sE-selectin coupled to the determination of sialyl Lewis-a/x determinant on tumor biopsies might become a useful and quick assay to evaluate the metastatic potential of a tumor.

Overall, E-selectin-mediated endothelial adhesion seems to have a key role for cancer cells in metastasis, which opens new avenues for therapeutic interventions aim at inhibiting this fatal complication of cancer.

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Eag

►Ether à-go-go Potassium Channels

EAOC (Endometriosis Associated Ovarian Cancers)

Definition

Ovarian cancers, predominantly endometrioid and clear cell subtypes, which are found co-existing with endometriosis, and may have arisen by malignant transformation of endometriosis.

►Endometriosis
►Ovarian Cancer

EAP1

►Securin

E2A-PBX1

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Definition

E2A-PBX1 is an oncogenic transcription factor expressed in neoplastic cells consequent to a somatic chromosomal translocation (t(1;19)) in some cases of ►acute lymphoblastic leukemia (ALL).

Characteristics

Leukemia cells commonly contain somatic chromosomal translocations that contribute to the induction and, presumably, perpetuation of the disease. They do this by altering proto-oncogenes that reside close to the involved ►breakpoints on the participating chromosomes. Translocation (1;19)(q23;p13.3) is the second-most frequently observed recurrent translocation in ALL, detectable in approximately 5% of cases using conventional cytogenetics. In the vast majority of instances, t(1;19) fuses coding regions from two genes, *E2A* and *PBX1*, that reside respectively on chromosomes 19 and 1. Subsequent transcription and pre-mRNA splicing lead to expression of E2A-PBX1, an abnormal, ►chimeric transcription factor with potent oncogenic activity. Abundant evidence supports the notion that E2A-PBX1 contributes to the abnormal accumulation of primitive lymphoid progenitors that characterizes ALL by deregulating the transcription of key target genes.

Clinical Aspects

ALL is primarily a pediatric disease, so most patients with t(1;19)-positive, E2A-PBX1-expressing leukemia are children. Although the neoplastic cells in ALL most often have immunophenotypic (►Flow cytometry in cancer diagnosis) and genotypic (►Molecular pathology) features characteristic of the very early stages of B-lymphoid development, prior to the initiation of immunoglobulin gene rearrangement or expression, the vast majority of cells associated with t(1;19) generally manifest characteristics typical of more mature "pre-B cells," including rearrangement of the immunoglobulin heavy chain gene locus and expression of cytoplasmic, but not surface, immunoglubulin heavy chain protein. Other clinical features associated with t(1;19)-positive ALL include: especially high leukocyte counts at presentation, non-caucasian race and central nervous system involvement. Although the translocation was originally associated with unfavorable clinical outcomes, the more intensive treatment regimens used currently have largely or completely abrogated this association.

Wild-type E2A and PBX1 Gene Products

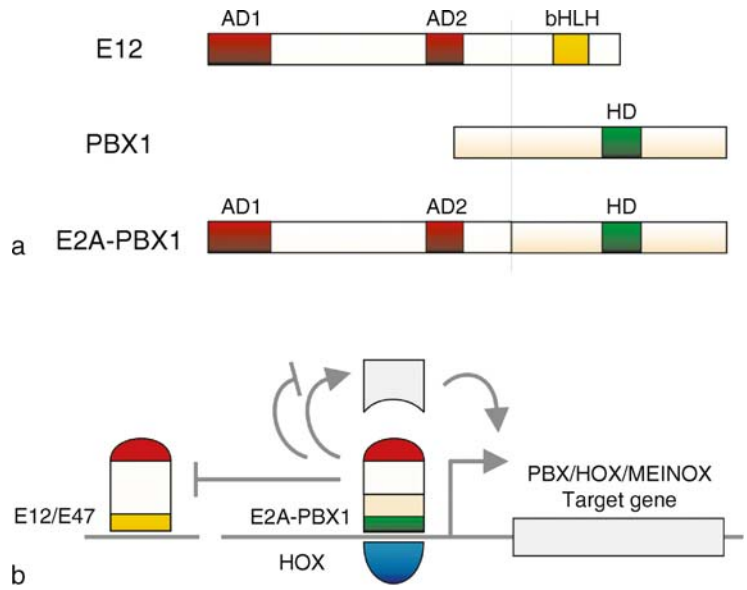
The *E2A* gene resides at chromosome band 19p13.3 and encodes two proteins, called E12 and E47, which are generated by alternative splicing of exons (Fig. 1). E12/E47 (or "E2A proteins") possess a C-terminal ►basic helix-loop-helix (bHLH) domain that mediates homo- or hetero-dimerization as well as binding to DNA at sites that contain the consensus sequence CANNTG (the ►E-box). E12/E47 function as transcriptional activators, inducing the transcription of genes that lie in the general vicinity of the E-boxes to which they bind. The E2A proteins have important roles in regulating lineage-specific cellular differentiation; their contributions to various aspects of lymphocyte development have been especially well delineated.

The *PBX1* gene, at chromosome band 1q23, was identified through its involvement in the 1;19 translocation. Its protein products, PBX1a and PBX1b, contain a homeodomain, an evolutionarily ancient domain involved in DNA binding and protein-protein interactions (►Homeobox genes and cancer). PBX1 binds to DNA and regulates the transcription of target genes in cooperation with other homeodomain-containing proteins of the HOX and MEINOX classes. These physical and functional interactions have important roles in embryonic development and tissue homeostasis. In particular, important roles in hematopoiesis are well documented.

Structure and Function of E2A-PBX1

The recombination of exons brought about by t(1;19) essentially fuses the amino-terminal two thirds of the E2A proteins (a portion that is invariant between E12 and E47) to most of PBX1. The *E2A*-encoded portion includes two transcriptional activation domains capable of inducing target gene transcription by recruiting transcriptional co-activators (►Chromatin remodeling in cancers), whereas the *PBX1*-derived portion includes the DNA-binding homeodomain.

E2A-PBX1 can function as a potently transforming oncoprotein in several cellular lineages. For example, enforced expression induces lethal lymphoproliferative diseases in transgenic mice and aggressive myeloproliferative diseases in a murine bone marrow transplantation model. The impaired cellular differentiation and accelerated proliferation that are associated with E2A-PBX1 expression result from the cumulative or cooperative effects of physical or functional interactions between E2A-PBX1 and other macromolecules. The available experimental evidence supports a general model the basis of which was originally suggested by the nature of the functional domains that are brought together in the oncoprotein (Fig. 1). Largely by means



E2A-PBX1. Figure 1 Structure and function of E2A-PBX1. (a) Translocation 1;19 results effectively in fusion of the amino-terminal two thirds of the E2A proteins (this portion is identical in the E12 and E47 isoforms) with most of PBX1. The vertical line indicates the point of fusion. AD1 and AD2, transcriptional activation domains 1 and 2; bHLH, basic helix-loop-helix domain; HD, homeodomain. (b) A model illustrating hypothetical mechanisms of neoplastic transformation by E2A-PBX1. The oncoprotein may deregulate the expression of critical genes normally controlled by PBX/HOX/MEINOX complexes, alter the function of transcriptional coactivators, or impair the function of wild-type E12/E47.

of its *PBX1*-encoded portion, E2A-PBX1 retains the ability to bind cooperatively with HOX proteins and cognate PBX/HOX binding sites on the DNA. This probably results in the abnormal expression of target genes whose transcription is normally regulated by PBX/HOX/MEINOX complexes. The retention of potent transcription-inducing potential by the E2A portion of the oncoprotein, and the documented involvement of HOX proteins (and, by implication, the target genes that they regulate) in normal and leukemia-associated hematopoiesis, are consistent with this mechanism. In a perhaps complementary mechanism, the interaction with E2A-PBX1 may alter the function of transcriptional co-activator proteins, including the histone acetyltransferases p300 and CREB-Binding Protein (CBP) (Chromatin remodeling in cancers), so as to promote neoplastic transformation in a manner at least superficially analogous to oncoproteins encoded by DNA tumor viruses such as ►Simian virus 40. Finally, E2A-PBX1 may exert dominant-inhibitory effects on the wild-type E2A proteins, as these possess tumor suppressor activities.

The *E2A* locus is involved in another translocation, t(17;19)(q22;p13), seen relatively rarely in cases of ALL. Here, a similar portion of the E2A proteins is fused to a portion of the transcription factor ►hepatic leukemia factor (HLF), including the DNA binding domain. These observations indicate the promiscuity of the *E2A* locus with respect to translocation partners and suggest the involvement of common oncogenic mechanisms in cases of ALL associated with t(1;19) or t(17;19).

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Early B-cell Factors

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Synonyms

Early B-cell factors; EBF; Olfactory neuronal transcription factor; Olf; Olfactory/Early B-cell factors; O/E; Collier-Olf-EBF; COE

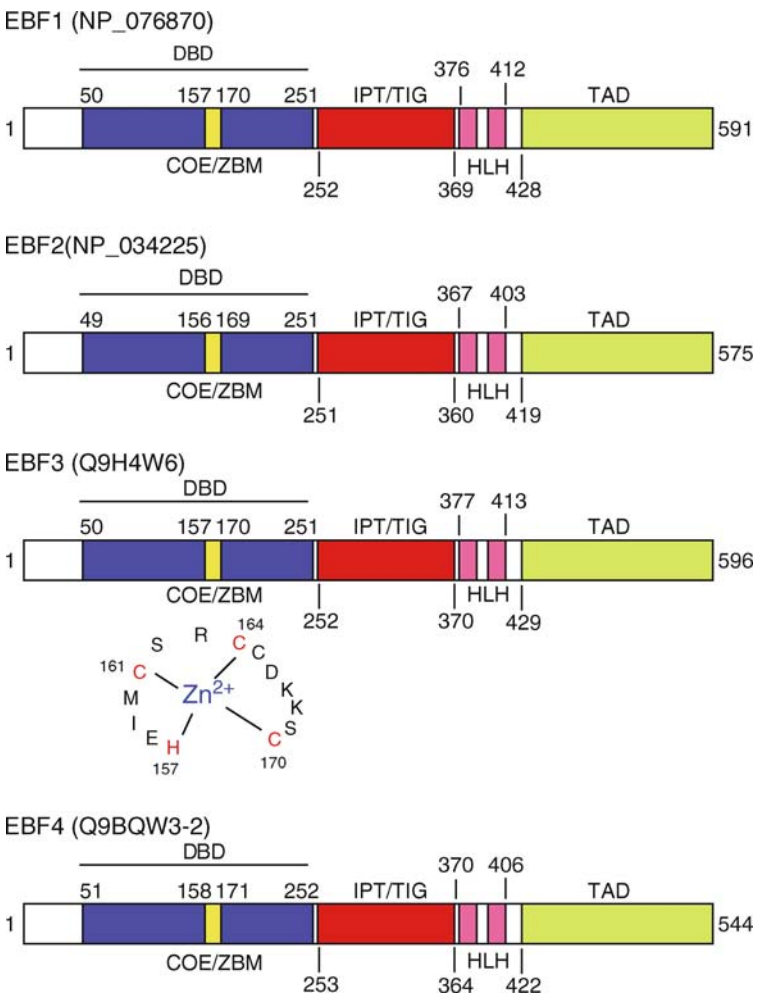
Definition

Early B-cell factors are a group of DNA-binding transcription factors containing the ►helix-loop-helix (HLH) domain. Within their highly conserved DNA-binding domain (DBD), a sequence motif consisting of an atypical zinc finger (H-X_3-C-X_2-C-X_5-C) is unique to this family of proteins.

Characteristics

Early B-cell factor 1 (EBF1) was initially isolated in 1993 from the nuclear extracts of a murine pre-B-cell line through oligonucleotide affinity chromatography. The DNA sequence of the oligonucleotide was derived from the promoter of the *mb-1* gene, encoding an immunoglobulin-associated protein that is only expressed in the early stages of B-lymphocyte differentiation. *EBF1* is essential for B-cell development, as mice lacking *EBF1* gene do not produce functional B cells and immunoglobulins. The cDNA encoding a related factor called Olf-1 was identified in the same year by screening rat cDNA clones that could activate reporter gene in yeast under the control of a synthetic promoter containing DNA elements derived from olfactory specific genes.

The EBF family is found only in the animal kingdom from *Caenorhabditis elegans* to humans. In the mouse and human genomes, there are four ►paralogous genes of the *EBF* family (EBF1–4). In humans, they localize at chromosomes *5q34*, *8p21.2*, *10q26.3*, and *20p13* for EBF1–4, respectively. Like a typical DNA-binding transcription factor, EBF proteins contain well-defined modular structural and functional domains (Fig. 1).



Early B-cell Factors. Figure 1 The structural features of EBF family of transcription factors. Shown are the four paralogs (EBF1–4) and their corresponding accession numbers (GenBank or SwissProt databases). Specific domains are shown in different color. Numbers refer to the position of amino acid residue in each protein. The signature zinc-finger motif of the EBF family of proteins in complex with zinc ion is also depicted. The different domains are DBD, DNA-binding domain; TAD, transactivation domain; COE, the signature sequence of EBF family; ZBM, zinc-binding motif; IPT/TIG, the immunoglobulin-like fold domain; HLH, helix-loop-helix motif.

The DBD near the N terminus consists of approximately 200 residues whose sequence is exceedingly well conserved throughout evolution with >75% sequence identity between distant species. This family of proteins binds directly to DNA sequences with a consensus of 5′-*CCCNNGGG*-3′ as ►homo- or heterodimers. The region following the DBD resembles the conserved domains called ►TIG-IPT (*I*mmunoglobulin-like fold, *P*lexins, *T*ranscription factors, or *T*ranscription factor *I*mmunoglobin). The function of this region in EBF has not been determined, although the TIG-IPT domain may be involved in homo- or heterodimerization in other transcription factors containing this sequence. Located next to TIG-IPT is the HLH domain that includes two helices with similar sequence. This feature distinguishes the EBF family from other HLH-containing transcription factors such as Myc, Max, and MyoD that usually contain two dissimilar amphipathic helices. The EBF HLH domain is probably involved in dimerization, as proteins with the deletion of this domain could not form stable dimer in solution. The C-terminal domain is highly rich in serine, threonine, and proline residues. It is less conserved, but is important for transcriptional activation; nonetheless, mutant without this domain could still activate transcription.

Widespread expression of *EBF* is detected in diverse cell types such as adipocytes and neuronal cells, and during different developmental stages such as limb buds and the developmental forebrain. The EBF ►orthologs in both *Drosophila melanogaster* and *Caenorhabditis elegans* is implicated in neurogenesis. During mouse embryogenesis, *EBF* members are expressed in early postmitotic neurons from midbrain to spinal cord and at specific sites in the embryonic forebrain, indicating that EBF proteins may be involved in regulating neuronal maturation in the central nervous system (CNS). Interestingly, EBF1 is abundantly expressed in the ►striatonigral medium spiny neurons (MSNs). *EBF1* deficiency in mice results in markedly reduced number of striatonigral MSNs at postnatal day 14 (P14), although such neurons are properly specified in $EBF1^{-/-}$ mice by P0. Thus, EBF1 appears to be a lineage-specific transcription factor essential to the differentiation of striatonigral MSNs. Targeted deletion of mouse *EBF2* results in defects in peripheral nerve morphogenesis, migration of hormone-producing neurons, projection of olfactory neurons, and cerebellar development. In addition to B-cell development and neuronal differentiation, EBF transcription factors are involved in other developmental processes. For example, EBF2 is a regulator of osteoblast-dependent differentiation of osteoclasts and *EBF2*-deficient mice have reduced bone mass. Furthermore, EBF1 induces adipogenesis in NIH-3T3 fibroblasts. The expression of EBF proteins in multiple tissues and their involvement in diverse developmental pathways suggest that they have fundamental cellular functions and their roles in lineage determination may be achieved through cooperation with other tissue-restricted factors.

The four paralogs of EBF members are highly similar to each other at the amino acid sequence level. It is therefore surprising that they have quite distinct functions, as *EBF3*-deficient mice exhibit neonatal lethality before postnatal day 2, and *EBF2*-null mice are much smaller than their wild type littermates, with body weight of the former being less than one half of that of the latter 30 days after birth. The N-terminal 50 amino acid residues and the entire C-terminal transactivation domain are the most divergent regions among these paralogs, although these regions are essentially identical among corresponding orthologs in different mammalian species. Therefore, functional specificity of individual EBF member may be determined by these divergent domains or through specific regulation of their expression. Indeed, the promoter sequences of the four paralogs appear quite distinct.

Potential Roles in Cancer

The *EBF3* locus at chromosome *10q26.3* is biallelically altered by genomic deletion and/or ►promoter hypermethylation in most cases of high-grade brain tumors. In a small number of examined clinical samples, the *EBF3* locus is inactivated in 50% of grade II, 83% of grade III, and 90% of grade IV (►glioblastoma multiforme) brain tumors. *EBF3* is expressed in normal brain cells, but is silenced in brain tumor cells. Thus, it is likely that EBF3 may be a tumor suppressor in the brain. EBF3 might also restrict abnormal proliferation in cancer of other tissue origins, as epigenetic silencing of *EBF3* occurs in cancer cell lines derived from breast, colon, bone, and liver. Consistent with epigenetic silencing of the *EBF3* locus, *EBF3* expression can be reactivated by treating cells with 5-aza-2′-deoxycytidine, a demethylating agent, and trichostatin A, an inhibitor of histone deacetylases. Ectopic expression of *EBF3* causes cell cycle arrest and apoptosis in cancer cells through regulating the expression of genes involved in cell cycle control. Specifically, EBF3 directly activates genes encoding the *Cip/Kip* family of ►inhibitors of cyclin-dependent kinases such as $p21^{cip1/kip1}$ and $p27^{kip1}$. Conversely, EBF3 can repress the expression of genes responsible for cell proliferation and survival such as ►*cyclins A* and *B*, *CDK2*, *Daxx*, and *Mcl-1*. Therefore, EBF3 may act as a tumor suppressor by regulating expression of specific set of genes.

In mouse B-cell lymphomas, retroviral insertions occur frequently in two genetic loci encoding Evi3 (ectropic viral integration site 3) and EBFAZ (EBF-associated zinc finger protein, also known as OAZ). Such viral integration results in heightened expression of EBFAZ or Evi3. These two proteins are highly similar to each other, with each containing 30 Krüppel-type

zinc fingers. Via several zinc fingers near its C terminus, EBFAZ or Evi3 binds to EBF and it is suggested that overexpression of EBFAZ or Evi3 in B-cell leukemias and lymphomas causes aberrant expression of EBF1 target genes that might contribute to tumorigenesis in B cells. In support of this notion, *Evi3* is significantly expressed in most human acute myelogenous leukemias. *EVi3* expression is abundant in human hematopoietic progenitors and declines rapidly during cytokine-driven differentiation. Interestingly, Evi3 or EBFAZ could either repress or activate EBF-mediated transcription, depending on cell type and gene promoter. Therefore, the precise implications of the EBFAZ–EBF pathway in cancer development remain to be determined. Finally, focal deletions of the *EBF1* locus have been detected in significant cases of B-progenitor ALL (acute lymphoblastic leukaemia).

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Early Detection

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Definition

Refers to testing of a subject or an asymptomatic population (screening) to determine the presence of a particular disease or risk factors associated with the disease. This definition has now been extended to incorporate new thinking on early detection that incorporates (i) determination of the potential of precancerous lesions, i.e. whether or not the lesion is likely to progress and become invasive; and (ii) detection of recurrence.

Characteristics

Sustained research and clinical efforts are needed to further reduce the burden of cancer. For instance, detecting cancer in its earliest stages could provide an opportunity to treat the disease before it spreads while cancer prevention strategies could reduce a person's risk of developing cancer. One of the best ways to decrease the number of cases and deaths from cancer is to prevent the disease before it starts. Cancer prevention, however, means more than avoiding behaviors that may increase a person's risk for the disease: cancer prevention also involves identifying cells that may develop into cancer and individuals who are most likely to have these types of susceptible cells. Early cancer detection and risk assessment allow interventions to be focused on the very early stages of cancer growth, before the disease has spread beyond it site of origin. (►Preneoplastic lesions).

Biomarker Consortium

The work presented in this essay comes from investigators who are part of the National Cancer Institute's Early Detection Research Network (EDRN). The EDRN is structured around four main components: (i) Biomarkers Developmental Laboratories (BDL), which develop and characterize new biomarkers or refine existing biomarkers, (ii) Biomarkers Reference Laboratories (BRL), which serve as a resource for clinical and laboratory validation of biomarkers, including technological development, standardization of assay methods and refinement, (iii) Clinical Epidemiology and Validation Centers (CEVC) which conduct and support the early phases of clinical and epidemiological research on the application of biomarkers, and (iv) Data Management and Coordinating Center (DMCC) which provides statistical, logistical, information support and develops the theoretical statistical approaches to pattern analysis of multiple markers simultaneously. The National Aeronautics and Space Administration's Jet Propulsion Laboratory serves as the Informatics Center.

Approaches

Early Detection Markers

Ovary: Ovarian cancer is highly lethal and, thus far, has no approved or marketed early detection tests. (Transvaginal ultrasound and CA-125 are under study in large screening trials). Recent work within the Early Detection Research Network (EDRN) is leading to a molecular test that is promising and may be widely available soon. Those molecular tests include, proteomic approaches, such as serum protein profiling, testing a panel of serum markers (CA 72-4, CA 15-3, CEA, CA 19-9, SMRP-1, OV-1.10, HE-4, Osteopontin, HK-11, HK-10, Spondin-2, Prolactin and CA-125), and identification and testing of autoantibodies in cancer patients as diagnostic markers. A great focus is on development of noninvasive or minimally invasive test for earlier detection of ovarian cancer. (►Ovarian cancer).

Colon: Colon cancer is the third most frequently diagnosed cancer in the United States and the third most frequent cause of cancer death (►Colon cancer). Successful prevention of deaths from colorectal cancer depends on early detection. The widespread use of current screening technologies (fecal occult blood test, sigmoidoscopy, colonoscopy, and barium enema) could reduce deaths from the disease, but many people avoid these tests due to their discomfort. Alternate strategies to screen for colorectal cancer could identify those at greatest risk or likelihood of disease versus those who need not submit to an invasive test. EDRN investigators have identified genetic, epigenetic and protein biomarkers that correlate with the presence of colorectal cancer using serum, stool, and urine. Investigators at Evanston Northwestern Healthcare Research Institute are using cutting edge optical technologies to create a device that evaluates the anatomical architecture of the cells lining the colon. Using this technology, a doctor would be able to assess whether or not changes in the overall structure of the cells indicate a risk of developing colorectal cancer. In many cancers, small changes occur in all the tissues exposed to potential cancer-causing compounds, a concept known as field carcinogenesis. Their plan to develop a free-standing optical probe will allow a primary care physician to determine the need for colonoscopy during a digital rectal exam. Spectral markers based on two optics technologies are being employed. In a study of more than 254 people, the spectral-assisted approach was able to detect 100% of the people with cancer (sensitivity) and 88% of those without the disease (specificity). The test has ►positive predictive values and ►negative predictive values of 71 and 100%, respectively, highlighting the ability of this technique to accurately detect patients with adenomas or colon cancer.

The Drexel University EDRN BDL is collaborating with the Great Lake New England Consortium to determine whether urine can be used as a source of DNA for early colon cancer detection. Their data indicate that mutant K-ras DNA derived from colorectal cancer cells is present in human urine. While they can reproducibly detect mutant K-ras in urine, this assay does not have sufficient sensitivity and specificity. This issue is being addressed by adding multiple proto-oncogene markers to the assay. Additionally, the use of DNA methylation markers is being explored, based on the observation that the methylation status of several genes in the polyps removed during the first colonoscopy could be used to predict those patients who would have polyps during their follow-up colonoscopies.

Other Gastrointestinal Sites: Despite advances in surgical technique and multimodal therapy, the 5-year survival rate for esophageal cancer remains dismal at 5–15% (►Esophageal cancer). Advanced stage of disease at initial diagnosis and high rates of recurrence contribute to this low survival. Developing and refining methods for early cancer detection is a key to improving survival in this deadly disease. Chronic reflux of acidic gastric contents can cause gastroesophageal reflux disease or GERD. Long-term GERD, in turn, can cause Barrett esophagus, a premalignant condition that increases a patient's risk of developing esophageal adenocarcinoma. Because of this increase in cancer risk, patients with a known diagnosis of Barrett's esophagus undergo endoscopic surveillance at regular intervals, usually every two to three years. Patients may undergo these surveillance endoscopies for the rest of their lives, sometimes submitting to as many as ten in a lifetime. However, most patients with Barrett's esophagus do not progress to cancer, and a biomarker test to predict those likely to progress could reduce the number of endoscopies and improve surveillance.

Methylation of DNA is a common alteration in cancer-related genes and is often associated with complete or partial repression of transcription (►Methylation). This mechanism is an alternative pathway for inactivation of tumor suppressor genes such as *p16* and *APC* in variety of cancers. An EDRN investigator at Johns Hopkins University has developed a three-tiered risk model that incorporates both epigenetic (methylation of tumor suppressor genes) and clinical parameters to improve the efficiency of Barrett esophagus surveillance. As progression-free survival differed significantly among the three risk groups, clinicians may be able to base the frequency of endoscopies on an individual patient's risk calculated using these epigenetic and clinical parameters. A related project also involves analyzing levels of methylated DNA in plasma from patients with Barrett's esophagus or esophageal adenocarcinoma. Among 24 patients with esophageal adenocarcinoma studied to date, 70% had hypermethylated HPP1 in their blood, compared with only 13% of control subjects. Thus, DNA methylation in sera may be useful for early detection of esophageal adenocarcinoma and as prognostic or recurrence biomarkers for this deadly disease.

Markers of Preinvasive Cancer: Prostate cancer is the most frequently diagnosed non-skin cancer in men in the United States (►Prostate cancer clinical oncology). The prevalence of the diagnosis makes the disease a major health burden. While many men will die from prostate cancer, a majority of them will survive the disease as it is not uniformly fatal. Identification of aggressive forms of the disease is needed to spare men who might not need extensive treatments. Promoter methylation of several genes is a common feature of prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN), the noncancerous

growth of cells lining the internal and external surfaces of the prostate gland which may increase the risk of developing prostate cancer. The team assembled a panel of methylated genes as a new molecular marker for early cancer detection. The assay is based on the percentage of methylated alleles (PMA). PMA values of *APC* and *RARβ2* are higher than those of *GSTP1* in HGPIN, carcinoma and normal prostate tissue; the median PMA value for all three genes is higher in prostate cancer.

Predictive Markers: Breast cancer is highly prevalent and has well-established early detection strategies available: mammograms and clinical breast exams. Molecular tests exist to help determine treatment options following breast cancer diagnoses, but prospects for blood tests to detect the disease are scant. The Food and Drug Administration has approved a micro-array-based test that looks at the expression of 70 genes linked to breast cancer which can accurately assess a patient's risk of recurrence or death. The correlations of this are vastly superior to those obtained with standard prognostic markers (►Microarray cDNA technology). The usefulness of this test is supported by the fact that the 70 genes in a woman's tumor analyzed by MammaPrint predict the 10-year survival of the patient at a significance level over three times greater than existing methods and with an accuracy level of 96.7%.

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Early Detection Biomarkers

Definition

Markers to screen patients to find cancer early.

Early Genes of Human Papillomaviruses

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E

Definition

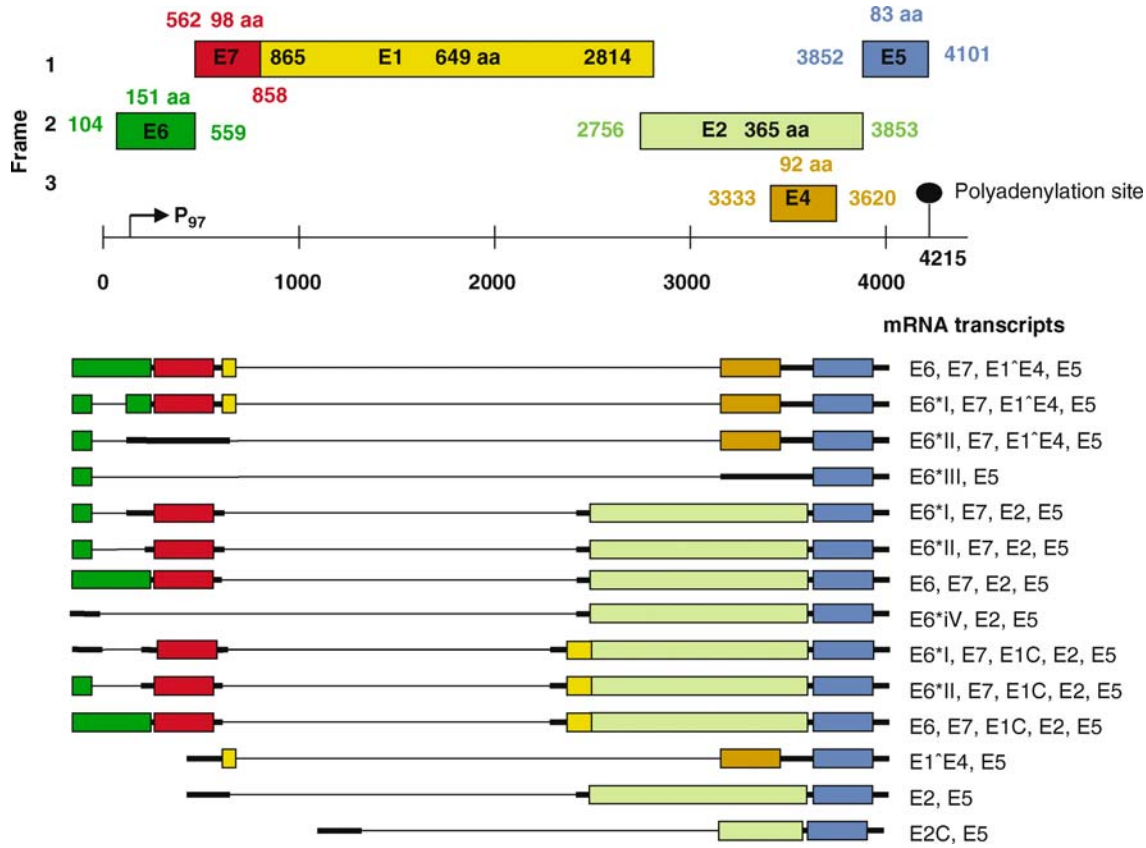
►Human papillomavirus (HPV) 16 has six early genes that are transcribed from the same DNA strand. As in other viruses, the function of the early proteins is to alter several cellular events to guarantee the completion of the virus life cycle. In addition, three early proteins, E5, E6, and E7 are also involved in the induction of malignant transformation of the infected cells. The cutaneous HPV types have a similar organization of the early region, with exception that the majority of these types lack the E5.

Characteristics

HPV constitutes of a heterogeneous group of viruses from the *Papillomaviridae* family. The HPV phylogenetic tree has been designed based on the homologous nucleotide sequence of the L1 capsid protein. So far, 92 HPV types have been fully sequenced, of which 60 belong to the alpha genera and the other 32 belong to the beta and gamma genera. Based on their tissue-tropism, HPVs can be divided in cutaneous and mucosal HPV types. The mucosal HPV types are included in the genus alpha together with certain benign cutaneous HPV types, whereas the beta and the gamma genera exclusively consist of cutaneous HPV types. Biological and epidemiological studies have clearly demonstrated that certain mucosal HPV types are associated with ►cervical cancer. On the contrary, the role of cutaneous types in carcinogenesis is still under debate, although several lines of evidence support their association with nonmelanoma skin cancer (NMSC). The mucosal HPV type 16 (HPV16) is the most frequently found HPV genotype in cervical cancers worldwide, and thus its early gene products are the best studied and characterized. (Fig. 1).

HPV16 E6 and E7 are the major transforming proteins. Three different lines of evidence have demonstrated the involvement of E6 and E7 in cervical carcinogenesis.

1. The first indication came from the analysis of HPV-infected cells, which showed that viral DNA is randomly integrated in the genome of the majority of cervical carcinomas. Integration leads to the



Early Genes of Human Papillomaviruses. Figure 1 Organization of the early region of HPV16. The six early genes are represented by using different colors. The nucleotide positions of each early gene and the predicted size in amino acids are also shown. Several polycistronic transcripts have been identified, which comprise of 2–3 early genes in different combinations and are most likely transcribed from the P_{97} promoter. The function of each transcript is not known, but some of them are only transcribed at different stages of differentiation. Several lines of evidence indicate that the alternative splicing in E6 and E7 transcripts play an important role in the translational regulation of the viral proteins.

disruption of several viral genes with preservation of only the E6 and E7, which are actively transcribed.

2. The discovery that E6 and E7 proteins are able to induce cellular transformation in vitro confirmed their oncogenic role. Immortalized rodent fibroblasts can be fully transformed by expression of HPV16 E6 or E7 protein. These rodent cells acquire the ability to grow in an anchorage-independent manner and to be tumorigenic when injected into nude mice. In addition, HPV16 E6 and E7 together are able to immortalize primary human keratinocytes, the natural cellular host of the virus. In agreement with the in vitro assays, transgenic mice coexpressing both viral genes exhibit epidermal hyperplasia and various tumors. Similar to the mucosal HPV types, E6 and E7 from certain cutaneous HPV types of the genus beta (e.g., HPV8 and 38) display transforming properties in in vitro and in vivo models. Independent studies have demonstrated that HPV16 E6 and E7 proteins do not induce cellular transformation via a "hit and run" mechanism, but continuous expression of both proteins is required for the maintenance of the malignant phenotype.
3. Finally, biochemical studies have clarified the mechanism of action of E6 and E7. The viral oncoproteins are able to form stable complexes with cellular proteins and alter, or completely neutralize, their normal functions. These events lead to the loss of control of cell cycle checkpoints, apoptosis, and cellular differentiation.

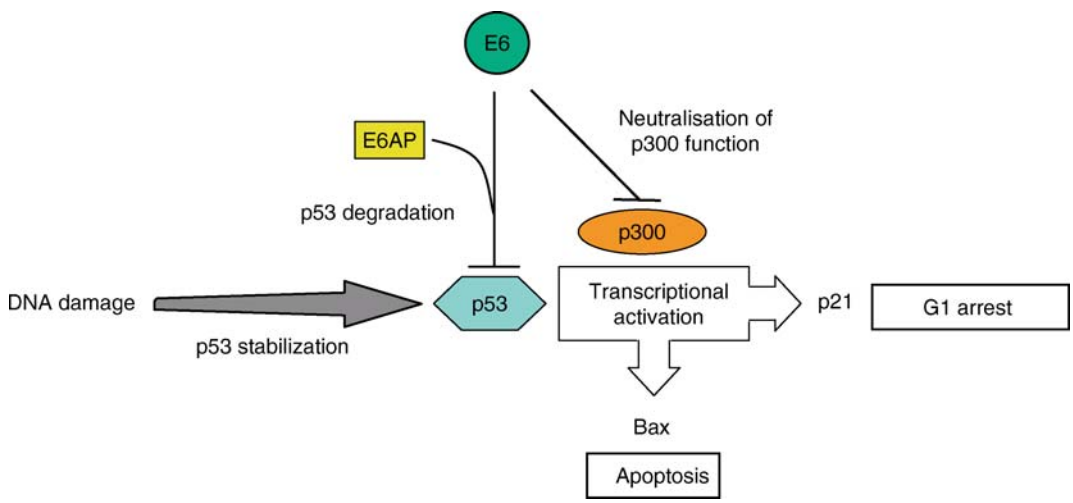
E6 protein. HPV16 E6 is a small basic protein of 151 amino acids. The major structural characteristic of E6 is the presence of two atypical zinc fingers. At the base of these zinc fingers are two motifs containing two cysteines (Cys-X-X-Cys), which are conserved in all E6 HPV types. The best characterized HPV16 E6 activity is its ability to induce degradation of the tumor suppressor protein p53 via the ubiquitin pathway. This cellular protein is a transcription factor that can trigger

cell cycle arrest or apoptosis in response to stress or DNA damage. E6 binds to a 100 kDa cellular protein, E6AP (E6-protein), which functions as a ubiquitin protein ligase (E3). The E6/E6AP complex then binds p53, which becomes very rapidly ubiquitinated and as a consequence is targeted to proteasomes for degradation. Since the major role of p53 is to safeguard the integrity of the genome by inducing cell cycle arrest or apoptosis, cells expressing HPV16 E6 show chromosomal instability, which greatly increases the probability that HPV-infected cells will evolve towards malignancy. Additional findings have demonstrated that HPV16 E6 also associates with the transcriptional regulators, CBP and p300, with resulting inhibition of p53-driven transcription. Thus, HPV16 E6 neutralizes p53 by two distinct mechanisms; the first is mediated by the p300/CBP association, while the second occurs via binding to E6AP to promote p53 degradation (Fig. 2).

Interestingly, the E6 protein from cutaneous HPV types is not able to induce degradation of p53. In fact, it has recently been shown that the beta HPV38 can inactivate p53 function by inducing accumulation of its antagonist, ΔNp73. HPV16 E6, as well as E6 from certain cutaneous HPV types, can also interfere with the apoptotic pathways via its association with Bak, a member of the Bcl-2 family. Analogously to its effect on p53, E6 induces Bak degradation via the ubiquitin-mediated pathway.

Several p53-independent cellular pathways, which are altered by the E6 molecule, have been identified. HPV16 E6 is able, through its association with E6AP, to promote the degradation of the transcriptional repressor NFX1-91, and consequently to activate the transcription of the *hTERT* (*human Telomerase Reverse Transcriptase*) gene encoding the catalytic subunit of the telomerase complex. This effect directly results in telomerase activity upregulation, a key event in the immortalization of primary keratinocytes. In addition, HPV16 E6 is able to interfere with cell mobility, through interaction with the human homologue of the Drosophila discs large protein (DLG). Also in this case, E6 binding leads to degradation of the cellular protein. However, different E6 domains are required to induce degradation of p53 and DLG. Deletion of the carboxy terminus of HPV16 E6 abolishes its binding to DLG without influencing its ability to promote p53 destabilization.

Another cellular target of HPV16 E6 is paxilin, a protein involved in transducing signals from the plasma membrane to focal adhesions and the actin cytoskeleton. The fact that E6 from the oncogenic HPV16, but not E6 from the low risk HPV types 6 and 11, is able to bind paxilin, suggests that this interaction has a role in the carcinogenesis of HPV infection. Several factors have been described to minimize or prevent exposure of HPV to the immune system. It has also been shown that HPV16 E6 interacts with interferon regulatory factor-3 (IRF-3), a positive transcriptional regulator of the INFβ promoter, which is activated in response to virus infection. E6 binding inhibits IRF-3 transactivation function. Thus, this E6-induced event may enable the virus to circumvent the antiviral response of the infected cell. The adhesion between keratinocytes and antigen presenting cells, i.e., Langerhans cells, in the epidermis



Early Genes of Human Papillomaviruses. Figure 2 p53 pathways targeted by HPV16 E6. When cells are exposed to DNA damaging agents, e.g., X-rays, the half-life of p53 can be greatly increased by posttranslational modifications (phosphorylation). In turn, p53 can either activate the transcription of the cyclin-dependent kinase (CDK) inhibitor, $p21^{WAF1/CIP1}$, leading to a G1 arrest and DNA repair before replication, or activate the transcription of the proapoptotic gene Bax with consequent induction of apoptosis. Cells expressing HPV16 E6 protein are resistant to the cell cycle arrest or apoptosis induced by DNA damaging agents.

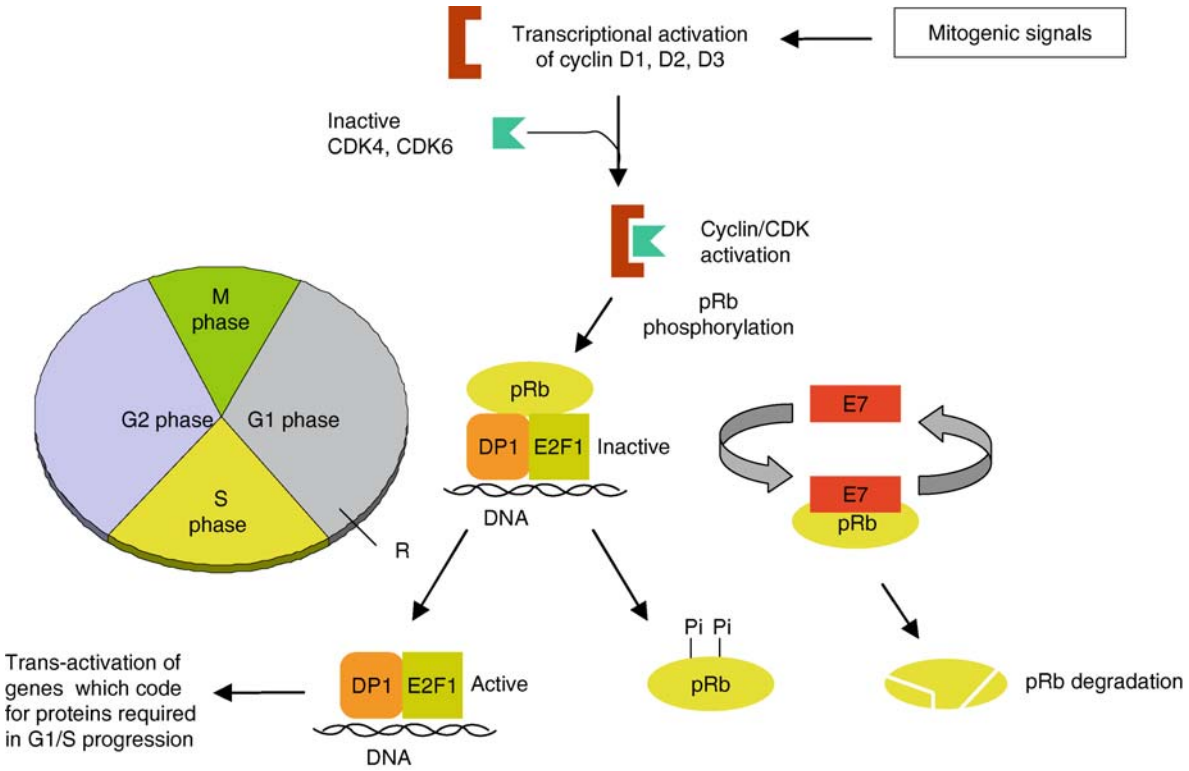
is mediated by E-cadherin. It has been shown that E6 can reduce the levels of cell surface E-cadherin on keratinocytes thereby limiting the presentation of viral antigens to the Langerhan cells and promoting viral survival. Furthermore, HPV16 E6 has been shown to suppress innate immune responses mediated by a family of toll-like receptors (TLRs) that are key sensors of evading pathogens. E6 is able to block the promoter of TLR9 which has been identified to recognize dsDNA sequences. Finally, E6 associates with ERC 55, a putative calcium binding protein located in the endoplasmic reticulum. However, the biological significance of this interaction is still unclear.

E7 protein. HPV16 E7 is an acidic phosphoprotein of 98 amino acids, which is structurally and functionally related to a gene product of another DNA tumor virus, the adenovirus E1A protein. On the basis of the similarity in primary structure between the two viral proteins, they can be divided into three domains: conserved region 1–3 (CR1–3). Mutational analysis of HPV16 E7 has demonstrated that all three regions are important for the in vitro transforming activity of the molecule. CR3 contains two CXXC motifs involved in zinc binding and essential for the stability of the protein. Independent studies have demonstrated that this viral protein is located in the nucleolus, nucleus, and cytoplasm. Indeed, it has been shown that the E7 molecule associates with cytoplasmic and nuclear proteins. The best understood interaction of E7 with a cellular protein is that involving the "pocket" proteins, pRb, p107, and p130. The pocket proteins are central regulators of cell cycle division. They negatively regulate, via direct association, the activity of several transcription factors, including members of the E2F family (E2F1–5), which are associated with their partners, DPs. Under normal cell cycle regulation, phosphorylation of pRb, which is mediated by cyclin-dependent kinase (CDK) activity, leads to the disruption of pRb/E2F complexes, with consequent activation of E2Fs. HPV16 E7 binds the pocket proteins and, analogously to the phosphorylation, results in the release of active E2Fs which in turn activate the transcription of a group of genes encoding proteins essential for cell cycle progression, such as cyclin E and cyclin A. As described for the interaction between E6 and p53, HPV16 E7 protein is able to promote the destabilization of pRb through the ubiquitin–proteasome pathway (Fig. 3). Similarly, the beta HPV38 E7 binds to pRB and promotes its degradation. This property is not shared by all E7s from the different HPV genotypes. Indeed, E7 from the benign HPV1 can efficiently associate with pRb without inducing its degradation. It is likely that the E7-induced pRb degradation represents a more effective way to neutralize the function of the cellular protein. The other two members of the pocket protein family, p107 and p130, are involved in controlling additional cell cycle checkpoints; p130 exerts its transcriptional regulatory function during the G0/G1 transition, while p107 is active in the G1/S transition and in the G2 phase. Analogously to pRb, HPV16 E7 protein associates with p107 and p130, inactivating key cell cycle checkpoints.

Besides targeting the pocket proteins, E7 can alter cell cycle control by additional mechanisms. The HPV16 E7 protein is able to associate with the CDK inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ causing neutralization of their inhibitory effects on the cell cycle. Cells coexpressing HPV16 E7 and $p21^{WAF1/CIP1}$ or $p27^{KIP1}$ are still able to enter S phase, while in the absence of E7 cells are arrested in G1 phase. HPV16 E7 can also directly and/or indirectly interact with cyclin A/CDK2 complex. The biological function of this interaction remains to be elucidated, but it is possible that E7 may act by redirecting the kinase complexes to a different set of substrates.

Other cellular proteins involved in transcriptional regulation have been identified as HPV16 E7 targets. HPV16 E7 binds the TATA box binding protein (TBP) and the TBP-associated factor TAF110, indicating that the viral protein is able to interfere with the basic transcriptional machinery of the host cell. Furthermore, E7 associates with AP1 complex, activating its transcriptional activity.

Similar to other stimulators of proliferation (e.g., c-myc or adenovirus E1A), the HPV16 E7 protein, besides the ability to deregulate the cell cycle, also promotes apoptosis. Expression of HPV16 E7 in normal human fibroblasts (NHF) or in human keratinocytes results in a cytocidal response, which displays the typical features of apoptosis and is much more evident in the absence of mitogenic signals. This E7-induced apoptosis requires pRb inactivation and is mediated by p53-dependent and -independent pathways. It is likely that the E7-induced apoptosis represents a cellular response elicited by the loss of cell cycle control. Interestingly, E6 protein is able to completely abrogate the E7 activity in promoting apoptosis. Thus, both viral proteins are required to induce transformation of the host cells. Although E7 is constitutively expressed in HPV16-associated lesions and therefore appears as candidate antigen for a specific immune response, the immune system fails to produce an efficient defense against tumor outgrowth in affected patients. As in the case of E6, E7 has also evolved to escape immune surveillance. E7 has the ability to bind and prevent the transcription factor IRF-1, from activating the INFα and β promoters. E7 is also capable of downregulating innate responses via suppressing TLR9 expression. Furthermore in HPV16 E6 and E7 transgenic mice, the transgene product E7 does not induce an immune response. However, upon vaccination with E7, anti-E7 antibodies were produced without causing signs of autoimmune disease. In contrast, E7-specific cytotoxic T lymphocytes (CTL) were not detected after immunization. Therefore the E7



Early Genes of Human Papillomaviruses. Figure 3 Deregulation of the restriction point (R) by HPV16 E7. E2F transcription factors form heterodimer complexes with members of the DP family and regulate the transcription of several genes during the cell cycle. In quiescent cells, pRb is present in a hypophosphorylated form and associates with E2F molecules, thereby inhibiting their transcriptional activity. When quiescent cells are exposed to mitogenic signals, genes encoding the G1-specific D-type cyclins (D1, D2, and D3) are activated. Subsequently, cyclins associate with a catalytic subunit, CDK4 or 6, and after transport into the nucleus, the kinase complexes phosphorylate pRb in mid-G1 phase causing release of active E2F/DP1 heterodimer complexes and progression through the restriction point (R). E7 binding to pRb mimics its phosphorylation. Thus, E7 expressing cells can enter S phase in the absence of a mitogenic signals.

transgene expression induces specific immunological tolerance at the CTL level.

E5 protein plays an early role in the HPV-induced transformation. Studies on ▶bovine papillomavirus (BPV) have provided evidence that E5 is a potent oncoprotein. Recently, it has also been shown that HPV16 E5 is able to induce cellular transformation, although with less efficiency than BPV E5. HPV16 E5 is a small hydrophobic protein, which is located in the endoplasmic reticulum (ER), nuclear membrane, and cytoplasmic vesicles. E5 is able to enhance growth factor-mediated signal transduction to the nucleus, resulting in stimulation of cellular proliferation. BVP1 and HPV16 E5 associate with the 16 kDa subunit c of the vacuolar H + -ATPase, which is responsible for acidification of membrane bound organelles, such as Golgi, endosomes, and lysosomes. It has been recently shown that BPV1 E5 induces alkalinization of Golgi, and that this activity is linked to its in vitro transforming activity. Mutations in E5 that abolish the interaction with 16 kDa subunit c abrogate Golgi alkalinization and cellular transformation. HPV16 E5-mediated immune evasion also involves suppressing the expression of the major histocompatibility complex class I (MHC I) and antigen processing via the TAP pathway, reflecting the lack of antigen presentation to CTL.

Since the integration of viral DNA, which occurs in tumor cells, results in a loss of *E5* gene expression, it is clear that E5 is involved in early events during the multistep process of cervical cancinogenesis, and that its function is no longer required after the establishment of the transformed phenotype.

E1 and E2 are involved in the regulation of viral DNA replication. The function of E1 is to control the replication of viral DNA. E1 contains the cyclin-binding RXL motif and is able to associate with cyclin E and A. Consistent with these findings, E1 is phosphorylated by cyclin E or A-associated kinase. Mutation of E1 phosphorylation sites results in a reduction of HPV

DNA replication, supporting the idea that the E1/cyclin association plays an important role in viral DNA replication. Moreover, it has been shown that E1 has an ATPase and helicase activity. E1 forms a stable complex with E2 and binds to the replication origin of HPV in order to recruit cellular factors essential for DNA replication. Furthermore, E1 has been found associated with components of the cellular DNA replication machinery, e.g., DNA polymerase α.

E2 regulates the transcription of the early genes. In addition to controlling viral DNA replication together with E1, E2 is able to negatively or positively regulate the transcription of the early genes. Like all transcription regulatory factors, E2 has an amino-terminal transacting domain and a carboxy-terminal DNA binding domain, which recognizes four *cis* elements (ACCN6GGT) in the long control region (LCR) of the HPV genome. These two domains are separated by a central region (hinge), which is important, together with the amino-terminal domain, for the nuclear localization of the molecule. Whether E2 binding results in repression or activation of the promoter of the early genes is dependent on the position of the E2-binding site in the LCR. E2 binding to the promoter-distal or -proximal elements leads to a positive or negative regulation of the promoter, respectively.

E4 is probably involved in virus maturation and/or replication. E4 is a late protein expressed from the early region of the genome. Most of the studies have been performed on E4 from the cutaneous HPV type 1. In these lesions E4 is present at very high levels and several E4-derived proteins have been detected. The primary product is a 17 kDa protein, which is expressed from E1^E4 transcript. E4 associates with and disrupts the cytoplasmic keratin network. The biological significance of this E4-induced event is not fully understood. It has been proposed that E4 plays a role in the productive phase of the infection establishing a favorable condition for viral maturation.

Clinical Relevance

HPV16 infection results in the induction of a benign proliferation, which after a long latent period, can progress to invasive cancer. Persistent HPV infection is necessary for the development of the malignant lesion. This requirement is explained by the fact that viral proteins, in order to induce full malignant transformation of the host cells, have to cooperate with an activated cellular oncogene. Accumulation of mutations in cellular genes, which possibly lead to activation of an oncogene, requires continuous proliferation. This is achieved by the abilities of E6 and E7 to respectively neutralize apoptotic pathways and to induce unscheduled proliferation. Therefore, a possible approach to induce regression of a HPV-positive lesion is to target the biological functions of E6 and E7. This possibility is supported by findings, which clearly demonstrate that continuous expression of the two viral genes is necessary for the maintenance of the host cell transformed phenotype. Thus, we can predict that a blocking of the activity of E7 can lead to a rapid exit from the cell cycle. Neutralization of E6 function should result in an even more efficient way to induce regression of the HPV-lesion. As described above, E7 has a dual activity, being able to induce proliferation and apoptosis. E6, acting upon p53-dependent and -independent pathways, completely abolishes the E7-induced apoptosis. Thus, we could imagine that in cells expressing *E6* and *E7* genes, the block of only E6 functions may push the balance between proliferation and apoptosis in favor of the latter causing regression of the HPV-lesion.

Alternative targets are E1 and E2, which are involved in viral transcription and replication. Several approaches to neutralize the early viral proteins are under investigation. These include strategies to block the transcription or translation of viral genes or to identify small molecules able to specifically associate with and inactivate the viral proteins.

In addition, HPV E6 and E7 are able to downregulate the innate and adaptive immunity. Identification of strategies to reactivate the immune response in HPV-infected cells may favor the clearance of the infection preventing the development of cervical diseases.

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Early Menopause

Definition

Menopause at the age younger than 45 years.

►Menopausal Symptoms After Breast Cancer Therapy

Early Virus Genes

Definition

Are genes whose expression occurs before the onset of replication of the viral genome. Early proteins are usually required for replication of the virus nucleic acid. In contrast to the structural ("late") proteins, early proteins are usually not incorporated into the virus particle.

►Adenovirus
►Human Papillomavirus

Eaton-Lambert Syndrome

Definition

Is a disease seen in patients with ►lung cancer and characterized by weakness and fatigue of hip and thigh muscles and an aching back; caused by antibodies directed against the neuromuscular junctions.

►Extrapulmonary Small Cell Cancer

EBF

►Early B-cell Factors

Ebnerin (rat)

►Deleted in Malignant Brain Tumours 1

E-Box

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Synonyms

E-box; E motif

Definition

E-box is the collective term for DNA motifs with the consensus sequence CANNTG. E-boxes appear in a broad variety of promoters and enhancers and serve as protein binding sites. Proteins with affinity to this motif belong to the basic helix-loop-helix (bHLH) class of transcription factors, which can act as activators of transcription as well as repressors. More than 240 bHLH proteins are known to date in eukaryotes ranging from yeast to human, and the number continues to grow. Their binding specificity depends on both the nature of the "NN" nucleotides and sequences in the vicinity of the E-box. Genes containing E-boxes are activated during important developmental processes and some are described to be involved in cancer development and/or progression.

Characteristics

The DNA sequence "CAGGTGGC" was originally identified in 1985 within the sequence of the immunoglobulin enhancers, the first enhancers found to be activated in a tissue-specific manner. Later the name E-box, (with "E" for enhancer), was used as a common term for all motifs with the consensus sequence "CANNTG" and in 1989 the first two E-box binding proteins, E12 and E47, were identified. Both bind their recognition site as dimers, and dimerization is promoted by the helix-loop-helix (HLH) domain. HLH domains were subsequently found in other transcription factors such as MyoD and Myc proteins.

Genes Containing E-Boxes and the bHLH Proteins that Control them

To date an overwhelming number of E-box containing genes, regulated by bHLH proteins have been identified (Table 1), which include:

- Muscle (smooth and skeletal) specific genes that control myogenesis and are regulated by the ►bHLHs of muscle regulatory factors (Mrf).
- Genes involved in heart development that are regulated by the gene products of the ►Hand gene family, Hand1 and Hand2.
- Genes involved in neuronal development and differentiation are regulated by different bHLH proteins such as the Achaete-Scute family, Atonal family, E12/47 family, Hen family as well as by Nex, Hairy/E(Spl), and Id (►Id proteins).
- Insulin-inducible genes controlling pancreatic development are regulated by a member of the Atonal family, NeuroD. The latter is also a key player in neurogenesis.
- Genes involved in B- and T-cell development, regulated by bHLHs of the E12/E47 family.

E-Box. Table 1 Genes that contain E-boxes and that are involved in cell growth control or cancer development

Gene	Function/role in cancer development	E-box regulating protein
Bcl-6	Proto-oncogene coding for Krüppel-like zinc finger transcription factor, genetic rearrangements frequently found in lymphoma	?
Carbamoyl-phosphate synthase/aspartate carbamoyltransferase/dihydroorotase (CAD)	Enzyme involved in de novo pyrimidine biosynthesis, essential for cell growth	Myc
Cathepsin B	Protease, overexpression causes ability of cancer cells to metastasize	?
Cathepsin D	Estrogen induced protease, same mechanism as cathepsin B but mainly in breast cancer	USF1, USF2
Cdc2	Kinase, associates with Cyclin A and Cyclin B1, regulator of cell-cycle progression through G2 and M phase	USFs, myogenin
Cdc25A	Oncogene coding for cdk activating phosphatase, cell-cycle progression	Myc
c-Fos	Proto-oncogene coding for a transcription factor	E12, E47, myogenin
Cyclin B1	Activates cdc2 kinase, which regulates cell-cycle progression through G2 and M phase, increased expression often found in cancer cell lines	Myc
Cyclooxygenase-2 (COX-2)	Enzyme involved in prostaglandine synthesis in inflammatory processes, overexpression is correlated with tumor promotion	USFs
Fatty acid synthase (FAS)	Main enzyme in lipogenesis, overexpressed in wide variety of cancer types, mainly breast and prostate cancer, identical with prognostic molecule OA-519	USF
Heparin-binding epidermal growth factor like growth factor (HB-EGF)	Growth factor, member of the EGF family, postulated role in development of hepatocellular carcinoma, prostate cancer, breast cancer, esophageal, and gastric cancer	MyoD
3-hydroxy-3-methylglutyl coenzme A reductase (HMG-CoA reductase)	Rate-limiting enzyme in isoprenoid biosynthesis, inhibition blocks Ras activation and inhibits growth of ras-transformed cells	SREBP
Ornithine decarboxylase (ODC)	Rate-limiting enzyme in polyamine synthesis, essential for cell growth	Myc, MycN
Prothymosin alpha	Protein of unclear function, related to cell growth	Myc, MycN
Pulmonary surfactant protein A (Sp-a)	Lung-specific phospholipid-associated glycoprotein, mediates pathogen defense, frequently overexpressed in lung adenocarcinomas	USF1
Regulator of chromosome condensation-1 (Rcc1)	Guanine exchange factor, necessary for cell proliferation	Myc
Telomerase reverse transcriptase (Tert)	Catalytic subunit of telomerase which maintains chromosome ends and is an immortalizing enzyme	Myc

Genes and Gene Products that are Related to Growth Control and Cancer

- The proto-oncogenes ►BCL6 and ►FOS that encode transcription factors.
- CDC2 and CYCLIN B1 gene products, involved in cell-cyle regulation.
- Enzymes Cathepsin B and Cathepsin D, fatty acid synthase, and HMG-CoA reductase appear to be involved either in the process of tumor cells invading healthy tissue (Cathepsins) or seem to support the growth of cancer cells (fatty acid synthase and HMG-CoA reductase).

- Overexpression of the HB-EGF gene, encoding a growth factor, promotes cancer cell growth.
- COX-2 and SP-A gene products may be involved in tumor promotion. Underlying mechanisms are still unknown.

Many E-box-containing genes that are thought to be involved in cancer development provide binding for members of the Myc protein family, which play an important role in many cancer types. Being transcription factors they can activate transcription of the genes *CAD*, *CDC25A*, *CYCLIN B1*, *ORNITHINE DECARBOXYLASE*, *PROTHYMOSIN ALPHA*, *RCC1*, *ID2*, and *TERT*.

Members of the Usf (upstream transcription factor) protein family also contain the bHLH motif and bind to a variety of genes correlated with cancer. However, to date no oncogenic behavior has been described for these proteins and since Usfs are ubiquitously expressed, they may only play a less specific role in mechanisms of gene regulation (Table 2).

How do bHLH Proteins Bind to E-Boxes?

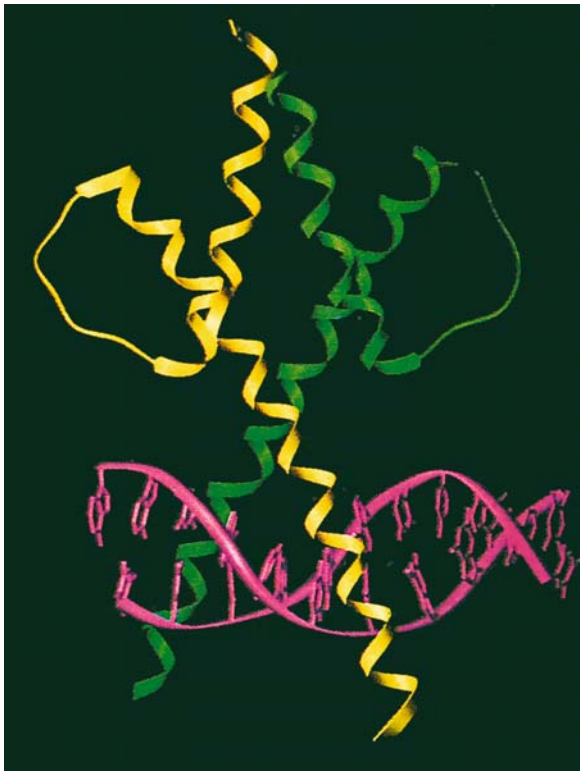
bHLH proteins bind to the E-box only as dimers, either as homodimers or heterodimers with other members of the bHLH protein family. The HLH motif within the protein allows dimerization: Two amphipathic α-helices are separated by a short stretch of amino acids, forming one or more β-turns, the "loop." Similar to the leucine-zipper motif, hydrophobic amino acid residues on one face of the helix interact with similar, also hydrophobic, residues of the helix from a second protein thus stabilizing the dimer. Some proteins possess a leucine-zipper as a second dimerization motif; these include members of the Mad, Srebp, Tfe, and Myc family (Fig. 1).

Dimerization is necessary but not sufficient for DNA binding. A sequence specific E-box recognition is mediated by a pattern of basic amino acids (Table 3). Since this basic region is localized N-terminal to the HLH region, it was this order which gave the name to a whole class of proteins, the "bHLH proteins."

E-Box. Table 2 List of bHLH protein families in animals and their E-box binding specificity

E-box sequence	bHLH family	Family members	Function
CAG (CTG)	Achaete/ Scute	Achaete, Scute, Mash-2, Hash-2, Fash-2	Neurogenesis
	Atonal	Atonal, Lin-32, Hath1, Math1, Math2, NeuroD, NeuroD2, NeuroD3, NeuroM	Neurogenesis; pancreatic development
	Delilah	Delilah	Differentiation of epidermal cells into muscle in Drosophila
	Hand	Hand1, Hand2	Cardiac morphogenesis; trophoblast cell development; neural crest development
	E12/E47	E12, E47, Pan1, Pan2	Ubiquitously expressed; myogenesis, neurogenesis; immunoglobulin gene expression
	Hen	Hen1, Hen2, Nhlh1, Nhlh2	Neurogenesis
	Lyl	Lyl-1, Nscl1, Nscl2, Scl/Tal-1, Tal-2	Hematopoietic proliferation and differentiation
	Mrf	MyoD, Myogenin, Myf5, Myf6, Mist1, Mrf4	Myogenesis
	Nex	Nex-1	Neurogenesis
	Twist	Twist, Paraxis, Scleraxis, Dermo-1, Tcf21	Specification of mesoderm lineages, myogenesis
CAC (GTG)	bHLH/ Pas	Arnt, Arnt2, trh, Hif-1α, Sim, ahr, BMAL1, clock, tim, per	Reaction to aromatic hydrocarbons; regulation of circadian rhythm; hypoxia response
	Hairy/E (Spl)	Hairy, E(Spl), Deadpan, Hes1, Hes5, Her1, Her4, Hesr-1, Sharp-1, Sharp-2	Neurogenesis; segmentation
	Mad	Mad, Mad3, Mad4, Mnt, Mxi1	Regulation of cell proliferation
	Myc	Myc, Mycn, Mycl, Max	Cell proliferation; differentiation
	Srebp/ Add	Srepb1, Srebp2, Add1, HLH106	Cholesterol homeostasis; sterol synthesis; adipocyte determination
	Tfe	Tfe3, Tfeb, Tfec, Mitf, Mi	Placenta vascularization; development of melanocytes, osteoclasts, masts cells
	USF	Usf1, Usf2, Spf1	Ubiquitous transcription factors
No binding	Id	Id1, Id2, Id3, Id4, TId1, TId2, XIdl, XIdx, XId2, emc	Negative regulators of myogenesis, neurogenesis

Extended, updated, and modified after Atchley and Fitch (1997).



E-Box. Figure 1 Shown is an E47 bHLH dimer bound to an E-box element (sequence: CACCTG). Each monomer consists of two α-helices that are separated by a loop; hydrophobic interactions between the α-helices stabilize dimerization. The basic region of each bHLH monomer makes contact with the major groove of the DNA molecule, each covering one half of the provided DNA binding site. [From Ellenberger et al. (1994) with permission].

E-Box. Table 3 Amino acid sequence comparison of bHLH proteins that bind the DNA sequences CAC GTG and CAG CTG

CAC GTG	bHLH	BB—N–ER-R—
	Myc	KRRTHNVLERQRRNE
	Max	KRAHHNALERKRRDH
	Usf	RRAQHNEVERRRRDK
	Tfe3	KKDNHNLIERRRRFN
CAG CTG	MyoD	RRKAATMRERRRLSK
	E12	RRVANNARERLRVRD
	E47	RRMANNARERVRVRD

B: basic residue.

bHLH proteins that rather bind to the "CAGCTG" DNA motif have different set of basic residues than those recognizing the DNA sequence "CACGTG." The change of only a single amino acid residue can alter binding specificity. The MyoD basic region usually recognizes the DNA sequence "CAGCTG." Replacement of the leucine at position 13 by an arginine within the basic MyoD region changed its binding preference to the "CACGTG" sequence.

Regulation of E-box-containing genes is also determined by the composition of the bHLH dimer bound to the DNA. Max, a member of the Myc family, can heterodimerize with Myc and activate target genes. If Myc is replaced by one of the Mad family members, the resulting heterodimer now represses the same target genes. Whereas Max is an ubiquitously expressed protein, the expression pattern of its potential partners are tissue-, developmental-, or cell cycle-dependent. Consequentely, transcriptional activation or repression of the target gene is determined by the ratio of Myc to Mad proteins, competing for Max dimerization.

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4E-BP1

Definition

A 21 kDa peptide, that when hypophosphorylated, binds eIF4E preventing formation of the translation preinitiation complex.

►Rapamycin

4EBP-1

Definition

eIF4E-binding protein 1; A protein that inhibits protein synthesis by binding to the eukaryotic translation

initiation factor 4E (eIF4E) in a phosphorylation-dependent manner.

►BCL3

EBV

Definition

►Epstein–Barr virus.

►Malignant Lymphoma: Hallmarks and Concepts
►Childhood Cancer
►Hodgkin Disease

EC 2.7.11.1

►B-Raf Signaling
►Raf Kinase

EC_{50}

Definition

The concentration of an agonist that produces 50% of the maximum possible response for that agonist.

►Small Molecule Screens

Ecchymosis

Definition

The skin discoloration caused by the escape of blood into the tissues from ruptured blood vessels. Ecchymoses can similarly occur in mucous membranes as, for example, in the mouth.

►Rituximab

ECM

Definition

►Extracellular matrix
►Maspin
►Stromelysin-1

ECMRIII

►CD44

ECOG Performance Status

Definition

Eastern Cooperative Oncology Group performance status is a zero to five scale used by healthcare professionals to evaluate disease progression and determine appropriate treatment and prognosis

►Nutrition Status

Ecological Study

Definition

A study in which the unit of observation are populations rather than individuals.

►Cancer Epidemiology
►Epidemiology of Cancer

ECSA

►Erythropoietin

Ecteinascidin 743

►Trabectedin

Ectodermal

Definition
Derived from ectoderm, the embryonic germ cell layer that forms the skin and nervous system.

►Bone Tumors

Ectodermal Dysplasias

Definition
Refer to inherited human disorders characterized by abnormalities in two or more ►ectodermal organs, including hair, teeth, skin, craniofacial region, and limbs.

Ecto-Enzymes

Definition
Enzymes at the cell surface that face the external environment, which can either be embedded in the membrane or be bound to separate anchoring proteins.

►Adenosine and Tumor Microenvironment

Ectopic Expression

Definition
Referring to the expression of a gene in an abnormal place or at abnormal time of an organism or a cell. This can be caused by a disease, or it can be artificially produced by introducing a transgene with a modified promoter into the target organism or cell. The ectopic expression then will provide an idea of the function of the gene, depending on the new phenotype the organism or cell will develop.

Eczema

►Allergy

Edelfosine

Definition
►ET-18-OCH3.

Edible Salt

►Salt Intake

EDN

►Endothelins

EDTA

Definition
Abbreviation for the chemical ethylenediaminetetraacetic acid. EDTA is a chelating agent widely used to sequester di- and trivalent metal ions, like Mn(II), Cu(II), Fe(III), and Co(III).

►Chelators as Anticancer Drugs

EEN

Definition
Extra eleven-nineteen gene.

►Endocytosis

EF-Hand

Definition
A highly conserved helix-loop-helix motif, which binds a Ca^{2+} ion with high affinity. It is usually present in pairs in cytosolic Ca^{2+}-signaling proteins, as well as some extracellular proteins.

►Secreted Protein Acidic and Rich in Cysteine (SPARC)

EF-Hand Proteins

Definition
Consist of single or multiple pairs of the helix-loop-helix motif. It consists of two perpendicularly placed α-helices (e.g., helices E and F in parvalbumin) and an interhelical loop, which together form a single Ca^{2+}-binding site. In many proteins, EF hands exist as a pair, with a short antiparallel P-sheet interaction between the two Ca^{2+}-binding loops.

►Calcium-Binding Proteins

Effectors

Definition
Proteins that specifically interact with an activated GTPase and transmit signals to other molecules. Effectors can be enzymes themselves with the activation of an enzymatic cascade, such as in the case of the Raf kinase, or with the generation of second messengers, such as the PI3K, which generates polyphosphoinositides.

►GTPase
►B-Raf Signalling
►P13K Signalling

Efflux Pumps

Definition
Are active enzymatic transporters localized in the cytoplasmic membrane of cells.

►Xenobiotics

Efflux Ratios

Definition
Are the ratio of how fast a drug cross the gastrointestinal (GI) tract in the absorptive direction divided by how fast the drug is effluxed out of the GI tract back into the GI tract lumen (or its Caco-2 mimic). An efflux ratio greater than one indicates drug is being pumped out of the GI tract wall faster than it is coming in.

►ADMET Screen

Eg5

►KSP Mitotic Spindle Motor Protein

EGF

Definition
Epidermal growth factor is a potent mitogenic factor for a variety of cells, promotes epithelial cell proliferation and has a profound effect on the differentiation of

specific cells in vivo. EGF is also a strong inhibitor of gastric acid secretion.

►Urogastrone (URG)
►Securin
►EGFR
►Epidermal Growth Factor-like Ligands

EGF-CFC Protein Family

Definition
A family of structurally related proteins identified only in vertebrates that perform an essential role during embryogenesis ad tumorigenesis. Human Cripto-1 is the founding member of the EGF-CFC family.

►Cripto-1

EGF Family

►Epidermal Growth Factor-like Ligands

EGF-like Ligands

►Epidermal Growth Factor-like Ligands

EGFR

Definition
►Epidermal growth factor receptor. The protein found on the surface of some cells and to which epidermal growth factor (►EGF) binds, causing the cells to divide. It is found at abnormally high levels on the surface of many types of cancer cells, so these cells may divide excessively in the presence of epidermal growth factor. Also known as ErbB1 or HER1.

►Huntingtin Interacting Protein 1 (HIP1)

EGFR Transactivation

Definition
Activation of the EGFR by stimuli that do not directly interact with the EGFR ectodomain.

►Amphiregulin

EGP-2

►EpCAM

EGP34

►EpCAM

Egr-1 (Early Growth Response-1)

Definition
Is a transcription factor, commonly activated by growth factors, and that recognizes and binds to the DNA sequence 5′-CGCCCCCGC-3′, whereby it activates transcription of target genes whose products are often involved in mitogenesis and differentiation.

►MIC-1

EH

Definition
Epoxide hydrolase, also known as epoxide hydrotase, converts highly reactive eposides to trans-dihydrodiols which can be conjugated and excreted from the body.

►Phase 2 Enzymes

Eicosanoid Signaling

►Arachidonic Acid Pathway

Eicosanoids

Definition

Eicosa-, Greek for "twenty" are signaling molecules derived by oxygenation from omega-3 (ω-3) or omega-6 (ω-6) fatty acids. They exert complex control over many bodily systems, especially in inflammation, immunity, and as messengers in the central nervous system. The networks of controls that depend upon eicosanoids are among the most complex in the human body. There are four families of eicosanoids – the ►prostaglandins, the prostacyclins, the thromboxanes, and the leukotrienes. For each, there are two or three separate series, derived either from an ω-3 or ω-6 essential fatty acid.

►Arachidonic Acid Pathway
►Leukotrienes
►Lipid Mediators

Eicosapentaenoic Acid

Definition

EPA; is one of the principle omega-3 fatty acids from fish oil.

►Cachexia

eIF4A

Definition

Eukaryotic translation initiation factor 4A, a component of translation initiation factor, eIF4F, RNA helicase.

►Programmed Cell Death 4 (PDCD4/Pdcd4)

Eker Rat

Definition

Harbors a naturally occurring mutation (insertion of a 6.3 kb intracisternal A particle) in one allele of the ►tuberous sclerosis complex (*Tsc2*) gene and was first described as an autosomal dominant, hereditary model of predisposition to renal adenoma and carcinoma in 1954. Kidney lesions vary in morphology and include pure cysts, cysts with papillary projections and solid adenomas which can be seen as early as 4 months. A small minority of these tumors become malignant, with nuclear atypia and expand to include the entire kidney and metastasize to the lungs, pancreas and liver. Eker rats also develop pituitary adenomas, uterine leiomyomas and leiomyosarcomas, splenic hemangiomas and, at a low frequency, brain hamartomas resembling human ►tuberous sclerosis complex (TSC) subependymal nodules.

ELAM-1

►E-Selectin-Mediated Adhesion in Cancer

Electrical Coupling

Definition

Is the passing of electric charges between adjacent cells through gap junctions.

►Gap Junctions

Electrolytes

Definition

Are mineral nutrients for which a major physiological role is to provide electrical charge in biological fluids. The main biologically important electrolytes include sodium, potassium, and chloride and, along with bulk minerals, are sometimes referred to as macrominerals.

►Mineral Nutrients

Electromagnetic (EM) Heating

Definition

Energy deposited as heat in tissue by ohmic (i.e. resistive) loss. Ohmic loss is associated with the oscillating current generated by the electric field component of the Electromagnetic field.

►Hyperthermia

Electromagnetic Fields

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Definition

Magnetic fields are generated by the movement of any electrical charge. A continuous electric current passing through a conductor creates a static magnetic field, while an electric current changing in time creates a variable magnetic field, which radiates electromagnetic waves spreading around the surrounding space at light speed. These electromagnetic fields enter living tissue but are known as non-ionizing radiation since they are weak and unable to break molecular bonds. Metals such as iron, zinc, manganese and cobalt are sensitive to electromagnetic fields that may exert their effects on proteins and cellular components containing these metallic elements.

Characteristics

Few environmental issues are as contentious as the question of whether exposure to electromagnetic fields affects biological systems. Considering the widespread use of electromagnetic radiation generating applications such as radio, television, wireless communications etc., the continuing change in the frequencies used, the health hazard implications of any connection between electromagnetic fields and cancer risk have raised a growing interest in the potential biological effects of electromagnetic fields on the mammalian cell growth, viability and response to genotoxic injury. This topic is still a subject of repeated argument, and caution in the interpretation of the effects of static and variable magnetic fields on cellular behavior needs to be claimed. A measurable magnetic field is created even by the residential electric current. It is noteworthy that we are pervaded by the Earth's static magnetic field that is hundreds of times greater than the low-frequency electromagnetic fields created by current within homes.

Epidemiological and Clinical Evidence

The first connection between human disease and electromagnetic fields was suggested by the observation of a higher incidence of cancer in children (►Childhood cancer) living near power distribution lines. Afterwards, major power lines have been held responsible for the occurrence of different cancer varieties. Results of different studies of a possible link between exposure to electromagnetic fields and childhood cancer, namely leukemia, have been rather inconsistent. One large study found no association between electromagnetic field exposure and an increased risk of childhood leukemia, in contrast to previous reports showing that the exposure to electromagnetic fields resulted in nearly a 20% increase in the risk of leukemia. This case–control investigation (►Case control association study) did not find a significant link between the risk of childhood leukemia and the actual measurement of magnetic fields in children's current and former homes, including homes their mothers lived in during pregnancy of the affected subjects. Electromagnetic field exposure has also been associated with the risk of breast cancer, mainly in men. Epidemiological studies have shown that in industrialized countries, where the electromagnetic field generating devices are in use on a large scale, breast cancer risk is higher. It has been suggested that electromagnetic field exposure might promote breast neoplasm through inhibition of ►melatonin release. Different occupational epidemiological studies have shown an increased incidence of breast neoplasm in women employed in occupations with high electromagnetic field exposure as well as in male electrical workers. However, other investigations, not producing any significant correlation, failed to confirm these suggestive data from occupational studies. In a large Swedish cohort study, an ~10% increase in the risk of cancer was documented in people in the medium and high exposure levels. Several types of cancer, including skin, digestive, respiratory, reproductive and urinary organs, were linked with occupational magnetic field exposure, suggesting an involvement of the endocrine and immune systems. Discrepancies in epidemiological studies dealing with this matter have involved different estimates of electromagnetic field exposure, measurement and characteristics; the statistical analysis performed with data obtained in such epidemiological reports is another Achilles heel and considerable biases can create misleading conclusions. Higher exposure has been associated with an increase in the cancer risk even though care needs to be taken in

drawing any conclusion, because no dose–response relation has been documented so far. New technologies have been introduced on a large scale only in more recent years and the possible short lag period between exposure and disease manifestation needs to be considered when examining the available data. Children are increasingly heavy users of communication sources (mobile phones) and they are likely to accumulate many years of exposure during their lives. They should be thoroughly monitored in study population to detect possible effects involving long induction periods or effects from long-term exposure.

Experimental Evidence

In case of high-frequency magnetic fields, biological effects and health risks are related to the thermal effect associated with sources emitting fields high enough to cause a significant temperature rise in living tissue. Carcinogenesis is a multistep process of accumulating mutations and promoting events. It has been proposed that electromagnetic field exposure might enhance the effects of other carcinogens, provided that both exposures are chronic. The potential for genotoxicity of electromagnetic fields has been investigated and several negative studies in several exposure categories have presented sound and independent, reproducible data. Using in vivo animal models of carcinogenesis the assessment of the potential carcinogenic activity of electromagnetic fields have yielded negative results in different studies, while using the rat mammary carcinoma model results seem to be conflicting. According to available data it is unlikely that long-term exposure to electromagnetic fields is carcinogenic per se in animal models. However, a promoting effect in the development of cancer under certain exposure conditions cannot be ruled out. Since exposure conditions vary widely in the different models thus far proposed, independent replication of experimental results is absolutely crucial. Exposure to electromagnetic field, alone or in combination with ionizing radiation, appears to induce an insult at the cellular level; to inhibit DNA synthesis and the growth of human tumor cell lines in vitro. However, controversies still exist about the possibility that electromagnetic fields may influence tumor promotion. Different in vitro studies have failed to demonstrate any detectable effect of electromagnetic fields on the rate of DNA synthesis and cultured cell growth. Moreover, exposure of cultured mammalian cells to electromagnetic fields has not resulted in the production of detectable DNA lesions and has not affected intracellular ►ATP levels, suggesting that electromagnetic fields are not genotoxic and cytotoxic. On the other hand, investigating the genotoxic potential of electromagnetic fields using in vitro experiments, statistically significant and suggestive positive results have been reported. Following electromagnetic field exposure, enzymatic activity induction, DNA mutation in human and non-human cells and DNA strand breaks in rat brain cells have been demonstrated. The static magnetic field has been shown to induce a remodelling and differentiation of human neuronal cells in the absence of any alteration of DNA, thus ruling out a direct effect of the magnetic field on DNA stability. Investigating the effects of a static magnetic field on the ability to proliferate of human breast cancer cells in vitro, it has been observed that magnetic field exposure only temporarily slows down cellular growth, which then eventually fully recovers. The reduced cell growth caused by the magnetic field could be explained by a temporary effect on some cellular metabolic events leading to the reduced DNA synthesis. Alternatively, it could be ascribed to a transient cellular differentiation, since induction of differentiated phenotype often correlates with decreased cell proliferation. These results are consistent with the observation that magnetic field induces time-dependent developmental effects on the process of differentiation of the chick cerebellar cortex. Human skin fibroblasts exposed to electromagnetic fields, generated by mobile phones, show alterations in cell morphology and increased expression of mitogenic signal transduction genes (►MAP kinase kinase 3 [MAP kinase], G2/mitotic-specific cyclin G1 [►Cyclin G-associated kinase]), cell growth inhibitors (transforming growth factor-beta [►Transforming growth factor]) and genes controlling apoptosis (bax) [►Apoptosis signaling]; a significant increase in DNA synthesis and intracellular mitogenic second messenger formation [►Signal transduction] matches the high expression of MAP kinase family genes.

Clinical Relevance

In conclusion, different studies have given no consistent or convincing evidence of a causal relation between electromagnetic fields and cancer. Available data from other reports suggest that the exposure to electromagnetic fields brings about a weak increase in the risk estimates of neoplasm. However, these studies generally lack statistical power and they have too many deficiencies to rule out an association. Considering that a weak association is not synonymous with a negligible or negative effect, additional more methodologically rigorous studies are warranted.

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Electrophiles

Definition

Are molecules that are deficient in electrons. These molecules seek out an electron pair and react. In cancer electrophiles are responsible for carcinogenesis by binding to DNA or proteins causing changes that can lead to cancer. Cofactors like glutathione mediate this by providing electrons to interact with the electrophile.

►Glutathione Conjugate Transporter RLIP76

Electroporation

Definition

Introduction of biomolecules, such as DNA or proteins, into a cell or a tissue by applying an electric current.

Electrospray Ionization (ESI)

Definition

One of the "soft ionization" techniques used in mass spectrometry, whereby samples are analyzed to produce primarily molecular weight information. ESI is especially suitable for the mass measurement of most small organic molecules as well as high molecular mass biomolecules.

►Surface Plasmon Resonance

Electrostatic Interaction

Definition

An electrostatic interaction or bond results from the attraction of a charged group on one molecule with an oppositely charged group on another molecule.

►Lactoferricin Antiangiogeneis Inhibitor

Elimination

Definition

Of a drug comprises both metabolism and excretion.

►Irinotecan

ELISA

Definition

*E*nzyme-*l*inked *i*mmunosorbent *a*ssay is a biochemical analysis technique used to detect the presence of a protein in a fluid sample. The assay uses two antibodies, one that specifically detects the protein of interest, and a second antibody that reacts to the first antibody-protein complex. The second antibody is usually linked to an enzyme that produces a chromogenic or fluorogenic substrate, thus providing the read-out for the assay. ELISAs are often used to measure cancer biomarkers in the blood of cancer patients.

►Osteopontin

ELISPOT

Definition

Enzyme-linked immunospot (ELISPOT) assay is an antibody capture-based method for enumerating specific T or B cells that secrete a certain protein. Basically, cells are placed on a membrane coated with an antibody specific for a given protein. The protein is captured

directly around the cell (on the membrane) and is detected with a secondary antibody. Using colorimetry, protein-producing cells can be visualised as spots and counted.

Elk-1

Definition

The transcription factor which is a component of the ternary complex that binds the serum response element, phosphorylated by MAP kinase in response to growth factors.

►Major Vault Protein
►MAP Kinases

Elongin

Definition

Transcription factor that stimulates the rate of elongation by RNA polymerase II that is composed of subunits A, B, and C.

►Suppressors of Cytokine Signaling

Elongin BC Complex

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Definition

The mammalian elongin BC complex is a heterodimer, composed of the 118 amino acid, ubiquitin-like elongin B protein and the 112 amino acid elongin C protein. The elongin BC complex interacts through a short, degenerate BC-box motif with multiple proteins, including the transcription factor elongin A, the von Hippel-Lindau (VHL) tumor suppressor protein and members of the SOCS-box protein family.

Characteristics

The elongin B and C proteins were initially identified as positive regulatory subunits of the three-subunit elongin complex, which is one of several transcription factors capable of controlling the activity of the RNA polymerase II elongation complex. The three-subunit elongin complex was originally purified from rat liver nuclei by its ability to activate the overall rate of elongation by RNA polymerase II, by suppressing transient polymerase pausing at many sites within transcribed sequences. The elongin complex is composed of a transcriptionally active A subunit of approximately 770 amino acids and the elongin BC subcomplex. The latter positively regulates the activity of the elongin complex by binding to a BC-box motif in the elongin A elongation activation domain and potently inducing elongin A transcriptional activity. The BC-box is an ~10 amino acid sequence motif with consensus Lxxx(C,A,S)xxx(A,I,L,V) Elongins B and C perform different functions in regulation of elongin A transcriptional activity. Elongin C functions as the inducing ligand and is capable of binding directly to the BC-box and maximally activating elongin A transcriptional activity in the absence of elongin B. Elongin B binds to elongin C and promotes stable binding of elongin C to elongin A.

Shortly after the discovery of the elongin complex, a large collection of BC-box motif-containing proteins were identified and found to function together with the elongin BC complex as integral components of members of two closely related classes of multiprotein E3 ubiquitin ligases (►Ubiquitination). In addition to a BC-box protein and the elongin BC complex, these E3 ubiquitin ligases include a heterodimeric submodule composed either of Cullin family member Cul2 and RING-H2 finger protein Rbx1 (also known as ROC1 or Hrt1) or the related Cul5 and Rbx2 proteins. In the context of these E3 ubiquitin ligases, the BC-box protein functions as the substrate recognition subunit. The elongin BC complex functions as an adaptor that links the BC-box protein to the Cullin/Rbx submodule, which in turn functions to recruit E2 ubiquitin conjugating enzymes of the Ubc5 family to the ubiquitin ligase complex and to activate ubiquitination of target proteins. Interaction of elongin BC with the BC-box is governed by interaction of a highly conserved leucine found at the N-terminus of the BC-box with a hydrophobic pocket created by residues in the C-terminal half of elongin C. A short N-terminal elongin C region binds to the N-terminal ubiquitin-like domain of elongin B. Additional sequences near the BC-box motif in BC-box proteins specify selection of the particular Cul2/Rbx1 or Cul5/Rbx2 submodule present in a given elongin BC-containing E3 ubiquitin ligase.

The VHL Tumor Suppressor Complex

The founding member of the family of elongin BC-containing E3 ubiquitin ligases is the VHL tumor suppressor (►Von Hippel-Lindau tumor suppressor

gene, ►Tumor suppression) complex, which is composed of the VHL BC-box protein, elongin BC, and a Cul2/Rbx1 submodule. The VHL gene on chromosome 3p25.5 is mutated in the majority of sporadic clear cell renal carcinomas, in familial erythrocytosis-2, and in the VHL disease, an autosomal dominant familial cancer syndrome that predisposes affected individuals to a variety of tumors including clear cell renal carcinomas, cerebellar hemangioblastomas and hemangiomas, retinal angiomas, and pheochromocytomas. A substantial fraction of VHL mutations found in sporadic clear cell renal carcinomas and in VHL kindreds results in mutation or deletion of the VHL BC-box and disruption of the VHL-elongin BC interaction and of the VHL ubiquitin ligase complex.

A major function of the VHL E3 ubiquitin ligase complex is to regulate transcription of genes such as vascular endothelial growth factor (VEGF), platelet derived growth factor β (PDGFβ), and E-cadherin by controlling the cellular levels of ►hypoxia-inducible transcription factors 1–3 by ubiquitin-dependent proteolysis. Under normoxic cell growth conditions, HIF transcription factors are rapidly ubiquitinated and destroyed by the proteasome. Under hypoxic conditions, ubiquitination of HIF transcription factors is inhibited and their concentrations rise to sufficient levels to activate hypoxia-inducible genes. Targeting of HIF transcription factors by the VHL ubiquitin ligase requires that HIFs are first post-translationally modified by hydroxylation of a critical proline in the HIF oxygen-dependent degradation domain (ODD) by a prolyl hydroxylase enzyme of the dioxygenase family. In light of evidence that the prolyl hydroxylase uses molecular oxygen as the oxygen donor, it has been proposed that the prolyl hydroxylase might serve as an oxygen sensor (or oxygen-dependent switch) for regulation of expression of HIF-dependent hypoxia-inducible genes.

Although it is well established that the VHL protein plays a crucial role in regulation of transcription of hypoxia-inducible genes through its control of cellular HIF levels, renal carcinoma cells expressing VHL mutants lacking a functional BC-box exhibit a collection of additional phenotypes, including

- defects in regulation of mRNA stability resulting in misregulation and constitutive expression of hypoxia-inducible genes;
- cell-cycle defects resulting at least in part from misregulation of the degradation of the Cdk inhibitor p27;
- defects in ubiquitin-dependent degradation of improperly processed or folded proteins;
- extracellular matrix defects resulting from failure of cells to secrete and/or properly assemble fibronectin.

At least some of these phenotypes result from defects in a VHL-dependent, HIF-independent pathway that remains to be defined. In addition, future studies are needed to determine whether these additional phenotypes are due to defects in the VHL E3 ubiquitin ligase complex or whether they result from other VHL defects not related to its function in the ubiquitin–proteasome pathway.

SOCS-box Proteins and the Elongin BC Complex

In addition to the VHL E3 ubiquitin ligase complex, which includes a Cul2/Rbx1 submodule, a growing collection of elongin BC-containing E3 ubiquitin ligases, which include Cul5/Rbx2 submodules, have been identified and found to play roles in human disease. Among the BC-box proteins that assemble with elongin BC and Cul5/Rbx2 are the so-called SOCS-box family of proteins and various virally-encoded proteins such as the Adenovirus E4orf6 and HIV-1 VIF proteins. In addition, elongin A assembles with elongin BC and Cul5/Rbx to form a potential E3 ubiquitin ligase that still possesses RNA polymerase II elongation factor activity.

SOCS-box proteins include the SH2 domain-containing suppressors of cytokine signaling (SOCS) proteins and more than 30 additional members of the ras, WD repeat, ankyrin repeat, and SPRY domain families. SOCS-box proteins are modular and are composed of an N-terminal SH2, ras, WD repeat, ankyrin repeat, or SPRY domain and a C-terminal SOCS-box. The SOCS-box is an ~50 amino acid motif composed of an N-terminal consensus BC-box and a short C-terminal L/P-rich region that helps specify interaction of SOCS-box proteins with Cul5/Rbx2. The founding members of the SOCS-box protein family were the SH2 domain-containing SOCS proteins, which assembly with elongin BC and Cul5/Rbx2 to form E3 ubiquitin ligases that function as negative regulators of cytokine-induced Jak/STAT signaling, at least in part by targeting Jak or receptor tyrosine kinases and inhibiting phosphorylation and activation of STATs.

The Adenovirus E4orf6 and HIV-1 VIF proteins, function together with cellular elongin BC and Cul5/Rbx2 as substrate recognition subunits of E3 ubiquitin ligases that promote virus growth. The E4orf6 ubiquitin ligase assembles shortly after Adenovirus infection of susceptible cells and targets the p53 tumor suppressor for ubiquitination and degradation by the proteasome, thus inhibiting apoptosis of infected cells and promoting viral infection. The VIF ubiquitin ligase targets the cellular APOBEC3G cytidine deaminase for ubiquitination and degradation by the proteasome. The APOBEC3G enzyme acts as a cellular antiretroviral factor by inducing hypermutations in newly synthesized HIV-1 minus-strand DNA.

Clinical Relevance

Although mutation of the elongin A or SOCS genes have not yet been identified in human disease, mutation of the VHL gene on chromosome 3p25.5 is responsible for the majority of sporadic clear cell renal carcinomas, for familial erythrocytosis-2, and for VHL disease, an autosomal dominant familial cancer syndrome that predisposes affected individuals to a variety of tumors including clear cell renal carcinomas, cerebellar hemangioblastomas and hemangiomas, retinal angiomas, and pheochromocytomas. Given their contributions to the adenoviral and retroviral life cycles, the E4orf6 and Vif ubiquitin ligases offer potential therapeutic targets for antiviral agents.

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ELR+ /ELR-chemokines

►CXC Chemokines

Emboli

Definition

Pieces of thrombus (commonly known as blood clots) or other material, which form at one site (in the cardiovascular system), breaks off and goes through the blood stream and lodges at another site, usually leading to significant problems, including sudden death. A common example is pulmonary thromboemboli.

►Cardiac Tumors

Embolization

Definition

Refers to the therapeutic introduction of various substances into the circulation to occlude vessels, by purposely introducing emboli. A treatment that clogs small blood vessels and blocks the flow of blood, such as to a tumor. Aim is to either to arrest or prevent hemorrhaging, to devitalize a structure, tumor, or organ by occluding its blood supply, or to reduce blood flow to an arteriovenous malformation.

Embryo-specific α-Globulin

►Alpha-Fetoprotein – Modern

Embryo-specific Alpha-Globulin

►Alpha-Fetoprotein – Modern

Embryonal Serum α-Globulin

►Alpha-Fetoprotein – Modern

Embryonal Serum Alpha-Globulin

►Alpha-Fetoprotein – Modern

Embryonic Stem Cells

Definition
Primitive cells from the embryo with pluripotential capacity. These cells are capable of proliferating indefinitely in a pluripotent state and have the potential to differentiate into all somatic cell types.

►Adult Stem Cells
►Stem Cell Markers
►Stem Cells and Cancer

EMI Domain

Definition
Is first named after its presence in proteins of the EMILIN family and is associated with other domains, such as C1q, laminin-type EGF-like FN3, WAP, ZP, or FAS1.

►Periostin

Emphysema

Definition
An abnormal, permanent airspace dilation with destruction of alveolar walls, with no evidence of fibrosis.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

EMS1

►Cortactin

EMT

Definition
►Epithelial-to-mesenchymal transition

Enchondroma

Definition
Benign central cartilaginous tumor. Enchondromas arise in the medullar cavity of bone. A benign tumor composed of hyaline cartilage-producing tumor cells.

►Bone Tumors
►Chondrosarcoma

End Replication Problem

Definition
Is where all organisms with linear chromosomes show loss of telomeric DNA with each cell division.

►Telomerase
►Senescence and Immortalization

Endocan

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Synonyms
Endothelial cell specific molecule-1; Endocan; ESM-1

Definition
Endocan is a soluble ►proteoglycan of 50 kDa carrying a single dermatan sulfate chain. This dermatan sulfate proteoglycan, which is expressed by the vascular endothelium, has been found freely circulating in the blood of healthy subjects. Expression of endocan has been shown to be upregulated in inflammatory diseases and during progression of cancer.

Characteristics
Endocan, synonym endothelial cell specific molecule-1 (ESM-1), was originally cloned from a human endothelial cell cDNA library and described as being secreted by human umbilical vein endothelial cells (HUVEC). Endocan transcripts were detected from a variety of cultured endothelial cells of different origins,

i.e., in human coronary artery endothelial cells (HCAEC), human pulmonary artery endothelial cells (HPAEC), human dermal microvascular endothelial cells (HDMVEC) and human capillary endothelial cells (HUCE) purified from adipose tissues. Moreover, there have been rising evidences of endocan expression in cultured cell lines that are not of endothelial origin as in human adipocytes, in melanoma cells involved in ►vasculogenic mimicry process, in renal carcinoma cells and more recently in ►glioblastoma cells. Structurally, endocan is a soluble proteoglycan of 50 kDa, constituted of a mature polypeptide of 165 amino acids and a single dermatan sulfate chain covalently linked to the serine residue at position 137. Experimental evidence implicates endocan as a key player in the regulation of major processes such as cell ►adhesion or proliferation in ►inflammation disorders and tumor progression. Inflammatory cytokines such as ►TNF-alpha and growth factors involved in ►angiogenesis process such as ►vascular endothelial growth factor (VEGF) and ►fibroblast growth factor (FGF-2) strongly increased the expression, synthesis, or release of endocan by human endothelial and/or tumor cells. Endocan has then been shown to be clearly overexpressed in various human tumors, with elevated serum levels being observed in late-stage ►lung cancer patients, as measured by enzyme-linked immunoassay, and with its overexpression in tumors being evident by immunohistochemistry. Upregulation of endocan have also been recognized as a significant molecular signature of a bad prognosis in several types of cancers.

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Endocannabinoids

►Cannabinoids and Cancer

Endochondral Ossification

Definition

Process in which hyaline cartilage serves as a precursor model for bone, which gradually replaces the cartilage. This occurs in the epiphyseal growth plate during elongation of long bones.

►Chondrosarcoma

Endocrine Ablation

Definition

Is the surgical removal of organs that produce hormones

►Estrogenic Hormones

Endocrine or Antihormonal Therapy

Synonyms

hormone theraphy; antihormone theraphy

Definition

Therapy used in breast cancer based in the blockade of steroid hormone receptor activity. The manipulation of hormones in order to treat a disease or condition.

►Fulvestrant
►Progestin

Endocrine-Related Cancers

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Synonyms

Malignant neoplasias originating in endocrine tissues or their target organs; Malignant tumors of the endocrine

glands; hormone-related cancers; Reproductive system cancers; cancers of hormone-responsive organs or tissues; Hormone-induced cancers; Endocrine-responsive cancers

Definition

The term endocrine-related cancers refers mainly to malignant tumors in either the endocrine glands or the endocrine target tissues. The endocrine system comprises the glands in the body that make and secrete hormones. All endocrine glands, such as the ovaries and testes, secrete hormones directly into the bloodstream, where they travel to a target organ or cell, such as the breast and prostate, to trigger a specific reaction. These glands are primarily involved in controlling a wide range of activities and functions in the body, such as metabolism, growth, and reproduction.

As is true for other types of malignant neoplasias, endocrine-related cancers are characterized by potentially out-of-control growth that either expands locally by invasion or spreads systemically by ►metastasis. In many cases, hormone production is abnormal when cancers are found in the endocrine glands, and hormone response can become disrupted when cancers occur in target tissues.

Other malignancies are also associated with the endocrine system. Carcinoids (►carcinoid tumors), a group of slow-growing but often malignant tumors, originate in the hormone-producing cells of the diffuse ►neuroendocrine. A large variety of other cancers that are not primary endocrine cancers also occur in endocrine tissues, including meningiomas, astrocytomas, and lymphomas. ►Metastases from other cancers can be found in such endocrine organs as the adrenal glands, pituitary, and thyroid gland. Malignancies such ►lung cancers can also produce hormones and cause patients to display clinically ectopic hormone syndromes.

Characteristics

The subject of endocrine-related cancers encompasses a vast area of clinical medicine and cancer biology. This essay mainly describes endocrine gland cancers. ►Breast cancers and ►prostate cancers, hormone target tissue cancers with a very high prevalence, will be described elsewhere.

Pathogenesis

The special natures of the origin and pathogenesis of endocrine tumors derive from a number of special properties of endocrine cells. As a result of environment risk and the stepwise accumulation of genetic defects, normal cells are transformed into malignant cells by a failure of normal growth regulation. The great majority of endocrine cells are stable, having a low growth rate and a long lifespan in contrast to the renewing tissues that are maintained by stem cells and have a short intermitotic life. In endocrine tissues, both function and growth are closely linked and are stimulated by one trophic hormone. In contrast to the renewing tissues, which undergo frequent cell divisions during which mutations commonly occur, endocrine tissues divide slowly and mutations are unlikely, but those that do occur are retained for a long time. In most types of endocrine cells, the mutation rate is low, but mutations accumulate; the chance of mutations is increased by any stimulus leading to mitotic growth in the endocrine tissues.

There are a number of possible explanations for the pathogenesis of endocrine cancers.

One possibility is the activation of ►oncogenes or inactivation of ►tumor suppressors. Defects in a variety of oncogenes and ►tumor-suppressor genes such as ►*ras*, ►p53, TGFβ, and ►IGF-I have been described in thyroid carcinomas.

Another possibility is that abnormal physiologic regulation disrupts the balance between stimulation and inhibition, and the resultant hyperfunction in the gland results in adenoma.

Abnormal stimulation may also induce gene disregulation, including oncogene activation or tumor-suppressor deactivation or damage, which has been described in the carcinogenesis of a number of human endocrine tumors.

Prevalence

Endocrine gland tumors are not common. There is a relative paucity of epidemiological investigations of endocrine cancers mainly because of the rarity of individual cases and the occult nature of the diseases (including asymptomatic, cases and their responses to therapy). In addition the reported incidence is also affected by variable levels of recognition or registration of the tumors in different clinical settings, including endocrinology, oncology, surgery, neurology, and even out-patient settings. In the United States, approximately 25,520 new cases of endocrine system cancer were diagnosed in 2004, and endocrine cancers have been estimated to account for approximately 4% of all new cancer cases each year. The most common malignancies of the endocrine system are gonadal tumors and thyroid tumors. In United States, ovarian cancer accounts for 44% of all endocrine system cancers, with an annual incidence of 8 per 100,000 women. Testicular cancer has an annual incidence of 2 per 100,000 men. In the United States, thyroid cancer has an annual incidence of approximately 6 per 100,000 persons (accounting for 36% of all endocrine cancers), whereas the annual incidence in the United Kingdom is around 0.6–1.5 per 100,000. The other endocrine tumors are less common. ►Adrenocortical cancer, carcinoids tumors, and other ►carcinoid tumors are all extremely rare, having incidences as low as 0.5 per 100,000 per year in the

United Kingdom. Parathyroid tumors are relatively more common, with an incidence of 28 per 100,000 per year in the United Kingdom, but the malignancy is still rare.

Clinical Presentation and Pathology

Most endocrine tumors are not malignant, although certain types when they do occur are likely to be cancerous. Endocrine tumors affect all age groups.

The clinical manifestations vary. Some patients are asymptomatic, with tumors incidentally discovered at autopsy or during sophisticated imaging techniques; others have multiple hormone-related symptoms or severe symptoms at late malignancy. Severity of symptoms depends on the site and the malignancy. Most endocrine tumors of childhood are benign or are low-grade malignancies. A small percentage of gonadal and ►germ cell tumors, thyroid cancers, and ►adrenocortical tumors are high grade.

Clinical manifestations of endocrine gland tumors are often associated with abnormalities of hormone secretion. For example, patients with insulin-producing islet cell tumors of the pancreas may have severe effects of low blood sugar due to the excessive insulin in the blood secreted by tumors. In addition to the hormone-producing tumors or functional tumors, there is another category of nonfunctional tumors that do not secrete hormones. Both types of tumors have the potential to be malignant.

Endocrine system tumors involve all anatomic areas from the head to the pelvic area and include tumors in single and multiple sites: pituitary cancer, thyroid cancer, parathyroid cancer, adrenal gland cancer, and endocrine ►pancreatic cancers, as well as ►multiple endocrine neoplasia (MEN), ectopic endocrine tumors, and neuroendocrine tumors, and metastases in the endocrine organs from other primary cancers.

Pituitary Tumors

Pituitary tumors are classified by the type of hormone they secrete. They are rarely malignant but, because of the production of a diverse spectrum of excess hormones varying with the cell type of origin, can cause a variety of health problems including ►Cushing syndrome, ►acromegaly, disturbance of milk secretion (prolactinoma), and local symptoms such as visual complication, headache, and injury to cranial nerves. In certain cases, tumors may cause pituitary hormone insufficiency due to pituitary tissue destruction.

Thyroid Tumors

Although only 5% of the tumors found on the thyroid are malignant, their biological behaviors may vary substantially, ranging from well-differentiated tumors to aggressive anaplastic cancers. A classification of thyroid tumors proposed by the World Health Organization (WHO) takes into account the histological features and clinical behavior of the tumor and classifies malignant thyroid tumors into four types. (►thyroid carcinogenesis).

Parathyroid Tumors

Around 5% of parathyroid tumors are malignant. Overproduction of parathyroid hormone, a condition known as hyperparathyroidism, is a common condition associated with both benign and malignant tumors. Untreated, hyperparathyroidism can result in osteoporosis (causing bones to become brittle and fracture easily), kidney stones, peptic ulcers, and nervous system problems.

Endocrine Pancreatic Tumors

Pancreatic islet-cell tumors are rare, with malignant tumors even more rare. The most common types of tumors are gastrinomas, which are associated with ►Zollinger–Ellison syndrome. Pancreatic islet-cell tumors include functioning and nonfunctioning varieties. Islet cells are multipotential in respect to peptide production, so functioning tumors can be classified into entopic-hormone tumors (e.g., insulinoma, glucagonoma, and somatostatinoma) and ectopic-hormone tumors (gastrinoma, VIPoma). Benign tumors are often treatable with surgery combined with medicine. For treatment of malignant tumors, in addition to surgery and radiation, cytotoxic ►chemotherapy has also been used.

Adrenal Tumors

There are two adrenal glands, one above each kidney in the back of the upper abdomen. The adrenal gland consists of two layers: an outside layer (the adrenal cortex) and an inner layer (the adrenal medulla).

Cancer of the adrenal cortex is also called ►adrenocortical cancer. The cells in the adrenal cortex make hormones that help the body work properly. When cells in the adrenal cortex become cancerous, they may make too much of one or more hormones, which can cause symptoms such as high blood pressure, weakening of the bones, and diabetes. If male or female hormones are affected, the person may undergo changes such as a deepening of the voice, growth of facial hair, swelling of the genitals, or swelling of the breasts.

Tumors that start in the adrenal medulla are called pheochromocytomas. About 10% of pheochromocytomas are malignant.

Most adrenal tumors are disorders of hormonal excess, but rarely malignant disease can cause adrenal insufficiency.

Ovarian Tumors

►Ovarian cancer can develop in the egg cells inside the ovary (germ cell tumors), but most occur in the cells

lining the outside of the ovary, and most of these tumors are benign.

Testicular Tumors

►Testis cancer can occur in one or both of the testes. Most testicular tumors are malignant and account for approximately 1% of all cancers in men. Over 90% develop in the germ cells and are generally malignant. Only 4% involve the endocrine cells of the testes, and these tumors are rarely malignant.

Multiple Endocrine Gland Tumors

Some disorders result in the simultaneous occurrence of tumors on several endocrine glands. Many of these are inherited disorders, including ►multiple endocrine neoplasia syndromes, ►Von Hippel–Lindau syndrome, and ►Von Recklinghausen disease (►neurofibromatosis types).

Carcinoids

►Carcinoid tumors a group of slow-growing but often malignant tumors, originate in hormone-producing cells of the diffuse neuroendocrine system and have been found in almost every organ of the human body. Approximate 10% of carcinoids secrete excess amounts of hormones, most notably the peptide serotonin (5-HT). Presenting symptoms including flushing, diarrhea, wheezing, abdominal cramping, and peripheral edema. The only curative therapy for carcinoid tumor is surgery.

Secondary Endocrine Malignancies

In addition to primary endocrine cancers, a large variety of other cancers—including ►meningiomas, ►astrocytomas, and ►lymphomas—occur in endocrine tissues. Metastases from other cancers can be found in such endocrine organs as the adrenal glands, pituitary, and thyroid gland.

Ectopic Hormone Syndromes

Nonendocrine tumors can also produce hormones, causing patients to display clinically ectopic hormone syndromes. For example, lung cancers produce parathyroid hormone-related protein and ►ACTH. Ectopic hormone syndromes are important because of several clinical reasons. Such syndromes may appear as the first signal of a malignancy. Ectopic hormone production may serve as a tumor marker even before the tumor mass is apparent and may serve as follow-up evidence for a response to therapy.

Treatment

Many endocrine cancers can be treated and some can be cured. However, given the complex nature of endocrine tumors, therapy for such cancers is unlike treatment for many other types of cancer. Targeting of these cancers as well as normalizing hormone levels requires the cooperation of medical endocrinologists and endocrine surgeons to apply both surgical and biochemical therapies. Surgery is generally the treatment of choice for most endocrine cancers. Chemotherapy is frequently used and is most effective in germ cell tumors of the ovary and testis. Although some tumors are resistant to radiation, radiation therapy may nonetheless decrease the chance of recurrence and halt the spread of cancer cells. ►Hormone replacement therapy is frequently used.

Prognosis

Many benign and malignant endocrine tumors are treatable with a combination of surgery and medication, and the survival rate for many patients with endocrine cancers is good. The best outcome is for patients with ovarian or testicular germ cell tumors, with most achieving disease-free long-term survival. Prognosis for patients with pituitary and thyroid tumors and adrenal adenoma and MEN is generally good. The survival rate for patients with adrenal carcinoma is poor, with an average survival of approximately 1.5 years.

In the United States, 25,520 new cases of endocrine system cancer were diagnosed in 2004, with an estimated 2,440 deaths, for a ratio of deaths to incidence of 0.096 for endocrine system cancers.

Cancers of Endocrine Target Tissues

Hormones have wide effects on a variety of cellular processes, including cell growth and differentiation, metabolic activity, and the metabolism of substances. In fact, the endocrine system directly or indirectly influences all mammalian organs and tissues. Unbalance of endocrine hormones is an endogenous factor that may sensitize the cells to the carcinogenic insult, thereby promoting cancer development. It has been well established that sex hormone-induced cell proliferation plays a critical role in corresponding hormone-initiated carcinogenesis, as is reflected in the fact that the many types of human cancers, including breast cancer, prostate cancer, and ►endometrial cancer, occur in sex-hormone target tissues.

More than 25% of human tumors originate in the endocrine organs or their target tissues. However, with aspect to prevalence, the major entity in the spectrum of endocrine-related cancers is endocrine target organs such as the breast and prostate. In the Western world, breast cancer is the most common tumor in women, and prostate cancer is the most prevalent malignancy in men and the second most common cause of male cancer deaths. They have attracted great attention in endocrinology because of not only their extremely high incidence but also their endocrinological nature. Specifically, breast cancer and prostate cancer as instance start as estrogen and androgen dependent-noninvasive disease, respectively, and are sensitive to

endocrine agents and treatable at an early stage with hormone therapy and surgery. However, if left undetected or untreated, these cancers eventually develops into a more aggressive, hormone-independent, highly invasive disease and the patients die of their diseases after tumor cells metastasize to distant sites in the bodies. These high-incident cancers of endocrine target tissues are described elsewhere.

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Endocrine-responsive Cancers

►Endocrine-Related Cancers

Endocurietherapy

►Brachytherapy

Endocytosis

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Synonyms

Internalization; Receptor-mediated endocytosis; Clathrin-mediated endocytosis; Pinocytosis; Fluid phase endocytosis

Definition

A process in eukaryotic cells consisting of a progressive invagination of a small region of the plasma membrane which is subsequently pinched off to form a cytoplasmic vesicle.

Characteristics

Eukaryotic cells use endocytosis to internalize plasma membrane, surface receptors and their bound ligands, nutrients, bacterial toxins, immunoglobulins viruses, and various extracellular soluble molecules. The molecular machinery of endocytosis is also largely overlapping that of ►synaptic vesicle recycling. Once an intracellular vesicle is formed, following the endocytic process, its content is trafficked through the ►endosomal compartment and normally destined to either degradation in the lysosomal compartment, or to ►recycling to the cell surface. The definition of endocytosis should be limited to the process leading to the formation of vesicles of 100–200 nm, which is also known with the general name of pinocytosis.

On the contrary, phagocytosis is the intake of large particles, such as bacteria or parts of broken cells. It is used by many protozoans to ingest food particles and by blood cells (macrophages) to take in and destroy pathogens and dead-cell debris. After the binding of the target particle to the cell surface, the plasma membrane expands along the surface of the particle and eventually engulfs it. Vesicles formed by this process are much larger than those formed by endocytosis (1–2 μm).

Four different types of endocytic processes are described below:

1. Fluid phase endocytosis or macropinocytosis is the simpler and nonspecific form of endocytosis in which any fluid extracellular material is taken up at a rate that is simply proportional to its concentration in the extracellular fluid. Fluid phase endocytosis does not require a specific interaction of extracellular material with surface-bound receptor structures. Macropinocytosis shares similarities with phagocytosis as both require activation of GTPases that, in turn, stimulates actin polymerization and depolymerization events necessary for membrane protruding. After the collapse of the ruffles onto the plasma membrane, macropinosomes are formed with a mechanism that is independent of the fission-inducing protein ►dynamin.
2. ►Clathrin-mediated endocytosis occurs through the formation of clathrin-coated pits at the plasma membrane, followed by the generation of clathrin-coated vesicles of 100–150 nm in diameter. The process requires, in addition to clathrin, the presence of the adaptor protein complex ►AP2, or of other adaptors. The general function of the adaptors is that of establishing a physical link between plasma

membrane receptors and clathrin. The formation of clathrin-coated pits at the plasma membrane shares structural and molecular similarities with the budding of clathrin-coated vesicles from the Golgi apparatus, which also requires clathrin and a different adaptor complex, AP1. Clathrin-mediated endocytosis requires interaction of an extracellular ligand with a surface receptor, and takes the name of receptor-mediated endocytosis (or internalization). Two types of receptor-mediated endocytosis are known: (a) constitutive and (b) ligand-induced.

(a) In constitutive endocytosis, membrane receptors are continuously internalized and after sorting in the endosomal compartment, they are recycled back to the cell surface. When a ligand binds to the receptor, the ligand is also internalized and can undergo different metabolic destinies. Two paradigmatic examples are provided by the constitutive endocytosis of the low-density lipoprotein (LDL) receptor and of the transferrin receptor. In the former case, LDL complexed to cholesterol is internalized with its receptor. In the endosomes, the LDL receptor dissociates from the LDL–cholesterol complex and it is redirected to the cell surface for more cycles of internalization. The LDL–cholesterol complex is routed to the lysosomes, where LDL is degraded and free cholesterol is made available to the cell. The cycle of the transferrin receptor is more complex. Transferrin, bound to iron, is also internalized together with its receptor. In the endosomal compartment, the acidic pH causes the dissociation of iron. Iron-free transferrin (apotransferrin) remains, however, bound to the receptor, and it is recycled to the plasma membrane. The process of constitutive internalization is thus used by the cell mostly for the uptake of nutrients.

(b) In the ligand-regulated process, internalization is triggered by the interaction of a ligand with its surface receptor. Both ligand and receptors are normally routed to the lysosomal compartment with ensuing degradation. However, a fraction of the internalized receptor can be redelivered to the plasma membrane, in a recycling process not dissimilar to that of constitutive endocytosis. The process of induced internalization therefore serves, in the majority of cases, as a down-regulation mechanism to extinguish signals originating on the plasma membrane from signaling receptors, for instance receptor tyrosine kinases (►Tyrosine kinase receptors). It has, however, become increasingly clear that endocytosis is also required to propagate signals originated from surface receptors, thus not merely constituting an attenuation mechanism (see below).

3. Caveolae-mediated endocytosis occurs through flask-shaped, nonclathrin-coated surface structures called caveolae. The vesicles formed in this process are of 50–85 nm in diameter and ►caveolin-1 is an essential component, as caveolin-1-null mice lack caveolar structures. By this process, cells are able to internalize certain glycosylphosphatidylinositol-linked proteins, albumin, bacterial toxins as well as membrane-associated receptors like MHC class I, TGFβR, or E-cadherin. Caveolae remain for long periods at the plasma membrane, but their internalization can be stimulated by various agents. These include the SV40 virus which uses caveolae for entry into cells and stimulates caveolar budding, as well as sterols and glycosphingolipids. Although the molecular circuitries underlying caveolar internalization are not completely understood, critical requirements for dynamin, Src kinases, protein kinase C (PKC), and actin recruitment have been demonstrated. It is important to point out that caveolae represent one type of cholesterol-rich microdomain on the plasma membrane and that in the absence of caveolin, lipid-raft-dependent trafficking occurs (see below).
4. Clathrin- and caveolin-independent endocytosis probably encompasses more than one pathway. Given the poor molecular definition, the mechanisms that govern caveolin- and clathrin-independent endocytosis remain in large part unknown, as illustrated by the fact that these pathways are described only in negative terms. These internalization routes have been shown to mediate the internalization of interleukin-2 receptor-β (IL2Rβ), some GPI-anchored proteins, and receptor tyrosine kinases.

It is likely that these different pathways have evolved so that pinocytosis can be coordinated with more complex aspects of cell physiology, such as signal transduction, development and modulation of the cell responses to, and interaction with, its environment.

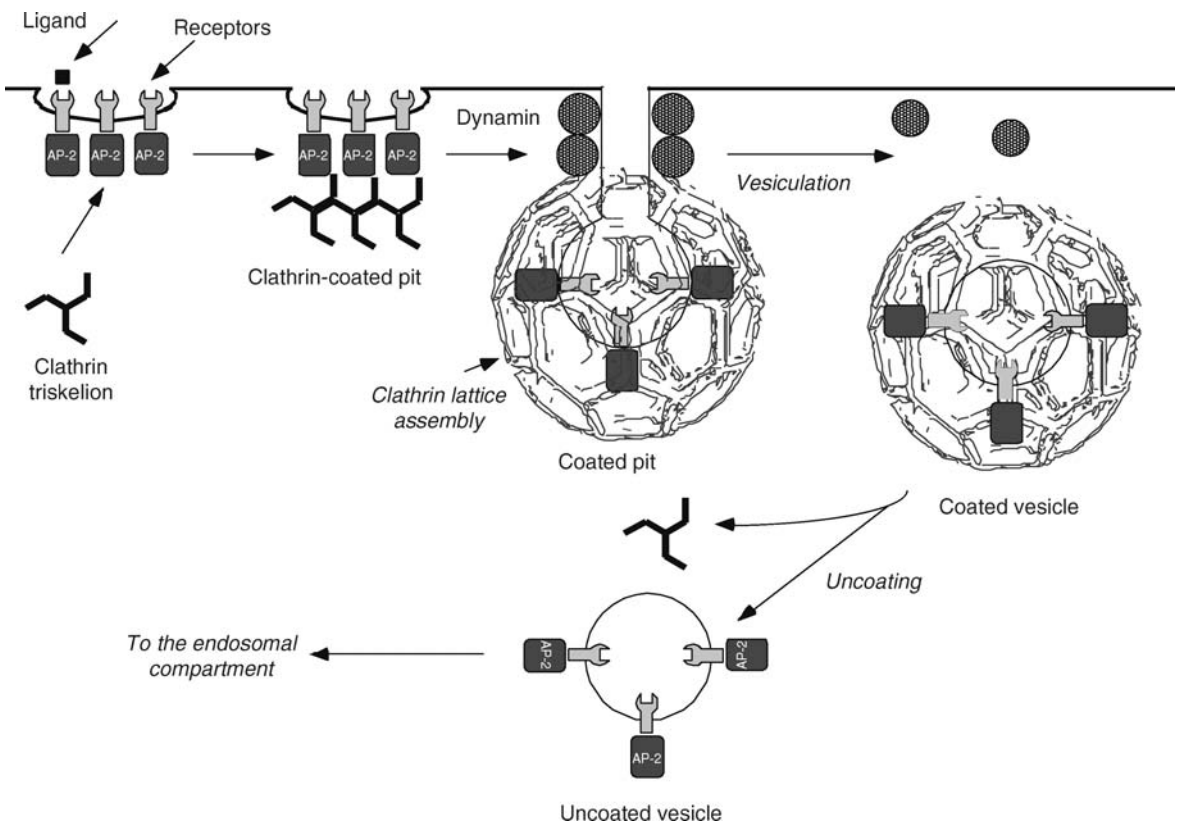
Endocytosis and Signaling

One aspect of endocytosis that has attracted much attention in recent years is the relationship between internalization of surface receptors and their ability to transduce signals within the cell. Many surface receptors (including, but not limited to, receptor tyrosine kinases) act as molecular transducers, by binding to extracellular ligands and delivering their signal to the cell, with various mechanisms. By-and-large these receptors are removed from the cell surface through endocytosis and destined to lysosomal degradation. Thus, for many years, endocytosis has been considered the major form of receptor attenuation leading to signal extinction. It has, however, become clear that signaling receptors continue to signal while en

route through the endosomal compartment, and that in many cases they gain access, in this compartment, to interactors and/or substrates, not available at the plasma membrane. In addition, the recycling of a fraction of the receptor to the cell surface might have a profound effect on signaling. It is becoming apparent that recycled signaling receptors are not simply redelivered to the plasma membrane, but might, in many cases, be delivered to specific regions of the membrane where local signaling is necessary. Therefore, endocytosis provides spatial and temporal dimension to signaling, ensuring prolonged signaling in the endosomes and local redistribution of signaling molecules. In this framework, endocytosis should not be considered merely as an attenuator of ►signal transduction, but rather as an integrator of signaling and attenuation. This complex interplay is at the basis of numerous cellular functions, including motility, proliferation, and determination of cellular fate/differentiation.

Molecular Mechanisms

The structural and regulatory mechanisms of the clathrin-mediated endocytosis are being elucidated. Four major structural components are required in the formation of an endocytic vesicle (see Fig. 1): clathrin, AP2, receptor tails, and dynamin. Polymerization of clathrin into a hexagonal/pentagonal array forms a cagelike lattice around the internalizing pit and provides the organizing framework of the pit. The AP2 complex drives the polymerization of clathrin and serves as a recruiter of receptors to the forming pit, due to its ability to simultaneously interact with the receptor intracytoplasmic tails and with clathrin. Receptor tails contain endocytic codes, i.e., amino acid sequences capable by themselves of sustaining internalization. Endocytic codes are thought to be cryptic in receptor tyrosine kinases and to be unmasked by conformational changes that follow receptor activation and autophosphorylation. In other types of receptors, such as the transferrin receptor, endocytic codes are probably continuously exposed, thus determining constitutive internalization. In many cases, endocytic codes contain a critical tyrosine residue, thus being known as "tyrosine-based" signals. There is ample evidence that tyrosine-based signals bind directly to the clathrin adaptor protein complex AP2, thus allowing receptor recruitment into the pit. Once a clathrin-coated pit is formed, dynamin is responsible for its release into the cytosol as a vesicle



Endocytosis. Figure 1 Schematic of clathrin-mediated endocytosis. From left to right, the various temporal phases of endocytosis are depicted: recruitment of receptors into pits, clathrin assembly, vesiculation, uncoating, and trafficking to the endosomal compartment.

(vesiculation). Vesiculation is caused by conformational changes of dynamim, which require its intrinsic GTPase activity, and which lead to pinch off of the vesicle from the plasma membrane. Once a vesicle is formed, it appears as a coated vesicle, still being surrounded by a clathrin lattice. Subsequent shedding of clathrin and AP2 leads to the formation of an uncoated vesicle. Uncoated vesicles fuse with other vesicles in the endosomal compartment, where decisions are made whether to recycle the content of the vesicles (or part of it) to the plasma membrane or to further process it in the lysosome. Several other proteins (AP180/CALM, epsins, Eps15 and Eps15R, amphiphysins, synaptojanins etc.), globally referred to as accessory or regulatory proteins, participates to the various phases of the endocytic process, frequently entering in contact with a forming pit through AP2, in a precise hierarchical fashion. Finally posttranslational modifications, such as phosphorylation, dephosphorylation, and ►ubiquitination, are known to play a role in the various cycles of assembly and disassembly of structural and regulatory endocytic proteins.

Clinical Relevance

Pathogenesis of a number of membrane receptor-linked diseases can be directly traced to primary defects in endocytosis. In Familial Hypercholesterolemia, genetic defects in the LDL receptor result in the absence of cholesterol internalization into the cell. Both receptor absent and receptor-defective mutants occur. Among the latter, internalization-defective mutants can be caused by nonsense or frameshift mutations or by single amino acid substitutions. In insulin-resistant diabetes mellitus, mutations of the insulin receptor gene occur that fall into various categories, among which those involving accelerated receptor degradation and impaired transport of receptors to the cell surface. Another genetic disease that might be linked to the processes of endocytosis and of vesicle transport is Huntington disease. The disease is associated with increased length of a glutamine stretch, located in the NH2-terminal region of the protein huntingtin. Abnormal protein interactions have been proposed as a pathogenetic mechanism. ►Huntingtin localizes to vesicles of the secretory and endocytic pathway and interacts with proteins involved in vesicle trafficking. Recent data suggest that huntingtin is a regulator of these pathways, through association with clathrin-coated vesicles.

A remarkable overlap of molecular events between endocytosis and vesicle recycling in synapses is emerging with increasing relevance for those conditions, of neurological interest, in which alterations affecting components of the synaptic vesicles were shown. Among those are the Lambert-Eaton myasthenic syndrome, in which synaptotagmin might be a target of autoimmunity; the Stiff-Man syndrome, in which amphiphysin and the glutamic acid decarboxylase (GAD) (both of which are associated with the cytoplasmic surface of synaptic vesicles) are targets of autoimmunity; and possibly even Alzheimer's disease, in which the progressive cognitive loss is associated with synaptic loss in the cortex and decrease in the levels of synaptobrevin and synaptophysin.

Alterations of the processes of endocytosis and intracellular vesicular sorting might play a role in ►cancer. For instance, several receptor tyrosine kinases, including Met, EGFR, and ErbB-2, display alteration of their endocytosis in human cancers. In addition, translocations are described in leukemia, which involve accessory endocytic proteins. The *Eps15* and ►*EEN* genes (►*extra eleven-nineteen genes*) are translocated with the ►*MLL* gene (myeloid/lymphoid, or mixed lineage, leukemia), resulting in the production of fusion proteins. The *CALM* gene is rearranged with the *AF-10 gene* (ALL1 fused gene from chromosome 10), which in turn is a partner in *MLL*-involving translocations. Tumor suppressor and/or cancer predisposition genes are also linked to endocytic/sorting pathways, including *ATM*, the cancer predisposition gene mutated in ataxia-teleangiectasia, and the two genes *TSC1* and *TSC2*, encoding hamartin and tuberin respectively, identified in tuberous sclerosis.

Another emerging field in cancer research concerns plasma membrane receptors that signal to the cell in a negative fashion, by transducing antiproliferative signals. In such cases, endocytosis could represent a mechanism to attenuate these signals, leading to increased proliferation. This is exemplified by studies of E-cadherin, a transmembrane adhesion molecule that, by establishing homophilic interactions at sites of cell-to-cell contacts, downregulates the β-catenin pathway and participates in the tight control of epithelial tissue homeostasis. There is strong evidence that loss of the tumor-suppressor function of E-cadherin represents a common event in human cancers and causes cell invasiveness in vitro and tumor progression in vivo.

Finally, recent data demonstrated that endocytic proteins are involved in cell-fate determination. This is particular relevant considering the emerging concept of cancer ►stem cell compartments. Signaling by the Notch receptor affects cell-fate specification, proliferation, apoptosis, and migration, and it is frequently deregulated in human malignancies. Both the Notch receptor and its ligands undergo ubiquitin-regulated internalization and degradation, and functional subversion of the endocytic/trafficking events is predicted to have profound impact in the regulation of this receptor. Another endocytic protein, involved in tumors, is Numb, which is also linked to the Notch pathway. Numb is a negative regulator of Notch, and loss of Numb expression is a frequent event in human breast tumors. This, in turn, results, in increased

Notch signaling, an event that contributes to cellular transformation.

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Endodermal

Definition

Derived from endoderm, the embryonic germ cell layer which forms the linings of hollow parenchymal organs and the substance of some solid organs.

►Bone Tumors

Endogenous

Definition

Originating from within the cell or organism, opposed to exogenous.

Endogenous Retrovirus

Definition

A provirus, usually defective for replication, integrated into germline cells of an organism and transmitted vertically from parent to offspring.

►Retrovirus
►Transduction of Oncogenes

Endolysosomal Pathway

►Endosomal Compartments

Endometrial Cancer

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Definition

Endometrial carcinoma is the most common malignancy of the female genital tract, and it is estimated to account for approximately 40,880 new cases and more than 7,310 deaths in 2005 in the United States and a similar figure in Europe. It is more frequent than ovarian cancer, but can be treated successfully more often by surgery and radiation therapy.

Characteristics

Risk Factors

Several risk factors for the development of endometrial carcinoma are related to an extended exposure to unopposed estrogen action, such as in nulliparity, late menopause, obesity, diabetes mellitus, estrogen replacement therapy, and tamoxifen treatment (►Estrogenic hormones and cancer; ►hormones and cancer; ►obesity and cancer risk; ►progestin and cancer; ►tamoxifen). Tamoxifen has proven to be highly effective in the treatment of all stages of ►estrogen receptor (ER) positive breast cancer, however, it has a partial estrogen agonist effect in the human uterus. In the uterus of postmenopausal women, the estrogen-agonistic activity of tamoxifen results in an increased risk for the development of endometrial hyperplasia and endometrial cancer. The National Surgical Adjuvant Breast and Bowel Project-P1 study showed that tamoxifen increases the risk of endometrial cancer in postmenopausal women by four- to fivefold. It is important to stress, however, that the stage and grade of endometrial cancers observed in postmenopausal women were the same as in the general population. There is also evidence that the increase risk of endometrial cancer continues for years after tamoxifen therapy is discontinued.

Classification

Endometrial carcinomas are classified into two types on the basis of biological and histopathological variables:

1. Type I tumors, are usually well-differentiated and endometrioid in histology, and are associated with a history of unopposed estrogen exposure or other hyperestrogenic risk factors such as obesity.
2. Type II tumors, which often poorly differentiated, non-endometrioid and are not associated with hyperestrogenic factors. These tumors are more likely to be metastatic and can recur even after clinical intervention.

Pathogenesis Mechanisms

The pathogenic mechanisms of endometrial cancer are poorly understood. However, as in other malignancies, accumulation of genetic abnormalities and epigenetic alterations is thought to cause the transformation of normal endometrium to cancerous tissue. These changes disrupt cellular signaling networks that govern processes such as cell proliferation, apoptosis and angiogenesis. So far, no specific gene or genes have been linked to the majority of cases of endometrial cancer. However, molecular analyses have implicated several well-characterized oncogenes and tumor-suppressor genes in endometrial carcinogenesis. Current data indicate that type I tumors are more commonly associated with abnormalities in the DNA-mismatch repair genes KRAS, PTEN (phosphatase and tensin homologue) and β-catenin, whereas type II tumors seem to be linked to abnormalities in TP53 and ERBB2 (also known as HER2/neu). These can be mutations, deletions and amplification/overexpression of genes or/and epigenetic deregulation.

Approximately 10% of EC cases are associated with hereditary nonpolyposis colorectal cancer (HNPCC), a dominantly inherited syndrome with germ-line abnormalities in one of five DNA-mismatch repair genes with resultant micro-satellite instability. Females with HNPCC have a ten-fold increased lifetime risk of EC compared with that of the general population and the lifetime risk of EC (42%) is higher than that for colorectal carcinoma.

Treatment

Treatment for uterine cancer depends on the stage of the disease and the overall health of the patient. Removal of the tumor (surgical resection) is the primary treatment. Radiation therapy, hormone therapy, and/or chemotherapy may be used as adjuvant treatment (i.e., in addition to surgery) in patients with metastatic or recurrent disease.

Surgery

Treatment for uterine cancer usually involves removal of the uterus, including the cervix (called total hysterectomy), and removal of the fallopian tubes and ovaries (called bilateral salpingo-oophorectomy). Surgery may be performed through an incision in the abdomen or through the vagina (called transvaginal hysterectomy).

Surgical treatment is required to determine the degree of myometrial invasion. The following surgical staging has been adopted by the International Federation of Gynecology and Obstetrics (FIGO) and by the American Joint Committee on Cancer (AJCC):

1. Stage I endometrial cancer is carcinoma confined to the corpus uteri:
   (a) Stage IA: tumor limited to endometrium
   (b) Stage IB: invasion to less than one half of the myometrium
   (c) Stage IC: invasion to greater than one half of the myometrium
2. Stage II endometrial cancer involves the corpus and the cervix, but has not extended outside the uterus:
   (a) Stage IIA: endocervical glandular involvement only
   (b) Stage IIB: cervical stromal invasion
3. Stage III endometrial cancer extends outside of the uterus but is confined to the true pelvis:
   (a) Stage IIIA: tumor invades serosa and/or adnexa and/or positive peritoneal cytology
   (b) Stage IIIB: vaginal metastases
   (c) Stage IIIC: metastases to pelvic and/or para-aortic lymph nodes
4. Stage IV endometrial cancer involves the bladder or bowel mucosa or has metastasized to distant sites:
   (a) Stage IVA: tumor invasion of bladder and/or bowel mucosa
   (b) Stage IVB: distant metastases, including intra-abdominal and/or inguinal lymph nodes

Radiation Therapy

Radiation uses high-energy x-rays to destroy cancer cells and shrink tumors. This treatment may be used prior to surgery (called neoadjuvant therapy) or after surgery to destroy remaining cancer cells. Radiation also may be used in patients who are unable to undergo surgery.

Hormonal Therapy

Endometrioid endometrial cancer has long been associated with states of estrogen excess. As a result, a variety of antiestrogens have been used for systemic treatment.

Progestogens

Following the theory of estrogen excess as a carcinogenic promoter, progestogens have been used in the treatment of endometrial cancer for their antiproliferative effects on the endometrium.

Aromatase Inhibitors

Another approach to reducing the estrogen stimulation of the tumor is to use aromatase inhibitors. Aromatase inhibitors are known to reduce levels of circulating estrogen by reducing estrogen production.

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Endometrial Carcinoma

Definition

►Endomentrial Cancer

Endometrioma

Definition

A benign ovarian cyst containing ►endometrium-like cells. It is commonly referred to as a chocolate cyst. It has the potential to become malignant so is generally removed after diagnosis.

►Endometriosis

Endometrioma-benign Endometriotic Ovarian Cysts

►Endometriosis

Endometriosis

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E

Synonyms

Endometrioma-benign endometriotic ovarian cysts; DIE; deeply infiltrating endometriosis

Definition

Endometriosis is a disease characterized by the presence of ►endometrium-like epithelial and stromal tissue outside their normal location in the uterus, commonly on the ovaries and peritoneum. It is benign but has been linked to the development of ►ovarian cancer and may be a ►preneoplastic lesion.

Characteristics

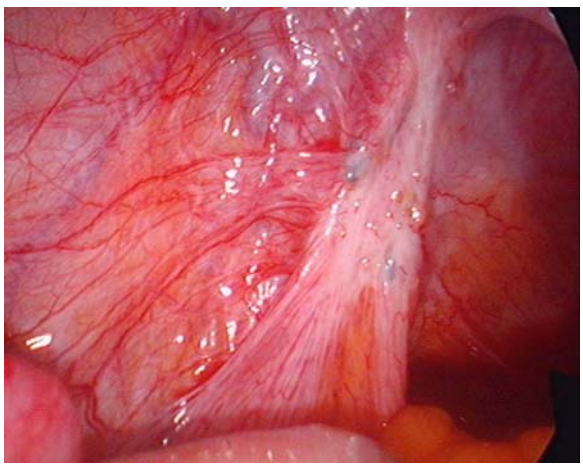
Endometriosis affects ~10% of women of reproductive age. Its symptoms include pelvic pain, ►dysmenorrhea and subfertility, although it may also be asymptomatic. The main pathological processes associated with endometriosis are peritoneal inflammation and fibrosis, and formation of adhesions and ►endometriomas. It is hormone-dependent, and is more common in women of low ►parity. Most endometriosis occurs sporadically, although it can be inherited as a complex genetic trait.

Surgery is required to make a formal diagnosis of endometriosis, e.g. at ►laparoscopy (Fig. 1), when laser or diathermy ablation of lesions can be carried out. Medical treatments act by inhibiting hormonal stimulation of the lesions, and include the oral contraceptive pill (OCP), ►progestogens and ►gonadotrophin-releasing hormone analogues.

The pathogenesis of endometriosis is unclear; one theory is retrograde menstruation, whereby endometrial tissue spills out of the Fallopian tubes during menstruation and implants on the ovaries and peritoneum. However, endometriotic lesions can be found in sites outside the peritoneum; hence, alternative theories include epithelial metaplasia of tissues into endometrium, and lymphovascular spread.

Interestingly, endometriosis shows many characteristics of tumorigenesis (Fig. 2). Endometriosis may invade tissue, traversing the basement membrane, and ►metastasise to other locations. Accordingly, ►matrix metalloproteinases are up-regulated and there is a deregulation of cell adherence signaling molecules such as β-catenin, ►E-cadherin and integrins.

Tumors may show self-sufficiency of growth and insensitivity to anti-proliferative signals. Similarly, endometriosis increases local ►estrogen production and its own responsiveness to estrogen, and the disease is associated with progesterone resistance related to an overall reduction in the levels of ►progesterone receptors (PRs) and the lack of a specific PR isoform – progesterone receptor B (PR-B). These aberrant mechanisms set up a positive feedback cycle of growth.



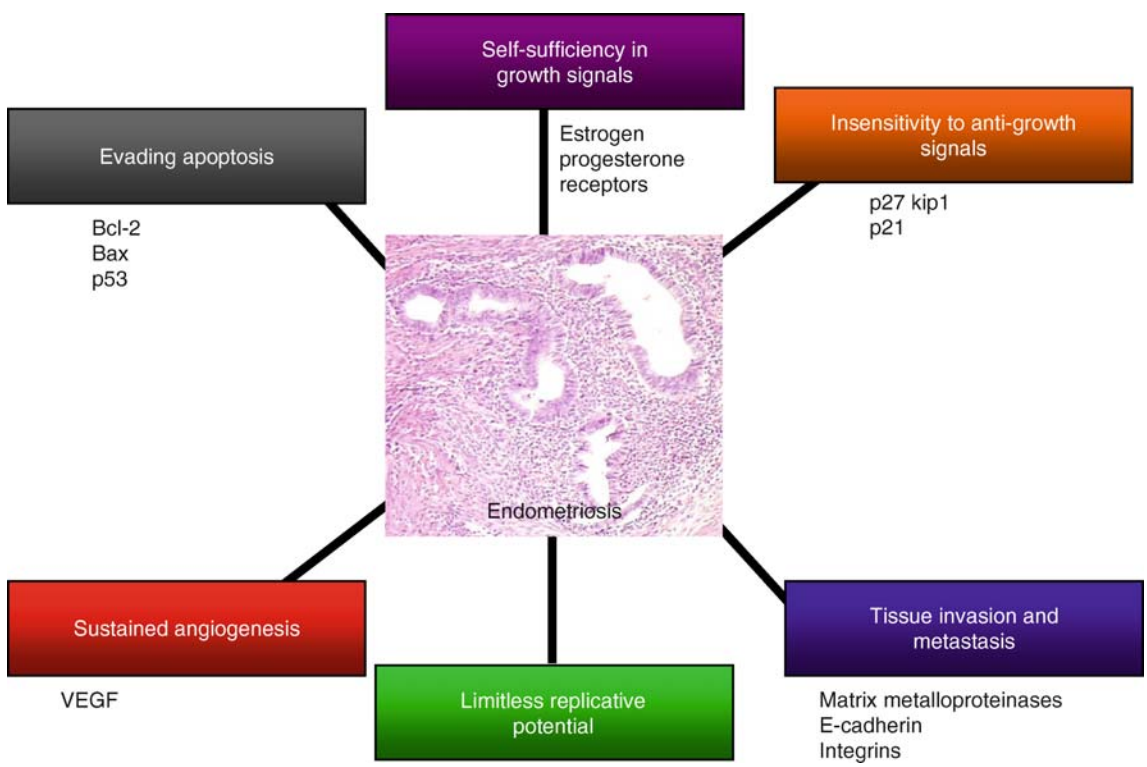
Endometriosis. Figure 1 Tumor phenotype.

Furthermore, endometriosis may differ from normal endometrium in its expression of ►transforming growth factor-β, ►insulin-like growth factors and ►cyclin dependent kinase inhibitors. It has increased ►Bcl-2 and decreased Bax expression, suggesting a degree of resistance to ►apoptosis.

►Angiogenesis is thought to be one of the mechanisms in tumor development, and indeed it seems to be important for the survival of endometriosis. In mouse models using transplanted endometrium, administration of ►endostatin and ►VEGF antibodies is effective at treating the "endometriotic" lesions.

Tumors are formed by the somatic acquisition of multiple genetic alterations producing a clone with a selective advantage. We would expect endometriosis to show some of these alterations if it is a neoplasm, albeit a benign one. Most importantly, it appears that endometriosis can be monoclonal in nature, a key feature of cancer.

It has been reported that endometriosis increases the risk of various cancers, namely ►breast cancer, ►ovarian cancer, ►non-Hodgkin lymphoma and ►melanoma, although the nature of this link has not been completely elucidated. In fact the risk of breast cancer may be decreased when endometriosis is diagnosed at an early age, possibly due to exposure to drugs with antiestrogenic effects. This suggests that a complex interplay of hormonal factors



Endometriosis. Figure 2 Tumor-biological qualities.

may influence the development of the two pathologies concomitantly.

Research has mainly focused on the relationship between endometriosis and ovarian malignancies. Endometriosis has been associated with the development of two histological subtypes of ovarian cancer: endometrioid and clear cell. The possibility that endometriosis may act as a precursor to these types of ovarian cancer, by malignant transformation of endometriosis, has attracted the most interest. Endometriosis may therefore be an early step in a neoplastic process which culminates in ovarian cancer.

Evidence supporting the link between endometriosis and ovarian cancer comprises:

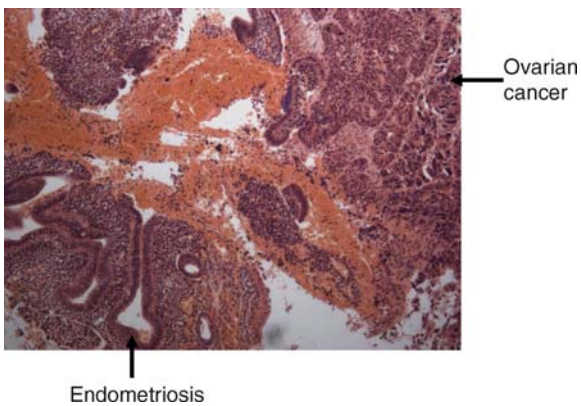
Pathological Studies

It has been observed for some time that ovarian tumors may be found on a background of endometriosis, leading to the description ►EAOC (Endometriosis-associated ovarian cancer) (Fig. 3). Sixty per cent of these cancers are found arising from within the endometriotic lesion itself; in the remaining 40% the endometriosis is at a distant site. This physical proximity suggests that there might be a causal relationship between endometriosis and ovarian cancer, rather than endometriosis existing merely as an epiphenomenon.

In 60–80% of these EAOCs the endometriosis found is histologically atypical, in that its cytological or architectural appearance is abnormal. This is in contrast to a 12–35% prevalence of atypia in endometriosis without co-existing malignancy.

The histology of EAOCs differs considerably from that of ovarian tumors in general, in that more than 90% are clear-cell or endometrioid carcinomas (which respectively make up 6 and 14% of all ovarian tumors). Conversely, endometriosis is found in a much higher proportion of operations for endometrioid and clear-cell tumors than for ovarian cancer in general. Location of endometrioid carcinoma and endometriomas follows the left-sided preponderance of endometriosis, whereas ovarian cancer in general does not.

Large pathology series have found ovarian cancer in 5–10% of endometriomas Other studies have found endometriosis in 21–54% of clear cell and endometrioid ovarian cancers. The presence of malignant tumors appearing on a background endometriosis is likely to be underestimated. Most women with endometriosis are asymptomatic and so unaware that they have the disease; by the time they develop ovarian cancer in the post-menopausal years, the endometriosis has atrophied and no longer visible. In younger women, the tumor may have obliterated the area of original endometriosis by the time it is removed.



Endometriosis. Figure 3 Histopathology.

Epidemiological Studies

The risk of developing ovarian cancer is increased by a factor of 1.7 in women with endometriosis, when confounding factors such as parity, infertility and use of the oral contraceptive pill (►OCP) are taken into account. Patients hospitalized for endometriosis are almost twice as likely to go on to develop ovarian cancer. A long standing history of ovarian endometriosis increases the risk of ovarian cancer by a factor of more than four, supporting the concept of a continuum from benign endometriosis to carcinoma.

Women with endometriosis who develop EAOCs are younger at diagnosis; they present with less advanced disease and they have longer disease-free survival than women with epithelial ovarian cancers. Conceivably, this occurs because women with endometriosis have frequent hospital appointments, ultrasound scans and possibly even increased awareness of ovarian cancer.

It is difficult to be able to draw firm conclusions from these epidemiological data. Endometriosis and ovarian cancer are hormonally-influenced diseases in which it is hard to separate causation from epiphenomena. For example, treatments for endometriosis such as ►danazol may increase the risk of ovarian cancer, and in the most part have not been confounded for. Alternatively, women who have a tendency to retrograde menstruation may be at risk of both endometriosis and ovarian cancer, due to increased exposure to environmental carcinogens transported into the peritoneal cavity. Thus, endometriosis and ovarian cancer, rather than being at different ends of a neoplastic spectrum, could be different endpoints brought about by similar risk factors or a common genetic predisposition, which therefore often co-exist.

A further explanation for the link is that endometriosis is itself a risk factor for ovarian cancer because of its hormonal and inflammatory effects, acting locally and systemically. Inflammation can promote cancer development by increased cell division in tissue repair,

which increases the potential for mutations to occur. Indeed, protective factors for ovarian cancer such as OCP use and parity are thought to act by suppressing ►ovulation, which involves inflammation and repair, and in ovarian cancer cell lines, anti-inflammatory drug administration is protective.

Molecular Genetic Studies

In contrast to the pathological and epidemiological studies discussed above, which cannot exclude an indirect link involving common environmental, immunological, hormonal or genetic factors, molecular genetic studies can go some way towards proving causation.

Research has focused on endometriosis as a precursor of ovarian cancer, in which endometriosis could be an early stage of a ►multi-step development process. By cumulative acquisition of aberrant genetic changes, endometriosis might progress through atypical stages to endometrioid and clear cell ovarian cancer (Fig. 4). This is analogous to the adenoma-carcinoma sequence in ►colon cancer, or the neoplastic progression of Barrett's esophagus to esophageal adenocarcinoma. Indeed, in these examples molecular genetic studies have been used to demonstrate clonal progression from precursor lesion to cancer.

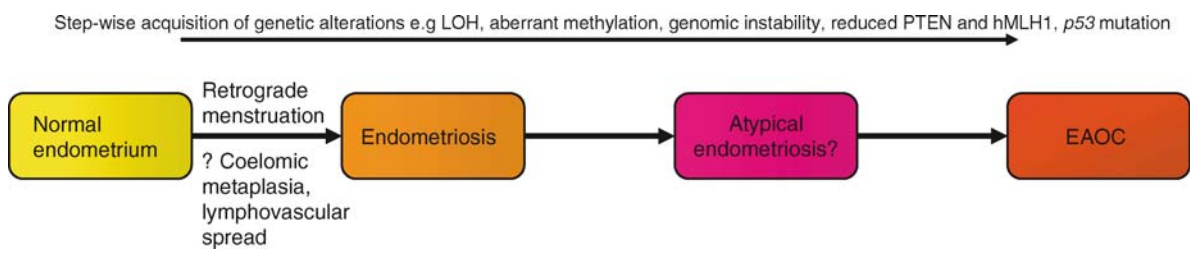
To prove that endometriosis is a precursor of EAOCs, one would expect them to have common genetic (and epigenetic) alterations. EAOCs are very often found in association with endometriosis which is metaplastic or atypical. Studies have compared the molecular genetics of endometriosis and EAOCs in an attempt to prove that malignant transformation of endometriosis occurs.

Advancing tumor stages are associated with accumulation of genetic alterations leading to gain of ►oncogene, and loss of ►tumor suppressor gene (TSG), function. ►Loss of heterozygosity (LOH) (which can indicate loss of TSG function) has been compared in various genomic regions in endometriosis co- existing with EAOCs, and these studies have consistently found common LOH events in the EAOCs and benign endometriotic tissue. For example, in a study using ►microsatellite markers across the genome, a third of LOH events detected in EAOCs were also detected in endometriosis samples from the same patient; the same allele was lost in each case indicating a common pathway, as it is very unlikely that the same allele was affected due to independent events. No LOH events were found only in the endometriotic tissue. These data strongly suggest that ovarian endometriosis can be a clonal precursor to EAOCs.

As would be expected from the "multi-hit" model of tumorigenesis, LOH was detected more frequently in EAOCs than endometriosis. Fluorescence in situ hybridization (►FISH) analysis has also been used to compare chromosomal aberrations in ovarian endometriosis compared to EAOCs. Abnormalities, specifically trisomies 1 and 7 and monosomies 9 and 17, were detected in a larger percentage of cells in EAOCs than in ovarian endometriosis, which in turn had a larger percentage of these chromosomal aberrations than normal endometrium. This suggests that there may be an expansion of the aberrant cell clones in carcinogenesis, which supports the theory of malignant transformation of endometriosis. Interestingly, many fewer chromosomal aberrations were found in extragonadal endometriosis compared to ovarian endometriosis; this suggests that it might be ovarian endometriosis alone which can progress to ovarian cancer, perhaps due to the ovarian environment inducing genetic changes, and/or a different aetiology for the two types of endometriosis.

The analysis of specific oncogenes and tumor suppressor genes has also provided weight to the malignant transformation theory. ►TP53 mutations are found with increasing frequency through the spectrum of normal endometrium, endometriosis, endometriosis co-existent with cancer, and ovarian cancer. In terms of ►epigenetic alterations, hyper-►methylation of the promoter region of hMLH1, which has a role in DNA ►mismatch repair, has been found in endometriosis. Notably, there is decreased hMLH1 expression in a greater proportion of cases of aggressive endometriosis associated with cancer than in lower stage endometriosis.

Further supporting a "multi-hit" theory, a mouse model of ovarian endometriosis has shown that both K-►RAS and ►PTEN mutations are necessary for the development of endometrioid ovarian cancer. Alone, each mutation in the ►ovarian surface epithelium cells



Endometriosis. Figure 4 Tumor evolution.

caused the development of endometriosis-like lesions, but it was only when the mutations were in combination that carcinoma resulted. Clearly the development of ovarian cancer as a result of two mutations is oversimplified, but the study adds strong weight to the malignant transformation theory.

Interestingly, there is some evidence for a role of PTEN in the progression of ovarian endometriosis to EAOCs in humans; LOH of the same allele has been detected in co-existing endometriomas and EAOCs, and LOH, reduced expression and mutation of PTEN have been detected in solitary endometriomas. In addition, PTEN lies near a chromosomal region of significant linkage for familial endometriosis, identified using 1,176 affected sister pairs (although unfortunately there are no data regarding how many have had ovarian cancer). However, in a recently published large case-control study, there was no evidence that genetic variants in the PTEN gene are contributing to genetic susceptibility for endometriosis.

Pathology, epidemiology and molecular studies have added weight to the theory of malignant transformation of endometriosis to EAOCs. Endometriosis can be clonal; in addition, the disease is associated with genetic and epigenetic alterations in genes previously implicated in tumorigenesis. Therefore, endometriosis may be a useful model for understanding early events in tumorigenesis, particularly ovarian cancer. However, it is still not known what proportion of endometrioid and clear cell ovarian cancers are EAOCs, and what proportion have arisen *de novo* or from other precursor lesions. There is extensive work to be done to elucidate the molecular alterations involved in endometriosis, EAOCs and endometrioid and clear cell ovarian cancers in general. Only then can we ascertain the relevance of endometriosis in ovarian cancers, and the potential for screening and targeted therapies geared towards early detection and treatment of ovarian cancer.

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Endometrium

Definition

The tissue which lines the uterus. It changes in thickness, vascularity and structure during the menstrual cycle to prepare for potential implantation of an embryo, and is shed during menstruation.

►Endometriosis
►Estrogenic Hormones

Endoplasmic Reticulum (ER)

Definition

An ►organelle in ►eukaryotic cells that is an interconnected network of ►tubules, ►vesicles and ►cisternae and that is responsible for several specialized cellular functions including ►protein translation, folding, and transport of proteins to be used in the ►cell membrane or to be secreted from the cell. The ER is an intracellular tubular or cistern-like membrane system partly arranged in tight contact with the nuclear membrane and with tubular structures reaching the cell's periphery. Depending on the presence of ribosomes/polysomes on the ER surface, it is classified as either rough or smooth ER; the former is responsible for synthesis of about a third of the cellular proteins while the latter is the metabolic compartment of lipids and ►xenobiotics.

Endoplasmic Reticulum Stress

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Synonyms

Endoplasmic reticulum stress response; ER stress

Definition

The endoplasmic reticulum (ER) is an organelle with several essential functions in eukaryotic cells. The ER is both a major intracellular calcium store and the place where proteins entering the secretory pathway are synthesized, folded, modified and delivered to their final cell surface or extracellular destination. Moreover, in

mammalian cells the ER is the site of sterols and lipids synthesis. Disturbance in any of these functions, which results in the disruption of the proper folding and secretory capacity of the ER and increased load of unfolded proteins in its lumen, defines a condition known as "ER stress." ER stress activates a complex and multifaceted intracellular signal transduction pathway that is essentially designed to re-establish ER homeostasis. Inability to restore ER functions induces cell death, which is usually in the form of ►apoptosis. ER stress contributes to the etiology of several human pathologies, including diabetes, neurodegeneration and cancer.

Characteristics

Proteins that are expressed at the cell surface or secreted extracellularly, as well as resident proteins of the organelles along the secretory pathway, are first co-translationally translocated into the lumen of the ER as unfolded polypeptide chains. The unique highly oxidizing ER environment, which is equivalent to the extracellular space, is required to sustain a variety of post-translational and co-translational modifications to which proteins are subjected after entering the ER. These modifications include the formation of intra- and intermolecular disulphide bonds and N-linked glycosylation. In the Ca^{2+}-rich ER lumen several resident chaperones and Ca^{2+} binding proteins, including the glucose-regulated proteins (GRPs) and the ►lectins, ►calreticulin and ►calnexin critically assist and monitor proper folding, maturation and stabilization of the nascent protein. These ATP-requiring processes are part of the stringent ►ER quality-control mechanisms that allow only proteins adopting a correctly folded or ►native conformation (Protein), or a proper oligomeric assembly in case of multi-subunit proteins, to be exported to the Golgi complex and move on through the secretory pathway. Terminal failure in protein folding or in oligomer assembly results in ►ER-associated protein degradation (ERAD), in which non-native conformers are retrotranslocated to the cytosol and degraded by the ►26S proteasome. ER stress is set off by various intracellular and extracellular perturbations, which alter the protein folding capacity of the ER. These include glucose deprivation, which interferes with N-linked glycosylation and affects ATP levels, redox unbalance and depletion of the ER Ca^{2+} store. Perturbation in ER homeostasis activates an evolutionarily conserved stress response, collectively called the Unfolded Protein Response or UPR. The UPR is primarily a pro-survival response initiated to restore normal ER homeostasis.

Molecular Mechanisms

The UPR consists of three major mechanisms: (i) translational attenuation to limit the biosynthetic load of the ER; (ii) transcriptional activation of cytoprotective genes encoding ER resident chaperones to increase the ER folding capacity; and (iii) increase clearance of unfolded proteins through the upregulation of ERAD. When these mechanisms fail to restore normal ER homeostasis, ER stress promotes the cell death program, which is likely activated to protect the host from the accumulation of dysfunctional cells.

At the molecular level the UPR is characterized by the activation of three ER transmembrane receptors: the pancreatic ER kinase (PKR)-like ER kinase (PERK), the inositol-requiring enzyme 1 (IRE1), and the activating transcription factor 6 (ATF6). These transmembrane proteins contain a lumenal domain, which functions as a sensor of the ER folding capacity, and a cytosolic effector domain that provides a signaling bridge connecting the ER to other cellular compartments. In unstressed cells the luminal domain of the transmembrane receptors is bound to a 78-kDa ER resident chaperone referred to as the glucose-regulated protein (GRP)78 or immunoglobulin binding protein, BiP. BiP belongs to the heat-shock protein (►HSP) 70 class of ►molecular chaperones and can form complexes with heterologous proteins that are processed through the ER. Similar to other HSP70 members, BiP binds both ADP and ATP, which serve to regulate its binding and release from nascent polypeptide chains. BiP cycles between an oligomeric and monomeric state, the latter of which is thought to bind preferentially to unfolded proteins. Association of the ER transmembrane receptors with BiP lock them into an inactive, monomeric conformation that prevents their oligomerization or trafficking to other compartments. On accumulation of unfolded proteins, which promotes a dramatic increase in the luminal pool of monomeric BiP, BiP is competitively titrated away from the luminal domains of the three receptors. BiP dissociation triggers the activation of these proximal ER stress sensors and initiates the first molecular event of the complex transcriptional and translational program defining the UPR.

Signal Transduction by the Three Arms of the UPR

Adaptation to ER stress involves the sequential activation of three major arms of the UPR: the PERK-eIF2α-ATF4, ATF-6 and IRE1 pathways.

- PERK is a ►type I transmembrane Ser/Thr protein kinase. Dissociation of BiP from PERK drives PERK homo-oligomerization and self-activation by *trans*-autophosphorylation. PERK mediates the specific phosphorylation of the ►eukaryotic initiation factor-2α (eIF2α) at Ser51. Phosphorylation of eIF2α interrupts the translation of most mRNAs, thereby reducing the load of newly synthesized proteins in the ER. However, while global translation is transiently repressed, the PERK-eIF2α pathway results in the preferential translation of a subset of mRNAs containing regulatory sequences in their 5′

untranslated regions, that allow them to bypass the eIF2α translational block. One of these mRNAs encodes ATF4, a member of the bZIP family of transcription factors. ATF4 promotes cell survival through the induction of several genes involved in restoring ER homeostasis. Under conditions of sustained ER stress, ATF4 prompts the expression of the transcription factor C/EBP homologous protein (CHOP) and of one of its target genes, the growth arrest and DNA damage-inducible gene 34 (GADD34). GADD34 is a protein phosphatase 1 (PP1) regulatory subunit that promotes eIF2α dephosphorylation, which ends the translational block and restores protein synthesis in the ER.

- ATF6 is a ►type II transmembrane protein that contains a transcription factor in its cytoplasmic domain. Under ER stress conditions the dissociation of BiP frees ATF6 to translocate to the Golgi apparatus, where it is cleaved by Golgi-resident proteases. The limited proteolysis of ATF6 releases the transcription factor domain into the cytosol and allows its migration into the nucleus, where it binds DNA and activates gene expression. AT6-responsive genes are activated rapidly after the induction of ER stress, and include the ER molecular chaperones BiP and GRP94, as well as ►protein disulphide isomerase (PDI). ATF-6 also leads to the transcriptional upregulation of the X-box binding protein (XBP1) mRNA, which is converted into a stable transcription factor by the endonuclease activity of IRE1.
- IRE1 contains a Ser/Thr kinase domain and an endonuclease domain facing the cytosol. Like PERK, IRE1 is a type I transmembrane receptor that is activated by oligomerization-induced *trans*-autophoshorylation in the ER membrane upon BiP dissociation. IRE1 autophosporylation stimulates its endonuclease activity and does not result in the propagation of a phosphorylation cascade. Active IRE1 catalyses the removal of a 26-nucleotide intron from the XBP1 mRNA, generating the $XBP1^s$ frameshift splice variant which encodes a stable and active transcription factor. $XBP1^s$ translocates to the nucleus and transactivates several cytoprotective genes involved in ER quality-control. $XBP1^s$ can also induce a negative feedback loop which, by relieving the PERK-mediated translational block, returns the ER function to normal once the UPR has been successful. The IRE1 pathway is thought to be the final branch of the UPR to be activated, subsequent to the rapid induction of PERK and ATF6 signaling which have a major cytoprotective role. The IRE1 signal plays a dual role in the UPR and if the ER stress persists, $XBP1^s$ initiates pro-apoptotic protein synthesis, thereby facilitating the induction of apoptosis.

Cell death after ER stress. The adaptive responses initiated by the UPR restrain cell death to the extent that ER homeostasis and protein folding capacity can be re-established. If, in spite of this, protein aggregation persists, the UPR shifts into a proapoptotic response, whose molecular effectors are still poorly defined.

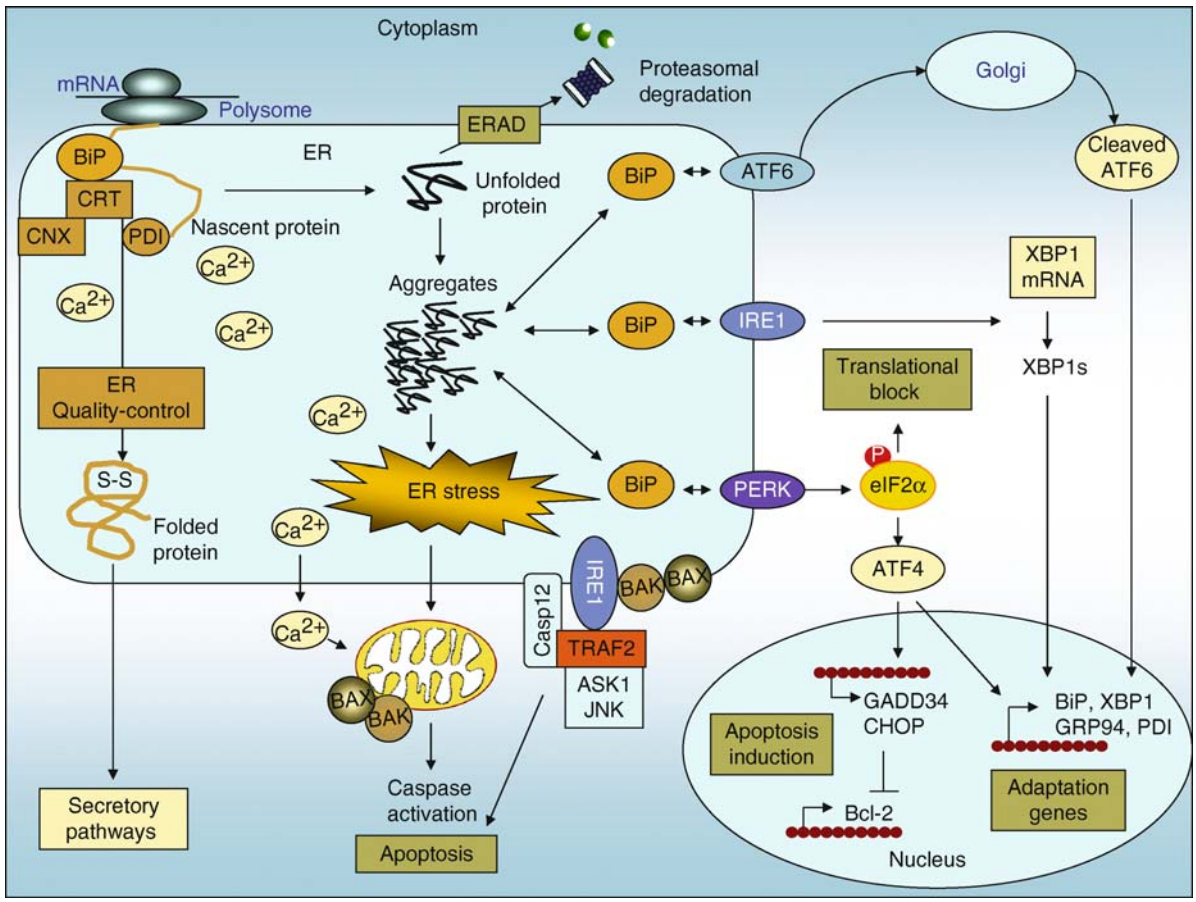
- *CHOP and IRE1.* Sustained CHOP induction, resulting from the late phase of ATF-6 activation and the PERK-eIF2α-ATF4 axis, tips the balance towards apoptosis under conditions of persistent ER stress. Several CHOP target genes contribute to the proapoptotic effect of CHOP induction. One mechanism entails CHOP-mediated suppression of *bcl-2* transcription, which increases the sensitivity of the cell to apoptotic cell death. Also, restoration of protein synthesis by the CHOP-mediated induction of GADD34 can promote the expression of proapoptotic effectors when the ER folding capacity cannot be restored. The IRE1 pathway leads to activation of the ►MAPK family member c-Jun N-terminal kinase (JNK) through the adaptor molecule TNF receptor-associated factor 2 (TRAF2) and apoptosis signal-regulating kinase-1 (ASK1). JNK can favor apoptosis induction following ER stress by increasing the proapoptotic activity of some ►BCL2 proteins, such as BIM.
- *Ca^{2+} signaling and BCL2 proteins.* The physiological ER Ca^{2+} content is maintained by the balance between the active Ca^{2+} uptake, controlled by ►SERCA (sarco-endoplasmic reticulum Ca^{2+}-ATPase) pumps, and its release, through opening of IP_3R (inositol 1,4,5-triphosphate receptor) or RyR (ryanodine receptor) ►Ca^{2+}-release channels. Various stimuli that cause depletion of ER Ca^{2+} store and result in cytosolic Ca^{2+} overload also stimulate the UPR, since the vast majority of ER resident chaperones involved in ER quality-control are Ca^{2+} binding proteins, and can induce cell death. Excessive Ca^{2+} uptake by mitochondria induces changes in the permeability of the inner mitochondrial membrane, which can result in the breakdown of the mitochondria membranes triggering apoptosis. Increased Ca^{2+} leak from the ER activates Ca^{2+}-dependent enzymes in the cytosol, including the Ca^{2+}/calmodulin-dependent phosphatase calcineurin and ►calpains, which can regulate apoptosis induction by modulating the activity of BCL-2 proteins. In addition to having a crucial role as regulators of mitochondria function during apoptosis, proapoptotic BAX, BAK and antiapoptotic BCL2 proteins regulate ER Ca^{2+} homeostasis and the UPR machinery. For instance, interaction of BAX and BAK with IRE1 during ER stress is required for IRE1-mediated JNK activation and apoptosis induction.
- ►*Caspases.* The execution phase of apoptosis following ER stress involves the activation of caspases. Both murine caspase 12 and human caspase 4 associate with the ER and are proteolytically processed after ER stress. The general relevance of this

E

event for the initiation of apoptosis following ER stress is however still dubious as catalytically active caspase 12, which is found only in rodents, and caspase 4 belong to the subclass of inflammatory caspases with primary role in inflammation and innate immunity rather than in ER stress (Fig. 1).

Clinical Relevance

Activation of the UPR is observed in several ►conformational diseases, including Alzheimer's and Creutzfeldt–Jakob diseases, and involves the accumulation of protein aggregates in the ER. In general, it is becoming increasingly clear that a range of pathological conditions are associated with ER stress, including diabetes, ischemia, lysosomal storage disease, cardiovascular diseases, atherosclerosis, neurodegeneration, viral infection as well as cancer. With respect to cancer, tumors often exhibit activated molecular effectors of the UPR. Also, conditions found in the tumor microenvironment – which, due to insufficient vascularization, often include glucose starvation, hypoxia and acidosis – can promote ER stress in tumors. Although the UPR is generally considered a protective response, prolonged ER stress can activate apoptosis and therefore its role in the disease process can be dualistic. The activated UPR could either



Endoplasmic Reticulum Stress. Figure 1 ER stress signaling pathways. After import in the ER lumen a nascent unfolded protein undergoes different post-translational modifications monitored by molecular chaperones and folding enzymes (CRT; calreticulin, CNX; calnexin, BiP, PDI), prior to its export from the ER through the secretory pathways. Incorrectly folded proteins are retrotranslocated to the cytosol and degraded by the proteasome through the ERAD. Perturbations in the ER environment resulting in the accumulation of unfolded proteins in the ER lumen, promotes the dissociation of BiP from three proximal ER-stress sensors. This triggers PERK and IRE1 oligomerization/activation and ATF6 release from the ER membranes. The PERK-eiF2α-ATF4 signal is responsible for the initial translational block, whereas the ATF-6 and the IRE1 axis mediate the rapid transcriptional activation of cytoprotective genes, including several ER resident chaperones. If ER stress persists, apoptosis is activated through several mechanisms involving Ca^{2+} signaling and the transcriptional upregulation of CHOP, which represses *bcl-2* expression. BAX and BAK proteins regulate the IRE1-TRAF2-ASK1 pathway which leads to JNK activation and processing of murine caspase-12.

limit tumor growth (by the induction of apoptosis in conditions of chronic stress), or instead facilitate it (by increasing the survival of cancer cells). Also, further research will be necessary to determine the extent to which UPR induction in tumors plays a role in chemotherapy resistance, which is currently a major obstacle for the treatment of human cancer.

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Endoplasmic Reticulum Stress Response

Definition

Synonymous: unfolded protein response (UPR); A series of ►endoplasmic reticulum (ER) alterations such as calcium depletion, protein misfolding and impairment of protein trafficking to the Golgi triggers this response, which involves attenuation of protein synthesis and selective transcription and translation of a series of genes, mainly involved in favoring correct protein folding. When these ER alterations cannot be repaired by the ER stress response, the damaged cells undergo ►apoptosis. Several stimuli, including ischemia, viral infection and drugs such as ►tunicamycin or ►cisplatin induce apoptosis through this pathway.

►Endoplasmic Reticulum Stress

Endoproteolysis

Definition

Is the directed digestion of the internal regions of ►proteins as opposed to exoproteolysis that is the cleavage of the terminal regions of proteins.

Endoscopic-Ultrasound Guided Fine Needle Aspiration (EUS-FNAB)

Definition

The use of endoscopic-ultrasound (EUS) for real-time guidance of an FNA biopsy needle into a lesion within or adjacent to the gastrointestinal tract (GI tract). EUS-FNAB is valuable for diagnosing and staging GI and mediastinal malignancies.

►Fine Needle Aspiration

Endosomal Compartments

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Synonyms

Receptor-mediated endocytosis; Endosomal protein sorting; Endolysosomal pathway

Definition

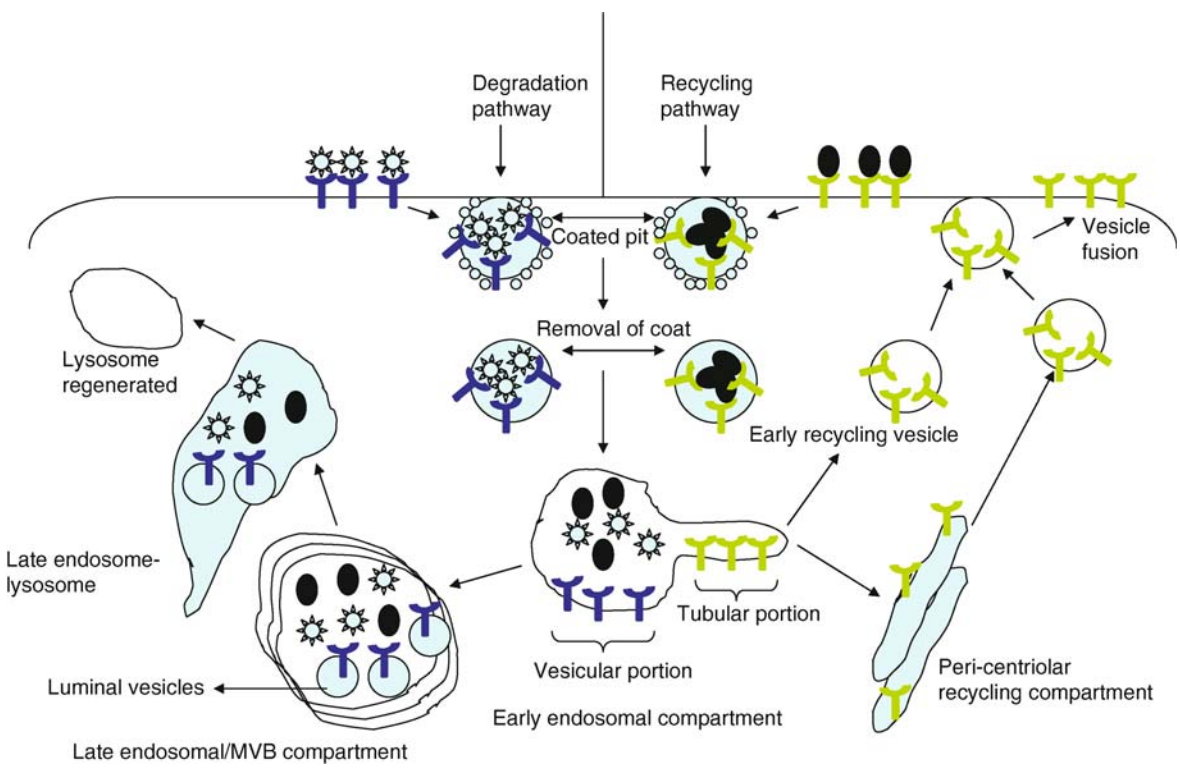
In eukaryotic cells, receptor-mediated ►endocytosis releases cargo-loaded vesicles at the plasma membrane. The vesicle content pass through a series of discontinuous closed membrane systems called ►endosomal compartments. In strict sense, ►endosomal compartments are temporal sorting stations where the fate of endocytosed cargo is determined.

Characteristics

Eukaryotic cells internalize a variety of extracellular material (cargo) either nonspecifically by phagocytosis and pinocytosis, or selectively through receptor-mediated endocytosis. In phagocytosis, specialized cells (e.g., ►macrophages and neutrophils) engulf large particles such as cell debris, bacteria, and viruses. Pinocytosis, exhibited by all cell types, involve uptake of extracellular fluid continually as tiny droplets (micropinocytosis) or sometimes as large droplets (macropinocytosis). By contrast, receptor-mediated endocytosis involves selective uptake of cargo by cognate cell surface receptors. Pinocytosis and receptor-mediated endocytosis are closely related, while phagocytosis is mechanistically distinct and therefore is not considered as endocytosis. In phagocytosis, plasma membrane

evaginates to wrap around the particle. Additionally, exocytosis adds membrane material to phagocytic vacuoles and compensates for loss of plasma membrane. In phagocytosis and pinocytosis, hydrolytic enzymes packed in Golgi-derived vesicles are directly delivered to the vesicle/vacuole and subsequent fusion with lysosomes result in degradation of contents. On the other hand, in receptor-mediated endocytosis, not all cargo is degraded. To illustrate with familiar examples, transferrin (Tf, carry iron) and low-density lipoprotein (LDL, carry cholesterol) are endocytosed along with their respective receptors, TfR and LDL-R. After internalization, iron is released inside the cell and both Tf and TfR are recycled to the plasma membrane for additional iron uptake. However, LDL is degraded to release cholesterol but LDL-R is recycled to the plasma membrane for further LDL uptake. Ubiquitinated growth factor receptors, on the other hand, are degraded to abolish receptor signaling. To achieve this, the incoming cargo is delivered to endosomal compartments where they are sorted either away from (to be recycled) or towards lysosomes (to be degraded). Besides their roles in differentiated cells, endosomal compartments are important during development, for example, in interpretation of morphogen gradients. In light of the details provided later, the illustration in Fig. 1 is self-explanatory and offers a basic view of the endosomal compartments.

The endosomal compartments are (i) early endosome (EE), (ii) recycling endosome (RE), (iii) late endosome (LE)/multivesicular bodies (MVBs), and (iv) lysosomes. Endosomal compartments are distinguished by (i) morphology in electron and fluorescence microscopy, (ii) differential density in Ficoll gradients, (iii) pH differences, and (iv) molecular signature provided by marker proteins. Morphologically, EEs are recognizable as tubulovesicular. LEs are tubular, multilamellar, and multivesicular (with internal vesicles). MVB contain internal vesicles (up to 200) because of protein sorting into the lumen by invagination of outer endosome membrane. Electron tomography studies suggest that the internal vesicles are free-floating and discontinuous with outer endosomal membrane. Distinct endosomal subpopulations can be enriched by density centrifugation and has allowed study of membrane fusion events between endosomal compartments. Also, molecular probes sensitive to pH changes can identify individual endosomal compartment as the luminal pH decreases along the degradative but increase along the recycling pathway (cytosolic pH is ~7.2). EEs are mildly acidic (pH ~ 6.0), recycling compartment is towards neutral (pH 6.4–6.5), LE/MVB is at pH ~ 5.0–5.5



Endosomal Compartments. Figure 1

and lysosomes at pH < 5.0. Finally, proteins recruited to specific endosomal compartments, such as Rab GTPases, serve as markers for identification of the compartment in fixed or live cell imaging experiments. The maturation theory suggests that EE progress towards lysosome by sequential addition of some material and removal of other material. The vesicular transfer theory proposes permanent existence of discrete compartments through which passage of cargo occurs by vesicle-mediated transfer.

Our current knowledge of endosomal compartments is mostly from studies of clathrin-dependent endocytosis (100–200 nm). Other mechanisms, classified as clathrin independent, exist and involve smaller vesicles (40–80 nm). These are mediated by ►caveolins (caveolae) and lipid rafts. Upon cargo binding, receptor adaptor proteins mediate polymerization of clathrin or caveolin on the cytosolic side causing plasma membrane invaginations (pits). The receptors and their cargo concentrate in these pits. Receptors and the initially assembled proteins provide binding sites for several proteins, such as Eps15, epsin, amphiphysin, intersectin, endophilin, SNX9, dynamin, and Rab5. Mostly, dynamin activity constricts and snip such invaginations to release receptor/cargo-loaded primary endocytic vesicles. The released vesicles are coated with clathrin or caveolin. Studies of cholera toxin-B (CTB) and Simian Virus-40 (►SV40) endocytosis revealed clathrin- and caveolin-independent pathways. Interestingly, dynamin and Arf6 are not required in this pathway.

After vesicle release, chaperone-mediated removal of the bulky clathrin or caveolin coat enables vesicles to deliver cargo to EEs. The EE compartment (also called sorting endosome) is the first compartment to receive all incoming cargo and is characterized by marker proteins such as Rab5 and EEA1 and the absence of coat. GTP-bound Rab5 interacts with at least 20 different proteins and along with EEA1, Rabex-5, and SNAREs mediates homotypic fusion and fusion of primary vesicles with early endosomal compartment. Until recently, it was thought that all endocytic cargos fuse with a homogenous early endosomal compartment, where individual cargo is separated via recognition of specific determinants (protein domains, a ubiquitin moiety, or other modifications) and shunted accordingly. Recently, however, EEs have been subdivided into dynamic and static EEs (DEE and SEE). This so-called preendosomal sorting mechanism suggests that the type of protein coat and adaptor proteins assembled around the primary vesicle (at the plasma membrane) predetermines whether the cargo is delivered to DEE or SEE. The cargo to be degraded is delivered to DEE and the cargo to be recycled effectively concentrates in the more abundant SEE.

Within the early endosomal compartment, most receptors disengage from their ligands and the receptors to be recycled move into tubular portions, while proteins to be degraded are retained in the vesicular portion. Although specific sorting mechanisms remain unknown for many receptors, ►ubiquitinated growth factor receptors are retained in the vesicular portion by sequential assembly of ubiquitin-binding Hrs, Escrt-1, -II, and -III complexes and later sorted into intraluminal vesicles (ILV) for degradation by lysosomes. Additional proteins such as STAM, AMSH, and UBPY may positively and negatively regulate the ILV sorting steps. From the tubular portions, Rab4 and Rab11 generate recycling vesicles that fuse with plasma membrane, returning receptors (mostly nonubiquitinated) directly back to cell surface. Such recycling vesicles are called early (fast) REs. A tubular compartment (Rab11-positive but Rab4-negative) exists, which is known as pericentriolar recycling endosomal compartment. It performs similar functions, i.e., returning endocytosed proteins to cell surface. However, its significance is apparent where recycling is important for membrane domain maintenance and membrane protein mobilization. For instance, in migrating fibroblasts, recycling TfRs are routed through the pericentriolar area and reach the plasma membrane of the leading lamella. In neurons, with distinct axonal and somatodendritic plasma membrane domains, polarized sorting of TfR is mediated in pericentriolar recycling compartment. In polarized epithelia, apical–basal polarity is reinforced by endocytosis-mediated recovery of missorted proteins or transcytosis and localized to correct membrane domain by traversing through the pericentriolar recycling compartment.

Beginning from EE, a process of endosome maturation ensues. This involves increasing acidification of the compartments and changes that permit fusion with next endosomal compartment, thereby progressively moving nonrecycled cargo towards lysosomes. LEs acquire Rab7 and lose Rab5, a process called Rab5–Rab7 conversion. Rab7 defines the functional identity of LE and makes the compartment competent for fusion with lysosome (degradation competent). Live-cell imaging shows that Rab5 is either replaced by Rab7 in early endosomal microdomains or Rab7 initially assembles on Rab5-positive EE and bud-off, taking cargo with it to become late endosomal compartment. Thus, the early compartment gradually convert/mature to LEs. An important feature is that LE membranes are highly tubular or multilamellar and contain an unusual lipid, called lysobisphosphatidic acid (LBPA). LEs become MPR-positive, allowing vesicles from the *trans*-Golgi network to deliver degradative enzymes to the compartment. Resident lysosomal proteins are also delivered by this mechanism through LEs. Some protein and lipid cargo are degraded in LEs as the pH-sensitive degradative enzymes are not fully activated. MPR is eventually carried away by vesicles back to *trans*-Golgi (to be used again later) following which fusion with

lysosome occur. LEs are identified by presence of both Lamp-1 and MPR.

Sorting of ubiquitinated cargo into ILVs results in the characteristic appearance of MVBs. A clear distinction between MVB and LE is difficult and thus mentioned as LE/MVB. In specialized cell types or under specific conditions a few typical characteristics of MVB assume prominence. In MVB that have a nondegradative function, the LBPA-containing ILVs undergo retrofusion with outer endosomal membrane, releasing their contents into cytosol (such as vascular somatitis virus and anthrax lethal factor). Also, MVBs serve as precursors for antigen-processing compartments (nonendosomal compartment), exosome release, T-cell secretory granules and melanosomes. Interestingly, ubiquitinated proteins are sorted to MVBs that are distinct from MPR- or LBPA-positive MVB. Since MPR is recycled and LBPA-containing internal vesicles can undergo retrofusion, this mechanism may represent separation of distinct classes of receptors and explain why not all proteins and lipids are degraded in LE/MVB. Finally, the cargo in LE/MVB is delivered to lysosomes. Lysosomes are the endpoint of endocytosis and contain all enzymes necessary for degradation of proteins and lipids. Syntaxin 7 and Rab7 appear to be critical for LE/MVB to deliver lipid and protein material to lysosomes. The cargo is transferred by both complete fusion and transient membrane interactions (kiss and run). In case of complete fusion, a hybrid LE–lysosome is observed. The resultant products are utilized by cells and in mammals, lysosomes reform after endosome–lysosome fusion and degradation of cargo.

Clinical Aspects

Defects in protein trafficking influence many aspects of tumor growth and ►metastasis. Tumorigenic mutations in receptor domains (EGFR, FGFR, PDGFR), adaptor proteins (AP-2), or E3 ubiquitin ligases (oncogenic forms of Cbl) that prevent receptor endocytosis may not represent direct defects in the endosomal compartment, but nevertheless influence normal trafficking of activated membrane receptors through the endolysosomal pathway. By contrast, activation of ►oncogenes such as V-Src and K-Ras promote increased growth factor uptake by stimulating macropinocytosis, contributing to tumor growth and metastasis. Hip1, an endocytosis regulator is directly implicated in tumor formation but its precise role remains unknown. Defects in the endosomal compartments are known to promote tumor progression by multiple mechanisms. Genetic defects leading to fusion of ALL/HRX, AF-10, and PDGFR with protein components of the endocytic pathway (Eps15, CALM, and Hip1, respectively) create abnormal oncogenic protein fusion forms and is detected in certain forms of leukemia. Recently, increased recycling of metastasis-promoting integrins (integrin β1, integrin α6β4, and integrin αvβ3) was directly attributed to Rab11 overexpression. As Rab11 expression is regulated by HIF, a transcription factor found elevated in many human cancers, this phenomenon may have significant implications. Not surprisingly, Rab11 and one of its family member, Rab11c, is linked to skin carcinogenesis, Barrett's dysplasia and aggressiveness of breast and ovarian cancers. Additionally, mutants of Rab5 prevent receptor endocytosis, while mutations in ubiquitin sorting machinery (Hrs, Tsg101, GGA, Escrt complexes) inhibit sorting of ubiquitinated receptors and prolong ►receptor tyrosine kinase activity of EGFR, PVR, and Torso by preventing degradation. Cadherins are Ca^{2+}-dependent homophilic cell–cell adhesion molecules (forming ►adherens junctions, AJ) and are critical determinants of tissue architecture. ►Epithelial to mesenchymal transition (EMT), hallmark of malignant tumors, is promoted by AJ disruption, prominently seen in breast, colon and gastric carcinomas. Cadherin internalization-recycling cycle has recently emerged as a major route for maintenance of AJ adhesion strength. MDM2, a E3 ubiquitin ligase found overexpressed in brain, breast, lung, and prostate cancers, interferes with this cycle by promoting sorting of E-cadherin towards lysosome for degradation thus inhibiting cadherin recycling and compromising AJ integrity. Finally, Rab GTPases control both endocytic and exocytic pathways. Abnormal activities of Rabs or their regulatory proteins and effectors lead to several human diseases, including many types of cancers.

►Endocytosis

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Endosomal Protein Sorting

►Endosomal Compartments

Endostatin

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Definition

Endostatin is a proteolytic fragment derived from the carboxyterminal, non-collagenous domain 1 (NC1) of ►collagen Type XVIII. Endostatin inhibits ►angiogenesis by blocking ►endothelial cell proliferation and ►migration.

Characteristics

Discovery

Endostatin was originally identified in the culture medium of a murine endothelioma cell line by Michael S. O'Reilly et al., from the laboratory of Dr. Judah Folkman. The same group of investigators has also discovered another angiogenesis inhibitor, ►angiostatin. The search for angiogenesis inhibitors began with the premise that the primary tumor secretes an inhibitor which suppresses the growth of metastatic cells at secondary sites. Biochemical characterization of endostatin showed a molecular mass of 20 KDa containing 184 amino acid residues. Endostatin selectively inhibits ►endothelial cells and does not affect the growth of non-endothelial cells, including tumor cells. Recombinant mouse endostatin was initially produced in E. coli in insoluble form. Treatment of mice with recombinant endostatin inhibited >99% of tumor growth. Soluble forms of both mouse and human endostatins are currently produced in yeast, insect and mammalian cells.

Structure and Function

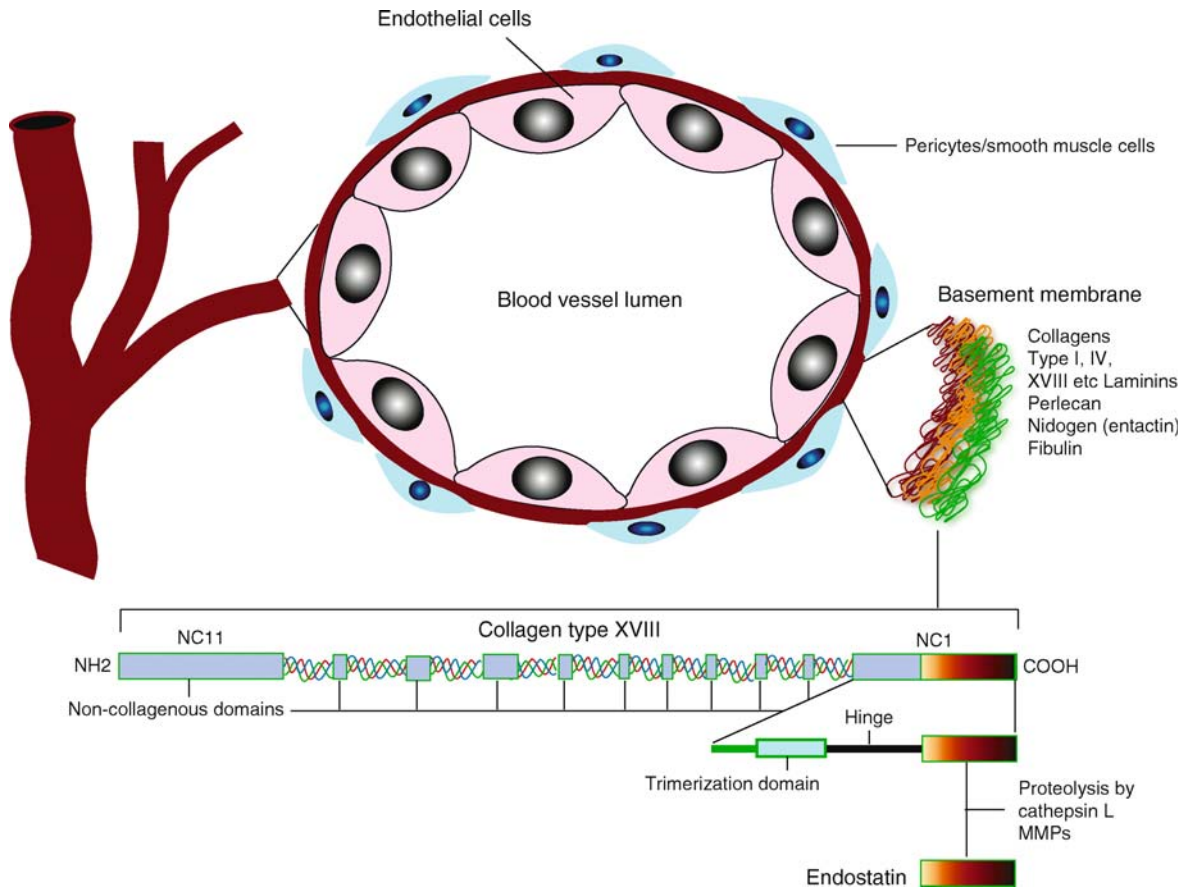
The precursor for endostatin is collagen Type XVIII, a special kind of collagen found at the basement membrane of endothelium and epithelium. Collagen XVIII along with collagen XV belong to a subfamily called multiplexin. Collagens normally have a contiguous, long stretch of triple helical region (collagenous domain) which ends at the carboxyterminus with a globular, non-collagenous domain (NC 1). Collagenous domains are characterized by repeated sequence of Glycine–Hydroxylysine–Hydroxyproline amino acid residues. The multiplexin subfamily of collagens has multiple interruptions in the collagenous domain. Collagen type XVIII, for example, has eleven non-collagenous (NC1–NC11) domains flanking ten collagenous domains (Fig. 1). Endostatin is generated from the NC1 domain, which contains a trimerization domain at the amino terminus followed by a protease-sensitive hinge region which culminates at the carboxyterminus as endostatin. Cathepsin L and, to some extent, matrix metaloproteinases cleave at the hinge region of NC1 domain to release endostatin. X-ray crystallographic studies have shown that endostatin is a compact globular molecule.

Schematic diagram shows the complex architecture of vascular basement membrane/extracelluar matrix. In addition to providing the scaffold for vascular assembly, the matrix contains a number of sequestered growth factors which can be locally released to modulate angiogenic response. The domain organization of collagen type XVIII is shown. Endostatin is generated by the proteolysis of the carboxyterminal NC1 domain.

A number of positively charged arginine residues are located at the major alpha helix aligning towards the surface of endostatin. Substitution of arg residues affects heparin binding but does not alter the biological activity. A single point mutation at proline 125 to alanine has been found to increase the biological activity of human endostatin. Comparison of amino acid sequences between various organisms reveals substitutions of proline to a different amino acid residue at this site. Endostatin sequence from chicken and *Xenopus* show proline to alanine substitution. Japanese puffer fish and Drosophila (fruit fly) have glycine at this position. *C. elegans* has an aspartic acid at this location. However, no polymorphism has been found in human collagen XVIII/endostatin at this position. Some of the recent studies suggest even shorter fragments of endostatin are biologically active and inhibit angiogenesis.

Mechanism of Action

Endostatin binds to α5β1 ►integrin and induces clustering within the lipid rafts. In addition, ►glypican 1, a heparin sulphate glycosaminoglycan, serves as a low affinity binding site for endostatin. Two phenylalanine residues (F31 and F34) are found to be critical for glypican binding. Mutation of these two residues affects endostatin binding to glypican 1. The Integrin binding site of endostatin has not yet been identified. Clustering of integrin-bound endostatin activates ►*Src* kinase and inhibits RhoA activity leading to the disruption of ►focal adhesion. Associated changes in the actin stress fibers ultimately affect endothelial cell migration. Endostatin is also known to affect a second signaling pathway, the ►Wnt signaling pathway, leading to the proteosomal degradation of ►β-catenin. Reduced levels of cytoplasmic β-catenin blocks T cell factor (TCF) mediated transcription of ►cyclin D1 and ►c-myc. Repression of cyclin D1, one of the critical mediators of cell cycle progression, arrests endothelial cells at the G1 phase of the cell cycle. Other reports have identified cell surface-bound tropomyosin, ►vascular endothelial growth factor (VEGF) receptor 2 and ►matrix metalloproteinase-2 (MMP-2) as potential targets for endostatin.



Endostatin. Figure 1 Generation of endostatin from the vascular basement membrane.

Genetic Variations

Mutations in collagen XVIII/endostatin gene has been identified in humans. Absence of collagen XVIII/endostatin is associated with ►Knobloch syndrome. In mice, collagen XVIII/endostatin gene knock out did not affect developmental angiogenesis but however showed progressive loss of vision. Other mutation, such as D104N substitution in endostatin, has been observed in Knobloch syndrome patients (heterozygous, with one allele truncated and the other with a mutation leading to D104N substitution) as well as in many cancer patients. D104N endostatin does not affect the biological activity of endostatin but showed reduced affinity to ►laminin. Cancer patients having D104N mutation in endostatin did not show any correlation with the disease progression and survival. These studies suggest that loss of collagen XVIII/endostatin does not paralyze the entire vascular system but leads to some selective changes associated with the eye. In contrast, increased expression of collagen XVIII/endostatin has been postulated to reduce the risk of cancer development. Normal serum levels of endostatin in human is between 25 and 30 ng/ml. Higher levels (about 70%) of endostatin has been observed in Down's syndrome patients who have an extra copy of chromosome 21 (trisomy). Collagen XVIII gene is located in chromosome 21. In comparison to age matched, control healthy population, a decreased risk for cancer development has been noticed in the Down syndrome patients. Folkman and Kalluri have hypothesized a physiological tumor suppressive role for endostatin based on these observations.

Preclinical Studies

Endostatin inhibits tumor growth in several model systems. More than fifteen different types of human tumor cell lines (e.g. ►ovarian cancer, breast cancer, glioblastoma cancer and renal cancer) transplanted into immunodefficient mice were inhibited, to varying degrees by endostatin treatment. Endostatin treatment was successful when initiated at an early stage of tumor growth. In a few studies, endostatin treatment induced the regression of established tumors. Furthermore, twice daily injections of endostatin were found to be more effective than daily bolus injections. Serum half-life (►Pharmacokinetics) and bioavailability are

responsible for the schedule dependent differences seen in the efficacy of endostatin treatment. This notion was further supported by the observation that tumor growth was better inhibited when endostatin was delivered by mini-osmotic pumps and in slow release formulations. Endostatin is well tolerated in mice and does not show any toxicity.

Clinical Trials

In 1999 human trials were initiated at three institutions, Dana-Farber Cancer Institute, Boston, University of Wisconsin, Madison and MD Anderson Cancer Center at Houston. Recombinant endostatin expressed in yeast was used in these Phase I trials. A total of 61 patients with advanced disease of different tumor types were enrolled into these studies. Recombinant endostatin was administered either as a 20 min or 1 h intravenous infusion. Dosages ranged from 15 to 600 mg/m^2/day. There was no dose limiting toxicity observed in all the three trials. Serum half-life of endostatin was found to be between 10 and 11 h. In two of the 25 patients treated at the MD Anderson Cancer Center, there was evidence of minor anti-tumor effect. Out of the total of 15 patients enrolled in Dana-Farber Cancer Institute there was a minor response in a patient with a pancreatic neuroendocrine tumor while two of the other patients showed stabilization of the disease. In the University of Wisconsin trial, there was no objective clinical response in any of the 25 patients treated with endostatin. Dynamic CT scans of the patients treated with endostatin showed some evidence of changes in the microvessel density. Other imaging methods to study functional changes in tumor vasculature indicated changes in blood flow and metabolism following endostatin treatment. A definitive ►antiangiogenic effect in treated patients could not be documented due to the lack of suitable biomarkers to validate changes in tumor angiogenesis. Another Phase I study was undertaken at the Vrije Universiteit Medical Center in the Netherlands. Thirty-two patients received recombinant endostatin as a continuous infusion for 4 weeks. Treatment was continued after 1 week of rest by twice daily subcutaneous injections. This trial also noted no adverse side effects in the treated patients and again there was no objective clinical response. However, two patients had a long-lasting stable disease.

Since one of the patients with pancreatic neuroendocrine tumor showed partial response in Phase I trial, a Phase II trial was initiated at multiple centers. Forty patients with advanced neuroendocrine tumors were treated with recombinant endostatin by daily subcutaneous injections. Even though a steady state level of potentially effective serum concentration of endostatin was achieved in these patients, no partial or clinical response was observed. While it is disappointing to note that the anti-tumor effects seen in experimental animals could not be replicated in these early clinical trials, a lot has been learnt on the pharmacological properties of endostatin. Future studies will focus on combining endostatin treatment with other modalities to improve the anti-tumor effects. In deed, the inhibition of tumor growth by ►radiotherapy and ►chemotherapy are potentiated by endostatin treatment. Furthermore, ►gene therapy approaches using viral vectors have shown promising effects in experimental animals. In fact, Adeno associated virus (AAV)-mediated expression of endostatin is in the early phases of clinical development. Surgical removal of tumors followed by chemotherapy in combination with endostatin treatment is a promising strategy for cancer treatment.

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Endothelial Cell Specific Molecule-1

►Endocan

Endothelial Cells

Definition

A specialized epithelial cell which lines the interior of the entire circulatory system, including the capillaries, arteries, veins, and heart. The inner most layer of a blood vessel. Endothelial progenitor cells are found in the bone marrow. Endothelial cells proliferate and migrate when stimulated with growth factors to form tube like structures. The primitive vasculature is pruned and matured under the influence of angiopoietins

►Endostatin
►Angiogenesis

Endothelial Derived Gene-1

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Synonyms

Magicin; Med28

Definition

Endothelial-derived gene EG-1 was discovered in 2002. Although first cloned from a human endothelial cell cDNA library (►Angiogenesis), EG-1's transcript has been shown to be present in other cell types as well, particularly in epithelial cells (►Epithelial tumors). The calculated mass of EG-1 is 19,520.2 Da based on amino acid sequence alone, whereas the native complete protein is ~22 kDa. The homology between the human EG-1 peptide to its mouse counterpart is 94.9%, 95.5% to its rat counterpart, and 31% to the Drosophila one.

Characteristics

EG-1 is strongly associated with cellular proliferation. Over-expression of EG-1 achieved by transfection results in increased proliferation of multiple human cell lines in culture. When these cells are injected subcutaneously into immunodeficient mice, EG-1 over-expressed cells develop into larger xenograft tumors [1].

Mechanisms

Over-expression of EG-1 results in activation of the MAPK (mitogen-activated protein kinase) pathway (►MAP kinase-01015), which has been shown to be crucial in promoting cellular proliferation. This manifests as increased levels of phosphorylated p44/42 MAP kinase, phosphorylated JNK (Jun-terminal kinase) (►JNK subfamily and cancer), and phosphorylated p38 kinase.

EG-1 overexpression also results in c-Src activation. c-Src is a member of the Src family of cytoplasmic tyrosine kinases that regulate cell growth, differentiation, cell shape, migration and survival (►SRC). c-Src has been reported to be over-expressed and to play a role in human carcinomas of the breast, colon, and others. Src family tyrosine kinases are often activated by receptor tyrosine kinases (►Receptor tyrosine kinase), such as EGF-R (epidermal growth factor receptor) (►EGR ligands) or PDGF-R (platelet derived growth factor receptor) (►PDGF). Via its proline-rich region, EG-1 binds to the Src family of protein tyrosine kinases c-Src and Yes, and possibly FYN and Hck (Hemopoietic cell kinase). As a result of EG-1 binding, c-Src then becomes catalytically active. However, EG-1 is not a direct substrate of c-Src, nor does it increase c-Src expression [2].

Two proteins with identical sequences to EG-1 have been identified: Magicin for Merlin and Grb2 interacting cytoskeletal protein in 2004 [3], and Med28 in 2004 [4]. Magicin is described to associate with the actin cytoskeleton, and is proposed to have a role in receptor-mediator signaling at the cell surface. Magicin binds directly to Grb2 (growth factor receptor bound 2 protein). Med28 is a member of the Mediator, a multiprotein transcriptional coactivator that is expressed ubiquitously in eukaryotes for induction of RNA polymerase II transcription by DNA binding transcription factors. As Med28, this protein is one subunit of the "adaptor" that bridges RNA polymerase II with its DNA binding regulatory proteins and transduces both positive and negative signals.

In summary, EG-1 is an important protein that has multiple interactions with crucial cellular pathways involved in cellular proliferation, cytoskeletal function, and transcriptional regulation.

EG-1 in Human Cancer

Immunohistochemistry of human samples demonstrates that EG-1 is present in the nucleus as well as in the cytoplasm, and possibly in the cell membrane. There are significantly higher levels of EG-1 peptides in cancer specimens of the breast, colon (►Colon cancer) and prostate (►Prostate cancer, basic characteristics and experimental models; clinical oncology), in comparison with their benign counterparts [5] (►Cancer). EG-1 has been detected at elevated levels in sera from breast cancer patients (►Serum biomarkers). In human urine, EG-1 appears primarily at a larger molecular weight, suggesting that these peptides may co-aggregate or associate with other moieties in urine (►Biomarkers). Thus, EG-1 may be secreted or it may be shed with cell death/turnover.

Translational Aspects

More recent studies have shown that endogenous EG-1 can be targeted to inhibit breast tumor growth [6]. This inhibition, whether delivered via siRNA lentivirus (►siRNA) or polyclonal antibody, results in decreased cellular proliferation in culture and smaller xenograft tumors in mice (►Mouse models). The effects are shown in both ER (estrogen receptor)-positive human breast cancer MCF-7 cells (►Estrogen receptor), as well as in ER-negative MDA-MB-231 cells. As breast cancer is the most common malignancy diagnosed in women and as one-third of these patients will die of their disease, a novel target for breast cancer therapeutic development such as EG-1 would be very

useful (►Breast cancer). Because EG-1 is unique, its use may not be redundant to other gene products/potential targets involved in other molecular pathways (►Molecular therapy; ►small molecule drugs). Further pre-clinical studies are warranted to explore the usefulness of targeting EG-1 for future cancer therapy.

►Chemotherapy of Cancer, Progress and Perspectives; ►neoadjuvant Therapy

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Endothelial Leukocyte Adhesion Molecule-1

►E-Selectin-Mediated Adhesion in Cancer

Endothelial Progenitor Cells (EPCs)

Definition

EPCs constitute a unique population of peripheral blood mononuclear cells derived from bone marrow that are involved in postnatal ►angiogenesis, wound healing, limb ischaemia, post-myocardial infarction, atherosclerosis and tumor vascularization. EPCs are derived from a precursor called a hemangioblast, and in the bone marrow these cells share many antigenic determinants with hematopoietic stem cells, including CD34, CD133, Sca-1, c-Kit, Tie-2 and Flk-1. However, once in the circulation, these cells express markers of endothelial commitment including ►von Willebrand factor (vWF) and VE-cadherin. The primitive EPC is probably best defined as $CD133^+$ $CD34^+$ $Flk\text{-}1^+$.

►Stem Cell Plasticity

Endothelial Transglutaminase

►Transglutaminase-2

Endothelin (ET)

Definition

A family of three similar 21 amino acid peptides which are amongst the strongest vasoconstrictors known and hence modulate blood pressure and flow.

►Endothelins

Endothelin-2

►Endothelins

Endothelin Converting Enzyme (ECE)

Definition

Two enzymes (ECE-1 and ECE-2), which catalyze the processing of the preproendothelins to the mature peptide.

►Endothelins

Endothelins

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Synonyms

Endothelin; ET; EDN; Endothelin-2; ET-2; vasoactive intestinal contractor or VIC

Definition

►Endothelins (ETs) are a family of three similar, small peptides that are among the strongest ►vasoconstrictors known and play a key part in vascular homeostasis. Endothelins have numerous roles in tumors including modulating ►angiogenesis and blood flow, inducing mitogenesis and ►invasion of tumor cells, immune activation, and protecting cells from ►apoptosis.

Characteristics

Endothelins (ETs) are a family of small, structurally related, vasoactive peptides that have a variety of physiological roles in many tissues, notably vascular homeostasis. The "ET axis" consists of three peptides, two receptors and two activating enzymes Table 1. Some examples of the roles of ETs in both normal physiology and pathological conditions are shown below:

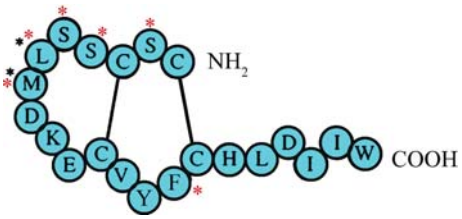
1. *Blood vessels:* Maintain basal level of vasoconstriction ("contraction of blood vessels" which controls blood pressure). Involved in the development of hypertension and atherosclerosis.
2. *Heart:* Affect the force and rate of contraction of the heart. Mediate hypertrophy and remodeling in congestive heart failure.
3. *Lungs:* Regulate the tone of airways and blood vessels. Involved in pulmonary hypertension.
4. *Kidney:* Controls water and sodium excretion, and acid-base balance. Participate in renal failure.
5. *Brain:* Modulates cardio-respiratory centers and hormone release.
6. *Cancer:* Numerous tumors – including carcinomas of the breast, lung, prostate and ovary – produce one or more of the ETs and their receptors, and there are many potential roles of the "ET axis" in cancer.

Mitogenesis: Endothelins have a mitogenic ("growth promoting") effect on both tumor and ►stromal (i.e. the non-cancer cell component of a tumor including blood vessels, immune cells and fibroblasts) ►(stroma) cells and enhance tumor growth. *Tumor angiogenesis*: Angiogenesis is the growth of new blood vessels and is critical for the growth of a solid tumor. ETs stimulate angiogenesis within solid tumors by acting directly on endothelial cells to modulate proliferation, migration, invasion and morphogenesis. ETs also modulate angiogenesis indirectly through induction of the angiogenic cytokine ►vascular endothelial growth factor (►VEGF) expression. ETs also affect blood flow through the established tumor vasculature due to their vasoactive nature. However, the effect of ETs on blood flow appears to be tissue- and tumor-specific. *Tumor invasion and metastasis*: Invasion is the process by which cancer cells spread beyond the border of the tumor enabling the tumor to grow and spread. ETs stimulate invasion of several types of tumor cells including ►ovarian carcinoma cells, Ewing's sarcoma and neuroblastoma cells and ►breast cancer cells. Stimulation of, for instance, breast tumor cell lines with ETs leads to an invasive phenotype via several autocrine and paracrine mechanisms including induction of ►matrix metalloproteinases (MMPs) and ►chemokine receptors, and the activation of macrophages. *Protection from apoptosis*: ETs can protect several cell types – including tumor cells, macrophages and endothelial cells – from apoptosis ("programmed cell death") induced by cellular stresses including ►hypoxia, serum-starvation and chemotherapeutic agents. *Immune modulation*: Trafficking, differentiation and activation of tumor-infiltrating immune cells are all modulated by ETs.

The Endothelin Axis. The ET axis consists of three 21 amino acid (aa) peptides (ET-1, ET-2 and ET-3) (Fig. 1),

Endothelins. Table 1 Genes and peptide sequences of ET axis members. Amino acids that differ in ET-2 and/or ET-3 from ET-1 are marked in Bold/Red

Gene	Mapping position	Peptide or protein	ET isoform peptide sequence
EDN1	6p24	ET-1	CSCSSLMDKECVYFCHLDIIW
EDN2	1p34	ET-2	CSCSS**WL**DKECVVFCHLDIIW
EDN3	20q13	ET-3	C**TC**F**TYK**DKECVY**Y**CHLDIIW
EDNRA	4q31	ET-RA	–
EDNRB	13q22	ET-RA	–
ECE1	1p36	ECE-1	–
ECE2	3q28-29	ECE-2	–



Endothelins. Figure 1 *Structure of ET-1.* ET-1 is a 21 amino acid peptide with a hydrophobic C-terminus and two disulphide bonds at the N-terminus. ET-2 and ET-3 are structurally similar to ET-1, differing by two and six amino acids respectively. The amino acids which differ in the ET-2 sequence are indicated by " *," while those which differ in ET-3 marked by " *."

two G-protein-coupled receptors (►ET-RA and ►ET-RB), and two membrane-bound ►endothelin-converting enzymes (ECE-1 and ECE-2). ET-1 was initially found in the conditioned medium of cultured endothelial cells and its activity as a potent vasoconstrictive peptide was described. ET-2 and ET-3 were rapidly described following ET-1's discovery, and further roles in a variety of tissues have been described.

The three ET isoforms – which are highly conserved in human, rat and mouse – derive from three separately regulated genes yet have a similar structure. Human ET-1 derives from a 212 aa precursor, preproendothelin-1. Removal of the signal sequence generates the 195aa proendothelin-1, which is further processed to release the intermediate 38aa "Big ET-1." ECEs hydrolyze Big ET-1 to yield the active 21aa ET-1. The gene for each ET has a distinct pattern of tissue expression: ET-1 is expressed by endothelial cells of many organs, ET-2 in the ovary and intestine, and ET-3 is found in the brain. There is a relatively low basal level of synthesis of ETs but these genes are readily inducible by inflammatory stimuli.

Endothelin Receptors. Two receptors for ETs have been characterized: ET-RA (also known as EDNRA or ET_AR) and ET-RB (EDNRB, ET_BR). Both receptors are expressed in a wide variety of tissue types. ETs bind these receptors with varying affinity: ET-RA binds ET-1 $\geq$ ET-2 > ET-3, but ET-RB shows no selective affinity for any ET subtype. Binding of the ligands to these ►G protein-coupled receptors (GPCR) modulates several overlapping signaling pathways resulting in the activation of phospholipase C and MAPK pathways, an increase in intracellular calcium and the induction of immediate early genes.

Induction of Endothelin Expression by the Tumor Microenvironment. One region of tumor, compared to another, may differ in the levels of hypoxia, cytokine concentration, immune infiltrate, vascularization, necrosis *etc.* The ►"tumor microenvironment" – particularly hypoxia and soluble factors such as cytokines – modulates expression of numerous "pro-tumor" genes, including those of the ET axis. Transcriptional regulation of numerous hypoxia-responsive genes is via the hypoxia-induced transcription factor, HIF-1, which initiates transcription of genes whose promoter contains a hypoxia response element (HRE). Hypoxia induces ET axis transcription in several cell types including endothelial and tumor cells. There is a functioning HRE in the anti-sense strand of the promoter of ET-1 and induction of ET expression by hypoxia is via HIF-1.

Endothelin Receptor Antagonists. The role of ETs in vasoconstriction has led to the development of several antagonists of the ET receptors that are currently under investigation for the treatment of hypertension, heart failure and renal disease. Several are now in phase II and III trials for the treatment of various neoplasms, particularly ►prostate cancer. ET receptor antagonists hold the attractive possibility that they will "hit" several different cell types and mechanisms of cancer progression.

Of the antagonists available, it is the modified peptide-based antagonists BQ123 (ET-RA antagonist) and BQ788 (ET-RB antagonist) that have been used extensively both *in vitro* and *in vivo*. Small molecule antagonists such as ►atrasentan, a highly selective ET-RA antagonist, have been used clinically. These antagonists can be administered orally, are well tolerated and have few toxic side effects. ET receptor antagonists inhibit proliferation of Kaposi's sarcoma cells, Ewing's sarcoma and neuroblastoma cells, melanoma cells and ovarian carcinoma cells.

As well as the commercially produced antagonists, it is of interest that ET activity may be modified by dietary factors. An extract of red wine polyphenols causes inhibition of ET-1 synthesis in endothelial cells; this is associated with modifications in phosphotyrosine staining, indicating that the active components of red wine cause specific modifications of tyrosine kinase signaling. Green tea polyphenol epigallocatechin-3-gallate inhibits the ET axis and downstream signaling pathways in ovarian carcinoma.

Endothelin Expression in Cancer

Numerous types of tumors produce one or more of the ETs and their receptors. However, the expression and actions of ETs in cancer are incompletely described and are tumor-type specific. ET axis expression is increased in many types of tumor, yet in several types of tumor, expression of the ET axis – particularly the receptors – is *decreased* in neoplastic tissue. For instance, in carcinomas of the breast both ET-RA and -RB are increased, yet in prostate cancer ET-RB is decreased while in lung cancer ET-RA is down-regulated. The function of the ET-RB receptor in tumors is particularly enigmatic; in some cases, such as breast

cancer, ligand-binding to ET-RB initiates several pro-tumor actions, such as promoting invasion, yet in prostate cancer the loss of ET-RB expression is postulated to increase ET-1 peptide in the tumor due to the loss of ET-RB's ET-clearance function.

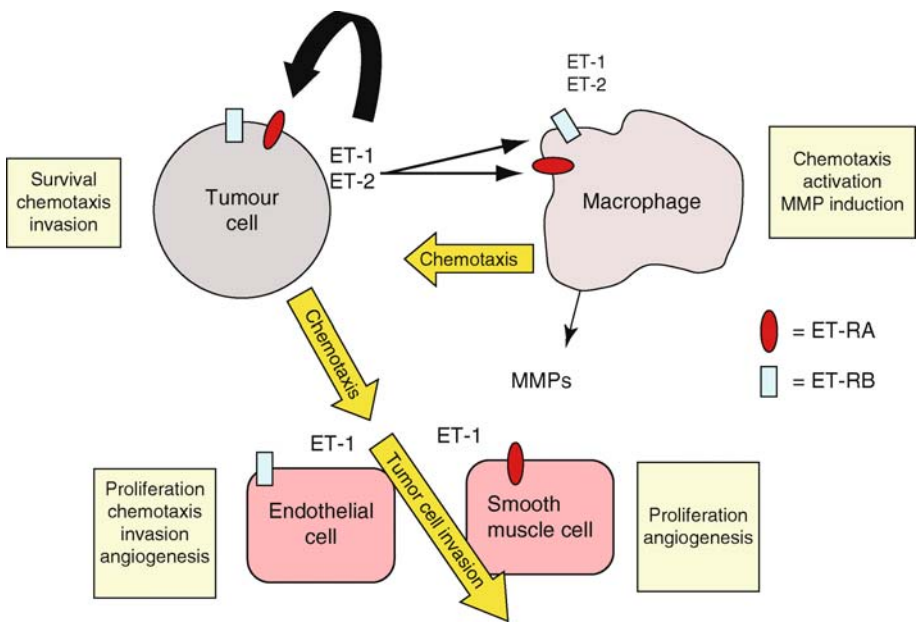
Ovarian Carcinoma. ETs have several roles in ovarian tumors including promoting growth and invasion, and ETs stimulate both tumor and stromal cells. Ovarian carcinoma cells secrete ET-1, which acts as an autocrine growth factor via ET-RA and also has a paracrine growth effect on the fibroblastic cells via both receptors. ETs acting through ET-RA promote invasion of the tumor cells by up-regulating secretion and activation of MMPs.

ET-RA is found in both tumor cells and intratumoral vessels, whereas ET-RB is expressed mainly in endothelial cells and ETs induce angiogenesis via hypoxia inducible factor (HIF)-1α and VEGF. Atrasentan decreases growth of ovarian xenografts in mice and this is associated with decreased angiogenesis and MMP expression, and increased apoptotic tumor cells.

Prostate Cancer. The role of ETs has been studied in both the normal and transformed prostate. ETs are produced in the normal prostate gland by epithelial cells and are found in high concentrations in seminal fluid (up to 5 μg/l). ET-RA and ET-RB are found in normal prostate tissue, but in the malignant prostate there is a loss of ET-RB and increased levels of ET-1. Roles for ETs in prostate cancer include growth promotion, apoptosis inhibition, and bone formation. Atrasentan delays time to progression in prostate cancer in phase III clinical trials. ETs modulate nociception ("sense of pain") and ET-RA antagonism attenuates prostate carcinoma-induced pain.

Breast Cancer. There are numerous potential consequences of ET expression in breast tumors that may lead to a more aggressive tumor cell phenotype (Fig. 2). These include the induction of invasion and angiogenesis via stimulation of tumor cells and tumor-associated macrophages (►TAMs). There is increased expression of the ET axis in ►invasive ductal carcinoma (IDC) of the breast compared to the normal breast; ►lymph node metastases (►Metastases) have a higher degree of ET staining still. Cells expressing ETs and their receptors in IDC include the tumor cells, the $CD68^{+}$ macrophage infiltrate and the endothelial cells.

ETs have a role in recruiting TAMs – macrophages express both ET receptors and chemotax towards ETs via ET-RB and a MAPK-mediated signaling pathway. Exposure of macrophages to ETs leads to an "activated" phenotype and cytokine secretion. Macrophages not only react to ETs but also produce ETs themselves and the TAMs contribute to the ETs in the breast tumor microenvironment.



Endothelins. Figure 2 *Putative roles of ETs in breast tumors.* TAMs express inflammatory cytokines that may induce ET expression by both tumor cells and macrophages, which release ETs and express both receptors. Microenvironmental factors, including hypoxia and the ETs themselves, further stimulate the ET axis. Stimulation of tumor cells and macrophages with ETs leads to chemotaxis of these cells, induction of MMP activity and cytokine expression, and invasion of the tumor cells. ET-2 also protects tumor cells from apoptosis and activates macrophages. Endothelial cells and VSMCs express ET-RB and ET-RA respectively. Stimulation of endothelial cells and VSMCs with ETs may stimulate angiogenesis.

ETs induce expression of chemokine receptors including CCR7 and potentiate the response of breast tumor cells to chemokines including CXCL12 and CCL21, which modulate the organ-specificity of breast cancer metastasis. A further potential function of ETs in breast cancer is the modulation of angiogenesis. ►Bosentan (a mixed ET-RA/B antagonist) inhibits tumor vascularization and bone metastasis in a murine model of breast carcinoma cell metastasis. Expression of ET-RA predicts unfavorable response to neoadjuvant chemotherapy in locally advanced breast cancer.

►*Melanoma*. The ET axis may be a promising therapeutic target for the treatment of melanomas. Activation of ET-RB promotes melanocyte precursor cell proliferation while inhibiting differentiation, two hallmarks of malignant transformation. In melanoma cell lines, ETs prevent apoptosis and ET-RB antagonists cause an increase in cell death. ETs are also involved in angiogenesis in mouse models of melanoma. *In vivo,* BQ788 slows growth of human melanoma tumors in nude mice. A Phase II study of Bosentan as monotherapy in patients with stage IV metastatic melanoma showed disease stabilization in 6 of 32 patients.

►*Lung Cancer*. ET-1 has been proposed as a prognostic marker in non-small cell lung carcinoma (NSCLC). There is higher expression of ET-1, ET-RA and ECE-1 in lung tumors compared to the normal tissue, whilst ET-RB is decreased. Interestingly, ET-1 is increased in the breath condensate of NSCLC patients and this could potentially be used as a non-invasive test for early detection of NSCLC.

►*Bladder Cancer*. The ET axis, particularly ET-RB, is over expressed in bladder cancer. Patients with ET-RB expression tend to have organ-confined tumors and no vascular invasion, and as such ET-RB is associated with *favorable* disease-free survival. When metastatic bladder carcinoma cells were injected into mice treated with Atrasentan, there was a dramatic reduction of metastases to the lungs.

►*Nasopharyngeal Carcinoma*. Elevated plasma big ET-1 is associated with distant failure in patients with advanced-stage nasopharyngeal carcinoma.

►*Cervical Cancer*. In human papillomavirus-positive cervical cancer cells, ET-RA mediates an ET-induced mitogenic effect. Atrasentan inhibits growth and angiogenesis in cervical cancer xenografts.

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Endothelium

Definition

Is the layer of epithelial cells that lines the cavity of the heart, and the lumina of the blood and lymph vessels. ►Endothelial cells are at the center of the angiogenic process.

►E-selectin-Mediated Adhesion in Cancer

Endotoxins

Definition

Are part of the outer membrane of the cell wall of Gram-negative bacteria such as *E. coli, Salmonella, Shigella, Pseudomonas, Neisseria*, and other pathogens. Another name for endotoxin is lipopolysaccharide (LPS). The lipid component (Lipid A) of endotoxin gives it its toxicity and other biological effects. The polysaccharide component acts as an immunogen. Endotoxins produce a variety of responses in animals including the production of ►cytokines and other inflammatory molecules.

►Kupffer Cells

Enediynes

Definition

A very potent and naturally occurring antibiotic from bacteria that acts by cleaving DNA. The adverse effects of enediynes on cells include mutagenicity, halting ►mitois by arresting the ►cell cycle, and inducing ►apoptosis. Enediynes were first isolated in the 1960s by the fermentation of microbes.. Enediynes are characterized by a nine- and ten-membered ring containing two triple bonds separated by a double bond. The enediyne group is often called a warhead because it is ready to cyclize, forming benzene via a highly reactive 1,4 benzeniod diradical intermediate. This diradical intermediate is responsible for the oxidative DNA cleavage. This cyclization process is named the Bergman cycloaromatization reaction. The enediyne group readily cyclizes via a diradical

intermediate that cleaves the DNA, giving rise to enediynes' powerful antitumor activity.

Energy of Photon

Definition

Is proportional to the frequency of the electromagnetic wave associated with the photon. For particles with non-zero mass and non-zero charge (such as the proton), the particle energy is associated with motion energy (the kinetic energy). Unit of energy that is often used in radiation biology or therapy is electron volt (eV). 1 eV = 1.602×10^{-19} Joule. Other energy units frequently encountered in radiological sciences are the ►MeV = 10^6 eV and the ►keV = 10^3 eV.

►Radiation Oncology

Engineered Antibody

►Diabody

Enhancer

Definition

A specific DNA sequence that binds transcriptional regulatory proteins that increase the expression of a gene. The enhancer can be positioned to either side of or within the gene.

►Retroviral Insertional Mutagenesis
►Prostate-Specific Membrane Antigen (PSMA)

eNOS

Definition

Endothelial nitric oxide synthase.

►Nitric Oxide

Enterohepatic Recirculation

Definition

Is the process whereby a chemical is reabsorbed in the gastrointestinal tract following biliary excretion.

►Irinotecan

ENU

Definition

N-ethyl-N-nitrosurea. A highly potent mutagen in mice.

Enucleate

Definition

Removal of the nucleus from the cell.

►Microcell-Mediated Chromosome Transfer

Enucleation

Definition

Removal of the eye from the orbit leaving the orbital tissues, extraocular muscles, conjunctiva and eyelids. Performed for certain intraocular malignancies, blind painful eyes, and in certain cases of severe trauma.

►Uveal Melanoma

Environmental Tobacco Smoke

Definition

►ETS (Environmental Tobacco Smoke).

Enzyme

Definition
Is any of several complex proteins that are produced by cells and act as catalysts in specific biochemical reactions.

Enzymic Mouth to Mouth Feeding

►Substrate Channeling

EOC

Definition
Epithelial Ovarian Cancer.

Eosinophil

Definition
A white blood cell that contains granules filled with chemicals damaging to parasites, and enzymes that damp down ►inflammation reactions.

Eosinophilic Granuloma

Definition
Is a benign form of Langerhans cell histiocytosis characterized by single or multiple bone lesions.

►Langerhans Cell Histiocytosis

Ep

►Erythropoietin

EPA

Definition
US Environmental Protection Agency.

►Benzene and Leukemia

EpCAM

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Synonyms
Epithelial cell adhesion molecule; Ep-CAM; 17-1A; GA733-2; EGP-2; EGP34; HEA125; MK-1; KSA; TROP-1; ESA

Definition
Epithelial specific cell adhesion molecule. A membrane protein found on all simple epithelia to varying degrees; is a type I membrane protein. It is a pan-epithelial differentiation antigen expressed on the basolateral surface of all ►carcinomas, varying in density and glycosylation.

As a homotypic cell adhesion molecule it is intimately integrated within the ►cadherin-►catenin and ►WNT signaling pathways. It modulates the expression of protooncogenes such as ►Myc oncogene. Its status as a pan-carcinoma antigen has rendered it an attractive target for cancer ►immunotherapy.

Characteristics
Structure and Function
Structure
This 37 kDa protein is formed from 314 amino acids (aa) of which only 26 aa face the cytoplasm.

The extracellular component contains three domains: the first is novel and is the site to which most of the

antibodies developed are targeted (323~A3, 17-1A and others). The second is similar to EGF-binding proteins 1 and 6 and ►thyroglobulin. The third has a novel structure that also has similarities with ►EGF. The intracellular portion of the antigen has a tyrosine phosphorylation site, the significance of which is uncertain.

Tissue Morphogenesis

EpCAM is essential for stable adhesion formation and tissue morphogenesis similar to adhesion molecules: carcino-embryonic antigen ►(CEA) and ►ICAM-1. The mechanism by which cytoskeletal and intracellular elements mediate this function are being characterised. EpCAM inhibits intercellular adhesion mediated by ►E-cadherins, in turn interacts with α-, β- and γ-►catenins forming the cadherin-catenin complex.

Catenins link cadherins with the actin cytoskeleton and form complexes with other proteins. Cadherins are crucial for the establishment and maintenance of epithelial cell polarity, morphogenesis of epithelial tissues, and regulation of cell proliferation and apoptosis. Their association with β-catenin is particularly interesting as this is a component in the ►Wnt Signalling pathway that regulates the expression of proto-oncogenes such as c-myc: fundamentally associated with tumor development. Wnt glycoproteins are signaling molecules that regulate cell-to-cell interaction during embryogenesis. Wnt proteins bind to receptors of the Frizzled family. Through several cytoplasmic relay components, the signal is transduced to β-catenin, which is stabilized, accumulates in the cytoplasm, and enters the nucleus, where it binds a lymphoid enhancer factor/T-cell factor transcription factor. Together, β-catenin and lymphoid enhancer factor/T-cell factor activate expression of many target genes, such as Myc Oncogene, ►VEGF, ►*cyclooxygenase-2* all associated with neoplasia.

EpCAM directly impacts the cell-cycle by up-regulating c-myc and cyclin A/E. Human epithelial cells expressing EpCAM reduce growth factors dependency, increase metabolism and colony formation. Inhibition of EpCAM expression with ►antisense nucleic acid reduces proliferation and metabolism in human carcinoma cells. The intracellular domain is essential for these effects.

EpCAM adhesive properties promote calcium independent homotypic cell sorting. Cells transfected to express EpCAM are sorted from cells of the same line that do not normally express EpCAM. It also inhibits invasive growth in cell colonies. Both activities are inhibited by anti- EpCAM antibodies.

The function of EpCAM thyroglobulin domain is being actively investigated. These domains commonly inhibit cathepsins: cysteine proteases frequently produced by tumor cells and known to be involved in metastasis.

Pattern of Tissue Expression

Normal Tissue

EpCAM is present on all normal epithelia excluding stratified squamous epithelia. Within the gastrointestinal (GI) tract colonic expression is greatest and gastric lowest. Glandular GI epithelium displays a marked expression gradient from crypts to the apex of villae.

Abnormal Tissue

Carcinomas and actively proliferating tissues show increased and differential expression of EpCAM.

Expression correlates with differentiation in gastric lesions. Immunochemical and mRNA studies show well-differentiated tumors are more expressive than those less differentiated. Normal background mucosa shows weak expression but interestingly areas of ►Barrett oesophagus or metaplasia are highly expressive.

Ninety percent of Colorectal carcinoma cells express EpCAM but in a differential form. Modifications include variable glycosylation analogous to tumor specific antigens such as colonic tumor antigen ►MUC1. EpCAM exists in the cell membrane of colon carcinoma cells as a high affinity noncovalent *cis*-dimer. Dimers on opposing membranes can associate *via* a head-to-head interaction to form tetramers with moderate affinity consistent with reversible intercellular associations. It is not known how exactly ►antibody binding correlates with variable glycosylation or oligomerization in a functional or structural sense, further investigation is required.

Tissue microarray assessment of EpCAM expression in 3,900 tissues of tumor of stratified stages and grades of 134 different histological subtypes sourced from head and neck, lung, gastrointestinal, breast, urogenital and mesenchymal tumors showed 75% tumor categories expressed EpCAM. At least weak EpCAM expression in >10% of tumors was observed in 87 of 131 different tumor categories. ►Colon cancer (81%), ►gastric cancer, ►pancreas cancer (78%) and ►lung cancer revealed a high proportion of strongly positive tumors suggesting EpCAM is an attractive target for pan-carcinoma ►immunotherapy.

Paradox of Expression with Advance in Carcinomas

A simple linear pattern of an increase in EpCAM expression with the progression of all tumors is not seen. The exact nature of EpCAM temporal expression vis a vis the grade of different tumor types remains to be stratified.

The functionally paradoxical up-regulation of EpCAM with disease progression in colorectal, breast, prostate and upper-GI carcinomas remains unexplained. This is intriguing in metastatic carcinoma in which degradation of intercellular adhesions is a primary feature. Perhaps, EpCAM is up-regulated in response to

other intra and extracellular processes that promote destruction of tissue adhesion and morphology, maintaining its constitutional stabilising function. Conversely in some tumors, e.g., colorectal cancer a loss of EpCAM expression is associated with increased local recurrence risk and a diffusely infiltrative morphology but not distant recurrence. In prostatic cancer reduced EpCAM expression correlates with a higher Gleason score but expression is higher on hormone-refractory tumor tissue than at earlier stages. In cholangiocarcinomas, squamous cell carcinoma of the head and neck and oesophagus increased expression correlates with reduced survival.

The relative loss of EpCAM expression in patients with gastric cancer is associated with a significant reduction in survival indicating that loss of Ep-CAM-expression identifies aggressive tumors especially in patients with stage I and II disease. Data from a Dutch study compared ►p53, ►CD44, E-cadherin, Ep-CAM and c-erB2/neu in tumors of 300 patients, investigating the extent of lymph node clearance. Patients without loss of EpCAM -expression of tumor cells (19%) had a significantly better 10-year survival compared to patients with any loss: 42% versus 22%. The prognostic value was stronger in stages I and II, and independent of the TNM stage. Similarly, in breast cancer, a relative reduction in EpCAM expressing disseminated tumor cells in the bone marrow of patients is associated with a relatively poorer prognosis whereas an increase in EpCAM positive tumor cells in lymph nodes and peripheral blood is associated with reduced survival.

In ►prostate cancer patients staged as M0 (no metastasis), BR (biochemical ►PSA relapse), and M1 (established metastasis), the presence of bone marrow EpCAM expressing tumor cells significantly increased with progression from M0 to BR, and M1 stages from 9 to 16–33%. These cells had double the ►chromosomal aberrations compared to cytokeratin positive tumor cells. There was only a small overlap between $EpCAM^+$ and CK^+ DTC populations of 9.5%. EpCAM marked a cohort of DTC in ca prostate patients that unlike CK^+ DTC expanded during biochemical relapse and had a phenotype different from that of CK^+ tumor cells. This differential expression of EpCAM as compared to cytokeratin indicates a specific functional role for EpCAM in the development of metastatic precursors over and above the simple role of an adhesion molecule once thought. The possible interaction of EpCAM with immune escape remains to be elucidated.

Once neoplastic transformation has taken place, a reduced EpCAM expression is an indicator of a more aggressive tumor phenotype with increased ►invasion, ►metastasis and mortality. This seemingly contradicts a recent study that suggested EpCAM silencing leads to reduced invasive potential of tumor cells. Breast cancer cell-lines were grown in ►Matrigel invasion/migration chambers. Cells in which EpCAM expression was silenced with ►SiRNAs showed a reduction of 35–80% in proliferation, 92% in cell-migration and 96% in cell-invasion without increase in cell-death or apoptosis. There was however an increase in E-cadherin, α-catenin, and β-catenin, This may be due to silencing of the inhibition that EpCAM exerts on E-Cadherin. Alternatively, EpCAM gene silencing may lead to decreased cytoplasmic β-catenin through an increase in its association with the E-cadherin adhesion complex. Hence, reducing EpCAM may decrease β-catenin availability for the wnt pathway and activation of its target genes downstream.

A process whereby EpCAM expression increases up to a point after which destabilising factors predominate and its expression is no longer stimulated is plausible. Once this point is reached, which may be variable according to the particular tumor type, tumors are less stable and display greater invasive and metastatic potential. A comprehensive study of the temporal expression of EpCAM during tumor progression is currently lacking. This would be useful as a predictor of the efficacy of immunotherapy in individual patients according to their tumor stage.

A 10-fold reduction in expression of EpCAM is seen in ►circulating tumor cells compared to primary tumors from whence they emerged and established metastases. Absence of homotypic adhesions stimulating EpCAM expression in the vascular microenvironment may be the causal link. EpCAM targeted immunotherapy may be more effective in established tumors or metastases as opposed to fluid borne disease. This does not preclude ascites due to peritoneal metastases for which treatment with ►trifunctional antibodies is effective. However the efficacy of destroying blood borne circulating tumor cells in a hope of eradicating minimal residual disease may be limited.

EpCAM Targeted Immunotherapy

Immunotherapy manipulates competent host immune system inducing tumor growth inhibition, regression or cytolysis. Approaches include the use of monoclonal antibodies and their derivatives, hybrid bispecific (trifunctional) antibodies, tumor cell vaccines, anti-idiotypic antibodies and dendritic cell vaccines. Pure immunomodulatory cytokines have been used to enhance the effect of MAbs.

Mechanism of Tumor Inhibition

The mechanisms by which anti–EpCAM antibodies exert tumor inhibition in-vivo remain controversial. Cytotoxic mechanisms include antibody dependent cell cytotoxicity (►ADCC) mediated by natural killer cells and T-lymphocytes, Complement mediated cytolysis

(CMC) and opsonization promoting ►phagocytosis mediated by PMNs.

The question of whether anti- EpCAM antibodies directly inhibit tumor cell proliferation remains unanswered. It could be postulated that EpCAM antibodies directly interfere with the activation of the Wnt pathway causing downregulation of c-myc: this remains untested. The majority of anti- EpCAM antibodies produced are specific for epitopes within the first of two EGF-like domains in the extracellular segment of EpCAM (Fig. 1) none have been shown to mimic the dimerization/tetramerization that EpCAM undergoes on ligation or to interfere with downstream gene activation or cell proliferation in-vivo. EpCAM antibodies do obliterate EpCAM mediated homotypic cell-sorting activity in-vitro; this effect may be a competitive event preventing dimerization alone. Although it is unlikely that a similar competitive effect takes place in established tumors, an investigation to see any effect on the establishment of metastases would be interesting.

A comparison between any difference in the cytotoxicity of antibodies according to the functional EpCAM domain targeted is awaited. It is possible that the majority of these antibodies work to opsonize cells alone – inducing the cytolytic mechanisms mentioned above – particularly as no physiological ligands for the extracellular domain of EpCAM other than EpCAM itself have been identified.

EpCAM forms a complex with the tight junction protein Claudin-7 within its intra-membranous segment the physiological significance of this is not yet known although an effect on apoptosis resistance in tumors is intriguing.

A flurry of interest followed the assertion that ►LAIR-1, a member of the inhibitory group of the immunoglobulin-like receptors, was a novel receptor for EpCAM. Speculation that neoplastic cells escape immunological surveillance and clearance by interacting with LAIR-1 via EpCAM gaining selective advantage for their growth, spread and dissemination was nullified when the original paper by Meyaard et al. was retracted because the observed binding of the LAIR-1 to EpCAM transfected cells was an artefact, attributed to the contamination of the LAIR-1 fusion protein preparation with an anti-human EpCAM monoclonal antibody.

Clinical Trials (Table 1)

Monospecific Murine Antibody

The murine Ig2a anti-human 17-1A monoclonal antibody Ederecolomab was the first immunotherapeutic agent licensed for use in large scale human anti-tumor immunotherapy trials. Initial trials in patients with advanced colorectal cancer showed little improvement in morbidity or mortality. Augmentation with ►interferm and ►GM-CSF increased ►ADCC with associated tumor lymphocyte infiltration and complement deposition. Patients with greater ADCC survived longer.

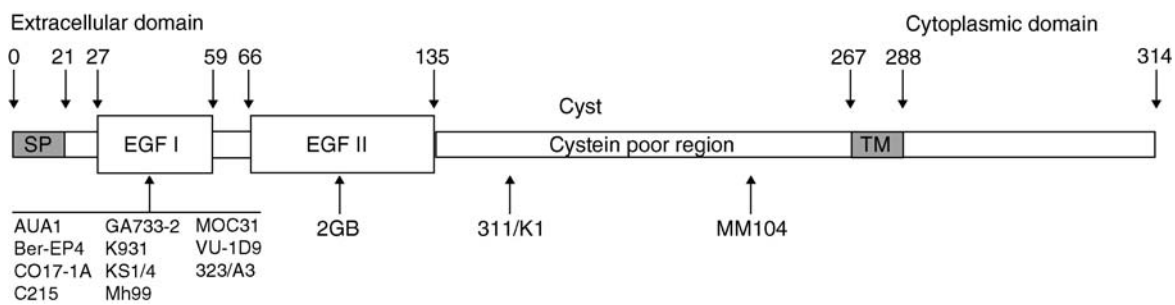
In 1994, 189 patients with ►Dukes C CRC were randomly assigned to adjuvant therapy with Ederocolomab or resection alone. Survival at 3 years was 72% for the Ederocolomab cohort and 62% for surgery alone. Further follow up at 7 years showed significantly reduced mortality (32%), disease recurrence (23%) and metastases leading to further phase II and III trials.

In 2002, Punt published results of a trial of 2,761 patients randomized to MAb 17-1A monotherapy, 5-FU and Folinic acid or 5-FU + Ederocolomab. No additional benefit was seen by adding immunotherapy to the standard chemotherapy regimen at 26 months. Immunotherapy alone was associated with significantly shorter disease free survival. Ederecolomab was removed from circulation.

The discrepancy between pre-clinical and clinical findings has lead to much debate. What are the reasons for this discrepancy?

EpCAM expression density varies at different stages of tumor growth suggesting patient antigen positivity should be assessed prior to clinical use. EpCAM density is a proven predictor of survival in breast cancer patients.

As a murine antibody, Ederecolomab induces a neutralising humoral response in humans resulting in a

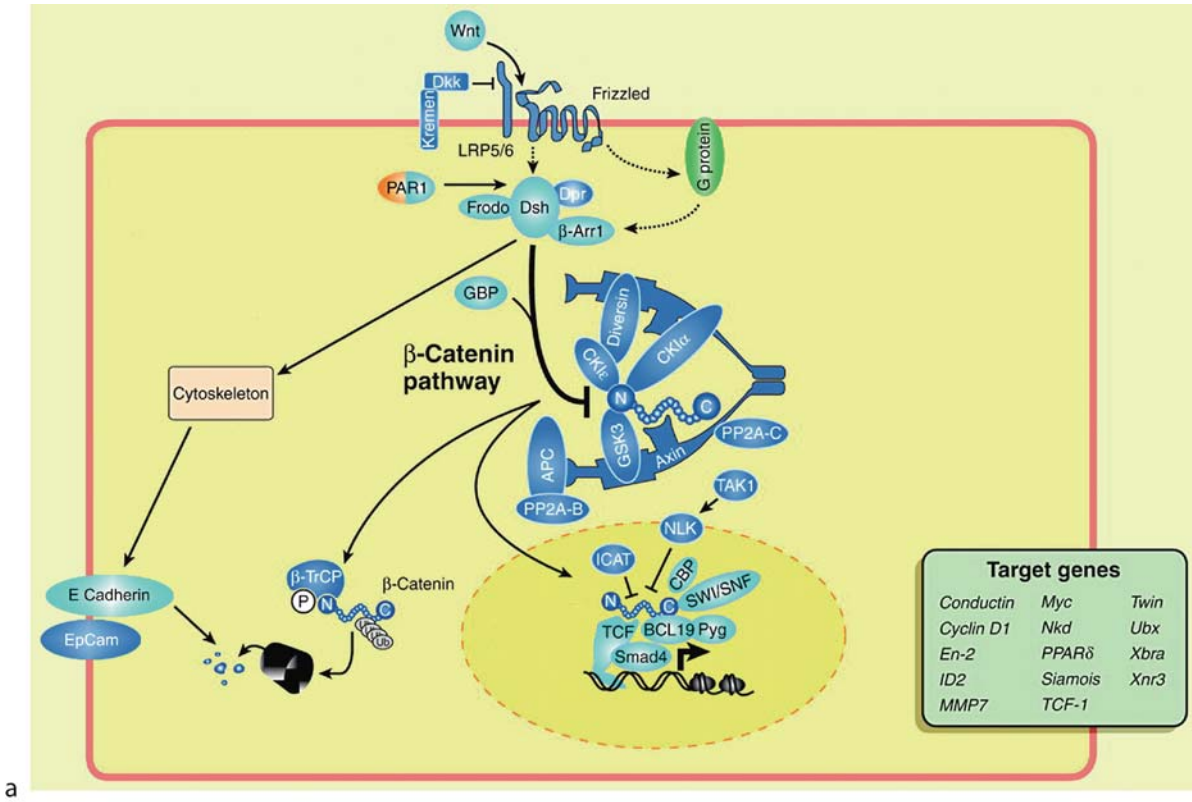


EpCAM. Figure 1 Protein domain structure.

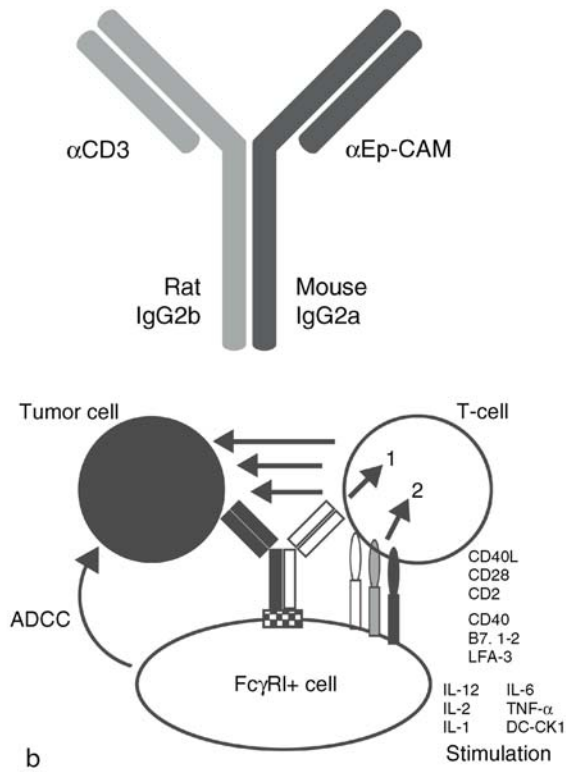
EpCAM. Table 1 Trials to assess efficacy of ►EpCAM targeted Immunotherapy for intra-abdominal carcinomas

Author	Patients	Treatment	Results	Conclusions
Weiner et al. (1986)	27 Metastatic adeno carcinoma colon or pancreas	Passive MAb17-1A preceded by 4 days γIFN	No objective clinical markers. Serum tumor markers reduced in 36%. 11 developed Ab3 response	MAb 17-1A safe for clinical use. Evidence of anti-idiotypic response
Herlyn et al. (1994)	Nine CRC	Active anti-idiotypic CO17-1A aluminium hydroxide precipitated	Three patients developed Ab3 response to Ab2 determinants	Marginal success
Herlyn et al. (1994)	54 CRC	Active polyclonal goat and monoclonal rat anti-idiotypic CO17-1A	Majority developed Ab3 response 30% developed delayed type hypersensitivity	Anti-idiotypic CO17-1A effective in stimulating log-term immunity in cohort
Fagerberg et al. (1995)	6 CRC	Active anti-idiotypic CO17-1A	Six patients developed T-cell immunity 5 mounted Ab3 response	Small study evidence of anti-idiotypic response
Ragnhammar et al. (1995)	86 Adv CRC	Passive murine MAb17-1A (76) or chimeric MAb17-1A (10)	All patients developed anti-idiotypic Abs increased by GM-CSF; c-MAb less response and more allergic side effects than Mab	Patients with Ab2 response – median survival 9/12
Riethmuller et al. (1998)	189 Dukes C	Passive observation or MAb17-1A adjuvant	7-year evaluation, mortality decreased by 32% and recurrence by 23%	Therapeutic effect maintained after 7 years, mortality/recurrence reduced
Shetye et al. (1998)	20 Adv CRC	Passive single infusion MAb17-1A +GM-CSF	Increased tumoral and PMN, monocytes and T lymphocytes	Increased TILs representing ADCC and CTLs
Hjelm et al. (1999)	20 Adv CRC	Passive MAb17-1A + IL-2 +GM-CSF	One patient partial remission 2 patients stable disease for 7 and 4 months	No augmentation of effect of MAb 17-1A
Punt et al. (2002)	2761st III CRC	Passive multicenter; (1) 17-1A MAb/5FU/LV or (2) 5 FU/LV or (3) 17-1A MAb	3-year surv DFS (1) 74.7% 63.8% (2) 76.1% 65.5% (3) 70.1% 53.0%	Addition of ederocolomab to standard therapy does not improve the disease outcome Panorex withdrawn
TRION Pharma, Fresenius 2003	23 symptomatic ascites Ca ovary	Passive trifunctional multicentre open label intraperitoneal Removab	Well-tolerated 22 of 23 patients ascites free at day 37	Effective treatment of malignant ascites phase III for all-cause malignant ascites underway
Heiss 2005	Eight peritoneal carcinomatosis	Passive trifunctional 4–6 applications intraperitoneal	Seven of eight patients no further paracentesis needed. Eradication of tumor cells in ascites	

ADCC = antibody-dependant cell cytotoxicity.
CRC = colorectal cancer.
CTL = cytotoxic T cells.
DFS = disease-free survival.
GM-CSF = granulocyte-macrophage-colony stimulating factor.
IFN = interferon.
MAB = monoclonal antibody.
PMN = polymorphonuclear cells.
TIL = tumor infiltrating lymphocytes.



a



b

EpCAM. Figure 2 Structure rationale for the development of bispecific antibodies.

short serum half-life. Foreign MAbs are rapidly cleared as immune complexes depositing in the liver, greatly reducing bioavailability. Reduced compatibility with human effector cells may also be significant.

EpCAM-targeted immunotherapy to date has targeted advanced disease: its value weighed against classic adjuvant treatments. The effect of such immunotherapy on earlier, less established disease or cancer models is unknown.

What are the Solutions?

►*Humanized Antibody*

A human IgG antibody, MT-201 (Adecatumumab) combines binding affinity similar to Ederecolomab with considerably enhanced ADCC potency with human gastric carcinoma cell lines. Addition of human serum containing IgG or human peripheral blood monocytes halves MT201 ADCC but abolishes that of Ederecolomab: indicating the importance of human anti mouse antibodies (HAMA) and compatibility of syngeneic effector cells.

MT201 reduces tumor growth in xenotransplanted HT-29 CRC cells in nude mice but only to a level similar to Ederecolomab. It is hoped human effector cells with greater type specificity of FCγ receptors will facilitate amplified tumor inhibition clinically. Three clinical trials are currently underway: two phase II studies with metastatic breast cancer and early-stage prostate cancer patients, respectively, and a phase I study testing the safety of a combination with taxotere.

►*Bispecific Antibodies*

Structure and Rationale for Development (Fig. 2)

Normal IgG molecules compose of Fc and FAb segments. The monospecific FAb segment binds to specific epitopes on antigens whereas the Fc portion recruits cells expressing Fc receptors (e.g. FcγR) such as macrophages. These are described as being bifunctional and monospecific. In trifunctional antibodies, the two halves of the FAb segment have different specificity: they are bispecific and trifunctional.

Both Ederecolomab and MT201 are bifunctional antibodies: IgG1 and IgG2A respectively with active components being the anti light chains and Fc portions. Zeidler (1999) successfully constructed a bispecific/trifunctional targeting both and CD3 (BiUII or Removab). The rationale being that ADCC is complemented by the presence of CD3 + T-lymphocytes in addition to Macrophage/Monocytes, NK and dendritic cells: known to express FcγR binding to the Fc portion of the antibody. This antibody consists of a Murine IgG2a associated with an anti light chain and Rat IgG2b associated with anti CD3.

In-vitro Cytotoxicity

in-vitro experiments of ADCC with cell-lines, effector-cells and BiUII demonstrated increased production of ►interleukins IL-1β, IL-2, IL-6, IL-12 and DC-CK1. simultaneous stimulation of accessory cells and T lymphocytes leads to antigen presentation to T-lymphocytes inducing immunomodulation and cytotoxicity. BiUII

EpCAM. Table 2 EpCAM targeting trials underway

Therapeutic (Alternative name)	Class	Ongoing or recently completed trials	Company
Catumaxomab (Removab®)	Trispecific antibody; mouse IgG2a/rat IgG2b hybrid	Phase II/III in ovarian cancer Phase II in gastric cancer	Trion Pharma/ Fresenius Biotech
Proxinium® Vivendium® (VB4-845)	Immunotoxin; single-chain antibody pseudomonas exotoxin fusion	Phase II/III in head and neck cancer Phase I/II in bladder cancer	Viventia
IGN-101 (edrecolomab)	Vaccine for induction of anti-idiotypic antibody response	Phase II in various adenocarcinoma Phase II/III in non-small cell lung cancer	Aphton
Adecatumumab (MT201)	Fully human IgG1 mAb	Phase II in metastatic breast and early-stage prostate cancer Phase I in metastatic breast, plus Taxotere	Micromet, Inc./ Serono
EMD 273 066 (huKS-IL2)	Fusion of humanized mAb KS1/4 with human IL-2	Phase I in hormone-refractory prostate cancer	Lexigen, Inc./ Merck KGa

EpCAM = epithelial cell adhesion activating molecule.
i.p = intraperitoneal.
i.v = intravenous.
MTD = maximum tolerated dose.
s.c = subcutaneous.

induces production of IL-2 in the presence of + cells activating accessory and T-cells without the requirement of exogenous IL-2. An immunologically self-supporting tri-cell complex is formed which is efficient for immune cell activation.

Cytolysis occurs within 1–3 days. The mode of cell-death is characteristically necrotic and not apoptotic. Lymphocytes with pore forming perforin proteins surround the tumor cells causing cytolysis.

Prolonged Anti-Tumor Immunity in-vivo

Another bispecific antibody: BiLu induces long-lasting antitumor immunity consisting of both humoral and cell mediated responses when administered intra-peritonealy in a murine syngeneic model It targets *murine* CD3 and human EpCAM. The Fc portion is identical to.

The human CD3 counterpart of BiLu: Catumaxo-mab/Removab has shown promising results.

Clinical Trials (Table 2)

A Phase I/II study for the treatment of ovarian cancer patients with symptomatic ascites has now been completed (23 patients) showing that Removab was safe and effectively reduced ascitic flow and tumor-cell content. A substantial Phase II/III trial assessing efficacy in patients with all causes of malignant ascites including primary gastrointestinal tumors commenced in September 2004 (250 patients) and a Phase IIa study of platinum refractory ovarian cancer patients is also underway. Finally, a Phase I/II study of patients with peritoneal carcinomatosis due to GI tumors but without symptomatic ascites is underway. Preliminary results are promising indicating the utility of Removab in the treatment of minimal fluid borne disease and micro-metastases.

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Ep-CAM

►EpCAM

Ependyma

Definition

Is a single layer of cells that line the cerebral ventricles and the central canal of the spinal cord.

►High-flow microinfusion
►Interstitual microinfusion
►Intracerebral microinfusion
►Intracerebral clysis
►Brain cancer drug delivery
►Convection Enhanced Delivery (CED)

Ependymoma

Definition

A tumor of ependymal cells.

►Brain Tumors

Eph Receptors

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Definition

Eph receptors are tyrosine kinases (RTK) bound to the extracellular membrane that function as "switches" that upon activation by Ephrin (EFN) ligands initiate signaling cascades that regulate numerous developmental processes, particularly in the vasculature and nervous system.

Characteristics

The name of the Eph receptors is derived from the name of the cell line used to characterize them initially (erythropoietin-producing hepatocellular carcinoma cell line). These receptors and their Ephrin (EFN) ligands constitute the largest family of ►receptor tyrosine kinase (RTK) known to date. Eph receptors are integral membrane proteins with a conserved N-terminal domain responsible for ligand binding, followed by a cysteine

rich region and two fibronectin type III repeats which are essential for dimerization and interactions with other proteins. The intracellular region of these receptors contains a juxtamembrane domain, a conserved kinase domain, a sterile alpha motif (SAM), and a PDZ-binding motif. There are at least 15 Eph receptors in the human genome. Based on sequence homology and the preferred type of Ephrin ligands that they bind, the Eph receptors can be divided in two subclasses, EPHA and EPHB. The ligands of the Eph receptors (Ephrins) are also bound to the extracellular membrane. Ephrins of the A subclass are bound to the membrane through a GPI anchor (*Glycosyl Phosphatidyl Inositol*), whereas members of the B subclass have a transmembrane domain. At least five Ephrins of the A subclass and three of the B subclass have been described.

The signaling cascade is initiated by the binding of a membrane-bound Ephrin and an Eph receptor in neighboring cells. This leads to the autophosphorylation of the Eph receptor on several tyrosine residues and the activation of the tyrosine kinase activity. In addition to the "forward" signaling elicited by the Eph receptors, the Ephrin ligands are also able to transduce a signal upon interaction with their cognate Eph receptor. This is often referred to as "reverse" signaling. The signaling cascade initiated upon ligand binding regulates many developmental processes and has an important role in tumor initiation and progression in different tissues.

Normal EPH Signaling

The role of Eph signaling in the nervous and vascular systems during normal development has been characterized in detail. During normal embryogenesis, Eph signaling has an important role in the development of the nervous system. Eph signaling regulates the migration of neural crest cells, the formation of the corticospinal tract, the boundary formation between hindbrain segments (rhombomeres), the establishment of neural topographic maps, and the formation and functional properties of neuronal synapses. Eph signaling also plays an important role in the angiogenic process by restricting arterial and venous endothelial mixing. In addition, interactions between Eph receptors and Ephrin ligands mediate cytoskeleton organization, cell migration, and substrate attachment. The EPH family also has an important physiologic role in the normal intestinal epithelium. EPHB2 and EPHB3, together with their ligand Ephrin-B1, regulate proliferation and cell positioning within the intestinal crypts.

EPH Signaling in Cancer

Deregulation of the levels of expression and normal Eph signaling are commonly observed in tumors of various origins. This is not surprising since aberrant Eph signaling can interfere with processes that are crucial during malignant transformation such as cell attachment, ►migration, proliferation, cytoskeleton organization, and ►angiogenesis. Eph/Ephrin activation has been implicated in several ►signal transduction pathways contributing to the tumorigenic process. For instance, Akt/PI3K (phosphatidylinositol 3-kinase) has been shown to be implicated in the increase in proliferation and migration of endothelial cells after activation of EphB4 with its ligand Ephrin-B2. In addition, Eph signaling can regulate cell ►motility by modulating the activity of other signal transduction proteins such as ►focal adhesion kinase (FAK) and ►Rho. The important role of Eph signaling in the modulation of the cellular attachment to the extracellular matrix seems to be regulated through the modulation of integrin-mediated adhesion.

Most of the studies in the literature report increased levels of expression of Eph receptors and/or Ephrin ligands in most of the tumor types studied, compared with the respective normal tissue. For instance, EphA2 is overexpressed in melanomas, EphA1 shows elevated levels in breast, liver and lung tumors, and EphB4 has been reported to be overexpressed and significantly contribute to tumor progression in prostate, bladder, breast, and head and neck tumors. Although the general view emerging is that Eph receptors and Ephrins may function as ►oncogenes in the sense that elevated levels or kinase activity, promote tumor formation and/or progression, Eph receptors may be important ►tumor suppressor genes in some tissues. This is the case of EphB2 and EphB4 in the intestine. The expression of these two EphB receptors is reduced in colorectal tumors compared to the normal intestinal cells and the premalignant lesions. The levels of expression of EphB2 and EphB4 negatively correlate with tumor progression and mechanisms of inactivation of these receptors include somatic mutations and hypermethylation of ►CpG islands situated within the promoter regions regulating their expression. Moreover, animal studies clearly support the tumor suppressor role of EPHB2 in colorectal tumors and germline mutations in this gene seem to predispose to prostate and possibly to colorectal cancer.

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Epidemiologic Studies

Definition

Studies designed to identify or measure the associations between the effect of specific risk factors or exposures and the risk of a disease. The common types of (analytic) epidemiologic study are case-control studies, cohort studies, and cross-sectional studies.

►Cancer Epidemiology
►Epidemiology of Cancer

Epidemiology of Cancer

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Synonyms

Cancer epidemiology

Definition

Is the study of the incidence, distribution, and ultimately, the prevention and control of cancer within the general population.

Characteristics

The discipline of cancer epidemiology is a relatively young one, with much of the methodology developed over the past 50 years. Prior to the advent of formal methods of collection and analysis of cancer incidence and risk factor data, associations were generally the result of reports or observations of astute clinicians or scientists. The literature is full of fascinating stories of early attempts at epidemiologic cancer studies such as that of the nineteenth-century physician, Alfred Haviland, who created elaborate maps of cancer deaths in England and Wales using national mortality statistics. One of the first and probably most well-known reports of a relationship between a risk factor and the occurrence of cancer occurred in 1950, with the publication of several case-control studies detailing the association between cigarette smoking and the development of lung cancer.

Study Design

There are a number of basic study designs in cancer epidemiology. Descriptive cancer epidemiology examines how cancer incidence and mortality rates vary according to demographic characteristics of the study population such as geographic location, race and sex. One may further analyze such information by categories of age as well as by birth cohort and time period. Ecological studies generally examine aggregate measures of risk and cancer outcome such as median income and cancer incidence across counties of a given state in an effort to identify an association between the two. Alternatively, many epidemiologic studies are based on the use of individuals as the study unit rather than larger groups, or populations of study subjects. Within this category of analysis there are essentially three study designs (with several variations) including the cross-sectional, case/control and cohort study design. Cross-sectional study designs allow for the consideration of a reference population at a given point in time. In a case-control study, the frequency of a particular risk factor among individuals with a cancer of interest (cases) is compared with that among individuals without cancer (controls). Case/control studies have been instrumental in the identification of numerous important cancer risk factors including the association between family history and breast cancer as well as between tobacco and lung cancer. In a prospective or cohort study, researchers assemble a cohort of healthy individuals who provide information on risk factors of interest at a baseline point in time. The study subjects are then followed prospectively until they develop cancer or the study is completed. An advantage of this type of study design is that risk factor data is collected before any cancer is diagnosed, thus reducing the amount of recall bias associated with disease status in the reporting of risk factor data such as family history information. There are many well-known examples of cancer cohort studies including the Atomic Bomb Casualty Commission established in 1947 to study the effects of exposure to radiation with outcomes such as leukemia, the British physicians cohort from the 1950s that examined the association between smoking and lung cancer and the Nurse's Health Studies I and II, started in 1976 and 1991, respectively, which have examined a wide range or hormonal and dietary risk factors (among others) in the development of breast and other cancers.

Risk Factors

Categories of cancer risk factors are many and include infectious agents, diet and lifestyle factors, endogenous and exogenous hormonal components and genetic factors, to name a few. It is currently popular to divide cancer risk factors into two broad categories defined as genetic and environmental. This has come about because of the many laboratory-based advances in the identification of genetically transmitted or regulated diseases such as cancer, leading to the emergence of a new field of

investigation, that is, the genetic epidemiology of cancer. For cancers, a small subset of cases exist that are attributable to rare inherited cancer susceptibility genes. The majority of cases appear to be due to sporadic mutations that may be a result of genetic or environmental events or the result of an interaction between genetic and environmental factors. Much of traditional epidemiologic methodology has been adopted for use in genetic epidemiology. In addition, new methods specific to genetic epidemiology have been developed including the twin, adoption, and pedigree study designs as well as segregation and linkage analyses.

The many advances in genetic testing, as well as in some instances, prevention or treatment options associated with particular cancer diagnoses, has led to a surge of interest in the availability of personalized risk estimates in the clinical setting and hence the development of cancer risk assessment models used to generate these estimates. Cancer risks may be presented in both relative and absolute terms and may define risk for a discrete period of time or over a lifetime. A wide variety of statistical methods exist to estimate cancer risk, with the most fully developed existing in the area of breast and ovarian cancer. Although the concept of risk assessment is not new to the fields of medicine or genetics, the use of detailed genetic information on a large population-based scale is, with all the associated difficulties of presentation and interpretability.

The field of cancer epidemiology is an exciting scientific discipline, which is able to adapt well to new information and technology. New developments in the field include the integration of biomarkers into exposure data, the inclusion of both molecular genetics and environmental risk factor data into study designs in an effort to explore the complex interaction between genotype and the environment, the creation of international data-bases via the Internet, and the merging of large data-bases that combine risk factor information with cancer incidence and mortality data. All of these advances should continue to assist scientists and health care-professionals in the identification of individuals at increased risk of developing cancer so that screening and prevention regimes as well as treatment plans may be developed.

►Alcoholic Beverages Cancer Epidemiology
►Obesity and Cancer Risk

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Epidermal Growth Factor Inhibitors

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E

Definition

The epidermal growth factor receptor (EGFR) is a member of the HER family of receptor tyrosine kinases that includes the EGFR itself (ErbB1/HER1), ErbB2 (HER2/*neu*), ErbB3 (HER3), and ErbB4 (HER4). These proteins are classic membrane-bound tyrosine kinase receptors whose activation is typically ligand dependent. The principal ligands for the EGFR are EGF and TGF-α. Other ligands include amphiregulin, heparin-binding EGF, the poxvirus mitogens, epiregulin, and β-cellulin.

Characteristics

EGFR Activation and Downstream Signaling

Receptor activation results in homo or heterodimerization and autophosphorylation of c-terminal tyrosine residues. Receptor activation enables the docking of cytoplasmic proteins that bind to specific phosphotyrosine residues, and initiate several cell signaling pathways. These pathways include the Ras-Raf-MAPK pathway, the PI3K-AKT pathway, the protein kinase C pathway, the STAT pathway, and the src kinase pathway all of which play important roles in tumor cell proliferation, invasion, migration, and inhibition of apoptosis. Recent reports have also demonstrated that the EGFR can be found in the nucleus where it can act as transcription factor. EGFR activation does not initiate linear downstream pathway signaling, but rather can activate multiple pathways that cross-connect intracellularly. The pattern of activation is often cell/tissue specific and likely contributes to the rich variety of biological responses to EGFR activation. Ligand activation of the EGFR also results in receptor down-regulation, mediated through endocytosis and ultimately Cbl-mediated EGFR ubiquitination and degradation [1]. However, the EGFR can be activated in a ligand-independent manner. This aberrant EGFR activation can result from receptor overexpression, gene amplification, activating mutations or loss of regulatory mechanisms.

Molecular Mechanisms of Targeted Therapies

Anti-EGFR therapies include monoclonal antibodies (mAbs) that recognize the EGFR and small molecule inhibitors of EGFR tyrosine kinase activity (TKIs). The mAb Cetuximab prevents receptor dimerization through steric inhibition of the extracellular domain.

Cetuximab also promotes receptor internalization and degradation without receptor activation, resulting in receptor down-regulation and reduced cell surface expression levels of the EGFR. Cetuximab also blocks the transport of EGFR into the nucleus, thus inhibiting any direct affects on DNA transcription and/or repair. Finally, Cetuximab has the potential to kill target cells by mediating antibody-dependent, cell-mediated cytotoxicity (ADCC) and complement fixation.

The TKIs are competitive inhibitors of adenosine triphosphate (ATP). They block the enzymatic activity of the intracellular domain of the EGFR. Because of their mechanism of action, these TKIs can block EGFR mutants that lack an extracellular domain, and can block ligand independent receptor activation.

EGFR Mutations

A number of genetic mutations of the EGFR have been found in cancer cells. These mutations generally result in constitutive activation of receptor signaling and can, by themselves be oncogenic, but also often confer responsiveness to targeted therapy.

The somatic gene mutations associated with an increased response to the TKIs are clustered within the receptor kinase domain, in exons 18–21 near the ATP binding region. These mutations result in structural changes that confer exquisite sensitivity to the TKIs, wherein the presence of EGFR mutations in patient's tumors results in increased responsiveness and thus progression free survival compared with patients with no EGFR mutations. Conversely, mutations in exon 20, including the T790M point mutation confer resistance to the TKIs. Furthermore, TKI resistance may also result from coexistent mutations in ras, raf, PI3K or loss of the tumor suppressor PTEN.

EGFRvIII (also referred to as del2-7 EGFR or ΔEGFR) is a constitutively active EGFR mutant variant that is the most common mutation found in glioblastoma (GBM). The mutation arises from an in-frame deletion resulting in a loss of the majority of the extracellular ligand binding domain leading to a constitutively active, ligand-independent tyrosine kinase. The variant constitutively activates the phosphatidylinositol 3′ kinase (PI3K) signaling pathway. Interestingly, while only about 10–20% of patients with GBM respond to EGFR inhibitors (despite the fact that most GBMs overexpress the EGFR) ~43% (13/30) of the patients with tumors that expressed EGFRvIII responded the TKIs. This percentage was increased to 78% (11/14) if the tumors coexpressed EGFRvIII and PTEN. PTEN is a tumor suppressor whose expression is frequently lost in GBM and acts as an inhibitor of the PI3K/AKT pathway. Loss of PTEN confers resistance to EGFR inhibitors, presumably because of the persistent activation of the PI3K pathway *downstream* of the EGFR. Nevertheless, only about 25% of the patients (14/59) actually exhibited this co-expression of EGFRvIII and PTEN. In fact, mutations are often cancer-type specific. For example, while ~50% of GBMs and head and neck cancers exhibit expression of EGFRvIII, only ~1.5% of NSCLC and 0% of metastatic colorectal cancers express EGFRvIII). By contrast, 10–25% of patients with NSCLC, very few head and neck cancers, and no GBMs contain EGFR TK gene mutations. (There has surprisingly been no assessment of EGFR TK mutations in CRC).

EGFR Inhibitors in GI Tumors

The Epidermal Growth Factor Receptor is implicated in the pathogenesis of several cancers including colorectal, pancreatic, NSCLC, Head and Neck, Breast, Brain cancer, and overexpression of EGFR has been associated in metastasis, chemotherapy resistance and poor outcome. The receptor has emerged as a rational target for anticancer treatment in these tumors. Monoclonal antibodies to EGFR and tyrosine kinase inhibitors to EGFR kinase have been developed to inhibit EGFR signaling.

Cetuximab and Panitumumab are the two monoclonal antibodies to EGFR approved by the US Food and Drug Administration (FDA) in the treatment of colorectal cancer. Cetuximab was FDA approved in Feb 2004 in combination with irinotecan in irinotecan refractory disease, or as a single agent in patients intolerant to irinotecan. Cetuximab has demonstrated efficacy in Phase II clinical trials in patients with refractory, metastatic colorectal cancer, either alone or in combination with irinotecan (Table 1). About 20% of patients with irinotecan refractory patients responded to the combination of cetuximab and irinotecan treatment, however only 10% responded to cetuximab treatment alone. The safety and efficacy of cetuximab plus irinotecan were compared with Cetuximab as a single agent (Table 1). The response rate for the combination arm was 22.9% (95% confidence interval (95% CI), 17.5–29.1%), and the medium time to progression was 4.1 months. The response rate for single-agent cetuximab as monotherapy was 10.8% (95% CI, 5.7–18.1%), and the median time to progression was 1.5 months. Cetuximab in combination with irinotecan reversed the irintecan resistance in over 20% of patients who progressed on prior irinotecan therapy.

Although the response rate from cetuximab is only around 10%, from recent press release in November of 2006 on the Phase III study (NCIC-CO.17) cetuximab demonstrated survival benefit over best supportive care as a single agent in the third-line treatment of colorectal cancer. These are the first data of an EGFR targeted antibody to demonstrate overall survival in the third-line treatment setting.

In September of 2006, FDA approved panitumumab, a recombinant human monoclonal antibody that binds

Epidermal Growth Factor Inhibitors. Table 1 Early data on efficacy of cetuximab as a single agent or in combination with irinotecan in patients with irinotecan refractory colorectal cancer

	Saltz, ASCO 2001	Cunningham, N Engl J Med 2004		Saltz, J Clin Oncol 2004	Lenz, ASCO 2004
Phase of trial	II	III		II	II
Treatment	Irinotecan + cetuximab	Irinotecan + cetuximab	Cetuximab	Cetuximab	Cetuximab
No. of patients	121	218	111	57	350
Prior treatment	Irinotecan	Irinotecan		Irinotecan	Irinotecan and oxaliplatin
PR	19%	23%	11%	9%	12%
SD	27%	32%	22%	35%	32%

the human EGFR, for the treatment of patients with EGFR-expressing metastatic adenocarcinoma of the colon or rectum (MCRC) after disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. The efficacy of panitumumab was demonstrated in a phase III randomized, open-label, controlled trial comparing panitumumab plus best supportive care (BSC) versus BSC alone in a total 463 patients with 5-FU, irinotecan and oxaliplatin refractory disease. Nineteen of 231 (8%) subjects in the panitumumab arm had a partial response, and there was significant prolongation of progression free survival in the panitumumab arm.

Clinical benefits of monoclonal antibodies to EGFR (Cetuximab and Panitumumab) combined with irinotecan and oxaliplatin have been tested for first-line treatment of MCRC in phase II clinical trials. In the phase II studies, the response rate of the combination therapy of EGFR inhibitor and cytotoxic chemotherapy reaches as high as 77%, disease control rate as high as 96%, rate of metastatses resection is over 20%. The high response rate of the combination therapy not only enable rapid improvement of MCRC associated symptoms but also improve the long term survival to the subgroup of patients whose tumor becomes resectable after combination therapy. The data from the phase II studies requires confirmation by randomized Phase III clinical trials. Large ongoing randomized phase III studies will establish the clinical the benefit of Cetuximab and Panitumumab as first-line in colorectal cancer. The role of cetuximab in adjuvant treatment of CRC is being evaluated with two randomized Phase III clinical trials, NCCTG/INT N0147 and PETACC-8.

Survival advantage of cetuximab to radiation therapy was demonstrated in a randomized phase III study comparing concurrent cetuximab radiation with radiation alone in locoregionally advanced head and neck squamous cell carcinoma. The role of cetuximab in concurrent chemoradiation has been tested in esophageal/gastric cancer, rectal cancer and pancreas cancer in phase II clinical trials. Patients with localized esophageal/gastric cancer received radiation and concurrent chemotherapy of cetuximab, carboplatin and paclitaxel. 67% of the patients had clinical complete response, 43% of the patients were found to have pathologic complete response at surgery. A confirmatory phase III study is being planned. Neoadjuvant chemotherapy and chemoradiation in rectal cancer treatment decreases local relapse rate of rectal cancer with higher sphincter preservation rate compared to post-operative chemoradiation. Cetuximab has been evaluated in combination with capecitabine, or oxaliplatin based CapOx regimen in concurrent neoadjuvant radiotherapy for the patients with rectal cancer in two phase II studies. The combination therapy was found be feasible and resulted in pathologic complete response (CR) in some patients. Currently the role of Cetuximab in the neoadjuvant chemoradiation is tested in the phase III EXPERT-C clinical trial.

Management of borderline resectable pancreas cancer is challenging and neoadjuvant chemotherapy or chemoradiation is controversial in those patients. The role of cetuximab in combination with gemcitabine in concurrent neoadjuvant chemoradiation was examined in a phase II clinical trial. Two of ten patients exhibited partial response. Six patients went on to margin (−) resection, including one patient each with borderline resectable and unresectable disease prior to therapy. The role of cetuximab with neoadjuvant chemoradiaiton in downstaging pancreas cancer for resection needs to be confirmed by phase III randomized study.

More than 80% patients treated with cetuximab experiences acneiform rash. Her1/EGFR is expressed in epidermis, sebaceous glands and hair-follicle epithelium. Her1/EGFR plays a role in the normal differentiation and development of skin follicles and keratinocytes. From the study of mice with Her1/EGFR gene knock out or with dominant negative mutation, it is predicted that EGFR inhibition in human is feasible,

but may be associated with cutaneous toxicity. Indeed skin rash become the class toxicity of EGFR inhibitors including monoclonal antibody to EGF receptor and small molecule inhibitor to EGF receptor kinase. Can the presence and intensity of skin rash predict the response to EGFR inhibitors? Sub-group analysis has been carried out to correlate the skin reaction and efficacy for the BOND study. The RR, mTTP and mOS increase with higher grade of skin toxicity to cetuximab. The same phenomena are observed with pantitumumab treatment. To take a step further, should the dose escalation of anti-EGFR antibody continue until skin rash is detectable? In patients who have had no or mild skin reactions in response to standard cetuximab dose, dose escalation (up to 500 mg/m^2) improved tumor response rate in a phase II study.

Is there any molecular marker that predicts Cetuximab efficacy in GI malignancy? Both the trials of Cunningham and Saltz have failed to show a significant correlation between EGFR expression based on immunohistochemistry (IHC) and response to treatment with either cetuximab and CPT-11, or cetuximab alone. Different from the positive association between Her-2 gene amplification and response to Herceptin (monocloncal antibody to Her-2) in breast cancer, Epidermal growth factor gene amplification is not frequent and cannot account for antitumor activity of cetuximab plus chemotherapy in advanced colorectal cancer patients. Unlike the co-relation between EGFR mutation and positive response to erlotinib in NSCLC, 50 primary untreated CRC tumors were sequenced. No mutation was identified. Multiple molecular markers including EGF A61G polymorphism and Cyclin D1 A870G polymorphism, have been proposed as molecular markers that predict response of CRC to cetuximab. However evaluation of these markers in larger data set is required to determine their usefulness in patient care.

Small Molecule inhibitors to EGFR tyrosine kinase (TKI) and Monoclonal Antibodies to EGFR are developed to target EGFR and its signaling transduction pathway. However due to different chemistry and mechanism of action, their clinical activities differ among different disease. Despite the clinical activities and safety data of anti-EGFR antibodies (cetuximab, panitumumab) in colorectal cancer, TKI as single agent showed minimal activity in MCRC. While patients with MCRC were treated with combination of TKI and fluoropyrimidine-, oxaliplatin-, and irintecan-based regimen, the clinical response rate was observed from 24 to 74% in phase II studies. However, TKI was found to increase grade 3, four toxicities and some of the trials had to be closed prematurely due to adverse effects. To confirm the clinical benefit of TKI to chemotherapy, a Phase III study of an Optimized chemotherapy plus bevacizumab with or without Erlotinib in MCRC is ongoing with a target accrual of 640 patients.

Erlotinib in combination with gemcitabine has demonstrated benefit in patients with pancreatic cancer in a Phase III study. Results from that trial are the first to our knowledge that demonstrated a clinical benefit from EGFR TKI used in combination with chemotherapy. Cetuximab, the anti-EGFR antibody was found to have encouraging activities while combining with gemcitabine and concurrent radiation in localized pancreas cancer, in addition cetuximab showed clinical efficacy in combination with gemcitabine/oxaliplatin (GEMOXCET) in the treatment previously untreated metastatic pancreatic cancer. Further evaluation in a phase III trial is warranted.

Treatment for Hepatocellular Carcinoma (HCC) is limited due to low response rate and transient response from most cytotoxic chemotherapy. Although cetuximab showed very modest activity in HCC, Erlotinib demonstrated clinical efficacy in patients with HCC. Thirty-two percentage of the 38 patients with HCC treated with erlotinib at 150 mg daily were found progression-free at 6 months. Disease control was seen in 59% of the patients. Median overall survival time was 13 months. Role of EGFR TKI needs to be confirmed with phase III study.

EGFR Inhibitor in Lung Cancer

Lung cancer could be divided into small cell lung cancer and non-mall-cell lung cancer (NSCLC). The development of epithelial growth factor receptor (EGFR) inhibitor as a therapeutic option is mainly in NSCLC. Both tyrosine kinase inhibitors (TKIs) and monoclonal antibodies of EGFR have demonstrated activity in NSCLC. Gefitinib (Iressa, AstraZeneca), a small molecule inhibitor of the EGFR, was the first selective EGFR inhibitors received accelerated approval from U.S. Food and Drug Administration after phase II clinical studies showed promising clinical response in NSCLC patients. However, subsequent large phase III trials failed to show survival benefit with gefitinib, which led to FDA restricting gefitinib use to only those patients who has been on this medication with good response. Erlotinib (Tarceva, Genentech) is a very similar molecule to gefitinib, has the same mechanism of action by competing with ATP for the ATP-binding site of EGF receptor. Its efficacy has been shown in phase III trial with prolonging overall survival by 2 months in advanced NSCLC. As a result of this, FDA approved erlotinib for second-line therapy of NSCLC. The different destinies of these two similar drugs elicited a lot discussion among investigators. Some hypothesized that the different results due to erlotinib was given to maximally tolerated dose while gefitinib was not, and patient population/genetic may be

different in those clinical trials. Both Gefitinib and erlotinib are oral medication, usually well tolerated. The main toxicities of these TKIs are acneiform rash, diarrhea and occasional interstitial lung disease. In addition to TKIs, anti-EGFR monoclonal antibody (MoAb), such as cetuximab (Erbitux) has also been tested in NSCLC with cytotoxic chemotherapy. The role of anti-EGFR MoAb in lung cancer is not very clear as these studies are still ongoing.

NSCLC patients respond differently to EGFR inhibitors, especially to TKIs as suggested by several clinical trials. Women, Asian, never smoked, and adenocarcinoma histology were found correlated with higher response rates to TKIs. Molecular analysis has given us mixing results on defining molecular predictors to TKIs. Several phase II studies demonstrated that mutations in Exon 18–21 of the EGFR correlated with responsiveness of NSCLC to gifitinib and erlotinib. While in a large randomized, placebo- controlled, phase III NSCLC study reported by NCI Canada, clinical response to erlotinib was associated with amplification of EGFR gene, not mutations of EGFR. K-ras is a downstream molecule in EGFR signally pathway. K-ras mutation has been consistently shown related to resistance to TKIs. Other molecules such as Her2 gene amplification, Akt phosphorylation have been reported to correlate with clinical response to TKIs (Cappuzzo, Eberhard). Like other target therapy in oncology, the molecular target for EGFR inhibitor need to be further defined in NSCLC, so we could individualize this therapy to achieve the best outcomes.

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Epidermal Growth Factor Receptor

Definition

Synonym ERBB, EGFR, ERBB1 and HER-1, is a protein of 1210 aa and 134 kD that mediates the biological signal of ►epidermal growth factor (EGF), also of transforming growth factor [transforming growth factor β] (TGF). The binding of EGF to EGFR leads to the internalization of the EGF-EGFR complex, induction of the tyrosine kinase activity associated with EGFR, stimulation of DNA synthesis and cell proliferation. The gene maps to 7p12, and is amplified or rearranged in glioblastoma multiforme and prostate carcinoma.

►HER-2/neu
►Herceptin

Epidermal Growth Factor-like Ligands

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Synonyms

EGF-like ligands; EGF family; Growth factors; EGF

Definition

►Epidermal Growth Factor (EGF)-like family members bind to and activate EGF receptor tyrosine kinases ErbB-1, -2, -3 and -4, also named HER1–4. This triggers the activation of intracellular signal transduction pathways resulting in cellular proliferation and differentiation. All members of the EGF-like family are produced as membrane-anchored precursors and are processed and released through the action of specific membrane-bound proteolytic enzymes of the sheddase family.

The Epidermal Growth Factor (EGF)-like family consists of at least 12 members: Epidermal Growth Factor (EGF), Transforming Growth Factor alpha (TGFα), Heparin binding-EGF like growth factor (HB-EGF), Amphiregulin (AR), Betacellulin (BTC), Epiregulin (EPR), Epigen, Cripto, and Neuregulins 1–4 (NRG1–4). These EGF-like members are defined by three characteristics: (i) they display high affinity binding to membrane-bound epidermal growth factor tyrosine kinases receptors ErbB1–4, (ii) they are eliciting a mitogenic response in EGF-sensitive cells and (iii) they possess at least one primary EGF structural motif of 50–60 amino acid

residues with the general sequence $X_nCX_7CX_{2-3}$-$GXCX_{10-13}CXCX_3YXGXRCX_4LX_n$ embedded in the transmembrane precursor molecule. Usually present as multiple structural units in the extracellular domain of EGF-like ligands, this conserved cysteine-rich EGF-like motif is crucial for the binding to ErbB receptors. The EGF-like ligands are proteolytically cleaved and liberated from the extracellular region of the transmembrane precursor. Ligands, either membrane-bound or soluble, can bind to homo- and heterodimer combinations of the four currently known membrane-anchored tyrosin kinase receptors ErbB1 (EGFR, HER-1), ErbB2 (Neu antigen, HER-2), ErbB 3 (HER-3), and ErbB4 (HER-4). Of all possible combinations, ErbB2 homodimers appear not to be stable. EGF, AR and TGFα bind preferentially to EGFR, while BTC, HB-EGF and EPR bind to both EGFR and ErbB4. NRG1 and NRG2 preferentially bind to ERbB3 and ERbB4, whereas NRG3 and NRG4 bind to ErbB4. Growth factor binding induces homo- or heterodimerization of the receptors and stimulates intracellular protein-tyrosine kinase activity resulting in ►auto- and cross-phosphorylation of key ►tyrosine residues in the C-terminal ►domain in the ErbB homo- or heterodimer. This autophosphorylation sites are docking stations for several proteins that associate with the phosphorylated tyrosine residues through ►SH2 domains (Src homology 2). This initiates downstream ►signal transduction cascades, among them being the ►MAPK, ►AKT and ►JNK pathway, which leads to ►DNA synthesis, cell proliferation, alterations in ►apoptotic pathways and ►cell migration and ►adhesion.

►Epidermal Growth Factor (EGF) was originally isolated from the mouse submaxillary gland as a stimulatory of eyelid opening and tooth eruption in newborn rodents. Other sources of (pro)EGF are the kidney*, epidermis* (*mainly membrane-bound proEGF), pancreas, small intestine, and brain. Human EGF precursor (preproEGF) is a large protein consisting of 1,207 amino acids (aa) containing nine EGF motifs in the extracellular proEGF region. The EGF motif closest to the transmembrane domain corresponds to soluble EGF. The enzyme responsible for cleavage of the extracellular proEGF domain is ADAM 10 (A Disintegrin and Metalloprotease domain 10). EGF null mice do not show a significant phenotype. Transgenic overexpression of mouse EGF targeted to villous enterocytes of the jejunum and ileum causes increased villous height and crypt depth in these intestinal parts. Transgenic mice over-expressing human proEGF-driven by the β-actin promoter have revealed that (pro)EGF is the active ligand for the EGFR in germ cells and proper EGF expression is important for the completion of spermatogenesis.

►Transforming Growth Factor alpha (TGFα) derives from a 160 aa precursor (preproTGFα). This protein was originally characterized by its capacity to induce ►oncogenic transformation in rat kidney fibroblasts. TGFα is targeted preferentially to the basolateral compartment of polarized epithelial cells where it is cleaved by TNF Alpha Converting Enzyme (TACE)/ADAM17. TGFα mRNA and protein have been detected in various adult tissues, including pituitary gland, brain, skin keratinocytes and macrophages. Transgenic animals with overexpression of TGFα display hypertrophy of skin and hyperkerotosis with alopecia. These animals also have stunted hair growth and psoriasis-like lesions. TGF α null mice show a very mild phenotype which includes wavy fur and whiskers.

►Heparin binding-EGF like growth factor (HB-EGF) derives from a 204 aa molecule. HB-EGF was identified in conditioned media of human monocytes/macrophages and was purified from phorbol ester-treated human U-937 macrophages as a 22-kDa factor with strong affinity for heparin. HB-EGF is expressed in a wide variety of hematopoietic cells, endothelial cells, vascular smooth muscle and epithelial cells. Cleavage of proHB-EGF may involve ADAM9, 10, 12, and 17. Over-expression of human HB-EGF decreases the weight and growth rate in these transgenic mice and this may involve alternation of insulin-like growth factor binding protein (IGFBP) pathways. Moreover, histological analysis showed over-expression of HB-EGF exclusively in kidney, liver, lung and stomach. Employing several mutant mice lacking HB-EGF expression revealed a critical role for HB-EGF in cardiac valve formation and normal heart function. HB-EGF null mice develop heart failure as a result of grossly enlarged ventricular chambers.

►Amphiregulin (AR) is synthesized as a 252 aa transmembrane precursor. AR was originally isolated from the phorbolester-treated human breast adenocarcinoma cell line MCF7. ProAR is expressed in many human tissue including ovary, placenta, pancreas, testes, lung, cardiac muscle, breast, kidney, spleen and colon. In polarized epithelial cells, cleavage of basolateral proAG is facilitated by TACE/ADAM17 to release a 78–84 aa growth factor. Posttranslational modification results in several different cell surface and soluble isoforms. Keratin-14 promoter-driven overexpression of AR in the basal epidermal cell layer in transgenic mice induces severe, early-onset skin pathology, resembling psoriasis. Histological examination of the skin in these AR over-expressing mice revealed hyperkeratosis, focal parakeratosis and acanthosis, mixed leukocyte infiltration, including CD3-positive T cells and neutrophils in the epidermis and dermis. These mice also display tortuous dermal vasculature. AR null mice similar to EGF null mice, do not display any gross or histological abnormalities.

►Betacellulin (BTC) has been isolated from conditioned media from a pancreatic β cell tumor cell line. BTC is also expressed in a variety of mesynchymal and epithelial cell lines and in many tissues including

pancreas, liver, kidney and small intestine. Membrane-bound proBTC is processed by ADAM10 to release soluble BTC. Transgenic chicken actin promoter-driven BTC over-expression in mice causes bony deformations of the skull, pulmonary hemorrhage syndrome, and complex eye pathology. Trangenic animals showed decrease in the weight of pancreas and increase weight of the eye, lung and spleen.

►Epiregulin (EPR) was initially purified from conditioned medium of the mouse fibroblast-derived tumor cell line NIH-3T3 clone T7. EPR is expressed in adult pancreas, liver, kidney and small intestine. Human epiregulin encodes a 46 aa growth factor which exhibits 24–50% identity with the sequences of other EGF-like ligands. Epiregulin exhibits a bifunctional regulatory property in that it inhibits the growth of several epithelial cell lines, like NIH-3T3, and stimulates the growth of fibroblasts, primary rat hepatocytes, human smooth muscle cells and various other cell types, including COS-7 (monkey) and Swiss 3T3 (mouse). Epiregulin can bind to and activate EGFR and ErbB4. EPR can also transactivate ErBB2 and ErbB3. Although EPR displays lower affinity toward EGFR, EPR-mediated receptor activation is more sustained when compared with EGF or BTC. The lower affinity of EPR to EGFR results in enhanced dissociation of EPR from the ligand-receptor complex during receptor endocytosis and renewed EGFR activation by recycled EPR. EPR-deficient mice develop chronic dermatitis.

Neu differentiation factors (NDF, heregulins, Neuregulins, Glia Growth Factors-GGFs, Acetylocholine Receptor Inducing Activity-ARIA) were isolated from mesenchymal cells based on their ability to elevate phosphorylation of ErbB proteins At least four different genes of neuregulins are known. NRGs are widely expressed in neurons of the central and peripheral nervous system. They are known to play important functions during neuronal development and migration. Furthermore, NRG-1 is also involved in heart development. Mice defective in genes encoding either NRG-1 or the receptors ErbB-2 or ErbB-4 display identical failure of trabecular formation in the embryonic heart, consistent with the notion that trabeculation requires NRG-1-mediated activation of ErbB-2/ErbB-4 heterodimers.

►Epigen encodes a protein of 152 amino acids that contains EGF-like features. Epigen has 24–37% identity with EGF, TGFα, and Epiregulin. In epithelial cells, Epigen stimulates the phosphorylation of ErbB1 and causes activation of MAP kinase signaling pathways. Epigen also activates genes under the control of the SRE (Serum Response Element). Epigen is a mitogen for the human keratinocyte cell line HaCaT and this activity can be significantly reduced by a blocking antibody to the receptor ErbB-1.

►Cripto-1 (Cr-1) is a glycoprotein member of 188 aa. Cr-l has been implicated in development. Expression of Cr-l was reported in trophoblast and embryoblast of 4-day old mouse blastocysts, and the myocardium of developing heart tubes in 8.5 old mice embryos. Transgenic murine embryos lacking Cr-l are devoid of cardiac specific gene expression (α and β- myosin heavy chain, myosin light chains 2A and 2V). Antisense RNA silencing studies showed that inhibition of Cr-1 expression inhibits the growth of human colon carcinoma cell lines expressing this factor.

E

EGF-Like Ligands and Cancer

Members of the EGF-like ligand receptor system are often amplified in various cancers. Over-expression of EGFR in brain tumors (oligodendrogliomas, glioblastomas) and in carcinoma of the stomach, thyroid, lung and breast (for the latter two also Neu/ErbB2 amplification) identifies these tumors as targets for the actions of EGF-like ligands and anti-cancer drugs directed at inhibiting ligand-mediated ErbB1/2 activation. EGF-like ligands affect tumor cell growth, differentiation and metastasis. Amplification and ligand-induced activation of EGFR correlates with increased *in-vitro* tumor cell migration, matrix degradation and enhanced *in-vivo* tissue invasiveness. EGFR transactivation as a result of the prior activation of ►G protein coupled receptors (GPCRs) involves the proteolytic cleavage of membrane-anchored EGF-like ligands by GPCR-mediated activation of members of the metalloproteinase superfamily. GPCR-mediated EGFR transactivation in COS-7, HEK-293, and breast cancer cells involves cleavage of proHB-EGF, while in colon epithelial cells and head and neck squamous cell carcinomas proTGFα has been implicated.

Brain Cancer

Malignant human gliomas are the most common form of primary tumors of the central nervous system and display an invasive growth pattern. Among the genetic alternations found in these tumors, p53 inactivation and PDGF/PDGFR activation represent early events, whereas the loss of chromosome 10, gene amplification, and rearrangement of genomic EGFR are late events in glioma carcinogenesis. The coamplification of TGFα and EGFR in human glioma cell lines and primary glioma tissues coincides with sustained glioma proliferation and suggests an autocrine growth loop promoted by this EGF-like ligand-receptor pair, especially in high grade glioma. Antisense-mediated down-regulation of TGFα expression was shown to inhibit glioma growth.

Breast Cancer

TGFα stimulates growth and differentiation of mammary epithelial cells and is implicated in the pathogenesis of human breast cancer. High expression of AR has been detected in several human breast cancer cell lines and primary human breast carcinomas. AR acts as an

autocrine/juxtacrine growth factor in human mammary epithelial cells transformed with an activated c-Ha-*ras* proto-oncogene or overexpressing Neu/ErbB2. EGF and AR may modulate invasion of metastatic breast cancer cells by increasing the expression of matrix-metalloproteinases. EGFR transactivation by membrane-anchored G protein coupled estrogen receptor GPR30 in breast cancer cells involves the MMP-2- and MMP-9-mediated release of HB-EGF.

Carcinoma of the Lung

EGF-like growth factors play an important role in the pathogenesis and progression of non-small cell lung cancers (NSCLC) comprising large cell cancer, squamous cancer and adenocarcinoma, and small cell lung cancer (SCLC). The amplification of TGFα combined with the presence of cytoplasmic, but not its membrane-anchored, EGFR and/or enhanced expression of EGFR in its cytoplasmic or membrane-bound form significantly associate with poor patient survival rates.

Colorectal Cancer

Most human colon cancer cell lines express TGFα, AR, and Cr-1. AR and Cr-1 appear to be suitable markers for human colorectal cancer tissues since transcripts for both EGF-like ligands are detected in 60–70% of primary or metastatic human colorectal cancers but are present in only 2–7% of normal human colonic mucosa. Immunoreactive AR was reported in primary and metastatic colorectal tumors but not in normal colon. Agonists to the GPCR prostaglandin E_2 receptor and the M3 muscarinic receptor lead to a metalloproteinase-/TACE-dependent processing of TGFα resulting in EGFR transactivation and proliferation of colon cancer (Caco-2, LoVo and HT-29) cell lines.

Head and Neck Squamous Cell Carcinoma (HNSCC)

GPCR-mediated EGFR transactivation via GPCRs for gastrin releasing peptide, lysophosphatidic acid (LPA) and carbachol was shown *in vitro* to result in a matrix-metalloproteinase-dependent enhancement in growth and invasiveness of HNSCC cells. This coincides with the release of TGFα and AR, but not EGF or HB-EGF, into the supernatant of HNSCC. Enzymatic processing of proAR and proTGFα upon treatment with lysophosphatidic acid (LPA) or carbachol involves the activation of TNF-converting enzyme (TACE/ADAM17) and the downstream activation of EGFR-induced MAPK signaling pathways in HNSSC.

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Epidermodysplasia Verruciformis (EV)

Definition

An inherited condition, characterized by the appearance of multiple flat papillomatous lesions on the skin which have a high propensity for malignant transformation. Associated with a specific subset of ►human papillomaviruses.

►Squamous Cell Carcinoma

Epidermoid

Definition

Similar to squamous or differentiated epidermal cells.

►Mucoepidermoid Cancer

Epidermoid Carcinoma

►Squamous Cell Carcinoma

Epigallocatechin

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Synonyms

Green tea polyphenol; Catechin

Definition

Epigallocatechin is one of several biologically active ingredients that make up the bulk of the potent antioxidant polyphenols known as catechins, which are found in green tea.

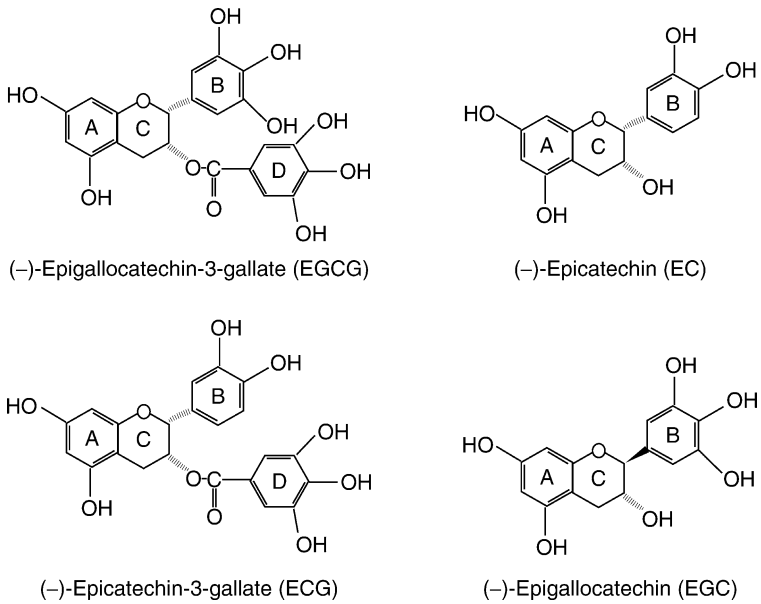
Characteristics

Next to water, tea is the second most widely consumed beverage in the world. Green tea, like oolong and black teas, is derived from the *Camellia sinensi* plant, but is processed immediately from fresh leaves and is protected from oxidation. The biologically active ingredients in green tea are a family of polyphenols (catechins) and flavonols, which are very strong antioxidants. The catechins comprise about 90% of the bulk of green tea and include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Fig. 1). The catechins are characterized by the presence of a di- or tri-hydroxyl group substitution on the "B"- ring and the meta-5.7-di-hydroxyl substitution at the "A"-ring (Fig. 1). EGCG appears to account for 50–80% of the total catechins found in green tea and is considered to be the most biologically active. A cup of green tea (2.5 g of dried green tea leaves brewed in 200 mL of water) usually contains about 90 mg of EGCG. In addition, green tea contains a similar or slightly smaller amount (65 mg) of EGC, about 20 mg each of ECG and EC, and about 50 mg of caffeine. However, physiologically achievable tissue levels of EGCG appear to be in the low micromolar range (i.e. 1–7 μM).

Epidemiologic and laboratory studies suggest that consumption of green tea might be associated with a decreased risk of developing skin, lung, bladder, esophageal, stomach, liver, duodenum and small intestine, pancreatic, colorectal, prostate, or breast ►cancer. Many chronic diseases, including cancer, are associated with ►oxidative DNA damage produced by free radicals. Much of the effectiveness of green tea has been attributed to its potent antioxidant activity, which is suggested to be greater than that of vitamin C or E or equivalent servings of most vegetables and fruits. Research data also suggest that some of the effects of green tea might be due to its ability to generate ►reactive oxygen species as a pro-oxidant molecule. However, direct unequivocal evidence for either mechanism of action *in vivo* is lacking. On the other hand, an accumulating number of research studies suggest that EGCG may specifically target and modulate distinct cancer genes or proteins, with little or no effect on normal molecules, in order to exert its anticancer effects.

Cellular and Molecular Targets of EGCG

Cells respond to their environment through a process known as signal transduction in which information from a stimulus outside a cell is transmitted from the cell membrane into the cell and along an intracellular chain of signaling molecules to perpetuate a response. The development of cancer is a complex, several-step process (i.e. initiation, promotion, progression) that affects innumerable genes and proteins, including signaling molecules that are critical in the regulation of many cellular functions but especially proliferation or growth. The initiation step is an irreversible period involving a



Epigallocatechin. Figure 1 Chemical structures of the major green tea catechins.

process in which a normal cell becomes changed so that it has the capacity to form a tumor (i.e. preneoplastic). The change results from DNA damage that can be induced by a number of "initiators," including radiation, ultraviolet irradiation, chemical carcinogens, or retroviruses. Under normal conditions, the DNA damage is either repaired or if the damage is too extreme, the cell is eliminated by a tightly controlled process of cell death called "►apoptosis." If the damaged DNA is allowed to duplicate, the cell may be predisposed to cancer. The promotion step is a potentially reversible process by which actively proliferating preneoplastic cells accumulate and begin to develop characteristics, including growth factor independence, lack of contact inhibition, and resistance to apoptosis, all of which enhance the cells' ability to proliferate by escaping normal control mechanisms. This step generally occurs over a period of many years and environmental tumor promoters or host factors may play roles in cancer promotion and latency. The final step of cell transformation is progression in which preneoplastic cells acquire increased metastatic potential and the ability to spread to other tissue sites of the afflicted organism.

Research findings have shown that the dysfunction or deregulation of various cellular signaling molecules is a major factor in cancer development and prevention. The prevailing idea today is that cancer may be prevented or treated by targeting and modulating the activity of specific cancer genes or signaling proteins. Each step of cancer development could be a potential target for anticancer agents, but especially the promotion step because of its length and reversible nature. In addition, an increased interest in discovering and developing natural, non-toxic compounds as chemopreventive agents now exists. The molecular mechanisms explaining how normal cells undergo transformation induced by tumor promoters are rapidly being clarified and the mechanisms by which natural compounds such as the green tea polyphenol EGCG, can act as chemopreventive agents are also being elucidated.

General Anticancer Effects of Green Tea Polyphenols

Green tea polyphenols have been reported to suppress cancer cell proliferation, enhance apoptosis, decrease ►angiogenesis and suppress oncoprotein activation. In particular, the mitogen-activated protein or ►MAP kinase signaling pathways are activated differentially by various tumor promoters. The MAP kinases generally transmit signals initiated by tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) and are also strongly stimulated by stresses such as ultraviolet (UV) irradiation or arsenic. The activation of these signaling cascades can result in a multitude of cellular responses including apoptosis, proliferation, inflammation, differentiation and development. MAP kinases activate a variety of target proteins that are important in tumor development, including activator protein-1 (►AP-1) and nuclear factor-kappaB (NF-κB), which in turn may promote transcription of a variety of cancer-related genes such as *cyclooxygenase-2* (*cox-2*).

A substantial body of evidence suggests that EGCG or other green tea polyphenols inhibit the phosphorylation and activation of the MAP kinases and various components of another critical cancer-associated pathway, the phosphatidylinositol-3 kinase (►PI3K)/►Akt pathway. Green tea polyphenols have also been reported to suppress tumor promoter- or growth factor-induced cell transformation and AP-1, NF-κB or COX-2 activation. EGCG has also been shown to inhibit the phosphorylation of the upstream epidermal growth factor family of proteins. Furthermore, consumption of green tea polyphenols has been associated with suppression of numerous markers of angiogenesis and ►metastasis, including expression of ►matrix metalloproteinases 2 and 9 and ►vascular endothelial growth factor. Other studies indicate that EGCG also inhibits ►telomerase activity to induce cell ►senescence and suppress DNA methyltransferase resulting in the re-activation of the ►tumor suppressor gene $p16^{INK4a}$.

Direct Molecular Targets of EGCG

Identifying EGCG receptors or high affinity proteins that bind to EGCG is a key step in understanding the molecular and biochemical mechanism of this polyphenol's anticancer effects. The structure of EGCG (Fig. 1) facilitates its ability to bind with varying affinity to a number of proteins. Proteins that have thus far been reported to directly bind with EGCG include ►fibronectin, laminin and the 67-kDa laminin receptor, ►insulin growth factor-1 receptor (IGF-1R), ►Bcl-2 and Bcl-x_L, vimentin, the glucose-regulated protein 78 (GRP78) chaperone protein, apoptosis-associated Fas, and fatty acid synthase. However, not all of these proteins (i.e. Fas, 400 μM; fatty acid synthase, 52 μM) show a high binding affinity and the consequences or details of the binding with EGCG are not fully understood.

EGCG was first reported to inhibit cancer cell ►adhesion to fibronectin by directly binding with this protein. However, the inhibition of cancer cell adhesion was not caused by EGCG binding to the cell-binding domain because EGCG interacted with the adjoining domain. EGCG was also reported to bind to another extracellular matrix protein, laminin, and resulted in an inhibition of ►melanoma cell adhesion. EGCG was later found to inhibit cell growth by binding with the invasion- and metastasis-associated 67-kDa laminin receptor (67LR), which is expressed on various cancer cell types. Importantly, the K_d value for binding was

39.9 nM suggesting possible *in vivo* relevance. The intermediate filament protein, vimentin, which has an important functional involvement in cell division and proliferation, was also identified as an EGCG-binding protein. Vimentin displayed an even higher affinity (K_i = 3.3 nM) for binding with EGCG and the association also appeared to have a regulatory role in controlling cell proliferation. Other work has suggested that EGCG (20 μg/mL) blocks anchorage-independent growth and human breast and cervical cancer cell phenotype expression through inhibition of IGF-1R downstream signaling. Thus EGCG appears to interact with surface proteins to suppress cancer cell proliferation.

EGCG may also interact with key proteins to modulate apoptosis. The pro-survival Bcl-2 proteins are over-expressed in many cancer types and thus contribute to resistance of cancer cells to apoptosis. EGCG was reported to directly bind to the BH3 pocket of Bcl-x_L (K_i = 490 nM) or Bcl-2 (K_i = 335 nM), resulting in the suppression of the anti-apoptotic activity of these proteins. EGCG was also shown to directly interact with GRP78, which is associated with the multidrug resistance phenotype of many types of cancer cells. EGCG suppressed GRP78's function and caused an increased etoposide-induced apoptosis in cancer cells. These results strongly suggest that EGCG can enhance apoptosis of cancer cells. Data suggest that EGCG has multiple targets and identification of novel EGCG-binding proteins could facilitate the design of new strategies to prevent cancer and hopefully help translate the effectiveness of EGCG observed in cell and animal models to humans.

Clinical Relevance

Tea polyphenols have attracted a great deal of interest because of their perceived ability to act as highly effective chemopreventive agents. EGCG has been reported to cause growth inhibition, G1-phase arrest, and apoptosis in a variety of human cancer cells. Notably, EGCG's effects appear to target only cancer cells with little or no effect on normal cells. This apparent specificity suggests that EGCG can be used in combination with traditional chemotherapeutic agents to enhance cancer cell death without harming normal cells. For example, treatment of lung cancer cells with EGCG plus ►celecoxib, a COX-2 inhibitor, has been shown to synergistically induce apoptosis. EGCG has also been shown to increase the toxicity of the chemotherapeutic drug, ►cisplatin, by several-fold in ovarian cancer cells and showed IC_{50} values for EGCG in the μM range even for ovarian cancers that are known to be resistant to cisplatin. Furthermore, the combination of EGCG with radiotherapy has been suggested to improve the efficacy of ionizing radiation in treating glioblastoma cells.

Although animal and cell culture data suggest a potent anticancer effect for EGCG, reports of anticancer activity of tea polyphenols in humans are less dramatic. Phase I and II clinical trials have been performed to test the anticancer effects of oral administration of green tea but results are still inconclusive. Laboratory data clearly indicate that EGCG and other green tea polyphenols are very unlikely to have only a single target or receptor to account for all its observed activities and effects. Furthermore, based on limited bioavailability, experimental concentrations of EGCG greater than 20 μM may not be relevant to the *in vivo* situation. Understanding the molecular mechanisms of tea in anti-tumor promotion may reveal additional high affinity molecular targets for the development of more effective agents with fewer side effects for the chemoprevention of cancer. A continuing emphasis on obtaining rigorous research data and critical analysis of those data regarding tea polyphenols and other food factors is vital to determine the molecular basis and long-term effectiveness and safety of these compounds as chemopreventive agents. Large-scale and comprehensive studies using combined approaches of biochemical, molecular, animal and clinical studies are needed to address the bioavailability, toxicity, molecular target, signal transduction pathways, and side effects of tea polyphenols for translation to humans.

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Epigastric

Definition

Pertaining to the upper and middle abdomen.

►Hepatic Epithelioid Hemangioendothelioma

Epigen

Definition

Encodes a protein of 152 amino acids that contains EGF-like features. Epigen has 24–37% identity with EGF, TGF , and ►Epiregulin. In epithelial cells, Epigen stimulates the phosphorylation of ErbB1 and causes activation of MAP kinase signaling pathways. Epigen also activates genes under the control of the SRE (Serum Response Element). Epigen is a mitogen for the human keratinocyte cell line HaCaT and this activity can be significantly reduced by a blocking antiBody to the receptor ErbB-1.

►Epidermal Growth Factor (EGF)-Like Ligands

Epigenetic

Definition

Heritable changes in genome function that occur without a change in DNA sequence. DNA-►methylation at ►CpG-islands and posttranslational histone modifications are the molecular basis for epigenetic information. Epigenetic changes of DNA influence the phenotype without altering the genotype. They consist of changes in the properties of a cell that can be transmitted through cells cycle divisions but do not represent a change in genetic information. The main epigenetic changes that target gene regulatory regions and modulate gene expression are methylation and acethylation.

►Histone Deacetylases
►Epigenetic Gene Silencing
►Epigenetics
►Epigenetic Therapy
►Nucleoporin

Epigenetic Asymmetry

Definition

Two genomes with identical DNA sequences, but with different forms of reversible but heritable modifications that regulate gene expression and repression.

►Epigenetic

Epigenetic Changes

Definition

Alterations in gene promoter such as hypermethylation that lead to a reduced expression of a specific protein.

►Epigenetic

Epigenetic Event

Definition

A chemical change that occurs to DNA, such as methylation of the cytosine residues of DNA, which does not change the sequence of DNA bases, but changes the transcription of genes in the DNA. In the case of methylation, methylation of the promoter of a specific gene causes that gene to become transcriptionally silent.

►Epigenetic

Epigenetic Gene Silencing

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Definition

Epigenetic: A heritable change in gene expression that is not accompanied by changes in DNA sequence. The two most studied ►epigenetic phenomena are ►DNA methylation and modifications of histone tails.

Characteristics

The term epigenetics was originally coined to describe the development of phenotype from genotype. The field of epigenetics now encompasses DNA methylation, covalent modifications of histones, nucleosome–DNA interactions, and most recently, small inhibitory RNA molecules. While it is well documented that genetic changes, such as mutations and deletions, play a functional role in silencing tumor suppressor genes in

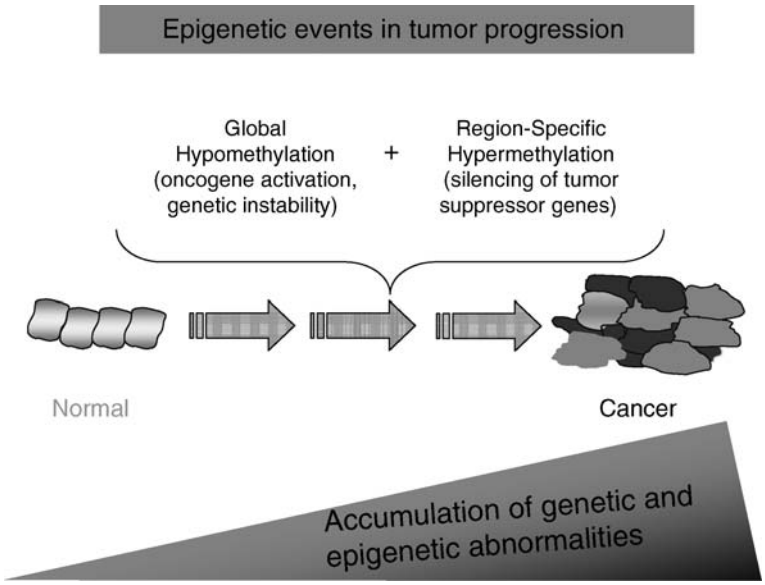
cancer cells, the functional importance of covalent epigenetic modifications, such as DNA methylation and histone post-translational modifications, is becoming increasingly recognized as an important early event during carcinogenesis and tumor development.

Epigenetics and Cancer

Epigenetic alterations are widely observed in cancer, and it has been shown that epigenetic mechanisms can be important to all phases of the cancer process, including tumor initiation, tumor progression and maintenance of the malignant state of cancer cells (Fig. 1). Tumors exhibit two characteristic changes in DNA methylation patterns. One is genome-wide hypomethylation, primarily in repeat elements and pericentromeric regions, which is responsible for genomic instability. The other is promoter hypermethylation of normally protected ►CpG islands near the transcription start site of genes, which is responsible for transcriptional inactivation. Another characteristic of tumors includes hypoacetylation of histones, and ►HATs have been reported as up- or down-regulated in a number of tumors. In this essay, both DNA methylation and histone modifications are discussed in the context of cancer.

DNA Methylation

The transfer of a methyl group to the carbon 5 position of a cytosine residue within the context of a CpG dinucleotide is the only known epigenetic modification of DNA itself. The 5-methylcytosine is the best-studied epigenetic modification and often referred to as the "fifth base" present in DNA. Although 5-methylcytosine comprises approximately 1% of the human genome, it is underrepresented in the bulk of the genome due to spontaneous deamination to thymine, which is not recognized by DNA mismatch repair systems. The CpG dinucleotides that are present are almost always methylated in normal cells, as 5-methylcytosine is widely believed to act to "silence" expression and/or retrotransposition of parasitic repeat sequences, such as Alu repeats and long-interspersed elements (LINEs) found throughout the genome. Aberrations in DNA methylation patterns are firmly associated with cancer, as evidenced by vast alterations in methylation patterns that occur during tumorigenesis, including global loss of methylation at CpG dinucleotides and region specific hypermethylation of distinct regions of 5-methylcytosine, located within specific CG-rich sequences known as CpG islands, often found within the promoter and associated with active genes. About 60% of human genes are associated with unique CpG islands, and recently it has been estimated that the human genome contains about 29,000 CpG islands. These normally unmethylated CpG islands may become methylated in cancer cells, and the event is associated with loss of expression of flanking genes. It has also been estimated that aberrant methylation can accumulate in as many as 10% of the CpG islands during tumor development. Thus, abnormal *de novo* methylation of CpG islands in human cancer cells represents one of the most prevalent molecular markers yet identified, and the list of methylated genes identified in various tumor types continues to grow. DNA is methylated by ►DNMTs, a family of proteins. ►DNMT1



Epigenetic Gene Silencing. Figure 1 Schematic representation of cancer progression. Characteristic epigenetic features of cancer are listed. During tumor progression, accumulation of both genetic and epigenetic abnormalities contributes to carcinogenesis.

is responsible for the maintenance of DNA methylation after each round of replication. De novo methylation of DNA is the responsibility of DNMT3a and DNMT3b.

Histone Modifications and Chromatin Remodeling

While DNA methylation is currently the best-studied epigenetic modification, other well-known chromatin alterations play critical roles in normal and aberrant physiological processes, including cancer. Histones are the major components of chromatin, the complex of DNA and protein found within the nucleus of eukaryotic cells. Early structural studies of chromatin revealed that 146 base pairs of DNA are "wrapped" around a core nucleosome, the fundamental unit of chromatin. A nucleosome consists of an octamer of histones, with 50 base pairs of "linker" DNA between repeating octamers. The N-terminal "tail" regions of histones extend from the nucleosome core octamer and are subject to various posttranslational modifications. Histone tails can be acetylated and deacetylated by HATs and ►HDACs, respectively. These enzymes are gene activators and repressors, and histone acetylation/deacetylation is important in transcriptional regulation. Additional histone modifications include methylation, by HMTs, phosphorylation, sumoylation, and ubiquitination. Overall, these and other modifications of histone tails play a critical role in chromatin compaction (e.g., tightly vs. loosely compacted chromatin), which can determine the access of various factors involved in transcription and gene expression and whether a gene is switched on (activated) or off (repressed). Furthermore, establishing transcriptional silencing of a gene involves a close interplay between histone modifications and DNA methylation, and loss and gain of DNA methylation, histone acetylation and methylation are observed in cancer cells. The sum total of these covalent alterations in the ►epigenome has been referred to as the "histone code", which can be "written" by the various modifying enzymes and "read" by various binding proteins that act to further modify chromatin and/or alter gene expression. According to the histone code hypothesis, the combination of DNA and histone modifications allows genes to go from the activate or inactive state interchangeably. The relatively new discipline of ►epigenomics promises to reveal novel insights into the histone code and a better understanding of normal development and human disease, including cancer.

Clinical Relevance

Unlike genetic changes, the epigenetic changes in cancer are potentially reversible. The ability to reactivate ►epigentically-silenced tumor suppressor genes and key control pathways and reverse the cancer cell phenotype is a promising strategy. Epigenetic drugs include both DNMT inhibitors ("demethylating" agents) and HDAC inhibitors (agents that cause "hyperacetylation" of histones). These agents can be used singly or in combination with currently available cancer chemotherapies. Epigenetic therapies themselves are now approved for various hematological malignancies and currently being studied in clinical trials for solid tumors. Furthermore, the combination of DNMT inhibitors with HDAC inhibitors has shown synergistic re-expression of epigenetically silenced genes and inhibition of tumor growth. Moreover, many investigators have shown that DNMT inhibitors with HDAC inhibitors can act to re-sensitize drug-resistant cancer cells to standard chemotherapeutic and hormonal agents. The ability to measure biochemical responses to epigenetic drugs, such as demethylation of previously hypermethylated genes and correlate this with clinical responses has also been shown, and epigenetic therapies for chemoprevention in individuals with aberrant epigenetic alterations but have not yet developed cancer is an exciting possibility.

Distinct CpG island methylation profiles for various cancers continue to emerge; consequently, aberrant DNA methylation and histone modification patterns are now being investigated as potential biomarkers and pathway-specific therapeutic targets. Thus, epigenetic profiling of tumors could provide new therapeutic targets and epigenetic biomarkers for prognosis, such as predicting therapy outcome in patients with cancer or, ideally, early cancer detection. Clearly, attractive and promising clinical possibilities exist for epigenetic-based therapies

In summary, perhaps all human cancers are at least partially associated with epigenetic dysregulation of gene expression, forming a rational basis for future treatment strategies designed to alter this fundamental processes in cancer. Comprehensive elucidation of epigenetic modifications, in both normal and diseased tissues, will allow for an extensive understanding of gene regulatory networks that control both normal and cancer phenotypes. The realization of the Human ►Epigenome Project (HEP), proposed as an exhaustive annotation of all histone and deoxcytosine modifications throughout the human genome, should have a major impact on cancer.

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Epigenetic Mechanism

Definition

A mechanism that modifies gene expression without directly affecting DNA sequence.

►Epigenetic

Epigenetic Modifications

Definition

Reversible, heritable changes in gene regulation that occur without a change in DNA sequence.

►Epigenetic

Epigenetic Reprogramming

Definition

Epigenetic reprogramming can be defined as modifications of the DNA that do not alter DNA sequence (e.g. DNA methylation and changes of the chromatin).

►CCCTC-Binding Factor (CTCF)
►BORIS

Epigenetic Therapy

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Definition

Epigenetic therapy refers to therapy using inhibitors of DNA ►methylation and histone deacetylation, which reverse these epigenetic modifications. These compounds inhibit tumor growth in vitro and in vivo, which is thought to be due to reactivation of epigenetically silenced ►tumor suppressor genes by inhibition of promoter DNA methylation and histone deacetylation of these genes.

Characteristics

DNA Methylation and Histone Deacetylation

►Epigenetic modifications regulate heritable changes in gene expression without changing the primary DNA sequence. The best-studied epigenetic mechanisms are DNA methylation and posttranslational histone modifications. DNA methylation is the covalent addition of a methyl group to the DNA, predominantly to the base cytosine 5′ to guanine, also called a CpG dinucleotide. CpG dinucleotides are clustered in small stretches of DNA called ►CpG islands, often located in or near the promoter region of approximately half of all human genes. Methylation of CpG dinucleotides, which occurs nonrandomly, is an important ►epigenetic gene silencing mechanism. Most methylation in the human genome occurs in the noncoding DNA, preventing the transcription of repeat elements, inserted viral sequences, and transposons. In contrast, CpG islands are largely unmethylated in both expressing and nonexpressing tissues under normal conditions. Exceptions to this unmethylated state of CpG islands involve the silenced gene alleles for imprinted genes and genes located on the inactive X chromosome of females. DNA methylation is catalyzed by DNA methyltransferases (DNMTs). DNA methylation can induce gene silencing through several mechanisms. By sterically hindering the binding of activating transcription factors to gene promoters, DNA methylation can directly repress gene transcription. Another mechanism is through recruitment of several methyl-binding domain proteins (MBDs) that recognize methylated DNA, including MeCP2, MBD1–4, and Kaiso. These proteins themselves can repress gene transcription, or bind proteins which cause gene silencing. The DNA helix is wrapped around a core of ►histone proteins. The basic amino-terminal tails of histones are subject to various posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, glycosylation, biotinylation, and carbonylation. The best-characterized histone modification is histone acetylation, which is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetylation generally correlates to active gene transcription, whereas ►histone deacetylation is associated with transcriptional repression, by blocking accessibility of transcription factors to their binding sites.

DNA methylation and histone deacetylation are interconnected in gene silencing. Methyl-binding domain proteins are components of HDAC complexes or recruit these complexes to methylated DNA,

resulting in transcriptional silencing. Furthermore, a much more direct connection between DNA methylation and histone deacetylation exists by direct interactions between DNMTs and HDACs. DNA methylation and histone deacetylation are pivotal in X chromosome inactivation, ►imprinting, and establishment of tissue-specific gene expression. However, aberrant epigenetic regulation of gene expression also plays a major role in the development of human cancer.

Epigenetic Abnormalities in Cancer

Aberrant epigenetic silencing of tumor suppressor genes by promoter DNA hypermethylation and histone deacetylation plays an important role in the pathogenesis of cancer. According to ►Knudson's two-hit model, complete loss of function of a tumor suppressor gene requires loss of function of both gene copies. Epigenetic silencing of the wild-type allele of a tumor suppressor gene by aberrant promoter hypermethylation and histone deacetylation can be considered as the second hit in this model, resulting in complete loss of function of the gene. It has become apparent that many genes, located across all chromosome locations, are epigenetically silenced in cancer cells. Examples are genes involved in cell cycle regulation and apoptosis (*p14ARF*, *p15INK4b*, *p16INK4a*, *APC*, *RASSF1A*, *HIC1*), DNA repair genes (*hMLH1*, *GSTP1*, *MGMT*, *BRCA1*), and genes related to ►metastasis and invasion (*CDH1*, *TIMP-3*, *DAPK*, *p73*, *maspin*, *TSP1*, *VHL*).

DNA Methyltransferase and Histone Deacetylase Inhibitors

In contrast to genetic alterations, which are almost impossible to reverse, ►chromatin remodeling in cancers is potentially reversible. This resulted in the development of pharmacologic inhibitors of DNA methylation and histone deacetylation. By inducing DNA demethylation and histone acetylation, DNMT- and HDAC inhibitors can reverse epigenetic silencing of tumor suppressor genes, resulting in reactivation of these genes in tumor cells and restoring of crucial cellular pathways. The most extensively studied DNMT inhibitors are 5-azacytidine and ►5-aza- 2′-deoxycytidine [5-aza-2′deoxycytidine and Cancer], which were initially developed as chemotherapeutic agents. These ►nucleoside analogs are incorporated into DNA in place of the natural base cytosine during DNA replication, and are therefore only active during S phase. Once incorporated into the DNA, a complex is formed with active sites of DNMTs, thereby covalently trapping these enzymes. This results in the depletion of active enzymes and the demethylation of DNA after several cell divisions. 5-Aza- 2′-deoxycytidine is the most commonly used DNMT inhibitor in assays with cultured cells. This compound reactivates dormant tumor suppressor genes by demethylation of their hypermethylated promoter, thereby restoring their normal function. This seems to be a widespread effect of 5-aza- 2′-deoxycytidine, because all cancer cell lines studied so far are sensitive to the DNA demethylating effects of this agent. Reactivation of silenced tumor suppressor genes might be the mechanism by which this compound suppresses growth and induces differentiation of human tumor cell lines. Examples of other DNMT inhibitors are the cytidine analogs 5,6-dihydro-5-azacytidine, 5-fluoro-2′-deoxycytidine and zebularine, the small molecule RG108, which blocks the DNMT active site, and MG98, an antisense oligonucleotide that specifically inhibits DNMT1 mRNA.

By inhibiting histone deacetylation, HDAC inhibitors cause accumulation of acetylated histones, leading to increased transcription of previously silenced tumor suppressor genes in malignant cells. Both naturally existing and synthetic HDAC inhibitors have been characterized. The effects of HDAC inhibitors on gene expression in transformed cells are selective; only about 2–10% of all known genes are affected by these agents. One gene most consistently induced by HDAC inhibition is *CDKN1A*, which encodes the cell cycle inhibitor ►p21. HDAC inhibitors can also relieve inappropriate transcriptional repression mediated by ►chimeric oncoproteins, such as ►PML-RARα, thereby inducing differentiation in cells harboring these translocations. HDAC inhibitors have many antitumor effects including induction of cell cycle arrest, differentiation, and/or apoptosis in virtually all cultured transformed cell types and in cells from different tumors.

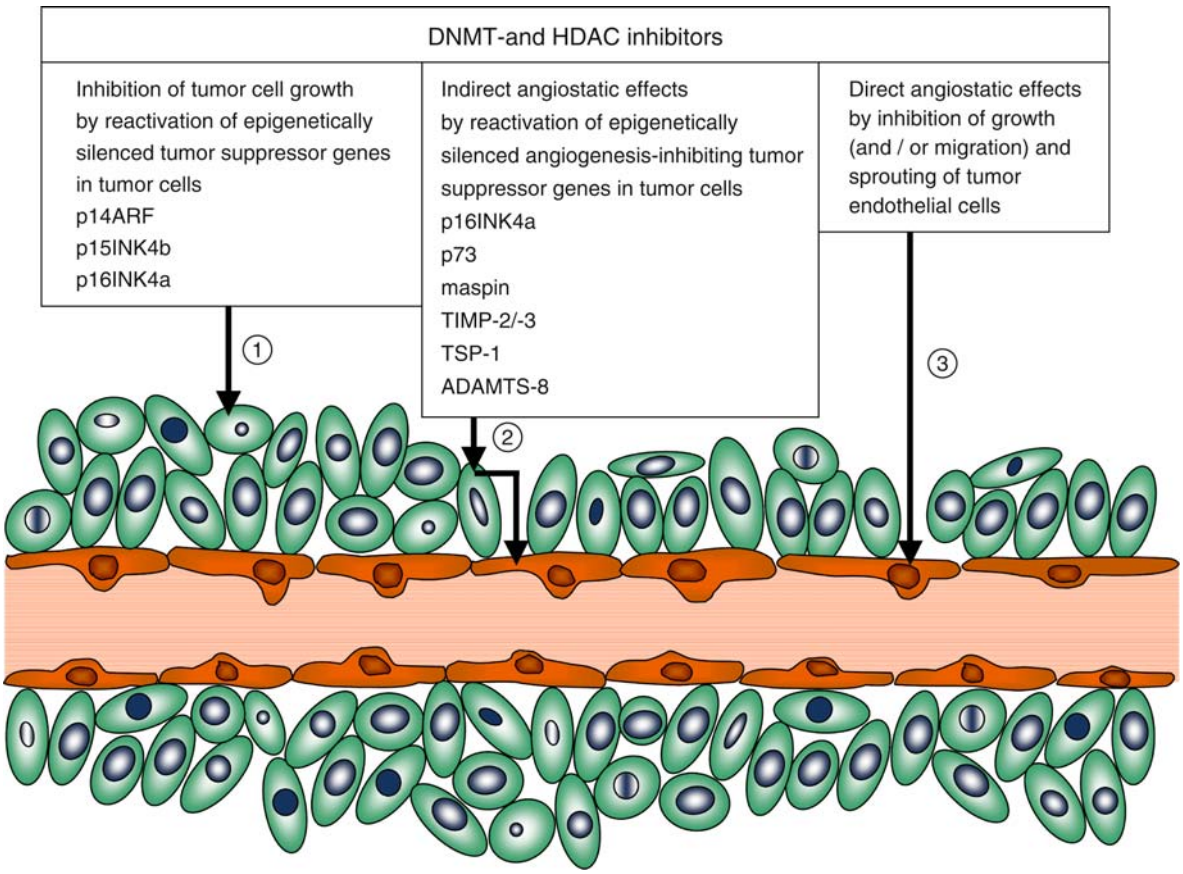
Epigenetic Therapy in Cancer

It is clear from in vitro and preclinical studies that the clinical application of reversing epigenetic aberrations in tumor cells, called epigenetic therapy, is an exciting strategy for cancer treatment. Many agents have been discovered that inhibit DNA methylation or histone deacetylation, and the value of these compounds will be established by ongoing ►clinical trials.

5-Azacytidine (Vidaza) and 5-aza- 2′-deoxycytidine (Decitabine) represent the two most prominent DNMT inhibitors that are being used in clinical practice, and these drugs have been approved by the ►FDA for the treatment of myelodysplastic syndrome (MDS). The use of DNMT inhibitors in the treatment of MDS results from the knowledge that epigenetic gene silencing of, in particular, *p15INK4b* is present in poor-risk MDS subtypes and often predicts transformation to acute myeloid leukemia (AML). Multiple HDAC inhibitors are currently being tested in patients through intravenous or oral administration. Suberoylanilide hydroxamic acid (SAHA) is one of the HDAC inhibitors most advanced in development. Encouraging results were obtained in Phase I, II, and III clinical trials for patients

with both hematologic and solid tumors. Other examples of HDAC inhibitors undergoing clinical testing in a range of solid and hematological malignancies are ►valproic acid, PXD101, NVP-LAQ824, LBH589, depsipeptide, MS-275, and CI-994. Complete targeting of epigenetic gene regulation might require a combination of ►chromatin modifying agents. The synergy between demethylating drugs and HDAC inhibitors in reactivation of epigenetically silenced tumor suppressor genes in vitro makes combined treatment with DNMT and HDAC inhibitors a promising epigenetic therapy. Reduction of individual doses should minimize toxic effects and optimize the therapeutic response of such combination. Encouraging anticancer activity of epigenetic therapy has been shown particularly in the treatment of hematologic disorders, but their effectiveness in solid tumors largely remains to be determined.

In addition to the inhibitory effects of DNMT and HDAC inhibitors on tumor cells, by reactivation of epigenetically silenced tumor suppressor genes, these compounds have also been described to inhibit ►tumor endothelial cell growth and ►tumor angiogenesis. ►Angiogenesis is required for tumor growth to a size of approximately 2 mm^3, but is also instrumental for tumor cells to metastasize to other locations in the body. Angiogenesis is considered to be a promising target of anticancer treatment. By influencing the gene expression profile of tumor cells, DNMT and HDAC inhibitors target genes, which are regulating angiogenesis. Among the epigenetically silenced tumor suppressor genes in tumor cells are genes with angiogenesis inhibiting properties. By re-expression of these genes in tumor cells, DNMT and HDAC inhibitors might indirectly – via the tumor cells – exhibit angiostatic effects in vivo. An example of these genes is *p16INK4a*, which can regulate angiogenesis by modulating ►vascular endothelial growth factor (VEGF) expression by the tumor. Another epigenetically silenced tumor



Epigenetic Therapy. Figure 1 Anti-tumor effects of DNA methyltransferase and histone deacetylase inhibitors in vivo. 1. DNMT- and HDAC inhibitors decrease tumor cell growth by reactivation of epigenetically silenced tumor suppressor genes in tumor cells. 2. Release of transcriptional repression of angiogenesis inhibiting tumor suppressor genes in tumor cells might result in indirect angiostatic effects of DNMT- and HDAC inhibitors. 3. DNMT- and HDAC inhibitors directly decrease endothelial cell growth and angiogenesis, thereby exhibiting direct angiostatic effects.

suppressor gene that inhibits angiogenesis by down-regulation of VEGF is *p73*. The tumor suppressor ►maspin, which is often silenced in tumors by epigenetic promoter modifications, is also an effective inhibitor of angiogenesis. Methylation-associated inactivation of the angiogenesis inhibiting factors tissue inhibitor of metalloproteinase-2- and -3 (*TIMP-2/3*) is frequent in many human tumors. Thrombospondin-1 (*TSP-1*) has been described to be repressed by epigenetic promoter modifications in several adult cancers, and can be reactivated by 5-aza- 2′-deoxycytidine. The secreted protease ADAMTS-8 (METH-2) has anti-angiogenic properties, which can specifically suppress endothelial cell proliferation, and significant down-regulation of *ADAMTS-8* by promoter hypermethylation, which has been described in different tumor types. Besides the indirect effects of DNMT and HDAC inhibitors on tumor angiogenesis, these compounds also directly inhibit endothelial cell growth and angiogenesis in vitro and in vivo. Potent anti-angiogenic activity has been described for several HDAC inhibitors, such as trichostatin A (TSA), SAHA, depsipeptide, valproic acid, butyrate, apicidin, LBH589, and NVP-LAQ824, as well as for the DNMT inhibitors 5-aza- 2′-deoxycytidine and zebularine. These drugs suppress spontaneous or VEGF-induced angiogenesis in different in vitro, ex vivo, and in vivo angiogenesis assays.

Clearly, the dual targeting of epigenetic therapy in cancer treatment, inhibiting both tumor cells as well as tumor angiogenesis, makes them suitable combinatorial anticancer therapeutics (Fig. 1). By targeting multiple genes and pathways in tumor cells, as well as endothelial cell biology and angiogenesis, DNMT and HDAC inhibitors decrease the development of resistance that is associated with many of the current chemotherapeutic- and ►anti-angiogenic drugs. Despite the promising data from clinical trials, there are several pitfalls regarding the clinical application of epigenetic therapy. An important side effect that should be taken into account in the use of these drugs is induction of global hypomethylation, which might induce tumorigenesis by aberrant activation of repetitive DNA sequences, transposons and ►oncogenes, induction of chromosomal instability and mutagenesis. Furthermore, the existence of many different DNMTs and HDACs makes the development of selective inhibitors that target individual enzymes imperative.

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Epigenome

Definition

The comprehensive collection of genome-wide epigenetic phenomena, DNA-methylation patterns and chromatin modifications

►Epigenetic Gene Silencing and Cancer
►Epigenetic

Epigenomics

Definition

A new field based on the comprehensive analyses of the entire epigenome using high-throughput technologies.

►Epigenetic Gene Silencing and Cancer
►Epigenetic

Epilepsy

Definition

A disease of the brain produced by the uniform firing of groups of neurons such that the normal generation and processing of directed action potentials in the affected area is disrupted and the function of the brain altered by this firing is interrupted, if the affected area controls muscle function, the uniform firing may result in repetitive movements.

►Oligodendroglioma

Epiphysis

Definition

The end of a bone, that lies between the joint surface on one side and the epiphyseal plate (growth plate) on the other.

►Cryosurgery in Bone Tumors

Epipodophyllotoxins

Definition

Substance class of anticancer drugs that are isolated or derivatives of natural products from *Podophyllum peltatum*, e.g., ►etoposide.

►ABC-Transporters

Epiregulin

Definition

EPR, was initially purified from conditioned medium of a mouse fibroblast-derived tumor cell line. EPR is expressed in adult pancreas, liver, kidney and small intestine. Human epiregulin encodes a 46 aa growth factor which exhibits 24–50% identity with the sequences of other EGF-like ligands. Epiregulin exhibits a bifunctional regulatory property in that it inhibits the growth of several epithelial cell lines and stimulates the growth of fibroblasts, primary rat hepatocytes, human smooth muscle cells and various other cell types, including COS-7 (monkey) and Swiss 3T3 (mouse). Epiregulin can bind to and activate ►EGFR and ErbB4. EPR can also transactivate ErBB2 and ErbB3. Although EPR displays lower affinity towards EGFR, EPR-mediated receptor activation is more sustained when compared with EGF or BTC. The lower affinity of EPR to EGFR results in enhanced dissociation of EPR from the ligand-receptor complex during receptor endocytosis and renewed EGFR activation by recycled EPR. EPR-deficient mice develop chronic dermatitis.

►Epidermal Growth Factor (EGF)-Like Ligands

Epirubicin

Definition

Is 4′-epi-isomer of the anthracycline antineoplastic antibiotic adriamycin.

►Adriamycin

Epistaxis

Definition

Is profuse bleeding from the nose.

►Nasopharyngeal Carcinoma

Epithelial Cadherin

►E-Cadherin

Epithelial Cell

Epithelial cells line and protect both the outside and the inside cavities and lumen of the body. They regulate selective permeability and transcellular transport between the compartments they separate and are involved in secretion, absorption and sensation detection.

►E-Cadherin

Epithelial Cell Adhesion Molecule

►EpCAM

Epithelial Growth Factors

Definition

Growth factors are proteins that bind to cell membrane receptors, including tyrosine kinase receptors, and activate cellular proliferation and/or differentiation. Growth factors can act in an ►autocrine, paracrine, or endocrine manner. Many growth factors can stimulate cellular division in numerous cell types, while others are specific to a particular cell type. Examples of growth factors include: fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor I and II (IGF-I, IGF-II), and ►platelet-derived growth factor (PDGF). Growth factor expression and subsequently expression of the corresponding cell receptors are altered during ►progression of cancers.

►Aging and Cancer
►Fibroblast Growth Factors
►Insulin-like Growth Factors

Epithelial Tumorigenesis

Definition

Epithelial tumorigenesis relates to the process of developing and/or progressing of a tumor, originating from ►epithelial cells. Malignant tumors of this origin are known as ►carcinoma, whereas malignant tumors derived from cells of the connective tissue are known as ►sarcoma.

►Stromagenesis

Epithelial-to-Mesenchymal Transition

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Definition

Phenotypic alterations in which ►epithelial cells adopt characteristics of mesenchymal cells. Epithelial-to-mesenchymal transition (►EMT) is a physiological process during normal development and a pathological process during cancer progression and fibrosis.

Characteristics

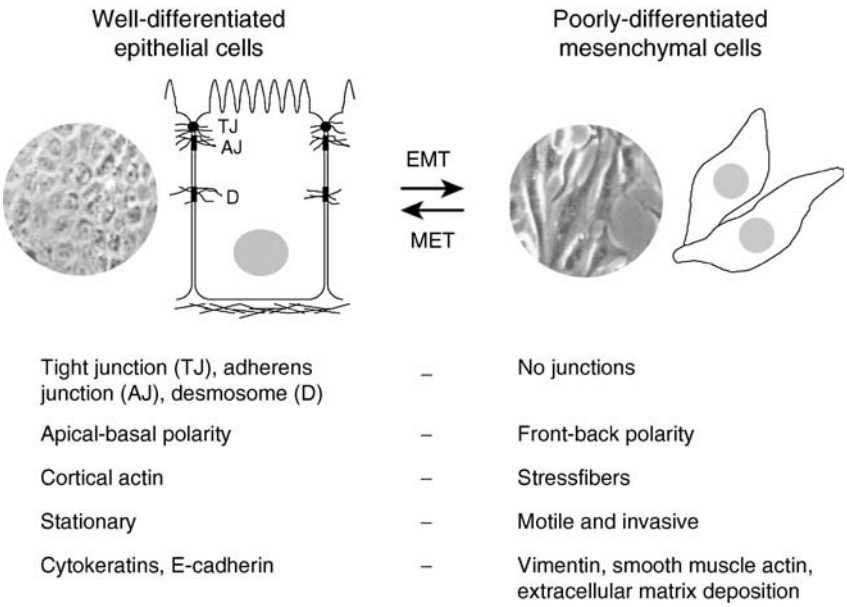
Epithelial cells line the external surfaces and internal cavities of the body. A distinguishing characteristic of epithelial cells is the presence of junctional complexes, such as ►tight junctions, ►adherens junctions, and desmosomes and segregation of the plasma membrane into apical and basolateral domains. These features promote adhesion, restrict motility, facilitate intercellular communication, and permit individual cells to function as a cohesive unit. The phenotype of epithelial cells cultured in vitro or in tissues is often described as well-differentiated (Fig. 1, left panel).

Mesenchymal cells are spindle-shaped with fibroblast-like morphology, lack adhesiveness, and are highly motile. They do not have junctional complexes and specialization of the plasma membrane into apical and basolateral domains. When cells of epithelial origin show a mesenchymal phenotype under in vitro culture conditions or in tissues they are often described as poorly differentiated (Fig. 1, right panel).

The plasticity of epithelial cells enables them to convert between the epithelial and mesenchymal phenotypes. These phenotypic transformations are highly regulated by specific signaling events and molecules. Conversion of the epithelial cell to a mesenchymal phenotype is known as EMT and vice versa as mesenchymal-to-epithelial transition (►MET). EMT and MET occur during normal development as well as in cancer progression.

EMT provides a mechanism for epithelial cells to overcome the physical constraints imposed upon them by intercellular junctions and to adopt a motile phenotype. The process was originally identified during specific stages of embryogenesis in which epithelial cells migrate and colonize various embryonic territories to form different organs. EMT is critical for the formation of ectoderm, mesoderm, and endoderm during gastrulation as well as for the differentiation of neural crest cells into neurons and glia of the peripheral nervous system. During embryogenesis EMT is spatially and temporally regulated in a subtle manner that is essential for normal organ development.

EMT during cancer progression occurs in an aggressive and uncontrolled fashion and might facilitate the invasive and metastatic potentials of cancer cells. The phenotypic conversion of epithelial cells to mesenchymal cells involves a series of events that includes dissolution of tight junctions, adherens junctions, and desmosomes, the suppression of molecules involved in restricting invasiveness and motility, and induction of factors that promote invasiveness and motility. A characteristic feature of cells undergoing EMT in culture is the change in the organization of the ►actin



Epithelial-to-Mesenchymal Transition. Figure 1 Transitions between well-differentiated epithelial cells and poorly differentiated mesenchymal cells during EMT. *Left panel*, phase contrast microscopy and schematic diagram of well-differentiated polarized epithelial cells with characteristic apical-basolateral polarity and junctional complexes. *Right panel*, cells that have undergone EMT display mesenchymal morphology with no junctions and are highly motile and invasive. The process of reversion of mesenchymal cells to an epithelial phenotype is called mesenchymal to epithelial transition (MET).

cytoskeleton. In most cases stress fibers are induced with a concomitant loss of the cortical actin ring. Although it is established that such morphological changes accompany EMT, the chronology of these events is still not deciphered. It is also not known whether all these changes are essential for induction of EMT and the metastatic potential of cancer cells.

Mechanisms

EMT is in part achieved by downregulation of epithelial specific molecules and induction of proteins expressed in mesenchymal cells. One of the epithelial cell molecules extensively studied that change during EMT is the cell–cell adhesion molecule ►E-cadherin. During epithelial morphogenesis, E-cadherin regulates the establishment of adherens junctions, which form a continuous adhesive belt below the apical surface. The extracellular domain of E-cadherin mediates calcium-dependent homotypic interactions with E-cadherin molecules on adjacent cells and the intracellular domain binds cytosolic catenins and links the E-cadherin complex to the actin cytoskeleton. A stable E-cadherin complex at the plasma membrane is essential for the cell–cell adhesion function of this protein.

Several studies have shown that expression of E-cadherin is reduced during EMT, associated with the loss of junctional complexes and the induction of a mesenchymal phenotype of carcinoma cells. It is believed that the decrease in adhesive force following reduced expression of E-cadherin facilitates invasion and dispersion of carcinoma cells from the primary tumor mass. Methods to abolish E-cadherin function promote epithelial cell invasion into a variety of substrates, as determined by a number of in vitro and in vivo experimental systems. Loss or reduced expression of E-cadherin is also accompanied by expression of mesenchymal markers such as vimentin, smooth muscle actin (►SMA), γ-actin, β-filamin, and talin and extracellular matrix (►ECM) components such as fibronectin and collagen precursors. Upregulation of these proteins facilitates cytoskeletal remodeling and promotes cell motility (Table 1).

The diverse molecular mechanisms mediated by growth factors and ECM proteins contribute to EMT. Growth factors such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), or insulin-like growth factor II (IGF-II) promote signaling cascades through their cognate receptor tyrosine kinases, which in turn signal through various downstream effector molecules such as Ras, Src, phosphatidylinositol-3-kinase (PI3K) and MAPK leading to EMT. In addition, signaling pathways essential for stem cell function during development such as the ►Wnt, ►Notch, and ►Hedgehog signaling pathways are activated during EMT. The role of Wnt signaling has been well established during normal development as well as in EMT. Binding of the soluble ligand Wnt to its receptor frizzled inhibits β-catenin degradation and facilitates its nuclear

Epithelial-to-Mesenchymal Transition. Table 1 Markers of EMT

Increased abundance/activity	
Epithelial/mesenchymal markers	N-cadherin
	Vimentin
	Fibronectin
	Smooth muscle actin
	γ-Actin
	β-Filamin
	Talin
	Collagen precursors
	MMPs
	Stress fibers
Signal transduction molecules/pathways	Epidermal growth factor (EGF)
	Hepatocyte growth factor (HGF)
	Insulin growth factor (IGF-I)
	Transforming growth factor (TGF-β)
	Ras
	Src
	PI3K
	Wnt
	Notch
	Hedgehog
	GSK-3β
	MAPK
	TNFα
	NFκB
	Smurf-1
Transcriptional regulators	Snail
	Slug
	ZEB-1
	ZEB-2
	TCF/LEF
	Smads
	Twist
	E12/E47
Decreased abundance/activity	
Epithelial/mesenchymal markers	E-cadherin
	Cytokeratin
	Claudin
	Occludin
	Desmoplakin
	Desmoglein

translocation together with the TCF/LEF transcription factors to activate transcription of target genes such as cyclin D1 and Myc. The transcriptional activity of β-catenin is increased in a wide variety of cancers as well as in growth factor-induced EMT of cultured cells.

A key molecule involved in the induction of EMT and extensively studied is the ►transforming growth factor-β ►(TGF-β). The ►TGF-β growth factor superfamily comprises TGF-βs, bone morphogenetic proteins (BMPs), activins, and other related proteins. TGF-β induces EMT in epithelia either through transcriptional or transcription-independent mechanisms. Cooperation between TGF-β and Ras/Raf/MEK/MAPK signaling is involved in the induction and maintenance of EMT. TGF-β has been shown to stimulate ERK1/2 activity in cell culture models of EMT that is required for the disassembly of junctional complexes and the induction of motility. TGF-β also activates PI3K in a RhoA-dependent manner, which has been implicated in the disassembly of tight junctions. In keratinocytes and several epithelial cell types, TGF-β treatment activates the Notch pathway by inducing the Notch ligand *jagged1* and the Notch target genes TLE3, HEY1, HEY2, HES1 at the onset of EMT. TGF-β, in cooperation with oncogenic Ras, induces EMT by the activation of the transcription factor ►nuclear factor κB (NF-κB). Constitutive activation of NF-κB induces EMT and metastasis, whereas inhibition of NF-κB by inhibitory IκBa suppresses EMT and metastasis in a breast tumor model. Although in different cell types TGF-β induces various signaling pathways, these signals subsequently target E-cadherin expression and the disassembly of epithelial junctional complexes to induce EMT. For example, the TGF-β type I receptor is localized to tight junctions through the tight junction protein occludin allowing for efficient ►TGF-β-dependent dissolution of tight junctions during EMT. The epithelial polarity protein Par-6 interacts with the TGF-β type I receptor and TGF-β binding initiates Par-6 phosphorylation and activation of the E3-ubiquitin ligase, Smurf-1. Activated Smurf-1 promotes degradation of local RhoA resulting in tight junction dissociation, inhibition of cell adhesion and transition to a mesenchymal phenotype.

The signaling cascades described earlier induce two major types of transcriptional regulators that mediate EMT, zinc finger (►Snail, Slug, ZEB-1, ZEB-2), and basic helix-loop-helix (Twist, E12/E47) proteins. The transcription suppressors SNAI1 (Snail) and SNAI2 (Slug) play a central role in the induction of EMT. These zinc finger proteins recognize E-box elements in the cognate target promoters and SNAI1 represses the transcription of the E-cadherin gene during EMT as well as embryonic development. Factors that regulate SNAI1 by phosphorylation, subcellular localization, and transcription have been well-described in development and EMT. While phosophorylation of SNAI1 in the two GSK3β phosphorylation consensus motifs targets it for export from the nucleus (motif 2) and ubiquitinylation and degradation (motif 1), phosphorylation of SNAI1 at Ser^{246} by p21-activated kinase (PAK1) results in its accumulation in the nucleus and induction of EMT.

LIV-1, an estrogen-regulated member of the LZT subfamily of zinc transporters is activated by STAT3, which is essential for nuclear localization of SNAI1 and suppression of E-cadherin expression during gastrulation in zebrafish embryos. Further, SNAI1 expression is transcriptionally suppressed by metastasis-associated gene 3 (MTA3), a subunit of the Mi-2/NuRD transcriptional corepressor, thereby establishing a mechanistic link between estrogen receptor status and invasive growth of breast cancers. While there is great deal of knowledge about SNAI1 regulation, much less is known about SNAI2. It has been shown that SNAI2 suppresses E-cadherin expression when ectopically expressed in well-differentiated epithelial cells. HGF and FGF induce SNAI2 to suppress desmoplakin and desmoglein thereby destabilizing desmosomes. The SMAD-interacting repressors SIP-1/ZEB2 and δEF1/ZEB1 that can be induced by TGF-β bind to the E-cadherin promoter to suppress its transcription. The basic helix-loop-helix transcription factors involved in the induction of EMT are E12/E47 (E2A gene product) and Twist, both of which have been shown to repress E-cadherin expression and induce EMT. The mechanisms by which these factors suppress E-cadherin expression are not well-established.

Clinical Relevance

Although EMT represents a fundamentally important process for tumor dissemination and is widely believed to be an essential event involved in cancer metastasis, there are several lines of evidence to suggest that many invasive and metastatic carcinomas have not undergone a complete transition to a mesenchymal phenotype. Many advanced carcinomas of prostate, breast, squamous cell carcinomas derived from a variety of origins, including the esophagus, oral epithelium, lung, cervix, and salivary neoplasms possess molecular and morphological characteristics of well-differentiated epithelial cells, with the presence of epithelial junctions and apical-basolateral plasma membrane asymmetry. High E-cadherin expression was also observed in a wide variety of carcinomas and E-cadherin levels did not correlate with invasiveness and metastasis. These results are consistent with the idea that complete EMT might not be necessary for cancer cell metastasis or that cancer cells redifferentiate to an epithelial phenotype following metastasis.

While EMT is well established in cultured cells there is no evidence for EMT in vivo and if EMT occurs it is not known at what stage of tumor progression. There are several possibilities by which cancer cells could spread without undergoing complete EMT: (i) Incomplete EMT by which epithelial cells partially convert to a mesenchymal phenotype acquiring invasive and metastatic potential. (ii) Cohort migration in which well-differentiated epithelial cells migrate as a cluster and cause metastasis. (iii) Reversion of poorly differentiated cells to a well-differentiated phenotype by MET at the site of metastasis. These diverse mechanisms might be regulated by the tumor microenvironment and/or signaling pathways distinct from the molecular machinery of EMT. Thus, there are several mechanisms by which cancer cells could metastasize and EMT may represent one of the global changes associated with malignant transformation of epithelial cells.

Recognizing EMT as a fundamentally important process for tumor dissemination together with the increasing knowledge about the molecular pathways leading to EMT may offer new targets for therapeutic intervention. Inhibitors of the TGF-β, ERK1/2 and PI3K/Akt pathways have shown encouraging results in the suppression of tumor progression. Further understanding of the molecular requirements of EMT will allow for more effective approaches for future therapeutic intervention.

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Epithelioid

Definition

Like ►epithelial cells which form the skin and line inside the lumens of the human body.

►Hepatic Epithelioid Hemangioendothelioma

Epithelioid Hemangioendothelioma (EH)

Definition

Is a neoplasm of vascular origin involving soft tissue and visceral organs such as liver, spleen, bone, brain,

meninges, breast, heart, head and neck, soft tissue, stomach, and lymph nodes.

►Hepatic Epithelioid Hemangioendothelioma

Epithelioid Sarcoma

Definition

Sarcoma of unknown origin with nodular growth pattern and predominantly epithelioid morphology that arises more frequently in distal extremities. The cells are positive for epithelial markers.

►Uncertain or Unknown Histogenesis Tumors

Epitope

Definition

Is a site on an antigen recognized by an antibody or an antigen receptor; epitopes are also called antigenic determinants. A T-cell epitope is a short peptide derived from a protein antigen. It binds to an ►MHC molecule and is recognized by a particular T cell. B-cell epitopes are antigenic determinants recognized by B cells and are typically discontinuous in the primary structure.

Epitope Spreading

Definition

Describes the fact that responses to ►autoantigens tend to become more diverse as the response persists. This is also called determinant spreading or antigen spreading.

Epo

►Erythropoietin

Epoetin

►Erythropoietin

Epothilone B Analogue

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Synonyms

Aza-epothilone B; BMS-247550; Ixabepilone; NSC 710428

Definition

The epothilone B analogue, ixabepilone, is the term used to denote one specific agent of a new class of anticancer drugs, the epothilones. The epothilones A and B are derived from fermentation of the myxobacteria *Sorangium cellulosum*. They have been found to have potent cytotoxic activity, which, like that of the taxanes, has been linked to stabilization of cellular microtubules resulting in the blocking of cell division at the ►G2/M transition portion of the cell cycle. The epothilone B analogue ixabepilone is a semi-synthetic analog of the natural product epothilone B, made by replacing the lactone oxygen of epothilone B with a lactam (azo-epothilone B) designed to overcome the metabolic instability of the natural product.

Chemical Name: [1S-[1R*,3R*(E),7R*,10S*,11R*,16S*]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-17-oxa-4-azabicyclo[14.1.0] heptadecane-5,9-dione.

Molecular Formula: $C_{27}H_{42}N_2O_5S$ M.W.: 506.7 g/mole

Characteristics

Introduction

The epothilones A and B, are a new class of anticancer agents isolated in the mid 1990's. They are in the macrolide class of drugs but have a mechanism of action similar to the taxanes. ►Paclitaxel, the first taxane to be widely used, was found in 1971 in a screening assay for antitumor agents. It is a complex diterpene compound. Its cytotoxic activity derives from its binding to cellular microtubules stabilizing them and preventing the dynamic growth and shrinkage that

occurs during normal cellular processes. In dividing cells, this leads to mitotic arrest and cell death by causing a block at the transition between G_2 and M phase of the cell cycle. Paclitaxel has been found to have important clinical activity against breast, ovarian, lung, and head and neck cancers. However some tumors such as colorectal cancer have innate resistance to this agent. Other tumors develop resistance to paclitaxel through the multidrug resistance mechanism in which ►P-glycoprotein removes cytotoxic agents from the tumor cell, or by genetic mutations leading to altered tubulin protein. Side effects such as ►neutropenia and ►peripheral neuropathy can limit its activity and usefulness in the clinic. Its low solubility requires that paclitaxel be administered in a Cremophor vehicle which itself can induce hypersensitivity reactions. Modifications to paclitaxel to improve its solubility or reduce side effects are difficult because of its complex ring structure. Therefore when the epothilones A and B were found to have a mechanism of action similar to the taxanes, it was hoped that they might lead to more effective anticancer agents. It was found that the epothilones did show important cytotoxic activity against tumor cells when tested in *in vitro* cell assays, but when tested in *in vivo* models of cancer, only modest antitumor activity was present. This was found to be due to poor metabolic stability, and other unfavorable characteristics. To overcome these problems, multiple semisynthetic analogues of the epothilones were made and tested. One of these analogues, BMS-247550, epothilone B analogue, or ixabepilone, was found to be the most effective epothilone in a variety of laboratory assays and was also active in paclitaxel resistant tumor models. It has now been tested in the clinic and has been found to have activity in a variety of clinical cancers.

Preclinical Testing

Ixabepilone has shown potent cytotoxic activity when tested against a broad panel of tumor cell lines *in vitro*, including human ►ovarian carcinoma, ►colon carcinoma, breast, prostate, lung, ►squamous cell carcinoma, human leukemia, and mouse ►lung carcinoma. The concentration of drug at which 50% of the tested cells were killed (IC_{50}) ranged from 1.4–34.5 nM. Included in this panel were cell lines resistant to paclitaxel by either Multi-Drug Resistance (MDR) due to P-glycoprotein overexpression, or due to mutations of β-tubulin, the two most common mechanisms of resistance to paclitaxel. Ixabepilone is more potent than paclitaxel in causing tubulin polymerization and has similar activity to that of the parental compounds, epothilones A and B. Ixabepilone maintained its activity in whole animal systems. Using paclitaxel sensitive human ovarian or colon tumor cell lines in the nude mouse models, ixabepilone produces comparable ►log cell kill and tumor growth delay as paclitaxel while in nude mouse or rat tumor models using paclitaxel resistant human ovarian, breast, colon, or ►pancreatic cancer cell lines, ixabepilone produced much greater tumor log cell kill and tumor growth delay than did paclitaxel. In contrast to paclitaxel which is usually ineffective when given orally, ixabepilone was also active in paclitaxel resistant human ovarian cancer and paclitaxel sensitive human colon cancer nude mouse models when given by the oral route.

E

Phase I Studies in Cancer Patients

In animal studies, the main toxicities of ixabepilone were related to the gastrointestinal tract, peripheral neuropathy, and bone marrow toxicity. Similar to the taxanes, when tested in cancer patients, ixabepilone required the same solvent for i.v. use, a Cremophor-based formulation (ethanol plus polyoxyethylated castor oil) which can lead to hypersensitivity reactions. After this type of reaction occurred in a patient, subsequent patients were prophylactically treated with histamine-1 (H1) and histamine-2 (H2) blockers. Schedules tested included an every 21 day cycle, weekly administration, a daily times 5 every 21 day cycle, and a daily times 3 every 21 day cycle. In each of these schedules, the drug was administered by the intravenous route. All of the phase I trials showed antitumor responses. These occurred in patients with breast, non-small cell lung, and ovarian cancer, and ►melanoma. Some of these patients had previous treatment with paclitaxel or ►docetaxel.

The 21 day cycle trials consisted of a 60 min i.v. infusion on day 1 repeated every 21 days. The dose limiting toxicities for this schedule were neutropenia and sensory neuropathy. One study recommended a phase II dose of 50 mg/m^2 and another trial of the same schedule suggested 40 mg/m^2 as the phase 2 dose. Most subsequent trials have used the lower dose. Other toxicities commonly seen with this schedule included fatigue, arthralgias, myalgias, and vomiting.

With treatments using weekly infusions or daily times five or daily times three infusions, maximum tolerated doses were lower. Neutropenia, sensory neuropathy, fatigue, and hypersensitivity reactions were seen with the weekly schedules. With the daily times five or three schedules, dose limiting toxicity was neutropenia with fewer hypersensitivity reactions and less severe neurotoxicity.

In patients given a 1-h infusion of ixabepilone, there was found to be bundling of microtubules in the peripheral blood mononuclear cells and this correlated with the plasma area under the curve, the concentration of drug measured in the blood multiplied by the time it is present. Similar microtubule bundle formation was seen in ►breast tumor cells obtained from a chest wall mass in a patient who showed a partial response after receiving drug on the 1 h infusion schedule. This

patient was taxane refractory and the tumor expressed multi-drug resistance protein. Cell death occurred in these tumor cells 23 h after the peak formation of microtubule bundles.

Phase II Studies in Cancer Patients

There is data from some phase II trials of ixabepilone in cancer patients. With ixabepilone given by a 1 or 3 h infusion on an every 21 day schedule, responses have been seen in a modest number of ►gastric or ►breast cancer patients previously treated with a taxane or ►non-small cell lung cancer patients who had previously received a platinum-based regimen. In breast cancer patients who were previously treated but had not received a taxane, a higher response rate of 34% was seen. A trial in colorectal cancer patients who had previously received an ►irinotecan-based regimen did not show any responses.

Multicenter phase II trials in the Southwest Oncology Group of ixabepilone in previously untreated patients with advanced pancreatic cancer or chemotherapy-naïve patients with hormone-refractory ►prostate cancer have shown encouraging results that suggest that further testing is warranted.

Summary and Future Outlook

The epothilone B analog ixabepilone is a cancer therapeutic agent with a mechanism of action similar to the taxanes, stabilization of cellular microtubules leading to mitotic arrest and cell death. However it demonstrates anti-tumor effects against both taxane-sensitive and taxane-resistant tumors and is clinically active against a broad spectrum of tumor types. Further testing as a single agent or in combinations in previously untreated patients is needed. Phase III trials in which it is compared to standard regimens will further define its role in cancer treatment. Ixabepilone is felt to be an important new anti-cancer agent that may surpass the taxanes in usefulness.

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Epothilones

Definition

Are a group of microtubule-targeting agents, like ►taxanes, and ►vinca alkaloids. Epothilones were originally identified as metabolites produced by the common soil myxobacterium *Sorangium cellulosum*. They were found initially to have a narrow antifungal spectrum, but they also were found too toxic for use as an antifungal. Subsequently, their anticancer properties were detected. They are important and powerful options in the management of breast cancer and prostate cancer. The epothilones are a new class of cytotoxic molecules, including epothilone A, epothilone B, and epothilone D, identified as potential chemotherapy drugs. Early studies in cancer cell lines and in human cancer patients indicate superior efficacy to the taxanes. Their mechanism of action is similar to that of the taxanes, but their chemical structure is simpler and they are more soluble in water. Although taxane-based therapy has been used successfully, its effectiveness is often compromised by the emergence of ►drug resistance. Efforts to overcome drug resistance have led to the discovery of several novel anti-►microtubule agents, including the epothilones. Epothilones exhibit broad antitumor activity similar to that of the taxanes, but they are less sensitive to known resistance mechanisms. The ongoing development of microtubule-targeting agents provides new strategies for overcoming taxane resistance and may improve clinical efficacy and patient outcomes.

Epoxide Hydrolase

Definition

Is an enzyme that catalyses the trans-addition of water to alkene epoxides and arene oxides

►Detoxification

Epoxides

Definition
Chemicals with a 3-membered ring containing one oxygen atom.

►Carcinogen Metabolism

EPR

Definition
Enhanced permeability and retention effect due to gaps in the endothelium with extended accumulation in the interstitial space of the tumor.

►Liposomal Chemotherapy

EPR Effect

Definition
Tumor vasculature is leaky and lymphatic vessels are poorly developed. Therefore, drugs with widths of ~1–200 nm size penetrate and remain in cancer cells for longer periods.

►Drug Delivery Systems for Cancer Treatment

EpRE

Definition
Electrophile response element, is a synonym of antioxidant response element (ARE).

►Phase 2 Enzymes

EPSCC

Definition
►Extrapulmonary Small Cell Cancer.

Epstein-Barr Virus

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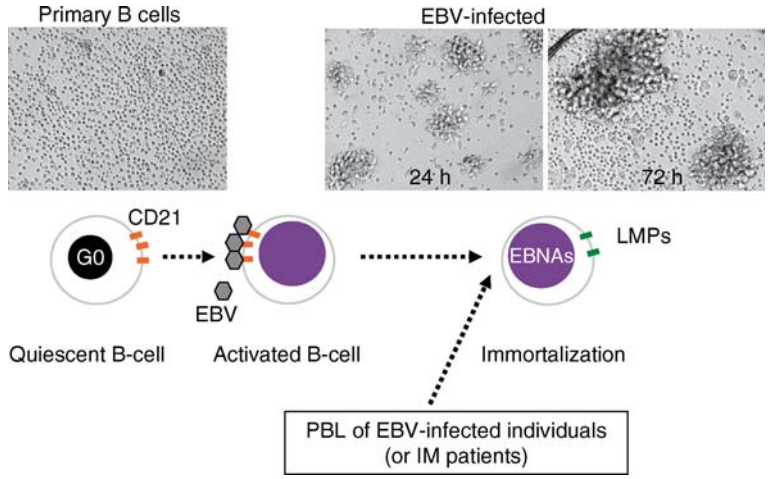
Synonyms
Human Herpes virus 4; HHV4

Definition
Epstein-Barr Virus (EBV) was the first virus isolated from a human tumor, the Burkitt's lymphoma (BL). EBV is a lymphotropic γ-herpesvirus widely spread in the human population: 90–95% of adults have antibodies against the virus. In the majority of cases, the primary infection occurs within the first 3 years of life and is asymptomatic. When EBV infection occurs later in life, usually during adolescence, it results in the symptomatic illness known as Infectious Mononucleosis (IM). Infected individuals carry the virus all their life, in a very low number of lymphoid B cells (probably resting B cells) in their peripheral blood and lymphatic organs. Intermittent viral shedding occurs into the saliva, due to viral replication in the oropharyngeal lymphoid or epithelial tissues: saliva is the main transmission route of the virus. Since its first discovery in 1964 in a Burkitt's lymphoma tumor, EBV has been found to be associated with several other human malignancies including the undifferentiated ►nasopharyngeal carcinoma (NPC), ►Hodgkin's disease, rare T-cell and Natural killer (NK)-cell lymphomas, gastric carcinomas and B and T cell lymphomas in immuno-compromised individuals. A characteristic unique to EBV is its capacity to induce the indefinite proliferation or immortalization of quiescent B lymphocytes, upon their infection *in vitro*.

Characteristics
In vitro Immortalization of Primary B Cells by EBV: The Growth Transcription Program
In vitro, infection of B cells by EBV is not productive but results in the outgrowth of latently infected ►lymphoblastoid cell lines (LCL) (Fig. 1). Such cell lines can also be obtained by culture of peripheral blood



Epstein-Barr Virus. Figure 1 Immortalization of B cells by EBV. EBV infection of resting primary human B-cells *in vitro* causes cell cycle entry of the infected cells, establishment of latent viral infection and conversion of the cell culture into permanently growing lymphoblastoid cell lines (LCLs), which progress to a fully immortalized state. Such LCLs can also be obtained by culture of PBL from individuals infected by EBV (mostly IM patients). The first step of infection is the interaction of the EBV gp350 glycoprotein with its receptor, the CD21. This induces a decondensation of the chromatin, a prerequisite for expression of a subset of the viral genes that cooperate to induce B cell proliferation and the expression of several cellular activation markers and adhesion molecules. These latency genes code for three nuclear proteins, the Epstein-Barr virus nuclear antigens (EBNA1, -2, -3A, -3B, -3C, -LP), three integral membrane proteins (LMP1, LMP2A, LMP2B) plus two non-polyadenylated small nuclear RNAs (EBERs). The EBV genome is maintained in these proliferating cells as multiple extrachromosomal episomes and is replicated by cellular proteins, concomitantly with the cellular genome, via a DNA replication origin called ►*oriP*.

Epstein-Barr Virus. Table 1 EBV gene latency transcription programs

Transcription program	Type of latency	EBV genes expressed	Occurrence
Growth	III	EBNA1, 2, 3a, 3b, 3c, LP, LMP1, LMP2a and LMP2b	PTLD, Immunoblastic lymphoma (HIV)
Default	II	EBNA1, LMP1 and LMP2a	HD, NPC, Gastric carcinoma
EBNA 1 only	I	EBNA1	BL, PEL
Latency	0	None	Memory B cells in peripheral blood

PTLD: Post transplant lymphoma disease; HD: Hodgkin's disease; NPC: Nasopharyngeal carcinoma; BL: Burkitt's lymphoma; PEL: Primary effusion lymphomas.

lymphocytes (PBL) from naturally infected individuals. The phenotype of LCLs (i.e. morphology and cell surface markers) is very similar to that of antigen activated B cells, and a limited set of viral gene products is expressed: six nuclear proteins, the Epstein-Barr Nuclear Antigens (EBNA-1, -2, -3A (or -3), -3B (or -4), -3C (or -6) and -LP (or -5), three integral membrane proteins (LMP-1, -2A (or TP-1) and -2B (or TP-2) and two small non-polyadenylated nuclear RNAs (EBER-1 and EBER-2). This expression profile is referred to as the growth transcription program or ►latency III (Table 1). In such immortalized cell lines, the viral DNA (a 172 kpb double-stranded DNA molecule) is maintained in the nucleus as multiple extrachromosomal copies of the viral episome. Among the nine proteins expressed in latency III, EBNA-1, -2, -3A, -3C, -LP and LMP-1 are essential for efficient transformation/immortalization of B-lymphocytes *in vitro*. These proteins are thought to cooperate for the initiation and maintenance of B cell proliferation *in vitro*.

EBNA-1 is a sequence-specific DNA-binding protein which binds to the EBV origin of replication (►*OriP*). This interaction is required for viral DNA replication and for equilibrated distribution of the EBV episomes to the daughter cells during cell division. EBNA-1 may have other roles in EBV-induced oncogenesis as EBNA-1 transgenic mice display an increased incidence of B-cell lymphoma.

EBNA-2 is a transcriptional activator which regulates the expression of all EBV genes expressed in

►latency III as well as certain cellular genes including CD21, CD23 and cfgr. EBNA-2 does not bind DNA directly but is recruited to EBNA2-responsive elements by the cellular sequence-specific DNA-binding factor RBP-Jκ (also called CBF-1). As RBP-Jκ is part of the Notch signalling pathway, EBNA-2 may mimic part of notch signal transduction (►NOTCH/JAGGED Signaling in Neoplasia). Although the exact function of EBNA-LP is still unknown, this nuclear factor has been found to cooperate with EBNA-2 for the transcriptional activation of the LMP-1 gene. Furthermore, co-expression of EBNA-2 and EBNA-LP in primary resting B cells previously activated through binding of the EBV glycoprotein gp350 to the EBV receptor CD21, induces Cyclin D2 expression and drives resting B lymphocytes into the G1 phase of the cell cycle.

EBNA-3A, -3B and -3C are related proteins but only -3A and -3C are essential for B-cell immortalization by EBV *in vitro*. However, these proteins have at least one common function: repression of EBNA2-activated transcription by directly contacting RBP-Jκ and inhibiting its binding to DNA. Furthermore, EBNA-3C is able to cooperate with activated (Ha)-Ras (►RAS), to induce the proliferation of primary rat fibroblasts.

LMP-1 (►Epstein-Barr Virus Latent Membrane Protein 1) is an integral membrane protein with a very short 24 aa N-terminal cytoplasmic domain, six membrane spanning hydrophobic segments and a 200 aa cytoplasmic C-terminal domain. LMP-1 transforms rodent fibroblast cell lines and Rat-1 cells expressing LMP-1 are tumorigenic in nude mice. LMP-1 acts as a constitutively activated member of the tumor necrosis factor receptor (TNRF) superfamily. It activates NF-κB transcription factor activity through a pathway that involves the recruitment of TNF-RI receptor-associated factors (TRAFs). It also induces ►AP-1 transcription factor activity via triggering of the c-jun N-terminal kinase (JNK). The STAT-1 transcription factor has also been shown to be a target of LMP1 which induces STAT-1 phosphorylation and thus its subsequent transfer to the nucleus.

LMP-2A is an integral membrane protein with a 119 aa hydrophilic N-terminal cytoplasmic tail which contains Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) followed by 12 transmembrane domains and a 27 aa hydrophilic C-terminus. LMP-2B differs from LMP-2A by the lack of the N-terminal cytoplasmic domain. Although dispensable for in vitro immortalization of B-lymphocytes LMP-2A has an important role in the biology of EBV *in vivo* (see below) in mimicking the presence of a B-cell receptor (BCR) and providing important survival signals for B-cells.

EBV Biological Cycle in Vivo

Although understanding of EBV infection biology *in vivo* is still rudimentary, it is believed to mimic the normal B cell response to environmental antigen. EBV transits the epithelium and infects naïve B cells in the underlying tissue. Expression of the latency genes (transcription growth program: Table 1) causes the cell to become activated, proliferate and migrate to the follicle. After this initial clonal expansion, some EBV-infected cells undergo germinal center reactions. EBV transcription is then limited to EBNA-1, LMP-1 and LMP-2 expression (the default transcription program: Table 1). These germinal center B cells will then differentiate into memory B cells in which EBV expression is turned off (latency program: Table 1). These cells constitute the long-term reservoir of EBV. The lytic viral cycle can be reactivated in these cells by signals that cause B cells to differentiate into antibody-secreting plasma cells (through antigen stimulation) and migrate to the mucosal epithelium allowing the release of viral particles in the saliva, the main route for transmission of the virus between individuals.

Although EBV is considered to be a B-lymphotropic virus, it can also infects epithelial cells *in vitro* and is found in several EBV-associated carcinomas *in vivo*. A role of the epithelial cells of the oropharynx in the amplification of virus production *in vivo* has long been suspected but not yet demonstrated.

Viral Productive Cycle

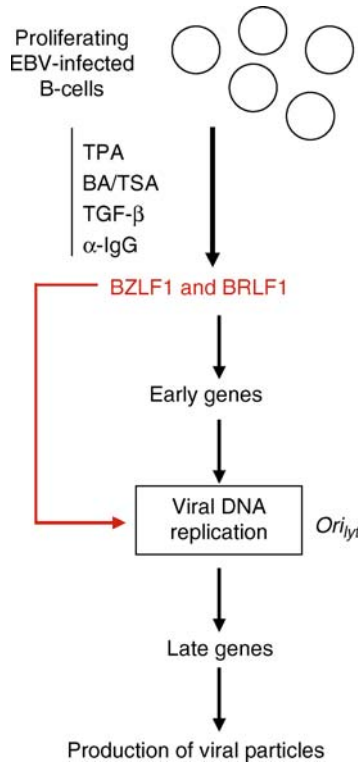
In vitro, the lytic productive cycle can be induced in EBV-infected cell lines (either LCL or cell lines established from Burkitt's lymphoma) by treatments of the cells with various agents such as the phorbol ester TPA, butyric acid (BA) or cross-linking of surface immunoglobulin etc. A key mediator of the entry into the productive cycle is the viral-encoded transcription factor BZLF1 (also called EB1, Zta and Zebra) which activates both transcription of all the EBV early genes and DNA-replication from the replication origins (►Ori_{lyt}) active during the lytic cycle (Fig. 2).

Clinical Relevance

EBV is associated with several human malignancies both in immuno-compromized and immuno-competent individuals.

In immuno-depressed individuals – post-transplant patients or AIDS (Acquired Immunodeficiency Syndrome) patients – EBV is probably directly involved in the appearance of immunoblastic B-lymphomas (in AIDS patients) or posttransplantation lymphoproliferative diseases (PTLDs) (in patients undergoing organ transplantation) due to the loss of normal cytotoxic T cell surveillance. These lymphomas are monoclonals or polyclonals and the cells usually express the full set of EBV genes found in LCLs proliferating in culture (latency III, Table 1).

In immuno-competent individuals EBV is associated with several cancers. Endemic Burkitt's lymphoma (BL) is found in certain parts of Africa and South



Epstein-Barr Virus. Figure 2 The EBV replicative cycle. The EBV lytic replication program can be induced *in vitro* by treatment of proliferating EBV-infected B cells with various chemicals, crosslinking of the surface immunoglobulins or by expression of the EBV transcriptional activator EB1 (also called Zta or ZEBRA), product of the BZLF1 gene. In cooperation with another viral transcriptional factor (also called R or Rta), product of the BRLF1 gene, EB1 activates the expression of all the EBV early genes. EB1 also directly stimulates viral DNA replication which is dependent on viral proteins (a DNA-polymerase, a polymerase processivity factor, a single-stranded DNA binding protein, a primase and a helicase/primase associated protein). This viral DNA replication is initiated at replication origins, called ori_{lyt}, which are different from the one used during latency. This lytic replication program leads to amplification of the viral genome, synthesis of structural viral proteins and the assembly of infectious virus particles.

America where malaria – which appears to act as a cofactor – is also endemic, and affects mainly children from 7 to 9 years old. In these regions, BL is associated with EBV in more than 90% of cases. In lower incidence regions the association is only found in 20–30% of cases. BL is a monoclonal tumor characterized by a translocation of the c-myc gene (►Myc Oncogene) to one of the immunoglobulin loci which results in an altered regulation of c-myc. The expression of EBV in the tumor cells is limited to EBNA-1, the EBER RNAs, plus several transcripts from the BamHI A region of EBV, also called Complementary Strand Transcripts (CST). This profile of expression is defined as ►latency I (Table 1). ►Burkitt's lymphoma cell lines can be readily established from tumor biopsies. On the contrary to LCLs, these cells are tumorigenic in nude mice. However, after several passages of these cells in culture, the expression profile of the EBV genes has been shown to derive towards a latency III profile.

In ►Hodgkin's disease, EBV is present in the Reed-Sternberg cells (►Hodgkin and Reed-Sternberg cells), in about 40% of cases, mostly of the mixed cellularity type. Expression of EBV in Hodgkin's disease is characteristic of latency II (Table 1).

EBV is also found associated with rare but specific types of nasal T-cell lymphomas more common in Southeast Asian populations and also natural-killer (NK) cell (►Natural killer cells) lymphomas. These types of lymphomas seem to arise either after acute primary infection or in some cases of chronic active EBV infection. The EBV expression profiles in these tumors are characteristic of latency I/II (Table 1).

EBV is also associated with a variety of carcinoma and in particularly the undifferentiated nasopharyngeal carcinoma (►Nasopharyngeal carcinoma) (NPC). NPC is associated with EBV in almost 100% of cases worldwide and is particularly common in areas of China and South-east Asia. Genetic disposition as well as environmental cofactors such as dietary components are thought to be important in the aetiology of NPC. EBV gene expression in NPC epithelial cells consists of EBNA-1, the EBER RNAs and LMP-1, LMP-2A/-2B (in 65% of cases) plus the CSTs (Complemetary Strand transcripts). This profile of expression is similar to latency II (Table 1). Several factors suggest that a reactivation of EBV (i.e. entry into the lytic cycle) precedes or accompanies the development of NPC. EBV is also found in about 10% of gastric adenocarcinomas (►Gastric cancer) with a pattern of expression of EBV genes similar to that observed in NPC.

The exact role of EBV in the development of these different tumors is not yet understood and both environmental and genetic cofactors also contribute. However, the fact that the EBV genome is present in the great majority of the cells in EBV-associated malignancies and the demonstration that the virus is present in the tumor cells at a very early stage, argue for a causative role for EBV in these cancers.

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Epstein–Barr Virus Latent Membrane Protein 1

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Definition

The latent membrane protein 1 (LMP1) of the ►Epstein–Barr virus (EBV) is an ►oncogene that is expressed during latent EBV infection. LMP1 sufficient for the transformation of rodent fibroblast cells and is essential for efficient B ►cell transformation. LMP1 mimics a constitutively active tumor necrosis factor (TNF) receptor and interacts with and deregulates the ►signal transduction network of the host cell leading to altered cell survival, differentiation, and phenotypic changes.

Characteristics

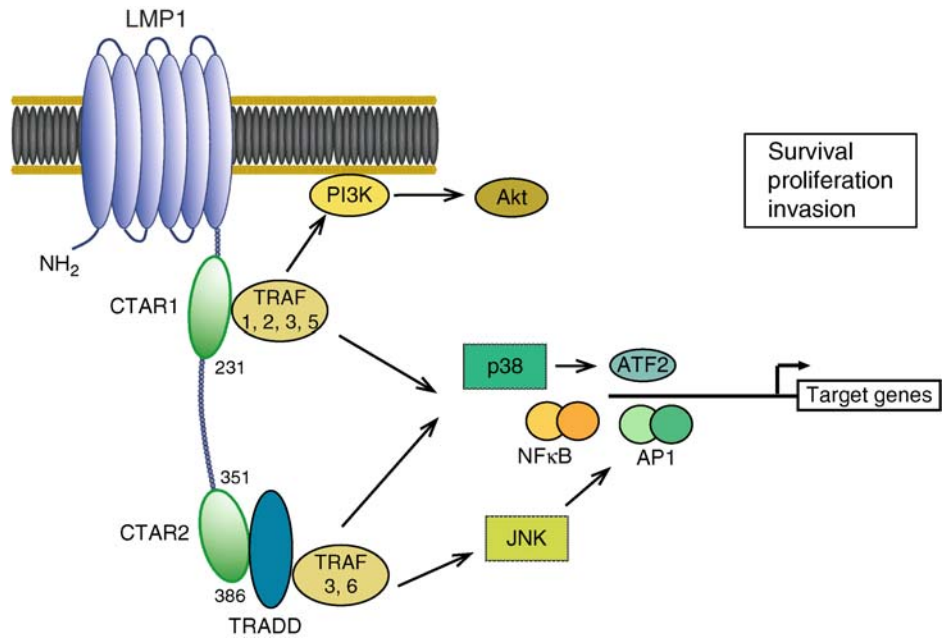
EBV belongs to the family of gamma herpes viruses; it infects humans and establishes a latent infection for the lifetime of the individual. Infection in childhood is usually asymptomatic, but in young adults can lead to infectious mononucleosis. Due to its association with ►B cell tumors as Burkitt lymphoma (BL), ►Hodgkin disease/lymphoma (HL), posttransplant lymphoproliferative disorder, and epithelial tumors as nasopharyngeal carcinoma (NPC) and gastric carcinoma, EBV was the first human tumor virus to be discovered and is now classified as a Group 1 carcinogen by the WHO. In vivo, EBV does usually not replicate in B lymphocytes, but instead establishes a latent infection with defined expression of the virus latent genes. In vitro, EBV infection leads to continuously proliferating lymphoblastoid cell lines (LCLs) and a different expression pattern of virus latent genes. The virus genes influence both viral and cellular transcription in the host cell. Among them LMP1 is essential for B cell transformation in vitro and behaves as a classical oncogene in rodent fibroblast transformation assays. When expressed in the B cell compartment of transgenic mice, LMP1 induces the development of B cell lymphoma whereas its expression in the murine epidermis results in hyperplasia. It is interesting to note that expression of LMP1 is variable among EBV-associated tumors, being always present in EBV-associated Hodgkin lymphoma, almost always absent in virus-positive Burkitt lymphoma and variably present in NPC.

Structurally, LMP1 is a 66kDa integral membrane protein consisting of 386 amino acids. It can be divided into three domains: A short cytoplasmic amino-terminus of 24 amino acids; a transmembrane domain consisting of six transmembrane helices which oligomerize to form membrane patches; and a carboxy terminal cytoplasmic domain of 200 amino acids. The cytoplasmic domain contains two carboxy terminal activating regions (CTARs), also known as transformation effector sites (TES). The CTARs are critical for EBV transforming activity; while CTAR1/TES1 is essential for initial B cell transformation, CTAR2/TES2 mediates growth factor-like signals which are required for long-term outgrowth of EBV infected cells. The region between CTAR1 and CTAR2, sometimes referred to as CTAR3, is not required for B cell ►immortalization.

Mechanisms

Cell transformation requires activation of the host cell signaling machinery. LMP1 mediates this activation by recruiting molecules of the TNF receptor and ►toll-like receptor family (Fig. 1). CTAR1 interacts with the TNF receptor-associated factors (TRAF) 1, 2, 3, and 5 through the PxQxT binding motif of LMP1. Recruitment of TRAF molecules to CTAR2 may be mediated indirectly through binding of the TNF receptor-associated death domain protein (TRADD), although much of CTAR2-mediated ►nuclear factor kappa B (NF-κB) activity has been shown to be TRADD-independent (Fig. 1).

These adaptor proteins subsequently recruit multiprotein complexes containing the NF-κB-inducing kinase (NIK) and the I-κB kinases (IKKs). This results in the activation of the canonical I-κB-dependent NF-κB pathway (involving p50-p65 heterodimers) and the noncanonical pathway, leading to the processing of p100 to generate p52-p65 heterodimers. While activation through the CTAR1 domain of LMP1 is mediated by the noncanonical NF-κB pathway, CTAR2 appears to activate the canonical pathway by utilizing TRAF6 and TAK1. CTAR2 is also important in activating the c-Jun N-terminal kinase (►JNK) leading to activation of the transcription factor ►AP-1. The phosphatidylinositol 3-kinase (►PI3K) pathway is triggered through CTAR1 leading in epithelial cells to actin polymerization and cell ►motility.



Epstein–Barr Virus Latent Membrane Protein 1. Figure 1 Structure of LMP1 and its functional domains. The signaling domains recruit TRAFs and TRADD which results in the activation of host cell signal transduction pathways and the regulation of host cell target genes.

Other signaling pathways activated by LMP1 are the p38 ►MAP kinase pathway and the ►signal transducers and activators of transcription (STAT) pathway.

The NF-κB pathway plays a key role in the activation of many genes and is essential for the transformation of B cells by EBV. NF-κB mediates induction of antiapoptotic genes (►*bcl-2*, *bfl-1*, *A20*, *c-IAPs*, ►*c-FLIP*) and downregulates proapoptotic genes as *Bax* (*Bcl-2*-associated protein x). LMP1 expression in B cells also results in the upregulation of activation markers and ►adhesion molecules.

LMP1 also modulates the communication between EBV-infected cells and its cellular environment by upregulating the expression of a large number of cytokines (►interleukin-6, -10, TNF-α) and ►chemokines (RANTES, IP-10, interleukin-8). It is also involved in migration and invasion processes as it activates proangiogenic factors as ►vascular endothelial growth factor, ►matrix metalloproteinases, and modulators of the cytoskeleton.

Although LMP1 shows no homology with any cellular protein it functionally mimics an activated CD40 receptor, which is a costimulatory receptor required for B cell proliferation. Although LMP1 and CD40 both recruit TRAF molecules and regulate an overlapping pattern of signaling pathways and target genes, they have divergent roles in B cell development. For example, in CD40-deficient LMP1 transgenic mice, LMP1, like CD40 can induce extrafollicular B cell differentiation, but in contrast to CD40, LMP1 leads to a defective germinal center reaction, characterized by splenomegaly and lymphadenopathy.

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ER

Definition

►Endoplasmatic Reticulum
►Estrogen Receptor

ER-Associated Protein Degradation

Definition

The mechanism whereby unfolded or misfolded proteins are re-exported from the endoplasmic reticulum lumen into the cytosol to undergo proteasomal degradation.

►Endoplasmic Reticulum Stress

ER Quality-Control

Definition

The molecular apparatus that monitors the maturation and transport of proteins in the endoplasmic reticulum and targets non-native conformers for degradation.

►Endoplasmic Reticulum Stress

ER Stress

►Endoplasmic Reticulum Stress

ERBB2

►HER-2/neu

ERG

Definition

Ets (E26 – E twenty-six) related gene: member of the *Ets* gene family encoding sequence specific transcription factors; closest relative to the Fli-1 gene.

►Ewing Sarcoma

Erionite

Definition

Is a zeolite silicate that in its native form, contains aluminum, calcium, and magnesium but no iron. The crystals resemble and feel like wool. They are sieve-like and, following inhalation, accumulate large amounts of iron. Over time, ►mesothelioma arises at sites of erionite deposition.

►Asbestos

ERK

Definition

Extracellular signal-regulated kinase. ERK1 and ERK2 are members of the ►MAP kinase family. MAP kinases act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes. Upon activation, this kinase translocates to the nucleus and peripheral adhesion sites, where it phosphorylates target substrates.

►Calpain
►Trefoil Factors

ERK/MAP Kinase (ERK/MAPK)

Definition

Mammalian MAP Kinase can be divided into four groups based on their structure and function: ►ERKs (Extracellular signal-Regulated Kinases), p38MAPKs, JNKs (c-Jun NH2-terminal Kinases) and ERK5 (Extracellular signal-Regulated Kinase-5) or BMK. The MAPK/ERK signaling cascade is activated by a wide variety of receptors involved in growth and differentiation including GPCRs (G-Protein Coupled Receptors), RTKs (►Receptor Tyrosine Kinases), integrins, and ion channels. The specific components of the cascade vary greatly among different stimuli, but the architecture of the pathway usually includes a set of adaptors like SHC, GRB2 (Growth Factor Receptor Bound protein-2), Crk, etc. linking the receptor to a GEF (Guanine nucleotide Exchange Factor) like SOS (Son of Sevenless), C3G, etc. transducing the signal to small GTP binding proteins (Ras, Rap1), which in turn activate the core unit of the cascade composed of a MAPKKK (Raf), a MAPKK (MEK1/2 (MAPK/ERK Kinase-1/2)) and MAPK (ERK). An activated ERK dimer can regulate targets in the cytosol and also translocate to the nucleus where it phosphorylates a variety of transcription factors regulating gene expression.

►Sprouty

Erlotinib

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Synonyms

Tarceva

Definition

Erlotinib is a potent and selective ►epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Erlotinib is commercially available in tablets of 25, 100, and 150 mg formulations. Erlotinib is currently approved for use in previously treated nonsmall cell ►lung cancer and in frontline management of ►pancreatic cancer in combination with gemcitabine chemotherapy.

Characteristics

Rationale for Targeting the EGFR. The EGFR is frequently dysregulated in epithelial cancers. Overexpression of EGFR can result in malignant transformation of cells. Patients whose tumors have overexpressed or dysregulated EGFR and/or ligand expression may have a worse prognosis. Activation of the EGFR initiates dimerization of the receptors leading to activation of the tyrosine kinase domain. The kinase in turn phosphorylates (►Phosphorylation) and activates proteins in the signal transduction cascade promoting cell proliferation, angiogenesis, invasion, and survival. In preclinical models, erlotinib selectively inhibited EGFR tyrosine kinase activity in human cancer cell lines and resulted in inhibition of various tumor growths and induction of apoptosis. Erlotinib potentiated the activity of cytotoxic agents and radiation in cancer cell line as well as in animal models.

Phase I Trials. The maximal tolerated dose of erlotinib was 150 mg once daily in Phase I trial. The most commonly observed toxicities were an ►acneiform rash and diarrhea.

Experience in Nonsmall Cell Lung Cancer (*NSCLC*). Three randomized trials evaluated the efficacy of erlotinib in advanced NSCLC. In the first trial, patients with previously treated advanced NSCLC were randomized to erlotinib or best supportive care. The results revealed a significant improvement in median survival (6.7 vs. 4.7 months, $p = 0.001$) and survival at 1 year (31.2% vs. 21.5%) in favor of erlotinib. The median time to progression and response rates in the erlotinib and best supportive care arms were 9.7 versus 8.0 weeks ($p < 0.001$) and 8.9 versus 1.0%, respectively. The incidence of grade 3 and 4 rash and diarrhea in the erlotinib arm was 9 and 6%, respectively. A very important finding, in a multivariate analysis, was that nonsmokers and those with adenocarcinoma histology benefited most from erlotinib. Based on this trial, erlotinib was approved in patients with previously treated NSCLC.

Two trials compared conventional chemotherapy to the same chemotherapy and erlotinib in patients with newly diagnosed advanced NSCLC. The chemotherapy regimens evaluated were ►gemcitabine/carboplatin (TALENT) and ►paclitaxel/cisplatin (TRIBUTE). No significant difference was observed between patients receiving chemotherapy or erlotinib and chemotherapy with respect to objective response rate, survival, or time to progression. Therefore, erlotinib is not used in combination with chemotherapy to treat patients with NSCLC.

Experience in Pancreatic Cancer. A randomized trial compared gemcitabine to gemcitabine and erlotinib in patients with advanced pancreatic cancer. The trial resulted in a significant improvement in median survival (6.37 vs. 5.91 months, $p = 0.01$) and 1 year survival (24 vs. 17%) in favor of erlotinib. Significant predictors for favorable outcome were performance status 0–1, locally advanced disease, and normal albumin. Treatment with erlotinib resulted in an improvement of progression free survival (3.75 vs. 3.55 months, $p = 0.009$). The incidence of grade 3 and 4 toxicities were higher in the erlotinib arm with respect to rash (6 vs. 1%), and diarrhea (7 vs. 2%). The Food and Drug Administration (FDA) approved erlotinib in combination with gemcitabine for previously untreated advanced pancreatic cancer. More recently a similar approval was granted in Europe for this particular indication.

Trials in Other Tumor Types. Erlotinib has been evaluated in ►bile duct, gastric, ►esophageal, ►hepatocellular, and ►colorectal (CRC) and ►head and neck cancers. In bile duct cancer, a multiinstitutional phase II trial evaluated single agent erlotinib in 42 patients with advanced disease. The trial met its primary endpoint with a progression free survival at 6 month of 17%. Three patients had a partial response. The Southwest Oncology Group (SWOG) performed a phase II trial of erlotinib in advanced gastric and gastroesophageal (GEJ) tumors. The response rate was 9%. All responses were observed in the GEJ patients. Interestingly, no responses were seen in the patients with gastric cancer. Philip et al. reported the results of 38 patients with advanced hepatocellular cancer treated with erlotinib. Forty seven percent of the patients had received prior chemotherapy. The study met its primary endpoint with a 6 month progression free survival of 32%. Three patients had partial response. Erlotinib has been evaluated in CRC as a single agent and in combination with ►FOLFIRI, ►capecitabine, capecitabine/oxaliplatin, and ►FOLFOX. As a single agent, erlotinib was evaluated in patients with previously treated CRC.

Erlotinib. Table 1 Ongoing trials of erlotinib in combination with targeted agents

Agent	Target	Phase (disease)
Isoflavone	Akt/NF-kB	II (Pancreatic)
Sorafanib	VEGFR, PDGFR, Raf	I
Dasatinib	Src	I
Cetuximab	EGFR	I
Celecoxib	Cyclooxygenase 2	II (NSCLC)
RAD001	mTOR	Phase I

Thirty nine percent of patients had stable disease no responses were observed. The combination of FOLFIRI and erlotinib resulted in excessive toxicity and the trial was discontinued. Erlotinib and capecitabine were well tolerated, and in the phase I trial, 2 of the 9 patients with CRC had a partial response. The phase II trial is still ongoing. Two trials evaluated erlotinib and capecitabine/oxaliplatin in patients with previously treated CRC. The partial response rates and stable disease were 20–22%, and 61–64%, respectively. There is still uncertainty whether erlotinib plays any role in CRC. Currently research in CRC is focused on the use of monoclonal antibodies that target the EGFR. Erlotinib in combination with FOLFOX is currently in a phase II trial. Modest activity was observed with erlotinib in head and neck cancer patients with a response rate of 4% and disease stabilization rate of 38%. In conclusion, erlotinib has demonstrated promising activity in cholangiocarcinoma, hepatocellular, and GEJ tumors. The role of erlotinib in these diseases requires further randomized trials.

Future Directions. Erlotinib has demonstrated a significant but modest activity in a number of carcinomas. The future challenge is how to improve the activity of erlotinib. Two approaches are being evaluated in clinical trials. The first is to select patients with a higher likelihood of benefit from erlotinib. For example, patients with NSCLC and either activating mutations of the EGFR or no smoking history have demonstrated an increased benefit from erlotinib therapy. Additional trials are evaluating the efficacy of erlotinib in previously untreated patients whose tumors have EGFR mutations. On the other hand, a trial is evaluating the combination of erlotinib with chemotherapy in previously untreated patients with NSCLC and no smoking history. The results of these trials will determine whether erlotinib should be used in the frontline management of NSCLC in selected patient populations.

The second approach is based on combining erlotinib with other targeted agents because of the redundancy of the signaling pathways and existence of independently activated survival pathways. Since the inhibition of the EGFR results in inhibition of angiogenesis, the combination of erlotinib and ▶bevacizumab is being evaluated in a number of malignancies including: hepatocellular, cholangiocarcinoma, NSCLC, and pancreatic cancer. The combinations of erlotinib with other agents targeting the signaling cascade are currently at different stages of development (Table 1).

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ERM Proteins

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Synonyms

Villin 2; Cytovilin

Definition

The ERM family consists of three closely-related proteins, ezrin, radixin, and moesin. ERM proteins are cell membrane and cytoskeleton linker proteins.

Characteristics

History, Structure, and Sequence

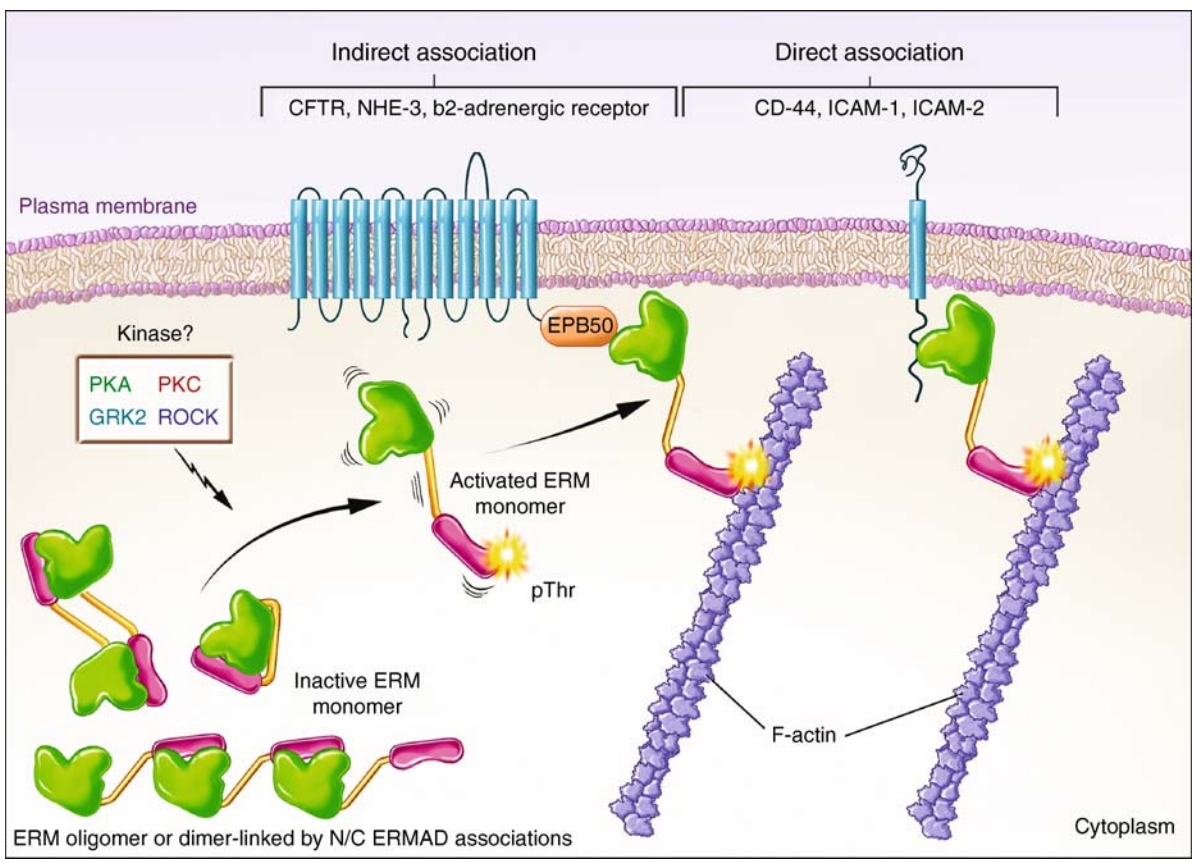
Ezrin, the prototype ERM protein is a 585-amino acid polypeptide, first identified as a constituent of

microvilli and shown to be present in actin-containing surface structure on a wide variety of cells. ERM proteins share homology in sequence structure and function. They are composed of three domains: an N-terminal globular domain; an extended α-helical domain; and a charged C-terminal domain. The N-terminal domain of ERM proteins is highly conserved and is also found in ►merlin, band 4.1 proteins, and members of the band 4.1 superfamily. This domain called FERM (four.one protein, ezrin, radixin, moesin) domain. The crystal structure of moesin revealed the FERM domain is composed of three structural modules that, together, form a compact clover-shaped structure. The C-terminal domain can extend across the FERM domain surface, potentially masking recognition sites of other proteins. Ezrin and radixin also contain a polyproline region between the helical and C-terminal domains. The cDNA sequence of radixin encodes a protein of 583 amino acids with 77% identity to ezrin. Moesin, isolated as a heparin binding protein, consists of 577 amino acids with 74% identity to ezrin.

Regulation

ERM proteins are conformationally regulated. ERM proteins exist in proposed dormant forms in which the C-terminal tail binds to and mask the N-terminal FERM domain (Fig. 1). The activation of ERM protein is mediated by both C-terminal threonine phosphorylation (T567 in ezrin, T564 in radixin, T558 in moesin) and exposure to PIP2. It is likely that phosphorylation at other residues in ERM proteins are needed to maintain an open activated conformation for ezrin and to direct ezrin-specific effects in cells. It is also unclear what functions are ascribed to the so-called "inactive" closed conformation of ERM proteins. Several protein kinases have been found to phosphorylate the C-terminal threonine residue of the ERM proteins. Examples include PKCα (►protein kinase C family), PKCθ (protein kinase C family), Rho kinases/ROCK, G protein-coupled receptor kinase 2 (GRK2), and Myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK).

Dephosphorylation of C-terminal Thr of moesin has been suggested to be a crucial step for lymphocyte adhesion and transendothelial migration. The disassembly of microvilli on lymphocyte cell surfaces caused by dephosphorylation of moesin facilitates the cell—cell (lymphocyte–endothelium) contact. Protein phosphatase 2C is involved in the dephosphrylation of moesin through the activation of Rac1 small ►GTPase.



ERM Proteins. Figure 1

Function, Distribution, Localization

ERM proteins either directly associate with the cytoplamic domains of adhesive type I membrane proteins, such as ►CD44, CD43, ICAM-1, -2, and -3, or indirectly associate with membrane proteins via PDZ-containing adaptors EBP50 and E3KAP. Regulated attachment of membrane proteins to F-actin is essential for many fundamental cellular processes, including the determination of cell shape, polarity, and surface structure, cell ►adhesion, ►motility, cytokinesis, phagocytosis, and integration of membrane transport with signaling pathways. There is functional redundancy between ERM proteins. This is best exemplified by the phenotype of the ezrin knock-out mouse. This mouse is viable at birth, suggesting the ability of radixin and moesin to fill the role of ezrin during development. Interestingly, the fatal phenotype of this mouse is characterized by intestinal villous malformations seen at day 13 postpartum. The normal intestinal epithelial cells nearly exclusively express ezrin. Although ezrin, radixin, and moesin are coexpressed in most cultured cells, they exhibit a tissue-specific expression patterns. Ezrin is highly concentrated in intestine, stomach, lung, and kidney although moesin is prominent in lung and spleen, radixin in liver and intestine. Ezrin is expressed in epithelial and mesothelial cells while moesin is expressed in endothelial cells. As indicted, the brush border of intestinal epithelial cells express only ezrin, and hepatocytes express only radixin.

The Expression and Functions of ERM Proteins in Cancer

Ezrin has been recently shown to be expressed in most human cancers and linked to progression in several cancers, including carcinomas of endometrium, breast, colon, ovary, in uveal and cutaenous ►melanoma, ►brain tumors, and most recently soft tissue sarcomas. cDNA array (►Microarray (cDNA) Technology) analysis of highly and poorly metastatic ►rhabdomyosarcoma and ►osteosarcoma, ezrin was indicated as a key metastatic regulator. In several murine and human cancer models, suppression of ezrin protein and disruption of ezrin function significantly reduced the metastatic phenotype despite the expression of other ERM proteins. This suggests that the redundancy provided by the other ERM proteins for ezrin does not extend to ►metastasis and that ezrin contributes a unique and necessary function to cells undergoing metastasis. Comparing lung adenocarcinoma with normal lung tissue, the expression of ezrin, radixin, and moesin were decreased on mRNA level as well as the protein level. Interestingly, the high expression of ezrin was observed in the invading tumor cells in lung adenocarcinoma.

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ERp57

Definition

Is a member of the protein disulfide isomerase (PDI) family and catalyzes the disulfide oxidation, isomerization, and reduction of proteins in the ER. It is distinct from other PDI family members in that it associates noncovalently with ►calreticulin or ►calnexin.

►Calreticulin

ERp60

►Calreticulin

Erythema

Definition

Refers to redness of the skin caused by capillary congestion. It can be caused by infection, massage, electrical treatments, allergies, exercise or ►solar ultraviolet light (sunburn). Erythema is a common side effect of ►radiation therapy due to patient exposure to ►ionizing radiation.

Erythema Multiforme Major

Definition
Synonym Stevens-Johnson syndrome; is an unusual, life-threatening reaction characterized by fever and flu-like symptoms followed by a severe, blistering rash on the skin and/or mucous membranes, that may occur after a respiratory infection (e.g., herpes simplex and mycoplasma infections) or as an allergic reaction to drugs (e.g., sulfonamides, penicillins, barbiturates, and phenytoin).

►Rituximab

Erythematous Pruritic Macropapular Rash

Definition
A red, itchy, and raised rash.

►Taxotere

Erythrocytosis

Definition
An excess of erythrocytes, or red blood cells (RBCs).

►Erythropoietin
►Polycythemia

Erythroid Colony Stimulating Activity

►Erythropoietin

Erythroleukemia

Definition
A form of acute myeloid leukemia where the myeloproliferation is of abnormal, immature red blood cells

►Erythropoietin

Erythroplakia

Definition
A red patch on the mucosa that is not attributable to any obvious cause. Generally, these lesions have a well-defined border and a soft, velvet-like appearance. Their atrophic nature contributes to the red coloration, as underlying vasculature is more prominent. Around 90% show signs of severe dysplasia or carcinoma-in-situ and may progress to invasive squamous cell carcinoma.

►Squamous Cell Carcinoma

Erythropoiesis

Definition
The development of mature red blood cells (erythrocytes).

►Erythropoietin

Erythropoiesis Stimulating Factor

►Erythropoietin

Erythropoietin

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Synonyms

Epo; Ep; epoetin; ESF; Erythropoiesis stimulating factor; ECSA; erythroid colony stimulating activity

Definition

Erythropoietin (Epo) (from Greek erythro for red, and poietin to make) is a small glycoprotein hormone that is essential for the production of red blood cells. Epo promotes the survival, proliferation and differentiation of erythroid progenitor cells (►BFU-E, CFU-E) to mature erythrocytes and initiates hemoglobin synthesis.

Characteristics

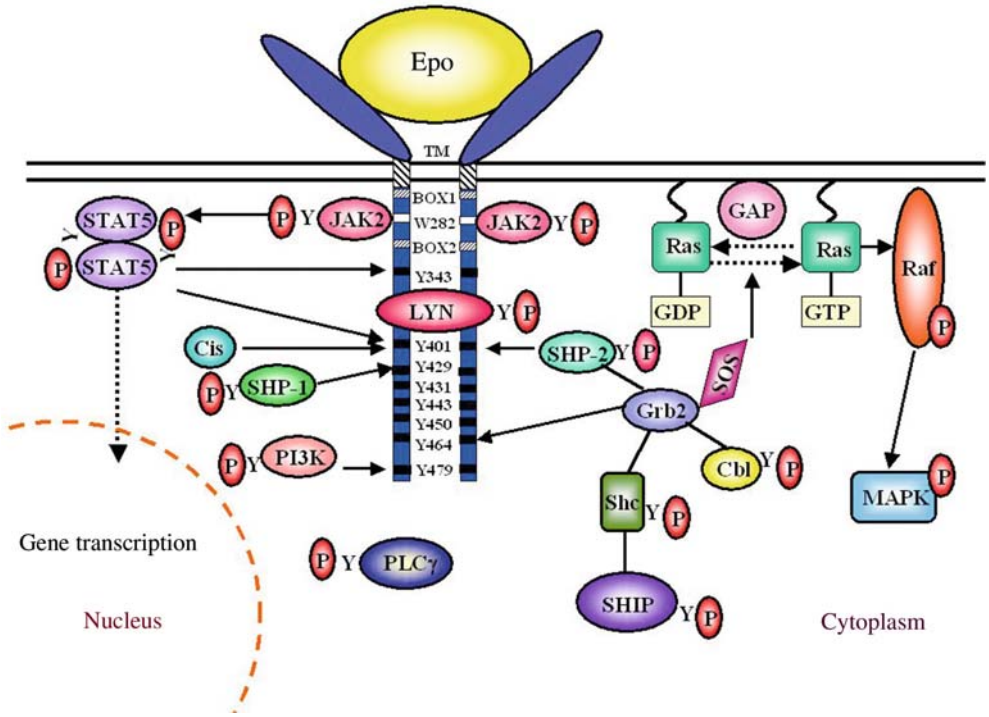
The Epo gene contains at least five exons and resides on chromosome 7q21-q22 in humans and chromosome 5 in mice. DNA sequences from monkey and mouse display 90 and 80% homology to human Epo, respectively. Epo is produced primarily in the kidney, and to a lesser extent in the liver. It is an acidic glycoprotein hormone with a molecular weight of 34–37 kD, and circulates in the blood plasma at a very low concentration (about 5 pmol/l). It is composed of a single chain polypeptide and is resistant to denaturation by heat, alkali or reducing agents. Epo is synthesized as a 193 amino acid precursor that is cleaved to yield an active protein of 165 amino acids. It is N-glycosylated at asparagine residues 24, 36 and 83 and O-glycosylated at serine 126. Epo is also sialylated and contains two disulfide bonds at positions 7/161 and 29/33. The alpha form of the hormone consists of 31% carbohydrates while the beta form consists of 24%. These two forms of Epo have similar biological and antigenic properties. The carbohydrate moiety of Epo plays an important role in the mediation of its full biological effect and the pharmacokinetic behavior of the protein in vivo; non-glycosylated Epo has a very short biological half-life. Epo is fully synthesized in its active form prior to secretion into circulation. Epo, already known as the stimulating hormone for ►erythropoiesis, has displayed different and interesting pleiotropic actions. It not only affect erythroid cells, but also myeloid cells, lymphocytes and megakaryocytes. This hormone can enhance phagocytic function of polymorphonuclear cells and reduce the activation of macrophages, thus modulating the inflammatory process. Epo also exerts diverse biological effects in many nonhematopoietic tissues and is involved in the wound-healing cascade, functions as a proangiogenic cytokine during physiological angiogenesis in the embryo and uterus, and exerts tissue-protective effects as part of the innate response to stressors.

Cellular and Molecular Regulation

The synthesis of Epo in the kidney is under the control of an oxygen-sensing mechanism. Transcriptional response of the Epo gene to hypoxia is mediated partly by promoter sequences but mainly by a 24 bp hypoxia-response element located at the 3′ flanking region of the Epo gene bound to the hypoxia inducible factor-1 (►HIF-1). Epo production is also modulated by several other factors such as hypoglycemia, increased intracellular calcium, insulin release, estrogen, androgenic steroids, and various cytokines.

The biological activity of Epo is mediated by its specific receptors present at 300–3,000 copies per cell, that undergo phosphorylation in response to Epo. The Epo receptor (EpoR) belongs to the class I cytokine receptor superfamily. The mouse EpoR consists of 507 amino acids with an extracellular domain, a single hydrophobic transmembrane domain and a cytoplasmic domain. The human EpoR is a 66 kD protein comprised of 508 amino acids. It consists of eight exons spanning some 6 kb on human chromosome 19p13.3.

The interaction of Epo with its receptor results in the formation of a homodimer and its subsequent internalization (Fig. 1). Dimerization of the receptor results in the autophosphorylation of Janus kinase 2 (JAK2), a protein kinase that is tightly associated with the EpoR. Once activated, JAK2 phosphorylates eight tyrosine residues located in the cytoplasmic domain of the EpoR. Phosphorylation of the EpoR leads to the recruitment and phosphorylation of a number of signal transduction proteins. One such protein is ►STAT5, a transcription factor that plays an important role in the regulation of in vivo erythropoiesis. Once phosphorylated by binding to tyrosine 343 and 401 of the EpoR, STAT5 translocates to the nucleus to activate the expression of several downstream target genes. Other signaling cascades triggered by Epo binding to its receptor include phosphatidylinositol 3-kinase (PI3K) that binds to tyrosine 479 and is involved in erythroblast survival, and Grb2 that binds to tyrosine 464 and is involved in the activation of the Ras pathway. Ras pathway may be required for the synergistic expansion of erythroid progenitors and precursor cells in response to Epo and stem cell factor (SCF). EpoR mediated activation of phospholipase A2 and C also leads to the release of membrane phospholipids, the synthesis of diacylglycerol and increase in intracellular calcium



Erythropoietin. Figure 1 Schematic diagram of the EpoR depicting the positions of tyrosine (Y) residues (black bars; Y) in the cytoplasmic domain and attachment sites of signal transduction proteins such as STAT5, SHP-1 and SHP-2. Binding of Epo to its receptor results in the autophosphorylation and activation of JAK2, which in turn phosphorylates eight tyrosine residues in the cytoplasmic domain of the EpoR.

levels and pH. Since phosphorylation of the EpoR by Epo is diminished after 30 min of stimulation, a number of tyrosine phosphatases have been identified that are involved in attenuating the signal. The tyrosine phosphatase ►SHP-2 binds to tyrosine 401 of the Epo-receptor and stimulates erythroid proliferation, while ►SHP-1 binds to tyrosine 429 and inhibits proliferation.

Expression of the EpoR is not restricted to hematopoietic cells and exhibits a multi-tissue distribution that includes vascular endothelial cells, muscle cells, and neurons, therefore, Epo is believed to play a physiological role in angiogenesis, cardiac and brain development. Abnormal regulation of Epo-EpoR signaling in hematopoietic cells has been associated with proliferative disorders of the bone marrow, such as ►polycythemia vera, a disorder characterized by ►erythrocytosis, as a consequence of an active mutation in the EpoR. Additionally, prolonged activation of STAT5 has been observed in cells transfected with mutant (tyrosine 429) EpoR, suggesting that STAT5 DNA binding activity may play a role in the pathogenesis of erythrocytosis. A point mutation at position 129 of the mouse EpoR gene results in constitutive activation of the receptor without stimulation with Epo. Mice infected with a retrovirus expressing this aberrant receptor develop ►erythroleukemia and splenomegaly. Taken together, these provide evidences that the precise control of Epo-EpoR signaling are critical for the normal proliferation and differentiation of erythroid progenitor cells.

Clinical Relevance

The synthesis of Epo is subject to a complex circuit that links the bone marrow and kidney in a feedback loop. Its reference interval in the blood plasma ranges between 3.3 and 16.6mIU/ml Patients suffering from most ►anemias display higher than normal concentrations of serum Epo; whereas, those suffering from anemia associated with chronic renal disease have values either low or within the normal range. Epo levels are disproportionately low in anemic patients with chronic disorders as well, such as rheumatoid arthritis, AIDS, and cancer, in which inhibition of Epo production and erythroid progenitor proliferation by inflammatory cytokines, such as IL-1 and TNF, are thought to play major causative roles. Abnormally high concentrations may also be induced by renal neoplasms, benign tumors, polycystic kidney disease, renal cysts, and hydronephrosis. The pathophysiological excess of Epo leads to erythrocytosis that is accompanied by an increase in blood viscosity and may cause heart failure and pulmonary hypertension.

Chronic kidney disease causes the destruction of Epo-producing cells resulting in hyporegenerative normochrome normocytic anemias. Epo is therefore clinically used for the treatment of patients with severe kidney insufficiency. In uremic patients, treatment with recombinant human Epo (rhEpo) effectively reactivates the bone marrow to produce erythrocytes, and also improves platelet adhesion and aggregation. Hypertension is an important complication in the treatment of renal anemia with rhEpo. rhEpo is also used to treat non-renal forms of anemia caused by chronic infections, inflammation, radiation therapy and chemotherapy. Beyond ameliorating anemia, rhEpo has been shown to restore radiosensitivity and increase cytotoxity of chemotherapy in the treatment of cancer-related anemia. However, clinical trials have shown increase in the relative risk of thrombo-embolic complications and lower survival, which raises concerns about the potential adverse effects of rhEpo in cancer patients. Additional studies show that Epo and EpoR expression also occurs in tumor cells, suggesting the potential for the generation of an autocrine or paracrine growth-stimulator Epo-EpoR loop in cancer cells. Further studies will be required to investigate the effects, if any, of rhEpo therapy on disease progression and survival. For its role in stimulating the production of erythrocytes, an important application of Epo is the pre-surgical activation of erythropoiesis allowing for the collection of autologous donor blood. rhEpo has emerged as a novel anti-inflammatory and cytoprotective agent, as evidenced by its physiological response to various forms of tissue injury. Accordingly, the therapeutic potential of Epo has been shown in acute renal failure, diabetic neuropathy, myocardial infarction and cerebral ischemia. The recent characterization of Epo variants, such as asialo-Epo and carbamylated-Epo, that retain nonhematopoietic, tissue-protective properties of Epo without stimulating erythropoiesis has uncovered new areas of research into the mechanisms of Epo-mediated signaling in nonhematopoietic tissues as well as novel clinical applications for rhEpo and its derivatives in disorders other than anemia.

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Erythropoietin Receptor

Definition

Is a transmembrane protein capable of specifically engaging ►Erythropoietin by the extracellular N-terminal domain and of activating selective metabolic pathways by its intracellular C-terminal end. The C-terminal domain is mutated in some forms of polycythemia.

►Polycythemia

ES

Definition

Ewing sarcoma; largely undifferentiated small-round-cell tumor of bone and soft tissue in children and young adults.

►Ewing Sarcoma

ESA

Definition

Antigen expressed on the baso-lateral cell surface in most human simple epithelia and a vast majority of carcinomas.

►Pancreatic Cancer Stem Cells
►EpCAM

ESF

►Erythropoietin

ESFT

Definition

Ewing sarcoma family tumor; generic term for Ewing sarcoma, peripheral primitive neuroectodermal tumor, and Askin tumor; synonymous to Ewing tumor (ET).

►Ewing Sarcoma

ESM-1

►Endocan

Esophageal Cancer

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Definition

Esophageal cancer comprises two main types of malignant epithelial neoplasms: squamous cell carcinoma, originating from the lining squamous epithelium of the esophagus and adenocarcinoma (also called Barrett adenocarcinoma), originating from metaplastic columnar epithelium in the lower part of the esophagus.

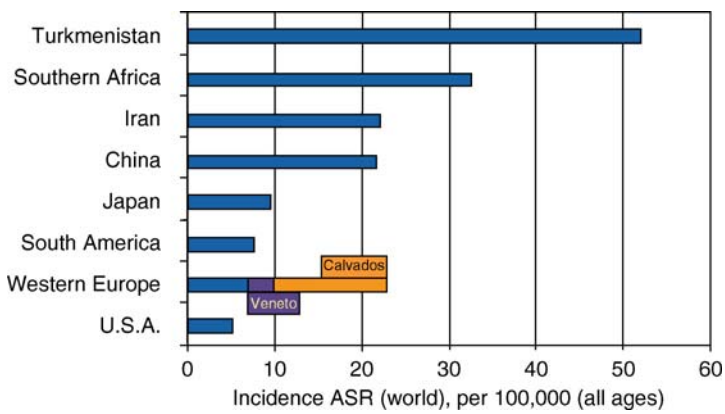
Characteristics

Esophageal cancer is the sixth most frequent cancer worldwide. In 2001, the estimated number of deaths due to esophageal cancer amounted to about 338,000 out of a total of 6.2 million cancer deaths. Of those, more than 80% occurred in developing countries, the majority being squamous cell carcinomas. The occurrence of this cancer varies greatly in different part of the world, with areas of high mortality rate per year in regions of South Africa, North east of Iran and China (30 or more per 100,000 in males and 10 per 100,000 in females). In Europe or USA, the age standardized annual mortality of squamous cell carcinoma is no more than 5 in males and 1 in females per 100,000. There are however, areas in Europe, namely in Normandy and Brittany in France and North east of Italy, where the mortality rates, at least in males, are as high as those observed in China.

Adenocarcinoma of the esophagus is less frequent and occurs mainly in industrial countries. However, recent epidemiological data show increasing numbers of adenocarcinoma cases and this type of cancer accounts for more than 50% of all oesophageal cancer in USA.

The 5-year survival rate for patients with squamous cell carcinoma and adenocarcinoma of the esophagus is similarly poor (~10%), with no difference between industrial and developing countries. This is mainly due to their late detection and the poor therapy efficacy. No reliable prognostic markers are available.

Epidemiological studies have clearly shown that tobacco smoke (►Tobacco carcinogenesis) and alcohol, together with a low intake of fresh fruits, vegetables, and meat, is causally associated with squamous cell carcinoma. It is estimated that in industrial countries approximately 90% of this cancer is attributable to tobacco and alcohol consumption. Other risk factors are chewing of betel, consumption of pickled vegetables, and hot mate drink in South East Asia, China, and South America, respectively.



Esophageal Cancer. Figure 1 Incidence of esophageal cancer in males by selected world regions. [From Parkin et al. (1999)].

Adenocarcinoma of the esophagus arises from Barrett's esophagus, a condition in which the normal squamous epithelium is replaced by metaplastic columnar epithelium. This condition is frequently present in patients with chronic gastroesophageal reflux and these patients have a more than 100-fold higher risk than the general population to develop adenocarcinoma.

Squamous cell carcinoma and adenocarcinoma of the esophagus show multiple genetic alterations (point mutations, allelic loss, and gene amplification) of several oncogenes and tumor suppressor genes. The most interesting observation in both cancer types is the high prevalence of mutations (up to 80%) of the tumor suppressor gene *p53*. In addition a distinct pattern of p53 mutations, namely a high prevalence of G>A transitions at CpG sites in adenocarcinoma and a higher prevalence of G>T tranversions and mutations at A:T base pairs in squamous cell carcinoma. There is good evidence that the mutations in squamous cancer types are attributable to carcinogens present in tobacco smoke. In both types of cancers, p53 mutations occur very early and are followed by the accumulation of other genetic alterations during the process of esophageal carcinogenesis. It is evident that these genetic alterations are relevant not only in the understanding of the multifocal monoclonal origin of this cancer but also to the elucidation of his multifactorial etiology.

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ESR2

►Estrogen Receptor

Estradiol

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Definition

Estradiol or Estradiol-17β is biologically the most active estrogen and circulating estrogens are mainly originated from ovarian ►steroidogenesis in premenopausal women and peripheral aromatization of ovarian and adrenal androgens in postmenopausal women.

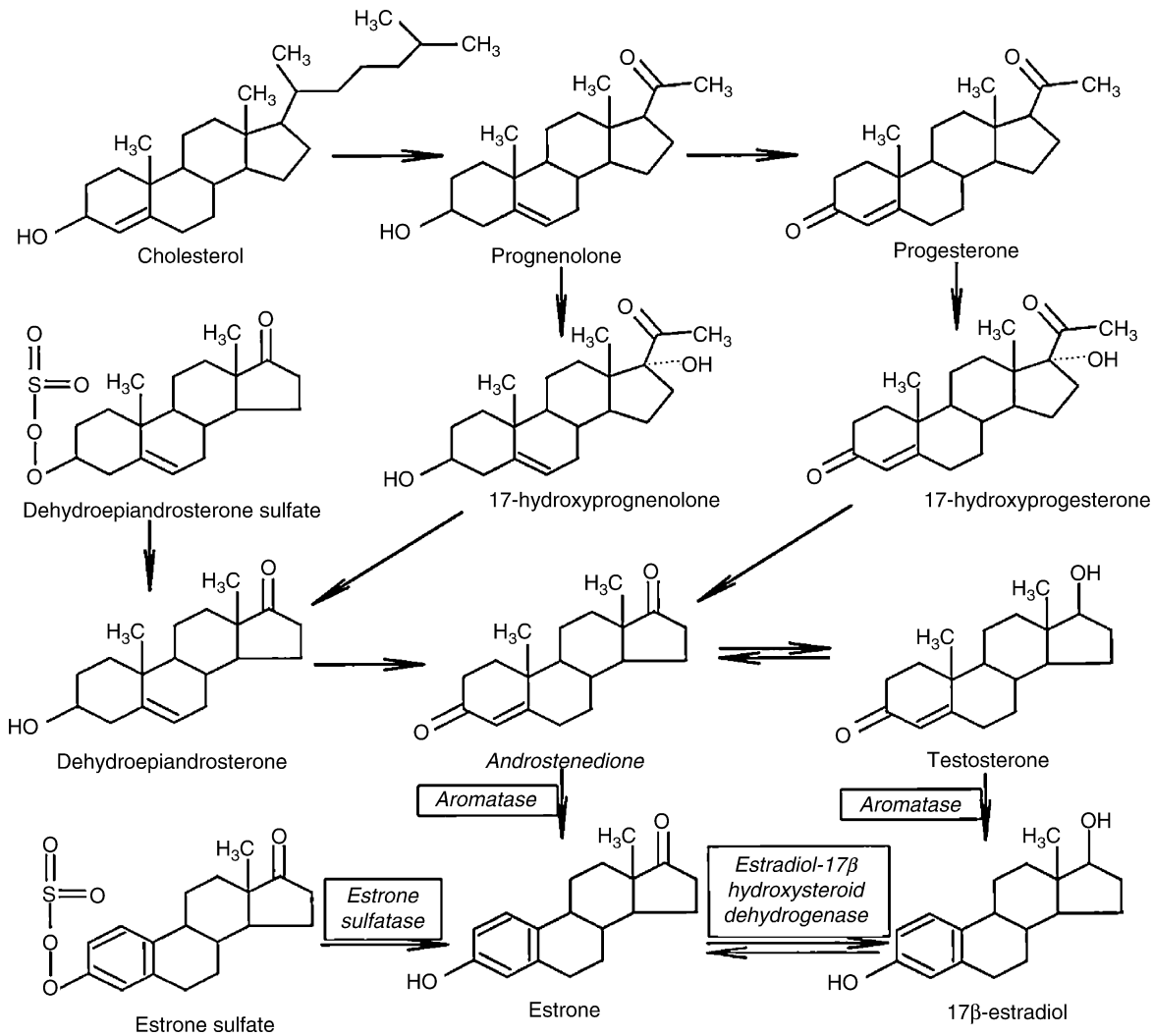
Characteristics

Mechanism of Action

It is generally accepted that the biological activities of estrogens are mediated by nuclear ►estrogen receptors (ER) which, upon activation by cognate ligands, form homodimers with another ER–ligand complex and activate transcription of specific genes containing the estrogen response elements (ERE). According to this classical model, the biological responses to estrogens are mediated by the ER universally identified until recently, which has been termed as ERα after the discovery of a second type of ER (ERβ). The presence of ERα in target tissues or cells is essential to their responsiveness to estrogen action. In fact, the expression levels of ERα in a particular tissue have been used as an index of the degree of estrogen responsiveness. For example, human breast carcinomas are initially positive for ERα, and their growth can be stimulated by estrogens and inhibited by ►antiestrogens. The ERβ has been cloned from the rat, mouse, and human. ERβ and ERα share high sequence homology, especially in the regions or domains responsible for specific binding to DNA and the ligands. ERβ can be activated by estrogen stimulation, and blocked with antiestrogens. Upon activation, ERβ can form homodimers as well as heterodimers with ERα. The existence of two ER subtypes and their ability to form DNA-binding heterodimers suggests three potential pathways of estrogen signaling: via the ERα or ERβ subtype in tissues exclusively expressing each subtype and via the formation of heterodimers in tissues expressing both ERα and ERβ. In addition, estrogens and antiestrogens can induce differential activation of ERα and ERβ to control transcription of genes that are under the control of an AP1 element.

Sources of Estrogens

Circulating estrogens are mainly originated from ovarian steroidogenesis in premenopausal women and peripheral aromatization of ovarian and adrenal androgens in postmenopausal women. Three main



Estradiol. Figure 1 Steroidogenic pathways leading to the biosynthesis of estrogens. [Reprinted from: Russo, Russo IH (2004) Biological and molecular basis of breast cance. Springer–Verlag, Heidelberg].

enzyme complexes that are involved in the synthesis of biologically active estrogen (i.e., estradiol-17β) (Fig. 1): (i) aromatase that converts androstenedione to estrone, (ii) estrone sulfatase that hydrolyses the estrogen sulfate to estrone, and (iii) estradiol-17β hydroxysteroid dehydrogenase that preferentially reduces estrone to estradiol-17β in tumor tissues.

Role of Estrogens in Human Breast Carcinogenesis

There are three mechanisms that have been considered to be responsible for the carcinogenicity of estrogens: receptor-mediated hormonal activity, which has generally been related to stimulation of cellular proliferation, resulting in more opportunities for accumulation of genetic damages leading to carcinogenesis, a cytochrome P450 (CYP)-mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates, and the induction of aneuploidy by estrogen. There is also evidence that estrogen compromises the DNA repair system and allows accumulation of lesions in the genome essential to estrogen-induced tumorigenesis.

Receptor-Mediated Pathway

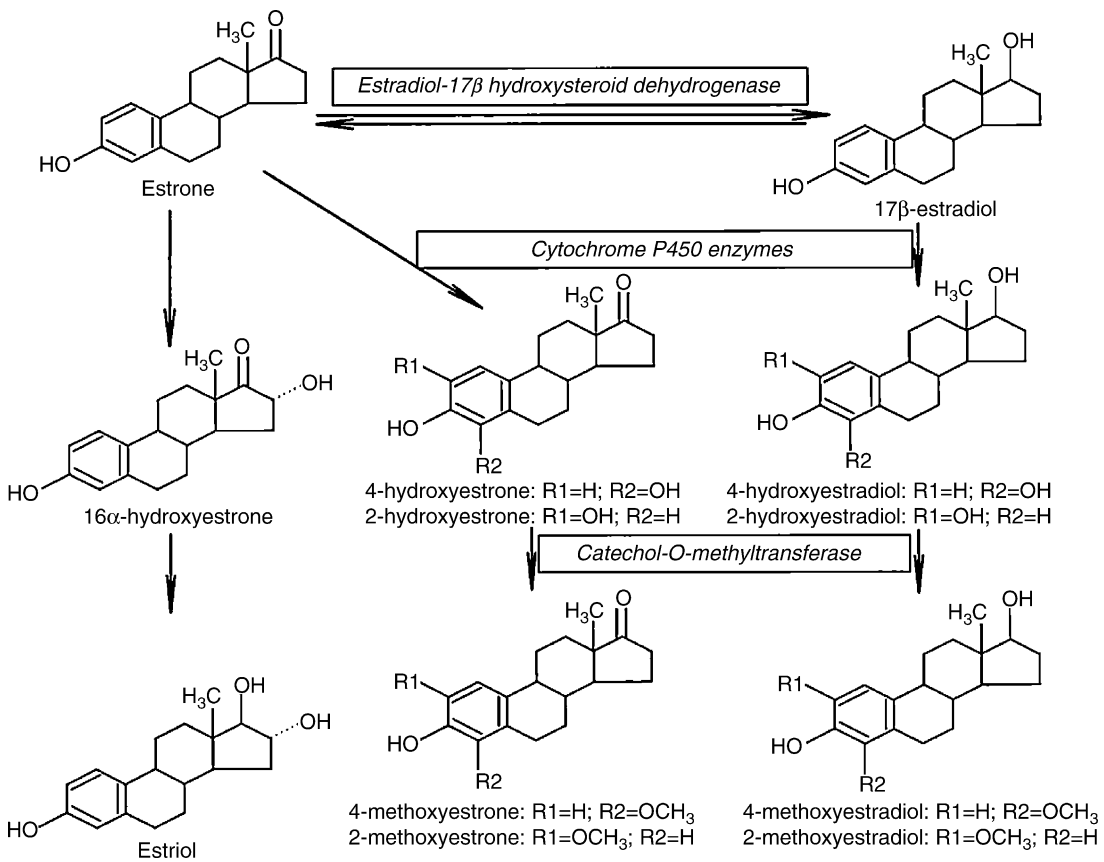
The receptor-mediated activity of estrogen is generally related to induction of expression of the genes involved in the control of cell cycle progression and growth of human breast epithelium. The biological response to estrogen depends upon the local concentrations of the active hormone and its receptors. The proliferative activity and the percentage of ERα-positive cells are highest in ►Lob 1 in comparison with the various lobular structures composing the normal breast. These findings provide a mechanistic explanation for the

higher susceptibility of these structures to be transformed by chemical carcinogens in vitro, supporting as well the observations that Lob 1 are the site of origin of ▶ductal carcinomas. The presence of ERα-positive and ERα-negative cells with different proliferative activity in the normal human breast may help to elucidate the genesis of ERα-positive and ERα-negative breast cancers. Even though it is now generally believed that alterations in the ER-mediated signal transduction pathways contribute to breast cancer progression toward hormonal independence and more aggressive phenotypes, there is also mounting evidence that a membrane receptor coupled to alternative second messenger signaling mechanisms are operational, and may stimulate the cascade of events leading to cell proliferation. This knowledge suggests that ERα-negative cells found in the human breast may respond to estrogens through this or other pathways. The biological responses elicited by estrogens are mediated, at least in part, by the production of autocrine and paracrine growth factors from the epithelium and the stroma in the breast. In addition, evidence has accumulated over the last decade supporting the existence of ER variants, mainly a truncated ER and an exon-deleted ER. It has been suggested that expression of ER variants may contribute to breast cancer progression toward hormone independence. Although more studies need to be done in this direction, it is clear that the findings that in the normal breast the proliferating and steroid hormone receptor positive cells are different open new possibilities for clarifying the mechanisms through which estrogens might act on the proliferating cells to initiate the cascade of events leading to cancer.

E

Oxidative Metabolism of Estrogen

There is evidence that the oxidative catabolism of estrogens, which is mediated by various CYP complexes, constitutes a pathway of their metabolic activation to reactive free radicals and intermediate metabolites that can cause oxidative stress. Estradiol-17β and estrone, which are continuously interconverted by estradiol-17β hydroxysteroid dehydrogenase (or 17β-oxidoreductase), are the two major endogenous estrogens (Fig. 2). They are generally metabolized via two major pathways: hydroxylation at C-16α position



Estradiol. Figure 2 Biosynthesis and steady-state control of catechol estrogens in human breast tissues. [Reprinted from: Russo J, Russo IH (2004) Biological and molecular basis of breast cancer, Springer–Verlag, Heidelberg].

and at the C-2 or C-4 positions (Fig. 2). The carbon position of the estrogen molecules to be hydroxylated differs among various tissues and each reaction is probably catalyzed by various CYP isoforms. For example, in MCF-7 human breast cancer cells, which produce ►catechol estrogens in culture, CYP 1A1 catalyzes hydroxylation of estradiol-17β at C-2, C-15α, and C-16α, CYP 1A2 predominantly at C-2, and a member of the CYP 1B subfamily is responsible for the C-4 hydroxylation of estradiol-17β. CYP3A4 and CYP3A5 have also been shown to play a role in the 16α-hydroxylation of estrogens in human.

The hydroxylated estrogens are catechol estrogens that will easily be autooxidated to semiquinones and subsequently quinones, both of which are electrophiles capable of covalently binding to nucleophilic groups on DNA via a Michael addition and, thus, serve as the ultimate carcinogenic reactive intermediates in the peroxidatic activation of catechol estrogens. In addition, a redox cycle consisting of the reversible formation of the semiquinones and quinones of catechol estrogens catalyzed by microsomal P450 and CYP-reductase can locally generate superoxide and hydroxyl radicals to produce additional DNA damage. Furthermore, catechol estrogens have been shown to interact synergistically with nitric oxide present in human breast generating a potent oxidant that induces DNA strand breakage.

Steady-state concentrations of catechol estrogens are determined by the CYP-mediated hydroxylations of estrogens and monomethylation of catechols catalyzed by blood-borne catechol *o*-methyltransferase (Fig. 2). Increased formation of catechol estrogens as a result of elevated hydroxylations of estradiol-17β at C-4 and C-16α positions occur in human breast cancer patients and in women at a higher risk of developing this disease. There is also evidence that lactoperoxidase, present in milk, saliva, tears, and mammary glands, catalyzes the metabolism of estradiol-17β to its phenoxyl radical intermediates, with subsequent formation of superoxide and hydrogen peroxide that might be involved in estrogen-mediated oxidative stress. A substantial increase in base lesions observed in the DNA of invasive ductal carcinoma of the breast (34) has been postulated to result from the oxidative stress associated with metabolism of estradiol-17β.

The breast is an endocrine organ and can synthesize E_2 in situ from precursor androgens via the enzyme aromatase. Breast tissue contains aromatase and produces amounts of E_2 that exert biologic effects on proliferation. The effects of local production exceed those exerted in a classical endocrine fashion by uptake of E_2 from plasma. One critical factor is excessive synthesis of E_2 by overexpression of CYP19 in target tissues and/or the presence of excess sulfatase that converts stored E_1 sulfate to E_1. The observation that breast tissue can synthesize E_2 in situ suggests that much more E_2 is present in some locations of target tissues than would be predicted from plasma concentration. A second critical factor might be high levels of 4-CE due to overexpression of CYP1B1, which converts E_2 predominantly to 4-OHE_2. This could result in relatively large amounts of 4-CE and, subsequently, more extensive oxidation to their CE-3, 4-Q. A third factor could be a lack or low level of COMT activity. If this enzyme is insufficient, either through a low level of expression or its low activity allele, 4-CE will not be effectively methylated, but will be oxidized to the ultimate carcinogenic metabolite, CE-3, 4-Q. Fourth, a low level of GSH and/or low levels of quinone reductase and/or CYP reductase can leave available a higher level of CE-Q that may react with DNA.

The effects of some of these factors have already been observed in analyses of breast tissue samples from women with and without breast cancer. The levels of E_1 (E_2) in women with carcinoma were higher. In women without breast cancer, a larger amount of 2-CE than 4-CE was observed. In women with breast carcinoma, the 4-CE were 3.5 times more abundant than the 2-CE and were 4 times higher than in the women without breast cancer. Furthermore, a statistically lower level of methylation was observed for 2-CE and 4-CE in cancer cases versus controls. Finally, the level of CE-Q conjugates in women with cancer was three times that in the controls, suggesting a larger probability for the CE-Q to react with DNA in the breast tissue of women with carcinoma. The levels of $E_1(E_2)$ ($p < 0.02$) and quinone conjugates ($p < 0.01$) are highly significant predictors of breast cancer, and the levels of methylated CE ($p < 0.02$) are significant predictors of protection against breast cancer. Altogether, these data are supporting the concept that estrogen and its metabolites can be found at high concentration in the breast tissue indicating a direct carcinogenic effect in the breast epithelial cells.

Estrogens as Inducers of Aneuploidy

Breast cancer is considered the result of sequential changes that accumulate over time. DNA content changes, i.e., loss of heterozygosity (LOH) and aneuploidy, can be detected at early stages of morphological atypia, supporting the hypothesis that aneuploidy is a critical event driving ►neoplastic development and progression. Aneuploidy is defined as the gain or loss of chromosomes; it is a dynamic, progressive, and accumulative event that is almost universal in solid tumors. The extensive array of altered gene expression observed in tumors and the numerous altered chromosomes detected by comparative genomic hybridization provide striking evidence, that aneuploidy can totally disrupt cell homeostatic control. The main question is whether aneuploidy is a consequence of neoplastic development or a cause of

neoplastic development. One of the several mechanisms proposed for the development of aneuploidy is the failure to appropriately segregate chromosomes. For example, interference with mitotic spindle dynamics, abnormal centrosome duplication, altered chromosome condensation and cohesion, defective centromeres, and loss of mitotic checkpoints. Functional consequences of centrosome defects may play a role during neoplastic transformation and tumor progression, increasing the incidence of multipolar mitoses that lead to chromosomal segregation abnormalities and aneuploidy. In considering estrogen as a carcinogenic agent, there is evidence that it affects microtubules and a recent report indicates that progesterone may facilitate aneuploidy. The importance of these findings is magnified with the recent publications that demonstrate women on ►hormone replacement treatments that include progesterone have increased mammographic breast density and increased breast cancer risk than women taking only estrogen.

In the center stage of the research endeavor on aneuploidy are the centrosomes that are organelles that nucleate microtubule growth and organize the mitotic spindle for segregating chromosomes into daughter cells, establishing cell shape and cell polarity, processes essential for epithelial gland organization. Centrosomes also coordinate numerous intracellular activities, in part by providing a site enriched for regulatory molecules, including those that control cell cycle progression, centrosome and spindle function, and cell cycle checkpoints. Although the underlying mechanisms for the formation of abnormal centrosomes are not clear, several possibilities have been proposed and implicated in the development of cancer such as alterations of checkpoint controls initiating multiple rounds of centrosome replication within a single cell cycle and failure of cytokinesis, cell fusion, and cell cycle arrest in S-phase uncoupling DNA replication from centrosome duplication.

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17β-Estradiol

Definition

The major sex hormone present in females.

►Estrogenic Hormones

Estramustine

Definition

A ►nitrogen mustard linked to ►estradiol; used to treat ►prostate cancer. Estramustine and its major metabolite estramustine bind to microtubule-associated proteins (MAPs) and ►tubulin, thereby inhibiting ►microtubule dynamics and leading to ►anaphase arrest in a dose-dependent fashion. Also has radiation-protective properties.

Estrogen Receptor

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Synonyms

ER; Estrogen receptor alpha ESR1; Estrogen receptor beta; ESR2

Definition

Estrogen receptors are represented by two main members (estrogen receptor alpha, ERα and estrogen receptor beta, ERβ), which bind ►estrogens. These receptors are mainly nuclear, even though membrane receptors are also suspected to exist. Nuclear estrogen receptors belong to a large family of ►nuclear receptors, which are ligand-activated ►transcription factors able to modulate the expression of different genes.

Characteristics

History

The mediators of estrogens, namely estrogen receptors, remained elusive until the synthesis of radiolabeled ►estradiol by Jensen and Jacobsen in 1960, which allowed the identification of such receptors. But it

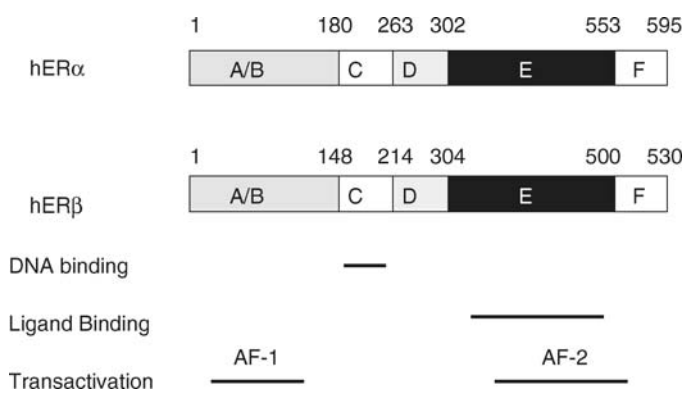
took more than 20 years, with the development at a high rate of molecular biology techniques to have the first cDNA encoding estrogen receptor (α) cloned by the group of Chambon in Strasbourg. In 1996, when most of nuclear receptors had already been cloned, a great surprise arose with the fortuitous isolation of a second estrogen receptor (β) cloned from a prostate library.

Molecular Mechanisms of Action

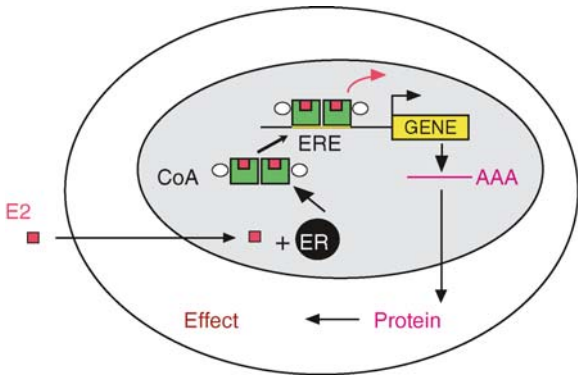
The genes coding for estrogen receptors ERα and ERβ are located on two distinct chromosomes (ERα on chromosome 6q25.1, and ERβ on chromosome 14q22–24). ERα and ERβ proteins have a respective size of 595 and 530 amino acids (Fig. 1). As indicated by their membership to nuclear receptors, estrogen receptors are mainly present in the nucleus of cells, even in the absence of estrogens. In addition, a small pool of ERs localize to the plasma membrane and signal mainly through coupling to G-proteins, but we will not discuss this issue here. Upon binding to estrogens, nuclear estrogen receptors dimerize, bind ►coactivators and interact with DNA or DNA-bound proteins to modulate the transcription of estrogen target genes (Fig. 2). Estrogen receptors share the common structure of nuclear receptors composed of 5 domains (A, B, C, D, E, and F) (Fig. 1). The C domain (DBD) is responsible for DNA binding. Two distinct synergistic transcriptional activation functions (AFs) have been identified: the ligand-independent AF-1 located in the N-terminal A/B region, and the ligand-dependent AF-2 encompassing region E (the LBD). Both ER AF-1 and AF-2 were found to act in promoter context and cell-specific fashions. Coactivators are mainly interacting with the E domain, but an increasing number of coactivators are also able to interact with the AB domain and the DBD. Estrogen receptors modulate the transcription either by binding to classical estrogen receptor element (ERE) or by interacting with ►AP-1, SP-1, or NF-kB-bound transcription factors. Many EREs consist of two inverted, palindromic half sites and are frequently present in the upstream regulation region of estrogen target genes. On the majority of ERE promoters, ERβ is less potent activator than ERα. Interestingly, ERα and ERβ form preferentially heterodimers rather than homodimers when they are both expressed in the same cell, which enables ERβ to decrease ERα transcriptional potential. Both estrogen-bound receptors mediate gene transcription similarly through the ERE pathway but have opposite effects when signaling through the AP-1 pathway. Indeed, ERα activates AP-1 gene transcription, whereas ERβ inhibits this pathway. Moreover, Tamoxifen-liganded ERα is inactive on AP-1 elements, but ERβ is able to activate the transcription response.

What have We Learned from ER Knock-Out Mice?

Disruption of the ERα gene (αERKO animals) is not lethal, but rather the animals develop normally and exhibit a life span comparable to their wild-type litter mates. αERKO mice exhibit several abnormalities and deficiencies, most notable of which are the phenotypic syndromes that result in infertility in both sexes. The mammary phenotype of αERKO female mice demonstrates that embryonic mammary gland development is independent of ERα but ERα is required for ductal elongation during puberty and complete mammary gland development in the mature mouse. The female reproductive tract of αERKO undergoes normal pre- and neonatal development but is insensitive to estrogens during adulthood. Ovaries undergo normal pre- and neonatal development, but are anovulatory during adulthood, exhibit multiple hemorrhagic cysts, and no corpora lutea. There is also a 30–40% incidence of ►ovarian tumors by 18 months of age. Male reproductive tract of αERKO displays dilation of Rete testis, atrophy of the seminiferous epithelium, and decreased sperm counts, leading to infertility.



Estrogen Receptor. Figure 1 Schematic representation of ERα and ERβ proteins.



Estrogen Receptor. Figure 2 Mechanism of action of estrogen receptors. CoA: coactivator; E2: ►estradiol; ERE: estrogen responsive element.

Mice lacking ERβ (βERKO) display a much less pronounced phenotype than αERKO. They develop normally and are indistinguishable grossly and histologically from their littermates. Sexually mature βERKO females are fertile and exhibit normal sexual behavior, but they produced substantially fewer litters as well as significantly less number of pups per litter when compared with their wild-type littermates. This reduction in fertility is the result of reduced ovarian efficiency. The mutant females have normal breast development and lactate normally. However, lactating glands display alveoli which are larger and there is less secretory epithelium in ERβ than in wild-type mice. Ovaries undergo normal pre- and neonatal development, but do not exhibit normal frequency of spontaneous ovulations during adulthood, exhibit a severely attenuated response to superovulation treatment with reduced number of oocytes and multiple trapped preovulatory follicles. In addition, male βERKO mice develop prostate hyperplasia.

ER disruption has also distinct effects on sexual behavior of the animals. αERKO male mice, although they rarely ejaculate and are infertile, show almost normal levels of mounts and just reduced levels of intromissions. In contrast, all three components of sexual behaviors are present and robust in βERKO males. Aggressive behavior is greatly reduced in αERKO male mice. In particular, male-typical offensive attacks are almost completely abolished, whereas lunge and bite attacks are still present. On the other hand, the aggressive behavior of βERKO is not reduced but rather elevated depending on age and social experiences.

Involvement of Estrogen Receptors in Physiology and Cancer

Estrogens play a critical role in many physiologic processes, including reproduction, cardiovascular health, bone integrity, immune system, cognition, and behavior. But estrogens are also involved in many pathologies including for instance osteoporosis, endometriosis, or neurodegenerative diseases, but also cancer (breast, ►ovarian, ►endometrial, ►prostate, ►colorectal).

ERα and ERβ display disparity in their tissue distribution. Studies on rat have shown that ERα mRNA is predominant in the uterus, mammary gland, testis, pituitary, liver, kidney, heart, and skeletal muscle, whereas ERβ transcripts are significantly expressed in the ovary and prostate. In human, ERα and ERβ are both present in the brain, breast, cardiovascular system, and bone. In addition, ERα is much more abundant than ERβ in uterus and liver, whereas the opposite is true for ERβ in ovary, prostate and gastrointestinal tract. This differential distribution suggests that the two receptors could play distinct roles.

The most characterized cancer location involving estrogen receptors is definitely the breast. The cumulative exposure of the breast epithelium to estrogens is one of the first risk factor for ►breast cancer incidence. Estrogens are not only believed to be involved in the development and growth of breast tumors but also the late course of the disease, in their ►metastasis. The potential carcinogenic properties of estrogens are also suspected for endometrial and ►ovarian cancers. Two current hypothesis could account for estrogen carcinogenic action. First, estrogen metabolism could lead to the production of genotoxic products which directly alter DNA integrity. Second, by stimulating cell proliferation, estrogens could increase the number of cell divisions and thus the risk of replication errors, leading possibly to selective advantages for the mutated cells, which would exhibit defects in terms of apoptosis, proliferation, and DNA repair. It is interesting to note that this second hypothesis can be further divided into two subhypothesis. Indeed, ERα is generally expressed at low levels in normal breast epithelium cells (approximately 10–20% depending on the phase of menstrual cycle). Moreover, in the normal breast, it seems that there is a discordance between the cells which express ERα and the one that proliferate. For this reason, some people believe that estrogens act directly on epithelial cells to stimulate the proliferation and others suggest that estrogens act on stromal cells, which in turn secrete soluble growth factors stimulating epithelial cell growth.

In contrast to the situation observed in normal breast, breast cancer cells express frequently ERα and the same cells are actively proliferating. Breast cancers are first divided into two subtypes based on whether or not the tumor cells express ERα. ERα-positive cancers represent about two thirds of all breast cancers. The reason for analyzing ERα status is based on the facts that estrogens constitute one of the major mitotic signals for ERα-positive breast cancers. Moreover, antiestrogen drugs such as tamoxifen are commonly used as first line therapy against ERα-positive breast cancers. Unfortunately though, the use of tamoxifen is rarely associated with long-term remissions in metastatic diseases.

In contrast to the situation described for ERα, it has been reported by several groups that ERβ expression was lower in cancerous tissues compared with normal tissues. This is true for breast, ►ovarian, ►prostate, lung, and colon cancers, suggesting that this could be a general trend. Moreover, the exogenous delivery of ERβ to breast and prostate cancer leads to an inhibition of proliferation of tumor cells both in vitro and in vivo. In addition, ERβ is able to reduce the ►invasion potential of tumor cells. Moreover, ERβ knock-out animals display prostate hyperplasia suggesting the possible proliferation gatekeeper role of ERβ. Unlike ERβ, ERα levels are frequently correlated to tumor grade. Patients whose tumors express ERα have a longer interval to recurrence and an improved survival. Concerning ERβ, the prognostic value of this receptor is more controversial, even though several studies have shown that ERβ was associated with a longer disease-free survival.

At the present time, it is not completely understood why tumor cells in some instances lose the expression of ERα or ERβ. Several studies suggest that promoter hypermethylation of both genes could silence the activity of ERα and ERβ promoters. On the other hand, the hyperexpression of ERα in the vast majority of breast cancers is not at all clarified. In addition, the role of the numerous splice variants identified for ERα and ERβ transcripts is not well established. The alternative splicing of ERα mRNA occurs frequently in breast tumors, but the expression of these transcripts is also usually conserved not only in primary tumors but also in distant metastases. In the same line, several single nucleotide polymorphisms (SNPs) in ERα gene have been and associated either with an increased or a decreased risk of breast cancer, whereas the situation is much less clear for SNPs found in ERβ gene.

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Estrogen Receptor Alpha ESR1

►Estrogen Receptor

Estrogen Receptor Beta

►Estrogen Receptor

Estrogen Synthase

►Aromatase and its Inhibitors

Estrogenic Hormones

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Definition

Estrogens are steroid sex hormones produced chiefly in the ovary and responsible for development and function of the female reproductive tissues such as uterus and mammary gland. Smaller amounts are also are produced by the testis in the male and the adrenal gland in both sexes, and contribute to maintenance of bone density and function of cardiovascular and neurological tissues. Estrogenic hormones play a role in both the genesis and treatment of several types of cancer. In general, estrogen-related tumors are those involving tissues of the female reproductive tract, although estrogens produce liver cancers in the hamster and are probably a factor in their occurrence in humans.

Characteristics

Etiology

Carcinogenesis is known to be a multistep process (►multistep development), involving both initiation (alteration of DNA) and promotion (proliferation of the altered cells). It is generally agreed that the principal effect of estrogenic hormones is on the promotion stage, especially in tissues where growth and function are normally regulated by estrogen. It has been controversial whether estrogens, especially in physiological amounts, also cause genetic changes in a manner similar to the action of chemical carcinogens such as dimethylbenzanthracene or nitrosourea.

Breast Cancer

The human malignancy most studied in relation to estrogen is carcinoma of the breast, both because

of its high incidence and because its involvement with estrogens is especially striking. Much evidence indicates that estrogenic hormones play an important role in the appearance of mammary cancer, both in experimental animals and in the human. It has long been known that early menarche and/or late menopause increases the risk of ►breast cancer and that artificial menopause induced by ovariectomy or radiation reduces the risk, suggesting a cumulative effect of the number of ovulatory cycles on the incidence of the disease. It is also known that full-term pregnancy before the age of 20 years confers a significant protective effect, whereas nulliparous women have an increased susceptibility to breast cancer, but the basis of this phenomenon is not clear. The effect of ►hormone replacement therapy has been the subject of much investigation and some controversy, but from the most recent studies it appears that for breast cancer the risk from unopposed estrogen is small, and the addition of progestin to the regimen makes little difference.

The putative involvement of estrogens in the genesis of breast cancer has afforded an approach to its prevention that is currently under investigation. The ►antiestrogen tamoxifen was shown to prevent both the induction of mammary tumors by dimethylbenzanthracene in the rat and the appearance of cancer in the contralateral breast after ►mastectomy in the human. Following this, a large clinical trial has demonstrated that this agent, and related antiestrogens, can lower the incidence of breast cancer in women who are at high risk for developing this disease.

Uterine, Cervical and Ovarian Cancer

Exposure to estrogen, unopposed by progestin, is a major factor in the occurrence of cancer of the uterine ►endometrium. Unopposed estrogen-replacement therapy for more than 5 years results in an elevated risk of endometrial cancer, which persists for several years after the medication has been discontinued. The addition of progestin to the estrogen-replacement regimen substantially reduces this risk. In comparison with cancers of the breast and uterus, involvement of estrogens in the etiology of cervical neoplasia is less clear. Most studies have been of the effect of oral contraceptives and generally have shown some correlation between the prolonged use of these agents and the incidence of cervical cancer. As in the case of breast cancer, late menopause, which gives rise to a longer period of ovulatory activity, results in an increased risk of ovarian cancer. Similarly, pregnancy and the use of oral contraceptives, which decrease the number of ovulatory cycles, are protective.

Vaginal Adenocarcinoma

During the period 1945–1955, large doses of estrogenic hormones were often administered to pregnant women with a history of miscarriage in the belief that this would protect against spontaneous abortion. Because orally-active steroidal hormones were not available at that time, a synthetic estrogen called diethylstilbestrol (DES) was used. In the early 1970s, a previously rare cancer, clear cell ►adenocarcinoma of the vagina, began to appear in the daughters born to the DES-treated mothers, leading to the impression that estrogens in general, and DES in particular, are carcinogens and should not be used for human medication. However, the amounts administered (0.5–1.0 g) were 5,000–10,000 times the hormonally active dose, and the cancers produced were not in those persons receiving the estrogen but in their offspring, indicating that this is an in utero phenomenon and the action of the hormone is better described as ►teratogenic than carcinogenic. Longer follow-up has demonstrated a slightly increased incidence of breast cancer among the DES-treated mothers, but not what might be expected from such high doses of a true carcinogen. Genital abnormalities were produced in the male offspring, in keeping with a teratogenic phenomenon.

Hepatoma

In certain animal species such as the hamster, liver cancer can be induced by the simple administration of estrogens. In the western world, primary liver cancer in humans is a relatively rare phenomenon, except for individuals with cirrhosis. However, it is a major cause of death in Asia and South Africa. The introduction of oral contraceptives has led to an increased incidence of liver tumors after long-term use of preparations containing substantial amounts of estrogen. It has been reported that hepatomas can arise from the use of prolonged ►diethylstilbestrol (DES) therapy for prostatic cancer.

Therapy

When cancer occurs in tissues where growth and function depends on estrogenic hormones, in some instances the malignant cells retain their hormone dependency while others lose the need for continued stimulation. It is not clearly established what is the exact basis for hormone dependency, and whether escape from this regulation takes place on neoplastic transformation or during subsequent tumor progression. For cancers that retain hormone dependency, depriving them of estrogen provides an effective palliative treatment, less traumatic than ►cytotoxic chemotherapy, which is the only recourse for the majority of non-hormone-dependent ►metastatic cancers.

Breast Cancer

Hormone-dependent mammary tumors can be deprived of supporting estrogen either by removing the organs in which the hormone is produced, administration

of substances that inhibit estrogen ►biosynthesis, or by giving a so-called antiestrogen that prevents the hormone from exerting its growth-stimulating effect in the cancer cells. More than a century ago, before it was known what estrogens are or that they are produced in the ovary, it was found that removal of the ovaries from young women with advanced breast cancer caused remission of the disease in some patients. But the majority of breast cancers occur in postmenopausal women, where the ovaries are no longer functional, and it was long suspected that in the older patient the adrenal glands are the source of supporting estrogen. When cortisone became available, first for the treatment of inflammatory diseases, it became possible to remove the adrenal glands or the pituitary gland which controls them and maintain the patient on ►glucocorticoid replacement therapy. Subsequent clinical experience showed that about one-third of all the patients have mammary tumors that undergo remission when deprived of supporting hormone by any of these procedures, and ►endocrine ablation became first-line therapy for advanced breast cancer, especially after methods were developed to predict which patients will or will not respond to endocrine manipulation.

When it was demonstrated that estrogens, like steroid hormones in general, exert their physiological actions in combination with specific receptor proteins, it was established that patients whose tumors contain low or negligible amounts of ►estrogen receptor (ER) rarely respond to any kind of endocrine therapy, whereas most, but not all, patients with ER-rich cancers benefit from such treatment. Determination of estrogen receptor on excised breast cancer specimens, either by immunological or hormone-binding procedures, is now standard clinical practice.

As an alternative to endocrine ablation, hormone deprivation can be effected by inhibiting the enzymes involved in estrogen biosynthesis. This approach has the advantage that it eliminates not only estrogen arising from the ovary or adrenal gland, but also that which, in some cases, appears to be produced by the tumor itself. The first successful agent for this purpose was aminoglutethimide, which inhibits the key enzyme, ►aromatase, but its clinical utility has been limited by undesirable side effects. Several improved compounds have been developed recently including fadrozole, letrozole, vorozole and arimidex, which show promise of increased activity with reduced toxicity.

With the advent of ►tamoxifen, the first antiestrogen to be tolerated on prolonged administration, this reversible treatment has largely replaced the irreversible endocrine ablation as first-line therapy for ►endoplasmic reticulum (ER)-rich breast cancers. Although there are side effects from prolonged treatment, as well as a slightly increased risk of endometrial cancer, the benefits greatly outweigh the drawbacks. Tamoxifen and related non-steroidal compounds such as torimifene, raloxifene and droloxifene, show curious pharmacology in that depending on species, tissue and dose they can act either as stimulators or inhibitors. A limitation of tamoxifen therapy is the development in many patients of an "acquired tamoxifen resistance" in which the medication no longer inhibits but actually stimulates the growth of the cancer. More recently, steroidal antiestrogens such as faslodex (ICI 182,780) and RU 58668 have been developed, which show only inhibitory (antagonist) but not stimulatory (agonist) action.

Uterine and Cervical Cancers

Because growth and development of the uterus are stimulated by estrogen, attempts have been made to treat ►endometrial cancer with ►tamoxifen in a manner analogous to mammary cancer, but the response rate is low and variable. The most widely used hormonal therapy for this malignancy is treatment with ►progestin. Cervical cancer is especially sensitive to radiation and does not metastasize agressively and so surgery and/or radiotherapy are the usual therapeutic procedures, and endocrine therapy has found little application.

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Estrogens

Definition

Are female hormones. The most important estrogen is ►estradiol which increases breast cancer risk.

►Alcohol Consumption

ET

Definition
Ewing tumor; generic term for ►Ewing sarcoma, peripheral primitive neuroectodermal tumor, and Askin tumor; synonymous to Ewing sarcoma family tumor (ESFT).

►Endothelins

ET-18-OCH3

Definition
Synonym edelfosine; is an anticancer drug with a structure similar to a potent phospholipid mediator, the Platelet-activating-Factor (PAF). The difference between these two molecules resides in the presence of an ether-linked non-hydrolysable methyl- moiety instead of an acetyl- ester-liked group at the sn-2 position of glycerol in ET-18-OCH3.

►Lipid Mediators

ET-2

►Endothelins

ET-743

►Trabectedin

ET-RA

Definition
Endothelin Receptor A. One of two G-protein coupled receptors for the endothelins.

►Endothelins

ET-RB

Definition
Endothelin Receptor B. The second receptor for the endothelins, which is structurally similar to ET-RA.

►Endothelins

Eta-1

►Osteopontin

Ethanol

Definition
Synonym ethyl alcohol, chemical compound having the formula C_2H_5OH found in alcoholic beverages.

►Hepatic Ethanol Metabolism

Ether à-go-go Potassium Channels

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Synonyms
Eag; Kv10.1; KCNH1

Definition
Ether à-go-go potassium channels are transmembrane proteins opening in response to changes in ►membrane potential and allowing the movement of potassium ions.

Characteristics
Ion transport is crucial for maintaining proper cellular function; this important task is performed by several ►membrane transporters including ►ion channels.

Among other functions, ion channels play key roles in neurotransmission, muscle contraction, metabolism, sensory transduction, ►apoptosis and cell cycle progression. Because of their pivotal role in cellular function, altered expression and/or activity of ion channels leads to several diseases including cardiac arrhythmias, diabetes and epilepsy, turning these proteins into major drug targets. Participation of ion channels in cell proliferation and cell death makes them not only fundamental elements for the understanding of ►cancer but also potential clinical tools both for diagnosis and therapy of cancer.

Ether à-go-go (Eag) comprises a family of voltage-gated potassium channels opening in response to changes in membrane potential. Some members of the Eag family, namely, *human Eag1* (h-Eag1) and *human-eag-related gene* (h-Erg) are linked to major diseases. H-Erg channels have an essential role in the cardiac action potential; h-Erg mutations produce the long Q-T syndrome type 2 leading to cardiac arrhythmias and eventually – in many cases- death of the patient. A huge number of very different drugs inhibit h-Erg channels producing cardiac arrhythmias as a non-desirable side-effect, thus h-Erg has become an intensively studied ion channel when designing new drugs. In addition, both Eag1 and Erg have been found to be over-expressed in a variety of tumors. H-Erg channels have been found in leukemic cells and biopsies from ►colorectal cancer and ►endometrial cancer; hence, h-Erg expression has been suggested as a molecular marker for human neoplastic hematopoietic cells and a potential prognostic factor for colorectal cancer. H-Eag1 is the other member of the EAG family involved in cancer. In addition to be over-expressed in many human tumors, h-Eag1 possess oncogenic properties, has a more restricted distribution in normal healthy tissues in comparison to h-Erg channels and specific inhibition of h-Eag1 gene expression reduces tumor cell proliferation. In the following, only oncogenic Eag1 channels will be discussed.

Oncogenic Potential of h-Eag1 Channels

Eag1 channels display oncogenic properties. Some characteristics of tumor cells are that they are able to grow in very low serum concentration, lose ►contact inhibition and induce tumor formation when injected into immune-deficient mice. ►Cell lines that normally do not display these characteristics, acquire properties of tumor cells when forced to express h-Eag1 channels. The oncogenic potential given to the cells by Eag1 channels is specific because the expression of another type of voltage gated potassium channel in the same cell type does not induce tumor properties like those induced by Eag1. In addition, cell lines forced to express Eag1 have a higher metabolic activity and DNA synthesis than cells lacking Eag1 or expressing a different potassium channel. Findings of these oncogenic properties of Eag1 raised immediately research on the distribution of Eag1 in normal human tissues and tumor samples and its potential use as a cancer ►biomarker.

Eag1 as a Diagnostic Marker

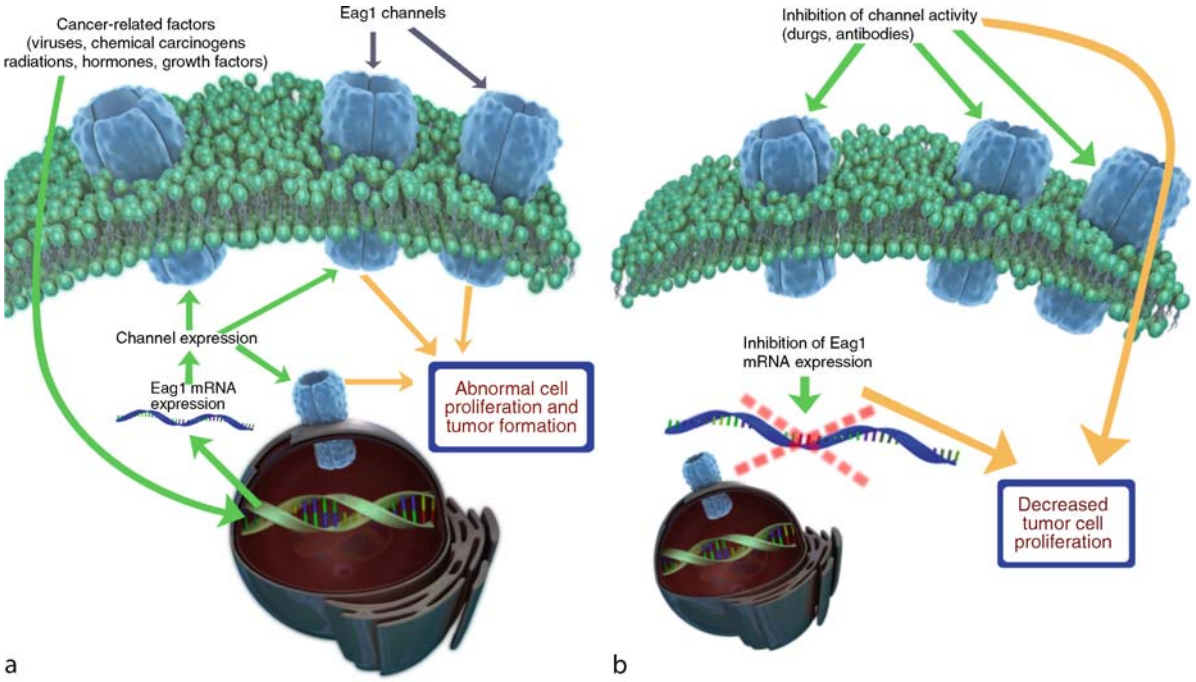
Eag1 mRNA is distributed in a very restricted manner in healthy tissues. It is mainly expressed in brain, although few amounts are found in testis, adrenal gland, placenta and is also transiently expressed in myoblasts. Actually, Eag1 expression before myoblasts fusion is the only known role of Eag1 in normal non-tumor cells; the precise participation of Eag1 in brain function remains elusive. In sharp contrast, Eag1 mRNA, protein expression (detected by specific antibodies) and/or protein activity (studied with the ►patch-clamp technique) have been found in several tumor cell lines and in a wide variety of biopsies from human tumors including lung, mammary gland, prostate, colon and uterine-cervix.

Eag1 mRNA expression has been also suggested as a potential early indicator of tumor formation. ►Cervical cancer studies revealed Eag1 mRNA expression in all of the samples from carcinomas but also in some control samples from patients with normal cervix (diagnosed by pap smears studies); these patients presented either ►human papilloma virus (HPV) infection or alteration in nearby regions (for example an ovarian cystadenoma or endometrial hyperplasia) suggesting Eag1 as a potential sign of early cellular alterations. Cancer etiological factors like viruses -including HPV-, chemical carcinogens, radiations (including ►UV radiation), ►estrogens, etc. are potential inducers of Eag1 expression with the subsequent altered cellular proliferation and tumor formation (see Fig. 1). Besides, Eag1 mRNA is present not only in mammary gland tumors but also in some free-tumor tissues from the vicinity of the tumors, while Eag1 is not found in commercially available RNA from normal mammary epithelium.

Diagnostic methods based on Eag1 expression are promising because in some cases (for example cervical cancer) cells can be obtained from the patients and tested for Eag1 protein expression with specific antibodies. On the other hand, it has been shown that fluorescent labeled antibodies against Eag1, show the presence of the protein in lymph nodes that had not been clinically evident; this approach represents a non-invasive optical technique to detect Eag1. The restricted distribution of Eag1 in normal tissues, the abundant and ubiquitous expression in tumors and the potential regulation by cancer-associated factors convert Eag1 expression in an attractive cancer biomarker.

Eag1 as a Therapeutic Target

Several approaches have demonstrated that Eag1 expression in tumor cells has an important role in cell



Ether à-go-go Potassium Channels. Figure 1 Clinical relevance of Eag1 channels. (a) Eag1 channels are overexpressed in tumor cells favoring cell proliferation, some cancer related factors are potential inducers of Eag1 channels which are expressed at the plasma membrane but also probably in the nucleus. (b) Inhibition of either channel activity or RNA expression decreases tumor cell proliferation. Therefore, Eag1 channels represent powerful tools both for cancer diagnosis and therapy.

proliferation. Eag1 channels are inhibited by several non-specific potassium channel blockers, however, at the moment there are no drugs inhibiting specifically Eag1 channel activity. Imipramine, a common antidepressant drug inhibiting several ion channels including Eag1, decreases tumor cell proliferation of different cell lines expressing Eag1, strongly suggesting that Eag1 channel activity is necessary in proliferation of human tumor cells.

More recent molecular biology strategies like ►RNA silencing have been proved to be very useful for inhibiting specific gene expression. This approach has been used to study the role of Eag1 in proliferation of tumor cells. Specific inhibition of Eag1 gene expression by small interfering RNA (►siRNA) caused a marked decrease in proliferation of several tumor cell lines. In some cases, specific Eag1 RNA silencing inhibited tumor cell proliferation in more than 80%.

Targeting Eag1 for cancer therapy (see Fig. 1) offers at least two important advantages:

1. Because of Eag1 restricted distribution in normal tissues, targeted cells would be mainly cancer cells and the side-effects in normal cells should be almost insignificant. In healthy tissues Eag1 is mainly expressed in the brain which is already protected by the ►blood brain barrier.
2. The well-known drug-resistance showed by some cancer cells via membrane transporters would be bypassed when using specific blockers of channel activity targeting the plasma membrane Eag1 channels.

Mechanisms of Oncogenicity

Molecular mechanisms explaining the oncogenic potential of Eag1 channels remain unknown, however, there are several current hypothesis which can be divided into two groups, general and particular. General hypothesis include the following:

1. Eag1 channels establish a negative membrane potential required for cell cycle progression from the G1 to the S phase. Potassium channel inhibition arrest the cells in the G1 phase of the cell cycle.
2. The negative membrane potential increases the electromotive force for calcium entry which in turn might trigger several transduction pathways.
3. Potassium movement regulates cell volume and volume changes are associated to cell proliferation for instance by altering nutrient concentration.

Particular current hypothesis are based on more structural features. Like other voltage gated potassium channels, Eag1 protein is composed by four subunits (see Fig. 1) having six transmembrane spanning

segments numbered S1–S6; the S4 segment forms the voltage sensor due to its positively charged amino-acids residues, and the loop between the segments S5–S6 forms the pore of the channel. Nevertheless, Eag1 has special sequences not shared as a whole with other potassium channels that provide potential clues on its oncogenic mechanisms and include the following:

1. Eag1 has a nuclear targeting signal presumably to direct the channel to the nucleus. It has been demonstrated for a calcium channel that a segment of the channel encodes a transcription factor regulating the expression of several genes. Something similar should be expected to happen with Eag1 if the channel or at least part of the protein was expressed in the nucleus (see Fig. 1).
2. Epsin is a protein participating in the ►endocytosis of growth factor receptors. Epsin binds to Eag1, probably this binding changes the free-epsin levels deregulating endocytosis of growth factor receptors and allowing growing signals to proceed.
3. Calmodulin binding sites (integrating calcium signals), a nucleotide binding domain and a Pern-Arnt-Sim domain (PAS domain involved in responses to ►hypoxia) as well as putative phosphorylation sites by ►protein kinase C and mitogen activated kinase (►MAP kinase) described for Eag1 might also act in concert to regulate channel activity or interaction with other proteins and favor cell proliferation.

Outline

Expression of Eag1 potassium channels confers oncogenic properties to mammalian cells. Because of their very restricted distribution in normal human tissues but more general distribution in tumor samples, Eag1 mRNA and/or protein expression offer potential tools for the diagnosis of a wide variety of neoplasms. Moreover, the potential regulation of Eag1 by cancer etiological factors like human papilloma virus, suggest Eag1 as a possible indicator of early cellular transformation. Specific inhibition of Eag1 produces a drastic decrease in tumor cell proliferation, making Eag1 a promising target for cancer therapy. Despite the molecular mechanisms of Eag1 oncogenicity are unknown, Eag1 represents a hopeful tool for cancer diagnosis and therapy.

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Etiology

►Cancer Causes and Control

Etk

Definition

Synonym Bmx, a Btk family tyrosine kinase, mediates cellular transformation by linking Src to STAT3 activation, binding to the FERM domain of FAK

►Focal Adhesion Kinase (FAK)

Etoposide

Definition

Synonym VP-16, with common trade names etopophos (etoposide phosphate) and vepesid. Etoposide is a cytotoxic chemotherapeutic. Most commonly it is used to treat ►Ewing sarcoma, testicular cancer, ►lung cancer, lymphoma, non-lymphocytic leukemia and ►glioblastoma multiforme. Etopososide inhibits DNA synthesis, through inhibition of ►DNA topoisomerase II.

Ets

Definition

Family of transcription factor genes with similar DNA-binding domain named after the first member, the avian erythroblastosis virus oncogene E26 (E twenty-six).

►Ets Transcription Factors

ETS (Environmental Tobacco Smoke)

Definition

Is the complex mixture of chemicals formed from the smoldering of a tobacco product; smoke exhaled by the smoker, smoke that escapes while the smoker inhales, and some vapor-phase components that diffuse into the environment. To date, over 50 compounds in ETS have been identified as carcinogens. Exposure to ETS is also known as passive smoking or involuntary tobacco smoke.

►Tobacco-Related Cancers
►Tobacco Carcinogenesis

Ets Transcription Factors

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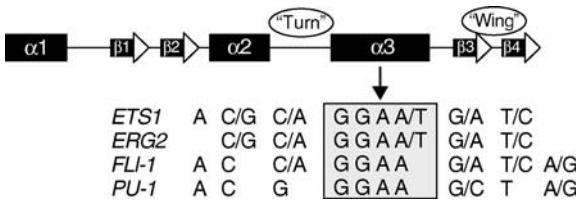
Definition

Ets transcription factors are defined by a unique DNA binding domain, the ETS domain, which specifically interacts with an ~10 bp long DNA sequence containing a 5′-GGAA/T-3′ core motify (Fig. 1) Ets stands for E26 transformation specific or E26, as the Ets sequence (v-ets) was first identified in the genome of the avian retrovirus E26. c-Ets1, closely related to v-Ets, was the first cellular Ets protein that was discovered. More than 30 different Ets proteins have been identified, found throughout the metazoan world including mammals, sea urchins, worms and insects. Currently, 27 human Ets proteins are known. The Ets family is subdivided into subfamilies based on the similarity in the ETS domain (Fig. 2).



Ets Transcription Factors. Figure 1 The ETS domain. This winged helix-turn-helix domain binds DNA by a loop-helix-loop scaffold, composed of the helix (α2)-turn-helix(α3) motif and the loops between α2 and α3 (turn) and between the β strands β3 and β4 (wing). All direct contacts with specific bases of the DNA are made by residues in the α3 recognition helix while residues of the two loops contact the phosphate backbone. The resulting neutralization of the phosphate charges is likely to induce DNA bending, as observed in Ets protein-DNA complexes. In contrast to the helices, the loops are not strictly conserved among members of the Ets family. They may, therefore, be responsible for the preference of an individual Ets protein for the sequences flanking the conserved GGAA/T binding motif.

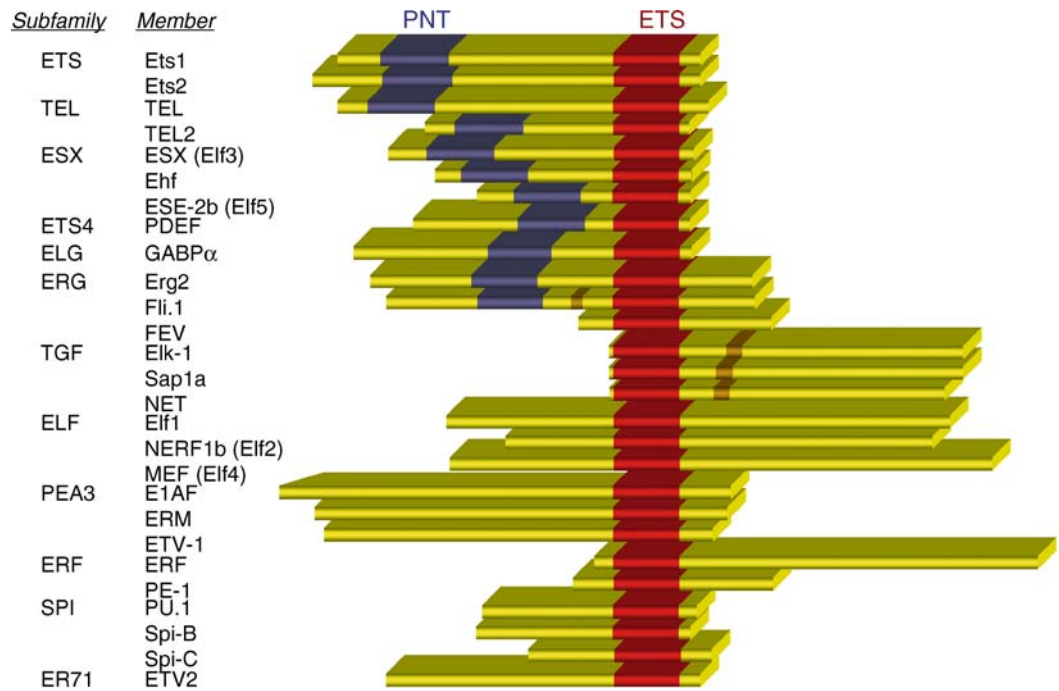
Characteristics

In contrast to many other transcription factors, Ets proteins bind to DNA as monomers. Most eukaryotic cells express a variety of Ets proteins at the same time. To achieve functional specificity, Ets proteins display differences in preference for certain nucleotides flanking the core motif in the Ets responsive DNA element and, more important, for certain cooperating partners. A strong interaction with a cooperating partner may even force Ets proteins to bind to an unfavorable DNA binding site, such as GGAG (Pax5/Ets1 partnership). In many cases, interactions with other proteins depend upon particular protein domains. E.g., for the cooperation with ►SRF, the so-called B-domain is required, which is found in the proteins of the TCF subfamily and the Fli-1 protein. The Pointed domain, named after the Drosophila Ets Protein Pointed and shared by many Ets proteins of different subfamilies, shows similarities to the sterile alpha motif (SAM) domain and is an interface for homotypic and heterotypic protein-protein interactions. In Ets1 and Ets2 proteins, the Pointed domain is the docking site for ERK1/2 allowing these kinases to phosphorylate Ets1 and Ets2 at an N-terminal threonine. In contrast, the Pointed domain of the TEL protein mediates homo-oligomerization. Most Ets proteins are transcriptional activators, others (ERF, NET, Tel, Drosophila YAN, Caenorhabditis lin-1) act as repressors. Some, such as Elk-1 and Net, can undergo activator-repressor switching (Fig. 2).

Ets proteins play an important role in transcriptional regulation. Many eukaryotic genes contain Ets DNA binding sites and are responsive to Ets proteins. Ets-responsive genes are found among critical genes that regulate fundamental cellular processes such as proliferation, differentiation, ►invasion and ►adhesion.

Ets Factors and Development

Some Ets factors, including Ets2, Esx, Ese-2, Fli-1, Pu.1, GABPα and Tel, are essential for embryonic development. Disruption of the ets2, ese-2, fli-1, pu.1, gabpα or tel gene in mice results in early death of the embryo. Lack of Ets2 or Ese-2 leads to defects in trophoblast development and to the absence of extraembryonic ectoderm markers. Ese-2 is also involved in mammary



Ets Transcription Factors. Figure 2 Members of the Ets transcription factor family in humans. The DNA binding domain (ETS), the Pointed (PNT) domain and the SRF-interacting B-domain are marked. Note that most Ets proteins have several different names. The Esx and Elf proteins are grouped into two separate subfamilies. Splicing variants of the different Ets proteins are not listed. Tel – translocation, Ets, Leukemia, Esx – epithelial-restricted with serine box, Ehf – Ets homologous factor, ESE – epithelium-specific Ets, PDEF – prostate derived Ets factor, ELG – ets like gene, GABP – GA-binding protein, Erg – ets-related gene, Fli – Friend leukemia integration, FEV – Fifth Ewing variant, TCF – ternary complex factor, Elk – ets like gene, Sap – SRF accessory protein, NET – new ets transcription factor, Elf – E74-like factor, NERF – new ets related factor, MEF – myeloid elf-1 like factor, PEA3 – polyoma enhancer A3, E1AF – adenovirus E1A factor, ERM – Ets related molecule, ETV – Ets translocation variant, ER81 – Ets related clone 81, ERF – Ets2 repressor factor, PE – PU-Ets-related, Spi – SFFV provirus integration site, PU – recognizes purine-rich sequences.

alveolar morphogenesis. Tel null mutant embryos fail to develop a vascular network in the yolk sac. Pu.1 is necessary for B- and T-cell development, erythropoiesis, terminal myeloid cell differentiation and maintenance of hematopoietic stem cells. Fli-1 null embryos die of aberrant hematopoiesis and hemorrhaging. Deficiency of GABPα which is expressed in embryonal stem cells leads to embryonic death prior to implantation. GABPα is also required for the function of neuromuscular junctions. Mice lacking Esx die early after birth. Their intestinal epithelial cells fail to differentiate and polarize as result of reduced levels of the TGFβII receptor (►transforming growth factor β). Ets1 deficiency leads to defects in B- and T-cell development. In ER81 null mice, two types of mechanoreceptors, muscle spindles and the Pacinian corpuscles, are either absent or degenerated. MEF is involved in osteogenic differentiation.

Regulation of Ets Protein Activities

The activities of Ets proteins are controlled transcriptionally and post-translationally. The expression of many Ets genes are restricted to certain cell types and/or can be induced by specific extracellular stimuli. E.g., the transcription from the Ets1 gene can be activated by a variety of factors including phorbol ester, ►AP-1, ►TP53, ►retinoic acid, ERK1/2 (►MAP kinase) and HIF-1 (►hypoxia Inducible Factor-1). Many Ets proteins undergo post-translational modifications, which have an impact on their activities. The most common post-translational modification of Ets proteins is phosphorylation by MAP kinases, such as ERK1/2. Phosphorylation by MAPK leads to activation of activating Ets proteins, such as Ets1, Ets2, Er81, Erm, Sap1, Elk1, Pea3 or GABPα, and loss of activity of repressing Ets proteins, such as Tel or Erf. When phosphorylated by ERK1/2, Net even switches from a repressor to an activator phenotype. It seems that MAPK-dependent phosphorylation (►phosphorylation of proteins) ►shifts the balance between Ets-dependent activation and repression toward activation. In the case of Ets1, MAPK-dependent phosphorylation enhances the transcriptional activity by recruitment of the coactivator CBP/p300 (►p300/CBP Co-Activators). Some Ets proteins are also targets of ►PKA, PKC (►protein kinase C Family), CaMKII (►Calcium binding proteins and Cancer), ►CKII and cyclin A-dependent

cdk2 (►cyclin-dependent kinase). CKII increases the activity of Pu.1 and Spi-B and PKCα activates Ets1. In contrast, PKA inhibits the DNA binding activities of Er81 and Erm, whereas CaMKII and cdk2 act inhibitory on Ets1 and GABPα, respectively. CaMKII phosphorylates Ets1 on serines of a serine-rich region flanking an autoinhibitory module that regulates Ets1 DNA binding activity. The inhibitory effect of CaMKII on the Ets1 protein increases with each serine that is phosphorylated within the serine-rich region allowing fine-tuning of Ets1-dependent transcription.

A few Ets proteins, Ets1, Elk-1 and Tel, have been shown to undergo ►sumoylation. This post-translational modification inhibits transcriptional activity of Ets1 and Elk-1 and abrogates the repressing activity of Tel. When sumoylated, the activating Elk-1 protein even transforms to a repressor. ►Acetylation is another means nature uses to modify the activities of Ets proteins, such as Ets1 and Er81. Er81 becomes acetylated and phosphorylated in response to Her2/neu-stimulated signaling. Acetylation takes place on two lysines within the transactivation domain of Er81 increasing its DNA binding affinity and protein stability. Elf-1 is an example of an Ets protein that becomes glycosylated. ►Glycosylation affects the subcellular localization and DNA binding activity of Elf-1.

Ets Proteins and Cancer

The Ets proteins Ets1, Ets2, Fli-1 and Erg are able to transform murine cells. These and other Ets proteins are also involved in human carcinogenesis and/or tumor progression. This is in line with the fact that many of these Ets proteins are targets of the ►Ras/Raf/MEK/ERK signaling pathway which is often deregulated in human tumors. The Ras-responsive Ets1 protein is found in different types of solid tumors, including ►carcinomas and ►sarcomas. Its overexpression often correlates with increased invasion, higher tumor microvessel density, higher grading and unfavorable prognosis. Ets1 has been linked to the regulation of key proteases, such as ►matrix metalloproteases, involved in the degradation of the ►extracellular matrix. In tumors, Ets1 is expressed by tumor cells as well as by stromal cells. By its ability to convert endothelial cells to an angiogenic phenotype, Ets1 is involved in tumor-dependent ►angiogenesis. A number of other Ets proteins, such as ERG, PEA3 and E1AF, are capable of upregulating proteases and supposed to be involved in tumor progression. PEA3 has particularly been linked to mammary gland development and oncogenesis. Fli-1 and Ets1 has been shown to regulate tenascin C (►tenascin and cancer), an extracellular matrix protein, associated with tumor progression. In some tumors, Ets genes are subject to mutations and recombinations. The inhibitory Ets protein Tel2 has been shown to induce myeloproliferative diseases in mice by cooperating with the ►Myc oncogene and stimulating proliferation. Elf-1 has been implicated in tumor-associated angiogenesis. A target of Elf-1 is Tie2 (►receptor tyrosine kinases), a receptor tyrosine kinase involved in the activation of endothelial cells. Chromosomal translocations leading to fusion proteins containing Ets proteins are observed in Ewing tumors and certain types of leukemias. EWS-Ets fusion proteins (►EWS-FLI (ets) Fusion Transcripts), most often containing Fli-1 or Erg, rarely ETV-1, E1AF or FEV, are critically involved in the development of Ewing tumors. The fusion protein presumably acts as a transcription factor that binds through the Ets domain to Ets-responsive genes. In addition, EWS-Ets proteins have been suggested to interfere with RNA splicing. Ets fusion proteins, as found in leukemias, harbor either Tel or Erg2. Erg2 is fused to TLS, a protein structurally related to EWS. Hence, TLS-Erg2 chimeric proteins are supposed to have similar functions as EWS-Ets proteins. Tel is frequently fused to tyrosine kinases, such as PDGFRβ (►platelet-derived growth factor), Abl (►BCR-ABL1) or Jak2 (►Signal Transducer and Activators of Transcription in Oncogenesis). The Pointed domain of Tel mediates homo-dimerization resulting in constitutively active kinases. In another fusion protein, Tel is linked to the DNA binding factor AML-1 (►runx) which together with CBFβ forms the transcription factor CBF. CBF activity is often inhibited in leukemic cells. As a result of Tel-dependent dimerization, CBF function is also blocked when AML-1 is fused to Tel.

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ETV6

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Synonyms

ETS variant gene 6

Definition

A gene encoding an ►*ets* domain transcription factor located on human chromosome 12 band p13. It is a frequent target of ►chromosomal translocations.

Characteristics

Discovery

The short arm of chromosome 12 is a hot spot for chromosomal rearrangements in diverse types of hematological malignancies. These rearrangements include balanced translocations with a great number of different chromosome partner bands as well as unbalanced translocations and deletions. The latter two rearrangement leads to the loss of genetic material from 12p. Molecular cytogenetic studies showed that more than half of the observed balanced translocations of 12p have breakpoints that involve the *ETV6* gene. There are currently more than 40 different 12p translocations described that involve the *ETV6* gene.

ETV6 was originally identified as the fusion partner of the ►platelet derived growth factor receptor beta gene (*PDGFRB*) in a balanced t(5;12)(q31:p13) translocation from a case with chronic myelomonocytic leukemia (CMMoL). Initially, *ETV6* was called ►*TEL* (translocation *ets* leukemia gene), but was later renamed as *ETV6* (*ets* variant gene 6) to avoid confusion with the abbreviation for telomere.

Protein Domains

ETV6 is a member of the *ets* (E-26 transforming specific) family of ►transcription factors. All *ets* family proteins share a very conserved protein domain of about 88 amino acids in length, the so called *ets* domain (Fig. 1). The *ets* domain is a sequence specific DNA binding domain but also mediates protein-protein interaction. It is evolutionarily highly conserved and found in invertebrates such as *Drosophila* and *C. elegans*. The *ets* domain of *ETV6* is more closely related to the *ets* domain of the Drosophila protein *yan* than to *ets* domain of the human ►*ETS1* or *SPI1* (PU.1) genes.

The other evolutionarily conserved domain in ETV6 is the N terminally located *pointed* or SAM (sterile alpha motif) domain. This domain is even more highly conserved in evolution than the *ets* domain, and is found in many *ets* family members as well as in many other transcription factors and signal transduction proteins. The *pointed* domain serves as a homo- and heterodimerization module.

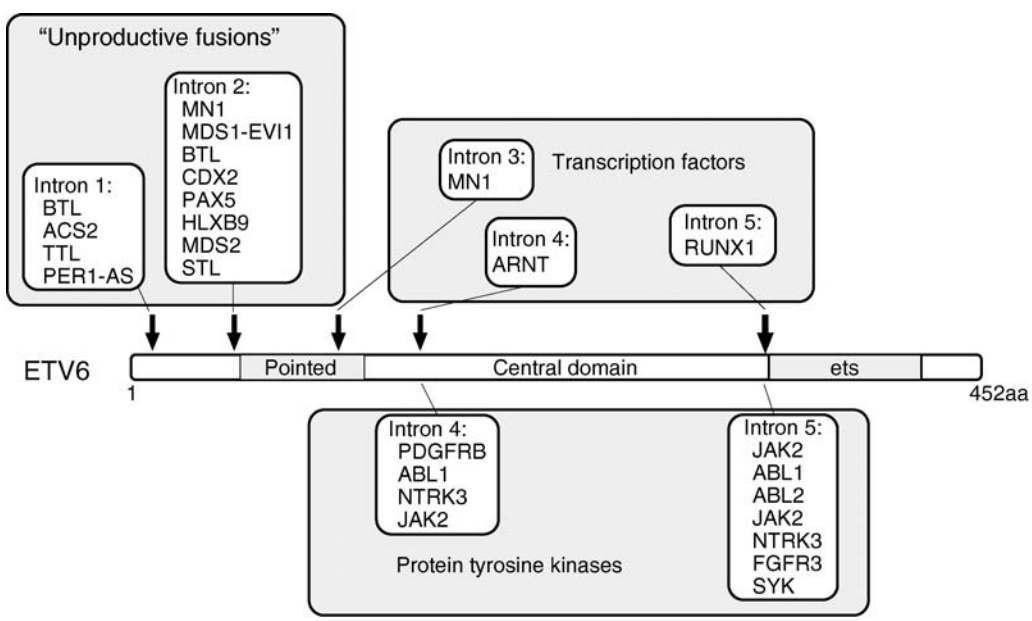
ETV6 Fusion Partners in Cancer

After the initial cloning of *ETV6*, several other fusion partners of *ETV6* were identified in quick succession. There are now well over 20 fusion partners of *ETV6* described, and the list is still growing.

The various *ETV6* fusions can be assigned to three groups: (i) protein ►tyrosine kinases (PTK); (ii) transcription factors and others; (iii) "unproductive" fusions, i.e. fusion that do not result in an obvious fusion protein.

Protein Tyrosine Kinase Fusion Partners of ETV6

The first identified fusion partner of *ETV6* was the protein ►tyrosine kinase (PTK) platelet-derived growth



ETV6. Figure 1 Diagrammatic representation of the ETV6 protein with the position of the breakpoints of the various fusion partner genes.

factor receptor beta gene. The ETV6/PDGFRB fusion protein is a constitutively active PTK and is the critical product of this translocation. In the ETV6/PDGFRB fusion protein, the N terminal portion of ETV6, which includes the *pointed* domain, is fused to the C terminal two thirds of the PDGFRB protein, which includes the tyrosine kinase domain of PDGFRB. This general structure, i.e. the *pointed* domain of ETV6 in the N terminal half and the tyrosine kinase domain of the fusion partner in the C terminal half of the fusion protein, is characteristic of all ETV6/PTK fusions. Many studies have shown that the *pointed* domain of ETV6 serves as a dimerization module for the fusion protein. Dimerization of the fusion protein leads to the constitutive activation of the PTK domain, which results in autophosphorylation of the fusion protein as well as phosphorylation of cellular proteins like rasGAP, Shc, SH-PTP2, SH-PTP1, CRK-L, CBL, paxillin, and STATs. Expression of ETV6/PTK fusions in the interleukin 3 dependent hematopoietic cell line Ba/F3 leads to factor independent growth. Several ETV6/PTK fusion proteins have also been assayed in murine bone marrow transplantation or transgenic mouse models where they lead to various hematological diseases like myelo- or lympho-proliferative syndromes. The different protein tyrosine kinase genes that have been found to be fused to ETV6 are listed in Table 1. It should be noted that most of these fusions are rather rare. For several partners just one or a handful of cases have been described in the literature.

ETV/PTK fusions are not only found in various types of leukemia but the ETV6/NTRK3 fusion has also been found in solid tumors such as congenital fibrosarcoma, mesoblastic nephroma and secretory breast carcinoma.

Transcription Factors and Other Fusion Partners of ETV6

There are only two fusions of *ETV6* with non PTKs for which the transforming potential of the fusion protein could be established. This is the very common ETV6/►RUNX1 and the much rarer MN1/ETV6 fusion.

The ETV6/►*RUNX1* Fusion

The ETV6/RUNX1 (TEL/►AML1) fusion was the second fusion of ETV6 that was identified. It was soon recognized that the ETV6/RUNX1 fusion is the most common fusion gene found in childhood ►acute lymphoblastic leukemia, which is present in up to 25% of all childhood B-ALL cases.

The ETV6/RUNX1 fusion protein, which is the critical fusion in this translocation, comprises the *pointed* domain of ETV6 as well as the DNA binding and transactivation domain of RUNX1.

Many ETV6/RUNX1 positive ALLs have interstitial deletions (detectable by Fluorescence in situ hybridization, ►FISH) of the short arm of the non-rearranged chromosome 12, which encompass the ETV6 locus. Even in cases in which no interstitial deletion of the non-rearranged ETV6 allele can be detected by FISH, there is no expression of the wild-type ETV6 allele suggesting a very important role for ETV6 loss of function in the pathogenesis of ►ALL.

Little is known about mechanisms by which the ETV6/RUNX1 fusion protein causes leukemia. Several attempts to establish ETV6/RUNX1 transgenic or bone marrow transplant leukemia models have failed or yielded leukemia only after a very long latency. There is some evidence that leukemogenesis by ETV6/RUNX1 is accelerated if cell cycle regulation is compromised by other mutations.

Some hints as to the function of ETV6/RUNX1 have been gleaned from reporter gene assays. In these experiments, the ETV6/RUNX1 fusion protein behaves as a strong transcriptional repressor on AML1 target genes. The *pointed* domain and the central domain of ETV6 have been shown to recruit transcriptional corepressors like SMRT, N-CoR and mSin3A.

The MN1/ETV6 Fusion

The MN1/ETV6 fusion is found in some patients with ►acute myeloid leukemia (AML) or ►myelodysplastic syndrome with a t(12;22)(p13;q11) translocation. *MN1* was originally found as a gene disrupted by a translocation in ►meningeoma. The critical MN1/ETV6 fusion protein, which is able to transform murine NIH3T3 fibroblasts in vitro (►NIH3T3 transformation assay) contains transcriptional activation domains derived from MN1 and the *ets* DNA binding domain of ETV6. This is the only known example in which the *ets* domain of ETV6 seems to be critical for transformation.

The ETV6/ARNT Fusion

Another rare ETV6/transcription factor fusion is the ETV6/ARNT fusion gene reported in one patient with AML and a t(1;12)(q21;p13), which links the N-terminal portion of ETV6 including the *pointed* domain to almost the complete ►aryl hydrocarbon receptor nuclear translocator (ARNT).

"Unproductive" ETV6 Fusions

A large number of chromosomal translocations involving *ETV6* have been cloned, in which the breakpoints lie in intron 1 or 2 of *ETV6*. These translocations result in *ETV6* fusions that contain only the 54 amino terminal amino acids of ETV6 and lack any of the important protein domains of ETV6 like the *pointed* or *ets* domain (Table 1). Almost all of these translocations have been identified in only one or at the most a handful of leukemia cases.

It is very likely that most, if not all, of these translocations do not result in the formation of a

ETV6. Table 1 Translocation partners of ETV6

ETV6 fusion partner	Translocation	Disease
Tyrosine kinases		
PDGFRB	t(5;12)(q31;p13)	CMMoL
ABL1	t(9;12)(q34;p13)	AML, ALL
ABL2	t(1;12)(q25;p13)	AML-M3,-M4, ►T-ALL
JAK2	t(9;12)(p24;p13)	Pre B cell ALL, T-ALL
NTRK3	t(12;15)(p13;q25)	Congenital fiborsarcoma, mesoblastic nephroma, secretory breast carcinoma, AML
FGFR3	t(4;12)(p16;p13)	Peripheral T-cell lymphoma
►SYK	t(9;12)(q22;p12)	►MDS
Transcription factors and cofactors		
RUNX1	t(12;21)(p13;q22)	ALL
MN1	t(12;22) (p13;q11)	AML and MDS
ARNT	t(1;12)(q21;p13)	AML-M2
"Unproductive fusions"		
MDS1/EVI1	t(3;12)(q26;p13)	MDS, ►CML
BTL	t(4;12)(q11-q12;p13)	AML-M0
CDX2	t(12;13)(p13;q12)	AML
PAX5	t(9;12)(q11;p13)	ALL
HLXB9	t(7;12)(q36;p13)	AML
MDS2	t(1;12)(p36.1;p13)	MDS
STL	t(6;12)(p13;q23)	B-ALL
ACS2	t(5;12)(q31;p13)	MDS-►RAEB, AML, ►AEL
TTL	t(12;13)(p13;q14)	ALL
PER1 anti sense	t(12;17)(p13;p13)	AML

transforming ETV6/other fusion protein, but rather that they lead to the transcriptional up-regulation of genes adjacent to the translocation breakpoints. This has been elegantly shown for the t(12;13)(p13;q12) which results not only in the formation of a fusion between *ETV6* and the caudal-related homeobox gene *CDX2,* but also in the upregulation of a transcript that codes for the full length CDX2 protein. In a bone marrow transplant model, the upregulation of the wild type CDX2 protein and not the expression of the ETV6/CDX2 fusion protein is critical for the development of leukemia in a murine bone marrow transplantation model.

Putative Tumor Suppressor Gene and Physiological Function

There are several lines of evidence that suggest that *ETV6* might function as a ►tumor suppressor gene. In up to 70% of childhood ALL cases with ETV6/RUNX1 fusions, there is concomitant deletion of the non-rearranged *ETV6* allele. Deletions of the short arm of chromosome 12 are frequently found in a broad spectrum of hematological malignancies, and the common region of deletion was mapped to a small genomic region including *ETV6* and *CDKN1B*. In addition, several studies showed that even if no deletion of the *ETV6* locus can be detected there was no expression of *ETV6* at the mRNA level or absence of the ETV6 protein.

In vivo and in vitro studies provide additional evidence that *ETV6* might function as a tumor suppressor gene. *ETV6* expression inhibits growth in soft agar of *RAS* transformed NIH3T3 fibroblasts cells and leads to the differentiation of erythroleukemia cells into erythrocytes. Additionally, the expression of *ETV6* in serum-starved NIH3T3 cells induces apoptosis. Reporter gene assays have demonstrated that ETV6 is a strong transcriptional repressor, which requires corepressors like N-Cor, mSin3 and SMRT.

Targeted deletion of the murine *Etv6* gene demonstrated that *Etv6* is essential for yolk sac angiogenesis and the establishment of definitive hematopoiesis in the bone marrow, while hematopoiesis in the yolk sac and fetal liver was not affected. $Etv6^{-/-}$ mice die between day 10.5 and 11.5 of embryonic development due to a defect in yolk sac ►angiogenesis and widespread apoptosis of mesenchymal and neural cells.

Furthermore, *Etv6* could be shown to be an essential regulator for the maintenance of hematopoietic stem cells in adult murine bone marrow.

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Euchromatin

Definition

Is the lightly packed form of chromatin that commonly is under active transcription. Regions of chromatin that contain genes that are active or are poised for activity and replicates early during cell cycle.

►Histone Modifications

Eukaryotic Initiation Factors (eIF)

Definition

Translation rates are primarily regulated at the initiation level, a complicated multistep process involving a large number of initiation factors such as the eukaryotic translation Initiation Factor 4 group (e.g. eIF4F), which facilitates the recruitment of ribosomes to the mRNA 5υ end, and the ►eIF2 protein, which is composed of three subunits (Alpha, Beta, Gamma), and is one of the key molecules in the initiation of translation.

►Anoxia and Cancer
►Endoplasmic Reticulum Stress

Eutectic Temperature

Definition

Temperature at which both solute and solvent will become solidified.

►Cryosurgery in Bone Tumors

Evans Syndrome

Definition

An autoimmune disease in which an individual's antibodies attack their own red blood cells and platelets, resulting in autoimmune hemolytic anemia and immune thrombocytopenic purpura.

►Rituximab

Everolimus

Definition

Analog of Rapamycin

►Rapamycin

EVI-1

Definition

Ecotropic viral integration site 1 (Evi-1) protein is a nuclear zinc-finger protein involved in leukemic transformation of hematopoietic cells.

►Smad Proteins in TGFβ Signaling

Ewing Sarcoma

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Synonyms

Ewing tumor; Ewing sarcoma family tumors; Peripheral primitive neuroectodermal tumor; Neuroepithelioma

Definition

Aggressive small-round-cell tumor affecting bone and soft tissue in children and young adults. Ewing's sarcoma (►ES) and peripheral primitive neuroectodermal tumor (►pPNET), also called ►neuroepithelioma are currently defined as biologically closely related tumors along a gradient of limited neuroglial differentiation. ►Askin tumor is the historical designation of Ewing's sarcoma of the chest wall. Today, all these neoplasms are summarized as Ewing tumors (►ET), or Ewing sarcoma family tumors (►ESFT). Although first described in 1866 and 1890 by Lücke and Hildebrand, respectively, the disease carries the name of the American pathologist James Ewing who, in 1921, was the first to recognize the tumor as a separate entity, which he defined as diffuse endothelioma of the bone.

On the genetic level, Ewing sarcoma family tumors are defined by the consistent presence of a reciprocal ►chromosomal translocation between the long arms of chromosome 22 and either chromosome 11 (85%) or 21 (10%). In rare cases, alternative rearrangements of chromosome 22 with either chromosome 7, 17 or 2 have been reported. These aberrations result in a gene fusion that serves as a diagnostic criterion allowing to discriminate Ewing sarcoma family tumors from osteomyelitis and childhood malignancies with a similar small-round-cell phenotype, including ►neuroblastoma, ►rhabdomyosarcoma, non-Hodgkin lymphoma and small cell osteosarcoma. Immunohistochemically, Ewing sarcoma family tumor cells are defined by the abundant presence of the cell surface marker ►CD99. The exact histogenesis of the disease is not known. An origin from a very primitive, ectodermally derived, migrating cell from the neuroepithelium has been suggested based on ultrastructural and histological signs of limited neural differentiation. More recent experimental evidence indicates that at least part of the neural resemblance is a functional consequence of the characteristic gene fusion in Ewing sarcoma family tumors. Based on these molecular biological findings, a mesenchymal origin is currently discussed for the disease.

Characteristics

Ewing sarcoma family tumors comprise about 10–15% of malignant bone tumors with a yearly incidence of 0.6 per million in the caucasian population. The disease is rarely observed among black Africans, African Americans and Chinese people. It typically occurs in adolescence, in the second decade of life (average age at diagnosis is 13.5 years) with a slight male prevalence. Nevertheless, infants less than 5 years of age as well as adults up to 60 years of age are occasionally diagnosed with Ewing sarcoma family tumors. The tumor usually presents as a painful swelling, rapidly increasing in size. The duration of symptoms prior to the definitive diagnosis can be weeks to months, or rarely even years, with a median of three to nine months. In patients with metastatic disease, nonspecific symptoms such as malaise and fever may resemble symptoms of septicemia. The most frequent tumor localizations are pelvis, the long bones of the extremities, the ribs, the scapula and the vertebrae. Less frequently, Ewing sarcoma family tumors arise from extraosseous locations. Unlike ►osteosarcomas, Ewing sarcomas tend to arise from the diaphyseal rather than the metaphyseal portion of the bones and is frequently accompanied by tumor-related osteolysis, detachment of the periosteum from the bone, and spiculae of calcification in soft tissue tumor masses.

About 20–25% of Ewing sarcoma patients present at diagnosis with gross, clinically detectable metastases in the lung and/or in bone and/or bone marrow. Metastases to lymph nodes or other sites like liver or central nervous system (CNS) are rare. In contrast to patients with localized disease, cure of this group of patients is very difficult to achieve.

Classic Ewing sarcoma is composed of a monotonous population of small round cells with high nuclear to cytoplasmic ratios arrayed in sheets. The cells have scant, faintly eosinophilic to amphophilic cytoplasm, indistinct cytoplasmic borders, and round nuclei with evenly distributed, finely granular chromatin and inconspicuous nucleoli. Mitotic activity is usually low. By means of immunohistochemistry, the tumor cells occasionally stain positive for neuroglial markers, such as neuron specific enolase, S100 protein, chromogranin A and B or the gene product PGP9.5. This is in addition to CD99, which is highly expressed in Ewing sarcoma family tumors with consistency. The inclusion of glycogen can frequently be observed in the tumor cells. Infrequently, Ewing sarcoma is focally immunoreactive for cytokeratins.

Cytogenetics and Gene Alterations

The most consistent marker of Ewing sarcoma family tumors is the rearrangement of the Ewing sarcoma gene ►EWS on chromosome 22 (band q12) with a gene encoding for an ►Ets transcription factor. These proteins are characterized by a unique structure of their DNA binding domain determining target gene specificity. In the majority (85%) of Ewing

sarcoma family tumors EWS is rearranged with ►Fli-1, which is located on chromosome 11 (band q24). The second most frequent translocation partner of EWS in this disease is the ►Ets family member ►ERG on chromosome 21 (band q22) (10%). In rare cases of Ewing sarcoma family tumors, complex or interstitial chromosomal rearrangements fuse EWS to related Ets transcription factor genes located on chromosomes 7 (band p22), 17 (band q12), and 2 (band q36). These gene rearrangements are currently monitored for diagnostic purposes, either on the chromosomal level using fluorescent in situ hybridization (►FISH) or on the RNA level using reverse transcriptase polymerase chain reaction (►PCR) (►RT-PCR). The latter method also allows for high sensitivity detection of minimally disseminated disease in blood, bone marrow and peripheral blood progenitor cell (►PBPC) collections. The prognostic impact of RT-PCR detectable tumor cells in these samples is currently under prospective evaluation in several clinical studies.

As a result of the gene fusion a potent novel transcription factor with altered structural and functional features is expressed in the tumor cells. EWS-Fli-1 and EWS-ERG fusion proteins have been shown to render mouse fibroblast cell lines and bone marrow-derived mesenchymal progenitor cells tumorigenic in animal models. Using antagonistic agents, generated by means of gene technology, involvement of these aberrant gene products in Ewing sarcoma tumor cell proliferation has experimentally been demonstrated. It is commonly assumed that the EWS-Ets chimeric transcription factors mediate their transforming properties by inappropriately activating or repressing other genes. In-vitro gene transfer experiments into a number of different cell types indicated that the spectrum of genes responsive to EWS-Ets fusion proteins is context-dependent. EWS-Fli-1 is toxic to most primary human cell types with only very few exceptions. Among tissues tolerant of the expression of the Ewing sarcoma oncogene are bone-marrow derived mesenchymal progenitor cells. Here, EWS-Fli-1 interferes with the differentiation potential of these cells. With the advent of new technologies to modulate EWS-Ets expression in Ewing sarcoma cells such as ►RNA interference (RNAi), we are just starting to understand the mechanisms underlying malignant transformation by this oncogene. Large scale gene expression profiling studies have identified a specific signature of EWS-Fli1 within the Ewing sarcoma ►transcriptome. Current functional studies attempt to separate malfunctions essential for tumorigenesis from collateral damage.

Due to variable chromosomal breakpoint locations in the individual tumors the EWS-Ets translocation products vary in size. A prognostic impact of certain EWS-Fli-1 gene fusion types for localized disease has been suggested and is currently under prospective evaluation.

Otherwise, no reliable genetic indicators of prognosis have been identified for Ewing sarcoma family tumors, so far. Cytogenetically, trisomy 8 and 12 accompany the characteristic rearrangement of chromosome 22 in about 44 and 12% of tumors, respectively. Additional structural changes affect chromosomes 1 and 16 in about 20% of tumors, most frequently leading to a gain of 1q and a loss of 16q and the formation of a derivative chromosome. A possible prognostic impact of these cytogenetic alterations has been discussed controversially. Genetic aberrations associated with unfavorable disease in many human malignancies such as mutations of the tumor suppressor gene p53 and of the Ras oncogene are infrequent in Ewing sarcoma family tumors. Besides the EWS-Ets gene rearrangement the only molecularly defined recurrent genomic alteration occurring in 20–30% of primary Ewing sarcoma family tumors is the homzygous loss of the ►INK4A gene located on chromosome 9 (band p21). This aberration most frequently occurs as a small interstitial deletion undetectable with classical cytogenetic means. Preliminary retrospective data suggest an adverse prognostic impact of this aberration and of rare p53 mutations. However, in the absence of any prospectively confirmed molecular prognostic marker, the extent of disease monitored by clinical imaging techniques at the time of diagnosis (computed tomography of the chest to document or exclude intrathoracic metastases and 99m-Technetium whole body radionuclide bone scans to search for skeletal metastases), microscopically detectable bone marrow micrometastases, and the histopathologically determined tumor response to initial chemotherapy, still serve as the only accepted criteria for treatment stratification.

Aetiology

The aetiology of Ewing sarcoma family tumors is not known. Neither is there evidence for genetic predisposition nor for a role of environmental exposure. Except for an interethnic polymorphism within a EWS gene region frequently affected by the Ewing sarcoma specific chromosome translocation, the molecular basis for differences in Ewing sarcoma incidence between Caucasians, Asians and Africans have only poorly been investigated. Due to its tight association with the disease, the EWS-Ets gene rearrangement is considered the primary event during Ewing sarcoma pathogenesis. No specific recombinogenic activity has been identified as responsible for this aberration and although involvement of a viral agent in generating the chromosomal translocation has been suggested, it has not been confirmed.

Therapy

In the pre-chemotherapy era, less than 10% of Ewing sarcoma patients survived the disease despite the

well known radiosensitivity of the tumor and despite its radical resection, calling for systemic treatment to eradicate disseminated tumor cells. Today, patients with Ewing sarcoma family tumors are treated by multimodal therapeutic regimens including radiotherapy and chemotherapy (combinations of vincristine, actinomycin D, cyclophosphamide, doxorubicin, ifosfamide, etoposide) as well as surgical resection whenever possible. By using this treatment strategy together with optimized schedules and dose intensities, the results for patients with localized disease was improved to an overall survival rate of 60–70% in recent years. The treatment of Ewing sarcoma patients worldwide is organized in cooperative trials, aiming to further improve treatment outcome. In contrast, the management of primary metastatic disease and early relapse remains a clinical challenge that is currently assessed by myeloablative approaches, combining high-dose chemotherapy and total-body irradiation with stem cell reinfusion. The efficacy of this therapeutic approach for high-risk Ewing sarcoma patients remains to be established. Novel therapeutic approaches that are currently under investigation in pilot clinical studies target the tumor vasculature. To avoid the toxic side effects of chemotherapy, future biologically tailored therapy may target the EWS-Ets fusion protein or genes downstream of this tumor specific aberration.

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Ewing Sarcoma Family Tumors

►Ewing Sarcoma

Ewing Tumor

►Ewing Sarcoma

EWS

Definition

Ewing sarcoma gene; also termed EWSR1 (Ewing sarcoma breakpoint region 1); identified as a gene on chromosome 22 consistently disrupted by chromosomal translocation in Ewing sarcoma family tumors, and frequently in myxoid liposarcoma, clear cell sarcoma, myxoid chondrosarcoma, and some acute leukemias.

►Ewing Sarcoma

EWS-FLI (ets) Fusion Transcripts

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Definition

The *EWS-FLI1* fusion ►transcript is the result of a ►balanced reciprocal chromosomal ►*translocation* between chromosomes 11 and 22, which fuses the *EWS* gene in chromosome 22 to the *FLI1* gene in chromosome 11. This fusion transcript is detected in approximately 85% of cases of the ►Ewing sarcoma family of tumors, and is considered a tumor-specific molecular rearrangement, therefore useful for diagnosis, prognosis, and presumably for specific therapeutics. Approximately 10% of Ewing tumors have fusions involving *EWS* and *ERG* genes. An additional 5% bear fusions between EWS and other less frequent genes.

Characteristics

Structure

Chromosomal translocations result in the genesis of chimeric genes, encoding hybrid transcripts and novel fusion proteins. Many fusion proteins contain juxtaposed functional domains usually found in separate proteins.

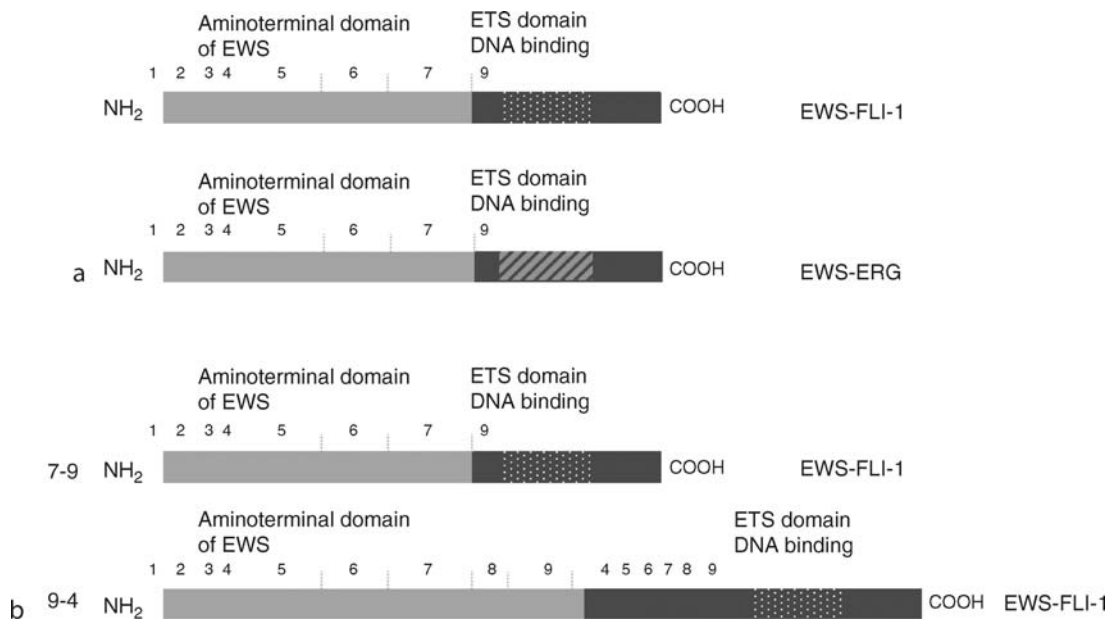
The EWS-FLI1 fusion protein contains the aminoterminal domain of EWS and the carboxyterminal region of FLI1. *EWS* gene is an RNA-binding protein, which is believed to mediate mRNA transcription probably through its interaction with RNA polymerase II complex. Several forms (at least 12 types) of EWS-FLI1 exist because of variations in the location of the EWS and FLI1 genomic breakpoints. They contain different combinations of exons from EWS and FLI1, the most frequent being the fusion of EWS exons 1–7 to FLI1 exon 6–9 (type 1) and fusion of EWS exons 1–7 to FLI1 exon 5–9 (type 2).

Properties of the *EWS-FLI1* Fusion Transcript and Protein

EWS is widely expressed in most tissues, and because the genomic structure of the fusion, the EWS promoter drives the expression of EWS-FLI1. The aminoterminal domain of EWS, included in the fusion, has strong transactivating properties. The *FLI1* gene encodes a member of the ETS family of ►transcription factors and its expression is highly restricted to hematopoietic, endothelial, and mesodermal cells as well as to neural crest cells. The ETS DNA-binding domain of FLI1 is included in the fusion. The resulting EWS-FLI1 protein is therefore an aberrant transcription factor. The chimeric product EWS-FLI1 can transform some cell lines in culture and can inhibit or activate diverse cellular pathways. For example, EWS-FLI1 protein can suppress transcription of transforming growth factor beta type 2 receptor gene leading to TGF-beta resistance; can activate MFNG, a member of fringe family, related to somatic development, and other genes. The action of EWS-FLI1 as a transcription factor is probably related to the cell context in which this fusion is detected. This cellular context is probably influenced by the cell type, stage of differentiation, and microenvironment, and could include expression of several growth factors, for example, IGF1 and its receptor. On the other hand it is likely that *FLI1* gene is developmentally regulated, being expressed at certain times and places, and this can confer some site and tissue specificity to the transcriptional activity generated by the EWS-FLI1 fusion protein.

EWS and Other Specific Human Translocations

EWS can fuse after chromosomal translocation to other genes, including several members of the ETS transcription factor family. In an analogous way to FLI1, EWS can be detected fused to *ERG* gene through a t(21;22), to *ETV* gene through a t(7;22), to *E1A-F* gene through a t(17;22) or to *FEV* gene through a t(2;22) ►chromosomal translocation. All of these *EWS-ETS* gene fusion transcripts can confer a common tumorigenic phenotype of small round cells and can be found in the Ewing family of tumors. But, interestingly, EWS can fuse to other genes and be detected in other tumor types. For example, in desmoplastic small round cell tumor, EWS is fused to the tumor suppressor gene *WT1* through a t(11;22), in clear cell sarcoma of soft tissue is fused to the ATF1 through a t(12;22), in myxoid and round cell liposarcoma can also be found fused to the CHOP



EWS-FLI (ets) Fusion Transcripts. Figure 1

gene, and to CHN gene, located in chromosome 9, in extraskeletal myxoid chondrosarcoma.

Genesis of the Translocation

The mechanism by which the translocation is generated is another field worth to be studied. In other words why do the breakpoints always occur in the same introns? Is that a random event or are there certain areas of these particular genes particularly prone to recombine? There is some evidence that several chromosomal translocations may not be random events, but may be specifically promoted by the presence of certain DNA sequence motifs at or around certain target genes. A number of recombinogenic sequences have been described, like topoisomerase-binding sequences in leukemias or lymphomas, and translin sequences in alveolar rhabdomyosarcoma and in myxoid liposarcoma. Furthermore it has been suggested that mobile elements or endogenous retroviruses may take part in gene rearrangements. In Ewing tumors, in which ►illegitimate recombination has been reported to occur, recombinogenic sequences have not been described in a large study of genomic breakpoints.

The EWS-FLI1 Fusion and the Pathogenesis of ►Ewing Sarcoma

A problem to study sarcoma pathogenesis, in general, and Ewing tumor genesis, in particular, is the absence of preneoplastic lesions, as well as the lack of animal transgenic models. Therefore, to study the mechanism by which EWS-FLI1 induces ►Ewing sarcoma there are two complementary approaches:

1. Induced expression of the fusion in different cellular models. For example, induced expression in NIH3T3 fibroblastic cells accelerates tumor growth in immunodeficient mouse models, while expression in alveolar rhabdomyosarcoma or neuroblastoma cell lines induces a shift in differentiation, which becomes closer to that of Ewing tumor. The same experiment performed in other cell lines induces, however, cell death, showing that the cellular context matters in the pathogenesis of Ewing tumor.
2. By small interference RNA studies blocking EWS-FLI1 mRNA, cell cycle arrest and a decrease in tumor growth in animal models, is observed.

Both types of experiments confirm that EWS-FLI1 functions as an aberrant transcription factor, although the target genes are still relatively unknown. The availability of expression microarrays in the last years, coupled to the experiments described earlier, has been useful to suggest possible target genes. EWS-FLI1 participates in Ewing tumor pathogenesis controlling cell proliferation and survival by promoting expression of IGF1, MYC, CCND-1, PDGFC, DAX1-NR0B1, and NKX2-2, and by repressing genes such as $p21^{WAF1}$, $p57^{kip}$, *TGFbRII*, and *IGFBP3*. The genes related to the induction of the undifferentiated phenotype of Ewing tumor are largely unknown.

In addition, EWS-FLI1 exerts its function with the help of other proteins such as RNA helicase A, which binds to the promoters targeted also by EWS-FLI1, enhancing its function.

Clinical Relevance

EWS-FLI1 (ets) in Diagnosis

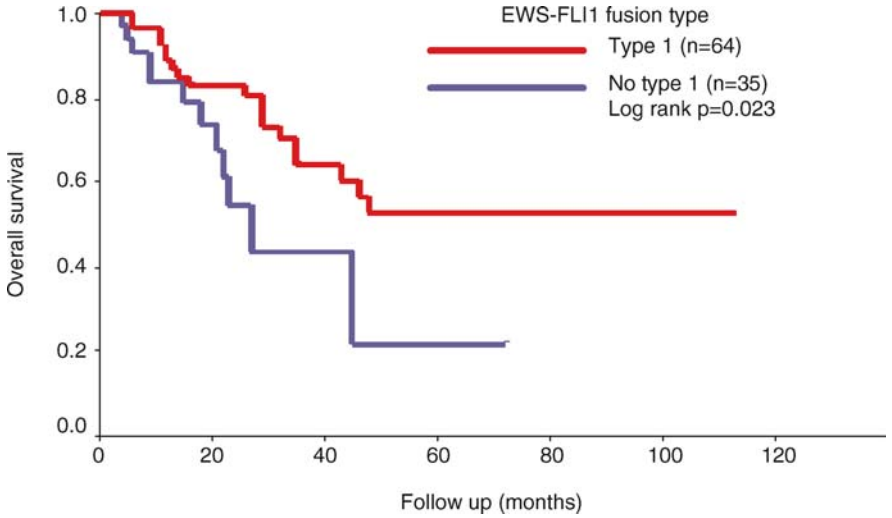
The detection of the chimeric product *EWS-FLI1*, in the adequate morphologic and immunophenotypic context, is diagnostic of a ►Ewing family tumor. The fused product can be studied by fluorescence in situ hybridization (FISH) or by ►RT-PCR in very small samples of tissue or cytological specimens. Frozen tissue is the preferred source of RNA, which can also be extracted from ►formalin-fixed paraffin-embedded tissue with variable success. *EWS-FLI1* detection is particularly useful in cases in which a Ewing family tumor arises in unusual locations (kidney, skin, lung, ovary, pancreas), or in patients over 40 years, or shows atypical features (epithelial differentiation, no ►CD99/MIC2 expression).

EWS-FLI1 (ets) Fusion Type and Prognosis

Ewing tumors display a moderate level of molecular heterogeneity. The variability in the chimeric transcript structure may help to define clinically distinct risk groups of Ewing tumors. In fact, two independent groups have found that the type 1 *EWS-FLI1* fusion transcript is associated with less aggressive clinical behavior than the patients carrying tumors with other *EWS-FLI1* fusion types (Fig. 2), regardless stage at diagnosis, tumor location, or tumor volume. A recent study has shown that this particular gene fusion (type 1) encodes for a chimeric protein that functions as a weaker transcription factor than chimeric proteins encoded by other fusion types. In fact tumors with *EWS-FLI1* type 1 fusions have a lower proliferative rate than their counterparts with other fusion types. So far, no clinical differences have seen between patients with tumors bearing *EWS-FLI1* fusions, and those having *EWS-ERG* transcripts. These results are still pending of confirmation; a European prospective study is being conducted to assess for clinical differences among tumors having the most prevalent fusion types.

EWS-FLI1 (ets) and Detection of Minimal Residual Disease

EWS-ETS fusion transcripts can be detected by RT-PCR in peripheral blood and bone marrow. That



EWS-FLI (ets) Fusion Transcripts. Figure 2

demonstration may contribute to patient management and staging, although clinical relevance is still unclear. A French study has shown in 2003 that presence of circulating Ewing tumor cells and/or Ewing tumor cells in the bone marrow at the time of diagnosis is a significant predictor of tumor relapse.

EWS-FLI1 (ets) and Therapeutics

Current efforts are focused on directly inhibit chimeric proteins (or their downstream targets) and on immunotherapy directed at tumor cell-specific epitopes derived from chimeric products. A potentially useful approach is the delivery of siRNA in the appropriate vectors. The results of an animal model of metastatic Ewing tumor in which EWS-FLI1 siRNA has been injected intravenously is encouraging, and could potentially be used in the future treatment of patients with this neoplasm.

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Ex Vivo Culture

Definition

►Histocultures.

Excentric Cleavage

Definition

Is the assymetric cleavage of ►carotenoids at positions outside of the central double bond to yield two β-apocarotenals of differing chain lengths (e.g. cleavage of carotenoids at their 7′,8′; 9′,10′; 11′,12′; or 13′,14′ double bonds). Excentric cleavage of carotenoids at the 9′,10′ double bond has been shown to be an oxidative pathway for both provitamin A and non-provitamin A carotenoids. Current research aims to characterize additional excentric cleavage enzymes and their affinities for specific carotenoids.

►Carotenoids

Excess Relative Risk (ERR)

Definition
Formally, ERR is RR-1. In regression analyses, where exposure is a continuous rather than a dichotomous variable, ERR can be given by unit exposure. This reflects the increase of risk by increase of exposure.

►Uranium Miners
►Radon

Exchange Factors

Definition
Proteins that catalyze the exchange of GDP for GTP on GTPases. They can be regulated by direct interaction with adaptor molecules as in the case of Sos which is activated by Shc/Grb2, or by second messengers, as in the case of DAG for Ras-GRP activation, or hetero-trimeric G-proteins, as in the case of Ras-GRF1.

►GTPase

Exchangeable Apolipoprotein

Definition
The protein component of plasma lipoproteins.

►Synuclein

Excipient Culture

Definition
►Histocultures.

Excitation

Definition
Is the propulsion of an electron to a higher level of orbit in an atom.

►Proton Beam Therapy

Excretion

Definition
Is the process by which a chemical is removed from the body.

►ADMET Screen

Excretory Urography

Definition
A test to evaluate the kidneys, urinary tract and bladder. Medicine is given through an intravenous line (IV). This medicine moves through the body and is ultimately concentrated in the kidneys. A series of X-rays are taken, and are viewed by the radiologist to evaluate the kidneys, urinary tract and bladder. ►Bladder cancer; ►Transitional Cell Carcinoma.

Exfoliation of Cells

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Synonyms
Shedding of cells; Sloughing of cells

Definition
Cell exfoliation (Latin – *exfoliare* – to strip of leaves) is a process of spontaneous or induced complete

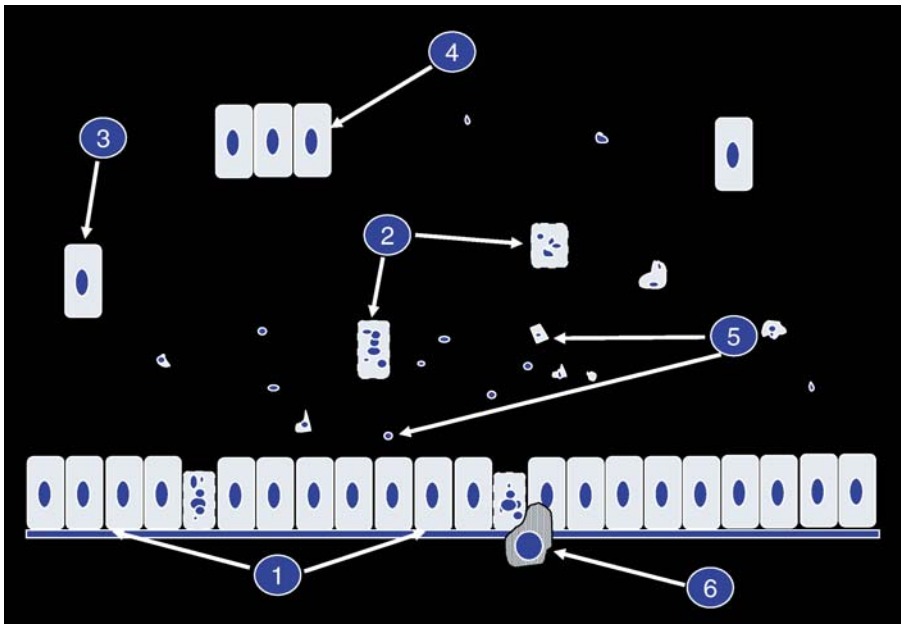
detachment of single epithelial cells or groups of cells from an epithelial layer.

Characteristics

Exfoliation of cells is one of the main mechanisms of cell loss participating in the homeostatic control of cell population size. Cell exfoliation is a characteristic feature of epithelial tissues forming epithelial layers covering both external body surface (skin epidermis and skin appendages) and surfaces of internal cavities and passages (gastrointestinal tract, respiratory system, and genitourinary system) as well as major exocrine glands and glandular ducts (mammary gland, exocrine pancreas, biliary system, etc). Cell exfoliation process in normal physiological conditions is closely associated with terminal differentiation and orderly loss of dying cells compensated by permanent cell population renewal. This relationship is exemplified by the structure of stratified squamous (epidermal type) epithelia where layers of maturing cells are being moved towards the surface due to continuous arrival of younger counterparts produced by proliferation in the basal layers of the epithelium. Eventual death, keratinisation (in the epidermis) and inevitable exfoliation of cell remnants is the normal destiny of these terminally differentiated cells. Less is known about cell exfoliation in the epithelia of internal organs; however, studies of well-structured columnar colonic epithelium can be chosen as the most reliable source of information. Obvious links between cell differentiation and eventual shedding exist in this dynamic cell population with extremely high rates of cell proliferation and loss. The progeny of stem cells located at the base of the colonic crypt constantly migrate in the direction of the lumen. During this migration colonocytes gradually lose their proliferative capacity and undergo differentiation, thus terminally differentiated cells reaching surface (luminal) epithelium should be promptly eliminated to be replaced by future generations of their counterparts. Two ways of cell loss coexist in the colonic epithelium under normal conditions (Fig. 1).

1. Exfoliation of single colonocytes or colonocyte groups into the lumen of the gut. Strictly speaking, the shed cells enter the ►mucocellular layer covering colonic mucosa rather than the gut contents.
2. Apoptosis *in situ* followed by the engulfment of apoptotic cells by adjacent colonocytes or subepithelial macrophages.

Physiological changes or development of pathological processes can significantly shift proportions of cells eliminated by each of these mechanisms. There are two principal variants of post-exfoliation cell fate (Fig. 1). Some colonocytes undergo immediate apoptosis (detachment-induced apoptosis or ►anoikis) following exfoliation, whereas other exfoliated cells and especially groups of cells maintain their structural integrity and possibly viability for minutes or even



Exfoliation of Cells. Figure 1 Schematic representation of cell exfoliation in normal physiological conditions. Colon model, surface epithelium, crypts are not shown. 1. Normal epithelium (two apoptotic cells shown). 2. Anoikis immediately following exfoliation. 3. Exfoliated normal epithelial cell preserving its structure. 4. Group of exfoliated normal epithelial cells preserving their structure. 5. Cellular and nuclear debris. 6. Subepithelial macrophage.

hours following exfoliation. In the human colon, exfoliated cells enter the mucocellular layer overlaying colonic mucosa and can be transferred distally, protected by the mucus, without being incorporated into the faeces. The movement of the cell-containing mucocellular layer is mostly driven by its close contact with moving faecal flow as well as by constant peristaltic movements of the gut. There is no doubt that some proportion of exfoliated colonocytes can reach the gut contents, but these cells should be rapidly destroyed in the anaerobic bacteria-dominated faecal milieu rich in bile acids and other cytolytic agents. Observations of the presence of well-preserved epithelial cells in human faecal samples mostly concern colonocytes excreted together with the mucocellular layer fragments or exfoliated squamous cells of the anal epithelium sometimes misidentified as colonocytes.

Similar exfoliation models with tissue-specific corrections can be applied to the epithelial populations of other internal organs. Preservation of cellular structure after exfoliation is described for cells of bronchial epithelium in sputum samples, bladder, urethral and prostate epithelium in urine samples, gastric epithelium in gastric lavage liquid, cervical, vaginal, and endometrial epithelium in cervical smears, mammary gland epithelium in nipple aspirates and pancreatic duct epithelium obtained endoscopically. Exfoliated cell migration or passive transport occurs in all internal passages or ducts, being facilitated by contents flow, peristaltic movements or cell-driven mucus transport (►*cilia* of the bronchial epithelium). Shed cells can be commonly found in human body excretions (cells of bladder, urethra and occasionally prostate epithelium in urine, colonocytes and squamous anal epithelium in faeces, bronchial, nasopharyngeal and oral cavity epithelium in sputum, etc.). Exfoliated epithelial cells should be distinguished from non-epithelial free cells (leukocytes, macrophages, lymphocytes, etc) that are also often present in the excreted materials, but are unrelated to the process of cell exfoliation.

Mechanisms

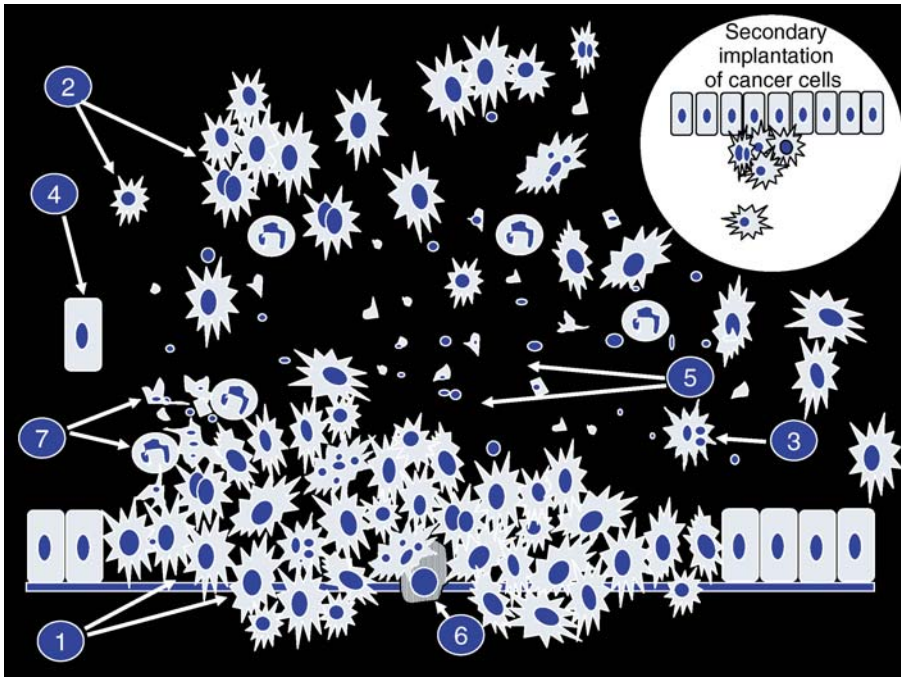
Molecular mechanisms underlying cell exfoliation in normal conditions are poorly investigated and remain largely unknown. Extrapolation of the information obtained *in vitro* might be misleading; therefore, only tentative suggestions can be made. At the cellular level, pathways leading to cell exfoliation should involve considerable structural and functional changes of the cytoskeleton, cell membrane and membrane-bound subcellular structures responsible for the preservation of contacts between neighboring cells (►adherens junctions, ►tight junctions, ►gap junctions) as well as between cells and basal membranes (►focal adhesions). A number of proteins associated with these structures (adherens junction–associated cadherins and catenins, tight junction-associated claudins and occludin, gap junction-associated connexins and focal adhesion-associated integrins and focal adhesion kinase) are likely to participate in exfoliation induction. In particular, the ►integrin system appears to be intimately involved in the homeostatic regulation of cell differentiation, providing communication between epithelial cells and the underlying stromal extracellular matrix. The loss of contact with the extracellular matrix can trigger anoikis through integrin-mediated signaling, but it is evident that many exfoliated cells are able to evade this fate. Mechanisms of this phenomenon remain obscure and need profound investigation as well as other aspects of cell exfoliation *in vivo*.

Cell Exfoliation in Neoplasia

Cancer development is usually associated with a dramatic increase in cell exfoliation from tumor surface (Fig. 2). The increased exfoliation may reflect a compensatory response to the impairment of the apoptotic mechanism of cell elimination *in situ* commonly observed in malignant tumors. Anoikis induction appears to be the ultimate homeostatic goal of the enhanced exfoliation. Although this mechanism of cell elimination may be partially functional in early tumors, resistance to anoikis is now regarded as a hallmark of metastatic cancer cells, which tend to survive exfoliation. Loss of cell adhesion (often associated with E-cadherin function impairment) and disruption of normal interactions with underlying stroma associated with malignant progression, also strongly contribute to the enhancement of exfoliation from tumors. Cancer cells are better adapted to survival in the conditions of oxygen deficiency; therefore their prolonged post-exfoliation persistence on the surfaces of internal cavities, passages and glandular ducts can be expected. These cells shed by tumors are often immature and retain proliferative capacity. Therefore their metastatic potential and ability for secondary implantation can be manifested. Reports describing the occurrence of secondary cancers or peritoneal carcinomatosis following surgical interventions on tumors of several sites (colorectum, ovary, gallbladder) attribute these secondary metastatic cancers to the direct re-implantation of malignant cells exfoliated from the primary tumors. There is a strong probability that cell exfoliation without immediate anoikis in physiological conditions presents a normal "prototype" of metastatic behavior.

Clinical Aspects

The possibility of using exfoliated epithelial cells for various clinical and research purposes is highly attractive because analysis of this material often allows avoiding highly invasive sampling (►biopsy) of



Exfoliation of Cells. Figure 2 Schematic representation of cell exfoliation from tumor surface. Colon model, surface epithelium, crypts are not shown. Inset demonstrates secondary implantation of malignant cells distally from the primary tumor. 1. Tumour (malignant cells). 2. Exfoliated tumor cells and cell groups preserving their structure. 3. Occasional anoikis of a tumor cell immediately following exfoliation. 4. Exfoliated normal epithelial cell preserving its structure. 5. Cellular and nuclear debris. 6. Subepithelial macrophage. 7. Neutrophilic leukocytes and necrotic tumor fragments (focal necrosis and inflammatory reaction).

normally inaccessible tissues. Exfoliated cells can be collected either non-invasively (from sputum, urine, faeces etc) or by simple and minimally invasive procedures (cervical smears, buccal swabs, direct cell collection from the surface of rectal mucosa during proctoscopy, etc.). Although exfoliated cells became widely employed for some specific medical, forensic and research purposes (e.g. buccal mucosal cells are routinely used for DNA isolation for genotyping), clinical oncology remains the main area where their use is already common and looks even more promising with the introduction of modern molecular methodologies.

Cytological diagnostic approaches based on the examination of exfoliated cells were developed first. Exfoliative cytology analysis of smears prepared from cervical epithelium (examination of ►PAP smears) has become a standard screening procedure for cervical cancer. Diagnostic cytology is used for endometrial carcinoma, bronchogenic lung cancer, tumors of the bladder and prostate, and gastric cancer.

The recent introduction of molecular assays targeting cancer biomarkers created another major direction, aiming to employ exfoliated cells as the material of choice for the molecular diagnosis and screening of oncological conditions. Approaches to colorectal cancer screening occupy a leading position among problems addressed in this direction. Significant efforts were concentrated on the analysis of exfoliated colonocytes (or DNA derived from these cells) present in human stool samples. Although cancer-specific molecular changes are often detected by such analysis, especially when ►multi-marker panels of PCR-based assays were applied, the sensitivity of the method has not reached desirable levels. The main reason for this problem is likely to be the relatively low presence of exfoliated colonocytes in the faecal samples. Human DNA found in this material can also derive from exfoliated anal epithelium or free cells (leukocytes, lymphocytes, macrophages, etc), which certainly do not contain tumor markers. Moreover, strong faecal presence normally decreases PCR efficiency. Direct collection of exfoliated cells from the surface of rectal mucosa appears to provide much more abundant and contamination-free material that can be used for different analytical procedures. Even simple detection of unusually high DNA yield in samples of exfoliated material directly collected at standardized conditions can be interpreted as a warning signal indicating a high probability of colorectal tumor presence.

Multiple other applications of exfoliated cells for cancer diagnosis by molecular biomarker detection are being developed, but these studies remain research

projects rather than proven clinical approaches. The present absence of a single biomarker allowing 100% positive identification of cancer presence is the most important obstacle interfering with the development of molecular diagnostic procedures based upon the use of exfoliated cells.

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Exfoliative Erythroderma

Definition

An ►inflammation of the skin with erythema and scales involving over 80% of the body surface.

►Sezary Syndrome

Exobiotics

►Xenobiotics

Exon

Definition

Part of the pre-mRNA that is retained in the mRNA and is used to encode the protein and regulate its translation

►pre-mRNA Splicing

Exonic Splicing Enhancer (ESE)

Definition

Sequence elements within the pre-mRNAs that bind to specific splicing accessory molecules to promote exon recognition and inclusion in the mRNA.

►pre-mRNA Splicing

Experimental Carcinogenesis

►Toxicological Carcinogenesis

Experimental Metastasis

Definition

Tumor cells are injected into the blood of a mouse and their movement to distant organs through the blood, and subsequent invasion into and colonization of an organ is monitored.

►Syk Tyrosine Kinase
►Metastasis

EXT Genes

Definition

Tumor suppressor genes causing multiple osteochondromas. The gene products exostosin-1 en -2 catalyse heparan sulphate biosynthesis.

►Chondrosarcoma

External Granular Layer (EGL)

Definition

A secondary transient germinal matrix zone from the rhombic lip, containing vast numbers of densely packed

neurons. The EGL disappears in humans during the first 2 years of life when all external granular cells have migrated inwardly toward the internal germinal layer.

►Medulloblastoma

Extra Eleven-Nineteen Gene

Definition

The EEN gene encodes a partner of the ►Mixed lineage leukemia (MLL) gene in a case of t(11;19)(q23;p13) translocation. The 19p13 band is where two other partners of MLL also map, ENL and ELL/MEN. For this reason, the new fusion gene was defined EEN (extra eleven-nineteen gene).

►Endocytosis

Extracellular Domain (ECD)

Definition

Part of a molecule that is on the outer surface of the cellular membrane.

►Herceptin

Extracellular Matrix

Definition

Is a meshwork of glycoproteins, proteoglycans and glycosaminoglycans that serves as support for adhesion and growth of cells in coherent tissues. It is the connective tissue that fills up the space between cells, consisting of a network of protein fibers in a polysaccharide matrix. The compounds making up the extracellular matrix are mainly secreted by fibroblasts. The ECM serves as a structural element of tissues to which cells attach through cell-substrate adhesion molecules. Interaction of cells with the extracellular matrix is important for a variety of processes such as adherence, cell migration, differentiation and the regulation of inflammation.

►Adhesion
►Cell Adhesion Molecules
►Calpain
►Migration
►Stromagenesis
►Extracellular Matrix Remodelling
►Tissue Inhibitors of Metalloproteinases (Timps)

E

Extracellular Matrix Remodeling

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Definition

Extracellular matrix (ECM) remodeling is a series of quantitative and qualitative changes in ECM during neoplastic transformation facilitating tumor growth and ►metastasis.

Characteristics

ECM is produced and assembled by the cells it is surrounding. The main components of ECM include glycosaminoglycans (with predominant hyaluronic acid) and proteoaminoglycans (e.g., perlecan, aggrecan), noncolagenous glycoproteins (such as ►fibronectin, laminins, tenascin), collagens and many other biologically important molecules involved in cell–cell and cell–matrix interactions as well as in matrix remodeling. ECM provides not only the mechanical support for the attachment and organization of cellular structures but is also actively involved in the exchange of information with cells, and therefore in the regulation of many important processes such as cell proliferation, migration, differentiation, and survival. Cell–cell and cell–matrix interactions are mediated via adhesive proteins such as cadherins and integrins – adhesive membrane receptors localized on cell surface.

Matrix anchors cells however, it is also physically confining them. This restriction becomes a problem as organs are growing. To some extent, it can be endured due to the inherent plasticity of a matrix, but after a certain point, structural changes are necessary for a proper functioning of the organ and organism. The cells of a given organ must therefore posses an ability to change/remodel its surroundings. Hence, during physiological processes, such as embryonic development,

tissue morphogenesis, ►angiogenesis, cartilage, and bone remodeling, a degradation of old elements of matrix and a synthesis of new ones are taking place. The process is governed by signals from regulatory proteins of ECM that are ensuring the divisions and differentiation of cells according to the needs of organism. The mechanisms that are designated for physiological processes are adapted for the needs of tumor cells. Tumors can be viewed as functional tissues with cells surrounded by ►microenvironment of ECM. Tumor cells must remodel the matrix to establish the communication between tumor cells and ECM and break barriers of the controlling mechanisms of the host cells. Local host stroma plays an important role in the transition from normal to malignant tissue. It has been established that stroma and tumor cells can interchange growth factors, cytokines, angiogenesis factors, and proteases to activate surrounding ECM and facilitate the expansion of tumor cells. Stromal cells by the release of specific molecules can change cell phenotype and induce neoplastic transformation in the neighboring cells. It has been demonstrated that tumor associated fibroblasts have altered properties in comparison to fibroblasts from normal epithelial cells. Tumor cells are changing the composition of ECM either by forcing the production of ECM components in an altered form or by stimulation or inhibition of the expression of some other compounds. Hyaluronic acid, which is promoting cell ►migration via its surface receptor, is very often overexpressed in malignant tissues. Laminin, which is essential for the integrity of tissue, is produced in an altered form and in lower quantities. A desmoplastic reaction, which accompanies many solid tumors, is characterized by altered expression of many proteins (such as α-smooth muscle actin, smooth muscle myosin, and desmin) in desmoplastic fibroblasts as well as altered production of some ECM components (such as collagen types III and IV, tenascin, ►matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), other proteases involved in ECM degradation, and growth factors).

One of the ways tumors are facilitating their migration is by suppressing cell–cell adhesion. A key molecule in maintaining cell–cell adhesion is ►E-cadherin. In human epithelial cancers, a downregulation of E-cadherins and upregulation of another form called N-cadherin has been observed. Downregulation of E-cadherin promotes the invasive and metastatic phenotype of transformed epithelial cells.

Other adhesive receptors – integrins – are engaged in specific binding of the cells and components of ECM. They are heterodimeric proteins composed of α and β subunits. There are several types of both α and β subunits, and therefore a certain number of combinations. Overexpression of many of them has been observed in a number of cancers. Integrins are engaged in signal transmission from the ECM into the cells and regulation of genes expression, for example, of enzymes participating in ECM degradation. They also mediate cell migration. Some integrin receptors are recognizing a RGD sequence (arginine–glycine–asparagine) – a conserved element, which is present in many ECM components. Peptides containing this sequence are implicated in inhibition of metastasis. The manner in which it is conducted is still unclear. It is speculated that they can selectively inhibit either adhesion of tumor cells to structural proteins, the production of proteases or the migration of tumor cells.

Degradation of ECM is a key event in ECM remodeling. It is conducted by a number of hydrolytic enzymes such as metalloproteinases, cysteine proteases (cathepsins B and L), aspartic proteases (►cathepsin D), serine proteases (elastase), sulfatases, and glycosidases. These enzymes function both under physiological and pathological conditions and most of them is produced in a form of inactive zymogens activated by proteolytic processes. Some of the proteases (e.g., cathepsins) are considered lysosomal enzymes but in case of cancer cells a change in cellular distribution with significantly elevated expression in cytosolic fraction has been observed. Cathepsins are working at acidic pH and are involved in intracellular proteolysis, while serine proteases and MMPs act at neutral pH and are mostly responsible for extracellular proteolysis. The proteases can either directly degrade ECM components or indirectly by activation of other proteases, which in turn will also degrade ECM. It seems that the enzymes act in a determined order resulting in a cascade of proteolytic processes. Cathepsin D is produced in inactive form as procathepsin D. The zymogene undergoes autocatalytic activation in an acidic environment. Compared to normal tissue, the extracellular pH in tumors is usually more acidic. The second cathepsin, cathepsin B, also produced in a form of zymogene, can be activated either by cathepsin D or other proteases (elastase, cathepsin G, uPA, tPA). Active cathepsin B can in turn activate prourokinase-type plasminogen activator (pro-uPA). uPA activates plasminogen into plasmin. Both cathepsin B and plasmin are subsequently ready to cleave zymogens of MMPs, producing their active forms. The sequence of the degradation of ECM components seems to be determined as well. Glycoproteins that surround collagen molecules and protect them from proteolysis are degraded first by the action of cathepsins and plasmin. This permits the degradation of collagens by MMPs. As a result, ECM becomes destabilized and the barriers preventing migration of neoplastic cells are removed. Especially difficult to penetrate by cancer cells is a basement membrane with

collagen type IV as a main component. This obstacle can be, however, removed with a help of leukocytes. Leukocyte proteases act on basement membrane, degrade it, and thus facilitate cancer cells migration. Additionally, the contact of leukocytes with neoplastic cells influences the synthesis of other proteases, which further promote the degradation of ECM.

Among other enzymes implicated in ECM remodeling are also ►heparanase and sulfatases, which together with MMPs participate in the alternations of heparin sulfate proteoglycans (HSPGs). HSPGs are interacting with many effector molecules such as FGF, IL-8, and ►VEGF acting as coreceptors, and therefore involved in regulation of biological activities of cells. HSPGs are overexpressed in many cancers. Additionally, the changes occurring in proteoglycan structure upon the action of the three mentioned groups of enzymes result in altered affinity for growth factors and growth factor receptors dramatically affecting transmission of signals. The accelerated hydrolysis observed in some conditions, including cancers, leads to such changes in cell surface proteoglycans that hinder their ability to mediate cell adhesion. Moreover, the shedded fragments were demonstrated to promote tumor growth and metastasis.

The elevated activity of enzymes is an interplay between enzymes and their inhibitors – it can result either from enhanced expression of enzymes or from the reduction of the available inhibitors. Cathepsin B, for example, not only directly activates metalloproteinases but also further enhances their activity by cleaving and thus inactivating their inhibitors TIPM-1

Extracellular Matrix Remodeling. Table 1 Proteases participating in degradation of ECM components

Protease family	Protease	Protease function	Protease inhibitors
Aspartyl protease	Cathepsin D	Degradation of ECM components	
		Conversion of cysteine procathepsins into cathepsins	
Cysteine proteases	Cathepsin B, L, H, K	Degradation of ECM components	Cystatins, stefins, kininogen
		Conversion of pro-MMPs into MMPs	
Serine proteases	Plasmin	Degradation of ECM components	α_2-Antiplasmin
		Activation of uPA	α_2-Macroglobulin
	Urokinase-type plasminogen activator (uPA)	Conversion of inactive elastase into elastase	PAI-1, 2, 3
		Conversion of plasminogen into plasmin	
Neutrophil serine proteases	Elastase	Degradation of ECM	α_2-Antiplasmin
	Cathepsin G	Components	α_2-Macroglobulin secretory leukoprotease inhibitor
Matrix metalloproteinases		Degradation of ECM components	TIMP-1, 2, 3, 4
	Collagenases [MMP-1, 8, 13]	Activation of other pro-MMPs into MMPs	α_2-Macroglobulin
		Degradation of collagens: I, II, III, VII, X and gelatins	
	Stromelysins [MMP-3, 10]	Degradation of proteoglycans, laminin, gelatins collagens: III, IV, V, IX, fibronectin, entactin and collagenases-1	
	Gelatinases [MMP-2, 9]	Degradation of gelatins, collagens: I, IV, V, VII, X, fibronectin, elastin and procollagenase-3	
	Membrane type [MMP-14, 15, 16, 17, 24, 25]	Degradation of collagens: I, II, III, gelatins, aggregan, fibronectin, laminin, MMP-2, 13, tenascin, nidogen	
	Others [MMP-7, 11, 12, 19, 20, 23]	Degradation of proteoglycans, laminin, gelatins, fibronectin, collagens: IV, elastin, entactin, tenascin, α_1-antiproteinase	

From Skrzydlewska et al. [1]

and TIMP-2. Cathepsin B itself can be inhibited by cystatins and ►stefins, and it has been demonstrated that in many pathological conditions, including cancer, the concentration of cystatins has been reduced. An interesting example of interplay between proteases is ►plasminogen activator system. Beside direct degradation of ECM components, it seems to be implicated in tumor cell mobility and ►invasion. It is composed of proactivators, plasminogen, their cell surface receptors, inhibitors, and antiplasmins. uPA activates plasmin, and pro-uPA – its precursor – is activated not only by cathepsin B and elastase, but also by plasmin. uPA and plasmin are inhibited by serpins. The principal role in degradation of ECM is played by MMPs. They are a group of 28 Ca^{2+}- and Zn^{2+}-dependent proteases. Based on their structure and substrate specificity, MMPs were originally classified as collagenases, gelatinases, stromelysins, and matrilysins. Taking into account common functional domains, they are currently divided into eight groups. Under physiological conditions, MMPs are produced in low quantities in zymogene forms. Their expression is induced during ECM remodeling processes by cytokines (IL-4 and IL-10), growth factors (TGF-α, TGF-β, FGF), and cell–cell or cell–matrix interactions. The activation of transcription of MMPs genes can involve either of the three mitogen-activated protein kinases pathways: extracellular signal-regulated kinase (ERK), stress-activated protein kinase/Jun N-terminal kinases (SAPK/JNKs), and p38. Zymogens are activated either by autoproteolysis or by another MMP, or by a serine protease. MMPs are specifically inhibited by TIMPs and small molecules containing TIMP-like domains such as NC1 domain of collagen type IV. Besides inhibiting MMPs, TIMPs themselves express various antioncogenic properties and TIMP-3 is suggested to participate in tumor cell death. A list of the main proteases participating in ECM degradation and their inhibitors is presented in Table 1.

Degradation of ECM is necessary for the migration of neoplastic cells but it also serves other purposes important in tumor cells expansion. It results in unmasking of cryptic sites, production of functional fragments, and in the release of signaling factors. Proteolysis of ECM components reveals new binding sites for interaction with cell surface receptors and in this way increases tumor metastatic potential. Cleavage of ECM and cell surface molecules produces active fragments influencing tumor growth and spread. It has been documented that the degradation of ECM components by MMPs leads to the production of proangiogenic molecules. MMPs are also cleaving E-cadherin producing a fragment that is inhibiting E-cadherin and thus induces tumor cell invasion. On the other hand, the degradation of collagen XVIII by elastase releases a C-terminal fragment, which is a potent inhibitor of angiogenesis and tumor growth. Signaling factors, such as TGF-β, PDGF, or b-FGF, are in many cases stored in an inactive form, bound to ECM components. Hence, elevated activity of matrix proteases results in the release of increased number of growth factors, which can after binding to their receptors activate various signaling pathways. These processes, initiated by tumor cells, are taking place in host tissue and result in altered regulation of intracellular signaling facilitating tumor growth and metastasis.

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Extracellular Nucleic Acids

►Circulating Nucleic Acids

Extracellular Signal-Regulated Kinase (ERK)

Definition

Extracellular signal-regulated kinase (ERK signaling pathway that is preferentially activated in response to growth factors and ►phorbol ester (a tumor promoter), and regulates cell proliferation and cell differentiation.

Extracorporeal Photochemotherapy

Definition
Irradiation of white blood cells with ultraviolet A light in the presence of 8-methoxypsoralen.

►Sezary Syndrome

Extrahepatic

►Klatskin Tumors

Extralesional Excision

Definition
Tumor surgery by which the excision takes place outside the pseudo capsule of the tumor.

►Cryosurgery in Bone Tumors

Extramedullary

Definition
Located or taking place outside the bone marrow.

►Acute Lymphoblastic Leukemia

Extramedullary Hematopoiesis

Definition
Hematopoiesis outside bone marrow such as in spleen and liver. Massive extramedullary hematopoiesis follows the deterioration of normal hematopoietic environment of bone marrow such as in myelofibrosis and myelogenic leukemia.

►Rap1 and Sipa-1

Extrapulmonary Small Cell Cancer

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Synonyms
Oat cell carcinoma; Small cell neuroendocrine carcinoma; Microcytoma; Reserve cell carcinoma; Kulchitsky cell carcinoma; Carcinoma with amine precursor uptake decarboxylation cell differentiation

Definition
Extrapulmonary small cell carcinoma (EPSCC) is a high grade epithelial cancer of neuroendocrine origin composed of small, round to fusiform cells with minimal cytoplasm that arises at various anatomical sites in the absence of a primary lung neoplasm.

Small cell carcinoma (SCC) is a distinct clinicopathological entity first described in the lung. It represents approximately 20% of all bronchogenic carcinoma. Extrapulmonary small cell carcinoma (EPSCC) indistinguishable from small cell ►lung cancer was first reported in 1930. Since its first description, EPSCC has been reported in virtually all anatomical sites. The primary sites most frequently involved are gynecologic organs, especially the cervix; genitourinary organs, especially the urinary bladder; the gastrointestinal tract, especially the esophagus; and head and neck region.

EPSCC often represents a diagnostic and therapeutic challenge. Limited data is available about its clinical behavior and outcome. The available literature is predominantly based on reviews of published cases or analysis of institutional data. The clinicopathological features and general management of EPSCC will be reviewed here, followed by a brief description of SCC specific to the more common sites.

Characteristics
Epidemiology
SCC arising from extrapulmonary sites represents 2–4% of all SCC. Approximately 1,000 cases per year have been reported in the United States, which represents an overall incidence of between 0.1% and

0.4% of all cancer. Patients with EPSCC are generally middle-aged or older similar to SCC of lung, however, women with SCC of cervix tend to be younger. Both genders are affected and predominance of either gender varies according to the primary site of involvement. For example, SCC of esophagus, urinary bladder, and head and neck region are more common in men whereas female preponderance has been noted in patients with SCC of gallbladder. Although cigarette smoking appears to be associated with EPSCC especially of the head and neck region, it has not been clearly identified as a risk factor for EPSCC and role of smoking in the development of malignancy remains speculative.

Pathology

The histologic criteria are the same as those for the pulmonary neoplasm. SCC is composed of sheets and nests of round to fusiform cells with minimal amounts of cytoplasm and granular nuclear chromatin. Nucleoli are absent or inconspicuous. The typical organoid architectural patterns of low-grade ►neuroendocrine neoplasms such as ►carcinoid tumor are generally absent. Mitotic rates are high and necrosis of individual malignant cell is common. It may contain non-SCC elements, varying in type depending on the location.

The pathogenesis of SCC is largely unknown and remained speculative. It exhibits several neuroendocrine features characterized by the presence of enzymes such as of dopa decarboxylase, ►calcitonin, neuron-specific enolase, chromogranin A, and CD56 (neural cell ►adhesion molecule). SCC is thought to originate from totipotent ►stem cells present in all tissues. Others have suggested that it may arise from more differentiated tumors during the clonal evolution of a carcinoma as a late-stage phenomenon.

Clinical Features

The clinical presentation is determined by the site of involvement and extent of the disease. Systemic symptoms, such as anorexia and weight loss, are common especially in patients with advanced disease. Focal symptoms are mostly site-specific and are usually indistinguishable from those of other neoplasms arising from that anatomical site. Though uncommon, similar to SCC of the lung paraneoplastic syndromes such as ectopic ►ACTH production or inappropriate antidiuretic hormone secretion may be the dominant presenting feature. Eaton–Lambert syndrome, thyoxine intoxication, and hyperglucagonemia, have also been reported but are rare.

Diagnosis and Staging

The diagnosis of SCC is primarily rested on morphological assessment. However, ►immunocytochemistry plays an important role and electron microscopy can be of value in difficult cases. The malignant cells are immunoreactive for keratin and epithelial membrane antigen in virtually all cases. Thyroid transcription factor-1 (TTF-1) immunostaining has been proposed by several investigators to differentiate small cell lung carcinoma from EPSCC. However, TTF-1 expression is not specific for SCC of pulmonary origin and should not be used to distinguish primary from metastatic SCC in extrapulmonary sites.

Although no specific staging system for EPSCC has been established most authors have adopted "Two Stage System" originally introduced by the Veterans' Affairs Lung Study Group. This staging system consists of two categories: limited disease (LD) defined as tumor contained within a localized anatomic region, with or without locoregional lymphadenopathy; and extensive disease (ED), defined as tumor outside the locoregional boundaries. Information provided by the "Tumor-Node-Metastases (TNM)" staging system may be valuable in certain anatomical sites such as SCC of large bowel.

The diagnosis of EPSCC requires a normal computed tomographic (CT) study of chest. A primary lung tumor should be excluded. Some investigators have suggested routine bronchoscopy, but this is not widely adopted. Abdominal and pelvic CT scan is a useful test to determine primary site and to assess the extent of the disease. Although there is a lack of data regarding the role of ►positron emission tomography (PET) scan in the management of EPSCC, it can be a useful tool for the detection of primary tumor in SCC of unknown primary site. In the absence of neurologic symptoms, CT scan of head is not routinely performed. Bone marrow biopsy is indicated if there is cytopenia without other evidence of disseminated disease. Other studies such as endoscopic examination are aimed to assess the affected sites and vary accordingly.

Management

Limited data are available about the optimal management of EPSCC. As in pulmonary SCC, the survival of untreated patients is poor. Treatment goals for extensive stage and limited stage disease are different and they should be treated differently. Treatment for LD is potentially curative, whereas that of ED is palliative.

SCC is sensitive to both ►radiation therapy and ►chemotherapy. The unfavorable prognosis and the chemosensitivity of its pulmonary counterpart have persuaded many clinicians to use combined-modality therapy including surgery, radiation and chemotherapy. The response rate varies from 48 to 100%. However, the optimal integration of these modalities and precise sequence is remained to be defined. Whereas chemotherapy can induce major regression of localized disease and concurrently treat occult metastases, surgery and/or radiation therapy represent the best option for ►locoregional therapy at majority of the anatomical

sites. In carefully selected patients with LD and small tumor volume, surgery can be curative. Although the role of ►adjuvant therapy remains to be defined platinum-based adjuvant chemotherapy may be beneficial given the chemoresponsiveness of the disease and the high rate of systemic recurrence. The possible synergism between chemotherapy and radiotherapy supports combined ►chemoradiotherapy and for many patients with LD at various anatomical sites the combination of chemotherapy and radiation therapy can be an effective treatment. Cranial irradiation is not routinely used in the management of these patients who achieved a complete response.

Patients with ED of any site are best managed with systemic chemotherapy. Responses to therapy occur in 60–90% of patients, however, most response are partial and of short duration. The use of surgery or radiotherapy in these patients is restricted for palliation of local symptoms.

Prognosis

SCC follows an aggressive course with early propensity for metastases. EPSCC of various anatomical sites behave differently and outcome varies according to the primary site of the disease involvement. In general the prognosis of EPSCC is comparable to SCC of lung and that extent of disease is an important factor predicting survival. Poor performance status and abnormal white blood cell count are the other important variables that correlate with survival.

In reported series patients with EPSCC are not uniformly treated or comparably staged. The median overall survival of all patients with EPSCC is 9–15 months and 5-year survival is 10–15%. Patients with LD have median overall survival of 25–34 months and 5-year survival of 31% whereas patients with ED have a median overall survival of 2–12 months and 5-year survival of 2%. Despite the generally aggressive behavior of SCC, long-term remission or cure can be achieved in selected patients with a tailored therapy.

Genitourinary Tract

Urinary Bladder

SCC has been reported in the urinary bladder, prostate, and kidney. Although urinary bladder is the most common site of EPSCC in the genitourinary tract, it accounts for less than 1%t of all ►bladder cancers. Patients are usually between the ages of 40 and 60 years, and it is three times more common in men than women. SCC may coexist with ►transitional cell carcinoma and other types of bladder tumors. Majority of patients present with locally advanced or disseminated disease. Surgery is generally recommended for patients with localized disease often followed by adjuvant chemotherapy. Combination of chemotherapy and radiation have been given concurrently in an effort to preserve the bladder in many cases. The overall median survival in most reported series is about 2 years.

Prostate

The incidence of prostate SCC is less than 1% of the total of ►prostate cancer. The median age of the patients is approximately 65 years, which is similar to that of patients with adenocarcinoma of prostate. Prostate SCC may present at initial diagnosis or appear later in the evolution of an adenocarcinoma. Approximately 30% of patients present initially with prostatic adenocarcinoma, 20% present with combined adenocarcinoma and SCC, and 50% of patients presented with SCC. ►Prostate specific antigen (PSA) is not elevated in majority of the patients with prostate SCC and they respond poorly to antiandrogen therapy. Most of the patients have advanced disease at diagnosis and median survival is about 15 months. Patients presenting initially with an adenocarcinoma have a median survival of 25 months compared with a median survival of 5 months for patients presenting with SCC.

Gynecological Sites

Cervix

SCC most commonly involve cervix but may also develop in the endometrium, ovary, vagina, and vulva. SCC represents 0.4–1.4% of all ►cervical cancer. The cervix should always be considered as the site of origin in a woman with a SCC of unknown primary site. Women with cervical SCC tend to be younger with median age of diagnosis is about 40–50 year. The prognosis varies with the stage of the disease. Survival is poor with hysterectomy alone and most patients are treated with a multimodality approach, using chemotherapy regimens that are typically used for small cell lung cancer. Patients with cervical SCC treated with combination of ►chemoradiotherapy had 3-year survival of 60%.

Gastrointestinal Tract

Esophagus

Primary SCC involving the esophagus appears to be the most frequently reported digestive tract site of EPSCC. Stomach, pancreas, ampulla of Vater, gallbladder, small intestine, and colon and rectum are the other sites in gastrointestinal tract where SCC have been reported. SCC of esophagus is rare and incidence has been estimated to range from 0.8 to 2.4% of all ►esophageal cancers. Most cases occur between the ages of 50 and 70 years, and EPSCC is twice as common in men compared with women. Combination chemoradiotherapy is effective against esophageal SCC and may improve survival. ►Adjuvant systemic chemotherapy is recommended following surgery for localized disease although long-term survival has been reported in a few

cases. The reported overall median survival is approximately 5 months, with a median survival for patients with LD is about 8 months and for patients with ED is about 3 months.

Colon and Rectum

SCC arising in the colon and rectum is rare making up approximately 0.2% of all colorectal neoplasms. The epidemiology is somewhat similar to that of adenocarcinoma with a slight male predominance. The majority of the cases are diagnosed between the ages of 50 and 70. Within the large bowel, the most frequent site is the rectum, followed by the cecum and sigmoid. Tumors with mixed histology are often present. The overall prognosis is poor with a median survival of 6 months. Surgery is the primary treatment for localized disease. ►Adjuvant radiation for local control and systemic chemotherapy to treat ►micrometastases are recommended, however, in the absence of clinical trials, the individual contribution of each component to the survival cannot be determined.

Head and Neck Region

Larynx

Although larynx is one of the most common extrapulmonary sites, laryngeal SCC account for only 0.5% of all primary ►laryngeal carcinoma. Most patients are between the ages of 60 and 80 years, and there is a male predominance. Smoking, tobacco chewing, and excess ►alcohol consumption have been associated with SCC of the larynx. The supraglottic region is the most commonly reported site. The majority of patients of localized SCC of the head and neck have been treated with local modality of treatment. Although optimal management for these patients is undefined, several investigators have reported that the use of concurrent chemoradiotherapy regimens for limited-stage disease offer potential for long-term survival. Median survival of patients with primary SCC of the larynx, hypopharynx, and trachea is between 7 months and 11 months.

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Extrarenal

Definition

Arising outside of the kidney, most often in soft tissues

►Rhabdoid tumor

Extrarenal Rhabdoid Tumor

Definition

Sarcoma of children. The neoplastic cells have abundant eccentric cytoplasm with eosinophilic "inclusions" and co-express epithelial and mesenchymal markers. Genetic study has shown mutations, deletions or translocations of chromosome 22.

►Uncertain or Unknown Histogenesis Tumors

Extraskeletal Myxoid Chondrosarcoma

Definition

Sarcoma with a multinodular pattern, myxoid matrix and malignant chondroblast-like cells arranged in cords and nests. The thigh is the most common region affected. There is no evidence of cartilaginous differentiation and the cells are only positive for vimentin.

►Uncertain or Unknown Histogenesis Tumors

Extravasation

Definition

In inflammation, the movement of leukocytes from the capillaries to the tissue is called extravasation. In cancer, it refers to circulating tumor cells exiting the microvasculature to the surrounding tissue. The passage of cancer cells from blood or lymph vessels to the

surrounding tissues. Escape of a particle from the bloodstream into the surrounding tissues.

►Tumor-Endothelial Cross-talk
►Circulating Tumor Cells

Extreme Hypoxia

►Anoxia and Cancer

Extrinsic Membrane Proteins

Definition

►Peripheral Membrane Proteins

Extrinsic Pathway of Apoptosis

Definition

One of the two archetypal pathways of caspase activation, known also as "the death receptor pathway." This pathway of programmed cell death is initiated through the stimulation of the transmembrane death receptors.

►APAF-1 Signaling
►Apoptosis

Ezrin-Radixin-Moiesin (ERM) Family

Definition

Family of intracellular structural proteins.

►ERM Proteins

4F9

►Metastasis Suppressor KAI1/CD82

F-box and Leucine-rich Repeat Protein 1 – Fbxl1

►Ubiquitin Ligase SCF-Skp2

FAB Classification

Definition

French-American-British Classification; Standardized classification system for acute leukemias based on morphology.

►Chromosomal Translocation t(8;21)

Fab Fragment

Definition

A proteolytic fragment of the immunoglobulin molecule resulting from digestion with the enzyme papain.

►Anti-HER2/Neu Peptide Mimetic

FAC

Definition

*F*ocal *a*dhesion *c*omplex is a multiprotein complex that is transiently formed inside the cell in response to ligand binding to integrins. FAC is attached to actin filaments that allow the cell to exert the mechanical tension required for cell movement. FAC can also activate other downstream signaling pathways that can alter cell ►migration.

►Osteopontin
►Integrin Signalling

FACS

Definition

Individual cells can be characterized and separated in a machine called a fluorescence-activated cell sorter (FACS) that measures cell size, granularity, and fluorescence due to bound fluorescent antibodies as single cells pass in a stream past photodetectors. The analysis of single cells in this way is called flow cytometry and the instruments that carry out the measurements and/or sort cells are called flow cytometers or cell sorters.

Factor Ia

Definition

►Fibrin

Factor V

Definition

Proaccelerin, Labile Factor; Cofactor of the blood coagulation cascade without enzymatic activity.

►Proteinase-activated Receptors

Factor VII

Definition

Proconvertin; Serine proteinase that is involved in blood coagulation. It binds to tissue factor, where it is activated by thrombin, factor Xa or another activated factor VIIa molecule, bound to tissue factor. It can stimulate proteinase-activated receptor 2.

►Proteinase-activated Receptors

Factor VIII

Definition

Procofactor of the blood coagulation cascade. It is produced by vascular endothelial cells. It is mainly bound to ►von Willebrand factor (vWF) in the circulation. Upon conversion by thrombin or factor Xa, the active cofactor (factor VIIIa) is released from vWF and forms a complex with factor IXa which in turn activates factor X.

►Proteinase-activated Receptors

Factor IX

Definition

Christmas Factor; Serine proteinase of the coagulation pathway. It activates factor XI and prekallikrein

►Proteinase-activated Receptors

Factor X

Definition

Stuart-Prower Factor; Serine proteinase of the coagulation pathway. It is produced by the liver and circulates in the blood stream. It is activated to form factor Xa by factor IXa and factor VIIa. Together with factor Va, it activates prothrombin. It can also stimulate proteinase-activated receptors 1 and 2.

►Proteinase-activated Receptors

Factor XI

Definition

Plasma Thromboplastin Antecedent; Serine proteinase of the blood coagulation cascade. It is produced by the liver and is converted by factor XIIa and thrombin to enzymatically active factor XIa.

►Proteinase-activated Receptors

Factor XII

Definition

Hageman Factor; Serine proteinase of the coagulation pathway. It activates factor XI and prekallikrein.

►Proteinase-activated Receptors

D-factor

►Leukemia Inhibitory Factor

FAD

Definition

Abbreviation for Final Appraisal Document.

►National Institute for Health and Clinical Excellence (NICE)

FADD

Definition

Fas-associated protein with death domain (FADD) is a protein that bridges Fas that belongs the ►tumor necrosis factor (TNF) family and other death receptors

to ►caspase-8 to form death inducing signaling complex (►DISC) during cell death.

►Methoxyestradiol
►FLICE Inhibitory Protein
►Apoptosis

FADD-like Antiapoptotic Molecule 1

►FLICE Inhibitory Protein

FADD-like ICE

►Caspase-8

FAE

Definition

Fumaric acid ester.

►Dimethylfumarate

FAK

►Focal Adhesion Kinase

Familial Adenomatous Polyposis

Definition

FAP; Is an autosomal dominant disease characterized by the presence of at least one hyndred polyps in the large bowel and by several extracolonic manifestations, which include polyps in other organs, desmoid tumors, dental abnormalities, osteomas, and congenital hypertrophy of the retinal pigmented epithelium. An inherited disease of the large intestine marked by the formation of numerous glandular polyps that typically become malignant if they are left untreated. In most cases it is caused by inactivation of the APC gene (►APC gene in Familial Adenomatous Polyposis).

►Colorectal Premalignant Lesions
►APC Gene in Familial Adenomatous Polyposis

Familial Breast Cancer

Definition

Familial clustering of breast cancer due to inherited germ line mutations in breast-cancer risk genes, BRCA1, BRCA2, and probably in additional genes that so far have no been identified.

►BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk.

Familial Cancer

Definition

If a cancer is familiar, it is more common in relatives of an affected individual than in the general population. The level of aggregation is usually measured as a relative risk in affected families as compared to all families. A relative risk of 2.0 implies a two-times higher risk for the offspring of an affected parent.

Breast cancer, for example, is two-times more common in daughters whose mothers had breast cancer. The risks can be measured between parents and offspring, between siblings or between any first-degree relatives.

Familial aggregation is found in all types of cancer. The highest familial risks are observed for thyroid and testicular cancers, with familial risks between 5 and 10. Higher than 3.0 are the risks for ►melanoma, ►prostate cancer, ►endometrial cancer and squamous cell ►skin cancer. The breast and colon cancer the risk is about 2.0.

The reasons for familial cancer are inherited susceptibility. A shared environment and common patterns of behavior also play a role. Familial aggregation of ►lung cancer and ►cervical cancer (relative risk about 2.0) can be partially explained by environmental factors. Inherited cancers are a subgroup of familial cancers where the genetic component is obvious. In

many cases the underlying genes have been identified and gene tests are available for some cancer-related genes. Inherited cancers are often monogenic and confer a high risk in those family members who have inherited the defective gene. The frequency of disease is described by penetrance, which is 100% if all carriers of the defective gene contract cancer. Inherited cancers include cancers at many sites and syndromes at multiple sites.

►Colon Cancer
►Breast Cancer Genes BRCA1 and BRCA2
►Familial Adenomatous Polyposis

Familial Cancer Syndrome

Definition

Occurrence of specific heritable cancer types in a familial manner.

►Familial Cancer

Familial (constitutional) Panmyelocytopathy (type) Fanconi

►Fanconi Anemia

Familial Hypoplastic Anemia Fanconi

►Fanconi Anemia

Familial Polyposis Coli

Definition

FAP.

►Familial Adenomatous Polyposis
►APC Gene in Familial Adenomatous Polyposis

FAMP

►Fludarabine

FANC Genes

Definition

Designation of genes, whose ►homozygous or ►hemizygous mutations cause ►Fanconi anemia.

FANCJ

►BACH1 Helicase

Fanconi Anemia

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Synonyms

Familial (constitutional) panmyelocytopathy (type) Fanconi; Familial hypoplastic anemia Fanconi

Definition

Fanconi anemia (FA) is a human inherited disorder clinically characterized by variable congenital anomalies, bone marrow failure and cancer susceptibility. Cellular features include genomic and chromosomal instability and hypersensitivity to DNA-crosslinking agents. FA is a rare recessive disease with an overall prevalence of about 1 in 300,000, but much higher prevalence in selected populations.

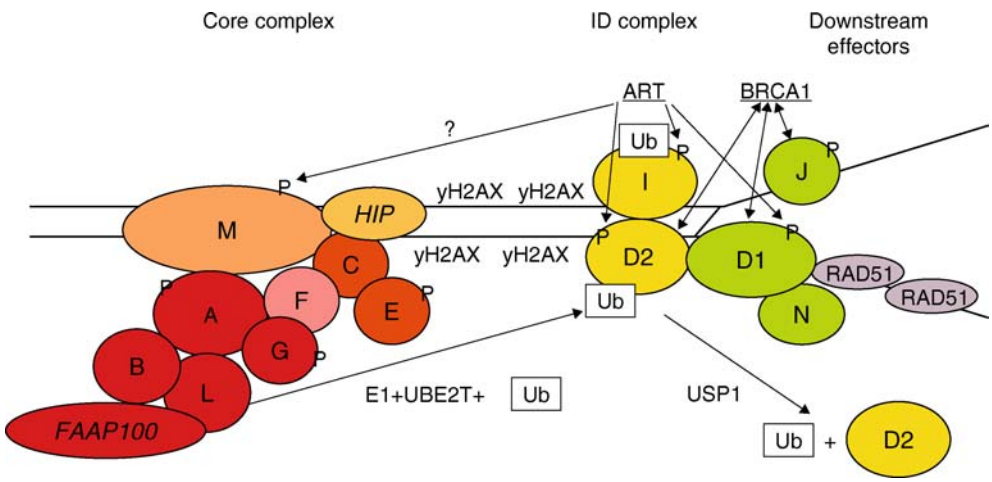
Characteristics

Approximately 70% of FA patients display variable presence of (intrauterine) growth retardation, hyper- and hypopigmentations of the skin, radial ray and external ear defects, microcephaly, microphthalmia, and malformations of the inner organs including most

frequently kidney, gastrointestinal tract, heart and brain. FA is genetically heterogeneous comprising at least 13 ►complementation groups (A to N). Underlying these subtypes are biallelic (in case of the X-chromosomal group FA-B hemizygous) mutations in 13 different genes (*FANCA*, *-B*, *-C*, *-D1*, *-D2*, *-E*, *-F*, *-G*, *-I*, *-J*, *-L*, *-M* or *-N*). The products of the FA genes interact with themselves and with a number of other proteins in a common pathway. The FA pathway for genomic maintenance, also called FA/BRCA pathway (Fig. 1), is a DNA damage-activated signaling pathway, important for the recognition of ►DNA-interstrand crosslinks and the initiation of repair of these lesions by nucleotide excision repair, ►translesion synthesis and ►homologous recombination. FA patients have defects in the FA pathway and share a high risk for the occurrence of characteristic malignancies at relatively young age. Approximately 23% of FA patients develop one kind of neoplasm or more during lifetime. In most cases, there are sharply increased risks for ►acute myeloid leukemia (AML) during childhood and ►squamous cell carcinomas (SCC) becoming effective during young adulthood. These risks are associated with defects of any of the FA genes except *FANCD1* and *-N*. Associated with defects of the latter is a different risk combination with earlier onset. *FANCD1* is also known as ►*BRCA2* and *FANCN* as ►*PALB2*. ►Biallelic mutations in either of them consistently result in a type of FA with strong risks for developmental types of cancer during infancy including ►medulloblastoma and ►nephroblastoma (►Wilms tumor), and AML. In particular defects of *FANCD1* and *-N* include a number of cases with multiple tumors. FA-D1 and -N patients invariably succumb to their malignancies at early age. Given the high degree of genomic instability and elevated mutation rates seen in FA cells, there is little doubt about a causal relationship between the FA-specific defect of genomic maintenance and the emergence of cancer in FA patients.

Myelodysplastic Syndromes (MDS)

In ►myelodysplastic syndromes, bone marrow precursor cells fail to differentiate properly such that not enough mature erythrocytes, leukocytes and thrombocytes arrive at the periphery. MDS is a frequent hematological disorder in FA. It occurs in ~7% of all patients. MDS is generally considered a premalignant condition, but less so in the context of FA. FA-related MDS progresses to ►leukemia more slowly compared to MDS in non-FA patients; it obviously follows a different natural course. Thus, FA patients may suffer from MDS for many years without developing overt leukemia. Nevertheless, the presence of MDS affects the prognosis of FA: The estimated 5-year-survival rate of FA patients with MDS amounts to only 0.09 as opposed to 0.92 in patients without MDS. Wherever



Fanconi Anemia. Figure 1 Model of the FA/BRCA signaling network. A DNA interstrand-crosslink stalls a replication fork. ATR is activated and phosphorylates FANCM, FANCD2, FANCI and FANCD1/BRCA2. The FA core complex (FANCA, -B, -C, -E, -F, G, -L and -M, *orange* to *red*) is assembled and loaded onto double-stranded DNA through FANCM and the Hef-interacting protein, HIP (FAAP24). The active FA core complex translocates along DNA and, as a E3 ligase, monoubiquitates FANCD2 and FANCI by means of UBE2T as the E2 ligase and FANCL as the E3 catalytic subunit. The ID complex (FANCI and -D2, *yellow*) is directed to chromatin at the stalled replication fork via γH2AX. The active ID complex, supported by BRCA1, recruits downstream effector proteins (*green*). While FANCJ/BRIP1 is thought to stabilize the replication fork and enable translesion synthesis, FANCD1/BRCA2 in combination with FANCN/PALB2 uploads RAD51 (*violet*) and is attributed a role in restart of the replication fork after homologous recombination. All of the latter proteins colocalize in repair foci. Adapted from Wang (2007).

possible, MDS is treated by ►hematopoietic stem cell transplantation (HSCT) to diminish the risk of transition to AML.

Leukemia

The most common malignancy arising in FA patients is leukemia. Its overall cumulative incidence is 37% (33% by age 40). The median age of onset is 14 years, thereafter the incidence rate of leukemia levels out at <1% per year. With 94% of all leukemias, AML predominates in FA with the relative risk being about 500- to 800-fold increased. ►Acute lymphoblastic leukemia (ALL) amounts to only 6%, in contrast to 84% of leukemias in the general population being ALL. Chronic myelomonocytic leukemia (CMML) or ►Burkitt lymphoma have been reported in single cases.

All FAB subclasses are represented in FA-associated AML, albeit with different proportions compared to the general population. There are more FA patients in the M4 and M6 and fewer in the M1 and M2 subclasses than in non-FA AML patients. Monosomy of chromosome 7 and partial tri- or tetrasomies of chromosome 3q, occurring as clonal aberrations in bone marrow and detectable also in peripheral blood leukocytes, are proven risk factors for the development of MDS and AML. AML may be the first and only manifestation of FA in otherwise unsuspicious patients.

Solid Tumors

The overall risk of FA patients for the emergence of solid tumors exceeds that of the general population by nearly 50-fold, with higher risks for selected tumors. The rate of solid tumors rises more than linearly and reaches 10% per year by age 45. If the competing death risks of anemia and leukemia could be removed, the estimated cumulative probability of solid tumors in FA patients would amount to 76% by that age.

The median age of onset of cancer in FA, including solid tumors and leukemia, is 16 years, whereas it is 68 years in the general population. In up to 25% of FA cases with all types of malignancies, the diagnosis of neoplasm precedes the diagnosis of FA, an extreme example being a 49-year-old patient with bilateral carcinoma of the breast, who developed MDS and AML following chemotherapy.

The incidence of solid tumors in FA patients increases beyond disease-associated rates after ►HSCT with the rate of tumor development in the HSCT group being 2.8-fold the rate in the untransplanted FA group. Conditioning, especially following regimens including total body irradiation, but also immunosuppression or posttransplant complications such as chronic graft versus host disease or infections, sharply enhance the risk for malignancies with poor survival. Most of these tumors are head and neck cancers, often involving the tongue.

Squamous Cell Carcinoma of the Head and Neck (HNSCC)

The most typical solid tumors in FA are ►HNSCC and ►SCC of the genital region in females. HNSCC in FA includes tumors of the tongue, gingiva, oropharynx and larynx. The cumulative lifetime incidence of HNSCC is about 700-fold increased with a median patient age of 31 years. In addition, there is an ~2,300-fold increased risk compared to the general population to develop carcinoma of the esophagus. FA patients with HNSCC are usually young, non-smokers and non-drinkers. Females are more often affected than males, particularly among those patients, in whom the diagnosis of FA was made after the diagnosis of cancer. This may reflect the overall survival advantage of female FA patients. FA-associated HNSCC is not fundamentally different from sporadic HNSCC, except a potential sensitivity to crosslinking agents. HNSCC initially presents as chronic ►inflammation or just red or white mucosal spots or minor lesions such that the diagnosis is often delayed. This delay leads to poor therapeutic success and no better than 50% 2-year-survival rates with this aggressive tumor type. A sensitive brush method for obtaining cells from the tongue and oral cavity of FA patients may facilitate monitoring of early cell changes and thereby contribute to the prevention of this devastating type of malignancy.

SCC of the Vulva and Cervix

The cumulative probability to develop carcinoma of the vulva is 4,300-fold, that to develop carcinoma of the cervix is 200-fold increased in FA patients. The occurrence of SCC of the vulva, cervix and anus may not only be promoted by FA, but additionally be triggered by human papilloma virus infections. It has been reported that 54% of all FA patients with these tumors had HPV-associated ►condylomas before the occurrence of cancer.

Liver Tumors

Liver tumors are frequent in FA and in the great majority follow androgen therapy for aplastic anemia. They are often multiple and mostly include adenomas (benign focal nodular hyperplasia or peliosis), seldom ►hepatocellular carcinomas (HCC). In rare cases, both adenomas and HCC can be found in the same patient. Adenomas without liver cell dysplasia are not considered premalignant changes and usually regress upon discontinuation of androgen therapy. The median age of onset of these tumors is 13 years and the cumulative incidence is 46% by age 50. The development of liver tumors due to androgen therapy is not specific for FA patients. The risk for HCC may depend on the length of term of therapy and on the specific

type of androgen used. Oxymetholone and methyltestosterone appear to be associated more frequently with the occurrence of HCC, whereas patients treated with danazol develop adenomas, but may be less affected by HCC.

Other Tumors

Solid neoplasms occasionally found in FA patients include ►brain tumors and ►renal carcinomas, ►breast cancer, ►basal cell carcinoma, ►neuroblastoma, ►desmoid tumors, gonadoblastoma, ►melanoma, neurilemmoma and ►osteosarcoma. A major factor thought to promote tumor development in FA is oxidative stress. *In vitro* experiments suggest that FA cells are more sensitive than non-FA cells towards the effects of reactive oxygen species.

FA Gene Involvement in Neoplastic Disease of Non-FA Individuals

Among the known ►breast cancer risk genes, three are authentic FA ►genes. These include the downstream effector genes of the FA/BRCA pathway, *FANCD1* = *BRCA2*, *FANCN* = *PALB2* and *FANCJ* = ►*BRIP1*. *BRCA2* is considered a high-penetrance breast cancer susceptibility gene with heterozygous mutations increasing the risk more than tenfold. Heterozygous *BRCA2* mutations are associated with female and male breast cancer, ovarian cancer and pancreatic carcinoma in familial forms. All of these cancers show allelic loss at the *BRCA2* locus consistent with the tumor suppressor function of the gene.

FANCN = *PALB2* has been identified as a low-penetrance susceptibility gene for female and male breast cancer with ►monoallelic truncating germline mutations causing a 2.3 times higher risk for female breast cancer at all ages and a relative risk of 3.0 for women under 50 years of age. This low-penetrance susceptibility is surprising since PALB2 is functionally associated with ►BRCA2 and biallelic mutations in both genes cause a similar, severe type of FA.

FANCJ = *BRIP1* interacts with the ►BRCT domain of ►BRCA1. Heterozygous inactivating mutations in ►*BRCA1* predispose to breast cancer and inactivation of ►BRIP1 abrogates certain BRCA1 functions. Consistently, heterozygous truncating germline mutations in the *FANCJ* gene *BRIP1* have been shown to represent low-penetrance breast cancer susceptibility alleles and confer an approximately twofold increased risk at all ages and a relative risk of 3.5 for carriers aged less than 50 years.

Studies of *FANCA*, *-C*, *-D2*, *-E*, *-F* and *-G* in familial breast cancer cases did not identify any truncating mutations. However, there are not enough data to date, which would exclude that certain mutations in some of these FA genes might enhance, for instance, breast cancer risk, as has been proposed for three different common mutations in *FANCC* by study of FA families.

Inactivation of the FA/BRCA pathway through hypermethylation of the *FANCF* gene has been reported for a variety of spontaneous malignancies including cervical, ovarian, bladder and ►non-small-cell lung cancer, AML, and HNSCC. The proportion of cases affected by *FANCF* silencing in each of these tumor types is relatively low, a maximum being reported 21% *FANCF*-methylated ovarian carcinomas in one study. Of note, the *FANCF* gene is located adjacent to a hot-spot region for hypermethylation on chromosome 11p15. Functional suppression of imprinted genes in this region has been found in many tumor types. Thus, *FANCF* ►hypermethylation may well occur incidentally due to spreading of the ►epigenetic modification.

The occasional involvement of germline or rare somatic FA gene mutations and ►LOH has also been reported in some sporadic cancers. As with *FANCF* hypermethylation, only a minority of tumors in non-FA individuals reveals mutations in FA genes, with *BRCA2* predominating. In pancreatic carcinoma cell lines, the proximal FA pathway was inactivated in 9.5%, and *BRCA2* in 7% of cases. One explanation is that sporadic disruption of the FA pathway causing genomic instability might be an early event during malignant transformation in a subset of tumors, preferentially among FA-typical cancers that occur in non-FA patients. Alternatively, it could be a later phenomenon during tumor advancement studies of microsatellite instability were incompatible with selective pressure for functional loss of BRCA2 in tumor evolution, but fitted closely with the absence of selection. This suggests that *BRCA2* inactivation occurs at a time when positive or negative selection for or against such event has been lost, implying that disruption of the FA/BRCA pathway in sporadic cancer is rather a stochastic phenomenon with neutral genetic drift during tumor progression. This resembles silencing of *FANCF*. Where it occurs, it is often found in only some proportion of tumor cells. Cancer rates in obligate FA heterozygotes comparable to those in the general population (apart from carriers of mutations in the FA genes mentioned above) also argue against disruption of the FA/BRCA2 pathway as an event initiating tumorigenesis.

The functional consequences of disruption of the FA pathway in sporadic cancer have not fully been explored. Nevertheless, drug-induced inhibition of the FA/BRCA pathway has been suggested as a potential route to sensitization of tumors to DNA-crosslinking agents. It has been proposed as a means of targeted chemotherapy. However, once the cell death pathway is compromised, e.g. by silencing of ►TP53, and disconnected from the FA pathway in tumor cells, no benefit of such therapy is to be expected.

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FAP

Definition

►Familial Adenomatous Polyposis

Farnesyl Lipid

Definition

C15 isoprenoid lipid, an intermediate in the ►HMG-CoA reductase mevalonate biosynthetic pathway, used in the biosynthesis of ►farnesylated proteins.

Farnesylated

Definition

A molecule that has a posttranslational modification that adds a fifteen carbon fatty acid residue.

Farnesyltransferase

Definition

Enzyme that adds farnesyl residues to protein molecules as a posttranslational modification.

FAS

Definition I

Synonym APO-1, CD95 Fas, also known as APT1, tumor necrosis factor receptor superfamily member 6 (TNFRSF6), CD95, APO-1 and FAS1 is a type I membrane protein of about 40 kD. It was originally identified by mouse monoclonal antibody called anti-Fas or anti-APO-1. In the 6th Workshop and conference on Human Leukocyte Differentiation Antigens (Kobe, 1996), Fas was designated as CD95. The gene maps to 10q24 and is mutated in Autoimmune Lymphoproliferative Syndrome. The protein mediates apoptosis through sequential activation of ICE-like caspases (Casp 4, Casp 5, Casp 3) and may also be involved in autoimmune diabetes.

►Apoptosis
►Chemokine

Definition II

►Fatty Acid Synthase

Fas-associated Death Domain

►FLICE Inhibitory Protein

Fas Associated with a Death Domain

Definition

FADD; Is a 208 amino acid protein that is encoded by two exons located on chromosome 11q13.3. It consists of an N-terminal death-effector-domain (DED) and a C-terminal death domain (DD). FADD functions as cytosolic adaptor molecule critical for apoptotic signaling by death receptors of the TNF receptor I (TNFR1) superfamily.

►FADD
►Caspase-8

FAS I

Definition
Type I fatty acid synthase found in mammals and fungi; a multifunctional polypeptide.

►Fatty Acid Synthase

FAS II

Definition
Type II fatty acid synthase found in prokaryotes, plants, and mitochondria. It consists of an acyl carrier protein and seven structurally independent monofunctional enzymes.

►Fatty Acid Synthase

Fas Ligand

Definition
FasL is a cell-surface member of the TNF family of proteins. Binding of Fas ligand to Fas triggers apoptosis in the Fas-bearing cell.

►FAS
►Apoptosis
►Apoptosis-Induction for Cancer Therapy

Fasciclin I

Definition
FASI; contains sequences that allows binding integrins and glycosaminoglycans in vivo.

►Periostin

Faslodex

Definition
Trade name for ►fulvestrant, an estrogen receptor antagonist used in the treatment of advanced ►breast cancer, and marketed by AstraZeneca.

FASN

►Fatty Acid Synthase

FAT

Definition
The focal adhesion targeting domain of focal adhesion kinase mediates paxillin and talin binding.

►Focal Adhesion Kinase

Fatty Acid Synthase

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Synonyms
FAS; FASN

Definition
Fatty acid synthase is a key enzyme which regulates the de novo biosynthesis of long-chain fatty acids.

Characteristics
Structure and Function
Fatty acid synthase (FAS) is a key enzyme that regulates the de novo biosynthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA in the presence of

NADPH. There are two types of FAS. ►FAS II, found in prokaryotes, plants, and mitochondria, consists of an acyl carrier protein (ACP) and seven structurally independent monofunctional enzymes. Each of these enzymes is encoded by a separate gene that produces a unique protein which catalyzes a single step in fatty acid synthesis. Mammalian FAS, named ►FAS I, consists of two identical multifunctional polypeptides. Each monomer of FAS I contains seven distinct catalytic domains starting from the N-terminal. These catalytic domains including β-ketoacyl synthase (KS), malonyl/acetyltransferase (MAT), dehydrase (DH), enoyl reductase (ER), β-ketoacyl reductase (KR), acyl carrier protein (ACP), and thioesterase (TE).

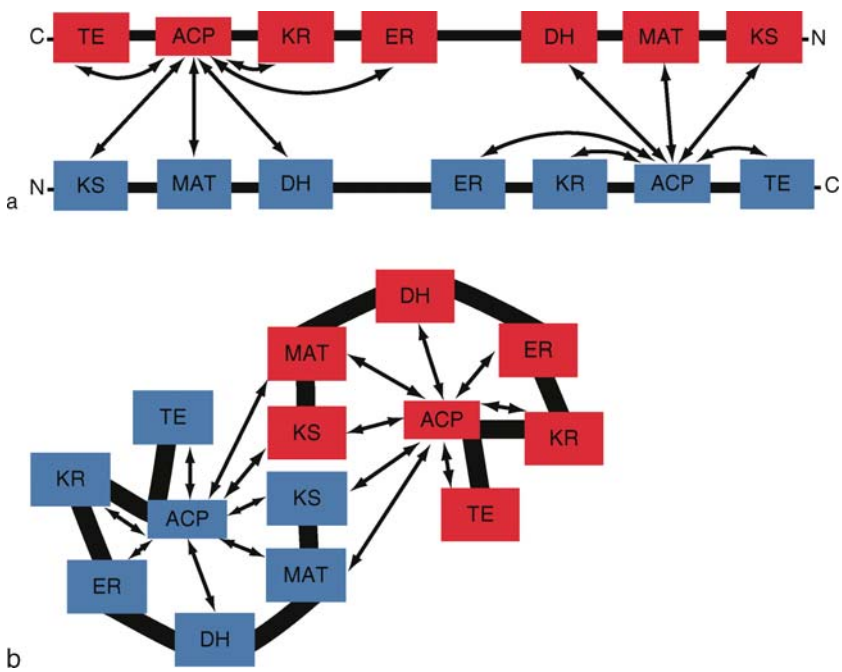
Two models have been proposed for the structure of FAS. The conventional model is based on crosslinking studies with the bifunctional reagent, 1, 3-dibromopropanone (DBP). In this model, two identical monomers are arranged in a head to tail orientation that allows functional interactions across the monomer interface (Fig. 1a). The second model is based on functional mutant complementation and improved DBP crosslinking studies. These studies suggest that the FAS monomers form a coiled conformation that allows for a variety of intra- and intermonomer functional domain interactions (Fig. 1b). In this model, both of KS and MAT domains are located in the center of the FAS dimer and can interact with the ACP groups on either of the monomers.

FAS in Normal Tissues

FAS I plays an essential role during embryonic development and a key role in energy homeostasis in adult mammals. Most normal tissues preferentially use circulating dietary fatty acids for the synthesis of new structural lipids. Thus, FAS expression is generally low to undetectable in most human normal tissues and is high only in the lipogenic tissues, such as liver and adipose tissues. In liver and adipose tissues, fatty acid synthesis occurs when it is necessary to store excess calories from carbohydrates as ►triglycerides. FAS expression is tightly controlled by nutrients through transcriptional induction and regulated by insulin, glucagon, glucocorticoids, and thyroid hormone. During starvation, fatty acid oxidation is activated to produce free fatty acids for survival, and FAS activity is rapidly downregulated. The effect of nutrients and ►hormones on the expression of lipogenic genes is mostly mediated by the sterol regulatory element-binding protein-1 (►SREBP-1). In adipose tissue, the ►transcription factor, ►peroxisome proliferator-activated receptor γ PPARγ, is critical for regulating both adipogenesis and lipogenesis. Activation of PPARγ stimulates fatty acid synthesis and storage.

FAS and Cancer

Elevated expression of FAS has been observed in many human tumors, including carcinoma of the breast,



Fatty Acid Synthase. Figure 1 Two models for the organization of FAS [1].

prostate, colon, ovary, endometrium, mesothelium, lung, thyroid, and stomach. Moreover, overexpression of FAS in breast, prostate, and thyroid ►cancers has been associated with more aggressive malignancies. The differential expression of FAS in normal and tumor tissues has led to FAS being considered as a target for anticancer therapy. Abnormally active endogenous fatty acid metabolism appears to be important for cancer cell proliferation and survival. The data from clinical studies and animal models indicate that tumor cells constitutively express high levels of FAS and undergo significant endogenous fatty acid biosynthesis. In contrast to liver and adipose tissues, the function of elevated endogenous synthesis of long-chain fatty acid in most human cancers is not for energy storage; the new synthesized fatty acids are converted into ►phospholipids, not triglycerides. In cancer cells, overexpression of FAS seems to be independent of the regulatory signals found in normal tissues. Recent studies indicate that ►mitogen-activated protein kinases (MAPKs) and ►PI3 kinases pathways ►(PI3K signaling) are likely involved in FAS regulation via SREBP-1c. Inhibition of MAP and PI3 kinases downregulates SREBP-1 levels, thereby reducing FAS expression and fatty acid synthesis in transformed human cancer cells. Deletion of the major SREBP-binding site from the FAS promoter abrogates the transcription activity of FAS. The constitutive activation of the MAPK and PI3 kinase AKT signaling pathways in cancer cells leads to elevated levels of FAS and sustained fatty acid synthesis.

FAS Inhibitors as Potential Cancer Chemotherapeutic Agents

Several FAS inhibitors have been developed to study the loss of FAS function in tumor cells. These inhibitors include ►cerulenin, the cerulenin derivative, ►C75, the β-lactone, ►orlistat, and the ►green tea polyphenol, epigallocatechin-3-gallate (EGCG). Cerulenin, C75 and orlistat are selective inhibitors of tumor cell growth.

Cerulenin, (2R, 3S)-2, 3-epoxy-4-oxo-7, 10-trans, *trans*-dodecadienamide, was the first identified "specific" inactivator of FAS. It is a natural antibiotic product of the fungus *Cephalosporium ceruleans*. Cerulenin irreversibly inhibits FAS by binding covalently to the active site cysteine thiol in the β-ketoacyl-synthase domain. Cerulenin is selectively cytotoxic to a number of established human cancer cell lines including leukemias, breast, colon, brain, ovary, and prostate. The inhibition of fatty acid synthesis by cerulenin has been shown to be dose-dependent. The cytotoxic effect generally parallels the level of endogenous fatty acid synthesis in human breast tumor cells. FAS inhibition by cerulenin leads to apoptotic cell death in ►breast cancer, ►prostate cancer, ►brain cancer, and ►colon cancer cells. However, cerulenin's chemical instability renders it ineffective as a systemic anticancer agent.

C75, a potent derivative of cerulenin, is a more stable FAS inhibitor. Structurally, it is a cell-permeable α-methylene-γ-butyrolactone, designed to be less reactive and potentially safer than cerulenin. In vivo and in vitro studies have confirmed the selective toxicity of C75 against tumor cells. C75-mediated inhibition of FAS increases malonyl-CoA levels and inhibits carnitine palmitoyltransferase 1 (CPT-1) activity, preventing the oxidation of newly synthesized fatty acids. High levels of malonyl-CoA and low levels of CPT-1 may represent mechanisms whereby FAS inhibition leads to tumor cell death. C75 treatment of mesothelioma and prostate tumor (►Prostate cancer, clinical oncology) ►xenografts in ►nude mice leads to significant inhibition of tumor growth. Subcutaneous xenografts of MCF-7 ►breast cancer cells in nude mice treated with C75 showed fatty acid synthesis inhibition, ►apoptosis, and reduced tumor growth with no normal tissue toxicity. C75 has also been used as an antiobesity treatment in animal models. Treating obese mice with C75 produces a profound reduction in body weight and food intake.

►Orlistat (also known as Xenical or tetrahydrolipstatin), a US Food and Drug Administration (FDA)-approved drug used for treating ►obesity, is a saturated derivative of lipstatin and works by inhibiting pancreatic and gastric lipases in the lumen of the gastrointestinal tract to decrease systemic absorption of dietary fat. Orlistat is also a rather potent and selective inhibitor of FAS. It inhibits the thioesterase domain of FAS which is responsible for releasing palmitate from the ACP of the enzyme. Orlistat has been reported to have antitumor activity in many tumor cell models because of its ability to block the activity of FAS. Cell cycle arrest induced apoptotic cell death and downregulation of the ►Her2/neu (erbB-2) ►oncogene have been observed in orlistat treated breast cancer cells. Orlistat is also able to effectively inhibit the growth of prostate tumor ►(prostate cancer, clinical oncology) ►xenografts implanted in nude mice.

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Fatty Acid Transport

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Definition

Is the process through which free fatty acids pass through the membranes of cells. The term may include: uptake of fatty acids from the blood or the extracellular space; release of fatty acids from the cell interior to the extracellular space or blood; and transport of fatty acids within the cell across intracellular membranes during biosynthesis and metabolism. While all three types of transport may occur in tumor cells this essay concentrates on the characteristics and mechanisms for free fatty acid entry into tumor cells.

Characteristics

Fatty acids serve multiple functions in cells and their transport from arterial blood plasma to the cell interior is necessary for cell growth and survival. Fatty acid oxidation is a major source of energy for heat production and generation of ATP for biosynthesis and growth. During metabolism fatty acids are incorporated into several complex lipids required for cell structure and function. Fatty acids also play important roles in cell signaling; they are anchors for attachment of specific signaling proteins to membranes and they are converted to several types of ►lipid mediators involved in intra- and extracellular cell signaling mechanisms. The essential long chain polyunsaturated fatty acids, linoleic (C18:2n6), linolenic (C18:3n3), eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3) acids, which are obtained from dietary sources, play particularly important roles in cell signaling, membrane structure, growth and control of fatty acid transport. If dietary fat consumption exceeds the need, fatty acids are stored in white adipose tissue as triacylglycerols for subsequent release during postprandial and longer periods of food deprivation.

Fatty acids greater than 8 carbons in chain length are not very water soluble and are transported in the blood bound to plasma albumin. The plasma albumin-fatty acid pool is maintained by release of free fatty acids from plasma lipoproteins (catalyzed by endothelial lipoprotein lipase) and by release of free fatty acids from lipid stores. Fatty acids bound to plasma albumin dissociate from the albumin binding sites to maintain a plasma free fatty acid concentration of 7–10 nM. This plasma free fatty acid pool is a major source of free fatty acids for cell consumption. Accumulation of high free fatty acid concentrations inside cells, however, is toxic for cell structure and function and specific intracellular fatty acid binding proteins (FABPs) are present in cells to maintain low free fatty acid concentrations. Specific isoforms of these FABPs are expressed in different organs and tissues.

Characteristics - Tumor

Transplantable solid rodent tumors and human cancer xenografts grown in nude rats have ready access to the free fatty acid pool in arterial blood plasma. Cancers that arise spontaneously undoubtedly also have access to plasma fatty acids. Arteriovenous difference measurements in arterial and tumor venous blood plasma samples collected across solid tumors in vivo or during perfusion in situ indicate that substantial rates of fatty acid uptake occur. Thirty-five to 45% of the total fatty acids in arterial blood plasma is removed during one pass of the arterial blood through a fast-growing rat hepatoma. Similar uptakes (35 to 45%) occur for each of the seven major plasma fatty acids in rat arterial blood: myristic, palmitic, palmitoleic, stearic, oleic, linoleic and arachidonic acids. Consequently, larger amounts of the more abundant plasma fatty acids, oleic, linoleic and arachidonic acids are removed. Since tumor fatty acid uptake is proportional to the arterial plasma fatty content of each fatty acid, there is no preferential uptake of any single fatty acid. Each of five different human cancer xenografts, ER^+ and ER^- MCF-7 breast, PC3 prostate, CFDT1 renal transitional and FaDu pharyngeal carcinomas, grown in nude rats removes fatty acids from the arterial blood plasma. Fatty acid uptake by human tumors also occurs in proportion to the concentration of the individual fatty acid in arterial blood plasma. However, the rates and efficiencies of fatty acid uptake in human cancer xenografts are different and are directly proportional to the tumor growth rate.

Growth and incorporation of [^{3}H]thymidine into DNA in rodent tumors and human cancer xenografts in vivo or during perfusion in situ are directly dependent on the availability and uptake of plasma ►linoleic acid. Plasma linoleic acid concentrations, tumor linoleic acid uptakes and growth rates are increased in tumor-bearing rats fed diets enriched in linoleic acid. Similar stimulations of tumor growth occur when tumor-bearing rats are subjected to an acute fast, except that the elevation in plasma linoleic acid content in the acutely fasted rats is derived from lipolysis of host fat stores. In contrast, the absence of linoleic acid in arterial blood plasma, as occurs in tumor-bearing rats fed a linoleic acid-deficient diet, inhibits growth and the rate of [^{3}H]thymidine incorporation into tumor DNA. Linoleic acid-dependent growth in rodent tumors and human cancer xenografts results from the conversion in the tumor of linoleic acid to

13-hydroxyoctadecadienoic acid (13-HODE). 13-HODE, a mitogen in these tumors, is a product of lipoxygenase activity and is released into the tumor venous blood plasma. Depending on the tumor type 1 to 10% of the linoleic acid removed from the arterial blood is converted to 13-HODE. Thus, tumor growth rates are directly proportional to both linoleic acid uptake and 13-HODE release.

Arterial blood plasma long chain polyunsaturated fatty acids affect tumor growth differently; whereas the n6 fatty acid, linoleic acid, increases tumor growth, the presence of ►n3 fatty acids in arterial blood plasma inhibits tumor growth. In tumor-bearing rats fed a diet containing both linoleic acid and n3 fatty acids, the rates of linoleic acid uptake, 13-HODE production,[^{3}H]thymidine incorporation into DNA and growth are suppressed. Each of the n3 fatty acids, α-linolenic, stearidonic, eicosapentaenoic and docosahexaenoic acids, inhibits fatty acid transport; a 50% inhibition occurs at a plasma n3 fatty acid concentration of about 0.15 mM. Most interesting, uptake of all plasma saturated, monounsaturated and n6 polyunsaturated fatty acids is depressed by n3 fatty acids. However, tumor uptake of n3 fatty acids is not inhibited. Other dietary agents also inhibit tumor fatty acid transport and growth. These include, ►melatonin and some conjugated linoleic acid isomers and *trans* fatty acids. As with n3 fatty acids, the inhibition of tumor fatty acid transport is directly proportional to the arterial blood plasma content of the inhibitor. Melatonin, which is present in the diet and is the natural hormone of the pineal gland, is the most potent inhibitor of tumor fatty acid transport yet discovered. A plasma concentration of 0.1 nM melatonin causes a 50% inhibition of fatty acid transport. In addition to inhibitions of both fatty acid transport and 13-HODE release, these agents cause significant reductions in the intratumor cAMP content, [^{3}H] thymidine incorporation and phosphorylated-MEK and -ERK1/2 (►MAP Kinase). Significantly, these inhibitions caused by n3 fatty acids, melatonin, CLA isomers and *trans* fatty acids are reversed by the addition of either 8-bromo-cAMP or pertussis toxin to the arterial blood containing these agents. [Pertussis toxin catalyzes the ADP-ribosylation of the α subunit of inhibitory heteromeric guanine nucleotide G proteins and reactivates the inhibited adenylyl cyclase activity. 8-bromo-cAMP is a cell-permeable analog of cAMP that is resistant to hydrolysis by phosphodiesterases.] Addition of 13-HODE to the arterial blood restores phosphorylated-MEK and -ERK1/2 and [^{3}H]thymidine incorporation but not fatty acid transport. The results strongly suggest that fatty acid transport and growth in rodent tumors and human cancers are dependent on an elevated intratumor cAMP content and that the inhibition of fatty acid transport is mediated by an inhibitory ►G protein-coupled receptor(s).

Mechanisms

The mechanism for fatty acid transport in eukaryotic cells is not yet clearly resolved. Two hypotheses, transport by passive diffusion and transport by protein–mediated carriers, have been presented and studied extensively in model membrane vesicles and cultured cells. Lipophilic non-ionized free fatty acids rapidly penetrate membrane vesicles and red cells, a process that has been termed flip-flop to describe movement of the fatty acid across the membrane leaflet barrier. The barrier to flip-flop is increased by ionization of the fatty acid and may be further increased by changes in the lipid phases in cell membranes of different cells. These findings strengthen the hypothesis for fatty acid transport via protein-mediated carriers. Three groups of membrane protein carriers that contribute to fatty acid transport have been identified in mammalian cells. Fatty Acid Transport Proteins (FATP1–6) are a family of proteins that have distinct tissue distributions. Several but not all of the FATP isoforms transport fatty acids across cell membranes; several also have long chain fatty acid Co-A synthetase (LCACS) activity, which may be important for retention of the fatty acid inside the cell. Evidence also indicates that the plasma membrane protein Fatty Acid Translocase (FAT/CD36) is involved in fatty acid uptake in heart, skeletal muscle and adipose tissue. Control of fatty acid transport by FATP isoforms and FAT/CD36 may involve movement of the carriers between intracellular membranes and the plasma membrane. A Fatty Acid Binding Protein in plasma membranes (FABPpm) that is present in heart, intestine, liver and adipocytes may also play an important role in fatty acid transport. Since all of these proteins may be present in cells, cooperative activities (as yet undefined) may occur among the different protein carriers.

The requirement for high intratumor cAMP is a unique property that appears to control fatty acid transport in solid tumors. The mechanisms that maintain the high intratumor cAMP content are not known but may be catalyzed by stimulatory G_sPCR(s) (►G proteins). This cAMP requirement is not easily reconcilable with the current understanding of fatty acid transport mediated by either passive diffusion or by protein carriers. Depletion of intratumor cAMP and inhibition of fatty acid transport caused by n3 fatty acids, melatonin or CLA isomers is dose-dependent and complete at high inhibitor concentrations. Despite the fact that arterial blood plasma fatty acid concentrations remain available to the tumor, fatty acid transport into the tumor is inhibited. However, addition of either 8-bromo-cAMP or pertussis toxin to the

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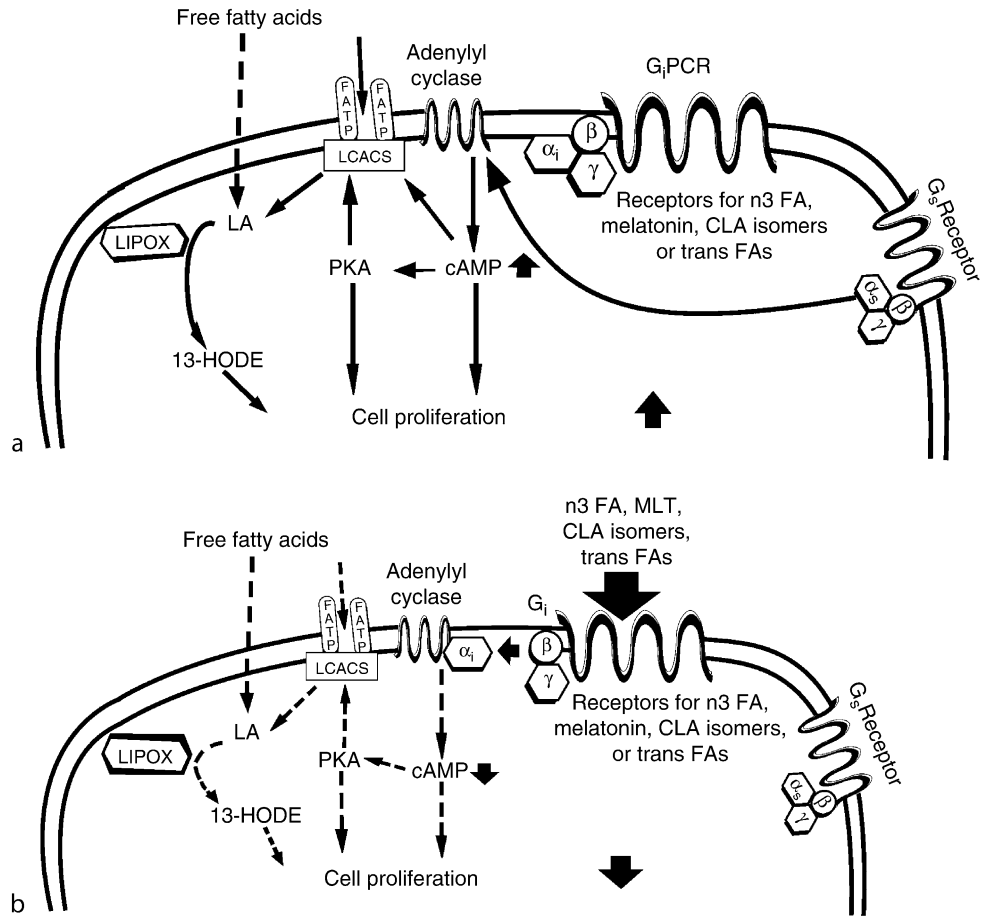

Fatty Acid Transport. Figure 1 Schematic representations for fatty acid transport (a) and control of transport (b) by dietary inhibitors. (a) The sequence of events associated with elevated intratumor cAMP, uptake of linoleic acid, 13-HODE production and cell proliferation are designated by solid arrows. (b) Attenuation of intratumor cAMP and fatty acid transport caused by binding of n3 fatty acids, melatonin, CLA isomers or *trans* fatty acids to inhibitory G protein-coupled receptors is designated by dashed arrows. Abbreviations are: G_iPCR, inhibitory G protein-coupled receptors; G_sPCR, stimulatory G protein-coupled receptor; 13-HODE, 13-hydroxyoctadecadienoic acid; LCACS, long chain fatty acid acyl-Co A synthetase; LIPOX, lipoxygenase, LA, linoleic acid; PKA, protein kinase A.

inhibitor-containing arterial blood restores intratumor cAMP and fatty acid transport. Reversal of the inhibition by these agents indicates that inhibitory G_iPCR(s) (►G proteins) for n3 fatty acid, melatonin and CLA isomers mediate the reduction of intratumor cAMP and inhibition of fatty acid transport. Rat hepatoma 7288CTC and the human cancer xenografts contain one or both of the inhibitory G protein-coupled melatonin receptors, MT_1 and MT_2. Compound S20928, a specific antagonist for melatonin receptors MT_1 and MT_2, increases intratumor cAMP and mitigates the melatonin inhibition. There is as yet no direct evidence that inhibitory G protein-coupled receptors for n3 fatty acids, CLA isomers or *trans* fatty acids exist in solid tumors. mRNA for FATP1 is over-expressed in a rat hepatoma suggesting that FATP1 may mediate fatty acid transport in hepatomas and other tumors. If that is the case, the activity of FATP1 as a fatty acid transport carrier in these tumors may require cAMP (Fig. 1).

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n3 Fatty Acids

Definition

n3 long chain polyunsaturated fatty acids (n3 PUFAs), also known as ω3 PUFAs, are a group of dietary fatty acids that have important roles in several physiological functions. They are characterized by the position of the terminal *cis*-olefinic bond, which is located 3 carbon atoms from the methyl end of the carbon chain. The n3 PUFA, α-linolenic acid (C18:3n3), is present in several plant oils and is a precursor in animal tissues of stearidonic (C18:4n3), eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3) acids. Marine fish and oils derived from them are important dietary sources for the C18 through C22 n3 PUFAs. The position of the terminal cis-olefinic bond in n3 PUFAs distinguishes them from the n6 or ω6 PUFAs in which the location of the terminal *cis*-olefinic bond is 6 carbons from the methyl end of the carbon chain. Dietary n3 PUFAs (►Fatty acid uptake) have a negative effect on growth of established solid tumors, whereas the dietary n6 PUFA, linoleic acid (C18:2n6) [►linoleic acid] has a positive tumor growth effect. The mechanisms for interaction among n3 and n6 PUFAs are a conundrum in cancer biology.

►Fatty Acid Transport

Fbl1

►Ubiquitin Ligase SCF-Skp2

FcR

Definition

Fc Receptor; Cell surface receptor which binds the Fc moiety of some immunoglogulin classes. Fc stands for crystallizable, nonantigen binding fragment of an immunoglobulin molecule obtained by papain digestion.

►Autoantibodies

FDA

Definition

The Food and Drug Administration is an agency of the United States Department of Health and Human Services and is responsible for regulating food, dietary supplements, drugs, cosmetics, medical devices and radiation emitting devices, biologics, and blood products in the United States.

FDG

Definition

^{18}F-Fluorodeoxyglucose; Glucose analog labeled with the positron emitter ^{18}F enabling detection of tumor manifestation sites due to altered glucose metabolism. FDG represents the most important biomarker for positron emission tomography.

►Positron Emission Tomography
►Salivary Gland Malignancies

Fecal Immunochemical Test

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Synonyms

Immunochemical fecal occult blood test; Immunological fecal occult blood test; iFOBT; Fecal occult blood test; FIT

Definition

A fecal immunochemical test for hemoglobin is a test designed to detect hemoglobin in feces using an antibody raised against human hemoglobin. It is one of a class of technologies referred to as ►fecal occult blood test (FOBT).

Characteristics

Fecal immunochemical tests (FIT) incorporate an antibody specific for human hemoglobin; such react with the globin protein moiety of hemoglobin.

Fate of Hemoglobin in the Gastro-Intestinal Tract

The globin moiety of hemoglobin is a protein and as such is rapidly digested in stomach or small intestine such that it is no longer detectable by immunochemical methods. Ingestion of blood volumes of up to 100 mL has not resulted in detectable immunoreactive hemoglobin in feces, based on reports using some of the earlier types of FIT. In other words, FIT are selective for colorectal bleeding, especially where the bleeding is occult (i.e. not visible to the naked eye). This is different from the chemical, specifically guaiac-based, FOBT which can return a positive result for blood derived from the proximal as well as distal ►GI tract.

FIT technology differs from chemical-based FOBT that react to the peroxidase activity of heme (Fig. 1). In feces, hemoglobin is subject to degradation by endogenous digestive enzymes (albeit at a much slower rate than in small intestine and stomach) and by bacterial enzymes. As shown in Fig. 1, this releases heme from globin. FIT technology detects globin and not heme and so is not subject to the factors that interfere with methods that depend on detection of heme. Heme itself is degraded by bacterial enzymes. Globin itself is progressively degraded at widely varying rates in feces.

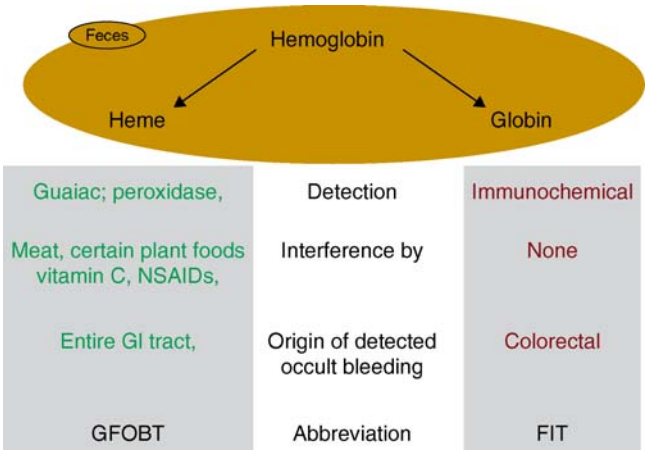
Because fecal immunochemical test technology is so different from that of the chemical methods, because it reacts to a different analyte (i.e. globin not heme) and because it provides the option of quantification of fecal hemoglobin rather than being limited to qualitative assessment as is the case for GFOBT, it is recommended that the terminology "fecal immunochemical test" be applied and abbreviated as FIT, to emphasize that it is a quite different technology and analyte from chemical FOBT.

Uses for FIT

The primary use of FIT is in screening for colorectal cancer. Chemical FOBT have been proven to reduce mortality and incidence in four randomized controlled trials. Comparative studies have shown FIT to be more sensitive for cancer and more sensitive for colorectal adenomas than guaiac-based FOBT. An hemagglutination-FIT has been shown to be superior to a high-sensitivity guaiac-FOBT in that it achieved the same higher sensitivity for cancer but required less than half as many colonoscopies to achieve this. In other words, FIT improve sensitivity for colorectal cancer and adenomas but not at the cost of unacceptable deterioration in specificity.

FITs are not subject to interference by diet or drugs and maintenance of an adequate specificity is not dependent on proscription of certain foods or drugs as is the case for guaiac-FOBT. This, together with the improved stool-sampling methods characteristic of FIT, makes it easier for screenees and improves participation rates in screening.

It is not advisable to use FIT as an all-purpose test for fecal blood as it is selective for colorectal bleeding. If used in this setting, such as in the context of iron-deficiency, it should be combined with a chemical test. However, it is important to note that the negative predictive value of either guaiac-FOBT or FIT is not 100% and many consider their use should be confined to screening for colorectal cancer where they act as an aid to early detection; as such they serve to identify those most likely to have colorectal neoplasia and so in need of a diagnostic procedure such as ►colonoscopy.



Fecal Immunochemical Test. Figure 1 Fate of hemoglobin in feces and detection of products by the various fecal occult blood test (FOBT) technologies.

Quantifiable FIT

Several of the latest FIT allow quantification of fecal hemoglobin. Results show that fecal hemoglobin concentration corresponds to the "stage" of neoplasia. People with cancer have the highest levels on average, those with a normal colon the lowest levels while those with adenomas have intermediate levels. Studies such as these show that patients with advanced adenomas (size greater than 1 cm, villous change or high grade dysplasia) do have occult bleeding. In other words, the higher the level of fecal hemoglobin the more likely that neoplasia is present.

The advantage of fecal hemoglobin measurement is that it returns full control of sensitivity and specificity to the doctor managing screening. It enables the doctor to set the level of fecal hemoglobin that would trigger follow-up diagnostic colonoscopy. At the population level, such an approach allows flexible choice of a test specificity:sensitivity ratio, so controlling the colonoscopy rate to that which is feasible for a health care system.

Types of FIT Currently Available

A variety of test technologies are employed in the currently available tests. This includes latex agglutination, membrane diffusion devices for detecting immunogold- or latex-labeled antibodies, hemagglutination, and magnetic particle agglutination. The end point for some tests are read by eye while others may also be read by a machine designed specifically for the particular technology and its endpoint. Some devices are designed for simplicity and development in an office-setting while others are designed for large scale development in a laboratory – automated processing, development and reading may be available.

Internationally, there are no set criteria that a test manufacturer should follow when setting the specifications for a test. As such, different tests may behave differently with differences in the quantity of hemoglobin to which they react as well as the nature of the hemoglobin antigen that they detect.

A test result may be quantitative, providing a measure of the concentration of hemoglobin in feces, or qualitative, providing an indication as to whether the fecal hemoglobin is considered to have reached a level likely to reflect the presence of neoplastic pathology in the colon or rectum.

Stool-Sampling Procedures

The nature of the fecal sample is an important component of a test. Each test comes with its own characteristic stool-sampling device. These include devices that keep the sample dry and so more likely to keep the antigen stable and others that use devices that include liquid and in which hemoglobin degradation might proceed during specimen transit unless samples are kept cold.

Most devices require the stool to be kept clear of toilet bowl water when sampling while a few require sampling to be undertaken from a stool kept clear of water. The range of sampling devices include wooden spatulas that smear feces onto a card, probes that absorb or wick materials from feces when poked into the stool, probes that trap feces into grooves when poked into the stool, and brushes that sample toilet bowl water from the surface of the immersed stool. Some provide precise control of stool sample quantity while others do not provide such control.

Most manufacturers provide packaging that enables samples to be sent by mail to a processing laboratory.

As hemoglobin can be rapidly degraded in feces especially at room temperature or above, whole stools should never be sent to a laboratory for sampling by the laboratory. Rather, the appropriate stool-sampling device provided for a test must be used. It is likely that different tests vary in their capacity to detect partially degraded hemoglobin. This will be dependent on the antibody used but there is little published material to guide users.

Performing an in-office GFOBT as part of a digital rectal examination has been common practice especially in the USA. However, usually only one rather than the recommended two or three stool samples is obtained. Recently this approach has been evaluated in a large group of asymptomatic subjects where it was found to be unsatisfactory. Screening based on digital sampling of stool as part of a rectal examination cannot be recommended and manufacturer's recommendations for obtaining stool samples should be followed.

Biology of Bleeding from Colorectal Neoplasia

The fact that microscopic bleeding may arise from cancers or adenomas, provides the basis for the use of FOBT to aid early detection of colorectal neoplasia. Blood loss is variable from day-to-day and may fall within the normal range. Furthermore, blood of colorectal origin is not uniformly distributed within a stool and often is found on the surface. Consequently, it is usually recommended that when screening for colorectal cancer, samples from at least two separate stools be collected. It is also recommended that the stool surface be sampled rather than the stool interior or an homogenate.

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Fecal Occult Blood Test

Definition

FOBT; A fecal occult blood test finds blood in the stool by placing a small sample of stool on a chemically treated card, pad, or wipe. Then a special chemical solution is put on top of the sample. If the card, pad, or cloth turns blue, there is blood in the stool sample. A fecal occult blood test may be used to check for ►colon cancer, but it is never used to diagnose this condition. Other tests for ►colon cancer include a digital rectal examination, sigmoidoscopy, colonoscopy, or CT scan.

►Fecal Immunochemical Test

Felty Syndrome

Definition

Is a rare disorder that involves rheumatoid arthritis, a swollen spleen, decreased white blood cell count, and repeated infections. The cause of Felty syndrome is unknown. It is more common in people who have had rheumatoid arthritis for a long time. People with this syndrome are at risk of infection because they have a low white blood cell count.

Female Sex Hormones

Definition

Estrogens and progestagens.

►Estrogenic Hormones

Feminization

Definition

Rarely occurs in ►adrenocortical carcinoma and is the result of an increased production of estrogen by the tumor cells. Males usually develop breasts (gynecomastia), and girls present with precocious telarche (isolated unilateral or bilateral breast development).

►Childhood Adrenocortical Carcinoma

Fenestration

Definition

The architecture of the endothelial cells comprising the lymph capillaries carries large windows allowing entrance of lymph fluid, particles, and cells into the lymphatic system.

►Sentinel Node

FERM

Definition

There is a homology to Protein 4.1, ezrin, radixin, and moesin, which are members of the 4.1 superfamily of actin binding proteins. It is the N-terminal domain of FAK, which interacts with growth factor receptors and other transmembrane proteins.

►Focal Adhesion Kinase
►ERM Proteins

FERM Domain

Definition

Is name of the protein 4.1 family domain and stands for F (four point one) E (ezrin) R (radixin) M (moesin). It is a unique module involved in the linkage of cytoplasmic proteins to the membrane.

►Neurofibromatosis 2

Fermentation Products

Definition

Dietary fiber including resistant starches, complex carbohydrates and cellulose, have been put forward as cancer protective food components. The theory is that dietary fiber may protect against ►colon cancer through absorption of risk factors and especially through secondary events resulting from the fermentation of carbohydrates by the microflora. These will lead to fecal bulking, increased speed of colon transit, increase of nitrogen metabolism, increased bacterial load in the colon, acidification and finally to the production of short chain fatty acids (SCFA). Butyrate, one of the major SCFA, is considered to be beneficial due to its trophic effects as an essential nutrient to the colon epithelium. Suggested mechanisms of cancer prevention at a cellular level have been reported to be the promotion of differentiation, induction of apoptosis and inhibition of proliferation in colon tumor cell lines. These mechanisms are classified as suppressing activities of cancer preventing agents

►Biomarkers

Ferredoxin Reductase

Definition

A 54 kDa flavoprotein, synonym adrenodoxin reductase, a mitochondrial enzyme responsible for transferring electrons from NADPH to ►cytochrome P450, via ferredoxin, to substrates, during steroidogenesis.

►Fragile Histidine Triad

Ferritin

Definition

Is a crystalline lattice protein, 450 kDa, that can sequester up to 450 atoms of iron. As the metal accumulates within ►macrophages, increasing amounts of ferritin are synthesized to safely contain the toxic metal. Ferritin is a main intracellular iron storage protein and consists of 24 light and heavy subunits. Each ferritin complex can store about 4,500 iron (Fe^{3+}) ions.

►Asbestos
►Phase 2 Enzymes

Ferruginous Bodies

Definition

Are ►asbestos fibers that, in respiratory tract tissues, have become coated with iron-rich material derived from ferritin/hemosiderin. The bodies typically are associated with ►macrophage clusters. Over time, catalytic iron is released from the ferritin coat, possibly by action of macrophage hydrogen peroxide.

F

FET

Definition

^{18}F-Fluoroethyltyrosine; Amino acid (tyrosine) labeled with the positron emitter ^{18}F enabling detection of tumor manifestation sites (especially brain tumors) due to increased transport of amino acids and increased protein biosynthesis.

►Positron Emission Tomography

Fetal Hamartoma of the Kidney

►Mesoblastic Nephroma
►Hamartoma

α_1-fetoglobulin

►Alpha-Fetoprotein – Modern

α-Fetoprotein

Definition

AFP; 70 kD glycoprotein synthesized in early embryonal development by cells of the yolk sac, liver and

gastrointestinal tract. A tumor marker for hepatocellular carcinoma.

►Hepatoblastoma

α-feto-protein

►Alpha-Fetoprotein – Modern

Feto-specific Proteins

►Alpha-Fetoprotein – Historical

Fetuin

►Alpha-Fetoprotein – Modern

Fetuin-A

►Alpha-Fetoprotein – Modern

FEV1

Definition

Forced expiratory volume in one second. Following a maximal inspiration, the volume of air exhaled in the first second during a maximally forced expiration.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

Fever

Definition

Is defined as an elevation of the body temperature that exceeds the normal daily variation.

►Fever of Unknown Origin

Fever of Obscure Origin

►Fever of Unknown Origin

Fever of Unexplained Origin

►Fever of Unknown Origin

Fever of Unknown Origin

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Synonyms

Fever of unknown origin; Fever of unexplained origin; Pyrexia of unknown origin; Fever of obscure origin

Definition

Fever of unknown origin is a clinical syndrome of ►fever that does not resolve spontaneously within 3 weeks and the cause remains unknown after extensive workup. Fever is defined as an elevation of the body temperature that exceeds the normal daily variation. The hypothalamus controls the body temperature and a normal body temperature is ordinarily maintained because the hypothalamic thermal regulatory centre balances the excess heat production derived from metabolic activities in the muscles and the liver with heat dissipation from the skin and lungs. Normally, healthy adult individuals have a mean oral temperature of 36.8°C ± 0.4°C. The temperature is a little bit higher in the

evening. Fever of unknown origin is an important clinical challenge. In 1961 Petersdorf and Beeson [1] defined it in their first report as an illness characterized by fever of more than 3 weeks' duration, with temperatures of more than 38.3°C and failure to identify the origin of the fever despite 1 week of hospitalization. Because of the development of diagnostic procedures, increasing costs of hospital care and the increasingly outpatient work-up, the definition was modified in 2003 and a hospital setting was no longer needed for assessment of the illness.

Characteristics

Over the last decades, several case-series have examined the underlying diseases for fever of unknown origin. More than 200 causes have been reported to cause fever of unknown origin.

After work-up, fever of unknown origin has generally been considered to be caused by infections, neoplasms, inflammatory or a heterogeneous group of other diseases. The most common infections reported in the literature are tuberculosis and intra-abdominal abscesses. Other important causes are temporal arthritis and other collagen vascular diseases and venous thromboembolism. One is unable to establish the causal diagnosis in 9–25% of the cases.

In the early case-series, the proportion of cancers varied between 7 and 24%. Although most causes of fever of unknown origin resolve spontaneously, the risk of occult cancer remains a concern. It is important to emphasize that most studies on the origin of fever of unknown origin did not include any control groups or a reference standard and criteria and the final diagnosis was defined in many different ways.

Cancer and Fever of Unknown Origin

Although several studies have been conducted on the association between fever of unknown origin and a subsequent diagnosis of cancer, the studies have been small, based on 40–300 patients, several studies have been based on referral centers and almost all studies lack control groups.

Nearly all common cancers in the literature had a link to fever of unknown origin. In the referral centre, case-series of a proportion of cancer diagnosed in patients with fever of unknown origin has decreased from about 30% of cases in the 1970s to 9–20% in the 1990s. It has been suggested that improved diagnostic imaging with e.g. CT and MRI has improved the detection of otherwise occult solid tumors.

In 2005 a large, Danish population-based study based on health care databases assessed the risk of cancer in 43,205 patients hospitalized from 1977 to 1997 with fever of unknown origin. Data on patients with a discharge diagnosis of fever of unknown origin were linked to the Danish Cancer Registry. The incidence rate of cancer was compared with the expected cancer incidence rate in the general population. The median follow-up was 6.3 years: 9,932 of the patients were more than 60 years old, 399 cancer cases were diagnosed during the first year of follow-up among the 43,205 patients with a relative risk (standardized incidence rate ratio) of 2.3 (95% CI: 2.1–2.5). The relative risk was raised for various types of cancer. Especially for ►Hodgkin disease (relative risk 27.8 95% CI: 15.9–45.1), non-Hodgkin ►lymphoma (relative risk 9.9 95% CI: 7.1–13.3), ►leukemia (relative risk 5.6 95% CI: 3.9–7.8) and ►multiple myeloma (relative risk 4.4 95% CI: 2.1–8.0), as well as sarcoma (relative risk 6.6 95% CI: 3.6–11.1) and solid tumors in the liver (relative risk 6.1 95% CI: 3.2–10.7), gall bladder (relative risk 2.7 95% CI: 0.9–6.3), brain (relative risk 4.1 95% CI: 3.5 – 6.2), kidney (relative risk 2.6 95% CI: 2.6; 95% CI: 1.4 – 4.5), colon (relative risk 2.7 95% CI: 2.0–3.5), and pancreas (relative risk 3.7 95% CI: 2–3–5.6).

During 1–19 years for hospitalization for fever of unknown origin 1,097 cancer cases were observed compared with 977.8 cancer cases expected. During the follow-up period, there was still an increased relative risk of hematological cancer as Hodgkin disease (relative risk 2.1 95% CI: 1.1–3.8), non-Hodgkin lymphoma (relative risk 1.4 95% CI: 1.0–1.9), leukemia (relative risk 1.7 95% CI: 1.3–2.2) and multiple myeloma (relative risk 1.6 95% CI: 1.0–2.4), liver (relative risk 1.9 95% CI: 1.1–2.9), brain (relative risk 1.4 95% CI: 1.0–1.9) and kidney cancer (relative risk 1.5 95% CI: 1.1–2.1). Until publication of the Danish study, the former studies on cancer risk had included a total of 1,200 fever of unknown origin patients. The absolute risks of cancer during the first year of follow-up were low, much lower than those reported in former studies.

Diagnosis

There are no randomized clinical trials in the literature about the clinical utility of different diagnostic strategies. Mourad et al. [2] has suggested the following minimal to qualify as fever of unknown origin: comprehensive history, physical examination, complete and differential blood cell count,, blood film reviewed by hematopathologist, routine blood chemistry, urinanalysis and microscopy, blood and urine cultures, antinuclear antibodies, rheumatoid factor, human immunodeficiency virus antibody, cytomegalovirus IgM antibodies, heterophil antibody test, Q-fever serology, chest radiography, hepatitis serology. To obtain a cancer diagnosis abdominal CT scan is the most central diagnostic test, but further diagnostic work up depends of symptoms and findings.

Prognosis of Cancer Associated with Fever of Unknown Origin

The underlying disease is the main predictor for the outcome of fever of unknown origin. Four studies have shown that 52–100% of patients with fever of unknown

origin and cancer will die within 5 years after the diagnosis. In the Danish database study of the 399 cases of cancer, the extent of cancer was compared for cases associated with fever of unknown origin and similar cancer 3,958 cases that did not have fever of unknown origin. Cancer diagnosed in patients with fever of unknown origin during the first year of follow-up was associated with higher prevalence of metastases and an increased mortality ratio of 1.4. After 1 year of follow-up, the cumulative survival among cancer cases was about 50% and after 2 year about 35%.

A diagnosis of cancer subsequent to hospitalization because of fever of unknown origin does not necessary imply an extremely poor prognosis.

Conclusion

The existing literature has consistently shown that fever of unknown origin is a marker of occult cancer. The last Danish study showed that this association in a population-based setting is weaker than reported in former studies, but the relative risk remains increased many years subsequent to the hospitalization for fever of unknown origin for hematological cancer, liver, brain and kidney cancer. Fever of unknown origin is associated with more advanced cancer disease and a poor prognosis compared with similar cancer patients without fever of unknown origin, but the prognosis is not extremely poor.

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FEV1/FVC Ratio

Definition

The ratio of the forced expiratory volume in one second to the forced vital capacity.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

FGD1

Definition

Faciogenital dysplasia 1 was the first identified gene product responsible for this disease. It functions as an activator for the small GTPase Cdc42, which in turn serves to activate N-WASp and other actin-related events.

►Cortactin

FGF

Definition

Fibroblast growth factor.

►Fibroblast Growth Factors

FGFR3

Definition

Fibroblast Growth Factor Receptor 3; A receptor for fibroblast growth factors, oncogenically activated by point mutations in ►urothelial carcinoma, ►cervical cancer, and certain benign skin tumors.

FH

►Fumarate Hydratase

FHIT

Definition

►Fragile Histidine Triad Gene; The *FHIT* gene is a member of the histidine triad gene family and is considered to be a tumor suppressor gene. The common fragile site FRA3B is located within this gene.

►Fragile Sites

Fibrin Degradation Products

Definition

FDP; Fibrin or ►fibrinogen is proteolytically degraded into fibrin degradation products (fibrinolysis). ►Plasmin, a major extracellular serine protease, plays a role in this process. Fragments D and E are major fibrin degradation products. Fragment D contains C-terminal globular domains (γC of the γ chain and βC of the β chain). Fragment E contains the central portion of fibrinogen.

Fibrin

Definition

Factor Ia; A protein that is involved in blood clotting. It is produced by the liver as a soluble precursor, fibrinogen, which circulates in the blood stream.

►Proteinase-Activated Receptors

Fibrinogen

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Definition

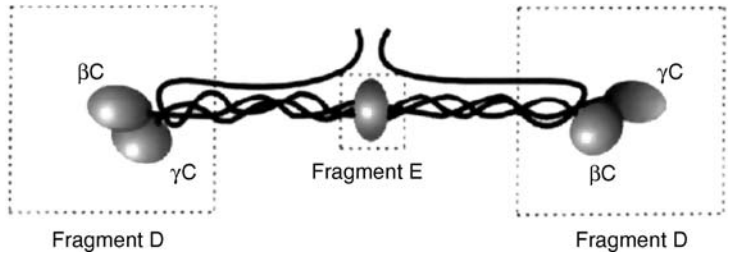
Is a major plasma glycoprotein (350 kDa) that plays an important role in blood clotting, cellular and matrix interactions, ►inflammation, wound healing, and neoplasia.

Characteristics

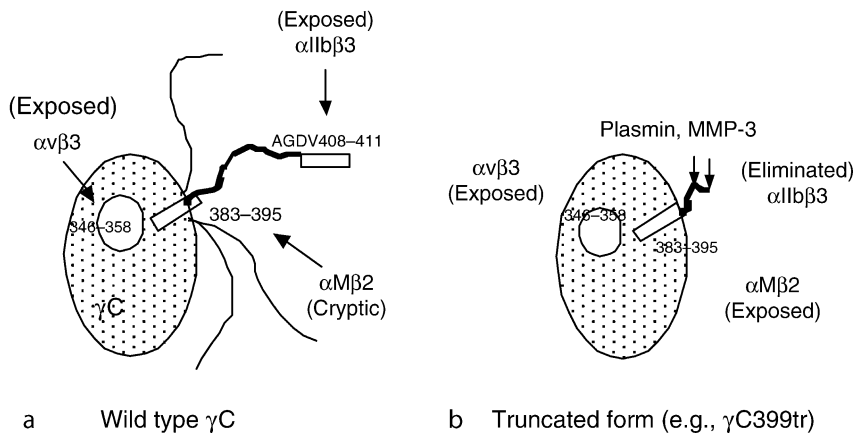
Fibrinogen is a 340 kDa glycoprotein that consists of two identical disulfide-linked subunits. Each subunit is composed of three nonidentical polypeptide chains: alpha, beta, and gamma. The beta and gamma chains have conserved C-terminal domains of about 250 amino acid residues (designated βC and γC, respectively). The crystal structure of the isolated γC domain, as well as that of the γC and βC domains in the fibrinogen D fragment, revealed that both domains are similarly folded. A minor 420 kDa form of fibrinogen (fibrinogen-420) has an alternative extended form of the alpha chain (αE), which has a C-terminal domain (designated αEC) whose sequence and fold are highly homologous to those of the βC and γC domains. Fibrinogen binds to CD54 (ICAM-1) through the frist IgG domain (8KVILPRGGSVLVTC21 of CD54). CD54 binds to Fibrinogen γ-chain (117NQKIVNLKEKVAQLEA133).

Fibrinogen is polymerized into fibrin network, and this process is triggered by thrombin. Polymerized fibrin is further cross-linked by factor XIII. Fibrin or fibrinogen is proteolytically degraded into ►fibrin degradation products (FDP) (fibrinolysis). Plasmin, a major extracellular serine protease, plays a role in this process. Fragments D and E are major fibrin degradation products. Fragment D contains C-terminal globular domains (γC of the γ chain and βC of the β chain). Fragment E contains the central portion of fibrinogen (Fig. 1). The γC domain has been shown to interact with several ►integrin cell adhesion receptors (αvβ3, α5β1, αIIbβ3, αXβ2 (p150,95), and αMβ2 (Mac-1)) and the αEC domain binds to avβ3 and aMβ2 (Fig. 2).

Integrins are a family of cell ►adhesion receptors that recognize extracellular matrix ligands including fibrin (ogen) and cell surface ligands. Integrins are transmembrane α–βheterodimers, and at least 18α and 8β subunits are known. It has been well established that integrins transduce signals inside the cells upon ligand binding, and integrin functions are regulated by the signals from inside the cells. According to the crystal structure of the human γC domain, γC has a C-terminal fibrin-polymerization domain, a single calcium-binding site, and a deep binding pocket. Several integrins recognize γC. Integrin αIIbβ3 in platelets, αvβ3 in endothelial cells, and αMβ2 in leukocytes recognize γC and play an important role in thrombus formation, angiogenesis, and inflammation; respectively. This domain does not have an RGD motif, a prototype integrin recognition sequence. αIIbβ3 recognizes the C-terminal sequence ($HHLGGAKQAGDV^{400–411}$) of γC. Deletion of the QAGDV sequence at the C-terminal region of the γC domain in mice induces a defect in platelet aggregation. Integrins αMβ2 and αXβ2 (p150,95) in leukocytes recognize residues 190–201 and 383–395 of γC. The αM I-domain directly interacts with this sequence. αvβ3 is a fibrinogen receptor in endothelial cells and αvβ3-fibrinogen interaction may play a key role in angiogenesis associated with wound healing and tumorigenicity. αvβ3 also recognizes γC. Although αIIbβ3 requires $HHLGGAKQAGDV^{400–411}$ of γC for binding, this sequence is not important for recognition by αvβ3. The residues 190–201 and 346–358 in γC sequences are involved in αvβ3 binding. Although they are spatially distinct sequences, they are adjacent in the three-dimensional structure. Residues 346–358 do not support



Fibrinogen. Figure 1 Domain structure of fibrinogen, and its proteolytic fragments D and E.



Fibrinogen. Figure 2 Integrin binding to the γC domain of fibrinogen.

the binding of αIIbβ3 or αMβ2 to fibrinogen. Thus αvβ3 binds to γC in a manner distinct from αMβ2 and αIIbβ3.

Fibrin(ogen) is found deposited in the majority of human and experimental animal tumors. The deposition of fibrin(ogen), along with other adhesive glycoproteins, into the extracellular matrix (ECM) serves as a scaffold to support the binding of growth factors and to promote the cellular responses of adhesion, proliferation, and migration during ►angiogenesis and tumor growth. Fibrin(ogen) degradation products (FDPs) generated during fibrinolysis have been implicated in tissue inflammation associated with the adult respiratory distress syndrome, disseminated intravascular coagulation, and septic shock. Plasma concentrations of FDP fragment D are markedly elevated in these disorders. Activation of fibrinolysis and the resulting generation of FDPs, including fragment D, contribute to lung vascular injury. Fragment D detaches endothelial monolayers from the substratum, and that fragment D increased endothelial monolayer permeability. FDP fragment D increases secretion of endothelial plasminogen activators and that fragment D may be a critical mediator linking activation of fibrinolysis to vascular endothelial injury in inflammatory disorders.

The isolated γC or its truncation mutant γC399tr, which lacks the αIIbβ3-binding site, induces ►apoptosis of endothelial cells, while native fibrinogen or fragment D does not have such effects. γC or γC399tr blocks tube formation by endothelial cells on matrigel through inducing apoptosis. Colon carcinoma cells that were engineered to secrete γC grow much slower than non-secreting cells in vivo while they grow at comparable rate in vitro, suggesting that γC secretion affects tumor growth through blocking angiogenesis in vivo. γC399tr effectively blocks tumor growth, and suppresses intratumoral microcirculation. Soluble native fibrinogen and fragment D do not bind to fully activated αvβ3, but soluble γC and γC399tr truncation mutant does. Thus the αvβ3-binding site is cryptic in native fibrinogen and fragment D, but is exposed in γC and γC399tr. These findings shed a new light on fibrinogen functions, and γC and γC399tr are potential therapeutics.

At least three integrins bind to the γC domain. The C-terminal AGDV sequence (408–411) is required for αIIbβ3, residues 383–395 are recognized by αMβ2, and residues 346–358 are uniquely involved in binding to αvβ3. In the C-terminal, approximately 20 residues are missing in the crystal structure of the γC domain,

probably because these residues take various conformations due to their flexibility. Truncation of the C-terminal 12 residues (400–411) of the isolated γC domain (γC399tr) eliminates the AGDV motif required for αIIbβ3 binding and exposes the αMβ2-binding site. Our preliminary results suggest that the αvβ3-binding site is not accessible in native fibrinogen and in fragment D, but is exposed in the isolated recombinant γC domain and γC399tr. It is unclear whether the αvβ3-binding site becomes further accessible by the truncation.

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Fibroadenoma

Definition

Benign tumor characterized by the proliferation of fibrous (stromal) and glandular tissue.

►Cowden Syndrome

Fibroblast

Definition

A cell of the mesenchymal lineage, which is a connective tissue cell.

Fibroblast Growth Factor 2

Definition

Synonym FGF2 basic FGF; is a broad spectrum and pleiotropic mitogen for growth and differentiation, affecting epithelial and endothelial cells, smooth muscle cells, and osteoblasts. FGF2 incites tumor angiogenesis, is expressed by various tumor cell lines, and seems to be biologically important in tumor progression and metastasis.

►Fibroblast Growth Factors

Fibroblast Growth Factors

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Synonyms

HBGF; Heparin-binding growth factors

Definition

Fibroblast growth factors (FGFs) constitute a large family of growth and differentiation factors. The family comprises two prototypic members, acidic FGF (aFGF, FGF1) and the basic FGF (bFGF, FGF2), as well as 21 additionally related polypeptide growth factors that have been identified to date. The designation "FGF" is historical and refers to the fact that the first members of the family stimulated fibroblast proliferation. In fact, FGFs can act on a wide range of cells, especially of mesodermal and neuroectodermal origin.

Characteristics

Common characteristics of members of the FGF protein superfamily are a high affinity for heparin and heparin-like glycosaminoglycans as well as a high sequence homology within a central core domain of 120 amino acids. During embryonic development, FGFs are curcially involved in embroyonic development, and in many processes such as wound healing, hematopoiesis, and particularly angiogenesis. FGFs are also implicated in the maintenance of an undifferentiated state in embryonic stem cells. Most FGFs interact with a family of high-affinity cell surface receptor tyrosine kinases (RTK). Although more than 20 FGFs with different effects on various target cells have been identified, only four different FGF receptor isotypes (FGFR1–4) exist. By expression of different splice variants of FGFR1–3, four *FGFR* genes encode a broad variety of different isoforms which differ in their FGF ligand-binding profiles. For instance, one particular splice variant, FGFR2IIIb, is mainly expressed on epithelial cells and represents the receptor for FGF7 (also named

keratinocyte growth factor, KGF). Within the classical FGF family, FGF11–14, also known as FGF homologous factors (FHF1–4), represent a subclass of FGFs. This subclass is characterized by the fact that, although FHFs can bind heparin like the classical FGFs, they are unable to activate FGFRs and interact with intracellular target proteins.

Regulation

Due to their affinity for heparin-like glycosaminoglycans, the majority of secreted FGFs are bound to ►extracellular matrix (ECM) components. Locally stored FGFs can be released from this extracellular reservoir by at least two mechanisms. One mechanism involves enzymatic degradation of ECM, e.g., during wound repair or tumor cell invasion. Alternatively, the carrier protein FGF-BP (FGF-binding protein) binds FGFs in a noncovalent manner and shuttles them from their ECM storage site to their cell surface receptors.

Clinical Relevance

Besides their ability to regulate proliferation and differentiation, some FGFs are important angiogenic inducers. Increased FGF activity in malignant tumors results from stromal as well as cancer cells and in turn acts on cancer cells as well as the surrounding stroma, and especially the vasculature. In selected cancer types, activating mutations in specific FGFRs or altered expression of isoforms have been described. Due to their multiple effects on tumor growth, FGFs, their receptors, and FGF-BP have been analyzed in various tumor cell types as potential tumor and/or progression markers. Moreover, in the future, selective inhibition of FGF/FGFR-signaling, e.g., by thalidomide, could exploit the crucial function of FGF-signaling in cancer for therapy.

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Fibroblasts

Definition

Are a mesenchymal cells that secrete growth factors and ►extracellular matrix. Tissue-specific fibroblasts are uniquely suited to maintain connective tissues of a given organ.

►Desmoplasia
►Chemical Carcinogenesis
►CXC Chemokines
►Metastasis

Fibrofolliculomas with Trichodiscomas and Acrochordons

►Birt–Hogg–Dubé Syndrome

Fibrogenesis

Definition

Refers to the scarring that takes place in the liver (or any organ) where connective tissue replaces the normal cells within the injured organ (the cells are commonly hepatocytes in the liver).

Fibroid

Definition

Refers to benign (non-cancerous) tumors that grow in, on or outside of the wall of the uterus. They usually range in size from as small as a pea to as large as a grapefruit. About 20–25% of all women have fibroids, and they are very common in women over 30. Women over the age of 35 have between a 20 and 40% chance of having fibroids. Synonym fibromyoma, ►uterine leiomyoma or myoma, uterine fibroids only exceptionally undergo a malignant change towards uterine cancer, but almost never develop into cancer.

Fibromas

►Uterine Leiomyoma

Fibromyoma

►Uterine Leiomyoma, Clinical Oncology

Fibronectin

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Definition

Is a large adhesive glycoprotein and a normal constituent of extracellular fluids, extracellular matrices, most basement membranes and certain cell types. It is implicated in a variety of different biological phenomena such as cell ►adhesion, establishment and maintenance of normal cell morphology, cell ►migration, differentiation, transformation, hemostasis, thrombosis, wound healing, ►transformation and ontogeny.

Characteristics

The molecule consists of two similar but non-identical polypeptide subunits, which are disulphide-linked at the carboxyl terminus. The longer α-chain and shorter β-chain each contain two free SH-groups and 60 cysteine residues, many of which appear to be involved in intrachain disulphide loops. Fibronectin is glycosylated at about 4–6 sites within the protein via arginine (N-linked), which account for 4–5% of the molecular weight of the molecule. Major sugar residues in glycosylation units are mannose, galactose, glucosamine and sialic acid.

Both α and β chains consist of similarly ordered globular domains:

1. Amino-terminus
2. Gelatin-binding domain
3. Cell adhesive/attachment domain
4. Heparin-binding domain
5. Carboxy-terminus (Fig. 1)

Forms of Fibronectin

Cellular fibronectins are secreted by various cell types, such as fibroblasts, epithelial and endothelial cells, chondrocytes, ►macrophages and platelets, which organize them into extracellular matrices. Cellular fibronectins appear to be more interesting, in the context of neoplastic disease, than plasma fibronectin because their expression is affected by oncogenic transformation.

Functions

Fibronectin is implicated in a variety of different biological phenomena such as cell adhesion via its receptors (Fig. 2) and the establishment and maintenance of normal cell morphology as a component of the ►extracellular matrix (ECM).

Fibronectin is also implicated in hemostasis and thrombosis. Fibronectin contains binding domains for cells, gelatin (collagen), heparin and some other proteoglycans, DNA, hyaluronic acid and fibrin.

Structure

Each polypeptide chain consists of internal repeats of three types of homologies termed type I, II, and III repeat. There are 12 type I repeats, 2 type II repeats and 15–18 type III repeats (Fig. 1). Each exon of the single gene encodes one type I or type II repeat. Most type III repeats are encoded generally by two exons, but this pattern is altered at three positions that exist as a single exon, and where alternative splicing occurs. Type I repeats are ~40 amino acids long, type II are ~60 and type III are ~90, as originally shown in bovine plasma fibronectin.

Fibronectin Polymorphism

The diversity observed among fibronectins is due to both alternative splicing of the single primary transcript and due to post-translational modifications.

Post-Translational Modifications

Fibronectin undergoes glycosylation that appears to stabilize fibronectin molecules against proteolysis. In addition, the degree of glycosylation may influence the interaction of the protein with cells; for instance, higher amounts of glycosylation decrease the avidity for collagen. Other post-transcriptional modifications of fibronectin include phosphorylation and sulfation. Fibronectin is phosphorylated on a serine residue near the carboxy terminus. Certain fibronectin variants are found to be tyrosine-linked sulfated.

Alternative Splicing

►Alternative splicing is a widespread gene regulation mechanism able to generate diversity in a reversible way and without requiring the expression of new genes. Some eukaryotic genes, such as the gene encoding for

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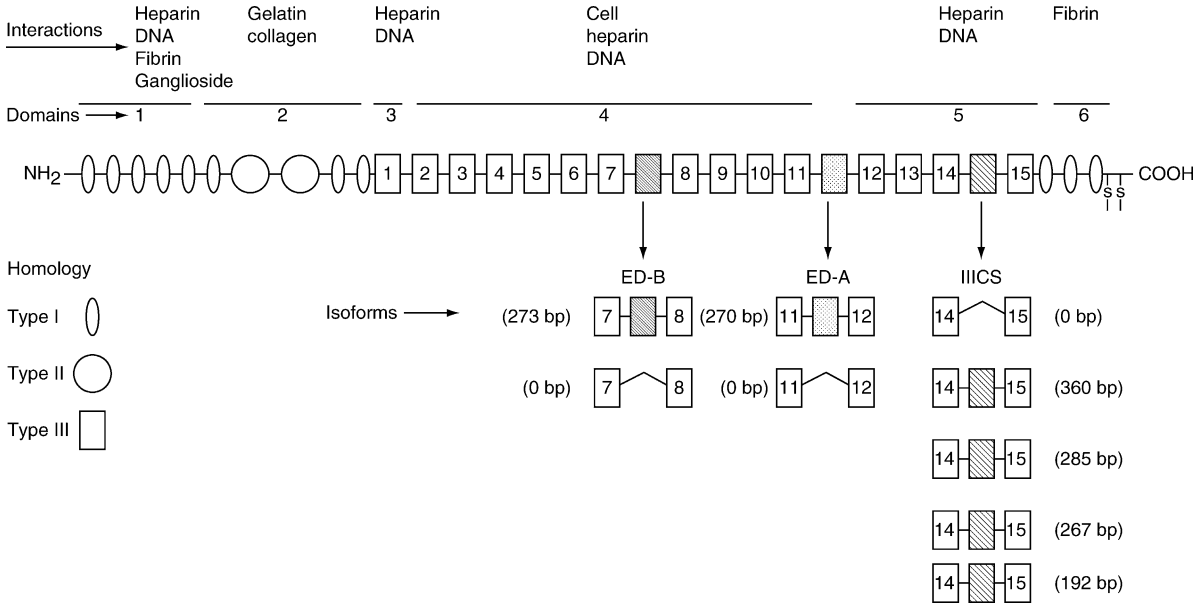
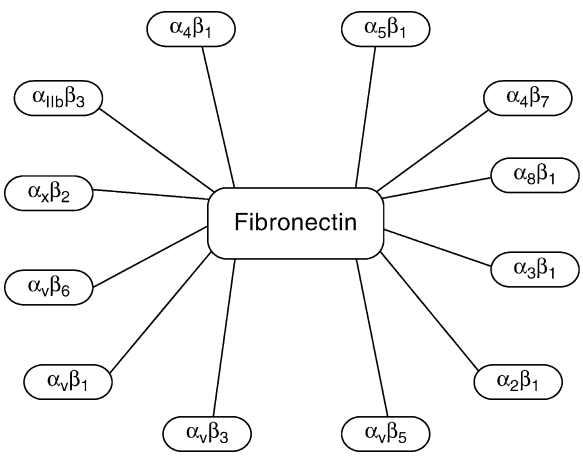


Fibronectin. Figure 1 Fibronectin molecule. Diagrammatic representation of the domain structure of fibronectin. Shown are homology repeats and different sites of alternative splicing (ED-B, ED-A and IIICS), giving rise to different isoforms of fibronectin.



Fibronectin. Figure 2 Fibronectin interactions with integrins. Different integrins that interact with fibronectin.

tenascin, produce multiple isoforms by alternative mRNA splicing. In general the exon changes caused by alternative mRNA splicing do not produce radically different proteins. Rather, they produce a set of similar proteins called protein isoforms.

Alternative splicing in fibronectin occurs in three regions of the primary transcript and generates 20 different isoforms that differ in the number of internal repeats. The regions of variation are: type III constant region (IIICS), extra domain A (ED-A) and extra domain B (ED-B). The same regions in rats are known as V, EIIIA and EIIIB, respectively.

Alternative splicing of fibronectin mRNA is regulated in a cell-, tissue- and developmentally-specific manner. Furthermore, it has been demonstrated that the splicing pattern of fibronectin mRNA is deregulated in transformed cells and in malignancies. The fibronectin isoforms containing the IIICS, ED-A and ED-B sequences are expressed more in transformed cells and in tumor tissues than in their normal counterparts. Because of the preferential expression of ED-B and de novo glycosylated fibronectin variants in fetal and tumor tissues, these fibronectin isoforms are known as oncofetal or embryonic fibronectins.

- IIICS region is situated between the last two type III homology repeats. This region of fibronectin undergoes complex patterns of alternative splicing; it may be totally included, partially included or excluded and may yield up to five different isoforms. IIICS domain is also subject to glycosylation. This fibronectin variant results from the addition of an α-N-acetyl-galactosamine to a threonine residue in a hexapeptide segment (Val-Thr-His-Pro-Gly-Tyr) located in the IIICS domain. De novo glycosylation of fibronectin is associated with cellular immaturity, cancer formation and the malignancy of breast, gastric and oral carcinomas.
- ED-A is situated between 11th and 12th type III homology repeats and it is the second region of alternative splicing. It is a single exon that is either included or omitted from the mature mRNA. This variation is tissue specific, and ED-A is not found in the mRNA of liver where plasma fibronectin is

synthesized. In human adult tissues, ED-A fibronectin has a restricted expression in certain tissues such as renal and colonic mucosa. ED-A is also present in normal adult myocardium where it is deposited as spots in the interstitium.

- The third region of variation is ED-B that is localized in the middle of the cell binding domain 4, between seventh and eighth type III homology repeats. ED-B is itself a complete type III homology repeat composed of 91 amino acids and coded by a single exon, which is either included or omitted from the mature mRNA. ED-B is the most conserved fibronectin region with 100% and 96% homology with rat and chicken fibronectin, respectively. ED-B containing fibronectin, with very few exceptions (superficial synovial cells, intima of some vessels and areas of interstitium of ovary, functional layer of endometrium during the proliferative phase and isolated areas of basement membrane of celomic epithelium), is reported to be absent in normal tissues. It has a greater expression in the intima of vessels of fetal brain cortex, stomach, jejunum, thymus and lungs of 8- to 12-week-old fetuses and tumor tissues as established by immunohistochemistry using the monoclonal antibody BC-1 (specific for ED-B containing fibronectin). In tissues from older fetuses (20–22 week old) only basal portions of gastric and duodenal glands were found positive for ED-B fibronectin. This suggests that ED-B fibronectin undergoes a programmed expression during ontogenesis.

Biological Significance of ED-B

The biological significance of ED-B is unknown. Its proximity to the cell-binding region suggests a role in cell adhesion and migration. This is also supported by the expression of ED-B in fetal tissues and during angiogenesis. In both the cases, cell migration and interactions are required. So far, no binding peptide is located to the ED-B repeat. But the ED-B domain seems to enhance cell adhesion and spreading. This adhesion effect of the ED-B may be mediated via conformational changes. It has been reported that insertion of the ED-B in fibronectin causes conformational changes in the molecule. The conformational changes induced by the ED-B in the downstream segment of the fibronectin may improve the access to the integrin binding sites in the ninth and tenth type III repeats. It has been shown that ED-B domain with its neighboring type III repeats is important in promoting cell adhesion.

Fibronectin Isoforms in Disease

Expression of fibronectin isoforms differs in disease. Although the expression of ED-A, ED-B and de novo glycosylated fibronectin is not disease specific, it is correlated with tissue modulation processes and particularly with connective tissue formation (fibroplasia).

Dilated cardiomyopathy (DCM) is a heart muscle disease in which alterations to cardiomyocytes result in changes in the composition of the ECM, including increase in the deposition of fibronectin, laminin and collagens in the intercellular spaces and in the vicinity of blood vessels. In patients with DCM, ED-A is detected as spots in the interstitium like in normal hearts. In contrast, ED-B and de novo glycosylated fibronectin, which are not seen in normal adult myocardium, are expressed in DCM. Re-expression of the ED-B and de novo glycosylated fibronectin in the adult heart was also found in myocardial hypertrophy in the rat. ED-A is synthesized by the synovial lining fibroblast-like (type B) cells. The expression of ED-A seems to be correlated with activated or transformed states of synovium. De novo glycosylated fibronectin was present in the synovial fluid of patients with rheumatoid arthritis. Patients with osteoarthritis also produce the same variant of fibronectin although at lower levels. De novo glycsylated fibronectin was also proposed as a predictor of preterm birth. High levels of this fibronectin variant were detected in the cervicovaginal secretion of women during the second and third trimesters of pregnancy.

Fibronectin Isoforms and Cancer

Fibronectin isoforms are detectable at higher levels in tumors, whereas they are least or not at all expressed in normal adult tissues. In pituitary adenomas the presence of ED-A and ED-B has been reported. They were localized in the adenoma neovasculature, especially in the endothelium and smooth-muscle layers of the vessel walls. Expression of ED-A and ED-B fibronectins was also shown in human colorectal carcinomas and in tumors derived from rat colon carcinoma. ED-A labeling was detected in all the samples of human carcinomas in the stroma separating the tumor glands but also in normal colon stroma. Apart from ED-A and ED-B, the glycosylated isoform of fibronectin is also expressed in colorectal cancer but is absent from the normal tissue. Increased expression of ED-B fibronectin mRNA was observed in different types of ►lung cancer including adenocarcinoma, ►squamous cell carcinoma, small cell carcinoma and large cell carcinoma. These results suggest that in lung cancer alternative splicing of fibronectin mRNA in ED-B domain occurs irrespective of type of cancer or degree of differentiation. In liver cancer both ED-A and ED-B mRNAs are increased in malignant tumors whereas ED-B mRNA was also detected in benign neoplasms.

►Tissue Inhibitors of Metalloproteinases (Timps)

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Fibrous Dysplasia

Definition

A tumor-like space-occupying process characterized by the production of immature bone and sometimes of hyaline cartilage by fibrous tissue elements.

►Bone Tumors

Fibulins

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Synonyms

BM90, FBLN-1; FBLN-2; S(1-5), T16, EFEMP1, FBLN-3; MBP1, UPH1, H411, EFEMP2, FBLN-4; DANCE, EVEC, UP50, FBLN-5; Hemicentin, him4, FIBL6, HMCN1, FBLN-6

Definition

Are a family of secreted glycoproteins that are deposited in various extracellular structures such as basement membranes, elastic fibers, proteoglycan aggregates and fibronectin microfibrils. Fibulins have been shown to play an important role in organogenesis and vasculogenesis. Current evidence suggests that fibulins may acts as tumor suppressors or tumor promoters.

Characteristics

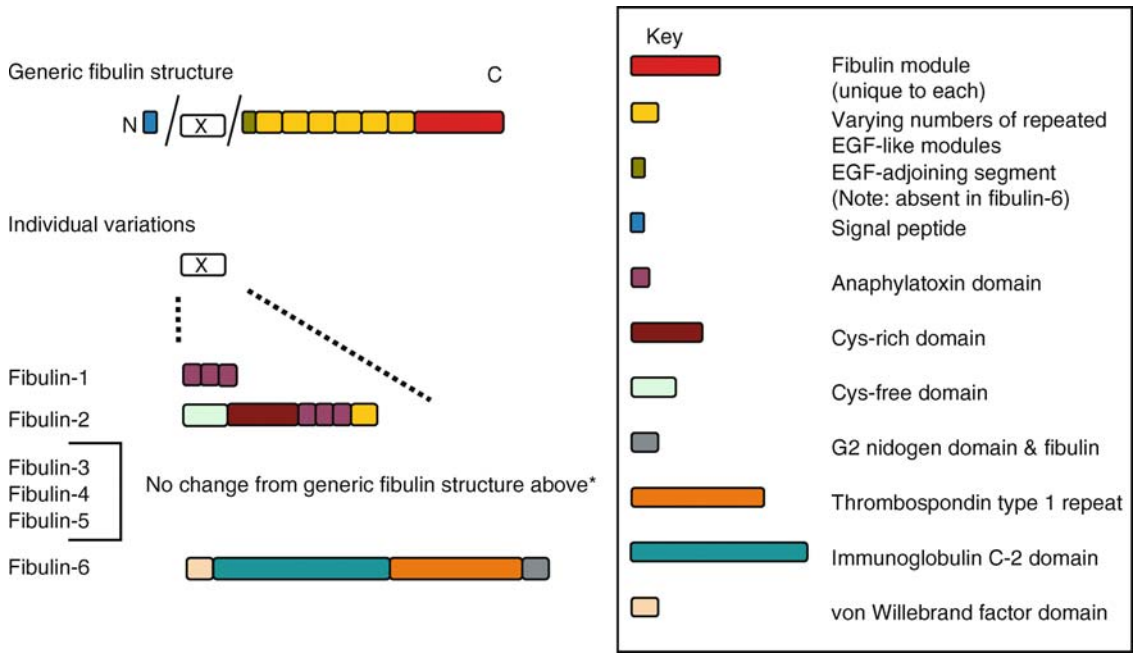
Fibulins arise as products from six distinct genes, termed fibulin 1-6, each residing on separate chromosomes. The fibulin isoforms vary in size from 50 to 200 kDa and are comprised of a collection of repeated calcium-binding epidermal growth factor (cbEGF)-like domains, which consist of rod-like elements with globular domains at the ends, and a unique C-terminal fibulin-type module (Fig. 1). Fibulins are prominently expressed in the blood and blood vessels but have also been found to reside in the lung, heart, liver, brain and other bodily tissues. Of the six fibulins identified to date, cancer-associated linkages have been documented for fibulin-1, -3, -4 and -5.

Fibulin-1

Fibulin-1 was the first member of the fibulin family identified. It consists of an N-terminal signal sequence, three anaphylatoxin (AT) repeats, nine EGF-like domains, eight of them processing the consensus sequence for calcium ligation, and an alternatively spliced fibulin-type C-terminal domain (FIB), giving rise to four possible variants, fibulin-1A to -1D. Variants-1C and -1D have been found to reside in most tissues and cell lines, whilst variants-1A and -1B have been detected at low levels in the human placenta. Current evidence suggests that fibulin-1 may act as a tumor suppressor or ►oncogene, depending on cellular context and/or which splice variant is expressed. Overexpression of fibulin-1D in human fibrosarcoma-derived cell lines suppresses anchorage-independent growth, motility and matrigel invasion *in vitro* and delays tumor formation *in vivo*. Similarly, elevated expression of fibulin-1D or addition of purified protein inhibits cells ►adhesion, spreading and motility *in vitro*. In addition, ectopic overexpression of fibulin-1D inhibits transformation by the papillomavirus E6 gene, possibly through a protein-protein interaction mechanism. Fibulin-1 can interact with positive (fibronectin and laminin) and negative (aggrecan and veriscan) regulators of cell motility, thus influencing cell movement and tumor progression. Elevated expression of fibulin-1 in ovarian and breast carcinomas has been reported (►Ovarian cancer, ►breast cancer), with there being an increased level of fibulin-1C:-1D seen in the former tumor tissue type. Serological screening of cDNAs identified fibulin-1 protein as an immunogen in patients with breast cancer. Similarly, dendritic cells obtained from fibulin-1 seropositive patients demonstrated a T cell response to the presence of fibulin-1. DNA microarray studies of lung adenocarcinomas demonstrated a close association of fibulin-1 and -2 with matrix metalloproteinase-2 expression, a promoter of ►metastasis and tumor invasion. The mechanism by which fibulin-1 promotes or inhibits tumorigenesis is unclear at present. However, available data suggest that there are specific binding functions for fibulin-1D that are not shared by fibulin-1C.

Fibulin-2

Fibulin-2 consists of an additional 400 amino acid long N-terminal domain, which is composed of



Fibulins. Figure 1 Schematic representation of the fibulin family structure. The structure of a generic fibulin is shown, with individual variations between family members represented underneath. The six secreted glycoproteins comprising the family are characterized by a unique fibulin-type module at the C-terminus and repeated epidermal growth factor (EGF)-like domains. Splice variants (known to exist for fibulins-1 to -4) are not illustrated. *Fibulins -3 and -4 have five EGF-like modules followed by a sixth with an insertion. There is no insertion in the sixth EGF-like domain of fibulin-5. Fibulins -3, -4 and -5 share ~50% amino acid homology.

cysteine-rich and cysteine-freesubdomains and nine EGF-like domains, which are a similar type to the EGF domains of fibulin-1. The role of fibulin-2 in cancer is unknown at present; however, recent studies have identified fibulin-2 as an overexpressed metastasis-associated gene in solid tumors of diverse types.

Fibulin-3

Fibulin-3 consists of five ►cbEGF-like modules and a carboxy-terminal domain III. Various alternatively spliced fibulin-3 transcripts have been identified which show a partial or complete deletion of the amino-terminal domain I. Fibulin-3 has been shown to positively and negatively regulate cell proliferation *in vitro*. Expression of fibulin-3 mRNA has been reported during the processes of cell growth arrest and senescence and is also upregulated in transformed cell lines. Microinjection of *in vitro* translated fibulin-3 mRNA into normal human fibroblasts stimulated DNA synthesis of injected cells, as well as some surrounding cells. Endogenous levels of fibulin-3 have been shown to inhibit proliferation, invasion, and angiogenic sprouting of endothelial cells in the presence of vascular endothelial growth factor. The ability of fibulin-3 to inhibit these tumorigenic variables suggests a possible role in reducing tumor angiogenesis and consequently tumor growth both *in vivo* and *in vitro*. Lastly, the rat homologue of fibulin-3 has been shown to associate with DA41, as determined by yeast two-hybrid assay. The DA41 protein interacts with the tumor-suppressor protein DAN, indicating that fibulin-3 might have an indirect role in the regulation of cell growth through a network of molecular interactions. Further work is required to determine the role of fibulin-3 in cancer.

Fibulin-4

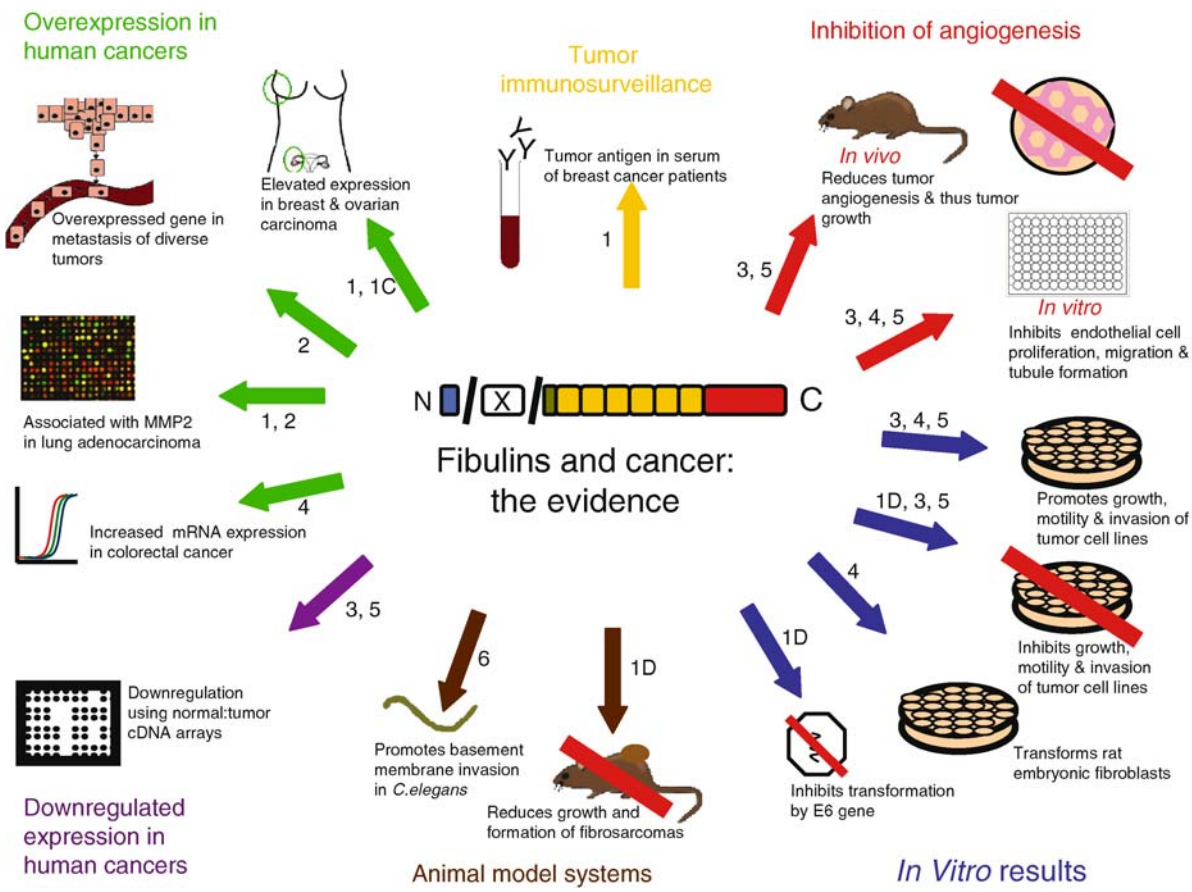
Fibulin-4 also consists of five cbEGF-like modules and a carboxy-terminal domain III. The human fibulin-4 gene is localized to 11q13, a region commonly amplified in a variety of human cancers. Mouse fibulin-4, originally identified as a specific protein partner for mutant p53, displays both mutant p53-dependent and -independent oncogenic properties. Fibulin-4 has been shown to induce cell proliferation and increase rates of neoplastic transformation by synergizing with mutant p53 protein. Elevated expression of fibulin 4 mRNA has been reported in a panel of paired normal/tumor human colon tissue biopsies (►Colon cancer). The exact mechanism responsible for the increased fibulin-4 levels in colorectal tumors is unknown at present. Further work is necessary to determine whether altered fibulin-4 expression is prevalent in other tumor types.

Fibulin-5

Similar in structure to fibulin-3 and -4, fibulin-5 is composed of six cbEGF-like domains followed by a globular C-terminal module which differs from other fibulins by the presence of integrin-binding RGD motif. Recent studies suggest that fibulin-5 may suppress or promote tumorigenesis. In human tissues derived from 68 patients, fibulin-5 mRNA expression was altered in 65% of the tumors, of which 95% showed down-regulation and 5% demonstrated up-regulation. The reduction of fibulin-5 expression was most prominent in cancers of the kidney, breast, ovary and colon. Importantly, fibulin-5 was expressed aberrantly in 68% of metastatic human malignancies (17 of 25 cases), of which 100% showed down-regulated expression of fibulin-5. Paradoxically, fibulin-5 expressing HT1080 cells exhibite enhanced DNA synthesis and increased cell ►migration to fibronectin as compared with control cells. In the presence of synthetic basement membranes, fibulin-5 expressing HT1080 cells invaded significantly better than control HT1080 cells. In addition, vascular smooth muscle cells derived from fibulin-5 knockout mice exhibited increased proliferation and migratory responses to mitogenic stimulus, with these effects being inhibited by the overexpression of fibulin-5. In short, the ability of fibulin-5 to enhance the malignancy of human fibrosarcoma cells and to down-regulate mRNA expression in human tumor tissue suggests that aberrant fibulin-5 may affect cancer cell growth in a context and/or tumor-specific manner.

Fibulin-6

Fibulin-6 was first identified in *Caenorhabditis elegans* (*C. elegans*), with orthologues in human and mice. It consists of a series of predicted cbEGF-like domains, followed by a single unusual EGF-like domain at the carboxy terminal. The ability of cells to invade through basement membranes and enter new tissues is crucial to the spread of cancer. To date, there is no direct evidence linking fibulin-6 to cancer. However, the ability of fibulin-6 to promote invasiveness *in vivo* has been reported using *C. elegans* as a model system. Further studies are necessary to elucidate fibulin-6 participation in invasiveness and possibly in ►carcinogenesis.



Fibulins. Figure 2 Fibulin family and cancer: the evidence. A summary of the available evidence linking members of the fibulin family with cancer. The individual fibulins linked to each process are shown in bold beside an arrow leading to a graphic representation of the findings.

Conclusion and Perspective

Fibulins are emerging as key contributors to carcinogenesis as indicated by their proposed involvement in tumorigenic mechanisms such as proliferation, adhesion, motility and invasion (Fig. 2). Their relevance to diagnosis and therapeutic implications in cancer is not fully clear at present. Fibulin-1, which is highly expressed in the blood and has been identified as an immunogen in patients with breast cancer, could aid in the detection of specific variants or processed forms of this protein via the development of a highly sensitive serum-based assays. Analysis of fibulin levels in more accessible biological materials, such as serum and urine, is warranted. Therapeutically, recombinant forms of fibulin-1, -4 and -5 might have potential as tumor suppressive or anti-angiogenic agents. Fibulin-1 protein can inhibit the adhesion and motility of a broad range of tumor cell types with minimal effects against primary cells. Using a retroviral-based gene-therapy approach, overexpression of fibulin-5 *in vivo* promotes wound closure. Lastly, the ability of recombinant fibulin-4 protein to inhibit angiogenesis *in vitro* suggests a possible therapeutic role in the treatment of cancer. In summary, further work is needed to fully resolve the complex role of fibulins in tumor development and progression.

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Field Cancerization

Definition

A concept to explain how multiple primary tumors and recurrences occur in a given tissue. First described for head and neck ►squamous cell carcinoma, but also recognized to occur in many other organs systems including skin, lung, breast, anogenital, colon, bladder. Stem cells exposed to carcinogens may become genetically altered, giving rise to clones of altered daughter cells. These undergo subsequent rounds of cell division, with each individual cell becoming a potential target for further genetic events and, consequently, malignant progression.

Field Defect

Definition

A phenomenon where grossly normal-appearing tissue which has pre-cancerous genetic changes. This tissue can be considered at risk for becoming a pre-malignant lesion and is hypothesized to occur in cancers which are commonly multifocal or locally recur after surgical removal of previously found growths.

►Carcinoid Tumors

Field Effect

Definition

Explanation for induction of multiple abnormalities within a single tissue. Where a "field" of cells is exposed to a given carcinogen, multiple unrelated lesions may arise.

►Malignancy-associated Changes

FIGO

Definition

International Federation of Gynecology and Obstetrics (FIGO); www. figo.org.

►Ovarian Cancer

Filipodia

Definition

Long thin protrusions at the periphery of cells and neuronal growth cones. They are composed of F-actin bundles and play a role in cell polarization and motility.

Finger-like membrane protrusions which are used by cells for ►migration. Thin, spike-like cytoplasmic protrusions containing actin filament bundles and generated on the leading edge of a crawling cell.

►Calpain
►Plexins

Final Appraisal Document

Definition

Final on guidance on a new health technology produced for by the UK's National Institute for Health and Clinical Excellence for the NHS.

►National Institute for Health and Clinical Excellence

Finasteride

Definition

Is an antiandrogen that acts by inhibiting type II 5-alpha reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT). It is used as a treatment in benign prostatic hyperplasia (BPH) in low doses, and ►prostate cancer in higher doses.; is marketed as Proscar, Propecia, Fincar, Finpecia, Finax, Finast, Finara, Finalo, Prosteride, Gefina, Finasterid IVAX).

Fine Needle Aspiration Biopsy

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Synonyms

Fine needle aspiration (FNA); Fine needle aspiration cytology (FNAC); Needle biopsy; Needle aspiration biopsy (NAB); Skinny needle; Thin needle; Aspiration cytology

Definition

Is defined as the removal of cells using a fine gauge needle from a lump or mass for diagnostic purposes.

Characteristics

Background

The diagnosis of cancer requires analysis of tissue or cell samples under the microscope. Although physical exam, radiological imaging, and laboratory tests are useful in determining which lumps might be malignant, these tests are not specific and all lesions must be sampled and examined under a microscope to determine the precise nature. The removal of a sample for examination under a microscope is called a *biopsy.* One of the biopsy methods used for cancer diagnosis is called a *needle biopsy.* There are 2 types of needle biopsies: core needle biopsy and fine needle aspiration biopsy (FNAB). *Core needle biopsy* removes a small cylinder of tissue and involves microscopic examination of intact tissue as a slice, called ►histology. FNAB withdraws cells and microscopic pieces of tissue which are evaluated under the microscope as individual cells and clusters of cells. FNAB is easier to obtain, causes less discomfort, is less invasive, and is more cost effective than core needle biopsy. The disadvantage of FNAB is that it cannot always provide the level of information afforded by core biopsy. In many clinical situations, however, both methods are equivalent.

This essay explains the FNAB method.

What is FNAB?

Fine needle aspiration biopsy (FNAB) is a non-surgical diagnostic technique used for the diagnosis of mass lesions. FNAB is not new; it has been successfully practiced in Europe since the 1950s. From Europe, FNAB spread to other parts of the world and is now an established technique used worldwide. The beauty of FNAB is in its simplicity. It requires only needles, syringes, glass slides, stains, a microscope and a skillful operator. Skinny ("fine") hollow needles attached to a syringe are used to extract ("aspirate") cells from a suspicious mass for microscopic examination. Although the procedure is simple, it is not easy, and successful results are obtained only when the procedure is performed and interpreted by a team of experts trained in the art of FNAB. The team optimally consists of a ►cytopathologist, a ►cytotechnologist and for lesions requiring image-guidance, a radiologist. The cytopathologist, a medical doctor with subspecialty training in ►cytology, interprets the results and, in the case of superficial masses, also procures the specimen. The radiologist procures the samples from deep-seated lesions aided by image guidance. The cytotechnologist is responsible for assisting with the FNAB procedure,

the initial microscopic screening, and preliminary diagnosis of slides. He/she may also be responsible for specimen collection, preparation and staining and record keeping. When performed by such a team, FNAB is very sensitive and specific with a diagnostic accuracy approaching that of histology.

FNAB is versatile and can be performed at any body site. Common superficial sites include thyroid, breast, lymph node, salivary gland and subcutaneous tissue. Common deep internal sites include liver, pancreas, kidney, lung, mediastinum, pleura, pelvis, retroperitoneum, bone, brain, retina and deep soft tissue. More recently, a technique called ►endoscopic-ultrasound guided fine needle aspiration (EUS-FNAB) was developed to diagnose tumors in and around the digestive tract.

By using FNAB, a major ►surgical biopsy (►open biopsy) can often be avoided.

What Cellular Characteristics Do Cytologists Study in Order to Make a Microscopic Diagnosis of Cancer?

►Cytologists distinguish normal cells from cancer cells, and recognize the different types of cancer cells by studying the morphologic features of cells. The two components of the cell that cytologists study are the nucleus and the cytoplasm. The nucleus tells the cytologist about the health of the cell and the cytoplasm tells about what kind of cell it is. In cancer, the nucleus looks abnormal. It usually takes up more cell volume and may be darker than a healthy nucleus. It can also have an irregular shape. The abnormal changes in the nucleus are due to the fact that they often contain too much DNA. The cytoplasm gives the cytologist clues about the type of cancer present-whether it is an adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinoma, lymphoma, or sarcoma. The way the cell clusters relate to each other assist the cytologist decide the type of cancer present. Sometimes studying the morphology does not yield enough information to allow the cytologist to decide the type of cancer. In these instances the cytologist uses ancillary ►immunocytochemical stains and other molecular markers on the cellular material obtained from the FNAB procedure to assist in making a definitive diagnosis and distinguish the type of cancer.

Method

Palpation-Guided Aspirates

For superficial palpable lumps, the procedure is optimally performed in a dedicated FNAB outpatient clinic by a cytopathologist with the assistance of a cytotechnologist (Fig. 1). The procedure takes approximately 15 minutes and is performed while the patient is awake. The patient is examined, the mass is localized, and the skin over the mass is cleansed with antiseptic. Local anesthetic may be injected into the skin over the nodule, although many practitioners prefer to perform aspirations without any anesthetic. A fine bore needle (approximately 23–25 gauge) is inserted through the skin and into the mass. A syringe attached to an aspiration device is commonly used to apply suction and the needle is rapidly moved up and down inside the nodule until enough cells and small tissue fragments have been dislodged and drawn up into the needle. The needle is then removed, and a gauze pad is applied with pressure to the biopsy site. After the fine needle aspiration biopsy is completed, a band-aid is applied and the patient is discharged home and can resume usual daily activities.

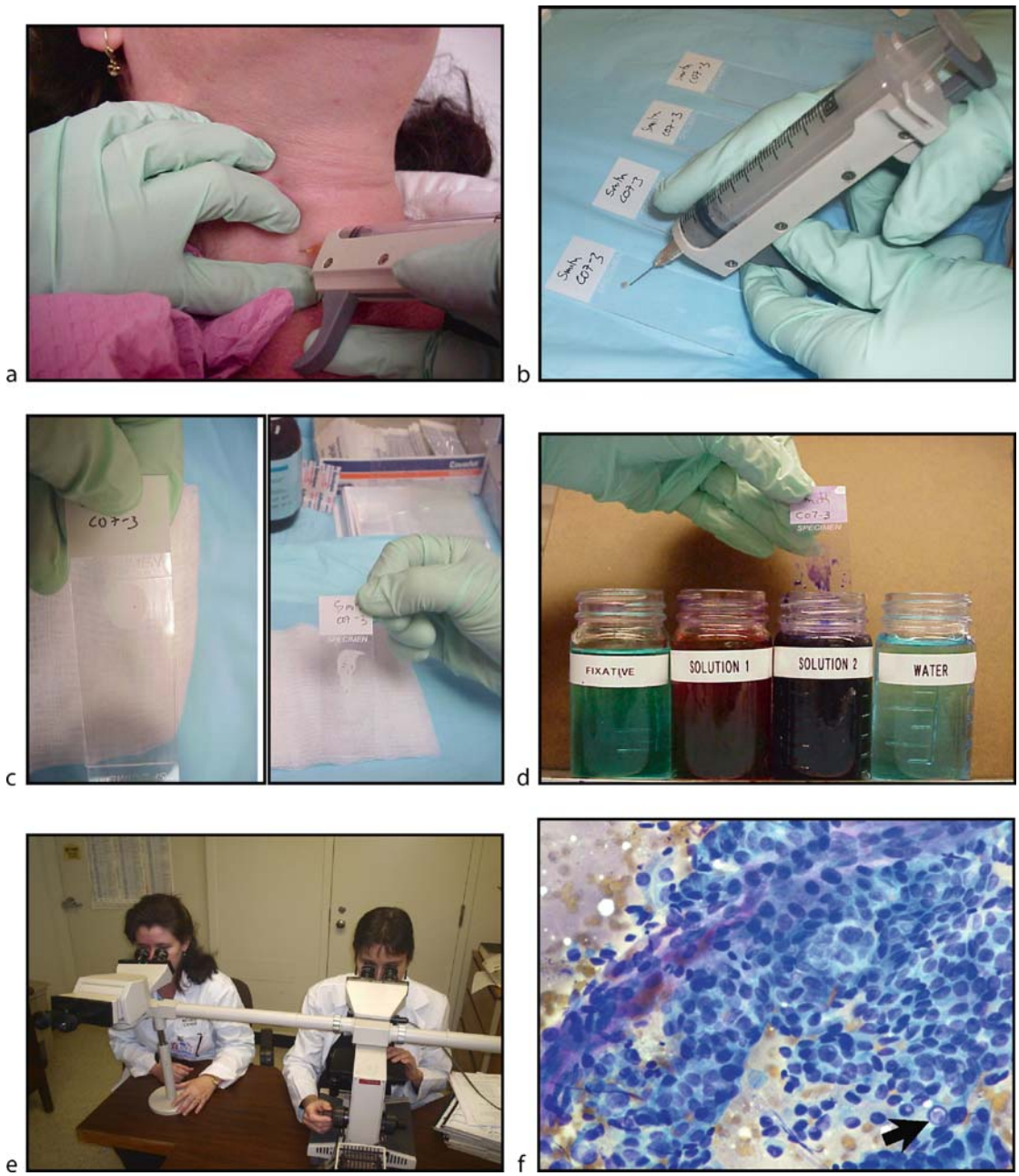
The biopsy material is expelled onto glass slides and smeared into a thin film. A number of samples are usually taken in separate aspirations or "passes" to increase the likelihood that an adequate sample will be obtained. In some facilities, representative smears are selected, stained and microscopically evaluated immediately for adequacy of the sample and for preliminary diagnosis prior to discharging the patient. This is optimal as it assures adequate sampling and appropriate sample triaging. If sufficient diagnostic material is obtained the procedure is terminated, and the patient is discharged. If the immediate microscopic evaluation demonstrates a lesion for which ancillary studies are needed, additional material is obtained immediately to fulfill the specimen requirements. This may include molecular studies (such as flow cytometry, immunocytochemistry, FISH and PCR), microbiology stain and culture, electron microscopy or other studies, as needed. ►Wright-Giemsa and ►Papanicolaou stained smears are routinely prepared in all cases. Cellular material can also be made into a "►cell block" and processed similar to histology.

Deep Seated Aspirations

Deep-seated soft tissue and visceral lesions are amenable to percutaneous FNAB using radiographic image-guidance (CT or ultrasound), which serves to guides the tip of the needle into the mass (Fig. 2). Image guidance can also be useful for difficult to palpate superficial masses or superficial masses located adjacent to vital structures. The technique for deep-seated aspirations is similar to that for superficial masses except that modified longer needles are required and a radiologist, rather than a cytopathologist usually does the FNAB. Optimal diagnostic yield is achieved when a cytologist is present during the procedure. Having the cytologist present allows for microscopic evaluation for adequacy and appropriate triage of the material. As in palpation-guided FNAB, smears are routinely made and if needed, material procured for ancillary studies as determined by the on-site microscopic evaluation.

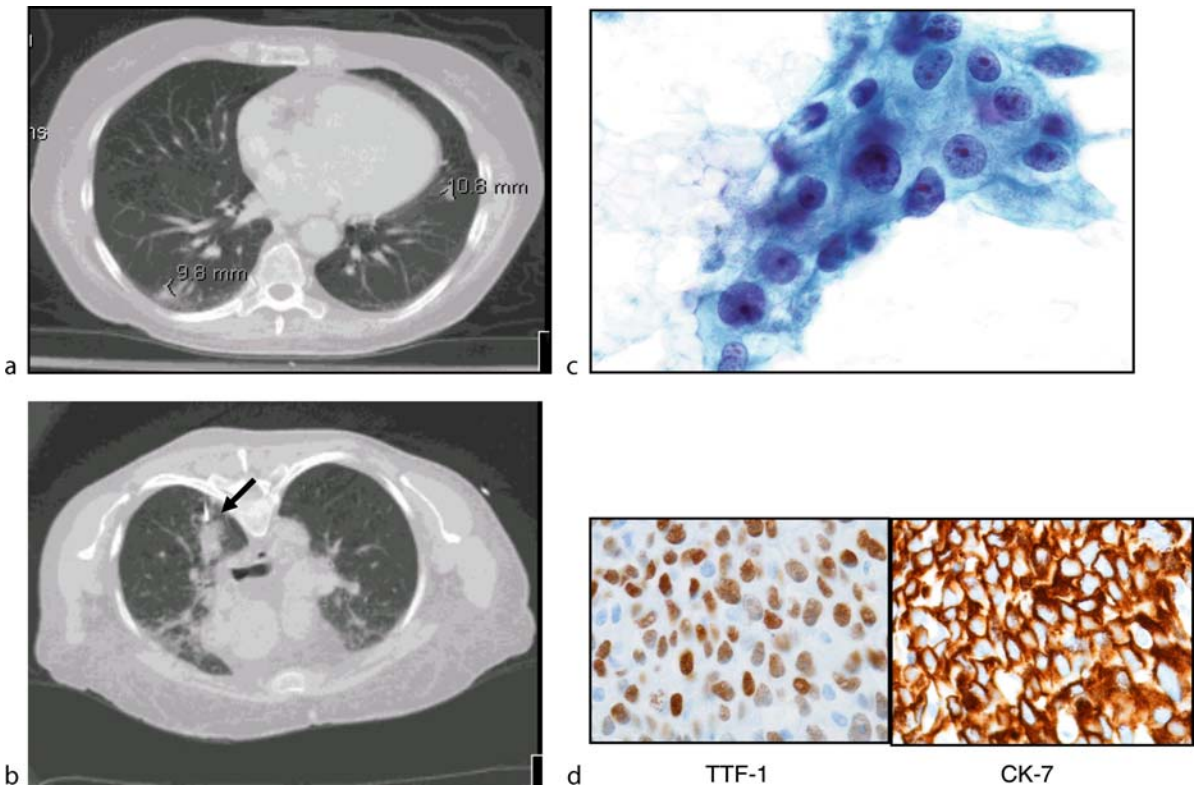
Complications and Contraindications

Complications associated with superficial palpable masses are rare and most commonly consist of minor



Fine Needle Aspiration Biopsy. Figure 1 Example of a palpation-guided FNAB procedure of a 1 cm right thyroid nodule in a 46-year-old female. (a) The needle is shown within the nodule. (b) The needle has been withdrawn from the nodule and the sample is placed in a drop onto a microscopy slide. (c) The specimen is smeared out using a two-slide smearing technique. (d) Representative slides are rapidly stained with a Wright-Giemsa stain. (e) The slides are evaluated microscopically for assessment of adequacy and preliminary diagnosis. Microscopic evaluation in this case demonstrated a papillary thyroid carcinoma. (f) Microscopic view showing the characteristic features of papillary carcinoma. Malignant thyroid cells are arranged in papillary configurations. Note the intranuclear hole (arrow) characteristic of this tumor type. (400× magnification, Wright-Giemsa stain).

bruising at the site. Deep-seated organ aspirations have higher complication rates which include infection, bleeding, peritonitis and pneumothorax. Uncorrected coagulopathy is an absolute contraindication for deep-seated FNAB. Needle tract seeding by tumor cells has also been rarely described in both superficial and deep FNAB; this complication is more commonly seen with core needle biopsy due to the larger needle caliber.



Fine Needle Aspiration Biopsy. Figure 2 Example of a CT-guided FNAB of a lung nodule. This patient was a previously healthy 65-year-old female with multiple pulmonary nodules noted incidentally on a CT scan done for other purposes. (a) CT scan shows bilateral, to numerous to count, pulmonary nodules thought to be suggestive of diffuse involvement of the lung by metastatic carcinoma. (b) CT scan showing the placement of the needle (arrow) within in a right posterior upper lobe nodule. (c) Microscopic view of a smear from the lung nodule demonstrates adenocarcinoma. Note the large malignant appearing cells with abundant cytoplasm and round to irregular nuclei with prominent nucleoli (1000× magnification, Papanicolaou stain). (d) Immunostaining results performed on the cell block shows the tumor to have strong positive nuclear immunostaining for TTF-1 (a marker for lung cells) and cytoplasmic staining for CK7 (also a marker for lung cells). The immunostains confirm that the cancer is actually a primary lung adenocarcinoma and not metastatic.

Generally, there are no absolute contraindications to FNAB of superficial masses.

Diagnostic Accuracy and Yield

When performed and interpreted by a dedicated FNAB team of experts a greater than 95% overall sensitivity and 99% specificity for the detection of cancer can be expected. The failure rate in obtaining a definitive diagnosis is less than 10% in experienced hands. This outcome may occur either when the specimen does not contain enough cells or when the specimen is adequate but overlapping cytologic features between benign and low-grade malignant entities precludes a definitive diagnosis. For such cases, further diagnostic steps such as core or open tissue biopsy are required.

Diagnostic Utility of FNAB

FNAB can provide specific diagnoses for many types of cancers. It is superb for the diagnosis of primary, recurrent and metastatic carcinoma, usually yielding enough information about the carcinoma subtype to start definitive treatment. FNAB is also sensitive for the detection of metastatic melanoma, lymphoma and many sarcomas.

The clinical value of FNAB is not limited to cancers. Most lumps and mass lesions are not cancers and FNAB is very valuable in diagnosing benign tumors, infections, cysts, inflammatory conditions and benign physiological alterations.

Limitations of the FNAB Method of Study

FNAB is a cytological method. In other words, it studies cells rather than intact tissue slices. The study of cells does not allow for evaluation of the relationship of the malignant cells to the supporting tissue as well as histology does. In addition, sometimes the FNAB cannot harvest sufficient cells/tissue for ancillary studies. Due to these limitations,

a histologic diagnosis may sometimes be preferred over a cytologic one.

Examples:

1. Some thyroid tumors require evaluation of the tumor capsule in order to decide if the tumor is benign or malignant; this can only be achieved by histological evaluation.
2. Although ►lymphoma is readily diagnosable by FNAB, sometimes the lymphoma subtype cannot be classified by FNAB to the extent required for optimal oncologic treatment. In this case, a surgical biopsy may be needed.
3. In some centers core needle biopsy is preferred over FNAB for the evaluation of prostatic and non-palpable mammographically detected breast lesions due to the purported higher sensitivity of core biopsy in these instances.

Despite these limitations, FNAB remains the initial diagnostic test of choice in most patients presenting with mass lesions. FNAB has the advantage of decreasing healthcare costs and improving patient care by providing a highly accurate and rapid diagnosis compared to alternative methods.

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Fine-Needle Aspiration Cytology

Definition

FNAC; A simple, inexpensive and well-tolerated method that consists in the aspiration of cells present using a fine needle. Smears, containing cells, are prepared immediately and microscopically examined by a cytopathologist. This method has a high sensitivity and a high specificity.

►Fine Needle Aspiration Biopsy

Firestorm Pattern

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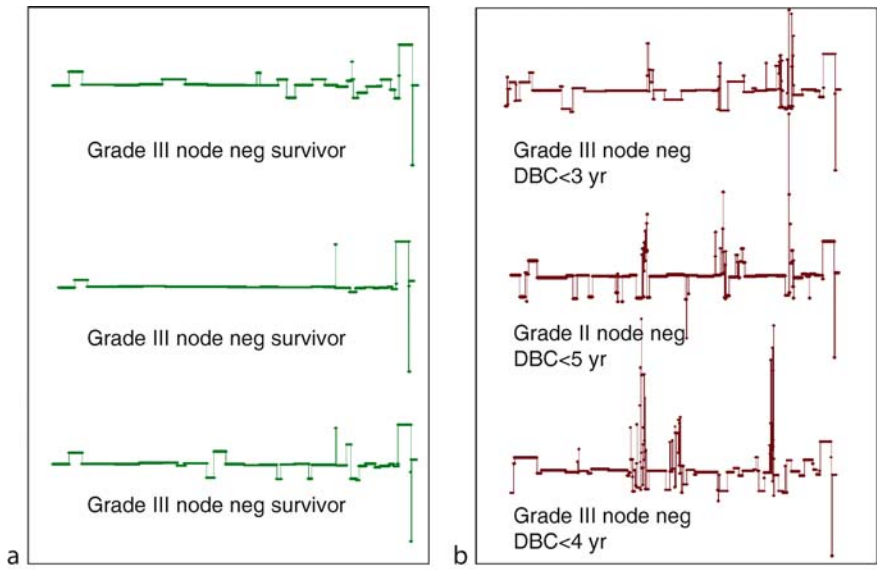
Definition

Firestorm refers to a type of genomic rearrangement, originally identified in breast cancer, characterized by multiple, narrow amplicons on a single chromosome arm usually coupled with deletion of the intervening regions. Firestorm amplicons are usually highly amplified (up to 30x) ►amplification and are maintained contiguously on a single chromosome, but not necessarily associated with their original centromere. There is evidence that firestorms are a marker for poor outcome in breast cancer in patients with otherwise good prognoses.

Characteristics

The term “firestorm” was coined to describe special cases of chromosome rearrangement revealed through high resolution microarray profiling in breast cancer genomes where multiple amplifications and deletions have occurred on a single chromosome arm. Firestorms appear as a series of tightly grouped peaks and valleys in genome profiles of copy number, with the peaks very often containing known or suspected oncogenes. As many as five separate firestorms may be observed in a single tumor genome, yet each one is clearly concentrated on a single arm. This structure is easily contrasted with so-called “simplex” and “saw-tooth” profiles that are characterized by whole chromosome or chromosome arm deletions and duplications distributed among many (“saw-tooth”) or a select few (“simplex”) chromosomes (Fig. 1). Additional information can be found in entries for amplification and amplified in breast cancer in this volume.

Firestorms are characterized by their structure and their potential significance for cancer gene identification as well as for diagnosis and prognosis. Based on the association of amplification and deletion in firestorms, along with their limitation to a single arm, it appears that firestorms are the result of a mechanism based on chromosome mechanics rather than over-replication of a single locus or a genome-wide destabilization of chromosome integrity. Firestorm peaks are frequently associated with known ►oncogenes (e.g. ►MYC, ►CCND1, ►ERBB2) but that the adjacent peaks are non-randomly distributed and may be pointers to novel oncogenes. Finally, based on an analysis of clinical outcomes in Swedish patients from 1985 to the present, there is evidence that a measure of firestorm activity, the



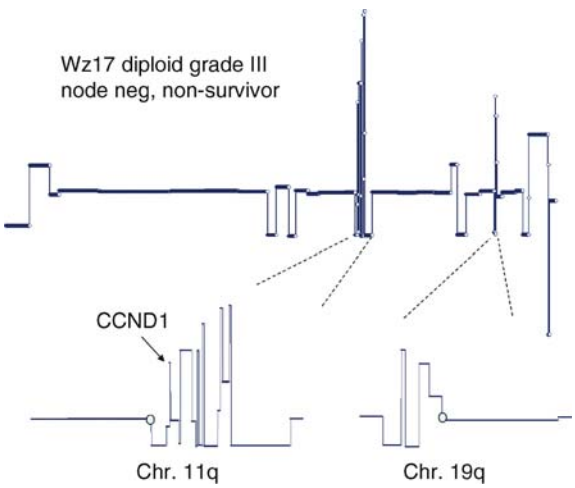
Firestorm Pattern. Figure 1 Comparison of simplex and "firestorm" genomic profiles from patients with similar pathology profiles. DBC: died of breast cancer.

"firestorm index" (F) may be a useful prognostic measure of metastatic disease recurrence.

Contrast with Other Forms of Gene Amplification

Two distinct forms of gene or regional amplification have been known since 1970s. Independently segregating replicons carrying the dihyrofolate reductase gene (DHFR), known as ►"double minutes" (DMs), were described in 1978 by Alt and Schimke and coworkers in murine cells as a result of selection with methotrexate. At about the same time, chromosomal regions carrying tandem copies of a single cytoband (►Homogenously staining region or HSR) were identified in drug resistant lines and later in transformed cell lines and solid tumors In both cases, the interpretation was on amplification of a locus either to overcome drug resistance or, presumably, to advance the replication of a cancer cell.

Firestorms are similar to HSR in that the amplified regions are carried on chromosome arms rather than replicating independently as double minutes. The main distinctions with HSR were revealed through the detailed resolution available with microarray analysis of copy number revealing that firestorms exhibit multiple narrow peaks of amplification and that those amplifications are tightly coupled to deletions of the intervening regions (Fig. 2). Using the ROMA microarray technique on arrays containing 85,000 probes coupled with a breakpoint analysis algorithm it is possible to observe breakpoints in copy number at 50 kbp resolution and to distinguish separate amplifications and deletions spaced less than 100 kbp apart. Thus, HSR refers to a single locus amplified in tandem



Firestorm Pattern. Figure 2 Firestorm amplifications are associated with deletion of the non-amplified regions.

on a chromosome, while firestorm refers to multiple HSR like events on a single chromosome arm.

Figure 1 provides examples of the microarray genome profiles of six pseudo-diploid, node-negative breast tumor samples that contrast the simplex and firestorm types. The genomes are presented in chromosome order, left to right, and represent copy number relative to a standard male primary fibroblast line. The X and Y chromosomes on the right provide scale, the XX/X ratio showing as an increase (duplication) and the Y/0 ratio as a loss. Figure 1a shows typical simplex profiles, most often associated with good prognosis and

long-term survival. Figure 1b provides examples of firestorm profiles in tumors of similar histopathologic status. Irrespective of which chromosome suffers the firestorms there is a similar risk for recurrence, consistent with the common notion that there are multiple pathways toward metastasis.

Figure 2 provides a magnified view of a typical pseudo-diploid genome containing two firestorms along with expanded views of the two firestorms on chromosomes 11 and 19. The expanded views demonstrate the typical pattern in firestorms where multiple, narrow regions are amplified, and, concomitantly, the non-amplified regions are actually deleted. The combination of amplification and deletion has been validated by interphase FISH in all cases tested, revealing one normal chromosome arm and one completely rearranged chromosome arm that is made up of only the amplified regions as has been demonstrated in metaphase chromosomes from cell lines by Gisselsson and co-workers. Thus, firestorms not only result in the gain of up to thirty or more copies of certain loci, but also the loss of the intervening regions, the combination of which may provide both increase in oncogene activity and deletion of tumor suppressors in a single multi-step process.

Mechanism of Firestorm Formation

The genesis of firestorms is very likely through a combination of chromosome breakage and rejoining events of the type first described by Barbara McClintock, based on her cytogenetic observations in maize. She noted that unprotected chromosome ends were likely to fuse after replication creating either a dicentric chromosome or a "ring" chromosome, either of which result in a visible "anaphase bridge" between nuclei at the end of mitosis. These bridged chromosomes would break, either randomly or at preferred sites, resulting in another set of broken ends and a repeat of the process, which she named the breakage-fusion-bridge (BFB) cycle. Elements of this process have been historically been observed in tumor cells as ring chromosomes and other abnormalities and have been implicated in the generation of altered chromosomes visualized cytogenetically.

Breakage-fusion-bridge and related mechanisms such as break induced replication have the power to introduce both amplifications and deletions through repeated rounds of recombination and replication. One of many variations of the way such a mechanism could introduce firestorm patterns is shown in a highly simplified cartoon in Fig. 3. DNA breaks may occur anywhere in the genome, but degradation of telomeres is one way to generate such an unprotected end. Two broken ends may fuse, or a single end may invade its sister (or another chromosome) (Fig. 3a). If linked centromeres separate at nuclear division, the chromosome will eventually break and the daughter nuclei will obtain an unequal complement of loci, along with "invasive" broken ends (Fig. 3b). Subsequent rounds of invasion at random loci, fusion and breakage can lead to repeated amplification of multiple sites (Fig. 3c). Chromosome invasion can also result in pairing and unauthorized DNA replication leading to further amplification of regions near the recombination site (not shown in the figure). Finally, invasion of an unrelated chromosome, perhaps at repeated sequences, may lead to a translocation of the rearranged loci (Fig. 3d).

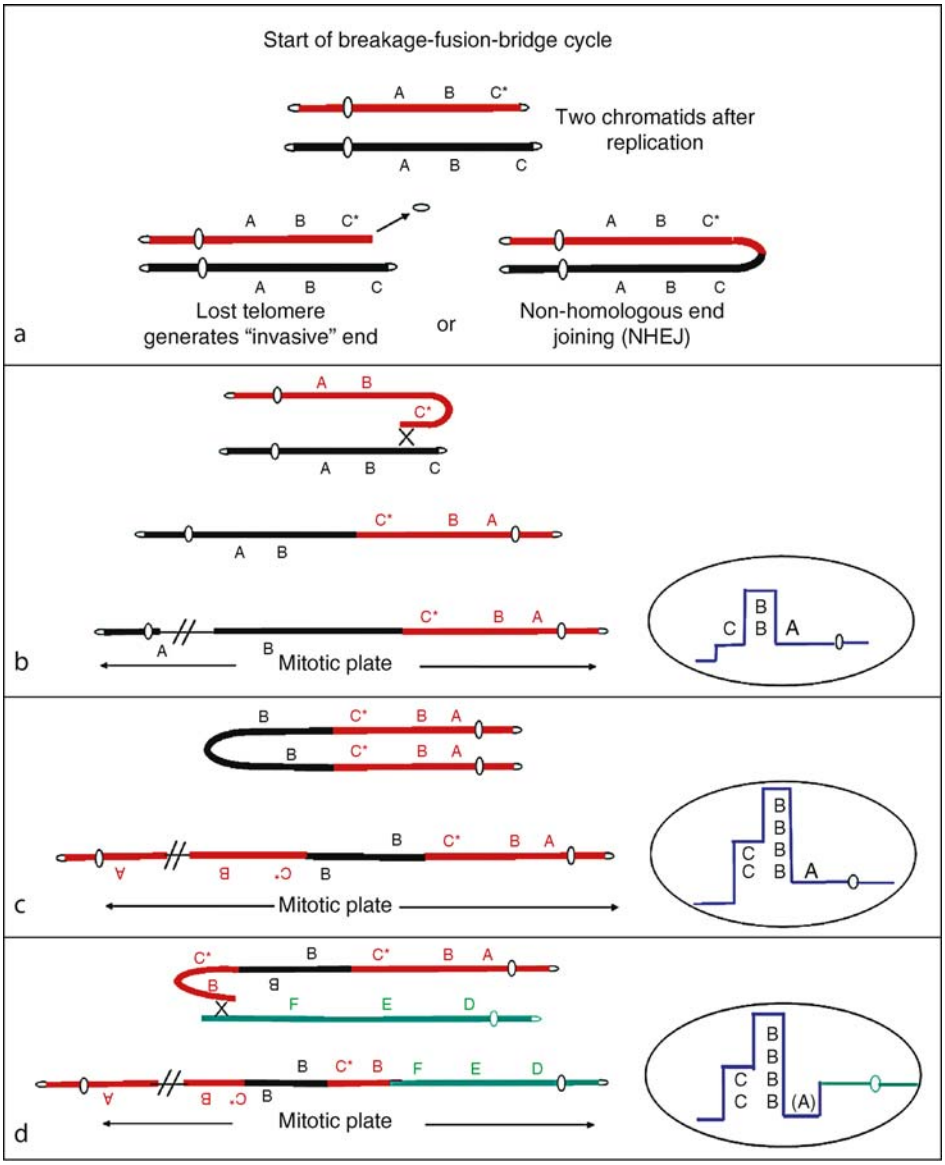
Relation of Patterns to Clinical Outcome

It has long been postulated that complexity of genome rearrangement could serve as a marker for malignant "progression." On first inspection, the highly rearranged "sawtooth" and "firestorm" patterns appeared to correlate with shorter survival in the diploid tumors, presumably due to selection of novel genetic combinations afforded the cancer cells by the opportunity for accelerated recombination. This was documented by developing a mathematical measure of complexity suitable for statistical analysis. Simply using the total number of segments, or events, as a measure does not clearly distinguish a sample with a single firestorm from the simplex pattern with a similar number of events, but the effects of the firestorm appeared by inspection to be much more deleterious to survival. Therefore a measure was chosen that would separate the firestorm patterns from the simplex patterns by scoring the close-packed spacing of the firestorm events, while at the same time incorporating the total number of events. The resulting sum of the reciprocals of the mean of lengths of all adjacent segment pairs ("Firestorm index" or F) accomplished this goal:

$$F = \sum_i \frac{2}{l_i^L + l_i^R} \qquad (1)$$

where i enumerates all the discontinuities with a magnitude above a numerical threshold of 0.1 in the segmented profile, and where $l_i^R (l_i^L)$ denotes the number of probes in the closest neighboring discontinuity on the right (left), or to a chromosome boundary, whichever is closer. The measure works equally well if absolute position in the genome is substituted for probe number. Using this algorithm the firestorm patterns achieve high F values even if only a single arm is affected because of the contribution of event proximity. It has been suggested that adding a factor for amplitude as well as spacing provides an even more effective measure (Anne-Lise Borressen-Dale, personal communication).

Preliminary analyses of a large diploid tumor collection indicated that prognoses in primary breast cancer, measured by the probability of overall survival, was correlated with the morphology of the gene copy



Firestorm Pattern. Figure 3 Example of a mechanism for firestorm formation using the breakage-fusion-bridge (BFB) cycle.

number signature as reflected in F value. Within the balanced group of the samples tested, the magnitude of the signature was independent of such established clinical markers as node status, histologic grade and primary tumor size. It is thus reasonable to expect that the firestorm signature will contribute to the prediction of outcome, perhaps in combination with other known factors.

A clear potential application of such a measure is in the determination of prognosis, with a focus on the identification of patients with such excellent prognoses that systemic therapy is not required or, conversely, such poor prognoses – in spite of clinical measurements that might be misleading in this regard – that systemic treatment is absolutely indicated. For example, a patient with a small, estrogen-receptor positive, node-negative primary breast cancer – all factors that usually indicate a good prognosis – might have an especially poor prognosis as predicted by F.

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First-echelon Node

►Sentinel Lymph Nodes

First-tier Node

►Sentinel Lymph Nodes

FISH

Definition

►Fluorescence in situ hybridization; molecular cytogenetic technique; fluorescently labeled small DNA or RNA probes are hybridized to chromosomes on interphase or metaphase spreads on slides; enables microscopic visualization of genes to define their number and chromosomal localization.

►Amplification
►Aneuploidy

FIT

►Fecal Immunochemical Test
►Fecal Occult Blood Test

Five Elements

Definition

The five elements (wood, fire, earth, metal, and water) concept is a philosophical theory developed in ancient China to explain the composition and phenomena of the physical universe. It is used in Chinese medicine to expound the unity between human and nature, as well as the physiological and pathological relationship and interconnection among the internal organs. The five elements match the five viscera, in which liver, heart, spleen, lung, and kidney correspond to wood, fire, earth, metal, and water respectively. The five elements concept explains the interpromoting and interacting relations, as well as the encroachment and violation in illness condition between the five viscera.

►Chinese versus Western Medicine

FK-506

Definition

Tacrolimus is a macrolide antibiotic. It reduces peptidyl-prolyl isomerase activity by binding to the immunophilin FKBP-12 (FK506 binding protein) creating a new complex. This FKBP12-FK506 complex inhibits both T-lymphocyte signal transduction and IL-2 transcription.

►Rapamycin

FKHL-16

►Forkhead Box M1

FLAME-1

►FLICE Inhibitory Protein

Flavin Monooxygenases

Definition

A family of microsomal ►flavoproteins (FMOs) that catalyze the oxidation of numerous drugs. They possess an NADPH and flavin binding site. The FMO system does not act to oxidize carbon atoms.

►Lead Optimization

Flavonoids

Definition

Are plant secondary metabolites with a diphenylpropane-containing structure. Flavonoids are normal constituents of the diet, as they are present in many fruits, vegetables and beverages. Numerous preclinical studies have shown that flavonoids have anticancer properties, yet some studies have revealed that high doses of some flavonoids can produce carcinogenic effects.

►Resveratrol
►Polyphenols
►Thioredoxin System

Flavopiridol

Definition

Is an experimental drug, derived from a medicinal plant from India, that has been used for centuries in many indigenous medicines. Is a ►cyclin-dependent kinase (cdk) inhibitor, can cause cell cycle arrest, induce apoptosis in cancer cells, and inhibit tumor cell growth in vivo.

Fli1-1

Definition

Friend leukemia virus integration site 1: gene on chromosome 11 encoding for an Ets transcription factor; rearranged with EWS in 85% of ►Ewing sarcoma family tumors.

►Ets Transcription Factors

FLICE

►Caspase-8
►FLICE inhibitory protein

FLICE Inhibitory Protein

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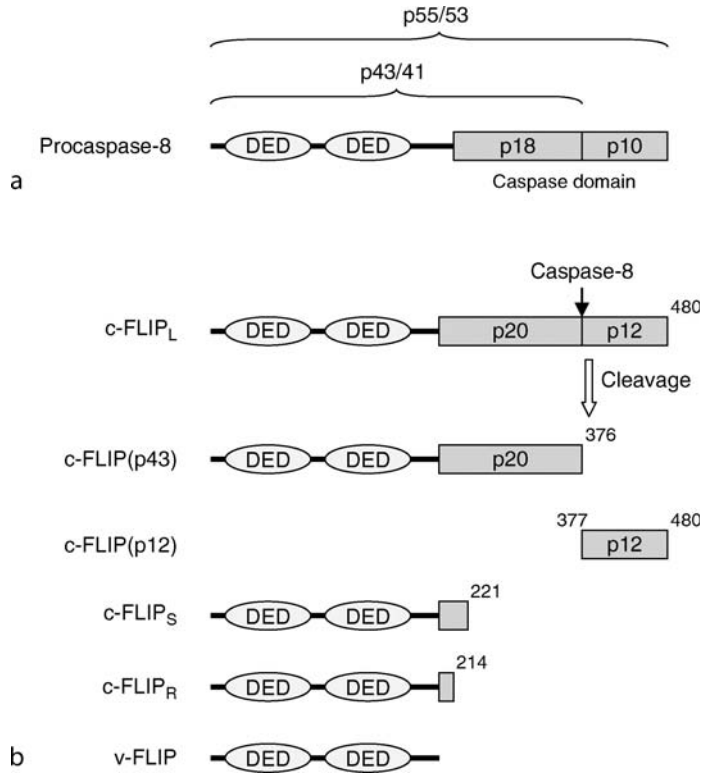
Synonyms

Caspase-eight-related protein; CASPER; Caspase homologue; CASH; Caspase-like apoptosis-regulatory protein; CLARP; Inhibitor of FLICE; I-FLICE; Fas-associated death domain; FADD-like antiapoptotic molecule 1; FLAME-1; MACH-related inducer of toxicity; MRIT

Definition

Human cellular FLICE inhibitory protein (c-FLIP) gene shares sequence homology with ►FADD, procaspase-8 and procaspase-10 and is located on chromosome 2q33–34. c-FLIP has eleven splice variants at the mRNA level. Two of these ►splice variants are considered major splicing variants detectable at the protein level in various types of cells and have been extensively studied thus far. One splice variant is designated the long isoform of c-FLIP (c-FLIP$_L$), which is a 55 kDa protein consisting of 480 amino acids, and the other major splice variant is the short isoform of c-FLIP (c-FLIP$_S$), which is a 26 kDa protein consisting of 221 amino acids. The third form of c-FLIP, designated c-FLIP$_R$, which is a 23 kDa protein consisting of 214 amino acids, is primarily expressed in T and B cells. Both c-FLIP$_S$ and c-FLIP$_R$ contain two ►DEDs, whereas c-FLIP$_L$, has two DEDs and a catalytically inactive caspase-like domain and thus shows overall homology with procaspase-8. c-FLIP$_L$ can be processed at Asp376 by caspase-8, yielding the N-terminal 43 kDa and C-terminal 12 kDa fragments which are termed c-FLIP(p43) and c-FLIP(p12), respectively (Fig. 1).

Some viruses such as herpesvirus saimiri, human herpesvirus 8 and moluscum contagiosum virus also produce DED-containing proteins, which are called viral FLICE-inhibitory protein (v-FLIP). Similar to c-FLIP$_S$, v-FLIP consists of two DEDs (Fig. 1).



FLICE Inhibitory Protein. Figure 1 Structures of procaspase-8 (a) and c-FLIP (b). Two major splice variants of c-FLIP, c-FLIP$_L$ and c-FLIP$_S$, have been extensively studied at the protein level. c-FLIP$_R$ and v-FLIP function in a similar manner to c-FLIP$_S$. c-FLIP$_L$ is processed by caspase-8 at Asp376, generating the N-terminal fragment c-FLIP (p43) and the C-terminal fragment c-FLIP (p12).

Characteristics

Function

c-FLIP primarily functions as a specific inhibitor of ►death receptor-mediated ►apoptosis. c-FLIP proteins can be recruited to the ►DISC by DED interaction. Both short forms of c-FLIP isoforms, c-FLIP$_S$ and c-FLIP$_R$, block death receptor-induced apoptosis by inhibiting procaspase-8 activation at the DISC. The role of c-FLIP$_L$ at the DISC is controversial. Some reports demonstrate that c-FLIP$_L$ functions as an anti-apoptotic protein in a way similar to c-FLIP$_S$, whereas others describe that c-FLIP$_L$ acts as a proapoptotic molecule in certain cellular contexts to facilitate the activation of procaspase-8 at the DISC. Endogenous c-FLIP$_L$ functions primarily as an inhibitor of death receptor-mediated apoptosis because ►siRNA-mediated knockdown of c-FLIP$_L$ augmented DISC recruitment, activation, processing, release of caspase-8, and apoptosis in several ►cancer cell lines. In general, c-FLIP$_S$ completely inhibits cleavage of procaspase-8 by preventing the initial cleavage step of procaspase-8 between the p18 and p10 subunit of the caspase homology domain, whereas c-FLIP$_L$ allows the first cleavage of procaspase-8 into caspase-8 (p43/41) but prevents the final cleavage between the prodomain and the p18 subunit of the p43/41 intermediate to generate active caspase-8 (Fig. 1). v-FLIP can function in a similar way to c-FLIPs to bind to the DISC and inhibit death receptor-induced apoptosis.

Besides inhibition of apoptosis, c-FLIP modulates the activity of ►nuclear factor-κB (NF-κB), ►MAP kinase and ►Wnt signaling pathways, suggesting that c-FLIP may play a critical role in many cellular activities. c-FLIP plays an essential role in heart development since disruption of mouse c-FLIP is prenatally lethal probably due to cardiac failure, resembling the phenotype of caspase $8^{-/-}$ and $FADD^{-/-}$ mice.

Modulation of c-FLIP Level

c-FLIPs are short-lived proteins subjected to ubiquitin (►ubiquitination)/proteasome (►protease)-mediated degradation. Many anticancer agents modulate c-FLIP levels through such a mechanism. However, the underlying mechanism including ►E3 ubiquitin ligases responsible for c-FLIP ubiquitination has not been fully elucidated. A recent study has demonstrated that N-terminal c-Jun kinase (JNK)-dependent phosphorylation and activation of the E3 ubiquitin-protein ligase ►Itch specifically ubiquitinates c-FLIP$_L$ and induces its proteasomal degradation.

In addition to the posttranslational modification, c-FLIP expression can be regulated at the transcriptional level. In this regard, the MAP kinase, PI3 kinase (►PI3K signaling)/Akt, NF-κB, p53 (►p53 protein, biological and clinical aspects) and c-Myc (►Myc oncogene) and JAK/STAT pathways have been suggested to be involved in regulation of c-FLIP expression. c-Myc directly represses c-FLIP transcription while other pathways such as PI3 kinase/Akt, NF-κB and p53 increase c-FLIP transcription. Thus, activation of c-Myc sensitizes cancer cells to death receptor-induced apoptosis.

Role in Carcinogenesis

c-FLIP is expressed abundantly in some tissues, such as heart, skeletal muscle and lymphoid tissues. However, c-FLIP is also expressed in a broad spectrum of normal cells such as endothelial cells, keratinocytes, motoneurons, muscle cells, pancreatic cells, dendritic cells and macrophages, suggesting that c-FLIP is an important regulator of many physiologic processes. Accordingly, dysregulation of c-FLIP might lead to development of many diseases including cancer. c-FLIP is up-regulated in many human tumor cells, including colon, gastric, hepatocellular, ovarian, endometrial carcinoma cells, melanoma, leukemia, and Hodgkin lymphoma cells. Increased c-FLIP/►caspase-8 ratio in ►Epstein-Barr virus-transformed cells results in resistance to death receptor-induced apoptosis. High c-FLIP protein expression is associated with a poor clinical outcome in Burkitt lymphoma. Moreover, transfection of c-FLIP into cancer cells promotes cancer ►metastasis. Thus, elevated c-FLIP levels in tumor tissues may allow tumor cells to escape from the death receptor-mediated apoptotic death. Some studies have shown that c-FLIP is involved in mediating the immune escape of tumors *in vivo*.

The Wnt signaling pathway is involved in ►carcinogenesis as mutations in Wnt signaling are associated with many human cancers. It has been shown that c-FLIP$_L$, together with FADD, boosts the Wnt-signaling pathway through inhibiting β-catenin ubiquitination and proteasomal degradation, thereby contributing to carcinogenesis.

Implications in Cancer Therapy

c-FLIP might be a promising target for cancer therapy. In general, c-FLIP expression correlates with resistance against death receptor-induced apoptosis in a variety of cancer types and c-FLIP-transfected cancer cell lines develop more aggressive tumors *in vivo*. Conversely, downregulation of c-FLIP by antisense oligonucleotides (►antisense DNA therapy), siRNA, or ►small molecule drugs sensitize cells to death receptor-mediated apoptosis. Some small molecule drugs with anticancer activity including DNA-damaging agents, ►histone deacetylase (HDAC) inhibitors, COX-2 (►cyclooxygenase in colorectal cancer) inhibitors, and PPARγ (►peroxisome proliferator-activated receptor and cancer) agonists reduce c-FLIP levels in cancer cells. Given that the death ligand ►TRAIL and agonistic anti-TRAIL receptor DR4 and DR5 antibodies (►TRAIL receptor antibodies) are being tested in clinical trials for treatment of cancer, inhibition of c-FLIP expression in cancer cells is of particular importance for TRAIL- or TRAIL receptor-based cancer therapies. Anticancer agents with c-FLIP-inhibitory activity will greatly sensitize cancer cells to TRAIL- or TRAIL receptor-based cancer therapies.

Recent studies have shown that overexpression of c-FLIP also protects cancer cells from apoptosis induced by certain anticancer drugs including chemotherapeutic agents (►chemotherapy of cancer, progress and perspectives) and the COX-2 inhibitor ►celecoxib. In agreement, siRNA-mediated knockdown of c-FLIP sensitizes cancer cells to apoptosis induced by chemotherapeutic drugs including ►cisplatin, 5-fluorouracil (►fluorouracil) and ►taxol. Thus c-FLIP may also play an important role in mediating cell resistance to chemotherapy.

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Flow Cytometry

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Definition

FCM; Is a method for counting, analyzing and sorting particles e.g. cells in a single cell suspension by their light absorbing or fluorescing properties. Flow

cytometry permits assessment of physical (cell size, shape and internal complexity), chemical (expression of proteins and other molecules) and biological (metabolic pathways e.g. calcium ion flux) attributes of single cells in suspension. (►FACS)

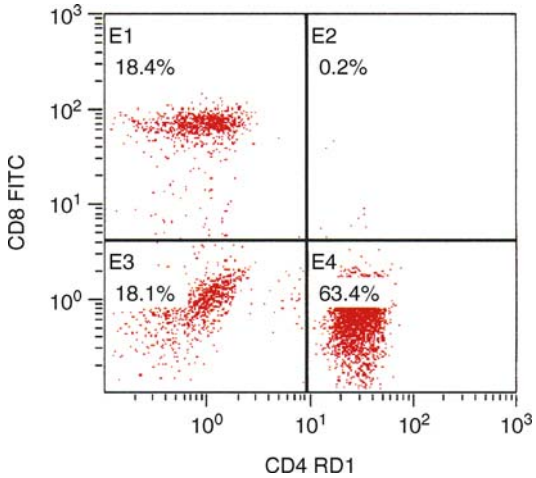
Characteristics

1. The Technology
2. Applications in cancer

The Technology

There are three essential components of a flow cytometer, fluidics, optics and electronics. *Fluidics:* A single cell suspension in a highly controlled fluid stream is forced through a nozzle to induced laminar flow at a high flow rate (meters/sec). A higher-pressure sheath fluid stream surrounds the sample in isotonic fluid and the cells are examined (interrogated) at a point by the excitation light, usually a laser beam. *Optics:* A laser light source generates a monochromatic light beam that passes through the sample stream and gets scattered when it encounters a cell. A simple light scatter plot can identify cells based upon their size, shape and internal complexity. If ►fluorochromes are attached to the cells (either by direct labeling or through conjugated monoclonal antibodies), these absorb the laser light and re-emit a light of longer wavelength (specific to each fluorochrome). Multiple wavelengths can be collected and analyzed at the same time (multicolor analysis). Minimal overlap between generated wavelengths is established prior to collection of the light by a system of filters and dichroic mirrors and later by electronic compensation. ►Epitope density is a major determinant of fluorochromes to be used for conjugating antibodies e.g. low density epitopes can be detected by antibodies bound to phycoerythrin (PE) and high density epitopes would be easily detectable by fluorescein isothiocyanate (FITC). *Electronics:* The light is then collected by photomultiplier tubes and converted into analog electric signals, either in log or linear domains followed by digitization of the signals. Attribution of all the signals associated with a specific cell permits multiparameter analysis. The digital data is collected in a list mode format usually since this allows for complicated analysis and the data can be processed many times. The computer analysis of the data can be performed either by stand-alone software packages or on-line systems with file transfer abilities.

Selection of a subpopulation for further analysis is called gating and can be performed at multiple time-points e.g. live gating- selecting the population at the time of data collection, analysis gating – after the data has been collected in the list mode; other gating strategies include forward and back gating. The data are displayed in graphic formats including histograms, dot plots, contour plots or isometric plots (Fig. 1). Analysis



Flow Cytometry. Figure 1 A dot plot display of two parameters (CD4 and CD8 ►antigens) from a patient with a lymphoproliferative disorder permitting assessment of the expression pattern and the number of positive and negative cells for each ►antigen as well as any co-expression, if present.

of the data requires careful interpretation of the generated histograms and determining an overall pattern of expression of multiple ►antigens. Assessing negative or positive expression of an antigen and viewing co-expression as color-coded histograms requires some basic understanding of binary logic and Boolean gating. Proper instrument setup, calibration, and compensation along with use of appropriate external and internal controls are necessary for misinterpretation of findings.

Flow sorters allow physical separation (sorting) of cells with up to 99% purity for any additional studies such as molecular or functional assays. A comprehensive quality assurance and quality control program has to be established for pre-analytic, analytic and post-analytic stages.

Specimen Issues. Peripheral blood, tumor masses (fine needle aspirates, core biopsies, and incision or excision biopsies) and bone marrow and various body fluids are common specimens analyzed by FCM. Fresh samples are preferred to obtain the cellular population for further analysis, although paraffin embedded tissues have also been analyzed successfully by flow cytometry. An assessment of representative morphology and viability of the single cell suspension allows for a more meaningful interpretation of flow data e.g. by ensuring that the critical tumor cell population has not been lost due to cellular fragility. Dead cells can non-specifically bind antibodies and affect data interpretation.

Applications

Flow cytometry provides an objective measure of multiple attributes of single cells at a great speed

(thousands of cells per second) allowing determination of not only average characteristics of a tumor's cellular population but tumor cell heterogeneity as well. In addition to size, shape and internal complexity determination, Fluorochromes can be used either to directly label molecules inside a cell (e.g. DNA, RNA, thiols) or label monoclonal antibodies directed against specific cell surface or intracellular proteins/molecules. FCM also permits functional assessment e.g. viability, oxidative burst, mitochondrial membrane potential, cell activation using intracellular calcium ion flux, DNA synthesis and cell cycle phase analysis and apoptosis etc. Green fluorescent protein labeling can be used as a gene reporter.

The applications of flow cytometry in cancer include; (i) ►Immunophenotyping of ►hematologic malignancies for diagnosis and prognosis and monitoring ►Residual Disease, (ii) Organ transplantation support, (iii) DNA analysis of S-phase fraction of solid tumors and (iv) determining treatment efficacy.

Immunophenotyping (IP)

Immunophenotypic patterns of cells correspond to different stages of differentiation and function and complement morphologic assessment of tumor cells for correct diagnosis. IP is essential for initial diagnosis and classification, determining certain prognostic variables (e.g. cytokine receptors, multidrug resistance), identifying potential treatment targets and post-treatment follow-up including minimal residual disease assessment.

For lymphoid lesions the FCM provides lineage information (B vs. T-lymphoid) by using antibodies against pan-B antigens (e.g. CD19, ►CD20, CD22) and pan-T antigens (e.g. ►CD3, CD5, CD7). FCM provides information on whether a population is likely to be neoplastic or not. This latter aspect is determined by identifying light chain restriction (clonality) for B-cells and detecting antigen deletion, subset expansion, or aberrant antigen expression for T-cells. Once a population is determined to be neoplastic identifying a relatively unique pattern of antigen expression can be used either to make the diagnosis or complement the diagnostic process by integrating the information with other diagnostic data e.g. morphology and genetics. For lymphoid neoplasms composed of smaller lymphoid cells, immunophenotyping is often considered the gold standard for diagnosis. Expression of certain antigens is related to prognosis e.g. ZAP-70 in ►chronic lymphocytic leukemia and this is optimally measured by FCM.

For ►acute myeloid leukemias, FCM is used in a similar fashion to determine the expression of "immature" (e.g. CD34, TdT, CD117, HLA-DR), "differentiation-related" antigens e.g. CD11b for acute leukemias with monocytic differentiation and "lineage-related" antigens e.g. CD41 and CD61 for megakaryocytic leukemias. The role of FCM in acute leukemia diagnosis includes, a) distinguish acute myeloid from acute lymphoid leukemia, b) correlation with other diagnostic findings such as morphology and genetics, c) recognition of acute biphenotypic subtype and d) prognostic value of antigen expression (e.g. CD7 in AML confers poor prognosis). FCM is also useful in the diagnosis of ►myelodysplastic syndromes e.g. by demonstrating abnormal antigen pattern of myeloid, erythroid and megakaryocytic antigens and accurate blast quantitation.

The presence of antigens on tumor cells is useful in instituting targeted therapy e.g. ►rituximab for CD20+ B-cell lymphomas, alemtuzumab for CD52 expressing chronic lymphocytic leukemia.

The immunophenotype is also useful in follow-up of patients in order to assess residual disease, recurrence and progression/transformation. FCM is extremely useful in differentiating leukemic blasts and hematogones (reactive B-cell precursors), a critical distinction in follow-up marrows of children with B-lineage acute lymphoblastic leukemia. FCM by virtue of its specificity, sensitivity, reliability and established standardization is useful in identifying smaller populations of tumor cells in post treatment specimens, which are below the detection limit of conventional methods (minimal residual disease-MRD); the clinical significance of MRD is established for certain malignancies e.g. childhood acute lymphoblastic leukemia and is being evaluated for others. For follow-up of myeloma patients, abnormal plasma cells (CD19−, CD56+) can be assessed in bone marrow samples, although this technique is somewhat limited by sampling error due to focal distribution of plasma cell clusters.

FCM is not without its limitations. Interpretive errors may be caused by antigens are not always being lineage restricted, non-classic immunophenotypic profile of a tumor, sampling issues (e.g. non-representation of an abnormal plasma cell clusters in the bone marrow aspirate). A better way to interpret the data is not only pay attention to the numbers of cells positive or negative but also the pattern of antigen expression and the composite profile of expression of multiple antigens. The best was is to determine the pattern of antigen expression rather relying on one antigen alone. In expert hands the method is reliable, objective and fast.

Transplant Support

Assessing the numbers and purity of CD34+ stem cells is necessary for stem cell transplant patients since the number of viable CD34+ cells is a crucial factor determining rapid and long-term multilineage engraftment. FCM on ►apheresis products, cord blood or bone marrow ensures adequate numbers of CD34+ cells are present. Harvest products can also be assessed for

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contamination by tumor cells. Other roles of FCM include, assessment of immune reconstitution by determining numbers of B, T and NK cells and their subsets, graft rejection, graft versus host disease and graft versus leukemia effect. FCM is a highly sensitive technique for pre-transplant ►cross matching, HLA ►antibody screening and monitoring post-transplant antibody levels for solid organ transplantation patients.

DNA Ploidy and S Phase Fraction

DNA content of cells can be determined by determining ►fluorescence intensity of DNA binding dyes such as propidium iodide (PI); three populations G0/G1, S and G2/M are easily discernible. FCM is useful when material cannot be obtained for karyotyping, however, it cannot detect balanced translocations and partial chromosome deletions. Since the measurement is that of total DNA content, rather than actual chromosome numbers, the results are in reference to a normal "diploid" population. The DNA histogram plots fluorescence intensity (x-axis) versus the number of cells (y-axis). A majority of the malignancies have an abnormal DNA content (other than diploid) and aneuploid tumors generally have a bad prognosis with a few exceptions. ►S phase fraction: The proportion of cells in S phase – an estimate of the tumor's proliferative activity- can be determined either by PI labeling or by using a dot plot of anti-bromodeoxyuridine and propidium iodide. S phase fraction generally correlates with increasing DNA amount in tumor cells and the grade of tumors e.g. in lymphomas and a higher S phase fraction is generally considered an adverse prognostic variable. Enthusiasm for DNA ►ploidy and S phase fraction determination in routine oncologic practice has been somewhat limited in view of the technological variations and prognostic and predictive information available from other parameters. Improvement and standardization in methodology may lead to enhanced use of DNA ploidy data by FCM in routine clinical practice. Flow karyotyping refers to the use of FCM to analyze or separate chromosomes on the basis of their DNA content.

Determining Treatment Efficacy

The in vitro prediction of cellular sensitivity to treatment can be assessed by FCM by microdrop encapsulation assay and BrDU incorporation proliferative survival assay. Development of treatment protocols may also take into FCM determination of apoptosis.

Newer and Future Applications

Detection of rare circulating tumor cells and circulating endothelial cells may help in assessing tumor progression and response to treatment, however this application with great potential, at present, is not applicable in routine clinical practice, mainly due to technical issues. Cellular RNA content may be useful for the discrimination of acute leukemias and ►multiple myeloma. As the notion of personalized medicine gets further impetus identification antigens against which treatment can be directed will increase in clinical practice. Biological microbeads are useful for flow cytometric immunoassays.

A relatively recent development has been exploitation of a hybrid technology between flow cytometry and image cytometry, the laser scanning cytometry, to quantify fluorescence intensity of individual cells on glass slides. From a dot plot display populations can be selected and the cells that generated the data can be relocated and then visualized with either bright field or epifluorescence microscopy.

The future of flow cytometry appears to be bright. Technological advances will continue to positively impact the use of FCM. Semiconductor quantum dots (Qdots) are likely to be used as fluorophores in view of their exceptional brightness and large Stokes shifts. Multicolor analysis is likely to expand to use additional colors. Photothermal flow cytometry can be used for detection of label-free detection of cells. Rare event detection including identification of single cells is likely to improve with the use of additional approaches to spectral analysis such as Raman spectroscopy and fluorescence resonance energy transfer measurements.

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FLT

Definition

^{18}F-Fluorothymidine; Radionucleoside that accumulates predominantly in proliferating tissues and can therefore be used for non-invasive imaging of proliferation with Positron Emission Tomography (PET).

►Positron Emission Tomography

Fludarabine

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Synonyms

9-β-D-Arabinosyl-2-fluoroadenine (F-ara-A) monophosphate; FAMP; NSC-312887; Fludarabine phosphate

Definition

Is the ►prodrug of 9-β-D-arabinosyl-2-fluoroadenine (F-ara-A), a synthetic halogen-substituted analogue of deoxyadenosine. Fludarabine and the two other related compounds ►cladribine (2-chloro-2′-deoxyadenosine) and ►pentostatin (2′-deoxycoformycin) are collectively referred to as ►purine analogues, although pentostatin is not actually an analogue, but an inhibitor of ►adenosine deaminase. Fludarabine is widely used in the treatment of indolent lymphoproliferative malignancies, ►acute myeloid leukemia, and as a part of the conditioning regimen for ►non-myeloablative allogeneic stem-cell transplantation.

Characteristics

Clinical and Cellular Pharmacology

The development of fludarabine stemmed from the success of the cytosine analogue ►cytarabine (ara-C) in the treatment of acute myeloid leukemia. A similar adenine analogue, Ara-A (vidarabine), was a successful anti-viral agent that had limited anti-cancer activity due to its rapid clearance by adenosine deaminase. Subsequent structural modifications by Montgomery and Hewson resulted in the development of 9-β-D-arabinosyl-2-fluoroadenine (F-ara-A), an adenosine deaminase-resistant derivative of ara-A. Because F-ara-A is poorly soluble, its 5′-monophosphate prodrug – designated fludarabine – was synthesized as the preferred compound for clinical development. *In-vivo*, fludarabine is rapidly and reliably dephosphorylated to F-ara-A, which is then taken up into cells and phosphorylated to its triphosphate (F-ara-ATP), the active compound mediating the anti-neoplastic properties of fludarabine (Fig. 1). The rate limiting step in this conversion process is the phosphorylation of F-ara-A to its monophosphate (F-ara-MP) by ►deoxycytidine kinase.

The current standard dose and administration of fludarabine is 25–30 mg/m^2 via a short infusion (15–30 min); this is substantially lower than the potentially neurotoxic doses used in the initial phase I trials of fludarabine in advanced malignancies, which was up to 260 mg/m^2 over 2–5 min. Pharmacokinetic data of F-ara-A from this phase I study showed a volume of distribution of 44.2 L/m^2, clearance of 4.08 L/h/m^2 and a triphasic elimination phase comprising a rapid distribution phase (5 min) followed by an intermediate elimination phase (1–2 h) and a prolonged terminal elimination phase (10–30 h). Similar kinetics were observed after the typical 30 min infusion. F-ara-A elimination is predominantly renal, with approximately 60% of a bolus recovered in the urine within 24 h. Dose modification for impaired renal function is required.

In circulating leukemic cells, peak F-ara-ATP concentrations are reached at about 4 h after the start of fludarabine infusion. There is considerable inter-individual variation in F-ara-ATP elimination: in patients with ►chronic lymphocytic leukemia (CLL), the intracellular F-ara-ATP half-life ranged from a few hours to several days with a median of 15 h. Based on this data, daily administration schedules are commonly employed in clinical practice.

New oral preparations of fludarabine are available. Their bioavailability range between 55% (tablets) to 75% (liquid).

Mechanisms of Anti-Cancer Activity

The main elements of fludarabine action are illustrated in Fig. 1. Most classical anti-metabolite agents, such as cytarabine, are most active against rapidly dividing cells, where they are readily incorporated into newly synthesized DNA and cause chain termination. The initial expectation was that a similar mechanism of action would apply to fludarabine, and therefore the initial phase I-II trial was conducted in patients with acute myeloid leukemia or ►acute lymphocytic leukemia, diseases with rapidly proliferating cell populations. Although fludarabine showed some anti-leukemic activity, the doses explored (up to 150mg/m^2/day) were associated with prohibitive delayed neurotoxicity including cortical blindness and coma. Later, fludarabine was tested in patients with CLL at substantially lower doses (20–30 mg/m^2/day). CLL was traditionally considered be a disease of slowly proliferating, long-lived leukemic cells, and it was therefore somewhat unexpected when fludarabine, an anti-metabolite agent, was found to be highly effective. This clinical observation led to extensive re-consideration of possible mechanisms accounting for fludarabine activity in non-dividing cells, summarized in Fig. 1. Currently the dominant mechanism of fludarabine in indolent lymphoproliferative disorders is unknown. It is likely that most of the indolent lymphoproliferative disorders, including CLL, are more proliferative than initially thought. There is also evidence to suggest that quiescent lymphocytes are continually breaking and rejoining their DNA, and F-ara-ATP may inhibit DNA repair in this situation.

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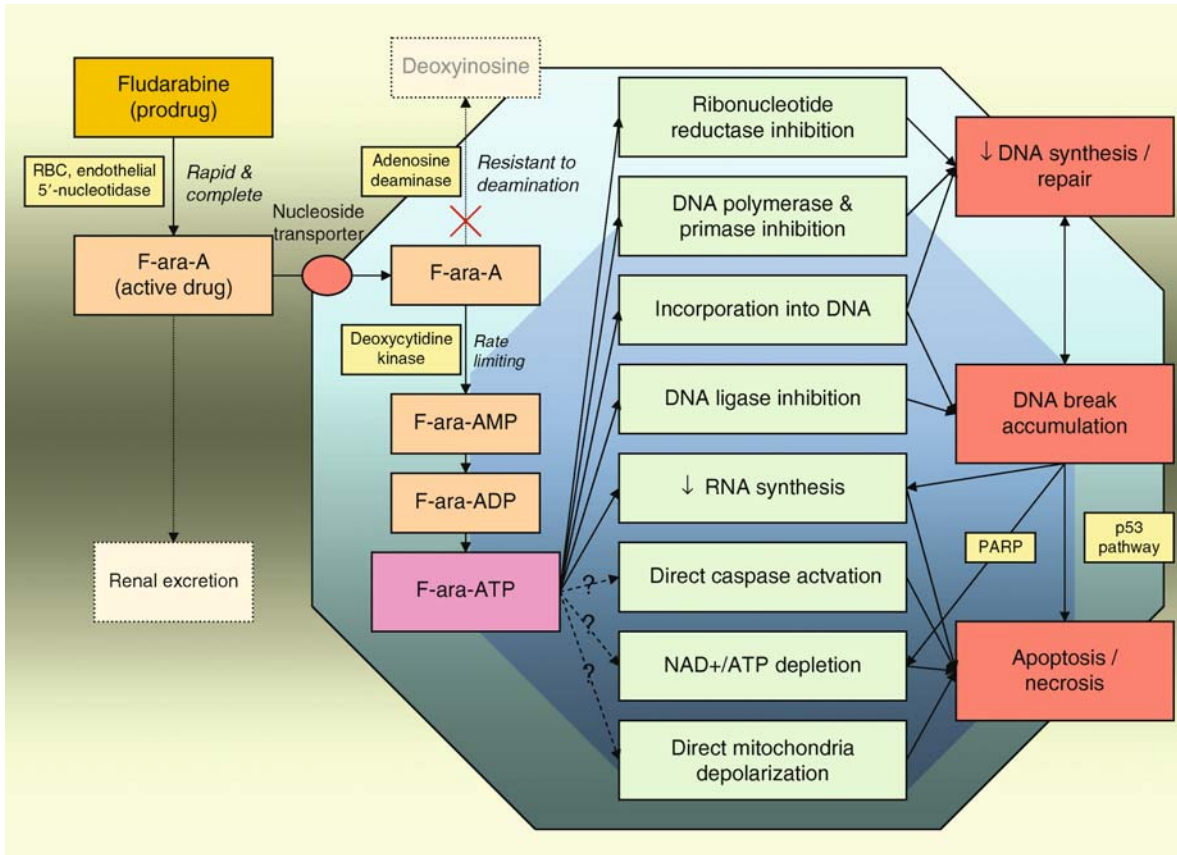

Fludarabine. Figure 1 Major components of Fludarabine metabolism and action. Differential toxicity in leukemia cells may be due to relative abundance of the high-affinity nucleoside transporter, efficient phosphorylation of nucleosides to triphosphate, and increased DNA turnover in tumour cells.

Lastly, it is possible that intracellular accumulation of dATP or its analogues (such as F-ara-ATP) activate ►apoptotic pathways directly without the requirement for DNA damage.

There is mounting clinical evidence that *in-vivo* activity of fludarabine depends strongly on the presence of an intact ►p53 pathway. Patients with mutation and/or deletion of the p53 gene respond less well to fludarabine, and any responses achieved are associated with substantially shorter remissions. ►Microarray experiments have shown that *in-vivo* exposure to fludarabine results in activation of genes associated with the p53 pathway.

Clinical Applications

Fludarabine is primarily used in the treatment of indolent lymphoid malignancies, particularly CLL. In CLL, three large randomized trials have established fludarabine as being the most active single agent, achieving more complete remissions and longer responses than ►chlorambucil or alkylator-based combination regimens. Subsequent *in-vitro* studies showed that the combinination of fludarabine and ►cyclophosphamide was synergistic, primarily due to fludarabine inhibition of the repair of cyclophosphamide-induced DNA damage. Translation of this combination of fludarabine and cyclophosphamide (FC) into the clinic has confirmed this preclinical synergy, with FC established as being more effective than fludarabine in three large randomized trials. More recently, the addition of ►rituximab (a monoclonal antibody directed against the CD20 molecule expressed on the majority of indolent lymphoid malignancies) to FC has resulted in a regimen capable of achieving complete response in 70% of patients treated.

Based on the favorable experience with CLL, fludarabine was also tested in patients with other indolent lymphoproliferative maligancies, particularly ►follicular lymphoma and related ►non-Hodgkin lymphomas. In follicular lymphoma, the combination of fludarabine and ►mitoxantrone achieves superior CR rates and remission durations to ►CHOP, an alkylator-based combination commonly employed in patients with histologically aggressive lymphomas.

Analogous to the experience with CLL, combinations with rituximab are being explored in patients with indolent B-lymphomas with promising activity.

In acute myeloid leukemia, fludarabine has inadequate activity at the maximally tolerated dose to be clinically useful as a single agent. However, Gandhi and Plunkett showed through a series of elegant pre-clinical and translational pharmacologic studies that exposure to fludarabine may increase the effectiveness of cytarabine (ara-C), the most important agent in acute myeloid leukemia therapy. Ara-C is converted to its active metabolite, ara-CTP, in leukemic blasts with the rate-limiting step being conversion to ara-CMP by deoxycytidine kinase. A major cellular target of F-ara-ATP is ►ribonucleotide reductase, a critical enzyme for sustaining intracellular levels of dNTPs, and reduction in intracellular dCTP may reduce the negative feedback on deoxycytidine kinase. Indeed, clinical studies have shown that exposure to fludarabine significantly increases leukemic blast accumulation of ara-CTP following administration of cytarabine. The combination of fludarabine and cytarabine is currently being explored in patients with acute myeloid leukemias associated with the t(8;21) or inversion 16 chromosomal abnormalities, the subtypes where dose-intensity of cytarabine are most crucial.

One of the side effects of fludarabine is lymphocyte depletion and associated predisposition to ►opportunistic infections. Indeed, CD4 and CD8 T-cells may be suppressed for 12 months or more following therapy with fludarabine. This immunosuppressive effect of fludarabine has been successfully exploited in allogeneic stem cell transplantation. Traditionally, large ("myeloablative") doses of chemotherapy ►myeloablative megatherapy and/or radiation were required in stem cell transplantation to suppress host immunity sufficiently to prevent graft rejection; such conditioning regimens are highly toxic, and limit the applicability of stem cell transplantation to younger and fitter patients. New approaches to stem cell transplantation using less intensive regimens ("non-myeloablative" conditioning) incorporate fludarabine with ►cyclophosphamide and/or other agents to achieve similar degrees of transient immunosuppression required for successful engraftment, with substantially less toxicity.

The major side-effect of fludarabine at currently clinically used doses (25 – 30 mg/m^2/day) is ►myelosuppression and pre-disposition to infection. Delayed neurotoxicity due to diffuse widespread demyelination, previously a major problem seen in early trials employing high doses of fludarabine (>100 mg/m^2 bolus, or >40 mg/m^2/day continuous infusion), is not encountered at currently used doses. In addition to infections resulting from neutropenia, the use of fludarabine has been associated with a number of "atypical" infections including *Pneumocystis jiroveci* (PCP) pneumonia, systemic listeriosis and reactivation of herpes family viruses. These are commonly attributed to the CD4 lymphopenia caused by fludarabine, but concurrent ►corticosteroid exposure and the effects of previous chemotherapy are also contributory, as these infections are very rarely seen with single-agent fludarabine.

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Fludarabine Phosphate

►Fludarabine

Fluid Phase Endocytosis

►Endocytosis

Fluorescein Angiography

Definition

Technique involving the systemic injection of sodium fluorescein which allow for the evaluation of the retinal circulation and provides further information on disorders of the choroid, retina, retinal pigment epithelium, and the optic nerve. The fluorescence pattern including staining, leakage, blockage, and pooling provide characteristic patterns that allow for diagnosis and monitoring of multiple disease conditions.

►Uveal Melanoma

Fluorescence

Definition

Fluorescence is secondary photon emission by molecules, which are excited to an energetically higher state

by primary photons. The emission of electromagnetic radiation by a substance, especially of visible light, by the absorption of incident radiation and persisting only as long as the stimulating radiation is continued.

►Photodynamic Therapy
►Flow Cytometry

Fluorescence Cystoscopy

Definition

A type of photodynamic diagnosis that uses endoscopy to investigate the urinary bladder via the urethra.

►Hypericin

Fluorescence Diagnostics

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Synonyms

Tissue spectroscopy; Laser-induced fluorescence diagnosis LIFD; Photodynamic diagnostics

Definition

Fluorescence diagnostics are procedures designed to detect neoplastic tissues based upon spectroscopic recognition of tissue-specific accumulations of natural or artificial fluorescing molecules (fluorophores).

Characteristics

Certain fluorescing dyes selectively accumulate in cancer cells and may, since fluorescing molecules are very rare in the biological environment, serve for sensitive detection of early cancerous lesions in human tissues without interfering with tissue integrity. Some of the dyes used for ►photodynamic therapy present a strong fluorescence, and since they localize to malignant tumors, early attempts were made to use these dyes for tumor diagnosis. Polyporphimer sodium (Photofrin®), for example, is a more effective purified fraction of the classical hematoporphyrinderivate (HpD), which has now obtained clinical approval for various indications in several countries. Polyporphimer sodium has to be applied intravenously and reaches a peak tumor:normal-tissue ratio 48–72 h after injection. The possible wavelengths for stimulation are 405 nm (Soret), 505, 525, 565, and 630 nm (Q-bands). The main advantage of this substance lies in the large clinical experience with its use for Photodynamic Therapy. The disadvantages are a long-lasting ►photosensitivity of the skin, still unsatisfactory tumor selectivity, and the relatively unfavorable longer activation wavelength of 630 nm. New dyes have entered the field, one of which is ►5-aminolevulinic acid (►ALA). This substance is unique in the group of photodiagnostic agents, since it is not fluorescent by itself and has to be transformed by a cellular enzymatic reaction to the natural cellular fluorophore protoporphyrin IX. Since ALA can be absorbed after topical application, it is possible to locally apply by endoscopic spraying. The fast photobleaching kinetics of ALA is a disadvantage, however, this substance has actually gained importance as a photodiagnostic drug.

The autofluorescence features of certain tumors may also allow sensitive detection of early cancers. Background subtracting technology has contributed to sensitivity and specificity of point measuring and imaging systems. However, to date, there are few organ sites where such diagnostic modality is truly established.

Application

Detection of Early Lung Cancer by Fluorescence Bronchoscopy

The bronchoscopic detection of severe dysplasia and early invasive ►lung cancer generally requires the expert analysis. Hematoporphyrin-mediated specific fluorescence was early investigated to improve diagnostic sensitivity during bronchoscopy. A group in Vancouver, trying to implement background subtracting techniques realized that the autofluorescence background differed significantly between tumorous and healthy mucosa. Malignant tumors are characterized by a reduction in elastin- and connective tissue-mediated autofluorescence. Thus, instead of using an exogenous tracer, they developed an imaging system based on autofluorescence characteristics only. This system was commercialized in the mean time under the name ►light-induced fluorescence endoscopy (LIFE) and is gaining rather widespread acceptance. In a recently published multicenter evaluation, the relative sensitivity of white light bronchoscopy enhanced by LIFE versus white light bronchoscopy alone was 6.3 for intraepithelial neoplastic lesions and still 2.7 for invasive cancers. The positive predictive value was 0.33. The authors conclude in that report that autofluorescence bronchoscopy, when used as an adjunct to standard white light bronchoscopy, enhances the ability of the bronchoscopist to localize small neoplastic lesions, especially intraepithelial lesions that

may have significant implication in the management of lung cancer in the future.

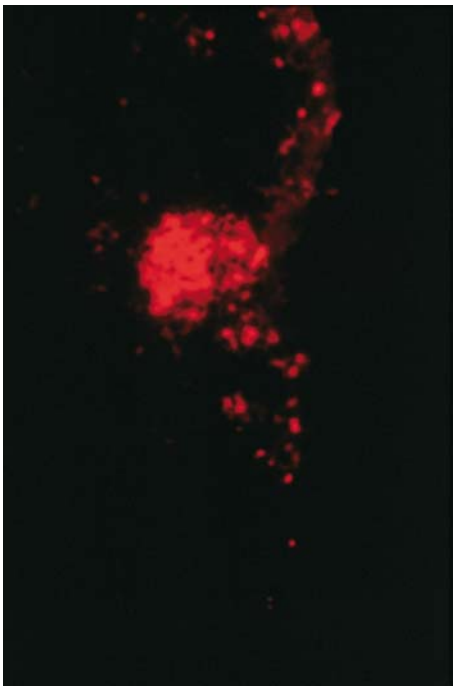
Urinary Bladder

If the success of autofluorescence detection originates from the bronchi, the success of ALA-induced fluorescence detection originates from the urinary bladder. While early attempts had been undertaken with hematoporphyrin fluorescence imaging, the topical use of ALA to induce protoporphyrin IX fluorescence has rapidly set a standard. In their latest assessment, a group from Germany reported in 208 consecutive patients with superficial bladder cancer a best estimate for the sensitivity of ALA-induced fluorescence endoscopy of 93.4% (95% confidence intervals 90–97.3) compared with a value of 46.7% (95% confidence intervals 39.4–54.3) for white light endoscopy. Some autofluorescence based approaches do not find the same acceptance as yet.

Other Organ Sites

Sensitive detection of premalignant conditions or early tumor stages may be considered as the general aim of fluorescence enhanced endoscopic approaches. Based on endoscopic accessibility and upon the presence of a risk group with sufficient incidence of dysplastic or cancerous lesions, research was directed towards patients with ►Barrett esophagus or with ►ulcerative colitis. However, both predisposing conditions are associated with an extended, inhomogeneous chronic inflammatory process that seems to interfere with the homogeneity of the background fluorescence. Neither autofluorescence nor ALA-enhanced fluorescence techniques have been reported to be a worthwhile adjunct to conventional diagnostics. The differentiation of small colonic polyps may be possible by autofluorescence; it is, however, questionable, whether the clinical benefit will justify the effort.

The procedures discussed so far have all dealt with the tissue surface. If microscopic tumor deposits are to be detected at a certain depth in the tissue, more sophisticated techniques are required to isolate the specific fluorescence signal from the abundant autofluorescence originating from the interpositioned normal tissue. Single point measurements may be advantageous over imaging techniques. Using a single point background subtracting measuring device with an excitation wavelength in the red (630 nm), Mang et al [1] were able to detect very small tumor deposits in the skin of breast cancer patients and lymphatic micrometastases (Fig. 1) in a rat model.



Fluorescence Diagnostics. Figure 1 Fluorescence micrograph of a lymph node micrometastases after systemic application of polyhematoporphyrin as photodiagnostic agent. The fluorescence intensity demonstrates the strong accumulation of the photodiagnostic agent within the metastatic deposit [image courtesy to TS Mang, Buffalo, USA].

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Fluorescence in Situ Hybridization

Definition

►FISH; Detection of specific chromosomal structures by hybridization of fluorescence dye-conjugated probes to DNA.

►Leukemia Diagnostics
►Minimal Residual Disease
►Chromosomal Translocations

Fluorescence Resonance Energy Transfer

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

Fluorochrome

Definition

Fluorescent compound used to label biomolecules (protein, nucleic acids) with a fluorescent label.

►Flow Cytometry
►FISH

Fluorodeoxyglucose-Positron Emission Tomography

Definition

FDG-PET; is a nuclear medicine imaging method that produces a three-dimensional functional map of the body. To perform this imaging study, patients are injected intravenously with a radioactive tracer that decays by emitting a positron. The most commonly used molecule is fluorodeoxyglucose (FDG). After allowing the tracer to concentrate in organs or tumors (1 h for FDG), patients are placed in the scanner and the images are obtained.

►Childhood Adrenocortical Carcinoma

Fluorouracil

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Synonyms

5-Fluorouracil; 5-FU

Definition

Is a drug primarily used in cancer treatment. It is considered a ►pyrimidine analog belonging to the class of drugs known as ►antimetabolites. This ►chemotherapy agent has been used against cancer for about 40 years and appears to have multiple sites of action. As a pyrimidine analog it is activated inside the cell to a cytotoxic metabolite that is incorporated into RNA and DNA. This action causes ►cell cycle arrest and, in many instances, induces ►apoptosis. Because its main actions involve DNA synthesis, this agent is considered an S-phase specific drug and is active during this phase of the cell cycle in actively dividing cells.

Characteristics

Fluorouracil continues to have wide use in the treatment of cancer. Research over the past several years has increased our understanding of how 5-FU inhibits cell growth and this has led to the development of new strategies that have increased the utility of this anti-cancer drug. However, drug resistance continues to limit the usefulness of this antimetabolite as well as limit the use of some of the newer antimetabolite derivatives of 5-FU.

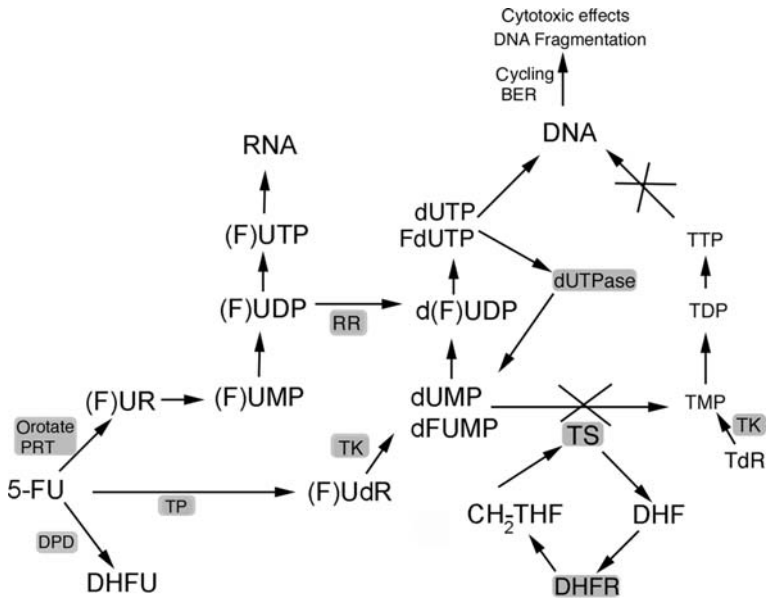
Mechanisms of Action

One of the key actions of 5-FU is the inhibition of thymidylate synthase (TS). Once in the cell 5-FU can be converted to fluorodeoxyuridine by ►thymidine phosphorylase (TP) and then to the monophosphate, FdUMP by thymidine kinase (Fig. 1). FdUMP in the presence of a reduced folate cofactor forms a stable covalent complex with TS, inhibiting activity and blocking de novo thymidylate (TMP) synthesis. Cytotoxic determinants of this mechanism include diminished levels of TMP and TTP. This results in the inhibition of DNA synthesis.

Other pathways that appear to contribute to the cytotoxic action of 5-FU include extensive incorporation of the ribonucleotide metabolite, FUTP into both cytoplasmic and nuclear RNA, disrupting normal RNA homeostasis. The contribution of the RNA pathway to cellular toxicity varies greatly and depends on cell type and the experimental setting. 5-FU can affect ►pre-mRNA processing such as ►polyadenylation as well as mRNA translation, ultimately leading to decreased expression of protein, including key proteins involved in metabolizing 5-FU.

Determinants of Cell Sensitivity to 5-FU

Cancer cells vary widely in their sensitivity to this chemotherapeutic agent, and numerous determinants appear to play a role in whether a cell is sensitive or resistant to 5-FU. Preclinical as well as clinical studies have demonstrated that TS expression is a key



Fluorouracil. Figure 1 Potential pathways for the metabolism of fluorouracil. RR, ribonucleotide reductase; TK, thymidine kinase; DHFR, dihydrofolate reductase; PRT, phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; dUTPase, dUTP nucleotidohydrolase; TS, thymidylate synthase; TP, thymidine phosphorylase.

determinant of 5-FU sensitivity. Quantitation of TS expression in tumors has revealed that low levels of TS correlate with improved response to 5-FU based therapy. Metabolic enzymes also play a role in 5-FU response rates. The catabolic enzyme, dihydropyrimidine dehydrogenase (DPD) initiates the degradation of 5-FU by converting the fluoropyrimidine to dihydrofluorouracil. High levels of DPD in colorectal tumors lead to elevated resistance to 5-FU. Other determinants that play a role include thymidine phosphorylase (TP), which converts 5-FU to an active metabolite known as FUdR (Fig. 1). The ►tumor suppressor ►p53, which regulates gene transcription of a number of key enzymes, is also known to alter cellular sensitivity to 5-FU. Research efforts continue in an attempt to further elucidate the mechanisms of action of 5-FU in the hope of improving its efficacy in the clinic. Results from a number of laboratories have uncovered an additional cytopathic mechanism that is downstream of TS inhibition. This mechanism contributes to 5-FU cytotoxicity through a self-defeating base-excision repair process. Along with the depletion of TTP pools (because of a decrease in de novo TMP synthesis) there is also an increase in dUMP, the normal substrate of TS. Metabolism of dUMP to the triphosphate, dUTP (as well as the conversion of FdUMP to FdUTP) results in elevated pools of these atypical precursors for DNA synthesis. Normally the enzyme, dUTP nucleotidohydrolase (dUTPase) which hydrolyzes dUTP to dUMP, prevents significant dUMP incorporation into DNA. However in the presence of FU metabolites, the efficiency of dUTPase appears to be diminished. It appears that FdUTP effectively ties up the enzyme, allowing dUTP pools to build up. Under these conditions there is a destructive cycle of dUMP (and FdUMP) incorporation into DNA, removal of uracil (and fluorouracil) by the base-excision repair enzyme, uracil-►DNA glycosylase and reincorporation of (F)dUMP, during the synthesis phase of ►DNA repair. The end-point is loss of DNA integrity and cell death. Studies have revealed that levels of dUTPase vary significantly between cell lines. These studies show that elevated dUTPase activity in the cell correlates with lower dUTP pools and diminished DNA fragmentation. Several investigators have also demonstrated that elevated dUTPase correlates with increased resistance to the fluoropyrimidine antimetabolite. Introduction of recombinant vectors, expressing dUTPase, into sensitive cells decreased sensitivity to FUdR (an active metabolite of 5-FU) as well as decreasing dUTP pools and DNA fragmentation. Utilizing ►small interfering RNA (siRNA) strategies, it was shown that suppression of dUTPase, resulted in a significant enhancement of dUTP pools, enhanced DNA fragmentation and a decrease in IC_{50} for FUdR. Clinical evidence points to an association between dUTPase levels, survival and response to 5-FU in colorectal cancer. Higher levels of dUTPase correlate with poorer response to 5-FU therapy. Published studies also provide evidence that dUTPase levels vary widely between individual cancer tumor specimens.

While considerable data has accumulated on the expression characteristics of dUTPase in a number of

cancer cell lines as well as cancer tissues, very little information exists on the expression characteristics of the DNA repair enzyme, uracil-DNA glycosylase (UDG) in cells in relation to 5-FU or its metabolites. It has been estimated that as many as 400 uracil residues could arise in the human genome per day as a result of spontaneous cytosine deamination occurring under physiological conditions. The mutagenic potential of uracil is particularly high because, unlike many other lesions, it can be efficiently used as a template. Like thymine, uracil can base pair with adenine and yield a stable duplex structure. During occurrences of cytosine deamination to uracil, C to T and G to A base substitutions arise. Subsequent rounds of DNA replication lead to transition mutations. Uracil-DNA glycosylases and related enzymes initiate base-excision repair pathways that function to correct misincorporation of dUMP and to correct deaminated cytosine residues in DNA. This process is necessary to prevent the accumulation of mutations, which may lead to genomic instability and pathologic outcomes such as cancer. There are two isoforms of UDG, one located in the nucleus, nUDG (also known as UNG2 or UDG1A) and one that is targeted for ►mitochondrial DNA repair, mUDG (also known as UNG1 or UDG1). Recent evidence indicates that both the nuclear and mitochondrial isoforms of UDG are modulated by 5-FU (and FUdR) treatment in certain cell lines but not in others. Significant modulation occurs at the transcriptional and at least for nUDG at a post-translational level. In normally cycling cells in culture, nUDG protein expression occurs in the G_1 phase of the cell cycle and that during the S to ►G_2 phase transition, nUDG is degraded by a ►ubiquitin-mediated process. Recent evidence also indicates that 5-FU mediates an atypical turnover of nUDG in late G_1/early S phase of the cell cycle. Additional data indicate that for cell lines that do not down regulate nUDG (in response to 5-FU), siRNA-mediated knockdown of nUDG increases resistance to the cytotoxic effects of the FUdR metabolite, increasing the IC_{50} by sixfold. A derived speculation from these findings is that cells can tolerate uracil in DNA (especially base-paired to adenine) much better than they can tolerate DNA strand breaks. So if the cell maintains uracil in DNA until pools of dUTP (and FdUTP) decrease, subsequent DNA repair would be much less deleterious. Therefore it can be inferred that in certain cell lines, damage induced by 5-FU instigates a ►checkpoint mechanism that culminates in the down regulation of UDG. This mechanism protects cells from the destructive, cyclic base-excision repair process created by elevated pools of dUTP. Conversely cells that lack this mechanism, and maintain elevated levels of UDG, are more sensitive to the effects of cyclic base-excision repair and show increased toxicity to fluoropyrimidines. Strategies directed at stabilizing nUDG in cancer cells may provide clinically significant approaches for novel drug development.

Toxic Side-Effects

Toxicity to fluorouracil manifests in several ways. Early symptoms include nausea and anorexia. This is followed by ►stomatitis and diarrhea. Mucosal ulcerations of the gastrointestinal tract may lead to fulminant diarrhea, shock and death. This is most prominent in patients receiving continuous infusion of 5-FU or a combination of 5-FU and ►leucovorin (LV). The major toxicities of IV bolus regimens is ►myelosuppression. Toxicities of infusional 5-FU are a function of dose and duration of exposure. DPD catalyzes the initial step in degrading 5-FU. In a small percentage of the population DPD activity is below average levels for the general population. Administration of 5-FU to these individuals can cause significant life-threatening toxicity. Unfortunately, in most cases this genetic predisposition is not uncovered until administration of 5-FU.

Capecitabine, an Orally Administered ►Prodrug of Fluorouracil

Statistical analyses have indicated that prolonged low-dose infusional 5-FU resulted in higher response rates with some improvement in survival when compared to bolus injection. Because of the labor-intensive nature of prolonged infusional therapy new modes of oral delivery of 5-FU have been formulated. ►Capecitabine is an orally administered fluoropyrimidine carbamate prodrug that is activated to 5-FU by a series of enzymes. Studies suggest that the latter activating enzymes in this series are higher in the tumor relative to normal tissue, rendering a certain degree of selective activation of the prodrug in tumor tissue.

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5-Fluorouracil (5-FU)

Definition

A cytotoxic agent; it blocks the ►methylation reaction of deoxyuridylic acid to thymidylic acid.

►Fluorouracil

Fluoxetine

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Synonyms

Prozac; Selective serotonin reuptake inhibitors SSRIs

Definition

Fluoxetine hydrochloride is a drug used in the clinic for the treatment of depression and for a broad spectrum of psychiatric disorders. Fluoxetine has also been shown to inhibit multidrug resistance extrusion pumps expressed in a variety of cancer cells.

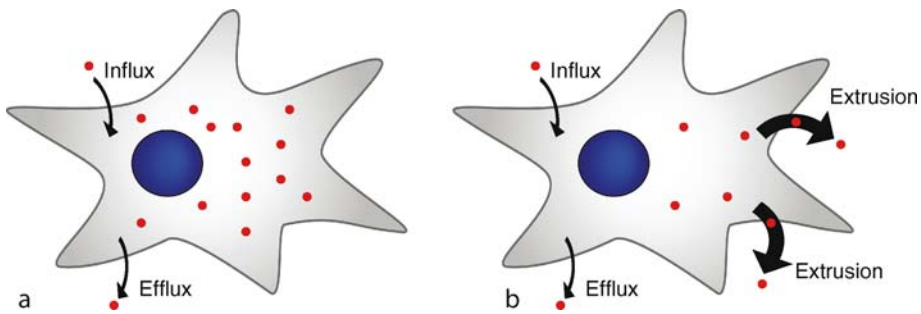
Characteristics

Fluoxetine hydrochloride is a drug used clinically in depression, and in a wide spectrum of psychiatric disorders. Fluoxetine was derived from an antihistamine (diphenhydramine), and found to inhibit reuptake of the neurotransmitter ►serotonin. Fluoxetine is also used (off-label) to treat many other conditions, including Attention-Deficit Hyperactivity Disorder (ADHD). The mechanism by which the drug operates is still unclear. Fluoxetine has been shown to be effective in cancer therapy in two areas; As an anticancer effector inducing apoptosis, as reported *in vitro* for ►Burkitt lymphoma (a type of ►B cell lymphoma), ►glioma and ►neuroblastoma cells, and as a non-selective inhibitor of the ►cancer multidrug resistance (MDR) extrusion pumps.

Multiple Drug Resistance

Multiple drug resistance (MDR) is one of the principal mechanisms by which chemotherapy treatment fails many cancer patients. It affects patients with a variety of blood cancers and solid tumors, including ►breast cancer, ►ovarian cancer, ►lung cancer, lower gastrointestinal tract, liver and ►brain tumors. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. In a drug-sensitive tumor cell the drug can accumulate to a sufficient level that culminates in cell death (Fig. 1a). The molecular basis of cancer drug resistance is complex and has been correlated to elevated enzymes that can neutralize chemotherapeutic drugs, but more often it is due to the overexpression of extrusion transporters from the ►ABC (ATP-Binding Cassette) Superfamily. These transporters actively pump chemotherapeutic drugs out of the cell, thus reducing the intracellular drug doses below lethal thresholds (Fig. 1b). Chemotherapy kills drug-sensitive cells, leaving behind a higher proportion of drug-resistant cells. As the tumor is recurred, chemotherapy may fail as a consequence of residual drug resistance cells dominating the tumor population.

Among the ►ABC transporters, the most investigated proteins are: ►P-glycoprotein (ABCB1); the multidrug resistance associated proteins (MRPs) of



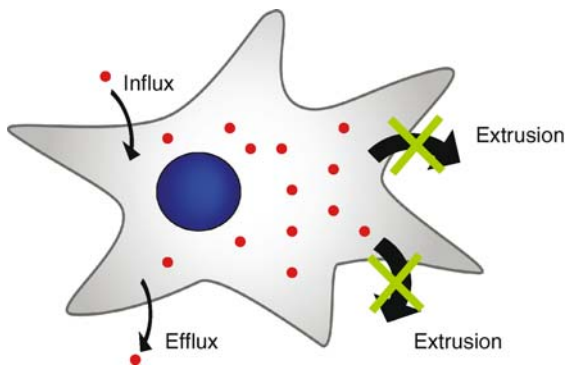
Fluoxetine. Figure 1 Illustration of drug efflux and accumulation in drug-sensitive and in drug-resistant tumor cells. (a) A drug-sensitive tumor cell. Drug molecules (red dots) diffuse across the cell membrane. The influx of the drug is superior to the drug efflux due to the direction of the drug's electrochemical gradient, allowing sufficient drug accumulation inside the cell. The cell nucleus is in blue. (b) A resistant tumor cell combines drug diffusion across the cell membrane with extrusion pumps that expel the drug out of the cell and thus reducing the drug accumulation inside the cell.

which the most studied is the MRP1 (ABCC1); and the breast cancer resistance protein (ABCG2). Those proteins share a similar function – they all expel chemotherapy from the cells, however their structure is different from one another.

Clinical MDR appears in two modes, inherent (intrinsic) and acquired. When the tumor is not responding to the chemotherapeutic treatment, even in the first cycle of administration, it can be defined as an inherent (intrinsic) resistance. The acquired form of drug resistance is defined when the tumor response to chemotherapy treatment is reduced over time (usually after several cycles of administration). Recent findings from cancer ►stem cell research may shed light into our understanding of the biology behind the different modes. High levels of ABC transporters in stem cells (particular ABCG2 in normal stem cells) and the recognition and retention of ABC transporters in cancer stem cells that intrinsically and consistently over-express ABC transporters contribute to the understanding of the phenomena, but still leave the tumor biologist with a vital question: How come some tumors are intrinsically resistance to chemotherapy while others remains sensitive? The role of cancer stem cells in cancer recurrence and in intrinsic vs. acquired MDR needs further studies. It is clear, however, that the impact of cancer stem cells on MDR will become a major factor in designing strategies to overcome drug resistance.

MDR Reversal by Chemosensitization

Drug resistance mediated by extrusion pumps is a problem that holds the key to its solution. In the most direct view, arresting the pump action (Fig. 2) should restore drug accumulation inside the MDR tumor cell to levels comparable to those of a drug sensitive tumor cell (Fig. 1a).



Fluoxetine. Figure 2 A chemosensitized drug-resistant tumor cell. By blocking the extrusion pumps, the drug is allowed to accumulate inside the cell, comparable to the case of a drug-sensitive tumor cell.

Several different names have been given to an agent capable of pump arrest: MDR reversal agent, MDR modulator, chemosensitizer, and pump inhibitor. The ongoing search for chemosensitizers that can apply in the clinic is into its third generation. First-generation chemosensitizers were found among drugs already approved for different indication, such as ►verapamil, cyclosporine A and ►progesterone. Today those drugs are used as *in vitro* benchmarks. They cannot be employed clinically due to dose related adverse effects, toxicity and, in some cases, solubility limitations.

The second and third generations of chemosensitizers were drawn from chemical derivatization of the first-generation molecules and from combinatorial chemistry designed predominantly against ABCB1 (P-glycoprotein). Examples include many compounds that are under development in pharmaceutical companies such as VX-710, XR9051, XR9576, MS-209, GF120918, LY335979, ONT-093, and PSC833 (Valspodar). Some of the compounds reached clinical trials; although they are less toxic and more potent then the first generation compounds, they are still prone to adverse effects, poor solubility, and unfavorable changes in ►pharmacokinetics of the anti-cancer drug. Not all of them fit to blocking different pump proteins. Most of them usually function with one or two pumps and thus are not "multi-pump" blockers. PSC833 (Valspodar) was one of the prominent candidates that reached clinical trials. Valspodar has been extensively investigated both pre-clinically and clinically and found to function as a single-pump blocker (ABCB1). Recently (2006), it has been reported that Valspodar did not improve treatment outcomes and increased the toxicity in a randomize phase III clinical trial in patients with recurring or refractory multiple myeloma. Valspodar treatment was in combination with the chemotherapeutic drugs vincristine, ►doxorubicin and the steroid dexamethasone, compared with treatments that exclude the chemosensitizer.

The diversity among pumps proteins, the heterogeneity of cells in a given tumor as well as variability between patients will probably require treatment with more than one chemosensitizer. Multi-pump blockers, will have distinct self-explanatory advances over single-pump ones. Given the above-discussed need for additional chemosensitizers, it is important to contemplate what it takes to qualify a candidate molecule as an effective chemosensitizer for which pump inhibition is a single or a major mechanism.

We propose that a candidate molecule should meet three *in vitro* criteria prior to investigating its potential *in vivo*. The *in vitro* criteria are based on both functional and mechanistic principles. The most prominent one is cell demise: treatment of a given MDR tumor cell line, with a combination of the candidate molecule

and a chemotherapeutic drug, should significantly enhance cell death, compared with similar treatment with drug alone.

Mechanistic criteria include drug efflux and drug accumulation. Incubating MDR cells with a chemotherapeutic drug, with or without the candidate chemosensitizer, should result in higher intracellular drug accumulation in the latter case. Efflux of a chemotherapeutic drug from MDR cells, preferable under unidirectional conditions, should be significantly faster in the absence of the chemosensitizer candidate, than when it is present. If a candidate chemosensitizer meets all those criteria, there is merit in expanding the evaluation in several directions: testing, in the same cell line, different chemotherapeutic drugs drawn from those known to be substrates of a given MDR pump; testing different additional MDR cell lines; testing MDR cell lines with their parent sensitive lines (upon availability). Comparative studies to known chemosensitizers used as benchmarks, can stringent the evaluation of the candidate molecule.

Fluoxetine as a Multi-pump Chemosensitizer

Fluoxetine belongs among the first-generation of chemosensitizers, namely drugs approved for non-cancer indications and found to act as MDR modulators. Yet, one critical factor sets fluoxetine apart from other first generation members and indicates it may merit a separate category, possibly fourth-generation chemosensitizers. Unlike first-generation chemosensitizers with their drawbacks mentioned above, fluoxetine exerts its ability to chemosensitize MDR cells at low safe doses, well below its human safety range.

In Vitro Studies

Fluoxetine was tested in ABCB1/MDR tumor cells, in ABCC1/MDR tumor cells and in drug-sensitive tumor cells. Test drugs were the chemotherapies: ►Adriamycin (doxorubicin), ►mitomycin C, ►paclitaxel and ►vinblastine. The multi-pump chemosensitizers verapamil and cyclosporine A, shown to affect the ABCB1 and ABCC1 pump proteins, were used as benchmarks. All three criteria were tested.

Cytotoxicity – two measures are useful for evaluation of whether a chemosensitizer candidate meets the *in vitro* cytotoxicity criterion: IC_{50}, the drug concentration that generates 50% inhibition of cell proliferation which, in the case of MDR, is measured in the absence and the presence of a fixed chemosensitizer concentration; RF, the fold change in drug sensitivity, is calculated from the ratio of IC_{50} in the absence to that in the presence of the candidate. Fluoxetine, verapamil, and cyclosporine A were kept at low doses, where they do not affect cell viability. Fluoxetine had no effect on the response of drug-sensitive cells to chemotherapeutic drugs. RF values obtained for human breast cancer, colon cancer, and ►leukemia cell lines as well as mouse leukemia cell line were between 0.9 and 1.1. For doxorubicin, mitomycin C, and paclitaxel, fluoxetine-induced RF values in human ►pancreas cancer cell line and in human brain tumor (both expressing ABCC1 pumps), were in the range of 10–80. The same experiment using the benchmarks yielded RF values of 2–19. In mouse ►melanoma cell lines, and in resistant forms of ►breast carcinoma and ►leukemia (from human source) the RF values with the same drugs span from 20 to 70, where the benchmarks yielded only a minor RF numbers between 2 and 5.

Drug Efflux – The efflux of drugs from drug-sensitive cells was quite slow. Complete depletion of intracellular ►doxorubicin from human breast carcinoma took more than 10 h and was unaffected by fluoxetine or the other benchmarks. Drug efflux from other MDR-expressing pumps (both ABCB1 and ABCC1 proteins) was found to be rather fast. For example, under unidirectional flux conditions it took between 0.5 and 2 h for complete depletion of such cells from intracellular drugs (►doxorubicin, ►vinblastine, ►paclitaxel, and ►mitomycin C). The benchmarks slowed down drug efflux, increasing the time span for complete depletion up to 3–5 h. Fluoxetine showed a remarkable effect by slowing down the efflux, thereby increasing the time for complete depletion, to the range of 8–12 h, close to that of drug-sensitive cells.

Drug Accumulation – Drug accumulation was measured with fluorescent substrate like Rhodamine-123 and actual drugs. The benchmarks were found to increase intracellular drug accumulation compared to cells exposed to drugs alone. This effect was found in inherent and acquired MDR cells, whether the pump-protein was ABCB1 or ABCC1. Fluoxetine increased drug accumulation by three- to sevenfold better than the benchmarks.

In Vivo Studies

Following the *in vitro* findings, fluoxetine was studied in several different mouse tumor models with doxorubicin as the test drug. A number of parameters were unique to these studies: fluoxetine dose was well below human safety limits; it was administrated orally in the drinking water; it was given continuously from tumor inoculation until termination of the experiment. Most of the efforts were on efficacy, but it was initially verified that fluoxetine did not alter the drug's pharmacokinetics, a problem encountered with some previous-generation chemosensitizers. Biodistribution was also tested showing that while fluoxetine generated a 12-fold increase in doxorubicin accumulation in ►lung tumors, it had no effect on drug accumulation in liver, spleen and kidneys of tumor-bearing mice. Several

tumor-bearing mice models have been tested to confirm efficacy. Those included inherent (intrinsically inherent) tumors expressing ABCB1 and separately ABCC1; models of lung metastasis as well as lung tumors (as primary source); human tumors inoculated to immunodeficiency mice and mouse tumors. In all cases fluoxetine attenuated tumor progression and in human tumors it even caused complete regression in 40% of the mice while the other mice from this group showed dramatically decreased tumor size and ►metastatic burden in lung tumors that was mirrored in increased survival rate. The survival rate of animals bearing tumors that was treated with a combination treatment of both chemotherapy and fluoxetine was two- to fourfold more than their controls (including the mice that got only chemotherapeutic drug) in different tumor-bearing mice.

Conclusions

The need to provide cancer patients with an arsenal of clinically approved chemosensitizers that will address the different MDR pump-proteins is still a remaining challenge. Despite the complexity and the multifactorial nature of cancer MDR, encouraging prospects do exist with respect to maturation of the third generation chemosensitizers into established clinical modalities.

Fluoxetine met all three *in vitro* criteria for acting as a chemosensitizer. It is important to emphasize that one criterion may be inconclusive. Fluoxetine also acted as a chemosensitizer *in vivo*. The data indicated that fluoxetine belongs to the category of multi-pump blockers, showing the capacity to reverse MDR generated by two major proteins ABCB1 (p-glycoprotein) and ABCC1 (MRP1), but further studies are required to determine whether MDR reversal is the only or the major mechanism by which fluoxetine works and whether fluoxetine can inhibit other pump proteins such as ABCG2 and other members of the MRP family.

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Fluvastatin

Definition

Member of the ►statins family.

FOBT

Definition

►Fecal Occult Blood Test

Focal Adhesion

Definition

Large complex of proteins that link the cytoskeletal elements of the cell to ►extracellular matrix via the integrin mediated biochemical signaling. Focal adhesions translate the biochemical signals into mechanical signals that are necessary for cell movement. Stable and elongated adhesion plaques (several μm^2 in area) that mediate strong adhesion to the ►extracellular matrix (ECM). Rho-dependent actomyosin contractility is required for maturation of focal adhesions from initial focal complex.

►Endostatin
►Focal Adhesion Kinase
►Exfoliation of Cells
►Tensional Homeostasis

Focal Adhesion Kinase

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Synonyms

FAK

Definition

The nonreceptor ►protein tyrosine kinase focal adhesion kinase (FAK) was originally isolated as a tyrosine-phosphorylated 125-kDa protein in ►v-Src-transformed

chicken embryo fibroblasts. FAK is a signal transducer of integrins and interact with certain soluble growth factors and chemokines. This kinase is involved in cellular processes like cell ►adhesion and spreading, ►migration, proliferation, and cell survival.

Characteristics

FAK consists of a central kinase domain, a large N-terminal ►FERM domain (protein 4.1, ezrin, radixin, and moesin homology), and of a large C-terminal ►FAT domain (focal adhesion targeting domain). Additionally, it comprises three proline-rich regions. The FERM-domain mediates interactions of FAK with growth factor receptors ►EGFR (epidermal growth factor receptor) and ►PDGF (platelet-derived growth factor receptor), which are often overexpressed in tumors and promote cell ►motility and ►invasion. The activation of the non receptor tyrosine kinase Etk (also called Bmx) by extracellular matrix proteins is regulated by binding to the FERM-domain of FAK. The FAT-domain mediates the binding of integrin-associated proteins such as paxillin and talin leading to the formation of focal contacts. An important C-terminal domain of FAK is the FAK-related nonkinase domain (►FRNK) which functions as a negative regulator of kinase activity. Overexpression of the FRNK segment that is separately expressed in certain cell types inhibits cell spreading and cell migration. The kinase domain induces adhesion-induced activation of FAK through phosphorylations of tyrosine residues at tyrosine residues Tyr397, Tyr576, and Tyr577. Additional phosphorylation sites are Tyr861 and Tyr925 in the C-terminal domain. Two proline-rich regions function as binding sites for ►Src-homology domain containing proteins.

FAK Phosphorylation and Regulation

Following attachment of the cell to extracellular matrix, subsequent integrin clustering results in rapid phosphorylation of FAK at Tyr397 and leads to the formation of phosphotyrosine docking sites for several classes of signaling molecules. So the ►phosphorylation of tyrosine residue 397 of FAK creates a high-affinity binding site for the ►SH2 domain of ►Src family tyrosine kinases and leads to the recruitment and activation of ►Src kinase and of p85 subunit of PI3-kinase, phospholipase C, and the adapter protein Grb7. The association of FAK and ►Src follows further phosphorylations of Tyr576, Tyr577, Tyr407, Tyr861, and Tyr925. A number of signaling proteins are phosphorylated and they are involved in adhesion, invasion, and survival signaling of tumor cells. Proline-rich recognition sites for ►SH3 domain-containing proteins provide binding motifs, for example ►Cas, an adaptor protein, or for ►GTPase activating proteins GRAF and ASAP1, which are involved in regulation of small GTP-binding proteins such as ►Rho and Arf proteins that play important roles in cytoskeletal reorganizations (Fig. 1).

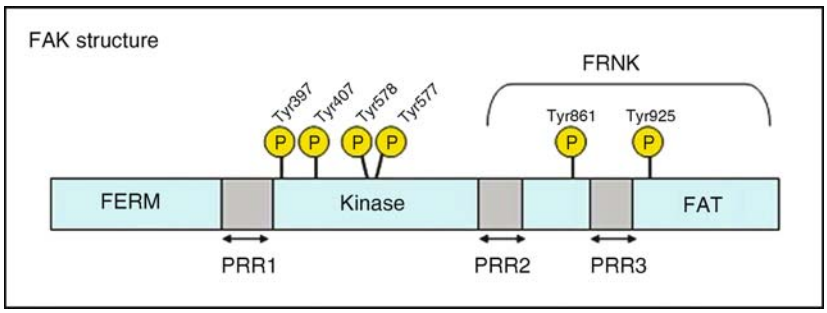
FAK functions in different downstream pathways and some are changed in tumors (Fig. 2).

Focal Contact

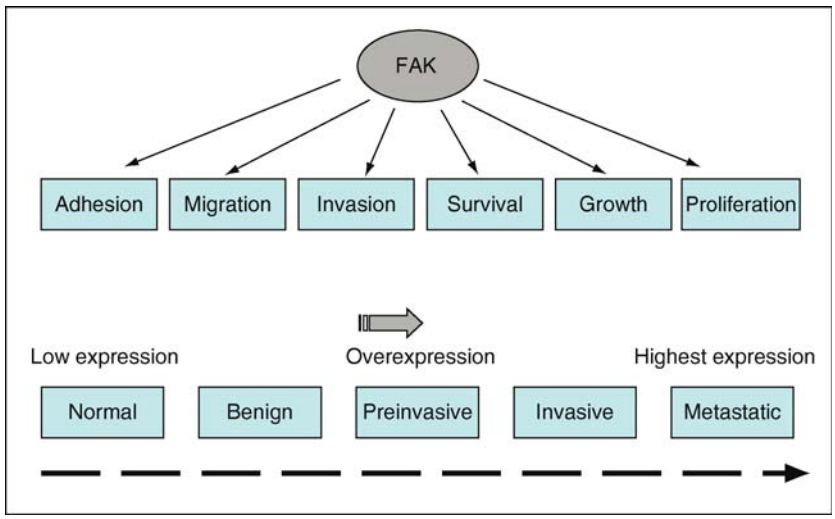
The actin cytoskeleton of cells interacts through integrins with the extracellular matrix. These sites of contact are named focal contacts. Focal contacts are composed of structural and regulatory proteins such as paxillin and talin, which recruit FAK and vinculin to focal contacts. α-Actinin, phosphorylated by FAK, binds to vinculin and crosslinks actomyosin stress fibers, which are further connected to the focal contacts. External signals are transduced into the cell interior that can generate additional cytoskeletal rearrangements.

Regulation of Focal Contacts

The regulation of assembly and disassembly of focal contacts is very important for the motility and migration



Focal Adhesion Kinase. Figure 1 The structure of focal adhesion kinase and tyrosine phosphorylation sites. The schematic illustration demonstrates the important N-terminal (FERM domain), central (kinase domain) and C-terminal domains (FAT and FRNK domains) of FAK with localization of their tyrosine (Tyr) phosphorylation sites and the three proline-rich regions (PRR1–3).



Focal Adhesion Kinase. Figure 2 Overview about the role of FAK signaling in different cellular processes and its overexpression in tumor progression.

of cells. The formation of strong or weak focal contacts is an important determinant whether a cell can migrate or not. It seems to be a cycle between binding of FAK to focal contacts and its release from these structures. The Src-mediated phosphorylation of FAK at Tyr925 leads to a SH2 binding site for ►GRB2 following Ras and ERK2 activation. The ►ERK activation results in the phosphorylation of FAK at Ser910, which subsequently leads to decreased paxillin binding and finally in release of FAK from focal contacts and to its turnover. In contrast, the Erk2-mediated phosphorylation of ►paxillin results in activation and binding of FAK to paxillin at new or growing focal adhesions.

Signaling Pathways in Cancer

Cell Migration

FAK is an important regulator of cell motility and cell migration, which are prominent processes in tumor ►progression and development of ►metastasis. To promote cell movements the FAK/Src complex phosphorylates Cas leading to recruitments of further ►SH2-containing proteins such as the adaptor protein ►Crk. The interaction of Crk with the ►DOCK180/ELMO complex results in ►Rac activation, a small ►Rho family GTPase. ►Rac1 is responsible for plasma membrane protrusions and formation of ►lamellipodia. Paxillin phosphorylation at tyrosines 31 and 118 also leads to Crk binding promoting Rac activation and paxillin is involved in modulation the dynamics of cell adhesion. The activation of RhoA promotes cell contractility and formation of stress fibers, contractile actin-myosin filaments, which are important for focal adhesion assembly. The regulation of RhoA and Rac1 activity is an important step in FAK signaling.

Invasion

To become invasive tumor cells have to degrade the ►basement membrane and migrate into the underlying ►stroma. The invasion of tumor cells through vessel walls and into the surrounding stroma requires the degradation of the ►extracellular matrix. FAK signaling is linked to the production and activity of matrix-degrading metalloproteinases (MMP), which represent important members of degradative proteases that can promote local tissue destruction and tumor-induced neoangiogenesis. For example, the activation of Rac1 and JNK (JUN N-terminal kinase) through the Src–Cas–Crk–DOCK180 complex results in increased MMP2 and MMP9 expression. The focal adhesion turnover is also facilitated by ►calpain-2-mediated cleavage of focal contacts. This protease is activated by ►EGF-induced ►MAPK (mitogen-activated protein kinase) and promotes the migration and invasion of tumor cells. There appears to be a correlation between FAK overexpression in tumors and their invasive and metastatic properties.

Cell Survival

Another role of FAK signaling is to promote the resistance to ►anoikis, a form of ►apoptosis resulting from loss of attachment to the extracellular matrix. The ability to survive in the absence of adhesion is an important characteristic of tumor cells to progress to a malignant phenotype. Several studies showed that inhibition of FAK signaling caused increased apoptosis. In addition, overexpression of FAK increases the adhesion independent growth of several tumor cell types. In contrast, analysis of nontumor cells did not found comparable effects on cell survival. The phosphorylation of tyrosine 397 and the kinase activity

of FAK are required for this process. Binding to the p85 subunit of PI3-kinase and following AKT kinase activation result in prevention of apoptosis. The suppression of anoikis is mediated by binding of FAK to the receptor-interacting protein (RIP), which consequently cannot bind to the dead receptor complex.

Growth and Proliferation

The adhesion-independent growth of tumor cells is an important step in tumor progression. For example, it has been shown that inhibition of FAK reduces the ability of breast cancer cells to grow in an adhesion-independent manner. Furthermore, it was demonstrated that FAK is overexpressed with increased tumor stages. This result was associated with increased rates of cell proliferation. Therefore, FAK may promote tumor growth through inhibition of apoptosis and acceleration of tumor cell proliferation.

Tumor Progression

A ►DMBA/TPA mouse skin carcinogenesis model was used, among others, to investigate the role of FAK in tumor ►progression. The results suggest that FAK expression and FAK-dependent cell signaling are increasingly enhanced with advanced stages of tumor progression and malignant progression. Normal epithelium and benign hyperplastic lesions have low FAK expression, whereas in preinvasive and invasive stages an overexpression of FAK was observed. In metastatic tumors the highest FAK expression was found. This overexpression seems to be a marker for an invasive and metastatic tumor phenotype.

Metastasis

►Metastasis is a process in which circulating tumor cells usually invade into potential host organs by breaking through vessel walls at the primary and secondary tumor site. In the blood flow the cells are exposed to shear forces requiring stabilization of their adhesive interactions under dynamic conditions in fluid flow. Similar to endothelial cells tumor cells can respond to external forces with increased integrin clustering and FAK activation. Exposed to this shear stress, motile cells can show enhanced FAK phosphorylation at the leading edge promoting a number of FAK-mediated tumor cell characteristics, such as migration and survival. This pathway appears to enable metastatic cells to resist shear forces within their target organs, to adhere and successfully colonize target organs.

FAK Overexpression

Many studies reported overexpression of FAK in various tumor types using different detection methods at mRNA, DNA, and protein level. Increased expression was found in patients' specimens of epithelial tumors of colon, liver, breast, ovary, stomach, thyroid, prostate, and oral cavity. Elevated FAK expression was also detected in many tumor-derived cell lines. These expression levels correlate with tumor progression.

Cancer Therapy

Once modified, the involvement of FAK in many cellular processes qualified FAK as a determinating and possibly rate-limiting pathway for tumor progression making this kinase an interesting target for anticancer therapy. In preclinical models, the inhibition of FAK signaling already demonstrated promising therapeutic effects by prevention of tumor cell migration and invasion resulting in higher survival rates. However, further preclinical investigations and clinical studies are necessary to establish FAK as a novel treatment strategy.

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Focal Complex

Definition

Initial and small (<1 μm^2 in area) adhesion plaques formed between integrin receptors of cells and the ►extracellular matrix (ECM). It contains ►integrins and actin binding proteins such as vinculin, talin, and paxillin, as well as actin.

►Tensional Homeostasis

Focal Contact

Definition

A type of anchoring cell junction forming a small region on the surface of a cell that is anchored to the

►extracellular matrix. Attachment is mediated by transmembrane proteins such as integrins, which are linked, through other proteins, to actin filaments.

►Migration

Folate

Definition

The salt of folic acid, a B vitamin containing the amino acid glutamate as part of its chemical structure.

FOLFIRI

Definition

Combination chemotherapy of ►irinotecan, ►leucovorin, and 5-flourouracil (►5-FU). The regimen is commonly used in the treatment of ►colorectal cancer.

►Erlotinib (Tarceva®)

FOLFOX

Definition

Combination chemotherapy of ►oxaliplatin, ►leucovorin, and 5-flourouracil (►5-FU). The regimen is commonly used in the treatment of ►colorectal cancer.

►Erlotinib (Tarceva®)

Folinic Acid

Definition

►Leucovorin

Follicle Stimulating Hormone

Definition

FSH; and luteinizing hormone (LH) are called ►gonadotropins because stimulate the gonads - in males, the testes, and in females, the ovaries. They are not necessary for life, but are essential for reproduction. These two hormones are secreted from cells in the anterior pituitary called gonadotrophs. Most gonadotrophs secrete only LH or FSH, but some appear to secrete both hormones.

Follicular Adenoma, Oncocytic Variant

►Hurthle Cell Adenoma and Carcinoma

Follicular Adenomas

►Follicular Thyroid Tumors

Follicular Carcinoma

►Follicular Thyroid Tumors

Follicular Carcinoma, Oncocytic Variant

►Hurthle Cell Adenoma and Carcinoma

Follicular Lymphoma

Definition

FL; is a common low grade ►B-cell lymphoma, which accounts for ca. 40% of B-cell lymphomas. Typically these lymphomas carry a ►translocation t(14;18), which brings the rearranged ►BCL-2 gene (chromosome 18) under the control of the immunoglobulin enhancer (chromosome 14). Tumors usually express B-cell markers

and surface immunoglobulin. Many patients do not require treatment for a long time. A characteristic feature is a spontaneous "waxing and waning" of the enlarged lymph nodes, which may reflect residual but inefficient attempts of the immune system to eliminate the cancer. FL is not considered curable, except for patients in very early stages of disease. Often this lymphoma transforms into a more aggressive form of lymphoma, diffuse large B-cell lymphoma, which carries a poor prognosis.

►B-cell Tumors

Follicular Thyroid Tumors

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Synonyms

Follicular adenomas; Papillary carcinoma; Follicular carcinoma; Anaplastic carcinomas

Definition

Are tumors derived from the thyrocyte, the cellular unit of ►thyroid follicle. These tumors include benign adenomas, papillary and follicular carcinomas, two entities considered as differentiated carcinomas, and undifferentiated or anaplastic carcinomas.

Characteristics

Clinical thyroid nodules, that are the consequence of abnormal localized growth of the thyroid tissue, are detected in 4–7% of the general population in a iodine sufficient area. The prevalence is higher in countries affected by moderate or severe iodine deficiency. Infraclinical nodules are even more common, depending on the techniques used for screening, and ultrasonography detects nodules in more than 50% of women older than 60 years. About 5% of thyroid nodules are malignant. The annual incidence of thyroid carcinoma, the most common malignancies of endocrine organs, ranges from 1.2 to 3.8 per 100,000, being twice as high in females as in males. The annual mortality rate ranges from 0.2 to 2.8 per 100,000.

Classification

The clinico-pathological classification of thyroid nodules distinguishes: non-neoplastic nodules, found in patients with either hyperplasia or inflammatory and ► autoimmune thyroid diseases, benign follicular thyroid adenomas, including non-functioning or cold nodules (80% of all nodules) and functioning or hot nodules, and thyroid carcinomas. Most thyroid nodules are benign lesions: a follicular adenoma is defined as a benign encapsulated tumor with evidence of follicular cell differentiation.

Less than 10% of cold nodules are malignant follicular thyroid tumors, i.e. thyroid carcinomas. Among these, 75% present evidence of follicular differentiation and thus are designated as differentiated cancers; they include two distinct histotypes, papillary carcinomas (PTC), characterized by distinctive nuclear features, and follicular carcinomas (FTC). Undifferentiated or anaplastic carcinomas are very rare and defined as highly malignant tumors with undifferentiated cells.

Etiology

Several risk factors for thyroid carcinoma have been identified from epidemiological studies. About 3–5% of Papillary thyroid carcinoma (►thyroid carcinogenesis) have a familial component history, and can occur in the context of rare familial syndromes (►familial polyposis coli, ►Cowden disease). Pre-existing benign thyroid diseases increase risk of developing a thyroid carcinoma. In areas with iodine deficiency compared to those with sufficient iodine intake, the prevalence of nodules is higher but the incidence of thyroid carcinoma is identical; follicular and anaplastic carcinomas are more frequent and papillary carcinoma is less frequent. The role of other dietary factors, such as fish consumption, cyanogens and smoking remains unclear.

Radiation exposure during childhood increases the risk of developing ►PTC later in life, probably by inducing chromosomal rearrangement. This can be related to an exposure to external radiation or to radioactive iodine, as a consequence of Chernobyl accident.

Diagnosis

Thyroid nodules are usually asymptomatic. Most of them are found by the patient himself or during a physical examination. The diagnostic evaluation of thyroid nodules essentially relies on serum TSH determination, ultrasonography which is useful for establishing the size of the lesion and its solid or cystic nature and of ► Fine-Needle Aspiration Cytology. In some cases, cytology cannot differentiate adenoma from carcinoma and the use of immunohistochemical markers contributed only marginally to better defining FTC.

Thyroid scan is performed only in patients with a low serum ►TSH level, to ascertain the presence of a hot nodule. Computerized tomography scans and magnetic resonance imaging are performed only in the rare patients with extensive disease. Thyroid carcinomas may release amounts of thyroglobulin in blood but high levels are also found in a variety of benign thyroid diseases and should not be measured.

Treatment

In the vast majority of cases, follicular adenomas are simply followed up with no therapy. Surgery is indicated only when compression or discomfort is present.

Initial treatment of thyroid carcinomas is based on total thyroidectomy with central lymph node dissection in patients with a PTC. Radioiodine is administered post operatively only in patients with either persistent disease or with poor prognostic indicators, such as age $\geq$ 45 years, poorly-differentiated carcinomas and extended disease.

All patients with thyroid cancer are treated post-operatively with levothyroxine.

Follow-up aims to detect persistent or recurrent disease at an early stage and is based on the combined use of thyroglobulin testing following recombinant human TSH stimulation and of neck ultrasonography. The majority of patients are cured, as demonstrated by the work-up performed at 1 year.

Genetics

The putative relationships between adenomas and carcinomas and the mechanisms of ►thyroid carcinogenesis are not clearly understood. Activating point mutations of the *RAS* gene are detected in 30–40% of benign and malignant follicular thyroid tumors. Chromosomal imbalances are also frequent in FTCs, particularly the PAX8-PPARγ1 rearrangement that is found in about 30% FTCs but also in some follicular adenomas. These observations suggest that FTC proceeds from at least two distinct oncogenic events, namely *RAS* mutations and PAX8-PPARγ1 rearrangement. Papillary thyroid carcinomas are characterized in 70% of cases by the presence of either a ►RET/PTC rearrangement, or an activating point mutation of *RAS* or *BRAF* genes. These genetic alterations are independent and nonoverlapping, and induce a constitutive activation of the MAP-kinase pathway. The higher frequency of RET/PTC rearrangements in radiation-associated PTC and also in some adenomas suggest that they may be directly induced by radiation exposure. Overexpression of several growth factors, such as ►MET, ►VEGF, ►FGF1 and 2 and ►IGF1, is believed to be secondary to other oncogenic events. Finally, inactivating mutations of the ►p53 gene are found only in ►anaplastic thyroid carcinomas.

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Folliculin

►Birt–Hogg–Dubé Syndrome

Food-derived Heterocyclic Amines

Definition

Are mutagens formed by the condensation of creatine with amino acids when protein rich foods like meats are cooked at high temperatures. These compounds have been implicated as risk factors for a number of human cancers and cause tumors in animal models.

►Arylamine *N*-Acetyltransferases

Food for Specified Health Use

►Nutraceuticals

FOR

►WWOX

Foreign Substances

►Xenobiotics

Forkhead

Definition

FKHR; ►FOX; ►Forkhead box M1.

Forkhead Box M1

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Synonyms

HFH-11B; Trident; WIN; FKHL-16; MPP2

Definition

FoxM1 is a transcription factor of the Forkhead family. The Forkhead family consists of about 50 transcription factors that share a conserved 100 amino acid (Forkhead or winged-helix) DNA binding domain, which is responsible for DNA binding via the consensus TAAACA site. The human FoxM1 gene contains ten exons, and it expressed as three alternatively spliced variants (a, b, c). FoxM1 is generally expressed in dividing cells and it is required for proper cell proliferation. Furthermore, FoxM1 is essential for the execution of ►mitosis, and it is overexpressed in most human cancers. FoxM1 may directly induce tumorigenesis because of its dominant role in cell proliferation. Therefore, FoxM1 is an attractive target for small molecule inhibition in cancer treatment.

Characteristics

FoxM1 expression correlates with the proliferative state of cells. It is expressed in all embryonic tissues and in proliferating cells of epithelial and mesenchymal origin. FoxM1 expression is not detectable in quiescent cells, but it is induced when these cells are stimulated to re-enter cell cycle. Along these lines, FoxM1 is also expressed in the majority of dividing mammalian cells, but its expression is turned off in terminally differentiated, non-dividing cells. Developmental studies suggest that FoxM1 may play a role in the development of nervous system and it is critical during organogenesis. FoxM1 is required for hepatoblast differentiation toward biliary epithelial cell lineages and for embryonic development of pulmonary vasculature. FoxM1 expression is also induced in proliferating cells following lung and liver injury suggesting that FoxM1 is essential for ►tissue regeneration and repair. Similarly, endothelial cell-restricted FoxM1-deficient mice exhibited a marked impairment in endothelial barrier repair and a significant increase in mortality following acute lung injury. Following vascular injury, FoxM1$^{-/-}$ lungs displayed diminished cell proliferation, increased expression of ►CDK inhibitor p27 and decreased expression of ►cyclin B1 and ►Cdc25. Deletion of FoxM1 in endothelial cells led to decreased expression of cell cycle promoting proteins and Cdk activities suggesting that FoxM1 plays a critical role in the restoration of endothelial barrier function following vascular injury.

FoxM1 is induced early during the G1 phase of the cell cycle and its expression continues through S phase and mitosis. Transcriptional activity of FoxM1 is dependent on its phosphorylation because cyclin-CDK1/2-dependent phosphorylation of FoxM1 during the cell cycle is required for the recruitment of the transcriptional co-activator ►p300/CBP. FoxM1 activation is also dependent on Ras-MAPK pathway, which regulates FoxM1 subcellular localization and transcriptional activity. Overall, it is appears that multiple FoxM1 phosphorylation events may regulate its activity during cell cycle progression. FoxM1 is required for the execution of the mitotic program because FoxM1-depleted cells fail to progress beyond the prophase stage of mitosis. FoxM1 transcriptionally activates ►cyclin B, ►survivin, ►Aurora B kinase, ►Cdc25 phosphatase and Plk1, all of which are implicated in mitosis. In addition, FoxM1 induces cell cycle progression through transcriptional induction of Skp2 and Cks1 genes (specificity subunits of Skp1-Cullin1-F-box ubiquitin ligase complex) that leads to the degradation of cell cycle/►cyclin-dependent kinase inhibitors p21^{WAF1} and p27^{KIP1}. FoxM1c also transactivates ►c-myc, c-*fos*, ►*hsp70* and histone *H2B/a* genes all of which are involved in proliferation and tumorigenesis.

FoxM1 is one of the most significantly upregulated genes in human solid tumors. FoxM1 is overexpressed in non-small cell lung carcinomas, anaplastic astrocytomas, glioblastomas, basal cell carcinomas, hepatocellular carcinomas and intrahepatic cholangiocarcinomas. Furthermore, FoxM1 is overexpressed in primary breast tumors, and its depletion by RNA interference (RNAi) in these cancer cells leads to mitotic catastrophe. Since FoxM1 expression is specifically increased in breast carcinomas relative to normal tissue FoxM1 could be involved in promoting cellular transformation of breast epithelial cells. Approximately 60–80% of all breast cancers overexpress estrogen receptor (ER) and ER plays a key role in breast cancer development. It was shown that FoxM1 is a transcriptional regulator of ER expression in breast cancer cells and silencing of FoxM1 expression in breast cancer cells using RNAi resulted in strong inhibition of ER expression. FoxM1 expression is essential for the

development of liver cancer because it was demonstrated that FoxM1$^{-/-}$ mice livers are highly resistant to developing hepatocellular carcinomas in response to a Diethylnitrosamine (DEN)/Phenobarbital (PB) liver tumor induction protocol. Inhibition of FoxM1 transcriptional activity by a cell-penetrating peptide containing amino acids 24-46 of FoxM1►p19^{ARF} also reduced anchorage-independent cell growth and inhibited development of liver cancer in DEN/PB mouse models.

FoxM1 also contributes to cellular transformation by human papillomavirus HPV-16 by interaction with oncogenic HPV-E7 protein. This interaction increases the transforming activity of HPV-E7 and enhances the transcriptional activity of FoxM1. Increased levels of FoxM1 accelerated prostate cancer development and progression in mouse models. Knockdown of FoxM1 by RNAi in several prostate cancer cell lines led to a significant reduction in cell proliferation and anchorage-independent cell growth in soft agar. FoxM1 was significantly overexpressed in basal cell carcinomas (BCCs), but not in ►squamous cell carcinomas. Constitutive expression of the transcription factor ►Gli1, the known target of ►Sonic Hedgehog (Shh) signaling, led to induction of FoxM1 mRNA expression, and coexpression of Gli1 and FoxM1 proteins was observed within the same BCC tumor sample. Since Shh plays a key role in the development of BCC, FoxM1 may be a putative target of Shh pathway. Similarly, in gliomas where Shh is also implicated in tumorigenesis FoxM1 protein has been overexpressed. The levels of FoxM1 expression in human glioma tissues was directly correlated with the glioma grade and inversely correlated with patient survival. Enforced FoxM1 expression in non-tumorigenic glioma cell lines caused them to form xenograft tumors in nude mice, while inhibition of FoxM1 expression by RNAi suppressed their anchorage-independent growth in vitro and tumorigenicity in vivo.

Since FoxM1 is overexpressed in many types of cancer where it may be required for proliferation of cancer cells, it is an attractive target for drug development. To obtain a screening system for the identification of small molecule inhibitors of FoxM1 transcriptional activity in a high-throughput fashion, we developed a cell line that stably expresses doxycycline/tetracycline-inducible FoxM1-GFP, firefly luciferase under the control of multiple FoxM1 response elements, and a control renilla luciferase under the CMV promoter. We used this cell line to screen for FoxM1 transcriptional inhibitors in libraries of small molecules (Challenge Set and Diversity Set) obtained from NCI. We selected only those compounds that specifically inhibit FoxM1 and we identified a well-known thiazole group antibiotic ►siomycin A (NSC-285116) as an inhibitor of the transcriptional activity of exogenous and endogenous FoxM1. In addition, using quantitative real-time RT-PCR, we found that the mRNA levels of the transcriptional targets of FoxM1, such as ►Cdc25, ►survivin and CENPB were sufficiently reduced after ►siomycin A treatment. Furthermore, Siomycin A may antagonize FoxM1 function by at least two distinct mechanisms – one by blocking its phosphorylation, thereby leading to its reduced transactivation ability, and the other by down-regulating its mRNA and protein levels. Using clonogenic assay, siomycin A may act as an effective inhibitor of FoxM1-based cellular transformation. In addition, siomycin A induced potent apoptosis in human breast and liver cancer cell lines, that correlated with inhibition of FoxM1 expression. Now, it is necessary to determine if inhibition of FoxM1 by siomycin A leads specifically to apoptosis of cancer cells, and to test this drug in animal models of human cancer. FoxM1 inhibitors such as siomycin A or other small molecules may have potential as anticancer drugs.

In summary, FoxM1 is often overexpressed in many types of human tumors and it appears that FoxM1 expression is critical in the process of cellular transformation. FoxM1 function is inhibited by several tumor suppressors, such as ►p19 ARF, pRb, p16 and p53 and may be activated by multiple oncogenic signaling pathways suggesting that FoxM1 may be classified as a proto-oncogene. However, it remains to be elucidated if overexpression of FoxM1 in cancer cells is a result of cell transformation that leads to increased proliferation and FoxM1 expression, or whether FoxM1 directly collaborates with other oncogenes to induce cell transformation. In any case, if cancer cells require high levels of FoxM1 expression for their survival, then FoxM1 appears to be an attractive target for anticancer therapy. Identification of the FoxM1 inhibitor siomycin A suggests that targeting of FoxM1 by small molecules is a feasible alternative and in a short time this approach may help to identify novel anticancer compounds for clinical trials.

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Formalin-fixed Paraffin-embedded Tissue

Definition

The usual process needed for a tissue to be studied under the microscope in ►Pathology. Because of this process most high molecular weight DNA is destroyed as well as almost all RNA molecules. Therefore it is a good source of small-sized DNA molecules, and an inconsistent way to get RNA.

Formation of New Blood Vessels

►Angiogenesis

Forward Chemical Genetics

Definition

An approach to studying biology in which a compound that induces a particular biological phenotype is first detected. Subsequently, the affected target and its relationship to the phenotype are identified.

►Small Molecule Screens

FOS

Definition

v-fos FBJ murine osteosarcoma viral oncogene homolog, is a leucine-zipper protein of 380 amino acids and 40 kD. The human FOS gene locus maps at 14q24.3 and the mouse fos gene locus at chromosome 12 (40.00 cM). FOS is a protooncogene and c-Fos is a nuclear phosphoprotein that forms a tight but non-covalently linked complex with c-Jun to the ►AP-1 transcription factor. c-Fos expression increases rapidly upon growth factor stimulation or wounding of cultured cells.

Fossil Tree

►Ginkgo Biloba

F

FOX

Definition

Refers to Forkhead box genes; Encode a family of ►transcription factors that play important roles in regulating the expression of genes involved in cell growth, proliferation, differentiation, and longevity. Many FOX proteins are important to embryonic development and some are implicated in tumorigenesis by their expression level.

►Forkhead box M1

FOXC2

Definition

A forkhead family transcription factor that is expressed in human lymphatic vessels. Studies in mice indicate this transcription factor regulates lymphatic capillary and valve development as deletions of this gene cause aberrant lymphatic vessel development that resembles the human disease ►lymphedema-distichiasis.

►Lymphangiogenesis

FOXO1A

Definition

Is the acronym for forkhead box O1A (►rhabdomyosarcoma), also known as FKHR.

►Beckwith-Wiedemann Syndrome Associated Childhood Tumors

FOXO 3A

Definition
Forkhead box O3A, a member of the forkhead family of transcription factors.

►PUMA

FPC

►Familial polyposis coli
►APC Gene in Familial Adenomatous Polyposis

FRA3B

Definition
The fragile site or region at chromosome region 3p14.2. The constitutive fragile site at 3p14.2 (FRA3B) is the most frequently activated constitutive fragile site, with up to 50% of normal lymphocyte derived metaphases showing the characteristic gaps or decondensation at 3p14.2 after ►aphidicolin treatment. Common or constitutive fragile sites are now known to be fragile regions; i.e. the position of the gaps can occur at many different sites within a fragile region, which may extend up to a megabase in size.

►FHIT
►Fragile Sites

FRA16D

Definition
The fragile site or region at chromosome region 16q23.3.

►WWOX
►Fragile Sites

Fragile Histidine Triad

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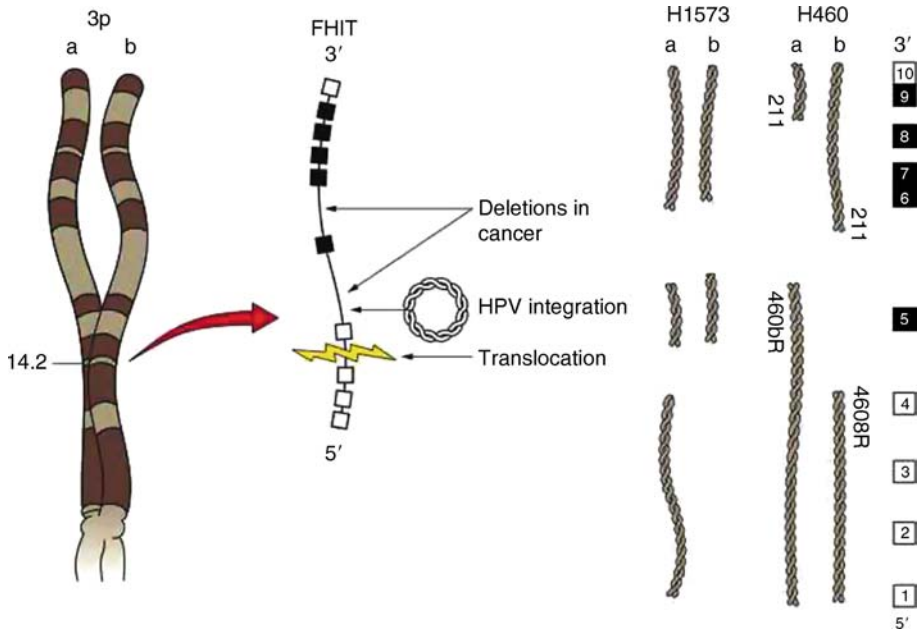
Synonyms
FHIT

Definition
The Fragile Histidine Triad (*FHIT*) gene, at human chromosome region 3p14.2 encompasses a familial ►clear cell renal cancer chromosome translocation breakpoint and the most active chromosomal constitutive ►fragile site, ►*FRA3B*. The gene is inactivated in a large fraction of most types of human cancers, through intragenic ►hemizygous or ►homozygous deletions, ►hypermethylation of regulatory region ►CpG islands, or a combination of these mechanisms, probably as a result of ►DNA damage to the *FRA3B* fragile region.

Characteristics
The Gene
The *FHIT* genomic locus spans >1.6 Mb of DNA, while the *FHIT* cDNA is 1.1 kb. Fig. 1 illustrates the structure of this ►tumor suppressor gene and the positions of fragile region landmarks such as the familial renal cancer translocation and the ►human papillomavirus integration site. The right side of Fig. 1 illustrates the types of biallelic deletions observed in this locus in lung cancer and other cancer-derived cell lines. Sequencing of the gene and isolation of cancer cell deletion endpoints revealed that many deletion endpoints are within recombined LINE-1 repeats, suggesting that ►carcinogen damage to the fragile region is repaired through recombination between LINE-1 (►LINE element) sequences flanking the damaged region, with concomitant loss of portions of the *FHIT* gene. The murine *Fhit* locus is also fragile and the mouse Fhit protein is more than 90% identical to human Fhit. One *Fhit* allele was inactivated in murine ►embryonic stem (ES cells) to create *Fhit* ►knockout mice in which the gene is functionally inactive. Mice heterozygous or homozygous for the mutant *Fhit* locus are fertile, long-lived and exquisitely sensitive to tumor induction by carcinogens.

The Protein
The amino acid coding region begins in exon 5, ends in exon 9 (Fig. 1) and encodes a protein of 147 amino acids.

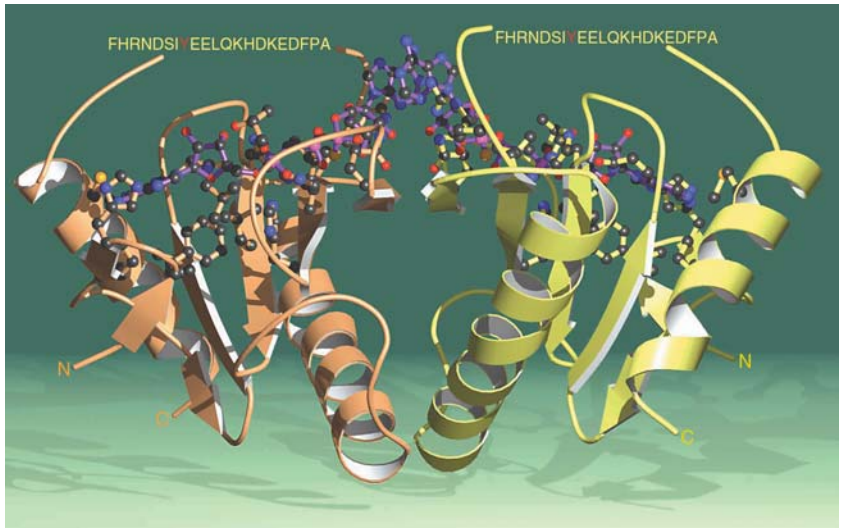


Fragile Histidine Triad. Figure 1 The *FHIT* gene at chromosome region 3p14.2. The gene encompasses *FRA3B* where numerous chromosomal alterations are observed in cancer cells. This locus is highly susceptible in all individuals to carcinogen-induced breakage. As a consequence chromosomal alterations including deletions, insertions and translocations occur. The chromosome alterations frequently lead to loss of parts of the Fhit protein-coding sequence and the consequent loss of Fhit protein expression (on the right are examples of alleles in lung cancer-derived cells). The *FHIT* gene is hemi- or homozygously deleted in the majority of lung, stomach, esophageal and kidney cancers and in a large fraction of many other cancers; point mutations are almost never observed. Fhit protein is absent or reduced in lung, stomach, kidney and other cancers, consistent with a role as a tumor suppressor and fulfilling the prediction that fragile site alterations contribute to the neoplastic process.

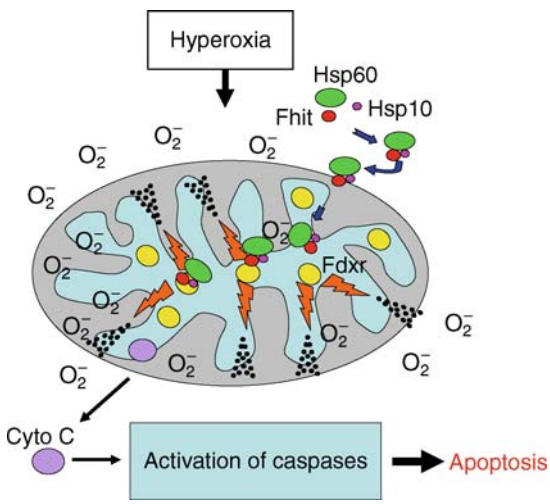
The Fhit protein is a member of a large Histidine Triad (HIT) gene family of nucleotide binding proteins with four conserved histidine residues, three of which occur in a histidine triad, HxHxHxx (where "x" is hydrophobic). There are more than thirty HIT genes in species representing all branches of life. Fhit and its orthologs form a closely related subfamily of HIT proteins, found only in eukaryotes, that bind and hydrolyze dinucleoside oligophosphates, especially diadenosine tri- or tetraphosphates (Ap3A, Ap4A), to produce a nucleoside monophosphate plus a nucleoside di- or tri-phosphate. Site-directed mutagenesis of the conserved Fhit histidine residues to asparagines results in decreased enzymatic activity for each mutation, with the largest change occurring with replacement of the central histidine, H96. Thus, H96 is essential for hydrolysis of the diadenosine tri- or tetra-phosphate but the Fhit H96N mutant still acts as a tumor suppressor, in accord with the proposal that the Fhit-substrate complex sends the tumor suppression signal. Fhit is constitutively dimeric, existing as a highly curved, ten-stranded antiparallel-sheet, which presents nucleotides on the top surface. Amino acids 107-127, following the catalytic site, form an unstructured loop (Fig. 2) within the loop, amino acid Y114 can be phosphorylated by ►Src family kinases and FhitY114 mutant proteins lose apoptotic and tumor suppressor functions. Protein cross-linking and ►proteomics methods were used to characterize a Fhit protein complex involved in triggering Fhit-mediated apoptosis. This complex includes ►Hsp60 and Hsp10 that mediate Fhit import into mitochondria, where it interacts with ►ferredoxin reductase (Fdxr), responsible for transferring electrons from NADPH to cytochrome P450 via ferredoxin (Fig. 3). Fhit restoration significantly increases the production of ►reactive oxygen species (ROS) and the rate of ►apoptosis of lung cancer cells under oxidative stress conditions; conversely, Fhit-negative cells escape apoptosis, carrying serious oxidative DNA damage that may contribute to an increased mutation burden.

Bioactivity

The biological functions of Fhit have been studied in human cancer cell lines stably over-expressing exogenous Fhit. Tumorigenicity was suppressed in gastric, lung, renal and breast cancer lines. Similarly, cancer cell lines infected with adenovirus carrying the *FHIT* gene were killed by apoptosis. Fhit sends signals for apoptosis



Fragile Histidine Triad. Figure 2 Architecture of Fhit–substrate complex. View of the Fhit dimer with bound substrate. Monomers of Fhit are yellow and copper; substrate molecules are purple. Atoms are colored as follows: carbon, black; nitrogen, blue; oxygen, red; phosphorus, magenta; sulfur, orange. The amino acid sequence of the unstructured loop is also shown. (adapted from Pace et al. (1998) Proc Natl Acad Sci USA 95:5484–5489).



Fragile Histidine Triad. Figure 3 Model depicting proposed Fhit signal pathway induced by hyperoxia. Fhit protein binds the Hsp/Hsp10 complex in the cytosol, and is carried to the mitochondria; in the mitochondria Fhit interacts with Fdxr protein and in the presence of H_2O_2, which creates hyperoxic conditions, ROS is generated, leading to release of cytochrome C, followed by apoptosis. Reduced Fhit leads to reduced ROS and reduced apoptosis.

in response to various types of cellular stress: in response to DNA damaging agents such as ►UVC light and ►mitomycin C, Fhit-deficient cells escape apoptosis and accumulate mutations; over-expression of wild-type Fhit leads to reduced expression of activated ►Akt and ►Survivin, reduced expression of cyclophilin A, and reduced expression of phospho ►Chk1; Fhit, when over-expressed, enters mitochondria, interacts with Fdxr protein and causes an increase in Fdxr protein level, associated with generation of ROS and followed by programmed cell death (Fig. 3). Fhit does not affect the *FDXR* transcriptional level but may affect stability of the protein. Fhit localizes in both cytosol (where it interacts with Hsp60 and Hsp10) and mitochondria where it interacts with main mitochondria chain respiratory proteins. Interestingly, Fhit restoration significantly increases the production of ROS in lung and colon cancer-derived cells in response to stressful treatments. Study of Fhit deficient mouse tissue-derived and human cancer-derived cells *in vitro* has led to several important conclusions: Fhit is not a cell cycle regulated gene; repair protein-deficient cancers are more likely to be Fhit-deficient; Fhit-deficient cells show enhanced resistance to UVC, ►mitomycin C and ►ionizing radiation-induced cell killing, possibly due to strong activation of the ►*ATR/CHK1* pathway following DNA damage; Fhit-deficient cells show higher efficiency of homologous recombination repair, a major double-strand break repair pathway. The DNA damage-susceptible *FRA3B/FHIT* chromosome fragile region paradoxically encodes a protein that is necessary for protecting cells from accumulation of DNA damage, through modulation of ►checkpoint proteins and inactivation of Fhit contributes to accumulation of abnormal checkpoint phenotypes in cancer development.

Clinical Relevance

More than 640 reports describe the involvement of Fhit loss in development in nearly every type of cancer. Important findings are that: (i) large fractions of kidney, lung, gastric, colon, pancreatic, breast, cervical and other cancers show absence of expression of Fhit protein, suggesting that its inactivation is critical in the progression of many human cancers, especially cancers of organs directly exposed to environmental carcinogenic agents; (ii) loss of Fhit expression in cancers can result from hemi or homozygous deletion or translocation within the *FHIT* gene or hypermethylation of CpG islands in the promoter region or a combination of these mechanisms; (iii) Fhit loss is a very early step in development of some types of cancers and can be associated with prognostic features or survival; (iv) loss of Fhit protein is associated with loss of other fragile genes in breast cancer; (v) an ►SNP within the *FHIT* locus is associated with development of ►prostate cancer in some families with increased prostate cancer susceptibility; (vi) *FHIT* restoration in a number of malignant cell lines triggers ►apoptosis *in vitro* and in preclinical models; (vii) *Fhit* knockout mouse strains show approximately tenfold enhanced susceptibility to carcinogen-induced tumors and have been useful in development of tumor models for prevention and therapeutic studies; oral Fhit gene therapy can prevent and reverse tumor growth in Fhit-deficient mice; (viii) Fhit enhances ROS-related apoptotic effects of chemotherapeutic agents.

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Fragile Sites

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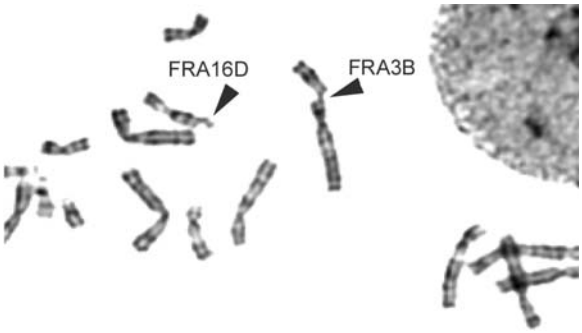
Definition

Fragile sites are specific chromosomal loci that are especially prone to forming gaps and break as seen on ►metaphase chromosomes under conditions of replication stress.



Fragile Sites. Figure 1 Examples of the common fragile sites FRA3B (3p14.2) and FRA16D (16q23) on human chromosomes.

Characteristics

Expressed fragile sites are seen as gaps and breaks on metaphase chromosome preparations of cultured cells. Fragile sites are not usually expressed on chromosomes under normal replication conditions, but rather become detectable under conditions that partially inhibit DNA replication. The formation of gaps and breaks at fragile sites is thought to represent an underlying unusual aspect of chromosome structure or function.

Categories of Fragile Site

Fragile sites are broadly classified into two main categories, rare and common based on their population frequency, pattern of inheritance and method of induction.

Rare Fragile Sites

►Rare fragile sites are seen in a small proportion of individuals and are inherited in a Mendelian manner. Some, such as FRAXA, are associated with human genetic disorders, and their study led to the identification of nucleotide-repeat expansion as a frequent mutational mechanism in humans. There are currently 31 known rare fragile sites. Nine of these have been cloned: FRAXA (Xq27.3), FRAXE (Xq28), FRAXF (Xq28), FRA10A (10q23.3), FRA10B (10q25.2), FRA11B (11q23.3), FRA12A (12q13.1), FRA16A (16q13.11), and FRA16B (16q22.1).

The major group of rare fragile sites is the folate-sensitive group, associated with CGG-repeat expansion. This group includes FRAXA, in the *FMR1* gene, which is responsible for the ►fragile-X syndrome, and FRAXE in the *FMR2* gene, which is associated with nonspecific mental retardation. In addition, an autosomal folate-sensitive fragile site, FRA12A, in the *DIP2B* gene has recently been associated with mental retardation. Other non-folate sensitive rare

fragile sites, characterized by expanded AT-rich minisatellite repeats, are induced by ►bromodeoxyuridine or ►distamycin-A.

Common Fragile Sites

►Common fragile sites (CFSs), comprise the largest class of fragile sites. These sites are found in all individuals and are considered to be a normal component of chromosome structure. Unlike rare fragile sites, CFSs are not caused by expansion of ►trinucleotide repeats. CFSs form gaps and breaks on metaphase chromosomes when DNA synthesis is partially inhibited by folate stress or using compounds such as ►aphidicolin, an inhibitor of DNA polymerases. In addition to forming gaps and breaks on metaphase chromosomes, CFSs exhibit additional features of unstable DNA. They are hot spots for deletions, sister ►chromatid exchanges, ►chromosomal translocations, integration of transfected and viral DNA sequences, and chromosome breaks associated with initiation of gene ►amplification.

There are over 76 different CFSs with one or more seen on most human chromosomes. However, just 20 of these sites account for more than 80% of all gaps and breaks seen in aphidicolin-treated lymphocytes. The most highly expressed of these sites are FRA3B (3p14.2) and FRA16D (16q23). CFS regions span several hundred kilobases to over a megabase of DNA. Despite their size, a number of these sites are fully contained within large genes such as the tumor suppressor genes ►*FHIT* (fragile histidine triad gene) at 3p14 and ►*WWOX* (WW domain containing oxidoreductase) at 16q23.

CFSs appear to be highly conserved during mammalian evolution. ►Orthologs of human CFSs have been found in the ►syntenic regions of a number of other mammalian species, including other primates, cat, dog, pig, horse, cow, Indian mole rat, deer mouse, and laboratory mouse strongly suggesting an underlying function for these sites in the normal functioning of the cell.

Molecular Basis of Fragile Site Expression

Rare Fragile Sites

The expression of all rare fragile sites studied at the molecular level is dependent on an expansion of repeat DNA sequences. Most, such as the folate sensitive fragile site FRAXA (Xq27.3) in the 5′ non-coding region of the *FMR-1* gene, are caused by an expansion of CGG trinucleotide repeats. Normal individuals have anywhere from 1–50 CGG repeats. Whereas, in the disease state the number of repeat units increases dramatically, to 200–2,000 repeats. Trinucleotide repeats are inherited in a Mendelian manner but also follow a phenomenon known as ►anticipation whereby an increase of severity of symptoms and an earlier age of onset of disease is seen in succeeding generations. Other rare fragile sites are associated with an expanded AT-rich minisatellite repeat sequence. The rare fragile sites FRA16B (16q22.1) and FRA10B (10q25.2) are examples in which expansion of the existing AT-rich minisatellite repeats, to 33 and 42 base pairs respectively, is the cause of fragility.

Common Fragile Sites

Less is currently known about the molecular basis of expression of common fragile sites. While similar in appearance to rare fragile sites at the cytogenetic level, common fragile sites are not caused by expansion of trinucleotide or other simple repeat sequences. All common fragile sites are AT-rich and contain numerous repeat elements such as, LINES, SINES, and other long repeat elements. Common fragile sites contain clusters of sequences with a potential for high ►DNA flexibility and formation of unusual DNA structures that could impede DNA replication. Furthermore, these sites are among the last sites in the genome to replicate and late or incomplete DNA replication may contribute to their fragility. Despite these findings there is no clear answer as to why these sites are more sensitive to replication stress than other none fragile loci.

Cellular Regulation of Common Fragile Site Instability

Mechanisms of fragile site expression provide important clues about the consequences of stalled or perturbed replication in mammalian cells. It has been shown that the ►ATR (►ataxia-telangiectasia and rad3-related) protein kinase is crucial for maintaining fragile site stability. ATR is a major cell cycle checkpoint protein that responds to replication stress and stalled replication forks by blocking cell cycle progression. Deficiency of ATR leads to a spontaneous expression of fragile sites that is amplified 8–10 fold when cells are treated with aphidicolin. Consistent with these findings, it has been shown that individuals with the genetic disease ►Seckel syndrome 1 have a hypomorphic mutation in *ATR* and show increased fragile site breakage following exposure of cells to aphidicolin. It has also been shown that the ►*BRCA1*, the ►Fanconi anemia family gene *FANCD2*, *CHK1*, *HUS1* and *SMC1* also play important roles in sensing and ►repair of DNA damage at common fragile sites and that loss of any of these genes leads to an increase in common fragile site expression.

Once broken, CFSs are repaired by a variety of cellular mechanisms. It has been shown that ►*RAD51*, *DNA-PKcs*, and *Ligase IV* are all important for repair of broken sites and that loss of any of these genes causes an increase in detectable gaps and breaks on metaphase chromosomes.

It is hypothesized that increased ►replication stress leads to collapsed or delayed replication forks,

incomplete replication of the associated regions, and large single-strand gaps, some of which can escape ►G2/M checkpoints. CFSs are more prone to forming gaps and breaks under replication stress than are other sites in the genome. When checkpoints are perturbed or absent, as is the case when ATR is lost, instability increases dramatically through out the genome but common fragile sites are more strongly affected. Furthermore, loss of genes that would normally function to repair damaged sites can also lead to an increase in visible gaps and breaks at CFSs on metaphase chromosomes. Thus, breaks at CFSs can serve as "signatures" of increased replication stress and are valuable cytological markers for the study of checkpoint and repair pathways that respond to replication stress.

Clinical Relevance

Rare Fragile Sites

The FRAXA rare fragile site at Xq28 is a cytogenetic manifestation of the expanded CGG repeat mutation in the promoter region of the *FMR1* gene associated with the ►fragile-X syndrome, a leading cause of mental retardation in humans. Large repeat length leads to methylation and transcriptional inactivation of *FMR-1* and thus phenotypic expression of any associated disease. Individuals with a moderate repeat length, 50–100 repeats, are considered to have premutations of the disease. In this moderate repeat expansion state clinical manifestations of the associated disease are possible, including the recently described FXTAS (fragile-X tremor ataxia syndrome) however, symptoms are less severe and less frequent than in the full mutation state. A similar fragile site on the X chromosome, FRAXE (Xq28), is associated with the genes *FMR2* and *FMR3* and can lead to mild mental retardation in some families. FRA11B (11q23.3) on the long arm of chromosome 11 is believed to lead to terminal deletions of chromosome 11 in a small number of cases of Jacobsen syndrome. In addition, an autosomal folate-sensitive fragile site, FRA12A, in the *DIP2B* gene has recently been associated with mental retardation. It is likely that other rare fragile sites will be found to be associated with specific genes and genetic diseases.

Common Fragile Sites

At least two of the thirteen cloned common fragile sites lie within known ►tumor suppressor genes. FRA3B (3p14.2) spans over 500Kb of sequence and is centered on exon 5 of the *FHIT* gene. Deletions within FRA3B (3p14.2) have been found in solid tumors and are associated with loss or alteration of the *FHIT* gene. Specifically, alteration or deletion of the *FHIT* gene has been found to frequently occur in ►Barrett esophagus, gastric, colon, lung and other types of cancer cells. FRA16D (16q23) is centered on exons 6, 7, and 8 of the *WWOX* gene. The *WWOX* gene has been implicated in both ►apoptosis and tumor suppression, and deletions of *WWOX* have been found in a number of gastric adenocarcinomas and multiple myelomas. Consistent with the finding that fragile sites are sensitive to replication stress, there is evidence that deletions at common fragile site regions appear very early during tumorigenesis and precede more global genomic deletions and ►LOH in cells with activated DNA-damage checkpoint response genes such as *ATR* and ►*ATM*. In addition, two groups have reported that the ATR checkpoint pathway is activated very early during tumorigenesis in response to unknown factors that lead to replication stress

These and many similar data suggest that common fragile sites are frequently unstable in cancers and can lead to the deletion or alteration of tumor suppressor genes associated with them. Lower expression or loss of tumor suppressor genes such as *FHIT* or *WWOX* may increase susceptibility for development of certain cancers. Because of this, it is probable that the expression of common fragile sites plays a significant role in the early stages of tumor formation. Additionally, due to the frequency of deletions at common fragile sites early in tumorigenesis, deletions at these sites can be used as signatures of increased replication stress and early tumorigenesis.

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Fragile-X Syndrome

Definition

A genetic disease associated with the rare fragile site FRAX and caused by an expanded CGG trinucleotide repeat in the *FMR1* gene. This is the most common cause of inherited mental retardation.

►Fragile Sites

Fragilomics

Definition

Referring to a systematic large scale study to identify the repertoire of ►fragile sites of the genome, to define the genes disrupted by fragile site activation and to determine the role they might have in human chronic diseases including cancer; the term fragilome was coined in the early 2004s as a linguistic equivalent to the concept of genomics.

Fragment Analysis

Definition

Method for the detection of ►polymerase chain reaction (PCR) fragments. Fluorescence labeled primers enable the detection and exact length calculation of PCR amplificates on sequencing machines.

►Leukemia Diagnostics

Frameshift Mutation

Definition

Is a genetic mutation that inserts in or deletes from a DNA sequence a number of nucleotides not evenly divisible by three. Consequently, the insertion or deletion disrupts the reading frame, or the grouping of the codons, resulting in a completely different translation from the original DNA.

Free Radicals

Definition

Are chemical species that possess at least one unpaired electron and are therefore unstable and highly reactive. The presence of unpaired electrons confers a considerable degree of reactivity upon a free radical. Free radicals can cause cellular damage by reacting with proteins, lipids, carbohydrates and DNA, and they are thought to be involved in the etiology of many human diseases including cancer, cardiovascular disease, and age-related diseases.

►Carotenoids
►Particle-induced Cancer
►Oxidative Stress
►Nonsteroidal Anti-Inflammatory Drugs
►Reactive Oxygen Species

Freeze Surgery

►Cryosurgery in Bone Tumors

FRNK

Definition

FAK-related nonkinase domain, the C-terminal domain of focal adhesion kinase, which functions as a negative regulator of kinase activity. Overexpression of FRNK inhibits cell migration.

►Focal Adhesion Kinase

FTC

Definition

Follicular Thyroid Carcinoma; ►Follicular Thyroid Tumors.

5-FU

►Fluorouracil

Fucosylation

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Definition

Is one of the most popular modifications with ►oligosaccharides on glycoproteins or glycolipid, which is involved in cancer. Fucosylation involves the attachment of a fucose residue to *N*-glycans, *O*-glycans, and glycolipids. *O*-fucosylation, which is a special type of fucosylation, is very important for ►Notch signaling. Increased levels of fucosylation have been reported in a number of pathological conditions, including ►inflammation and cancer. Therefore, certain types of fucosylated glycoproteins or antibodies, which recognize fucosylated oligosaccharides, have been used as tumor markers. The regulatory mechanisms of fucosylation are complicated.

Characteristics

Regulation of Fucosylation

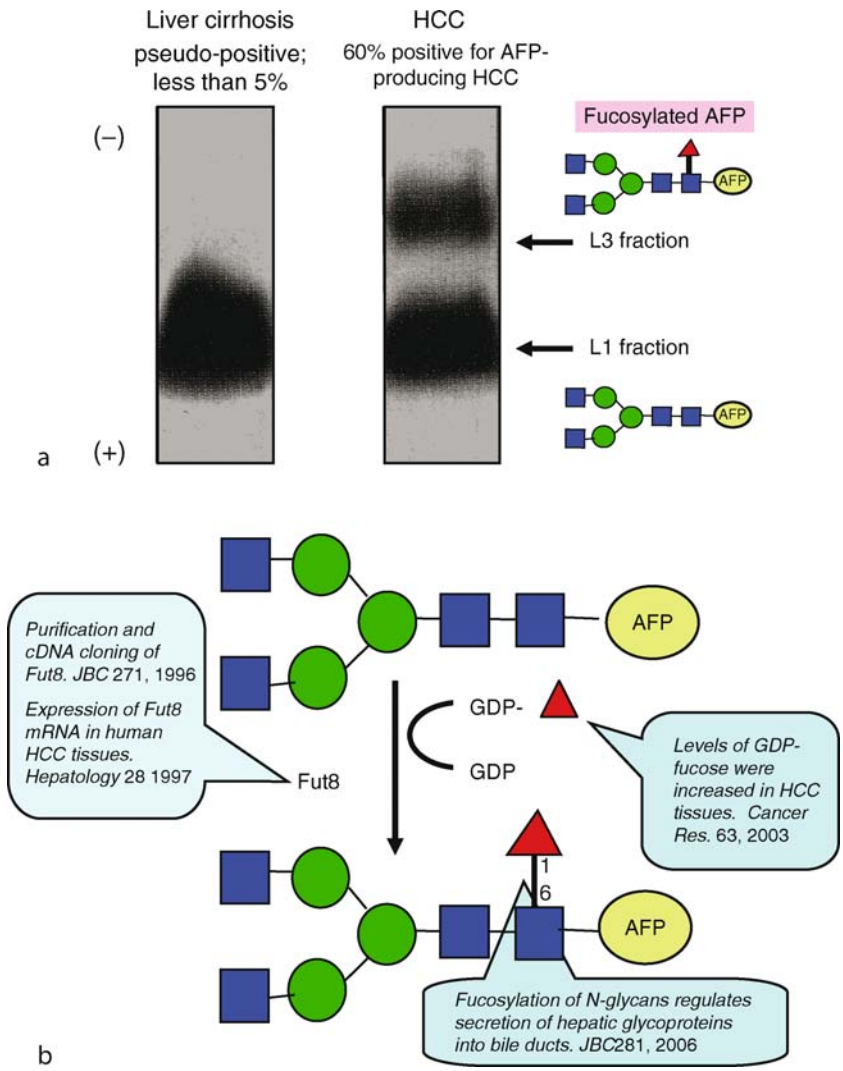
The regulation of fucosylation appears to be complicated, and depends on the type of cells or organs under consideration. Basically, fucosyltransferases, ►GDP-fucose synthetic enzymes, and GDP-fucose transporters are involved in the fucosylation pathway. There are 11 different, known fucosyltransferases (Fut) that have been identified to date, and they are divided into four groups. Fut1 and Fut2 are involved in the synthesis of α1-2 fucose, Fut3, 4, 5, 6, 7, and 9 in the synthesis of α1-3/α1-4 fucose, ►α1-6 fucosyltransferase (Fut8) in the synthesis of α1-6 fucose (►core fucose), and enzymatic activities as fucosyltransferases have not been confirmed in both Fut10 and Fut11. Fut8 has little homology with other fucosyltransferase members and plays an essential role in many biological phenomena because most Fut8 knockout mice die after birth. This drastic change is not observed in other Fut1~9 knockout mice. Core fucose, a product of Fut8 regulates the functions of many growth factor receptors and adhesion molecules. For example, depletion of the core fucose on the EGF receptor inhibits binding to its ligand, leading to the suppression of cell growth and the depletion of core fucose from α3β1 integrin suppresses that attachment of cells to laminin.

►Core Fucosylation and α-Fetoprotein

The expression of Fut8 is quite low in normal liver and increased levels of fucosylated proteins in serum can be used as a tumor marker because numerous serum proteins are produced in the liver. A representative of such a case is fucosylated ►α-fetoprotein (AFP), which can be detected by LCA (lens culinaris) lectin affinoelectrophoresis. AFP is a well-known tumor marker for ►hepatocellular carcinoma (HCC), but it is sometimes also increased in benign liver diseases such as chronic hepatitis and liver cirrhosis. In contrast, fucosylated AFP, referred to ►AFP-L3 (Fig. 1a), is very specific for HCC and was approved as a tumor marker for HCC by the FDA (Food and Drug Administration) in 2005. The molecular mechanisms underlying the production of fucosylated AFP in HCC are complicated. The enhancement of Fut8 is required to produce fucosylated AFP, but this enhancement is insufficient for the production of fucosylated AFP in HCC. GDP-fucose, a donor substrate for many fucosyltransferases is also a key factor in the production of fucosylated proteins. There are two different synthetic pathways for producing GDP-fucose in hepatocytes. One is a de novo pathway and the other is a salvage pathway. The de novo pathway is particularly dominant in hepatocytes and ►FX and GMD plays a pivotal role in the de novo pathway. While the expression of Fut8 is increased in both HCC and liver cirrhosis, the levels of FX are significantly increased in HCC tissues. The levels of GDP-fucose in cells or tissues can be measured using a novel ►HPLC assay and significant increases in GDP-fucose are observed in HCC tissues, compared to the surrounding tissue. In terms of the fucosylation pathway, many factors including fucosyltransferases, GMD, FX, and the GDP-fucose transporter appear to be coregulated. More importantly, fucosylated glycoproteins produced in hepatocytes are secreted into the bile, which is on the apical side of hepatocytes. When the oligosaccharide structures of bile and serum glycoproteins are compared by a lectin blot or 2D mapping, dramatic increases in fucosylation are observed in bile glycoproteins. Fut8 ►knockout mice show a decreased level of hepatic glycoproteins such as α1-acid glycoprotein and α1-antitrypsin in their bile, suggesting that fucosylation regulates the secretion of certain types of hepatic glycoproteins into the bile. The disruption of this system could be one of the mechanisms underlying the increases in fucosylated protein levels, including AFP-L3 in the serum of patients with HCC (Fig. 1b).

Fucosylation and Lewis Antigen

Fut3, 4, 5, 6, 7, and 9 are involved in the synthesis of the Lewis antigen. They function in a cell- or organ-specific manner. Sialyl Lewis X or sialyl Lewis A are used as tumor markers for certain types of cancer. Increases in these Lewis antigens in cancer tissues are correlated with a poor prognosis in colon cancer, due to the high incidence of liver metastasis. The reason for this is that Sialyl Lewis X is a ligand for selection which is expressed in endothelial cells. The first step of tumor metastasis is weak binding through an oligosaccharide and a lectin, followed by strong binding via integrins.



Fucosylation. Figure 1

Although these ►Lewis antigens are fucosylated oligosaccharides, other glycosyltransferases except fucosyltransferases are involved in its synthesis. The donor substrate, GDP-fucose is also important for the synthesis, but the Km values for these Lewis enzymes are different from that of Fut8. This type of fucosylation is also a signal for sorting hepatic glycoproteins into the bile. In the human liver, Fut6 is involved in the synthesis of Lewis types of fucosylation and hepatic glycoproteins with this oligosaccharide structure are present in the bile. In the case of mice, Fut6 is a pseudogene, and therefore the secretion of certain kinds of hepatic glycoproteins into the bile is disrupted in Fut8 knockout mice.

Fucosylated Haptoglobin and Pancreatic Cancer

In terms of glycomics, fucosylated glycoproteins are recognized by several types of ►lectins. These lectins include AAL, UEA, LCA, and AOL. AAL recognizes α1-3/α1-4 and α1-6 fucose, UEA recognizes α1-2 fucose, LCA recognizes the native form of α1-6 fucose with a mannose arm, and AOL recognizes α1-6 fucose more specifically. These lectins could be applicable to a diagnosis of cancer. In western blotting of the AAL lectin using serum from patients with ►pancreatic cancer, approximately 40 kD proteins were found to be highly fucosylated. The N-terminal sequence revealed that this protein was the haptoglobin β chain. The positive rate of fucosylated haptoglobin is 60–80% and the ratio is increased with the progression of the stage pf the disease. Increases in fucosylated haptoglobin levels have been observed in several types of cancer (20–40%) and, it was reported that high levels of fucosylated ►haptoglobin were produced in the advanced stage of ovarian cancer, lung cancer, and breast cancer. Basically, haptoglobin is produced in the

liver and has a low level of fucosylation on its glycans, since the expression of Fut8 is quite low in a normal liver. The ectopic expression of haptoglobin is observed in special conditions such as infections, inflammation, and cancer. The question herein is where fucosylated haptoglobin is produced in patients with pancreatic cancer. A special pancreatic cancer cell, PSN-1 expresses haptoglobin mRNA and produces fucosylated haptoglobin into conditioned medium. However, this case is very rare in comparison with the positive rate for fucosylated haptoglobin (60–80%). If white blood cells infiltrated around pancreatic cancer cells produce haptoglobin, they would be fucosylated, because blood cells express high levels of Fut8. The third hypothesis is that fucosylated haptoglobin produced in the liver is miss-sorted into the blood due to a factor that is secreted from pancreatic cancer cells. To determine which theory is correct, detailed analyses of oligosaccharide structures including the site-directed analysis of haptoglobin oligosaccharides need to be performed. Collectively, fucosylation is highly associated with cancer and additional, detailed information regarding its mechanism would be highly desirable, since it could be a target for novel cancer therapy.

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α1-6 Fucosyltransferase

Definition

Fut8; A glycosyltransferase that transfers fucose onto the innermost *N*-acetylglucosamine in *N*-glycans via an α1–6 linkage. It has no homology with other fucosyltransferases.

►Fucosylation

Fucosyltransferases

Definition

Enzymes that catalyze addition of fucose monosaccharides.

►Lewis Antigens
►Fucosylation

Fucoxanthin

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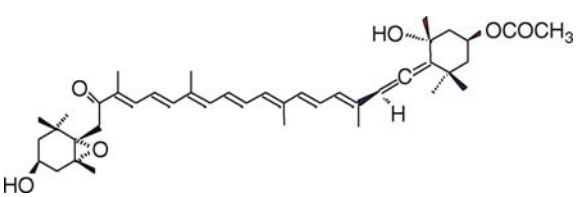
Definition

Is a xanthophyll that contains allenic bond, two hydroxy, keto, and epoxy groups. It is one of the most abundant ►carotenoids accounting for >10% of estimated total natural production of carotenoids. Further, fucoxanthin is the characteristic pigments of brown seaweeds (phaeophyceae) which are the largest occurring group among seaweeds. In South East Asian countries, some brown seaweeds containing fucoxanthin are often used as a food source (Fig. 1).

Characteristics

Effect on Cancer Cell Growth

Cell proliferation is the key in promoting and further progression of carcinogenesis. In a study screening the antiproliferative activity of seaweed extracts on tumor cells, fucoxanthin from the brown seaweed,



Fucoxanthin. Figure 1 Structure of fucoxanthin.

Undaria pinnatifida, is found to be the active principle. When several cancer cells are cultured with fucoxanthin, the cell viability decreases. The antiproliferative activity of fucoxanthin on human cancer cells is generally higher than other carotenoids. Fucoxanthin exhibits the higher activity than β-carotene and astaxanthin on human ►colon cancer cells (Caco-2, HT-29, DLD-1) and human leukemia cell (HL-60). Treatment of Caco-2 cells with fucoxanthin induces morphological changes such as a diminished size and rounded shape. Also, the cell membrane has shrunk with a condensed cytoplasm. The stronger inhibitory effect of fucoxanthin is found in human prostate cancer cells (PC-3, DU 145, LNCap). In this case, the effect of 15 kinds of carotenoids (phytoene, phytofluene, ξ-carotene, lycopene, α-carotene, β-carotene, β-cryptoxanthin, canthaxanthin, astaxanthin, capsanthin, lutein, zeaxanthin, vioaxanthin, neoxanthin, and fucoxanthin) present in foodstuffs is evaluated on the grown of the cancer cell lines. Among the carotenoids, neoxanthin and fucoxanthin cause a remarkable reduction in the growth of prostate cancer cells.

Apoptosis

There is a wealth of information pertaining to apoptosis in anticancer research. ►Macrophages recognize the cells undergoing apoptosis and engulf them without adversely affecting or damaging the neighboring cells. Apoptosis-inducing activities provide a novel means of ►chemoprevention and chemotherapy in the treating cancer. In an investigation on the apoptosis-inducing activity of fucoxanthin, a DNA ladder, which is a characteristic feature of apoptotic cells, is clearly visible in HL-60 cells treated with fucoxanthin. Similar results can be obtained with ►camptothecin, which is known to be a strong apoptosis-inducing agent. The fragmented DNA content designated as the enrichment factor as estimated by sandwich ►ELISA, increases with the concentration of fucoxanthin in the medium. DNA fragmentation, indicating by in situ ►TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling), reveals that fucoxanthin reduces cancer cell viability by inducing apoptosis. Some apoptosis-inducing agents are known to arrest a specific cell phase. Therefore, it can be presumed that fucoxanthin affects cell cycle.

Mechanism

Fucoxanthin suppresses expression of ►Bcl-2 protein, which is responsible for suppression of programmed cell death as a survival factor. This is indicative of the fact that downregulation of Bcl-2 protein may contribute to fucoxanthin-induced apoptosis in cancer cells. DNA fragmentation induced by fucoxanthin is partially inhibited by a ►caspase inhibitor Z-VAD-fmk. Further, fucoxanthin also regulates the redox signals, and then facilitates the progression of apoptosis through Bcl-2 protein suppression, and caspase-dependent and-independent pathway.

Combination with Troglitazone

►Troglitazone is known to inhibit cell growth and induce apoptosis through the activation of ►PPARγ. Oral administration of troglitazone inhibits the early stage of colon tumorigenesis. On the other hand, preincubation of cancer cells with fucoxanthin remarkably enhances the effect of troglitazone. Therefore, the combined action of PPARγ ligand such as troglitazone and fucoxanthin is more effective on chemoprevention of cancer than troglitazone, and possibly other agents, alone.

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Fulvestrant

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Synonyms

Faslodex

Definition

Was originally known as ICI 182,780 and is now marketed by AstraZeneca under the trade name Faslodex®. The chemical formula of fulvestrant is 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17β-diol.

Fulvestrant, a steroidal 7α-alkylsulfinyl analog of 17β-►estradiol, is an ►estrogen receptor antagonist with no agonist effects. It is used as an endocrine treatment for postmenopausal women with hormone-sensitive ►advanced breast cancer.

Characteristics

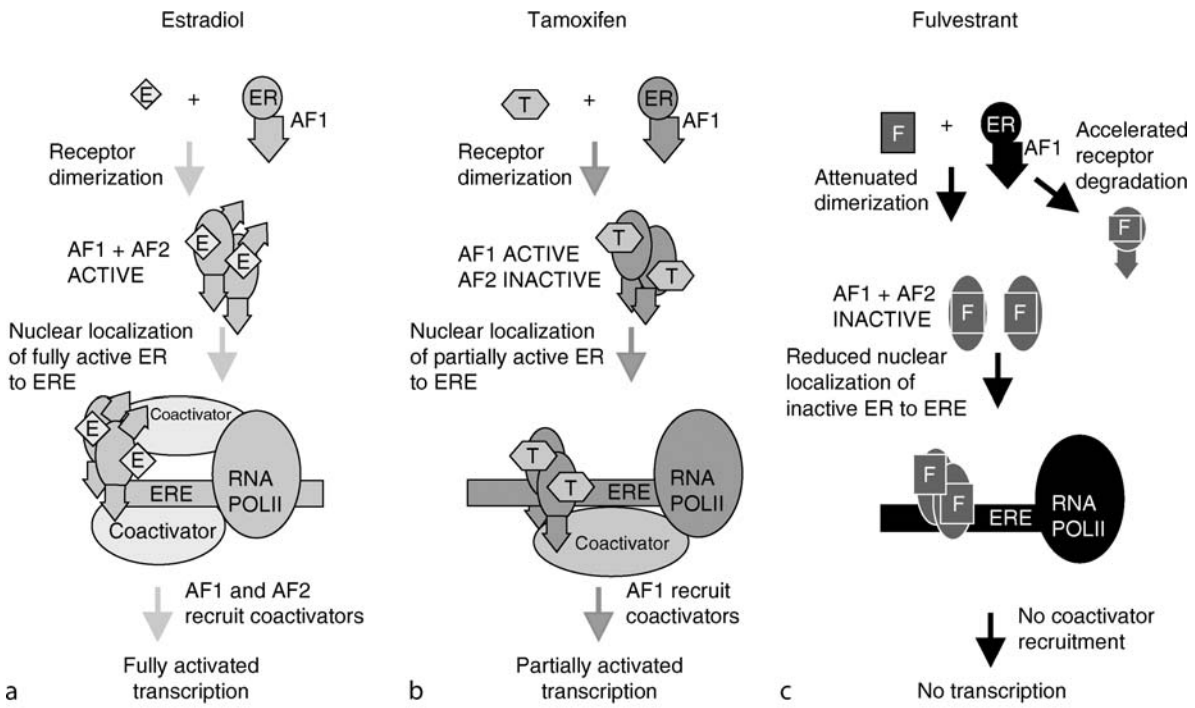
Mode of Action

Currently, more than one million women worldwide are diagnosed with ►breast cancer each year. In postmenopausal women, approximately 75% of breast tumors are hormone sensitive, expressing the estrogen receptor and/or progesterone receptor, and are stimulated to grow in the presence of estrogen. To understand the treatments for hormone-receptor positive breast cancer, we must first understand the role of ►estrogen, a natural circulating hormone that has been shown to drive tumor growth. Once estrogen has bound to the ►estrogen receptor, the receptors dimerize, before translocation to the nucleus, where the complex binds to specific DNA sequences (estrogen response elements) in target genes. Activating functions on the estrogen receptor (AF1 and AF2) recruit protein cofactors, allowing the transcription and expression of the target genes, resulting in increased cell division and tumor progression (Fig. 1).

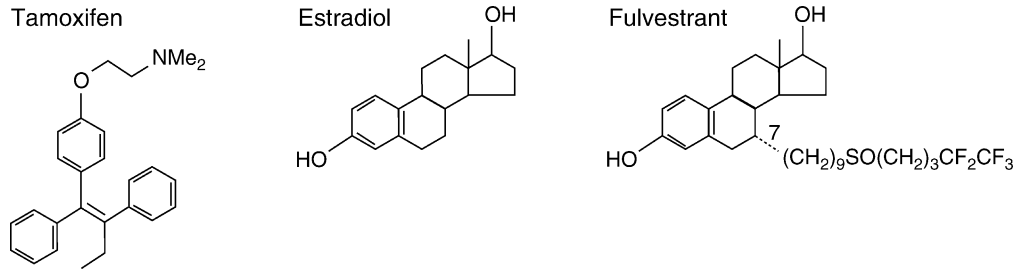
As an estrogen receptor antagonist, fulvestrant exhibits a high estrogen receptor binding affinity and produces a complete receptor blockade. Following binding of fulvestrant, dimerization of the estrogen receptor is impaired and the bound receptor is rapidly degraded, a process unique to fulvestrant amongst the ►antiestrogen receptor agents. Nuclear localization is also disrupted, and both AF1 and AF2 are inactivated, leading to complete abrogation of estrogen signaling through the estrogen receptor (Fig. 1). This also means that fulvestrant has no estrogen agonist activity, which is important, since even partial agonist activity can lead to an increased incidence of endometrial abnormalities and cancer.

As a steroidal analog of estradiol, fulvestrant is structurally distinct from the non-steroidal ►tamoxifen, a selective estrogen receptor modulator, which is also used in the treatment of hormone receptor-positive breast cancer (Fig. 2). Although tamoxifen binds to the estrogen receptor, and permits dimerization and translocation, AF2 is not activated and so the transcription of estrogen-responsive genes is blocked (Fig. 1). However, this block is not complete, since AF1 continues to function, and therefore tamoxifen retains partial agonist activity, with the associated endometrial risks.

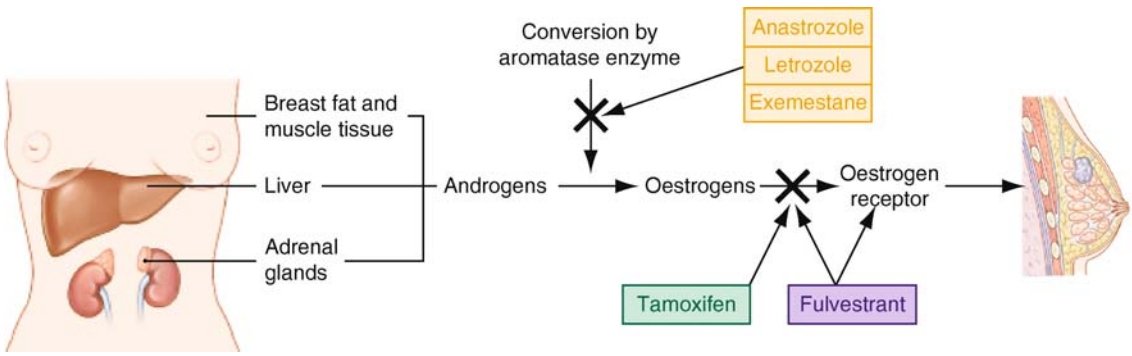
Fulvestrant, with its unique mode of action, is fundamentally different from the third-generation ►aromatase inhibitors such as anastrozole, letrozole or exemestane, which are used to treat breast cancer in an increasing proportion of postmenopausal women.



Fulvestrant. Figure 1 The mode of action of fulvestrant, tamoxifen and estradiol. (Reproduced from Dowsett et al. [2, Fig. 1]. Breast Cancer Res Treat 93:S11–S18 (2005). With kind permission of Springer Science and Business Media.)



Fulvestrant. Figure 2 Chemical structure of fulvestrant.



Fulvestrant. Figure 3 Scheme showing how different endocrine therapies (fulvestrant, tamoxifen, aromatase inhibitors) work in breast cancer.

Following the menopause, most endogenous estrogen is produced via the conversion of adrenal androgens in peripheral tissues. Aromatase inhibitors work by blocking the aromatase enzyme, which catalyzes this conversion, thus reducing the levels of circulating estrogen available to bind to the estrogen receptor (Fig. 3).

Current Utilization of Fulvestrant

While treatment for hormone-sensitive early breast cancer aims to remove the tumor by surgical or radiological techniques followed by adjuvant ►endocrine therapy, treatment for advanced disease is essentially palliative rather than curative, with the emphasis on extending life and preserving quality of life amongst patients. In postmenopausal women with hormone-sensitive advanced breast cancer, it is now standard practice to employ a sequence of endocrine agents, to slow the progression of the disease and delay for as long as possible the requirement for cytotoxic chemotherapy treatment. Consequently, novel endocrine agents that are both effective and lack cross-resistance with existing therapies are required to extend the duration of the sequential treatment regimens.

Fulvestrant is a new therapeutic option that can be added to the hormonal treatment sequence. Results from Phase III clinical trials showed that in postmenopausal women with hormone-responsive advanced breast cancer who had progressed on previous antiestrogen therapy, fulvestrant was at least as effective as anastrozole, in terms of time to progression, objective response rates and survival. This evidence led to its regulatory approval and fulvestrant is currently licensed for use as a second-line endocrine treatment agent for advanced breast cancer after progression or recurrence on an antiestrogen. More recently, Phase III trial data have confirmed fulvestrant activity in the post-aromatase inhibitor setting. Reflecting all these data, fulvestrant is considered in the National Comprehensive Cancer Network guidelines as an option after the failure of first-choice endocrine treatment (tamoxifen or an aromatase inhibitor). Thus, fulvestrant is a valuable addition to the endocrine armory.

Importantly, due to its unique mechanism of action, analyses of patients progressing on fulvestrant have demonstrated continued sensitivity to subsequent endocrine therapies, indicating that fulvestrant lacks cross-reactivity with the ►aromatase inhibitors and tamoxifen.

Administration and Tolerability

Instead of the daily oral dosing used with other endocrine therapies, fulvestrant is given as a monthly

250 mg/5 mL intramuscular injection, which provides slow release of the drug and sustained pharmacologic activity over the dosing interval (28 ± 3 days). The injection is well tolerated locally and may also help to assure treatment compliance. Once in the body, fulvestrant is predominantly bound to plasma proteins and metabolized by the liver, with negligible renal excretion, and it is not implicated in clinically significant drug–drug interactions, making it suitable for use in patients receiving polypharmacy for co-morbid conditions.

Fulvestrant is well tolerated, with most adverse events being mild to moderate in intensity. In clinical trials, the most commonly reported adverse events were nausea, asthenia and pain. Fulvestrant has potential tolerability benefits over some existing treatments, e.g. it is associated with less hot flashes than tamoxifen, and a lower incidence of joint disorders than anastrozole.

Future Uses of Fulvestrant

In recent years, the third-generation aromatase inhibitors, have been shown to be superior to tamoxifen for the treatment of both early and advanced breast cancer. Treatment guidelines currently recommend that an aromatase inhibitor should be used as either the primary endocrine therapy in postmenopausal women, or after 2–3 years of tamoxifen. However, even in this estrogen-deprived environment, some tumors will become resistant to treatment and begin to progress. Therefore, if cytotoxic chemotherapy is to be further delayed, an alternative endocrine therapy must be used. Indeed, as aromatase inhibitors continue to replace tamoxifen in the first-line setting, it is becoming increasingly important to identify agents that are effective after recurrence or progression on these drugs. As previously described, results from both Phase III and Phase II studies suggest that fulvestrant may be active after progression on aromatase inhibitors.

Fulvestrant's unique mode of action and lack of cross-reactivity also invites the possibility of potentially synergistic combinations with other treatment agents. Preclinical data suggest that the combination of fulvestrant with aromatase inhibitors will offer a more effective anti-tumor effect than either agent alone, and Phase III trials are underway to fully evaluate this treatment strategy.

In addition, the epidermal growth factor (EGF) receptor-mediated pathway of gene transcription, which can provide an alternative growth stimulus for breast tumors in the absence of hormone receptors, has also been shown to cross-talk with the estrogen receptor-mediated pathway. This, in turn, has important implications for the development of resistance to endocrine therapy. As fulvestrant increases degradation of estrogen receptors, it may limit the potential for cross-talk with other pathways. Combination treatment with fulvestrant and an agent targeting growth factor receptors (such as gefitinib, lapatinib or trastuzumab) may therefore further limit cross-talk and potentially delay the time of onset of treatment resistance. Currently, several clinical trials are ongoing to investigate the activity of such combination therapy in patients with advanced breast cancer.

Summary

Fulvestrant, a steroidal analog of ►estradiol, is an effective treatment for postmenopausal women with hormone-sensitive advanced breast cancer who have progressed on previous endocrine therapy. With its unique mode of action, fulvestrant provides a valuable addition to the endocrine treatment sequence, with significant benefits for patients. It is administered as a monthly intramuscular injection, and is well tolerated and associated with few adverse events. Fulvestrant may also have a potential use in combination treatment strategies, as the partner of choice with ►EGF receptor inhibitors.

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Fumarase

►Fumarate Hydratase

Fumarate Hydratase

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Synonyms

FH; Fumarase

Definition

Fumarate hydratase is an enzyme that functions in the mitochondrial citric acid cycle, catalyzing the reversible hydration/dehydration reaction in which fumarate is converted to malate.

Characteristics

The human gene encoding fumarate hydratase is located in the chromosome segment 1q42.3-q43. It consists of ten exons that span over 20 kb of genomic DNA. The transcript is approximately 1.8 kb long and is predicted to encode a 510 amino acid polypeptide. The first exon of *FH* encodes a signal peptide that directs the protein to the mitochondrion. There the signal peptide is cleaved, and the remaining mature FH protein forms a functional homotetramer in the mitochondrial matrix. Some processed FH is also present in the cytosol, although the function of this cytosolic FH is unclear. In addition to the mitochondrial signal, the processed FH contains other domains such as alpha-helical and lyase domains. The alpha-helixes form a superhelical core for the tetramer. The functionally active FH enzyme converts fumarate to malate. This hydration reaction is a part of the citric acid cycle (also known as the tricarboxylic acid cycle or the Krebs cycle) which is an essential component of cellular carbohydrate metabolism. In the cytosol, fumarate is produced in the urea cycle and therefore FH is connected to protein metabolism as well. FH is well conserved, human FH sharing a 57% amino acid identity with the *Escherichia Coli* FumC protein, and it belongs to a protein superfamily which includes mostly other enzymes such as aspartase, adenylosuccinate lyase, and argininosuccinate lyase.

The first clues as to the role of FH in human disease came from the identification of two siblings that presented with progressive encephalopathy, dystonia, ►leucopenia, and ►neutropenia. They had elevated levels of lactate in their cerebrospinal fluid and high fumarate excretion in their urine. A ►homozygous mutation was discovered in a conserved region of the *FH* gene in both of these patients. Also, FH deficiency was shown to be present in all tissues studied in the patients, and their healthy parents were shown to carry the mutation in a heterozygous form. Since then, about 20 families with FH deficiency have been reported in the literature. The symptoms are severe and the affected individuals usually die within a few months of birth.

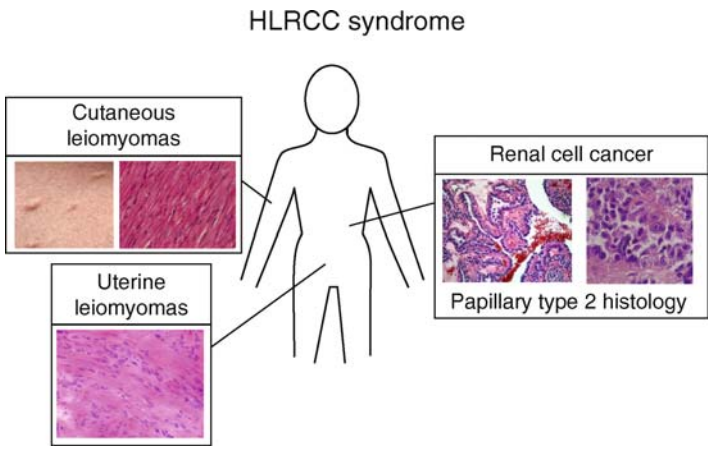
Evidence for yet another role of FH in human disease came from quite a different line of research. The genomic locus harboring the *FH* gene was independently mapped by genetic linkage analysis to segregate with inherited predisposition to ►leiomyoma and ►renal carcinoma; ►Hereditary leiomyomatosis and renal cell cancer (HLRCC), and to ►multiple cutaneous and uterine leiomyomatosis (MCUL). Soon after, these two conditions were shown to be variants of the same syndrome and the underlying gene was identified as *FH*. The tumors showed loss of heterozygosity (►LOH) and retention of the mutated allele, therefore suggesting that the gene acted as a ►tumor suppressor. Also, FH enzyme activity was shown to be reduced in the leukocytes and absent in the tumors of mutation carriers.

Clinical Features of HLRCC/MCUL

Since the first reports indicating the involvement of FH in tumorigenesis, more than 100 families with the HLRCC/MCUL phenotype have been reported in the literature (Fig. 1). Although no population-based studies have been carried out, it seems clear that the prevalence of HLRCC/MCUL is very low. There are reports of HLRCC/MCUL from all around the world, and there seem to be population differences in the phenotype. For example, HLRCC seems to be more common in Finland and North America, whereas in the UK, renal cell cancer is rarely detected in the families segregating heterozygous *FH* mutations. The most common manifestation of HLRCC/MCUL is cutaneous and/or ►uterine leiomyomas. Early-onset renal cell cancer is significantly rarer and is typically of the papillary type II histology.

Cutaneous leiomyomas are small benign tumors of the skin that show as multiple 0.5–2 cm skin-colored nodules, and their tissue of origin is thought to be the arrectores pili muscle of the hair follicle. They can manifest clinically as pain and paresthesias already in childhood, and the age of onset ranges from 10 to 50 in the HLRCC/MCUL families.

Uterine leiomyomas (also known as ►myomas or ►fibroids) are smooth muscle cell tumors that arise within the smooth muscle lining of the uterus, the myometrium. They are some of the most common neoplastic tumors of women, and estimates of affected individuals range from 25% to up to 77% depending on the methods used for the diagnosis. Even though they are benign, they can cause severe morbidity such as aberrant bleeding, abdominal pain and even infertility. In families affected by HLRCC/MCUL, the onset of leiomyomas seems to be earlier than in the general



Fumarate Hydratase. Figure 1 Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant tumor predisposition syndrome caused by germline mutations in the *fumarate hydratase* gene. The most common features are cutaneous and/or uterine leiomyomas. Predisposition to renal cell carcinoma is present in a subset of families. Typically, HLRCC renal cell carcinomas display papillary type 2 histology.

population. In these families, leiomyomas are also more often symptomatic and therefore they more commonly result in hysterectomy (i.e. the surgical removal of the uterus). In addition to typical leiomyomas, some HLRCC/MCUL patients develop atypical leiomyomas. These are rare variants of leiomyomas which are sometimes hard to discern from uterine leiomyosarcomas. Whether HLRCC/MCUL predisposes to malignant leiomyosarcoma is still a somewhat open question, although some studies suggest that this might indeed be the case.

Familial clustering of ►renal cell cancer was the key finding in the identification of the syndrome HLRCC. In the first family reported, four patients aged 33–48 were identified. Since then, additional cases of renal cell cancer have been detected in some HLRCC/MCUL families, the median age of onset being around 40. The natural history of HLRCC/MCUL renal tumors is malignant with early metastasis often leading to the demise of the patient. In the early reports, all renal cell cancers related to HLRCC/MCUL displayed a distinctive papillary type II histology, although other types of renal tumors, such as collecting duct and clear-cell carcinoma, have been later associated with HLRCC/MCUL as well. HLRCC/MCUL renal tumors are typically unilateral, which is in contrast to other inherited forms of renal cell cancer such as von ►Hippel-Lindau Syndrome (VHL), Hereditary Papillary Renal Carcinoma, and ►Birt-Hogg-Dubé Syndrome (BHD), in which tumors often affect both kidneys.

FH-mutation carriers might be at risk of developing ►Leydig cell tumors and ovarian cystadenomas. Incidental cases of other tumors, such as breast and prostate cancer and some hematological malignancies, have also been reported in HLRCC/MCUL families, although it remains unclear whether any of these are true manifestations of the germline *FH* mutations.

Mutations in the *FH* Gene

The syndrome HLRCC/MCUL is transmitted in an ►autosomal dominant manner, and germline *FH* mutations have been detected in ~85% of all the families displaying the HLRCC/MCUL phenotype. Altogether, ~60 different mutations have been identified. The vast majority (~70%) are single base pair substitutions, of which ►missense mutations comprise about 60%; the rest are non-sense mutations. Small deletions and insertions as well as ►splice site changes have been reported. In addition, whole gene *FH* deletions have been detected in some families. Mutations occur throughout the gene. The mutation R58X has been detected in four families of diverse ethnic and geographical backgrounds in North America. ►Haplotype analysis has suggested that the mutation has occurred independently in these families, indicating that this might represent a mutational hot spot. The same mutation has also been detected in families from the United Kingdom and Australia. Other mutations that have been detected in several families of different geographical backgrounds are, for example, N64T and R190H, and these may represent mutational hot spots as well. The mutations in families with the renal cell cancer phenotype do not differ from those seen in families without these malignant tumors and, in fact, the same mutations have been detected in families with either of the two phenotypes. This has raised the question of whether an additional genomic locus could act as a modifier together with *FH* mutations.

A ►founder effect has been detected at least in populations of the Finnish and Iranian origin. Two

mutations, H153R and a 2-bp deletion in codon 181, have both been identified in three different families in Finland. Similarly, a splice site mutation IVS6-1G>A has been detected in a common haplotype in several families of Iranian origin. Most tumors arising in HLRCC/MCUL families have a second somatic inactivating hit in the *FH* gene. This is often acquired through the loss of the wild-type *FH* by a partial or whole genomic deletion of chromosome arm 1q.

FH mutations are also rarely seen in sporadic tumors. Inactivation of the *FH* gene has been detected in three tumors from the Finnish population, one soft-tissue sarcoma of the lower limb, and two uterine leiomyomas, all showing loss of the wild-type *FH*. However, despite mutation screens comprising hundreds of tumor specimen, no other somatic changes in *FH* have been detected in various tumor types including prostate, breast, colorectal, lung, ovarian, thyroid, head and neck cancers, pheochromocytomas, gliomas, and melanomas. Therefore, it is safe to say that, in general, somatic inactivation of the *FH* gene is a very rare occurrence in human tumors.

As determined by microarray based gene expression analysis, as well as by traditional immunohistochemical methods, ►uterine fibroids carrying *FH* mutations have distinct biological properties which seem to require two hits in the *FH* gene. The molecular mechanisms through which mutations in *FH* lead to tumorigenesis are still far from being well understood. Some evidence suggests that the disruption of FH activity would lead to the stabilization of the ►hypoxia inducible factor-1 (HIF1) under normoxic conditions, thus activating several growth-promoting signaling cascades.

The mutations detected in the recessively inherited developmental disorder FH deficiency are also mostly missense mutations, and they occur throughout the *FH* gene. The mutational spectrum of FH deficiency does not seem to be different from that of HLRCC/MCUL and, indeed, a phenotype compatible with HLRCC/MCUL has been reported in some of the parents of the children affected by FH deficiency.

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Functional Foods

►Nutraceuticals

Functional Vascular Stabilization

►Vascular Stabilization

Fungus

Definition

Member of a class of relatively primitive vegetable organisms. Fungi include mushrooms, yeasts, rusts, molds, and smuts.

Funnel Factors

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Definition

Is a molecule where several oncogenic signals converge and drive the proliferative signal downstream. This transformation-inducing signal inexorably passes and is canalized through the funnel factor. Funnel factors provide a clear reflection of the tumor's transforming potential regardless of the triggering genetic alteration upstream. The level of expression of these factors should correlate with the degree of malignancy of the tumor and the most relevant clinical parameters, such as ►metastasis and survival. Therefore, they may reflect the molecular information and transformation potential for each tumor.

Characteristics

Background of Molecular Human Carcinogenesis

Funnel factors are those final effectors that channel the malignant cellular growth signals, which are transduced through pathways or cascades that induce and mediate changes into the cell physiology. Several of these pathways or cascades are redundant, that is they can trigger a similar cellular effect. There are six acquired capabilities considered to be necessary for the malignant cellular growth: ►self-sufficiency in growth signals, insensitivity to anti-growth signals, limitless replicative potential, resistance to ►apoptosis, sustained ►angiogenesis and, finally, the ability to infiltrate the surrounding tissue and metastasize. Each of these changes in cellular physiology can be brought about through dozens of signaling pathways or cascades, each implicating various genes or proteins. Many different oncogenic alterations may be involved in each biochemical route. This intricate molecular background and its biochemical consequences can help us to understand the great heterogeneity observed in tumors, with over 250 types of malignant human tumors with distinctive clinical and pathological characteristics and thousands of morphological and pathological tumor subtypes.

So far, up to 300 mutated genes implicated in oncogenesis have been identified as human cancer genes. Many oncologists and pathologists ask whether all this information is really important for the management of individual cancer patients. The answer is unknown because only a few molecular targets have been identified in a small number of tumor types. For example, ►amplification of ERBB2 is seen in 25–30% of ►breast carcinomas, ►*EGFR* mutations in less than 10% of ►lung carcinomas, and ►*c-KIT* in the rare ►gastrointestinal stromal tumors; but in most carcinomas, there is no distinctive oncogenic target. In the near future, technological advances will allow us to study the complete genetic background, ►mRNA profiling, and protein expression of individual tumors, and identify a myriad of genetic and biochemical alterations. But even then, attempts to inhibit or counteract single genetic alterations with the use of multiple specific agents would probably be chaotic. Nevertheless, dissection of the biochemical pathways is progressing. We now know which factors are the final growth signaling effectors that can control ►transcription and protein synthesis. Then, it is logical to think that the level of expression of these final effectors, which channel the proliferation signal, can be associated with the real oncogenic role of a pathway in individual tumors.

Cell Signaling in Human Tumors

Among the acquired capabilities of tumor cells, a funnel factor has been described for self-sufficiency in growth signals. This essential oncogenic capability is one of the most extensively studied characteristics of tumor cells, and one that is constitutively activated in nearly all tumors. The process of converting extracellular signals into cellular responses, in this case cell growth and division, is called signal transduction. The growth signal transduction pathway is comprised of ►growth factors, ►growth factor receptors, factors transmitting the growth signal, and the final effector factors, some of which are located in the nucleus to activate ►transcription factors and some in the ►ribosomes to activate protein synthesis. The neoplastic cell, however, may be able to generate signals for survival or proliferation through various mechanisms without depending on exogenous signals. These mechanisms include alterations in the growth factors or receptors, or in the signaling pathways, themselves. Among the latter, the most highly recognized and important are the ►RAS-►RAF-►MAPK (ERK1/2) and ►PI3K-►AKT pathways, which regulate ►mTOR. Specific molecular alterations are detected in these signaling cascades in the majority of tumors. Usually these are single alterations with an oncogenic impact, such as growth factor mutations or RAS mutations; other concomitant genetic alterations are not usually found in these biochemical pathways.

Searching for Funnel Factors: p-4E-BP1

In studies performed in various tumor types, the expression of key cell-signaling factors, including Her1 and Her2 growth factor receptors, as well as the RAS-RAF-MAPK and the PI3K-AKT-mTOR pathways were correlated with the associated clinico-pathological characteristics of these tumors. The downstream factors p70, S6, 4E-BP1, and EIF4E, which play a critical role in the control of protein synthesis, survival, and cell growth, were also analyzed. It was found that ►phosphorylated ►4E-BP1 (eukaryotic ►translation initiation factor 4E binding protein 1) levels in breast, ovary, and prostate tumors were associated with malignant progression and an adverse prognosis, regardless of the upstream oncogenic alterations. Thus, p-4E-BP1 seems to act as a funnel factor for an essential oncogenic capability of tumor cells, self-sufficiency in growth signals, and could be a highly relevant molecular marker of malignant potential. The results showing that 4E-BP1 is associated with the prognosis in breast, ovary and prostate tumors, are supported by other data. In breast cancer, ►phosphorylation of AKT, mTOR and 4E-BP1 has been associated with tumor development and progression; and in prostate cancer, one of the best biomarkers of the mTOR pathway is 4E-BP1, since overexpression of this factor has been highly associated with this type of tumor. Moreover, experimental studies have shown that 4E-BP1 is essential for cell transformation. Transfer of 4E-BP1 phosphorylation site mutants into breast carcinoma cells suppressed their tumorigenicity.

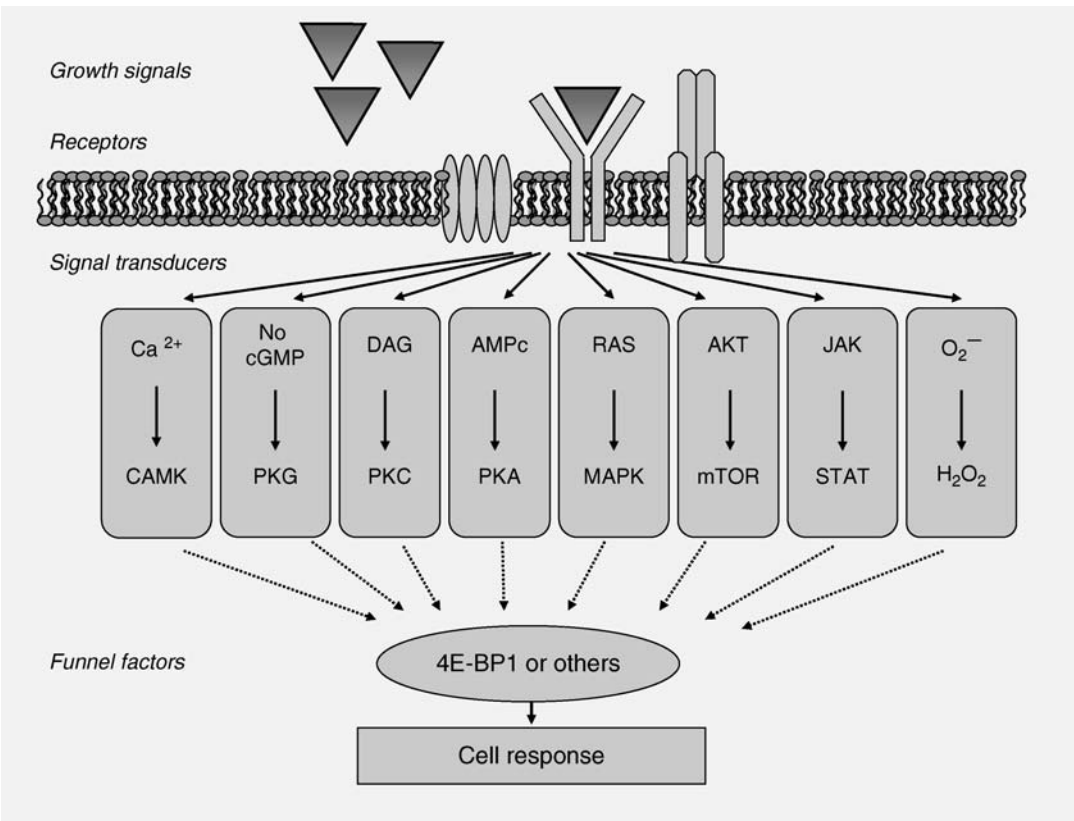
4E-BP1 is a eukaryotic translation initiation factor 4E (EIF4E)-binding protein that plays a critical role in the control of protein synthesis, survival, and cell growth. During ►cap-dependent translation, EIF4E binds to the mRNA ►cap structure and promotes formation of the ►translation initiation complex and ►ribosome binding. When 4E-BP1 is active (non-phosphorylated 4E-BP1) it binds to EIF4E and impedes formation of the initiation complex; translation is then blocked, favoring apoptosis. However, when 4E-BP1 is phosphorylated, the affinity for EIF4E binding is reduced, EIF4E is released, and cap-dependent translation can initiate.

4E-BP1 has seven phosphorylation sites. It is likely that mTOR is the main phosphorylation pathway of 4E-BP1, although other ►kinases may be implicated, such as ►CDK1, ►ATM, ►PI3K-AKT, ►ERK1/2, and perhaps other, still unidentified, kinases. Therefore, 4E-BP1 phosphorylation can be the consequence of many different oncogenic events occurring in several biochemical pathways, including amplification or mutation of growth factor receptors, loss of function or mutations in ►PTEN, ►ATM, ►p53, ►PI3K or ►RAS, or other collateral mechanisms of cellular oncogenic activation (Fig. 1). Because of the elevated number of genetic alterations that regulate 4E-BP1, we propose that the phosphorylated form of this protein can act as a "bottleneck" or funneling factor through which the transforming signals converge, channeling the oncogenic proliferative signal regardless of the upstream specific oncogenic alteration.

The role of other 4E-BP ►isoforms, such as 4E-BP2 and 4E-BP3, in human tumors is still unclear, and it is not known whether they can be activated in 4E-BP1-negative tumors. Study of the EIF protein family will also be determinant when reliable antibodies allow us to analyze their expression in large series of tumors. Recent data have provided novel perspectives into the proliferative and oncogenic properties of EIF4E, since it has been shown to have an impact on nearly every stage of cell cycle progression. Earlier studies have shown that EIF4E levels are substantially elevated in several types of cancers.

Funnel Factors in Other Oncogenic Pathways

Extending the concept of funnel factor, there might be several funnel factors where the final biochemical effect converges for each of the oncogenic capabilities of tumor cells. For example, in the apoptosis pathways,



Funnel Factors. Figure 1 Schematic diagram showing how funneling factors channel the proliferation signal.

where the expression of certain proteins that inhibit apoptosis, such as survivin and livin, might be associated with resistance to apoptosis regardless of the activation of other antiapoptotic or proapoptotic genes that might be present.

Study of the expression profiles of funnel factors from all the cell transformation pathways would allow us to obtain an individual functional-molecular signature for each tumor. This signature, combined with clinical and pathological data would help us to establish the malignant potential of each individual tumor and deduce its potential resistance to conventional chemotherapy and radiotherapy. Obviously, in addition to molecular characterization of tumors for prognostic purposes, it is necessary to study factors that might be potential therapeutic targets, currently one of the most promising areas in the field of cancer treatment. With this functional approach it seems worthwhile to investigate whether these funnel factors can be critical targets for cancer treatment.

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Furin

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Synonyms

Furin; PACE; SPC1; PCSK3; Dibasic processing enzyme; Prohormone convertase; Paired basic amino acid cleaving enzyme

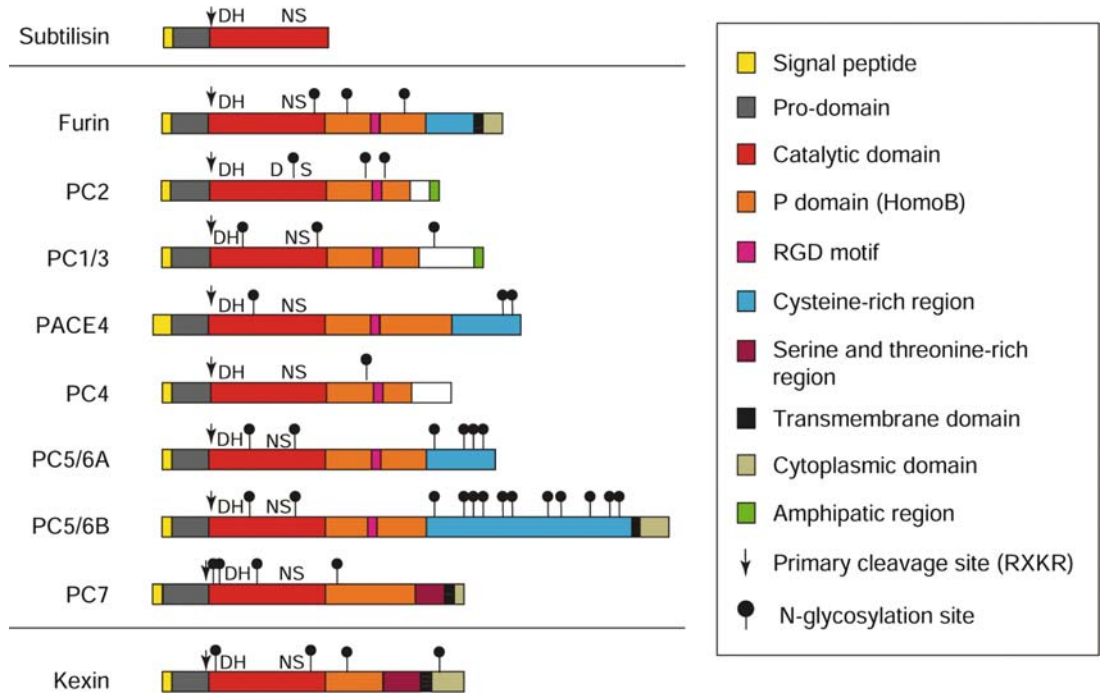
Definition

Furin (E.C. 3.4.21.75) is a highly specialized proteinase that cleaves the unique sequence motifs in a variety of proteins. Normally, following furin cleavage, the target protein is activated and, therefore, it can exhibit its biological activity. Because furin has been discovered first, currently it is the most studied enzyme of the proprotein convertase (PC) family of serine proteinases. Seven distinct proprotein convertases of this family (furin, PC2, PC1/3, PC4, PACE4, PC5/6, and PC7) have been identified in humans, some of which have ►isoforms generated as the result of ►alternative splicing. Structurally and functionally, furin resembles its evolutionary precursor: the prohormone-processing enzyme, kexin (EC 3.4.21.61), which is encoded by the KEX2 gene of yeast *Saccharomyces cerevisiae*. The polypeptide sequence of the furin ►catalytic domain is homologous to that of *Bacillus* subtilisin, an evolutionary precursor of PCs. Furin and related PCs are involved in the limited ►endoproteolysis (►protease activated receptor) of inactive precursor proteins which occurs at the sites marked by paired or multiple basic amino acids.

Characteristics

A wide variety of proteins are initially synthesized as parts of higher molecular weight, but inactive, precursor proteins. Specific endoproteolytic processing of these ►proproteins is required to generate the regulatory proteins in a mature and biologically active form. A large majority of these active proteins, including ►matrix metalloproteinases, growth factors, and ►adhesion molecules are essential in the processes of cellular transformation, acquisition of the tumorigenic phenotype, and metastases formation. The enzyme furin, which is encoded by the *fur* gene, was the first and can be considered the prototype of a mammalian subclass of subtilisin-like proteases. The localization of the gene immediately upstream from the FES ►oncogene (V-FES feline sarcoma viral oncogene homolog) generated the name FUR (for FES upstream region). Furin is similar to other PCs in that it contains a signal peptide, a prodomain, a subtilisin-like catalytic domain, a middle P domain, a cysteine-rich region, a transmembrane anchor, and a cytoplasmic tail (Figs. 1 and 2). Furin and PCs are normally N-glycosylated ►glycoproteins (►glycosylation). Phosphorylation of the cytoplasmic tail is required for the trans-Golgi localization of furin which in vivo exists as di-, mono- and non-phosphorylated forms. Propeptide cleavage is a prerequisite for the exit of furin molecules out of the ►endoplasmic reticulum. The second cleavage in the propeptide occurs in the ►trans-Golgi network, which is followed by the release of the propeptide bound to furin and the activation of furin.

Furin is expressed in all examined tissues and cell lines and is mainly localized in the trans-Golgi

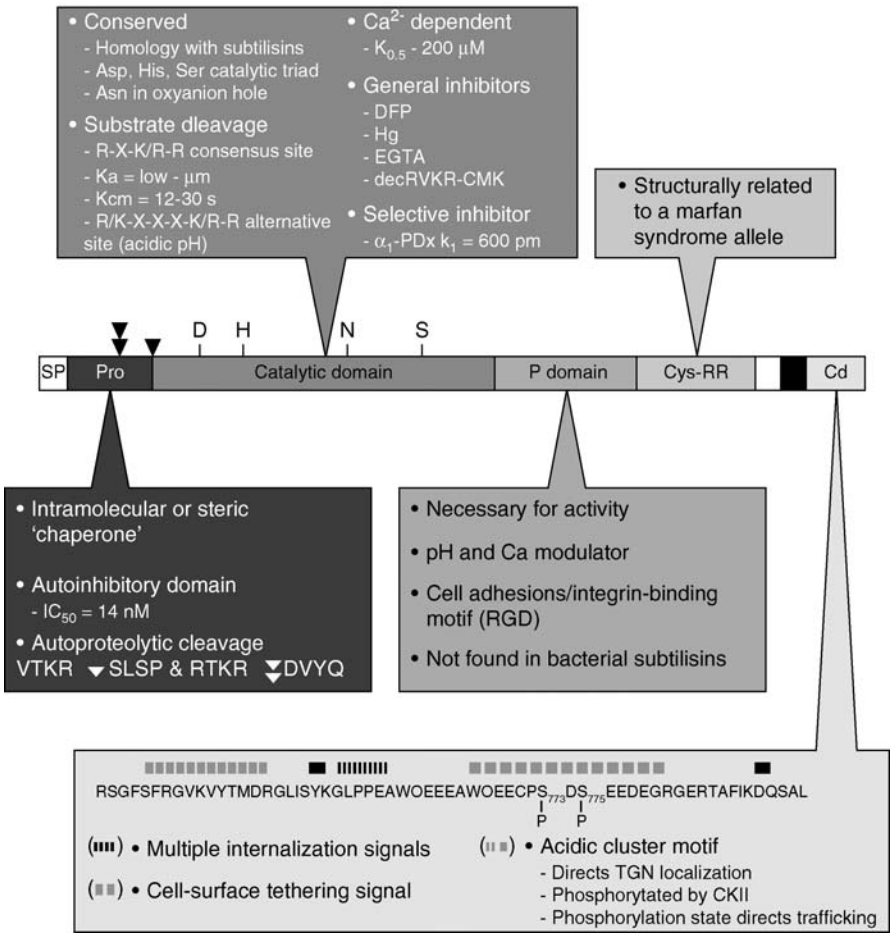


Furin. Figure 1 A modular domain structure of furin and six related PCs. The A and B isoforms of PC5/6 are encoded by the same gene. The structure includes (i) the N-terminal signal peptide, which directs proteins into the secretory pathway, (ii) a pro-domain, which maintains the inactive zymogen state of PCs and which also acts as an intramolecular chaperone for the proper folding, (iii) a catalytic domain with the active site that exhibits an Asp (D)-His(H)-Ser(S) catalytic triad and an additional Asn(N), (iv) a barrel-like structured P domain that regulates enzyme stability, (v) a C-terminal domain that contains membrane attachment sequences, a Cys-rich region and intracellular sorting signals. Adapted from [4].

network. Some proportion of the furin molecules cycles between the trans-Golgi and the cell surface. Furin represents the ubiquitous endoprotease activity within constitutive secretory pathways and normally it is capable of cleaving the Arg-X-(Lys/Arg)-Arg consensus motif, where X is any amino acid type (Table 1). Furin and related PCs are activating proteases and normally they do not inactivate polypeptides. Because of the overlapping substrate preferences and cell/tissue expression, there is a substantive level of redundancy in the PC functionality, albeit certain distinct functions of the individual PCs have also been demonstrated. Furin knockout, however, is lethal in mice. Furin null embryos die because they fail to accomplish ventral closure successfully and to form a looping heart tube. These processes require cellular migration and proliferation, both of which are regulated by furin. Through regulation of cellular migration and proliferation, furin plays an important, albeit incompletely understood role, in cellular transformation, acquisition of the tumorigenic phenotype, cancer progression and metastasis. The expression of furin, however, discriminates sharply between small cell lung cancers, which have no expression, and non-small cell lung cancers, in which furin is overexpressed.

The roles of furin and other PCs in cancer have been characterized as the result of many studies of gene expression and enzyme inhibition. Because of the redundancy, it is not always clear if all PCs present in cancer cell/tissue are directly relevant to tumorigenicity. An enhanced expression of furin and related PCs in cancer is not necessarily an indicator of a poor clinical outcome. There is, however, evidence that high levels of furin-related PCs contribute to tumor growth and metastasis by controlling the activation of key cancer-associated proteins, including matrix metalloproteinases and growth factors such as ►VEGF, ►TGFβ and ►PDGF. The multiple effects of PCs on cell proliferation, motility, adhesion and invasion have led to a concept that in the course of tumor development and progression PCs act as "master switches" of the key tumorigenic protein functionality. If this concept is valid, then PCs could be identified as important therapeutic targets in a number of cancer types. The challenge remains to identify the functionally-relevant, target PC in each cancer type, because it is unlikely that broad-range PC inhibitors would have significant clinically beneficial effects.

No natural protein inhibitors of furin are known. D-Arg-based peptides, α1-anti-trypsin Portland and,



Furin. Figure 2 Furin structure. The 794-residue pre-profurin sequence contains a 24-residue signal peptide, an 83-residue prodomain, a 330-residue subtilisin-like catalytic domain, a 140-residue middle P domain (also termed the "homo B," a 115-residue cysteine-rich region, a 23-residue transmembrane anchor, and a 56-residue cytoplasmic tail. Adapted from [1].

especially the synthetic peptidic inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone, are used to inhibit furin in the cleavage reactions in vitro and in cell-based tests. Inhibition of furin results in a significant reduction in tumor cell invasion. This reduction appears to be associated with a processing blockade of proteins directly involved in the mechanism of invasion including matrix metalloproteinases, growth factors and adhesion signaling receptors such as integrins.

PCs including furin are implicated in many pathogenic states because they process to maturity membrane fusion proteins and pro-toxins of a wide variety of both naturally occurring and weaponized bacteria and viruses, including anthrax and botulinum toxins and H5N1 bird flu, Marburg and Ebola viruses. After processing by furin and the subsequent internalization inthe complex with the respective receptor followed by acidification of the ►endosomal compartment (►endocytosis), the processed, partially denatured, infectious proteins expose their membrane-penetrating peptide region and escape into the cytoplasm. The intact toxins and viral proteins, however, are incapable of accomplishing these processes. Normally, the low pathogenicity viral subtypes have mutations in the cleavage site sequence and thus a reduced sensitivity to furin. Accordingly, proteolytic processing by furin is an important determinant in the overall pathogenicity of viruses and bacterial toxins. Based on these data, PCs, including furin, are promising targets for drug design in a variety of acute and chronic diseases including cancer and infectious diseases.

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Furin. Table 1 Furin targets and the sequence of the cleavage sites

Proteinases		P6 P4 P1↓P1′	
FURIN	Furin, two autolytic cleavage sites	RGVTKR↓SLSP	75–76
		KRRTKR↓DVYQ	107–108
MMP-11	Matrix metalloproteinase 11, stromelysin-3	RNRQKR↓FVLS	97–98
MMP-14	Matrix metalloproteinase 14, MT1-MMP	NVRRKR↓YAIQ	111–112
MMP-15	Matrix metalloproteinase 15, MT2-MMP	RRRRKR↓YALT	131–132
MMP-16	Matrix metalloproteinase 16, MT3-MMP	HIRRKR↓YALT	119–120
MMP-17	Matrix metalloproteinase 17, MT4-MMP	QARRRR↓QAPA	125–126
MMP-24	Matrix metalloproteinase 24, MT5-MMP	RRRNKR↓YALT	155–156
MMP-25	Matrix metalloproteinase 25, MT6-MMP	VRRRRR↓YALS	107–108
ADAM-9	A desintegrin and metallopeptidase domain 9	LLRRRR↓AVLP	205–206
ADAM-12	A desintegrin and metallopeptidase domain 12	ARRHKR↓ETLK	207–208
ADAM-19	A desintegrin and metallopeptidase domain 19	PRRMKR↓EDLN	105–206
ADAMTS1	A desintegrin and metalloproteinase with thrombospondin type-1 motif, 1	SIRKKR↓FVSS	252–253
ADAMTS4	A desintegrin and metalloproteinase with thrombospondin type-1 motif (aggrecanase-1), 4	PRRAKR↓FASL	212–213
ADAMTS13	A desintegrin and metalloproteinase with thrombospondin type-1 motif. 13	RQRQRR↓AAGG	74–75
BMP1	Bone morphogenetic protein 1	RSRSRR↓AATS	120–121
BMP4	Bone morphogenetic protein 4	RRRAKR↓SPKH	292–293
Meprin-A	Meprin A alpha	PSRQKR↓SVEN	653–654
BACE1	Beta-site APP-cleaving enzyme 1	GLRLPR↓ETDE	45–46
Serum proteins			
	Albumin	RGVFRR↓DAHK	24–25
VWF	von Willebrand factor	SHRSKR↓SLSC	763–764
F9	Coagulation factor IX	LNRPKR↓YNSG	46–47
PROC	Protein C	RSHLKR↓DTED	199–200
Extracellular matrix			
FBN1	Fibrillin 1	RGRKRR↓STNE	2731–2732
ZPC3	Zona pellucida glycoprotein 3	ASRNRR↓HVTE	301–302
Chaperone			
7B2	Secretogranin V	QRRKRR↓SVNP	181–182
Receptors			
ITGA3	Integrin alpha chain, alpha 3	PQRRRR↓QLDP	875–876
ITGA6	Integrin alpha chain, alpha 6	NSRKKR↓EITE	902–903
LRP1	Low density lipoprotein-related protein 1	SNRHRR↓QIDR	3943–3944
NOTCH1	Notch1	GGRRRR↓ELDP	1665–1666
INSR	Insulin receptor	PSRKRR↓SLGD	762–763
DSG3	Desmoglein 3	KRRQKR↓EWVK	49–50
MET	Hepatocyte growth factor receptor c-met	EKRKKR↓STKK	307–308
CUBN	Cubilin/vitamin B-12 receptor	LQRQKR↓SINL	35–36
SORL1	Sortilin-related receptor	PLRRKR↓SAAL	81–82
HGFR	Hepatocyte growth factor/scatter factor receptor	EKRKKR↓STKK	307–308
Growth factors and hormones			
IGF-1a	Insulin-like growth factor 1a/somatomedin C	PAKSAR↓SVRA	119–120
NTF3	Neurotrophin 3	TSRRKR↓YAEH	138–139
VEGFC	Vascular endothelial growth factor C	HSIIRR↓SLPA	227–228
NPPB	Natriuretic peptide B	TLRAPR↓SPKM	102–103
PTH	Parathyroid hormone	KSVKKR↓SVSE	31–32

Furin. Table 1 Furin targets and the sequence of the cleavage sites (Continued)

Proteinases		P6 P4 P1↓P1′	
TGFB1	Transforming growth factor, beta 1	SSRHRR↓ALDT	278–279
TNFSF12–TNFSF13	Tumor necrosis factor (ligand) member 12-member 13/proliferation-inducing ligand APRIL	RSRKRR↓AVLT	104–105
EDA-A2	Ectodysplasin a isoform	VRRNKR↓SKSN	159–160
NGFB	β-Nerve growth factor	THRSKR↓SSSH	179–180
	Semaphorin 3A	KRRTRR↓QDIR	555–556
Viral envelope glycoproteins			
HO	Hemagglutinin type H5	RRRKKR↓GLFG	344–345
F	Newcastle disease virus F fusion protein	GRRQKR↓LIGA	116–117
F	Parainfluenza HPIV3 F fusion protein	DPRTKR↓FFGG	109–111
P130	Sindbis virus structural polyprotein p130	SGRSKR↓SVID	328–329
prM	Flaviviral prM protein	SRRSRR↓SLTV	215–216, West Nile Virus
		HRREKR↓SVAL	205–206, Dengue virus
UL55	Cytomegalovirus/herpesvirus 5 protein UL55/glycoprotein B	THRTKR↓STDG	460–461
gp160	HIV-1 glycoprotein-160	VQREKR↓AVGL	498–499
Fo	Measles virus fusion protein	SRRHKR↓FAGV	115–116
E2	Infectious bronchitis spike protein	TRRFRR↓SITE	537–538
GP	Marburg virus spike glycoprotein	YFRRKR↓SILW	435–436
env	Ebola envelope glycoprotein	GRRTRR↓EAIV	501–502
BALF4/GP110	Epstein-Barr virus/herpesvirus 4	LRRRRR↓DAGN	432–433
Bacterial endotoxins			
ExoA	Pseudomonas aeruginosa exotoxin A	RHRQPR↓GWEQ	304–305
PA83	Anthrax protective antigen	NSRKKR↓STSA	196–197
α-Toxin	Clostridium alpha-toxin	KRRGKR↓SVDS	398–399
DT	Diphtheria toxin	GNRVRR↓SVGS	218–219
Aerolysin	Aeromonas aerolysin	KVRRAR↓SVDG	455–456
Shiga toxin	Shigella shiga toxin I subunit A	ASRVAR↓MASD	273–274

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Fusin

►Chemokine Receptor CXCR4

Fusion Genes

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Synonyms

Fusion proteins; fusion oncogenes; chimeric genes; chimeric oncogenes; chimeric transcripts; hybrid genes

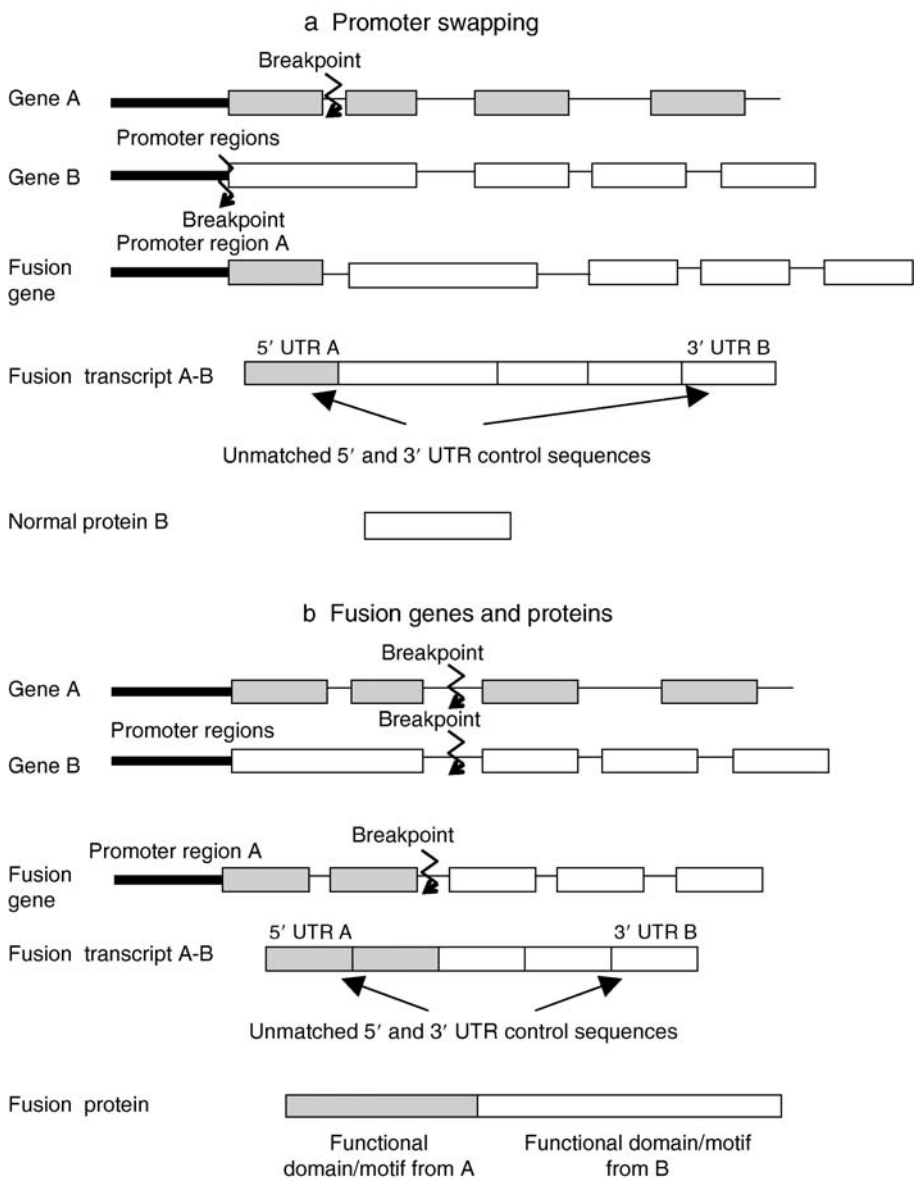
Definition

A hybrid gene created by joining portions of two different genes (to produce a new protein) or by joining a gene to a different promoter (to alter or deregulate a gene transcription).

Characteristics

A wide variety of recurrent molecular alterations has been associated with cancer including ►polymorphisms, changes in gene copy number (amplifications and deletions), point mutations, ►epigenetic modifications and gene fusions due to structural ►chromosomal rearrangements, such as translocations and less frequently inversions. As for these last ones, the genes located at the breakpoints of the rearrangement may be structurally changed with dramatic effects on their products. Molecularly, two events of structural aberrations can be generated: "promoter swapping" (the exchange of promoter control regions) or fusion gene.

In detail (Fig. 1a) "promoter swapping" occurs when the regulatory elements of a gene (promoter and/or enhancer) become aberrantly juxtaposed to a proto-oncogene, thus driving deregulated expression of an oncogene. Molecularly, the breakpoints of the rearrangements occur upstream from the coding region of the partner gene resulting in two chimeric genes which have exchanged their promoter regions, and less frequently noncoding exons. At the genomic level, the



Fusion Genes. Figure 1 A schematic representation of two events of stuctural aberations: (a) Promoter swapping and (b) Fusion genes and Fusion proteins.

3′ partner gene B is placed downstream of the 5′ gene A promoter region. The chimeric transcript contains 5′ ►untranslated regions (UTR) from the A gene and a coding region B that is intact and encodes a normal protein B. This mechanism can be exemplified by the three translocations that characterize Burkitt lymphoma: t(8;14), t(8;22), and t(2;8). All these rearrangements lead to the activation of *MYC*, located on 8q24, by juxtaposing the coding sequences of the gene to one constitutively active immunoglobulin (Ig) genes promoter or regulatory regions (IgH at 14q32, IgK at 2p12, and IgL at 22q11).

Fusion genes (Fig. 1b) arise when the coding regions of the two genes are juxtaposed, resulting in a chimeric transcript that produces a fusion protein with a new altered activity. In detail, in the majority of cases, fusion genes are formed when DNA breaks occur within two different genes mainly within the introns, A and B, and the gene fragments are joined in erroneous combinations. In most cases, the results are two fusion genes: A-B and B-A. On genomic level, the 3′partner gene B is placed under the 5′ gene A promoter control region which dominates the transcription control of the fusion gene. As a result, in the fusion protein the functional domains from the A and B proteins are brought together in a new abnormal combination. In cancer, the genes that are often interrupted by a chromosomal rearrangement are oncogenes , thus harboring fusion oncogenes. An appropriate example of a fusion oncogene is ►BRC/ABL characterizing chronic myelogenous leukemia which is driven by t(9;22)(q34;q11), also known as the ►Philadelphia chromosome, the first translocation to be molecularly characterized. In particular, the translocation fuses the *ABL* gene normally located on 9q34, with the *BCR* gene at 22q11. The BCR/ABL fusion created on the derivative chromosome 22 encodes a chimeric protein with an increased ►tyrosine kinase activity and abnormal localization. Table 1 enlists molecularly characterized recurrent chromosomal rearrangements found in cancers.

Formation Of Fusion Genes

Several factors influence the formation of fusion oncogenes and their role in tumorigenesis. Firstly, the rate at which fusion genes are formed is important. Literature suggests that at least some fusion genes are found in healthy individuals, implying that at least some gene fusions emerge at a notable rate. The mechanisms behind fusions are unknown but the occurrence of several double strand breaks that coincide in time and space are important. The proximity of damaged partner genes at the moments of repair is critical and the localization of chromatin and genomic regions in the interphase nuclei may be critical. Secondly, the presence of a fusion gene in a cell is not enough to cause cancer. Additional genetic or epigenetic changes are also needed and the risk for these additional events to occur affects the outcome. Thirdly, once the fusion is formed, its penetrance, i.e. the proportion of fusion carriers that develop tumors, is determined by selected mechanisms. Interestingly, many fusion oncogenes demonstrate a strict specificity for tumor type. The risk of getting a certain translocation could depend on cell type-specific processes that make the specific genes or DNA regions involved vulnerable to the translocation. It is clear that tumor development in different cell types and tissues locations involves many pathways, distinct genes, and also exogenous factors. A common mechanism for early genetic changes can however be distinguished in a number of different tumor types by specific chromosome rearrangements.

Moreover, the transcriptional orientation of fusion partner genes is essential in order to harbor functional fusion genes. At times, the partner genes are not oriented in the correct direction with regards to their transcriptional orientation, and more complex rearrangements are needed to fuse the partner genes into functional fusion genes. For instance, the EWS-ERG fusion is found in about 10% of Ewing sarcomas and it is the result of a complex rearrangement, a ►translocation and an ►inversion, given that the genes involved are not transcribed in the same centromeric/telomeric direction. This requirement and the necessary presence of critical functional protein parts seem to influence how frequently variant fusion genes are present in tumors. Moreover, to produce a functional fusion gene is necessary that the exons flanking the breakpoints can give rise to splicing events that maintain their reading frames. Overall, the factors that generate double-strand breaks are largely unknown.

Clinical Relevance

Studies over the past decades have revealed that recurring chromosome rearrangements leading to fusion oncogenes are specific features not only of leukemias and lymphomas, but also of certain epithelial tumors. Presently, over 600 recurrent balanced tumor-associated chromosomal rearrangements have been molecularly characterized. However, the data are strongly biased in favor of hematologic malignancies and sarcomas. An important example of a recurrent rearrangement which leads to the development of a targeted therapy is the t(15;17)(q22;q21) in ►acute promyelocytic leukemia which fuses the ►*PML* gene (15q22) with *RARα* gene at 17q21. The PML protein contains a zinc-binding domain called a “ring” finger that may be involved in protein–protein interaction. RARα protein encodes the retinoic acid alpha-receptor protein (►retinoic acid receptors a member of the nuclear steroid/thyroid hormone receptor superfamily. Although retinoic acid binding is retained in the fusion protein, the PML/RARα may confer altered DNA-binding specificity to the RARα ligand

Fusion Genes. Table 1 Molecularly characterized recurrent chromosome rearrangements and fusion genes in cancer

Disease	Affected gene	Rerrangement
Hematopoietic tumor		
Lymphoid		
►Anaplastic Large Cell Lymphoma	NPM-ALK	t(2;5)(q23;q35)
	TPM3-ALK	t(1;2)(q25;p23)
	TFG-ALK	t(2;3)(p23;q21)
	ATIC-ALK	inv(2)(p23q35)
	MSN-ALK	t(X;2)(q11-12;p23)
	CLTCL-ALK	t(2;17)(p23;q23)
►Burkitt Lymphoma, B-cell acute lymphoid leukemia	MYC (relocation of IgH locus)	t(8;14)(q24;q32)
	MYC (relocation of IgK locus)	t(2;8)(p12;q24)
	MYC (relocation of IgL locus)	t(8;22)(q24;q11)
B-cell precursor ALL	E2A-PBX1	t(1;19)(q23;p13)
	E2A-HLF	t(17;19)(q22;p13)
	TEL-AML1	t(12;21)(p12;q22)
	BCR-ABL	t(9;22)(q34;q11.2)
	MLL-AF4	t(4;11)(q21;q23)
	IL£-IgH	t(5;14)(q31;q32)
►Diffuse large B-cell lymphoma	BCL2-IgH	t(14;18)(q32;q21)
	BCL6- variant partners	t(3;v)(q27;v)
	BCL8-IgH	t(14;15)(q32;q11-13)
	FCGR2-Igλ	t(1;22)(q22;q11)
	MUC1-IgH	t(1;14)(q21;q32)
	NFKB2-IgH	t(10;14)(q24;q32)
Extranodal mucosa-associated lymphoid tissue	MALT1-API2	t(11;18)(q21;q21)
	MALT1-IgH	t(14;18)(q32;q21)
	BCL10-IgH	t(1;14)(p22;q32)
	BCL10-Igκ	t(1;2)(p22;p12)
Plasma cells myeloma	FGFR3-IgH and MMSET	t(4;14)(p16;q32)
	MAF-IgH	t(14;16)(q32;q23)
	MAF-Igλ	t(16;22)(q23;q11)
	CCND1-IgH	t(11;14)(q13;q32)
	MUM/IRF4-IgH	t(6;14)(p25;q32)
Pre-T cell lymphoblastic leukemia, lymphoma	MYC (Relocation to TCR α/δ locus)	t(8;14)(q24;q11)
	LYL1 (Relocation to TCRα/σlocus)	t(7;19)(q35;p13)
	TAL2 (Relocation TCRβ locus)	
	SCL (Relocation to TCR α/δ locus)	t(1;14)(p32;q11)
	OLIG2 (Relocation to TCR α/δ)	t(14;21)(q11;q22)
	LMO1(RBTN1) (Relocation to TCR α/δ)	t(11;14)(p15;q11)
	LMO2 (RBTN2) (Relocation to TCR α/δ)	t(11;14)(p13;q11)
	HOX11 (Relocation to TCR α/δ)	t(10;14)(q24;q11)
	HOX1-1L2	t(5;14)(q35;q32)
	CALM-AF10	t(10;11)(p13;q21)
	NUP98-RAP1GDS1	t(4;11)(q21;p15)
Myeloid		
►Acute promyelocytic leukemia	PML-RARα	t(15;17)(q21;q21)
	NPM-RARα	t(5;17)(q35;q21)
	PLZF-RARα	t(11;17)(q23;q21)

Fusion Genes. Table 1 Molecularly characterized recurrent chromosome rearrangements and fusion genes in cancer (Continued)

Disease	Affected gene	Rerrangement
►Acute myeloid leukemia	ETV6- variant partners	t(12;v)(p13;v)
Acute myeloid leukemia	NUP98-variant partners	t(11;v)(p13;v)
	MLL-variant partners	t(11;v)(q23;v)
	AML1-ETO	t(8;21)(q22;q22)
	CBFB-MYH11	inv(16)(p13q22)
	FUS-ERG	t(16;21)(p11;q22)
	CEV14-PDGFRB	t(5;14)(q33;q32)
	P300-MOZ	t(8;22)(q33;q32)
	MOZ-TIF2	inv(8)(p11q13)
	MOZ-CBP	
	DEK-NUP214	t(6;9)(p23;q34)
	RBM15-MKL	t(1;22)(p13;q13)
	MLF1-NPM1	t(3;5)(q25;q34)
	AML1-EVI1	t(3;21)(q26;q22)
Solid tumors		
Sarcomas		
Alveolar rhabdomyosarcoma	PAX3-FKHR	t(2;13)(q3?;q14)
	PAX7-FKHR	t(1;13)(q36;q14)
►Alveolar soft-part sarcoma	TFE3-ASPL	t(X;17)(p11;q25)
Angiomatoid fibrous histiocytoma	FUS-ATF1	t(12;16)(q13;p11)
Dermatofibrosarcoma protubeans	COL1A1-PDGFB	t(17;22)(q13;q13)
Desmoplastic small round cell tumor	EWS-WT1	t(11;22)(p13;q12)
Endometrial stromal sarcoma	JAZF1-JJAZ1	t(7;17)(p15;q21)
►Ewing sarcoma	EWS-FLI	t(11;22)(q24;q12)
	EWS-ERG	t(21;22)(q22;q12)
	EWS-ETV1	t(7;22)(q22;q12)
	EWS-E1AF	t(2;22)(q33;q12)
	EWS-FEV	t(17;22)(q12;q12)
	FUS-ERG	t(16;21)(p11;q22)
Infantile fibrosarcoma	ETV-NTRK3	t(12;15)(p13;q25)
Inflammatory myofibroblastic tumour	TPM3-ALK	t(1;2)(q22;p23)
	TPM4-ALK	t(2;19)(p23;p13)
	CLTC-ALK	t(2;17)(p23;q23)
Low grade fibromyxoid sarcoma	FUS-CREB312	t(7;16)(q33;p11)
Myxoid chondrosarcoma	EWS-CHN	t(9;22)(q22;q12)
	TAF2N-CHN	t(9;17)(q22;q11)
	TCF12-CHN	t(9;15)(q22;q21)
►Myxoid liposarcoma	FUS-CHOP	t(12;16)(q13;p11)
	EWS-CHOP	t(12;22)(q13;q12)
►Synovial sarcoma	SYT-SSX1	t(X;18)(p11;q11)
	SYT-SSX2	
	SYT-SSX4	
Soft-tissue clear cell sarcoma	EWS-ATF1	t(12;22)(q13;q13)

F

Fusion Genes. Table 1 Molecularly characterized recurrent chromosome rearrangements and fusion genes in cancer (Continued)

Disease	Affected gene	Rerrangement
Carcinomas		
►Follicular thyroid carcinoma	PAX8-PPARγ	t(2;3)(q13;p25)
►Papillary thyroid carcinoma	H4-RET (PTC1)	inv(10)(q11.2;q21)
	RIa-RET (PTC2)	t(10;17)(q11.2;q23)
	ELE1-RET (PTC3,4)	inv(10)(q11q22)
	RFG5-RET (PTC5)	
	TPM3-NTRK1 (TRK)	inv(1)(q21q22)
	TPR-NTRK1 (TRK-T1)	inv(1)(q21q25)
	TFG-NTRK1 (TRK-T3)	t(1;3)(q21;q11)
►Prostate cancer	TMPRSS2-ERG	inv(21)(q22.2;q22.3)
	TMPRSS2-ETV1	t(7;21)(p21.2;q22.3)
	TMPRSS2-ETV4	t(17;21)(q21;q22.3)
►Renal-cell carcinoma	PRCC-TFE3	t(X;1)(p11;q21)
	ASPSCR1-TFE3	t(X;17)(p11;q25)
	SFPQ-TFE3	t(X;1)(p11;p34)
	NONO-TFE3	inv(X)(p11;q12)
►Salivary gland tumors (malignant)	CTNNB1- PLAG1	t(3;8)(p21;q12)
	TORC1-MAML2	t(11;19)(q21;p13)
Secretory breast carcinoma	ETV6-NTKR3	t(12;15)(p13;q25)
►Non-small cell lung carcinoma	EMLH-ALK	inv(2)(p21;p23)

complex. Leukemia patients with the *PML/RARα* gene fusion have an excellent response to the all-trans retinoic acid treatment, which stimulates the differentiation of promyelocytic leukemia cells. Similarly, the molecular characterization of the t(9;22)(q34;q11) in chronic myelogenous leukemia, which generates the fusion oncoprotein BCR/ABL, lead to the development of a successful targeted treatment of imatinib.

In contrast to hematological neoplasia, our knowledge regarding fusion genes in solid tumors is very limited, due to the complexity and poor quality of their ►cytogenetic karyotypes, yet they constitute only the 10% of known recurrent balanced chromosome rearrangements. However, fusion oncogenes may be more common in epithelial tumors than previously thought. Usually, translocations in solid tumors result in gene fusions that encode chimeric oncoproteins. The first chromosome abnormalities to be molecularly characterized in solid tumors were an inv(10)(q11.2; q21.2), as the more frequent alteration, and a t(10;17)(q11.2;q23), in ►papillary thyroid carcinoma. These two abnormalities represent the cytogenetic mechanism which activate the proto-oncogene ►*RET* on chromosome 10, by generating the fusion genes forming the oncogene RET/PTC1 and RET/PTC2, respectively. Moreover, other chromosomal rearrangements leading to *RET* activation were recently described and listed in Table 1. A great impact in the study of solid tumors is foreseen by the recent identification of a large subset of ►prostate cancer harboring ►TMPRSS2/ERG, fusions, TMPRSS2/ETV1 and TMPRSS2/ETV4, generated by inv(21)(q22.2;q22.3), t(7;21)(p21.2;q22.3) and t(17;21)(q21;q22.3) respectively. In particular, the gene *fusion* of the 5′ UTR of TMPRSS2 (a prostate-specific gene) to *ERG* or *ETV1* (genes of the ►ETS family), was identified in the majority of prostate cancer. Although the clinical significance of those fusions is unknown, recent investigations indicate that the expression of TMPRSS2/ERG among prostate cancer patients is a strong prognostic factor for disease progression.

Although fusion proteins play an important role in oncogenesis, additional genetic alterations are essential in order to transform cells. Silencing the specific fusion genes that play fundamental roles for the corresponding tumor, blocking targets of fusion proteins and repressing the cooperating events are all promising therapeutic strategies that need to be further investigated. The detection of the intracellular targets of these fusions will harbor new and important insights into molecular pathways that underlie tumor development. Ultimately, a combination of these approaches with

conventional treatments may provide a powerful new approach to treat these fusion-positive tumors.

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Fusion Oncogenes

►Fusion Genes

Fusion Proteins

►Fusion Genes

FVC

Definition

Forced vital capacity. The volume of air that can be forcibly exhaled following maximal inspiration.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

FX

Definition

Human homologue of GDP-4-keto-6deoxymannose-3, 5-epimerase-4-reductase. This enzyme is rate-limiting in the GDP-fucose synthetic pathway.

►Fucosylation

FZD

Definition

Frizzled; seven-pass transmembrane Wnt receptors closely related to G protein-coupled receptors.

►Wnt Signaling

G1/S Transition

Definition

Of the ►cell cycle is when the cellular decision to start duplicating its DNA is initiated. Mediators regulating this process include ►cyclins, ►cyclin-dependent kinases (CDKs), and CDK-inhibitors together with the transcription factors ►E2F and ►pRb that control the ►restriction point after which DNA replication is initiated.

►*Myc* Oncogene
►Retinoblastoma Protein, Cellular Biochemistry

G Antigen

►GAGE Proteins

G2 Checkpoint Abrogation

Definition

A concept of anti-cancer therapy that relies on the inhibition of the G2 DNA damage ►checkpoint by small molecules in the presence of ►DNA damage. G2 checkpoint abrogation results in mitotic cell death.

►UCN-01 Anticancer Drug

G-protein Couple Receptor

Definition

GPCR; seven transmembrane receptor, 7TM receptor, is the largest protein family of transmembrane receptors. They transduce an extracellular signal (through ligand binding) into an intracellular signal (G protein activation) and are involved in a wide variety of stimulus-response pathways, from intercellular communication to physiological senses.

►Endothelins
►Protease Activated Receptor Family
►Adrenomedullin
►G-Proteins
►Receptor Cross-Talk
►Protease Activated Receptor
►Chemoattraction
►RHO Family Proteins

G-Proteins

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Synonyms

Heterotrimeric GTP-binding proteins; Heterotrimeric guanine nucleotide-binding proteins

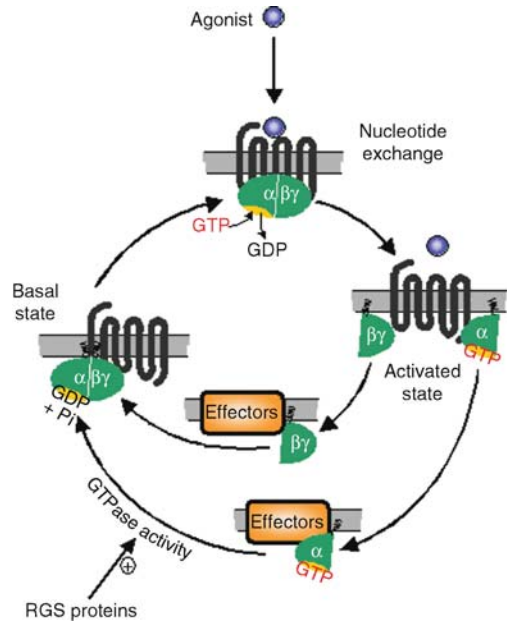
Definition

G-proteins are named for their ability to bind and hydrolyze the guanine nucleotide ►GTP. In the widest sense, the superfamily of guanine nucleotide-binding proteins comprises two structurally distinct classes, the monomeric GTP-binding proteins (also called ►monomeric GTPases) which are involved in a variety of cellular processes, and the heterotrimeric GTP-binding proteins which are primarily involved in transmembrane ►signal transduction by coupling membraneous receptors to various effector molecules. Traditionally, the term "G-protein" is only applied to the latter group, the heterotrimeric GTP-binding proteins.

Characteristics

Cellular functions in a living organism are regulated and coordinated by a huge variety of extracellular

signals including ►hormones growth factors, paracrine factors, neurotransmitters, or sensory stimuli. Many of these signals are received by cells through receptors on the plasma membrane which convey the incoming information by coupling to G-proteins which are attached to the inner side of the plasma membrane. More than 1,000 genes coding for G-protein-coupled receptors (►GPCR) have been identified making GPCRs one of the largest gene families of the mammalian genome. G-proteins activated through GPCRs regulate various effectors like enzymes and ion channels which produce intracellular signals resulting in specific cellular responses. G-protein functions are highly diverse due to their composition of different α-, β-, and γ-subunits, each of which are products of different genes. The α-subunit of the heterotrimeric G-protein possesses structural and functional homologies to other members of the guanine nucleotide-binding protein superfamily. The β- and γ-subunits of heterotrimeric G-proteins form an undissociable complex and represent a functional unit. Some G-proteins are very specialized like those expressed only in sensory cells; others appear to have functions in a wide variety of cells and tissues. Many G-proteins seem to have overlapping distributions and functions indicating that complex functional relationships exist among different G-proteins.



G-Proteins. Figure 1 The G-protein cycle. Upon activation of a G-protein-coupled receptor by binding of an agonist, GDP is released from the α-subunit of the heterotrimeric G-protein and replaced by GTP. This in turn leads to the dissociation of the α-subunit and the βγ-complex which are now able to interact with effector proteins. A GTPase activity inherent to the G-protein α-subunit terminates the G-protein activation. The hydrolysis of GTP to GDP can be accelerated by regulators of G-protein signalling (RGS) proteins as well as by various effectors.

Molecular and Cellular Regulation

In order to convey a signal from an activated receptor to an effector, the heterotrimeric G-protein undergoes an activation–inactivation cycle which allows the G-protein to function as a regulatable molecular switch (Fig. 1). In the basal state, the βγ-complex as well as the GDP-bound α-subunit is associated. In this form, the G-protein can be recognized by an appropriate activated receptor which interacts with the G-protein heterotrimer.

This interaction results in the dissociation of GDP from the α-subunit of the heterotrimeric G-protein. GDP is then replaced by GTP. Binding of GTP to the α-subunit induces a conformational change, which in turn leads to the dissociation of the α-subunit and the βγ-complex. The GTP-bound α-subunit as well as the βγ-complex is now able to interact with effector proteins. A ►GTPase activity inherent to the G-protein α-subunit terminates the G-protein activation. The formed GDP remains bound to the α-subunit which now reassociates with the βγ-complex. The reassociation of the heterotrimeric G-protein induced by the hydrolysis of GTP represents the inactivation mechanism for the βγ-complex. Two bacterial toxins specifically interfere with the G-protein activation–inactivation cycle and have been useful tools in studying G-protein-mediated signaling. ►Pertussis toxin blocks the interaction of activated receptors and various G-proteins whereas ►cholera toxin leads to the constitutive activation of some G-proteins. A physiological regulation of the GTPase activity of the α-subunit occurs by several effector proteins which interact with the GTP-bound α-subunit and accelerate their GTPase activity leading to G-protein inactivation. In addition, a family of proteins called "regulators of G-protein signaling" (►RGS proteins) are also able to increase the GTPase rate of the G-protein α-subunit.

More than 20 G-protein α-subunits have been described in the mammalian system, and they can be divided into four subfamilies based on structural and functional homologies (Table 1). The main properties of individual G-proteins appear to be primarily determined by the identity of the α-subunit of the heterotrimeric G-protein. While some G-protein α-subunits show a very restricted expression pattern others are expressed in a wide variety of tissues and some G-protein α-subunits like $G\alpha_s$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, and $G\alpha_{13}$ appear

G-Proteins. Table 1 Mammalian G-protein α-subunits

Class	Subtype	Expression	Effectors
$G\alpha_s$	$G\alpha_s$[a]	Ubiquitous	►Adenylyl cyclase ↑
	$G\alpha_{olf}$	Brain, olfactory epithelium	Adenylyl cyclase ↑
$G\alpha_{i/o}$	$G\alpha_{i1}$	Widely distributed	Adenylyl cyclase ↓
	$G\alpha_{i2}$	Ubiquitous	Adenylyl cyclase ↓
	$G\alpha_{i3}$	Widely distributed	Adenylyl cyclase ↓
	$G\alpha_o$[a]	Neuronal, neuroendocrine cells	VDCC ↓[b], GIRK ↑[b]
	$G\alpha_{gust}$	Taste cells, brush cells	►Phosphodiesterase ↑ ?
	$G\alpha_{t\text{-}r}$	Retinal rods, taste cells	Phosphodiesterase ↑
	$G\alpha_{t\text{-}c}$	Retinal cones	Phosphodiesterase ↑
	$G\alpha_z$	Neuronal, platelets	Adenylyl cyclase ↓
$G\alpha_q$	$G\alpha_q$	Ubiquitous	►Phospholipase C-β ↑
	$G\alpha_{11}$	Almost ubiquitous	Phospholipase C-β ↑
	$G\alpha_{14}$	Kidney, lung, spleen, testis	Phospholipase C-β ↑
	$G\alpha_{15/16}$[c]	Hematopoietic cells	Phospholipase C-β ↑
$G\alpha_{12}$	$G\alpha_{12}$	Ubiquitous	PDZ-RhoGEF, LARG
	$G\alpha_{13}$	Ubiquitous	p115-RhoGEF, PDZ-RhoGEF, LARG

VDCC, voltage-dependent Ca^{2+} channel; GIRK, G-protein-regulated inward rectifier potassium channel.
[a]Various splice variants.
[b]Effector is regulated by βγ-subunits.
[c]Species variants ($G\alpha_{15}$, mouse; $G\alpha_{16}$, human).
?, Regulation not shown directly.

to be expressed more or less ubiquitously. An individual cell expresses up to ten different G-protein α-subunits.

Five G-protein β-subunits and 11 γ-subunits have been described in the mammalian system. With the exception of the β_5-subunit which is expressed mainly in the central nervous system, the currently known β-subunits exhibit a high level of sequence homology (79–90%). In contrast, G-protein γ-subunits are much more heterogeneous. Similar to GTP-bound Gα, βγ-complexes can also regulate various effectors. The best examples of βγ-regulated effectors are particular isoforms of ►adenylyl cyclase and ►phospholipase C, as well as ion channels and ►phosphoinositide-3-kinase isoforms. With a few exceptions, there appear to be no major differences between different βγ-combinations with regard to their ability to regulate effector enzymes.

Stimulatory regulation of adenylyl cyclases through GPCRs involves G-proteins of the G_s-family of which two main members are known, G_s and G_{olf}.

The $G\alpha_{i/o}$-family members have been shown to mediate receptor-dependent inhibition of adenylyl cyclases. Since the cellular levels of these G-proteins are usually relatively high, they also represent an important source for βγ-complexes which can regulate a variety of cellular effectors. The G-protein G_o is the most abundant G-protein in the mammalian nervous system. G_o is involved in the inhibitory regulation of voltage-dependent Ca^{2+} channels, a process which appears to be mediated by the βγ-complex of G_o.

Several G-protein α-subunits are primarily expressed in sensory cells and have been involved in the signal transduction of sensory stimuli. Rod-transducin ($G_{t\text{-}r}$) and cone-transducin ($G_{t\text{-}c}$) play well established roles in the phototransduction cascade in the outer segments of retinal rods and cones where they couple light receptors to downstream signaling components of the retinal phototransduction cascade. In contrast to the transducins, the function of gustducin ($G\alpha_{gust}$) in taste cells is less well understood. Among the five taste qualities (sweet, umami, bitter, sour, and salty), sweet, umami, and bitter tastes appear to be transduced through heterotrimeric G-proteins.

$G\alpha_q$-family members mediate the pertussis toxin insensitive regulation of phospholipase C β-isoforms. The G_q-family consists of four members whose α-subunits are expressed from individual genes with different expression patterns. $G\alpha_q$ and $G\alpha_{11}$ appear to be expressed more or less ubiquitously and are primarily responsible for coupling of receptors to phospholipase C β-isoforms. In contrast, the murine G-protein α-subunit $G\alpha_{15}$ and its

human counterpart $G\alpha_{16}$ are only expressed in a subset of hematopoietic cells, and the expression of $G\alpha_{14}$ is restricted to several organs, e.g., kidney, testis, and lung. Receptors activating G_q-family members in mammalian systems do not discriminate between G_q and G_{11}.

The G-proteins G_{12} and G_{13} constitute the G_{12}-family and appear to be expressed ubiquitously. $G_{12/13}$ regulate the actin cytoskeleton through the activation of the monomeric GTPase Rho (►Rho-family proteins). This activation is mediated by a subgroup of guanine nucleotide exchange factors (►GEFs) for Rho including p115-RhoGEF, PDZ-RhoGEF, and LARG.

In recent years, genes of almost all G-protein α-subunits have been inactivated in mice. The resulting phenotypes of Gα-deficient animals have provided insights into the biological role of G-proteins demonstrating that G-protein-mediated signaling processes are crucially involved in multiple processes during development as well as in the adult organism.

Clinical Relevance

G-protein-mediated signaling processes are operating in all cells of the human organism. They are involved in many physiological and pathological processes. Many clinically relevant drugs function as agonists or antagonists of GPCRs and exert their effects through G-protein-mediated signaling pathways. Some diseases have been found to be caused by distinct defects in single G-protein α-subunits. Emerging experimental data indicate that GPCRs, e.g., the receptors for ►thrombin (►Protease-activated receptor), ►prostaglandin E_2 (PGE_2) (►Prostaglandins), lysophosphatidic acid (LPA), and sphingosine-1-phosphate (S1P), are crucially involved in tumor growth and ►metastasis.

►Gain-of-function mutations of the gene encoding $G\alpha_s$ (*GNAS*) give rise to the *gsp* oncogene (►Oncogene) which has been found in almost 30% of thyroid toxic adenomas as well as in some thyroid carcinomas (►Thyroid carcinogenesis) and growth hormone producing pituitary adenomas. The sporadic somatic mutation leads to the substitution of Arg201, the same residue which is ADP-ribosylated by cholera toxin, and results in a constitutively active form of $G\alpha_s$ by blocking its GTPase activity. This leads to activation of adenylyl cyclase independent of receptor agonists. The same sporadic mutation occurring early in embryogenesis results in a ►mosaicism which is responsible for the ►McCune-Albright syndrome characterized by polyostotic fibrous dysplasia of the bone, precocious puberty, and café-au-lait pigmentation of the skin.

An analogous mutation of the gene encoding $G\alpha_{i2}$ (*GNAi2*) (the *gip2* oncogene) has been found in human ovarian sex cord stromal tumors and adrenal cortical tumors (►Adrenocortical cancer).

In vitro studies indicate that expression of constitutively active forms $G\alpha_q$ and $G\alpha_{11}$ can lead to transformation of fibroblasts when expressed at low levels. No transforming mutants of $G\alpha_q$ and $G\alpha_{11}$ have been detected in human tumors, but various G_q/G_{11}-coupled receptors have been shown to be involved in the stimulation of proliferation in small lung cancer cells. The ►Kaposi sarcoma-associated herpesvirus (KSHV/HHV8) is suppose to encode a G_q/G_{11}-coupled receptor and signaling via this receptor has been shown to lead to cell transformation, tumorigenicity, and angiogenesis, and thus to critically contribute to KSHV-mediated oncogenesis.

GNA12 the gene encoding $G\alpha_{12}$ was identified as an oncogene (the *gep* oncogene) in soft tissue sarcoma. Constitutively active mutants of $G\alpha_{12}$ and $G\alpha_{13}$ have been shown to exhibit potent transforming activity in different systems. No activating mutation of $G\alpha_{12}$ or $G\alpha_{13}$ has been found in human tumors so far, however, increased expression levels of both proteins have been detected in various human cancers. In addition to their role in the regulation of cellular growth and their transforming ability, signaling via $G\alpha_{12/13}$-proteins has recently been implicated in the control of ►invasion and metastasis of breast and prostate cancer cell lines. By regulating a subgroup of RhoGEF-proteins, both $G\alpha_{12}$ and $G\alpha_{13}$ can activate the small GTPase RhoA which has been suggested to play an important role in cancer ►progression and invasion.

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GA733-2

►EpCAM

GABA

Definition

►Gamma-Aminobutyric Acid
►Photodynamic Therapy

GADD45

Definition

Growth arrest and DNA damage, a ►p53-responsive protein that induces a ►G2/M ►checkpoint of the cell cycle.

►Daxx
►G2/M Transition

GADD153

Definition

Growth Arrest DNA Damage 153 is a small nuclear protein that is capable of dimerizing with various ►transcription factors. Under normal cellular conditions this protein is not expressed in detectable levels, but is highly upregulated during times of cellular stress such as ►anoxia.

Gadolinium

Definition

A rare earth metal (lanthanide), gadolinium ions are chelated to form paramagnetic contrast agents for use in ►magnetic resonance imaging (MRI).

►Dynamic Contrast-Enhanced Magnetic Resonance Imaging

GAGE Proteins

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Synonyms

CT4; G antigen

Definition

Belong to the ►cancer testis antigen (CTA) family and consist of at least 16 highly homologous proteins (GAGE 1, GAGE2A-E, GAGE10, GAGE12B-J, GAGE13).

Characteristics

The 16 genes that encode GAGE proteins are located in an equal number of tandem repeats on chromosome X (p11.2-p11.4 region). GAGE 1 is unique among the GAGE proteins because of an exclusive C-terminal encoded by an exon that is interrupted in the other GAGE genes. The remaining GAGE members are composed of five exons encoding 116–117 amino acids with 98% identity, while GAGE1 consist of 138 amino acids. The molecular size of GAGE proteins is 26–29 kDa.

In contrast to other CTAs, the expression of which in adult normal tissues is restricted to germ cells of testis, GAGE is also expressed in a subset of oocytes of resting primordial follicles and in maturing oocytes of ovaries. In the testicular seminiferous tubuli, GAGE expression is restricted to the spermatogonia and, to a lesser degree, the primary spermatocytes. GAGE gene transcripts have been found in numerous cancers, most frequently ►malignant melanoma (24–42%), ►lung cancer (19–54%) ►thyroid cancer (30%) ►breast cancer (26%) ►hepatocellular cancer (38%) and ►ovarian cancer (30%) sarcomas (19%) (►Osteosarcoma, ►rhabdomyosarcoma), and ►bladder cancer (12%). Immunohistochemical studies have also identified GAGE in many cancers, but generally at a lower frequency than that observed by ►RT-PCR, including bladder cancer (40%) [00187], ►malignant melanoma (17%), ►lung cancer (16%) ►breast cancer (12%), and ►thyroid cancer (10%). The staining pattern varied significantly among and within specimens, and most GAGE-positive tumors also contained cancer cells lacking GAGE expression. GAGE has been correlated to poor prognosis in ►gastric cancer, ►esophageal carcinoma and ►neuroblastoma.

G

In vitro studies have shown that GAGE expression in tumors can be induced by the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (►Methylation), but the transcription of individual GAGE genes seems to be differently regulated, since the GAGE members are not always co-expressed.

GAGE proteins are immunogenic and lead to anti-GAGE antibody and ►cytotoxic T lymphocyte (CTL) responses in some cancer patients. The GAGE 1 gene was originally identified as encoding an antigenic peptide, YRPRPRRY, which was presented on a human melanoma by the MHC class I molecules HLA-Cw6 and recognized by a CTL clone derived from the melanoma patient. From the same patient, another CTL clone recognizing the peptide YYWPRPRRY, which is encoded by GAGE2A-E and presented by HLA-A29 molecules, was also isolated. Anti-GAGE antibodies are present in 6% of melanoma patients, but not in pancreas cancer patients.

Subcellular localization of GAGE in normal cells (e.g. germ cells) and cancer cells is similar; exhibiting weak cytoplasmic staining and variable nuclear staining. This suggests that GAGE, when expressed in cancer cells, is expressed in the natural context and thus may play a functional role therein. The nuclear localization of GAGE in spermatogonia and cancer cells suggests that GAGE may be a regulator of germline gene expression.

Due to the restriction of GAGE expression to immunoprivileged normal tissues and their relatively frequent expression in different types of cancer, GAGE proteins are considered attractive candidates for T cell-mediated, cancer-specific ►immunotherapy. However, the lack of GAGE expression in subsets of cancer cells within GAGE-positive tumors, as also observed for other CTAs, may limit its value as a therapeutic target. Combining different CTA targets may compensate for the heterogeneous expression of each CTA within cancers and improve the therapeutic potential.

The function of GAGE members remains largely unknown, although one study has reported anti-apoptotic properties of GAGE12 (formerly known as GAGE7). GAGE12-transfected cells were shown to be resistant to ►apoptosis induced by ►interferon-gamma or by the death receptor ►Fas/APO-1/CD95. In the Fas pathway, the anti-apoptotic activity of GAGE12 maps downstream of ►caspase-8 activation, and upstream of poly (ADP-ribose) polymerase (PARP) cleavage. Furthermore, GAGE12 renders the cells resistant to the chemo- and radio-therapeutic agents (►Chemotherapy of cancer, progress and perspectives, ►radioimmunotherapy), ►Taxol and gamma-irradiation.

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Gain of Function p53

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Definition

Gain of function is the property of some proteins whose alterations determine the acquisition of novel functions mainly opposite to those exerted by the ►wild-type counterparts.

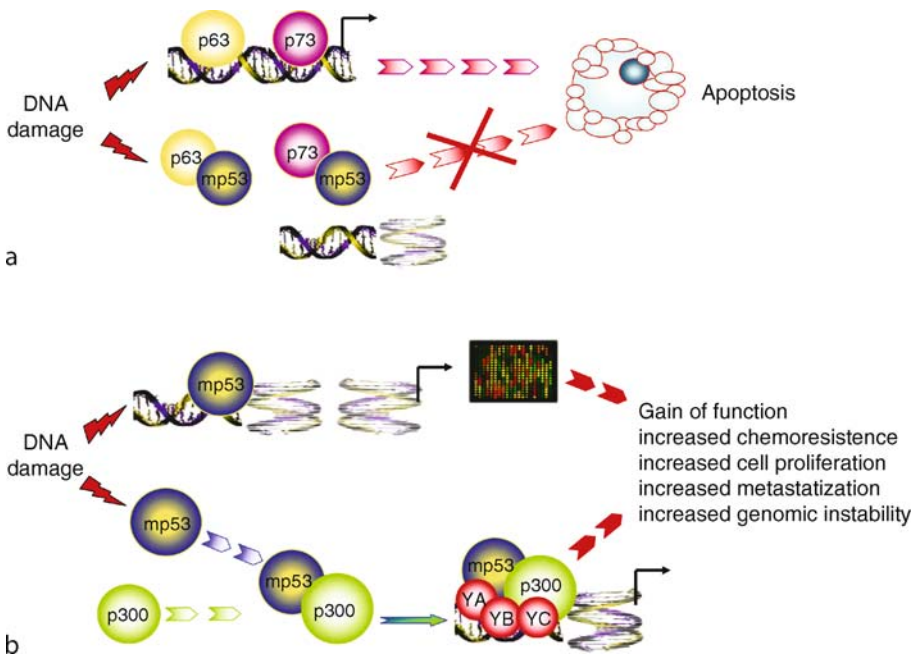
Characteristics

The gene product of the tumor suppressor ►p53 represents a paradigm of a protein whose alterations, mainly missense mutations, cause the acquisition of novel functions that contribute to the insurgence, the maintenance, the spreading and the ►chemoresistance of certain tumors. p53 is a ►transcription factor that can be roughly divided in three functional domains: (i) the N-terminus where resides the transcriptional activity; (ii) the central DNA binding domain that is responsible for the specific recognition of p53 binding sites; and (iii) the C-terminus domain that exerts oligomeric and autoregulatory activities. Half of human cancers bear p53 mutations that mainly occur within the specific DNA binding domain. The resulting proteins are characterized by the loss of the antitumoral activities of wild-type p53 (wt-p53) and, at least, for some of them by the gain of novel oncogenic activities. Many in vitro and, very recently, in vivo studies have shown that, at least, some of these mutant p53 proteins gain novel oncogenic functions that range from increased proliferation to enhanced chemoresistance to anticancer treatments. To date, the diverse mutant p53 proteins have been classified accordingly to their structural features. Indeed, they can be divided in two large classes: (i) DNA contact defective mutants whose missense mutation impacts on the region of the protein that contacts the DNA. The prototypes of this class of mutants are p53His273 and p53Trp248 that are also the

most frequent p53 mutations found in human cancers. (ii) Defective structure mutants whose missense mutation resides within the region of the stabilization of the internal loops (L2 and L3) of p53 and consequently their overall structure is quite different than that of wt-p53. Despite many ongoing attempts, a functional classification of p53 mutations that resembles their structures features has not been identified yet. By comparing wild-type versus mutant p53 two peculiar differences can be revealed. While wt-p53 is a short-living protein capable to activate target genes transcriptionally through the recognition of specific binding sites on their regulatory regions, mutant p53 is a very stable protein that is unable to bind wt-p53 consensus. There is scarce evidence on the molecular basis of the prolonged half-life of mutant p53 compared with that of wt-p53, but this peculiar feature might play a key role in gain of function.

Molecular Mechanisms

The molecular mechanisms underlying gain of function of mutant p53 proteins need to be clarified yet. To date, two scenarios can be proposed as molecular basis for mutant p53 gain of function. The first one relies on the prolonged half-life of mutant p53 proteins that are consequently very abundant in tumor cells. Thus, mutant p53 proteins can be engaged in many and multiple physical protein–protein interactions. One of the most studied is that with the newly discovered ►p53 family members, p73 and p63 (Fig. 1a). The latter, despite their critical roles in development and differentiation, have been shown to recapitulate most of the wt-p53 tumor suppressor activities when exogenously expressed in $p53^{+/+}$ and p53 null cells. Floating protein complexes involving mutant p53 and p73 or p63 have clearly evidenced in diverse tumor cell lines. The net result of these protein complexes is the severe impairment of p73/p63-mediated growth suppression and ►apoptosis. Chromatin immunoprecipitation experiments have clearly shown that mutant p53 severely impairs the in vivo recruitment of both p73 and p63 to the regulatory regions of their target genes. The polymorphism at position 72 of mutant p53 has been shown to be a critical determinant of the strength of the protein complex mutant p53/p73. Indeed, the 72R (Arg) forms of p53Ala143 and p53His175 mutants bind to p73 and impairs p73-mediated gene target transcriptional activation more efficiently than the equivalent 72P (Pro) mutants. The biochemical analysis of the protein complex mutant p53/p73 has revealed that the interaction surface is composed by the core domain of mutant p53 and the specific DNA binding



Gain of Function p53. Figure 1 Schematic representation of two molecular scenarios underlying gain of function of mutant p53. (a) Mutant p53 proteins form protein complexes with the newly discovered p53 family members, p73 and p63 (►p53 protein biological and clinical aspects). This results in the impairment of p73 or p63 recruitment on the regulatory regions of their target genes and of p73/p63-mediated apoptosis in response to DNA-damaging agents. (b-*upper part*) Mutant p53 binds directly to DNA through a consensus that remains to be identified yet; (b-*lower part*) Mutant p53 takes part to large protein complex facilitating the recruitment of the acetylase, p300. The final outcome is the transcriptional modulation of target genes responsible for the indicated biological effects.

domain of p73. These findings have discovered a novel function for the core domain of mutant p53, such as, its ability to function as a protein–protein interaction platform.

Is there any link between the selective pressure for p53 missense mutations in the core domain and gain of function activity of mutant p53 proteins? There is no sufficient evidence to make a conclusion. The vast majority of p53 mutant proteins are unable to bind wt-p53 binding sites present on the regulatory regions of its target genes and consequently are inefficient in the activation of wt-p53 mediated antitumoral effects. Opposite to such loss of function, mutant p53 proteins may acquire, through its mutated core domain, new properties that contribute to their gain of function. Despite many efforts, the presence of wt-p53/p73 protein complexes has not been identified in tumor cells. This might suggest that mutant p53 binds to a pool of selected proteins diverse than those bound by wt-p53. The presence of mutant p53 or wt-p53 in large and multiple protein complexes might represent a key determinant of their biological outputs. A paradigmatic example has been recently provided by the analysis of the transcriptional cross-talk between mutant p53 and the transcription factor NF-Y. The latter binds to both wt- and mutant-p53 but the transcriptional effects on NF-Y target genes are repression or activation, respectively. Is mutant p53 a bona fide transcription factor? (Fig. 1b) There is growing evidence accounting for transcriptional activity of mutant-p53 as molecular mechanism underlying its gain of function activity. This possibility mainly resides on the assumption that the N-terminus of mutant p53 in functionally intact and might exert specific transcriptional activity. To date, one of the key features of a bona fide transcription factor such as a specific DNA binding site for mutant p53 proteins has not been identified yet. This might suggest that mutant p53 can exert its transcriptional activity by engaging with DNA binding proteins, acetylases, deacetylases, and other proteins in the context of large protein complexes. If this hypothesis will be further demonstrated, as occurred for the transcriptional cross-talk mutant p53/NF-Y, it will mean that the plethora of the putative mutant p53 target genes is rather large, thereby providing the molecular basis underlying the diverse mutant p53-mediated oncogenic effects. There is scarce evidence on the role of mutant in these large protein complexes and specifically the contribution of its N-terminus transcriptional activation domain. It was originally shown that mutant p53 proteins, whose residues 22 and 23 were mutated, lost their oncogenic activity as well as their ability to transactivate specific target genes. Despite the molecular details need to be clarified yet, these findings strongly support a direct involvement of the N-terminus of mutant p53 in the activity of the large transcriptional competent protein complexes. The biochemical analysis of the spatial and temporal events regulating the cross-talk between mutant p53 and NF-Y has revealed a different transcriptional contribution of mutant p53 proteins (p53 protein biological and clinical aspects). Indeed, it was found that mutant p53 facilitates the recruitment of p300 acetylase, thereby indicating that it might serve as scaffold protein whose contribution results in the stabilization and proper activation of the transcriptional competent protein complex. Recent evidence has shown an additional molecular mechanism for mutant p53 gain of function activity. It is based on the tight association of mutant p53 with the nuclear matrix in vivo, and with high affinity to nuclear matrix attachment region (MAR) DNA in vitro. These findings suggest that mutant p53 interacting with key structural components of the nucleus, exerts its gain of function activity through the perturbation of the nuclear structure and function. As described earlier, the molecular scenario(s) underlying gain of function of mutant p53 are rather complex. It might be reasonable that a combination of specific protein–protein interaction and direct transcriptional activity takes place in driving gain of function of mutant p53. Many questions regarding the contribution of cell context, type of p53 mutations, posttranslational modifications are still unanswered and might be determinant in the final outcome of mutant p53 activities.

Clinical Aspects

Many in vitro and in vivo evidences have shown that the status of p53 is a key determinant of tumor aggressiveness and chemosensibility to common anticancer treatments. Tumors carrying p53 mutations are more resistant to the killing of anticancer agents and relapse more frequently than those bearing wt-p53. As a consequence of it, the overriding of mutant p53 gain of function might be extremely useful for treating mutant p53 tumors. Diverse approaches ranging from reactivation of mutant p53 to its wt-conformation as well as the elimination of mutant p53 have been undertaken in the last few years. In addition to them, the use of short peptides capable to specifically disassemble oncogenic protein complexes involving mutant p53 protein might be attempted to increase the chemosensibility of tumor cells. The well-established mutant p53/p73 protein complex could be a preferential target to be tackle with such approach. The amount of free and available p73 to be recruited in activating proapoptotic pathways in response to different anticancer treatments might be significantly increased by pretreating mutant p53 cells with short interfering peptides capable to disrupt the protein complex mutant p53/p73. These effects might render mutant p53 cells more prone to the killing of anticancer treatments.

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Gain-of-Function Mutation

Definition

Is any mutation of a gene that causes increased function and/or activity of its encoded protein or of a protein that is directly or indirectly regulated by the mutated gene.

►Gain of Function P53
►Gastrointestinal Stromal Tumor

GAK

►Cyclin G-Associated Kinase

Galactorrhea

Definition

Breast milk secretion at a time other than normal lactation.

►Prolactin

Galectin-3

Definition

Protein with an amino-terminal non-lectin domain and a carboxy-terminal lectin domain. Expressed in many cell types and binds glycoconjugates on the cell surfaces and ►extracellular matrix.

►CEA Gene Family

Gallbladder Cancer

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G

Definition

The biliary tract consists of an interconnected system of intra- and extrahepatic ducts that transport ►bile secreted from the liver to the digestive tract. The gallbladder is an important organ of the biliary system lying just under the liver, receiving, storing and then releasing the bile through bile ducts into the duodenum to help digesting fat. Gallbladder cancer (GC) is the most common type of cancer of the biliary tract.

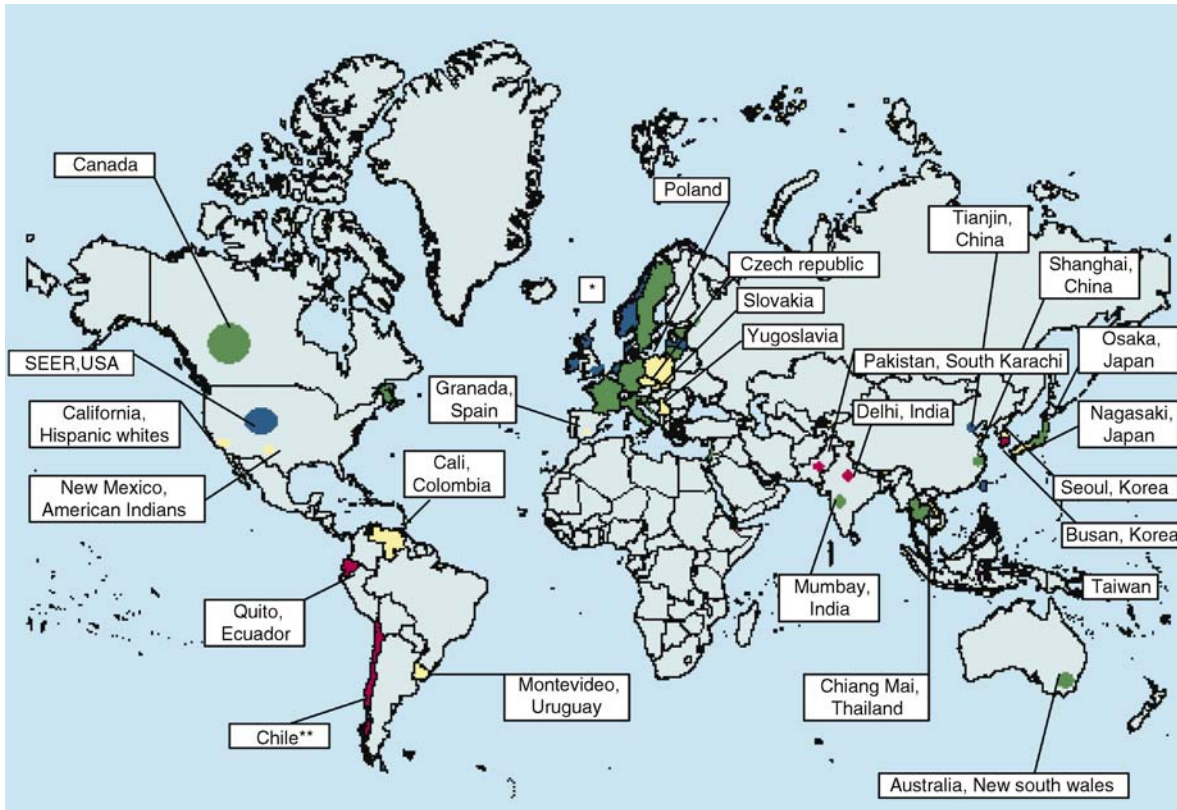
Characteristics

GC is a relatively rare neoplasm and despite being a non sex-related cancer is several-fold more frequent among women than among men. Detection of GC is quite difficult because symptoms and signs of GC are not specific and often appear late in the clinical course of the disease. For this reason, diagnosis is generally made when the cancer is already in advanced stages, and prognosis for survival is less than 5 years in 90% of cases.

Descriptive Epidemiology

GC incidence is characterized by a wide worldwide variation (Fig. 1) being low in several European countries and the United States of America (USA), relatively high in selected central European countries, and very high in some countries of Latin America and Asia. GC has been shown to be the first cause of cancer death among women in some areas of Chile.

According to ►incidence rates recorded by cancer registries in mid-1990s, the highest incidence rate worldwide was shown by women from Delhi, India (21.5/100,000), followed by South Karachi, Pakistan (13.8/100,000) and Quito, Ecuador (12.9/100,000). Cancer registries reporting high GC incidence rates were in Far East Asia (Korea and Japan), Eastern Europe (Slovakia, Poland, Czech Republic and Yugoslavia), and South America (Colombia). In Western Europe, elevated incidence rates were shown in Granada, Spain. Although



Gallbladder Cancer. Figure 1 Worldwide incidence of gallbladder cancer among women. Geographic areas according to incidence rates of mid-1990s from 40 selected cancer registries: areas in red are characterized by very high incidence rates (age-standardized rates ≥ 10 per 100,000 women); areas in yellow are characterized by high incidence (age-standardized rates between 6 and 10 per 100,000 women); areas in green are characterized by medium incidence rates (age-standardized rates between 2 and 6 per 100,000); areas in blue are characterized by low incidence rates (age-standardized rates < 2 per 100,000 women). *Countries in Europe were not specified because of space reasons: Germany, Italy, France, Slovenia, Croatia, Lithuania, Russia, Estonia shown medium incidence rates; Latvia shown low incidence rate. **No official incidence rate was available for Chile, but GC has been shown to be the first cause of cancer death among women in some areas of the country.

systematically lower than in women, high incidence rates among men (ranging between 4.4 and 8.0/100,000) were found in some areas of Asia and Eastern Europe.

Low incidence rates (below 3/100,000 women and 1.5/100,000 men) emerged for most registries from Northern Europe, with the partial exception of Sweden, and from the USA (SEER), and Canada.

The female to male (F/M) incidence ratio of GC incidence rates varied greatly: it was more than 5 in several high-risk areas (e.g., Pakistan, India, Colombia and Spain) as well as in low-risk areas (e.g., Denmark), but was typically between 2 and 3 in the majority of countries. F/M ratio was close to 1 in Korea, Japan and some part of China.

Incidence rates of GC in various ethnic groups from selected cancer registries in the USA confirmed the worldwide pattern, as GC was substantially more frequent among Hispanic than non-Hispanic white women and remarkably elevated among Korean and Chinese men. Very high incidence was also reported by men and women among American Indians in New Mexico. Also the F/M ratio reflected the worldwide situation being high among Hispanic whites, and close to 1 among Koreans, Filipinos, Japanese and Chinese.

Risk Factors

►Carcinogenesis of gallbladder is still poorly understood, and limited number of ►epidemiologic studies have been published on this issue because of (i) the rarity of GC in countries where most medical research is funded and performed, (ii) the difficulties of histological identification of GC, (iii) the lack of relevant animal models and tumor cell lines for GC, (iv) the lack of comprehensive national or international registries

for information on GC cases. ►Risk factors for GC include genetic predisposition, geographic variation and ethnicity, increasing age, female gender, chronic inflammation, congenital abnormalities, low socio-economic status, low frequency of ►cholecystectomy for gallbladder diseases and exposure to certain chemicals.

History of ►gallstones and ►cholecystitis are considered the major risk factors for GC. Several cohort and case-control studies found strong association between history of benign gallbladder diseases (mainly gallstones) and GC risk. Cholesterol and mixed gallstones (containing more than 50% of cholesterol) account for 80% of the all gallstones found, and pigment stones (composed largely of calcium bilirubinate) account for the remaining fraction. The etiology of cholesterol gallstones is thought to involve the interaction of genetic factors (e.g. modification of MDR3 and CYP7A1 genes, and numerous lithogenic genes) and several environmental factors (age, female gender, ►obesity, multiple pregnancies, a family history of gallstones and low levels of physical activity).

The worldwide distribution of gallstone prevalence shows a strong geographic and ethnic variation, and a positive correlation with the incidence rates of GC. High gallstone prevalence (≥50%) among women was found among American Indians in the USA, and among Mapuche Indians in Chile, both populations presenting very high GC incidence rates. Other areas with high or medium prevalence of gallstones were identified in South America, in Eastern and Western Europe. Very few is known about some regions of the world like India where high incidence of symptomatic gallstones has been observed, but results from ultrasound-based studies are not available. Low-risk areas for gallstones (i.e., prevalence <10% among women) included African countries, but also Thailand, China, Korea and Japan that reported high GC incidence rates.

Only a small proportion (1–3%) of patients with gallstones develops GC, thus other risk factors have been proposed to play a role.

Obesity and overweight are major risk factors for gallstones and large cohort studies showed that the association of GC with obesity is one of the strongest seen for any cancer sites. The influence of obesity, however, like the influence of belonging to certain ethnic groups, seemed to be at least in part mediated by an increased predisposition to develop gallstones.

The overall increased frequency of GC in women suggests a possible role for hormonal factors, especially in the formation of cholesterol gallstones. According to the results published by epidemiologic studies, high parity and high number of pregnancies, again recognized risk factors for gallstones, have been related to increased GC risk. Among parous women, older age at first birth or pregnancy has been associated with reduced risk of GC. The effect of oral contraceptive use was not materially related to GC risk, neither were duration of use and time since first and last use. Inconsistent results were obtained for the association of GC risk with menopausal status and HRT use. The exact role of women's hormones is thus still not clear.

Chronic infection of the gallbladder may contribute to the onset of GC, *per se* or via gallstone formation. Most available evidence implicates ►*Salmonella typhi* and *paratyphi* and *Helicobacter* species. All epidemiologic studies on *S. typhi* and *paratyphi* and GC based on ►biological markers, such as serum Vi antigen or the presence of the bacteria in bile specimens, found a significant positive association between carrier state of *S. typhi* and *paratyphi* and GC risk. Also *Helicobacter bilis* and *Helicobacter pylori* have been identified in bile specimens and associated with risk of biliary tract cancer. Unfortunately, most studies of infection and GC to date have been small (no more than 15 exposed cases), have lacked well matched controls (with or without gallstones) and have been hampered by the lack of standardized and non invasive methods for the detection of these infectious agents in large epidemiologic studies.

The association between dietary factors and GC has been also evaluated in some studies. Strong direct association has been found between risk of GC and total carbohydrate intake and total energy intake, and inverse one with fibers, vitamin V6, E, and C. However, apart from obesity, there is no nutritional nor dietary factor consistently related to GC risk. Life-style factors such as smoking and drinking alcohol have been found to be associated with obesity, gallstones and the risk of developing GC in some studies. Due to paucity of results and complexity of the issue, it is still difficult to derive consistent associations.

It has been proposed that two main pathways to GC exist worldwide. The predominant pathway involves gallstones and resultant cholecystitis and affects women to a greater extent than men. The other pathway involves an anomalous pancreatobiliary duct junction (APBDJ), a congenital malformation of the biliary tract that is more frequent in Japan, Korea, and possibly China, than in Western countries. With APBDJ, the pancreatic and common bile ducts join together before reaching the duodenal wall allowing reflux of secretions of the esocrine pancreas into the gallbladder. APBDJ seems specifically related to papillary carcinoma of the gallbladder, rarer in Western countries than in Japan, less invasive and fatal than other carcinomas of the gallbladder.

Conclusions and Perspectives

GC is a highly lethal and aggressive disease with a poor prognosis, but radical surgery can be curative when

proper investigations are done. For the time being, diagnosis of gallstones and removal of gallbladder currently represent the keystone to GC prevention in the majority of the population at high risk. Indeed, a strong inverse association between number of cholecystectomies and GC incidence and mortality rates can be found in many countries.

Behavioral interventions meant to prevent overweight and obesity are difficult to implement, but have the added benefit of preventing diabetes mellitus, cardiovascular diseases and a range of cancer sites in addition to GC, especially among women. If the etiologic role of *S. typhi* and *paratyphi*, *Helicobacter* species or other agents were better demonstrated, the benefits of prevention and treatment of these infections may be substantial.

►Bile Duct Neoplasms

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Gallstones

Definition

Are crystalline bodies formed within the gallbladder by accretion or concretion of normal or abnormal bile components. There are three main types: cholesterol gallstones, mixed gallstones, and pigment gallstones. Cholesterol and mixed gallstones account for 80% of the total, and pigment stones comprise the remaining 20%. Gallstones form when cholesterol and minerals in the bile clump together and form "stones" in the gallbladder. If a gallstone is large enough it can block the bile duct causing severe pain and cholestasis.

►Gallbladder Cancer
►Bile Duct Neoplasms

GALT

Definition

The gut-associated lymphoid tissues (GALT) are lymphoid tissues closely associated with the gastrointestinal tract, including the palatine tonsils, ►Peyer patches, and intraepithelial lymphocytes. GALT have a distinctive biology related to their exposure to antigens from food and normal intestinal microbial flora.

►Sjögren Syndrome

Gametic Phase Disequilibrium

Definition

►Linkage Disequilibrium

Gametogenic

Definition

Refers to the tissue types (ovary and testis) involved in the production of gametes.

►Cancer-Germline Antigens

Gamma-Aminobutyric Acid

Definition

GABA; Is an amino acid. As a neurotransmitter, it induces inhibition of postsynaptic neurons.

►Photodynamic Therapy

Gamma Delta (γδ) T Cells

Definition

γδ T cells, which represent only 5% of the total ►T cell population, use the gamma (γ) and delta (δ) chains to

form their heterodimeric T cell receptor (TCR). Unlike αβ T cells, they are not ►MHC restricted, recognize a variety of molecular species and have very limited TCR diversity. Like αβ T cells, they develop in the thymus but migrate from there into body tissues, especially epithelia and reside there. They are thought to be a first line of defense against pathogens that invade through the skin lung and intestine and are involved in skin wound repair.

►Immunoediting
►Chimeric T Cell Receptors

Gamma-Glutamylcysteine Synthetase

Definition

Is the first enzyme in the ►glutathione biosynthesis pathway. It catalyses the ATP-dependent condensation between the gamma-carboxylate group of glutamate and the amino group of cysteine to form the dipeptide gamma-glutamylcysteine.

►Alkylating Agents

Gamma Knife

Definition

A device used to treat ►brain tumors. A type of high-energy radiation that can be tightly focused on small tumors or other lesions in the head or neck, so very little normal tissue receives radiation. The gamma rays are aimed at the tumor from many different angles at once, and deliver a large dose of radiation exactly to the tumor in one treatment session.

►Glioblastoma Multiforme

Gamma-Ray Detection Probe

Definition

A device that can discern sites that emit a certain amount of gamma rays.

►Sentinel Lymph Nodes

Gamma-Ray Induced Cancer

►Radiation Carcinogenesis

Ganciclovir

►HSV-TK/Ganciclovir Mediated Toxicity

Ganglioneuroblastoma

Definition

Is a tumor consisting of Schwannian stroma with individually distributed neuronal elements.

►Neuroblastoma

Ganglioneuroma

Definition

Is a tumor consisting of intermingled microscopic foci of neuroblastic elements in an expanding Schwannian stroma, comprising more than 50% of the tumor volume.

►Neuroblastoma

Ganglioside Secretion

Definition

The release of ►gangliosides (acidic glucosphingolipids) from the surface of tumor cells into extracellular fluid. These lipids inhibit the growth of ►dendritic cells in their immediate vicinity, thus protect the cancer cells from immunological attack by the host.

►Sphingolipid Metabolism
►Gangliosides

Gangliosides

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Synonyms

Glycosphingolipids (sialic acid-containing)

Definition

A family of complex amphiphatic molecules, consisting of a carbohydrate and a lipid portion. The carbohydrate portion is comprised of one or more sialic acids linked to an oligosaccharide. The complex carbohydrate in turn is linked to ►ceramide, a lipid that consists of ►sphingosine and a fatty acyl residue.

Characteristics

Gangliosides, in addition to being ubiquitous components of normal cell membranes, are highly associated with tumors. A diverse group of molecules, marked structural differences characterize many gangliosides of tumor origin in comparison with gangliosides of normal tissues. Ganglioside biological activities can be highly dependent upon ganglioside molecular structure.

Structure

The ganglioside molecule consists of an oligosaccharide core to which is attached one or more sialic acids, and a hydrophobic lipid (ceramide) consisting of sphingosine (Sph, a long-chain base) and a fatty acid (FA) (Fig. 1), and is a product of ►sphingolipid metabolism. As an example, GM1, and major brain ganglioside, is shown in Fig. 1.

Individual carbohydrate species can be classified in ganglioside biosynthetic pathways. Their biosynthesis, in a sequential order of glycosylations, occurs via two main and several minor pathways. The major pathways are "a" (GM2, GM1a, GD1a) and "b" (GD3, GD2, GD1b, GT1b, GQ1b), stemming from a common precursor (GM3) derived from lactosylceramide. Each ganglioside is structurally more complex than its precursor molecule, and the stepwise addition of monosaccharide or sialic acid residues by specific membrane-bound glycosyltransferases in the Golgi apparatus, is catalyzed by the same glycosyltransferases in both pathways.

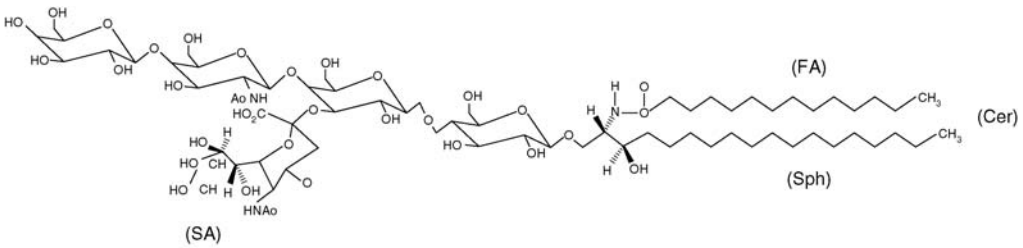
Oligosaccharide heterogeneity is complemented by structural diversity of the ceramide portion of the molecule, especially of tumor gangliosides. Ceramide structural differences may occur both in the sphingosine and in the fatty acyl group, and consist of at least three types of variations: length of the carbon chain, degree of unsaturation, and substitution by hydroxyl groups. All these have been shown to influence ganglioside biological activities. The predominant alteration in tumor ganglioside ceramide structure is significant variation in fatty acyl chain length. This complexity of structure, especially in tumor gangliosides, has implications for biological activity, and the particular importance of ceramide structure is suggested by studies showing differential functional effects of ceramide subspecies of tumor gangliosides.

Cellular Ganglioside Expression in Cancer

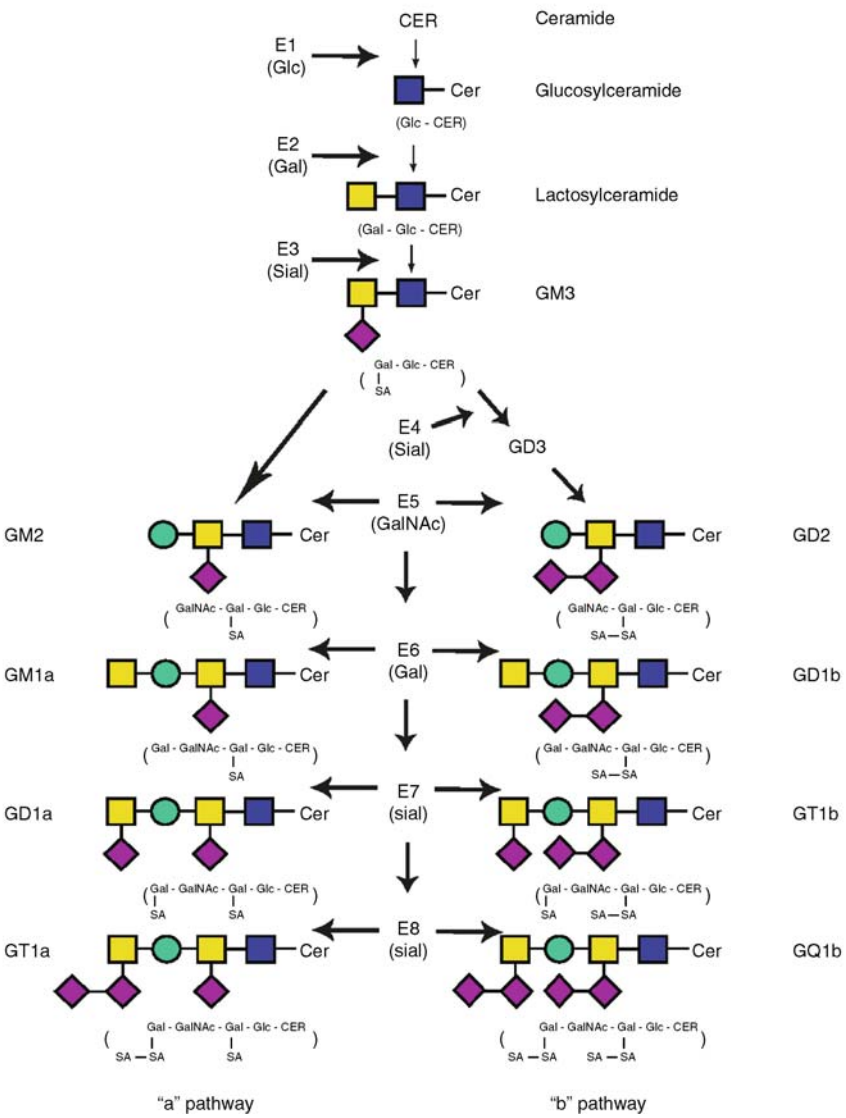
Gangliosides are normally found in all tissues and cells, in varying concentrations. They are present in highest concentrations in brain tissue. In cancer, there are both quantitative and qualitative changes in gangliosides (compared to analogous normal tissues) and these may serve as markers for tumors. The highly heterogeneous expression of gangliosides in normal and tumor tissues raises the possibility of their value as cell surface epitopes. Many monoclonal antibodies against specific gangliosides have been raised, one of the early examples being a monoclonal antibody to ganglioside GD2. GD2 was subsequently shown to be a universal marker for ►neuroblastoma as well as for other neuroectodermal tumors. Antibodies to gangliosides aid in identification of tumor cells and immunohistochemical analysis of tissues. When the expression of a ganglioside is operationally unique to a tumor type (such as GD2 in neuroblastoma), this may also have therapeutic application, such as ►monoclonal antibody therapy for the elimination of residual disease in partially treated patients. Finally, delineation and quantification of expression of specific ganglioside molecules on tumor cell surfaces (such as GD2 in neuroblastoma) has established their value as diagnostic and prognostic markers.

Ganglioside Shedding in Cancer

A striking characteristic of tumor cell ganglioside metabolism is the rapid rate of shedding from the cell surface. This is characteristic of many types of tumor cells, including, among others, neuroblastoma, lymphoma, melanoma, leukemia, and brain tumors. In humans, shed gangliosides can be detected in the peripheral circulation (e.g., in neuroblastoma, in which shedding of tumor-derived gangliosides correlates with the subsequent incidence and rate of tumor ►progression) and in cerebrospinal fluid (e.g., ►medulloblastoma and ►astrocytoma). Also extensively characterized in in vitro systems, this shedding process alters the tumor ►microenvironment: The release of specific gangliosides from tumor cells results in ganglioside enrichment of this ►microenvironment. In turn, these gangliosides



GM1 (Monosialoganglioside)



Gangliosides. Figure 1 The major pathways of ganglioside biosynthesis. GM3, derived from lactosylceramide, is the common precursor for both "a" and "b" pathway gangliosides. Each ganglioside consists of a ceramide back bone (CER), and a carbohydrate chain (glc = glucose, gal = galactose, GalNAc = N-acetylgalactosamine) containing one or more sialic acid (SA = sialic acid) residues. Parallel steps in both pathways are catalyzed by the same glycosyltransferases: (E1): Glucosylceramide synthase, (E2): Lactosylceramide synthase, (E3): GM3 synthase, (E4): GD3 synthase, (E5): GM2/GD2 synthase, (E6): GD1b/GM1a synthase, (E7): GT1b/GD1a synthase, (E8): GQ1b/GT1a synthase.

can be intercalated into the membrane of normal cells and alter their function.

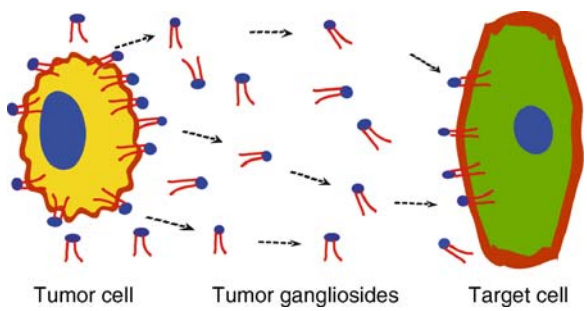
Functional Properties

Elucidating the functional properties of gangliosides has been a challenge facing investigators for over half a century. A role in brain function has long been suspected because of the high concentrations in brain tissue, and the critical role of gangliosides in brain development has been clearly shown through the use of ►knock-out mouse models, in which ganglioside biosynthetic enzymes shown in Fig. 1 have been deleted. Resulting consequences include a myriad of defects ranging from subtle neurologic defects to embryonic lethality. Defined human genetic ganglioside metabolic defects, with one recent exception, are catabolic defects, resulting in ganglioside storage. These diseases (e.g., Tay-Sachs disease) also have serious central nervous system consequences, including seizure disorders, mental retardation, etc.

More subtle functional properties of gangliosides have been uncovered through the study of the modulation of cell function by the addition of exogenous gangliosides, or by their depletion in cultured cells, using pharmacological inhibitors. These studies at the cellular level are delineating functional properties of gangliosides that may relate particularly to cancer progression, by the presence of tumor gangliosides in the tumor microenvironment (Fig. 2). Three such functional properties are highlighted:

Immunosuppression

More than two decades ago inhibition of mitogen and antigen-induced lymphoproliferative responses by normal brain gangliosides was observed. The first studies of tumor-derived gangliosides, in a mouse model, showed that tumor gangliosides markedly suppress cellular immune responses in vitro. A number of specific effects of exogenous gangliosides on the immune response have subsequently been characterized. A partial list includes inhibition of: ►antigen-presenting cell (e.g., ►dendritic cell) function, cytokine production, dendritic cell costimulatory molecule (e.g., CD80/CD86) expression and ►IL-12 production, ►T cell responses including cytotoxic effector function and proliferation of mitogen-activated lymphocytes, and interference with nuclear translocation of ►NFκB in monocytes and lymphocytes. The immune response to tumor in vivo is also significantly affected, immunosuppressive activity is influenced by ganglioside structure, and certain ganglioside structures, which are only minor components in normal tissue but are frequently major species in tumors (such as GD3 and GD2 ganglioside in neuroectodermal tumors), are in fact biologically very active ganglioside species. It is quite possible that certain shed tumor gangliosides function as intercellular signaling molecules, downregulate the cellular immune response, and thereby enhance tumor formation and progression according to the model in Fig. 2.



Gangliosides. Figure 2 Tumor cells rapidly synthesize gangliosides and shed them into their microenvironment, where they interact with, bind to, and affect the function of, normal cells within this tumor microenvironment.

Growth Factor-Induced Cell Signaling

It is modified by gangliosides because of the specific localization of gangliosides within the cell membrane, in glycosphingolipid-enriched domains. Existence in these domains puts gangliosides into close proximity with numerous biologically active cell surface molecules (such as growth factor receptors) with which they can then interact. The modification of cell surface ganglioside content, by the addition of exogenous gangliosides or by the inhibition of their synthesis and therefore depletion from cell membranes, has resulted in modification of ►signal transduction triggered by a number of different growth factors. Given the diversity of ganglioside molecules and the large number of biologically active growth factor receptors in the membrane, it is not surprising that a comprehensive unified understanding of the mechanisms of these interactions and their effects is still lacking. Nevertheless, some general characteristics have emerged, and these included that some simple gangliosides, such as GM3, may be inhibitory in their effects on growth factor signaling, whereas more complex gangliosides, such as GD1a, have an enhancing activity. In the tumor microenvironment, these effects may be highly significant with respect to promoting (or impeding) tumor growth, since a substantial number of ►receptor tyrosine kinases (►EGFR, ►VEGFR, ►FGFR, ►insulin receptor) have been affected by ganglioside modification of cell membranes, clearly pointing to a role of gangliosides as modulators of cell signaling.

Cell Proliferation and ►Angiogenesis

These are examples in which ganglioside modulation of cell signaling may affect a biological function critical

to tumor formation and progression. In this case, enrichment of normal fibroblast and vascular endothelial cell membranes by certain gangliosides, as they would be derived from tumors by shedding, results in significant upregulation of normal cell EGF and VEGF receptor dimerization, activation, and downstream signaling. In turn, this caused enhancement of EGF-induced fibroblast proliferation and ►VEGF-induced vascular endothelial cell proliferation and ►migration. Ganglioside enrichment of human umbilical vein vascular endothelial cells also caused even very low, normally barely stimulatory, VEGF concentrations to trigger robust VEGFR dimerization and autophosphorylation, downstream signaling, and cell proliferation and migration. By dramatically lowering the threshold for growth factor activation of contiguous normal stromal cells, shed tumor gangliosides may promote tumor progression by causing these normal cells to become increasingly autonomous from growth factor requirements.

Conclusions

In addition to being generally recognized as important in the central nervous system and its development, gangliosides, especially as derived from tumors, may be viewed as a new class of intercellular signaling molecules, associating with antigen-processing/presenting cells to inhibit accessory cell function interacting with lymphokines to inhibit cytokine-mediated lymphocyte proliferation, altering the expression of cell surface receptor molecules such that the cell has altered responsiveness to proliferative stimuli, and enhancing receptor-mediated cell signaling and function. These potentially protumorigenic effects of gangliosides suggest that targeting glycosphingolipid synthesis with pharmacologic inhibitors might impede tumor progression, a hypothesis currently being tested in view of striking inhibition of tumor progression observed using inhibitors of glycosphingolipid synthesis, which have been shown to decrease ganglioside synthesis and shedding.

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Gankyrin

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G

Synonyms

PSMD10; Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10; 26S proteasome regulatory subunit p28; p28; dJ889N15.2

Definition

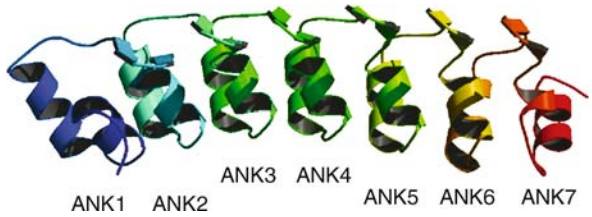
Gankyrin (Gann ankyrin-repeat protein; "Gann" in Japanese means cancer) is a small, 25kD, 226 amino acid protein originally identified as the product of an oncogene commonly overexpressed in human ►hepatocellular carcinoma (►HCC). Independently, it was purified as the p28 component of the 19S regulator (PA700 complex) of the ►26S proteasome. The gene is localized on human chromosome Xq22.3, and produces at least nine transcript variants encoding nine distinct proteins. Gankyrin associates with cyclin dependent kinase 4 (►CDK4) to control the phosphorylation of the ►retinoblastoma protein pRB and with the ►E3 ubiquitin ligase ►MDM2 to control the ubiquitylation and degradation of ►p53 (and possibly ►pRB).

Characteristics

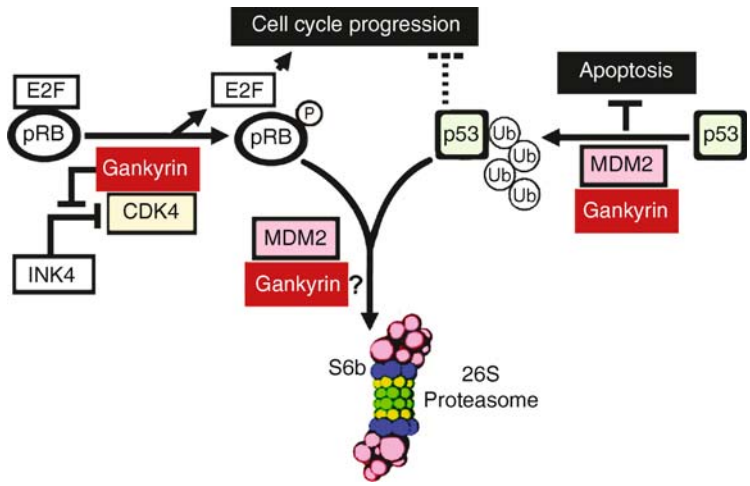
Gankyrin is a small cytoplasm–nucleus shuttling protein highly conserved throughout evolution (~40% identity to yeast Nas6p). Structurally, gankyrin consists of seven, not five or six as originally suggested by its amino acid sequence, ►ankyrin repeats (Fig. 1). Ankyrin repeat is the functional domain involved in protein–protein interactions, and gankyrin has been shown to interact with multiple proteins including the S6b ATPase (also known as Rpt3, TBP7, or PSMC4) of the 19S regulator of the 26S proteasome.

pRB and Gankyrin

Gankyrin plays a key role in regulating the cell cycle. Gankyrin contains the pRB-recognition motif LxCxE (178LACDE182) in the C-terminal domain, and binds pRB, but not pRB-related protein p107 or p130, in vitro and in vivo. Enforced expression of gankyrin in immortalized mouse fibroblasts and human tumor cells



Gankyrin. Figure 1 Structure of gankyrin: A ribbon representation. The polypeptide chain is color-ramped from its N-terminus in blue to the C-terminus in red. ANK1–7 indicates the seven ankyrin repeats.



Gankyrin. Figure 2 Activities of gankyrin on cell cycle control and apoptosis: In the presence of gankyrin, CDK4 is protected from the inhibitory effect of INKs (p16INK4A and p18INK4C). pRB is hyperphosphorylated and degraded, whereas E2Ftranscription factors are released to trigger expression of DNA synthesis genes and progression of cell cycle. p53 is polyubiquitylated by MDM2 and degraded to inhibit p53-dependent apoptosis and cell cycle arrest. P, phosphate; Ub, ubiquitin.

confers growth in soft agar and tumor formation in ►nude mice. The transforming activity of gankyrin correlates with its ability to bind pRB (e.g., the LxCxE point mutant E182A is inactive). Binding of gankyrin to pRB increases the rate of pRB degradation, suggesting that increased expression of gankyrin promotes tumorigenicity by targeting pRB to the ►proteasome. Gankyrin deactivates the pRB tumor suppressor pathway at multiple levels (Fig. 2). Gankyrin also binds CDK4, competing with p16►INK4A and p18INK4C, inhibitors of cyclin-dependent kinases (INKs), for binding to CDK4. Gankyrin displaces INKs, which results in active CDK4, hyperphosphorylation of pRB (together with other G1/S kinases), and release of the ►E2F transcription factor to activate DNA synthesis genes. Gankyrin does not bind to CDK6.

Gankyrin seems to play a role in cell cycle progression in normal cells as well. The upregulation of gankyrin correlates with cell cycle progression in normal rat primary hepatocytes and oval cells after mitogenic stimulation. Upregulation of gankyrin is also observed in the liver tissue of patients with fulminant hepatic failure, a model of active proliferation of human hepatocytes associated with liver regeneration.

p53 and Gankyrin

When overexpressed, gankyrin inhibits ►apoptosis in tumor cells that have been exposed to DNA-damaging agents. This antiapoptotic activity is, at least partly, due to increased degradation of p53, resulting in the reduced transcription of p53-dependent proapoptotic genes. Gankyrin binds to the E3 ubiquitin ligase MDM2 in vitro and in vivo, which increases p53–MDM2 association, thereby increasing the ubiquitylation and subsequent proteasomal degradation of p53. The mechanism by which gankyrin increases the binding of p53 to MDM2 and facilitates the ►ubiquitylation of p53 is currently unknown. However, it is known that gankyrin binds to a region of MDM2 (amino acids

411–438) just N-terminal to the ►RING finger domain. This puts the gankyrin-binding region in a central position, as the N-terminus of MDM2 binds p53 and the C-terminus contains a RING site that enables association with an E2 ubiquitin conjugating enzyme. Although not definitively proven, the fact that gankyrin binds to both the proteasomal S6b ATPase and MDM2 suggests a mechanism for the degradation of p53 by linking the ubiquitin ligase (MDM2) via gankyrin directly to the 19S regulator of the 26S proteasone. Such a mechanism could extend to other key cell-cycle regulators including pRB.

Gankyrin also controls MDM2 autoubiquitylation, especially in the absence of p53. Gankyrin does not bind to other p53 ubiquitylating enzymes, Cop1, Pirh2, p300 or the HECT ubiquitin ligase E6 AP.

Miscellaneous

In the yeast *Saccharomyces cerevisiae*, gankyrin (known as Nas6p) is a part of the multisubunit complex containing Sem1p (►DSS1 in mammals). Sem1p is also a proteasome subunit. Together with ►BRCA2 (breast cancer susceptibility gene 2), DSS1 regulates the activity of ►RAD51 recombinase and the formation of RAD51-positive DNA repair foci in response to ►DNA damage. These findings suggest a role of gankyrin in the ►DNA repair response.

In the hyperplastic liver of the transgenic zebrafish larvae overexpressing either one of the Bcl-2-related genes, zfBLP1 and zfMcl-1a, gankyrin is one of several genes that are upregulated.

Gankyrin upregulation is implicated in ►RAS-mediated transformation in rodent cells. Gankyrin is a promiscuous small ankyrin-repeat protein. In addition to binding S6b ATPase, pRB, CDK4, and MDM2 described earlier, gankyrin binds to or form a complex with MAGE-A4, Rel A, Tat, Integrase, and Vif of the ►human immunodeficiency virus 1, and probably others. Biological significance of these bindings is presently unknown.

Clinical Aspects

Gankyrin was discovered by constructing subtracted cDNA libraries from human noncancerous liver and ►HCC. The initial and subsequent studies have demonstrated that gankyrin mRNA is overexpressed in most HCCs.

The importance of the dual inactivation of both the pRB and p53 pathways for oncogenesis has been demonstrated, and gankyrin inhibits both of them. In a rodent hepatocarcinogenesis model, hypermethylation of the p16INK4A gene and p53 mutation appear at a late stage, whereas gankyrin is overexpressed from early stage after carcinogen treatment, preceding the loss of pRB protein and adenoma formation. Clinically, gankyrin mRNA is upregulated in early HCCs compared with dysplastic nodules in ►HCV ►cirrhosis. p53 mutation is not so frequent in HCCs, especially in low-grade or low-stage HCCs. These results suggest that gankyrin has important roles from early stages of hepatocarcinogenesis.

Whether gankyrin expression is deregulated in cancers other than HCC has not been extensively studied, but the ►SAGE analyses suggest its ubiquitous low expression in normal tissues and overexpression in several tumors including those of the brain, breast, lung, ovary, prostate, and stomach.

Overexpression of gankyrin is suggested to be of use as one of the diagnostic and subclassification markers for ►hyperdiploid ►acute lymphoblastic leukemia (ALL) with more than 50 chromosomes. Pediatric ALL is a heterogenous disease, with individual leukemia subtypes differing in their response to chemotherapy. Patients with hyperdiploid ALL with more than 50 chromosomes, one of the six prognostic subtypes, have an excellent prognosis.

As a dual-negative regulator of pRB and p53, gankyrin is a rational cancer therapeutic target. Overexpression of ►MAGE-A4, one of the melanoma antigens and a gankyrin interactor of unknown function, suppresses the tumorigenic activity of gankyrin. When overexpressed, gankyrin has antiapoptotic activity. Conversely, downregulation of gankyrin expression by small interfering RNA (►siRNA) or ►antisense oligonucleotides increases p53 protein levels and function, increases ►caspase activities, and promotes ►apoptosis of tumor cells in vitro. Growth of human HCC cells transplanted to nude mice is suppressed by intratumoral injection of ►adenovirus delivering siRNA against gankyrin ►Drug Delivery Systems for Cancer Therapy. These findings illustrate the importance of gankyrin functions for proliferation of HCC. Currently, response rate of HCC to systemic chemotherapy is only 0–25%, and has never been shown to prolong survival of the patients. Blocking expression and/or function of gankyrin might be a valuable therapeutic and preventive strategy against human HCC.

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GAP

Definition

GTPase-activating proteins. A type of regulatory proteins that bind to Ras or Ras-related GTP-binding proteins and inactivate them by stimulating their ►GTPase activity.

►Semaphorin
►RAS Activation

Gap Junctions

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Definition

Are intercellular channel structures located in the ►plasma membranes of neighboring cells that mediate transfer of materials up to a molecular weight of 1,000 Da.

Characteristics

Transferred materials comprise ions, growth factors, and second messengers, among others. This transfer seems to be passive and nonselective. Channels (►Ether à–go–go potassium channels) are formed by two hemichannels or ►connexons, every one embedded in the plasma membrane of adjacent cells and connected to each other to form hydrophilic, tube-like pores that allow transfer of material from one cell to another. In some cases, only one hemichannel is present allowing material to pass from the cell into the intercellular space. Individual channels accumulate in regions of the plasma membrane forming the typical ►plaques observed in the microscope. One plaque may contain up to 1,000 channels.

The connexons are formed by six molecules of ►connexin proteins, each of which contains four transmembrane regions. Connexins are a family of at least 20 members in humans, with similar structures and different molecular weights. Both, the N-terminal and the C-terminal end of the proteins are located intracellularly, with the C-terminal end being of different length in the different connexin types.

The capacity of gap junctions to mediate cell–cell communication (coupling) can be measured by ►metabolic cooperation, ►electrical coupling, or by estimating the transfer of a diffusible dye from a cell to their neighbors. Dye transfer is the most commonly used method of gap–junction analysis. In this method, one cell is microinjected with a solution of a low molecular weight fluorescence material like ►Lucifer yellow and diffusion to neighbor cells is recorded under the microscope after defined times.

Coupling between cells is dependent on connexin phosphorylation. In general it can be postulated that increased phosphorylation results in decreased coupling.

The main function of gap junctions is probably to maintain ►homeostasis in cells and tissues. This includes growth control, differentiation, or death through the pass of signaling molecules from one cell to their neighbors.

Gap junctions play the most important role during experimental carcinogenesis as well as in primary human tumors. Essentially, cellular ►transformation is associated with a decrease in gap junctions-mediated cell–cell communication. This decrease is due not only to the failure of formation of channels, but also to the downregulation of connexin expression at the protein and RNA levels. Some tumoral cell lines seem to display average amounts of connexins and show regular cell–cell communication between the malignized cells, but not between malignant and normal cells. This implies that malignant cells will grow independently of the homeostatic control mechanisms displayed by the normal, neighbor cells.

A similar reduction of gap junction-mediated cell–cell communication is observed in cells treated with carcinogens or tumor promoters, but not in cells treated with mutagenic (noncarcinogenic) substances. ►Oncogenes like ►ras or ►src or growth factors like ►EGF close gap junctions through different molecular mechanisms. Consequently, growth inhibitors like ►retinoids or dexamethasone increase cell–cell communication.

In some cases, a noticeable reduction in gap–junction communication is observed in ►preneoplastic changes, being the reduction in connexin expression stronger with increased malignancy of the lesion, as found in prostate tumors. In other cases, like the larynx, no such differences have been found between normal and premalignant lesions. In some experimental models, restoration of gap junction is associated with loss of malignancy and cell differentiation, which suggests that connexin proteins are tumor suppressors. In vitro transfection of connexin genes into malignant cells results in restoration of the gap junctions and growth inhibition. Nevertheless, under in vivo conditions, and using mice deficient in connexin expression, no direct correlation between gap–junction communication and spontaneous malignant growth has so far been established.

Most of these studies have been performed using cell lines derived from human tumors but reliable information concerning the situation in primary human tumors is not yet available. In several of these studies, the

amount and localization of connexins has been taken as a measure for cell–cell communication, an assumption that is not always valid.

Clinical Relevance

The property of the cells to communicate through gap junctions has been used as the basis for a cancer treatment strategy. The theory behind this strategy is that cells may pass toxic, low molecular weight substances to their neighbors through gap junctions. This will result in a bystander effect increasing the efficiency of the standard therapies. Although under *in vitro* conditions the strategy seems to be promising, no results have so far been obtained in primary human tumors.

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Gαq

Definition

Alpha subunit of heterotrimeric ►G protein q (G protein activating ►phospholipase C).

►Calcitonin

Gardner Syndrome

Definition

A rare hereditary disorder that is characterized by the presence of multiple polyps in the colon. Patients may also develop bone and soft tissue tumors. The co-existence of ►familial adenomatous polyposis (FAP) with the specific extra-intestinal manifestations epidermoid cyst, ►osteoma and ►desmoid tumor. Advances in the understanding of the genetics of ►FAP and careful analysis of the phenotype have shown that Gardner syndrome is neither genetically nor clinically distinct from FAP.

►APC Gene in Familial Adenomatous Polyposis
►Familial Adenomatous Polyposis

Gαs

Definition

Alpha subunit of stimulating ►G protein (heterotrimeric G protein that activates ►adenylyl cyclase).

►Calcitonin

Gastric Cancer

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Synonyms

Stomach cancer; Gastric carcinoma

Definition

Gastric cancer includes a variety of tumors that develop in the stomach. The vast majority of these tumors, more than 95%, are from epithelial origin and are named as gastric carcinomas. There are also less frequent none-pithelial tumors in the stomach, including ►lymphomas, and ►mesenchymal tumors.

Characteristics

Carcinomas of the stomach are heterogeneous tumors from the morphologic standpoint. This heterogeneity is reflected in the histopathologic classifications of the tumors. The most common classifications are the Lauren classification and the World Health Organization (WHO) classification.

The Lauren classification recognizes two major types of gastric carcinomas, the “intestinal” and the “diffuse,”

which display different clinicopathological characteristics and occur in different epidemiologic settings. Microscopically, the "intestinal" type gastric carcinoma retains a glandular structure and cellular adhesion/polarity. Grossly, it usually displays a sharp margin and an expansive growth. The "diffuse" type gastric carcinoma often displays an invasive growth pattern. Scattered clusters of malignant cells frequently infiltrate the submucosa. However, it has little glandular formation. The "diffuse" type shows an infiltrative growth pattern which often results in the absence of mass. Endoscopically, this carcinoma may be difficult to identify without the presence of ulceration or mass. In advanced forms of this carcinoma, infiltration of the gastric wall leads to linitis plastica (leather-bottle-like stomach).

The WHO classifies gastric carcinoma in several groups. The WHO papillary and tubular groups correspond with the intestinal type of gastric carcinoma in the Lauren classification. The mucinous and signet-ring cell WHO groups correspond with the diffuse type in the Lauren classification. The undifferentiated type lacks features that further identify its origin.

Epidemiology

Worldwide, there are currently over 900,000 new diagnosis of gastric cancer each year making it the third and fifth most common form of cancer in males and females, respectively. There is a tenfold variation in incidence between the highest and lowest risk populations. Populations with high incidence rates (more than 40 cases/100,000/year) occur in Eastern Asia, South America, and Eastern Europe. The Lowest incidence rates (around 15 cases/100,000/year) occur in North America, Northern Europe, and most countries in Africa. Incidence rates in most populations show a declining trend over several decades. There is also a variation in incidence among different ethnic groups within the same area. Blacks, Hispanics, and Native Americans in the US are 1.5–2.5 times more likely than whites to develop a gastric cancer. Migration studies show that the risk of cancer changes within two generations when people move from high-incidence to low-incidence countries. For example, Japanese immigrants to the US maintain their original risk of gastric cancer, whereas the subsequent generations show a decline in incidence reaching the incidence rate of the host country.

Etiology

A variety of host-related, environmental, and infectious causes have been implicated in sporadic gastric cancer. Environmental factors, particularly early in life, appear to influence the risk of the disease. The main identified environmental risk factor for gastric cancer is the infection by the bacteria ►*Helicobacter pylori*. *H. pylori* infection is a prevalent chronic infection in humans. The infection is generally acquired during childhood and in most individuals the infection results in asymptomatic gastritis. Later in life, chronic infected individuals can progress through chronic atrophic gastritis, intestinal metaplasia, and dysplasia, towards gastric carcinoma, especially in high-incidence areas. Patients with *H. pylori* infection have three- to fivefold increased risk for noncardial cancers as indicated by prospective studies. Progression to more severe diseases occurs in a fraction of infected individuals and seems to depend on several factors, including host susceptibility, environmental factors, and differences in *H. pylori* strains. Recent studies in animal models, particular the development of gastric cancer in Mongolian gerbils infected with human isolates of *H. pylori* have also provided conclusive evidence to the etiologic role of *H. pylori* in gastric cancer development.

Eradication of *H. pylori* has been shown to reverse premalignant lesions and significantly decreases the rate of development of gastric cancer providing additional evidence for the role of *H. pylori* in gastric carcinogenesis. *H. pylori* exhibit a high level of genetic diversity among strains which contribute to different degrees of virulence among strains. Two of the most studied virulence-associated genes are *vacA*, present in all *H. pylori* strains and encoding a bacterial toxin; and cagA, not present in all strains and a marker for the cag pathogenicity island (PAI). The genotyping of these *H. pylori* genes allowed the identification of *H pylori* strains that are associated with a higher degree of inflammation and epithelial damage in the gastric mucosa, as well as with increased risk for development of gastric carcinoma.

Several host-related factors have also been shown to be associated with the development of gastric carcinoma. Human ►genetic polymorphisms conditioning host variable expression of ligands for bacterial adhesins may be crucial for successful and persistent infection. In addition, polymorphisms in proinflammatory ►cytokine genes, such as *IL-1B, IL-8, IL-10,* and ►*TNF*-α, and ►mucin gene *MUC1*, have been shown to be associated with the risk of development of gastric cancer and its precursor lesions. In summary, infections with certain *H pylori* strains combined with host genetic susceptibility lead to increased risk of developing severe gastritis, chronic atrophic gastritis, and gastric carcinoma.

Additionally, other factors have been implicated. Inadequate consumption of fresh fruits and vegetables,

high salt intake and consumption of smoked or cured meat, and fish are considered dietary risk factors. There is also evidence that refrigeration of food may contribute for the protection against this cancer by facilitating year-round consumption of fresh fruits food and by reducing the use of salt and exposure to various carcinogens present in smoked and cured meat and fish. Finally, various studies suggest a twofold increased risk in smokers.

Molecular Biology of Gastric Cancer

The large majority of gastric carcinomas are sporadic. There is evidence that indicates that sporadic gastric carcinoma appears after a carcinogenesis process that begins with chronic *H. pylori* infection and a series of histopathological changes in the gastric mucosa that often develops over a long period of time. The available evidence support the existence of two main histogenetic pathways of carcinogenesis: one leading to the "intestinal" carcinoma developing after a pathway which includes chronic atrophic gastritis, intestinal metaplasia, particularly the incomplete type III intestinal metaplasia, and adenomatous dysplasia; and the other leading to the "diffuse" carcinoma, either de novo or via hyperplastic changes. Both pathways appear to develop in the setting of *H. pylori*-associated gastritis.

H. pylori might contribute for the development of gastric carcinomas by the converging effects of two main types of molecular events: (i) collateral damage of inflammatory by-products causing mutational events in gastric epithelial cells and (ii) direct effects on gastric epithelial cells by *H. pylori* organisms or released bacterial products. At the molecular level this events include: direct toxic effects on epithelial cells, alteration of the apoptosis–proliferation balance, alterations on transduction pathways and gene expression, impairment of DNA mismatch repair, and alterations in cell adhesion.

Various genes have been analyzed in attempts to understand the molecular basis for human gastric carcinoma development, but only a few with frequent alterations have been identified.

The homeobox *CDX2* gene has been identified as crucial for the transdifferentiation process leading to intestinal metaplasia, a precursor lesion observed in the gastric carcinogenesis pathway that is characterized by the a modified expression of ►*mucin* genes. The use of mucins as molecular differentiation markers allows the identification and classification of various subtypes of intestinal metaplasia, contributing for the identification of subtypes with higher risk of evolving to gastric carcinoma.

In addition, ►amplification of ►ERBB2 and the ►MET genes have been found in ~10% of intestinal and diffuse types of gastric carcinoma. Oncogenic activation of K-ras (0–18% in both histological types) have also been found. In addition, mutations in the tumor-suppressor gene ►*p53* have been reported in the diffuse type (0–21%) and intestinal type (36–43%). ►*APC* tumor-suppressor gene mutations are usually found in gastric adenomas but are rarely found in gastric carcinomas. This is different from the frequent mutations of APC in both colorectal adenomas and carcinomas. Furthermore, somatic mutations of ►E-cadherin are observed in 33–50% of the diffuse type of gastric carcinoma.

►Microsatellite instability (MSI) is observed in 5–10% of diffuse type and in 15–40% of intestinal type of gastric carcinomas. The major mechanism for the MSI in gastric carcinomas is inactivation of the mismatch repair gene *hMLH1* resulting from the ►hypermethylation of its ►promoter.

The presence of ►Epstein-Barr virus (EBV) has been observed in some cases of gastric cancers. EBV is a ubiquitous human herpesvirus which establishes a lifelong infection of B-cells in the human population. While in the vast majority of individuals the infection remains asymptomatic, a small proportion of individuals may develop virus-associated tumors, mainly ►lymphomas. EBV has also been detected in a range of tumors on nonlymphoid lineages. EBV is found in the tumor cells of the undifferentiated type of gastric carcinoma often with lymphoid stroma.

Familial Clustering of Gastric Cancer

A small number of gastric cancer patients show a heritable risk gastric cancer, with familial predisposition and apparent independence of environmental factors. Familial clustering has been reported in ~1% of gastric cancers.

Hereditary Diffuse Gastric Cancer

►Germline mutation of the E-cadherin gene (*CDH1*) in families with history of gastric cancer was first described in 1998 by Guilford et al. describing a germline truncating E-cadherin mutations in three Maori kindreds. Since then, multiple families with a history of gastric cancer ►HDGC and *CDH1* mutations have been identified, and the HDGC syndrome has been established. E-cadherin is a cell adhesion molecule predominantly expressed in epithelial tissues. E-cadherin molecules are generally localized at the basolateral surface of the epithelial cell, in regions of cell–cell contact. Truncating and ►missense germline mutations in the *CDH1* gene have been reported in HDGC. Affected individuals inherit one copy of the defective

E-cadherin gene. Somatic mutation, deletion, or promoter ►methylation inactivates the other copy. The cancer appears with an ►autosomal dominant pattern with high ►penetrance. When prophylactic gastrectomy is performed, multifocal early gastric diffuse gastric carcinoma is often found in the resected specimen.

Diagnosis and Detection

Gastric cancer often does not cause major symptoms in the early stages. Most patients with gastric cancer are diagnosed when they undergo endoscopy and biopsy after exhibiting symptoms. About 50% of patients may have nonspecific gastrointestinal complaints, such as dyspepsia. This is one of the reasons why about 80% of patients are diagnosed with advanced tumors. Diagnosis may also be obtained by double-contrast Barium X-ray. Other methods for diagnosis include endoscopic ultrasound, computed tomography, positron emission tomography, and magnetic resonance imaging. Serological assays screening, including serum pepsinogen, is a potentially useful method for detection of gastric cancer. Other molecular mechanisms for early diagnosis are currently under investigation.

Management

Surgical resection remains the primary treatment for gastric cancer. Prognosis of GC is poor with a 5 year survival rate closely related to the stage of the carcinoma when diagnosis and surgical resection is performed. Surgery with curative intent remains the only way to improve survival. Poor results in survival may be explained by the fact that when symptoms occur, and diagnosis is made, the cancer has often already spread. Presently, only a small percentage of patients are eligible for curative surgery. Management of advanced stage cancer also includes surgical resection, with chemotherapy. Novel drugs and treatments are currently under investigation.

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Gastric Carcinoma

Is a form of ►Gastric Cancer

Gastrin

Definition

A gastrointestinal peptide hormone that regulates gastric acid secretion and motility

►Gut Peptides

Gastrin-Releasing Peptide

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Synonyms

Bombesin amphibian gastrin-releasing peptide

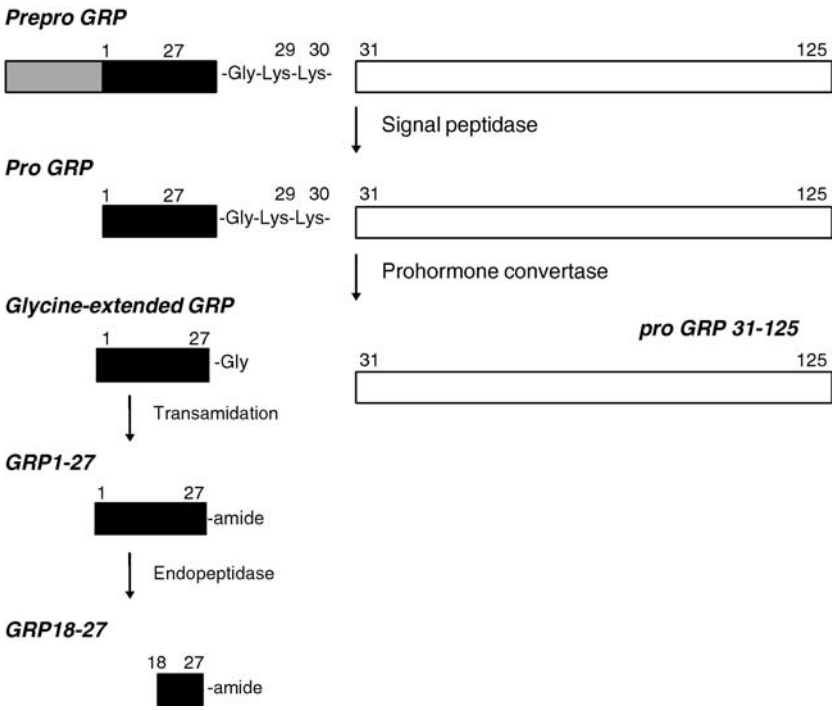
Definition

GRP; Is a peptide hormone of 27 amino acids, with the sequence VPLPAGGGTVLTKMYPRGNHWAVGHL-Mamide.

Characteristics

The tetradecapeptide ►bombesin was first isolated and characterized from the skin of the frog *Bombina bombina* in 1970. A decade later, the mammalian counterpart of bombesin was identified using an anti-bombesin antibody and named gastrin-releasing peptide (GRP). The name GRP arises from the first known activity of inducing gastrin secretion from G cells in the gastric antrum.

The human GRP gene maps to chromosome band 18q21. The three GRP mRNAs, which arise from a single nascent mRNA by alternate splicing at the junction between exons 2 and 3, encode peptides of 148, 141, and 138 amino acids. Like many neuropeptides mature amidated GRP arises by a series of well-defined processing steps from these initial large translation products. All three GRP mRNAs encode



Gastrin-Releasing Peptide. Figure 1 Processing of proGRP. PreproGRP (148 amino acids in human) is converted to proGRP (125 amino acids) by removal of the signal peptide. The sequential actions of a prohormone convertase that cleaves on the C-terminal side of the sole pair of lysine residues, and a carboxypeptidase B-like enzyme that removes the lysines, convert proGRP to glycine-extended proGRP1–27. The C-terminus of proGRP1–27 is then amidated to form mature GRP1–27. An additional cleavage between amino acids 17 and 18 yields GRP18–27.

GRP1–27 and GRP18–27, but differ in sequence at their C-termini. Multiple cleavages of the C-terminal extension peptide proGRP31-125 also occur in a tissue specific manner (Fig. 1).

GRP Receptors

The three different receptor subtypes (GRP-R, NMB-R and BRS-3) for the GRP family of peptides all belong to the seven-transmembrane family. The human genes encoding GRP-R, NMB-R and BRS-3 are located at chromosome bands Xp22.2-p22.13, 6q21-qter and Xq26-q28, and encode proteins of 384, 390 and 399 amino acids, respectively.

The different GRP receptor subtypes can be readily distinguished on the basis of agonist and antagonist affinities. The GRP-R has high affinity for bombesin and GRP and very low affinity for NMB. Conversely the NMB-R has a higher affinity for NMB than GRP. No natural ligand has been found for the BRS-3 receptor which has low affinity for both GRP and NMB. All the GRP receptors characterized to date are coupled to guanine nucleotide binding proteins (►G-proteins), and activate ►phospholipase C to increase intracellular concentrations of inositol phosphates, diacyl glycerol and calcium. Other intracellular mediators activated by GRP receptors include ►mitogen-activated protein kinase, ►focal adhesion kinase, and ►phosphatidylinositol 3-kinase.

Physiological Functions of GRP

One of the first biological actions of GRP described was the stimulation of ►gastrin release, which in turn results in the stimulation of gastric acid secretion. In addition to gastrin, GRP stimulates the release of a variety of hormones including secretin, ►somatostatin and cholecystokinin, but the physiological relevance of such changes is not known. GRP also stimulates pancreatic exocrine secretion, smooth muscle contraction, gastrointestinal motility, and the healing of damaged gastric mucosa, and delays gastric emptying.

The availability of potent and selective receptor antagonists and of blocking antibodies, together with the generation of ►knock-out mice lacking individual members of the GRP-R family of receptors, has allowed further investigation of the physiological functions of GRP. Such studies have demonstrated that GRP improves learning and memory performance in rats and mice and alters appetite. In addition growth of the colonic mucosa was attenuated in GRP-R deficient mice, in agreement with previous reports that

administration of GRP stimulated growth of the gut mucosa and pancreas.

GRP and Cancer

Apart from its physiological roles, GRP has been shown to stimulate growth of a number of cancerous tissues. The field was initiated by the demonstration that a monoclonal anti-GRP antibody blocked the binding of GRP to cellular receptors and inhibited the growth of ►small cell lung cancer (SCLC) cells both *in vitro* and as ►xenograft *in vivo*. The conclusion that GRP might be an ►autocrine growth factor in SCLC prompted various research groups to investigate the role of GRP in cancer development and progression.

GRP and Lung Carcinoma

Human ►SCLC cells as well as tumors have been shown to express both the GRP peptide and the GRP-R mRNA. GRP18-27, proGRP31-98 (the C-terminal flanking peptide of proGRP) and GRP mRNA have been detected in several small cell, but not in ►non-small cell, ►lung carcinoma cell lines. In patients with SCLC, GRP mRNA and peptide were detected in samples of blood, marrow, and ►pleural effusion. Several groups have reported increased circulating concentrations of proGRP31-98 in SCLC patients and suggested that proGRP may be useful as a prognostic marker. GRP-R mRNA is commonly expressed in both small cell and non-small cell lines.

GRP is ►mitogenic for SCLC cell lines *in vitro* and *in vivo,* but not for cell lines derived from either a squamous lung carcinoma or a lung adenocarcinoma. Subcutaneous GRP treatment of nude mice xenografted with a human SCLC cell line significantly increased tumor weight and DNA content and the increase was blocked by a GRP antagonist.

GRP and Prostate Carcinoma

GRP expression was detected by ►immunohistochemistry in both biopsy and radical prostatectomy specimens from patients with prostatic adenocarcinomas and benign prostatic hyperplasia (►prostate cancer). Circulating proGRP31-98 was increased in some patients with prostatic cancer although there was some overlap with patients with benign prostatic hyperplasia. Using ►RT-PCR and in situ hybridization, widespread but variable GRP-R mRNA expression was demonstrated in specimens of prostate carcinoma and benign prostate hypertrophy, while normal tissue consistently displayed a low GRP-R mRNA concentration. Using ^{125}I-Tyr^{4}-bombesin as a radioligand, GRP-R was detected in prostatic intraepithelial neoplasms and invasive prostate carcinomas. GRP receptors have also been demonstrated on several prostate carcinoma cell lines.

GRP antagonists inhibit the growth of a prostate adenocarcinoma in rats and of human prostate cancer cell lines *in vitro* and as xenografts in mice. An anti-bombesin antibody significantly inhibits both bombesin-stimulated and basal growth of these cell lines. Invasion and migration of several prostate cancer cells is also accelerated by GRP.

GRP and Breast Cancer

GRP immunoreactivity has been detected in human breast carcinoma biopsies. Similarly GRP mRNA was detected in primary breast carcinomas but not in breast cancer cell lines. Using radiolabeled bombesin the GRP receptor was shown to be expressed by neoplastic mammary epithelial cells in many invasive ductal carcinomas and ductal carcinomas in situ.

Bombesin increases cell growth in both estrogen-dependent and -independent human breast cancer cell lines. These effects are blocked by a GRP antagonist. In addition, the GRP antagonists significantly decrease the final tumor volume and weight of xenografts of breast cancer cell lines in female nude mice.

GRP and Gastric Carcinoma

Although there have been no reports of GRP expression in gastric adenocarcinoma, GRP-R expression has been detected in 40% of non-antral gastric adenocarcinomas, and in human gastric carcinoma cell lines. Growth of some gastric cancer cell lines is significantly inhibited by treatment with a GRP-R antagonist. Bombesin significantly increases the incidence of peritoneal metastases from gastric cancers induced in rats by treatment with a chemical carcinogen.

GRP and Pancreatic Carcinoma

GRP immunoreactivity was detected in over 50% of pancreatic ductal adenocarcinomas, and in both well- and poorly differentiated pancreatic carcinoma cell lines. Both GRP-R mRNA and protein were detected in peri-tumoral small veins, but not in the tumor tissue of ductal adenocarcinomas. The mRNAs encoding both GRP-R and NMB-R have been detected by RT-PCR in several ►pancreatic cancer cell lines.

Several groups have reported that GRP stimulated growth of human pancreatic cancer cell lines and that the stimulation was reversed by a GRP antagonist. The presence of an autocrine loop involving GRP and the GRP-R was suggested by the observation that GRP antagonists inhibited xenograft growth of human pancreatic adenocarcinoma cell lines.

GRP and Colorectal Carcinoma

GRP and GRP-R are expressed in many colorectal adenocarcinomas, but not in uninvolved normal mucosa or in tumor metastases. GRP and GRP-R mRNAs have also been detected in many colon cancer cell lines, although functional receptors are frequently absent.

GRP significantly increases the number of ►colorectal cancers and peritoneal metastases induced in rats by treatment with chemical carcinogens. GRP is a mitogen for several colorectal cancer cell lines *in vitro* and GRP antagonists block the proliferative effect of GRP. GRP may also have a morphogenic role in colorectal cancer. A recombinant peptide derived from the C-terminal region of proGRP (proGRP42-98) has been shown to be biologically active in colon cancer cells.

GRP and Gastrointestinal Carcinoid Tumors

Gastrointestinal ►carcinoid tumors are low grade, malignant epithelial neoplasms showing neuroendocrine differentiation. GRP is frequently expressed in carcinoids from the stomach, small intestine and appendix, but rarely in colonic carcinoids.

Clinical Significance

A large proportion of human small-cell lung, prostate, gastric, breast, pancreatic and colorectal cancers express GRP receptors. Sixty-three percent of all human cancers of the types mentioned are GRP-R-positive by PCR-based techniques, and this figure compares favorably with the figure of 56% from ligand binding studies. The frequent expression of GRP-R in tumors makes the receptor an attractive target for cancer treatment.

The observations that GRP stimulates ►angiogenesis, and that GRP-R antagonists block tumor growth and angiogenesis, present new therapeutic options. GRP-R ligands are also receiving considerable attention as vehicles to deliver cytotoxic agents. Cytotoxic bombesin analogues have been used to inhibit the growth of cancers and cell lines of different origins including ovarian, gastric, prostate, small-cell lung and pancreatic carcinoma, and glioblastoma. Tumor imaging and detection is the other area which has exploited the fact that GRP receptors are over-expressed in a number of tumors. GRP-like peptides labeled with β- and/or γ-emitting radionuclides are currently being investigated for their ability to bind to cell-surface GRP receptors, and hence for their potential utility for detection and/or therapy of tumors.

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Gastrinoma

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Synonyms

Zollinger-Ellison syndrome; Islet cell tumor

Definition

Gastrinomas are functioning ►gastrin-producing tumors of the neuroendocrine cells that are located in the duodenum, pancreas and/or "gastrinoma triangle" and cause clinical ►Zollinger-Ellison syndrome (ZES).

Characteristics

Gastrinoma is a functioning ►neuroendocrine tumor of high malignant potential that follows an indolent clinical course. Neuroendocrine tumor cells release gastrin into the blood stream causing clinical symptoms, i.e., ZES.

Annual incidence of ZES in the US is estimated at 1 in 2.5 million people. It may occur in sporadic and familial forms. Twenty to 25% of ZES cases are associated with familial ►multiple endocrine neoplasia type 1 (MEN1; ZES-MEN1) (►Endocrine-related cancers; ►Carcinoid tumor; ►Hereditary cancer syndromes). Sporadic ZES can develop at any age, but the majority of cases are diagnosed between the fourth and sixth decade of life (mean age 50.5 years). ZES-MEN1 patients have multiple gastrinomas and are younger than those with sporadic ZES. Sixty percent of the patients are men.

Diagnosis

The diagnosis of ZES is usually established clinically and then confirmed by demonstration of the specific tumor type (gastrin-positive neuroendocrine tumor) in a pathology specimen. Clinical diagnosis of ZES is based on the finding of concurrent ►hypergastrinemia (increased serum gastrin produced by the tumor) and ►hyperchlorhydria (increased gastric acid secretion by hyperplastic parietal cells secondary to the trophic action of gastrin) in patients with ulcer disease (►Gastrin-releasing peptide; ►Gut peptides). Because ZES symptoms overlap with those of much more common peptic ulcer disease, the diagnosis of gastrinoma (ZES) may be delayed for many years. Multiplicity and unusual (low duodenal and intestinal) location of ulcerations, resistance to medical therapy, frequent and early recurrence of ulcers, prolonged and unexplained diarrhea or steatorrhea, enlarged gastric or duodenal folds, and negative tests for Helicobacter

pylori, when they occur raise a suspicion for ZES. The diagnosis is established by positive results with two or more of the following: elevated fasting serum level of gastrin (>100 ng/l), abnormal secretin stimulation test (increment in fasting serum level of gastrin >200 ng/l after intravenous secretin), and an elevated basal level of gastric acid output (>10 mEq/h).

Gastrinomas are thought to originate from gastrin-producing (G) cells that are normally dispersed in gastric antral and upper duodenal mucosa (►Gastrointestinal tumors; ►Gastrointestinal hormones). However, there is no direct correlation between the distribution of G cells in the digestive system and frequency of gastrinoma location. The tumors commonly arise in ectopic sites such as pancreas (the second most common location) that is normally devoid of G cells. More than 50% of gastrinomas arise in the duodenum. Ninety percent of all gastrinomas are located in the "gastrinoma triangle" (the anatomic junction of cystic and common bile ducts, second and third portion of the duodenum, and neck and body of the pancreas). Solitary pancreatic or duodenal gastrinomas are characteristic for sporadic ZES cases. Location of the tumors in proximal duodenum and their multiplicity are common in ZES-MEN1 patients. Cases of gastrinoma located solely in periduodenal and peripancreatic lymph nodes have been reported, and clinical cure has been achieved following surgical excision of regional lymph nodes in some of such cases.

Microscopically, gastrinomas are composed of small, uniform cells with abundant eosinophilic cytoplasm, and small uniform nuclei with inconspicuous nucleoli. Correlation between histologic features and malignant behavior is poor. Mitotic activity and nuclear pleomorphism, when rarely present, are unreliable predictors of prognosis in gastrinoma. Despite their small size and lack of invasion into adjacent organs, duodenal gastrinomas demonstrate high malignant potential with propensity for regional lymph node and distant metastases.

Because gastrinoma may be an initial manifestation of ►MEN1 in up to one third of familial ZES cases, it is currently recommended to evaluate all ZES patients for medical and family history of the endocrine neoplasms and assess their parathyroid and pituitary function. The differentiation of patients with familial ZES-MEN1 from patients with sporadic ZES is important because of the differences in natural history of ZES, need for family screening, difficulty in controlling acid hypersecretion, and need for exploratory laparotomy for cure.

Gastrinomas are reported to be malignant in 60–90% of cases. About 34% of patients with ZES have metastatic disease at the time of diagnosis. Periduodenal and peripancreatic lymph nodes, liver, and bone are the most common sites of metastases. Gastrinomas in MEN1-ZES and sporadic ZES patients have similar rates of metastases.

Therapy

Since ZES can be cured only by excision of a gastrinoma in the early stage of the disease, the exact tumor localization may be crucial in successful management of ZES patients. Duodenal gastrinomas are frequently small in size (less than 1.0 cm) and are difficult to find preoperatively. Tumor localization studies (►CT, ►MRI, and ►octreotide scanning) are necessary for preoperative localization. Exploratory ►laparotomy with intraoperative ultrasonography, transduodenal endoscopic illumination, duodenotomy, and surgical resection of the tumor is currently recommended for patients with sporadic ZES patients. The usefulness of surgery varies for patients with ZES-MEN1 who usually have multiple tumors.

Treatment of patients with ZES is also directed at reducing gastric acid hypersecretion. Total gastrectomy or vagotomy has been used. However, over the past 15 years a highly potent antisecretory proton pump inhibitor, ►omeprazole, has been effective in suppression of gastric acid output and has significantly decreased the early mortality of patients due to complications of ulcer disease. With the increased ability to control hyperchlorhydria, the progression of gastrinoma and metastases are becoming the primary factor in long-term survival of patients with ZES. The clinical course of gastrinoma is indolent and the prognosis is directly related to the spread of the tumor. Patients with liver metastases have only a 20–30% chance of surviving for 5 years, whereas patients without liver metastases have an excellent long-term prognosis (>90% 5-year survival rate).

Genetics

The role of the *MEN1* gene as an early event in gastrinoma tumorigenesis has been established in *MEN1*-associated gastrinomas as well as in 33% of sporadic gastrinomas regardless of metastases. In both familial and sporadic gastrinomas the *MEN1* gene, located on chromosome 11q13, is thought to act as a tumor suppressor based on the presence of inactivating mutations in the normal tissue/blood DNA, accompanied by the loss of the wild type allele in the tumor. Either ►homozygous deletion or ►hypermethylation at the 5′ region of the *p16/MTS1* or ►$p16^{INK4a}$ tumor suppressor gene on chromosome 9p21 was demonstrated exclusively in sporadic gastrinomas and not in other pancreatic neuroendocrine tumors. The finding suggests that *p16/MTS1* or $p\ 16^{INK4a}$ defect is restricted to gastrin-producing tumors. Somatic genetic changes associated with the development of most sporadic gastrinomas and as well as with the progression to malignancy are currently unknown.

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Gastroesophageal Reflux Disease

Definition

GERD; The reflux from acidic gastric content into the esophagus mostly due to a gastric hernia results in esophagitis, favoring cancer risk in the lower esophagus.

►Alcohol Consumption
►Esophageal Cancer

Gastrointestinal Hormones

►Gut Peptides

Gastrointestinal Stromal Tumor

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Synonyms

GIST

Definition

GastroIntestinal Stromal Tumor (GIST) is a type of sarcoma (i.e., a connective tissue neoplasia). GIST is a rare cancer affecting the digestive tract or nearby structures within the abdomen.

Characteristics

Epidemiology and Clinical-Pathological Features of GIST

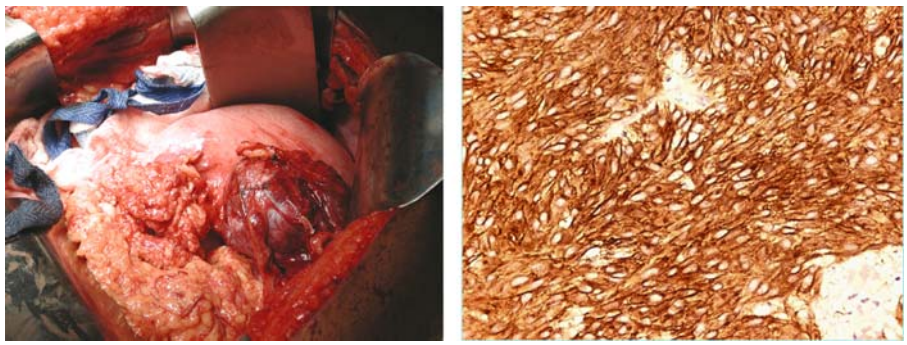
GISTs are believed to arise from the ►Interstitial Cells of Cajal (ICCs), the pacemaker cells for the autonomous movement of the GI tract. Other studies have suggested that GISTs arise from interstitial mesenchymal precursor ►stem cells; however, pinpointing the progenitor cell has been difficult. Although it is considered a rare tumor, nearly 4,500–6,000 new cases are diagnosed annually in the United States. The peak incidence of GIST occurs later in life with a median age of 58 years; however, there are also reports of pediatric GISTs. The most common sites of origin for GIST are the stomach (39–70%) and small intestine (31–45%). Other primary sites include the large bowel, rectum, appendix, and rarely the esophagus. A small percentage of GISTs arise outside the tubal gut, i.e., within the mesentery, gallbladder, and omentum. These tumors are known as extra-gastrointestinal GIST. The symptoms of GIST include bloating, gastrointestinal bleeding, or fatigue related to anemia. The common metastatic sites for GIST include the liver and omentum, and less frequently, the lung and bone.

Diagnosis

Approximately 95% of GISTs express the antigen CD117 (better known as ►KIT) when examined by ►immunohistochemistry (IHC) (Fig. 1). The c-*KIT* gene is the normal cellular homologue of a viral oncogene (v-Kit, Hardy Zuckerman 4 feline sarcoma viral oncogene homologue). There is a small subset of GISTs that lack KIT expression.

Oncogenic Signaling Pathways in GIST

KIT is a 145-kDa transmembrane glycoprotein and is a member of the tyrosine kinase family of receptors. Other members in this family include PDGFRα and β (Fig. 2a). KIT is normally expressed in hematopoietic stem cells, ►mast cells, melanocytic cells, germ cells, and the ICC. The normal function of KIT is dependent on binding to its ligand, ►Stem Cell Factor (SCF), and is essential in embryonic development. Acquired mutations in the c-*KIT* gene are referred to as "►gain-of-function" or "activating" mutations, which lead to constitutive ligand-independent activation of the tyrosine kinase activity of the receptor. Molecular genetic studies have shown that the vast majority of GISTs (70–80%) possess a c-*KIT* mutation in either exon 9, 11, 13,



Gastrointestinal Stromal Tumor. Figure 1 GIST. *Left*: Surgically resected gastric GIST; *right*: Immunohistochemical staining of a paraffin-embedded GIST tissue section for KIT protein expression.

or 17, and that a subset of GIST (~10%) possesses a *PDGFRα* mutation in either exon 12, 14, or 18. Even though c-*KIT* or *PDGFRα* mutations are detected in GIST, approximately 10% of GISTs lack mutations in either of these genes. Therefore, other yet to be discovered genetic and potentially epigenetic mechanisms, independent of c-*KIT* and *PDGFRα* activation, may contribute to the pathogenesis of GIST.

KIT gain-of-function mutations result in autoactivation of the receptor, and consequently transmit the KIT oncogenic signal to downstream targets such as phosphatidylinositol 3-Kinase (►PI3K), ►AKT (as known as protein kinase B), and mitogen-activated protein kinases (►MAPK). These signaling proteins influence proliferation, ►apoptosis, differentiation and/or cell ►adhesion (Fig. 2b). Clinical observations and laboratory research have shown that different gain-of-function mutations within the same receptor can affect different down-stream pathways and clinical response to therapy.

Cytogenetics and Molecular Cytogenetic Alterations

Even though mutations in c-*KIT* or *PDGFRα* appear to be the primary driving force in the pathogenesis of GIST, several other genetic and genomic changes have been documented which contribute to the development of this disease. ►Monosomy for chromosome 14 is one of the most frequent (>60%) genomic alterations detected in GISTs. Other common changes in metastatic GISTs include loss of chromosome arms 1p, 9p, 10, 14q, 15q, and 22, and gains involving 5p and 20q.

Therapy

►Cytoreductive surgery is the standard of care for patient with primary GIST. This surgery seeks to remove the entire gross tumor, and may require total or subtotal organ resection, depending on tumor location and size. However, surgery has limited success for locally recurrent or metastatic GIST and clinical response of patients to systemic therapy using conventional chemotherapies is abysmal. ►Chemotherapy response rates in patients with metastatic disease are less than 5% for all tested cytotoxic agents with a median survival of 10–20 months.

►Imatinib mesylate (STI571 or Gleevec™), is an oral 2-phenylaminopyrimidine derivative that acts as a selective inhibitor against several ►receptor tyrosine kinases including KIT, PDGFRα, and ►BCR-ABL (which is the causative ►chromosomal translocation in ►chronic myelogenous leukemia). Based on a high percentage of metastatic GIST patients demonstrating clinical response to imatinib in phase I/II ►clinical trials, the FDA granted Novartis approval of imatinib for the treatment of advanced GIST in 2001. Clinically, it has been reported that patients with metastatic or localized GIST possessing a mutation in exon 11 (i.e., involving the juxtamembrane domain of the receptor) of c-*KIT* mutations have a longer ►progression free survival and overall survival when treated with imatinib as compared to patients with tumors with other types of c-*KIT* mutations. Likewise, mutations in exon 12 (juxtamembrane domain) of *PDGFRα* are more sensitive to imatinib treatment than patients with other types of mutations in *PGDFRα*.

In summary, GISTs are the most common mesenchymal tumors of the digestive tract and possess mutations in either c-*KIT* or *PDGFRα*. For patients with localized resectable GIST, surgery remains the treatment of choice, whereas, for patients with metastatic disease, imatinib-based therapy is providing benefit for long-term disease control.

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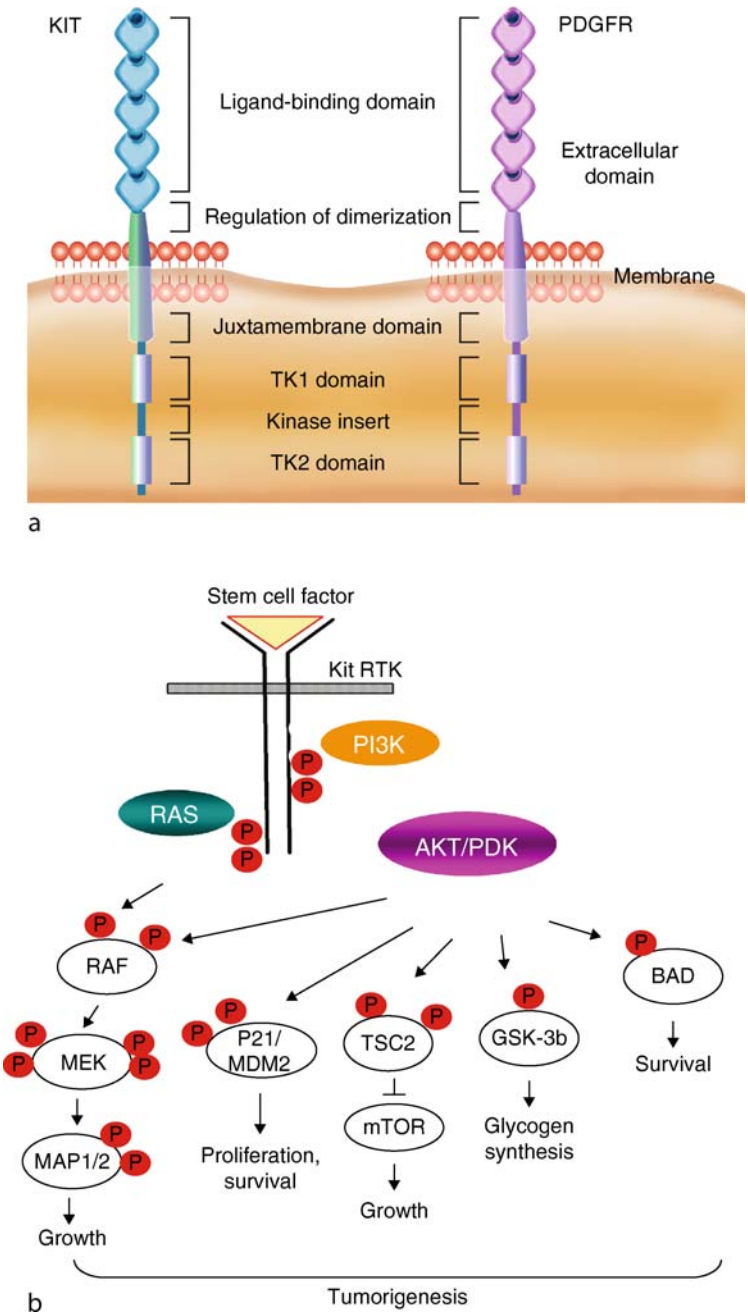

Gastrointestinal Stromal Tumor. Figure 2 Structure and signaling pathways of KIT and PDGFRα. (a) Schematic structure of KIT and PDGFR*α*. (b) Signaling pathways down-stream of KIT activated by stem cell factor or by oncogenic mutations. Shown are some of the most studied signaling events that are set in motion in response to KIT activation. *Red circles* with a "P" indicate phosphorylation of the protein. Phosphorylation of proteins by kinases is a means of controlling the activity of the recipient protein.

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Gastrulation

Definition

Is the formation of a gastrula from a blastula during embryogenesis.

►Homeobox Genes

Gatekeeper

Definition

Type of tumor suppressor gene; Refers to a subgroup of gene products, whose major cellular function is in the control of cell division, death or lifespan. Inactivation of gatekeeper genes favors, through a variety of mechanisms, the unrestrained growth typical of cancer cells. Multiple gatekeeper mutations may be required for the neoplastic transformation of a cell. Gatekeeper gene products often participate in the control of ►cell cycle (for example, the Rb gene product [►retinoblastoma protein, cellular biochemistry]) or in the signals that regulate proliferation (for example, the ►APC gene product). It is possible that large proteins with several functional domains (pleiotropic genes), such as ►BRCA1 and ►BRCA2 proteins originally described as gatekeepers may also have caretaker functions.

►von Hippel-Lindau Tumor Suppressor Gene
►Breast Cancer Genes BRCA1 and BRCA2
►Caretakers Genes

Gatekeeper Position

Definition

Is a protein residue located at the back of the ATP binding site, whose properties (size, charge and hydrophobicity) regulate the binding of inhibitors.

►Nilotinib

Gaucher Disease

Definition

An inborn genetic disease due to a defective β-glucosidase that normally hydrolyzes glucosylceramide. In another form of the disease, the protein (saposin C) that activates the glucosidase is absent.

►Sphingolipid Metabolism

GBM

►Glioblastoma Multiforme

GBP28

►Adiponectin

GCL

Definition

Glutamate cysteine ligase, also known as γ-glutamylcysteine synthetase (GCS), comprises a catalytic subunit and a regulatory subunit. It is the rate-limiting enzyme in ►glutathione biosynthesis.

►Phase 2 Enzymes

GC-MS

Definition

Gas chromatography-mass spectroscopy (GC-MS) has the advantage of unequivocally identifying the analytes in a mixture. It is used to identify ►adducts to DNA or

altered DNA bases, which result for instance from ►oxidative DNA damage.

GC/Post-GC Type B-Cell Tumors

Definition

Since somatically mutated V genes (►immunoglobulin genes) are a hallmark of ►B cells that have left the ►germinal center (GC), studies of V genes in B-cell tumors provide information on their stage of maturation; post-GC type tumors that carry mutated V genes include ►DLBCL, ►Burkitt lymphoma, ►marginal zone lymphoma and classical ►Hodgkin lymphoma. The pattern of mutations in ►follicular lymphoma reveals intraclonal heterogeneity, indicating ongoing mutation (GC-type); ►chronic lymphocytic leukemia and ►mantle cell lymphoma cells harbor unmutated V genes (pre-GC type).

►BCL6 Translocations in B-cell Tumors

GCSF

Definition

►Granulocyte Colony-Stimulating Factor

GCV

Definition

►Ganciclovir

GDEPT

►HSV-TK/Ganciclovir Mediated Toxicity

GDF

Definition

Synonym growth differentiation factor-11; ►BMP-11 and member of the ►TGF-β superfamily.

GDF15

►MIC-1

GDP

Definition

Guanosine diphosphate.

►RAS

GDP-Fucose

Definition

A common donor substrate for fucosyltransferases.

►Fucosylation

GEF

Definition

Guanine nucleotide exchange factors (GEFs) are proteins that stimulate the exchange (replacement) of guanine nucleoside diphosphates for guanine nucleoside triphosphates bound to ►G proteins.

►RAS Activation

Gefitinib

Definition

An orally available tyrosine kinase inhibitor that has the chemical structure of 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline and is capable of selectively inhibiting the epidermal growth factor receptor kinase. Gefitinib is used to treat patients with advanced ►non-small cell lung carcer, and it is most effective for patients who have specific mutations within the catalytic domain of the epidermal growth factor receptor kinase drug target.

►Drug Design
►Tyrosine Kinase Inhibitors

Gelatin-Binding Protein 28

►Adiponectin

Gelatinase B

►Serum Biomarkers

Geldanamycin

Definition

GM; A benzoquinone ansamycin antibiotic from *Streptomyces hygroscopicus* var. Geldanus that binds to Heat Shock Protein 90 (►Hsp90) and thereby blocks its function as an important (►Ansamycin Class of Natural Product Hsp90 Inhibitors) chaperone. From a tumor biology perspective, the more than 50 ►HSP90 client proteins include ►TP53, the kinases ►SRC, Raf-1, B-Raf, HER-2/neu/ErbB2 and ►Bcr-Abl as well as several members of the ►steroid hormone receptor family, which become de-stabilized and subsequently degraded upon treatment with Geldanamycin or its clinically more relevant derivatives 17-AAG (17-allylamino-17-demethoxygeldanamycin) and 17-DMAG (17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin).

►Ansamycin Class of Natural Product Hsp90 Inhibitors
►B-Raf Signaling
►Hsp90

Gelsolin

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Definition

Is a widely expressed 82–84 kDa ►actin-binding protein that is found inside the cells and also in a secreted form in extracellular fluid. Gelsolin severs actin filaments ►actin filament severing by breaking the ionic interactions between actin molecules within the filament and it caps the fast growing (+) end of actin filaments. Filament severing and capping are regulated by Ca^{2+}, the ►phosphoinositide phosphatidylinositol 4,5 bisphosphate (PIP2) and pH.

Characteristics

Cytoplasmic gelsolin was discovered in 1979 and named for its ability to activate gel–sol transformation of actin filaments in a Ca^{2+}-dependant manner. Gelsolin is the founding member of a ►gelsolin family of ►actin filament severing and/or capping proteins. Other members include villin, scinderin, adseverin, CapG, and flightless I. Among these, gelsolin and CapG have been implicated as ►tumor suppressor.

The ►actin cytoskeleton is remodeled dynamically during cell movements. Gelsolin contributes to dynamic remodeling by promoting the disassembly and subsequent reassembly of the actin cytoskeleton in response to changes in intracellular signals. Disassembly is mediated through filament severing and reassembly is mediated by filament uncapping. Gelsolin is activated by micromolar Ca^{2+} to bind to the side of actin filaments and to sever them. After severing, gelsolin remains attached to the (+) ends of these short filaments

even as Ca^{2+} level decreases to submicromolar level. Gelsolin is detached from the (+) ends (uncapping) by PIP2. It is hypothesized that the combination of severing to generate large number of short capped actin filaments and subsequent uncapping can account for explosive actin filament growth. The importance of gelsolin for cell motility has been demonstrated by gelsolin overexpression and depletion by RNA interference in tissue culture cells and by gelsolin gene knockout. For example, although the gelsolin ►knockout mice are viable, their ►neutrophils and ►fibroblasts have decreased motility.

Gelsolin has also been implicated in ►apoptosis, either as an enabler or a protector. Gelsolin is cleaved by ►caspase-3 into two halves and the resulting amino terminal half severs actin filament in a Ca^{2+}-independent manner. This may contribute in part to the morphologic changes associated with apoptosis in some type of cells. However, a protective role of gelsolin has been suggested in Jurkat cells and in neuronal cells. Thus, blockage or enhancement of gelsolin cleavage might retard or enhance apoptosis depending on the cellular context.

The relation between gelsolin, apoptosis and tumorigenesis probably reflects a complex balance between the multiple effector functions of gelsolin.

Gelsolin and Cancer

The role of gelsolin in reorganizing the actin cytoskeleton suggests that it may be involved in promoting tumor cell ►invasion and dissemination. The first study investigating the distribution of gelsolin in ►breast cancer showed that, in ►ductal carcinoma, it is notably downregulated in epithelial cells compared with normal breast tissue. However, gelsolin is expressed at a high level in stromal ►myofibroblasts. Therefore, cytoplasmic gelsolin is expressed in normal epithelial cells, in some tumor cells and importantly in intratumoral and peritumoral stromal myofibroblasts, and in vessel smooth muscle cells. The function of gelsolin in these highly contractile cells is not yet understood.

Since the initial study, there have been many other investigations to determine if gelsolin has a role in tumorigenesis. These studies suggest that depending on the type of tissue and the stage of cancer growth, gelsolin may act as a tumor suppressor or a tumor enhancer. In some cases, gelsolin is a reliable prognostic marker. In this general and short chapter, it is not possible to review of all reports in the field. We therefore took the liberty of selecting only studies that investigate both in vitro and in vivo conditions; these studies should be most relevant for understanding the role of gelsolin in tumor development.

Gelsolin Considered as a Tumor Suppressor

►Immunohistochemistry studies showed that gelsolin expression is downregulated in many types of cancer including breast, stomach, colon, bladder, prostate, lung, kidney, and ovary. For example, 78% of ►bladder tumor tissues and six bladder cancer cell lines displayed low or undetectable gelsolin compared with their normal counterparts. Furthermore, transfection of exogeneous gelsolin cDNA into a bladder cancer cell line reduced colony-forming ability and tumorigenicity in vivo. Loss of gelsolin was also observed in human ►ovarian cancer cell lines and in ovarian carcinoma compared with normal ovaries and benign adenoma. As in the case of bladder cancer cells, reexpression of gelsolin in ovarian tumor cell lines resulted in a reduction in colony formation.

Knockdown of gelsolin using small interfering (si) RNA has given contradictory results. For example, gelsolin depletion in the human immortalized mammary epithelial cell line MCF10A unexpectedly increased cell motility and induced overexpression of the small ►GTPase ►Rac, which has been implicated in dynamic ►lamellipodia formation. Gelsolin depletion also converted ►cadherin from E- to N-type via the induction of the ►transcription factor Snail, whose expression is inversely correlated with E-cadherin mRNA levels in several epithelial tumor cell lines. These unexpected results suggest how a decrease in gelsolin expression can nevertheless lead to enhanced tumor ►cell motility.

The molecular basis for the decrease in gelsolin expression in tumor cells is not known. There are suggestions that gelsolin mRNA level may decrease due to ►epigenetic modifications resulting from DNA ►methylation and ►histone deacetylation, in ►ovarian cancer and in ►breast cancer cell lines. Paradoxically, although gelsolin can suppress tumor progression, it was also shown that overexpression of gelsolin in malignant tumors was associated with poor prognosis. Gelsolin has been suggested to be a motility marker for tumor cells, although its prognostic value has so far only been examined in a small number of malignant tumors. Furthermore, there is surprisingly little information about the possibility that other members of the ►gelsolin family may substitute for the depleted gelsolin in cancer cells.

Gelsolin Considered as a Tumor Enhancer

Besides acting as a tumor suppressor, gelsolin has also been implicated as a tumor enhancer, particularly in lung and breast carcinomas. For example, ►non-small cell lung carcinoma (NSCLC), which comprises 70–80% of all lung carcinomas and has a 5-year survival rate of about 15%, is usually associated with decreased gelsolin level in about 70% of the cases

examined. However, the decrease in gelsolin is not associated with adverse prognostics. Instead, the small percent of cells with high focal gelsolin expression was correlated with the highest risk of cancer recurrence compared with tumors that had no or low gelsolin expression.

Likewise, the expression of gelsolin in breast cancer is variable and may involve only a few cells in the issue. Although gelsolin is absent in many breast cancers, about one third of cases have detectable gelsolin. In some cases, gelsolin expression was focally localized and accentuated in cell clusters or in single invasive cells that show transendothelial migration or are embedded in the stroma. Interestingly, an overexpression of gelsolin, when associated with ERBB2 and EGFR expression, resulted in a more agressive tumor phenotype. Altogether, these results suggest that although gelsolin can be extinguished during cell transformation, its focal high expression in a subpopulation of tumor cells can facilitate tumor dissemination and metastasis by promoting tumor cell locomotion.

In summary, the current findings are consistent with the hypothesis that gelsolin functions in a complex manner in the development and progression of tumors.

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Gelsolin Family

Definition

A superfamily of ►gelsolin-like proteins that has a conserved modular motif. Gelsolin is the founding member; other members include villin, scinderin, adseverin, supervillin, CapG, and flightless I. With the exception of flightless I, all are shown to be Ca^{2+}-activated ►actin severing and/or binding proteins. Some of these proteins have also been implicated in transcriptional regulation.

Gemcitabine

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Synonyms

dFdC; 2′,2′-Difluoro-2′-deoxycytidine; GEMZAR

Definition

Is an antitumor drug belonging to the class of antimetabolites. Antimetabolites are agents that interfere with normal metabolism due to their structural similarity to normal intermediates in the synthesis of RNA and DNA precursors. Due to differences in metabolism between normal and cancer cells, antimetabolites have the potential to act with a certain degree of specificity in cancer cells. Antimetabolites target the synthetic pathway of pyrimidines (uracil, cytosine, thymine) and purines (guanine, adenosine) nucleotides, where they can serve as substrates for enzymes, or can inhibit enzymes, or both. As a consequence of their interference, either by incorporation or inhibition, antimetabolites induce cell damage leading to apoptotic cell death.

Several cytidine and deoxycytidine analogs have been synthesized in the past and tested for antitumor efficacy. The first successful agent in clinical practice was ara-C (1-β-D-arabinofuranosylcytosine) which is currently used as first-line treatment in acute myeloid leukemia and ►non-Hodgkin lymphoma.

Characteristics

Mechanism of Action and Resistance

Is a potent and specific fluorine substituted ara-C analog with pronounced activity against solid tumors. Gemcitabine presents advantages in ►pharmacokinetic and ►pharmacodynamic properties over ara-C. Its mechanism of action is well characterized. Gemcitabine exhibits cell-phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the ►G1/S transition. Membrane transport is mediated by facilitated diffusion catalyzed by human equilibrative nucleotide transporter 1, a carrier for physiologic nucleosides. The molecule itself is inactive and needs to be metabolized

intracellularly to its mononucleotide by deoxycytidine kinase, and then by nucleotide kinases to the active diphosphate (dFdCDP, gemcitabine-DP) and triphosphate (dFdCTP, gemcitabine-TP) nucleotides. The activity of gemcitabine is strongly correlated with the extent of gemcitabine-TP. Once incorporated into the DNA strand, an additional natural nucleotide is added, masking gemcitabine, preventing ►DNA repair by base pair excision, and eventually resulting in chain termination. Such process is self-potentiated by the inhibitory effect of gemcitabine-DP on the reaction generating normal deoxycitidine-TP, thus favoring the incorporation of gemcitabine-TP into DNA. Gemcitabine is also incorporated into RNA and induces cell ►apoptosis. Ribonucleotide reductase can be inhibited by gemcitabine-DP, resulting in depletion of deoxycitidine-TP and deoxiadenosine-TP. Deoxycitidine-TP is a feedback regulator of nucleotide kinase, thus favoring the activation of gemcitabine. The depletion of deoxiadenosine-TP will inhibit DNA repair. Collectively, the various effects of gemcitabine result in a unique pattern of self-potentiation.

Inactivation of gemcitabine can occur by deamination into its inactive metabolite difluoro-deoxyuridine by cytidine deaminase (CDD) or dephosphorylation of nucleotide-MP by 5′-nucleotidase (5′-NU), which exerts the opposite function of deoxycitidine kinase.

Three general mechanisms of resistance to nucleotide analogs have been described. The first one arises from insufficient intracellular concentration of nucleotides-TP, which may result from inefficient cellular uptake, reduced levels of activating enzymes, increased degradation by increased 5′-NU or CDD activity, or expansion of the natural deoxynucleotide-TP pool. The second mechanism may be due to inability to achieve sufficient alterations in DNA strands or deoxynucleotide-TP pool. The third mechanism may be a consequence of defective apoptotic pathways, which can impair the activity of antitumor drugs of various mechanistic classes.

Clinical Profile

For clinical use gemcitabine is administered intravenously. The standard administration of gemcitabine is a 30 min infusion of 1,000 mg/m^2/week up to 7 weeks, followed by 1 week rest.

Toxicity

The dose limiting toxicity is ►myelosuppression, with 25 and 5% of patients experiencing severe (G3-4) ►neutropenia and ►thrombocytopenia, respectively. At the standard regimen hematological toxicity is of short duration, and mild gastrointestinal toxicities (nausea, vomiting), alteration in liver and renal functions, diarrhea and stomatitis are also observed. Serious renal or liver toxicities are rare but not reversible. Other toxicities include edema, cutaneous toxicity associated to pruritus, fever, neurotoxicity and dyspnea (uncommon but severe).

Indications

Gemcitabine is approved for the treatment of several solid tumors such as pancreatic cancer, advanced ►non-small cell lung carcinoma (NSCLC), ►bladder cancer, ►ovarian cancer and ►breast cancer.

NSCLC. Gemcitabine is approved for use in combination with ►cisplatin for first-line treatment of patients with inoperable, locally advanced (Stage IIIA or IIIB) or metastatic (Stage IV) NSCLC. Two schedules have been investigated and the optimal schedule has not been determined. The combination with cisplatin was investigated on the basis of preclinical studies showing inhibition of repair of platinum-induced DNA damage, and of the results of Phase II studies with single agent gemcitabine in more than 400 patients producing response rates consistently above 20%. Promising results are reported in a Phase III study combining gemcitabine to carboplatin, a platinum-analog less myelotoxic than cisplatin. The combination produced better results, in terms of response rate and time to progression than gemcitabine alone. Several Phase II trials have investigated the combination with non-platinum derivatives such as ►docetaxel, ►paclitaxel or vinorelbine, but no one has proven advantages when compared to the standard platinum-based regimen. Due to the low toxicity profile gemcitabine is under investigation in elderly patients with advanced NSCLC, either as single-agent or in combination with other drugs.

Pancreatic Cancer. Gemcitabine is approved for the treatment of patients with locally advanced (non-resectable Stage II or III) or metastatic (Stage IV) ►adenocarcinoma of pancreas both for first-line treatment and for patients previously treated with a ►5-fluorouracil-containing regimen. The approval for first-line treatment was based on the results of a randomized study showing improvement in median survival, 1-year survival and clinical benefits over weekly 5-fluorouracil. Several randomized trials have compared gemcitabine with a gemcitabine-based combination, none of which having shown any incremental benefit in terms of survival or quality of life. Improvement in survival might be achieved by prolonged infusion strategy of gemcitabine and is currently under evaluation.

Breast Cancer. Gemcitabine is approved for use in combination with ►paclitaxel for the first-line treatment of patients with metastatic breast cancer after failure of prior ►anthracycline-containing adjuvant chemotherapy. The combination of gemcitabine and ►taxanes (docetaxel or paclitaxel) was tested in clinical

trials based on the good single-agent activity of each drug. In a Phase III study gemcitabine in combination with paclitaxel significantly improved response rate, time to progression and survival versus paclitaxel in metastatic breast cancer, with no significant increase in toxicity. The best schedule for administration of gemcitabine-taxane combination and the relevance of the combination in ►Her-2/neu positive patients are currently under investigation.

Bladder Cancer. Systemic chemotherapy has reached a plateau in the treatment of ►transitional cell carcinoma of the bladder. Gemcitabine is approved for such disease in combination with cisplatin. Such regimen has equivalent response rate and survival, but improved toxicity profile, compared to the previous standard regimen including ►methotrexate, ►vinblastine, ►adriamycin and ►cisplatin. Promising results have been observed with intrabladder infusion of gemcitabine.

Ovarian Cancer. Gemcitabine is approved in combination with ►carboplatin in several European countries for the treatment of recurrent epithelial ovarian cancer. It has been recently approved also in the USA on the basis of a randomized trial comparing the combination against carboplatin alone in 356 women with recurrent ovarian cancer, whose tumors had previously responded to first-line therapy. The median progression-free survival was 8.6 months for patients taking carboplatin and gemcitabine, and 5.8 months for patients taking carboplatin alone. Quality-of-life was comparable in the two groups of patients. Multicenter Phase III trials are ongoing comparing paclitaxel plus carboplatin with gemcitabine plus carboplatin. The results of a single-institution Phase II study in 24 patients, indicate an acceptable toxicity (with bone marrow toxicity as dose-limiting) with high overall response rate (91%) for the combination gemcitabine plus carboplatin.

Clinical Pharmacology

Pharmacokinetic studies indicate that the drug is eliminated in the urine. Gemcitabine is rapidly converted to the inactive uracil metabolite (recovered in the urine for more than 90%), which has a long half life. Gemcitabine pharmacokinetic is linear and described by a 2-compartment model. ►Clearance and ►volume of distribution vary with duration of infusion, age and gender. Gemcitabine plasma protein binding is negligible.

Future Perspective

Due to its low bone marrow toxicity, gemcitabine might be a suitable drug to be combined with ►immunotherapy, and preclinical studies support such expectation. Gemcitabine can induce massive tumor-cell apoptosis both in in vitro and in vivo systems. Uptake of dead cells by ►antigen presenting cells, still alive after gemcitabine treatment, will result in increase of cross presenting tumor antigens to T lymphocytes in tumor draining lymph nodes. Moreover gemcitabine, which is particularly toxic for B lymphocytes, by impairing the antitumor antibody response might favorite the generation of ►cytotoxic T lymphocytes, which have to be generated for immunotherapy to be effective. Again, chemotherapy may upregulate cellular death receptors, which are used by T cells to kill targets. Gemcitabine followed by CpG-oligodeoxynucleotide, a modulator of the natural ►immune response, results in a strong therapeutic synergism in a pancreatic tumor model.

Thus, in addition to more combination studies with established or novel antitumor drugs, future clinical studies should address the role of gemcitabine in combination with various immunotherapies for the treatment of cancer.

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Gemtuzumab Ozogamicin

Definition

Is a humanized IgG4κ anti-►CD33 antibody linked to the anti-tumor antibiotic calicheamicin. Gemtuzumab ozogamicin is approved for treatment of relapsed ►acute myeloic leukemia in patients older than 60 years.

►Monoclonal Antibody Therapy

GEMZAR

►Gemcitabine

Gene Amplification

Definition
Amplification of the numbers of copies of one or more genes; synthesis of additional copies of an original gene.

►Amplification
►Neuroblastoma

Gene Battery

Definition
Refers to a group of genes regulated by a particular transcription factor.

►Dioxin

Gene Directed Enzyme-Prodrug Therapy

►HSV-TK/Ganciclovir Mediated Toxicity

Gene Expression Cassette

Definition
Stretch of recombinant DNA typically comprising a ►promoter, a gene and a polyadenylation signal; often used in ►gene therapy.

Gene Expression Profile

Definition
Messenger-RNA from relevant cells is copied to labeled (with fluorescence dye or radioactive nucleotide) complementary cDNA probes. The probes are incubated on a DNA array containing spots of DNA from known genes being investigated. Hybridization of a given probe signifies the presence of the mRNA in question in the cell or tissue studied, i.e., the gene is active. The method allows estimation of activity genes in one assay. Firstarray many was developed in early 1980 as "Oncogip" for profilin of ►oncogene expression.

►Microarray (cDNA) Technology

Gene Expression Profiling

Definition
A microarray-based methodology to study gene expression (RNA) patterns. Parallel analysis of the expressed transcriptome of a given cell line or tissue sample, first developed in early 1980ies for ►oncogene profiling.

►Microarray (cDNA) Technology

Gene Gun

Definition
Is a way for in vivo transformation of cells or organisms used for gene therapy and genetic immunization. This gun uses particle bombardment where DNA- (or RNA-) coated gold particles are loaded into the gun. A low pressure helium pulse delivers the coated gold particles into virtually any target cell or tissue.

►DNA Vaccination

Gene Knockout

Definition
Is a genetically engineered organism that carries one or more genes in its chromosomes that have been made

inoperative (have been "knocked out" of the organism). This is done for research purposes. Also known as knockout organisms (►knock-out mice) or simply knockouts, they are used in learning about a gene that has been sequenced, but which has an unknown or incompletely known function. Researchers draw inferences from the difference between the knockout organism and normal individuals.

Gene Profile

Definition

A extensive survey of genes leading to RNA encompassing known coding sequences in the genome.

►Gene Expression Profile

Gene Therapy

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Definition

Directed introduction and expression of new genetic information in cells of an organism for therapeutic purposes. Only somatic gene therapy is presently permitted, and the introduction of genetic material into germ-line cells is not allowed.

Characteristics

The science of gene therapy has its beginnings in the early 1980s, subsequent to the identification of several disease-related genes and the development of technologies for gene isolation, purification and transfer to cells in culture. Many different human diseases represent theoretical targets for gene therapeutic intervention and the strategies developed depend on the nature of the disease to be treated. Table 1 illustrates several classes of human disease and the respective treatment strategies currently being followed. It goes without saying that detailed knowledge of the molecular mechanisms of disease development and progression is a prerequisite for the development of gene therapeutic intervention strategies.

Transfer of Genes

Very many different methods have been developed to transfer therapeutic genes to patient cells. A consideration which is central to the choice of gene transfer vehicle (►vector) is whether stable or only transient gene expression is required. Stable gene expression is necessary for the treatment of hereditary genetic defects whereas transient gene expression is sufficient, and may even be desirable, when employing genes encoding toxic gene products e.g. in the treatment of cancer. Many gene transfer vehicles employ components of viruses (viral vector-mediated gene transfer) since viruses have evolved to efficiently transfer their own genes to cells and to express the respective gene products at high levels. Since it may not be possible to completely eliminate potential safety problems with viral vectors, non-viral gene transfer vehicles have been developed in parallel. These transfer vehicles are based on lipids/►liposomes (lipoplexes), on polycations (polyplexes), on branched polymer structures (dendriplexes) or consist simply of naked DNA (►Non-viral vectors for cancer therapy). In general, the transfer efficiencies and gene expression levels achieved with non-viral vectors are poorer than with viral vectors. The commonly used gene transfer vectors, their advantages and their disadvantages are summarized in Table 2.

In many situations, in addition to high gene transfer efficiency, it is necessary that the transfer vector is selective i.e. mediates expression of the therapeutic gene exclusively in targeted diseased cells. Achieving selectivity is a major hurdle which is being approached in several ways. Gene vectors are being manipulated (on the surface of the gene vector particle) such that targeted cells are exclusively accessed. Selectivity can be achieved by ensuring that, even in the situation in which many cells have been accessed, expression of the therapeutic gene can only occur in specific targeted cells (e.g. by employing tissue-specific promoter/enhancer elements to control therapeutic gene expression).

Clinical Relevance

Therapeutic genes can be introduced into patient cells either ex vivo or in vivo (see Fig. 1). In the ex vivo application, a patient biopsy is genetically manipulated outside the body and subsequently reinjected or transplanted. This procedure has the advantage that only the cells in the biopsy come in contact with the transfer vector. Furthermore, the circumstances in cell culture generally allow more efficient gene transfer than on in vivo application. In addition, in some cases it is possible to expand the cells in the biopsy and create a pool of genetically modified cells which can be

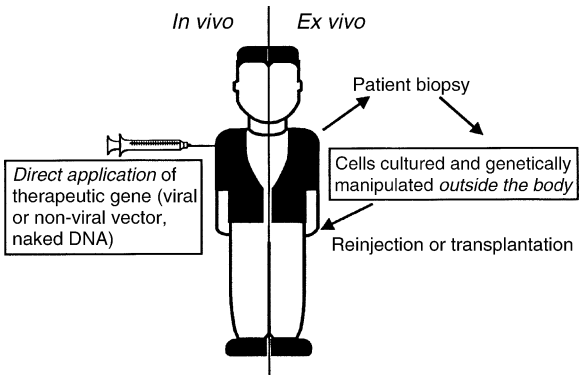
Gene Therapy. Table 1 Examples of gene therapeutic strategies for different disease groups

Disease group	Genetic basis of disease	Strategy
Hereditary monogenic disease e.g. haemophilia, cystic fibrosis	Defective gene which results in a single gene product being missing or non-functional	Introduction of wild-type gene (e.g. into liver or muscle for the production of a protein in the blood circulation, into lung in the case of cystic fibrosis)
Multifactorial disease e.g. cardiac disease, diabetes	Many genes and their gene products directly or indirectly involved	At present too complex. However, restenosis. (i.e. tissue proliferation after surgical dilation of a blood vessel) can be treated by locally expressing genes which inhibit cell proliferation
Cancer	Somatic mutation of cellular genes (several)	1. Induction of cell death employing genes encoding toxic gene products 2. Stimulation of the immune system to recognise and destroy cancer cells. (►Cancer vaccines ►DNA vaccination) 3. Inhibition of "cancer genes." (►Cell-cycle targets for cancer therapy) 4. Prevention of tumor ►angiogenesis
Infectious diseases e.g. AIDS	New genetic material from the infectious agent (e.g. virus) induces pathogenic processes	1. Inhibition of expression of genes encoded by the infectious agent employing e.g. antisense, ribozyme or small-interfering (si) RNA approaches. (►Antisense DNA therapy) 2. Vaccination against viral gene products expressed from transferred heterologous viral vectors or from naked DNA i.e. infection prevented or eliminated by the patient's immune system

Gene Therapy. Table 2 Gene transfer vehicles, their advantages and disadvantages

Gene vehicle	Advantages	Disadvantages
Retrovirus vectors including those based on lentiviruses	1. Integration of vector into host genome leading to stable gene expression 2. No cellular toxicity 3. Lentiviral vectors also infect (transduce) differentiated non-dividing cells	1. Titer not as high as e.g. adenoviral vectors 2. Vector integration into host genome is potentially mutagenic
►Adenovirus vectors	1. Very high titers (10^{13}/ml)	1.Only transient gene expression
	2. Very high, but transient, gene expression	2. Immune response to vector and transgene
Adeno-associated virus (►AAV) vectors	1. AAV is not pathogenic in humans	1. lower coding capacity than with retroviral and adenoviral vectors
	2. Non-toxic	2. Gene expression in proliferating tissue only transient
	3. Infects non-dividing cells	
Lipoplexes, polyplexes and dendriplexes	No viral genes, no toxic effects	Mostly lower and transient gene expression
Naked DNA	No viral genes, no toxic effects	Mostly lower and transient gene expression

reapplied to the patient as required. The main disadvantage of the ex vivo procedure is that it is technically cumbersome, time-consuming and very expensive. The most straightforward and desirable situation would be to apply the gene transfer vehicle in vivo either directly into specific tissues or organs (e.g. directly into tumor tissue) or by injection into the blood circulation. In vivo application requires that the transfer vector efficiently



Gene Therapy. Figure 1 Application routes for gene therapy.

reaches the appropriate diseased cells and at present, this is very often not the case. In fact, major problems hampering gene therapeutic approaches today concern the efficiency and the selectivity of the transfer vectors when applied in vivo. Further problems are related to the induction of host immune reactions towards the (viral) vector and the transgene.

Permission for the first clinical gene therapy study was granted in 1989 and since then numerous ►clinical trials, for the most part with only relatively small numbers of patients, have been carried out. Lack of sufficient selectivity and efficiency of transfer as well as lack of stability of transgene expression, the results of most of these trials did not establish any statistically verified positive effects on disease progression or mortality. Recently, however, more favorable results have been obtained. Thus, as a result of a gene therapeutic intervention with a retroviral vector, several individuals were cured of severe X-linked combined immunodeficiency. Unfortunately, however, within this same trial, severe adverse side-effects were observed in three treated patients. In the meantime, the reasons for these adverse effects have been largely elucidated and subsequent protocols will be adjusted accordingly. At present, basic research is focussed on improving gene vector properties and on gaining a better understanding of the interactions between the gene vector, the transgene and the patient's immune system. It is to be anticipated that the combined knowledge gained from these efforts will allow gene therapeutic protocols to be developed which will represent valid treatments for diseases which have been difficult or impossible to therapy up until now.

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Gene Transfection

Definition

Synonym Gene Transduction, Gene Transfer; Introduction of genes into cells.

►Transfection

Gene-Environment Interaction

Definition

The combined effect of genetic susceptibility and exposure to non-genetic factors, broadly defined as "environmental."

►Cancer Epidemiology

Genetic Association

Definition

Studies of genetic association test whether ►allele or ►genotype frequencies are different between groups of individuals (for example, subjects with and without cancer).

►Linkage Disequilibrium

Genetic Association Study

►Case Control Association Study

Genetic Epidemiology

Definition

The branch of epidemiology which aims at elucidating and quantifying the genetic causes of cancer. Genetic epidemiology investigates the aggregation of cancers in

families (linkage studies), and the presence of high-risk genetic variants in sporadic cases (association studies).

►Cancer Epidemiology
►Epidemiology of Cancer

Genetic Haplotype

Definition

Is a term used in the context of ►case–control association study where instead of analyzing the association between trait and each genetic marker singly, multiple markers are analyzed simultaneously for a combined effect. These multiple markers when combined together are called a haplotype. There are two rationales for examining haplotypes. First, using haplotypes allows multiple potentially causal markers to be tested simultaneously for association. However, for haplotypes to be superior to individual markers, multiple functional markers must have a strong interaction when combined and yet have no detectable effect when considered individually. Second, haplotypes can be tested for association because they may be a proxy for untyped causal markers. Some have pointed out that the additional association tests entailed in haplotype-based analyses can actually result in a loss of power, and hence lower efficiency, once corrections for multiple testing are taken into account.

►Case–Control Association Study
►Haplotype

Genetic Immunization

►DNA Vaccination

Genetic Instability

Definition

Synonym genomic instability, seems to be the hallmark of cancer cells, in contrast to the normal cells that, with few exceptions such as e.g. telomeres (►telomerase) or ►V(D)J-recombination, are genetically stable. The principal concept here is that when genes involved in the maintenance of genetic stability of the cellular genome undergo mutation, the repair of ►DNA damage often will be incomplete, due to impaired DNA repair (►repair of DNA) systems. As the consequence, during tumor ►multistep development, tumor cells assume a ►mutator phenotype and will accumulate mutations leading to the evolution, by Darwinian selection, of tumor cells that escape cellular growth signals and become more and more malignant. This process is not necessarily related, as often postulated, to very rapid tumor cell proliferation. Cells of many types of tumors actually multiply rather slowly and, in contrast, normal cells in various sites, like the epithelium of the intestine, multiply very rapidly but still remain normal. The ensuing genetic instability drives tumor ►progression by generating mutations in ►oncogenes and ►tumor-suppressor genes. These mutant genes provide cancer cells with a selective growth advantage, thereby leading to the clonal outgrowth of a tumor.

Genetic instability can be at the level of the chromosome (►chromosomal instability; CIN) or can be expressed as ►microsatellite instability (MIN; also referred to as MSI). Chromosomal instability (CIN) is a defining characteristic of most human cancers. Mutation of CIN genes increases the probability that whole chromosomes or large fractions of chromosomes are gained or lost during cell division. The consequence of CIN is an imbalance in the number of chromosomes per cell (►aneuploidy) and an enhanced rate of ►loss of heterozygosity. Microsatellite instability is a condition manifested by damaged DNA due to defects in the normal DNA repair process. MSI is a key factor in several cancers including ►colon cancer, ►endometrial cancer, ►ovarian cancer and ►gastric cancer. For colon cancer, a prominent form is hereditary non-polyposis colorectal cancer (HNPCC) or ►Lynch Syndrome, where an inherited mutation in a ►mismatch repair gene causes ►microsatellite instability. The replication error results in a ►frameshift mutation that inactivates or alters ►tumor suppressor genes.

Genetic changes in genetically unstable cells will occurs at random possibly affecting, in humans, any of the estimated 30,000 genes present in the genome. Because of this randomness, genomic instability eventually will lead to tumor cell populations that are genetically different, both within a given tumor in the individual patient and in the same tumor type of different patients. Because the biological behavior of tumor cells is dictated by the profile of genetic changes, the heterogenous populations of cells will respond differently to therapeutic treatments, such as ►chemotherapy or any other form of therapy. Very often, large populations of tumor cells, due to the pattern of genetic changes, may disappear by therapy-induced ►apoptosis. Still, minor populations, again because of their

particular genetic pattern, may be inherently ►drug resistant and may develop into a new tumor that now is fully resistant against therapeutic drugs. For the same reason, tumors in different patients may respond differently.

►Chromosomal Instability

Genetic Knock-out

Definition

A gene knockout is a genetically engineered organism that carries one or more genes in its chromosomes that have been made inoperative (have been "knocked out" of the organism). Also known as knockout organisms or simply knockouts (►knock-out mice), they are used in learning about a gene that has been sequenced, but which has an unknown or incompletely known function. Researchers draw inferences about gene function from the difference between the knockout organism and normal individuals.

►Orphan Nuclear Receptors

Genetic Polymorphism

Definition

Genetic polymorphism is the presence of multiple inheritable forms of a gene within the population; a genetic trait where the least common ►allele is found in approximately at least 1% of the population

►Detoxification
►Modifier Loci
►Pharmacogenomics in Multidrug Resistance

Genetic Recombination

Definition

Is the process whereby DNA from one chromosomal location is exchanged for, or replaces, another region of the genome.

Genetic Susceptibility

Definition

An inherited increase in the risk of developing a specific disease.

►Mutagen Sensitivity

Genetic Toxicology

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Synonyms

Chemical mutagenesis; Clastogenesis; Chemical carcinogenesis

Definition

The study of the processes and mechanisms by which chemicals or radiations cause damage, including ►mutations, DNA single-strand breaks and double-strand breaks, ►gene rearrangements and gene ►amplifications, and ►chromosome damage in prokaryotes or eukaryotic cells. This includes aspects of mutagenesis, i.e., the process by which specific chemicals induce changes in the sequence of DNA bases in genomes. It includes also ►chemical carcinogenesis, i.e., the molecular mechanisms by which chemicals carcinogens induce mutation in ►tumor suppressor genes, inactivating them, and induce mutations, rearrangements, or amplifications of ►oncogenes, deregulating them, causing fifteen such changes. These five to eight changes, consisting of inactivation of tumor suppressor genes and activation of oncogenes, leads to further derangements in the expression of ►numerous genes, which results in the cell acquiring a tumorigenic phenotype and the eventual development of cancer.

Characteristics

Genetic Toxicology

Genetic Toxicology, or the science of the study of chemicals and radiations (►Radiation-induced cancer) that can cause damage to genes and the genome, is now a very advanced science. There are now many

assay systems, both in bacteria, yeast, *Drosophila*, mice, and in cultured murine and human cells, which can be used to detect ►DNA damage. Such damage includes mutations induced by chemicals or radiation, gene rearrangements, gene amplification, chromosome damage (gaps, breaks, fragments, dicentrics, and satellites), single-strand breaks in DNA, and double-strand breaks in DNA. Important assay detection systems include the ►Ames assay that detect reversion of His − *Salmonella* back to His + *Salmonella*, and which also utilizes addition of exogenous ►cytochrome P450 to metabolically activate premutagens, such as ►polycyclic aromatic hydrocarbons to epoxides and diol epoxides. Mutation and recombination can also be studied in yeast. In addition, there are assays to study mutation in mice, using the "spot test." Plasmids can also be added to mice and retrieved from mice treated with suspect chemical mutagens, and then analyzed to detect mutations in these plasmids.

There is now an extensive body of data indicating that specific chemicals and radiations can be assayed in mammalian cells, both rodent cells (murine, rat, and hamster cells) and cultured human cells, for their ability to cause mutation. Examples of these assays include the assay for mutation to 6-thioguanine (or 8-agazguanine) resistance, which detects base substitution, frameshift, and deletion mutations caused by chemicals and radiations. There is an assay for mutation to ouabain resistance, which detects base substitution mutations in the (Na, K) adenosine triphosphatase (ATPase) in rodent and human cells. There is also an assay in L5178Y mouse lymphoma cells that detects large chromosomal mutations and also point mutations, in which the cells are assayed that are resistant to bromodeoxyuridine. These cells have mutations in or deletions of, the thymidine kinase gene. There is also a simple assay in which cells are exposed to chemical mutagens or radiations, and then the cells are lysed, and DNA damage is assayed in a gel electrophoretic assay ("►COMET assay").

From results of these assays, we now know that we can easily detect chemicals that cause genetic toxicity, in the form of DNA damage, mutations, gene amplification, or breaks in the single- or double-strands of DNA, or damage to chromosomes (gaps, breaks, fragments, dicentrics, satellites) (►Clastogenesis). In terms of correlations of genetic toxicity with cancer-causing ability, it has also been established that ~45% of all chemical carcinogens are mutagens. Hence, these assays detect mutagenesis/DNA damage, and will therefore detect mutagenic chemical carcinogens and radiations. Many chemical carcinogens are also clastogenic agents. The other classes of carcinogens involve those that cause ►methylation of tumor suppressor genes, rendering them transcriptionally quiescent, or demethylation of oncogenes rendering them transcriptionally active at inappropriate times. Another group of carcinogens are certain hormones (►Hormonal carcinogenesis), such as ►diethylstilbestrol, estrogen, and ►testosterone, which act by inducing cell division. Inappropriately high numbers of cell division can lead to spontaneous mutations in cells, or can drive cell division to replicate the DNA of cells that have been adducted by chemical mutagens or damaged by radiations, leading to fixation of chemical mutations.

Chemical, Viral, and Radiation Carcinogens

Broadly defined, a ►carcinogen is any material, whether radiation, chemical, or virus, which can induce tumors in lower animals or in humans. ►Tobacco contains ~4,500 different chemicals, and over 20 different known strong human carcinogens, as well as ►tumor promoters and ►cocarcinogens. Exposure to tobacco and tobacco products is thought to account for ~30% of all human cancers. These include ►lung cancer, ►bladder cancer, ►oral cancer, ►pharyngeal cancer, ►laryngeal cancer, ►nasopharyngeal cancer, ►esophageal cancer, ►kidney cancer, ►liver cancer, and ►pancreatic cancer, according to the latest US Surgeon General's report. ►Second-hand tobacco smoke, or environmental tobacco smoke, also causes cancer in exposed individuals.

A second grouping of human cancers is thought to be due to exposure to excess levels of normal hormones, including ►estrogens and ►testosterone. Excess estrogen is believed to lead to ►breast cancer by causing an excessive stimulation of cell division, leading to spontaneous mutations in the oncogenes and tumor suppressor genes of breast epithelial cells. An excessive level of cell division in the epithelial cells of the prostate, driven by testosterone and its metabolites, is thought to contribute to the induction of prostate cancer. Human ►endometrial cancer is thought to be caused by estrogens without progestogens. ►Ovarian cancer can be induced by ovulation and the accompanying hormonal changes. Exposure to excess levels of hormones is estimated to induce ~30% of new human cancer cases.

Dietary influences are very important in human cancer induction. Dietary influences are estimated to contribute to ~15% of human cancers. Cancers influenced by diet include ►stomach cancer, ►colorectal cancer, and nasopharyngeal cancer. Consumption of an excess of animal fat and excess caloric intake predispose to cancer induction. Conversely, high consumption of green leafy and yellow vegetables and fresh fruits and a diet high in fiber inversely correlate with cancer induction. There are also a number of dietary mutagens formed by pyrolysis of foods. These include the tryptophan metabolites, TRP P1 and TRY P2, and other metabolites such as PhiP and MeIQX. ►Colon cancer in particular is thought to be induced by an excess of animal fat and deficiency of dietary fiber. Caloric excess in the diet correlates with

endometrial cancer induction. Excessive ►alcohol consumption correlates with increased risk of induction of pharyngeal cancer, laryngeal cancer, liver cancer esophageal cancer, oral cancer, colon cancer, and breast cancer (►Alcohal-rediated Cancer). Another specific example of a known human chemical carcinogen occurring during food consumption is ►aflatoxin B1, a biocidal metabolite of the mold, *Aspergillus flavus*. *A. flavus* biosynthesizes and then utilizes aflatoxin B1 as a biocide against other microorganisms in order to establish its own ecological niche. Aflatoxin B1 is metabolized by cytochrome P450 in mammals to a mutagenic metabolite, aflatoxin B1 2,3-epoxide, which binds covalently to DNA and induces mutations in the ►*p53* tumor suppressor gene and in other genes. This process leads liver cancer in humans. Aflatoxin B1-induced liver cancer is common in The People's Republic of China, Taiwan, Africa, and Mozambique, where hot, wet climates favor the growth of *A. flavus*, and this fungus contaminates fruits and grains, which are ingested by humans, leading to liver cancer.

Exposure to oncogenic viruses is estimated to induce ~10% of all human cancers. Notable and well-studied viruses that are oncogenic include the ►human papillomaviruses, including HPV16 and 18, which induce human ►cervical cancer. ►Hepatitis B virus and ►hepatitis C virus induce human liver cancer in Taiwan, in The People's Republic of China, and in Mozambique. The ►human T cell leukemia virus (HTLV) induces human T cell leukemias in Japan and the Caribbean. Infection of humans with the human immunodeficiency virus (HIV) is known to lead to ►lymphomas. Exposure to ►Epstein-Barr Virus can lead to benign conditions such as mononucleosis, and also at lower incidences, to Burkitt Lymphoma in Africa and to ►nasopharyngeal carcinoma in The People's Republic of China.

A fifth category of agents that induce human cancer are drugs, ►x-rays, and ►UV radiation. This combined category of carcinogens is estimated to induce 10% of all human cancers. Certain medically approved drugs, such as a fraction of cancer chemotherapeutic agents, including ►alkylating agents, ►adriamycin, and ►tamifoxen, can induce secondary malignancies (►secondary tumor). Certain analgesics, such as phenacetin, can induce renal pelvic cancer. Prominent examples of radiation carcinogens include ►ultraviolet light associated with ►skin cancer and ionizing radiations – gamma rays, x-rays, beta particles, alpha particles, and neutrons. Studies of the atomic bomb blasts in Hiroshima and Nagasaki during World War II have provided the best estimates of dose–response curves for radiation-induced carcinogenesis. UV light from the sun and in tanning salons can induce skin cancer.

The sixth broad category of carcinogens that can induce human cancer occurs in the occupational setting. Occupationally induced cancer is estimated to contribute ~5% of all human cancers. In this context, exposure to ►benzene has been linked in epidemiological studies to the induction of ►acute myelogenous leukemia, ►non-Hodgkin lymphoma, ►chronic myelogenous leukemia, and many other types of leukemia in rubber workers and in workers in shoe factories in the past. Exposure of nickel refinery workers to mixtures of soluble and insoluble ►nickel compounds as aerosols has been correlated with increased incidences of nasal sinus and respiratory cancers. Similarly, exposure of workers to hexavalent ►chromium compounds in the chromate manufacturing industry and in the chrome plating industry correlates with induction of lung cancer. Vinyl chloride exposure has led to human lung, liver, and brain cancer, and to the immune-mediated disease, vinyl chloride disease, which is similar to scleroderma.

Other examples of occupational carcinogens include bis-chloromethyl ether (lung cancer), ►asbestos (lung cancer and ►mesothelioma), and trichloroethylene (cancer at multiple sites) and perchloroethylene. ►Dioxin (TCDD, or 2,3,7,8-tetrachloro-p-dibenzo-dioxin), a by-product of paper manufacturing, is a potent carcinogen and tumor promoter. ►Arsenic, a by-product of copper smelting, is also found as an environmental carcinogen in drinking water contaminated by arsenate leached from iron sulfide-bearing rocks.

Environmental carcinogenesis is thought to be important also. However, at the present time, no solid estimates of the amount of human cancer that environmental pollution with carcinogens causes can be made with confidence due to our lack of knowledge in this area.

Chemical Carcinogenesis

Chemical carcinogenesis is the process by which chemical carcinogens (and radiations) can induce tumors in lower animals or in humans. Broadly viewed, chemical carcinogenesis begins as the process in which the very complicated series of ►signal transduction pathways mediating cellular growth and proliferation and their negative regulators are disrupted and deregulated. Chemical carcinogenesis is clearly a multistep process.

Chemical carcinogenesis has been divided conceptually and experimentally in the l930s by Isaac Berenblum, of the Weizmann Institute in Israel, into two stages, initiation and promotion (►Tumor promotion), when studying carcinogenesis on the backs of shaved mice. Berenblum used small doses of the ►polycyclic aromatic hydrocarbon 7,12-dimethylbenza(a)anthracene (►DMBA) as the initiator and the mixture croton oil, a biocide isolated from the plant, *Euphorbia lathyris*, as a promoter. Initiation is thought to be a mutation, likely in an oncogene such as the ►*RAS* cellular oncogene. Stimulation of the initiated

cells bearing mutations in the *RAS* or other cellular oncogenes with croton oil stimulates the cells to grow. With repeated croton oil stimulation, the initiated cells form a benign tumor called a ►papilloma. With further croton oil stimulation, the cells convert into a malignant tumor, called a ►carcinoma. Further damage to the cells of this carcinoma can result in a metastatic carcinoma. For many decades, investigators have used tetradecanoyl-phorbol acetate (►TPA), a chemical purified form croton oil, as the tumor promoter. TPA is one of the most potent tumor promoters known, and it binds to ►protein kinase C to stimulate cell division of initiated cells. Studies of how tumor cells interact with fibroblasts and immune effector cells to enable them to continuously grow and break free of the homeostatic mechanisms of the host that mediate tissue integrity are subjects of intense current interest.

Chemically Induced Morphological and Neoplastic Transformation and the Molecular Biology of Carcinogenesis/Cell Transformation and Human Cancer

At the cellular level, chemical carcinogenesis first involves the conversion of a normal cell into a tumor cell. There are a number of in vitro cell culture systems in which this process can be studied in a systematic way. They involve studying the loss of contact inhibition of cell division, the loss of anchorage dependence of cell division, escape from calcium ion-induced terminal differentiation, and the eventual acquisition of the property of tumorigenicity. These include C3H/10T1/2 Cl 8 mouse embryo fibroblastic cells, Balb/c 3T3 cells, Syrian hamster cells, mouse epidermal keratinocytes, and rat tracheal hamster epithelial cells among the rodent cell systems. Additional human cell systems involve diploid human fibroblasts and human epidermal keratinocytes.

This process of malignant cell transformation proceeds first through a series of mutations, rearrangements, or amplifications of oncogenes, or in epigenetic changes involving methylation status of oncogenes, leading to inappropriate expression of the normal oncogene product, or to expression of a mutated oncogene product. Such expression can lead to inappropriately high expression of various proteins involved in signal transduction pathways downstream of the oncogene product in the signal transduction pathway. A number of oncogenes contribute to cellular progression chemical carcinogenesis. Oncogenes are a set of genes controlling cellular growth and proliferation, and consist of a large number of gene families. Prominent members of these families include the ►*RAS* gene family, the ►*MYC* gene family, ►*ABL*, ►*FOS*, ►*JUN*, ►*ERBB2*, *FMS*, ►*KIT*, *RAF*, *SIS*, *ERBA*, *ETS*, *REL*, ►*HER2/neu*, ►*SRC*, ►*BCL-2*, *BCL-3*, *BCL-6*, *HOX1*, *RHOM-1*, *RHOM-2*, *TAL-1*, *TAL-2*, *TAN-1*, and many others.

Secondly, this process involves the inactivation of a number of ►tumor suppressor genes. Tumor suppressor genes are negative regulators of cell growth and proliferation. Prominent members of this large group of genes include the genes ►*RB1*, ►*FHIT*, ►*VHL*, ►*APC*, ►*WT1*, ►*NF1*, ►*NF2*, ►*TP53*, ►*MEN1*, ►*PTEN*, and many others. Inactivation of the *RB1* tumor suppressor gene leads to release of the transcription factor EF-2 from the Rb–EF2 complex, which can cause cell cycle progression. Mutational inactivation or deletion of *TP53* can lead to failure to stop cell cycle progression and allow DNA repair to proceed, leading to an accumulation of mutations in cells. Additionally, inactivation of *TP53* can lead to a failure of cells bearing many mutations to undergo ►apoptosis. This allows accumulation of cells bearing mutations, and allows these cells to progress toward malignancy.

The accumulation of mutations, gene amplifications, gene rearrangements, or deletions is thought to lead to conversion of a normal cell into a malignant cell. These include events that activate a number of cellular oncogenes, and that inactivate tumor suppressor genes. Hence, a number of cellular oncogenes would be mutated and activated, or amplified, or rearranged and placed under the control of strong promoters. This would lead to expression of mutant oncogene product, or to higher steady-state levels of normal oncogene product. These protein products then would impact upon signal transduction pathways to stimulate these pathways. In the same manner, mutational inactivation of tumor suppressor genes, methylation of the promoters of these genes to transcriptionally inactivate them, and breakage and loss of part of the chromosome bearing these genes, or loss of the entire chromosome bearing these genes, can also contribute to carcinogenesis. It is thought that fifteen events, including activation of oncogenes and inactivation of tumor suppressor genes, lead to carcinogenesis. What has only recently become known is that fifteen such events can have far-reaching effects on global gene expression, leading to aberrant expression of 100–300 genes in cells, which results in the malignant phenotype. This is because mutation or overexpression of each oncogene can lead to increased expression of ~10 additional genes in signal transduction pathways in which this gene participates. Similarly, each tumor suppressor gene may control expression of approximately an additional 10 genes. When this suppressor gene is inactivated, stimulation of its expression of these additional 10 genes is also lost. Hence, physiologically, tumor cells suffer global derangement of gene expression. Our knowledge of this complexity paradoxically provides many opportunities for therapeutic intervention to kill tumor cells, cause them to apoptose, or to cause them to differentiate into nontumorigenic cells.

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Genetically Engineered Mice

►Mouse Models

Genetically Engineered Model

Definition

Cancer models created by genetically altering an animal (usually a mouse) to make it more susceptible to cancer. The genetic alteration can cause the animal to overexpress a gene that promotes tumor development or inactivate a gene that suppresses tumor development.

►Ultrasound Micro-Imaging
►Gene Knockout
►Transgenic
►Knock-out mice

Genistein

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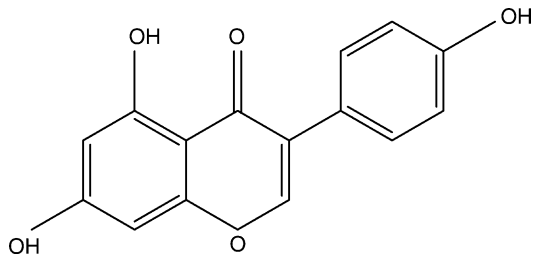
Synonyms

4′,5,7-Trihydroxyisoflavone; Soy phytoestrogen



Genistein. Figure 1 The chemical structure of Genistein.

Definition

Genistein is an ►isoflavone, derived principally from soybeans, that has cancer preventive and therapeutic effects (see Fig. 1).

Characteristics

Soy and Cancer

Numerous epidemiological studies have indicated that a diet high in soy products can decrease the risk of developing cancers, including ►breast cancer, ►prostate cancer, ►colon cancer, ►lung cancer and ►skin cancer. In countries where soy products are frequently consumed, there is a lower incidence of these and other cancers. A study of more than 600 women in Singapore (420 healthy controls and 200 women with confirmed breast cancer) demonstrated that ingestion of soy products correlated with a decrease in cancer risk. Moreover, a similar study with men showed that soy consumption decreased prostate cancer mortality.

Two isoflavones, genistein and daidzein, appear to be the major mediators of the cancer preventive activity of soybeans. For genistein, which is probably the primary active component, a body of work related to its cancer preventive and therapeutic effects, as well as to its mechanisms of action, has emerged.

Cancer Prevention by Genistein

Studies of ►chemical carcinogenesis and cancer development in transgenic animals have demonstrated that administration of genistein can decrease the incidence of cancer and decrease the multiplicity of tumors that develop. For example, treatment of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice with 100–500 mg genistein/kg diet reduced the incidence of advanced-stage prostate tumors in a dose-dependent manner. A high-isoflavone diet also inhibited methylnitrosourea-induced prostate tumors in Lobund-Wistar rats. Treatment of mouse mammary tumor virus (MMTV)-neu mice with diets containing 250 mg/kg of genistein or daidzein increased the latency for spontaneous breast tumors but did not affect tumor size or multiplicity. Topically applied to ►DMBA-initiated and ►TPA-promoted Sencar mice,

genistein reduced the incidence and multiplicity of skin tumors by 20% and 50%, respectively.

In addition to its chemopreventive effects, genistein can be used therapeutically. It increases the survival of animals bearing ►xenograft, chemically induced and spontaneous (transgenic) tumors and improves the response to conventional therapies. Genistein can act additively or synergistically with chemotherapeutic agents, hormone therapy, ►immunotherapy or ►radiation therapy. Epidemiological studies with cancer patients suggest that increased soy consumption may increase survival. Moreover, while randomized trials are still ongoing (or have yet to be performed for many types of cancers), genistein appears to have little or no toxicity.

Mechanisms of Action of Genistein

Although the mechanisms of action for the anticancer effects of genistein have not been established, there are a number of candidate pathways. Reported *in vitro* and/or *in vivo* activities of genistein include estrogen agonism/antagonism; inhibition of protein tyrosine phosphorylation; ►topoisomerase inhibition; suppression of ►angiogenesis; scavenging of free radicals; and inhibition of ►matrix metalloproteinases, ►NF-κB and oncogenes, such as ►MDM2. Any or all of these effects can decrease carcinogenesis and tumor progression, lead to the induction of apoptosis and cell differentiation, and suppress ►metastasis.

Bioavailability and Safety

The ►bioavailability of genistein appears to be greater than that of many other natural compounds. Plasma concentrations of genistein in tumor-bearing ►nude mice fed a diet containing 1 mg/g genistein were 3.4 μmol/L; in humans, a single oral dose of 460 mg resulted in peak plasma concentrations of 20–25 μmol/L. In most commercially available products, isoflavones are present as glycosides, which may require hydrolysis by intestinal beta-glucosidases. After absorption, ►phytoestrogens are primarily converted to glucuronic acid derivatives. Extensive metabolism, accomplished by bacteria in the gut, leads to the formation of equol and lignans.

Although genistein is generally regarded as safe, and toxic effects of genistein do not occur when cells are exposed to physiologically relevant levels of the compound, there are some undesirable effects, most of which have been observed in animal models and are due to its hormone-like properties. Because there is evidence from *in vitro* and animal studies that it can stimulate the growth of hormone-dependent cancers (especially ►estrogen receptor alpha positive breast cancers), use of genistein to treat these cancers is controversial.

Other Indications

Because of its estrogenic effects, genistein has been used to ameliorate the effects of menopause. Studies so far have been inconclusive, although some suggest that genistein can improve the symptoms. Future studies, especially those involving use of randomized placebo controls, will be needed to determine its efficacy.

Based on its estrogenic, anti-oxidant and other properties, genistein has also been suggested to prevent osteoporosis, improve cognitive function, decrease cholesterol, and improve cardiovascular health.

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Genomic Imbalance

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Definition

Refers to a genome showing any loss or gain of DNA sequences compared with the reference DNA whole sequence of the genome of interest. The term usually indicates extensive disequilibrium in the number of chromosomes or chromosome segments per cell.

Characteristics

Genomic Imbalance Concept

The relative dosage of genes governing cell physiology, is the product of evolution. Human somatic cells have evolved their "genome balance" as ►diploid, and variation in the number of chromosome copies as compared to the euploidy which has evolved, leads to genomic imbalance. This affects gene dosage and, consequently, promotes imbalances in cellular pathways. Most cancer cells acquire genomic imbalance as a consequence of ►aneuploidy, i.e. an abnormal copy number

of genomic elements, identified as gain and/or loss of whole chromosome(s) (aneuploidy) or chromosome segment(s) (partial aneuploidy). Genomic imbalance reflects the ►karyotype complexity of the cancer cells.

Cancer as a Genetic Disease

Cancer as a genetic diseasse involving genomic imbalance is a concept that has been explored since the end of the nineteenth century. In 1890 Paul von Hansemann (1858–1920), noticed that chromosomes in carcinomas segregate in unbalanced fashions, and at the beginning of the twentieth century, in 1914, Theodor Boveri (1862–1915) proposed his "chromosome theory of cancer," according to which cancer results from abnormal chromosomal composition within cells. Nowadays, this concept is widely accepted, based on molecular evidence, according to which cancer is a biological process characterized not only by accumulation of chromosome changes, but also by gene mutations, all contributing to the cell transformation process. The loss of critical cellular checks and balances throws proliferation processes into disorder by means of sequential steps, broadly including ►initiation, ►progression, ►invasion and ►metastasis.

Cause of Genomic Imbalance in Cancer

Genomic imbalance is the product of a phenomenon called ►chromosome instability (CIN), resulting in the increased probability that during cell division, whole chromosomes or chromosome segments will be acquired or lost. CIN is actually an ongoing process, responsible for variation in the rate of chromosome changes according to Darwinian selection, and may end with the production of stable aneuploid cells having proliferative advantages. A certain degree of cytogenetic heterogeneity within a tumor is maintained by CIN, and cytogenetically related or unrelated clones often persist alongside the favored one, contributing to intratumoral genomic imbalance. Many aggressive cancers, such those of the bladder, brain, breast, bone, cervix, colon, gallbladder, head and neck, liver, lung, ovary, pancreas, prostate, and testis show an accumulation of complex rearranged chromosome patterns, which may include, in addition to aneuploidy, ►double-minutes and the formation of ►homogeneously-staining regions. CIN, and the consequent genomic imbalance, calls for the interaction of various outcomes, including, abnormal mitotic segregation, abnormal shortening of ►telomeres and ►mitotic checkpoint deficiency.

- *Abnormal mitotic segregation*. Chromosome segregational defects may be due to different mechanisms, such as ►multipolar spindles formation, loss of ►sister chromatid cohesion, ►kinetochore defects during spindle attachment.

Multipolar spindles may develop in the presence of supernumerary ►centrosomes. Centrosome amplification is frequently seen in almost all types of solid tumors, and to a lesser extent in leukemia and lymphoma. Its occurrence is strongly associated with a high degree of aneuploidy. Amplification of *STK15/* aurora kinase*A* gene, inactivation of tumor suppressor genes, such as TP53 (Tumor Protein 53), *RB1* (Retinoblastoma), and *BRCA1* (breast cancer1) as well as overexpression of cyclins D and E, play a prominent role in this process.

Loss of sister chromatid cohesion is crucial in the metaphase-to-anaphase transition and the correct separation of genetic material in the cell. Cohesion is maintained by proteins called ►cohesins, which have to be degraded to lead to sister chromatid separation. Cohesins are released through a number of catabolic steps driven by the anaphase-promoting complex (APC), a ►ubiquitin ligase, targeting proteins for degradation. In yeast, APC acts on ►securin proteins, which in turn prevent ►separin (a cysteine protease, also called separase) from promoting sister chromatid separation. Human homologs of yeast separin and securin are respectively ESP1 (Extra Spindle Poles-like1) and ►PTTG1 (Pituitary Tumor-Transforming Gene1) proteins, and their inactivation interferes with the correct distribution of genetic material at ►anaphase.

►Kinetochore defects during spindle attachment may also have a major impact on chromosome malsegregation. Kinetochore is a highly-complex proteinaceous structure located in centromeric DNA and providing dynamic attachments to spindle microtubules, mediating the chromosome bi-orientation necessary to facilitate accurate segregation. Disturbances in this process result in aneuploid cells.

- *Abnormal shortening of telomeres*. This phenomenon is observed in both benign and malignant tumors. Shortening of ►telomeres during normal cell development and aging is the rule; however, in somatic cells that have lost proliferation control, it gives rise to genome instability and abnormal cell proliferation. Such abnormal shortening causes telomeres to lose their ability to protect chromosome ends from fusion. The products of chromosome fusion contribute to CIN by the formation of anaphase bridges, leading to structural and numerical chromosome changes or, through cell division inhibition, to poliploydization and subsequent multipolar spindle formation.
- *Mutations at mitotic checkpoint*. The regular mitotic phase of the ►cell cycle involves an orderly set of events, subdivided into phases: the prophase, prometaphase, metaphase, anaphase and telophase. In a normal cell, anaphase can start only when all chromosomes have congressed to the metaphase plate and achieved bipolar attachment to the mitotic

spindle. This scenario requires a powerful ►mitotic checkpoint, based on a dynamic balance of ►ubiquitination (by APC) and deubiquitination (possibly by ►USP44, Ubiquitin-Specific Protease44) of target proteins. Breakdown of mitotic checkpoint signaling brings on cell death, whereas its weakening, i.e. minor changes in checkpoint protein levels, does not compromise cell viability but promotes aneuploidy, since the mitotic checkpoint does not recognize a single or a few chromosomes lacking spindle attachment.

Although the role of genomic imbalance in tumorigenesis is recognized, and for some tumors diverse genomic imbalances correlate with prognosis, whether or not it may actually initiate tumorigenesis is still being debated.

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Genomic Imprinting

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Definition

Is an ►epigenetic alteration (i.e., not involving a change in base sequence) of a specific parental ►allele of a gene, or the chromosome on which it resides, in the gamete or zygote, leading to differential expression of the two alleles of the gene in somatic cells of the offspring. Genomic imprinting challenges two assumptions of conventional Mendelian genetics applied to human disease: that the maternal and paternal alleles of a gene are equivalent, and that two functional copies of a gene always are associated with health (Table 1).

Characteristics

Imprinted genes probably account for many examples of developmental malformations in humans, as uniparental disomy (UPD) of several chromosomes is associated with a variety of recognized defects, but UPD affecting most or all of a chromosome is rarely seen. Imprinting may have arisen in mammals as a result of an evolutionary conflict between maternal and paternal genomes. There is a strong and sometimes surprising relationship between imprinted genes and growth, including both prenatal growth and postnatal growth related to nurturing ability. Imprinting is also thought to underlie some quantitative trait loci for growth, with considerable potential commercial application in animal husbandry, and imprinting may be a potential barrier to stem cell transplantation. For all these reasons, genomic imprinting has generated intense interest.

Imprinted Genes and their Regulation

Despite this interest, the comprehensive identity of all imprinted genes remains unknown. At present over 80 imprinted genes are suspected in one or more species (see http://www.geneimprint.com). Imprinted genes appear to be organized within genomic domains. Evidence for this idea comes from studies of the region of 15q11-13 involved in Prader-Willi and Angelman syndromes, which cause mental retardation and neurological problems. This region harbors at least six imprinted genes and the region of the IGF2R gene contains at least three. Similarly, band 11p15 contains a domain of imprinted genes distributed over ~1 Mb. These genes play diverse roles including hormone-mediated growth stimulation (insulin-like growth factor II, or IGF2), control of the cell cycle (p57KIP2) and ion trafficking (KVLQT1). Both cis-acting and trans-acting factors appear to be important in the regulation of genomic imprinting. It is believed that cis-acting sequences can regulate imprinting over large distances. Evidence for this idea comes from the identification of patients with microdeletions involving upstream exons of the small nuclear ribonucleoprotein N gene (SNRPN), in the Prader-Willi region of chromosome 15. These deletions lead to disrupted imprinting extending over several megabases. This deletion site has therefore been termed an "imprinting center" for this chromosomal region. However, the molecular basis for the function of the imprinting center is as yet unknown.

Genomic Imprinting. Table 1 Key ideas of genomic imprinting and cancer

Genomic imprinting	An epigenetic alteration in the gamete leading to differential allele expression in the offspring
Examples of imprinting in disease	UPD with birth defects
	Specific disorders (PWS, AS)
	Human cancer
Imprinting in cancer	Loss of imprinting (LOI) in childhood and adult tumors
	Both gene silencing and gene activation
	Link to cancer risk
Mechanisms	DNA methylation
	Conserved regulatory elements and genomic domains
	Modifier genes

Imprinting and DNA Methylation

Cytosine DNA ►methylation also appears to be important in the regulation of genomic imprinting, as loss of the cytosine DNA methyltransferase I gene (Dnmt1) disrupts normal genomic imprinting in mice. DNA methylation is a covalent modification of DNA in which a methyl group is transferred from S-adenosyl methionine to the C-5 position of cytosine. DNA methylation occurs almost exclusively at CpG dinucleotides. CpG islands, which are sequences unusually rich in ►CpG dinucleotides, are usually found in the vicinity of imprinted genes.

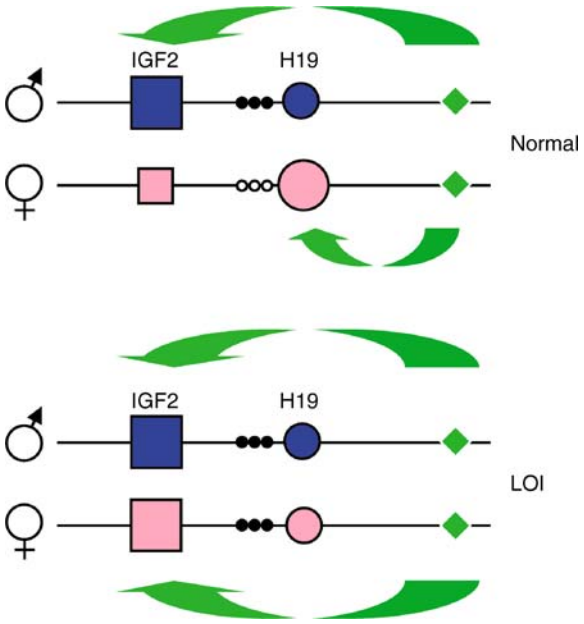
Recently, one mediator of imprinting was discovered. It is the CCCTC-binding factor (►CTCF), a transcription factor that binds to an unmethylated, GC-rich sequence ~2kb upstream of the H19 gene. The latter has previously been shown to be necessary for normal imprinting of H19 and ►IGF2. The same CTCF does not bind to methylated DNA. CTCF is known to be an insulator binding protein, and its binding prevents access by IGF2 to a shared enhancer 3′ to H19 (and about 200 kb telomeric to IGF2 itself).

Loss of Imprinting (LOI) in Cancer

In 1993, it was discovered that IGF2 can undergo loss of imprinting (LOI) in cancer, with abnormal expression of the normally silent maternal allele. LOI of IGF2 has been found in a wide variety of tumors, including embryonal tumors and adult malignancies. In the case of ►colon cancer, LOI was found not only in the tumors but in the normal tissues of patients with colon cancer. Furthermore, multiple well-controlled studies have now shown an association of LOI with a positive family history and personal history of colorectal tumors, both benign and malignant. Studies of mouse models with LOI and a gene mutation causing intestinal tumors show that LOI increases tumor initiation, and acts by increasing and altering the tissue stem cell compartment. Thus, tests for LOI may eventually prove useful for identifying patients at risk of cancer, thereby reducing overall cancer mortality (Fig. 1).

In the case of embryonal tumors, this activation of the maternal allele of IGF2 is coupled to silencing of the maternal H19 allele and methylation of a normally unmethylated maternal H19 ►CpG island. However, LOI of IGF2 occurs independently of H19 in adult tumors, such as ►cervical cancer and ►brain cancer. LOI of IGF2 is now recognized as one of the most common genetic alterations in human cancer. LOI of IGF2 is also found in about 15% of patients with ►Beckwith-Wiedemann syndrome (BWS). BWS is a disorder of prenatal overgrowth, birth defects and cancer, which is transmitted as an ►autosomal dominant trait, although most cases arise sporadically. In addition, LOI of the LIT1 gene, also on 11p15, is found in about 40% of BWS patients. This gene is particularly interesting, as it is an antisense transcript normally expressed from the paternal allele, that lies entirely within the maternally expressed KVLQT1 gene. A link between LOI and DNA methylation is also suggested by studies using the drug 5-aza-2′-deoxycytidine, which inhibits DNA methylation. This drug can restore a normal pattern of imprinting to tumor cells with LOI, suggesting that this or other pharmacological agents might eventually be used to treat cancers specifically with LOI or as chemopreventive agents in patients with LOI in their normal tissues.

Frequent loss of heterozygosity (►LOH) of 11p15 has also been observed generally in embryonal tumors, including ►Wilms tumor, ►rhabdomyosarcoma and ►hepatoblastoma. LOH of 11p15 is one of the most common genetic changes in cancer and is found in many adult malignancies, including those of the stomach bladder, ovary, breast and lung. Further strong support for the existence of an embryonal ►tumor suppressor gene on 11p15 derives from genetic complementation experiments. ►Microcell-mediated chromosome transfer of a human chromosome 11 suppresses the growth of rhabdomyosarcoma cells



Genomic Imprinting. Figure 1 Upper panel shows a normally imprinted IGF2 and H19 gene, with IGF2 expressed (drawn large) from the paternal allele, and H19 from the maternal allele. A shared enhancer (green) cannot cross an unmethylated CpG island (small open circles) upstream of the maternal H19 allele, presumably because of binding of CTCF to this island. The lower panel shows loss of imprinting (LOI) in cancer, with a switch of the paternal chromosome to a maternal epigenotype. Here the H19 CpG island is also methylated on the maternal chromosome, allowing the enhancer to interact with IGF2, with activation of the maternal IGF2 allele and silencing of the maternal H19 allele.

in vitro. Furthermore, subchromosomal transferable fragments limited to 11p15 do suppress tumor cell growth. However, in the investigation of an 11p15 tumor suppressor gene it is puzzling that in virtually all cases of LOH of 11p15 in embryonal tumors it is the maternal allele that is lost, an observation made before the discovery of human imprinted genes or such genes on 11p15 in particular. To address this problem, Sapienza argued that a gene that is not normally imprinted might become so aberrantly. According to Sapienza, a specific parental allele (in this case the paternal) would become silenced in some individuals. One or more of the maternally expressed genes on 11p15 might fulfill this role.

One of the most exciting frontiers in the study of genomic imprinting and its role in cancer is the identification of sequences that lie between genes, which might serve a regulatory role in the maintenance of normal imprinting of large genomic domains, such as on 11p15. One recent approach to identifying such sequences is by comparative genomics, in which the mouse sequence is obtained and compared to human sequence, in order to identify species conserved orthologous elements. These elements can then be tested in functional assays and be analyzed for mutations or deletions in patients with cancer or ►BWS. Proteins that interact with such sequences could include as yet unidentified modifiers of DNA methylation and/or genomic imprinting. Thus, one of the most important implications of the study of imprinting is that the lessons learned may be applicable to understanding the regulation of genomic domains generally, and their dysregulation may provide novel insights into the mechanism of cancer.

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Genomic Instability

Definition

►Genetic Instability

Genomics

Definition

Denotes the complete study of the hereditary material of living beings: both the coding and noncoding portions. Structural genomics involves creating "maps" of genomes, carrying out DNA sequencing, and determining the localization and the regulatory regions of genes on ►chromosomes. Functional genomics is the branch of genomics that studies the biological function of the

genes and their products, the coded proteins, their expression and regulation, as well as the interactions between different genes.

Genotoxic

Definition

Causes damage to DNA. Examples of genotoxic agents are chemicals or radiations that cause mutation to cells, chromosome breakage in cells, gene ►amplification in cells, or single-stranded or ►DNA-double strand breaks.

►Chemically Induced Cell Transformation
►Chemical Carcinogenesis
►DNA Damage

Genotoxic Carcinogen

Definition

A carcinogen capable of causing a change to the structure of the genome.

►Toxicological Carcinogenesis

Genotoxicity Tests

Definition

These represent a range of standardized assays designed to test chemicals for interaction with DNA, i.e. genotoxicity or mutagenicity. These comprise a number of different tests both *in vitro* and *in vivo*. The most usual study conducted early in the development of a new drug is the ►Ames Assay that utilizes mutants of *Salmonella typhimurium*. Various mammalian cell culture systems are also employed such as Chinese hamster ovary (CHO) cells. Clastogenic activity is assessed by exposing cells to chemicals and examining cells microscopically for chromosome damage. An *in vivo* study is also often performed examination of the bone marrow in treated rodents.

►Preclinical Testing
►Clastogen

Genotype

Definition

Catalog of individual's two alleles at a particular DNA location. Refers to the complete genetic composition of a single cell or organism. It also can refer to the specific allelic composition of a particular gene or group of genes.

►Arylamine *N*-Acetyltransferases (NAT)
►Biomonitoring
►Linkage Disequilibrium

Geranylgeranyl

Definition

C20 isoprenoid lipid, an intermediate in the HMG-CoA reductase mevalonate biosynthetic pathway, used in the biosynthesis of geranylgeranylated proteins.

►Rho Family Proteins

Geranylgeranylation

Definition

Post-translational modification of proteins by the attachment of an isoprenoid to C-terminal cysteine residues.

►Statins

Germ Cell Layers

Definition

Collection of cells formed during embryogenesis into the mesoderm, endoderm and ectoderm that go on to form specific organs of the body.

►Cowden Syndrome
►Germ Cell Tumors

Germ Cell Tumors

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Synonyms

Testicular tumors; Gonadal neoplasms; Dysgerminomas

Definition

►Testicular cancer represents a group of histologically heterogeneous neoplasms typically arising in gonadal tissue and, uncommonly, arising in extragonadal sites such as the retroperitoneum or mediastinum. A disease of young men which, when metastatic was previously uniformly fatal, testicular cancer is now usually cured.

Characteristics

Incidence

Germ cell tumors represent the most common cancer in young men between the ages of 20 years and 40 years. These tumors have a bimodal age distribution being most common in men ages 15–25 with a second, smaller peak at about age 60. It is estimated that ~8,000 new cases of testicular cancer were diagnosed in the United States in 2005. During the past century, the worldwide incidence of testicular neoplasms has nearly doubled, with the highest increases reported in the United States, Great Britain and Northern Europe.

Risk Factors

Numerous case-control and cohort studies have established cryptorchidism as the major identifiable risk factor, although only about 10% of cases are associated with this phenomenon. This increased risk is true for the contralateral testicle even if it is normally descended. Additional risk factors include a personal history of testicular cancer as well as the presence of a first degree relative with the disease. Scrotal trauma and toxic exposures have not clearly been associated with the occurrence of germ cell tumors.

Histological Classification

The main histological categories of germ cell tumors are seminomas (►Seminomatous Germ Cell Tumor) and non-seminomas (►Non-seminomatous Gene Cell Tumor). Non-seminomas are further subcategorized as embryonal carcinomas, endodermal sinus tumors (also known as yolk sac tumors), choriocarcinoma and ►teratoma. Tumors that contain more than one histological subtype are termed mixed germ cell tumors. For classification and treatment purposes, any tumor not histologically a pure seminoma is classified as a non-seminoma.

Clinical Presentation and Diagnosis

The majority of patients with testicular cancer presents with a painless testicular swelling or a palpable mass. In many cases, testicular swelling can be accompanied by pain secondary to bleeding or infarction within the tumor. Systemic symptoms at presentation such as abdominal pain, decreased appetite with or without associated weight loss, night sweats, chest pain, shortness of breath or hemoptysis usually indicate either an advanced stage of disease or an extragonadal primary tumor. If the diagnosis of a germ cell tumor is suspected, a bilateral high-resolution testicular ultrasound should be performed. In addition, the tumor markers human chorionic ►gonadotropin (hCG), ►alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH) have unique diagnostic and prognostic significance in germ cell tumors and levels should be obtained on all patients with suspected germ cell tumors. Though not specific for germ cell tumors, AFP is produced by tumors with endodermal sinus or embryonal components as well as by immature teratomas. HCG is a hormonal product of syncitiotrophoblasts and can be expressed by choriocarcinomas, mixed germ cell tumors and, sometimes, by seminomas. LDH is a cellular protein expressed in numerous tissues and can be produced by non-seminomas. These markers are important for ►staging of tumors, monitoring therapy, deciding when to apply surgical consolidation as well as to sensitively detect residual or recurrent disease. Additional preoperative workup typically includes a chest radiograph and discussion of sperm banking with the patient. When a testicular mass is found on ultrasonography, a radical inguinal ►orchiectomy should be performed, unless the patient is ill, and initiation of systemic therapy is deemed urgent. In this instance, it would be appropriate to proceed with ►chemotherapy without tissue diagnosis, if the markers are elevated and the clinical picture is compatible with the diagnosis of germ cell tumor. Trans-scrotal biopsies should be avoided as they can disrupt regional lymphatics, potentially altering the typically predictable lymphatic spread of these tumors. Postoperatively (or preoperatively if this will not delay surgery) an abdominal and pelvic computed tomography (CT) scan should be obtained and, if clinically indicated, brain magnetic resonance imaging (MRI) and a bone scan. CT scans can reveal clinically significant adenopathy

that will be important in clinical decision-making. The preferred sites of initial spread for right-sided tumors are typically the infra-renal paracaval, inter-aortocaval and possibly paraortic nodes. In contrast, left-sided tumors preferentially spread to the infra-renal paraortic nodes initially.

Staging and Risk Stratification

As with all staging systems, the purpose is to classify patients with respect to prognosis and to allow one to offer treatments having a toxicity profile commensurate with the burden of disease and risk of recurrence. Given the importance of tumor markers in the management of germ cell tumors, the staging for germ cell tumors takes these into account along with histology, site of origin and anatomic extent of disease. The standard risk stratification used for these tumors is that developed by the International Germ Cell Cancer Consensus Group (IGCCCG). Through a retrospective multivariate analysis of nearly 6,000 patients with germ cell tumors, this group identified the clinical features strongly associated with prognosis: primary site of disease, the presence of non-pulmonary visceral metastases and marker levels at the initiation of therapy. Using these features, they divided non-seminomatous germ cell tumors into good-, intermediate- and poor-risk categories and seminomas into good- and intermediate-risk categories (Table 1). This risk stratification system provided the basis for the most recent American Joint Commission on Cancer (AJCC) TNM staging system for germ cell tumors. In general, Stage I disease is confined to the testis, stage II disease is disease that does not spread beyond the retroperitoneum and stage III disease involves the nodal regions beyond the retroperitoneum or non-nodal metastatic disease. Elevated serum tumor markers can help to define higher stages of disease.

Management

As the biology and management of seminomas and non-seminomas are quite different, we will consider the management of these distinct histologies separately.

Seminomas

By IGCCCG risk stratification, all patients with seminomas without non-pulmonary visceral metastates are categorized as having good-risk disease. Amongst these patients are those with Stages I and II disease and some with Stage III disease. The majority of patients with Stage I disease will be cured with radical inguinal orchiectomy alone, however, ~15–20% of patients with Stage I seminoma will have recurrent disease. As a result, there exists a need to identify those features of Stage I disease that would indicate a higher risk of relapse so that such patients could be offered adjuvant therapy. It has been reported that there is a subset of patients with Stage I seminoma with a primary tumor

Germ Cell Tumors. Table 1 International germ cell cancer consensus group classification prognostic risk stratification

	Seminoma	Nonseminoma
Good risk	Any primary site	Testis/retroperitoneal primary and
	No non-pulmonary visceral metastases	No non-pulmonary visceral metastases and
	Normal AFP, any hCG, any LDH	AFP < 1,000 ng/mL and
		hCG < 5,000 mIu/mL and
		LDH < 1.5 × upper limits of normal range (ULN)
	82% 5-year PFS; 86% 5-year OS	86% 5-year PFS; 90% 5-year OS
Intermediate risk	Any primary site	Testis/retroperitoneal primary and
	Non-pulmonary visceral metastases	No non-pulmonary visceral metastases and
	Normal AFP, any hCG, any LDH	AFP 1,000–10,000 ng/mL and
		hCG 5,000–50,000 mIu/mL and
		LDH 1.5–10 × ULN
	67% 5-year PFS; 72% 5-year OS	75% 5-year PFS; 80% 5-year OS
Poor risk	–	Mediastinal primary or
		Non-pulmonary visceral metastases or
		AFP > 10,000 ng/mL or
		HCG > 50,000 mIu/mL or
		LDH > 10 × ULN
		41% 5-year PFS; 48% 5-year OS

less than 4 cm in size and without rete testis involvement who have a relapse free survival of 88%, and thus may constitute a group of patients most appropriate for surveillance. At present, surveillance is considered a reasonable option for highly motivated patients with Stage I seminoma after orchiectomy, though given the fact that nearly one third of patients with recurrent disease will require systemic chemotherapy, this has not been the most commonly used approach. At present, the majority of patients with Stage I seminoma are treated with infra-diaphragmatic radiotherapy (20 Gy) to the para-aortics. Such treatment results in 5-year survival rates of 98–99%. Data exists to support the use of a single dose of ►carboplatin as ►adjuvant therapy in Stage I seminoma, but this practice has not been widely adopted at present. Recently published retrospective data from the Princess Margaret Hospital in Toronto, Canada, showed comparable long-term results for patients with stage I seminoma treated with radiation prophylactically versus those with stage I who elected to be followed with active surveillance with initiation of therapy at the time of recurrence.

Patients with Stage II disease are divided into those with non-bulky disease (those with nodal metastases no larger than 5 cm) and those with bulky disease (those with nodal metastases greater than 5 cm). For patients with non-bulky disease (Stage IIA-B), infra-diaphragmatic radiation therapy (20 Gy) to include the para-aortics and ipsilateral iliac nodes with a boost (6–10 Gy) to the involved site is the standard therapy. Residual abnormalities are sometimes encountered following radiation therapy, but observation is generally recommended.

For patients with advanced seminoma, defined as having Stage IIC or Stage III disease, chemotherapy is the treatment modality of choice. For those patients with good-risk disease, chemotherapy with four cycles of ►etoposide and ►cisplatin (EP) is generally offered. ►Bleomycin is generally excluded as the risk of pulmonary toxicity outweighs the small incremental benefit afforded by its use. In the same light, ►carboplatin has been demonstrated to be inferior to cisplatin and is thus not substituted in this scenario. For those patients with intermediate-risk disease, chemotherapy is usually administered with four cycles of ►bleomycin, ►etoposide and ►cisplatin (BEP). After completion of chemotherapy, patients with advanced seminoma are restaged with chest, abdominal and pelvic CT scans as well as serum tumor markers. If no residual mass is found, or the residual mass measures <3 cm in size and tumor markers are normal, a surveillance schedule is initiated. If the residual mass measures >3 cm in size, a ►positron emission tomography (PET) scan is performed, if available, to assess for the presence of viable tumor. In the presence of a positive PET scan, salvage radiation therapy is offered at our institution. For those patients unable to undergo PET scan, the size of the residual mass guides post-chemotherapy management, with masses greater than 3 cm having a higher risk of relapse. In this case, a surgical biopsy or consolidative radiation therapy are the interventions of choice. Patients who are found to have progressive disease after initial therapy are generally treated with salvage chemotherapy.

Non-Seminomatous Germ Cell Tumors (NSGCT)

NSGCT are risk stratified according to the IGCCCG classification schema based on the location of the primary tumor, the presence of non-pulmonary visceral metastases and the level of tumor marker elevation. Treatment options vary by stage and can include observation, chemotherapy and/or retroperitoneal lymph node dissection (RPLND).

Patients with Stage IA NSGCT (tumor limited to the testis and epididymis without lympho-vascular invasion and normal post-orchiectomy tumor markers) are generally managed with either surveillance (in reliable patients) or RPLND. The reason for surveillance as an option is that the majority of patients with Stage I NSGCT will not have recurrence and of those approximately 30% who do have recurrent disease, there is effective chemotherapy which can result in long-term survival for most patients. RPLND is often used because it usually leads to accurate staging and can be curative in the majority of patients. The presence of N1 or N2 disease found at RPLND can then be managed either with surveillance or two cycles of EP or BEP chemotherapy. Patients with N3 disease found at RPLND are typically managed as good-risk advanced stage patients and are treated with EP for four cycles or BEP for three cycles. Patients with Stage IB NSGCT are generally managed with RPLND, although either active surveillance (for T2 disease only) or chemotherapy with two cycles of BEP is also appropriate. Post-RPLND management is as stated above for Stage IA disease. Patients with Stage IS disease (persistent marker elevation after orchiectomy) are managed with chemotherapy (either four cycles of EP or three cycles of BEP).

Stage IIA NSGCT in the presence of negative post-orchiectomy tumor markers are generally approached with either RPLND or primary chemotherapy with EP for four cycles or BEP for three cycles. Those patients with Stage IIA disease who have persistent tumor marker elevations are managed with chemotherapy alone. Patients with Stage IIB disease and negative markers may undergo RPLND as long as lymph node metastases are within lymphatic drainage sites. Post-RPLND management is the same as for those patients who have RPLND for Stage I disease. If the patient

has multi-focal lymph node metastases, adjuvant chemotherapy is considered the management option of choice. For patients who have received chemotherapy and have a residual retroperitoneal mass post-treatment, RPLND is generally performed to look for viable tumor, or teratoma.

Patients with advanced disease (Stage IIC and III) disease are risk stratified by the IGCCCG into three categories (good, intermediate and poor). Those with good-risk disease (testis or retroperitoneal primary; no non-pulmonary visceral metastases as well as AFP <1,000 ng/mL, hCG <5,000 mIU/mL and LDH <1.5 times upper normal range) are initially managed with chemotherapy (EP for four cycles or BEP for three cycles). Patients with intermediate-risk (testis or retroperitoneal primary; no non-pulmonary visceral metastases and AFP 1,000–10,000 ng/mL, hCG 5,000–50,000 mIU/mL or LDH 1.5–10 times upper normal range) or poor-risk disease (mediastinal primary or non-pulmonary visceral metastases or AFP ≥10,000 ng/mL, hCG ≥50,000 or LDH ≥10 times upper normal range) are managed initially with four cycles of BEP chemotherapy. For patients with advanced stage disease, who have a complete response to chemotherapy, the approach at MD Anderson Cancer Center is surveillance as the next step in management. If there is any residual disease (as long as tumor markers have normalized), all sites of residual disease are resected. The presence of non-viable tumor or teratoma allows for standard observation. For those patients with residual viable tumor >10%, salvage chemotherapy is initiated, generally with either a combination of vinblastine, ifosfamide and platinum (VeIP) or paclitaxel, ifosfamide and platinum (TIP). Approximately one-third of patients with persistently elevated tumor markers after multiple chemotherapy regimens may achieve long-term survival with "desperation surgery," if they undergo complete resection of their residual disease.

Conclusions

Germ cell tumors were amongst the first solid neoplasms to show remarkable response rates with chemotherapy. The successful management of most patients with testicular cancer has established a paradigm for the use of multi-agent chemotherapy regimens integrated with surgery, radiation and tumor marker evaluation. It has also allowed for risk-adapted treatment strategies, sparing patients with lower risk disease the therapies with greater short- and long-term risk, while treating patients with higher risk disease more aggressively. However, it must be remembered that despite remarkable successes in the management of germ cell tumors, there remains a population of patients with advanced, chemo-refractory disease for whom more effective salvage therapies are still needed.

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Germinal Center

Definition

Secondary lymphoid follicle present in the lymph node cortex that serves as the site of B cell maturation to antibody-producing cells. Germinal centers are histological structures in lymphoid organs like lymph nodes, spleen, and ►Peyer patches. They are mainly composed of proliferating ►B cells and smaller numbers of ►T cells, ►macrophages and ►dendritic cells. In germinal centres, T cell-dependent immune responses take place, giving rise to high affinity memory B cells and plasma cells. Many mature B cell lymphomas derive from malignant transformation of germinal center B cells.

►Hodgkin and Reed/Sternberg Cell
►Hodgkin Disease, Clinical Oncology

Germinal Stem Cells

Definition

Pluripotent stem cells derived from so-called primordial germ cells, which are the cells that give rise to the gametes (sperm and eggs) in adults.

►Adult Stem Cells

Germinoma

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Definition

Germinomas are rare malignant primary ►brain tumors thought to be arising from primordial germ cells of the yolk sac endoderm. They form part of the spectrum of ►germ cell tumors (GCTs) and are the commonest type of GCT in the central nervous system (CNS). in the CNS the histopathological appearances are termed germinoma and account for approximately 60% of all primary intracranial GCTs. Intracranial GCTs are histopathologically identical to their gonadal counterparts with a testicular primary being referred to as a ►seminoma and an ovarian primary as a ►dysgerminoma.

Characteristics

Germinomas account for 1–3% of all intracranial neoplasms and commonly arise in midline structures adjacent to the third ventricle. The most common regions involved are the pineal (~45%) and suprasellar (~35%) areas. In around 15% of cases concurrent lesions are detected in both the pineal gland and the suprasellar area or very rarely the basal ganglia or the brainstem. These tumors are termed bifocal or multiple midline GCTs.

Germinomas have a tendency to spread through the subependymal lining and cerebrospinal fluid (CSF). Approximately 5–10% of patients present with either microscopic or macroscopic metastatic disease in the CSF at the time of diagnosis. Extraneural metastases at diagnosis are extremely uncommon.

There is a marked geographical variation in the incidence of germinomas. In the West, they account for 0.4–3.4% of primary central nervous system tumors while in Japan and the Far East they are five to eight times more frequent.

Germinomas are more commonly found in males with a male to female ratio of approximately 2:1 and predominantly affect patients in their teens with approximately 75% of patients' diagnosed with a primary CNS GCT being in the age range of 10–20 and are a recognized tumor entity in CNS ►childhood cancer. The WHO classification divides germ cell tumors into subtypes depending on the final differentiated cell. With regards to their management they are divided into three major subgroups: pure germinomas, secreting (malignant) non-germinomatous germ cell tumors and teratomas. Classically Germinomas consist of large uniform cells with clear cytoplasm. Syncytiotrophobastic cells can be present and may secrete low levels of beta human chorionic gonadotrophin (β-HCG). Despite this, germinomas are often referred to as non secreting germ cell tumors if β-HCG levels are ≤50 IU/l in either serum or CSF. There is no evidence to suggest that a slight elevation of β-HCG levels is associated with a worse outcome.

Presenting symptoms of a germinoma depend on the anatomical site of the primary tumor and its growth rate. The classical symptom of pineal primaries is Parinaud syndrome (paralysis of upward gaze, headache and impaired pupillary constriction to light with preservation of accommodation). In addition tumors in this location frequently compress the Sylvian aqueduct leading to obstructive hydrocephalus, indicated by symptoms of raised intracranial pressure (diurnal headache, vomiting and lethargy). The commonest presenting symptoms of suprasellar tumors are obstructive hydrocephalus, endocrine and visual field defects and/or reduced visual acuity. The frequent involvement of the pituitary stalk and the proximity of the tumor to the hypothalamopituitary axis leads to diabetes insipidus (DI). This symptom can precede the radiological and pathological diagnosis of a suprasellar germinoma sometimes by up to a few years. Other symptoms include hypothalamic damage e.g. anorexia or weight gain, somnolence, mood swings, disrupted sleep pattern, electrolyte imbalances, temperature disregulation, failure to thrive, precocious puberty, secondary amenorrhea, and panhypopituitarism.

Diagnosis

When a germinoma is suspected based on the clinical history and physical examination, ►magnetic resonance imaging (MRI) with intravenous contrast enhancement of the brain and spine is the diagnostic imaging of choice to establish the precise location and extent of the disease. The classic appearance of a germinoma on MRI is an iso- or hyperdense well defined lesion that uniformly enhances with contrast. If the clinical picture and radiological findings are suspicious of a primary CNS GCT, tumor marker analysis is important to differentiate between secreting GCTs and germinomas as pathologically elevated tumor markers establish a diagnosis without the requirement for tissue diagnosis. When the tumor marker assays are negative, a histopathological verification of primary tumor is mandatory. In addition an examination of the CSF must be performed in all patients, to confirm or refute microscopic tumor dissemination (M1 disease), and establish the presence or absence of elevated CSF tumor markers (αFP, β-HCG). An isolated elevation of ►CSF tumor markers in the presence of negative serum markers establishes the diagnosis of a secreting germ cell tumor and

subsequently defines the appropriate management strategy. If CSF cannot be obtained intraoperatively a lumbar puncture should only be performed at diagnosis, if the ventricles are not obstructed or an adaeuate CSF diversion has been performed in patients with obstructive hydrocephalus. Alternatively, a lumbar puncture should be performed within 1–2 days of the surgical intervention to relieve raised intracranial pressure as the half-life of βHCG is only 5 days after complete macroscopic resection. It has to be acknowledged that there is currently no uniformly validated laboratory test for the measurement of CSF tumor markers although this is an internationally agreed mandatory requirement for staging CNS germinomas. To complete staging, a CSF analysis for cytology should be performed 14 days after the surgical intervention to avoid false positive results.

The differential diagnosis of a pineal mass includes pinealoblastoma, pineocytoma, glioma or benign cysts. At a suprasellar location, the possible differential diagnoses include, predominantly, optic chiasm and hypothalamic gliomas, craniopharyngiomas and Rathke's pouch cysts.

Treatment

Surgery

In an emergency situation, an endoscopic ventriculostomy or ventriculo-peritoneal shunt with or without a prior external ventricular drainage (EVD) will alleviate raised intracranial pressure, secondary to obstructive hydrocephalus, and/or could be used to obtain diagnostic tissue. Otherwise the standard diagnostic neurosurgical procedure is an open biopsy of the mass lesion. There is, however, increasing use of less invasive techniques such as stereotactic and endoscopic biopsy. Modern neuro-surgical techniques using a stereotactic approach are deemed safe and mortality is in the region of 0.5% in experienced hands with similar diagnostic yield rates.

There is no advantage in attempting a gross total resection in intracranial germinomas as it does not alter management or outcome, but it can be associated with significant surgical related morbidity. Radical surgery is reserved for the very few patients with a significant residual mass following completion of treatment which usually represents a residual mature teratoma component on histopathological assessment.

Histological subtype is the single most important prognostic factor for outcome as the natural history and treatment of different histopathological subtypes are quite distinct.

Radiotherapy

In excess of 90% of germinomas are radiocurable when treated in a tertiary specialist neuro-oncology unit with close links to an endocrine service. Historically the gold standard treatment for germinomas has been conventionally fractionated craniospinal ►radiotherapy (using external beam ►ionizing radiation therapy) followed by a boost to the primary site given the extremely high ►radiation sensitivity of these tumors. The pattern of relapse in localized germinomas is dominated by ventricular recurrences and it is unusual to develop an isolated spinal cord relapse. A recent literature review confirmed that there is good evidence to suggest that in completely staged patients with localized germinomas irradiation of the ventricles, followed by a boost to the primary tumor area gives equivalent long term control rates compared to wide field craniospinal radiotherapy. Originally the craniospinal axis was treated with a dose of 30–35 Gy followed by a boost of 10–20 Gy in 7–12 fractions to the primary site. Over the last two decades consecutive studies have demonstrated that a reduction of the dose to the craniospinal axis to 24 Gy is equivalent with respect to long term disease free survival. In addition, there was no loss of local control when reducing the primary tumor dose from 50–54 to 40–45 Gy. The use of three-dimensional planning and conformal radiotherapy, in conjunction with reduced volumes is likely to minimize the amount of normal tissue irradiated to high doses of radiotherapy. This is likely to reduce treatment related late sequelae e.g. growth impairment, neurocognitive disabilities, cerebrovascualr events and second malignant neoplasms.

There is some evidence to suggest that the primary tumor dose can be further reduced if a complete response can be demonstrated following multiagent induction chemotherapy in primary localized germinomas. However, there is controversy as to what volume requires irradiation in these patients. In addition controversy exists how to classify bifocal tumors. In Europe bifocal tumors are regarded as localized disease whilst in the States these patients are treated using strategies designed for primary metastatic disease.

There is currently no controversy over the volume that should be irradiated in patients with proven evidence of metastatic germinoma at diagnosis. CSF-positive disease is a risk factor for spinal seeding but it does not predict for recurrence when treated with craniospinal radiotherapy. Long term control in excess of 90–95% at 5 years is achieved even in the presence of widespread macroscopic metastatic disease (M 2/3) when craniospinal irradiation to dose levels of 24–30 Gy, and boosts to all sites of macroscopic disease up to a dose of 40–45 Gy is used.

Chemotherapy

Germinomas are inherently chemosensitive. As primary radiotherapeutic strategies in very young children are associated with noticeable late morbidity, focus in the scientific pediatric oncology community for a long time has been to develop chemotherapeutic strategies

for germinomas. This is either as a primary treatment strategy or in combination with risk adapted reduced doses or irradiation. However, despite being chemosensitive, only a limited number of patients with localized germinoma are primarily chemocurable as the delivery of conventional chemotherapy is to a degree limited in these patients by the ►blood brain barrier. Most patients require salvage treatment including radiotherapy. In addition a chemotherapy only approach comes at the expense of significant treatment related morbidity and mortality, not associated with radiotherapy alone. It might be anticipated that with continuing improvements in research and supportive care, as well as the availability of novel chemotherapeutic agents, some of the current limitation maybe overcome in the future.

At present chemotherapy has been successfully used in a number of combined modality treatment approaches (►chemoradiotherapy) for patients with localized disease. This aims to reduce the volume and/or the dose of radiotherapy with a reduction of late morbidity associated with radiotherapy in very young children. There is no proven benefit for using chemotherapy with respect to event free or overall survival in patients with metastatic disease at diagnosis.

The backbone of most reported multiagent chemotherapy are platinum derivatives (particularly ►carboplatinum), epipodophyllotoxins (e.g. ►etoposide), alkylating agents (e.g. cyclofosfamide, ifosfamide) and/or antibiotics (e.g. ►bleomycin). The commonest chemotherapy associated side effects are short term but can be life threatening. These include hematological morbidities with the risk of bleeding and infection (particularly neutropenic sepsis), renal and hearing impairment, hemorrhagic cystitis, electrolyte disturbances (particularly in patients with known DI) and infertility to name a few. While recurrences are rare, after combined modality treatment (~15%) they are potentially salvageable by further chemo and/or radiotherapy.

Genetics

Little data is available regarding critical genetic mutations in pathogenesis of intracranial GCTs. Speculation regarding a genetic link has arisen due to case reports of intracranial GCTs occurring in children with Downs syndrome. Cytogenetic studies have repeatedly demonstrated abnormalities of chromosomes 1 and 12, mirroring the changes seen in extracranial GCTs. Very rarely, patients with intracranial GCTs have been reported to subsequently develop gonadal GCTs, perhaps indicating an unidentified genetic predisposition. CNS germinomas, in particular, often display sex chromosome abnormalities, usually an increased number of copies of chromosome X. Along with the increased incidence in peri-pubertal patients and its association with the site of origin being near the diencephalic nuclei, which regulates gonadotropin activity, has formed the basis for a speculated link to gonadotropins. For example an excess of intracranial GCTs has been reported in patients with Klinefelter's syndrome, which is associated with high levels of gonadotropins. However, this association may possibly be more likely linked to the underlying chromosomal anomalies rather than the endocrine stimuli.

Recent studies demonstrated frequent mutations of in germinomas (~25%). ►KIT is a ►tyrosine kinase whose mutations may result in constitutive activation and has been implicated in ►chronic myelogenous leukemia (CML) and ►gastrointestinal stromal tumors (GISTs). Such a mutation may offer new therapeutic opportunities in this tumor group in the future.

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Germline Mutation

Definition

Is a heritable variation in the DNA that occurred in the lineage of germ cells (in the egg or the sperm). Mutations in germline cells when transmitted to offspring, gets incorporated in every cell of the body while those in somatic cells are not.

GI

Definition

►Gastrointestinal

Giant Cell Tumor

Definition

A locally destructive primary ►bone tumor in skeletally mature individuals consisting of proliferating mononuclear cells and large multinucleated ►osteoclast-like giant cells.

Gilbert Syndrome

Definition

Is frequently seen in Caucasians and is an inherited disorder, normally without serious consequences, that affects the glucuronidation of bilirubin in the liver. This results in an increase in the level of (unconjugated) bilirubin levels in the bloodstream and can lead to ►jaundice, in particular a yellow skin and eyes.

Ginkgo Biloba

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Synonyms

Salisburia adiantifolia; *Pterophyllus salisburiensis*; *Salisburia macrophylla*; Ginkgo tree; Maidenhair tree; Kew tree; Fossil tree; Japanese silver apricot; Yinxing

Definition

Ginkgo biloba is originally from Asia and has been utilized as an herbal remedy for thousands of years in the traditional Chinese pharmacopoeia. *G. biloba* is known for its antioxidant, anti-inflammatory, and neuroprotective properties. Extract from *G. biloba* leaves is commonly prescribed medicine to improve circulation and cardiovascular health.

Characteristics

Ginkgo, a unique medium-large deciduous tree with no close living relatives, is classified in the division of Ginkgophyta, consisting of the single class Ginkgoopsida, order Ginkgoales, family Ginkgoaceae, genus *Ginkgo* and the only one species – *G. biloba*. Ginkgo, the world's oldest living tree on earth for 270 million years (the Permian period), was referred to as a living fossil by Charles Darwin in 1859. They spread throughout Laurasia during the middle Jurassic and Cretaceous, but became much rarer thereafter. Ginkgo was discovered in China in 1691 and is now widely dispersed throughout the world. Ginkgo trees are common and widespread in Asia, Europe and North America. Once thought to be extinct, ginkgo trees disappeared from North America about 7 million years ago and from Europe about 2.5 million years ago.

Ginkgo is characterized by its unique two-lobed fan-shaped leaves and naked seeds (gymnosperms). Unlike most gymnosperms, ginkgo is a dioecious tree, male and female are separate plants. Ginkgo trees grow at a moderate rate, and can reach a height of about 100–130 ft and a spread of 25–30 ft at full maturity. The trunk can become approximately 10–13 ft wide in diameter. Ginkgo trees are straight columnar and sparingly branched. The leaves have a unique open dichotomous venation pattern. They are bright to dark green during the summer, and turn golden yellow in the fall. The seed has a silvery shine and is called silver apricot (nut). The seeds have been considered as a delicious food by the Chinese and Japanese. Green algae live in symbiosis with the ginkgo embryo and reproductive tissue, which is not known to occur in any other tree and only occurs in the animals. Ginkgo has a long life span, 1,000 or more, and may live as long as over 3,000 years. The oldest ginkgo is about 3,500 years old in China. *G. biloba* has been widely utilized in herbal medicine and as a dietary supplement. In the Far East, ginkgo has been used as a health-promoting supplement for over 3,000 years. The seeds are used in traditional Chinese medicine, and standard extract of the leaves is used as a phytomedicine in Europe and as a dietary supplement in the United States.

G. biloba Seeds

G. biloba seeds are called bai-guo (white nut) in Chinese or ginnan in Japanese. The seeds are considered to be beneficial to asthma, cough, irritability of the bladder, and uterine flux. Ginkbilobin, a protein isolated from *G. biloba* seed, has potent antifungal activity. Recently, *G. biloba* seeds have been discovered to have anticancer activity. *G. biloba* seed polysaccharide, isolated by ethanol and purified by Sephadex G-200 chromatography, potentially stimulates ►apoptosis of human hepatoma SMMC-7721 cells. However, 4-*O*-methylpyridoxine, a ginkgotoxin of *G. biloba* seed, is poisonous when a large amount is consumed.

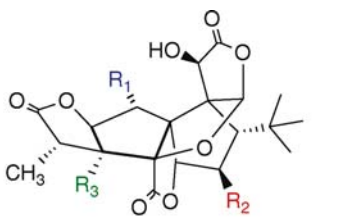
G. biloba Extract

Ginkgo leaf, known as bai-guo-ye in Chinese medicine, was first mentioned in 1436 for the external use

to treat skin and head sores as well as freckles, and for internal use in 1505 during the Ming dynasty of China. *G. biloba* extract has been available in Europe since the early 1990s. Dried *G. biloba* leaves are extracted by organic solvent, concentrated under reduced pressure, washed by liquid–liquid extraction, and finally dried. Extract is composed of 1 part extract from 50 parts raw leaves. The German Commission, one of Europe's most respected herbal standard authorities, has defined the active components of *G. biloba* extract (trade names: EGb 761, Ginkgold, Cerenin, Ginkocer Ranbaxy, Tebonin, LI 1370, Rökan, and Tanakan) as 22–27% ginkgo-flavone glycosides (primarily glycosides of kaempferol, quercetin, and isorhamnetin with glucose or rhamnose) and 5–7% terpene lactones (2.8–3.4% ginkgolides and 2.6–3.2% bilobalide) (Fig. 1) At least seven flavonoid components, including quercetin, isorhamnetin, kaempferol, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin, have been isolated from *G. biloba* extract using reversed phase HPLC. Ginkgolides are only present in *G. biloba* and not in other living species, and are composed mostly of A, B, C, and a very small quantity of J and M, which are only different in the number and position of hydroxyl groups. Ginkgolide B and bilobalide account for about 0.8 and 3% of the total extract, respectively. Other components include proanthocyanidins (4.2–5%), glucose, rhamnose, organic acids, D-glucaric acid, and ginkgolic acid (anacardic acid, less than 5 ppm).

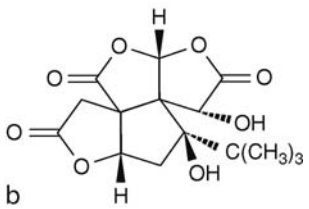
G. biloba extract is well known for its antioxidant activity to scavenge free radicals, especially oxygen-centered free radicals, such as $OH^{\bullet}$, $O_2^{\bullet -}$, $RO^{\bullet}$, and $ROO^{\bullet}$, and to neutralize ferryl ion-induced ►lipid peroxidation. The antioxidant activity can contribute from its ginkgo-flavone glycosides and bilobalides. *G. biloba* extract and bilobalide exert their protective effects by upregulating mitochondrial NADH dehydrogenase gene expression, decreasing state 4 respiration and increasing respiratory control ratio, which leads to the elimination of oxidative damage. Additionally, terpene lactones in *G. biloba* extract are potent antagonists of platelet activating factor (PAF) by blocking the binding of PAF to its receptors. The overall actions of terpene lactones inhibit platelet aggregation and improve blood circulation of the brain and other tissues. Of these ginkgolides, ginkgolide B has shown to be the most potent PAF inhibitor (10 times more potent) with the longest duration of action. Due to the antioxidant and antiaggressive actions, *G. biloba* extract exerts its therapeutic activity primarily in cerebrovascular dysfunctions and peripheral vascular disorders, such as age-related dementia, Alzheimer's disease, failing memory, poor cerebral and ocular blood flow, congestive symptoms of premenstrual syndrome, as well as the prevention of altitude sickness. Additionally, *G. biloba* extract may provide a useful preventive or therapeutic treatment for peptic ulcer, intermittent claudication, drug-induced retinopathies, injured retinal cells from spontaneous hypertension, gentamycin-induced cochlear damage, and reperfusion-induced ventricular fibrillation.

Besides the antioxidant and antiaggressive actions of *G. biloba* extract, it has been reported to have antiviral and anti-inflammatory properties. Ginkgetin, a biflavonoid from *G. biloba* extract, has antimicrobial effect on inhibiting the growth of the fungi, the influenza virus sialidase, and herpes simplex virus type 1, and anti-inflammatory effect on suppressing the expression of proinflammatory molecules, such as ►phospholipase A2, ►cyclooxygenase-2, 5-lipoxygenase, inducible nitric oxide synthase, and interleukin-1β, to protect against inflammatory diseases, such as arthritis and chronic skin ►inflammation.



a

	R_1	R_2	R_3
Ginkgolide A	H	H	OH
Ginkgolide B	OH	H	OH
Ginkgolide C	OH	OH	OH
Ginkgolide J	H	OH	OH
Ginkgolide M	OH	OH	H



b

Ginkgo Biloba. Figure 1 Structure of (a) ginkolides and (b) bilobalide.

G. biloba extract may be considered a possible chemopreventive or anticancer agent and useful in preventing or treating cancer invasiveness and ►metastasis. In vitro or animal studies have shown that *G. biloba* extract inhibits proliferation of cancer cells, such as ►breast cancer, ►glioblastoma, ►colon cancer, ►ovarian cancer, and ►hepatocellular carcinoma, and induces ►apoptosis of cancer cells, such as oral cavity cancer, ►ovarian cancer, and ►hepatocellular carcinoma. A clinical study has demonstrated that oral administration of *G. biloba* extract in the patients with upper digestive tract malignant tumors decreases tumor size, improves the patients' clinical symptoms, and prolongs the survival period. Additionally, *G. biloba* extract alleviates the inhibited hematopoietic function and weight loss due to ►chemoradiotherapy. The anticancer mechanisms of *G. biloba* extract may involve (i) the inhibition of cell proliferation through decreasing the overexpression of peripheral-type benzodiazepine receptor and proliferating cell nuclear antigen, (ii) the induction of ►apoptosis through stimulating ►caspase-3 activity and ►p53 protein expression, (iii) the cytotoxic effect through modulating the ►apoptosis and/or ►necrosis, and (iv) the stimulation of carcinogen metabolism through increasing the detoxifying enzyme activity of ►cytochrome P450, ►glutathione S-transferase, and quinine reductase in the liver. Certain components in *G. biloba* extract have been identified to participate in the anticancer action. Quercetin, an important flavonoid of *G. biloba* extract, suppresses cell proliferation of human ►hepatocellular carcinoma cell lines BEL-7402, HuH-7, and HLE with a peak inhibition at 50 μM. Ginkgetin inhibits the proliferation of human ►ovarian cancer OVCAR-3 cells via the induction of ►apoptosis in a dose-dependent manner. Ginkgolide B inhibits cell proliferation of highly aggressive human ►breast cancer MDA-231 cells but not affects nonaggressive ►breast cancer MCF-7 cells. Ginkgolic acid not only reduces the growth of tumor cells in vitro without the influence the growth of normal cells, but also exerts the cytotoxic effect to induce death by ►apoptosis and necrosis possibly via the mediation of protein phosphatase 2C.

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Ginkgo Tree

►Ginkgo Biloba

Ginseng

Definition

A natural product with anti-cancer, cardiovascular disease and neurologic disease properties. Active ingredients include ginsenosides. American and panax ginseng have potent anti-inflammatory properties.

►Inflammation

GIST

►Gastrointestinal Stromal Tumor

GISTs

Definition

Gastrointestinal stromal tumors are the most common mesenchymal tumors (sarcomas) specific to the GI tract, generally defined as ►KIT (CD117)-positive tumor with a characteristic set of histologic features including spindle cell, epithelioid or rarely pleomorphic features. Ten to 15% of patient mutations occur in exon 9 KIT.

►Mesothelin
►Kit/Stem Cell Factor Receptor in Oncogenesis

GJIC

Definition

Gap junctional intercellular communication.

►HSV-TK/Ganciclovir Mediated Toxicity

GlcCer

Definition

Glucosylceramide, the β-linked glucoside of ceramide.

►Glucocerebroside
►Sphingolipid Metabolism

Gleason Grading

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Definition

The Gleason grading system of prostate adenocarcinoma is the principal grading system recommended by a the WHO consensus conference. The system is based on the histological pattern of arrangement of glandular architecture of carcinoma cells in hematoxylin&eosin; (H&E)-stained sections (►H&E stain). Five basic histological patterns or grades are recognized to generate histological scores ranging from 2 to 10. ►Gleason score is directly correlated with virtually pathological variables, including tumor volume and margin status in radical prostatectomy specimens, as well as serum ►prostate-specific antigen (PSA) levels. Gleason score is a powerful predictor of clinical and pathological stage, progression (►Tumor progression) to metastatic disease, and survival. Models are used to predict responses to specific therapies, such as surgery, radiotherapy, and androgen ablation therapy.

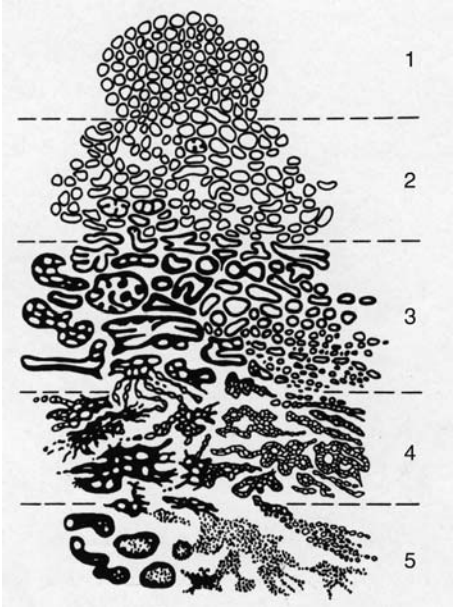
Characteristics

The Gleason grading system is the one predominantly used in both research and daily clinical practice, and is routinely used for grading of all prostate tissue samples, including needle-core biopsies and samples from radical prostatectomy. The Gleason grading system was devised by Dr. Donald F. Gleason, a pathologist, and a member of the Veterans Administration Cooperative Urological Research Group.

The Gleason grading system is based on the histological pattern of arrangement of glandular architecture of carcinoma cells in H&E sections. The system defines five histological patterns or grades with decreasing differentiation illustrated in a standard drawing observed at relatively low magnification (10–40×) (Fig. 1). The primary and secondary patterns, i.e., the most prevalent and second most prevalent patterns on simple visual inspection, are added to obtain the Gleason score or sum.

Gleason pattern 1 (Grade 1) is composed of very well circumscribed nodular growth of separate, closely packed glands without any infiltration into adjacent normal prostate tissue. The glands are uniform, rounded to oval, and of intermediate size (Fig. 2). This pattern, which is usually seen in the transition zone in incidental cancers, is exceedingly rare. Atypical adenomatous hyperplasia should be excluded before diagnosing a pure pattern of this type.

Gleason pattern 2 (Grade 2) features less well-defined masses that are not as circumscribed as pattern 1, and is composed of round or oval glands (Fig. 2). Compared to those of pattern 1, pattern 2 glands display increased variability in size and shape,



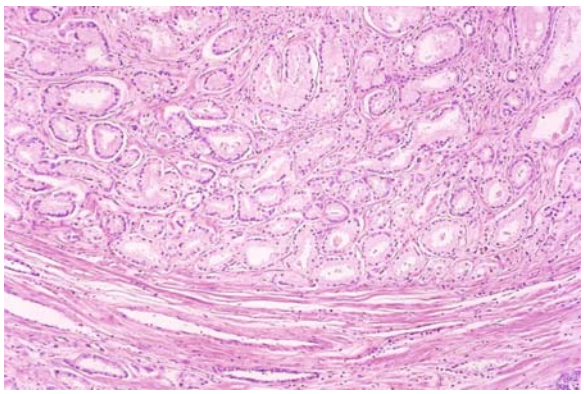
Gleason Grading. Figure 1 Five histological patterns or grades with decreasing differentiation illustrated in a drawing by Dr. Gleason.

with a degree of separation by stroma. There is minimal and limited invasion by neoplastic glands into the stroma. Pattern 2 is usually found admixed with pattern 3 to yield a Gleason score of 5. Gleason pattern 2, like pattern 1, is often found in transition-zone carcinomas, but may occasionally be found in the peripheral zone.

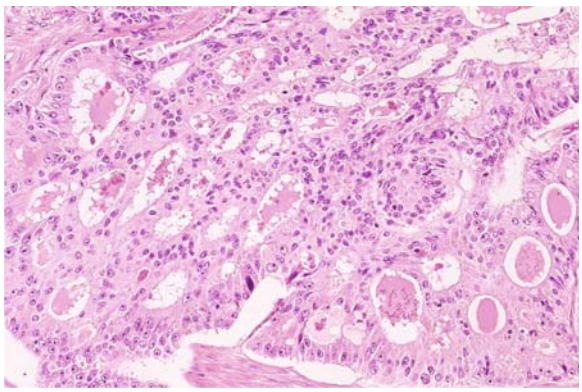
Gleason pattern 3 (Grade 3) is the most common pattern of growth of prostate adenocarcinoma, and is found in pure form in the large majority of cases. The glands are more infiltrative, and the distance between them is more variable than in patterns 1 and 2. Pattern 3 is characterized by small- to medium-sized single glands of irregular shape extending into the stroma (Fig. 3). Small glands forming glandular luminal spaces are typical of pattern 3. Each gland has an open lumen and is circumscribed by stroma. Shape, especially of medium-sized glands, is quite variable with angular and elongated forms. Cribriform or papillary growth, which is rarely observed in pattern 3, is recognized by the smooth, pushing edges of the invasive periphery.

Gleason pattern 4 (Grade 4) involves poorly differentiated growth of high-grade tumor with fused or cribriform glands appearing as raggedly infiltrative masses. The hypernephroid or hypernephromatoid pattern is a rare variant of fused glands with clear cytoplasm. Cribriform pattern 4 glands are large or irregular, and are recognized by their ragged edges compared to the smooth borders noted in pattern 3 (Fig. 4). Most cribriform invasive adenocarcinomas should be considered pattern 4 rather than pattern 3. Pattern 4 is often combined with pattern 3 to yield a score of 7, which is currently one of the most commonly assigned Gleason scores.

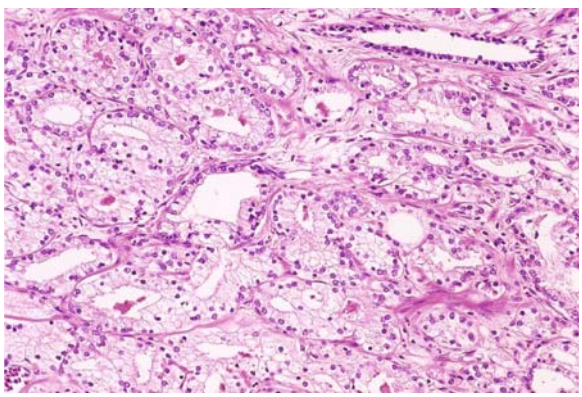
Gleason pattern 5 (Grade 5) involves the most poorly differentiated growth of tumor, which is almost completely lacking in glandular lumina, with only occasional lumina observed. The tumor cells form ragged sheets or exhibit anaplastic single cell proliferation invading the stroma (Fig. 5). Comedo appearance in pattern 5 is associated with smooth, rounded masses, cords, or cylinders of carcinoma with the presence of



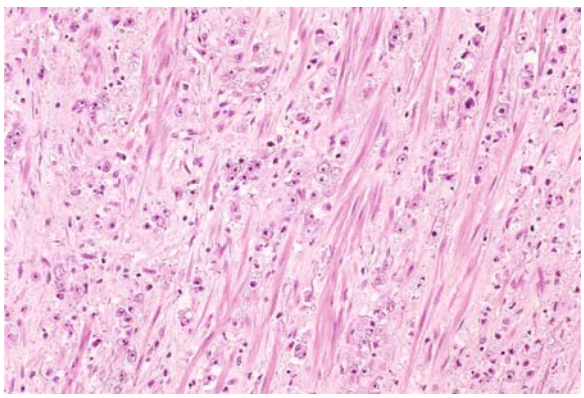
Gleason Grading. Figure 2 Gleason grade 2 + 1 = score of 3 adenocarcinoma.



Gleason Grading. Figure 4 Gleason grade 4 adenocarcinoma showing cribriform pattern.



Gleason Grading. Figure 3 Gleason grade 3 adenocarcinoma.



Gleason Grading. Figure 5 Gleason grade 5 adenocarcinoma comprised of ragged sheets of anaplastic tumor cells.

necrosis. Gleason score 9 (Gleason grade 4 + 5) and Gleason score 10 (pure pattern 5) are less common, and noted in association with ►prostate cancer of high clinical stage.

Clinical Aspects

Increasing Gleason grade is directly associated with pathological and clinical end points, including lymphatic and vascular invasion, tumor size, local recurrence, and lymph node or distant metastasis in patients receiving no treatment, radical prostatectomy, radiation therapy, or other types of treatment. Although Gleason grading by needle biopsy can be used to predict pathological stage, it is not completely accurate in each patient. Several studies have addressed the discrepancies between Gleason score in needle biopsies and corresponding radical prostatectomy specimens. Perhaps the most important factor in such discrepancies is sampling error, which is related to the small amount of tissue removed by thin-core needle biopsies. In order to predict pathological stage clinically, needle-biopsy Gleason grade needs to be combined with other pretreatment factors, such as serum PSA, local clinical T stage, and amount of tumor included in the needle biopsy.

The Gleason score assigned to the tumor at radical prostatectomy is the most powerful predictor of progression after resection. Gleason scores 2–4 are rarely noted in stages T1c or T2. All patients with Gleason scores of only 2–4 are cured. Gleason scores 5–6 are associated with relatively low rates of progression after definitive therapy. Most patients with tumors with these Gleason scores are cured, whether they exhibit extraprostatic extension or positive margins or not. Tumors with Gleason scores 7–10 are associated with a worse ►prognosis. Tumors with a Gleason score of 7 have a significantly worse prognosis than those with a Gleason score of 6. Several studies have addressed whether Gleason scores 3 + 4 or 4 + 3 have different prognoses, with somewhat conflicting results. Patients with Gleason score 8–10 tumors present at an advanced stage, and are not amenable to local treatment. The National Comprehensive Cancer Network (NCCN) has presented guidelines for the management of recurrence of prostate cancer, and recommends that decisions regarding treatment be based on risk stratification. Patients are characterized as at low, intermediate, high, or very high-risk of recurrence according to their clinical stage, Gleason score, and PSA value. The low-risk category includes men with T1 to T2 disease, Gleason score 2–6, and a PSA value φ 10 ng/ml. The intermediate-risk category includes those with any T2b to T2c disease, or any Gleason 7 score, or PSA value 10–20 ng/ml. Treatment decisions in both of these categories are stratified by life expectancy. The high-risk category includes men with T3a to T3b disease, Gleason score 8–10, or PSA value >20 ng/ml. The treatment strategy for these patients is based on expected patient survival. Finally, the very-high-risk category includes all patients with T3c or T4 disease or nonlocalized disease. Radical prostatectomy is not considered for these patients, and treatment should consist of hormone therapy or observation.

Gleason score is the most powerful predictor of clinical and pathological stage, progression to metastatic disease, and survival, and should be routinely reported for adenocarcinoma of the prostate. In the future, markers discovered by gene expression profiling will be prognostically useful if they yield added value beyond established prognostic factors including Gleason grade.

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Gleevec

Definition

►Glivec
►Imatinib
►STI-571

GLFG Bodies

Definition

Glycine–leucine–phenylalanine–glycine (GLFG) repeats. These FG rich areas are found on ►Nucleoporin 98 (NUP98) and NUP98 chimeric proteins arising from translocations in leukemia. They act as docking sites for importins, exportins and transportins involved in nuclear pore function. GLFG repeats have transcriptional-activation potency that is mediated in part by its

interaction with the transcriptional co-activator CBP/p300. The GLFG domain itself is responsible for targeting NUP98 from the nuclear pore to novel GLFG bodies in the nucleus. The GLFG motif may also regulate the localization of NUP98 chimeric proteins.

►NUP98-HOXA9 Fusion
►Nucleoporin Family
►p300/CBP Co-Activators

Gli

Definition

Synonym Glioma1; The three vertebrate *Gli* genes are orthologues of the one *Drosophila* gene *Cubitus interruptus (ci)*. The Gli molecules are zinc-finger transcription factors that mediate ►hedgehog signaling. Gli1 and 2 are primarily involved in transcriptional activation, while Gli3 is cleaved to from a truncated transcriptional repressor.

►GLI Proteins

GLI-Kruppel Family Member

►GLI Proteins

GLI Proteins

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Synonyms

GLI-Kruppel family member; Glioma-associated oncogene homolog 1 (zinc finger protein) (GLI1); Tax helper protein (GLI2)

Definition

GLI proteins belong to the family of zinc finger transcription factors and act at the distal end of the Hedgehog (HH) signaling pathway to control HH target gene transcription.

Characteristics

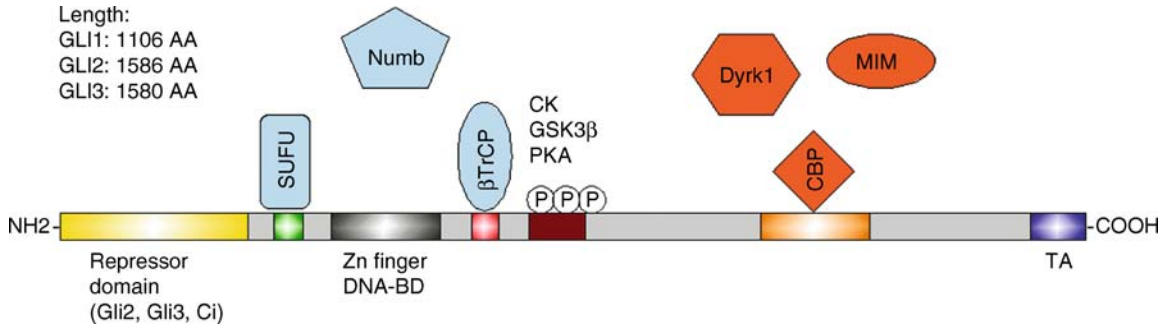
In vertebrates, three GLI homologs, GLI1, GLI2 and GLI3 have been identified.

GLI proteins belong to the family of zinc finger transcription factors and play key roles in development and disease as they mediate HH signaling in numerous embryonic processes such as pattern formation and cell fate decisions as well as in cancer and developmental anomalies.

All GLI proteins including the fly homolog Cubitus interruptus (Ci) have in common a highly conserved DNA binding domain composed of five zinc finger domains of the C2-H2 class, which mediates specific binding to the consensus GLI binding sequence GACCACCCA in promoters of HH target genes (Fig. 1). GLI1 and GLI2 encode the main activators of HH-target genes, while GLI3 acts mainly as transcriptional repressor in the absence of HH-signaling. Transcriptional activation of GLI target genes involves the C-terminal trans-activation domain. Repression of target genes by GLI3 requires proteolytic processing of full-length GLI3 into a fragment encompassing the N-terminal repressor and the DNA binding domain, a process that depends on phosphorylation by multiple kinases such as ►PKA, CK and ►GSK3β.

Regulation of GLI Activity in Response to HH Signaling

Activation of GLI transcription factors is initiated by binding of HH protein to its trans-membrane receptor ►Patched (PTC). This allows another trans-membrane protein, Smoothened (SMO), to relay the signal towards the nucleus. The question of how GLI proteins are activated by SMO has not yet been answered completely. Unlike GLI1, which is a direct transcriptional target gene of the pathway, GLI2 and GLI3 are considered latent transcription factors whose activation requires pathway activity. The identification of several intraflagellar transport (IFT) proteins as regulators of GLI protein activity has shed new light on the mechanisms of signaling and GLI activation. IFT proteins play a critical role in the growth and maintenance of cilia. In fact, several components of the HH signaling pathway including GLI are specifically localized in the primary cilium, a microtubule-based organelle that protrudes from the surface of most mammalian cells. In the absence of HH ligand, the latent activator forms of GLI2 and GLI3 are kept inactive by SUFU (see below) and another protein termed Iguana. Further, absence of ligand promotes the generation of GLI3 repressor, which also depends on proper function of the cilium. Binding of HH protein to its receptor PTC leads to re-localization of SMO to cilia, where it activates



GLI Proteins. Figure 1 Schematic illustration of the basic domain architecture of GLI proteins and interacting proteins. Based on sequence comparison studies, GLI1 neither contains a CBP binding domain, nor a N-terminal repressor domain; as the GLI domains that interact with Numb, MIM, Dyrk1 have not yet been mapped, these GLI binding proteins are not shown as directly associated with GLI. Negative regulators of GLI activity are shown in light blue, positive regulators in orange; phosphorylation by Protein Kinase A (PKA), Casein Kinase (CK) and Glycogen Synthase Kinase-3β (GSK3β) is critical for proteolytic processing of GLI3, Ci and probably of GLI2. DNA-BD: DNA-binding domain; TA: transactivation domain; AA: amino acids.

GLI proteins in a cilia-dependent manner, though the molecular details are not fully understood. Proteolytic processing of GLI3 into its repressor form is also inhibited in the presence of HH ligand.

Regulation of GLI activity at the post-translational level also involves interactions with co-factors, integration with other signaling systems and signals affecting protein stability.

Suppressor of Fused (SUFU) encodes a key negative regulator of GLI proteins. SUFU has been shown to bind GLI proteins and loss of SUFU function results in constitutive HH signaling and tumor development. Further, interactions of GLIs with co-factors such as Creb binding protein (CBP), Dyrk1 or Missing in Metastasis (MIM) are crucial for full transcriptional activation of HH targets.

The complexity of regulation of GLI proteins is further underlined by studies showing that signal cross-talk between HH/GLI and major intracellular signaling cascades such as RAS/RAF/MEK/ERK and PI3K/AKT modulates the transcriptional activity of GLI proteins.

The activity of GLI proteins is also tightly controlled by several mechanisms regulating GLI protein stability. Binding of beta-TrCP to GLI marks GLI for degradation by the ubiquitin-proteasome pathway. Similarly, binding of Numb, a developmental protein involved in binary cell fate decisions, to GLI1 protein triggers itch-mediated ubiquitination and degradation of GLI1 via the proteasome machinery, thereby promoting growth arrest and differentiation of neuronal cells.

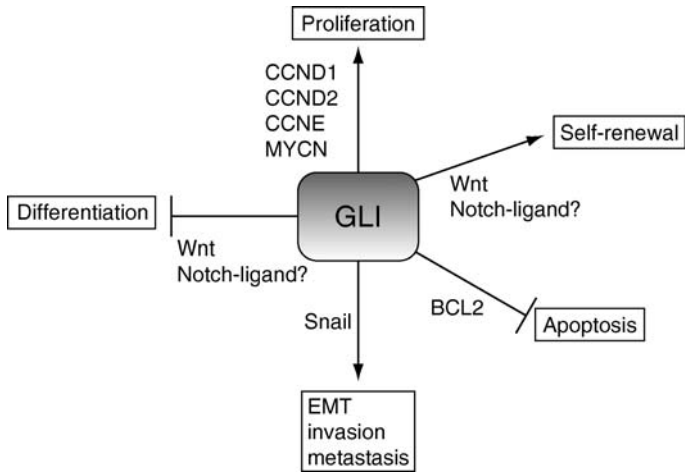
GLI Proteins in Cancer

Aberrant activation of the Hedgehog/GLI signaling pathway leads to the development of a number of human malignancies ranging from semi-malignant tumors of the skin (Basal cell carcinoma) to fatal cancers of the brain, lung, upper gastrointestinal tract, pancreas and prostate.

HH-associated cancers display elevated expression levels of one or more GLI transcription factors, particularly GLI1 and GLI2, suggesting a key role for both GLI proteins in HH-driven cancers. The oncogenic activity of GLI1, and probably also that of GLI2, has multiple facets (Fig. 2). GLI1 can transform rodent cells and promote cell cycle progression of several epithelial cell types. In agreement with these oncogenic properties, inhibition of GLI1 or GLI2 function has been shown to slow down tumor cell proliferation and to increase apoptosis. Enhanced expression of GLI1 also augments the metastatic potential of prostate cancer cells.

The identification of direct GLI target genes with a putative role in cancer development and growth has shed light on the molecular mechanisms underlying Hedgehog-driven tumorigenesis. GLI1 and GLI2 have been shown to increase the expression of key regulators of G1/S and G2/M transition of the cell cycle. The Cyclin D genes CCND1 and CCND2 are up-regulated in HH-associated cancers and both are likely to be directly controlled by GLI proteins. In Drosophila, Cyclin E is directly regulated by the fly GLI homolog Ci. HH-induced proliferation also involves direct activation of the N-MYC (►MYCN) proto-oncogene. In neuronal cells, overexpression of MYCN mimics the proliferative effect of Hedgehog, while inhibition of MYCN abrogates Hedgehog-induced proliferation.

GLI transcription factors also promote cell survival by directly activating transcription of the key anti-apoptotic factor BCL2. Of note, Wnt and Notch ligands have also been described as direct GLI targets, which may play a role in stem cell renewal and inhibition of cell differentiation.



GLI Proteins. Figure 2 GLI proteins regulate multiple oncogenic mechanisms. Direct GLI target genes that have been implicated as key mediators of the respective oncogenic process are listed. EMT: Epithelial-mesenchymal transition.

Furthermore, GLI proteins have been implicated in the control of the invasive and metastatic behavior of prostate cancer cells, as overexpression of GLI can convert cells with low metastatic potential into highly malignant cells. This may well be mediated through direct transcriptional activation of Snail, a transcription factor with a critical role in ►epithelial-to-mesenchymal transition (EMT) and in the down-regulation of the ►cell adhesion molecule ►E-cadherin, two hallmarks of increased invasiveness and metastasis.

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Glioblastoma

Definition

A member of the group of ►brain tumors, the most malignant astrocytic tumor, composed of poorly differentiated neoplastic astrocytes with areas of vascular proliferation and/or ►necrosis. Typically affects adults and is preferentially located in the cerebral hemispheres. Glioblastomas may develop from low-grade diffuse or anaplastic ►asrocytoma, but most frequently they present after a short clinical history with no evidence of a less malignant precursor lesion.

►Glioblastoma Multiforme

Glioblastoma Multiforme

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Synonyms

Glioblastoma; GBM; Grade IV astrocytoma; WHO grade IV astrocytoma

Definition

Glioblastoma multiforme (GBM) is a highly aggressive central nervous system (CNS) tumor derived from glial cells.

Characteristics

Introduction

GBM is the most aggressive and life-threatening CNS tumor. Although predominately found in the brain, it can also develop in the other tissues of the CNS, such

spinal cord and optic nerves. Primary GBM tumors usually develop de novo. However, sometimes a lower grade, less malignant ►brain tumor can evolve into a higher grade secondary GBM. GBM tumors are the most malignant primary brain tumor, accounting for about 60% of brain tumor cases in adults over 50 years old. Rapid proliferation, aggressive ►invasion, infiltration, and destruction of neighboring normal tissues are the dominant characteristics of GBM tumors. ►Metastasis of the tumor to other tissues beyond the CNS is rarely, if ever, observed clinically. GBMs occur predominately in the subcortical white matter of the cerebral hemispheres. Tumor infiltration often extends into the adjacent cortex, basal ganglia, and contralateral hemisphere. The occurrence of GBMs in the brainstem is rare in adults, but not in children.

The Genomic Abnormalities in GBM Tumors

Similar to other malignant tumors, ►genomic instability, such as chromosomal abnormalities that result in the ►loss of heterozygosity (►LOH), and gene mutations are frequently detected in GBM tumor cells. The most frequently observed mutations are found in the genes of ►p53 (►TP53), ►EGFR, ►mdm2 and ►PTEN. EGFR is a regulatory protein involved in the control of cell proliferation. Amplification/overexpression of EGFR has been identified as one of the most common characteristics of GBM tumors. The truncated, ligand-independent and constitutively activated isoforms of EGFR are frequently found in GBM tumors. It has been demonstrated that the overexpression of EGFR correlates positively with increased tumor growth rate and decreased survival. Approximately half of GBM patients have elevated EGFR expression.

Mutations in the ►tumor suppressor gene, p53 (TP53), were one of the first genetic abnormalities identified in gliomas. Frequently, p53 mutations have been observed in low-grade gliomas and secondary GBM tumors, suggesting that inactivation of p53 (TP53) is an early event in GBM pathogenesis. ►Amplification of the gene mdm2 is also frequently detected in GBMs. This protein forms a complex with p53 (TP53), resulting in an inhibition of p53 (TP53) function. Thus, amplification of mdm2 in tumor cells constitutes an alternative mechanism for facilitating uncontrolled cell growth. Clinically overexpression of mdm2 is observed in 10–15% of GBM patients, and is usually associated with poor prognosis.

The most frequent gene alterations occur on chromosome 10 for both primary and secondary GBM tumors. These mutations appear to be specific for GBMs, are rarely found in the lower grade brain tumors, and are generally accepted as a ►biomarker of poor prognosis. The tumor suppressor gene, PTEN, located at 10q23.3 encodes a tyrosine phosphatase and plays a significant regulatory role in the AKT signaling pathway. Loss of the PTEN function causes increased phosphorylation of the inositol phospholipids that activate the AKT serine/threonine-kinase, leading to enhanced cell cycle entry and aberrant proliferation. The PTEN mutation is found in about 20–40% of GBM patients.

Treatment of GBM Tumors

Nearly all GBM tumors are treated with a combination of surgery, ►radiotherapy, and ►chemotherapy. All of these treatments are difficult because the location of GBM tumors in the brain and spinal cord present unique challenges that require a balance between the safety and effectiveness of the treatments. The development of sophisticated noninvasive techniques such as ►computed tomography (CT), ►positron emission tomography (PET), and ►magnetic resonance imaging (MRI) has allowed surgeons to locate the tumor more precisely so that stereotactic surgery can remove more of the tumor without damaging nearby critical normal brain structures. This has not only led to increased survival, but also to improved posttreatment quality of life.

Even when it appears that the entire tumor has been surgically removed, GBM tumors usually regrow from the few GBM tumor cells that remain in the surrounding brain tissue. Postoperative ►radiotherapy is usually recommended in an attempt to kill the remaining tumor cells that could not be surgically removed. Both conventional radiotherapy (fractionated radiotherapy) and ►stereotactic radiosurgery (single fraction radiotherapy) are used to kill the remaining tumor cells. Conventional radiotherapy uses external radiation beams to treat either large volumes of the brain or the whole brain. This approach is usually recommended for large infiltrating tumors or those that have multiple foci throughout the brain. Stereotactic radiosurgery and ►Gamma Knife radiosurgery, have been developed to allow one to precisely target radiation directly to small, well localized tumors. These radiosurgery techniques can deliver large doses of radiation to an area no bigger than 4–5 mm in diameter in a single treatment, thereby increasing tumor cell kill while drastically reducing the radiation damage to the surrounding normal brain tissue. These radiosurgery techniques have not only led to an increase in the median survival of GBM patients, but also improved their posttreatment quality of life.

Chemotherapy has also been used as a primary treatment for GBM tumors, and as an adjuvant to surgery and radiotherapy for recurrent GBM tumors. However, the existence of the blood–brain barrier (BBB) complicates the use of chemotherapeutic agents for treating CNS tumors. Water soluble (hydrophilic) molecules and molecules with molecular weights greater than 600 do not cross the BBB very efficiently, if at all. While this is unimportant for treating cells in the center of a GBM tumor where there is no BBB, it is

critical for treating the cells at the edge of the tumor where the BBB is still intact.

For many years, the lipophilic nitrosoureas, BCNU and CCNU, were the most effective systemic chemotherapeutic agents for treating GBM tumors. When used as single agents, they increased the lifespan of GBM patients by 2–3 months. In combination with other chemotherapeutic agents and/or radiation, they increased the lifespan of GBM patients by 3–6 months. Nevertheless, until recently, the best combinations of nitrosourea-based chemotherapy, radiotherapy, and surgery resulted in an increase in the median lifespan to only about 12 months, probably due to very little of the systemically administered drugs getting to the viable tumor cells in the CNS.

To overcome the problem of delivering substantial concentrations of these chemotherapeutic agents to the tumor, a biodegradable polymer wafer containing BCNU was approved by FDA in 2002. These wafers can be inserted during surgery so that they line the entire tumor cavity. As these wafers degrade, they release BCNU in a controlled fashion to maintain a higher concentration of the drug in the tumor over a longer period of time than can be achieved if the drug is administered systemically. A phase III randomized trial that included 240 GBM patients compared the results from implanting the BCNU-containing polymer wafers with the results from implanting a placebo wafer. All patients received the standard treatment consisting of tumor resection followed by fractionated external radiation. The median lifespan of the group treated with the BCNU-containing polymer wafers was 13.9 months, while the median lifespan of the group treated with placebo wafer was 11.6 months. Although the wafer results were better than the results obtained with systemic administration of BCNU, the increase in the medium lifespan was not as dramatic as expected, suggesting that new chemotherapeutic agents need to be developed for the treatment of GBM tumors.

In 2005, temozolomide (Temodal, Temodar, TMZ), an alkylating agent that can be given orally, was approved by the FDA for the treatment of GBM tumors. After oral administration, TMZ is rapidly absorbed with almost 100% bioavailability. It readily crosses the blood–brain barrier and achieves effective concentrations in the cerebrospinal fluid. A phase III clinical trial performed by the European Organization for the Research and Treatment of Cancer (EORTC) used temozolomide combined with radiation to treat patients with new diagnosed GBM. GBM patients in the arm treated with temozolomide and radiation had a significant increase in their median lifespan (14.6 months) over those treated in the control arm (12.1 months). More importantly, the patients treated with temozolomide and radiation had a large increase in their two-year survival rate (26.5%) compared to the control group (10.4%). Consequently, the current standard of care for GBM patients is now surgery followed by treatment with temozolomide and radiation.

Current Status of GBM Research

Despite the best efforts of neurosurgeons, radiation oncologists, and medical oncologists, the median lifespan of patients with GBM tumors has not been changed very much. Although the 2 year survival of GBM patients has more than doubled using surgery, temozolomide chemotherapy, and radiotherapy, the 5 year survival is still less than 1%. Thus, new therapeutic approaches that can selectively enhance tumor cell killing while sparing normal brain cells are still urgently needed. Currently, researchers are studying new therapeutic modalities including ►immunotherapy, stem cell therapy, ►gene therapy and anti-angiogenesis therapy. Many researchers have been successful in treating GBM tumors in animal models, but there has been only limited success when these new treatments have been translated to the clinic. The key factors that have hindered clinical therapeutic progress are the lack of appropriate animal models that predict the response of human GBM tumors to treatment, the inability to deliver effective doses of chemotherapeutic agents to the tumor, and the genetic heterogeneity of human GBM tumors that make them among the most difficult tumors to treat.

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Glioma

Definition

Is a primary brain tumor derived from transformed glial cells. Malignant glioma (GBM) is the most common primary brain tumor and is uniformly fatal despite aggressive surgical and adjuvant therapies.

►Brain Tumors

Glioma Retinae

►Retinoblastoma

Glivec

Definition

The first of a new class of antiproliferative agents that interfere with growth signaling pathways in tumor cells. Is targeted to kill cells that harbor the specific leukemia-causing ►BCR-ABL1 fusion protein found predominantly in ►chronic myeloid leukaemia (CML), while having little effect on the growth of normal cells that do not carry the mutant protein.

►Gleevec
►Imatinib
►STI-571

γ Globulins

Definition

Plasma proteins can be separated on the basis of electrophoretic mobility into albumin and the α, β, and γ globulins. Most antibodies migrate in electrophoresis as γ globulins (or gamma globulins), and patients who lack antibodies are said to have ►agammaglobulinemia.

►Sjögren Syndrome

Glucagon

Definition

Has a major role in maintaining normal concentrations of glucose in blood, having the opposite effect of ►insulin. That is, glucagon has the effect of increasing blood glucose levels.

Glucagonoma

Definition

Is a functioning ►islet cell tumor that produces excessive amounts of the hyperglycemic hormone glucagon, causing elevated blood sugar levels. This tumor can be associated with characteristic skin lesions (necrolytic migratory erythema).

►Neuroendocrine Carcinoma

Glucocorticoids

Definition

Are a class of steroid hormones, characterized by an ability to bind with the cortisol receptor and trigger many biological effects. Glucocorticoids are distinguished from mineralocorticoids and sex steroids by the specific receptors, target cells, and effects.

►Anti-Inflammatory Drugs
►Estrogenic Hormones

Glucose

Definition

A 6-carbon monosaccharide used by cells as a source of energy and metabolic intermediate.

►Warburg Effect

Glucuronic Acid

Definition

Is a polar, very water soluble sugar containing a carboxlic acid group that is chemically attached to a drug in a phase II metabolism reaction.

►ADMET Screen

β-Glucuronidase

Definition

β-Glucuronidases are enzymes produced by common intestinal microflora and are capable to deglucuronidate glucuronidated compounds, such as SN-38G. β-glucuronidases may play a role in the pathogenesis of ►irinotecan-induced delayed-type diarrhea.

Glucuronidation

Definition

Is the formation of hydrophilic glucuronides catalyzed by uridine diphosphate-glucuronosyltransferases. Glucuronidation facilitates the excretion of both endogenous and exogenous (lipophilic) molecules.

Glut1

Definition

Glucose Transporter 1. Expressed in fetal tissues and adult blood-brain barrier endothelia and erythrocytes. One of the carrier proteins of glucose along the cellular membrane. It is the first glucose transporter that has been characterized. In the adult, Glut 1 is expressed at highest levels in red cells and in the endothelial cells of barrier tissues such as the blood-brain barrier.

►Polycythemia

Glut4

Definition

Insulin-regulated glucose transporter. Found in adipose and skeletal muscle tissues, it is responsible for insulin-regulated glucose disposal.

►Insulin Receptor

Glutamate Carboxypeptidase II

Definition

A type of carboxypeptidase that is a transmembrane protein oriented such that the C-terminus of the protein is in the extracellular space outside of the cell membrane (i.e. type II transmembrane protein) and that is capable of sequentially hydrolyzing peptide bonds between adjacent glutamate amino acids starting at the carboxy-terminus of a peptide.

►Prostate-Specific Membrane Antigen

γ-Glutamylcystein Synthetase

Definition

Enzyme that catalyzes the first step in which an amide linkage is formed between the amino group of cysteine and the γ-glutamyl group of glutamate.

►Pharmacogenomics in Multidrug Resistance

Glutathione

Definition

A tripeptide of glutamic acid, cysteine, and glycine, which exist in reduced (GSH) and oxidized (GSSG) forms and functioning in various redox reactions, in the destruction of peroxides and free radicals, as a cofactor for enzymes, and in the ►detoxification of harmful compounds. Glutathione is an antioxidant and protects cells against free radicals. It is also involved in the formation and maintenance of disulfide bonds in proteins and in transport of amino acids across cell membranes.

►Alkylating Agents
►Chemoprotectants
►Pharmacogenomics in Multidrug Resistance
►Xenobiotics

Glutathione Conjugate Transporter RLIP76

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Synonyms

Ral interacting protein 76kDa; RLIP76

Definition

RLIP76 is a multifunctional protein important for utilizing the energy currency adenosine triphosphate (►ATP) to transport various chemical materials outside the cell. Through this transport process this transporter protein converge many cell signaling processes through the modulation of substrate levels or the contribution of protein–protein interactions to stimulate cell signaling. Overexpression of RLIP76 has been correlated to contribute to the phenomenon known as ►multidrug resistance. This protein is also known to have a clinical impact in addition to multidrug resistance toward ►inflammation, heat and ►oxidative stress, radiation, and a culmination of these stressors allowing it to contribute to general stress resistance.

Characteristics

►Ral Interacting Protein 76 kDa (RLIP76), a known Ral effector, is a 655 amino acid long protein capable of the ATP-dependent transport of ►glutathione conjugates (GS-E, adducts of glutathione and electrophiles) and amphiphilic ►xenobiotics. In addition to this ATP-dependent transport this protein has been well established as a known Ral-binding protein and ►Rho-►GAP protein that participates in ►endocytosis. RLIP76 is the predominant GS-E transporter and current studies have established a link with this protein between glutathione metabolism and a myriad of signaling pathways (Fig. 1).

Expression of RLIP76 is everywhere in mammalian tissues including kidney, liver, brain, muscle, eye, erythrocytes, and leukocytes. Its presence has also been demonstrated in cancer cell lines of various tissue origins. In cells, RLIP76 is primarily associated with the membrane but its presence in cytosol has been demonstrated, which is consistent with its known functions.

The primary structure of RLIP76 using N-terminal sequencing revealed that RLIP76 possess a GAP activity for cdc42 and the Ral-binding domain. In addition, it also contains a GTP binding domain. RLIP76 was demonstrated to function as a Ral GEF (►guanidine exchange factor), Rho-►GAP (GTP-activating protein) and is responsible for signal propagation downstream to proteins such as POB1 (partner of RalBP1).

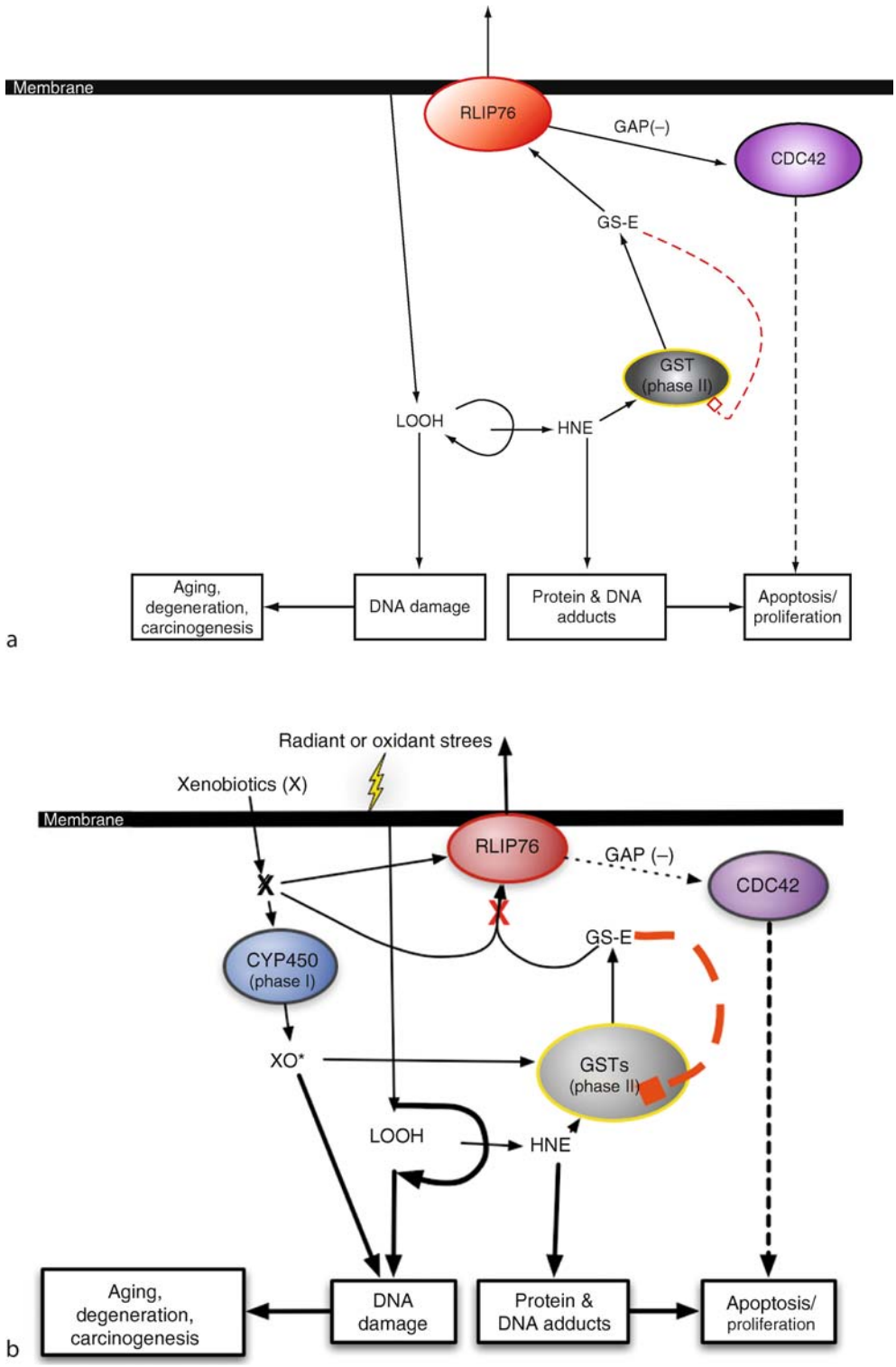
Discovery of RLIP76

A protein initially discovered as an ATP-dependent GS-E transporter was designated as DNP-SG ►ATPase. This protein stimulated ATPase activity in the presence of DNP-SG (dinitrophenyl *S*-glutathione a known conjugate of the cofactor glutathione and 1-chloro-2, 4-dinitrobenzene). ATP-dependent transport of DNP-SG and other GS-E was demonstrated in liposomes reconstituted with purified DNP-SG ATPase protein. Immunoscreening of a human bone marrow cDNA library using antibodies specific to DNP-SG ATPase resulted in cloning of a previously described protein RLIP76. It was established that that the structural, immunological and kinetic properties of tissue purified DNP-SG ATPase were identical and that in addition to DNP-SG, other GS-E including LTC4, GS-HNE, and various chemotherapeutic drugs such as doxorubicin also stimulated the ATPase activity of RLIP76. Proteoliposomes reconstituted with recombinant RLIP76 catalyzed the ATP-dependent transport of these chemicals with kinetic parameters similar to that of DNP-SG ATPase. Additional immunological studies with tissue-purified DNP-SG ATPase and recombinant RLIP76 established the identities of these two proteins and that this transporter was distinct from the known transporters including the classical family of transporters that are members of the ATP binding cassette transporter superfamily (►ABC transporter proteins).

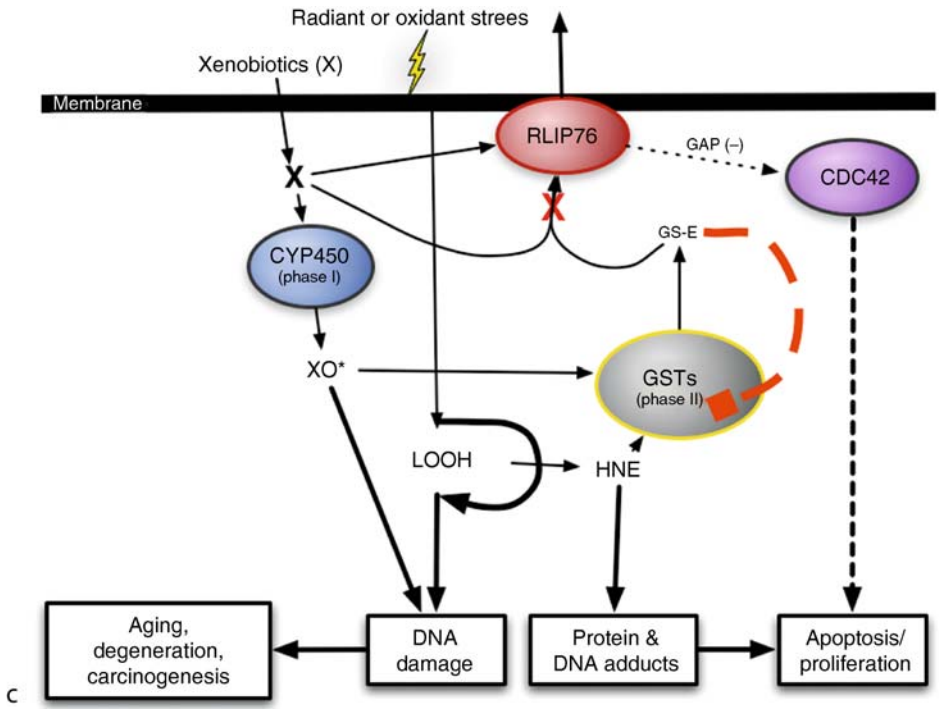
Clinical Significance

Drug Resistance

The role of RLIP76 in ►drug resistance of cancer cells is suggested by studies showing that its over-expression confers resistance and drug accumulation by increasing the rate of transport from cancer cells. In contrast, inhibition of RLIP76 by antibodies reverses the protein's overexpression effects. More importantly, depletion of the normal levels of RLIP76 in cancer cells by ►small interfering RNA (siRNA) sensitizes these cells to chemotherapy treatment and promotes ►apoptosis. The ability of RLIP76 to transport foreign material as well as their metabolites suggests that it is one of the major



Glutathione Conjugate Transporter RLIP76. Figure 1 (Continued)



Glutathione Conjugate Transporter RLIP76. Figure 1 (a) At normal levels or absence of increased stress, a baseline level of lipid hydroperoxide generation from membrane lipids gives rise to radicals. 4-hydroxynonenal (4-HNE) and other lipid aldehydes as well as peroxides are processed by GSTs to glutathione conjugate (GS-E) which are transported in an ATP-dependent manner by RLIP76 and other transporter proteins. The concentration of GS-E is low. Because of low concentration of GS-E, RLIP76 is balancing between transport and GAP (toward cdc42), thus inhibiting this proapoptotic pathway. Under conditions of low HNE, formation of DNA adducts is kept low and cell proliferation (marked bold) is favored. (b) Under the conditions of stress, lipid peroxidation is increased resulting in greater amount of toxicity and greater amount of HNE formation, which is catalyzed to GS-E by the various GSTs. Because of the rise in GS-E product, some product-inhibition of GSTs occurs until RLIP76 activity is increased. If xenobiotic toxins (chemotherapy drugs, carcinogens) are also present under these condition, a competition arises between lipid metabolites and activated P450 metabolites (XO*) for GST-mediated conjugation with GSH, the concentration of XO* rises more rapidly and genotoxicity is enhanced, particularly because XO* can propagate themselves via lipid peroxidation. Because of increased transport substrate, GS-E, the transport activity of RLIP76 is favored over its GAP activity toward cdc42, partially releasing that inhibition. The net effect, depending on the severity of stress would result in decreased proliferative signals and increased tendency to apoptosis (marked bold), both as a result of loss of cdc42 inhibition, and as a result of increased electrophilc lipid or xenobiotic oxidation products. (c) If RLIP76 is inhibited under conditions of stress, not only is there rapid accumulation of GS-E, but inhibition of GSTs by GS-E results in accumulation of XO*, which are also formed more rapidly because of increased substrate xenobiotic due to lack of the xenobiotic efflux activity represented by RLIP76. Under these conditions, genotoxicity and apoptosis signaling are greatly increased and proliferation is diminished.

components of the phase III ►detoxification systems. This contention is supported by studies showing that in mice as well as in cancer cells in culture, RLIP76 is responsible for more than 70% of the total ►doxorubicin transport as opposed to ►MRP1 and other transporters which are responsible for transporting only less than 30% of the drug.

Heat-shock and Oxidative Stress

Increases in oxidants can cause genomic insult leading to carcinogenesis. ►Oxidative stress usually leads to enhanced generation of endogenous electrophiles, which are metabolized to ►GS-E by ►GSTs. Besides being toxic, GS-E metabolites are known to be important contributors to cell signaling. Competitive inhibition of GS-E transport by xenobiotics implies that cell signaling pathways may be modulated by such compounds, particularly during oxidative stress-induced signaling. Alternatively, pharmacological effects of such xenobiotics could be modulated by oxidative stress. A major role of RLIP76 in defense against oxidative stress is suggested by studies showing that short exposure

of mild heat shock, oxidative stress, or ►UV radiation, to human cell lines leads to induction of RLIP76 as an early stress responder even prior to the proteins known for stress response (e.g., the ►heat-shock proteins). Under stress conditions, formation of electrophilic products (e.g., ►4-HNE) from the breakdown of lipids is observed and consequently GS-E levels rise. With induced expression of RLIP76, the stress-preconditioned cells acquire at least threefold higher capacity to transport endogenous GS-E as compared with untreated controls. This increased efflux of GS-E can be blocked by coating the cells with antibodies against RLIP76, indicating that GS-E are specifically transported by RLIP76. Cells, preconditioned to stress, display an enhanced ability to exclude GS-E due to induced RLIP76. Preconditioned cells are resistant to ►4-hydroxynonenal (4-HNE), superoxide anion (O_2-), ►hydrogen peroxide (H_2O_2), or ►UV radiation-mediated toxicity. These cells also acquire resistance to apoptosis by suppressing ►Jun N-terminal kinase and ►caspase-3. Inhibiting GS-E transport can abrogate the protective effect of this stress preconditioning. These studies suggest that the intracellular concentrations of endogenous GS-E, such as the conjugate between glutathione and 4-hydroxynonenal (GS-HNE), can affect stress-mediated signaling and that RLIP76 and GSTs are the major determinants of the intracellular levels of 4-HNE and GS-HNE.

Radiation Toxicity

Generation of ►reactive-oxygen species is one of the common effects of using high-energy radiation on biological tissues. RLIP76 is capable of providing protection against oxidative stress, primarily due to its ability to remove various electrophilic toxicants generated during ►lipid peroxidation caused by the reactive oxygen species. This implies that RLIP76 should provide protection against radiation toxicity because lipid peroxidation is an unavoidable consequence of exposure to radiation. It has been demonstrated that purified RLIP76 incorporated into liposomes is eagerly taken up by cultured cells resulting in increased cellular RLIP76 levels. When lung cancer cell lines in culture were treated with RLIP76 liposomes prior to the exposure to radiation, these cells with increase levels of RLIP76 were found to be more resistant to radiation toxicity as compared with the control cells. Efflux of GS-E formed from the reactive products of lipid peroxidation contributes significantly to radiation toxicity and that the increased efflux of GS-E from RLIP76-enriched cells rendered these cells resistance to radiation. Significance of GS-E efflux and role of RLIP76 against X-radiation has also been confirmed in the in vivo model.

The ability of RLIP76 to modulate stress from chemical, heat, radiation sources in addition to amphiphilic xenobiotic toxins such as ►vinca alkaloids used in chemotherapy indicates that RLIP76 plays a positive role in preventing oncogenesis but a negative role as the major contributor to resistance in cancerous cells.

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Glutathione Conjugates

Definition

GS-E; Are the end product for the reaction of electrophiles with the nulceophile glutathione. ►Glutathione-*S* transferase is responsible for the catalysis of this reaction.

►Glutathione Conjugate Transporter RLIP76

Glutathione-S Transferase

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Synonyms

Glutathione *S*-transferases; Glutathione transferases

Definition

GSTs; Refer to a family of ►Phase 2 detoxification enzymes that catalyse the conjugation of glutathione

(GSH) to a wide variety of endogenous and exogenous electrophilic compounds, thereby decreasing their reactivity with cellular macromolecules. This detoxification ability plays a pivotal role in cellular protection from ►oxidative stresses.

Characteristics

GSTs are divided into three distinct super-families: cytosolic, mitochondrial, and membrane-bound microsomal. Microsomal GSTs play a key role in the endogenous metabolism of leukotrienes and prostaglandins. The mammalian cytosolic GSTs are highly polymorphic and could be divided into six classes: alpha (A), mu (M), omega (O), theta (T), sigma (S) and pi (P). Classification is based on sharing greater than 60% identity within a class and focuses mainly on the more highly conserved N-terminal domain that contains a catalytically active tyrosine, cysteine or serine residue. The catalytic residue interacts with the ►thiol group of glutathione (G-site), and crystal structure has confirmed a substrate binding site (H-site) that facilitates catalysis and is in proximity to the G-site. GSTs have been found in virtually almost every living species, including mammals, plants and bacteria.

Catalytic function

GSTs catalyze a general reaction:

$$GSH + R - X \rightarrow GSR + HX$$

The function of the enzyme is to bring the substrate into close proximity with GSH by binding both GSH and the electrophilic substrate to the active site of the GSTs, and active the sulfhydryl group of ►glutathione, then allowing for nucleophilic attack of GSH on the electrophilic substrate (R-X). Lots of diverse chemicals and compounds serve as substrates for GSTs, and differences in expression of specific GSTs isoforms could be an important determinant of target organ and species sensitivity.

Signaling regulation

Though GSTs were early investigated because of their pivotal role in detoxification of ►reactive oxygen species (ROS) and maintenance of the cellular redox state, they were then discovered as "ligandins" owing to their abilities to interact covalently and noncovalently with various compounds that were not substrates for enzymatic activity, including steroids, thyroid hormones, prostaglandins and bile acid. As the ligand-binding characters of GSTs were confirmed, the regulatory effects of GSTs on stress- and anticancer drugs-activated cellular signaling pathways were revealed in various studies.

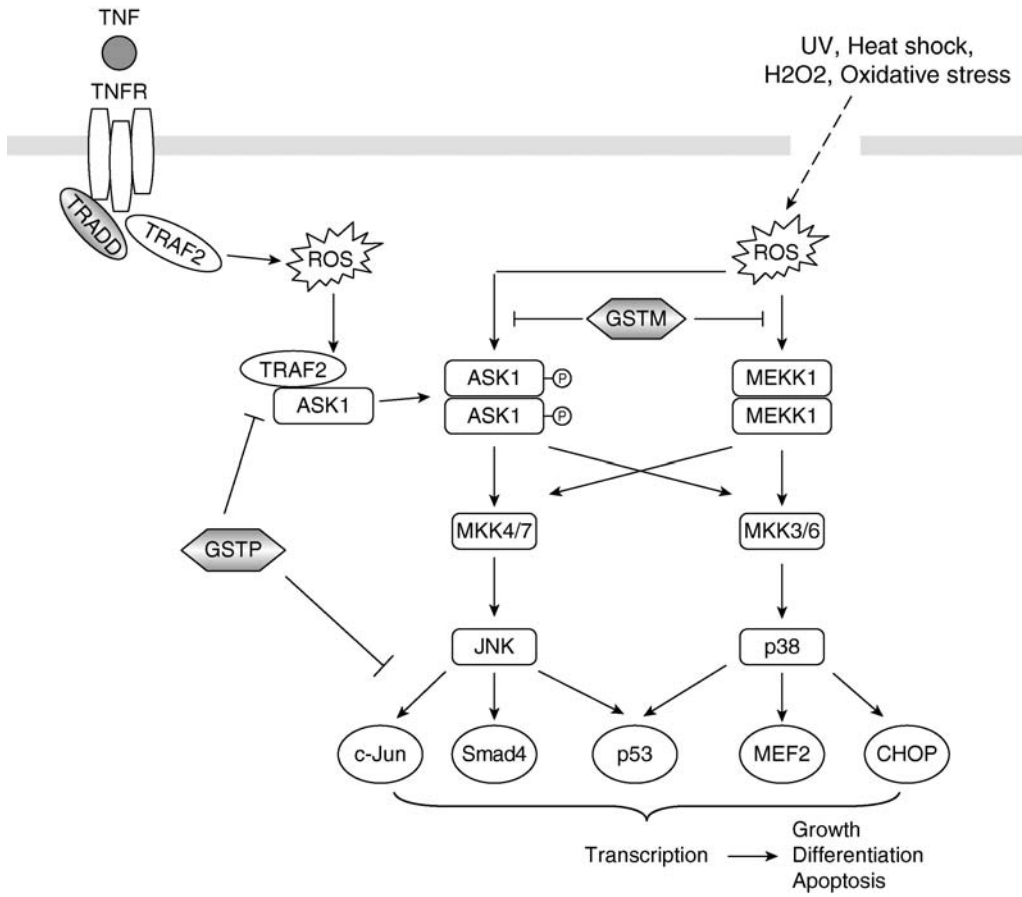
The Pi and Mu classes play an important role in regulating mitogen-activated protein kinase (►MAPK) signaling pathway via their noncatalytic, ligand-binding activity. GSTP functions as an endogenous inhibitor of ►JNK, which interact with the C-terminal of that kinase. GSTP also regulates TNF-α-induced MAPKs signaling by forming ligand-binding interactions with ►tumor necrosis factor – associated factor and suppressing TRAF2-enhanced activation of ►apoptosis signal-regulating kinase1, thereby attenuated TNF-α-TRAF2-ASK1-triggered cancer cell apoptosis. GSTM also has been identified as a negative regulator of ASK1 through direct protein-protein interaction (Fig. 1). All of these findings suggested that the cell death inhibition role of GSTs may provide a novel strategy for antitumor therapy.

Cancer related

For more than a decade, GSTs have generated interest as a neoplastic marker based on its elevated expression in many tumor tissues relative to matched normal tissue. Elevated expression of GSTs have been implicated in cellular response to oxidative stress and protect tumor cells from apoptosis elicited by a variety of cytotoxic agents, such as ►hydrogen peroxide, ►UV radiation, ►cisplatin, ►arsenic trioxide and ►etoposide. Chemotherapeutic-resistant tumor cell lines also have been shown to overexpression GST isozymes. A primary cause of cancer treatment failure and patient relapse is an acquired or intrinsic resistance to anticancer therapies. Acquisition of drug resistance can be attributed to various factors that include avoidance of apoptotic cell death, altered expression of multidrug resistance-associated proteins, altered drug metabolism or uptake, and/or overexpression of GSTs.

Deletion of GSTM1 and GSTT1 genes results in 'null' genotype and increased risk for malignancies as a consequence of a decreased capacity to detoxify possible carcinogens. GSTM1 and GSTT1 phenotypes were also been examined in patients with ►acute myelogenous leukemia (AML). Patients null for GSTT1 exhibited an increased toxicity and reduced survival rate following chemotherapeutic treatment. In contrast, patients null for GSTM1 or GSTT1 showed a twofold reduction in cancer relapse during remission.

The literature is replete with correlative epidemiological reports, where small sample size precludes definitive conclusions of cause and effect between GST expression and cancer incidence. Many studies have provided epidemiological linkage of polymorphic expression of GSTs with cancer incidence and prognosis. As a single enzyme, GST is unlikely to act in isolation, nevertheless, the elevated expression of GSTs in many tumor tissues and cause of cancer treatment failure is well accepted and GSTs have been selected as plausible pharmaceutical targets.



Glutathione-S Transferase. Figure 1 GSTP and GSTM act as regulators of cellular signalings. GSTP interacts and inhibits JNK activity by sequestering JNK/c-Jun, whereas GSTM binding MEKK1 and ASK1 directly. These protein–protein interactions negatively regulate oxidative stresses-induced cell growth, differentiation and apoptosis. GSTP also inhibit TNF-α-triggered cancer cell apoptosis through TRAF2, thereby represses the TRAF2–ASK1 complex.

GSTs have emerged as a promising therapeutic target because specific isozymes are overexpressed in a wide variety of tumors and may play a role in the etiology of other diseases, including neurodegenerative diseases, multiple sclerosis, and asthma. An initial approach was the design of GST inhibitors to act as reasonably non-toxic modulatory agents, useful in situations where conventional anticancer agents are detoxified by GSTs. A variety of GST inhibitors were shown to modulate drug resistance by sensitizing tumor cells to anticancer drugs. The first clinical modulatory studies focused on an approved drug, ethacrynic acid (EA). The therapeutic value of EA as a chemosensitizer has been used in patients. TLK199 is a peptidomimetic GSH analogue that is a low micromolar inhibitor of GSTP. TLK199 was shown to potentiate the toxicity of numerous anticancer agents in different tumor cell lines. Alternatively, compounds designed to disrupt the protein–protein interactions of GSTs with TRAF2 or stress kinases also provide a possible therapeutic approach with respect to modifying proliferative responses.

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N-Glycan Chain

Definition

Glycan covalently linked to an asparagine residue of a polypeptide chain in the consensus sequon-Asn-X-Ser/Thr.

►Mucins

Glycans

Definition

Are composed of carbohydrate units covalently linked together via glycosidic bonds.

►Glycobiology

Glycobiology

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Definition

A discipline of natural sciences covering studies of the structure, biosynthesis and biology of glycans and glycoconjugates, and proteins that specifically interact with these. It is involved in almost every aspect of cancer; transformation, progression, ►metastasis, diagnosis, monitoring, and therapy. Still, the general awareness of the potential and clinical use of discoveries in glycobiology among oncologists, or in oncology among glycobiologists, is limited.

Characteristics

Glycan Structural Diversity is Theoretically Immense but Genetically Restricted

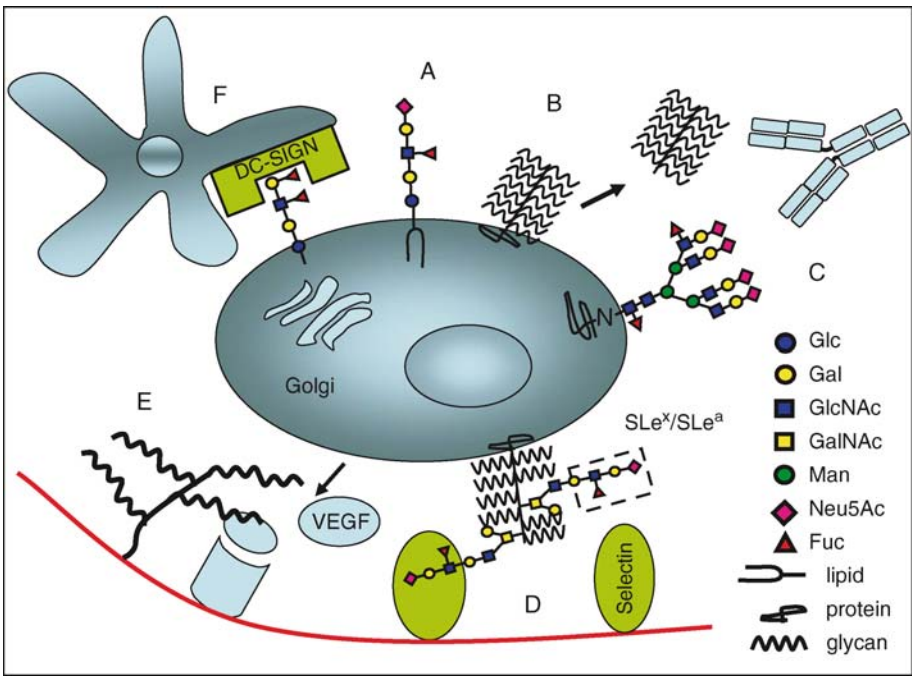
The field of glycobiology has evolved from mainly structural characterization of naturally occurring carbohydrates (sugars or ►glycans) to functional studies of the biology and interaction of such compounds with both endogenous and exogenous proteins, ►lectins typically equipped with carbohydrate recognizing domains (CRD). The glycans (Fig. 1) may occur as free oligosaccharides but are usually covalently linked at the reducing end to an aglycone part being either lipid (in ►glycolipids) or protein (in ►glycoproteins, ►mucins, or ►proteoglycans). The aglycone part as well as the glycan part is of fundamental and often mutual importance for the biosynthesis, localization, and function of the resultant ►glycoconjugates. Carbohydrates of proteins are either O-linked or N-linked and may, as in glycolipids, be either neutral or acidic due to presence of carboxyl and/or sulfate groups.

The structural variability of glycans in nature is, although theoretically immense compared with proteins and oligonucleotides due to the many possible monosaccharides and the many ways these may be linked together, restricted by the number of genes responsible for biosynthesis, and degradation of such glycans. The biosynthesis of glycans is typically localized to the Golgi system whereas preassembly and trimming typically appear in the cytoplasm and the ►endoplasmic reticulum (ER). Some biosynthetic steps even occur in the nucleus. The general requirement for enzymatic ►glycosylation, i.e., initiation or extension of a carbohydrate chain, is a glycosyltransferase, a nucleotide activated sugar donor, an acceptor molecule and an appropriate divalent metal ion. Enzymatic glycosylation is, in contrast to oligonucleotide and protein synthesis, not template driven. Instead glycosylation is characterized by the specificity, redundancy, and promiscuity of the enzymes and the local competition between enzymes which together determine the final glycan structures.

Glycans Evoke Antibody Responses

Since glycans decorate the cell surface of all living cells they may be used both as tags for identity and as molecular sensors of the environment. Glycan structures are cell- and species-specific and typically change during embryogenesis, differentiation, and growth. Cancer development, progression, and spread are distorted or unforeseen series of events that, under the pressure of the innate and acquired immune systems, continuously selects the most aggressive malignant clones. Thus, successful malignant cells will have to adapt to the host physiology, evade the immune defense and take advantage of common cellular and molecular processes that may increase their possibility for survival. Glycans play important parts in such phenomena as will be exemplified later.

The human ABO(H) histo-blood group system, originally identified on red blood cells but now known to occur on many other cells, is of carbohydrate nature. This system, detected due to transfusion complications caused by ►naturally occurring antibodies toward nonself structures, has been the raw model for how ►tumor associated carbohydrate antigens (►TACA) may be utilized to evoke B-cell-mediated antibody



Glycobiology. Figure 1 Tumor cell glycan-mediated interactions with antibodies and lectins. Glycans are biosynthesized by enzymes in the Golgi system, using activated monosaccharides (Glc, Gal, etc.) and typically show aberrant glycosylations not normally found in differentiated cells of that cell type. Glycans appear as membrane bound glycolipids (a), mucins (b, d), glycoproteins (c), or as secreted mucins (b). Antibodies raised against tumor-associated carbohydrate antigens (TACA) are used for serum tests, immunohistochemistry, nuclear imaging, and radioimmunotherapy. Tumor cells overexpress specific glycans, e.g., SLe^x and SLe^a, and use these in the first steps of selectin-mediated adhesion to vascular endothelium facilitating haematogenous metastasis (d). Angiogenesis, defined as the sprouting of blood-vessel endothelial cells, is crucial for local growth of tumor cells in tissues and is dependant on the interactions of proangiogenic factors (e.g., VEGF), proteoglycans, and endothelial cell receptor tyrosine kinases (e). Recognition of TACA by antigen-presenting cells is mediated by C-type lectins (e.g., DC-SIGN) interacting with specific glycans (f). Glc: glucose, Gal: glactose, GlcNAc: *N*-acetylglucosamine, GalNAc: *N*-acetylgalactosamine, Man: mannose, Neu5Ac: neuraminic, Fuc: fucose, SLe^x/SLe^a: Sialyl Lewis x or Sialyl Lewis a structures, VEGF: vascular endothelium growth factor, DC-SIGN: dendritic cell-specific ICAM-3 grabbing nonintegrin.

response. In the classical literature there were encouraging reports of how illegitimate or ►aberrant glycosylation occurred in various adenocarcinomas. The most spectacular clinical report was perhaps the description in 1951 by Levine et al. of a woman of blood group *pp*, i.e., a rare subgroup of the glycan-based histo-blood group P system, who survived for 25 years after a minor incompatible blood transfusion and an incomplete resection of a gastric adenocarcinoma, later shown to contain glycolipids reactive with her own hyperimmune serum (anti-Tja).

A long-standing debate has been whether aberrant glycosylation is a result or a cause of cancer. Many recent reports indicate that some, if not all, aberrant glycosylations are part of the initial oncogenic transformation as well as key events in defining the malignancy (invasive and metastatic phenotype) of tumors. Apart from the examples given in some detail below glycans also play important parts in apoptosis, motility, growth, and angiogenesis (Fig. 1).

Glycans are Clinically Useful as Cancer Markers

With the advent of monoclonal antibody techniques many groups challenged mice with human tumor cells in order to produce antitumor antibodies of potential use for diagnosis and therapy (Fig. 1). Many tumor-associated antigens have been shown to be carbohydrates typically expressed both in carcinomas but also on other, often embryonic or immunocompetent cells (►CA19–9 and SPan-1 recognizing SLe^a; CA50, and DU-PAN-2 recognizing SLe^c, the nonfucosylated precursor of SLe^a; CSLEX-1, and FH6 recognizing ►SLe^x and $SdiLe^x$; B72.3 and CC49 recognizing STn etc). Some of these antibodies are in clinical use worldwide for screening, diagnosis, prognosis, and follow-up after tumor treatment. Only in Japan almost

10 million serum tests for CA19–9 are performed each year, comparable to the number of tests for ►CEA and more than those for ►α-fetoprotein. Other well-known biomarkers of malignancy such as ►PSA for ►prostate cancer and ►CA125 for ►ovarian cancer are also glycoproteins. Thus, although ►TACA appearing in plasma is of considerable advantage for diagnostic use, circulating glycans being either shed or secreted by the tumor cells and mainly as large mucins, may also block antibodies injected for therapy. The clinical use of therapeutic antibodies has so far been limited, partly also due to the immune responses raised against mice monoclonal antibodies, but new strategies for humanizing such antibodies are now under way which will facilitate the use of labeled antibodies e.g., for ►nuclear imaging and for ►radioimmunotherapy.

TACAs are Recognized by Lectins on Antigen-Presenting Dendritic Cells

►Dendritic cells (DC) are specialized ►antigen-presenting cells that express a wide variety of receptors for antigen presentation and uptake. The cells play a key role in controlling ►T-cell responses directing them either to activation or to tolerance. Recent reports reveal that TACA (e.g., CEA or MUC-1 mucin) interact with the calcium dependant proteins (►C-type lectins), DC-SIGN (Dendritic Cell-specific ICAM-3 grabbing nonintegrin), MR (mannose receptor), and MGL (macrophage galactose-type lectin) on dendritic cells (Fig. 1). Both tumor cell-associated and circulating mucins may become internalized, processed, and presented on ►MHC class II molecules, provided their glycans show the aberrant glycosylations recognized by these lectins (e.g., Le^x, Le^y, and Tn antigens). This system may seem to be adequate for early detection and removal of malignant cells. However, TACA may manipulate dendritic cells through their C-type lectins to allow tumor cells to evade the immune system if no other danger signals are provided in parallel. A further understanding of these processes will probably be necessary for understanding why *in vivo* immune responses against TACA are so difficult to obtain but also to pave the way for new antitumor vaccine strategies.

Human Retrovirus HTLV-1 (►Human T-cell Leukemia Virus) Induces Specific Glycan Biosynthesis

Fundamental questions are why certain glycans typically appear in tumors, what advantages do they give the tumor cell and what proteins are there to recognize such glycans? ►DNA methylation or ►histone deacetylation is supposed to mediate decreased expression of glycosyltransferases and errors in alternative splicing has been described for the ABO gene as a plausible explanation for the appearance of TACA in tumor tissues. Several viral infections have been shown to drastically alter the expression of host glycans and corresponding glycosyltransferases in infected cells. Of particular interest is the elevated expression of SLe^x in leukemia cells of patients with adult ►T-cell-leukemia (ATL), a rare but fatal malignancy of helper T-cells. This disease is an endemic leukemia induced by the virus ►HTLV-1. The leukemia cells typically infiltrate various organs and tissues including the skin and the level of infiltration correlates significantly with the level of SLe^x expression on the surface of the leukemia cells. The *pX* gene of HTLV-1 codes for an oncogenic protein, called ►Tax, which has a transactivating effect on several important cellular genes including *hFUT7* which actually codes for the glycoyltransferase responsible for the final step in the biosynthesis of SLe^x. The description of this mechanism is a significant contribution and was the first proof of principle where aberrant glycosylation was directly linked to a genetic mechanism of malignant transformation. The significance of genetic regulation of glycosyltransferase genes in the many cancers of epithelial origin, where the SLe^x and its structural analog SLe^a are characteristically overexpressed, awaits further elucidation.

Selectin–Glycan Interactions Initiate Adhesion of Malignant Cells to Vascular Endothelium

The infiltration of tumor cells from the circulating blood stream into the tissues, as exemplified by leukemia cells in ATL-patients, is for white blood cells (granulocytes and lymphocytes) a physiological homing process which starts with the tethering, rolling, adhesion to, and finally infiltration through the endothelium lining the vessel wall. This process is strictly sequential and initiated by specific calcium-dependant protein–carbohydrate interactions mediated by a family of three ►C-type lectins called E-, P-, and L-selectins and their glycan counter-receptors. These lectins all recognize a common carbohydrate structure, SLe^x, although their receptor specificities vary as to the detailed glycan structures and to the dependence of critical sulfate groups on the core protein or on the glycan itself. E-selectin is typically expressed on endothelial cells at sites of inflammation. P-selectin is constitutively expressed on platelets, on endothelium of lung and brain choroid plexus microvessels and on activated endothelial cells. L-selectin is typically expressed on leukocytes i.e., myeloid cells, naïve T-cells, and some activated and memory T-cells. The revelation of the homing process of myeloid cells, necessary for a normal immune defense, has been possible only through the combined efforts of many outstanding groups using both *in vitro* and *in vivo* studies particularly single and double knock-out mice defective for various cytokines, selectins, glycosyltransferases, mucins, or mucin-like selectin ligands.

Drugs may Inhibit Metastases by Blocking Selectin–Glycan Interactions

Malignant cells misuse the selectin–glycan interactions to metastasize into vascularized distant tissues by presenting at their surfaces increased levels of mucins or mucin-like proteins with multivalent expression of SLe^x, SLe^a, and related glycans (Fig. 1). In a study over a 10-year period it was shown that, 80% of colorectal cancer patients died if there were high levels of SLe^a in their primary tumors, whereas 90% of these patients survived if their tumors lacked expression of SLe^a. The process of metastasizing should not be regarded only as a 1:1 cell interaction since platelets, monocytes, neutrophils, and malignant cells circulating in the blood often produce microparticles which allow for additional networks of selectin–ligand interactions. Many studies have successfully demonstrated the use of blocking the selectin–glycan process for treatment of inflammatory and malignant conditions in animals but so far there has been no break-through in humans. A particularly remarkable effect was however recently shown in a 10-year follow-up of ►colon cancer patients, expressing high levels of SLe^a and postoperatively treated with cimetidine, a histamine-2 receptor blocker usually prescribed for the therapy of gastroduodenal ulcers, where survival increased from 20 to 90%, probably due to the suppressive effect of cimetidine on E-selectin expression on vascular endothelial cells. Glycomimetics, drugs of noncarbohydrate nature but imitating the bioactive conformation of native glycans, are now being tested to selectively block selectin–glycan interactions. Other ways of strategies may be through the antisense cDNA and more recently through the ►siRNA approach.

Concluding Sweet Remarks

The field of glycobiology has generally been considered "harder and less accessible" than other fields dealing directly with proteins and their genes in defining cancer cell phenotypes. This may have been caused by the structural complexity, nomenclature, and methodology of glycans being less straightforward than linear protein or genetic profiling. However, glycoconjugates are intimately involved in most processes of cancer development and research and should be considered essentials for future prevention and treatment of cancer. Improvements in methodology and mutual transfer of basic and clinical knowledge will undoubtedly bridge the gaps between the different fields.

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Glycocalyx

Definition

Is a general term referring to extra cellular polymeric material (protein and carbohydrate) produced by cells. The term was first used to describe the polysaccharide matrix excreted by epithelial cells and forming a coating on their surface. A pericellular coat of polysaccharides including hyaluronan, which surrounds the plasma membrane.

►Hyaluronan Synthases
►Carcinoembryonic Antigen

Glycoconjugates

Definition

Type of compounds consisting of one or more ►glycans covalently linked to other types of chemical constituents, such as lipids and proteins.

►Glycobiology

Glycoforms

Definition

Different molecular forms of a ►glycoprotein, resulting from variable glycan structure and/or glycan attachment site occupancy.

►Mucins

Glycogen Synthase Kinase-3

Definition

Is a serine/threonine kinase, which phosphorylates certain cellular substrates involved in various signaling pathways usually causing functional inhibition. Phosphorylation of the substrates often leads to their inactivation.

►BCL3
►Protein Kinase C Family

Glycolipids

Definition

Common name for membrane bound ►glycans linked to a lipid being either ►ceramide (glycosphingolipids) or diacyl-glycerol (glycoglycerolipids).

►Glycobiology

Glycolysis

Definition

Is a series of biochemical reactions by which a molecule of glucose is oxidized to two molecules of pyruvic acid and can occur in the absence of oxygen. A catabolic cellular cascade generating ►ATP and various other metabolites from glucose.

►Warburg Effect

Glycoprotein

Definition

Is a protein modified by the addition of relatively short sugar chains. Is a macromolecule composed of a protein and carbohydrate(s). The carbohydrate is attached to the protein during ►post-translational modification. The addition of sugar chains can happen either at asparagine and is termed N-glycosylation, or at hydroxylysine, hydroxyproline, serine, or threonine, and is termed O-glycosylation. The sugar group(s) can assist in protein folding or improve proteins stability.

►Mesothelin
►Glycobiology
►Gastrointestinal Stromal Tumor

Glycoprotein-340

►Deleted in Malignant Brain Tumours 1

Glycosaminoglycans

Definition

GAG; Gel-forming polysaccharides of the ►extracellular matrix. These polysaccharides are composed of repeating disaccharide units in which one sugar is either N-acetylgalactosamine or N-acetylglucosamine. Typically each disaccharide carries a carboxyl group and often one or more sulfates so that most glycosaminoglycans have a high density of negative charges. The negatively charged molecules can bind strongly to positively charged regions of proteins in a manner similar to heparin. The glycosaminoglycans are often combined with protein to form proteoglycans. Glycosaminoglycans are an important component of the extracellular matrix in vertebrates.

Glycoside

A form of a molecule with a sugar moiety attached to its anomeric carbon via an *O*-glycosidic bond. Plants frequently store molecules as glycosides, which are inactive until they are enzymatically processed.

►Genistein

Glycosphingolipids

Definition

Are ►ceramides with one or more sugar residues.

►Lipid Mediators

Glycosyl Phosphatidyl Inositol

Definition

A GPI anchor or glycosylphosphatidylinositol is a phosphate containing glycolipid that can be attached to a protein during ►post-translational modification. It is linked to the C-terminal amino acid of a mature protein.

►Carcinoembryonic Antigen

Glycosylation

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Synonyms

Carbohydrate part of glycoconjugates

Definition

Glycosylation is defined as the covalent attachment of a carbohydrate to a protein, lipid, carbohydrate, or other organic compound, catalyzed by glycosyltransferases, utilizing specific sugar donor substrates.

Characteristics

The type of glycosylation of the different biomolecules can be used to classify the following families of glycoconjugates: The asparagine-linked (*N*-linked) oligosaccharides of many glycoproteins; The serine or threonine-linked (*O*-linked) oligosaccharides that are present on many glycoproteins and that predominates on secreted or membrane-bound ►mucins; the glycosaminoglycans (GAG), which are ►glycans present as free polysaccharides or as part of proteoglycans (such as heparin sulfate and chondroitin sulfate); the ►glycosphingolipids, which consist of oligosaccharides linked to ceramide; the glycosylphosphatidylinositol (GPI)-linked proteins, which are proteins that bear a glycan chain linked to phosphatidylinositol; and nuclear and cytoplasmic proteins, which bear the monosaccharide *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) linked to serine. Glycans can exist as membrane-bound glycoconjugates, for example, forming the glycocalix, or as secreted molecules, for example, as components of the ►extracellular matrix. Gycans mediate key cellular functions such as cell–cell interactions, ►adhesion and ►motility, as well as intracellular signaling events.

Glycosylation Alterations and Functions in Cancer

Altered glycosylation is a common feature of cancer cells. These modifications have been observed both histologically using lectins or monoclonal antibodies, and by mass spectrometry. Changes in glycosylation in cancer include both under- and overexpression of naturally occurring glycans, as well as novel expression of glycans normally restricted to other adult tissues, or structures that arise during development in embryonic tissues. The biosynthesis of these altered glycans generally arises from changes in the expression of the glycosyltransferases. Glycosyltransferases are Golgi-resident enzymes that are responsible for the biosynthesis of oligosaccharide chains. Modification of the set and the level of expression of glycosyltransferases can lead to changes in the structures of protein *N*-linked and *O*-linked gycans as well as glycolipids. Glycans play main roles in the biology of the tumor cells.

Glycosylation and Cell Signaling. *N*-linked glycans participate in the control of protein folding, and play a crucial role in the protein half-life and quality control systems. *N*-glycans may also interfere cellular receptors and tumor growth. *N*-glycosylation on insulin-like growth factor 1 receptor (IGF1R) affects its phosphorylation and therefore influences cell-surface receptor translocation, leading to modification of the signaling and affecting the growth and survival of melanoma and sarcoma cells. Inhibitors of *N*-glycosylation can reduce the survival of cells from tumors that depend on IGF1R signaling.

Glycosphingolipids, particularly ►gangliosides frequently overexpressed in tumors, are also involved in cell signaling and tumor cell growth. Gangliosides are capped with sialic acid, a negatively charged acidic saccharide, on the terminal tip of the glycan. In normal cells, gangliosides are often associated with certain cell membrane ►tyrosine kinases receptors, such as EGF receptor and ►IGF receptor, modulating their phosphorylation and interfering with their growth-promoting functions. The ganglioside G_{M3}, which is commonly expressed in lung cancers, melanomas, and neurogenic tumors, is able to regulate growth signaling through the receptor tyrosine kinases. Gangliosides also contribute for the molecular organization of receptor complexes in lipid rafts. Ganglioside G_{M1} contributes for ERBB3 and ERBB4 receptors to form heterodimers

on lipid rafts on ►breast cancer cell membranes, facilitating ERBB signaling and functions.

Protein *O*-Glycosylation. Another good example of glycoconjugates interfering with cell signaling and promotion of proliferation comes from mucins. Mucins are secreted or membrane-associated glycoproteins that contain clusters of *O*-glycans in a central domain of the core protein. The ►mucin (*MUC*) gene family includes several members of glycoproteins that are expressed in a tissue- and cell-specific manner. Most carcinoma expresses mucins with altered *O*-glycan chains. Membrane-associated mucins can interfere with signal transduction. MUC4 mucin is overexpressed in mammary tumors, contains an epidermal growth factor (EGF)-like motif on its extracellular (juxtamembrane) domain which interacts with the ►ERBB2, initiating phosphorylation of the receptor tyrosine kinase in the absence of other ligands. In breast carcinomas, ERBB2 is frequently overexpressed or mutated in an active form. Experimental studies have showed that MUC4 overexpression increases cell growth and that MUC4 overexpressing cells can autophosphorylate ERBB2, contributing for the inhibition of ►apoptosis. *O*-glycans on MUC4 play an important role in the protein folding leading to the exposure of the EGF-like domain and its activity in cancer cells. Another membrane-associated mucin, ►MUC1 shows an altered *O*-glycosylation carrying several cancer-associated carbohydrate antigens. The cytoplasmic tail of MUC1 can be phosphorylated following mucin interactions with other partners. This may modify signaling pathways and affect β-catenin signal transduction by two mechanisms: MUC1 might be cleaved in the cytoplasm and move with β-catenin to the nucleus thereby influencing transcription, or it might sequester β-catenin in the cytosol and therefore prevent it from interacting with the cadherins or other complexes, thereby influencing ►WNT signaling. Mucin *O*-glycans are also involved in the invasive and metastatic properties of adenocarcinomas by simultaneously configuring the adhesive and antiadhesive cell-surface properties of tumor cells. Mucin *O*-glycans contain domains that mediate both antiadhesion and adhesion effects, and might block proteolytic activity.

Proteoglycans may function as coreceptor for soluble growth factors. The structure of ►Proteoglycans consists of a core protein and one or more GAGs. GAGs are linear polysaccharides composed of repeating disaccharide units (GlcNAc in heparan sulfate or GalNAc in chondroitin sulfate) linked to uronic acid (GlcA or IdoA). Most mammalian cells produce proteoglycans that may be secreted to the extracellular matrix, inserted into the plasma membrane or stored in secretory granules. GAG binding to a protein ligand may cause: (i) immobilization of the protein ligand at site of production, (ii) interference with enzyme activity, (iii) interaction with signaling receptors, (iv) protection of ligands against degradation, and (v) reservation of ligand for future mobilization. These interactions lead to profound physiological effects, affecting processes such as: cell–matrix (interaction with ►fibronectin, ►laminin, and ►thrombospondin), growth (interaction with ►fibroblast growth factors, ►transforming growth factor-β), ►angiogenesis (interaction with vascular endothelium growth factor), and ►inflammation (interaction with selectins, ►interleukin-8, and others). The tumor ►microenvironment often shows significant alterations in content of proteoglycans. Cancer cells seem to usurp GAG mediated functions to promote tumor growth, progression, and ►invasion. For example, ►breast cancer, ►ovarian, ►pancreatic cancer, and ►hepatocellular cancer cells modulates sulfation of proteoglycans in a manner that promotes their binding capability for growth factors and inducing receptor tyrosine kinase activation.

Glycans in Tumor Cell Adhesion and Invasion. *N*- and *O*-glycosylation of glycoproteins controls different tumor cell biological properties. One of the best studied modification observed in cancer cells is the increased expression of complex β1,6-branched *N*-linked glycans on their cell surface. This alteration is caused by an increased expression of the enzyme *N*-acetylglucosaminyltransferase V (GnTV). This enzyme transfers a GlcNAc residue onto growing *N*-linked glycans so that the subsequently glycosylation results on multiantennary chains. This increased branching creates additional sites for terminal sialic acid residues, which, together with the corresponding upregulation of sialyltransferases, may lead to an increased sialylation. The expression of complex β1,6-branched *N*-glycans has been shown on tumor cell ►E-cadherin – an adhesion molecule that mediates cell adhesion through homotypic interactions and β1-integrin. Increased β1,6-branched *N*-glycans on tumor cell E-cadherin reduces cell–cell adhesion, and promotes tumor cell detachment and invasion. The coordinated control of expression of GnTV and other molecules, such as E-cadherin, contributes for the modulation the adhesion and invasion phenotype of tumor cells.

Alterations in *O*-glycosylation are common feature observed in glycoproteins expressed in tumors cells. These alterations include the expression of simple mucin-type *O*-linked carbohydrate antigens, such as the Tn, sialyl-Tn, T, and sialyl-T antigens, which are rarely expressed in normal cells. The biosynthesis of these glycans may occur due to different reasons, such as: altered expression and cellular mislocalization of glycosyltransferases, imbalanced competition among glycosyltransferases for acceptor substrates, and overexpression of acceptor substrates. The sialyl-Tn antigen is a good example of a simple *O*-linked glycan that is abnormally expressed in membrane-associated and

secreted glycoproteins, mainly mucins, from tumor cells. Sialyl-Tn expression is associated with an aggressive phenotype. ►Transfection of ►gastric cancer and ►breast cancer cells with the cDNA that encodes the sialyltransferase responsible for sialyl-Tn biosynthesis (ST6GalNAc-I) modifies cell–cell aggregation, and increases migration and the cell invasion potential. These observations are in agreement with the association of sialyl-Tn expression and the increased metastatic potential and poor prognosis observed in colorectal, gastric, and ovarian carcinoma.

In addition to changes in the core structures of glycans, alterations of terminal structures are also associated with malignant phenoptype. Glycosyltransferases involved in the biosynthesis of terminal structures, such as sialyltransferases and fucosyltransferases, frequently show altered expression in tumors. The altered expression of these enzymes leads to overexpression and/or neoexpression of terminal glycans, namely: sialyl-►Lewis X (S-Le^x), sialyl-Lewis A (S-Le^a), among others. These terminal structures can be carried on *O*- and *N*-linked glycans on proteins as well as on glycolipids.

The expression of S-Le^x and S-Le^a in cancer cells can promote ►metastasis through the interaction with Selectins. Selectins normally mediate adhesion between ►leukocytes (which express L-selectin), ►platelets (P-selectin) and ►endothelial cells (E- and P-selectins) that bear the appropriate glycosylated ligand. This ligand consists of oligosaccharides containing the terminal S-Le^x structure. The presence of such ligand on blood cells (such as monocytes or neutrophils) or in endothelial cells induces the transmigrations of leukocytes to sites of inflammation and/or infections, as well as of platelets to sites of vascular damage. In contrast to their normal counterpart, tumor cells from ►gastric cancer, ►colon, ►pancreatic cancer, and ►lung carcinoma, frequently overexpress S-Le^x, or S-Le^a on glycoproteins and glycosphingolipids. The expression of these glycans is associated with poor prognosis for the patients. These glycans have been shown in metastatic tumor cells in the blood of colon carcinoma patients and demonstrated to bind to ►E-selectin. Likewise, overexpression of E-selectin in a transgenic mouse liver induced redirection of the metastatic patterns of syngenic carcinomas that normally metastasizes the lung. Furthermore, the use of a specific disaccharide decoy that acts as a competitive substrate of glycosyltransferases reduced the levels of S-Le^x on colon carcinoma cells. In comparison to untreated cells, these S-Le^x-deficient cells showed decreased interactions with selectins, increased susceptibility to leukocyte-mediated lysis, and reduction of lung ►metastasis in a murine tumor model. These studies, together with the clinical data, indicate that interactions between glycans and selectin molecules play a role in the metastatic process of some carcinoma cells. In summary, this may fit with the classical observation that cancer cells entering the bloodstream tend to form thromboemboli with platelets and leukocytes, probably facilitating the transport to distant sites, contributing to evasion of the immune system, and finally participating in the process of extravasation in the distant metastatic site.

Alterations in the levels of sialylation, namely oligosaccharide chains containing terminal sialic acid are also often observed in tumor cells. This is the case of sialic acid capping terminal galactose in *N*-linked glycans by the enzyme ST6Gal-I, which is upregulated in colon and breast cancers. Hypersialylation of glycans on β1-integrins augment colon tumor progression by altering cell preference for certain extracellular matrix milieus, as well as by stimulating cell migration. In addition, Polysialilation controlled by polysialyltransferases can modulate the sialylation of molecules, such as NCAM in human tumor cells, which has been linked to cancer development and dissemination.

Glycans Used as Serological Markers in Cancers

The most widely used serum diagnostic assays for carcinomas are based on serum measurement of certain glycans. These serological assays are currently used to facilitate diagnosis, track tumor recurrence or tumor burden or provide a surrogate measure for therapeutic response. For example, the serological marker ►CA125 detects the MUC16 glycoprotein and its detections in the serum of ovarian cancer patients is associated with tumor burden and prognosis. Another useful serological assay is the ►CA19-9, which recognizes the S-Le^a carbohydrate antigen present on mucins and glycolipids. CA-19-9 can be used in pancreatic, gastric, and colorectal carcinomas. The CA15-3 assay, which recognizes the MUC1 mucin glycoprotein, is also a useful marker commonly overexpressed in breast carcinomas with serum levels also associated with tumor burden and prognosis. In an attempt to improve their clinical application of the serological assays, a combination of the existing assays against carbohydrate structures detected in the serum have improved the sensitivity with a concomitant loss of specificity. Other assays with higher specificity are under investigation.

Therapeutic Applications of Glycans in Cancers

Various therapeutical approaches are being developed targeting glycans. These strategies include the targeting of glycans that affect tumor proliferation, such as the interference with the coreceptor activity of proteoglycans on tumor cells. In addition, various approaches are targeting glycan during invasion using methods to block specific *N*- and *O*-glycosylation steps as well as the use of competitors of proteoglycans that, once again interfere with tumor cell invasion.

Tumor cell dissemination is another step in which glycans can be targeted. Agents that can interfere with selectin – carbohydrate interactions have been considered for the treatment of metastatic disease. These interactions can be targeted through the administration of neutralizing antiselectin antibodies or small molecules that mimics the SLeX or SLeA selectin ligands. The applicability of such experimental therapies to clinical tumor biology and metastasis remains challenging but the importance of this strategy warrants further development of this class of therapeutic agents, which might work best in combination with conventional chemotherapy.

In addition, several immunotherapy based approaches are being tested in preclinical and ►clinical trials targeting cancer-associated mucin glycans expressed on adenocarcinomas. These trials include ►monoclonal-antibody-based therapies – either ►radioimmunoconjugates or passive ►immunotherapy and ►antitumor vaccines.

Monoclonal-antibodies directed to cancer associated glycans, such as S-Tn, and glycan carriers, such as MUCl mucin have been used as radioimmunoconjugates. Second generation approaches are under development, such as the use of humanized forms of the murine antibodies as well as recombinant single-chain variable fragments, which have a much smaller mass than full antibodies and therefore are capable of reaching most cells in a tumor.

Another immunotherapy based approach aims at augmenting the immunity against tumor mucin glycans. A tumor's ability to generate and overexpress unique mucins and glycans is now being used to improve or augment the immune system's ability to recognize and destroy tumor cells. Methods to destroy tumor cells using vaccines that target the tumor mucins MUC1 or MUC16, or the cancer-associated S-Tn glycan, have been introduced. Vaccines of this type are typically prepared by conjugating the glycan to a carrier protein, and then injecting the compound into the patient together with an adjuvant that boosts T-cell responses. Vaccines that contain these epitopes are currently being used in breast, colorectal, and ovarian cancer patients to augment antitumor immune responses. In addition, peptide mimetics of tumor carbohydrates, such as SLeX, SLeA, or SLeY, have also been shown to stimulate tumor immunity in animal models. In another promising strategy, antitumor immunity has been generated by making unique alterations to tumor-cell surface sialic acids (which are normally tolerated by the immune system). This might be accomplished by introducing unnatural sialic acid precursors to tumors whereby metabolic incorporation leads to the generation of unnatural sialic acid epitopes, forming the basis for the induction of novel antitumor immune responses.

Other approaches have targeted at augmenting immunity against tumor glycosphingolipids. Some tumors express very high levels of immunogenic gangliosides, and often shed them into the bloodstream. Paradoxically, this can lead to immune silencing, by inhibition of costimulatory molecule synthesis and by the arrest of dendritic-cell maturation, resulting in the inability to generate effective antitumor T-cell immune responses. Although glycosphingolipids are relatively poor immunogens, certain glycosphingolipids might be manipulated to generate both passive and active immunity by eliciting a host response to a tumor glycan. Immunization with purified GD2 or GM2 gangliosides are being used in current clinical trials against melanoma (using GD2–KLH), neuroblastoma (using anti-GD2 small immunoproteins), breast (using NeuGcGM3 proteoliposomes that contain the *N*-glycolyl form of sialic acid), and prostate carcinomas (using a conjugate between KLH and Globo-H hexasaccharide, a glycan expressed on prostate and breast cancer glycolipids).

Finally, liposomal drug-delivery techniques such as ►antisense oligodeoxynucleotides directed to inhibit the synthesis of ►oncogenes have been delivered to human ►neuroblastomas by encapsulation in cationic ►liposomes that have been covalently coupled to monoclonal antibodies against the GD2 ganglioside expressed in the tumor cells.

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Glycosylation, Aberrant

Definition

Altered pattern of glycans typically found in tumor cells as a function of incomplete or altered biosynthesis (or trimming) of ►glycans.

►Glycobiology

N- and O-Glycosylation

Definition

►Post-translational modification of newly synthesized proteins by enzymatic cross-linking of carbohydrate moieties to the polypeptide backbone. N- and O-►glycosylation occur on asparagine and serine/threonine residues, respectively, according to defined sequence motifs in the polypeptide

►Cystatins

O-Glycosylation

Definition

All mucin-bound **O**-glycans are built up in a sequential step-by-step process in the Golgi apparatus starting with the addition of a GalNAc residue to a serine or threonine residue. Subsequently, elongation of **O**-linked sugars is achieved by the stepwise transfer of saccharide units to the GalNAc-glycosylated protein.

►Mucins
►N- and O-Glycosylation

Glycosylphosphatidylinositol Linkage

Definition

GPI linkage is a glycolipid modification localized at the C-terminus of proteins and anchors the protein to the cell membrane. ►Phospholipase C is an enzyme that cleaves GPI-linked proteins, releasing them for the cell membrane.

►Cripto-1
►Glycosylation

Glycosyltransferase

Definition

Enzyme that catalyzes the transfer of a sugar moiety from a sugar nucleotide donor to a substrate.

►Mucins
►Glycosylation

Glycyrrhetinic Acid

Definition

A triterpenoid from ►licorice.

►Betulinic Acid

Glypican 1

Definition

Cell surface heparin sulfate ►proteoglycan that functions as a co-receptor for heparin binding growth factors such as ►fibroblast growth factor (FGF) and transforming growth factor beta (►TGF-β)

►Endostatin

G2/M Arrest

Definition

Stop of ►cell cycle at G2 or M phase.

►Thiazolidin
►G2/M Transition

G_2/M Checkpoint

Definition

Is a point in the G2 phase of the ►cell cycle where cells become arrested in response to ►DNA damage. The G2/M ►checkpoint is regulated by proteins that modify or sequester the components of the ►Cyclin B/Cdc2 kinase complex, the principal enzymatic activity responsible for the initiation of ►mitosis. It is one of two major checkpoints (G1/S and G2/M) at which the transition from one phase of the cell cycle to the next can be stopped. The initiation of mitosis is subject to a number of checkpoint controls. These controls serve to arrest cell-cycle progression, if progression

would otherwise result in progeny with incomplete or damaged genomes. To progress into mitosis, the nuclear DNA of a cell has to be completely replicated and be in an undamaged state so it can condense into complete chromosomes. Cells in G2 phase that sustain DNA damage will arrest prior to entering mitosis. This arrest continues until their DNA is sufficiently repaired. If damage cannot be repaired, ►apoptosis is induced. Other conditions such as separation of ►centrosomes to allow spindle pole formation are also necessary for a cell to begin the G2/M transition. As transformed cells typically lack some G2/M checkpoint mechanisms and most studies have been carried out using such cells, it is likely that there exist additional checkpoint conditions yet to be identified.

►G_2/M Transition
►Oxidative DNA Damage
►Trefoil Factors
►G2/M Transition

G_2/M Cyclins

Definition

The ►cyclin family members can be divided into G1/S and G2/M subfamilies depending on when their associated ►cyclin-dependent kinase (CDK) activity peaks. Cyclins B1, B2 and A are all G2/M cyclins. In humans these cyclins localize CDK1 to different parts of the cell as it enters ►mitosis. This probably confers some degree of substrate specificity and allows them to be differentially regulated, by keeping CDK1 away from or in contact with its respective regulators. Cyclin A/CDK2 is localized to the nucleus. Its role in inducing G2/M events is unclear, however its activity peaks at this point of the cell cycle.

Cyclin B1/CDK1, translocated to the nucleus at G2/M, can initiate ►NEBD, inactivate the nuclearly localized inhibitory kinase wee1 and activate nuclearly localized phosphatase cdc25C. The abundance of this complex (cyclin B1/CDK1) enables it to efficiently phosphorylate many other substrates at the G2/M transition.

Cyclin B2CDK1, present at much lower amounts within the cell as compared to cyclin B1/CDK1, is localized to the Golgi apparatus and is involved in breaking up of this organelle during mitosis.

►G_2/M Transition

G_2/M Transition

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Definition

The G2/M transition is a decisive point in a cells life cycle. The point at which, after successfully completing a second growth phase (G2 phase) following the replication of its DNA (S phase), it begins ►mitosis (M phase), the phase during which it physically separates itself into two daughter cells (Fig. 1).

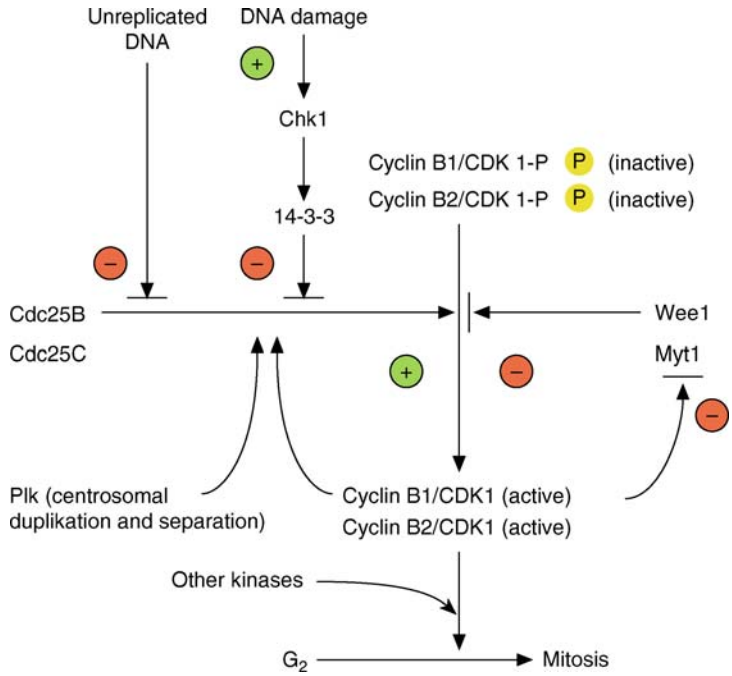
Characteristics

To divide itself equally into two daughter cells a cell must fundamentally change almost all of its structures. These dramatic structural changes become apparent at the G2/M transition. In addition to these structural changes, RNA processing stops and majority of mRNA ►translation is repressed (there are some specific mRNA sequences that can overcome this inhibition allowing them to be translated during mitosis). The G2/M transition occurs because of a pivotal shift in the balance of kinase and phosphatase activities within the cell. A cell will not usually commit to such big changes unless a number of criteria have been met, such as accurate replication of its DNA or separation of its centrosomes. Such criteria are sensed and acted upon via ►G2/M checkpoint pathways.

Structural Changes at the G2/M Transition

One of the first visible signs that a cell is about to enter mitosis is the increasing compaction of its DNA. Cells stained with fluorescent DNA-binding dyes typically show a more granular nuclear staining pattern than is normally seen. As DNA becomes more condensed (a process known as ►chromosome condensation), the nuclear envelope surrounding it disassembles (a process called ►nuclear envelope breakdown or NEBD). NEBD effectively breaks the down nuclear-cytoplasmic compartmentalization within the cell. The ►nucleolus within the nucleus also disassembles causing RNA synthesis and processing to stop.

In the cytoplasm, membrane trafficking and sorting between the ►endoplasmic reticulum, Golgi apparatus and ►plasma membrane ceases. At the same time the endoplasmic reticulum and Golgi apparatus fragment, enabling these organelles to distribute themselves easily between the two daughter cells. Anchored cells grown in culture begin to round up at G2/M; this is due to profound changes in the ►cytoskeleton seen in all cells as they enter mitosis. Actin and intermediate filament networks are reorganized. ►Microtubules undergo a



G_2/M Transition. Figure 1 Regulating the G2/M transition.

dramatic change in their dynamic stability i.e. the rates at which they grow and shrink in length. The number of microtubules nucleated from each centrosome increases approximately tenfold, but the length of these more abundant microtubules decreases by tenfold. This change in microtubule dynamics together with the separation and moving apart of the two centrosomes allows the formation of a ►spindle apparatus. The spindle apparatus functions to organize the chromosomes into an aligned configuration, a stage in mitosis known as metaphase. Following metaphase, the pairs of ►sister chromatids can separate from one another and migrate to the opposite poles of the spindle apparatus (a stage known as anaphase), allowing the final stage of mitosis (telophase) to proceed.

Triggering G2/M Transition

Many kinases and phosphatases are needed for a cell to complete its G2/M transition. The best characterized and most important of these being the cyclin B1/cyclin-dependent kinase (►CDK1), sometimes called ►maturation promoting factor (MPF). Cells injected with active cyclin B1/CDK1 can be induced to enter mitosis. Cyclin B1/CDK1 activity, often taken as a measure of the mitotic state of a cell lysate, can be measured by its ability to phosphorylate exogenously added histone H1 as this is a very good in vitro substrate of cyclin B1/CDK1. The importance of cyclin B1/CDK1 in its activity for driving a cell into mitosis is reflected in the many ways in which its activity is regulated.

Members of the cyclin protein family each bind to a specific member of the ►cyclin-dependent kinase (CDK) family of serine/threonine kinases. CDK's alone are inactive, however when bound to a cyclin, the conformation of CDK changes to an active one. Thus the amount of cyclin within the cell regulates the amount of potential CDK activity. Cyclin B1 is the most abundant ►G2/M cyclin protein and it associates with only one member of the CDK family, CDK1. It accumulates throughout S and G2 phase, rapidly associating with CDK1 as it is synthesized (CDK protein levels are usually in excess of that of cyclin B1). This would cause a slow increase in CDK1 kinase activity as the cell progresses through S and G2 phases, but CDK1 is rapidly made inactive by an inhibitory phosphorylation that blocks the active site of the CDK. This inhibitory phosphorylation is carried out by two kinases, wee1 and myt1, which are localized in the nucleus and cytoplasm, respectively. Wee1 and myt1 kinases allow the cyclin B1/CDK1 complex to stockpile in an inactive state. Removing the inhibitory phosphate masking the active site, rapidly activates accumulated cyclin B1/CDK1 causing the cell to enter mitosis. Cdc25C is the major phosphatase that removes the inhibitory phosphate on CDK1 and thus triggers cyclin B1/CDK1 activation. Cdc25B, another cdc25 family member, is also activated at G2/M, although there is much less of this in the cell relative to Cdc25C. Positive and negative feedback loops reinforce the quick activation of cyclin B1/CDK1; cdc25 phosphatase activity and wee1 kinase activity can be activated and

inhibited respectively by phosphorylation by cyclin B1/CDK1. It is also likely that the timely destruction of the wee1 kinase helps to trigger and maintain activation of cyclin B1/CDK1.

Cells in G2 phase that sustain DNA damage delay activation of their cyclin B1/CDK1 and thus their progression into mitosis. G2/M checkpoint controls converge to a point where the phosphorylation state of CDK1 complexed to ►G2/M cyclins is regulated. Cells expressing a mutated CDK1 that cannot be inhibited by Cdc25 can partially by-pass a DNA damage induced checkpoint arrest. Chk1 and cds protein kinases are two conserved nuclearly localized kinases needed for the DNA replication and DNA damage G2/M ►checkpoints. When activated by the presence of inappropriate DNA breaks or incomplete DNA replication, both phosphorylate cdc25C phosphatase and in doing so create a ►14–3–3 protein-binding site. 14–3–3 protein bound to cdc25p blocks cyclinB1/CDK1 activation by inhibiting the phosphatase's activity and exports nuclearly localized cdc25C protein to the cytoplasm.

The dynamic localization of key enzymes involved in the G2/M transition is important in regulating entry into mitosis. During interphase, cyclin B1 shuttles between the nucleus and the cytoplasm, but at steady state it is primarily cytoplasmically localized. A defining moment in the G2/M transition is the rapid nuclear translocation of cyclin B1/CDK1. This is brought about both activating its rapid nuclear import and inhibiting its nuclear export. Shifting cyclin B1/CDK1 into the nucleus is thought to expose it to nuclearly localized activating cdc25 protein. Thus the movement of cdc25 protein out of the nucleus by 14–3–3 protein stops cyclin B1/CDK1 from being activated in the nucleus.

Although Cyclin B1/CDK is the most important kinase in triggering the G2/M transition, many other kinases and phosphatases are active upon mitotic entry making it difficult to ascribe specific substrates to each kinase. ►Polo-like kinase (Plk) functions in multiple stages of mitosis. During G2 phase of the cell cycle and early phases of mitosis it is localized to centrosomes and spindle poles where its activity is necessary for centrosome separation and bipolar spindle formation. It may also play a role upstream of cyclin B1/CDK1 activation by helping to activate the cdc25 phosphatase.

►Decatenation G2 Checkpoint

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GMEM

►Tenascin-C

GnRH

Definition

►Gonadotropin-Releasing Hormone

GnRH Analogs

Definition

Gonadotropin-Releasing Hormone Analogs; Over 1,000 analogs (agonists and antagonists) were synthesized. Both groups are more stable and display higher affinity to the GnRH receptor. The agonists display a higher biological activity, while the antagonists compete with endogenous GnRH for the receptor and block its activity.

►Gonadotropin-Releasing Hormone

Goldberg-Hogness Box

Definition

►TATA Box

Gompertzian Growth Curve

Definition

Discovered by Benjamin Gompertz, a nineteenth century actuary, the Gompertzian growth curve describes the complex pattern of tumor growth. The curve has an early, almost exponential growth rate followed by slower growth rate which reaches a plateau as tumors grow larger in size.

►Norton-Simon Hypothesis
►Log-Kill Hypothesis

Gonadal Mosaicism

Definition

A variable proportion of the eggs or sperm in a transmitting individual contain a genetic defect. The risk of passing along the disease or trait varies with the percentage of abnormal gametes. Most often implied when a non-affected parent has one or more affected children with the disease.

►Mosaic
►Mosaicism

Gonadal Neoplasms

►Germ Cell Tumors

Gonadotropes

Definition

A pituitary cell type (5–10% of the cells), expressing ►Gonadotropin-Releasing Hormone (GnRH) receptors and responding to Gonadotropin-Releasing Hormone (GnRH) with ►gonadotropin synthesis and release.

Gonadotropin-Releasing Hormone

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Synonyms

LRF, luteinizing hormone (LH)-releasing factor; LHRH, LH-releasing hormone; GnRH, gonadotropin-releasing hormone

Definition

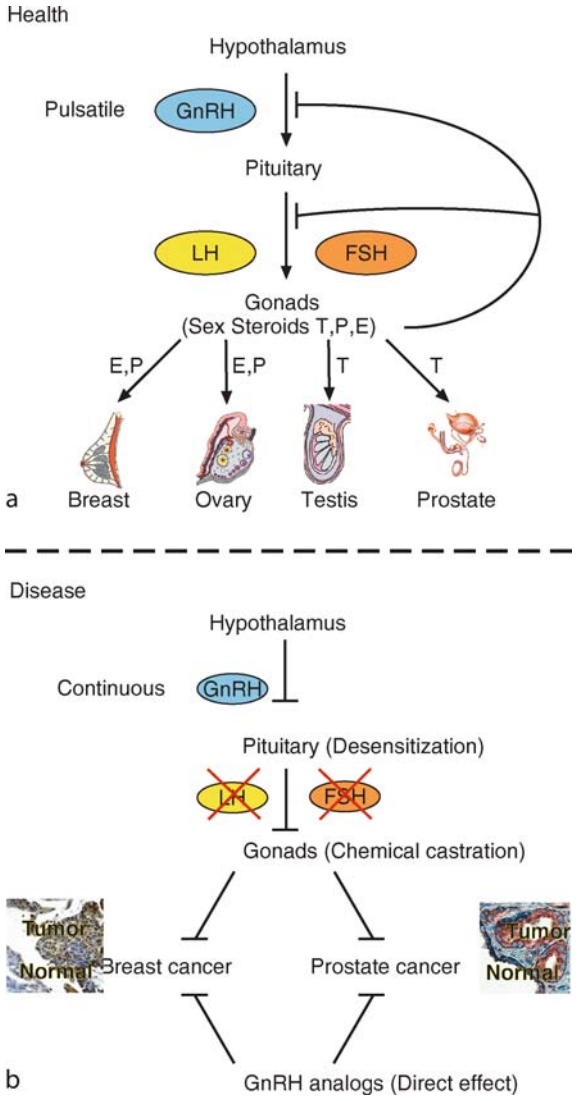
GnRH is the central regulator of reproduction. GnRH binds and activates its cognate receptor in pituitary ►gonadotropes, resulting in the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) that control ovulation and spermatogenesis.

Characteristics

To isolate GnRH, Andrew Schally's group collected 2.5 million pig brains, while Roger Guillemin's group collected 5 million sheep brains, and while competing, both groups have announced in 1971 the isolation and structure of GnRH as pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly.NH_2. For their outstanding work, both Guillemin and Schally received the Nobel Prize for Medicine in 1977.

The pulsatile nature of hypothalamic GnRH secretion is absolutely required for physiological maintenance of ►gonadotropin (LH and FSH) secretion. Continuous exposure of the pituitary to GnRH results in desensitization of gonadotropin secretion. This homologous desensitization will result in reduction in LH and FSH levels and gonadal steroid sex hormone (►progesterone, ►estrogen, and ►testosterone) levels. Since the steroid sex hormones are required for the functioning of the gonads and many of their cognate cancers, their removal causes malfunction of dependent organs (breast, ovary, testis, and prostate). This phenomenon is known as ►chemical castration (Fig. 1) and is the basis for the wide use of stable and long acting ►GnRH analogs (GnRH-A) in fertility regulation, precocious puberty, ►uterine leiomyoma, ►endometriosis and ►sex hormone-dependent cancers, such as ►prostate cancer and ►breast cancer.

As "chemical castration" agents, GnRH-A (e.g., Leuprorelin, Buserelin, Goserelin, Histrelin, Deslorelin, Nafarelin, and Triptorelin) have a drawback since they initially stimulate gonadal functions before desensitization occurs (castrate levels of testosterone (<50 ng/dl)



Gonadotropin-Releasing Hormone. Figure 1 GnRH actions in the pituitary, the normal physiological actions (a), the "chemical castration effect" and the direct effects of GnRH analogs upon cancer cells (b) are depicted in this model. LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone; P, progesterone; E, estrogen.

take at least 15 days of treatment). This "tumor flare" effect has first promoted the additional use of antiandrogens for prostate cancer (e.g., Bicalutamide, Flutamide, Nilutamide, Megestrol, and Cyproterone) and later the use of several generations of GnRH peptide antagonists (e.g., Abarelix, Antarelix, Cetrorelix, Ganirelix, Antide, Teverelix, FE 200486, and Nal-Glu), which compete with endogenous GnRH for binding to its receptor (castrate levels of testosterone can be achieved after 4 days of treatment). For example, abarelix (the first GnRH antagonist that was approved for clinical use in the USA), became a good alternative to surgical castration, or GnRH agonists plus antiandrogen. The high dose of the antagonists required for cancer treatments, the high cost, side effects (allergic reactions), and low solubility prompted the synthesis of further generations of peptide antagonists, long acting delivery systems and the development of nonpeptide, orally active GnRH antagonists, which are in clinical trials (e.g., TAK-013, TAK-810 and NBI-42902). GnRH agonists are mainly created by substituting the Gly6 with a D-amino acid (Leu, or Ser, or His, or Trp) and the Gly-NH_2 at the 10 position with N-Et-NH_2. The GnRH antagonists require substitution of the 1–3, 6, and the 10 positions in order to be pharmacologically effective. The nonpeptide, orally active GnRH antagonists are based on the structure and/or derivatives of tetracyclic benzodiazepine, ketoconazole, spiroamines, thienopyridines, indole, quinolone, erythromycin A, and imidazolpyrimid-5-ones, which conform to a basic structure of a basic protonable nitrogen group, one or two aromatic groups, and an aliphatic lipophilic group. These nonpeptide GnRH antagonists show marked species selectivity, which is a hindrance in the actively ongoing drug discovery progress. Unlike surgical castration, the use of GnRH agonists plus antiandrogens, or the use of GnRH antagonists, or the treatment with nonpeptide, orally active GnRH antagonists is reversible. This is of great importance in the treatment of young adults. For example, in breast cancer patients who are estrogen and progesterone receptor positive, treatment with GnRH agonists (e.g., goserelin) was found to be as effective as surgical oophorectomy in premenopausal women.

Sex hormone ablation by GnRH-A is achieved during prolonged treatments. However, prostate cancer, which is initially hormone-dependent, may become eventually hormone-refractory, and is no longer sensitive to the GnRH-A-mediated reduction in androgen levels. Similarly, removal of sex hormones by GnRH-A is not relevant to hormone-insensitive breast and ovarian cancer (►Hormones). GnRH-A may also be useful in treating these types of tumors by direct binding to them. The actions of GnRH are mediated by the GnRH receptor (GnRHR), a type A ►G-protein coupled receptor (►GPCR), (►G-proteins) that is expressed in both the pituitary and extrapituitary sites, including normal tissues and tumors. Expression of GnRH and GnRHR, as well as direct growth regulatory effects of GnRH-A in sex hormone-dependent tumor cells, has been reported for cancers of the breast, ovary, and prostate. Interestingly, in these cells, GnRH-A reduce proliferation and induce ►apoptosis, which is mediated by the GnRHR via ►signal transduction mechanisms that are distinct from the classical pituitary pathways. The understanding that GnRH-A can be used not only to reduce androgen levels but also as a direct antiproliferative factor, as well as the binding of

GnRH-A to some cancer cells, may open a new vista into the design of novel GnRH-A for the treatment of hormone-refractory cancers.

The direct effects of GnRH-A may influence a large portion of sex hormone-dependent cancers. Some 85% of human prostate cancers, 80% of endometrial cancers, and 50% of breast cancers express GnRH and GnRHR (type I, the same as in pituitary gonadotropes). Obviously, hypothalamic GnRH does not reach the tumors. It is thought that GnRH is expressed in the tumors and might act in an autocrine/paracrine fashion by binding to local GnRHR. In these tumor cells, the activated GnRHR transmits its signal via Gαi and activation of the ►protein kinase C (PKC) and the ► Map kinase (MAPK) pathways, culminating in an antiproliferative and/or apoptotic effect. Hence, cancer cells are under the influence of opposing survival (e.g., ►EGF, ►IGF-1), versus apoptotic (e.g., GnRH) signaling pathways, which are elicited by hormones produced in the tumors and which are acting in an ►autocrine/►paracrine mechanism. As the cancer progresses, it is obvious that the survival arm is prevailing. It is the researcher role to find the means to increase the apoptotic arm. GnRH-A seem ideal for this task as they can act as "chemical castration" effectors in sex hormone-dependent tumors, or by inducing either direct or toxin-amplified antiproliferative and/or apoptotic actions in sex hormone-independent tumors.

One way that is currently used to employ the direct binding of GnRH-A to tumor cells is the GnRH-toxin therapy. As mentioned above, the expression of GnRHR in tumor cells provided the option for GnRH based targeted toxins. Conjugates of GnRH were prepared by adding various toxins such as: *Pseudomonas* exotoxin, diphtheria toxoid, glutaryl-2-(hydroxymethyl)-anthraquinone, and 2-pyrrolinodoxorubicin, and were shown to be effective in animal models and initial clinical trials in humans. This approach is based on the assumption that the number of GnRHRs in the targeted tumors will be higher than in normal tissues. The second assumption is that the GnRH-toxin conjugate will bind to GnRHRs, followed by internalization of the hormone-receptor complex and release of the hormone-toxin in the cell to exert its killing effect. The third assumption is that the GnRH-toxin conjugate will be provided once to destroy pituitary gonadotropes and cancer cells, a great advantage over the life-long therapy with GnRH-A. Obviously, pituitary gonadotropes have the highest receptor density and will be the prime target of the GnRH-toxin. The conjugate will therefore destroy the gonadotropes and lower gonadotropin (►LH and ►FSH) levels, resulting in "chemical castration" due to lowering of gonadal sex steroids (progesterone, estrogen, and testosterone) (Fig. 1). Thus, the GnRH-toxin conjugate will act at both the pituitary levels and also directly on the GnRHR-expressing cancer cell. This is of advantage during the hormone-sensitive state of the cancer (breast and prostate), while the direct effect of the conjugate will be beneficial during the hormone-resistant phase. The direct effect will be important also in ovarian cancer, since ovarian cancers are less dependent on gonadal steroids as compared to breast and prostate cancer. GnRH-toxin conjugates can be prepared by chemical synthesis or by recombinant fusion proteins (e.g., GnRH + *Pseudomonas* exotoxin). However, while natural GnRH is used for the fusion protein, GnRH-A with D-amino acid (e.g., Lysine) are used for chemical synthesis. Various studies have utilized GnRH-toxin conjugates in rat and ovine pituitary cells, as well as in human cancer cell lines. Once established in cells, the conjugates were tested successfully in various nude mice xenograft models, in dogs (to demonstrate the ability of the GnRH-conjugates to destroy pituitary gonadotropes), and initial clinical trials in humans with advanced prostate cancer have been initiated.

Studies in various laboratories are underway to evaluate the effects and signaling involved in the direct growth suppressive effects of GnRH-A in cancer cells. It seems that GnRH stimulates a "life" signal in pituitary gonadotropes and a "death" signal in cancer cells. Identification of the "death" signal, in particular for sex hormone-insensitive cancer cells, may enable an increase of the beneficial effect of GnRH-A in cancer patients by the combined treatment with GnRH-A and the "death" signal regulator. As second messengers mediate hormone action, the idea is to use GnRH-A and to "kill the messenger," particularly for advanced sex hormone-insensitive cancers.

In summary, GnRH-A can be multifaceted drugs in the cure of ►sex hormone-dependent tumors. GnRH-A can act as "►chemical castration" effectors, or by inducing either direct or toxin-amplified antiproliferative and/or apoptotic and killing actions in sex hormone-independent tumors. However, more studies are required in order to optimize and design future generations of GnRH agonists and antagonists for the treatment of hormone-independent tumors, for which there is no cure to date.

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Good Laboratory Practices

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Definition

GLPs; Are the legal requirements delineated by the FDA in 21 CFR Part 58 governing testing laboratories to ensure the quality of nonclinical laboratory studies related to safety of regulated health care products.

Characteristics

Principles

Following incidents of scientific fraud and laboratories not following their protocols in drug testing, the FDA issued Good laboratory Practices in December 1978 as 21 CFR Part 58 (Major Revision 1987) to govern nonclinical safety studies for food, drugs and medical devices. The US Environmental Protection Agency adopted two sets of GLPs in 1979 as part of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), which was codified as 40 CFR part 160, and as part of the Toxic Substances Control Act (TSCA), codified as 40 CFR 792. In 1980 the Organization for Economic Cooperation and Development (OECD, www.oecd.org) also issued a set of international GLPs (revised in 1997) applicable to non-clinical safety studies, pesticides and chemicals. Similar to the US, Japan has three sets of GLPs, covering drugs, industrial chemicals and pesticides. Fortunately, the basic principles of GLPs are similar and accurate documentation is the foundation of GLPs and other GXPs. As with GMPs, when working with a contract laboratory the ultimate responsibility for GLP compliance resides with the study sponsor.

The core of the FDA's GLPs are divided into the following major sections in 21 CFR Part 58 that individually address the general requirements for GLP operations:

Subpart A: General Provisions

Subpart B: Organization and Personnel

To be GLP compliant, a laboratory has to have a quality assurance unit, qualified and properly trained personnel and a study director must be appointed for each nonclinical study. Personnel should have adequate education, experience and training to carry out their function. Additionally, it is important that the management organizational structure be defined and personnel aware of their position and responsibilities within the organization. A study director must be appointed for each study and they have the responsibility for the conduct of the study and for the interpretation and review of the results. The Quality Assurance unit is charged with the interpretation and enforcement of GLPs and ensuring that studies are performed in compliance with standard operating protocols and GLPs. Internal audits carried out by Quality Assurance are a major part of a good quality system verifying compliance with GLPs.

Subpart C: Testing Facility

Facilities must be adequate in size and construction to be able to perform the studies. Special attention is paid to animal care facilities to ensure studies are not compromised by cross contamination, either from diseased animals, infestations or other poor animal handling.

Subpart D: Equipment Design

Laboratory studies generally rely on sophisticated equipment and this equipment should be suitably designed and used to perform its lab function. For example, weighing to an accuracy of ± 1 mg of a compound on a balance that is only accurate to 0.01 grams would not be allowed. Additionally, this equipment must be cleaned, maintained and calibrated at regular intervals and this maintenance must be documented.

Subpart E: Testing Facility Operation

As with GMPs, the main business mechanism for compliance with GLPs is through the drafting of well-written Standard Operating Protocols (SOPs) that define how processes are to be performed. This includes management processes, animal care and handling, handling of samples, standards and reagents, maintenance and calibration of equipment, performance of tests and analyses, and handling of data with an emphasis on ensuring the quality and integrity of data generated by a study.

Subpart F: Test and Control Article Characterization

Samples that are to be placed on a test and the control articles are to be characterized as to their identity, strength, purity, composition and method of fabrication as well as other considerations such as stability. Since GLP testing is often performed at contract laboratories, the contract laboratories generally include a statement in their report that these characterizations are the responsibility of the study sponsor.

Subpart G: Protocol and the Conduct of the Non-clinical Laboratory Study

Each study is required to have an approved study protocol or study plan that clearly indicates the purpose of the study and describes the methods used to conduct the study, including reference to the appropriate SOPs. The study needs to be performed in accordance with its study protocol, all data recorded promptly and according to good documentation practices. Any changes or deviations to an approved study protocol need to be documented, with reasons for the changes, and approved by the study director. If data is to be collected or maintained in an electronic format, then this should be done according to 21CFR Part 11, although compliance with Electronic Documents Part 11 is an area of ongoing revision.

Subpart H & I: (Reserved)

Subpart J: Records and Data Reporting

The accuracy and integrity of GLP study records are crucial for the validity of future work performed on the test article. A final report is prepared for each study and approved by the study director and the quality assurance unit. Protocols, data, specimens (if possible), and all records and reports for the study must be securely archived, in a fashion that insures future retrieval.

Subpart K: Disqualification of Testing Facilities

The FDA conducts routine periodic inspections of GLP laboratories (Surveillance Inspections) and "for cause" audits if there is a perceived problem (Directed Inspections). If non-compliance with GLPs is found, then the FDA has the right to disqualify laboratories.

21CFR Part 58 is a relatively short document and should be read in its entirety. The most recent version is available at http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr58_07.html. Access to this and other related documents is easily obtained through the Internet as most regulatory agencies post these documents at their web site, so readers are encouraged to refer to the original documents. The FDA web site (www.FDA.gov) also provides access to regulations, guidance papers and warning letters. Guidelines for FDA Office of Regulatory Affairs inspectors are also available (www.fda.gov/ora/compliance). Other documents are available from OECD (www.oecd.org) and the European Agency for the Evaluation of Medicinal Products (EMEA, www.emea.europa.eu) web sites.

Basic good practices are the foundation of both GLPs and GMPs. Staff should be qualified and well trained. Only established, approved procedures should be used and all activities should be recorded. Records and samples should be archived and available. As with GMPs, the future will see increased automation and use of computers in analytical techniques and the use of quality management systems to ensure improvements in quality and compliance with GLPs.

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Good Manufacturing Practices

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Definition

GMPs; Are the legal requirements governing manufacturers of regulated health care products. For drug products, GMPs are the regulations defining the documentation, procedures and process controls that must be followed so the manufacturers can assure that the product produced has the required safety, identity, strength (or potency), purity and quality. Pharmaceutical Quality Systems will play an increasingly important role in the future of GMPs.

Characteristics

The purpose of GMPs is to protect patients by insuring that drug products are safe and effective. In most countries, GMPs are legally proscribed with legislation delegating the interpretations and enforcement to an administrative "inspecting authority" within the government. In the United Kingdom this agency is the Medicine Control Agency (MCA), similar in function to the FDA in the United States (US). In the US the main statute underlying GMPs is the Federal Food, Drug and Cosmetic Act (FD&C Act of 1938). USFDA, International Conference on Harmonisation

(ICH, www.ich.org), World Health Organization (WHO, www.who.int), European Agency for the Evaluation of Medicinal Products (EMEA, www.emea.europa.eu) and Pharmaceutical Inspection Cooperation Scheme (PIC/S, www.picscheme.org) all have versions of GMPs, generally with similar approaches. Although there is not yet an agreed on worldwide version of GMPs, the FDA and other agencies are pursuing international harmonization of GMPs through ICH and PIC/S.

Under the FD&C Act, the Federal Food and Drug Administration (FDA) has promulgated the Current Good Manufacturing Practices (CGMP) regulations as found in 21 CFR 210-226 of the Code of Federal Regulations. The FDA emphasizes the "current" aspect of GMPs and so the regulations are often structured as broad expectations, lacking in specific details. This is done to encourage manufacturers to continually update and improve practices and methods as better systems are developed to control the manufacturing process.

The regulations for pharmaceuticals are covered in 21 CFR Parts 210 and 211, with additional regulations for biological products in Parts 600 and 610. The GMPs as found in 21 CFR 211 for finished pharmaceuticals are divided into the following major sections that individually address the general requirements for GMP operations:

- Subpart A: General Provisions
- Subpart B: Organization and personnel
- Subpart C: Buildings and facilities
- Subpart D: Equipment
- Subpart E: Control of components and containers
- Subpart F: Production and process control
- Subpart G: Packaging and labeling
- Subpart H: Holding and distribution
- Subpart I: Laboratory controls
- Subpart J: Records and reports
- Subpart K: Returned and salvaged drug products

Failure to follow GMPs will be interpreted by the FDA as equivalent to adulteration of the product, as improperly made drugs have killed people.

Quality Assurance

It is the manufacturer's responsibility to turn the quality principles of GMPs into business practices. In most companies the "Quality Assurance" organization is charged with the interpretation and enforcement of GMPs. A major part of a good quality system is the use of internal audits carried out by Quality Assurance, which is recorded, with a follow-up CAPA (Corrective And Preventative Actions) system to make sure deficiencies are noted and fixed. There are three main concepts to any quality CAPA system: (i) Remedial action to correct the problem, (ii) a root cause analysis to determine the cause(s) of the problem, and (iii) based on the analysis, including a preventative action to avoid recurrence of the problem. The FDA does not have access to internal audit files but will want evidence that they are taking place. Audits by Quality Assurance and contract auditors are also good training of personnel for FDA inspections.

Documentation and SOPs

In the GMPs "written procedures shall be established and followed for…" appears at least 100 times. This means that a documentation control system is a standard GMP expectation. The main business mechanism for compliance with GMPs is through the drafting of well-written Standard Operating Protocols (SOPs) that define how processes are to be performed. Approximately half of all FDA warning letters are for GMP violations and the most common GMP violations are for the absence of a critical SOP or that SOPs were not being followed. SOPs are equivalent to commitments by the company and the expectation is that personnel will follow their instructions. By following well-written SOPs, workers are adhering to GMPs as interpreted by their company. Change control is another fundamental of cGMPs that requires thorough evaluation (CAPA) and documentation to assure that any process or equipment changes do not have negative unintended consequences. If records or data are to be collected or maintained in an electronic format, then this should be done in accordance with 21CFR Part 11, although compliance with Electronic Documents Part 11 is an area of ongoing revision.

FDA Quality Initiatives

In the last few decades there has been an increase in the number of drugs manufactured using biological processes, with inherently more process variability. This gradually led to a realization that the basis of GMPs suitable for the production and purification of small molecules may not be optimum for the governing of processes for biologicals. Along with this has been a drive for cost-effective ways to improve quality in every phase of a pharmaceutical product. Quality systems stemming from the management studies of Juran and Deming have been used in other manufacturing sectors for process improvements for several years. The FDA recognized the value of quality systems in the Quality System Regulations for Medical Devices (21 CFR Part 820, 1996). The FDA Modernization Act of 1997 further stimulated a review in the thinking of cost-effective ways for quality improvement, followed by changes in policies governing GMPs. This led to a number of initiatives and guidance documents to guide drug manufacturers into a modern more flexible pharmaceutical quality systems approach to GMPs embracing Quality by Design (QbD), risk assessment and risk management. Once it is understood what is

critical to quality in a process and its acceptable limits or design space, then quality can be designed into the manufacturing process. Continual improvement to process (via CAPA) and product understanding can lead to continual process improvement. The major themes running through these guidances are process understanding, understanding risk, and continual improvement. All the details of these documents cannot be abstracted into a concise review, so readers are encouraged to refer to the original documents. All these initiatives, guidance papers and also warning letters are available at the FDA web site (www.FDA.gov).

Pharmaceutical Quality for the Twenty-First Century

In 2002, the FDA announced a significant 2-year initiative entitled "Pharmaceutical cGMPs for the twenty-first century – A Risk-Based Approach" (final report issued in 2004) to modernize the FDA systems for reviews and inspections of manufacturing practices for drugs and vaccines. Risk-based and science-based approach to quality with an emphasis on using quality systems and risk management approaches. In an era of increased numbers of pharmaceuticals and decreasing resources, the FDA went through a reappraisal of how they review product quality regulations to change to a focus on manufacturers' quality systems. This document encourages innovation by manufacturers such as applying the concepts of risk management and quality systems approaches to the manufacture of pharmaceuticals. This requires a thorough understanding of the manufacturing process to evaluate the procedure variables to determine which critical variables are of highest risk to product quality and allow manufacturers to selectively spend more effort controlling critical parameters, with the goal of continually improving the process. These ideas have been further defined in ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality Systems (currently at Step 2).

Process Analytical Technology (PAT)

The US FDA began encouraging use of automated process control technology with the issue of its 2004 "*Guidance for Industry, PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*" document (http://www.fda.gov/cder/guidance/6419fnl.pdf). Until the PAT guidance was issued, product quality was monitored by testing of process intermediates and final products and the optimum strategy for pharmaceutical manufacturing was to duplicate the steps performed in a process as closely as possible to eliminate variables. PAT calls for a change from end-stage testing and monitoring of a process to the use of analytical techniques for continuous monitoring. With PAT technology, the focus is on both the process and the product. Instead of unchangeable processes, the process variables can be monitored, and controlled, in real-time to define the quality of the product. With the understanding that in biological systems there is always a degree of variability in the process, the idea behind PAT is to promote automated process control to monitor the important process variables in real-time and make adjustments to keep critical process variables at their optimum level as the process proceeds, thus allowing process changes that improve final product quality while reducing overall manufacturing costs. This idea of designing quality into the process has come to be known by the term Quality by Design or QbD. Principles of PAT are being implemented internationally (see EMEA web site http://www.emea.europa.eu/Inspections/PAThome.html) and included in later initiatives and guidances such as ICH Q8: Pharmaceutical Development.

ICH Q8 Pharmaceutical Development

http://www.fda.gov/cder/guidance/6746fnl.pdf Adopted by the FDA May 2006 under Revision, Jan 2008, …/8084dft.pdf

During pharmaceutical development the limits of the process parameters that produce a quality product are defined to establish the "*design space*." Once the manufacturer's design space has been accepted by the FDA, working within the design space would not be considered a process change. Movement of the process outside the design space would be considered a process change and would require regulatory approval as a post-approval change.

Essentially pharmaceutical development would perform extensive robustness experiments on the process variables using design of experiments (DoE) to determine the limits to the variables that produce product of consistent quality. Once the acceptable range for the critical operating parameters is known, then process controls within this design space would not require regulatory review or approval. The idea of design space complements PAT, as it allows use of process technology to monitor and control the process parameters within the design space variables.

ICH Q9 Quality Risk Management

http://www.fda.gov/cder/guidance/7153fnl.pdf Adopted by the FDA June 2006

Q9 provides the Quality Risk Management tools [such as Failure Modes and Effects Analysis (FMEA), hazard analysis critical control point (HACCP)] that can be applied across the product lifecycle. Risk management means taking a systematic approach to assess, control and review possible risks to the quality of a product. This process works with quality by design methods to remove common process errors that could affect product quality.

Quality System Approach to Pharmaceutical Good Manufacturing Practices

This FDA Guidance for Industry, issued in 2006, is one of the major outcomes of the Pharmaceutical Quality for the twenty-first century initiatives. The guidance document recommends that manufacturers meet CGMP requirements by using a comprehensive quality approach based on continuous improvement and risk management.

ICH Q10 Pharmaceutical Quality Systems

http://www.fda.gov/cder/guidance/7891dft.pdf Currently in Draft (ICH Step 2). Similar to the Quality System Approach Guidance, Q10 provides guidance on the quality system framework to continually improve process understanding and for process improvement. The ideal Pharmaceutical Quality System would include knowledge management and quality risk management, process performance monitoring (PAT), CAPA, change management and management review. The FDA has applied a quality systems approach to the way that inspections are done. Their Systems-Based Inspections are now focused on the following six manufacturing systems: Quality System, Production System, Facilities and Equipment System, Laboratory Controls System, Materials System, and Packaging and Labeling System.

To summarize, regulatory agencies are taking a new approach to GMPs as exemplified in ICH Q8, Q9, and Q10, and are shifting their focus to expecting compliance by demonstration that that manufacturers have quality systems in place to prevent and resolve issues of quality, safety and efficacy.

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Gordon-Kosswig Melanoma System

►Xiphophorus

gp170

►P-glycoprotein

Gorlin Syndrome

Definition

Synomously used term for ►basal cell nevus syndrome (BCNS) also called ►naevoid basal cell carcinoma syndrome (NBCCS). Named after the first person to describe typical patients, Robert J. Gorlin DDS.

►Medulloblastoma

Government Drug Regulatory Agencies

Definition

These are government departments that govern the marketing licence applications and monitor the adverse effects of marketed drugs. In the United States, this agency is the Food and Drug Administration (FDA) and the Ministry of Health and Welfare (MHW) in Japan. In the European Union, each country has its own agency but there is also an agency in the European Union, the European Medicines Agency (EMEA) based in London. Often these government agencies may be responsible for monitoring of Good Laboratory Practice (GLP) regulations in their respective territories.

►Preclinical Testing

gp36

►Podoplanin

gp90^{Hermes}

►CD44

GPCR

Definition
The ►G-protein coupled receptor family is the largest group (over 800) of membrane receptors. GPCRs span the membrane seven times and are also known as seven transmembrane receptors (7TM). They are involved in numerous physiological and pathological functions and are currently the target for over 60% of existing drugs and the major group for future drug design.

►Gonadotropin-Releasing Hormone
►G-Proteins

G6PD

Definition
Glucose-6-Phosphate Dehydrogenase.

►Lead Exposure

gp-Fy

►Duffy Antigen Receptor for Chemokines

GPI PLD

Definition
A glycosyl-Phosphatidyl-inositol-specific phospholipase D (GPI-PLD) specifically cleaves GPI anchor to generate phosphatidic acid and the soluble protein. GPI-PLD physiological role is completely unknown. Up-regulation in GPI-PLD expression has been observed in immortalized and malignant tumor cell lines and appears to correlate with malignancy and tumor progression.

►Mesothelin

GPI-Anchored Protein

Definition
A GPI (glycosyl-phosphatidyl-inositol) anchored protein is a protein that was post-translationally modified at its C-terminus by a glycolipid called GPI anchor. GPI anchors are hydrophobic and serve to attach membrane proteins to the lipid bilayer of cell membranes.

►Mesothelin

GPI-Linked

Definition
Refers to a type of membrane association whereby proteins are anchored to the cell surface through glycosyl-phosphatidylinositols attached to their carboxy terminus.

►Semaphorin

Gp38k

►Serum Biomarkers

Grade IV Astrocytoma

►Glioblastoma Multiforme

Grading of Tumors

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Synonyms
Tumor grading; Histologic grading; Assessment of the degree of tumor differentiation; Assessment of anaplasia of a tumor

Definition

Grading is a system of histopathologic tumor analysis in which a numerical or semiquantitative descriptive value ("*grade*," or *gradus* in Latin) is assigned to a neoplasm, taking into account its histologic level of ►differentiation and/or resemblance to the tissue of its origin, as well as the extent of ►anaplasia.

Characteristics

Basic Principles

More than one hundred years ago it was noticed by several pathologists that some tumors resemble histologically their tissue of origin, whereas others have lost most of such tissue specific traits and have become anaplastic or undifferentiated. It was suggested by von Hansemann in the 1890ies that one could predict the prognosis of many tumors by examining them microscopically and by taking into account their microscopic properties. This basic concept was refined subsequently by numerous pathologists worldwide until the current grading systems were developed for most malignant tumors.

The tumor grade is formulated by the pathologist who compares the microscopic features of the tumor with those of the tissue from which it has arisen. On the basis of such subjective evaluation, or by following specific systems based on points assigned to each morphologic variable, the pathologist may classify a tumor as well differentiated, moderately differentiated, or poorly differentiated. *Well-differentiated tumors* resemble to a great extent the tissue of their origin. *Moderately differentiated tumors* have retained some of the features of the tissue of their origin, but have also acquired some anaplasia, or differ significantly in some respects from the tissue of their origin. *Poorly differentiated tumors* are anaplastic neoplasms that have retained only some vague resemblance to their tissue of origin. Alternatively, the pathologist may assign to the tumor under study a numerical grade. For example, in the above three tiered system grade 1 would correspond to well differentiated, grade 2 to moderately differentiated, and grade 3 to poorly differentiated tumors. Other more complex systems have been developed for specific tumors.

Methods of Grading

Tumor grading is performed by pathologists analyzing various characteristics of tumors under the light microscope. The most common characteristics evaluated are cytologic features and architectural features.

Cytologic features include size and shape of the nucleus, the ratio of the volume of the nucleus to the volume of the cytoplasm, and the relative number of dividing cells called the mitotic index. *Architectural features* include the histologic organization of the tumor (e.g., percentage of glands on one side and solid areas on the other, as a part of the entire tumor in an adenocarcinoma), and the boundaries of the tumor. A well-differentiated tumor typically has small and regular-shaped nuclei, small nuclear volume relative to the rest of the cellular volume, a relatively low number of dividing cells, shows orderly organization with well formed glands, and has well defined tumor boundaries. A poorly differentiated tumor has large and pleomorphic nuclei (i.e., irregularly shaped nuclei of variable size and shape), large nuclear volume compared to the rest of the cellular volume (i.e., high nucleus: cytoplasm (N/C) ratio), a high number of dividing cells, disorganized tumor architecture including broad solid areas, and poorly defined tumor boundaries.

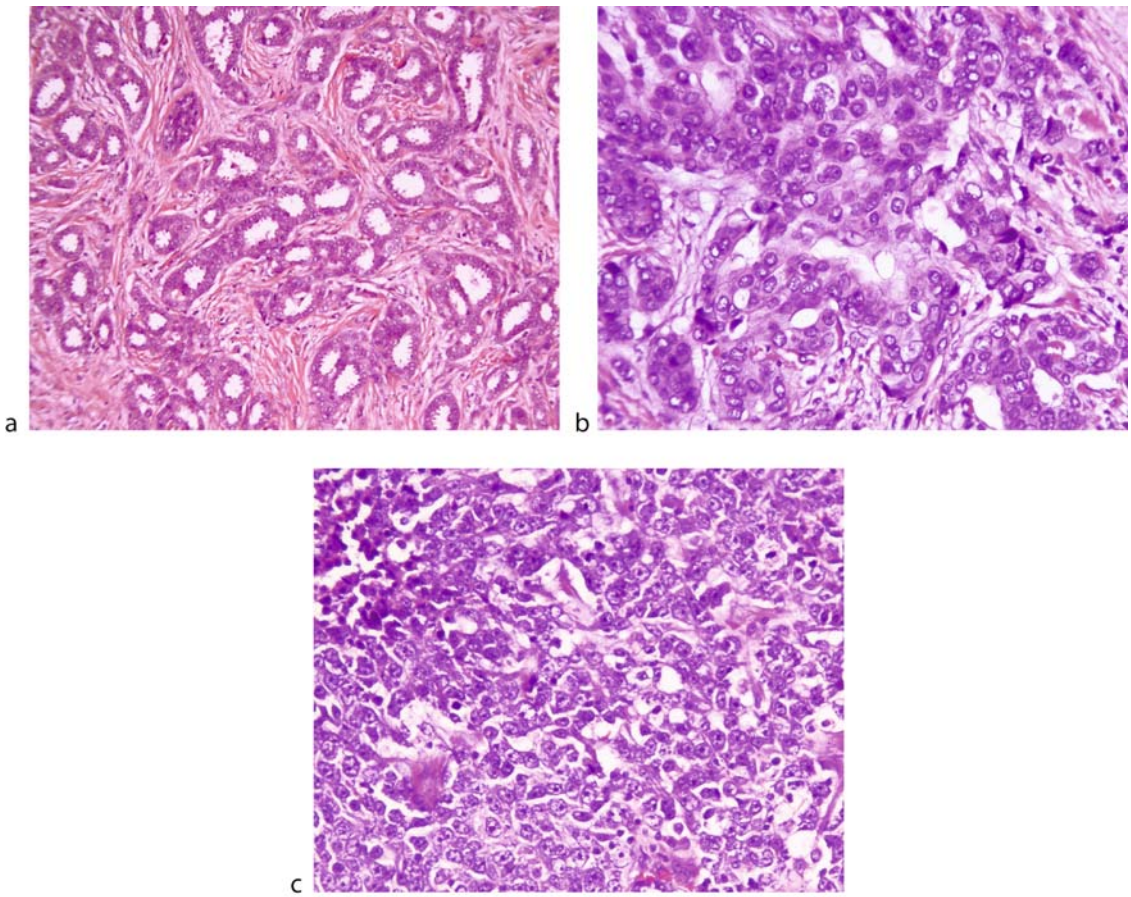
Depending on the number of malignant characteristics present, the American Joint Commission on Cancer (AJCC) has recommended that most malignant tumors be given a grade using a scale from well differentiated on one end to undifferentiated on the other. The most widely used scale is the one from GX through G4, with equivalent descriptors as follows:

- Grade X (GX) – Grade cannot be assessed
- Grade 1 (G1) – Well differentiated (Low-grade and less aggressive)
- Grade 2 (G2) – Moderately differentiated (Intermediate-grade and moderately aggressive)
- Grade 3 (G3) – Poorly differentiated (High-grade and moderately aggressive)
- Grade 4 (G4) – Undifferentiated (High-grade and aggressive)

Similar systems have been recommended by the experts of the World Health Organization (WHO) for most tumors in the human body. Alternatively, Roman numerals I through IV may be used, as is common in the WHO classification of brain tumors. For some tumors grade 3 and grade 4 are combined, and thus a three tiered system may be more practical.

Special grading systems are used for several types of cancer, most notably ►prostate cancer and ►breast cancer. Adenocarcinoma of the prostate is graded by most pathologists using the ►*Gleason grading system*. Gleason score, ranging from 2 to 10, is obtained by separately grading the most prevalent ("primary") and the next most common ("secondary") pattern and then adding the two values. *Scarff-Bloom-Richardson system* is the most widely used semiquantitative system for grading breast carcinomas. It is based on giving points on a scale from 1 to 3 to each of the following features: nuclear grade, tubular/glandular differentiation, and mitotic count (Fig. 1a–c).

To achieve consistency and uniformity, and to assure that the tumors are properly registered for epidemiologic purposes, the pathologists grade tumors by following



Grading of Tumors. Figure 1 Invasive breast carcinoma. (a) Well-differentiated carcinoma (grade 1) composed of well formed glands lined with cells that have uniform small nuclei. (b) Moderately differentiated carcinoma (grade 2) composed of glands and some solid sheets. Tumor cell nuclei are slightly enlarged and show mild pleomorphism. (c) Poorly differentiated carcinoma (grade 3) composed predominantly of solid cords and sheets. Tumor cell nuclei are enlarged and show pleomorphism.

the guidelines and criteria recommended by WHO or JCCC. Such tumor grades are entered into the data base of tumor registries. The pathologists should specify which grading system they have used to avoid confusion and achieve greater comparability of data from one medical center or country to another. Certain forms of preinvasive carcinoma in situ and closely related epithelial dysplasias, such a cervical intraepithelial neoplasia, are also routinely graded.

Grading is of limited use for tumors of endocrine glands such as the parathyroids, adrenals or ►islets of Langerhans. For some tumors, such as ►melanoma, ►seminoma, embryonal carcinoma or ►retinoblastoma, there are no useful grading systems and accordingly, these tumors are not graded histologically. Some tumors are by definition high grade and are automatically given the designation G4, which is typically used for cancer registry purposes. This group of tumors includes small cell carcinomas of the lung or any other site, large cell carcinoma of the lung, and several sarcomas such as ►Ewing sarcoma, ►rhabdomyosarcoma and malignant fibrous histiocytoma. Tumors that have been treated are not graded.

Future Perspectives

Currently, tumor grading is based on a subjective evaluation of routine histologic sections by a trained pathologist. In some instances, as is the case with carcinomas of the breast or prostate, semiquantitative methods have been devised. Still, all these approaches are subjective and there is considerable intra- and interobserver variation. It is hoped that this variation will be reduced in the future, by a more extensive use of immunohistochemical, molecular biologic methods, and the application of image analysis and computer technology.

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Graft Acceptance and Rejection

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Definition

Refers to the fate of cells, tissues or organs transplanted between different individuals or from one individual to oneself.

Characteristics

The fate of grafts, whether they are accepted or rejected, is a subject of relevance for cancer. Grafts of various types such as bone marrow, stem cell or organ grafts may be carried out for the treatment of cancer and because the rules governing the outcome of grafts explains in part the survival and surveillance of tumors.

Graft acceptance and rejection depends on two factors. One factor reflects the extent to which the source of the graft and the recipient differ genetically. The second factor reflects whether the graft consists of isolated cells or tissue or an intact organ.

The genetic relationship between the donor and the recipient determines whether or not a graft is accepted. Grafts from one individual to themselves are referred to as ►autografts. Autografts are always accepted if they are placed in the correct location. Grafts between genetically identical individuals are called ►isografts. Isografts are usually accepted unless the donor has acquired antigen through infection, chemical modification or mutation. Grafts between different individuals of the same species are referred to as ►allografts. Allografts are almost always rejected unless the immune system of the recipient is defective or the donor and recipient are highly inbred and closely related. Grafts between individuals of different species are called ►xenografts. Xenografts are always rejected unless the recipient of the graft is immuno-incompetent.

Graft Acceptance and Rejection. Table 1

Graft type	Source of vasculature	Source of growth factors
Cells	Recipient	Recipient
Tissues	Recipient and donor	Recipient and donor
Organs	Donor	Donor

Xenografts may also fail if growth factors of the recipient do not support engraftment.

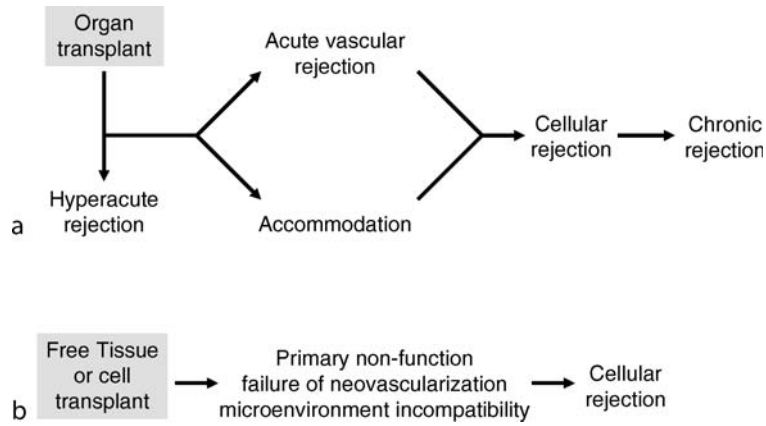
The susceptibility of a graft to rejection and the type of rejection that occurs depend on how the graft receives its supply of blood. Transplanted cells, such as bone marrow cells or hepatocytes, derive their vascular supply, nutrition and growth factors mainly by the in-growth of blood vessels of the graft recipient (Table 1). Transplanted tissues, such as tumor fragments, pancreatic islets and skin, are in part by in-growth of blood vessels of the recipient and in part by spontaneous joining of blood vessels of the recipient with existing blood vessels in the tissue. Transplanted organs are fed entirely by blood vessels formed by the joining of graft and recipient blood vessels.

The ability of cell and tissue grafts to undergo ►neovascularization and to be supported by growth factors of the recipient determines graft acceptance over a period of days to weeks. The acceptance of grafts also depends on the location and the biological compatibility between the donor and the recipient. For example, bone marrow cells may survive transplantation into some locations, such as in bone marrow or in the blood stream, but not others, such as into muscle. As another example, bone marrow cells may fail to engraft across species because the growth factors of the recipient are incompatible with the graft.

The biological responses to transplantation are summarized in Fig. 1. Cell and tissue grafts are subject to ►graft primary non-function or failure of engraftment, owing to the resistance mounted by ►natural killer cells, ►macrophages or other leukocytes. If this hurdle is bypassed, cell and tissue grafts are subject to cellular rejection mediated by ►T lymphocytes. Organ transplants may be subject to immediate or ►graft hyperacute rejection caused by antibodies specific for the transplant donor that bind immediately upon transplantation and activate the complement system. Antibodies forming after transplantation of organ grafts may cause ►graft acute vascular rejection or ►graft chronic rejection. Organ grafts between different individuals are, like cell and tissue grafts, subject to ►graft acute cellular rejection.

Cellular and Molecular Mechanisms

Differences between the ►major histocompatibility antigens of a graft donor and graft recipient are the most



Graft Acceptance and Rejection. Figure 1 The immunological response to transplantation. The immune response to transplantation can be classified according to whether the graft consists of isolated cells or free tissues, such as islets of Langerhans or of a primarily vascularized organ such as the kidney or heart. (a) Vascularized organ grafts are subject to hyperacute and acute vascular rejection caused by the action of antidonor antibodies on donor endothelium. If hyperacute or acute vascular rejection are averted, the graft may undergo accommodation, a condition in which the graft appears to resist injury despite the return of anti-donor antibodies to the circulation and the presence of an intact complement system. A vascularized organ graft may also be subject to cellular rejection and chronic rejection more or less like the corresponding types of rejection observed in allografts. (b) Free tissue grafts are subject to failure caused by primary non-function, failure of neovascularization or failure of the microenvironment to support the survival and function of the foreign tissue. If the free tissue or isolated cells engraft, they are then subject to cellular or humoral rejection.

important determinant of acceptance or rejection ►(MHC). Major histocompatibility antigens are highly polymorphic; thus, all individuals other than identical twins in an outbred population are likely to differ in major histocompatibility antigens, and these differences trigger potent immune responses. Major histocompatibility antigens can be recognized directly by T lymphocytes; thus, intact foreign cells can provide a strong stimulus. Recognition of major histocompatibility antigens by T lymphocytes provokes a severe and very rapid immune response, which accounts for the inevitable rejection of allografts if the recipient does not receive immunosuppressive therapy (T-cell response). Major histocompatibility antigen may also stimulate the production of antibodies. Alloantibodies, as such, can cause vascular rejection of organ grafts and can be used in tissue typing (Fig. 1), but antibodies have little impact on most cell and tissue grafts unless they are placed in the circulation.

Antigens that provoke slower and less severe responses are referred to as ►minor histocompatibility antigens. Any protein or carbohydrate that distinguishes two individuals can potentially serve as a minor histocompatibility antigen. Minor histocompatibility antigens are generally processed by antigen-presenting cells and presented to a very small fraction of T cells, thus accounting for the slower immune response.

Bone marrow transplants engender several distinct aspects of graft acceptance and rejection. Engraftment of bone marrow depends to some extent on the number of hematopoietic stem cells transferred, more stem cells hastening and increasing the frequency of engraftment. When the bone marrow cells are from a non-autologous (that is allogeneic) source, the cells can be rejected by T cells of the recipient. Natural killer cells are particularly important in resisting foreign bone marrow (►Natural killer cells). Natural killer cells are lymphocytes that can act against a variety of foreign cells that lack self-MHC molecules or are targeted by antibodies.

A special type of immune response known as ►graft versus host disease can occur after transfer of mature T cells from one individual to another, as in bone marrow transplantation. The mature T cells attack the recipient of the transplant, damaging skin, intestine, liver and other organs affected by graft versus host disease. Graft versus host disease may be directed against major or minor histocompatibility antigens.

One basis for graft acceptance may be immunological ►graft tolerance. Tolerance refers to the specific non-responsiveness of the immune system when an individual is confronted with a graft or an antigen that is generally considered capable of triggering immunity. Tolerance to self-antigens is acquired by developing T cells in the thymus and developing B cells in the bone marrow and by maturity in B cells through various mechanisms and in various locations. Tolerance to foreign cells and antigens may be induced by various means; however, the induction of tolerance often requires severe treatments that lower the ability to respond broadly to foreign antigens.

Clinical Relevance

Cell, tissue and organ grafts are often carried out as to address the consequences of organ and tissue failure brought about by chemotherapy, cancer, or the surgical removal of tumors. Bone marrow or hematopoietic stem cell transplantation is frequently undertaken to enable chemotherapy. Organ transplantation may be undertaken if the removal of the primary tumor or the complications of chemotherapy dictate; however, organ transplantation is avoided as much as possible because the immunosuppression needed to retain an organ allograft may hasten the recurrence of a tumor.

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Graft Acute Cellular Rejection

Definition

Severe damage or destruction of engrafted organs, cells and tissues, usually within days to months after transplantation, caused by T-cell-mediated immune responses.

►Graft Acceptance and Rejection

Graft Acute Vascular Rejection

Definition

Severe damage or destruction of engrafted organs, usually from days to several months after transplantation, probably caused by several humoral factors including antibodies and complement, which may result in unwanted activation of donor endothelium.

►Graft Acceptance and Rejection

Graft Chronic Rejection

Definition

Severe damage and ►inflammation to engrafted organs, usually from several months to several years after transplantation, caused by humoral and cellular responses to donor endothelium.

►Graft Acceptance and Rejection

Graft Hyperacute Rejection

Definition

The destruction of engrafted organs, usually within 24 h of transplantation, caused by preformed antibodies and activation of complement on donor cells.

►Graft Acceptance and Rejection

Graft Primary Non-Function

Definition

Failure of cells and tissues to engraft due to harmful interactions with recipient ►leukocytes.

►Graft Acceptance and Rejection

Graft Tolerance

Definition

A specific non-responsiveness of the recipient's immune system to graft antigens that would normally elicit a rejection reaction.

►Graft Acceptance and Rejection

Graft-Versus-Host Disease

Definition

GVHD; is a life-threatening complication of allogeneic hematopoietic stem-cell transplantation in which donor T cells attack the tissues of the transplant recipient after perceiving host tissues as antigenically foreign. GVHD is mainly directed against epithelial tissues of the skin, liver, and gastrointestinal tract.

►Allogeneic Cell Therapy
►Graft Acceptance and Rejection

Graft-Versus-Leukemia/Tumor

Definition

(GvL/GvT) effects, in adoptive immunotherapy, involve the recognition and elimination of the recipient's residual leukemia/tumor cells by donor lymphocytes derived from hematopoietic cell transplantation. The targets of GvL/GvT responses are the major and minor histocompatibility antigens, which differ between donor and patient.

►Immunotherapy

Granulocyte Elastase

►Neutrophil Elastase

Granulocyte-Colony Stimulating Factor

Definition

G-CSF, is a cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and hematopoietic stem cells. In ►hematopoietic stem-cell transplantation, G-CSF is used to mobilize hematopoietic stem cells into the peripheral circulation where they can be harvested by ►apheresis.

►Allogeneic Cell Therapy
►Cytokines
►Macrophages

Granulocytes

Definition

White blood cells filled with granules containing potent chemicals that allow the cells to digest microorganisms, or to produce ►inflammation reactions. ►Neutrophils, ►eosinophils, and ►basophils are examples of granulocytes.

Granulocytopenia

►Neutropenia

Granulosa Cell Tumors

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Definition

Granulosa cell tumors (GCTs) are rare ovarian tumors derived from the somatic cells that immediately surround the developing ►oocyte. Initial prognosis is favorable, yet a propensity for late relapse requires prolonged observation. GCTs represent only 2–5% of all ovarian tumors, yet are the most common type of malignant sex cord-stromal cell tumor. This latter, broad classification of heterogeneous tumors includes epithelial cells derived from the ►ovarian stroma, the ►rete ovarii and developing ovarian follicles.

Characteristics

GCTs typically represent slow growing neoplasms, with 5–25% of these tumors exhibiting malignant

potential. In particular, GCTs can be differentiated from cancers originating from the ►ovarian surface epithelium (OSE) in that the former typically exhibit enhanced steroidogenic potential associated with gonadotropin-responsiveness, and thus are considered an endocrine tumor. GCTs frequently produce elevated levels of estrogen that initiates symptoms typical of estrogen excess, such as ►metrorrhagia and/or endometrial ►hyperplasia. Patients with GCTs also experience an increased risk of developing ►breast cancer or ►uterine cancer. Occasionally, GCTs may produce ►androgen that promote ►virilization or ►hirsutism. Although there are no known risk factors (including genetic) for developing GCTs, there may exist an association with infertility and treatment for infertility.

In addition to monitoring steroid (estradiol or testosterone) production, elevated serum levels of ►inhibin (inhibin A and B), and ►Mullerian Inhibiting Hormone (MIH) can serve as useful diagnostic indicators of this disease. The predominant factor associated with overall survival is detection at ►FIGO stage I. More advanced stages (FIGO stages II-IV) have a poor prognosis, with a five-year survival rate similar to ►OSE cancer. Significantly, relapses are common after a mean of 6 years from initial diagnosis, and 5% of relapses will occur following 20–30 years without symptoms. Rupture of the initial tumor is a primary factor related to propensity for relapse.

From histological and etiological perspectives, GCTs include distinct juvenile and adult types. The juvenile type typically occurs during premenstrual ages, often before age 10 and almost always before age 30. Prepubertal patients present with abdominal pain and swelling together with precocious pseudo puberty, while after puberty symptoms of juvenile GCTs include menstrual irregularities or ►amenorrhea. Relapses from the juvenile type are almost always observed within 3 years of the original diagnosis. By comparison, adult-type GCTs occur predominantly during or subsequent to ►menopause (median age of diagnosis is 50–55 years), and represent the major incidence (95%) of GCTs. Even more rarely, testicular GCTs of both juvenile and adult type have been documented in XY genotypic males.

Clinical Aspects

The low incidence and consequent paucity of information pertaining specifically to the genetics and cellular biology of GCTs has limited the development of optimal and targeted treatment protocols, particularly compared to cancers of OSE origin. A unilateral presence occurs in greater than 95% of adult and juvenile type GCTs. Accordingly, the standard treatment following early detection is ►surgical debulking. There is currently little evidence to support more effective outcomes combining surgery with adjuvant radiation or hormonal therapies. ►Platinum-based ►chemotherapy is commonly utilized for relapsing or metastatic GCTs, with some of the highest reported response rates obtained with ►cisplatin, ►vinblastine and ►bleomycin (PVB) therapy. While the positive response to platinum-based therapies is estimated at 63–80%, the well recognized persistence of relapse is indicative of the need for more efficacious adjuvant therapies.

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Granzymes

Definition

Granzymes are a family of serine ►proteases, that are stored in the granules of ►cytotoxic T lymphocytes and ►natural killer cells (NK cells). They are components of the immune system that protect higher organisms against viral infection and cellular transformation. Following receptor-mediated conjugate formation between a granzyme-containing cell and an infected or transformed target cell, granzymes enter the target cell via ►endocytosis and induce ►apoptosis. Granzyme B is the most powerful pro-apoptotic member of the granzyme family.

Grape Seed Extract

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Definition

Is a ►nutraceutical rich in ►grape seed proanthocyanidins. It is widely consumed for its beneficial health effects attributed to its high proanthocyanidin(s)

content. Extract is prepared from the seeds of the grapes (*Vitis vinifera*), and seeds are usually procured from wine or juice industry where they are generated as major by-product and/or waste product. Standardized preparations are sold throughout the world and are usually marketed as health supplement.

Characteristics

►Grape seed extract is a complex mixture of ►polyphenols (includes phenolic acids, colored ►anthocyanins, ►flavonoids, and complex flavonoids). It is particularly rich in proanthocyanidins. Chemically, proanthocyanidins in turn encompass two major chemical constituents: procyanidins and procyanidin gallates. Procyanidins are basically oligomeric or polymeric flavonols, consisting of (+)-catechin and (−)-epicatechin, whereas, procyanidin gallates are mainly (−)-epicatechin acetylated with gallic acid. The degree of polymerization (process in which monomers link together to form long chains or network) in proanthocyanidins in grape seed extract falls in the range of 2–15.

Biological Activity

The chemical constituents such as phenolic acids, flavonoids, and proanthocyanidins present in grape seed extract exhibit antiradical (scavenge free radicals), antioxygen (scavenge oxygen free radicals), antilipid peroxidation (halt the process which makes fatty acids/lipids go rancid), as well as metal chelating (bind metal ions) properties and are largely responsible for strong antioxidant capacity of this extract. It has been found that grape seed extract is even more potent scavenger of oxygen-free radicals as compared to known antioxidants, such as ►vitamin C and ►vitamin E succinate.

Health Beneficial Effects

Grape seed extract possesses health beneficial effects, which in part are due to its high antioxidant capacity especially in those diseases where ►reactive oxygen species (ROS) are involved in the pathogenesis of the disease. Even though, grape seed extract is widely consumed as health supplement throughout the world, its health beneficial effects have been proven scientifically and conclusively only in animal models of various human diseases. Such studies have not only unraveled the beneficial effects, but also provided insight into the mechanism of action of grape seed extract in particular disease scenario as listed later.

Cardiovascular Diseases

Grape seed extract has been shown to prevent the development of aortic atherosclerosis in animal model by scavenging the ROS in plasma and interstitial fluid of the arterial walls leading to inhibition of oxidation of LDL (►Low density lipoproteins, in common terms, as bad cholesterol, oxidized LDL contributes to development of atherosclerosis). Additionally, proanthocyanidins from grape seed have been shown to exert antithrombotic effect, which can be of benefit in disease conditions such as venous thrombosis, atherosclerosis, and coronary thrombosis (thrombosis: formation of blood clot). It even protects cardiomyocytes from exogenous as well as endogenous oxidative stress by scavenging ROS and chelation of iron. Grape seed extract not only prevents atherosclerosis (plaques formed by lipids inside the artery) but also has been found to improve the cardiac recovery from reperfusion-induced injury by free radicals generated during reperfusion (restoration of blood flow) after ischemia (restriction in blood supply/flow). Similarly, it exerts protective effect against ischemia-reperfusion-induced renal damage.

Other Health Conditions

Grape seed extract prevents the progression of cataract formation in hereditary cataractous rats as well as experimental cataract induced by selenite treatment, and thus can be used as an effective preventive agent against the development and progression of cataract formation.

Use of grape seed extract has been scientifically proven to treat stress-induced disorders. It exerts nootropic (improvement in brain functions such as cognitive ability and mental agility) effects in animal models, thereby underscoring the importance of using grape fruits and seeds as advocated in traditional system of medicine “ayurveda” for treatment of stress-related disorders.

Grape seed extract exerts inhibitory effects on the fat-metabolizing enzymes, pancreatic lipase and lipoprotein lipase (enzymes which aid in digesting fat), which have important implication in limiting the dietary absorption of fat, and thus consumption of grape seed extract can be an effective, safe, and cost effective weight control strategy. In gastrointestinal disorders, grape seed extract exhibits the antiulcer activity as studied in rats. It also affords protection against acute and chronic stress-induced gastrointestinal injury, primarily by scavenging ROS.

Another important biological activity of grape seed extract is its capacity to lighten the UV-induced pigmentation of skin in guinea pigs. When applied topically, grape seed extract improves dermal wound healing.

Grape seed extract also exhibits endothelial-dependent relaxing activity, which can have profound effect in conditions where nitric oxide/nitric oxide synthase system dysfunction is involved in pathogenesis, such as atherosclerosis, erectile dysfunction.

Owing to antioxidant capacity, it prevents gentamicin-induced nephrotoxicity and bone marrow chromosomal

aberrations in animal model. Grape seed extract also inhibits the production of ►nitric oxide (NO) and ROS along with expression of inducible nitric oxide synthase (iNOS) in ►macrophages stimulated with lipopolysaccharide (LPS) of periodontopathogens; thereby it can also act as a potential preventive agent against periodontal diseases.

Oxidative stress is one of the major contributory factors in the process of aging. Consumption of grape seed extract reduces the accumulation of age-related oxidative damages in central nervous system in experimental animals. It even protects and maintains membrane integrity of erythrocytes in aged animals.

Another important activity associated with grape seed extract is its ability to act as insulinomimetic, and it can activate glycogen and lipid synthesis.

Grape seed extract affords multi organ protection against toxicities of various chemicals such as chemotherapeutic drugs, carcinogens, and acetaminophen.

Grape seed extract also exhibits anti-HIV effects possibly by downregulating the expression of HIV-1 entry coreceptors in normal peripheral blood monocytes and lymphocytes.

Anticancer and Cancer Preventive Efficacy

Another important biological activity of grape seed extract is its anticancer activity against cancers of varying phenotype. Grape seed extract shows cytotoxic effects (killing of cells) against human ►breast cancer, ►prostate cancer, ►skin cancer, ►lung cancer, gastric adenocarcinoma, ►chronic myelogenous leukemic and ►colonic carcinoma cells, importantly here, without affecting the viability of other type of normal cells, such as normal gastric mucosal cells and murine macrophages. Anticancer effects were also observed with grape seed extract against carcinogen-induced liver tumorigenesis. In two-stage skin carcinogenesis mouse model (in this model, development of tumor requires two critical events; initiating event which usually occurs with chemical carcinogen and promoting event which usually occurs with chemicals known as tumor promoters), grape seed extract exerted antitumor promoting effects as evident by significant inhibition of tumor incidence, tumor multiplicity (average number of tumors per tumor bearing mouse), and tumor volume (measure of size also).

Another very important biological activity in context to its anticancer activity is its ability to act as an inhibitor of enzyme ►aromatase. This enzyme converts ►androgen into ►estrogen, and is normally expressed in many tissues such as ovary, placenta, bone, and adipose. Most of the breast tumors are sensitive to estrogen, and there is an increased expression of enzyme aromatase in breast cancer tissues as compared with normal breast tissue. Targeting of this enzyme either by inhibiting its activity or by inhibiting its expression can have important implications in arresting the growth of estrogen-sensitive breast tumors. Additionally, estrogen is primarily synthesized by ovaries, however after menopause, its synthesis declines tremendously, therefore in those women, who have breast cancer after menopause, aromatase inhibitors are much more effective in controlling the progression of this disease. Studies conducted with grape seed extract in preclinical ►xenograft model and breast cancer cell line have revealed that it can be a potential chemopreventive agent against breast cancer because of its ability to inhibit the activity as well as expression of this enzyme.

Aberrant activation of signaling pathways is frequently observed in cancer. In prostate cancer, ►epidermal growth factor receptor (EGFR) and ►insulin-like growth factor receptor-type-I (IGF-IR) are constitutively active (do not need signal/stimuli for activation) along with the increased production of growth factors. This provides an autocrine loop for constitutive activation of both mitogenic and survival signaling in these cancer cells, thereby causing their abnormal proliferation. Grape seed extract inhibits the growth and induces apoptotic death of both androgen-dependent and -independent prostate cancer cells; in part by via inhibition of EGFR-mediated mitogenic signaling. Another important event involved in the growth and spread of cancer is ►angiogenesis (formation of new blood vessels from preexisting ones) and neovascularization. Grape seed extract inhibits the growth of prostate cancer cells tumor xenograft by decreasing the proliferation of these cells, increasing the apoptotic index, and by strong inhibition of tumor angiogenesis. Grape seed extract strongly inhibited the release/secretion of proangiogenic factor ►vascular endothelial growth factor (VEGF) in this preclinical xenograft model. It also inhibited the constitutively activated IGF-I (ligand)/IGF-IR signaling in these cells, thus shutting off the mitogenic and survival mechanism for these cells.

Grape seed extract has shown anticancer activity against not only carcinogen-induced intestinal and colorectal cancers in animal models but also against colorectal carcinoma cell lines of human origin. Even growth of colorectal cancer cells as tumor in xenograft model was strongly inhibited by grape seed extract. Uncontrolled proliferation due to loss of cell cycle checkpoint controls is one of the underlying defects in cancer. Grape seed extract inhibited this abnormal growth of colorectal cancer cells by upregulating the levels of ►cyclin-dependent kinase inhibitors (cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors control the cell cycle progression) to halt these cells at G1 phase (normal cell goes through different phases before next division of cell occurs). In colorectal tumors grown as xenograft in ►nude mice, grape seed extract inhibited the markers of cell

proliferation and induced apoptotic death leading to overall inhibition of tumor growth. Additionally, cancerous cells have growth advantage over normal cells due to increased levels of nucleotide synthesis by using the salvage (alternate pathway to procure precursors for nucleotide synthesis) pathway. Extract from whole grapes including seed inhibited the activity of enzymes involved in the salvage pathway, thus diminishing the survival advantage of colon cancerous tissues, and this might be plausible mechanism of anticancer effects of grape seed extract in general.

Clinical Studies

Even though all the earlier listed beneficial effects have been proven scientifically in animal models, there are few studies that have explored the mechanism of health beneficial effects in humans. In this context, grape seed extract reduced the inflammatory response as well as oxidative stress in systemic sclerosis (a systemic connective tissue disease) patients as evidenced by decreased expression of ►adhesion molecules (ICAM-1, VCAM-1, E-selectin: endothelial adhesion molecules and their expression is upregulated in this disease) and reduced levels of malondialdehyde (end product of peroxidation of lipids by ROS) in the plasma of the patients indicating a beneficial effect of consuming grape seed extract in this disease.

Hypercholesterolemia is a major risk factor for cardiovascular diseases. Consumption of grape seed extract in conjunction with niacin-bound chromium altered the lipid profile of hypercholesterolemic patients, especially lowered LDL levels and decreased the circulating levels of autoantibodies to oxidized LDL (auto antibodies to oxidized LDL are involved in the pathogenesis of coronary artery disease, atherosclerosis) and thus can have potential role in lowering the risk of cardiovascular diseases in such patients. Consumption of grape seed extract-supplemented meal by humans reduced the postprandial (time after meal consumption) oxidative stress by increasing the plasma levels of antioxidants, thereby decreasing the oxidant levels in the plasma and consequently protecting LDL from deleterious oxidative modification to some extent. Similarly, consumption of grape seed extract by young healthy volunteers improved the total serum antioxidant activity, which may have beneficial effects especially in hypercholesterolmic patients who are at increased risk of cardiovascular diseases.

Grape seed extract consumption in humans has been shown to lower the 24-h energy intake without affecting the satiety, mood, or tolerance, and thus can be an effective therapy to control body weight.

Grape seed extract, not only by itself, but in conjunction with other drugs can have beneficial effects, such as it exerted protective effect on various symptoms of Syndrome X when given in conjunction with niacin-bound chromium complex. Similarly, when given orally to patients with chloasma, it improves on the symptoms of chloasma (a skin condition involving hypermelanosis).

Results of preliminary study conducted in patients with chronic pancreatitis symptoms revealed that oral consumption of grape seed extract reduced the index of pain and incidence of vomiting in such patients. Together, it is evident that grape seed extract possesses multiple health beneficial effects, and thus can be an excellent health supplement.

Conclusions

With the increased acceptance of anything natural by patients or human population in general, recent years have seen rapid growth of nutraceutical industry. These ►nutraceuticals are commonly used as health supplements and/or as alternative complementary medicine. In this context, grape seed extract with high ►antioxidant capacity has shown excellent health beneficial effects especially in those disease conditions where oxidative stress is involved in the etiology. Even though numerous reports are available regarding the health beneficial effects of consuming grape seed extract, yet comprehensive studies in humans are required to be conducted further to completely understand the mechanism of its action under different disease scenarios and to term its usage as absolute safe in humans, especially under those disease conditions where adverse drug interactions when used in combination with this extract are suspected.

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Grape Seed Proanthocyanidins

Definition

GSP; A mixture of oligomeric procyanthocyanidins extracted from grape (*Vitis vinifera*) seeds. GSP exhibits

potent antioxidant, anti-inflammatory, anticarcinogenic and antiatherogenic activities.

►Chemoprotectants
►Grape Seed Extract

Graves Disease

Definition

Is a thyroid disorder characterized by goiter, exophthalmos, "orange-peel" skin, and hyperthyroidism. It is caused by an antibody-mediated auto-immune reaction, but the trigger for this reaction is still unknown. It is the most common cause of hyperthyroidism in the world, and the most common cause of general thyroid enlargement in developed countries. In some parts of Europe the term Basedow disease or Graves-Basedow disease is preferred to Graves disease.

►Thyroid Carcinogenesis

GRB2

Definition

Growth factor receptor bound protein 2, plays a central role in signaling by receptor protein tyrosine kinases, where its ►SH2 domain binds to the receptor and the two ►SH3 domains link to effectors.

►Focal Adhesion Kinase

Grb2 Adaptor Protein

Definition

An adaptor protein comprised only of one ►SH2 domain and two ►SH3 domains. For example, upon activation of ►EGF, SH2 domain of Grb2 is used to bind to the receptor through its, while SH3 domain binds to SOS, the guanine nucleotide exchange factor for ►Ras, causing Ras activation leading to activation of the Raf-MAPK(Erk) pathway.

►Membrane-Linked Docking Protein

Green Tea

Definition

A natural product with anti-cancer, cardiovascular disease and neurologic disease properties. Active ingredients include polyphenolic ►catechins. Green tea has potent anti-inflammatory properties.

►Inflammation
►Anti-Inflammatory Drugs

Green Tea Catechins

Definition

Are the flavan-3-ols found in green tea leaves (*Camellia sinensis*). The major four catechins in green tea leaves are (−)-►epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC). The potential health benefits of green tea catechins include antioxidant, anticarcinogenic, anti-inflammatory, antiatherogenic and antimicrobial activities.

►Chemoprotectants

Green Tea Polyphenol

►Epigallocatechin

Groucho

Definition

Class of proteins that are co-repressors of a vast number of transcription factors. The prototype of this conserved family is the Drosophila Groucho protein, which is expressed throughout development and plays important roles in various developmental processes.

Growth Arrest

Definition

Is the inhibition of cell division of cells. Growth arrest can be induced by e.g. chemotherapeutics by modulating proteins essential for cell cycle division.

►Apoptosis-Induction for Cancer Therapy

Growth Factor

Definition

A growth factor binds to and signals, through a plasma membrane receptor, to stimulate cellular growth and/or cell division. They bind to specific cell surface receptors and stimulate cellular proliferation, e.g. ►PDGF or ►TGF. Growth factors are important for regulating a variety of cellular processes. Growth factors typically act as signaling molecules between cells.

►Signal Transducers and Activators of Transcription in Oncogenesis
►Stress Response

Growth Factor Receptors

Definition

Cell surface receptors that transduce growth factor signals into the cell interior. They often carry tyrosine kinase activities in their cytoplasmic domains and are overexpressed in many cancers. As a result, cancer cells are stimulated to proliferate in the presence of very low concentrations of growth factor molecules.

►Receptor Tyrosine Kinases

Growth Guidance Cue

►Semaphorin

Growth Hormone

Definition

(GH); synonym somatotropin; Is a protein hormone of about 190 amino acids that is synthesized and secreted by cells called somatotrophs in the anterior pituitary. It is a major participant in control of several complex physiologic processes, including growth and metabolism. Stimulates growth and cell reproduction in humans and animals.

G-SE

Definition

Glutathione (GSH) Conjugates of Electrophiles.

GSH

Definition

►Glutathione
►Hepatic Ethanol Metabolism

GSK3

Definition

►Glycogen synthase kinase 3; serine/threonine protein kinase important in ►Wnt signaling and insulin signaling; phosphorylates ►β-catenin, ►Axin, ►APC and ►LRP5/6.

GSL

Definition

Glucosphingolipid, a class of glycosphingolipids based on glucosylceramide.

►Sphingolipid Metabolism

GSP

Definition
Guanine nucleotide binding adenylate cyclase-stimulating protein; ►G-proteins. GNAS1 (guanine nucleotide binding protein, alpha stimulating activity polypeptide 1).

►Thyroid Carcinogenesis

GST

Definition
►Glutathione S-transferase, is a family of drug-metabolizing enzymes. Its main function is to catalyze the conjugation of glutathione with electrophilic ►xenobiotics and endobiotics, giving rise to usually less electrophilic, more water soluble, and more disposable products.

►Phase 2 Enzymes

GTP

Definition
Guanosine triphosphate.

►RAS

GTP-Binding

Definition
GTP, guanosine triphosphate, is a phosphate-group transferring coenzyme that activates ►RAS proteins and other GTP-binding proteins. RAS proteins possess an intrinsic hydrolytic activity which cleaves guanosin-triphosphate (GTP) to guanosindiphosphate (GDP) and a phosphate group. GDP-bound RAS proteins are inactive.

►RAS Transformation Targets

GTP-Binding Proteins

►GTPase

GTPase

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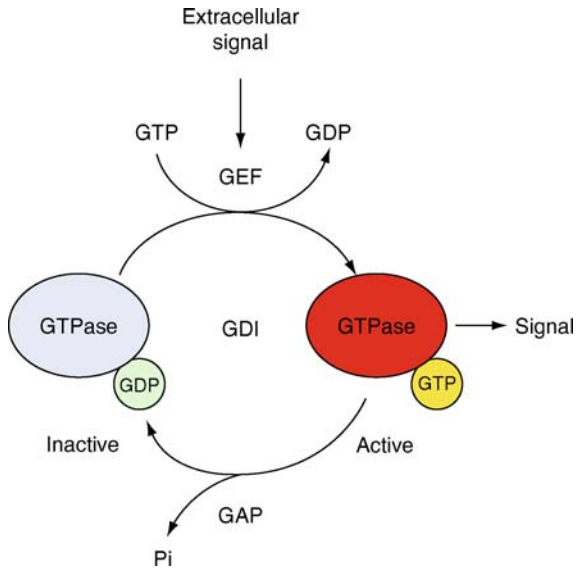
Synonyms
GTP-binding proteins

Definition
GTPases, also known as GTP-binding proteins, ►G-proteins or small GTP-binding proteins are members of the ►Ras superfamily. They are enzymes that catalyze the hydrolysis of GTP to GDP and inorganic phosphate. They are important intermediaries in signal transduction.

GTPases are proteins that work as molecular switches in the regulation of cell responses to extracellular signals. Their function is regulated by GDP/GTP-cycling, where GDP/GTP exchange promotes formation of the GTP-bound protein and GTP hydrolysis promotes formation of the GDP-bound protein. The intrinsic GDP/GTP exchange and GTP hydrolytic activities are typically low and are further accelerated by regulatory proteins. For some GTPases, the GTP-bound form represents the active form (e.g., ►Ras and ►Rho family proteins), whereas for others GDP/GTP cycling is required for activity.

Characteristics
The function of GTPases is regulated by an alternating cycle of on/off states depending on their binding to either GTP or GDP, respectively (Fig. 1). Thus, when bound to GTP, these molecular entities acquire a structural conformation that enables them to interact with other proteins called ►effectors. Effectors are then able to activate specific signaling pathways. When bound to GDP, the conformation of GTPases return back to an inactive state and the signal is interrupted. Frequently, a specific GTPase has the ability to regulate several effectors making their role plastic, versatile and complex (Fig. 2).

Ras protein is the founding member of a large superfamily of small GTPases composed of ~150 members identified with some degree of similarity to the Ras protein. All these molecules have been grouped into at least five major branches according to their

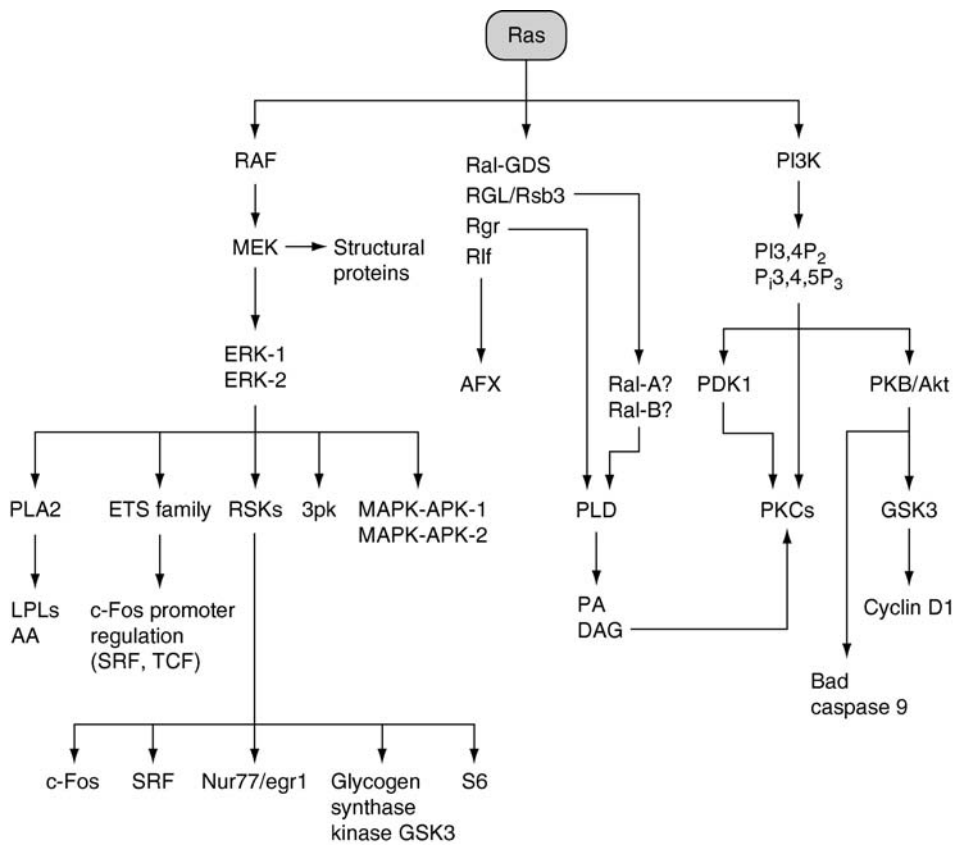


GTPase. Figure 1 Activation/inactivation cycle of GTPases.

sequence homology and functional similarities: Ras, Rho, Rab, Ran and Arf (Table 1). Three of these branches, Ras, Rho, and Rab, include most of the best characterized members of the superfamily. Variations in structure, post-translational modifications that dictate specific subcellular locations and the proteins that serve as their regulators and effectors allow these small GTPases to function as sophisticated modulators of a remarkably complex and diverse range of cellular processes.

The Ras Family

The Ras sarcoma (Ras) oncoproteins are the founding members of the Ras superfamily. This family comprises 36 members, and has been the subject of intense research, in large part because of their critical roles in human oncogenesis. Ras proteins serve as signaling nodes activated in response to diverse extracellular stimuli. Once activated, Ras proteins interact with multiple downstream effectors, which regulate cytoplasmic signaling networks



GTPase. Figure 2 Specific GTPases can interact with a number of different effector molecules. Here mammalian effectors of the Ras protein and their activated signaling pathways are depicted.

GTPase. Table 1 The Ras superfamily

Ras family (36)					
H-Ras	E-Ras	Rit1	Rheb1	RalA	RRP22
N-Ras	Rap1A	Rit2	Rheb2	RalB	RasL10B
K-Ras	Rap1B	Rem1	Noey2	NKIRas1	RasL11A
R-Ras	Rap2A	Rem2	Di-Ras1	NKIRas2	RasL11B
TC21	Rap2B	Rad	Di-Ras2	RasD1	Ris/RasL12
M-Ras	Rap2C	Gem	Rerg	RasD2	FLJ22655
Rho family (22)					
RhoA	Rac1	Rnd1	Cdc42	Wrch1	
RhoB	Rac2	Rnd2	TC10	Wrch-2	Miro-1
RhoC	Rac3	Rnd3	TCL	RhoBTB-1	Miro-2
RhoD	RhoG	Rif	RhoH	RhoBTB-2	
Rab family (61)					
Rab1A	Rab5A	Rab9A	Rab18	Rab27B	Rab38
Rab1B	Rab5B	Rab9B	Rab19	Rab28	Rab39A
Rab2A	Rab5C	Rab10	Rab21	Rab30	Rab39B
Rab2B	Rab6A	Rab11A	Rab22A	Rab32	Rab40A
Rab3A	Rab6B	Rab11B	Rab22B	Rab33A	Rab40B
Rab3B	Rab6C	Rab12	Rab23	Rab33B	Rab40C
Rab3C	Rab7A	Rab13	Rab24	Rab34	Rab41
Rab3D	Rab7B	Rab14	Rab25	Rab35	Rab42
Rab4A	Rab8A	Rab15	Rab26	Rab36	Rab7L1
Rab4B	Rab8B	Rab17	Rab27A	Rab37	RabL4
					RasEF
Arf family (27)					
Arf1	Sar1a	Arl4	Arl9	Ard1	
Arf3	Sar1b	Arl5	Arl10A	Arf4L	FLJ22595
Arf4	Arl1	Arl6	Arl10B	ArfRP1	LOC339231
Arf5	Arl2	Arl7	Arl10C	ArfRP2	
Arf6	Arl3	Arl8	Arl11	Arl2L1	
Ran family (1)					
Ran					

that control gene expression. Ras proteins govern cell proliferation, differentiation, and survival.

The best characterized Ras signaling pathway is activation of Ras by the epidermal growth factor receptor tyrosine kinase. Activated Ras binds to and promotes the translocation of the ►Raf serine/threonine kinase to the plasma membrane, where additional phosphorylation events promote full Raf kinase activation (Fig. 2). Raf phosphorylates and activates the MEK1/2 dual specificity protein kinase, which phosphorylates and activates the ERK1/2 mitogen-activated protein (MAP) kinase. Activated ERK translocates to the nucleus, where it phosphorylates Ets-family transcription factors, which in turn activate Ets-responsive promoters.

Other Ras family proteins, including Rap, R-Ras, Ral and Rheb proteins also regulate signaling networks. Finally, although biochemically similar to Ras, several Ras family proteins appear to act as ►tumor suppressors, rather than as ►oncogenes (e.g., Rerg, Noey2 and D-Ras).

The Rho Family

Ras homologous (Rho) proteins control key signaling and structural aspects of the cell in response to diverse stimuli. They regulate actin organization, cell cycle progression and gene expression. Currently available information from the human genome sequence project has suggested the presence of a total of 22 Rho family members, RhoA, Rac1 and Cdc42 being the best characterized. Rho GTPases are key regulators of actin reorganization. Consequently, these proteins have been implicated in the regulation of cell polarity, cell

movement, cell shape, and cell-cell and cell-matrix interactions, as well as in regulation of ►endocytosis and exocytosis.

The Rab Family

First described as Ras-like proteins in brain (Rab), Rab proteins constitute the largest branch of the superfamily, with 61 members. Rab GTPases are regulators of intracellular vesicular transport and the trafficking of proteins between different organelles of the endocytic and secretory pathways. These proteins facilitate each of the major steps in membrane traffic: vesicle formation and budding from the donor compartment, transport to the acceptor compartment, and vesicle fusion and release of the vesicle content into the acceptor compartment.

Rab proteins localize to specific intracellular compartments consistent with their function in distinct vesicular transport processes. This localization is dependent on prenylation, and specificity is dictated by divergent C-terminal sequences.

The Arf Family

The ADP-ribosylation factor (Arf) family proteins have been found to be ubiquitous regulators of membrane traffic and phospholipids metabolism. The biological effects of Arfs are thought to occur on membranes and to result from their specific interactions with a large number of effectors that include coat complexes, adaptor proteins, lipid-modifying enzymes, and others. The Arf family includes 27 members arranged into three different groups: the Arfs, Arf-like (Arls), and SARs. Arf1 is the best characterized member. Arf1 regulates the formation of vesicle coats at different steps in the exocytic and endocytic pathways.

The Ran Family

The Ras-like nuclear (Ran) protein is the most abundant small GTPase in the cell and is essential for the translocation of RNA and proteins through the nuclear pore complex. The Ran protein is also involved in control of DNA synthesis and cell cycle progression. Ran regulates formation and organization of the microtubule network independently of its role in the nucleus-cytosol exchange of macromolecules. Although related to the Rab proteins in sequence, it has features that distinguish it. Unlike other small GTPases, Ran function is dependent on a spatial gradient of its GTP-bound form. This results in a high concentration of Ran-GTP in the nucleus, which facilitates the directionality of nuclear import and export.

Regulation of GTPases

Regulation of GTPases is achieved both by positive and negative mechanisms (Fig. 1). GDP/GTP cycling is controlled by two main classes of regulatory proteins. Specific guanine-nucleotide-►exchange factors (GEFs) promote formation of the active, GTP-bound form, catalyzing the exchange of pre-bound GDP for GTP (more abundant in the cell cytoplasm). Exchange factors are regulated by extracellular signals such as receptor tyrosine kinases for growth factors, receptors coupled to ►heterotrimeric GTP-binding proteins or lipid metabolites-sensitive exchange factors (such as RasGRP). There are exchange factors with a highly specific activity towards members of the superfamily of GTPases, and other exchange factors with a broad spectrum, able to activate efficiently several members of the superfamily.

►GAPs (►GTPase activating proteins) catalyze, by a factor of at least 100-fold, the intrinsic ability of GTPases to hydrolyse GTP into GDP+Pi, rendering the protein inactive and ready for the next cycle of activation.

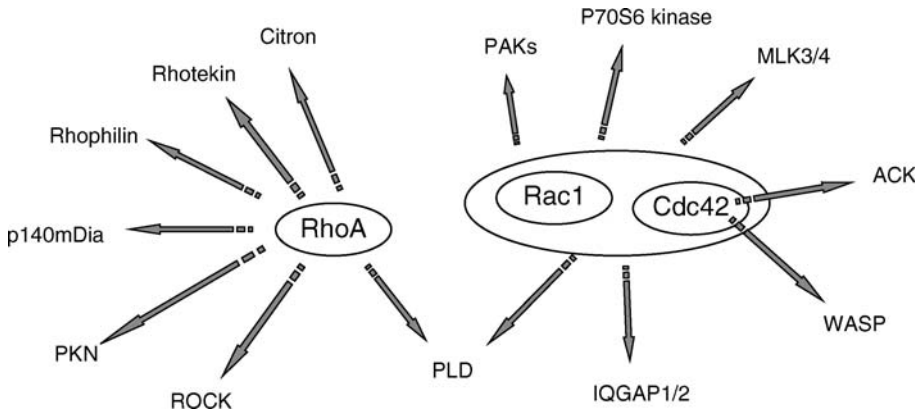
In some cases, cycling of GTPases is modulated by other proteins in addition to exchange factors and GTPases, such as GDI (GDP dissociation inhibitor) that can block both nucleotide hydrolysis and exchange, and participate in Rho GTPase movement between cytosol and membranes. In this case, exchange of GDP by GTP must be accompanied by removal of this inhibitor.

Although the GTP-bound form is the active form for all Ras superfamily GTPases, the cycling between the GDP-bound and GTP-bound states, in which distinct functions are associated with each nucleotide-bound form, is also critical for the activities of Rab, Arf and Ran GTPases.

Clinical Implications

The activity of several GTPases have been implicated in human diseases like cancer, immunodeficiency such as Wiscott-Aldrich syndrome, developmental disorders such as Aarskog-Scott syndrome or X-linked mental retardation. Alterations at both upstream regulatory elements (exchange factors) and defects on the inactivating process (GTPases) have been described as responsible for the dysfunction of the pathway.

Alternative mechanisms for activation of the oncogenic potential of GTPases have been found. ►Point mutations that reduce their GAP-stimulated GTPase activity would render proteins with a much lower efficiency to catalyze GTP hydrolysis. Such point mutations are found frequently in human tumours where members of the Ras branch are involved. Alternatively, cancer-promoting mutations can also affect components of the regulatory cycle, such as GAPs and exchange factors. This is found in proteins involved in the regulation of Ras such as in the case of ►neurofibromatosis type 1 (neurofibromin or NF1) and Rho activity (Dbl, Vav, Ost, Ect2, etc). In some cases, point mutations can promote Ras proteins to



GTPase. Figure 3 The main effectors for the archetypes of Rho proteins, RhoA, Rac1 and Cdc42, are represented. Most effectors are shared by both Rac1 and Cdc42.

spontaneously exchange GDP for the more abundant GTP. In all cases, the endpoint result of these alterations is the same: a larger proportion of the GTPase, and for longer periods of time, will be bound to GTP, rendering the protein permanently in their activated state. Thus, it can interact and activate the corresponding signaling effectors. Recently, it has become evident that Rho family participates in the carcinogenic process by either overexpression of some of the members of the family with oncogenic activity, or by down-modulation of those members with tumor suppressor activity.

Signaling Pathways

GTPases control a large number of signaling pathways involved in regulation of cell growth, cytoskeleton structure, secretion, transcription and ►apoptosis. Specific effectors that are under the control of the specific GTPase mediate all these cellular functions. The GTPases most closely related to the carcinogenic process belong to the Ras and Rho branches.

In the case of Ras proteins, at least three major effectors have been identified in mammalian cells (Fig. 2). The Raf kinase, which is connected to the MEK/ERK kinases pathway, impinges on transcriptional regulation of factors mediating cell proliferation such as ETS. Also, ►PI3K is involved in the regulation of survival signaling through activation of the PDK/PKB pathway. Finally, the Ral-GDS family of exchange factors, including Ral-GDS, Rlf, Rgr and Rgl/Rsb3 participate in important signaling processes such as transcriptional regulation (AFX transcription factors) and lipid-derived signaling through the Ral/PLD pathway, which have implications in cell growth control. Evidence has been generated that a combination of any of two from these three signaling pathways may be sufficient to acquire the transforming phenotype. In addition, cross talking among Ras-dependent and Rho-dependent signaling is an absolute requirement for full transformation.

Regarding the Rho branch, which is also implicated in the carcinogenic process, several effector molecules have been identified (Fig. 3). These include the kinases PKN, ROKα/Rho kinase (Rock), PAKs, p70-S6K, ACKs and MLKs, lipid-related enzymes such as ►phospholipase D (PLD) and PI3K, and other molecules such as p140mDia, citron, rhophilin, rhotekin or WASP. Activation of these effector molecules results in changes in cell morphology and ►motility, as well as transcriptional activation of the ►AP-1, SRF, ►NFκB and STAT transcription factors (►signal transducers and activators of transcription in oncogenesis). Finally, Rho proteins play a role in the acquisition of the metastatic phenotype. The precise effector that is involved in each of these biological effects and in particular its relationship to cancer, has yet to be assigned.

►RAS
►Nucleoporin

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GTPase-Activating Protein

Definition

Negative regulatory protein that accelerates the intrinsic ►GTP hydrolysis activity of a GTP-binding protein, to convert the protein from its active, GTP-bound form to the inactive GDP-form.

►Rho Family Proteins

Guanine Nucleotide Exchange Factor

Definition

Positive regulatory protein that accelerates the intrinsic guanine nucleotide exchange activity of a GTP-binding protein to favor formation of the active GTP-bound form of the protein.

►Rho Family Proteins

Guanylate Cyclase

Definition

GC; Activation of GC leads to formation of cyclic guanosine monophosphate (cGMP).

►Nitric Oxide

Guidance Cues

Definition

A group of secreted or membrane associated proteins that function in the steering of axonal growth cones. The known guidance cues belong to one of four different gene families: the slit, netrin, ephrin and ►semaphorin gene families. Many growth guidance cues are involved in cancer.

Gut Peptides

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Synonyms

Neuropeptides; Gastrointestinal hormones; Secretagogues

Definition

Are chemical messengers that regulate gastrointestinal (GI) functions such as secretion, motility, absorption, digestion, and cell proliferation. These ►polypeptides are produced by endocrine cells in the stomach, pancreas, or intestine and act locally through ►autocrine or ►paracrine mechanisms, or at distant sites in a classical endocrine manner. The gut peptides are also produced in the central and peripheral nervous systems, where they function as neurotransmitters.

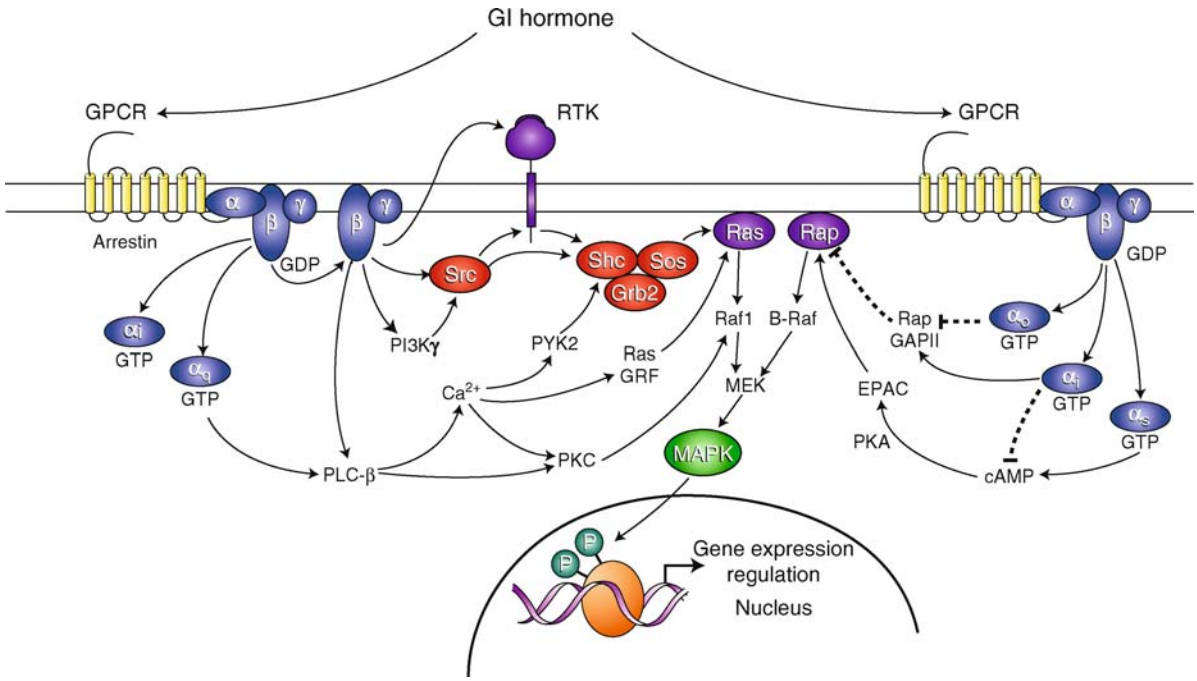
Characteristics

Overview

The best characterized gut peptides include ►gastrin, ►gastrin-releasing peptide (GRP)/►bombesin (BBS), ►somatostatin, ►neurotensin (NT), and ►cholecystokinin (CCK). Glucagon-like peptide 2, ghrelin, vasoactive intestinal peptide, and peptide YY are other examples in this class of dual-function hormones/neurotransmitters. Most of the gut peptides have more than one active form which results from initial synthesis as a large prohormone, and subsequent cleavage to shorter polypeptides.

Messages from the gut peptides are converted to intracellular signals through heterotrimeric ►G-protein coupled receptors (GPCRs), also known as seven-transmembrane domain receptors. The complex functioning of these receptors is incompletely understood, but mechanisms such as heterodimerization of receptors, activation of varying G-proteins, and receptor internalization and desensitization probably allow for the diversity in effects seen in different tissues by each hormone. There are 4 known G_α subunits, 6 G_β subunits, and 12 G_γ subunits; the β and γ subunits form a heterodimer.

Upon peptide binding to the extracellular portion of the receptor, the intracellular G-protein subunits G_α and $G_{\beta\gamma}$ dissociate to activate phospholipases, adenylyl cyclases, protein kinases, membrane ion channels, and the Ras family of small ►GTPases. The proliferative effects of the gut peptides are mediated predominantly through the ►mitogen-activated protein kinase/extracellular-regulated kinase (MAPK/ERK) family of



Gut Peptides. Figure 1 Overview of gut peptide signaling cascades.

proteins. After phosphorylation by serine-threonine kinases, the activated MAPK product translocates to the nucleus where it phosphorylates transcription factors, thereby influencing expression of genes regulating growth (Fig. 1). In cancer, activation of $G\alpha_q$-type GPCRs may transactivate tyrosine kinase pathways (e.g., Src, FAK, EGFR) to further contribute to the mitogenic effects.

Each gut peptide has a unique role in regulating gastrointestinal function and growth of normal and neoplastic tissues.

Gastrin

Gastrin regulates gastric acid production and gastric cell proliferation. Gastrin is produced in the pituitary gland and by G cells in the antrum of the stomach. Its release is stimulated by certain amino acids (AAs) in food and inhibited by the presence of acid in the stomach. The precursor forms of gastrin are 101 and 80 AAs in length, and the active forms are 34 and 17 AAs (G-34 and G-17). The gastrin receptors are the same as those for the peptide CCK and are therefore called CCK-A and CCK-B (also CCK-1 and CCK-2). There is a third receptor which binds gastrin with low affinity, CCK-C.

Gastrin has a role in promoting tumor growth in some neoplasms of the GI tract. Gastrin (G17), glycine-modified forms (Gly-G17), and both of its receptors have been found in human ►gastric cancers, including mutated gastrin receptors. Patients with gastric cancer have higher serum levels of G17; hypergastrinemia is also known to occur as a result of ►*Helicobacter pylori* infection of the stomach, which carries an increased risk for gastric adenocarcinoma. Pancreatic adenocarcinomas generally express both CCK-A and CCK-B. A vaccine directed at G17 (Gastrimmune) has been tested in Phase II trials for advanced pancreatic cancer and as an adjuvant to cytotoxic chemotherapy in patients with gastric and gastroesophageal carcinoma.

The relation between gastrin and ►colorectal cancer (CRC) is less clear and is an area of active investigation. Elevated gastrin levels increase the risk of colon adenocarcinoma. While relatively few CRCs express gastrin receptors or G17, many demonstrate increased expression of the prohormone. Gly-G17 promotes tumor cell growth in CRC cells through a unique and as of yet, uncharacterized receptor which is not blocked by pharmacologic inhibitors of CCK-A or CCK-B receptors. Activation of the gastrin gene is induced by the ►RAS oncogene, and has also been recently related to activation of the ►β-catenin pathway. The gastrin gene may therefore play a role in early CRC tumorigenesis.

Aberrant expression of gastrin receptors may also play a role in GI cancers. A CCK-2 ►splice variant has been detected in colon and pancreatic cancers. This receptor is constitutively active, it triggers intracellular signals without requiring activation by one of its ligands, gastrin or CCK, and may represent an autocrine type of growth stimulation in neoplastic tissue. Its role in human cancers requires further investigation.

Gastrin-Releasing Peptide

Gastrin-releasing peptide (GRP) is the mammalian homolog of the hormone bombesin, originally isolated from the skin of the frog *Bombina bombina*. GRP is found in nerves throughout the GI tract and other organs such as lungs, thymus, prostate, and the pregnant uterus. GRP has stimulatory effects throughout the GI tract; it stimulates gastric acid, gastrin, and pancreatic secretion, intestinal motility, and growth of the gastrointestinal mucosa and pancreatic cells. The precursors to GRP consist of 148 and 125 AAs, and some of the final products are amidated or modified with a glycine residue. The active forms are composed of peptides 1–27, 18–27, and 18–27gly.

GRP stimulates growth and activates nuclear oncogenes in many human cancers and holds potential as a therapeutic target in certain neoplasms. Small cell lung cancers (►SCLC) often both over-express GRP receptors and secrete GRP—the hallmark of autocrine signaling which feeds neoplastic growth. GRP receptor antagonists slow the growth of SCLC cells and enhance efficacy of treatment with cytotoxic chemotherapy. Similarly, androgen-insensitive prostate cancers often demonstrate elevated expression of GRP receptors and also secrete active peptide.

Certain ►neuroblastomas express GRP and their growth is inhibited by receptor antagonism *in vitro*. GRP receptors are expressed in a subset of ovarian cancers, but the role of this peptide is unclear since the tumors have not been found to produce GRP.

Neurotensin

Neurotensin (NT) was originally isolated from brain tissue and subsequently found in the intestine, particularly the distal small bowel. NT is secreted in response to ingested fat and stimulates pancreatic secretion and fat absorption, inhibits gastric and intestinal motility, and stimulates growth of normal intestinal mucosa as well as colorectal and pancreatic cancers with NT receptors (NTR).

NT functions as an endocrine or autocrine/paracrine factor in some of the most prevalent and least curable types of human cancer. Many neoplastic tissues express NTRs, but fewer also possess the ability to produce active NT peptide. Studies suggest that activation of the ERK pathway mediates the trophic effects of NT in various cancers.

NTRs are expressed in 75–90% of pancreatic cancers, but not normal pancreas; *in vitro* and *in vivo* data demonstrates increased pancreatic cancer cell growth in the presence of NT. This gut peptide also plays a role in a subset of breast and colon cancers. While normal breast and colon tissues do not express NTRs, many cancer cell lines express the receptors (e.g., 50% of colon cancer cell lines), and NT expression has been detected a subset of breast and colon cancer tissues. There is limited data to suggest that NT is a growth factor in other GI neoplasms such as stomach and liver; NT may be synergistic with carcinogens in promoting tumorigenesis based on animal studies, but its effect in human stomach and liver cancers is not known.

The autocrine effects of NT are well-established in cancers containing neuroendocrine cells, including a subset of prostate and lung cancers. In particular, many small cell lung cancers and androgen-insensitive prostate cancers both produce NT peptide and express high-affinity NTRs, and *in vivo* studies suggest that inhibition of this trophic pathway holds significant therapeutic potential.

Somatostatin

Somatostatin is universally recognized as an inhibitory GI hormone. It inhibits release of growth hormone, all known GI hormones, inhibits GI motility, gastric acid secretion, intestinal absorption, pancreatic secretion, splanchnic blood flow, and growth of normal and neoplastic tissues. It is produced throughout the central and peripheral nervous systems, in the D cells of pancreatic islets of Langerhans, and mucosa of the stomach and intestine. Peptides of 14 and 28 AAs as well as precursor forms of more than 120 AAs have varying potency. There are five sub-types of somatostatin receptors.

Somatostatin generally has an anti-proliferative effect in neoplastic tissues, but little is known about the specific mechanisms. Its effect has been studied in pancreatic and colon cancers, where its role in tumor cell growth inhibition may depend on the specific receptor subtypes present. It may also modulate growth by inhibiting secretion of the trophic gut peptides described in this review, or by influencing expression of growth factor receptors. The somatostatin analog, octreotide, has a well-established role in the treatment of ►carcinoid tumors.

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Gynaecomastia

►Gynecomastia

Gynecomastia

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Synonyms

Gynaecomastia; Gynecomazia; Hypertrophy of male breast

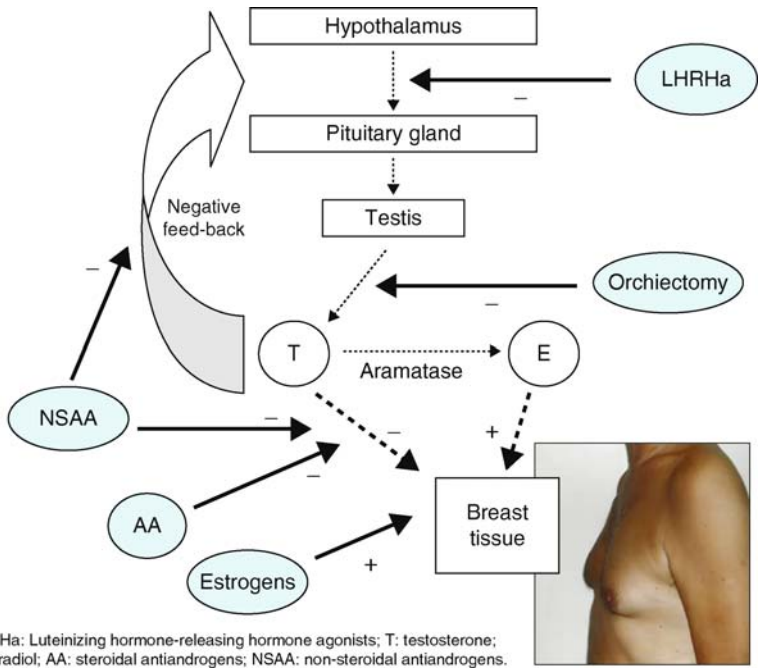
Definition

Gynecomastia is a benign proliferation of the glandular component of the male breast caused by an increased ratio of estrogen to androgen activity. Gynecomastia can be unilateral or bilateral and consists of a palpable mass of tissue with a diameter of at least 0.5 cm, usually presenting as a disk of tissue underlying the nipple. This condition is diagnosed clinically and radiologically, even if there is no consensus on which method should be used to define gynecomastia. Therefore, a careful history of the patient and a good clinical examination represent the first approach to diagnosis. Radiographic investigations using mammography or ultrasound are recommended to confirm the diagnosis. The severity of gynecomastia can be evaluated by using a four-grade score on the basis of the largest diameter: grade 1, <2 cm; grade 2, 2–4 cm; grade 3, 4–6 cm; and grade 4, >6 cm. Recently, Van Poppel et al. introduced a questionnaire (Breast Symptom Questionnaire, BSQ) that correlates well with patients' subjective perceptions of gynecomastia.

Characteristics

Gynecomastia is the most common breast abnormality in the male population. It can result from physiological changes in growth and development, or it can be caused pathologically by a chronic disease (e.g., chronic liver disease due to alcohol abuse) or a tumor (e.g., choriocarcinoma). Gynecomastia also can be induced by drug treatments affecting the balance between estrogens and androgens, such as hormonal therapies for ►prostate cancer.

The incidence of gynecomastia following treatments for prostate cancer varies. Antiandrogen monotherapy is associated with a higher incidence of gynecomastia than other prostate cancer treatments. The mechanisms of induced gynecomastia in prostate cancer patients are shown in Fig. 1.



Gynecomastia. Figure 1 Mechanisms of induced gynecomastia in prostate cancer patients.

The profile of patients with prostate cancer has significantly changed in recent years. Men are being diagnosed at earlier stages of the disease and at younger ages. Consequently, prostate cancer patients are still physically and sexually active, and quality of life has become an important issue in their management.

Hormonal therapies that have a more favorable impact on quality of life than castration are clearly required. Antiandrogen monotherapy with ►bicalutamide (150 mg) offers potential quality-of-life benefits over other treatment modalities for prostate cancer. Gynecomastia with or without breast pain is a recognized adverse effect of bicalutamide, with an incidence of ~70% of patients within the first 6–9 months of hormonal therapy. This adverse event can be a major concern for the patient, sometimes causing treatment withdrawal. Because gynecomastia may compromise treatment outcome, simple and safe methods of managing this condition are needed.

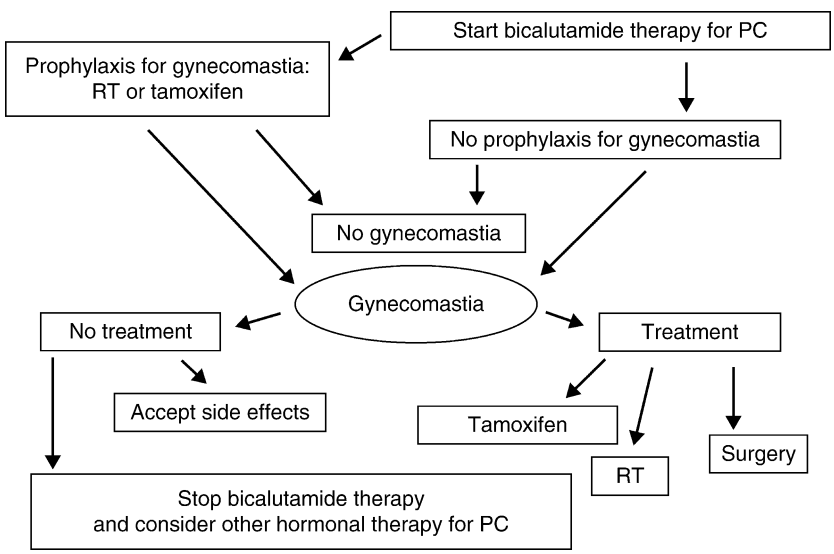
Therefore, clinicians who treat ►prostate cancer need information on the management of gynecomastia in their patients. In this setting, three options can be considered: surgery, ►radiation therapy, and medical therapy as prophylaxis or treatment. Although surgery has been used prophylactically, it is generally reserved for patients who have advanced gynecomastia. Recent data have shown that prophylactic, low-dose (10–12 Gy) breast irradiation is an effective and well-tolerated strategy for prevention or treatment of bicalutamide-induced gynecomastia.

In an interesting comparative study by Perdonà et al., the efficacy of ►tamoxifen (10 mg/day) versus ►radiotherapy (12 Gy) in the management of gynecomastia/breast pain due to bicalutamide (150 mg) monotherapy was investigated. Tamoxifen was extremely effective in preventing the development of bicalutamide-induced gynecomastia/breast pain and was significantly more effective than radiotherapy. These positive findings were in agreement with previous reports of different treatment schedules, such as those by Boccardo et al. and Saltzstein et al. using 20 mg tamoxifen daily and the report by Eaton et al. using 20 mg tamoxifen weekly.

Fradet et al. have conducted a trial to define the optimal tamoxifen dose for treating gynecomastia without compromising disease control. In this double-blind, parallel-group, multicenter trial, 282 patients with PC were randomized to receive bicalutamide (150 mg/day) plus either daily tamoxifen (1, 2.5, 5, 10, or 20 mg) or placebo for 12 months. At 6 and 12 months, tamoxifen decreased the incidence of breast events in a dose-dependent manner. These findings suggest that the optimal prophylactic dose of tamoxifen is 20 mg/day.

However, other issues remain unresolved:

- There may be some concerns regarding the use of tamoxifen in PC. Although blocking the effects of estrogen may effectively prevent or treat gynecomastia, the consequences of such treatment are unknown. Indeed, such therapy might be expected to increase androgen secretion by blocking the negative feedback of estradiol on the hypothalamic-pituitary axis. However, it is reassuring that most studies have reported no detrimental effects of tamoxifen on bicalutamide-induced ►PSA suppression.
- The optimal duration of medical therapy is unknown. Clinical trials addressing longer treatment duration and follow-up are necessary, although ethical concerns may be raised.



Gynecomastia. Figure 2 Management options for bicalutamide-induced gynecomastia. PC, prostate cancer; ►RT, radiation therapy.

- Tamoxifen is generally well tolerated at all doses. However, there are some minor, reversible side effects in tamoxifen-treated patients, and this should be discussed with the patient before therapy is initiated.
- There are financial considerations associated with the use of tamoxifen to prevent gynecomastia because the cost is not reimbursed by health insurances/systems in Europe. However, this is not a serious problem for most patients because tamoxifen is available as a relatively cheap generic drug.
- At present, a consensus on the most suitable method to assess gynecomastia is lacking. In the absence of a standardized parameter of evaluation, any comparison among different trials is of questionable value.

In conclusion, two approaches for the management of gynecomastia are recommended, a prophylactic and a therapeutic approach. The first approach aims to prevent this embarrassing condition, and the second avoids unnecessary treatment of those patients who would not have suffered from gynecomastia. Radiotherapy and medical therapy with tamoxifen are both applicable and effective. When unsatisfactory results are obtained with one therapy, the other can be offered as a second-line option. In this setting, the role of surgery remains limited. Switching to a different hormonal treatment for prostate cancer is another option that can be considered by clinicians and patients (Fig. 2).

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Gynecomazia

►Gynecomastia

Gyrus

Definition

plural: gyri, is a normal anatomical feature of the brain and represents the convex fold of brain matter seen on gross inspection.

►Convection Enhanced Delivery (CED)

H

H-2

Definition
The major histocompatibility complex of the mouse is called H-2 (for histocompatibility-2). Haplotypes are designated by a lowercase superscript, as in $H\text{-}2^{b}$.

►Sjögren Syndrome

H60

Definition
Minor histocompatibility antigen 60, a murine NKG2D ligand.

►NKG2D Receptor

H-CAM

►CD44

H Enzyme

Definition
A product of the *H1* gene, also named fucosyltransferase1 (FUT1), that catalyzes addition of fucose in α1,2 position onto type 2 chains.

►Lewis Antigens

H731 (Human PDCD4 Variant 2, Cancer-related)

►Programmed Cell Death 4

H3 (Rhesus Monkey)

►Deleted in Malignant Brain Tumours 1

HA

Definition
*H*yaluronic *a*cid is a glycosaminoglycan (or mucopolysaccaride) composed of repeating dimeric units of glucuronic acid and N acetyl glucosamine. The major function in the body is lubrication of joints, although it has also been shown to be associated with cancer.

►Osteopontin
►Hyaluronan Synthases

HACBP

►Calreticulin

Hageman Factor

Definition
►Factor XII

Hairy Cell Leukemia

Definition

HCL; Is a chronic lymphoid leukemia, a B-cell disease, and the abnormal cell has hairlike cytoplasmic projections on its surface. The disease is easily controlled, and about 10% of patients never require therapy. In cases where the disease progresses, 5-year survival rate after therapy with cladribine or ►Interferon-α (IFN-α) is approximately 85%.

Half-life

Definition

Biological half-life of a chemical refers to the time required for an organism to eliminate half of the amount of that chemical originally contained in that organism. Also, the time required for the activity of a radioactive isotope to decay to half of its original value.

►Brachytherapy
►Dioxin

Halsted Radical Mastectomy

Definition

►Radical Mastectomy

Hamartin

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Synonyms

TSC1; Tuberous sclerosis complex 1

Definition

One of the two tumor suppressor proteins (the other is called ►tuberin or TSC2) forming an intracellular ►heterodimeric complex is called ►tuberous sclerosis complex (TSC). Both proteins are needed for the normal activity of TSC. Mutation of the hamartin gene with the consequence of altered protein leads to the development of a neurological disorder ►tuberous sclerosis, associated with the formation of usually benign tumors, or ►hamartomas, in different organ systems.

Characteristics

TSC is responsible for the inhibition of ►mammalian target of rapamycin (mTOR) kinase, whose pathway is involved in numerous cell processes linked to cell growth control, like cell cycle progression, transcription, and translation control as well as nutrient uptake. mTOR detects signals of nutrient availability, hypoxia, or growth factor stimulation and, in favorable environmental conditions, its activity leads to phosphorylation of ►eIF4E-Binding Protein 1 (4E-BP1, PHAS-I) and ribosomal ►p70 S6 kinase (S6K1), having direct effect on mRNA translation efficiency. Thus, mTOR controls cell growth through general protein biogenesis. When TSC complex is not functional, mTOR is hyperactive and leads to potentiation of cell growth and formation of hamartomas.

The TSC complex does not act directly on mTOR, but inhibits a ►G protein, ►Ras homologue enriched in brain (►Rheb). Rheb is a member of the Ras superfamily of GTPases, sharing the highest homology with Ras and Rap. ►GTPase-activating protein (GAP) region inhibiting Ras-related family of small G proteins, such as Rap1, Rab5, and Rheb, has been found in tuberin, thus explaining the link between tuberin mutations and strong phosphorylation of mTOR target proteins (S6K1 and 4E-BP1).

Although the GAP domain is only found in tuberin, mutations of hamartin result in the same clinical presentation of tuberous sclerosis, although in this case the severity of the disease may be slightly lower. Thus, although the specific activity is exerted by tuberin, hamartin can also influence signal transduction from Rheb to mTOR.

The *TSC1* gene is located on chromosome 9q34. It has 23 exons and an 8.6 kb mRNA transcript. Protein product, hamartin, is an 1164-amino-acid/130 kDa protein expressed in most human tissues, including the brain, kidney, heart, liver, small and large intestine, prostate, or testes. The protein is hydrophilic and has a single ►transmembrane domain at amino acids 127-144, and a predicted coiled-coil region at residues 719-998.

Cytosolic hamartin forms ►homomeric complexes. Whether this interaction is important for the proper formation of the TSC complex or for the development of the disease is not clear. It seems, however, that

tuberin acts as a chaperone, preventing formation of homomeric hamartin complexes. It is postulated that hamartin inhibits tumor formation by regulating actin dynamics and cellular adhesion through the actin-binding proteins of the ►ezrin-radixin-moiesin (ERM) family as well as the small G protein Rho. Importantly, interaction of hamartin with the ►ERM proteins is required for the activation of Rho by serum or lysophosphatidic acid. Moreover, hamartin was found to bind neurofilament-light chain (NF-L) and colocalize with NF-L and ERM proteins in neuronal growth cones of primary cortical neurons. As the binding of NF-L and ERM proteins by hamartin may play an important role in neuronal migration which could explain disturbances of this process that are often postulated to underlie characteristic brain lesions found in tuberous sclerosis, sometimes defined as "neuronal migration disorder."

Hamartin can be phosphorylated by ►cyclin-dependent kinase 1 (CDK1 or cdc2), activated in late cell cycle phases, at three residues: T417, S584, and T1047, one of which lies in the hamartin/tuberin interaction domain. It seems that complexing with tuberin protects hamartin from being phosphorylated, although on the other hand, such a phosphorylation does not influence the interaction with tuberin. Hamartin phosphorylation by CDK1 activates TSC, which strongly suggests specific regulation of this complex during cell cycle progression.

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Hamartoma

Definition

Benign tumor composed of abnormal "normal" tissue that is found at the site of interest but is disorganized in nature.

►Uveal Melanoma
►Cowden Syndrome
►Hamartin

Hamartomatous Polyposis

Definition

A benign tumor like malformation resulting from faulty development in an organ and composed of an abnormal mixture of tissue elements that develop and grow at the same rate as normal elements but are not likely to compress adjacent tissue.

►Peutz-Jeghers-Syndrome

HAND Gene Family

Definition

Is a group of bHLH proteins comprising Hand1 and Hand2, which are involved in heart and neural crest development.

►E-box

Hand–Schüller–Christian Disease

Definition

Is one form of Langerhans cell Histiocytosis characterized by the clinical triad of lytic bone lesions, exophthalmos and diabetes insipidus.

►Langerhans Cell Histiocytosis

Haploinsufficiency

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Definition

Reduced expression levels of a protein as a result of a mutation in one allele of the corresponding gene, leading to phenotypic effect.

H

Characteristics

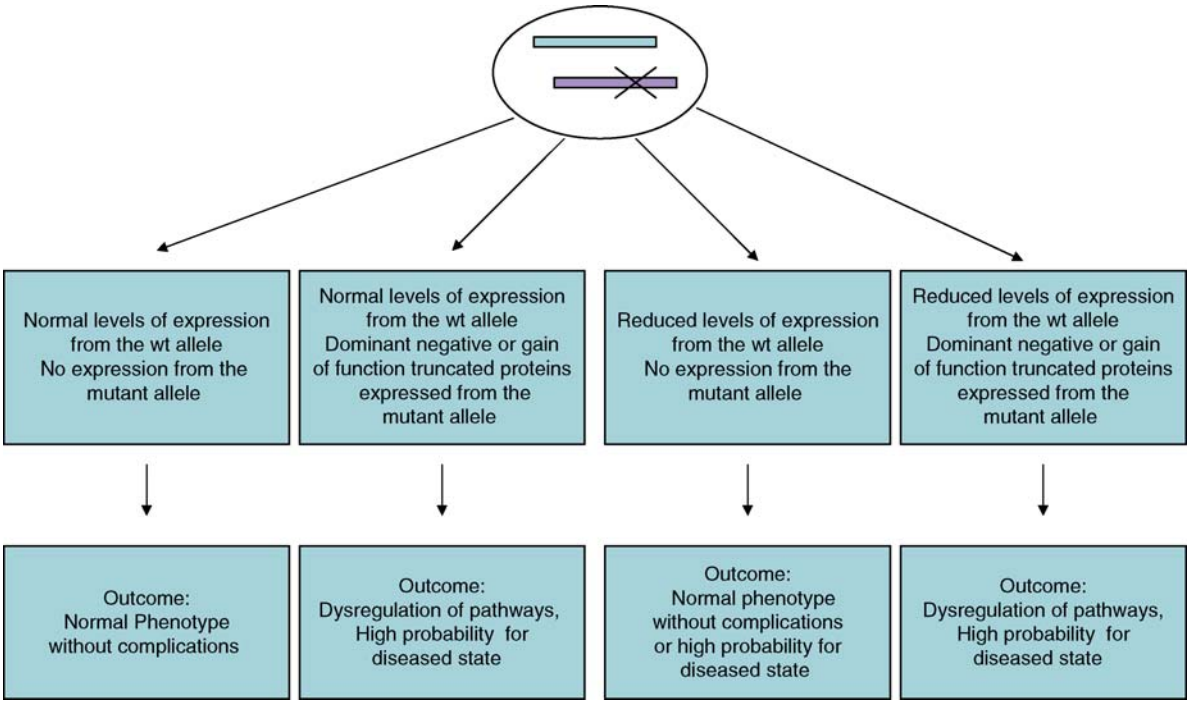
In the early 1920s, shortly after Morgan's chromosome theory of heredity became generally accepted, Calvin Bridges from Columbia University characterized a mutant type of *Drosophila melanogaster* known as "Diminished". He showed that the "Diminished" character is not due to the mutation of an ordinary gene, but to haploidy of the entire fourth chromosome. The elimination of one allele of the genes residing on this chromosome resulted in a complex of mutant characters, including somatic changes, and high rates of sterility and mortality.

During the next two decades several studies were focused on the role of gene haploidy in the generation of specific phenotypes. It became clear that gene dosage plays an important role in cell function and organism development. The word "haploinsufficiency" was credited to Otto Mohr by Curt Stern in the latter's seminal article "Genic Action as studied by means of the effects of different doses and combinations of alleles," published in 1943.

Despite this early evidence for the significance of haploinsufficiency, the dominant thinking from Mendel to the last decade of the twentieth century was that gene expression is a discrete event, i.e. genes are either expressed or not, either dominant or recessive. A general assumption also was that mutations in one allele of a gene does not have a phenotype. Even as late as 1971, these concepts were central to Knudson's two hit theory of carcinogenesis, which suggested that inherited mutations in one allele of a ►tumor suppressor gene increase the probability of early onset of tumors, but do not functionally contribute to it. Initial characterizations of Rb and p53 mutations in human tumors appeared to confirm that heterozygote mutations did not have a phenotype. However, with the development of knockout ►mouse models in the 1990s, it became obvious that mutation in one allele of a tumor suppressor gene could indeed have phenotypes, in some cases significantly different from the wild type.

Mutations in one allele of a tumor suppressor gene (the most studied case), or in any other gene, may result in reduced expression level of the corresponding protein. These mutations may lead to a complete absence of a protein product from the mutant allele (null genotype) or to the synthesis of a truncated protein. Since many of the regulatory proteins are complex, consisting of different functional domains, a truncated protein may retain some functional domains, therefore competing with the wild type protein for some functions (dominant negative or gain of function mutations). Therefore a mutations in one allele may lead to four principle outcomes, illustrated in Fig. 1.

Dominant negative or gain of function mutations often result in early onset of disease state. These are the well documented studies, for example, of the consequences of haploinsufficiency for *WT1*, *p53*, *BRCA1* and *BRCA2* genes. An expanding number of



Haploinsufficiency. Figure 1 Possible effect of mutations in one allele of a gene.

epidemiological studies, as well as mouse models, show that haploinsufficiency in null allele background is also often a major factor in tumor formation. For example, haploinsufficiency for the tumor suppressor genes *APC*, *BML*, *FEN1*, *MSH2*, *p27*, *p53*, *Rb*, *SMAD4* and *NF1* has been shown to be associated with increased incidence of sporadic human cancers including colorectal and gastric cancers, leukemia and lymphoma, breast cancer, retinoblastoma, and others (for a more complete list see Refs. [3] and [4]).

The difficulties in the estimating of the functionality of proteins coded by mutant genes as well as the problems associated with precise defining of the genetic background in large epidemiological studies have been a strong motivating factor in the development of mouse models. The role of haploinsufficiency was particularly well demonstrated in 1996, when three laboratories independently created *p27* null and heterozygous mice. The $p27^{-/-}$ mice had increased growth rate, as well as early mortality as a result of benign pituitary adenoma. As might be expected based on gene dosage concepts, $p27^{+/-}$ heterozygous mice had intermediate growth rate and intermediate mortality compared with the wild type and null genotype. Expression level analysis for the p27 protein also show that it is haploinsufficient in heterozygous animals. These results clearly show that the expression level of p27 may determine phenotype, a conclusion also reached from mouse model studies for *p18*, *PTEN*, *Plk4*, *TGF-b1*, and other genes.

Heterozygous mice typically also have an intermediate response to DNA damaging agents, such as ionizing radiation or some chemical carcinogens, compared with the wild type and null genotype. For example, tumor development in *p27* deficient mice induced by a 1 Gy X-ray dose show distinctive characteristics based on the genotype. $p27^{-/-}$ mice had significantly reduced life span as a result of multiple tumor types. $p27^{+/-}$ mice again show intermediate phenotypic susceptibility to tumors and survival. These tumors in $p27^{+/-}$ mice retain the wild type allele and were less severe than those from the knockout background. Similar results have been obtained in mouse models haploinsufficient for *p53*, *Nbn*, *DMP1* and *TGF-b1*.

Another remarkable result from these mouse-model experiments is that different mouse strains which haploinsufficient for the same protein developed different type of tumors; for example, p53 haploinsufficient mice developed strain-specific tumors after chemical carcinogen treatment. Such observations show the critical importance of the genetic background. In a related set of experiments, it was shown that mice double heterozygous for two genes (for examples *Fen/Apc*, *Cdh1/Apc* and *Xpc/Trp53*) show an increased frequency of tumor incidence in comparison with single heterozygous mice. Such results point to the importance of the genetic background for familial cancers known to arise as a result of heterozygosity for *BRCA1*, *BRCA2*, *ATM*, *CBFA2*, *Smad4* and *Cdh1*. The key conclusion is that heterozygosis for a single gene can contribute to tumor formation.

There are indications that in the general population the number of individuals who are heterozygote for some genes is quite substantial; for example, 0.5–3% of the US population are heterozygote for the *ATM* gene, and recent large-scale epidemiological studies suggest that relative risk of breast cancer in ATM heterozygotes is about 2 compared to the general population, and about 5 in women under 50 years of age. To date, *BRCA1*, *BRCA2*, *TP53* (high risk), and *CHEK2* and *ATM* (moderate risk), have been shown to be involved in enhanced breast cancer predisposition in humans. To what extent these and other heterozygous carriers may have increased cancer risks in other sites is a key question which can probably only be resolved through a better understanding of the mechanisms underlying the role of heterozygosity in tumor formation.

Mechanism of Disease Initiation Involving Haploinsufficiency

Defining the role of haploinsufficiency in the cases where there is expression of dominant negative or gain of function protein from the mutant allele is much easier than when there is no protein expression. Generally, the truncated protein competes with the wild type protein for specific function, or as shown for p53, it may form non functional complexes with the wild type proteins. This can result in partial or complete lost of function for the wild type protein, and often accelerates progress toward a disease state. The mechanisms acting in a null allele background are less well understood. In this case, the only differences between the wild type and haploinsufficient organism are the expression levels of the corresponding proteins. In fact, expression levels of a single protein may have negative effect on the efficiency of a pathway, and haploinsufficiency for two or more proteins can have further negative effects. The effects of this type of haploinsufficiency shows significant interdependence between the proteins involved in cell regulation and tumor formation. Thus, realistic analysis of possible mechanisms inevitably involves analysis of biological networks in which many (if not all) regulatory proteins are linked with each other. Understanding such networks is not easy, even for the known pathways where, for example, the exact yields and activities of the involved proteins are not easy to estimate. Nevertheless, it is now clear that biological networks are complex, hierarchical and, on a local level, are usually modular or motif based. The relatively new discipline of systems biology hold a great deal of promising for elucidating these networks, and one particular mode of organization, the scale free

network, has been shown to be a powerful descriptor of the interrelationships between different cellular components. Scale free networks involve different levels of connectivity between their nodes – a few highly interconnected nodes (hubs) communicate with the remaining larger number of less interconnected nodes. This hierarchy is highly tolerant of inactivated nodes, since the likelihood that one of the few hubs will be affected is small. Even if such an event occurs, the network may not completely lose its connectedness. In analyzing haploinsufficiency, the nodes would represent the status of proteins, and low concentrations of only one of the highly interconnected hub proteins could negatively influence the network response as a whole. The general principles that apply to complex networks require some modifications for biological networks. Mathematical or computer networks are usually analyzed as already connected structures of elements, whereas biological networks are often self assembly/disassembly networks. The requirement for assembly in response to an event at an unknown location in a relatively large (on molecular scale) region, introduces spatial and quantitative constraints. DNA double-strand breaks (DSB), for example, are local events that may appear at any place in the nucleus. In response to a DSB, a network has to be assembled locally in order to signal and initiate the repair. Proteins that are potential members of such local networks have to be in close proximity, or be able to rapidly translocate to the site. Several experiments confirm this scenario. Immunofluorescence analyses of cells subject to radiation induced DSB show that many DNA repair proteins, such as ATM, p53, MRE11, Rad50 and NBS1, ATR, colocalize and form discrete foci near the sites of DNA damage. Migration of DNA repair proteins toward the site of DNA damage has also been analyzed by FRAP. By measuring diffusion rates, it has been shown that translocation and transient immobilization of RAD51, RAD52, RAD54, as well as the NER repair complex ERCC1-XPF, occurs near DNA repair sites in mammalian cells. Haploinsufficiency for ATM or BRCA1 (which recruits proteins toward the BRCA1-associated surveillance complex, BASC) can lead to incomplete assembly of the BASC complex, and diminution or loss of its function. As a result, such cells may contain more unrepaired or misrepaired DSB, or may be more prone to failure of apoptosis, all of which could lead to mutation accumulation and subsequent disease progression. This mechanism suggest an important role for haploinsufficiency in tumor development. Furthermore, the role of combined haploinsufficiency emphasizes that individual predisposition to tumor may be a result of a combination of haploinsufficiencies for different genes. These observations may, in part, account for the higher than predicted rate of cancer in humans.

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Haplotype

Definition

A haplotype is a linked set of genes associated with one haploid genome. The term is used mainly in connection with the linked genes of the major histocompatibility complex, which are usually inherited as one haplotype from each parent. Some MHC haplotypes are overrepresented in the population, a phenomenon known as linkage disequilibrium.

►Linkage Disequlibrium
►HLA Class I

Haptens

Definition

Are molecules that can bind antibody but cannot by themselves elicit an adaptive immune response. Haptens must be chemically linked to protein carriers to elicit antibody and ►T-cell response.

Haptoglobin

Definition

One of the acute phase proteins produced in the liver. Haptoglobin binds to oxidized hemoglobin, which is

produced in hemolytic diseases. Haptoglobin is also involved in arteriosclerosis and the immune system. The ectopic production of haptoglobin was observed in several diseases, including infections, ►inflammation and cancer.

►Fucosylation

Haptotaxis

Definition

Haptotaxis is type of chemoattraction where the chemical gradient of the ligand is presented to the cell bound to the substrate. Movement of cells in response to a biochemical gradient on a surface. Attraction indicates on a positive haptotaxis whereas repellence indicates on negative haptotaxis.

►Laminin Signaling
►Chemoattraction

Hardy–Weinberg Law

Definition

First described by G. H. Hardy and Wilhelm Weinberg, it mentions that, under certain conditions, after one generation of random mating, the genotype frequencies at a single gene locus will become fixed at a particular equilibrium value. It also specifies that those equilibrium frequencies can be represented as a simple function of the allele frequencies at that locus.

In case of a single locus with two alleles A and a with allele frequencies of p and q, respectively, it predicts that the genotypic frequencies for the AA homozygote to be p^2, the Aa heterozygote to be $2pq$, and the other aa homozygote to be q^2. In other words, it is predicting genotypic frequencies from allele frequencies.

Deviations from the HWE can be due to Genotyping errors.

HARP

►Pleiotrophin

HAS

►Hyaluronan Synthases

HAS1

Definition

Hyaluronan Synthase 1 – An integral multipass plasma membrane protein that catalyses hyaluronan synthesis, and is associated with maintenance of a basal level of hyaluronan. Exhibits low catalytic activity and synthesises high molecular weight hyaluronan.

►Hyaluronan Synthases

HAS2

Definition

Hyaluronan Synthase 2 – An integral multipass plasma membrane protein that catalyses hyaluronan synthesis. High expression levels are observed in developmental processes involving tissue expansion and growth which may require rapid changes in matrix composition. Exhibits high catalytic activity and synthesises high molecular weight hyaluronan.

►Hyaluronan Synthases

HAS3

Definition

Hyaluronan Synthase 3 – An integral multipass plasma membrane protein that catalyses hyaluronan synthesis and is associated with high catalytic activity and synthesis of low molecular weight hyaluronan. Produces hyaluronan that contributes to the glycocalyx and initiates cell signal transduction via interaction with hyaluronan-specific cell surface receptors.

►Hyaluronan Synthases

Hashimoto Thyroiditis

Definition

Is an thyroid ►autoimmune disease in which the immune system attacks and destroys the cells of the thyroid gland. The thyroid helps set the rate of metabolism, which is the rate at which the body uses energy. Hashimoto hyroiditis stops the gland from making enough thyroid hormones for the body to work the way it should.

►Chronic Lymphocytic Thyroiditis
►Marginal Zone B-Cell Lymphoma
►Chronic Lymphocytic Thyroiditis

HAT

Definition

1. Histone acetyltransferases. Enzymes that transfer acetyl groups from acetyl-CoA to histone tails. Transcriptional coactivators often have HAT activity. Three superfamilies of HATs are known: GNAT, MYST and p300/CBP.
   ►Histone Deacetylases
   ►Epigenetic silencing
   ►P300/CBP Co-Activators

2. Hypoxanthine-aminopterin-thymidine.
   ►Microcell-Mediated Chromosome Transfer

HAUSP

Definition

Herpes-associated ubiquitin-specific protease, a deubiquitinase that interacts with p53 and promotes its accumulation.

►HAUSP De-ubiquitinase

HAUSP De-Ubiquitinase

►Herpesvirus-Associated Ubiquitin-Specific Protease De-ubiquitinase

γH2AX

Definition

A histone variant called H2AX is phosphorylated at serine 139 when double- stranded breaks (DSBs) occur in cells. This phosphorylated form of H2AX is called γH2AX, a hallmark for DSBs.

►BRIT1 Gene

Hay Fever

►Allergy

HBBM

►Pleiotrophin

HB-EGF

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Definition

Heparin-binding epidermal growth factor-like growth factor; is a high-affinity, activating ligand for the ►epidermal growth factor receptor (EGFR/HER1/ErbB1) and for the related receptor tyrosine kinase, ►ErbB4. HB-EGF was originally identified by a combination of biochemical and bioactivity-based in vitro assays. Following biochemical purification, the protein was recognized as a member of the epidermal growth factor (EGF) family by molecular cloning. The HB-EGF gene resides on chromosome 5 (5q23) in the human genome.

Characteristics

HB-EGF is a ►mitogen, morphogen, motility and cell survival factor. The protein is initially expressed in a

membrane-anchored form (proHB-EGF), with a single membrane-spanning domain, a structural characteristic shared by other ErbB family ligands. HB-EGF was initially observed as a mitogenic activity recognizable in cell culture medium conditioned by macrophage-like cells. The protein was recognized to have a high affinity for immobilized heparin and heparin-affinity chromatography was used in its purification. The heparin-binding property of HB-EGF, which is conferred by a region of basic residues that precedes (reading from the N-terminus) the EGF-like motif, distinguishes it biochemically from EGF and most other EGF-like growth factors. HB-EGF most closely resembles one other EGF-like growth factor and EGFR ligand, ►amphiregulin, which exhibits a similar affinity for heparin and has a similar overall primary structure. The results of a variety of studies indicate that cell surface heparan sulfate proteoglycans play a role in the control of HB-EGF bioactivity. Hence, the heparin-binding property of the protein is likely to be physiologically relevant. The heparin-binding region may also serve as a nuclear localization motif.

The soluble form of HB-EGF is mobilized from the cell surface form by regulated proteolytic cleavage. Several membrane-associated proteases, including ►matrix metalloproteinase (MMP)-3, MMP-7, and several proteases of the disintegrin and metalloproteinase (►ADAM) family have been implicated as HB-EGF sheddases in various cell types and physiologic contexts. The specific protease involved in a particular physiologic or pathologic process is likely to reflect cell- and tissue-dependencies that may not apply to other settings.

An interesting property of HB-EGF, unique among the EGF growth factor family, is that the ectodomain and mature form of the protein bind diphtheria toxin with high affinity. The membrane form of the protein (proHB-EGF) is the physiologic receptor for diphtheria toxin. ProHB-EGF is internalized by normal endocytic processes after toxin binding. Hence, HB-EGF is a mediator of the clinical effects of diphtheria toxin by serving as a means of transporting the active toxin into cells.

Clinical Relevance

Experimental and translational studies indicate that HB-EGF plays a role in formation of the vascular system during development, in muscle hypertrophy, in angiogenic tissue reactions, and as an autocrine, paracrine and juxtacrine signaling molecule. Published information suggests that HB-EGF may play a functional role in ►bladder cancer and ►ovarian cancer.

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HB-GAM

►Pleiotrophin

HBGF

►Fibroblast Growth Factors

HBGF-8

►Pleiotrophin

HBNF

►Pleiotrophin

HBNPF

►Pleiotrophin

HC-11 Cells

Definition

Are mouse mammary epithelial cells that are able to differentiate *in vitro* in response to prolactin producing the milk protein β-casein.

►Cripto-1

HC gp39

►Serum Biomarkers

HCAEC

Definition
Human coronary artery ►endothelial cells.

HCC

Definition
►Hepatocellular carcinoma
►Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma
►Hepatic Ethanol Metabolism

HCV

►Hepatitis C Virus

HD

Definition
►Hodgkin Disease; Hodgkin lymphoma.

HDAC Inhibitors

Definition
Histone beautylase inhibitors Class of natural and synthetic molecules that abrogate or decrease the activity of histone deacetylases.

►Histone Modifications
►Histone Deacetylases

HDACs

Definition
Histone deacetylases; Enzymes that remove acetyl groups from histone tails at lysine residues.

►Epigenetic Gene Silencing and Cancer
►Histone Deacetylases

HDM

►MDM Genes

HDMVEC

Definition
Human dermal microvascular ►endothelial cells.

H&E Stain

Definition
Hematoxyline&eosine; histological stain of tissue sections, to detect tissue organization by microscopic inspection.

►Gleason Grading

HEA125

►EpCAM

Health Technology Appraisal

Definition

HTA has been defined as the structured evaluation of the properties or effects (or both) of a health technology, designed to answer such questions as:-

- Does the treatment work?
- Who does it work best for?
- At what costs do we get the results?
- How does the treatment compare with the alternatives?

►National Institute for Health and Clinical Excellence

Heat Shock Protein 90

►Hsp90

Heat Shock Protein 70

Definition

HSP70; A family of proteins important in protein folding and protecting cells from stress. HPS70s can aid in protein re-folding during thermal or oxidative stress, which tends to denature vital cellular proteins. This term originally referred to proteins whose expression was greatly induced by elevated temperatures. Expression of HSPs is also known to increase by cellular stress.

►Hyperthermia
►Hsp90

HECT

Definition

Homology to the E6-associated protein C terminus; ►Itch.

Hedgehog

Definition

Abbreviated Hh in mouse, HH in human; In vertebrates there are three hedgehog genes (*Sonic*, *Indian* and *Desert*) that encode secreted proteins involved in embryonic patterning of a wide range of tissues.

►Hedgehog Signaling

Hedgehog Signaling

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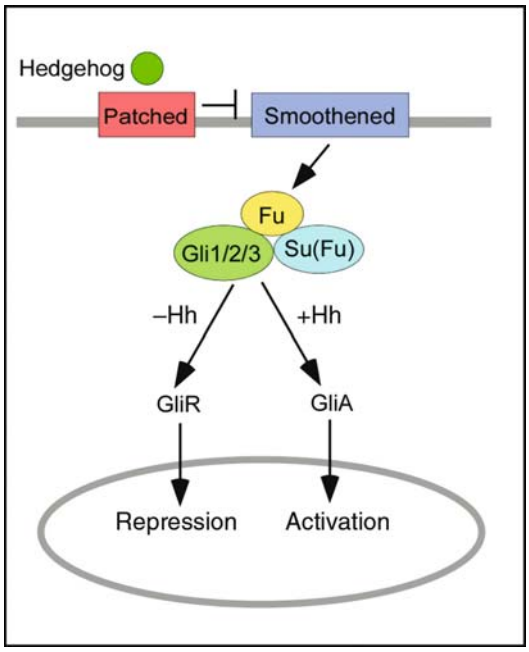
Definition

The Hedgehog pathway is a signaling cascade that plays a crucial role in the embryonic development of a wide range of vertebrate organs and systems. Mutations in members of the hedgehog pathway have been found in a number of tumor types including ►basal cell carcinoma (BCC) and ►medulloblastoma. More recently, ligand-dependent activation of the pathway has been demonstrated in a wider range of tumors. Although some differences exist, the overall pathway has been conserved from *Drosophila* through to human.

Characteristics

The ►Hedgehog proteins are secreted molecules that act through a receptor complex involving two additional proteins ►Patched and ►Smoothened. The current model for hedgehog signaling suggests that, in the absence of Hedgehog, Patched inhibits Smoothened and thus prevents signaling. When Hedgehog binds to Patched, the inhibition of Smoothened is released and intracellular signaling is activated. The ultimate regulation of Hedgehog target genes in the nucleus is mediated in mammals by the three ►Gli zinc finger transcription factors. Gli1 and 2 act primarily as transcriptional activators, while Gli3 is also involved in transcriptional repression. The repressor function of Gli3 is mediated by cleavage of the full-length activator form of the protein into a truncated repressor form (see Fig. 1 for model).

Recent years have seen the identification of many additional proteins that regulate Hedgehog signaling both at the cell surface and at various points in the intracellular signaling cascade. Likewise cilia, microtubule base



Hedgehog Signaling. Figure 1 Simplified representation of the Hedgehog signaling pathway in vertebrates. In the absence of Hedgehog, the transmembrane protein Patched inhibits signaling from the Smoothened molecule. In this state full-length Gli is cleaved to its repressor form (GliR) and enters the nucleus where it represses transcription of target genes. Of the three vertebrate Gli genes, Gli3 plays the major role as a transcriptional repressor in vivo. On Hedgehog binding to Patched, inhibition of Smoothened is relieved, cleavage of Gli is inhibited and the full-length activator (GliA) form enters the nucleus and activates transcription of target genes. A complex of molecules including Suppressor of fused (Su(Fu)) and Fused (Fu), regulates the cytoplasmic/nuclear translocation of Gli, although the precise role of these proteins in vertebrates remains unclear. A number of other molecules have been shown to regulate signaling at various points in the pathway, but these have not been shown for simplicity.

organelles present in virtually all vertebrate cells, have recently been linked to hedgehog signaling in higher vertebrates. For simplicity these aspects of hedgehog signaling will not be addressed in detail here.

Hedgehog Signaling and Cancer

The pivotal role of the Hedgehog signaling pathway in tumor formation was first realised with the discovery that the *Patched* gene is mutated in a rare inherited cancer predisposition syndrome known as ►naevoid basal cell carcinoma syndrome (NBCCS) or Gorlin's syndrome. NBCCS is characterized by a range of developmental defects in addition to cancer predisposition. While the major tumors seen in NBCCS patients are BCCs, other neoplasms that occur at an increased incidence in this syndrome include medulloblastoma, ovarian fibroma, meningioma, fibrosarcoma, rhabdomyosarcoma and cardiac fibroma.

In addition to being responsible for NBCCS, mutations in the *Patched* gene also occur in a large percentage of the common sporadic forms of BCC that occur in the general population. *Patched* acts as a tumor suppressor gene with inactivation of both copies required for tumor progression. NBCCS individuals are born with one copy of the *Patched* gene inactivated either by mutation or genomic deletion in every cell of their bodies, and tumor formation results when the other copy is inactivated in a given cell throughout life. By chance this will occur more frequently and at a younger age than inactivation of both copies in the same cell during the life of a non-NBCCS individual. In addition to BCCs, mutations in *Patched* have also been demonstrated in a subset of sporadic medulloblastomas, tricoepitheliomas (TEs), esophageal squamous cell carcinomas and transitional cell carcinomas of the bladder. Inactivating mutations in the ►*Suppressor of Fused* (*SU(FU)*) gene, which like *Patched* encodes a negative regulator of Hedgehog signaling, have also been detected in medulloblastomas and BCCs. Contrary to the action of *Patched* and *SU(FU)*, *Smoothened* has been shown to act as an oncogene, with activating mutations detected in both BCCs and medulloblastomas. The net result of all of these mutations is the ligand-independent activation of the Hedgehog pathway.

Until relatively recently our knowledge of the involvement of Hedgehog signaling in cancer was restricted primarily to tumors associated with Gorlin's syndrome. However, a wider spectrum of tumors has now been shown to be associated with ligand-dependent activation of the pathway. These tumors include small cell lung cancer, oesophageal, stomach, biliary tract, pancreatic, and prostate cancer.

Hedgehog, Stem Cell Renewal and Cancer

The underlying mechanisms linking the Hedgehog pathway to cancer remain elusive but a number of theories exist. Recent evidence supports the view that tumors arise from ►cancer stem cells, a minor population of cells within a tumor that are characterized by an inherent capacity for self renewal. In this context a number of signaling pathways, including the Hedgehog cascade, have been implicated in tissue regeneration and stem cell renewal. In a number of tissues, injury is associated with stem cell expansion and Hedgehog pathway activation. It has been suggested that cancer may result when this state of tissue repair remains unregulated, thus retaining the stem cell in a continuously activated state.

Targeting Hedgehog Signaling for Therapeutics

A number of studies have investigated antagonists of Hedgehog signaling as potential cancer therapeutics.

In particular, the plant alkaloid ►cyclopamine inhibits signaling at the level of Smoothened and has been shown to inhibit growth of certain Hedgehog-associated tumors in mouse models. Synthetic small molecule antagonists of Hedgehog signaling have also been investigated as potential therapeutics. These studies have demonstrated that inhibition of Hedgehog signaling can block tumor growth and suggest this approach as a promising treatment for a number of cancers. However, issues surrounding blockage of a key developmental pathway re-activated in tissue repair need to be carefully considered before safe effective therapies are developed.

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HEK 293

Definition

A cell line derived form human embryonic kidney. Frequently used for adenoviruses production.

►Oncolytic Adenovirus

HeLa Cells

Definition

Are ►immortalized human epithelial cells from a cervical carcinoma transformed by ►human papillomavirus 18 (HPV18). The name of the cell line is derived from the woman who had developed the cancer, Henrietta Lacks. HeLa cells are adherent cells (they stick to surfaces) and maintain contact inhibition *in vitro*, i.e. as they spread out across the culture flask, when two adjacent cells touch, this signals them to stop growing. Loss of contact inhibition is a classic sign of cancer cells, i.e. cells which form tumors in experimental animals.

Helicase

Definition

Is an enzyme that separates the complementary strands of a DNA duplex.

Helicobacter Pylori

Definition

A noxious bacterium responsible for gastrointestinal injury including duodenal ulcer and ►gastric cancer.

►Chemoprotectants
►Gastric Cancer

Helix-Loop-Helix Domain

Definition

HLH domain; A structural motif consisting of two helices connected by a loop sequence. It is found in diverse transcription factors and is involved in homo- or heterodimerization.

►Early B-cell Factors

Helper CD4 T Cells

Definition

Are CD4 T cells that can help B cells to make antibody in response to antigenic challenge. The most efficient helper T cells are also known as Th2 cells, which make the cytokines IL-4 and IL-5. Some experts refer to all CD4 T cells, regardless of function, as helper T cells; we

do not accept this usage because function can be determined only in cellular assays, and some CD4T cells kill the cells they interact with.

Helper T (Th) Cells

Definition

Like CTL, helper T cells bear an αβ TCR that recognize fragments of antigenic proteins. However, these fragments are bound to MHC class II molecules, which are usually found only on cells of the immune system and specifically on specialized antigen presenting cells such as B cells, macrophages and dendritic cells Helper T cells bear CD4 receptors that also bind to MHC class II. Activated CD4 T cells can differentiate into two main phenotypes that differ depending on the cytokine subset they secrete. Th1 cells secrete IFN-γ and are important in activating and maturing CTL whereas Th2 cells secrete cytokines important in maturing antibody secreting B cells. Both Th1 and Th2 are required to maintain long lived memory CTL or B cells. They thus function to help other arms of the immune response, hence their "helper" designation.

►Immunoediting

Hemagglutinating Virus of Japan

Definition

►HVJ

Hemangioblastoma

Definition

Is a benign tumor of the nervous system that may occur sporadically or in association with ►von Hippel-Lindau disease. It accounts for approximately 2% of intracranial tumors, arising most frequently in the cerebellar hemispheres and vermis.

►Angioblastoma

Hemangioendothelioma

Definition

A tumor of unpredictable behavior comprised of proliferating endothelial cells. It may be multifocal in the same bone or even in the same limb bud.

►Bone Tumors

Hemangioma

Definition

Benign tumor composed of newly formed blood vessels.

►Hepatic Epithelioid Hemangioendothelioma
►Bone Tumors

Hemangiosarcoma

Definition

Malignant tumor of mesenchymal origin arising from endothelial cells.

►Bovine Papillomavirus

Hematological Malignancies, Leukemias and Lymphomas

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Definition

Hematological malignancies are a collective term for neoplastic diseases of the hematopoietic and lymphoid tissues, with clinical presentation as leukemia, lymphoma or myeloma.

Characteristics

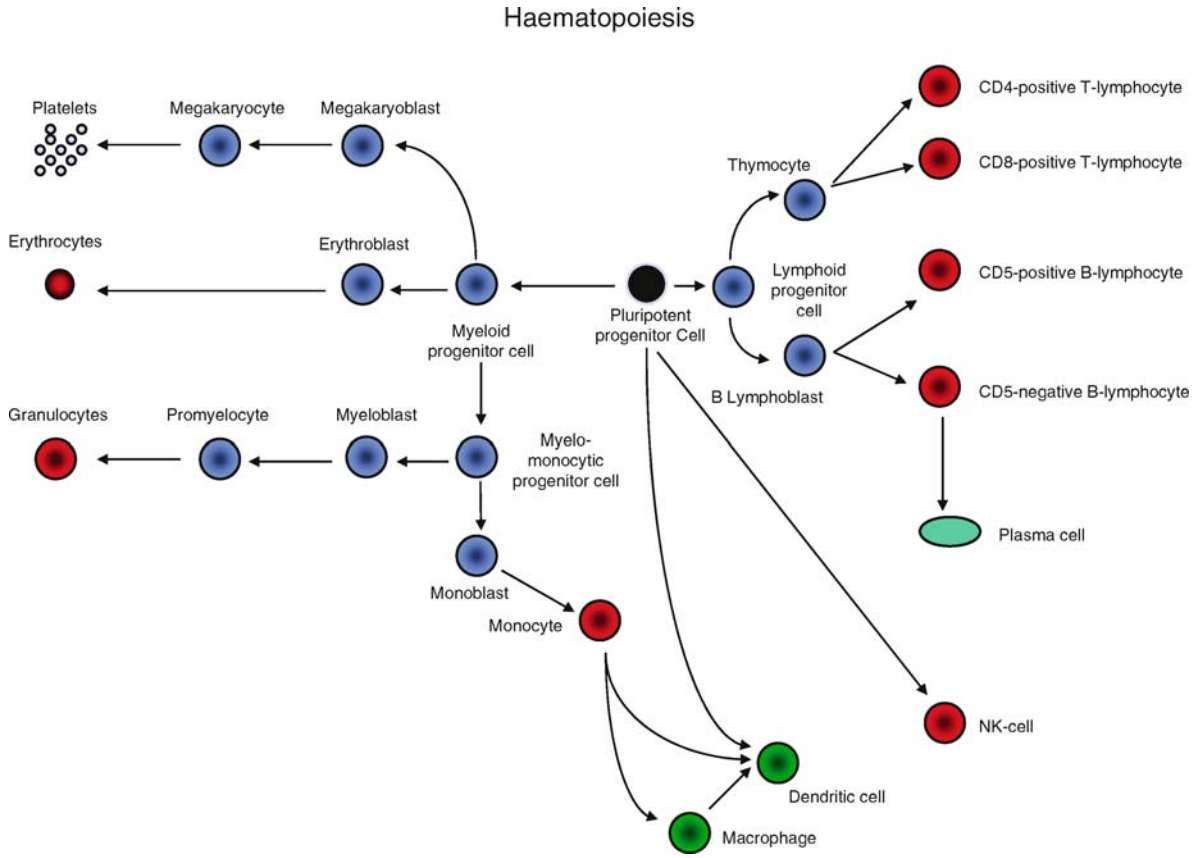
The Hematopoietic System

The blood contains a number of different cell types, which can be divided into cells with and without a nucleus. The unnucleated cells are red blood cells (erythrocytes) and platelets (thrombocytes). The nucleated white blood cells consist of three major subclasses: granulocytes, lymphocytes and monocytes, which can be further categorized as eosinophil, basophil or neutrophil granulocytes, T-lymphocytes, B-lymphocytes and ►natural killer cells (NK-cells). These cells are all derived from a common ancestor, the ►pluripotent progenitor cell in the bone marrow. In the bone marrow, different lineages are derived from this pluripotent progenitor. Precursor cells proceed through specific maturation steps before they, as mature circulating cells, leave the bone marrow in order to circulate in the bloodstream. Fig. 1 shows a simplified schematic representation of these processes. The pluripotent progenitor cell is shown in black in the middle, cells which are normally found only in the bone marrow (or thymus) are in blue, cells normally circulating in the blood are in red and cells found in tissues are in green.

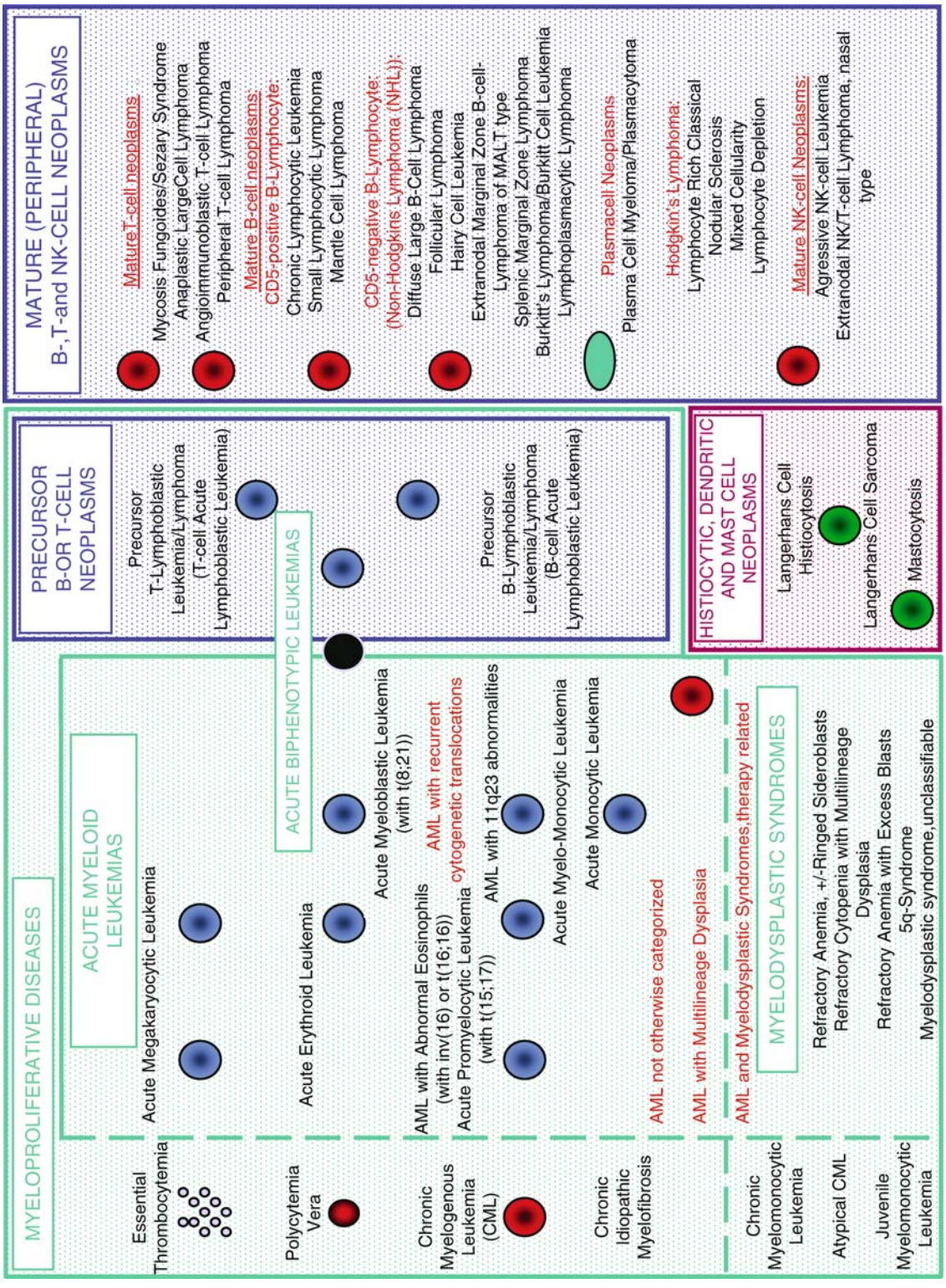
Two major lineages can be defined: The myeloid lineage (shown in green on the left side) and the lymphoid lineage (shown in blue on the right side). Platelets, erythrocytes, granulocytes, monocytes and ►dendritic cells are derived from the myeloid lineage. T- and B-lymphocytes, plasma cells and NK-cells are derived from the lymphoid lineage. The lineages, and the maturation steps involved, can be recognized using morphologic, cytochemical and immunophenotypic features. Similarly, hematologic malignancies are categorized according to such features.

The WHO Classification

The World Health Organization (WHO) has published a unified classification of neoplastic diseases of the hematopoietic and lymphoid tissues. In relation to the development of hematopoietic cells, the two major WHO classes can be viewed as disease "domains", based on the cell types primarily affected in each class and specific subclasses. The principles of this classification are shown in Fig. 2. The domains of the myeloid neoplasms are on the left side, rare histiocytic, dendritic or mast cell neoplasms are at the bottom and the domains of lymphoid neoplasms are on the right side. The WHO classification adopts the REAL (Revised European American Lymphoma) classification of lymphoid neoplasms, whereas the classification of myeloid neoplasms



Hematological Malignancies, Leukemias and Lymphomas. Figure 1 Hamatopoiesis.



Hematological Malignancies, Leukemias and Lymphomas. Figure 2 Leukemic Diseases.

has been revised in several categories compared to the FAB (French American British) classification.

Each of these two major classes consists of subclasses which subsequently categorize the specific disease entities, the most common of which are shown in Fig. 2. Myeloid neoplasms (Fig. 2, left side) consist of three major subclasses: chronic myeloproliferative disorders, ►myelodysplastic syndromes and acute myeloblastic leukemias, but also include more rare myeloid disorders with both proliferative and dysplastic features and disorders of histiocytes, mast (►Mastocytosis) cells and dendritic cells. Lymphoid neoplasms consist of two major subclasses, precursor and mature neoplasms, with specific disease entities of each subclass defined according to the involved cell type, that is, B-, T- or NK-cells. (Fig. 2, right side). Neoplasms of B-cell origin are exceedingly more common than disorders arising in T- and NK-cells (►Malignant lymphoma).

Major Myeloid Subclasses within the WHO Classification

Chronic Myeloproliferative Disorders (MPD's) are disorders of effective hematopoiesis initially presenting with increased levels of one or more of the circulating myeloid cell types: erythrocytes, platelets and granulocytes. On the basis of specific genetic events they can be subdivided into ►Philadelphia chromosome positive disorders, carrying the t(9;22) (►BCR-ABL1) hallmark of Chronic Myeloid Leukemia (►CML), and ►Philadelphia chromosome negative MPD's (Philadelphia chromosome negative myeloproliferative disorders), described as essential thrombocytemia, polycytemia vera and chronic idiopathic myelofibrosis in the WHO classification. The Philadelphia chromosome negative MPD's are better classified according to the presence or absence of activating mutations in the JAK2 protein kinase (►JAK2 mutations). Also recognized within the subclass of chronic MPD's, are rare disorders as chronic neutropilic leukemia, chronic eosinophilic leukemia and unclassifiable myeloproliferative diseases. The "domain" of MPD's is larger than the peripheral blood, since all of these disorders have the potential to transform into acute leukemia (►Blast crisis), including leukemias with a lymphoid phenotype. Furthermore, a small but distinct class of myeloproliferative diseases also show dysplastic features. Therefore the WHO classification has defined a class of myelodysplastic/myeloproliferative diseases, shown in the lower left corner of Fig. 2. This class includes chronic myelomonocytic leukemia, atypical chronic myelogenous leukemia and juvenile myelomonocytic leukemia.

The ►*myelodysplastic syndromes* (MDS) are diseases of ineffective hematopoiesis, resulting in decreased levels of one or more of the circulating myeloid cell types. As the myeloproliferative diseases, the myelodysplastic syndromes can also transform but only to acute myeloid leukemias, suggesting that the "domains" of these diseases may be somewhat smaller than that of myeloproliferative disorders. The class includes refractory anemia (with or without ringed sideroblasts), refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts, 5q-syndrome (MDS: 5q-syndrome) and unclassifiable myelodysplastic syndromes.

The acute myeloid leukemias (AML) (Acute myeloblastic leukemia) can be divided into sublasses (as shown in red in Fig. 2): AML's with recurrent cytogenetic ►translocations (►Chromosomal aberrations including ►acute promyelocytic leukemia, AML with multilineage dysplasia (with or without preceeding myelodysplastic syndrome), therapy-related AML and myelodysplastic syndromes and AML not otherwise categorized. Conversion from myelodysplastic syndrome to ouvert AML is defined by a blast count exceeding 20% of the cells in the bone marrow. Finally acute biphenotypic leukemias are included among myeloid neoplasms in the WHO classification.

Major Lymphoid Subclasses within the WHO Classification

The lymphoid precursor neoplasms are the acute lymphoblastic leukemias (ALL) of either B- or T-lineage origin, but also include the presentation of these diseases as lymphoblastic lymphomas.

The Mature T and NK-cell neoplasms include mycosis fungoides (and the leukemic variant of this lymphoma; ►Sezary syndrome), ►anaplastic large T-cell lymphoma angioimmunblastic T-cell lymphoma, peripheral T-cell lymphoma not otherwise specified (NOS) and a number of more rare diseases. NK-cell neoplasms are rare, but include both lymphoma and leukemia subtypes (Fig. 2, bottom right).

The mature B-cell neoplasms are historically and based on histology divided into of ►Hodgkin disease and ►Non-Hodgkin Lymphoma (NHL). ►Hodgkin disease is further subclassified as either nodular lymphocyte predominant Hodgkin lymphoma or classical Hodgkin lymphoma with the four specific subtypes: nodular sclerosis, lymphocyte-rich, mixed cellularity and lymphocyte depletion. The non-Hodgkin lymphomas are subclassified according to morphology, immune phenotype, rearrangement of the immunoglobulin genes and recurrent ►chromosomal aberrations. ►Chronic lymphocytic leukemia/small lymphocytic leukemia and ►mantle cell lymphoma are characterized by obligatory expression of ►CD5 in the cell membrane. The most common NHL is ►diffuse large B-cell lymphoma, which is not a single uniform entity

as demonstrated by gene expression profiling and molecular genetics (►Bcl-6 translocations in B-cell tumors), but may contain several related disease entities with variable clinical course. The most common small cell lymphoma is ►follicular lymphoma, characterized by the t(14;18) recurrent translocation resulting in rearrangement of ►Bcl-2. Several other small cell lymphoproliferative syndromes, often referred to as ►indolent lymphomas, exists including extranodal ►marginal zone ►B-cell lymphoma of ►MALT (mucosa associated lymphoid tissue) type, splenic marginal zone lymphoma and lymphoplasmacytic lymphoma (with the clinical syndrome of ►Waldenström macroglobulinemia). ►Burkitt lymphoma is a very aggressive disease characterized by the rearrangement of the ►MYC oncogene exists plus a number of rarer conditions and variants. The non-Hodgkin lymphomas have a very heterogenous clinical course and biology, from highly aggressive but potentially curable diseases such as diffuse large cell lymphoma and Burkitt lymphoma to indolent but incurable diseases as CLL and follicular lymphoma. Even these seemingly indolent diseases may over time acquire additional molecular changes, resulting in transformation to large cell lymphomas (Richters syndrome).

The plasma cell neoplasms are dominated by ►multiple myeloma and related variants as monoclonal gammopathy of unknown significance to localized plasmacytoma or disseminated plasma cell leukemia.

Diagnosis

The complexity of the hematopoietic system (Fig. 1) is reflected by the existence of a wide spectrum of distinct tumors, with numerous variants (Figs. 1 and 2). Optimal treatment of a given condition is dependent on exact classification of the disease. It is not sufficient to rely solely on morphology, cytochemistry and immunophenotype, since some specific conditions, which require specific treatments, can only be identified based on cytogenetics (►Chromosomal aberrations) or even more sensitive molecular methods as fluorescent in-situ hybridization (►FISH), polymerase chain reaction (►PCR) or DNA-sequencing of specific genes. The classification of hematological malignancies described here is based on these features, but also recognizes the clinical course of specific entities, such as the establishment of MDS- or therapy-related AML's as diseases with particularly poor prognoses compared to AML's with recurrent cytogenetic translocations. The continued elucidation of molecular mechanisms underlying hematological malignancies and the application of new technologies, such as ►microarray (cDNA) technology ►differential display or ►microRNA analysis, in this research will establish new entities and create a need for further revisions of the dynamic classification scheme in the near future.

Treatment

Biological risk prediction and targeted therapy, using antibodies alone, or in combination with chemotherapy or as intelligent probes carrying a radionucelotide to the very center of the tumor (►Radioimmunotherapy), are now being applied for the treatment of hematopoietic neoplasms. The indications for ►immunotherapy using ►allogeneic ►bone marrow transplantation are steadily expanding. A multitude of new designed drugs are under systematic development (►Drug design).

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Hematopoiesis

Definition

Is the formation of blood cellular components. All of the cellular components of the blood are derived from haematopoietic stem cells.

►Acute Myeloid Leukemia

Hematopoietic Niche

Definition

Specific ►microenvironment to support the maintenance of multipotent stem cell activity of hematopoietic

stem cells. In bone marrow, osteoblasts are considered to provide major niche for the stem cells.

►Rap1 and Sipa-1

Hematopoietic Progenitors

Definition

Stem cells capable of differentiating into all cell types of the various blood lineages.

►AAV

Hematopoietic Stem Cell

Definition

HSC; A rare cell comprising <0.01% of normal bone marrow. It is normally quiescent in the osteoblast stem cell niche of the marrow but upon cell-cycle activation undergoes asymmetric self-division generating additional stem cells (self-renewal) and committed progenitors of multiple lineages (pluripotentiality). It is a target for oncogenic transformation in leukemia.

►NUP98-HOXA9 Fusion
►Stem Cell Telomeres

Hematopoietic Stem Cell Transplants

Definition

HSCT; A procedure also referred to as bone marrow transplantation, to deliver bone marrow stem cells to a patient, which for example suffers from cancer. The transplanted cells can originate from another individual (allogeneic) or from the same individual, which are transplanted back after removal (autologous) to stimulate blood cell production. An allogeneic transplant can be matched or mismatched for HLA self-antigens.

►Natural Killer Cell Activation
►Minimal Residual Disease

Hematopoietic System

Definition

The network of tissues, located principally in the bone marrow, that functions throughout life to replenish and replace the different cellular components of the blood.

►Chemokine Receptor CXCR4

Hematoporphyrin Derivative

Definition

HPD; Hematoporphyrin is a photosensitizer consisting of porphyrin derivatives. It is used in photodynamic therapy and fluorescence diagnosis due to its photochemical and fluorescent properties.

►Photodynamic Therapy

Hematoxylin and Eosin

Definition

Most used staining method in histology that uses a basic dye (hematoxylin) that stains basophilic structures such as nucleic acids in blue and an acid dye (eosin) that stains acidophilic structure such as proteins in red. Also known as H&E stain.

►Hurthle Cell Adenoma and Carcinoma

Hematuria

Definition

Excretion of blood in urine.

►Chemoprotectants

Heme

Definition
Is the prosthetic group of hemoglobin, myohemoglobin, cytochromes, and metalloproteins. It is composed of a heteocyclic organic ring with a central iron atom and formed in mitochondria. It is responsible for the red color of the blood (erythrocytes) and mediates the binding of oxygen.

►Photodynamic Therapy

Heme-Oxygenase 1

Definition
An inducible enzyme with potent anti-inflammatory and anti-cancer activities.

►Inflammation

Hemichannel

Definition
Is used to designate what is essentially a half gap junction channel; this is referred to as a "connexon" if composed of connexins, and a "pannexon" if composed of pannexins.

►Pannexins

Hemihypertrophy

Definition
Congenital muscular or osseous hypertrophy of one side of the face or body.

►Nephroblastoma

Hemizygous

Definition
Having only one copy of a gene as opposed to the two normally seen in a diploid genome.

►Allele

Hemizygous Deletion

Definition
In a diploid organism, loss of one ►allele or portion of an allele from the genome.

Hemochromatosis

Definition
A hereditary condition associated with iron overload. Because of increased reactive and nitrogen oxide species in the liver, there is a high risk of liver cancer in people with uncontrolled hemochromatosis.

►Inflammation
►Liver Cancer, Molecular Biology

Hemoglobin

Definition
Is a 64.5 kDa protein with heme as the oxygen-binding prosthetic group. It is responsible for binding and transport of oxygen by erythrocytes from the lung to the peripheral organs and tissues of the body.

►Photodynamic Therapy

Hemoglobinopathy

Definition
Refers to a range of genetically inherited disorders of red blood cell hemoglobin and includes sickle cell disease and the thalassemias.

►Adult Stem Cells

Hemosiderin

Definition
Is an insoluble amalgam of degraded ferritin plus ferric hydroxide. As ferritin becomes satiated with iron, it is transformed into hemosiderin. Excessive accumulation of hemosiderin is associated with macrophage cell death.

►Asbestos
►Macrophages

Henle–Koch Postulates

►Koch's Postulates

Hensin (Rabbit)

►Deleted in Malignant Brain Tumours 1

Heparan Sulfate

Definition
HS; is a linear sulphated polysaccharide found as part of proteoglycans on the cell surface or extracellular matrix, in all animal tissues. HS consists of alternating hexuronate and glucosamine units. The hexuronate can be either d-glucuronate (GlcA) or l-iduronate (IdoA). The amine of the glucosamine is usually acetylated (GlcNAc) or sulfated ($GlcNSO_3$), but it may also be unsubstituted. Within *N*-sulfated domains of HS, 2-hydroxyl groups of GlcA and IdoA, and 6-hydroxyl groups of $GlcNSO_3$ may be sulfated. Sometimes there are also 3-*O*-sulfate groups present on $GlcNSO_3$ units. There is evidence for a temporally and spatially controlled expression of HS epitopes within different organs and tissues.

Heparan Sulfate Proteoglycans

Definition
Consist of a core protein to which chains of the negatively charged glycosaminoglycan, heparan sulfate, are attached. The combination of various classes of core proteins and highly polymorphic heparan sulfate chains creates a superfamily of macromolecules that are widely distributed in mammalian tissues. Among their many activities, heparan sulfate proteoglycans are able to influence cell growth and differentiation by interacting with various proteins such as growth factors.

►Lactoferricin Antiangiogeneis Inhibitor

Heparanase

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Definition
Heparanase is a mammalian enzyme (endo-β-glucuronidase) degrading heparan sulfate (HS), a ubiquitous strongly anionic linear polysaccharide associated with the cell surface and ►extracellular matrix (ECM) of a wide range of cells of vertebrate and invertebrate tissues. The enzyme cleaves glycosidic bonds in HS with a hydrolase mechanism and is thus distinct from bacterial eliminases, called heparinases and heparitinase. The heparanase enzyme facilitates cell migration and egress from blood vessels and hence plays a role in tumor ►metastasis, ►angiogenesis, ►inflammation

and autoimmunity. Heparanase activity has been identified in a variety of human tumors (i.e., melanoma, carcinoma of the breast, liver, colon, prostate and pancreas; myeloid leukemia) and certain normal cells (i.e., cytotrophoblasts, platelets, neutrophils, activated T-lymphcytes). HS chains (Mr ~30,000) are cleaved by heparanase at only a few sites, resulting in HS fragments of still appreciable size (Mr ~5,000). Thus, the enzyme recognizes a particular and quite rare HS structure. A 2-O sulfate group on hexuronic acid residue located two monosaccharide units away from the cleavage site appears essential for substrate recognition by heparanase (Fig. 1). Heparanase activity (pH optimum in solution ~6.0) has been demonstrated in both lysosomal and endosomal cellular compartments and in the cell membrane. In human neutrophils the enzyme is localized in tertiary granules.

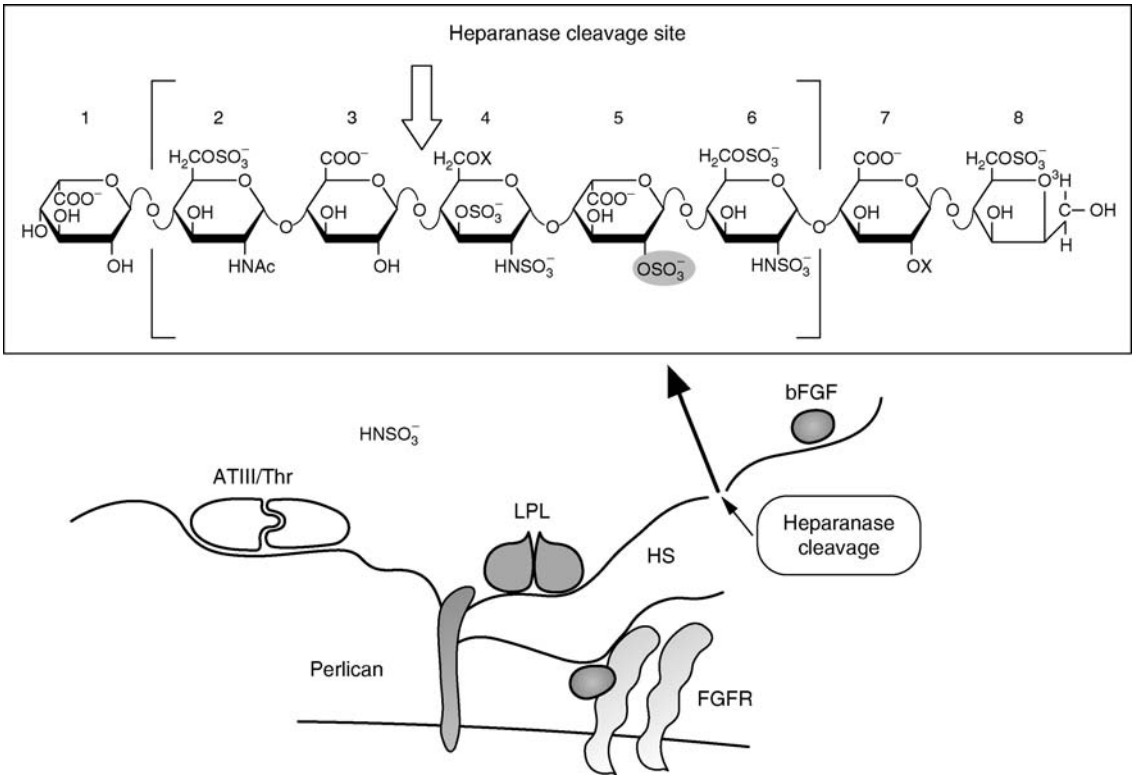
Characteristics

Heparan Sulfate Proteoglycans (HSPGs)

The basic HSPG structure consists of a protein core to which several linear heparan sulfate (HS) glycosaminoglycan (GAG) chains are covalently O-linked. The HS chains are typically composed of repeating hexuronic (L-iduronic or D-glucuronic acid) and D-glucosamine disaccharide units that are substituted to a varying extent with N- and O-linked sulfate moieties and N-linked acetyl groups (Fig. 1). The HS chains generally consist of clusters of sulfated disaccharide units separated by low or non-sulfated regions. Studies of the involvement of extracellular matrix (ECM) molecules in cell attachment, growth and differentiation revealed a central role of HSPGs in embryonic morphogenesis, angiogenesis, metastasis, neurite outgrowth and tissue repair. The HS chains, unique in their ability to bind a multitude of proteins, ensure that a wide variety of effector molecules (i.e., heparin-binding growth factors, chemokines, lipoproteins, enzymes) (Fig. 1) cling to the cell surface and ECM and thereby exert localized cellular effects. Moreover, transmembrane (syndecan) and membrane anchored (glypican) HSPGs have a co-receptor role in which the proteoglycan, in concert with other cell surface molecules, comprises a functional receptor complex that binds the ligand and mediates its action.

Molecular Properties

Cloning and expression of a single human heparanase cDNA sequence was achieved using amino acid sequences derived from heparanase enzymes purified from human platelets, placenta, hepatoma cells and



Heparanase. Figure 1 Scheme of heparan sulfate and the heparanase cleavage site (*top*), and of a basement membrane HSPG (perlecan) (*bottom*). Cleavage is associated with release of HS-bound growth factors and enzymes.

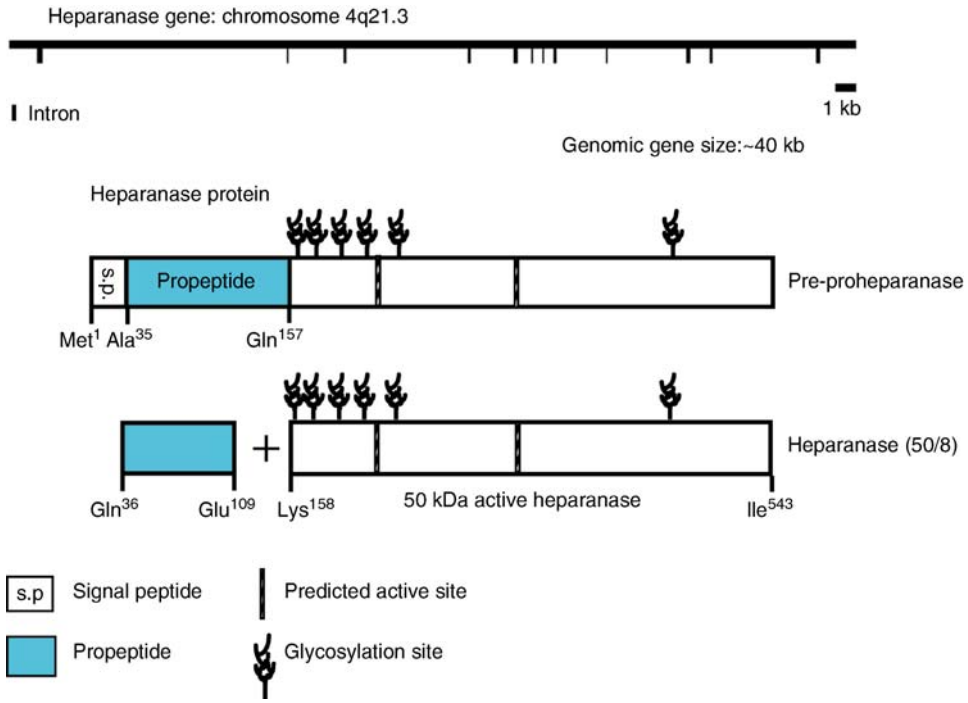
transformed embryonic fibroblasts. The heparanase cDNA contains an open reading frame of 1629 bp, which encodes for a 61.2 kD latent polypeptide of 543 amino acids (Fig. 2). This pro-enzyme is post translationally cleaved into 8 and 50 kDa subunits that non-covalently associate to form the active heparanase (Fig. 2). Heterodimer formation is essential for heparanase activity. Site-directed mutagenesis revealed that similar to other glycosyl hydrolases, heparanase has a common catalytic mechanism that involves two conserved acidic residues, a putative proton donor at Glu^{225} and a nucleophile at Glu^{343} (Fig. 2). The sequence also contains a putative N-terminal signal peptide sequence (Met1 to Ala35) and a candidate transmembrane region (Pro515 to Ile534; Fig. 2). Alignment of the human, mouse and rat heparanase amino acid sequences, corresponding to the 50 kD human mature enzyme (Lys158 to Ile543), demonstrated 80.0%, 79.7% and 92.7% identity between the human and mouse, human and rat, and mouse and rat heparanases, respectively. A 58–60% homology was found between these enzymes and the chicken heparanase. The fact that highly homologous cDNA sequences were derived from different species and types of normal and malignant cells is consistent with the notion that one dominant endoglucuronidase is expressed by all mammalian cells. Thus, unlike the large number of proteases that can solubilize polypeptides in the ECM, it appears that only one heparanase is used by cells to degrade the heparan sulfate side chains of HSPGs. The genomic locus that encodes heparanase spans ~40 kb. It is composed of 12 exons separated by 11 introns and is localized on human chromosome 4q21.3.

Preferential Expression in Human Tumors

Expression of the heparanase mRNA and protein in normal tissues is restricted to the placenta, activated immune cells, platelets and keratinocytes. Heparanase is preferentially expressed at early stages of carcinoma progression. Immunohistochemistry, *in situ* hybridization, RT-PCR and real time-PCR analyses revealed that heparanase is up regulated in essentially all human tumors examined. These include carcinomas of the colon, thyroid, liver, pancreas, lung, bladder, cervix, breast, gastric, prostate, head and neck, salivary gland, nazopharyngs, as well as leukemia, lymphoma and multiple myeloma. Patients exhibiting high levels of heparanase mRNA and/or protein had a significantly shorter postoperative survival time than patients whose tumors contained relatively low levels of heparanase. There is also a correlation between heparanase expression levels and tumor vascularity in cancer patients, indicating a significant role in tumor angiogenesis.

Involvement in Tumor Metastasis

A critical event in the process of cancer invasion and metastasis is degradation of various ECM constituents including collagen, laminin, fibronectin and HSPGs. The ability of HSPGs to interact with various ECM macromolecules and with different attachment sites on plasma membranes suggests a key role for this proteoglycan in



Heparanase. Figure 2 Scheme of the human heparanase gene and protein.

the self-assembly and insolubility of ECM components, as well as in cell adhesion and locomotion. Cleavage of HS may therefore result in disassembly of the subendothelial ECM and hence play an important role in fundamental biological phenomena involving cell migration and ranging from pregnancy, morphogenesis and development to inflammation, angiogenesis and cancer metastasis. Expression of HS degrading heparanase correlates with the metastatic potential of tumor cells. Moreover, elevated levels of heparanase were detected in sera of metastatic tumor-bearing animals and cancer patients, in tumor specimens and in the urine of patients with aggressive metastatic disease. A direct role of heparanase in tumor metastasis was provided by the increased lung, liver and bone colonization of melanoma, lymphoma, myeloma and prostate carcinoma following transfection and over-expression of the heparanase gene. A marked decrease in the metastatic potential of cells expressing high levels of endogenous heparanase was noted in response to administration of anti-heparanase siRNA, indicating that heparanase is causally involved in cancer progression.

Involvement in Tumor Angiogenesis

Angiogenesis represents a coordinated multicellular process that requires the functional activity of a wide variety of molecules including growth factors, ECM components, adhesion receptors and matrix-degrading enzymes. HSPGs and HSPG-degrading enzymes have been implicated in a number of angiogenesis-related cellular events including cell invasion, migration, adhesion, differentiation and proliferation. The heparin affinity of fibroblast growth factors (FGFs) and other heparin-binding growth factors appears to be the basis for their storage in ►basement membrane (BM) and ECM, where they are bound to HS and can be released in an active form by HS degrading enzymes. The released angiogenic factors may then stimulate endothelial cell proliferation and migration associated with neovascularization.

An important early step in the angiogenic cascade is degradation of the subendothelial capillary BM by proliferating endothelial cells and formation of vascular sprouts. Heparanase, degrading the polysaccharide scaffold of BM, markedly contributes to the invasive ability of endothelial cells and their migration through the ECM toward the angiogenic stimulus. Apart from direct involvement in BM invasion by endothelial cells, the heparanase enzyme elicits an indirect angiogenic response by releasing HS-bound angiogenic growth factors from ECM and BM (Fig. 1) and by generating HS degradation fragments that stabilize and promote the mitogenic and angiogenic activity of heparin-binding growth factors (i.e., bFGF, ►VEGF). In fact, recombinant mammalian heparanase, as well as heparanase secreted by platelets, tumor cells and inflammatory cells, release FGF as a complex with HS fragments. This yields a highly active form of FGF that readily interacts with high affinity receptors on the surface of endothelial cells and thus elicits an angiogenic response. The molecular size of the HS chain required for optimal stimulation of FGF receptor binding, dimerization and signaling is similar to that of HS fragments released by heparanase. A profound angiogenic response is elicited in vivo by lymphoma cells overexpressing the heparanase gene and embedded in a reconstituted BM (Matrigel). It appears that cooperative interaction between heparanases from tumor, inflammation and endothelial sources play an important role in the angiogenic cascade. Apart of the well studied catalytic feature of the enzyme, heparanase was noted to exert biological functions apparently independent of its enzymatic activity. Non enzymatic functions of heparanase include enhanced cell adhesion and induction of p38 and Akt phosphorylation. Moreover, enzymatically active and inactive heparanase were noted to induce VEGF expression, thus providing, among other mechanisms, a molecular basis for the potent angiogenic capacity of heparanase. The anti-cancerous potential of heparanase inhibitors is, therefore, not restricted solely to suppression of the invasive metastatic phenotype, but also to suppression of tumor angiogenesis.

Clinical Relevance

Heparanase inhibiting molecules (e.g., non-anticoagulant species of heparin, polysulfated polysaccharides, polyanionic molecules) markedly reduce (>90%) the incidence of lung colonization induced by various tumor cells, in correlation with their anti-heparanase activity. The occurrence of a single heparanase species and its ability to promote both tumor angiogenesis and metastasis, the most critical steps in tumor progression, make it a promising target for cancer therapy. The heparanase inhibiting pentasaccharide, phosphomannopentaose sulfate (PI-88) is being tested in cancer patients as it reduces the vascularity, primary tumor growth and metastasis of mammary adenocarcinoma in rats. A recently developed ELISA method indicates that determination of heparanase levels in body fluids (e.g., plasma, urine, saliva, pleural effusuions) may have a prognostic value in detection of cancer and response to treatment.

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Heparanase Inhibitors

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Definition

Heparanase inhibitors are a group of compounds inhibiting/decreasing heparanase enzymatic activity, which abolish degradation of heparanase on heparan sulfate (HS) of extracellular matrix (ECM) and thus subsequential cascade.

Characteristics

►Heparanase, a single functional HS-degrading endoglycosidase, is a mammalian endo-β-*D*-glucuronidase cleaving HS chains at a limited number of sites. The enzyme is synthesized as a latent 65-kDa precursor that undergoes subsequent proteolytic cleavage, yielding 8-kDa and 50-kDa subunits. These subunits heterodimerize to form a highly active enzyme. The enzyme is thought to participate in the cleavage of HS chains from HS-containing proteoglycans (HSPGs), leading to ECM remodeling that facilitates the cell invasiveness and ►angiogenesis associated with ►cancer metastasis. The development of ►heparanase inhibitors for the treatment of highly malignant tumors is therefore of considerable interest.

In view of its enzymatic characteristics, much effort has devoted in drug development targeting the substrate as mimetics based on the high-affinity interaction between the enzyme and its substrate. These include heparin, chemical derivatives of heparin, nonanticoagulant heparin, and other polyanionic molecules such as sulfated phosphomannopentaose (PI-88), oligomannurarate sulfate (JG3), laminaran sulfate, and suramin. Others targeting heparanase itself includes small molecules, peptides, and neutralizing antibodies. In addition, effort also has been made on several fronts to inhibit heparanase gene expression.

Inhibitors Targeting the Heparanase Substrate

Heparin, Chemically Modified Derivative and Nonanticoagulant Heparin

As an analog of the natural substrate of heparanase, heparin is commonly considered to be a potent inhibitor of heparanase. This activity is attributed, in part, to the high affinity heparin–enzyme interaction and the limited degradation of heparin. Heparin thus serves as an alternative enzyme substrate. Early reports showed that heparin and some of its derivatives, as well as other sulfated polysaccharides inhibiting tumor cell heparanase, also inhibited experimental metastasis in animal models. Other related compounds lacking heparanase-inhibiting activity failed to exert an antimetastatic effect. No significant differences in tumor inhibition were found between the currently used unfractionated heparins and low molecular weight heparins (LMWH). Regardless of the mode of action, heparin and LMWH were reported to exert a beneficial effect in cancer patients. The use of heparin as an antimetastatic agent is, however, limited due to its potent anticoagulant activity. This drawback has stimulated research on the potential use of modified, nonanticoagulant species of heparin and HS in cancer therapy.

Glycol-split *N*-acetyl heparin, which shows dramatically increased heparanase-inhibiting activity compared with unmodified heparin, is a valuable compound. Notably, glycol-splitting causes substantial loss of the anticoagulant activity of heparin due to the complete removal of heparin affinity for antithrombin. The main reason for such an effect is the cleavage of C-2–C-3 bonds of the GlcA residue of the pentasaccharidic sequence, which is essential for binding to antithrombin. Encouragingly, glycol-split *N*-acetyl heparins also exhibit marked decreases in the ability to release basic fibroblast growth factor (bFGF) from the ECM. Mitogenic activity in the ECM is thus inhibited.

Work on heparanase inhibition by glycol-split heparin is mostly recent. The data from this nonanticoagulant heparin species applying on animals gave us clues that it is possible to separate the antimetastatic and anticoagulant activities of heparin. We thus predict that nonanticoagulant heparin species may have clinical potential, because they can be given in doses higher than those used for heparin, thereby exploiting fully the antimetastatic component of heparin species. Together, the combination of potent heparanase inhibition, the lack of anticoagulant activity, and the low release of ECM-bound bFGF, promises that *N*-acetylated glycol-split heparins will be highly effective and specific antiangiogenic and antimetastatic agents.

Sulfated Phosphomannopentaose

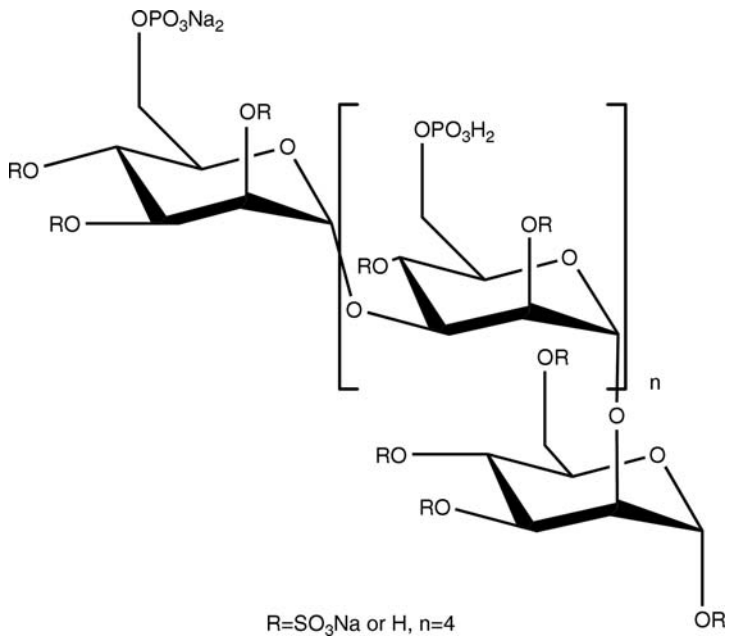
Heparanase enzyme activity also can be efficiently inhibited by shorter but more extensively sulfated oligosaccharides. The use of such small molecules may overcome intractable side effects caused by

administration of larger inhibitors. Researchers have therefore sought small saccharides potently inhibiting heparanase activity but without side effects. The first such molecule, PI-88, showed good safety and tolerability in Phase I trials and the Food and Drug Administration has now cleared PI-88 for a Phase III clinical trial.

PI-88, a highly sulfated phosphosulfomannan (Fig. 1), is a hemisynthesized product obtained by sulfation of a saccharide from the yeast *Pichia holstii*. PI-88 is a mixture of chemically sulfated oligosaccharides, ranging from disaccharides to hexasaccharides, with the majority (60%) being pentasaccharides. PI-88 exerts its biological effects by blocking the enzymatic activity of heparanase and by interfering with the action of HS-binding angiogenic growth factors such as aFGF, bFGF, and VEGF. In an animal model, PI-88 was shown to inhibit the primary tumor growth of the highly invasive rat mammary adenocarcinoma 13762 MAT by ~50%, to inhibit metastasis to the draining popliteal lymph node by ~40%, and to reduce the vascularity of tumors by ~30%. All these effects are highly significant. Acute hematogenous metastasis assays also demonstrated that PI-88 was a potent (>90%) inhibitor of blood-borne metastasis. The data from a completed Phase II clinical trial showed that PI-88 was especially well-suited to postoperative use. In particular, PI-88 was effective in postoperative liver cancer patients in whom tumor burdens were low. PI-88 is one of a new class of multi-targeted cancer drugs that have an antiangiogenesis effect. A particular concern with PI-88 systemic treatment is drug interference with a relatively broad range of protein–HS interactions. An interpretation of drug specificity is thus difficult.

Oligomannurarate Sulfate-JG3

The success of PI-88 inspired a surge in the development of additional oligosaccharide-based innovative heparanase inhibitors, using substrates mimicking GAG as prototypes. Oligomannurarate sulfate, a new, hemisynthesized, structurally novel, sulfated oligosaccharide derived from marine oligomannurarate blocks (Fig. 2), showed promising potential substrate-based heparanase inhibitor. JG3 is a highly sulfated oligosaccharide with molecules ranging in size from tetrasaccharides to decasaccharides. JG3 significantly inhibited tumor angiogenesis and metastasis both in vitro and in vivo. As a nondegradable substrate mimetic, JG3 specifically binds to the KKDC and QPLK domains of heparanase, with high affinity, and significantly inhibited heparanase activity (the IC_{50} was 6.55 ng/ml). The JG3–heparanase interaction was competitively inhibited by LMWH but not by other GAGs. In addition, JG3 abolished heparanase-driven invasion, inhibited the release of HS-sequestered bFGF from the ECM, and repressed subsequent angiogenesis. Moreover, JG3 inactivated bFGF-induced FGFR and ERK1/2 phosphorylation, and blocked bFGF-triggered angiogenic events by directly binding to bFGF. Thus, JG3 appears to inhibit both major heparanase activities by simultaneously acting as both a substrate mimetic and a competitive inhibitor of heparan sulfate. In vivo, the B16F10 ►melanoma lung metastasis model, orthotopic xenografts of human breast carcinoma MDA-MB-435, and ►hepatocellular carcinoma BEL-7402 in athymic mice, have been used to evaluate the effects of JG3 on tumor growth, angiogenesis and metastasis. JG3



Heparanase Inhibitors. Figure 1 Phosphomannopentaose (PI-88).

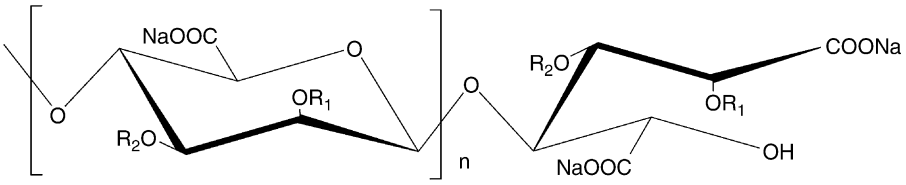
dramatically decreased the numbers of B16F10 metastases, with an inhibition rate of 82.2%. Also, JG3 suppressed MDA-MB-435 tumor growth by 37.6%, abated tumor metastasis by 88.3%, and inhibited tumor-related angiogenesis. In addition, JG3 inhibited hepatocellular carcinoma BEL-7402 primary tumor growth by 44.2%. Encouragingly, unlike other polyanionic compounds, JG3 showed very low toxicity, probably due to its weak anticoagulant activity (~13-fold less than that of heparin, and nearly threefold less than that of PI-88). These data all suggest that JG3 is potentially valuable as an ►anticancer agent.

Suramin

Suramin (Fig. 3) is a polysulfonated naphthyl urea, inhibiting heparanase with an IC_{50} of 48 μM. Suramin inhibited B16 melanoma cell invasion (IC_{50} = 10 μM) through reconstituted basement membrane, but had no effects on melanoma cell growth. Suramin has not been widely used because it has significant toxic effects in humans, including neurotoxicity, renal toxicity, adrenal insufficiency, and anticoagulant-mediated blood dyscrasias. In efforts to avoid these side effects, analogs of suramin have been synthesized and are undergoing evaluation. Compounds NF 227, NF 145, and NF 171 are three such analogs, all of which possess heparanase inhibitory activities more potent than that of suramin (the IC_{50} values were ~20–30 μM). These compounds effectively inhibited heparanase-mediated angiogenesis in an animal model.

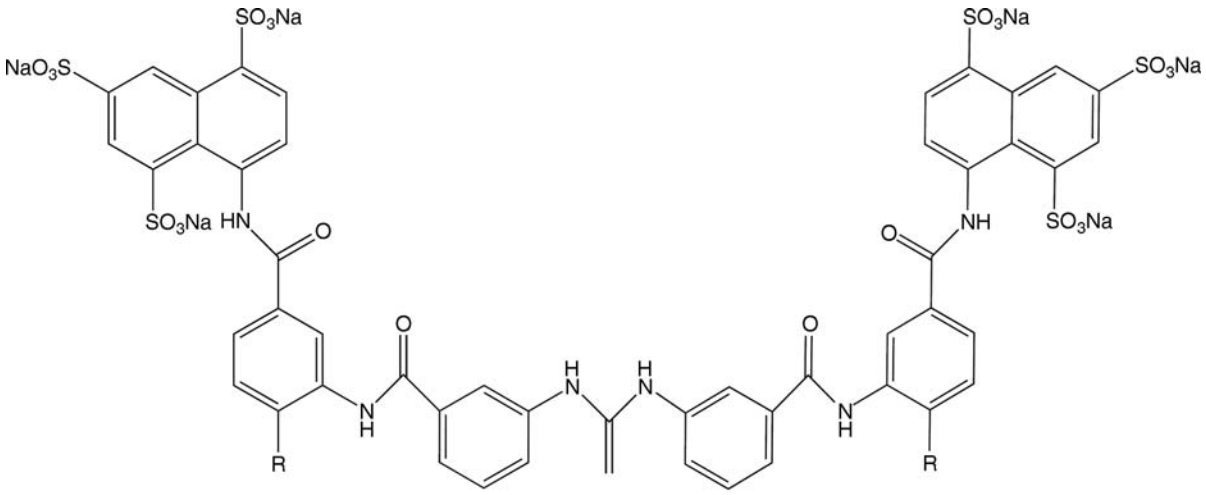
Noncarbohydrate Substrate Mimetic Polymers

Synthetic, linear, noncarbohydrate polyanionic polymers have also been studied as heparin mimetics. The materials were initially tested for inhibition of heparanase-mediated degradation of HS in the subendothelial ECM. One series of polymers, synthesized by polymerization of phenol-based monomers, showed high inhibitory effects on heparanase activity. Of these, RG-13577, polymerized from 4-hydroxyphenoxy monomers, has a molecular weight of ~5,800, and showed almost complete inhibition of heparanase activity at 2.5 μg/ml.



R_1=SO_3Na, R_2=SO_3Na or H, n = 3–9

Heparanase Inhibitors. Figure 2 Oligomannurarate sulfate (JG3).



R=Me

Heparanase Inhibitors. Figure 3 Suramin.

Another group of polymers, derived from *N*-acryl amino acid monomers bearing charged functional groups, range from ~3,000 to 6,000 Da in size, and also showed potent inhibition of heparanase. Polymers with hydroxyl groups, when sulfonated, showed ~70–80% inhibition of heparanase activity even at 1 μg/ml. This activity is comparable to that of heparin.

Inhibitors Targeting Heparanase

Small Molecules

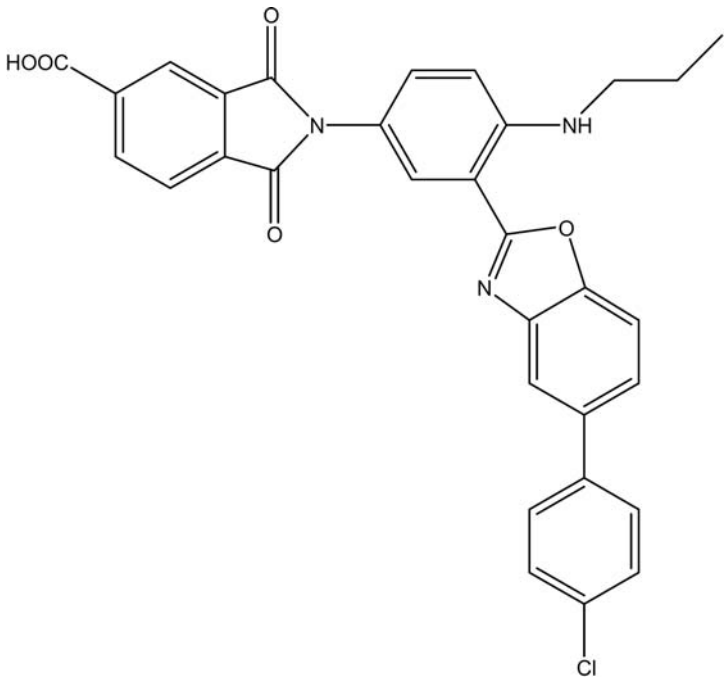
Besides the efficacy of heparanase substrate mimetics, development of small-molecular weight compounds directly inhibiting heparanase activity is still required. Several heparanase inhibitors have been discovered among microbial metabolites or have been obtained by organic synthesis. This group of compounds does not contain any sulfate moieties and the group members have the clearly defined chemical structures. Compounds showing high potency against heparanase in vitro and in animal models, which would be developed as therapeutic leads.

A class of inhibitors, exemplified by 1*H*-isoindole-5-carboxylic acid, was reported. The structure activity relationship study of this class compound led to 2-[4-propylamino-5-[5-(4-chloro)phenyl-benzoxazol-2-yl] phenyl]-2,3-dihydro-1,3-dioxo-1H-isoindole-5-carboxylic acid (Fig. 4), which displayed potent heparanase inhibitory activity (IC_{50} = 0.2 μM) and a much improved antiangiogenic effect (IC_{50} = 1 μM) when compared to other derivates.

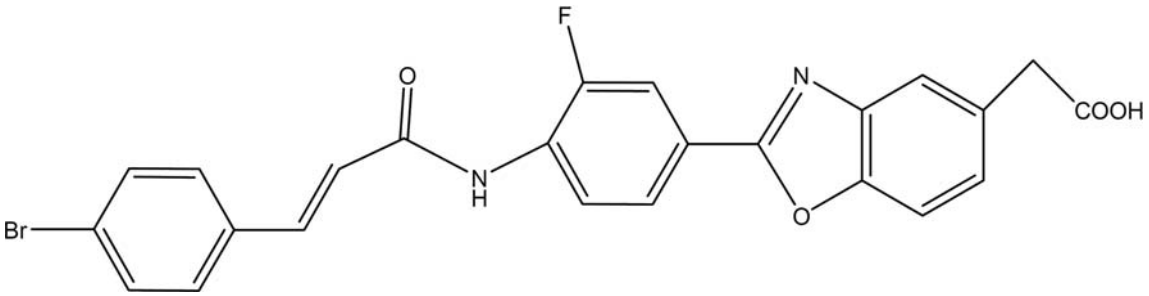
Other valuable small molecules are furanyl-1, 3-thiazol-2-yl-acetic acids and derivatives developed as potent heparanase and angiogenesis inhibitors. Although they showed promising in vitro potency against heparanase, this series of compounds suffered drawbacks such as synthetic difficulties and poor DMPK properties. On its structure basis, the changed chemical structure (benzoxazol-5-yl-acetic acids) showed much more activity and potency on DMPK. The most powerful inhibitor in this class (Fig. 5) showed antiheparanase activity with an IC_{50} of 0.4 μM and antiangiogenesis with an IC_{50} of 1 μM. More importantly, the material attained a plasma concentration ca. tenfold above its IC_{50} value for heparanase inhibition.

Novel 1-[4-*H*-benzoimidazol-2-yl]-phenyl]-3-[4-(1*H*-benzoimidazol- 2-yl)-phenyl]-ureas were recently described as potent inhibitors of heparanase. Among these compounds, 1,3-bis-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea (Fig. 6) had high heparanase inhibitory activity (IC_{50} = 0.075 μM) in vitro and was effective in a B16 experimental metastasis model.

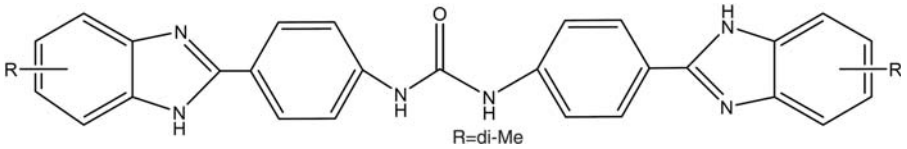
Two other types of small inhibitory molecules are those in the RK-682 series and the KI-105 series. Compound RK-682 was isolated from a *Streptomyces* species and showed anti-heparanase activity (IC_{50} = 17 μM). The derivative 4-Bn-RK-682, showed better selectivity for heparanase than did RK-682. Compound KI-105 was synthesized to mimic the anionic groups of HS using nitro and benzoic acid moieties.



Heparanase Inhibitors. Figure 4 2-[4-propylamino-5-[5-(4-chloro)phenyl-benzoxazol-2-yl]phenyl]-2,3-dihydro-1, 3-dioxo-1H-isoindole-5-carboxylic acid.



Heparanase Inhibitors. Figure 5 Benzoxazol-5-yl-acetic acids.



Heparanase Inhibitors. Figure 6 1,3-bis-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea.

Peptides Competing with the Heparin/HS Binding Domains of Heparanase

It has been reported that a peptide with the KKDC motif of the 50 kDa heparanase subunit region (Lys158–Asn171) physically associated with both heparin and HS, and inhibited heparanase enzymatic activity in a dose-dependent manner.

Antiheparanase Antibodies

Very limited work has been performed in this area. In one study, a rabbit antibody against recombinant heparanase demonstrated dose-dependent inhibition of heparanase activity. The antibody inhibited ovarian carcinoma cell invasion at a concentration of 0.67 μM.

Conclusions and Perspectives

As the predominant enzyme in tumor cells, heparanase represents an attractive target for the development of novel anticancer agents. Most research on heparanase inhibitors remains, however, at the in vitro or early preclinical stages. Currently, all heparanase inhibitors raise specificity concerns, because they may cross-react with other biological molecules including heparin-binding proteins or even tyrosine kinases. The phenotypes of animals with heparanase gene knockouts should provide great insights into the roles of heparanase in the complex in vivo environment. Studies on enzyme kinetics will help to obtain complete information on heparanase biochemistry. The determination of heparanase crystal structure will tremendously benefit rational drug design, leading to the development of more specific and more potent inhibitors. Also, researchers continue to focus on further defining heparanase substrate specificity, exploring both catalytic and noncatalytic enzyme activities. This work, in combination with enzyme crystal structure determination, allows us to hope that a more "rational" approach to the development of effective and highly specific heparanase inhibitors will soon be possible. We predict that future data will further reinforce the idea that heparanase represents a tractable and highly attractive target for novel types of anticancer agents.

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Heparin

Definition

Is a negatively charged polysaccharide that is a derivative of heparan sulfate.

►Lactoferricin Antiangiogeneis Inhibitor

Heparin-affin Regulatory Peptide

►Pleiotrophin

Heparin-binding Brain Mitogen

►Pleiotrophin

8-kDa Heparin-binding Glycoprotein

►Serum Biomarkers

Heparin-binding Growth Associated Molecule

►Pleiotrophin

Heparin-binding Growth Factor 8

►Pleiotrophin

Heparin-binding Growth Factors

►Fibroblast Growth Factors

Heparin-binding Neurite Promoting Factor

►Pleiotrophin

Heparin-binding Neurotropic Factor

►Pleiotrophin

Hepatic Acinus

Definition

Is the functional unit of the liver that consists a mass of liver tissue cells (hepatocytes) aligned around the hepatic arterioles and portal venules.

►Hepatic Epithelioid Hemangioendothelioma

Hepatic Carcinoma

►Hepatocellular Carcinoma

Hepatic Epithelioid Hemangioendothelioma

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Synonyms

Sclerosing endothelial tumor; Sclerosing angiogenic tumor; Sclerosing epithelioid angiosarcoma; Sclerosing interstitial vascular sarcoma

Definition

►Epithelioid hemangioendothelioma (EH) is a neoplasm of vascular origin involving soft tissue and visceral organs such as liver, spleen, bone, brain, meninges, breast, heart, head and neck, soft tissue, stomach, and lymph nodes (Table 1).

Characteristics

The term EH was first recognized as soft tissue vascular tumors of endothelial origin with clinical course between benign ►hemangioma and ►angiosarcoma. The incidence of EH is less than 0.1 per 100,000 population. Hepatic EH (HEH) affects more commonly adult females and is characterized by an ►epithelioid or ►histiocytoid morphology and a growth pattern with evidence of endothelial histogenesis. Two different types of HEH have been described so far, the nodular type in the early stage of HEH; and the diffuse type that reflects the advanced stage of the disease with coalescence of the lesions which are associated with hepatic vascular invasion (Fig. 1a). The clinical manifestation of HEH is heterogeneous varying from cases with no symptoms to hepatic failure. In the symptomatic patients, the most common clinical manifestations include right upper quadrant pain, ►hepatomegaly, and weight loss. Weakness, ►anorexia, ►epigastric mass, ►ascites, nausea/vomiting, ►jaundice, and fatigue are the other common presenting manifestations. The majority of cases have a multifocal tumor involving both liver lobes. The right lobe is affected more than the left lobe in both multifocal and unifocal presentations. Lung, regional lymph nodes, ►peritoneum, bone, spleen, and diaphragm are the most common sites of extrahepatic involvement at the time of diagnosis. ►Pleura, ►mediastinum, ►retroperitoneum, ►myocardium, ►pericardium, brain, cervical lymph nodes, ►common bile duct, pancreas, and uterus are the other reported sites with HEH ►metastases.

Etiology

The definitive etiology of HEH is unclear. Some possible etiologic factors include oral contraceptives, vinyl chloride, ►asbestosis, thorotrast, major trauma to the liver, viral hepatitis, ►primary biliary cirrhosis, and alcohol consume. In contrast to many other types of primary liver tumors, HEH does not arise upon a background of chronic liver disease.

Diagnosis

Due to the rarity of the tumor, only strong suspicion can help to perform necessary evaluations and establish an appropriate diagnosis.

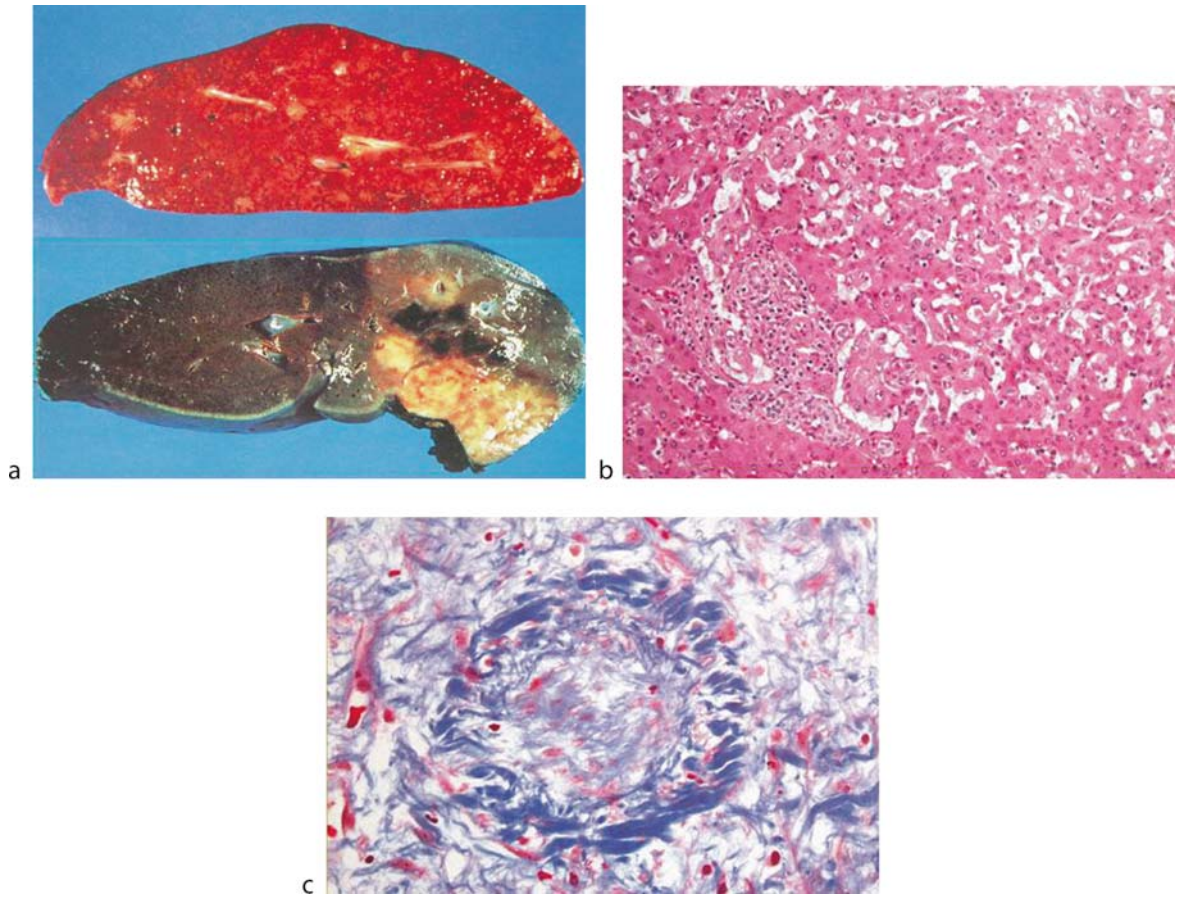
Laboratory. The majority of the cases have abnormal liver laboratory parameters such as elevated alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT) and bilirubin. ►Tumor markers are mostly within normal range and only suitable to rule out other primary or metastatic tumors.

Imaging. Imaging studies include ultrasonography, computed tomography (CT) scan, magnetic resonance imaging (MRI), ►scintigraphy, and angiography. On ultrasonography the most common finding is ►hypoechoic lesions. On CT scan, ►low-density pattern is the most common abnormal feature. Moreover, the majority of cases show contrast enhancement in CT images. Two major manifestations of HEH in CT images are multiple, nodular lesions, and/or large masses which can be the result of coalescence of smaller nodules. The nodular type is relatively nonspecific, whereas the diffuse type is very suggestive. On MRI, HEH is usually hypointense on T1-weighted and hyperintense on T2-weighted images (►T1- and T2-weighted images) (►hypointense, ►hyperintense). One of the characteristic feature of the HEH is the target shape of the lesions which could be due to the presence of a central sclerotic zone and a peripheral region of cellular proliferation (Fig. 2). On scintigraphic

Hepatic Epithelioid Hemangioendothelioma. Table 1 Comparison of epithelioid hemangioendothelioma in different sites

Organ	Age	Gender	Distribution	Angiocentricity	Mortality (%)
Soft tissue	2nd–9th Dec.	m~f	Solitary, rarely multifocal	50% Arise from vessel	13
Bone	1st–8th Dec.; peak 2nd/3rd Dec.	m>>f	50% Multifocal	?	?
Lung	Median 40y	f>m	>50% Multifocal	Intravascular; spread common	65
Liver	2nd–9th Dec.	f>m	>50% Multifocal	Intravascular; spread common	35

The majority of primary epithelioid hemangioendothelioma arises from soft tissue, bone, lung, and liver. The worst outcome is seen in the lung and hepatic tumors. There is a difference in age and gender distribution of EH in various sites [1].

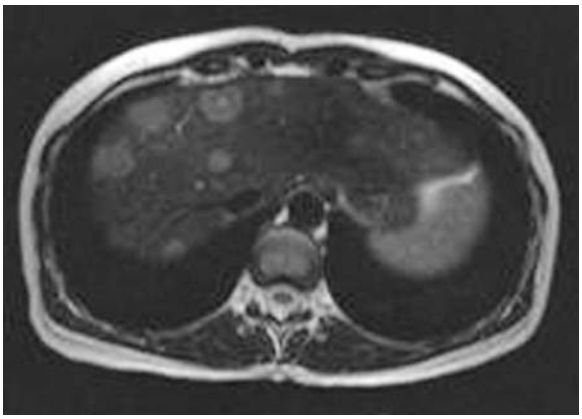


Hepatic Epithelioid Hemangioendothelioma. Figure 1 Hepatic epithelioid ►hemangioendothelioma. (a) Macroscopy: Multifocal growth pattern of nodules and coalescence of them to form confluent masses specifically in the periphery with extension to and retraction of liver capsule. (b) Hematoxylin–eosin stain: The tumor cells form intracytoplasmic lumina containing erythrocytes. (c) Tissue stain for factor VIII-related antigen: In addition to the intracytoplasmic lumens, the pleomorphic cells of tumor are immunoreactive for factor VIII related Ag in this tissue section.

imaging, ►low uptake is the major finding, which can be useful for the follow-up of patients under therapy. Angiographic findings are nonspecific ranging from hypo- to hyperperfusion. It is noteworthy that imaging studies can only lead to a strong suspicion regarding the presence of HEH and its pattern.

Histopathology. Definitive diagnosis requires a histopathological examination. Often, a laparoscopic wedge or core biopsy is sufficient to depict architectural features of the HEH such as the intravascular characteristics. The diagnosis is mostly confirmed with ►immunohistochemical (Immunohistochemistry) evidence of endothelial differentiation (Fig. 1b and c).

Differential Diagnosis. More than two thirds of HEH cases may be initially misdiagnosed. The most common misdiagnoses are ►cholangiocarcinoma, angiosarcoma, ►hepatocellular carcinoma (HCC), ►metastatic carcinoma (►Metastasis), and sclerosing hemangioma.



Hepatic Epithelioid Hemangioendothelioma. Figure 2 MRI sections of HEH T2-weighted hyperintense lesions and central necrosis showing a target appearance.

Some important features for differential diagnosis include the infiltrative growth pattern with preservation of the hepatic acinar (►Hepatic acinus) landmarks such as portal areas, the characteristic vascular invasion with tufting of portal vein branches and terminal hepatic venules, the identification of epithelioid tumor cells especially with intracytoplasmic lumina, and the delay of staining for epithelial differentiation markers.

Therapy

There is no generally accepted strategy for the treatment of HEH due to its heterogeneous dignity and variable clinical outcome. Theoretically, ►liver resection is the first choice for curative treatment of HEH, but in the majority of the cases an oncological resection is impossible due to multicentricity of the lesions or anatomical difficulties. ►Liver transplantation is generally the most common treatment modality. According to the Pychlmayr classification of hepatic malignancies, HEH is placed among the favorable indications for liver transplantation. Life expectancy of the patients with HEH is potentially good. Long-term disease-free survival after liver transplantation is reported in patients with disseminated disease at the time of diagnosis; conversely some patients with disease confined to the liver developed rapid recurrence and metastases following liver transplantation. The experience with other therapeutic modalities such as systemic or regional chemotherapy, ►transarterial chemoembolization (►Chemotherapy), and ►radiotherapy (►Chemoradiotherapy) is limited and variable, therefore, generally these treatments are of limited value especially as the first line therapy. Considering only follow-up without any treatment as a management option is controversial. There are some cases with long-term survival or even complete spontaneous tumor regression without receiving any treatment but until now, it is impossible to reliably identify HEH patients with a nonaggressive tumor and to consider them for a "wait and see" strategy. The mode of hepatic involvement and presence or absence of extrahepatic involvement are the main factors in the decision of the treatment modality.

When less than one lobe is involved and there is no extrahepatic involvement, liver resection could be done as the first choice in treatment of HEH. The therapeutic strategy in the presence of extrahepatic involvement is especially controversial. When extrahepatic involvement exists, independent of performing liver resection or not, ►adjuvant chemotherapy (►Adjuvant chemoendocrine therapy) may be considered. In the case of massive involvement of the liver, liver transplantation is the best therapeutic choice. Extrahepatic involvement by itself does not exclude liver transplantation. Although chemotherapy in this situation is questionable, it may control the growth of the extrahepatic tumor. It is noteworthy that the clinical course of HEH is variable, ranging from a favorable disease with prolonged survival, even without therapy, to a rapidly progressive disease with a grave outcome. Therefore, the decision of the treatment strategy has to be tailored for each case, and the individual rate of progression, severity of signs and symptoms, and response to other treatment modalities may be important determinants for decision making.

Clinical Outcome

Three main causes for tumor recurrence and treatment failure after liver transplantation are error in the pretransplant evaluation, enhanced tumor growth under immunosuppressive therapy, and lack of effective anticancer therapy following surgery. The 5-year survival in different reports varies from 45 to 70%. Generally, the surgical therapies such as liver resection or transplantation have the best survival rates and chemoradiotherapy or no treatment lead to the worst clinical outcome. The presence of tumor necrosis may be associated with poor outcome, while typical indicators of biologic aggression such as ►nuclear atypia, capsule penetration, and number of ►mitosis are found to be unrelated to clinical outcome. The unpredictable natural course and prognosis of HEH makes it difficult to give a correlation between the morphological grading or clinical staging and outcome.

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Hepatic Ethanol Metabolism

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Definition

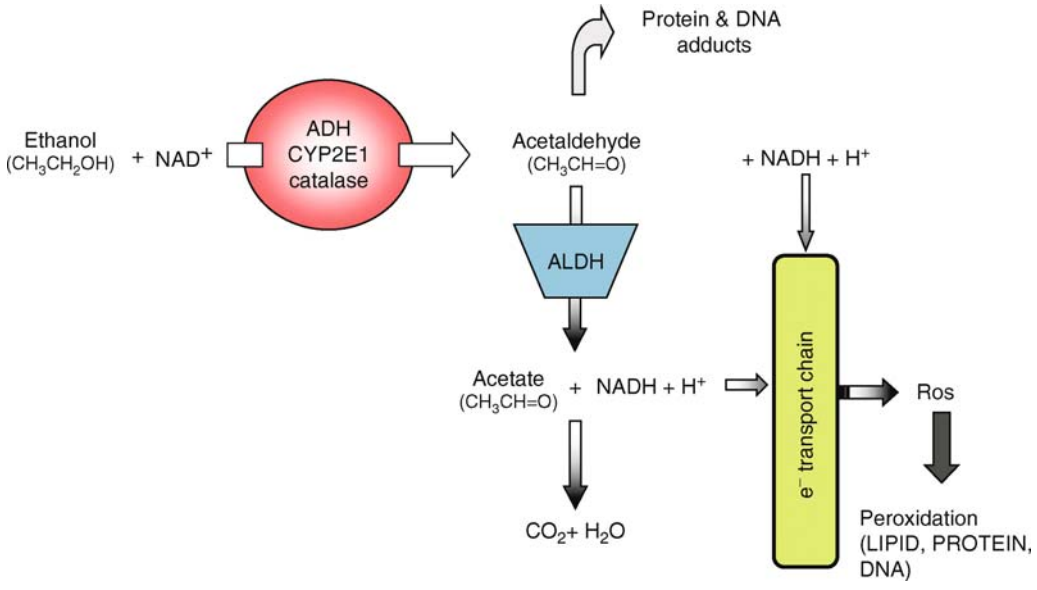
The majority of ►ethanol metabolism occurs in the ►liver following the ingestion of alcoholic beverages.

The enzymatic reactions involved in ethanol metabolism can in turn lead to a variety of deleterious effects on cells both within the liver and at the systemic level which has led to chronic ethanol consumption being identified as a major risk factor in the development of ►liver cancer and a significant risk factor for the development of non-hepatic tumors.

Characteristics

The liver demonstrates a "dual circulation" vasculature in which oxygenated blood is delivered *via* the hepatic artery and deoxygenated blood, containing substances that have been absorbed from the gastro-intestinal tract, via the hepatic portal vein. Following ingestion ethanol is rapidly absorbed and enters the hepatic portal vein where it is delivered to the functional subunits of the liver termed the hepatic lobules. As blood flows through the hepatic vasculature (sinusoids) specialized epithelial cells, termed ►hepatocytes, that line the vasculature process materials absorbed in the GI tract to maintain normal physiological homeostasis. In addition to performing essential physiological functions such as protein, lipid and carbohydrate metabolism, bile production and regulation of vitamin A storage the liver also plays a central role in drug and hormone metabolism.

Following ethanol consumption metabolism occurs in the hepatocyte via three main enzymatic pathways; ►alcohol dehydrogenase (ADH), ►cytochrome p450 2 E1 (CYP2E1) and catalase. The metabolism of ethanol is a two step process, the first involves the conversion of ethanol to ►acetaldehyde (via ADH, CYP2E1 or catalase) and the second requires the conversion of acetaldehyde to acetate by the ►aldehyde dehydrogenase (ALDH) enzyme (Fig. 1). In both instances, these reactions lead to the production of the reduced form of nicotinamide dinucleotide (►NADH). These metabolic pathways for ethanol directly contribute to many of the deleterious effects of ethanol intake on normal hepatic and systemic physiology. While the metabolism of acetaldehyde by ALDH is an efficient metabolic pathway, acetaldehyde is a highly reactive species that, if allowed to accumulate, can cause significant protein damage and formation of ►adducts to DNA and ►DNA damage. In addition to the damaging effects of acetaldehyde/NADH, once formed, must be recycled to the oxidized NAD+ form by the electron transport chain in the mitochondria of hepatocytes. This process requires increased oxygen demand and can in turn lead to the production of reactive oxygen species (►ROS) and increase cellular ►oxidative stress that can cause ►oxidative DNA damage and protein/lipid peroxidation. In the instance of moderate ethanol consumption the ADH–ALDH system is sufficient to metabolize ethanol. However, following chronic, excessive ethanol intake the CYP2E1 enzyme becomes induced leading to increased acetaldehyde/NADH production and further elevating ROS synthesis and hepatic oxidative stress (Fig. 1). Although ethanol metabolism is localized primarily in the hepatocytes, the nonparenchymal cells of the liver including ►hepatic stellate cells (HSCs) and ►Kupffer cells (KCs) have also been shown to express ethanol metabolizing genes. Human HSCs express both ADH and ALDH, and CYP2E1 expression has been observed



Hepatic Ethanol Metabolism. Figure 1 Hepatic ethanol metabolism as it pertains to potential mechanisms of liver damage. Ethanol is metabolized by a two-step process to acetaldehyde that, in turn, is metabolized by acetaldehyde dehydrogenase (ALDH) to acetate.

in both HSCs and KCs, therefore further contributing to the detrimental effects of ethanol metabolism in the liver. Depending on the level and period of ethanol consumption normal hepatic function becomes increasingly compromised with complications ranging from moderate steatosis (fatty liver) to acute alcoholic hepatitis and eventually the development of fibrosis and cirrhosis. In addition, epidemiological data indicates that chronic ethanol consumption acts synergistically to accelerate the progression of ►HCC in patients exposed to other common risk factors including exposure to ►aflatoxins and ►hepatitis virus associated HCC.

The damage caused to the liver through acetaldehyde adduct formation and/or ROS generation/oxidative stress is thought to be a major risk factor for ethanol related liver cancer development. Greater than 80% of all primary tumors diagnosed in the liver arise as a result of hepatocyte cell transformation (►hepatocellular carcinoma; HCC). Unlike other common malignancies HCC occurs most commonly in the absence of familial patterns and largely within the realms of known risk factors. Chronic ethanol consumption, metabolism and the cellular and genetic damage associated with these events thus represent a significant risk factor for hepatic damage, cell transformation and the development of HCC. In addition to the direct effects of ethanol metabolism on the hepatocyte integrity, such as impaired microtubule polymerization, several other direct and indirect factors can combine to augment the effects of ethanol consumption in the liver and the body as a whole. Glutathione (►GSH) is an antioxidant important in reducing the oxidative stress caused by the synthesis and release of ROS. In the mitochondria, GSH is the only source of hydrogen peroxide metabolism and becomes significantly depleted following chronic ethanol ingestion due, at least in part, to decreased GSH transport into the mitochondria. While these detrimental effects on hepatocyte function directly increase the probability of cell transformation, increasing evidence suggests that the sequential nature of tumor formation and/or other systemic effects may play an increasing role in the effects of ethanol during the development and progression of HCC.

Ethanol abuse accounts for the majority of liver fibrosis and cirrhosis cases in the western world; however, even though the clinical progression is well-described, the ►molecular pathology is less well understood. Current models propose that alcoholic liver disease is initiated by an inflammatory response due to the activation of KCs as well as the infiltration of leukocytes including macrophages, neutrophils and lymphocytes. The activation of these inflammatory cells has been shown to be due to elevated gut-derived endotoxin plasma levels. Ethanol alters gut permeability to macromolecules, decreases gut motility and increases growth of Gram-negative bacteria in the intestinal microflora which ultimately leads to the introduction of endotoxin into the portal microcirculation. The activation and recruitment of inflammatory cells lead to the production of several profibrogenic cytokines. These cytokines likely induce and perpetuate the activation of HSCs leading to increased deposition of extracellular matrix (ECM) like type I collagen. The HSCs themselves can also exacerbate the inflammatory response through the secretion of chemoattractants and adhesion molecules necessary for leukocyte adhesion and infiltration.

The presence and activation of KCs, other infiltrating inflammatory cells and damaged hepatocytes, lead to increased ROS resulting in the activation of quiescent HSCs. The quiescent HSCs reside in the perisinusoidal space of Disse, and one of their primary functions in the healthy liver is the storage and homeostasis of vitamin A, namely retinol, retinal and retinoic acid. Upon a fibrogenic stimulus, including ethanol, the HSC transdifferentiates from a quiescent, vitamin A storing cell to that of an activated myofibroblast-like cell which proliferates, migrates to the site of injury and is responsible for the excessive accumulation of ECM. This continued deposition of ECM leads to scarring of the liver and ultimately liver dysfunction. Reduced levels of serum and hepatic vitamin A have been reported in persons with alcoholic liver disease (►ALD). Ethanol exposure to HSCs inhibits retinoic acid production and intracellular retinol levels. Possible mechanisms which interfere with retinoid metabolism in the cell may include reduced vitamin A uptake and enhanced degradation of vitamin A.

In addition to oxidative damage incurred directly by the hepatocyte, liver damage also affects cell membrane integrity due to ethanol metabolism via a nonoxidative pathway leading to the generation of fatty acid ethyl esters (FAEE). Fatty acid ethyl esters accumulate in the cell plasma membrane as well as in the mitochondrial membrane leading to organelle dysfunction. Accumulation of FAEE, particularly linolenic acid ethyl ester (LAEE) has been reported to activate signaling pathways important in regulating collagen expression which may further contribute to ethanol-induced fibrosis and subsequently HCC progression.

In addition to the effects of ethanol and ethanol metabolism on hepatocytes and other cell populations other factors must also be considered as to how ethanol can affect tumorigenesis. For example, the high incidence of cigarette smoking in ethanol dependent patients, regional differences in dietary intake, the type of beverage consumed and the socio-economic status and relative balance of diet in ethanol dependent patients can all affect the incidence and rate of hepatic tumor development and progression. Similarly, the induction of CYP2E1 (following chronic ethanol intake) has also been demonstrated to play a significant role in pro-carcinogen and ►carcinogen metabolism, many of which are present in cigarette smoke and alcoholic beverages.

Since oxidative stress has been linked to the development of ALD, the use of antioxidants as possible therapeutic strategies has been explored. The addition of antioxidants such as vitamin E, superoxide dismutase, GSH precursors such as *S*-adenosyl-L-methionine (SAMe) and the green tea extract, (–)-epigallocatechin-3-gallate (EGCG), have been shown to prevent or ameliorate ethanol-induced liver injury in a variety of animal models of ALD and HCC. However, the use of antioxidants should be approached with caution due to the possible toxic properties of antioxidants under certain conditions. Similarly, the use of "over the counter" antioxidants raises the possibility of drug–drug interactions and altered endogenous and exogenous agent metabolism caused by antioxidant intake, many of which are currently poorly studied and reported.

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Hepatic Excretion

Definition

Is the excretion of a drug by the liver by way of the gall bladder emptying into the intestine and ultimately into the feces.

►ADMET Screen

Hepatic Leukemia Factor (HLF)

Definition

Is a basic leucine zipper protein defined by a PAR domain that plays a critical role in hematopoietic specific expression of the LMO2 gene. A role for HLF in HSC self-renewal is supported by studies showing that ectopic expression of HLF enhanced HSC engraftment and inhibited apotosis.

►NUP98-HOXA9 Fusion
►E2A-PBX1

Hepatic (Liver) Fixed Macrophages

►Kupffer Cells

Hepatic Stellate Cell

Definition

HSC; Pericytes located in the perisinusoidal space of the liver responsible for vitamin A storage. In the damaged liver activation of HSCs leads to depleted Vitamin A storage and synthesis of extracellular matrix proteins (scarring).

►Hepatic Ethanol Metabolism

Hepatitis

Definition

Is liver inflammation and necrosis of varying severity and etiologies, such as toxic (drugs, alcohol), metabolic, autoimmune or viral.

►Hepatitis C Virus
►Hepatic Ethanol Metabolism

Hepatitis B Virus

Definition

HBV; is a member of the Hepadnaviridae family (hepatotropic DNA viruses). The HBV genome is a partially relaxed double-stranded circular DNA of ~3,200 base pairs, and encodes four partly overlapping open reading frames (ORFs). Thirty to fifty percent of persons who acquire the infection before the age of 5 years develop chronic HBV infection. HBV is a high-risk factor for ►hepatocellular carcinoma. ►Hepatocellular Carcinoma – Clinical Oncology; Hepatocellular carcinoma – etiology, risk factors and prevention; ►liver cancer – molecular biology.

►Hepatocellular Carcinoma
►Hepatitis Virus Associated Heptocellular Carcinoma

Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma

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Synonyms

Liver cancer; Hepatocellular carcinoma; HCC

Definition

The term hepatitis B x antigen, or HBxAg, refers to the gene product of hepatitis B virus (HBV) that contributes importantly to virus replication, the pathogenesis of chronic liver disease, and to the development of HCC.

Characteristics

There are an estimated 350 million people worldwide who are chronically infected with HBV and often replicate virus for many years or decades. These people are at high risk for the development of chronic hepatitis, which may progress onto cirrhosis (end stage liver disease) and then HCC. Although the pathogenesis of infection is immune mediated, this is characterized most often by responses that trigger hepatocellular damage and destruction but do not clear virus.

An important characteristic of ►chronic liver disease (CLD) is that ►liver cell regeneration provides opportunities for integration of virus DNA into the replication forks of cellular DNA. It turns out that the region encoding HBxAg is the most frequently integrated region of HBV DNA into the host genome. This region often encodes HBxAg, which is a ►*trans*-activating protein that alters the expression of cellular genes in many chromosomes throughout the host genome. This is quite an accomplishment for a small protein of only 17 kDa, and is achieved by constitutively activating a number of signal transduction pathways in the cytoplasm (such as JAK/STAT [►signal transducers and activators of transcription in oncogenesis], ►NF-κB, ►AP-1, AP-2, ►ras, src, PI3K/Akt [►PI3K signaling; ►Akt signal transduction pathways in oncogenesis] and β-catenin [►Wnt signaling], among others) and by binding to transcription factors in the nucleus (such as Oct-1, CREB, ATF-2, b-zip, TBP, and other basal transcription factors) or sequestering them in the cytoplasm (e.g., p53). HBxAg also alters gene expression at the post-transcriptional level by blocking the activity of the ►proteasome, which normally degrades proteins, and by altering the integrity of translation. Some of these pathways have been shown to be operative in the mechanism(s) whereby HBxAg promotes cell growth in culture dishes and anchorage independent growth in soft agar (a property often associated with cancer cells). The findings that HBxAg is capable of conferring tumorigenicity upon nonmalignant liver cells, and that the sustained over-expression of HBxAg in transgenic mice gives rise to HCC, further underscores the centrality of HBxAg expression to the development of HCC.

It is likely that inadequate immune responses permit the persistence of infected hepatocytes during chronic infection. However, there is increasing evidence that HBxAg inhibits a number of ►apoptotic pathways (►apoptosis signaling) (e.g., Fas and tumor necrosis factor alpha (TNFα), both of which trigger programmed cell death) during chronic infection, thereby promoting the persistence of infected cells during CLD. HBxAg is also promotes ►fibrogenesis, in that it promotes the expression of extracellular matrix proteins such as ►fibronectin, promotes the cross-linking of collagen, and potentiates ►TGFβ1 signaling, in part, by inhibiting expression of the TGFβ1 binding partner, α-2-macroglobulin. In this context, it is not surprising to find a strong direct correlation between intrahepatic HBxAg expression and CLD. This relationship would protect virus infected cells from immune destruction, but these same features also promote carcinogenesis, since tumor cells are also resistant to apoptosis and demonstrate constitutively active signaling pathways that had been previously altered in preneoplastic tissue by HBxAg.

There are few natural effectors of HBxAg that are known to be responsible for these profound biological changes that ultimately result in cancer. HBxAg appears to stimulate ►insulin-like growth factor 2 (IGF2), which promotes hepatocellular growth, activates wild type β-catenin (and its effector, c-*myc*), *trans*-activates several unique "oncogenes," and promotes ►angiogenesis. HBxAg also binds to and inactivates the ►tumor suppressors, ►p53 and ►PTEN, down-regulates expression of the natural cell cycle inhibitor, ►$p21^{WAF1/SDI1/CIP1}$, and promotes phosphorylation (inactivation) of the Rb tumor suppressor protein (►retinoblastoma protein), suggesting the molecular basis upon which HBxAg stimulates unchecked cell growth. HBxAg also binds to and inactivates a novel ►senescence factor, $p55^{sen}$, suggesting that HBxAg contributes to carcinogenesis, in part, by overcoming cellular senescence. The observation that HBxAg also down-regulates ►E-cadherin expression, the latter of which is involved in cell adhesion, provides at least part of the explanation as to how HBxAg stimulates cell motility (metastasis) in carcinogenesis.

►Oxidative stress is a common feature of CLD, resulting in the activation of HBxAg in CLD and HCC. Over-expression of HBV envelope associated polypeptides, cytokine signaling, and cell mediated immunity against HBV infected cells, all promote the development

of oxidative stress. This was associated with decreased intrahepatic levels of superoxide dismutase (Cu/Zn), which normally protects cells from ►reactive oxygen species. These conditions stimulate HBxAg, which compromises transcription coupled DNA repair and ►nucleotide excision repair, resulting in increased chromosomal alterations (genetic instability) and micronuclei formation (where metaphase plates separate prior to complete DNA replication). In addition, HBxAg is associated with the outer mitochondrial membrane, where it binds to the voltage dependent anion channel (VDAC3), resulting in decreased mitochrondrial membrane potential and the further development of oxidative stress. In this way, oxidative stress augments HBxAg function in propagating chronic infection and in stepwise hepatocarcinogenesis.

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Hepatitis C Virus

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Synonyms

HCV

Definition

►Hepatitis C virus (HCV) belongs to the flaviviridae family (Hepaciviruses genus) and is a pathogenic human RNA virus, which causes chronic liver disease and hepatocellular carcinoma (►liver cancer, molecular biology) (HCC).

Characteristics

The size of this enveloped virus is ~60 nm. Its nucleocapsid contains a single-stranded RNA genome of ~9.6 kb genome (Fig. 1) of plus(+) strand polarity that carries a single open reading frame. At least six major genotypes (1–6) with up to three subtypes (a to c) exist, which differ not only in their nucleic acid sequence but also in their pathophysiological properties. All structural and non-structural viral proteins are processed from a polyprotein precursor of 3,010–3,033 amino acids in the cytoplasm or endoplasmic reticulum of the infected cell.

Untranslated Regions

The 5′- and 3′-untranslated regions (UTR) are highly conserved within the different genotypes. The 341–344 nucleotides 5′-UTR (Fig. 1) forms extensive stem-loop structures, which are important in translation initiation. It also harbors an internal ribosomal entry site. The 3′-UTR is composed of a poorly conserved variable region (28–42 nucleotides), a variable polypyrimidine stretch and conserved 98 nucleotides at the 3′ end (x region). Both UTRs seem to be crucial for regulation of HCV translation and possibly also for controlling HCV replication, and are therefore candidate targets for experimental antiviral strategies.

Structural Proteins

The core protein C (21 kD) polymerizes to an icosaedric capsid and binds RNA to form the nucleocapsid. The envelope proteins E1 (31 kD) and E2 (70 kD) form heterodimers whose formation is mediated by the chaperon calnexin. These dimers are embedded in a host-derived lipid bilayer. Within the E2 sequence there are two hot spots of mutations (hypervariable regions, HVR1 and HVR2). Mutations occurring during HCV infections are due to a selection driven by the host immune system and account for the regular existence of a variety of ►quasispecies. The E2 protein is produced as two precursors, E2/NS2 and E2/p7 that differ in their C-terminus. The hydrophobic p7 is probably important for membrane anchoring of E2. The function of p7 or E2/p7 during the HCV life cycle is not known. All structural protein processing is performed by cellular signal peptidases.

Non-structural Proteins

The non-structural protein NS2 (23 kD) is released from the polyprotein precursor both by host signal peptidases at the C-terminus of p7 and by a NS2/3 proteinase at the NS2-NS3 junction. NS3 (68 kD) has

Aminoacids	Protein	Function
1–191	C	Core
192–383	E1	Envelope glycoprotein
384–746	E2	Envelope glycoprotein
747–809	p7	?
810–1026	NS2	Zn auto - protease
1027–1657	NS3	Ser protease
		RNA helicase
1658–1711	NS4A	NS3 Ser protease cofactor
1712–1972	NS4B	?
1973–2420	NS5A	Interferon-α-resistance
2421–3010	NS5B	RNA polymerase

Hepatitis C Virus. Figure 1 Genomic organization and designation of HCV RNA, and amino acid positions and function of HCV proteins.

three different functions: It is part of the NS2/3 proteinase, its N-terminal third contains a serine proteinase and at the C-terminus a RNA-dependent NTPase/helicase was discovered. Together with NS4A (6 kD) as a stable complexed cofactor, the N-terminal NS3 serine proteinase catalyses the cleavage between NS3-NS4A, followed by cleavage between NS5A-NS5B, NS4A-NS4B and NS4B-NS5A. The NS3 helicase can unwind double-stranded (ds)RNA, dsDNA and RNA/DNA heteroduplex molecules. For this purpose any NTP or dNTP is used as source of energy. NS4B is a 26 kD membrane associated protein of unknown function. NS5A (56–58 kD) represents two cytoplasmatic proteins, p56 and p58, which are both phosporylated at serine residues. This process may be essential for the viral replication cycle, but the biological functions of these proteins are not understood. There is some evidence that NS5A mediates interferon-alpha resistance of HCV. The NS5B (65 kD) protein is responsible for HCV RNA replication; it represents an RNA-dependent RNA-polymerase that is not found in humans and may therefore be another attractive target for antiviral strategies. The RNA minus(−) strand is transcribed in the host cytoplasm into a plus(+) strand RNA. This serves as a template to produce new minus(−) strands for packaging into the envelope. Both steps are accomplished by the NS5B protein.

Cellular and Molecular Regulation

Although the HCV genome does not harbor acutely transforming oncogenes, the HCV core as well as the NS3 gene are candidate genes whose products may mediate malignant hepatocyte transformation.

Oncogene complementation assays using HCV core and H-►ras or c-►myc oncogenes showed that HCV core cooperates with these oncogenes and transforms primary rat embryo fibroblasts to the tumorgenic phenotype. Focus formation, soft agar growth and tumor development was also shown in nude mice using Rat-1 fibroblasts. Also in this model a loss of contact inhibition, morphological changes and anchorage- and serum-independent growth occurred when HCV core and v-H-ras were cooperatively expressed. The most striking evidence of an oncogenic potential of HCV comes from an HCV core-transgenic mouse model, in which hepatic tumors arose in up to 30% of animals of two different strains. Interestingly, all tumors occurred in male animals. However, other mice transgenic for HCV core or C-terminally truncated (amino acid 384–715) HCV core failed to develop histological or biochemical signs of liver disease.

The postulated underlying molecular mechanisms of HCV core-induced hepatocyte transformation are manifold. They comprise sequestration of LZIP in the cytoplasm leading to a loss of CRE-dependent

transcription and regulation of cell proliferation and subsequently to morphological transformation of NIH 3T3 cells. Other mechanisms may be interference with ►apoptosis or ►transactivation of cellular c-fos, c-myc, p53 or β-interferon promoters.

Stable expression of the 5′-portion of the NS3 gene was able to induce focus formation and soft agar growth in NIH 3T3 cells. Only recently it was demonstrated that internal cleavage of the NS3 protein occurs at cleavage sites FCH(1395)//S(1396)KK and IPT(1428)//S(1429) GD within the HCV RNA helicase domain in presence of NS4A. These findings were confirmed in two different isolates of HCV of genotype 1b. The 5′-portion of NS3 was more oncogenic than the full-length NS3 protein.

Clinical Relevance

An estimated 2% of the world's population is chronically infected with HCV. It accounts for 20% of acute and 70% of chronic hepatitis cases. HCV is mainly transmitted by blood. Since screening and treatment of blood products for HCV has been routinely performed, post-transfusion hepatitis has become extremely rare. Intravenous drug addiction is today a major way of HCV transmission. Acute hepatitis is often clinically inapparent, and in chronic hepatitis symptoms occur mainly in later stages of disease. A high chronicity rate of up to 75% of acute infection and its silent course account for the pathogenic potential of this virus, which includes extrahepatic manifestations. Twenty percent of chronically infected patients develop ►liver cirrhosis and 2–5% per year will progress to hepatocellular carcinoma (HCC).

Therapy for HCV infection comprises the combined use of 3–6 MU of α-interferon administered subcutaneously three times a week and 800–1200 mg of the nucleoside analog ►ribavirin p.o daily for 6–12 months dependent on the genotype and viral load. Roughly 40–45% of previously untreated or relapse patients benefit from the treatment, i.e. eliminate HCV and normalize liver enzymes. In addition, this treatment is capable of improving histological signs of liver damage. Its effect on HCC prevention can not clearly be judged at present.

Recent therapeutic innovations comprise ►pegylated interferons that allow single weekly dosing due to slow effector release from subcutaneous depots and a decrease of systemic side effects. Sustained response rates up to 70 % can be reached. A synthetic consensus interferon-alpha showed promising effects in monotherapeutic use in a preliminary trial and is presently being evaluated in combination with ribavirin. Major future achievements are expected from the development of inhibitors of HCV protease, helicase or RNA-dependent RNA polymerase, which may improve effectivity of antiviral treatment similarly to the situation in HIV infection, but are not yet available for clinical use. Therapeutic nucleic acids such as antisense oligodeoxynucleotides, RNAs or ribozymes may be another experimental concept for the treatment of HCV infection in the future.

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Hepatoblastoma

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Definition

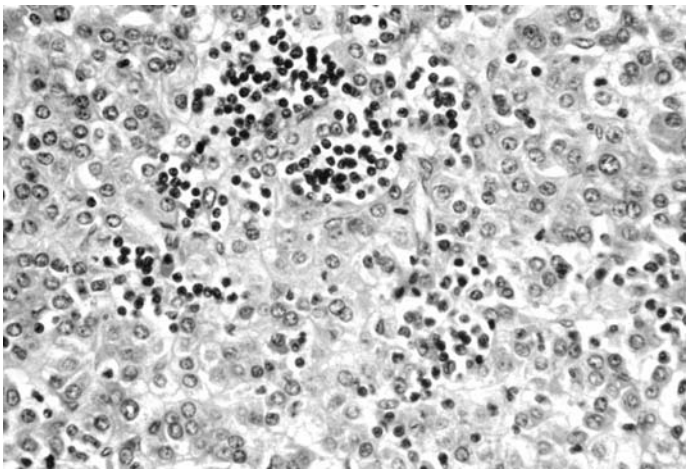
Heptoblastoma is a childhood malignant embryonal liver tumor consisting of immature epithelial cells with or without additional mesenchymal component.

Characteristics

Hepatoblastomas are the most frequent malignant liver tumors of childhood. Their annual incidence is 0.5–1 cases per million children under 15 years of age in Western countries. The affected children are most frequently between 6 months and 3 years old. Hepatoblastoma has also been detected in utero by prenatal ultrasound examination. Since liver tumors lack early clinical symptoms, hepatoblastoma patients often present with locally extended tumors at diagnosis. However, distant metatases usually occur very late in the disease progression. The patients frequently have highly elevated ►α-fetoprotein levels. In these cases this ►oncofetal antigen can be useful as a sensitive diagnostic marker and also as a marker for the monitoring of treatment response.

Pathological Classification

Hepatoblastomas always consist of immature epithelial liver cells resembling fetal or embryonal liver cells. Approximately one third of the cases contain additional mesenchymal components and are then termed "mixed" hepatoblastomas. According to Weinberg and Finegold (1983) the epithelial component is further classified into



Hepatoblastoma. Figure 1 Histopathology of an epithelial hepatoblastoma showing foci of hematopoietic cells.

well differentiated "fetal," less differentiated "embryonal" and undifferentiated "small cell anaplastic" categories. The latter is rare, as are "macrotrabecular" or "teratoid" variants. Small cell anaplastic as well as macrotrabecular variants indicate a bad prognosis. A histopathological hallmark of hepatoblastomas, in particular of the fetal differentiated cases, is the occurrence of hematopoietic foci mainly consisting of erythropoietic or thrombopoietic progenitor cells mimicking fetal hematopoiesis in the liver (Fig. 1).

Staging

In the last 30 years the treatment modalities and outcome of hepatoblastoma patients has significantly improved. It turned out that hepatoblastomas are responsive to chemotherapy. Today, most hepatoblastoma patients are enrolled in multicenter studies and receive a stage and risk adapted multimodal therapy. Different staging systems are used including the American and German four-graded postoperative staging system, the Japanese TNM classification and the European SIOP PreTreatment Extent of Disease (PRETEXT) grouping system. The patients are stratified into standard and high risk patients, the latter often presenting with extended, multifocal or metastatic disease.

Therapy and Outsome

In current protocols most patients receive a ►neoadjuvant chemotherapy with ►cisplatin and ►doxorubicin, in some studies combined with ifosphamid, 5-fluorouracil or carboplatin. The combination carboplatin and etoposide has also been effective. The overall survival after chemotherapy and resection is 70–80%. Unfortunately, approximately one quarter of the patients still die from the disease, so that predictive factors are important to early recognize these high risk patients and to offer them an intensified therapy. High risk patients have been treated with prolonged preoperative chemotherapy (SIOP, USA) or with mega-therapy (Germany).

Predictive Factors

Several histological, clinical as well as serological factors have been evaluated for their predictive value. In particular, the extension of the tumor in the liver, multifocality, vascular invasion and the presence of metastases have been of predictive importance in most studies. The decline of alpha-fetoprotein levels under chemotherapy predicts the clinical response. In contrast, the impact of distinct histological features such as mixed versus epithelial, or fetal versus embryonal differentiation and chromosome ►ploidy of the tumors is still under discussion and the data is controversial.

The etiology of hepatoblastomas is unknown. Environmental factors do not seem to play a major role in the pathogenesis of hepatoblastomas. Preterm infants may have an elevated risk for the development of hepatoblastomas. Although most hepatoblastomas occur sporadically, familial cases have been described. The incidence is highly elevated in families with ►adenomatous polyposis coli (FAP [►APC Gene in Familial Adenomatous Polyposis]) and ►Beckwith-Wiedemann Syndrome.

Genetic and Molecular Characteristics

Cytogenetic analyses have revealed several recurrent numerical aberrations as well as structural alterations. Most frequently trisomies of chromosomes 1, 2, 8 and 20 have been described as well as a recurrent translocation t(1;4)(q12;q34) in ~15% of hepatoblastomas. Approximately 50% of cases show gain of material of chromosome 2q. A few cases have an ►amplification of material from chromosomal band 2q24, indicating the location of a hepatoblastoma related oncogene. ►Microsatellite analyses have uncovered

frequent allelic losses of chromosomal regions of chromosome arms 1p and 11p. The losses of chromosomal region 11p15.5 are always of maternal origin indicating the existence of one or more imprinted hepatoblastoma associated gene(s) expressed from the maternal ►allele.

Studies on the mutational status of the ►TP53 gene resulted in conflicting data. Whereas TP53 mutations were described in a small Japanese tumor collection, these were absent in other studies. Similarly, somatic missense mutations were described in one study but not confirmed by others. The activation of the ►WNT signaling pathway by activating mutations in the ►β-catenin gene is a frequent event in hepatoblastomas. Approximately 50% of the hepatoblastomas save point mutations or deletions of exon 3 encoding the protein degradation targeting site of the β-catenin protein, resulting in a destruction of N-terminal phosphorylation sites that are necessary for protein degradation. This leads to the accumulation of a mutated protein, which transfers oncogenetic signals to the nucleus and increases transcription of specific target genes of the ►TCF/LEF family of transcriptions factors such as ►cyclin D1 and ►c-myc. Similar mutations have been described in other tumor entities including colorectal cancer. However, hepatoblastoma represents the malignant tumor with the highest incidence for β-catenin mutations.

Cellular Characteristics

Hepatoblastoma cells resemble liver progenitor cells during embryonic and fetal development suggesting that hepatoblastomas are derived from such progenitors. Hepatoblastoma cells are still dependent on growth factors and use specific growth factor signaling systems. The important fetal mitogen, insulin-like growth factor-II, has been demonstrated to be highly over-expressed in hepatoblastomas. The encoding ►IGF2 gene maps to chromosome 11p15.5, a region frequently altered in hepatoblastomas and related embryonal tumors. They also produce several hematopoietic ►cytokines such as ►interleukin-1, ►stem cell factor, ►erythropoietin and ►thrombopoietin that induce hematopoietic foci in hepatoblastomas. Hepatoblastoma cells over-express the receptor ►Met for hepatocyte growth factor (HGF; ►scatter factor) and proliferate in response to HGF in vitro. Concentrations needed for this effect are usually found in the serum of patients after liver surgery. This may explain why hepatoblastomas often show rapid regrowth after partial resection. Therefore it is now common sense that the tumors should undergo primary resection, only when they can be removed without residual tumor cells.

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Hepatocellular Carcinoma

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Synonyms

Liver cancer; Hepatoma; Hepatic carcinoma; Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma; Primary liver cancer; Primary hepatic carcinoma; Liver cell carcinoma

Definition

Hepatocellular carcinoma (HCC) is a primary cancer that arises from hepatocytes, the major cell type of the liver. Most cases of HCC are secondary to either hepatitis virus (usually type B or C) infection or cirrhosis.

Characteristics

Epidemiology

HCC is the fifth most common cancer in men and the eighth most common cancer in women worldwide. An estimated more than half a million new cases are diagnosed annually, while there are major geographical differences in incidence. The annual incidence rates in Eastern Asia and sub-Saharan Africa exceed 15/10,000 inhabitants, while the figures are intermediate (between 5/10,000 and 15/10,000) in the Mediterranean Basin and Southern Europe, and very low (<5/10,000) in Northern Europe and North America. In high-risk countries, HCC can develop before the age of 20 years, whereas in low-risk countries, HCC is rare before the age of 50 years. The incidence of HCC is increasing in several developed countries and the increase will likely continue for some decades. This trend is related to the

increasing incidence of ►hepatitis B virus (HBV) and ►hepatitis C virus (HCV) infections in those areas. In selected areas of some developing countries, the incidence of HCC has decreased, possibly as a result of the introduction of the HBV vaccine. The incidence of HCC worldwide varies according to the prevalence of HBV and HCV infections.

There are gender differences in the incidence of HCC, with a male predominance ranging between 2:1 and 4:1.

Risk Factors

In more than 80% of patients with HCC, it has developed as a consequence of chronic liver disease, usually (but not always) in association with cirrhosis. Exceptions include chronic HBV infection and certain types of HCC in sub-Saharan Africa, where aggressive and massive tumors can develop before the onset of cirrhosis. The major causes of cirrhosis, and hence HCC, are HBV, HCV, and alcohol, but less prevalent conditions such as hemochromatosis, primary biliary cirrhosis, nonalcoholic steatohepatitis, and Wilson disease have been also associated with the development of HCC. The attributable risk estimates (proportion of a disease due to a specific factor) for the combined effects of HBV and HCV infections account for well over 80% of HCC cases worldwide. The risk among those with cirrhosis increases in parallel with the impairment of liver function and is higher in males, patients older than 50 years, and subjects with increased ►alpha-fetoprotein (AFP) concentration.

►Aflatoxin has also been implicated as a factor in the etiology of HCC in parts of the world where this mycotoxin (a toxin produced by a fungus) occurs in high levels in food.

Diagnosis

Groups of subjects at higher risk for HCC can be identified. The major risk factors for HCC are chronic HBV infection, chronic HCV infection when associated with advanced fibrosis or cirrhosis, and cirrhosis regardless of cause. The surveillance tools are AFP concentration and liver ultrasonography (US). Other tumor markers such as lectin-bound AFP and ►des-gamma-carboxy prothrombin (DCP) have been proposed to surpass the efficacy of AFP, but their clinical efficacy is yet to be unequivocally proven. In patients with a high suspicion of HCC, dynamic enhanced computed tomography (CT) or magnetic resonance imaging (MRI) are generally used as confirmatory tools for diagnosis. A consensus statement from the European Association for the Study of the Liver (EASL) has been formulated to standardize diagnostic approaches in patients with HCC as below. Since nodules less than 10 mm in size were considered not feasible to be confidently diagnosed, close observation was recommended. For nodules between 10 and 20 mm in size, positive result of the biopsy was needed to establish the diagnosis, while for those larger than 20 mm a specific radiologic profile observed by two imaging techniques was considered to make the diagnosis if the patient was known to present with underlying cirrhosis.

Prognosis

The natural history of HCC depends on the stage of the tumor and the severity of associated liver disease at the time of diagnosis. For example, a patient with a 1 cm tumor with no cirrhosis has a >50% chance of surviving 3 years, even without treatment. In contrast, a patient with multiple tumors involving both lobes of the liver with decompensated cirrhosis is unlikely to survive more than 6 months, even with treatment.

AFP levels have been shown to be prognostically important, with the median survival of AFP-negative patients significantly longer than that of AFP-positive patients. Other prognostic variables include performance status (measure of general wellbeing), liver functions, and the presence or absence of cirrhosis and its severity in relation to the ►Child-Pugh classification.

Prognostic Staging System

A clinical staging system provides guidance for patient assessment and appropriate therapy. It is useful for the decision to treat certain patients aggressively while avoiding the overtreatment of other patients who would not tolerate therapy or whose life expectancy rules out any chance of success. Clinical staging is also an essential tool for comparison between groups in therapeutic trials and between different studies.

The current classifications most commonly used for HCC are the ►Okuda staging system, the ►Child-Pugh staging system, tumor node metastasis (TNM) staging, and Cancer of the Liver Italian Program (CLIP) score (Table 1). Among these, the CLIP score is currently the most commonly used integrated staging score, for both tumor and liver disease stages.

The Japan Integrated Staging (JIS) system, a new system that is based on a combination of the Child-Pugh system and the Liver Cancer Study Group of Japan (LCSGJ) system – the LCSGJ system is also concordant with TNM classification for HCC by the International Hepato-Pancreato-Biliary Association and the International Union Against Cancer (UICC) – has recently been proposed in Japan (Table 2). The stratification ability of the JIS scoring system is much better than that of the CLIP scoring system. The JIS scoring system also performed better than the CLIP scoring system in selecting the best prognostic patient group.

Treatment

There is no worldwide agreement on a common treatment strategy for patients with HCC, and several proposals have been published (Fig. 1).

Hepatocellular Carcinoma. Table 1 Definitions of the CLIP score

Variables	Score		
	0	1	2
Child-Turcotte-Pugh stage	A	B	C
Tumor morphology	Unilobular and extension ≦50%	Multinodular and extension ≦50%	Massive or extension >50%
AFP (ng/ml)	<400	≧400	–
Portal vein thrombosis	No	Yes	–

Hepatocellular Carcinoma. Table 2 Japan Integrated staging (JIS) scoring system

Variables	Score			
	0	1	2	3
Child-Pugh grade	A	B	C	
TNM stage by LCSGJ	I	II	III	IV

Definition of TNM stage by the Liver Cancer Study Group of Japan (LCSGJ)

T factor	I. Single	II. <2 cm	III. No vascular involvement
T1		Fulfilling three factors	
T2		Fulfilling two factors	
T3		Fulfilling one factor	
T4		Fulfilling zero factors	
Stage I		T1 N0 M0	
Stage II		T2 N0 M0	
Stage III		T3 N0 M0	

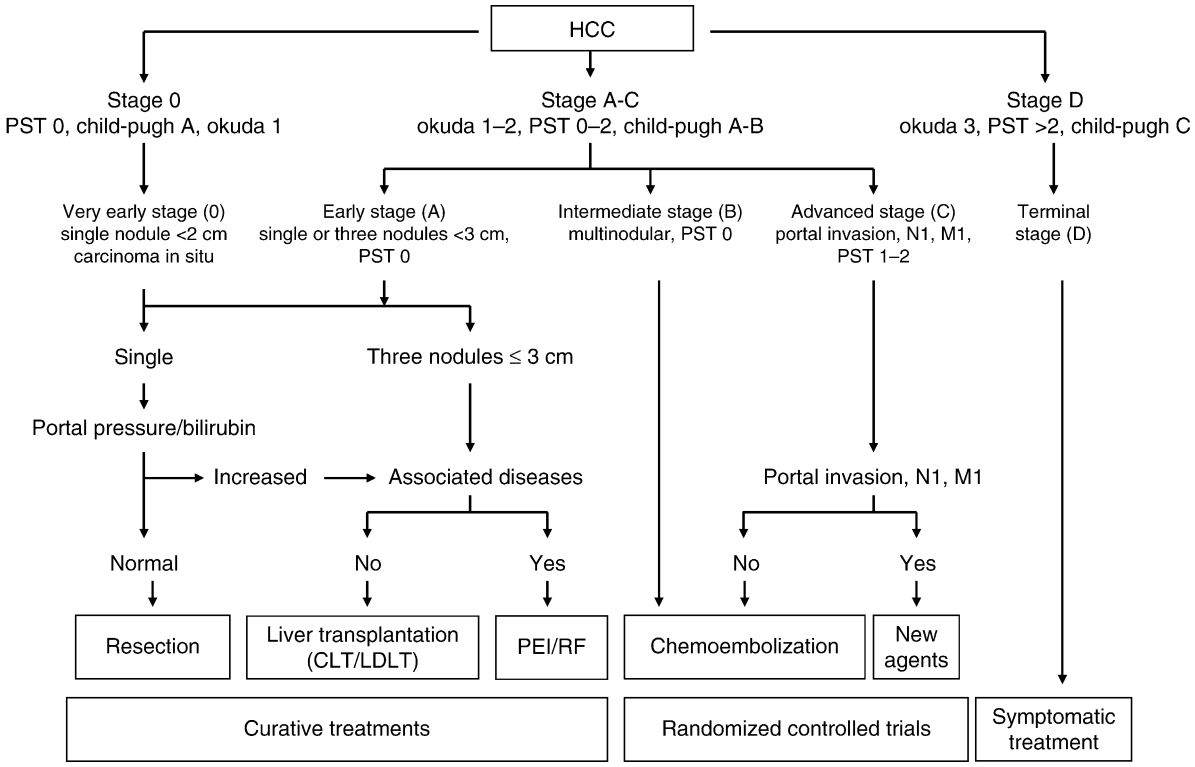
The three major curative therapies –surgical resection, liver transplantation, and percutaneous treatments – compete as first-line treatment options for single small HCC in patients with liver function.

Surgical resection yields good results in candidates who have single tumors and excellent liver functional reserve. Five-year survival in well-selected patients with substantial HCC is around 50%, reaching a 70% rate in those with normal bilirubin concentration who do not have portal hypertension. The main problem of surgical resection as compared with liver transplantation is the high recurrence rate that may exceed 50% at 3 years and 70% at 5 years.

Transplantation is the first choice for patients with small multinodular tumors (three nodules ≤3 cm) or those with advanced liver dysfunction. These are patients with single HCC ≤5 cm or up to three nodules ≤3 cm (the Milan criteria), who in major Liver and Transplantation Units achieve 70% survival at 5 years with a recurrence rate below 15%.

Percutaneous treatment is a therapeutic option that has become quite widely-used during the last decade. Destruction or ablation of tumor cells can be achieved by the injection of chemical substances (e.g., ethanol, acetic acid, and boiling saline) or by the insertion of a probe that modifies local tumor temperature (e.g., radiofrequency ablation, microwave, laser, and cryotherapy). This procedure can be done percutaneously with minimal invasiveness or during laparoscopy, and it's currently considered the best option for patients with early HCC who are not candidates for surgery.

Transcatheter arterial chemoembolization (TACE) is usually performed in the treatment of intermediate-advanced stages of HCC. TACE is the delivery of high concentrations of chemotherapeutic agents directly to the HCC tumors via the hepatic artery, which provides the tumor with most of its blood supply. Embolizing agents such as cellulose, microspheres, lipiodol, and gelatin form particles (Gelform) are used to deliver intra-arterial chemotherapy (e.g., doxorubicin,



Hepatocellular Carcinoma. Figure 1 Barcelona Clinic Liver Cancer (BCLC) staging and treatment algorithm. PST, performance status test; CLT, cadaveric liver transplantation; LDLT, live donor liver transplantation; PEI, percutaneous ethanol injection; RF, radiofrequency ablation.

epirubicin, ►mitomycin, or ►cisplatin) to the tumor via the hepatic artery. In general, patients with portal vein thrombosis, encephalopathy, or biliary obstruction are not candidates for TACE. Objective response, as reflected by intratumoral necrosis and reduced tumor burden on dynamic CT or MRI, is associated with delayed tumor progression, and as a whole, results in an improvement of survival. According to the prospective cohort study by the Liver Cancer Study Group of Japan, the survival rate of over 8,000 patients with unresectable (inoperable) HCC treated by TACE were 82% at 1 year, 47% at 3 years and 26% at 5 years.

Systemic chemotherapy has no effect (<10% objective responses) and is frequently associated with toxicity and hence is usually not recommended.

Radiation therapy has some effect, but the impact on survival is still unknown.

Prevention

Vaccination against HBV at birth has decreased the incidence of HCC in areas with high prevalence of this viral agent. Unfortunately, there are no similar means of preventing hepatitis C, alcoholic liver disease, or nonalcoholic steatohepatitis. Therapy for the underlying liver disease to prevent progression to cirrhosis may be the best approach for prevention of HCC. Antiviral treatment for chronic hepatitis decreases the proportion of patients developing cirrhosis and this should reduce the long-term incidence of HCC. When cirrhosis is already established, there is no evidence supporting a preventive effect of drugs such as interferon.

►Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma

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Hepatocellular Carcinoma – Etiology, Risk Factors and Prevention

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Synonyms

Primary liver cancer; Liver cancer; Hepatoma

Definition

Hepatocytes and cholangiocytes are the most common types of liver epithelial cell, and their malignant transformation can lead to hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC), respectively.

Characteristics

Etiology

Oncogenic and Environmental Factors

The radioactive contrast medium thorotrast was used to detect arterial injury during World War II. This medium can become trapped in the reticulo-endothelial system of exposed individuals, and can release weak γ-rays over a prolonged period of time. As a result, HCC, angiosarcomas, and CCC can develop ≥10 years after exposure. For this reason, the clinical use of thorotrast was recently prohibited.

Aflatoxin B1, which is found in the fungus *Aspergillus flavus*, acts as an oncogenic factor of HCC. In 1979, the World Health Organization (WHO) reported the prevalence of HCC to be 13 cases per 100,000 individuals among the Mozambique population. Estimate uptake volume of aflatoxin was 222.4 ng/kg in adult which was most amount of consumption in the Africa.

Anabolic steroids are also hepatocarcinogenic substances, and have been closely linked to both HCC and liver cell adenoma; this association was first reported in 1971 in a patient with Fanconi anemia who was treated with oxymetholone.

Recently, oral contraceptives that contain synthetic estrogen and progestogen have been linked to an increased risk of HCC in a large cohort study in Europe; the relative risk was found to be 503 times greater for liver cell adenoma and 20 times greater for HCC after >8 years of administration.

High-Risk Non-viral Liver Diseases

Patients with Budd–Chiari syndrome, hemochromatosis, glycogen-storage disease, and α-antitrypsin deficiency represent high-risk groups for HCC.

Viral Hepatitis-Induced HCC

The most important factor influencing hepatocarcinogenesis is continuous infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV). In a clinical setting, continuous infection with HCV can induce chronic hepatitis concomitant with necroinflammation and disruption of the repair systems of the liver; this can lead to hepatic injury and further interference with the repair system for DNA damage, eventually resulting in hepatic fibrosis, liver cirrhosis, and, finally, HCC. HBV infection can lead to the same hepatocarcinogenic process, although an additional mechanism allows HBV integration to occur without chronic hepatitis and liver cirrhosis.

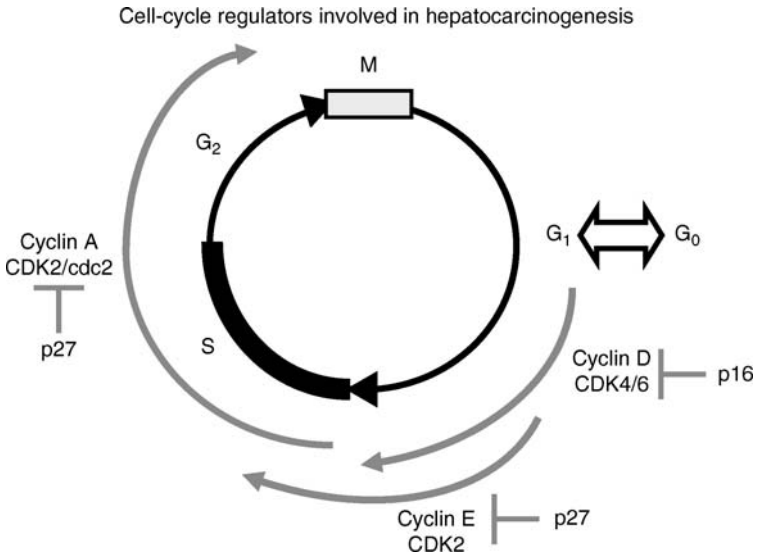
Traditionally, south-east Asian countries, particularly Korea, China, and Japan, have shown high infection rates for both HBV and HCV. Moreover, the incidence of HCC has been relatively high throughout Asia. An increased incidence of HCV among HCC cases was more recently reported in Japan, with prevalence rates of ∼75% compared with 17% for HBV infection. The maternal transmission of HBV has now been limited by a national HBV vaccination program, which is causing the prevalence to decrease. However, the prevalence of HCV is continuing to increase throughout Asia, and especially in Japan, because of its high incidence in past blood transfusions.

Alcohol intake, obesity, male gender, and old age are all recognized progression factors for HCC in chronic cases of HBV and HCV infection.

Molecular Aspects of Hepatocarcinogenesis (Fig. 1)

The mechanism of hepatocarcinogenesis is not yet completely understood; however, advances in molecular biology methods have revealed many altered genes in human HCC. A loss of heterozygosity (LOH) at chromosome arms 1p, 1q, 4q, 6q, 8p, 9p, 13q, 16p, 16q, and 17p has been found in HCC with a frequency of >20%, and chromosome arms 6q, 9p, 13q, 16p, and 17p LOH have been linked to inactivation of the tumor-suppressor insulin-like growth factor 2 receptor (IGF2R), p16, retinoblastoma (RB1), axin 1, and p53.

The genetic changes involved in hepatocarcinogenesis can be divided into at least five pathways: the p53 pathway, which is involved in the response to DNA damage or genomic instability; the p16/p27/RB1 pathway, which is involved in cell-cycle control; the transforming growth factor-β (TGF-β) pathway, which is involved in growth inhibition and apoptosis of hepatocytes; the Wnt/β-catenin/APC pathway, which is involved in intercellular interactions; and the E-cadherin/integrin and extracellular signal-regulated kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathways, which are involved in cancer cell migration and metastasis.



Hepatocellular Carcinoma – Etiology, Risk Factors and Prevention. Figure 1 Possible mechanisms of hepatocarcinogenesis. Cell-cycle regulators involved in hepatocarcinogenesis.

Among the different signaling pathways involved in hepatocarcinogenesis, deregulation of the cell-cycle machinery is thought to be closely involved in the progression of HCC. Cell-cycle progression in mammalian cells is mainly governed by cyclin A, cyclin D, and cyclin E, along with cyclin-dependent kinase 2 (CDK2) and CDK4. The kinase activities of these proteins are negatively regulated by the CDK inhibitors (CDKIs) p16 and p27. RB1, which is phosphorylated by CDKs and activates the E2F family of transcription factors, is mutated in ~15% of HCCs. The cyclin D1 gene, which is involved in the G1 progression and G1/S transition phases of the cell cycle, has been shown to be amplified in 10–20% of HCCs. By contrast, both p16 and p27 are frequently inactivated in HCCs. P16 is inactivated in ~50% of HCCs, mainly due to the *de novo* methylation of the DNA-promoter region. Somatic mutation of the p16 gene has also been described in some cases of HCC. Recently, p16 DNA methylation was identified in preneoplastic liver tissues of individuals with chronic hepatitis virus infections. P27 is a member of the KIP family of CDKIs, and its expression is reduced in 40–60% of HCCs. Decreased p27 expression is closely associated with poor prognoses of individuals with HCCs, indicating that it is an adverse prognostic factor. In some cases of HCC, increased cell proliferation has been noted despite relatively high levels of p27 expression, suggesting the inactivation of p27 by sequestration into cyclin D1–CDK4-containing complexes.

Although many genes appear to be altered in HCC, the frequencies of individual mutations are low, and the patterns of genetic alteration differ among patients. Connections between different pathways might thus be plausible mechanisms underlying hepatocarcinogenesis.

Diagnosis

Serum alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin, which is also known as protein induced by vitamin K antagonist (PIVKA II), are useful serological markers of HCC. Although AFP exists in normal human tissues (both fetal and adult), serum levels are sensitive to the presence of HCC. The overall diagnostic accuracy of AFP is ~80%. By contrast, PIVKA II is only present in HCC tissues, so its specificity is relatively high, and its overall diagnostic accuracy is ~55%. The simultaneous measurement of AFP and PIVKA II could therefore be useful for the detection of HCC. However, as elevated AFP levels can be the result of liver cirrhosis, it is important to determine whether they are caused by active liver cirrhosis or by HCC that has arisen from liver cirrhosis. The elevation of serum AFP reflected as regeneration of hepatocytes.

The levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are also often elevated in the serum of patients with HCC. However, the specificities of these markers are lower than those of AFP and PIVKAII.

Total imaging is important for detecting small HCCs. The majority of HCCs arise from chronic hepatitis and liver cirrhosis; periodical checking using ultrasound scanning and computed tomography (CT) is therefore crucial to detect the early stages of HCCs.

Recent advances in the use of imaging tools, such as CT scanning with contrast medium and magnetic resonance imaging (MRI), have enabled the precise detection of HCCs. Accuracy levels have reached >90% using enhanced CT and MRI techniques, and the current limit of detection for HCCs is ~10 mm diameter.

HCCS can develop from small nodules into large nodules when given access to an arterial blood supply rather than a portal blood supply. Small HCCs with poor arterial blood supplies are difficult to distinguish from adenomatous hyperplasia, large regenerative nodules, or angiomyolipoma. Direct tumor biopsy guided by ultrasound with a fine needle is therefore useful for making a final diagnosis in such cases.

Treatment

There are several treatment modalities for HCC. Surgical treatment is divided into two categories: hepatic resection and liver transplantation. The former is based upon the hepatic functional reserve, tumor location, and tumor numbers, and includes many procedures ranging from partial resection to extended right or left hepatic lobectomy. The latter is based on the tumor size and tumor numbers alone. The Milan criteria (that is, the presence of one nodule <5 cm in size or three nodules <3 cm in size, with no vascular involvement or extrahepatic metastasis) is applied worldwide to HCCs for both deceased and live donor liver transplantation. The current 5-year-survival rate for HCCs that meet the Milan criteria is >80%, compared with <30% for those that do not meet the criteria.

Medical treatment includes ethanol injection, microwave coagulation, and radiofrequency ablation (RFA) by ultrasound guidance. Recently, RFA has become popular for the treatment of HCC, because it offers complete necrosis, and is relatively safe and easy to perform. The 5-year-survival rate after RFA is >80%; however, the recurrence of nodules in non-treated lobes in cases of multicentric carcinogenesis is relatively common, especially in HBV-positive and HCV-positive HCCs. In addition, the differential diagnosis of intrahepatic metastasis and multicentric nodules can be difficult, although the rate of local recurrence is lower after RFA. Several reports have described a genetic approach for detecting multicentric and intrahepatic metastatic nodules, although this technique remains controversial. Only small nodules with an arterial blood supply are diagnosed as intrahepatic metastatic nodules during the early stages of HCC, because multicentric nodules still possess a portal blood supply at this time.

Transcatheter arterial embolization (TAE) has been developed as a technique for the treatment of advanced HCCs with high-volume arterial blood supplies. The blood supply to the normal liver is 25% arterial and 75% portal. By contrast, advanced HCC tissues have an arterial blood supply of 99%. The intensive embolization of a segment with advanced HCC can lead to 99% ischemic change in HCC tissues and 25% ischemic damage in non-cancerous tissues. Consequently, a major ischemic effect occurs in the HCC tissues with only a minor ischemic effect in the liver tissues. A meta-analysis and cohort study indicated that TAE combined with an anti-cancer agent containing lipiodol was an effective tool for the treatment of advanced HCC. An additional cohort study of 8,510 cases of advanced HCC reported 3-year and 5-year survival rates of 47 and 26%, respectively. These data were taken from the Japanese Liver Cancer Study Group during the past 10 years.

Recently, good results have been produced using chemotherapy along with an interferon injection. Advanced HCCs with portal vein tumor thrombi that were treated with intra-arterial continuous infusion of 5-fluorouracil (5-FU) via a reservoir, along with a daily subcutaneous injection of interferon alpha, showed a 45% response rate. Cisplatin (CDDP) is another eagerly anticipated new drug for use in chemotherapy against advanced HCC.

Prognosis and Prevention

The prognosis of HCC is dependent upon the tumor development and associated hepatic functional reserve. Several prognostic schemes have been developed for HCC, including the CLIP score from Italy, the Barcelona Clinic of Liver Cancer (BCLC) system from Spain, and the Japanese Integrated System (JIS) and modified JIS.

The natural course of HCC is usually studied over time; however, data from this type of study are not yet available for recently developed methods. The ultimate treatment modality for HCC without recurrence is liver transplantation, yet the problem of how to manage subsequent recurrent HCV infection persists. Rapid progression towards liver cirrhosis is also a major problem throughout the world.

The prevention of HCC has become an important medical issue. A program for the eradication of HCV by Peg interferon with ribavirin has been applied worldwide, with a current success rate of 50–60%. Patients who have undergone a virological response due to treatment with Peg interferon and ribavirin have shown no potential for subsequent hepatocarcinogenesis. Biochemical responder as normal transaminase with reappearance of HCV after combination therapy is also reduced the potential of hepatocarcinogenesis.

This preventative procedure has also been applied for HBV using lamivudine, adefovir, and entecavir. However, this approach has failed to eradicate HBV, and has only succeeded in reducing the HBV DNA titer. This reduction of HBV DNA and normal transaminase suggests the inhibition of hepatocarcinogenesis. Another chemoprevention material is not available until now in clinical.

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Hepatocyte

Definition
Predominant cell type in the liver of epithelial origin. Involved in the metabolism of endogenous and exogenous substances and the maintenance of physiological homeostasis.

►Hepatic Ethanol Metabolism
►Hepatocellular Carcinoma

Hepatocyte Activator Inhibitor-1

Definition
HAI-1; Is a transmembrane serine protease inhibitor that regulates the conversion of latent to active ►hepatocyte growth factor (HGF).

Hepatocyte Growth Factor

Definition
HGF.

►Scatter Factor

Hepatocyte Growth Factor-like Protein

►Macrophage-Stimulating Protein

Hepatocyte Stimulating Factor

►Interleukin-6

Hepatocyte Stimulating Factor-III

►Leukemia Inhibitory Factor

H

Hepatolithiasis

Definition
Stone formation within the intrahepatic bile ducts.

►Cholangiocarcinoma

Hepatoma

►Hepatocellular Carcinoma – Etiology, Risk Factors and Prevention
►Hepatocellular Carcinoma

Hepatomegaly

Definition
Enlarged liver.

►Hepatic Epithelioid Hemangioendothelioma

Hepatosplenomegaly

Definition
Enlarged liver and spleen.

Hepsin

Definition

Is a serine protease (type II) spanning the plasma membrane, highly expressed on the surface of hepatocytes. The physiological function is not known, although in vitro studies indicate that hepsin plays a role in the initiation of blood coagulation and in hepatocyte growth.

►TMPRSS1

HER2

Definition

Synonym ErbB2 or Neu; Human epidermal growth factor receptor 2. A member of the human epidermal growth factor receptor (HER or EGFR) family that is amplified/overexpressed in ~25% of invasive breast cancers and is the molecular target for the therapeutic antibody trastuzumab (Herceptin).

►Basal-like Breast Cancer
►Herceptin

HER-2/neu

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Synonyms

ERBB2; c-erb-B2; p185neu; v-erb-B2; Avian erythroblastic leukemia viral oncogene homolog 2

Definition

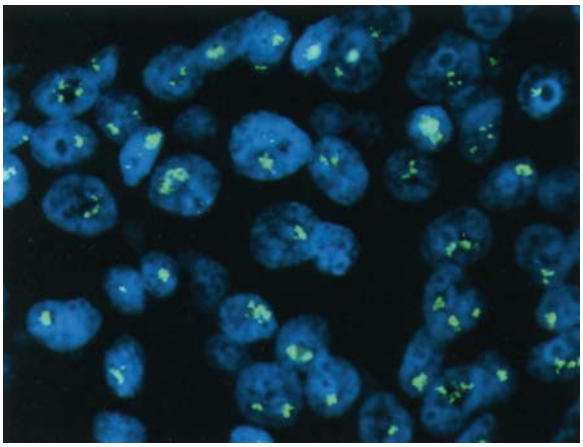
The proto-oncogene HER-2/neu (ERBB2) is located on chromosome band 17q21.1 and encodes a transmembrane ►receptor tyrosine kinase. The name for the HER-2 protein is derived from "Human Epidermal growth factor Receptor" as it features substantial homology with the ►epidermal growth factor receptor (EGFR).

Characteristics

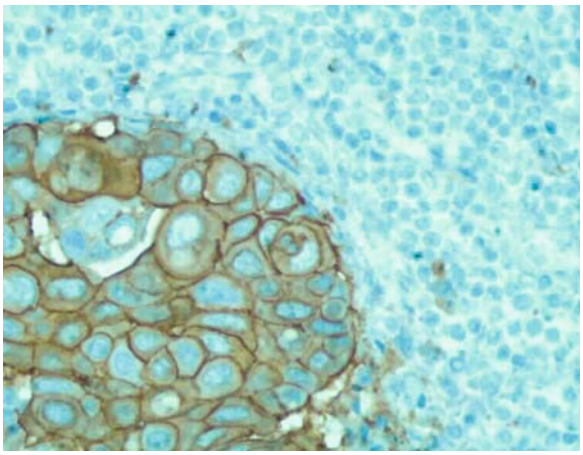
The HER-2/neu (p185neu) protein belongs to a family of closely related growth factor receptors including.

- HER-1 (ERBB1), EGFR
- HER-2 (ERBB2)
- HER-3 (ERBB3)
- HER-4 (ERBB4)

HER-2/neu gene ►amplification and protein overexpression have been identified in 10 to 34% of breast cancers, and patients whose breast cancers contain HER-2/neu aberrations have a poor prognosis. The prognostic impact of HER-2/neu amplification was initially most convincing in patients with axillary



HER-2/neu. Figure 1 HER-2/neu gene amplification detected by FISH. Note clusters of signals in each nucleus.



HER-2/neu. Figure 2 HER-2/neu protein overexpression detected by immunohistochemistry. Note intense continuous membrane staining pattern.

HER-2/neu. Table 1 Reagents for HER-neu analysis

Product name	Source	Method	Indication
Inform	VentanaMedical	FISH	Prognosis
HercepTest	Dako	IHC	Herceptin response
PathVysion	Vysis	FISH	Prognosis, taxotere response
CB11	VentanaMedical	IHC	Herceptin response
Serum test	Bayer Diagnostics	ELISA	Prognosis, therapy response

lymph node metastases. By contrast, a prognostic role was less compelling in patients with localized (lymph node negative) breast cancer. Various studies came to different conclusions regarding a link between HER-2/neu gene or protein abnormalities and survival in lymph node negative patients. Reviews of these non-correlating studies have focused on the immunohistochemical methods used to identify over-expression of the HER-2/neu protein. Technical inconsistencies in the detection of HER-2/neu protein by ►immunohistochemistry have included both the differing sensitivities and specificities of the commercially manufactured anti-HER-2/neu antibodies and the several antigen retrieval techniques used in formalin-fixed paraffin embedded tissues. Also, the lack of a standardized interpretation protocol results in significant inter-observer variability. Notably, fluorescence in situ hybridization technique (►FISH) evaluations have consistently demonstrated a relationship between HER-2/neu gene amplification and breast cancer recurrence and disease-related death in both lymph node-negative and -positive cases (Figs. 1 and 2)

►ELISA-based measurements of HER-2/neu protein in breast cancer cytosols also correlate with disease outcome. Serum-based HER-2/neu evaluation can also be accomplished using the ELISA method, although consensus as to the clinical utility of this technique has not been achieved. Southern- and slot-blotting methods are less effective since the DNA of tumor cells extracted from the primary carcinoma sample are diluted by DNAs from benign breast tissue and inflammatory cells. More recent studies support an association between HER-2/neu gene amplification or protein over-expression with poor clinical outcome in gastrointestinal, pulmonary and genitourinary neoplasms.

Clinical efficacy of the drug, ►trastuzumab (Herceptin™), has stimulated significant expansion of HER-2/neu testing in the management of breast cancer. Tratsuzumab is a humanized monoclonal antibody administered intravenously that is particularly effective when combined with cytotoxic agents (either ►taxotere or ►Adriamycin plus Cytoxan). A 27% response rate with these treatment approaches has been described for patients with advanced metastatic breast cancer refractory to conventional treatment. In 1998, the US FDA approved the use of ►tyratsuzumab for the treatment of metastatic HER-2/neu positive breast cancer (IHC 2+ or 3+). In 2006, the US FDA also approved trastuzumab in combination with cytotoxic chemotherapy for the adjuvant treatment of lymph node positive primary breast cancer. On going clinical trials of trasuzumab plus cytotoxic therapy have, to date, failed to achieve significant results for patients with prostate, lung, ovarian and pancreatic cancers. The various products available for HER-2/neu testing are shown in the Table 1.

In addition to its role in predicting prognosis and response to trastuzumab therapy, HER-2/neu testing in breast cancer has also been used to select other treatment options including the use of the dual kinase oral HER1/HER2 inhibitor, ►lapatinib. Although the ability of HER-2/neu status to predict response to anti-estrogen therapy has not achieved consensus, the enhanced response of HER-2/neu positive tumors to chemotherapy regimens containing anthracyclines is generally well-accepted in clinical oncology practice. However, cardiotoxicity is the major side-effect of trastuzumab and this has limited the use of trastuzumab in combination with anthracylines especially in the metastatic disease setting.

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HER3

Definition

Synonym ErbB-3; A member of the EGFR family of receptor tyrosine kinases. It is overexpressed in breast, stomach, pancreatic and colon cancer.

►ADAM17
►ErbB-3
►ADAM17

Herceptin

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Synonyms

Trastuzumab; RhumAb Recombinant humanized anti-HER-2 monoclonal antibody; Anti-human p185neu receptor immunoglobulin G1

Definition

Herceptin is a recombinant humanized monoclonal antibody that selectively binds with high affinity to an extracellular domain of the HER-2 receptor ►tyrosine kinase, and is approved by the United States Food and Drug Administration for therapeutic use in patients with early-stage and metastatic breast cancers that overexpress the HER-2 protein.

Characteristics

Approximately 20–30% of human breast cancers overexpress the ►HER-2/neu receptor tyrosine ►kinase protein. These cancers are extremely aggressive with an increased tendency to ►metastasize, decreased time to disease progression, and reduced overall survival. In 1998, the United States Food and Drug Administration approved Herceptin, a monoclonal antibody targeted against an extracellular portion of HER-2, for clinical use in metastatic breast cancers that show ►amplification of the *her-2* oncogene and overexpress the HER-2 protein. Recent studies also demonstrated that Herceptin-based treatment prevents recurrence of approximately half of all early-stage localized breast cancers that overexpress HER-2.

Clinical Activity

As a single agent Herceptin has a response rate of 12–34% in metastatic breast cancer, depending on the method used for assessing HER-2 status in the cancer cells and the number of prior therapies. These rates are significantly improved to about 60% when combined with chemotherapeutic agents of the ►taxane class. The duration of response is one year or less, indicating that resistance develops rapidly. The main side effect associated with Herceptin-based therapy is ►cardiotoxicity.

In the ►adjuvant setting, Herceptin shows significant benefit in early stage HER-2-overexpressing breast cancer. Randomized phase III clinical trials showed that addition of Herceptin to postoperative chemotherapy (either in sequence or in combination) reduces the risk of recurrence by half. However, ~15% of women still develop metastatic disease despite receiving Herceptin-based adjuvant therapy.

Mechanisms of Activity

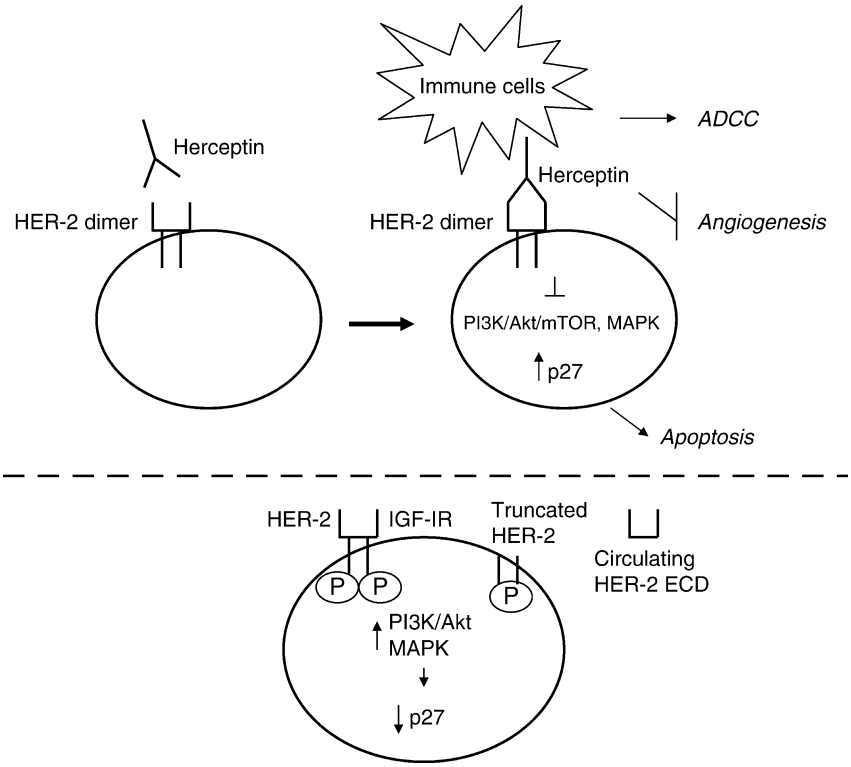
Molecular Level

Herceptin physically binds to the HER-2 receptor, and inhibits downstream signaling through the ►phosphatidylinositol-3-kinase (PI3K) and ►mitogen-activated protein kinase (MAPK) pathways (Fig. 1). This induces expression of the ►cyclin-dependent kinase (cdk) inhibitor p27kip1 and increases association of p27kip1 with cdk2, promoting G0/G1 cell cycle arrest and blocking cellular proliferation. In addition, Herceptin disrupts association between HER-2 and the non-receptor tyrosine kinase ►Src, thus, reducing Src activity such that the ►phosphatase PTEN is dephosphorylated and translocated to the plasma membrane where it is activated. Signaling from the PI3K downstream effectors ►Akt and ►mTOR are then inhibited, resulting in reduced proliferation and enhanced ►apoptosis.

Cellular Level

In vivo studies show that Herceptin activates an immune process called ►antibody-dependent cellular cytotoxicity (ADCC), which results in the lysis of cells to which Herceptin is bound. Mice deficient for immune cells mediating this process are less responsive to Herceptin, and patients treated with Herceptin show a higher infiltration of ►leukocytes and ADCC activity.

In addition, cellular survival is abrogated by Herceptin due to inhibition of ►angiogenesis, which is achieved by both reduced expression of pro-angiogenic



Herceptin. Figure 1 Herceptin: Mechanisms of Action. (Upper portion) Herceptin binds to the extracellular domain of the HER-2 protein, which is in a homodimer or heterodimer with another receptor. Upon binding, several molecular mechanisms become activated. Immune cells recognize the antibody-bound cells and initiate a process called antibody-dependent cellular cytotoxicity (ADCC), which results in lysis of the cells to which Herceptin is bound. Herceptin also decreases expression of factors that induce formation of blood vessels, a process called angiogenesis, and increases expression of anti-angiogenic factors. In addition, signaling from the downstream pathways including PI3K/Akt/mTOR and MAPK is inhibited leading to a block in proliferation and increased cell death, or apoptosis. (Bottom portion) Resistance to Herceptin is an important clinical problem. Preclinical studies show a unique interaction between HER-2 and the insulin-like growth factor-I receptor (IGF-IR) in resistant cells with cross talk occurring from IGF-IR to HER-2 resulting in heightened signaling and reduced p27kip1 levels. Other studies have shown increased Akt signaling due to other unknown mechanisms, which may include loss of the Akt negative regulator PTEN phosphatase. Truncated forms of HER-2 have also been discovered such that the cytoplasmic and transmembrane domains remain bound to the cell membrane with constitutive kinase activity, and the extracellular domain (ECD) is released and can be detected circulating in serum.

growth factors and increased expression of anti-angiogenic factors, resulting in decreased ►endothelial cell migration and formation. Combination of Herceptin with chemotherapeutic agents may actually rely upon this ability of Herceptin to block angiogenesis, as tumor vasculature and circulation of anti-neoplastic therapies may become normalized.

Mechanisms of Resistance

The majority of metastatic breast cancer patients who initially respond to Herceptin begin to demonstrate disease progression again within one year. Even in the adjuvant setting, where trastuzumab improves survival rates in patients with early-stage breast cancer, ~15% of patients eventually develop metastatic disease. Preclinical studies have provided some information regarding potential molecular mechanisms contributing to trastuzumab resistance, although further work is required to achieve an improved response rate in the HER-2-overexpressing population, and to identify novel agents that will benefit patients whose cancers are refractory to trastuzumab.

Insulin-like Growth Factor-I Signaling

Increased signaling from the ►insulin-like growth factor-I (IGF-I) receptor (IGF-IR) is associated with Herceptin resistance. Increased signaling may occur through two different mechanisms. Overexpression of

total IGF-IR levels resulted in resistance in two cell culture models. In addition, a unique physical interaction between IGF-IR and HER-2 resulting in increased IGF-IR signaling and receptor ►cross talk to HER-2 was observed in a separate model of Herceptin resistance developed subsequent to chronic drug exposure. Herceptin sensitivity was restored in this model when IGF-IR was inhibited. Further in vivo studies will determine whether this mechanism of resistance translates to patients and whether in fact IGF-I signaling is an appropriate target for drug development in Herceptin-resistant cancers.

Akt Signaling

Activation of the HER-2 tyrosine kinase secondary to protein overexpression results in increased signaling from the PI3K/Akt/mTOR pathway. Constitutive PI3K/Akt/mTOR signaling is observed in some Herceptin-resistant cells, perhaps due to deficient expression of the Akt negative regulator, the PTEN phosphatase. Although the mechanism by which Akt signaling may be heightened in Herceptin-resistant cancers remains unclear, this pathway is being developed as a putative novel target in this patient population.

Extracellular Domain and Truncated Forms of HER-2

The full-length HER-2 protein is cleaved into two fragments in some cancer cells, a 95 kDa truncated receptor that remains bound to the membrane with constitutive kinase activity, and a 110 kDa fragment consisting of the ►extracellular domain (ECD), which can be found circulating in cell culture media or in patient sera. Herceptin may bind the ECD, decreasing interaction between intact membrane HER-2 receptors and Herceptin, and thus reducing response. In addition, the truncated 95 kDa receptor lacks the ECD and thus cannot bind Herceptin. Hence, in cases where circulating HER-2 ECD levels are elevated, resistance to Herceptin may develop. A decline in ECD levels after exposure to Herceptin has in fact been correlated with response to treatment, suggesting that changes in ECD levels over time may serve as a biological predictor for response to Herceptin.

The mechanism by which the full length receptor becomes cleaved in some cases and not in others is unknown. Cleavage may occur due to the activity of ►matrix metalloproteinases (MMPs), as MMP inhibitors prevent ECD shedding. Alternatively, or in addition, recent evidence suggests that truncated forms of HER-2 may be a result of alternative initiation of translation using different start codons in the *her-2* gene. Understanding the mechanism of cleavage and the value of circulating ECD in predicting Herceptin response is an important focus of ongoing clinical investigation.

New Directions

The use of Herceptin has now expanded into the adjuvant setting to significantly benefit patients with early-stage, localized HER-2-overexpressing breast cancer. In the metastatic setting, Herceptin is being tested in combination with various other targeted agents to improve response rates and duration and limit cytotoxic effects. Lapatinib, a dual inhibitor of both the ►epidermal growth factor receptor (EGFR) and HER-2 tyrosine kinases, is being tested in patients whose breast cancer has progressed on trastuzumab, and appears to be a promising agent in this setting and in trastuzumab-naive patients. In addition, studies are examining the efficacy of targeting the IGF-I and Akt/mTOR signaling pathways in patients whose cancers are refractory to trastuzumab. Identification of novel therapeutic targets will guide the development of more effective treatments and trastuzumab-based combinations for the HER-2-overexpressing population of breast cancer patients.

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Hereditary Breast Cancer

Definition

►Familial Breast Cancer

Hereditary Hamartosis Peutz-Jeghers

►Peutz-Jeghers-Syndrome

Hereditary Leiomyomatosis and Renal Cell Cancer

Definition

HLRCC; A dominantly inherited tumor predisposition syndrome caused by mutations in the *fumarate hydratase* gene.

►Fumarate Hydratase

Hereditary Nonpolyposis Colon Cancer

Definition

HNPCC, synonym ►Lynch syndrome; is an autosomal dominantly inherited disease associated with a marked increase in cancer susceptibility. The incidence in the population is around 1 in 500. It is characterized by an early onset of colon cancer, with a predominant location of the tumor in the proximal side of the colon and an excess of synchronous and metachronous colorectal cancer. A variety of extracolonic tumors (such as endometrium, stomach, small bowel, urinary tract, biliary system, and ovary) are also reported in HNPCC. Population-based epidemiological studies indicate that the disease accounts for around 5% of total cases of colorectal cancer in Western countries.

►Mismatch Repair in Genetic Instability

Hereditary Nonpolyposis Colorectal Cancer

►Lynch Syndrome

Heregulin

Definition

Synonym Neuregulin. A ligand for the erbB-3 and erbB-4 receptors.

►ADAM17

HERG

Definition

The human ether-a-go-go related gene encodes for the pore forming subunit for the rapid component of the delayed rectifier potassium channel Ikr. This target is the primary target of Vaughn-Williams Class III antiarrythmics.

►Lead Optimization
►Ether-à-go-go Potassium Channels

H

Herniorrhaphy

Definition

Surgical repair of a hernia.

►Pseudomyxoma Peritonei

Herpes Simplex Virus

Definition

A large DNA virus causing typical lip lesions. Herpes viruses have a large double stranded DNA genome and form enveloped particles with an icosahedral capsid. Genetically modified strains are used in ►oncolytic virotherapy.

►Kaposi Sarcoma
►Human Herpesvirus 6

Herpesvirus-Associated Ubiquitin-Specific Protease

Definition

►HAUSP De-Ubiquitinase

Herpesvirus-Associated Ubiquitin-Specific Protease De-ubiquitinase

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Institute for Cancer Genetics, and Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Synonyms

HAUSP de-ubiquitinase

Definition

The cysteine protease ►de-ubiquitinase (DUB) Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) has been fairly well characterized in recent years and has emerged as an important component of the ►p53-►Mdm2 signaling pathway.

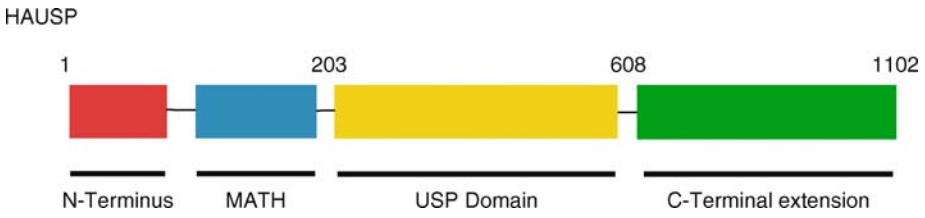
Characteristics

De-ubiquitinases are specific protease enzymes that catalyze the removal of ubiquitin from substrates by cleaving the isopeptide bond between ubiquitin and substrate. The de-ubiquitination enzyme family (DUBs) fall within two classes of proteases: the metalloproteases and cysteine proteases, though most DUBs fall within the latter. Further classification subdivides the cysteine proteases into four sub-classes: ubiquitin C-terminal hydrolases (►UCH), ubiquitin-specific proteases (►USP), ►Machado-Joseph disease proteases (MJD), and Otubain proteases (OTU). The MJD and OTU subclasses were more recently identified through a bioinformatics approach. The UCH human subclass consists of four proteins that are thought to function mainly for the recycling of ubiquitin or for the processing of newly synthesized ubiquitin that forms as either a polyubiquitin chain precursor or fused to ribosome precursors. The largest and best characterized sub-category is by far the USP family, with proteins having diverse cellular functions from oncogenesis to transcriptional regulation. USPs contain a well conserved catalytic site consisting of a Cysteine-Histidine motif that is critical for its enzymatic activity. However, aside from this core region, USPs are quite diverse in both their size and additional domains, a point that may be important for their range of functions and substrate specificities.

One particular cysteine protease DUB, the Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP), has been fairly well characterized in recent years and has emerged as an important component of the p53-Mdm2 signaling pathway. HAUSP was first identified as a 135 kD cellular factor that associated with the Herpesvirus regulatory protein Vmw110. It contains 1,102 amino acids and can be loosely divided into three functional domains: an N-terminal domain consisting of a meprin and TRAF homology motif (MATH), the core cysteine-histidine catalytic domain, and a C-terminal region (Fig. 1). HAUSP functions as a specific de-ubiquitinase for several substrates by removing ubiquitin monomers from them causing diverse functional consequences. De-ubiquitination of the mammalian proteins p53, Mdm2, and MdmX causes ►protein stabilization and significant effects to the p53-Mdm2 signaling pathway as detailed below. HAUSP-mediated de-ubiquitination of the transcription factor FOXO4 causes transcriptional repression in response to oxidative stress. HAUSP also has direct interactions with several viral proteins including the herpesvirus (HSV-1) ICP0 and Epstein-Barr Virus (EBV) EBNA1. In the case of the HSV-1 protein ICP0, a shared reciprocation occurs with HAUSP where ICP0 can also ubiquitinate HAUSP. However, the most well characterized functions of HAUSP to date are within the p53-Mdm2 pathway and indicate the growing complexity of the physiologic functions of HAUSP.

p53-Mdm2 Pathway

Regulation of the p53-Mdm2 pathway is of utmost importance for the development, formation, and progression of tumors. The tumor suppressive properties of the sequence-specific transcription factor p53 is underscored by the fact that it is frequently mutated in over 50% of all human tumors. The ability of p53 to quickly respond to cellular stress such as DNA



Herpesvirus-Associated Ubiquitin-Specific Protease De-ubiquitinase. Figure 1 Schematic representation of the HAUSP protein structure. HAUSP can be sub-divided into three main functional domains: the N-Terminus, which includes the meprin and TRAF-like homology motif (MATH), the core Cysteine-Histidine catalytic motif, and the C-terminal region.

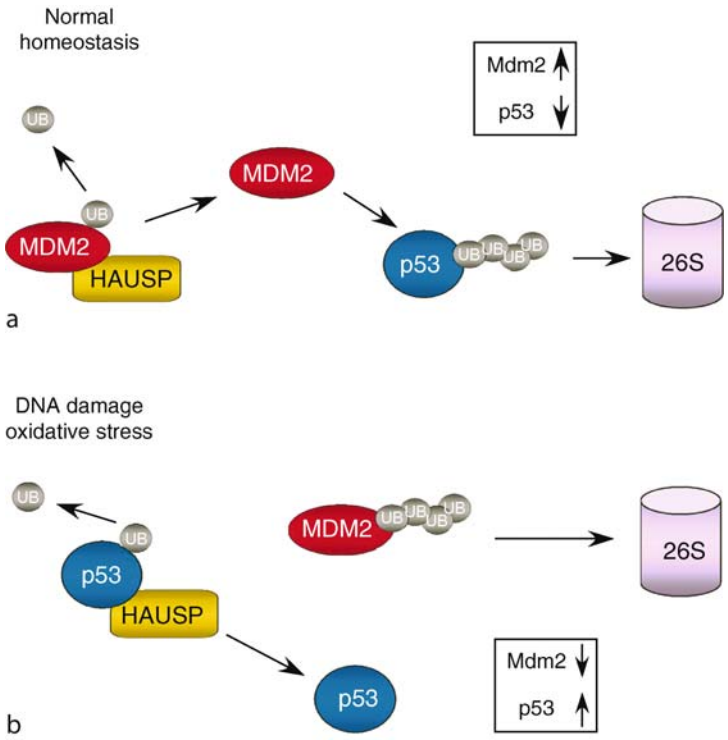
damage, oxidative stress, and aberrant ►oncogene expression occurs in part due to the multitude of mechanisms that exist to quickly stabilize and activate p53 to a level sufficient for it to activate genes involved in cell growth arrest and apoptosis. Without this pathway, the cell loses the tumor suppressive "brake" and increases the probability that cell growth will occur in an unregulated and chaotic fashion. p53 regulation is therefore a critical step in cellular homeostasis. The predominant negative regulator of p53 is the ►E3 ubiquitin ligase, ►Mdm2. This E3 ligase specifically ubiquitinates p53 and promotes ►proteasome-mediated degradation through the catalysis of ubiquitin chains on the substrate via isopeptide linkages. Mdm2 therefore ensures the correct spatial and temporal induction of p53 protein levels to prevent expression of this potent transcription factor when it is not needed. p53 and Mdm2 also exist in a negative feedback loop with each other, whereby p53 acts as a specific transcription factor for the *mdm2* gene and promotes its induction. When p53 is activated and protein levels are high, Mdm2 is induced, which in turn drives the reduction of p53. The balance of protein levels for p53 and Mdm2 is therefore a critical step in maintaining proper p53 expression and function.

HAUSP was first shown to function within this pathway when it was discovered that it could specifically interact with p53. HAUSP acts as a specific de-ubiquitinase for p53 in a dose-dependent manner by removing ubiquitin moieties and stabilizing the protein. It can also antagonize Mdm2-mediated ►ubiquitination of p53 and rescue the protein from degradation. Ubiquitination of p53 by Mdm2 and the subsequent degradation by the 26S proteosome is a critical regulatory mechanism for maintaining p53 at low protein levels during times of cellular homeostasis. By antagonizing this function, however, HAUSP serves as an important positive regulator of p53 and may be important for its function during times of cellular stress. Having a way for reversing protein degradation provides the cell with a mechanism for quick stabilization of p53 when needed. HAUSP provides this mechanism by blocking the degradation of p53 and allowing the protein to quickly accumulate.

In addition to p53, HAUSP can also de-ubiquitinate and stabilize Mdm2 protein. Mdm2 has inherent auto-ubiquitination capability, and as such ubiquitinates itself creating an unstable protein with a half-life of approximately 30 min. HAUSP can specifically de-ubiquitinate Mdm2 and block the self-ubiquitination. This stabilization effect in turn leads to more Mdm2 that is able to ubiquitinate and degrade p53, a finding that is contrary to the direct effects HAUSP has on p53. The mechanism underlying these seemingly different results seems to depend on the levels of HAUSP protein and suggests that HAUSP interacts with both proteins in a selective or guided manner. Complete ablation of the HAUSP gene using a somatic knockout approach in the colorectal cancer cell line HCT116 has a profound stabilizing effect on p53 and destabilizing effect on Mdm2. However, when the gene product is reduced marginally by transient siRNA in several different cell types, p53 reduction is also seen. The ability of HAUSP to de-ubiquitinate both proteins suggests an intrinsic "switch," whereby HAUSP is selectively triggered to de-ubiquitinate one substrate or the other. A provocative possibility is that HAUSP is DNA damage responsive and may switch substrate specificity upon receiving various stress signals. It has been shown that treatment with the ►radiomimetic drug ►neocarzinostatin (NCS) blocks the interaction between HAUSP and Mdm2. In addition, crystal structures have shown that the Mdm2-HAUSP complex has a higher binding affinity and makes more extensive interactions than the p53-HAUSP complex. The data to-date suggest a provocative model where HAUSP provides an important and delicate balance of proteins within the p53-Mdm2 pathway (Fig. 2). Under normal, unstressed conditions, the preferential interaction is the HAUSP-Mdm2 complex, where HAUSP can maintain sufficient levels of Mdm2 protein to keep p53 ubiquitinated and degraded. However, during times of cellular stress, the interaction between HAUSP and Mdm2 is blocked, possibly due to post-translational modification such as phosphorylation. The release of the HAUSP-Mdm2 complex creates a pool of free HAUSP monomers that can then interact with p53 to de-ubiquitinate and stabilize the protein. Free Mdm2 protein is then subject to auto-ubiquitination and degradation. The negative feedback loop of these 2 proteins will then drive higher Mdm2 expression and the subsequent downregulation of p53 when sufficient time has allowed for the cell to either repair the DNA damage or initiate an apoptotic response. The oscillating responses of p53 and Mdm2 protein levels together with the instability of these proteins through the ubiquitin-proteosome pathway indicate the important need of HAUSP in balancing these proteins for appropriate p53 responses to quickly occur.

Implications in Cancer Therapy

The reasoning for intense study of the p53-Mdm2 pathway and proteins that have functional impact on the pathway is clear when considering the large ►chemotherapy potential it could have for various cancer treatments. The p53 gene is frequently mutated in over 50% of all human tumors, and in addition, a subset of tumors retains a functional p53 gene and requires these tumor cells to circumvent this pathway. These tumors are prime candidates for chemotherapeutics that would induce p53 expression and subject the cells to an abundance of stabilized and activated p53 protein. Drug



Herpesvirus-Associated Ubiquitin-Specific Protease De-ubiquitinase. Figure 2 Model for the function of HAUSP in the p53-Mdm2 pathway. HAUSP preferentially interacts with Mdm2 during times of cellular homeostasis, which stabilizes Mdm2 through deubiquitination and results in Mdm2-mediated ubiquitination and degradation of p53. During times of cellular stress when p53 is needed, the HAUSP-Mdm2 interaction in blocked, resulting in Mdm2 destabilization and subsequent p53 stabilization through the direct interaction with HAUSP.

design has focused on blocking the interaction between p53 and its predominant negative regulator Mdm2 as a mechanism for stabilizing the protein. Indeed, moderate success has been seen with a small molecule inhibitor of Mdm2, Nutlin 3A, in a variety of cancer cell lines. Treatment with this drug significantly blocks the p53-Mdm2 interaction and yields a robust p53 response by stabilizing the protein. Still, other approaches of drug design in this pathway may need to be considered for two reasons. First, E3 RING ubiquitin ligases are not classical enzymes in that they only mediate the transfer of activated ubiquitin from an ►E2-conjugating enzyme to the substrate. Classical targeting of enzymes with small molecule inhibitors generally requires the presence of specific amino acids within the catalytic site of the protein that use energy for a defined enzymatic cascade. The ►RING domain of E3 ubiquitin ligases only mediates the transfer of ubiquitin and as such provides a poor target for drug inhibition. Second, the mechanism of action for Nutlin 3A is to block the protein-protein interaction of p53 and Mdm2. However, the precise binding site for p53 on Mdm2 is ill defined and it remains possible that the proteins are capable of making more than one interaction. This would require an inhibitor that could block multiple binding sites to efficiently and effectively inhibit the p53-Mdm2 interaction. An alternative approach of drug design for stabilizing and activating p53 in this setting would be to target the inactivation of HAUSP. It is clear that removing HAUSP completely causes a profound stabilization and activation of p53 protein. HAUSP also has a classic enzymatic motif that can be targeted for inhibition by small molecule inhibitors. An inhibitor of HAUSP would only have to target its specific de-ubiquitinase activity and not the protein-protein interactions occurring between p53-HAUSP and p53-Mdm2. Inhibition of its activity would create an environment where Mdm2 was severely unstable allowing for the accumulation and activation of p53.

The de-ubiquitinase HAUSP has emerged as an important regulator of the p53-Mdm2 signaling pathway. The process of de-ubiquitination has global consequences within the cell and the ability to reverse the ubiquitin-proteosome pathway has important implications for the quick regulation of protein levels. Further studies of HAUSP will elucidate regulatory mechanisms for the protein itself as well as develop further understanding of how it selectively chooses particular substrates.

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Heterochromatin

Definition

Regions of chromatin that contain silenced genes and replicates late during cell cycle.

►Histone Modifications
►Sirtuins

Heterocromatic Regions

Definition

Regions of a chromosome that are highly condensed, transcriptionally inactive, and late-replicating.

►Replication Factories and Foci

Heterocyclic Amines

Definition

Chemicals with fused aromatic rings (similar to aromatic amities) but with one or more carbon atoms replaced with nitrogen atoms.

►Carcinogen Metabolism
►Food-Derived Heterocyclic Amines

Heterodimer

Definition

A protein composed of two polypeptide chains which differ in composition.

►Taxotere

Heterodimeric Complex

Definition

Complex composed of two different subunits.

►Hamartin

Heteroduplex Analysis

Definition

Method applied for mutation screening. Following Polymerase Chain Reaction (PCR) of a region with a suspected mutation products are denaturated and renaturated allowing four products in case of a mutation (normal double strand, mutated double strand, two heteroduplices). Detection can be performed on renaturation gels.

►Leukemia Diagnostics

Heterokaryon

Definition

Cell containing two or more nuclei having different genetic constitutions.

►Microcell-Mediated Chromosome Transfer

Heterologous Growth Control

►Contact Normalization

Hetero-oligomers

Definition

Refer to complexes consisting of several different protein subunits.

Heteroplasmy

Definition

Single cells contain different mtDNA populations. It could occur due to mutations in mtDNA.

►Mitochondrial DNA

Heterotrimeric G-Proteins

Definition

G proteins are guanine nucleotide binding proteins involved in signal transduction pathways. One subfamily of G proteins is the membrane-associated heterotrimeric G proteins. These proteins are activated by G protein-coupled receptors and are made up of alpha (α), beta (β) and gamma (γ) subunits.

►RAS Activation
►G-Proteins

Heterotrimeric GTP-binding Proteins

►G-Proteins

Heterotrimeric Guanine Nucleotide-binding Proteins

►G-Proteins

Heterozygosity

Definition

Is the condition of having two different ►alleles at one or more homologous chromosomal loci.

►Birt–Hogg–Dubé Syndrome

Heterozygous

Definition

Referring to the configuration of a genetic locus in which the two copies of the gene are different versions ►homozygous.

►Alleles

Hexabrachion

►Tenascin-C

Hexavalent Chromium

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Synonyms

Chromate; Cr(VI); Chromium(VI); Cr^{6+}

Definition

Chromium is a metallic element with oxidation states ranging from +2 to +6. Hexavalent chromium rarely occurs naturally, but is produced from anthropogenic sources. Chromium in the hexavalent state occurs naturally only in the rare mineral crocoite ($PbCrO_4$).

Characteristics

Numerous regulatory agencies worldwide have concluded that all ►hexavalent chromium compounds should be considered carcinogenic among exposed populations although strong evidence has been presented that water-insoluble (also known as particulate) hexavalent chromium compounds are the more potent carcinogens.

Uses and Sources

The metallurgical, refractory and chemical industries are the major users and producers of hexavalent chromium. The compounds are used for chrome plating, the manufacture of dyes and pigments, leather tanning and wood preserving. Smaller amounts are used in rust and corrosion inhibitors, drilling muds, textiles and toner for copying machines. Hexavalent chromium compounds are brightly colored particularly with reds, yellows and oranges and are commonly seen in paints, dyes and inks of those colors.

Hexavalent chromium enters the environment mostly through human activities, particularly as chemical industry waste. It is emitted as mists and particulate matter during manufacture and use of metal chromates. Waste streams from electroplating, leather tanning and textile industries as well as those that make dyes and pigments can discharge hexavalent chromium into waters. The levels of hexavalent chromium in soil come mainly from disposal of commercial products containing chromium, chromium waste from industry and coal ash from electric utilities.

Epidemiology

Hexavalent chromium was first associated with human ►cancer more than one hundred years ago (in 1890) when a case report described an adenocarcinoma in the nasal turbinate bone of a Scottish chrome pigment worker. Since that report, numerous studies of the ►epidemiology of cancer showed that occupational exposure to hexavalent chromium can cause an 18–80 fold increased risk of cancer of the respiratory system, particularly bronchogenic and nasal cancers. These reports were primarily retrospective mortality studies of workers in factories involved in chromate production, chrome pigment production and chrome plating from countries around the world including the United States, United Kingdom, Germany, Norway and Japan. Further epidemiological work has considered other industries such as stainless steel welding, ferrochromium alloy production and leather tanning, but the results have been inconclusive due in part to mixed exposures. Tumors in organs other than the respiratory system have not generally been reported though sporadic reports have been made of stomach and immune-related cancers. This lack of evidence has led to the conclusion that hexavalent chromium is only carcinogenic at sites of contact, but few studies have focused on tumors at other sites and so such a conclusion may be premature.

Human pathology studies support the hypothesis that the water insoluble hexavalent chromium compounds are the more carcinogenic species. Specifically, they show that hexavalent chromium accumulates and persists at bifurcations in the bronchiole tubes where models show that insoluble particles impact and deposit (soluble compounds would likely dissolve in the mucosal lining and be distributed throughout the lung). Hexavalent chromium-induced tumors occur at these bronchial bifurcation sites.

Animal Studies

Animal studies have supported the findings of epidemiology studies and show that hexavalent chromium compounds are carcinogenic in animal assays producing the following tumor types: intramuscular injection site tumors in rats and mice and intrapleural and intrabronchial implantation site tumors in rats. Only hexavalent chromium compounds were found to be carcinogenic in animal studies. Trivalent chromium compounds were not carcinogenic in animals, and so it has been concluded that only hexavalent chromium should be classified as a human carcinogen. In addition, in general only the particulate hexavalent chromium compounds induced tumors in experimental animals.

Animal studies also show that hexavalent chromium compounds are genotoxic (i.e. damage DNA). Specifically, these studies show that hexavalent chromium compounds induce chromosomal aberrations, sister chromatid exchanges (SCEs), mutations, DNA strand breaks and DNA-protein crosslinks.

Cell Culture Studies

Cell culture studies also support the conclusion that hexavalent chromium compounds are carcinogenic. These studies show that hexavalent chromium compounds can transform normal cells into tumor forming cells (i.e. induce neoplastic transformation), though again this effect was specific to the water insoluble compounds, and both water insoluble and soluble compounds can change the morphology of cells. Extensive cell culture work also shows that hexavalent chromium compounds are highly genotoxic inducing DNA damage in cells from numerous species. The

damage includes a variety of DNA lesions including ►aneuploidy, chromosomal aberrations, ►centrosome amplification, SCEs, DNA double strand breaks, mutations, DNA single strand breaks, ►adducts to DNA, DNA-protein cross-links and DNA-DNA cross-links.

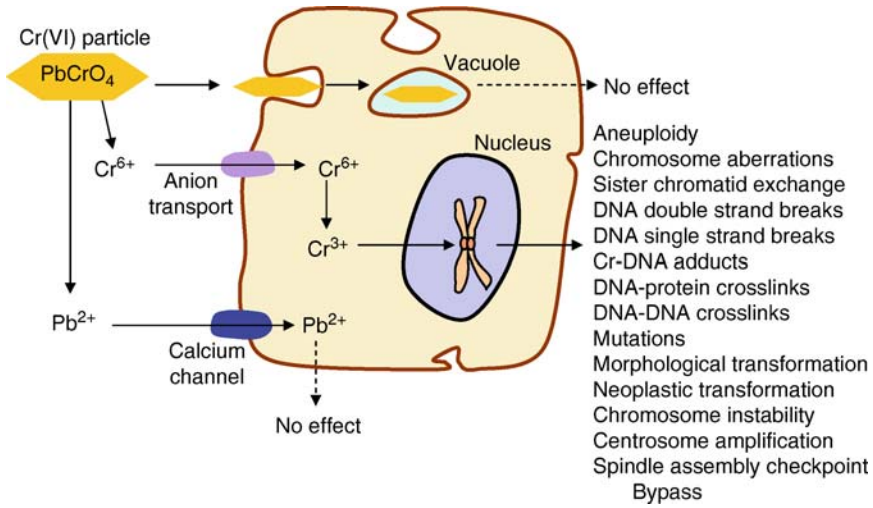
Role of Valence State, Uptake and Metabolism

Hexavalent chromium compounds are carcinogenic and trivalent chromium compounds are not considered to be carcinogenic. The explanation for this difference is related to their modes of uptake. Hexavalent chromium compounds are rapidly and well-absorbed by cells though a facilitated diffusion process involving an anion transport protein. By contrast, trivalent chromium compounds are slowly and poorly absorbed by simple diffusion. This diffusion is further slowed and reduced because trivalent chromium binds to many molecules in the extracellular environment.

Once inside the cell, it is clear that hexavalent chromium itself does not cause damage directly. Instead, it is almost immediately reduced inside the cell to trivalent chromium. *In vitro* studies using naked DNA show that hexavalent chromium cannot bind to or damage DNA itself, but that adding a reducing system does induce breaks and chromium binding to DNA. These studies also show that trivalent chromium can damage and bind to DNA. The reduction of hexavalent chromium to trivalent chromium involves a number of intermediate species. These include pentavalent chromium, tetravalent chromium and reactive oxygen species. *In vitro* studies show that each of these species can interact with and damage DNA. Thus, one or some combination of these species is the ultimate carcinogen. It is still unclear which species are the most important as methods to detect them within biological systems are limited by sensitivity and specificity. Current technology (e.g. electron paramagnetic resonance or spin trapping) can only detect these species at very high doses and data do suggest that the metabolism of hexavalent chromium may be different at lower concentrations. Moreover, pentavalent chromium is generally active in assays that measure reactive oxygen species (e.g. dye activation methods), and thus, one cannot distinguish between the various species in cells.

Role of Solubility

Epidemiological, whole animal and cell culture studies indicate that water solubility plays a key role in ►carcinogenesis caused by hexavalent chromium with water-insoluble compounds the most potent form as discussed above. The potency of the particulate form appears to be related to its ability to impact at bronchial bifurcation sites and continually dissolve over time releasing hexavalent chromium ions that can be taken into cells. Hexavalent chromium particles can clearly enter cells but do not seem to have an effect and the particles do not dissolve inside the cell. Instead, the water-insoluble particles partially dissolve in the extracellular environment. This dissolution releases both a hexavalent chromium anion and a cation (e.g. lead, barium, zinc etc.). The cations do enter cells, but again do not appear to reach high enough levels to induce biological effects. Instead, at this time, all of the toxicological effects of the water-insoluble compounds seem to result from the transport of hexavalent chromium into the cell and its subsequent reduction to trivalent chromium. This mechanism is illustrated in Fig. 1. Future research may reveal a



Hexavalent Chromium. Figure 1 Mechanistic model of particulate hexavalent chromium genotoxicity. This figure is a schematic diagram showing the hypothesized mechanism for particulate chromate carcinogenicity.

yet undiscovered effect of the internalized particles or cation.

Mechanism of Carcinogenicity

The mechanism of carcinogenicity is uncertain and poorly understood with several potential steps emerging. Lung cancers are characterized by aneuploidy (an abnormal number of chromosomes), particularly with a triploid or tetraploid chromosome complement. Recent data indicate that chronic exposures to the water-insoluble hexavalent chromium compounds induce this type of aneuploidy that is caused by centrosome amplification and bypass of the spindle assembly checkpoint. The centrosome amplification and spindle assembly checkpoint bypass may result from hexavalent chromium-induced DNA double strand breaks leading to a prolonged G2/M phase cell cycle arrest or alternatively direct effects on genes involved in centrosome duplication and the spindle assembly checkpoint or both.

A second mechanism is that chromium can bind to both DNA bases and the phosphodiester backbone potentially through coordinate covalent binding or electrostatic/ionic interactions or induce oxidative damage to the bases. These lesions may then result in base substitution mutations, which if unrepaired could lead to carcinogenesis if these mutations occur in key genes such as proto-oncogene or tumor suppressor genes.

A third mechanism involves hexavalent chromium-induced DNA double strand breaks. These breaks may be caused directly by either pentavalent chromium or reactive oxygen species produced by the intracellular reduction of hexavalent chromium or be caused indirectly by chromium-induced adducts to DNA or crosslinks resulting in collapse of replication forks. These double strand breaks then may lead to chromosome aberrations, and chromosome instability described above or may indirectly cause mutations through their repair.

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Hexavalent Chromium-induced Carcinogenesis

►Chromium Carcinogenesis

HFH-11B

►Forkhead Box M1

HGF

Definition

Hepatocyte growth factor (HGF), initially identified as a circulating factor implicated in hepatic regeneration, displays pleiotropic actions in many different tissues. HGF/Met signaling controls cell migration, growth and differentiation in several embryonic organs and is implicated in human cancer.

►Signal Transducers and Activators of Transcription in Oncogenesis
►MET Signaling
►Scatter Factor

HGF Activator

Definition

Is a 34 kD serine protease that is produced as an inactive pro-enzyme and proteolytically cleaved to its active form. Unlike plasminogen activators, which cleave and activate scatter factor (SF; HGF) in stoichiometric

ratios, HGF activator functions to convert proSF to mature active SF in enzymatic quantities.

►Scatter Factor

HGFL

►Macrophage-Stimulating Protein

γH2AX

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Synonyms

Histone H2AX phosphorylated at serine 139

Definition

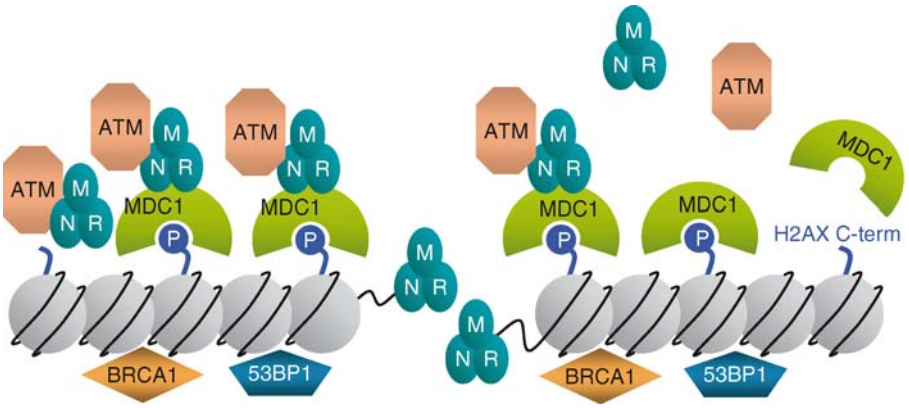
H2AX is one of the histone proteins that is systematically found and ubiquitously distributed throughout the genome. DNA double-strand breaks (DSBs) induce rapid phosphorylation of H2AX (142 amino acids) at serine 139, a highly conserved serine residue located in the C-terminus.

Characteristics

In eukaryotes, DNA is highly condensed and packaged into chromatin within the nuclei. This condensed chromatin forms a structural barrier for DNA processing during ►DNA repair, replication, transcription, and recombination. A fundamental subunit of the chromosome is the nucleosome, which is composed of 146 bp of DNA wrapped in two complete turns around an octamer of the core histones H2A, H2B, H3, and H4, and there are varying lengths of linker DNA connecting these subunits. The core histone octamer forms a 100-kDa protein complex. Histone H2A has been conserved throughout eukaryotic evolution. There are three H2A subfamilies: H2A1-H2A2, H2AZ, and H2AX. Histone variants are non-allelic isoforms that replace major histones within the nucleosome. In a normal human fibroblast, H2AX usually composes about 10% of the H2A histone complement, and the cell contains about 6 × 10^6 molecules of H2AX per cell. H2AX is ubiquitously distributed and expressed throughout the genome. H2AX contains a C-terminal region longer than those seen in the bulk H2A species. The phosphorylated form of histone H2AX, *i.e.* γH2AX, which is phosphorylated at serine 139 within the conserved C-terminal region, has attracted considerable attention. The detection of γH2AX has been estimated by Western blotting, flow cytometry, and imunocytochemistry. Various physical, chemical, and biological factors induced the phosphorylation of H2AX.

Mechanisms

The γH2AX is detectable within 3 min after ionizing radiation (IR), and the amount of γH2AX increases until a plateau is reached 10–30 min after IR. A proposed model could explain how H2AX phosphorylation is regulated and how various DNA repair proteins are recruited to γH2AX-containing chromatin. First, unrepaired DSBs are recognized directly by various proteins, including the MRE11 (meiotic recombination 11)/RAD50/NBS1 (►Nijmegen breakage syndrome 1; NBN; nibrin) (MRN) complex (Fig. 1). The MRN complex then recruits ►ATM (ataxia telangiectasia mutated) to the free DNA ends by mechanisms that include a direct interaction between ATM and the NBS1 C-terminus. ATM thus becomes activated and phosphorylates various target proteins at the site of the DNA damage, including H2AX molecules that are located proximal to the lesion. Next, MDC1 (mediator of DNA damage checkpoint 1) recognizes the phosphorylated H2AX proximal to the lesion *via* its tandem BRCT domain, and in so doing helps to counter γH2AX dephosphorylation by PP2A (protein phosphatase 2A) and possibly by other phosphatases. Concomitantly, MDC1 acts as a mediator/adaptor protein, thus providing a "landing-platform" for more molecules of the MRN complex to bind. Interactions between ATM and MRN and/or between ATM and MDC1 could then bring about the recruitment of further activated ATM molecules to the chromatin regions flanking the lesion: DNA-PKcs (DNA-dependent protein kinase catalytic subunit) and its interaction partner Ku (Ku70 and Ku80) might also be recruited by analogous mechanisms. This further PIKK (phosphatidylinositol 3-kinase-related protein kinase) recruitment then in turn leads to the phosphorylation of additional H2AX molecules that are located more distal to the initiating lesion. A positive feedback loop thus emerges, "fuelled" by MDC1 and its ability to associate with γH2AX; concomitantly protecting γH2AX from dephosphorylation and mediating the recruitment of the MRN complex, active ATM and associated proteins to the chromatin flanking the DNA lesion. As a consequence, γH2AX "spreads" further and further away from the initial break, and a visible γH2AX "focus" starts to form in the nucleus. Thus, many



γH2AX. Figure 1 H2AX phosphorylation.

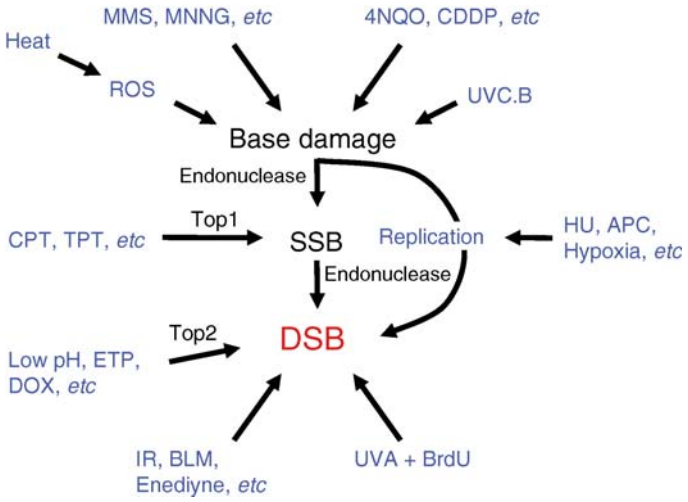
components of the DNA damage response, including ATM, ►BRCA1 (breast cancer 1), 53BP1 (►p53 binding protein 1), MDC1, RAD51, and the MRN complex, form IR-induced foci that co-localize with γH2AX foci. These nuclear micro-domains are thought to contain hundreds to thousands of molecules that accumulate in the vicinity of a DSB.

Among the variety of possible DNA-damage events, DSBs are the most lethal events. Previously, DSBs in cellular DNA have been quantified by pulse-field gel electrophoresis (PFGE) assays, neutral single cell gel electrophoresis (the comet assay), and the DNA elution assay. Since these methods all indicated that the efficiency of DSB detection was very low, the lower limit of DSB detection had been about 100 DSBs per cell nucleus. Because of the rapid induction and amplification of γH2AX, and the 1:1 correspondence between the number of γH2AX foci and the number of DSBs, γH2AX recognizing antibodies have become a gold standard to detect the presence of DSBs.

γH2AX foci were detected, not only after exposure to IR, a ultraviolet light (UV) A-laser microbeam in the presence of bromodeoxyuridine (BrdU) and heavy charged-particles, but also after exposure to classical DSB-inducing agents such as bleomycin (BLM), tirapazamine, etoposide (ETP) and doxorubicin (DOX) (Fig. 2). BLM and tirapazamine produce DSBs *via* production of free radicals. ETP and DOX are thought to induce DSBs by preventing Top2 (DNA topoisomerase II)-mediated DNA religation, because Top2 rewinds the superhelix by cutting DNA double strands and religating them. Low pH also induces the formation of γH2AX foci and DSBs by preventing Top2-mediated DNA relegation.

DNA lesions, other than DSBs, produced by mono-functional alkylating agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and adozelesin, can also lead to γH2AX foci. The γH2AX foci induced by other alkylating agents such as methyl methanesulfonate (MMS) were depressed by DNA repair mechanisms containing Polβ (DNA polymerase β) activity. Since the majority of MMS-induced foci detected were present in PCNA (proliferating cell nuclear antigen) positive cells, these foci might be attributed to replication-mediated DSBs. DSBs are formed in response to single strand breaks (SSBs) being repaired *via* base ►excision repair (BER). This idea is supported by the fact that 4-nitro-quinoline-*N*-oxide (4NQO) and hydrogen peroxide, which produce primarily SSBs, can produce DSBs, and an increase in γH2AX foci is seen in response to this agent. The enediyne antibiotics calicheamicin γ1 and C-1027, which directly produce DSBs at a high frequency, efficiently induced H2AX phosphorylation. Exposure to DNA cross-linking agents such as cisplatin (CDDP) and actinomycin D also result in the production of phosphorylated H2AX. SSBs are induced by DNA cross-links *via* nucleotide excision repair (NER), and the induced SSBs can be converted to DSBs. CDDP induces DSBs and CDDP lethality is reduced by DSB repair mechanisms such as non-homologous end joining repair (NHEJ). Although actinomycin D action is usually considered to be unrelated to DNA damage and to cause an inhibition of RNA polymerase, actinomycin D has been shown to prevent the religation step of Top1 (DNA topoisomerase I) action. When an RNA polymerase molecule progresses through a transcription bubble, the generated topological stress must be released by the DNA nicking and closing activities of a Top1. The production of SSBs would be especially prevalent at transcriptionally active sites. When a replication fork collides with a covalently bound Top1 cleavage complex, the extension of the leading strand is terminated at the 5′-end of the template strand, which generates a DSB. Therefore, actinomycin D can induce the formation of γH2AX foci. Additional support for this idea is provided by the fact that the induction of γH2AX foci occurs only in S-phase cells with active replication fork progression after treatment with Top1 inhibitors such as camptothecin (CPT) and topotecan (TPT).

In response to replication stress induced by UVC, UVB, hydroxyurea (HU), aphidicolin (APC),



γH2AX. Figure 2 DSB formation.

adozelesin, MMS and hexavalent chromium (Cr(VI)), H2AX molecules are phosphorylated by ATR (ataxia telangiectasia and Rad3 related) and ATM through Top1 activities at multisteps. An alternative model suggests that the S phase-dependent DSBs could be generated from SSBs. The formation of DSBs induced by UVC, HU and APC was found by using PFGE and the alkaline comet assay.

DSBs are generated endogenously during regulated DNA transposition events in which H2AX has also been demonstrated to play a role. Such processes include meiotic recombination, V(D)J recombination, heavy chain class switching, apoptotic DNA fragmentation, senescence and dysfunctional telomeres. In addition, the background expression of γH2AX in S/G_2-phase cells could be a response to DSBs or stalled replication forks at damage sites introduced during normal DNA replication.

Hyperosmotic stress and heat stress have also been reported to induce the formation of γH2AX foci by PFGE and the comet assay. The number of existing DSBs is increased when DNA repair is inhibited under hyperosmotic conditions because transient DNA strand breaks are continuously created during transcription and replication, and then hyperosmotic stress induces ►apoptosis.

Heat stress induces ATM activation, and ATM is known to be activated by the presence of DSB lesions. Heat induces DSBs through a different pathway and not in the same manner as that seen in replication arrest in the presence of HU with ATR activation. The inhibition of base BER of base damage is induced by heat stress through the production of reactive oxygen species (ROS), and leads to an increase in the number of existing DSBs. Because DNA synthesis enzymes such as DNA Polβ are heat sensitive when compared with incision enzymes, theoretically providing a mechanism which could account for increased numbers of DNA breaks in heated cells. Therefore, there might be a mechanism to explain the possibility that heat indirectly induces nick formation through enzymatic repair processes. DSBs could then be generated when nicks form in close proximity to each other on opposite strands. The inhibition of ►poly(ADP)ribose polymerase, which is involved with BER and SSB repair, also induces γH2AX foci, providing support for the above hypothesis.

Although hypoxia did not induce apoptosis or any DNA damage detectable with the comet assay, phosphorylation of H2AX occurs with much slower kinetics in response to hypoxic stress. It is still necessary to clarify if hypoxia-induced γH2AX depends on the presence of well-known factors such as DSBs or replication arrest, or on the presence of still unknown factors.

Although there is a report that γH2AX foci formation results from disorganization of chromatin structure, this possibility has not been directly shown to occur. No γH2AX foci were detected after chromatin-modifying treatments (hypotonic conditions, or exposure to chloroquine or tricostatin A). Although γH2AX foci are also induced by replication arrest, it is well established that the formation of γH2AX foci depends on the formation of DSBs, and not just the presence of S-phase.

Clinical Aspects

Hereditary diseases affecting the cellular response to DSBs include ataxia telangiectasia (AT), Nijmegen breakage syndrome and ►Bloom syndrome. The hallmarks of these disorders are growth defects, immunodeficiency, hypogonadism, hypersensitivity to specific DNA damaging agents, chromosomal fragility, and cancer predisposition. Mouse models in which specific components of the DSB repair/signaling pathway are disrupted recapitulate most of the pleotropic features found in ►genomic instability syndromes.

H2AX-deficient mice resemble chromosomal instability disorders in that they show radiosensitivity, male specific infertility, small size, reduced levels of secondary immunoglobulin isotypes, and tumor susceptibility.

The histone H2AX gene, located 11 Mb telomeric to ATM at 11q23.3, is in a region commonly deleted or translocated in several human hematological malignancies and solid tumors. Heterozygous deletion of chromosome bands 11q22–q23 is detected at a particularly high frequency in some types of lymphoma/leukemia with rapid disease progression and poor survival. Since somatic disruption of ATM only accounts for a distinct subset of tumors involving alterations in 11q, other tumor suppressor genes in this chromosomal region are likely to play a pathogenic role. Given that H2AX deficient mice present all of the features of genomic instability syndromes and that the H2AX gene is located in close proximity to the ATM locus, it remains possible that the molecular basis for some AT-like patients that have been classified on the basis of physical mapping may reside in mutations in H2AX.

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HHV4

►Epstein-Barr Virus

HHV8

Definition

Human herpesvirus 8.

►Childhood Cancer

HHV-8

Definition

HHV-8 is a herpes virus found in Kaposi's sarcoma lesions. Following infection, the virus is normally efficiently controlled by the immune system, but under conditions that compromise immune function (aging, AIDS, pharmacologic immune suppression) the control of HHV-8 replication is lost and KS lesions ensue.

►TAT Protein of HIV
►Childhood Cancer

HIF1

Definition

►Hypoxia-inducible factor-1 is a critical regulator of cellular and systemic responses to low oxygen levels.

►Signal Transducers and Activators of Transcription in Oncogenesis

HIF-1

►Hypoxia Inducible Factor-1

HIF1α

Definition

Abbreviation for ►hypoxia-induced factor 1α.

►Thiazolidin

High Affinity Ca^{2+}-binding Protein

►Calreticulin

High Content Screen

Definition

A procedure in which molecules are systematically tested for their ability to activate, perturb, or modify a target or a biological process of interest that is detected *via* techniques that combine automated digital microscopy and flow cytometry.

►Small Molecule Screens

High-dose (Myeloablative) Chemotherapy

►Myeloablative Megatherapy

High-Dose Therapy with Stem-Cell Support

Definition

The access to frozen, autologous stem cells allows the administration of high-dose, myeloablative therapy aiming to eradicate the last tumor cell. Subsequently, the bone marrow function is restored by the infusion into the blood stream of thawed autologous stem cells, which will home to and repopulate the bone marrow.

►Mantle Cell Lymphoma

High Endothelial Venules

Definition

HEVs; are specialized venules found in lymphoid tissues. Lymphocytes migrate from blood into lymphoid tissues by attaching to and migrating across the high endothelial cells that make up the walls of these blood vessels.

►Sjögren Syndrome

High-flow Microinfusion

►Convection Enhanced Delivery

High-frequency Ultrasound Imaging

►Ultrasound Micro-Imaging

High-Grade

Definition

Referring to an advanced tumor that has progressed through ►multi-step tumorigenesis and has become highly malignant.

High Grade/Undifferentiated MEC

►Mucoepidermoid Cancer

High Mobility Group

Definition

HMG group; is a family of chromosomal proteins involved in transcription, replication, recombination, and DNA repair. They are named according to their electrophoretic mobility in acrylamide gels. HMG proteins are subdivided into three superfamilies each with a characteristic functional sequence: HMGB family, the HMGN family, and the HMGA family.

►Stem Cell Markers

High Throughput Screen

►Small Molecule Screens

High Throughput Screening

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

High Throughput Screens

Definition

HTS; Rapid and Large Scale Procedures. The process of using automated assays to search through large numbers of compounds to identify desired biological activities in order to begin (Lead generation). High throughput screens are rapid screens involving parallel assays used in pharmaceutical research to identify potential targets or drugs from large combinatorial libraries of reagents.

►Chromophore-Assisted Laser Inactivation
►Microarray (cDNA Technology)
►Lead Optimization

HILDA

►Leukemia Inhibitory Factor

Hindgut

Definition

The section of the gastrointestinal system between the distal third of the transverse colon and the rectum

►Carcinoid Tumors

HIPK2

Definition

Homeodomain-interacting protein kinase 2.

►Daxx

HIP1r

Definition

Huntingin Interacting Protein 1-related. The only known mammalian relative of HIP1.

►Huntingtin Interacting Protein 1 (HIP1)

Hirschsprung Disease

Definition

HSCR; Is an inherited disease caused by absence of intramural ganglia of Meissner and Auerbach in the intestinal tract. The lack of these enteric ganglia leads to absence of innervation which results in intestinal obstruction. The disease can affect either only a part of the colon (most common) or the entire intestinal tract (very rare). HSCR usually occurs as an isolated case, however, HSCR can also be familial. Evidence suggests that the genetics of long-segment Hirschsprung disease and short-segment Hirschsprung disease is different. To date, more than ten genes (►*RET*, *GDNF*, *NTN*, *EDNRB*, *EDN3*, *SOX10*, *ECE1*, *PHOX2B*, *SMADIP1*, *HSCRM1*, and *HSCRM2*) have been identified to play some role in the pathogenesis of HSCR. The major susceptibility gene for HSCR is *RET*.

►RET

Hirsutism

Definition

Excessive growth of facial or body hair in women.

►Granulosa Cell Tumors

Histiocytoid

Definition
Like histiocytes which are part of the human immune system.

►Hepatic Epithelioid Hemangioendothelioma

Histiocytosis X

►Langerhans Cell Histiocytosis

Histo-blood Group Lewis Antigens

►Lewis Antigens

Histocompatibility

Definition
Histocompatibility is literally the ability of tissues (Greek:histos) to get along with each other. It is used in immunology to describe the genetic systems that determine the rejection of tissue and organ grafts resulting from immunological recognition of histocompatibility (H) antigens.

►MHC

Histocompatibility Antigens

Definition
Synonym H antigens are known as major histocompatibility antigens when they encode molecules that present foreign peptides to T cells and as minor H antigens when they present polymorphic self peptides to T cells.

►Sjögren Syndrome

Histocompatibility Testing

Definition
A method of matching the self antigens (►HLA) on the tissues of a transplant donor with those of the recipient. The closer the match, the better the chance that the transplant will take.

Histocultures

Definition
Ex vivo Culture or Excipient Culture; a piece of tissue or tumor is excised and cultured in vitro usually on collagen. Used to study therapy resistance, cell-cell interactions, tumor markers and drug penetration in a more realistic tumor microenvironment than monolayer culture.

►Three-Dimensional Tissue Cultures

Histogram

Definition
Is a graphical illustration showing the frequency distribution of a measured parameter.

►Oxygenation of Tumors

Histologic Grading

►Grading of Tumors

Histology

Definition
The microscopic study of thin intact slices of tissue. A histologic section allows for detailed study of the cells making up the tissue and the architectural relationship of the cellular components to each other.

►Fine Needle Aspiration
►Molecular Pathology
►Pathology

Histone

Definition
A group of simple alkaline proteins found in the chromosomes of eukaryotic cells.

►Histones

Histone Acetylation

Definition
Functional histone modification of the N-terminal tails at lysine residues by histone acetyltransferases. The source of the acetyl group in histone acetylation is acetyl-coenzyme A.

Histone Acetyltransferases

Definition
HATs; Family of proteins that acetylate conserved lysine amino acids on histone proteins by transferring an acetyl group from acetyl CoA to lysine to form ε-N-acetyl lysine. Lysine acetylation neutralizes the positive charge normally present, thus reducing affinity between histone and (negatively charged) DNA which renders DNA more accessible to transcription factors. Also, histone acetylation and other posttranslational modifications generate binding sites for specific protein-protein interaction domains.

►Lunasin

Histone Deacetylases

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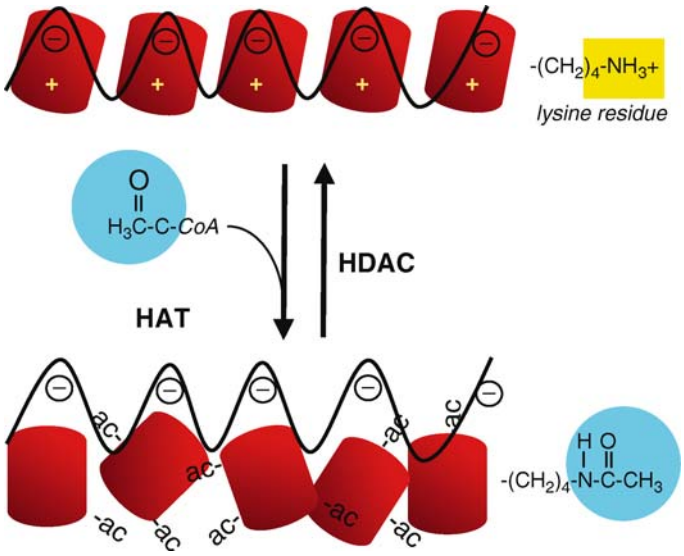
H

Synonyms
HDACs

Definition
►Histone deacetylases (HDACs) are a family of protein deacetylating enzymes that remove acetyl-groups from lysine residues of histone proteins and also non-histone proteins, such as transcription factors.

Characteristics
Functional Roles of Histone Deacetylases (HDACs)
HDACs regulate the acetylation status of histone and also non-histone proteins. They are ubiquitously expressed in all major tissues investigated. By controlling the acetylation level of histone proteins, HDACs are major players in the regulation of ►chromatin structure and transcriptional activities in the genome. Besides this role in the control of chromatin function, HDACs regulate posttranslational acetylation of transcription factors, cytoskeletal proteins and signal transduction molecules thereby modulating their cellular activities. Thus, two major functions of HDACs can be defined:

1. Regulation of chromatin structure and transcriptional activity. Together with histone acetyl transferases (►HATs), HDACs regulate the acetylation status of histone proteins. HATs transfer acetyl-groups from acetyl-CoA to ε-amino residues of lysines within histone proteins, resulting in a neutral charge of its amino acid residue. HDACs catalyse the opposite reaction and remove acetyl-groups from lysines, resulting in a positively charged, free amino-group (Fig. 1). The balance of these counteracting enzyme systems regulate the charge of histone proteins and their interaction with the negatively charged DNA. High activity of HDACs causes deacetylation of lysine residues, increase of positive charge of histone proteins, increase of histone-DNA-interaction and finally, a condensed chromatin structure (Fig. 1).



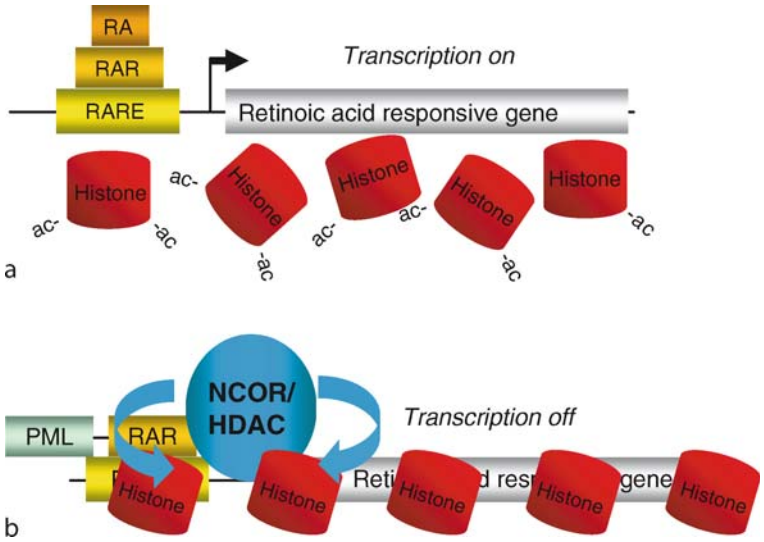
Histone Deacetylases. Figure 1 Histone acetyl transferases (HATs) and histone deacetylases (HDACs) control the level of histone acetylation. Histone proteins within nucleosomes are depicted as red cylinders interacting with the DNA (black line). The depicted chemical structures illustrate the underlying acetylation and deacetylation reaction in more detail. HATs transfer acetyl-groups (blue circle) from acetyl-Coenzyme A (CoA) to the ε-amino groups of lysine residues. This results in loss of positive charges of histone proteins, reduced binding with the negatively charged DNA and finally, an open chromatin structure (bottom panel). The opposite reaction is catalyzed by HDACs: removal of acetyl groups from lysine residues (blue circle, bottom panel) results in an increase in positive charges (yellow box, top panel) with subsequent increased interaction of histones with DNA leading to a condensed chromatin structure (top panel). Thus, HDAC activity favors a condensed, transcriptionally inactive chromatin structure. ac: acetyl-groups.

Therefore, deacetylated histones are found in silenced, transcriptional inactive chromatin regions and acetylated histones are found in transcriptional active chromatin regions. HDACs are recruited to specific chromatin sites as components of multi-protein ►repressor complexes. For example, HDAC1 and 2 are components of the Co-REST complex which inactivates the expression of neuronal genes in non-neuronal tissues. Other complexes which contain HDAC1 and 2 are the NURD and SIN3A repressor complexes. HDAC 3 is found within the NCOR/SMRT repressor complex.

2. HDACs are involved in the regulation of non-histone protein functions. In addition to the regulation of the acetylation status of histone proteins, HDACs deacetylate several non-histone proteins such as transcription factors, signal transduction proteins and cytoskeletal proteins thereby regulating their protein-stability, protein-protein interactions, cellular localization and DNA-binding activity. Among the growing list of non-histone proteins identified as substrates for HDACs are ►p53, ►E2F, GATA1, STAT3, TFIIE, TFIIF, HMGB1, ►HSP90, NF-kB, tubulin and importin. For example, the acetylation status of the oncosuppressor p53 regulates its stability, interaction with DNA and its transactivation activity.

HDACs and ►Cancer

Aberrant recruitment of HDACs to target genes with subsequent ►transcriptional silencing plays an important role in the malignant transformation of cells. In general, it is believed that aberrant recruitment of HDACs primarily affects silencing of genes involved in cell cycle control, ►apoptosis and differentiation. Recruited HDACs induce a compact chromatin structure and subsequent ►epigenetic inactivation of the involved genes by the mechanism depicted in Fig. 1. The best studied molecular model in this respect is ►acute promyelocytic leukemia (APL). In most APLs, ►the retinoic acid receptor-α (RAR) gene is fused to the PML gene through a translocation process involving chromosomes 15 and 17. Expression of the resulting fusion protein PML-RAR results in aberrant recruitment of HDACs to retinoic acid target genes (Fig. 2). This in turn leads to aberrant transcriptional silencing of retinoic acid regulated genes resulting in a differentiation block of myeloid precursor cells giving rise to leukemia (Fig. 2). This differentiation block can be overcome by high doses of retinoic acid and also by HDAC inhibitors. Other examples of aberrant recruitment of HDACs by deregulated transcription factor activities are ►AML1-ETO and TEL-AML1 in ►acute myeloid leukemias and ►BCL6 in ►B-cell lymphomas.



Histone Deacetylases. Figure 2 HDACs are involved in transcriptional silencing of retinoic acid responsive genes in acute promyelocytic leukemia (APL). (a) In normal myeloid precursor cells, retinoic acid (RA) binds to its retinoic receptor (RAR) which recognizes the retinoic acid response element (RARE) in the promoter of genes involved in myeloid differentiation. Thus, in the presence of retinoic acid, these retinoic acid responsive genes are actively transcribed. (b) In APL, the retinoic acid receptor (RAR) is fused to the promyelocytic leukemia (PML) protein (PML-RAR). PML-RAR also binds to the promoter, but has much lower affinity for retinoic acid and recruits HDAC containing repressor complexes (NCOR/HDAC) to retinoic acid responsive genes. Recruited HDAC activity in turn leads to histone deacetylation and chromatin condensation via the mechanism depicted in Fig.1. Subsequently, retinoic acid responsive genes become epigenetically silenced resulting in a differentiation block of myeloid precursor cells giving rise to leukemia.

H

HDAC Family Members

The first HDAC was affinity purified and cloned from cells using trapoxin, an inhibitor of HDAC activity, in 1996. Since then, 18 HDAC family members have been identified. The classification of the human HDAC family is based on their homology to the three yeast histone deacetylases Rpd3, Hda1, and Sir2. Hence, three major classes of human HDACs have been defined (Table 1). Whereas class I and II HDACs can be inhibited by HDAC-inhibitors such as trichostatin A, class III enzymes are resistant to these agents and also differ in their absolute dependency on NAD+ for their activity. HDAC11 appears to be a destinct family member and it has been suggested as a class IV enzyme (Table 1). As opposed to sirtuins, HDAC1–11 are currently referred to as "classical" HDACs by most authors. Class I HDACs are found almost exclusively in the nucleus, whereas class II HDACs are able to shuttle in and out of the nucleus in response to certain cellular signals.

Little is known about the regulation of the expression of HDAC genes themselves. SAGE analysis revealed that HDACs are widely expressed in almost all tissues investigated. No major differences were observed between the expression pattern in normal and malignant tissues at the mRNA level. However, significant variation in expression levels between individual HDAC family members was observed within a given tissue type. The enzymatic activity of HDACs can be regulated by posttranslational modifications such as phosphorylation.

Histone Deacetylases. Table 1 Classes of histone deacetylase enzymes

HDAC classes	HDAC family member
Class I yeast Rpd3 homologues	HDAC1
	HDAC2
	HDAC3
	HDAC8
Class II yeast Hpa1 homologues	HDAC4
	HDAC5
	HDAC6
	HDAC7
	HDAC9
	HDAC10
Class III (sirtuins) yeast Sir2 homologues	SIRT1–7
Class IV (?)	HDAC11

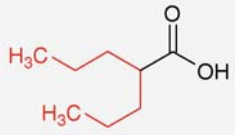
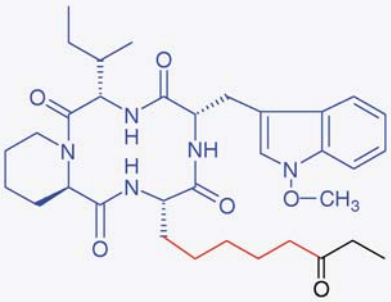
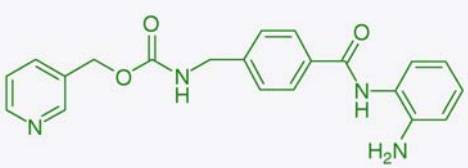
HDAC Inhibitors

Several compounds have been identified that inhibit the enzymatic activity of histone deacetylases. Exposure

of cancer cells to HDAC inhibitors results in inhibition of cell proliferation, induction of apoptosis, induction of differentiation, inhibition of ►invasion and ►migration, inhibition of clonogenic growth and also ►anti-angiogenic effects in a variety of cultured cancer cells. Based on ►microarray analysis, it has been estimated that approximately 2–10% of genes are modulated in their expression following exposure of cancer cells to HDAC inhibitors. Among the genes frequently altered in expression are the CDK inhibitors ►p21, p16 and p27, ►cyclin A and D, the actin-binding protein gelsolin, involved in morphologic and cytostructural changes and apoptotis modulating genes such as Fas and TRAIL receptors. Interestingly, it appears that non-transformed are less sensitive to HDAC inhibitors.

To date, several structurally divergent compounds with inhibitory activity against HDACs are known with an ongoing list of novel variants. HDAC inhibitors bind to the ►catalytic pocket of HDACs and chelate the zinc ion at its base, thereby inhibiting the enzymatic activity. Based on their chemical structure, HDAC inhibitors are grouped into four categories (Table 2): (i) short

Histone Deacetylases. Table 2 Structural classes of HDAC inhibitors

HDAC inhibitor class	Compounds	Concentration range	Example with chemical structure
Short chain fatty acids	►Valproic acid, butyric acid, phenyl butyric acid	mM	Valproic acid 
Hydroxamic acids	SAHA, TSA, CBHA, scriptaid, pyroxamide	nM-μM	SAHA 
Cyclic tetrapeptides	Apicidin, depsipeptide, trapoxin, CHAP, HC-toxin	nM-μM	Apicidin 
Benzamides	MS-275, Cl-994	μM	MS-275 

Histone Deacetylases. Table 3 Selection of clinical studies using HDAC inhibitors

Compound	Trial	Results	Toxicity
►Valproic acid	Phase II Acute myeloid leukemia, MDS	Partial and complete remissions in combination with retinoic acid; minor hematological improvements in extended Phase II study	Neurological toxicity
MS-275	Phase I/II Refractory solid tumors, leukemias and lymphomas	Long half life (39–80 h), once every 14 d best oral schedule	Nausea, vomiting, anorexia and fatigue dose-limiting toxicities
SAHA	Phase I/II Refractory solid tumors, leukemias and lymphomas	Good oral bioavailability, partial and complete responses in hematological and solid malignancies FDA approval for cutan out T-cell lymphoma	Dehydration, fatigue, diarrhoea, anorexia, thrombocytopenia, anemia
FK-228 (depsipeptide)	Phase I/II T-cell lymphoma (cutaneous), leukemias, solid tumors	Partial and complete responses in ►T-cell lymphomas	Bone marrow toxicity, cardiac arrhythmia, fatigue, nausea, vomiting

chain fatty acids,(ii) hydroxamic acids, (iii) cyclic tetrapeptides, and (iv) benzamides. Whereas short chain fatty acids require millimolar concentrations in order to exert anti-tumoral effects *in vitro*, the other classes of HDAC inhibitors act in the micro- to nanomolar range (Table 2). Hence, short chain fatty acids are considered to induce "off-target" effects besides HDAC inhibition, whereas hydroxamic acids, cyclic tetrapeptides and benzamides act in a more specific manner. The selectivity of HDAC inhibitors for targeting individual members of the HDAC family is largely unknown. Whereas TSA has been described as a pan HDAC inhibitor, other compounds have been shown to vary in their selectivity against individual HDAC family members up to 3 orders of magnitude.

In several animal models, anti-tumoral activities of HDAC inhibitors have been described. Hence, clinical trials using HDAC inhibitors as anti-cancer compounds have been initiated. So far, pilot studies have shown promising results with moderate toxicities. Currently, HDAC inhibitors are evaluated in several ►phase I and II clinical trials in patients with leukemias and solid tumors (Table 3).

Epigenetic Therapy

Besides HDAC inhibitors, DNA-methyltransferase (DNMT) inhibitors (i.e. 5-aza-deoxycytidine and 5-aza-cytidine) are being used as epigenetically active compounds in cancer therapy. Hence, the term "Epigenetic Therapy" of cancer has been introduced to indicate that HDAC and DNMT inhibitors target epigenetic regulatory mechanisms in cancer cells. Whereas, DNMT inhibitors are thought to act epigenetically in the strict sense by inducing DNA de ►methylation at ►CpG islands thereby activating genes silenced in cancer (i.e. ►tumor suppressor genes), it is currently not known, whether HDAC inhibitors act solely through epigenetic mechanisms (i.e. histone and chromatin modifications) or also by modulating the activity of transcription factors and other non-histone proteins. Both, DNA-methylation and histone acetylation are linked processes. For example, methylated CpG islands are bound by methyl-cytosine binding proteins (MeCP2) which in turn recruit HDACs to promoter sites to suppress transcription.

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Histone Deacetylation

Definition

Is a process that removes the acetyl groups from lysine residues on histone proteins, resulting in net positive charge of histones. This increases the affinity of histones for the negatively charged DNA and blocks the access of transcription factors. Histone acetylation is associated with active gene transcription, whereas histone deacetylation is associated with transcriptional silencing.

Histone Demethylases

Definition
Group of enzymes that remove methyl groups from target proteins.

►Histone Modifications

Histone H4

Definition
Histone genes encoding five classes of histone protein are clustered on chromosomes 1 and 6. The H4 gene consists of a single exon flanked by histone gene-specific motifs. The H4 promoter sequence contains consensus binding sites for histone nuclear factor D (HiNF-D) and interferon regulatory factor 2. HiNF-D is a multicomponent protein containing cyclin A, CDC2 and an RB-related protein, which links H4 gene regulation directly to cell cycle control.

►BCL6 Translocations in B-cell Tumors

Histone H2AX Phosphorylated at Serine 139

►γH2AX

Histone Methylation

Definition
Functional histone modification of the N-terminal tails at lysine residues by ►histone methyltransferases. The enzymes catalyze the transfer of one to three methyl groups from s-adenosylmethionine to lysine.

►Class II Tumor Suppressor Genes

Histone Methyltransferase

Definition
An enzyme that mediates the methylation of lysine or arginine residues onto histones. This mark behaves as an index for chromatin, so that cellular memory and identity can be retained after mitosis.

►Methylation
►Histone Modification

Histone Modification

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Definition
Histones are negatively charged proteins which assemble into a multi-subunit protein octamer complex around which the DNA of eukaryotic cells is wrapped. The ►posttranslational modification of histones, along with ►DNA methylation, has been commonly termed an ►epigenetic process, although the heritable nature of histone modifications has yet to be clearly demonstrated. Histones are aberrantly modified in cancers, leading to deregulated expression of ►tumor suppressors and ►oncogenes, events important in tumorigenesis and cancer progression. In addition, cellular levels of histone modifications are altered in cancer and are predictive of clinical outcome in a number of cancer types.

Characteristics
Histones and Chromatin
The DNA of eukaryotic cells associates with numerous different proteins to form ►chromatin, the physiologically relevant form of DNA. The most abundant proteins in chromatin are the histone proteins which are small, basic proteins that are highly conserved among eukaryotic organisms. There are four canonical histone proteins, H2A, H2B, H3 and H4 which interact with each other and DNA to form a ►nucleosome. Histones H2A and H2B interact with each other to form a heterodimer while two copies each of H3 and H4 interact to form a heterotetramer. The H3-H4 heterotetramer associates with two H2A-H2B heterodimers to assemble into a histone octamer with a roughly cylindrical core in which the N-terminal tails of each of the histones extends outwards in a relatively unstructured manner.

The intimate association of DNA with histones in the context of nucleosomes has significant functional consequences on DNA-templated processes, including DNA replication, DNA repair and transcription. The nucleosome presents an efficient barrier to these processes, thereby imposing the need for a dynamic chromatin structure. This principle is reflected partly in the heterogeneous distribution of nucleosomes and histone modifications throughout the human genome giving rise to the nucleosome rich, transcriptionally inactive ►heterochromatin and the relatively nucleosome depleted and transcriptionally active ►euchromatin structure.

Histone Modifications

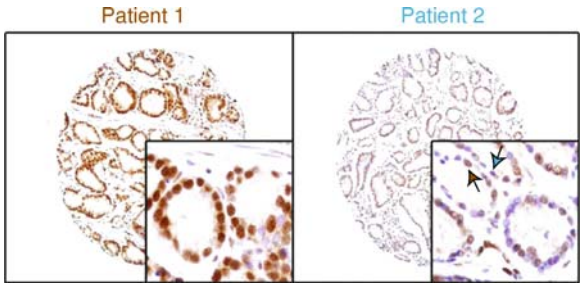
Chromatin structure is also affected by the covalent posttranslational modification of the histone proteins, particularly at their N-terminal tails. The modification of histones occurs at lysine, arginine and serine amino acid residues by the covalent attachment of chemical moieties including – but not limited to – acetyl, methyl, and phosphoryl groups. In general, acetylation of lysine residues is associated with an open chromatin conformation that allows for active gene transcription. Methylation of lysines and arginines tends to be more ambiguous in its effects on chromatin structure and transcription, with the specific functional output depending on the particular amino acid residue that is methylated.

Large families of enzymes, collectively termed ►histone-modifying enzymes, catalyze the modification of histones. ►Histone acetyltransferases (HATs) utilize acetyl-CoA to attach acetyl groups to lysine residues while ►histone deacetylases (HDACs) remove this mark. Methylation of lysine and arginine residues is catalyzed by a family of ►histone methyltransferases (HMTs) using S-adenosyl methionine (SAM) as a substrate. Methyl groups are removed from histones by a family of ►histone demethylases (HDMs). Examples from each class of histone-modifying enzymes have been found to be altered in cancer. ►Translocation of the HAT ►p300 has been found in ►certain hematological malignancies while inactivating mutations of a closely related HAT, ►CBP, is associated with the onset of Rubenstein-Taybi syndrome which predisposes to cancer. Elevated levels of the HMT SMYD3 occur in colorectal and ►hepatocellular carcinomas due to an increase in the number of repeats of an ►E2F transcription factor binding site while another HMT, MLL, is found translocated in various forms of leukemia. A number of HDACs and HDMs are also found mis-expressed in multiple cancer types, including GASC1, a gene originally identified as being amplified in oesophageal squamous carcinomas and which was only later shown to possess demethylase activity. Changes in levels of expression for the histone-modifying enzymes seem to be a common feature of cancer and may explain why expression patterns of select groups of histone-modifying enzymes can be used to distinguish cancer cells from normal cells and also have the ability to specify cancer type.

►Histone modifying enzymes are targeted to specific gene promoters by sequence-specific transcription factors or through specialized domains such as the ►bromodomain and ►chromodomain that recognize and bind to existing histone modifications. In this way, histone modifications are regulated at the level of individual genes which contributes to regulation of gene expression at these genes. In cancer, the aberrant recruitment of histone modifying enzymes establishes altered patterns of histone modifications at specific genes including oncogenes and tumor suppressors. For example, the cyclin E gene – which has an important role in the G_1 to S phase transition – is normally silenced by the deacetylation of lysine residues catalyzed by HDAC1. HDAC1 is recruited to this locus by the ►retinoblastoma (Rb) tumor suppressor protein. Mutations in Rb are common in cancer and can result in aberrant recruitment of HDAC1 to genes such as cyclin E that are involved in cell cycling with subsequent changes in the levels of gene expression. Similarly, an altered pattern of lysine methylation at the ►CDKN2A locus – which encodes the p16INK4A and p14ARF tumor suppressors – is found concurrent with silencing of this gene. The upregulation of oncogenes by epigenetic means is less well-documented than the silencing of tumor suppressors, but examples have been noted. For instance, the MLL HMT is translocated in certain leukemias, leading to the upregulation of the developmentally important Hox genes, a step that may be necessary for leukemic transformation. Other than gene promoters, lower levels of acetylation of lysine 16 and methylation of lysine 20 on histone H4 have been found at DNA repetitive elements in hematological malignancies and colorectal adenocarcinomas.

Histone Modifications and Cancer Prognosis

Differences in levels of histone modifications at the DNA repetitive elements, and at individual genes, have not proven to be predictive of clinical outcome. However, in addition to alterations at specific gene promoters, histone modifications also show large-scale deregulations in cancer. When examined at the level of individual nuclei (i.e. cellular level), cells within a tumor tissue display dissimilar levels of histone modifications, where cells have relatively high or low levels, revealing an epigenetic heterogeneity at the cellular level within cancers (Fig. 1). This cellular epigenetic heterogeneity exists not only within individual tumors, but also between patients who have varying levels of cells with relatively high or low cellular levels of histone modifications. In fact, patterns of cellular levels of various histone modifications can be used to



Histone Modification. Figure 1 Cancer tissues exhibit cellular epigenetic heterogeneity. ►Immunohistochemical (IHC) analysis of histone modifications in malignant prostate glandular epithelial cells from tumors of similar ►grade and ►stage reveals heterogeneity in cellular levels of specific modifications. In a given patient's tissue, some cells have higher levels of certain histone modifications (brown nuclei indicated by the *brown arrow*) than other cells (blue nuclei indicated by the *blue arrow*). Increased prevalence of cells with low levels of histone modifications (i.e., cells with blue nuclei) indicates poorer prognosis. In the case shown here, patient 2 has increased number of cells with low levels of histone acetylation at histone H3 (lysine 18) and therefore poorer prognosis compared to patient 1.

group patients into separate classes that exhibit significantly different clinical outcomes. Generally, patients with increased prevalence of cells with lower levels of histone modifications have poorer outcome. Although the biological significance and the underlying molecular mechanism of the cell-to-cell variability in histone modifications is poorly understood, the clinical data suggest that cellular levels of modification may serve as a valuable ►biomarker in the near future.

Epigenetic Therapy: HDAC Inhibitors

The reversible nature of histone modifications has raised the possibility that changes in gene expression due to altered patterns of modifications can be effectively treated. An altered pattern of histone acetylation as a result of mis-targeting of HDACs is a common phenomenon seen in hematological malignancies. This type of epigenetic aberration has received much attention due to the discovery of a group of natural and synthetic molecules known to inhibit HDACs, termed ►HDAC inhibitors (HDACi). These molecules have been shown to inhibit HDACs by occluding access of the substrate to the active site of the enzyme. HDACi are currently utilized in chemotherapeutic regimens and can be grouped into several classes based on their molecular structure. Some HDACi inhibit specific classes of HDACs (which are grouped into three different classes based on regions of homology) while others are known to inhibit all three classes. However, the specificity of a given HDACi for a specific HDAC is poorly defined and continues to be a topic of investigation.

The treatment of transformed cells with HDACi promotes differentiation of tumor cells and leads to growth arrest and ►apoptosis in both cultured cell lines and animal models. HDACi exert their function selectively on cancer cells which display a tenfold higher level of sensitivity to these drugs than do their non-transformed counterparts. The use of HDACi in cancer treatment relies on the ability of these molecules to affect the gene expression program, silencing the expression of oncogenes while promoting the re-expression of silenced tumor suppressors. Perhaps surprisingly, the use of HDACi affects the expression of only a small subset of the genome, with some genes being upregulated and some being downregulated. While the exact mechanism of HDACi has not been elucidated, it is thought that they work in part by affecting chromatin structure. This has been shown to occur at tumor suppressor genes, such as the ►$p21^{WAF1}$ gene where treatment with HDACi has been shown to increase levels of acetylation on both histones H3 and H4. In addition to increasing acetylation the $p21^{WAF1}$ locus demonstrated a more open chromatin conformation upon treatment with HDACi. This observation has raised the idea that HDACi may also work by inducing changes in chromatin structure leading to ►genomic or chromosomal instability. Increased genomic instability may activate the cell cycle checkpoint, leading to cell cycle arrest.

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Histone Modifying Enzymes

Definition

Group of enzymes that collectively catalyze the posttranslational modifications of histone proteins.

►Histone Modification

Histone Proteins

Definition
Small, basic DNA-binding proteins of eukaryotes. Together, histones and DNA form chromatin.

►Epigenetic Therapy

Histones

Definition
DNA-binding proteins that form the basic subunit of chromatin in eukaryotes, the nucleosome. A nucleosome consists of eight core histone proteins (two of each H2A, H2B, H3, H4) forming the central histone octamer and a stretch of DNA wrapped around it. The interaction of histone proteins with the DNA occurs through side chains containing the basic amino acids lysine and arginine, which are subject to reversible acetylation. Other posttranslational modifications of histone side chains are methylation, phosphorylation and ubiquitination.

►Histone Deacetylases
►Histone Modification

Histopathological

Definition
Human tissues embedded in paraffin and sectioned as a part of the diagnostic process reveal cellular differences that can be used to classify different grades of cancer.

►Pathology

HIV

Definition
The human immunodeficiency virus (HIV) is the causative agent of the acquired immune deficiency syndrome (AIDS). HIV is a ►retrovirus of the lentivirus family that selectively infects macrophages and CD4 T cells, leading to their slow depletion, which eventually results in immunodeficiency. There are two major strains of the virus, HIV-1 and HIV-2, of which HIV-1 causes most disease worldwide, HIV-2 is endemic to West Africa but is spreading.

►TAT Protein of HIV

HLA

Definition
The acronym for Human Leukocyte Antigen, is the genetic designation for the human ►MHC. Individual loci are designated by uppercase letters, as in HLA-A, and alleles are designated by numbers.

►Sjögren Syndrome

HLA Class I

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Definition
The major histocompatibility complex (►MHC) is a system of genes encoding molecules expressed on the cell surface that are required for ►antigen presentation to the immune system. In humans, this system is called ►Human Leukocyte Antigen or HLA genes, since it was discovered through antigenic differences among white blood cells from different individuals in the search for polymorphic antigens to match for transplantation. HLA class I molecules are of key importance in the cell-mediated anti-tumor immune response against viral infections and transformed cells by presenting peptide antigens to ►cytotoxic T lymphocytes.

Characteristics
The classical HLA class I and class II molecules (HLA-ABC and HLA-DR, DP, DQ, respectively) are cell-surface glycoproteins closely related in structure and function. HLA class I molecules consist of two poly-

peptide chains, a heavy chain of 340 amino acids encoded in chromosome 6, and a light non-polymorphic chain, β2-microglobulin (β2-m), encoded on chromosome 15. The class I and the class II molecules have a distinct distribution among cells with a functional role in the immune response. MHC class I molecules are expressed on the surface of nearly all nucleated cells with various levels of expression. MHC class II molecules are expressed on ►antigen-presenting cells (APC). Structurally, HLA molecules are cell surface-bound glycoproteins that contain four immunoglobulin-like domains. The two external domains form a peptide-binding groove consisting of two parallel α-helix amino acid chains on an eight β-pleated polypeptide sheet.

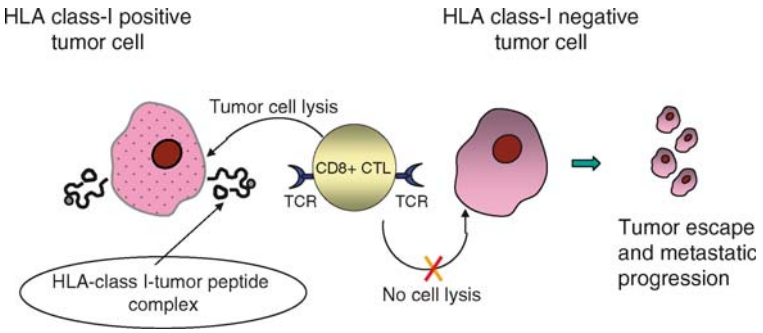
Bioactivity

HLA class I molecules bind peptide fragments derived from proteolytically degraded proteins that are endogenously synthesized by a cell. The processing of antigens is accomplished by a complex series of intracytoplasmic events involving antigen-processing machinery (APM). Processed foreign or self-antigen in a complex with HLA class I or II molecules is recognized by a specific TCR on the surface of T cells. The major function of HLA class I molecules is to alert T cells to the presence of intracellular extraneous peptides derived from intracellular pathogens (viruses, some bacteria) and tumor cells. HLA class I molecules can also interact with natural killer cells (►NK cells) via inhibitory ►receptors or activating ligands to prevent NK-mediated cell lysis. Two classes of HLA molecules are specialized to present antigens of different origin. HLA class I molecules present endogenously synthesized antigens (e.g., tumor associated antigens) to CD8+ CTLs (Fig. 1), whereas HLA class II molecules present exogenously derived proteins (e.g., bacterial products) to CD4+ helper cytotoxic T lymphocytes. Among the different reasons for a poor immunogenicity of tumor cells, the most frequent one is the loss of the expression of HLA class I molecules, which prevents the presentation of tumor antigen peptides to T cells (Fig. 1).

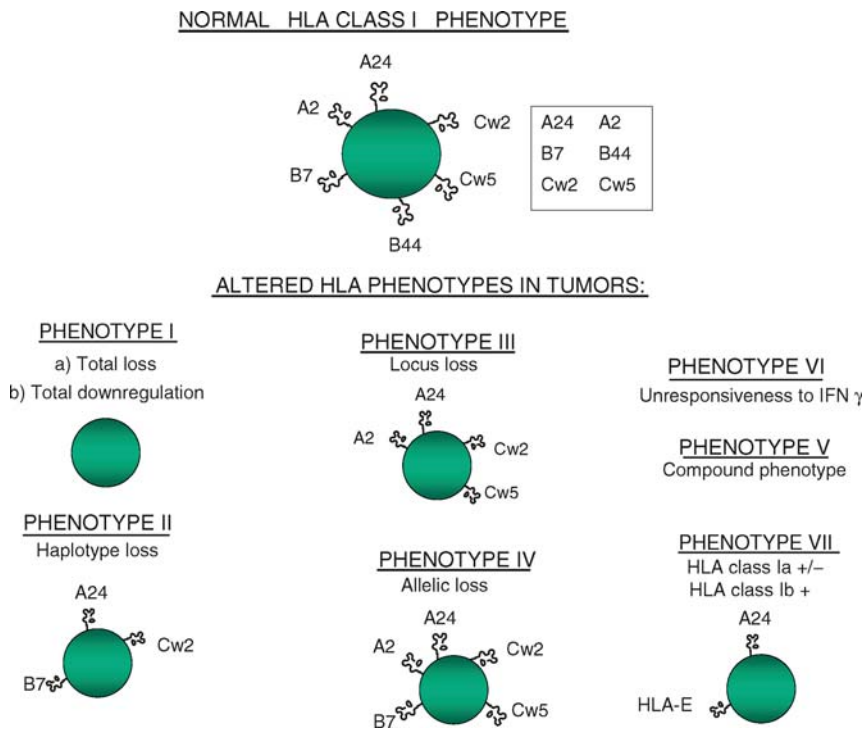
Altered HLA Class I Phenotypes in Tumors

The data obtained over the past 15 years lead us to classify altered HLA class I tumor phenotypes into seven groups (Phenotypes I to VII) (Fig. 2):

- *Phenotype I*: (a) total HLA class I loss, which is associated with structural defects in the β2-microglobulin gene. These defects have been identified in ►melanoma, colon carcinomas (►colon cancer) and other types of malignancy, and range from single base mutations to partial gene deletion. (b) Total HLA class I downregulation, when the class I molecules are not absent but the level of their expression is significantly reduced. It may be caused by defects in the regulation of transcriptional activity of HLA class I heavy chain genes, a dysfunction of the components of the antigen processing and presentation pathway, or some epigenetic (►epigenetics) events such as hypermethylation of cis-acting regulatory elements of the HLA ►promoter.
- *Phenotype II*: HLA ►haplotype loss. The loss of an HLA haplotype associated with the loss of a full chromosome 6 (LOH, loss of heterozygocity) or deletion of a large genomic region has been shown practically in all types of tumor analyzed to date. Chromosomal non-disjunction or mitotic recombination has been proposed to underlie this phenotype.
- *Phenotype III*: HLA ►locus loss. The loss of class I locus expression of HLA-A or HLA-B has been documented in many types of tumor, with HLA-B loss being the most frequent type. The mechanisms of locus downregulation may be transcriptional and can frequently be reversed by ►interferon-gamma (IFN-gamma).
- *Phenotype IV*: HLA allelic loss. It is difficult to define the precise frequency of tumors that show the loss of



HLA Class I. Figure 1 Schematic representation of the HLA class I–mediated interaction between cytotoxic T lymphocytes (CTL) and tumor cell. Tumor cell presents a "peptide/HLA class I" complex to CD8+ CTL via TCR (T-cell receptor). Binding of this complex to TCR initiates a chain of reactions leading to proliferation of CTLs and tumor cell lysis. Tumor cells with lost HLA class I escape T-cell lysis and proliferate leading to cancer progression.



HLA Class I. Figure 2 Altered HLA class I phenotypes in tumors.

only one HLA ►allele because of the limited availability of allele-specific antibodies. The molecular mechanisms underlying the selective allele loss might result from point mutations, partial deletions of HLA class I genes, or splicing defects.

- *Phenotype V*: compound phenotype. It involves multiple events and may be the result of immunoselection during tumor development.
- *Phenotype VI*: Unresponsiveness to IFN-gamma due to various defects in IFN receptor activation or signal transduction.
- *Phenotype VII*: expression of non-classical HLA class I molecules, such as HLA-E, in tumor deficient for classical HLA class I molecules (HLA-A, B, C).

All of these phenotypes can be found in various types of tumor, regardless of the tissue origin or of the carcinogen inducing the tumor. However, there are differences in the distribution of the phenotypes and the combinations of molecular mechanisms leading to each phenotype vary (Table 1).

HLA Class I. Table 1 Frequency of HLA class I expression defects in various types of tumor detected by immunohistological examination using anti-HLA class I specific antibodies

Tumor type	Total
Breast	88
Cervix	63
Colon	81
Larynx	79
Melanoma	69
Pancreas	39
Prostate	85

The results are expressed as percentage of alterations of HLA, including total or partial losses.

Functional Significance of HLA Class I Loss: Implications for Immune Evasion

Tumor cells often express new antigens because of multiple genetic alterations that are associated with malignant transformation. These antigens are recognized by T cells in association with HLA class I molecules, and the activated T cells consequently eliminate tumor cells. The process that involves the recognition and destruction of cancer cells by numerous immune effector cells and molecules is known as immunosurveillance. Nevertheless, cancer cells escape from immunosurveillance through the outgrowth of poorly immunogenic tumor cell variants, which emerge due to growth advantages created by a combination of features in tumor cells and factors in the tumor environment. Therefore, cancer progression may be considered as a result of a balance between tumor immunosurveillance and tumor escape. Several mechanisms have been discovered that enable malignant

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tumors to evade immune surveillance. Loss of antigens, costimulatory signals and/or adhesion molecules (►cell adhesion molecules), expression of immuno suppressive factors like ►Fas ligand and deficiencies in the signal transduction pathway of CD8+ cytotoxic T cells (CTL) have all been reported. HLA class I downregulation is one of the major tumor ►escape mechanisms, and it represents a significant challenge for a successful application of cancer ►immunotherapy.

A key contributor to the appearance of HLA class I-negative tumor clones is T-cell immunoselection. Cells that are highly immunogenic and express high levels of HLA class I are eliminated by CTLs, while cancer cells with no HLA class I expression proliferate and result into a metastatic colonization (Fig. 1). On the other hand, malignant cells with total HLA class I loss are susceptible to NK cell lysis because of inactivation of inhibitory receptors on NK cells. Another immunoselection route is provided by the partial loss of HLA class I antigens that allows tumor cells to escape both CTL and NK attack. Thus, tumor evolution may involve multiple steps, thereby generating heterogeneity of HLA expression even within an individual cancer. For instance, the metastatic capacity (►metastasis) of different tumor clones measured in spontaneous metastasis assays has been correlated with high MHC class I expression and low NK sensitivity in some tumor models.

A normal expression of HLA class I molecules on the tumor cell surface is crucial for a successful outcome of peptide-based cancer therapy (►peptide vaccines for cancer), since cytotoxic T cells can only recognize tumor-derived peptides in a complex with self-MHC class I molecules. Peptide-based immunotherapy is an established approach of cancer treatment, with the aim of boosting anti-tumor T-cell reactivity by stimulation with tumor specific peptides. However, the overall clinical outcome of this type of treatment is poor. In many cases the failure of this therapy and the progression of the cancer are associated with total loss of HLA class I tumor expression.

When the mechanism underlying total HLA class I loss is at a transcriptional level, the expression of surface HLA class I antigens can be reversed by cytokine treatment and T-cell based therapy can be successfully applied. Evidence for immunoselection of tumors that have lost the expression of several HLA molecules under selective pressure to escape T-cell cytotoxicity was recently demonstrated in patients who had undergone immunotherapy. In patients with structural HLA loss, the defects are irreversible and peptide-based vaccines will be of limited value. It is, therefore, important to develop strategies to classify altered HLA phenotypes and identify the molecular mechanisms underlying these alterations.

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HLH Domain

Definition

►Helix-Loop-Helix Domain

hMAWD: Human MAPK Activator with WD Repeats

►Serine-Threonine Kinase Receptor-Associated Protein

HMEC-1

Definition

Endothelial cell line derived from human microvessel endothelial cells

►Calcitonin

HMG-CoA Reductase

Definition

3-Hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) is the first enzyme of the HMG-CoA reductase pathway,

the metabolic pathway that produces cholesterol and various other biomolecules. HMG-CoA reductase inhibitors are a group of prescription drugs used to lower cholesterol, a white waxy substance that can stick to the inside of blood vessels, resulting in clogged arteries, heart disease, and strokes. These medicines work by slowing down the body's ability to make cholesterol. Drugs in this class include atorvastatin (brand name Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), pravastatin (Pravachol), and simvastatin (Zocor). Collectively, they are known as ►statins.

HMG-I/Y

Definition

High-mobility group (HMG) I/Y non-histone nuclear proteins.

►Cannabinoids and Cancer

HMGA1

Definition

High mobility group A1 protein, a distinct member of the HMG protein family. HMGA1 is a small basic protein that binds to AT-rich regions of DNA and functions mainly as a specific cofactor for gene activation. HMGA1 by itself has no intrinsic transcriptional activity; rather, it can transactivate promoters through mechanisms that facilitate the assembly and stability of stereospecific DNA–protein complexes that drive gene transcription. Stabilization of transcription factor-DNA interactions by HMGA1 plays a critical role in gene regulation. In particular gene contexts, HMGA1 acts to stabilize transcription factor-DNA and protein–protein interactions through a combination of its own binding to a proximal minor groove AT-rich sequence and through direct interaction with an adjacent transcription factor through one of its additional AT-hook motifs or through direct effects on the conformation of multisubunit proteins.

►Insulin Receptor

HMGA2

Definition

High Mobility Group A2 is a member of the HMGA subfamily of high mobility group (HMG) proteins. HMG proteins are abundant, non-histone chromosomal proteins. The HMGA subfamily is composed of four members: HMGA1a/b/c which are proteins encoded by alternatively spliced mRNAs of the *HMGA1* gene, and the HMGA2 protein, which is derived from a different gene, located at human chromosome region 12q15. HMGA2 contains three DNA-binding domains (AT-hooks) followed by an acidic carboxyterminal tail and functions as an architectural transcription factor: it has no intrinsic transcriptional activation/repression capacity, however, it bends DNA in such a way that other transcription factors can bind more easily to promoters and/or enhancers of their target genes.

►Lipoma Preferred Partner

HMTs

►Histone methyltransferases

4-HNE

Definition

4-Hydroxynonenal; Is found throughout animal tissues, and in higher quantities during ►oxidative stress due to the increase in the ►lipid peroxidation chain reaction, due to the increase in stress events.

HNPCC

Definition

Hereditary nonpolyposis colorectal cancer (HNPCC); ►Lynch syndrome.

►Colon Cancer
►Lynch syndrome

hnRNP M

Definition

The hn RNP proteins are RNA binding proteins that are multifunctional. hnRNP M comprises of 4 related proteins that are involved in RNA processing and can also act as a receptor for *N*-acetyl glucosamine in the thyroid and for ►CEA in Kupffer cells.

►Carcinoembryonic Antigen (CEA)

HO-1

Definition

Heme oxygenase-1, is the inducible form of heme oxygenases. It cleaves the heme ring at the alpha methane bridge to form biliverdin, which is the precursor of antioxidant bilirubin. HO-2 is constitutively expressed, and HO-3 is catalytically inactive.

►Phase 2 Enzymes

Hoarseness

Definition

Is one form of dysphonia that is defined as a rough or noisy quality of voice. However, hoarseness is often used interchangeably with dysphonia. It is a symptom of both local laryngeal pathology and systemic disease. It is not only a distressing symptom for patients, but is also often the early presenting symptom of serious disease such as cancer of the larynx.

►Nasopharyngeal Carcinoma

Hodgkin Disease

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Definition

Hodgkin disease (HD) is unique among the neoplasms derived from lymphoid tissues, i.e., malignant lymphomas. In fact, many characteristics of HD are controversial, in particular the epidemiological, virological, genetic, histopathological, immunological, and biological findings. It is now clear that HD is not a single disease with variants, but rather a group of at least two diseases, namely nodular lymphocyte predominant Hodgkin disease (nLPHD), and classic Hodgkin disease (cHD).

Characteristics

While nLPHD is a B-cell neoplasm, it is not clear whether the B-cell-derived displastic cells, i.e., the L-H or popcorn cells are monoclonal or polyclonal. Moreover, it is not clear whether nLPHD should be treated in as it shows slow or no progression at all. In contrast, cHD contains four histotypes, i.e., NSHD (nodular sclerosis HD), MCHD (mixed cellularity HD), LDHD (lymphocyte depletion HD), and cLRHD (lymphocyte-rich HD) that require treatment because otherwise they are fatal.

Immunological and molecular biological studies of cHD at the single cell level suggest that HRS (Hodgkin and Reed–Sternberg) cells in most cases are monoclonal derivatives of late germinal center B-cells, but in few cases are derivatives of cytotoxic T-cells and although less likely of NK-cells. The major event in the pathogenesis of B-cell related cHD is the blockage of apoptotic pathway. Epstein–Barr virus (►EBV) might be involved in the postulated dysfunction of the apoptotic pathway, leading to the genesis of classic HRS cells.

The essential elements for diagnosis of HD are the following:

- Lymphomegaly, single or multiple, not painful, often monostational and with variable dimensions over time
- Frequent presence of one or two systemic symptoms (fever, night sweat, loss of weight)
- Presence of the Reed–Sternberg cell in the lymphonodal biopsy

The most characteristical clinical presentation of HD is a young adult, showing an asymptomatical lymph node swelling. The enlarged lymph gland, which is usually not tender, occurs most widely in the neck, often in the sopraclavicolar fossa but it may also be discovered in the mid- or right neck or in the axilla. Another common presentation of the illness is the discovery of an anterior mediastinal mass on the routine chest radiographic examination.

Treatment of patients with HD has been one of the most significant successes in twentieth century clinical medicine. This was once uniformly a fatal disease, now is curable in approximately 75% of patients at many major medical centers worldwide. The management of these patients, however, is often difficult and requires particular attention to details of the staging and

treatment program. This is a necessary procedure in order to obtain good results by keeping the potential serious toxicities and morbidities of the therapy to a minimum. Most of the serious side effects of the therapy of HD are not evident for at least 5–20 years or more after treatment is completed. These might be described as problem of success, since they require many years of survival, free of HD recurrence to be recognized. As they have become evident, treatment programs have been modified in an effort to reduce their incidence and severity to a minimum. Therefore, the management of patients with HD continues to evolve. Treatment recommendations however, may differ somewhat among physicians and investigators with great experience in the treatment of HD.

A therapeutic program for a patient with HD should not be initiated without definitive diagnosis by an experienced hematopathologist. Appropriate diagnostic studies and stage determination should be made before start of a therapy. Almost all patients benefit from consultation with both an experienced medical oncologist or hemathologist and a radiation oncologist to jointly plan a treatment program, although not all patients require both modalities, chemotherapy and radiation therapy, in their initial management. Generally, the standard recommended treatment for patients with HD depends on the stage of the disease. There are special clinical situations and settings in which the standard approach must be modified, for example, in patients with HIV infection. Briefly, patients with stage IA and IIA supradiaphrammatic may be managed with full dose extend field radiation without chemotherapy. Those patients with bulky disease request management with combined chemo- and radiation therapy modality. Patients with stage IA and IIA infradiaphrammatic may be cured with radiation therapy alone. Patients with stages I and IIB are usually treated with chemotherapy alone or with a combined modality approach, i.e., chemotherapy and radiation therapy. Patients with stage IIIA may be treated with a combined modality approach, while those in stage IIIB are generally treated using chemotherapy alone as in a those with stage IV. ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) is the most effective single chemotherapy regimen in HD.

There are a lot of clinical problems that require the intervention of experienced medical oncologists. In particular, pulmonary infiltrates, epidural cord compression, herpes zooster, and postsplenectomy sepsis. Moreover, there are clinical problems after therapy including complications and late effects of the therapy, either from radiation therapy or from chemotherapy, e.g., sterility, second-tumors, coronary heart, lung, and artery diseases from radiation therapy, etc. Recently, in order to avoid these serious long-term side effects, treatment centers try to use a combined chemo- and radiation therapy approach with a short but intensive chemotherapy regimen in combination with limited field, low-dose radiation therapy. This approach could be successful, in order to maintain the high rate of cures decreasing the potential serious side effects in the follow-up. Finally those patients who relapsed or become resistant may be salvaged by high dose chemotherapy with stem cell support. This approach has been shown to cure some of the patients who otherwise would have died from HD.

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Hodgkin Disease, Clinical Oncology

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Synonyms

Hodgkin lymphoma

Definition

Hodgkin disease is a form of cancer – a type of ►malignant lymphoma. It is a malignancy of ►Reed-Sternberg cells and variants (Hodgkin cells, collectively). It occurs in about 3–4 per 100,000 population, and represents approximately 25% of all cases of ►lymphoma.

Characteristics

Pathology

Hodgkin disease is identified by the recognition of Reed-Sternberg cells and variants (collectively called Hodgkin cells) in the appropriate milieu, which usually consists of varying numbers of small lymphocytes, histiocytes, eosinophils, and plasma cells. Typically, the cellular milieu consists of greater than 99% of the reactive non-neoplastic elements, with the Hodgkin cells representing a rare dispersed population within involved tissues (and therefore difficult to study, except by single-cell dissection). Hodgkin disease can be separated histologically into classical type and nodular lymphocyte predominance type. The classical type is generally subdivided into nodular sclerosis, lymphocyte-rich, mixed cellularity, and lymphocyte

depletion subtypes, also based on their histologic appearance. Cases of nodular sclerosis are characterized by the presence of thick fibrous bands that traverse the affected lymph node and a variant of Hodgkin cell, the lacunar cell, which shows artifactual retraction of the cytoplasm in formalin-fixed sections. Cases of the lymphocyte-rich subtype have a marked predominance of small lymphocytes and have few identifiable Reed-Sternberg cells, while cases of lymphocyte depletion have a relative paucity of small lymphocyte and often show diffuse fibrosis. The mixed cellularity subtype lacks thick fibrous bands and has an intermediate number of Hodgkin cells. The Reed-Sternberg cells of classical Hodgkin disease are unique polyploid or multinucleated cells, with large inclusion-like nucleoli, while variants consist of similar mononucleated cells with large nucleoli. In nodular lymphocyte predominance, a nodular pattern is often seen in the absence of broad fibrous bands, and there is a predominance of small lymphocytes. The Hodgkin cells in the nodular lymphocyte predominance type are known as ►L&H cells, and have multilobated nuclei with less prominent nucleoli than the cells in classical Hodgkin disease. Classical Hodgkin cells have a characteristic immunohistochemical profile, being ►$CD30^+$, ►CD15 (X carbohydrate hapten)$^+$, ►CD20 (B lineage marker)$^{+/-}$ and ►CD45 (leukocyte common antigen)$^-$, while the L&H cells in the nodular lymphocyte predominance subtype are generally $CD30^-$, $CD15^-$, $CD20^+$, and $CD45^+$. CD30 is a member of the ►tumor necrosis factor receptor superfamily and is a late activation marker of lymphoid cells. The reactive lymphocytes are usually helper T cells in classical Hodgkin diseases. In nodular lymphocyte predominance, B cells make up the predominant cell type in the nodules; however, the L&H cells are usually surrounded by a collar of ►$CD57^+$ ►helper T cells.

Basic

Studies of the variable-region genes of the immunoglobulin receptors have demonstrated that the vast majority of cases of Hodgkin cells are derived from ►germinal center B cells. In nodular lymphocyte predominance Hodgkin disease, the precursor cells are usually mutating germinal-center B cells, and the pattern of suggests that the cells have been selected for expression of functional immunoglobulin receptors. In classical Hodgkin disease, the Reed-Sternberg cells are heavily somatically mutated, but ongoing mutations are not identified; in fact, some of the mutations identified result in stop codons that would ordinarily lead to elimination of the cell in the germinal center through apoptosis. This suggests that the Reed-Sternberg cells of classical Hodgkin disease are preapoptotic, crippled, germinal center B cells. There is some data to suggest that escape from apoptosis may arise through deregulation of ►NFκB, possibly due to mutations in the ►IκBα molecule that is the natural inhibitor of NFκB, ►amplification of the ►REL gene, overexpression of the tumor necrosis factor receptor–associated factor 1(►TRAF-1) 1 molecule, or stimulation of the CD30 or CD40 expressed on Hodgkin cells. Escape from ►apoptosis may also result in some cases from inactivation of the ►CD95 death receptor pathway, possibly by mutations in the CD95 gene or by expression of $cFLIP_L$. In rare cases, Hodgkin cells may be derived from T cells. Hodgkin cells produce a variety of cytokines, including tumor necrosis factor, interleukins 1–10 and 13, lymphotoxin-alpha, tumor necrosis factor, transforming growth factor-beta, interferon-gamma, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and ►TARC chemokine. Since Hodgkin cells produce both interleukin-13 and its receptor, this may provide an ►autocrine proliferation stimulus. These cytokines probably account for the abundant cellular milieu as well as some of the unusual clinical findings, such as systemic symptoms. For example, the secretion of TARC may be the main reason for the predominance of T-helper cells in involved tissues. Although high levels of ►p53 protein have been identified in Hodgkin disease, only a minority of case has detectable mutations of the p53 gene. About 40% of cases of the classical form of Hodgkin disease are associated with the ►Epstein-Barr virus. In these cases, monotypic episomes of the Epstein-Barr virus can be identified in the Hodgkin cells; these cells have a latency pattern II. Epstein-Barr virus-associated cases are particularly found in younger patients and with mixed cellularity and lymphocyte depletion subtypes, particularly in economically disadvantaged populations. There are few cytogenetic studies of Hodgkin disease, because Hodgkin cells are rare in involved tissues, they are difficult to grow in culture, and few bona fide cell lines exist. Most reported karyotypes are extremely complex, with frequent polyploidy; no characteristic abnormalities have been consistently identified. Single cell studies have demonstrated the absence of the t(14;18) that is the hallmark of ►follicular lymphoma.

Clinical

Patients with Hodgkin disease usually present with an enlarged lymph node. Patients with the nodular sclerosis subtype have a predilection for disease above the diaphragm, particularly mediastinal node involvement. Patients with lymphocyte depletion often present with abdominal nodal involvement, while patients with nodular lymphocyte predominance often present with isolated disease in the upper neck. Some patients may also have systemic, or "B," symptoms – weight loss >10% during the previous six months, documented fever, and night sweats. There is a slight male

predominance and a bimodal age distribution in Western populations, with peaks of incidence in young adulthood and old age. Among economically disadvantaged populations, the first peak of incidence occurs earlier in childhood, particularly for males. There is a relatively low incidence of Hodgkin disease among Asian populations. Hodgkin disease usually starts at a single site and progresses in an orderly manner through the lymphatic system to local lymph nodes before disseminating hematogenously to distant sites, particularly the bone marrow, spleen, or liver. Hodgkin disease is typically staged from I to IV. Stage I represents involvement of a single lymph node structure, stage II is involvement of two or more lymph node regions on the same side of the diaphragm, stage III is involvement of lymph node regions or structures on both sides of the diaphragm, and stage IV is involvement of extranodal sites. Prognosis is directly related to stage. Hodgkin disease is typically treated by multidrug chemotherapy and/or radiotherapy, with an overall 5-year survival of greater than 80%. Patients who relapse usually do so within the first three years after treatment, and have a significantly worse prognosis. High-dose chemotherapy with ►autologous bone marrow transplantation or peripheral blood stem-cell transplantation has become a standard therapy for patients who fail conventional chemotherapy regimens. Novel immunotherapies have been proposed, including treatment with interleukin-2, bi-specific antibodies, immunotoxins, and radioimmunoconjugates. Patients with the nodular lymphocyte predominance subtype may behave differently than the classical forms of Hodgkin disease, with a better overall survival, but a greater number of recurrences, which are independent of time after treatment.

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Hodgkin Lymphoma

Definition

►Hodgkin disease
►Hodgkin Disease, Clinical Oncology

Hodgkin and Non-Hodgkin Lymphomas

►B-cell Tumors

Hodgkin and Reed/Sternberg Cell

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H

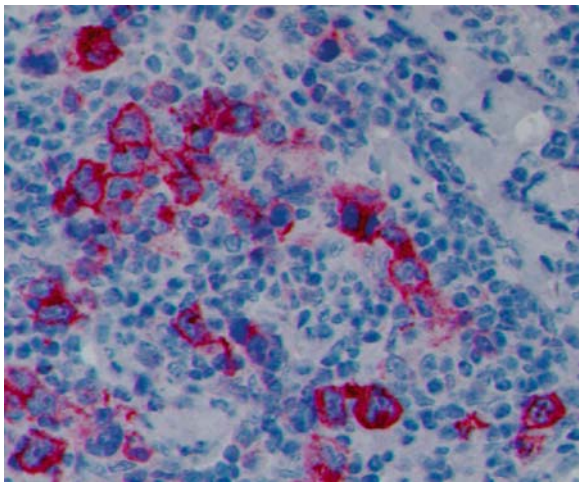
Definition

Hodgkin and Reed/Sternberg (HRS) cells are large cells with a peculiar morphology and immunophenotype that does not resemble any other normal cell in the body. The cells are called Hodgkin cells when they are mononucleated and Reed/Sternberg cells when they are multinucleated. HRS cells in nearly all instances derive from B lymphocytes, but in rare cases they originate from T cells. HRS cells are the hallmark cells of Hodgkin lymphoma, in which they represent the tumor cell clone.

Characteristics

Associated Pathologies

The first cases of Hodgkin lymphoma (HL) were described by Thomas Hodgkin in 1832. A peculiar type of cells that is a hallmark of HL was first characterized in detail about 100 years ago by Dorothy Reed and Carl Sternberg. These cells are called Hodgkin cells when they are mononucleated and Reed/Sternberg cells when they are bi- or multinucleated. HRS cells are large cells ten times or more of the size of small lymphocytes with prominent nucleoli. Reed/Sternberg cells most likely derive from Hodgkin cells by endomitosis, i.e., nuclear division without cell division. In HL, the HRS cells usually represent less than 1% of cells in the tumor tissue (Fig. 1). They are surrounded by a mixture of various types of cells, including T cells, B cells, plasma cells, macrophages, eosinophils, and others. In about 40% of cases of HL in the Western world, the HRS cells are latently infected by the ►Epstein–Barr virus (EBV). HL is one of the most frequent malignant lymphomas in the Western world, with an incidence of about 2.5–3 new cases per 100,000 people per year. Nowadays, about 90% of the patients can be cured by chemo- and/or radiation therapy. Based on differences in the histological picture and the cellular



Hodgkin and Reed/Sternberg Cell. Figure 1 HRS cells in Hodgkin lymphoma tissue. CD30 immunostaining (red) of a HL lymph node tissue section with CD30-positive HRS cells. Nuclei of the cells are stained in blue with haemalaun.

composition of the lymphoma, HL is subdivided in four types of classical HL (nodular sclerosis, mixed cellularity, lymphocyte depletion, and lymphocyte-rich classical) and lymphocyte-predominant HL. This latter type represents about 5% of cases. In lymphocyte-predominant HL, the tumor cells have a distinct morphology and immunophenotype and are normally not multinucleated. These cells are not called HRS cells but lymphocytic and histiocytic ►(L&H) cells.

It is very difficult to grow HRS cells in culture, and only a few cell lines could be established from HL biopsies. Nevertheless, these cell lines are valuable models for the functional analysis of HRS cells. The cell lines still available and considered as HRS cell-derived include the B cell lineage lines L428, L1236, KMH2, L591 (the latter is the only EBV-positive line) and the T cell lineage HL lines HDLM2 and L540.

Although HRS cells are the pathognomonic cells in HL, HRS or HRS-like cells are also occasionally observed in other lymphomas. These include some ►diffuse large B cell lymphomas and ►B cell chronic lymphocytic leukemia (B-CLL). In B-CLL, the HRS-like cells can either be clonally related to the B-CLL lymphoma clone, or they can represent an independent cell clone. HRS cells are also regularly found in an infectious disease, i.e., ►infectious mononucleosis. This disease is caused by EBV and can occur if the primary infection of an individual by EBV does not occur in young children (when it is usually asymptomatic) but is delayed into adolescence or adulthood. In infectious mononucleosis many B cells are infected by EBV and can expand to large clones. Some of the EBV-infected B cells for unknown reasons acquire the morphology and to some extend the immunophenotype of HRS cells.

As HRS cells have most extensively been analyzed in HL, the following discussion of HRS cells refers to HRS cells in HL.

Cellular Origin

As the HRS cells usually represent only a small fraction of the cells in the HL tissue and as they have an unusual immunophenotype that does not resemble any normal hematopoietic cell type (see later) the cellular origin of these cells was unclear for a long time. However, about 10 years ago, the molecular analysis of isolated HRS cells revealed that these cells derive from B lymphocytes in nearly all cases. HRS cells carry rearranged immunoglobulin (Ig) genes which is specific for B cells. These rearranged Ig genes carry with few exceptions a high load of somatic mutations. The acquisition of such mutations through the process of ►somatic hypermutation takes place in antigen-activated B cells in the course of T cell-dependent immune responses in specific histological structures of lymph nodes and other lymphoid organs – the ►germinal centres. Therefore, it is very likely that HRS cells derive from such mature germinal centre B cells. Curiously, HRS cells frequently carry destructive somatic mutations that prevent expression of a functional B cell antigen receptor. As normal germinal centre B cells acquiring such mutations undergo ►apoptosis, HRS cells may represent transformed preapoptotic germinal centre B cells. The detection of rearranged T cell receptor genes in a few cases of HL indicates that in rare instances (less than 2% of cases), HRS cells may also derive from T cells.

Immunophenotype

HRS cells show an unusual immunophenotype with coexpression of markers of distinct hematopoietic cell lineages. B cell antigens (e.g., CD19, CD20) or T cell antigens (e.g., CD3, CD4) are detectable on variable proportions of HRS cells in about 10–20% of cases. Several markers of dendritic cells are regularly expressed (e.g., thymus and activation regulated chemokine (TARC), fascin, and restin), but also markers of myeloid cells are found (CD15). As HRS cells are in nearly all cases derived from B cells, it is evident that many of these markers are aberrantly expressed by HRS cells. It also became clear that HRS cells lost expression of most typical B cell markers. For the diagnosis of HL, it is important that HRS cells in all cases of classical HL express CD30, an activation marker and member of the tumor necrosis factor receptor family.

Cell Differentiation and Function

HRS cells can be considered as hyperactivated cells, as a multitude of signaling pathways is chronically

activated in these cells. HRS cells show constitutive activity of the transcription factor NFκB, which regulates the expression of many genes, including important antiapoptotic factors. Indeed, inhibition of NFκB in HRS cell lines induced apoptosis of the cells. Also the PI3 kinase/AKT signaling pathway and the activator protein 1 (AP-1) are active in HRS cells and contribute to cell survival and/or proliferation. Remarkable is Notch-1 activity in HRS cells, as this transcription factor is normally active only in T cells and suppresses expression of B cell-specific genes. Thus, the deregulated activity of Notch-1 in HRS cells may contribute to the downregulation of B cell genes, as discussed earlier. In HL tissues, many cytokines are produced by HRS cells and surrounding cells. On this basis, it is perhaps not surprising that HRS cells have an active Jak/STAT pathway, as many cytokines signal through this pathway. There appears to be even an autocrine interleukin stimulation in HRS cells, as they express interleukin 13 and the interleukin 13 receptor. Three of the STAT transcription factors have been identified as constitutively active in HRS cells, STAT3, 5 and 6. Another important family of signaling molecules is receptor tyrosine kinases. In several cancers, specific members of this family show deregulated activity that contributes to pathogenesis. In HL, multiple receptor tyrosine kinases are expressed and active, which is in its extent unique among human tumors.

Transforming Mechanisms of HRS Cells

As mentioned earlier, in about 40% of cases of HL in the Western world, HRS cells are infected by EBV. In some instances (e.g., in childhood cases in Central America) the association can be even up to 90%. In EBV-positive cases, three latent viral proteins are expressed, the EBV nuclear antigen EBNA1, and the latent membrane proteins LMP1 and LMP2A. EBNA1 is essential for the replication of the viral genome in proliferating cells. Interestingly, LMP1 and LMP2A functionally mimic two main survival signals for germinal centre B cells, i.e., CD40 signaling and B cell receptor signaling. In this way, EBV can rescue the HRS cell precursors from apoptosis and contributes to the malignant transformation.

HRS cells usually show multiple numerical and structural chromosomal abnormalities. Some of these are shared by all HRS cells, while others are found only in fractions of cells of a tumor clone, indicating a significant genomic instability. Due to the multitude of genomic aberrations, it has been difficult to identify events causally involved in the transformation of HRS cells. However, some genomic imbalances occur recurrent and are therefore candidates for pathogenetic events. These include, for example, amplification of the genomic region including the *c-Rel* gene, a member of the NFκB family. HRS cells frequently also harbor translocations involving the immunoglobulin loci, but the partner genes involved in these translocations have not yet been identified.

Isolated HRS cells were also analyzed for mutations in proto-oncogenes and tumor suppressor genes. Mutations in the tumor suppressor genes *p53* and *CD95* were found in less than 10% of cases investigated. Inactivating mutations in a main inhibitor of the NFκB transcription factor, IκBα, are present in about 20% of cases. Rare cases may also carry mutations in IκBε, another inhibitor of NFκB. Mutations in these NFκB inhibitors contribute to the constitutive activity of this factor, which has an important role as an antiapoptotic factor in HRS cells (see earlier). Mutations were recently also found in the *SOCS1* gene, which is an inhibitor of STAT transcription factors. Thus, these mutations likely contribute to the constitutive activity of STATs in HRS cells.

In summary, the transforming events involved in the generation of the malignant HRS cells in classical HL have only partly been revealed, and it appears so far that multiple different combinations of genetic aberrations can cause the pathogenesis of HL.

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Hoechst-33342 Dye

Definition

Is a UV-excitable nucleic acid stain that is readily taken up by all cells, except those cells that express the transporterABCG2. Hoechst-dim cells can be detected by flow cytometry or by fluorescence microscopy.

►Stem Cell Markers
►FISH

Hollow Fiber Assay

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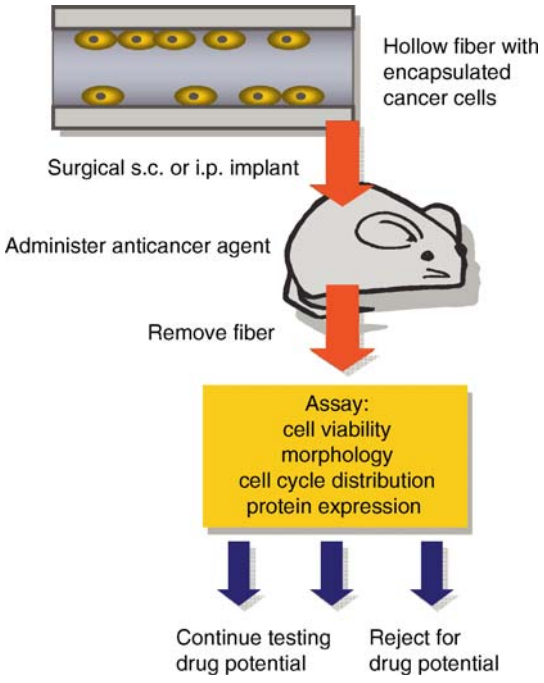
Definition

The hollow fiber assay (►HFA) is a fast in vivo assay to determine the cytotoxic effect of drugs, as well as their pharmacodynamic (►Pharmacodynamics) effects on human tumor cell lines grown in hollow fibers that are implanted subcutaneously or intraperitoneally in mice or rats.

Characteristics

Various in vivo models exist to asses the efficacy of potential anticancer agents, including subcutaneously implanted ►xenografts, ►orthotopic models, and the HFA. The HFA has been optimized at the National Cancer Institute (►NCI) and is a unique in vivo model for ►drug discovery. It permits the simultaneous evaluation of the efficacy of anticancer agents against multiple tumor cell lines in two physiological compartments of one animal. In this assay, semipermeable biocompatible fibers are used, which can be filled with cancer cells. These cancer cells can be derived from different tissue origins and can have different cellular characteristics. The fibers are made of polyvinylidene fluoride (PVDF) and have an internal diameter of 0.5–1 mm and are 2 cm long. The fiber has a molecular weight exclusion of 500,000 Da. Therefore soluble bioactive agents (e.g., proteins, such as growth factors produced by tumor cells or host) can bypass the fiber, but tumor cell to host cell contact is not possible. The fibers are mostly implanted in mice, but it is also possible to implant the fibers in rats and other animals for ►preclinical testing of anticancer agents. One mouse can support the growth of up to six cancer cell lines, and therefore enables to test more than one cell line simultaneously in one animal. The fibers can be transplanted in both immunocompromized and in immunocompetent mouse strains, since the immune cells of the host can not infiltrate into the hollow fiber and therefore can not activate an immune response or impede the growth of the tumor cells. The fiber can be implanted either intraperitoneally (►i.p.) or subcutaneously (►s.c.), therefore differences of intravenous (►i.v.), oral, i.p., and s.c. administration of drugs can aid in assessing the effects of hepatic pass through, thus providing information for dose estimations and administration routes for more extensive in vivo testing of both cytotoxic and antiangiogenic ►chemotherapy. After treatment, active cell proliferation and cellular characteristics, including ►cell cycle distribution, ►DNA damage induction, cell death induction (►Apoptosis), protein expression levels and cell morphology can easily be studied (Fig. 1). Besides this, in vivo pharmacokinetic (►Pharmacokinetics) parameters, including drug transport, pH and pO_2 can be analyzed.

The NCI currently uses a panel of 12 tumor cell lines for routine hollow fiber screening of anticancer drug activities. It is used as the initial in vivo assessment to determine potential activity of agents that have reproducible activity in the in vitro anticancer drug screen. The HFA prioritizes compounds for secondary ►xenograft screening and helps to reduce the large number of active compounds generated by the in vitro ►NCI 60 cell line screen that were forming a bottleneck for entry into secondary xenograft testing. The NCI HFA requires 24 mice for testing of one compound against a panel of 12 cancer cell lines in contrast to the NCI xenograft model, where three xenograft models are used to test one agent with each xenograft model, which requires about 50 mice. The use of the HFA in early ►preclinical drug screens fits excellently with the philosophy of replacement, refinement and reduction (3Rs) of animals in research, controlling the use of laboratory animals.



Hollow Fiber Assay. Figure 1 Schematic overview of the HFA procedure for preclinical testing the anticancer activity of a compound using cancer cell lines transplanted subcutaneously (s.c.) or intraperitoneally (i.p.) in mice. Method is described according to NCI guidelines, http://dtp.nci.nih.gov/branches/btb/hfa.html.

Since the development of the HFA for ►drug screening, other groups have used this assay to independently demonstrate drug activity. Compound efficacy has been demonstrated using the HFA, using both primary human tumor cells and characterized tumor cell lines. Although the NCI uses the HFA successfully to quantitatively define anticancer activity, the role of this in vivo assay could be extended. The HFA has already been adapted for evaluation of antiviral agents and it may also be adapted to use it in other medical fields.

The in vivo HFA has demonstrated a good predictivity of xenograft activity. Anticancer activity in xenograft models is defined by a reduction in relative tumor volume. In the HFA the activity is defined as a reduction of the number of viable cells, measured with an ►MTT assay. Any compound found to be active in xenograft models is often also active in the HFA.

A xenograft model does not easily permit elucidation of the mechanism of action of a compound in a tumor. Retrieval of tumor cells from xenograft models for pharmacodynamic analysis is complicated by host cell contamination. Cells grown in a hollow fiber can easily be harvested for mechanistic studies as a single cell suspension without contamination of host cells. When the HFA is used for preclinical pharmacodynamic investigations, compounds may progress to the clinic much faster. On the other hand, more information is required on tumor cell–stroma interaction. The development of such a model using the HFA is a new challenge in its application.

The HFA has several advantages over standard animal efficacy models. First, the assay has demonstrated the ability to provide quantitative indices of drug efficacy with minimum expenditures of time (procedure takes less than 2 weeks) and materials (e.g., test compounds). It is not limited by the high costs that are associated with large-scale animal testing using xenograft tumor models. Second, conducting studies in animal models requires substantial amounts of time and resources for the testing procedure. Even when it is possible to conduct such studies, it is possible that the experimental agents will exhibit only minimal antitumor activity. Thus, the HFA will prevent to test inactive compounds in a xenograft model. Third, cancer treatments that appear promising in tissue culture are often less effective in solid tumors. This can be partially because of the proliferative and microenvironmental heterogeneity that develops in these tumors during their growth in a ►three-dimensional structure; cells in a hollow fiber also grow in a three-dimensional structure. Fourth, this procedure of testing takes the pharmacokinetic behavior of the drug into account, as well as potential biotransformation. Fifth, the HFA is excellently suited to perform initial in vivo experiments to study combinations, because the compounds will have a dynamic in vivo interaction. Sixth, the HFA prevents that the mouse suffers from the tumor induced cachexia.

The HFA was not developed to replace the classic xenograft model. Complex interactions which occur when the transplanted tumor cells are growing in and interacting with the host's tissue can not be analyzed. Cells can not metastasize to secondary organs of the animal, since the fiber prevents cellular ►migration. Although ►angiogenesis in the tumor will not be induced when these fibers are used, neovascularization occurs to supply the fiber with nutrients. This is possibly induced by growth factors that are released by the tumor cells in the fiber. The hollow fiber can be used perfectly as a preliminary tool to asses the capacity of a compound to reach tumor cells growing in two distinct physiologic compartments (s.c. or i.p.) and to asses whether the drug can reach pharmacologically active concentrations in the tumor cells. Using modern ►bioluminescent techniques, it will be possible to perform dynamic growth of the cells in the fibers.

Methodology

The HFA procedure is as follows (Fig. 1): tumor cells are harvested at the desired cell density from a log phase growing in vitro culture. The cell suspension is flushed into the hollow fibers, which are subsequently heat-sealed so that the cells can not escape from the fiber. The density is dependent on the cell line and may vary from 1 to 100×10^5 cells/fiber. Prior to transplantation into the host, fibers are placed into tissue culture medium and incubated at 37°C in 5% CO_2 atmosphere for 24–48 h. On the day of implantation, samples of each tumor cell line preparation can be quantified for viable cell mass by a stable endpoint MTT assay. Mice can then be treated with anticancer agents starting few days after fiber implantation and for a maximum of 2 weeks. Following treatment, the fibers are collected from the host and subjected to the stable endpoint MTT assay. The percent net growth for each cell line is calculated and compared to the percent net growth in the vehicle treated controls. Furthermore, at the treatment endpoint, various other cellular analyses can be performed.

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Homeobox

Definition

A short usually highly conserved DNA sequence found within genes that are involved in the regulation of development (morphogenesis) of animals, fungi and plants. Genes that have a homeobox are called homeobox genes and form the homeobox gene family.

►Nucleoporin

Homeobox Genes

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Definition

Homeobox genes are a class of developmental regulatory genes that encode homeoproteins. Homeoproteins function as transcription factors to regulate downstream targets, turning on (activating) or turning off (repressing) other genes that in turn regulate developmental processes. Aberrant expression of homeobox genes has been implicated as causal factors in leukemia and solid tumors.

Characteristics

Homeobox genes are often referred to as master control genes. They are active during embryonic development and regulate important processes such as ►morphogenesis and cellular ►differentiation. They were first identified in Drosophila as the homeotic cluster (HOM-C) genes and were later found to have homologs in many species. They are now known to be highly evolutionarily conserved and are present in animals, plants and fungi.

Homeobox genes share a common sequence motif (the homeobox), which is 180 nucleotides in length and encodes a 60 amino acid region (the homeodomain). The homeodomain mediates DNA binding to sites containing a ►TAAT sequence that are found in the transcriptional regulatory elements of target genes. Upon DNA binding, homeoproteins are thought to act as transcription factors to regulate downstream targets.

There are two major subclasses of vertebrate homeobox genes:

- The clustered genes, or HOX genes
- The non-clustered genes

The non-clustered genes are sub-divided into many subfamilies, which are classified on the basis of sequence similarities within their homeobox regions. The HOX family of homeobox genes, as well as most other families of homeobox genes, is thought to control cellular proliferation and differentiation during development. The HOX genes are expressed beginning in ►gastrulation and are involved in patterning the body axis from the branchial arches to the tail.

What is most notable about HOX genes is their positioning on four chromosome clusters. Not only are the sequences of the individual genes highly conserved across species, the order of the genes on the chromosomes is conserved as well. Moreover, the physical order of the genes on the chromosome correlates with their spatial and temporal expression patterns along the anteroposterior axis of the embryo.

Many studies have reported a link between deregulated homeobox gene expression and abnormal cellular proliferation, which implicates homeobox genes not only as development regulators, but also as potential protooncogenes and tumor suppressor genes. The downstream targets of homeobox genes are postulated to include extracellular matrix proteins, adhesion molecules and growth factors. Because these target genes are likely to be important for tumorigenesis as well as development, their misexpression may upset the delicate balance of cell proliferation, differentiation and apoptosis, thereby contributing to carcinogenesis.

Hox Genes and Leukemia

In addition to their functions during development, homeobox genes also play important roles in adult tissues. For instance, the HOX genes are expressed in specific patterns during lineage determination in ►hematopoiesis. HOX genes have been demonstrated to be important for normal blood cell formation; in addition, abnormal expression of HOX and other homeobox genes contributes to the development of leukemia and lymphoma.

A common mechanism by which abnormal gene expression contributes to leukemia is through translocation of two chromosomal regions, which may

produce fusion proteins with novel properties or impaired activities. For example, in human acute myeloid leukemia (AML) a translocation between chromosomes 7 and 11 fuses the nucleophorine gene NUP98 in frame with HOXA9. It is thought that the resulting chimeric protein is no longer able to interact with HOXA9 target genes. Another example of a homeobox translocation in leukemia is the fusion of PBX1 homeobox gene and the E2A gene. Normally, PBX1 forms transcriptionally active protein complexes with HOX proteins; its fusion with E2A alters such interactions, which is thought to direct homeoproteins to different targets, thereby inducing leukemogenesis.

Like PBX, other divergent homeoproteins interact with specific HOX proteins to potentiate or modulate their effects, and their genes are potential targets for deregulation in carcinoma. For example, it now appears that the MEIS1 homeobox gene is co-activated with HOXA9 in human myeloid leukemia. In addition, translocation of the MLL gene is a common event having been found to be fused to more than 25 other genes in leukemia. While MLL is not a homeobox gene, it is homologous to a Drosophila protein that is an important regulator of HOM-C genes. The leukemogenic MLL fusion proteins are thought to disrupt HOX gene expression in hematopoietic progenitor cells, thereby contributing to myeloid or lymphoid acute leukemias.

Homeobox Genes and Solid Tumors

A common feature of HOX gene expression in certain adult organs, such as kidney, lungs and colon, are the significant differences in expression pattern between normal and cancer tissue. For example, in the kidney HOXC11 is present in tumors but not in normal tissue. Conversely, HOXB5 and HOXB9 are normally expressed in the kidney but expression is often lost in tumors. Other HOX genes, such as HOXD4, are expressed in both normal kidney and in kidney tumors, although they express a different-sized transcript in the tumors versus the normal tissue. In each of these cases the significance of these differential gene expression patterns remains undetermined.

Similar observations have been made in the colon. Misexpression of some HOX genes (HOXA9, HOXB7 and HOXD11) is found in primary colon cancer and metastatic lesions originating from colorectal tumors. In addition, HOXB6, HOXB8, HOXC8 and HOXC9 are misexpressed at specific stages of colon cancer progression. And, while HOXB7 and HOXD4 are both expressed in normal and neoplastic colon, the size of the transcripts differ. Again, the significance of these observations for disease initiation or progression is unclear.

CDX1 and CDX2 homeobox genes are expressed in normal colon, while their expression is reduced in colon tumors. Interestingly, there appears to be an inverse correlation between the levels of protein expression and the severity of the dysplasia. This correlation, in conjunction with other data, suggests that expression of CDX1 and CDX2 is important for maintaining normal colon differentiation and that their loss of expression may promote a cancer phenotype. These observations have been supported by mutant mouse models in which loss of CDX genes results in colon tumors.

Misexpression of certain homeobox genes has also been implicated in prostate cancer. For example, the GBX2 homeobox gene is overexpressed in several metastatic prostate cell lines, suggesting a possible role in cancer progression. Conversely, loss of function of the NKX3.1 homeobox genes has been implicated in prostate cancer initiation. Notably, NKX3.1 maps to a hotspot that is frequently deleted in many prostate cancer samples, and mutant mice lacking NKX3.1 display prostatic epithelial dysplasia. Like, the CDX genes, these mutant mouse models provide excellent support for a functional role of these homeobox genes in cancer.

At this point much of the data is rather circumstantial and based on altered expression patterns. Nonetheless, the available evidence suggests that homeobox genes may provide an important link in understanding the delicate balance of development, cell-cycle control and cancer. The specific molecular events that occur between the misexpression of homeobox genes and the progression to cancer remains a topic of active investigation.

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Homeostasis

Definition

Is one of the fundamental characteristics of living things. It is the maintenance of the internal environment within tolerable limits. Homeostasis is maintained by

means of multiple dynamic equilibrium adjustments, controlled by interrelated regulatory mechanisms, resulting in a stable, constant condition.

►Apoptosis-Induction for Cancer Therapy

Homeotic Genes

Definition

Genes involved in developmental patterns and sequences.

►Polycomb Group

Homer Wright Rosette

Definition

Is a circular or spherical grouping of dark tumor cells around a pale, eosinophilic, central area that contains neurofibrils but lacks a lumen; seen in some cases of ►medulloblastoma, ►neuroblastoma, and ►retinoblastoma. A rosette structure is also an important morphologic finding in the diagnosis ►Ewing sarcoma/►pPNET.

Homing Peptides and Vascular Zip Codes

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Definition

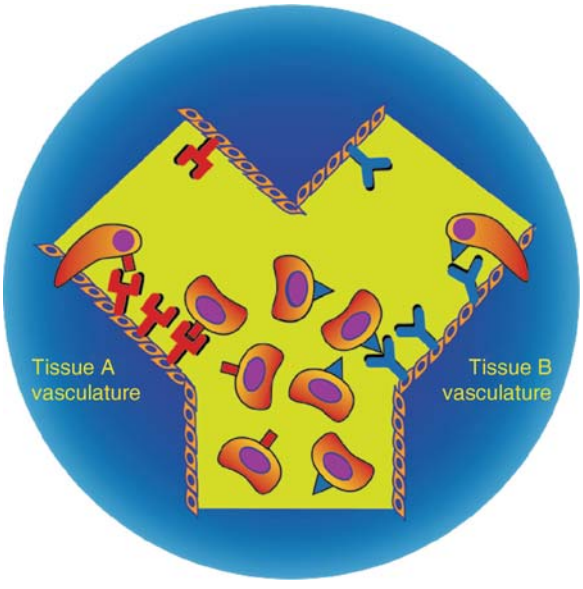
Vascular Zip Codes refers to tissue- and disease-specific molecular differences in vessels.

Characteristics

Peptide libraries displayed on phage can be screened *in vivo* to derive peptides capable of homing to selected target tissues through the circulation. The resultant peptides bind to endothelial (and possibly pericyte) surface proteins that are selectively expressed in the vasculature of individual tissues (Fig. 1). Homing peptides have been obtained for the vasculature in a large number of individual normal tissues that reveal a previously unsuspected degree of endothelial specialization. Screening for tumor homing has produced a collection of peptides that home to tumor vasculature. Atherosclerotic lesions have also been targeted in this manner.

The vessels of some normal organs have been known to express specific marker molecules, but *in vivo* phage has revealed an unprecedented degree of diversification in endothelia. The tissues for which vascular homing peptides have been described in the literature include brain, kidney, lung, heart, skin, pancreas, retina, intestine, muscle, uterus, prostate, fat tissue, and the adrenal gland. Success with so many tissues indicates that many, perhaps all, organs modify the endothelium of the vasculature, and that it is justified to refer to this heterogeneity as "vascular zip codes."

The vasculature of tumors is also specialized. Tumors depend on ►angiogenesis to grow and undergo ►metastasis. The actively growing tumor vessels are biochemically and structurally different from normal resting blood vessels. Integrins αvβ3, αvβ3, and α5β1as well as receptors for various vascular endothelial growth factors, are examples of molecules expressed at elevated levels in tumor blood vessels. Significantly, each of these receptors plays a critical role in angiogenesis. Other markers of tumor vasculature include an alternatively



Homing Peptides and Vascular Zip Codes.
Figure 1 Homing peptides direct phage binding to specific sites in the vasculature.

spliced form of fibronectin, endosialin, certain aminopeptidases, an anthrax toxin receptor, and intracellular proteins aberrantly expressed at the cell surface (nucleolin, annexin A1). Melanoma-associated proteoglycan NG2 is a marker of pericytes in tumor vessels.

Phage library screening *in vivo* has resulted in the identification of several peptide motifs that selectively direct phage into tumors. One of these motifs contains the sequence RGD (arginine-glycine-aspartic acid) embedded in a peptide motif previously shown to bind selectively to αv integrins. A number of additional tumor-homing peptides and receptors for some of them have been identified. Examples include peptides that recognize aminopeptidase N, cell surface nucleolin and clotted plasma proteins in tumor vessels. Homing peptides have also been used to show that the blood vessels of pre-malignant lesions differ from those of the corresponding normal tissue and from the vessels of fully developed tumors in that tissue. Further, lymphatic vessels in tumors differ molecularly from the lymphatics of normal tissues.

Biological Significance

Vascular zip code molecules are functionally important in the vessels that express them. A prime example is various angiogenesis markers, many of which are involved in the angiogenesis process. One known function of zip code molecules in the vessels of normal tissues is to serve cell trafficking. Tumor cells appear to make use of such molecules in homing to preferred sites of metastasis.

Clinical Significance

Coupling of drugs, ►apoptosis-inducing peptides, protein therapeutics, or imaging agents to tumor-homing peptides enhances the activity of these compounds in mice and lowers their toxicity. The homing peptides are also useful for the targeting of nanoparticles and potentially also cells to tumors. Delivery strategies similar to the tumor targeting should be applicable to targeting of pathological conditions other than tumors.

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Homing receptor

►CD44

Homo Sapiens Mitotic Arrest Deficient-like 1 Protein

►Mitotic Arrest-Deficient Protein?

H

Homodimer

Definition

A type of protein interaction in which two of the same protein bind together to form a complex, often necessary for the normal function of receptors.

►Prostate-Specific Membrane Antigen

Homo- or Heterodimers

Definition

A protein complex consisting of two identical (homo) or different (hetero) subunits.

►Early B-cell Factors

Homogeneously Staining Region

Definition

HSR; is a region within a chromosome lacking the typical banding pattern after staining with Giemsa, indicative of DNA amplification. In tumor cells indicating oncogene amplification, in some cases also amplification of genes encoding proteins for drug metabolism.

►Amplification

Homogenous Assays

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

Homogenous Time Resolved Fluorescence

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

Homolog

Definition

Genes that share a high degree of sequence identity or similarity, indicating their common ancestor or function.

Homologous Recombination Repair

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Definition

Homologous recombination repair is a DNA repair process that includes the invasion of an undamaged DNA molecule by a damaged molecule of identical or very similar sequence. Resynthesis of the damaged region is accomplished using the undamaged molecule as a template.

Characteristics

Homologous recombination repair has been found in all organisms examined from bacteria to man. It has an important role in repairing DNA damage with high fidelity by correcting damage with the use of information copied from an homologous undamaged molecule. Sister chromatids (duplicated chromosomes following DNA replication) or the paternal and maternal copies of chromosomes provide the required homology (sequence identity or near-identity over a few hundred DNA base pairs). In somatic cells, if the homologues have some sequence differences the copying process may alter the sequence of the damaged chromosome to the same as that of the undamaged chromosome, potentially revealing mutations (►loss of heterozygosity). Additionally, recombination between repeat sequences may lead to high frequencies of sequence variation as seen in certain ►minisatellite sequences. In germ cells, homologous recombination is vital for the reassortment of chromosomes during meiosis to create genetic diversity in organisms.

Cellular Regulation

The proteins that mediate homologous recombination repair have functions and often structures that have been conserved in evolution. These proteins have to seek out homologous regions of chromosomes, exchange DNA strands, copy sequence from the undamaged strand and finally resolve the DNA structures arising from exchange. This complex series of events is best understood in bacteria but recently many of the components and functions of homologous recombination repair have been identified in mammalian cells. Evidence suggests that DNA double-strand breaks commonly trigger repair by homologous recombination; these breaks may be caused by the interaction of DNA with chemical radicals, produced as a consequence of cellular metabolism, or by external damaging agents such as ionizing radiations. Meiotic recombination is also driven by DNA double-strand breaks, but these are formed enzymatically at specific sites in DNA.

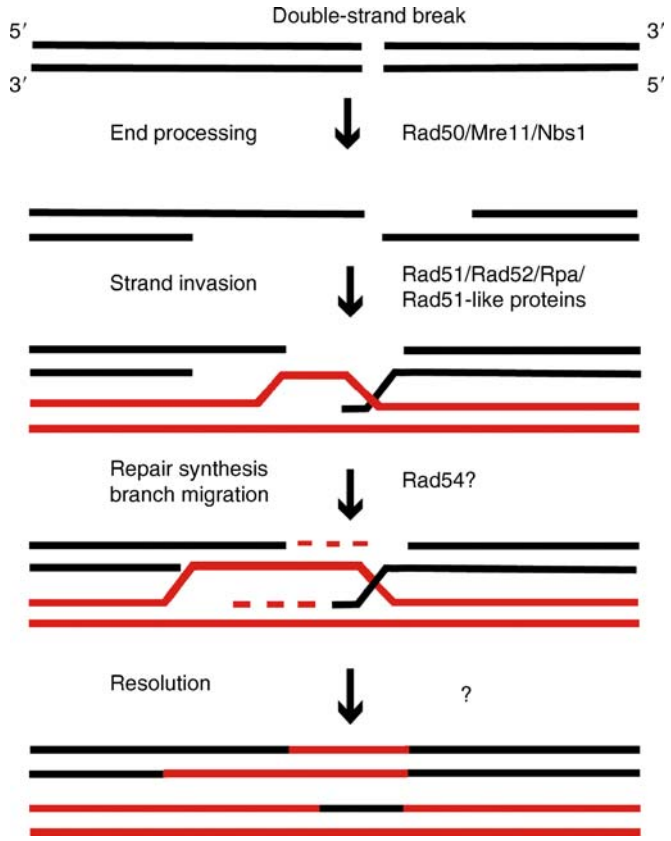
In bacteria, the ►RecA protein is central to homologous recombination through its ability to search for homologous regions of DNA and promote strand exchange. RecA forms a polymer on DNA to give a nucleoprotein filament, acting as a DNA-dependent ATPase. Filament formation occurs very rapidly on single-stranded DNA; in purified solutions the rate can approach 1,000 RecA monomers assembled per minute. RecA will also polymerize on double-stranded DNA but needs a short single-stranded gap to start the process, potentially targeting the protein to sites needing repair. On binding RecA and in the presence of homologous double-stranded DNA, strand exchange occurs to form junctions between the two DNA molecules. A number of other proteins are involved in the early stages of homologous recombination repair in bacteria; to generate the single-stranded DNA required for RecA-mediated strand exchange different proteins (RecBCD, RecJ, RecQ, or RecE) may be used depending on the initial state of the DNA. For example, during bacterial conjugation the RecBCD protein will unwind DNA from a break and cut the unwound DNA at a specific sequence-recognition site,

forming a long 3′ single-stranded region suitable for invading homologous double-stranded DNA. Other proteins assist in the loading of RecA onto single-stranded DNA and/or strand-invasion activities (RecF, RecO, RecR and single-stranded DNA binding protein). During recombination the junctions formed at sites of exchange migrate along the DNA molecules to complete gap repair, and finally the junctions must be cut to free the participating DNA molecules. Again some of these functions are supplied by different proteins, in this case either RuvABC or RecG, indicating the presence of more than one pathway for homologous recombination.

While much of the mechanistic detail of homologous recombination repair has been described in bacteria, it is clear that the principles of this mechanism have been retained by all organisms. RecA-like proteins have been found in eukaryotes; in particular the ►Rad51 protein from the yeast *Saccharomyces cerevisiae* (Rad51p) has both structural and functional similarities to RecA. Rad51p leads to the formation of nucleoprotein filaments on DNA and the promotion of homologous pairing and strand exchange reactions in vitro. However, in addition to Rad51p there are three other ►RecA/Rad51-like proteins in yeast; Rad55p, Rad57p and Dmc1p. Biochemical studies suggest that these RecA/Rad51-like proteins do not have redundant functions but rather have distinct roles to play in the early stages of repair. The Dmc1 protein functions exclusively in meiosis, with loss of its function leading to sterility, while the other RecA-like proteins operate in both mitosis and meiosis. Rad55p and Rad57p exist as a dimer and appear to act as a cofactor for the assembly of Rad51p onto single-stranded DNA. A three-protein complex (►Rad50p/►Mre11p/Xrs2p) has been found that promotes the early stages of homologous recombination but is also involved in a number of other repair and DNA maintenance functions; the Mre11 protein in particular has nuclease activities that may process double-strand breaks to form single-stranded regions associated with strand invasion. Other yeast recombination proteins, such as ►Rad52p and ►Rad54p, interact with Rad51p and promote Rad51-mediated strand exchange. Rad52p interacts with single-stranded DNA binding protein (Rpa) and facilitates the loading of Rad51p onto single-stranded DNA lacking secondary structure. Rad54p is structurally related to a family of putative DNA helicases but its precise function is unknown.

Rad51 also occurs in human cells. Despite millions of years of evolutionary divergence, the human protein is remarkably similar in structure and function to the yeast Rad51 protein. Additionally, six further RecA/Rad51-like proteins have recently been identified in humans. Two of these, Xrcc2 and Xrcc3, were found through their ability to complement the sensitivity of certain mammalian cell mutants to DNA-damaging agents. The remainder, including a protein closely similar to the yeast Dmc1, were found by searching for RecA/Rad51-like proteins in the human protein sequence databases. These proteins are presently the subject of intensive study but there is already evidence that some may function like the yeast Rad55 and Rad57 proteins in forming dimers that help Rad51 to function efficiently. The increase in the numbers of RecA/Rad51-like proteins in higher organisms also suggests that certain specialized functions may have evolved to deal with specific types of DNA damage or with tissue-specific recombination events. The yeast Rad50 and Mre11 proteins are also conserved in mammalian cells, but a structural homologue of the yeast Xrs2 protein has not been found in humans. Instead the Nbs1 (►Nijmegen breakage syndrome) protein (also known as p95 or nibrin), defective in the radiosensitive and cancer-prone human disorder Nijmegen breakage syndrome, appears to be a functional analogue of Xrs2. The features of Nbs1-deficient cells are very similar to those of ataxia-telangiectasia cells, and recently the human Mre11 protein has been shown to be mutated in individuals with an ataxia-telangiectasia-like disorder. Proteins similar to the yeast Rad52 and Rad54 have also been found in humans, and in the case of Rad54 it has been shown that the human protein is able to function in place of the yeast protein. The Rad52 protein is not so well conserved in structure between yeast and humans, although it is possible that another Rad52-like protein remains to be discovered in humans. In biochemical experiments, however, the human Rad52 protein has been shown to stimulate Rad51-mediated DNA-strand transfer reactions, probably at an early stage of Rad51 filament formation. Proteins involved in the later stages of recombination repair have yet to be identified in yeast or mammalian cells. The interactions of these proteins in the early stages of repair of a DNA double-strand break by homologous recombination are illustrated in the Fig. 1.

To study the effects of loss of recombination genes in mammals, several of the genes have been disrupted in mice (“knockout mice”). Surprisingly, disruption of Rad51 in mice is lethal for embryonic development and cells could not be cultured from tissue derived from the knockout animal. This finding suggests that the mammalian Rad51 gene has an important role in essential aspects of DNA metabolism or in development. It also suggests that the other Rad51-like genes cannot replace the function of Rad51 itself. Disruption of some of the Rad51-like genes in mice also leads to embryonic lethality, showing that these similarly have important functions in the organism. In contrast, knocking-out other recombination genes in mice is not necessarily lethal. Disruption of the meiosis-specific Dmc1 gene, as well as similar knockout



Homologous Recombination Repair. Figure 1 A model for the repair of a DNA double-strand break by homologous recombination in human cells. The main steps are noted to the left, and the proteins involved (where known) to the right. The Rad51-like proteins include Xrcc2 and Xrcc3. The broken DNA molecule (black) is processed to give long 3′ single stranded regions; these invade an undamaged homologous molecule (red). The branched-out undamaged strand acts as a template to repair the break. Repair synthesis is accompanied by branch migration. Resolution involves cutting the junctions between the two molecules; here it is shown without crossing-over, potentially leading to gene conversion where the homologous molecules differ in sequence.

experiments with the mouse Rad52 and Rad54 genes, yielded viable progeny. With the possible exception of the Rad52 knockout mouse, each has severe defects in aspects of mitotic and/or meiotic processes, consistent with roles in homologous recombination repair.

The potential importance of homologous recombination repair genes in genetic stability and in development has been highlighted by several recent discoveries. Firstly, it has been found that the human Rad51 protein interacts physically with the tumor-suppressor protein p53, which has a central role in the control of the cell cycle and apoptosis. Additionally, Rad51 knockout embryos survived longer in a p53-deficient background, suggesting at least part of the growth problems experienced by Rad51 knockouts arises from unrepaired damage triggering ►cell-cycle checkpoints leading to growth arrest. Equally important, Rad51 interacts with the breast cancer-susceptibility gene products ►Brca1 and ►Brca2. Disruption of the Brca1 or Brca2 genes in mice gives embryonic lethality and shows a similar elevation of chromosomal aberrations as that found in Rad51-disrupted cells. The link between the Brca proteins and homologous recombination repair has been extended by showing that Brca1 and Brca2-deficient cells have a large reduction in ability to repair double-strand breaks in homologous substrates integrated into the genome. Brca1 is phosphorylated in response to DNA damage and this event is dependent on the protein kinase mutated in the cancer-prone disorder ataxia-telangiectasia (Atm). Atm may act as a sensor of DNA damage, especially double-strand breaks, and there is evidence supporting a model in which Brca1 phosphorylation triggers the activity of Rad51 and Brca2 to initiate homologous recombination repair. However, Brca1 also associates with the human Rad50 protein and has a role in the ►transcription-coupled repair pathway, so its action is likely to be broader than this model suggests.

The role of recombination proteins in influencing development in multicellular organisms has been illustrated by recent findings in the fruit fly, Drosophila melanogaster. Mutations in the spindle class of genes lead to patterning defects in the oocyte and embryo, apparently due to mis-localization and/or failure to accumulate normal levels of oocyte signaling molecules. The cloning and sequencing of these genes showed that they are homologues of RAD51 or RAD54. Mutation in these genes leads to the accumulation of DNA damage, which triggers a meiotic recombination checkpoint that down-regulates some of the genes essential for embryonic development. Interestingly, oocytes that are mutant for both spindle-class genes and the Drosophila homologue of ATM (mei-41) can bypass this meiotic arrest and show normal developmental patterning, suggesting that loss of ability to signal damage fails to trigger this checkpoint. The downside of this checkpoint loss is that DNA damage may be carried through into later cell divisions, resulting in genomic instability.

The mechanisms of homologous recombination repair are still being investigated, but it is clear that this repair pathway is of considerable significance in all organisms. Other pathways can repair certain types of DNA damage; for example, in mammalian cells, ►non-homologous end joining is an alternative way to repair DNA double-strand breaks. However, simple end joining of broken DNA is prone to loss of sequence (DNA deletion) at the damage site, while homologous recombination repair can rejoin breaks with high fidelity. In bacteria it is clear that a major role of homologous recombination repair is to sort out problems arising during DNA replication, in particular where a replication fork stalls due to damage in its path. In yeast and mammals there is also evidence that homologous recombination proteins are particularly active during DNA replication. Additionally, certain types of DNA damage such as ►DNA interstrand cross-links formed by agents such as mitomycin-C may be resolved only by the homologous recombination repair pathway. In support of this idea, cell lines lacking homologous recombination repair genes are very sensitive to mitomycin-C and other DNA cross-linking agents.

Clinical Relevance

Loss of homologous recombination repair leads to unrepaired damage in the genome, which in turn can give rise to genomic instability. Both Mre11 and Nbs1 have been found to be associated with rare human disorders with complex phenotypes, including radiation sensitivity and cancer-proneness (ataxia-telangiectasia and Nijmegen breakage syndrome, respectively). The association of homologous recombination repair proteins with p53 and the Brca proteins also suggests that this repair pathway has potentially important connections with predisposition to cancer. The links are strongest with breast cancer, through the Brca proteins and possibly the Atm protein. Additionally, loss of one of the human Rad51-like proteins (Rad51L1) has been associated with uterine leiomyomas.

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Homologue of Synaptopodin

►Myopodin

Homomeric Complex

Definition

Complex composed of two or more identical subunits.

►Hamartin

Homophilic and Heterophilic Adhesion

Definition

Adhesion between two undefined cell types can be mediated by either identical (homophilic) or different (heterophilic) adhesion molecules.

►Cell Adhesion Molecules

Homoplasmy

Definition

In normal conditions all copies of mtDNA are identical within coding region.

►Mitochondrial DNA

Homotypic and Heterotypic Adhesion

Definition

Adhesion mediated by undefined adhesion molecules between identical cell types (homotypic) or two different cell types (heterotypic).

►Cell Adhesion Molecules

Homozygous Deletion

Definition

In a diploid organism, loss of both alleles or portion of both alleles from the genome of a cell, tumor or organism. Physical loss of both copies of the same gene or of the same chromosomal segment of a pair of homologous chromosomes.

►Fragile Histidine Triad

Horizontal Gene Transfer (HGT)

Definition

In this process, an organism transfers its genetic material to another cell that is not its offspring. The horizontal transfer of genetic material was originally detected in prokaryotes, but recent data show that it occurs not only in unicellular, but also in multicellular eukaryotes and might be an important evolutionary mechanism.

►Circulating Nucleic Acids

Hormonal Carcinogenesis

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Definition

The process by which a normal cell is transformed into a cancer cell is called "Carcinogenesis". When the carcinogenic event is either potentiated or promoted by hormones (i.e., natural or synthetic) (►diethylstilbestrol, estradiol) is considered as "Hormonal Carcinogenesis" (►Estrogenic hormone and cancer) (►Hormones and Cancer).

Characteristics

There are three major classes of hormones based upon their chemical structures. These are (i) peptide/protein hormones (e.g., leptin, angiotensin II, interleukins, ACTH, gastrin and others), (ii) amino acid or fatty acid-derived hormones (e.g., epinephrine, acetylcholine, prostaglandins and others, and (iii) steroid hormones (e.g., estrogen, progesterone, testosterone and others). Each hormone has its unique features and plays specific role under normal physiological conditions. Normally, it requires very minute amounts to exert its biological function through its receptor or other signaling pathways. However, excess or alter form of a hormone or its receptor sometimes exhibits carcinogenic behavior and distort the regulated state of the cells, which ultimately activate the carcinogenic switch or growth of the tumor or aggressive properties of a tumor or all the above events. The ideal example of carcinogenic hormone is natural or synthetic estrogens such as 17β-estradiol (E2) and diethylstilbestrol (DES). In animal system (i.e., mice, rats and hamsters), these two estrogenic compounds induces tumors in various estrogen-depended organs or tissues and the incidence rate is 100%. Moreover, a strong links between estrogens and the development cancer in human have been evident over 300 years ago that showed a protective effect of pregnancy on the development of breast cancer and also underscored that the higher rates of breast cancer among catholic nuns. This finding was reconfirmed by several epidemiological studies that showed a single full-term pregnancy reduces the risk of development of breast cancer by 50% or more. Subsequent births, especially at an early age, further reduce the risk. Moreover, breast cancer risk in the presence of estrogen can be determined by several factors including early menarche, late menopause and obesity in postmenopausal women.

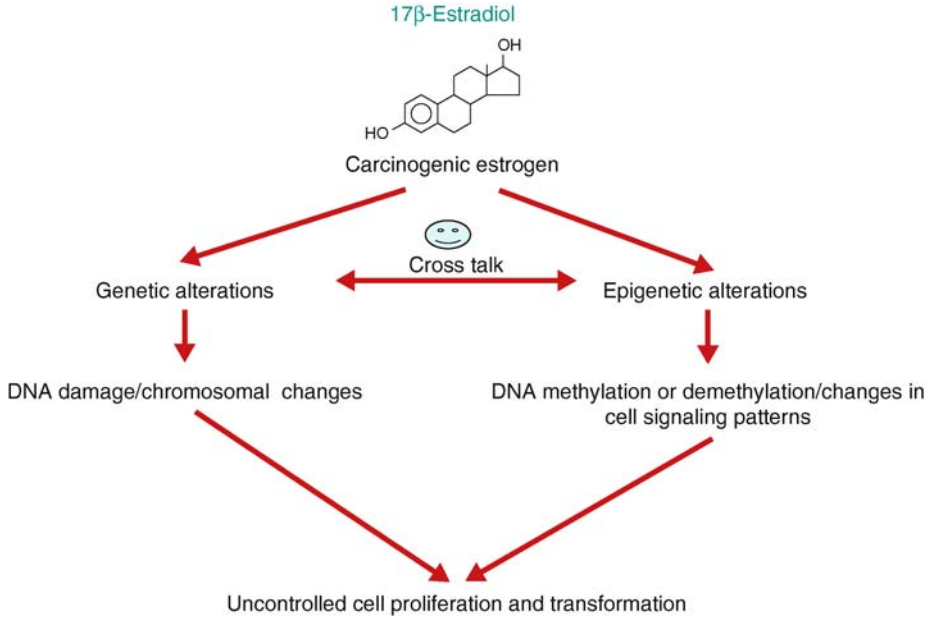
Several cancers have been found to relate with the status of carcinogenic hormones in the body. For instance, breast or prostate cancer development may depend on the supply of estrogen or androgen, respectively, or their cognate receptors in the tumor cells. Therefore, the removal of the source glands (ovary, testis or pituitary) or targeting the receptors by antagonists (i.e., tamoxifen) of the hormones is used to prevent the growth of these tumors.

Mechanisms

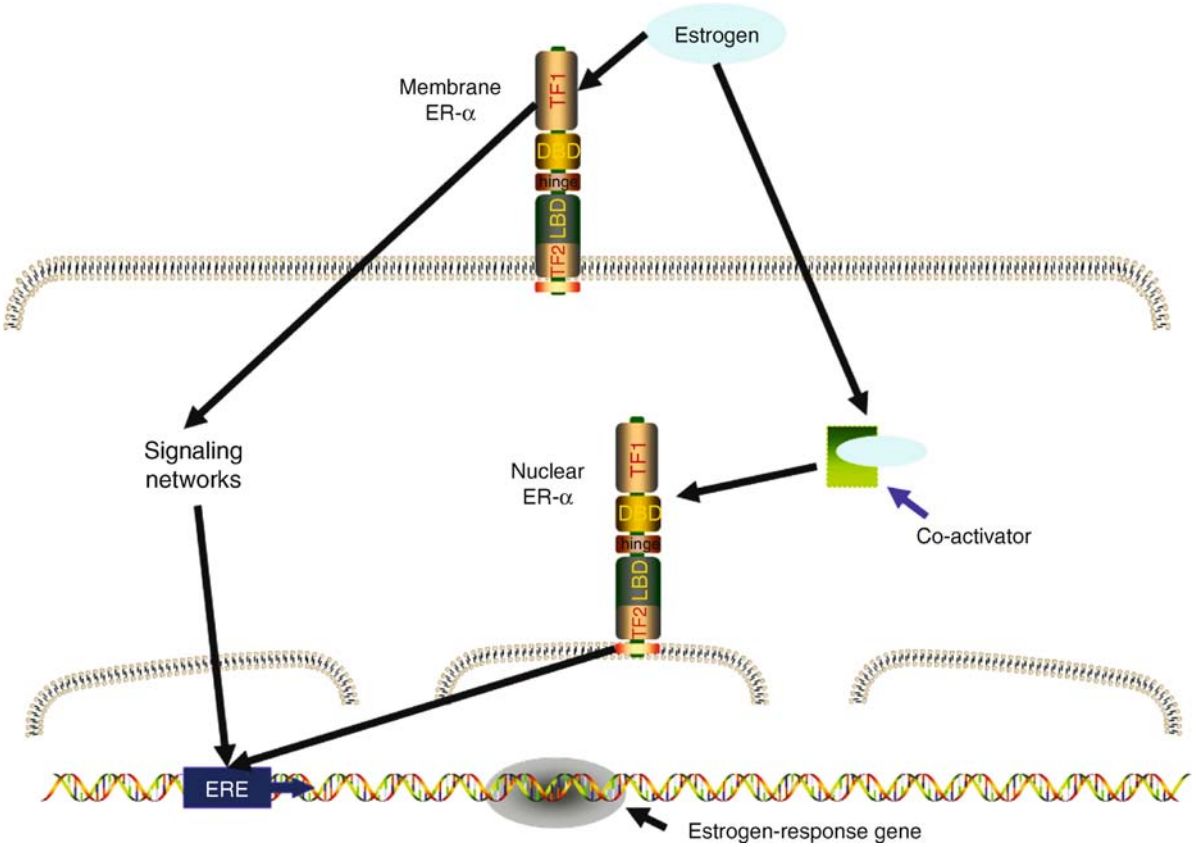
Cancer is a mixed tissue of abnormal cells, and the etiology of this disease remains uncertain. After decades of studies it has been apparent that cancer begins with multiple genetic and epigenetic insults that included mutations of regulatory genes, activation of oncogenes or inactivation of tumor suppressor genes. These molecular insults facilitate the cells to grow abnormally, gain motile behavior and other features needed to grow the cancer cells and invade to distant parts of the body. These fatal abnormalities are probably built up in the normal cells through endogenous or exogenous carcinogens.

Estrogens have been used for last several decades to explore the molecular mechanisms of hormonal carcinogenesis by several investigators. These studies found chronic exposure of estrogens involve interplay and cross-talk between genotoxic and epigenetic changes that eventually lead to an uncontrolled cell proliferation and cellular transformation (Fig. 1). The genetic alterations are accomplished by estrogens through the formation of DNA adducts and chromosomal abnormalities including chromosome breaks, aneuploidy and telomeric association, while epigenetic alterations are potentiated through DNA methylation/demethylation or through the alterations in cell signaling molecules such as WISP-2/CCN5 and others. These epigenetic changes through the molecular cross-talk may precede genetic changes in normal or premalignant cells and foster the accumulation of additional genetic and epigenetic hits.

How estrogens induce genetic and epigenetic abuses in some cells? Despite decades of investigations, an absolute answer of this question is elusive. Because estrogen-induced carcinogenesis can be blocked by estrogen antagonists (i.e., Tamoxifen or ICI 182,780) or estrogen withdrawal, the estrogen receptors (nuclear or cytoplasmic) may be involved in estrogen-induced carcinogenic switch (Fig. 2). The normal physiological effects of estrogen are usually mediated through two classical estrogen receptors, estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β). These receptors are encoded by two separate genes though they have some common domains and have similar binding affinity with the ligands. Nevertheless, these two receptors play redundant roles in estrogen signaling. In cells where both



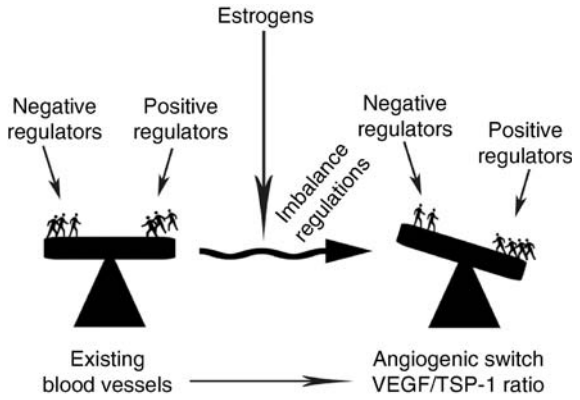
Hormonal Carcinogenesis. Figure 1 Putative Pathways involved in hormonal carcinogenesis. Carcinogenic estrogens-induced neoplastic transformation is mediated through genetic and epigenetic abuses in the target tissues or cells. Presumably genetic and epigenetic changes by carcinogenic hormones are interdependent and cross-talk or side-talk with each other to enhance uncontrolled proliferation and genomic instability which are the hallmarks of carcinogenic switch.



Hormonal Carcinogenesis. Figure 2 The role of nuclear and cytoplasmic receptors in hormonal carcinogenesis. The endogenous or exogenous estrogen binds with either cytoplasmic estrogen receptor or nuclear receptor and subsequently activates estrogen-response genes associated with cellular proliferation and transformation events.

receptors are expressed, the estrogen responsiveness is determined by the ER-α: ER-β ratio. The studies shown that isoforms of ER-β heterodimerize and inhibit the activity of ER-α, which suggest a modulatory role of ER-β. In hormonal carcinogenesis, participations of these two receptors are considered to be crucial. However, some investigators believe that non-receptor pathways are also involved in hormonal carcinogenesis. Like estrogens, other hormones which are having carcinogenic potential exhibit identical effect on the cells for the induction of transformation.

The formation of new blood vessels surrounding the tumors from preexisting blood vessel, which is also known as tumor angiogenesis, is the hallmark of cancers. It is necessary for increased nutrient and oxygen supply to the tumor cells. This process is controlled by several positive and negative angiogenic factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor-alpha (TGF-α), TGD-β, thrombospondin-1 (TSP-1) and statin (►Antiangiogenesis). Some of these angiogenic factors such as PDGF and TGFs are proteins/peptide hormones. Normally, angiogenesis is a highly controlled



Hormonal Carcinogenesis. Figure 3 Angiogenic switch during hormonal carcinogenesis. Estrogen plays dual roles in tumor angiogenic switch. It activates positive regulator of angiogenesis and simultaneously silenced the expression of negative regulator.

process. Uncontrolled and persistent changes in angiogenic process are occurred at different stages of tumor progression. This phenomenon is occurred by imbalance expression of positive and negative angiogenic

regulators. Multiple studies have shown that tumor angiogenic switch can be regulated by steroid hormones including estrogen and progesterone. These hormones modulate both positive and negative angiogenic regulators for angiogenic switch (Fig. 3).

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Hormonal Therapy

Definition

►Endocrine Therapy

Hormonal Treatments

Definition

Treatment that adds, blocks, or removes hormones. To slow or stop the growth of certain cancers (such as prostate cancer and breast cancer), synthetic hormones or other drugs may be given to block the body's natural hormones.

►Temsirolimus
►Hormones

Hormone-induced Cancers

►Endocrine-Related Cancers

Hormone-Refractory Prostate Cancer

Definition

HRPC; Synonym androgen-independent prostate cancer (AIPC); Prostate cancer that has become refractory, that is, it no longer responds to hormone therapy.

Hormone-related Cancers

►Endocrine-Related Cancers

Hormone Replacement Therapy

Definition

HRT; Is a medical treatment for menopausal, perimenopausal, and postmenopausal men and women, based on the assumption that it may prevent discomfort and health problems caused by diminished circulating estrogen and progesterone hormones in the female. The treatment involves a series of drugs designed to artificially boost hormone levels. The main types of hormones involved are estrogens, progesterone or progestins, and sometimes testosterone in the case of men.

►Estrogenic Hormones
►Estradiol
►Progestin

Hormone-Responsive Element

Definition

A specific *cis*-regulatory DNA sequence for a certain hormone that acts by binding to a receptor. This hormone–receptor complex can act as a transcription factor by binding to the hormone responsive element.

Hormones

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Definition

Hormones are complex chemical messengers that bind with high affinity to specific cellular receptors to activate single or multiple ►signal transduction pathway(s) leading to growth, ►differentiation, embryogenesis or other biological processes. More than one hormone may work in concert to fulfill a physiological function. Hormones, through their capacity to influence cell growth and differentiation, may further modify the response of the body to carcinogens, the biological behavior of established tumors, and overall ►cancer risk. Hormones, therefore, play an important role in both the genesis and treatment of cancer.

Characteristics

Hormone-dependent malignancy has been most extensively studied in steroid hormone responsive organs. The steroid hormones, androgens, estrogens (►Estradiol; estrogenic hormones and cancer) and progestins (►Progestins and cancer), are implicated as key regulators in the development and the cancer process in the breast (►Breast cancer), prostate (►Prostate cancer, clinical oncology), ovary (►Ovarian cancer) and endometrium (►Endometrial cancer). More recently, however, hormonal influences in cancer are also becoming apparent in steroid hormone independent organs such as the colon. Likewise, non-steroid hormones participate, directly or indirectly, as modifiers of cancer, not only in endocrine system responsive organs, but also at other sites.

Mechanisms

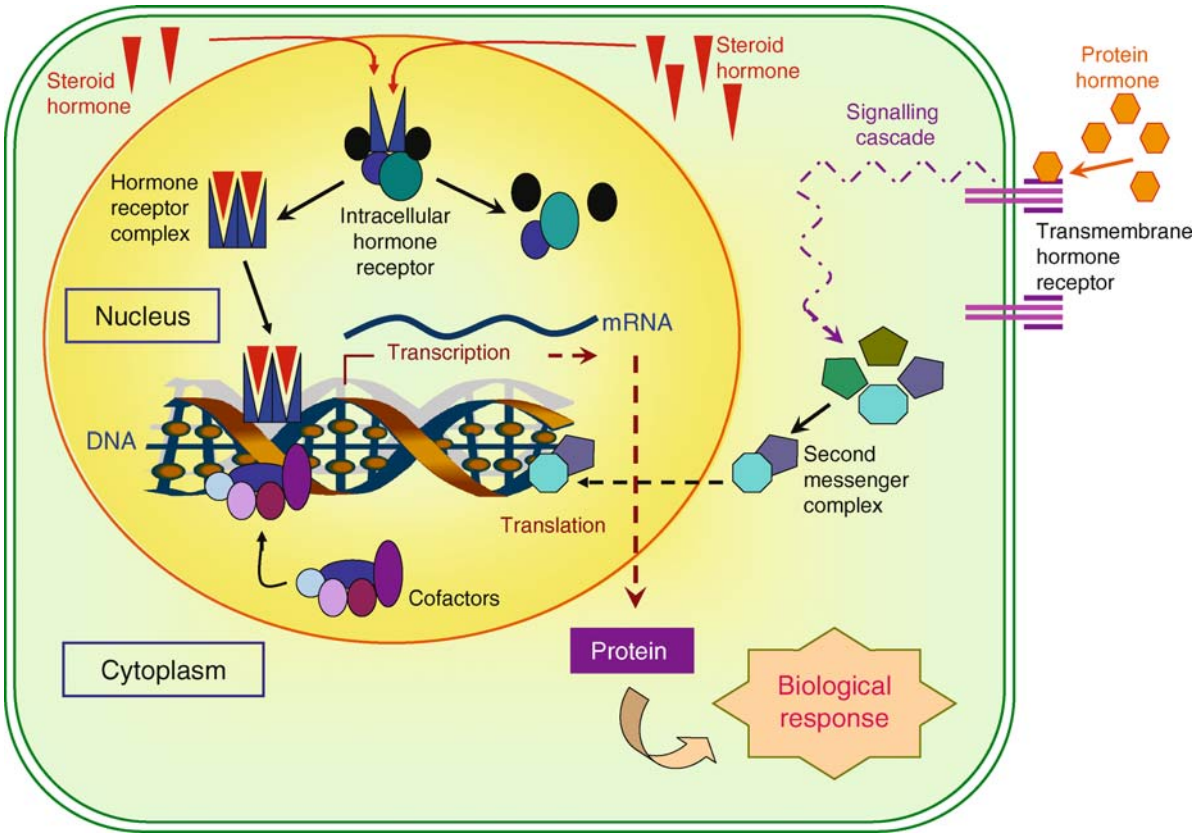
The precise mechanisms of hormonal influences on cancer are being elucidated. In the context of the multistage theory of ►carcinogenesis (►Multistep development), it has been generally recognized that the principal effects of a given hormone are during the ►cancer promotion stage, occurring predominantly in tissues where normal cellular growth and function are regulated by the specific hormone. Single or multiple hormones may interact to modify carcinogenesis. The tumor promoting effects of hormone(s) may be a result of indirect interaction of the hormone with the genome, culminating in epigenetic (►Epigenetics) consequences. Recent evidence, however, implicates hormonal modulation during the ►cancer initiation stage as well by direct interaction of certain hormonal metabolites with the genetic material.

The Receptor Concept

A receptor-mediated mechanism for hormone action during normal physiological functions is now widely accepted (Fig. 1). Hormones play a role as ligands that interact with specific receptors: the protein type binds to trans-membrane receptors, while the steroidal type binds to intracellular receptors. In the case of a protein hormone, a cascade of receptor-ligand mediated cytoplasmic responses is triggered leading to activation of post-receptor secondary messengers. On the other hand, a steroid hormone on entry into the cell forms cytoplasmic – receptor complexes that bind to DNA as a dimer. In both cases, recruitment of other co-factors ultimately regulate the activities of the cell's general transcription apparatus assembled at the gene's promoter. The mRNA produced is eventually translated into proteins which elicit and achieve a complex array of cellular responses such as alterations in growth and differentiation of the target tissue.

An interplay of a variety of factors can interfere with the normal development and function of the ►hormone receptors in target tissues, culminating in pathological conditions such as cancers. Thus, hormone receptors may be activated non-specifically by hormone-like endogenous metabolites or xenobiotic (►Xenobiotics) ligands found as dietary constituents (e.g. ►phytoestrogens), environmental pollutants, or food contaminating chemical or veterinary drug residues. Inherited genetic polymorphisms or mutations in hormone receptors and/or hormone metabolizing enzymes may further affect normal hormonal function.

In the case of steroid hormones, estrogens function as growth promoters through the nuclear receptors, estrogen receptor-α (ER-α) (►Estrogen receptor) or ER-β by signal transduction-mediated increase in cellular proliferation, decrease in ►apoptosis and regulation of growth factor production. ER-α and ER-β are products of two different genes, and ER-α may predominate in breast tumors. Likewise, androgen action on physiological processes such as cellular differentiation, secretory function, metabolism, morphology, proliferation and survival, is exerted in target tissues via binding to another nuclear receptor transcription factor, the ►androgen receptor (AR). Testosterone, produced mainly in the testis, is able to directly bind to and activate the AR. However, the androgens produced in the adrenal glands, androstenedione and dehydroepiandrosterone, are converted to testosterone in peripheral tissues prior to their interaction with the AR. Similarly, in the prostate, testosterone is first metabolized to a more potent ligand dihydrotestosterone, before its interaction with the AR. The effects of progestins as



Hormones. Figure 1 Schematic presentation of two types of receptor mediated hormonal action. Hormone binding to a trans-membrane or an intracellular receptor activates a cascade of events which eventually regulate the cell's transcription and translation machinery to elicit a complex array of functional responses by the cell.

well are mediated through two isoforms of progesterone receptor in a tissue-specific manner.

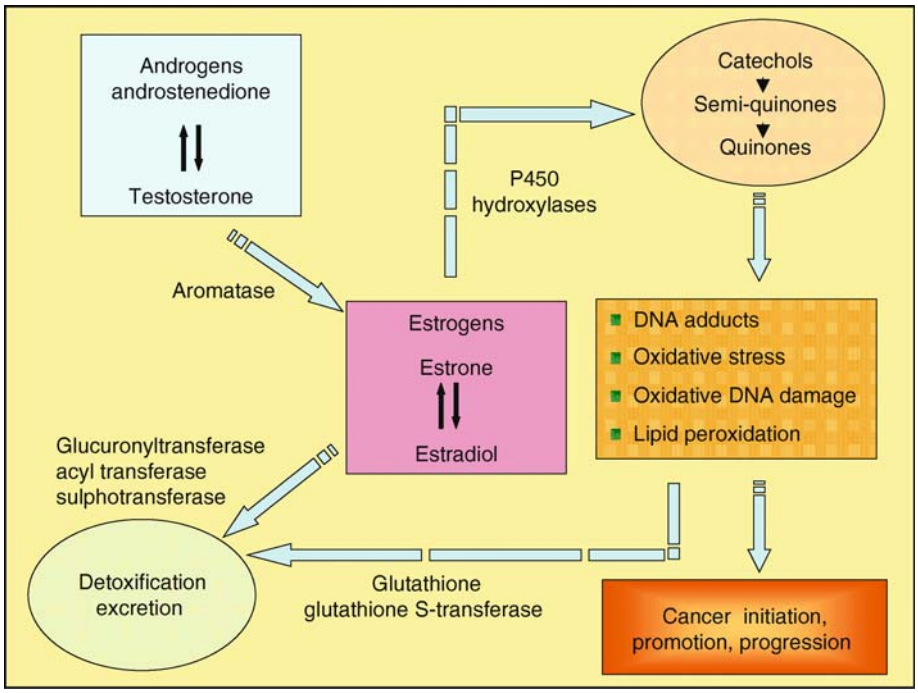
Prolactin exhibits dual characteristics of a circulating hormone and a ►cytokine, both of which utilize receptor-mediated mechanisms. In its role as a cytokine, prolactin is secreted and regulated in extrapituitary sites where its binding to cytokine superfamily receptors not only can promote cell proliferation and survival, but also increase cell motility, suppress apoptosis, upregulate BRCA1 (►Breast cancer genes BRCA1/BRCA2), induce both ER-positive and ER-negative tumors, increase tumor growth rates (►Prolactin and cancer), support tumor vascularization and enhance tumor metastases.

Insulin and ►insulin-like growth factors (IGF-I) likewise act through a receptor (►Insulin receptor) -mediated mechanism to regulate energy metabolism. The bioactivity of IGF-I is increased through its enhanced synthesis and by a decrease in several of its binding proteins (IGFBP; IGFBP-1 and -2). Insulin and IGF-I both stimulate ►anabolism based on the amount of available energy and the necessary substrates such as amino acids. The anabolic signals by insulin or IGF-I can promote tumor development by inhibiting apoptosis, and by stimulating cell proliferation. Furthermore, both insulin and IGF-I stimulate the synthesis of sex steroids, and inhibit the synthesis of sex hormone-binding globulin, a binding protein that regulates the bioavailability of circulating sex steroids to tissues. Receptor-mediated mechanisms also intercede the action of thyroid hormone, and the gastrointestinal hormones gastrin, cholecystokinin, neurotensin and ►gastrin-releasing peptide.

Hence, the receptor concept provides one common mechanism whereby a steroid or a non-steroid hormone may potentially mediate or modulate cancer under the conditions that an aberration in its receptor-mediated signal transduction system favors an enhancement or prevention of neoplasia.

Endogenous Metabolic Activation of Steroids

A second mechanism of action implicated in hormonal modification of carcinogenesis involves endogenous metabolism of the steroid hormones, estrogen and androgens by tissue-specific enzymes (Fig. 2). A similar mechanism for non-steroid hormones has not been described so far. Estrogen is biotransformed by several ►cytochrome P450 isoforms and various peroxidases



Hormones. Figure 2 Metabolic activation of steroid hormones. Aromatase-mediated metabolism of androgens to estrogens, and further oxidative metabolism of estrogens lead to genotoxic effects that may contribute to cancer induction.

to produce estrogenic metabolites that may be protective through their antioxidant properties or growth and ►angiogenesis inhibitory activities. On the contrary, the more reactive quinone metabolites of estrogen may directly form ►adducts with DNA and/or cause oxidative damage to lipids and DNA (►Oxidative DNA damage) through redox cycling processes that produce ►reactive oxygen species (ROS). Increased production of ROS may further alter regulation of gene expression through effects on transcription factor function. The androgens testosterone, androstenedione and dehydroepiandrosterone may act as additional cancer modifiers *in situ* in their respective target organs when they are metabolized first to estrogens by aromatase, another cytochrome P-450 enzyme, followed by their metabolic activation to quinones.

Epidemiology and Animal Studies

Steroid Hormones

Breast cancer risk is associated with prolonged exposure to predominantly estrogens. Thus, increased *in utero* exposure to estrogens, early onset of ►menarche, late ►menopause, hormone replacement therapy and postmenopausal obesity enhance breast cancer incidence. Androgens, through their metabolism to estradiol by the aromatase enzyme expressed in epithelial cells of both normal and neoplastic breast, act synergistically with estrogens to directly stimulate tumor growth and increase breast cancer risk in postmenopausal women. Androgens are further implicated as a causative factor in prostate cancer induction, though with limited epidemiological evidence (►Epidemiology of cancer). Human plasma levels of androgens do not always correlate with prostate cancer susceptibility. Animal studies, however, support a strong tumor promoting role for androgens acting via androgen receptor-mediated mechanisms, and following a tumor-initiating stimulus. Exposure of the fetus to higher androgen concentrations is a potential ►risk factor for prostate cancer in later life. However, as in the breast, malignant changes to the prostate gland depend upon both androgenic and estrogenic responses that are accomplished through aromatase mediated conversion of testosterone to estradiol and via estrogen receptors found in both human and rat prostate. Similar synergistic links between androgens and estrogens have been reported in uterine cancer in experimental animals, and clinically, in ovarian and endometrial cancers.

Estrogen receptors are likewise expressed in several ►gastrointestinal cancers such as those of the esophagus, gallbladder, stomach and colon (►Colon cancer). Estrogens may further interact with additional growth factors and polyamines to modulate growth and progression (►Cancer progression) of colorectal tumors. However, the epidemiological evidence for a role for estrogens in colorectal cancers is currently inconsistent.

Loss or down-regulation of progesterone receptor observed in ovarian carcinoma, and antiproliferative

and apoptotic effects of progesterone in the ovary, suggest that progestins protect against ovarian cancer. On the contrary, synthetic progestins, globally used as contraceptive agents, are implicated as tumor promoters or ►cocarcinogens in rodent liver, pituitary and mammary gland.

Prolactin

The neuroendocrine hormone prolactin may play a role in several types of cancers in reproductive and non-reproductive tissues. Its possible role in breast cancer has been widely considered because of its physiological action in the stimulation of mammary gland development and differentiation during puberty, pregnancy, and lactation. While experimental data support a relationship between increased prolactin levels and breast cancer, epidemiological data is discrepant.

Insulin

Physiologically, the growth hormone insulin-like growth factor-I (IGF-I)-axis is an important modulator of growth and development, as well as a regulator of a wide range of biological functions. Their potent mitogenic and anti-apoptotic effects, for example, play a critical role in the development of the breast, and in regulation of rapidly renewing epithelial cell populations such as those in the colon. In cancer patients, increased insulin production is commonly observed during cancer development. Some types of tumors produce constituents that stimulate insulin secretion by the pancreas, while other more aggressive tumor types produce insulin ectopically. Global variations in cancer incidence rates provide evidence for diet and associated factors such as nutritional energy balance, macronutrient composition of the diet, physical activity and body size (►Obesity and cancer risk) as among the most important lifestyle factors that influence cancer risk (►Nutritional status and cancer). Recent studies further suggest that these dietary and related factors may influence the risk of cancers of the colon, pancreas, endometrium, breast, ovary and prostate by affecting the levels of circulating serum insulin and increasing the bioavailability of IGF-I. IGF-I has been positively associated with the risk of colorectal cancer in human studies. *In vitro,* IGF-I elicits mitogenic and anti-apoptotic actions on colorectal cancer cells.

Gastrointestinal Hormones

The gastrointestinal hormones (►GI hormones), gastrin, cholecystokinin, neurotensin and gastrin-releasing peptide (►Gut peptides), are localized mainly in the pancreas and in the mucosa of the gastrointestinal tract from the stomach to the rectum. Certain gastric (►Gastric cancer), pancreatic (►Pancreas cancer, basic and clinical parameters) and colorectal cancers possess specific G-protein-coupled receptors through which the gastrointestinal hormones stimulate cancer cell growth. Gastrointestinal hormone receptors have also been noted in breast, lung and prostate cancers where they may modify neoplastic growth and aggressive behavior of the lesions.

Thyroid Hormones

An association between thyroid hormones and cancer has been considered extensively with conflicting results. In experimental models, perturbations in thyroid hormones affect estrogen and prolactin secretion, and cause pathological alterations in the ovary, endometrium and breast that are consistent with precancer. However, clinical evidence for the role of thyroid hormones in cancers in these organs is not definitive due to difficulties in differentiating thyroid hormone-induced cellular alterations from neoplasia-induced cellular atypia.

Hormonal Management of Cancer

Recognition of the role of estrogens in the stimulation of breast tumor growth has led to the development of several therapeutic endocrine agents. In premenopausal women ►gonadotrophin-releasing hormone (GnRH) agonists suppress ovarian oestrogen synthesis and reduce estradiol close to postmenopausal levels. Inhibition of aromatase, the terminal step in estrogen biosynthesis, provides another way of treating hormone-dependent breast cancer in older patients. Currently available ►aromatase inhibitors are effective in the management of hormone-dependent breast cancer in post-menopausal women failing antiestrogen or tamoxifen therapy (►Tamoxifen) in locally advanced or metastatic disease. GnRH agonists used in combination with an aromatase inhibitor suppress estrogen levels even further. Similarly, hormone therapy for prostate cancer has evolved from the use of estrogens to GnRH agonists. Recently, investigational GnRH antagonists have been discovered that cause the inhibition of luteinizing hormone production, eventually leading to a suppression of testosterone and dihydrotestosterone on which continued growth of prostate cancer cells is dependant. Steroid hormone receptors are another well established therapeutic target in cancer, and drug development has continued to focus on agents that either block steroid hormone production or bind to and modulate their receptors. The implication of insulin and IGF-I as additional factors in cancer modulation may further advance development of newer cancer prevention drugs that restore sensitivity to insulin and reduce hyperinsulinemia.

►Celecoxib
►Estrogenic Hormones
►Hormonal Carcinogenesis

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Hornstein–Knickenberg syndrome

►Birt–Hogg–Dubé Syndrome

HOX

Definition

The HOX homeodomain (HD) proteins are DNA-binding transcription factors that are key regulators of development and hematopoiesis. In vertebrates, there are 39 *HOX* genes that are organized into four chromosomal clusters (*HOXA*, *B*, *C* and *D*), which can be classified into 13 paralogous groups based on their extensive sequence homology within the HD and their relative chromosomal locations within a cluster. *HOX* genes are expressed at various stages during hematopoietic development. Perturbation of orderly HOX gene activation and inactivation results in hematological abnormalities. Increased expression of HOXA and B genes has been documented in human acute myeloid malignancies.

►NUP98-HOXA9 Fusion

HOXA5 gene

Definition

Is part of the A cluster of *HOX* genes on chromosome 7 and encodes a DNA-binding transcription factor that may regulate gene expression, morphogenesis, and differentiation. ►Methylation of this gene may result in the loss of its expression and, since the encoded protein up-regulates the tumor suppressor p53, this protein may play an important role in tumorigenesis.

►Pleiotrophin
►P53 Family

HOXA9

Definition

Is a member of the abdominal-B subclass of HOX genes and plays important roles in normal hematopoiesis as well as in leukemia development.

►NUP98-HOXA9 Fusion

HpaII Tiny Fragments (HTF) Islands

►CpG Islands

HPAEC

Definition

Human pulmonary artery ►endothelial cells.

HPD

Definition

►Hematoporphyrin derivative

HPRT

Definition
Hypoxanthine phosphoribosyl transferase.

►Microcell-Mediated Chromosome Transfer

hPTTG1

►Securin

HPV

►Human Papillomaviruses

HRPC

Definition
►Hormone refractory prostate cancer

HS1

Definition
Hematopoietic Specific protein 1 is a protein found exclusively in leukocytes that is functionally similar to cortactin. It is a component of the ZAP 70 and Syk tyrosine kinase signaling pathways activated during the immune response.

►Cortactin

HSC

Definition
Hematopoietic stem cell. HSCs are multipotent stem cells found in the bone marrow and give rise to all the cell types of both the myeloid and lymphoid lineages.

►Adult Stem Cells

HSD18

►Methylation-Controlled J Protein

HSF

►Interleukin-6

HSF-III

►Leukemia Inhibitory Factor

hSNF5

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

HSNF5/INI1

Definition
►Tumor Suppressor hSNF5/INI1.

hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

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Synonyms

hSNF5; Human non sucrose fermenting 5; SMARCB1; SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily b, member1; BAF47; BRG- and BRM-associated factor, 47 kDa

Definition

hSNF5/INI1 is a member of the highly conserved ►SWI/SNF complex, involved in the ATP-dependent ►chromatin remodeling. hSNF5/INI1 acts as a ►tumor suppressor gene.

Characteristics

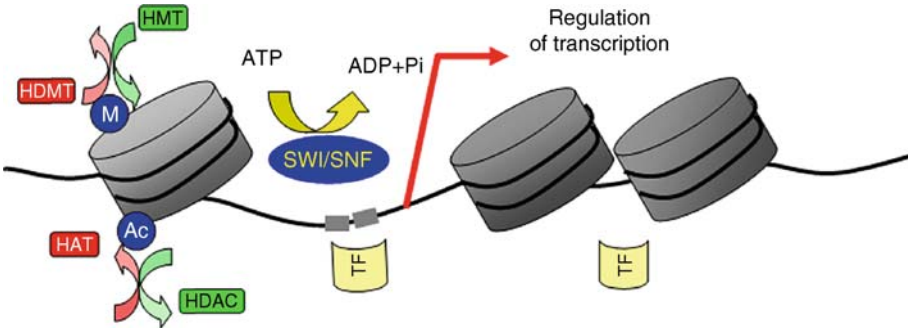
The Chromatin Remodeling and the SWI/SNF Complex

In the nucleus of eukaryotic cells, DNA is wrapped around histones, constituting a dense histone–DNA complex referred to as nucleosomes. The closed conformation of DNA in nucleosomes precludes the binding of transcription factors to their target promoters. By regulating the compaction degree of nucleosomes, cells can allow interactions between promoters and transcription factors. This regulation relies on two main mechanisms: (i) stable covalent modifications of histones, including actelyation, methylation, ubiquitination, etc. (ii) ATP-hydrolysis-dependent chromatin remodeling, capable to affect the spatial organization of the chromatin and the stability of nucleosomes in a dynamic way. The SWI/SNF complex, first described in *Saccharomyces cerevisia*, is the prototype of ATP-dependent chromatin remodeling complex. Approximately 5% of the genome is affected by the SWI/SNF-dependent regulation of the transcription, either by induction or by repression (Fig. 1).

The SWI/SNF complexes are conserved throughout the evolution. In humans, chromatography has lead to identify two fractions of SWI/SNF complexes, PBAF or SWI/SNF-B, characterized by the presence of polybromo protein, and BAF or SWI/SNF-A, which may be the actual homolog of yeast SWI/SNF. The BAF complexes are variably composed of approximately ten subunits, in a cell-type-specific manner. However, all BAF complexes contain one of the two ySnf2 human homologs, hBRM or hBRG1 (Fig. 2). Either of them brings the ATPase activity of the complex. In vitro, hBRG1 and hBRM are sufficient to induce a chromatin remodeling. Nevertheless, the addition of other members of the core complex increases the efficiency of the remodeling. Furthermore, the integrity of the entire complex seems to be indispensable in vivo. Among the BAF proteins, at least three are ubiquitously expressed, demonstrating a critical role in the function of the complex: BAF155, BAF 170 and BAF47. Altogether, these indispensable subunits constitute the core complex of SWI/SNF. The latter, BAF47, maintains the stability of the whole complex in yeasts but might not play such a role in mammals. Since its interaction with the HIV1 integrase IN was its first role identified, it has been called "INI1."

hSNF5/INI1 Gene and Protein Structures

In human, hSNF5/INI1 gene is located in 22q11.2. It is composed of nine exons and spreads over ~50 kb (Fig. 3a). An alternative splicing in exon 2 produces two



hSNF5/INI1/SMARCB1 Tumor Suppressor Gene. Figure 1 Chromatin remodeling and regulation of transcription. The chromatin is wrapped around histones. Acetyl or methyl radicals are added or removed by histone acetyltransferases (HAT) or histone deacetylases (HDAC) and histone methyl transferases (HMT) and histone demethylases (HDMT) respectively. ATP hydrolysis (ATP = ADP + Pi) also participates to a direct chromatin remodeling through the ATPase activity of remodeling complexes such as SWI/SNF, regulating the accessibility of the chromatin to transcriptions factors (TF).

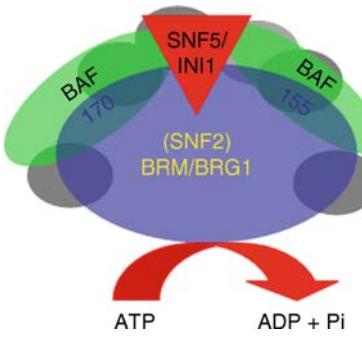
different transcripts of 1155 and 1128 nucleotides. hSNF5 protein has strong homologies with other SWI/SNF proteins in various species (yeast Snf5, *Drosophila* dSNR1, *Caenorhabditis elegans* CeSnf5). The homology domain encompasses 193 amino acids and two highly conserved repeated sequences, Rpt1 and Rpt2 (Fig. 3b). This repeated domain brings a nuclear export signal, consistent with the subcellular localization of the protein. Rpt1 directly interacts with several partners, such as HIV IN, c-MYC, hBRM or ALL1. A coiled-coil domain at the C-terminal extremity plays a role in the binding to interaction partners.

Biological Roles of *hSNF5/INI1*

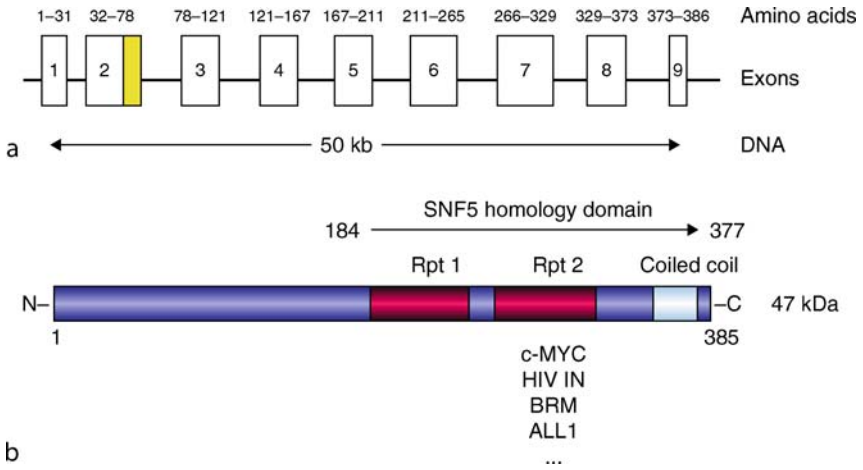
Studies of Snf5 inactivation in cell and mouse models have brought numerous insights on Snf5 biological functions. First, there are some strong evidences of Snf5 being involved in cell survival. Indeed, complete inactivation of Snf5 leads to early embryonic lethality and decreases cell survival in vitro. In MEFs, loss of Snf5 also impairs the cell cycle progression and, consistently, alters the expression of various Rb-E2f-responsive genes. Furthermore, the defect of Snf5 enhances sensitivity to DNA damage and subsequent apoptosis through a p53-dependent manner. These paradoxical roles in survival and apoptosis might be time-, cell-type- and stress-dependent. However, the most relevant observation is that conditional inactivation of Snf5 leads to the development of a tumor in 100% of the mice. Moreover, the short median delay of 11 weeks before tumor development is rarely observed with the inactivation of a single gene, and indicates a tremendously potent tumor suppressor role for SNF5.

To which extent the tumor suppressor function of SNF5 depends on SWI/SNF remains unclear. However, reexpression of hSNF5/INI1 in rhabdoid cells has demonstrated its critical role in the control of the ►G1–S transition. This effect could rely either on a repressive effect upon ►Cyclin D1 expression, or on a strong induction of ►p16/INK4a. Interestingly, a functional RB protein is required for INI1-dependent cycle control, whereas INI1 is dispensable for normal RB-mediated growth arrest (Fig. 4). Hence, INI1 definitely acts upstream RB.

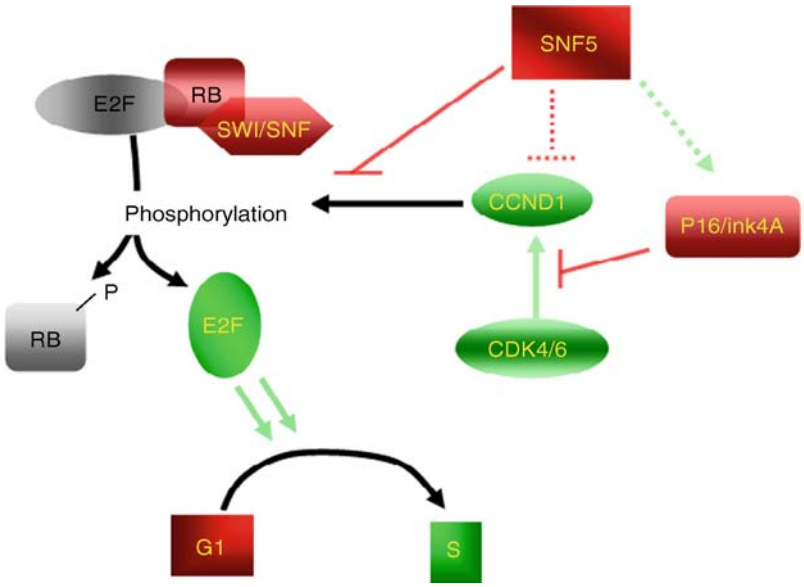
However, the control of the G1–S transition may not fully explain the antioncogenic role of hSNF5/INI. Indeed, missense mutations do not affect the replication checkpoint, but might rather lead to polyploidy by promoting chromosomal instability and compromising the mitotic checkpoint. Furthermore, there has been evidence for the involvement of hSNF5/INI1 in



hSNF5/INI1/SMARCB1 Tumor Suppressor Gene. Figure 2 The SWI/SNF complex. SWI/SNF complexes are composed of up to 12 proteins. Alternatively hBRM or hBRG1 carries the ATPase activity. The other proteins of the core complex are BAF155, BAF170, and BAF47.



hSNF5/INI1/SMARCB1 Tumor Suppressor Gene. Figure 3 *hSNF5/INI1* gene and protein structure. Interacting proteins are indicated in front of the Rpt2 domain. a. Structure of the gene: 9 exons encompassing ~50 kb. b. Structure of the protein with the highly conserved hemology domain.



hSNF5/INI1/SMARCB1 Tumor Suppressor Gene. Figure 4 hSNF5/INI1 and the G1–S transition. Arrows in green and red indicate respectively a positive and negative regulation. Dot lines indicate not fully consensual links. SWI/SNF has a physical interaction with RB and directly coregulates E2F1 transcription. hSNF5/INI1 on its own represses the G1 to S transition. This effect can be due either to *CCND1* downregulation or to *p16/INK4a* over expression. hSNF5/INI1 acts upstream RB.

dynamic regulation of the cytoskeleton. The restoration of the hSNF5/INI1 gene in rhabdoid cells alters the expression of Rho-family genes, likely to modulate the cytoskeleton, and obviously modifies the organization of actin stress fibers. hSNF5/INI1 might therefore play a role in cellular adhesion and migration properties.

Finally, taking into consideration the critical role of chromatin remodeling in the activation of cell-type-specific genetic programs, the role of hSNF5/INI1 in driving cell differentiation has also been investigated. INI1-dependent differentiating effect has been observed in hepatocytes. Conversely, reexpression of hSNF5/INI1 in rhabdoid cell lines drives distinct differentiation phenotypes according to the cell anatomic origin. A critical impairment of INI1-dependent differentiation programs may account for the highly undifferentiated phenotype of hSNF5/INI1-deficient tumors.

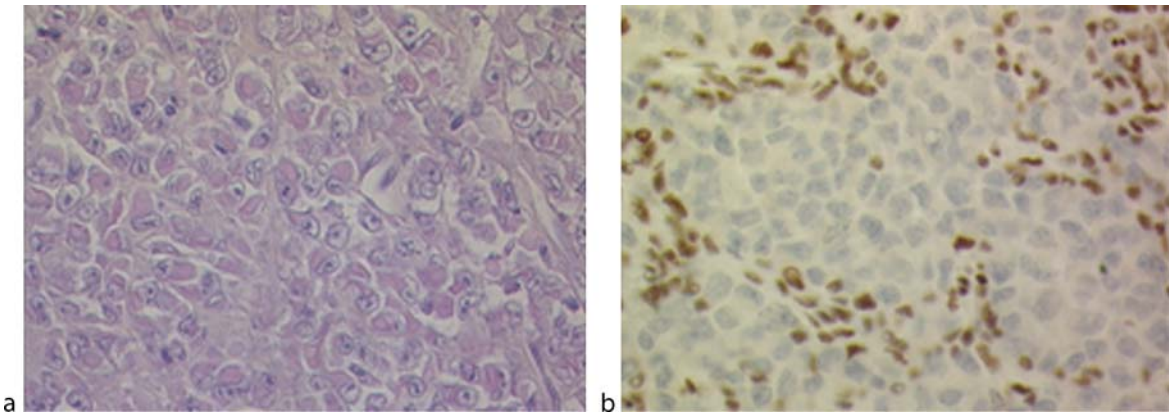
Genetic Alterations of *hSNF5/INI1* in Human Malignancies

Deletions encompassing the hSNF5/INI1 locus are encountered in many tumor types, including ►rhabdoid tumors (RTs), ►proximal epitheliod sarcomas (PES), meningiomas, schwannomas. Biological effects of hemizygous INI1 deletions are questionable, since loss of one allele results only in a 15–20% reduction in total *INI1* mRNA levels due to transcriptional compensation by the remaining allele. Hence, "two-hit" events only are likely to drive transformation. Strikingly, biallelic inactivation is encountered in about 80% of rhabdoid tumors. Karyotypic analyses in RTs usually show no or few other alterations than deletions or translocations of 22q11. Altogether, this indicates a very strong association between hSNF5/INI1 extinction and RTs. Both homozygous deletions and hemizygous deletions with point mutations are encountered.

In RTs cell lines, mitotic recombinations of chromosome 22q and nondisjunction/duplication, lead to partial or complete uniparental disomy. This mechanism may account for most homozygous changes and result in the total or partial loss of one chromosome associated with the duplication of the remaining chromosome carrying the deleted or mutated allele. The association of a point mutation with a whole gene deletion is less frequently encountered, but seems to be preponderant in ►brain tumors. Point mutations, consisting in nucleotides replacement, deletion or insertion and leading to truncating nonsense codons, are spread all over the nine exons with no obvious hotspot. Missense mutations are much rarely reported.

Studies using immunohistochemistry with anti-SMARCB1 antibody have confirmed the total loss of protein expression in a high proportion of RTs as a direct consequence of the genetic alterations. Whether hSNF5/INI1 inactivation is specific for RT or could be involved in miscellaneous other tumors remains not fully consensual (see next section).

Congenital multifocal rhabdoid tumors and familial cases strongly supported the existence of constitutional mutations in a predisposing syndrome. Indeed, about 40



hSNF5/INI1/SMARCB1 Tumor Suppressor Gene. Figure 5 pathological aspects of *hSNF5/INI*-deficient ►rhabdoid tumor. (a) "Rhabdoid" cells: discohesive polygonal or round cells with abundant cytoplasm, juxtanuclear globular eosinophilic cytoplasmic inclusion, large nucleus with one prominent nucleolus. (b) negative staining of rhabdoid cells with anti-hSNF5/INI1 antibody, resulting from a complete loss of the protein and the biallelic inactivation of *hSNF5/INI1* gene. Positive staining is retained in normal stromal cells.

germline mutations have been reported so far, associated to the development of a tumor during early childhood in almost all patients. Hence, the penetrance is very high, though not full. Nucleotides or whole gene deletions, point mutations and splice site changes have all been observed at the constitutional level.

Pathological and Clinical Aspects of hSNF5/INI-Deficient Tumors

RTs were initially described as highly aggressive variants of Wilm tumors occurring in infants (►Nephroblastoma). "Rhabdoid" cells were defined by key morphologic histological and immunophenotypical features: polygonal or round shape with abundant cytoplasm, juxtanuclear globular eosinophilic cytoplasmic inclusion, large nucleus with one prominent nucleolus (Fig. 5a), coexpression of vimentin and epithelial markers such as epithelial membrane antigen and/or cytokeratins. Such features were then observed in other pediatric malignancies, in brain (Atypical Teratoid/Rhabdoid Tumors) (Brain tumors) and miscellaneous soft-tissues. Presently, the cell origin of RTs remains unknown. The actual incidence of RTs may still be underestimated since those rare tumors are frequently misdiagnosed. However, immunohistochemistry with a monoclonal anti-hSNF5/INI1 antibody is very sensitive and highly specific for the detection of hSNF5/INI1 loss-of-function (Fig. 5b), and should now facilitate the diagnosis of most RTs.

hSNF5/INI1 biallelic genetic inactivation has also been reported in other types of ►childhood cancers such as central PNET (►Medulloblastoma, deleted in malignant brain tumors), choroid plexus carcinomas, and in a small subset of undifferentiated malignant tumors without evidence of "rhabdoid" morphological features. In adults interestingly, in which RTs are thought to be much rarer, hSNF5/INI1 complete defect has also been observed in some "proximal-type" of epithelioid sarcomas (►Proximal-type epithelioid sarcomas), and, marginally, in composite tumors with "rhabdoid" component. hSNF5/INI1 deficiency might therefore account for a wider spectrum of tumors. Nevertheless, it still remains unclear whether these different malignancies should be considered as phenotypical variants of a same biological entity. In accordance with this hypothesis, they usually share with typical RTs a highly aggressive clinical behavior.

Conclusion

There are increasing evidences of chromatin remodeling being a critical process in oncogenesis. hSNF5/INI1 offers a convincing example as a tremendously potent tumor suppressor gene. In humans, hSNF5/INI1 inactivation is strongly associated with rhabdoid tumors, but may not be as restricted to these children malignancies as previously thought. Identification of hSNF5/INI1 involvement in human cancers has given helpful diagnostic tools, based on molecular screening and specific anti-hSNF5/INI1 immunohistochemistry. Better knowledge on hSNF5/INI1 roles in oncogenesis should lead to more efficient targeted therapies, and improve the dramatically poor prognosis of hSNF5/INI1-deficient tumors.

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HSP

Definition

Heat shock protein. HSP89α, HSP90β; the HSP89α and HSP90β genes are composed of 11 and 12 exons, respectively. The regulation of HSP gene expression in eukaryotes is mediated by the conserved heat shock transcription factor (HSF). HSF acts through heat shock elements (HSEs) composed of three contiguous inverted repeats of a 5 bp sequence, nGAAnnTTCn; upon heat stress, HSF binds to HSEs as a trimer.

►Hsp 90

Hsp60

Definition

Heat shock proteins that acts as molecular chaperones in complex in bacteria and eukaryotes; the Hsp60/Hsp10 complex is important for import and folding of key proteins into mitochondria.

►Molecular Chaperones

Hsp90

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Synonyms

Heat shock protein 90

Definition

Hsp90, the 90-kDa heat shock protein, is a ►molecular chaperone that regulates the degradation, folding and/or transport of a diverse set of critical cellular regulatory proteins. Most Hsp90 clients, i.e. those proteins that require its "chaperoning" activity for appropriate function, participate in some aspect of signal transduction including a wide variety of protein kinases, hormone receptors, and transcription factors. Moreover, Hsp90 can stabilize mutated proteins allowing them to maintain normal function despite genetic abnormalities. This ability to buffer genetic changes and serve as a capacitor of phenotypic variation has implicated Hsp90 in evolutionary and oncogenic processes.

Characteristics

Hsp90 is an ATP-dependent molecular chaperone and is one of the most highly expressed proteins in eukaryotic cells. There are two major isoforms of Hsp90, the major form Hsp90α and the minor form Hsp90β. The two hsp90 genes differ primarily in their non-coding and regulatory regions. Both the α and β proteins consist of four basic structural domains. The N-terminal domain is the site of ATP binding and is essential for its chaperone function. Inhibitory agents, at least those developed to date, specifically target this site preventing ATP binding and consequently Hsp90 function. The N-terminal domain is connected to the middle domain by a small highly charged linker region that is thought to be the site for co-chaperone binding. The larger middle domain contains the site for client protein binding and the C-terminal domain provides the dimerization site, which is essential for Hsp90 activity. The Hsp90 proteins function as homodimers of α/α and β/β. Whereas there does appear to be functional differences between the isoforms with β being associated with development, there is also considerable overlap.

Although the majority of Hsp90 is located in the cytoplasm, this molecular chaperone can also be found in the nucleus, albeit at considerably lower levels, where it has been associated with the regulation of gene expression and the ►DNA damage response to radiation. In addition, Hsp90 is located on the cell surface where it plays a role in antigen processing and the immune response. Finally, recent studies have shown that Hsp90 can be secreted into the extracellular space. Although the specific function has not been clearly defined, in this location Hsp90 has been suggested to play a role in blood clotting, cell migration and tumor metastatic processes.

Mechanism

Hsp90 chaperone function is mediated by an ATP-dependent cycling between two conformations, which regulates its interactions with specific co-chaperones

and co-factors and drives the loading and off loading of client proteins. The Hsp90 dimer typically exists in a multi-protein chaperone complex that includes the co-chaperones Hsp70 (70-kDa heat-shock protein) and Hsp40 (40-kDa heat-shock protein), the adaptor protein HOP (hsp co-chaperone organizing protein) and the cofactor p23. In addition, there are specific immunophilins and other co-chaperones that regulate substrate binding. For example, the co-chaperone ►Cdc37 is involved in Hsp90-mediated stabilization of protein kinases. A client protein initially binds to the Hsp70/Hsp40 complex, which links via HOP to an ADP-bound Hsp90. Exchanging ADP with ATP alters Hsp90 confirmation such that HOP and Hsp70/Hsp40 are released and p23 and other co-chaperones such as Cdc37 are recruited to the complex. In the ATP-bound confirmation and associated with these co-chaperones Hsp90 folds and stabilizes a client protein maintaining it in a responsive conformation. Although the specific processes have not been completely defined, in the absence of the appropriate stimulus or ligand binding to the client protein, Hsp90 through its ATPase activity cycles back to its ADP-bound conformation recruiting the initial set of co-chaperones, which ultimately leads to client protein degradation. Considerable insight into the mechanism of Hsp90 chaperone activity has been generated through the use of the inhibitor ►geldanamycin. This benzoquinone ansamycin binds to the nucleotide binding site of Hsp90 resulting in a conformation that resembles the ADP-bound conformation. The inability of Hsp90 to cycle to its ATP-bound conformation then maintains the chaperone complex in a state that favors client protein degradation. Studies to date indicate that in cells treated with geldanamycin or one of its analogs the half lives of the Hsp90 client proteins are uniformly and significantly reduced.

Clinical Aspects

As a Single Modality

Hsp90 has received considerable attention as a potential target for cancer therapy. Because of an increased understanding of the mechanisms and molecules that mediate malignant transformation, recent strategies in cancer therapy have begun to focus on a target-based approach. The putative advantages of this strategy include tumor selectivity sparing normal cells and the availability of markers indicative of tumor susceptibility. Most attempts to apply target based therapy have entailed the use of agents that target a single molecule. For tumors in which their phenotype is driven by a single oncogenic molecule, such as the targeting of Bcr-Abl by ►STI571 in chronic myelogenous leukemia and ►Her2/neu by ►Herceptin is certain breast tumors, this approach has demonstrated clinical activity. However, most tumors, especially solid neoplasms, contain multiple genetic abnormalities and are subject to a high degree of genomic instability. The result is that their malignancy/survival is driven by variety of molecules existing in a number of different survival pathways. Under these circumstances, targeting a single molecule is likely to be of limited consequence. As an alternative to targeting a single molecule, inhibiting Hsp90 provides a multi-target approach to cancer treatment. Hsp90 client proteins include a large number kinases, receptors and transcription factors that have been implicated in transformation and maintenance of the malignant phenotype. Examples of such proteins dependent on Hsp90 include ErbB2 (►Her2/Neu), Src, ►Akt, ►c-Raf-1, cyclin dependent kinases Cdk4 and Cdk6, HIF1-α, estrogen and androgen receptors and hTERT. Thus, Hsp90 inhibition provides a means of simultaneously targeting multiple proteins critical to a malignant cell. In addition, Hsp90 can stabilize mutant proteins (e.g. ►p53) allowing for at least some functional activity. Given the level of genomic abnormalities and instability of tumor cells, the ability to buffer against such genetic variation suggests another avenue through which Hsp90 contributes to tumor cell survival.

In laboratory studies exposure of tumor cells to Hsp90 inhibitors such as geldanamycin and its analogs 17-allylaminogeldanamycin (17-AAG) and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) results in the combinatorial decrease in its client proteins mentioned above as well as additional client proteins, although there is some cell type specificity. With respect to a potential cancer therapy, inhibition of Hsp90 induces tumor cell death or significantly slows their proliferation in a number of in vitro and in vivo experimental systems, although the critical target or targets (i.e., client proteins) for the most part have not been defined. An additional characteristic that supports the application of Hsp90 inhibitors in cancer treatment is their relative selectivity for tumor cells over normal cells. The molecular basis for this selectivity has not been clearly defined. However, Hsp90 is typically expressed at higher levels in tumor cells than normal cells suggesting that tumors may be more dependent on its chaperoning activity. Regardless of the specific mechanisms involved, the ability of Hsp90 inhibitors to preferentially kill tumor cells over normal has led to the ongoing evaluation of 17AAG and 17DMAG in clinical trials as single modality agents.

In Combination with Radiotherapy

Hsp90 has also been identified as a determinant of tumor cell ►radiosensitivity. Among Hsp90 clients are included a number of proteins (e.g. Raf-1, Akt and ErbB2/Her2/Neu) that have been associated with protection against radiation-induced cell death; a reduction in their individual activities has been shown to result in radiosensitization in some, but not all tumor cell lines. Tumor cell radiosensitivity is regulated by a wide variety of signaling

molecules with their specific contributions often determined by cell type. Because Hsp90 inhibitors induce the simultaneous loss of a number of these molecules that can potentially affect radiosensitivity, the use of these agents allows for implementing a multi-target approach to ►radiosensitization. The putative advantages of such a multi-target strategy are increases in the degree and probability of radiosensitization. Indeed, inhibitors of Hsp90 such as geldanamycin, 17AAG, 17DMAG and radicicol have been shown to significantly enhance the radiosensitivity of a number of tumor cell lines derived from a variety of histologies. As for Hsp90 inhibitor treatment alone, the inhibitors have little to no effect on the radiosensitivity of normal cells evaluated in vitro. This lack of normal cell radiosensitization occurs despite a similar reduction in client proteins suggesting that it is not the difference between Hsp90 per se in tumor and normal cells, but the actions of its client proteins.

Mechanistic studies of the tumor cell radiosensitization induced by 17DMAG have implicated Hsp90 in two components of the DNA damage response – DNA double strand break repair and ►cell cycle checkpoint activation. The inhibition of double strand break repair could be traced to the loss of ErbB2 (Her2/neu) in 17DMAG treated cells and the consequent reduction in ErbB1 (EGFR) activity, which leads to a reduction in the ErbB1 interaction with DNA-PKcs and the subsequent attenuation of ►DNA-PK activation after irradiation. The abrogation of cell cycle checkpoint activation by 17DMAG was associated with a reduction in radiation-induced activation of ►ATM, which was the result of a reduced interaction between ►NBS1 and ATM. Whereas most studies regarding Hsp90 as a target for cancer treatment have focused on its cytoplasmic activities, these radiation studies indicate that this chaperone has a critical role within the nucleus. The contribution of Hsp90 to double strand break repair and cell cycle checkpoint activation in tumor cells suggests that its inhibition may also be of benefit in combination with chemotherapeutic agents that kill tumor cells through DNA damage.

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HSPG

Definition

Heparan sulfate proteoglycan; extracellular matrix protein containing heparan-sulfate polysaccharide chains.

►Wnt Signaling
►Proteoglycans

HSR

Definition

Homogeneously staining region.

►Amplification

HSV-TK/Ganciclovir Mediated Toxicity

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Synonyms

Suicide gene therapy; Gene directed enzyme-prodrug therapy; GDEPT

Definition

A form of ►suicide gene therapy in which the cDNA for a viral enzyme, the herpes simplex virus thymidine kinase (HSV-TK), is transferred to tumor cells followed by treatment with the antiviral drug ganciclovir (GCV). Expression of HSV-TK enables cells to phosphorylate GCV to a monophosphate derivative. Cellular enzymes convert the monophosphate to GCV triphosphate, which elicits toxicity through incorporation into DNA.

Characteristics

HSV-TK/GCV mediated killing of tumor cells, and indeed suicide gene therapy in general, has been developed as a mechanism to improve the selectivity of cancer chemotherapy. Since traditional antitumor drugs target rapidly dividing tissues, such as tumor cells, they also can destroy normal dividing host tissue

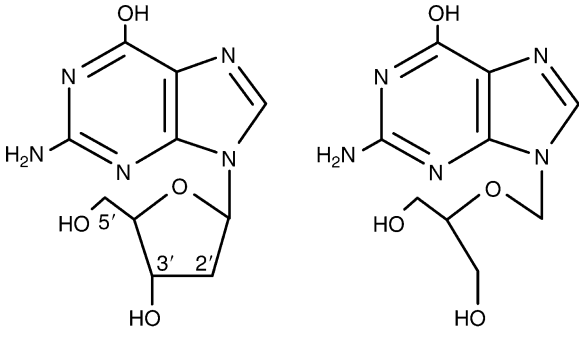
such as cells in the bone marrow, gastrointestinal tract, or hair follicles. Normal tissue toxicity is the major impediment to traditional chemotherapy. With HSV-TK/GCV therapy, a foreign gene (encoding HSV-TK) which can activate a normally innocuous ►prodrug (GCV) to a toxic product is transferred selectively to the tumor. When the patient is treated with the prodrug, only the tumor cells expressing the foreign gene will be affected (thus the designation of "suicide" gene therapy); since normal host tissues cannot activate the prodrug, they are spared from toxicity.

Toxicity to HSV-TK-Expressing Cells

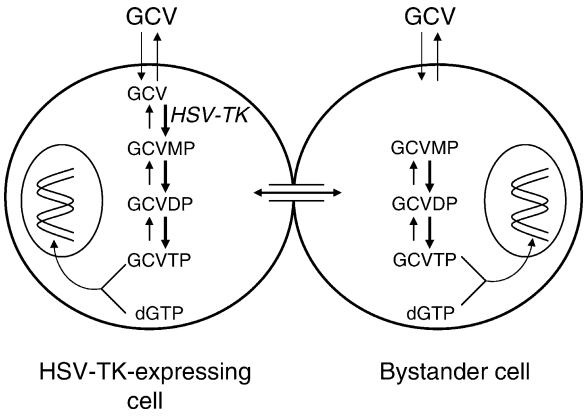
GCV is an acyclic analog of 2′-deoxyguanosine that requires phosphorylation for biologic activity (Fig. 1). It was originally discovered as an antiherpesvirus agent, and it is used clinically in the treatment of cytomegalovirus infection. When herpes simplex virus infects human cells, a number of proteins are expressed from the viral genome to facilitate virus replication and spread. One of these proteins, HSV-TK, contrasts with mammalian thymidine kinase in that it can phosphorylate purine as well as pyrimidine nucleoside substrates and their analogs. GCV serves as a substrate for HSV-TK with a Km value of ~50 μM, but it is not an efficient substrate for any of the mammalian nucleoside kinases thus accounting for its selectivity in herpesvirus-infected cells. Following subsequent phosphorylation by mammalian kinases to its triphosphate metabolite, the drug is incorporated into the viral DNA leading to cessation of replication. Based on this mechanism of selectivity, it was proposed that tumor cells genetically engineered to express HSV-TK would be killed when treated with GCV. Proof of this principle was first shown in murine sarcoma cells, and since then numerous reports have demonstrated similar results in many different cell types.

At the triphosphate level, GCV can compete with the endogenous 2′-deoxyguanosine 5′-triphosphate (dGTP) for incorporation into DNA by mammalian DNA polymerases. Since GCV has the equivalent of both the 5′ and 3′ hydroxyls of deoxyguanosine, it can be incorporated into DNA and allow further extension of the DNA strand (internucleotide addition). In contrast, the structurally related ►acyclovir lacks the equivalent of a 3′ hydroxyl group, and thus is an obligate DNA chain terminator. GCV and its metabolites do not interfere with RNA or protein synthesis. The incorporation of GCV monophosphate into DNA is the primary lesion that results in cell death. For some cell types, GCV induces cell death through ►apoptosis (Fig. 2).

Compared to other substrates for HSV-TK, such as acyclovir, GCV is significantly more toxic and more mutagenic to cells that express HSV-TK. The action of acyclovir is primarily ►cytostatic, whereas GCV induces cell killing at low, clinically achievable concentrations. Although GCV triphosphate accumulates in cells to a relatively low level of 10–20 μM, this is sufficient to produce several logs of cell death. This high toxicity may be attributable to the avid incorporation and lengthy retention of GCV into DNA. GCV triphosphate and its incorporation into the nascent DNA strand do not produce strong inhibition of DNA synthesis, so cells incorporate high levels of this drug, complete DNA replication and go on to divide. Daughter cells become irreversibly blocked when they enter S-phase, suggesting that GCV monophosphate cannot serve as a template for DNA replication. A



HSV-TK/Ganciclovir Mediated Toxicity. Figure 1 Structure of GCV.



HSV-TK/Ganciclovir Mediated Toxicity. Figure 2 Mechanism of Cytotoxicity for GCV in HSV-TK-Expressing Cells and Bystander Cells. GCV is selectively phosphorylated to the monophosphate in HSV-TK expressing cells. Further phosphorylation can be accomplished by cellular enzymes. GCV triphosphate competes with the endogenous dGTP for incorporation into DNA, leading to cell death. GCV at the mono-, di- or triphosphate level can be transferred directly to bystander (non-HSV-TK-expressing) cells via GJIC channels, resulting in its incorporation into DNA of bystander cells with subsequent cytotoxicity.

strong G2/M block has also been observed in some cell types after GCV treatment. The cell cycle position in which cells become blocked may depend on the concentration of GCV.

Toxicity to Non-HSV-TK-Expressing Cells (►Bystander Effect)

With current gene transfer technologies, only a small percentage of tumor cells will express the foreign gene. For this approach to be successful in cancer treatment in patients, there must be a mechanism by which cells that do not express the ►transgene (bystander cells) can be killed. It was noted early on that when only a fraction of the cell population expressed HSV-TK, treatment with GCV resulted in killing of both the HSV-TK-expressing and HSV-TK-nonexpressing bystander cells. The strong cell killing ability of GCV in HSV-TK-expressing and neighboring bystander cells has resulted in complete regressions of experimental tumors in animals, spurring clinical interest in this approach.

In suicide gene therapy, bystander cell killing generally occurs through the transfer of a toxic metabolite, produced in the transgene-expressing cell, to bystander cells. The bystander cell killing with HSV-TK/GCV was an unexpected finding since the toxic metabolite, GCV triphosphate, is negatively charged and therefore would not readily pass through cell membranes to kill bystander cells. However, GCV mono-, di- and triphosphate accumulate in bystander cells when co-cultured with HSV-TK-expressing cells and GCV. The primary mechanism that appears to account for transfer of GCV phosphates is ►gap junctional intercellular communication (GJIC). GJIC allows the direct exchange of small molecules (<1,000 D) between neighboring cells. With a molecular weight ranging from ~350–510 D, GCV mono-, di- or triphosphate could potentially be transferred between cells through gap junctions. Tumor cell lines show varying levels of GJIC activity, in which some are totally devoid of GJIC whereas others show nearly 100% communication between cells, yet transfer of GCV phosphates can occur even between low GJIC-expressing cells. Increasing the GJIC activity of tumor cells increases the transfer of toxic GCV metabolites to bystander cells thus enhancing bystander cell killing, however this lower the amount of GCV phosphates in the HSV-TK-expressing cells with a concomitant reduction in cell killing.

Characteristics in Vivo

Experimental Tumors in Animals

Human tumor xenografts that stably express HSV-TK exhibit strong tumor growth delay and complete regressions after treatment with GCV. However, this is an idealized system in which all cells can activate GCV, whereas engineering an existing tumor to express HSV-TK is an inefficient process even when mediated by a viral vector (retrovirus, adenovirus, herpes simplex virus, adeno-associated virus, lentivirus) to transfer the cDNA for the transgene. Although viral ►transduction of tumors *in vivo* represents the best of our current gene transfer approaches, the percentage of cells that express the transgene is typically less than 10%. Nevertheless, *in vivo* transduction of tumors in animals has produced excellent tumor growth delay with some complete regressions, suggesting an impressive bystander effect *in vivo*. These successful animal studies fueled in large part the clinical interest in HSV-TK/GCV, and to date it is one of the most commonly used gene therapy approaches in cancer treatment.

Mechanism of the Bystander Effect in Vivo

With demonstration of transfer of phosphorylated GCV between HSV-TK-expressing and non-expressing cells *in vitro* as an important component of the bystander effect, it was expected that a similar mechanism would explain the bystander effect noted *in vivo*. While this may explain a portion of the bystander cell killing in experimental tumors, there is ample evidence implicating an immune component as well. Tumors in immune-competent animals usually exhibit more regressions than the same tumor in immune-deficient animals, and following tumor regression immune-competent animals reject the same tumor upon rechallenge. Further evidence of the involvement of the immune system is shown by the ability of GCV treatment to regress two separate tumors in an animal when only one expresses HSV-TK. It has been postulated that the dying tumor cells expressing HSV-TK are immunogenic, mediating the antitumor response. The assistance of the immune system *in vivo* in promoting tumor regression is a powerful addition to the HSV-TK/GCV approach.

Clinical Relevance

Since the initial reports of tumor regression in animals treated with HSV-TK/GCV, many clinical trials utilizing this approach have been initiated. Based on reports of brain tumor regressions in experimental tumors in animals, glioblastoma was targeted initially for HSV-TK/GCV treatment in clinical trials. Other trials have focused on treatment of ovarian cancer and prostate cancer with HSV-TK/GCV. Recently HSV-TK/GCV has been evaluated in the treatment of graft vs. host disease in allogeneic bone marrow transplant recipients. These trials have employed primarily retrovirus or adenovirus vectors to transduce tumor cells to express HSV-TK, and the results have shown that these vectors are safe to use in humans.

While HSV-TK/GCV has been well tolerated in patients, Phase I/II clinical trials have not demonstrated strong antitumor activity with this approach. There are

several possible reasons that the results have not been more dramatic. First, most patients in these trials had been previously treated with surgery, chemotherapy and/or radiation therapy, so these trials were treating tumors that were already resistant to initial therapy and therefore less likely to respond to the gene therapy. Second, despite the administration of high numbers of virus particles and multiple administrations of the vector in some trials, it is likely that less than 1% of the cells in the tumors expressed HSV-TK, and thus tumor eradication relied almost entirely on the bystander effect. Recently, promising results have been reported in clinical trials in men with prostate cancer, in which the combination of HSV-TK/GCV with another suicide gene therapy, cytosine deaminase to activate 5-flucytosine to the anticancer drug 5-fluorouracil, produced evidence of tumor control. Considering that cancer is usually treated with more than one drug, it is reasonable to expect that the optimal use of HSV-TK/GCV will be in combination with other agents and modalities.

Improving Cancer Therapy with HSV-TK/GCV

In view of the low efficiency of gene transfer for current methodologies, the major limitation to the therapeutic efficacy of HSV-TK/GCV is the inability to express the transgene in a larger percentage of the tumor. Improved vector delivery systems are needed to enhance tumor transduction. Replication competent adenovirus vectors may increase transduction somewhat, although they have only transient expression. Newer approaches include nonviral methods, such as nanoparticles, to deliver the transgene. Structural modifications to HSV-TK that improve affinity for GCV have been shown to be efficacious in preclinical models and are undergoing clinical study, and this approach will be most useful in combination with superior transduction methods.

One aspect of this treatment that has not received much attention is optimizing the administration of GCV. The current dosing regimen is based on that used for treating patients with herpes virus infections. Given that most current gene transfer approaches produce only transient expression of HSV-TK, increasing dose intensity to match the duration of expression of the transgene may improve therapy. ►Pharmacodynamic studies are needed to determine the optimal dose and schedule that will maximize the activation of GCV in tumors expressing HSV-TK.

With the current low efficiency of gene transfer regardless of the vector used, methods to improve bystander cell killing could make a major impact. Preclinical models have shown that increasing GJIC through gene transfer of cDNAs for ►connexins can improve bystander killing. However, to increase bystander killing by this mechanism requires connexin expression in a large percentage of both the bystander and HSV-TK-expressing cells, and thus with the low level of gene transfer by current methodologies this may not impact tumor therapy significantly. Recent reports demonstrate that the addition of inhibitors of ►ribonucleotide reductase, such as hydroxyurea or gemcitabine, to HSV-TK/GCV regimens can synergistically increase bystander cell killing both *in vitro* and *in vivo* in animal models. This appears to work through increasing the GCV triphosphate:dGTP value resulting in greater GCV incorporation into DNA. Pharmacologic modulation to increase bystander cell killing would be expected to make a more dramatic improvement in efficacy than methods requiring gene transfer, and merit testing in clinical trials.

Suicide gene therapy in cancer treatment is still in its infancy. Although the excellent responses in preclinical models have not been realized yet in clinical studies, promising evidence of efficacy is emerging, particularly in the treatment of prostate cancer. In addition, new technologies and approaches that enhance response in preclinical systems may have clinical impact. Improving gene transfer, optimizing drug administration regimens, increasing bystander cell killing through pharmacologic means, and combining HSV-TK/GCV with other therapies may translate to greater clinical efficacy with this novel approach to cancer treatment.

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hTERT

Definition

Human telomerase reverse transcriptase (hTERT) is a catalytic subunit of telomerase.

►Telomerase

HTLV-1

Definition

►Human T-cell leukemia virus-1 is the first human ►retrovirus that was discovered by American and Japanese scientist in the early 1980s. HTLV-1 infection is associated with the clonal expansion and transformation of T lymphocytes which lead to adult T-cell leukemia (ATL), an aggressive hematologic malignancy.

►Mitotic Arrest-Deficient Protein 1 (MAD1)
►T-cell Leukemia/Lymphoma 1 (TCL1)

HUCE

Definition

Human capillary ►endothelial cells.

Human Cancer Epidemiology

Definition

Study of the distribution and, in some instances, the causal factors of cancer in communities and populations.

►Toxicological Carcinogenesis

Human Cartilage Glycoprotein-39

►Serum Biomarkers

Human ESP1-associated Protein 1

►Securin

Human Herpes Virus 4

►Epstein-Barr Virus

Human Herpesvirus 6

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Definition

The family Herpesviridae is comprised of viruses characterized by a distinct morphology. Virion particles are enveloped and have a diameter of ~200 nm. The icosahedral capsid contains a double stranded linear DNA molecule with a molecular weight of 80–150 million daltons.

Characteristics

According to their biologic and molecular properties, ►herpesviruses are classified in three subfamilies (alphaherpesvirinae, betaherpesvirinae, gammaherpesvirinae). Humans are the natural host for eight herpesviruses: herpes simplex 1 (HSV-1), herpes simplex 2 (HSV-2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), ►Epstein Barr virus (EBV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8), also known as ►Kaposi sarcoma associated herpesvirus.

All herpesviruses have the important biologic characteristic of establishing life-long latent infections within specific cells of the host after primary infection. Latent virus may occasionally reactivate and produce recurrent infections. Both animal and human herpesviruses have been implicated in the etiology of animal or human neoplasms. The human herpesviruses with oncogenic potential include EBV and HHV-8. In addition, a potential oncogenic role has been suggested also for HHV-6, but no firm association has been established.

HHV-6 is a betaherpesvirinae encoding ~100 genes and has a selective tropism for T-lymphocytes and macrophages. Viral strains can differ in biological properties such as pathogenic potential, in vitro cell tropism, reactivity with monoclonal antibodies, and are consequently divided in two variants: HHV-6A and HHV-6B. Infection with HHV-6, usually acquired in early infancy, is highly prevalent in humans. The virus

causes Exanthem Subitum, a benign childhood disease, but primary infection can remain asymptomatic or result in febrile episodes without relevant symptoms. As a result of primary infection the virus establishes latent infection and resides silently in T-lymphocytes and in macrophages. In fact, small amounts of viral DNA are commonly detected in peripheral blood mononuclear cells of healthy adults, in the absence of viral replication. Occasionally, the virus may reactivate, especially in immunocompromised individuals (HIV-infected, transplant recipients). HHV-6 reactivation has been linked to disease especially in bone marrow transplant recipients. HHV-6 is present also in salivary glands and may be shed with saliva.

Clinical Aspects

A possible role of HHV-6 in human oncogenesis has been suggested since its original isolation from patients with B- and T- cell lymphoproliferative diseases. Subsequently, HHV-6 DNA sequences have been detected in a variable number of non-Hodgkin lymphomas, in sporadic cases of leukemia, angioimmunoblastic lymphoadenopathy, cerebral lymphoma and also in neuroglial tumors, oral carcinoma, cervical carcinoma. However, an etiological role of the virus in these cases is not likely, whereby only a small number of cells are infected by HHV-6, even in the case of clonal lymphoproliferations. The stronger potential association is with ►Hodgkin disease. Viral sequences are present in about 30% of cases and viral antigens can be detected in Reed-Sternberg cells, which appear to be the neoplastic element in this non-clonal lymphoproliferative disease. Furthermore, HHV-6 can reactivate EBV and enhance its gene expression. This synergistic interaction could be significant in the development of EBV-associated oncogenesis. However, the ubiquitous nature of the virus, the persistance of latent infection in virtually all healthy adults and the ability to integrate in the host cell chromosomal DNA complicate the interpretation of viral footprint in neoplastic lesions. HHV-6 is detected in some neoplastic lesions of the uterine cervix and virus infection enhances the tumorigenic potential of human papillomavirus. However, current evidence suggests that a role of HHV-6 in cervical oncogenesis is unlikely.

The tumorigenic role of HHV-6 is supported by in vitro studies that show a transforming potential on murine and human cells. Three different fragments of viral DNA immortalize primary cells or morphologically transform continuous cell lines. These cells injected in nude mice develop tumors. The protein encoded by the viral gene DR7, encoded by one of the transforming fragments, has the ability of transactivating heterologous promoters and binds to the antioncogene p53, altering its ability to regulate genes important in growth control.

In conclusion, the etiologic role of HHV-6 in human neoplasias is still undefined. The observation that only a small proportion of cells shows footprints of viral infection, even in neoplasias with a clonal origin, suggests the possibility that the virus might simply be a passenger in the tumor tissues. On the other hand, the detection of viral sequences in lymphoproliferative diseases and the in vitro transforming potential of selected fragments of viral DNA support the hypothesis that the virus might be involved in the oncogenetic process, at least in a fraction of cases.

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Human Immunodeficiency Virus

Definition

►HIV; The virus that causes AIDS.

Human Kallikrein 3

►PSA

Human Lymphocyte Antigen

Definition

The HLA system is also referred to as the human major histocompatibility complex (►MHC). HLA expression on cells defines “self” and protects the individual from

an autologous natural killer cell attack; HLA antigens are genetically defined and display an enormous diversity.

►Natural Killer Cells
►Activated Natural Killer Cells

Human Non Sucrose Fermenting 5

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

Human Papillomaviruses

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Synonyms

Human papillomavirus; HPV; Human wart virus

Definition

Human papillomaviruses (HPV) are members of a heterogenous group of viruses; to date, there are more than 100 different viruses (HPV 1, 2, etc.) identified by DNA sequence analysis (genotypes). Papillomaviruses of a number of animal species have been found (e.g., bovine papillomavirus, canine oral papillomavirus). With very few exceptions, papillomaviruses infect only their natural hosts.

Characteristics

Virus Particle

Human papillomavirus consists of an icosahedral virus capsid (without a lipid-containing envelope) of 55 nm in diameter that is built up of 360 copies of the major structural protein (L1) and 12 copies of the minor capsid protein (L2). Enclosed in the protein shell is one molecule of a circular double-stranded DNA of about 8,000 bp, which constitutes ~20% of the virus particle by weight. In addition to the genes L1 and L2 coding for the structural proteins, the genome contains also 6–8 "early" genes. These code for "nonstructural" proteins required for the regulation of viral gene expression (E2), for DNA replication (E1, E2, E6, E7), and virus maturation (E4). Transcription of the entire genome proceeds in one direction, i.e., only one strand contains the coding information. The major promoters are located in a 1 kb region ("upstream regulatory region"; URR), which contains the origin of replication.

Virus Replication

Papillomaviruses infect basal cells of stratified epithelia. Binding to and uptake into target cells appears to be mediated by a specific receptor(s), although the precise mechanism is not known. Low levels of DNA replication occur within basal cells, which appears to be necessary for persistence of papillomavirus infection. In suprabasal cells, vegetative DNA replication is initiated. By the aid of the viral proteins E6 and E7, the cells of the stratum spinosum that have entered the program of epithelial differentiation reinitiate the S-phase of the cell cycle. This switch provides the virus with the cellular machinery that is required for its own DNA replication. The E7 protein was found to bind to the hypophosphorylated form of the retinoblastoma (►Retinoblastoma protein, cellular biochemistry) protein (pRB), a critical molecule in the control of the G1/S boundary during the cell cycle. As a consequence, transcription factors of the E2F family are released from a complex with pRB and trigger expression of genes that are necessary for DNA replication and cell division, such as Myb and cyclin A and cyclin E. There is evidence that E7 can also override cell cycle control at the G2/M checkpoint via interaction with and functional inactivation of inhibitors of cyclin-dependent kinases (p21Waf1/Cip1 and ►p27). Additional effects of the E7 have been described that may lead to cell proliferation, such as binding to members of the ►AP-1 transcription factor family.

The papillomavirus protein E6 binds to the product of the tumor suppressor gene p53, leading to its ►ubiquitination and rapid degradation. This gene, which by deletion or mutation is involved in more than 50% of human cancers, is crucial for cell cycle control, like the members of the RB family. Activated in response to stress (e.g., by DNA damage), the p53 protein leads to cell cycle arrest and eventually to ►apoptosis. It is assumed that one of the functions of the E6 protein is to counteract the side effect of E7-dependent hyperproliferation, i.e., p53-induced apoptosis. Additional cellular proteins were found to be targeted by E6, e.g., ►telomerase, ERC-55 (a putative calcium binding protein), paxillin (a cytoskeleton protein), and the interferon regulatory factor-3 (IRF-3). The consequences of such interactions are still unknown, but in the latter instance it is tempting to speculate that the E6 protein interferes with activation of interferon (as an antiviral principle) as the transcriptional activity of IRF-3 is inhibited after binding. The interaction between the E6 and E7 and cellular proteins was most intensively studied in case of the HPV type 16, which is the prototype of a cancer-associated papillomavirus. It is

unclear, however, whether the role of the E6 and E7 proteins in virus replication are comparable between different HPV types. The interaction of these proteins with cellular proteins could not be demonstrated for certain HPV types.

Although it was observed that papillomavirus particles bind to a variety of different cells in culture, there is no evidence for persistence or replication of papillomaviruses other than in skin or mucosa. The conditions of differentiating epithelia are very difficult to reconstitute in cell culture, thus the propagation of papillomaviruses in experimental systems was impossible until recently. By the aid of ►organotypic cultures established from human keratinocytes, the functions of papilloma virus genes can be studied. This system, however, is still insufficient to generate papillomavirus progeny on a preparative scale. Thus the molecular analysis of papillomavirus mostly depends on the molecular cloning of the DNA of the viral genome. Recent progress has been made by characterization of virus-like particles (VLP) that are generated by expression of the major structural protein L1 (with or without L2) in recombinant vectors (e.g. baculovirus or yeast). VLP were also used to study early steps of the virus life cycle.

Pathogenesis of Papillomavirus Infection

Papillomaviruses are mostly transmitted by direct skin contact (e.g., during sexual intercourse), but also infection via contaminated objects has been reported. Replication of HPV induces benign proliferations (skin warts, genital warts, laryngeal papillomas) within the affected skin or mucosa. Depending upon the site of infection and the condition of the patient (e.g., age), such lesions often regress spontaneously. Malignant progression of HPV-induced lesions is uncommon and depends upon the site of infection and on the HPV type. Despite the plethora of HPV types, there is a striking association of certain types to particular disease (e.g., HPV 1–4 in skin warts, HPV 6 and HPV 11 in genital warts and laryngeal papillomas).

Papillomavirus infection of the genital tract is very common. It is detected in women and in males at high frequency (in 5–50% of women depending on age and sexual behavior). Those infections remain frequently subclinical and are diagnosed only by detection of viral DNA in cervical swabs. Clinical manifestations of genital papillomavirus infections are genital warts (condylomata acuminata) or intraepithelial neoplasias of the uterine cervix (CIN), vulva (VIN), penis (PIN), or the perianal region (AIN). CIN is considered a precursor to cervical cancer. However, the risk for malignant progression varies greatly (1–30%) depending upon the degree of severity defined as CIN I-III, which have been recently reclassified as low grade (CIN I) and high grade (CIN II + III). The second important parameter is the presence of particular HPV types. Thus papillomaviruses infecting the genital tract are classified as low-risk types (e.g., HPV 6, 11) and high-risk types (e.g., HPV16, 18, 31, 45).

Bioactivity

The role of certain (high-risk) HPV types in the development of anogenital cancers, most notably tumors of the uterine cervix, has been demonstrated by a large number of epidemiological studies and experimental observations.

1. Viral DNA of 14 different HPV types (most frequently HPV 16, followed by HPV 18, 45, 31) was found in more than 99% of large numbers of cervical cancer biopsies analyzed in worldwide studies. Also, cells established from cervical cancer and kept in culture for many years as cell lines, such as the HeLa line, contain HPV genomes; virus-specific mRNA and some proteins can be detected in cell lines and in biopsies, although fewer samples have been analyzed for gene expression.
2. An infectious etiology of cervical cancer has been suspected for many decades; recently the risk factors for cervical cancer and infection by high-risk HPV types (e.g., number of sexual partners) were shown to be identical.
3. Follow-up studies demonstrated that there is no development of high-grade CIN (the end-point of the follow-up) without the persistence of high-risk HPV.
4. CIN-like changes can be induced experimentally in organotypic cultures of human keratinocytes and in the epithelia of ►transgenic mice expressing the E6 and E7 proteins.
5. Cells of rodent and human origin can be transformed in culture by the expression of the E6 and E7 genes. Following inoculation, such cells grow as malignant tumors in small laboratory animals. The E6 and E7 proteins are required not only during the initial steps of transformation but also for the maintenance of the transformed state of the cell. Suppression of gene expression (by as yet undefined host factors or by introduction of an E6/E7-specific ►antisense nucleic acid) leads to reduction of proliferation or even to loss of the malignant phenotype. The constant expression of the E6 and E7 proteins within the infected epithelium favors the accumulation of mutations in the cellular genome leading to the activation of oncogenes or inactivation of negatively regulating factors and ultimately to the development of cancer.

Despite the causative role of papillomaviruses, it is obvious that only a minority of infected women (1–5%, depending upon geographic region) develop cervical

cancer, which with few exceptions occurs at least two to three decades after primary infection. Cervical cancer is several-fold more prevalent in developing countries than in affluent societies, whereas the prevalence of HPV infection is comparable across societies. Hence it is likely that other influences such as personal hygiene and sexual infections (e.g., chlamydia) may act as cofactors. Besides the recently developed HPV-specific prophylactic vaccines the regular attendance in cervical cancer screening programs (not available in poor resource countries) that are based on the detection of abnormal cells obtained from cervical swabs (►Papanicolaou test) is the most effective protection against the disease. Other widely used cofactors such as smoking or use of oral contraceptives seem to be of minor influence.

HPV may play a role in the development of other cancer types as well (e.g., some cancers of the respiratory tract) but the evidence is less complete. Non-melanoma skin cancer is another candidate for being associated with papillomavirus infection, but the types that may be involved (e.g., HPV 20) are as yet only scarcely characterized.

Clinical Relevance

Papillomavirus infections, at least in their initial stages, are usually unrecognized by the immune system. In terms of expression of cytokines and the presence of immune cells, there is no difference between a wart and normal skin. However, based on animal experiments and by studying the natural history of papillomavirus infections, it was concluded that the immune system plays an important role both in preventing papillomavirus infections and in eliminating the clinical manifestations of infections (regression of warts). For instance, patients suffering from immunosuppression (e.g., by receiving immunosuppressive drugs after renal transplantation) have an increased prevalence of HPV-associated disease. It is not clear which event triggers HPV-specific immune responses during the natural courses of infection and why eventually such immune responses are ineffective (cervical cancer occurs in immunological normal women).

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Human Pituitary Tumor-transforming Gene 1

►Securin

Human Podoplanin

►Podoplanin

Human Symbols for Snail1 and Snail2: SNAI1,SNAI2

►Snail Transcription Factors

Human T-cell Leukemia Virus

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Synonym

Human T-lymphotropic virus (HTLV)

Definition

The human T-lymphotropic virus (HTLV), also known as human T-cell leukemia virus, was first identified in the early 1980s and is associated with adult T cell leukemia (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neurologic condition. Two related but distinct types of HTLV exist; HTLV-I and HTLV-II. HTLV-I is somewhat more common worldwide and is the only type associated with ATL. Both HTLV-I and HTLV-II have been linked to HAM/TSP (►T-cell leukemia/lymphoma 1 (TCL1)).

Characteristics

►Retroviruses are 100 nm, lipid-enveloped RNA viruses. HTLV is a deltaretrovirus with a 9 kb genome.

The genome consists of two identical RNA molecules located within the virus capsid. The viral genes include the *gag* (core region), *pol* (polymerase), *env* (the envelope) and certain other genetic regions that produce proteins associated with transformation or pathogenesis (e.g., X region). The X region encodes the viral proteins Tax and Rex that are involved in up-regulating virus expression. Both HTLV-I and II infect CD4+ lymphocytes; however, HTLV-II appears to preferentially infect CD8+ T cells. Although lymphocyte subsets are not altered to the same extent as in HIV infection, certain studies have demonstrated that HTLV-II, and to a lesser degree HTLV-I, are associated with an increased incidence of pneumonia, bronchitis and urinary tract infections. These conditions may reflect an immunodeficiency resulting from direct infection of CD8+ lymphocytes, the induction of CD4+ CD25+ T regulatory cells that suppress the immune response, or from inflammatory responses to HTLV-II in these organ systems.

HTLVs are spread by blood, genital secretions and by mother to child transmission often via maternal milk. This transmission appears to be primarily by cell to cell contact since low progeny production occurs with very little virus release from the infected cell. In this regard, HTLV particles are not generally observed in biopsies from ATL or TSP patients in vivo but the virus can be induced to replicate in cell culture. Instead, the HTLV-I provirus is propagated by lymphocyte division, resulting in oligoclonal infection with the occasional emergence of monoclonal proliferation. This latter event can result in frank leukemia.

Currently around 20 million individuals are infected worldwide with HTLV-I. This virus is most prevalent in southwestern Japan, the Caribbean, parts of South America and in central Africa. HTLV-II is endemic among Indian tribes in North, Central and South America and in central African pygmies. Within the last 30–50 years a low level epidemic of HTLV-II has affected injection drug users and their sexual partners in the United States and Europe.

Leukemia

Clinical Presentation

ATL occurs in 2–4%of HTLV-I-infected persons and takes up to 30 years to develop. Infection in childhood is thought to predispose to the development of adult T cell leukemia (ATL), since the mothers of ATL cases are more likely to be HTLV-I seropositive than the mothers of healthy HTLV-I carriers. ATL can be distinguished from other human T cell leukemias by its occurrence predominantly in adulthood with an acute course, the morphologic properties of the leukemic cells (indented and lobulated nuclei), the generalized lymphadenopathy with an absence of mediastinal lymph node involvement, frequent lytic changes in the bone associated with hypercalcemia and in some cases infiltration of the skin. Chemotherapy can reduce the tumor mass but long-term survival has not been achieved. Hypercalcemia can be managed by standard protocols. There have been case reports of hairy cell leukemia, large granular cell leukemia and CD8+ cutaneous lymphomas occurring in HTLV-II infected persons, with the latter found particularly in whose with concomitant HIV infection. However in contrast to HTLV-I, an etiological role for HTLV-II in hematologic malignancy has not been shown in epidemiologic studies.

Pathology

HTLV, by infecting CD4+ helper T cells, establishes a chronic infection that over time develops into leukemia. The glucose transporter 1 (GLUT-1) protein is most likely the receptor for HTLV-1 and is expressed on the surface of many different cells. This finding can explain the ability of HTLV to infect a wide variety of human cells. The mechanism for cell transformation is not fully explained but appears to be related to expression of the X region viral proteins particularly Tax. This transcriptional transactivator protein enhances the activity of cellular transcription factors and activates various promoters including NFkB. Tax can also block the activity of the tumor suppressor protein p53 and inhibit apoptosis. These effects result in immortalization of cells in culture and eventual tumor development. Some investigators believe that the production of the lymphocyte growth factor interleukin-2 (IL-2) drives the CD4+ lymphocytes to proliferate, particularly since there is an up-regulation of IL-2 production and expression of the IL-2 receptor on the infected cell surface. The leukemic cells, once proliferating, spread through the body and can induce a variety of syndromes including bone lesions due to osteoclastic activity. Hypercalcemia is frequently found in patients with acute ATL in association with an increased number of osteoclasts possibly induced through cytokine effects on hematopoietic precursor cells. ATL differs from cutaneous T cell leukemia by the absence of leukemic cell infiltration of the epidermis despite presence of tumor cells in the dermis and subcutaneous tissue. Moreover, the bone marrow and lungs are usually not involved in ATL. ATL treatment includes IFN-α and zidovudine, as well as inhibitors of NFkB activity, but the median survival with ATL is about a year even with therapy.

Neurologic Disease

Clinical Presentation

Cases of tropical spastic paraparesis (TSP) have occurred in the Caribbean (e.g., Jamaica, Dominican Republic, Martinique, Trinidad) in Latin America, Africa and India. In Japan, a similar disease, called HTLV-associated myelopathy (HAM), is frequently diagnosed in HTLV-I endemic areas. This neurologic

disease occurs in up to two percent of infected individuals, most commonly between ages 35 and 45; it is observed more often in females than in males. This finding can reflect either sexually acquired infection or a female predilection for the immunologic basis for its pathology. A neurologic syndrome identical to TSP/HAM is found in HTLV-II infected persons, although the occurrence of neurologic disease may be less common than with HTLV-I. When first observed, TSP may suggest transverse myelopathy or multiple sclerosis. TSP can occur rapidly (within 3–4 months after blood-borne HTLV-I infection) but generally also takes several years to develop in infected people. Symptoms include bilateral weakness of the legs, back pain, and loss of vibration sensation in the feet. Bladder and bowel dysfunction is common. Mental capacity is usually normal and examination of the cerebral spinal fluid does not usually reveal abnormal findings, although tests for HTLV antibody and provirus are often positive.

Pathology

Because of the very high titer of antibody to HTLV-I and the presence of lymphocytes within the central nervous system, TSP has been considered to result from an autoimmune-like response of the host. The neuropathology can be caused by inflammation of nerve roots from HTLV-1 infected cells infiltrating the spinal cord, or by direct infection of glial cells by HTLV-1. Cellular and cytokine-mediated inflammatory responses directed at HTLV-I initiate the damage to neural tissue, followed by gliosis in advanced cases. Treatment with corticosteroids has shown some improvement but generally TSP has a slow unremitting course.

►Cancer, cancer causes and control, transduction of oncogenes, virology

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Human T-lymphotropic Virus (HTLV)

►Human T-cell Leukemia Virus

Human Umbilical Vein Endothelial Cells

Definition

HUVECs; Primary human endothelial cells isolated from the umbilical cord. HUVECs are used to study ►angiogenesis.

Human Wart Virus

►Human Papillomaviruses

Humanized Antibodies

Definition

Hybrid immunoglobulins in which the murine residues that conform to specific complementarity determining regions and others of possible structural relevance are “transplanted” to a human antiBody framework. From here, the corresponding regions and residues have been eliminated to try to abrogate MAMA and gain human effector functions. *Applications:* AntiBody therapy when effector and other Fc-associated functions and properties are needed.

►Diabody

HUMARA

►Androgen Receptor

Humoral Immune Response

Definition

Humoral refers to the extracellular fluid, such as serum, plasma, lymph, etc, and the humoral immune response is synonym of an antibody-mediated immune response

►Autoantibodies

Humoral Immunity

Definition

Is the antibody-mediated specific immunity made in a humoral immune response. Humoral immunity can be transferred to unimmunized recipients by using immune serum containing specific antibody.

Huntingtin Interacting Protein 1 (HIP1)

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Definition

►HIP1 is the first example of an endocytic adapter protein that is altered in many human cancers. HIP1 represents a novel type of oncoprotein that hijacks ►endocytosis to increase signaling from multiple receptors in parallel to transform normal cells into cancer cells.

Characteristics

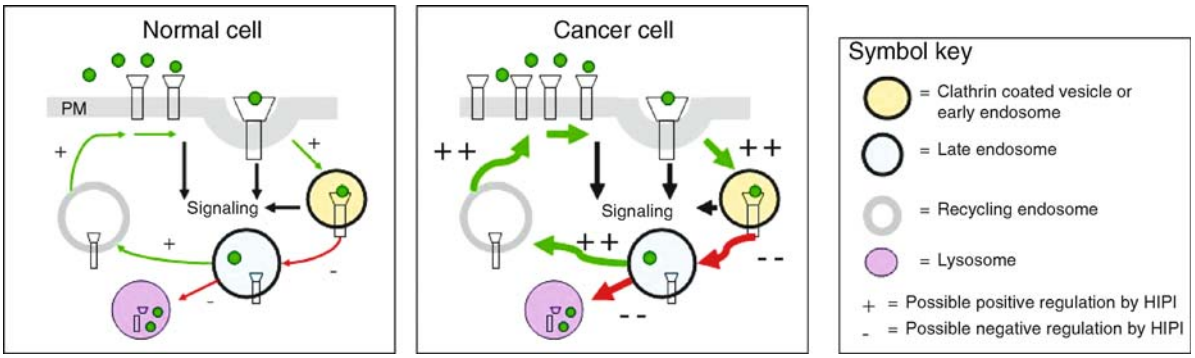
HIP1.

HIP1 is a 116 kDa cytosolic protein that was originally identified to interact with huntingtin, the protein whose gene is mutated in Huntington disease. HIP1 and its only known mammalian relative, HIP1-related (►HIP1r), contain ►clathrin-binding domains and carboxyl terminal ►actin-binding TALIN homology domains. HIP1 and HIP1r also contain amino terminal epsin/AP180 N-terminal homology (E/►ANTH) domains. These domains bind specific inositol lipids and have been shown to be important in epsin- and AP180-mediated modulation of the growth factor receptor uptake phase of clathrin mediated endocytosis.

HIP1's Original Link to Cancer. HIP1 was cloned independently after it was identified it as part of a t(5;7) ►chromosomal translocation in a patient with myeloid leukemia. The t(5;7) breakpoint encoded a fusion protein between HIP1 and PDGFßR, designated HIP1/PDGßR. The HIP1/PDGFßR fusion protein oligomerizes, has constitutive kinase activity, and transforms hematopoietic cells to IL-3-independent growth. Mutation analysis of HIP1/PDGßR indicated that constitutive kinase activity was not sufficient for transformation. Rather, transformation required HIP1 functions aside from the ability to oligomerize with the fusion protein. The fact that HIP1 sequences independent of the oligomerizing domain(s) were necessary for transformation was the first indication that over-expression of HIP1 alone may contribute to tumorigenesis through novel mechanisms. HIP1 is a specific marker for a variety of other cancer types and is associated with a negative prognosis (►Autoimmunity and prognosis) in prostate cancer.

How Might Hip1 and Hip1r Use Clathrin-Mediated Trafficking to Promote Cancer? Both structural (lipid-, clathrin-, and actin-binding domains) and functional data suggest that HIP1 and HIP1r are involved in membrane trafficking. One simple possibility of how HIP proteins use clathrin-mediated membrane to promote the survival of cells is that altered expression of HIP1 and HIP1r could alter growth factor receptor density and signaling, affecting cellular proliferation, survival, and differentiation (Fig. 1). Over-expression of HIP1 and HIP1r inhibits ligand stimulated degradation of growth factor receptors, leading to elevated receptor levels.

Targeted Mutations of Murine Hip1 and Hip1r. To understand the normal function of HIP1/HIP1r and how these proteins may interact with cancer pathways, phenotypes that result from mutant Hip1 and Hip1r alleles in gene targeted mice have been examined. Based on these studies, it is clear that deficiency of either HIP1 or HIP1r alone does not severely affect embryonic or early adult development. However, HIP1 (but not HIP1r) is required for maintenance of adult spinal, testicular, and hematopoietic tissues. Combined HIP1r and HIP1 deficiency accelerates (earlier onset and increased severity) spinal defects and leads to dwarfism and premature death. Remarkably, transgenic ubiquitous expression of the human HIP1 protein in the double Hip1/Hip1r mutant mice corrects these defects. This transgenic rescue is important since it indicates that studying the role of mouse HIP1 or human HIP1 in the mouse will yield valuable insights into the role of the human HIP1 protein family in human diseases such as cancer. The findings from these studies, particularly the rescue experiments, underscore the power of using



Huntingtin Interacting Protein 1 (HIP1). Figure 1 Altered membrane trafficking and cellular transformation. The left-hand panel displays the steps in membrane receptor uptake, recycling, and degradation. The middle panel displays how increased uptake, decreased degradation, or increased recycling could lead to increased signal transduction and tumor-cell proliferation and survival.

mouse genetics to study human disease. That is, the conclusions derived from these studies are especially rigorous compared to studies that use cultured cells or cell lines because mouse experiments are performed under physiological conditions *in vivo* .

Hip1 and Hip1r have Restricted Patterns of Expression and are not House-Keeping Genes. Since testicular degeneration is observed in *Hip1* mutant mice and HIP1 is specifically expressed in post-meiotic spermatids, the cells that exhibit increased apoptosis in the Hip1 knockout testis, it is likely that HIP1 expression is cell-intrinsically required for the survival of progenitors at a specific stage of spermatogenesis. Single and double knockout mice present with abnormalities only in early adulthood, emphasizing that HIP1 family members are not housekeeping genes that are widely expressed or widely required for the survival of most cells. Both HIP1 and HIP1r are expressed in restricted patterns in many different tissues. Even within tissues affected by Hip1-deficiency, HIP1 and HIP1r appear to regulate the survival or proliferation of only certain cell types within these tissues. Therefore, transient inhibition of HIP1 or HIP1r as a cancer therapy could potentially be well-tolerated.

HIP1 and Solid Tumors. Using monoclonal antibodies against human HIP1, it has been found that benign colon and prostate epithelia do not express detectable HIP1, yet malignant colon and prostate epithelia usually do. Examination of patients with prostate cancer indicates that HIP1 expression in prostate tumors is associated with cancer progression and a poor prognosis (►Autoimmunity and prognosis). These data raise the intriguing question of whether over-expression of HIP1 or mutated HIP1 in other tissues might promote tumorigenesis. HIP1 participates in numerous pathways (PtdIns signaling, clathrin trafficking, actin dynamics), and future use of human HIP1 transgenic mice and cellular transformation assays to determine whether dysfunction of all, or only a subset, of these pathways contributes to tumorigenesis will be important. A separate, but equally important question is whether HIP1 is necessary for cancer initiation or progression. In fact, the prostate cancer-prone TRAMP mice that are deficient for HIP1 are resistant to prostate cancer progression but not initiation.

Humoral Response to HIP1 Overexpression. The observation that HIP1 expression is necessary for the full tumor phenotype in TRAMP mice and that HIP1 expression is elevated in mouse and human prostate cancers raised the question of whether aberrant HIP1 expression could be monitored in the serum. As one would expect of a cytoplasmic protein, HIP1 itself was difficult to detect in serum. However, anti-HIP1 antibodies in the serum of mice and humans with lymphoid, brain and prostate cancer are detectable. This is not unprecedented, as ►autoantibodies to other solid tumor antigens as well as leukemia antigens have been detected in patient sera. Additionally, antibodies to HIP1r have been found in patients with colon cancer. In addition to use of this test in humans, this newly-developed test specifically detects HIP1 changes in sera from TRAMP mice and p53 deficient mice with prostate cancer and lymphoid cancers, respectively. Given the fact that some cancer-bearing mice as well as humans with prostate cancer and lymphoma develop antibodies to HIP1, it will be important to expand these findings to larger cohorts of patients with different types of tumors at different stages of the disease.

Over-expression of HIP1 is Sufficient to Transform Fibroblasts. Since abnormalities in proliferation are hallmarks of cancerous cells and HIP1 is over-expressed in tumor cells, HIP1 over-expression may be directly oncogenic. To begin to test this, full-length HIP1-overexpressing 3T3 cell lines have been constructed and assayed for anchorage-independent growth in soft agar and for their ability to form tumors in nude mice. In both assays, HIP1-overexpressing cells were transformed, while control cells were not. An important

clue as to how HIP1 may achieve these transforming effects came from the finding that EGFR (►epidermal growth factor receptor ligands) levels are elevated in the HIP1 transformed cells and inhibition of the EGFR (►epidermal growth factor receptor ligands) tyrosine kinase with the specific small molecule inhibitor, CI-1033 (Pfizer), inhibited HIP1-mediated transformation. These data suggest that HIP1 over-expression transforms fibroblasts by increasing signaling by receptor tyrosine kinases. A mutant form of HIP1 in which the lipid binding-ANTH domain was deleted was unable to transform cells. HIP1 transforming activity should be next tested *in vivo* and in additional cell types (epithelial and hematopoietic cells) by transgenically expressing high levels of human HIP1 or mutants of human HIP1 in different murine tissues.

Future

How abnormalities in the trafficking of membranes and their components lead to cancer initiation and evolution is a major question to answer as a result of HIP1's looming role in cancer. HIP1 represents a novel type of oncogene that we believe transforms cells by dysregulating membrane trafficking and by upregulating signaling through multiple pathways. Although the link between endocytosis and cellular signaling has been well established, the link between clathrin-mediated membrane trafficking and cancer has only been referred to as an interesting possibility due to anecdotal leukemic alterations or as it relates to monoubiquitination and degradation targeting of growth factor receptors by the E3 ligase, Cbl. It had not been experimentally verified in humans prior to the discovery of HIP1 abnormalities in a variety of cancer types.

The aim of future research will be to not only gain a deeper understanding of HIP1, but also to expand work beyond the HIP1 pathway to determine if other proteins involved in membrane trafficking are linked to tumorigenesis. By combining *in vitro* cell culture and *in vivo* (mouse and human) work, the paradigm of trafficking and tumorigenesis will move to a new level. It is anticipated that changes in trafficking will not only upregulate signaling for relevant pathways (such as the EGFR (epidermal growth factor receptor ligands) tyrosine kinase) that promote survival and proliferation, but that these changes in membrane dynamics will also influence yet to be discovered transcriptional regulators through novel mechanisms.

Future research should also expand the analysis of the HIP1 antibody response. Testing this further with immunization of mice with HIP1 antigens as well as testing more patients for antibody levels is necessary. The need for serum biomarkers for clinical cancer care is great. By analyzing the humoral response to abnormal HIP1 in controlled animal models, as well as screening less controlled but direct human serum samples from patients with pathologic or clinically linked data (Brca mutation (►BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk), p53 loss, Androgen Receptor, Estrogen Receptor, Progesterone Receptor, Her2/neu, survival, relapses and time to relapses), use of a humoral response to HIP1 may or may not be deemed be a useful screening tool.

The antigen that is expressed in cancer samples could be a mutated or a posttranslationally altered form of HIP1/HIP1r. A good example is the t(5;7) chromosomal translocation that results in the expression of HIP1/PDGFβR in CMML. Analysis of the HIP1 and HIP1r genes in primary human breast, lymphoid, brain and prostate cancer samples using Southern blot and sequence analysis will answer this. If cancers frequently harbor mutant forms of HIP1 or HIP1r, then this information will guide investigators in their design the HIP1 antigens, which should then be used to detect serum humoral response.

In addition to altered clathrin-mediated membrane trafficking in cancer there are other interesting cancer-related pathways that have not yet been investigated for links to HIP1 abnormalities and that may be functionally important. For example, mutations in the BRCA1 and BRCA2 genes are the main causes of hereditary breast and ovarian cancers. BRCA1 mutant tumors are distinct from BRCA2 mutant and sporadic tumors in that they are Estrogen Receptor negative, Progesterone Receptor negative, Her2/neu negative, and have upregulated EGFR (epidermal growth factor receptor ligands). Could BRCA1 deficiency lead to mutations at the HIP1 locus that affect the levels of EGFR (epidermal growth factor receptor ligands)? Could inhibition of HIP1 be a way to target BRCA1 mutant tumors? By screening humans with these mutations for anti-HIP1 antibodies an investigation of these interesting questions may yield novel mechanistic insights into human cancers.

Significance

HIP1 proteins are clearly important to normal and neoplastic physiology. Ongoing work is focused on understanding mechanisms of transformation by HIP1 and its mutants and why deficiency of both HIP1 and HIP1r leads to premature degenerative phenotypes in adult mice. With this work, ways to monitor and modulate the HIP1 family to alleviate neoplastic and degenerative diseases in patients can be easily imagined. Longer term goals are to expand the idea that abnormalities in the movement of cell membranes and their components can cause cancer and that modulating membrane trafficking will lead to effective cancer therapies. Continuous use of mouse models and patient materials to experimentally pursue these future goals is needed. By discovering a role for disrupted membrane trafficking in cancer and by working toward a better

understanding of how this dysregulation contributes to tumorigenesis, establishment of a new field of study that integrates cancer biology, clinical oncology, and basic molecular biology will be achieved.

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Hurthle Cell

Definition

Oncocytic cell arising from the follicular epithelium and characterized by an eosinophilic granular cytoplasm and a vesicular nucleus with a large nucleolus. Named after Karl Hurthle German histologist born 1860-died 1945. This is a misnomer since Askanazy was the first to describe these cells.

►Hurthle Cell Carcinoma

Hurthle Cell Adenoma and Carcinoma

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Synonyms

Follicular carcinoma, oncocytic variant; Follicular adenoma, oncocytic variant

Definition

►Hurthle cell adenomas and carcinomas are respectively benign and malignant epithelial tumors arising from the follicular epithelium of the thyroid gland, containing at least 75% of ►oncocytes and lacking the characteristic nuclear features of papillary thyroid carcinoma (i.e. irregular clear nuclei with grooves, overlapping and pseudo-inclusions). The oncocytes in Hurthle cell adenomas and carcinomas are characterized by an eosinophilic granular cytoplasm on ►hematoxylin and eosin sections with a vesicular nucleus and a large centrally located nucleolus. The oncocytic nature of the cytoplasm is due in the vast majority of the cases to its high content in mitochondria.

Characteristics

Clinical and Histopathologic Characteristics. Hurthle cell carcinomas is a rare tumor accounting for only 3–4% of the ~33,550 newly and yearly diagnosed thyroid malignancies in the United States. The median age at diagnosis for Hurthle cell carcinomas is ~61 years and the female to male ratio is 2:1 while it is 8:1 for Hurthle cell adenomas. The exact incidence of Hurthle cell adenoma is unknown but probably much higher than the incidence of its malignant counterpart. Most patients with Hurthle cell adenoma and carcinoma present with a painless thyroid mass. Patients with carcinoma tend to present at an older age and usually have larger tumors than adenoma patients. Rarely patients with Hurthle cell carcinoma present with distant metastases usually to lung and bone. Hurthle cell carcinomas should be separated from Hurthle cell adenoma on the basis of presence or absence of invasion of the tumor capsule (capsular invasion) as well as presence or absence of vascular invasion. The latter is defined as invasion of a vessel within or beyond the tumor capsule in the surrounding thyroid or extrathyroid tissue. Hurthle cell adenoma and carcinoma cannot be separated on the basis of growth pattern whether follicular (i.e. making glands) or solid. The size of the tumor cell and its nuclear-cytoplasmic ratio cannot also be used to diagnose malignancy. Therefore, the differentiation between benign and malignant Hurthle cell tumors is impossible in smears from fine needle aspiration since it is not possible to assess capsular or vascular invasion in cytology specimens. The diagnosis of malignancy can be a very difficult exercise in tissue sections, especially after fine needle aspiration. In addition to causing infarction, the needle biopsy can lead to artefactual irregularities of the tumor capsule rendering the evaluation of capsular and vascular invasion very difficult. Hurthle cell carcinomas should also be differentiated from the ►tall cell variant of papillary carcinoma and ►papillary carcinoma, oncocytic variant. These two entities share with Hurthle cell carcinoma the presence of an oncocytic cytoplasm but differ from it by displaying the nuclear features of papillary carcinoma (i.e. irregular clear

nuclei with grooves, pseudo-inclusions and nucleoli apposed to the nuclear membrane). Hurthle cell carcinoma are divided into minimally invasive and widely invasive tumors The minimally invasive carcinoma, also termed encapsulated, is totally surrounded by a fibrous capsule and displays capsular invasion and/or foci of vascular invasion. It is unusual for these foci of invasion to be detected grossly. In contrast, widely invasive Hurthle cell carcinoma are defined by extensive areas of invasion at both the macroscopic and microscopic level and the capsule remnant is often difficult to identify. This classification correlates very well with outcome, such that minimally invasive tumors have an overall excellent prognosis, whereas widely invasive tumors have a much poorer outcome with a 55% mortality rate according to one series. However, there are cases of encapsulated minimally invasive follicular carcinoma that recur and metastasize. Identifying these cases at the time of diagnosis is crucial because a minimally invasive tumor will be treated by lobectomy alone followed by observation in some centers. This approach will risk undertreating those few minimally invasive tumors with poor outcome. At variance with the minimalist surgical approach, many surgeons will perform a total ►thyroidectomy followed by radioactive iodine therapy on any minimally invasive Hurthle cell carcinoma, most likely overtreating a large number of cases. In view of the limitations of the above subcalssification, many authors have attempted to revise this classification. Indeed, some researchers will exclude from the minimally invasive category any tumor with vascular invasion, even if microscopic while we will exclude from the minimally invasive group any tumor with ≥4 foci of microscopic vascular invasion since the latter has a significant recurrence rate. Within widely invasive Hurthle cell carcinoma, adverse predictors of survival are extrathyroidal extension, nodal metastasis, positive margin, and a solid growth pattern. On multivariate analysis, extrathyroidal extension and nodal metastases are independent predictors of outcome within widely invasive Hurthle cell carcinoma, present in 59 and 21% of cases respectively.

Immunohistochemistry. Hurthle cell tumors are typically positive for the thyroid markers thyroid transcription factor 1 (►TTF-1) and ►thyroglobulin. In carcinomas with a solid growth pattern, thyroglobulin positivity can be very focal. Various cell cycle proteins have been used to differentiated between benign and malignant Hurthle cell tumors and stratify patients with Hurthle cell carcinoma. Only Ki67 was found to be significantly elevated in widely invasive Hurthle cell carcinoma in comparison to Hurthle cell adenoma and minimally invasive Hurthle cell carcinoma. But this marker is not an independent prognostic marker since it correlates very well with the extent of vascular invasion and the presence of extra-thyroid extension.

Genetics. Hurthle cell tumor frequently show chromosomal DNA imbalance. Somatic mutations have been identified in the mitochondrial DNA of Hurthle cell carcinoma. These findings have no clinical use in these tumors.

Imaging. It is important to note that the follow up imaging of Hurthle cell carcinomas include radioactive iodine scans and positron emission tomography (PET). Hurthle cell adenomas can also be positive by both modalities. This is important to know since PET performed for non-thyroid malignancies (like melanoma) can lead to the discovery of Hurthle cell tumors whether benign or malignant. These are referred to as PET incidentalomas. The presence of PET incidentalomas should lead to a thyroid nodule work up.

Treatment. All authors agree that non-minimally invasive Hurthle cell carcinoma should be treated by total thyroidectomy followed by radioactive iodine administration. For minimally invasive carcinomas, there is disagreement between authors. While some recommending full therapy for these patients (similar to widely invasive tumors), we believe minimally invasive tumors should be treated by ►thyroid lobectomy alone as long as they display no more than two foci of capsular and or vascular invasion. All authors agree that lobectomy is sufficient for the treatment of Hurthle cell adenoma.

Current and Past Controversies

Hurthle cell tumors have generated a large number of controversies since their original description. One controversy regards the cell type. While most physicians, especially in North America go by the term Hurthle cell, it is a misnomer. It is ►Askanazy and not Hurthle who provided the description of these follicular oncocytic cells. A much more debated issue is whether Hurthle cell carcinoma is a distinct tumor entity. Some authors believe this tumor should be separated from conventional non-oncocytic follicular carcinoma. Indeed, compared to non-oncocytic follicular carcinoma, Hurthle cell malignancies generally take up less radioactive iodine, have a higher frequency of extrathyroid extension, local recurrence and nodal metastases. Hurthle cell carcinoma are overall more aggressive tumors. Since many of the differences are not evident when patients are stratified by extent of invasion, other authors believe Hurthle cell carcinoma are simply a morphologic variant of follicular carcinoma. This is the official position emanating from the last world health organization classification of endocrine tumors. Another controversy arose in the 1970's when some authors suggested that all Hurthle cell tumors are malignant. This view is no longer accepted and all current thyroid pathologists believe it is possible to

differentiate benign from malignant Hurthle cell tumors on histologic grounds. As mentioned above, there is still controversy as to what constitutes a minimally invasive tumor and there is still a debate regarding the optimal therapy for minimally invasive Hurthle cell carcinomas.

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Hutchinson-Weber-Peutz-Syndrome

►Peutz-Jeghers-Syndrome

HUVEC

Definition

►Human Umbilical Vein Endothelial Cells

HVEM

Definition

HVEM is a member of the tumor necrosis factor (►TNF) receptor superfamily. Binding of herpes simplex virus viral envelope glycoprotein D (gD) to this receptor protein has been shown to be part of the viral entry mechanism. HVEM also mediates the immune response of ►LIGHT.

►Herpesvirus Entry Mediator

HVJ

Definition

Hemagglutinating virus of Japan (HVJ), synonym Sendai Virus, is a negative-strand RNA virus that is a member of the paramyxovirus family. HVJ is best known for its membrane fusion activity mediated by two surface glycoproteins, which are termed F (fusion) and HN (hemagglutinin). This virus belongs to the mouse parainfluenza virus family and is not a human pathogen.

►Non-viral Vector for Cancer Therapy

HVJ-Envelope Vector

Definition

Ultraviolet light (UV) inactivated ►Sendai virus (synonym HVJ) particles are mixed with therapeutic molecules such as DNA, synthetic oligonucleotides, proteins, or anticancer drugs in the presence of a mild detergent. By centrifugation of the mixture, such molecules are incorporated into the particles, which are called HVJ envelope vector and can deliver molecules to both cultured cells and tissues by membrane fusion activity. The vector itself can induce T cell-mediated antitumor immunity through activation of dendritic cells and inhibition of regulatory T cells.

►Non-viral Vector for Cancer Therapy

HVJ-Liposomes

Definition

These are viral liposomes with a fusigenic envelope derived from ►HVJ. HVJ-liposomes are formed by the

fusion of liposomes containing therapeutic molecules with inactivated ►Sendai virus particles.

►Non-viral Vector for Cancer Therapy

Hyaluronan

Definition

A polymeric linear glycosaminoglycan consisting of repeating disaccharide units of N-acetyl glucosamine and glucuronic acid linked by glycosidic bonds. A ubiquitous component of the extracellular matrix that has various roles in maintenance of tissue hydration, cellular proliferation, motility and adhesion.

►Hyaluronan Synthases

Hyaluronan Receptor

►CD44

Hyaluronan Synthases

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Synonyms

Hyaluronic acid synthases; HAS; Hyaluronic acid; HA

Definition

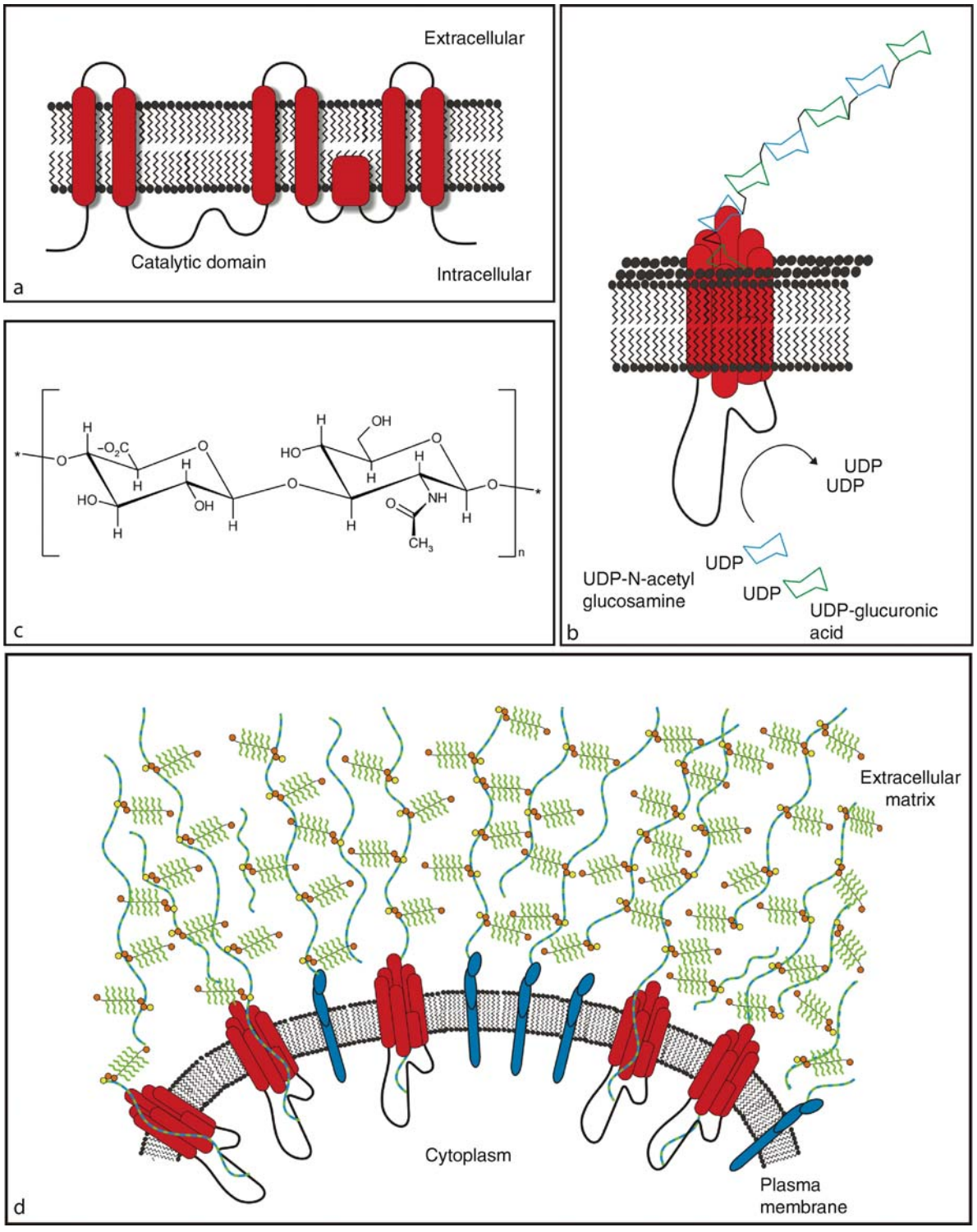
Hyaluronan synthases (HAS) are multi-isoform transmembrane proteins which catalyse the synthesis of the high molecular weight carbohydrate, ►hyaluronan (HA). High expression of hyaluronan synthases in a neoplasm or associated peritumoral ►stroma is often a poor prognostic indicator in cancer. Hyaluronan is a ubiquitous component of the ►extracellular matrix where it has various roles in matrix hydration, promotion of cellular growth and proliferation, induction of malignant transformation, and invasion and metastasis.

Characteristics

Hyaluronan Synthases and Hyaluronan

Hyaluronan synthases (►HAS1, ►HA2, ►HAS3) are integral plasma membrane proteins that utilize UDP-glucuronic acid and UDP-N-acetyl glucosamine substrates to alternately add monosaccharide units to a growing chain of hyaluronan. Three isoenzymes (designated *HAS1*, *HAS2*, and *HAS3*) are localized to separate chromosomes and exhibit varying enzymatic properties despite having relatively high amino acid sequence homology. Each isoform however, is topologically and structurally similar with predicted clusters of transmembrane domains seen at either end of the protein which vary from 1 to 2 in number at the amino terminal and from 4 to 6 at the carboxyl terminus. A large cytosolic region separates the transmembrane domains of the amino- and carboxyl termini and contains the active sites and substrate binding residues to fulfill HA synthetic capacity. All isoforms catalyse the polymerisation of the ►UDP-monosaccharides at the internal face of the plasma membrane, where catalysis occurs within the large cytoplasmic domain which constitutes approximately half of the overall protein. The growing *HA* chain is extruded through the plasma membrane into the ►extracellular matrix (ECM) or onto the ►pericellular surface where it can remain bound to *HAS* or associates with cellular *HA* receptors to form a ►glycocalyx. The expansion of the *HA* chain into the extracellular matrix allows the size constraints that would normally be imposed by cellular dimensions to be overcome, and thus permits unrestrained polymer growth. Upon release of *HA* into the extracellular milieu, it is retained within the matrix via interaction with ►proteoglycans and receptor proteins which typically contain a *HA*-binding structural motif (Fig. 1).

Hyaluronan (►HA) exhibits a diverse range of physiological functions despite its simple and unadorned structure of repeating disaccharide units of N-acetyl glucosamine and glucuronic acid. The disaccharide building blocks are linked by glycosidic bonds (in which a single oxygen atom links the two sugar units) into a macromolecule which typically contains 2,000–25,000 disaccharides and ranges in molecular weight between 10^5 and 10^7 Da. It is highly negatively charged due to the prevalence of carboxyl groups contributed from the glucuronic acid moiety. Within an aqueous environment, the hyaluronan becomes heavily hydrated primarily due to the formation of large solvation cavities which are a result of electrostatic repulsion between intra-hyaluronan anionic groups. Hyaluronan adopts a random coil structure in solution where molecules entangle to form a viscous, gel-like meshwork that can contract in response to external pressure, but will relax and expand when released as a result of charge repulsion. In this way a *HA*-rich extracellular matrix is malleable, elastic and flexible,



Hyaluronan Synthases. Figure 1 Hyaluronan and hyaluronan synthases. (a) The HA synthase is predicted to be a multipass transmembrane protein with a large cytoplasmic domain, which holds key substrate binding and catalytic residues. (b) HA is extruded through the plasma membrane following polymerisation of UDP-glucuronic acid and UDP-N-Acetyl Glucosamine. (c) HA is composed of repeating units of glucuronic acid and N-acetyl glucosamine linked by glycosidic bonds. (d) HA chains can associate with HA Synthases (*red*), cell surface receptors such as CD44 and RHAMM (*blue*), and interact with other matrix components including proteoglycans (*green*), forming a structured, malleable and hydrated pericellular and extracellular matrix.

and provides an ideal medium for growth, expansion and cushioning of tissues.

The broad range of physiological activities of *HA* are typically dependent on molecular weight and concentration as these impact on the physical and mechanical properties, viscosity, hydration, matrix forming capacity and the interactions between *HA* and cellular receptors. Therefore as each of the *HAS* isoenzymes differ in catalytic rate and molecular weight of the synthetic products, the differential regulation and control of HAS expression are important determinants in HA function. HAS1 characteristically drives the synthesis of high molecular weight *HA* (ranging from 2×10^5 to $\sim 2 \times 10^6$ Da), however exhibits a relatively low catalytic rate suggesting a role in maintenance of a basal level of cellular HA. HAS2 produces higher molecular weight HA and is more catalytically active; increased expression of this isoenzyme is observed in many developmental and repair process involving tissue expansion and growth. ►HAS3 is most active and synthesizes lower molecular weight HA (10^5 to 10^6 Da) that has been associated with providing HA for release into the ►pericellular matrix, or for interaction with cell surface receptors.

The molecular weight of native HA can be degraded through cleavage mediated by ►hyaluronidase enzymes; there are six hyaluronidases that exhibit varying cellular locations and properties, of which Hyal-1 and Hyal-2 hyaluronidases are the predominant forms responsible for HA catabolism in somatic tissues. Hyal-2 is anchored to the plasma membrane and can cleave HA to 20 kDa fragments, enabling it to be internalized for lysosomal degradation into disaccharides in a process mediated by Hyal-1. The concurrent activity of *HAS* and hyaluronidase enzymes results in significant polydispersity of HA within the extracellular matrix. Whilst originally though to be an inert, space-filling macromolecule, the diverse sizes of HA molecules can bind to specific cell surface receptors such as ►CD44 and ►RHAMM (Receptor for Hyaluronan Mediated Motility) to induce transduction of various size-dependent cell signals, thereby highlighting the importance of action of HA synthetic and degradative enzymes.

Hyaluronan Synthases and Hyaluronan in Cancer

The extracellular environment within and surrounding a tumor mass can have a profound effect on tumorigenesis, progression and invasion. This is perhaps most evident in metastasis where cell migration and invasion must be mediated by manipulation and breakdown of the extracellular matrix; inevitably alteration and disruption of the matrix composition is characteristic of cancer initiation and progression. Increased HAS expression and deposition of HA in comparison to that in normal and benign tissue is one such morphological change in matrix composition that is commonly observed in many malignancies. The correlation between high levels of hyaluronan and tumor aggressiveness and invasiveness is long established and in many malignancies, elevated intra-tumoral HA is associated with poor prognosis and low survival rates. Elevated intra-tumoral HA can be resultant of higher expression levels of HAS within the tumor cells, and/or through the HA synthesized by stromal cells that surround a tumor. There is a dynamic interplay between stromal and malignant tissue, where paracrine signaling by tumor cells can induce upregulation of expression of HAS in stromal cells such as fibroblasts or smooth muscle cells. Paracrine stimulation of HAS activity can be attributed to tumor release of growth factors such as ►TGF-β, ►FGF and ►PDGF, or by inflammatory cytokines such as ►TNF-α.

These observations have established a clear link between HAS, HA and tumorigenesis; the mechanisms by which HA production augments the malignant process have begun to be elucidated by manipulation of expression levels of the *HAS* enzymes, and disruption of the endogenous interactions that occur between HA and tumor cells via receptors such as CD44 and RHAMM.

H

Proliferation and Tumor Growth

Increases in ►pericellular HA are observed in proliferating and migrating cells during normal physiological processes such as morphogenesis, growth and wound healing, so it is not surprising that elevated hyaluronan also correlates to pathologies like cancer that are defined by rapid and inappropriate cell division and motility. Structurally, increased synthesis and deposition of HA results in hydration and expansion of the tissue volume creating less dense connective tissue that differs significantly from the architecture of the extracellular matrices associated with non-diseased cells and normal tissue. This flexibility and malleability of HA-rich matrices provides a medium that can readily surround rapidly dividing cells concomitantly accommodating changes in shape that occur during mitosis. Extracellular matrices rich in HA can also promote proliferation through the facilitation of diffusion of nutrients, growth factors and cytokines to a tumor, and creation of a concentrated reservoir of various growth effectors.

Expression of the hyaluronan synthases fluctuate within the cell cycle progression, with maximal activity occurring during mitosis where HA facilitates partial cell rounding and detachment. The interaction of HA and the centrosomally localized RHAMM receptor results in stabilization of the mitotic spindle during mitosis. Cell division is inhibited when the pericellular HA matrix that forms immediately prior to mitosis is disrupted, thereby demonstrating the multifaceted roles that both HA and HAS play in cell cycle regulation and cellular proliferation.

Experimentally induced overexpression of each HAS isoenzyme enhances the growth kinetics of a wide range of tumor cell lines; conversely, the

disruption of interactions of HA with ►CD44 or ►RHAMM induces cell cycle arrest or apoptosis, suggesting that the role of HA in a tumor is structurally permissive but also has more complex roles in the initiation of cell signaling cascades promoting proliferation. Very high overexpression of HAS isoenzymes and high concentrations of high molecular weight HA can conversely inhibit tumor growth, indicating that there is a tightly controlled active regulation of HAS expression during the development of malignancy. The increased expression of hyaluronidase in many malignancies is suggestive of a cooperative balance between increased HA synthesis and degradation in tumors, where an imbalance in expression may lead to growth inhibition. Controlled regulation of HAS and hyaluronidase expression is evident in induction of angiogenesis. When diffusion of nutrients within a high molecular weight HA rich matrix is no longer sufficient for growth and proliferation, the HA can be degraded to low molecular weight products which stimulate endothelial cell proliferation, migration and tumor vascularization.

Production of HA can also contribute to the acquisition of an anti-apoptotic phenotype in cancer thereby promoting tumorigenesis. Normal cell survival requires anchorage to the substratum; cancer cells can avert this requirement and grow independently in a manner dependent on binding of native HA to CD44. This interaction can facilitate cell survival by promoting the activation of the ►PI3K signaling/►AKT pathway and stimulating phosphorylation of pro-survival effectors, ►FAK and BAD. Hyaluronan binding to CD44 is also required for complex assembly of a number of factors critical for signaling in the ►HER-2/neu pathway; when this signaling pathway dysfunctions there is a direct correlation with malignancy development in a number of solid tumors. RHAMM-HA interaction similarly initiates signaling cascades directing phosphorylation of BAD, and appears implicated in aberrant mitosis through disruption of RHAMM binding to microtubules leading to chromosomal misaggregation.

Invasion and Metastasis

Hyaluronan synthase expression and production of hyaluronan can augment the invasion and metastasis of tumor cells in several ways:

- By creation of hydrated, expanded matrix pathways which are compatible with cell migration and facilitate tissue penetration
- Promotion of anchorage independent growth
- Stimulation of cell locomotion and migration mediated by HA interaction with CD44 and RHAMM: CD44 has the capacity to interact with cytoskeletal proteins such as ►actin and ankyrin. The activation of CD44 induced by HA binding can initiate signaling cascades involving ►Src kinases and Ras/Rho GTPases that lead to cytoskeletal rearrangement, membrane ruffling and migration. Cellular locomotion also requires turnover of focal adhesions (cell substratum adhesions); this process appears to require RHAMM-mediated activation of Src kinase and transient phosphorylation of focal adhesion kinase dependent on HA-RHAMM interaction
- HA-CD44 interaction stimulates the expression of ►matrix metalloproteinases (MMPs): MMPs are required for proteolytic degradation of the extracellular matrix; this occurs when cells invade normal tissue and penetrate through the basement membrane into the surrounding vasculature. Association of *HA* and CD44 can promote expression of MMP-9 and MMP-2 and memprane-type1-matrix metalloproteinase (MT1-MMP). MT1-MMP additionally mediates the proteolytic cleavage of CD44 from the cell surface and the detachment from underlying hyaluronan during cell locomotion. Shedding of CD44 promotes CD44 expression and the continual receptor cycling allows continued adherence and detachment from the matrix as a tumor cell migrates
- *Induction of* ►epithelial-mesenchymal transition *(EMT)*: The acquisition of an invasive and metastatic phenotype in carcinomas is akin to EMT seen in embryogenesis. Increased HAS expression and production of high molecular weight HA can be sufficient to induce stimulation of ErbB and PI3K signaling/Akt pathways and EMT. Elevated *HA* production can interfere with formation of intercellular and matrix adhesions increasing cellular autonomy, and inducing anchorage independent growth, MMP expression and cytoskeletal rearrangement which are typical characteristics of mesenchymal tissue

Multidrug Resistance

Multidrug ►resistance hinders the administration of an effective chemotherapy regime. Acquisition of resistance can be from mutation of drug binding sites, enzymatic deactivation or decreased permeability of drug entry, however is most commonly associated with increased drug efflux by ATP-binding pumps such as MDR (multi-drug resistance) proteins. Overproduction of hyaluronan promotes multidrug resistance, observed when upregulation of HA synthesis renders drug sensitive cancer cells resistant. Administration of hyaluronidase as an adjunct to chemotherapy regimes increases the sensitivity of tumors to various therapeutic agents by reducing barriers to drug penetration, and degrading the HA-rich matrix which typically facilitates cytokine diffusion. Hyaluronan synthesis also stimulates the expression of MDR1 and acquisition of drug resistance via HA-CD44 mediated activation of the ErbB2 signaling complex; this leads to downstream activation of PI3 Kinase signaling which subsequently stimulates both HAS and MDR1 expression. Hyaluronan additionally stimulates MDR1 in an unknown mechanism independent of PI3 Kinase activation.

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Hyaluronic Acid

Definition

HA; High molecular weight sugar consisting of multimers of three repeats of a basic disaccharide repeat (glucuronic acid and N-acetyl glucosamine joined in β1Π 3). The hexamers are joined in β1Π 4 formation between N-acetyl glucosamine and glucuronic acid. HA is present in high concentration in extracellular spaces and promotes cell migration in embryogenesis and neoplastic transformation. The aminoterminal region of CD44 can bind to HA.

►CD44
►Hyaluronan Synthases

Hyaluronic Acid synthases

►Hyaluronan Synthases

Hyaluronidase

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Definition

HAases are endoglycosidases that degrade hyaluronic acid (HA). HA is a non-sulfated glycosaminoglycan. Exhaustive digestion of HA with HAase generates tetrasaccharides, whereas, limited digestion generates HA fragments, some of which induce angiogenesis.

Characteristics

Six HAase genes are present in the human genome and these occur in two linked triplates. HYAL-1, -2 and -3 genes are clustered in the chromosome 3p21.3 locus, whereas, HYAL-4, HYAL-P1 and PH20 (encodes testicular HAase) reside in the chromosome 7q31.3 locus [1, 2]. Based on their pH activity profiles, HAases are divided into two categories. HYAL-1, -2 and -3 are considered acidic HAases because they are active at acidic pH. For example, HYAL-1 has a pH optimum around 4.0–4.2 and the enzyme is inactive above pH 5.0. It is normally expressed in serum and urine. On the contrary, PH20 or the testicular HAase is a neutral HAase as it is active at pH 7.0 (pH activity profile 3.0–9.0) and is involved in ovum fertilization.

Among the six mammalian HAases, HYAL-1 is the major tumor-derived HAase and is expressed by a variety of tumor cells. HYAL-1 was initially purified from the urine of patients with high-grade bladder cancer and was shown to be expressed in bladder epithelial cells, prostate tumors and in head and neck squamous cell carcinoma cells.

HAase Expression in Cancer

(►Cancer, ►biomarkers) Using a modified HAase ELISA-like assay (referred to in this review as the HAase test) Lokeshwar et al. found that HAase levels are elevated in prostate cancer tissues, when compared to normal prostate and benign prostatic hyperplasia tissues. HAase levels were also shown to be elevated in high-grade bladder tumor tissues, in the urine of patients with high-grade bladder cancer and also in the urine of patients with Wilms tumor. These studies have established a link between HAase and the tumor invasive/metastatic phenotype. In addition to genito-urinary tumors, HAase levels are elevated in head and neck squamous cell carcinoma, breast tumors, metastatic tumors and glioma cells.

For example, in head and neck squamous cell carcinomas the expression of HYAL-1 protein and mRNA is elevated, along with the expression of PH20 mRNA. In breast cancer, the less invasive cells appear to express HA-synthase (HAS)-3 and HYAL-3, whereas, highly invasive cells express HAS-2 and HYAL-2. However, how and why the HA production by HAS-2 and HA degradation by HYAL-2 promote tumor cell invasion, but HA production by HAS-3 and HA degradation by HYAL-3 associates with low-invasive phenotype is unclear. It is noteworthy that in these studies, the expression of HAS and HYAL isoforms was studied only at the transcript level by real time RT-PCR. Given that functionally inactive splice variants of HYAL-1 and HYAL-3 are previously reported [3], the expression

of HYAL genes at the mRNA level may not translate into HAase activity produced by breast cancer or any other cell type. Similar observations regarding HYAL-2 and HYAL-3 mRNA expression were reported for endometrial carcinoma.

Contrary to the findings regarding elevated expression of one or more HAases in tumors, based on real time RT-PCR studies Bertrand et al. reported that HYAL-2 expression correlates with lymphoma diagnosis, but the expression actually decreases in high-grade lymphomas, when compared to low-grade lymphomas.

Taken together, HAase expression appears to be elevated in many carcinomas and the expression correlates with tumor invasiveness. However, in some carcinomas Haase expression may inversely correlate with tumor grade.

HAase Functions in Cancer

HAase a Tumor Promoter. The angiogenic (3–25 disaccharide units) (Angiogenesis) HA fragments have been shown to stimulate endothelial cell proliferation, adhesion and capillary formation. Such angiogenic HA fragments are found in the urine of patients with high-grade bladder cancer, in the tissue extracts of high-grade prostate tumors, and in the saliva of patients with head and neck squamous cell carcinoma, suggesting that the HA-HAase system is active in high-grade invasive tumors.

cDNA transfection studies have shown that HYAL-1 is involved in tumor growth, infiltration and angiogenesis [4]. Lokeshwar et al. showed that blocking HYAL-1 expression in bladder and prostate cancer cells decreases tumor cell proliferation due to cell cycle arrest in the G2-M phase and decreases their invasive activity. In xenografts, inhibition of HYAL-1 expression results in decreased tumor growth. Furthermore, while HYAL-1 expressing tumors infiltrate muscle, blood vessels and lymphatics, tumors lacking HYAL-1 expression resemble benign neoplasms and have a significantly low microvessel vessel density and smaller capillaries [4, 5]. Interestingly, HA production by the tumor stroma correlates with HYAL-1 levels in tumor cells, suggesting crosstalk between the tumor and the tumor-associated stroma [4, 5]. Such crosstalk between HA and HYAL-1, with respect to tumor growth and angiogenesis, was recently confirmed by Simpson who tested tumor growth and angiogenesis following the expression of HAS-2 and HYAL-1, either individually or together, in a non-invasive prostate cancer cell line. While HAS-2 or HYAL-1, when expressed individually in a prostate cancer cell line caused increased tumor growth and angiogenesis, their co-expression had a synergistic effect on this increase. In addition, HYAL-1 expression in prostate cancer cells induces both lymph node and lung metastasis in orthotopic tumor models.

HAase a Tumor Suppressor. In some epithelial carcinomas, the 3p21.3 locus is deleted, and although, the tumor suppressor gene (►Tumor suppression) in this locus is not a HYAL gene (i.e., HYAL-1, -2, or -3), it was previously suggested that HYAL-1 is a tumor suppressor [5]. In support of HAase being a tumor suppressor, Jacobson et al. reported that while HAS-2 expression in a rat colon carcinoma line promoted tumor growth, the over-expression of HYAL-1, at levels (220–360 mu/10^6 cells) that are not found in tumor tissues and tumor cells, inhibited tumor growth and generated necrotic tumors. Furthermore, Shuster et al. showed that administration of super high concentrations of bovine testicular HAase (300 units) caused a ~50% regression in breast tumor xenografts. However, Lokeshwar et al. recently showed that while HYAL-1 levels that are expressed in tumor tissues and cells promote tumor growth, invasion and angiogenesis, HAase levels exceeding 100 mU/10^6 cells), i.e., at levels that are not naturally expressed by tumor cells, significantly reduce tumor incidence and growth due to induction of apoptosis [5]. Therefore, the function of HAase as a tumor promoter or a suppressor is a concentration-dependent phenomenon, but in tumor tissues, the tumor cell-derived HAase acts mainly as a tumor promoter.

Regulation of HAase Activity

Except for HYAL-2, the promoter region of other HAases has not been cloned, and therefore, very little is known about the regulation of HAases at the genomic level. Alternative mRNA splicing is another mechanism by which HAase activity is regulated. For example, in some tumor cells internal splicing within the 5′ untranslated region in exon 1 correlates with HYAL-1 protein levels and HAase activity. HYAL-1 protein is not detected in tumor cells which express a HYAL-1 transcript that retains the 5′ untranslated region. However, it is unclear how and why the 5′-untranslated region in the HYAL-1 mRNA prevents translation. Lokeshwar et al. have reported several alternatively spliced variants of HYAL-1 and HYAL-3 transcripts. All of these variants are enzymatically inactive [3]. Recent data on one of the HYAL-1 variants, HYAL1-v1, shows that the expression of HYAL1-v1 is higher in normal bladder tissues than in bladder tumor tissues. Furthermore, HYAL1-v1 expression reduces HAase activity secreted by bladder cancer cells because of a complex formation between HYAL-1 and HYAL1-v1. HYAL-1-v1 expression induces apoptosis in bladder cancer cells and reduces tumor growth, infiltration and angiogenesis. This suggests that a critical balance between the levels of HYAL-1 and HYAL-1 variants may regulate HYAL-1 function in cancer.

Clinical Importance of HAase

HAase as a Diagnostic and Prognostic Indicator. The diagnostic and prognostic potential of HAase, either

alone or together with HA has been extensively explored in cancer of the prostate and bladder. For example, the HA-HAase test, which measures urinary HA and HAase levels, has 91.2% sensitivity, 84.4% specificity and 88.3% to detect bladder cancer, regardless of the tumor grade and stage. More importantly, the HA-HAase test appears to detect bladder cancer recurrence even before the tumor becomes clinically detectable. Thus, a false positive HA-HAase test (i.e., test positive but no tumor is detected) carries a tenfold risk of developing bladder cancer within 5 months. In a side-by-side comparison, the HA-HAase test was also superior to a variety of FDA-approved bladder tumor markers. In addition to urinary HAase levels, measurement of HYAL-1 mRNA levels in exfoliated cells found in urine also appears to be a marker for bladder cancer. For example, Eissa et al. found that HYAL-1 mRNA expression determined by RT-PCR has >90% accuracy in detecting bladder cancer. Furthermore, HYAL-1 mRNA levels measured in exfoliated cells are elevated in patients with invasive and poorly differentiated carcinoma. These studies show that HAase is a highly accurate marker for detecting high-grade bladder cancer, and when it is combined with HA, it detects both low-grade and high-grade bladder cancer with ~90% accuracy.

The prognostic potential of HYAL-1 has been explored in prostate cancer. By performing immunohistochemistry on radical prostatectomy specimens, on whom there was a minimum 5-year follow-up, Posey et al. and Ekici et al. found that HYAL-1 staining in radical prostatectomy tissues is an independent predictor of prostate cancer progression (local recurrence or distant metastasis). Furthermore, HA-HYAL-1 staining has an 87% accuracy in predicting disease progression. It is noteworthy that in prostate cancer specimens, while HYAL-1 is exclusively expressed by tumor cells, HA expression mainly results from the tumor-associated stroma.

In a limited number of studies, HAase expression has also been studied in other carcinomas. For example, there is some evidence that HYAL-1 may be a marker for head and neck squamous cell carcinomas, as salivary HAase levels are elevated in head and neck cancer patients. PH20 mRNA levels are elevated in primary and lymph node metastatic lesions of laryngeal carcinoma when compared to normal laryngeal tissues. In contrast to the observations in many carcinomas, increased HYAL-2 expression inversely correlates with invasion in B-cell lymphomas and may serve as a prognostic indicator (►Chemotherapy of cancer; Progression and perspectives).

HAase and Cancer Therapeutics. Testicular HAase has been added in cancer chemotherapy regimens to improve drug penetration. Tumor cells growing in three-dimensional multicellular masses, such as spheroids *in vitro* and solid tumors *in vivo* acquire resistance to chemotherapeutic drugs (i.e., multicellular resistance). But this acquired chemoresistance can be abolished by the addition of testicular HAase. In limited clinical studies, HAase has been used to enhance the efficacy of vinblastin in the treatment of malignant melanoma and Kaposi's sarcoma, boron neutron therapy of glioma, intravesical mitomycin treatment for bladder cancer and chemotherapy involving cisplatin and vindesine in the treatment of head and neck squamous cell carcinoma. It is noteworthy that the HAase concentrations (1×10^5–2×10^5 IU) used in these clinical studies far exceed the amount of HAase present in tumor tissues, and therefore, it is unlikely that at these concentrations the infused HAase will act as a tumor promoter.

Summary

HAase appears to be an important molecular determinant of tumor growth, infiltration and angiogenesis. At concentrations that are present in tumor tissues, HAase acts as a tumor promoter. HAases either alone, or together with HA are potentially accurate diagnostic and prognostic indicators for cancer detection and tumor metastasis. We are only beginning to understand the complex role that this enzyme plays in cancer. Nonetheless, it is already proving to be a useful target for developing novel cancer therapeutics and diagnostics.

►Hyaluronidases
►Early Detection

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Hybrid Genes

►Fusion Genes

Hybrid Positron Emission Tomography/Computed Tomography

►Positron Emission Tomography

Hybridomas

Definition

Are fusion cells consisting of antibody-producing B cells and non-secreting cultured myeloma cells. After isolation of antibody-producing B cells from the spleen of immunized animals these polyclonal B cells are fused with myeloma tumor cells by permeabilization of the cell membranes. Hybridoma cells grow and multiply rapidly and produce large amounts of the desired antibodies.

►Monoclonal Antibody Therapy
►Bispecific Antibodies

Hydrogen Dioxide

►Hydrogen Peroxide

Hydrogen Nuclei

Definition

Contain a single proton with a net charge and spin. They therefore possess a magnetic moment. They are abundant in the human body in water and fat and are the Magnetic Resonance Imaging (MRI) active nuclei used in all clinical and most research MRI.

►Dynamic Contrast-Enhanced Magnetic Resonance Imaging

Hydrogen Peroxide

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Synonyms

Dihydrogen dioxide; Hydrogen dioxide

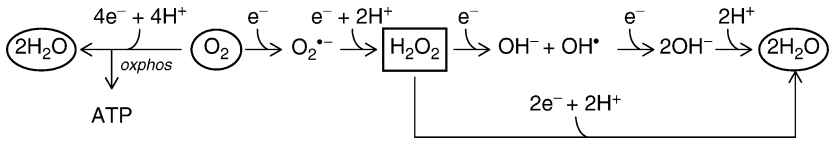
Definition

Hydrogen peroxide (H_2O_2) is a ►reactive oxygen species (ROS) generated from molecular oxygen (O_2). Although the controlled cellular production of H_2O_2 plays an important physiological role, high cellular levels of H_2O_2 can produce carcinogenic effects and induce cell death.

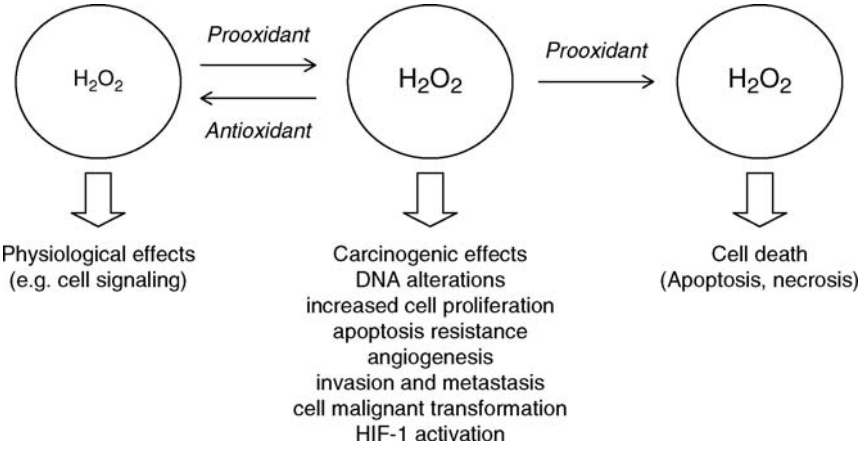
Characteristics

H_2O_2 is a pale-blue liquid first isolated in 1818 by Louis Jacques Thénard. H_2O_2 has industrial and domestic uses (e.g. paper bleaching, chemical synthesis, laundry detergents, antiseptic for wound cleaning, etc.) and it is manufactured today through an autoxidation reaction using O_2 from the air. Cells of aerobic organisms also generate H_2O_2 from O_2. Most of the energy (ATP) that aerobic cells need to live is obtained through a process called ►oxidative phosphorylation (oxphos). In this process, ATP generation is coupled with a reaction in which O_2 is reduced [►reduction/oxidation] to water (H_2O) by a mitochondrial protein complex called cytochrome oxidase. In this reaction, four electrons and four protons are added to O_2 to form two molecules of H_2O. But when a molecule of O_2 gains only one electron to form superoxide anion ($O_2^{\bullet-}$), this highly reactive oxygen species tends to gain three more electrons and four protons to form H_2O; this process involves several reactions and results in the production of other ROS such as H_2O_2 (Fig. 1).

ROS are generated in multiple compartments within the cell (e.g. mitochondria, cytosol, plasma membrane, peroxisomes, endoplasmic reticulum, etc.) and by numerous enzymes (e.g. cytochrome oxidases, NADPH oxidases, cyclooxygenases, cytochromes *P*450, xanthine oxidase, etc.). ROS can be eliminated by endogenous antioxidant systems. For instance, glutathione and thioredoxin systems decrease the cellular levels of H_2O_2 by catalyzing a reaction in which H_2O_2 is reduced to H_2O. Likewise, the enzyme catalase eliminates H_2O_2 by transforming two molecules of H_2O_2 into two molecules of H_2O and one molecule of O_2. Antioxidant agents can reduce the cellular levels of ROS by preventing their generation or by favoring their elimination. For instance, some polyphenols (e.g. ►flavonoids) can prevent the generation



Hydrogen Peroxide. Figure 1 Aerobic cells generally use O_2 to generate energy (ATP) through a process called oxidative phosphorylation (oxphos), but they can also use O_2 to generate reactive oxygen species such as hydrogen peroxide (H_2O_2).



Hydrogen Peroxide. Figure 2 While the controlled generation of H_2O_2 has an important physiological role, a sustained increase in the cellular levels of H_2O_2 can produce carcinogenic effects, and an excessive increase in the levels of H_2O_2 can induce cell death. Antioxidant agents can reduce the cellular levels of H_2O_2 and prevent the carcinogenic effects induced by H_2O_2; these agents may therefore exert a cancer-preventive activity. Prooxidant agents can increase the cellular levels of H_2O_2 and may induce carcinogenic effects. A sufficient increase in the cellular levels of H_2O_2 induced by prooxidant agents may trigger cell death and be therapeutically useful.

of H_2O_2 by scavenging $O_2^{\bullet-}$; and selenium compounds can favor the elimination of H_2O_2 by providing selenium atoms, which are essential components of the H_2O_2-detoxifying enzyme glutathione peroxidase. Prooxidant agents, on the contrary, can increase the cellular levels of ROS by increasing their generation or by reducing their elimination. For instance, arsenic can increase ROS generation by activating the enzyme NADPH oxidase, and buthionine sulfoximine (an inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme of glutathione synthesis) can reduce ROS elimination by decreasing glutathione-mediated H_2O_2 decomposition.

The controlled generation of ROS plays an important role in the physiological control of cell function. For instance, cells under hypoxic conditions generate H_2O_2 and use it to activate hypoxia-inducible factor 1 (HIF-1) [►hypoxia and tumor physiology], a transcription factor that codes for many proteins that help cells adapt to low oxygen levels. An uncontrolled or excessive cellular production of H_2O_2, however, can produce carcinogenic effects and cell death (Fig. 2).

Carcinogenic Effects

Cancer cells from different tissues have been observed to produce high amounts of H_2O_2. High cellular levels of H_2O_2 have been associated with DNA alterations, including DNA damage, mutations, and genetic instability. For instance, transition metals such as iron or copper can react with H_2O_2 to produce hydroxyl radical ($OH^{\bullet}$) via the Fenton reaction; $OH^{\bullet}$ is a highly reactive species known to produce ►oxidative DNA damage. The production of DNA alterations by H_2O_2 may play an important role in ►carcinogenesis, as cancer is considered to be a genetic disease caused by DNA alterations.

Cell proliferation, ►apoptosis resistance, ►angiogenesis, invasion and ►metastasis are key events of the carcinogenesis process. It is well known that, in order for cancer to develop, tumor cells must proliferate. Accumulating data have shown that H_2O_2 stimulates cell proliferation; in fact, the cell proliferation induced by different stimuli can be decreased by H_2O_2-detoxifying enzymes (e.g. catalase). Under

physiological conditions, cells with irreparable damages usually commit suicide by triggering a programmed process called apoptosis. Since tumor cells have important damages in many of their components, the formation of a cancer requires that tumor cells develop apoptosis resistance. Interestingly, it has been found that non-cytotoxic concentrations of H_2O_2 can produce apoptosis resistance in cancer cells. Angiogenesis – the generation of new blood vessels – is also necessary for the formation of a solid tumor; without vascular growth, the tumor mass is restricted to within a tissue-diffusion distance of approximately 0.2 mm. Recent results support that H_2O_2 has an important function in angiogenesis. For instance, angiopoietin-1 plays an important role in angiogenesis and it has been found that its angiogenic effect is mediated by H_2O_2. It is recognized that the metastatic spread of primary tumors accounts for approximately 90% of all cancer deaths. The process by which cells from a localized tumor invade adjacent tissues and metastasize to distant organs is therefore the most clinically relevant processes involved in carcinogenesis. H_2O_2 has been found to modulate the activity of several processes (e.g. cell ►motility, migration, adhesion) and molecules (e.g. ►MET, ►matrix metalloproteinases) involved in tumor invasion and metastasis. Indeed, studies carried out in animal models have revealed that the targeted delivery of catalase can inhibit tumor metastasis.

Although the transcription factor HIF-1 plays an important role in the physiological control of cell function, HIF-1 overexpression is commonly observed in most human cancers and has been associated with increased patient mortality in several cancer types. HIF-1 increases the transcription of a variety of genes that code for proteins involved in processes intimately related to cancer, including apoptosis resistance, angiogenesis or invasion and metastasis. H_2O_2 can both activate HIF-1 and mediate the activation of HIF-1 caused by different stimuli; in fact, the presence of enzymes that reduce the cellular levels of H_2O_2 prevents the activation of HIF-1 caused by different triggers (e.g. hypoxia, TNF-alpha). The key role of H_2O_2 in carcinogenesis is also supported by experimental data that have demonstrated that H_2O_2 can cause and mediate cell malignant transformation. For instance, it has been reported that the expression of Nox1 (a homologue of gp91phox, the catalytic moiety of the $O_2^{\bullet-}$-generating NADPH oxidase of phagocytes) in normal NIH3T3 fibroblasts resulted in cells with malignant characteristics that produced tumors in athymic mice. These transformed cells showed a 10-fold increase in H_2O_2 levels. When catalase was overproduced in these transformed cells, H_2O_2 concentration decreased, and the cells reverted to a normal appearance, the growth rate normalized, and cells no longer produced tumors in athymic mice.

Clinical Relevance

Despite some important advances in cancer therapy, the number of cancer deaths has not decreased in the last three decades. New strategies to control this disease are required. It is recognized that cancer chemoprevention (the use of chemicals to prevent, stop or reverse the process of carcinogenesis) is an essential approach to controlling cancer. Since H_2O_2 seems to have an important role in carcinogenesis, a chemical capable of preventing or decreasing excessive cellular levels of H_2O_2 might be useful in cancer chemoprevention.

Some complementary and alternative medicine practitioners have used H_2O_2 and other "hyperoxygenation" therapies for the treatment of cancer. In 1993, the American Cancer Society studied the available literature and found no evidence that treatment with H_2O_2 and other "hyperoxygenation" therapies was safe or resulted in objective benefit in the treatment of cancer. Today it is accepted that the direct administration of H_2O_2 is not an appropriate strategy for the treatment of cancer, as H_2O_2 can produce toxicity by oxidizing macromolecules such as DNA, proteins or lipids. Indeed, as discussed above and represented in Fig. 2, a large body of research strongly supports that H_2O_2 can produce carcinogenic effects. However, an increasing number of reports indicate that a sufficient increase in the cellular levels of H_2O_2 may be an effective therapeutic strategy. For instance, it is recognized that many anticancer drugs currently used in the clinic produce their antitumor activity by inducing apoptosis in cancer cells, and H_2O_2 is an effective inductor of apoptosis in cancer cells. In addition, some studies have shown that specific concentrations of H_2O_2 can kill cancer but not normal cells; it has been proposed that the increased levels of H_2O_2 found in tumor cells may account for their increased susceptibility to H_2O_2. This selectivity for cancer cells is in accordance with animal experiments that have shown that the use of H_2O_2-generating systems can deliver H_2O_2 to sites of malignancy and produce anticancer effects with little toxicity to the host. The therapeutic potential of H_2O_2 is also supported by the fact that the anticancer activity of several drugs commonly used in the clinic (e.g. paclitaxel [►taxol], arsenic trioxide) is mediated, at least in part, by an increase in the cellular levels of H_2O_2. Recent data have also shown that high concentrations of vitamin C (only achievable through the i.v. route) can produce selective killing of cancer cells through a H_2O_2-dependent mechanism. Therefore, although the direct administration of H_2O_2 does not seem an appropriate anticancer approach, any strategy capable of increasing the levels of H_2O_2 in cancer cells might be therapeutically useful.

Accumulating evidence suggests that the modulation of the cellular levels of H_2O_2 may be an important

approach for the development of cancer chemopreventive and therapeutic strategies. Chemicals with antioxidant properties may reduce the cellular levels of this oxidant and produce cancer chemopreventive effects; indeed, most cancer chemopreventive agents have antioxidant properties. Chemicals with prooxidant properties may induce a sufficient increase in the cellular levels of H_2O_2 and produce cell death in cancer cells; these drugs may produce a chemotherapeutic effect. Many chemicals (e.g. vitamin C, vitamin E, β-carotene, ►curcumin, ►sulforaphane, ►epigallocatechin, etc) induce either an antioxidant or prooxidant effect mostly depending on the concentration at which they reach the cells. Low concentrations of these agents would therefore produce chemopreventive effects, while high concentrations would produce chemotherapeutic effects. However, these agents may produce carcinogenic effects when used at concentrations that increase the cellular levels of H_2O_2, but not sufficiently to induce cell death (Fig. 2). Antioxidant/prooxidant drugs may act as cancer preventive, therapeutic or carcinogenic agents mostly depending on their dose and route of administration.

►Oxidative Stress

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Hydrophilic

Definition

Literally means water loving. These are drugs that much prefer to dissolve in water than in fat.

►ADMET Screen

Hydroquinone

Definition

Product of two-electron reduction of quinone ($C_6H_4(OH)_2$).

►Mitomycin C

Hydroxyapatite

Definition

Hydroxyapatite crystals constitute the major and essential component of normal bone and teeth. Hydroxyapatite crystals represent the mineral matrix of bone and teeth and give rigidity to bones and teeth. The chemical formula of basic hydroxyapatite is $Ca_{10}(PO_4)_2OH_6$ and these crystals can be physiologically substituted with magnesium, fluor, or carbonate.

►Zoledronic Acid
►Bisphosphonates

Hydroxybutyrate Dehydrogenase

►Serum Biomarkers

5-HydroxyIndoleacetic Acid (5-HIAA)

Definition

A breakdown product of serotonin that is eliminated in the urine. Urinary levels of 5-HIIA are used as a diagnostic and monitoring tool in carcinoid tumors.

►Neuroendocrine Carcinoma

Hydroxyl Group

Definition
Functional group consisting in an atom of oxygen joined to a hydrogen atom by a single bond.

►Bisphosphonates

Hydroxylapatite

Definition
Synonym ►hydroxyapatite $Ca_5(PO_4)_3(OH)$.

14-Hydroxyldaunorubicin

►Adriamycin

4-Hydroxynonenal

Definition
An unsaturated hydroxyalkenal that is generated by the breakdown of arachidonic acid during stress in cells.

►Glutathione Conjugate Transporter RLIP76

13-Hydroxyoctadecadienoic Acid (13-HODE)

Definition
A primarily growth stimulatory signaling molecule resulting from the metabolism of LA by 15-lipoxygenase-1 in cancer cells.

►Melatonin

2-Hydroxyoleic Acid

Definition
Synonym α-hydroxyoleic acid and 2-hydroxy-9-cis-octadecenoic acid. Synthetic derivative of oleic acid with anticancer activity against a wide variety of cancer types, oral administration and negligible side effects.

►Membrane-Lipid Therapy

7-Hydroxystaurosporine

►UCN-01 Anticancer Drug

7-Hydroxystaurosporine, NSC 638850

Definition
►UCN-01 Anticancer drug

Hydroxysteroid Dehydrogenases

►Reductases

Hyperalgesia

Definition
Type of pain that is frequently associated to cancer chemotherapy or cancer itself. Hyperalgesia is an increased sensitivity and lowered threshold to a stimulus – such as burn of the skin – that is normally painful. Allodynia is caused by a stimulus – such as touch, pressure and warmth – that does not normally provoke pain.

►Cannabinoids and Cancer
►Allodynia

Hypercalcemia

Definition
An excess of calcium in the blood (normal range: 9–10.5 mg/dL or 2.2–2.6 mmol/L). Can be due to excessive skeletal calcium release, increased intestinal calcium absorption, or decreased renal calcium excretion. Symptoms of hypercalcemia include fatigue, vomiting, depression, confusion, anorexia. Severe hypercalcemia (range above 15–16 mg/dl) may lead to coma and cardiac arrest.

►Vitamin D
►Bone Loss, Cancer Mediated

Hyperchlorhydria

Definition
Is the increased gastric acid secretion.

►Gastrinoma

Hyperdiploidy

Definition
Having a chromosome number that is more than the normal diploid number.

►Acute Lymphoblastic Leukemia

Hypergastrinemia

Definition
Is increased serum gastrin.

►Gastrinoma

Hyperglycemia

Definition
Is a condition of elevated glucose levels in the bloodstream. It is a hallmark of metabolic syndrome and diabetes.

►Adiponectin

Hypericin

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Synonyms
Hypericum extract; Hypericum red

Definition
Hypericin is a natural ►photosensitizer present in the plant, *Hypericum perforatum*, commonly known as St John's wort. It belongs to the class of phenanthroperylenequinones and napthodianthrone, has a molecular weight of 504.45. St John's wort has been used for centuries to treat mental disorders and nerve pain.

In ancient times, herbalists wrote about its use as a sedative and a treatment for malaria, as well as a balm for wounds, burns, and insect bites.

Today, St John's wort is used by some for depression, anxiety, and/or sleep disorders. Hypericin is used in photodynamic diagnosis (►PDD) and photodynamic therapy (►PDT) for diagnosis and treatment of cancers.

Characteristics
Physical Properties
Hypericin yields red fluorescence when excited with a specific wavelength of light by lasers such as 442 nm (He-Cd), 488 nm (Ar), or 543 nm (He–Ne), and light by xenon-arc lamp with a band-pass filter of 380–450 nm or 375–400 nm. It has a high extinction coefficient near 600 nm. The two main maximum absorption and ►photoactivation peaks of hypericin occur near 550 and 600 nm. The two fluorescence peaks of hypericin are near 600 and 650 nm. Hypericin has very low water solubility. The aggregated hypericin is not fluorescent. It generates a high quantum yield of singlet oxygen and superoxides.

H

Clinical Applications

PDD of Cancers

The initial presentations of bladder cancer in 70–80% of the cases are superficial and limited to the urothelial lining of the bladder mucosa and submucosa. Visual differentiation between normal tissue and transitional cell carcinoma is relatively easy but not for carcinoma in situ or nonmalignant diseases such as cystitis due to radiotherapy, chemical or bacterial origins. They are often invisible to the naked eye. ►Fluorescence cystoscopy is a form of PDD of cancer and has significantly improved the diagnosis and early detection of cancer. In bladder cancer detection, hypericin has several advantages over conventional photosensitizer, ►5-aminolevulinic acid (►5-ALA, a prodrug) (Table 1). A human clinical trial had found that the use of hypericin showed higher sensitivity (82%) than just white-light cystoscopy (62%). This report justifies the use of hypericin-PDD for bladder cancer in clinical settings. Hypericin was also shown to accumulate in patients with stomach cancer, and detected stomach cancer with 85% specificity.

►PDT of Cancers

►Basal cell carcinoma and ►squamous cell carcinoma are the most common cancers among the Caucasians. The effect of hypericin-PDT had been investigated in these skin cancers. It was found that 1 mg or more of hypericin was acceptable as an alternative treatment for basal cell carcinoma with clinical remission achieved in 2 out of 11 patients. One out of eight patients with squamous cell carcinoma achieved clinical remission without sign of recurrence observed after 5 months of treatment with 1.5–3 mg of hypericin. Mild to transient erythema and edema at the tumor lesions were observed when the cancers were treated with hypericin and there appeared to be a correlation between the degree of tumor reduction and occurrence of erythema and edema at the tumor lesions. As this is the first reported clinical trial on the use of hypericin-PDT in the reported skin cancers, the PDT treatment protocol and dosage were transposed from results with animal models and the trial was restricted to 4–6 weeks because of ethical reasons. Thus, long-term remission rates are needed to further evaluate the clinical usefulness of hypericin with additional trials.

Another hypericin-PDT use in cancer treatment was for local application of hypericin in a patient with recurrent malignant mesothelioma. The patient had previously been treated with radiotherapy, chemoimmunotherapy and subsequently PDT using hematoporphyrin derivatives (Photosan III). Topical hypericin was applied to the patient and a month later, the combination of hypericin and Photosan III was applied. It was found that complete remission was achieved histologically with a light dose at 90 J/cm^2 together with PDT using combined systemic Photosan III and topical application of hypericin.

Preclinical Investigations

The chemical groups reported to cause the photodynamic activities of hypericin are semiquinone, singlet oxygen, and superoxide anion radicals. Light triggers the photosensitization (photoactivation) of hypericin for photodynamic reactions. Hypericin-PDT effect is a function of drug, light, and oxygen present. There are three PDT effects of the photosensitizers on biological systems. Firstly, it can damage the vasculature, causing thrombosis and blood flow stasis. Secondly, PDT kills tumor cells via generation of free radicals that damage mitochondria, plasma membrane, and other organelles, hence inducing apoptosis or necrosis of the tumor. Thirdly, PDT modulates the immune system against cancer cells. Successful cancer treatment is usually a result of the combination of all these effects. With the appropriate use of light and adjuvant therapy such as heat or ►antiangiogenesis agents, better therapeutic outcomes can be expected. Table 2 shows the reported methods to improve the efficiency of hypericin for PDD and PDT in preclinical investigations. As

Hypericin. Table 1 Comparison of 5-ALA and hypericin for detecting human superficial bladder carcinoma

Characteristics	5-ALA	Hypericin
Form	Prodrug, need to be converted to the active form	Active form
Sensitivity	75–100%	82–94%
Specificity	Very low specificity, 43–68.5% (with many false-positives)	High, 91–98.5%
Stability	Easily photobleached during process	Greater stability, no significant photobleaching
Permeability across biological membrane	Charged molecule – difficult to cross biomembranes	Hydrophobic – good permeability

Hypericin. Table 2 Methods for enhancement of hypericin-PDD/PDT efficacy

Concept	Methods
Chemical modifications	Improve physicochemical properties of hypericin. Analogs with better water solubility, absorption and fluorescence properties, and singlet oxygen quantum yield
Physical methods	Optimize light conditions (fractionated light dose), oxygen perfusion, hyperthermia
Pharmaceutical formulations	Prepare liposomes, nanoparticles, topical applications
Combination with adjuvant therapy	Add oxygen carrier, antiangiogenesis agents, drugs that are able to synergize with hypericin for cancer treatment

administration of hypericin to patients remains a challenging task due to its poor aqueous solubility and lipophilic nature, the most popular approach is by a formulation approach to enhance the efficacy of hypericin-PDT. There is a trend to develop formulations of hypericin without plasma proteins. Despite the use of cutting-edge technology approaches, such as preparing transferrin-mediated targeted delivery liposomes and nanoparticles of hypericin, such preparations did not always confer the desired enhanced treatment effects. It appeared that the use of an adjunct with hypericin, especially those prepared without using plasma proteins, was more successful in enhancing the delivery of hypericin with both in vivo and in vitro systems.

Using a pharmaceutical solvent and penetration enhancer, *N*-methyl pyrrolidone, delivery of hypericin was shown to be better than the conventional formulation of hypericin with albumin. Using chick chorioallantoic membrane implanted with human bladder cancer cells, *N*-methyl pyrrolidone was demonstrated to be an excellent alternative to plasma proteins as adjuvant with hypericin. The improved *N*-methyl pyrrolidone–hypericin formulation enabled more efficient delivery and uptake of hypericin at the target site. This will not only allow a lower drug dose to be used potentially but will also significantly reduce patients' waiting time.

The exact mechanism for the tumor selectivity uptake of hypericin has not been fully understood. The uptake/transport/delivery of hypericin is dependent on several competing or interrelated factors, which include the type of incubation medium (with or without serum proteins), cell type, delivery system, and biological testing method. It is likely that both active and passive transport mechanisms contribute to the overall uptake of hypericin but passive diffusion is likely to be the more dominant mechanism.

Apart from its photosensitizing properties, hypericin was found to be a ►protein kinase C inhibitor. It was also reported to involve in the modulation of immune system such as expression of cytokines. Hypericin-PDT triggered the expression of angiogenic factors, ►matrix metalloproteinases, and various signaling pathways. The rapid advancement in genetic research had shown that the effects of hypericin-PDT could be independent of the status of ►*p53*, the tumor suppressor gene. Many reports have attributed the failure of conventional chemotherapy and radiotherapy to the mutated ►*p53*, due to apoptosis failure caused by the nonfunctional p53. Therefore, hypericin-PDT provides an alternative pathway in treating cancers.

In the absence of light, toxicity of hypericin was found to be very low. Apart from its potent photosensitizing properties that are light-dependent, hypericin shows unique activities even in the dark. In complete darkness, hypericin has cytostatic activities that were able to prolong the survival of mice with high-grade squamous carcinoma tumors. This is attributed to hypericin inhibiting several key steps of the angiogenesis.

Many different types of cancers including leukemia, glioblastoma, and childhood rhabdomyosarcoma have been treated with hypericin-PDT and in vitro results were promising in many of the cases. Continual effort in basic and translational research will lead to a better understanding of the contributing factors responsible for effective hypericin-PDT applications.

►Photodynamic Therapy

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Hyperlipidemia

Definition
Refers to an elevation of lipids (fats) in the bloodstream.

►Cachexia

Hypermethylation

Definition
Increased ►methylation of cytosine residues in cytosine-guanine pairs in regulatory regions of DNA of specific genes or of global DNA within a cell or tissue.

►Fragile Histidine Triad
►Methylation-Controlled J Protein (MCJ)

Hyperplasia

Definition
Consists of an increase in the number of tissue cells, without changes in their volume. This alteration, generally secondary to prolonged growth stimuli, is reversible and does not evolve to malignancy.

►Preneoplastic lesions

Hyperplastic growth

Definition
Tissue overgrowths that still retain their normal characters and ability to differentiate.

Hypersensitivity

►Allergy

Hypersensitivity Reaction

Definition
A serious and sometimes life-threatening reaction to a drug or other chemical or venom.

►Paclitaxel

Hyperthermia

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Definition
Hyperthermia refers to the elevation of temperature above physiological levels, typically to values of 40–45°C.

Characteristics
Hyperthermia utilizes elevated tissue temperatures, typically between 40°C and 45°C, to alter the tumor and normal tissue environment. The goal of hyperthermia in cancer treatment is to create an environment that will aid in eradicating tumor while sparing normal tissue. Hyperthermia accomplishes this by causing direct cytotoxic effects and a variety of physiologic effects, including the alteration of blood flow and oxygenation status. Clinically, hyperthermia can work synergistically with both radiation and chemotherapy.

Cytotoxic Effects
Hyperthermia elicits cytotoxic effects in tissue through a variety of mechanisms, inducing both ►apoptosis and ►necrosis. Above 40°C, protein is a dominant molecular target, with protein denaturation exhibiting a similar heat of inactivation as thermal cell kill and damage (130–170 kcal/mole). Other cellular targets include the cytoskeleton, which controls many signal transduction pathways, cellular respiration enzymes, and DNA repair processes. These components of the cell are particularly heat sensitive and lead to increased cytotoxic effects.

Physiologic Effects
Hyperthermia also has an intricate relationship with tumor physiology, as the degree of physiologic response varies with temperature. An initial temperature increase to 41–41.5°C will result in elevated blood flow and increased vascular permeability.

Generally, muscle and skin perfusion increase by at least tenfold, while tumor perfusion increases only 1.5- to 2-fold. This difference in response between normal and cancerous tissue is one of the most exploited benefits of hyperthermia in cancer treatment. At these initial hyperthermia temperatures, edema may result from the increase in vascular permeability, and as higher temperatures are reached, vascular stasis and hemorrhage may develop. Additionally, higher temperatures may result in vascular damage, although typically these temperatures are neither utilized nor often achievable in the clinic.

With respect to normal tissue, thermal damage from hyperthermia exhibits varying degrees of severity depending on temperature and tissue type. Some normal tissues are more heat sensitive than others, yet the sensitivity is not easily predicted by classical cytotoxicity or radiotherapy principles. While these principles correlate proliferative potential with radiation or chemo-sensitivities, this is not predictive of thermal sensitivity. For example, the brain and testes both exhibit high thermal sensitivities, yet the brain has almost no proliferative potential and the testes are highly proliferating. Furthermore, in addition to showing no correlation between thermal sensitivity and proliferative potential, there is also no tissue-specificity. For instance, the spinal cord, peripheral nerves, and brain all demonstrate varying sensitivity to heat, yet these are all nervous tissue.

In addition to physiologic changes in blood flow and vascular permeability, ►tumor metabolism and oxygenation can also be altered substantially with hyperthermia. As mentioned earlier, certain enzymes may be highly heat sensitive. Along metabolic pathways, enzymes involved in aerobic tumor metabolism exhibit much greater heat sensitivity than those involved in anaerobic metabolism. This lends itself to a theory that hyperthermia treatment may cause reductions in tumor metabolism and respiration. Reported effects in support of this theory have been decreased ATP production and increased lactate concentration following hyperthermia.

Furthermore, alteration in tumor metabolism has additional downstream effects, particularly with respect to tumor oxygenation status. As a tumor shifts from aerobic to anaerobic metabolism following hyperthermia, the tumor may experience decreased oxygen consumption rates; this decrease in oxygen consumption may consequently lead to significant improvement in the tumor oxygenation status. Studies have shown oxygenation increases following hyperthermia in rodent and canine tumors, as well as human tumors. However, this oxygenation effect is most pronounced at temperatures between 41°C and 43°C, decreasing at higher temperatures where vascular damage occurs.

Immunological Effects

Hyperthermia can augment the body's innate immunity as well as increase immune activity specifically towards tumors. In general, the body increases in temperature as a response to infection; many bacterial or viral pathogens are heat sensitive and thus an elevation in body temperature serves as a primary method of defense against invading agents. Furthermore, as body temperature rises, cellular metabolism does as well, which can aid in accelerating immunologic responses.

With regards to immune response towards tumor, hyperthermia can induce maturation of ►dendritic cells, cause increased lymphocyte trafficking, and engage in ►heat shock protein 70 (HSP70) mediated immune response, which in turn increases T-cell specific antitumor activity and further stimulates dendritic cells. These physiologic responses augment the body's natural immune response against tumors.

Hyperthermia Physics

Delivery Modalities

Hyperthermia may be delivered using either invasive or non-invasive sources. Non-invasive techniques are generally preferred, and consist of two main methods of delivering heat to tissues: ►electromagnetic (EM) heating or ►ultrasound. While the underlying physics principles of heat deposition differ between EM and ultrasound modalities, they are similar in a variety of ways. Both are sensitive to tissue heterogeneity as well as blood flow geometry, and are susceptible to difficulties of energy coupling into tumor tissue.

Materials with finite electrical conductivity experience energy dissipation with the flow of electric current; this is the principle behind ohmic, or resistance electromagnetic heating. Delivery of EM heating is dependent on both frequency and wavelength. As EM frequency increases over the radio frequency (RF) and microwave (MW) range, electrical conductivity also increases. Since energy deposition in tissue is proportional to the local tissue conductivity and the magnitude of the electric field, these higher frequencies result in greater energy deposition and shorter penetration depths. Additionally, in the design of antennae or electrodes to deliver the EM field, there is a general constraint that limits the ability to localize heating at depths greater than 2–5 cm. This arises from the relation between EM frequency and wavelength to the physical size of the antenna or electrode source. Longer wavelengths will penetrate deeper into tissue, but require physically larger heating antennae, thus preventing localization of the EM field. This tradeoff between localization and penetration yields a critical limitation in electromagnetic heating: localized heating is limited to depths less than 2–5 cm. At depths greater than 2–5 cm EM heating will result in regional energy deposition, potentially involving large volumes of normal tissue.

In accordance with these depth limitations, electromagnetic heating devices are typically divided into two categories: superficial applicators which provide focused heating at depths less than 2–5 cm and deep heating devices which distribute heat to a deeper penetration but over a broader range. Superficial devices consist of waveguides and microstrip or patch antennas which operate in typical microwave band frequencies (433, 915, or 2,450 MHz) and usually require a water bolus to couple energy into the tissue. The deep heating devices typically used are magnetic induction, capacitive coupling, and phased array fields. These devices operate at lower frequencies, between 5 MHz and 200 MHz. A schematic illustration of a phased array device for deep heating is shown in Fig. 1.

Similarly to electromagnetic heating, ultrasound also experiences decreased penetration with increasing frequency. As an advantage over EM, however, ultrasound operates with much lower wavelengths and consequently avoids the severity of problems associated with focusing at greater depths. Unfortunately, ultrasound is limited by resolution, since it is particularly sensitive to the reflective and absorptive effects of air and bone, respectively. This imposes severe limitations of ultrasound heating in regions of tissue heterogeneity and changing geometry.

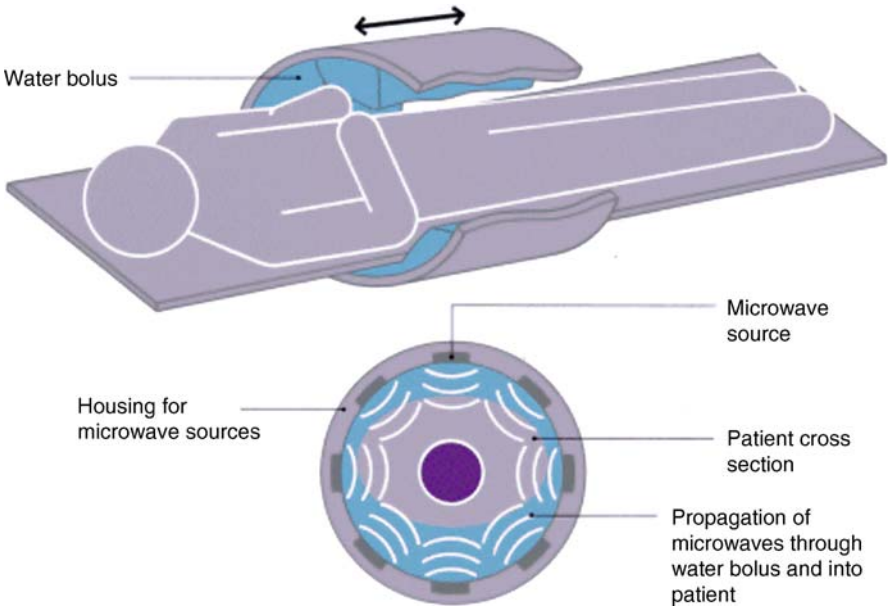
Ultrasound devices are also separated into categories of superficial and deep heating. Single and multiple transducer plane wave devices operating with frequencies between 1 MHz and 3 MHz have been designed for superficial tumors, while at depths greater than 2–5 cm scanned focused transducers, phased arrays, or multiple scanned focused transducers in the 0.5–2 MHz range are used. Ultrasound devices require a temperature controlled water bolus for energy coupling.

Thermometry

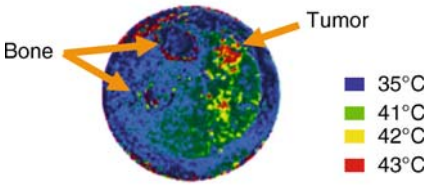
One of the greatest challenges facing clinical hyperthermia is the difficulty in obtaining accurate and noninvasive thermometry. Currently, most thermometry is performed through placement of ►catheters into the tumor via ultrasound or CT guidance; thermometers are then placed within the catheters. Temperature readings are attained statically with multipoint thermometers or dynamically by moving a single point thermometer through the catheter during heating.

There are many disadvantages to the common method of invasive thermometry. For one, there is a greater risk to the patient. Invasive methods are uncomfortable and present a risk for infection and hemorrhage. Additionally, the procedure is lengthy, requiring image registration and extra time of the physician. Perhaps most crucially, the data obtained from invasive methods is limited and often does not provide much spatial data, which in turn limits the control and uniformity of the hyperthermic treatment.

In an attempt to gain better uniformity of treatment, noninvasive thermometry methods have been developed; these methods rely mostly on ►Magnetic Resonance Imaging (MRI) techniques, although a variety of technologies are being pursued. MR imaging has many temperature sensitive parameters that can be exploited for thermometry purposes, including relaxation times T_1 and T_2, bulk magnetization, and proton resonance frequency. Of these four parameters, the proton resonance frequency is the most commonly



Hyperthermia. Figure 1 Illustration of an annular phased microwave array for heating deep situated tumors.



Hyperthermia. Figure 2 Example of noninvasive thermometry data acquired with phase difference (chemical shift) MRI in a sarcoma of the lower leg. This method can yield temperature data with a resolution of 1.0°C.

used, and has demonstrated superior temperature sensitivity and resolution. Clinical studies have illustrated resolutions of 0.5–1°C with MRI thermometry. Figure 2 shows a temperature distribution image obtained from an MRI of a sarcoma in the lower leg.

Despite the many advantages hyperthermia affords, clinical implementation has proved challenging. As mentioned above, hyperthermia heating regimens can be difficult to reproduce, utilizing invasive thermometry methods that have limited spatial coverage. Additionally, the definition and calculation of thermal dose used in hyperthermia is not well defined, making clinical trial comparisons between institutions difficult. However, the technology associated with clinical hyperthermia continues to improve, and many of these challenges are being addressed in ongoing research.

Thermal Dosimetry

The Arrhenius relationship is used to describe the rate of cell kill for a given temperature over time, and provides the basis for thermal dosimetry. Hyperthermia cell survival curves decrease exponentially, with higher rates of cell kill at increased temperatures. Arrhenius plots can be determined from in vitro data by taking the log of the slope of cell survival curves as a function of temperature. A typical graph of surviving fraction with temperature and its resulting Arrhenius plot is given in Fig. 3.

Arrhenius plots are characterized by their biphasic nature, with the temperature at which the slope changes commonly referred to as the "breakpoint;" this phenomenon is derived from tissues developing thermal tolerance. For human tissue, the "breakpoint" occurs at ~43.5°C. Below the breakpoint, the rate of cell kill decreases twofold to fourfold for each 1.0°C decline in temperature. Above the breakpoint, the rate of cell kill doubles for each 1.0°C elevation of temperature.

Achieving constant temperatures over time is difficult in tumor tissue, leading to non-uniformity in hyperthermia delivery between patients. Since the in vivo slopes of the Arrhenius plots are practically identical to the in vitro slopes, and they provide a correlation between rate of cell kill and temperature, the Arrhenius relationship is used as a method for normalizing thermal data between different patients or treatments. This is expressed as Cumulative Equivalent Minutes at 43°C (CEM 43°C) and is given by the equation:

$$\text{CEM } 43^{\circ}\text{C} = \text{tR}^{(43-\text{T})}$$

Where t = time of treatment in minutes, T = average temperature in Celsius during treatment, and R is a constant, equal to 0.5 above the breakpoint and 0.25 below. In more complex situations with large temperature variations, the thermal data may be broken into shorter time intervals of 1–2 min where the temperature remains relatively constant. In these instances the total CEM 43°C for the treatment is attained through summation of CEM 43°Cs for each interval period. Also referred to as the thermal isoeffect dose, the CEM 43°C has been a valuable predictor of treatment efficacy and standardization of heat treatment in clinical trials.

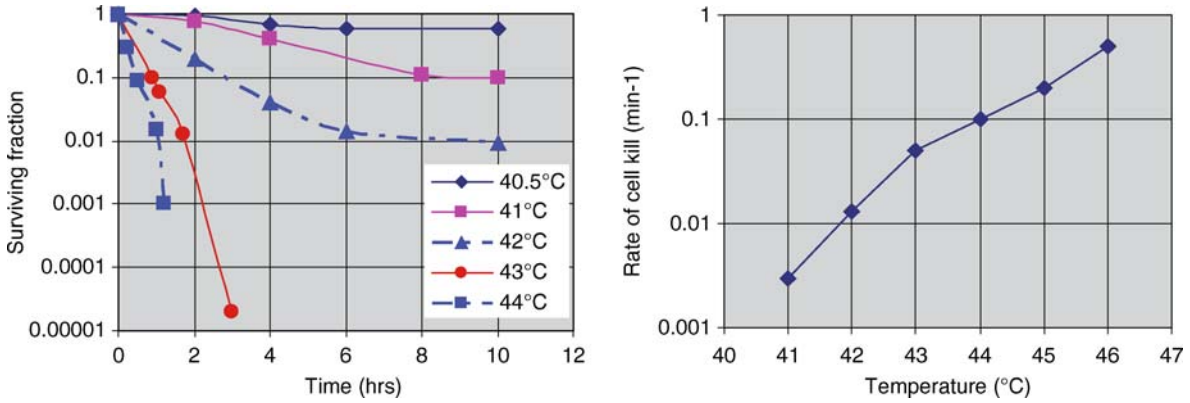
Clinical Hyperthermia

Hyperthermia has been used to treat superficial tumors as a lone method of treatment. However, while the occasional response is observed, in general heat treatment alone is not associated with long term tumor control. While the practice of using solely hyperthermia is still performed in some clinics, this use is not supported by peer-reviewed published scientific data. Instead, hyperthermia is best utilized as a synergistic adjunct modality to oncology treatments, including radiation and chemotherapy. Figure 4 underscores the important physiological processes of hyperthermia in augmenting radiation and chemotherapy efficacy.

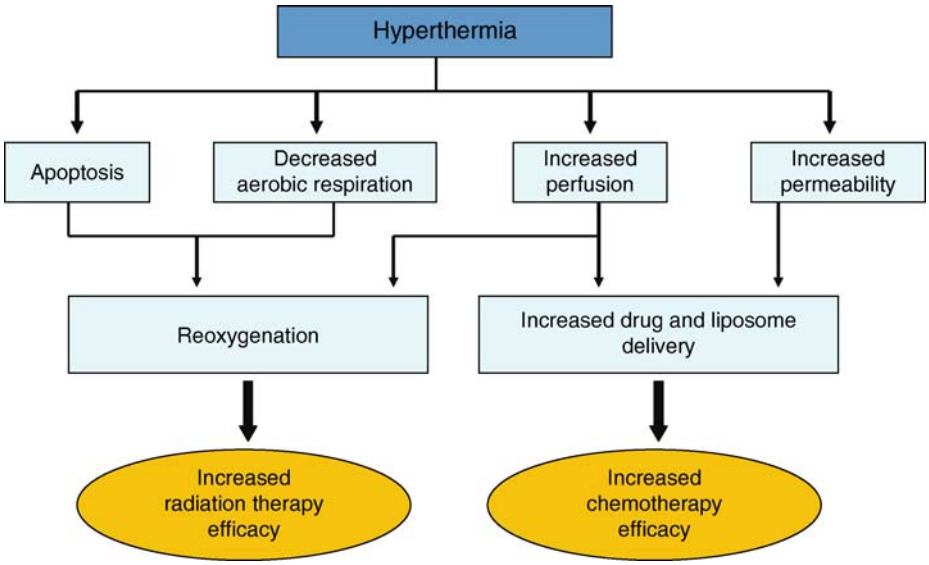
Radiation Therapy and Hyperthermia

The rationale for combining hyperthermia with radiation therapy is based on a variety of synergistic effects. For one, the areas of radiation resistivity and heat sensitivity are complimentary. While cells in ▶S-phase are typically radioresistant, this is the most heat sensitive phase of the cell cycle. Also, hypoxic cells are typically three times more resistant to radiation than normoxic cells, whereas there is no difference in thermal sensitivity based on cellular oxygenation status. Furthermore, hyperthermia can augment radiation response by causing reoxygenation of tumor tissue and inhibiting sublethal repair through inactivation of critical DNA repair pathways.

Clinically, the therapeutic gain of combining radiation therapy and hyperthermia is determined by the Thermal Enhancement Ratio, or TER. The TER is defined as the ratio of radiation dose to achieve an isoeffect for radiation divided by the radiation dose to achieve the same effect with combined radiation and hyperthermia. Typical TER values for local tumor control are greater than one, and are typically smaller



Hyperthermia. Figure 3 Example of thermal cell survival curves (on the left), plotted as surviving fraction over time at a specific temperature. On the right, the Arrhenius plot is shown for the data. The breakpoint is observed at 43°C.



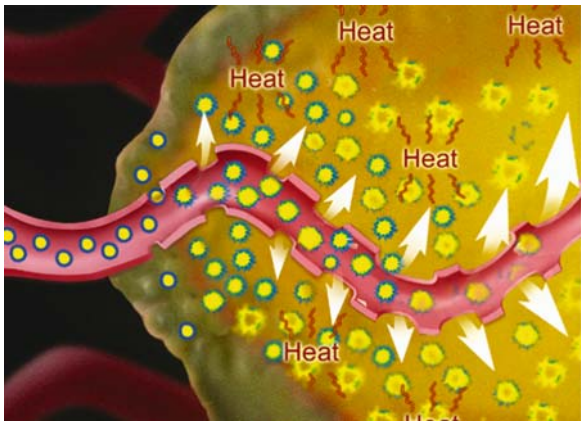
Hyperthermia. Figure 4 Physiologic benefits of low temperature (40–43°C) hyperthermia and the rationale behind synergistic radiation and chemotherapy uses.

for normal tissue damage, suggesting therapeutic gain for a combined hyperthermia and radiation course over radiation alone.

Chemotherapy and Hyperthermia

Hyperthermia also exhibits synergism with some chemotherapeutic agents, through increasing cellular uptake, oxygen radical production, and DNA damage, while inhibiting DNA repair. Chemotherapeutic drugs that have been shown to have heightened efficacy when combined with hyperthermia include ►cisplatin, ►nitrogen mustards, doxorubicin (►Adriamycin), ►nitrosoureas, ►bleomycin, ►mitomycin C, and hypoxic cell sensitizers. While this list of drugs that are synergistic with hyperthermia are many, there are also those that have not shown interaction when combined with heat, such as ►etoposide and the ►vinca alkaloids.

In addition to showing additive effects with traditional chemotherapeutic agents, hyperthermia also offers an avenue for exploitation of innovative drug delivery strategies. Liposomes (►Liposomal Chemotherapy) are nanoparticle lipid vesicles that can be loaded with a variety of high concentration chemotherapeutics. These liposomes show enhanced extravasation following hyperthermia due to increased permeability of the vasculature. Furthermore, this enhanced accumulation is tumor specific, due to the discrepancy in degree of permeability increase between



Hyperthermia. Figure 5 Illustration of the principles underlying thermosensitive liposome efficacy. Thermosensitive liposomes release their contents upon heating to a desired temperature, and release drug both within the tumor vasculature and after extravasation into tumor tissue.

tumor and normal blood vessels. Not only does hyperthermia increase overall liposomal extravasation, but novel thermosensitive liposomes have been developed with lipid bilayers that degrade upon reaching a thermal transition temperature, typically designed to release in a range of 40–43°C. This allows for both targeted and triggered drug release into tumor tissue. Figure 5 shows an illustration of thermosensitive liposomes extravasating into tumor tissue and releasing their contents upon heating.

There are a variety of factors that should be considered when combining hyperthermia and chemotherapy. For one, response may vary based on the hypoxic and pH parameters of the tumor. Additionally, a very notable effect of hyperthermia is its influence on tumor resistivity to certain drugs; hyperthermia can induce at least partial reversal of drug resistance with a variety of drugs, most notably cisplatin, ►melphalan, nitrosoureas, and doxorubicin. Finally, sequencing of hyperthermia and drug delivery may greatly influence efficacy of treatment. Most drugs exhibit optimal results when heat and drug are delivered concomitantly, or when drug is given immediately prior to heating; some exceptions include 5-FU (►Fluorouracil) and a few other antimetabolites.

Clinical Trials

Phase III clinical trials, particularly those comparing radiotherapy (RT) alone to RT + HT, have yielded significant positive results for a variety of cancers. Both superficial and deep tumor sites have been investigated, as well as both palliative and curative treatment goals. Statistically significant improvement in complete response (CR) rates with HT + RT versus RT alone have been demonstrated with many cancers, most notably chest wall recurrences of breast cancer, stage III and IV head and neck, metastatic melanoma, bladder, and cervical carcinoma. Most importantly, improvements in survival have also been shown in cervix, head and neck, glioblastoma, esophageal cancers.

While many trials have been performed validating the use of hyperthermia with radiotherapy, fewer exist for chemotherapy (CT) and hyperthermia. However, phase II trials have illustrated improved treatment efficacy of CT + HT in sarcomas and breast carcinomas, compared with historical controls. Additionally, the use of tri-modality (CT, RT, and HT) treatments are being investigated, with encouraging results in phase II trials of locally advanced rectal and cervical cancers and a phase III trial for esophageal cancer.

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Hypertrophy

Definition

Increase in size of an organ or tissue due to an increase in the size of cells while the number stays the same.

►Peroxisome Proliferator-Activated Receptor

Hypertrophy of Male Breast

►Gynecomastia

Hypervariable Region

Definition
Regions of the heavy or light chains of ►immunoglobulins in which there is considerable sequence diversity within that set of immunoglobulins in a single individual. These regions specify the antigen affinity of each antibody.

Hypodiploidy

Definition
Having a chromosome number that is less than the normal diploid number.

►Acute Lymphoblastic Leukemia

Hypoechoic

Definition
The echo of the tissue is low compared to the adjacent area.

►Hepatic Epithelioid Hemangioendothelioma

Hypogammaglobulinemia

Definition
Abnormally low levels of immunoglobulins.

Hypomethylation

Definition
A reduced level of CpG island ►methylation relative to the normal tissue specific pattern.

►Methylation-Controlled J Protein (MCJ)
►CpG Islands

Hypomethylation of DNA

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Synonyms
DNA demethylation; DNA undermethylation

Definition
DNA hypomethylation refers to the loss of the methyl group in the 5-methylcytosine nucleotide. Methylation is a natural modification of DNA, and mainly affects the cytosine base (C) when it is followed by a guanosine (G) in mammals ►(Methylation). The term hypomethylation can be applied to describe the unmethylated state of most CpG sites in a specific sequence that is normally methylated, or as a general phenomenon affecting the bulk of the genome; this is a decrease in the proportion of methylated versus unmethylated cytosines.

Characteristics
In human, DNA methylation mainly occurs at CpG sites. Up to 80% of all CpG sites in human DNA are methylated. However, this methylation occurs primarily in areas where CpG density is low, or at repeat DNA sites, such as Alu elements. CpG islands are regions where CpG density is high and most of them are unmethylated. Patterns of DNA methylation have been linked to control of gene expression, maintenance of chromosomal integrity, and in regulation of DNA recombination in mammals. Methylation in a gene promoter region is generally associated with gene silencing. Heavily methylated DNA replicates later than nonmethylated DNA, and late replication is associated with the formation of inactive chromatin, which facilitates transcriptional silencing of noncoding regions.

Global hypomethylation is typical in aging cells, as well as in neoplasia, where it is an early event. Early studies in the eighties already identified a depletion of the methyl-cytosine content as a landmark in colorectal cancer and other types of tumors. DNA hypomethylation has been shown to promote tumorigenesis in murine colon and liver and was included as an early event in a genetic model for colorectal tumorigenesis. Different investigations sustain a causal link between DNA hypomethylation and genetic instability, reporting an association between defects in DNA methylation and aneuploidy in human colorectal cancer cell lines, increased chromosomal rearrangements in hypomethylated centromeric regions in mitogen-stimulated cells from individuals affected with immunodeficiency, centromere instability and facial anomalies (ICF

syndrome), and an increased mutation rate owing to DNMT1 deficiency in murine embryonic stem cells and in murine somatic cells. Moreover, DNMT1 deficiency also results in constitutive chromosomal instability in a human colon cancer cell line. Decreased methylation levels in LINE sequences correlate with losses of heterozygosity on discrete chromosomal loci in colorectal carcinomas and other studies have demonstrated that DNA hypomethylation precedes genomic damage in human gastrointestinal cancer.

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Hypospadias

Definition

A congenital defect of the ventral surface of the penis which causes abnormal urethral opening proximal to its normal location.

►Nephroblastoma

Hypothalamus

Definition

A region of the brain below the cortex and the cerebrum that regulates numerous important body functions and makes hormones that impact pituitary function.

►Prolactin

Hypoxia

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Synonyms

Anoxic; Anaerobic

Definition

Being oxygen deprived.

Characteristics

Cellular hypoxia is a common stress in normal development and numerous pathological conditions, including cancer. By the time a tumor has grown to a detectable size, poor and disordered ►angiogenesis, leaky vessels, and high interstitial tumor pressure all result in significant tumor hypoxia. Studies in human tumor ►xenografts reveal a mean pO_2 of <5 mmHg 70–80 μm from a vessel wall. Both non-invasive measurements and direct assessment of tumor oxygenation in patients have demonstrated the presence of profound hypoxia in a marked variety of solid tumors, including, ►melanoma, ►prostate cancer, head and neck, and ►cervical cancer as well as in ►hematological malignancies including myeloma. In one study, using oxygen electrodes to assess breast cancer oxygenation in 36 patients and controls, the average pO_2 was 30 mmHg in tumors as compared to 65 mmHg in normal breast tissue and 67 mmHg in benign fibrocystic breast disease. Areas of severe hypoxia or ►anoxia (<5.0 mmHg) were noted in >30% of the measurements. There was no correlation between tumor hypoxia and tumor size or stage.

For over 30 years, hypoxic tumors have been known to be relatively chemo-resistant and radio-resistant. More recently, tumor hypoxia has been correlated with poor clinical response to radiation in cervical and breast cancer, chemo-radiation in patients with head and neck cancer, and chemotherapy in myeloma. Proteins up-regulated in hypoxic cells predict a poor prognosis when identified in a resected tumor or, in some cases, in patient serum. Intriguingly, prospective studies of head and neck and cervical cancer patients show that tumor hypoxia correlates with local/regional progression irrespective of whether surgery or radiation is applied as primary treatment. This suggests that while hypoxic tumors are less responsive to cytotoxic therapy, potentially due to drug delivery issues, they are also *inherently* more aggressive than non-hypoxic tumors.

Phenotypes of Hypoxic Cells

Several hypotheses could explain why hypoxic tumors may be less responsive to therapy and inherently more aggressive than non-hypoxic tumors (Fig. 1). These hypotheses are based primarily on the phenotypes of hypoxic neoplastic cells, for the most part studied *in vitro*. One hypothesis of why hypoxic tumors are more aggressive than non-hypoxic tumors is that hypoxia selects against cells with a pro-apoptotic phenotype. Hypoxia is a potent stimulus for apoptosis in a variety of tumor cell lines. Hypoxia-induced ►apoptosis can be blocked by ►Bcl-2 over-expression or a variety of other mutations (including, in some

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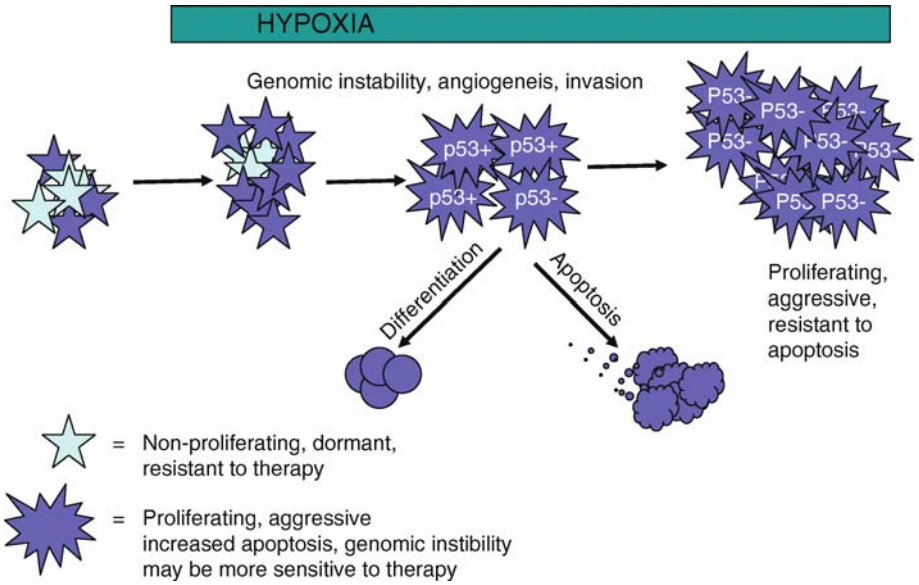


Hypoxia. Figure 1 A model of how tumor hypoxia may affect the aggressiveness of these tumors. Hypoxia may promote de-regulated proliferation and apoptosis, genomic instability and differentiation, and thus select for aggressive tumors.

cases, ►p53 mutations). Thus, only hypoxic cells with a strong anti-apoptotic advantage survive, resulting in the selection of cells that are less responsive to therapy and a more aggressive tumor.

A second hypothesis for the aggressiveness of hypoxic tumors is that hypoxic cells are more prone to ►genomic instability, which may lead to inactivation of tumor suppressor genes or conversely, to activation of ►oncogenes. Several studies have suggested that hypoxia down-regulates several ►mismatch repair genes, and that some hypoxic cells have an increased rate of mutagenesis. Genomic instability appears to be increased in cells that are rendered hypoxic and then re-exposed to oxygen, perhaps due to DNA damage from the generation of ►reactive oxygen species. This hypoxia-reoxygenation pattern is known to occur in stroke and myocardial infarctions (i.e. re-perfusion damage) but may also play an important role in the dynamic tissue bed of tumors.

An additional hypothesis of why hypoxic tumors are more aggressive than non-hypoxic tumors is based on *in vivo* and *in vitro* data demonstrating that while most non-transformed, differentiated cells do not proliferate when hypoxic, a variety of tumor cells can proliferate in these conditions. While hypoxia-induced growth arrest has been noted in several transformed, neoplastic cell lines, hypoxic proliferation can occur in some cervical cancer, osteosarcoma, and transformed rodent cell lines. ►p53 status does not affect proliferation of hypoxic cells, but other mutations in the ►Retinoblastoma (Rb) axis allow cells to proliferate when moderately hypoxic (~1% environmental oxygen); these mutations are well documented in a majority of tumors and include elevated CDK2 activity (due to cyclin E over-expression in breast cancer, increased cyclin D activity in lymphoma, and decreased p27 found in many cancers) and mutations in Rb. Thus it is conceivable that hypoxic cells which can proliferate have ►cell cycle checkpoint abnormalities, and these abnormalities render these tumors more aggressive.

A lack of differentiation is a hallmark of cancer, and hypoxia has also been demonstrated to regulate differentiation. These effects vary, as do proliferation and apoptosis, depending on the cell type. Some hypoxic cells, including some hematopoietic stem cells, are resistant to pharmacologically-induced differentiation. Other cells undergo increased differentiation when hypoxic. Therefore, it is plausible that hypoxia correlates with absence of differentiation, hence aggressive tumorigenesis. A final hypothesis to explain the aggressiveness of hypoxia is that hypoxic tumors, through up-regulation of ►vascular endothelial growth factor (VEGF) and other pro-►angiogenic factors, are able to metastasize more rapidly than non-hypoxic tumors. Indeed, studies in cervical cancer have shown more metastatic disease in those patients with resected hypoxic tumors. Hypoxic cells have also been shown to up-regulate genes that may play an important role in digesting the extracellular matrix and tumor cell invasion.

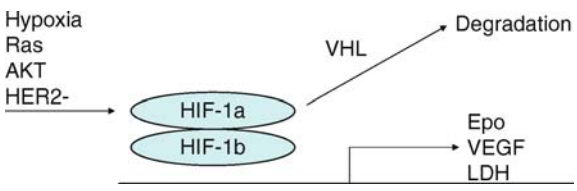
Gene Regulation in Hypoxic Cells: Implications for Tumorigenesis

In an attempt to better understand the biology of hypoxic cells and hypoxic tumors, much work has

focused into the delineation of the mechanisms by which cell signaling and gene regulation are altered in hypoxic cells, and whether this may be extrapolated to hypoxic tumors. Several signaling events and ►transcription factors are generated in hypoxic cells, including ►NFκB, activation of p38, and fluxes in reactive oxygen species. However, the best studied regulator of hypoxia-induced genes is the transcription factor ►Hypoxia Inducible Factor 1 (HIF-1), a heterodimer HIF-1α and HIF-1β. HIF-1β may also heterodimerize with the HIF-1α-related proteins HIF-2α and HIF-3α, and while the individual roles of each of these are still being evaluated, HIF-2 may play an important role in tumorigenesis. HIF-1 was first characterized as the transcription factor responsible for up-regulating erythropotietin, a growth factor for red blood cells that is induced during periods of anemia (and thus systemic hypoxia).

Under normoxic conditions, HIF-1α is hydroxylated by a prolyl hydroxylase, ubiquitinated, and degraded by the ►proteasome. In hypoxic cells, prolyl hydroxylases are inactive, and HIF-1α is stabilized. HIF-1α is over-expressed in renal cancer when the ►E3 ubiquitin ligase responsible for its degradation, the ►Von-Hippel-Lindau protein VHL, is absent. In fact, renal cell cancers are often characterized by increased vasculature and associated with erythrocytosis, both features of HIF-1 target gene activation. HIF-1α is also stabilized by ras, AKT, Her-2 over-expression and other oncogenic alterations often found in cancer (Fig. 2). Over-expression of HIF-1 is an independent indicator of poor prognosis in many malignancies.

Complete ►knockout of HIF-1α is embryonic lethal in mice. Original experiments done on embryonic stem cells suggested that apoptosis may be increased in hypoxic cells lacking HIF-1 function. In a mouse model of astrocytomas, HIF-1 was required for growth in a hypoxic environment, and HIF-1 in endothelial cells also seems necessary for solid tumor growth. Although the complete functional significance of HIF-1 in both normal cells and in cancer remains unclear, most consider HIF-1 activity as important in promoting the survival and adaptation of hypoxic tumors. Thus, the interference of HIF-1 activity is being actively pursued as a therapeutic target in cancer therapy.



Hypoxia. Figure 2 The hypoxia-induced transcription factor, HIF-1, may be highly expressed in both hypoxic as well as normoxic tumors. This over-expression leads to the up-regulation of a variety of genes that allow a tumor to survive and grow.

Because of the important, although unclear, role of HIF-1 in cancer, a major challenge in the field is to determine what genes are regulated by the HIF-1 transcription factor. The full range of HIF-1 targets is not known, but knock-out studies have predicted anywhere from 100 targets to 2.6% of the genome. It is clear that HIF-1α transactivates many genes necessary for hypoxic adaptation, including glucose transporters, glycolytic enzymes, and growth factors important for angiogenesis. It has recently been demonstrated that many HIF-1 targets such as VEGF and erythropoietin are protective against apoptotic cell death. Common HIF-1 targets are vital for tumor invasion, metastasis and angiogenesis, and many of these targets, such as VEGF and LDH, are elevated in many aggressive cancers.

One well-recognized result of hypoxia, and HIF-1 up-regulation, is an increase in anaerobic (glycolytic) metabolism (►glycolytic metabolism). The cancer cells preference to utilize anaerobic metabolism even in the presence of ample oxygen was first noted by Otto ►Warburg. This property of many malignant cells is an inefficient method of energy production, requiring increased utilization of glucose. In fact, the increased utilization of glucose by hypoxic and non-hypoxic tumors forms the basis for ►positron emission tomography (PET) scanning in cancer, where tumor cells may be recognized non-invasively through their high up-take of labeled glucose. Recent studies indicate that this increased metabolism of glucose even in normoxic tumors is due to increased activity of HIF-1, which is known to increase the expression of many of the enzymes involved in glycolysis. HIF-1 also inhibits flux through the Krebs cycle and thus suppresses aerobic metabolism directly.

In addition to the well recognized role hypoxia has on transcription, through HIF-1 and other transcription factors, there are other mechanisms that have been described for altering the expression of proteins important in the neoplastic process in hypoxic cells. The mRNA of an undetermined number of transcripts, including VEGF, are stabilized in hypoxic cells, although the mechanism(s) have not been entirely clarified. More recent studies have documented that hypoxia can suppress protein translation through the ►mTOR pathway and others. Misfolded proteins in the hypoxic cell's endoplasmic reticulum also activate the unfolded protein response (UPR). The UPR is an efficient mechanism to halt protein translation, while paradoxically *increasing* the translation of a select number of mRNAs, including the transcription factor ATF-4. The hypoxic suppression of protein translation is thought to be a protective mechanism by which hypoxic cells conserve energy.

Intriguingly, recent data suggests that some of these regulatory mechanisms may be altered in hypoxic neoplastic cells, potentially providing cancer cells another mechanism to manipulate the hypoxic environment.

►Oxygenation of Tumors
►Hypoxia and Tumor Physiology
►Anoxia and Cancer
►Metastasis
►Reactive Oxygen Species

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Hypoxia Inducible Factor

Definition

HIF; A molecule induced by ►hypoxia, and up-regulated in inflammation and many cancers. It regulates the expression of genes involved in inflammation, tumor progression, invasion and ►metastasis and is therefore a target for cancer ►chemoprevention.

►Inflammation

Hypoxia Inducible Factor-1

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Synonyms

HIF-1

Definition

►Hypoxia is widespread within most solid tumors. This stress situation is sufficient to activate the key transcription factor, hypoxia inducible factor (HIF)-1 that mediates the activation of the survival pathways in cancer cells. HIF-1 can also be induced by oxygen-independent genetic deregulations that activate a variety of ►oncogene signaling pathways or inactivate ►tumor suppressors. Increased tumor HIF-1 is often correlated with increased ►angiogenesis, malignant cancer ►progression, poor patient prognosis, and resistance to cancer therapies. HIF-1 is a proangiogenic transcription factor that interacts with a specific promoter sequence, called hypoxia response elements (HREs) in the regulatory region of the target DNA to stimulate transcription of multiple angiogenic factors including ►vascular endothelial growth factor (VEGF). In addition, HIF-1-targeted genes also include genes involved in cell proliferation, survival, ►invasion, and ►drug resistance. Due to its role in several key events of cancer progression, HIF-1 is an important molecular target for cancer therapy.

Characteristics

Mechanisms

Hypoxia, or lack of oxygen, has been recognized as a major driving force of solid tumor progression. Microregions of heterogeneous cell environments are associated with the development of abnormal vasculature formation in malignant tumors, which often consist of distended capillaries with leaky walls and sluggish flow, as compared with the regular, ordered vasculature of normal tissues. Despite the constant effort of tumor cells to recruit new blood vessels, there are significant gradients of critical factors for cell growth such as oxygen, glucose, other nutrients, and growth factors. Hypoxia occurs in tumor cells that are 100–150 μm away from the nearest blood vessel and tend to be widespread in solid tumors. The anaerobic metabolism of glucose provides a major energy source for tumor cells in hypoxic regions. The ability of tumor cells to endure profound hypoxia indicates that their adaptation to hypoxic conditions is a crucial step in tumor progression. HIF-1 is the intrinsic survival factor of tumor cells to overcome O_2 and nutrient deficits during tumor proliferation and progression. Regardless of oxygen tension, cancer cells sustain high aerobic glycolytic rates and produce high levels of lactate and pyruvate. This phenomenon, known as the Warburg effect after its discoverer was first described in cancer more than seven decades ago. Expression of HIF-1 is indirectly upregulated via "gain-of-function" mutations in ►oncogenes and "loss of function" mutations in ►tumor suppressor genes that result in increased HIF-1 transcriptional activity. As a result of genetic alterations together with the intratumoral hypoxia, HIF-1 activation is enhanced in the majority of common human cancers, which is strongly correlated with tumor grade, vascularity, ►metastasis, prognosis, and overall

survival. HIF-1 can induce various gene products that control energy metabolism, neovascularization, survival, and cell ►migration; all of which are promoters of tumor growth. HIF-1 is a heterodimeric transcription factor composed of two subunits, HIF-1α and HIF-1β. The HIF-1α subunit is rapidly degraded under normal O_2 conditions (normoxia) while the β subunit is constitutively expressed. Under low oxygen concentrations, HIF-1α is stabilized and dimerizes with HIF-1β to form an active transcription factor. Both HIF-1 subunits are members of the basic helix–loop–helix (HLH)-containing family of transcription factors. The HLH domain mediates heterodimer formation between the HIF-1 and HIF-1 subunits, which is necessary for DNA binding by the basic domains. HIF-1 is constitutively produced and degraded under normoxia while HIF-1 expression is unaffected by O_2 levels. Oxygen-dependent hydroxylation of three amino acid residues within HIF-1 affects both the stability of HIF-1 and its function as a ►transcription factor. The hydroxylated protein is then recognized by von Hippel Lindau protein (►pVHL), which is a part of an E3 ubiquitin ligase complex, ubiquitinated and targeted for proteasomal degradation. Under hypoxic conditions, HIF-1 remains unhydroxylated and does not interact with pVHL but preferentially binds to p300/CBP transcription coactivators. Following this hypoxic stabilization, HIF-1 translocates to the nucleus where it heterodimerizes with HIF-1 to bind to the DNA sequence 5′-RCGTG-3′ (hypoxia responsive element), located in the promoter of target genes. This subsequently leads to upregulation of factors that promote tumor growth and angiogenesis, including glycolytic enzymes and VEGF. HIF-1 transcriptionally upregulates VEGF production as part of the oxygen-regulation system that stimulates the production of new blood vessels to support tumor growth. Overexpressed VEGF leads to increased capillary leakiness and vascular permeability, which is followed by vascular hyperplasia, leading to increased vessel density. Solid tumors universally develop large areas of hypoxia as tumor growth exceeds the existing vascular supply, which promotes growth of new vasculature through HIF-dependent and -independent mechanisms. These cancers are particularly lethal; hypoxic cells in solid tumors are able to remain viable because they are resistant to both radiation and chemotherapy.

Clinical Aspects

HIF-1 activity is tightly regulated by the stability of HIF-1 in both hypoxic and normoxic conditions in normal tissues. It is deregulated by the activation of oncogenic pathways and the loss of tumor suppressor function (e.g., pVHL, ►PTEN, and ►p53) in many cancers, which causes HIF-1 to accumulate regardless of oxygen tension. The extensive ►immunohistochemical data in multiple human tumors demonstrate unequivocally that the HIF pathway is a major contributor to tumor development and progression in ►prostate cancer, ►breast cancer, ►lung cancer, ►colon cancer, ►bladder cancer, head and neck cancers, ►ovarian cancer, ►renal cell carcinoma, ►hepatocellular carcinoma, ►gastric cancer, ►brain tumors, and ►pancreatic cancer. In some diseases, HIF-1 is an independent prognostic factor and could be used to predict clinical outcome and survival or alternately used to assist in decisions for ►adjuvant therapies. Evidence of the importance of HIF as a key factor in tumorigenesis, ►angiogenesis and tumor progression from the immunohistochemical data of human tumors, and the growing knowledge of the molecular mechanism of its regulation will reveal novel anticancer therapies in the near future. Currently, a number of drug candidates that inhibit HIF-1 accumulation are in clinical trials (http://clinicaltrial.gov) including heat shock protein ►HSP90 inhibitors, ►mTOR inhibitors, and ►2-methoxyestradiol.

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Hypoxic

Definition

►Referring to hypoxia.

Hysterectomy

Definition

Refers to an operation to remove a woman's uterus. Sometimes, the ovaries and fallopian tubes also are taken out. Hysterectomies are very common – one in three women in the United States has had one by age 60.

►Uterine Leiomyoma

IA4

►Metastasis Suppressor KAI1/CD82

IAP

Definition

►Inhibitor of Apoposis Family

IARC

Definition

International Agency for Research on Cancer.

IARC *TP53* Database

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Definition

The ►IARC ►TP53 mutation database is a public web-based resource for the analysis and interpretation of the biological and clinical impacts of ►*TP53* gene variations in human cancers (http://www-p53.iarc.fr/). It contains data that are compiled from the scientific literature and includes all *TP53* gene variations that have been reported in human cancers, with related information on tumor pathology, patients demographics, and predicted or experimentally assessed functional impacts of mutations. It can be searched and analyzed online and is useful to draw hypotheses on the nature of the molecular events involved in ►*TP53* mutagenesis and on the natural history of cancer.

With over 25,000 somatic and 400 germline mutations and 2,000 citations in the world literature, this database is recognized as a major source of information on *TP53* mutation patterns in human cancer. The database is meant to be a resource for a broad range of scientists and clinicians who work in different research areas: basic research, to study the structural and functional aspects of the ►p53 protein; ►molecular pathology of cancer, to understand the clinical significance of mutations identified in cancer patients; molecular epidemiology of cancer, to investigate links between specific exposures and ►mutation patterns and to make inferences about possible causes of cancer; molecular genetics, to study genotype/phenotype associations.

Useful Links: TP53 OMIM: 191170; LFS OMIM: 151623; GenBank: X54156; IARC TP53 database: http://www-p53.iarc.fr.

Characteristics

TP53 Gene Mutations in Human Cancers

The tumor suppressor gene *TP53* (OMIM #191170) encodes a transcription factor with multiple, anti-proliferative functions activated in response to several forms of cellular stress. Somatic *TP53* gene alterations are frequent in most human cancers and inherited *TP53* mutations predispose to a wide spectrum of early-onset cancers (►Li-Fraumeni Syndrome, OMIM#151623). More than 72,000 tumor samples have been screened for *TP53* mutations with an overall mutation frequency of 30% (ranging from 5% to 70% depending on the type and stage of cancer), which makes data on *TP53* mutations the largest dataset of mutations in any human gene.

In contrast to other ►tumor suppressor genes that are mainly inactivated by truncating mutations, the most frequent alterations in *TP53* gene are single nucleotide substitutions leading to the production of full-length proteins which differ from the wild-type protein by a single amino-acid substitution (►missense mutation). *TP53* missense mutations cluster within the central

portion of the gene encoding the DNA-binding domain of the protein. Nine missense mutations account for about 30% of all mutations found in human cancers, but more than 1,500 distinct mutant proteins have been found in human cancers. A great amount of data has been generated on the functional impact of p53 missense mutations. In particular, one study has performed a systematic analysis of the trans-activation activity on eight different p53 response-elements (p53-RE) in yeast cells of all possible missense mutations in the entire sequence of *TP53* (2314 mutations). This study showed that a great proportion of missense mutations retain partial trans-activation activity on one or more p53 response-elements and that there is an overall good correlation between complete loss of trans-activation and frequent occurrence in cancer.

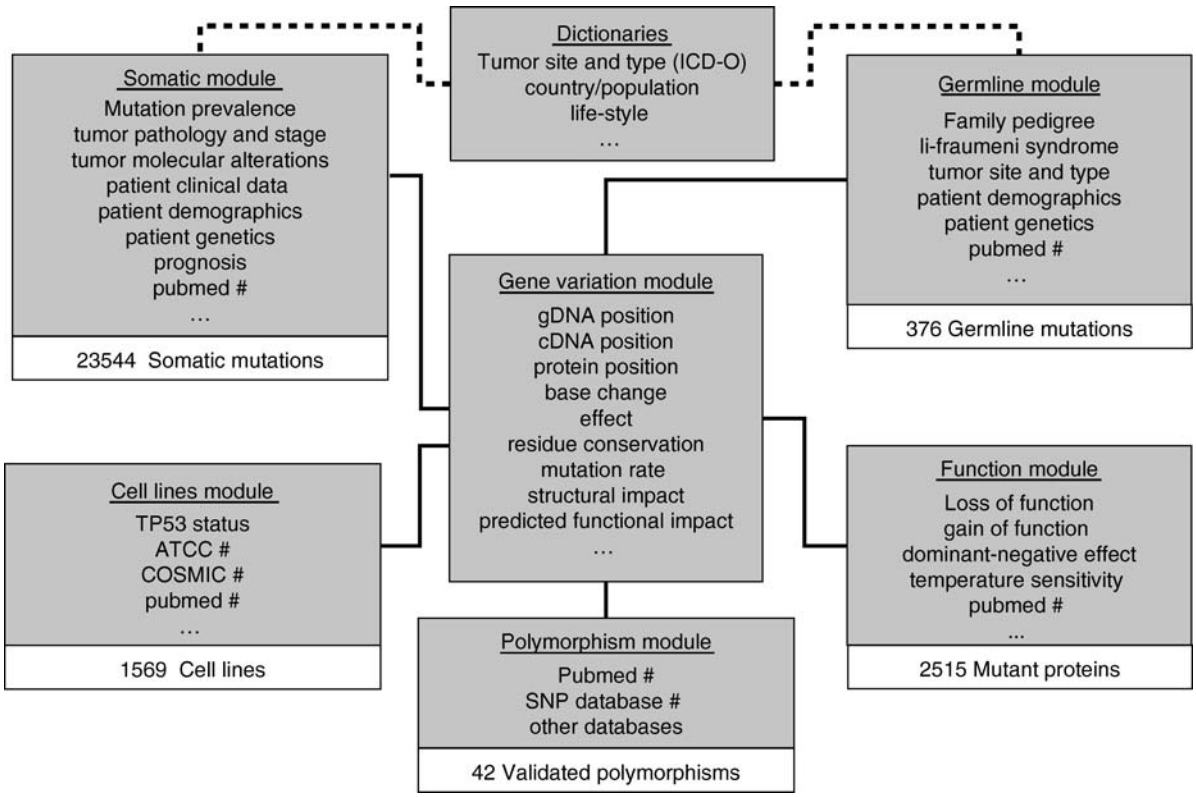
The diversity of mutations observed in cancers is a source of information on the natural history of cancers. The factors that influence the formation of a specific mutation pattern can be seen as a succession of "filters" that select for particular mutations. First, the capacity of exposed cells and tissues to metabolize carcinogens determines the capacity of a given mutagen to induce DNA-damage. Second, the type of damage caused by a mutagen can be specific in its nature and DNA-sequence context. Third, the inherent capacity of cells to repair DNA corrects for most alterations and only those that escape repair will result in fixed mutations. Fourth, biological selective mechanisms favor cells that have acquired a proliferative advantage as a consequence of the mutation. Weighing the contribution of these successive filters in shaping a tumor-specific mutation pattern provides interesting clues on the molecular mechanisms involved in the etiology and pathogenesis of human cancers. The nine frequent mutants are found in all types of cancers and result from transitions at highly mutable ►CpG dinucleotides, at codons encoding residues that play essential structural of chemical roles in specific DNA-binding activities of p53 protein. These mutations result in a complete loss of DNA-binding activity and trans-activation capacity, on which rely the tumor suppressor function of *TP53*. Underlying mutagenicity and loss of function are thus the main driving force for the selection of these mutations during cancer development. In some cancers, characteristic patterns of *TP53* mutations have been observed and linked to exposure to environmental carcinogens, e.g., ►aflatoxin B_1 (AFB_1) exposure and G>T mutations at codon 249 in ►hepatocellular carcinoma of ►HBV chronic carriers; ►ultraviolet radiation and CC to TT tandem mutations in non-melanoma skin cancer; and cigarette smoke and G>T transversions in ►lung cancer of smokers (►tobacco-related lung cancer). Specific mutation patterns in *TP53* may thus help identify environmental exposures that may be involved in ►carcinogenesis.

Database Structure and Content

The IARC TP53 Database contains data that are extracted from peer-reviewed publications or bioinformatics databases and curated manually. The core database is a relational database that integrates data on somatic mutations in sporadic cancers, on germline mutations in familial cancers, on *TP53* gene status of cell-lines, on polymorphisms in human population and on functional assessment of mutations. It is organized around a "gene variation module" to which five modules are connected: a "somatic module," a "germline module," a "function module," a "polymorphism module" and a "cell-line module" (Fig. 1).

The database is a relational database in which different sets of data are integrated in a single scheme. The structure and content presented corresponds to the R11 (October 2006) release of the database that is maintained in SQL sever. This database design and content allow, for every single gene variation, to retrieve data on its functional and structural impact, and data related to its expression in a somatic, cell-line or germline context. Details on database contents and annotations are available at http://www-p53.iarc.fr/Help.html.

The "gene variation module" contains all possible single nucleotide substitutions in the coding sequence and splice sites of *TP53*, plus all other sequence alterations that have been reported in human cancers, each alteration being a unique entry. Gene variation are described at the DNA and protein levels and are annotated with structural and functional information related to the position of the mutation in the protein sequence (functional domain, residue function, structural motif, solvent accessibility of residue). For missense mutations, classifications based on the predicted or experimentally assessed functional impacts are also available. The "►polymorphism" includes common gene variations reported in publications or extracted from ►SNP databases, with links to databases that provide information on population frequency or disease associations. The "somatic and germline modules" contain data related to the somatic or germline expression of mutations respectively, with common set of dictionaries used to annotate pathological, clinical and patient information. The "cell-line module" contains *TP53* gene status of human cell-lines with links to the ATCC catalog (cell-line provider). The "Function module" contains experimental data obtained on the activities and properties of p53 mutant proteins when transfected and over-expressed in human or yeast cells. Each entry in this module corresponds to the results of a set of experiments performed with one mutant in a specific cell-type. Experimental assays that have been included were performed in yeast or human cells and were designed to (i) measure the transactivation activities of mutant proteins on reporter genes placed



IARC *TP53* Database. Figure 1 Structure and contents of the IARC TP53 database (R11, 2006).

under the control of various p53 response-elements, (ii) test the capacity of mutant proteins to induce cell-cycle arrest or apoptosis, (iii) exert dominant-negative effect over the wild-type protein, be temperature sensitive, or display various activities that are independent and unrelated to the wild-type protein (gain of function). A specific vocabulary has been implemented to describe experimental results in a standard format. The detailed annotation system used in all modules is described in the online user guide at http://www-p53.iarc.fr/Help.html.

Web Site and New Search Tools

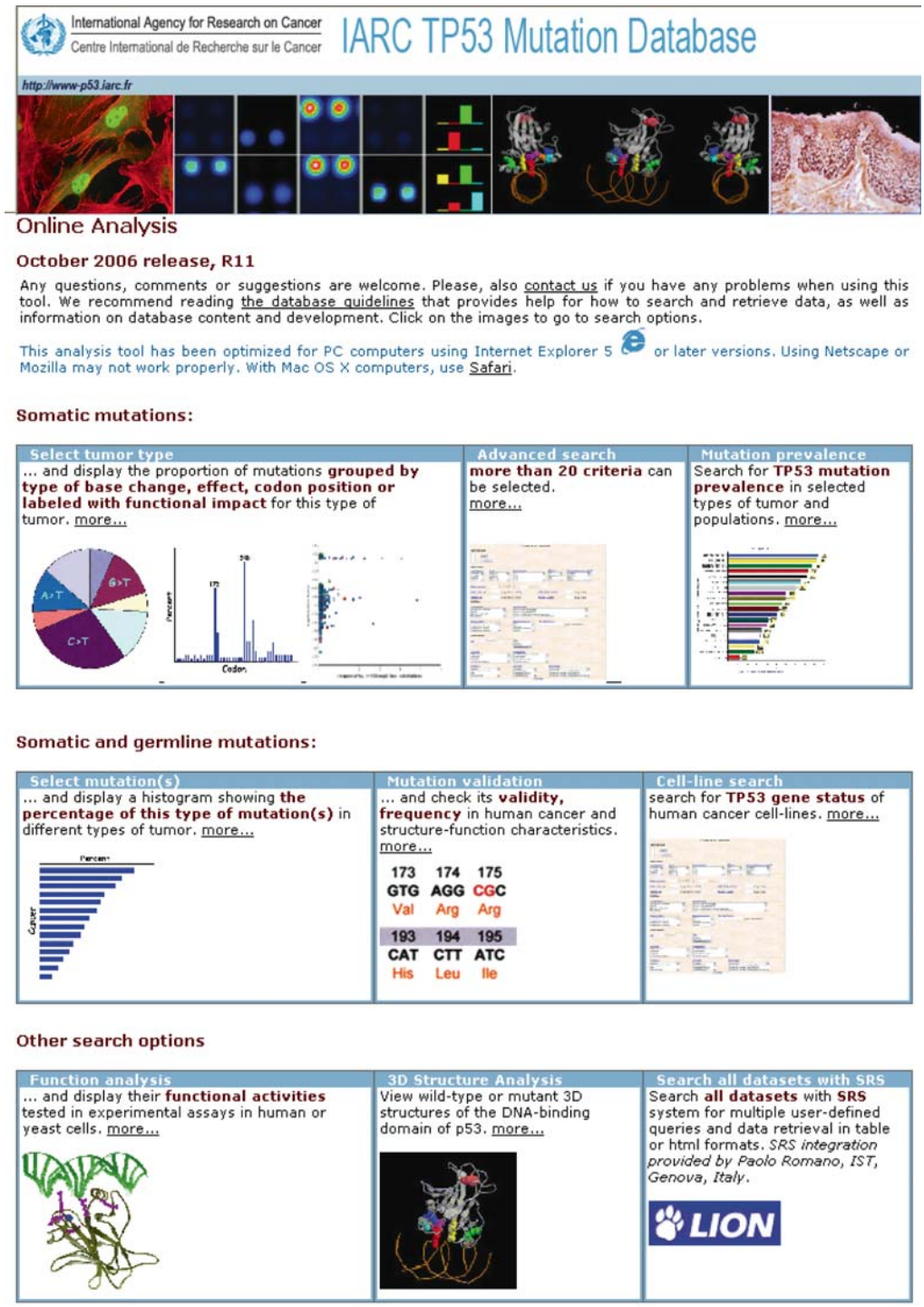
The IARC *TP53* web site (http://www-p53.iarc.fr/) provides a search interface for the core database and includes a comprehensive user guide, a slide-show on *TP53* mutations in human cancer, protocols and references for sequencing *TP53* gene, and links to relevant publications and entries to bioinformatics and cancer databases.

The database interface allows the download of all modules and propose various tools for the selection, analysis and downloads of specific sets of data according to user's query (Fig. 2). Mutations reported in specific types of cancers and/or population groups can be analyzed with graphs that display their type by base substitutions (mutation patterns), codon distribution, position within the 3D structure of p53 DNA-binding domain, and predicted or observed effect on protein (function patterns). Other graphs can be drawn to display the types of tumor associated with specific mutations expressed in a somatic or germline context, and to display the prevalence of mutations found in specific tumor types and/or population groups. Other tools include: a mutation validation tools that allows the retrieval of all data available in the database for a specific DNA variation (frequency as a somatic or germline mutation, predicted and observed functional impact); a search option to retrieve experimental data on functional properties of mutant proteins; a search option to retrieve cell-lines for which *TP53* gene status is known.

Several options and tools are available to retrieve and analyze specific sets of data from the core database according to user defined queries. Different types of graphs are implemented to analyze data and raw data can be downloaded as tables. All datasets can be entirely downloaded as tab-delimited text files. The search page is available at http://www-p53.iarc.fr/P53main.html.

Recommendation to Users

The IARC TP53 database is exclusively based on peer-reviewed publications. Trends in reporting and



IARC *TP53* Database. Figure 2 Search system for the analysis of the IARC TP53 database.

publishing mutations have thus a strong influence on the data included in the database. Studies from which data are extracted have diverse designs and diverse way of reporting mutations and related information. This diversity requires an effort of standardization of annotations and affect database analysis. It thus important that users are aware of the limitations and possible biases that may affect their analysis of the database. These bias are described in details at http://www-p53.iarc.fr/Help.html#Recommendations.

The IARC TP53 database is a free service to the scientific community, and contributions from researchers and journal editors are most welcome to help us develop this important resource for cancer research.

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IκB

Definition

Are initially described as proteins that inhibit the function of transcription factor ►NF-κB (inhibitor of κB). Meanwhile, IκBs extended to a protein family of seven members primarily structural related by the presence of several ►ankyrin repeats in their central domain. Through these motifs, they interact with and regulate NF-κB function in various ways.

►BCL3
►Nuclear Factor kappa-B

IκBα

Definition

MAD-3, pp40, RL/IF-1 and ECI6; Is one of a family of proteins that are the natural inhibitors of ►NF-κB. It acts by complexing to NF-κB in the cytoplasm of unstimulated cells. When IκBα is inactivated, NF-κB is allowed to enter the nucleus.

►Hodgkin Disease, Clinical Oncology
►Nuclear Factor kappa-B

IC_{50}

Definition

50% inhibitory concentration; Refers to the concentration of a drug that reduces a biochemical activity (e.g. an enzymatic activity) or cellular parameter (such as cell multiplication) to 50% of its normal value in the absence of the inhibitor.

►Small Molecule Screens

ICAMs

Definition

Intercellular ►adhesion molecules are cell-surface ligands for the ►leukocyte integrins and are crucial in the binding of lymphocytes and other leukocytes to certain cells, including ►antigen-presenting cells and ►endothelial cells. They are members of the immunoglobulin superfamily. ICAM-1 is the most prominent ligand for the integrin CD11a:CD18 or LFA-1. It is rapidly inducible on endothelial cells by infection, and plays a major role in local ►inflammatory response. ICAM-2 is constitutively expressed at relatively low levels by endothelium. ICAM-3 is expressed only on leukocytes and is thought to play an important part in adhesion between T cells and antigen-presenting cells, particularly ►dendritic cells.

►Integrin Signaling

Id1

Definition

Member of the group of ►Id Proteins.

Id Proteins

Definition

Inhibitor of DNA (Id) binding proteins are a family of related nuclear ►helix-loop-helix-proteins implicated

in the control of differentiation and cell cycle progression. Id nuclear proteins interact with ►transcription factors and prevent them from binding to DNA. The primary targets of Id proteins are the basic helix-loop-helix (bHLH) transcription factors, which regulate cell-type-specific gene expression and expression of ►cell cycle regulatory genes. Generally, they act as positive regulators of cell growth and as negative regulators of differentiation. Id proteins lack a basic DNA-binding domain; therefore, heterodimers between Id and bHLH proteins cannot bind to DNA. This mode of regulation is referred to as dominant-negative. Id proteins act as dominant-negative antagonists of other helix-loop-helix transcription factors; Id1, Id2, Id3, Id4, E-box.

- Id1, inhibitor of DNA binding 1, is a protein of 154 amino acids and 16 kD. The gene maps to chromosome 20 band q11.
- Id2, inhibitor of DNA binding 2, is a protein of 134 amino acids and 14 kD. It is expressed in most early fetal tissues but not in the corresponding mature tissues. The gene maps to 2p25.
- Id3, inhibitor of DNA binding 3, also known as Heir-1 is a protein of 119 amino acids and 12 kD. It is expressed abundantly in lung, kidney, and adrenal gland, but lacking in adult brain. The gene maps to 1p36, a region frequently deleted in human cancers including neuroblastoma.
- Id4, inhibitor of DNA binding 4, is a protein of 161 amino acids and 16 kD. The gene maps to 6p22-21.

►E-box
►Helix-Loop-Helix Domain

Idarubicin

Definition

Is a semisynthetic ►anthracycline antibiotic derived from ►daunorubicin.

►Adriamycin

Idiopathic Bone Infarction

Definition

Localized bone ►necrosis and its associated bone marrow for unknown reasons.

►Bone Tumors

Idiopathic Myelofibrosis

►Primary Myelofibrosis

Idiotype

Definition

Is the collection of all antigenic determinants (idiotopes) contained within the variable regions of both heavy and light chain of an antibody molecule. The sum of the antigenic determinants of epitopes that are encoded in the variable regions of the ►immunoglobulins heavy and light chain.

►Idiotype Vaccination
►B-cell Tumors

Idiotype Vaccination

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Synonyms

Anti-idiotype vaccination; Idiotypic vaccination

Definition

►Idiotype vaccination is an ►immunotherapy procedure based on the fact that save for its very early stage of differentiation, each clone of B lymphocytes features a specific antibody on the cell surface. The most variable portion of this antibody is a unique feature of the corresponding clone. Its natural function is that of specifically recognizing an antigen, but it can also be used as an antigen (idiotype) itself, that is as a potential vaccine target and tool. The most relevant context in which this second function is exploited is idiotype vaccination as a treatment for human ►B-cell lymphoma.

Characteristics

Field of Application

The vast majority of B-cell lymphomas consist of the clonal expansion of neoplastic B-cells, all featuring the same surface antibody and consequently the same

idiotype. However, each patient's tumor presents with a different, patient- and tumor-specific idiotype. Therefore, an individualized, custom-made idiotype vaccine must be produced for each patient. Most of what we know about idiotype vaccination in human B-cell lymphoma derives from studies conducted in an indolent subset called ►follicular lymphoma. Far less has been instead concluded so far as to whether or not the same approach could be successful in other B-cell malignancies, such as ►mantle cell lymphoma, ►multiple myeloma, and ►chronic lymphocytic leukemia, or even in certain solid tumors, whenever an anti-idiotype ►monoclonal antibody structurally mimics tumor-associated antigens other than antibodies. Nevertheless, idiotype vaccination stands out as the most successful human cancer vaccine, since over the last 20 years has provided the first formal proofs of principle concerning biological efficacy, clinical efficacy, and clinical benefit of such a procedure.

Formulation

Although idiotypic vaccination has been tested in humans with different formulations, such as soluble protein idiotype associated with an immunogenic carrier and an immunologic adjuvant, ►dendritic cells pulsed with the soluble protein idiotype, or idiotype ►DNA vaccine, most clinical results and all proofs of principle have been obtained using the first of these three options. In particular, the most successful idiotype vaccine formulation consists of soluble protein idiotype, that is the patient- and tumor-specific antigen, which is conjugated with keyhole limpet hemocyanin (KLH), the immunogenic carrier, and administered together with granulocyte-macrophage colony-stimulating factor (GM-CSF), the immunologic adjuvant.

Production

While KLH and GM-CSF are commercially available, soluble protein idiotype needs to be produced one patient at the time through either hybridoma or ►recombinant technology. In the former case, each patient's tumor cells, which are capable of producing the idiotype, but not to release it, are fused with a cell line that vice versa can release an idiotype, but is unable to produce it. The ►hybridoma resulting from the fusion process features both functions, can be cultivated in vitro, and releases therapeutic amounts of the patient- and tumor-specific, soluble protein idiotype. In the latter scenario, the genetic information encoding for the idiotype is introduced by means of a vector inside mammalian, insect, plant, or bacteria cells, which will ultimately replace the hybridoma as a factory for soluble protein idiotype production.

Clinical Aspects

Idiotype vaccination has provided the first formal proof of biological efficacy in 1992, when scientists at Stanford University showed that patients with follicular lymphoma were capable of developing a specific, anti-idiotype immune response after receiving idiotype vaccination. Seven years later, researchers at the National Cancer Institute proved clinical efficacy of idiotype vaccination by showing that in most follicular lymphoma patients who had received it, the immune system had become capable of killing in vivo tumor cells that had survived prevaccine ►chemotherapy. Finally, in 2006 clinical efficacy of idiotype vaccination was proved by showing that all follicular lymphoma patients who respond to the vaccine from an immunologic standpoint experience a significant prolongation of their disease-free survival.

Idiotype vaccination's procedure typically consists of a monthly, subcutaneous injection of 0.5 mg of idiotype conjugated with 0.5 mg of KLH, administered together with 125 mcg of GM-CSF. The same dose of GM-CSF alone is then given daily over the following 3 days at the same site of the complete vaccine formulation delivery. After 5 or 6 monthly doses, further boosts every 2–3 months are becoming increasingly popular and recommended.

Idiotype vaccination's side effects are mild and mostly local, if at all present. Flu-like symptoms are rare and self-limiting.

Open Questions

To date, a number of questions remain open with respect to idiotype vaccination.

For instance, from a clinical standpoint, we do not know whether this form of active ►immunotherapy has the potential to cure or just to control the disease in lymphoma patients who respond to it. Nor do we know whether patients with B-cell malignancies other than follicular lymphoma may benefit from it. The former question implies that it remains currently impossible to determine whether the administration of boosts should or could be stopped in patients who have responded to the vaccine from an immunologic point of view, irrespective of whether that immune response persists detectable, and remain free of disease. The latter question implies instead that only through well-designed ►clinical trials it might be possible to test idiotype vaccination in settings other than follicular lymphoma. In particular, it has to be reminded that typical randomized studies may fail to serve the purpose of proving the clinical benefit of a customized form of immunotherapy. In this respect, surrogate endpoints such as those defining minimal residual disease also need to be used with caution. For instance, in the very case of follicular lymphoma, both ►bcl-2 rearrangement assessed by molecular fingerprinting through qualitative and quantitative polymerase chain reaction and tumor cell phenotype assessed through ►flow cytometry not always correlate with immune responses and clinical outcome. All in all, it is possible that the currently ongoing randomized clinical trials will be at least able to shed some light on whether it may be possible to match the financial concerns of pharmaceutical companies

with the peculiar logistic requirements of a customized treatment like idiotype vaccine.

As for the adequate timing for treating patients with this immunotherapeutic approach, it seems to have become ever clearer that the best clinical setting to use idiotype vaccination is a good quality clinical complete response. This prevaccine result can be achieved nowadays through a variety of old and new therapeutic approaches such as standard or high-dose chemotherapy, cold or passive immunotherapy using ►radioimmunoconjugates. However, it is likely that in lymphoma patients selected to later undergo idiotype vaccination, prevaccine treatment should privilege agents and procedures with the lowest immune suppressive potential. At the opposite side of the widening spectrum of possible applications of idiotype vaccination stand the anecdotal attempts conducted with healthy donors undergoing this totally safe procedure with the myeloma-specific soluble protein idiotype obtained from their sibling recipient before ►hematopoietic stem cell allotransplant. Clinical and biological results remain incompletely understood, though extremely appealing from a purely scientific point of view.

Another crucial context in which idiotype vaccination specialists still struggle is that of defining the relevance of idiotype vaccine-induced humoral and cellular responses. In fact, it is not yet clear whether, though both desirable, just either of them is required for patients to experience clinical benefit. The question is of no little importance, considering that in nearly half of the patients with any vaccine-induced, idiotype-specific immune response, either type of immune response is not detectable. Currently, humoral responses, that is those featuring vaccine-induced anti-idiotype antibodies, are assessed and monitored in the lab by a single, relatively standardized method, whereas at least half a dozen of nonstandardized techniques are used in different labs worldwide to assess and monitor vaccine-induced cellular responses

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Idiotypic Vaccination

►Idiotype Vaccination

IDO

►Indoleamine 2,3-Dioxygenase

I-FLICE

►FLICE Inhibitory Protein

IFN

Definition

►Interferon are ►cytokines that can induce cells to resist viral replication. Interferon-α (IFN-α) and interferon-β (IFN-β) are produced by ►leukocytes and ►fibroblasts, respectively, as well as by other cells, whereas interferon-γ (IFN-γ) is a product of CD4 T_H1 cells, ►CD8 T cells, and ►NK cells. IFN-γ has its primary action the activation of ►macrophages.

iFOBT

►Fecal Immunochemical Test

IgE-Mediated Hypersensitivity

►Allergy

IGF

Definition

►Insulin- Like Growth Factors.

►Insulin Receptor

IGF-2

Definition

Insulin-like growth factor 2.

►Insulin Receptor
►Insulin-Like Growth Factors

IGF-I

Definition

Insulin-like growth factor-1.

►Insulin Receptor-1
►Insulin-Like Growth Factors

IGs

Definition

►Immunoglobulin Genes.

IHC

►Immunohistochemistry

IISRE

Definition

A type IIS "stretch" restriction endonuclease (IISRE) is a restriction enzyme that binds double-stranded DNA at a particular sequence and then cleaves double-stranded DNA at a fixed distance and direction from its binding site without regard to sequence specificity. Examples include *Bsg*I, *Bpm*I, *Eco*57I, *Fok*I, and *Hph*I.

►Combinatorial Selection Methods

IL-1

Definition

►Interleukin-1

►Photodynamic Therapy

IL-12

Definition

►Interleukin-12 is the product of ►monocytes such as ►macrophages, ►neutrophils and ►dendritic cells. It is an early component of the innate immune response and serves to activate ►natural killer cells and assist in the differentiation of Th cells to the Th1 phenotype. Since both these cells are a source of ►IFN-γ, IL-12 is a key regulator of IFN-γ production by the immune system.

►Immunoediting

Illegitimate Recombination

Definition

Recombination with nonhomologous regions of DNA, each of the polynucleotide strands of dsDNA being

religated with DNA from different regions of the same or different chromosomes.

►Chromosomal Translocation
►Fusion Genes

Image Cytometry

Definition

Involves measurement of the shape and density of cellular structures to assess anatomic pathology of a given cell. Modern approaches to image ►cytometry incorporate computational analysis to infer disease state based on hundreds of measurements from each individual cell.

►Malignancy-Associated Changes

Imaginal Discs

Definition

Monolayer sac-like epithelial tissues in *Drosophila* larvae that later give rise to adult structures.

►Lats in Growth Regulation and Tumorigenesis

Imatinib

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Synonyms

Imatinib mesylate; Gleevec; Glivec; STI571; ►STI-571; CGP57148

Definition

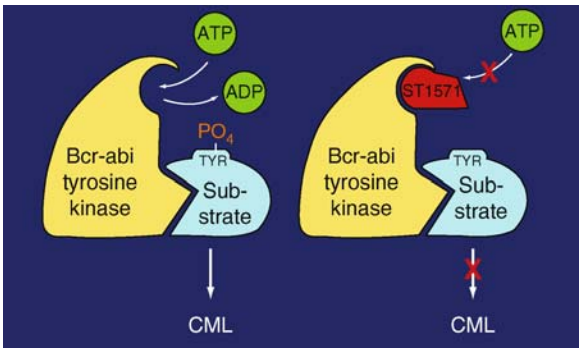
Is a small molecular weight compound that inhibits ►tyrosine kinases including ►ABL, ►KIT (►Kit/stem cell factor receptor in oncogenesis) and PDGFR (►Platelet-derived growth factor receptor). It has significant anti-tumor activity in chronic myeloid leukemia and ►gastrointestinal stromal tumors.

Characteristics

Imatinib (Gleevec, Glivec, formerly STI571 or CGP57148) is a tyrosine kinase inhibitor with activity against all of the ABL tyrosine kinases including ►BCR-ABL, ABL, v-ABL, and ARG (Abelson-related gene). Besides the ABL tyrosine kinase, other kinases inhibited by imatinib are the platelet-derived growth factor receptor alpha (PDGFRA) and beta (PDGFRB) and KIT. Given the critical role of tyrosine kinases in the regulation of cellular growth and known activating mutations that cause several cancers, it was hypothesized that specific inhibitors of these protein kinases might be effective anticancer agents. Beginning in the late 1980s, scientists at Ciba Geigy (now Novartis), under the direction of N. Lydon and A. Matter, performed high throughput screens of chemical libraries searching for compounds with kinase inhibitory activity. From this time-consuming approach, a lead compound was identified. Its inhibitory activity against the PDGFR was optimized by synthesizing a series of chemically related compounds and analyzing their relationship between structure and activity (►Drug design). The most potent molecules in the series were dual inhibitors of the PDGFR and ABL kinases. Of the several compounds generated from this program, imatinib emerged as the lead compound for clinical development based on its superior in vitro selectivity against ►CML cells and its drug-like properties, including pharmacokinetic and formulation properties (►Pharmacokinetics; ►pharmacodynamics).

BCR-ABL and CML as a Target for Imatinib

CML is characterized by the presence of the BCR-ABL ►fusion gene and protein. It arises from a ►reciprocal translocation between the long arms of chromosomes 9 and 22 (►Chromosome translocations) that generates a shortened chromosome 22 termed the ►Philadelphia chromosome. This was the first consistent chromosome abnormality associated with a human cancer. The (9;22) translocation results in fusion of the *ABL* ►oncogene from chromosome 9 with sequences from chromosome 22, the breakpoint cluster region (*BCR*), giving rise to a chimeric *BCR-ABL* gene. This fusion gene is transcribed and translated into a protein that functions as a constitutively activated tyrosine kinase. BCR-ABL induces a CML-like syndrome when introduced into bone marrow cells of mice (►Mouse models), confirming its causative role in the human disease. All of the transforming activities of BCR-ABL are dependent on its tyrosine kinase activity, thus a specific inhibitor of



Imatinib. Figure 1 Schematic representation of the mechanism of action of the BCR-ABL tyrosine kinase and its inhibition by STI571 (imatinib).

this kinase would be predicted to be an effective and selective therapeutic agent for CML (Fig. 1).

In a pivotal set of ►preclinical testing, imatinib was shown to suppress the proliferation of BCR-ABL-expressing cells in vitro and in vivo. In colony-forming assays of peripheral blood or bone marrow from patients with CML, imatinib caused a 92–98% decrease in the number of BCR-ABL colonies formed, with minimal inhibition of normal colony formation.

Activity of Imatinib in CML

Given the central role of the BCR-ABL tyrosine kinase in causing CML and the favorable preclinical profile of imatinib, CML was selected as the initial disease in which to test imatinib. In a standard dose-escalation Phase I study (►Clinical trial) conducted in patients with CML, imatinib was well tolerated. Significant clinical benefits were observed at doses of 300 mg per day and above. In patients with chronic-phase disease who were interferon resistant or intolerant, 53 of 54 (98%) had their previously abnormal blood counts return to normal, typically within 4–6 weeks after beginning imatinib. Ninety percent of these responses lasted beyond 1 year. In patients with myeloid ►blast crisis, the most advanced phase of the disease, 21 of 38 (55%) patients responded, with 18% having responses lasting beyond 1 year. Imatinib rapidly advanced through Phase II and III testing for patients with CML and was FDA-approved in May 2001. It is now the standard initial therapy for patients with CML. A 5-year update of imatinib as initial therapy for patients with CML demonstrates an overall survival of 89%. At 5 years, an estimated 93% of patients remain free from disease ►progression to advanced phases of the disease: accelerated phase or blast crisis. Most of the side effects of imatinib are classified as mild to moderate and include low blood counts, ►Myelosuppression, fluid retention, diarrhea, nausea, muscle and joint aches, and skin rashes.

Mechanisms of Relapse/Resistance to Imatinib

Response rates to imatinib in CML patients with chronic-phase disease are quite high and, thus far, have been durable. Response rates are also quite high in patients with advanced-phase disease, but relapses, despite continued therapy with imatinib, are common. In the largest studies of resistance or relapse, several consistent themes have emerged. In the majority of patients who respond to imatinib but subsequently relapse while remaining on therapy, the BCR-ABL kinase has been reactivated. BCR-ABL kinase activity was analyzed by assessing tyrosine phosphorylation of CRKL, a direct substrate of the BCR-ABL kinase, and the major tyrosine phosphorylated protein in samples from patients with CML. In these studies, between 50 and 90% of relapsed patients have a BCR-ABL point mutation located in one of over 40 different amino acids scattered throughout the ABL kinase domain. Some other patients have amplification of *BCR-ABL* at the genomic or transcript level. In contrast, in patients with primary resistance – that is, patients who do not respond to imatinib therapy – BCR-ABL-independent mechanisms are most common. In patients who relapse as a consequence of reactivation of the BCR-ABL kinase, the BCR-ABL kinase remains a good target. Alternate ABL kinase inhibitors are already in clinical trials (►Nilotinib, ►dasatinib) and dasatinib is FDA-approved for patients with CML with imatinib resistance. There remains at least one imatinib-resistant mutation, T315I, that is not inhibited by either drug.

Activity of Imatinib in Other Diseases

Given the success of imatinib in CML, it was logical to try imatinib in other diseases where activated tyrosine kinases targeted by imatinib have causative roles. Thus, imatinib also has significant activity in patients with ►acute lymphoblastic leukemia who are *BCR-ABL*-positive. Another tumor in which imatinib has shown activity is ►gastrointestinal stromal tumor (GIST). GISTs are mesenchymal neoplasms that can arise in any organ in the gastrointestinal tract or from the mesentery or omentum. The majority of GISTs express KIT, and in 90% of cases, KIT activation is linked to mutations, usually involving exons 9 or 11. Published data suggest that the response rate of GISTs to single- or multi-agent ►chemotherapy is less than 5%. In contrast, the response rate to imatinib as a single agent in patients with advanced GIST was 53–65% with another 19–36% of patients having disease stabilization. Mutational status helps predict response to imatinib in GIST patients. Patients whose tumors contain the most common activating KIT mutation exon 11 have a significantly higher partial response rate (83.5%) to imatinib therapy

than did patients whose tumors had no detectable mutations in *KIT*. Patients whose tumor harbored a *KIT* exon 9 mutation did less well than those with an exon 11 mutation but responded better (response rate of 48.7%) and had a longer time-to-treatment failure than those with no detectable mutation.

Similar to the situation in CML, many imatinib-resistant tumors have acquired kinase mutations in the kinase domain of KIT. However, resistance mechanisms in GISTs are more heterogeneous than those seen in chronic-phase CML as some tumors actually lose KIT expression and other tumors become imatinib-resistant without acquisition of secondary kinase mutations. Again, similar to the situation with CML, novel KIT kinase inhibitors are being tested in patient with imatinib-resistant GIST and one of them, sunitinib, has been FDA-approved for this indication.

Imatinib also has activity in neoplasms that are caused by oncogenic activation of PDGFRs (Platelet-derived growth factor receptor). This includes the subset of patients with chronic myelomonocytic leukemia that results from fusion of the ►*EVT6* (*TEL)* and *PDGFRB* genes. Similarly, dermatofibrosarcoma protuberans, a low-grade sarcoma of the dermis, is characterized by a (17;22) translocation involving the *COL1A1* and *PDGF-B* genes, which results in over-production of fusion COL1A1-PDGF-BB ligand and consequent hyperactivation of PDGFRB. Patients with this tumor also respond to imatinib. Hypereosinophilic syndrome is another example of an imatinib-sensitive disease. In this case a *PDGFRA* fusion gene product (FIP1L1-PDGFRA) is the activated target.

Conclusion

The development of imatinib and its clinical application demonstrate an emerging paradigm in cancer therapy where the tumor is defined by molecular genetic abnormalities instead of the tissue of origin. It further demonstrates that effectiveness of cancer therapy when treatments target events critical to the growth and survival of specific tumors. (►Molecular therapy).

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Imatinib Mesylate

►Imatinib

Imiquimod

Definition

A small molecular drug that activates TLR7 and/or TLR8 agonist that is used as a prescription medication to treat ►skin cancer (►basal cell carcinoma, ►Bowen disease, superficial squamous cell carcinoma, some superficial malignant melanomas and actinic keratosis) as well as genital warts.

Immature B Cells

Definition

Are ►B cells that have rearranged a heavy- and a light-chain V-region gene and express surface IgM, but have not yet matured sufficiently to express surface IgD as well.

Immediate Early Genes

Definition

IEGs; A class of approximately 100 structurally and functionally unrelated genes, whose transcription is induced through the phosphorylation and activation of pre-existing transcription factors such as ►ELK-1 by intracellular signaling pathways like ►MAP kinase. A hallmark of immediate early genes (IEGs) is that their transcription is independent from *de novo* protein

synthesis, which distinguishes them from delayed early genes. Examples for IEG products include ►FOS, a component of the ►AP-1 transcription factor complex, and several members of dual specificity phosphatases, which act as negative feedback regulators of MAP kinases.

►B-Raf Signaling

Immediate Early Stress Response

►Stress Response

Immortalization

Definition

Describes the acquisition by a eukaryotic cell line of the ability to grow through an indefinite number of divisions in culture. Cells are capable of indefinite proliferation (or unlimited lifespan) without any other changes in the phenotype necessarily occurring. In long lived multicellular organisms, immortality may be thought of as an abnormal escape from cellular ►senescence.

►Telomerase
►Senescence and Immortalization

Immortalized

Definition

Refers to the status of ►immortalisation of cells.

Immortalized Cells

Definition

Transformed cells that can grow and divide indefinitely in vitro.

►Adult Stem Cells
►Immortalization

Immune Adjuvants

Definition

Substances that are typically added to vaccines to enhance their immunogenicity.

►Peptide Vaccines for Cancer
►Adjuvant

Immune Complex

Definition

The binding of antibody to a soluble antigen forms an immune complex. Large immune complexes form when sufficient antibody is available to cross-link the antigen; these are readily cleared by the reticuloendothelial system of cells bearing Fc and complement receptors. Small, soluble immune complexes that form when antigen is in excess can be deposited in and damage small blood vessels.

Immune Deviation

Definition

Is the deliberate polarization of an immune response from one dominated by T_H1 to one dominated by T_H2, or vice versa.

Immune Escape

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Definition

Is one of the hallmarks of cancer development and ►metastasis. It is characterized by the lack of ability of

the immune system to eliminate transformed cells prior to and after tumor development.

Characteristics

Several mechanisms have been proposed and tested to explain cancer immune escape. It is now evident that both the host as well as the tumor play important roles in this phenomenon. The host's contribution is manifested by the host's ability to recognize to antigens expressed by tumor cells, a phenomenon known as "Host Ignorance". It happens because of defects in both the innate and adaptive arms of the immune system. The tumor's contribution is manifested by the adaptation of tumor cells to evade the immune systems or developing a ►microenvironment that suppresses the immune system.

Host Ignorance

The innate arm of the immune system forms a first line of defense against cancers. ►Macrophages, ►dendritic cells, and ►monocytes are essential in eliminating cancers. Another type of cell involved in the elimination of cancers is ►NK cells. These cells are capable of recognizing and attacking cells that have down-regulated ►MHC class I expression. This phenomenon is known as "missing self-recognition." NK cells can effectively eliminate cells that have been altered due to cellular transformation, infection, or other carcinogens and cannot present new antigens. If these players involved in the ►innate immune system are defective, cancer cells may escape tumor ►immunosurveillance from not only innate immunity but also ►adaptive immunity as NK cells, macrophages, NKT cells, play a role in the induction and enhancement of ►adaptive immune responses.

The adaptive arm of the immune system has also been shown to play a crucial role in recognizing and eliminating cancer cells. However, several defects have been shown to hamper the ability of this arm to combat tumor cells.

1. Defects in ►antigen-presenting cell (APC). To elicit an effective immune response, antigens must be processed first by the APCs and presented to the immune system. Dendritic cells (DCs) are professional APCs and play an important role in regulation of adaptive immune response. Many tumor patients have shown defects in the DC compartment in terms of cell number and/or altered function. Tumor infiltrating DCs have defective surface expression of HLA and B7 costimulatory molecules, thus are likely to induce anergy rather than to stimulate tumor-specific T cells. Additionally, the immunosuppressive enzyme indoleamine-2,3-dioxygenase (IDO), which has been implicated as one mechanism that helps to maintain maternal ►tolerance toward the fetus during pregnancy, was recently recognized as a potential mechanism of tolerance in malignancies. Expression of ►IDO on APCs was observed in tumor draining lymph nodes, which may be indicative of the creation of a tolerogenic microenvironment. The molecular mechanism by which ►IDO suppresses T cells is still being elucidated. It was suggested to be mediated by depletion of the essential amino acid tryptophan and by the generation of pro-apoptotic metabolites.
2. Negative immune regulation by suppressive cells. There are several types of suppressive cells in the immune system, including ►regulatory T cells, ►myeloid suppressor cells (MSCs) and NKT cells. They exist to keep the immune response under control and prevent autoimmunity. In addition, these cells have been shown to inhibit immune responses against tumor cells. (i) *Regulatory T (Treg) cells* are a subpopulation of $CD4^+$ ►T cells. Tregs are crucial for controlling autoreactive ►T cell responses. At least two distinct subsets of Tregs have been identified: natural and inducible Tregs. Natural Tregs are T cells that arise in the thymus. They require direct cell contact with their target cells in order to exert their regulatory functions. Inducible Tregs arise in the periphery and exert their regulatory functions by the soluble cytokines IL-10 and TGF-β. The exact mechanisms by which Tregs exert their regulatory functions remain largely unknown. However, there is sufficient evidence, both in humans as well as in rodents, demonstrating that Tregs play crucial roles in suppressing anti-tumor responses. (ii) *Myeloid suppressor cells* were first identified by the expression of surface marker CD11b and Gr-1. Their suppressive activity was shown by inhibiting lymphocyte proliferation and inducing apoptosis in CD8 T cells. (iii) *NKT cells* are a unique sub-lineage of T cells. However, in contrast to conventional and regulatory T cells that recognize peptides in the context of MHC class I and II molecules, NKT cells recognize glycolipids presented by CD1d, a non-classical antigen presenting molecule. This difference in recognition means that NKT cells can recognize a class of antigens that conventional T cells cannot recognize. NKT cells have been shown to promote as well suppress immune responses to cancer. Currently, it is still unclear what factors determine their function.
3. Defective anti-tumor T cells. Another factor that leads to a failed immune response to tumor is cells dysfunction in ►tumor-infiltrating lymphocytes (TILs). In a number of studies, TILs have been shown to have defective anti-tumor killing effect. This dysfunction may result from the ineffective granule exocytosis. TILs also have been reported to have defective cytokine production and low expression of B7.1 and B7.2 costimulatory molecules, suggesting anergy could occur.

Tumor Cell Adaptation

To escape immune surveillance, tumor cells deploy several strategies.

1. *Downregulation of MHC class I expression on cell surface.* The presentation of antigen by ►MHC class I is crucial for immune responses. By downregulating MHC class I molecules on the surface, tumor cells may become elusive targets for T cells thereby escape the destruction by ►cytotoxic T lymphocytes (CTLs).
2. *Suppression of the Immune System*. Tumor cells have several "counterattack" methods to fight the immune system. This include: (i) *Secretion of immunosuppressive cytokines such as IL-10, TGF-β, IL-13, etc*. IL-10 interferes with the induction of anti tumor responses. It has been shown to block dendritic cell-mediated priming of T cells into CTL effectors in vitro, and its expression in serum appears to be associated with negative prognosis in certain cancers. ►TGF-β is another cytokine that inhibits activation, proliferation, and activity of T lymphocytes. This ►cytokine is often found at high levels in malignancies and is associated with a poor prognosis as well as lack of response to tumor immunotherapy. Both cytokines can be produced by the tumor cells themselves or by infiltrating stroma cells. Interestingly, TGF-β has also been shown to drive expansion of regulatory T cells, thus bringing together two evasion mechanisms. (ii) *Up-regulation of tumor cells of Fas ligand on the surface to induce apoptosis of tumor killing effector cells.* The death-inducing ►FAS/APO-1/CD95 ligand (FasL/CD95L) has been reported to be expressed on many human tumors of various origins. Expression of FasL in tumors implies that cancer cells are resistant to Fas-induced cell death, which preserves them from extermination by cytotoxic T cells and actively induces death of effector T cells. In addition to local defense, accumulating evidence suggests that the FasL expression may also be relevant for tumor progression and formation of metastasis. It was further described that tumor cells are able to release membrane vesicles (MV) or exosomes containing FasL which induce ►apoptosis of activated T cells. This apoptosis-inducing pathway, via the release of FasL-positive MV, may indeed play a significant role in eliminating the most effective component of the anti tumor T cell response, and provides an explanation for the observed spontaneous T cell apoptosis in the peripheral circulation of cancer patients. (iii) *Inhibition of effector cells by inhibitory ligands including PD-L1, CTLA-4 and LAG-3.* Evidence suggests that tumor cells may evade T and NK cell recognition by receptor ligand interaction. CTLA-4 is a receptor predominantly found on T lymphocytes interacting with CD86 ligand. CTLA-4 is related to CD28 on T lymphocytes, but plays an opposite role. CTLA-4 may act at the level of TCR or CD28 signaling to inhibit T cell activation. Several studies provide evidence that CTLA-4 can up-regulate TGF-β expression and can attenuate CD28 signals on Treg expansion and survival. The precise mechanism of T cell inactivation by CTLA-4/CD86 signaling, however, remains uncertain. The PD-1 – PD-L pathway has been postulated to regulate the immune response in both lymphoid and non-lymphoid organs. PD-Ls are expressed in many tumors such as ►ovarian cancer, ►esophageal cancer, ►kidney cancer and ►brain cancer. It is suggested that tumor associated PD-Ls may promote T cell apoptosis and thus affect immune responses. Another inhibitory ligand associated with ►MHC class II interactions is LAG-3 (lymphocyte activation gene-3). LAG-3-MHC class II could enhance the cross-talk between T cell and DC. In human cells, LAG-3 serves as a negative regulator of activated T cells. A comprehensive understanding of these inhibitory pathways will facilitate the design of effective ►immunotherapy in the future.

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Immune Escape of Tumors

Definition

Many tumors grow progressively despite an ongoing tumor-specific immune response. It has been experimentally shown that tumor cells adopt a variety of

strategies to "escape" both ►innate immunity as well as ►adaptive immunity. For example, tumor cells down-regulate or loose tumor antigens or important components of the antigen-processing machinery so that ►T cells are no longer able to recognize peptides bound to major histocompatibility complex molecules on the cell surface. Tumor cells may also express ►cytokines (such as IL-10 or ►TGFβ) or cell surface molecules (such as ►FAS or PD-1) which actively inhibit cellular anti-tumor immunity including the induction of T cell ►apoptosis, T cell anergy and regulatory T cells.

►Melanoma Vaccines
►T-Regulatory Cells

Immune Response

Definition

Is the response made by the host to defend itself against a pathogen.

Immune Responses to Autoantigens

►Autoimmunity and Prognosis

Immune Surveillance of Tumors

Definition

The concept that the immune system is able to recognize and eliminate cancerous or pre-cancerous cells was first postulated in 1909 by Paul Ehrlich and was further propagated by McFarlane Burnet in the 1950s. Studies in genetically modified, immunodeficient mice have led to a refinement of this theory by Robert Schreiber and co-workers and has been called "cancer ►immunoediting." It describes three phases: (i) The elimination phase in which nascent tumor cells are destroyed by elements of the ►innate immunity and ►adaptive immunity; (ii) the equilibrium phase, in which tumor cells persists but are "equilibrated" by the immune system which prevents tumor progression; and (iii) the escape phase, in which tumors actively disable immune recognition by co-opting immune cells for growth, ►angiogenesis and ►invasion.

►Melanoma Vaccines
►Immunoprevention
►Allergy

Immune System

Definition

Refers to the tissues, cells, and molecules involved in ►adaptive immunity, or sometimes the totality of host defense mechanisms.

Immunoassay

Definition

A test using antibodies to identify and quantify substances. Often the ►antibody is linked to a marker such as a fluorescent molecule, a radioactive molecule, or an enzyme.

Immunoblotting

Definition

Is a common technique in which proteins separated by gel electrophoresis are blotted onto a nitrocellulose membrane and revealed by the binding of specific labeled antibodies.

Immunochemical Fecal Occult Blood Test

►Fecal Immunochemical Test

Immunocompetent

Definition
Capable of developing an ►immune response.

Immunocytochemistry

Definition
synonym: ►Immunohistochemistry; Is a method of identifying cell types based on the demonstration of a specific cytoplasmic or nuclear protein or antigen *in situ*. It does this by detecting specific antibody-antigen interactions where the antibody has been tagged with a visible label, most commonly an enzyme. It is very useful for differentiating and subclassifying ►carcinomas, ►sarcomas, ►melanomas and ►lymphomas.

►Fine Needle Aspiration
►Pathology

Immunodeficient Nude Mice

Definition
Are athymic hairless mice without T-cells, that are unable to generate an effective ►immune response. This makes possible the transplantation of human cancer cells without rejection.

►MIC-1
►T cell

Immunodiffusion

Definition
Is the detection of antigen or antibody by the formation of an antigen:antibody precipitate in a clear agar gel.

Immunoediting

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Definition
Changes in the immunogenicity of tumors due to the anti-tumor response of the immune system that result in the emergence of immune-resistant variants.

Characteristics
History
The concept of immunoediting is predicated on the insight that the immune system can recognize tumor cells. The notion that the immune system monitors the host, not only for pathogen invasion but also for neoplastic changes, arose early in the history of Immunology, was first proposed by Paul Ehrlich in 1909 and then resurrected 50 years later by Burnet and Thomas. These early immunologists proposed that the immune system recognizes cells that have undergone neoplastic changes and eliminates them before they can form tumors, a concept known as immune surveillance. However, although this notion has been around for nearly a century it was only quite recently unequivocally demonstrated in murine models when Schreiber and colleagues, in 2001, examined the incidence of adenomas and carcinomas in aging wild type mice compared to mice lacking the ►recombinase activating gene-2 (RAG2). The RAG2 gene controls the ►V(D)J recombination of genes required to generate ►B cell and ►T cell antigen-specific receptors. In its absence, or the absence of its partner in this process, RAG1, the development of lymphocytes that bear these receptors is aborted. Thus in mice that lack RAG1 or RAG2, T cells, B cells and NKT cells fail to develop and the mouse has no adaptive immune response. The Schreiber lab showed that these immunodeficient mice had a higher incidence of spontaneous cancer and were more susceptible to carcinogen induced sarcomas. These tendencies were increased in mice lacking not only the RAG2 gene but in addition the ►*STAT-1* gene, which controls the ►interferon–gamma ►signal transduction pathway. Thus both adaptive immune effector cells and the multifunctional lymphokine IFN-γ were shown to play a clear role in immunosurveillance. However, in the process of studying ►immunosurveillance the Schreiber group made the interesting observation that a high proportion of tumors that emerged in carcinogen treated RAG2 deficient mice failed to grow in wild type mice indicating that within the population of tumor cells that

arose in the immunodeficient mice were cells that could be recognized and eliminated by an intact immune system. In contrast tumors that arose in wild type mice were less immunogenic and would grow when transplanted into either wild type or RAG2 deficient mice. Schreiber termed this process by which the immunological environment alters the immunogenicity of tumors "immunoediting."

The Role of Interferons and Other Immune Components

A large body of work provides evidence that both ►adaptive immunity and ►innate immunity play a role in controlling the immunogenicity of tumors that develop in the mouse. Most of these studies have focused on the formation of carcinogen induced sarcomas in mice genetically manipulated to lack the expression of genes required for the generation of lymphocytes and cytokines. Thus in terms of innate immune cells, ►γδ (gamma delta) T cells, ►Natural Killer cells and NKT cells have been shown to be involved in controlling tumor growth as have conventional αβ (alpha beta) ►T cells, the hallmark of the adaptive cellular immune response. The importance of cytotoxic lymphocytes, such as T cells and ►NK cells, is highlighted by the inability of mice that lack the specific toxic molecules ►perforin and ►Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), expressed by cytotoxic cells, to control both carcinogen induced and spontaneous tumors. That at least a subset of the lymphocytes that recognize and control tumor growth are ►MHC class I restricted, tumor-specific, ►cytotoxic T cells (CTLs) is evidenced by the fact that mice lacking the ►LMP2 (Low Molecular Mass Protein 2) ►proteasome subunit, involved in processing antigen for recognition by CTL, develop spontaneous ►uterine tumors. The adaptive immune response has evolved to ignore its own tissues, by eliminating self-reactive lymphocytes during their development, in order to avoid ►autoimmunity or "horror autoxicus" as Paul Ehrlich termed it. Thus the participation of CTLs in tumor immunoediting implies that tumors must express ►tumor-associated antigens.

The original observation of immune editing identified a key role for the type II interferon, IFN-γ, in the process. This pleiotropic cytokine is the product of lymphocytes of both the innate (γδ T cells, NK and NKT cells) and adaptive ($CD4^+$, MHC class II restricted ►helper T (Th) cells and $CD8^+$ MHC class I restricted CTLs). It seems likely that both innate and adaptive lymphocytes are the source of the IFN-γ that shapes the immune response to tumors. The effects of IFN-γ on the immunogenicity of tumors could occur though multiple processes since it is known as a key regulator of adaptive and innate immune responses. There is a great deal of evidence that some of its effects are mediated through host cells in addition to tumor cells. However, given that many tumor cells express IFN-γ receptors, it can directly interact with tumor cells and there is evidence that it stimulates tumor cells to increase MHC class I expression, downregulate ►angiogenesis and promote the infiltration of CTLs into the tumor mass. Another cytokine, ►IL-12, has also been shown to promote tumor immunoediting. IL-12 is intimately involved in the regulation of IFN-γ production early in the immune response and probably mediates its effects through its influence on IFN-γ expression.

In addition to IFN-γ, the ►type I interferons have a profound influence on tumor growth. Early studies in mice using type I interferons for tumor ►immunotherapy have been translated into several clinical applications for these cytokines in the therapy of ►melanoma, ►CML, ►follicular lymphoma, ►hair cell leukemia and ►Kaposi sarcoma. There are several important differences in mice and humans between the type I interferon, IFN-γ, which is the sole molecular species, and the type II interferons, IFN-α for which there are at least 12 variants, and IFN-β, which is unique. Unlike IFN-γ, which is the product only of cells of the lymphocytic lineage, type I interferons can be produced by all nucleated cells when stimulated by products of viral infection and also by infection with some bacteria. As such they are a first line of defense against pathogen invasion of the host. In addition, although tumor cells can express receptors for type I interferons, it appears, that unlike IFN-γ, the type I interferons do not act directly on tumor cells and mediate their anti-tumor effects though host cell responses. Exactly which cells are involved requires further study but they appear to be cells of the hematopoietic lineage. Similar to IFN-γ, there are several points at which type I interferons might influence the immune response to tumors since they can activate ►dendritic cells, ►macrophages and ►NK cells and are involved in the priming and survival of T cells. In addition, similarly to IFN-γ they can act on stromal cells within and surrounding tumors to down regulate angiogenic factors. Finally, there is evidence that type I interferons may inhibit the transformation of normal cells by upregulating the expression of the tumor-suppressor molecule, ►p53. The importance of this class of cytokines in shaping the immune response to tumors was confirmed recently by studies in mice lacking the IFN-α receptor. Carcinogen induced sarcomas were found to arise more frequently in these mice and were also shown to be more immunogenic when transplanted into wild type mice.

The Role of Induced Immune Pressure

The editing of tumors in response to immune pressure is exacerbated when the immune system has been specifically primed to a ►tumor-associated antigen.

In a ►mouse model for ►breast cancer in which ►HER-2/neu, a member of the ►epidermal growth factor receptor family, is overexpressed on breast tissue, resulting in the emergence of breast tumors, it was shown that mice immunized with ►cancer vaccines expressing fragments of HER-2/neu could control tumor outgrowth for a significant length of time but eventually succumbed. When HER-2/neu was isolated from the emerging tumors they were found to have accumulated a significant number of mutations in the precise region of the molecule the particular vaccine targeted. In addition, it was verified for one of the fragments that each mutation occurred in a region recognized by CTLs and that this mutation abrogated CTL recognition. This is a clear demonstration of how the host immune system can sculpt a tumor-associated antigen to evade the immune system. Transplanted, syngeneic mouse tumors have also been shown to mutate in a region of an antigen expressed by the tumor and recognized by CTLs after adoptive transfer of T cells that recognize that region.

Evidence of Immunoediting in Human Cancer

Although there is an abundance of evidence for immunoediting in murine models of cancer, the evidence that this occurs in human cancer is largely circumstantial. It has long been known that cancer patients develop immune responses to their own tumors. Indeed, both tumor-specific T cells and antibodies isolated from cancer patients have been harnessed to identify tumor-associated antigens by methods such as ►SEREX. In addition the ability of a patient to mount a response to their tumors, particularly if there is evidence of tumor infiltration of immune effector cells, is a strong predictor of a favorable prognosis. That the immune system sculpts tumor immunogenicity in tumors that arise in cancer patients is supported by the emergence of tumors in patients undergoing antigen-specific immunotherapy that have down regulated the tumor antigen to which the therapy is directed, or components of the cellular machinery that generates the cell surface complex of ►HLA I and antigen recognized by CTLs. These include LMP proteasome subunits and transporter molecules that chaperone antigen peptides from the cytosol to the Golgi apparatus for loading onto HLA class I molecules. Sometimes the HLA I molecule itself is lost, usually by mutation of the beta2 microglobulin subunit, common to all HLA class I molecular complexes. In some cases, however, lack of HLA class I on the surface of a tumor is due not to mutational events in the HLA class I genes themselves but to down regulation of expression of the protein. This can often be reversed by cytokines, such as IFN-γ but it is interesting to note that human tumors have been shown to arise that lack the IFN-γ receptor. The phenomenon of HLA class I loss from the surface of tumor cells has been documented in numerous clinical cancer vaccine trials for ►melanoma, ►prostate carcinoma and for HER-2/neu positive tumors. In addition, even in the absence of immunotherapy, HLA class I expression has been shown to be lost or down regulated in all types of tumors especially in patients with advanced disease. Indeed, the frequency of deletion or down regulation of these cell surface molecules has been found to be as high as 15% in primary melanoma lesions and 50% in primary ►prostate carcinoma lesions. A final piece of evidence for immunoediting in human cancer is that patients that are seriously immunosuppressed, for example post organ transplant, have a higher incidence of cancer than healthy individuals.

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Immunogenicity

Definition

The ability of a foreign substance to confer a certain level of immunity to the host.

►T-Cell Response

Immunoglobulin

Definition

Ig; The immunoglobulin molecule, composed of two light and two heavy protein chains, comprising the antibody.

►Immunoglobulin Genes

Immunoglobulin E (IgE)

Definition

An antibody characteristic of the atopic allergic immune response.

►Allergy
►Immunoglobulin Genes

Immunoglobulin Genes

Definition

Cluster in multimember gene families and are located on chromosome 14 (heavy chain gene segments), 2 (κ light chain) and 22 (λ light chain): V genes (variable region genes), D genes (diversity region genes) and J genes (joining region genes) for the heavy Ig heavy chains, V and J region genes for κ and λ light chains. Ig gene rearrangement brings together one representative of these gene families (V, D and J for the heavy chain, V and J for κ and λ light chains). ►V(D)J recombination is a remarkable process since it entails double strand DNA breaks, loss or DNA and re-ligation of DNA strands. Joining of the V-D-J segments is imprecise and involves insertion of non-template nucleotides (N-additions) and trimming back of the beginning/ends of the gene segments. Ultimately, the rearrangement leads to a unique sequence of VH-N-D-N-JH, which generates diversity for antigen recognition, and which acts a marker for each individual B-cell and its progeny.

►B-Cell Tumors

Immunoglobulin Libraries

Definition

Recombinant nucleic acid libraries of natural or synthetic origin that encode antiBody binding sites (Fab or scFv) for which the products (and corresponding genes) can be selected against an antigen of interest and subsequently modified to improve their affinity. Applications: Fast selection of new antiBody binding sites from different species. Modification of pre-existing binding site affinity.

Immunoglobulins

Definition

Immunoglobulins (Ig): All antibody molecules belong to a family of plasma proteins called immunoglobulins (Ig). Membrane-bound immunoglobulin serves as the specific antigen receptor on B lymphocytes. Different immunoglobulin isotypes are called IgM, IgD, IgG, IgA, and IgE.

Immunoglobulin A (IgA): Immunoglobulin A is the class of immunoglobulin characterized by α heavy chains. IgA antibodies are secreted mainly by mucosal lymphoid tissues.

Immunoglobulin D (IgD): Immunoglobulin D is the class of immunoglobulin characterized by δ heavy chains. It appears as surface immunoglobulin on mature naïve B cells but its function is unknown.

Immunoglobulin E (IgE): Immunoglobulin E is the class of immunoglobulin characterized by ε heavy chains. It is involved in allergic reactions.

Immunoglobulin G (IgG): Immunoglobulin G is the class of immunoglobulin characterized by γ heavy chains. It is the most abundant class of immunoglobulin found in the plasma.

Immunoglobulin M (IgM): Immunoglobulin M is the class of immunoglobulin characterized by μ heavy chains. It is the first immunoglobulin to appear on the surface of B cells and the first to be secreted.

Immunohistochemistry

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Synonyms

IHC; Immunohistology; Immunocytochemistry; Molecular Morphology

Definition

Immunohistochemistry (IHC) is a technique to detect and localize specific proteins in tissue sections by labeled antibodies that bind specifically to the investigated antigen.

Characteristics

History

Since the introduction of microscopy into the diagnosis of diseases by Bennett in 1842 and Virchow in 1858,

pathologists have searched for better and more specific stains. In 1934, Marrack was the first to employ modified antibodies to visualize cholera and typhoid agents. Nearly a decade later, Coons (1941) used a primary antibody that was labeled with a fluorescent dye, fluorescein isothiocyanate, and utilized it on human tissue. However, as a specialized dark-field microscope and use of frozen sections were needed for the fluorescent dyes, the technique remained mainly a research tool until other labels were developed. In 1974, Taylor and Burns demonstrated that IHC could be performed on routinely processed tissue using immunoperoxidase techniques with chromogenic dyes. This step enabled the use of IHC in routine clinical laboratories.

Techniques

Immunohistochemistry combines the principles of immunology with histochemistry, and involves two basic steps: first, an ►antibody binds to its specific target antigen at the cellular location in the tissue sample. The antigen-antibody binding is then detected by labeling techniques. IHC therefore not only enables pathologists to detect whether or not particular antigens are present within a given tissue, but also allows marking its cellular location.

There are two major types of antibodies in use: polyclonal antibodies or antisera and monoclonal antibodies. Polyclonal antibodies are produced by an injection of an antigen or antigen fragments into a host animal. The most common host animals are rabbit, horse, mouse and goat. If a small antigenic molecule or antigen fragment (hapten) is used as immunogen, immunogenicity is typically enhanced by coupling the hapten with a compound such as keyhole limpet hemocyanin. In the resulting immunologic reaction, host lymphocytes are activated and ultimately plasma cells initiate production of antibodies. As antigens contain various immunogenic determinants, the resulting antiserum will be a heterogeneous mix that recognizes several epitopes. Such potentially cross-reacting specificities are unwanted for use in IHC and must be removed by further purification. Monoclonal antibodies are developed by the hybridoma technique or through molecular engineering using bacteriophages. In the hybridoma technique, activated (antibody-secreting) B-lymphocytes are isolated from the immunized animal (in the past usually mouse, now increasingly common rabbit) and fused with cultured myeloma cells which have limitless proliferative potential. Each of the resulting hybrid cells (hybridoma) produces a single type of antibody, thus being 'monoclonal' in nature.

The detection methods include the 'direct' and the 'indirect' alternatives. In the direct conjugate-labeled-antibody method, the label (such as a fluorescent agent or an enzyme) is chemically attached to the primary antibody. The localization of this fluorescent label is detected by ►immunofluorescence microscopy or if the label is an enzyme (typically, horseradish peroxidase) coupled to antibody, it is detected by its action on a chromogenic substrate. The indirect method uses an unlabeled primary antibody and a labeled secondary antibody (second layer) directed against the primary antibody. Several secondary antibody molecules bind to each primary antibody molecule resulting in signal enhancement, thus in increased sensitivity.

Furthermore, methods involving a third layer for signal amplification can be employed. These include the PAP (peroxidase-antiperoxidase) method, the biotin-avidin mediated procedures (such as those employing the avidin-biotin conjugate or ABC procedure, biotin-streptavidin systems), immunogold or polymer based labels, catalyzed signal amplification and alkaline phosphatase-based methods.

Several efforts have been made to improve the sensitivity, the reliability and the standardization of IHC. A major step was the development of antigen retrieval. The antigen retrieval process breaks protein cross linkages formed during formaldehyde fixation of a tissue and enables these proteins to be accessible for antibodies. Antigen retrieval is performed by exposing tissue to heat for various lengths of time in retrieval buffer solutions with specific pH. Several protocols are available but these often have to be adjusted for optimal results which can be done standardized for various temperatures and durations in a 'test battery' approach.

Several automated immunostaining systems are commercially available. While they significantly increase the reproducibility and standardization, they certainly do not guarantee optimal results. Quality control issues as well as the need for the validation of the results apply for staining carried out with both the automated systems and manual IHC. Interpretation of IHC staining results necessitates a highly trained pathologist and even among experts is subject to inter-observer discrepancies. The development of automated image analysis systems addresses this problem. The currently available systems can both assess the intensity as well as the percentage positivity of antigenic markers. The limitations of the human eye to differentiate colors restrict the use of multiple markers on the same slide. The spectral imaging technology allows to differentiate different chromogens reporting on multiple markers by serially, digitally "erasing" a given chromogen image from the microscope image by a specially designed software.

Uses

IHC plays an essential role in modern pathology. Its most common use is the identification of tumor origin

and tumor type by tissue-specific markers. By IHC a pathologist can specify tumor entities and evaluate tumors of unknown origin as well as stage lymph nodes and bone marrow for the presence of metastatic disease.

More recently, several markers have been identified that can predict disease prognosis, treatment response and serve as therapeutic target.

Use in Research

In a research setting, the expression of relevant markers is investigated. This demands studies on well-defined samples across various tumor stages and clinical outcome. The tissue microarray technique allows an acceleration of such studies in a high-throughput manner. Tissue microarrays (TMA) consist of compendia of 0.6 mm cylindrical biopsy cores from paraffin-embedded tissue that are transferred to defined array coordinates in a recipient block. These multi-tissue-core blocks can then be processed similar to a routine single-tissue block. A difference from the conventional IHC is the high level of standardization since all slides in one TMA experiment are incubated together, ensuring identical reagent concentrations and incubation temperatures.

Clinical Uses

Diagnosis

Intermediate Filaments

The presence of intermediate filaments, which function as cytoskeletal components in both normal and malignant cells, is useful in the initial classification and diagnosis of neoplasms. Five major classes of intermediate filaments exist: ►Cytokeratin (CK), vimentin, desmin, neurofilament and glial fibrillary acidic protein (GFAP). Most neoplasms show a predominant expression of one or more of these intermediate filaments. Based on their molecular weight and isoelectric point, the cytokeratins are subdivided into more than 20 types. Carcinomas are typically CK positive, whereas sarcomas, melanomas, and lymphomas are generally vimentin positive. Tumors of myogenic origin characteristically express desmin and/or muscle actins and vimentin. Glial tumors are predominantly positive for GFAP.

Nuclear Transcription Factors

Transcription factors are proteins involved in the regulation of gene expression. By binding to promoter elements upstream of genes they either facilitate or inhibit transcription. Though not exclusively found in a specific tumor type, transcription factors are highly tissue specific and can be useful in determining the primary site of a tumor.

Carcinoma

Essentially all cells of epithelial origin express cytokeratins, which are therefore highly sensitive markers for carcinomas. However, other tumors such as mesotheliomas and non-seminomateous germ cell tumors also stain positive for CK. More specific markers and sub-typing of keratins have to be applied to further differentiate CK-positive cells and to assess the site of origin of the carcinoma. The profile of cytokeratin sub-types is useful to determine the tumor type. As for example, hepatocellular carcinomas are positive for CK antigens that can be detected by antibodies AE3 and CAM5.2 but are negative for antibody AE1, which is directed against a different set of CK. Recently, the use of CK7 and CK20 profile has proven a useful tool in distinguishing cellular origin. Nuclear transcription factors are useful in the detection of carcinomas. Thyroid transcription factor-1 (TTF-1) is found in the thyroid and lung. CDX-2 is specific for colorectal epithelium. For some tissue types, tissue-specific tumor markers (e.g. prostate specific antigen (PSA) for prostate, or thyroglobulin for thyroid) or tissue-associated markers (e.g. gross cystic disease fluid protein 15 (GCDFP-15) and mammaglobin in breast, uroplakins for transitional urothelial cells, OC 125 for ovarian cells, synaptophysin for neuroendocrine lesions, etc.) are available. It is important to remember that these markers are often not uniquely found in the specific tissue. For instance, the detection of PSA has also been described for samples of salivary glands and breast.

Melanoma

For melanomas, which usually are cytokeratin-negative and vimentin-positive, a sensitive tissue marker, S-100, is available. HMB-45, Melan A and tyrosinase are used to confirm the diagnosis.

Sarcoma

The common IHC pattern for all forms of sarcoma includes vimentin positivity. Generally speaking, sarcomas are CK-negative, but some may yield positive reactions especially for low molecular CKs following antigen retrieval and highly sensitive labelling techniques. Additional stains are then employed to identify the tumor type. Rhabdomyosarcoma and Leiomyosarcoma express markers that are typical for muscular tissue such as desmin and muscle specific actin.

Lymphoma

Lymphomas show a wide variety of morphologic appearances, making IHC especially important in their diagnosis. Membranous staining for ►CD45 can typically be seen in lymphoid tissue. CD3 and ►CD20 are more specific markers and are used to

further confirm lymphomas. ►CD antigens are essential in subclassifying lymphoid tissue.

Infectious Agents

Since the early days of its development, IHC has been used for the detection of infectious agents. Nowadays, microbiologic cultures are the gold standard but for pathogens that are difficult to culture such as cytomegalovirus, mycobacteria, toxoplasmosis, pneumocystis carinii, histoplasma capsulatum, ►Helicobacter pylori and ►human papillomavirus IHC methods are at least as effective.

Tumor Stage and Occult Metastases

The most important factor determining outcome of patients with cancer is the presence of regional and/or systemic dissemination (metastases). While routine pathologic work-up cannot detect small metastatic deposits in many instances, IHC is a highly sensitive way to detect such occult metastases in blood, lymph nodes and bone marrow. This technique is based on the fact that monoclonal antibodies can reveal cells of different histogenesis in the investigated tissue. As normal lymph nodes, bone marrow or blood do not contain cells with epithelial antigens, the finding of CK-positive cells suggests metastases of a carcinoma. The presence of occult metastases has been shown to influence clinical outcome for several cancer types such as breast, lung and prostate cancer. In breast cancer, IHC is able to show the presence of occult metastases in bone marrow in 10–40% of patients with low-stage disease. Lymph node metastases occult to routine pathologic work-up have been described for several tumors including breast cancer in up to 20% of patients and in more than 10% of patients with prostate cancer. The reported sensitivity of this technique ranges from the detection of 1 epithelial cell in 10,000 to 2–5 epithelial cells in a million hematopoietic cells.

Prognosis

Mutations and overexpression of oncogenes and tumor suppressor genes play a vital role in tumorigenesis. The presence or absence of their protein products may predict the biological behavior of tumors more accurately than clinical and pathologic criteria. Since antibodies are available for many of these gene products, tumors with different prognosis can be differentiated by means of IHC.

The best investigated proteins include ►p53, the protein product of the ►Retinoblastoma gene (pRb), p27, ►p21 and ►p16. RB gene alterations resulting in reduced expression are known to characterize tumors with a higher risk for metastatic disease. p21, p16 and p27 are the three major cyclin-dependent kinase inhibitors which negatively regulate pRb activity and are normally required for cell cycle suppression. The loss of *p27* expression is associated with colon, breast, prostate, and gastric cancer progression. Changes in protein expression of p21 have been associated with higher tumor grade and worse prognosis in patients with bladder cancer. p53 plays an important role in cell cycle progression, and apoptosis in response to DNA damage, and is expressed by all human cells. Normal (wild-type) p53 protein has a short half-life of only 6 to 30 minutes because of its ubiquitin action-mediated degredation dictated by its binding with another regulatory protein, ►MDM2. Therefore p53 does not accumulate in normal cells. Overexpression of p53 (by altered p53 gene or because of ineffective MDM2 protein) in the nuclear compartment therefore indicates a dysfunctional p53 pathway, a characteristic of tumors. Alterations in p53, p21 and pRb act in cooperative or synergistic ways to promote cancer progression and simultaneous overexpression has been linked to worse prognosis.

Treatment Response

An increasingly important application of IHC is to identify specific targets for therapy. Consequently, this will allow better selection of patients who will benefit from certain treatment modalities. In several tumor types, treatment decisions already are influenced or determined by molecular findings. An early example is hormonal therapy for breast cancer patients depending on estrogen and progesterone expression of the tumor. Because of its vital role in the apoptotic pathway ►p53 alterations are likely to influence response to chemotherapy. p53 alterations confer increased chemosensitivity on tumors such that combining agents with different actions may have synergistic effects on tumor cell killing.

Targeted Therapy

In breast cancer Her-2/neu overexpression was linked to resistance to tamoxifen therapy and commonly used adjuvant chemotherapy. However, it has been shown that the HER2/neu receptor tyrosine kinase can serve as a therapeutic target. Treatment with the monoclonal antibody ►trastuzumab (brandname: Herceptin) which is directed against this protein is effective only for patients whose tumors show overexpression of Her2/neu. In invasive ►bladder cancer there is evidence to suggest that patients who harbor p53 alterations respond to adjuvant chemotherapy that contains DNA-damaging agents such as cisplatinum.

Immunohistochemistry has allowed for identification of tumor origin, tumor prognosis, and likelihood of response to therapy for an increasing array of tumors. The ability to determine which tumors are most likely

to progress (and thus need further therapy), coupled with the ability to predict specifically the response of individual tumors to chemotherapeutic agents and the ability to identify specific targets of therapy will have a profound effect on the way treatment decisions for patients with cancer are made. It is not difficult to envision the day when drug selection is based on the presence of specific targets and on the resistance patterns of individual tumors to specific agents. Treatment decisions will become less organ based and will reflect the biology of the tumors. This has already helped us to approach patient-specific (as opposed to disease specific) management of care.

►Gastrointestinal Stromal Tumor

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Immunohistology

►Immunohistochemistry

Immunoliposomes

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Definition

Are ►liposomal drug formulations possessing antibody molecules conjugated to the liposomal surface. This allows for a ►targeted drug delivery to tumor cells or other tumor-associated structures (active targeting).

Characteristics

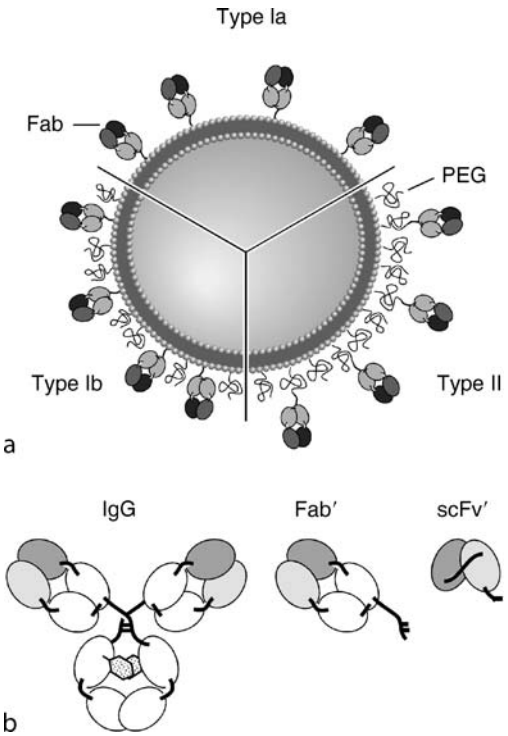
►Liposomes are vesicular particles composed of a lipid bilayer enclosing a hydrophilic inner phase. Liposomes can be used as carrier systems for cancer drugs (►Drug delivery systems) by encapsulating hydrophilic drugs into the interior or by incorporating lipophilic drugs into the lipid bilayer. Liposomes have normally a size (diameter) of 100–200 nm. Several liposomal formulations of therapeutic drugs are approved for cancer therapy, e.g., liposomal doxorubicin (Doxil/Caelyx, Myocet) (►Liposomal chemotherapy). Delivery of these drugs to tumors is a passive process and efficacy depends on long circulation and enhanced permeability and retention in the tumor tissue (EPR effect). New formulations of liposomal drugs (e.g., Doxil/Caelyx) have polyethylene glycol (PEG) chains incorporated into the lipid bilayer to further increase stability and pharmacokinetic properties and to decrease elimination of the liposomes by phagocytic cells. The coupling of antibody molecules to the lipid surface allows for an active targeting by recognition of antigenic structures expressed by the tumor. Thus, immunoliposomes are designed to increase selectivity and efficacy of liposomal drugs.

Immunoliposome Types and Antibody Formats

Immunoliposomes are generated by chemically coupling antibodies or antibody fragments to the liposomal surface. Besides whole ►antibody molecules (e.g., IgG molecules), antibody fragments such as Fab′ fragments or ►single-chain Fv fragments can be utilized for the generation of immunoliposomes. The use of whole antibodies is less favorable since these immunoliposomes are recognized by phagocytic cells via Fc receptors. Consequently, Fab′ or scFv′ fragments are the formats of choice to generate immunoliposomes. Immunoliposomes can be classified depending on the position of coupled antibodies and the liposomal composition. In type I immunoliposomes, the antibodies are coupled directly to the lipid bilayer either in the absence (type Ia) or presence of PEG chains (type Ib). Coupling of the antibodies to the distal end of incorporated PEG chains results in type II immunoliposomes (Fig. 1a).

Coupling of antibodies is facilitated by the use of functionalized lipids, e.g., lipids possessing an amino-reactive succinimidyl moiety or a sulfhydryl-reactive maleimide group. Antibody or antibody fragments can be generated by established ►hybridoma technology and biochemical means or by genetic engineering as recombinant molecules. For example, Fab′ fragments can be produced by enzymatic cleavage of IgG molecules with pepsin resulting in $F(ab')_2$ fragments, which after reduction are separated in Fab' fragments exposing a free sulfhydryl group at the end of the molecule (Fig. 1b). Furthermore, the implementation of antibody engineering allows for the generation of

small antibody molecules with desired properties, e.g., scFv molecules exposing a genetically introduced cysteine residue at the C-terminus used for site-directed coupling (Fig. 1b).



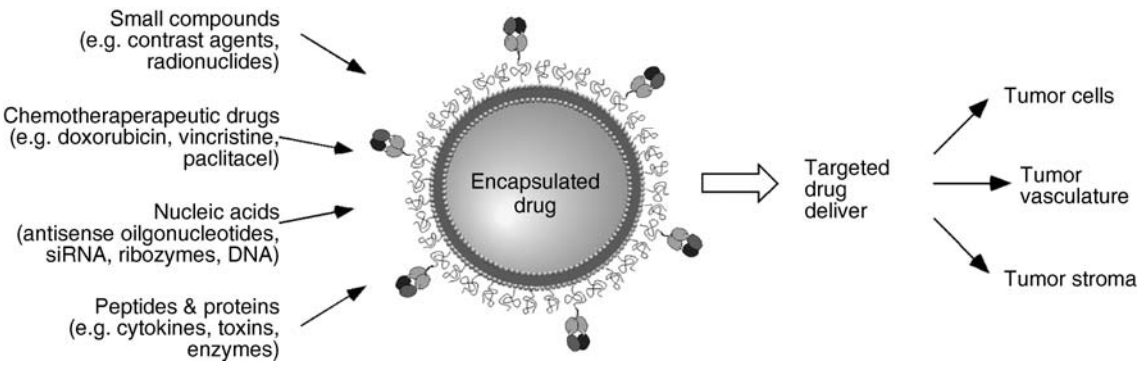
Immunoliposomes. Figure 1 Classification of immunoliposomes and antibody formats. (a) Three types of immunoliposomes can be distinguished. Type I immunoliposomes have the antibody molecules coupled directly to the lipid bilayer, either in the absence (type Ia) or presence (type Ib) of polyethylene glycol (PEG) chains. In type II immunoliposomes, the antibodies are coupled to the distal end of PEG chains. (b) Antibodies formats used for the generation of immunoliposomes. The two variable domains (V_H, V_L) forming the antigen-binding site are shown in dark and light gray.

Drugs and Targets

Immunoliposomes can be combined with a wide variety of different drugs. Besides small compounds and chemotherapeutic drugs, immunoliposomes can also be used for the delivery of nucleic acids (e.g., ►siRNA) or therapeutically useful peptides and proteins (Fig. 2). Encapsulation of drugs into liposomes alters their pharmacokinetic properties, e.g., reduces rapid renal elimination of small molecular weight drugs. In addition, drug encapsulation has been shown to reduce side effects and to increase stability of the drug within the body.

Several modes of action have been described for immunoliposomes. Delivery to extracellular structures may lead to increased accumulation of liposomes in the tumor tissue and slow release of drug, which then can enter the target cell. Alternatively, binding of immunoliposomes to cell surface receptors results in internalization of immunoliposomes (►Endocytosis) and intracellular release of the drug. Several studies have shown that internalization of immunoliposomes leads to increased cytotoxicity and may also bypass drug resistance mechanisms.

Main targets of immunoliposomes are molecules expressed by tumor cells. Thus, various immunoliposomal formulations of chemotherapeutic drugs (e.g., ►doxorubicin, ►vincristine) have been generated using antibodies against tumor-associated antigens including CD19, ►CD20, Her2/neu, ►epidermal growth factor receptor (EGFR), and disialoganglioside GD2 for therapy of lymphoma and solid tumors. In addition, research has been focused on targeting of tumor blood vessels (►vascular-targeting adjents) as well as targeting of extracellular tumor stroma components or tumor stroma fibroblasts, which are more easily accessible for circulating liposomes. Efficacy of immunoliposomes is influenced by several factors. Besides lipid composition, which has an influence on stability and release of drug, and the antibody format used, efficacy depends also on the kind of target molecule as well as its density on the cell surface and sensitivity of target cells for the encapsulated drug.



Immunoliposomes. Figure 2 Active compounds combined with immunoliposomes. Immunoliposomes allow for a targeted delivery of various kinds of active molecules, including small compounds, chemotherapeutic drugs, nucleic acids, and peptides and proteins.

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Immunologic Tolerance

Definition

The lack of an ►immune response.

Immunological Fecal Occult Blood Test

►Fecal Immunochemical Test

Immunophenotype

Definition

The overall expression of proteins that identify a specific cell type as determined by using specific antibodies reacting against surface proteins on cell membrane. The collective repertoire of proteins expressed on the outer surface of a particular type of cell.

►CD Antigens

Immunophenotypic Determinants

►CD Antigens

Immunophenotyping

Definition

Determining the expression of antigens on or in cells.

►Flow Cytometry
►Leukemia Diagnostics

Immunophilins

Definition

Are a group of intracellular proteins that bind to the anti-inflammatory agents ►cyclosporine A and FK506. The complexes interfere with calcineurin-mediated transcriptional activation and inhibit the transcription of genes encoding several proinflammatory molecules. Proteins that bind immunosuppressant drugs.

Immunoprevention

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Synonyms

Immunoprophylaxis of cancer

Definition

Prevention of cancer onset or of early cancer development and ►progression by means of immunological treatments, such as vaccines, antibodies or cytokines.

Characteristics

Immunoprevention of cancer can be applied to tumors caused by viruses (and other infectious agents) or to tumors unrelated to infectious agents. In both cases the aim is the same, however the underlying concepts and the advancement of clinical development are different. Prevention of viral tumors is based on vaccines against viral antigens, whereas immunoprevention of tumors unrelated to infectious agents targets antigens expressed by early neoplastic cells.

Immunoprevention of Viral Tumors

About 18% of all human tumors are directly caused by infectious agents or indirectly by persisting inflammation accompanying chronic infection. In such cases the application of immunological strategies to prevent infection is a type of ►primary cancer prevention because it aims at removing a risk factor that can cause cancer. The first success in this direction was the demonstration that vaccination programs implemented in the 1980s against ►hepatitis B virus (HBV) significantly reduce the incidence of hepatitis virus associated ►hepatocellular carcinoma. Current vaccines are highly effective (90–95%) in preventing chronic HBV infection. Studies in countries where HBV infection is frequent demonstrated that vaccination reduced by half infantile hepatocellular carcinoma incidence and mortality. HBV is responsible for one third (developed countries) to two thirds (less developed countries) of all cases of hepatocellular carcinoma, and ►hepatitis C virus (HCV) causes about one fourth. HCV vaccines under development are expected to contribute a further substantial decrease in the worldwide incidence of hepatocellular carcinoma.

►Human papillomaviruses (HPV) are the most prevalent carcinogenic viruses in humans; various types of HPV cause more than half a million new cases of ►cervical cancer and other tumors worldwide. The first human vaccine against HPV was approved in 2006 in the US, EU, and various other countries. It is a quadrivalent vaccine against HPV types 6, 11, 16 and 18. A divalent vaccine against HPV 6 and 18 is undergoing approval in 2007. Both vaccines are made of ►virus-like particles (VLP). Clinical trials of both vaccines demonstrated 89–100% protection of vaccinated women from persistent HPV infection, 100% protection from histologic evidence of cervical cancer, and no serious adverse events. These vaccines are thus expected to have a major impact on the incidence and mortality of HPV-related cancers. Four critical issues are (i) current vaccines do not include HPV types causing one third of cervical cancers worldwide, hence screening programs should not be abandoned until cross-protection by current vaccines has been demonstrated or novel multivalent vaccines have been developed; (ii) vaccines are currently very expensive, and only a few national or regional health systems are prepared to subsidize the costs; (iii) the matter is further complicated by the need to vaccinate prepuberal (9–12-year-old) girls against a sexually-transmitted virus, an issue that is causing considerable ethical debate in some countries; (iv) the follow-up of vaccinated persons is still too short to estimate the long-term duration of immunity and the need for periodic boosts.

Attempts toward the development of vaccines against ►*Helicobacter pylori*, the third major infectious risk factor of human cancer, until now did not produce a credible candidate for mass vaccination.

On the whole it can be estimated that a full-scale deployment of the approaches described above for HBV, HCV and HPV could lead to the immunoprevention of two thirds of infectious human cancers, or more than 10% of all human cancers. This is a very important result which however leaves open the question of what immunoprevention can do for the majority of human cancer not caused by infectious agents.

Immunoprevention of Tumors Unrelated to Infectious Agents

Immunoprevention of non-infectious tumors is a relatively recent development and is currently at the level of ►preclinical testing. This type of immunoprevention targets early neoplastic cells, hence it can be classified as a kind of ►secondary cancer prevention because it aims at preventing the evolution of incipient tumors into clinically-evident, symptomatic masses.

The ►immune surveillance theory posits prevention of tumor onset as a fundamental function of the immune system, on a par with prevention of infection. In fact cancer incidence in knockout mice lacking ►adaptive immunity and ►non-adaptive immunity is much higher that in immunocompetent mice. However the very existence of progressive, malignant tumors demonstrates that the efficiency of spontaneous immune surveillance is lower than 100%. Thus the aim of cancer immunoprevention is to enhance immune surveillance of tumors by means of treatments that elicit protective antitumor immune responses and/or decrease immunosuppressive components.

Immune targeting of ►preneoplastic lesions or of early neoplastic cells has several advantages with respect to conventional cancer ►immunotherapy, which instead must necessarily target advanced tumors. The efficacy of immune defenses is higher against smaller tumor deposits, a property shared by most antitumor approaches, including chemotherapy. Nascent neoplastic lesions are less protected from immune effectors by ►stromagenesis and ►angiogenesis, tumor progression caused by the accumulation of multiple genetic alterations is still at an early stage, and genomic instability has not yet generated a wide array of heterogeneous tumor variants, eventually leading to the selection of immunoresistant phenotypes.

Demonstrations of cancer immunoprevention were mainly obtained in ►cancer-prone genetically modified mouse models and in some ►chemical carcinogenesis systems. Three different strategies were effective in inhibiting and/or delaying tumor onset in mice: (i) monoclonal antibodies directed against membrane tumor antigens; (ii) immunostimulants, such as recombinant ►cytokines (►interleukin-12), plasmids containing CpG sequences (►CpG islands), or bacterial

derivatives (α-galactosylceramide); (iii) vaccines containing whole cells, recombinant proteins, synthetic peptides or plasmids encoding the target antigen. Using standard categories borrowed from immunotherapeutic jargon, the first approach can be classified as *passive immunoprevention*, because it is based on the administration of a preformed immunological "drug" directly acting on tumor cells, the second as *active, antigen nonspecific immunoprevention* and the third as *active, antigen specific immunoprevention*.

Early attempts provided proof-of-principle demonstrations that stimulation of the immune system in healthy, cancer-prone hosts can effectively delay or reduce tumor incidence later in life. Second generation studies aimed at increasing preventive efficacy through the combination of different strategies, much as it happened in chemotherapy (►Chemotherapy of cancer, progress and perspectives). Various combinations of antigen specific vaccines and powerful immunostimulants completely prevented tumor onset in very aggressive mouse models of cancer development, thus demonstrating that cancer immunoprevention can effectively halt a genetic predisposition to cancer. Microscopic analysis of tumor-free aged mice showed that tumor progression is blocked at the stage reached when vaccination begins. For effective immunoprevention of tumor onset vaccination must start before tumor progression reaches specific critical stages, such as *in situ* carcinoma.

Immune Mechanisms of Cancer Prevention

Highly active vaccines like those used for complete cancer immunoprevention elicit simultaneously many overlapping immune responses in immunocompetent mice, hence vaccination of mice with selective immunodepressions is the only way to dissect protective mechanisms from less important immune components. The most important immune mechanisms at work in cancer immunoprevention comprised both ►T cell responses, including the release of cytokines (interferon-γ, IFN-γ) and, less frequently, ►cytotoxic T lymphocytes (CTL), along with antibodies whenever the target antigen was expressed on the cell surface.

The importance of antibody responses clearly distinguishes cancer immunoprevention from cancer immunotherapy, the latter being mainly based on CTL rather than antibodies. The largely different time scales involved justifies this discrepancy, because cancer immunoprevention requires a long-term protection from tumor onset, ideally extending for the entire life of the host, whereas a rapid destruction of tumors or metastases is the goal of cancer immunotherapy. CTL activity must be of short duration to avoid severe toxicity for the host, whereas protective antibodies persisting for a long time are harmless. The same dualism applies to viral immunity in which acute infection is mostly resolved by CTL, whereas long term immunity from reinfection and protection elicited by vaccination are provided by neutralizing antibodies.

The direct interaction of cytokines like IFN-γ and of antibodies with early neoplastic cells results in multiple molecular blocks of cell growth and tumor progression combining cytostatic and cytotoxic mechanisms. Inhibition of cell proliferation is a logical part of the set of antiviral activities of IFN-γ that can directly block tumor cell growth, moreover it inhibits the release of ►matrix metalloproteinases involved in tumor cell invasiveness (►Invasion) and induces the production of chemokines with antiangiogenic activity. Antibodies binding to surface tumor antigens mediate tumor cell killing via ►complement-mediated cytotoxicity and ►antibody-dependent cell-mediated cytotoxicity (ADCC). Whenever the target antigen is involved in mitogenic signaling, antibodies can directly inhibit tumor cell proliferation without the need of further molecules or cells of the immune system, in practice by acting as "receptor antagonists." In the case of the ►HER-2/neu oncogene, specific antibodies induced by preventive cancer vaccines inhibit dimerization of surface HER-2/neu proteins (a key step required for the initiation of signal transduction) and induce HER-2/neu internalization and recycling, eventually leading to a complete depletion of HER-2/neu surface expression. In HER-2/neu-addicted cells (►Oncogene addiction) a prolonged loss of HER-2/neu expression and signaling blocks cell proliferation and can trigger ►apoptosis.

Target Antigens of Cancer Immunoprevention

The inhibitory mechanisms targeting HER-2/neu can be ineffective against most other tumor antigens, either because the target is not a surface molecule and antibodies cannot bind tumor cells, or because tumor progression leads to a loss of antigen processing machinery (including major histocompatibility complex molecules), required for antigen recognition by T cells. The latter is a very frequent event affecting 80–90% of all human tumors. For cancer immunoprevention it was proposed that the immune recognition of ideal target antigens should persist even if defects in antigen processing impede T cell recognition, hence the antigen should be expressed on the cell surface and recognized by antibodies. A second desirable property is a direct involvement of the target antigen in oncogene addiction or in the maintenance of tumorigenicity, as is the case of HER-2/neu, because this prevents tumor escape from immune defenses through loss of antigen expression, again a frequent phenomenon for most other tumor antigens. Tumor antigens fulfilling both requirements were named ►oncoantigens. Members of this new class of tumor antigens ideally suit the

concepts of cancer immunoprevention, in particular for what concerns the need of persistent, lifelong antitumor responses, however oncoantigens make also attractive new targets for cancer immunotherapy because they are not prone to relevant mechanisms of immunoresistance and therapeutic failure.

Clinical Developments

The mass of preclinical data demonstrating the efficacy of cancer immunoprevention in mouse models warrants the translation of this approach to humans, however this will require a precise definition of the subjects who can benefit from this type of intervention and consequently of the design of clinical trials. The analysis of these issues reveals key scientific issues to be investigated and suggests possible developments. Immunoprevention of hereditary cancer syndromes would be a straightforward translation from preclinical models, however the definition of suitable target antigens in most hereditary tumors is currently lacking and will require adequate immunological studies. Analogous problems face the application of cancer immunoprevention to other human groups at increased risk of cancer due to exposure to carcinogens and/or presence of preneoplastic lesions, moreover the lower level of risk (as compared to hereditary cancer) implies the recruitment of a large number of subjects. Lessons for the clinical deployment of cancer immunoprevention might come from the development of ►tamoxifen, which was first used for therapy of advanced ►breast cancer patients, followed by ►adjuvant therapy *i.e.* for prevention of tumor relapse and ►metastasis; the finding that tamoxifen also prevented the appearance of additional primary tumors eventually led to large prevention trials that demonstrated its efficacy in preventing breast cancer. Early clinical testing of preventive vaccines in advanced cancer patients will also provide much needed data concerning safety and risks of adverse effects.

The Risks of Cancer Immunoprevention

The lack of severe adverse effect registered in the clinical trials leading to the approval of HPV vaccines, along with similar results from worldwide HBV vaccination programs, indicates that the immunoprevention of virus-related cancers will share with other vaccines for infectious diseases an intrinsic high level of biosafety. The main reason is that the target antigens are not expressed by normal tissues of the host, hence the risk of triggering autoimmune reactions is very low.

On the contrary, virtually all tumor antigens unrelated to infectious agents are also expressed by some normal cells during life. In some cases normal cells express a cross-reacting molecule with minor aminoacidic differences from the tumor version, but more frequently the antigens are structurally identical, and the only differences are either quantitative or topographical. The implication is that some autoimmune responses will frequently accompany successful prophylactic or therapeutic vaccination, even though development of autoimmune diseases is not an automatic consequence.

A major difference between cancer therapy and prevention is that both physicians and patients accept inherently high risks of serious adverse effects when dealing with an existing life-threatening disease, whereas preventive treatments to be administered for long periods to healthy individuals need to minimize not only severe, but also mild adverse reactions.

Preclinical studies of cancer immunoprevention did not reveal major risks of ►autoimmunity, however the use of transgenic mouse models implies that in most instances protective immune responses were actually directed against transgenic products rather than against endogenous molecules, thus minimizing "real" autoimmunity. Therefore early clinical trials of cancer immunoprevention in humans will require intensive analyses to discover early signs of autoreactive immune responses and a special attention to the long-term risks of triggering autoimmunity.

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Immunoprophylaxis

►Immunoprevention

Immunoreceptor

►Chimeric T Cell Receptors

Immunoreceptor Tyrosine-based Activation Motif

Definition

ITAM; A common cytoplasmic motif, YxxL/I-6-8-YxxL/I, that activates signaling pathways; it is found for example in adapter molecules (DAP12) that associate with NK activation receptors that lack cytoplasmic domains (for example NKp44), which is recognized by downstream signaling molecules of the tyrosine kinase family. This conserved amino acid sequence was originally discovered in receptors found in immune cells, but has also been found in other types of non-hematopoietic, non-receptor proteins. Phosphorylation of two tyrosine amino acids within this motif creates a binding site for other proteins and activates complex signaling pathways.

►Natural Killer Cell Activation

Immunoregulatory Aberrations

►Autoimmunity and Prognosis

Immunoscreening cDNA Expression Libraries

Definition

This method utilizes the exquisite specificity of antibodies to recognize a given antigen in the midst of thousands of antigens in a cDNA library. It lends itself to study structure and function of the expressed autoantigen in cDNA expression libraries of the desired cancer, built into a virus such as λgt11, T7, or other virus which are grown in bacteria. The viral coat displays the universe of potential autoantigens selected by autoantibodies in sera which can be arrayed forming a library. The microarray can be probed with multiple sera from patients and controls and finally, associations are sought between positive phages, diagnosis, or other clinical parameters

►Autoantibodies

Immunostimulatory Molecules

Definition

Are molecules that provide signals for antigenic specificity and immune response of T cells. Examples include ►T cell receptor (TCR), CD28, CD40, B7-1 and ►cytokines.

►T-Cell Response

Immunosuppressed

Definition

A state of reduced immune function.

►Immunosuppression
►Allergy

Immunosuppression

Definition

Is a state in which the ability of the body's immune system to fight infections or disease is decreased. A process or act that leads to a reduced activation or efficacy of the immune system.

Immunosuppressive Drugs

Definition

Compounds that inhibit adaptive immune responses are called immunosuppressive drugs. They are used mainly in the treatment of graft rejection and severe autoimmune disease.

►Adaptive Immunity
►Allograft Rejection
►Graft Acceptance and Rejection

Immunotherapy

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Synonyms

Biological therapy

Definition

Immunotherapy is the treatment of cancer or inflammatory/autoimmune disease by inducing, enhancing or suppressing an immune response. Immunotherapy can be nonspecific or (antigen)-specific. Nonspecific immunotherapy aims to enhance the overall host immune response, whereas specific immunotherapy targets the immune system against a particular tumor or increases tolerance towards a specific allergen. There are four main categories of specific immunotherapy: ►adoptive immunotherapy, antibody-based immunotherapy, cancer ►vaccine therapy and ►allergen-specific immunotherapy. From these, adoptive and antibody-based immunotherapies are passive approaches, whereas cancer vaccine therapy and allergen-specific immunotherapy are active approaches.

Characteristics

Despite advances in oncological research, cancer remains a leading cause of death throughout the developed world. Nonspecific approaches to cancer treatment, such as surgery, radiotherapy and generalized chemotherapy, have been successful in the management of a distinct group of leukemias and slow-growing solid cancers. However, many solid tumors show considerable resistance to such approaches, and the prognosis in these cases is correspondingly poor. Immunotherapy is an emerging alternative area of cancer treatment. Cancer immunotherapy includes both passive and active strategies. Passive immunotherapy involves the *ex vivo* creation of established tumor-immune elements (antibodies, immune cells) that are administered to patients to mediate anti-tumor activity directly or indirectly, and which do not stimulate the host immune system. In contrast, active immunotherapy induces a tumor-specific immune response in the patient, leading to the production of specific immune effectors (antibodies and T-cells). Historically, cancer immunotherapy has focused on nonspecific immune stimulants. Pioneer work began more than 140 years ago, when Wilhelm Busch observed that tumors show temporary regression during an infection. Two decades later, Sir William Coley developed and improved this therapeutic concept by vaccinating a large number of sarcoma patients with attenuated mixed bacterial extracts (►Coley toxin).

Nonspecific Immunotherapy

1. ►Bacillus Calmette-Guerin (BCG) is the most effective intravesical nonspecific immunotherapeutic agent, and is used for the prevention and treatment of superficial ►bladder cancer. The proposed anti-tumor mechanism of BCG involves activation of the immune system and the promotion of a local acute nonspecific ►inflammation in the bladder lumen. Immune cell activation in response to BCG is mediated by a family of transmembrane recognition receptors called ►Toll-like receptors (TLRs). Intravesical, BCG-induced inflammation facilitates the infiltration of a broad range of immune cells (►macrophages, ►lymphocytes and ►natural killer cells) and the activation of pro-inflammatory cytokines such as ►interleukin-1 (IL-1), ►interleukin-6 and ►tumor necrosis factor-alpha (TNF-α).
2. ►Cytokines are low-molecular-weight, soluble proteins that regulate the innate and adaptive immune systems. The anti-tumor activity of cytokines is mediated by one of two general mechanisms: first, a direct anti-tumor effect, and second, indirect enhancement of the anti-tumor ►immune response. It has been hypothesized that both the cytokine-activated lymphocytes and their secretory products such as interferon-gamma and tumor necrosis factor-beta (TNF-β) may contribute to the lysis of tumor cells *in vivo*. The exogenous administration of ►interleukin-2 (IL-2) is efficient in a broad spectrum of experimental tumors, including sarcomas, carcinomas, hemoblastoses, melanomas and hepatomas. In humans, IL-2 and interferon-α2b are approved for the treatment of advanced melanoma and for use with ►adjuvant therapy.

Specific Immunotherapy

1. Adoptive Immunotherapy involves the infusion of immunologically-competent, *ex vivo*-expanded, donor-derived lymphocytes (DLI), which specifically destroy tumor cells by ►graft-versus-leukemia (GvL) or ►graft-versus-tumor (GvT) effects. In addition, peripheral blood-derived ►lymphokine-activated killer (LAK) cells and ►tumor-infiltrating lymphocytes (TILs) derived from tumor sections have proven to be effective anti-tumor agents. To address MHC and exogenous cytokine-independent activation of anti-tumor effector functions, T cells can be engineered to express ►chimeric T-cell receptors.

I

Chimeric receptors are composed of a recognition unit (antibody fragment) and an intracytoplasmic signaling molecule. Such receptors can be used to target various types of effector cells including cytotoxic T-cells towards any tumor-associated antigen for which there is a suitable antibody.

2. Antibody-based immunotherapy exploits the highly specific binding between antibodies and their corresponding ►tumor-associated antigens (TAAs), resulting in some significant clinical responses. Tumor-associated antigens are structures presented predominantly by tumor cells, thereby allowing antibodies to distinguish tumors from non-malignant tissue. Therapeutic ►monoclonal antibodies can destroy tumor cells directly by inducing ►apoptosis or indirectly through immunologic mechanisms such as ►antibody-dependent cell-mediated cytotoxicity (ADCC) and/or ►complement-dependent cytotoxicity (CDC). In addition, the natural function of antibodies can be enhanced by conjugating them to toxins (►immunotoxins), radionucleotides (radioimmunoconjugates), liposomes (►immunoliposomes) and cytotoxic drugs. Host immune responses can be enhanced through the induction of ►anti-idiotypic antibodies or through the use of ►bispecific antibodies containing arms with different specificities. Monoclonal antibodies are the largest class of biotechnology-derived proteins, with 19 monoclonal antibodies already approved for human use by the United States Food and Drug Administration (FDA).
3. ►Cancer vaccine therapy represents an active, systemic, tumor-specific immune response of host origin. It is used either to treat existing cancers (►therapeutic vaccines) or to prevent cancer development (►prophylactic vaccines). There are several types of cancer vaccine: isolated whole cell cancer vaccines or tumor cell lysates, protein- or peptide-containing vaccines, viral vector vaccines and anti-idiotype vaccines. Following the administration of a vaccine-antigen that resembles a specific target, the patient's humoral and T-cell-specific immune response induces defense mechanisms to combat the target *in vivo*.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

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Immunotoxin

Definition

A toxic substance that is attached to a cell binding ligand and used to destroy a specific target cell.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy
►Monoclonal Antibody Therapy

Importin-Alpha-3

Definition

Is a nuclear localization signal receptor subtype that helps in translocation of certain proteins from cytosolic compartment to the nuclear compartment.

►Transglutaminase-2

Importins

Definition

A group of proteins involved in transporting molecules from the cytoplasm through the nuclear pores of the nuclear envelope into the cell nucleus.

►Modular Transporters

Imprinted Gene

Definition

A gene that is marked in the germline; this denotes its maternal or paternal origin and influences its expression in the developing embryo. Refers to the parent-of-origin-specific monoallelic expression of a gene.

►Imprinting

Imprinting

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Definition

►Genomic imprinting; Describes a phenomenon in which a gene is expressed either from the paternal or form the maternal ►allele and thus discriminates these genes from the majority of genes that are expressed from both alleles.

Characteristics

Normally genes are expressed from both the maternal and the paternal allele. Genomic imprinting results in allele specific expression of certain genes from either the paternal or the maternal allele. These genes are marked before fertilization in a way that either the maternal or the paternal allele is transcriptionally silenced in the offspring. One of the first indications that certain autosomal regions are subject to genomic imprinting came from mouse genetic studies using Robertsonian and ►reciprocal translocations. In these studies, uniparental duplications or deficiencies for certain chromosomal regions were analyzed. The failure of a disomy or duplication from one parent to complement a corresponding nullisomy or deficiency from the other parent constituted the genetic evidence for the occurrence of imprinting effects. In addition, embryos that contain either two copies of the maternal or the paternal genomes fail to survive in early development indicating the complementary need for both the maternal and paternal genome. More than 25 imprinted genes have been identified in mice and humans and there are estimates for about 100 imprinted genes in the mammalian genome. Certain characteristic features have been identified for imprinted genes. Most of the imprinted genes have important roles in early development. Interestingly, imprinted genes tend to occur in clusters suggesting a common regulatory mechanism. One of the best studied cluster of imprinted genes is located on mouse distal chromosome 7 (human 11p15.5), encompassing 1.5 Mbp and including the maternally expressed genes p57KIP2, Kvlqt1, Mash2 and H19 as well as the paternally expressed genes Ins2 and Igf2. It is now well accepted that imprinting could be regulated in a tissue specific manner in a way that only some tissues express the gene from one allele while others show biallelic expression. Here, unknown mechanisms exist that allow to by-pass the regulation of imprinting. It is interesting to note that a number of imprinted genes encode for RNAs but do not have an open reading frame and are not translated. It is believed that these RNAs play a role in the regulation of the imprinting process. Interestingly, short GC-rich repeat sequences were identified in the vicinity of many imprinted genes usually located in or near so called differentially methylated sites, ►CpG island-like sequences that are methylated on only one allele.

Cellular and Molecular Aspects

Regulatory mechanisms underlying genomic imprinting are under intense investigations in many laboratories but only incompletely understood. The features of the imprinting signal and the mechanism are unknown, but strong evidence suggests the involvement of DNA ►methylation. Several requirements for the underlying mechanisms can be postulated. First, the imprinting signal, or imprint mark, in the imprinted region must be established before fertilization. Second, the imprint mark must be an ►epigenetic modification and must directly or indirectly affect the transcription of a gene by silencing one allele and leaving the other active. Third, the imprint mark must be stable in ►mitosis and must be transmitted during cell division. Finally, the imprint mark must be reversible in a passage through the opposite germline. At present, DNA methylation is the only mechanism that conforms with the above requirements. Several lines of experimental data support the assumption that DNA methylation plays an important role in imprinted regulation. In mammals, ►DNA methylation occurs only at the cytosine residue of ►CpG dinucleotides. It was shown that DNA methylation in promoter regions can turn off the transcription of a gene. Most genes are subject to a process of demethylation directly after fertilization with most of the CpG sites unmethylated at the 16 cell stage. However imprinted genes are exceptions in this demethylation process by maintaining small regions that show allele specific methylation. DNA methyltransferase generates methylation patterns that are transmitted correctly following DNA replication and cell divisions. Studies of the expression of imprinted genes in DNA methyltransferase-deficient mutant mice indicated that normal level of DNA methylation are required for the control of allele specific expression. Studies with transgenic mice suggested that methylation is the epigenetic modification underlying genomic imprinting. A direct correlation between paternal inheritance, ►transgene, ►hypomethylation and tissue specific expression of the transgene was shown, while the maternally derived copy is methylated and not expressed.

Clinical Relevance

Imprinted genes are involved in critical steps during normal embryonic development. A growing body of

evidence implicates genomic imprinting in the pathogenesis of certain human disorders, inherited tumor syndromes and sporadic tumors. At least ten genetic disorders have been found to be associated with genomic imprinting effects. In some cases, the trait is transmitted exclusively (or mainly) from one parent (either father or mother) or the disease is particularly severe when transmitted from one parent. In other cases the disease is associated with uniparental disomies or parent-of-origin specific aberrations.

The best studied examples of imprinted genetic diseases are the Prader–Willi-Syndrome (PWS) and Angelman syndrome (AS). PWS is characterized by mild to moderate mental retardation, individuals are slow moving and overweight due to severe hyperphangia. Patients with AS show severe mental retardation are thin, hyperactive and show disorders of movement and uncontrolled laughter. Both syndromes are linked to abnormalities on human chromosome 15q11–13. The first hint for a possible imprinting effect in these syndromes came from the finding that the deleted fragments in both syndromes are from opposite parental origins. In PWS the deletion occurred in the paternal copy and in cases of AS the maternal copy was deleted. Additional evidence came from the finding of maternal disomy of chromosome 15 in PWS patients and paternal disomies of chromosome 15 in AS. These data suggest that the PWS gene(s) is transcribed from the paternal allele only and the AS gene(s) is expressed from the maternal allele. Several imprinted genes were identified in the critical region for PWS/AS including paternally expressed SNRPN and maternally expressed UBE3A.

There is also evidence that some of the imprinted genes have oncogenic or tumor suppressor function. Loss of tumor suppressor function of an imprinted gene could be achieved by ►loss of heterozygosity (LOH) involving the usually active copy, as shown for the cyclin dependent kinase inhibitor, p57KIP2, in lung cancers, H19 in ►Wilms tumor and NOEY2, a member of the ►RAS superfamily, in breast and ovarian cancers. Alternatively, uniparental disomy including the normally silent allele could lead to inactivation of an imprinted tumor suppressor gene. Activation of a growth supporting gene such as IGF2 (►Insulin-like Growth Factors) could occur by uniparental disomy involving the normally active copy. In addition, relaxation of imprinting control, also called loss of imprinting (LOI), could lead to biallelic expression and thus overexpression of an imprinted oncogene, as shown for IGF2 in Wilms tumor. The first evidence for the involvement of DNA methylation in LOI came from the finding of complete methylation of the CpG island located immediately upstream of H19 transcription start site. Usually this ►CpG island shows allele specific methylation on the maternal allele. This epigenetic change correlated with LOI in IGF2 and silencing of H19.

Another human disease is the ►Beckwith–Wiedemann Syndrome (BWS) that is characterized by a number of growth abnormalities including gigantism. Between 5% and 10% of BWS patients are prone to Wilms tumor, adrenocortical carcinoma, hepatoblastoma or embryonal rhabdomyosarcoma. Wilms tumors have been shown to exhibit preferential loss of maternal alleles at chromosome 11p. A cluster of at least seven imprinted genes was identified in 11p15.5 including the paternally expressed IGF2 and the maternally expressed H19. The most common abnormality in BWS patients is LOI of IGF2 without any detectable chromosomal abnormalities.

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In Situ Breast Cancer

►Ductal Carcinoma In Situ

In Situ Cancer or Carcinoma

►Dormancy

In Situ Carcinoma

►Dormancy

In Vitro

Definition
Describes experiments undertaken outside of a living organism (e.g. in Petri dish).

►Malignancy-Associated Changes

In Vitro Genetics

Definition
Is an umbrella term encompassing a variety of ►combinatorial selection methods that involve large pools of nucleic acid sequence variants, a selectable function (e.g. protein binding or ribozyme-catalyzed chemical transformation), and ►PCR amplification.

In Vivo

Definition
Inside of a living organism.

Inbred Lines

►Mouse Models

Inbred Strain

Definition
A strain of animal that has no genetic differences (►polymorphisms) with other animals of the same strain.

►Mouse Models

Incidence

Definition
The number of new cases of a disease or condition occurring in a specific population during a given period of time.

►Incidence Rate

Incidence Rate

Definition
Is the number of new disease cases per population at risk measured over a given time interval (high incidence rate implies high disease occurrence).

►Epidemiology of Cancer
►Cancer Epidemiology

Independent Prognostic Factor

Definition
An indicator used to estimate the risk of disease recurrence and death in an individual patient. Multivariate statistical analysis determines whether a prognostic factor exhibits a new, independent value as compared to established prognostic factors.

►Prognosis
►Prognostic Biomaker

Indian

Definition
Gene in ►Hedgehog Signaling.

Indirubin and Indirubin Derivatives

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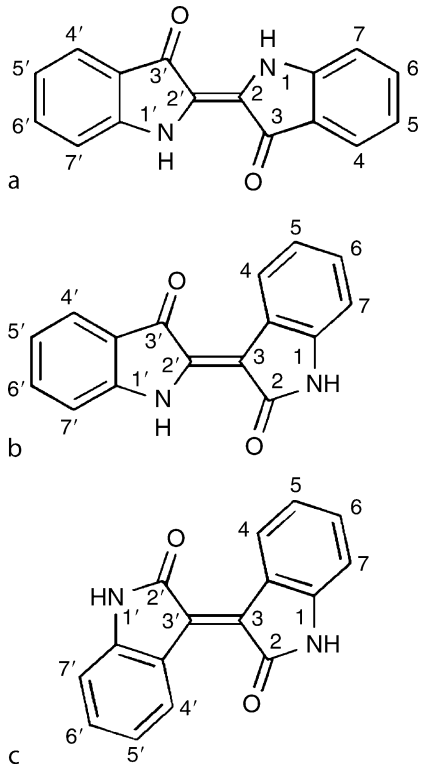
Synonyms

3-(1,3-Dihydro-3-oxo-2H-indol-2-ylidene)-1,3-dihydro-2H-indol-2-one; 2′,3-Biindolinylidene-2,3′-dione: 3-(3-Indolinone-2-ylidene)-indolin-2-one

Definition

Indirubin is the parent compound of a spectrum of 2′,3-bisindoles synthesized to improve the biological activity of this natural 2′,3-bisindole lead structure. Indirubin and its isomers indigo and isoindigo are composed of two indolinone ring systems, linked through a double bond to make up 2,2′-(indigo), 3,2′-(indirubin) and 3,3′-(isoindigo) bisindoles, respectively (Fig. 1).



Indirubin and Indirubin Derivatives. Figure 1
Structures and numeration of indigoid bisindoles. (a) Indigo, (b) Indirubin (E211), (c) Isoindigo.

Characteristics

History

While indigo is one of the oldest dyes known, with a history of use dating back to bronze age, the two other isomers, especially indirubin, have gained reputation as components of traditional medications used against a variety of human diseases.

The discovery of the anticancer activity of indirubin can be traced back to a traditional Chinese medication, consisting of 11 ingredients, mostly of herbal origin, with the name of Danggui Longhui Wan. The preparation is used traditionally for a variety of chronic and acute diseases including chronic myelocytic leukemia (►CML). Chinese scientists achieved the identification of the active ingredient of this medication, Quing Dai, which corresponds to natural indigo, prepared from the leaves of indigo-producing plants. Furthermore, the Chinese scientists discovered that not only the blue dye indigo, but a minor byproduct, the red colored trace constituent indirubin was the active antileukemic principle. In a clinical trial, synthetic indirubin was given orally to CML patients at dosages of 150–450 mg/day. A total of 314 patients participated in the study. Complete remissions were observed in 26%, partial remission in 33%, and some beneficial response in 28% of patients. Treatment was well-tolerated, without major side effects. Studies exploring the mechanism of action reported a spectrum of relatively unspecific biological effects, not really convincing to fully explain the respectable anti-CML activity of the compound.

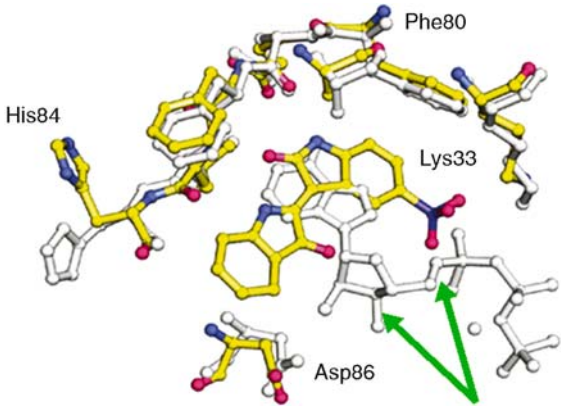
Mechanism

Research on indirubins gained momentum, when in 1999 it was discovered that such 3,2′-bisindoles act as potent inhibitors of serine/threonine kinases, especially of ►cyclin-dependent kinases (CDKs) and of glycogen-synthase kinase 3β (GSK3β). Later it was found that indirubins also inhibit ►receptor tyrosine kinases, such as ►vascular endothelial growth factor receptor (VEGFR) or ►Src kinase and block Stat-3 signaling. Moreover, indirubins have also been discovered to activate the ►aryl hydrocarbon receptor transduction pathway. Thus indirubins exert, in addition to their multimodal kinase inhibitory activity, further bimolecular effects contributing to anticancer activity.

The relative kinase inhibitory profile of individual representatives of these 3,2′-bisindoles, as well as their pharmacokinetic properties is markedly influenced by the type and pattern of substituents attached to the 3,2′-bisindole basic scaffold. Within cancer targets, VEGFR2 and c-Src, as well as specific CDKs are of prime interest, since their respective partner proteins, activators or inhibitors, are aberrantly expressed in many human malignancies.

Structure/Activity

Chemical improvement of the parent molecule initially was driven by the aim to improve solubility and bioavailability without compromising anticancer activity.



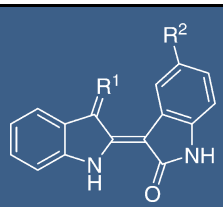
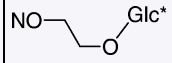
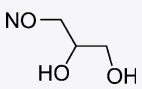
Indirubin and Indirubin Derivatives. Figure 2
Superposition of crystal structures of CDK2–E226 (yellow) and CDK2–AMPPNP (white). Green arrows point to 5- and 3′-position of indirubin scaffold. (Reprinted from Davies et al (2001), Structure 9:389; with permission from Elsevier).

Indirubins act as ATP-competitive inhibitors of ATP-dependent kinases. Crystal structures of CDK2–-indirubin complexes have been described in detail. It was shown that the flat disk shape of the 3,2′-bisindole molecule slides easily into the binding pocket and is tightly bound, mainly by virtue of hydrogen bonding and lipophilic interactions with the ATP-binding cleft, situated in the hinge region connecting the amino terminal with the carboxy terminal part of the kinase. The strong binding affinity of indirubin derivatives to the ATP site is practically not affected by additional binding of the activating partner cyclin A to CDK2. A superposition of the structures of the binding complexes of the indirubin derivative, E 226, and of a nonhydrolyzable ATP mimic, AMPPNP, with CDK2, unraveled molecular positions of the indirubin scaffold preferably to be exploited for chemical improvement of the molecule. Thus, positions 5 and 3′ (arrows in Fig. 2) were identified as those of first choice for molecular modifications by attaching appropriate substituents.

The chemistry to achieve the synthesis of such derivatives has been described in detail. A selection of results from comprehensive structure/activity studies is summarized in Tables 1 and 2.

The results show that the inhibitory activity of the parent molecule, indirubin (E211) on isolated

Indirubin and Indirubin Derivatives. Table 1 Inhibition of CDK/cyclin complexes (IC_{50}-Werte, μM) by indirubins in comparison with roscovitine

Substance						
E-Nr	R^1	R^2	CDK1/Cyclin B	CDK2/Cyclin A	CDK2/Cyclin E	CDK6/Cyclin D
E211	O	H	10[a]	2.2[a]	7.5[a]	–
E226	O	So_3^-	0.055[a]	0.035[a]	0.15[a]	–
E231	NOH	H	5.1 ± 2.9[b] 0.18[a]	2.2 ± 0.2[b] 0.44[a]	0.33 ± 0.05[b] 0.25[a]	0.08 ± 0.03[b]
E729		OCH_3	5.6 ± 1.3[b]	0.8 ± 0.1[b]	0.09 ± 0.01[b]	0.4 ± 0.1[b]
E804		H	1.7 ± 0.4[b]	0.5 ± 0.1[b]	0.2 ± 0.04[b]	0.06 ± 0.01[b]
Roscovitine			7.9 ± 0.4[b] 0.65[a]	3.5 ± 1.3[b] 0.7[a]	1.0 ± 0.4[b] 0.7[a]	>10[b]

[a]Method: Meijer et al. (1997).
[b]Method: Jakobs et al. (2005) Glc: β-D-glucopyranosyl –: not determined.

Indirubin and Indirubin Derivatives. Table 2 Solubility in water and human tumor cell growth inhibition of indirubin derivatives

Substance[a]	Solubility in water (mg/L)	LXFL-529L	MCF-7	HCT116
E211	<0.1	9.9	4.0	–
E226	>>100	≥100	≥100	–
E231	0.12	3.0	3.3	–
E729	42	0.5	1.2	0.5
E804	1.6	0.9	0.1	<0.1

SRB-assay, incubation 3d, IC_{50}-values in μM, Solubility in mg/L.
[a]Structures see Table 1–: not determined.

CDKs could be improved dramatically. While indirubin (E211) itself was somewhat less active than roscovitine, a standard purine-type of CDK inhibitor, several 3′ or 5-substituted derivatives achieved IC_{50} values, especially toward CDK2/cyclin A, CDK2/cyclin E, and CDK6/►cyclin D down to the low nanomolar range (Table 1). At the same time, solubility in water and thus bioavailability could be improved by factors of up to about 400, as compared with the parent compound. In addition, the potency to inhibit human tumor cell proliferation was also strongly improved (Table 2). These novel compounds not only induce arrest in G1/S and G2/M phases of the cell cycle of various human tumor cells but also strongly trigger ►apoptosis and cellular necrosis by several mechanisms, including inhibition of antiapoptotic proteins, such as ►Mcl-1 and ►survivin, and induction of endoreplication.

Thus, within this family of bisindoles, molecular permutations entail major effects on biological activities and allow generation of novel derivatives that lend themselves to clinical application. Since the major cellular targets of these compounds are aberrantly expressed in many human tumors, it is reasonable to expect selective activity toward such human tumors. These encompass amongst others, ►breast cancer, ►renal cancer, ►pancreatic cancer, and ►prostate cancer as well as ►leukemias. However, the state of clinical testing and verification has not been reached at present.

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Individual Susceptibility

Definition

Marked variability in the manner in which individuals will respond to a given exposure to a toxic agent. A person’s body size, age, gender, genetics, and health status can affect individual susceptibility.

►Biomonitoring

Individualized Treatment

►Personalized Cancer Medicine

Individually Mutated Tumor Antigens

Definition

Tumor antigens that are only expressed by malignant cells of a particular tumor. These antigens result from

tumor-specific mutations, which creates new peptides that enable antigen-specific T cells to differentiate between normal and malignant cells. Mutations may enhance the binding affinity of peptides to major histocompatibility complex molecules or may engage a different T cell repertoire than the corresponding normal peptide.

►Melanoma Vaccines

Indoleamine

Definition

An aromatic heterocyclic organic compound consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring and containing an amine group.

►Polycyclic Aromatic Hydrocarbons

Indoleamine 2,3-Dioxygenase

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Synonyms

IDO

Definition

Indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.42) is an enzyme catalyzing the initial and rate-limiting step in the catabolism of tryptophan. This enzyme induces ►immunosuppression and ►tolerance, and is involved in the ►immune escape of tumor cells, leading to cancer progression.

Characteristics

Enzymatic Properties, Tissue Distribution, and Regulation of Expression

IDO is a heme-containing intracellular enzyme that catalyzes the initial and rate-limiting step of tryptophan catabolism by cleavage of the pyrrole ring of L-tryptophan (Fig. 1). While only a small amount of tryptophan from food is converted to serotonin and further converted into melatonin, most (more than 95%) dietary tryptophan is metabolized by IDO along the kynurenine pathway, leading finally to the biosynthesis of nicotinamide adenine dinucleotide (NAD).

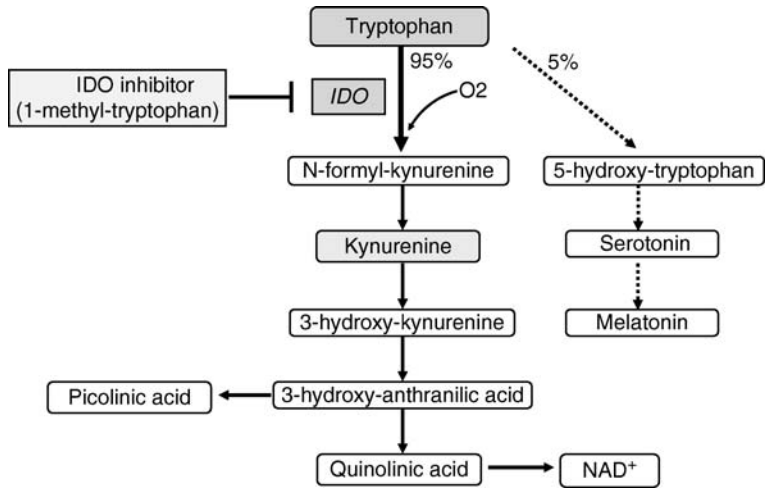
IDO is expressed in a wide range of tissues such as the lung, intestine, brain, and placenta, although within these tissues, expression occurs only in a limited range of cell types. In contrast, in the liver, tryptophan is catabolized by a structurally distinct liver-specific enzyme, tryptophan 2,3-dioxygenase (TDO; EC 1.13.1.12), but not by IDO.

A cDNA encoding human IDO has been cloned and its deduced primary structure is obtained. Human IDO cDNA encodes a protein of 403 amino acids with a molecular weight of approximately 45 kDa. The IDO protein is encoded by a tightly regulated gene that -responds to inflammatory mediators such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and lipopolysaccharide (LPS). In humans, IDO is encoded by the *INDO* gene, which is comprised of ten exons spanning approximately 15 kb at chromosome site 8p11-p12. IFN-γ is a major inducer of IDO expression. Transcriptional induction of the *INDO* gene is mediated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, specifically JAK1 and STAT1α. NF-κB also contributes to IDO induction. It has been shown that ►IFN-γ and ►TNF act synergistically through ►NF-κB activation to induce expression of interferon regulatory factor (IRF)-1, which leads to upregulation of IDO expression. In addition, ►prostaglandin E_2 (PGE_2) strongly upregulates IDO.

IDO and Immune Suppression

Accumulating evidence indicates immunosuppressive function of IDO. First, IDO is expressed in placental trophoblasts and macrophages during pregnancy and prevents rejection of the allogeneic fetus, thereby suggesting involvement of IDO in fetal–maternal tolerance. This is based on the findings that a pharmacological inhibition of IDO activity by 1-methyl-tryptophan (1-MT) results in rejection of the fetus in pregnant mice. Subsequent studies clarified the mechanism of IDO ►immunosuppression by locally depleting tryptophan and by producing toxic tryptophan metabolites (e.g., kynurenine, see Fig. 1), which causes arrest of proliferation and the apoptosis of alloreactive T-cells. It has been similarly demonstrated that enzymatic activity of IDO correlates with reduced T-cell-mediated immune responses in several experimental systems, including models of inflammatory diseases, autoimmune diseases, and organ/tissue transplant rejection.

Secondly, IDO is expressed in ►antigen-presenting cells (APCs), especially certain subsets of ►dendritic cells (DCs), and it regulates immune response



Indoleamine 2,3-Dioxygenase. Figure 1 Catabolic pathway of trytophan and kynurenine by IDO.

and induces tolerance. For example, exposure of ►monocyte-derived DCs to a cocktail of cytokines for the purpose of maturation results in strong upregulation of IDO expression. Mature DCs that express functional IDO can be potent suppressors of ►T-cell response and promote tolerance in vivo and in vitro. Expression of functional IDO requires ligation of B7-1/B7-2 (CD80/CD86) molecules on DC cell membranes by cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) expressed by CD4 + CD25- ►regulatory T cells. This binding of CTLA-4 to B7 receptors on DCs activates IFN-α-mediated STAT1-dependent IDO upregulation. Similarly, the synthetic soluble fusion protein CTLA4-immunoglobulin (CTLA-4 Ig) is a potent inducer of IDO expression by DCs. IDO-expressing DCs may also promote the development of regulatory T cells. These findings therefore suggest that IDO-expressing DCs and ►T regulatory cells may cooperate to induce antigen-specific T-cell anergy and create a state of peripheral immune tolerance.

While the mechanisms of IDO-dependent inhibition of T-cell function have been elucidated, less is known about the possible role of IDO in regulating ►natural killer cell activity. NK cell function is also suppressed by IDO, and IDO inhibits the proliferation of NK cells in vitro. Furthermore, L-kynurenine, a tryptophan-derived catabolite produced by IDO activity, inhibits the cytokine-mediated expression of the specific triggering receptors, NKp46 and NKG2D, which are responsible for the induction of NK cell-mediated killing. As a consequence, L-kynurenine-treated NK cells display an impaired ability to kill target cells recognized via NKp46 and NKG2D. These findings suggest that IDO can suppress both T-cell and NK cell responses.

IDO and Cancer

Although many tumor-associated antigens have been identified in various tumor cells, the reason why tumor antigen-specific host T-cells fail to control tumor progression remains obscure. Tumors are known to successfully escape the host immune system by two possible mechanisms; a loss of surface antigens that renders them invisible to immune cells, or an attack on the immune cells that disables their antitumor functions. The accumulated evidence that IDO is physiologically important for establishing peripheral tolerance to alloantigens prompts a hypothetical mechanism that tumors could create a state of tolerance through tryptophan catabolism carried out by IDO. If IDO is actually present in tumor tissues, it could induce local immune suppression, resulting in the protection of tumor cells from host T-cell-mediated rejection and/or attack by NK cells, and subsequently leading to tumor growth and progression.

IDO is expressed in various human cancer tissues, and that it is expressed not only by immune cells, but also by the tumor cells themselves. Tumors expressing IDO can resist immune rejection by tumor-associated antigen-specific host cytotoxic T-cells in mouse models. This effect is accompanied by a lack of accumulation of T-cells at the tumor site, resulting from arrest of T-cell proliferation caused by IDO-mediated local tryptophan depletion. When the IDO inhibitor 1-MT is dissolved in the drinking water given to tumor-bearing mice, the growth of IDO-expressing tumors is significantly reduced.

In contrast, it is shown that IDO is expressed by CD19 + plasmacytoid DCs in tumor-draining lymph nodes in mice, and these specific IDO-expressing DCs potently suppress host antitumor T-cell responses and

induce tolerance to tumor-derived antigens. In humans, IDO + cells of host origin are also present in draining lymph nodes of patients with ►melanoma, ►breast cancer, and other tumors.

Taken together, two possible mechanisms for the immunosuppressive action of IDO in tumor-bearing hosts are proposed. IDO expressed by the tumor cells themselves can create a localized immunosuppressive status within the tumor microenvironment either by suppressing effector T-cell function and proliferation due to tryptophan depletion, or by directly killing tumor-infiltrating T-cells and NK cells using toxic metabolites of tryptophan. Alternatively, host DCs expressing IDO can pick up tumor-derived antigens and migrate into tumor-draining lymph nodes, where these IDO-expressing APCs cannot effectively prime naïve T-cells, resulting in T-cell deletion, failure of clonal expansion, or perhaps induction of regulatory T-cells.

Clinical Relevance

IDO as a Prognostic Indicator for Human Cancer

Historically, it was known that decreased serum tryptophan levels as well as increased serum tryptophan metabolites could be detected in some patients with advanced cancer, suggesting elevated tryptophan catabolism by IDO. Expression of IDO, either by host cells or by tumor cells, is associated with poor outcome in various clinical settings. In patients with malignant melanoma, the presence of IDO-positive cells in sentinel lymph nodes is correlated with a significantly worse clinical outcome. In a gene expression profiling study, IDO is overexpressed in chemotherapy (►paclitaxel)-resistant ►ovarian cancer tissues, and patients with high IDO expression have poor clinical outcomes in serous-type ovarian cancer. Similarly, high IDO expression is also correlated with the advanced disease stage and liver metastasis in ►colon cancer, as well as a reduced number of CD3 + lymphocytes infiltrating into tumor tissues. High IDO expression in tumor cells is found in 46.3% of ►endometrial cancer patients, and is positively correlated with surgical stage, myometrial invasion, lymph-vascular space involvement, and lymph node metastasis. Furthermore, patients with high IDO expression have significantly impaired overall survival and progression-free survival compared to patients with weak or no expression of IDO. Even in early-stage disease (stage I and II), the survival rate of patients with high IDO expression is significantly reduced. On multivariate analysis, IDO expression is an independent prognostic factor for progression-free survival in endometrial cancer. These findings indicate that IDO may be a reliable and promising prognostic indicator for the treatment of human cancers.

Targeting IDO as a Therapeutic Strategy for Cancer Treatment

The idea that IDO has a role in enabling tumors to escape the host immune system could make IDO an attractive new target for cancer ►immunotherapy, and suggests that IDO inhibitors might have utility as anticancer agents.

Small-molecular inhibitors of IDO including 1-MT potentiate the antitumor activity of clinically relevant chemotherapeutic agents in mice. The combination of 1-MT with ►cyclophosphamide, ►cisplatin, ►doxorubicin, or ►paclitaxel regresses tumors synergistically in mouse spontaneous breast cancer models, suggesting that 1-MT could be utilized to block host-mediated immunosuppression and to enhance antitumor activity in the setting of combined chemoimmunotherapy. Furthermore, of the three stereoisomers of 1-MT, 1-methyl-L-tryptophan, 1-methyl-DL-tryptophan, and 1-methyl-D-tryptophan, the D-isomer has been shown to be most effective in reversing the suppression of T-cells generated by IDO-expressing DCs, and is most efficacious as an anticancer agent in chemoimmunotherapy regimens in mouse melanoma and breast cancer models.

In addition to the use of IDO inhibitors, the antitumor efficacy of silencing IDO by siRNA is reported. IDO–siRNA treatment of B16F10 murine melanoma cells prevents catabolism of tryptophan, and inhibits apoptosis of T-cells in vitro. In vivo treatment of B16F10 tumor-bearing mice with IDO–siRNA successfully postpones tumor formation and decreases tumor size, with a concomitant recovery of T-cell responses and enhancement of tumor-specific killing. These findings indicate that IDO–RNA interference has the potential to enhance cancer therapy by reinstalling anticancer immunity.

Implication of IDO for DC-based Cancer Immunotherapy

With respect to peptide-pulsed DC-based immunotherapy protocols, the currently used DC maturation cocktail contains IDO-inducing cytokines such as PGE_2. PGE_2 sensitizes DCs to TNF-α-mediated IDO activation, leading to the development of DC-mediated T-cell tolerance, which is the opposite effect of what is intended. It may be possible to include an IDO inhibitor in the DC maturation cocktail, generating mature DCs with inactivated IDO, and thus increasing the efficacy of DC-based antitumor peptide vaccination. Alternatively, application of ►cyclooxygenase-2 (COX-2) inhibitors such as ►celecoxib, which inhibits the production of PGE_2, in combination with a DC-based ►cancer vaccine significantly augments vaccine efficacy by reducing tumor burden, preventing metastasis, and increasing survival in a mouse breast

cancer model. The improved vaccine potency by COX-2 inhibitors is associated with an increase in the number of tumor-specific cytotoxic T cells, which may be attributed to a significant decrease in levels of tumor-associated IDO activity. These findings suggest a potential clinical relevance of IDO in DC-based therapeutic vaccines, and may be helpful in designing future cancer vaccines.

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Indolent Lymphoma

Definition

Lymphoma that tends to progress slowly. Indolent lymphomas include ►chronic lymphocytic leukemia/small lymphocytic lymphoma and follicular small cleaved cell lymphoma.

►Rituximab
►Malignant Lymphoma, Hallmarks and Concepts

Induction

Definition

Happens when levels or activities of enzymes involved in metabolism of parent ►xenobiotic are increased as a response to xenobiotic exposure.

Induction Chemotherapy

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Synonyms

Neoadjuvant chemotherapy (►Neoadjuvant Therapy); Preoperative chemotherapy)

Definition

Induction chemotherapy is chemotherapy given before the initiation of another treatment, which can be but is not limited to radiation, surgery or ►chemoradiotherapy. At least in the case of solid tumors, the theoretical benefit is provided by the ability to suppress distant metastases and to shrink the tumor, which in turn facilitates subsequent intensified ►local therapy. Its use is limited to tumors that are loco-regional, often bulky, but where cure remains possible.

Characteristics

The term "induction chemotherapy" differs to some degree when considering solid tumors vs leukemia.

Solid Tumors

When considering overall schemes to cure solid tumors, there are three major types of regimens, which are distinguished by their timing relative to one another and, to some degree, purpose:

1. induction or neoadjuvant therapy – i.e., therapy which precedes ►definitive therapy;
2. ►definitive therapy – i.e., therapy which is potentially curative by itself and forms the backbone of the curative regimen;
3. ►adjuvant therapy – i.e., therapy which follows definitive therapy.

Chemotherapy or radiotherapy may be components of any of these three types of regimens. When chemotherapy comprises an induction regimen, it is called "induction chemotherapy." By contrast, surgery may be a component only of definitive therapy.

The rationale behind most induction chemotherapy rests on the hypothesis that, in delivering ►systemic therapy as early as possible:

1. The tumor may shrink, thereby making subsequent ►local treatment more effective. For example, if the tumor shrinks with induction chemotherapy, the surgeon may have an easier time removing the entire tumor while minimizing the potential for damaging neighboring organs.

2. The development of distant metastases may be suppressed.

A potential advantage of including induction chemotherapy as part of a treatment approach is that the tumor's response to it may predict response to subsequent therapy. This fact is exploited, for example, in patients with bulky ►laryngeal cancer so as to avoid larynx removal. Patients whose tumors shrink substantially with induction chemotherapy (often ►cisplatin and ►5-fluorouracil) receive subsequent chemoradiation and thus avoid laryngectomy. Patients whose tumors do not shrink undergo immediate larynx removal.

Induction regimens are used in many cancer types, such as those in the head, neck, lung, or pancreas. In patients with ►esophageal cancer who have locoregional disease, a common induction regimen in the United States uses chemotherapy (often cisplatin and 5-fluorouracil) concurrent with radiotherapy. This is followed by ►definitive therapy, which in this case is surgical resection of the tumor. In Europe, the more common induction regimen uses chemotherapy alone.

The disadvantages of induction regimens rest on the fact that definitive (i.e., most effective) treatment is delayed. If induction therapy is unsuccessful, the tumor may continue to grow to such an extent that definitive therapy is no longer possible or is compromised. A further concern is that the patient, weakened from the side effects of chemotherapy, becomes a suboptimal candidate for definitive therapy.

Leukemia

In acute leukemia (►Acute Myeloid Leukemia, ►Acute Lymphoblastic Leukemia, and ►Acute Promyelocytic Leukemia), a cancer of white cells in the blood, all adult patients who can tolerate such chemotherapy are given induction chemotherapy. The goal of such therapy is to induce "►remission" – to reduce the number of leukemia cells in the body to undetectable levels and restore normal bone marrow function. It is generally believed that some leukemia cells persist undetected even after successful induction chemotherapy, leaving the possibility of ►relapse if further therapy is not administered.

This further therapy is called "consolidation," and is designed to remove all remaining leukemia cells with a view toward cure. Consolidation therapy usually incorporates further chemotherapy and/or hematopoietic cell transplantation.

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Infection

Definition

Invasion of the body by microorganisms (bacteria, viruses, parasites, etc.).

Infectious Mononucleosis

Definition

Is the acute primary infection of humans by ►Epstein–Barr virus. This disease mainly occurs when primary infection by Epstein–Barr virus takes place in adolescence or adulthood. Infectious mononucleosis is a self-limiting disease that is resolved mainly by an efficient ►T cell response against virus-infected B cells.

►Hodgkin and Reed/Sternberg Cell

Infiltration

►Invasion

Inflammation

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Definition

Is an immune response to infection or irritation. Inflammation is characterized by the following quintet: redness (rubor), heat (calor), swelling (tumor), pain (dolor) and dysfunction of the organs involved (functio laesa). Acute inflammation is short-lasting, lasting only a few days. If it is longer lasting however, then it is referred to as chronic inflammation. Chronic inflammation is a prolonged inflammatory response (weeks,

months, or even indefinitely). It is prolonged by the persistence of the causative stimulus to inflammation in the tissue. The inflammatory process unavoidably causes tissue damage and is accompanied by concurrent healing and repair.

Characteristics

Overall, chronic inflammation is bad for human health. There is a close association between chronic inflammation and prevalent diseases found in the developed world (cardiovascular disease/CVD, cancer, diabetes, and Alzheimer disease/AD to name a few). Evidence for this comes from epidemiological studies; clinical trials, showing decreased risk of disease with the use of anti-inflammatory drugs; and animal studies which show increased disease incidence with genetically (knock-out nice/transgenic animals) or environmentally (chemical/physical/biological) induced chronic inflammation (Table 1).

Extensive laboratory and clinical evidence shows that chronic inflammation drives cancer. At the molecular level, ►reactive oxygen species (RONS) and ►reactive nitrogen species (NOS) and ►aldehydes, produced during chronic inflammation, can induce cancer gene ►mutation and ►posttranslational modifications of key cancer-related proteins. Other products of inflammation, including ►cytokines, growth factors and transcription factors such as nuclear factor-kappa B (►NF-κB), control the expression of cancer genes (e.g. tumor suppressor genes and oncogenes) and key inflammatory enzymes such as ►nitric oxide synthases (NOS) and ►cyclooxygenases (COX). These enzymes in turn directly influence RONS and ►eicosanoid levels. The pro-cancerous outcome of chronic inflammation is increased ►DNA damage, increased DNA synthesis, cellular proliferation, the disruption of ►DNA repair pathways and cellular milieu, the inhibition of ►apoptosis, and the promotion of ►angiogenesis and ►invasion.

Chronic inflammation is also associated with ►immuno-suppression, which is a risk factor for cancer. Alternatively, a careful balance in ►innate immunity and ►adaptive immunity is critical to controlling chronic inflammation. An imbalance in this system (e.g. hyper-active effector T cells or under-active suppressor T cells) can perpetuate inflammation. Because of these and other proposed mechanisms associated with inflammatory-mediated ►carcinogenesis, many potential targets for therapeutic intervention exist.

Current treatment strategies for high cancer risk, ►reactive species overload diseases (Table 2) are frequently aimed at treating or preventing the cause of inflammation.

Although these strategies have led to some progress in combating reactive species overload diseases and associated cancers, exposure often occurs again after eradication, treatment to eradicate the cause fails, or the treatment has long-term side effects. Therefore, the identification of molecules and pathways involved in chronic inflammation and cancer is critical to the design of agents that may help in preventing the progression

Inflammation. Table 1 The evidence linking chronic inflammation to disease

Disease	Evidence
Cardiovascular disease (CVD)	• ►Aspirin reduces ►C-reactive protein levels which is a predictor of cardiovascular disease
	• Anti-inflammatory drugs reduce risk of CVD
	• High inflammatory load is associated with increased CVD risk
	• Induced chronic inflammation in animals increases CVD
	• Genetically knocking out pro-inflammatory molecules (►cytokines) decreases animal CVD
Cancer	• Chronic inflammation in specific human tissues increases risk of cancer in that tissue (Table 2)
	• ►Anti-inflammatory drugs reduce the risk of cancer
	• Inflammatory load in serum is associated with high risk of cancer
	• Low level of antioxidant enzymes in cancer patients
	• Induced chronic inflammation in animals increases cancer in the area where chronic inflammation occurs
Diabetes	• ►Diabetes patients have a high inflammatory load
	• Risk of diabetes increases with a specific serum inflammatory profile
	• Animal models of diabetes have a high inflammatory load
Alzheimer Disease (AD)	• High inflammatory load in AD
	• Anti-inflammatory drugs reduce risk of AD
	• Inducing brain inflammation in animals leads to AD

Inflammation. Table 2 Examples of high cancer risk, reactive species overload diseases

Disease	Organ with high cancer risk
Inherited	
►Hemochromatosis	Liver
IBD[a]	Colon
Hereditary pancreatitis	Pancreas
Acquired	
Viral	
Viral ►Hepatitis B	Liver
Viral Hepatitis C	Liver
Bacterial	
►*H. Pylori* infection	Stomach
IBD	Colon
Urinary bladder catheterization	Bladder
Prostatitis	Prostate
Parasitic	
Schistosoma haematobium	Bladder
Liver fluke (*Opisthorchis viverrini*)	Colon
Chemical/Physical	
Barrett Esophagus	Esophagus
Non-hereditary chronic pancreatitis	Pancreas
Metabolic syndrome/►obesity	Multiple[b]
►Lung cancer/►mesothelioma	Lung

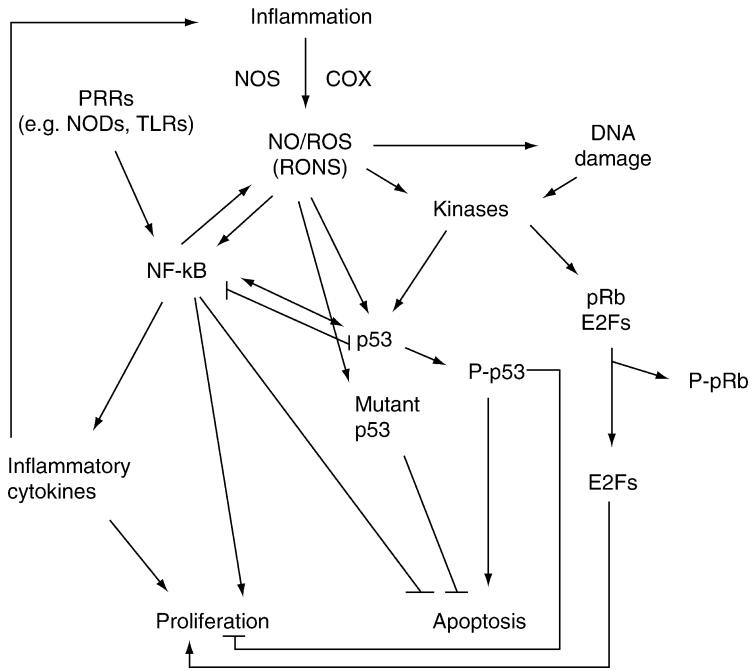
[a]Indicates ►inflammatory bowel diseases. These include ►ulcerative colitis and ►Crohn disease.
[b]Evidence suggests that obesity is associated with chronic inflammation and an increased risk of colon, breast, endometrium, kidney, esophagus, pancreas, liver, gallbladder, stomach.

of reactive species overload disease and cancer associated with disease progression (Fig. 1).

There are many specific and general mediators of these targets that have strong potential to be used as chemo-preventive agents in inflammation-driven cancer. Successful applications include the use of ►tumor necrosis factor-α (TNF-α) inhibitors (monoclonal antibodies) for inflammatory bowel disease and ►interferon-α (IFN-α) for hepatitis. More general medicines that have consistently been found to inhibit many diseases associated with chronic inflammation (cancer, CVD, diabetes) are non-steroidal anti-inflammatory drugs (NSAIDs), such as ►acetylsalicylic acid (ASA). A derivative, 5-ASA, has been used with remarkable success in ameliorating high colon cancer risk inflammatory bowel disease. The mechanisms of 5-ASA are not fully understood, but it is a weak ►COX inhibitor, it activates apoptosis, inhibits proliferation and ►NF-κB, scavenges ►RONS, and inhibits RON-associated base damage.

To this end, there are many natural food and herbal products that target multiple players in the inflammatory cascade. These include red wine (resveratrol), raw fruits and vegetables, and fiber. Many others, such as ►green tea, ►curcumin, ginkgo biloba (►gingko tree), ►ginseng, ►grape seed extract, and garlic have anti-inflammatory properties.

It is unclear which agents will be better in inhibiting inflammatory-mediated carcinogenesis: those acting ubiquitously against inflammatory pathways (e.g. 5-ASA) or specific inhibitors of key inflammatory molecules and pathways (e.g. NF-κB antisense). Perhaps advances in our knowledge of factors that selectively control RNA expression (e.g. through the use of microRNAs and small modulatory RNAs) and their ability to bind a broad spectrum of distinct mRNAs will advance our therapeutic potential for reactive species overload diseases and associated cancers. Although these would equate to a multi-targeted biological "smart-bomb," a better understanding of the efficacy of these molecules in an inflammatory microenvironment is warranted. Multi-targeting might also be accomplished by combination treatment strategies. Future studies should also be aimed at identifying new key molecular targets, molecular signatures in inflammation and intermediate ►biomarkers of inflammatory-mediated



Inflammation. Figure 1 Some key players involved in the inflammation-to-cancer sequence. PRR, pattern recognition receptors; TLR, toll-like receptors; NOD, nuclear binding oligomerization domain; NF-kB, nuclear factor-kappa B; NO, nitric oxide; ROS, reactive oxygen species; RONS, reactive nitrogen and oxygen species; NOS, nitric oxide synthase; COX, cyclooxygenase; E2F, E2 promoter binding factor.

carcinogenesis. The latter, combined with identifying polymorphisms in key inflammatory genes, will help identify specific populations at risk for free radical-driven cancers and provide insight into individuals sensitive or resistant to anti-inflammatory treatment.

Finally, understanding the mechanisms by which key cancer genes and proteins are altered in reactive species overload diseases will provide important insight into the protection against cancer associated with reactive species overload (Fig. 1). ►p53 is mutated in ►ulcerative colitis and ►hemochromatosis. The p53 pathway is also activated in ulcerative colitis (a type of ►inflammatory bowel disease). Other cancer pathway molecules such as ►hypoxia inducible factor-alpha (HIF-1α), ►signal transducer and activator of transcription (STAT)1, STAT3, ►heme-oxygenase-1, ►poly (ADP-ribose) polymerase (PARP), ►extracellular matrix molecules (►integrins), and ►cyclin D1 and cyclin E are up-regulated during free radical stress. Although the specific consequences of this up-regulation are not fully understood, targeting these molecules holds promise for future research into ►chemoprevention in reactive species overload diseases. Inhibiting PARP has been shown to ameliorate experimental colitis; inhibiting integrins improves ►crohn disease and ►ulcerative colitis; germline p53 disruption inhibits helicobacter-induced pre-malignant lesions and invasive ►gastric carcinoma; and ►myc induces free radical stress, and disables a ►p53-mediated ►DNA damage response, implicating p-53 ►Inflammatory Response and Immunity as a potential target for treatment chronic inflammation and associated cancers. To this end, it is also clear that emphasis should be placed on examining the influence of promising drugs in patients with high cancer risk, reactive species overload diseases in prospective randomized control trials. Only then will we know the true value of targeting inflammation and affected cancer pathways for chemoprevention.

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Inflammatory Response and Immunity

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Definition

►Inflammation is the first response of the immune system to infection or irritation, and may also be elicited by tissue damage due to an uncontrolled/abnormal growth of self-cells. Various immune system cells and their secreted mediators and products are actively involved in this response. Inflammation is characterized by the following quintet of symptoms: redness, heat, swelling, pain, and dysfunction of the organs involved. The inflammatory response could be acute or chronic.

Characteristics

An Overview of Inflammation

The immune system is composed of a large variety of cells and soluble mediators that interact in a complex and dynamic network to ensure protection against foreign pathogens and elimination of infected tissue or abnormally growing autologous tumor cells. The immune system is divided into two arms, ►innate immunity and ►adaptive immunity, both participating in the generation of acute and chronic inflammation. Acute inflammation is a short-lasting (several days) immunological process that is mediated by activation of innate and adaptive immune cells. In most cases, this response is beneficial to the host as it results in clearance of invading pathogens. Following acute inflammation, the antigenic/pathogenic stimulus is eliminated; immune cells are no longer triggered, and thus, return to their quiescent state. An acute response could be also induced under sterile conditions, in response to tissue damage. Such a response is accompanied by a multifactorial network of chemical signals that initiate and maintain a host response, designed to "heal" the afflicted tissue. This involves activation and directed migration of leukocytes (neutrophils, basophils, monocytes and eosinophils) from the circulation to sites of damage. These cells, while clearing the damaged tissue, secrete ►chemotactic factors that guide various cells such as ►macrophages to the site of tissue injury. There, they engulf the damaged tissue constituents and help activate the adaptive immune system. Mast cells are also important in acute inflammation due to their release of stored and newly synthesized inflammatory mediators, such as histamine, ►cytokines and ►proteases.

In contrast, chronic inflammation is an inflammatory response of prolonged duration (weeks, months, or even indefinite) whose extended time course is provoked by persistence of the initiating stimulus. Chronic inflammation may develop either as the progression of acute inflammation, if the original stimulus persists, or after repeated episodes of acute inflammation. Chronic inflammation may be local or systemic, caused by spreading of inflammatory cells and mediators to the entire body via the blood stream and the lymphatic system. In a variety of pathologies such as autoimmune diseases, chronic infections and cancer that are characterized by the development of chronic inflammation, local and/or systemic ►immunosuppression accompanied by ►opportunistic infections was reported. Immunosuppression observed under chronic inflammatory conditions is evident both in the adaptive and innate immune systems, as T lymphocytes (►T-cell Response) and natural killer (NK) cells (►Activated Natural Killer Cells), respectively, become dysfunctional. This phenomenon is associated with reduced ►ζ chain expression by both cell types, and was shown to be induced by ►myeloid suppressor cells (MSCs). The ζ chain is part of key functional receptors in the immune system; the ►T cell antigen receptor (TCR) and NK killing receptors (NKP30, NKP46 and CD16).

An Overview of Cancer

The multiple factors leading to malignant transformation (oncogenic process) involve mutations in somatic cells and subsequent alterations of morphology and growth characteristics, ultimately resulting in transformation, local invasion, and metastasis. Genes controlling cell growth, DNA repair, and programmed cell death (apoptosis) are prime targets for the oncogenic process. DNA alterations are irreversible and can persist almost indefinitely in otherwise normal tissue until the exposure of cells to chemical irritants such as phorbol esters, factors released at the site of wounding, partial organ resection, hormones or chronic irritation provide the "final hit" leading to abnormal cell growth. A prerequisite for the survival and expansion of cancer cells is the development of strategies for escaping immune system surveillance. The term "cancer immuno-editing" represents a dynamic process comprised of three phases: elimination, equilibrium, and escape. Elimination represents the classical concept of immuno-surveillance against cancer, in which the immune system recognizes and eliminates tumor cells. Equilibrium describes the period of immune-mediated latency after incomplete tumor destruction during the elimination phase, and escape refers to the final outgrowth of tumor cells following their release from the immunological restraints of the equilibrium phase. Tumor growth, characterized by the abnormal expansion of modified self-cells within a normal tissue site, leads to

destruction of surrounding tissue. In addition, the inner part of an overgrowing tumor often becomes necrotic due to inefficient ►angiogenesis, resulting in poor tumor perfusion and cell death. In the course of cell death and tissue necrosis, a sterile inflammatory response ensues, aimed at clearing the dead cells and injured tissue. However, if the tumor persists, with continuing tissue destruction, chronic inflammation develops.

Interrelationship Between Inflammation and Cancer

The link between inflammation and cancer promotion was first observed in the nineteenth century, but only in recent years has it become a generally accepted phenomenon. Epidemiological studies showed that chronic infection and inflammation are two of the most important epigenetic and environmental factors contributing to tumorigenesis and tumor progression. Inflammatory mediators generally contribute significantly to the process of transformation by inducing DNA damage, activating oncogenes, or inducing loss of anti-oncogenic activity. For example, various ►reactive oxygen species (ROS) produced in inflamed tissues (►Oxidative Stress), whose main role is to attack invading infectious agents and foreign elements could cause injury to host cells and induce ►DNA damage and mutations, when produced at excessive levels.

Innate immune cells also contribute to cancer promotion through paracrine regulation of intracellular pathways, in which factors secreted by specific immune cells (cytokines, such as TNFα and IL1β) or released from damaged tissue (necrotic, apoptotic) in the tumor microenvironment, affect other cells in their surroundings via the corresponding ►cytokine receptors and ►Toll-like receptors (TLRs), respectively. Components of invading pathogens, such as gram negative bacterial LPS and double-stranded viral RNA are also involved in the activation of TLR-expressing cells. In the cells affected through paracrine pathways (normal immune cells, pre-cancerous, or cancerous cells), intricate processes are initiated involving changes in intracellular signal transduction pathways that, in turn, modulate expression of transcription factors, such the ►nuclear factor-kappa B (NFκB). This factor is responsible for the transcription of a specific array of genes (pro-inflammatory cytokines, chemokines, growth factors, and anti-apoptotic factors) that are involved in cancer promotion and support of growth. TLRs are among the major activators of NF-κB transcription factor and are the front-line receptors that trigger inflammation in response to microbial infection or tissue damage, aiming at clearing the pathogen or affected tissue. However, the activation of NF-κB through TLR stimulation in local chronic inflammation may also serve as an initiating event, enabling the infected or damaged cells to evolve into cancer cells, with uncontrolled proliferation. Moreover, since NF-κB activity leads to inhibition of programmed cell death (apoptosis), which can eliminate defective cells, NF-κB activation could contribute both to cancer survival/development and resistance of the tumor to drug and radiation therapies that induce cell elimination by apoptosis.

Another mechanism by which chronic inflammation could support transformation is the promotion of angiogenesis by angiogenic factors such as ►vascular endothelial growth factor (VEGF) and ►matrix metalloproteinases (MMPs). These factors are released from the surrounding inflamed tissue or by the transformed cells, thereby contributing to tumor progression. Increased expression of ►cyclooxygenase-2 (COX-2) and subsequent ►prostaglandin production has been shown to promote tumor growth, ►invasion, and ►angiogenesis, and to suppress the anti-tumor ►adaptive immune response. In addition, a variety of secreted inflammatory cytokines and chemokines (e.g ►IL-2, CXCL13) have also been shown to support cell growth and ►migration. Such factors not only confer survival and growth advantages to susceptible tumor cells, but also affect the degree of local cell invasion, thereby enabling metastastatic spread. Inflammatory characteristics are observed in most pre-cancerous and cancerous tissues, as reflected by the migration of innate immune cells into the affected target tissue and the release of specific inflammatory mediators.

Thus, in the course of preexisting chronic inflammatory diseases such as ►autoimmune disease and chronic infections, activated innate and adaptive immune cells could form an optimal environment to induce and support arising tumors by the release of DNA damaging compounds and/or potent pro-survival molecules, both involved in the initiation of neoplastic cell development and enabling uncontrolled cell growth. In addition, in the course of chronic inflammation, immunosuppression of both the innate (NK cells) and the adaptive (T cells and B cells) immune responses is induced, resulting in unresponsiveness of the immune system to abnormal growing tumors. Thus, the initial step of an inflammatory response aimed at destroying pathogens or abnormal self-cells, often leads to a chronic inflammatory response, which in many cases is associated with immunosuppression and cancer. In parallel, activation of innate and adaptive immune cells also elicits anti-tumor responses through T and NK cell-mediated toxicity, antibody-dependent cell-mediated cytotoxicity, and antibody-induced complement-mediated lysis. During the anti-tumor immune response (►Tumor Suppression), an inflammatory tumor microenvironment is generated. When the inflammatory response becomes chronic due to the inefficient clearance of the tumor or due to damage of the surrounding tissue, the proinflammatory factors both support tumor growth and lead to immunosuppression of ►NK cells, ►T cells

and ►B cells, resulting in tumor overgrowth and ►metastasis. Moreover, immunosuppression in tumor-bearing host could also result from the increased frequency of regulatory T cells (Treg) (►T Regulatory Cells) within the peripheral blood of cancer-bearing hosts and/or among tumor-infiltrating lymphocytes and tumor-draining lymph nodes. Tregs are able to inhibit proliferation and cytokine production by effector T cells, as well as NK cell-mediated cytotoxicity. Thus, the delicate balance between anti-tumor immune response, activity of Tregs and developing inflammation, dictates whether a tumor will develop or be rejected (Fig. 1).

Examples of Inflammation-Associated Cancers

Over the past 15 years, numerous cancers have been shown to be associated with local chronic inflammation. Studies on liver cancer suggested that patients with persistent ►hepatitis B virus infections experience inflammation and scarring of liver tissue and thus, are at increased risk of liver cancer (Hepatocellular Carcinoma). Other disease characterized by chronic inflammation including ulcers caused by the bacterium, ►*Helicobacter pylori*, and an immune disorder known as ►ulcerative colitis, predispose patients to ►stomach cancer and ►colon cancer. ►Cervical carcinoma, which is caused by infection with ►human papilloma virus (HPV) has also been suggested to have an inflammatory component. Innate immune cells, most prominently ►mast cells and ►granulocytes, infiltrate the pre-malignant epithelial tissues, followed by the recruitment of macrophages as the cancer develops further. Innate immune cells drive a chronic inflammatory process that promotes overgrowth of epithelial cells, tissue remodeling and angiogenesis, eventually followed by invasive carcinoma. Other examples include the associations between chronic bronchitis and ►lung cancer, schistosomiasis (►schistosomas, gematobition) and ►bladder cancer, chronic pancreatitis and pancreatic cancer, and chronic cholecystitis and gall bladder cancer. In addition, epidemiologic studies showed that regular intake of ►non-steroidal anti-inflammatory drugs (NSAIDs) lowers the risk of developing several types of cancers. However, in the absence of specific risk factors, NSAIDs are not recommended at present, since many of these drugs, such as ►aspirin, can cause severe gastrointestinal complications.

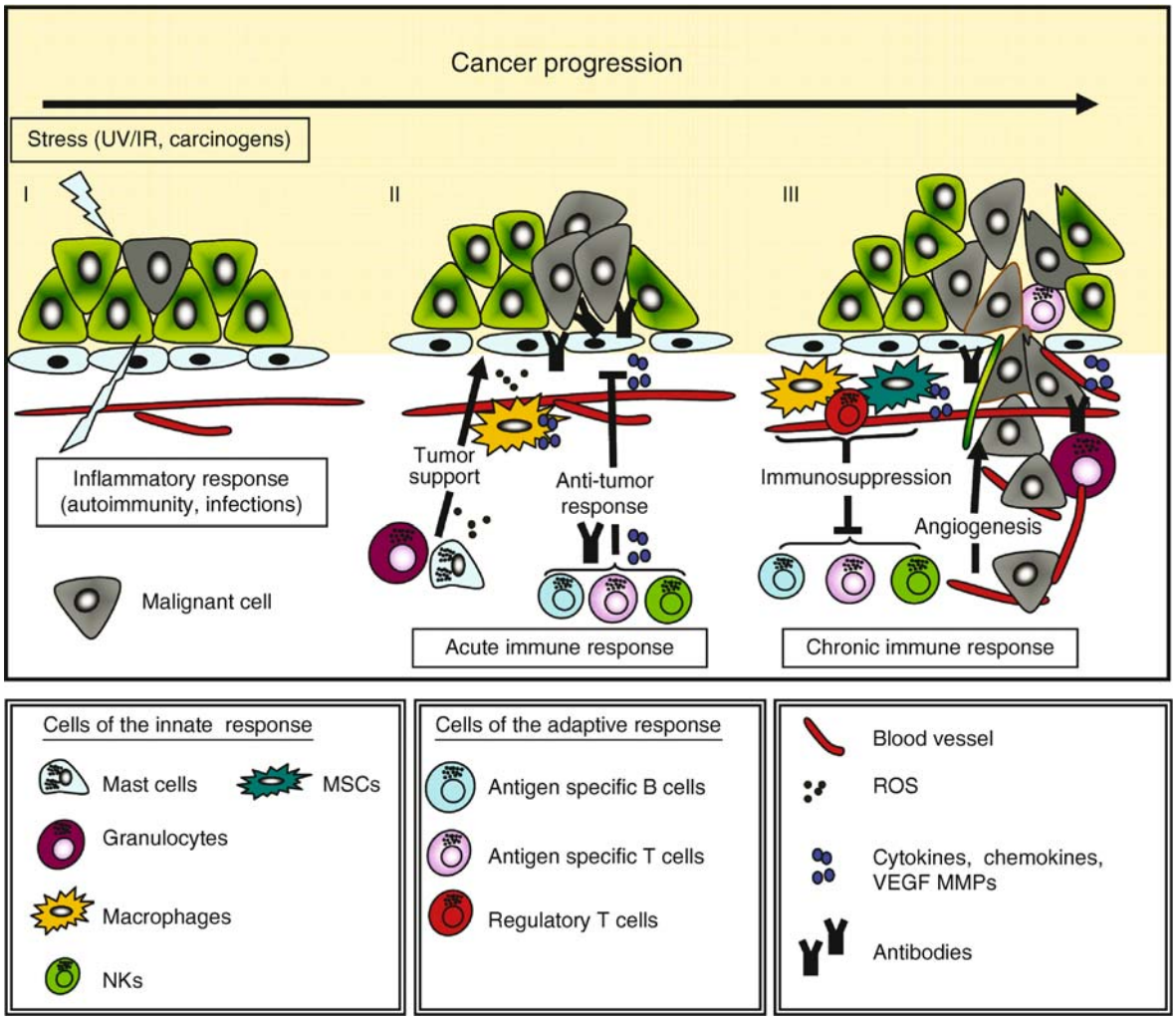
Based on the knowledge accumulated during the last two decade it has been estimated that inflammation contributes to the development of at least 15% of all cancers.

Immunotherapeutic Strategies Against Cancer

One of the major goals in cancer immunotherapy research is to sever the destructive association between chronic inflammation and cancer. Cancer ►immunotherapy is a developing strategy to buttress the immune system, augmenting its ability to destroy tumors. The identification of tumor-associated antigens recognized by the effector arm of the adaptive immune system has opened new approaches towards cancer immunotherapy. However, chronic inflammation predisposes individuals to cancer, and cancer promotes chronic inflammation; under both scenarios, chronic inflammation-dependent immunosuppression is evident in association with tumor overgrowth. Therefore, to attain an effective therapy, anti-inflammatory and immunotherapeutic approaches will have to be combined, as described bellow.

Currently, several forms of immunotherapy are being tested against cancer; these approaches fall into two categories, which are not mutually exclusive: The first category involves a passive attack against the tumor. This could be mediated by administration of ►monoclonal antibodies (Mabs) that are directed against various tumor associated antigens (when the tumor is immunogenic) and are conjugated to anti-cancer drugs, toxins, radioisotopes, or other biologic response modifiers (►Cytokine Receptor as Target for Immunotherapy and Immunotoxin Therapy). When the antibodies bind to the antigen-bearing cells, they deliver their cytotoxic load with the aim of tumor destruction. Mabs could also be directed against specific inflammatory compounds that support tumor growth and suppress the immune system. Thus, by using specific Mabs, inflammatory compounds(s) could be neutralized, leading to inhibition of tumor expansion and recovery of the immune response (►Monoclonal Antibody Therapy). In addition, immune response modifiers such as interferons could potentially stimulate immune system cells to act against the tumor. Other strategies that use anti-angiogenic agents, ►MMP-inhibitors and anti-inflammatory drugs have also met with some success.

The second category is based on inducing an active attack against the tumor. This could be mediated by several approaches: (i) ►Dendritic cell (DCs)-dependent immunotherapy that uses the patient's own DCs, which initiate the adaptive immune response through their role in antigen presentation. DCs isolated from patient blood are loaded with tumor proteins (antigens) and injected back into the patient to initiate a potent specific immune response directed against the cancer cells. This approach is currently being investigated in phase IIb/III clinical trials for ►brain cancer and ►prostate cancer. (ii) T cell immunotherapy that utilizes T cells activated against defined tumor antigens or genetically modified to express ►TCRs that recognize a specific tumor antigen, aiming at efficiently destroying the tumor. In both cases, T cells are taken from the cancer patient, selected or modified, and then injected back to the same patient where they are expected to



Inflammatory Response and Immunity. Figure 1 The interrelationship between cancer and inflammation. (I) Tumors can emerge as a result of stress or excessive inflammatory responses during an infection or autoimmune response. (II) Tumor/pathogen or self antigens presented by DCs in secondary lymphoid organs activate adaptive immune responses, resulting in both tumor-promoting and tumor-inhibitory effects. Activated innate and adaptive immune cells promote tumor development by the release of potent pro-survival molecules and/or DNA damaging ROS, both involved in the initiation of neoplastic cell development. In contrast, activation of cells of the innate and adaptive immune system also elicits anti-tumor responses through T and NK-cell-mediated toxicity, antibody-dependent cell-mediated cytotoxicity, and antibody-induced complement-mediated lysis. The delicate balance between the two types of responses will dictate whether tumor will develop or be rejected. (III) Inflammatory cells induce/support tissue remodeling and angiogenesis. Tissues suffering from chronic inflammation exhibit an increased risk of tumor development due to immunosuppression of NK, T and B cells mediated by MSCs and Treg.

mediate their cytotoxic function. (iii) Vaccine-based immunotherapy that depends upon the administration of a tumor specific component (antigen), or inactivated cancer cells to elicit an immune response (►Cancer Vaccines). For vaccine use, the immunogen is injected into the body in a formulation that targets and activates local dendritic cells, which in turn activate the endogenous immune system.

While each of these active immunotherapeutic approaches have shown some promise, the general success rates are limited due to some critical obstacles. One of the obstacles to effective active tumor immunotherapy is the chronic inflammation and associated immunosuppression that often characterizes developing tumors. Moreover, the increased ►Treg activity in tumor-bearing host may be associated with

poor immune responses to tumor antigens and contribute to immune dysfunction. It is expected that the immunosuppressive environment generated in tumor-bearing hosts due to chronic inflammatory response and the increased Treg activity will limit the response to an active immunotherapy, affecting the endogenous host immune system, as well as suppressing any newly administered immune modulators. Immunotherapy using immune response modifiers such as ►cytokines and ►chemokines also requires a responsive immune system, and therefore these approaches are expected to fail under an immunosuppressive environment. Moreover, it is possible that adjuvants (►Adjuvant Therapy) used to enhance a patient's immune response to weakly immunogenic tumors or pathogenic antigens might even result in activation of chronic inflammation and thus negatively affect the specific anti-tumor response. With this knowledge in mind, the timing of tumor active immuno-therapy must be reassessed to maximize its efficacy. It is therefore critical to develop monitoring strategies that may allow the identification of patients who could benefit from cancer immunotherapies and that would help optimize the timing of these interventions. One such strategy could be based upon measurements of ζ chain expression levels in the host's peripheral T cells. Such measurements could serve as a prognostic marker for the appearance of a chronic inflammation-dependent immunosuppressive environment (associated with ζ chain down-regulation) and for measuring the impact of the disease on the patient's immune system. Accordingly, anti-inflammatory drugs are expected to neutralize the immunosuppressive environment and thus could serve a dual purpose; they could be used in cancer prevention or as a treatment to optimize the patient's response to immunotherapy. In addition, strategies that deplete Tregs, inhibit their function or block their migration, rather than enhance or restore their function, are likely to be advantageous for cancer immunotherapy.

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Inflammatory Bowel Disease-associated Cancer

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Definition

Inflammatory bowel diseases (IBDs) result from the combined effect, of environmental factors, e.g., endogenous microbial flora, smoking status, and on the other hand of inherited variants of disease susceptibility genes conferring risk of disease and disease phenotype genes influencing response to treatment and location of disease. Their interaction leads to an aberrant, relapsing, and/or sustained intestinal inflammatory and immune response. Two major forms of IBD, ►ulcerative colitis (UC) and ►Crohn disease (CD), have been described. Ulcerative colitis is characterized by a diffuse but usually superficial inflammation of the colonic mucosa, affecting the rectum and extending proximally along a variable length of the colon. Crohn disease is a chronic granulomatous inflammation which may affect the entire gastrointestinal tract with predominant locations at the terminal ileum and/or colon. By contrast to UC, inflammatory lesions in CD patients are often distributed heterogeneously as a patchwork of inflamed and noninflamed fields of intestinal mucosa.

Both UC and CD patients are at higher risk of cancer development, in particular colorectal cancer (CRC) in UC and colonic CD, and less frequently small bowel carcinoma in CD patients. In addition, lymphoma may occur in UC and CD patients as the consequence of the treatment with immunosuppressive agents.

Characteristics

IBD-Associated CRC

The single-layered intestinal epithelium at the interface between the mucosa and the luminal content is an essential actor of the interactions between immunoregulatory factors of the mucosa and environmental factors, mainly enteric bacteria, while being disrupted during ulceration and then repaired at remission. Epithelial repair requires tightly regulated changes in cell functions including increased cell proliferation, apoptosis, and migration. So far, disease susceptibility genes have been described in CD (NOD2/CARD15; IL-23R) and several loci are suspected in CD (OCTN1 and OCTN2 on chromosome 5) and UC (e.g., the IBD2 locus on chromosome 12). NOD2/CARD15 is

expressed both in lymphocytes and in Paneth epithelial cells, and controls the ►NFκB pathway involved in the immune response.

IBDs represent one of the higher risk conditions for CRC (►colon cancer), together with the genetically linked situations of hereditary nonpolyposis CRC (►Lynch syndrome) and ►familial adenomatous polyposis (►APC gene in familial adenomatous polyposis). In contrast to sporadic ►CRC that almost invariably outgrow the mucosa as polyps, morphological changes linked to IBD-associated cancers are highly heterogeneous with flat lesions or elevated lesions generally referred to as dysplasia-associated lesions or masses (DALM). Flat lesions and sometimes raised lesions look like gross abnormalities encountered in IBD, making them difficult to identify during endoscopic examination. Also remarkable is the multicentricity of neoplasms in IBD-associated cancers, reflecting "►field cancerization" unlike sporadic cancers.

Genetic alterations (►chromosome instability and/or ►microsatellite instability) and epigenetic changes (modifications of the ►methylation patterns of DNA) are similar in IBD-associated cancers and in sporadic CRC, suggesting that chronic inflammation plays a causative role for the increased risk of cancer development. However the sequence of the genetic alterations differs. Whereas the loss of function of APC (►APC/β-catenin pathway) and ►p53 are respectively considered as an early event linked to cancer initiation and a late event associated to the adenoma-to-carcinoma transition in sporadic cases, p53 chromosomal loss or mutations are already present in early stages in IBD-associated cancers, while APC alterations occurring later. Moreover, chromosomal rearrangements involving the p53-encoding locus can even be detected at a significant rate (∼20%) in nondysplastic colitis epithelium. The relatively late involvement of APC alterations in IBD-associated cancers may explain the flat morphology of the dysplasia, in contrast to the polyp-like lesions found in sporadic cancers.

Chronic inflammation produces a favorable ►microenvironment for cancer development. It contains elevated amounts of proinflammatory cytokines like ►TNFα and ►interleukins-1β, ►interleukin-4, ►interleukin-6, and ►interleukin-8 secreted by infiltrating mononuclear cells, ►prostaglandins resulting from the induction of ►COX-2 in epithelial cells and in fibroblasts of the lamina propria as well as reactive oxygen and nitrogen species (►RONS). These oxyradicals target a large number of molecules including DNA, RNA, proteins and lipids. In particular, they induce the formation of ►adducts to DNA, generating point mutations in genes like p53 and in ►CpG islands involved in DNA methylation. The mutagenic effect of RONS is thought to participate in the neoplastic transformation of epithelial cells. Moreover, the environment rich in proinflammatory cytokines and prostaglandins prevents apoptosis and sustains cell proliferation, which further facilitates tumor progression. This provides a way to explain how chronic inflammation results in malignant transformation and tumor growth from the resident intestinal epithelium. In addition, recent experimental data obtained in mouse models of gastric cancer linked to ►*Helicobacter pylori*-induced gastritis suggest that chronic inflammation may locally recruit circulating bone marrow stem cells that would further evolve into carcinomas. Although this observation requires confirmation and further studies to investigate if it can be extrapolated to human IBD-associated cancers, it opens new avenues in the field of chronic inflammation-induced cancers.

Compared with the general population, patients with long-standing IBD are at an increased risk for the development of CRC. Early data estimated the risk of CRC at 0.5% per year after 10 years of UC diagnosis, and 1% per year after 20 years. More recently, a meta-analysis reviewing 116 studies representing a total of 54,478 UC patients, determined the cumulative risk of CRC to be 2% at 10 years after diagnosis of UC, 8% at 20 years, and 18% at 30 years. Patients with Crohn's colitis, like UC patients, also are at greater risk (approximately at the same level) for CRC than the general population.

Strategies aimed at preventing IBD-associated CRC need to define the most relevant target population at risk. Several factors have been identified among which duration of colitis (>8 years) and anatomical extent of colitis [standardized incidence ratio (SIR): 1.7, 95% confidence interval (CI): 0.8–8.2 for proctitis; SIR: 2.8, 95% CI: 1.6–4.4 for left-sided colitis; SIR: 14.8, 95% CI: 11.4–19.9 for pancolitis], appear as the most important and reproducible from one study to another. Other factors like family history of sporadic CRC (twofold higher risk), young age of IBD onset, backwash ileitis, and more recently degree of inflammation in the involved colon, are also considered as risk factors. Finally, association to primary sclerosing cholangitis (PSC), although rare (approximately 2–7.5% of IBD patients, more frequently in UC patients), appears as a very important risk factor and has conducted to a specific surveillance strategy in these particular patients since the cumulative incidence of patients presenting both UC and PSC was 33% at 20 years in a population-based Swedish study. By contrast, a few other factors have been suggested to be protective, such as folate, ursodeoxycholic acid, and with the highest degree of evidence, treatment by 5-aminosalicylates (5-ASA).

The goal of surveillance strategies (i.e., surveillance ►colonoscopy) is to detect by using a safe and effective intervention, neoplasia at a curable stage (ideally as ►dysplasia). Dysplasia is defined as an unequivocally

but noninvasive (intraepithelial) neoplasia. Despite the theory proposing that IBD-associated colon carcinogenesis progresses from no dysplasia to indefinite dysplasia, and then to low grade dysplasia (LGD), high grade dysplasia (HGD), and finally invasive cancer, in reality, this conceptually useful model is by no means absolute. This uncertainty thrives on much of the controversy regarding treatment of LGD. Except in the case of dysplasia in DALM which usually is not too difficult to detect for experienced practitioners, macroscopic identification of flat dysplastic lesions (either of low or high grade) appears particularly difficult during conventional colonoscopic surveillance. Nevertheless, this early identification represents a major challenge, as the presence of HGD in the colonic epithelium is associated with concurrent, macroscopically-undetectable CRC in 42–67% of patients (in case of colectomy performed shortly after HGD diagnosis at colonoscopy), and to the finding of a synchronous CRC in up to 19% of patients in case of LGD diagnosis at colonoscopy.

Considering these facts, recommendations for colonoscopic surveillance have been established. Screening ►colonoscopy should be initiated after 8–10 years of disease, including biopsies of each macroscopic lesion (with additional biopsies in the flat mucosa surrounding the DALM) and random biopsies of the macroscopically normal appearing mucosa (four-quadrant biopsies every 10 cm, some authors considering sampling every 5 cm in the rectosigmoid). In patients with PSC, colonoscopic surveillance should begin at the time of diagnosis of PSC and IBD. At each surveillance examination, the whole colon should be examined, and biopsies processed in separate clearly identified specimen containers. Recently, new endoscopic technologies have been suggested to improve the diagnosis yield of early malignancy, especially dysplasia detection in flat mucosa. This is the case for high-resolution and magnifying endoscopy, chromo (or dye) endoscopy based on vital staining with methylene blue or contrast staining with indigo carmine, magnifying chromoendoscopy, narrow-band imaging, and confocal laser endoscopy. These techniques open a new world of endoscopic imaging, but their usefulness for improving dysplasia detection in IBD needs to be carefully assessed before they will be recommended in routine screening. Nevertheless, the most recent data indicate that dye endoscopy with indigo carmine or methylene blue have to be seriously considered in daily practice.

If no dysplasia is identified, surveillance examination is recommended every 1–3 years (some authors proposing a subsequent screening at 3 years between 8 and 20 years of disease, every 2 years between 20 and 30 years, and each year after 30 years of IBD diagnosis), except for IBD patients with concurrent PSC (surveillance colonoscopy recommended each year regardless of IBD duration). In case of LGD detection in flat mucosa, sample analysis by a second pathologist is recommended, and if LGD is confirmed, a surveillance colonoscopy should be performed 6 months later, although some authors propose to perform colectomy. Detection of HGD in flat mucosa indicates colectomy. Decision after LGD or HGD diagnosis in a polypoid lesion should integrate additional information: (i) if the polypoid lesion has been completely removed and no dysplasia has been detected elsewhere in the colon, a control colonoscopy with multiple random biopsies is recommended 6 months later; (ii) if this is not the case (incomplete removal and/or dysplasia detected in other biopsies), colectomy is indicated.

A number of studies have examined the chemopreventive potential of several medications. Until now, 5-ASA have been the most thoroughly studied as potential IBD-associated CRC preventing agents. In fact, at least in vitro but also in some in vivo studies, 5-ASA have been shown to inhibit cell proliferation, to induce apoptosis, to act as potent RONS scavengers, and to enhance DNA repair. Although the optimal dose and duration of 5-ASA treatment to prevent IBD-associated CRC are unclear, data suggest that chronic systemic administration of 5-ASA at a dose of at least 1.2 g/day is the most likely to prevent IBD-associated CRC development.

Small Bowel Adenocarcinoma in Crohn Disease

The incidence of small bowel adenocarcinoma (SBA) is very low. In CD, its relative risk has been reported to be up to 50 times higher than in the general population. In a recent study, its cumulative risk in patients with ileal CD has been estimated to be 0.2% after 10 years of disease, and 2.2% after 20 years, with a median age at diagnosis of 47 years compared with 68 years for patients with SBA de novo. It occurs in a median time of 15 years after CD diagnosis arising from long-standing inflammation. CD-associated SBA is difficult to diagnose and causes premature mortality in early-onset CD patients.

Cancer risk Associated to Immunosuppressive Therapy in IBD

In the recent years, the efficacy of "classical" immunosuppressive drugs [azathioprine, 6-mercaptopurine (6-MP), or methotrexate], as well as that of biological agents (i.e., in current practice, anti-TNFα antibodies such as infliximab) led to their increased use in IBD. This emphasizes the question of their potential carcinogenic effects, in particular considering the risk of lymphoma which has been previously reported in patients treated by azathioprine or 6-MP after renal or hepatic transplantation (although used at doses even higher than in IBD). This question is not definitively resolved, probably due to the heterogeneity

of the different studies, in particular when considering population-based cohorts on one hand and hospital-based cohorts on the other hand. Discrepancies may result from varying factors such as cohort sizes, duration and dosage of treatment, and/or duration of follow-up. Nevertheless, some general statements based on the most relevant studies in the field can be suggested: (i) the absolute risk of ►lymphoma in the general IBD population appears extremely low, (ii) lymphoma risk in IBD patients treated by azathioprine or 6-MP is probably not more than two- to fourfold increased, although the respective responsibility of treatment and underlying disease has to be more accurately investigated, (iii) particular attention needs to be brought to the ►Epstein–Barr virus positive lymphoma risk in azathioprine or 6-MP treated IBD patients, and finally, (iv) the issue of lymphoma risk is likely to become more relevant in the future with the growing number of immunosuppressive and/or biological agents being used (or tested) in IBD, sometimes concurrently. This has been emphasized by the report of hepatosplenic T-cell lymphomas in young petients treated both with purine analogs and infliximab.

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Inflammatory Cytokines

Definition

Small proteins that are released primarily by activated immune cells. Inflammatory ►cytokines act by binding to specific cell membrane receptors that are involved in amplification of inflammatory reactions. Cytokines can act in an ►autocrine, ►paracrine, or ►endocrine manner. ►Interleukins, ►lymphokines, and ►interferons are all cytokines.

►Aging and Cancer
►Inflammation

Inflammatory Response

Definition

Refers to the ability of the innate component of the immune system to react to an infection or irritation. An orchestrated cellular and biochemical response of the body to injury or infection. Can be classified into acute and chronic. The response consists of cellular and exudative components. These involve the movement of white cells and fluid containing proteins and antibodies into the tissue to repair damage and inactivate a foreign agent.

►Inflammation
►Inflammatory Response and Immunity

Infratentorial Primitive Neuroectodermal Tumor

►Medulloblastoma

Inherited Human Polycystic Kidney Disease

►Polycystic Kidney Disease

Inhibin

Definition

Dimeric peptide hormones (designated inhibin A and inhibin B) secreted by the follicular cells of the ovary

and the Sertoli cells of the testis. Inhibits production and secretion of ►follicle stimulating hormone (FSH) by the pituitary.

►Granulosa Cell Tumors

Inhibitor of Apoptosis Family

Definition

IAPs are a family of proteins that are important regulators of ►apoptosis. IAPs function by binding to and inhibition of activated ►caspases. IAPS are characterized by having one or more protein domains of 70 amino-acids called baculovirus IAP repeat (►BIR domain).

►Apoptosis-Induction for Cancer Therapy

Inhibitor of Cyclin-Dependent Kinase

Definition:

Protein that inhibits the kinase activity of ►CDK.

►Early B-cell Factors
►Cyclic Dependent Kinases

Inhibitor of FLICE

►FLICE Inhibitory Protein

Inhibitors

Definition

Proteins or chemicals capable of blocking the activity of an enzyme. Endogenous inhibitors are natural products of cells and tissues as opposed to exogenous inhibitors, which require administration to cells and tissues by various routes.

►Cystatins

Initial Area Under the Gadolinium Contrast Agent Concentration–Time Curve

Definition

IAUGC; A model-free biomarker derived from contrast agent concentration data that is used as a biomarker in clinical trials of angiogenesis inhibitors.

►Dynamic Contrast-Enhanced Magnetic Resonance Imaging

Initiation

Definition

In carcinogenesis, the first step in skin carcinogenesis is initiation, which is a reversible process during genetic mutations, gene activation or inactivation occur. Examples of initiation are mutations in the ►RAS oncogene or inactivation of the p53 tumor suppressor gene

►Skin Carcinogenesis

Initiation and Promotion

Definition

In carcinogenesis, the process in which an animal is treated with a low dose of a carcinogen, then this is followed once per week with a tumor promoter, and leads to a synergistic induction of tumors.

►Chemical Carcinogenesis
►Tumor Promotion

Initiation Codon

Definition

The mRNA sequence AUG, which specifies methionine, the first amino acid used in the ►translation process.

Initiation Factors

Definition

Initiation of ►translation involves the small subunit of the ribosome binding to 5′ end of mRNA with the help of initiation factors.

Initiator Caspases

Definition

Are activated independent of cleavage by dimerization of the monomeric zymogen at multiprotein complexes, to which the ►caspase zymogens are recruited by virtue of their N-terminal recruitment domain. In the extrinsinc pathway, ►caspase-8 and -10 are activated at the death-inducing signaling complex (►DISC), whereas in the intrinsinc pathway, the site of activation of caspase-9 is the ►apoptosome.

INK4a

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Definition

Inhibitor of ►cyclin-dependent kinase 4; proteins are cyclin-dependent kinase inhibitors that block the action of cyclin-dependent kinase to induce cell cycle arrest. The four INK4 family proteins (p16INK4a, p15INK4b, p18INK4c, and p19INK4d) contain four tandemly repeated ►ankyrin motifs and have similar biochemical phenotypes. The p16INK4a is an alternative reading frame product (►ARF) of p16INK4a and p15INK4b, which are coded at the human chromosome 9p21 region. The significance of the p16INK4a, ARF, and p15INK4b in tumorigenesis are confirmed by the high frequency of the genetic and epigenetic modifications of these genes in tumor samples.

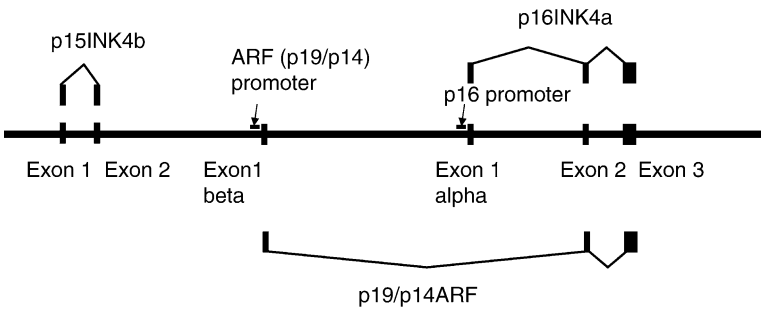
Characteristics

Structure of *INK4b/ARF/INK4a* Locus and Molecular Functions of INK4s/ARF

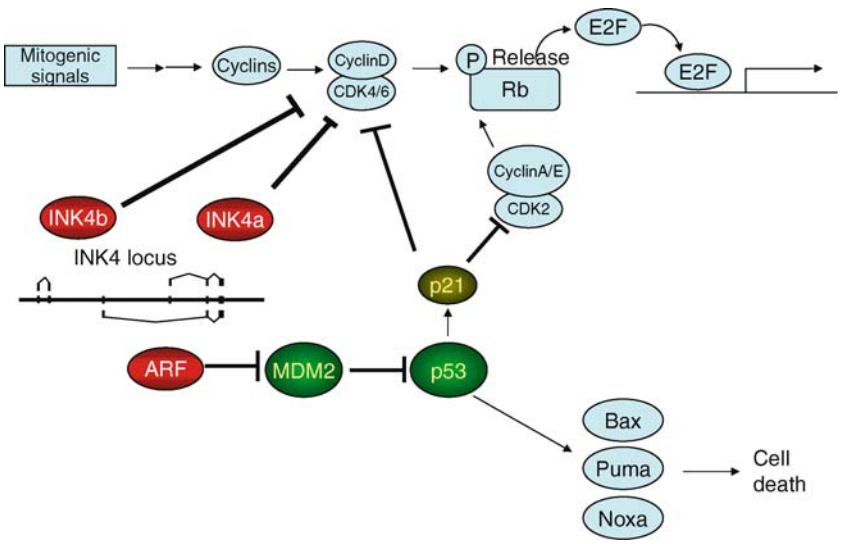
The *INK4b/ARF/INK4a* gene locus is located at the human chromosome 9p21 region. Chromosome region 9p21 is involved in chromosomal ►inversions, ►translocations, and ►deletions in a variety of malignant cell lines and primary tumor samples, including those from ►melanoma, pancreatic adenocarcinoma, ►non-small cell lung cancer, ►leukemia, and ►glioma. These findings indicate that 9p21 contains a tumor suppressor locus that may be involved in the tumorigenesis of several tumor types. In a region of less than 40 kb of the human genome, three related genes, *p15INK4b*, *ARF*, and *p16IN4a* are encoded (Fig. 1).

The INK4 class of ►cell cycle inhibitors, p15INK4b and p16IN4a are homologous inhibitors of the cyclin-dependent kinases, CDK4 and CDK6, which inactivate the tumor suppressor RB1 protein via phosphorylation of its c-terminal region. The association of the INK4 proteins to CDK4/CDK6 induces an allosteric modification that abrogates the binding of these kinases to ►cyclin D, resulting in inhibition of CDK4/6-mediated phosphorylation of retinoblastoma family member proteins. Hence, the existence of p15INK4b and p16INK4a maintains retinoblastoma family member proteins in a hypophosphorylation state, which facilitates binding of ►E2F to induce a cell cycle arrest in the G1 phase (Fig. 2).

ARF and *INK4a* have different first exons, exon 1 beta and exon 1 alpha, respectively. These first exons are spliced to a common second exon and third exon. Although exons 2 and 3 are shared by p16INK4a and ARF, these proteins are encoded in alternative reading frames. The predicted 132-amino acid p14 (ARF) is shorter than the corresponding mouse protein, p19(ARF), and the 2 proteins share only 50% identity. However, both proteins have the ability to elicit a p53 response, manifest in the increased expression of both p21Cip1/Waf1 and several p53-downstream proapoptotic molecules, resulting in a distinctive cell cycle arrest in the G1/G2M phases and apoptotic cell death, respectively. Previous reports showed that ARF binds to ►MDM2 and promotes the segregation of MDM2 in the nucleolus. This interaction is mediated by the exon



INK4a. Figure 1 Genomic stucture of INK4b/ARF/INK4a locus. Residing on chromosome 9p21 in humans and chromosome 4 in mice, the *INK4b/ARF/INK4a* locus includes 2 different genes. *INK4b* and *INK4a/ARF*. *INF4a* and *ARF*, which have the independent exon1 alpha and exon1 beta, respectively, and common exon2/exon3, encode different proteins via an alternative splicing mechanism. In this commentary, p14ARF (murine p19ARF) will be referred to as ARF.



INK4a. Figure 2 Physiological roles of INK4s/ARF. Mitogenic signals activated cyclin D-dependent kinases, which phosphorylate RB and RB family proteins to facilitate entry into S phase. Consititutive oncogenic signals can activate the INK4a/ARF locus. By antagonizing the activity of cyclin D-dependent kinases, p16INK4a and p15INK4b prevent entry into S phase. MDM2 is a p53-inducible gene that normally acts to terminate the p53 response. The ARF protein inhibits MDM2 to induce p53, leading either to p53-dependent apotosis or to induction of the CDK inhibitor p21Cip1, inhibition of cyclinE/Cdk2, and RB-dependent cell cycle arrest.

1-beta-encoded N-terminal domain of ARF and a C-terminal region of MDM2.

Roles in Tumorigenesis

Human cancers frequently harbor ►homozygous deletions of the *INK4b/ARF/INK4a* locus that abrogate expression of all p15INK4b/ARF/p16IN4a. In a large number of human cancers, specific somatic loss of p16INK4a, through ►point mutation or small deletion, has been reported. Furthermore, silencing of p16INK4a through promoter methylation is reported at high frequency in numerous types of human malignancies. Therefore, p16INK4a is an important tumor suppressor in human malignancies.

In the case of p15INK4b, specific ►epigenetic gene silencing by ►hypermethylation of the p15INK4b promoter has been described in ►hematological malignancies including ►leukemia and ►myelodysplastic syndrome and rare cases of glial tumors. In myelodysplastic syndrome, hypermethylation of p15INK4b has been reported in the absence of p16INK4a hypermethylation. p15INK4b seems to be an important tumor suppressor in specific lineages of human malignancies, e.g., hematological malignancies.

Selective inactivation of ARF, in the absence of additive loss of p16INK4a and p15INK4b, has only been reported in a small number of human malignancies, e.g., familial melanoma/astrocytoma patients and somatic ARF-specific mutations and promoter methylations in colon carcinoma patients. Therefore, it seems that ARF cooperates with p16INK4a and p15INK4b in tumorigenesis of human malignancies and their relative and combinational importance in any given tumor types remains to be elucidated.

Roles in Senescence

Cellular ►senescence is a fundamental cellular program that is activated in various situations of stress and acts to prevent further cell proliferation. Senescence induced by extrinsic stress, such as DNA damage or oncogene activation, occurs relatively rapidly, in a matter of days. As a population of cells is propagated in culture, cells are exposed to various extrinsic and intrinsic stresses and the population gradually stops dividing. These findings have led to a distinction between "stress-induced premature senescence," a term referring to rapid senescence triggered by extrinsic stress, and "replicative senescence," a term referring to senescence that occurs following extended proliferation, presumably triggered by various stresses. Cellular senescence is thought to play an important role in tumor suppression and contribute to organismal aging. In fact, the two definitive tumor suppressor pathways, ARF/MDM2/p53 and p16INK4a/Rb, have been shown to play critical roles in the induction of cellular senescence. In tissue culture of primary cells, the accumulation of one or more *INK4b/ARF/INK4a* locus genes can eventually lead to cell cycle arrest through the mechanisms presented in Fig. 2. The cellular senescence is cancelled by downregulation of expression caused by gene deletion or epigenetic regulation, by inactivation of the gene products by mutation, or by cellular resistance to those cell cycle inhibitors. Considering the significance of the *INK4b/ARF/INK4a* locus genes in the cellular life span, p16INK4a plays a central role in senescence in human cells, whereas ARF assumes a prominent role in mouse cells. For mice, p19ARF and p16INK4a both accumulate significantly after passage, but spontaneous escape from senescence occurs through deletion of INK4/ARF or p53 mutation in wild type MEFs. Consistent with this, ARF-null MEFs do not have proliferation failure, but p16INK4a-null and p15INK4b-null MEFs indicate limited proliferation. Whereas senescence generally occurs in the setting of increased expression of p16INK4a, but not ARF, and enforced Ras–Raf pathway activation also appears to induce only p16INK4a, along with senescence in cultured human cells. Furthermore, only p16INK4a induction has been reported with human aging, although ARF expression studies in human aging have not been reported.

Activators of the p15INK4b/ARF/p16INK4a

Ras–Raf–p38MAPK Pathway

Involvement of the Ras signaling pathway into the regulation of the *INK4b/ARF/INK4a* locus-encoded genes has been reported in detail. Expression of oncogenic Ras in primary human or rodent cells results in a permanent G1 arrest of the cell cycle. The arrest induced by ►Ras is accompanied by accumulation of ►p53 and ►p16, and is phenotypically indistinguishable from cellular senescence. Constitutive activation of MAPKK induces both p53 and p16, and is required for Ras-induced senescence of normal human fibroblasts. Ras signaling pathway activation can result in phosphorylation and enhance binding of ►Ets transcription factor to the p16INK4a promoter. These results imply that premature senescence via *INK4b/ARF/INK4a* locus activation acts as a fail-safe mechanism to limit the transforming potential of excessive Ras mitogenic signaling. Whereas oncogenic Ras induces ARF transcription in MEFs, similar effects have not been detected in human cells. One of the key molecules activated by Ras signaling pathway is DMP1, which binds directly to the p19ARF promoter region. Although there are putative binding sites in the human ARF promoter region, the effects of DMP1 have not been demonstrated in human cells.

E2F

The ►E2F transcriptional factors are divided into two groups; (i) transcriptional activating E2F1, E2F2, and E2F3; (ii) transcriptional repressing E2F4, E2F5, and E2F6. Several E2Fs have been detected at the endogeneous ARF promoter by chromatin immunoprecipitation assay and directly activate ARF transcription without association with DP-1. Since ARF transcription is not regulated by a cell cycle-dependent manner, the unphysiological level of E2F seems to be required to induce ARF. Some studies described the inverse correlation between pRb status and p16INK4a expression in tumor cells. However, the physiological relation remains to be elucidated in terms of the relationship between the pRb/E2Fs and INK4 pathway.

Myc Oncogene

►Myc Oncogene has the ability to control p16INK4a transcription in human cells. Myc protein binds to the promoter and first intron of human p16INK4a, which is in line with the reports that Myc upregulates p16INK4a transcription in human cells. However, Myc has little effect on p16INK4a expression in mouse cells.

In mouse cells, establishment of MEFs as continuously growing cell lines is normally accompanied

by loss of the p53 or p19ARF, which act in a common biochemical pathway. Myc rapidly activates ARF and p53 transcription in MEFs and triggers replicative crisis by inducing apoptosis. MEFs that survive Myc overexpression sustain p53 mutation or ARF loss during the process of establishment and become immortal. These observations are consistent with the cooperation of -Myc and Bmi1 in mouse lymphomagenesis, suggesting that the ARF/p53 pathway is a physiological safeguard system against Myc-induced oncogenic stresses.

Suppressors of p15INK4b/ARF/p16INK4a

AML1/ETO

►AML1/ETO chimeric protein results from the t(8;21) translocation in human ►acute myelogenous leukemia. AML1/ETO can bind directly to the ARF promoter regions as well as the POZ/BTB domain protein, ZBT7B. The translocation seems to convert the wild-type AML1 from being an inducer of ARF to a repressor.

p53

One of the most important tumor suppressors, *p53* seems to have a significant role in ARF transcriptional suppression because ARF is generally transcriptionally upregulated in ►p53-inactivated cells. However, the mechanism of the transcriptional repression of ARF by reintroduction of wild-type p53 into p53-null cells remains to be elucidated.

►*Polycomb* Proteins

Traditionally, cancer has been viewed as a genetic disease that is driven by the sequential acquisition of mutations, leading to the constitutive activation of proto-oncogenes and loss of function of tumor suppressor genes. However, it has become increasingly evident that tumor development also involves "►epigenetic changes" patterns of altered gene expression that are mediated by mechanisms that do not affect the primary DNA sequences. The *INK4b/ARF/INK4a* region is known to be regulated by not only the genetic alteration but also by epigenetic modifications.

►Polycomb group (PcG) genes were first identified in Drosophila as a group of genes required for maintenance of stable repression of Hox cluster genes during development. There are increasing lines of evidence that PcG proteins themselves affect cellular proliferation and replicative senescence. Targeted disruption of Bmi1, Mel18, rae28, and M33, which are members of the class II PcG complex, leads to proliferation defects in hematopoietic stem cells and mouse embryo fibroblasts, indicating that inactivation of these PcGs results in cell proliferation failure. Replicative senescence of MEFs derived from Bmi1-, M33-, and Phc2-null mice has been shown to be mediated by derepression of the central mediators of senescence signals, p19ARF and p16INK4a, which are encoded by the *INK4b/ARF/INK4a* region. The molecular mechanism underlying the transcriptional regulation of these genes by mammalian PcG complexes, however, has not yet been appropriately addressed except for physical interactions of Bmi1 and Phc2 gene products to *ARF* and *INK4a* genomic regions.

Future Directions

The significant role of the INK4b/ARF/INK4a region and its products, p15INK4b/p14(p19)ARF/p16INK4a, in suppression of tumor development is well-established. However, regulatory mechanisms controlling these genes remain to be elucidated. The entire genomic region, which covers the genomic region from p15INK4b to p16INK4a (Fig. 1), is frequently deleted in a wide spectrum of tumors as described previously. However, the precise molecular mechanism of the biallelic gene deletion has not been addressed previously. In mouse MEFs, c-Myc induces the accumulation of p19ARF/p53 at the transcriptional level and has a significant proportion of the cells undergo apoptosis, whereas Myc-induced cell cycle accelerators, e.g., cyclin E, cyclin A, and CDC25A, are upregulated. In the face of Myc overexpression, there was a strong selective advantage for cells that sustained p53 mutations or the INK4b/ARF/INK4a deletion, and once such variants emerged, these soon predominated and were able to continuously proliferate. These results suggest that genes encoded by the *INK4b/ARF/INK4a* region are deleted by oncogene-derived stress-induced unknown molecular mechanisms and the safe guard machineries-broken cells continuously proliferate by oncogene-derived stimulations. The precise mechanisms of the locus deletion should be addressed to understand the immortalization and malignant transformation of normal cells.

A particularly important recent finding with regard to the *INK4b/ARF/INK4a* regulation was a coordination of transcription at the locus and DNA replication. The coordination between silencing of the locus and DNA replication was reported. In detail, a DNA replication origin in close proximity to the locus appears to transcriptionally repress p15INK4b/ARF/p16INK4a expression in a CDC6-dependent manner. Obviously, these findings suggest a novel molecular connection between DNA replication and p15INK4b/ARF/p16INK4a transcription. Further analysis of the cooperation between this CDC6-dependent regulation and other known regulatory mechanisms of this locus, both of genetic and epigenetic regulations, seems to be worthwhile.

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Innate Immune System

Definition

Comprises the cells and mechanisms that defend the host from infection by other organisms or tumor growth, in an immediate and non-specific manner. The recognition of tumor cells by ►natural killer cells is part of an innate immune response and does not depend upon the adaptive immune system; it does not confer long-lasting or protective immunity to the host.

►Toll-Like Receptors
►Adaptive Immunity

Innate Immunity

Definition

Used together with the term ►adaptive immunity to describe the two functional parts of the immune system. The original strict separation of the two parts of the immune system is currently reconsidered because a deep and intimate interconnection and cross-talk between the humoral and cellular parts of innate and adaptive immunity does exist. Nevertheless, innate immunity describes the immediate (within hours to a few days) reaction of the immune system in response to a given challenge. Typically involves, but is not limited to, (antimicrobial) phagocytes and substances, natural killer cells, certain cytokines and the complement system. Innate immune cells recognize common molecular patterns on infectious agents, cells infected with viruses, and transformed cells. Cells of the innate immune response include ►macrophages, ►dendritic cells, mast cells, neutrophils, eosinophils, natural killer (NK) cells, natural killer T (NKT) cells and γδ cells, and the cytokines they secrete on activation, as well as antimicrobicides that are made by specialized cells and tissues.

►Immunoediting
►Bacillus Calmette-Guérin
►DNA Vaccination

Innexins

Definition

Are the invertebrate gap junction proteins.

►Pannexins
►Gap-Junctions

iNOS

Definition

Inducible ►nitric oxide synthase.

►Nitric Oxide
►Nitric Oxide Synthase

Inosine

Definition

A nucleoside; the breakdown product of adenosine through the enzyme adenosine deaminase (ADA).

►Adenosine and Tumor Microenvironment

Inositol Lipids

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Definition

Are a class of ►phospholipids where inositol is the polar headgroup. The simplest inositol phospholipid is ►phosphatidylinositol (ptdlus). The inositol moiety can be phosphorylated at several different positions giving rise to a number of other molecular species.

Characteristics

Among different inositol lipids, the importance in ►transmembrane signaling and regulation of cell functions are best documented for PtdIns (4,5)P2 and PtdIns (3,4,5)P3. There are several ways in which these low abundance inositol lipids (less that 1% of membrane phospholipids) could provide a signaling link or fulfill other roles in different cellular processes.

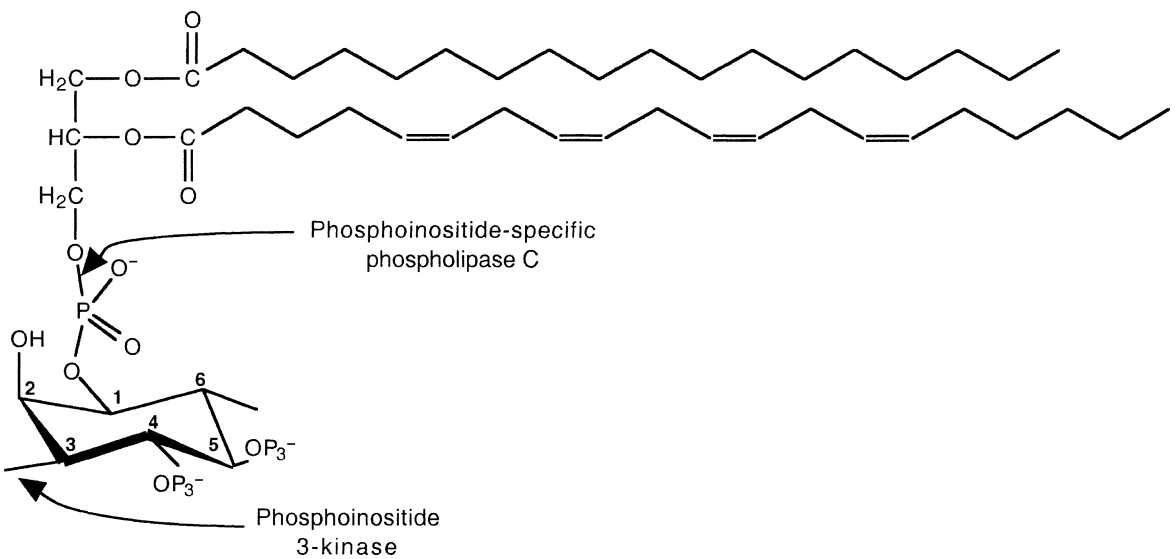
Hydrolysis of PtdIns(4,5)P2 to Generate Second Messenger Molecules

Hydrolysis of PtdIns(4,5)P2 occurs in response to a large number of extracellular signals and generates two ►second messenger molecules, inositol (1,4,5) trisphosphate (Ins(1,4,5)P3) and diacylglycerol (DAG) molecules. The reaction is catalyzed by phosphoinositide-specific ►phospholipase C (PI-PLC) (Fig. 1). There are several ►isoforms of this enzymes (PLCβ, PLCγ, PLCδ, and PLCε) linked to and activated by different cellular receptors. For example, PLCγ is regulated through tyrosine kinase receptors such as receptors for ►epidermal growth factor, ►fibroblast growth factor, and ►platelet-derived growth factor, while PLCε could be a novel target for ►RAS proteins.

The second messengers generated from PtdIns(4,5)P2 interact with specific intracellular targets and, in turn, cause their activation. Ins(1,4,5)P3 binds to specific receptors in the endoplasmic reticulum causing a release of calcium from this intracellular store into the cytoplasm. Membrane resident diacylglycerol (DAG) is required for activation of several isoforms of ►protein kinase C (PKC). These second messengers act as a common component in different signaling pathways, contributing to diverse cellular responses. Specificity of the pathways is provided at the level of a receptor and downstream components (e.g., calcium-regulated proteins and PKC substrates) present in a specific cell type or state.

Binding of PtdIns(4,5)P2 to Specific Proteins

In addition to its role as a precursor of Ins(1,4,5)P3 and DAG, PtdIns(4,5)P2 has emerged as a highly versatile signaling molecule in its own right. These other functions are mediated through direct binding of PtdIns(4,5)P2



Inositol Lipids. Figure 1 Structure of phosphatidylinositol (4,5) bisphosphate (PtdIns (4,5)P2) shows a typical phospholipid containing an inositol ring as a headgroup. The positions on the inositol ring are designated 1–6 and two phosphate groups are present at positions 4 and 5. Phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3) is generated in a phosphorylation reaction; the third phosphate group is added at position 3 of the inositol ring. Hydrolysis of PtdIns (4,5)P2 at the C-bond separates the hydrophobic part that contains two lipid chains, from water-soluble inositol that contains phosphates at the positions 1, 4, and 5.

to specific protein targets, and include fundamental processes in membrane trafficking and plasma membrane-cytoskeleton linkages. Many proteins that regulate actin cytoskeleton (e.g., gelsolin and profilin) and proteins involved in ►endocytosis (e.g., dynamin and AP-2 adaptor) bind PtdIns(4,5)P2. The binding involves different positively charged protein surfaces that in some proteins are present within the modular ►pleckstrin homology domain (PH-domain). The result of the binding could be a direct change in the protein function or a regulated membrane targeting. For example, the PH domain of phospholipase Cδ1 associates with membranes of many cell types, but after PLC stimulation and the reduction in PtdIns (4,5)P2 concentrations, it translocates to the cytoplasm. Concentration of PtdIns(4,5)P2 is not only regulated at the level of hydrolysis by PLC but also through regulation of several types of inositol lipid kinases and phosphatases.

Generation of PtdIns(3,4,5)P3 and Other 3-Phosphorylated Inositol Lipids and Their Binding to Specific Intracellular Targets

Inositol lipids phosphorylated at the 3-position of the inositol ring (PtdIns(3)P, PtdIns(3,4)P2, PtdIns (3,5)P2, and PtdIns(3,4,5)P3) are generated by ►phosphoinositide 3-kinase (►PI3-K). PI3-Ks are grouped into three classes on the bases of their structure and according to the inositol lipid they preferentially utilize as a substrate. For example, the class I PI3-Ks are receptor-regulated ►signal-transducer proteins that preferentially phosphorylate PtdIns(4,5)P2 in vivo and generate PtdIns(3,4,5)P3 (Fig. 1). Several target proteins for PtdIns(3,4,5)P3 and PtdIns(3,4)P2 have been described and they include protein kinases such as PKB/Akt, PDK1, and Btk. In the case of PKB/Akt, the direct binding to the PH domain (with high affinity and specificity toward 3-phosphorylated inositol lipids compared with more abundant PtdIns(4,5)P2) results in both membrane targeting and conformational changes that lead to phosphorylation and activation of this protein kinase. Activated kinases, in turn, phosphorylate and regulate downstream targets and thus propagate the signal. The 3-phosphorylated inositol lipids also participate in diverse cellular functions including cell survival, proliferation, migration, and vesicle budding. In addition to regulation of PI3-K, the levels of PtdIns (3,4,5)P3 are also controlled by a 3-phosphatase.

Clinical Relevance

There is considerable experimental evidence that the key enzymes involved in the control of inositol lipids, ►PI-PLC and ►PI3-K, play an important role in processes critical for tumor development and spreading, including cell proliferation, survival, and ►migration. However, oncogenic or constitutively active mutants of either PI-PLC or PI3-K have not yet been isolated from human tumors. In the case of PI3-K, an oncogenic form (v-p3k) of the class I PI3-K has been isolated from a chicken ►retrovirus that causes ►hemangiosarcomas. Another oncogenic form of class I PI3-K (mutation in the regulatory subunit) has been isolated from transformed murine lymphoid cells. Nonetheless, the importance of the control of inositol lipid levels in human cancers have been emphasized by the findings that the tumor suppressor protein ►PTEN is a 3-phosphatase that dephosphorylates PtdIns(3,4,5)P3. The *PTEN* gene is deleted or mutated in a wide variety of human cancers. Many human tumors have also been found to express increased levels of PI-PLC or PI3-K.

The role of a signaling protein in generation and spreading of a malignant tumor is not limited to its function as an oncogene or a tumor suppressor gene. For example, it has been documented that the activation of PLCγ is a rate limiting step in breast and prostate tumors that overexpress growth factor receptors. This type of tumor is associated with a poor prognosis. PLCγ seems to be required for cell migration but not for proliferation, and the motility and invasiveness of cancer cells are strongly inhibited either after treatment with a chemical PLC inhibitor (U73 122) or in the presence of molecular inhibitors.

►Polyphosphoinositides

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Inositol Polyphosphates

Definition

IPs; Inositol derivatives with various numbers of inorganic phosphate groups attached at each of the carbon atoms.

►Pentakisphosphate

Inositol Tetrakisphosphate

Definition

Inositol Polyphosphate (IP) containing four phosphate groups within the inositol ring.

►Pentakisphosphate

Inositol 1,4,5-trisphosphate

Definition

Natural compound containing three phosphate groups at the 1, 4 and 5 positions within the inositol ring. Its production is a common step seen in cell signaling.

►Pentakisphosphate

Inositoltrisphosphate

Definition

IP3; is generated by ►phospholipase C from phosphatidylinositol and is instrumental in Ca^{2+} mobilization required in signal transduction.

►Lipid Mediators

Insertional Mutagenesis

Definition

Is the alteration of a gene by integration of a foreign, often exogenous, DNA sequence. For instance, a virus DNA can integrate into a gene or in the vicinity of a gene.

►Retroviral Insertional Mutagenesis

Insulator

Definition

Is a DNA sequence that acts as a barrier to the influence of neighboring genes.

►CCCTC-Binding Factor

Insulin

Definition

Is a natural hormone produced in the pancreas by the beta cells of the ►islets of Langerhans; controls the level of the sugar glucose in the blood. Insulin permits cells to use glucose for energy. Cells cannot utilize glucose without insulin. People who are Type 1 ►Diabetes mellitus must use manufactured insulin, usually in an injectable form, to replace the natural insulin that is no longer produced by their body (for instance as the result of beta-cell degeneration). People with Type 2 sometimes need to use insulin when their cells become too resistant to the insulin that they produce naturally and when oral medications are no longer working.

Insulin Receptor

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Definition

IR; Is a phylogenetically ancient ►receptor tyrosine kinase protein embedded in the ►plasma membrane of virtually all cells. When the peptide hormone ►insulin binds to the IR, the receptor becomes activated and induces a cascade of intracellular events that will lead to several metabolic and growth promoting effects.

Characteristics

The IR belongs to the tyrosine kinase growth factor receptor family and functions as an enzyme that transfers phosphate groups from ►ATP to tyrosine

residues on intracellular target proteins. The IR consists of two identical extracellular alpha subunits (130 kDa) that house insulin binding domains, and two transmembrane beta subunits (95 kDa) that contain ligand-activated tyrosine kinase activity in their intracellular domains (Fig. 1). When insulin binds to the alpha subunits, the receptor is first activated by tyrosine autophosphorylation, and then the IR tyrosine kinase phosphorylates various intracellular effector molecules (e.g. ►IRS proteins and ►Shc) which in turn alters their activity, thereby generating a biological response. The IR exists as two splice variant isoforms: the IR-B isoform that is responsible for signaling metabolic responses involved mainly in the regulation of glucose uptake and metabolism by increasing glucose transporter (►Glut4) molecules on the plasma membrane of the insulin-responsive tissues muscle, liver, and fat, and the IR-A isoform that is capable of binding ►IGF-2 with high affinity and signals predominantly mitogenic responses. As a consequence of these cellular activities, abnormalities of IR expression and/or function can facilitate the development of several metabolic and neoplastic disorders in humans as well as in animal models.

Regulation

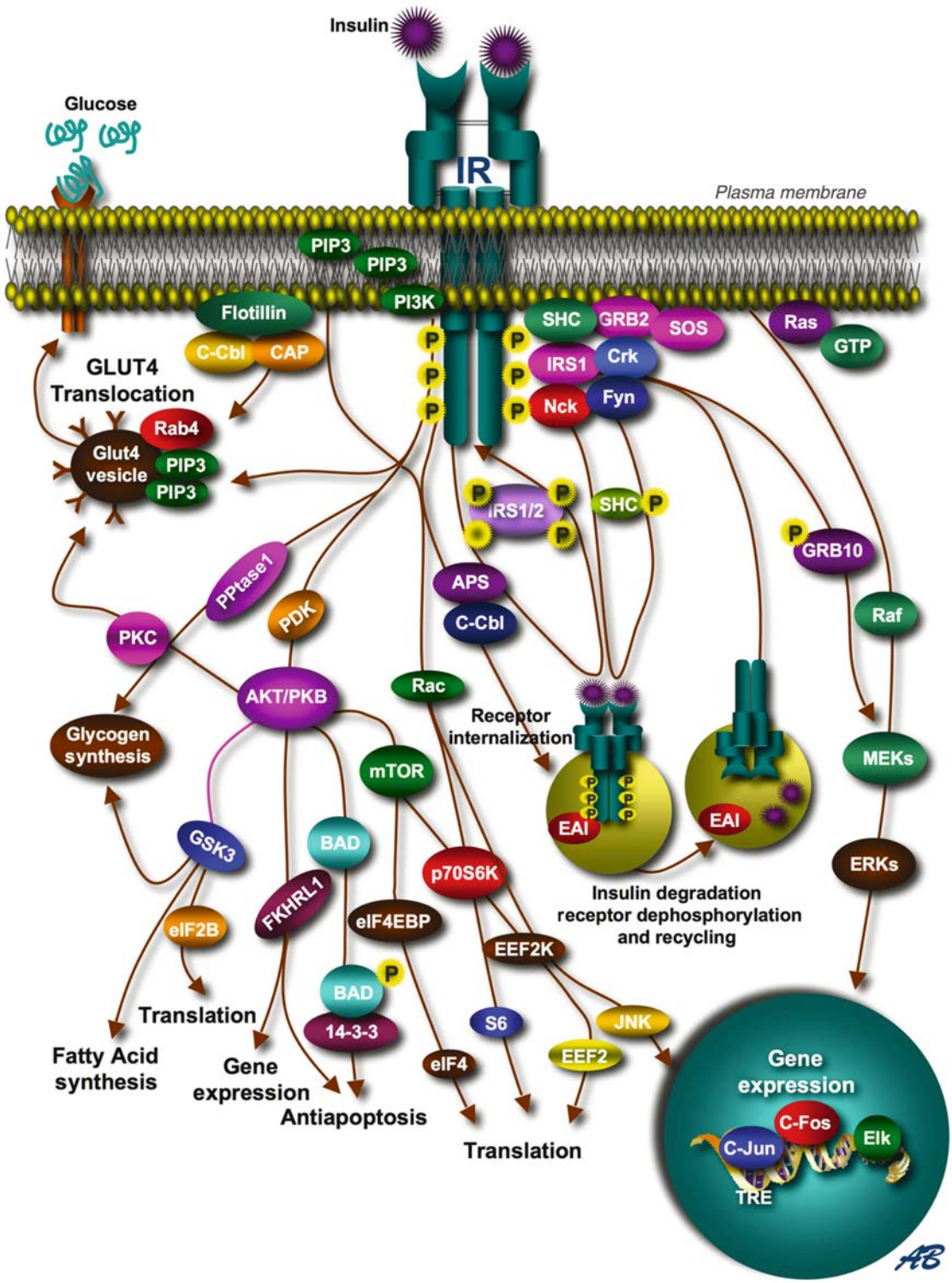
Gene expression in eukaryotic cells is controlled by nuclear regulatory proteins (*trans*-acting factors) that modulate the transcription of genes by binding to specific *cis*-acting transcriptional elements in the promoter of target genes. The IR gene promoter extends over 1,800 bases 5′ upstream from the IR gene ATG codon, contains a series of GGGCGG repeats that are putative binding sites for the mammalian transcription factor Sp1, and has neither a ►TATA box nor a CAAT box, reflecting the common features for the promoters of constitutively expressed genes (so-called housekeeping genes). Like other housekeeping promoters, the IR gene promoter confers a basal level of transcriptional activity common to all cells, whereas significantly higher transcriptional activity is induced in the muscle, liver, and fat, at which levels the IR has been shown to be under the regulation of hormones, metabolites, and differentiation. Promoters of genes that are activated in a tissue specific manner are often regulated by a combination of tissue specific and ubiquitous transcription factors, where the ubiquitous element facilitates or enhances the action of one or more tissue-specific transcription factors. The molecular mechanisms regulating IR gene expression are being elucidated and evidence has been provided showing that the architectural transcription factor ►HMGA1 is required for proper transcription of the IR gene in cells expressing IRs. HMGA1 acts on the IR gene promoter as an element necessary for the formation of a transcriptionally active multiprotein–DNA complex involving, in addition to the HMGA1 protein, the ubiquitously expressed transcription factor Sp1 and the CCAAT-enhancer binding protein beta (C/EBP-beta). By potentiating the recruitment and binding of Sp1 and C/EBP-beta to the IR promoter, HMGA1 greatly enhances the transcriptional activities of these factors in the context of the IR gene. Conversely, repression of HMGA1 function in cells and tissues adversely affects transactivation of the IR gene promoter by Sp1 and C/EBP-beta, and considerably reduces IR protein expression.

Clinical Relevance

The IR is of major importance in certain states of ►insulin resistance in humans, in which abnormalities of the receptor may lead to defective transmembrane signaling. In this respect, dysfunctional IR signaling is implicated in certain common dysmetabolic disorders, including ►obesity, type 1 and type 2 ►diabetes, the dysmetabolic syndrome X, and the polycystic ovary syndrome (PCOS). Also, clinical syndromes due to mutations in the IR gene have been identified in patients with genetic forms of severe insulin resistance (i.e. leprechaunism, type A insulin resistance, and the Rabson–Mendenhall syndrome). Many of these patients have point mutations in the coding sequence of the IR gene, leading to reduced or absent IR expression in target tissues. Recently, defects in IR gene regulation have been reported in individuals with insulin resistance and type 2 diabetes, in which the generation of IR mRNA was considerably impaired, although the IR genes were normal. In these individuals, cell-surface IRs were decreased and the expression of HMGA1 was markedly reduced.

Even though it is an open question whether IR plays a critical role in aging and longevity in mammals, disturbance of the neuronal IR seems to be of pathogenetic relevance in human Alzheimer's disease and depressive disorders, suggesting a neurotrophic role of IR in the brain. According to recent studies, IR in the brain begins to disappear early in Alzheimer's and continue to decline as the disease progresses. It has been shown that stimulation in the brain of a receptor that mediates insulin responses can halt or diminish the neurodegeneration of Alzheimer's disease. Also, a high prevalence of insulin resistance has been reported in patients with depression, and an increased risk of cognitive decline has been found in women with insulin-resistant PCOS.

A relation between IR and cancer has been established following the observation that overexpression of functional IRs can occur in human ►breast cancer and other epithelial tumors including ovarian and colon cancer, in which the IR may exert its oncogenic potential via abnormal stimulation of multiple cellular signaling cascades, enhancing growth factor-dependent proliferation and/or by directly affecting cell metabolism.



Insulin Receptor. Figure 1 In the absence of insulin, most of the IRs in the plasma membrane are in a non-tyrosine phosphorylated inactive state, and only a very small proportion of receptors are lightly phosphorylated and subjected to constitutive ►endocytosis and recycling. Upon binding of insulin, the IR undergoes autophosphorylation which enables the receptor to have a kinase activity and phosphorylates various cytoplasmic substrates, such as IRSs. From this point, signaling proceeds via a variety of signaling pathways (i.e. PI3K signaling pathway, Ras and MAP kinase cascade) that are responsible for the metabolic, growth-promoting and mitogenic effects of insulin.

An explanation for increased IR expression in epithelial tumors has been recently provided for several human breast cancers, in which overexpression of the transcription factor ►AP2-alpha accounts for increased IR expression in neoplastic breast tissue. In these cases, it has been demonstrated that transactivation of the IR gene by AP-2 alpha needs direct physical association of AP-2 with HMGA1 and Sp1, which represents a fundamental prerequisite for AP-2 alpha to activate IR gene transcription in neoplastic breast tissue. Epidemiological and clinical evidence points to a link between insulin resistant syndromes such as obesity and type 2 diabetes and cancer of the colon, liver, pancreas, breast and endometrium. The mechanistic link between insulin resistance and cancer is unknown, but constitutive activation of the tyrosine kinase activity of IR and related downstream signaling pathways by chronic sustained hyperinsulinemia (a hallmark of insulin resistance) in these clinical syndromes appears to have a role in the neoplastic transformation process.

Thus, the IR plays a major role in the pathophysiology of a wide range of metabolic, neurodegenerative and proliferative disorders in humans. Selective modulation of IR expression and/or function may represent a useful therapeutic strategy for these diseases. Also, measures to decrease insulin resistance in insulin resistant patients may offer a general approach to prevention of cancer in these predisposed individuals.

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Insulin Receptor Substrate Proteins

Definition

IRS proteins; Large intracellular ►docking proteins through which the ►insulin receptor and IGF-1Recepror propagate their signals. Receptor tyrosine phosphorylation of IRS proteins yields sites for Src-homology 2 (SH2) domain containing proteins to bind and transmit signals downstream.

►Receptor Cross-Talk
►Insulin Receptor
►SH2/SH3 Domains

Insulin Resistance

Definition

Occurs when the body does not respond properly to ►insulin, a hormone made by the pancreas. It is characterized by the diminished ability of cells to respond to the action of insulin in transporting glucose from the bloodstream into muscle and other tissues.

►Adiponectin
►Diabetes

Insulin-like Growth Factor Binding Proteins

Definition

IGFBPs are a family of secreted proteins that bind the ►insulin-like growth factors, IGF-I and IGF-II, with affinities comparable to their respective receptors. To date, six insulin-like growth-factor-binding proteins (IGFBP-1 to IGFBP-6) have been identified.

►Kallikreins
►Diabetes

Insulin-like Growth Factors

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Definition

The insulin-like growth factors (IGF-I and IGF-II) are structurally related molecules that play essential roles in the regulation of cell survival, growth, proliferation,

differentiation and metabolism. The IGF family is comprised of (i) ligands (IGF-I, IGF-II and insulin), (ii) six well characterized high affinity binding proteins (IGFBP-1 through -6), (iii) IGFBP proteases and (iv) cell surface receptors that mediate the biological functions of IGFs. These transmembrane receptors include the IGF-I receptor (IGF-IR), IGF-II/mannose 6-phosphate receptor (IGF-II/M6PR), ►insulin receptor (IR) and insulin receptor-related receptor (IRR). In many tumor cells the IGF-IR is often upregulated and/or hyperactivated. Furthermore, increased circulating IGF-I levels are considered a significant risk factor for the development of various types of cancers. Although the oncogenic role of IGFs (i.e. their ability to initiate ►carcinogenesis) is still under the debate, numerous lines of evidence suggest that these powerful growth factors enhance tumor growth and ►progression.

Characteristics

IGFs

The insulin-like growth factors (IGF-I and IGF-II) are ubiquitously expressed growth factors that are structurally related to insulin. However, in contrast to insulin and other peptide hormones, they are not stored within cells of a specific tissue but are produced by numerous cell types in the body and circulate in approximately 1,000-fold higher concentrations than most other known peptide hormones. These properties suggest more universal functions of the IGFs in the body compared to the more specific metabolic role of insulin. The IGFs are critical in a broad range of functions during pre- and postnatal life. In adult tissues, IGFs are important trophic factors that support normal differentiated functions of various tissues and prevent programmed cell death (►apoptosis). IGF-I is known as a major regulator of postnatal growth. Most of the circulating IGF-I is produced by the liver, although other tissues are capable of synthesizing this peptide locally. Thus, IGF-I has characteristics of both a circulating hormone and a tissue growth factor. In contrast to IGF-I, IGF-II plays a fundamental role in embryonic and fetal growth, whereas due to ►imprinting of the *Igf2* gene, its role in postnatal period of life is less important, especially in rodents.

IGF Receptors

Most of the actions of both IGF-I and IGF-II are mediated *via* the IGF-IR, which is expressed by virtually all cell types except adult hepatocytes. The IGF-IR and IR belong to the large family of ►receptor tyrosine kinases. The two receptors are structurally related and are composed of two α-subunits localized entirely extracellularly and two β-subunits spanning the membrane and localized primarily intracellularly. Both subunits are linked together by disulfide bonds. They assemble a $\alpha_2\beta_2$-configuration with ligand binding primarily mediated by the α-subunits, which form a binding pocket. Binding of the ligand to the α-subunit leads to conformational changes resulting in stimulation of the β-subunit intrinsic tyrosine kinase activity with subsequent multisite phosphorylation of the β-subunit. The current concept is that insulin and the IGFs act as bivalent ligands, both IGF-IR and IR are capable of binding insulin and IGF-I or -II, though each receptor binds its own ligand with a 100–1,000-fold higher affinity than the heterologous peptide. In cells expressing both receptor genes, hybrid insulin/IGF-I receptors can form. The hybrid receptors have ligand specificity profiles more comparable to the IGF-IR than to the IR since they bind IGF-I with an affinity similar to the IGF-IR, but insulin with a much lower affinity. Moreover, the IR is also responsible for some of the mitogenic actions of IGF-II. IGF-II is an agonist of the A isoform of the IR. This splice variant of the IR is expressed at high levels in fetal and neoplastic tissues. IRR and hybrid IR/IRR have not yet been extensively studied, and their ability to bind all the different insulin-like peptides as well as their biological significance remains unclear.

The IGF-II/M6PR is structurally distinct from the IGF-IR and is actually identical to the cation-independent mannose 6-phosphate receptor, which lacks tyrosine kinase activity and is not considered to have any role in IGF ►signal transduction. The IGF-II/M6PR functions as a scavenger receptor and is involved in uptake and degradation of IGF-II. The IGF-II/M6PR is strongly expressed during tissue differentiation and organogenesis, and high levels of the IGF-II/M6PR were found in fetal tissue, which decline in late gestation and in the early postnatal period due to genomic imprinting.

IGFBPs

Unlike insulin, the IGFs are present in the circulation and throughout the extracellular compartments almost entirely bound to a family of multifunctional, structurally related, high affinity IGF-binding proteins (IGFBPs), which can modulate biological effects of the IGFs. To date, six IGFBPs with high affinity have been cloned and sequenced. All share structural homology with each other and specifically bind the IGFs. They differ in molecular mass, binding affinities for the IGFs, posttranslational modifications such as phosphorylation and glycosylation as well as susceptibility to proteolysis. The IGFBPs act as carrier proteins in plasma, control the efflux of the IGFs from the vascular space, and prolong half-lives of the IGFs. They regulate metabolic clearance and tissue- and cell-specific localization of the IGFs thereby modulating

their biological actions in a negative or a positive manner. Finally, some IGFBPs also have intrinsic bioactivities that are independent of the IGFs (Fig. 1).

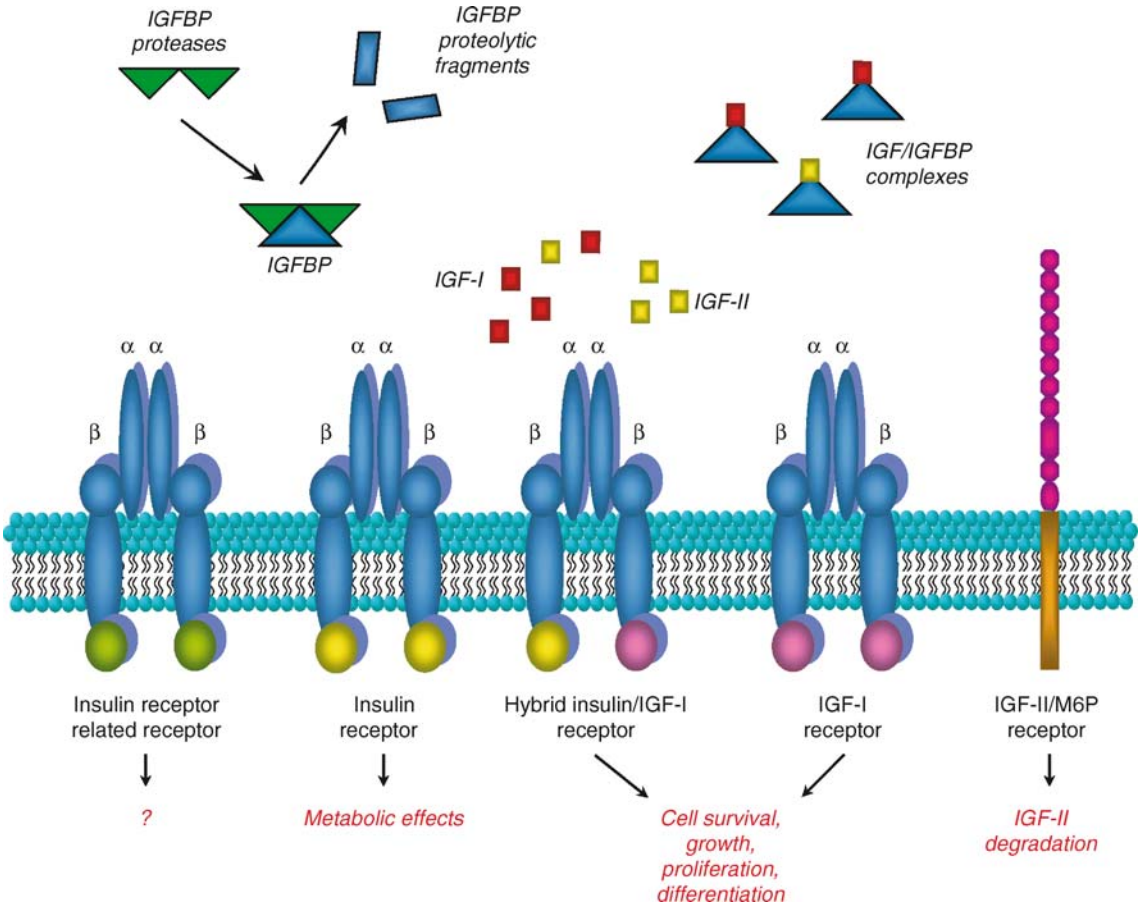
Mechanisms

The biological effects of IGFs are mainly mediated by the IGF-IR and two principal signaling pathways including ►mitogen-activated protein kinase (MAPK) pathway that plays a pivotal role in cell growth and proliferation, and ►phosphatidylinositol-triphosphate kinase (PI3K) pathway, which is mainly involved in mediating the metabolic, antiapoptotic and other more differentiated effects of IGF-I. Upon ligand binding and receptor autophosphorylation, the IGF-IR recruits and phosphorylates several adaptor proteins including ►Shc and members of the insulin receptor substrate (IRS) family of proteins. They bring together and coordinate the activity of other signaling intermediates, finally resulting in activation of the ►MAPK and PI3K pathways. Typically, the MAPK pathway is initiated by the recruitment of growth factor receptor bound 2 protein (Grb2) that *via* the guanine nucleotide exchange factor Son of Sevenless (SOS) stimulate the activity of the GTPases ►Ras and Rac, which, in turn, through the sequential phosphorylation of certain kinases finally lead to activation of terminal MAP kinases ERK1/2, ►JNK and p38 kinase. Although JNK and p38 kinase are primarily activated by environmental stressors, several lines of evidence suggest that they can also be activated in response to IGFs. The activated MAP kinases phosphorylate several important cytoplasmic substrates and also translocate to the nucleus where they phosphorylate transcription factors leading to immediate-early gene induction followed by progression of the cell cycle. Alternatively, signal transduction through the PI3K pathway results in the activation of protein kinase B also known as ►Akt, which is known to block apoptosis by phosphorylating numerous cellular substrates such as Bad, GSK-3, Foxo, Mdm2, CREB, IKK, caspase-9, p21 and p27. The PI3K pathway also activates p70S6 kinase involved in regulation of ribosome biogenesis as well as some isoforms of ►protein kinase C, which are capable of potentiating signal transduction through the MAPK pathway. Thus, IGFs induce cell proliferation both by enhancing cell cycle progression and by inhibiting apoptosis. Furthermore, evidence for both direct and indirect interaction between the IGF-IR and other growth regulatory signals has been demonstrated, thereby expanding the traditional view of highly specific IGF-IR/IGF interactions and rendering the IGF-IR central in cellular response. For instance, in many cell types IGF-IR and ►epidermal growth factor receptor family members physically interact and transphosphorylate each other. ►Estrogen receptor activation augments the IGF-I response in estrogen receptor-positive MCF-7 mammary carcinoma cells at multiple levels. Moreover, estrogens enhance the tyrosine phosphorylation of the IGF-IR and IRS-1 and eventually increase expression of cyclins and reduce the level of cdk inhibitors (Fig. 2).

Altered Expression of the IGF System Components in Tumor Cells

The expression of the components of the IGF system is often altered in malignant cells. In certain tumors, the ►imprinting of the *Igf2* gene is lost, and this results in increased IGF-II gene expression. In general, IGF-II is more commonly expressed by tumors than IGF-I, although increased IGF-I expression has been found in numerous tumors as well.

The IGF-IR is often upregulated or hyperactivated in tumor cells. Expression of the IGF-IR is regulated by tumor suppressors and growth factors in a negative and positive manner, respectively. Tumor suppressors such as ►Wilms Tumor-1 (WT1) and ►p53 bind to the *Igf1r* gene promoter and inhibit receptor gene expression. Mutations of these genes occur in various tumors and paradoxically enhance the activity of the *Igf1r* gene promoter. This explains the upregulation of the IGF-IR gene expression in Wilms tumor (a pediatric kidney tumor) and ►colon cancer, which is often accompanied by p53 mutations. Both WT1 and p53 also inhibit IGF-II gene expression. By analogy with the IGF-I receptor, IGF-II gene expression is increased when *WT1* and *p53* genes are mutated. Thus, the autocrine IGF-IR/IGF-II loop is turned on under these circumstances. Basic ►fibroblast growth factor and ►platelet-derived growth factor are capable of enhancing the IGF-IR gene expression. Since tumors often express these and other growth factors, this could also upregulate the IGF-IR gene expression. Certain ►oncogenes can also regulate the IGF-IR at posttranslational level. For instance, ►Src augments the phosphorylation state of the IGF-IR, thereby increasing its kinase activity. Neoplastic growth can be also enhanced by injections of recombinant human IGF-I into mice. In this case, the latency period is shortened and tumor growth is accelerated. This response is particularly enhanced in tumors with higher levels of IGF-IR expression. In contrast to the IGF-IR, the IGF-II/M6PR expression is often decreased or lost in tumor cells. The IGF-II/M6PR possesses properties of a ►tumor suppressor gene. Tumor cell growth is inhibited when the IGF-II/M6PR expression is restored and is increased when its expression is reduced. In addition to the IGFs and their receptors, the IGFBP expression is also altered in tumor cells. Various IGFBPs are expressed by numerous tumors and often in different combinations. For example, estrogen receptor-positive breast cancer cells release IGFBP-2, whereas estrogen receptor-negative breast tumors

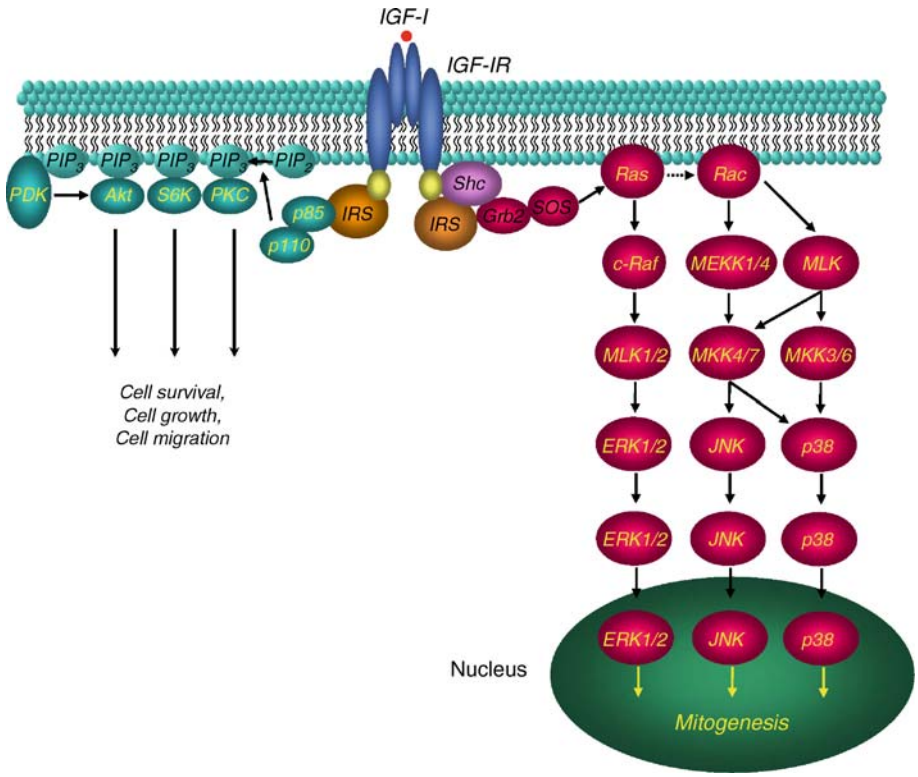


Insulin-like Growth Factors. Figure 1 The IGF family is comprised of (i) ligands (IGF-I, IGF-II and insulin), (ii) IGF-binding proteins (IGFBPs), (iii) IGFBP proteases and (iv) cell surface receptors. IGFBPs act as carrier proteins in plasma, control the efflux of the IGFs from the vascular space, prolong half-lives of the IGFs, regulate their metabolic clearance, provide tissue- and cell-specific localization of the IGFs, modulate biological actions of the IGFs, and some also have intrinsic bioactivities that are IGF-independent. The IGFs can be released from the IGF/IGFBP complexes by the action of IGFBP proteases. The insulin receptor (IR), insulin-like growth factor I receptor (IGF-IR) and insulin receptor-related receptor (IRR) are heterotetrameric complexes composed of extracellular α-subunits that bind the ligands, and β-subunits that anchor the receptor in the membrane and contain tyrosine kinase activity in their cytoplasmic domains. Hybrids consist of a hemireceptor from both IR and IGF-IR. The IGF-II/M6PR is not structurally related to the IGF-IR and IR or the IRR, having a short cytoplasmic tail and no tyrosine kinase activity. IR is responsible for metabolic effects, whereas IGF-IR and hybrid IR/IGF-IR for cell survival, growth, proliferation and differentiation. The insulin-like growth factor II/mannose 6-phosphate receptor (IGF-II/M6PR) functions as scavenger receptor and is responsible for uptake and degradation of the IGFs. This receptor is not considered to have any role in IGF signaling.

release IGFBP-1 and -3. The IGFBP production can be altered by growth factors, steroid hormones, cytokines, and these changes in IGFBP levels may alter biological effects of the IGFs on tumor growth and progression. Interestingly, wild type p53 induces the expression of IGFBP-3 that seems to be critical for the inhibitory function of p53 on cell growth. Furthermore, enhanced IGFBP proteolysis is thought to contribute to carcinogenesis. Numerous IGFBP proteases produced by tumor cells mediate release of the IGFs from the IGFBP/IGF complexes that eventually leads to increased IGF-IR stimulation. For instance, prostate cancer cells secrete ►prostate-specific antigen (PSA), which exerts IGFBP-3 proteolytic activity thereby enhancing the local bioavailability of free IGFs.

Syndrome of Hypoglycemia

An emerging clinical syndrome is tumor-induced hypoglycemia. This phenomenon is often seen in terminally-ill, poorly nourished patients. In addition, it can be also observed in patients with large mesenchymal tumors in the abdomen or thorax that



Insulin-like Growth Factors. Figure 2 Signal transduction cascades initiated by the IGF-IR. Activation of the IGF-IR kinase results in receptor autophosphorylation and tyrosine phosphorylation of several docking proteins such as Shc and insulin receptor substrate (IRS) proteins. Once activated, IRS molecules recruit Src homology 2 (SH2)-domain containing molecules such as Grb2 and the p85 subunit of phosphatidylinositol-3′-kinase (PI3-K). Grb2 *via* SOS stimulates the activity of the GTPases Ras and Rac, which through the phosphorylation of numerous kinases finally lead to activation of terminal MAP kinases ERK, JNK and p38 kinase. Activated MAP kinases are translocated to the nucleus where they activate a variety of transcription factors. Alternatively, the binding of the p85 and p110 subunit of PI3-K to IRS proteins generates phospholipids that participate in the activation of 3-phosphoinositide-dependent kinase (PDK) 1 and 2. In turn, they phosphorylate several targets involved in the regulation of different biological processes including glucose transport, protein synthesis, glycogen synthesis, cell proliferation and cell survival.

secrete large quantities of IGF-II. In these patients, IGF-II is not fully processed, and therefore it is poorly bound to the circulating IGFBPs. This allows IGF-II to interact more readily with insulin receptors thereby causing hypoglycemia. Clinically, tumor-induced hypoglycemia can be diagnosed in cancer patients that have normal or elevated circulating IGF-II levels, whereas their insulin, growth hormone (GH), IGF-I and IGFBP-3 levels are suppressed. These patients usually have a poor prognosis. Surgical excision and ►chemotherapy or ►radiation therapy-induced reduction of tumor size is ►palliative, and GH and corticosteroid therapy provides symptomatic relief.

Clinical Aspects

An increasing body of evidence suggests that the IGF system is a promising target for adjuvant anticancer therapy. If chemotherapy inadequately ablates tumor cells, then blockade of the proliferative and antiapoptotic effects of the IGFs may be helpful. The IGF system could potentially be targeted at various levels. Reduction of circulating IGF-I levels can be achieved by GHRH antagonists or somatostatins as well as GH receptor antagonists. Application of inactive IGF molecules, small-molecule competitive binding antagonists or soluble IGF-I receptors may inhibit binding of endogenous IGFs to the receptor thereby abrogating their tumor-promoting effects. The IGF-IR represents another attractive therapeutic target. Approaches that are currently being tested in preclinical and clinical studies include the use of blocking ►monoclonal antibodies directed against the extracellular portion of the IGF-IR and ►small molecule drugs that inhibit the tyrosine kinase activity of the IGF-IR. Application of ►RNA interference and ►antisense therapy to reduce the IGF-IR expression, as well as overexpression of altered or

truncated IGF-IR that acts in a ►dominant-negative manner, represent additional approaches that have been effective in laboratory studies.

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Insulinoma

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Synonyms

β-Cell tumor of the islets

Definition

Insulinomas are functioning ►insulin producing tumors of the pancreatic ►islets of Langerhans, resulting in hypoglycemia.

Characteristics

Insulinomas arise from the β-Cell of the pancreatic islets. The nonregulated secretion of insulin from these tumor cells into the blood stream results in fasting hypoglycemia. Insulinomas are relatively rare, with approximately four cases per million person-years. However, they are the most common tumor of the pancreatic islets. Insulinomas may appear at any age, but the majority appears in the fifth decade. Insulinomas are evenly distributed along the pancreas. Insulinomas, associated with ►multiple endocrine neoplasia type 1 (MEN1), tend to appear one decade earlier. Insulinomas are usually solitary and their localization is even along the pancreas, exceptions are those associated with MEN1, where multiple tumors are the rule.

Diagnosis

The presence of ►Whipple Triad (hypoglycemia and neuroglycopenic symptoms that are corrected by the administration of carbohydrate) is the hallmark of the diagnosis of insulinoma. Fasting, therefore, is the major maneuver used in the diagnosis of insulinoma and has two important purposes. The first goal is to document hypoglycemia and its relationship to the patient's symptoms, and the second is to demonstrate inappropriate insulin concentrations in the face of hypoglycemia. The prolonged (48–72 h) fast is the gold standard for the diagnosis of hypoglycemia. This study should be conducted under supervised conditions (i.e., while hospitalized). Diagnosis of insulinoma has been centered on the 72-h fast that was introduced long before to measure insulin or insulin-related components. Now the insulin and proinsulin measurements are widely available, all of the necessary information from a fast can be derived in the first 48 h. Thus, the 48-h fast has become the new standard. The diagnosis is based on detectable insulin levels (≥6 μU/ml), detectable C-peptide, and elevated proinsulin levels, when the patient has symptoms of hypoglycemia and glucose levels <45 mg/dl (2.49 mMol). At the end of the study, a sulfonylurea screen must be obtained to rule out factitious hypoglycemia.

Other diagnostic tests that may be used are the C-peptide suppression test and the ►tolbutamide intravenous test. The C-peptide suppression test is based on the observation that exogenous insulin suppresses C-peptide more in normal islets compared to insulinoma islets. The tolbutamide intravenous test is rarely used today due to the risk of hypoglycemia.

Differential Diagnosis

There are several conditions that may result in hypoglycemia, and thus are part of the differential diagnosis of hypoglycemia.

1. Inadvertent or intentional (factitious) administration of insulin secretagogues or insulin
2. Other medications such as salicylates, haloperidol, beta blockers, pentamidine, trimethoprin – sulfamethoxazole, quinine, acute steroid withdrawal
3. Significant medical conditions such as renal, hepatic and cardiac failure, severe malaria, sepsis and lactic acidosis
4. Neoplastic syndrome associated with large tumors, secondary to secretion of ►insulin like growth factor 2

While the later two conditions are usually clinically obvious, the first is challenging. A properly conducted fasting test will usually be allowed for proper diagnosis.

Treatment

Surgical removal of the tumor is the most effective treatment for insulinoma. Localization of the tumor

prior to surgery may be beneficial. Selective pancreatic angiography with calcium stimulation allows localization of the tumor to the head, body, or tail of the pancreas. The study is based on the ability of calcium, injected into one of the feeding blood vessels of the pancreas, to cause the insulinoma to secrete insulin into the venous drainage of the pancreas. Imaging techniques such as ►magnetic resonance imaging (MRI), ►computed tomography (CT), and ultrasound (US) lack the sensitivity to detect tumors. Finally, the use of intraoperative ultrasonography allows for detection of virtually all tumors. The ability to detect most tumors has made the old practice of blind distal pancreatectomy obsolete.

Medical treatment is limited to diazoxide, calcium channel blockers and ►somatostatin. Medical treatment can be used to control symptoms while the patient is awaiting surgery or when a patient refuses or is not a candidate for surgical intervention.

Based on the experience at the National Institutes of Health, Bethesda MD, the following steps are recommended after the biochemical diagnosis of an insulinoma:

1. Perform an abdominal US and abdominal CT to evaluate the patient's anatomy and to rule out a metastatic disease.
2. Perform a hepatic venous sampling after Ca^{2+} stimulation. While some centers view this step as excessive and costly, because of the nature of the operation, we believe that accurate preoperative localization is valuable.
3. Operation to be performed by a surgeon experienced with the use of intraoperative US or with the assistance of a radiologist.

Insulinoma is a rare tumor, and most internists, endocrinologist, and surgeons will have limited experience with this condition. Thus it is advisable to refer subjects suspected of having this tumor to a center with expertise diagnosing, localizing, and treating pancreatic endocrine tumors.

Malignant Insulinoma

Only 5–12% of the reported cases of insulinoma are malignant. Most patients with malignant insulinoma have lymph node or liver ►metastasis. The prognosis of these patients is relatively poor with a median survival period of ~2 years. Diazoxide therapy for control of hypoglycemia has had the greatest long-term benefit. Some patients have gastrointestinal intolerance to the drug, and in very high doses it may produce ►anorexia. All the other forms of therapy have either had no benefit or relatively short-term benefit and most, such as chemotherapy regimens (such as streptozotocin), are toxic and of little benefit. ►Embolization may have the greatest benefit next to diazoxide.

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Integral Membrane Protein

Definition

Synonym transmembrane or intrinsic membrane proteins. Proteins irreversibly associated with membrane through transmembrane-spanning regions.

►Membrane-Lipid Therapy

Integrated Positron Emission Tomography/Computed Tomography

►Positron Emission Tomography

Integrin

Definition

A family of ►cell adhesion receptors. alphabeta heterodimers of transmembrane proteins that recognize ►extracellular matrix or cell surface ligands. The ►RGD motif is a prototype of the integrin-binding motifs in ligands. Integrins transduce intracellular signals upon ligand binding and are regulated by intracellular signals. Integrins are heterodimeric proteins (alpha and beta subunit) and facilitate cell attachment to the extracellular

matrix. About 18 different alpha and 8 beta subunits of integrins have been characterized. Combinations between these subunits provide a variety of integrins with unique functional properties. Integrin mediated signaling bridges communication between the extracellular matrix and intracellular microfilaments involved cell migration. Integrins bind ►extracellular matrix proteins, including laminins, on their extracellular domain and bind cytoskeletal elements, such as ►actin, on their cytoplasmic domain; integrins then provide a physical link between the extracellular and intracellular skeletons. Integrin signaling affects numerous biological outcomes including cell ►adhesion, ►migration, and ►invasion.

►Fibrinogen
►Integrin Signaling
►Tumor-Endothelial Cross-talk

β3 Integrin

Definition

Second subunit of the αvβ3 integrin receptor that binds to vitronectin and is expressed on endothelial cells during ►neovascularization and on metastazing ►melanoma cells.

►CEA Gene Family

Integrin-Mediated Death

►Anoikis

Integrin Signaling

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Definition

Integrins comprise a family of cell surface receptors that bind ►extracellular matrix proteins and cell surface counterreceptors, thereby linking the cell's ►microenvironment to the ►cytoskeleton.

Characteristics

Virtually all cells of multicellular animals express integrins, and most cells of adult mammals constitutively express multiple different integrins. These cell surface receptors play an important role in mediating cell ►adhesion to adjacent connective tissue proteins. Integrins are transmembrane heterodimers that consist of noncovalently bound glycoprotein subunits. Each heterodimer is composed of a single α and a single β subunit, both of which participate in ligand recognition. A total of 18 α and β subunits have been identified, some of which have multiple splice variants, and these combine to form 24 distinct integrin heterodimers. Most integrins (with the notable exception of α6β4) have small cytoplasmic domains that are connected to the cytoskeleton by multiple linkage proteins. They generally lack catalytic activity but participate in ►signal transduction by linking to a variety of cytoplasmic molecules. Cytoskeletal proteins, signaling molecules, and adaptor proteins bind directly to integrin cytoplasmic domains, linking integrins to other proteins and creating elaborate networks underlying the plasma membrane. These protein complexes are capable of undergoing rapid formation and dissolution, allowing rapid changes in the organization of the cytoskeleton and modulation of multiple downstream signaling pathways. In addition to integrin cytoplasmic domains, the transmembrane and extracellular regions of integrins can also interact with a variety of proteins that regulate integrin function.

Expression of particular integrins is cell-type dependent. Epithelial cells, the most common cell type of adult malignancies, generally express several integrins that bind extracellular matrix proteins. These include α6β4 and α3β1 (receptors for laminin isoforms), α2β1 (a collagen receptor), and α5β1 (a receptor for ►fibronectin). In malignant epithelial cells, integrin expression is generally decreased compared to benign cells, although increased integrin expression occurs in some cancer cells. There is also an altered distribution of integrins in malignant cells compared to benign cells. For example, α6β4 is present at the basal surface of benign epithelial cells within hemidesmosomes but is expressed more diffusely in malignant epithelium in vivo and is localized to the leading edge of invading carcinoma cells in vitro. Integrin signaling to a great extent accounts for the anchorage dependence of normal cell proliferation, and the ►anchorage-independent cell growth that characterizes cancer cells is to some extent a reflection of altered integrin signaling in cancer cells.

Signaling

Ligands regulate integrin function by inducing changes in the conformation of the cell surface heterodimers.

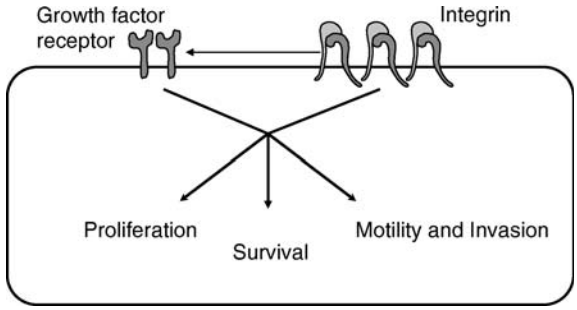
Upon binding to the heterodimer, the ligand opens up a pocket and separates portions of the integrin chains near the amino-terminal ends. This shift in conformation is transmitted along the length of the integrin protein towards the membrane, producing a change in conformation of the intracellular region. Ligands maintain the integrin in an open active configuration in which the integrin cytoplasmic tails are separated and there is high affinity for the ligand. In contrast, the closed inactive form has low ligand-binding affinity. A variety of cytoplasmic regulatory proteins exert their effects in the opposite direction by binding to the cytoplasmic domain, transmitting a conformational change along the integrin towards the amino-terminal ligand binding region, thereby altering ligand recognition. The aggregation or "clustering" of integrins in focal areas on the cell surface is a second important mechanism for regulating integrin function. Lateral migration and clustering of integrins in the plasma membrane enhances integrin-mediated signal transduction.

Integrin signaling regulates a variety of functions in benign and malignant epithelial cells. Epithelial cells are relatively non-motile and quiescent after embryogenesis and development but can be induced to undergo increased ►motility and proliferation under certain conditions, such as wound repair. Malignant epithelial cells usurp some of the integrin-mediated mechanisms underlying normal cell function to facilitate tumor growth and survival and to promote the motility, ►invasion and ►metastasis of cancer cells.

Growth and Differentiation

Full activation of the growth stimulatory pathways in normal epithelial cells requires not only integrin ligation but also cell adhesion to the extracellular matrix. The modulation of growth stimulatory pathways by integrin-mediated matrix adhesion provides a means whereby cell growth can be promoted in favorable environments and inhibited in areas where epithelial cell proliferation would be inappropriate. Cooperative signaling between integrins and growth factor receptors has been demonstrated for a number of growth factor receptors important for epithelial proliferation, including epidermal growth factor receptor (EGFR), ►HER-2/neu, and ►MET (Fig. 1).

Integrin ligation in adherent cells leads to activation of Erk, which translocates to the nucleus and produces increased ►cyclin D1. Integrin signaling through the PI3K-Akt-►mTOR pathway also raises cyclin D1 levels by increasing the translation of cyclin D1 mRNA. On the other hand, integrin signaling leads to degradation of the cyclin-dependent kinase inhibitors p21cip1 and p27kip1. Together, increased cyclin D1 and the degradation of cyclin-dependent kinase inhibitors result in hyperphosphorylation of the retinoblastoma gene product (Rb), leading to release of ►E2F, increased expression of cyclin A, and the formation of cyclin A/cdk2 complexes required for entry into S phase.



Integrin Signaling. Figure 1 Cooperative signaling between integrins and growth factor receptors. Schematic diagram depicting crosstalk between activated integrins and growth factor receptors, leading to integrin-mediated regulation of tumor cell proliferation, survival, and motility and invasion.

Activation of integrin-linked kinase (ILK) through the phosphatidylinositol 3-kinase pathway ►PI3K Signaling can also promote tumor cell proliferation. Activated ILK phosphorylates and inhibits glycogen synthase kinase-3 (GSK3), which relieves the negative regulation of activator protein 1 (►AP-1) and β-catenin/TCF transcription factors. Both of these transcription factors induce expression of cyclin D1, promoting cell cycle progression. Alternatively, integrin signaling through ►focal adhesion kinase (FAK) leads to activation of the small G-protein Rac and Rac-mediated activation and nuclear translocation of NF-κB. The Rac-PAK pathway downstream of FAK also leads to activation of the transcription factor Jun by Jun amino-terminal kinase (►JNK). Both NF-κB and Jun regulate genes involved in cell proliferation.

Integrin signaling not only regulates cell proliferation but also plays an important role in maintaining epithelial polarity and differentiation. Signaling through Rac appears to be critical for establishing epithelial polarity, which is disrupted in cancer cells as a result of altered integrin signaling. Malignant breast epithelial cells with overexpressed β1 integrins grow into non-polarized structures in 3-D culture, and inhibition of β1 signaling in these malignant cells results in reversion to a normal polarized phenotype.

Motility and Invasion

Migratory stimuli induce a relocalization of integrins to facilitate cell motility. Integrin signaling at the leading edge of migrating epithelial cells leads to

phosphorylation and activation of Rac through both ILK- and FAK-mediated pathways. Rac signaling through PAK downstream of ILK stimulates phosphorylation of ►Raf kinase, leading to activation of Erk. Activation of Erk leads to phosphorylation and activation of myosin light chain kinase (MLCK), which phosphorylates and activates myosin II, the main motor protein in eukaryotic non-muscle cells. Erk activation at the leading edge of motile cells, therefore, leads to actin-myosin contraction within sheet-like projections, or lamellipodia, and the generation of traction forces.

The α6β4 integrin plays a role in cell motility distinct from that of other integrins. In cells with hemidesmosomes, stimuli that induce migration, such as EGF, induce a relocalization of α6β4 from hemidesmosomes to the leading edge of migrating cells. α6β4 localizes initially within finger-like projections, or filopodia, anchoring them to laminin matrices. The stabilized filopodia then act as tracks to guide the subsequent spreading of lamellipodia. Signaling by α6β4 activates phosphodiesterases, which degrade cAMP, releasing the inhibitory effect of cAMP on ►Rho family proteins activity. α6β4-mediated Rho activation appears to stimulate the actin-myosin contraction necessary for the generation of traction forces at the leading edge of the cell. Rho downstream of α6β4, therefore, appears to play a role similar to that of Rac downstream of other integrins.

Integrin signaling through ILK in some types of epithelium induces downregulation of ►E-cadherin and loss of β-catenin from cell–cell ►adherens junctions. β-Catenin undergoes nuclear accumulation and activation, where it activates members of the T cell factor (TCF) family of transcription factors that regulate a variety of genes required for ►epithelial-mesenchymal transition (EMT), a process in which malignant epithelial cells undergo dedifferentiation and acquire mesenchymal-like properties, including the ability to undergo single cell invasion. Although ILK can be regulated downstream of other pathways such as the ►WNT signaling pathway, its direct linkage to β1 integrins implicates these integrins as important regulators of EMT. Signaling through ILK also promotes ►matrix metalloproteinase expression and secretion, facilitating cancer cell invasion of the surrounding connective tissues.

Survival

In stable adherent epithelial cells, integrins are clustered in focal adhesions. Focal adhesion kinase (FAK) binds to the β subunit of most integrin heterodimers in focal adhesions. FAK serves as a multifunctional adaptor linking integrins to multiple downstream signaling pathways, including the PI3K-Akt pathway. Signaling through Akt mediates cell survival in adherent epithelial cells by phosphorylating and preventing the pro-apoptotic activities of ►Bcl-2 family proteins BAD and Bax. Integrin signaling through the Erk pathway also promotes cell survival in adherent epithelial cells by downregulating the pro-apoptotic protein Bim. Although signaling through unligated β1 integrins can induce ►apoptosis by recruiting ►caspase-8 to the cell membrane, apoptosis generally results from loss of integrin-mediated survival signals, such as occurs in detached epithelium. In contrast to normal cells, cancer cells develop mechanisms for preventing apoptosis under anchorage-independent conditions.

The α6β4 integrin does not bind FAK but activates Akt-mediated survival pathways through an alternate mechanism, possibly through phosphorylation of the adaptor proteins IRS-1 and IRS-2. In addition, α6β4 has also been shown to activate the mammalian target of rapamycin (mTOR) downstream of Akt. Signaling through the mTOR pathway induces phosphorylation of 4E-binding protein (4E-BP1), a translational repressor that binds to and inhibits eukaryotic translation initiation factor 4E (eIF-4E). Phosphorylation of 4E-BP1 induces its dissociation from eIF-4E, and unbound eIF-4E promotes cap-dependent mRNA translation. Proteins induced through this pathway include ►vascular endothelial growth factor (VEGF), which also appears to promote PI3K-Akt signaling through an autocrine loop mechanism.

Integrin-mediated signal transduction regulates many cellular functions in addition to cell adhesion, including cell survival, proliferation, morphogenesis, differentiation, motility, invasion and metastasis. Although there remain significant gaps in our knowledge about the mechanisms underlying these various functions of integrins, abundant information is becoming available about specific pathways and mediators of integrin signaling in cancer cells. Detailed information about integrin signaling in cancer has important practical implications, not the least of which is the potential therapeutic targeting of integrin signaling pathways or growth factor receptor pathways modulated by integrin signaling.

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Intensity-modulated Radiation Therapy

Definition

IMRT; is a form of advanced ►radiation therapy. In IMRT, the beam intensity varies across the treatment field, and numerous small beams with modulated intensities are irradiated through multiple ports to the target to ensure that a prescribed dose is homogeneously delivered to the target while a reduced dose is given to adjacent critical organs.

►Proton Beam Therapy

Intercellular Junctions

Definition

Synonym cell-cell junctions, ►gap junctions, ►adherens junctions. Refers to protein structures that connect the ►plasma membrane of adjacent cells. There are several types of intercellular junctions. These include ►cadherins, ►connexins, ►mucins, ►selectins, and some ►integrins. These junctions mediate numerous processes including ►adhesion, signaling, metabolic coupling, homeostasis, and maintenance of cell morphology. Intercellular junctions are often disrupted in tumor cells, thereby disturbing functions related to these processes. Some intercellular junction proteins, such as connexins and cadherins, can act as ►tumor suppressors. In addition, intercellular junctions between transformed and nontransformed cells can help control tumor cell growth by ►contact normalization.

►Gap Junctions
►Adherens Junctions

Interferon

Definition

A protein substance produced by white blood cells and other types of cells. These substances have anti-viral properties, and have been used successfully in combating hepatitis and liver cancer associated with hepatitis.

►Inflammation
►Liver Cancer, Molecular Biology

Interferon-α

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Synonyms

B-cell interferon; Leukocyte interferon; Type I interferon

Definition

Interferons (IFNs) belong to the group of cytokines and are produced by various cells as part of an immune response to a viral infection or other immune trigger. IFNs are a family of molecules that include more than 15 expressed genes and a number of pseudogenes coded on chromosome 9. IFN-α belongs to the type I IFNs.

Characteristics

IFN-α is produced by monocytes/macrophages, lymphoblastoid cells, fibroblasts, and plasmacytoid ►dendritic cells (DC). It is detectable under physiological conditions but markedly enhanced during infections, especially viral infections. IFN-α binds to the IFN-α/β receptor CD118; binding to the Epstein–Barr virus receptor CD21 is also described. Binding to the IFN receptor activates the transcription of several different genes inducing the synthesis of host cell proteins that contribute to the inhibition of viral replication. In addition to its antiviral properties, IFN-α exhibits several other features that are of interest especially for the use in combination treatments of cancer, as there are: (i) direct inhibitory effects on tumor cell growth; (ii) ►radiosensitization and ►chemosensitization effects; (iii) ►antiangiogenic properties; (iv) enhancement of immunogenicity of tumors, and (v) modulation of the immune system.

IFN-α has a nearly 50-year long tradition in oncology. It has never fulfilled the overdrawn promises of the beginning but it has its niches; some has been lost during the last years but new entities like ►pancreas carcinoma show promising results as targets of IFN-based regimens.

Malignant Melanoma

High-dose IFN α-2b (HDI) is the only effective ►adjuvant therapy for ►melanoma patients at high risk for recurrence and death. Adjuvant therapy with HDI has been shown to increase significantly disease-free survival and overall survival in large, randomized, cooperative group trials compared with observation in patients with resected stage IIB and III melanoma.

These data are mature regarding the disease-free survival but less convincing regarding the overall survival as the large Intergroup trial E1690 failed to show an overall survival benefit. However, two large studies, the E1684 and the E1694 trial, were positive regarding the overall survival in the HDI arm. The approved regimen consists of intravenous induction therapy with 20 million international units/m^2/d, five times per week for 4 weeks, followed by 10 million international units/m^2 subcutaneously, three times per week for 48 weeks as maintenance. The toxicity associated with this regimen is substantial and can adversely affect acceptance of this regimen. With appropriate monitoring, dose modifications, and aggressive supportive care, HDI can be administered safely and is tolerable for the majority of patients. There have been no toxic deaths in the cooperative group trials of HDI over the past decade since the adoption of formal dose modification guidelines during the early phases of the first trials.

Pancreas Carcinoma

There are data showing that combined ►chemoradiotherapy plus Low-dose IFN-α (LDI) after curative resection of ►pancreatic ductal adenocarcinoma result in a 5-year survival of 50%, while chemoradiotherapy alone is described with a 5-year survival lower than 25%. However, a randomized controlled trial clearly proofing that IFN-α is responsible for this enormous difference is still outstanding. Recent data from translational research show strong immune responses in LDI-treated patients who develop first an unspecific immune response which later on result in a pancreas carcinoma-specific immune response.

Renal Cell Carcinoma/Leukemia/Multiple Myeloma

There is no evidence to support a benefit for ►adjuvant, ►Immunotherapy in high-risk patients who have undergone potentially curative surgery of ►renal cell carcinoma (RCC). At least five randomized trials failed to demonstrate a survival benefit from this approach. In the metastatic situation the role of IFN-α is unclear. The activity of monotherapy with IFN-α in metastatic RCC has been extensively evaluated, including several large, well-designed trials. Using a variety of preparations, doses, and schedules, the overall response rate may be as high as 15%; the median time to response is about 4 months, and most responses are partial and rarely persist beyond 1 year. Although no clear dose-response relationship has been shown, daily doses ranging from 5 to 10 million units appear to have the most favorable therapeutic index. Combinations with ►IL-2 and chemotherapy has been tested, the best results were obtained together with ►5-FU. Nearly one third of the patients responded. Nowadays, the tyrosine kinase inhibitors ►Sorafenib and ►Sunitinib seem to be better candidates for therapy of metastatic RCC.

IFN-α, initially partially purified IFN-α followed by recombinant IFN-α-2a, has been nontransplant standard therapy for chronic myeloid leukemia for several decades until the tyrosine kinase inhibitor Imatinib had its triumphal procession.

IFN-α has been used in ►multiple myeloma, however clinical activity is limited. In randomized studies no benefit of IFN-α maintenance therapy has been proven.

Hairy Cell Leukemia

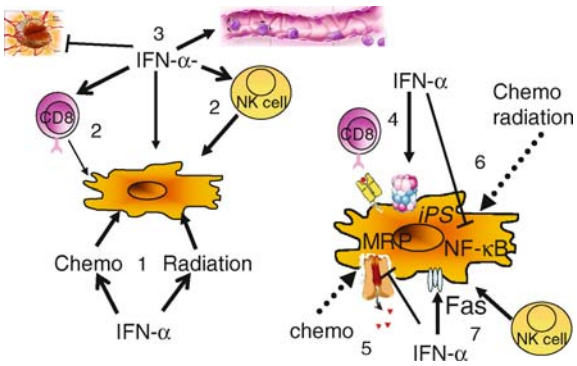
Since 1984, IFN-α is known to be an effective therapy for ►hairy cell leukemia, numerous large studies have confirmed this. The precise mode of action is not known but it might be mainly an immunomodulatory one, especially dealing with interleukin-6 production. IFN-α is administered with 2 million units/m² three times a week.

Direct Effects of IFN-α

IFN-α directly inhibits proliferation of tumor cells, prolongates the ►cell cycle, i.e., has a cytostatic mechanism. This is thought to be achieved by modulation of 2′,5′-oligoadenylate synthetase activity or modulation of cellular ►oncogenes. It has proapoptotic effects, downregulates oncogene expression, inhibits ►ornithine decarboxylase, an important biosynthetic enzyme, thus may resulting in depletion of metabolites required for tumor growth and induces tumor suppressor genes. However, at clinical achievable dosage concentrations these effects are not striking so other mechanism should contribute Fig. 1.

Radio- and chemosensitizing Effects

IFN-α acts synergistically with chemo- and radiotherapy. Treatment with IFN-α upregulates, for example, thymidine phosphorylase (TP) mRNA in tumor tissue.



Interferon-α. Figure 1 Pleiotropic mode of action of IFN-α.

TP is an enzyme which catalyzes the conversion of the pyrimidine antimetabolite ►5-fluorouracil (5-FU) into the active form. Furthermore, IFN-α prevents resistance mechanisms like ►NF-κB upregulation due to chemo- and radiotherapy. The transcription factor NF-κB is of great importance for cellular survival. By inducing certain antiapoptotic target genes, NF-κB is capable of conferring cellular resistance against various apoptotic triggers, including death receptor activation and DNA-damaging insults.

Antiangiogenic Effects

IFN-α, especially at low dosages, has antiangiogenic properties as most obviously in ►Kaposi sarcoma. ►Angiogenesis is a fundamental process that ensures adequate metabolic supply to tissues even in tumors. Angiogenesis starts with vasodilation and an increase in vascular permeability as a response to proangiogenic factors such as ►vascular endothelial growth factor (VEGF). This is accompanied by detachment of ►pericytes from the vessel wall and ►basement membrane and ►extracellular matrix degradation. In the next step, the underlying endothelial cells migrate into the perivascular space, multiply, and form new vascular sprouts. This ►neovascularization results in a hierarchical vascular network. Tumors lose the ability to form stable, hierarchically organized vessels. IFN-α has been shown to downregulate the expression of the proangiogenic molecules basic fibroblast growth factor (bFGF) and the matrix metalloproteinases, MMP-2 and MMP-9. Downregulation of angiogenesis-related genes and therapy of solid tumors is dependent on optimization of biological dose and schedule of IFN-α. In an animal model it was clearly shown that addition of IFN-α to 5-FU leads to tumor regression of pancreatic carcinoma. The animals had lower ►VEGF serum level and thus less VEGF receptor expression. Furthermore, vessel density was reduced and vessel structure and hierarchy normalized and pericyte marker decreased. Furthermore, after IFN-α therapy more leukocytes attached to the endothelium of the tumor vessels were able to migrate into the tumor.

Immunogenic Effects

IFN-α is described to enhance the immunogenicity of tumors. This phenomenon is provoked by an increase of ►MHC class I expression which enhance immune recognition. Both the increase in ►MHC molecules as well as a stabilization of MHC molecules formed has been described. Furthermore, IFN-α is described to induce the immunoproteasome. Intracellular proteins are degraded to peptides by the proteasome; a large proteolytic complex whose primary function is the controlled elimination of proteins marked for degradation by ubiquitin, and then presented to ►cytotoxic T lymphocytes (CTLs) by MHC class I molecules. It is well known that stimulation with IFN-γ induces the replacement of the three catalytic subunits of the proteasome by their homologous subunits LMP-2, MECL-1, and LMP-7, to form so-called "immunoproteasomes" (iPS). The efficiency of the modified proteasome to eliminate proteins remains unchanged, but its preferences for cleavage are modified. Several antigenic peptides, including tumor epitopes, were found to be processed differentially by the two proteasome types, thus implying a different ability to be recognized by ►CTLs. Treatment of carcinoma cells with IFN-α also resulted in a switch to the immunoproteasome thus determining the immunodominance hierarchy so that it has direct impact on ►CD8 ►T cell responses by modifying the repertoire of responding $CD8^+$ T cells. Presently it is discussed that the immunoproteasome is more competent at producing class I binding peptides. There are also several reports from tumor epitopes which are better processed by cells carrying immunoproteasomes. IFN-α pretreated tumor cells are getting more vulnerable to T cells.

Furthermore, IFN-α enhances production of antibodies to tumor cells thus resulting in an increased ►antibody-dependent cellular cytotoxicity (ADCC), linking ►natural killer cells (NK cells) to tumor cells and increasing ►complement-dependent cytotoxicity (CDC).

Immunomodulation

IFN-α acts on T, NK, and DC and monocytes thus releasing cytokines. IFN-α enhances the survival of T cells by expression of antiapoptotic genes, induces the generation of $CD8^+$ memory cells and it promotes differentiation of naïve T helper cells to T helper 1 cells ►T_H1 cells. IFN-α is also important for the proliferation and long-term survival of antigen-specific T cells. In clinical studies classical secretion of Th1 cytokines as interleukin-2 (IL-2), IFN-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and ►interleukin-12 (IL-12) in response to IFN-α administration were observed. Th1 cytokines are thought to confer protective immunity to tumors. IFN-α makes T cells more responsive to IL-12 thus promoting a Th1 immune response.

►NK cells which kill tumor cells by ►Fas-induced ►apoptosis as well as by ►perforin release are activated by IFN-α. ►FasL is upregulated on ►T cells and ►NK cells in response to IFN-α stimulation and therefore an enhanced ability of immune cells to kill via the Fas pathway is given.

Furthermore, IFN-α plays an essential role in the differentiation, maturation and functions of ►dendritic cells, and enhances ►macrophage activities. These

►antigen-presenting cells stand in the beginning of the immune cascade and enables that a switch from the unspecific immune system to the specific immune system occurs. Antigen-specific cells could uptake tumor material and prime naïve IFN-α stimulated T cells in an antigen-specific way.

IFN-α functions are as follows: (i) IFN-α acts as radio-and chemosensitizer. (ii) IFN-α enhances NK cell mediated cytotoxicity and to a lesser extent CD8 cells. (iii) IFN-α acts antiangiogenic and enhances leucocyte–endothelium interactions. (iv) Treatment with IFN-α induces the switch to the immunoproteasome resulting in an increased immunogenicity. (v) IFN-α prevents the induction of multidrug-resistance proteins. (vi) Chemo- as well as radiotherapy induces NF-κB, known to promote growth of carcinoma cells. IFN-α inhibits NF-κB upregulation or even downregulates NF-κB. (vii) IFN-α induces upregulation of Fas thus resulting in an increase in NK cell cytotoxicity.

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Interferon Gamma

Definition

IFN-Gamma, Type II IFN; A natural protein involved in the regulation of immune and inflammatory responses. IFN-gamma is produced in activated T-cells and ►natural killer cells, and has direct anti-viral and anti-tumor effects (generally weak), however it also potentiates the effects of the type I IFNs. IFN-γ released by Th1 cells recruit leukocytes to an infection site, resulting in increased ►inflammation. It also stimulates ►macrophages to kill bacteria that have been engulfed. IFN-γ released by Th1 cells is also important in regulating the Th2 response.

►GAGE Proteins
►Immunoediting

Interferons Type I

Definition

The type I interferons are a multi-member ►cytokine family that bear structural resemblance to other cytokines, including IFN-γ. Although there are a total of seven type I interferons in mice and five in humans from an immunological perspective IFN-α and IFN-β are the most important. There are 12 known varieties of IFN-α in mice and 13 in humans, whereas only one species of IFN-β exists for both species. Both IFN-α and -β bind to the same receptor, which consists of IFNAR1 and IFNAR2 subunits. Signaling occurs through the JAK/STAT pathway. Although all cells can produce type I interferons in response to pathogens, a specialized ►dendritic cell produces up to 1,000 times more IFN-α and -β than other cell types.

►Immunoediting

Interleukin

Definition

IL; Is a generic term for ►cytokines produced by ►leukocytes. In use is the more general term cytokine, but the term interleukin is used in the naming of specific cytokines such as ►IL-2.

►Interleukin-4
►Interleukin-6

Interleukin-1

Definition

IL-1; A cytokine, endogenous messenger of cells of the immune system, which promotes ►inflammation and destruction of chondral tissue.

►Interleukin

Interleukin-1 beta

Definition

IL-1β; is a potent immuno-modulator that mediates a wide range of immune and inflammatory responses including the activation of B- and T-cells.

►Interleukin

Interleukin-2

Definition

IL-2; Is the cytokine that is most central to the development of an ►adaptive immune response.

Interleukin-4

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Definition

IL-4; Is a prototypic immunoregulatory cytokine secreted by activated ►CD4 T cells of the ►T helper (Th) 2 subset and by ►basophils and ►mast cells. IL-4 has an important role in regulating antibody production, hematopoiesis, ►inflammation and the development of effector ►T-cell responses.

Characteristics

IL-4 is a 15–20 kD ►glycoprotein composed of 129 amino acids. IL-4 contains six cysteine residues that are all involved in intramolecular disulfide bridges. The secondary structure of IL-4 was shown to consist of a four-helix bundle with a unique up-up-down-down helix topology. The X-ray structure reveals that IL-4 is a highly compact and globular protein with a predominantly hydrophobic core.

Human IL-4 receptor (IL-4R) is found on ►T cells, ►B cells, ►mast cells, ►basophils, ►macrophages, ►fibroblasts, ►endothelial cells, hepatocytes, keratinocytes, stromal cells and neuroblasts. IL-4 receptor is a heterodimer composed of an α subunit, with IL-4 binding affinity, and the common γ subunit that is also part of other cytokine receptors. It functions to enhance the binding of IL-4 to its receptor, presumably by direct interaction with the ligand, and to induce intracellular signals transduction events such as ►JAK/STAT signal pathway. Janus kinases JAK1 and JAK3 and ►signal transducers and activators of transcription 6 (STAT6) have been found to play a unique role in IL-4 receptor-mediated intracellular signaling and hematopoietic cell development.

IL-4 is a highly tissue-specific gene that is expressed in the Th2 cell subset and not in the Th1 cell subset. The human IL-4 gene is a single copy gene in the haploid genome. It spans about 10 kb and is located on chromosome 5q23–31 along with the IL-3, IL-5, and GM-CSF genes. Several transcriptional factors such as STAT6, NFAT (nuclear factor of activated T cells), c-Maf and GATA3 also influence gene expression and production of IL-4. For example, STAT6-►knock-out mice are unable to produce Th2 cytokines. The Th2-specific transcription factor c-Maf promotes IL-4 promoter activity. NFAT1 is involved in termination of the late phase of IL-4 gene transcription, thereby inhibiting Th2 responses.

Generally, antigens and antibodies that cross-link the T-cell receptor induce IL-4 production. The initiation of IL-4 production, a key event in the control of IL-4 production may, in naïve T cells, require limited T-cell receptor (TCR) signaling in co-stimulation with surface CD28 and CD40 ligand. Once IL-4 production is initiated, IL-4 feeds back on the differentiating cells and accelerates Th2 commitment in an autocrine fashion.

Effects of IL-4 depend upon binding to and signaling through IL-4 receptor complex. The IL-4R, which lack intrinsic tyrosine kinase activity, uses multiple intracellular domains to induce proliferative and gene expression responses. The membrane proximal region of IL-4R contains sequences known as Box 1 and Box 2 for binding of Janus family kinases JAK1 (bound to IL-4Rα) and JAK3 (bound to γc). Distal to this region is the I4R motif for binding of insulin receptor substrate (IRS)-1 and IRS-2. IRS-1 and IRS-2 links the IL-4R to downstream signaling cascades including phosphoinositol 3′ kinase (PI-3-K) and mitogen-activated kinase to induce cell growth. A more distal region of the IL-4R, termed the gene expression domain, contains three tyrosine residues, which mediate activation of STAT6 through tyrosine phosphorylation. STAT6 phosphorylation is believed to induce transcriptional activation after the STAT dimers translocate from the receptor to the nucleus, where they bind to specific targeting sequences and influence gene transcription. In short, the JAK/STAT signal pathway connects activation of the receptor complexes directly to transcription of genes. Upon IL-4 receptor oligomerization induced by a polypeptide ligand, JAKs are activated,

presumably by trans-auto phosphorylation on tyrosines. Subsequently, JAKs phosphorylate STAT proteins, which form homodimeric or heteromeric complexes via their Src-homology 2 (SH2) domains. These complexes translocate to the nucleus and bind directly to response elements present in the promoters of target genes, thus triggering induction of transcription.

Bioactivity

IL-4 was initially called B cell growth factor-1 because of its role in the early steps of B cell activation. IL-4 has now been shown to regulate a wide spectrum of cellular functions in B cells, T cells, monocytes/macrophages and other hematopoietic and non-hematopoietic cells. IL-4 plays a central role in regulating the differentiation of antigen-stimulated naïve T cells. IL-4 causes such cells to develop into cells capable of producing IL-4 and a series of other cytokines including IL-5, IL-10 and IL-13. IL-4 controls the specificity of immunoglobulin class switching. IL-4 also has an important role in tissue adhesion and inflammation.

IL-4 has been shown to have antitumor and immunomodulatory activity against many human hematopoietic and solid tumor cells in vitro. In isolated cases, however, IL-4 was found to have mitogenic activity toward human T-cell leukemia cells. IL-4 receptors are expressed on solid human tumor malignancies. IL-4 inhibits the growth of many solid tumor cell types including malignant ►melanoma, ►breast carcinoma, ►ovarian carcinoma, ►mesothelioma, neurofibrosarcoma and ►renal cell carcinoma, ►non-small cell lung carcinoma and ►colon carcinoma. Antitumor activity of IL-4 has been explored in various animal models. For example, when injected around tumor draining lymph nodes directly into a solitary tumor nodule or intraperitoneally for systemic metastasis, IL-4 has been shown to have significant antitumor activity. Also, transfection of the IL-4 gene induces killing of many tumor types in vivo. In addition, IL-4 potentiates the antitumor effects of TNF or IFN-γ on a variety of tumor cell lines.

Clinical Relevance

IL-4 has diverse effects on health and many pathological states. IL-4-mediated Th2 cell response may avoid the extensive inflammatory tissue injury and prevent autoimmunity by downregulating Th1 cell response. IL-4 has also been shown to have potent antitumor activity in vitro. On the other hand, IL-4 and its receptor are the essential mediators in the development of allergic and inflammatory diseases. Inappropriately high IL-4 production has also harmful effects in infectious diseases. In addition, abnormality in IL-4 receptor/JAK/STAT signaling contributes to hematopoietic disorders and tumorigenesis.

IL-4 and Allergic and Inflammatory Diseases

IL-4 is mainly responsible for Th2-driven tissue ►inflammation. IL-4 has been implicated in allergic and inflammatory diseases including allergic rhinitis, urticaria, conjunctivitis, food allergies, asthma and systemic anaphylaxis. A dysregulation of IL-4 production or a change in IL-4 responsiveness may be the underlying abnormality in atopy, the tendency to produce excessive IgE in response to allergens. Allergen-specific T cells from atopic subjects preferentially develop into IL-4-producing Th2 cells and their CD4+ cells exhibit high IL-4 production when stimulated by antigens other than allergens. Moreover, IL-4-deficient mice develop attenuated airway inflammation compared with wild type mice. IL-4 plays a significant role in viral, bacterial and parasitic infections. IL-4 may be involved in the progression of acquired immune deficiency syndrome (AIDS) caused by ►human immunodeficiency virus (HIV-1).

Human Severe Combined Immunodeficiency (SCID)

Human ►severe combined immunodeficiency (SCID) is a set of primary immunodeficiency diseases characterized by profoundly impaired cell-mediated and humoral immunity. More than 50% of SCID cases are X-linked SCID, which manifests complete or profound defects of T cells and NK cells, but carry normal or slightly increased numbers of B cells, leading to death mostly within a year after birth if not treated with bone marrow transplantation. XSCID is commonly associated with mutations that chromosomally map to Xq13 in the γc receptor gene. Mutations occur throughout the entire γc gene, including the extracellular and cytoplasmic domains, which impair cytokine ligand binding as well as signal transduction. These mutations are manifested as deletions, insertions, splice junction defects, point mutations and premature stop codons in the γc gene. The complete dysfunction of the γc subunit in ligand binding and signal transduction results in the typical phenotype associated with XSCID. A form of autosomal SCID exists with clinical symptoms identical to XSCID in which the gene encoding JAK3 is affected.

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Interleukin-6

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Synonyms

B-cell differentiation factor; BCDF; B-cell stimulating factor-2; BSF-2; Hepatocyte stimulating factor; HSF

Definition

IL-6; Is a member of a family of cytokines that includes ►leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), ►oncostatin M (OSM), IL-11 and cardiotrophin-1 (CT-1). These cytokines are functionally redundant and structurally similar. They also share receptor subunit for signal transduction, and thus elicit similar and overlapping physiological responses. IL-6 is a multifunctional cytokine. In addition to its important roles in the immune response, inflammation and hematopoiesis, IL-6 is also involved in other important physiological processes such as promotion of osteoclast resorption of bone and regulation of the growth of many tumor cells.

Characteristics

The human IL-6 gene was cloned in 1986. It contains five exons in its 5 kb genomic DNA, which is mapped to human chromosome 7p21–p14. IL-6 is first synthesized as a precursor protein of 212 amino acids and the mature IL-6 is a glycoprotein composed of 185 amino acids. Different ►post-translational modifications (e.g., glycosylation and phosphorylation) of the protein results in various molecular masses of 22–28 kDa. The promoter of the IL-6 gene contains binding sites for various transcription factors, including binding sites for ►NFκB and C/EBPβ (CAATT-enhancer-binding protein β), allowing the transcriptional regulation (induction or repression) of the IL-6 gene in response to various stimuli such as ►estrogen, ►interleukin-1 (IL-1) or ►tumor necrosis factor (TNF). IL-6 is produced in various cell types. Major sources of IL-6 production include stimulated ►monocytes, ►fibroblasts and ►endothelial cells.

Genes

The receptor for IL-6 (IL-6R) is composed of IL-6Rα, an 80 kDa IL-6 binding protein and a signal transducer termed gp130. IL-6Rα is similar to other cytokine receptors in the type I cytokine receptor superfamily. Gp130 is a membrane-associated 130 kDa glycoprotein, which is shared among the receptors for CNTF, LIF, OSM, IL-11 and CT-1. The sharing of gp130 provides an explanation for the functional redundancy among the IL-6-related family of cytokines. Binding of IL-6 to IL-6Rα triggers the association of IL-6Rα with gp130. The signal is then transduced through activation of tyrosine kinases, Janus kinase (►JAK), and subsequent activation of downstream transcription factors ►STATs (►signal transducer and activator of transcription), resulting in activation of gene expression. There are a few members within the JAK family tyrosine kinases. Among them, JAK1, JAK2 and Tyk2 have been shown to associate with gp130 and to be activated by IL-6. STATs are tyrosine phosphorylated transcription factors that are activated by JAK tyrosine kinases. There are also a few members in the STATs family. Of these STATs, STAT1, STAT3 and STAT5 are activated in response to IL-6. Numerous studies indicate that STAT3 is essential in IL-6-induced, gp130-mediated signal transduction, regulating various important physiological processes including cell growth, survival and differentiation. Another pathway activated by IL-6 is the ►MAP kinase pathway, which is an important pathway involved in regulating cell differentiation, cell growth and death in response to IL-6 and other growth factors. IL-6 receptor is expressed in both immune- and non-immune tissues, such as prostate tissue and neuronal tissue. As a multifunctional cytokine, IL-6 is able to induce differentiation of B cells, T cells, myeloid leukemic cells and even neuronal cells. IL-6 is also able to induce cellular growth of myelomas, keratinocytes, mesangial cells, ►renal-cell carcinoma, ►Kaposi sarcoma and tumor cells such as ►prostate cancer cells. IL-6 is able to inhibit growth of myeloid leukemic cells and some other tumor cells, such as ►breast cancer, ►cervical cancer, human ►lung cancer cell lines and ►melanoma. The biological activity of IL-6 can be enhanced by other factors such as ►glucocorticoid, which has been recognized to be able to synergize the IL-6 response. In many cases, STAT3 plays essential roles in the synergy between IL-6 and other factors. Soluble form of IL-6Rα can also interact with gp130. Thus, cells lacking IL-6Rα are not responsive to treatment with IL-6 alone but are responsive to co-treatment with IL-6 and soluble IL-6Rα.

Clinical Relevance

IL-6 is related to various diseases. It has been found that abnormal IL-6 production and abnormal expression of IL-6 receptor are related to ►autoimmune disease. Serum IL-6 level may be related to various diseases. Low serum IL-6 levels are observed in some diseases (e.g., ►monoclonal gammopathy), while elevated serum IL-6 levels are associated with other diseases such as human ►prostate cancer. A growing number of clinical observations have revealed the frequent association of

high serum IL-6 levels with ►androgen-independent prostate cancers, suggesting the involvement of IL-6 in prostate cancer androgen-independent progression. IL-6 receptor is expressed in both prostate cancer tissues and prostate carcinomas cell lines. In the absence of androgen, IL-6 is able to activate the androgen receptor, which is involved in prostate cancer androgen-independent progression. STAT3 has been shown to be required for IL-6 to activate androgen receptor in prostate cancer cells. In addition to its role in prostate cancer progression, studies also indicate that IL-6 could function as a growth factor and could be involved in the oncogenic process of other human tumors such as ►renal-cell carcinoma, ►Kaposi sarcoma, ►lymphoma and ►breast cancer. IL-6 is also essential for the osteoporosis (accelerated bone loss) caused by estrogen deficiency. Estrogen has been shown to be able to inhibit IL-6 production. Thus, one of the most common forms of ►osteoporosis is postmenopausal osteoporosis found in women after menopause or ovariectomy, due to overproduction of IL-6 caused by loss of estrogen production. ►Estrogen replacement therapy has thus been used to prevent postmenopausal osteoporosis.

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Interleukin-8

Definition

IL-8, a potent neutrophil chemotactic peptide that elicits pleiotropic (►pleiotropy) biological effects, is secreted in large amounts by normal human osteoblastic and bone marrow osteoprogenitor stromal (HBMS) cells in response to IL-1β and tumor necrosis factor-α. It is also a chemokine that induces angiogenesis in cancer tissues.

►Sivelestat

Interleukin-12

Definition

IL-12; A ►cytokine, endogenous messenger of cells of the immune system, which induces and maintains TH-1 immune reaction and supports T-killer cells in tumor destruction.

Intermediate

Definition

Is a molecular entity that is formed from the reactants and reacts further to give the products of a chemical reaction.

►Xenobiotics

Intermediate Endpoint

►Surrogate Endpoint

Intermediate Junction

►Adherens Junctions

Internal Dose

Definition

The amount of a substance penetrating the absorption barriers (e.g., skin, lung tissue, gastrointestinal tract) of an organism through either physical or biological processes. The term covers the amount of a chemical recently absorbed and the amount of chemical stored in one or several body compartments or in the whole body.

►Biomonitoring

Internalization

►Endocytosis

International Agency for Research on Cancer

Definition

IARC; Is an organization under the World Health Organization. IARC evaluates the carcinogenic risks of chemicals, exposures to complex mixtures and other agents to humans. The evaluations are published in monographs in which data on carcinogenicity are critically reviewed. The evaluations result in a classification according to the evidence for carcinogenicity.

Interphase Cytogenetics

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Definition

Refers to the analysis of genes and chromosomes in non-dividing nuclei. The method does not require the preparation of metaphase chromosomes, which is necessary for chromosome banding analysis or spectral karyotyping. Interphase cytogenetics is particularly useful for the visualization of ►chromosomal translocations, ►aneuploidy, gene deletions or ►amplifications and structural and numerical chromosomal aberrations in tissue sections, cytological preparations, and other fixed specimen. The technique involves the hybridization of nucleic acid probes labeled with either fluorescent or chromogenic dyes, followed by microscopy and signal assessment.

Characteristics

Interphase cytogenetics employs fluorescence in situ hybridization (►FISH), in which nuclei acid probes tagged to fluorescent molecules are hybridized to cytological preparations from non-dividing cells or tissue sections (chromogenic dyes are used less commonly). The analysis requires a microscope equipped for epifluoresence, and, preferably, a digital imaging system, which can now be complemented with software for image acquisition and signal enumeration. For instance, a trisomy for chromosome 21 could be visualized with DNA probes that specifically recognize this chromosome. All cells that would carry this particular chromosomal aberration would reveal three fluorescent signals, as opposed to two that all normal cells have. And this pattern is independent from the cell cycle, because metaphase chromosomes maintain their structural integrity at all stages, and three signals in interphase cells indicate unambiguously the presence of an extra copy of this chromosome.

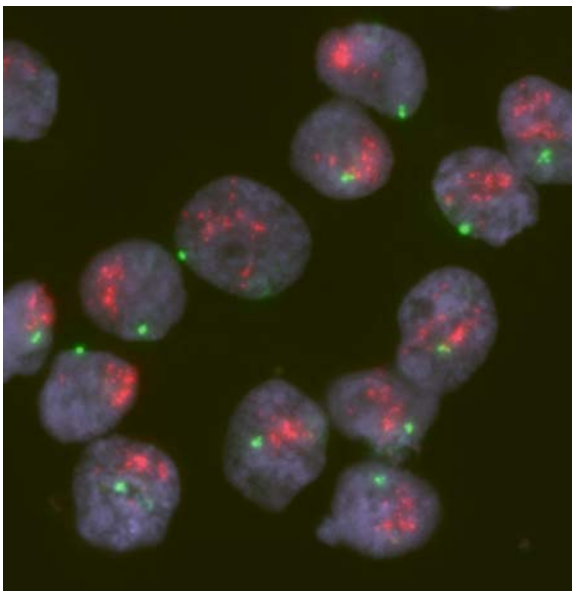
The application of chromosome banding techniques, and molecular cytogenetic analyses, including fluorescence in situ hybridization (FISH), ►comparative genomic hybridization (CGH), and ►spectral karyotyping (SKY) to the analysis of cancer genomes has firmly established that ►chromosomal aberrations are a defining feature of this disease. Chromosomal aberrations in cancer cells occur in different facets. Most of the ►hematological malignancies are characterized by ►chromosomal translocations, which generate an oncogenic fusion protein or cause deregulation of oncogenes. The cells of most solid tumors of epithelial origin (carcinomas), however, contain chromosomal ►aneuploidy that result in tumor type and tumor stage specific ►genomic imbalances. Chromosomal translocations in hematological malignancies comprise, e.g., the ►Philadelphia chromosome, which is a reciprocal, balanced translocation between chromosomes 9 and 22. The detection of this reciprocal translocation in interphase cells can be achieved using either whole chromosome painting probes, or, better, with locus specific probes that hybridize adjacent to the breakpoint. Fluorescence in situ hybridization techniques allow labeling of such probes with different colors, and hence the design of probe cocktails that specifically pinpoint the genetic aberration. For instance, the colocalization of red and green labeled probes in interphase cells would result in a merged yellow color that denotes the juxtaposition of two probes that are separated in normal cells. Similarly, the deletion of tumor suppressor genes can be assessed. Such deletions appear as a lack of hybridization signals. Another class of relevant chromosomal aberrations in cancer cells are chromosomal trisomies. Chromosomal ►trisomy are among the earliest genetic insults in the genesis of solid tumors. For instance, the progression risk of early dysplastic lesion in the uterine cervix can be predicted by the emergence of copy number increases

of the long arm of chromosome 3, which results in the genomic ►amplification of human ►telomerase gene. At advanced stages of carcinogenesis tumors can acquire multiple copies of oncogenes. These ►amplification events correlate in general with poor prognosis, however, can in some instances also stratify patients for specific therapeutic regimens, such as in the case of the ►*HER*-2/neu oncogene amplification in ►breast cancer. Gene amplifications can be very elegantly visualized and quantified by interphase cytogenetics (Fig. 1). A DNA probe that targets the particular oncogene will display multiple copies in interphase cells, and the copy numbers can be correlated to non-involved chromosomes detected with a second fluorochrome.

The application of interphase cytogenetics with sophisticated probe sets has become mainstream for routine diagnostic procedures in hematological malignancies. With the emergence of strictly conserved patterns of genomic imbalances in solid tumors it is conceivable that the visualization of such abnormalities in routine diagnostic samples, for instance in Pap smears or in fine needle aspirations of the breast, will become standard practice as well. One particular advantage of interphase cytogenetics is that the genetic information can be evaluated on individual cells together with such relevant parameters as histo- or cytomorphology. Interphase cytogenetics therefore serves as a tool that allows one to combine pertinent genetic information directly with morphological changes in the progression of solid tumors. This combination of genetics and morphology will improve diagnosis, prognosis and therapy planning, because more predictive information can be obtained. Interphase cytogenetics has the potential to not only systematically integrate tumor phenotype with genotype in hematological malignancies but also in solid tumors.

Future developments of interphase cytogenetics can be envisioned in several areas: (i) it would arguably be useful to increase the numbers of chromosomal targets that can be visualized simultaneously. This can be achieved by combining multiple probes each tagged with a different fluorochrome. Recent developments in fluorescent dye chemistry and in microscope hardware and software along with novel imaging tools, such as spectral imaging, are likely to increase the multiplicity of interphase cytogenetics; (ii) a combination of genetic and chromosomal markers for specific cancers and cancer stages with morphological markers, for instance markers for cell lineage in hematological malignancies, would increase the value of the respective approaches for cancer diagnostics; (iii) finally, automated digital imaging systems and image analysis algorithms for fully automated signal enumeration would facilitate the conversion of interphase cytogenetics towards a high-throughput genetic analysis.

In summary, interphase cytogenetics offers a unique approach for the visualization of genetic and chromosomal aberrations in cancer tissues and cytological preparations on a single cell level. The maintenance of the cellular morphology offers great promise that interphase cytogenetics could contribute significantly to the diagnostic enhancement of the mainly morphologically based evaluation of cancer and its precursor lesions.



Interphase Cytogenetics. Figure 1 The image shows cells from an invasive breast carcinoma after hybridization with a probe cocktail that contains the *HER* 2 oncogene (red). Interphase cytogenetics allows precise enumeration of copy numbers of this growth factor receptor. Only one signal is present for the probe specific for the tumor suppressor gene ►*TP* 53 (green). Such deletions are common in breast cancer.

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Interstitial Cells of Cajal

Definition

ICC; Is a type of cell found in the ►gastrointestinal tract. It serves as a pacemaker that triggers gut contraction. ICC serve as electrical pacemakers and generate spontaneous electrical slow waves in the gastrointestinal (GI) tract. Interstitial cells of Cajal embedded in the musculature of the gastrointestinal tract are involved in the generation of electrical pacemaker activity for gastrointestinal motility. This pacemaker activity manifests itself as rhythmic slow waves in membrane potential, and controls the frequency and propagation characteristics of gut contractile activity.

►Gastrointestinal Stromal Tumor

Interstitial Space

Definition

Is the extracellular space between cells of an organ.

►Convection Enhanced Delivery (CED)

Interstitual Microinfusion

►Convection Enhanced Delivery

Interstrand Cross Link

Definition

Covalent linking of opposite polynucleotide strands, which prevent DNA strands separation result in block of DNA ►replication and ►transcripition.

►BACH1 Helicase and Cancer

Intervention Study

Definition

A study aimed at testing a cause (or preventive factor) of cancer by modifying its distribution in the study population.

►Cancer Epidemiology
►Epidemiology of Cancer

Intestinal Absorption

Definition

Is the entry of the drug from the contents of the ►gastrointestinal tract into the tissue lining the GI tract.

►ADMET Screen

Intestinal Permeability

Definition

Is the process of a drug passing across the wall of the ►gastrointestinal tract. There are several mechanisms for this process. The most common is called passive intestinal permeability and depends on the drug dissolving in the gastrointestinal wall to gain passage across.

►ADMET Screen

Intestinal Type Adenocarcinoma

Definition

An appendiceal malignancy that histologically resembles the intestinal surface. This type of cancer is sometimes referred to as non-mucinous adenocarcinoma.

►Appendiceal Epithelial Neoplasms

Intrabodies

Definition
Antibodies and their fragments for which the genetic information is designed in a way that, after translation by the eukaryotic cell machinery, the resulting molecules will remain associated to different predefined cellular structures and compartments. *Applications:* Intracellular therapy.

Intracerebral Clysis

►Convection Enhanced Delivery

Intracerebral Microinfusion

►Convection Enhanced Delivery

Intraductal Carcinoma

►Ductal Carcinoma In Situ

Intraductal Papillary Mucinous Tumors

►Bile Duct Neoplasms

Intralesional Excision

Definition
Tumor surgery by which the excision takes place within the pseudo capsule of the tumor.

►Cryosurgery in Bone Tumors

Intraocular Melanoma

►Uveal Melanoma

Intraperitoneal Chemotherapy

Definition
Instillation of chemotherapy directly into the peritoneal cavity, usually performed immediately after a cytoreduction, before intestinal reconstruction, and before a fibrotic process within the abdomen begins.

►Appendiceal Epithelial Neoplasms
►Chemotherapy of Cancer

Intravasation

Definition
Is the entry of migrating cells in vessels. The invasion of blood vessels and lymphatics by cancer cells in a primitive tumor.

►Bone Tropism
►Circulating Tumor Cells

Intravesicular Instillation

Definition
Internal local administration of chemotherapeutic agent used in the treatment of superficial ►bladder cancer.

►Mitomycin C

Intrinsic Pathway of Apoptosis

Definition
One of the two archetypal pathways of ►caspase activation, known also as "the mitochondrial pathway."

It is activated by several forms of cytotoxic stress and involves release of ►cytochrome-c from ►mitochondria, binding of APAF-1, and activation of procaspase-9.

►APAF-1 Signaling

Intrinsically Disordered Proteins

►Intrinsically Unstructured Proteins

Intrinsically Unfolded Proteins

►Intrinsically Unstructured Proteins

Intrinsically Unstructured Proteins

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Synonyms

Intrinsically disordered proteins; Intrinsically unstructured proteins; Intrinsically unfolded proteins; Naturally disordered proteins; Naturally unstructured proteins; Naturally unfolded proteins; Disordered domain; Unstructured domain; Unfolded domain

Definition

Intrinsically unstructured proteins are entire proteins or proteins with large segments that lack a rigid three-dimensional structure under physiological conditions.

Characteristics

Proteins fall into a continuum of different structure types that include tightly folded single domains, multidomains interconnected with flexible disordered regions, compact but disordered molten globules, and highly extended unstructured domains. Entire proteins or large segments of proteins (often over 50 residues) may have backbone bonds and angles that vary significantly, and thus, lack a rigid three-dimensional structure.

Bioinformatics studies predict that a significant fraction of many species' genomes codes for intrinsically unstructured domains. These regions are highly conserved between species and the percentage of intrinsically unstructured regions increases with species complexity. Experimentally, over 100 proteins have been shown to be at least partially disordered. It was previously thought that unlike small unstructured proteins and unstructured polypeptide hormones, larger proteins had to be structured to be functional. However, recent work has shown that unstructured domains are more than just flexible linkers, having roles in the assembly of macromolecular arrays, transcriptional regulation, translation, and signal transduction. In fact, the increased intrinsic disorder in more complex organisms may be related to the corresponding increase in complexity in cell signaling and regulation.

In typical cell-signaling networks, most proteins have only one or two binding partners while a few "hub" or "signaling node" proteins have tens, hundreds, or even more partners. Unstructured domains are likely the key to hub promiscuity; hubs may possess unstructured domains that bind multiple proteins or structured hubs may bind unstructured domains in many different proteins. These unstructured domains typically undergo coupled folding and binding, adopting a more rigid three-dimensional fold as they bind their partners.

Many ►tumor suppressor genes and ►Oncogenes are signaling nodes, affecting a wide range of cellular processes. The identification and study of unstructured domains in cancer-related proteins (►Cancer) has revealed multiple roles for these domains in cancer pathophysiology.

Identification of Unstructured Proteins

Classical biochemical methods for identifying protein activities typically select against unstructured proteins; unfolded proteins are more sensitive to degradation by proteases in homogenates, and the low copies of regulatory proteins may provide for low assay activity. However, with the increasing availability of large-scale genome sequencing data and records of already-known unstructured proteins, the prediction of unstructured regions is becoming more efficient. Although one cannot predict the tertiary structure of globular proteins from sequence data (unless there is high sequence homology to a known protein/domain structure), the prediction of unstructured domains is much simpler. Disordered domains are typically composed of polar and charged amino acids (commonly glutamine, glutamic

acid, lysine, serine, and proline; less commonly alanine and glycine), possess a low proportion of bulky hydrophobic amino acids (isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, and valine), and often contain repetitive sequences. Computer programs available for the prediction of unstructured domains include DisEMBL, DISOPRED2, FoldIndex, GLOBPROT 2, and PONDR. DISPROT is a recently established database of protein disorder.

Experimentally, crystal-structure analysis can be used to identify the presence of unstructured states (through the absence of electron density), but long regions of disorder will not crystallize. NMR spectroscopy is the primary method currently used to study disordered proteins, although other methods may include circular dichroism, infrared spectroscopy, vibrational circular dichroism spectroscopy, Raman spectroscopy, fluorescence spectroscopy, size exclusion chromatography, or analytical ultracentrifugation.

Unstructured Proteins and Cancer

In a computational analysis of four protein datasets, ~79% of human cancer associated proteins are predicted to contain ≥30 consecutive disordered residues, compared to 66% for signaling proteins, 47% for eukaryotic proteins from ►SWISS-PROT, and 13% for nonhomologous protein segments with ordered three-dimensional structure. Furthermore, among the human proteins in SWISS-PROT, human cancer associated proteins are predicted to have approximately twofold greater extent of disorder than biosynthesis, degradation, G-protein-coupled receptor (►G-proteins), inhibitors, membrane, metabolism, kinase, or transport proteins. The role of many human cancer associated proteins as signaling hubs, which require selective binding of multiple different targets, points to the importance of disordered domains in cancer biology. Several examples that illustrate the diverse role of unstructured domains in cancer include:

- p53: (►*p53* gene family, ►p53 protein, biological and clinical aspects, ►TP53) The tumor suppressor p53 contains an N-terminal transcriptional-activation domain and a C-terminal regulatory domain. Both are intrinsically disordered (~70% of p53 is unstructured), and the structural flexibility is likely essential for allowing the wide variety of posttranslational modifications known to occur at these sites. Side chains are consequently exposed and regions can reconform to an acceptable structure for different modifying enzymes' active sites. Furthermore, coupled folding and binding likely allows p53 to interact with a variety of proteins, including CBP (cAMP-response element-binding protein) and p300 (►CBP/p300 coactivators). A study of ►*MDM2* –p53 N-terminal transactivation domain binding suggests that this unstructured p53 domain functions as a "fishing line" with a loosely folded "hook." The "hook" adopts a more folded structure after initial ►MDM2 binding; this Mdm2–p53 interaction can hence be regulated by posttranslational modifications.
- p21: (►p21 (WAF1/CIP1/SDI1)) The N-terminal portion of p21 lacks a stable secondary or tertiary structure in free solution. However, this fragment adopts an ordered, stable conformation when bound to cyclin-dependent kinase 2 (Cdk2) (►Cyclin-dependent kinases). The unstructured domain may therefore be responsible for p21 promiscuity, as p21 inhibits a diverse number of cyclin–Cdk complexes (including cyclin A–Cdk2, cyclin E–Cdk2, and cyclin D–cdk4 (►Cyclin D)).
- Cdk2: Cdk2 is almost completely ordered, with only two small regions of disorder. These two regions, however, overlap with the regions that interact with cyclin-dependent kinase inhibitor protein p27/1B ($p27^{Kip1}$).
- ►Bcl-2: This antiapoptotic protein contains a large unstructured loop. Loop phosphorylation can either enhance or inhibit Bcl-2. Upon stimuli such as DNA damage (►DNA damage-induced apoptosis), p53 can inhibit Bcl-2 by binding to its loop domain, and loop phosphorylation prevents this interaction. Taxol (►Paclitaxel, ►Taxol) induces unstructured loop phosphorylation and consequent inhibition of Bcl-2, and may also inhibit Bcl-2 via direct binding. Bcl-x_L, a member of the antiapoptotic Bcl-2 family, can be deamidated (and as a result inactivated) at two asparagines in its unstructured loop.
- WT1: In ►Wilms tumor suppressor protein, alternative splicing can lengthen an unstructured linker, which increases its flexibility and inhibits DNA binding. Both the function and subcellular localization of this protein is altered.
- ►SRC: In Hck and Src, C-terminal tyrosine phosphorylation induces the unstructured linker between the SH2 and SH3 domains (►SH2/SH3 domains) to clamp both domains. This inducible "snap lock" results in protein inactivation. Unstructured domain "snap locking" may also be the mechanism by which ►Cis_2His_2 zinc fingers have their helices stabilized, thereby allowing for the correct orientation to bind to DNA major grooves.
- ►E-cadherin: The cytoplasmic domain of E-cadherin is intrinsically disordered and contains motifs that function as signals for ubiquitin-proteasome-mediated degradation. β-catenin (►APC-β-catenin pathway) binding, however, renders these sequences inaccessible.

Other Roles in Cancer Biology

Disordered domains are involved in many other aspects of cancer pathophysiology, particularly in ►hypoxia-related proteins, histone function, and in other

cell-signaling proteins. Another layer of complexity is added by the fact that intrinsically disordered regions are often located at the sites of ►chromosomal translocations. It is likely that translocations in structured domains lead to the production of nonfunctional and rapidly degraded misfolded proteins, whereas translocations in unstructured domains may lead to a fusion protein with aberrant functions (e.g., joining two structured domains from different proteins). The multiple roles of unstructured proteins in cancer are only beginning to emerge, and further analyses both on a global-scale and on an individual protein basis will advance our understanding of these domains in cancer and disease.

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Intron

Definition

Non-coding DNA which separates neighboring exons in a gene. During gene expression, introns are transcribed into RNA but then intron sequences are removed from the pre-mRNA by splicing. Intervening sequence that is removed from the pre-mRNA by the splicing reaction.

►pre-mRNA Splicing

Invadopodia

Definition

Protrusive membrane regions containing ►matrix metalloproteinases that function to degrade ►extracellular matrix. Found on the ventral surface of cultured cells, they are enriched in cytoskeletal proteins and serve as sites of matrix internalization.

►Cortactin

Invasion

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Synonyms

Tumor cell invasion; Infiltration

Definition

Is a process in malignant cells consisting of penetration of and movement through surrounding tissues.

Characteristics

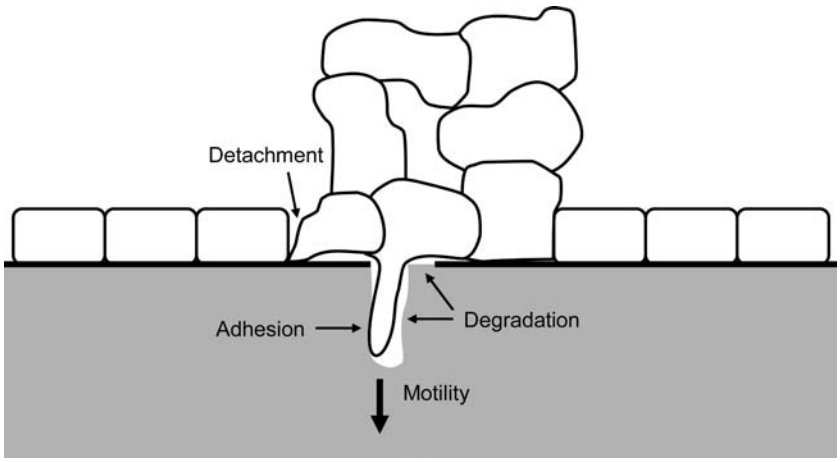
Following an initial period of cell proliferation at the tissue site of origin, malignant cells acquire the capacity to undergo invasion of the surrounding tissues. Some malignant neoplasms undergo extensive local invasion without metastasizing, but invasion is a prerequisite for the development of ►metastasis.

Although the process is not the same for all types of malignancies, the invasion of epithelial-derived malignant cells (the most common type) involves the following steps (Fig. 1):

- Detachment from adjacent cells and underlying ►basement membrane
- Disruption and degradation of surrounding matrix
- ►Adhesion to surrounding matrix components
- ►Migration through the adjacent tissue

Epithelial cells are normally connected to each other by means of ►tight junctions, ►adherens junctions, and ►desmosomes. Transmembrane ►adhesion proteins, particularly the ►cadherin family of membrane glycoproteins, are important components of these cell junctions. Epithelial cells are also adherent to an underlying basement membrane by means of focal adhesions and hemidesmosomes, and integrins are important components of these cell-matrix adhesion complexes. The detachment of tumor cells from adjacent cells and the underlying basement membrane involves disruption of such cell-cell and cell-matrix adhesion structures.

In order to invade the surrounding connective tissue, tumor cells must penetrate the underlying basement membrane. Tumor cells induce secretion of a variety of proteases that become activated and cleave basement membrane proteins, such as collagen IV and laminin. Disruption of the basement membrane provides the malignant cells access to the surrounding connective tissue. Further degradation of connective tissue components by proteases and other enzymes provides a



Invasion. Figure 1 Schematic representation of the steps involved in tumor cell invasion.

pathway that allows the tumor cells to traverse the connective tissue matrix.

Upon dissociating from adjacent cells and detaching from the basement membrane, malignant cells extend filopodia and ►lamellipodia, plasma membrane-covered protrusions of actin-rich cytoplasm, into regions of degraded connective tissue matrix. These protrusions adhere to the surrounding matrix components to initiate tumor cell motility. ►Migration through the connective tissues occurs through a repeating cycle of tumor cell extension at the front of the cell, adhesion to matrix, contraction of actin-myosin filaments, and release of cell attachments at the rear.

In addition to invading connective tissue stroma, malignant tumor cells can invade the perineurium. Particular types of malignant cells are more prone to do this than others. Invasion of the perineurium allows unimpeded tumor extension along the nerves. Invasion of ►lymphatic vessels and blood vessels results in lymphatic and hematogenous dissemination, respectively, with the production of distant metastases.

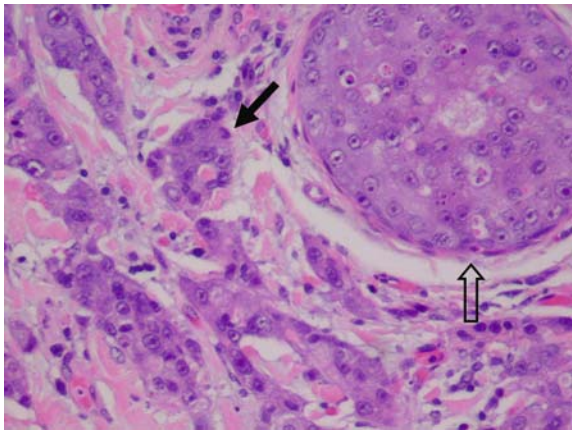
Mechanisms

Detachment of malignant cells from each other and from the underlying basement membrane, the first step in tumor cell invasion, is a consequence of altered cell-cell and cell-matrix adhesion. ►E-cadherin, a principal component of ►adherens junctions in epithelial cells, forms complexes with various members of the catenin family to promote cell-cell adhesion in normal cells. The catenins link E-cadherin to the actin filament network. Downregulation of cell-cell junctions occurs either through decreased E-cadherin expression or increased ►Wnt signaling, which depletes beta-catenin from junctional complexes.

Integrins within hemidesmosomes promote anchorage-dependent growth and survival of normal cells by signaling cooperatively with growth factor receptors to activate cell proliferation and survival pathways. Tumor cell detachment from the basement membrane results from downregulation of cell-surface integrins or dissociation of adhesion complexes.

To disrupt the underlying basement membrane and degrade surrounding matrix, tumor cells upregulate expression of a number of proteases, including serine-, cysteine-, aspartyl- and ►matrix metalloproteinases (MMPs). Plasminogen activators (►plasminogen-activating system) are among the group of serine proteases. Both MMPs and plasminogen activators have specific inhibitors involved in their regulation. Both are known to bind tumor cell surface receptors, thereby localizing matrix degradation to the invading front of the tumor, and protease-mediated cleavage of some cell surface receptors also contributes to tumor cell invasion. In addition to protease secretion by the tumor cells themselves, tumor-stromal interactions induce production of MMPs by surrounding fibroblasts and macrophages.

The migration of tumor cells is stimulated by secreted cytokines and growth factors. The cleavage products of some matrix components are also likely to promote tumor cell migration, as some are known to have chemotactic activity. In addition, alterations in Wnt and integrin signaling associated with cell-cell and cell-matrix detachment, respectively, also promote tumor cell migration. Growth factors can induce not only dissociation of adhesion complexes but also a relocalization of specific integrins to the leading edge of tumor cells. Integrins at the leading edge signal through ►RAS motility pathways to promote tumor cell migration. Wnt signaling not only leads to cell-cell detachment but also promotes tumor cell migration by inducing nuclear localization of beta-catenin with subsequent activation of genes involved in cell motility.



Invasion. Figure 2 Photomicrograph of an invasive ductal carcinoma of breast, the most common form of invasive breast cancer. The portion of the tumor undergoing invasion of the connective tissue stroma (closed arrow) is shown adjacent to a residual component of noninvasive ductal carcinoma in situ (open arrow).

Clinical Aspects

The size of the invasive tumor is used in ►tumor staging to predict the likelihood of tumor recurrence after treatment. The size of the invasive tumor is essentially a quantitative measure of invasion, and tumor size is one of the main determinants of the type and extent of therapy. Microinvasion (less than 1 mm of invasion) has minimal risk of tumor progression after treatment. Greater amounts of invasion increase the likelihood of both local and systemic recurrence (Fig. 2). Tumor invasion can extend beyond the area of involvement identified clinically, resulting in local recurrence following surgical excision. Invasion of lymphatic and blood vascular channels can lead to distant metastasis with widespread dissemination of tumor cells. Although distant metastasis is the cause of death for most cancer patients, extensive local invasion of a malignant neoplasm can compress adjacent vital structures and cause death in the absence of distant metastasis.

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Invasive Ductal Carcinoma

Definition

IDC; Ductal carcinoma refers to the development of cancer cells within the milk ducts of the breast. Invasive DC have the capability to invade into the surrounding tissue.

►Endothelins
►Ductal Carcinoma In situ

Inverse PCR

Definition

Has been used for the amplification of unknown DNA segments that lie outside the boundaries of the known sequence. Genomic DNA is digested with appropriate restriction enzymes and self-ligated at low DNA concentration. The circular DNA is used as a template for polymerase chain reaction (►PCR), using primers designed against known sequences. The resulting PCR product contains an unknown segment that is flanked by known sequences.

Inversion

Is a ►chromosomal rearrangement that occurs when a chromosome segment is clipped out, turned upside down and reinserted within the same chromosome.

Ion Channels

Definition

Are transmembrane proteins allowing the passage of ions. Most of ion channels require specific signals to switch from a closed to an open state. Activating signals include membrane potential, temperature, vibrations and a variety of ligands like neurotransmitters, hormones and ATP.

►Ether à-go-go Potassium Channels

Ionization

Definition

Is the departure of an electron from an atom.

►Proton Beam Therapy

Ionizing Radiation

Definition

Is any type of radiation with energy sufficient to eject one or more from outmost orbital electrons from the atom or molecule; this process is called ionization. Examples of ionizing radiation used in radiation therapy are protons, electrons, X- and γ-rays. The proton is the nucleus of the hydrogen atom. The hydrogen atom consists of one positively charged proton and one negatively charged external electron. The proton is about 1,800 times heavier than electron. Protons can be accelerated to high energy in a cyclotron. Hospital-based cyclotrons are being used to treat a broad spectrum of cancer patients. Electrons can be accelerated to high energy by such devices as linear accelerator. Linear accelerators are widely used in cancer therapy. A positively charged electron is called positron. ►Isotopes that emit positrons are used in a nuclear medicine imaging procedure called ►positron emission tomography (PET scan). X- and γ-rays rays are bundles of electromagnetic energy that travel with the speed of light. In many interactions X- and γ-rays act as particles without mass or charge (photons). X-rays are used in the imaging procedure called CT (►computed tomography) or CAT scan. γ-rays are used in specialized procedure called Gamma Knife stereotactic radiosurgery, an alternative to open brain surgery. The Gamma Knife consists of 201 beams of γ-radiation emitted by radioactive cobalt, focusing on one area of the brain. This new technique is very useful at treating certain malignant and benign tumors that could be inaccessible or inadequate for open surgery.

►Radiation Oncology
►Ionizing Radiation Therapy

Ionizing Radiation Induced Cancer

►Radiation Carcinogenesis

Ionizing Radiation Therapy

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Synonyms

Radiotherapy; Radio-isotope therapy; Targeted radioimmunotherapy

Definition

Ionizing radiation therapy, or radiotherapy, is the clinical use of ionizing radiations for the treatment of ►cancer.

Characteristics

►Ionizing radiation consist of photons or charged particles which interact with molecules to break covalent bonds. The aim of their use in radiotherapy is to cure cancers by killing tumor cells. This is done by the production of reactive free radicals that damage DNA, the genetic material within a cell, which is the main target. Multiple types of ►DNA damage are produced including ►DNA double-strand breaks where both strands of the helix are broken. Cells have mechanisms for ►repair of DNA double-strand breaks, but some will be repaired incorrectly or left unrepaired. Incorrectly repaired DNA damage may lead to mutations. Unrepaired double strand breaks lead to cell death from the formation of damaged chromosomes. In some situations irradiated cells can commit suicide via the process of ►apoptosis, or programmed cell death. Many things modify the effectiveness of ionizing radiations to kill cells. One important modifier is oxygen, which normally sensitizes cells to radiation. In the most solid tumors, due to poor blood supply, which is highly disrupted within a tumor, ►hypoxic regions lacking oxygen can occur which are more resistant to radiation treatment.

Radiation can be used as either a ►palliative or ►curative treatment. For palliative use, the requirement is not to cure the tumor but to reduce pain and swelling, prevent bleeding or prevent tissue damage and ulceration with a view to improving quality of life under conditions where the tumor is incurable.

For curative use the aim is to optimize the delivery of dose to the tumor whilst minimizing the dose delivered to surrounding normal tissues. Damage to surrounding normal tissues can lead to a risk of complications including loss of function and increased secondary cancer risk. In many cases radiation therapy is combined with other treatments including chemotherapy

and surgery as either an ►adjuvant, ►concomitant or ►neoadjuvant treatment.

Whole body or total body irradiation (►TBI) can also be used when bone marrow cells from another donor are to be transplanted, especially in cases of ►leukemia.

Radiation can be delivered from external beams which nowadays are electron linear accelerators (Linacs) of 6 or 18 MV. These use microwave technology to accelerate electrons onto a solid target to produce X-rays. The radiation exits the accelerator via a gantry which can move around the patient who is on a moveable treatment couch. The beams from these linacs can be directed to irradiate the tumor using either conformal or stereotactic approaches.

►Intensity modulated radiation therapy (IMRT) is becoming common where both the profile and intensity of the beam is modulated using sophisticated multileaf collimators. The aim is to shape the beam to deliver maximal dose to the tumor whilst avoiding or minimizing dose to critical tissues, such as the spinal cord.

Treatment planning is used to determine the site of the tumor and delivery regime for irradiations. The tumor size is defined by the gross tumor volume (GTV). Due to uncertainties on the extent of the tumor interaction with normal tissues at is edges, movement of the patient and organs during irradiation, a larger volume, termed the treatment planning volume (PTV) is used. Combinations of conventional diagnostic X-ray images, computed tomography (CT), magnetic resonance imaging (MRI) or more recently ►positron emission tomography (PET), which uses tracer radionuclides such as 18-Fluorodeoxy-glucose, are used to image the tumor volume to allow the PTV to be prescribed. A new approach is to use real-time imaging coupled with real-time adjustment of the beams to improve uncertainties via image guided radiation therapy (IGRT).

The dose to be delivered is dependent on the volume of the tumor, the ►radiosensitivity of the tumor cells and the surrounding normal tissue. Most radiotherapy schedules are given as fractionated doses typically varying from 1.8 to 3 Gy per day delivered over a 6–8 week period delivering total dose of up to 75–80 Gy. The aim is to maximize the effect in tumors whilst protecting normal tissues. For palliative treatments reduced numbers of fractions are typically given with higher dose per fraction.

►Brachytherapy involves the delivery of high dose within a tissue or organ using implanted seeds or wires containing radioisotopes. It is commonly used for accessible tumors such as cervix, head and neck and prostate. For example Iodine-125 seeds can be permanently placed into prostate tissue (interstitial brachytherapy) to treat prostate tumors. Radioactive wires, commonly Iridium-192 can also be placed in tissues using automatic (after-loading machines) procedures to minimize radiation exposure of personnel. The procedures aim to deliver localized high doses to tumors, minimizing dose to other critical organs and reducing side-effects.

Radioisotopes can also be infused into tumors via the blood supply to be selectively taken up at particular sites. These include using Strontium-89 to treat bone ►metastasis, Iodine-131 to treat ►thyroid cancer and radiolabeled ►somatostatin analogues or Meta-iodobenzylguanidine (MIBG) to treat ►neuroendocrine tumors. Radioisotopes can also be tagged to specific antibodies designed to recognize antigens or receptors expressed by tumors. This is called ►radioimmunotherapy. For example Iodine-131 and Ytrium-90 have been tagged to anti-►CD20 antibodies for the treatment of ►non-Hodgkin lymphoma. These have the advantage that they can be used for small tumors and disseminated diseases including metastasis but disadvantages in that uptake into tumors can be limited. The radioisotopes of choice are those which emit short-range electrons or alpha-particles to minimize killing of cells to localized regions. In the future it may be possible to combine PET imaging of specific targeted isotopes with therapy to get good estimates of dose-delivery.

Charged particles such as protons and carbon ions are used as alternative to electrons or gamma rays. Charged particles deposit their energy in a characteristic way leading to the production of a Bragg peak. This allows these radiation to produced defined dose delivery to depths within the body. This advantageous when tumors are very close to critical structures and in the use of radiotherapy in children. Protons have a similar biological effectiveness to electrons and X-rays, but carbon ions are more effective or damaging per unit dose. Carbon ions can also overcome the limitations of low oxygen concentration in tumors as they are equally effective in the presence or absence of oxygen.

Tissues respond in different ways to radiation exposure. Some react quickly, via what are termed “early effects” These can occur in weeks to a few months after exposure and mainly involve parenchymal cells. Others respond at much later times, months to years after radiation exposure, mainly involving connective tissue and are called “late effects.”

Examples of early effects include mucositis, dermatitis and depletion of the cellular compartments of the bone marrow. Many of these response can be transient in nature and dependent on the rate of turnover of epithelial cells.

Examples of late effects include radiation nephropathy, telangiectasia, lung or subcutaneous fibrosis, chronic myelopathy and bone necrosis. They tend to be restricted to slowly turning over tissues such as CNS, lung, liver, heart and kidney. Some tissues can show both early and late effects. For example, the skin can show early epidermal reactions followed by later fibrosis.

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IPL1

►Aurora Kinases

IPT/TIG Domain

Definition

A domain that has an immunoglobulin-like fold.

►MET

IQGAP1 Protein

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Synonyms

IQ motif-containing GTPase activating protein

Definition

IQGAP1 is the best-characterized member of the IQGAP family of eukaryotic proteins that were first identified in 1994 in metastatic human osteosarcoma cells. This family of proteins is found in a wide spectrum of species and is highly conserved throughout the evolution from yeast to human. The name refers to the presence of "IQ" motifs and a domain similar to Ras ►GTPase-activating proteins (GAP).

Characteristics

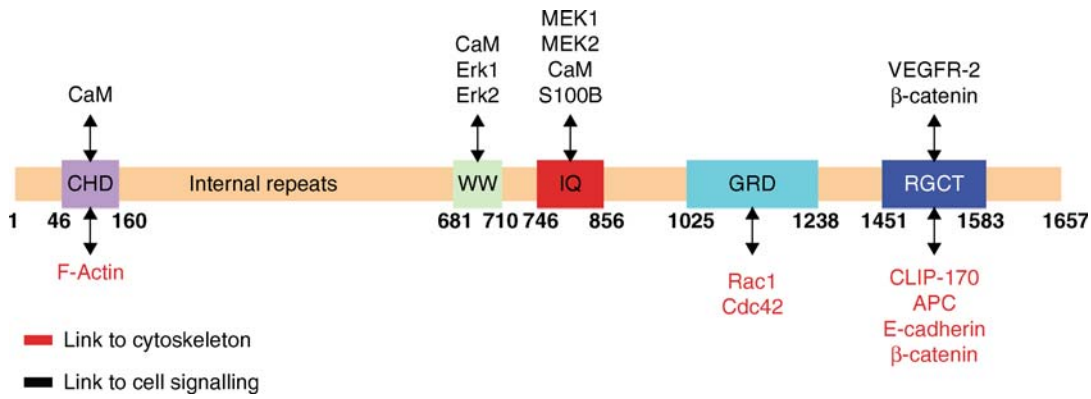
IQGAP1 is a member of IQGAP family that has been characterized in yeast, Hydra, worms, and mammals. The human IQGAP1 isoform is most similar to mouse IQGAP1 (94% identity) and has 62% identity to human IQGAP2. IQGAP1 is the prototype of this family and the most extensively studied member. IQGAP1 is a multi-modular scaffolding protein that binds to a diverse array of signaling and structural molecules which confers it many different roles in cell physiology. IQGAP1 domains and target proteins are presented in Fig. 1. IQGAP1 contains a calponin homology domain (CHD), four IQ motifs (IQ), a GAP-related domain (GRD) homologous to the GTPase-activating domains of Ras family GTPases, and a RasGAP C terminal (RGCT) domain. Moreover, if IQGAP1 presents a domain that has RasGAP homology (GRD), the catalytic activity is missing. Instead, IQGAP1 inhibits the intrinsic GTPase activity of Rac1 and Cdc42 Rho-family GTPases by stabilizing them in their active GTP-bound form.

The primary sequence of IQGAP1 contains numerous binding domains able to link many proteins involved in different biological responses. These domains are CHD, poly proline protein–protein domain (WW), IQ motif (IQ), Ras GTPase-activating protein related domain (GRD), and RGCT. Numbers represent amino acid residues. Different target proteins in red represent a link with cell cytoskeleton and, in black, a relationship to cell signaling.

Functions

For several years IQGAP1 has been demonstrated as one of the principal regulator of ►cytoskeleton architecture. More recently, IQGAP1 has been considered as a signaling crossroads by linking components of signaling cascades or membrane receptors which initiate them. Firstly, through its CHD domain or its partners as Rac1/Cdc42 Rho GTPases, IQGAP1 is able to act directly or indirectly on actin network to regulate cell ►migration and neurite outgrowth. Moreover, IQGAP1 is present at cell–cell contacts where it interacts with ►E-cadherin and β-catenin to modulate cell–cell ►adhesion. In epithelial cells, IQGAP1 captures and stabilizes ►microtubules through the microtubule-plus-end binding proteins CLIP-170 and APC near the cell cortex, leading to establishment of cell polarity and to a directional cell migration. Through its global impact on cell cytoskeleton IQGAP1 appears as a major coordinator of actin and microtubule networks which is primordial for a tuneful cell migration.

Secondly, IQGAP1 can influence a large range of cellular functions by participating in several signaling pathways and by interacting with a heterogeneous group of receptors. For example, in endothelial cells, the interaction between IQGAP1 and the cytoplasmic domain of ►VEGFR-2 (Flk-1) controls endothelial cell ►migration and proliferation. In other respects, IQGAP1 can interact with calmodulin and S100B both



IQGAP1 Protein. Figure 1 Structural binding domains and partners of IQGAP1 protein.

proteins susceptible to Ca^{2+} and with components of ►Mitogen Activated Protein Kinase (MAPK) signaling which impacts on underlying biological responses from ►migration to proliferation/differentiation processes. Surprisingly in light of the multiple functions that have been attributed to IQGAP1, *Iqgap1*-►knock-out-mice do not show any drastic defects during development or for most of their adult life. Their only obvious phenotype is a late onset gastric hyperplasia, suggesting that the protein may carry out a nonredundant function to maintain the integrity of the gastric mucosa in older animals.

IQGAP1 and Cancer

Convergent evidence hypothesize a putative role of IQGAP1 in ►carcinogenesis. IQGAP1 is overexpressed in numerous neoplasms, including ►gastric cancer, ►colorectal cancer, and ►ovarian cancer particularly at the ►invasion front. In advanced gastric carcinomas, IQGAP1 is located at cell–cell junctions where it inhibits ►E-cadherin-mediated cell–cell adhesion. These cell–cell contacts are important for self-renewal, differentiation, and regulation of cell homeostasis. Other interacting partners of IQGAP1 such as ►Rho, ►GTPases, ►calmodulin, and ►β-catenin are also known to be present and upregulated in several malignant and metastatic cancers to stimulate cell proliferation and/or to enhance cell ►migration. In human glioma cell lines IQGAP1 was overexpressed and concentrated at the leading edges of migrating cells. Recent studies provided evidence of the existence of a specific tumor subpopulation within a variety of tumor types with a tumorigenic potential that have a lot of common features with ►stem cells. The thorough characterization of these tumorigenic cells in a rat model of N-ethyl-nitrosourea (ENU, an ►alkylating agent) induced glioma and in human glioma gave rise to the identification of IQGAP1 protein as a reliable marker and allowed to discriminate ►glioblastoma (GBM) from ►oligodendroglioma. Glioma are the most common primary ►brain tumors which are subdivided into two major groups: oligodendroglioma and ►astrocytoma including the most malignant form, the GBM. In ►GBM, the expression of IQGAP1 characterizes a high mitotic cell population which forms perivascular niches. Moreover, this IQGAP1-positive cell subpopulation isolated from human GBM have numerous features of "►cancer stem-like cells" by growing in clonal tumor spheres in suspension culture and by expressing ►stem cell markers. The GBM "cancer stem-like cells" which have the exclusive ability to drive tumor formation, represent a minor population within the tumor bulk and could provide an effective target for therapies. Taking into consideration the well-known function of IQGAP1 in the regulation of cell migration, IQGAP1 might also play an essential role in the invasive character of malignant tumors by promoting the dissemination of these amplifying cancer stem-like cells.

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IRE1

Definition

Inositol requiring transmembrane kinase and endonuclease 1 (IRE1) is an ►endoplasmic reticulum (ER) transmembrane glycoprotein and it contains both kinase and RNase activities in the cytoplasmic domain. ►Endoplasmic reticulum stress and ►anoxia lead to its autophosphorylation and the subsequent activation of its RNase activity.

►Anoxia

IRES

Definition

Internal ribosome entry sites are *cis*-acting elements that recruit the small ribosomal subunits to an internal initiator codon in the mRNA with the help of cellular *trans*-acting factors.

►APAF-1 Signaling

IRF1

Definition

Interferon (►IFN) regulatory factor 1 serves as an activator of interferons alpha and beta transcription. IRF1 also functions as a transcription activator of genes induced by interferons alpha, beta, and gamma.

►Class II Tumor Suppressor Genes

Irinotecan

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Synonyms

CPT-11; Campto®; Camptosar®

Definition

An intravenously administered classical anticancer drug, registered for the first- and second-line treatment of ►colorectal cancer. Other administration routes, e.g., oral formulations, have been studied as well.

Characteristics

Colorectal cancer (►Colon cancer) includes cancer of the colon and the rectum. To give an impression of its burden of disease, in Europe about 375,000 new cases of colorectal cancer are diagnosed yearly, whereas its incidence in the US is estimated to be about 150,000 new cases per year, with a small overrepresentation of males (odds ratio, 1.1). Yearly, in Europe about 200,000 people die from colorectal cancer, making it the second most common cause of cancer death (►Aging). Over the last years, the death rate from colorectal cancer has gone down somewhat, which is in part attributed to a declining incidence. In addition, as a result of the emerging (systematic) use of screening tests in combination with higher symptom awareness, colorectal cancer is more often detected in an earlier stage (►Colorectal premalignant lesions), improving the chances of cure. Unfortunately, still more than 60% of cases are detected when the cancer is advanced, that is irresectable and/or metastasized to other parts of the body. As a result of the blood flow from the digestive system through the portal venous system directly to the liver, not infrequently metastases of colorectal cancer are confined to the liver. For most cancers, metastasized disease means a poor prognosis, and primary purpose of treatment is palliation (►Palliative therapy). However, there is increasing evidence that this picture is quite different for colorectal cancer when metastases are found solely in the liver. At present, for patients with metastases confined to the liver, resection is considered the standard of treatment. If surgery is possible, the 5-year survival rate is over 40%, otherwise median survival is about 6–9 months. Preferably, patients with irresectable and/or metastasized (not confined to the liver) colorectal cancer should be treated with anticancer drugs that have been found to be effective in the treatment of colorectal cancer, such as irinotecan.

Mechanism of Action

In the early sixties of last century, the cytotoxic potential of ►camptothecin, a plant alkaloid isolated from *Camptotheca acuminata* (family Nyssaceae), was discovered. Unfortunately, in the early development, severe and unpredictable adverse effects, in particular ►myelosuppression, diarrhea, and hemorrhagic cystitis, were seen in clinical studies, limiting its further clinical development.

Later on, these adverse effects were attributed to the rapid and full conversion of the pharmacologically active lactone form of camptothecin to its toxic and inactive carboxylate form in plasma. This finding resulted in a renewed interest in this drug and the development of various (semi-)synthetic and water-soluble derivatives, including 9-aminocamptothecin and 9-nitrocamptothecin, diflomotecan, topotecan, lurtotecan, and the ►prodrug irinotecan. Like other analogues of camptothecin, irinotecan and in particular its active metabolite ►SN-38 reversibly stabilize the "►cleavable complex," that is the covalent interaction between DNA and the nuclear enzyme ►topoisomerase I, by binding to it, thus preventing the resealing of single strand breaks. In this way, irinotecan prevents the replication fork to proceed, which ultimately results in its antitumor effect and its characteristic adverse effects on rapidly dividing tissues, such as bone marrow and intestinal mucosa.

Position of Irinotecan

After its introduction into clinical practice in the late fifties, ►5-fluorouracil has become the mainstay of chemotherapeutic treatment for advanced colorectal cancer. The introduction of irinotecan and oxaliplatin to the drug market in the nineties has resulted in significant steps forward. Indeed, in large prospective randomized trials (►Clinical trial), the addition of irinotecan or oxaliplatin to 5-fluorouracil and leucovorin has been shown to result in a significant clinical benefit in terms of response rate, progression-free survival, overall survival, and quality of life, irrespective of regimen. Both in the US and Europe, irinotecan has been approved for the first- and second-line treatment of irresectable and/or metastatic colorectal cancer in 2000. In trials investigating the benefits of the addition of irinotecan or oxaliplatin to 5-fluorouracil and leucovorin, it has been shown that overall survival increases with the number of patients who are able to receive second-line therapy. However, since only half of patients may actually be well enough to receive a second-line therapy, the choice of a most advantageous first-line regimen is critical. For the moment, taking into account all the published data regarding antitumor effects and adverse effects of irinotecan and oxaliplatin, a clear choice cannot be made for the preferential use of one of both drugs. Apart from colorectal cancer, responses to irinotecan therapy are also seen in various other types of cancer, including lung (►Non-small cell lung carcinoma), ►esophageal cancer, ►pancreatic cancer, and ►gastric cancer) malignancies. In Europe, irinotecan monotherapy is given at a dose of 350 mg/m^2 every 3 weeks, whereas in the US, irinotecan is administered on a weekly basis (125 mg/m^2). Irinotecan is also combined with both 'classical' and targeted anti-cancer agents.

Adverse Effects

Regardless of its schedule of administration, ►myelosuppression and delayed-type diarrhea are the most common severe adverse effects of irinotecan therapy (grade 3 or 4, according to the Common Terminology Criteria for Adverse Events (►CTCAE) Version 3.0). Myelosuppression is seen in about 15–20% of patients, whereas severe delayed-type diarrhea is characteristically seen in 20–25% of patients about 5 days after start of therapy. In particular the occurrence of diarrhea can be debilitating and potentially life-threatening and may have significant clinical implications, as it affects the dose that can be safely administered. The interindividual variability in both systemic and intestinal exposure to SN-38 has been associated with its occurrence. There is fairly strong evidence that patients with low capacity to detoxify SN-38, such as patients with ►Gilbert syndrome, have a higher risk of irinotecan-induced ►neutropenia as well as diarrhea. Given this association as well as the lack of sufficient information to recommend a relatively safe dose for patients with hepatic dysfunction, irinotecan should not be given to patients with Gilbert's syndrome, or serum bilirubin levels over 2.0 mg/dl and/or transaminase levels over three times the institutional upper limit of normal (in case of liver metastasis, over five times).

Other relatively frequently reported adverse effects of irinotecan include nausea, vomiting (►Emesis), anorexia, constipation, anemia, asthenia, abdominal pain/cramping, pain, and ►alopecia. If cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and early diarrhea occur, they are usually seen during, or shortly, after infusion. Since cholinergic symptoms are thought to be related to the anticholinesterase activity of irinotecan, they can be (prophylactically) dealt with intravenously or subcutaneously administered atropine.

Metabolism and Excretion

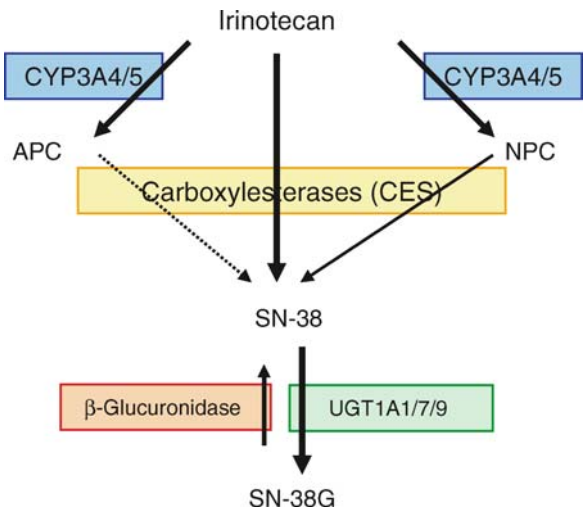
In humans, irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is hydrolyzed into its active metabolite SN-38 (7-ethyl-10-hydroxy-camptothecin) by ►carboxylesterases, present in serum, in the epithelial lining of the intestines, in tumor tissue, and in high content in the liver. It has been suggested that activation of irinotecan into SN-38 in the tumor itself might be even more important than systemic circulating SN-38 levels formed by hepatic carboxylesterases. Besides being hydrolyzed into SN-38, irinotecan is also sensitive to a Phase I oxidative reaction, mediated by ►cytochrome P450 3A (CYP3A) isoforms and resulting in the formation of inactive metabolites, including APC (7-ethyl-10-[4-N-(5-aminopentanoic

acid)-1-piperidino]carbonyloxycamptothecin) and NPC (7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin). Although in different extents, these substances are supposed to be further metabolized into SN-38 by carboxylesterases. The primary pathway of ►detoxification of SN-38 is a Phase II glucuronic acid conjugation reaction that results in the formation of a β-glucuronic acid conjugate (SN-38G; 10-*O*-glucuronyl-SN-38). This ►glucuronidation is mediated by uridine diphosphate-glucuronosyltransferase (UGT) isoforms, especially 1A1, in decreasing order of importance (Figs. 1 and 2).

Exposure to irinotecan and its active metabolite SN-38 is substantially reduced in patients concomitantly receiving CYP3A enzyme-inducing drugs, such as phenytoin, phenobarbital, carbamazepine, rifampin, and rifabutin. Indirectly lowering the exposure to SN-38 and thus reducing the potential antitumor effects of irinotecan, such pharmacokinetic (►Pharmacokinetics/►pharmacodynamics)-based drug–drug interactions may have major clinical ramifications. Since activity and expression of CYP3A are easily influenced, also complementary alternative medicines (►CAM), such as ►St. John's wort and garlic, may affect the pharmacokinetic and clinical profile of irinotecan. Conversely, inhibition of CYP3A by ketoconazole, for example, may result in lower formation of APC and NPC, leaving more irinotecan available for conversion into SN-38. Likewise, modulation of ►UGT1A1 by concomitantly taken inhibitors (such as atazanavir sulfate, indinavir, and ►genistein) or inducers (such as rifampicin and cigarette smoking) may influence systemic SN-38 levels. Although systemic circulating levels of SN-38 are relatively low, distinct relations between systemic exposure to SN-38 (defined as the area under the plasma-concentration vs. time curve; (►AUC) and risk and severity of toxicity have been demonstrated. Therefore, irinotecan should not be prescribed without taking into account the interacting potential of concomitantly used (prescribed) comedication, over-the-counter medication, and CAM-products which are often not mentioned by patients without being asked actively by their physician.

Both the parent compound irinotecan and its metabolites, including SN-38, are excreted by drug-transporting proteins from the adenosine-triphosphate (ATP) binding cassette (ABC) transporter superfamily (►ABC drug-transporters), including ABCC2 (cMOAT; MRP2), and to a lesser extent, ABCC1 (MRP1), ABCC4 (MRP4), ABCB1 (►P-glycoprotein), and ABCG2 (ABCP; MXR; BCRP), via a hepatobiliary pathway into the feces, and to a lesser extent into urine. Besides influencing the renal and hepatobiliary secretion, can play a role in acquired (multi) drug resistance, and can also affect intestinal reabsorption, since both SN-38 and irinotecan are thought to undergo reuptake from the intestines with resulting reentrance of the systemic circulation (►Enterohepatic recirculation).

In the intestines, hepatobiliary excreted irinotecan and NPC may be converted into SN-38 by local carboxylesterases, whereas SN-38G is subjected to endogenous bacterial ►β-glucuronidase-mediated deglucuronidation. In addition to a higher hepatobiliary excretion of SN-38 and a less effective detoxification of SN-38 into SN-38G in the intestinal mucosa itself, both processes are supposed to play a role in the occurrence of local toxic effects of SN-38 on the intestinal mucosa resulting in delayed-type diarrhea.



Irinotecan. Figure 1 Schematic representation of irinotecan metabolism.

Dosage Individualization

As is the case for most intravenously administered anticancer drugs, the traditional method of individualizing irinotecan dosage is by using a formula derived from weight and height (that is body-surface area; ►BSA) alone. In the US, single agent irinotecan is generally administered on a weekly basis (125 mg/m^2), whereas in Europe, irinotecan is normally administered once every 3 weeks (350 mg/m^2). However, since irinotecan pharmacokinetic parameters appear to be insufficiently related to BSA, the usefulness of normalizing irinotecan doses to BSA in adults has been questioned. The implementation of a flat-fixed dosing strategy, thus independent of BSA, has been suggested until better dosing methods become available, which might, for example, be based on factors known to impact on irinotecan ►elimination. Many attempts have been made to understand the complex pharmacokinetic profile of irinotecan. As mentioned before, the metabolic pathway of irinotecan involves various

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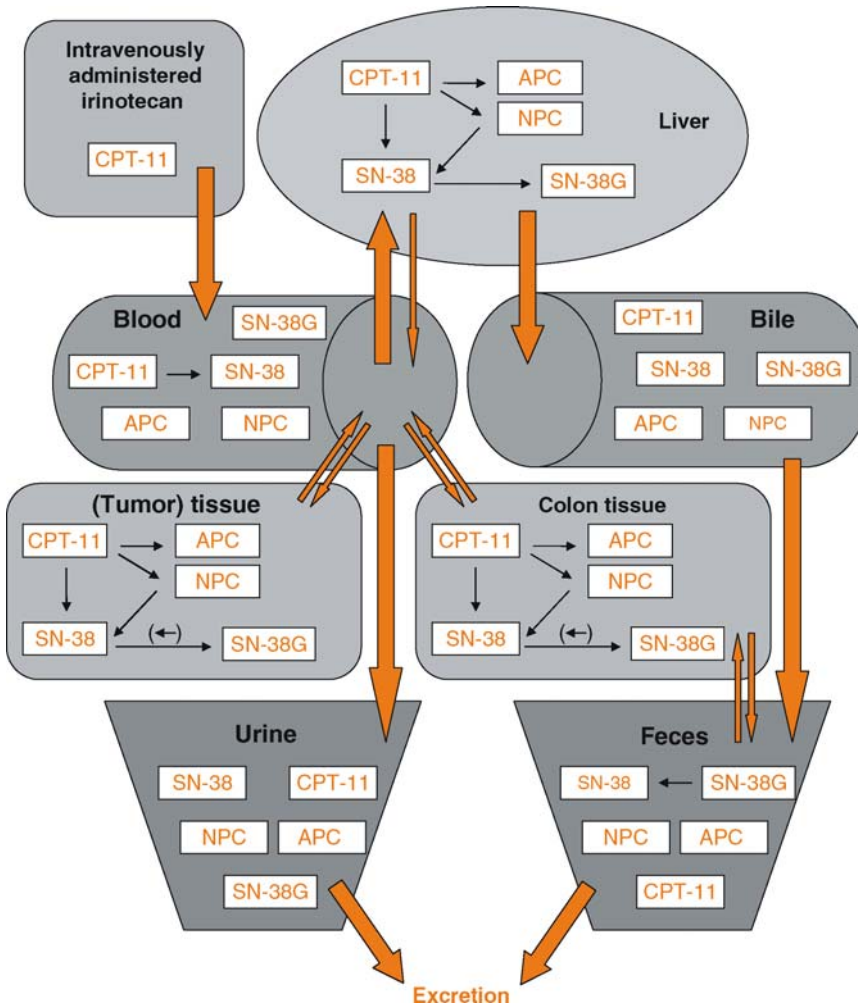

Irinotecan. Figure 2 Schematic representation of the ►disposition of intravenously administered irinotecan.

metabolizing enzymes and drug transporters. Since variations in their encoding genes may lead to a significantly altered expression and/or function, such variations may contribute to the variability in pharmacologic profiles between patients. Besides other factors, genetic variations in these genes may therefore be valuable to truly individualize and optimize irinotecan treatment.

Role of Genetic Screening

In particular, the ►*UGT1A1* gene contains many genetic variants influencing the expression and functional properties of its encoded protein (UGT1A1). Polymorphisms resulting in absent or low UGT1A1 activity, have been associated with three heritable unconjugated hyperbilirubinemia syndromes: ►Crigler-Najjar syndrome type 1 and 2, and Gilbert syndrome. Gilbert syndrome has normally no serious consequences. It is frequently seen in Caucasians and has been associated with the presence of an additional, seventh, dinucleotide (TA) insertion (*UGT1A1*28*) in the TATA-box of the *UGT1A1* promoter region, leading to a considerable reduced enzyme expression of about 30–50%. Besides being associated with decreased activity and SN-38 glucuronidation in humans, in particular the homozygous presence of *UGT1A1*28* is a well-known risk factor for the occurrence of severe toxicity. In 2005, the US Food and Drug Administration approved a molecular assay for use in identifying such patients. Following this, the package label of irinotecan was changed, now stating that "Individuals who are homozygous for the *UGT1A1*28* allele are at increased risk for neutropenia following initiation of irinotecan treatment. A reduced initial dose should be considered for patients known to be homozygous for the *UGT1A1*28* allele. Heterozygous patients (carriers of

one variant allele and one wild-type allele which results in intermediate UGT1A1 activity) may be at increased risk for neutropenia; however, clinical results have been variable and such patients have been shown to tolerate normal starting doses." Unfortunately, a precise dose reduction is not yet established, and subsequent dose modifications should be considered based on individual patient tolerance. In addition, showing the limitations and the clinical complexity of the use of ►pharmacogenetics (that is, dichotomous distributions) as basis for dosing strategies, the association between *UGT1A1*28* genotype and diarrhea is far less clear compared to neutropenia. For example, the presence of one or two *UGT1A1*28* alleles explains only about half of all cases of severe diarrhea following treatment with irinotecan, indicating its multifactorial origin. Furthermore, evidence is beginning to emerge that polymorphisms in other *UGT1A* genes may influence irinotecan-related toxicity. For example, variations in the gene encoding extrahepatically expressed UGT1A7 resulting in less transcriptional activity and lower enzyme activity have recently been associated with lower gastrointestinal irinotecan-induced toxicity and higher antitumor response.

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γ-irradiation

Definition

Gamma-(γ)-irradiation (γ-IR) is a treatment used to kill cancer cells by exposure to ionizing radiation.

►p53 Family

IRS

Definition

►Insulin receptor substrates (1–4) are targets of ►receptor tyrosine kinases.After receptor autophosphorylation, the IRS proteins are tyrosine phosphorylated and can therefore interact and activate ►SH2 domain-containing proteins. The IRS proteins can also bind to the p85 subunit of phosphatidylinositol (PI)-3 kinase and activate the serine kinase PKB/Akt pathway that regulates apoptosis.

►Insulin Receptor

Ischemia

Definition

Restriction in blood supply/flow.

ISEL

Definition

In situ end labeling; synonym to ►TUNEL.

Islet Cell Tumor

Definition

►Pancrease cancer that arises from the small clusters of cells called islets, which are scattered throughout the normal pancreas. A mass of abnormal cells that forms in the endocrine (hormone-producing) tissues of the pancreas. Islet cell tumors may be benign (noncancer) or malignant (cancerous). A pancreatic islet cell tumor is an uncommon tumor of the pancreas that arises from a cell in the pancreas, referred to as the islet cell. Normally, islet cells produce insulin and other hormones, but islet cell tumors can also produce hormones. Islet cell tumors include insulinoma, glucagonoma, and ►gastrinoma (►Zollinger-Ellison syndrome). A family history of ►multiple endocrine neoplasia type I (MEN I) is a risk factor for the development of islet cell tumors. In general, islet cell

tumors have a far better prognosis that other types of cancer of the pancreas.

►Islet of Langerhans tumor
►Pancreatic islet cell tumor
►Gastrinoma
►Pancrease Cancer
►Neuroendocrine Carcinoma

Islets of Langerhans

Definition

►Langerhans, islets of

Isochromosome

Definition

abbreviated "i" = mirror image chromosome consisting of two identical arms.

Isodose

Definition

The dose of radiation deposited in a radiation treatment field expressed either as a dose (Gy) or as a percentage of dose (%).

►Brachytherapy

Isoflavones

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Definition

Are nonsteroidal phenolic compounds, originally derived from plants, which mainly belong to the family of Leguminosae (soy bean, red clover, various beans, and sprouts). There, they play a role in plant–microorganism interactions, as part of the host's defense mechanisms. However, these molecules constitute a range of other activities as well, by which they may exert health-beneficial effects in humans.

Characteristics

History

Initial interest in isoflavones is based on the history of their discovery in the 1940s. Animals grazing on clover-rich pastures became infertile. Research revealed that isoflavones harbor estrogen-like activities, which lead to their classification into the group of phytoestrogens or plant-derived estrogens. This revelation attracted much interest of the scientific world, as conventional or classical ►hormone replacement therapy (HRTs) for women in the menopausal state were contra-indicated due to negative outcomes of long-term clinical trials.

Examples

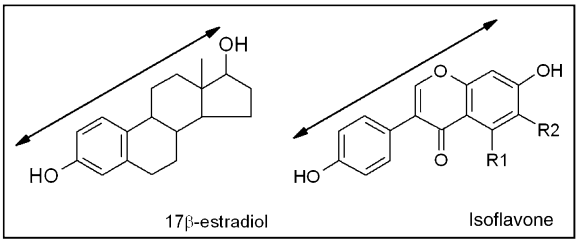
The most abundant isoflavones of soy bean include ►genistein (4′,5,7-trihydroxyisoflavone), daidzein (4′,7-dihydroxyisoflavone), and to a lesser extent, glycitein (4′,7-dihydroxy-6-methoxyisoflavone). Red clover, in contrast, contains mainly the 4′-methyl esters of genistein and daidzein, biochanin A and formononetin, respectively. As soy bean-derived products are widespread, humans are exposed to isoflavones via their diet. In addition, food supplements containing isoflavones exist, mainly manufactured to attenuate menopausal symptoms.

Metabolism

In plants, isoflavones predominantly exist in soluble, inactive sugar-conjugated forms (malonyl glucosides). After ingestion, bacterial and intestinal enzymes hydrolyze and metabolize them on their passage through the gastrointestinal tract. This allows absorption of aglycones into the blood stream and subsequent extensive biotransformations by tissue cells, possibly followed by enterohepatic circulation in case of excretion by bile. Unfortunately, knowledge of the metabolites and their potencies is scarce, as well as the active concentration to which the organs and tissues are exposed.

Mechanisms

Their potential to exert estrogen-like actions lies in their chemical structure, which resembles the planar shape of endogenous estrogen 17β-estradiol (see Fig. 1). Most importantly, the distance of 11–12 Å between the hydroxyl groups at the endings of both skeletons is virtually identical. These characteristics account for their potential to bind to the ligand binding pocket of ►estrogen receptors (ER), and subsequently induce conformation changes that translate in selective gene expression. A mechanistic model of estrogen actions



Isoflavones. Figure 1 Chemical structures of estrogen molecules 17β-estradiol and the isoflavone skeleton.

has emerged, based on the ligand-dependent conformation changes of the complex, which is dynamically modulated by recruitment of ►cofactors and ►post-translational modifications of ER and/or cofactors. The differential structure of the binding platform for cofactors between estrogens and isoflavones determines the specificity in ER-dependent gene transcription, which is furthermore context-dependent as a cell- or tissue-specific balance in coactivators or corepressors for ER exists. Due to their tissue-specific effects, isoflavones are categorized as ►selective estrogen receptor modulators (SERMs) and may be favorable as alternatives in HRT to combat ►osteoporosis and other menopause-associated complaints such as climacteric changes (night sweats, hot flushes), mood fluctuations, vaginal atrophy, etc.

Besides this hormonal function, most studies support their role in cancer prevention and treatment. Many different mechanisms have been reported, including induction of tissue differentiation, inhibition of tissue growth by induction of ►apoptosis, or inhibition of progression through the cell cycle. Isoflavones also diminish the risk on cancer as ►antioxidants, by scavenging radicals and as such, counteracting oxidative stress that intracellularly affects the integrity of DNA and proteins. By inhibiting enzymes in the hormone metabolism (aromatase), isoflavones lower the endogenous estrogen production, which is an essential growth factor for ER-dependent cancer cells. Another essential activity of isoflavones is their capacity to inhibit ►tyrosine kinases, which plays an important role in transmitting growth signals from the environment via growth factor receptors (such as ►EGFR, ►HER-2/neu, ►VEGFR, ►IGFR), to elicit intracellular responses. Also, cross-talk between these signaling pathways and ER in cancer cells leads to estrogen-independent activation of ER proteins, tumor growth, and antiestrogen therapy resistance. As such, interference of isoflavones on the level of tyrosine kinases may affect many growth processes.

At the molecular level, many studies revealed that isoflavones interfere with Rel-dependent transcription. These ►NFκB proteins (of the Rel family) control gene expression involved in apoptosis and ►inflammation. Most breast cancer cells amplify Rel genes or express constitutively active Rel transcription factors to elevate their survival capacity and metastatic potential. Rel inhibition by isoflavones may be multifaceted and acts at multiple levels on the signaling pathways towards activation of NFκB as well as ER.

Finally, mice fed with a soy isoflavone diet reveal changes in ►DNA methylation patterns in their offspring. As such, prevention or reversal of hypermethylation-induced inactivation of key tumor suppression genes by isoflavones may be an effective approach to protect against cancer.

Diseases

Besides their possible favorable effect on physiological systems that depend on a good endocrine balance, isoflavones may influence disease onset or pathology too. Epidemiological studies on the relation between isoflavone intake and cancer underscore the potentially protective function of isoflavones. These studies have observed large ethnic differences in breast and prostate cancer rates, among other cancer types. Relatively to US people, a significantly lower incidence and mortality of these hormone-dependent cancers exists in Asian populations, who consume higher amounts of soy bean-based foods. To determine the extent of genetic versus environmental factors, migrant studies have been performed. Emigration towards the West substantially significantly increases the risk on breast cancer and leads to cancer risks tending towards the levels of the native people in the destination country. These findings suggest that rather environmental, behavioral and lifestyle factors influence the cancer frequency between populations. Among these factors, isoflavones play a substantial role as molecular investigation has shown their anticarcinogenic and carcinoprotective feature (see mechanisms). However, controlled clinical trials with isoflavone food supplements are absolutely required to definitely rely cancer protection on isoflavones in man.

In 1863, Rudolf Virchow noted ►leukocytes in neoplastic tissues, and speculated that the chronic inflammatory infiltrate, observed in tumors, reflects the origins of cancer. It has been estimated that more than 15% of all cancers are initiated by chronic inflammatory diseases. This is exemplified by hepatitis or inflammatory bowel disease that predispose to liver cancer or colon cancer, respectively. However, for many inflammation-associated cancers, the initiating influence remains obscure. The finding that long-term use of ►nonsteroidal anti-inflammatory drugs (NSAIDs), such as ►aspirin, markedly decreases ►colorectal cancer risk and strengthens the proposed link between inflammation and cancer (Fig. 2). The proposed mechanism leading to neoplasia includes the ►respiratory burst from inflammatory cells that launches superoxide free radicals, which contribute to malignant transformation by peroxidating lipids in cellular and organellar membranes. Other reactions by free radicals with amino acids or DNA result in protein

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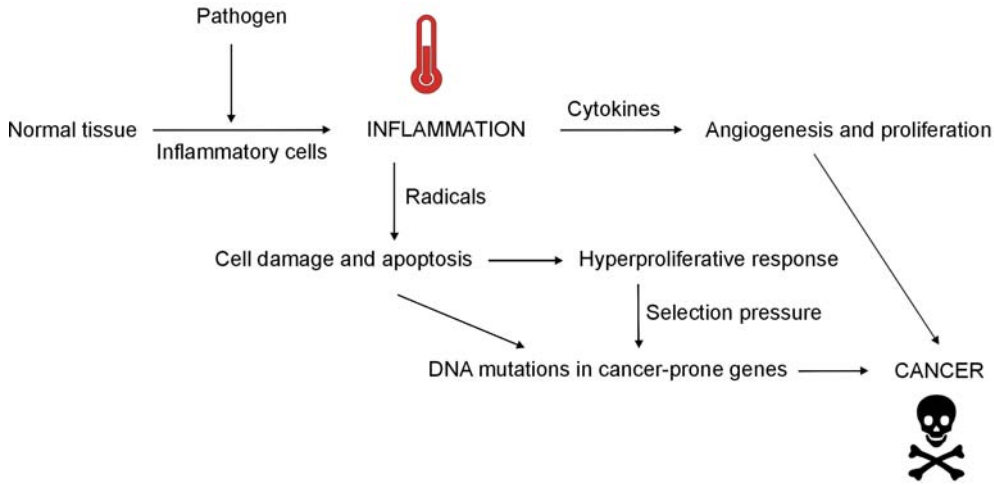

Isoflavones. Figure 2 Overview of the mechanisms and processes that provide the link between inflammation and cancer.

fragmentation or single-stranded breaks and genetic mutations, respectively. Such cellular damage stimulates apoptotic cell death which elicits a hyperproliferative response in the surrounding tissue and selects for outgrowth of cancer cells. Ultimately, populations of cells bearing survival advantages constitute the phase of malignant transformation and cancer progression, which is further enhanced by ►cytokine-mediated ►angiogenesis and growth. In turn, cancer cells avoid immunosurveillance through outgrowth of poorly immunogenic tumor cell variants (immunoselection) and through subversion of the immune system, by actively suppressing the immune response. Since isoflavones act as antioxidant and interfere with inflammatory cytokine expression, they may attenuate neoplastic transformation by interrupting the intimate connection between (chronic) inflammation and cancer initiation.

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Isoform

Definition

Is a structurally and functionally related form of a protein. Isoforms, also referred to as isozymes, are enzyme molecules that catalyze the same reaction but their other properties (e.g., domain organization or way of regulation) differ. Protein isoform is a version of a protein with some small differences, usually a ►splice variant or the product of ►posttranslational modification.

Isografts

Definition

The engraftment of organs, cells or tissues between genetically identical individuals.

►Graft Acceptance and Rejection

Isopeptide Bonds

Definition

Refers to a chemical bond between a carboxyl group and an amino group. Isopeptide bonds are similar to peptide bonds that are found in amino acids except for which amino group participates in the bond.

Isoprenoid

Definition
A major class of nonsaponifiable lipids that occur in plants, animals, and bacteria. They are characterized by chains consisting of modular groups of five carbon atoms.

►Rho Family Proteins

Isoprostanes

Definition
Are derived from the free radical non-enzymatic oxidation of arachidonic acid; the best characterized is F2-Isoprostane endowed with proinflammatory properties.

►Lipid Mediators
►Arachidonic Acid Pathway

Isothiocyanates

Definition
A group of naturally-occurring and synthetic compounds with strong pungency. Many of them occur in cruciferous vegetables. Isothiocyanates are electrophiles that readily bind covalently with proteins.

►Sulforaphane

Isotope

Definition
Contains the atoms of a chemical element that differ only in the number of neutrons but not in the number of protons. Every element contains stable or instable (radioactive) isotopes. Hydrogen for instance has two isotopes: deuterium whose nucleus has one proton and one neutron and tritium whose nucleus has one proton and two neutrons.

►Radon
►Radiation Oncology

ITAM

Definition
Immunoreceptor tyrosine-based activation motif, defined by the consensus sequence YxxL/I x_6 YxxL/I (whereby x denotes any amino acid), which mediates cellular activation.

►NKG2D Receptor
►Chimeric T-cell Receptors

Itch

Definition
Is the second HECT (homology to the E6-associated protein C terminus) domain ►E3 ubiquitin ligase found to control programmed cell death through ubiquitin-induced degradation of antiapoptotic proteins.

►FLICE Inhibitory Protein

ITIM

Definition
Immuno-receptor inhibitory motif that contains a phospho-tyrosine residue surrounded by valine and leucine residues.

►CEA Gene Family

Ixabepilone

►Epothilone B Analogue

J1 220/200

►Tenascin-C

JAK

Definition

Janus kinase, family of intracellular nonreceptor tyrosine kinases that transmit cytokine-mediated signals. JAK1, Janus kinase 1; JAK2, Janus kinase 2; JAK3, Janus kinase 3. Transduce cytokine-mediated signals via the JAK-STAT pathway.

►Interleukin-4
►Signal Transducers and Activators of Transcription in Oncogenesis
►Suppressors of Cytokine Signaling

JAK2

Definition

Janus kinase 2; is a cytoplasmic protein kinase associated with the receptors of many cytokines and growth factors, including erythropoietin and thrombopoietin receptors. Engagement of the cytokine receptor with ligand results in the phosphorylation of both receptor and associated JAK2, which, in turn, activates specific pathways.

►Polycythemia

Japanese Silver Apricot

►Ginkgo Biloba

Jasmonates in Cancer Therapy

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Definition

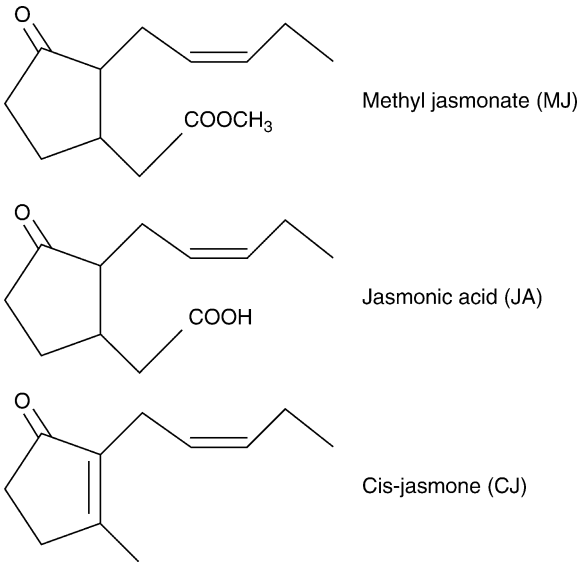
Jasmonates are plant stress hormones that exhibit anticancer activities.

Characteristics

Jasmonates are a family of plant stress hormones. They are lipid regulators that mediate responses to mechanical and infectious stresses in plants. These compounds are derived principally from linolenic acid, and are structurally similar to certain ►prostaglandins (Fig. 1). Programmed cell death often accompanies the antimicrobial response of plants, resulting in the formation of a zone of dead cells around the infection site. Thus, cellular suicide is a characteristic of the ►stress response in both plants as well as animals.

Anticancer Activities of Jasmonates In Vitro and In Vivo

Jasmonates including jasmonic acid and methyl jasmonate (►MJ) can suppress proliferation, induce necrosis and ►apoptosis in various types of ►hematological malignancies, ►leukemia, and ►lymphomas and solid cancers (►colon cancer, ►breast cancer, ►prostate cancer, ►lung cancer, hepatoma, ►melanoma). These compounds are effective against ►drug resistant cancer cells expressing mutant ►p53 or overexpressing ►P-glycoprotein. Interestingly, jasmonates bypass the mutant p53-induced apoptotic block by inducing necrosis. In addition, jasmonates were found to kill preferentially transformed cells, even when these are part of a mixture containing normal and leukemic cells in blood drawn from ►chronic lymphocytic leukemia (CLL) patients. Jasmonates synergize with various chemotherapeutic drugs in their cytotoxic activity. Furthermore, they are capable of suppressing cell motility (an ability essential for ►metastasis) at subtoxic concentrations. MJ prolonged significantly the survival of lymphoma-bearing mice (when



Jasmonates in Cancer Therapy. Figure 1 Members of the jasmonate hormones family occurring in plants.

administered orally as a monotherapy) and leukemia-bearing mice (when administered i.v. together with ►adriamycin). Furthermore, MJ suppressed the colonization of mouse lungs in an experimental metastasis melanoma model. Several studies compared various synthetic derivative of MJ and discovered some that exhibit superior activity in vitro, e.g., methyl 4,5-didehydrojasmonate, and even in vivo, i.e., 5,7,9,10-tetrabromo derivative of MJ.

The Mechanisms of Action of Jasmonates in Cancer Cells

Two major sources of cellular ATP, ►oxidative phosphorylation and ►glycolysis, as well as various mechanisms of ATP consumption, determine the steady state levels of ATP. ATP depletion can result in different settings in either necrosis or apoptosis. MJ induced a decrease in ATP cellular levels hours before signs of cytotoxic effect could be recorded, in a variety of cancer cell types. A positive correlation has been established between the susceptibility of a given cell type to the cytotoxic effect of MJ and the degree of ATP depletion induced in that cell. It was hypothesized that jasmonates induce cancer cell death via a pathway involving ATP depletion. MJ-treated B lymphoma cells exhibited a rapid time- and dose-dependent decrease in cellular ATP levels. When these cells were pretreated with oligomycin (►OM; an inhibitor of oxidative phosphorylation) before treatment with MJ, it did not increase ATP depletion induced by MJ. It thus appears that MJ and OM act on the same ATP biosynthetic source, namely, the ►mitochondria; and that MJ perturbs these organelles to an extent that renders the OM effect irrelevant. Inhibition of glycolysis (by ►2-deoxy-D-glucose) enhanced significantly the effect of MJ on ATP levels, yielding a drastic depletion in cellular ATP levels. Accordingly, high glucose levels protected the B lymphoma cells from MJ-induced ATP depletion, while high levels of pyruvate did not. These results suggest again that in the presence of MJ, mitochondria are unable to utilize pyruvate in order to generate ATP via oxidative phosphorylation. Thus, these findings support the hypothesis that ATP depletion is a major mechanism of MJ-induced cytotoxicity. Furthermore, oxidative phosphorylation in the mitochondria appears to be the target of MJ-induced bioenergetic perturbation. Consequently, the direct effects of jasmonates on mitochondria isolated from cancer cells were studied. Jasmonates induced mitochondrial membrane depolarization in leukemic cells. Further analysis focused on the potent jasmonate derivative MJ. Mitochondria were isolated from human leukemia and hepatoma cells, and their fate in the presence of MJ was determined. The permeability transition pore complex (►PTPC) regulates movement of compounds across the mitochondrial membrane. Abnormally long opening of this pore can be associated with cytochrome c escape into the cytosol, initiating a cascade of molecular events, which culminates in cell death. MJ induced the release of cytochrome c from mitochondria isolated from leukemia and hepatoma cells. However, MJ did not induce release of cytochrome c from mitochondria isolated from normal lymphocytes, suggesting that the preferential effects of jasmonates on cancer cells stem from differences at the mitochondrial level. Inhibitors of the PTPC opening that act via interaction with proteins in the mitochondrial inner membrane: cyclosporin A and bongkrekic acid inhibited the MJ-induced release of cytochrome c from mitochondria isolated from cancer cells. To summarize the bioenergetic mechanism, jasmonates are capable of perturbing directly and selectively mitochondria from cancer cells, thereby depleting their ATP generation capacity, resulting in cell death. This proposed mechanism of action fits the findings that MJ-induced cytotoxicity is transcription- and translation-independent. An essential issue that needs to be addressed is the basis for the selective effect on mitochondria from transformed cells. Several findings suggest that the composition and function of mitochondria in cancer cells and normal cells differ. These include a higher mitochondrial membrane potential, possible modulation of the expression of PTPC components, and enhanced rates of ATP generation through glycolysis rather than through oxidative phosphorylation (a phenomenon known as the ►Warburg effect) in cancer cells.

Two additional mechanisms of action were reported as instrumental in the anticancer activities of jasmonates. One such mechanism is based on the concept of

►redifferentiation, according to which an anticancer agent may suppress neoplastic growth by inducing a reprogramming of the cellular phenotype, resulting in a state closer to its normal tissue of origin. Several markers of differentiation were induced by MJ in HL-60 cells. These include nitroblue tetrazolium (►NBT) reduction, a typical marker of myelomonocytic differentiation (which was also induced by MJ in other myelomonocytic leukemia cells, e.g., U937 and THP-1); Morphologic differentiation into ►granulocytes with some properties of monocytes such as monocytic granules; Expression of both monocyte-specific surface antigen ►CD14 and granulocyte-specific antigen ►CD15; Finally, MJ induced α-naphthyl acetate esterase activity, a marker of monocytic differentiation. Known inducers of granulocytic and monocytic differentiation, all-*trans* retinoic acid and 1α,25-dihydroxyvitamin D_3, respectively, synergized with MJ in the induction of HL-60 differentiation. MJ induced mitogen-activated protein (MAP) kinase activity in HL-60 cells, and PD98059 (a ►MAP kinase inhibitor) suppressed differentiation (NBT reduction) induced by MJ. Thus, MAPK signaling is probably functional in the redifferentiation induced by MJ. MJ was reported to induce the expression of the transcription factor CCAAT/enhancer-binding protein (C/EBP) δ in HL-60 cells, which might contribute to MJ's ability to induce granulocytic differentiation in human myeloid leukemia cells.

A third mechanism of action is focused on ►reactive oxygen species (ROS). MJ-induced ►apoptosis in A549 human lung adenocarcinoma cells can be suppressed by certain antioxidants, including N-acetyl cysteine and catalase (specific for hydrogen peroxide), but not by inhibitors of hydroxyl radicals and superoxide ions. MJ induced an increase in the proapoptotic members of the ►BCL-2 family, Bax and Bcl-X_s, but not in the levels of antiapoptotic proteins, BCL-2 and Bcl-X_L. Catalase prevented the MJ-induced modification in the levels of BCL-2 family members. Thus, induction of apoptosis in A549 cells by MJ appears to be mediated by a cascade involving hydrogen peroxide generation, and an increase in the expression of proapoptotic members of the BCL-2 family of proteins. Since BCL-2 proteins affect mitochondria, and MJ treatment enhanced the levels of mitochondrial ROS in A549 cells, ROS may be involved in MJ-induced mitochondrial perturbation in these cells. This would further support the findings positioning mitochondria at the heart of the jasmonate anticancer mechanism of action.

Biochemical Outcomes Shared by Jasmonate-Treated Plant and Animal Cells

The basis for the study of jasmonates as anticancer agents is in fact part of a much larger scientific endeavor to identify plant-derived molecules as novel potential bio/chemotherapeutic drugs. It is therefore of great significance to identify biochemical outcomes shared by jasmonate-treated plant and animal cells, since such molecular events may further direct the search for new and promising drug candidates. Studies suggest that jasmonates can induce similar metabolic, i.e., ROS generation, signaling, i.e., MAP kinase induction, and stress-associated, i.e., heat shock proteins (HSP) expression, modifications in plant cells as well as in cancerous animal cells. Such alterations are known to mediate cell death and suppression of cellular proliferation.

Conclusions

Since 2002, several research groups in different countries have described the anticancer effects of the plant family of stress hormones called jasmonates. The search for new anticancer agents is mostly driven by the excessive toxicity of currently available chemotherapeutic drugs, as well as the resistance emerging sooner or later toward those drugs. Thus, jasmonates with their high level of selectivity toward neoplastic cells and their efficacy against drug resistant cancer cells, present a unique opportunity to become a novel class of anticancer drugs consisting of a structure dissimilar to any known family of cancer drugs. Furthermore, the most well studied mechanism of action of jasmonates, that of bioenergetic depletion, positions these compounds in the forefront of a very timely approach to cancer therapy based on manipulations of the energy metabolism peculiar to cancer cells. This approach has recently been the focus of leading research groups aiming at decreasing the ATP levels in cancer cells via modifications of its biosynthesis and/or catabolism. Detailed analysis of the effects of jasmonates on cancer cell energy metabolism will undoubtedly embolden our understanding of their mechanism of action, and should eventually lead to the application of jasmonates in clinical oncology.

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Jaundice

Definition
Is the yellowing of the skin, the whites of the eyes (conjuctiva) and mucous membranes caused by increased levels of bilirubin in the blood that may be due to liver damage as well as other diseases. Discoloration is the result of increased levels of bilirubin in the blood due to obstruction of biliary drainage from the liver through the biliary tree into the small intestine.

►Cholangiocarcinoma
►Bile Duct Neoplasms

Jaw Osteonecrosis

Definition
Presence of non-healing exposed necrotic bone in the mandible, maxilla or palate.

►Bisphosphonates

J-Domain

Definition
A conserved domain that stimulates the ATPase activity of the ►heat shock protein (Hsp70) family. First identified in the *E.Coli* ►co-chaperone DNAJ, it has since been observed in eukaryotic homologues i.e. Hsp40.

►Molecular Chaperones
►Hsp90

Jelly Belly

►Pseudomyxoma Peritonei

JFY1

►PUMA

JNK

►Jun N-terminal Kinase
►Mitogen-Inducible Gene 6 (MIG-6) in Cancer
►JNK Subfamily

JNK1

►JNK Subfamily

JNK2

►JNK Subfamily

JNK3

►JNK Subfamily

JNK Cascade

Definition
Is a ►map kinase MAPK phosphorylation cascade that results in JNK activation. JNK cascade consists of three kinases: MAPKKK-MAPKK-JNK (MAPK).

►JNK Subfamily

JNK Subfamily

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Synonyms

JNK1; SAPKγ; MAPK8; JNK2; SAPKα; MAPK9; JNK3; SAPKβ; MAPK10; c-Jun N-terminal kinase; Stress-activated protein kinase

Definition

JNK is an intracellular protein kinase that transmits rapidly and efficiently various different types of signals originating from outside of a cell in the process called ►signal transduction. Like other protein kinases, JNK is an enzyme that transmits its signals via phosphorylating its specific substrate proteins. The most studied JNK substrate is c-Jun, a component of the dimeric transcription factor ►AP-1, which gives rise to its name: c-Jun N-terminal kinase. Another name for JNK is a stress-activated protein kinase (SAPK). The p38 MAPKs are also called SAPKs and thus the term SAPK actually refers to both JNK and p38 subfamilies.

Characteristics

JNK subfamily consists of three ►isoforms: JNK1, JNK2, and JNK3. They are encoded by three separate genes: *MAPK8, MAPK9* and *MAPK10*. Alternative ►splicing of these genes can produce at least ten different JNK mRNAs (four JNK1, four JNK2, and two JNK3 splice variants). The JNK protein products that result from these mRNAs are ~46 and 55 kDa in size and differ from each other in their ability to bind to and phosphorylate their substrates like c-Jun (AP-1) and ►ATF2 (AP-1) and in the length of their carboxyl terminus. The JNK1 and JNK2 are ubiquitously expressed and the expression of JNK3 is almost exclusively restricted to heart, brain, and testis.

JNK is constitutively present in cells and its function is regulated by activation. JNK is activated via specific upstream kinase cascade known as ►JNK cascade or ►MAP kinase cascade and phosphorylation events resulting in the phosphorylation of the specific threonine and tyrosine motifs (Threonine–Proline–Tyrosine; TPY) close to the amino terminus of JNK. These motifs are identical in all the different JNK isoforms and splice variants. The specificity of the JNK signaling is believed to be achieved by spatiotemporal regulation of the association of JNK and its upstream kinases with the specific JNK ►scaffold proteins leading to controlled JNK activation. Additional level of specificity can be achieved by the existing isoforms, splice variants and substrates of JNK within the cell, the specific JNK upstream kinases and regulators involved, as well as by cross talk between JNK and other signaling pathways activated by the given stimulus.

JNK can transmit various different types of signals within the cell including inflammatory, developmental, growth-supporting, cancer-promoting, ►apoptosis-inducing, and other stress-related signals. The most studied JNK activators involve ►UV radiation and ►TPA as well as various growth factors and cytokines. JNK can also be activated by ►reactive oxygen species. One important evidence supporting for the role of JNK subfamily in cancer is the fact that JNK resides on the same signaling pathway or is known to signal via many known cancer-promoting ►oncogenes, ►tumor suppressors, and cellular survival proteins that protect cells from apoptosis or ►programmed cell death, including proteins such as ►RAS, ►Myc, ►Her2/neu, ►SRC, ABL (►BCR–ABL), c-Jun (AP-1), ►p53 protein, ►BCL-2, and ►AKT among others.

JNK transmits signals via phosphorylating its specific substrate proteins. The phosphorylation of substrate will change its function or availability by, for example, changing its conformation, activity or stability, and thereby leading to cellular response. JNK is a Serine/Threonine kinase and the consensus JNK phosphorylation site is Serine or Threonine followed by Proline (SP or TP). This sequence is, however, quite common in proteins and can not be used by itself to predict a JNK substrate. The first found and most studied JNK substrate is the protooncogene c-Jun, a part of the dimeric transcription factor ►AP-1. JNK can also phosphorylate other transcription factors, as well as proteins involved in signal transduction, protein degradation, ►migration and programmed cell death, cellular processes whose regulation is altered in cancer.

Experimental evidence suggests both ►tumor suppressor and tumor initiator role for JNK. Probably one of the strongest evidence for the tumor initiator role of JNK comes from the findings that the oncogenic ►RAS augments the activity of JNK and that JNK augments the oncogenic activity of AP-1. Supporting its tumor suppressor role, mutations in JNK3 that inactivate it have been found in human brain tumors. Furthermore, inactivating mutations of ►MKK4, a JNK upstream kinase, associated with reduced JNK activity have been found in human colon cancer, pancreas cancer, breast cancer, and prostate cancer.

Studies of JNK ►isoform-specific knockout mice (►Knockout mouse) and cells derived from them point for divergent biological functions for different JNK isoforms in respect to cancer as well as in respect to other diseases and cellular processes. For example, JNK2 knockout mice are more resistant to

►TPA-induced experimental skin cancer (►Skin carcinogenesis) and mice lacking JNK1 are less susceptible to diethylnitrosamine-induced hepatocarcinogenesis (►Hepatocelxlular carcinoma) than corresponding wild-type mice. Isoform-specific differences can also be observed in RAS transformation of immortalized (►Senescence and immortalization) murine fibroblasts. Experiments involving down regulation of isoform-specific messenger RNAs and subsequent protein levels by expression of antisense oligos or overexpression of ►dominant negative JNK isoforms for the competition of JNK substrates in cells demonstrate different roles for JNK1 and JNK2 in the proliferation of some human cancer cell lines. These studies strongly support for the development of JNK isoform-specific inhibitors. The data of the differences of the various JNK isoforms in cancer have just begun to emerge. The role of different JNK splice variants in cancer development and progression as well as their usability for the development of cancer therapeutics is a subject that will most likely be explored in the near future.

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JNK-Interacting Protein

Definition

JIP; Is a family of scaffold proteins that facilitate signaling through the ►JNK pathway. There are three JIPs such as MAPK8IP1 (MAPK8 interacting protein 1), MAPK8IP2 (JNK interacting protein 2), and MAPK8IP3 (mitogen activated protein kinase 8 interacting protein 3).

►Doublecortin

JUN

Definition

Is a proto-oncogene homologous to the avian sarcoma virus 17 oncogene. It is an early response gene of mitogenic and radiation stress signals. It encodes the transcription factor jun, which forms heterodimers with ►fos and related proteins that bind to the ►AP-1 consensus site of many gene promoters.

►Ubiquitination

Jun N-terminal kinase

Definition

JNK; Is a subfamily of mitogen-activated protein kinases (MAPKs) primarily activated in response to environmental stresses and proinflammatory cytokines. JNKs phosphorylate two regulatory amino acids (Ser63 and Ser73) in the N-terminus of the transcription factor c-Jun; however, many other important substrates for JNKs are now identified. One of them is doublecortin (DCX).

►Doublecortin
►MAP Kinase

Junctional Complex

Definition

The collection of various types of junctions, mainly found in epithelial cells. The junctions form a complex of ►adhesion molecules integrated with the ►cytoskeleton. In vertebrates, the most apical junction is the tight junction, followed by the ►adherens junctions and the ►desmosomes.

►Cell Adhesion Molecules
►Tight Junctions

K^{trans}

Definition

The volume transfer coefficient of contrast agent between the blood plasma and the extracellular extravascular space; used as a biomarker in clinical trials of angiogenesis inhibitors.

►Dynamic Contrast-Enhanced Magnetic Resonance Imaging

K Cells

►Activated Natural Killer Cells

K Lymphocyte

►Activated Natural Killer Cells

KAI1

►Metastasis Suppressor KAI1/CD82

Kallikrein-related Peptidases

►Kallikreins

Kallikreins

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Synonyms

Tissue kallikreins; Kallikrein-related peptidases

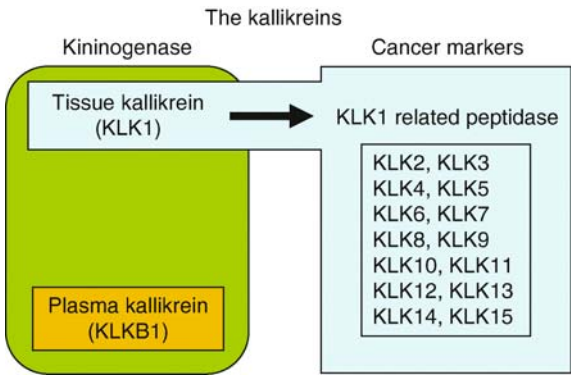
Definition

Kallikreins are a family of ►serine proteases encoded by genes clustered on chromosome 19q13.4. Their expression is associated with progression of several cancers, and they serve as ►biomarkers. Among the kallikreins, ►prostate specific antigen (PSA; kallikrein 3, KLK3) is the most well-known kallikrein.

Characteristics

Kallikreins play important roles in cancer development. For several decades, kallikreins have been known as enzymes that generate bioactive peptides, called kinins, involved in blood-pressure regulation. When the first kallikrein (kallikrein 1) was discovered in 1930, the enzyme was reported to have a hypotensive effect and be present at high concentrations in the pancreas, the organ known as *kallikreas* in Greek. It is now known that kallikrein 1 produces ►bradykinin from ►kininogen. In addition to kallikrein 1, another enzyme found in the blood plasma and encoded by the *KLKB1* gene in chromosome 4q35 also produces bradykinin. To distinguish these two different enzymes, kallikrein 1 is also known as tissue kallikrein and the second enzyme is known as plasma kallikrein. Of all the kallikreins, only kallikrein 1 (tissue kallikrein) and plasma kallikrein have significant kininogenase activity. Discoveries of kallikrein 1 related peptidases and their association with cancers have given new meaning to the term kallikrein (Fig. 1).

More than 20 kallikreins with high sequence similarity to kallikrein 1 were discovered in rat and mouse salivary glands in the 1980s and were assigned to a serine protease family known as the glandular kallikrein family. However, only two kallikreins with homology to kallikrein 1 have been identified in humans. For about two decades, the human kallikrein



Kallikreins. Figure 1 The kallikrein proteases. Kallikreins can be categorized based on their function or structural homology. The conventional kallikreins include tissue and plasma kallikreins, which are kininogenases. The tissue kallikrein-related peptidases are increasingly recognized as cancer markers.

gene family consisted of only three members: *KLK1* (tissue kallikrein, pancreatic/renal kallikrein), *KLK2* (glandular kallikrein), and *KLK3* (prostate specific antigen). Owing to the Human Genome Project and the increased numbers of available genome sequences, a cluster of serine protease genes adjacent to kallikrein 1 have been identified on chromosome 19q13.4. The chromosome location, sequence similarity, and similar expression patterns of these genes in many tissues indicate an extended kallikrein gene family. At present, the human kallikrein family consists of 15 structurally homologous genes.

The Kallikrein Genes

The kallikrein gene family members cluster consecutively on chromosome 19q13.4 and all have several common features in their gene structure. For example, all 15 kallikreins have five coding exons, the first of which contains the 5′-untranslated region. Kallikreins are serine proteases and contain the catalytic triad characteristic of serine proteases. The three amino acid residues – histidine, aspartate, and serine – that constitute the catalytic triad are conserved in the kallikreins. The catalytic triad residues are located on the same exons in all kallikreins: histidine near the end of the second exon, aspartate in the middle of the third exon, and serine at the start of the fifth exon.

Kallikreins are expressed in various tissues at different levels. The most ubiquitous kallikreins, *KLK10* and *KLK11,* are expressed in more than 30 organs. Most kallikreins are expressed in the salivary gland as well as endocrine-related tissues, and the mRNAs of all 15 kallikreins are detectable in the prostate, testis, thymus, and thyroid. All 15 kallikrein genes have more than one transcriptional variant. There are more than 50 splice variants of the 15 kallikrein genes. Some gene transcripts of kallikreins are tissue-specific. Kallikrein expression is regulated by many stimulators and inhibitors. The expression patterns of many kallikrein gene transcripts reflect the different degrees of malignancy and may have clinical diagnostic and prognostic values. The strong expression pattern of kallikreins in endocrine-related tissues prompted several investigators to conduct experiments on the effects of sex steroid hormones on kallikrein expression regulation. The investigations found that most kallikreins are regulated by steroid hormones such as androgens, estrogens, progestins, glucocorticoids, either individually or in different combinations. The results also showed that, in different tissues, different kallikreins are regulated by different steroid hormones. For example, the expression of *KLK4* is upregulated by androgen in prostate and breast cancer cells but by estrogen in ►endometrial cancer cell lines.

The Role of Kallikreins in Tumor Progression

Over the past several years, intensive research on the expression of kallikreins in several ►hormone-related cancers has shown that kallikrein expression levels are associated with cancer. Studies on hormonal cancers have shown that kallikreins may be downstream targets of signaling pathways activated by ►hormones. The study of kallikreins and their molecular mechanisms in tumor formation and progression is an emerging field. The roles of kallikreins in tumorigenesis have not been well characterized; however, it has been suggested that genetic aberrations in the *KLK* locus may account for altered expression of kallikreins during tumorigenesis. Several kallikreins have been found to have dual and disparate roles in tumor growth and invasion.

KLK1, KLK2, and KLK3 may promote tumor growth and survival by degrading ►insulin-like growth factor binding proteins (IGFBPs) to release the mitogenic growth factor ►insulin-like growth factor 1 (IGF1). Kallikreins could also stimulate tumor growth through the ►protease-activated receptors (PAR). Conversely, KLK3 is known to release ►transforming growth factor-β (TGFβ) from its latent form to suppress tumor growth.

Kallikreins have been found to activate tumor growth factor and promote ►angiogenesis via a proteolytic cascade that activates the ►urokinase plasminogen activator (uPA)–urokinase plasminogen activator receptor (uPAR) system. KLK1 stimulates the angiogenic response by releasing bradykinin from kininogen. Bradykinin promotes angiogenesis by upregulating the basic ►fibroblast growth factor (bFGF) or by stimulating the formation of ►vascular endothelial growth factor (VEGF). However, KLK3, KLK6, and

KLK13 are capable of generating an angiostatin-like fragment from plasminogen to inhibit angiogenesis.

Many kallikreins are known to degrade ►extracellular matrix (ECM) proteins either directly or indirectly through proteolytic cascades. Cancer ►metastasis consists of a series of linked, sequential steps, such as attachment of cells to the ECM, production of ECM-degrading enzymes, and increased cell motility; therefore, it is not surprising that proteases such as the kallikreins are reported to promote tumor ►invasion. The molecular mechanisms of kallikreins in tumor-cell invasion and metastasis have not yet been well characterized. Different kallikreins play different roles in tumor invasion and may act differently in different cancers. Although tumor-cell invasion may be due to the proteolytic activity of kallikreins, overexpression of some kallikreins results in suppression of invasion and a favorable prognosis. For example, KLK8 degrades ►fibronectin to disrupt the fibronectin-associated ►integrin signaling pathway. The altered integrin signaling retards tumor cell ►motility to confer a favorable prognosis. All kallikreins share common sequence and function features with proteases. The different roles of kallikreins in tumor growth and invasion may be due to the tumor type and its ►microenvironment.

Kallikreins are secreted proteases and can be found in body fluids or tissues, which allow them to be used as diagnostic and prognostic markers for cancers and for therapy efficacy assessments. Most of the research on kallikreins as markers for diagnosis and prognosis has involved hormonal cancers such as breast, ovarian, testicular, and prostate cancers. Over the past several years, investigators have extended the study of kallikreins to cancers other than hormone-dependent malignancies, including ►leukemia, ►lung cancer, and other cancers. Compared with hormone-dependent cancers such as ►breast cancer, ►ovarian cancer, and ►prostate cancers, much more work is needed to fully investigate the role of individual kallikreins in non-hormonal cancers and their clinical use as diagnostic and/or prognostic markers.

Kallikreins as Diagnostic and Prognostic Markers

Aberrant amounts of kallikrein gene transcripts and/or proteins are found in malignancies such as breast, prostate, testicular, and ovarian cancers. Of the 15 kallikreins, KLK1 and KLK12 have not been established as diagnostic or prognostic markers for many of the cancers investigated so far. KLK3, or prostate specific antigen (PSA), is the most well-characterized kallikrein for prostate cancer diagnosis. KLK2 and KLK11 complement KLK3 to give more accurate diagnosis of prostate cancer. The gene transcripts of many kallikreins are overexpressed in cancer tissues. Antibodies against several kallikreins have been developed for immunoassays. The kallikreins that have been clinically validated as diagnostic markers are listed in Table 1. Given the ubiquitous gene expression of kallikreins in many cancer tissues, kallikreins have great potential as diagnostic markers and further

Kallikreins. Table 1 Kallikreins as serological diagnostic and prognostic markers

Markers	Serological diagnosis	Favorable prognosis	Unfavorable prognosis
KLK1	–	–	–
KLK2	Prostate	–	–
KLK3	Prostate	Breast	–
KLK4	–	–	Ovary
KLK5	Breast, ovary	Prostate, testicular	Breast, ovary
KLK6	Ovary	–	Colorectal, gastric, ovary, RCC
KLK7	–	–	Breast, ovary
KLK8	Ovary	Ovary, lung	–
KLK9	–	Breast, ovary	–
KLK10	Ovary	Testicular	ALL, ovary, SCC
KLK11	Ovary, prostate	Ovary, prostate	Lung, RCC
KLK12	–	–	–
KLK13	–	Breast, ovary, testicular	–
KLK14	Breast, ovary	Ovary, testicular	Breast, prostate
KLK15	–	Breast	Ovary, prostate

Abbreviations: ALL, acute lymphoblastic leukemia; RCC, renal cell carcinoma; SCC, squamous cell carcinoma (head and neck); –, not established or not applicable.

investigation in more cancer types than those listed in Table 1 is warranted.

Different kallikreins either promote or inhibit tumor ►invasion and ►metastasis and can be markers of favorable or unfavorable ►prognosis. Different kallikreins have different roles in tumor progression and may act differently in different cancers. Although proteases are known to degrade ECM components to facilitate tumor-cell invasion, Several kallikreins are associated with a favorable prognosis in patients with the cancers listed in Table 1. Of the kallikreins, *KLK5*, *KLK10*, *KLK11*, *KLK14*, and *KLK15* are markers of favorable prognosis for some cancers but of unfavorable prognosis for other cancers. This shows that whether kallikreins promote or inhibit tumor progression depends on the tissue type and the tumor microenvironment. However, further investigation is needed to determine the roles of kallikreins in cancer biology.

Kallikreins are emerging ►biomarkers for cancers. As yet, use of kallikreins as diagnostic markers has been established for only a limited number of cancers. Further investigations are needed to characterize the roles of individual kallikreins in tumor growth, angiogenesis, ►extracellular matrix remodeling, invasion, and metastasis. The use of kallikreins as diagnostic and prognostic markers for more hormonal and non-hormonal cancers, and as therapeutic targets and predictive markers for therapy responses warrants further investigation.

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Kaplan–Meier Product Limit Estimator

►Kaplan-Meier Survival Analysis

Kaplan-Meier Survival Analysis

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Synonyms

Kaplan–Meier product limit estimator; Life table estimates; Time-to-event analysis

Definition

Kaplan–Meier survival analysis is a nonparametric method of summarizing survival event probabilities in tabular and graphical form.

Characteristics

Often, the focus of ►cancer epidemiology studies is on measurement of disease-free ►survival time (see also, ►epidemiology of cancer). For example, the success of a breast cancer treatment might be discussed in terms of time-until-relapse or cancer-free survival time. To measure time effectively, a meaningful origin is chosen such as birth, first diagnosis of cancer, beginning of treatment or last known occurrence of the disease. In a randomized study or ►clinical trial, we might begin measuring time at the point of ►randomization to treatment. Typically, time is measured prospectively in a survival study in contrast to the retrospective measurement of exposure in the ►case control association study.

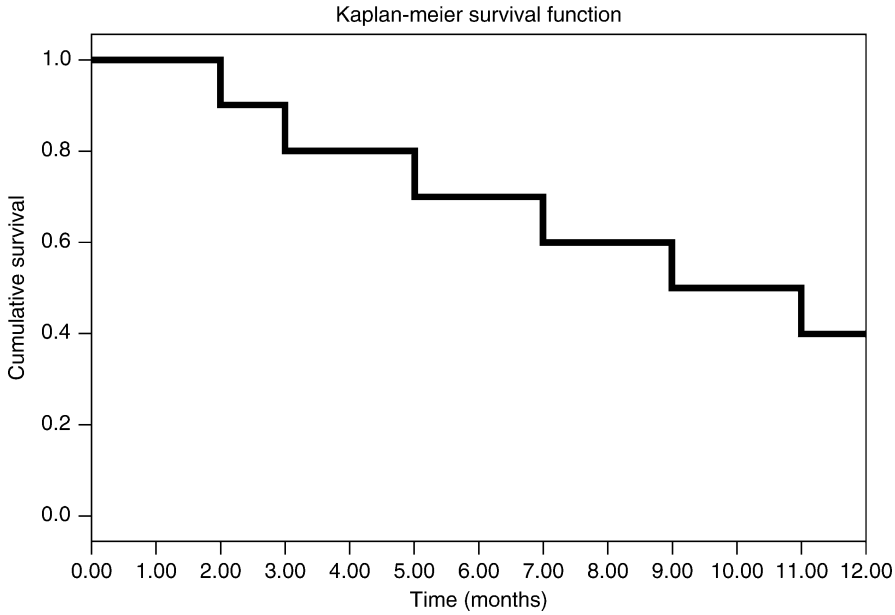
The outcome of interest is often measured as time-until-failure or some other well-defined event. In a cancer study, we might consider death, treatment intolerance or tumor recurrence as failure. Survival analysis methods allow us to discuss the event times in positive terms of survival rather than in negative terms of failure. If t represents the time to a failure or event, then

$$F(t) = P(T \leq t) = P\,(\text{failure occurs at or prior to time } t)$$

is called the cumulative distribution function of the failure times. The complement of the cumulative failure distribution is

$$S(t) = 1 - F(t) = P(T > t) = P(\text{surviving past time } t)$$

and is called the survival function. Note that although t measures the time-until-failure, it also represents the disease-free survival time. Fig. 1 illustrates a typical Kaplan–Meier survival function estimate.



Kaplan-Meier Survival Analysis. Figure 1 Kaplan–Meier survival curve.

K

Censoring

If all individuals in the study fail, we can precisely describe the survival distribution, $S(t) = 1 - F(t)$ Suppose we have decided to follow patients in a clinical trial for 5 years and that our outcome is recurrence at a specific tumor site. For those who are diagnosed with recurring tumor during the study period, exact failure times are recorded. Those who complete the 5-year follow-up but do not have a recurrence are said to be right censored. By focusing on survival instead of failure, we are able to conclude that the survival time was at least 5 years for the censored individuals without knowing the exact time of failure beyond that 5-year study period. In other words, we may not know the exact time of failure for individuals that survived longer than 5 years, but we do know they lived without recurrence for at least 5 years.

In addition to ►censoring by the limits of the study design, individuals may also be censored due to factors beyond the investigators control, such as death from other causes. Although the analysis itself does not distinguish between these types of censoring, one caveat in generalizing results is that intermittent censoring must be non-informative so that the reason for censoring should not be related to the either the time of failure or the treatment choice. In other words, this type of censoring should be random.

Parametric, Semi-Parametric and Nonparametric Survival

Three major divisions are made in the types of survival analyses; ►parametric, semi-parametric and ►nonparametric. If survival times are assumed to follow a specific mathematical distribution, the parameters of that distribution can be estimated from the sample. Perhaps the simplest parametric assumption in survival analysis is one of a constant hazard rate, λ, leading to the failure distribution, $F(t) = 1 - e^{-\lambda t}$, and the complementary survival distribution, $S(t) = e^{-\lambda t}$ that we recognize as exponential decay. Metabolism studies, for example, may exhibit exponential decay. The parameter, λ, is estimated from the data as $\hat{\lambda} = r/T$ where r is the total number of events and $T = \sum t$ is the total of all survival times. Other useful parametric survival distributions are the Weibull, lognormal and gamma distributions.

Another common approach introduced; by D. R. Cox in 1972 and called ►Cox proportional hazards modeling has become one of the most common methods of survival analysis and is based on semi-parametric estimation where the background hazard rate is not estimated. Cox assumed the survival distribution, $S(t) = [S_0(t)]^{\exp(\sum \beta x)}$ where $S_0(t)$ is a baseline hazard rate that is typically treated as a nuisance, x is some covariate of interest and β is the slope parameter associated with that covariate. In this type of analysis, we are usually interested in comparing covariate parameters so that S(t) itself is not estimated fully.

Finally, nonparametric methods including actuarial and life table methods are useful for summarizing survival distributions. Perhaps one of the nonparametric approaches to survival analysis that is most often used in the medical literature is that introduced by E. L Kaplan and P. Meier in 1958. Useful in visualizing

survival data, the Kaplan–Meier curve is plotted as a starting point in most survival analyses. Unlike the parametric and semi-parametric approaches to quantifying a survival distribution, the nonparametric Kaplan–Meier method requires few assumptions other than independence of failure time observations.

Kaplan–Meier Product Limit Estimator

Many of the survival analysis methods, such as Cox proportional hazards models and parametric models, require iterative solutions and specialized software. The Kaplan–Meier Product Limit Estimator, on the other hand, is easily calculated. The r event times are ordered from smallest to largest so that $t_1 < t_2 < t_3 < \cdots < t_r$. For example, assume the relapse times (in months) for ten cancer patients are recorded as (2, 3, 5, 7, 9, 11, 12, 12, 12, 12). If there is no censoring, the Kaplan–Meier estimator at any event time, j, is simply the proportion with event times greater than t_j. For example, at $t = 7$ months four have relapsed and six have not so that the probability of surviving more than seven months is $\hat{S}(7) = 6/10$. In the case of single right censoring at the end of the study, the calculations are similar. If the relapse times are (2, 3, 5, 7, 9, 11, 12+, 12+, 12+, 12+) where "+" indicates censoring, $\hat{S}(7) = 6/10$ is the proportion surviving longer than 7 months as before. In addition to the plot of these data in Fig. 1, we might choose to report summary statistics in the form of quantiles, medians or means. For these data, the median is 9 months since $\hat{S}(9) = 0.5$. The mean is related to the area under the survival curve and, for data sets in which the largest observation is censored, the mean is based on the truncated failure times so that the estimate of the mean is too low. In this case, the mean is 8.5 months.

The probability of surviving can be found using simple counting rules and the multiplication rule for joint conditional probabilities. At each distinct event time, there are n_j individuals at risk of whom d_j relapse and $n_j - d_j$ do not relapse. Assume the event times are recorded as follows: (2, 3, 5, 7, 9, 11, 12+, 12+, 12+, 12+). At 7 months, we find $n_j = 7$ individuals still at risk of relapse of whom $d_j = 1$ relapses and $n_j - d_j = 6$ do not. Applying the multiplication rule to the conditional probabilities for each distinct event time, the probability of surviving past 7 months is

$$\hat{S}(7) = \left(\frac{9}{10}\right)\left(\frac{8}{9}\right)\left(\frac{7}{8}\right)\left(\frac{6}{7}\right) = \frac{6}{10}$$

as we found previously.

If any censored times are less than any event times, we must alter the formula slightly. In essence, the Kaplan–Meier product limit estimator is still an application of counting rules and conditional probabilities but the numbers at risk at some event times are altered due to censoring. Suppose we find that one observation is censored prior to seven months and the event times are recorded as (2, 3, 5+, 7, 9, 11, 12+, 12+, 12+, 12+). In terms of an event, all we know of the individual who was censored at five months is that they at least survived longer than the previous event time of 3 months. The product of the conditional probabilities using only event times is

$$\hat{S}(7) = \left(\frac{9}{10}\right)\left(\frac{8}{9}\right)\left(\frac{6}{7}\right) = 0.6857$$

and is slightly different from the estimate when no censoring prior to seven months was observed. The censored observation provides additional information for the first two periods by increasing the overall sample size. The median survival time for these data is 11 months, illustrating the effect of censoring on the estimates. The mean is 9.1 months but, again, may be too low due to the censoring of all observations at 12 months. In general, the Kaplan–Meier estimator is defined as

$$\hat{S}(t) = \prod_{t_j < t} \left(\frac{n_j - d_j}{n_j}\right)$$

where the product is over the conditional probabilities of all event times prior to time t. The quantity in brackets is the estimated conditional probability of surviving to time $t_j{+}_1$ given the individual has survived to time t_j. The graph is a step function as illustrated in Fig. 1.

Comparing Survival Functions

In many survival studies, a major interest is in comparing survival experiences of two or more groups. For example, if $n_1 = 100$ women given hormone replacement and $n_2 = 100$ women given a placebo are followed for 10 years with age at first diagnosis of breast cancer is recorded, cancer-free survival time between the groups is an important outcome for study. The null hypothesis of interest is $H_0 : S_{\text{HRT}}(t) = S_{\text{Placebo}}(t)$ against the alternative $H_1 : S_{\text{HRT}}(t) S_{\text{Placebo}}(t)$, say. If no observations are censored, a nonparametric ▶Mann–Whitney U-test can be used to compare the distributions. For more than two distributions, the Wilcoxon generalization of the Mann–Whitney test is appropriate.

With censoring, however, several variations for comparing two or more groups have been proposed. Two of the most common tests for comparing two groups are the generalized Wilcoxon test and the log-rank test. The log-rank test, for example, is based on a chi-square statistic using the Observed and Expected number of failures

$$X^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2}$$

where $E_1 = \sum_{j=1}^{r_1} e_{1j}$ and $E_2 = \sum_{j=1}^{r_2} e_{2j}$ are summed over the ordered failure times for groups one and two,

respectively. At each failure time, the expected number of deaths for each group is calculated similar to the usual chi-square test of independence as $e_{ij} = n_{ij} d_j/n_j$ where n_{ij} is the number of deaths in group i at time j, d_j is the total number of deaths in both groups at time j, and n_j is the total number at risk in both groups at time j. The log-rank test is more sensitive to differences that occur later in time than the Wilcoxon test that gives more weight to the earlier failures.

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Kaposi Sarcoma

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Definition

KS; Is a vascular tumor, often affecting the skin. Discovered by Moritz Kaposi in 1872, Four epidemiological forms exist:

- Classic KS occurs mainly in elderly HIV negative male patients of Southern European and Middle Eastern origin.
- Endemic KS: In some African countries, KS has existed for many decades, long preceding HIV. Unlike classic KS, endemic KS also occurs in children, where they often present with lymphadenopathy, rather than skin lesions. Endemic KS is generally a more aggressive disease than classic KS, though less so than African AIDS-associated KS.
- Post-transplant or iatrogenic KS is also known to develop after an organ transplant. Patients of Mediterranean, Jewish or Arabian ancestry are over-represented among immunosuppressed patients who develop KS after a transplant, indicating that those born in countries where classic KS occurs continue to be at risk of developing KS even if they migrate to "low-risk" countries.
- AIDS-KS: In 1981 the occurrence of two rare diseases in young gay men from New York City (NY, USA) and California were reported: Kaposi sarcoma and Pneumocystis carinii pneumonia. This was the beginning of the AIDS epidemic and AIDS-KS is today the most common form of KS. In HIV-infected individuals the underlying immunosuppression can lead to an aggressive disease that starts with skin or mucosal lesions, but without treatment, often develops into disseminated disease affecting various organs including lung, liver, gut and spleen.

Characteristics

Histology

Histologically, KS is a complex lesion: in early KS lesions there are a collection of irregular endothelial lined spaces that surround normal dermal blood vessels and these are accompanied by a variable inflammatory infiltrate (patch-stage). This stage is followed by the expansion of a spindle-celled vascular process throughout the dermis. These spindle cells form slit-like, vascular channels containing erythrocytes (plaque-stage). The later nodular-stage KS lesions are composed of sheets of spindle cells. The spindle cells form the bulk of established KS lesions and are therefore thought to be the neoplastic component. Most of the spindle cells in KS lesions express endothelial markers, including CD31 and CD34. Recently, it was also shown that these spindle cells express markers associated with lymphatic, rather than vascular endothelial cells including vascular endothelial growth factor receptor (VEGFR)-3 and podoplanin. This suggests that these spindle cells might belong to the lymphatic lineage of endothelial cells. Early KS ("patch stage") is probably a non-clonal proliferation of lymphatic endothelial cells or endothelial precursors, with a prominent inflammatory and angiogenic response, whereas advanced disease can develop into a true clonal malignancy with metastases of clonally derived spindle cells to different sites.

Studies of AIDS case surveillance support the pre-AIDS data on the existence of a sexually transmissible KS cofactor: KS occurs predominantly in gay and bisexual men with AIDS, less commonly in those acquiring HIV through heterosexual contact and rarely in AIDS patients with hemophilia or in intravenous drug user.

Virus Involvement

Sequences of Kaposi sarcoma-associated herpesvirus (KSHV or human herpesvirus-8) were first identified in Kaposi sarcoma biopsies. KSHV is a gammaherpesvirus and related viruses are found in non-human

primates. The KSHV genome consists of an ~140 kb long unique coding region (LUR) flanked by ~800 bp noncoding tandemly repeated units. Over eighty five open reading frames (ORFs) have thus far been identified, including nearly seventy with sequence similarity to related gammaherpesviruses. Novel ORFs not present in other herpesviruses were designated K1–K15, although many of these now appear to be present in related viruses. KSHV encodes for a number of cellular homologs and these include a viral cyclin, bcl-2, IL-6, interferon regulatory factor (IRFs), FLICE inhibitory protein (FLIP) and chemokine homologs (vMIP I, II and III).

Viral Strains

ORF-K1 is use to subtype KSHV:subtypes A, B, C and D have been identified which display between 15 and 30% amino acid differences between their ORF-K1 coding regions. These subtypes have close associations with the geographic and ethnic background of individuals. Within these four subtypes, over 13 clades have now been described. Subtype B is found almost exclusively in patients from Africa, subtype C in individuals from the Middle East and Mediterranean Europe, subtype A in Western Europe and North America and subtype D has only so far been described in individuals from the Pacific Islands.

KSHV Latent Proteins

A number of KSHV ORFs are transcribed during latency and these proteins could be involved in oncogenesis:

- Latent nuclear protein (ORF 73/LNA) of KSHV encodes a latent immunogenic nuclear protein (LNA) detected as nuclear speckling by immunofluorescence using KSHV positive sera on ►PEL cells. LNA is expressed in all KS spindle cells latently infected with KSHV, in all the immunoblasts in KSHV associated Castleman disease and in all cells of (►primary effusion lymphoma (PEL)). Like EBNA-1, LNA is essential to maintain the KSHV episome (extrachromosomal persistence). Furthermore, LNA tethers KSHV DNA to chromosomes during mitosis to allow the segregation of viral episomes to all progeny cells. LNA therefore maintains a stable episome during mitosis. LNA also interferes with p53 andpRb pathways to deregulate the cell cycle. Strategies including the development of small molecules that interfere with the functions of LNA could be useful to abort latent KSHV infection and therefore prevent KSHV associated diseases.
- V-cyclin; cellular cyclins [►cyclin D] are critical components of the cell cycle: cyclins are regulatory subunits of a specific class of cellular kinases. By physically associating with an inactive cyclin-dependent kinase core (cdk), cyclins lead to the formation of active kinase holoenzymes which recognize and phosphorylate an array of cellular substrate molecules. The phosphorylating activity of these holoenzymes is responsible for regulating the passage of cells through the replication cycle. Cyclins associate with their partners (the cyclin dependent kinases, CDKs) to be fully active. The KSHV cyclin has highest sequence similarity to the cellular D-type cyclins. The KSHV cyclin is expressed during latency inferring a possible role in tumorigenesis. The KSHV-cyclin form active kinase complexes with CDK6 to phosphorylate the retinoblastoma protein (pRb). Furthermore, unlike cellular D cyclin/CDK6 complexes, KSHV-cyclin/CDK6 activity is resistant to inhibition by CDK inhibitors (CKI) p16, p21 and p27. Ectopic expression of v-cyclin prevents G1 arrest imposed by each inhibitor and stimulates cell-cycle progression in quiescent fibroblasts. KSHV cyclin/CDK6 also phosphorylates (inactivates) p27, the CKI known to be an effective inhibitor of cyclin E/CDK2 activity. This suggests that this viral cyclin can activate both pathways necessary for G1/S-phase progression (i.e. cyclin D/CDK6 and cyclin E/CDK2). The expression of the KSHV-cyclin in latently infected spindle and ►PEL cells, indicate a possible role in either the proliferation or the arrest of differentiation of these cells.
- ORF-K15 latent membrane protein. At the far right-hand end of the KSHV genome, the ORF K15 encodes a putative latent transmembrane protein. Two highly divergent forms of K15 have been identified; the predominant (P) and minor (M) forms. These two alleles have only 33% amino acid homology to each other, yet retain the 12 transmembrane spanning domains and a cytoplasmic signal-transducing carboxyl-terminus. K15 induces NFκB activation and this activity localises within the last 18 amino acids of K15, which contains the putative TRAF-binding motif. K15 is also able to activate JNK. This ability to activate JNK, like that of NFκB activation, is located within the last 18 aa of the K15 extreme C-terminus. The JNK signaling pathway is known to lead to the activation of ►AP-1, a pleotropic transcription factor implicated in cellular transformation and phenotypic changes.

Neoplasms Associated with KSHV

Three neoplasias are consistently linked with KSHV infection: Kaposi sarcoma, a subtype of ►Castleman disease and PEL. KSHV sequences have also been described in squamous carcinoma, multiple myeloma and other vascular tumors, however, most studies could not confirm any association between KSHV and these tumors. The detection of KSHV in some of these reports might be due to PCR contamination.

- Kaposi sarcoma. Four observations link KSHV to Kaposi sarcoma (although none of these findings on their own are sufficient to support a causative role):
  - KSHV DNA is present, by the polymerase chain reaction (PCR), in all four epidemiological forms of KS and in nearly all KS biopsies tested. However, KSHV DNA is rarely, if at all, detectable in other vascular tumors.
  - The detection of KSHV DNA by PCR in the peripheral blood of HIV infected individuals predicts who might subsequently develop KS, indicating that those at risk of KS have a higher viral load than those not at risk.
  - Seroepidemiological surveys show that general populations at risk of developing Kaposi sarcoma have a higher prevalence of KSHV infection. The incidence of classic KS and AIDS-KS in different populations correlates broadly with the prevalence of the virus in these populations.
  - In nodular KS lesions, KSHV is latently expressed in nearly all the tumor (spindle) cells. This is reminiscent of other viral driven cancers e.g. ►Epstein-Barr virus (EBV) latent infection in post-transplant lymphoproliferation or human papillomavirus infection in cervical cancer.
- Plasmablastic variant Multicentric Castleman's Disease; Castleman's disease (CD) is a lymphoproliferative disorder. Recently CD is more often diagnosed in HIV infected patients. A systemic variant of CD is associated with multiple organ involvement, especially spleen and lymph nodes with systemic symptoms such as weight loss and fever. This is called multicentric ►Castleman's disease (MCD). MCD has been associated with increased circulating IL-6 levels. KSHV DNA is found in some cases of MCD. KSHV is present in plasmablasts in MCD and these plasmablasts are not present in KSHV negative MCD. These KSHV positive plasmablasts belong to the B cell lineage. KSHV appears also to be present in all tumor cells of plasmablastic lymphoma that develops in patients with the KSHV positive plasmablastic variant of MCD. The development of plasmablastic lymphoma therefore appears to represent a further evolution of this disorder. Unlike KSHV positive primary effusion lymphoma cells, the plasmablasts in MCD are only positive for KSHV, and not for EBV.

Current studies suggest that KSHV positive MCD has a poorer prognosis that the KSHV negative cases. In contrast to KS lesions that can resolve by partly restoring the immune system (e.g. by HAART for HIV positive patients), KSHV positive MCD often continues to progress.

- ►Primary Effusion Lymphoma (PEL); PEL is a body cavity-based lymphoma that usually presents and persists as an effusion: pleural, pericardial or ascitic. The lymphoma cells in these cases are negative for most lineage-associated antigens, although immunoglobulin (Ig) gene rearrangement studies indicated a B-cell origin. These lymphomas occur predominantly in HIV+ individuals with advanced stages of immunosuppression, but are occasionally seen in HIV negative patients. Like KS which can occur in the same patient, PEL occurs primarily in gay men and not in other HIV-positive risk groups. PEL cells contain between 50 and 150 copies each of the KSHV genome. The majority, but not all, PELs are co-infected with EBV, suggesting that the two viruses may cooperate in neoplastic transformation. Terminal repeat analysis indicates that EBV is monoclonal in most cases, implying that EBV was present in the tumor cells prior to clonal expansion. PEL cells consistently lack molecular defects commonly associated with neoplasia of mature B cells including activation of the proto-oncogenes bcl-2, bcl-6, n-ras, and k-ras, as well as mutations of p53. KS, MCD and PEL have all been described in one patient and up to 30% of HIV infected patients with KSHV positive MCD, will also have or develop KS.

KSVH and Immunity

The introduction of aggressive anti-HIV therapies lead to a decline in the incidence of KS in AIDS patients and also in the resolution of KS in those already affected. This suggests that cellular immune responses, compromised in AIDS, but recovering after highly active antiretroviral therapy (HAART), could be important in the control of KSHV infection and in the development of KS. This is further supported by the observation that post-transplant KS lesions can regress when immunosuppressive treatment is stopped.

KSHV, like other herpesviruses, is able to elicit HLA class I restricted ►cytotoxic T cell (CTL) responses. In one pilot study, KSHV specific CTL responses were not present in most patients with KS, indicating that a decline in cellular immune responses against KSHV may be present in HIV+ patients with KS and could contribute to KS pathogenesis. This would be reminiscent of the lack of EBV specific CTLs seen in immunosuppressed patients which correlates with the onset of EBV driven lymphoproliferation.

Anti-Herpesviral Drugs

In vitro, KSHV replication is insensitive to ganciclovir and acyclovir, but is moderately sensitive to foscarnet (phosphonoacetic acid) and sensitive to cidofovir. These agents target lytic herpesviral infection and if lytic infection is necessary to drive tumor formation

or to recruit inflammatory cells to form KS lesions, these drugs might prove useful in the management of some patients. Foscarnet has been shown to induce KS lesion regression in one small study and to reduce the onset of KS in other studies. Foscarnet and cidofovir are however associated with significant toxicity and would seem to be inappropriate therapy for most KS patients.

Treatment for KS

In ►immunocompetent patients with single lesions, excisional biopsy is often all that is required. Patients with a few lesions, especially if these are confined to a limited region, is best treated with local radiotherapy. Total-skin electron beam therapy has also been used to treat more disseminated skin lesions. Patients with disseminated disease can be treated with systemic chemotherapy. Single agents that often lead to responses include ►bleomycin, ►vinblastine/vincristine, oral ►etoposide and ►doxorubicin. In HIV infected patients, these agents seldom lead to long-term control of the disease. A major disadvantage of doxorubicin and oral etoposide is ►alopecia. Taxol has also been used, but this agent is associated with significant toxicity. Liposomal doxorubicin is better tolerated and is associated with high response rates. Systemic interferon has also been used and responses have been seen for many months (even years). If tolerated, subcutaneous interferon injections remain a therapeutic option to treat KS. More experimental therapies include thalidomide, retinoic acid and human chorionic gonadotropin. However, these agents only produce transient responses in some patients.

KS often regress with the cessation or modification of immunosuppressive therapy in organ transplant recipients. Effective treatment of HIV infection should prevent KS and has already lead to remission of KS in some patients.

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Karnofsky Performance Score

Definition

A medically derived metric from 0 to 100 that reflects a person's ability to perform certain ordinary tasks in order to determine a patient's suitability for therapy or to evaluate a patient's progress after a therapeutic procedure with 100 being completely intact, 70 being unable to carry on these normal activities, 50 indicating a need for considerable assistance, 40 being totally disabled, and hospitalization recommended for scores of 30 or less.

Karyoplast

Definition

Cell nucleus surrounded by thin band of cytoplasm and enveloped by plasma membrane.

►Microcell-Mediated Chromosome Transfer

Karyotype

Definition

The complete set of chromosomes of one cell. Usually represented as a map with photographs of mitotic chromosomes arranged in homologues pairs. Chromosome complement of cells. In a karyotype, the total number of chromosomes is given first, followed by sex chromosome constitution and a description of numerical and structural aberrations. A normal male karyotype is thus written 46,XY; the normal female complement is 46,XX.

KCNH1

►Ether à-go-go Potassium Channels

KCS

►Sjögren Syndrome

Keap1

Definition
Kelch-like ECH-associated protein 1, is the principal repressor of ►Nrf2. Keap1 binds to Nrf2 and targets it for ►ubiquitin-mediated proteasomal degradation.

►Phase 2 Enzymes

Kennedy Disease

►Androgen Receptor

Keratinocyte

Definition
A major cell type of the epidermis that synthesizes keratin. It originates from the division of keratinocyte stem cells in the basal layer and undergoes gradual differentiation through different layers until reaching the stratum corneum and forming an effective barrier of dead cells against foreign material and infectious agents.

►Stefins

Keratoconjunctivitis Sicca

►Sjögren Syndrome

Keratodermas

Definition
Disorders involving thickening of the skin.

►Connexins

Keratosis

Definition
►Actinic Keratosis

Ket

►p53 Family

Kew Tree

►Ginkgo Biloba

Ki-1

►fALK Protein

Ki1 Lymphoma

►Anaplastic Large Cell Lymphoma

Ki-67

Definition

Is a proliferation-associated antigen expressed in all phases of the cell cycle except in G0. Antibodies against Ki-67 are used in tumor pathology to detect proliferating cells.

►Diffuse Large B-Cell Lymphoma
►Pathology

KIAA0413

►APAF-1 Signaling

Killer Cells

►Activated Natural Killer Cells

Kin-Cohort Study

Definition

A study design used to estimate ►penetrance. The sample of individuals is unselected for family history. Genetic testing is performed on the subjects, and the family histories of the mutation carriers are compared with the family histories of the non-carriers.

►Epidemiology of Cancer
►Cancer Epidemiology

Kinase

Definition

An enzyme that transfers a phosphate group from a donor, usually ATP or ADP, to a serine, threonine or tyrosine residue on an acceptor protein.

►Molecular Chaperones
►Signal Transduction
►Receptor Tyrosine Kinases
►Cyclin Dependent Kinases

In vitro Kinase Assay

Definition

Analytical method to measure the activity of protein or lipid kinases *in vitro*. Kinases are usually purified from cell extracts by immunoprecipitation and then mixed with ATP and an appropriate substrate. ►Kinase activity is then analyzed by measuring the phosphorylation status of the substrate, either by immunochemical methods (►ELISA or Western Blotting) using phospho-specific antibodies or by its labeling with radioactive phosphorus.

Kinesin

Definition

A large family of microtubule-dependent motor proteins with different functions. While some kinesins are responsible for transporting organelles, proteins, and mRNAs to specific destinations along ►microtubules by hydrolyzing ATP, others are required for mitotic spindle assembly and chromosome separation during mitosis and cytokinesis.

►KSP Mitotic Spindle Motor Protein
►Doublecortin

Kinesin-5

►KSP Mitotic Spindle Motor Protein

Kinesin Spindle Protein

►KSP Mitotic Spindle Motor Protein

Kinesin-like Protein KIF11

►KSP Mitotic Spindle Motor Protein

Kinetic Parameters

Definition

The properties of chemical agents or enzymes in the efficiency and speed of their action upon a chemical reaction.

►Biomonitoring

Kinetochore

Definition

Is the protein structure in eukaryotes that localizes on the ►centromere and connects the chromosome to microtubule polymers from the mitotic spindle during ►mitosis and meiosis. A region of the chromosome centromere to which microtubular spindle fibers attach. Each ►centromere contains two kinetochores, each receiving spindle fibers from one of the opposite poles.

►Micronucleus Assay
►Genomic Imbalance

Kinetochore Outer Plate

Definition

Disk-shaped proteinaceous structure forming on the outside of chromosomal centromeres during ►mitosis; involved in chromosome segregation.

Kininogen

Definition

There exist high and low molecular weight kininogen (HMWK and LMWK). Both are inhibitors of thiol proteinases. HMWK plays an important role in blood coagulation since it brings together prekallikrein, factor XI and factor XII. A physiologically active peptide, bradykinin, is released from HMWK by plasma kallikrein. ►Bradykinin is a potent vasodilator. In contrast, LMWK is not involved in coagulation and inhibits platelet aggregation.

►Proteinase-Activated Receptors

KiSS1

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Definition

KiSS1 (GenBank Accession No. U43527) is a human ►metastasis-suppressor gene that was discovered in human ►melanoma cells. The name is derived from SS for putative Suppressor Sequence, the Ki was added to remind people of its discovery in Hershey, Pennsylvania. Upon introduction into metastatic cells, which normally do not express the gene, the cells retain the ability to form tumors while losing their ability to metastasize.

Characteristics

As melanomas progress toward increasing malignancy, numerous nonrandom chromosomal changes occur. One particular change involving deletions involving the long arm of chromosome 6, tends to occur at a time coincident with acquisition of metastatic potential. This suggested that a metastasis suppressor gene was encoded at that site. This hypothesis was validated upon introduction of chromosome 6 into metastatic human melanoma cells using the technique of ►microcell-mediated chromosome transfer. Metastatic potential was lost but tumorigenicity was retained in the chromosome 6-melanoma hybrids. By comparing mRNA expression between parental (metastatic) cells and chromosome 6 hybrids (nonmetastatic) using subtractive hybridization, KiSS1 was identified as being expressed exclusively in the nonmetastatic cells. Transfection of KiSS1 into the metastatic melanoma cells also suppressed metastasis without affecting tumorigenicity.

The KiSS1 gene maps to chromosome 1q32, suggesting that a gene(s) on chromosome 6 regulates its expression. The regulatory gene CRSP3, encoded

on chromosome 6q, was deleted in the advanced, metastatic melanoma cells. Re-expression of CRSP3 restores KISS1 expression.

Does KiSS1 Suppress Metastasis of Other Human Cancers?

Transfection of KiSS1 into at least two human melanoma cell lines, a human breast carcinoma cell line and a human ovarian carcinoma cell line results in significant suppression of metastasis. Tumorigenicity (growth of injected cells at orthotopic sites) is not suppressed by KiSS1 expression.

How Does KiSS1 Suppress Metastasis?

The KiSS1 gene encodes a predominantly hydrophilic protein of 145 amino acids (approximately 15.4 kDa). A signal peptide regulates Golgi localization and processing before secretion. Nascent KiSS1 is proteolytically processed into a number of peptides, termed kisspeptins. A 54-amino acid polypeptide, termed metastin or kisspeptin-54, was found to bind to a G-protein coupled receptor (alternatively named GPR54, AXOR12 or hOT7T175). KiSS1 binding to GPR54 in the hypothalamic-pituitary axis to induce GnRH secretion which, in turn, induces puberty. KiSS1 (kisspeptin)–GPR54 interactions have recently been discovered not to be involved in metastasis suppression. Therefore, the molecular mechanism of action of KiSS1 remains unknown.

The cellular mechanism of action appears to be prevention of tumor cell growth at ectopic sites. KiSS1-expressing melanoma cells can complete every step of the metastatic cascade antecedent to colonization of lungs.

Is KiSS1 a Marker of Metastasis?

Using a series of cell lines representing the progression of normal melanocytes to metastatic melanomas, KiSS1 expression is lost as the cells convert from radial to vertical growth phase (benign to malignant transformation). Several additional studies show that high KiSS1 expression is generally correlated with good prognosis and reduced recurrence for multiple cancer types.

Clinical Relevance

►Metastasis is the most life-threatening attribute of cancer cells. Any gene/protein that could block or slow metastasis would benefit long-term survival rates among cancer patients. Likewise, any marker that could allow physicians to more accurately stage the disease would aid in treatment planning and monitoring. KiSS1 offers potential in both arenas.

To the extent that KiSS1 plays a role in the suppression of metastasis at late stages and blocks metastasis to multiple organs, it may provide or lead to therapeutic targets designed to inhibit the ability of the cells to form tumors at secondary sites.

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KIT

Definition

Activating mutations in the KIT receptor tyrosine kinase cause ligand-independent activation of its tyrosine kinase function which initiates a cascade of intracellular signaling involved in tumorigenesis. Exon 9 KIT defines a distinct subset of gastrointestinal tumours that are often located in the small bowel and have an aggressive clinical behavior. Kit is an example of a cancer target that has been successfully blocked with a new small molecule inhibitor, ►Gleevec (or ►imatinib). This drug blocks the receptor's tyrosine kinase activity in stromal tumors of the gastrointestinal tract. In these tumors, a mutation in *c-kit* constitutively activates the receptor tyrosine kinase independently of ligand binding.

►Mesothelin
►Kit/Stem Cell Factor Receptor in Oncogenesis
►AAMP

Kit/SCF-R

►Kit/Stem Cell Factor Receptor in Oncogenesis

Kit/Stem Cell Factor Receptor in Oncogenesis

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Synonyms

Stem cell factor; SCF; receptor; SCF-R; Kit/SCF-R; c-Kit; Mast cell growth factor; MGF; receptor; *Steel* ligand receptor

Definition

Kit/SCF-R is a receptor protein ►tyrosine kinase (RPTK) encoded by the ►proto-oncogene c-kit, which is allelic with the murine dominant white-spotting (W) locus on chromosome 5, and whose human homolog is located on human chromosome 4 (4 q11–12).

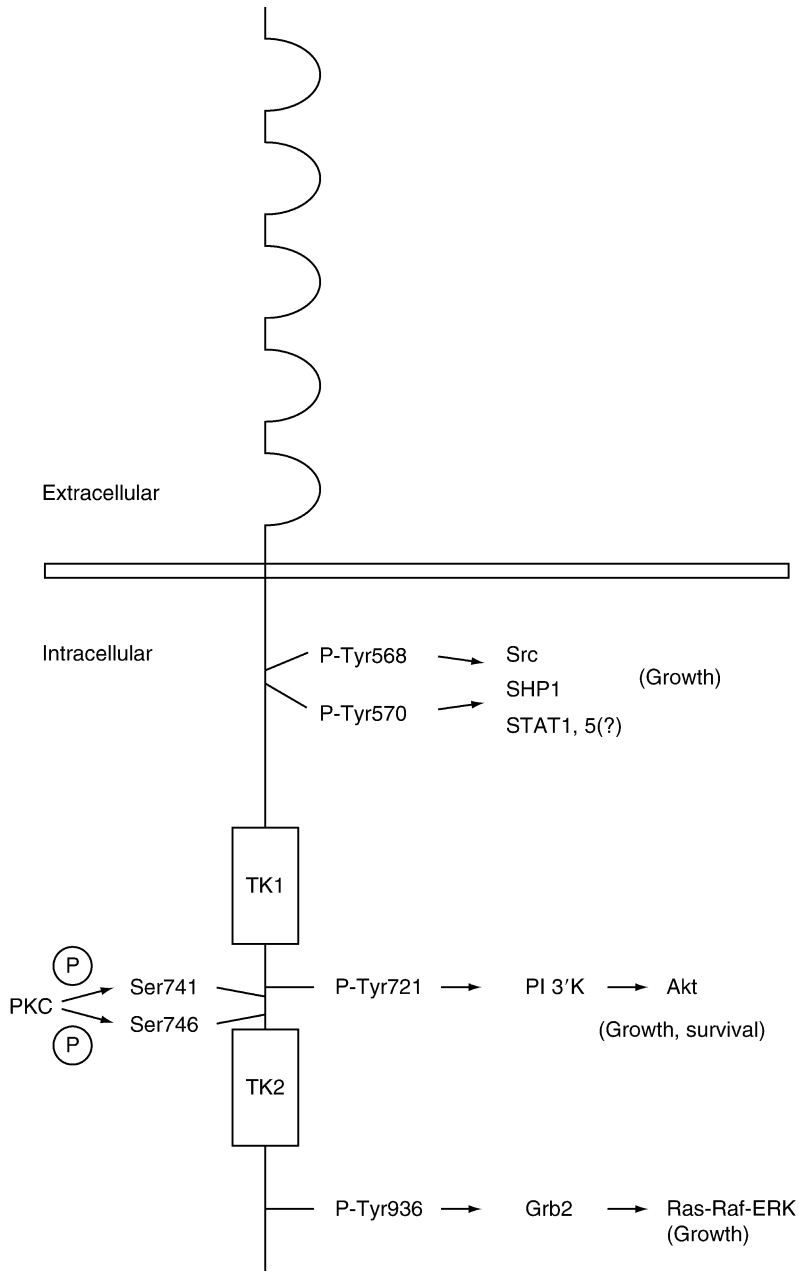
Characteristics

Going from the N-terminus towards the C-terminus, all RPTKs contain an extracellular ligand-binding domain, a single transmembrane-spanning domain and an intracellular domain composed of a hydrophilic juxtamembrane (JM) region, followed by the highly conserved tyrosine kinase domain and a hydrophilic C-terminus. Based on structural similarities, Kit/SCF-R belongs to the subclass III of RPTKs that includes the receptors for platelet-derived growth factor (►PDGF) α and β, colony-stimulating factor-1 (CSF-1) and the Flt-3/Flk-2 RPTK. These proteins are ∼950 to ∼1100 amino acids in length, with ∼550 extracellular amino acids and 400–550 intracellular amino acids. Their overall identity is 25–40%, but the identity in their intracellular domains is between 45 and 55%, and in the kinase domains proper between 63 and 85%. They are characterized by five immunoglobulin-like repeats in the extracellular ligand-binding domain and a long (75–100 amino acids) hydrophilic kinase insert region, which interrupts the tyrosine kinase domain between the ATP-binding region and the conserved catalytic base. The Kit/SCF-R is expressed in hematopoietic, melanogenic and gametogenic precursors and derivatives, and in interstitial cells of Cajal. The development of these cells depends on Kit/SCF-R and its ligand. However, Kit is expressed in numerous other tissues/cell types including mammary duct epithelial cells, thyrocytes and cerebellar basket cells. Two alternative RNA transcripts, resulting from alternate usage of 5′ splice donor sites, generate two forms of Kit/SCF-R in all tissues examined. The only difference is the presence or absence of four additional amino acids, GNNK, in the extracellular domain close to the membrane. When overexpressed, the shorter isoform of Kit/SCF-R (ΔGNNK-Kit/SCF-R), which is the most prevalent in most tissues examined, is constitutively tyrosine phosphorylated, causes tumorigenesis in nude mice and greater activation of ERK/MAPK. However, ΔGNNK-Kit/SCK-R has not been directly associated with any tumors in man or mouse.

Molecular and Cellular Regulation

The general mechanisms of ligand-induced activation of Kit/SCF-R and its downstream signaling have been fairly well characterized, and very similar principles seem to govern ligand-induced signaling by all subclass III RPTKs. In outline, binding of the bivalent ligand, SCF, induces dimerization of Kit/SCF-R leading to activation of its tyrosine kinase domain and subsequent autophosphorylation on specific tyrosine residues. The phosphorylated tyrosine residues and 3–5 amino acid residues immediately N- or C-terminal to each of these, in turn, create specific binding sites for intracellular signaling molecules leading to their recruitment from cytosolic compartments to the membrane, where the activated RPTK is located. The binding of signaling molecules to the phosphorylated tyrosine residues occur through conserved protein-protein interaction domains, such as Src homology 2 (SH2) and protein tyrosine binding (PTB) domains. The SH2 and PTB domains have a defined, conserved primary sequence of ∼100 and ∼110 amino acids, respectively, and they exist as independently folded domains in a variety of intracellular proteins including proteins possessing a catalytic domain, pure adaptor molecules, structural proteins and translocated transcription factors. The conformational changes induced in the signaling molecules upon binding to an RPTK can unmask other protein-protein interaction domains allowing for interaction, recruitment and activation of yet other intracellular signaling molecules. Several other protein-protein interaction domains that have been identified include proline-rich-binding SH3 and WW domains, phospholipid-binding PH (pleckstrin homology) domains, phosphoserine/phosphothreonine-binding 14-3-3 (►14-3-3 proteins)] and FHA (Forkhead-associated) domains. The specificity of signaling from different RPTKs is to a large extent determined by the primary sequence surrounding the autophosphorylated tyrosine residues in the receptors. This enables the activated RPTK to “select” a specific subset of signaling molecules within the cell, which, in turn specifically interact with, and/or activate, other signaling molecules, and a network of activated catalytic proteins and of multiprotein signaling complexes result. Examples of Kit/SCF-R-induced signaling molecules and pathways of importance for cell

growth control include the Ras-Raf-ERK and Src-activated pathways, which are mainly involved in cell proliferation and the phosphatidylinositol 3′-kinase (►PI3-Kinase) and JAK/STAT (►Signal transducers and activators of transcription in oncogenesis) signaling pathways involved in cell proliferation and cell survival (Fig. 1). Several signaling components within each of these pathways are able to interact with and/or



Kit/Stem Cell Factor Receptor in Oncogenesis. Figure 1 Schematic representation of known tyrosine autophosphorylation sites in human Kit/SCF-R together with the signaling molecules they recruit and activate. Several signaling molecules have been shown to bind to the juxtamembrane tyrosine autophosphorylation sites Y568 and Y570. Concentration differences of these molecules in different cell types, as well as differences in their subcellular distribution, may determine their recruitment to the autophosphorylation sites in vivo. Note that Grb2 has been shown to bind to the activated Kit/SCF-R in vivo, but that its binding to phosphorylated tyrosine 936 has only been mapped in vitro. Grb2 is an upstream activator of the Ras-Raf-ERK cascade. SHP1 is a protein tyrosine phosphatase, expressed in hematopoietic cells.

modify the activity of each other, allowing for so-called ►signal transduction cross-talk. This is important for fine tuning and modulation of signaling. It is becoming increasingly clear that such Kit/SCF-R-initiated signaling pathways eventually impinge on and regulate the cell cycle machinery including the ►p16-cyclin D-►Rb pathway, as well as the DNA damage response pathways including p19ARFand p53. It is perhaps exclusively through these latter effects that the signaling pathways ultimately regulate proliferation and/or anti-apoptosis, respectively. For some recent reviews on RPTK-initiated signal transduction and mitogenic/survival signaling pathways [2, 3].

Once the appropriate Kit/SCF-R-induced signaling pathways have been activated, it is crucial that further Kit/SCF-R signaling is down-regulated and inactivated to achieve a proper cell biological response. Several mechanisms prevail to achieve this, including negative feedback mechanisms, ligand-induced receptor internalization and degradation, and coordinated activation or organization of negative intracellular regulators including phosphatases and scaffolding proteins. In the case of Kit/SCF-R, SCF stimulation causes activation of ►PKC, which acts in a negative feedback loop to inhibit further Kit/SCF-R tyrosine kinase activity by directly phosphorylating two serine residues in the receptor (Fig. 1). SCF stimulation also induces Kit/SCF-R internalization with a $T½ < 0.5$ h, and it has been shown that the internalized receptors undergo regulated degradation, in part through ►ubiquitin-mediated proteolysis. The ligand-induced Kit/SCF-R down-regulation is likely to play an important regulatory physiological role in development, since SCF-expressing stromal cells interact with Kit/SCF-R-expressing parenchymal cells in gonads, bone marrow and cerebellum during development. In these tissues SCF exists in a mainly transmembrane form, which does not induce Kit down-regulation to the same extent as the soluble form of SCF, thus allowing for more sustained Kit signaling. There is some evidence that there are quantitative and qualitative differences in the activated signaling molucules induced by the two forms of SCF. The transmembrane form of SCF is important for stem cell self renewal and survival, while the soluble form is important for chemotaxis and cell proliferation. As part of a long-term mechanism to ensure proper Kit/SCF-R signaling, the Kit expression level is regulated in a cell- and tissue-specific manner at the transcriptional level as well. The c-kit promoter contains Sp1, SCL/Tal1 and AP-2-binding sites and its expression is tightly and positively regulated by each of these transcription factors.

Perturbation of the normal regulatory mechanisms governing Kit/SCF-R-induced signal transduction has serious clinical consequences. Accordingly, both in humans and mice, naturally occurring ►loss-of-function mutations and ►gain-of-function mutations in Kit/SCF-R have been reported that result in developmental defects or malignancies.

Clinical Relevance

W-mutant and Steel mutant mice have naturally occurring loss-of-function (LOF) mutations in Kit/SCF-R or in SCF, respectively, resulting in varying degrees of anemia, mast cell depletion, white-spotted depigmentation, decreased fertility and constipation. A similar phenotype is found in humans suffering from piebaldism, a syndrome caused by LOF mutations in Kit/SCF-R homologous to several of those found in W mutant mice. The W mutations provided the first reported examples of germ-line mutations in a mammalian proto-oncogene and the first in vivo evidence of the importance of RPTKs for not only cellular proliferation but also normal development and differentiation in mammals. Many of the W mutations are single base c-kit substitutions causing in-frame point mutations of highly conserved residues in the kinase domain of Kit/SCF-R, resulting in decreased or abolished kinase activity. Such mutations are dominant negative, which means that the phenotype in afflicted heterozygous individuals is more severe than would be expected from only one allele being mutated. A likely explanation for this is that SCF induces the formation of homo- and heterodimers of wild type (wt) and mutant (mut) Kit/SCF-R, and that only the wt/wt homodimers are fully signaling active, while the mutant receptor suppresses signaling from heterodimers. Since the ratio of wt/wt:wt/mut:mut/mut Kit/SCF-R dimers in a cell heterozygous for mutation in Kit/SCF-R is expected to be 1:2:1, only ~1/4 of all the Kit/SCF-R molecules are engaged in active signaling complexes, which is probably sub-threshold levels for most signaling pathways. An increased frequency of germ cell tumors and myeloid leukemias has been reported in some of the W mutant mice. This might indicate a possible role for Kit/SCF-R as an anti-oncogene, and again points to the importance of proper regulation of RPTK-induced signaling to ensure normal differentiation and development. For reviews on the physiological roles of Kit [4, 5].

The proto-oncogene c-kit was originally identified as the cellular counterpart of the ►oncogene v-kit, which encodes the transforming protein of the HZ4-feline sarcoma virus (FeSV) derived from a feline fibrosarcoma. HZ4-FeSV originated through transduction of feline c-kit sequence by feline leukemia virus. The protein encoded by the v-kit oncogene is essentially a doubly truncated version of Kit/SCF-R, where the extracellular, transmembrane and ~50 most C-terminal amino acids have been deleted. Its identification from a feline tumor immediately implicated v-kit as a tumorigenic oncogene. More recently numerous

gain-of-function (GOF) mutations have been identified in Kit/SCF-R that are strongly implicated in several kinds of human malignancies, including mast cell leukemia/mastocytosis, acute and chronic myeloid leukemias, gastrointestinal stromal tumors, germ cell tumors and possibly thyroid carcinomas. The mutations identified thus far are mainly located in exon 11, which encodes part of the juxtamembrane region, and in exon 17, which encodes part of the C-terminal half of the kinase domain. However, mutations have also been identified in other regions of Kit/SCF-R including the extracellular domain. All the GOF mutations in exons 11 and 17 cause ligand-independent dimerization of Kit/SCF-R resulting in its constitutive kinase activation. Future research into signal transduction by such mutated receptors should reveal more about the mechanisms responsible for transformation. For an overview of all Kit/SCF-R mutations associated with human malignancies identified to date, see Fig. 2.

Mastocytosis/Mast Cell Leukemia

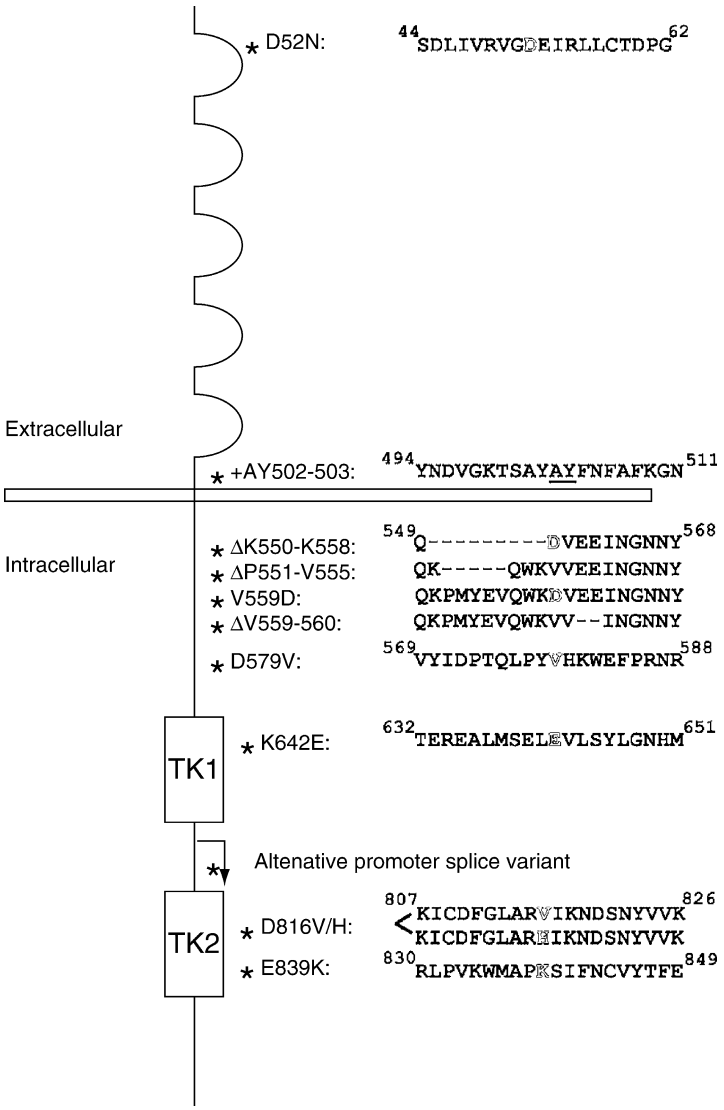
These are relatively rare conditions, characterized by mast cell hyperplasia in bone marrow, liver, spleen, lymph nodes, gastrointestinal tract and skin. There is often clinical evidence of mast cell activation, including urtication, pruritus, abdominal pain, nausea, vomiting, diarrhea, bone pain, flushing, vascular instability and headache. Mastocytosis is classified into four groups:

- Indolent forms
- ►Mastocytosis with associated hematological disorders, including myeloproliferative/myelodysplastic disorders
- Aggressive mastocytosis
- Mast cell leukemia

The prognosis is good for patients with indolent mastocytosis but generally poor for the other three groups, with mast cell leukemia having the most fulminant behavior. In 1993, two activating mutations in Kit/SCF-R, D816V in exon 17 and V560G in exon 11 were reported in the human mast cell line HMC 1, which was established from a patient with mast cell leukemia. Subsequent studies confirmed the presence of these mutations, in particular D816V, in Kit/SCF-R in mast cells from patients with mastocytosis. The D816V mutation has been associated with mainly sporadic, systemic mastocytosis in adults, while the G839K mutation was reported to be typically associated with cutaneous, mainly pediatric cases. Mutations at analogous sites have been identified in mast cells and mast cell lines from other species, including mouse, rat and dog. Mastocytosis is one of the most common tumors in dogs, is usually malignant and as a general rule associated with Kit/SCF-R mutations. The above mutations have been identified in such cases, but the most common mutations involve exon 11 and 12, including tandem duplications and other intragenic rearrangements. The mechanisms for transformation are unknown, but it is possible that a general increase in signaling caused by the Kit/SCF-R mutations is enough to cause mast cell transformation. Accordingly, mice reconstituted with bone marrow cells expressing another constitutively active RPTK v-ErbB, develop malignant mastocytosis with associated acute myeloid leukemia (AML) that can be transplanted to secondary recipients. The V559G and the D814V mutations in murine Kit/SCF-R cause constitutive tyrosine autophosphorylation in retrovirally transduced Ba/F3 and myeloid FDC-P1 cells, and leukemia in nude mice upon injection of the transduced cells. One study showed that in murine mast cells transfected with D816V-Kit/SCF-R there was an enhanced tyrosine phosphorylation of a 130 kDa protein and enhanced ubiquitin-mediated degradation of SHP-1, a cytoplasmic protein tyrosine phosphatase that negatively regulates Kit/SCF-R signaling. In conclusion, the D816V-Kit/SCF-R and to some extent exon 11 mutations, are recurrent mutations associated with, and supposedly causally involved in, aggressive forms of mastocytosis and mast cell leukemia.

Gastro-Intestinal Tumors

A strong association between Kit/SCF-R GOF mutations and ►gastrointestinal stromal tumors (GISTs) has been established. GISTs are the most common mesenchymal tumors of which ~1 out of ten cases is malignant. The primary tumors are located mainly in the ventricle and small intestine, and the highest incidence of new cases is in persons >40 years of age with an even sex distribution. Interestingly, >60% of GISTs are associated with exon 11 mutations in Kit/SCF-R, but none with mutations in exon 17, including of D816. Kit exon 11 GOF mutations occur mainly in malignant GISTs, which tend to be larger, with necrosis, hemorrhage, intra-abdominal spread and liver metastases and frequent recurrences. Hence, the exon 11 mutations portend poor prognosis with a 3 year survival <30% versus >65% for exon 11 mutation negative tumors, and it has been reported that Kit exon 11 mutations are an independent prognostic factor for GIST survival. The Kit/SCF-R (CD117) expression is diagnostic for GI stromal tumors versus ►leiomyomasa and gastric Schwannomas. Most cases examined are also CD34-positive, and ultrastructurally cells look like ►interstitial cells of Cajal (ICC), which is why it has been proposed that the ICC is the cell of origin for most GISTs. However, tumors phenotypically identical to GISTs (CD117+, most CD34+) occur as primary tumors in the omentum and mesentery as well, which indicates that GISTs might not all originate



Kit/Stem Cell Factor Receptor in Oncogenesis. Figure 2 Schematic representation of mutations in Kit/SCF-R, associated with human malignancies and dysplasias. Amino acid residues are denoted with single letters, numbers indicate the residue number in the human Kit protein sequence. Point-mutated amino acid residues are shown in outline, amino acid deletions with a dash and amino acid additions are underlined. The D52N mutation has been associated with myelodysplastic syndromes that include myelofibrosis and chronic myeloid leukemia. The AY duplication, the juxtamembrane mutations and the K642E mutations have mainly been associated with gastro-intestinal stromal tumors, but also with mast cell leukemias. The D816V and E839K mutations have been connected with mast cell leukemias. The D816H mutation has been connected with seminomas and dysgerminomas. Mutations, found in the hydrophilic region between the N- and C-terminal region of the kinase domain result -due to alternative promoter usage- in truncated versions of Kit. These isoforms have been identified in various cell lines which derived from colon carcinomas and hematopoietic malignancies.

from ICCs, but rather from a multi-potential precursor cell. A characteristic of GISTs is mitochondrion-rich ICCs, and some GISTs are gastro-intestinal autonomic nerve tumor (GANT)-like stromal tumors. An immunohistochemical and histological re-evaluation of archived paraffin-embedded esophageal tumor samples disclosed that ~25% of these were indeed Kit-positive GISTs with exon 11 mutations rather than leiomyomas or leiomyosarcomas, which was the original classification. This is important, since

esophageal GISTs are malignant, while leiomyomas, which are Kit-negative, are benign. Analysis of eight GISTs devoid of exon 11 mutations in Kit/SCF-R revealed exon 9 mutations in 6 and an exon 13 mutation (K642E) in 2, the latter causing constitutive tyrosine autophosphorylation of Kit/SCF-R. Finally, in colon carcinoma cell lines certain intron 15 alternative promoter splice variants, causing either a 78 bp deletion or a truncated Kit/SCFR with 25 unique amino acids fused to the C-terminal 257 amino acids, have been reported sporadically. The functional consequences are unknown. However, a potential autocrine loop of Kit/SCF-R and SCF might exist in colon cancer, since colonic mucosal cells usually express SCF, but areKit/SCF-R-negative. In conclusion, a majority of GISTs harbor GOF mutations in exon 11 of Kit/SCFR and there is overwhelming evidence that these are involved in the oncogenesis. Kit expression and exon 11 mutations are both of significant diagnostic and prognostic value for GISTs, the prognosis being significantly more severe if both are present.

Acute and Chronic Myeloid Leukemias

A vast number of publications have attempted to address the putative role of Kit/SCF-R in myeloid leukemias. While it is still unclear whether Kit/SCF-R is causally involved in these diseases, it does have an important diagnostic and prognostic value. While only 1–3% of normal mononuclear marrow blasts express Kit/SCF-R, a big European multi-center study concluded that 67% of all ►acute myeloid leukemia (AML) cases, ~30% of all biphenotypic acute leukemias and all undifferentiated acute leukemias express Kit/SCF-R. Kit/SCF-R is expressed mainly in M4 and M5 AML subclasses, but the highest expression levels are found on blasts in M1 and M2 subclass AML cases. A high proportion of megakaryocytic and erythroid leukemic cells are also positive for Kit expression, as is most blasts in chronic myeloid leukemia (CML). In general, Kit/SCF-R expression is useful in the differential diagnosis between AML (mostly positive) and acute lymphocytic leukemia (ALL; all negative). Negative expression for Kit/SCF-R in AML also identifies with some certainty two M5b subgroups. AML blasts express between 600 and 29,000 Kit molecules/cell, but there is no correlation between expression level and prognosis. Despite conflicting reports, it seems to be the consensus from the literature that there is no correlation between Kit expression and prognosis for AML in general, but that expression of Kit/SCF-R in the M1 subclass indicates a worse prognosis. This might be due to a strong correlation between expression of a non-P-glycoprotein multidrug resistance protein and Kit/SCF-R. Mutations in exon 8 of c-kit have been identified in a high proportion of AML cases, and all had the inv. 16 re-arrangement. Conversely, ~20% of inv. 16 AMLs had c-kit exon 8 mutations. All exon 8 mutations involved either deletion or replacement of the codon for D419. The functional consequences of these mutations for the kinase activity of Kit/SCF-R are unknown at present. However, retroviral transduction of murine hematopoietic precursors with D816V-Kit and transplantation of these cells into syngeneic hosts resulted in myeloid leukemias in a significant proportion of cases, showing that GOF mutations in Kit is sufficient for leukemic progression in mice. It has been suggested that constitutive association of ►BCR-ABL1 with activated Kit/SCF-R is responsible for the basophilia and myeloid growth in the chronic phase of ►CML. However, the ability of SCF to stimulate blast growth has also been utilized to mobilize peripheral CD34+/CD38– and other committed progenitors in patients about to undergo bone marrow transplantation for subsequent autologous transplantation. Addition of SCF together with G-CSF and with standard chemotherapy is superior to G-CSF and chemotherapy for this purpose.

Germ Cell Tumors

All carcinoma-in-situ (►CIS) testis, ~90% of seminomas and dysgerminomas, but only ~5% of non-seminomas express Kit/SCF-R. Isochrome 12p is a marker of ►germ cell tumors, and ►loss of heterozygosity on the long arm of chromosome 12 implicates SCF as a tumor suppressor. Furthermore, intersex gonads (45X/46XY and other cases with additional Y chromosome material) have delayed Kit expression and increased testicular cancer risk. This could indicate an anti-oncogenic role of Kit/SCF-R and its ligand for germ cell tumor development under some circumstances. However, other results might indicate that SCF and Kit/SCF-R can drive tumor progression. Hence, ectopic expression of Kit and SCF has been found in cervical and ovarian carcinomas and ovarian teratomas. Furthermore, there is an association between mediastinal germ cell tumors (MGCT) and hematological malignancies (e.g. acute leukemia and malignant histiocytosis), and often Kit-positive areas are found in these mediastinal tumors. This and other results have made it clear that Kit/SCF-R expression is a diagnostic aid for extragonadal seminomas. All classical seminomas are Kit-positive, aneuploid and positive for placental alkaline phosphatase, while 40% of spermatocytic seminomas are Kit-positive, and all are diploid or polyploid and negative for placental alkaline phosphatase. This indicates that some spermatocytic seminomas might originate from primordial germ cells. In line with this, experimental testicular teratomas can be generated by transplanting E12 male genital ridges to

testes of adult mice. Importantly, it was recently found that tumors of seminoma/dysgerminoma type had a D816H mutation in Kit/SCF-R causing its constitutive activation. In conclusion, Kit/SCF-R expression is of diagnostic help for seminomas/dysgerminomas, and GOF mutations in Kit/SCF-R might be oncogenic and involved in the generation of such tumors.

Malignant Melanoma

Normal melanocytes depend on ►bFGF, ►HGF and SCF in vitro. ►Melanoma cells become independent of these growth factors, in part through autocrine bFGF stimulation. Interestingly, Kit mRNA and protein are down-regulated in human and murine ►melanoma cell lines. This correlates with in vivo findings: While Kit/SCF-R is expressed in normal melanocytes, benign and dysplastic naevi and nontumorigenic melanomas, expression is lost in dysplastic naevi, tumorigenic primary melanomas and metastases. In addition, transfection of Kit/SCF-R into highly metastatic melanoma cell lines, induced slowed growth rate and fewer lung metastases in nude mice. The transcription factor AP-2 controls expression of c-kit and the gene for MCAM/MUC18 positively and negatively, respectively. AP-2 is down-regulated in melanomas and this is thought to be the reason for loss of Kit expression, allowing the malignant cells to escape SCF-induced apoptosis. Conversely, enforced AP-2 expression suppresses tumorigenicity and metastatic potential, possibly through c-kit transactivation and subsequent SCF-induced apoptosis. It has been proposed that AP-2 loss is a crucial event in malignant melanoma development.

Other Neoplastic/Malignant Lesions

Kit/SCF-R and its ligand have been found co-expressed in cells from ►small cell lung cancer and ►neuroblastoma, and it was reported that it might be involved in malignant progression in these cases. In ►neurofibromatosis 1 (NF-1) there is infiltration with Kit-positive mast cells in the neurofibroma tissue, which is composed mainly of Schwann cells with an increased SCF mRNA expression compared to normal skin. The mast cells produce collagen VIII, which might contribute to the fibrosis in this disease. There is an abnormally high expression level of Kit in NF-1-derived Schwann cell lines and decreased neurofibromin expression (Ras-GTPase). The proliferation is Kit-dependent. In myelodysplastic lesions, Kit mutations might be involved in the pathogenesis. A recurrent D52N-Kit/SCFR mutation has been reported in these cases. Finally, down-regulation of Kit/SCF-R has been reported in breast cancer and in ►thyroid carcinoma, despite expression by normal mammary duct epithelial cells and thyroid cells. It has been proposed that the Kit/SCF-R downregulation enables de-differentiation of the cells in these tumor types.

Gene Transfer, Immunotherapy, Vaccination

SCF might be useful in expansion of peripheral blood leukocytes and of hematopoietic progenitors in culture before retroviral transduction or re-introduction in conjuction with autologous bone marrow transplantation. Phase II and III trials are currently being conducted on advanced stages of breast cancer and certain leukemias for this purpose. SCF might also be useful in conjuction with immuno-therapy. For instance, following high-dose ►cyclophosphamide and IL-3, ►dendritic cells can be mobilized and expanded ex vivo from CD34+ cells in the presence of GM-CSF, TNFα, Flt3 ligand and SCF. Dendritic cells are competent antigen-presenting cells for CD8+ cytotoxic T cells, so they can be used to stimulate the host immune defense against undesirable antigens, including tumor antigens. Ongoing phase II trials are examining the use of such expanded dendritic cells for immunotherapy or vaccination. Finally, in the past five years several small molecule protein kinase inhibitors have been approved as drugs for the treatment of specific types of cancer. Among these are two that act as inhibitors of Kit tyrosine kinase activity. ►Imatinib (STI571/GleevecTM), an Abl, PDGFR and Kit inhibitor, was originally approved by the FDA for the treatment of chronic myelogenous leukemia (►CML) in 2001. Shortly thereafter, imatinib was shown to be efficacious for treatment of gastrointestinal sarcomas (GIST), which are caused by mutant forms of Kit and PDGFRalpha, and was approved by the FDA for treatment of GIST in 2002. In 2006, a second tyrosine kinase inhibitor (TKI), ►sunitinib (SU11248/SutentTM), was approved for the treatment of imatinib-resistant GIST. Several additional TKIs have either approved or are in clinical trials for the treatment of cancer and other diseases caused by mutant Kit and other activated tyrosine kinases, and the number of approved TKI drugs is likely to grow significantly in the next ten years.

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Klatskin Tumors

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Synonyms

(Peri-) Hilar Cholangiocarcinoma; Extrahepatic; cholangiocarcinoma; Cholangiocellular carcinoma; Bile duct carcinoma

Definition

Klatskin tumors are anatomically defined as extrahepatic ►cholangiocarcinomas that occur at the confluence of the left and right hepatic duct (Fig. 1a). Morphologically, most Klatskin tumors (hilar cholangiocarcinomas) exhibit a nodular-sclerosing growth pattern. Few are papillary tumors and mass-forming hilar lesions are rare. Histologically, the vast majority of these tumors are adenocarcinomas.

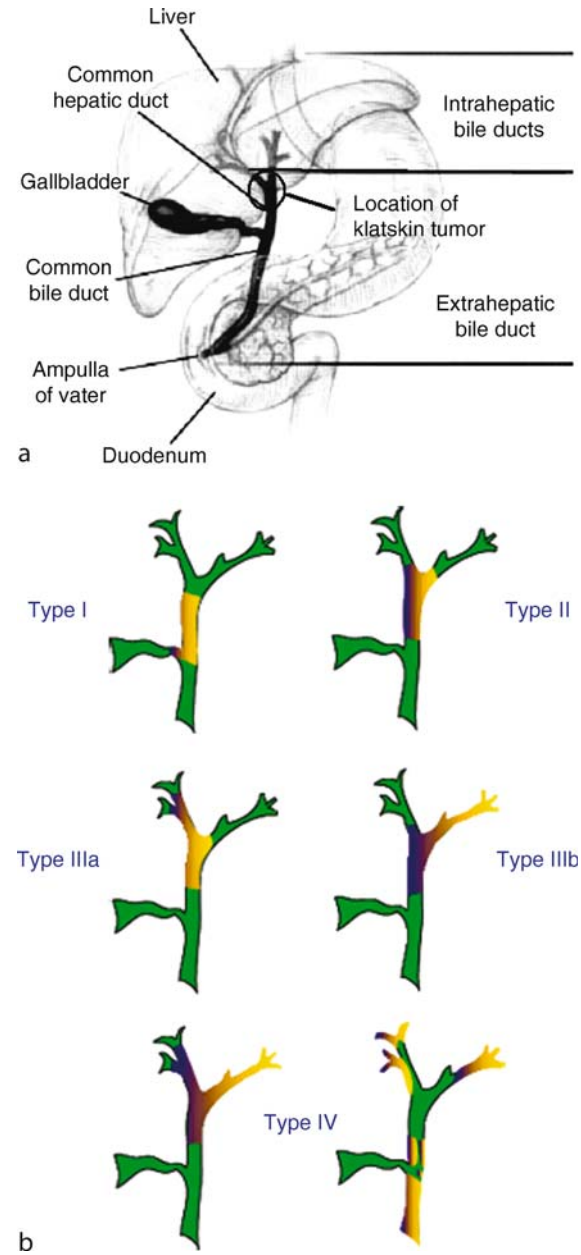
Characteristics

History and Classification

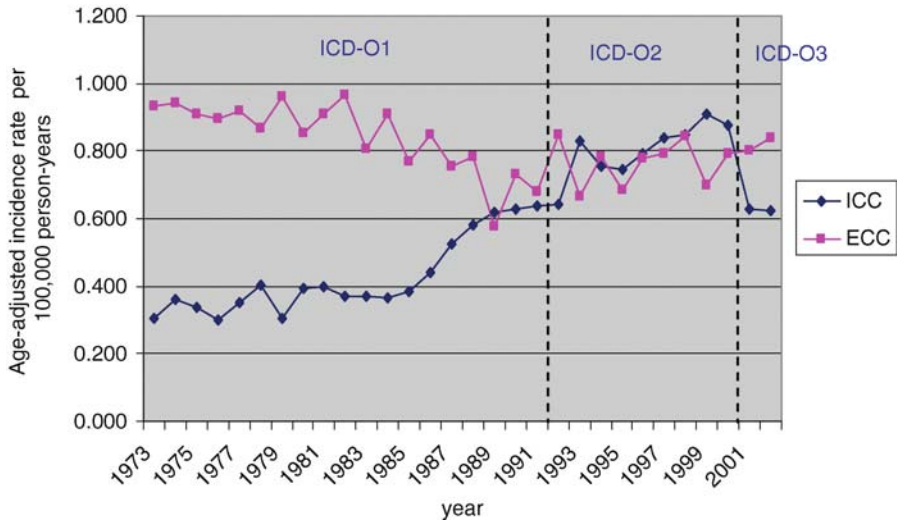
Hilar cholangiocarcinomas were first described by Klatskin and were further classified by Bismuth and Corlette (Fig. 1b): Bismuth type I tumors involve the common hepatic duct below the confluence of the right and left hepatic ducts; Bismuth type II tumors involve the confluence of the left and right hepatic duct; Bismuth type III tumors involve the common hepatic duct and the right (type IIIa) or left hepatic duct (type IIIb). Bismuth type IV tumors involve either the confluence of the hepatic ducts and the right and left hepatic duct, or are multicentric.

Epidemiology

Over the last decades, the incidence of intrahepatic cholangiocarcinoma increased in the United States, as it did worldwide. In contrast, the incidence of extrahepatic cholangiocarcinoma remained constant (Fig. 2). A recent study showed, that extrahepatic cholangiocarcinomas (including Klatskin tumors and distal extrahepatic lesions) represent ~50% of all cholangiocarcinomas in the Surveillance Epidemiology and End Results (SEER) cancer registries of the United States. A frequently cited study reported that 67% of cholangiocarcinomas were hilar tumors. However, the study was a retrospective examination of cholangiocarcinoma patients who underwent surgical exploration at a tertiary medical center. Due to the study design, the patients were unlikely to be representative of all cholangiocarcinoma patients in the United States. In addition, the International Classification of Diseases for Oncology (ICD-O) assigned Klatskin tumors a histology code rather than a topography code, and the histology code was cross-referenced to the topography code for intrahepatic rather than extrahepatic tumors. This misclassification has introduced error into the reporting of cholangiocarcinoma rates in the U.S. SEER cancer registries, making it impossible to define Klatskin tumor incidence on a population-based level.



Klatskin Tumors. Figure 1 (a) Anatomical location of Klatskin tumors. (b) Bismuth Corlette classification of Klatskin tumors (hilar cholangiocarcinoma).



Klatskin Tumors. Figure 2 Age-adjusted incidence rates for intrahepatic CC (ICC), extrahepatic CC (including Klatskin's tumors under 8162/3; ECC) diagnosed in SEER9 registries, 1973–2002. The dashed line represents the incidence of tumors reported in SEER to histology code 8162 (Klatskin). It must be noted that these tumors are unlikely to comprise all Klatskin tumors, since according to the ICD-O coding (1992–to date) could also be coded as ECC using other histology codes.

Etiology and Risk Factors

There is recent evidence that the molecular pathogenesis of Klatskin tumors/extrahepatic cholangiocarcinoma and intrahepatic cholangiocarcinoma may differ. Conditions that are associated with biliary inflammation increase the risk for cholangiocarcinogenesis. Well defined risk factors for Klatskin tumors and other extrahepatic cholangiocarcinomas include primary sclerosing cholangitis, infection with liver flukes (*Opisthorchis viverrini, Clonorchis sinensis*), choledochal cysts, ►Caroli Syndrome, biliary stones, smoking and ►Thorotrast exposure. Hepatitis C virus has been reported to be associated with intrahepatic cholangiocarcinoma and, in some reports, with hilar cholangiocarcinoma. Many patients do not exhibit any of the known risk factors, highlighting the need for further studies on etiopathogenesis of these tumors.

Biology

Diagnosis

Clinical Symptoms. Patients present with obstructive jaundice (icterus, dark urine, pale stools, pruritus) and weight loss, and frequently signs of bacterial cholangitis. Abdominal pain and ascites may be present in patients with advanced disease.

Biochemical Investigations. Parameters indicating *obstructive* cholestasis such as increased alkaline phosphatase, gamma glutamyltranspeptidase, serum bilirubin, and sometimes transaminase concentrations, may be found in patients with Klatskin tumors. ►Tumor markers such as ►CA 19-9 and ►CEA, may be elevated. These tumor markers, however, are not specific for Klatskin tumors/cholangiocarcinoma and CA19-9 may also be elevated in conditions with benign cholestatic disease.

Imaging Methods. Accurate diagnosis is key to delineate benign from malignant biliary strictures and to determine the resectability of Klatskin tumors. Ultrasound is the initial diagnostic test to evaluate hilar cholangiocarcinoma and may show intrahepatic duct dilatation and caliber changes of bile ducts, but may fail to show liver infiltration of smaller tumors. On ►computed tomography (CT), the primary obstructing hilar lesion may be invisible. On contrast enhanced CT, (infiltrative) lesions may show as thickening of the duct. Further signs include dilatation of the intrahepatic bile ducts or the atrophy-hypertrophy complex. Used to assess resectability, the accuracy of CT ranges from 60 to 75%. On ►magnetic resonance imaging, Klatskin tumors appear hypointense on T1- and hyperintense on T2-weighted images. Compared to the liver parenchyma, most Klatskin tumors are hypovascular. Gadolinium chelates and ferumoxide contrast agents can be used to assess liver invasion because of a good liver-tumor contrast. Magnetic resonance cholangiopancreatography (MRCP) can be used to visualize the obstructing lesion and intrahepatic bile duct dilatation and, in a small study, was shown to have an accuracy of 84% to assess the level of bile duct involvement.

Endoscopic retrograde cholangiopancreatography (ERCP) can be used to endoscopically determine the

location and extent of biliary tract obstruction. ERCP also allows palliative stent implantation for biliary drainage, and to obtain tumor cytology or biopsy. However, according to the specific growth pattern of Klatskin tumors, the sensitivity of brush cytology is 30%, and the combination of brush cytology/biopsy only ranges up to 70%.

Treatment

To date, the only curative approach to hilar cholangiocarcinomas is surgery with resection of the bile ducts and hepatojejunostomy, and/or partial hepatectomy to achieve tumor free margins. However, at the time of diagnosis, only few/a small proportion of patients have resectable disease. Resection is contraindicated for Bismuth Type IV tumors or in the presence of any of the following: vascular encasement, occlusion or invasion of the main portal vein or hepatic artery, distant or lymph node metastases, liver invasion, or invasion of extrahepatic organs. Surgery for hilar cholangiocarcinoma with curative intent is associated with 5-year survival rates of 40%. Recently, there has been some evidence that liver transplantation, together with neoadjuvant chemoradiation may improve survival in selected patients with unresectable hilar cholangiocarcinoma.

►Palliative approaches include the endoscopic placement of biliary stents or percutaneous biliary drainage to improve the symptoms of cholestasis. ►Photodynamic therapy with hematoprophyrin based photosensitizers, in addition to bilateral endoprosthesis insertion, has been shown to prolong survival by several months and improve quality of life in several randomized controlled trials. So far, ►radiation, as well as systemic ►chemotherapy has not led to a significant improvement of survival rates, however, several new protocols are currently under investigation.

Survival

The prognosis of hilar cholangiocarcinoma is poor, with 5 year survival rates ranging between 5 and 10%.

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KLK3

►PSA

KLRK1

►NKG2D Receptor and Cancer

Knobloch Syndrome

Definition

Is an autosomal recessive disease that is characterized by ocular defects leading to retinal detachment, macular degeneration and blindness.

Knock-Down

Definition

►Knockdown

Knock-in

Definition

A genetically engineered mouse that has a different version of a gene inserted into its genome.

►Mouse Models

Knock-Out

Definition

Gene invalidation resulting in the absence of expression of the corresponding protein. A commonly used model is the ►Knock-out mouse.

►Gene Knockout
►Mouse Models

Knock-out Mice

►Knock-out Mouse

Knock-Out Mouse

Definition

Knock-out mouse is a genetically engineered mouse carrying one or more of its genes made inoperable through a ►gene knockout (usually an inactivating mutation). Knockout is a technical approach to functionally characterize a gene that has been sequenced but has an unknown or incompletely known function. Because genes between mouse and humans are evolutionary conserved and, a priori, are assumed to have similar functions, the phenotype that a knock-out gene has in the mouse may be used to extrapolate its function in humans. For many genes, however, disease-associations in humans did not identify a similar phenotype in a knock-out mouse. Different strains of the mouse also may develop different phenotypes upon knock-out of a particular gene.

Knockdown

Definition

Gene knockdown refers to the experimental reduction of expression of a gene. This is commonly achieved by introducing small interfering RNAs (►SiRNAs) or other anti-sense oligonucleotides into cells.

►Antisense Nucleic Acid

Knudson Hypothesis

Definition

Originally described as a model where in retinoblastoma two genetic "hits" result in cancer; subsequently, the two "hits" were identified as occurring as inactivating mutations in the two alleles of a gene today known as the ►retinoblastoma gene (*RB1*). The two mutations can occur somatically in an individual. More often, one mutation inactivates one allele in a germ cell, the developing embryo then has one inactivated and one normal allele. Because of the ►recessive nature of the *RB1* gene, there will be no phenotype in the child, it does have however an increased risk. In most cases, the remaining normal allele will mutate in the retina during early childhood resulting in cells with both *RB1* alleles inactivated. As the consequence, the child will develop retinoblastoma.

This two-hit hypothesis has been generalized to entertain the idea that most cancers develop following this model. Accordingly, numerous ►loss-of-heterozygosity studies have been done on virtually every tumor entity to identify genomic regions deleted on one tumor cell chromosome with co-existing gene mutation on the other chromosome. Only in few models the two-hit model could be convincingly verified, and today the Knudson hypothesis more or less appears to be restricted to few cancer types. Instead, there are convincing data now to support the idea that cellular effects supporting the process of malignant cellular conversion can result from the inactivation of one of the alleles. The basic concept here is ►haploinsufficiency.

Koch's Postulates

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Synonyms

Henle–Koch postulates

Definition

A set of criteria used to identify the specific pathogen that causes an infectious disease.

Characteristics

Koch's postulates are attributed to Robert Koch, who received the 1905 Nobel Prize in Medicine or

Physiology "for his investigations and discoveries in relation to tuberculosis." Jakob Henle was a professor at the University of Göttingen when Koch enrolled as a student there in 1862, and Henle was one of the early proponents of the idea that contagious diseases were caused by microorganisms. In the early days of bacteriology there were numerous heated arguments over the identity of pathogenic agents. The presence of commensal microorganisms alongside pathogenic microorganisms often resulted in the misidentification of the real disease-causing organism. At that time there was also a school of thought which held that there were no true bacterial species, but rather that a bacterium could adopt nearly limitless morphologies and physiologies, which would allow it to cause tuberculosis, anthrax, food spoilage, or other problems depending on the particular form adopted by the bacterium. It was against this backdrop that Koch proposed the following three criteria (as translated by Thomas M. Rivers) for identifying a microbiological cause of disease.

1. "The parasite occurs in every case of the disease in question, and under circumstances which can account for the pathological changes and clinical course of the disease."
2. "It occurs in no other disease as a fortuitous and non-pathogenic parasite."
3. "It, after being fully isolated from the body and repeatedly grown in pure culture, can induce the disease anew."

Koch famously applied these principles as he isolated *Bacillus anthracis* and *Mycobacterium tuberculosis* and identified them as the causes of anthrax and tuberculosis, respectively. By establishing a theoretical framework for determining cause and effect, Koch's postulates had a profound effect on infectious disease research. Within a few decades, the identities of dozens of new pathogens were revealed. Yet these precepts had obvious limitations arising from the simple fact that not all pathogens can be propagated in pure form as autonomous living organisms. Viruses were the first such examples to be considered by the scientific community, and it was soon apparent that Koch's postulates had to be revised to accommodate the scientific discoveries of the twentieth century. Over the years since Koch's postulates were first postulated, many investigators have articulated a revised set of criteria to permit the systematic evaluation of viruses, prions, serum cholesterol, tobacco smoke, chromosomal translocations (►chromosomal translocation) and other factors as the underlying cause of a disease, contagious or otherwise.

In 1976, Alfred S. Evans proposed the following ten criteria as a "unified concept" for disease causality, which could be applied to either chronic or acute diseases.

1. *Prevalence* of the disease should be significantly higher in those exposed to the putative cause than in case controls not so exposed.
2. *Exposure* to the putative cause should be present more commonly in those with the disease than in controls without the disease when all risk factors are held constant.
3. *Incidence* of the disease should be significantly higher in those exposed to the putative cause than in those not so exposed as shown in prospective studies.
4. *Temporally*, the disease should *follow* exposure to the putative agent with a distribution of incubation periods on a bell shaped curve.
5. *A spectrum* of host responses should follow exposure to the putative agent along a logical biologic spectrum from mild to severe.
6. *A measurable host response* following exposure to the putative cause should *regularly appear* in those lacking this before exposure (i.e. antibody, cancer cells) or should *increase* in magnitude if present before exposure; this pattern should not occur in persons so exposed.
7. *Experimental reproduction* of the disease should occur in higher incidence in animals or man appropriately exposed to the putative cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or demonstrated in a controlled regulation of natural exposure.
8. *Elimination or modification* of the putative cause or of the vector carrying it should decrease the incidence of the disease (control of polluted water or smoke or removal of the specific agent).
9. *Prevention or modification* of the host's response on exposure to the putative cause should decrease or eliminate the disease (immunization, drug to lower cholesterol, specific lymphocyte transfer factor in cancer).
10. The whole thing should make biologic and epidemiologic sense.

The correct identification of the cause of a cancer is essential, because the cause can either be avoided, thereby preventing cancer, or the cause can be targeted through drug design, thereby curing the cancer. It is also clear that if one invests preventive or drug design efforts into the wrong cause, then one will have accomplished naught. Obvious limitations of Koch's postulates arise when one attempts to apply them to non-contagious diseases, when suitable animal models for reproducing the disease do not exist, or when there is an inordinately long latent period between initial exposure to the causal agent and the disease's manifestation. These circumstances often apply to the development of cancer. The ability of a pathogenic agent to cause cancer is also dependent upon the presence or absence of various tumor

suppressors, which causes some individuals to be more susceptible to developing cancer than others. For these and other technical reasons, it is often unwise to dogmatically invoke Koch's postulates in an effort to disprove a particular agent as a cause of disease. An infamous controversy arose in the late 1980s and early 1990s when Koch's postulates were invoked in an attempt to disprove that HIV-1 caused AIDS, although that pathogenic link is now universally accepted. Koch's postulates are of great historical significance because they marked the application of logic and reason to the field of pathology. As many scholars have noted, it is unwise to insist upon the application of the original postulates when we now know that there are pathogenic mechanisms that do not conform to the lifestyles of protozoa, fungi, or bacteria. It is the rigorous application of logic and reason to the elucidation of the cause of disease which remains as the lasting legacy of Koch's postulates.

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Kostmann Syndrome

Definition

Synonym: Severe Congenital neutropenia; Is an inherited disorder of the bone marrow. Children born with this condition lack ►neutrophils (a type of white blood cell that is important in fighting infection, also called granulocytes). These children suffer frequent infections from bacteria which in the past led to death in three-quarters of cases before 3 years of age. Children with hostmann syndrome have an increased risk of developing ►acute myeloid leukemia (CML) or ►myelodysplasia.

►myelodyplastic syndromes

Kringle Domain

Definition

Is a triple-disulfide-loop structure spanning ~80 amino acids and playing a role in protein-protein interactions. This domain was first found in bovine prothrombin.

►Macrophage-stimulating Protein
►Scatter Factor

KSA

►EpCAM

KSP

►KSP Mitotic Spindle Motor Protein

KSP Mitotic Spindle Motor Protein

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Synonyms

Eg5; Kinesin spindle protein; KSP; Kinesin-5; Kinesin-like protein KIF11

Definition

KSP mitotic spindle motor protein is a microtubule-associated molecular motor belonging to the ►kinesin superfamily, which plays an essential role in the separation of centrosomes, the assembly of bipolar spindles, and the faithful segregation of chromosomes into daughter cells during ►mitosis.

Characteristics

KSP is a plus-end-directed ►microtubule motor protein, well conserved from yeast to mammals. It can generate directed mechanical force by hydrolyzing ATP and move along microtubule filaments from the minus end (oriented towards the nucleus) to the

plus end (oriented towards the cell periphery). The KSP protein contains a highly conserved N-terminal motor domain encompassing the head and neck motifs, a coiled-coil stalk domain, and a tail region containing a characteristic BimC box close to the C-terminus. Based on the N-terminal motor domain sequence, the vertebrate KSP belongs to the BimC subfamily of ►kinesins, a large family of ►microtubule-based motor proteins. More recently, KSP was classified to the kinesin-5 subgroup according to a new nomenclature system. The globular motor domain of KSP is responsible for catalyzing hydrolysis of ATP, interacting with microtubules and nucleotides, and driving the movement along microtubules empowered by ATP hydrolysis, whereas the stalk domain mediates protein–protein interactions to form homotetramers. Active KSP is a homotetramer in which two polypeptides first dimerize to form a parallel coiled-coil and then two dimers form an antiparallel tetramer containing four motor domains with a dimeric motor unit at each end (Fig. 1). As a tetramer, KSP is able to crosslink and translocate along two adjacent microtubules with each dimeric motor unit interacting with a single microtubule fiber. Fluorescence studies suggest that when binding to two antiparallel microtubules, KSP can slide them apart, thereby pushing spindle poles apart. If binding to two parallel microtubules, KSP can mediate bundling of parallel microtubules, contributing to the assembly and integrity of mitotic spindles (Fig. 2).

Like other ►kinesins of the BimC subfamily, human KSP is required early in prometaphase for the separation of duplicated centrosomes and the formation of bipolar mitotic spindles. Inhibition of KSP with neutralizing antibodies, small molecule inhibitors, or small interfering RNAs (siRNAs) causes formation of the characteristic monopolar mitotic spindle (also termed monoaster), a radial array of microtubules surrounded by a ring of chromosomes, and cell cycle arrest at prometaphase. Further studies indicate that suppression of KSP activates the ►spindle assembly checkpoint, thereby preventing the onset of anaphase and causing mitotic arrest. Prolonged mitotic arrest induces apoptosis.

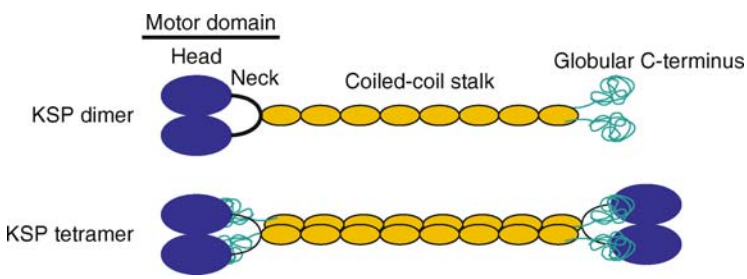
Inhibition of KSP does not affect postmitotic cells, indicating that KSP is only required for cell proliferation and that it functions exclusively in mitosis. Consistent with its exclusive role in cell division, KSP is preferentially expressed in proliferating tissues, such as thymus, bone marrow ,and intestine epithelium, with minimal or no expression in nonproliferating tissues such as the central nervous system. In addition, it displays much more abundant expression in actively proliferating tumor tissues than in normal adjacent tissues.

Regulation

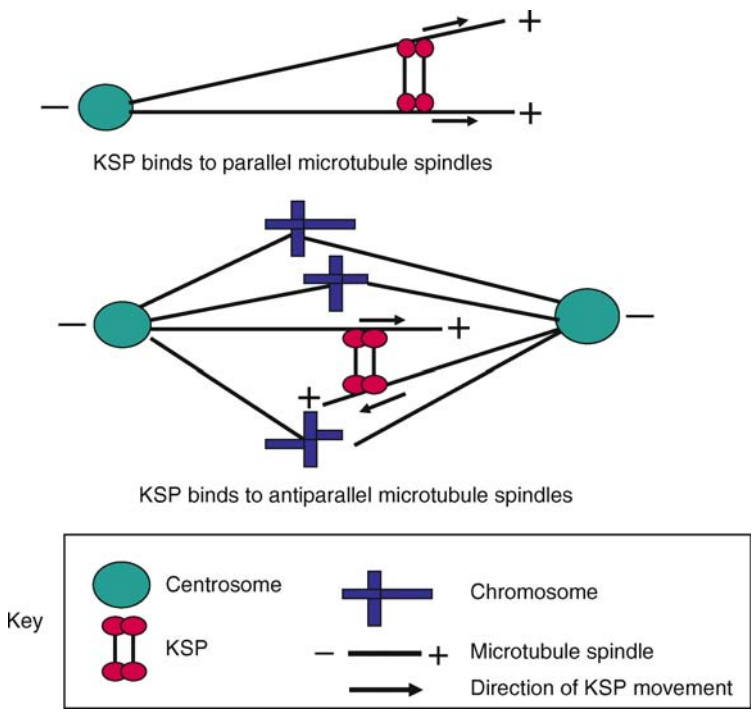
The ATPase activity of KSP is essential for its function and it is stimulated by the interaction with microtubules. The control of ATPase activity by interactions with microtubules is thought to prevent futile hydrolysis of ATP. It has been shown that there is an evolutionarily conserved $p34^{cdc2}$ phosphorylation site (Thr-927 in human KSP) located in the BimC box of KSP, and that $p34^{cdc2}$-mediated phosphorylation of this site controls the association of KSP with the microtubule spindles. Moreover, KSP can be phosphorylated by ►Aurora kinase, a mitotic kinase, but the functional importance of this phosphorylation remains to be determined.

Relevance to Cancer

The mitotic spindle is a protein complex consisting of multiple components including microtubules and motor proteins and it is a pharmaceutically validated target for cancer therapeutics. ►Vinca alkaloids and ►taxanes that interfere with the dynamics of microtubules by binding to β-tubulin perturb mitosis and have been used in the clinic for decades for the treatment of many human malignancies. However, since microtubules have essential functions beyond mitosis in nonproliferating cells, including intracellular transport



KSP Mitotic Spindle Motor Protein. Figure 1 Schematic representation of the structures of KSP dimmer and tetramer.



KSP Mitotic Spindle Motor Protein. Figure 2 Schematic illustration of KSP functions.

and organelle positioning, drugs that target microtubules cause microtubule-dependent side effects, such as peripheral neuropathy. In addition, an increasing number of tumors have acquired resistance to these antimicrotubule drugs via various mechanisms including acquired mutations on β-tubulin, altered expression of tubulin isoforms, changed microtubule dynamics as well as elevated expression of membrane drug efflux pumps like ►P-glycoproteins (P-gp). Thus, agents that are able to selectively disrupt the mitotic spindle via a novel mechanism of action and without P-gp liability are greatly desired for cancer therapy.

There are several important traits that make KSP an attractive target for cancer treatment: (i) KSP is a crucial component of the mitotic spindle and is essential for spindle bipolarity and proper segregation of chromosomes during mitosis. (ii) KSP functions exclusively in dividing cells and there is no evidence that inhibition of KSP affects nonproliferating cells. (iii) KSP is overexpressed in tumor tissues relative to adjacent normal tissues. (iv) Since KSP is an ATPase and the ATPase activity is required for its function, KSP is a druggable target. Indeed, a number of small molecule inhibitors of KSP ATPase with distinct chemical structures have been generated and currently at different stages of development. Some specific inhibitors of KSP have exhibited broad antiproliferative activity in tumor cell lines and significant antitumor efficacy in various murine tumor models. A few KSP inhibitors have entered clinical trials. Among them ispinesib demonstrated some activity in taxane-treated patients with metastatic breast cancer in a phase II study. As expected, the clinical trial data indicate that KSP inhibitors could not exhibit neurotoxicity because of their lack of effect on microtubule dynamics.

Although KSP inhibitors hold great promise for cancer treatment, their clinical antitumor activity remains to be determined. The clinical development of these novel antimitotic agents holds many challenges. A critical issue is how to identify the responders in the clinic, or what ►biomarkers could predict clinical response of tumors to KSP inhibitors. Preclinical studies suggest that tumor cells with a competent spindle assembly checkpoint are more sensitive to KSP inhibitor-mediated cell death than those with a deficient spindle checkpoint. This awaits confirmation in the clinic. Additionally, a better understanding of the mechanism that mediates the lethality of these agents or the link between KSP inhibition and induction of cell death is required to guide the selection of dosing schedules and the identification of determinants of drug sensitivity. Finally, since antimicrotubule drugs and KSP inhibitors may kill cancer cells through a partially overlapping pathway, elucidation of the mechanisms that cause resistance to microtubule inhibitors in the clinical setting would tell whether KSP inhibitors should be used for the treatment of antimicrotubule agent-resistant tumors.

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Ku70

Definition

The protein of 70-kDa molecular weight is a DNA-helicase involved in the DNA double-strand break repair system (►Double-strand break DNA repair), more precisely the nonhomologous end joining.

►Ku Antigen, 70-kDa Subunit
►Lupus Autoantigen p70
►Thyroid-Lupus Autoantigen (TLAA)
►Thyroid Autoantigen, 70-kDa (G22P1)
►Securin

Kulchitsky Cell Carcinoma

►Extrapulmonary Small Cell Cancer

Kunitz Trypsin Inhibitor

Definition

KTI; Antinutritional/allergen protein found in soybean that KTI acts as a inhibitor of pancreatin and chymotrypsin.

►Lunasin

Kupffer Cells

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Synonyms

Hepatic (Liver) fixed macrophages

Definition

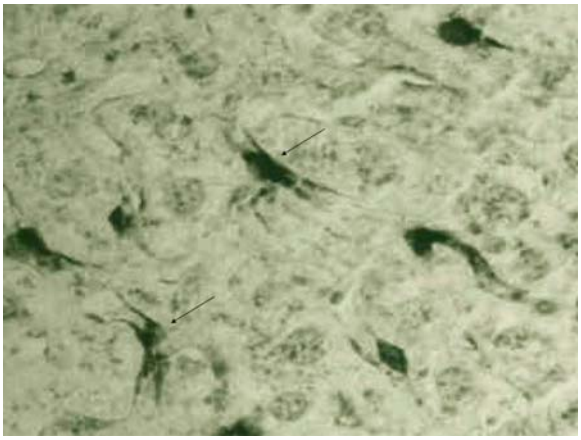
Kupffer cells are ►macrophages that are fixed within the sinusoids of the liver. They are the body's most abundant source of fixed macrophages.

Characteristics

Kupffer cells were first described in 1876 and are named for, the German pathologist Karl Wilhelm von Kupffer who called them the star cells (sternzellen) of the liver. Kupffer cells are tissue macrophages that are located in the hepatic sinusoidal blood flow attached to the endothelial cell lining. They represent 10% of all liver cells and are the body's largest population (approximately 80%) of fixed macrophages. They are the first macrophage population to contact bacteria, bacterial debris and ►endotoxins arising in the gastrointestinal tract. Kupffer cells are both highly endocytic and phagocytic and a major function is to remove particulate matter from the circulation. They are also an important component of innate immunity which is the most rapid response to dangerous stimuli and can modulate immune responses by antigen presentation and suppression of T-cell activation. Thus, the main role of Kupffer cells is to protect the body by removing potentially harmful substances from the blood. In addition Kupffer cells function to remove and destroy old red blood cells. Kupffer cells have also been implicated in cancer cell surveillance (see below). Kuppfer cells originate in the bone marrow. These bone marrow monocytes enter the circulation and become implanted in the liver where they differentiate into fixed tissue macrophages. Kupffer cells are considered terminally differentiated and the cells no longer divide (Fig. 1).

Kupffer Cells and Liver Disease

Kupffer cells are involved in the development of alcoholic liver disease. Kupffer cells become activated when they are exposed to the bacterial cell wall component endotoxin (Lipopolysaccharide, LPS). This results in the secretion of signaling molecules including ►cytokines and ►reactive oxygen species (ROS), which can be damaging to liver cells especially those that have been affected by alcohol ingestion and contain a large amount



Kupffer Cells. Figure 1 Section of mouse liver showing stellate shaped Kupffer cells (arrows). The cells are stained by the immunoperoxidase method for the presence of a breast cancer associated antigen, the gross cystic disease fluid protein (GCDFP-15), 15 min after intravenous injection of the purified protein.

of fat (alcoholic steatosis). Endotoxin is produced by gram negative bacteria in the large intestine and ingestion of alcohol alters the permeability of the intestine (leaky gut) to endotoxin causing an increased level in the portal blood entering the liver. To activate Kupffer cells and other macrophages endotoxins combine with a serum protein, lipopolysaccharide binding protein (LBP). This complex reacts with a cell surface receptor, ►CD14 which in turn interacts with the Toll like receptor ►TLR4 and the intracellular signaling protein ►MyD88. This causes a cascade of intra-cellular signals that activate transcription factors such as ►NFκB and result in the synthesis and secretion of cytokines and ROS.

Relationship of Kupffer Cells to Primary and Secondary Liver Cancers

The liver is a favored site for the secondary spread of many cancers including those of the large intestine and breast. The role of Kupffer cells in the development of cancers in the liver is controversial. There is no doubt that they play an important part in both the development of primary liver cancer (►hepatocellular carcinoma or hepatoma) and in the formation of ►metastasis from a distant site including cancers of the lungs, breast, colon and other gastrointestinal cancers. Some experimental studies have shown that elimination of Kupffer cells from the liver results in enhanced metastatic disease. Other studies have implicated Kupffer cells as agents that interact with the tumor cells entering the liver and their pro-inflammatory responses can induce tumor cell implantation and growth allowing increased metastatic disease. In primary liver cancer recent evidence has shown a role for Kupffer cell activation in carcinogenesis and progression of the cancer. Some liver carcinogens are able to activate Kupffer cells and induce cytokines and other factors that promote hepatocyte growth. These factors can also effect growth of pre-neoplastic cells and lead to the development of hepatoma. Evidence has accumulated that the pro-inflammatory cytokine IL-6 plays an important role in promoting hepatoma cell survival and proliferation. This tumor cell related increase in IL-6 is mediated through the MyD88/NFκB signaling cascade in Kupffer cells. In the case of colorectal cancer metastasis to the liver cytokines produced by Kupffer cells play a part in both tumor cell implantation and their subsequent survival and growth. During the development of colorectal cancer metastasis to the liver, Kupffer cells respond to the glycoprotein carcinoembryonic antigen (►CEA). The cells bind CEA via a pentapeptide that reacts with a surface protein that is identical to the RNA binding protein hnRNP M4. This results in Kupffer cell activation and a cytokine cascade that includes IL-1, IL-6, IL-10 and TNF-α. The high local concentration of cytokines produced within the hepatic sinusoids has multiple effects that influence cancer cell implantation, survival and proliferation. The initial effect of the Kupffer cell produced cytokines seems to be on the sinusoidal endothelial cells. They become activated and produce adhesion molecules such as ►E-selectin and ►ICAM-1 on their cell surfaces. This allows the cancer cells to attach to the endothelial cell layer and arrest in the liver sinusoids. When this happens the tumor cells can break through the endothelial cell layer and invade the parenchyma Cytokines, in particular IL-10 are also protective against the effects of ischemia/reperfusion (IR) injury caused by blockage of the blood supply by tumor cell infiltrates into the small sinusoids. IR causes oxidative stress and cell death. Secretion of anti-inflammatory cytokines such as IL-10 counteracts these effects and aids the initial survival of the cancer cells following implantation in the sinusoids. These studies were largely carried out using human colorectal cancer cells in culture or in metastasis models in immuno-suppressed mice and imply that Kupffer cells are important participants in metastasis development. However, other experiments using syngeneic animal tumors have shown a role for Kupffer cells in preventing liver metastasis. Experiments where Kupffer cells have been depleted prior to injection of tumor cells have often resulted in extensive tumor growth in the liver. Similarly when Kupffer cells have been activated prior to tumor cell injection reduced tumor growth in the liver is seen.

Kupffer Cell Cancers

Cancer originating in Kupffer cells is very rare and few cases have been reported in the literature. They are described as Kupffer cell ►sarcomas or hemangioendotheliosarcomas of the liver. The tumors contain

phagocytic giant cells and numerous other cells with morphology similar to Kupffer cells. These hemorrhagic tumors tend to be quickly fatal with abdominal discomfort, ascites and jaundice as symptoms. Kupffer cell cancers will metastasize to distant sites including the pancreas and lung. Similar tumors to those found in humans can be induced in rats by exposure to low doses of carcinogens during liver regeneration.

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Kuzbanian

Definition

►ADAM10

Kv10.1

►Ether à-go-go Potassium Channels

L-PAM

Definition
►Melphalan

L&H

Definition
Lymphocytic and/or histiocytic.

►Hodgkin Disease, Clinical Oncology

L&H Cells

Definition
Lymphocytic and Histiocytic Cells.

Label-Free Analysis

Definition
A biochemical analysis employing detection method without the need of label such as fluorescence or radioactive tag.

►Surface Plasmon Resonance

Labile Factor

Definition
►Factor V.

Lactate

Definition
A 3-carbon carboxylic acid also known as 2-hydroxypropanoic acid.

►Warburg Effect

Lactate Dehydrogenase

Definition
LDH is a ubiquitously expressed enzyme. Serum levels of LDH can be used to monitor treatment of testicular cancer, ►Ewing sarcoma, non Hodgkin lymphoma and some types of leukemia. Elevated levels of LDH can also be caused by a number of non-cancerous conditions, including heart failure, hypothyroidism, anemia, as well as lung and liver diseases.

►Serum Biomarkers
►Testis Cancer
►Hodgkin Disease

Lactoferricin Antiangiogenesis Inhibitor

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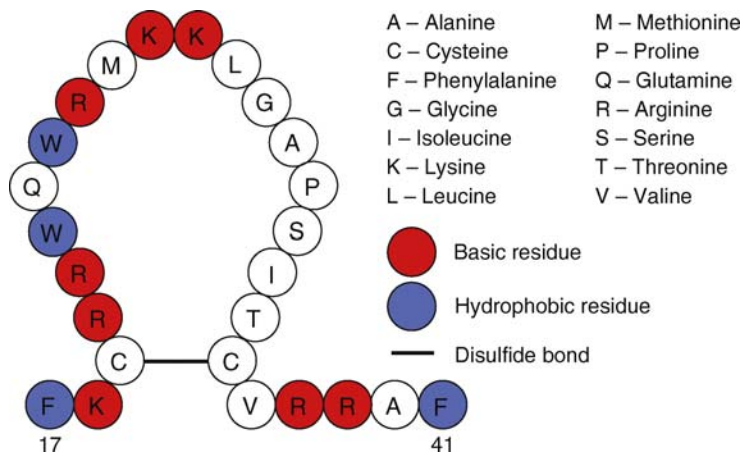
Definition
Lactoferricin is a cationic peptide that is generated by the acid-pepsin hydrolysis of mammalian ►lactoferrin present in the secretory granules of neutrophils (polymorphonuclear leukocytes), as well as in exocrine

secretions, including milk, tears, and saliva. Interestingly, substantial quantities of lactoferricin are generated in the stomach following the ingestion of lactoferrin-containing milk. Lactoferricin possesses potent antimicrobial, antiviral, immunomodulatory, and antitumor activities. In terms of anticancer activity, the best studied of the lactoferricins is bovine lactoferricin, which consists of amino acid residues 17–41 from the NH_2-terminal region of bovine lactoferrin (Fig. 1). A disulfide bond that forms between cysteine residues located at each end of the peptide creates a looped or hairpin structure. In an aqueous environment, bovine lactoferricin assumes an ►amphipathic, twisted β-sheet configuration with clear positively charged and hydrophobic faces. The relatively high proportion of asymmetrically clustered basic amino acid residues (arginine and lysine) and the hydrophobic tryptophan residues that in part comprise bovine lactoferricin are believed to be important for the peptide's biological activity.

Characteristics

The *in vitro* and *in vivo* anticancer activity of bovine lactoferricin has been attributed to the selective cytotoxic effect (either by membrane lysis or ►apoptosis induction) exerted by this cationic peptide on a broad range of cancer cell types, including leukemias, lymphomas, fibrosarcomas, and various carcinomas. However, recent studies indicate that bovine lactoferricin is also able to inhibit ►angiogenesis. A similar, but less potent, antiangiogenic activity has also been reported for bovine lactoferrin. Although ►neovascularization is normally tightly regulated by the opposing effects of proangiogenic and antiangiogenic factors, this process becomes dysregulated during tumor growth. The action of proangiogenic factors, in combination with basement membrane degradation by proteolytic enzymes, results in the proliferation, migration, and differentiation of tumor-associated endothelial cells. Neovascularization is an essential step in ►tumorigenesis since the new blood vessels provide oxygen and nutrients to rapidly dividing cancer cells and remove metabolic wastes from the tumor ►microenvironment. This allows solid tumors to grow in size, as well as promoting the development of metastatic disease. Diffusible, tumor-associated growth factors that stimulate angiogenesis include ►vascular endothelial growth factor$_{165}$, ►platelet-derived growth factor, ►heparin-binding epidermal growth factor-like growth factor, and basic ►fibroblast growth factor (also known as fibroblast growth factor 2).

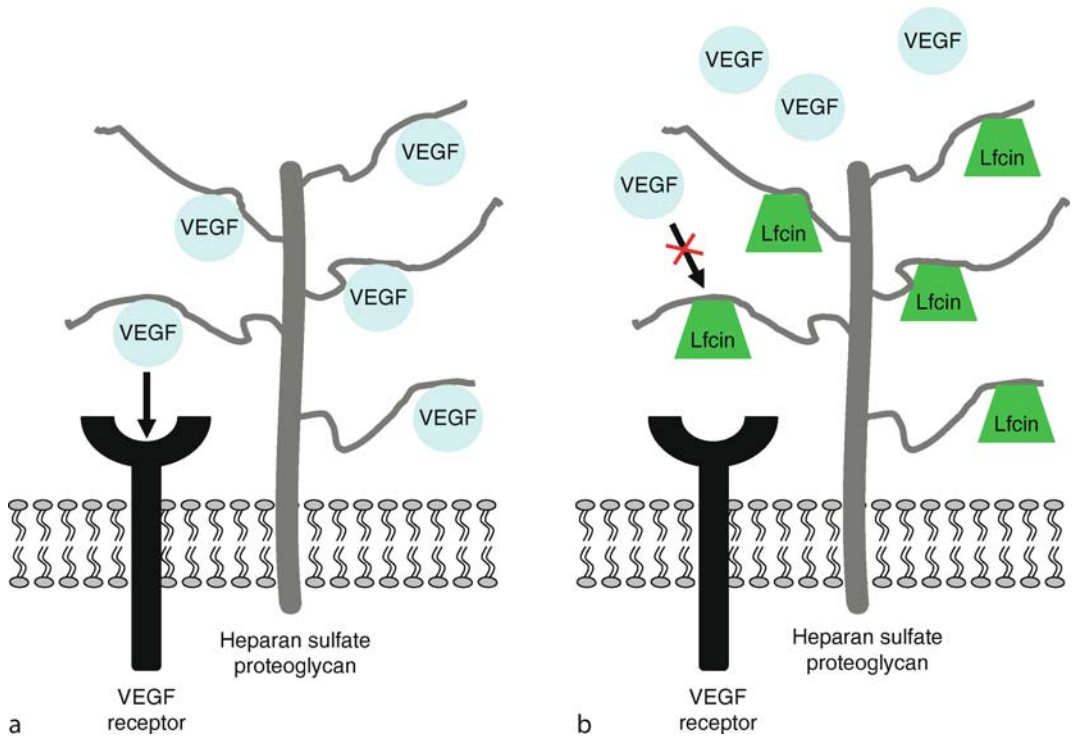
Bovine lactoferricin is a potent *in vivo* inhibitor of vascular endothelial growth factor$_{165}$- and basic fibroblast growth factor-induced angiogenesis in the mouse ►Matrigel-plug assay. This finding is consistent with the observation that administration of bovine lactoferricin by subcutaneous injection reduces the number of tumor-associated blood vessels in B16-BL6 melanoma-bearing mice. In addition, bovine lactoferricin exhibits a dose-dependent inhibitory effect on the *in vitro* proliferation of human umbilical vein endothelial cells in response to vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor, as well as inhibiting the *in vitro* migration of human umbilical vein endothelial cells across transwell membranes in response to vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor. Bovine lactoferricin therefore inhibits endothelial cell proliferation and



Lactoferricin Antiangiogenesis Inhibitor. Figure 1 Primary structure of bovine lactoferricin. The amino acid sequence of bovine lactoferricin is indicated by single-letter code (see the accompanying key). Basic and hydrophobic amino acid residues are important for peptide function and are colored red and blue, respectively. The disulfide bond, indicated by the solid bar between cysteine residues at opposite ends of the peptide, forms a loop consisting of 18 amino acids. Numbers indicate the sequence position in bovine lactoferrin.

migration, which are essential steps in neovascularization. Importantly, bovine lactoferricin does not affect the viability of human umbilical vein endothelial cells, suggesting that the antiangiogenic activity of bovine lactoferricin is independent of its membranelytic and apoptosis-inducing activity.

Vascular endothelial growth factor$_{165}$ and basic fibroblast growth factor must first interact with ►heparan sulfate proteoglycans on the surface of endothelial cells in order for these proangiogenic factors to bind to and trigger signal transduction through their respective cell-surface receptors. Other heparan sulfate proteoglycan-dependent proangiogenic factors that have been linked to tumor progression include platelet-derived growth factor and ►heparin-binding epidermal growth factor-like growth factor. Positively charged bovine lactoferricin mediates its inhibitory effect on basic fibroblast growth factor- and vascular endothelial growth factor$_{165}$-induced angiogenesis by interacting with negatively-charged heparin-like structures on the surface of human umbilical vein endothelial cells, thereby competing with basic fibroblast growth factor and vascular endothelial growth factor$_{165}$ for the heparan sulfate proteoglycans that are required for these proangiogenic factors to bind to and signal through their specific cell-surface receptors (Fig. 2). Although not yet formally proven, it is likely that bovine lactoferricin will have a similar inhibitory effect on angiogenesis induced by other heparin-binding tumor-associated proangiogenic factors. However, ►electrostatic interactions alone do not govern the binding of bovine lactoferricin to the heparan sulfate proteoglycans that are involved in cell-surface receptor signaling caused by heparin-binding proangiogenic factors. Thus, a scrambled form of bovine lactoferricin that retains the net positive charge of the native peptide is unable to inhibit the binding of vascular endothelial growth factor$_{165}$ or basic fibroblast growth factor to human umbilical vein endothelial cells. The primary and secondary structure that is conferred on bovine lactoferricin by its amino acid sequence is therefore an important factor in the selectivity of bovine lactoferricin for heparin-like structures that are involved in basic fibroblast growth factor and vascular endothelial growth factor$_{165}$ interactions with their respective receptors on human endothelial cells.



Lactoferricin Antiangiogenesis Inhibitor. Figure 2 Model of bovine lactoferricin-mediated blockade of heparin-binding growth factor-induced angiogenesis. (a) Heparin-binding proangiogenic growth factors such as vascular endothelial growth factor$_{165}$ (VEGF) must interact with heparin-like binding sites on heparan sulfate proteoglycans in order to bind to and signal through receptors on the surface of endothelial cells. (b) Bovine lactoferricin (Lfcin) complexes with heparin-like binding sites on cell-surface heparan sulfate proteoglycans, thereby competing with heparan sulfate proteoglycan-dependent proangiogenic growth factors for heparin-like binding sites and preventing proangiogenic growth factor receptor signaling from taking place.

Clinical Perspectives

Starving a solid tumor of its blood supply by preventing or interfering with tumor-induced neovascularization has generated considerable interest as an alternative to conventional chemotherapy for the prevention of tumor growth and metastasis. Indeed, several different inhibitors of angiogenesis are currently being used in the treatment of human cancers. However, the results obtained to date in clinical practice have been less than was hoped for on the basis of preclinical studies, most likely because the current generation of anti-angiogenic agents only target a single proangiogenic growth factor receptor, whereas multiple proangiogenic factors are typically associated with tumor-induced angiogenesis. In this regard, the ability of bovine lactoferricin to inhibit neovascularization in response to multiple heparin-binding growth factors may allow bovine lactoferricin to be more effective than current antibody-based antiangiogenic agents for the blockade of tumor-induced angiogenesis. However, the susceptibility of bovine lactoferricin to enzymatic degradation and inactivation through interactions with anionic serum components remains a major obstacle to any future use of this host defense peptide as an antiangiogenic agent. One possible solution is to synthesize an all-►D amino acid analogue of bovine lactoferricin since cationic peptides that consist of all-D amino acids exhibit dramatically increased stability in serum. Alternatively, tumor-targeted ►liposomes might be used to encapsulate and deliver bovine lactoferricin directly to tumor sites while retaining the peptide's ability to mediate antiangiogenic activity. Although preclinical studies have revealed that bovine lactoferricin is a potent inhibitor of angiogenesis, additional research will be needed in order to realize the potential of bovine lactoferricin as a novel antiangiogenic agent for the treatment of human cancers.

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Lactoferrin

Definition

Is an 80-kDa iron-binding glycoprotein belonging to the transferrin family. Lactoferrin has important multifunctional roles in host defense.

►Lactoferricin Antiangiogeneis Inhibitor

LAIR1

Definition

Leukocyte-associated immunoglobulin-like receptor 1.

LAK

►Activated Natural Killer Cells

LAMB

►Carney Complex

Lamellar Phase

Definition

The most common lipid secondary structure, also called the lipid bilayer, which defines most phospholipid membranes found.

►Membrane-Lipid Therapy

Lamellipodia

Definition

Areas at the edge of adherent cells that extend away from the cell body by the pushing of internal actin filaments as they polymerize. Flattened sheet-like structures composed of a crosslinked F-actin meshwork that project from the cell membrane. Often associated with the leading edge of migrating cells.

►Cortactin
►Migration

Laminin

Definition

LM are large, heterotrimeric, cruciform matrix glycoproteins composed of highly homologous α, β, and γ subunits. Five α (α1–5), four β (β1–4), and three γ (γ1–3) chains variably assemble to create 14 known isoforms that convey a variety of important biological functions. Specific LM isoform expression and post-translational processing can directly influence cellular response to growth factors, intracellular signaling, cell proliferation, susceptibility to apoptosis and migratory capacity. Changes in LM isoform expression in vessel walls have been shown to foster angiogenesis as well as leakage in vessels, rendering them attractive to tumor cells and susceptible to metastatic invasion. Laminins are expressed in both normal and malignant tissue, but different specific isoforms predominate in each case.

►Aging and Cancer
►Laminin Signaling
►Tissue Inhibitors Of Metalloproteinases

Laminin-Receptor

Definition

Are proteins that bind ►laminin and transduce a certain signal into the cell bearing the receptor.

►Laminin Signaling

Laminin Signaling

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Definition

►Lamininś are a family of glycoproteins with an apparent molecular weight between 400 and 900 kDa. They are heterotrimers of three subunits, α, β, and γ held together by disulphide bonds to form triple helical coiled coil in a shape of a cross. Five α chains, three β chains, and three γ chains have been identified and by combination they assemble to form over 14 laminin isoforms that have different tissue distributions and functions. Laminins are essential for basement membrane assembly, promote cell attachment and ►angiogenesis, induce neurite outgrowth, affect gene expression and are involved in cell proliferation, migration, and differentiation. Biochemical dissection related some of the laminin functions to specific parts of the glycoproteins. It appears that different parts in the molecules have different effects on cells. Some of these parts are cryptic and interact with cells only after proteolytic cleavage of the laminin molecules. In vitro, most structural and functional studies have been performed on laminin-1 (α1β1γ1).

Characteristics

Laminin Signaling

Laminins activate various ►signal transduction pathways. It was shown that ►chemotaxis induced by laminin-1 is pertussis toxin sensitive, indicating on the involvement of a pertussis toxin-sensitive G protein in the process, while ►haptotaxis seems to involve a different mechanism. It was shown that human osteoclast-like cells selectively recognize laminin isoforms. The cells adhered to laminin-2 but not to laminin-1, and a sharp increase in intracellular Ca^{2+} was detected upon addition of soluble laminin-2 to the cells. Another study showed that laminin-1 induced a rapid and transient mRNA expression of c-fos and c-Jun in PC12 cells, and stimulated the DNA binding activity of the complex of these proteins to the ►AP-1 site. In tumor cells, addition of laminin-1 resulted in a time- and dose-dependent activation of phospholipase D (PLD) followed by generation of ►phosphatidic acid that is involved in signal transduction events leading to the induction of ►MMP-2 and enhanced invasiveness of metastatic tumor cells. This effect of laminin-1 was not seen in normal cells in vitro. Laminins' signaling has

been shown to involve kinase/phosphatase cascades since bound laminin-1 and laminin-5 induce protein ►dephosphorylation in neural cells during process formation. A recent study performed in our laboratory showed that mitogen activated protein kinases (►MAPK) are involved in laminin signaling. Addition of exogenous soluble laminin-1 resulted in a significant decrease in the ►phosphorylation (activation) of ERK, JNK, and p38 after 30 min of incubation. This laminin-induced dephosphorylation of all MAPK was dose-dependent and transient. Another study demonstrated that incubation of macrophages with a peptide from the laminin-α1 chain, but not intact laminin-1, triggered protein kinase C-dependent activation of ERK1/2, leading to upregulation of proteinase expression. Several recent studies using laminin-5 have shown activation of ERK1/2 via focal ►adhesion kinase (FAK), while other studies have shown activation of Rac1 via phosphoinositide 3 kinase (PI3K). Although some functions may be common to all laminin variants, other may be unique and isoform-specific, depending on the tissue or organ in which they are expressed. In addition, various signal transduction pathways may be activated by different ►laminin receptors.

Laminin Receptors

The biological effects of laminins are mediated by numerous laminin receptors that are divided into two major groups: ►integrin and nonintegrin receptors (Table 1).

Integrins

Integrins are a large family of cell-membrane receptors for extracellular matrix proteins (Table 1). Integrins are heterodimeric combination of various α-subunits with various β-subunits. The ligand specificity for different integrins can be altered depending on the type of divalent cation present, the surrounding lipid environment, and various cell-specific factors. Inside the cell, the short cytoplasmic domains of integrins associate with various cytoskeletal proteins that mediate integrin signal transduction. At least eight integrins bind laminin; some of them bind additional extracellular matrix components as well. Integrins recognize mainly laminin-α chains and hence determine cell adhesion and response to laminin isoforms.

Two possible integrin-related signal transduction pathways have been identified. First is direct signaling, where binding to integrins by extracellular proteins triggers intracellular signaling events. The second is integrin modulation of mitogen-initiated signaling; in this case, integrin-mediated cell anchorage influences signaling pathways activated by growth factors. In general, integrin direct signaling activates FAK, small GTPases of the ►Rho family, and MAPK, resulting in accumulation of highly ►phosphorylated proteins and cytoskeletal molecules at the adhesion sites. Binding to integrins is followed by receptor clustering that initiates activation and autophosphorylation of FAK. Tyrosine-phosphorylated FAK can recruit Src family kinases to focal contact sites. This sets up additional tyrosine phosphorylation of proteins such as cytoskeletal proteins and adaptor proteins such as Grb2. Small GTPases of the Rho family (Rho, Rac, and Cdc42) are involved in ►integrin signal transduction and affect cytoskeleton arrangement. Rho contributes to cell adhesion to extracellular matrix. Rac and Cdc42, via PI3K, mediate the increase in cell motility and invasiveness induced by the integrin. Some integrins activate MAPK cascades. For example, laminin binding to the integrin α6β4 results in activation of an associated kinase and consequently tyrosine phosphorylation of the β4-subunit cytoplasmic domain, followed by association of the adaptor protein Shc with tyrosine-phosphorylated β4 integrin subunit. Shc is then phosphorylated on tyrosine residues, presumably by an integrin-associated kinase, and combines with the adaptor protein Grb2 which exists in a complex with the ras GTP exchange factor

Laminin Signaling. Table 1 Laminin receptors and their additional ligands

Receptor	Ligands
Integrins α1β1	Collagen (I,II,IV), laminin (1, 2)
Integrins α2β1	Collagen (I,II,IV), laminin (1, 2), chondroadherin
Integrins α3β1	►Fibronectin, collagen (I), laminin (2, 5, 8, 10, 11), nidogen, epiligrin, perlecan
Integrins α6β1	Laminin (1, 2, 5, 8, 10, 11)
Integrins α6β4	Laminin (1, 2, 5, 10)
Integrins α7β1	Laminin (1, 2, 8, 10)
67 kDa laminin receptor	Laminin[a]
Dystroglycan	Laminin (1, 2), agrin, perlecan
Heparan sulfate	Laminin (1, 2), collagen XVIII

Laminin-4 receptor interactions presumed to be similar to those of laminin-2.
[a]Most studies on laminin-1.

SOS. This leads to Ras activation followed by activation of a kinase cascade consisting of Raf, MEK (MAPK/ERK kinase), and ERK, resulting in increased cell motility and proliferation. In addition, integrin α6β4 activates the JNK cascade, via Rac1, resulting in jun protein expression. Jun associates with fos, whose expression is induced by ERK cascade, to form the AP-1 transcription factor. In human hepatocellular carcinoma cells, laminin-binding integrin α6β1 stimulation resulted in FAK tyrosine phosphorylation, leading to FAK–GRB2 association and ERK cascade activation, which promotes tumor cell migration. Interestingly, aggregation of integrin receptors, even in the absence of ligand occupancy, is sufficient to induce a prompt transmembrane accumulation of at least 20 signal transduction molecules, including Src, Rho, Rac1, Ras, ERK1/2, and JNK.

Nonintegrin Receptors

The 67kDa laminin receptor is a nonintegrin receptor. A highly conserved 37 kDa protein is the precursor of the receptor, but the exact manner by which it configures its mature form is not clear. The amino acid sequence of the 37 kDa precursor is extremely well conserved through evolution, however, it corresponds to that of additional proteins, suggesting a multifunctional protein. The cDNA of the 37 kDa precursor is virtually identical to a cDNA encoding the ribosomal protein p40. In addition, the 37 kDa precursor acts as a receptor for cellular prion protein and is involved in the life cycle of prions. It has also been found that the 37 kDa precursor is identical to the oncofetal antigen protein that is expressed by tumors.

The 67 kDa laminin receptor mediates cell attachment to laminin. Colocalization of the 67 kDa laminin receptor with the cytoskeleton constituents α-actinin and vinculin, and the focal adhesion plaque was found. The receptor is involved in several physiological processes such as implantation [56], invasive phenotype of trophoblastic tissue, angiogenesis, T-cell biology, and shear stress-dependent endothelial nitric oxide synthase expression. Increased expression of the 67 kDa laminin receptor correlates with cell proliferation, migration, and ►invasion capacity. Clinical data suggest a correlation between 67 kDa laminin receptor expression in tumor cells and tumor progression. Expression of the receptor has been shown to be upregulated in neoplastic cells compared to their normal counterparts and directly correlates with an enhanced invasive and metastatic potential in numerous malignancies. Malignant ►mesothelioma is one of the most aggressive human cancers, however, no tumor is less susceptible to distant ►metastasis and still associated with such high mortality rates. In a recent study, we wound frequent expression of 67 kDa mRNA but very rare expression of the protein in clinical malignant mesothelioma samples in contrast to metastatic breast or lung carcinomas. These findings suggest that the differences between malignant mesothelioma and carcinomas regarding expression of the 67 kDa laminin receptor may account at least in part for the reduced ability of MM to metastasize to distant organs, due to lack of the signaling mediated by the receptor.

By stable transfection of A375SM melanoma cells, we established lines expressing reduced or elevated 67 kDa laminin receptor. We found that stable antisense-transfected cells that expressed reduced 67 kDa laminin receptor demonstrated significantly less aggressive tumor phenotype, as reflected by their reduced invasiveness through Matrigel, diminished attachment to laminin, and decreased MMP-2 expression and activity. Further, the basal phosphorylation extent (activity) of ERK, JNK, and p38 was significantly higher in cell lines expressing reduced 67 kDa laminin receptor, compared to parental cells. The increase in MAPK phosphorylation in cells expressing reduced 67 kDa laminin receptor was accompanied by a significant reduction in MKP-1 mRNA level and a significant increase in PAC-1 mRNA level. It seems that the 67 kDa laminin receptor induces downregulation of MKP-1 expression that may contribute to the reduced activity (dephosphorylation) of MAPK induced by the receptor, which is followed by an upregulation of PAC-1 expression, possibly as a negative feedback.

67 kDa Laminin Receptor and Integrins

There are studies that indicate an association between the 67 kDa laminin receptor and the α6 integrin subunit that is a part of the laminin-binding integrins α6β4 and α6β1. Biochemical analyses indicate on coimmunoprecipitation of the 67 kDa laminin receptor with the α6 integrin subunit. Specific reduction of the α6 integrin subunit by an antisense was accompanied by a proportional decrease in the cell surface expression of the 67 kDa laminin receptor. Other studies targeting the 67 kDa laminin receptor, showed a significant reduction in one of the α6 integrin subunit isoforms. Analysis of α6 integrin subunit and of the 67 kDa laminin receptor in ►ovarian carcinoma samples showed no statistical correlation between the two.

►Dystroglycan

Dystroglycan consists of two subunits, which are translated from a single mRNA as a propeptide that is proteolytically cleaved into two noncovalently associated proteins. Dystroglycan was originally isolated from skeletal muscle as an integral membrane component of the dystrophin–glycoprotein complex (DGC). The exact function of the entire DGC is not completely determined but evidence indicates that it confers structural stability to the sarcolemma during contraction. In fact, mutations in components of this complex

lead to various types of muscle disorder such as Duchenne muscular dystrophy and limb-girdle muscular dystrophies.

Dystroglycan is also expressed in many other cell types and it plays important roles outside skeletal muscle. It has been implicated in early mouse development, structure and function of the central nervous system, myelination and nodal architecture of peripheral nerves, epithelial morphogenesis, cell adhesion, synaptogenesis, and signaling. In addition, several extra- and intracellular proteins are less tightly associated with the DGC, such as nitric oxide synthase [nNOS], dystrobrevin, and laminin-2. Molecules that bind to the cytoplasmic tail of β-dystroglycan include the signaling molecule Grb2, components of the ERK–MAP kinase cascade including MEK and ERK, and rapsyn. Binding of laminin-2 to dystroglycan induces phosphorylation of Grb2 followed by Sos binding. This phosphorylation initiates activation of Rac1 pathway that is further followed by MAPK activation.

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Langerhans, Islets of

Definition

Are groups of specialized cells in the pancreas that produce and secrete hormones. Named after the German pathologist Paul Langerhans (1847–1888), who discovered them in 1869, these cells are arranged in groups that Langerhans likened to little islands in the pancreas. There are five types of cells in an islet: alpha cells that produce ►glucagon, which raises the level of glucose (sugar) in the blood; beta cells that produce ►insulin; delta cells that produce ►somatostatin which inhibits the release of numerous other hormones in the body; and PP cells and D1 cells, about which little is known. Degeneration of the insulin-producing beta cells is the main cause of type I (insulin-dependent) diabetes mellitus.

Langerhans Cell

Definition

Langerhans' cells are immature ►dendritic cells found in skin containing Birbeck granules and expressing CD1a. They are the most efficient antigen processing and presenting cells of dendritic cell family.

►Langerhans Cell Histiocytosis
►Birbeck Granules

Langerhans Cell Histiocytosis

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Synonyms

Histiocytosis X

Definition

►Langerhans Cell Histiocytosis (LCH), previously referred to as Histiocytosis X, is a rare clonal disorder of Langerhans cell proliferation, involving the skin, bone and other organs. The disease family consists of the syndromes originally described as ►eosinophilic granuloma, ►Hand–Schüller–Christian disease, and ►Letterer–Siwe disease. Modern classification of LCH consists of single-system versus multisystem and unifocal versus multifocal.

Characteristics

Epidemiology

Most patients diagnosed with LCH are children with a peak percentage of diagnoses occurring between 1 and 3 years of age. The incidence of LCH has been estimated to be five cases per million per year in children. It appears to be more common in boys than in girls (1.2–2:1). The incidence of LCH in adults is thought to be one-half of that in children. Development of the disease is usually sporadic; however, the fact that about 1% of patients have relatives with LCH and monozygotic twin pairs are concordant for LCH suggests a genetic predisposition. A higher frequency of malignant disorders has been reported in patients with LCH than in the normal population. Acute lymphoblastic leukemia is the most common malignancy preceding or co-occurring with LCH.

Etiology

Whether LCH is a neoplasm or is reactive in nature has been a controversial issue. Pathological Langerhans cells (LCH cells) are monoclonal, and sometimes show chromosomal deletion or gain, suggesting a neoplastic etiology. While LCH lesions often regress spontaneously, there is no evidence that LCH cells are immortalized, supporting the possibility of a reactive nature. Although infection may play a role in the development or reactivation of LCH, no well-accepted environmental risk factors are associated with the disease, except for cigarette smoking in adult pulmonary LCH. It has been demonstrated that 77% of adult patients with LCH pulmonary lesions are smokers.

Pathophysiology

LCH lesions not only contain LCH cells but also various inflammatory participants, including T lymphocytes, macrophages, plasma cells, eosinophils, osteoclast-like multinucleated giant cells and neutrophils. These cells stimulate each other to produce abundant cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-4, IL-5, IL-7, IL-10, transforming growth factor (TGF)-ß, ►RANKL and ►osteoprotegerin. This cytokine storm plays a role in the proliferation of LCH cells and of other infiltrating cells, and is responsible for the various clinical features of LCH. LCH cells in the bone, lymph node and some skin lesions contain immature ►dendritic cells. These cells do not express CD83 or CD86, but do express intracellular the major histocompatibility complex (MHC) class II antigen. They also express the immature dendritic cell marker ►CCR6 and produce the ligand for CCR6 (►CCL20/MIP-3α) as well as ►CCL5/RANTES and ►CXCL11/I-TAC. These ligands may play a role in recruiting eosinophils and CD4 positive T cells into the lesions, respectively. LCH cells show a greater proliferative capacity and a lower antigen presenting capability, suggesting maturation is arrested at an activated state. It is hypothesized that IL-10 as well as TGF-ß could be key factors in the inhibition of maturation of these cells.

Clinical Manifestations

LCH affects a number of different organs, so clinical signs and symptoms may be extremely variable. Patients can present with either single-system or multisystem involvement. Single-system presentations most often occur in the bone with single-site or multifocal involvement, but can also occur in the skin. Most commonly, the initial manifestations include the occurrence of soft tissue mass, bone pain, skin rash and fever. Laboratory findings include normochromic and normocytic anemia and an elevated erythrocyte sedimentation rate. Elevations in IgM are also common.

The bone is involved in about 80% of patients with LCH. The skull is most often affected followed by the extremities, ribs, spine, and mandible and maxilla. Osteolytic "punched out" lesions with sharp margins are typically seen on X-ray. Bone lesions may be asymptomatic or accompanied with pain and soft tissue swelling, which may cause compression of adjacent structures such as the optic nerve or the spinal cord. The clinical course of LCH when localized solely to the bone is generally benign and it sometimes resolves spontaneously over a period of months to years. However, it may result in permanent sequelae including the collapse of vertebral bodies, orthopedic deformities and growth impairment.

Skin involvement is seen in approximately half of patients. Patients present with lesions that are seborrhea-like eruptions on the scalp or an erytematous rash on the trunk, abdomen and inguinal areas. Ulcerative lesions in the genital or inguinal region may also be present. There may be bleeding into the lesions, even in the absence of thrombocytopenia.

Lymph nodes in the cervical, axillary and inguinal areas are most commonly affected. Rarely, the nodes can become massive and cause upper airway obstruction.

Earinvolvement usually appears as an aural discharge caused by external otitis, which is often associated with the destruction of mastoid. Ossicle or vestibular damage of the middle ear may cause a loss of hearing.

Hepatosplenomegaly occurs in 20% of patients with the infiltration of histiocytes into hepatic sinuses. Various degrees of liver dysfunction may appear, including hyperbilirubinemia, hypoproteinemia, hypoalbuminemia, elevation of γ-GTP, alkaline phosphatase and/or transaminases, ascites and edema. Histological examination of the liver shows portal infiltrates which can cause bile duct destruction and periportal fibrosis (sclerosing cholangitis) leading to biliary cirrhosis with portal hypertension and ultimately secondary hypersplenism.

Pulmonary involvement in children is usually part of multisystem disease, but in adults the lung involvement may be solitary and frequently regresses after the cessation of smoking. The lung pathology is associated with tachypnea, dyspnea, cyanosis, cough, pleural effusion and recurring pneumothorax. High resolution computed tomography may reveal reticular or micronodular opacities as well as large nodules and honeycombing. Typical histological findings are alveolar destruction and diffuse interstitial infiltration of histiocytes. Pulmonary fibrosis develops in 10% of patients and can lead to respiratory failure.

Hematopoietic involvement is seen in disseminated LCH and defined by anemia (hemoglobin < 10 g/dl in the absence of iron deficiency), leukopenia (leukocytes $< 4.0 \times 10^9$/l) or thrombocytopenia (platelets $< 100 \times 10^9$/l)

with or without bone marrow involvement. In severe cases, serious anemia and thrombocytopenia may develop, often associated with a secondary hemophagocytic syndrome.

Oral mucosa infiltration may appear as ulcerations or swelling of gingiva resulting in the loss of teeth. Infiltration of the small bowel may occur and cause malabsorption of nutrients. Diarrhea with blood and/or mucus suggests involvement of the colon. Occasionally the pancreas is also involved.

In the central nervous system (CNS), infiltration and dysfunction of the pituitary gland and/or adjacent hypothalamus occurs in about 20% of cases in those with multisystem involvement. The most frequent manifestation is diabetes insipidus (DI), which may precede, co-occur, or follow other symptoms and signs of the disease. DI occurs more often among patients with skull involvement (known as "CNS risk lesions"). Infiltration of the anterior pituitary is less common and may cause growth retardation and panhypopituitarism. These pathologies usually develop in those with DI after a disease course of 10 years. Magnetic resonance imaging (MRI) findings of LCH involvement of the pituitary gland and the hypothalamus are demonstrated by the loss of the physiologic high intensity signal of the posterior pituitary lobe on T1-weighted images. There can also be thickening of the pituitary stalk or a hypothalamic mass.

Progressive, degenerative CNS disease may develop over the years after onset of disease, often when the disease is considered quiescent. CNS involvement causes ataxia, tremor, dysarthria, dysphagia and hyperreflexia. Changes in personal behavior, judgment and cognitive function may also develop. In this case, MRI studies using T2-weighted or FLAIR images may reveal bilateral symmetric lesions in the cerebellar white matter and basal ganglia. Histologically, the neurodegenerative LCH is characterized by the presence of CD8 positive T lymphocyte infiltration, microglia activation, gliosis, neuronal and axonal destruction with secondary demyelination. There may be a lack of ►CD1a positive LCH cells, as is seen in autoimmune encephalitis. Currently there is no established therapy for LCH-CNS disease.

Pathology and Diagnosis

A pathological examination is indispensable in the diagnosis of LCH. With hematoxylin-eosin staining, LCH cells have a distinctive homogeneously stained pink cytoplasm. The nuclei appear twisted with a longitudinal groove and a small nucleolus, often with a "coffee bean" appearance. Immuno-histochemical staining of ►S-100 protein and ►langerin (CD207) is helpful for detection of LCH cells. In active lesions of the disease, LCH lesions show granulomas caused by the aggregation of LCH cells as well as a number of various inflammatory cells. A definitive diagnosis can be made by either positive staining for CD1a or electron microscopic demonstration of ►Birbeck granules in the granulomatous lesional cells. In the later stages of LCH, macrophages are more predominant than LCH cells in the lesions, and xanthomatous and fibrotic changes are characteristic. It is not uncommon that lesions at different stages of disease may be mixed in the same organ simultaneously.

Prognosis

The clinical course of LCH varies quite widely depending on the extent of organ involvement. Multisystem disease can be separated into two categories based on whether or not "risk organs" are involved. Risk organs are defined as the liver/spleen, lung or the hematopoietic system. LCH may resolve spontaneously in patients with localized, unifocal disease. Patients with single-system disease or without risk of organ involvement have a mortality of less than 5%. Prognosis is worse in children with multisystem and risk organ involvement who often have fatal outcomes despite intensive treatment Mortality rates of 10–50% have been reported. Infants younger than 2 years at diagnosis tend to have risk organ involvement, more often than older children; however, a recent study revealed onset age itself is not a prognostic factor. A major, positive prognostic factor appears to be a favorable response to the first 6 weeks of systemic multi-agent chemotherapy. Patients without an initial response tend to have an extremely high mortality rate with reports of 15–70%. This is in contrast to that of less than 5% in patients with a good initial response. In adults, lung disease may be a life threatening complication; it has been reported to contribute to a mortality rate of approximately 25%. Reactivation can occur unpredictably in more than half of patients, even those treated with multi-agent chemotherapy. Reactivated lesions may sometimes resolve spontaneously but there is an increased risk of permanent sequelae.

Treatment

Treatment of LCH should be planned according to the clinical presentation and the extent of organ involvement.

In single-system LCH, the major aims of treatment are to lessen symptoms and to reduce the chance of permanent sequelae. In the case of a single bone lesion without symptoms, a wait-and-see approach or diagnostic curettage is the standard method of care. Steroids may be used for symptomatic bone lesions. Systemic chemotherapy with vinca alkaloids and corticosteroids for 6 or 12 months could be applied for patients with CNS-risk lesions or multifocal symptomatic bone disease. Radical operation of jaw lesions is discouraged as this often results in disfigurement and loss of

teeth. Radiation is rarely used because of the reported increased risk of secondary tumors. When there is skin involvement only a wait-and-see approach is considered optimal. Alternatively, patients can be provided therapies such as topical corticosteroids or thalidomide. In patients with isolated pulmonary LCH with functional impairment, systemic chemotherapy is indicated to reduce further parenchymal destruction.

In multisystem LCH, the major aims of treatment are to increase survival and to reduce the incidence of late sequelae. Systemic chemotherapy with vinca alkaloids and corticosteroids for 12 months is the most commonly used regimen. In cases with risk-organ disease, more aggressive chemotherapy combined with agents such as cytarabine (Ara-C), 6-mercaptopurine and methotrexate may be considered. Etoposide (VP-16) is no longer considered a reasonable therapeutic agent because there is no reported significant efficacy and it has been shown to cause therapy-related acute myeloid leukemia (t-AML).

In patients with refractory progressive disease, myeloablative therapy with a combination of high dose Ara-C and cladribine (2-CdA) is currently being tested. Immuno-suppressive therapy with cyclosporin A, anti-thymocyte globulin or anti-TNF agent, and immuno-modulation agents like thalidomide, IFN-α or bisphosphonate are also used on an investigational basis. Allogeneic hematopoietic stem cell transplantation has proved to be efficacious in some cases. Additionally, liver or heart and lung transplants have been performed successfully in patients with end-stage organ involvement.

Late Sequelae

Permanent sequelae are common events in many LCH patients. They are most often the result of the infiltrative nature of the disease itself which causes tissue destruction and granulomatous fibrosis or gliosis of various tissues. Seventy percent of patients with multisystem disease and 25% of single-system disease patients suffer one or more life-long sequelae, including DI (24%), orthopedic problems (20%), hearing loss (13%), neurologic problems (11%), growth hormone deficiency, loss of teeth, pulmonary fibrosis and biliary cirrhosis with portal hypertension.

t-AML may develop as a consequence of LCH treatment with chemotherapy, especially following the use of topoisomerase II inhibitors, such as VP-16. The cumulative incidence of t-AML in patients treated with VP-16 for LCH has been estimated to be around 1%. In addition, secondary solid tumors, particularly sarcomas and brain tumors may develop in irradiated areas.

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Langerin

Definition

Is a cell surface receptor that induces the formation of the ►Birbeck granule.

►Langerhans Cell Histiocytosis

LAP

Definition

Latency-Associated Peptide.

►Transforming Growth Factor Beta

Laparoscopy

Definition

Examination of the abdominal and pelvic structures within the peritoneum using an illuminated tubular instrument, which is passed through the abdominal wall via a small incision. It can be used for diagnosis and for certain operations.

►Endometriosis

Lapatinib

Definition

Is an anti-cancer drug, a ►tyrosine kinase inhibitor of human ►epidermal growth factor receptor type 2 (HER2, (synonym ►HER2/neu, also epidermal growth factor receptor (EGFR). Lapatinib is active in combination with capecitabine in women with HER2-positive metastatic breast cancer that has progressed after ►trastuzumab-based therapy.

Large Cell Calcifying Sertoli Cell Tumor

Definition

LCCST; Is a rare type of ►testis cancer.

Large Cell Medulloblastoma

Definition

Variant of medulloblastoma accounting ~5% of cases. Characterized by more abundant cytoplasm than seen in classic medulloblastoma and large areas of necrosis.

►Medulloblastoma

Large-Cell Undifferentiated Carcinoma

Definition

About 10–15% of ►lung cancer are this type. It can start in any part of the lung. It tends to grow and spread quickly.

Non-small cell lung cancer is a common disease. It is usually treated by surgery (taking out the cancer in an operation) or radiation therapy (using high-dose x-rays to kill cancer cells). However, chemotherapy may be used in some patients.

The prognosis (chance of recovery) and choice of treatment depend on the stage of the cancer (whether it is just in the lung or has spread to other places), tumor size, the type of lung cancer, whether there are symptoms, and the patient's general health. Patients unsuitable for surgery may be offered curative intent radiotherapy. ►Adjuvant therapy may be given to more advanced resected cases. For late stage cases, chemotherapy with or without palliative radiotherapy are the conventional options, although the long term survival rates are very low.

Large Granular Lymphocyte

►Activated Natural Killer Cells

Large Tumor Suppressor Gene

►Lats in Growth Regulation and Tumorigenesis

Laryngeal Carcinoma

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Definition

The vast majority of malignant neoplasms of the larynx arises from the surface epithelium and therefore classified as keratinizing or nonkeratinizing ►squamous cell carcinomas (SCC). The other rare malignant forms include verrucous carcinoma, adenocarcinoma, fibrosarcoma, and chondrosarcoma. Histopathologically, laryngeal SCC can further be classified into: well differentiated (more than 75% keratinization), moderately differentiated (25–75% keratinization), poorly differentiated (<25% keratinization).

Characteristics

Laryngeal carcinoma accounts for a small fraction (less than 2%) of all human malignancies, but the incidence varies among different countries. It is most common between the ages of 45 and 75 years. Men are four or

five times more frequently affected than women. The etiology is unknown, but exposure of the mucosa to a wide variety of ingested and inhaled exogenous carcinogenic agents, such as tobacco smoke (►Tobacco Carcinogenesis) and alcohol, greatly increases the risk of developing these tumors.

Laryngeal carcinoma infiltrates locally in the mucosa and beneath the mucosa and could metastasize via the lymphatic system and the bloodstream. According to their anatomical localization, laryngeal carcinomas could be subdivided into ►supraglottic carcinomas, confined to the supraglottic space and spreading interiorly into the preepiglottic space, ►glottic carcinomas, rarely spreading into the supraglottic area but rather into the subglottic space, and ►subglottic carcinomas, often showing an infiltrative growth pattern unrestricted by tissue barriers.

Carcinomas of the supra- and subglottic larynx are more likely to be non-keratinizing and poorly differentiated, and, in general, they have a more aggressive behavior and tend to ►metastasize (Metastasis) early (20–40% of the cases). In contrast, lesions of the true vocal cords are more often moderately to well differentiated, rarely metastasize, and tend to be associated with a better prognosis.

Genetic Changes in Laryngeal Carcinomas

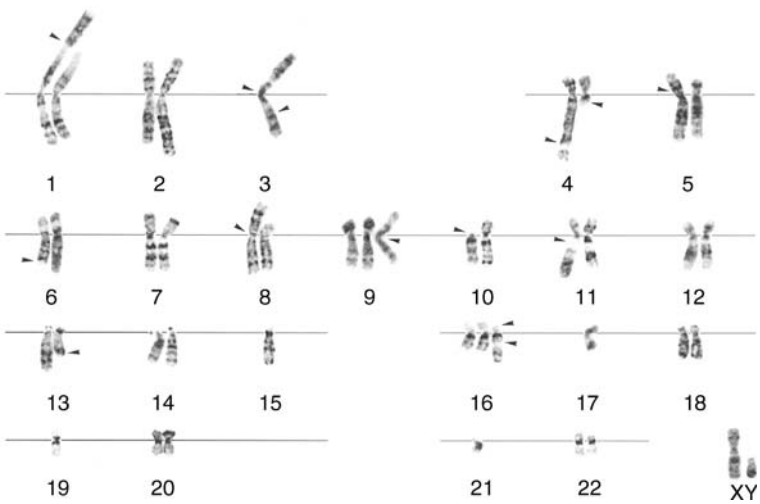
Chromosome Abnormalities in Laryngeal Carcinomas

One hundred fifteen laryngeal carcinomas with clonal ►chromosome abnormalities have been reported. In general, the ►karyotypes are relatively complex with a nonrandom pattern of deleted and amplified ►chromosome segments. This is in line with the notion that laryngeal carcinoma, like most other malignancies, develops through the accumulation of multiple genetic changes. The chromosomes most frequently involved in structural rearrangements are chromosomes 1, 2, 3, 4, 5, 7, 8, 11, 12, and 15, with breakpoints clustering to the pericentromeric regions, i.e., the ►centromeric bands p10 and q10 and the juxtacentromeric ►bands p11 and q11, accounting for 43% of the total breakpoints. The most common imbalances brought about by numerical and unbalanced structural rearrangements are loss of chromosomal region 3p21-pter, part of or the entire chromosome arms 4p, 6q, 8p, 10p, 13p, 14p, 15p, and 17p, and gain of chromosomal regions 3q21-ter, 7q31-pter, and 8q. A total of 17 recurrent structural aberrations, mostly in the form of whole-arm translocations (►Chromosome translocations), ►isochromosomes (i), and ►deletions (del), have been identified. The most common among them were i(8q), i(3q), i(5p), del(3)(p11), and ►homogeneously staining regions (hsr), a cytogenetically detectable sign of gene ►amplification, in band 11q13 (Fig. 1).

A subgroup of laryngeal SCC have had multiple, unrelated abnormal ►clones, with simple, often balanced structural rearrangements or numerical changes. These clones have always had near-diploid chromosome numbers. The finding of such ►cytogenetic polyclonality could be interpreted as evidence of "►field cancerization" but it can not be ruled out that the ►cytogenetically unrelated clones are united by a submicroscopic, pathogenetic mutation; the cytogenetic differences would then only reflect differences in clonal evolution. The third alternative is that some of the near-diploid clones actually represent preneoplastic lesions or genetically damaged, nonneoplastic epithelial or stromal cells in the tumor surrounding.

Fluorescence in Situ Hybridization (FISH)

FISH analysis has recently been undertaken to verify and in detail characterize the most common recurrent

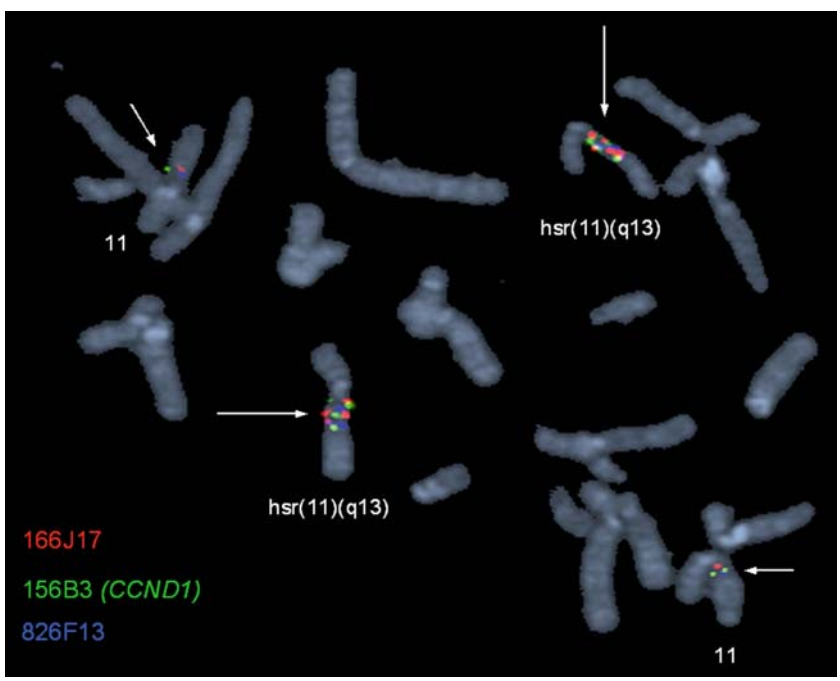


Laryngeal Carcinoma. Figure 1 Representative karyogram from a laryngeal SCC showing complex karyotypes with multiple numerical and structural rearrangements. Arrowheads indicate breakpoints in clonal aberrations.

chromosomal changes in head and neck SCC, including laryngeal SCC. FISH has demonstrated that cytogenetically detectable hsr in these tumors almost always corresponds to amplification of DNA sequences originating from 11q13, and that CCND1 (Cyclin D) is always included in the amplicon (Fig. 2). The amplicons mapped vary in size from 3.5 to 4.5 Mb with a core of 1.5–1.7 Mb and often many oncogenes in this region are coamplified, including CCND1, FGF3, FGF4, EMS1, and SHANK2. Another finding is that the amplification of 11q13 is often concomitant with deletion of distal 11q, indicating that not only the amplification of one or more dominantly acting oncogenes in 11q13, but also loss of a tumor suppressor gene in the distal part of 11q is critical for the development of laryngeal SCC. Detailed FISH characterization of pericentromeric rearrangements, in particular for chromosomes 1 and 8, with the use of YAC clones spanning the pericentromeric region of chromosomes, suggests that the essential outcome of these rearrangements at the DNA level is the resulting genomic imbalances, i.e., loss or gain of neoplasia-associated genes, and not rearrangement of genes in the euchromatin near the centromere. Furthermore, more precise mapping of breakpoints on chromosomal arms 1p and 8p has delineated critical regions for deletions within 1p11–p13 and the subtelomeric region of 8p.

Molecular Genetic Findings

A large scale effort has been devoted to the identification of tumor suppressor gene loci and amplified oncogenes in laryngeal carcinomas. Loss of heterozygosity (LOH) studies have pointed out the frequent loss of alleles from 3p, 8p, 9p, 13q and 17p in laryngeal SCC. A number of recent studies based on allelotyping or comparative genomic hybridization (CGH) indicate that head and neck SCC, including laryngeal SCC, display massive and widespread genomic imbalances and certain chromosome segments are lost more often than others. Apart from LOH from 3p, 9p, 13q, and 17p in more than 50% of the cases, deletions in 3q, 4p, 4q, 6p, 6q, 8p, 8q, 11q, 14q, 17q, 19q, and 20p have been found in 30–50% of the cases. Some candidate tumor suppressor genes in frequently deleted regions, e.g., FHIT in 3p, CDKN2A in 9p, RB1 in 13q, and TP53 in 17p, have been investigated with regard to homozygous deletions or inactivating mutations. Extensive analysis of TP53 (p53 gene family) mutation and the alteration of its protein in laryngeal SCC and its precursor lesions have shown that TP53 mutation occurs at an early stage of these neoplasms. Furthermore, correlation studies have shown that overexpression of mutated TP53 predicts poor disease-free and poor overall survival rates in patients with laryngeal SCC. Using various detection techniques such as immunohistochemistry and RT-PCR, it was shown that loss of CDKN24



Laryngeal Carcinoma. Figure 2 Metaphase plate from a laryngeal SCC hybridized with three bacterial artificial chromosome clones: 166J17 (red), 156B3 (*CCND1*, green), and 826F13 (blue), showing the amplicon of 11q13, including CCND1 locus. Short arrows indicate signals in normal chromosome 11, and long arrows indicate amplified signals in derivative chromosome 11 harboring hsr.

expression, through either homozygous deletion or promoter hypermethylation, was present in 52–82% of HNSCC including laryngeal SCC. A number of studies have further disclosed that the decreased expression of this gene was associated with poor survival in laryngeal SCC. FHIT was investigated in laryngeal SCC and its precursor lesions. In a recent study, decreased expression of this gene, through deletion or promoter methylation was detected in about 42% of SCC and 23% of dysplasia lesions. Although allelic loss of RB1 was shown in high frequency in laryngeal SCC in a few studies, expression analysis of RB1 revealed inconsistent results in different research groups. Thus, the role of this gene in laryngeal SCC has not been clearly established. Several candidate oncogenes in frequently gained regions, i.e., CCND1 in 11q13, EGFR in 7p, and MYC and PTK2 in 8q have been investigated. The most frequently amplified DNA sequences are located in chromosomal band 11q13; reported frequencies vary between 15 and 60%, with an average of 30% in primary head and neck SCC, including laryngeal SCC. FISH and molecular studies have implicated CCND1 as the prime target in this amplification process. Several attempts have been made to correlate cytogenetic or molecular genetic data with clinical outcome in patients with laryngeal carcinoma, and it has been shown that 11q13 rearrangements and amplification/overexpression of CCND1 seem to be associated with a poor prognosis.

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Laser

Definition

Acronym for Light amplification by the stimulated emission of radiation; Is a coherent light source used to deliver light energy of a single wavelength and high intensity; CALI.

►Chromophore-Assisted Laser Inactivation

Laser Capture Microdissection

Definition

Procedure in which a ►laser beam is used to dissect a patch of cells away from other cells present in a tissue section that has been mounted on a microscope slide.

Laser Diagnostics

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L

Definition

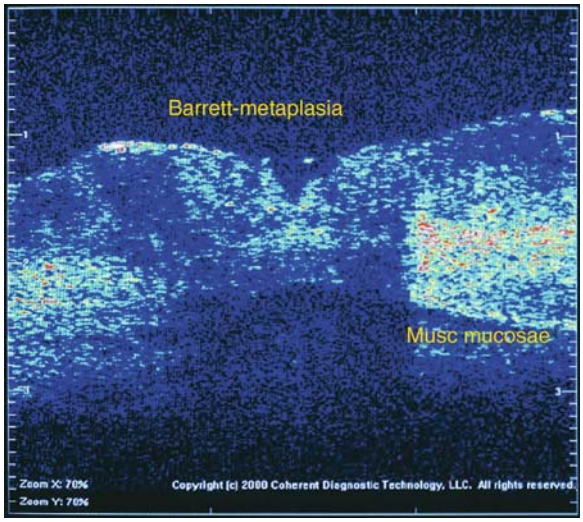
Laser diagnostics are procedures designed to detect or to differentiate neoplastic tissues based upon various forms of interaction of laser-emitted photons with tissues (Fig. 1).

Characteristics

The interaction of light with tissue may result in absorption or scattering of the photons. These phenomena may be directly observed for diagnostic purpose in a transillumination approach. Actual research in ►optical mammography focuses on time-resolved systems able to separate scattering from absorption. The thermal expansion of a tissue area absorbing more light that the surrounding tissue may be used for an acoustic recognition in so-called photoacoustic diagnostic systems (still at an early experimental phase).

Furthermore, fluorescence may result from absorption. Natural fluorophores are rare, thus, fluorescence spectroscopy may sensitively detect the presence of an exogenous or a specific endogenous fluorophore accumulating in malignant or premalignant lesions (►fluorescence diagnostics).

The phenomenon of backward scattering of incident photons is exploited for imaging of superficial tissues by optical coherence tomography (OCT). The backscatter intensity of any point in the tissue is determined



Laser Diagnostics. Figure 1 Optical coherence tomography of esophageal mucosa registered ex vivo with a clinical OCT-prototype (Coherence Diagnostic Technologies/Carl Zeiss). An island of Barrett esophagus epithelium is surrounded by normal squamous cell epithelium. The actual depth limitation enables imaging of the mucosal layer only (dimensions are not calibrated; the vertical scale represents about 3–4 mm, the lateral scale 12 mm).

by interferometry using very short coherence light sources, one of which are short pulsed lasers. Scanning vertical and lateral dimensions, tissue may be imaged at almost microscopic resolution. The vertical resolution of OCT-systems depends on the medium coherence length of the light source and is actually limited at 4–10 μm, which is at least 10-fold better than the limits of high-frequency ultrasonography. However, the strong absorption and random scattering of tissues limits OCT to superficial tissue layers with a maximum depth of about 2 mm. In oncology, OCT may thus be suitable for the recognition of early neoplastic lesions of the aerodigestive tract, the urinary tract and eventually of the skin.

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Laser-induced Fluorescence Diagnosis LIFD

►Fluorescence Diagnostics

Latent TGF-β Binding Protein

Definition

Four different gene products. Large glycoproteins that can associate with the extracellular matrices and connective tissues and target small latent TGF-β to tissues.

►Transforming Growth Factor Beta

Lateral Gene Transfer

Definition

LGT; synonym ►horizontal gene transfer.

►Circulating Nucleic Acids

Lats in Growth Regulation and Tumorigenesis

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Synonyms

Warts (wts) – Drosophila lats; WARTS (WTS) – mammalian LATS1; kpm – mammalian LATS2; Large tumor suppressor gene

Definition

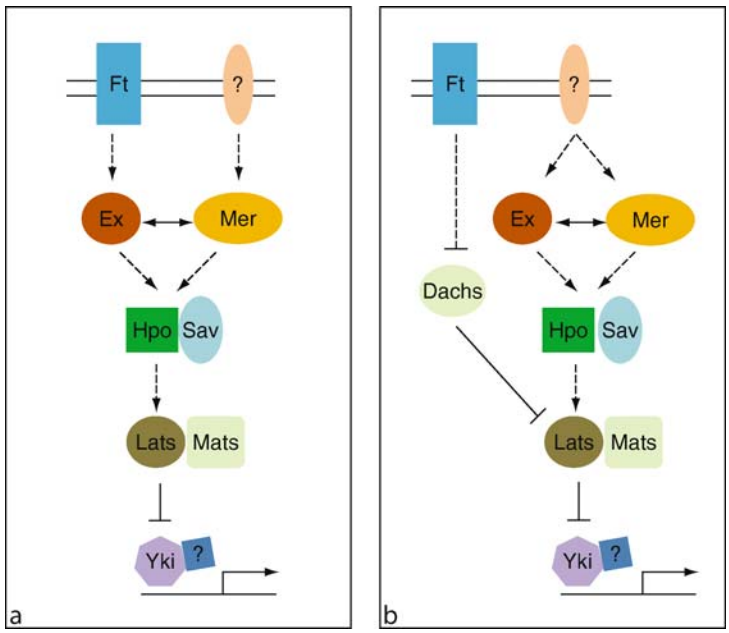
The *lats* (*la*rge *t*umor *s*uppressor) gene, also known as *warts* (*wts*), is a ►tumor suppressor gene originally discovered in the fruit fly *Drosophila melanogaster*. Loss-of-function mutations in the gene cause dramatic overproliferation including developmental defects in ►mosaic tissues within the larval ►imaginal discs (Fig. 1). The *lats* gene encodes a Serine/Threonine protein kinase with a catalytic domain that is highly similar to the human myotonic dystrophy protein kinase. Together with other related members, which include the *Saccharomyces cerevisiae* Dbf2, Dbf20 and Cbk1, *Schizosaccharomyces pombe* Sid2 and Orb6, *Neurospora crassa* Cot1, *Ustilago maydis* UKC1, *Caenorhabditis elegans* Sax-1, *Drosophila* Trc, and mammalian NDR1 and NDR2, they comprise the NDR kinase family, a subclass of the AGC protein kinases.

The *Drosophila* genome contains a single gene for *lats*, whereas there are two homologs, LATS1 and LATS2, in mammals and humans. The genes are functionally conserved since the expression of the human cDNA for either *LATS1* or *LATS2* in the fly rescues the *lats* mutant phenotypes. The overgrowth phenotype of *lats* mutant has been attributed to the coordinated deregulation of both cell cycle progression and ►apoptosis. Recent genetic analyses in *Drosophila* reveal Lats as a key component of the Hippo (Hpo) ►signal transduction pathway (Fig. 2). Mammalian orthologs exist for each component of the pathway and are likely to function in a similar manner. Studies so far indicate that this pathway plays a major role in the developmental regulation of cell proliferation, cell survival, as well as tissue growth and organ size, all of which are key aspects that are also important in tumorigenesis. Whereas knockout mice deficient for *LATS2* are lethal, mice deficient for *LATS1* develop soft-tissue sarcoma, ovarian stromal cell tumors, and pituitary dysfunction (Fig. 1b). In various human cancers, the *LATS1* and *LATS2* genes are found to be mutated or down-regulated. In addition, they are able to suppress tumor growth when overexpressed in human cancer cells. Furthermore, cells lacking LATS function display characteristic ►cancer hallmarks such as being multinucleated and having defects in cytokinesis, ►centrosome amplification, and genomic instability. Thus, like their fly counterpart, the *LATS* genes also function as tumor suppressors in mammals.

Characteristics

The *Drosophila* imaginal disc tissues afford an excellent *in vivo* context for studying growth, cell proliferation and survival. The ability to efficiently

Lats in Growth Regulation and Tumorigenesis. Figure 1 Lats in growth regulation and tumorigenesis. Lats has a tumor suppressor function in flies and mice. (a) Adult fly showing a clone of *lats* mutant cells which have overproliferated to form a large tumor outgrowth. (b) The homozygous LATS1 knockout mouse also develops tumors in the form of a soft tissue sarcoma.



Lats in Growth Regulation and Tumorigenesis. Figure 2 Alternative models of the Hpo-Lats tumor suppressor pathway. (a) Components function in a linear fashion. (b) Lats plays a central element for alternative inputs from upstream signaling events. On the one hand, Lats stability is regulated by Ft mediated by Dachs by-passing the requirement for Ex function. Alternatively, Lats activity is regulated by Hpo signaling. Solid lines indicate observed direct interactions (adapted from Pan 2007).

generate mosaic animals carrying clones of homozygous mutant cells in the developing imaginal discs and to follow their growth over time allow a powerful means to study the effects of mutations in essential genes. Such genetic mosaic approach has been used effectively in genetic screens to look for tumor suppressors or negative regulators of growth, which cause overgrowth when both copies of a gene are inactivated. This inactivation closely recapitulates the *in vivo* condition in human cancer patients carrying somatic mutant cells.

Using this approach, mutations in the *lats* gene were identified on the basis of the ►hyperplastic growth phenotype in mosaic flies. Somatic cells mutant for *lats* overproliferate cell autonomously to form large tumorous overgrowths in a wide variety of adult integumentary tissues (Fig. 1A). The tumors can be as large as 1/5 of the body size. Histological examination of the *lats* tumors reveals that they form lobes and folds in the epithelial structures, yet their tissue organization is still characteristically maintained as a monolayer and cell polarity is not affected. Although the mutant tissue is still capable of differentiation, defects are evident in the deposition of extra cuticle and malformed bristles and hairs. On the cellular level, mutant cells appear morphologically irregular such that the apical surface of the cells bulges away from cell body in what is known as "apical hypertrophy". In addition, mutant clones induced in the imaginal discs also appear starkly different from wild type clones in being rounded and having smooth edges rather than jagged and geometric. Such observation suggests a lack of directional cell division or a modification of cell ►adhesion properties. Since apical-lateral junctions are still present in the mutant, *lats* may potentially regulate some functional aspects of the ►adherens junctions.

When comparing the growth of clones that are mutant for other *Drosophila* tumor suppressors, such as *lethal(2) giant larvae*, *discs large* or *hyperplastic discs*, *lats* mutant clones exhibit several distinctive behaviors. When clones for other tumor suppressors are induced, the mutant cells appear to be unresponsive to stimulating growth signals. They grow much slower than wild-type cells and are unable to differentiate. As a result, these cells have a growth disadvantage and are competed away such that very small or no mutant tissues are eventually detected in the adult. Analysis of clone areas and cell numbers in *lats* mosaics, on the other hand, reveals that the mutant cells grow astonishingly faster than wild-type cells in sibling clones. Interestingly, there is no apparent change in the size of *lats* mutant cells, which means there must be a concomitant increase in cellular growth in order to support the faster pace of the cell cycle. Thus, the

dramatic expansion of the *lats* mutant clones is not only attributable to an increase in cell number, but also to an increase in tissue mass. This suggests that the deregulation of growth, as well as the inappropriate engagement of the cell cycle, represent key steps where many tumor suppressors may act in tumorigenesis.

The increased rate of proliferation of *lats* mutant cells is due to the shortened phases of the cell cycle which are equally affected. In contrast to proliferative defects seen with other tumor suppressors, *lats* mutant cells do not cycle indefinitely but exhibit a much delayed exit as compared to wild-type cells in the same developmental context. For example, in the developing eye disc, wild-type cells undergo two rounds of cell division and exit the cell cycle after the so-called second mitotic wave (SMW). However, in *lats* mutant clones, cells continue to divide and undergo mitoses at a much later time as indicated by BrdU incorporation and anti-phosphorylated H3 staining of cells posterior to the SMW. Another key finding is that cells within *lats* mutant clones are highly resistant to apoptosis. This is particularly evident during pupal eye development where normally there is a major wave of cell death to remove excess cells that have not been recruited for differentiation. In *lats* mutants, this scheduled cell death is absent leading to the appearance of extra interommatidial cells in the pupal retina. Thus, the effects of increased rate of cell proliferation together with growth and the inhibition of apoptosis contribute to the *lats* dramatic overgrowth phenotype.

Insights into the function of *lats* initially came from the identification of the LATS1 and LATS2 homologs in human and mouse where biochemical studies highlight their roles in the regulation of mitosis and apoptosis. The activity and phosphorylation status of the LATS proteins oscillate in a cell cycle-dependent manner. Both have been shown to interact with the cyclin-dependent kinase CDC2 and inhibit its kinase activity when they are overexpressed, resulting in cell cycle arrest in ►G2/M by LATS1 and in G1/S by LATS2 through the displacement of CDC2. LATS2 has also been shown capable of arresting cells at G1/S by downregulating Cyclin E/CDK2 kinase activity. The localization of the LATS proteins to the mitotic apparatus further points to its role in regulating mitotic events. For example, an interaction with the actin filament assembly factor Zyxin has been detected for LATS1. Recently, LATS1 has been shown to colocalize with and bind to the cytoskeletal protein kinase LIMK1 at the contractile ring during ►cytokinesis, the consequence of which is the inhibition of LIMK1-induced cytokinesis defects and phosphorylation of Cofilin. Interestingly, the mechanism of inhibition of both LIMK1 and CDC2 doesn't appear to involve LATS kinase activity. Nevertheless, consistent with these observations, the removal of LATS1 function leads to defects in mitotic exit and aberrant cytokinesis where the mutant cells are multinucleated, display centrosome amplification, and exhibit ►genomic instability. Similar defects are also observed with the expression of a kinase-dead LATS1, which delays mitotic progression through the activation of a spindle assembly check point. These mitotic defects are hallmarks of cancer cells and clearly support the function of the LATS proteins as tumor suppressors.

A role for LATS2 in the regulation of mitosis has also been documented. Its localization to the ►centrosome requires phosphorylation by the centrosomal ►kinase Aurora-A. LATS2 also functions in a checkpoint pathway to prevent ►tetraploidization via p53, which is activated upon binding of LATS2 to MDM2 and feeds back to upregulate *LATS2* expression in G2/M cells. Finally, a recent genetic screen has identified two miRNAs, *miR-372* and *miR-373*, which function as novel ►oncogenes in testicular germ cell tumors by downregulating *LATS2* through direct biding to sites in its 3′UTR, the effect of which is to relieve the CDK inhibition by p53.

Although no mitotic defects have been observed in *Drosophila* for *lats* mutants, its role in controlling mitotic events and progression is corroborated by the finding of genetic interactions between *lats*, *cdc2* and *cyclin A*. Mutations in either *cdc2* or *cyclin A* can suppress both the lethality and cell proliferation defects of *lats* mutants. Furthermore, the loss of *lats* function leads to the accumulation of Cyclin A, supporting the idea that Lats limits cell proliferation by negatively regulating Cdc2/Cyclin A activity. The studies above present evidence that may explain in part how mammalian LATS can contribute to tumorigenicity through the regulation of the cell cycle and mitotic events, but they cannot fully account for the effect in *Drosophila* of *lats* mutations on the expansive growth and cell survival. However, further clues do point to an additional role for LATS in regulating apoptosis to suppress growth. For example, the pro-apoptotic proteins Bax and Caspase-3 both have been shown to be upregulated in response to LATS1 overexpression. LATS1 has also been shown to be not only a substrate for the serine protease Omi/HtrA2, but whose activity is also dependent on LATS1 binding. The overexpression of LATS2, on the other hand, can induce cell death through the downregulation of the apoptosis inhibitors ►Bcl2 and Bcl-xL.

Thus far, although the kinase activities of LATS1 and LATS2 are clearly required for cell cycle regulation and play a role in tumorigenesis, we know relatively little of the mechanisms by which they act on cell cycle progression, cell growth and survival. This is hampered by the fact that substrates and effectors of LATS have not been identified, nor do we know what the nature of the upstream regulators is. In recent years, however, work in *Drosophila* has discovered several new tumor

suppressor genes that share similar overgrowth phenotypes as *lats*. The first breakthrough was the identification of the *salvador* (*sav*) gene in a genetic mosaic screen for mutations affecting tissue growth. The *sav* gene encodes a WW domain-containing protein and lacks any other enzymatic domains making it likely to serve as a scaffolding protein. Indeed, biochemical assays reveal that Sav protein interacts directly with Lats through the WW region. Mutant clones of *sav* display the same cell autonomous overgrowth phenotype as for *lats* clones. Like *lats*, this is attributed to an increase in cell number and suppression of cell death. These two aspects have been linked to elevated levels of Cyclin E and the inhibitor of apoptosis, Diap1, respectively, in mutant clones for either *lats* or *sav*. The mechanism by which *lats* and *sav* regulate these downstream targets at this point has not been elucidated, but they have been used as key signatures in examining other tumor suppressors and genes that might function similarly to *lats*. Of course, as discuss below, these are not the only targets since the overexpression of *cyclin E* alone does not impart any growth effects, and neither does its co-expression with *Diap1*. Of significance is the ability to coordinate both cell proliferation and cell death that represents a key common feature of the tumor suppressor function of genes such as *lats* and *sav*.

With the discovery of mutations in *hippo* (*hpo*), which also share similar loss-of-function overgrowth phenotypes as *lats* and *sav*, a signal transduction pathway begins to take shape since *hpo* also encodes a Ser/Thr kinase belonging to the Sterile-20/Mst kinase family (Fig. 2). In the budding yeast, the Cdc15 kinase is the closest homolog to Hpo and functions to phosphorylate Dbf2, a *lats*-related kinase. These kinases comprise a signal transduction pathway that regulates mitotic events and cell morphogenesis in the yeast. The conservation of these modular functions suggests that Hpo may also act upstream of Lats. Indeed, Hpo has been shown to phosphorylate and activate Lats in biochemical assays and in tissue culture. This activity requires Sav where it has been suggested to function as a scaffolding protein to bring Lats and Hpo together into an activation complex. The parallel to the yeast modular pathway also makes another prediction where the Dbf2 kinase requires a binding partner, namely Mob1, which has no obvious domains and has been postulated to function analogously to the cyclins for modulating CDK activities. The identification of *Mats* (*Mob as tumor suppressor*) confirms this notion. Mutations in *mats* behave identically to *lats*, *hpo*, and *sav*. Mats has been shown to bind to and modulate Lats intrinsic kinase activity. These four genes, thus, comprise the core components of the Hpo signaling pathway and function to negatively regulate the gene expression of *cyclin E* and *Diap1* (Fig. 2).

Through a yeast two-hybrid screen, Yorkie (Yki) was identified as an interacting protein with Lats, and encodes the *Drosophila* ortholog of the mammalian transcriptional coactivator yes-associated protein (YAP). The overexpression of Yki in clones completely reproduces all aspects of the overgrowth phenotypes of loss-of-function mutations in the Hpo core components, including the upregulation of target genes, which makes Yki a *bona fide* link in the pathway and, significantly, represents a critical transcriptional effector of the Hpo/Lats kinase cascade. Lats has been shown to directly bind and phosphorylate Yki, leading to its inactivation. Yki is capable of activating transcription; however, it lacks a DNA-binding domain, which suggests that it may require a transcription partner or complex to regulate gene expression. As mentioned before, *cyclin E* and *Diap1* are not the only target genes of Hpo signaling since both cannot confer the growth aspect of the pathway. In an overexpression screen, a ►microRNA has been found to stimulate imaginal disc growth, as well as to affect both cell proliferation and apoptosis. The microRNA is encoded by the *bantam* gene and has been shown to be a transcriptional target for Yki. However, the growth defect associated with *yki* loss-of-function can only be partially rescued by *bantam* overexpression and, likewise, the overgrowth phenotype of *yki* overexpression can only be partially suppressed by the loss of *bantam* function. The combined effect of *bantam*, *cyclin E*, and *Diap1*, however, still cannot fulfill all of the signaling output of the Hpo pathway as defects in differentiation and cell adhesion, for example, are not accounted for.

How the Hpo pathway is regulated has been an outstanding question until a candidate approach reveals ►Merlin (Mer) and Ex (Expanded), which encode related members of the FERM domain-containing protein family, as possible upstream components (Fig. 2). This is suggested by their function as adaptor proteins associated with plasma membrane proteins and the cytoskeleton. Mutations in either gene alone have been shown to have relatively weak effects on growth of imaginal discs. However, since Mer and Ex can heterodimerize and function redundantly, double mutants have characteristic phenotypes of Hpo pathway mutants.Genetic epistasis experiments have placed both genes upstream of *hpo* where their overexpression can influence the phosphorylation of Lats. The mechanism is unknown and additional intervening components remain to be identified since neither Mer nor Ex binds to Hpo. Interestingly, Ex expression is also regulated by Yki, thus, forming a regulatory feedback loop. Despite their membrane localization, Mer and Ex are still intracellular proteins and, therefore, the existence of a receptor for mediating physiological responses to effect Hpo signaling still remains to be seen.

The protocadherin Fat (Ft) recently has been identified as one candidate receptor since loss of its function causes phenotypes similar to *hpo* and *lats* mutants and they all share each other target gene expression. Ft has been known to function as a tumor suppressor, but exactly how it influences growth is not understood. Several studies have placed *ft* genetically upstream of *ex* and *hpo* in a linear pathway since the overexpression of either can bypass the requirement for *ft* (Fig. 2). Furthermore, Hpo and Lats are phosphorylated and activated in response to the overexpression of Ft in cell transfection, which also lead to the repression of Yki-dependent target gene expression. Interestingly, Ft is required for the localization and stability of Ex but not Mer, suggesting that the latter might function in parallel, consistent with the similar phenotypes displayed by *ft mer* and *ex mer* double mutants. Thus, there may be yet another receptor that can regulate Hpo activity through Mer. Although *ft* functions upstream of *hpo* and *lats*, there is an alternative line of evidence to suggest that the pathway may not be linear after all, since Ft can affect the stability of Lats protein specifically and not other downstream components (Fig. 2B). This function is mediated through Dachs, an unconventional myosin downstream of and acts antagonistically to Ft. *dachs* acts genetically upstream of *lats,* and their proteins coprecipitate in biochemical assays. In light of these observations, Lats remains the central element of the pathway but its activity and abundance may be affected differently by various upstream inputs. Nevertheless, further studies are required to resolve the biochemical relationships among the components of the Hpo pathway.

Clinical Relevance

The kinase cascade comprising Hpo and Lats is conserved from yeast to mammals. Many components of this pathway also have orthologs across species and may function in a physiological relevant manner as demonstrated by the ability of some human gene counterparts to rescue the corresponding *Drosophila* mutants. In *Drosophila*, loss-of-function mutations in many components of the Hpo pathway result in overgrowth of ►epithelial tissues, which in many ways resemble tumor formation in humans and mammals. Indeed, cells of these imaginal discs possess cellular behavior and properties that reflect closely those of cycling mammalian cells, an especially important consideration when extrapolating mechanistic insights to mammalian models of cancer. Importantly, in most cases cell cycle progression and kinetics in *Drosophila* are punctuated by growth phases characterized by similar G1-S and ►G2-M transitions as in mammals. In addition, developing disc tissue structures exhibit a polarized epithelium mirroring mammalian tissues that are highly prone to cancer.

These studies have revealed an emerging tumor suppressor pathway that has profound effects on growth with consequences on body, organ, and cell size as well as on cell number. The phenotypic consequences on cell growth coupled to the coordinated deregulation of both cell proliferation and survival provide a growth advantage to mutant cells in mosaics clones, but also very likely confer such traits to tumor cells. Thus, there is a strong link between these developmental processes and tumorigenesis. Consistent with the overgrowth phenotype in *Drosophila*, knockout mice for LATS1 develop soft-tissue carcinoma, ovarian stromal cell tumors, and pituitary dysfunction (Fig. 1B). Although mutations in either LATS1 or LATS2 have rarely been detected, epigenetic modifications of these genes, for example, downregulation by methylation, have been associated with human breast cancers. Many cancer cell lines derived from human melanoma or mouse mammary gland carcinoma have been shown to carry mutations in genes for *SAV1 (WW45)* and *MATS1 (MOBK1)*. The human ortholog of Mer, ►NF2 (►Neurofibromatosis 2), gene is notable in the Hpo pathway in being the only one so far that has been discovered as a *bona fide* tumor suppressor. Patients carrying mutations in the NF2 gene develop tumors mainly affecting the central nervous system as found in sporadic tumors as well as in the familial cancer syndrome neurofibromatosis type 2. The loss of NF2 also leads to other tumors such as malignant mesothelioma. From the *yki* overexpression phenotype in *Drosophila*, the mammalian YAP is predicted likely to function as an oncogene. Indeed, the mouse locus at 9qA1, which contains the YAP gene, has been found to be highly amplified in a mouse model of a liver carcinoma as well as mammary cancer. Similar finding has also been found for the human locus at 11q22 where amplification occurs in cancers of the liver, lung, ovary, pancreas, and esophagus.

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LD

►Linkage Disequilibrium

LD_{50}

Definition

Lethal dose 50. ►Preclinical Testing

LDL

Definition

►Low density lipoprotein.

LDL Receptor

Definition

►Low density lipoprotein receptor (LDLR); gene family consists of cell surface proteins involved in receptor-mediated ►endocytosis of specific ►ligands.

Lead Chromate

Definition

($PbCrO_4$); A water-insoluble chromium-containing compound consisting of lead, and hexavalent chromium in the chromate anion, that is only very sparingly soluble in water. This compound used to be used to manufacture paint used on aircraft and the yellow line in the middle of streets to demarcate traffic lanes. It gives the yellow and yellowish orange color to paints and pigments. When prepared in small particle sizes (<10 μm), this chromium compound is phagocytosed by mammalian cells and can cause cytotoxicity, chromosomal aberrations, and morphological transformation of mammalian cells, and lung tumors when lower animals inhale it.

►Chromium Carcinogenesis

Lead Exposure

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Definition

Lead (Pb) is a heavy, low melting, bluish-gray metal that occurs naturally in various mineral forms in the earth's crust. Lead compounds are substances in which lead is combined with two or more other elements. Organic lead refers to lead compounds which contain carbon, whereas inorganic lead refers to those substances that do not contain carbon and includes metallic lead.

Characteristics

Sources of Lead Exposure

Exposure to lead is predominantly due to ►anthropogenic activity, which has occurred since industrial lead production started millennia ago. The greatest potential for exposure has been experienced by industrial workers, and lead exposure is currently generally well controlled in major lead-using industries such as ►smelting and battery manufacture (Table 1). However, cases of clinical lead poisoning in certain industries still occur and workers using end-products containing lead, such as lead-based paints, continue to be exposed and little to no decreases in lead exposure levels have been observed in certain work environments such as the construction industry. It has been estimated that about one-fifth of the United States general population has a history of exposure on the job.

Historically, the largest source of environmental lead exposure in the United States has been through inhalation and ingestion of air, dust, soil, water and food contaminated from the use of lead in pipes, paints, food and drink cans, and gasoline. These uses have been phased out in many developed countries, including the United States, resulting in a decline in blood lead levels by more than five-fold. Nevertheless, segments of the general population continue to be exposed to excessive amounts of lead, especially from lead-based paints and

Lead Exposure. Table 1 Industrial processes and activities with risk of lead exposure

Industrial processes	Activities
Abrasive blasting	Ceramics making
Battery manufacturing	Drinking from a private well
Gas welding and cutting	Enameling
Metal preparation and pouring	Glassblowing
Metal thermal spraying	Home remodeling
Painting (pigments, binders, and biocides)	Ingesting an herbal remedy
Semiconductor manufacturing	Jewelry making
Silk-screen printing	Living in a house with old plumbing or old paint (pre-1979)
Smelting copper or lead	Living near a smelter
Soldering	Lost wax casting
Steel producing	Painting
Welding	Smoking cigarettes
Welding over coatings	Stained glass making

From HazMap: Occupational Exposure to Hazardous Agents (http://hazmap.nlm.nih.gov/).

contaminated soil in urban settings with an older housing stock. Additionally, drinking water continues to be an important source of lead exposure.

Lead accumulates in the body and may become biologically available long after the occupational or environmental exposure has ceased. Therefore, lead exposure remains a public health concern worldwide.

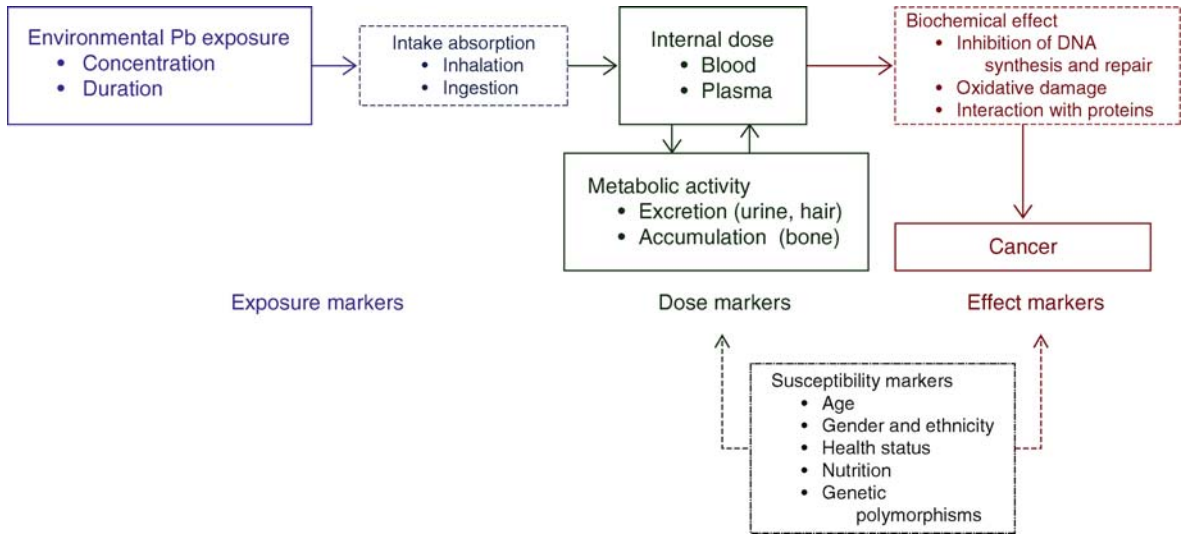
Biological Markers of Lead Exposure

A variety of ►biomarkers exists to measure exposure to lead, including lead in urine, blood, hair, nails, teeth and bone. Blood lead levels are the most common indicator of internal lead exposure in epidemiological studies. However, these levels tend to decline rapidly over time because of a half-life of about one month and therefore represent only recent exposures. For children aged <6 years, the U.S. Centers for Disease Control and Prevention (►CDC) has defined an elevated ►BLL as ≥10 μg/dl. For adults, the highest BLL acceptable by standards of the U.S. Occupational Safety and Health Administration (OSHA) is 40 μg/dl. In the United States, the state-based Adult Blood Lead Epidemiology and Surveillance (►ABLES) program tracks laboratory-reported adult BLLs in an effort to reduce the proportion of adults (age 16 or older) who have BLLs of 25 μg/dl or greater, whereas the CDC tracks children's BLLs by using both National Health and Nutrition Examination Surveys (►NHANES) and state and local surveillance data.

Lead accumulates in the skeleton and remains there for many years. In children, up to 80% of absorbed inorganic lead is stored in the bone, whereas in adults this figure rises to more than 90%. Organic lead compounds are oxidatively dealkylated in the body, and any inorganic lead produced in this way will be distributed like exogenous inorganic lead. The turnover rate of skeletal lead varies by compartments in bone. The half-life of lead in ►cortical bone (e.g. tibia), which constitutes about 80% of skeleton, can be several decades whereas a considerably shorter half-life of only several years has been reported for ►trabecular bone (e.g. patella, calcaneus) which constitutes about 20% of the skeleton. While blood lead levels reflect primarily recent exposures (ongoing steady-state or recently elevated exposures) and the mobilization of lead from the skeleton back into the circulation, bone lead levels have been shown to accurately reflect accumulated exposure. By providing a biological marker of cumulative dose, bone lead may be used in epidemiological studies of lead carcinogenicity to more accurately evaluate dose–response associations in studies of populations in which lead exposure is occurring through multiple possible pathways. Bone lead levels can be determined with a direct, non-invasive measurement using X-ray fluorescence (►XRF) spectroscopy.

Lead Carcinogenesis: Possible Etiologic Mechanisms

Experimental evidence demonstrates that various water-soluble and -insoluble lead compounds can induce ►renal carcinoma and ►brain tumors (gliomas) in rodents. Lead is known to be toxic to the peripheral and central nervous system as well as the kidney. However, there does not seem to be a direct carcinogenic effect of lead since it does not seem to directly result in ►DNA damage. Rather, current mechanistic evidence for a role of lead in ►carcinogenesis suggests a facilitative role involving inhibition of DNA synthesis and repair, ►oxidative stress, and interaction with DNA-binding proteins and ►tumor suppressor genes. This facilitative role suggests that lead exposure may interact with other possible risk factors in the etiology of cancer.



Lead Exposure. Figure 1 Conceptual framework for the association between environmental lead exposure and cancer of the brain and central nervous system.

A general conceptual framework for the carcinogenic effects of lead exposure is presented in the figure. Individual indicators of susceptibility may influence the nature of this relationship, such as age, gender, and genetic polymorphisms. For example, anemia due to glucose-6-phosphate dehydrogenase (►G6PD) deficiency and polymorphisms in the enzyme delta-aminolevulinic acid dehydratase (►ALAD) may affect the susceptibility to lead toxicity, including cancer, by affecting the toxicokinetics of lead in the body (Fig. 1).

Lead Exposure and ►Cancer Epidemiology

The relationship between cancer in human populations and exposure to lead from environmental or occupational sources has been considered in several epidemiological studies. These studies primarily consist of case–control and cohort studies. In ►case–control association studies, cases diagnosed with a disease of interest and controls without that disease are asked about lead exposure (on the job, from hobbies, or from living in older homes) at any point in their life. In cohort studies, cancer mortality or incidence rates are compared between groups of people with and without elevated lead exposure (based on high-lead jobs compared to the general population, or blood lead levels). This literature has been reviewed on several occasions. Most epidemiological studies evaluating the carcinogenic effects of lead exposure have generally suffered from limited statistical power due to a small number of cancer deaths or diagnoses, and the lack of biological measures of exposure. Most studies lack quantitative data on dose–response – one of the key parameters in determining causality – and only few studies divided individuals in high and low exposure groups. Nevertheless, occupational cohort studies have been considered particularly informative with respect to cancer risk because of high documented exposures. These studies were predominantly based on highly exposed battery or smelter workers who were exposed several decades ago (1940s–1970s). These studies have reported on a variety of cancer types, including those of the lung, stomach, kidney and brain. Most studies show a weak to moderate positive association between lead exposure and cancer, in particular for cancers of the stomach and brain, and to a lesser extent for lung and kidney cancer. Follow-up studies of general population samples in the United States and case-control studies shed little further light on these associations.

The International Agency for Research on Cancer (IARC) recently concluded that there is limited evidence in humans for the carcinogenicity of inorganic lead compounds, and inadequate evidence for an effect of organic lead compounds on human cancer.

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Lead Generation

Definition

Chemical modification of a structure to increase desired but non-optimized biological activities.

►Lead Optimization

Lead Optimization

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Definition

The synthetic chemical modification of a biologically active molecule to address pharmacokinetic, pharmacodynamic and toxicologic issues in order to enable its clinical utility.

Characteristics

Drug discovery activities that occur prior to lead optimization such as the choice of target, assay development, ►high throughput screening and early molecular optimization and testing, are typically referred to as ►lead generation. Leads typically have been somewhat optimized for their molecular properties (solubility, removal of covalent modifiers, chemical stability), have been subjected to *in vitro* assays to predict their (►Pharmacokinetic) properties (Caco-2 flux, liver microsomal stability, protein binding, inhibitory activity toward (►Cytochrome P450 isoenzymes) (CYP450s), possess reasonable *in vitro* and cellular potencies toward the molecular target, and have been subjected to some selectivity measurements (kinase selectivity screen and a broad receptor/transporter selectivity screen).

Lead Optimization, the subject of this entry, encompasses chemical modifications of the molecular structure that improve its druglikeness, particularly with respect to issues of potency, selectivity, safety/toxicity and pharmacokinetic (absorption, distribution, metabolism, excretion) parameters. Efficacy in an animal model of disease also is first evaluated during this phase. This essay focuses on optimization of (►Small molecule drug) candidates, though biologics leads are optimized as well.

During (►Preclinical testing), the candidate drug is further examined to assess its suitability for first-in-human safety trials (Phase 1 clinical trials). Development of medium-scale chemical synthesis, in order to produce (►Good manufacturing practices) (GMP) drug substance, is explored. Further assessment of the compounds pharmaceutical properties and formulation work is also undertaken. The initial pharmacokinetic studies are usually repeated in multiple species. This phase also further examines the safety of the candidate drug in non-human species. Target candidate drug exposure is then compared to the dose causing overt toxicity in a rodent, dog or monkey species to evaluate the safety window of the candidate in first-in-human trials. The typical procedure to assess toxicologic risk is based on the compound safety margin which is the ratio of the no adverse event level (NOAEL) in the most sensitive species to the expected therapeutic dose in man.

Chemotherapeutic agents constitute a significant subset of cancer drugs (as well as antibiotics) but are much less used for treatment of other diseases. Chemotherapeutic drugs rely on selective toxicity for cancer cells, either as a result of favorable differences in distribution or favorable differences in biochemistry.

Physicochemical Optimization

Intravenously administered drugs must have high solubility. Drugs administered *via* a peroral route should have adequate solubility in order to be able to reach the site of action within the organism. During *in vitro* testing solubility of a potent compound is usually not an issue, but when animals must be dosed with larger quantities of drug substance in order to measure efficacy or tolerability, absorption may be limited by the failure of the compound to dissolve in the gastrointestinal tract. Thus solubility, permeability and potency are in dynamic tension during optimization. For instance, high solubility can sometimes overcome low permeability with a drug of moderate to high potency.

Solubility can be measured in a number of ways. One way is to initially dissolve the compound in dimethylsulfoxide, a polar aprotic solvent, which overcomes crystal lattice energy. This is the characteristic of a kinetic solubility method. Some compounds soluble at aqueous 0.5–5% DMSO that would not easily or at all attain dissolution if tested with a thermodynamic method, which entails dissolution initially in a buffered aqueous medium. A thermodynamic method however, is perceived to be more relevant to the *in vivo* situation

and should be evaluated at a range of biologically relevant pHs. There are a number of strategies used to optimize the solubility of sparingly soluble drug candidates. The most important is to introduce solubility directly into the molecule during optimization, through introduction of water solublizing moieties at a permissible part of the structure. Another common approach is the formation of a suitable salt. Almost half of present day drugs are salts, and this strategy may be undertaken very early in the lead optimization process. Also, formulation with appropriate organic solvents, surfactants and lipids can be explored during lead optimization and during preclinical development.

Besides solubility, there are a number of other physiochemical parameters that begin to be evaluated during the lead optimization phase, namely chemical stability, morphic states (monomorphic vs. polymorphic), crystallinity and hygroscopicity.

It is also important during the lead optimization phase to bridge the chasm between the discovery chemistry (large number of compounds synthesized in milligram amounts) and the chemical development (few compounds in kilogram amounts) departments. This can occur *via* the formation of specialized teams that bridge that scalability gap, or by the presence of chemists in the discovery chemistry departments that work on process improvements, or both.

Pharmacokinetic Optimization

In years past, a substantial percentage of drugs failed in the clinic secondary to poor pharmacokinetic properties. Today, that number is greatly reduced, chiefly as a result of the focus on ADME (Absorption, Distribution, Metabolism, Excretion) properties during lead optimization. Ideal ADME properties for a drug candidate depends on the clinical target, but typically encompass good ►bioavailability, ►systemic clearance, and ►volume of distribution that may be expected to yield the desired dosing regimen. A low probability for drug-drug interactions and metabolism-based toxicity is also highly desirable.

Analysis of current orally administered drugs and drug candidates has been a primary guide to correlating physical properties with successful clinical developability. It would be useful to develop models that quantify to some extent known effects of easily calculated molecular properties on absorption. The Lipinski "Rule of Five" states that poor absorption frequently occurs with a molecular weight over 500, a logP over 5, and the number of hydrogen bond donors and acceptors each greater than 5. In addition to Lipinski's original rules concerning molecular weight, lipophilicity and the number of H-bond donors or acceptors; a number of studies have also discussed the negative absorption impact of water complexation by amide bonds, high molecular flexibility and high polar surface area.

Bioavailability is a term used to describe the rate and extent of absorption of an orally administered drug in comparison to intravenous administration. High oral bioavailability is almost always an important consideration for the development of good drugs. Oral bioavailability is affected by both the extent of absorption into the intestinal cells in the gut lumen as well as the compounds presystemic elimination by both the intestine and the liver. So structure-specific recognition factors do limit oral bioavailability, such as (►P-glycoprotein) (PGP) transport in the gut, CYP450 metabolism or other enzymatic clearance mechanisms in the gut or liver.

If absolute oral bioavailability is >20% in dogs and rodents then frequently oral bioavailability in humans is >20% as well. However, since there is reasonable correlation between clearance, volume of distribution and half-life data between rat and human as well, this may be tracked during Lead Optimization. It is widely assumed that monkeys are good predictors of human oral bioavailability but this is frequently not the case. They are a good predictor of the fraction absorbed (Fa) but as they have elevated metabolic clearances and hepatic enzyme activities, the oral bioavailability in monkeys is typically lower.

Consideration must be given to the ability of a compound to undergo *in vivo* biotransformation as this is an important component of systemic bioavailability and systemic clearance. It is frequently necessary to decrease the rate of hepatic oxidative metabolism and first-pass elimination in a chemical series during optimization. A number of *in vitro* methods are available to monitor progress. One of the most common and high throughput entail the use of liver microsomal preparations. These retain activity of key components of enzymes involved in drug metabolism such as cytochrome P450s and ►flavin monooxygenases. The drug candidate is incubated in the presence of such preparations, a half-life estimation is made and this can be compared to standards with known first-pass eliminations. The compound's metabolites should be identified in human, efficacy and tolerability species. This can frequently give ideas for means by which the structure can be modified to decrease oxidative metabolism and reduce clearance.

Metabolism of a lead compound may also be evaluated in liver slices or hepatocytes. When a compound induces an enzyme involved in its own metabolism, evidence for which is usually found during multiple-dose pharmacokinetic or efficacy studies, the use of the latter are quite useful. When ►Phase II metabolism is important, again the use of liver slices may be more appropriate as well as *in vitro* glucuronidation assays in microsomal preparations in the absence of NADPH.

Metabolism-based drug interactions comprise one of the major concerns during drug development. If two or more drugs are competing with each other for the same metabolic enzyme, the circulating therapeutic drug concentrations can become elevated and may cause undesirable toxic effects. These are even more clinically important when a drug has a narrow therapeutic index or when the pharmacokinetics are markedly changed. Cytochrome P450 proteins are a critical family of enzymes involved in the metabolism of drugs. Of these the most important are CYP1A2, 2C9, 2C19, 2D6 and 3A4.

CYP3A4 has primarily been associated with *in vivo* drug-drug interactions leading to the withdrawal of marketed drugs. CYP3A4 is responsible for 25% of total CYP content and has broad substrate specificity. CYP2D6 is responsible for basic amine functionality metabolism, and this is important because of the prevalence of these in marketed drugs. Strong inhibitors <1 μM for 3A4 and 2D6 (cDNA expressed CYPs) are certainly major concerns.

Mechanism-based inhibition of 3A4 (irreversible binding or covalent modification) is also a major concern. Furans, some amines and unsaturated distal groups (double and triple bonds) are common culprits. For instance, the triple bond on ethinylestradiol modifys the heme group, though the CYP protein may be modified as well. Care is required when evaluating compounds, as they may initially appear to be only weak inhibitors of CYP3A4 because the inhibition is time-dependent. High liver distribution can exacerbate this issue, as many drug candidates are highly distributed to liver, where CYP3A4 is found in high concentration.

Clearance is a measurement of the ability of the body to eliminate a drug from the body and is made up of hepatic (metabolic and biliary) and renal clearance. For the majority of compounds hepatic metabolism is a major clearance mechanism, so clearance may be expressed relative to hepatic blood flow in the species in which the pharmacokinetic measurement was obtained. The drug concentration seen in the plasma depends on the amount of drug present in the whole body as well as how extensively it is distributed to various compartments. The volume of distribution is a measure of this. High steady state volumes of distribution are associated with more binding in tissue than in plasma and are found frequently in basic drug candidates, while the reverse is true of a low volume of distribution which is frequently found in association with acidic drugs. Since the half-life of a drug is inversely proportional to the clearance, and directly proportional to the volume of distribution, optimization of these parameters is frequently necessary.

Plasma protein binding can be important when activity is driven by the free fraction of drug in plasma. This may be a significant issue when there is a target within the vasculature and a lead with a small volume of distribution, but may be less of an issue with an extravascular target with a high volume of distribution. In the blood, acid glycoprotein binds weakly basic drugs while albumin, which constitutes 50–60% of total serum protein, binds neutral drugs and weak acids.

Toxicological Optimization

Just as the number of compounds in development that fail for pharmacokinetic issues have decreased, the number of compounds that fail during development for toxicity issues have increased. Even so, Adverse Drug Reactions (ADRs) may account for up to 5% of all hospital admissions and 10–20% of hospital inpatients for marketed drugs.

Toxicity issues are frequently observed during lead optimization either when tolerability is being examined specifically, or when dosing pharmacokinetic or efficacy studies. Toxicity is typically first identified in rats or dogs. Toxicologic effects of a compound frequently do require optimization, especially in backup programs where such issues may be better understood as a result of the identification of toxicology in previously identified drug candidates. ADRs may generally be grouped into two classes. Predictable adverse drug reactions can be predicted based on the pharmacology of the drug and are dose-dependent, idiosyncratic drug reactions are unpredictable, cannot be correlated to the pharmacology of the drug and are typically non dose-dependent. Optimization for predictable toxicity normally occurs *via* the identification of a (►Biomarker) or *in vitro* readout for the toxicity followed by the development of a structure-activity relationship around the toxicity in order to remove it during optimization.

Idiosyncratic toxicity is more difficult to approach. Hepatotoxicity is the most common idiosyncratic reaction. These effects can be serious (hepatic necrosis) and skin dyscrasias can be as serious as Stevens-Johnson syndrome (toxic epidermal necrolysis). Mechanistically, the hapten hypothesis is that modification of an endogenous protein by a reactive metabolite or directly by a reactive parent drug generates a foreign protein that may lead to an immune-mediated adverse reaction. The alternative danger hypothesis stipulates that rather than the hapten formation triggering the immune system, cellular damage produced by the reactive metabolite generates a danger signal. If cells are resistant to the stress or the toxic metabolites cannot cause cell injury at all times, no idiosyncratic toxicity occurs. This accounts for the interindividual variations in idiosyncratic drug reactions and the nature of differential responses to various reactive metabolites.

The human liver and blood systems are the most active at forming reactive metabolites.

Several enzymes from ►Phase I metabolism are recognized as important in generating reactive intermediates such as CYP450s, ►myeloperoxidases, ►cyclooxygenases and flavin monooxygenases. CYPs are the most important because electrons are transferred. Phase II metabolism is potentially less of an issue although there are some reports on acyl glucuronide toxicity (these may bind to proteins with or without cleavage of the glucuronide). A large number of electrophiles (generated from *in vitro* experiments such as with HLMs) can be trapped as glutathione adducts or N-acetyl cysteine/N-acetyl lysine adduct mixtures. This method is unlikely to produce false negatives, since safe compounds normally do not generate glutathione conjugates.

Cardiovascular safety is another important consideration during lead optimization. Frequently ►HERG activity needs to be removed from a compounds list of off-target effects.

This channel is the target of a wide range of agents which increase the risk for ►Torsade de Pointes (TdP), as well as many which do not. The growing list of non-antiarrythmic drugs that cause this increased QT interval, and the increasing number of drugs that have been withdrawn from the market because of their ability to trigger TdP, has resulted in increased regulatory scrutiny for this particular safety issue. Unfortunately there is no single cell based assay, or *in vitro* heart preparation or animal model that is very strongly predictive for TdP in humans. Not all drugs which prolong the electrocardiographic QT interval cause TdP, nor is there a quantitative correlation between the magnitude of QT interval increases and the arrythmia. Because there is no gold standard test of the torsadogenic potential of a lead, the use of multiple *in vitro*, *ex vivo* and *in vivo* assays is currently recommended by most experts during the lead optimization phase.

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Leakage Radiation

Definition

Radiation coming from the source outside the useful beam.

►Radiation Oncology

Lean Body Mass

Definition

LBM; Mass of the body, including consists of water, bones, collagen, and muscle, minus fat mass.

►Nutrition Status

LECAM-2

►E-Selectin-Mediated Adhesion in Cancer

Lecithin

Definition

Phosphatidylcholine (PC) is a major class of glycerophospholipids present in all mammalian and some prokaryotic cells, which plays various roles in membrane structure and cellular signaling.

►Lysophosphatidylcholine

Lectin

Definition

Are proteins that interact with a sugar or sugar chain with Carbohydrates in general, but do not include enzymes related to carbohydrate metabolism, such as

glycosyltransferase and glycosidase. Lectins include galectin, mannose-6 phosphate receptor, the calnexin/►calreticulin families, and offers.

►Fucosylation
►Cell Adhesion Molecules
►Glycobiology

Leiomymatous Harmatoma of the Kidney

►Mesoblastic Nephroma

Leiomyoma

Definition

A benign neoplastic tumor composed of smooth muscle cells with variable amount of fibrous stroma. They appear most commonly in the smooth muscle lining of the uterus (Uterine leiomyoma), the myometrium.

►Fumarate Hydratase
►Uterine Leiomyoma, Cellular and Genetic Characteristics
►Uterine Leiomyoma, Clinical Oncology

Leiomyomata

►Uterine Leiomyoma, Clinical Oncology

Leiomyosarcoma

Definition

Belongs to a group of cancers called soft tissue sarcomas. Sarcomas are cancers that develop in the supporting or connective tissues of the body (such as muscle, fat, nerves, blood vessels, bone, and cartilage). They are rare. Leiomyosarcomas are one of the commoner types of sarcoma to occur in adults over the age of 50.

►Non-Rhabdomyosarcoma Soft Tissue Sarcomas

Lentigines

Definition

Are benign lesions that occur on the sun-exposed areas of the body. The backs of hands and face are common areas. The lesions tend to increase in number with age, making them common among the middle age and older population. They can vary in size from 0.2 to 2 cm. These flat lesions usually have discrete borders, are dark in color, and have an irregular shape.

►Liver Spots

Lentiginosis

Definition

Presence of ►lentigenes in very large numbers or in a distinctive configuration. Centrofacial lentiginosis, uncommon autosomal dominant syndrome of small hyperpigmented macules in a horizontal band across the centre of the face at one year, increasing in number up to ten years, and associated with skeletal and neural defects.

►Carney Complex

Lentiginosis polyposa Peutz

►Peutz-Jeghers-Syndrome

Leptomeningeal Carcinomatosis

Definition

Involvment of ►leptomeninges through seeding which occurs either by direct spread or via bloodstream. Any

cancer can cause this, but adenocarcinoma is most commonly involved. Patients usually have a known underlying malignancy but primary presentation can be with symptoms of meningeal involvement.

►Carcinomatosis

Leptomeningeal Disease

►Leptomeningeal Dissemination

Leptomeningeal Dissemination

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Synonyms

Leptomeningeal disease; Leptomeningeal seeding; Leptomeningeal metastasis; Neoplastic meningitis; Subarachnoidal spread

Definition

Tumor cell spread into leptomeningeal structures of the brain and/or spine.

Characteristics

Leptomeningeal spreading is a major disease complication in primary central nervous system (CNS) tumors, in solid tumors outside the CNS such as breast, lung, gastrointestinal cancer, melanoma, and as well in hematological malignancies. Leptomeningeal dissemination can occur at initial diagnosis (►primary dissemination) or during the course of the disease following initial therapy (►secondary dissemination), but it is usually a late manifestation of malignant diseases and almost always associated with active tumor disease. Primary leptomeningeal disease is found mostly in primary brain tumors, sarcomas, neuroblastomas, lymphomas and leukemia. Secondary dissemination occurs mostly in lung and breast carcinomas, but also in leukemia and lymphomas and primary CNS malignancies. The way of neuroaxis dissemination for primary CNS tumors and tumors outside the CNS is thought to be hematogenous via arachnoid vessels. Primary brain tumors may also disseminate throughout the neuroaxis by direct extension, by spreading along the nerves or by release of tumor cells into the ►cerebrospinal fluid (CSF). Due to the physiology of the CSF-flow leptomeningeal metastases can involve all structures of the neuroaxis.

In brain tumors, leptomeningeal dissemination is well known in primary CNS lymphomas and medulloblastomas, occurring in up to 33% of all patients at the time of initial diagnosis, in germ cell tumors and also glioblastomas. Patients with evidence of CSF spread are considered to have a poorer prognosis compared with CNS negative patients. In children with high-grade gliomas, the rate of leptomeningeal dissemination is reported to range between 7% and 28% and as with other tumors leptomeningeal spread is a sign of frank tumor dissemination and carries a worse prognosis. In patients with acute lymphoblastic leukemia initial CNS involvement is found in up to 7.4% of patients depending on the individual risk profile of the patient. Although CNS involvement was reported to be negatively correlated with event-free survival (EFS) in one study, this observation could not be confirmed by others. The percentage of initial CNS involvement in patients with acute myeloblastic leukemia (AML) ranges between 5.9% and 12.2%, patients with CNS-negative AML do better than those with initial CNS involvement.

Classification of cerebral leptomeningeal metastasis according to Chang's guideline for cerebellar medulloblastoma (Chang 1969):

Metastasis stage	
M0	No evidence for metastasis
M1	Tumor cells in CSF
M2	Gross nodular seeding of brain CSF spaces
M3	Gross nodular seeding of spinal CSF spaces

Symptoms

The most common symptoms in patients with proven or suspected leptomeningeal dissemination are headache, nausea, vomiting, fluctuating consciousness, cranial or spinal nerve palsy (palsies), and spinal radicular symptoms. Clinical presentation may fluctuate, because several levels of the neuroaxis are affected simultaneously. Duration of symptoms may vary from several days to weeks or even months. In patients with medulloblastoma, secondary diffuse CSF seeding can lead to sudden neurological deterioration and spinal

cord compression. Intracranial hypotension syndrome was described in diffuse meningeal melanomatosis. The symptoms could also mimic cerebral vasculitis, meningitis or viral encephalitis.

Diagnosis

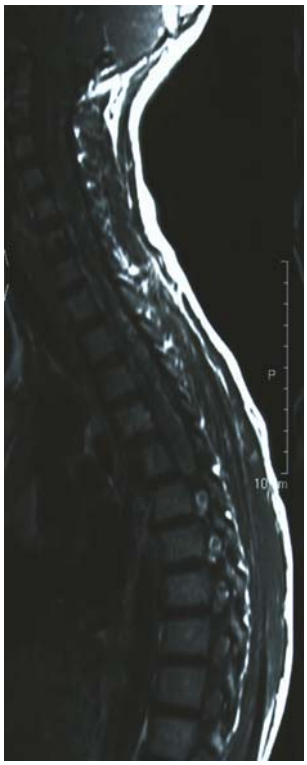
If leptomeningeal dissemination in a patient with an underlying malignant disease is suspected meningitis and viral encephalitis have to be excluded. Other rare diseases should also be considered such as granulomatous angiitis, histiocytosis, Lyme disease, multiple sclerosis, vasculitis, Wegner's granulomatosis, sarcoidosis, post-liquorpuncture changes, and opportunistic infections.

Neuroimaging

In former days conventional myelography and CT myelography improved detection of metastatic disease and can still be used today in cases in which MR imaging is not feasible; it could show nerve root thickening, nodularity, thecal sac irregularity and spinal cord enlargement. Nowadays magnetic resonance imaging (MRI) is the diagnostic tool of choice in patients with suspected leptomeningeal dissemination of a malignant disease and may show hydrocephalus, pial linear or nodular enhancement, subependymal and/or neural deposits, or dural enhancement; however MRI has low specificity (i.e. meningeal enhancement on MRI does not always indicate metastases). Strong methionin uptake on positron emission tomography (PET) may help to confirm diagnosis even before CSF became positive. The time-point of neuroradiographic evaluations should also be considered: False positive spinal MRI findings of subarachnoidal spread of primary CNS tumors have been described, when MRI studies were performed within two weeks after surgery. In medulloblastoma, metastases are generally located along the posterior margin of the spinal cord. More sophisticated MRI techniques such as FLAIR sequences were discussed to be the clue to detect leptomeningeal abnormalities. Contrast enhanced FLAIR imaging sequences seem to improve the detection rate of leptomeningeal disease compared to routine contrast-enhanced T1–weighted imaging by suppression of signal intensity of normal vascular structures on the surface of the brain and spine. Importantly, in patients with primary brain tumors, MRI imaging of the whole spinal axis should be done as a staging procedure before starting treatment and during follow-up (Fig. 1).

Laboratory Findings

Lumbal puncture for cytologic detection of malignant cells is an important diagnostic feature. In at least 50% an elevated opening pressure can be seen. An elevated protein in CSF is found in 80% of patients, whereas about one third of the patients have low CSF glucose levels.



Leptomeningeal Dissemination. Figure 1 Sagittal T1-weighted postgadolinium magnetic resonance imaging (MRI) in a 12-year old girl with glioblastoma multiform showing multiple nodular enhancing lesions of the entire spinal cord.

Detection of CSF seeding by means of cytopathological analysis of CSF specimens has low sensitivity, since only 15–60% of patients with leptomeningeal metastases have positive results. This could be explained by the paucity of viable tumor cells released in the CSF of patients with minimal disease and the presence of confounding reactive lymphocytes in more than one half of patients with leptomeningeal metastases, especially in patients with lymphoma and leukemia. Patients with low tumor burden who would probably most benefit from treatment are, therefore, more likely to be falsely negative. Several consecutive CSF samples and punctures may be required to identify malignant cells. Since positive CSF cytology correlates with a poor outcome regardless of the time of assessment, CSF cytology was found to be highly predictive for overall survival. In addition cytologic examination of lumbar CSF was shown to be superior to cytologic examination of ventriculoperitoneal shunt CSF for the detection of leptomeningeal spread in children with primary brain tumors. Of note MRI imaging and cytologic analysis CSF performed simultaneously might increase the detection rate of CSF dissemination.

Another diagnostic tool is the analysis of specific markers for leptomeningeal dissemination such as PSA for prostate cancer CA-125 for ovarian cancer and CA153-3 for breast cancer, and of non-specific markers such as beta-glucuronidase, lactate dehydrogenease (LDH), and carcinoembryonic antigen (CEA). These markers are helpful, when clinical symptoms strongly suggest dissemination in patients with negative neuroimaging and negative CSF cytological examination. These markers should be detected in serum and CSF levels and might be used for monitoring (in case of leptomeningeal dissemination: ratio <60:1).

Flow cytometric techniques of CSF in patients with lymphoma or leukemia allow the detection of meningeal dissemination with high sensitivity and PCR analysis of clonal immunoglobulin heavy chain (IgH) seems to have both high specificity and sensitivity.

Treatment

Management of leptomeningeal dissemination has to focus on both, treatment of symptoms and treatment of the malignant disease. ►Symptom management includes anticonvulsant treatment in case of seizures and pain medications in case of painful radiculopathy. Corticoids are not very helpful because edema in leptomeningeal metastases is not common.

Because leptomeningeal dissemination is often diagnosed in advanced disease stage, therapy is mainly palliative (except in leukemia and lymphoma, and germ cell tumors), aiming at preserving neurological function and potentially improving quality of life. As to whether the treatment of LM is ►palliative or ►curative depends on various clinical parameters, particularly on the underlying disease. Treatment of leptomeningeal metastases must be directed to the entire CNS, because leptomeningeal metastasis spreads throughout the neuroaxis by way of CSF flow. Treatment consists mainly of involved field irradiation, systemic and intrathecal chemotherapy.

►Intrathecal chemotherapy could be administered via lumbal puncture or Ommaya reservoir, which is the preferred route of administration due to a better distribution of the drug in the CSF and subarachnoid space. Only few chemotherapeutics are available for intra-CSF drug therapy (methotrexate, cytarabine, etoposide, mafosfamide and thiotepa). These agents were primarily tested in patients with primary brain tumors, leukemia and lymphoma. Liposomal formulation such as liposomal cytarabine may increase the therapeutic drug level over extended periods of time and are new approaches to enhance efficacy of drugs administered into the CSF. Other drugs are under investigation: i.e. darcarbazin, melphalan and topotecan. Except for hematopoetic neoplasms and, to even lesser extent, for breast cancer, intrathecal chemotherapy might be efficacious only in cases, where free tumor cells float within the CSF. It is, however, insufficient to treat solid tumor desposits or even bulky disease. Experimental intrathecal treatment includes immunotherapy (intrathecal administration of monoclonal antibodies, cytokines or lymphokine activated killer cells) or the use of targeted toxins.

The use of ►systemic chemotherapy is being re-evaluated for the treatment of leptomeningeal disease. It seems to be more effective when it is given as part of the initial treatment. In highly malignant medulloblastoma (and germ cell tumors) CNS-directed therapy is mainly based on craniospinal irradiation, whereas intrathecal chemotherapy with MTX via ventricular access devices is given to young children not amenable to CSI or to patients with leptomeningeal dissemination. In primary CNS lymphomas in which leptomeningeal involvement is common, intrathecal MTX is frequently used in combination with HD MTX. Nevertheless, patients with an initial diagnosis of meningeal leukemia are treated more intensively than CNS-negative patients, i.e. they will receive additionally intrathecal chemotherapy. In addition, intensive systemic chemotherapy may substitute for radiotherapy.

Even today with new ►radiation techniques radiation is almost strictly avoided in children younger 18 months. Overall in children, the attempt is to minimize radiation and its long-term sequelae by using more intensive chemotherapy. The total dose and fractions depend on tumor entity and age of patients, and could vary between 12–30 Gy. In children with ALL prophylactic cranial irradiation is administered to high risk patients to reduce the risk of leptomeningeal disease: Due to improved systemic and intrathecal chemotherapy, doses of radiotherapy could be lowered to 12 Gy and craniospinal irradiation is no longer necessary; whether patients with AML require prophylactic cranial irradiation is a matter of debate. Furthermore radiation is used to stabilize or improve neurologic function, pain and CSF flow. Additionally, chemotherapy was described to be more effective after radiation. In summary, radiotherapy in leptomeningeal metastases is almost only be used to treat symptomatic areas.

Summarizing the treatment of leptomeningeal dissemination, there is an urgent need to develop new drug-based or radiation-based treatment. For this purpose new and better surrogate markers for response must be developed to identify clearly the effectiveness of a therapeutic regimen.

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Leptomeningeal Metastasis

►Leptomeningeal Dissemination

Leptomeningeal Seeding

►Leptomeningeal Dissemination

Leptomeninges

Definition

The two innermost layers of tissues that cover the brain and spinal cord. The two layers are called the arachnoid mater and pia mater.

Lesion on Tongue, Lip and Other Areas in the Mouth

►Oral Cancer

LET

Definition

Linear energy transfer; Represents the average amount of radiation energy lost when traversing a small distance. It has units of energy divided by the short distance (keV/μm).

►Radiosensitization

Letterer–Siwe Disease

Definition

Disease is a fatal and disseminated form of Langerhans cell histiocytosis which is most commonly seen in children less than two years old.

►Langerhans Cell Histiocytosis

Leucopenia

Definition

A reduction in the circulating white blood cell count to less than 4,000/μL.

►Rituximab

Leucovorin

Definition

Synonym folinic acid. Is a reduced folate that can assist 5-FU in the inhibition of thymidylate synthase augmenting the toxicity of this drug.

►Fluorouracil

Leukemia

Definition

Leukemia is a group of cancers originated from blood cells. It can be divided into different types based on the

type of blood cells involved, the stage of differentiation when normal cells become leukemic cells and the speed of disease development. When leukemia arises from the lymphocyte precursors it is called lymphoblastic leukemia and when it arises from the myeloid precursors it is known as myeloblastic leukemia.

►STI-571
►Minimal Residual Disease
►Hematological Malignancies

Leukemia Diagnostics

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Definition

Leukemia diagnostics comprises the combined application of different methodologies in a standardized fashion, allowing the precise diagnosis, subclassification, and determination of prognostic parameters.

Characteristics

Introduction

The diagnosis of leukemias today is based on a comprehensive approach applying a variety of different methods in combination. While ►cytomorphology and cytochemistry have been the mainstay of diagnostics for decades and have been used to differentiate between acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphatic leukemia (CLL), and myelodysplastic syndromes (MDS) the current up-to-date approach includes ►immunophenotyping/Multiparameter flow cytometry (MFC) as well as ►cytogenetics and ►molecular genetics to correctly diagnose and subclassify leukemias.

►Multiparameter flow cytometry (MFC) is used for immunophenotyping of the leukemic cells which is essential particularly to diagnose and classify lymphatic leukemias. Karyotyping and ►fluorescence in situ hybridization (FISH) are applied to detect chromosomal alterations which are considered disease-defining lesions in an increasing number of entities. Different molecular genetic techniques (►PCR, ►fragment analysis (Genescan), ►heteroduplex analysis, ►melting point analysis, ►nested PCR, ►real time PCR, ►sequence analysis, ►Single stranded conformation polymorphism analysis (SSCP)) allow the identification of leukemia-specific fusion genes and gene mutations and are also an integral part of the diagnostic work-up of leukemias.

The basis for establishing this diversified diagnostic approach has been the growing body of insight into the genetics of leukemias, which not only allowed the definition of recurring genetic aberrations but also their strong correlation to distinct disease subtypes with specific clinical characteristics, including prognostic impact. As a consequence, today the decision to treat a patient suffering from a particular leukemia and the specific therapeutic option to select for him or her largely depends on the presence or absence of these genetic alterations.

The monitoring of minimal residual disease (MRD) increasingly gains clinical relevance in patients with leukemias. While conventional methods like cytomorphology can be used to assess the prognostically highly relevant achievement of complete remission, newly developed methods like multiparameter flow cytometry and quantitative real time PCR allow the exact quantification of the amount of residual malignant cells in patients with complete remission. The level of this MRD in many cases significantly correlates with the further course of the disease and is incorporated into the guidance of a risk-adapted therapy.

The present essay provides, in the first step, an overview of the methods applied in leukemia diagnostics and then focuses on the respective leukemia subentities and their specific diagnostic findings. The content is then summarized by comprehensive algorithms detailing the modern diagnostic work-up for the different leukemias.

Methods Applied in Leukemia Diagnostics

Cytomorphology and Cytochemistry

Cytomorphologic analysis and cytochemistry are performed on peripheral blood and bone marrow smears which are air-dried without fixation before staining. A panoptic staining (Pappenheim or May Gruenwald Giemsa) is used for the general assessment of cell characteristics. Cytochemistry applies myeloperoxidase staining and non-specific esterase staining for the identification of myeloid and monocytic cells, respectively, while the use of Schiff's reaction, acid phosphatase, and chloroacetate esterase (CE) have been substituted by immunophenotyping. Iron staining is applied in the diagnostic setting of MDS.

Immunophenotyping

Immunophenotyping by multiparameter flow cytometry allows the identification, quantification, and characterization of cell populations in peripheral blood or bone marrow samples. Cells are differentiated from each other, based on their light scatter features during their pass across a laser beam (higher cell sizes result in higher forward scatter signal [FSC]; higher heterogeneity of cell content results in higher side scatter signal [SSC]) as well as on their antigen expression patterns.

As 1,000–2,000 cells are analyzed per second, the simultaneous analysis of the expression of five antigens in 10,000 cells within seconds is current standard. Populations can be characterized even if their concentration is only around 1%. During the monitoring of minimal residual disease (MRD), 250,000 cells are analyzed.

While the detection of FSC and SSC signals is possible in unmanipulated cells, the detection of antigens requires the use of monoclonal antibodies against the respective antigens, which are coupled to a fluorescent dye. Five or even more different fluorescent dyes can be routinely used as the light emitted differs in wave length and is specifically recognized by different detectors. Sophisticated compensation programs, however, are necessary to account for overlaps in the spectrum of the emitted light.

Due to its expression on all peripheral blood and bone marrow at different levels, CD45 represents an ideal antigen for performing a differential count. Thus, monocytes feature the strongest expression of CD45 and may be separated from lymphocytes, which also express CD45 quite strongly, based on differences in the SSC signal. Granulocytes express CD45 at a lower level and feature the strongest SSC signal. Importantly, erythrocytes hardly express CD45 and blasts show a CD45 expression level similar to granulocytes; however, both the latter populations are easily separated from each other, based on their SSC signal (low SSC signal in blasts). Therefore, CD45 gating is useful for the analysis of different cell populations, particularly for the analysis of blasts.

The simultaneous detection of the expression of multiple antigens within one tube allows the comprehensive assessment of antigen expression patterns in different cell populations, which, in comparison to single and dual color approaches, leads to quick and valid results in diagnostics, particularly in the quantification of MRD. The panel of antibody combinations is selected, respectively, in consideration of the suspected diagnosis and may be supplemented based on primary results.

Cytogenetics

Importance of Chromosome Analysis, Indication

Chromosome analysis today is an essential part of the diagnosis in hematologic neoplasias. The results help in establishing the diagnosis. Most importantly, however, chromosome analysis provides prognostic information which is derived from the karyotype of the malignant cells. The neoplasia-associated chromosome aberrations are acquired genetic alterations and are limited to the malignant cells. Thus, the non-malignant cells in patients with hematologic neoplasias are cytogenetically normal.

Material

For chromosome analysis, bone marrow is preferred to peripheral blood because malignant cells are present at higher percentages and have a higher proliferative activity. If bone marrow cannot be obtained, the cytogenetic analysis may be done on peripheral blood cells. Since viable cells are needed for metaphase cytogenetics, the bone marrow should be shipped into the cytogenetic laboratory within 24 h. The cells must not be frozen.

Conventional Chromosome Analysis Using Banding Techniques

A sufficient number of good quality metaphases is needed for chromosome analysis. Bone marrow cells are arrested in the metaphase by the addition of colchicine either directly after drawing the sample or after short-term cultivation (24–72 h). To maximize the gain of metaphases the leukemic cells can be stimulated by cytokines during cultivation. The addition of hypotonic potassium chloride solution swells the cells and they are fixed in multiple steps by the use of a methanol acetic acid solution. Then the cells are dropped on to glass slides. To allow the unequivocal identification of each chromosome, a banding technique must be applied. The most frequently applied banding techniques are G- (Giemsa-), Q- (Quinacrin-), and R- (reverse) banding. These techniques lead to the appearance of light and dark bands on the chromosomes which are specific for each chromosome and allow an unequivocal identification of each chromosome. To allow a valid report a complete analysis of 20–25 metaphases is required according to the respective international consensus.

Nomenclature – ISCN Cytogenetics

Chromosomes are classified according to their size, the position of the centromere (which divides both arms of the chromosomes), and the characteristic banding patterns. Each chromosome has a short arm (p) and a long arm (q). Based on the banding pattern, each chromosome is divided into regions and bands, which are numbered from the centromere to the telomere. The internationally accepted cytogenetic system of nomenclature (ISCN: International System of Cytogenetic Nomenclature) allows the exact description of all numeric and structural aberrations in a karyotype formula. The karyotype formula in the first place gives the number of chromosomes followed by the sex chromosomes. Thus, the normal female karyotype is 46,XX and the normal male karyotype is 46,XY.

The numeric chromosome aberrations include monosomies (loss of a chromosome) and trisomies (gain of a chromosome). Furthermore, the complete set of chromosomes may be multiplied. In normal cells a double set of chromosomes (diploid chromosome set) is

present. Three- and fourfold sets of chromosomes are designated as triploid and tetraploid.

The most frequently occurring structural chromosome aberrations are deletions (losses of parts of chromosomes), translocations (exchange of parts of chromosomes between different chromosomes), inversions (twisting of a part of a chromosome by 180°), and isochromosomes (a chromosome consisting of either two short arms or two long arms while the respective other arms are lost).

In the karyotype formula a gain of a chromosome is indicated by a "+" and a loss of a chromosome is indicated by a "–"; e.g. 47,XX,+8 is a trisomy of chromosome 8 and 45,XY,−7 is a monosomy of chromosome 7. There are abbreviations for structural chromosome aberrationens which are internationally agreed on, e.g. "t" for translocation and "inv" for inversion: t(8;21)(q22;q22) indicates a break in chromosome 8 at band q22 and a break in chromosome 21 at band q22 as well as an exchange of the fragments between both chromosomes. Different chromosomes and breakpoints in different chromosomes are divided by in the karyotype formula, while breaks within a chromosome are listed without a separator, e.g. inv(16)(p13q22): breaks occurred in chromosome bands p13 and q22 of a single chromosome 16 with a fragment being twisted by 180°. Another example is del(5)(q13q31): breaks occured in the bands q13 and q31 of a single chromosome 5 with a loss of the area between q13 and q31.

Chromosome aberrations are designated clonal if an identical structural aberration or a gain of a chromosome is observed in at least two metaphases or if a loss of the same chromosome is observed in at least three metaphases.

Comparative Genomic Hybridization (CGH)

Comparative genomic hybridization allows a comprehensive analysis of the tumor genome with regard to over- and underrepresented DNA sequences (losses and gains of chromosomes, deletions, amplifications). The technique is based on the simultaneous staining of both test DNA of a healthy volunteer and tumor DNA using two different fluorescent dyes. Identical aliquots of both DNA samples are then mixed and hybridized on normal metaphases. Differences in the numbers of copies in different sequences between normal and tumor DNA are detected by the quantification of the ratio of fluorescence intensity (tumor DNA to normal DNA) in each region of the normal metaphase chromosomes. Balanced translocations, inversions, or other aberrations which are not accompanied by a change in the number of copies are thus not detectable by CGH. CGH may play an important role particularly if a chromosome analysis is not possible, e.g. if living tumor cells are not available or if tumor cells do not proliferate in vitro.

Fluorescence In Situ Hybridization (FISH)

The FISH technique relies on the hybridization of DNA probes which identify specific chromosomal structures. Probes can be used which are specific for the centromeric region of particular chromosomes, for genes, or for complete chromosomes. The DNA of both the applied probe and of the patient sample are denaturated, i.e. both DNA strands of the double helix are separated. During the following renaturation, the DNA probes attach to the complementary section of the patient DNA (hybridization). The DNA probes are either directly conjugated to a fluorescent dye or are analyzed using fluorescence conjugated antibodies. The respective chromosome structures therefore are assessable as fluorescence signals.

A significant advantage of the method lies in its applicability not only to metaphases but also to interphase nuclei. A disadvantage is that information is obtained only on chromosomes and genes for which probes are used.

The role of the FISH technique differs between the different leukemia subgroups (see respective chapters).

Interphase FISH

Due to the multitude of different chromosome aberrations, which are observed particularly in acute leukemias, a screening based on FISH on interphase nuclei covers only a fraction of potentially present aberrations and therefore cannot substitute the classic chromosome analysis. However, if a specific question should be answered, e.g. the detection of the translocation t(15;17)(q22;q12) when acute promyelocytic leukemia is suspected, the FISH technique represents a fast and reliable method, providing a result within 4 h.

In follow-up assessments during therapy the FISH technique can be used for the detection of residual disease if at diagnosis aberrations have been found by chromosome analysis for which FISH probes are available. The sensitivity for this method is higher than for the chromosome analysis; however, it is lower than for PCR.

Metaphase FISH

In addition to the probes applicable to interphase nuclei, so-called chromosome painting probes can be applied to metaphases which specifically bind to the complete DNA of a chromosome. This technique is used mainly for the confirmation of the conventional chromosome analysis in difficult cases.

The 24-color-FISH method allows the display of all 22 different pairs of chromosomes as well as of the sex chromosomes in one single hybridization. It is applicable to metaphase chromosomes only and helps in identifying complex structural aberrations.

Molecular Genetics

PCR

PCR is the most frequently applied method to detect molecular genetic changes in leukemia. By using oligonucleotides specific for certain sequences, regions of interest can be amplified and further analyzed. All the following methods are based on this standard method.

RT-PCR

In specific cases, especially when fusion genes are analyzed in which breakpoints are distributed over a wide genomic range, it is advantageous to use cDNA as the template for PCR.

Nested PCR

Using this method, both the sensitivity and specificity of a PCR can be increased. In hematology, it is frequently applied to detect small amounts of molecules, i.e. mainly for the detection of minimal residual disease. For a nested PCR a fraction of a finished PCR is used for a new PCR. Oligonucleotides that hybridize within the first amplificate are used as primers. This additional amplification increases the sensitivity significantly. Depending on the type of mutation and the initial material, one malignant cell in 10^4 to 10^8 normal cells can be detected. Thus, this is the most sensitive method currently available for most sequences targeted for the detection of minimal residual disease (MRD). However, due to the high sensitivity, this method also carries the highest risk of contamination. In order to minimize this risk, different precautions must be applied and control reactions must be performed in parallel.

Real Time PCR

Another method for the amplification and detection of PCR products is the real time PCR. Different from other methods of detection, this is not an end-point analysis, but the measurement is performed during the phase of PCR when a logarithmic amplification of PCR products occurs. This allows an exact quantification of the target sequences in the material which is assessed. The method is based on the addition of fluorescence-conjugated probes to the specific primers required for the PCR. These probes hybridize during the running PCR with the continuously increasing amplification products and release fluorescence signals, which are detected in an optical device specifically constructed for this approach. Thus, an increase of fluorescence intensity occurs during PCR. The time point (PCR cycle) at which a fluorescence higher than baseline is detected for the first time in a sample correlates with the number of targeted molecules in the sample. The fluorescence intensity of the targeted gene is normalized to a constantly present gene or transcript, based on which the number of malignant cells present in the sample can be calculated. Also, for this method of detection, the application of specific PCR machines equipped with optical devices is needed. Using these machines it is also possible to conventionally detect PCR products; however, the strength of real time PCR is its capacity for an exact quantification for follow-up analyses.

Mutation Screening

Different methods are available, which allow the screening of defined gene regions for mutations without the necessity of sequencing. Some examples are listed below:

Heteroduplex Analysis

As the first step in this analysis, a region with a suspected mutation is amplified by PCR. Since in man two alleles of each locus are normally present, two different PCR products occur. After completion of the PCR, these are denaturated (conversion to single-strand state) and then renaturated. Consequently, four renaturated products are possible: a normal double strand, a mutated double strand, and two heteroduplices, in which one base, respectively, is not paired to the other strand, i.e. a mismatch is present. These heteroduplices are detectable on specific gels or by a dHPLC device (►WAVE).

SSCP Analysis (Single Stranded Conformation polymorphism Analysis)

In this analysis denaturated single strands are separated using a non-denaturating surrounding by gel or capillary electrophoresis. It takes advantage of the fact that mutated single strands lead to other secondary structures as compared to unmutated ones and thereby have different features within the electric field.

Melting Point Analysis

In addition to the classic real time PCR, a further step is applied. Fluorescence-marked probes, which lie on a potentially mutated area, are slowly melted off the PCR products. In case of a mutation under the probes, a so-called mismatch occurs. Thus, the probes fit less well in the wildtype and faster melt off the PCR product.

Fragment Analysis (Genescan)

As an alternative to the gel analysis, PCR fragments can be detected by a fragment analysis which is also called Genescan-analysis. This method is performed using sequencing machines equipped with special software. To allow the detection, one of the two PCR primers is conjugated to a fluorescent dye. The advantage of this method is its capacity to determine the length of the amplificates exactly. In addition, with some limitations, it is possible to quantify the amplificates in comparison with another gene, which is generally the unmutated wild type. The method needs more extensive equipment

and resources, due to the use of primers conjugated to fluorescent dyes, as compared to the standard gel analysis. It is frequently used in the context of multiplex PCR reactions, i.e. reactions employing multiple primers with the parallel amplification of up to four different loci. An example of this application is the chimerism analysis after allogeneic stem cell transplantation with microsatellite markers.

Sequence Analysis

The exact delineation of the base sequence of a region of a gene is frequently needed. To allow this, sequencing reactions are performed with nucleotides being integrated during PCR based reactions. Each of the four different nucleotides is labeled to a fluorescent dye and a dideoxy group, respectively, and leads to a chain stop in the PCR reaction. Subsequently, the products are separated according to their length on a matrix (e.g. a gel or a polymer) and are detected by an optical device allowing the exact determination of the base sequence.

For some gene regions the sequence analysis is feasible as a primary approach in a limited number of patients. In case of a screening of multiple gene regions in one patient or of one gene in many patients, the direct sequencing requires large resources. In this instance, different screening approaches are available for the assessment of mutations in particular gene regions without the need for a full sequence analysis.

Diagnostic Procedures in Leukemia

Diagnostic Workup of AML

Cytomorphology

The examination of both bone marrow and peripheral blood smears by cytomorphology and cytochemistry is necessary to diagnose AML according to FAB criteria (Table 1). Based on the FAB (French-American-British) classification, which was established in 1976 and revised in 1985, AML is subdivided into 11 morphologically different groups. Some of these groups correlate with distinct genetically defined forms of AML;

Leukemia Diagnostics. Table 1 Definition of subgroups of AML according to FAB, association with genetic aberrations

FAB-Subtype	FAB-criteria				Association with		
	Granulopoiesis	Monopoiesis	Erythropoiesis	Immunologic marker	Cytogenetics	Molecular genetics	Frequency
M0	<10% POX <3%	<20%	<50%	Myeloid positive Lymphoid negative			
M1	<10% POX >3%	<20%	<50%		t(8;21)	AML1-ETO	1.7%
M2	>10% maturation	<20%	<50%		t(8;21)	AML1-ETO	12.5%
M3	Hypergranular Auer-rods	<20%	<50%	HLA-DR negative	t(15;17)	PML-RARA	98%
M3v	Microgranular Monocytoid nuclei	<20%	<50%	HLA-DR negative	t(15;17)	PML-RARA	
M4	>20%	>20%	<50%				
M4Eo	>20% Abnormal eosinophils	>20%	<50%		inv(16)/t(16;16)	CBFB-MYH11	100%
M5a	<20%	>80% Immature	<50%		11q23 Aberr.	MLL-Fusion	31%
M5b	<20%	>80% Mature	<50%		11q23 Aberr.	MLL-Fusion	17%
M6	>30% of NEC are blasts	variable	>50%				
M7	>30% megakaryoblasts	variable	<50%	CD41/CD61 positive			

Bold = typical finding; NEC = non-erythroid cells.

however, most of them do not. Increasing insights into the biology of AML as well as the identification of specific chromosome aberrations prompted the WHO to suggest a new classification in 2001, based on biology and cytogenetics.

The FAB classification subdivides AML based on cytomorphology and cytochemistry into different subgroups. With only a few exceptions, the diagnosis of AML requires bone marrow blasts of at least 30% of all nucleated cells; at least 3% of the blasts must react positive for myeloperoxidase. The threshold for blasts is 20% in the WHO classification.

The AML classification suggested by the WHO combines the methods previously used for the FAB classification, i.e. cytomorphology, cytochemistry, and immunophenotyping, with cytogenetics and molecular genetics as well as with clinical parameters (Table 2). The genetic characterization of AML, in particular, not only allows a classification according to the prognosis, but provides the basis for the definition of distinct subgroups with recurrent balanced translocations. Thus, the WHO classification divides AML into four groups:

1. AML with recurrent cytogenetic aberrations
2. AML with myelodysplasia-associated features
3. Therapy-related AML and MDS
4. AML not otherwise categorized

Leukemia Diagnostics. Table 2 WHO classification of AML

AML with recurrent cytogenetic aberrations
AML with t(8;21)(q22;q22), AML1(CBF-alpha)-ETO
Acute promyelocytic leukemia (AML with t(15;17)(q22;q11-12) and variants, PML-RARA)
AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q22), CBFB-MYH11)
AML with 11q23(MLL)-fusions
AML with multilineage dysplasia
AML following a myelodysplastic syndrome
AML without antecedent myelodysplastic syndrome
AML and myelodysplastic syndrome, therapy-related
Alkylating agent-related
Topoisomerase type II inhibitor-related (some may be lymphoid)
Other types
AML not otherwise categorized
Minimally differentiated AML
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic or monocytic leukemia
Acute erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis

The first group comprises biologically and clinically largely consistent subgroups of AML. The WHO classification thus represents a significant development of the FAB classification. The independent biologic and prognostic impact of groups 2 to 4, however, has not been demonstrated yet. Importantly, the subdivision of group 4 essentially reflects the FAB classification. A further new definition in the WHO classification is the deletion of MDS-RAEB-t and the reduction of the limit between MDS and AML from 30 to 20% bone marrow blasts.

Immunophenotyping

AML is diagnosed on the basis of cytomorphology and cytochemistry. The only exceptions are AML M0 and AML M7 in which immunophenotyping is also necessary. Furthermore, some genetically defined subgroups feature typical although not fully specific immunophenotypes which are therefore used to guide further diagnostics only.

According to the abundance of promyelocytes, the AML M3 shows characteristic findings in the scatter plot and in addition is negative for HLA-DR and has a strong unspecific fluorescence signal. However, some of these findings can be present also in AML M2.

Typically, in AML M2 with t(8;21) there is an aberrant coexpression of CD19 and CD56; this can also be found in some AML without t(8;21).

The same is true for the immunophenotype of AML M4Eo with positivity for CD2 and an asynchronous coexpression of CD15 and CD34 which, however, may be present in other AML subtypes as well.

AML M0 is undifferentiated and lacks myeloperoxidase positivity in cytochemistry. Thus, the diagnosis cannot be made based on morphology alone. Immunophenotyping allows the distinction from ALL based on the expression of CD13, CD33, and CD117 and other myeloid antigens and at the same time a lack of lymphatic antigens or, respectively, a low lymphatic score not sufficient to diagnose a biphenotypic acute leukemia. In half of the cases an expression of MPO can be detected although cytochemistry shows negativity for myeloperoxidase.

AML M7 is negative for myeloperoxidase in cytochemistry. The expression of CD41 or CD61 is detected flow cytometrically. Due to the frequently occurring myelofibrosis a cytologic examination may not be possible in some cases.

Monitoring of Minimal Residual Disease (MRD)

In patients with AML a significant prognostic impact of immunologically determined levels of MRD has been demonstrated. Due to the similarity of immunophenotypes between AML and normal bone marrow

in some cases early work focused on highly aberrant leukemia-associated aberrant immunophenotypes (LAIPs) including mainly the aberrant expression of lymphoid markers (CD19, CD7, CD56) and the asynchronous expression of progenitor cell and differentiation markers (e.g. CD34+CD117-CD15+).

For MRD levels determined after the achievement of complete remission and completion of consolidation therapy, a significant impact on both relapse-free and overall survival has been demonstrated, which has been largely independent of other prognostic parameters.

Newer studies have aimed at extending the immunologic MRD analysis to patients with less aberrant LAIPs and thus at the applicability of the method to each patient with AML. By the use of a comprehensive panel of monoclonal antibodies this task has been accomplished with a median sensitivity of 0.05%. Further analyses using this approach have confirmed the prognostic impact of MRD levels. Thus, as early as on day 16 of the induction therapy, patients can be divided into two prognostically differing groups based on the MRD level (2-year event-free survival: 53% vs. 19%, $p < 0.0001$; 2-year overall survival: 58% vs. 43%, $p = 0.0133$). Furthermore, the MRD levels determined after both achievement of complete remission and completion of consolidation therapy, demonstrate a similar prognostic impact, which is independent of other parameters.

In patients with AML, thus, multiparametric flow cytometry allows the quantification of MRD in virtually all cases, while molecular techniques allow this in half of the cases. Current studies will determine the role and place of both methods in AML. Table 3 shows a comprehensive panel of antibodies, which can be used to define a LAIP in AML.

Cytogenetics

More than 50 recurrent structural chromosome aberrations have been described in AML. The karyotype of the leukemic blasts is the most important independent prognostic parameter in AML.

50 to 75% in adult AML and 75 to 85% in childhood AML carry clonal chromosome aberrations. The incidences of the respective chromosome aberrations are age-dependent. However, the prognostic relevance of the karyotype is largely independent of age.

A variety of characteristic chromosome aberrations are known in AML, which define distinct entities with typical morphology and clinical course. The newly defined WHO classification of AML includes cytogenetic aberrations as central classification criteria. Thus, the classification is primarily based on specific cytogenetic rearrangements:

- AML with t(8;21)(q22;q22), AML1/ETO
- acute promyelocytic leukemia/AML M3/M3v with t(15;17)(q22;q11–12) and variants, PML/RARA
- AML with abnormal bone marrow eosinophils and inv (16)(p13q22) or t(16;16)(p13;q22); CBFB/MYH11
- AML with 11q23(MLL)-anomalies

Based on cytogenetics and pathogenesis AML can be separated into three groups according to the karyotype:

1. AML with normal karyotype (40 to 45%).
2. AML with balanced chromosome aberrations (20 to 25%), the most frequent ones being t(8;21)(q22;

Leukemia Diagnostics. Table 3 AML panel for the detection of leukemia-associated aberrant immunophenotypes (LAIP)

Combination	FITC	PE	ECD	PC5	PC7
1	Isotype	Isotype	Isotype	Isotype	Isotype
2	CD64	CD87	CD56	CD4	CD45
3	CD65	CD2	CD13	CD34	CD45
4	CD9	HLA-DR	CD33	CD34	CD45
5	CD11b	CD116	CD117	CD34	CD45
6	CD34	CD56	CD33	CD19	CD45
7	CD15	CD7	CD33	CD34	CD45
8	CD36	CD61	CD235a	CD14	CD45
9	CD4	7.1	CD13	CD14	CD45
10	CD38	CD135	CD90	CD34	CD45
11	CD15	CD133	CD117	CD34	CD45
12	Isotype	Isotype	Isotype	Isotype	Isotype
13	MPO	LF	cCD33	cCD34	cCD45
14	TdT	cCD22	CCD79a	cCD3	cCD45

C = cytoplasmic; combinations 12 to 14: cytoplasmic analysis; 7.1 = antibodies for the detection of the NG2 antigen (associated with 11q23 aberrations).

q22), inv(16)(p13q22)/t(16;16)(p13;q22), t(15;17)(q22;q12), and 11q23-rearrangements involving the MLL gene. Less frequent ones include inv(3)(q21q26), t(6;9)(p23;q34), and t(3;21)(q26;q22).
3. AML with unbalanced karyotype abnormalities (30 to 40%) including trisomies (e.g. +8, +11, +13, +21), monosomies (e.g. −7), and deletions (e.g. 5q-, 9q-) as well as the large group of complex aberrant karyotypes (3 and more chromosome aberrations, but none of the recurrent balanced aberrations).

With regard to prognosis AML are divided into three groups based on the karyotype:

1. Favorable karyotype: t(15;17)(q22;q12), inv(16)(p13q22)/t(16;16)(p13;q22), t(8;21)(q22;q22)
2. Intermediate karyotype: normal karyotype, all aberrations not grouped into 1 or 3
3. Unfavorable karyotype: complex aberrant karyotype, -5/5q-, -7/7q-, 17p aberrations, 11q23/MLL-rearrangements, inv(3)(q21q26), t(6;9)(p23;q34)

For a variety of infrequent karyotype aberrations the prognostic impact has not been clearly defined yet. Significant insights into correlations between genetic alterations and response to therapy led to the approach of increasingly selecting therapy according to the karyotype.

The prognostic relevance of the karyotype is valid within all age groups as well as in both de novo and therapy-associated AML.

Fluorescence In Situ Hybridization

In the diagnostic setting of AML, the FISH analysis is used mainly in addition to the classical chromosome banding analysis. Since the karyotype aberrations occurring in AML are highly heterogeneous, even a large-scale FISH screening on interphase nuclei would cover only a small part of these aberrations. Thus, FISH cannot replace the classic chromosome banding analysis.

However, in targeting a specific alteration, e.g. the translocation t(15;17)(q22;q12) (on the molecular level the rearrangement of the PML and RARA genes) when an acute promyelocytic leukemia is suspected, the FISH technology on interphase nuclei provides a fast and valid result within 4 h. Furthermore, the frequently occurring genetic aberrations, t(8;21)(q22;q22), inv(16)(p13q22) and rearrangements of the MLL gene are used for stratification in the newly defined WHO classification of AML. These alterations are detectable by FISH as are the frequently occurring deletions, e.g. deletions on the long arm of chromosomes 5 or 7, and monosomies (−7) and trisomies (+8, +11, +13, +21). A large portion of AML with complex aberrant karyotype is detectable by FISH with the help of a medium-scale set of probes.

The so-called chromosome painting using 1 to 3 or even 24 colors (24-color-FISH) on metaphase chromosomes is applied in addition to the classic chromosome analysis, if the karyotype cannot be fully resolved on the basis of the chromosome analysis after classic banding, which is not uncommon in complex aberrant karyotypes.

FISH can further be used during the course of therapy for the detection of residual disease. It is more sensitive and specific compared to cytomorphology or chromosome banding analysis; however, it is less sensitive compared to real time PCR and immunophenotyping. FISH has its role in the detection of residual disease, mainly in AML with complex aberrant karyotype, since this subgroup in general lacks genetic alterations detectable by PCR.

Molecular Genetics

Detection of Fusion Genes

The detection of fusion genes using RT-PCR plays an important role in the molecular diagnosis of AML. Reciprocal chromosome rearrangements are found in about 25% of all AML. The molecular correlates and fusion genes, respectively, of most reciprocal cytogenetically detectable rearrangements, are known. The most frequently occurring reciprocal translocations t(15;17), t(8;21), and inv(16)/t(16;16) are represented by the fusion genes PML-RARA, AML1-ETO, and CBFB-MYH11, respectively, on the molecular level. Rearrangements of the MLL gene are present in 5% of all AML with more than 50 different translocation partner genes. Furthermore, some reciprocal rearrangements occur infrequently and are present in less than 1% of AML; however, they may be useful for diagnostic purposes and defining prognosis. The most frequent fusion genes in AML are listed here.

The following fusion genes are detectable by RT-PCR (Table 4):

AML1-ETO, AML1-EVI1, BCR-ABL, ETV6-MDS/EVI1, CBFB-MYH11, PML-RARA, RARA-PML, MLL-ENL, MLL-ELL, MLL-AF6, MLL-AF9, MLL-AF10, MLL-AF1q21, MLL-AF17, MLL-MSF, MLL-p300, CALM-AF10, MLL-PTD, FUS-ERG, EFG-FUS, CHIC2-ETV6, NUP98-HOX9, DEK-CAN, MOZ-CBP.

For the fusion genes PML-RARA, AML1-ETO, and CBFB-MYH11 it has been demonstrated that the quantification of their expression at diagnosis is prognostically relevant. For all other fusion genes, the quantification at diagnosis is also useful since this evaluation may be used as the starting point for assessment during follow-up.

Detection of Molecular Mutations

In recent years a variety of mutations and small gene rearrangements, which are not detectable cytogenetically, have been described. Nonetheless, they play an important role in molecular diagnosis and estimation of prognosis in AML.

Leukemia Diagnostics. Table 4 Translocations and respective fusion genes in AML

			Frequency	
Cytogenetics	Fusion gene	Subtype	Children	Adults
t(8;21)(q22; q22)	*AML1-ETO*	M2/(M1)	10–15%	8–12%
inv(16) (p13q22)	*CBFβ-MYH11*	M4eo	6–12%	8–12%
t(15;17)(q22; q12)	*PML-RARA*	M3/M3v	8–15%	8–10%
t(6;11)(q27; q23)	*MLL-AF6*	M4/M5a	2–5%	<1%
t(9;11)(p22; q23)	*MLL-AF9*	M5a	8–10%	1–2%
t(10;11)(p13; q23)	*MLL-F10*	M5a	<1%	1–2%
t(11;19)(q23; p13)	*MLL-ENL*	M5a	<1%	<1%
t(11;19)(q23; p13)	*MLL-ELL*	M5a	<1%	<1%
t(3;21)(q26; q22)	*AML1-EVI1*	–	1%	<1%
t(6;9)(p23; q34)	*DEK-CAN*	M1/M2/M4	1–2%	<1%
t(8;16)(p11; p13)	*MOZ-CBP*	M4/M5	<1%	<1%
t(1;22)(p13; q13)	*OTT-MAL*	M7	2%	–
t(7;11)(p15; p15)	*HOXA9-NUP98*	M2	–	<1%
t(10;11)(p13; q14)	*CALM-AF10*	M0/M1/M5	–	<1%
t(16;21)(p11; q22)	*FUS-ERG*		–	<1%

Mutation	Most frequent subtypes	Frequency (total)	Prognosis
MLL-PTD	Normal karyotype (11%)	6.5%	Unfavorable
	trisomy 11 (20 to 50%)		
FLT3-LM	Normal karyotype (40%)	23%	Unfavorable
	t(15;17) (35%)		
FLT3-TKD	All AML	6.5–7%	Dependent on additional defects
KITD816	t(8;21) (12%)	1.5%	Unfavorable
KITexon8	inv(16) (10%)	<1%	Unfavorable
NRAS	inv(16) (45%)	10%	Intermediate
	inv(3)/t(3;3)		
KRAS	inv(16); t(8;21) (5–20%)	<1%	
	(in childhood AML)		
AML1	M0 (22%), trisomy 21 (30%), trisomy 13 (80%)	5%	Unfavorable
CEBPA	Normal karyotype (18%)	10%	Favorable

Among these molecular mutations are the partial tandem duplications of the MLL gene (MLL-PTD) and the length mutation of the FLT3 gene. These mutations are found mainly in AML with a cytogenetically normal karyotype and are associated with an unfavorable prognosis. The FLT3-LM is found in 10 to 15% of all childhood AML and in 20 to 25% of all adult AML. Furthermore, in another 6 to 7% point mutations are found within the tyrosine kinase domain of the FLT3 gene (TKD mutations). Thus, with a total of about 30% FLT3 is one of the most frequently mutated genes known so far in AML.

In an additional 10% of all AML, mutations are found in the transcription factors CEBPA and AML1, which play an important role in hematopoiesis. Furthermore, 30% of all AML and 55% of AML with normal karyotype, carry NPM1 mutations. Mutations of both CEBPA and NPM1 are considered prognostically favorable.

Some point mutations like those of KIT and RAS are not specific for AML; however, they contribute significantly to leukemogenesis, in particular in cooperation with AML1-ETO and CBFB-MYH11. Accordingly, a two-hit-hypothesis has been suggested for leukemogenesis. Mutations of tyrosine kinase genes like ABL, FLT3, and KIT as well as RAS mutations are designated type I mutations, which lead to an increased proliferation of hematopoietic cells. Fusion genes and mutations of transcription factors are designated type II mutations, which lead to a stop in differentiation. Only the cooperation of both types of mutations results in the clinically evident acute leukemia.

A variety of methods are available for the detection of these molecular mutations. Among others these comprise conventional RT-PCR (FLT3-LM, MLL-PTD), ►GeneScan analysis (FLT3-LM, NPM1), ►melting curve analysis (FLT3-TKD, NPM1, NRAS, KITD816), dHPLC/WAVE (FLT3, AML1, CEBPA, NPM1, KIT), and consequently sequence analysis (for all mutations).

In addition to the prognostic relevance in AML, these molecular markers may be used as targets for the PCR-based detection of minimal residual disease.

Monitoring of Minimal Residual Disease (MRD)

The detection of minimal residual disease using PCR-based methods is feasible for all markers with a sensitivity between 1:100 and 1:1,000. The methods mainly applied are conventional and nested RT-PCR, fragment analysis, and WAVE analysis.

An even higher sensitivity (1:10,000 to 1:1,000,000) is achieved by "real time PCR." This method is established for the fusion transcripts: AML1-ETO (applicable in 7–10% of all AML cases), CBFB-MYH11 (7–10%), PML-RARA (7–10%), DEK-CAN (1%), different MLL translocations (5%), MLL-PTD (6%), and NPM1 mutations Typ A, B, and D (25%). Furthermore, it is possible to build patient-specific real-time PCR assays for the FLT3-LM (23%) and rare NPM1 mutation types (5%).

Diagnostic Workup of ALL

Cytomorphology

Cytomorphology and cytochemistry are used to diagnose bone marrow blasts negative for myeloperoxidase and non-specific esterase in ALL; however, the exact diagnosis and further subclassification rely on immunophenotyping. The exception is the typical L3-morphology of blast cells in Burkitt's lymphoma and mature B-ALL.

Immunophenotyping

Acute lymphoblastic leukemias (ALL) are grouped into B-precursor- and T-precursor-leukemias, based on the immunophenotype. They are further subdivided according to the degree of maturation of the leukemic blasts into Pro-B-ALL, common-ALL, Pre-B-ALL, and mature B-ALL, and into Pro-T-ALL, Pre-T-ALL, cortical T-ALL, and mature T-ALL, respectively. In general, in case of the respective morphologic findings (negativity for MPO) a B-precursor-ALL is diagnosed if both cCD22 and CD19 are expressed. A T-precursor-ALL is present if c/sCD3 and CD7 are expressed. The definition of ALL with aberrant expression of myeloid antigens as a separate entity is not preferred since these findings in most cases are associated with genetically defined and clinically relevant aberrations. Table 5 provides the antigen expression patterns of the respective entities.

Monitoring of Minimal Residual Disease (MRD)

In T-precursor-ALL the coexpression of cCD3 and TdT is mainly useful as LAIP. In B-precursor-ALL it is the coexpression of CD19 and CD10. Furthermore, in many cases the aberrant coexpression of myeloid antigens as well as the expression of CD34 can be used.

The first area in which the significant prognostic impact of immunologically determined MRD levels has been demonstrated has been after the achievement

L

Leukemia Diagnostics. Table 5 Classification of ALL

	B-precursor-ALL				T-precursor-ALL			
Antigen	Pro-B-ALL	c-ALL	Pre-B-ALL	Mature B-ALL	Pro-T-ALL	Pre-T-ALL	Cortical T-ALL	Mature T-ALL
cCD22	+	+	+	+	−	−	−	−
CD79α	+	+	+	+	−	−	−	−
CD19	+	+	+	+	−	−	−	−
CD24	+/−	+	+	+	−	−	−	−
CD20	−/(+)	+/−	+/−	+	−	−	−	−
sIg	−	−	−	+	−	−	−	−
cIgM	−	−	+	+	−	−	−	−
cCD3	−	−	−	−	+	+	+/−	−
sCD3	−	−	−	−	−	−	−/+	+
CD7	−	−	−	−	+	+	+	+
CD5	−	−	−	−	−	+/−	+	+
CD2	−	−	−	−	−	+/−	+	+
CD1a	−	−	−	−	−	−	+	−
CD4	−	−	−	−	−	−/+	+/−	+/−
CD8	−	−	−	−	−	−/+	+/−	+/−
CD10	−	+	+/−	+/−	−/+	−/+	−/+	−
HLA-DR	+	+	+	+	−/+	−/+	−	−
CD34	+	+	+	−/+	−/+	−/+	−	−
TdT	+	+	+	−/+	+	+	+	+/(−)

c=cytoplasmic; s=membrane.

of complete remission in childhood ALL: the detection of residual leukemic cells was associated with a significantly increased risk of relapse. This association was independent of other prognostic parameters. Further analyses have demonstrated that as early as on day 19 of the induction therapy MRD levels are equally significant with regard to prognosis.

These results have been reproduced in adult ALL. Thus, patients with low-level MRD after completion of induction therapy have a longer relapse-free survival. Also in this context, the impact of MRD was independent of other prognostic parameters.

Multiparameter flow cytometry is, compared to molecular techniques, feasible for the quantification of MRD in virtually all patients with ALL. Current trials will define the respective roles of both methods, based on direct comparisons.

Cytogenetics

A large number of chromosomal aberrations with prognostic and therapeutic relevance have been described. On the one hand, ALL are subdivided into ploidy groups based on the karyotype, i.e. according to the number of chromosomes (Table 6). On the other hand, they are grouped according to structural aberrations (Table 7). The most frequent aberration in adult ALL is the t(9;22)(q34;q11) which is associated with a very unfavorable prognosis. With the availability of the tyrosine kinase inhibitor, imatinib, the detection of this Philadelphia-translocation and of its molecular genetic correlate, the BCR-ABL rearrangement, is even more important as treatment with this specific drug improves outcome. In addition, the detection of translocations involving the MLL-gene, which is located on the long arm of chromosome 11, is prognostically important and requires specific therapeutic approaches.

Cases with a t(8;14)(q24;q32), a t(2;8)(p12;q24), or a t(8;22)(q24;q11) – all of which come along with rearrangements of the CMYC gene – have a high chance of cure in case of the application of a specific therapeutic protocol which significantly differs from protocols applied in cases with other ALL subtypes.

Leukemia Diagnostics. Table 6 Frequencies of groups according to ploidy in adult ALL

Group	Frequency (%)
Normal karyotype	26–34
Hypodiploidy <46	2–8
Pseudodiploidy	7–59
Hyperdiploidy 47–50	7–17
Hyperdiploidy > 50	4–9
nearly triploid	3
nearly tetraploid	2

Fluorescent In Situ Hybridization

In the diagnostic setting of ALL FISH analysis is used mainly in addition to the classic chromosome analysis. Since multiple karyotype abnormalities occur in ALL, even an extensive FISH "screening" using interphase nuclei would detect only a part of these abnormalities. Therefore, the chromosome analysis cannot be replaced by FISH.

However, in case of focusing on a specific aberration, the FISH technique on interphase nuclei represents a fast and reliable method, giving the result within 24 h. The genetic aberrations occurring frequently in ALL, i.e. the Philadelphia-translocation t(9;22)(q34;q11) (rearrangement of BCR and ABL on the molecular level), an MLL-rearrangement, or a CMYC-rearrangement, all of which have therapeutic implications, can be identified using FISH on interphase nuclei.

A FISH-screening targeting the most frequent aberrations is useful in particular in cases with no valid result of the chromosome analysis. This should be considered also in cases with a normal karyotype with the selection of probes according to the immunophenotype.

The so-called "chromosome painting" with 1 to 3 or 24 colors (24-color-FISH) on metaphase-chromosomes is applied in addition to the classic chromosome analysis if the karyotype cannot be fully described after the classic banding approach, e.g. in complex aberrant karyotypes.

Furthermore, the FISH method can be used for the detection of residual disease during the course of therapy. It is more sensitive and more specific than cytomorphology; however, it is less sensitive than real time PCR and immunophenotyping. The FISH method

Leukemia Diagnostics. Table 7 Frequent chromosomal aberrations in adult ALL

Aberration		Gene	Frequency
t(1;19)(q23;p13)	Pre-B-ALL	E2A-PBX1	3%
t(4;11)(q21;q23)	Pro-B-ALL	MLL-AF4	6%
t(9;22)(q34;q11)	c-ALL	BCR-ABL	25–30%
t(8;14)(q24;q32)	Mature B-ALL	IGH-MYC	5%
t(10;14)(q24;q11)	T-ALL	HOX11-TCR	3%
t(12;21)(p13;q22)	Pre-B-ALL	ETV6-ALL1	<1%
9p	T, Pre-B	$p16^{INK4A}$	15%
6q	c, Pre-B,T		6%
14q11	T-ALL	TCR	6%

is used for the detection of residual disease mainly in ALL with unbalanced karyotypes as these cases lack genetic alteration detectable by PCR.

Molecular Genetics

Two types of genetic alterations are present in lymphatic leukemias and lymphomas: Somatic mutations which are involved in the pathogenesis and rearrangements of immunoglobulin genes and T-cell receptor genes as markers of clonality.

The molecular diagnostic approach in ALL primarily focuses on the detection of high-risk cases with t(9;22) and t(4;11) in which an RT-PCR for BCR-ABL and MLL-AF4 is performed as a substitute or supplement to cytogenetics and FISH.

In contrast, the ETV6-AML1-rearrangement, which occurs frequently in childhood ALL, and is associated with a favorable prognosis, can be identified exclusively on the molecular level and/or using FISH.

Activation of the MYC gene due to various Ig rearrangements (Tables 8 and 9) is found in 4% of adult ALL representing the leukemic form of Burkitt's lymphoma. The molecular detection is accomplished by Southern blot. The use of FISH makes this diagnostic step simpler and faster; however, it requires the smear of intact cells.

Between 4 and 7% of all T-ALL cases feature rearrangements of the HOX11-gene (Tables 10 and 11). HOX11 codes for a transcription factor. The transforming action of overexpressed HOX11 leads to an immortalization of T-cells.

In cases without fusion genes a characterization of clonal Ig- and TCR-gene-rearrangements should be performed. These can be used as markers for residual disease during the course of therapy. Since the latter alterations are identified in DNA while fusion genes are analyzed in RNA it is important to asservate both DNA and RNA at the time of diagnosis.

Leukemia Diagnostics. Table 8 Fusion genes in ALL

			Frequency	
Cytogenetics	Fusion gene	Subtype	Childhood	Adult
t(1;19)(q23;p13)	*E2A-PBX1*	Pre-B-ALL	5–6%	3%
t(4;11)(q21;q23)	*MLL-AF4*	Pro-B-ALL	2%	6%
t(11;19)(q23;p13)	*MLL-ENL*	Pro-B-ALL	<1%	<1%
		Pre-T		
t(9;22)(q34;q11)	*BCR-ABL*	c-ALL	2–5%	25–30%
		Pre-B		
t(8;14)(q24;q32)	*MYC-IGH*	Mature B-ALL	3%	5%
t(10;14)(q24;q11)	*HOX11-TCR*	T-ALL	1%	3%
t(12;21)(p13;q22)	*ETV6-AML1*	c-ALL	10–20%	<1%

Leukemia Diagnostics. Table 9 MYC-rearragements in ALL

			Frequency	
Cytogenetics	Fusion gene	Subtype	Childhood	Adult
t(2;8)(p12;q24)	*IGK-MYC*	B-ALL	<1%	<1%
t(8;14)(q24;q23)	*IGH-MYC*	B-ALL	3%	2–4%
t(8;22)(q24;q11)	*IGL-MYC*	B-ALL	<1%	<1%

Diagnostic Workup of CML

Cytomorphology

Cytomorphology typically reveals hypercellularity in both peripheral blood and bone marrow with an increase of immature myeloid cells as well as of eosinophils and basophils.

Immunophenotyping

MFC is applied in CML only in case of blast crisis in order to delineate the cell lineage, i.e. lymphatic or myeloid.

Cytogenetics

In 90 to 95% of all patients, a Philadelphia translocation is present on the cytogenetic level: t(9;22)(q34;q11). This is a translocation between the long arm of a chromosome 9 and a long arm of a chromosome 22. The remaining patients carry so-called variant Philadelphia translocations or a normal chromosome set. The variant Philadelphia translocations are divided into simple ones, in which the long arm of chromosome 22 is translocated to another chromosome (and not to chromosome 9), and complex ones, in which further chromosome are involved besides chromosoms 9 and 22. Patients with CML and a normal karyotype carry a BCR-ABL-rearrangement detectable by both FISH and RT-PCR. By applying FISH and using metaphases the BCR-ABL-fusion gene is detected either on chromosome 22 or, less frequently, on chromosome 9. Submicroscopic insertions are discussed as underlying mechanisms which are not detectable by classic cytogenetics. This group of CML is designated Philadelphia-negative BCR-ABL-positive CML. The clinical and hematologic course of patients with classic and variant Philadelphia-translocation does not differ from the

Leukemia Diagnostics. Table 10 T-cell receptor(TCR)-B-rearragements in ALL

			Frequency	
Cytogenetics	Fusion gene	Subtype	Childhood	Adult
t(1;7)(p32;q35)	*TAL1-TCRβ*	T-ALL	<1%	<1%
t(1;7)(p34;q35)	*LCK- TCRβ*	T-ALL	<1%	<1%
t(7;9)(q35)(q32)	*TCRβ−TAL2*	T-ALL	<1%	<1%
t(7;9)(q35;q34)	*TCRβ−TAN1*	T-ALL	<1%	<1%
t(7;10)(q35;q24)	*TCRβ−HOX11*	T-ALL	<1%	<1%
t(7;11)(q35;p13)	*TCRβ−RHOM2*	T-ALL	<1%	<1%

Leukemia Diagnostics. Table 11 T-cell receptor(TCR)-α- and -δ-rearragements in ALL

			Frequency	
Cytogenetics	Fusion gene	Subtype	Childhood	Adult
t(1;14)(p23;q11)	*TAL1-TCRδ*	T-ALL	<1%	<1%
t(8;14)(q24;q11)	*MYC-TCRα*	T-ALL	<1%	<1%
t(10;14)(q24;q11)	*HOX11-TCRδ*	T-ALL	<1%	1–3%
t(11;14)(p13;q11)	*RHOM2-TCRδ*	T-ALL	<1%	2–3%
t(11;14)(p15;q11)	*RHOM1-TCRδ*	T-ALL	<1%	<1%
inv(14)(q11q32.1)	*TCRα−TCL1*	T-ALL	<1%	<1%
inv(14)(q11q32.3)	*TCRα−IGH*	T-ALL	<1%	<1%

course in patients with Philadelphia-negative BCR-ABL-positive CML.

During the progression into the accelerated phase or into blast crisis in 75 to 85% of the patients, additional karyotype aberrations occur. The most frequently occurring aberrations (so-called clonal evolution) are trisomy 8 (+8), isochromosome of the long arm of chromosome 17 (i(17)(q10)), an additional Philadelphia-chromosome (+der(22)t(9;22)), as well as trisomy 19 (+19). The occurrence of additional karyotype aberrations is a prognostically unfavorable parameter and precedes the clinical manifestation of a blast crisis by 2–6 months in most cases.

In CML follow-up, assessments during therapy are prognostically most important with consequences for the further management of the disease. The classic chromosome analysis represents the gold standard for the assessment of a cytogenetic remission. At least 20 metaphases should be analyzed to obtain a valid result. A complete cytogenetic remission (no Ph+ metaphases) is differentiated from a "partial" remission (1 to 32% Ph+ metaphases), a "minor" remission (33 to 66% Ph+ metaphases), a "minimal" remission (67–95% Ph+ metaphases), and no remission (≥95% Ph+ metaphases). The complete and the partial remission are grouped together as "major" remission.

During therapy with imatinib, the occurrence of karyotype aberrations has been observed in some patients in clones lacking a Philadelphia translocation. Most frequently this aberration is the trisomy 8. Further aberrations are monosomy 7 as well as different translocations. The clinical impact of this phenomenon is yet unclear and is being evaluated in clinical trials.

Fluorescence In Situ Hybridization

Using FISH on interphase nuclei, the presence of a BCR-ABL rearrangement can be detected or ruled out within 24 h. The FISH analysis has some advantages as compared to the classic chromosome analysis: Using this method a CML can be diagnosed and monitored even in patients with Philadelphia-negative BCR-ABL-positive CML. Furthermore, it is more sensitive compared to the classic chromosome analysis in detecting residual disease. FISH may be applied on interphase nuclei but also on metaphases. Using the so-called "Hypermetaphase-FISH"-technique, up to 500 metaphases may be assessed for the presence of a BCR-ABL rearrangement, while only 20 to 25 metaphases are analyzed by the classic chromosome analysis. However, additional cytogenetic aberrations and Ph-negative clones cannot be detected using FISH alone and require conventional chromosome banding analysis.

Molecular Genetics

The molecular correlate of the Philadelphia translocation is the BCR-ABL fusion gene. The diagnosis of a CML

can be made only if the Philadelphia translocation is detected by cytogenetics or by FISH or if the BCR-ABL rearrangement is detected by RT-PCR. Since 5% of all CML cases carry a cryptic BCR-ABL rearrangement that is not detectable cytogenetically, a FISH and/or PCR assessment should be done in parallel to cytogenetics at diagnosis. Furthermore, a PCR-based quantification of the BCR-ABL expression provides a starting value for follow-up assessments.

The PCR-based monitoring of BCR-ABL during therapy is a standard procedure in CML. Independent of the therapy applied, a response to therapy is most effectively assessed by real time PCR with regard to both quantity of response and sensitivity. In contrast to most other leukemias, follow-up assessment in CML is possible using peripheral blood. It is recommended to do a peripheral blood evaluation every 3 months.

In patients under imatinib therapy, an association between an increase of the BCR-ABL expression and the occurrence of a resistance against imatinib has been described. In 2 to 5% of all CML cases, a primary resistance against imatinib is present. Many of these resistances are due to point mutations of the ABL gene within the BCR-ABL fusion gene. Other patients acquire secondary mutations in the tyrosine kinase loop of the BCR-ABL fusion protein during therapy with imatinib, which lead to a resistance against the drug. In some cases this resistance can be superseded by an increase of the dose of imatinib or by the combination with IFN-α. Therefore, the early detection of these resistances increasingly carries clinical importance. Mutations have been described in 23 different codons so far. Some of these lead to changes in conformation while others directly inhibit the binding of imatinib to the activating domain of the tyrosine kinase ABL. The type of mutation therefore provides information on the usefulness of an increase of the imatinib dose. The point mutations can be detected by sequencing the ABL part of the fusion gene.

Diagnostic Workup of CLL

Cytomorphology

The diagnosis of CLL per definition requires a lymphocytosis >5,000/µl, although a chronic elevation of the absolute lymphocyte count in the peripheral blood and the presence of both the typical morphologic findings and the typical immunophenotype are increasingly considered diagnostic. Cytomorphologically, CLL cells are small lymphocytes with a narrow cytoplasm and a dense nucleus without nucleoli. Furthermore, shadow cells of Gumprecht are present.

Immunophenotyping

CLL features a typical immunophenotype with a coexpression of CD5 and the B-cell-associated antigens CD19, CD20, and cCD79a. A weak surface expression of CD22 and Ig is present, with a clonal light chain restriction. The positivity of CD23 which is commonly present in CLL allows the distinction from mantle cell lymphoma. The latter is further characterized by a strong surface expression of Ig as well as by an expression of CD22 and FMC7. Overall, no single marker allows the diagnosis of CLL. Rather, the comprehensive evaluation of all the antigens assessed and the application of the Matutes-score have been proved useful (Table 12).

The presence of four or five of the findings characteristic for B-CLL was found in a large series in 87% of all B-CLL and in only 0.3% of other B-cell lymphomas. Conversely, only 0.4% of B-CLL and 72% of other B-cell lymphomas had none or only one of these criteria.

The expression of CD38, as well as the cytoplasmic expression of ZAP70, is prognostically important. Both findings are associated with an inferior prognosis.

If CLL/PL is diagnosed by cytomorphology (10 to 55% prolymphocytes in peripheral blood) often accompanied by a trisomy 12, which occurs more frequently in these cases, the immunophenotype may show a stronger expression of CD20 and of sIg as compared to CLL. Furthermore, there is a weaker expression of CD23 as well as a positivity for CD22 and CD79b.

In contrast to B-CLL, the co-expression of CD5 is not, or only weakly, present in B-PLL (>55% prolymphocytes in peripheral blood), the surface expression of Ig and of CD20 is stronger and CD22 and FMC7 are expressed.

Besides diagnosis, immunophenotyping allows the quantification of minimal residual disease (MRD). The CLL cells typically carry the phenotype CD19+ CD20+CD79-CD5+ and thereby differ from normal B-lymphocytes. The prognostic relevance of the MRD-level, which can be assessed with high sensitivity, has been demonstrated in multiple studies.

Cytogenetics

The classic chromosome analysis has played only a minor role in the past due to the difficulties in cultivating the cells in vitro. By using FISH analysis, the strong prognostic impact of chromosome aberrations could be demonstrated. Recently, a reliable cultivation of CLL cells has been shown to be feasible and chromosome aberrations have been detected by chromosome analysis at higher frequencies compared to FISH analysis.

Leukemia Diagnostics. Table 12 Matutes-score of B-CLL

Characteristics of B-CLL
CD5+
CD23+
FMC7−
sIgM(+)
sCD22(+) or CD79b(+)

Fluorescence in Situ Hybridization

FISH analysis detects the most frequent chromosomal aberrations in CLL in a targeted way. These comprise 13q deletions, trisomy 12, 11q deletion, and 17p deletion. The presence of a 17p deletion or of a 11q deletion indicates a more aggressive course of disease compared to a normal karyotype, while the sole presence of a 13q deletion confers a favorable prognosis. Furthermore, the assessment of t(11;14) is useful for the distinction between CLL and mantle cell lymphoma.

Molecular Genetics

Half of the patients with CLL carry so-called somatic mutations in the variable region of immunoglobulins. The presence of 2% or less mutations in this area of the immunoglobulins as compared to the original DNA sequence is designated "unmutated" while the presence of more than 2% mutations is designated "mutated." The unmutated status is associated with an unfavorable prognosis even in early stages of the disease.

Diagnostic Workup of MDS

Cytomorphology

During the cytomorphologic examination at least 200 bone marrow cells and 20 megakaryocytes should be evaluated. Dysplastic findings should be present in at least 10% of the cells. A particular diagnostic role is played by the so-called pseudo-Pelger neutrophils, ringed sideroblasts, mikromegakaryocytes, and augmented blasts. These morphologic aberrations correlate in part with clonal markers and show a low inter-observer variability. This is true particularly for the prognostically favorable and therefore clinically relevant 5q-syndrome. The assessment of hypogranulation in neutrophils should not be the only diagnostic criterion for dysplasia. Accordingly, an early stage of refractory anemia (RA) with cytopenia in only one lineage is often difficult to diagnose and requires the assessment of follow-up samples. With regard to the differentiation between hypoplastic MDS and aplastic anemia, it is important to notice that dysplastic findings in erythropoiesis may be present also in the latter. They therefore play no diagnostic role in this instance, unlike dysplastic findings in the other lineages and augmented bone marrow blasts. PNH should be considered as differential diagnosis. With regard to the separation of MDS and AML a cut-off of 20% bone marrow blasts has to be used according to the presently applied WHO classification.

Immunophenotyping

Multiparameter flow cytometry allows the qualitative assessment of dysplasia in the different cell lineages, granulopoiesis, monopoiesis, and erythropoiesis through the detection of aberrant antigen expression patterns. Furthermore, the quantification of blasts can be done with a high correlation to cytomorphologic findings. The flow cytometric findings are of particular diagnostic value in cases difficult to judge by cytomorphology. Further studies will define the role of multiparameter flow cytometry in comparison to cytomorphology and cytogenetics with regard to both diagnostics and prognostication.

Cytogenetics

In the context of diagnostic assessment of MDS the chromosome analysis plays a significant role by detecting karyotype aberrations typical for MDS and particularly so in cases difficult to judge by cytomorphology.

The typical aberrations, which are also considered for the prognostically highly relevant IPSS (Tables 13 and 14), include loss of Y chromosome, del(5q), del (20q), as well as complex aberrations and aberrations of chromosome 7. In cytomorphologically defined borderline cases a distinction between AML and MDS can be accomplished by the detection of t(8;21)(q22;q22), t(15;17)(q22;q11-12), or inv(16)(p13q22)/t(16;16)(p13;q22) which define an AML, respectively.

Fluorescence In Situ Hybridization

FISH analysis may be used in case of lack of adequate material for cytogenetics, e.g. in case of a dry tap, smears may be obtained from a bone marrow biopsy and analyzed by FISH. The analysis includes probes targeting the aberrations most relevant for determining the prognosis: -Y, del(5q), del(20q), aberrations of chromosome 7, del(17p).

Molecular Genetics

In contrast to AML there are no specific molecular markers for MDS. In case of rare reciprocal translocations

Leukemia Diagnostics. Table 13 IPSS, basis

			Points		
	0	0.5	1	1.5	2
% bone marrow blasts	5	5–10		11–20	21–30
Karyotype	Favorable	Intermediate	Unfavorable		
Cytopenias	0/1	2/3			

Karyotype favorable: normal, -Y sole, del(5q) sole, del(20q).
Karyotype unfavorable: complex aberrant (≥3 aberrations), aberrations of chromosome 7.

Leukemia Diagnostics. Table 14 IPSS, prognostic grouping

Points	0	0.5–1.0	1.5–2.0	≥2.5
Risk group	Low	Int-1	Int-2	High

fusion gene-specific PCRs can be applied which, however, do not play a major role in routine diagnosis. Some of the AML-specific mutations like the partial tandem duplication of MLL (MLL-PTD), FLT3 length mutations (FLT3-LM), RAS mutations, as well as mutations of AML1 and CEBPA may be present in MDS with high blast counts. They are indicative of a progress of MDS to AML. Similarly, an increasing expression of WT1 represents a marker for the progress of MDS.

Diagnostic Algorithms in Leukemia Diagnostics

Based on the data provided above, algorithms for the diagnostic work-up of leukemias have been proposed. These are outlined below and should be applied and evaluated in the context of large clinical trials. Leukemia diagnosis has undergone steady development and is expected to become even more refined within the next few years, particularly taking into consideration gene expression profiling with microarrays, which may be incorporated.

Diagnostic Algorithm in AML

In AML (Fig. 1) a combination of cytomorphology, cytochemistry, immunophenotyping, and cytogenetics should be applied in the first step. Results of cytomorphology and cytochemistry guide the selection of antibody panels to be used for immunophenotyping. Results of these three methods guide the selection of culture conditions for cytogenetics.

In case of specific cytomorphologic findings, the respective genetic alterations should be analyzed by FISH and PCR (AML M3/M3v: t(15;17)/*PML-RARA*; AML M4eo: inv(16)/*CBFB-MYH11*; AML M1/2 with characteristic long Auer rods: t(8;21)/*AML1-ETO*; AML M5a: 11q23/*MLL* rearrangements). The combined analysis of genetic alterations by both FISH and PCR provides a maximized diagnostic security as well as information on variant translocations or submicroscopic deletions, which are only detectable by interphase FISH.

Based on cytogenetic results, FISH analysis using specific probes is applied for numerical (e.g. +8, −7) and structural aberrations (e.g. 5q-, 7q-). Cases with complex aberrant karyotype can further be investigated by 24-color FISH. FISH may be used in infrequent cases without cytogenetic result to identify the most frequent and prognostically relevant aberrations.

RT-PCR is used, according to cytogenetic results, for the detection of fusion genes as well as for the analysis of *FLT*3-LM, *MLL*-PTD, or *NPM*1.

Follow-up assessment during complete remission should be performed by cytomorphology, FISH, PCR, and MFC, whenever a specific marker has been identified at diagnosis. The latter two highly sensitive methods are particularly useful for the quantification of the prognostically important MRD levels.

Diagnostic Algorithm in ALL

Cytomorphology and immunophenotyping should be applied in the first step in ALL (Fig. 2). Cytomorphology identifies acute leukemia negative for peroxidase and reveals cases suspicious for Burkitt's lymphoma/mature B-ALL. MFC allows the diagnosis of ALL as well as the subclassification into different B-precursor ALL and T-precursor ALL classes.

Cytogenetics follows the results of MFC with specific culture conditions for B- and T-precursor ALL.

FISH analysis for BCR-ABL and MLL rearrangements follows MFC in case of B-precursor ALL.

FISH analysis for CMYC rearrangements follows cytomorphology in case of findings typical for Burkitt's lymphoma as well as MFC in case of mature B-ALL.

In childhood B-precursor ALL, FISH analysis for ETV6-AML1 is applied.

In case of cytogenetics not yielding a result, FISH and PCR are applied for the detection of BCR-ABL and MLL rearrangements.

Real time PCR is applied for MRD monitoring, targeting either fusion transcripts or IgH receptor-/T-cell receptor rearrangements. MFC is also used for MRD monitoring.

A complete reevaluation should be performed at relapse.

Diagnostic Algorithm in CML

The diagnosis of CML is made by a combined approach of cytomorpholgy, cytogenetics, FISH, and PCR, identifying characteristic peripheral blood and bone marrow features as well as the t(9;22) and the BCR-ABL fusion gene (Fig. 3).

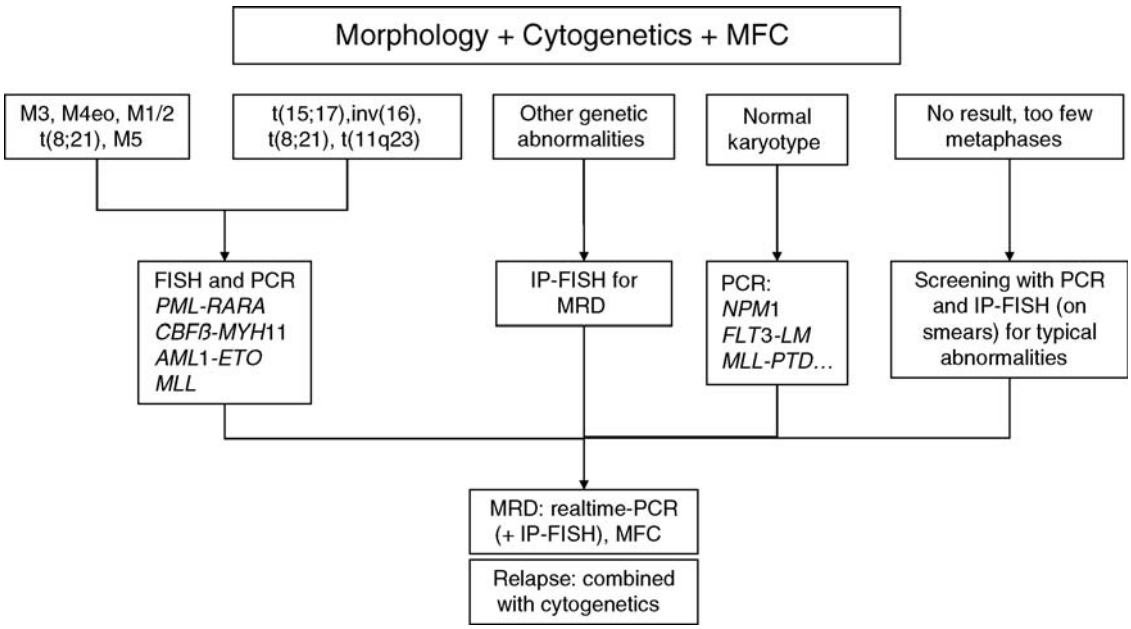
During therapy all methods are applied in combination to quantify the amount of residual disease as well as additional chromosomal abnormalities.

In case of failure or suboptimal response an analysis for BCR-ABL mutations conferring resistance to imatinib should be performed.

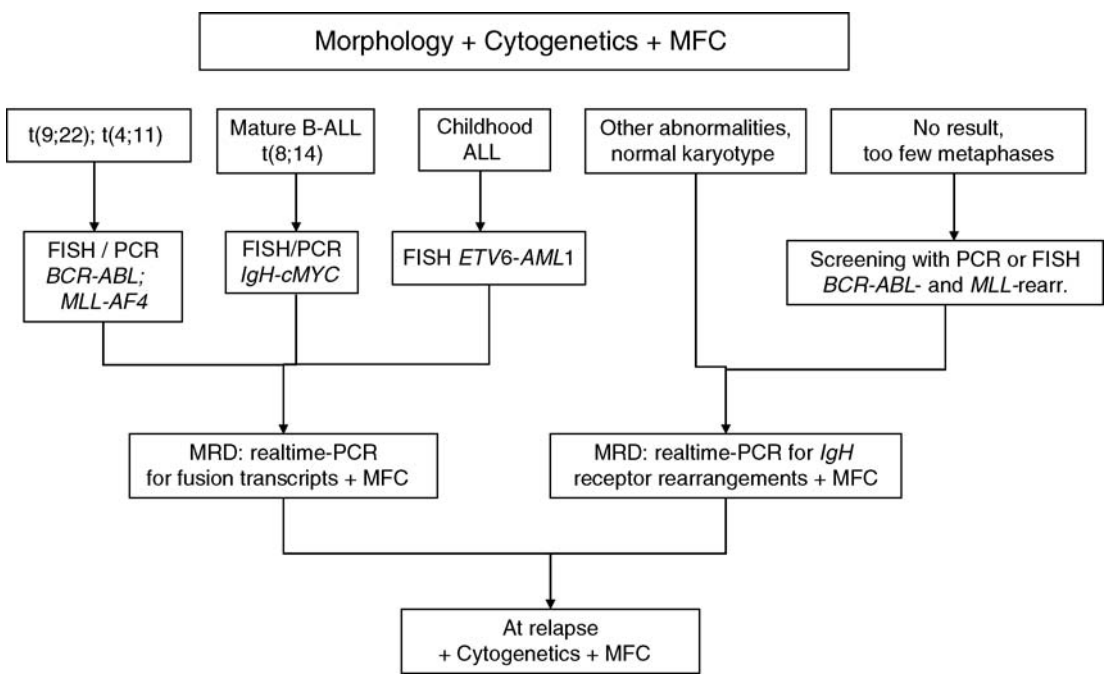
Immunophenotyping should be performed in blast crisis to delineate the lineage (lymphatic or myeloid).

Diagnostic Algorithm in CLL

Cytomorphology and immunophenotyping should be applied in the first step in CLL (Fig. 4). Cytomorphology identifies mature lymphocytosis. MFC allows the



Leukemia Diagnostics. Figure 1 Algorithm for diagnosis and follow-up in AML.



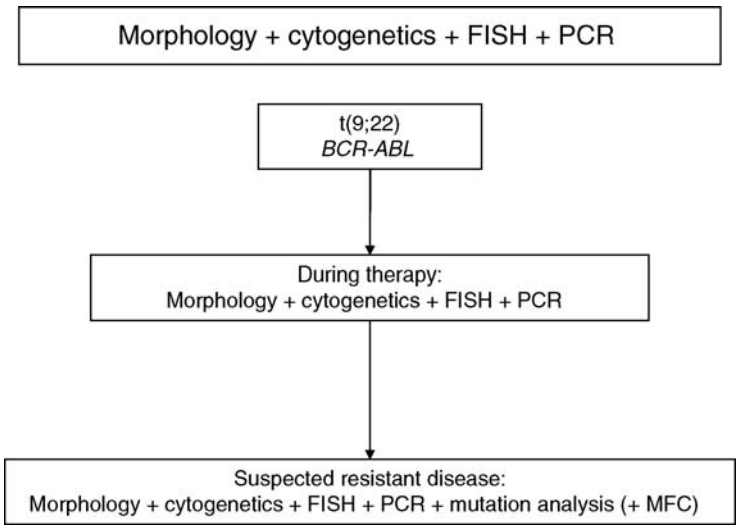
Leukemia Diagnostics. Figure 2 Algorithm for diagnosis and follow-up in ALL.

diagnosis of CLL and its discrimination from other indolent lymphomas according to the Matutes score.

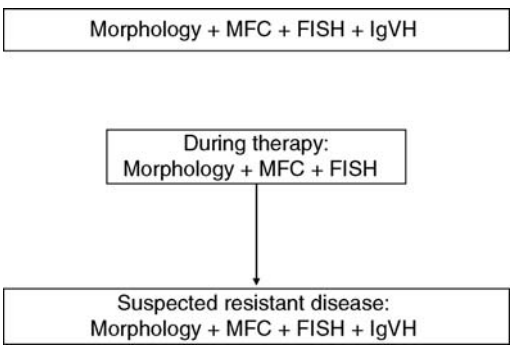
In addition, MFC is used for the determination of the expression of both CD38 and ZAP-70.

FISH analysis is performed to identify the most common and prognostically important chromosomal abnormalities, i.e. 6q-, 11q-, trisomy 12, 13q-, and 17p- as well as t(11;14). A cytogenetic analysis is capable of identifying additional chromosomal abnormalities which may yield further prognostic information.

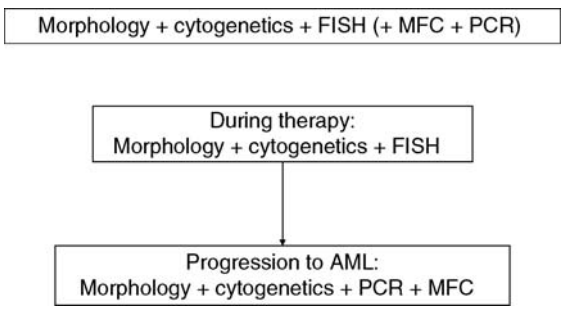
Molecular genetics is applied to determine the IgVH mutational status.



Leukemia Diagnostics. Figure 3 Algorithm for diagnosis and follow-up in CML.



Leukemia Diagnostics. Figure 4 Algorithm for diagnosis and follow-up in CLL.



Leukemia Diagnostics. Figure 5 Algorithm for diagnosis and follow-up in MDS.

Monitoring during therapy is performed by a combination of cytomorpholgy, MFC, and FISH.

Diagnostic Algorithm in MDS

The diagnosis of an MDS is based, besides the presence of cytopenias, on cytomorphology (Fig. 5). Cytogenetics is used to identify chromosomal abnormalities which subclassify MDS and yield prognostic information. In cases of equivocal findings in cytomorphology cytogenetics can assure the diagnosis. Immunophenotyping may be used to identify aberrant antigen expression patterns typical for MDS. PCR may be performed in MDS.

During the course of therapy, which may be limited to supportive measures, cytomorphology in combination with cytogenetics and FISH should be applied.

In case of progression to AML, cytomorpholgy, cytogenetics, PCR, and MFC should be applied.

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Leukemia Inhibitory Factor

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Synonyms

D-factor; Hepatocyte stimulating factor-III; HSF-III; Cachexia-inducing agent; DIA; DRF; CNDF; HILDA; MLPLI; LIF

Definition

►Leukemia Inhibitory Factor (LIF) is a cytokine belonging to a family of ►interleukin-6 related cytokines that signal through gp130 protein coupled receptors and whose inducible production (e.g. due to hypoxia) can occur in ►hypoxia-inducible factor-1 manner in multiple tissues. LIF is a pleiotropic cytokine that affects several biological processes, and its current name implicating "a leukemia inhibitory factor" has merely historical meaning.

Characteristics

LIF

LIF was purified from Krebs-II ascites tumor cell line and then cloned from a murine T-lymphocyte cDNA library and originally identified as a factor that inhibits the proliferation of undifferentiated and highly clonogeneic murine myeloid leukemic cell line M1 and induces its differentiation into macrophages. At the molecular level human and murine LIF is a glycoprotein with a 180-amino-acid single 4-α-helix polypeptide chain with a conserved disulfide bond. Natural occurring LIF is heavily glycosylated, showing an apparent molecular weight of 32–62 kDa, depending on the source. It is accepted that the absence of glycosylation appears not to affect its biological activity. Human and mouse LIF share 78% sequence homology. Human LIF can activate mouse cells, but vice versa mouse LIF cannot activate human cells.

LIF Receptor

LIF interacts with the heterodimeric human LIF-receptor (LIF-R) comprising a 190 kDa LIF-binding α-chain (130 kDa mouse) and a 130 kDa signal-transducing β-chain (gp130). LIF receptors have been identified on several cells, including monocytes, liver, neural cells, muscle cells, primordial germ cells, placenta and embryonic stem cells. Therefore LIF-R is a member of the gp130 superfamily of receptors. Accumulated data that the LIF-binding α-chain shows some level of "molecular promiscuity" and can be also part of the type II receptor for ►oncostatin M (OSM), and part of the receptors for ciliary neurotropic factor (CNTF) and carditrophin-1 (CT). Thus LIF in addition to LIF-R can also signal for example through the OSM receptor.

LIF Over Expression and LIF Knockouts

Prolonged over expression of mice to LIF leads to ►cachexia coupled with a hypermotile state, overgrowth of bone tissue and osteoblasts in the bone marrow cavities, loss of spermatocytes from the seminiferous tubules of the testis, absence of corpora lutea in the ovaries, a reduced number of Purkinje cells in cerebellum, thymus atrophy and an involution of the pancreas. Mice with LIF knocked-out ($LIF^{-/-}$) are viable, however they show abnormalities of the hippocampus and an accumulation of olfactory receptor neurons. Interestingly $LIF^{-/-}$ mice are unable to get pregnant, which is explained by a crucial role of secreted LIF in the uterus during the process of blastocyst implantation.

Pleiotropic Effects of LIF

LIF plays an important role in several biological processes in embryogenesis, tissue regeneration, metabolism and cancer ►metastasis. It activates in normal cells Jak-STAT-3, MAPKp42/44and PI-3K-AKT signaling pathways and in murine embryonic stem cells (ES) LIF also regulates expression of Oct3/4. Biological effects of LIF are controlled by circulating soluble LIF-R that blocks the action of any LIF in circulation. At the cellular level LIF signaling is negatively controlled by ►suppressors of cytokine signaling (SOCS).

LIF was identified as a factor that maintains totipotency of murine ES and is crucial for blastocyst implantation in mice. LIF is also one of the factors that promotes dedifferentiation of murine primordial germ cells into embryonic germ cells. In humans LIF has no effect on totipotency of human ES cells. However, LIF was recently demonstrated to reduce the vertical transmission of HIV-1 virus though the placenta. Finally, LIF enhances the proliferation of normal primordial germ cells and spermatocyte differentiation.

LIF is playing an important role in the development of hippocampal and olfactory receptor neurons. It stimulates the proliferation of skeletal muscle satellite cells and is a hyper trophic factor for myocardium. Therefore, LIF may play a role in regeneration of neural tissue, myocardium and skeletal muscle. In support of this LIF is up regulated in damaged brain, heart and skeletal muscle. It is possible that LIF in cooperation with other factors such as stromal derived factor-1 (SDF-1) and hepatocyte growth factor (HGF)/►scatter factor recruits circulating stem cells and macrophages to damaged organs.

In the hematopoietic system, LIF shows positive co-stimulatory effects on megakaryocyte maturation and platelet formation. LIF also stimulates proliferation of the human factor-dependent hematopoietic cell lines TF-1

and DA as well as proliferation of the murine factor dependent GB2 cell line. LIF together with Flk ligand has been shown to enhance blast colony formation by murine bone marrow cells. These blast colonies are enriched in macrophages and ►dendritic cells. LIF receptors on murine hematopoietic populations are mainly restricted to cells of the monocyte lineage. Thus, the potential influence of LIF on the compartment of hematopoietic stem cells requires further study.

LIF also exerts several metabolical effects such as inhibits lipid transport to adipocytes, enhances production of acute-phase proteins by the liver and was also identified as a factor capable of switching autonomic nerve signaling from an adrenergic to a cholinergic mode. LIF also has several endocrine effects being a major regulator of ACTH production in the pituitary and inhibits production of prolactin and growth hormone.

LIF and Oncogenesis

LIF appears to be an important developmental motto-morphogene that displays several pleiotropic biological effects in vitro and in vivo. Recent evidence has accumulated that this interesting cytokine affects survival, proliferation and differentiation of many cell types not only normal but malignant cells as well. For example while LIF stimulates proliferation or differentiation of several established malignant hematopoietic stem cell lines (TF-1, DA, GB2, or M1), it inhibits in vitro proliferation of ►breast cancer cells. LIF also may play some role in tumor vascularization and ►angiogenesis.

Recently, based on the observation that LIF stimulates proliferation of skeletal muscle satellite cells and myocytes, as well as cardiomyocytes we hypothesized that the LIF–LIF-R axis could be involved in progression of human ►rhabdomyosarcomas (RMS). We found that in RMS cells LIF stimulates (i) phosphorylation of MAPKp42/44, AKT and STAT3, (ii) ►adhesion and chemotaxis and (iii) increased resistance to cytostatics. Thus, we presented evidence for the first time that the LIF–LIF-R axis exerts chemotactic activity and may direct RMS metastasis. The role of this axis in progression of other non-hematopoietic malignancies requires further study.

Clinical Aspects

The LIF–LIF-R axis has emerged as an important axis regulating both the development and homeostasis of an adult organism. The novel recognized role of LIF in trafficking of normal and ►cancer stem cells may lead to the development of better therapeutic strategies aimed at directing ►migration of normal stem cells to regenerate damaged organs (e.g. heart or brain) but on the other hand inhibiting metastasis of LIF-responsive cancer stem cells. Thus, a new area of investigation on the role of gp130 signaling molecules in cancer metastasis has been established.

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Leukocyte Elastase

►Neutrophil Elastase

Leukocyte Endothelial Cell Adhesion Molecule-2

►E-Selectin-Mediated Adhesion in Cancer

Leukocyte Functional Antigens

Definition

LFAs; are ►cell-adhesion molecules initially defined with monoclonal antibodies: LFA-1 is a β_2 integrin; LFA-2 is a member of the immunoglobulin superfamily, as is LFA-3, now called CD58. LFA-1 is particularly important in T-cell adhesion to endothelial cells and antigen-presenting cells.

Leukocyte Homing

Definition

Refers to the process by which leukocytes, or white blood cells, are targeted to the sites at which they are

needed. This process involves active recruitment out of the blood stream and into tissues.

►CXC Chemokines

Leukocyte Interferon

►Interferon-α

Leukocytes

Definition

White blood cells found in peripheral blood and tissues, in particular the bone marrow and spleen.

►CD Antigens

Leukopenia

►Neutropenia

Leukoplakia

Definition

A white patch on the mucosa that does not rub off and is not attributable to any obvious cause. It is a diagnosis of exclusion and is not a disease entity in itself. It has a relatively low frequency for malignant transformation into squamous cell carcinoma.

►Squamous Cell Carcinoma

Leukotriene Receptors

Definition

Are seven transmembrane G-protein coupled receptors (GPCR). Two receptors for LTB_4, the high affinity receptor BLT_1 and the low affinity receptor BLT_2 are known. Two distinct receptors for the cysteinyl leukotrienes (CysLTs), the high affinity receptor $CysLT_1$ and the low affinity receptor $CysLT_2$ have been isolated and characterized. There are several antagonists for the $CysLT_1$ receptor, which are used in clinical treatment of asthma including Singulair™ (Monteluskast) and Accolate™ (Zafirlukast), but only one known antagonist for $CysLT_2$ receptor, Bay-U9773. Several antagonists are also known for the BLT_1 and BLT_2, such as U-75302, LY293111, and CP-105696, which block the BLT_1 receptor at low concentration and BLT_2 at higher concentrations.

►Leukotriene

Leukotrienes

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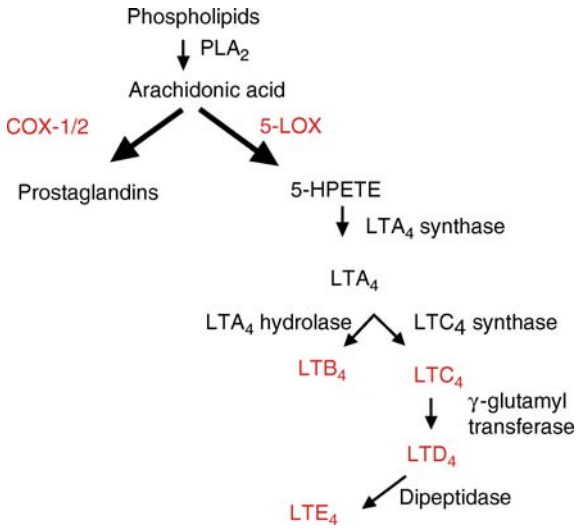
Definition

►Leukotriene, the dihydroxy leukotriene, ►LTB_4, is formed via the ►5-LO pathway through the enzyme leukotriene A_4 hydrolase. LTB_4 is one of the most chemotactic molecules known. It has a preferential action on leukocytes, producing chemotaxis and chemokinesis of neutrophils and mononuclear cells, as well as aggregation, degranulation and adherence of leukocytes to endothelial cells. The cysteinyl ►leukotrienes, including ►LTC_4, ►LTD_4, and ►LTE_4, are also known as the slow-reacting substance of anaphylaxis. ►CysLTs enhance contraction of smooth muscle, increase vascular permeability as well as migration and chemokine production in monocytic cells. CysLTs are produced in high amounts in activated eosinophils, basophils, macrophages, and mast cells. ►5-Lipoxygenase (5-LO) catalyzes the biosynthesis of proinflammatory leukotrienes that have both autocrine and paracrine mechanisms and may play an important role in ►inflammation-induced carcinogenesis. Inflammation is an important component of tumor progression. Many tumors start from the sites of infection and inflammation.

Characteristics

Leukotrienes and ►prostaglandins are metabolites of ►arachidonic acid that play major roles in various inflammatory conditions (Fig. 1). The release of these mediators, by cells recruited to or present at the site of



Leukotrienes. Figure 1 Biosynthetic pathway of prostaglandins and leukotrienes.

inflammation, modulates or influences the magnitude of the inflammatory response. An increased understanding of eicosanoids and how their receptors trigger intracellular signaling during inflammatory conditions is helping to elucidate the well-known connection between chronic inflammatory disease and neoplastic transformation. Here, we summarize the role of leukotrienes in cancer. In addition, we delineate how continuous ►leukotriene receptor activation and signaling can increase cell survival and proliferation as important early steps toward oncogenicity.

Today, it is clear that the tumor microenvironment, including inflammatory cells, is an important participant in the neoplastic process, proliferation, survival, and migration. A microenvironment rich in inflammatory cells, generating growth factors, and DNA-damage agents, potentiates the risk of neoplastic formation. Several mutations of key genes also seem to be important for the transformation of the ►inflammation–dysplasia–carcinoma sequence.

The leukotrienes potentiate biological activities in the pathogenesis of many diseases. In most chronic inflammatory conditions, such as inflammatory bowel disease, the levels of leukotrienes are increased. The strongest association of chronic inflammation with malignant disease is seen in colon carcinogenesis arising in individuals with inflammatory bowel disease. Indeed, patients suffering from inflammatory bowel disease have a 30–50% increased risk of developing colon cancer. In chronic inflammatory bowel diseases, elevated levels of leukotrienes are found, which increases the risk for development of cancer and thereby a reduced survival of these patients. As expected, it has been established that a cause-and-effect link between chronic inflammation and colon cancer, occurs via activation and overexpression of the two enzymes, 5-LO and COX (►Cyclooxygenase, COX-1 and -2), responsible for regulating the production of leukotrienes and prostaglandins, respectively (Fig. 1).

The expression of ►COX-2, is highly upregulated both during inflammatory conditions and cancer. Elevated prostaglandin production at the site of the tumor is a good indicator of increased COX-2 activity in colon cancer tissue. There are vast amounts of data suggesting that ►nonsteroidal antiinflammatory drugs (NSAIDs), ►COX inhibitors, reduce the risk of colon cancer. These inhibitors were shown to suppress the proliferation of intestinal cancer cell lines that express high levels of COX-2.

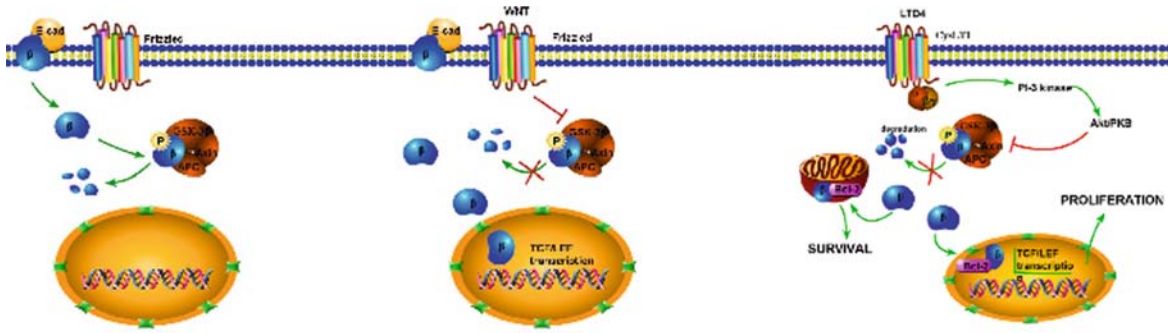
The production of different leukotrienes from arachidonic acid is dependent on the expression of 5-LO, an enzyme that regulates the first step in the synthesis of leukotrienes. Induction of experimental colitis in mice lacking the 5-LO protein significantly reduced the degree of infiltration of inflammatory cells and colonic injury. A number of other studies have shown that inhibition of 5-LO decreases growth and promotes cell death in several transformed cell lines.

In a recent tissue array study using colorectal cancer and control specimens, elevated levels of 5-LO and COX-2 were found in colorectal carcinomas. In accordance, similar observations were made in different colon carcinoma cell lines when these were compared with nontransformed intestinal epithelial cell lines. Interestingly, activation of ►$CysLT_1$ receptor signaling led to an increased COX-2-mediated production of prostaglandin E_2, PGE_2. Mediating COX-2 activation and PGE_2 production, has a major impact on intestinal epithelial cell survival.

5-LO metabolites may contribute to the development of several human tumors, including pancreatic, esophageal, prostate, breast, and colon cancer. COX-2 and 5-LO have been reported to be simultaneously upregulated in colorectal cancer. It is possible that blocking one of these enzymatic pathways may activate the other. Therefore combined treatment of these pathways could be a better treatment option. There is an additive effect of inhibiting proliferation, inducing apoptosis, decreasing Bcl-2 levels, and increasing Bax levels in cancer cells, after combined treatment with inhibitors of COX-2 and 5-LO.

►Leukotriene receptors are upregulated in colon cancer, and their signaling facilitates the survival and proliferation of cancer cells and reduces apoptosis. In agreement with this, an increased expression level of leukotriene receptors in different colon cancer cell lines correlates well with the ability of leukotrienes to increase the survival of these cells.

Apart from Bcl-2, there are a number of other cellular proteins that are closely connected to the regulation of cell proliferation, survival, and apoptosis. One of these is ►β-catenin. This protein became of interest in



Leukotrienes. Figure 2 A simplified signaling cascade leading to accumulation of free β-catenin and increased cell proliferation.

relation to LTD_4-induced survival signaling when a novel LTD_4-triggered association between Bcl-2 and β-catenin in the mitochondria from intestinal epithelial cell lines was identified. It could be hypothesized that LTD_4 may enhance cell survival via activation and association of β-catenin with Bcl-2 in the mitochondria. β-catenin activation and signaling execute an anti-apoptotic effect through protection of cytochrome c leakage from the mitochondria.

β-catenin is a protein with many roles, one well established as an effector molecule of the ►Wnt signaling pathway. The Wnt receptors, Frizzleds, belong to the same GPCR family of receptors as the leukotrienes. The presence of a Wnt signal allows β-catenin to translocate to the nucleus where it activates the transcription factors TCF/LEF. Normally, β-catenin is regulated by the adenomatous polyposis coli (►APC) complex to control the intracellular levels of β-catenin. LTD_4 can inactivate GSK-3β, known to phosphorylate β-catenin and induce its ubiquitination and degradation, and thus has the potential to increase the amount of free β-catenin if inactivated. LTD_4 induces inactivation of GSK-3β via PI3-kinase/Akt pathway allowing free β-catenin to be able to enter the nucleus and activate the transcription factor TCF/LEF, which activates potentially oncogenic genes, such as cyclin D1, c-myc, and COX-2 (see Fig. 2).

The infection–inflammation–dysplasia–carcinoma sequence is exemplified by *Helicobacter pylori* (HP) infection, a well-known risk factor for gastric adenocarcinoma. HP infection may also be a possible risk factor for respiratory system disease, which triggers a marked local inflammatory response and a chronic system immune response. Some of the possibly mediators involved in the pathogenesis of these infections include leukotrienes that may have a role in the development of lung cancer in association with HP infection. The risk of lung cancer in patients with gastric ulcers is around three times higher than in individuals without ulcers.

Activation of leukotriene receptor signaling pathways and subsequent effects on proliferation and survival of epithelial cells indicate that the inflammatory mediator, leukotrienes, can contribute to growth of cells during pathological inflammatory conditions. This, in turn, indicates that these receptors have an important role in neoplastic transformation and development of cancer.

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Leustatin

►Cladribine

Lewis Antigens

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Synonyms

Histo-blood group Lewis antigens

Definition

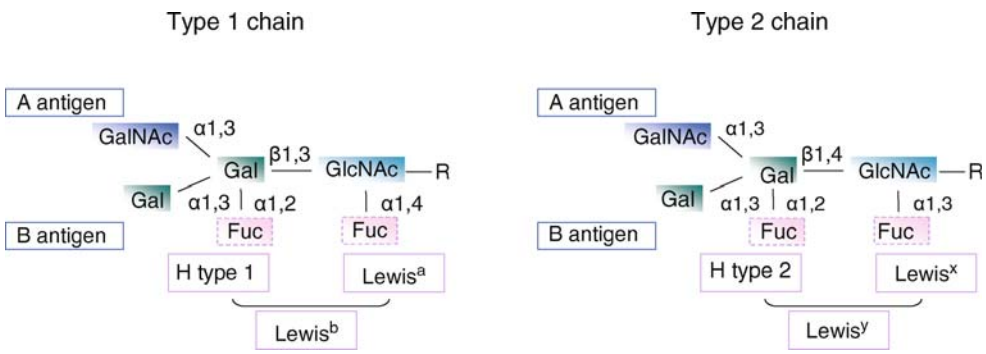
►Lewis antigens are fucosylated carbohydrates linked to lipids or to proteins and thus present as glycolipids or glycoproteins.

Characteristics

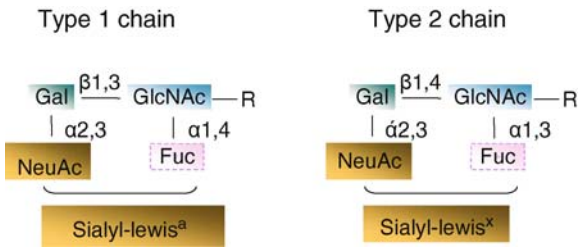
►Fucosylated ►Lewis antigens are expressed in normal tissues on two major carbohydrate type chains, type 1 and type 2, according to the linkage type between the galactose (Gal) residue and the N-acetyl-glucosamine residue (GlcNAc), β1,3 and β1,4, respectively (Fig. 1). Type 1 Lewis structures are widely expressed in endodermally derived tissues, such as glandular epithelia, in body fluids and saliva, and are adsorbed from plasma circulating glycolipids onto the surface of erythrocytes and lymphocytes. The presence or absence of type 1 antigens in a particular individual depends upon the presence of active enzymes responsible for the addition of the fucose monosaccharide. The α1,2 fucosyltransferase, the product of the secretor gene (*Se*) (►Secretor (Se) enzyme), acts on the terminal galactose and produces the H type 1 structure that forms the substrate for the α1,4 fucosyltransferase, the product of the Lewis gene (*Le*) (►Lewis (Le) enzyme), that synthesizes the difucosylated Le^b antigen. Individuals that have inactivating mutations of the *Se* gene are unable to synthesize the H type 1 structure and the Le^b antigen, are called nonsecretors and constitute 20% of human populations. Type 2 Lewis antigens are expressed in ecto- and mesodermally derived tissues, including skin and erythrocytes, and in a more restricted manner in endodermally derived epithelia like stomach glands. The α1,2 fucosyltransferase that acts on the terminal galactose and produces the H type 2 structure, is the product of the *H* gene, the ►H enzyme, that forms the substrate for the α1,3 fucosyltransferase, the product of the Lewis gene (*Le*), that synthesizes the difucosylated Le^y antigen. Other ►fucosyltransferases may be involved, in a tissue-specific manner, in the synthesis of Lewis antigens: an α1,3 fucosyltransferase activity has been described for FUT3, FUT4, FUT5, FUT6, FUT7, and FUT9, and an α1,4 activity was described for FUT3 and FUT5. The secretor and Lewis status of individuals are implicated in susceptibility to several diseases, mostly human infections, with the most dramatic example being the virtual absence of gastrointestinal infections by Calicivirus in nonsecretors.

Lewis Antigens in Cancer

Malignant cells frequently have abnormal ►glycosylation with expression of modified carbohydrate antigens, among which stand sialylated forms of the Lewis antigens – sialyl-Le^a and sialyl-Le^x (Fig. 2). Sialyl-$Le^{a/x}$ cell-surface molecules, linked to lipids and proteins, when tumor cells become invasive and depolarized gain access to circulation, either linked to the cell-surface or shed into the serum. The relevance of these sialylated structures in cancer was first revealed, in the 1980s, when monoclonal antibodies raised against cancer cells were shown to recognize sialyl-$Le^{a/x}$. They are widely used as tumor markers (CA19-9 for sialyl-Le^a and SLX for sialyl-Le^x) for initial serum diagnosis of cancer and for detection of cancer recurrence after surgery or treatment with radio- and chemotherapy. Later, in the 1990s, sialyl-$Le^{a/x}$ were identified as cancer cell-surface molecules involved in ►adhesion to endothelial cells, through ►E-selectin-mediated adhesion (and P-►selectin). Binding of tumor cells to endothelial cells, in a model that mimics leukocyte extravasation, contributes to the establishment of tumor growth at distant sites by hematogenous metastization. Several studies, analyzing cancers from different organ origins,



Lewis Antigens. Figure 1 Schematic representation of type 1 and type 2 ABH and Lewis antigens. Histo-blood group A is defined by the α1,3 terminal GalNAc, histo-blood group B is defined by the α1,3 terminal Gal, and the absence of further elongation of the H structure is characteristic of histo-blood group O individuals. Synthesis of type 1 Lewis antigens (Le^a and Le^b) and type 2 Lewis antigens (Le^x and Le^y) depends upon activity of fucosyltransferases (see text for details). GlcNAc, N-acetyl-glucosamine; Gal, Galactose; GalNAc, N-acetyl-galactosamine; Fuc, Fucose; A antigen, Histo-blood group antigen A; B antigen, Histo-blood group antigen B.



Lewis Antigens. Figure 2 Schematic representation of sialylated Lewis antigens – sialyl-Le^a and sialyl-Le^x. NeuAc, sialic acid (neuraminic acid).

showed that expression of these sialylated Lewis antigens correlated to the prognosis of the patients, reinforcing their role on metastatic behavior. They are more frequently overexpressed in carcinomas, mainly adenocarcinomas, but also in leukemia. Mechanisms underlying overexpression of sialyl-Le$^{a/x}$ in cancer cells have been recently clarified in some cancer models and essentially result from altered expression of α2,3 ►sialyltransferases and/or of α 1,3/4 fucosyltransferases, responsible for their synthesis (Fig. 2). A viral gene product that induces HTLV-1 viral-associated leukemias, was shown to transactivate fucosyltransferase VII, an α1,3 fucosyltransferase with rate-limiting activity for the synthesis of sialyl-Le^x in leukocytes, and therefore induces a strong constitutive expression of sialyl-Le^x in leukemic cells. Other mechanisms controlling gene expression, including methylation and identification of transcription factors, are under investigation.

The role of sialylated Lewis antigens in the metastatic process led to the development of several new candidate therapeutic approaches. Therapeutic strategies have been attempted to reduce the biosynthesis of sialylated Lewis antigens. Specifically, the synthesis of sialyl-Le^x was successfully inhibited in colon carcinoma cell lines by using a disaccharide competitive substrate as a decoy. An alternative approach is to use monoclonal antibodies directed to sialyl-Le$^{a/x}$ or analogs of the sialyl-Le$^{a/x}$ to block adhesion of tumor cells to endothelial cells and prevent metastization.

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Lewis Enzyme

Definition

A product of the Lewis gene, also named fucosyltransferase3 (FUT3), that catalyzes addition of fucose in α1,3/4 position onto type 1 and type 2 chains.

►Lewis Antigens

Lewis^x

Definition

Oligosaccharide corresponding to the histo-blood group antigen.

►CEA Gene Family

Lexatumumab

Definition

Is an anti- ►DR5 humanized antibody that was generated using a phage display and screening for DR5 binding properties. In contrast to ►mapatumumab, no data on specificity, selectivity and affinity of the antibody are publicly available. Lexatumumab exert pronounced apoptotic reactions in a wide array of malignant cell systems when used alone. The drug has proven efficacy on tumour growth in ►xenograft systems from renal cell carcinoma, non-small cell lung cancer, breast cancer and glioma. The combination of lexatumumab with various chemotherapeutic agents (camthotecin, ►cisplatin, carboplatin, ►paclitaxel, ►doxorubicin, bortezomib) or radiation increased the efficacy in cell lines and xenografts. The underlying mechanisms of sensitization are still not completely understood. However, it seems likely that the presence of the pro-apoptotic ►Bax molecule as well as the

up-regulation of the respective receptor participate in the increased efficacy of the combined approach.

►TRAIL Receptor Antibodies

LFS

Definition
Li-Fraumeni cancer family syndrome.

►Li-Fraumeni Syndrome

LH-RH

Definition
Luteinizing hormone-releasing hormone.

►Adjuvant Chemoendocrine Therapy

LHRH

Definition
LH-releasing hormone.

►Gonadotropin-Releasing Hormone

LHRH Agonists

Definition
Are analogues of gonadotropin releasing hormone; (LHRH = Luteinizing Hormone Releasing Hormone, also called: gonadothrophin-releasing hormone (GnRH)). They act as potent inhibitors of gonadotropin secretion, when given continuously, and ultimately results in near castrate testosterone levels; prostate cancer.

►Prostate Cancer, Clinical Oncology
►Gonadotropins

LI Element

Definition
►LINE

Li-Fraumeni Syndrome

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Definition
Li-Fraumeni syndrome (LFS) is a rare autosomal disorder characterized by a familial clustering of tumors, with a predominance of sarcomas, breast cancers, brain tumors and adrenocortical carcinomas, diagnosed before the age of 45 years. Other cancers, such as leukemia, lung cancer, gastric cancer, skin melanoma, pancreatic cancer, and ovarian cancer are also present in excess in some families and, in some cases, germ cell tumors, choroid plexus papilloma and Wilms' tumor have been reported as part of the spectrum.

Characteristics
Diagnostic Criteria
The clinical criteria used to identify a classic LFS family are:

- Proband with sarcoma before the age of 45.
- A first degree relative with any tumor before age 45.
- Another close relative (second or first degree) with cancer before age 45 or a sarcoma at any age.

Several clinical criteria for the diagnosis of LFS-like (LFL) families have also been proposed:

- *LFL-E1* (Eeles definition 1): two different tumors which are part of extended LFS in first or second degree relatives at any age (sarcoma, breast cancer, brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, pancreatic cancer).
- *LFL-E2* (Eeles definition 2): sarcoma at any age in the proband with two of the following (two of the tumors may be in the same individual): breast cancer at <50 years and/or brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, pancreatic cancer at <60 years or sarcoma at any age.
- *LFL-B* (Birch definition): proband with any childhood cancer or sarcoma, brain tumor or adrenocortical

carcinoma at <45 years, with one first or second degree relative with typical LFS cancer (sarcoma, breast cancer, brain tumor, leukemia, or adrenocortical carcinoma) at any age, plus one first or second degree relative in the same lineage with any cancer diagnosed under age 60.

- *Chompret*: (i) proband affected by a narrow spectrum cancer (sarcoma, brain tumor, breast cancer or adrenocortical carcinoma) before 36 years and at least one first or second degree relative affected by a narrow spectrum tumor (other than breast cancer if the proband is affected by breast cancer) before 46 years or multiple primary tumors; or (ii) a proband with multiple primary tumors two of which belong to the narrow spectrum and the first of which occurred before 46 years, whatever the family history; or (iii) a proband with ADC whatever the age at onset and the family history.

Genetics

The genetic origin of LFS was discovered in 1990 when four LFS families had been found to carry a germline mutation in the tumor suppressor gene ►TP53 (OMIM#191170). Since then, more than 400 families or individuals who are carriers of a TP53 mutation have been reported in the scientific literature. A database that compiles data from the literature and serves as a repository for data on patients and families with a TP53 germ-line mutation, or with LFS/LFL syndromes is maintained at IARC (http://www-p53.iarc.fr/Germline.html). Data from the IARC TP53 database show that in approximately 81% (116/143) of LFS cases, and 67% (97/145) of LFL cases, affected family members carry a germline mutation of one allele of the TP53 tumor suppressor gene. In some families, mutations in other genes have been reported. Two LFS and 3 LFL families had a heterozygous CHEK2 germline mutation, while one LFL family had a ►CDKN2A mutation and one LFS family a ►PTEN mutation. These genes are all connected to the p53 pathway. CHEK2 (OMIM#604373) (►Checkpoint Kinases) encodes a protein kinase that is involved in checkpoint control in response to DNA damage and acts upstream of p53 in the G1 checkpoint. CDKN2A (OMIM#600160) encodes two proteins, the cyclin-dependent kinase inhibitor p16(INK4) and the tumor suppressor p14(ARF). P14(ARF) is activated by oncogenic stress and promotes cell-cycle arrest via the inhibition of the neutralization of p53 by MDM2. PTEN (OMIM#601728) is a tumor suppressor gene encoding a protein phosphatase that negatively regulates the PI3-kinase/Akt (OMIM#164730) signaling pathway. PTEN is also a transcriptional target of p53 that acts downstream of p53. Alteration of the p53 pathway is thus the main cause of LFS/LFL syndromes, mutation of the TP53 gene being the most prevalent alteration. In fact, it is now recognized that CHEK2 does not predispose to LFS/LFL, but only to the breast cancers that have occurred in the context of these families. A study on 16 LFS families with wild-type TP53 has excluded PTEN, CHEK2 and CDKN2A as candidate genes in LFS.

The clinical criteria proposed by Chompret are currently recognized as the best criteria for predicting the presence of a TP53 germline mutation.

TP53 Germline Mutations

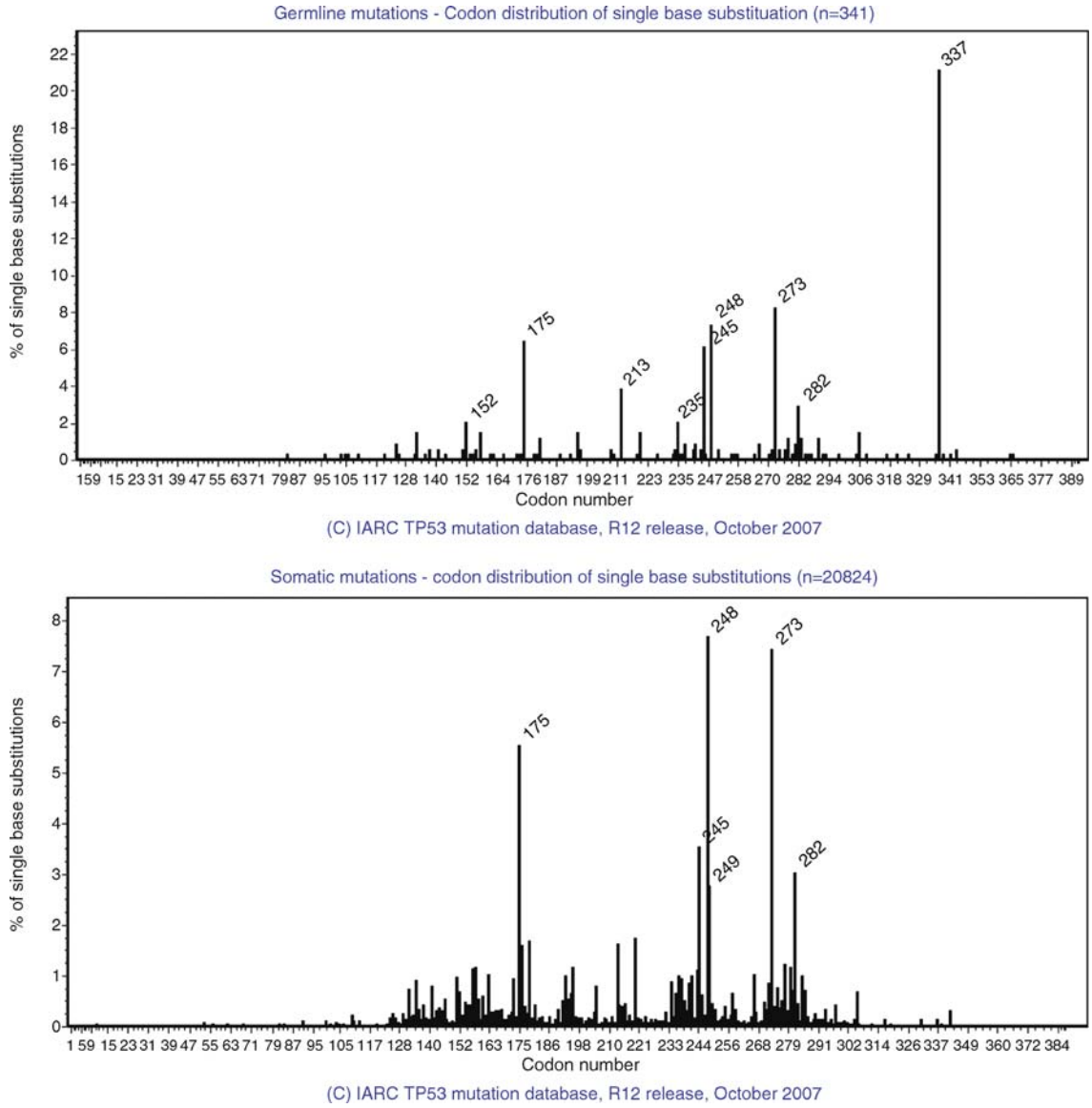
The tumor suppressor gene TP53 (OMIM:191170, synonym: P53), located on chromosome 17p13.1, has 11 exons that span 20 kb. Exon 1 is non-coding and exons 5–8 are remarkably conserved among vertebrates. The TP53 gene encodes an ubiquitous phosphoprotein involved in many overlapping cellular pathways that control cell proliferation and homeostasis, such as cell-cycle, apoptosis and DNA repair. The p53 protein is a transcription factor constitutively expressed in most cell types and is activated in response to various genotoxic and non-genotoxic stress signals. Loss of p53 function is thought to suppress a mechanism of protection against accumulation of genetic alterations.

Type and Origin of TP53 Mutations

Among TP53 germline mutations, point mutations are the most frequent (90%), followed by deletions (7%), insertions (2%) and other complex mutations.

As with somatic TP53 mutations, TP53 germline mutations are located in highly conserved regions of the DNA-binding domain (exons 5–8), with major hotspots at codons 175, 245, 248, and 273 (Fig. 1). These mutations correspond to G:C > A:T transitions at CpG sites that are considered to be of endogenous origin, i.e. formed as a result of deamination of 5-methylcytosine, a reaction that occurs spontaneously but which is usually corrected by DNA repair mechanisms. The occurrence of such mutations may be increased by factors that enhance the rate of methylation or the rate of deamination of 5-methylcytosine, as well as by defects in mismatch repair. Compared to somatic mutations, TP53 transitions at CpG sites are more frequent, representing 55% of all germline mutations compared to 25% in sporadic cancers. Overall, the spectrum of TP53 germline mutations is consistent with a formation through endogenous mutagenesis, rather than with causation by exposure to exogenous mutagenic carcinogens.

A germline-specific mutation hotspot has recently been described, R337H. It also results from a G:C > A:T transition at a CpG site but is located outside the DNA-binding domain, in the oligomerisation domain. This mutant has been found in Brazilian children affected by adrenocortical carcinomas and in Brazilian LFL families. It is considered of low penetrance and its prevalence in Brazil has been shown to be due to a founder effect.



Li-Fraumeni Syndrome. Figure 1 Similar to sporadic cancers, TP53 germline mutations preferentially occur in hotspot regions at codons 175, 245, 248 and 273. Specific to the germline, a mutation at codon 337 (R337H, in the conserved oligomerisation domain) is frequently found in the Brazilian population. (IARC TP53 database, R12, October 2007).

Tumor Spectrum in TP53 Mutation Carriers

Breast cancers, sarcomas (soft tissue sarcomas and osteosarcomas), brain tumors and adrenocortical carcinomas account for about 80% of all tumors arising in TP53 germline mutation carriers (Table 1). Childhood adrenocortical carcinomas (ADR, 14% of tumors) have been shown to be a hallmark for the presence of a TP53 germline mutation. Brain tumors (12% of all tumors) are mostly of astrocytic origin (65%). Pediatric brain tumors, including medulloblastomas and related primitive neuroectodermal tumors, and choroid plexus tumors are less frequent. This correlates with the occurrence of TP53 mutations in sporadic brain tumors, which prevail in astrocytomas and are considerably less frequent in medulloblastomas. In addition to the four main types of cancers strongly associated with TP53 mutation, seven types of cancers account for 14% of all cancers. They include leukemia/lymphomas, Wilms' tumor, malignant phyllodes tumor, carcinoma of the pancreas, stomach and colorectal cancers, that were found to be weakly to moderately associated with TP53 germline mutation, and lung and ovarian cancers. Although the frequent occurrence of lung and ovarian cancer might be due to the fact that they are frequent in

Li-Fraumeni Syndrome. Table 1 Tumor type, age at onset, and gender distribution in TP53 germ-line mutation carriers (IARC TP53 database, R12, October 2007)

Tumor type	Number N (%)	Median age at diagnosis		% male (ratio)	
		TP53 carriers	Sporadic[a]	TP53 carriers	Sporadic[a]
Breast cancer	217 (25.9)	33	63.1	0 (0/217)	0.7
Soft tissue sarcoma	142 (16.9)	14	61.3	43 (56/129)	53
Adrenocortical carcinoma	117 (13.9)	2	41.9	25 (29/116)	51
Brain tumor	101 (12.0)	15.5	57.4	64 (58/91)	56
Bone sarcoma	98 (11.7)	15	43.3	49 (43/88)	56
Leukemia/lymphoma	31 (3.7)	17	65.1	59 (13/22)	55
Lung cancer	22 (2.6)	41	68.7	50 (11/22)	66
Skin	20 (2.4)	50	–	19 (3/16)	–
Colorectal cancer	18 (2.1)	39	71.6	47 (7/15)	50
Stomach cancer	15 (1.8)	38	72.6	67 (10/15)	62
Ovarian ca.	13 (1.5)	39.5	64.3	0 (0/13)	0
Other	45 (5.4)	–	–	–	–

[a]Data based on cancer registries from United States, France, and United Kingdom compiled in Cancer Incidence in Five Continents v. 7, 1997.

the general population, they occur significantly earlier in TP53 germline carriers compared to sporadic cases (Table 1).

Age Distribution in Relation to Tumor Type

While the histopathologic characteristics of tumors associated with TP53 germline mutations are very similar to their sporadic counterparts, their age at onset show marked organ-specific differences (Fig. 2). Adrenocortical carcinomas associated with a TP53 germline mutation develop almost exclusively in children, while sporadic adrenocortical carcinomas have a broad age distribution with a peak beyond age 40 (Table 1). Bone sarcomas occur mainly in adolescents while soft-tissue sarcomas are more frequent in childhood. Brain tumors have a biphasic age distribution, with a first pick in childhood and a second pick between 20–40 years. Breast carcinomas are more prevalent in the 20–40 age range.

Gender Distribution of Patients with TP53 Germline Mutations

The gender distribution for these tumors (Table 1) shows an excess of males for brain tumor, hematopoetic cancers, and stomach cancer, whereas an excess of females was observed for adrenocortical carcinoma and skin cancer. All of the breast cancers were in females. This gender distribution is similar to the one of sporadic cancers observed in the general population with the exception of adrenocortical carcinoma, which occur significantly more frequently in females than in males in TP53 mutation carriers compared with sporadic cases in the general population.

Functional Consequences and Phenotype of Germline Mutations

The majority of TP53 germline mutations are missense mutations (80%), followed by nonsense, frameshift deletions and insertions, mutations in splice sites and other intronic or complex mutations. p53 mutant proteins resulting from missense mutations differ from each other in the extent to which they have lost transactivation activities, and in their capacity to inhibit wild type p53 in a dominant-negative manner. In addition, some p53 mutants appear to exert an oncogenic activity of their own, but the molecular basis of this gain-of-function phenotype is still unclear. It is thus expected that p53 germline mutants exert distinct biological activities that could influence tumor penetrance in term of organ sites or age at onset. Genotype/phenotype correlations have been reported in recent studies:

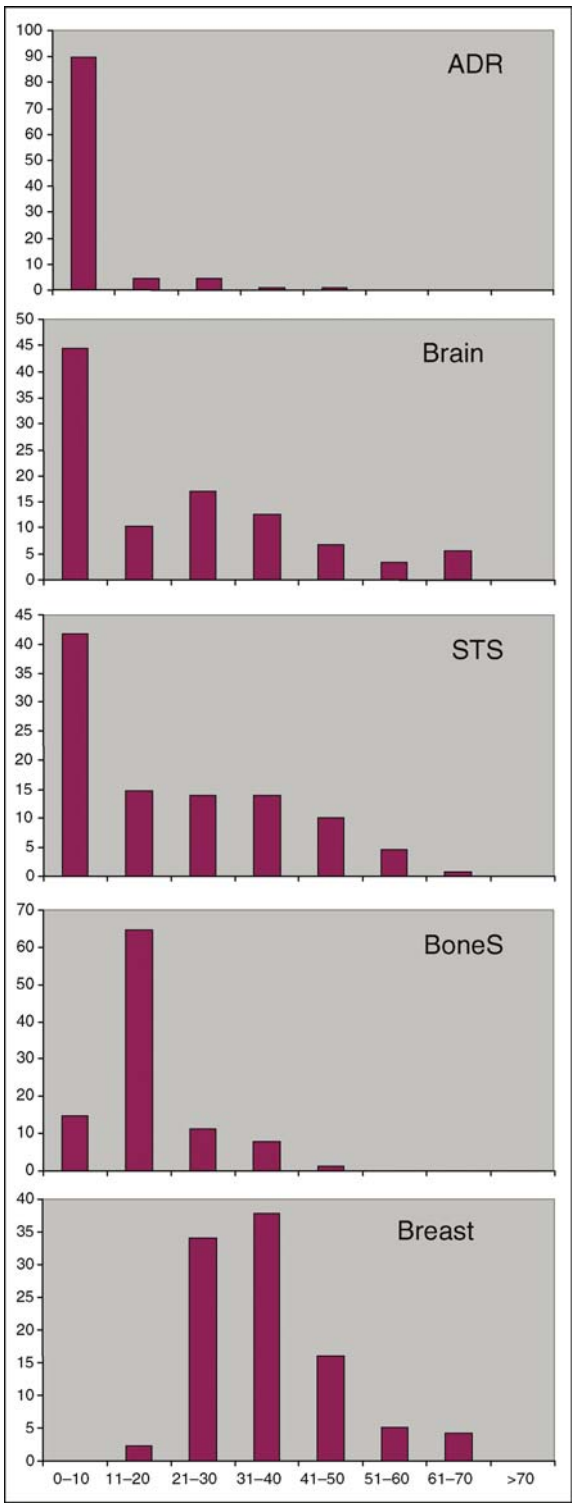
Brain tumors were more likely to be associated with missense mutations located within the DNA-binding surface of p53 protein that makes contacts with the minor groove of DNA.

Adrenocortical carnimoas were more likely to be associated with missense mutations located outside the DNA-binding surface.

Mutations with complete loss of transactivation activities were associated with earlier onset breast and colorectal cancers compared to mutations that retain some trans-activation capacities.

Truncating mutations were associated with earlier onset brain tumors compared to all other mutations.

Thus, the penetrance of a mutation may be related to its degree of loss of trans-activation capacities,



Li-Fraumeni Syndrome. Figure 2 Distribution of the age at onset of the five main types of tumors associated with TP53 germline mutations (IARC TP53 database, R12, October 2007). ADR, adrenocortical carcinoma; STS, soft tissue sarcoma; BoneS, bone sarcoma.

at least in some tissues. The notion that loss of function is in itself sufficient for causing LFS is substantiated by the fact that a large deletion encompassing the whole TP53 gene has been reported in an LFS family.

These observations show that the degree of loss of trans-activation capacities may affect the type and age at onset of tumors in LFS patients. It is not yet known how other functional properties contribute to mutation phenotypes.

Mutation Detection Methods and Results Interpretation

TP53 germline mutations are very diverse and scattered along the entire gene coding sequence, including splice sites in introns. These mutations have different biological effects that may affect their phenotype. Large deletions of the gene have also been reported in some LFS families. To fully assess TP53 status in an individual, it is thus recommended to (i) use sequencing techniques to precisely identify the mutation, (ii) screen all exons and splice junctions of the gene, and if no mutation is found, (iii) search for large gene deletions. A protocol for direct sequencing of TP53 is available in the IARC TP53 database (http://www-p53.iarc.fr/Download/TP53_DirectSequencing_IARC.pdf). A kit for detecting large deletions is available commercially (MLPA, http://www.mlpa.com/pages/p056pag.html).

For interpreting sequence analysis results, it is recommended to follow guidelines proposed by the ACMG (http://www.acmg.net/resources/policies/pol-027.pdf) working group and to consult the IARC TP53 Database to retrieve information on mutation prevalence and phenotype. If the mutation has already been reported as a germline mutation, the associated tumor spectrum can be retrieved (http://www-p53.iarc.fr/TumourCriteria.ASP). If the mutation has never been described in cancer families, data on its frequency in sporadic cancers and biological impacts (assessed in functional assays or predicted by sequence conservation models) can be retrieved (http://www-p53.iarc.fr/MutationValidationCriteria.asp) to estimate its functional severity.

If no alteration is found in TP53 gene, the family history may be carefully checked since certain families with LFS/LFL resemble families with hereditary breast cancer, who are candidates for BRCA1 or BRCA2 testing (see BRCA1/BRCA2 Hereditary Breast Cancer), while other families may resemble kindreds with familial brain tumors.

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Lichenification

Definition

Thickening of the skin with an exaggeration of the normal skin markings resulting from chronic rubbing and scratching.

►Sezary Syndrome

Lichenoid Dermatitis

Definition

A form of neurodermatitis, characterized by intense pruritus with exudative, weeping patches on the skin scattered irregularly over most of the body, many of which are of the eczematous type and undergo ►lichenification.

►Rituximab

Licorice

Definition

Liquorice; Is a shrub native to southern Europe and Asia, the roots of which have two primary desirable qualities: first, some varieties of licorice root are fifty times sweeter than sugar and may be chewed or eaten as a sweet and making it a useful component of candies and flavorings; second, licorice has been for thousands of years sought after for its reputed medicinal qualities.

LIF

►Leukemia Inhibitory Factor

Life Table Estimates

►Kaplan-Meier Survival Analysis

Ligament of Treitz

Definition

The suspensory ligament to the fourth part of the duodenum.

►Pseudomyxoma Peritonei

Ligands

Definition

From a chemistry viewpoint, it is an atom, ion, or molecule that generally donates one or more of its electrons through a coordinate covalent bond to, or shares its electrons through a covalent bond with, one or more central atoms or ions (these ligands act as a Lewis base). In biology, ligands are referred to small molecules (including peptides, amino acids etc…) that bind proteins and alter their function.

►Orphan Nuclear Receptors
►Gastrointestinal Stromal Tumor
►Dioxin

Steel Ligand Receptor

►Kit/Stem Cell Factor Receptor in Oncogenesis

Light-Induced Fluorescence Endoscopy

Definition
Is a bronchoscopy system for neoplasia detection based upon differential autofluorescence properties of early and invasive lung cancer.

►Fluorescence Diagnostics
►Laser Diagnostics

Light Tomography

►Optical Mammography

LIM Domain

Definition
Is a cysteine-rich domain composed of two tandem zinc fingers that are joined by a two-amino acid spacer. It functions as a modular protein-binding interface. The acronym "LIM" is derived from the first three proteins shown to harbor LIM domains: *C. elegans* LIN-11, rat Isl1, and ►C. elegans MEC-3. A single LIM domain consists of about 55 amino acids of which eight, mostly cysteines and histidine, are highly conserved. The latter bind two zinc ions, one for each zinc finger. The LIM consensus sequence has been defined as $CX_2CX_{16-23}HX_2CX_2CX_{16-21}CX_2$(C/H/D) (X represents any amino acid). LIM domains occur in a wide variety of eukaryotic organisms but are absent from prokaryotes. LIM proteins have a function in a wide variety of biological processes, such as cytoskeletal functions and control of gene expression.

►Lipoma Preferred Partner

LIM Domain Containing Preferred Translocation Partner in Lipoma

►Lipoma Preferred Partner

LIMD1

Definition
LIM domain containing protein 1; is a member of the ►zyxin family of proteins.

►Lipoma Preferred Partner

LINE

Definition
Synonym LI element; Long interspersed nuclear element; typically 6–8 kb pair long and frequent DNA sequences randomly distributed throughout the genome. In humans, LINE sequences make up approximately 20% of the genome, frequent are LINE-1, LINE-2, and LINE-3 elements. They mostly localize to AT-rich regions in the euchromatin. Because LINES move by copying themselves (instead of moving, like transposons do), they enlarge the genome. The human genome, for example, contains about 900,000 LINE sequences.

LINE sequences are transposable elements, which use RNA as an intermediary step. They encompass genes encoding two proteins: one RNA-binding, the other having activities of reverse transcriptase and endonuclease, which enable them to copy themselves. Their distribution pattern is unique for each individual genome, and their position therefore is diagnostic for the identity of individuals; ►Sticker sarcoma.

LINE-1 Elements

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Definition
Long interspersed nuclear elements (LINE-1 or L1) are a family of DNA repeat sequences with ~500,000 copies comprising ~17% of the human genome.

Characteristics
Structure
Full-length elements are ~6 kb, include a 910 bp 5′ untranslated region (UTR), two open reading frames

(ORF-1 and ORF-2) that are separated by a 63 bp inter-ORF region, a 205 bp 3′UTR, a variable length poly-A tail and are typically flanked by short direct target site duplications of 2–20 bp. The 5′UTR contains an internal promoter which drives L1 expression and also has an antisense promoter permitting transcription of the 5′UTR and its upstream genomic region. ORF-1 encodes a ~40 kDa protein (p40 or ORF1p) with RNA-binding affinity and nucleic acid chaperone activity. ORF-2 encodes a ~150 kDa protein (ORF2p) with endonuclease, reverse transcriptase and cysteine-rich domains (Fig. 1). The majority of L1 copies are either 5′ truncated, inverted or contain point mutations. An estimated 3,000–10,000 copies are full-length with only ~1% of these elements able to encode the factors needed for autonomous movement (►retrotransposition) enabling copying of L1 sequence and insertion into a new genomic location.

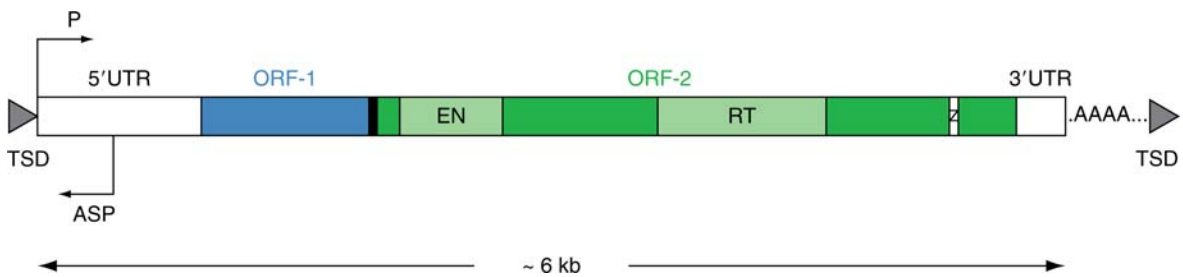
Genomic Impact

Although once considered "junk DNA" it is now realized that repetitive DNA families have influenced and shaped the human genome in both detrimental and beneficial ways. L1 elements can cause constitutional and somatic cell disease through not only retrotransposal insertion, but also as facilitators of recombination giving rise to genomic deletions, translocations and other chromosomal rearrangements. L1 elements have contributed substantially to the increase in DNA mass of human cells over evolutionary time. Over one-third of the human genome can be attributed to the activities of these elements either directly through self replication and insertion or indirectly by providing retrotransposal proteins enabling expansion of the nonautonomous ►Alu element, ►processed pseudogene and ►SVA repeat families. L1 elements contribute to reshuffling and relocation of DNA sequence through transduction of genomic sequence either 3′ or 5′ of the parent L1 during the retrotransposition process. Further proposed roles of L1 elements include involvement in ►X chromosome inactivation, downregulating or altering mRNA expression of the host gene in some cases of intronic insertion and repair of double strand DNA breaks by L1 insertion.

Role in Human Cancer

L1 element expression is normally restricted to developing germ cells. In somatic cells their activity is believed to be suppressed by a number of mechanisms including DNA ►methylation, TP53 activity, effects leading to L1 RNA instability and packaging into inactive chromatin. However, in some cancer cells L1 sequences become reactivated, largely through widespread ►hypomethylation of repetitive sequence. Cancer subtypes showing hypomethylation of L1 sequences identified so far include ►testicular, colon, liver, ovarian, bladder, prostate, breast and the hematological malignancies ►chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML, ►BCR-ABL1). In some instances hypomethylation is found early in the disease, for example in colon and bladder cancers. In other cases, including CML, CLL, prostate, ovarian and breast cancer, L1 hypomethylation is reported to occur as the disease progresses. Limited studies also suggest that L1 hypomethylation may be associated with clinical outcome.

It would be expected that with reactivation of L1 elements, assaults to the genome associated with retrotransposition i.e. mutagenesis, illegitimate recombination, altered transcriptional activity and gene regulation would occur. However, whereas there is considerable evidence pointing to L1 transcriptional reactivation through hypomethylation, there are limited documented examples of L1 elements associated with cancer. Rare cases have been reported and include L1 insertions into the ►*APC* and upstream of the ►*MYC* genes resulting in deregulation of these genes causing colon and breast cancer, respectively. L1 elements are found at breakpoint sites, for example inserted into the junction of a t(11;22) in a ►desmoplastic small round



LINE-1 Elements. Figure 1 Structure of a full-length human L1 element. The ~6 kb retrotransposon is flanked by target site duplications (TSD) and consists of (i) a 5′UTR with internal sense (P) and antisense (ASP) promoters; (ii) two open reading frames, ORF-1 encodes a 40 kDa RNA-binding protein, ORF-2 encodes a 150 kDa protein with endonuclease (EN) and reverse transcriptase (RT) activity, and contains a conserved zinc knuckle-like domain (Z) residing within a cysteine-rich region; (iii) a 3′UTR terminating in a poly-A tail.

cell tumor or are enriched at carcinoma genomic breakpoints e.g. 3p14.1 and 9p21 deletions.

Reasons for the small number of reports in this field of interogation could be due to either technical or biological factors. Current laboratory techniques used to investigate genetic alterations may have difficulty detecting subtle changes in L1 activity and limited numbers of cases are examined at the detailed level required to identify L1 insertions or other L1 mediated genomic rearrangements. There also may be additional safeguards within cells that provide protection against the activities of L1 elements. Therefore, it remains to be clarified the actual extent that reactivation of L1 elements in cancer cells plays in the initiation and progression of cancer.

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Lineage

Definition

The natural progression from an immature cell type to one or more differentiated cell types.

Lineage Differentiation Tumor Antigens

Definition

Tumor antigens that are expressed in a cell type-restricted manner by malignant cells and their normal counterpart. For example, proteins of the tyrosinase family of enzymes, which are responsible for the synthesis of melanin pigment in melanocytes, are frequently recognized by the cellular as well as the humoral immune system in melanoma patients.

►Melanoma Vaccines

Lineage Restriction

Definition

The inability of one lineage to give cell types of another, that is, to cross lineage boundaries.

Linear Accelerator

Definition

LINAC; is a device that accelerates electrons and other charged particles using electromagnetic waves. For radiotherapy applications, a high-energy electron beam is guided through a vacuum conduit to hit the metal foil in the path of the electron beam and to generate high energy X-rays.

►Radiation Oncology

Linearly Patterned Programmed Cell Necrosis

Definition

Is a kind of cell death. It shares morphological characteristics of traditional ►necrosis, but it is regulated by the ►apoptosis-related gene programme. Under the stimuli of ►hypoxia, a cluster of tumor cells exert this death and connect with each other by lines and networks, providing the space basement for vasculogenic Mimicry.

►Vasculogenic Mimicry

Lingual Cancer

►AAV

Linkage

Definition

Tendency for ►alleles at two genetic locations to be transmitted from parent to child in combination

►Linkage Disequilibrium

Linkage Disequilibrium

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Synonyms

Allelic association; LD

Definition

Linkage disequilibrium refers to the non-random association of ►alleles at two or more ►loci in a general population. When alleles are in linkage disequilibrium, ►haplotypes do not occur at the expected frequencies. Linkage disequilibrium between two alleles is related to the time of the mutation events, genetic distance, and population history. It can be used to improve the power of cancer ►genetic association studies.

Characteristics

Introduction to Linkage Disequilibrium

Two or more alleles are said to be in linkage equilibrium when they occur randomly in a population. Conversely, alleles are in linkage disequilibrium when they do not occur randomly with respect to each other.

Consider two neighboring polymorphic loci A and B with two alleles each (A_1 and A_2, and B_1 and B_2, respectively) having the allele frequencies shown in Table 1.

Under linkage equilibrium, the four haplotypes formed by these loci have the frequencies shown in Table 2. These are equal to the product of the component allele frequencies. The alleles are independent; for example, whether an individual carries allele A_2 at locus A is not dependent on whether this individual carries allele B_2 at locus B on this chromosome.

Under linkage disequilibrium, haplotypes do not occur at the frequencies expected when the alleles were independent. Positive linkage disequilibrium exists when two alleles occur together on the same haplotype *more* often than expected, and negative LD exists when alleles occur together on the same haplotype *less* often than expected (Table 3). For example, suppose linkage disequilibrium existed such that the alleles A_2 and B_2 tended to occur together, i.e., alleles A_2 and B_2 were in positive linkage disequilibrium. The probability that an individual carries allele A_2 at locus A is, to some extent, predictable based on whether this individual carries allele B_2 at locus B on this chromosome. The observed haplotype frequencies will differ from that expected as shown in Table 3.

Linkage Disequilibrium. Table 1 Allele frequencies

Locus	Allele	Observed frequency
A	A_1	p_1
A	A_2	p_2
B	B_1	q_1
B	B_2	q_2

Linkage Disequilibrium. Table 2 Haplotype frequencies under linkage equilibrium

Haplotype	Expected frequency
A_1B_1	$p_1 \times q_1$
A_1B_2	$p_1 \times q_2$
A_2B_1	$p_2 \times q_1$
A_2B_2	$p_2 \times q_2$

Measures of Linkage Disequilibrium

Deviation of the observed from expected haplotype frequencies can be quantified by several linkage disequilibrium measures. The basic linkage disequilibrium parameter, D, was introduced in 1918 and is defined as $D = x_{11} - p_1 \times q_1$. This difference between observed and expected haplotype frequencies is presented in Table 4. Although D is simple to calculate, its disadvantage is that it is sensitive to allele frequencies at the extreme values of 0 to 1. D maximizes when allele frequencies are both 0.5 and is not calculated if an allele frequency equals 0 or 1.

In 1964, Lewontin suggested D', a normalized D calculated by dividing D by its theoretical maximum for the observed allele frequencies ($D' = D/D_{max}$; Table 5). D' thus ranges from −1 to 1 and reflects both positive and negative linkage disequilibrium. D' is commonly used to characterize linkage disequilibrium, particularly, in the definition of haplotype blocks (see below).

A particularly useful metric of linkage disequilibrium is r^2 which is equivalent to the Pearson correlation coefficient. r^2 is calculated as $D^2/(p_1 \times p_2 \times q_1 \times q_2)$ and ranges from 0 to 1. Because it is less sensitive to extreme allele frequencies than D or D', r^2 is commonly

Linkage Disequilibrium. Table 3 Haplotype frequencies under linkage disequilibrium

Haplotype	Observed frequency	A_2 and B_2 in positive LD	A_2 and B_2 in negative LD
A_1B_1	x_{11}	$x_{11} > p_1 \times q_1$	$x_{11} < p_1 \times q_1$
A_1B_2	x_{12}	$x_{12} < p_1 \times q_2$	$x_{12} > p_1 \times q_2$
A_2B_1	x_{21}	$x_{21} < p_2 \times q_1$	$x_{21} > p_2 \times q_1$
A_2B_2	x_{22}	$x_{22} > p_2 \times q_2$	$x_{22} < p_2 \times q_2$

used to describe linkage disequilibrium in genetic epidemiologic studies of cancer. In addition, r^2 has utility in adjustment of estimates of statistical power in genetic association studies (see below) in which an assayed ►polymorphism may be in linkage disequilibrium with the truly causal polymorphism. Sample size must be increased by a factor of $1/r^2$. For example, if a sample size of 2,000 were required to detect a particular association between phenotype and a causal polymorphism ($r^2 = 1.0$), a sample size of 2,500 (= 2,000 × 1.25) would be required to detect the association if the causal polymorphism was in linkage disequilibrium with an assayed polymorphism at $r^2 = 0.8$ but was itself not assayed.

Estimation of Linkage Disequilibrium

Estimation of linkage disequilibrium between alleles at two loci requires observations of haplotype frequencies which is not currently technologically feasible in diploid organisms. Haplotype frequencies are, therefore, often estimated using statistical tools such as the expectation maximization (EM) algorithm. These methods take as input the observed combined ►genotype frequencies at the two loci (for example, the distribution of the nine possible combinations of A_1A_1, A_1A_2, and A_2A_2, with B_1B_1, B_1B_2, and B_2B_2).

Estimates of the observed haplotype frequencies are used for calculation of linkage disequilibrium measures. Numerous software packages facilitate these calculations ranging from EH (which provides estimates of observed haplotype frequencies) to Haploview (which calculates and graphically displays multiple linkage disequilibrium measures, Fig. 1).

Haplotype Blocks

Because of linkage disequilibrium in the human genome, there are regions in which only a reduced set of haplotypes occur relative to all possible haplotypes. These are referred to as haplotype blocks, and they can be defined in a variety of ways. A common definition for haplotype blocks is a region without "substantial" ancestral recombination; pairs of polymorphisms are considered to be in "strong LD" if the one-sided upper 95% confidence bound on D' is ≥0.98 and the lower bound is ≥0.70. Pairs are deemed to have "strong evidence of historical recombination" if the upper confidence bound on D' is ≤0.90. Based on this, haplotype blocks can be defined as regions over which ≤5% of comparisons among informative polymorphism pairs show strong evidence of historical recombination (Fig. 2).

Linkage Disequilibrium. Table 4 The linkage disequilibrium parameter D

Haplotype	Observed frequency
A_1B_1	$x_{11} = p_1 \times q_1 + D$
A_1B_2	$x_{12} = p_1 \times q_2 - D$
A_2B_1	$x_{21} = p_2 \times q_1 - D$
A_2B_2	$x_{22} = p_2 \times q_2 + D$

Linkage Disequilibrium. Table 5 The linkage disequilibrium parameter D'

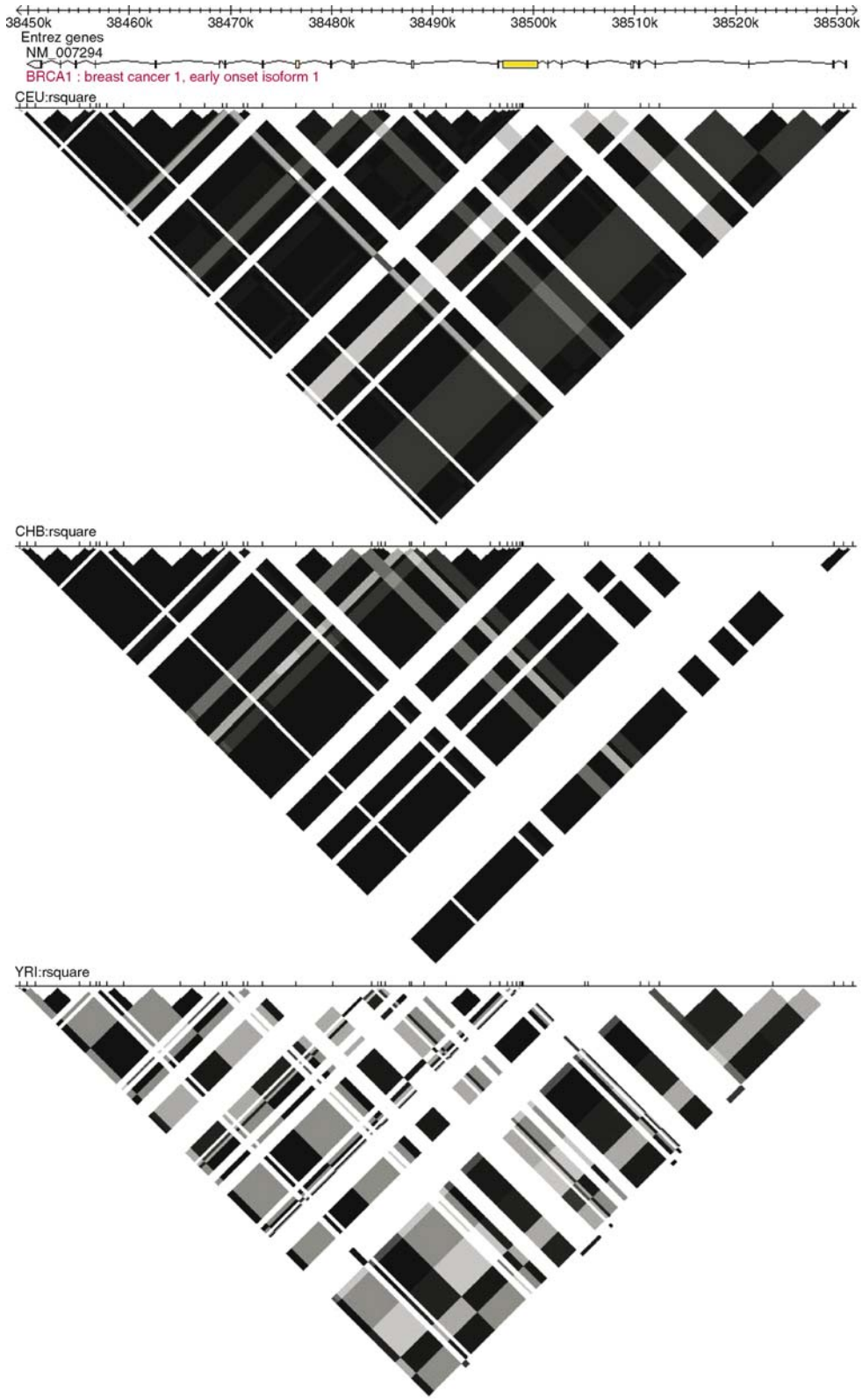
D	D′
$D > 0$	$D' = (x_{11} - p_1 \times q_1)/\min(x_{12}, x_{21})$
$D < 0$	$D' = (x_{11} - p_1 \times q_1)/\min(x_{11}, x_{22})$
$D = 0$	$D' = 0$

Linkage Disequilibrium *versus* Linkage

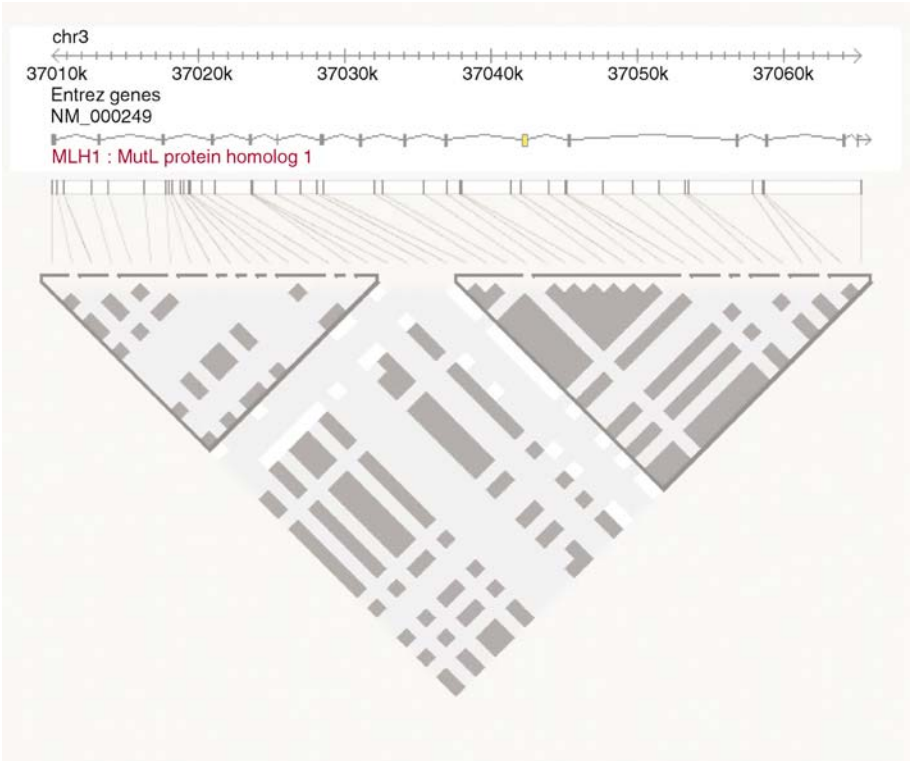
Genetic ►linkage exists when two alleles are co-inherited within a pedigree and this phenomenon is observed across multiple pedigrees. These loci are in linkage because they occur near enough to each other on the same chromosome such that the frequency of recombination (measured as θ) is relatively low. Linkage disequilibrium differs from linkage, in that the former describes alleles while the latter describes loci.

Origins of Linkage Disequilibrium

Linkage disequilibrium arises when a mutation event gives rise to a new allele on a particular chromosome in an individual. The new allele will be associated with the alleles already present on that individual's chromosome for all other loci. In time, as this person reproduces and the population grows, recombination between the new mutation and surrounding loci will return alleles in this



Linkage Disequilibrium. Figure 1 Plots of pairwise linkage disequilibrium (r^2) for polymorphisms in the ►*BRCA1* region genotyped in three populations by the International HapMap Project. CEU, Utah residents with ancestry from northern and western Europe; CHB, Han Chinese in Beijing, China; YRI, Yoruba in Ibadan, Nigeria; white, $r^2 = 0$; black, $r^2 = 1.0$; HapMap Release 22; chromosome 17 NCBI Build 36.



Linkage Disequilibrium. Figure 2 Plot of haplotype blocks among polymorphisms in the *MLH1* region (▶colon cancer) genotyped in Utah residents with ancestry from northern and western Europe by the International HapMap Project.Black, strong linkage disequilibrium; grey, uninformative; white, recombination; HapMap Release 22; chromosome 3 NCBI Build 35.

region to equilibrium, and the new mutation will occur on chromosomes regardless of the background of surrounding loci.

In stable populations, two factors inhibit this return to linkage equilibrium: time and genetic distance. The closer two loci are, the more time (number of recombination events) is required for linkage disequilibrium to break down, and the more recent the mutational event occurred, the larger the region of linkage disequilibrium.

Linkage Disequilibrium in Human Populations

Linkage disequilibrium can also reflect instability or changes in populations. Because modern human chromosomes represents the bottom generation of a very large *Homo sapiens* pedigree, patterns of linkage disequilibrium track with human migration patterns. The migration out of Africa into Europe and Asia is seen, for example, in the observation that modern-day Africans tend to have less linkage disequilibrium, generally, than Europeans or Asians. The relatively small number of "founding" chromosomes which migrated to new continents limited the variation induced by recombination. Therefore, more linkage disequilibrium is generally observed in populations which arose relatively recently.

The International HapMap Project is a large consortium formed in 2002 which seeks to characterize haplotypes and linkage disequilibrium in a variety of human populations. Researchers have performed genotyping on millions of genetic markers in individuals from four populations including Yoruba in Ibadan, Nigeria; Japanese in Tokyo, Japan; Han Chinese in Beijing, China; and Utah residents with ancestry from northern and western Europe. These data are publicly available to researchers who aim to analyze these genetic markers in relation to health conditions, including cancer.

An alternative way to characterize linkage disequilibrium in human populations involves resequencing genes of interest in a variety of DNA samples. Resequencing data has the advantage that a variety of polymorphism types may be detected (including insertions and deletions) and that rare alleles may be characterized. In 1998, the U.S. National Institute of Environmental Health Sciences embarked on the Environmental Genome Project including resequencing of key environmentally-responsive cancer candidate

genes in the populations also assessed by the International HapMap Project. These dense polymorphism data complement the genome-wide HapMap approach and allow researchers access to finer-scale linkage disequilibrium information in key genes involved in ►DNA repair, ►inflammation, ►apoptosis, ►cell cycle, and other pathways.

Using Linkage Disequilibrium to Select Informative Markers

Genetic association studies in ►cancer epidemiology aim to identify common inherited variants which are related to risk of cancer (►case–control studies), treatment response, survival, and other endpoints. These studies often focus on particular candidate genes (suspected because of known biological function related to cancer). Candidate gene studies may utilize linkage disequilibrium in that, rather than genotyping every genetic marker in a suspected gene or genomic region, only those markers thought to be independent are assessed. This "tagging SNP" approach can allow for greater gene coverage and cost efficiency. For example, in a study of 260 candidate genes related to the ►NF-κB pathway, 17,360 common polymorphisms were estimated to be captured by 2,181 tagging polymorphisms.

Numerous methods have been developed to identify subsets of tagging polymorphisms based on analysis of multiple polymorphisms in a genomic region. For example, pairwise-binning methods aim to identify the subset of polymorphisms which are linkage disequilibrium at a specific r^2 threshold with all available polymorphisms, and haplotype-tagging methods aim to aim to identify the subset of polymorphisms which tag all estimated haplotypes over a certain haplotype frequency threshold. ldSelect and Tagger are two commonly-used software tools for identifying tagging polymorphisms.

Data from the HapMap Project, the Environmental Genome Project, other public sources, as well as study participant data, can be used for selection of the optimal set of tagging polymorphisms. It is important to note that population differences in linkage disequilibrium (for example, due to ethnicity or sampling variation) between the more densely genotyped samples (for example, HapMap populations) and the target population (for example, a cancer genetic association study population) must be accounted for when using tagging polymorphisms.

Utility of Linkage Disequilibrium to Genome-wide Association Studies

Genetic association studies can also search the entire genome allowing for identification of cancer-related loci in unpredicted regions. Genome-wide association studies would not be feasible without capitalizing on linkage disequilibrium. This allelic association allows for a reduced set of markers to represent the over 12 million common polymorphisms thought to exist. Numerous commercial panels are available for high-throughput assessment of genome-wide polymorphisms, including from 300,000 polymorphisms to 1,000,000 polymorphisms. For example, a panel available in 2007 from Illumina Corporation (San Diego, CA) assessed approximately 550,000 polymorphisms but was estimated to offer as much genetic information (at $r^2 = 0.85$) as assaying 57%, 89%, 90% of the 3.7 million HapMap polymorphisms among the Yoruban, Asian, and Utah populations described above.

Other Uses of Linkage Disequilibrium in Cancer Research

Linkage disequilibrium also informs hypothesis testing in genetic association studies. The causal allele, if it exists, may be included in the set of assayed markers or it may lie on a haplotype defined by the set of assayed markers. Analysis of haplotypes may, therefore, provide additional information on association. Numerous methods exist for testing haplotype associations with disease (including score tests, logistic regression, and Bayesian methods). All methods first estimate haplotype frequencies (or probabilities) because haplotypes are usually not directly observed.

If an association signal is detected, linkage disequilibrium can be used in refinement of the signal where an association is detected (fine-mapping). A particular polymorphism found to be associated with disease may be in linkage disequilibrium with the truly causal allele. Thus, additional analysis of nearby polymorphisms may increase the association signal and provide a targeted region for functional genomics analysis. In summary, incorporation of linkage disequilibrium has the potential to improve the power and efficiency of genetic association studies leading to increased understanding of the genetic causes of cancer.

►Case–Control Association Study

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N-Linked Glycan

Definition

Asparagine (Asn/N)-linked glycan is a major structural type of sugar chain in glycoproteins. Oligosaccharides with Glc3Man9GlcNAc2 are initially transferred to Asn residues in the consensus Asn-X-Ser/Thr sequence in the newly synthesized glycoproteins in the endoplasmic reticulum (ER). The glycans are processed and modified in the secretory pathway in the ER and Golgi apparatus.

►Calreticulin

O-Linked Oligosaccharide

Definition

A glycan glycosidically linked to the hydroxyl group of the amino acids serine, threonine, tyrosine, or hydroxylysine. Regarding mucin glycosylation, the term **O**-glycan is used to denote the common linkage GalNAcα1-O-Ser/Thr.

►Mucins

Linoleic Acid

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Definition

Linoleic acid (LA) is an 18-carbon, polyunsaturated fatty acid (PUFA), which contains two *cis* double bonds. Because mammals cannot introduce a double bond at carbon atoms beyond *C-9* in the fatty acid chain, linoleic acid (18:2 *cis*-Δ^9,Δ^{12}) and linolenic acid (18:3 *cis*-Δ^9, Δ^{12},Δ^{15}) are two essential fatty acids. LA possesses low melting temperature and provides fluidity to cell membranes. LA is mainly contained in plant oils, such as safflower oil and corn oil.

Characteristics

The effect of linoleic acid (LA) on health is still controversial. LA is one of the two essential fatty acids, which means dietary supplementation of LA is necessary for maintaining cell activity. Saturated fatty acids have been implicated in obesity, heart disease, diabetes, and cancer while PUFAs generally have a positive effect on health; however, a high ω-6/ω-3 ratio, which is associated with today's Western diets, promotes the pathogenesis of many diseases, including cardiovascular disease, inflammatory diseases, and cancer. Several in vivo studies suggest that a high amount of ω-6 PUFA such as LA might enhance the incidence of some types of cancers via stimulation of epithelial cell proliferation. Experiments in animal models of mammary and colorectal carcinogenesis suggest that fatty acids promote tumor development; ω-6 PUFA generally stimulate tumor growth, while ω-3 fatty acids oppose this effect.

Other studies lead to opposite conclusions. The associations between monounsaturated fatty acids, trans fatty acids, PUFAs such as LA, alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), or ω-3/ω-6 ratio, and colorectal cancer are not convincing. Contrary to data from animal experiments, human studies do not show an association of breast cancer risk with ω-6 PUFA intake. High LA and arachidonic acid (AA) concentrations have been observed in insulin resistance-associated diabetic complications and in some tumors, but these are multifactorial processes that include many lifestyle determinants. It is therefore questionable to involve only ω-6 fatty acids in their etiology.

The controversial roles of LA might be due to the possibility that different metabolic pathways result in opposite effects. ω-6 PUFAs play a significant role in inflammatory and/or immune responses by bioactive molecules including PGE2.

LA is integrated into cytoplasmic membrane and is metabolized to AA through γ-linolenic acid, and dihomo-γ-linolenic acid (►Arachidonic acid-pathway and cancer). AA is stored as phospholipids in cytoplasmic membrane and serum, and is released by cleavage with phospholipase A2. AA is metabolized by cyclooxygenase-1 and cyclooxygenase-2 (COX-2) (►Cyclooxygenase-2 in colorectal cancer) to ►prostaglandins (PGs), thromboxanes (TXs), and ►leukotrienes (LTs). COX-2 is an inducible enzyme of COXs, which is absent in normal cells. In contrast, COX-1 is a constitutive enzyme expressed ubiquitously. COX-2 expression is induced in carcinogenic processes in many malignancies, involving colorectal, esophageal, breast, lung, pancreatic, and bladder cancers. Induced COX-2 expression in epithelial cells and also in stromal cells provides PGE2 in the local tissues. PGE2 possesses immunosuppressive and proinflammatory effects. COX-2-dependent overproduction of PGE2 is hypothesized to be an important part of sustained proliferative and chronic inflammatory conditions in colorectal epithelium, which are closely associated with

carcinogenesis (►Inflammation in cancer). PGE2 also has a strong association with colorectal cancer progression by promoting cell survival, cell growth, migration, invasion, and angiogenesis (►Colon cancer). The various biological effects exerted by PGE2 are through the G-protein coupled cytoplasmic membrane E-prostanoid receptors termed EP1 to EP4.

15-Lipoxygenase-1 (15-LOX-1) is known for its antiinflammatory properties and has a profound influence on the development and progression of cancers. 15-LOXs belong to the structurally and functionally related nonheme iron dioxygenases family. 15-LOXs are responsible for oxidative metabolism of ω-6 PUFAs, such as LA and AA to eicosanoids. Two isoforms are known in 15-LOXs; 15-LOX-1 (leukocyte type) and 15-LOX-2 (epidermis type). Both isoforms are expressed in normal and tumor tissues in various combinations. Different from other LOXs, such as 5-LOX and 12-LOX, 15-LOXs, 15-LOX-1 is revealed also as an ►apoptosis inducer in human cancers and inhibits cancer progression in several types of cancers, including colorectal, and breast cancers. By the contrary, reduction of 15-LOX-1 is correlated with the disease progression of breast and colon cancers.

For antitumor effects, 15-LOX-1 is closely associated with peroxisome proliferator-activated receptor γ (PPARγ) (►Peroxisome proliferator-activated receptor and cancer) activation. The oxidative metabolites of LA by 15-LOX-1 can function as endogenous activators and ligands of PPARγ. In particular, 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), and 13-oxooctadecadienoic acid (13-OXO) have biological effects as a PPARγ ligand. PPARγ is originally identified to induce adipocyte differentiation. PPARγ is a nuclear hormone receptor superfamily of ligand-activated transcription factors. PPARγ is dimerized with retinoic X receptor (►Retinoid receptor crosstalk in cancer), and binds specific responsive element within promoter DNA sequence to regulate gene expression. PPARγ initiates transcription of genes associated with energy homeostasis, cell growth, and anti-/proinflammatory effect. PPARγ is activated by endogenous secreted prostaglandins and fatty acids. 15-Deoxy-δ(12,14)-prostaglandin J2 is a strong endogenous ligand of PPARγ. Decrease in PPARγ expression is associated with cancer metastasis. PPARγ play a role in transcriptional regulation of cancer-related genes. Ligand activation of PPARγ in colorectal cancer cells attenuates colonic inflammation and causes a reduction growth via the induction of apoptosis. Conjugated LA (CLA), a strong ligand for PPARγ, has a substantial anticarcinogenic effect. Synthesized PPARγ ligands including troglitazone have been shown to be effective chemopreventive agents in a rat model of carcinogenesis and in AOM-induced colon cancer in mice. In in vitro transformation model, LA inhibits intestinal cell transformation. Inhibitory effect of PPARγ to cancer metastasis is also reported in several cancers, such as nonsmall cell lung cancer, colon cancer, thyroid cancer, and breast cancer. Downregulation of EGFR, TGF-α (►Epidermal growth factor receptor ligands), and upregulation of Bax, p21Waf-1 (►p21(WAF1/CIP1/SDI1)), ►E-cadherin by PPARγ activation induce antiproliferative, proapoptotic, and prodifferentiation effects. These alterations of gene expressions provide LA-induced anticarcinogenic, antitumor, and antimetastatic effects on cancer cells.

The sequential alteration of concurrence of COX-2 upregulation and 15-LOX-1 downregulation is found in the adenoma-carcinoma transition in colorectal neoplasia (►Colorectal premalignant lesions). Low grade adenomas express 15-LOX-1 but not COX-2; high grade adenomas and early carcinomas show decreased 15-LOX-1 expression and induction of COX-2 expression; and advanced carcinomas express COX-2 but not 15-LOX-1. It possibly shows close association of the switching of LA-metabolizing pathways with colon cancer development and progression. In expression of 15-LOX-1, several conditions play an important role. Cytokines, such as IL-4 (►Interleukin-4) and IL-13, high ratio of ω-3/ω-6 fatty acids, and ►nonsteroidal antiinflammatory drugs (NSAID), such as sulindac sulfone induce 15-LOX-1 expression and activation of PPARγ. Reciprocally, activated PPARγ represses COX-2 by inhibition of NFκB (►Nuclear factor κB) and ►AP-1. This negative regulation of COX-2 expression by PPARγ activation might be one of mechanisms of reverse expression between 15-LOX-1 and COX-2. Furthermore, promoter DNA methylation is responsible for silencing of 15-LOX-1 expression (►Epigenetic gene silencing). The epigenetic alteration might be a trigger to switch 15-LOX-1 repression and COX-2 upregulation along with malignant transformation and cancer progression in colon cancer.

Excess uptake of LA increases cancer metastasis by enhancing cell embedding into the target organs. LA-derived TXA2 accelerates platelets aggregation involving cancer cells. LA also affects cancer cell activity. Short-term treatment with LA induces apoptosis in cancer cells. In the nude mice peritoneal dissemination model, LA treatment inhibits formation of peritoneal metastasis. In contrast, cancer cells exposed to LA for long-term show quiescent condition in vitro and dormancy in transplanted animals. In these cells, decrease of EGFR, VEGF (►Vascular endothelial growth factor), and increase of ►BCL-2 are observed. Thus, LA might play a role in formation of cancer cell dormancy and delayed metastasis.

In summary, the effects of LA on human health are still not clearly figured out. As LA is an essential fatty acid, we cannot cease LA uptake. In further studies, it is

important to make use of the beneficial side of the double-edged sword of LA for prevention and treatment of cancer.

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Lipid Mediators

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Synonyms

Lipid second messengers; Bioactive lipids

Definition

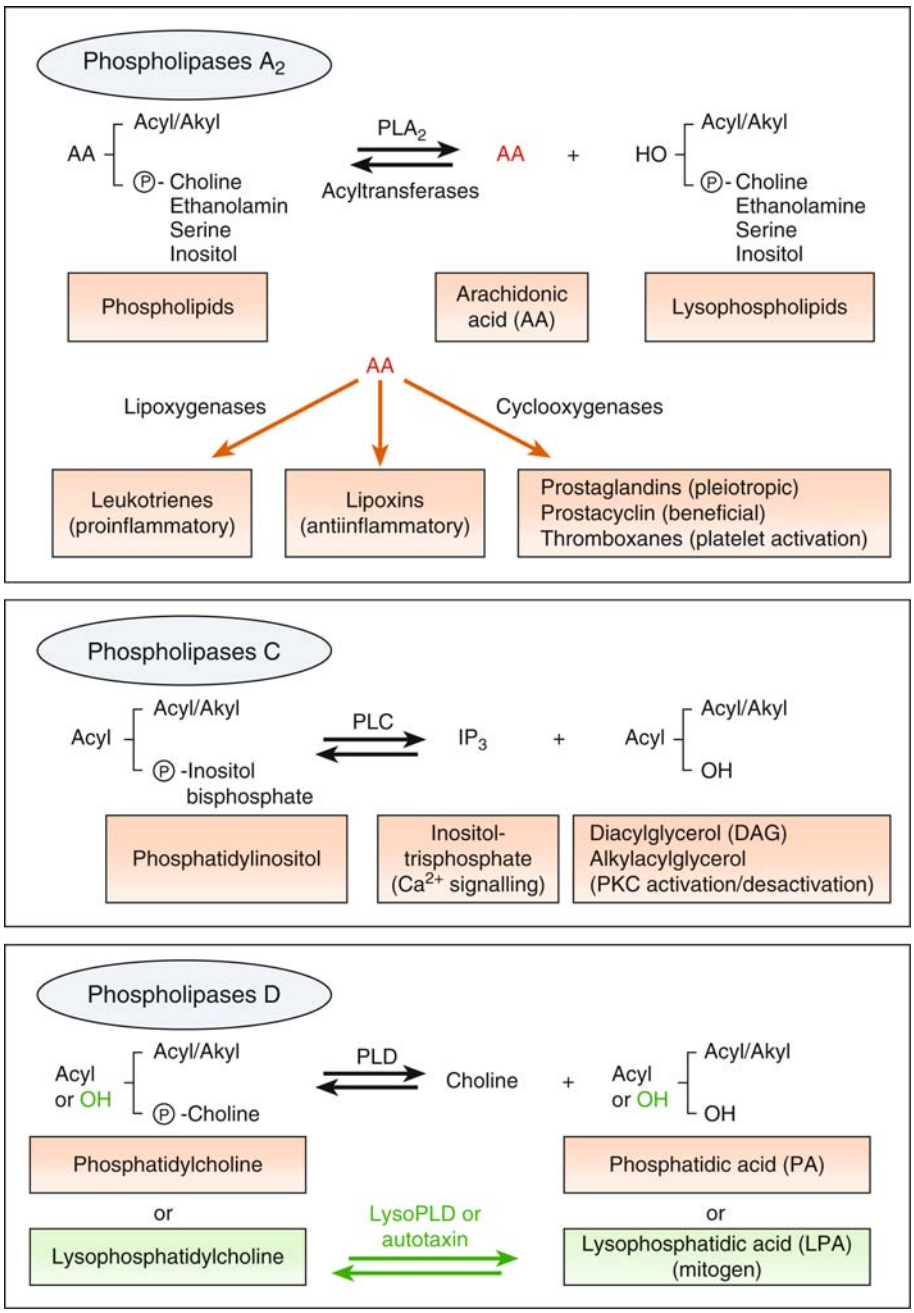
Lipid mediators are bioactive molecules rapidly produced upon cell activation either by enzymatic process or by oxidative fragmentation of polyunsaturated fatty acids attached to their glycerol backbone. The majority of lipid mediators are products of degradation and/or phosphorylation/dephosphorylation of glycerophospholipids by defined enzymes acting in concert and commonly designated as ►phospholipases (PLs), phosphokinases and phosphatases. Hydrolysis of ►phospholipids also generates free fatty acids, including arachidonate, a direct precursor of ►eicosanoids.

Characteristics

Within seconds after cell activation, certain phospholipids are either selectively hydrolyzed by defined phospholipases leading to highly bioactive molecules (lipids and/or fatty acids) or phosphorylated by specific kinases to bioactive ►phosphoinositides Inositol lipids. Essentially three phospholipase (PL) families, namely ►Phospholipase A2 (PLA2), ►Phospholipase C (PLC) and ►Phospholipase D (PLD) (Fig. 1) are involved in the degradation of structural phospholipids, leading either to direct formation of lipid mediators (►lysophosphatidylcholine in Cancer, ►inositoltrisphosphate (IP3) and ►diacylglycerol (DAG)) or to freeing of their immediate precursors (polyunsaturated fatty acids, including arachidonic (►Arachidonic acid-pathway and cancer) and ►Linoleic acid). Among PLA2s, the secretory group II PLA2 can produce (►Lysophospatidic acid (LPA) directly from phosphatidic acid. Recently described ►autotaxin, which is widely implicated in tumor progression, possesses lysoPLD activity and generates LPA from lysophosphatidylcholine (Fig. 1).

Free arachidonate, produced by degradation of phospholipids by PLA2, is a substrate for three major classes of lipid mediators (Fig. 1) (►Arachidonic acid-pathway and cancer): prostanoids/thromboxanes (►Prostaglandins), (►Leukotriene) and lipoxins, the latter being beneficial as they induce the resolution of the inflammatory response (►Inflammation). Upon (►Oxidative stress) arachidonate esterified to phospholipids at the sn-2 position of glycerol is oxidized either to ►isoprostanes, very potent proinflammatory mediators (►Bioactive lipid signaling) or to several ►oxidized phospholipids with xshort carbon chains resembling ►Platelet-activating-Factor (PAF) and endowed with potent capacity to modulate the immune response and (►Angiogenesis). Interestingly, an analogue of PAF containing a non-hydrolysable methyl group at the sn-2 position of glycerol, ►ET-18-OCH3 or edelfosine, is a selective antitumor phospholipid targeting (►Apoptosis) via endogenous activation of Fas/CD95 death receptor.

►Sphingolipids are a subclass of phosholipids which enter many metabolic pathways and constitute an interconnected network of signaling molecules with crucial roles in both cancer development and progression (►Cancer). ►Sphingomyelin, a structural phospholipid, is hydrolyzed by sphingomyelinases to a tumor suppressor lipid, ►ceramide, with proapoptotic and antiproliferative properties (Fig. 2) (►Ceramide). Ceramide may be either phosphorylated to ►ceramide-1-phosphate (C1P) with proinflammatory and antiapoptotic properties or may be degraded to ►sphingosine, a substrate for phosphorylation leading to S1P, a potent tumor-promoting lipid endowed with angiogenic and immunosuppressive properties. Additionally ceramide is glycosylated into ►gangliosides, bearing various sugars and endowed with highly tumorogenic properties (Fig. 2) (►Ganglioside). It is admitted today that the balance between the tumor suppressor molecule – ►ceramide – and its tumor-promoting metabolites is of prime importance in (►Carcinogenesis) and anticancer therapy.

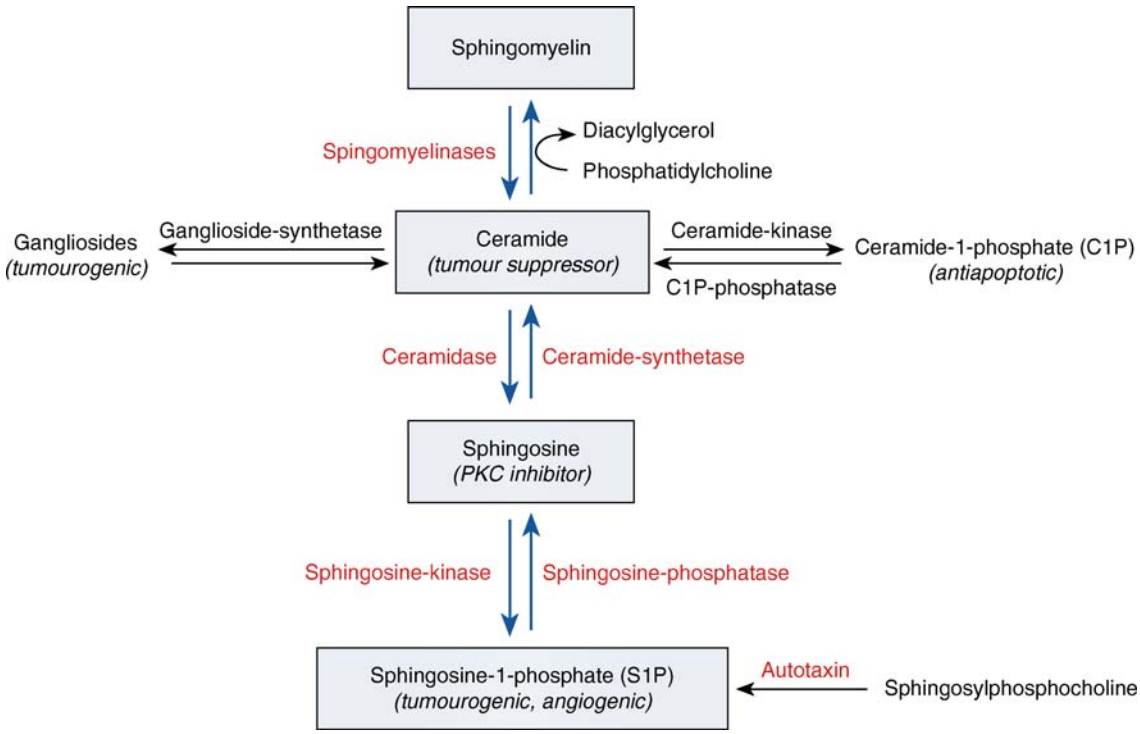


Lipid Mediators. Figure 1 Schematic representation of production of various lipid mediators by phospholipases.

►Conjugated linoleic acid (CLA), which is a mixture of positional and stereoisomers of octadecadienoate (18:2) found in foods derived from ruminants, may reduce carcinogenesis; however the data are still controversial. Equally, prostaglandin J2, which derives from oxidation of arachidonate by cyclooxygenases (COXs) is an emerging antineoplastic agent as it induces death receptor 5 (DR5), a specific ►receptor for TNF-related apoptosis-inducing ligand (TRAIL).

The majority of lipid mediators target distinct families of G-protein coupled receptors and activate vital cellular defenses usually defined as inflammatory reactions; however, some of these mediators, including LPA, modulate cell proliferation, growth, survival and (►Migration) enabling the expansion of defined cell subsets. LPA levels are elevated *in situ* and in circulation in ovarian tumors and in multiple myeloma, supporting its causal effect in proliferation and thus eventual use as a target and/or a diagnostic marker.

►Protein kinase C (PKC) isoformes are a family of serine/threonine protein kinases commonly divided into three subfamilies: classical, novel, and atypical. PKC



Lipid Mediators. Figure 2 Schematic representation of bioactive sphingolipid formation from sphingomyelin.

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isoformes, whose expression is cell type-specific and developmentally regulated, are key transducers in many agonist-induced signaling pathways (►Protein kinase C family). At least ten different PKC isoforms have been identified and are believed to play distinct regulatory roles. PKC isoforms are catalytically activated by several lipid cofactors, including DAG generated by receptor-mediated hydrolysis of membrane phospholipids. IP3 triggers Ca^{2+} release from internal stores, and the elevation of cytosolic Ca^{2+} acts synergistically with DAG to activate the relevant forms of PKC. PKC isoforms reside in the cytoplasm in an inactive conformation and undergo translocation to the plasma membrane or cytoplasmic organelles upon cell activation; however, PKC isoenzymes are also capable of translocating to the nucleus and certain isoforms can even reside within the nucleus. Certain PKC isoforms are often overexpressed in cancer. Tumor promoting phorbol esters and DAG activate classical and novel PKC isoforms. Naturally occurring retinoids (►Retinoic Acid), antisense oligonucleotides against specific PKC isoforms and specific PKC inhibitors can block this activation. Beta carotene and retinoid derivatives act as anticarcinogenic agents and can antagonize some of the biological actions of phorbol esters and oxidants.

One of the major inositol-containing lipid second messengers, the ►phosphoinositide-3,4,5-trisphosphate (PIP3) (►Phosphoinositide-3,4,5-trisphosphate-kinases (PI3-kinases)) is generated by the action of PI3-kinases activated in response to a variety of extracellular signals (►PI3K Signaling). Phosphatase ►PTEN, one of the most frequently mutated genes in human cancer, acts as a tumor suppressor by dephosphorylating PIP3, thus preventing both elevated levels of PIP3 and tumorogenesis (►PTEN). Loss or inactivation of PTEN leads to the formation of a variety of tumors, probably by deregulating ►the mammalian target of rapamycin (mTOR) signaling pathway. As mTOR activity can be suppressed by various compounds, including (►Rapamycin), it may be useful to treat tumors carrying inactivated PTEN with such drugs.

Inflammation has been associated with cancer recently. Beside cytokines and chemiokines incriminated already, lipid mediators are also implicated since the discovery that cyclooxygenase-2 (COX-2) inhibitors (►coxibs) (►Celecoxib) slow the progression of colorectal cancer (►Cyclooxygenase-2 in colorectal cancer) and that the COX-2 levels are increased in several tumors (colon, breast, ovarian and melanomas); for this reason the gene encoding for COX-2 is now considered an (►Oncogene). Equally, the products of 5-lipoxygenase may play a role in human tumors (brain, skin). Finally, the relatively recently discovered ►oxidized phospholipids, the analogues of the mediator PAF, are involved in the induction of immune suppression and might be instrumental in inducing cancer.

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Lipid Peroxidation

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Definition

►Lipid peroxidation is the metabolic process in which ►reactive oxygen species (ROS) result in the oxidative deterioration of lipids. This may significantly affect cell membrane structure and function.

Characteristics

Lipid peroxidation most often affects ►polyunsaturated fatty acids, because they contain ►methylene -CH2- groups which contain hydrogen that is especially reactive with ROS. Increased ROS production occurs in inflammation, during radiation, or during metabolism of hormones, drugs and environmental toxins. This can overwhelm endogenous protective ►antioxidant mechanisms and increase ROS-mediated damage to membrane structure and function. Such ROS reactions can also lead to protein damage, including DNA repair enzymes and polymerases, impairment, and production of aldehyde by-products such as malondialdehyde (MDA; β-hydroxy-acrolein) and 4-hydroxy-2-nonenal (HNE). MDA is formed during homolytic decomposition of lipid hydroperoxides that contain more than two double bonds. MDA reacts with DNA to form primarily a propane adduct with 2′-deoxyguanosine (M1G-dR). Although they have important physiological roles in cell proliferation, transformation, differentiation and ►apoptosis these aldehydes are also strongly carcinogenic. Mutagenicity of MDA and HNE, the major aldehyde products has been clearly demonstrated. These can promote the formation of DNA-adducts which are required to be repaired in order to maintain the fidelity of the DNA. If not, DNA ►mutations can occur. For example, the reaction between the epoxide of HNE with DNA leads to the formation of unsubstituted ethenodAdo adducts. Etheno adducts are mutagenic and have been detected in human tissue samples providing an important link between lipid peroxidation and in-vivo DNA-adduct formation. Alternatively, lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells. This suppression of cell cancer growth is enhanced by prooxidants and eliminated by antioxidants, and this elimination is proportional to the inhibition of lipid peroxidation products by antioxidants. Lipid peroxidation may also play an important role in the potential anticarcinogenic effects of other dietary factors including soy, marine n-3 fatty acids, isothiocyanates, green tea and vitamin D and calcium.

Mechanisms

As with any radical reaction the reaction consists of three major steps: initiation, propagation and termination. Initiation is the step whereby a fatty acid radical is produced. The initiators in living cells are most notably ROS such as hydroxyl radical, which combines with a hydrogen atom to make water and a fatty acid radical. The fatty acid radical is not a stable ►molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This too is an unstable species that reacts with another free fatty acid producing a different fatty acid radical and a ►hydrogen peroxide molecule or a cyclic peroxide molecule if it had reacted with itself. This cycle propagates itself as the new fatty acid radical reacts in the same way. This results in a chain reaction and the only way to stop a radical reaction is for two radicals to react and produce a non-radical species. This occurs when the concentration of radical species is high enough for there to be a high probability of two radicals actually colliding. However, in organisms there are a number of different molecules which bind and quench free radicals and so protect lipids from oxidation. These are usually lipid soluble vitamins such as alpha-tocopherol or vitamin E.

ROS-mediated formation of lipid hydroperoxides involves the initial abstraction of a bis-allylic methylene hydrogen atom. Lipid hydroperoxides can also be formed by the action of cyclooxygenases and lipoxygenases on polyunsaturated fatty acids (PUFAs). LOX and COX-mediated pathways of PUFA metabolism can potentially provide a rich source of lipid hydroperoxides.

Clinical Aspects

Chronic inflammation, part of the host immune response, has long been recognised to be associated with the development and progression of cancer. The combination of excess oxidant production and antioxidant depletion, and therefore, oxidative stress, may play a role in the development and progression of cancers. High ROS generation and persistent oxidative stress have been recognised as characteristics features of carcinoma cells both in vivo and in vitro. Also, it is widely accepted that patients with advanced cancer have reduced circulating antioxidant concentrations.

Therefore, in the cancer patient the risk for structural and functional damage of cell membranes is likely to be increased. Higher levels of circulating plasma MDA have been observed in different malignancies, including lung, gastrointestinal and hormone dependent cancers. However, whether such increased MDA concentrations are primarily due to the tumour, the ►inflammatory response or some other factors remain to be determined.

►Reactive Oxygen Species

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Lipid Phosphatases

Definition

A family of signaling molecules in the cells that includes MAPK and PI3K.

►Tight Junction
►MAP Kinases
►P13K signaling

Lipid Raft

Definition

Cholesterol and sphingolipid enriched microdomains in plasma membranes; they function as signaling platforms.

►Natural Killer Cell Activation

Lipid Second Messengers

►Lipid Mediators

Lipid Therapy

►Membrane-Lipid Therapy

Lipogenesis

Definition

Is the process that converts nonfat food materials into body fat.

►Cachexia

Lipoma

Definition

Is a benign neoplasm of adipose tissue. One of the most common soft tissue tumors in men and form part of the daily practice of every surgical pathologist. Like fat, lipomas are mainly composed of mature fat cells, but the cells vary slightly in size and shape and are somewhat larger. The tumors are usually thinly encapsulated and have a distinct lobular pattern. A minority of all lipoma patients has multiple lesions, but most patients only have one tumor. Most of these solitary lipomas cause few problems other than those of a localized mass.

►Lipoma Preferred Partner
►Cowden Syndrome

Lipoma Preferred Partner

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Synonyms

LIM domain containing preferred translocation partner in lipoma; LPP

Definition

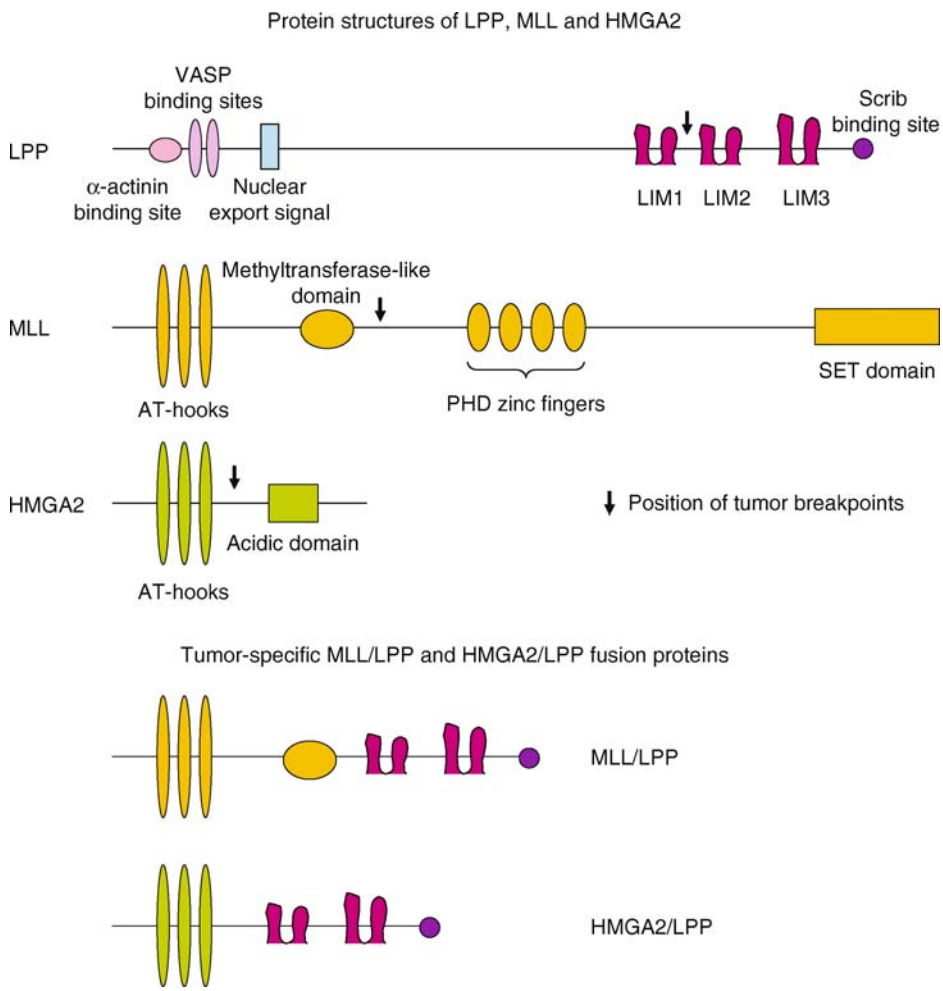
Lipoma preferred partner (LPP) is a ►LIM domain protein that belongs to the ►zyxin family of cytoskeletal

adaptor proteins. LPP is localized in cellular adhesion sites and is in certain conditions also detected in the nucleus where it acts as a transcriptional co-activator. It is highly expressed in smooth muscle cells and plays a role in cell migration. In particular tumors, aberrant LPP proteins are expressed in which the LIM domains are fused to DNA-binding domains. These tumor-specific LPP fusion proteins function as transcription factors.

Characteristics

LPP and Tumors

►Lipomas are benign tumors of adipose tissue and one of the most commonly occurring soft tissue tumors in humans. A minority of patients has multiple tumors, however most patients have only one tumor. More than 60% of human solitary lipomas have an abnormal ►karyotype. Two thirds of the latter carry chromosomal aberrations, mostly translocations, involving chromosomal region 12q15. The gene on 12q15 that is affected by these translocations is ►HMGA2. The HMGA2 protein consists of three AT-hooks (DNA binding domains) followed by an acidic carboxyterminal tail (see Fig. 1). By means of the translocation, the DNA binding domains are separated from the acidic tail. Multiple chromosomes have been identified as translocation partner of 12q15 in lipomas by cytogenetic analysis, indicating that many different genes are able to act as translocation partner of HMGA2 in these tumors. However, in a quarter of the cases, chromosome 3 at bands q27-q28 is found as the translocation partner of chromosome region 12q15. This means that the most consistent chromosomal aberration in lipomas is represented by t(3;12)(q27-q28;q15), being present in about 10% of all solitary lipomas. In 1996, the gene on 3q27-28 that is affected by the t(3;12) was discovered in the lab of Wim Van de Ven from the University of



Lipoma Preferred Partner. Figure 1 Upper part: Schematic representation of the LPP, MLL and HMGA2 proteins. Lower part: Schematic representation of the tumor-specific MLL/LPP and HMGA2/LPP fusion proteins.

Leuven, Belgium and named "*LPP*" for "lipoma preferred partner." After publication, the HUGO gene nomenclature commission advised to change the name of LPP in "LIM domain containing preferred translocation partner in lipoma." Indeed, the LPP protein contains three LIM domains in its carboxyterminus that are preceded by a proline-rich pre-LIM region (Fig. 1). Through the t(3;12), an *HMGA2/LPP* fusion gene is created encoding an HMGA2/LPP fusion protein consisting of the three DNA-binding domains of HMGA2 followed by the two most carboxyterminal LIM-domains of LPP (see Fig. 1). Fusion transcripts encoding identical HMGA2/LPP fusion proteins have also been detected in others tumors including pulmonary chondroid hamartomas, a ►parosteal lipoma, and a soft tissue chondroma. The HMGA2/LPP fusion protein localizes in the nucleus of cells and is a tumor-specific transcription factor: it binds DNA in the promoter of its target genes with the DNA-binding domains of HMGA2 and activates transcription through the LIM domains of LPP. In addition to benign tumors, rearrangements of the *LPP* gene have also been found in a case of malignant secondary acute monoblastic leukemia with a t(3;11)(q28;q23). In the latter, the two most carboxyterminal LIM domains of LPP are fused to the AT-hooks (DNA binding domains) of the mixed lineage leukemia (►MLL) protein (see Fig. 1). As such, as well in benign as in malignant tumors, tumor-specific fusions proteins are composed of AT-hooks, either from HMGA2 or from MLL, and LIM domains from LPP.

Cell Biological Characteristics of the LPP Protein

LPP is a member of the zyxin family of LIM domain proteins, which consists of seven members: zyxin, TRIP6/►ZRP1, LPP, ►ajuba, ►LIMD1, ►WTIP, and ►migfilin/Cal. LPP is most closely related to zyxin and ►thyroid receptor interacting protein 6 (TRIP6). All members of the zyxin family contain a proline-rich region (in LPP this region covers the amino-terminal 2/3 of the protein), which is followed by three LIM domains. LPP contains multiple protein-protein interaction domains. Known binding partners include the cytoskeletal proteins α-actinin, VASP (vasodilator-stimulated phosphoprotein) and palladin, the ETS domain transcription factor PEA3, and the tumor suppressor Scrib. In addition, LPP probably also interacts with a number of yet unidentified proteins that bind its three carboxyterminal LIM domains, which are modular protein binding interfaces.

In cultured fibroblasts and aortic SMCs, LPP colocalizes with vinculin at focal adhesions, which are attachment sites to the extracellular matrix. The three LIM domains of LPP cooperate to target the protein to these adhesion sites. LPP is also found in cell-cell contacts, and in transverse sections of bladder smooth muscle, an association of LPP with peripheral dense bodies is suggested. In addition to these cytoskeletal localizations, LPP also shuttles to the nucleus. Its nuclear-cytoplasmic localization is regulated in part by a nuclear export signal (NES) which is sensitive to the drug leptomycin B.

LPP as a Transcriptional Coactivator

In 2006, the lab of Andy Sharrocks from the University of Manchester, U.K., discovered a nuclear function for LPP as a coactivator for ►PEA3. PEA3 is an ETS domain transcription factor whose expression is regulated by a number of signaling cascades, including the mitogen-activated protein (MAP) kinase pathways. PEA3 is a transcriptional activator and regulates transcription of multiple genes, including the matrix metalloproteases (MMPs) MMP-1 and MMP-9. MMP's are enzymes that degrade the extracellular matrix during normal remodeling events and cancer metastasis. PEA3 is expressed during normal mammary gland development and is an important player in breast tumor metastasis. Also LPP is expressed in normal and cancerous breast tissue, and is recruited to the MMP-1 promoter in a PEA3-dependent manner.

LPP as a Smooth Muscle Marker

In 2003, two independent laboratories (the Somlyo lab from the University of Virginia, USA and the Lindahl lab from the University of Göteborg, Sweden) identified LPP as a novel smooth muscle cell marker. Although, in adults, LPP is ubiquitously expressed, it is highly expressed in vascular as well as visceral smooth muscle-containing tissues such as uterus, stomach, bladder, aorta and portal vein. Concerning the function of the LPP protein in smooth muscle cells, LPP is involved in the regulation of their migration, as shown in in vitro experiments.

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Lipomas

►Adipose Tumors

Lipomatous Tumors

►Adipose Tumors

Lipophilic

Definition

Literally means fat loving or greasy. These are drugs that much prefer to dissolve in fats than in water.

►ADMET Screen

Lipophilicity

Definition

Defines the molecular characteristic of being attracted to non-polar environments – literally the property of being "oil loving." This term is often used to describe a particular drugs preference for organic (non-polar), compared to water-based (polar) environments.

►Chelators as Anticancer Drugs

Lipoprotein

Definition

Biochemical assembly that contains both proteins and lipids.

►Wnt Signaling

Liposarcomas

►Adipose Tumors

Liposomal Chemotherapy

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Synonyms

Drug delivery vehicles; Drug carriers

Definition

The central problem in cancer chemotherapy (►Chemotherapy of cancer, progress and perspectives) is the severe toxic side effects of anticancer drugs on healthy tissues. The use of ►liposomes as drug delivery vehicles (►Drug delivery systems in cancer) for antitumor therapeutics has great potential to revolutionize the future of cancer therapy. As tumor architecture causes liposomes to preferentially accumulate at the tumor site, their use as drug carrier result in the localization of a greater amount of the drug load at the tumor site, thus improving cancer therapy and reducing the harmful nonspecific side effects of chemotherapeutics. In addition, targeting of liposomal anticancer drugs to antigens expressed or overexpressed on tumor cells provides a very efficient system for increasing the therapeutic indices of the drugs.

Characteristics

The Magic Bullet Concept

Within medical practice, there has long been the desire to achieve selective delivery of drugs to specific areas in the body in order to maximize drug action and minimize side effects. It is well-known that many drugs, while having a beneficial action, can also exhibit deleterious effects that may limit their clinical utility. Drugs used in cancer chemotherapy represent a clear example of this problem. Cytotoxic compounds can kill target cells, but also normal cells in the body.

The ►magic bullet concept, first expounded by the German physician Paul Ehrlich, represents an early description of the drug-targeting paradigm. Indeed, about one century ago Ehrlich coined the word "chemotherapy" to indicate the possibility to design and produce, by chemical synthesis, drugs, the so-called "magic bullets" as they were able to kill infectious agents

without affecting the human body. However, at that time, the recipe for the synthesis was based on four "G": Geld (money), Geduld (patience), Geschick (skill), and Glùck (luck). Indeed, until the last decade of the twentieth century, the most impressive progression in discovery of new chemotherapeutics has been due to "serendipity."

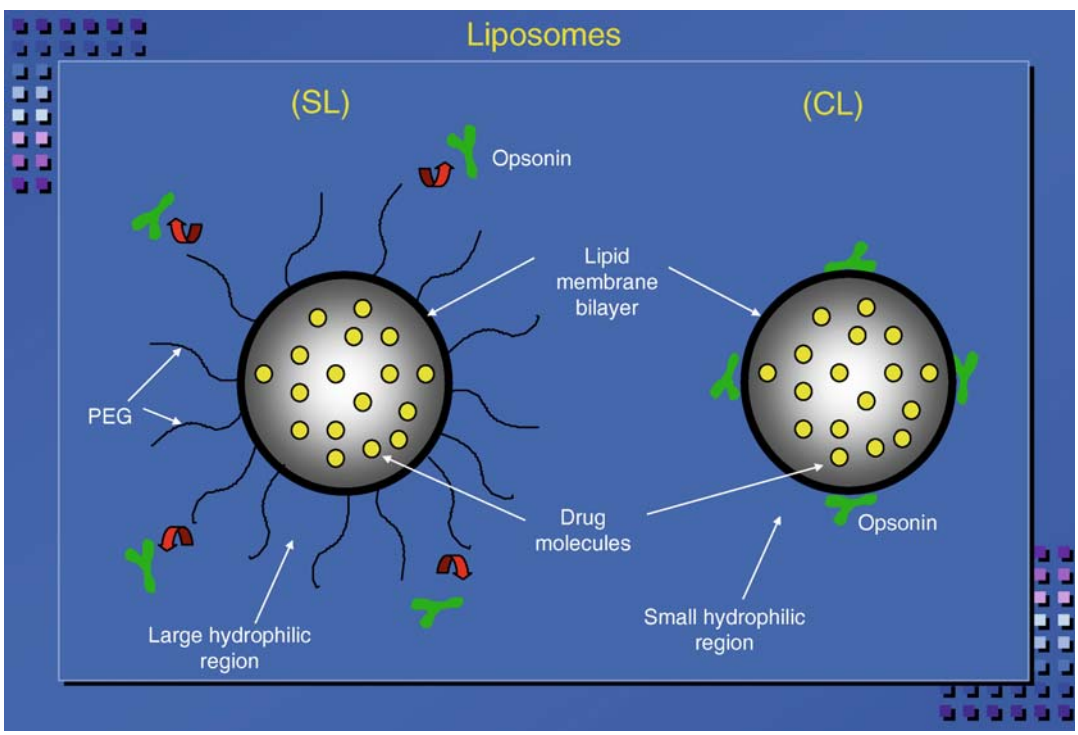
Drug Delivery Technology

The medical community has recently sought alternative therapies that improve selective toxicities against cancer cells. Nanobiotechnology, defined as biomedical applications of nano-sized systems, is a rapidly developing area within ►nanotechnology. ►Nanoparticles, such as liposomes, allow unique interaction with biological systems at the molecular level. They can also facilitate important advances in detection, diagnosis, and treatment of human cancers and have led to a new discipline of nano-oncology. Nanoparticles are being actively developed for tumor imaging in vivo, biomolecular profiling of cancer biomarkers, and targeted drug delivery.

Sterically Stabilized (Stealth) Liposomes

Several nanotechnological approaches have been used to improve delivery of chemotherapeutic agents to cancer cells with the goal of minimizing toxic effects on healthy tissues while maintaining antitumor efficacy. Among the most popular and well-investigated drug carriers are liposomes. Conventional liposomes (CL), first described by professor Alec D. Bangham in 1965, are made up of amphiphilic phospholipids and cholesterol, which, upon hydration, self-associate to form bilayers surrounding an aqueous interior (Fig. 1). Liposomes are artificial phospholipid vesicles with sizes varying from 50 to 1,000 nm, which can be loaded with a variety of water-soluble drugs (into their inner aqueous compartment) and sometimes even with water-insoluble drugs (into the hydrophobic compartment of the phospholipid bilayer).

One of the major drawbacks of conventional liposomes has been their rapid clearance from blood, due to adsorption of plasma proteins (opsonins) to the "naked" phospholipid membrane, triggering recognition and uptake of the liposomes by the mononuclear phagocytic system (MPS), also referred to as the reticuloendothelial system (RES). A major advance in the field of liposomes came with the development of sterically stabilized (Stealth®) liposomes (SL), which utilize a surface coating of a hydrophilic carbohydrate or polymer, usually a lipid derivative of polyethyleneglycol (PEG), to help evade MPS recognition (Fig. 1). The inclusion of PEG or other hydrophilic polymers extends the half-life of liposomes from less than a few minutes (classical liposomes) to several hours (Stealth liposomes).



Liposomal Chemotherapy. Figure 1 Comparison of the chemical structure of second generation liposomes (sterically stabilized, SL) liposomes.

Both conventional and Stealth liposomes rely on "passive" targeting to increase the localization of anticancer drugs to solid tumors. Growing solid tumors, as well as areas of infection and inflammation, have capillaries with increased permeability as a result of the disease process (e.g., tumor ►angiogenesis). Pore diameters in these capillaries can range from 100 to 800 nm. Drug-containing liposomes that have diameters in the range of approximately 50–200 nm are small enough to extravasate from the blood into the tumor interstitial space through these pores (►extravasation). Normal tissues contain capillaries with tight junctions that are impermeable to liposomes and other particles of this diameter. This differential accumulation of liposomal drugs in tumor tissues relative to normal cells is the basis for the increased tumor specificity of liposomal drugs relative to free drugs. In addition, tumors lack lymphatic drainage and therefore there is low clearance of the extravasated liposomes from tumors.

Passive targeting can result in increases in drug concentrations in solid tumors of several-fold relative to those obtained with free drugs. The mechanism of action of the liposomal drugs is thought to be due to sustained release of drug from the liposomes and diffusion of the released drug throughout the tumor interstitial fluid, with subsequent uptake of the released drug by tumor cells. This phenomenon has been termed the enhanced permeability and retention effect (►EPR).

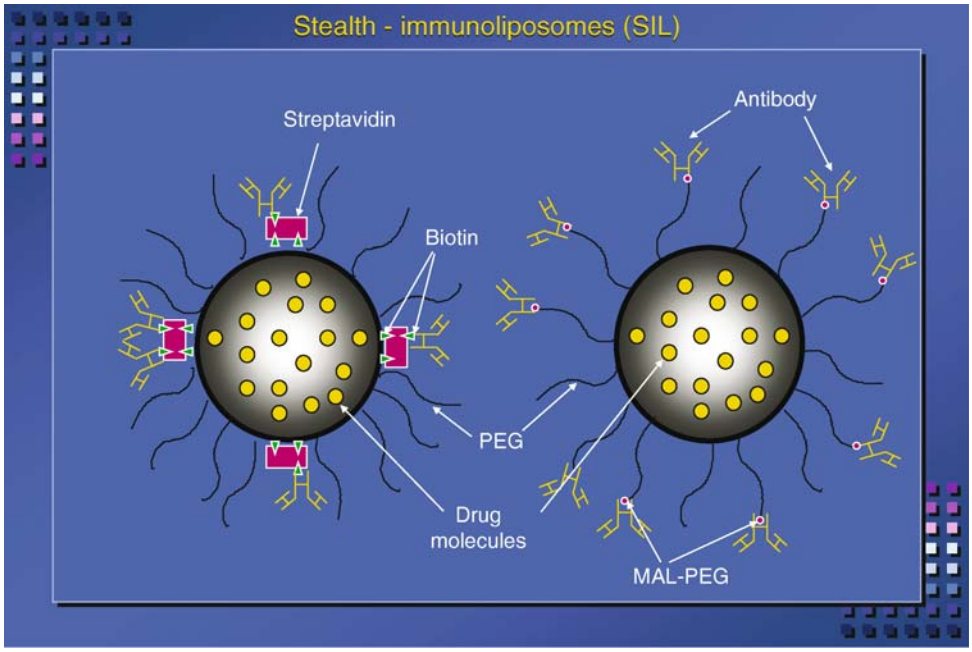
Liposomal formulations of few ►anthracycline anticancer drugs have received clinical approval. Three liposomal chemotherapeutic agents, all of which are nanoparticles measuring about 100 nm, are indeed being assessed in human cancer: liposomal daunorubicin, (Daunosome®), approved in the USA and Europe to treat AIDS-related Kaposi's sarcoma; liposomal doxorubicin, Myocet®, which, in combination with cyclophosphamide, is approved for the treatment of metastatic breast cancer in Europe; and pegylated liposomal doxorubicin, Doxil®/Caelyx®, approved for both Kaposi's sarcoma, refractory ovarian cancer, and metastatic breast cancer in Europe and USA. In addition, many other liposomal anticancer drugs are in clinical trials (i.e., liposomal cytosine arabinoside, Depocyt®, for the treatment of lymphomatous and neoplastic meningitis, liposomal cisplatin for patients with malignant pleural mesothelioma, sphingosomal vincristine for treatment of recurrent or refractory adult acute lymphocytic leukemia, liposomal muramyltripeptide phosphatidylethanolamine for patients with osteosarcoma).

Ligand-Targeted Stealth Liposomes

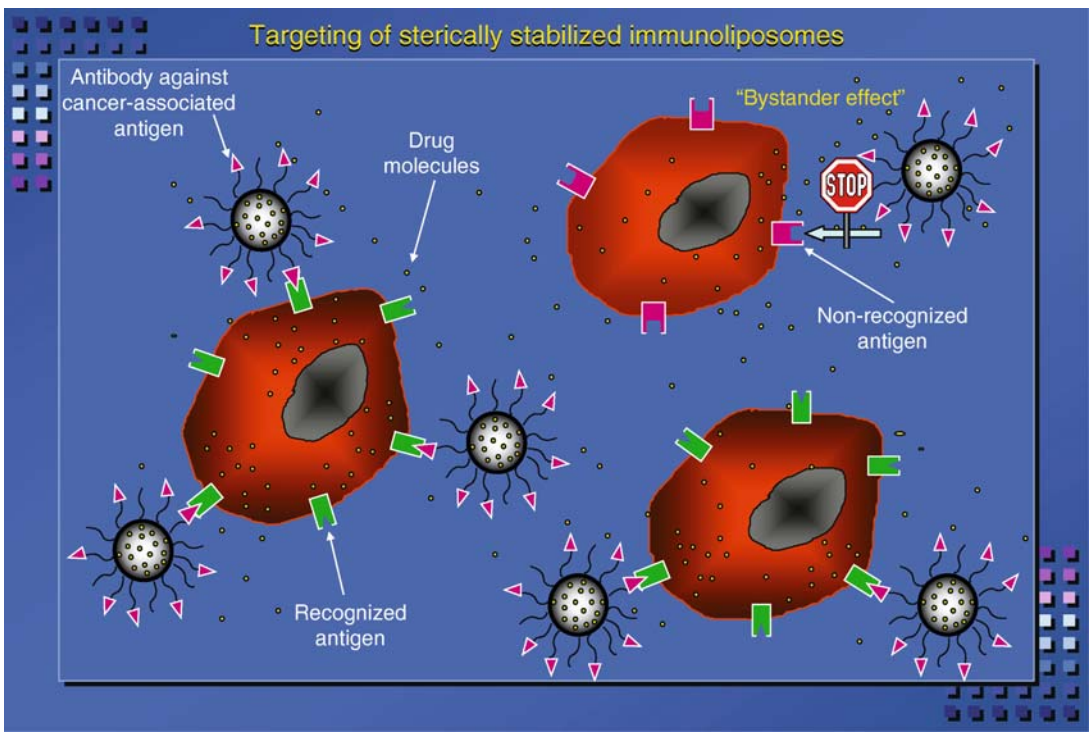
In the attempt of increasing the specificity of interaction of liposomal drugs with target cells and the amount of drug delivered to latter, recent efforts in the liposome field have been addressed to the development of ligand-targeted liposomes. These liposomes utilize targeting moieties coupled to the liposome surface to selectively deliver the drug–liposome package to the desired site of action (active targeting). Targeting moieties may include antibody molecules, or fragments thereof, small molecular weight, naturally occurring or synthetic ligands like peptides, carbohydrates, glycoproteins, or receptor ligands, i.e., essentially any molecule that selectively recognizes and binds to target antigens or receptors overexpressed or selectively expressed on cancer cells.

To date, liposomes chemically coupled to antibodies or antibody fragments have been the most extensively used ligand-targeted Stealth liposomes (stabilized ►immunoliposomes or SIL) (Fig. 2). This chemical complexation can be either noncovalent or covalent in nature. In the former case a biotin–streptavidin–biotin bridge between the liposome and the antibody is involved, while covalent binding can be achieved through the use of heterobifunctional cross-linkers or maleimide-derivatized PEG–lipid complexes (PEG–MAL). The great advantages of SIL encapsulating cytotoxic drugs over free drugs have been unquestionably demonstrated in a number of experimental models of cancer. The mechanism whereby SIL appears to act is related to localized release of the encapsulated drug at the targeted cell surface following binding of the drug carrying liposomes to the cell. With some particular antibodies, moreover, internalization of the liposomal drug package may occur and also contribute to the mechanism of cytotoxicity. Interestingly, it has been shown that approximately 400-fold more monoclonal antibody was required to achieve similar results with antibody–drug conjugates. Hence, high drug:antibody ratios can be achieved with SIL, thus decreasing the need for expensive and potentially immunogenic antibodies. Since most tumors are heterogeneous with regard to tumor-associated antigen expression, another advantage may be the "►bystander effect": specific binding of a SIL to a tumor cell, with release and diffusion of the drug and uptake by surrounding tumor cells may result in cytotoxicity of bystander cells lacking the specific epitope (Fig. 3).

In conclusion, liposomal formulation of chemotherapeutic drugs is considered a very promising modality of drug delivery because liposomes are biologically inert and completely biocompatible, they do not cause toxic or antigenic reactions, and the drugs included into liposomes are protected from the destructive action of the external environment. Association of drugs with carriers, such as liposomes has pronounced effects on the ►pharmacokinetic profile of the drug resulting in delayed ►drug absorption, restricted drug biodistribution, decreased volume of drug biodistribution, delayed drug clearance, and slower drug metabolism.



Liposomal Chemotherapy. Figure 2 Immunoliposomes: Liposomes chemically.



Liposomal Chemotherapy. Figure 3 Mechanisms of drug delivery of immunoliposomes: specific targeting to tumor cells.

Future Perspectives for Liposomal Chemotherapy

Monotherapy is not common in chemotherapy. A combination of drugs generally produces better therapeutic results. The rationale for use of multiple agents takes into consideration the heterogeneity of the tumor cells and differences in tumor cell sensitivity to individual drug classes. Treatment strategies generally include drugs with different mechanisms of action and

nonoverlapping side effects in order to attain maximum therapeutic benefit. The evaluation of targeted liposomes using drug or ligand combinations is only beginning to be explored, e.g., targeting either the same liposomal drug against different epitopes, targeting different drugs against the same epitope, or targeting different drugs against different epitopes. Combinations of liposomal drugs might also help to lower drug dosages and increase responses, which could help reduce drug toxicities. Given the exhaustive possibilities available to liposome chemistry, research will be quickly directed at multi-functional liposomes, combining tumor-targeting and tumor therapy in an all-in-one system, providing a useful multi-modal approach in the battle against cancer.

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Liposomes

Definition

Are artificial lipid bilayer vesicles formed from an aqueous suspension of phospholipids and cholesterol. These vesicles can be used to incorporate water-soluble materials, including drugs, proteins, and nucleic acids, enabling the delivery of such molecules to various cells. Synthetic cationic lipids can be used to form electrically charged (cationic) liposomes, which show robust association with DNA and enhance the efficiency of its delivery to target cells.

►Non-viral Vector for Cancer Therapy
►Drug Delivery Systems
►Immunoliposomes
►Liposomal Chemotherapy

Lipoxygenase

►Arachidonic Acid Pathway

5-Lipoxygenase (5-LO)

Definition

Key enzymes in the arachidonic acid metabolizing pathway into leukotrienes.

►Leukotrienes

Lisch Nodules

Definition

Melanocytic hamartomas in iris. Typical feature of neurofibromatosis Type 1.

►Neurofibromatosis Type 1

Liver

Definition

Large, glandular organ located in the upper right side of the abdominal cavity, divided by fissures into lobes and functioning in the secretion of bile and various metabolic processes.

►Hepatic Ethanol Metabolism

Liver Cancer

►Hepatitis B Virus x Antigen Associated Hepatocellular Carcinoma
►Hepatocellular Carcinoma – Etiology, Risk Factors and Prevention
►Hepatocellular Carcinoma

Liver Cancer – Molecular Biology

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Definition

Primary liver cancer includes four major histological tumor types. The most frequent form is hepatocellular carcinoma (HCC), a malignant epithelial neoplasm that develops from hepatocytes, the basal liver parenchymal cells. Hepatoblastoma is a rare embryonic liver tumor arising from immature hepatocytes, mainly in children under 2 years of age. Cholangiocarcinoma (CC) develops from the epithelium of intrahepatic biliary ducts, and angiosarcoma is a rare malignant mesenchymal (vascular) tumor. This chapter presents only the molecular biology of HCC.

Characteristics

Epidemiology

Liver cancer is one of the most common human cancers worldwide, with an estimate of 626,000 new cases diagnosed each year, and incidence is continuously rising in America and Europe. It ranks fifth in frequency in the world in terms of relative cancer incidence rates, but it shows heterogeneous geographical distribution, with the highest rates in Asia and Africa. HCC develops more frequently in males than in females, sex ratios ranging between 1.5 and 3 in most countries. It occurs predominantly in the second half of life, with increasing incidence between the ages of 40 and 80. Generally, HCC arises in the context of extensive liver lesions, including liver cirrhosis in 80% of cases, or chronic hepatitis.

Etiology

HCC is one of the few human neoplasms seroepidemiologically related to viral infections. More than 80% of HCC cases worldwide are associated with chronic infection with hepatitis B virus (HBV) (►hepatitis viruses) or ►hepatitis C virus (HCV). Other major risk factors include alcoholic cirrhosis, dietary intake of aflatoxin B1, a fungal metabolite which contaminates crops in some tropical areas, and inherited metabolic disorders such as tyrosinemia, hemochromatosis, and α1-antitrypsin deficiency. Recently, diabetes and obesity have also been recognized as significant etiological factors of HCC. The risk of developing HCC is greatly increased in chronic viral carriers exposed to other recognized risk factors, including aflatoxin B1, alcoholic cirrhosis, and diabetes.

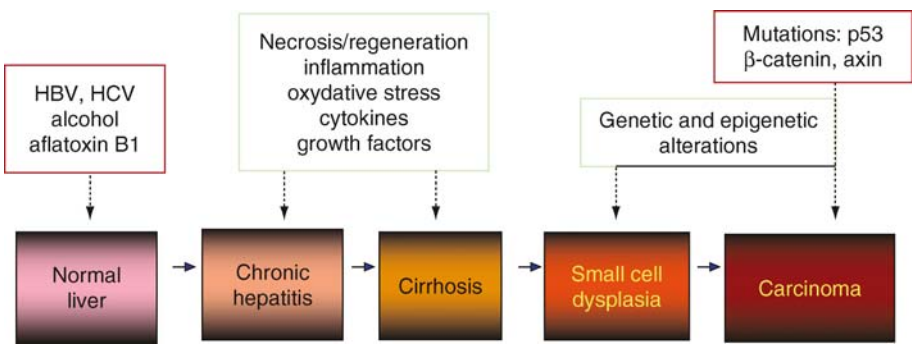
Role of Viral Factors

In HCC as in other human tumors, multiple steps involving independent lesions are required to reach the fully malignant phenotype. Chronic HBV infection plays a complex role in liver carcinogenesis, involving various cooperative mechanisms (Fig. 1).

Following liver injury by viral infections (HBV or HCV) or by excessive intake of alcohol or aflatoxin B1, a long period of necroinflammatory liver disease is generally followed by liver cirrhosis. Small cell dysplastic nodules are considered as direct precursor lesions of HCC. Accumulation of genetic and epigenetic changes, including mutations of the p53 and ►axin 1 tumor suppressors or the β-catenin oncogene lead to the fully malignant phenotype.

Direct Mutagenic Role

HBV DNA frequently integrates into host cell chromosomes. HBV integrations seem to occur randomly over the entire human genome, with no preferential site. Viral DNA integration is frequently associated with gross genetic alterations such as chromosomal translocations, deletions, or amplifications of large chromosomal regions. Therefore, it may promote genomic destabilization, in turn favoring the accumulation of genetic mutations at early steps of HCC development. Insertion of HBV DNA into cellular genes and



Liver Cancer – Molecular Biology. Figure 1 The multistep process of liver carcinogenesis.

subsequent modification of target gene expression or function (a process called ►insertional mutagenesis) is another potential oncogenic mechanism. Initial studies of four independent HCC cases identified viral DNA integration sites within cellular genes that play important roles in the control of cell growth, differentiation, or viability: retinoic receptor-beta, cyclin A2, mevalonate kinase, and SercA1 genes. These tumors produce viro-cellular chimeric proteins endowed with transforming capacities. The recent development of PCR-based technologies for rapid isolation of viral integration sites has allowed to better evaluating the prevalence of such oncogenic insertional events. Large scale analysis of HBV integration sites provided evidence for viral integration into transcribed regions in 62% of cases and for recurrent insertion sites in the human telomerase (hTERT) and mixed lineage leukemia (MLL) genes.

Another argument favoring a direct tumorigenic role of HBV comes from studies of animal models for virally induced liver cancer. Woodchucks chronically infected with the woodchuck hepatitis virus (WHV), a hepadnavirus closely related to HBV, develop frequent and early onset HCC. WHV DNA is found integrated in the vicinity of an oncogene of the ►*MYC* family (either *MYC* or ►*MYCN*) in more than 80% of woodchuck liver tumors. *MYC* genes act as important regulators of cell growth, death, and differentiation, and their abnormal expression has been implicated in the genesis of multiple human neoplasms. The critical role of WHV DNA integration in *MYC* oncogenes has been demonstrated in transgenic mice recapitulating the multistep process of liver carcinogenesis.

Oncogenic Potential of the HBx Transactivator

The HBV regulatory protein X (HBx) has pleiotropic functions and it can interfere with multiple cellular pathways controlling cell cycle, proliferation, DNA repair, and apoptosis. In particular, HBx has been reported to modulate calcium homeostasis, mitochondrial functions, proteasome activity, and signal transduction pathways. Described as a weak transcriptional activator, HBx exerts its functions by interacting with a variety of cellular partners such as DDB1 (a subunit of the Cul4A ubiquitin ligase complex), the histone acetyltransferases CBP/p300, and p53. In transgenic mouse strains, liver expression of HBx can either induce liver cancer or act as a cofactor and a tumor promoter in liver carcinogenesis.

Immunopathogenesis

There is a general consensus that hepatocellular damage in human hepatitis B is caused by the host immune response and not by the viral replication itself. Transgenic mouse models have provided evidence for an indirect role of HBV in cancer formation. Sustained viral replication and expression of most viral genes in the liver can be achieved in HBV transgenic mice with no pathological consequence. However, sustained expression of the large envelope protein of HBV in the mouse liver induces a process of necrosis and regeneration which ultimately leads to malignant transformation. Moreover, stimulation of cellular immune responses in mice transgenic for the HBV surface antigen (HBsAg) leads to frequent emergence of liver tumors. Thus, an important factor in tumorigenesis is the accelerated turnover of infected hepatocytes resulting from continuous cell death triggered by the host immune response and subsequent cell proliferation. Hepatocyte DNA lesions during persistent HBV infection may be induced by exposure to mutagenic products secreted by inflammatory cells, endogenous production of mutagens such as oxygen free radicals and nitrosamines, and impaired detoxification pathways or DNA repair mechanisms.

Chromosomal Aberrations

In the recent years, global insights into chromosomal alterations profiles have been provided by genetic studies of large sets of tumors. These studies have shown that a large variety of genetic and epigenetic alterations are present in different combinations among individual HCC cases. Therefore, HCC might rank among the most complex and heterogeneous types of human solid tumors, which is consistent with the multiple etiologies of HCC and the long period of chronic inflammatory disease that fosters accumulation of genetic and epigenetic defects.

Studies using PCR-based microsatellite marker analysis (MSA) and Comparative Genomic Hybridization (CGH) have identified the major genetic changes in HCC of various geographical and etiological origins. In MSA studies, the highest percentages of allelic deletions (LOH: ►loss of heterozygosity) were found at chromosomes 8p23, 4q22–24, 4q35, 17p13, 16p13–15, 16q23–24, 6q27, 1p36, and 9p12–14. The relative frequency of allelic losses may vary with the associated risk factors, implying that viral and chemical agents implicated in liver cancer may preferentially select some pathways rather than others. Importantly, genomic instability has been found at higher rates in HBV-related tumors than in tumors associated with HCV or other risk factors. CGH studies have confirmed prevalent chromosomal losses and demonstrated frequent DNA copy gains at 8q, 1q, 6p, and 17q.

Moreover, specific chromosomal gains and losses were found to occur at different stages of tumor progression, suggesting nonrandom chromosomal gains and losses occuring in an orderly fashion in liver cancer. The recent development of array-based CGH might allow more accurate evaluation of chromosomal changes and identification of cancer-related genes located on affected chromosomal arms.

In addition to genetic mutations, epigenetic mechanisms such as hypermethylation of promoters containing CpG island have been shown to modify gene expression patterns in HCC. A number of tumor suppressor genes including $p16^{INK4A}$, SOCS-1, APC, RASSF1A, GSTP1, and E-cadherin, are silenced by DNA methylation in a large proportion of liver tumors, and this process often starts at preneoplastic (cirrhotic) stages.

Tumor Suppressor Genes and Oncogenes

►*P53 is probably the most common molecular target involved in human carcinogenesis*. The p53 tumor suppressor protein is activated in response to DNA damage, inducing either cell cycle arrest to permit DNA repair, or apoptosis. Loss of p53 function occurs mainly through allelic deletions at chromosome 17p13, where the gene is located, and missense mutations within the DNA-binding domain. In HCC, LOH at chromosome 17p13 has been observed in 25–60% of tumors in different studies, and the worldwide prevalence of p53 mutations can be estimated to around 28%, with however important geographical variations. It is now well established that a mutation at codon 249 of the p53 gene is frequent in some regions of Africa (Mozambique, Senegal) and the southeast coast of Asia (Qidong, Vietnam) where chronic HBV infection is highly endemic and the aflatoxin B1 content of the diet is high. Thus, the specific "hot spot" 249 mutation appears to be a hallmark of dietary exposure to aflatoxin B1. In other countries, p53 mutations are seen at lower rate, and they are distributed over the coding exons.

The Retinoblastoma Gene. Allelic deletions at chromosome 13q14 have been associated with the inactivation of the RB tumor suppressor gene (►RB1). RB has been implicated in cell cycle control, and disruption of the RB pathway renders cells insensitive to antiproliferative signals. In liver cancer, LOH at the RB locus has been found in 25–48% of cases, and RB expression is strongly downregulated in 30–50% of tumors. While no mutation of the RB gene itself has been documented, inactivation of the RB pathway is achieved mainly by methylation-dependent silencing of $p16^{INK4}$, an inhibitor of cyclin-dependent kinases which blocks the cell cycle. In addition, overexpression of gankyrin, a new oncogene homologous to a subunit of the 26S proteasome, promotes RB degradation by the ubiquitin–proteasome pathway.

β-Catenin. Activation of the Wnt pathway has been implicated in liver oncogenesis by the finding of frequent mutations in the β-catenin gene. β-catenin is an important multifunctional protein involved in cell–cell adhesion and in transduction of differentiation signals during embryogenesis. Mutations in the β-catenin gene have been detected in about 22% of liver tumors. All mutant forms of β-catenin harbor missense mutations or short deletions in the amino-terminal domain (so-called "destruction box") and they are resistant to degradation. Thus, wild type β-catenin is expressed at the cell membrane in normal epithelial cells, whereas the mutants accumulate in the cytoplasm and nucleus of tumor cells. In about 7% of HCC cases, activation of β-catenin is also achieved in HCC by loss-of-function mutations of axin 1, a tumor suppressor protein known to bind β-catenin and promote its degradation. In contrast with colorectal cancer, another β-catenin partner, the adenomatous polyposis coli (►APC) tumor suppressor gene, is not mutated in HCC. Interestingly, β-catenin mutations are less frequent in HBV-related HCCs than in HCV-related or nonviral HCCs. No mutation of β-catenin could be evidenced in intrahepatic cholangiocarcinoma.

TCF1. The finding of frequent LOH at chromosome 12q24.2 in hepatocellular adenomas has led to identify mutations and bi-allelic inactivation of the TCF1 gene encoding the liver-enriched HNF1-α transcription factor. This finding links MODY 3 diabetes to benign liver tumors, and also occasionally to HCC.

►*Ras Family Oncogenes*. None of the genes of the ras family has been found to be mutated at significant rate in HCC, whereas K-ras mutations are frequent in intrahepatic cholangiocarcinoma.

Oncogenic Pathways

Deregulation of various signaling pathways has been reported in different HCC subtypes, comprising Wnt/β-catenin signaling and the $p14^{ARF}$/p53, $p16^{INK4A}$/RB, TGF-β, and PTEN/Akt pathways [3]. Evidence for activation of the Ras and Jak/Stat pathways in HCC by epigenetic silencing mechanisms has also been reported. Additionally, altered expression of growth factors such as HGF, IGFs, amphyregulin, and their receptors, as well as genes involved in angiogenesis have been involved in the development and progression of HCC.

Molecular characterization of HCC by gene expression profiling using DNA microarray technology has allowed to distinguish several tumor subtypes harboring significant biological differences. Importantly, these newly discovered subtypes may differ also by a number of clinicopathological features, such as tumor stage, etiological factor, association with recurrence or metastasis, and survival of the patients. New progress will come from integrating gene expression profiles and comprehensive genetic surveys of HCC.

Conclusions

Our understanding of the molecular pathogenesis of HCC has been obscured by the striking clinical and biological heterogeneity of this tumor. It is surmised that new tools provided by sequencing of the human genome and high-throughput screening of DNA, RNA, and protein alterations in HCC would permit a comprehensive view of the signaling networks

operating in liver cell transformation. Great insights will come from integrating the signals from different pathways at pretumoral and tumoral stages. Because HCCs carry a severe prognosis and remain refractory to current chemotherapy regimens, it is urgent to accurately evaluate new molecular targets for therapeutic treatments.

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Liver Cell Carcinoma

►Hepatocellular Carcinoma

Liver Cirrhosis

Definition

Cirrhosis is a chronic disease of the liver caused by damage from alcohol, chronic viral hepatitis, biliary disease, iron accumulation, etc. Normal parenchyma is replaced by delicate bands or broad scars of fibrous tissue, active parenchymal nodules created by hepatocyte regeneration, and passive nodules created by constrictive scarring, with disruption of liver architecture. These changes are irreversible and vascular intrahepatic system is reorganized with the creation of interconnections between vascular inflow and hepatic vein outflow channels. The end stage of cirrhosis is the complete loss of liver function. In some instances, cirrhosis predisposes to liver cancer.

►Preneoplastic lesions
►Liver Cancer, Molecular Biology

Liver Flukes

Definition

Parasites that are ingested and form cysts within the liver, causing chronic ►inflammation, predisposing to cholangiocarcinoma. Fluke eggs are eaten by snails and develop into cercaria which bore out of the snails and bore into the muscle of fish, where they form metacercaria. The metacercaria are then ingested by humans from raw or undercooked fish and adult parasites form liver cysts, feeding off bile.

►Cholangiocarcinoma

Liver Resection

Definition

Surgical removal of a part of liver.

►Hepatic Epithelioid Hemangioendothelioma

Liver Transglutaminase and G_h

►Transglutaminase-2

Liver Transplantation

Definition

An operation harvesting the liver from a donor and grafting to a recipient.

►Hepatic Epithelioid Hemangioendothelioma

LMP2

Definition

Low Molecular Mass Protein 2; The generation of antigenic protein fragments for (Low Molecular Mass

Protein 2) loading onto ►MHC class I molecules and subsequent display on the surface of the cell uses the cellular machinery for protein degradation, the proteasome. This is a large multi-subunit complex consisting of seven subunits and three proteolytic active sites. Although constitutively expressed in the cell, under the influence of IFN-γ, subunits are exchanged to create the immunoproteasome, which is more competent to produce peptides of appropriate size for MHC I binding. Two of the subunits, LMP2 and LMP7 are encoded by genes within the MHC locus and are components of the immunoproteasome.

►Immunoediting

Lob 1

Definition

Lobule 1; is also defined as the terminal ductal lobular unit of the human breast and the site of origin of breast cancer.

►Estradiol

Lobular Involution

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Synonyms

Senile involution

Definition

A descriptive term, involution refers to a progressive decrease in the size of an organ that is usually associated with a decline in its function. Organs that undergo involution include the post-partum uterus, the ovaries after menopause, the thymus gland, and breast. Except for the post-partum uterus, involutional changes are regarded as a manifestation of aging and are often irreversible.

Characteristics

Anatomy of the Human Breast

The adult human female breast consists of 15–20 irregular lobes that are separated by layers of connective tissue as they fan out from the nipple into the mammary gland proper. These lobes empty independently into the nipple through the lactiferous ducts, which conduct milk to the nipple. The ducts subdivide and terminate in multiple small lobules, which subdivide into multiple glandular acini or alveoli that produce milk during lactation. These lobules are the functional units of the mammary gland. If the terminal duct is also included, they are often referred to as terminal ductal lobular units. A system of branching intralobular ducts, which eventually converge with the larger lactiferous ducts, connects the individual lobules within each lobe. The highest concentration of lobules is usually found in the upper outer quadrant of the breast. The development of the breast begins at puberty. Its maximum development is reached sometime after the twentieth year with atrophic changes setting in by the age of 35. Lobules are not present in the male breast.

Involution

The breast continually undergoes structural changes depending on the menstrual cycle, pregnancy, and age. Thus, for normal development and function, a balance must exist between cell proliferation, differentiation, and death. A major change entails involution, which refers to two markedly different physiological processes that affect the lobules. The first relates to pregnancy and the production of milk. During pregnancy, in preparation for lactation, these lobules increase in size as a result of cellular proliferation. There is rapid epithelial proliferation with additional branching of the ducts and lobulo-alveoli growth. Following the cessation of lactation, the lobules shrink and return to their pre-pregnancy size, a process described as post-lactational involution. In animals, this process is very rapid occurring in a matter of days through a process of ►apoptosis. In humans, the time in which this occurs is somewhat uncertain because of the limitations on the opportunity for tissue sampling. This process repeats after each pregnancy. The second process, which greatly differs from the first, is not associated with pregnancy. It is referred to as age-related lobular involution and usually begins near the end of child bearing.

Age-Related Lobular Involution

In humans, information on age-related lobular involution is limited primarily to morphological descriptions. Involution is characterized by a gradual reduction in the number of the terminal lobular structures of the breast. The lobules progressively regress in size, that is atrophy, until they disappear ultimately being replaced

by fat and fibrous tissue. Nearly all of the lobular structures are eventually lost in the process of age-related involution. A few partially involuted lobules or glands persist in the breast years after menopause, usually surrounded by a dense fibrous tissue stroma.

Although it is a normal physiological process, the age of onset, rate, and extent of age-related lobular involution varies greatly among women. In most women, involution begins around age 35, at least 10–15 years before the time of menopause. In some women, however, it may start in the early 30s or even later. It is a relatively slow process often continuing for many years, even into menopause. By age 55, involution is practically complete and the breast has in a general way returned to its prepubertal condition. Involution is not uniform in the breast, but occurs in a patchy or focal distribution. Some areas of the breast may show nearly complete involution while others show only minimal change. Age 35 years appears to be a crucial year. A full term pregnancy before age 35 confers some degree of protection against future ►breast cancer. Pregnancy after age 35 has been associated with an increased risk of subsequent breast cancer.

The mechanism of lobular involution has not been extensively studied. Post-lactational involution involves apoptosis. Therefore, it can be presumed that age-related lobular involution also involves apoptosis, which in contrast occurs at a much slower rate than post-lactational involution.

Factors Affecting Involution

Very little is known about the factors that govern involution. The process does not follow the regular pattern of aging seen in other organs or tissues. Most likely, the physiological aging process in the breast is under the influence of various hormones. Although it is unknown what factors control the onset and rate of involution, the following associations exist. Oopherectomy at a young age leads to atrophy of the lobules and involution of the lobular epithelium, which resembles the atrophy normally seen in older women. This is of some interest, because oopherectomy also reduces the risk of breast cancer. Since involution predates menopause by many years, it is possible that early changes in ovarian function that precede menopause trigger the onset of involution.

There have been no international comparisons on the extent of physiologic involution in different populations. Surveys of the incidence of different histological tumor types of breast cancer in different geographic areas have suggested international variations exist. This possibility depends on the assumption that the incidence of different tumor types reflects lobular involution. Variation in lobular involution would offer one explanation for the substantial variability of breast cancer observed between populations.

More than 55 years ago, Clemensen showed that the age specific incident rate of breast cancer increased until age 50, then decreased for a few years, then increased again, but at a much slower rate than previously. Although many reasons were advanced for the decrease, it has been proposed that Clememsen's hook reflects involution with its loss of glandular epithelium. The second increase has been attributed to persisting ductal-lobular structures that are susceptible to malignant transformation.

Measurement of Involution

Currently, there is no way to assess quantitatively the extent of involution. Radiologically, dense ►mammographic parenchymal patterns have been associated with mammary gland mass, which apparently reflects the amount of epithelial tissue present. In general, there is an inverse association between the prevalence of dense parenchyamal patterns and age. However, parenchymal patterns are variable; they often vary in women who are the same age. There is no explanation as to why some women have dense breasts and others do not even at the same age. A decrease in the prevalence of dense parenchymal patterns with age most likely reflects the loss of epithelial lobular structures as a result of involution. Dense parenchymal patterns are also a predictor of breast cancer risk, the greater the density the greater the risk for breast cancer, which suggests a relationship between the amount of epithelial tissue and cancer.

Failure to Involute

It is tempting to speculate on the consequences of a delay or failure of involution to occur at the appropriate time. It is known that lobular epithelium can persist beyond the time of normal involution, which has lead to speculation about the association between abnormal lobular involution and the increased risk of breast cancer. Abnormal involution could involve incomplete involution, genetically abnormal involution, or normal but delayed involution.

In the only systematic examination of age-related lobular involution in the context of breast cancer, a strong inverse relationship was shown between the extent of lobular involution and breast cancer risk, independent of all known breast cancer risk factors investigated. Among women with benign breast disease, lobular involution was significantly associated with reduced breast cancer risk.

There are several possible biologic mechanisms by which aberrant involution could modify a woman's breast cancer risk. It has been suggested that the progressively smaller number of epithelial cells present

upon gradual lobular involution could lead to a reduction in breast cancer simply because a lesser amount of tissue is available for malignant transformation. It has also been suggested that abnormal involution may merely be a surrogate for an underlying susceptibility to breast cancer. Failure of timely involution allows for prolonged exposure of epithelial cells to mutagenic stresses thereby increasing breast cancer risk.

Certain known breast cancer risk factors may interfere with the process of involution. Women whose first full term pregnancy occurs after 35 years of age are at increased risk of breast cancer compared to both nulliparous women and women with earlier first full term pregnancies. It is likely that the ductal-alveolar epithelium proliferation that accompanies the late pregnancy may interrupt the process of involution. The persistent estrogen activity that results from a late age at menopause possibly explains the increased risk of breast cancer.

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Local Ablation Therapy

►Locoregional Therapy

Local Therapy

Definition

Treatment that affects cells in the tumor and in the surrounding area.

Local Treatment

Definition

Therapy which targets the tumor and surrounding regional area. Surgery and radiotherapy are forms of local treatment. The purpose of local treatment can be curative or ►palliative. Local treatment is distinguished from ►systemic treatment.

►Induction Chemotherapy

Locoregional Therapy

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Synonyms

Local ablation therapy

Definition

There has been no clear definition for locoregional therapy. Most often, locoregional therapy refers to various minimally invasive therapeutic procedures. With the aid of ultrasound guidance, an antitumoral device, drug or chemical is introduced directly into the tumor and causes tumor death.

Characteristics

For most human cancers, surgical resection remains the standard treatment modality. However, resection is sometimes possible in only a minority of patients. When surgical treatment is less likely to perform, physicians may choose other therapeutic procedures. Among the available methods, locoregional therapy has been frequently used to treat cancer. It is so named because the tumors can often be treated locally. The best example to demonstrate the role and usefulness of locoregional therapy is applying this kind of therapy in patients with ►hepatocellular carcinoma, or ►HCC, which is primary liver cancer originated from transformed liver cells. HCC is one of the common malignant tumors in the world, and most cases are found in Asia and Africa. Among the various locoregional interventions, ►transarterial chemoembolization, ►percutaneous ultrasound-guided therapy including injection of

►ethanol or ►acetic acid, and thermal ablation using ►radiofrequency and ►microwave energy, are the most commonly used methods (Table 1). These locoregional therapies possess the advantages of preserving the uninvolved liver parenchyma and avoiding potential morbidity and mortality of major hepatic surgery.

Transarterial Chemoembolization (TACE)

Most liver cancers, when formed, have a feeding artery that delivers nutrition and oxygen to tumor cells to enable their growth. TACE, using iodized oil and chemotherapeutic agents, combines the effect of targeted chemotherapy with that of ischemic necrosis induced by arterial embolization. Although TACE was originally used for ►metastatic liver tumors from colorectal cancers or other malignancies, it was later used to treat primary HCC because HCC nodule frequently has a high arterial blood supply (hypervascular lesion). In addition, TACE can target single- or multi-nodular HCCs in one treatment session and could be repeatedly administered. TACE is generally a palliative treatment for unresectable HCC, and a better outcome can only be expected in properly selected patients.

Percutaneous Ethanol Injection (PEI)

PEI was originally developed in the 1980s when the real time ultrasound-guided aiming became possible. The injected chemical, pure ethanol, induces local tumor necrosis as a result of direct protein denature, cellular dehydration and thrombosis of blood vessels. HCC usually is hypervascular and well-encapsulated by a tumor capsule that can limit the spread of ethanol. These characteristics have made PEI one of the most commonly used methods of local ablation therapy. Usually PEI is limited to patients with small-sized tumor nodules. Compared with the transarterial approach, PEI has the advantages of being safer, less expensive, and easy to perform. In addition, PEI allows selective treatment of HCC without significant damage to the adjacent liver parenchyma, and can be used for patients with moderately advanced cirrhosis. Side effects are mostly minimal.

Percutaneous Acetic Acid Injection (PAI)

Acetic acid induces profound tumor necrosis at a concentration of 15–50% through a similar mechanism as ethanol. It has a strong ability to penetrate cancer cells, and can dissolve lipids and extract collagen from intratumoral septa and capsule that frequently contain viable cancer cells. Therefore, acetic acid is at least equally effective compared with ethanol in treating HCC.

Radiofrequency Ablation (RFA)

RFA for human HCC was first reported in the mid-1990s. The puncture needle (or probe) has an insulated shaft and a noninsulated tip, which is inserted into the lesion under ultrasound or computed tomography guidance. The patient is part of the electric circuit with grounding pads on the thighs or the back. The radiofrequency energy emitted from the needle tip induces ionic agitation and frictional heat. The surrounding tissue, rather than the electrode itself, is the source of heat that destroys the cancer cells. RFA has now become one of the very efficient local ablative therapies for HCC due to its excellent necrotizing effect. While many investigators consider RFA a safe procedure, a few reports found that the complication rate of RFA could be quite high. When the location of tumor nodule is close to the major blood vessels, the radiofrequency energy will be carried away by the blood flow (the "heat sink" phenomenon) and result in a suboptimal treatment response.

Microwave Coagulation Therapy (MCT)

As a thermal ablation method for HCC, MCT utilizes a microwave coagulator that generates and transmits microwave energy to a needle electrode which is inserted into the lesion. MCT has been studied in patients with HCC as well as in liver ►metastasis. It can be applied with percutaneous or laparoscopic approach to ablate unresectable HCC, and is useful to control tumor

Locoregional Therapy. Table 1 Comparison of various locoregional therapies for HCC

Treatment	Advantages	Disadvantages
TACE	Target multiple tumors in one treatment session	No effect for hypovascular tumors, may induce liver or renal failure
PEI	Minimally invasive	Limited to small lesions, need multiple treatment
PAI	Minimally invasive, probably more effective than PEI	Limited to small lesions, need multiple treatment
RFA	Effective for small and medium sized HCCs	Probably higher complication rate, more expensive
MCT	Effective for small to medium sized HCC	Probably higher complication rate, more expensive

TACE: transarterial chemoembolization; PEI: percutaneous ethanol injection; PAI: percutaneous acetic acid injection; RFA: radiofrequency ablation; MCT: microwave coagulation.

bleeding from ruptured HCC or prevent massive blood loss in liver surgery.

Comments

Locoregional therapy has also been applied to other cancers other than HCC, but the overall experience is relatively limited. The choice and application of various locoregional therapies for HCC and other cancers may vary from center to center and is likely to depend on the experience, preference and facility of the referral center. Long-term survival in patients with unresectable HCC may be achievable in selected patients provided various locoregional therapies can be appropriately performed.

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Locus

Definition

Plural, **loci**; A fixed position or location in a DNA sequence.

►Linkage Disequilibrium

Log-Kill Hypothesis

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Synonyms

Skipper-Schabel-Wilcox model; Skipper-Schabel model

Definition

The ►log-kill hypothesis proposes a model for the effect of cytotoxic chemotherapy on tumor size. It states that a given dose of chemotherapy kills the same fraction of tumor cells regardless of the size of the tumor at the time of treatment.

Characteristics

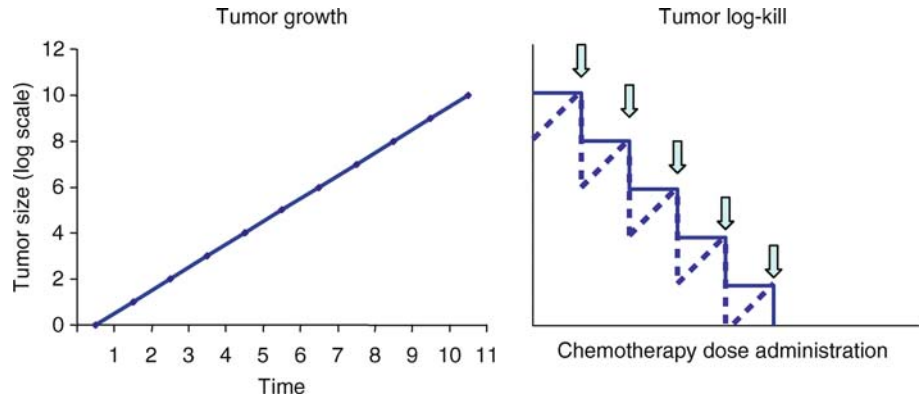
Experimental and mathematical models have attempted to describe the fundamentals of tumor cell growth and kinetics. From these systems arose an improved understanding of tumor growth characteristics, a foundation for the key principles of chemotherapy and eventually, recognition of the importance of dose scheduling. The Skipper-Schabel-Wilcox model is one of the most influential and pioneering experiments in the history of oncology.

Investigators at the Southern Research Institute developed a simple model of tumor growth using L1210 murine leukemia cells. This system had two notable features: a seemingly exponential growth pattern and the ability to spontaneously generate drug-resistant cells. These factors made it a reasonable reflection of the growth of rapidly fatal human malignancies and of the heterogeneity in drug sensitivity seen clinically in many cancers.

Leukemia in this murine model grows exponentially until it reaches a lethal volume of ~10^9 cells. A significant observation was that the doubling time remained constant regardless of the size of the tumor. This pattern of growth could be generalized to any fractional increase so that if it takes a certain amount of time (x) to grow from 10^3 cells to 10^4 cells, it will similarly take x amount of time for 10^7 cells to grow into 10^8 cells.

Building on the observations and assumptions above, the investigators hypothesized that when such a tumor is treated with chemotherapy, the fraction of cells killed is similarly constant. This fixed, relative cytoreduction suggests that a dose of drug active against a homogeneously sensitive population should always kill a constant proportion of the cells, regardless of the size of the tumor at the start of treatment. According to this model, if a dose of drug could improve survival by some period of time, then enough additional doses could delay death and lead to cure.

Graphically, when this fractional cell kill is expressed on a logarithmic scale, cytotoxicity occurs in the reverse of an exponential growth pattern (Fig. 1). Therefore, a drug dose that reduces 10^7 cells to 10^5 cells (a log-kill of 2) will also reduce the same tumor from 10^5 cells to 10^3 cells. Exponential cell growth is matched by exponential cell kill. Accordingly, enough cycles of enough drugs at high enough doses should be able to kill a high percentage of cells, if not all of them.



Log-Kill Hypothesis. Figure 1 The Skipper-Schabel-Wilcox "log-kill" model. Tumor growth appears exponential when plotted on a logarithmic scale (Left). With each dose of chemotherapy, a constant fraction of cells are killed (Right).

The log-kill model led to enthusiasm regarding the application of chemotherapy to the postoperative setting. ►Adjuvant therapy would theoretically cure patients of micrometastases as small volume tumors should be more easily eradicated as predicted by this model.

However, the Skipper-Schabel model does not accurately represent the entire clinical reality. The log-kill model presumes that growth is exponential between treatment doses and does not vary as a function of tumor size. Additionally, clinical experience with several solid tumors does not support this model. The observations that cure is rare for patients with advanced cancer, and that many patients with early stage cancer recur despite treatment suggest that this model does not completely predict or explain tumor cytokinetics.

Subsequent mathematical models based upon Gompertzian growth (►Gompertzian growth curve; ►Norton-Simon hypothesis) improved upon the concept established by the Skipper-Schabel-Wilcox model.

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LOH

Definition

►Loss of heterozygosity.

LOI

Definition

Loss of ►Imprinting.

Long Dispersed Nuclear Element

Definition

►LINE element.

Long Terminal Repeat

Definition

LTR; Regulatory sequence of the genome of a ►retrovirus.

►Retroviral Insertional Mutagenesis

Loss-of-Function Mutation

Definition

Is any mutation of a gene that causes decreased or abolished function and/or activity of its encoded protein or of a protein that is directly or indirectly regulated by the mutated gene.

Loss of Heterozygosity

Definition

Synonym LOH; or allelic loss can be experimentally demonstrated in cases in which the two alleles differ. It is the loss of an allele in tumor DNA compared to matched normal DNA from the same individual. LOH is a very frequent somatic genetic change in human tumors. When LOH is found to occur at high frequency in a particular chromosome region, it is generally considered indicative of the location of a tumor suppressor gene whose loss/inactivation occurs by a 'two-hit' mechanism (i.e., physical loss of one copy of an allele and mutation or other genetic/epigenetic alteration of the other copy of the same allele). LOH can be identified in cancer cells by a number of technical approaches that all are make use of the fact that the two alleles on the two homologous parental chromosomes differ in their DNA sequence. Consequently, normal cells can be shown to be ►heterozygous for a given chromosomal region. If cancer cells of the same person lack this heterozygosity, one of the two alleles must have been lost. In essence, determining LOH in a cancer cell is a technical approach to show the deletion of chromosomal material from one of the homologous parental chromosomes.

►Tumor Suppression
►Knudson Hypothesis
►CCCTC-Binding Factor
►Circulating Nucleic Acids
►WWOX

Lovastatin

Definition

Member of the statins family.

►Statins

Low Density Lipoprotein

Definition

LDL as a lipoprotein of the blood plasma transports triglycerides, cholesterol, and antioxidative vitamins from the liver and small intestine to peripheral cells. High LDL levels can lead to atherosclerosis.

►Photodynamic Therapy

Low Grade/Well Differentiated MEC

►Mucoepidermoid Cancer

Low Molecular Weight G-Proteins

►Rho Family Proteins

Low-Grade

Definition

Referring to a tumor that has progressed minimally and is still relatively benign.

LOX

►Arachidonic Acid Pathway

LPP

►Lipoma Preferred Partner

LRF

Definition
luteinizing hormone (LH)-releasing factor.

►Gonadotropin-Releasing Hormone

LRP

►Major Vault Protein

LRP5/6

Definition
Members of the ►low density lipoprotein (LDL) receptor superfamily; single-pass transmembrane receptors for Wnt and DKK proteins; recruit Axin to the membrane.

►Wnt Signaling

LS-80558

►Temozolomide

LTBP

Definition
Latent TGF-β Binding Protein.

►Transforming Growth Factor Beta

LTR

Definition
Long terminal repeat, the promoter of HIV genome transcription.

►TAT Protein of HIV

Lucifer Yellow

Definition
Is a low molecular weight (457 Da) hydrophilic fluorescent compound able to diffuse through gap junctions.

►Gap Junctions

Luciferase Reporter Gene Assays

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Synonyms
Bioluminescent reporter gene assays

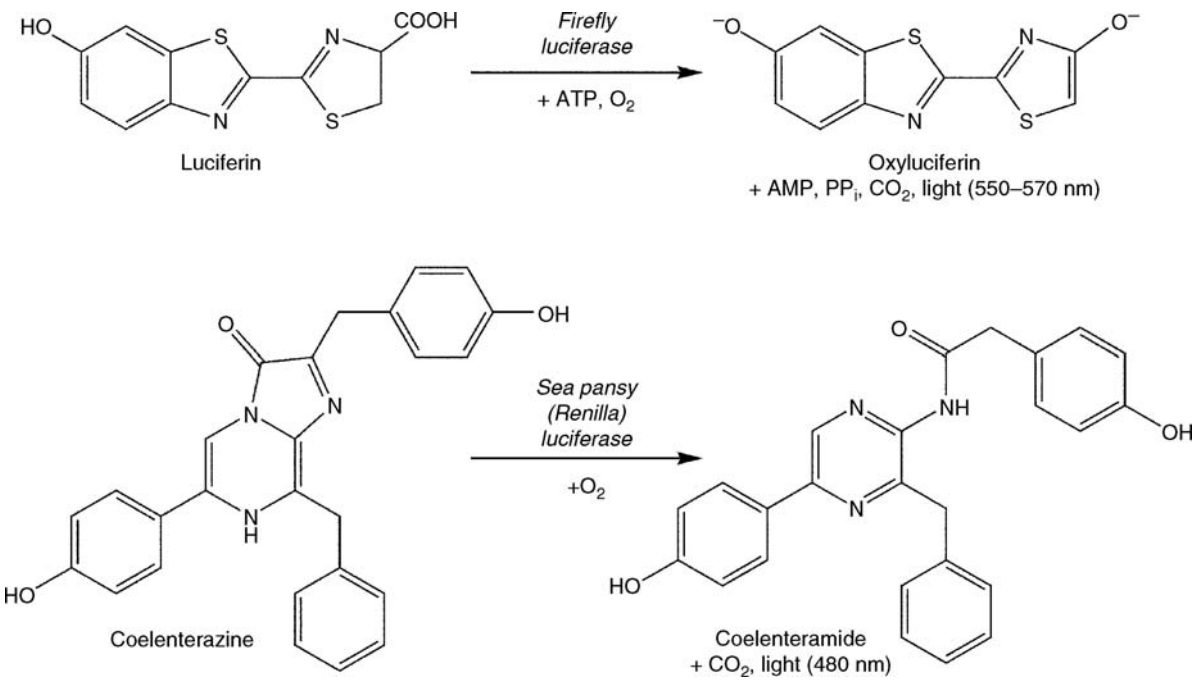
Definition
Luciferase ►reporter gene assays monitor the transcription of specific genes in cells and *in vivo* through the detection of light generated by the enzyme luciferase. Their primary uses are for mapping the control regions of genes, for measuring ►signal transduction pathways modulated by hormones or disease, as a tool in ►gene therapy and drug discovery, and for non-invasive whole-animal imaging.

Characteristics
Luciferase reporter gene assays are a type of reporter gene assay that utilize the light generated by one of a family of enzymes known as luciferases. Reporter genes are essential tools in the study of gene expression and regulation, as they are designed to function as surrogates for genes involved in key cellular processes and disease. To achieve this role, they are engineered

so that their expression is directly correlated with the expression of the gene to be studied, but they have the advantage that they are easier to measure. The characteristics of a good reporter gene include an easily measurable phenotype, low background activity in the cell under investigation, a large dynamic range, and high reliability and sensitivity. A number of such genes have been identified over the past 20 years, including members of the family of luciferases, β-galactosidase (β-Gal), chloramphenicol acetyltransferase (CAT), β-lactamase (β-lac), alkaline phosphatase (SEAP or SPAP), and green fluorescent protein (GFP) and derivatives. Each of these reporter genes has distinct advantages and disadvantages depending on the particular system being studied, but all are engineered in a similar way. The reporter gene is first cloned into an expression vector downstream of the regulatory elements of the cellular gene to be studied. The regulatory elements include the promoter, where specific transcription factors bind to modulate the gene's activity, and enhancers that can affect the promoter depending on the input from other signaling pathways in the cell. When this reporter gene expression vector is transfected into a cell, it becomes controlled by the same signals as the gene under study, and will function as a "reporter" of its transcriptional status. The proteins expressed by reporter genes can be measured directly by their intrinsic properties such as fluorescence, by enzymatic activities that generate fluorescent or luminescent products, or indirectly with antibodies. The primary advantages of reporter genes, as compared to direct measurements of gene transcription, are that they are easy to measure and allow for continuous monitoring of gene expression.

Luciferase is a general term for a family of enzymes that catalyze the oxidation of a number of substrates and in the process produce photons of visible light with emission spectra between 400 (purple) and 620 nm (orange). Luciferase genes have been cloned from a variety of bacteria, insects, and marine organisms, but the luciferases from firefly (*Photinus pyralis*), and sea pansy (*Renilla reniformis*), are most widely used as reporter genes in mammalian cells. The chemical reactions of the firefly and sea pansy luciferases are shown in Fig. 1.

Firefly luciferase is a monomeric protein of 61 kDa that produces an initial burst of light (seconds) followed by a sustained low level of luminescence (hours) when incubated with substrates. In the original assay, cell lysis was required prior to the addition of luciferin, but the development of membrane-permeable substrates has eliminated this step and also allowed imaging *in vivo*. Other improvements that have been introduced include modification of the reaction conditions to produce a stable luminescent output (important for applications that require consistent light output such as drug screening), and genetic changes that optimize expression and increase enzyme turnover. Light output is typically measured through the use of plate-based



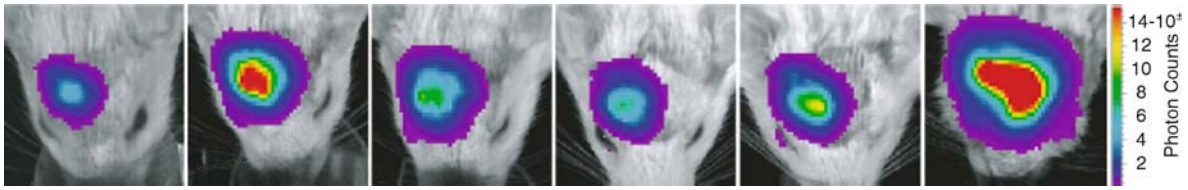
Luciferase Reporter Gene Assays. Figure 1 Chemical reactions catalyzed by (a) firefly (*Photinus pyralis*) and (b) sea pansy (*Renilla reniformis*) luciferases. The firefly luciferase reaction requires both ATP and O_2, while the sea pansy luciferase only requires O_2. The products of both luciferases include CO_2 and visible light.

luminometers or charged coupled device (CCD) cameras. Sea pansy luciferase (more commonly referenced as *Renilla*) is a monomeric protein of 36 kDa that uses the membrane-permeable substrate coelenterazine as a substrate and does not require ATP. While its structure is unrelated to firefly luciferase, it catalyzes a similar reaction and generates light of a shorter wavelength. The differences in substrate requirement and emitted wavelength have allowed the development of dual reporter systems that utilize both luciferase genes in a single assay. For example, cells have been transfected with firefly and *Renilla* reporter genes under the control of two separate signal transduction pathways, allowing the effects of compounds on each individual pathway to be monitored by the sequential addition of substrates. Similar dual reporter assays have been developed in living mice by taking advantage of the different emission wavelengths and kinetics of the two luciferases. Luciferases can also be utilized in ►protein-fragment complementation assays (PCAs) to measure the interaction between two proteins. In these assays, the luciferase gene is split into two parts, one of which is fused with the gene of the target protein and the other with its potential partner. The interaction of the target protein with its partner brings together the two fragments of luciferase and thus reconstitutes enzymatic activity. Such PCAs have been developed for a number of luciferases and function as highly sensitive detectors of protein–protein interactions in cells and *in vivo*. The primary advantages of either luciferase over the other reporter genes described above are their combination of high specific activity and low background (mammalian cells do not express light-generating enzymes), large and linear dynamic range (7–8 orders of magnitude), and the relatively rapid turnover of the enzyme. Highly stable reporter proteins can lead to a higher signal through accumulation, but their concentrations consequently become poorly linked to changes in transcription. The luciferase assay is also high-throughput, non-radioactive, non-toxic, relatively low cost, and easily adaptable to automation. The primary disadvantages of luciferase are the requirement for exogenous substrates and the 4–6 h delay from stimulus to response to allow transcription to occur.

Luciferase reporter gene assays have a number of important applications in biomedical science and cancer biology. Their initial uses were to map and characterize transcriptional control elements such as promoters, enhancers, silencers, and transcription factors, and these are still valuable applications today. In these experiments, an expression vector is developed with the control region of the gene to be studied inserted upstream of luciferase. Mutations or deletions are then made in the control region at different locations, and the effect of these changes on luciferase output are measured. Once the different regions are mapped in this way, they can be characterized further by removing them from the control region and inserting them immediately upstream of a constitutively active viral promoter driving luciferase. For example, tissue-specific enhancer elements inserted in either orientation should cause an increase in transcription only in the relevant cell types, and silencer elements should decrease expression. The contribution of individual transcription factors to the overall response can be characterized by monitoring the effect of the overexpression or ►siRNA-mediated inhibition of specific factors on the output of luciferase.

Another use of luciferase reporter gene assays is in the study of components of signaling pathways upstream of transcription. A wide variety of assays have been developed that can quantitate modifications of cell-surface proteins such as G-protein coupled receptors (GPCRs), signal transduction proteins such as protein kinases, and protein-protein signaling cascades. In the majority of cases, the particular protein to be studied is known and the assay is designed to respond to changes in its activation status. Specific examples include the site-directed mutagenesis of ►receptor tyrosine kinases, the identification of ligands for ►orphan GPCRs, and the involvement of particular proteins in different transcriptional activation pathways. One particularly important use for this type of assay is in the discovery of ►small molecule drugs. The sensitivity, convenience, and broad dynamic range of luciferase reporter gene assays make them especially suitable for cell-based high-throughput screening for inhibitors and activators of drug targets. Assays have been developed for nearly all classes of drug targets, including GPCRs, protein kinases, nuclear hormone receptors, and protein-protein interactions, and a number of successful drug discovery programs have been launched as a result. The assays are easily miniaturized to 384-well microtiter plate formats, and can be used to screen over 100,000 compounds a day with automated systems. Luciferase reporter gene assays also have applications in pathway identification and characterization when a specific target is not under study. These “black box” assays monitor the effect of a particular treatment on a cell which expresses a luciferase reporter gene linked to a general signaling cascade. Examples include their use in the identification of new drug targets through a ►chemical tool approach, and in genetic or chemical cytotoxicity screens for drug candidates or environmental pollutants.

An exciting and more recent use of luciferase reporter gene assays is in the visualization of gene expression *in vivo*, known as ►bioluminescent imaging. These assays allow the non-invasive, real-time monitoring of biological processes in living animals, and have already contributed to the development of better ways to diagnose and treat disease. The key observation that enabled these assays was that the light generated



Luciferase Reporter Gene Assays. Figure 2 The effect of chemotherapy on luciferase-expressing tumor cells implanted into mice. Tumor cells were implanted into the mice 16 days before the initiation of chemotherapy on day 0 (left panel), and luciferase activity was measured every 4 days thereafter (panels from left to right). Reference: Rehemtulla A, Stegman LD, Cardozo SJ, Gupta S, Hall DE, Contag CH, Ross BD (2000) Rapid and quantitative assessment of cancer treatment response using *in vivo* bioluminescent imaging. Neoplasia 2:491–495.

by luciferase could be transmitted through an animal's tissues and visualized by highly sensitive CCD cameras. Two general types of assays have been described: those where a normal animal is injected with cells or a virus engineered to contain a luciferase reporter gene, and those that utilize a luciferase reporter gene transgenic animal. The initial application for bioluminescent imaging was in animal models of bacterial infection. Pathogenic bacteria such as *Salmonella* and *Pseudomonas* were engineered to express bacterial luciferase without the need for exogenous substrate (other luciferases require the injection of luciferin or coelenterazine before imaging), and the process and distribution of infection was followed upon injection of the cells into an animal. This model has been used to identify those host cells that are important in defense against pathogens, and test possible treatments. A similar approach has been used to study the trafficking and fate of transplanted immune cells, carcinoma cells, or tumor xenografts that have been transfected with luciferase reporter genes. In these cases, the fate of the cells and the effects of different chemotherapeutic and immunotherapeutic regimens can be measured after injection (see Fig. 2). Finally, the effectiveness of viruses used for gene therapy can be monitored *in vivo* by engineering them to express luciferase reporter genes and measuring the degree and location of light output. This approach has been used to optimize the construction, delivery method, safety, and long-term efficacy of such viruses.

The use of transgenic animals in combination with luciferase reporter genes is another area of current development. For example, a number of groups have generated animals that express luciferase under the control of specific promoters (*e.g.* NFkB), or in particular tissues (*e.g.* pituitary). These animals are useful for determining the function of particular genes and the role of signal transduction pathways, for characterizing the effects of drug or other treatments on physiology and disease, and for measuring the tissue distribution and ►pharmacokinetics/pharmacodynamics of therapeutic drugs. One limitation of luciferase reporter gene assays *in vivo* is that they provide only a 2-dimensional view of expression patterns, but better detection technologies and the combination of bioluminescent imaging with other non-invasive technologies such as MRI or CT promise to improve resolution in the coming years.

Luciferase reporter gene assays are an essential *in vitro* and *in vivo* tool in the study of biology and medicine due to their simplicity, sensitivity, and ease-of-use. They have contributed greatly to the study of gene transcription and the elucidation of gene function, and are a valuable technology for drug discovery. Their application *in vivo* promises to facilitate the development of new treatments for cancer and other diseases.

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Lugol Unstained Lesion

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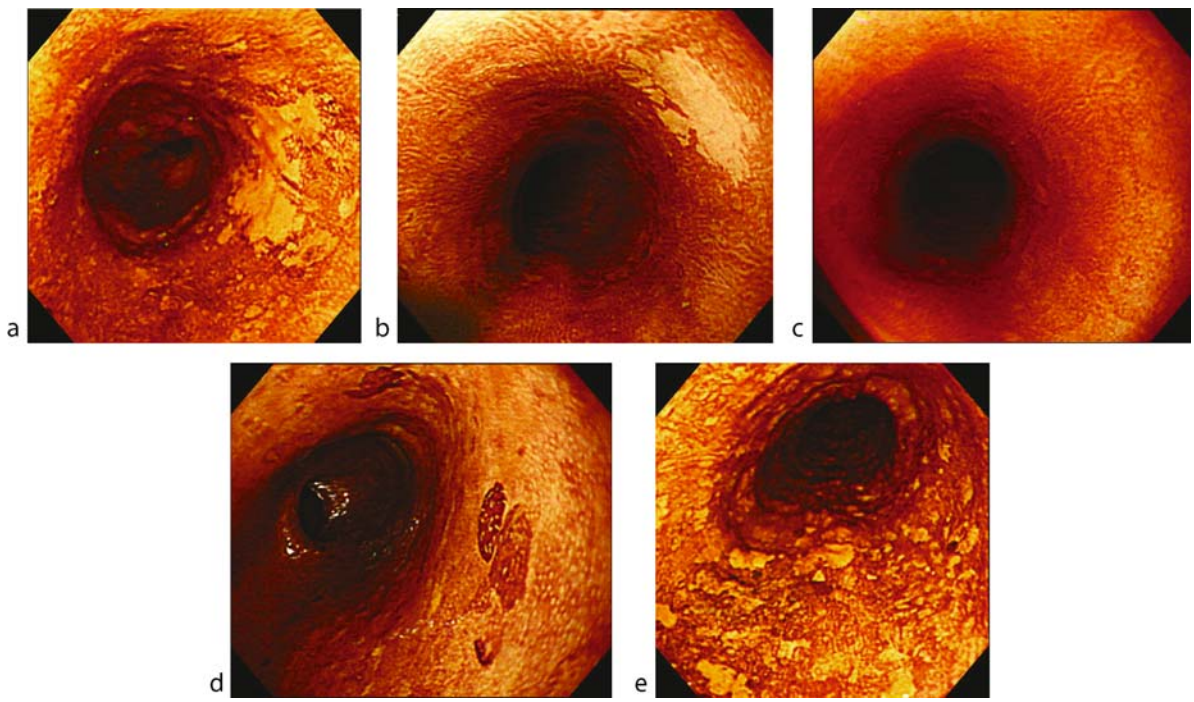
Definition

In 1933, the Lugol spraying method was introduced for the diagnosis of uterine cervical neoplasia using gynecologic colposcopy.

Since the 1980s, this technique has been used for screening of esophageal squamous cell carcinoma (ESCC) with Lugol chromoendoscopy. The Lugol spraying method has developed for early detection of ESCC from asymptomatic patients.

Characteristics

After ordinary endoscopic observation, 5–10 ml of 2.0% glycerin-free Lugol iodine solution, which is a brown liquid consisting of 2.0 g potassium iodine and 4.0 g iodine to 100 ml distilled water, is sprayed from the gastroesophageal junction to the upper esophagus using a plastic spray catheter passed through the biopsy channel of the endoscope. The whole esophagus is re-observed and epithelial areas are categorized as unstained (Fig.1a and b), normally stained (Fig. 1c), or overstained (Fig. 1d). Lugol unstained lesions (LULs) are defined as those areas either staining less intensely than normally stained epithelium, or completely unstained; this group of lesions includes carcinoma, dysplasia, and esophagitis. According to Lugol staining patterns, completely "unstained" areas are found in approximately 90% of high-grade dysplasia and carcinoma, while approximately 90% of areas, which stain less intensely than normally stained epithelium, represent non-dysplastic lesions and the remaining 10% are dysplastic. Therefore, LULs are detectable not only in dysplasias and carcinomas but also in non-dysplastic areas, for example with esophagitis, or in the setting of Barrett esophagus. Overstained areas represent glycogenic acanthosis (GA), which has been referred to as leukoplakia and hyperkeratosis of the esophagus. GA is a common benign lesion easily observed during ordinary endoscopic observations, usually varying in number and consisting of oval, slightly elevated, firm, gray-white plaques, measuring from 0.2 to a maximum of 1.5 cm in length. In addition, GAs stain a deep brown color when treated with Lugol solution. An iodine starch reaction occurs momentarily on esophageal epithelium when sprayed with Lugol solution. Therefore, since normal squamous epithelium contains glycogen that interacts with the iodine in the Lugol solution, normal esophageal epithelium becomes uniformly greenish-brown when stained (1). Dysplasia and esophagitis are not stained, since these regions have a reduced content or no glycogen. Therefore, these minute lesions that were not identifiable by conventional endoscopic observation, become visible when Lugol solution is used. Most individuals with LULs have only single or a few LULs, while multiple LULs (Fig. 1d) were found in some ESCC patients.



Lugol Unstained Lesion. Figure 1 (a) Endoscopic findings of a Lugol-unstained lesion, which was completely unstained. The lesion was 12 mm in diameter, and histologic finding was intraepithelial squamous cell carcinoma. (b) Endoscopic findings of a Lugol-unstained lesion, which was staining less intensely than normally stained epithelium. The lesion was 4 mm in diameter, and histologic finding was esophagitis. (c) Endoscopic findings of normally Lugol-staining epithelium without a Lugol-unstained lesion. (d) Endoscopic findings of an overstained lesion. (e) Endoscopic findings of multiple Lugol-unstained lesions. Many irregular lesions which were stained less intensely than normally Lugol-staining epithelium were located in one endoscopic view.

Carcinoma of the esophagus is considered to be a wide spread global disease estimated in 2000 to be responsible for 338,000 deaths worldwide ►(Squamous cell carcinoma). In Asia, some parts of Africa, South America, and South Europe, squamous cell carcinomas are the dominant form of esophageal cancer. Despite recent advances in the surgical treatment for ESCC, surgical outcome is not satisfactory, with an overall 5-year survival rate of 17–39% worldwide. In contrast, standard ►chemoradiotherapy has a curative potential for locally advanced ESCC, with inoperable cases having a 3-year survival rate of approximately 20%. The survival rate for advanced stage squamous cell carcinomas of the esophagus is poor, mainly due to their late detection and the poor efficacy of therapy containing surgery and chemoradiation. Conversely, the prognosis of patients treated endoscopically for carcinomas confined within the intraepithelium or proper mucosal layer is excellent ►(Tumor staging), with 5-year survival rates of 77–100% in Japan and Europe. These results indicate that endoscopically mucosal resection (EMR) for superficial ESCC can improve the prognosis of ESCC, highlighting the need to detect this disease at an early stage. Thus, the Lugol spraying method is a clinically important adjunct to endoscopic screening for ESCC, since two thirds of esophageal intraepithelial carcinomas are overlooked by conventional endoscopy alone. This simple technique of spraying Lugol solution in the esophagus is a highly sensitive tool for delineating areas of squamous dysplasia and carcinoma (1). In recent screening studies for ESCC, Lugol chromoendoscopy has been used worldwide.

Carcinogenesis (Precursor of ESCC)

Characterization of human esophageal precancerous lesions at the molecular level is of critical importance in our understanding of the etiology of ESCC and for the identification of useful ►biomarkers for prevention studies of this disease. The identification of ESCC precursors will ultimately lead to the prevention of poor-prognostic ESCC. Mutation analyses have demonstrated that *p53* gene mutations occur at an early stage of esophageal carcinogenesis, both in the setting of non-dysplastic lesions (i.e. basal cell hyperplasia (BCH), Lugol unstained lesions with non-dysplastic epithelium (LULs-NDE), chronic esophagitis) and in dysplastic lesions (i.e. low grade and high grade dysplasia, cancer in situ). Since *p53* mutations are frequently found in invasive ESCC, most of these lesions, both dysplastic and non-dysplastic, should be considered to be precancerous.

Mutations of the *p53* tumor suppressor gene ►(p53 protein, biological and clinical aspects) are the most common genetic abnormality in solid human cancers. Missense mutations are found in 78% of the 6,177 somatic *p53* mutations in exons 5–8, suggesting a correlation between the degree of evolutionary diversity and the structural or functional importance of individual amino acid residues. In contrast, *p53* gene mutations have been proposed to be concentrated in six hotspots (2). Based on the updated *p53* Gene Mutation Database containing 5,961 mutations, codons 175, 245, 248, 249, 273, and 282 have been identified as mutation hotspots in human cancers, and the incidence of the hotspot mutation is specific to the molecular alterations in solid human cancers (2). A hotspot can identify a relationship between the mutation, protein structure and function, and carcinogenesis. Furthermore, hotspot mutations in carcinomas represent protein alterations that provide a selective growth advantage to the cell, and missense mutations at six hotspots account for 25–30% of the mutations (2). In contrast, malignant transformation of human esophageal epithelium is a multistage progressive process from non-dysplastic lesions through dysplasia to carcinoma (3). The *p53* hotspot mutation was found in not only dysplasia or carcinoma but also Lugol unstained lesion with non-dysplastic epithelium (LUL-NDE) (4). Therefore, protein alterations that provide a selective growth advantage to the cell would have already occurred in cells of LULs-NDE before histologic transformation into dysplastic cells. Mutations at codon 175 and 273 have been shown to have transforming frequencies that are 22- and 8-fold, respectively, of the basal level for wild type p53 protein. The LUL-NDE or low-grade dysplasia containing mutations with high transforming activities, such as codon 175 and codon 273 mutations, might have growth advantages favoring progression to invasive ESCC with the acquisition of other genetic changes, and may acquire malignant potential before morphologically manifested cell proliferation at an early molecular level of carcinogenesis. In endoscopic biopsy samples, the *p53* missense mutation containing a hotspot mutation was found in NDE, which was identified as a LUL. No hotspot mutation was found in normal Lugol staining area or BCH. These findings suggest that some LULs-NDE may represent the earliest state of esophageal squamous cell carcinoma.

Multiple LULs

A single or few LULs were detected in one fifth of patients attending for routine endoscpy. Although subjects with multiple LULs (ten and more than ten LULs in one endoscopic view, Fig. 1d) were found in only 1% of the general population, dysplasia occurred frequently (60%) in these subjects. In contrast, multiple LULs were found in 27% of head and neck cancer patients, and secondary ESCCs were found in 72% of such cancer patients with multiple LULs (5). These results indicate essential information about field cancerization and malignant potential in respect of multiple LULs. The field cancerization phenomena

proposes that multiple squamous cell carcinomas occur either simultaneously with the primary lesion (synchronous) or after a period of time (metachronous) in the esophagus and the head and neck region. There is the possibility that widespread epithelial oncogenic alterations are found in patients with multiple LULs, indicating that esophageal epithelium with multiple LULs has unique characteristics in carcinogenesis.

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Luminal Epithelial Cells

Definition

One of the two major epithelial cell types in the breast that are located in an inner cell layer surrounding the lumen of the gland and produce milk.

►Basal-like Breast Cancer

Lunasin

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Definition

Lunasin is a novel seed peptide with ►cancer-preventive properties against chemical carcinogens and ►oncogenes.

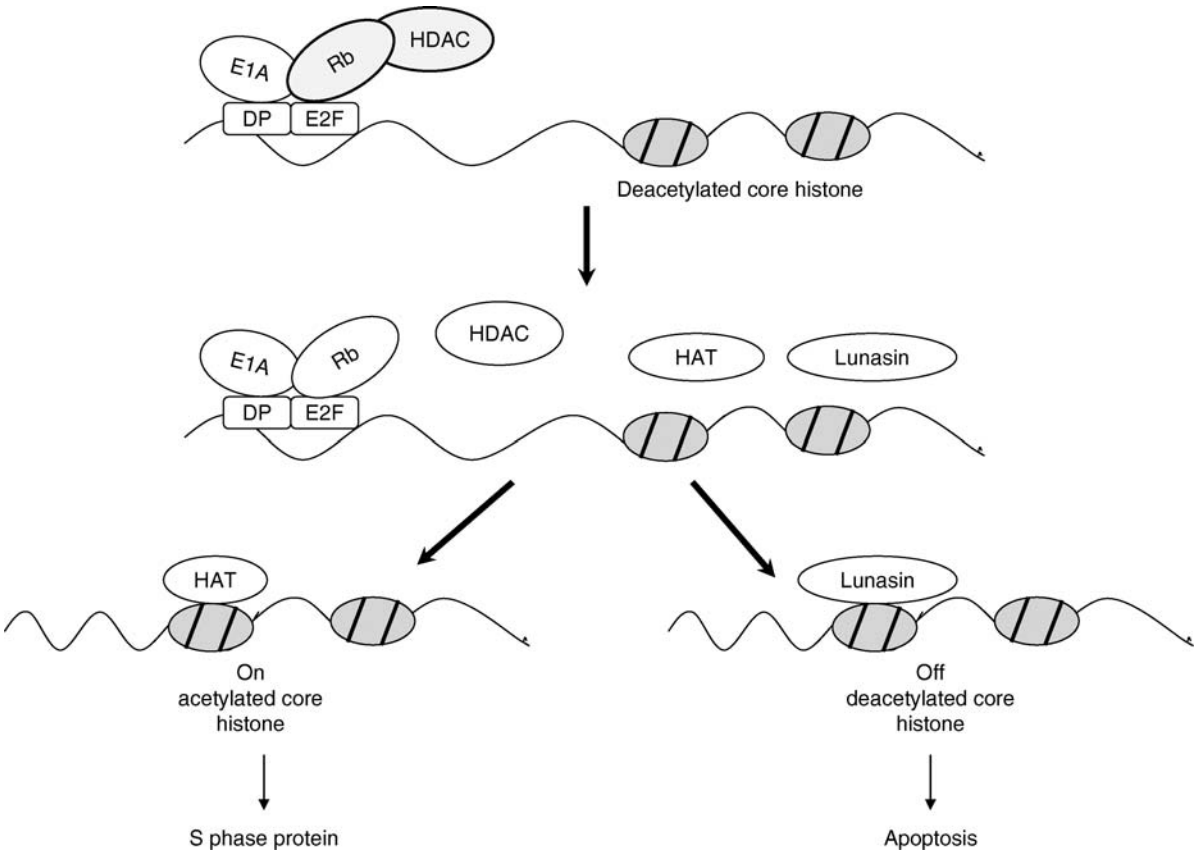
Characteristics

Lunasin is a unique 43-amino acid peptide that contains eight Asp (D) residues in its carboxyl end (bold) preceded by a cell adhesion motif Arg-Gly-Asp (RGD) (italics) and a predicted helix (underlined) with structural homology to a conserved region of chromatin-binding proteins: SKWQHQQDSCRKQKQGVNLTPC-EKHIMEKIQG-*RGD*-**DDDDDDDD**.

Initially identified in the soybean cotyledon, Lunasin has been recently found in barley, wheat, pepper, amaranth, and the *Solanaceae* family. It is likely that additional screening would reveal its presence in many other seeds. Lunasin has been demonstrated to have ►chemoprevention properties both *in vitro* and *in vivo*. In the absence of carcinogens, Lunasin does not seem to affect cell morphology and proliferation but prevents the transformation in the presence of carcinogens. At nanomolar concentrations, Lunasin added exogenously significantly suppresses chemical-carcinogen-induced (►DMBA and ►MCA) foci formation in mouse fibroblasts cells C3H10T1/2 (C3H). This effect is also produced by Lunasin in NIH3T3 cells, being fourfold more effective (on a molar basis) than the ►Bowman-Birk inhibitor (BBI), a known cancer-preventive agent from soy.

Lunasin also prevents transformation of mammalian cells by viral oncogenes. It inhibits, in a dose-dependent manner, foci formation in C3H cells and NIH3T3 cells transfected with the oncogene E1A, known to induce cell proliferation by inactivating the tumor suppressor protein Rb. Interestingly, Lunasin is effective even when added up to 15 days after transfection with E1A gene, suggesting its efficacy when applied even after the transformation event. Lunasin also suppresses colony formation induced by the ►RAS-oncogene in MCF-7 cells stably transfected with an inducible form of the oncogene.

►Chemical carcinogenesis and viral oncogenesis share common mechanism (s) involving changes in chromatin status that Lunasin disrupts to suppress cancer formation. Lunasin peptide added exogenously to mammalian cells treated with the ►histone deacetylases (HDAC) inhibitor sodium butyrate, internalizes into the cell and crosses the nuclear membrane, localizing in the nucleus. There, it binds specifically to deacetylated core histones H3 and H4, inhibiting their acetylation. The affinity of Lunasin for hypoacetylated chromatin and its inhibitory effect on histone acetylation is relevant to the proposed ►epigenetic mechanism of action (E1A-Rb-HDAC model; Fig. 1). This model stipulates that Lunasin selectively kills cells that are being transformed by disrupting the dynamics of histone acetylation-deacetylation when a transforming event occurs. The tumor suppressor protein Rb functions by interacting with E2F promoter and recruiting HDAC to keep the core histones in the deacetylated (repressed)



Lunasin. Figure 1 E1A-Rb-HDAC model to explain the ability of Lunasin to suppress E1A-induced transformation. *Top diagram*: Rb controls G1/S transcription by interacting with E2F promoter and recruiting HDAC to keep the core histones in the deacetylated (repressed) state. In a cell being transformed, E1A inactivates Rb and dissociates Rb-HDAC complex, exposing the deacetylated core histones in the E2F promoter (medium diagram). Lunasin competes with histone acetyltransferase (HAT) in binding to the deacetylated core histones. *Bottom diagram*: HAT binds and acetylates core histones, turning on E2F cell cycle transcription factors, leads to cell proliferation. Lunasin binds, turns off the transcription, perceived as abnormal by cell, results in apoptosis.

state. The inactivation of Rb by the oncoprotein E1A dissociates the Rb-HDAC complex, exposing the deacetylated core histones for acetylation by ►histone acetyltransferases (HATs). When this event occurs, Lunasin is triggered into action and binds to the deacetylated core histones, turning off transcription, which is perceived as abnormal by the cell and commits ►apoptosis. This epigenetic mechanism shows that Lunasin can influence regulatory pathways involving chromatin modifications that may be fundamental to carcinogenic pathways in general.

In the first animal model, Lunasin applied topically suppresses skin papilloma formation in SENCAR mice treated with DMBA and ►TPA. Tumor multiplicity is also reduced and the appearance of papilloma is delayed by this peptide.

Oral administration is an important feature of an ideal cancer preventive agent. To exert this effect, once orally ingested, the peptides have to survive degradation by gastrointestinal and serum proteinases and peptidases, reaching the target tissue or organ in an active form. Bioavailability studies have demonstrated that Lunasin, orally administered to rats, is protected from the gastrointestinal digestion by soy-naturally occurring protease inhibitors, such as BBI and ►Kunitz Trypsin inhibitor (KTI). Lunasin is absorbed and ends up in various tissues as an intact and bioactive peptide. This capacity and its *in vitro* demonstrated properties makes Lunasin the perfect candidate to exert an *in vivo* cancer-preventive activity. Animal studies demonstrating this preventive activity against different kinds of cancer, such as ►prostate, ►colon and ►breast cancer are currently being carried out.

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Lung Cancer

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Synonyms

Lung carcinoma; Bronchogenic carcinoma

Definition

The term lung cancer is used for malignant epithelial tumors arising from the respiratory mucosa (bronchi, bronchioles, and alveoli). Mesotheliomas, lymphomas, and stromal tumors (sarcomas) are distinct from epithelial lung cancers.

Characteristics

Lung cancer is the leading cause of cancer death worldwide with over 1 million deaths each year. The disease is largely preventable as the majority of cases are attributable to smoking. However, global statistics estimate that 15% of lung cancer cases in men and 53% in women occur in never smokers.

The 5-year overall lung cancer survival rate (15%) has nearly doubled in the past 30 years as a result of advances in combined-modality treatment with surgery, radiotherapy, and chemotherapy. Thus, primary carcinoma of the lung is a major health problem with a generally poor prognosis.

Pathology

Four major cell types make up 88% of all primary lung neoplasms according to the World Health Organization classification. These are ►squamous cell carcinoma, ►small cell carcinoma, ►adenocarcinoma (including bronchioloalveolar), and ►large cell carcinoma. The remainder includes rarer tumor types including carcinoids. The various cell types have different natural histories and responses to therapy, and thus a correct histologic diagnosis is essential. In the past 25 years, adenocarcinoma has replaced squamous cell carcinoma as the most frequent histologic subtype, and the incidence of small cell carcinoma is on the decline. These dramatic histologic shifts may be due to changes in the composition of cigarettes.

Etiology

Most lung cancers are caused by ►carcinogens and tumor promoters inhaled via cigarette smoking. The relative risk of developing lung cancer is increased about 13-fold by active smoking and about 1.5-fold by long-term passive exposure to cigarette smoke. Exposure to industrial pollutants and chemicals including ►asbestos, ionizing radiation, ►radon etc. contribute to ►pathogenesis, often exacerbating the effects of ►tobacco carcinogensis.

Biology and Molecular Pathogenesis

Molecular genetic studies have shown the acquisition by lung cancer cells of a number of genetic lesions, including activation of dominant oncogenes and inactivation of ►tumor suppressor genes or recessive oncogenes. Lung cells may have to accumulate a large number (perhaps 20 or more) of such lesions before developing a fully invasive/metastatic phenotype.

Activation of Dominant Oncogenes

Changes in dominant oncogenes, include point mutations in the coding regions of the RAS family of oncogenes (especially ►KRAS gene in adenocarcinoma of the lung); mutations in the tyrosine kinase domain of the ►epidermal growth factor receptor (EGFR) found in non-smoking adenocarcinomas (~10% in Caucasioans, with much higher rates in East Asians) with rates >50% in non-smoking East Asian patients); occasional mutations in ►BRAF and ►PIK3CA or activation of the PIK3CA/AKT/►mTor pathway; amplification, rearrangement, and/or loss of transcriptional control of myc family oncogenes are found in non-small cell cancers, while changes in all myc family members are found in small cell lung cancer); overexpression of bcl-2 and other anti-apoptotic proteins; and overexpression of other EGFR family members such as ►Her-2/neu, and ERBB3; and activated expression of the telomerase gene in >90% of lung cancers. Genome wide approaches are identifying other amplified or mutated dominant oncogenes (such as MET) which could be important new therapeutic targets.

Inactivation of Tumor Suppressor Genes

Selected study and genome wide approaches have identified a large number of tumor suppressor genes

(recessive oncogenes) that are inactivated during the pathogenesis of lung cancer. This usually occurs by a tumor acquired inactivating mutation of one allele (seen for example in the ►*p53* and retinoblastoma, ►*RB1,* tumor suppressor gene) or tumor acquired inactivation of expression by tumor acquired promoter DNA ►methylation (seen for example in the case of the *p16* and *RASSF1A* tumor suppressor genes) which are then coupled with physical loss of the other parental allele ("►loss of heterozygosity"). This leaves the tumor cell with only the functionally inactive ►allele and thus loss of function of the growth regulatory tumor suppressor gene. Genome wide approaches have identified many such genes involved in lung cancer pathogenesis including *p53*, *Rb*, *RASSF1A*, *SEMA3B*, *SEMA3F*, *FUS1*, *p16*, *LKB1*, *RARβ,* and ►*FHIT*. Several tumor suppressor genes on chromosome 3p appear to be involved in nearly all lung cancers. Allelic lost for this region occurs very early in lung cancer pathogenesis, including in histologically normal smoking damaged lung epithelium.

Autocrine Growth Factors

The large number of genetic and epigenetic lesions indicate that lung cancer, like other common epithelial malignancies, is a multistep process that is likely to involve both carcinogens causing mutation ("initiation") and tumor promoters. Prevention can be directed at both processes. Lung cancer cells produce many peptide hormones and express receptors for these hormones, which can act to stimulate tumor cell growth in an "►autocrine" fashion.

Highly carcinogenic derivatives of ►nicotine are formed in cigarette smoke. Lung cancer cells of all histologic types (and the cells from which they are derived) express nicotinic acetylcholine receptors. Nicotine activates signaling pathways in tumor and normal cells that block ►apoptosis. Thus, nicotine itself could be directly involved in lung cancer pathogenesis both as a mutagen and tumor promoter.

Clinical Presentation

There are multiple ways by which lung cancer presents, including weight loss, chronic cough, persistent lung infection, malaise, chest pain, hemoptysis etc. ►SCLC, in particular, may present with a wide variety of paraneoplastic syndromes. With the advent of computer tomography (CT) screening programs, a modest number of mostly early stage cancers are being detected as asymptomatic tumors.

Treatment

Two important guiding principles in the treatment of lung cancer are: (i) histologic diagnosis (especially the distinction between small cell or ►non-small cell carcinomas, NSCLC) and (ii) staging. For early stages of NSCLC, complete resections offer relatively high 5-year survivals. Patients unsuitable for surgery may be offered curative intent radiotherapy. ►Adjuvant therapy may be given to more advanced resected cases. For late stage cases, chemotherapy with or without ►palliative radiotherapy are the conventional options, although the long term survival rates are very low.

Small cell lung cancer (SCLC) is considered a chemotherapy sensitive disease. Patients with limited stage disease have high response rates to chemotherapy (60–80%), including a 10–30% complete response rate, although the response rates in extensive disease patients is somewhat lower (50%), and almost always consists of partial responses. Tumor regressions usually occur quickly, within the first two cycles of treatment, and provide rapid palliation of tumor-related symptoms. Thus, chemotherapy is the backbone of treatment of patients with SCLC.

Given the poor prognosis of most patients with lung cancer, targeted therapies offer the best long term prospects. ►Tyrosine kinase inhibitors (►erlotinib and ►gefitinib) have been associated with responses and improved survival in specific subsets of NSCLC, specifically adenocarcinoma histology, female gender, East Asian ethnicity and never smoking status. The presence of EGFR mutations (and/or increased gene copy number in some studies) in the tumors are often associated with dramatic responses and lengthened good quality life, although most responders eventually relapse. Many other targeted therapies are currently undergoing clinical trial.

As lung cancer is one of the most preventable of diseases, anti-smoking campaigns have been initiated in many countries. Avoidance of smoking for never smokers and cessation for current smokers offer the best hopes for prevention, although these aims have proven more difficult to implement than expected. However, as pointed out earlier, about 15% of lung cancer occurs in lifetime never smokers. The very different molecular changes in cancers arising in smokers and never smokers suggests that ►environmental tobacco smoke ("passive smoking") can only account for a minority of cancers arising in never smokers. Thus lung cancer will remain an important cause of morbidity and mortality long after a complete ban on the use of tobacco products is implemented on a world wide scale.

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Lung Carcinoma

►Lung Cancer

Lung Colony Formation Assay

Definition

Experimental model used in animals to assess the capacity of tumor cells to seed, survive and successfully initiate the formation of colonies in the lungs after intravenous (tail vein) injection. Also referred to as "experimental metastasis model" although this latter designation is misleading. ►Metastasis indeed is much more complex and involves many steps at the site of the primary tumor and local lymph nodes that are not covered by the lung colony formation assay

►Cystatins

Lung Resistance-related Protein

►Major Vault Protein

Lupus Erythematosus

Definition

A chronic autoimmune disease in which the immune system attacks the body's cells and tissues, resulting in inflammation and tissue damage, most commonly in the heart, joints, skin, lungs, blood vessels, liver, kidneys and nervous system.

►Rituximab

Luteotropic Hormone

►Prolactin

Luteotropin

►Prolactin

Lycopene

Definition

Is a bright red carotenoid pigment, a phytochemical found in tomatoes and other red fruits. Lycopene is the most potent carotenoid antioxidant in the human body.

►Chemoprotectants
►Carotenoids

Lymph

Definition

A transparent, slightly yellow fluid that carries lymphocytes, bathers the body tissues, and drains into to lymphatic vessels, which transport lymph to the immune organs and into the bloodstream.

►Lymphatic Vessels

Lymph Node

Definition

Is a component of the lymphatic system filtering the lymphatic fluid for bacteria, viruses, or foreign particles that can be recognized and eliminated by lymphocytes,

cells that rapidly multiple in response to infections in the highly specialized lymph node milleu.

►Sentinel Node

Lymph Node Metastases

Definition

Also known as nodal involvement, positive nodes, or regional disease. Cancer cells may spread to the regional lymph nodes near the primary tumor. Localized spread to regional lymph nodes is not normally counted as metastasis, but is a sign of worse prognosis.

►Endothelins

Lymphadenopathy

Definition

Enlarged lymph nodes.

Lymphangiogenesis

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Synonyms

Development of new lymphatic vessels; Growth of new lymphatic vessels

Definition

Lymphangiogenesis is the process whereby new lymphatic vessels develop within a tissue. Most commonly, lymphangiogenesis refers to the growth of lymphatic vessels by sprouting of new vessels from pre-existing lymphatic vessels. Additionally, lymphangiogenesis refers to the initial formation of the lymphatic system during embryonic development.

Characteristics

Lymphangiogenesis, the growth of new lymphatic vessels, occurs during embryonic development and in tumors and lymph nodes of tumor bearing animals. This key process also occurs in wounds and in inflamed tissues. Lymphangiogenesis in tumors has been linked to the formation of lymph node metastases. Importantly, lymph nodes are the initial or frequent sites of ►metastasis for many tumors, including human pancreatic, gastric, breast, and prostate carcinomas, ►melanomas and other tumors and recent studies indicate that lymphangiogenesis likely plays an important role in driving tumor metastasis.

The lymphatic system is comprised of a network of blind-ended, thin walled lymphatic capillaries, collecting vessels and specialized secondary immune organs, including lymph nodes, tonsils, Peyer's Patches and spleen. This system is connected to the vascular system through the thoracic duct. Lymphatic vessels drain protein-rich interstitial fluid and immune cells from tissues through lymph nodes. A thin network of lymphatic capillaries is found in the outer rim or capsule of the normal lymph node. Lymphatic capillaries are comprised of a single layer of lymphatic endothelial cells, which share many molecular characteristics with vascular endothelial cells. Collecting vessels in the lymphatic system are comprised of endothelia surrounded by a layer of ►pericytes. During early embryonic development, the lymphatic system forms by branching off of the cardinal vein and expanding into an alternate network of thin-walled vessels.

Lymphatic vessels differ from blood vessels in several ways. Large collecting lymphatic vessels contain vascular smooth muscle cells in their walls, as well as valves, which prevent the backflow of lymph. However, lymphatic capillaries, unlike typical blood capillaries, lack pericytes and a continuous basal lamina and contain large inter-endothelial valve-like openings. Lymphatic capillaries consist of loosely overlapping cells. Due to their greater permeability, lymphatic capillaries may be more effective than blood capillaries in allowing tumor cells to pass into the vessel lumen.

The recent identification of selective markers of lymphatic versus vascular endothelial cells has allowed identification of the mechanisms that regulate lymphangiogenesis. Lymphatic endothelia selectively express ►LYVE-1, a member of the ►CD44 hyaluronic acid receptor family, Prox-1, a lymphatic vessel specific homeobox transcription factor, ►podoplanin, a mucin-type glycoprotein, and ►VEGFR3, a receptor for ►VEGF-C and VEGF-D. Lymphatic capillaries, unlike typical blood capillaries, lack pericytes and a continuous basal lamina.

Tumor secreted factors such as VEGF-C and VEGF-A have been shown to promote lymphangiogenesis within tumors. These factors activate VEGFR3, a

tyrosine kinase VEGF family receptor that is expressed primarily on lymphatic endothelium. Expression of VEGF-C is correlated with increased lymph node metastasis and poor clinical outcome in a variety of tumors. Indeed, in animal models of metastasis, inhibitors of VEGF-C (soluble VEGFR3) inhibited tumor lymphangiogenesis and tumor metastasis to lymph nodes.

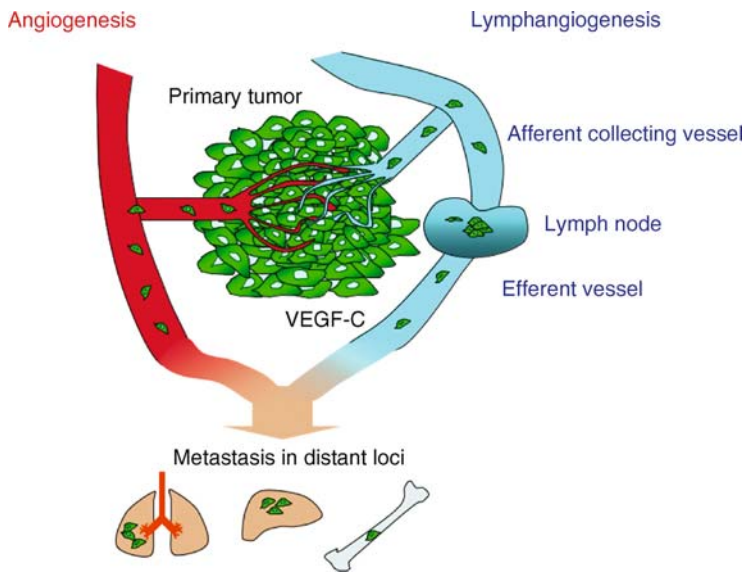
Tumors spread by lymphatic routes to lymph nodes but may also spread by hematogenous (vascular) or lymphatic routes to distant organs. Tumors secrete a number of factors including VEGF-C and others that induce both lymphangiogenesis and ►angiogenesis (Fig. 1).

Molecular Regulation

The molecular mechanisms that regulate lymphangiogenesis, the growth of lymphatic vessels, have recently begun to be understood. Two members of the VEGF family, VEGF-C and VEGF-D, stimulate lymphangiogenesis by binding to the receptors VEGFR-2 and VEGFR-3 on lymphatic endothelial cells. Indeed, homozygous deletion of VEGF-C genes in mice leads to a complete absence of the lymphatic system. VEGF-A, FGF and HGF can also stimulate lymphangiogenesis. The discovery of specific markers of lymphatic endothelium has facilitated analysis of the mechanisms regulating lymphangiogenesis. VEGFR3 is expressed only by quiescent lymphatic endothelial cells and not by quiescent vascular endothelial cells; however, both proliferating vascular and lymphatic endothelial cells express this protein. The homeodomain transcription factor Prox-1 is selectively expressed on lymphatic endothelium and not vascular endothelial cells. It is also expressed by developing neuronal cells in flies and mammals. Podoplanin, a mucin-type glycoprotein, is expressed by lymphatic endothelial cells and a few other cell types such as type I lung alveolar cells and kidney podocytes. Mice with genetic loss of podoplanin display paw lymphedema, or loss of fluid drainage from the paw. Importantly, the CD44 hyaluronic acid binding protein family member LYVE-1 is expressed only by lymphatic endothelium as well as liver and spleen sinusoidal endothelial cells. Interestingly, LYVE-1 is down regulated on pericyte-lined collecting vessels of the lymphatic system. Although the function of LYVE-1 is currently unknown, there is a clear association of the absence of LYVE-1 with the presence of pericytes. Expression of LYVE-1 is undetectable on lymphocytes, hematopoietic cells, or vascular endothelial cells. LYVE-1 is thus a useful marker to determine the localization of lymphatic endothelium by immunohistochemistry in vivo and to characterize and purify LEC in vitro.

While growth factors and their receptors play critical roles in angiogenesis and lymphangiogenesis, the integrin family of cell ►adhesion proteins controls cell attachment to the extracellular matrix and promotes the survival, proliferation and ►motility of many cell types. Angiogenesis, the development of new blood vessels, depends not only on soluble growth factors such as VEGF-A but also on survival and migratory signals transduced by the integrins αvβ3 αvβ5, α5β1 and/or α4β1. In contrast, only integrins α4β1 and α►9β1 have been shown to play roles in lymphatic vessel development, as animals lacking ►integrin α9β1 develop ►chylothorax and animals lacking α4β1in endothelia do not respond to VEGF-C.

Many tumors express VEGF-C and VEGF-D, growth factors that selectively regulate lymphangiogenesis.



Lymphangiogenesis. Figure 1 Model of tumor lymphangiogenesis.

While there are three known vascular endothelial growth factor receptors, VEGFR-1, VEGFR-2, and VEGFR-3, only one, VEGFR-3 is expressed predominantly on lymphatic vessel. Importantly, tumor-associated ►macrophages can release VEGF-C and can stimulate lymphangiogenesis in the absence of added growth factors. In fact, recent studies showed that macrophage secretion of VEGF-C and VEGF-D induces lymphangiogenesis, as well as lymphangiogenesis in tumors. Importantly, a number of studies have shown that antagonists of VEGF-C suppress tumor lymphangiogenesis and lymphatic metastases in animal models of tumor growth.

Clinical Relevance

Congenital or pathologically induced damage to the lymphatic system can result in lymphedema, a condition in which drainage of fluid from tissues is blocked, skin thickens and adipose tissue accumulates. Mutations in VEGFR3, the forkhead transcription factor ►FOXC2 and the transcription factor ►SOX18 each induce distinct forms of congenital or ►primary lymphedema. Recombinant VEGF-C was able to promote therapeutic lymphangiogenesis in animal models of lymphedema. Additionally, ►cancer surgery and radiation therapy, especially in breast cancer therapy, can induce ►secondary lymphedema by damaging the lymphatic system in normal tissues, such as the breast. Diseases of the lymphatic system include lymphedema, lymphangiosarcomas and lymph node metastasis. Primary lymphedema arises from congenital defects in molecules that regulate development of lymphatic vessels, such as VEGFR3, FOXC2 and SOX18. Secondary lymphedema arises as a consequence of surgery, infection (such as filariasis) or radiation therapy. In these disorders, the normal architecture of the lymphatic vessels and/or lymph nodes is disrupted. Removal of fluids from tissues can be disrupted in these disorders and tissue architecture is altered. An understanding of how lymphatic vessels grow and respond to environmental cues could help to develop therapies for these disorders. In over eighty percent of cancers, malignant tumor cells metastasize to the lymph nodes and travel through the lymphatic system. Tumor cell derived factors such as VEGF-C stimulate lymphangiogenesis in tumors and studies have shown that increased lymphatic vessel density is associated with increased metastasis. An understanding of the molecular mechanisms that regulate lymphangiogenesis may lead to new therapies for cancer metastasis and lymphedema.

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Lymphangioleiomyomatosis

Definition

LAM; is a disorder seen almost exclusively in females and is characterized by bronchiolar smooth muscle infiltration and cystic changes in the lung parenchyma. LAM patients often have angiomyolipoma of the kidneys and/or abdominal and hilar lymph nodes.

Lymphangitic Carcinomatosis

Definition

Diffuse malignant infiltration of the lungs with obstruction of the lymphatic channels that occurs most commonly in patients with carcinoma of the breast, lung, stomach, pancreas, prostate, cervix, or thyroid as well as in patients with metastatic adenocarcinoma from an unknown primary site.

►Carcinomatosis

Lymphatic Mapping

Definition

The process in which a radio-labeled tracer is injected in or near a primary tumor site to outline the way lymph drains to its corresponding lymph nodes. This process is visualized on lymphoscintigrams and serves, with or without the use of blue dye, as an intraoperative guide during sentinel node biopsy.

►Sentinel Lymph Nodes

Lymphatic System

Definition

A circulatory network of lymph vessels that transports lymph fluid and filters it in the lymph nodes. Lymph fluid coming from the entire body is collected in several large trunks that eventually drain into the venous circulation.

►Sentinel Lymph Nodes

Lymphatic Vessels

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Definition

Lymphatic vessels are the lymphatic capillaries, collecting vessels and ducts that form an integral part of the one-way, open-ended circulatory system known as the lymphatic system. In addition to lymphatic vessels, the lymphatic system consists of lymph nodes and other lymphoid organs.

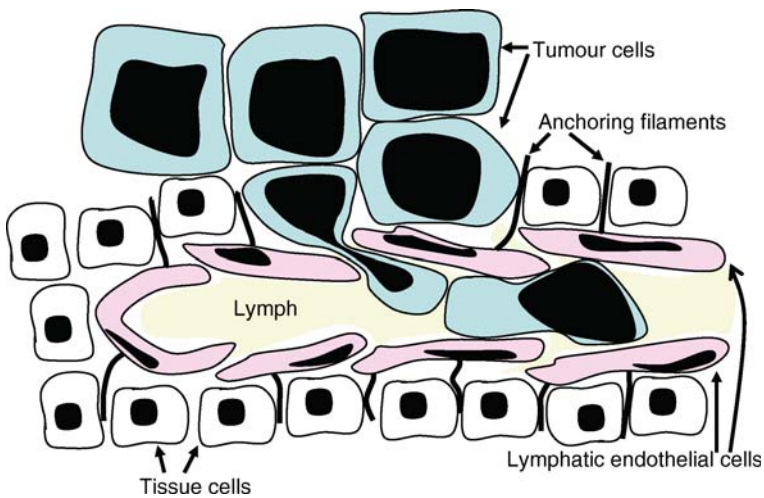
Lymphatic vessels have three interrelated physiological functions: (i) removal of excess fluids from body tissues, (ii) transportation of immune cells (including lymphocytes and dendritic cells) and, (iii) absorption of dietary fat and fat-soluble vitamins from the digestive system and the subsequent transport of fat (chyle) to the circulatory system.

Characteristics

Lymphatic capillaries are lined by a single layer of endothelial cells with overlapping intercellular junctional complexes (Fig. 1). They are devoid of ►pericytes and smooth muscle cells, and are surrounded by a discontinuous or absent basement membrane. Collecting lymphatic vessels typically possess a sparse smooth muscle cell layer, basement membrane and valves to facilitate unidirectional fluid transport. Lumen patency is maintained by anchoring filaments that connect the abluminal surface of the endothelial cells to the ►extracellular matrix. Lymphatic vessels are present in most organs, except avascular structures (such as epidermis and cartilage) and certain vascularized organs (the central nervous system and bone marrow).

Interstitial fluid in tissues drains into lymphatic capillaries and becomes ►lymph. It then flows into collecting lymphatic vessels away from tissues, passes through lymph nodes, and eventually reaches either the right lymphatic duct or the largest lymph vessel in the body, the thoracic duct. These vessels drain into the right and left subclavian veins respectively to return the processed lymph to the circulatory system.

The regulation of lymphatic vessel formation, growth and maturation is complex. The lymphatic system was first systematically described by Asellius in the seventeenth century (1627) as "milky veins" in the mesentery of a "well-fed" dog. The developmental origin of lymphatic vessels was first recognized by Sabin in the early twentieth century (1902), who proposed that endothelial cells bud off from veins in the jugular and



Lymphatic Vessels. Figure 1 Schematic representation of tumor cells invading a lymphatic capillary.

perimesonephric areas during early embryonic development and migrate to form primitive lymph sacs. Endothelial sprouting from these sacs subsequently forms lymphatic capillaries.

►Lymphangiogenesis is the *de novo* formation of lymphatic vessels, in a manner analogous to blood vessel ►angiogenesis. It plays an important physiological role in homeostasis, metabolism, immunity and wound healing. Aberrant lymphatic vessel formation has been implicated in a number of pathological conditions including tumor cell metastasis, edema, and inflammatory diseases.

Clinical Relevance

Defective lymphatic vessels result in a number of human pathologies. ►Congenital lymphedema is hereditary, and is often linked to mutation in the gene encoding vascular endothelial growth factor receptor-3, although other genes may also cause this condition. The hereditary disease ►lymphedema-distichiasis is caused by a mutation in the forkhead family transcription factor *FOXC2*. Lymphatic malformations are composed of defective cutaneous and subcutaneous lymphatic vessels. These lesions are not hereditary, and are thought to arise as a developmental defect where part of the developing lymphatic system becomes separated from the rest of the lymphatic system and subsequently becomes cystically dilated.

Secondary lymphedema occurs when the lymphatic system is damaged following trauma. Events that may cause damage include surgery and/or radiotherapy (typically during cancer treatment), infection, severe injury or burns. Secondary lymphedema may be transitory, recurring or a chronic condition.

Clinical findings have suggested that tumor-associated lymphatics play a key role in ►metastasis by providing a pathway for tumor cell dissemination, (Fig. 1). Indeed, the presence of metastatic tumor cells in regional lymph nodes is an important prognostic indicator for many human cancers.

Members of the ►vascular endothelial growth factor (VEGF) family, VEGF-A, VEGF-C and VEGF-D, signaling through VEGF receptors-2 and/or -3, have been shown to play a critical role in a number of *in vitro* and *in vivo* models of lymphangiogenesis. In cancer, the majority of clinical studies show positive correlations between VEGF-C and/or VEGF-D levels and tumor lymphatic vessel density, lymph node status and, in some instances, poor clinical outcome. In addition, several other growth factors, such as ►platelet-derived growth factors (PDFGs), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs) angiopoietins, and ►insulin-like growth factors (IGFs), have been demonstrated to promote lymphangiogenesis.

It is currently unclear whether preexisting lymphatic vessels are sufficient to permit tumor cell dissemination, or whether this function requires *de novo* lymphatic formation or an increase in lymphatic size. The relative contribution of each of these processes may also vary with tumor type and subtype. Furthermore, as intratumoral lymphatic vessels are typically collapsed due to the high interstitial pressure found in tumors, it is likely that in the majority of tumors peri-tumoral lymphatic vessels play a key role in tumor dissemination to lymph nodes.

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Lymphedema

Definition

Synonym lymphoedema; Refers to building up of lymphatic fluid in the soft tissues of the body, usually in an arm or leg, with the result of a swelling. As the fluid accumulates, the swelling continues. The lymphatic system consists of lymph vessels and lymph nodes that run through the body. Lymph vessels collect a fluid that is made up of protein, water, fats, and wastes from the cells of the body. Lymph vessels carry this fluid to the lymph nodes that filter waste materials and foreign products, and then return the fluid to the blood. If vessels or nodes become damaged or are missing, or when here is a blockage of the lymphatic system, the lymph fluid cannot move freely through the system. The fluids can then build up and cause swelling in the affected arms or legs.

Lymphedema-Distichiasis

Definition

Is an autosomal dominant disorder that classically presents as lymphedema of the limbs and double rows of eyelashes (distichiasis).

►Lymphatic Vessels

Lymphoblastoid Cell Lines

Definition

LCLs; ►Epstein Barr virus (EBV) immortalized B cell lines established by in vitro infection or culture of peripheral blood lymphocytes (PBL) from EBV-infected individuals. These cells are not tumorigenic in ►nude mice

Lymphocytes

Definition

All adaptive immune responses are mediated by lymphocytes. Lymphocytes are a class of white blood cells that bear variable cell-surface receptor for antigen. These receptors are encoded in rearranging gene segments. There are two main classes of lymphocyte – B lymphocytes (B cells) and T lymphocytes (T cells) – which mediate humoral and cell-mediated immunity, respectively. Small lymphocytes have little cytoplasm and condensed nuclear chromatin; on antigen recognition, the cell enlarges to form a lymphoblast and then proliferates and differentiates into an antigen-specific effector cell.

►Adaptive Immunity

Lymphocytic and Histiocytic Cells

Definition

L&H cells are the tumor cells in the lymphocyte predominant subtype of Hodgkin lymphoma. These are relatively large mononuclear lymphoma B cells, showing strong expression of the B cell maker CD20.

►Hodgkin and Reed/Sternberg Cell
►Hodgkin Disease

Lymphoepithelioma

►Nasopharyngeal Carcinoma

Lymphogenic Metastatic Spread

Definition

Small lymph capillaries surrounding or invading tumors may take up tumor cells and transport them via larger lymph vessels to regional lymph nodes.

►Senival Lymph Nodes

Lymphoid Organs

Definition

Are structured tissues where lymphocytes maturate, encounter their antigen and differentiate. They are divided into primary (bone marrow, thymus) and secondary (e.g., spleen, lymph nodes) lymphoid organs by providing sites for either lymphocyte maturation or activation and differentiation, respectively.

Lymphokine-Activated Killer

Definition

LAK cells are used in ►adoptive immunotherapy for the treatment of malignant diseases. The therapy involves the removal of peripheral blood from a patient, depletion of red blood cells from the blood to produce a lymphocyte-containing white blood cell fraction, incubating the blood fraction in culture medium with ►interleukin-2 (IL-2) to induce their transformation into tumor-destroying LAK cells, and injecting the LAK cells into the patient along with interleukin-2. LAK cells are thought to be similar to NK cells in that they lyse target cells in a non-major histocompatibility complex (►MHC)-restricted manner.

►Immunotherapy
►Activated Natural Killer Cells

Lymphokine-Activated Killer Cell

Definition

LAK; A white blood cell that is stimulated in a laboratory to kill tumor cells. If lymphocytes are

cultured in the presence of ►Interleukin 2, effector cells will develop that are ►cytotoxic to tumor cells.

Lymphokines

Definition

Soluble factors or cytokines that are produced by lymphocytes (mostly T cells), which have effects on the function of other cells expressing lymphokine receptors. Most common lymphokines belong to the interleukin family.

►Peptide Vaccines for Cancer

Lymphoma

Definition

Malignant lymphomas are defined as neoplasms consisting of cells of the lymphoid tissues. Contrary to most organs and tissues, and in spite of the fact that lymphomas show a wide range of aggressiveness from localized, indolent to highly aggressive, rapidly metastasizing tumors, no *bona fide* benign lymphomas have been defined. Distinct lymphoma entities in general show a characteristic clinical and biological behavior, but within these entities a broad spectrum of aggressiveness may be observed in individual cases, which is either due to disease progression in a multi-step process of lymphomagenesis and/or the involvement of specific risk factors.

►Malignant Lymphoma: Hallmarks and Concepts
►Hodgkin Disease

Lymphoscintigraphy

Definition

An imaging technique whereby a radio-labeled tracer is administered and its whereabouts in the lymphatic system are imaged using a gamma camera.

►Sentinel Lymph Nodes

Lymphotoxin

Definition

LT; Synonym tumor necrosis factor-β(TNF-β), a cytokine secreted by inflammatory CD4 T cells that is directly cytotoxic for some cells.

Lymphovascular Invasion

Definition

Presence of tumor cells invading blood or lymphatic vessel around the primary tumor.

►Adjuvant Chemoendocrine Therapy

Lymphovenous Shunt

Definition

Part of the lymphatic fluid entering the lymph node may be shunted to the blood capillaries without further transport in the serial system of lymph nodes. This direct access to the blood stream provides means of metastatic routes, where tumor cells may enter the blood stream at the site of the lymph node and spread by a hematogenously.

►Sentinel Node

Lynch Syndrome

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Synonyms

Hereditary nonpolyposis colorectal cancer; HNPCC

Definition

Lynch syndrome is an autosomal dominantly inherited cancer susceptibility syndrome, characterized by cancers

of multiple anatomic sites, of which colorectal cancer (CRC) is the most common. Mismatch repair (MMR) genes, inclusive of hMSH2, hMLH1, hPMS1, hPMS2 and hMSH6, in their mutant form are causal for the cancer phenotype. Lynch syndrome appears to show genotypic and phenotypic heterogeneity. hMSH2 mutations may predispose to a greater frequency of extracolonic cancers while mutations in hMSH6 may result in a predominance of gynecologic cancer, particularly endometrial carcinoma, so that CRC may not pose the primary basis for Lynch syndrome diagnosis. Lynch syndrome is the most commonly occurring hereditary CRC disorder.

Molecular genetic findings have enabled hereditary CRC to be divided into two groups: (i) tumors that show microsatellite instability (MSI), occur more frequently in the right colon, have diploid DNA, harbor characteristic mutations such as transforming growth factor β Type II receptor and BAX26 and behave indolently, of which the Lynch syndrome is an example; (ii) tumors with chromosomal instability (CIN), which tend to be left-sided, show aneuploid DNA, harbor characteristic mutations such as K-ras, APC, p53), and behave aggressively, of which familial adenomatous polyposis is an example.

Characteristics

Characteristics (clinical, molecular and pathology): Affected individuals inherit a mutation in one of the MMR gene alleles. When a second mutation is acquired in the wild-type allele, the target cell is less able to repair DNA mismatch errors. Tumors composed of such cells characteristically manifest microsatellite instability and are said to have replication error phenotype (RER+). Most of the tumors arising in the Lynch syndrome are MSI+, while about 15% of apparently sporadic CRCs are MSI+. This is exceedingly interesting in that those sporadic MSI + tumors have clinical pathologic features similar to those observed in the Lynch syndrome (1–3).

Prior to molecular genetic breakthroughs, one was required to depend upon the cardinal features of the Lynch syndrome, since there were no premonitory signs or biomarkers to guide diagnosis. These cardinal features are as follows:

- The inheritance pattern is autosomal dominant
- Gene penetrance is ~85–90%
- Gene carriers develop colorectal carcinoma at an early age (~45 years)
- Most (~70%) cancers arise proximal to the splenic flexure
- Multiple CRCs, both synchronous and metachronous, are common
- Accelerated carcinogenesis is present
- The prognosis is better than for sporadic colon cancer
- The pathology features of CRC are often distinguishable (but not pathognomic) and include poor differentiation, increased signet cells, medullary features, peritumoral lymphocytic infiltration, Crohn's-like reaction, and an increased frequency of tumor infiltrating lymphocytes (TILs) admixed with tumor cells

These clinical features characterize Lynch syndrome I. Lynch syndrome II is characterized by all of these same features and, in addition, shows an increased risk for malignancy at certain extracolonic sites, including the endometrium, ovary, stomach, small bowel, hepatobiliary tract, pancreas, ureter, renal pelvis and breast.

History

The history of the Lynch syndrome dates to an observation of Dr. Aldred Warthin, pathologist at the University of Michigan School of Medicine. He became deeply moved when his seamstress, in 1895, told him that she would likely die of cancer of the colon, stomach or her female organs, because of the enormous proclivity to these cancers in her family. Warthin listened intently, developed her pedigree, and along with other similar cancer prone families published this work in 1913. He updated the family in 1925. The seamstress's family has since been known as Family G. Lynch et al. [4] described the natural history and genetics of two large Midwestern kindreds (Families N and M) which subsequently were found to have features similar to Family G. Dr. A. James French, Warthin's successor as chairman of pathology at the University of Michigan, heard about Families N and M and recalled that his predecessor, Aldred Warthin, had discovered a similar family in 1895. Dr. French invited Dr. Lynch to take custody of all the detailed documents and pathology specimens which the meticulous Dr. Warthin had investigated, catalogued and published over a span of more than 30 years. Family G was then updated and published in 1971 [5]. A detailed review of the history of the Lynch syndrome has been published by Lynch et al. [6].

Management of the Lynch syndrome is predicated upon the cardinal features of its natural history, discussed above. Most importantly, given the proximal predilection for CRC, colonoscopy is mandatory. In fact, evidence is already in hand that colonoscopy will significantly reduce morbidity and mortality in Lynch syndrome patients [7, 8]. Approximately one-third of the cancers occur in the cecum, so that colon cleanout is necessary for good visualization of the cecum. Given its early age of onset, and accelerated carcinogenesis, we recommend colonoscopy be initiated between ages 20 and 25 and repeated every 1–2 years. In the Lynch syndrome II variant, in addition to the colon, attention for screening is focused on the endometrium and ovary. At age 30, transvaginal ultrasound of the endometrium

and ovary is performed and endometrial aspiration is considered.

The search for a germ-line mutation should be performed only on families with substantial evidence of a hereditary cancer syndrome. Therefore, to establish a syndrome diagnosis, collecting the patient's cancer family history is mandatory and may potentially constitute the most cost-beneficial component of a patient's medical workup. Once the Lynch syndrome diagnosis is established, high-risk patients are then presented with opportunities to search for the germ-line cancer-prone mutation. Herein, genetic counseling prior to DNA collection and at the time of disclosure of DNA test results is recommended. Once a Lynch syndrome MMR germ-line mutation is identified within a family, those who are positive for the mutation are provided genetic counseling at the time of disclosure of results and are afforded an opportunity to follow highly targeted surveillance and management recommendations, while those who are negative for the mutation will revert to general population guidelines.

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Lysolecithin

►Lysophosphatidylcholine

Lysophosphatidate

Definition

Lysophosphatidate (LPA) is a type of serum-derived lipid growth factor. This bioactive lipid phosphate is present outside the cell and signals through a series of cell surface receptors. LPA can be formed from phosphatidate by the action of phospholipase A1 or A2, or from circulating lysophosphatidylcholine by lysophospholipase D (autotaxin).

►Lysophosphatidylcholine
►Lipid Mediators

Lysophosphatidylcholine

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Synonyms

Lysolecithin

Definition

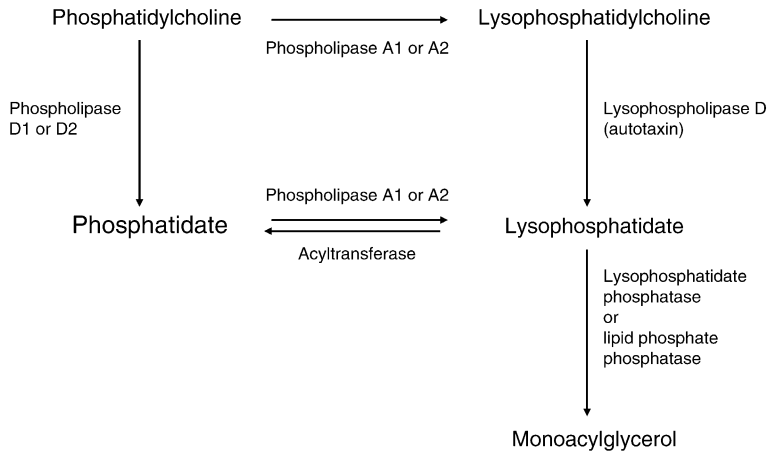
Lysophosphatidylcholine (LPC) is a major plasma lipid constituent that is produced from ►phosphatidylcholine (PC).

Characteristics

LPC is produced from PC under a variety of physiological and pathological conditions. LPC is present at high levels (about 100 μM) in plasma under normal conditions and exists mainly in albumin- or lipoprotein-bound forms. The biochemical conversion from PC to LPC is mediated by ►phospholipase A1 or ►phospholipase A2. Sequentially, LPC is converted to ►lysophosphatidate (LPA) by lysophospholipase D (autotaxin) (Fig. 1).

Signaling Mechanisms

LPC is a cell-signaling molecule. It acts as a ligand for a family of ►G-protein coupled receptors (GPCRs), e.g. G-protein coupled receptor 4 (GPR4) and G2A. Although our knowledge of LPC-sensitive GPCRs is



Lysophosphatidylcholine. Figure 1 Metabolism of lysophosphatidylcholine.

preliminary, circumstantial evidence suggests multiple roles in a variety of physiological and pathophysiological states, such as the development, regulation of the cardiovascular, immune and nervous systems, inflammation, arteriosclerosis and cancer.

GPR4 shows high-level expression in many tissues such as the lung, liver, kidney, ovary and lymph node. GPR4 has the Kd value for LPC = 159 nM in the binding experiments of GPR4-expressing cells. LPC induces transcriptional activation of the serum response element (SRE) in GPR4-transfected HEK293 cells. Gi and Rho (►Rho family proteins) signaling pathways are involved in SRE activation through GPR4. Rho-dependent activities induced by LPC also include actin rearrangement and cell migration. LPC-induced cell migration via GPR4 is sensitive to the C3-exoenzyme. GPR4 is also linked to the activation of the extracellular signal-regulated kinase (ERK) pathway in response to LPC, leading to the stimulation of DNA synthesis and cellular migration in Swiss 3T3 cells. In vascular endothelial cells, LPC upregulates adhesion molecules and growth factors and stimulates the secretion of chemokines and superoxide anions.

G2A is expressed in hematopoietic cells such as T- and B-lymphocytes, monocytes and macrophages and in tissues such as spleen and thymus. LPC activates the migration of T-lymphoid cell lines and peritoneal macrophages via a G2A/Rho dependent mechanism. G2A stimulates Rho via Ga13, resulting in actin rearrangement and SRE-dependent transcription activation. LPC-G2A activation also induces cell migration in Jurkat T cells. As a major component of oxidized low-density lipoprotein, LPC exerts a chemoattractive effect on T cells and macrophages in atherosclerosis and other chronic inflammatory diseases. G2A also displays distinct patterns of ERK activation.

As with other GPCRs, GPR4 and G2A induce ligand-dependent increase in $[Ca^{2+}]i$ (in particular, $[Ca^{2+}]i$ release from intracellular stores), by which they regulate cellular Ca^{2+} homoeostasis and the cytoskeleton, adhesion and migration, proliferation and survival.

Clinical Aspects

Although many lines of evidence support a causal relationship between LPC and atherosclerosis or other inflammatory diseases, its role in carcinogenesis has not yet been extensively investigated. The concentration of LPC (in particular, the ratios of palmitoyl-LPC to linoleoyl-LPC) is elevated in the sera of patients with ovarian cancer or multiple myeloma. In addition, LPC levels are elevated in the bile of patients with ►anomalous pancreaticobiliary ductal junction (APBDJ), which is one of the risk factors for biliary tract carcinomas, and that LPC inhibited cellular apoptosis by inducing ►cyclooxygenase-2 (COX-2) expression via a Raf-1 (►Raf kinase) dependent mechanism in human cholangiocytes. Therefore, LPC is considered to be involved in tumor development or progression, although this still needs to be clarified.

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Lysosome

Definition

Is a catabolic organelle in the cytoplasm of eukaryotes. It is characterized by a membrane proto-ATPase that maintains the low pH of 5–6. Lysosomes contain hydrolytic enzymes like proteases, nucleases, phosphatases, glucosidases and lipases. Proteins and other cellular material that is to be degraded enter the lysosome by endocytosis or autophagy. Together with the ubiquitin/proteasome system, the lysosome is the main site of intracellular protein degradation and also fulfills storage functions for ions and small molecules.

►Autophagy

Lyve-1

Definition

Is a transmembrane receptor for extracellular hyaluronic cid. It is a homologue of ►CD44 and mainly expressed on lymphatic endothelial cells.

►Podoplanin
►Lymphangiogenesis

Mϕ

Definition

Short for Macrophage; Tumor-associated Macrophages.

M&B 39831

►Temozolomide

MA-3, TIS (mouse Pdcd4)

►Programmed Cell Death 4

Mach

►Caspase-8

MACH-related Inducer of Toxicity

►FLICE Inhibitory Protein

Machado-Joseph Disease

Definition

MJD; synonyms spinocerebellar ataxia Type 3 (SCA 3), Portuguese-Azorean disease, Joseph disease, Azorean disease. Is a rare hereditary disorder affecting the central nervous system, especially the areas responsible for movement coordination of limbs, facial muscles, and eyes. The disease involves the slow and progressive degeneration of brain areas involved in motor coordination, such as the cerebellar, extrapyramidal, pyramidal, and motor areas. Ultimately, MJD leads to paralysis or a crippling condition, although intellectual functions usually remain normal. See also ►Spinocerebellar Ataxia Type 1.

Macro- and Microminerals

►Mineral Nutrients

Macroautophagy

►Autophagy

Macrolide

Definition

Macrolide antibiotics belong to the polyketide class of drugs. Their activity resides in the large lactone

ring (14- to 16-membered) to which one or more deoxy sugars, usually cladinose and desosamine, are attached.

►Rapamycin

Macrometastasis

Definition

Synonym metastasis derive from unrestrained proliferation of tumor cells far from the organ of origin (metastatic tumor cells).

►Circulating Tumor Cells
►Metastatic Colonitation

Macropain

Definition

Synonym ►Proteasome.

Macrophage Stimulating 1 Receptor

►Ron Receptor

Macrophage-Stimulating Protein

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Synonyms

Hepatocyte growth factor-like protein; HGFL; Macrophage stimulating protein; MSP; MST1; HGFL; DNF15S2

Definition

MSP is a plasminogen-related growth and motility serum protein that was discovered as a macrophage chemotactic factor (Fig. 1).

Characteristics

Structure

MSP is a heterodimeric protein (total mass 78 kD, 711 aa) composed of two chains, a 53 kD α and a 25 kD β chain that are linked together by a disulfide bond. Features of the α chain include an N-terminal domain corresponding to the plasminogen preactivating enzyme (PAP or hairpin loop), four kringle domains and a segment that terminates in the cleavage site for activation. The β chain has the serine protease-like domain that is devoid of enzymatic activity because of catalytic triad mutations. MSP is most closely related to hepatocyte growth factor/►scatter factor (HGF/SF), to which it has 45% sequence similarity, and the human gene maps to 3p21.

Biological Activities and Targets

- Murine resident peritoneal ►macrophages: involved in shape changes, ►chemotaxis and inhibition of inducible NO-synthase (iNOS).
- Epithelial cells: involved in enhanced ►adhesion to extracellular matrix, chemotaxis, proliferation, protection from ►apoptosis and increased beat frequency of ciliated epithelium.
- Hematopoetic cells: involved in proliferation, apoptosis and cytokine production.
- Osteoclast-like cells: involved in bone resorption.

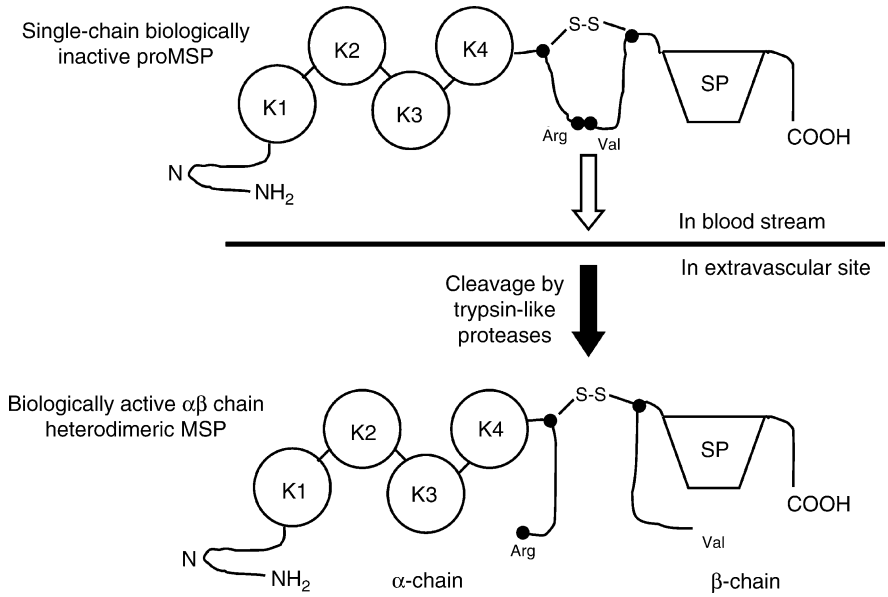
Cellular and Molecular Regulation

MSP is synthesized by hepatocytes as a biologically inactive single chain pro-MSP and is converted at extravascular sites to active MSP by trypsin-like proteases, which cleave at Arg483/Val484 to generate a disulfide-linked α/β chain heterodimer. The enzymes involved in the conversion of pro-MSP to MSP are:

- Purified coagulation system enzymes in vitro: factors XIa and XIIa, serum kallikrein;
- A trypsin-like proteolytic activity on the membrane of peritoneal macrophages;
- A trypsin-like protease in human wound fluid.

MSP Receptor Signaling

MSP mediates its biological activities by activating a cell receptor tyrosine kinase known as RON (Recepteur d'Origine Nantaise) in humans and STK (Stem cell-derived Tyrosine Kinase) in mice. RON/STK is a classical transmembrane receptor belonging to the Met-receptor tyrosine kinase family. The RON gene maps to 3p21, in close vicinity to the MSP gene. MSP-induced receptor activation involves, most likely, dimerization of receptors followed by autophosphorylation occurring in trans (between two receptors) of specific tyrosines in the cytoplasmic portion of the receptors. RON/STK receptors transmit a signal from the cell surface to the inside, which causes the cell to respond to MSP in a



Macrophage-Stimulating Protein. Figure 1 Structure and conversion of pro-MSP to MSP. MSP comprises an N-terminal domain (N) which includes a hairpin loop, four kringle domains (K1–K4) and a serine proteinase-like domain (SP). The interchain disulfide bridge is formed by the α chain Cys468 and the β chain Cys588 (*solid circles* connected by –S–S–). MSP circulates in the blood as single-chain biologically inactive pro-MSP. At extravascular sites, trypsin-like proteases convert pro-MSP to biologically active, disulfide-linked and αβ heterodimeric MSP by cleavage of the peptide bond between Arg483 and Val484 (shown as *solid circles*).

specific manner. MSP-activated signals and effects are cell type specific. MSP mitogenic effects are mediated via focal adhesion kinase (FAK), SRC and mitogen-activated protein kinase (MAPK). Phosphatidylinositol-3-kinase (PI-3K) mediates MSP-induced adhesion and motility. The PI-3K/AKT and the MAPK pathways transduce MSP anti-apoptotic effects, whereas activation of JUN N-terminal kinase (JNK) may induce apoptosis in selected hematopoietic cells.

Clinical Relevance

Active MSP is found in wound fluid, and a high level of RON receptor expression has been detected on the surface of epithelial cells in burn wound epidermis, suggesting a possible role for MSP in wound healing. Overexpression and constitutive ligand-independent RON activation are found in some breast carcinomas, suggesting a possible RON contribution to breast cancer progression. Transfection of a RON cDNA carrying activating mutations in the cytoplasmic domain at Asp 1232 or Met 1254 converts cells to tumorigenicity, raising the possibilty that mutated RON may contribute to tumor development in humans, although a primary role for mutated RON in causing human cancer has not been identified.

►Macrophages

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Macrophages

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Definition

Mononuclear phagocytic cells differentiated from bone-marrow derived monocytes and found in tissue.

Characteristics

Macrophages originally defined by their phagocytic ability, hence their name (large eater), are one of the major cell types in the body and are found in all tissues where they often constitute 10–20% of the cells. These phagocytic cells were initially defined as the reticuloendothelial system but this definition appears to include cells with different origins and now macrophages are considered part of the mono-nuclear phagocytic system that also includes the more differentiated specialist cells such as the bone-remodeling, ►osteoclasts and the antigen-presenting, ►dendritic cells. These cells have various origins including primitive embryonic macrophages formed in the yolk sac, those derived later in embryogenesis from hematopoietic stem cells (HSC) in the fetal liver and during the post-natal period from the bone marrow. Except in very early embryos, HSCs differentiate through several steps into ►monocytes that circulate and that after extravasation into tissues mature into macrophages. Monocytes appear heterogenous and it is probable that different subsets of macrophages differentiate from different monocytic precursors. HSCs are directed down the mononuclear phagocytic lineage under the influence of several growth factors of which the major one is ►colony stimulating factor-1 (CSF-1; also known as macrophage-CSF), a homodimeric growth factor that activates a class three trans-membrane tyrosine kinase receptor the product of the *c-fms* proto-oncogene expressed on all cells of the lineage. Other growth factors can also promote macrophage colony formation including ►granulocyte-macrophage colony stimulating factor (GM-CSF) and IL-3.

Macrophages perform diverse roles and often have specialized names such as the ►Kupffer cells of the liver, the ►microglia in the brain and the Langerhans cells in the skin. Considerable focus has been paid to their role in the immune system where they are central players in the innate response and the transition of this response to an acquired one. Indeed, macrophages are recruited by a wide range of danger signals to orchestrate these immune activities. They are recruited in response to locally synthesized factors, including the growth factors CSF-1 and ►VEGF, chemokines such as CCL-2, and bacterial products. They are also have important roles in tissue remodeling and wound healing responding to any damage to produce matrix remodeling molecules and trophic and angiogenic factors. Studies on mutant mice depleted in macrophages, particularly the ►osteopetrotic mouse mutant that is homozygous for an inactivating mutation in gene for CSF-1 ($Csf1^{op}$), have confirmed these functions but have also revealed important roles for macrophages during development. These studies have shown that macrophages are essential for the full and timely development of many tissues including the mammary gland, skin, pancreas, brain and bone. For example, in the absence of CSF-1 signaling the mono-nuclear phagocyte derived osteoclasts fails to develop with the consequence that the bone laid down by osteoblasts is not remodeled causing an osteopetrotic phenotype after which the mutation is named. In these mice a bone cavity fails to form adequately and bone marrow hematopoiesis is compromised. Similar trophic and remodeling capacities of macrophages are found for many tissues and their deficiency results in a wide range of phenotypes including small size, reproductive compromise, neurological deficiencies and immune malfunction.

These diverse roles have led to a categorization of different states. Initially a "classically activated" macrophage was defined that showed enhanced antimicrobial activities in a stimulus dependent, but antigen-non-specific manner. These macrophages are activated largely by products of specifically-activated T helper (TH-1) cells particularly interferon γ and have also been referred to as M1 type macrophages. In addition to their ability to kill pathogens through the generation of reactive oxygen and nitrogen species, they can also behave as antigen-presenting cells. In contrast, exposure to cytokines such as ►IL-4 and IL-13 result in an alternatively activated class of macrophages that is produced in a Th-2 type responses and which are involved in allergic reactions and humoral responses to parasites. This class also overlaps with a trophic and remodeling macrophage sub-set that has been referred to as M2-macrophages. This M1/M2 dichotomy has been applied particularly in the tumor context (see below) and represents two extremes of function. It seems likely however, given the wide-range of macrophages phenotypes that there is a continuous spectrum of activities many of which are overlapping and that for our constraining definitions, may appear contradictory. Indeed macrophages may be continuously and reversibly educated by their micro-environment to perform specific tasks.

Macrophages are also found in all solid tumors. Although the original concepts were that these cells were attempting to reject the tumor through their cytocidal and antigen-processing capacities, a recent meta-analysis indicated that in over 80% of studies their density was positively correlated with poor prognosis. Similarly, over-expression of CSF-1 or the macrophage chemoattractant, ►CCL-2 (monocyte chemoattractant protein-1) is correlated with poor prognosis in a variety of cancers. These data suggest that ►tumor associated macrophages (TAM) predominantly, although not exclusively, act to enhance tumor progression and metastasis. In fact, macrophage may also play a causal role in cancer since there is a growing body of evidence that chronic, sometimes called smoldering inflammation, produced by infection for example by Hepatitis virus, ►*Helicobacter pylori* and *Schistosomiasis* or by chronic irritants such as asbestos

or cigarette smoke, can lead to cancer. Since macrophages are central to the inflammatory response their activities in producing growth factors, reactive oxygen and nitrogen species can create a mutagenic and growth promoting environment that both cause oncogenic mutations and promotes the outgrowth of malignant epithelial cells.

Detailed studies of the role of macrophages in mouse models of cancer support the clinical data. For example, genetic depletion of macrophages in a mouse model of breast cancer resulted in a slower rate of tumor progression to malignancy and a dramatic inhibition of metastasis. In contrast, premature recruitment of these cells enhanced these processes. Furthermore, therapeutic approaches using SiRNA or antisense oligonucleotides directed against CSF-1 or treatment with anti-CSF-1 antibodies reduced macrophage accumulation and inhibited tumor growth in xenograft models of human cancer.

TAMs have several activities that can enhance tumor progression and promote metastases. Firstly, they can stimulate angiogenesis and recently they have been found to be responsible for the ►angiogenic switch that is closely associated with the onset of malignancy *in vivo*. This is likely to be at least in part due to their ability to synthesize vascular ►endothelial growth factor (VEGF), a CSF-1 regulated gene in macrophages. Secondly, they can enhance tumor cell migration and invasion *in vivo* through a paracrine loop that requires EGF and CSF-1 signaling in the tumor cell and macrophage respectively with the cognate ligand being produced by the reciprocal cell. Tumor cells also show greater motility when they are in close apposition to macrophages *in vivo*. Macrophages are largely found in the stroma surrounding tumors but they also align in clusters along the abluminal side of vessels. Imaging of living tumors in mice using multi-photon microscopy has revealed that these macrophage foci are the major sites for tumor cell intravasation. Macrophages are also significant producers of proteases such as uPA and ►MMP9, both of which have been associated with tumor progression and metastasis. These proteases can remodel extra-cellular matrix thereby allowing escape of tumor cells from the basement membrane and access into the surrounding stroma and its associated vasculature. These proteases can also release matrix-bound growth factors such as VEGF, TGFβ1 and heparin-binding EGF, growth factors that have all been shown to play a causal role in tumor progression.

It has also been suggested that cells of the mononuclear phagocyte lineage form part of a niche that forms the "soil" for metastatic cells to grow. This is consistent with growing evidence of macrophages in the primary tumor migrating to distant sites where they promote the establishment of metastases. These niches allow tumor cell homing, growth and subsequent vascularization.

Macrophages can also be involved in the rejection of tumors through their cytocidal and antigen presenting properties and there are undoubtedly cases when this occurs. However, there is growing evidence that the tumor microenvironment directs macrophages away from these "activated" macrophage properties (M1) towards an alternative state (M2) that causes trophic and repair roles that are characteristic of their normal functions during development and in wound healing. These properties are enhanced by an environment that contains growth factors such as CSF-1 and the cytokines IL-4 and IL-10. There is also a growing body of evidence that the tumor microenvironment causes differentiation of a class of mononuclear phagocytes, referred to as ►myeloid suppressor cells, that block the development of cytotoxic T cells whose activities can reject tumors. Thus, macrophages may also play an important role in the ►immunoediting that allows tumors to escape immune destruction despite the expression of alloantigens.

In summary, the evidence both from clinical and experimental studies strongly suggests that in the majority of cases tumor associated macrophages are recruited to promote tumor progression and metastasis. It would appear that once in situ the tumor microenvironment educates the macrophages to perform many trophic functions that stimulate tumor growth, with its concurrent selection of epithelial cells with an increasing number of oncogenic mutations.

►Metastasis

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Maculae Adherents

►Desmosomes

MAD

Definition

Encodes a nuclear protein (221 amino acids and 25 kDa) that belongs to the basic region helix-loop-helix (BHLH) family of proteins. It dimerizes with MAX and antagonizes transcriptional activity of the MYC protein; MAD/MAX dimers bind to DNA through the CAC GTG sequence (►E-box). MAD competes for MAX/MYC dimerization. The gene maps to chromosomal position 2p13. Mad family are a group of bHLH proteins comprising MAD, MAD3, MAD4, MNT and MXI1.

►Smad Proteins in TGFβ Signaling

MAD1

►Mitotic Arrest-Deficient Protein 1

MAD1L1

►Mitotic Arrest-Deficient Protein 1

Mad (Mxd)

Definition

Max dimerization protein is a transcription factor of the Myc/Max/Mad(Mxd) network of basic region-helix-loop-helix-leucine zipper proteins. Mxd is primarily expressed in differentiated cells where it is considered to act as a transcriptional repressor, preventing transcription through recruitment of histone deacetylase-containing complexes. The four proteins Mxd1-4 were originally named Mad1, Mxi1, Mad3, and Mad4.

►*Myc* Oncogene

MADH4

►Deleted in Pancreatic Carcinoma Locus 4

Magic Bullet

Definition

Any drug designed for killing foreign agents saving the self compartments of human body.

►Liposomal Chemotherapy

Magicin

►Endothelial Derived Gene-1

Magnetic Resonance Imaging

Definition

A basis of magnetic resonance imaging (MRI) method is the behavior of atomic nuclei of certain elements when subjected to a strong constant magnetic field plus superimposed weak variable magnetic fields and radio waves of a defined frequency. Hydrogen is the usual element investigated by MRI. Atoms of hydrogen make up about 65% of the body. Normally, the hydrogen nucleus (proton) spins around its own axis of rotation and a collection of protons within the patient's body has their individual axes spatially oriented at random. When the patient is placed inside a strong (constant) magnetic field, axes tend to line up parallel to the lines of magnetic field, but spin either clockwise or counter clockwise. Few unpaired atoms in the hydrogen assemblage inside the body can be "wobbled" by weak varying magnetic fields and radio waves producing signals of varying intensity. Through electronic and computer processing these varying signals are used to construct three-dimensional images of the various organs and tissues.

►Radiation Oncology
►MRI
►Nasopharyngeal Carcinoma
►Dynamic Contrast-Enhanced Magnetic Resonance Imaging
►Hyperthermia

Magnetic Resonance Imaging-Guided Focused Ultrasound Surgery

Definition

A method of noninvasive thermal ablation of pathological tissue. The ultrasound beam penetrates through soft tissues and can be focused to target sites causing localized high temperatures (55–90°C) for a few seconds. As a result, well-defined areas of protein denaturation, irreversible cell damage, and coagulative necrosis are produced, while overlaying and surrounding tissues are spared.

►Uterine Leiomyoma

Maidenhair Tree

►Ginkgo Biloba

Major Histocompatibility Antigens

Definition

Polymorphic proteins between individuals of a given species or different species that can be directly recognized by recipient T lymphocytes, eventually leading to severe and rapid rejection reactions.

►Graft Acceptance and Rejection

Major Histocompatibility Complex

Definition

MHC; Is a cluster of genes on human chromosome 6 or mouse chromosome 17. It encodes a set of membrane glycoproteins called the MHC molecules. The MHC class I molecules present peptides generated in the cytosol to CD8 T cells, and the MHC class II molecules present peptides degraded in intracellular vesicles to CD4 T cells. The MHC also encodes proteins involved in antigen processing and other aspects of host defense. The MHC is the most polymorphic gene cluster in the human genome, having large numbers of alleles at several different loci. Because this polymorphism is usually detected by using antibodies or specific T cells, the MHC molecules are often called major histocompatibility antigens.

Major Histocompatibility Complex (MHC) Class I-related Chain Molecules

►MIC Molecules

Major Life Event

Definition

Critical event which causes severe stress and requires adjustment to the new situation (e.g. death of a loved one, divorce, job loss)

►Stress

Major Microtubule Organizing Center

►Centrosome

Major Vault Protein

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Synonyms

Lung resistance-related protein; LRP

Definition

Major vault protein (MVP) is the major component of the vault complex, the largest ribonucleoprotein particle which is hypothesized to be a general carrier molecule for nucleocytoplasmic transport.

Characteristics

The Vault Complex

The vault complex is an evolutionarily conserved ribonucleoprotein particle with an estimated molecular mass of 13 MDa. The barrel-like structures of the vault, initially detected in preparations of clathrin-coated vesicles from rat liver in the mid 1980s, were named vaults due to their resemblance to the vaulted ceilings in cathedrals. The vault complex consists of two symmetrical halves which can unfold into flower-like structures, each consisting of eight distinct petals that are joined to a central ring. Cryoelectron-microscopic three-dimensional image reconstruction techniques revealed a smooth hollow barrel-like structure having an invaginated waist and two protruding caps. The entire mammalian vault complex is composed of ~96 molecules of the 100–110 kDa major vault protein (MVP), 8 molecules of the 193 kDa vault poly(ADP-ribose) polymerase (VPARP), 2 molecules of the 240 kDa telomerase-associated protein (TEP1), and at least six copies of a small untranslated RNA of 88–141 bases. Each petal of the two flower-like halves of vault is believed to consist of six MVP molecules, and the minor vault proteins; TEP1 and probably VPARP, as well as the vault RNAs were shown to be located in the two protruding caps.

The number of vaults per cell has been estimated to be 10,000–100,000 copies. Vaults are most numerous in ►macrophages and epithelial cells with secretory and excretory functions as well as cells chronically exposed to ►xenobiotics such as bronchial cells and cells lining the intestine. In mammalian cells, the vault complex is predominantly localized in the cytoplasm, but ~5% is localized to the cytoplasmic face of the nuclear membrane at or near ►nuclear pore complexes ►(NPC). Based on its typical structure and subcellular localization, the vault is hypothesized as a general carrier molecule for nuclear-cytoplasmic transport. Cryo-immunoelectron microscopy and immunogold staining have provided evidence for the passage of MVP/vaults through the NPC by locating MVP inside the NPC. Furthermore, immunofluorescence and ►immunohistochemistry experiments have demonstrated the association of vaults with cytoskeletal elements. In experiments using sea urchins, the caps of vaults were found to associate with microtubules *in vitro*. Another study demonstrated that β-►tubulin co-precipitated with MVP, and that destabilization of microtubules by nocodazole slowed down the vault mobility in fluorescence-recovery after photobleaching (FRAP) experiments. These data suggest that a subset of vaults move directionally, via microtubules towards the nucleus. Besides, the internal cavity of the vault complex is about 5×10^7 Å^3 in size, large enough to contain particles such as ribosomes or hundreds of proteins as cargoes. Indeed, the sea urchin MVP was demonstrated to copurify with ribosomes, and cryoelectron-microscopic images often show electron dense material within isolated vault particles. To date several proteins have been reported to interact with MVP making them potential cargoes for the vault. In stable transfectant MCF-7 cells, double-site mutations of bipartite ►nuclear localization signal (NLS)-like sequences in ►PTEN (phosphatase and tensin homolog deleted on chromosome 10) disrupted both the nuclear localization of PTEN and the interaction between PTEN and MVP, suggesting that MVP/vaults contribute to the nuclear transport of PTEN.

Major Vault Protein (MVP)

The main component of the vault complex is MVP, also known as ►lung resistance-related protein (LRP), which constitutes 75% of the total mass of the vault and determines its structure. The human MVP gene, located on the chromosome 16p, has 15 exons and encodes 893 amino acids. The human MVP protein contains two distinct domains: a coiled-coil domain at its C-terminal end responsible for the interaction between two MVP molecules and crucial for vault formation, and the N-terminal domain comprised of seven repeats of ~55 amino acids. Two, and possibly three, calcium-binding EF-hands have been identified in the N-terminal domain. The N-terminal part of MVP has been shown to interact with the C-terminus of VPARP.

Recent evidence, from many groups, suggests that MVP is involved in the regulation of signaling pathways including the ►PI3K (phosphatidylinositol 3-kinase)/Akt and ►Ras/►Raf/►MAPKs (mitogen-activated protein kinases) pathways, possibly by acting as a scaffold protein for signaling molecules. To date, several proteins have been reported to interact with MVP, including steroid receptors, PTEN, ►SHP-2, MAPKs, ►COP1 (constitutively photomorphogenic 1), and ►Src. MVP was coimmunoprecipitated with the estrogen receptor in MCF-7 cell nuclear extract. PTEN in HeLa cells was shown to interact with MVP *in vitro* in a Ca^{2+}-dependent manner. SHP-2 was shown to dephosphorylate MVP *in vitro*. Furthermore, in response to ►EGF (epidermal growth factor), SHP-2 associated with tyrosyl-phosphorylated MVP, MVP interacted with the activated MAPKs, and a constitutive complex between tyrosyl-phosphorylated MVP, SHP-2, and MAPKs was detected in MCF-7 cells. In MVP-deficient fibroblasts, MVP was shown to cooperate with Ras for optimal EGF-induced ►Elk-1 activation and

was required for cell survival. UV irradiation enhanced MVP's tyrosil phosphorylation, caused dissociation of COP1 from MVP, and attenuated the inhibitory activity of MVP on ►AP-1 (activator protein-1) transcription. The interaction between Src and MVP was shown to be dependent on EGF-induced Src activity and MVP tyrosil phosphorylation.

Accumulating data indicate that MVP plays a crucial role in cell survival. Serum deprivation significantly increased cell death of $MVP^{-/-}$ MEFs as compared to wild-type MEFs. Disruption of two MVP genes in the slime mold *Dictyostelium discoideum* resulted in growth defects under nutrient stress. Furthermore, anti-MVP polyclonal antiserum reduced viability of the mature monocyte-derived dendritic cells.

There are a couple lines of evidence that suggest the involvement of MVP in cell differentiation. Phorbol 12-myristate 13-acetate, lipopolysaccharide, and sodium butyrate (NaB), agents known to induce cell differentiation, up-regulate MVP in a variety of cell types. In addition, MVP is strongly up-regulated during dendritic cell (DC) maturation. In human DC cultures, antibodies against MVP reduced the expression of DC differentiation and maturation markers and impaired the capacity to induce antigen-specific T cell response. The exact role of MVP in DC maturation may be controversial as the MVP knockout mouse failed to substantiate the role of MVP in DC development and function.

During sea urchin embryogenesis, MVP accumulates in the nucleus at the mesenchyme blastula stage, the developmental stage marking the transition between proliferation and differentiation. This may suggest that MVP contributes to development through nuclear transport. This is further substantiated by the expression and distribution of MVP in mammalian oocytes and embryos at various stages of preimplantation development.

Taken together, these data implicate a role for MVP in cell survival, differentiation, and development through mediating cellular signaling pathways and/or nucleocytoplasmic transport.

Drug Resistance

Utilizing immunocytochemical methods in a screen of 61 human cancer cell lines, MVP was detected in 78% of the cell lines and expression levels predicted resistance against a variety of anticancer drugs. These data were confirmed in a study with eight cancer cell lines chosen from the panel which demonstrated a positive correlation between MVP mRNA level and broad chemoresistance. In astrocytic tumor cell cultures, a correlation between MVP expression and resistance against several drugs including anthracyclins, etoposide, and ►cisplatin was observed. In contrast, in ►non-small cell lung cancer (NSCLC) cells, basic MVP expression levels correlated with resistance to cisplatin, but not to several other drugs, including daunomycin, doxorubicin, etoposide, vinblastine and bleomycin. In pharyngeal epidermoid carcinoma KB-3–1 cells, however, elevated MVP levels correlated with decreased accumulation of doxorubicin in the nuclei.

Several studies have reported increased expression levels of MVP in drug-selected multidrug-resistant (MDR) cell models. Doxorubicin selected U-937 human leukemia cells have increased levels of both MVP mRNA and protein and acquired resistance against doxorubicin, etoposide, mitoxantrone and 5-FU, independent of several ►ABC transporters (ATP-binding cassette transporters). A selection of KB-3–1 cells with the carcinogen benzo[a]pyrene led to a selective up-regulation of MVP. In general, many drug-selected MDR cell lines have a 1.3–1.5-fold increase in MVP. The exact mechanism of MVP in MDR has not been elucidated, however recent findings suggest that MVP contributes to MDR by regulating nuclear drug export.

While MVP is thought to play a role in MDR, other studies indicate that MVP does not always play a role. For instance, in A2780 ovarian carcinoma cells, MVP overexpression did not confer drug resistance against doxorubicin, vincristine and etoposide, although these transfectants contained increased number of intact vault particles with increased levels of the minor vault components TEP1 and VPARP. In the MVP knockout mouse model, both embryonic stem cells and bone marrow cells did not show an increased sensitivity to a panel of cytostatic agents compared to wild-type cells. The activities of P-gp, MRP1 and BCRP1 were not altered in the vault-deficient cells, excluding the possibility of these ABC-transporters compensating for the loss of vaults. Moreover, both knockout and control mice responded similarly to the drug treatment. In a functional study, stable transfection of GFP-tagged MVP in the SW1573 NSCLC cell line did not alter daunorubicin efflux rate and intracellular distribution. No differences were detected in the handling of daunorubicin in MVP-knockout MEFs compared to wild-type MEFs. ►SiRNA-mediated inhibition of MVP in the drug resistant SW1573/2R120 NSCLC cell line did neither alter doxorubicin nuclear efflux, intracellular distribution, nor sensitivity to doxorubicin.

Altogether, while a direct role of MVP in MDR mainly by drug transport mechanisms was initially assumed, more recent findings suggest that MVP might be indirectly involved in drug resistance by modulating cellular signals for proliferation and survival. The interaction of MVP with key molecules in the PI3K/Akt and Ras/Raf/MAPK pathways may suggest a role as scaffold protein for signaling molecules, however this remains to be examined in great detail.

Prognostic Significance

The MVP expression has been detected in diverse types of human cancers, and numerous studies have been performed to examine the prognostic significance of MVP on chemotherapy response and patient prognosis. Overall, however, the current available data cannot provide any conclusive evidence regarding MVP's prognostic impact in human cancers mainly due to limited size of the patient groups and discrepancies among studies. Prospective clinical trials using larger size of cohorts and multivariate analyses are required to clarify the issue.

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Malignancy-Associated Changes

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Definition

Malignancy-associated changes (MACs) are subtle changes in the nuclear morphology and ►chromatin structure of seemingly normal cells located proximal or even distal to neoplastic or ►preneoplastic lesions.

Characteristics

MACs were first described nearly 100 years ago in the leukocytes of patients harboring malignant disease, though the term "MAC" was not applied until the 1950s when these features were observed in normal buccal mucosa. The hallmarks of MAC are a variety of changes in nuclear texture, including increases in the ratio of nucleus to cytoplasm, disappearance of nucleoli, emergence of specific arrangements for chromatin bands and nuclear proteins, and the presence of multinucleated cells.

Although MACs were observed in the nuclei of tumor-associated peripheral blood, sputum, bone marrow, uterus, pancreas, liver, and skin cells, the subjective approaches for determining these features limited their use in a clinical setting. The development of ►image cytometry techniques in the 1970s allowed objective measurement of this phenomenon, raising the potential for clinical application. ►Densitometric analysis of Feulgen-stained nuclei by a camera with a charge-coupled device was used to objectively assess MACs. This stain binds to DNA, allowing characterization of chromosomal features, while the camera's high resolution facilitates hundreds of precise independent measurements of nuclear size, shape, and volume. The spatial organization of genomic material within the nucleus is another metric for assessing MAC effect. This includes the assessment of whether DNA is dispersed to the edges of the nucleus or clumped at the center, the degree of contrast between condensed and non-condensed chromatin, and whether chromosomes are found in large irregular clumps or small uniform clumps. Ultimately, the development of high resolution imaging approaches has facilitated analysis of more than 60 different cell features – many of which cannot be detected by the human eye – to identify MACs.

Mechanisms

Two general mechanisms have been used to explain how MACs arise. The first is that MACs develop in normal tissue neighboring tumors due to exposure to the same carcinogens that induced the tumor. This "►field effect" explanation proposes that MACs arise independently from neighboring tumor tissue. The second proposed mechanism is that MACs arise in normal cells due to soluble factors that are released from tumor tissue. That is, the tumor induces changes in surrounding normal tissue.

One avenue of support for the second explanation is that the degree of MAC in normal cells escalates with increasing lesion severity (e.g. MACs in normal uterine cervical cells have been shown to increase with cancer ►progression). Further support for the idea that MACs arise in a tumor-dependent fashion is the fact that MACs in normal cells diminish after a tumor has been removed. Additional support for this tumor-dependent model can be found in the fact that MAC severity in normal cells can decrease depending on the distance from a given lesion and that ►*in vitro* experiments involving the maintenance of normal cells and tumor cells in the same culture flask show that the number of MACs in the normal cells increases when the concentration of co-cultured tumor cells increases.

Dysregulation of growth factors is well characterized in a variety of tumor types and ►autocrine signaling is understood to drive unchecked cell cycle progression. It is possible that neighboring normal cells are susceptible to the effects of this mode of activation; normal cells expressing growth factor receptors on their outer membrane could be susceptible to aberrant activation

through ►paracrine signaling. Redistribution of chromatin is also a hallmark of MAC. ►Chromatin remodelling in cancers has previously been described and is known to result in oncogenic activation. In normal cells exhibiting MACs, these changes may alter transcriptional activity in various parts of the genome, which may in turn lead to activation of genes that drive other observable MACs.

Clinical Aspects

High resolution genomic technologies have been used to uncover specific factors associated with MACs. For example, gene expression microarray studies of airway epithelial cells from smokers with and without lung cancer revealed specific changes associated with the presence of disease. Up-regulated genes were involved with ►inflammation and cell cycle progression processes, including multiple factors associated with the oncogenic ►*RAS* signalling pathway, suggesting that the presence of cancer mediates the behavior of normal airway tissue.

MACs could have utility as surrogate ►biomarkers in at-risk populations (e.g. smokers with risk of ►tobacco-related cancer). Where individuals are asymptomatic and have no detectable tumor mass, the presence of MACs may be used to identify patients requiring greater clinical vigilance, including regular screening for disease. For example, MACs have been shown to improve sensitivity for detecting early lung cancers when used in conjunction with conventional approaches to ►sputum cytology. (The ability to couple MACs with non-invasive diagnostic approaches also makes it an attractive tool for clinical application.) Discovery of additional MAC-associated factors will increase the likelihood that these changes will be adopted as screening tools in the clinic.

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Malignancy of Small Round Blue Cell Type

►Desmoplastic Small Round Cell Tumor

Malignant Adrenocortical Tumor

►Adrenocortical Cancer

Malignant Endocrine Tumors

M

►Neuroendocrine Carcinoma

Malignant Lymphoma: Hallmarks and Concepts

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Definition

Malignant lymphomas are defined as neoplasms consisting of cells of the lymphoid tissues. Contrary to most organs and tissues, and in spite of the fact that lymphomas show a wide range of aggressiveness from localized, indolent to highly aggressive, rapidly metastasizing tumors, no bona fide benign lymphomas have been defined. Distinct lymphoma entities in general show a characteristic clinical and biological behavior, but within these entities a broad spectrum of

aggressiveness may be observed in individual cases, which is either due to disease progression in a multistep process of lymphomagenesis and/or the involvement of specific risk factors.

Characteristics

Classification

The current WHO classification of the tumors of hematopoietic and lymphoid tissues greatly follows the rules of the REAL classification. According to morphological and biological criteria, Hodgkin lymphomas are distinguished from non-Hodgkin lymphomas, and neoplasias arising from immature precursor cells are set apart from those arising from peripheral mature cells. Distinct clinical and pathological entities are recognized by a combination of morphological, immunophenotypic, genetic and clinical features required for their specific diagnosis. Within each entity, morphological and clinical variants may be recognized that are important for diagnostic and/or prognostic reasons. Specific biological and/or clinical features may characterize distinct disease subtypes, e.g. mediastinal B cell lymphoma within the category of diffuse large B cell lymphoma.

The classification of lymphomas is organized primarily according to the functional differentiation pathways of lymphoid and immune-regulatory cell types and correlated to a suggested normal counterpart of lymphoma cells. The initially presenting clinical features, such as nodal or extranodal disease localization, and presence or absence of leukemic dissemination are important. Primary genetic abnormalities or specific etio-pathogenetic factors apply for only certain diseases.

Malignant non-Hodgkin lymphomas and their major variants are listed according to the main differentiated cell type as precursor or mature peripheral cell type tumors and their relationship to the B cell or NK/T cell systems (Tables 1 and 2).

In the Hodgkin lymphomas (HL), two entities are recognized: Nodular lymphocyte predominant HL and classical HL, with the latter harboring several variants (nodular sclerosis, mixed cellularity, lymphocyte-rich and lymphocyte-depleted (Table 3).

Malignant Lymphoma: Hallmarks and Concepts. Table 1 B cell neoplasms

Precursor B lymphoblastic leukemia/lymphoma
(Precursor B cell acute lymphoblastic leukemia)
Mature B cell Neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Mantle cell lymphoma
Follicular lymphoma
Cutaneous follicle center lymphoma
Marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT) type
Nodal marginal zone B cell lymphoma (± monocytoid B cells)
Splenic marginal zone B cell lymphoma (± villous lymphocytes)
Hairy cell leukemia
Diffuse large B cell lymphoma
Variants
Centroblastic
Immunoblastic
T cell or histiocyte-rich
Anaplastic
Subtypes
Mediastinal (thymic) large B cell lymphoma
Intravascular large B cell lymphoma
Primary effusion lymphoma
Burkitt lymphoma
Plasmacytoma
Plasma cell myeloma

The Molecular Genetic Basis of NHL

Primary recurring genetic alterations have been defined in many B cell NHL and in some T cell NHL (Table 4). The likely primary transforming event represents constitutive activation of a proto-oncogene that has been translocated into strongly active or cell-specifically expressed cellular genes, forming fusion transcripts that retain the oncogenic or transcriptionally active part of the proto-oncogenes. Their functional consequence in some B cell lymphomas characterized by a protracted and indolent clinical course is to confer resistance to apoptosis (e.g. follicular lymphoma or marginal zone B cell lymphoma of MALT type), that is considered to represent the most important regulatory mechanism in the clonal evolution of B cells. It is activated in selection processes establishing anti-self tolerance during early development as well as during antigen-driven selection occurring in germinal centers of B cell follicles. Immunoglobulin rearrangements and heavy chain switching, as well as immunoglobulin gene related hypermutation processes during these steps of evolution, may cause a state of temporary active genomic destabilization that carries an increased risk for the formation of translocations involving the immunoglobulin heavy chain genes in the long arm of chromosome 14. Hereby, activated B cells destined to die may be rescued from apoptosis.

The transcriptional deregulation of some of these genes probably suffice to confer the fully malignant

Malignant Lymphoma: Hallmarks and Concepts. Table 2 T cell neoplasms

Precursor T lymphoblastic leukemia/lymphoma
(Precursor T cell acute lymphoblastic leukemia)
Mature T-cell and NK-cell neoplasms
T cell prolymphocytic leukemia
T cell large granular lymphocyte leukemia
Aggressive NK cell leukemia
Extranodal NK/T cell lymphoma, nasal, and nasal type
Sezary syndrome
Mycosis fungoides
Angioimmunoblastic T cell lymphoma
Peripheral T cell lymphoma (unspecified)
Adult T cell leukemia/lymphoma (HTLV 1+)
Anaplastic large cell lymphoma (T and null cell types)
Primary cutaneous CD30 positive T cell lymphoproliferative disorders
Variants
Lymphomatoid Papulosis (Type A and B)
Primary cutaneous ALCL
Borderline lesions
Subcutaneous panniculitis-like T cell lymphoma
Enteropathy-type T cell lymphoma
Hepatosplenic γ/δ T cell lymphoma

Malignant Lymphoma: Hallmarks and Concepts. Table 3 Hodgkin lymphoma (Hodgkin disease)

Nodular lymphocyte predominance Hodgkin lymphoma
Classical Hodgkin lymphoma
Hodgkin lymphoma, nodular sclerosis
Classical Hodgkin lymphoma, lymphocyte-rich
Hodgkin lymphoma, mixed cellularity
Hodgkin lymphoma, lymphocyte depletion

phenotype (e.g. activation of the *MYC* gene in Burkitt lymphoma inferred by the translocation t(8;14)), whereas other translocations (e.g. the activation of the anti-apoptotic *BCL2* gene by the translocation t(14;18) in follicular lymphoma) seem to prolong the lifespan of the cells only, thus enabling the acquisition of secondary aberrations: Less than 10% of follicular lymphomas show this translocation as the sole genetic abnormality. Neoplastic transformation, therefore, in most cases is mediated as a multistep process involving loss or functional inactivation of important regulatory and/or tumor suppressor genes, and may in addition be responsible for the progression of indolent to highly aggressive lymphomas. Genes frequently targeted in the progression of malignant lymphomas are *TP53* in 17p, $p16^{INK4A}$ in 9p, and *MYC* in 8q.

Antigen Receptors in NHL

Signaling via the antigen receptor provides the most important pathway of lymphocyte differentiation and selection during ontogenesis and antigen related immune reactions, and is also of pivotal importance in the pathogenesis of malignant lymphomas.

Immunoglobulin Receptor of B Cells:

The unique clonal antigen receptor repertoire in B cells is formed by the rearrangement of VDJ and constant regions of the immunoglobulin heavy and light chain genes. The demonstration of a clonally rearranged, single receptor is the basis to determine the clonality of a lymphoproliferative process. V-gene usage and organization of VDJ genes provide important clues to aspects and differentiation of HL. For extranodal B cell lymphomas, a biased V-gene usage (with respect to the normal repertoire) and the demonstration and distribution of mutations in the complementary determining regions (CDR) and framework regions (FR) exemplifies the influence of antigen on the selection of neoplastic B cell populations. The presence of mutations in IG genes is an important clue to assess the pre-follicular B cell or follicular and post-follicular B cell origin of lymphoma cells. Whereas chronic lymphocytic leukemia and mantle cell lymphoma either show non-mutated or mutated Ig-receptors, most lymphomas show either non-mutated receptors (precursor B cell lymphoblastic lymphoma) or mutated receptors (follicular lymphoma, marginal zone B cell lymphoma and most aggressive B cell lymphomas including diffuse large B cell lymphoma and Burkitt lymphoma) exclusively. “Ongoing” somatic hypermutations indicating a still active process of immunoglobulin-related hypermutations were shown to represent the characteristic genetic fingerprint of follicular lymphoma, but also demonstrated in marginal zone B cell lymphoma and diffuse large B cell lymphoma. Therefore, most lymphomas originate in the antigen-driven germinal center cell reaction or by an extrafollicular post-germinal center lymphocyte activation process.

T Cell Receptors in Peripheral T Cell Lymphomas:

The specificity of α/β and γ/δ types of T cell receptors as well as the significance of the expression and functional activity of NK cell receptors in NK/T cell lymphomas is still poorly understood.

Gene Expression Profiling

The development of high throughput technologies and, in particular, of DNA microarrays led to a major advantage in the understanding of complex biological processes. They formed the basis towards a molecular

Malignant Lymphoma: Hallmarks and Concepts. Table 4 Primary genetic events and their effects in malignant lymphoma

NHL entity	Chromosomal aberration	Genes involved	Function
B-CLL	t(14;19)(q32;q13)	*IGH* *BCL3*	?
	t(10;14)(q24;q32)	*IGH* *NFKB2*	Induction of proliferation
Follicular lymphoma	t(14;18)(q32;q21)	*IGH* *BCL2*	Resistance to apoptosis
Mantle cell lymphoma	t(11;14)(q13;q32)	*IGH* *Cyclin D1*	Dysregulation of cell-cycle control
Extranodal marginal zone B-cell lymphoma of MALT-type	t(11;18)(q21;q21)	*API2* *MALT1*	Resistance to apoptosis
	t(1;14)(p22;q32)	*IGH* *BCL10*	Resistance to apoptosis
	t(14;18)(q32;q21)	*IGH* *MALT1*	Resistance to apoptosis
Splenic marginal zone B-cell lymphoma	t(2;7)(p12;q32)	*CDK6* *IGK*	Dysregulation of cell-cycle control
Lymphoplasmacytic lymphoma	t(9;14)(p13;q32)	*IGH* *PAX5 (BSAP)*	Enhanced response to proliferation signals
Diffuse large B-cell lymphoma	t(3;14)(q27;q32)	*IGH* *BCL6*	Transcriptional induction of proliferation Resistance to differentiation
Burkitt Lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	*IGH, IGL* *MYC*	Transcriptional induction of proliferation (G1)
Prolymphocytic leukemia of T-type	Inv(14)(q11q32) t(14;14)(q11;q32) t(X;14)(q28;q11)	*TCRA* *TCL1* *TCRA* *MTCP1*	Enhancement of cellular proliferation and survival
Anaplastic large cell lymphoma (T/0)	t(2;5)(p23:q35) t(1;2)(q25;p23) t(2;3)(p23;q21) Inv(2)(p23;q35) Others	*NPM-ALK* *TPM3-ALK* *TFG-ALK* *ATIC-ALK* *others*	Constitutive activation of ALK tyrosin kinase
Hepatosplenic γ/δ T-cell lymphoma	i(7)(q10)	?	?

Abbreviations: *IGH*, Immunoglobulin heavy chain gene; *IGL*, Immunoglobulin light chain gene; *NFKB2*, Nuclear factor kappa B2; *BCL2*, B-cell lymphoma/leukemia gene 2; *API2*, Apoptosis inhibiting protein 2; *MALT1*, Mucosa-associated lymphoid tissue gene 1; *BCL10*, B-cell lymphoma/leukemia gene 10; *CDK6*, Cyclin-dependent kinase 6; *IGK*, Immunoglobulin kappa light chain gene; *PAX5 (BSAP)*, B-cell specific activator protein; *BCL6*, B-cell lymphoma/leukemia gene 6; *MYC*, c-MYC oncogene; *TCRA*, T-cell receptor alpha chain gene; *TCO1*, T-cell lymphoma/leukemia gene 1; *NPM*, Nucleophosmin gene; *ALK*, Anaplastic lymphoma kinase; *TFG*, *TRK*-fused gene; *TPM*, Tropomyosin gene; *ATIC*, Aminoimidazole carboxamide ribonucleotide; formyltransferase/IMP cyclohydrolase gene.

classification of lymphomas, and gene expression-based survival predictors are envisaged to become highly useful in guiding future treatment decisions.

In diffuse large B cell lymphoma, gene expression profiling (GEP) led to the recognition of two molecular subgroups, the germinal center-like type of DLBCL (GCB DLBCL) and the activated B-cell-like type of DLBCL (ABC DLBCL). GCB DLBCL are characterized by high expression of genes that are typically expressed in B-cells at the germinal center stage of B-cell differentiation, while ABC DLBCL express genes at a high level that are upregulated in B-cell in response to mitogenic activation. From a clinical point of view, the varying molecular features of DLBCL had a strong impact on the clinical course of the disease with strikingly different 5-year survival rates between GCB

and ABC DLBCL patients. Also, the molecularly defined DLBCL subtypes differ in their genetic constitution: The chromosomal translocation t(14;18) involving *BCL2* and amplifications of the *c-rel* locus on chromosome 2p were assigned to GCB-type DLBCL, while the genetic hallmark of ABC DLBCL are the constitutive activation of the NFκB-pathway, and genomic gains in 3q and 18q. In MCL, gene expression profiling led to the unequivocal definition of a new Cyclin D1-negative MCL subgroup. More important, a gene expression-based predictor of survival in MCL patients was constructed providing prognostic information at the time of diagnosis. In this approach, a 'proliferation signature' was defined encompassing genes required for DNA replication, cell cycle progression and metabolic processes required for the proliferation of tumor cells. Ranking of the MCL patients according to the proliferation signature score of their tumor specimens yielded four quartiles with median survival times differing by as much as 6 years. From a biological standpoint, the proliferation signature in MCL can be viewed as an integrator of various oncogenic events in these tumors. Results of GEP analyses in Follicular lymphoma led to the recognition of two gene signatures, Immune-response 1 (IR-1) and Immune-response 2 (IR-2) signatures with striking importance in defining the patient's individual risk. In this model, IR-1 over-expression was associated with a more favorable outcome, while IR-2 over-expression was pointing at an adverse prognosis. Most importantly, flow sorting of CD19 + and CD19-cell subpopulations from primary specimens suggested the expression of IR-1 and IR-2 signatures in the non-malignant cell population, thus providing evidence that expression signatures associated with survival in FL mainly reflect biological properties of non-malignant tumor-infiltrating cells.

Etiology

Among the etiological factors for malignant lymphoma, chronic viral and bacterial infections play a major role. Insertional mutagenesis, transcriptional activation by viral genes as well as viral homologues of cellular oncogenes and growth factors may cause lymphomatous transformation directly or indirectly by increasing the risk for further cellular mutagenic events.

The ►Epstein-Barr virus (EBV) plays a major role in the pathogenesis of Hodgkin lymphoma (HL), endemic Burkitt lymphoma and many immunodeficiency related malignant lymphomas. It is also found in a variety of peripheral T cell lymphomas, especially in angioimmunoblastic T cell lymphoma and extranodal NK/T cell lymphoma of nasal type, as well as in about 5% of diffuse large B cell lymphomas in the Western hemisphere. Epstein-Barr virus in all these cases is clonally integrated in lymphoma cells, suggesting that the virus was already present in the tumor stem cell. The transformation processes appear to be various in different lymphoma types and are still not completely understood.

HTLV-1 is invariably linked to Adult T cell Lymphoma/Leukemia, which is endemic in Southern Japan, the Caribbean and certain regions in Africa. The transformation is related to the transcriptional activation of cellular genes by the viral transcription factor TAX in CD4-positive T-helper lymphocytes and other mutagenic events.

The human immunodeficiency virus (HIV) is associated with increased risk for aggressive B cell lymphomas, either of Burkitt lymphoma subtype or immunoblastic-plasmablastic lymphomas. 50% of Burkitt lymphomas and 80% of immunoblastic and plasmablastic diffuse large B cell lymphomas in HIV-infected individuals are caused by ►EBV, and its presence is related to the status of immunodeficiency. Rare specific subtypes of NHL seen in AIDS patients are the primary effusion lymphomas that are related to HHV8 (KSHV) frequently with co-infection of EBV. Whether HIV plays a direct role in EBV- and HHV8-negative lymphomas, or transformation in these cases is induced by other viruses or sporadic mutagenic events in hyperproliferative B lymphocytes, is unknown.

►Helicobacter pylori (H.p.) infection is a causative factor for extranodal marginal zone B cell lymphomas of MALT type in the stomach. Early stages of lymphoma development may be successfully treated by H.p. eradication. The molecular genetic analysis of H.p.-associated lymphomagenesis in the stomach shows that different factors are effective in the transformation processes.

- An indolent form of gastric MALT-type lymphoma does not show progression and is associated with the translocation t(11;18) (q21;q21). No transformation to high grade lymphomas and no further genetic imbalances are found in these cases.
- Low grade MALT type lymphomas show characteristic genetic imbalances in 3q;11q and 18q.
- Diffuse large B cell lymphomas show similar chromosomal imbalances but additional and more frequent changes in chromosomes 6q and 7q as well as ►LOH in the loci of the ►APC, ►$p16^{INK4A}$, the retinoblastoma ►RB1 and ►TP53 tumor suppressor genes.
- De novo diffuse large B cell lymphomas show genetic imbalances, losses and deletions as in the afore mentioned aggressive B cell lymphomas, but without the alterations seen in low grade tumors.

This scenario reflects the different pathways of lymphoma transformation: stable accumulative disease, a progressing genetically unstable disease

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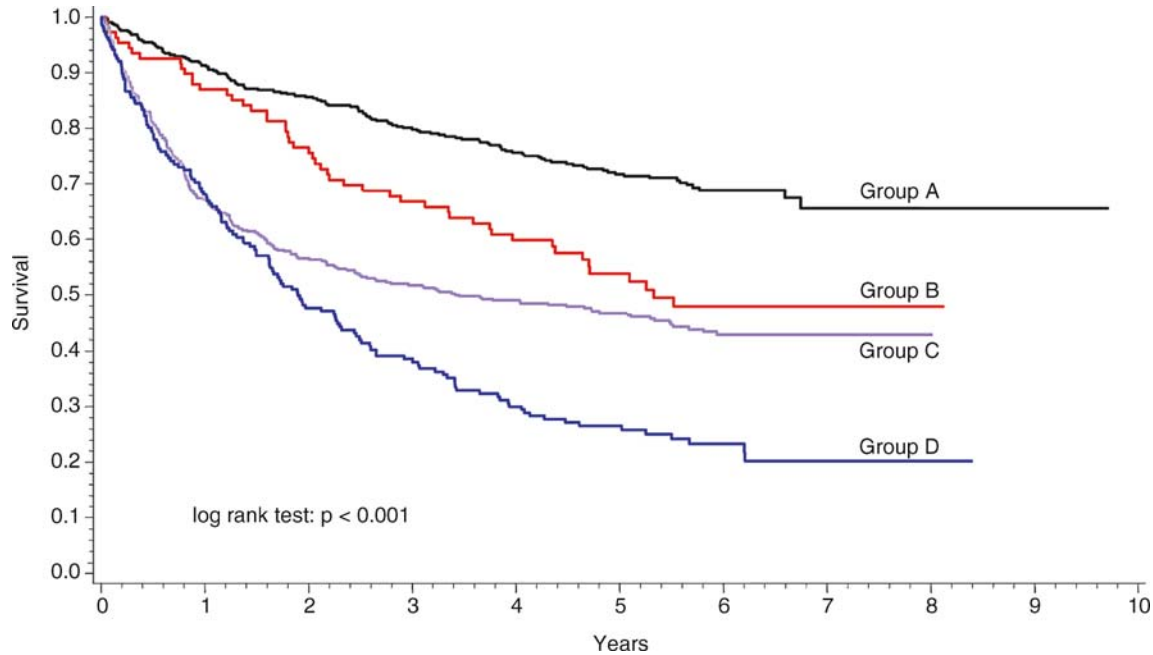

Malignant Lymphoma: Hallmarks and Concepts. Figure 1 Clinical groups of the International Lymphoma Classification Project according to different five year survival. Group A: 5 year Overall Survival > 70%: Indolent slow decline and/or few disease related deaths. (Anaplastic large cell lymphoma, marginal zone B cell lymphoma of MALT-type, follicular lymphoma).Group B: 5 year Overall Survival 50–70%: Continuous slow decline of survival. (Nodal marginal zone lymphoma, lymphoplasmacytoid lymphoma, small lymphocytic lymphoma). Group C: 5 year Overall Survival 30–50%: Rapid initial decline of survival plateau and few disease related deaths after 2 years. (Primary mediastinal large B cell lymphoma, diffuse large B cell lymphoma, Burkitt lymphoma, Burkitt-like lymphoma). Group D: 5 year Overall Survival < 30%: Rapid decline and low level plateau or rapid continuous decline. (Precursor T lymphoblastic lymphoma, peripheral T cell lymphoma, mantle cell lymphoma).

or aggressive de novo lymphomas, which are similar to sporadic non-H.p.-associated lymphomas of the B cell type.

Cell Biological Features of Hodgkin Lymphoma

All cases of Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and 98% of classical Hodgkin lymphoma (CHL) are B lymphocyte derived monoclonal lymphoproliferative disorders of (frequently) young adults. For the pathological diagnosis, their unifying feature is that scattered tumor cells (called Hodgkin cells and Reed-Sternberg cells in CHL and L&H cells in NLPHL) are found in a non-neoplastic background infiltrate of various cell types forming the lymph node tumors. A rosette-like arrangement of T lymphocytes around tumor cells is often characteristic, suggesting the functional interaction of tumor cells and T lymphocytes in the production of multiple cytokines and chemokines, which are responsible for the pleomorphic histological picture and the paraneoplastic clinical features well-known in this disease (so-called B symptoms). The tumor cells of both NLPHL and HL correspond to germinal center-derived activated B lymphocytes, however, they markedly differ in their antigenic phenotype and receptor status. Rare cases of HL may show a T cell phenotype and genotype.

The B cell nature of L&H-, HD- and RS cells has been elucidated by single cell microdissection from tumor tissue. L&H cells show transcription of intact immunoglobulin receptor genes, whereas in HD and RS cells of CHL, the transcription of immunoglobulin genes is blocked. In an earlier hypothesis, it was postulated that this feature was due to 'crippled' immunoglobulin genes due to hypermutations occurring in the germinal center, often leading to stop codons and deletions. This view, however, has been challenged by the demonstration of defective co-activators of immunoglobulin gene transcription (i.e. Oct 2 and BOB 1), considered to be the basis of this cellular defect.

The constitutive activation of the NFκB pathway by different molecular events (i.e. inhibitor inactivation of IκBα-2, hyperexpression of TRAF-1 signaling and other, yet unknown reasons) appears to be a central defect of cell regulation in Hodgkin lymphoma. The hyperactivity of this important transcription factor leads to resistance of apoptosis and increased transactivation of cytokine and chemokine genes and their receptors, respectively, stimulating unrestrained growth. This may

result in the survival of activated germinal center cell populations normally destined to die by apoptosis.

Basic Clinical Features of Malignant Lymphoma

Malignant lymphomas occur at a frequency of about 12:100,000 per year in the Western population, equivalent to about 10,000 new cases in Germany per year. The incidence rate of Hodgkin lymphoma, with a frequency of 4:100,000 per year, has been stable over the last decades, whereas non-Hodgkin lymphomas show a continuous increase over time. This increase is still evident if absolute data are corrected for the increase of mean lifetime expectancies. The reason for the increase of non-Hodgkin lymphomas is unclear.

For most NHL and NLPHL, there is a slight to marked male predominance, whereas classical Hodgkin lymphoma and mediastinal large B cell lymphomas more frequently occur in young females. The age range of malignant lymphoma is broad, from young children to the highest age groups, with a characteristic predilection for certain subtypes in young patients (Burkitt lymphoma, precursor lymphoblastic lymphoma, diffuse large B cell lymphoma of centroblastic type and anaplastic large cell lymphoma of T type). Other lymphomas are more frequent in the young adult population (Hodgkin lymphoma, mediastinal B cell lymphoma, anaplastic large cell lymphoma T type, extranodal diffuse large B cell lymphoma) or in higher age groups (indolent lymphomas, which apparently do not exist under the age of 20, diffuse large B cell lymphomas, and peripheral T cell lymphomas).

With the introduction of modern multi-agent chemotherapy regimens, the prognosis of HL and aggressive non-Hodgkin lymphoma has greatly improved, whereas other non-Hodgkin lymphomas still show an incurable, indolent or aggressive course (Fig. 1). For the aggressive NHL, the use of anti-CD20 antibodies has resulted in an apparently dramatic improve of cure rates.

Modern and refined classifications of lymphoma entities including newer molecular genetic classification approaches may better define and find new and more specific treatment options for the incurable diseases. A better definition of molecular events in malignant lymphoma (e.g. by gene expression profiling) may also help to define specific risk factors and prognostic features that can be used to modify present treatment protocols on a rational basis and adapt more specific treatment regimens to individual diseases.

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Malignant Melanoma

►Melanoma

Malignant Mesenchymal Nephroma of Infancy

►Mesoblastic Nephroma

Malignant Mesothelioma

►Mesothelioma

Malignant Neoplasias Originating in Endocrine Tissues or their Target Organs

►Endocrine-Related Cancers

Malignant Neoplastic Changes of the Colon

►Colon Cancer

Malignant Peripheral Nerve Sheath Tumor

Definition

MPNST; Cancer that arises from the cells of connective tissue sheath of a peripheral nerve. Synonyms: malignant neurolemmoma, neurofibrosarcoma. About half the cases are diagonosed in people with neurofibromatosis 1.

►Neurofibromatosis 1

Malignant Pleural Effusion

Definition

MPE; Presence of fluid in the pleural cavity due to direct infiltration of the pleura by cancer or to a disturbance in the mechanisms that normally move the fluid across the pleural space and reabsorb it.

►Pleural Effusion

Malignant Progression

Definition

The change of cells from benign to malignant behavior is called malignant ►progression or transformation. Malignant progression is characterized by the acquisition of progressive and uncontrolled growth of tumor cells. Malignant tumors have the ability to invade adjacent tissues and spread to distant sites in the body. Although there can be many causes for malignant progression, an underlying commonality is genetic mutation either by inheritance or more commonly by acquiring mutations in one's DNA over time.

Malignant Salivary Gland Cancer

►Mucoepidermoid Cancer

Malignant Tumor

Definition

A tumor that invades and destroys adjacent tissues and metastasizes to distant organs.

►E-Cadherin

Malignant Tumors of the Endocrine Glands

►Endocrine-Related Cancers

MALT Lymphoma

Definition

Mucosa associated lymphatic tissue (MALT) lymphoma is a low grade B cell gastric non-Hodgkin lymphoma [►Hodgkin disease]. In contrast to the lymph nodes or spleen it is not as anatomically distinct, but is embedded in the mucosae of various organs. Gut associated lymphatic tissue is called ►GALT and bronchus associated lymphatic tissue ►BALT.

►B-cell Tumors

Mammalian Target of Rapamycin

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Synonyms

mTOR

Definition

Mammalian target of Rapamycin, commonly known as mTOR, is a central kinase that integrates signals from cellular cues to regulate many processes, including protein translation, cell survival, proliferation, metabolism, ►autophagy, and the cytoskeleton.

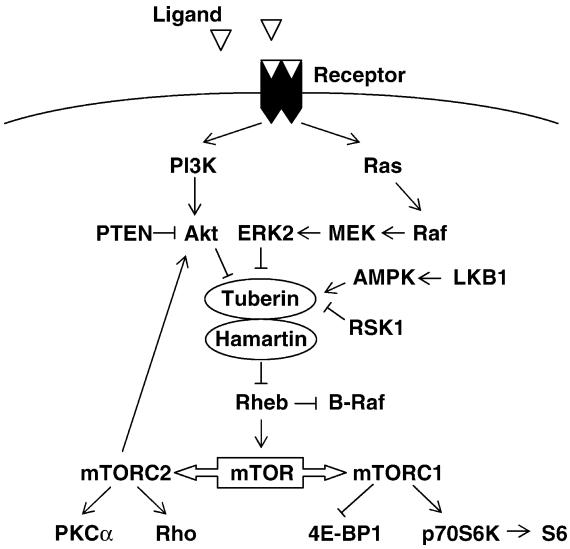
Characteristics

mTOR forms two functionally distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Both mTORC1 and mTORC2 are composed of mTOR and GβL. In addition, mTORC1 contains raptor while mTORC2 contains Sin1 and rictor. Functionally, the two complexes are very different. Activation of mTORC1 leads to the phosphorylation of downstream targets including p70 ribosomal protein S6 kinase (p70S6K), ribosomal protein S6 (S6), and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), that promote increased protein translation and cell growth. mTORC2 activation regulates Akt/PKB, PKCα, and Rho to control cell survival, proliferation, metabolism and the cytoskeleton.

Regulation

The mTOR signaling pathway is illustrated in Fig. 1. Upstream of mTOR are two ►tumor suppressor genes, *TSC1* and *TSC2*, that regulate mTOR activity via ►Rheb (Ras homologue enriched in brain). *TSC1* encodes hamartin, a 140 kDa protein that physically interacts with the protein product of *TSC2*, tuberin. Tuberin is a 200 kDa protein with a domain near the carboxyl terminus containing ►GTPase activating protein (GAP) homology. GAP proteins convert members of the Ras superfamily from their active, GTP-bound state to their inactive, GDP-bound state. Rheb, like other Ras family members, cycles between an active GTP-bound and an inactive GDP-bound state. Tuberin converts Rheb-GTP to Rheb-GDP, thereby inactivating Rheb and inhibiting mTOR. Conversely, loss of either tuberin or hamartin, or inactivation of tuberin by upstream regulators releases the brake on Rheb resulting in mTOR activation. At least three kinases are known to directly phosphorylate and inactivate tuberin: p90 ribosomal S6 kinase 1 (RSK1), Erk2 via the ►Ras signaling pathway, and Akt/PKB via the phosphatidylinositol 3 kinase (►PI_3K) signaling pathway. In addition, mTORC2 phosphorylates Akt/PKB on Serine 473, thereby activating Akt/PKB and inhibiting tuberin via a feedback mechanism. In contrast, LKB1/AMPK-mediated phosphorylation activates tuberin and inhibits Rheb-dependent mTOR activation.

The mTOR pathway is regulated by many intracellular and extracellular signals and integrates both positive and negative stimuli. mTOR is activated by insulin, growth factors, nutrients, and mitogens and inhibited by numerous stress conditions, such as cellular energy depletion and hypoxia. Pharmacologically, ►Rapamycin specifically inhibits mTORC1 thereby preventing phosphorylation of downstream effectors S6K and 4E-BP1 resulting in an inhibition of cell proliferation.



Mammalian Target of Rapamycin. Figure 1
Illustration of the mTOR signaling pathway. Tuberin and hamartin function as a complex to inactivate Rheb and inhibit mTOR. Inactivation of tuberin by upstream regulators, such as RSK1, Erk2 via the Ras signaling pathway, and Akt/PKB via the PI_3K signaling pathway, releases the brake on Rheb. In contrast, LKB1/AMPK-mediated phosphorylation activates tuberin. mTOR forms two functionally distinct protein complexes, mTORC1 and mTORC2. mTORC1 leads to the phosphorylation of p70S6K, S6, and 4E-BP1, that promote increased protein translation and cell growth. mTORC2 activation regulates Akt/PKB, PKCα, and Rho to control cell survival, proliferation, metabolism and the cytoskeleton. Arrowheads indicate activation and flat bars indicate inhibition of function.

Clinical Relevance

Unconstrained mTOR signaling has been noted in several human diseases. Inactivation of negative regulators of the AKT-mTOR pathway such as PTEN (associated with ►Cowden syndrome), TSC1 or TSC2 (►tuberous sclerosis complex), LKB1 (►Peutz–Jeghers syndrome), NF1 (►neurofibromatosis 1), and VHL (►von Hippel–Lindau disease), results in familial cancer syndromes (collectively called ►phakomatoses) with similar clinical features. In addition, Akt signaling is aberrant in many sporadic human cancers resulting in elevated mTOR activity. Collectively, these observations have provided a scientific basis for mTOR inhibition

using Rapamycin and its derivatives as a prospective treatment for these diseases.

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40 kDa Mammary Gland Protein

►Serum Biomarkers

Mammographic Breast Density and Cancer Risk

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Definition

The purpose of ►mammography screening is to reduce the mortality from breast cancer in the targeted population. Breast cancer is worldwide the most frequent cancer disease in women, and the highest incidence is found in countries with a high standard of living. Breast cancer is associated with the life long exposure to endogenous and exogenous hormones, and it is as such difficult to prevent. Mammography screening has therefore become an important tool in breast cancer control.

Screening is the examination of healthy people for the possible presence of diseases that has not given rise to symptoms. Screening might be undertaken in organized program targeting a given population at specified intervals, or opportunistically following the wish of a woman or advice from her physician. Mammography is a radiological examination of the breast for lumps and/or microcalcifications. Both invasive ►breast cancer and ►ductal carcinoma in situ (DCIS) can be detected by mammography. Mammography is used both as a diagnostic test for women with symptoms of breast cancer, and as a screening test for asymptomatic women. Mammography screening normally includes one- or two-view mammography of each breast at regular time intervals, and double reading may be recommended. Women testing positive are referred to assessment with clinical breast examination, additional mammography/ultrasound examination and needle biopsy, and in a small percentage of cases open biopsy may be needed to determine the final diagnosis.

Characteristics

Effect of Mammography Screening

With screening, the time of diagnosis of breast cancer patients is pushed forward. This period is called the lead time. The earlier diagnosis should allow for a better treatment, and a better prognosis. The effect of screening, however, cannot be measured by length of survival after diagnosis, as the survival time is by definition extended with the lead time. The effect of mammography screening can therefore only be measured by its effect on breast cancer mortality in the targeted population.

The effect of mammography screening has been studied in the 12 randomized controlled trials summarized in Table 1. Ten of the trials resulted in a reduced breast cancer mortality in the intervention group compared with that of the control group, but the reduction was statistically significant in only one of the trials. The size of the reduction varied depending on the targeted age group and the length of the follow-up period. The Two Canadian trials, both with a somewhat different design, formed an exception with no effect on breast cancer mortality. Following a detailed analysis of the data, the International Agency for Research on Cancer, IARC, concluded in 2002 that "There is sufficient evidence for the efficacy of screening women aged 50–69 years by mammography as the sole screening modality in reducing mortality from breast cancer." The calculated combined relative risk was 0.75 (95% confidence interval 0.67–0.85), and a 35% reduction was estimated for women accepting the invitation to screening. IARC further concluded that "There is limited evidence for the efficacy of screening women aged 40–49 years by mammography as the sole screening modality in reducing breast cancer mortality." The combined relative risk was 0.81 (95% confidence interval 0.65–1.01). Including data from the new UK trial, the combined estimate for all trials on women aged 40–49 is 0.84 (95% confidence interval 0.74–0.95). A 24% reduction in breast cancer mortality was estimated in the UK trial for women attending screening.

Mammographic Breast Density and Cancer Risk. Table 1 Randomized controlled trials of the effect of mammography screening on breast cancer mortality

Location	Year of start	Age at entry	Follow up in years	Relative risk, breast cancer mortality	95% confidence interval
United States; New York, Health Insurance Plan[a]	1963	40–64	18	0.78–0.82[b]	0.61–1.00[c]
Sweden; Malmø I	1976	45–70	19.2	0.81	0.66–1.00
Sweden; Malmö II	1978	43–49	9.1	0.65	0.39–1.08
Sweden; Kopparberg	1976	40–74	20	0.59	0.47–0.75
Sweden; Östergötland	1978	38–75	17.4	0.89	0.72–1.09
Sweden; Stockholm	1981	39–65	14.9	0.90	0.63–1.28
Sweden; Göteborg	1982	39–59	13.3	0.78	0.57–1.07
United Kindgom; Edinburgh	1978	45–64	14	0.79–0.87[b]	0.60–1.02[d]
Canada[e]	1980	40–49	11–16	0.97	0.74–1.27
Canada[f]	1980	50–59	13	1.02	0.78–1.33
Finland	1987	50–64	6	0.76	0.53–1.09
UK	1991	39–41	10	0.83	0.66–1.04

[a]Mammography and clinical breast examination (CBE) in the intervention group.
[b]Interval reflects different calculation methods.
[c]For 0.78.
[d]For 0.79.
[e]CBE and instruction in breast self-examination (BSE) before randomization. Mammography, CBE and BSE annually in intervention group.
[f]CBE and instruction in breast self-examination (BSE) before randomization. Mammography and CBE annually in intervention group, and CBE annually in control group.

Randomized trials are undertaken in dedicated centers with high quality equipment and quality assurance, and it is not a straightforward task to customize the trial results to routine health care. Continuous evaluation of the outcome of mammography service screening is therefore recommended. Such observational studies have been undertaken in England & Wales and the Netherlands using the population-based trends in breast cancer mortality. These studies indicated a 6% and 10%, respectively, reduction in breast cancer mortality following the introduction of organized mammography screening. A more rigid study design has been used in the Nordic countries where individual invitation and screening data can be linked with cancer register and death records. Following this methodology, a 25% reduction was seen in Denmark in women screened at age 50–69; a 27% reduction was seen in Sweden for women screened either at age 40–69 or at age 50–69; and a 42% reduction was seen in Finland for women screened at age 55–74. It should be noted that the participation rate in Finland was close to 90%, and the overall estimate for Finland is therefore expected to be close to the estimated effects for participants in Denmark and Sweden of 37% and 45%, respectively. The outcomes of the more rigid observational studies are well in agreement with the outcome of the combined estimated relative risk found in the randomized controlled trials. At present, both trial and observational data show that breast cancer mortality is reduced by about 25% in women offered screening at age 50–69, and trial data show that breast cancer mortality is reduced by about 16% in women offered screening at age 40–49. Modeling showed that both screening mammography and adjuvant therapy have helped to reduce the rate of death due to breast cancer in the United States from 1975 to 2000.

Benefits and Harms

Mammography screening is only a relevant tool in breast cancer control if it reduces breast cancer mortality. But mammography screening may also have other benefits and harms. The most important of these are summarized in Fig. 1.

On the benefit side, breast tumors are detected earlier by screening, which should allow for a more conservative treatment. Very accurate data on surgical procedures are needed to investigate this. Studies from Norway and Ireland indicate that an increasing proportion of breast cancer patients in screening regions have been treated with breast conserving surgery, but parallel developments took place in nonscreening regions.

On the harm side, the first thing to note is that patients with screen-detected cancers become cancer patients at an earlier point in time than if they have waited for their disease to become symptomatic. Two to three years of healthy life, depending on the length of the lead time, will be converted to life as a cancer patient.

Benefits	Harms
Reduced breast cancer mortality	Cancerpatient earlier in life
Conservative treatment	False positive/ false negative
	Overdiagnosis
	radiation

Mammographic Breast Density and Cancer Risk. Figure 1 Benefits and harms of mammography screening.

Mammography screening is not a perfect test. Some women will test positive but be declared disease free at assessment. The percent of false-positive tests depends on the cutoff point for abnormalities used by the radiologists. In the United States, about 45% of women undergoing ten rounds of screening will experience at least one false-positive test. In the Nordic countries, this percentage is 10–20%. Some breast cancers will also be missed at screening. As women with negative tests are not reexamined, this percentage of false-negative tests is not known. As a proxy, the proportional interval cancer rate is used, which is the number of breast cancer cases observed before the next scheduled screen in women with negative tests divided by the number of breast cancer cases expected in this cohort in the absence of screening. In European screening programs, the 2-year proportional interval cancer rates after first screen has varied from 0.34 to 0.49, clearly indicating that some breast cancers are missed at screening.

Overdiagnosis is screen detection of lesions that would otherwise not have become clinically manifest breast cancers in the women's life time. Overdiagnosis includes both invasive cancers and DCIS cases that if undetected would not have progressed to invasive cancer. Overdiagnosis is difficult to study because screening is intervention in the natural history of disease and therefore affects the incidence rate. As the time of diagnosis is pushed forward with screening, the incidence rate will go up when women start screening, the so-called prevalence peak. This peak is expected to be compensated by an incidence deficit when women stop screening, but this may not be easy to detect as it occurs at an older age where the background incidence is higher. Data from the randomized mammography screening trial in Malmö, Sweden, indicated a 16% overdiagnosis in women screened up to a maximum age of 78, whereas overdiagnosis was not seen in women screened up to the age of 70, but both estimated had broad confidence intervals. Data from the organized mammography screening program in Finland showed the same cumulative incidence of breast cancer at age 74 for women offered screening at age 50–59 as for women not offered screening. The cumulative incidence at age 74 was 8% higher in women offered screening at age 50–69, but a certain percentage of these women had probably continued screening after they left the organized program. Modeling data from the Netherlands estimated the life time cumulative risk to be 3% higher in women screened at age 50–74 than in nonscreened women. Based on the sparse data, overdiagnosis may in particular be an issue when screening is extended to women of older age.

Data from the UK mammography screening program showed an average radiation dose of 4.5 mGy to the glandular breast tissue from two-view mammography. Using this figure, it was estimated that a decade of annual screening starting at age 40 would result in 5 years of life lost from radiation-induced breast cancer per 1000 women screened. If screening led to a 20% reduction in breast cancer mortality among attendants, 25 years of life would be gained per 1000 women screened, giving a net gain of 20 years of life. The equivalent figures from a decade of screening every 3 years starting at the age 50 were estimated to be 1 year of life lost from radiation and 43 years of life gained from screening, resulting in a net gain of 42 years of life per 1000 women screened. The balance of benefit versus harm is thus considerably more favorable for screening starting at the age of 50 than for screening starting at the age of 40.

Public Health Screening Recommendations

The evidence for mammography screening benefits and harms form the basis for decisions at two levels. First, the individual woman has to decide whether or not she wants to undergo screening. Second, the health authorities have to decide whether or not mammography screening should be recommended as a public health policy. The first type of decision requires good information, but it is otherwise noncontroversial as it allows the individual woman to give different weights to benefits and harms. The second type of decision is much more complicated, because it involves responsibility for the life of other people, and for the allocation of public or health insurance resources.

The weighting of benefits and harms has resulted in two very different sets of screening recommendations from public health authorities. In the United States, the US Preventive Services Task Force recommends "screening mammography, with or without clinical breast examination, every 1–2 years for women aged 40 and older." In Europe, the Council of the European Union recommends "mammography screening for breast cancer in women aged 50—69 in accordance with European guidelines on quality assurance in mammography." The actual age range for screening varies across

European countries; for instance from age 40 to age 74 in parts of Sweden.

According to National Health Interview Survey data, 70% of US women aged 40 and above, and 79% of women aged 50–64, had a mammogram within the last 2 years. Statistics from the organized mammography screening program in England showed that 76% of women aged 53–64 had been screened at least once in the previous 3 years. Participation in the organized mammography screening programs in Europe has varied from about 90% in Finland and Northern Sweden to about 50% in Italy. There is overall an urban–rural gradient with higher screening participation in rural than in urban regions.

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Mammographic Density

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Definition

Mammographic density refers to the radio-lucent (white) appearance of breast tissue as seen on a mammogram. It is often expressed as percentage of the total breast area. A greater percentage is associated with a greater risk of breast cancer.

Characteristics

Mammographic Density and Risk of Breast Cancer

The radiological appearance of the breast varies among women because of differences in breast tissue composition and the x-ray attenuation properties of breast tissue. Fat is radio-lucent and appears dark on a mammogram, while stroma and epithelium attenuate x-rays more and appear light. It is the appearance of light tissue in a mammogram that is referred to as "mammographic density," and often expressed as the proportion of the total breast area occupied by radiologically dense tissue. These variations are illustrated in Fig. 1.

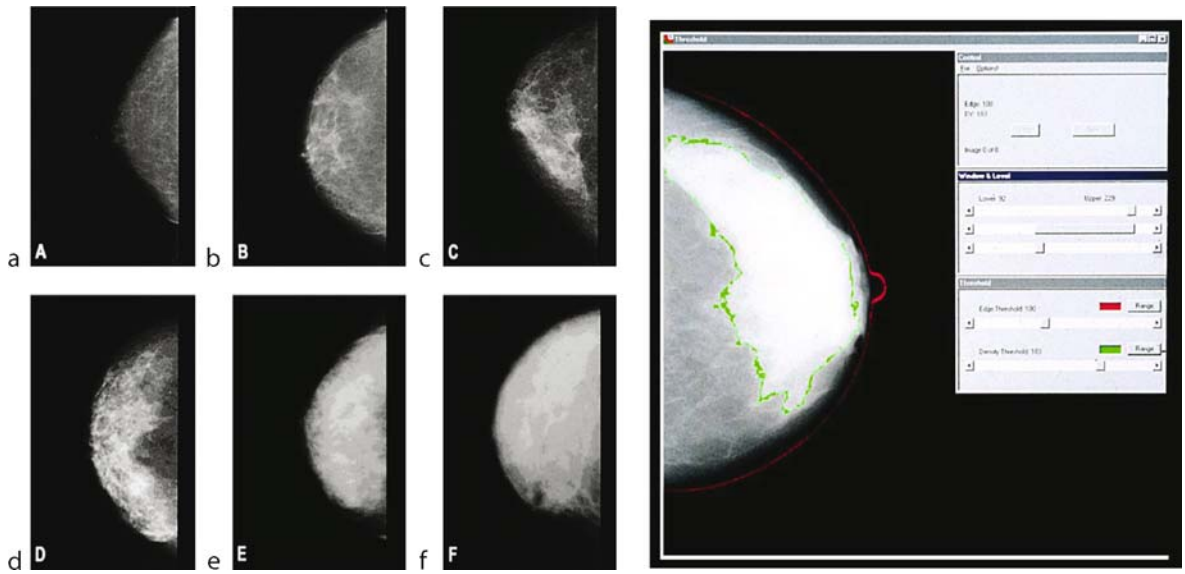
In 1976, Wolfe described a classification comprised of four categories: N1 in which the breast was mainly fat and risk of breast cancer lowest, DY in which the breast was mainly dense and risk of breast cancer highest, and P1 and P2 in which there were different degrees of linear density and cancer risk was intermediate. Most well designed epidemiological studies have found that this classification does identify women at different risks for breast cancer. The ▶Breast Imaging Reporting and Data System has described a qualitative method of classifying density.

Quantitative approaches that have been used to determine the proportion of the breast occupied by dense tissue include estimation by radiologists, planimetry, and computer assisted methods. Examples of the categories of the classification that have been used in estimation by radiologists are shown in Fig. 1, as is one of the computer-assisted methods. Planimetry and computer-assisted approaches have the advantage of generating a continuous measure that allows separate consideration of the dense and non-dense components of the image. Computer-assisted methods have also been shown to be highly reproducible.

In a systematic review of studies of mammographic features and breast cancer risk, McCormack and dos Santos Silva combined study specific data from more than 14,000 cases and 226,000 controls from 42 studies. Associations were stronger for studies in general populations than in symptomatic populations, and stronger for studies that used percent density than for those using Wolfe's classification or the Breast Imaging Reporting and Data System. Percent density in the prediagnostic mammogram was strongly associated with risk of breast cancer with a combined relative risk for incident breast cancer of 4.64 (95% confidence interval: 3.64, 5.91) in women with more than 75% of the breast occupied by density, compared to those with less than 5% density.

"Masking" of breast cancer by dense tissue does occur and is associated with an increased risk of breast cancer after a negative mammogram. Masking might inflate estimates of the risk of breast cancer estimates associated with mammographic density. However, the increased risk associated with density has been shown to persist for 10 years in one study, 8 years in another, and to increase risk of both screen detected and non-screen detected breast cancer. Masking does not therefore explain the increased risk of breast cancer associated with mammographic density. This risk factor both increases risk of the disease, and makes its detection by mammography more difficult.

All of the existing methods of assessing mammographic density have limitations. Non takes into account the thickness of the breast, and all are based on the



Mammographic Density. Figure 1 Examples of mammographic density and measurement. Left: examples of mammographic density; top left = 0% and bottom right = >75%. Right: illustrates computer assisted measurement.

projected area of the breast rather than volume. Current computer-assisted methods of measurement require that a dichotomous threshold be placed to distinguish dense from non-dense tissue, and do not allow for a gradual transition from one to the other, as is likely to exits in reality. Attempts to improve methods of measurement by addressing these and other limitations are in progress, and can be expected to improve risk prediction and to strengthen etiological associations.

Notwithstanding these limitations the relative risk of breast cancer associated with extensive mammographic density are larger than for almost all other risk factors for breast cancer. Although larger relative risks are associated with mutations in ►BRCA1 or ►BRCA2 genes, only a small proportion of the population carry these mutations, and the associated attributable risk is about 5%. By contrast, the attributable risk associated with mammographic density in 50% or more of the breast is about 30%.

Factors Associated with Variations in Mammographic Density

Mammographic density has consistently been found to be less extensive in older women, in women who are postmenopausal, in those who are parous and in those with a larger number of live births, and in those with greater body weight. Body weight and body mass index are strongly and positively correlated with the total area of the mammogram and the area of non-dense tissue, and weakly negatively correlated with the area of dense tissue, and it is therefore essential to adjust for measures of body size in analysis of the association between mammographic features and risk of breast cancer. Combined hormone therapy (HT) is associated with a small increase in risk of breast cancer, and increases mammographic density, effects that are not seen with estrogen alone. ►Tamoxifen, and a gonadotrophin hormone release agonist also reduce mammographic density.

Measurements of hormones and growth factors have shown that blood levels of growth hormone, ►IGF-I (in premenopausal women) and prolactin (in postmenopausal women), all mitogens in the breast, as well as sex-hormone binding globulin are associated with mammographic density. ►Estradiol has in general been found to be not associated, or associated inversely, with mammographic density in postmenopausal women. As levels of IGF-1 and prolactin have also been found to be associated with risk of breast cancer, respectively in premenopausal and postmenopausal women, they suggest potential mechanisms for the association of mammographic density with risk of breast cancer.

Heritability of Mammographic Density

The factors that are associated with mammographic density account for only 20–30% of the variation observed in the population. Early studies with limited numbers of mother-daughter sets, and a small twin study suggested that genetic factors might explain a proportion of the variation (i.e. the heritability) of mammographic density within a given population.

Twin pairs were aged 40–70 years and living in Australia or North America, and information was collected on the factors known to be associated with variations in mammographic density using the same questionnaires. Mammograms were obtained from each

member of each twin pair, digitized and percent density measured using the computer-assisted method shown above, by one observer blinded to zygosity and pairing. After adjusting for age, mammographic density had a symmetrical unimodal distribution, similar to that seen for other quantitative traits like height. After adjusting for age, age at menarche, parity, number of live births, menopausal status and body mass index, the variances in percent density were almost identical in both samples. The correlations between twin pairs in percent mammographic density in Australia and North America were, respectively, 0.61 and 0.67 for monozygotic twin pairs, and 0.25 and 0.27 for dizygotic twin pairs. The classic twin model assumes that variance for a given population can be partitioned into three components representing unmeasured effects, namely additive genetic effects, the effects of environmental factors that are shared by, or common to twin pairs, and individual specific environmental effects, that include measurement error. The proportion of the residual variation accounted for by additive genetic factors (heritability) was estimated to be 60% (95% confidence interval (CI): 54–66%) from Australian twins, 67% (95% CI: 59–75%) from North American twins, and 63% (95% CI: 59–67%) in the studies combined.

Analysis of the components of variance showed that the best fitting model included only components for the additive genetic factors, and person-specific environmental factors. Further details of the analysis, and additional references to the analysis of twin data, can be found in reference. These two twin studies thus replicate each other in providing compelling evidence that the wide variation in percent mammographic density among women aged 40–70 is strongly influenced by genetic factors.

Many of the factors associated with differences in mammographic density, including age at menarche and menopause, and BMI, are known to be at least in part heritable, but the estimates of heritability given for mammographic density were generated after adjusting for the effects of these factors. Heritability, however, refers to explaining variation within a population, and both twin studies were carried out in populations that are predominately European in origin, and living in "Western" environments, so our findings do not exclude the possibility of a greater influence of environmental factors at levels of exposure that lie outside the range usually seen in Western societies.

It is currently unknown whether, as with other quantitative traits, mammographic density variation at a given age, is determined by multiple genes, or variants in one or more major genes. Pankow et al. showed unadjusted sister-sister correlations in breast density of 0.27 ($p < 0.0002$), that are very similar to the correlation between DZ pairs seen in our twin studies. Segregation analysis of nuclear family data, assuming a single mode of inheritance of risk associated with one or more major genes, could not distinguish between dominant, recessive or co-dominant models.

Based on the relative risk of breast cancer associated with extensive mammographic density, and the observed correlation between dizygous twin sisters, we have estimated that the familial association in percent density would explain an increase in risk to first degree relatives by a factor of 1.05–1.08. Given that the increased risk to first-degree relatives of an affected woman is on average about twofold, this means that the genes that explain variation in mammographic density could explain 5–8% of familial aggregation on a population basis. Ziv et al. using a qualitative classification of density, have shown that subjects with first degree relatives with breast cancer do have greater amounts of density than those without such relatives.

Summary and Conclusions

There is now extensive evidence that mammographic density is a risk factor for breast cancer, independent of other risk factors, and associated with large relative and attributable risks for the disease. Mammographic density reflects variations in the tissue composition of the breast, and is positively associated with collagen and epithelium, and negatively associated with fat. Extensive mammographic density is relatively common, and estimates of attributable risk suggest that about a third of breast cancer may be attributable to density in 50% or more of the breast.

The epidemiology of mammographic density, notably the inverse association with age, is consistent with it being a marker of susceptibility to breast cancer, in a manner similar to the concept of "breast tissue age" in Pike's model of mammary carcinonogenesis. Cumulative exposure to mammographic density may thus be an important determinant of the age-specific incidence of breast cancer.

Mammographic density is a continuous quantitative trait that is highly heritable, and meets criteria for an intermediate phenotype. The genetic variants that influence mammographic density are now under investigation, and some of them may be associated with breast cancer risk.

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Mammotropic Hormone

►Prolactin

Mammotropin

►Prolactin

Mann–Whitney U-test

Definition

Is a nonparametric test for comparing the distributions for two groups of subjects and is based on replacing the original observations with an ordinal rank to form a test statistic.

Mantle Cell Lymphoma

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Synonyms

MCL; Centrocytic (mantle cell) lymphoma; Diffuse, small cleaved cell lymphoma; Intermediately or poorly differentiated lymphocytic lymphoma, diffuse or nodular

Definition

Mantle cell lymphoma is a B-cell neoplasia of monomorphic small to medium-sized lymphoid cells with irregular nuclei, morphologically most resembling centrocytes (According to WHO).

Characteristics

Incidence. MCL comprises ~6% of ►non-Hodgkin lymphoma.

Epidemiology. The majority of patients (2/3) are male, and the median age at diagnosis is 70 years. The etiology is unknown.

Pathogenesis. The causal molecular lesion is a ►reciprocal translocation t(11;14)(q13;q32), translocating the *CCND1* gene from chromosome 11 to chromosome 14, where it is brought under the control of the promoter of the constitutionally activated IgH gene. Therefore, overexpression of cyclinD1 is present in virtually all cases of MCL. A characteristic ►gene expression profile, however, allows the recognition of rare t(11;14)-negative, cyclin D1-neg. cases that in stead overexpress cyclinD2. More complicated cytogenetic changes occur, such as del13q14 and del17p. Also inactivation of the ATM gene on chromosome 11q22 may occur, leading to inhibition of the DNA-damage repair pathway.

Pathology. The normal architecture of the lymph node is replaced by a monomorphic lymphoproliferative process with a vaguely nodular, diffuse, or mantle-zone growth pattern. Two blastoid variants are recognized, the classical blastoid and the pleomorphic, both associated with high proliferation rate and poor prognosis. Transformation to diffuse large B cell lymphoma does not occur. Immunophenotype: The lymphoma cells are CD5+ B cells (CD19+, CD20+), which are CD23-negative, FMC7+, and strongly IgM+ often also IgD+.

Symptoms and Clinical Findings. The majority of patients have advanced disease with bone marrow involvement (Ann Arbor stage IV) (►Ann Arbor staging system) at diagnosis, often also with other extranodal involvement, typically of the gastrointestinal tract. The spleen is often enlarged. General symptoms as weight loss, night sweats, and fatigue are frequent.

Paraclinical Findings. The bone marrow involvement may lead to pancytopenia, and a minority of MCL cases are leukemic. LDH is often increased and some patients present with tumor lysis already at diagnosis.

Diagnosis and Differential Diagnosis. The first step of the diagnosis is the documentation of CD5+, CD23-neg. B-cell disease, but the hallmark of the disease is cyclinD1 overexpression or the t(11;14). The rare CD5-neg. or cyclinD1-neg. cases are always problematic to diagnose if gene-expression profile analysis is not available. The most important differential diagnosis to small-cell MCL is CLL, and to blastoid MCL, lymphoblastic lymphoma. CLL will be CD23+ with weak surface immunoglobulin expression, both CLL and lymphoblastic lymphoma will be t(11;14)-neg. and cyclinD1-neg., and in addition, lymphoblastic lymphoma will be TdT+. It was previously believed that some marginal-zone lymphomas could be t(11;14) pos., but such cases are now considered MCL.

Disease Staging. Standard work-up comprises physical examination including ear–nose–throat examination, CT scan of neck, chest, abdomen, and pelvis, bone marrow examination with flow cytometry and immunocytochemistry for cyclinD1 and ►fluorescent in situ hybridization (FISH) for t(11;14). There is increasing evidence that most cases of MCL are ►PET positive with intermediary specific uptake value, and particularly in this disease with frequent extranodal involvement this method may prove useful. Upper and lower endoscopy of the gastrointestinal tract is not indicated unless there are specific symptoms that require management, and will rarely have therapeutic consequences.

Prognostic Factors. Practically feasible prognostic factors are International prognostic Index (IPI), the proliferative index (proportion of Ki-67-pos. cells), and the cytomorphological subtype.

Course of Disease. Although MCL with indolent course of disease has been identified, both clinically and by proliferation-gene-profile studies, MCL generally presents itself with aggressive disease and symptoms of rapid tumor growth, and MCL has, until recently, been considered one of the lymphoma subtypes with the worst prognosis, with an expected median survival of 3½ years from the time of diagnosis following conventional anthracyclin-based chemotherapy. Newer results, however, suggest that long-term disease-free survival may be achieved by modern intensive immunochemotherapy with stem cell support at least in younger patients (<65 years).

Treatment. The choice of treatment must match the patients' age and performance status. In younger patients, induction therapy followed by stem cell harvest and ►high-dose therapy with stem-cell support is now standard treatment. CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) was for many years been considered the standard induction therapy, in spite of rather poor results (response and CR rates 75 and 25% respectively). Mantle cells are invariably CD20+ and the addition of rituximab to CHOP (R-CHOP) increases the complete and overall response rates to 35 and 95% respectively. The preferred 1st line induction treatment will often be R-CHOP that can be tolerated also by elderly patients. High-dose Ara-C has documented effect in MCL, and many consider it to be the most important drug in the treatment of MCL. Controlled trials are presently comparing R-CHOP with R-Ara-C in younger patients and with R-FC (Rituximab + Fludarabine + cyclophosphamide) in elderly patients.

►*Stem-Cell Harvest and Purging*. As most patients with MCL have bone marrow involvement, tumor-cell contamination of stem cell harvests from the bone marrow or the blood was previously considered a major problem and attempts to in vitro purge the autografts were not successful. However, intensive stem-cell mobilization with high-dose ara-C + concomitant rituximab (in vivo purging) has turned out to be highly effective leading to 90–100% PCR-negative grafts. Reinfusion of tumor cells with the autograft is therefore no longer of any significant concern.

High-Dose Therapy with Stem-Cell Support. Following induction therapy with CHOP, high-dose radiochemotherapy (TBI-CTX) leads to documented prolongation of progression-free survival. Intensive induction (R-hyperCVAD-MTX/Ara-C) without stem cell support is feasible but quite toxic, and, with the reduced risk of tumor-cell reinfusion with the graft, there seems to be no reason not to give the patients stem-cell support.

Total Body Irradiation (*TBI*). The importance of TBI in the conditioning regimen is disputed, and results based on pure chemotherapy, e.g., BEAM (BCNU, etoposide, Ara-C, and Melphalan) and HDS (high-dose sequential therapy with cyclophosphamide, Ara-C, melphalan, and mitoxantrone) leading to high proportion of long-term progression-free survival, contradict the necessity of TBI.

2nd-Line Treatment. Following relapse, systemic treatment with R-FC or R-Ara-C or involved field radiotherapy have proven efficacy, but all treatment will be palliative. Newer promising therapy modalities include proteasome inhibitors and, if the bone marrow is not too heavily infiltrated, radioimmunotherapy. In 2nd or later remission, autografting is not effective in MCL. ►Reduced intensity conditioning (RIC) allogeneic stem-cell transplantation is still experimental in this disease, but graft-versus-lymphoma has been documented and single-center reports on this modality in MCL have shown long-term disease-free survival.

Summary. A paradigm-shift is occurring in MCL, where – at least in younger patients – intensive immunochemotherapy with stem-cell support, in parallel with increasing biologic knowledge, may improve dramatically the poor prognosis traditionally associated with this disease.

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MAP Kinase

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Definition

Mitogen-activated protein (MAP) kinases are a family of protein kinases that mediate the transfer of phosphate from ATP to various proteins. They are evolutionarily conserved from yeast to mammals, and are expressed in nematodes, insects, slime molds and plants as well. Moreover, the MAP Kinases are ubiquitous enzymes, which are highly expressed in all cells of eukaryotic organisms. These kinases, operating within intracellular signaling pathways, play a key role in the transmission of extracellular signals from the plasma membrane to the nucleus. The MAP Kinases respond to a wide array of extracellular agents, including mitogens, hormones and cytokines, to regulate a variety of cellular responses such as proliferation, differentiation, ►adhesion, stress response and ►apoptosis.

Characteristics

In order to survive and perform their functions, cells need to respond to many extracellular signals such as mitogens, hormones, cytokines, physical changes of the environment and stress. These extracellular signals induce various distinct cellular processes, which are largely executed by induction of de-novo gene expression. Most of the extracellular agents bind to membranal receptors, and do not penetrate the cells in order to activate transcription. Rather, the extracellular signals are transferred from the membranes to the genes in the nucleus via several communication lines known as intracellular signaling pathways, which operate within a complex network. In many cases, the transmission of signals through these pathways involves sequential phosphorylation events by protein kinases, which are termed kinase cascades. Central building blocks in the signaling network are the MAP Kinases, which usually operate within signaling kinase cascades to connect receptors in the plasma membranes to nuclear transcription factors and other regulatory targets. They all respond to various extracellular factors and consequently regulate diverse cellular processes such as proliferation, differentiation, stress response, and apoptosis. These proteins, their mode of action and role in cancer are the subjects of this essay (►DNA damage response; G-protein; Insulin receptor; Integrin signaling and cancer).

Members of the MAP Kinase Family

The first MAP Kinases to be elucidated are the extracellular signal-regulated kinases 1 and 2 (ERK1/2) that have been identified as key components in growth factor signaling. Early studies on these kinases revealed that they are activated by upstream kinases, termed MAP Kinase/ERK kinases (MEKs). The activation occurs by phosphorylation of Thr and Tyr residues within a distinctive sequence known as Thr-Xaa-Tyr (TXY) motif, located in their activation loop. Subsequently, other protein kinases with a significant sequence homology to ERK1/2 (30–50%) were identified. Among them, the proteins that contain a TXY motif, and are activated by upstream kinases similar to MEKs, were grouped to the MAP Kinase family. Other protein kinases, demonstrating only some of the features, such as high sequence homology without activation by TXY phosphorylation downstream of a kinase cascade, or existence of a TXY motif without a sequence homology, are often considered as MAP Kinase-like proteins, and are not genuine members of the MAP Kinase family.

Presently, ten genuine MAP Kinases have been identified in mammals: ERK 1/2, c-Jun N-terminal kinase ►(JNK)1–3 (►JNK subfamily and cancer), p38 α–δ, and ERK5 (BMK1). Unfortunately, many names and acronyms have been attributed to each of the MAP Kinases over the years, and the terminology used in the field is still not unified. Details on the nomenclature and properties of the various MAP Kinases are summarized in Table 1. It should be noted that aside from the main gene products, many of the MAP Kinase genes encode for alternatively spliced forms that are designated by the addition of a letter after their name (e.g. ERK1b). Finally, the group of MAP Kinase-like proteins include: (i) ERK7 (ERK8 in human) that demonstrates a high homology to the other MAP Kinases, has a TXY motif, and yet is probably not activated by a kinase cascade. (ii) ERK3 and ERK4 that have high homology to other MAPKs but no TXY motif, and their mechanism of activation is not yet fully understood. (iii) Several other protein kinases, previously termed "cousins of MAPK," which do contain a TXY in their activation loop, but are not activated by kinase cascades. However, these kinases are functionally and structurally very different from genuine MAP Kinases, and therefore are currently regarded as a separate group of kinases.

Structure of MAP Kinase Cascades

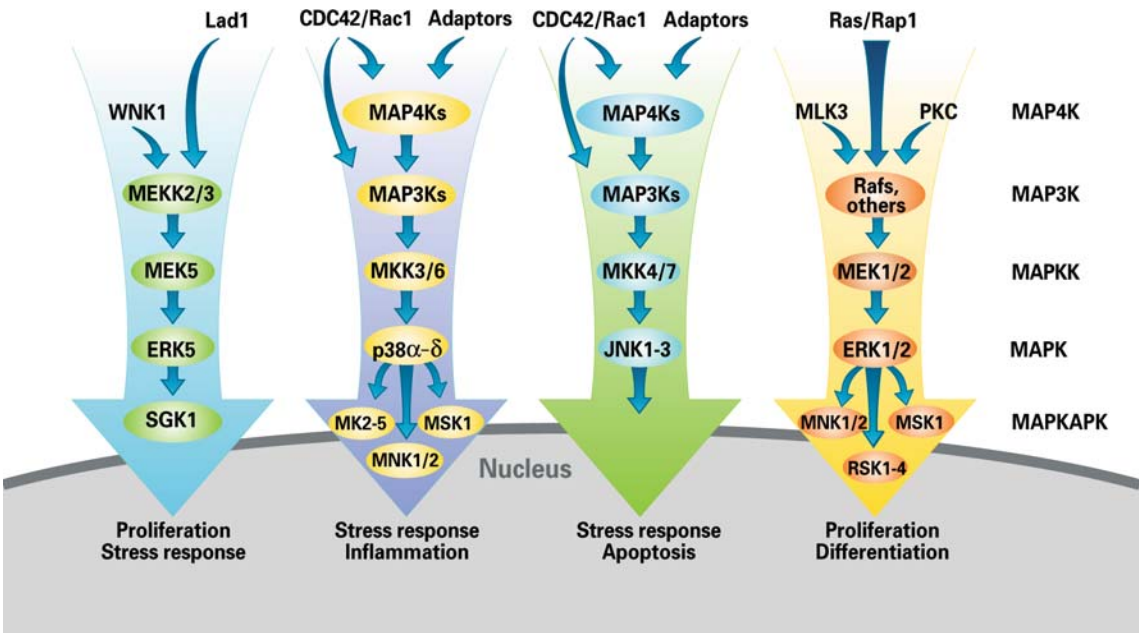
Transmission of signals via the MAP Kinase cascades is initiated by several mechanisms, including activation

of a small G protein (e.g. ►Ras), or interaction with adaptor proteins, which in turn, activate the upstream kinases of the cascades. Then, the signal is transmitted by three to five tiers of protein kinases in each cascade (Fig. 1):

- MAP4K (also termed MKKKK or STE20-like kinase), directly phosphorylates MAP3Ks
- MAP3K (also termed MKKK or MEKK), directly phosphorylates MAPKKs
- MAPKK (also termed MKK or MEK), directly and specifically phosphorylates MAP Kinases
- MAP Kinase (also termed MAPK) phosphorylates many targets including MAPKAPKs
- MAPKAPK (MAPK activated protein kinase, also termed MK).

MAP Kinase. Table 1 Nomenclature of members of the MAP kinase family

Name	Full name	Gene name	MW	Synonyms
MAP kinase				
ERK1	Extracellular signal-regulated kinase1	MAPK3	44 kDa	P44MAPK
ERK2	Extracellular signal-regulated kinase2	MAPK1	42 kDa	P42MAPK
P38α	P38αMAPK	MAPK14	43 kDa	SAPK2α, SAPK2A, CSBP1/2
P38β	P38βMAPK	MAPK11	42 kDa	SAPK2β, SAPK2B
P38γ	P38γMAPK	MAPK12	47 kDa	SAPK3, ERK6,
P38δ	P38δMAPK	MAPK13	46 kDa	SAPK4, Serk4
JNK1	c-Jun-N-terminal kinase1	MAPK8	46 kDa	SAPK1χ, SAPKχ, SAPK1
JNK2	c-Jun-N-terminal kinase2	MAPK9	54 kDa	SAPK1α, SAPKα, SAPK2
JNK3	c-Jun-N-terminal kinase3	MAPK10	49 kDa	SAPK1β, SAPKβ, Serk2
ERK5	Extracellular signal-regulated kinase5	MAPK7	110 kDa	BMK1
MAP Kinase-like				
ERK7	Extracellular signal-regulated kinase7	MAPK15	63 kDa	ERK8 in human (but only 69% identity)
ERK3	Extracellular signal-regulated kinase3	MAPK6	62/97 kDa	P97Mapk, ERK3α
ERK4	Extracellular signal-regulated kinase4	MAPK4	63 kDa	ERK3β, p63MAPK



MAP Kinase. Figure 1 Schematic representation of the MAP kinase signaling cascades. Activating phosphorylations are denoted by arrows.

Although MAPKAPKs can further phosphorylate and activate downstream protein kinases, these are usually not considered as components of the MAPK cascades. The three central tiers MAP3K, MAPKK, and MAPK are in fact the core signaling modules, whereas MAP4Ks and MAPKAPKs participate only in some instances. One or more kinase component in each of the tiers phosphorylates and activates components in the next tier, in order to transmit the signal downstream the cascade. Finally, a downstream component (MAP Kinases or MAPKAPKs) phosphorylates target regulatory molecules, either in the cytoplasm or the nucleus. This phosphorylation triggers changes in conformation and/or in molecular recognition of the target proteins, which lead to modulation of their activity, and consequently, initiation of the required physiological processes. Four distinct MAPK cascades have been elucidated thus far in mammals and those are named after their MAP Kinase components (ERK1/2, JNK, p38 and ERK5). The number of cascades, however, can be increased due to the existence of MAP Kinases that are not yet specified to any known pathway (e.g. ERK7/8). The components at the MAPKK are limited in number and usually selective to their cognate MAP Kinases. However, the upstream tiers contain many distinct components, and those can usually play a role in the activation of more than one cascade (e.g. Tpl2 was implicated in all MAP Kinase cascades). The cascades cooperate with each other, and along with other signaling components form a signaling network that determines the cell's fate upon distinct extracellular stimulations.

Signaling Via the MAP Kinase Cascades

The ERK1/2 Cascade

This cascade is a prototype of the MAP Kinase cascades, which is activated mainly by mitogens, hormones and differentiation factors. The extracellular factors stimulate cells by binding to their cognate membranal receptors that, via various mechanisms, induce activation of small ►GTPases (*e.g.,* Ras). These regulating proteins further transmit the signal to the MAP3K tier of the cascade that includes mostly ►Raf-1 (Raf kinase) and ►B-Raf (B-Raf signaling). Several other MAP3Ks of the ERK cascade (A-Raf, Tpl2, MOS and MEKK1) function under more specific conditions such as MEKK1 in stress. Although the MAP3K activation does not always require a phosphorylation by MAP4K, under some conditions ►protein kinase C and MLK3 can act at that tier to facilitate Rafs activation by phosphorylation. Thereafter, the signal is transmitted down the cascade through the MAPKKs MEK1 and MEK2, which are activated by phosphorylation of Ser residues in their activation loop. The activated MEKs are dual specificity protein kinases that demonstrate a high selectivity towards ERK1/2 in the MAP Kinase tier, activating them by phosphorylating their Thr-Glu-Tyr motif. ERK1/2, in turn, phosphorylate hundreds of regulatory proteins, either in the cytoplasm [e.g. ►PLA_2 (Phospholipase A2) and MAPKAPKs], or upon translocation they activate transcription factors such as Elk1 or ►c-Myc (Myc oncogene) in the nucleus. The MAPKAPK tier of the ERK cascade includes RSK1–4, which are specific to ERK1/2, and also MSK1, MNK1/2 and possibly MK3/5, which are activated by p38 as well.

The p38 and JNK Cascades

These cascades are also known as stress-activated protein kinase cascades and they posses considerable cross-talk between them. Their activation can be triggered not only by small GTPases but also by adaptor proteins, and both types of activators lead the signals to the MAP3K tier, either directly or via MAP4Ks. The kinases at these tiers seem to be common for the two cascades. There are at least 16 proteins at the MAP4K (e.g. GCK), and about 20 distinct proteins at the MAP3K (e.g. MEKKs, MLKs, ASKs) tiers. The funneling of the signals in these tiers towards the appropriate MAP Kinases is regulated mainly by scaffold proteins as described below. At the MAPKK tier, MKK3 and MKK6 are the main components of the p38 cascade, while MKK4 and MKK7 are the components of the JNK cascade, although some crosstalk between these components may occur. The MAPK tier of p38 is composed of products of four main gene products (p38α,β,γ,δ), all containing Thr-Gly-Tyr motif in their activation loop. Once activated, they either transmit the signal to the MAPKAPK tier (e.g. MK2), or phosphorylate regulatory proteins, such as transcription factors. On the other hand, three genes (JNK1–3) encode the JNK isoforms, which all contain a Thr-Pro-Tyr motif in their activation loop. Only a few cytosoplasmic targets, and no clear MAPKAPK, were identified for JNKs, but these kinases are considered to be major regulators of transcription, as they phosphorylate transcription factors such as c-Jun, and ATF (►Rho Family proteins).

The ERK5 Cascade

Another MAPK cascade is that of ERK5 (Big MAPK, BMK1), which is a 110 kDa protein that is activated by both mitogenic and stress signals. The mechanism of upstream activation of the cascade has not been fully elucidated as yet, but can involve either adaptor proteins (e.g. Lad), or MAP4K (e.g. WNK1). Those components activate the kinases at the MAP3K tier, consisting mainly of MEKK2/3. These kinases activate the only component in the MAPKK tier, MEK5, by phosphorylating Ser and Thr in its activation loop. MEK5 then activates ERK5 by phosphorylating both Thr and Tyr residues within its Thr-Glu-Tyr motif. Although this motif is identical to that of ERK1/2, there is no crosstalk between these cascades. Upon stimulation, ERK5 phosphorylates

several transcription factors (e.g. c-Myc), and one MAPKAPK, SGK, was identified thus far. Interestingly, ERK5 is unique in influencing transcription also through stabilization of transcription factors, and by a direct transcriptional activity mediated by its non-catalytic region.

Regulation and Specificity Determination of MAP Kinases

The activation of all MAP Kinases is reversible, as their activity peaks a few minutes (4 to 30) after stimulation and then declines back to basal levels. Fast decline (within 15–40 min) gives rise to an activation kinetics named "transient activation," while a slower decrease back to basal levels (40–180 min) is termed "sustained activation." These differences in signal duration often dictate the specificity and fate of the signals as described below. The main enzymes that regulate the duration as well as strength of the signals are protein phosphatases that counterbalance various activating signals. Because simultaneous phosphorylation of Tyr and Thr residues is required for the activity of MAP Kinases, their full inactivation can be achieved by the removal of phosphates from either one of the regulatory residues, or from both of them together. Thus, protein Ser/Thr phosphatases, protein Tyr phosphatases and dual specificity phosphatases all act as MAP Kinase phosphatases. One important group of regulatory phosphatases is the MAP Kinase dual specificity phosphatases (MKPs). The members of this group are products of induced genes and are rarely expressed in quiescent cells. Therefore, the immediate inactivation of MAP Kinases, 5–45 min after stimulation, is mainly regulated by Tyr phosphtases, and the MKPs take over at later stages after stimulation.

Another mechanism that participates in the regulation of MAP Kinases is the formation of multi-protein complexes, primarily due to interaction with scaffold proteins. The importance of scaffolds for MAP Kinase signaling was first demonstrated in yeast, whereby different scaffolds direct signaling components to regulate distinct processes. A similar mechanism is now known in mammals whereby scaffolds, such as KSR1 or ►IQGAP1 (IQGAP protein) for ERK, or JIP1 for JNKs facilitate the rate of MAP Kinase activation by bringing distinct components of the cascade to close proximity. In addition, the scaffolds may direct MAP Kinases to their proper subcellular localization, allowing interaction with proper target molecules upon distinct upstream signals, thereby securing their proper mode of action.

The ability of MAP Kinases to transmit different, and even opposing signals in the same cells raises the question as to how the specificity of the different signals transmitted by the MAP Kinase cascade is regulated. The duration and strength of the signal, as well as scaffolding with proper activators and targets, are two mechanisms suggested for the specificity determination. However, not less important are (i) subcellular localization of the cascade components that directs the signals to their proper destinations; (ii) presence of several similar isoforms in each tier of the cascade, each of which may possess distinct properties; (iii) extensive cross-talk and interplay between the MAP Kinases and other intracellular signaling pathways that modulate the output of each cascade, and (iv) combinatorial integration of the MAP Kinase signals at downstream levels. Although these mechanisms can independently determine signaling specificity, they often cooperate with each other in order to induce proper downstream effects.

Involvement of MAP Kinases in Cancer

MAP Kinase cascades provide viable targets for pharmaceutical intervention in various human diseases including cancer. All four MAP Kinase cascdes have been implicated to varying extents in the induction and progression of oncogenic ►cell transformation. Indeed, elevated phosphorylation and activity of the MAP Kinases is detected in many (but not all) cancers and metastasis. However, while ERK1/2 seem to be involved in the transformation of most cell types, the involvement of the other cascades is limited to specific cancers. The involvement of ERK1/2 in cancer is not surprising since this MAP Kinase lies downstream to many ►oncogene products such as Her2, ►v-Ras (RAS transformation targets) and B-Raf. In addition, ERK1/2 are key regulators of cellular proliferation, and therefore, the activation of ERK1/2 is usually required for the enhanced proliferation observed in cancer. These observations led to the development of MEKs and Rafs inhibitors that completely inhibit ERK1/2 activity. These inhibitors are now in clinical trials for several cancer types, and in spite of the wide range of ERK1/2 activity, it seems that these agents can become specific and potent anti cancer drugs.

Phosphorylation of c-Jun, which is induced primarily by JNKs, has been reported to play a critical role in Ras-induced tumorigenesis. In addition, JNKs and c-Jun appear to repress p53 gene expression and, thereby, induce mitogenicity in cancer cells. Therefore, JNKs may be involved in cancers induced by Ras and other oncogenes. However, in other cancers JNKs play a role in the induction of apoptosis, and therefore may have a tumor suppressive function. ERK5 can play a role in the regulation of proliferation as well, and it was shown that this kinase cooperates with ERK1/2 in ►oncogenesis, although very little is known today regarding its involvement in human cancer. Finally, p38 was implicated in the progression of only a few cancers, and is in fact considered to be a tumor suppressor in many cancer types. In view of the above information, it is likely that better understanding of

MAP Kinase role in oncogenic transformation and ►metastasis (Metastasis signaling) will lead to novel drugs and therapeutic strategies in combating cancer.

►Signal transducers and activators of transcription in oncogenesis.

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Mapatumumab

Definition

Mapatumumab is a fully ►humanized antibody. The antibody exerts anti-tumour activity in diverse preclinical tumours models including breast, gastrointestinal, lymphoma, ovarial carcinoma, and uterine cancers. Mapatumumab was shown to specifically recognize the TRAIL-Receptor 1 protein without any relevant interactions with the ►TRAIL decoy receptors. The efficacy of mapatumumab is increased by combinations with various cytostatic drugs including carboplatinum, cisplatin, camptothecin, topotecan, ►paclitaxel as well as radiation. ►Xenograft models for breast, colorectal, non-small cell lung cancer as well as uterine cancer revealed a high activity of either the drug alone or in combination with other cytotoxic treatment approaches including radiation.

►TRAIL-Receptor Antibodies

MAPK

Definition

Mitogen-activated protein kinases operate within signaling cascades, which consist of up to six tiers of protein kinases, which sequentially activate each other by phosphorylation. Four major MAPK cascades are known in mammals: ERK1-2, JNK1-3, p38 α-δ, and ERK5. The MAPK cascades are activated by growth factors, hormones, cytokines, and stress. The hallmark of the MAPK family is their ability to translocate to the nucleus and activate transcription factors. MAPKs are involved in growth, differentiation, development, transformation, metabolism, learning, survival, apoptosis, cell cycle control, and gene expression. MAPK inhibitors are being developed mainly for cancer therapy.

►MAP kinase

MAPK8

►JNK Subfamily

MAPK9

►JNK Subfamily

MAPK10

►JNK Subfamily

MAPs

►Microtubule Associated Proteins

MAR

►Melanoma-Associated Retinopathy

Marginal Excision

Definition

Tumor surgery by which the excision takes place just outside the pseudo capsule of the tumor.

►Cryosurgery in Bone Tumors

Marginal Zone B-Cell Lymphomas

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Definition

Marginal zone lymphomas (MZL) represent the neoplastic counterpart of the marginal zone. The latter is a distinct micro-anatomic compartment of the B-follicle, well developed in those lymphoid organs where an abundant influx of antigens is known to occur (spleen, mesenteric lymph nodes, and mucosa-associated lymphoid tissue or MALT). MZL account for approximately 10% of all non-Hodgkin lymphomas, being the third most frequent subtype after diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. They are subdivided into three entities by the World Health Organisation (WHO): splenic MZL, nodal MZL, and MALT lymphoma (or extranodal MZL). Despite histologic and genetic similarities, clinical differences were the main reason to consider these three MZL subtypes as distinct clinicopathological entities.

Characteristics

Histology

Diagnosis of an MZL is always made on a ►biopsy. Residual reactive ►germinal centers are a constant finding in all MZL occurring at any site. The architecture of a lymph node involved by primary nodal MZL is most frequently preserved, with the neoplastic proliferation extending into the interfollicular area and surrounding residual reactive germinal centers as distinct rings. In splenic MZCL, the white pulp is hyperplastic with broad marginal zones and variable invasion into the red pulp, resulting in a massive expansion of the spleen. In ►MALT lymphomas, the neoplastic cells may invade and destroy the epithelium, resulting in so-called lymphoepithelial lesions. The neoplastic proliferation itself may be predominantly composed of marginal zone B-cells ("centrocyte-like cells"), but is most frequently heterogeneous in cell composition. A polymorphous mixture of centrocyte-like cells, small lymphocytes, plasma cells and scattered large blast cells may be found in all MZL, regardless of the site. The immunophenotypes of extranodal, nodal and splenic MZL are almost completely similar and homologous to that of normal MZ B-cells. There is positivity for surface Ig (IgM > IgA, IgG), pan B-cell markers (CD20, CD19, CD79a) and complement receptors (CD21, CD35). IgD expression may be variable but has been considered by some authors as an important difference between nodal/extranodal MZL and splenic MZL, as they observed positivity in the latter and no expression in the former. In the differential diagnosis with other small B-cell lymphomas, absence of characteristic markers for those neoplasms is important: lack of CD5 is useful in distinction from mantle cell and small lymphocytic lymphomas, and lack of cyclin D1 in differential diagnosis with mantle cell lymphomas.

Pathogenesis

Growing evidence indicates that MZL, and MALT lymphomas in particular, are proceeded by chronic antigenic stimulation (chronic infection or autoimmune diseases) and involve deregulation of the ►nuclear factor κB (NF-κB) pathway. Long-standing (auto)antigenic stimulation explains how lymphoid infiltrates may appear in extranodal sites that are normally devoid of lymphoid tissue (e.g. the stomach, lung, the salivary and lachrymal glands). The list of microbial species associated with MZ lymphoproliferations now comprises at least five distinct members: ►*Helicobacter pylori*, *Campylobacter jejuni*, *Borrelia burgdorferi*, *Chlamydia psittaci*, and ►hepatitis C virus (HCV), which have been associated with gastric MALT lymphoma, immunoproliferative small intestinal disease (IPSID), cutaneous MALT lymphoma, orbital MALT lymphoma, and splenic MZL, respectively. Furthermore, some autoimmune diseases may also favor the development of a MALT lymphoma: patients with ►Sjögren syndrome have a 44-fold increased risk of developing a lymphoma and patients with ►Hashimoto thyroiditis have a 70-fold increased risk of thyroid lymphoma.

Besides inducing an initially polyclonal B-cell proliferation, sustained (auto)antigenic stimulation may also trigger inflammatory responses by attracting neutrophils, which release reactive oxygen species. The latter are genotoxic and cause a wide range of genetic abnormalities (see genetics). Remarkably, the genes targeted by most of these abnormalities are

involved in one and the same pathway leading to the activation of nuclear factor κB (NF-κB). The latter is a key transcription factor in the immune response as it regulates the expression of a number of survival- and proliferation-related genes in B-cells. As such, its constitutive activation by MALT lymphoma-related genetic abnormalities results in uncontrolled B-cell proliferation and thus subsequent neoplastic transformation of the B-cell clone.

In splenic and nodal MZ, the mechanisms determining lymphomagenesis are not clear yet.

Genetics

►Trisomy 3 and 18 have been detected at similar frequencies, ranging from 15% to 60%, in extranodal, nodal and splenic MZL. Other trisomies, such as trisomy 7 and 12 are less common.

The chromosomal translocations t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21) and t(3;14)(p13;q32) are all known to occur with variable frequencies in MALT lymphomas, resulting in *API2-MALT1*, *IGH-BCL10*, *IGH-MALT1* and *IGH-FOXP1* rearrangements respectively. The t(11;18)(q21;q21) is the most common structural chromosomal abnormality in MALT lymphomas. It is demonstrated in 10–50% of gastric MALT lymphomas, whereas this translocation rarely occurs in nongastric MALT lymphomas, with the exception of pulmonary MALT lymphomas. MALT lymphomas marked by t(11;18)(q21;q21) are resistant to *H. pylori* eradication treatment and only rarely evolve to a DLBCL. The t(11;18)(q21;q21) fuses the amino-terminus of the *API2*-gene (located at 11q21) to the carboxyl-terminus of the *MALT1*-gene (located at 18q21), hereby generating the fusion-protein API2-MALT1. The t(11;18)(q21;q21) has never been found in other lymphoma types and its presence in MALT lymphoma correlates with the absence of any further genetic aberrations. The t(1;14)(p22;q32) and t(14;18)(q32;q21) occur in a small minority of almost exclusively non-gastric MALT lymphomas, which typically display additional genomic aberrations and tend to be at advanced stage of disease at presentation. These translocations are both mediated by the *IGH*-gene enhancer and result in overexpression of the *BCL10*-gene (located at 1p22) and *MALT1*-gene (located at 18q21) respectively. Both BCL10 and MALT1 play a key role in the antigen-receptor signaling to NF-κB. The t(11;18)(q21;q21)- and (1;14)(p22;q32)-positive MALT lymphomas are marked by a respectively moderate to strong nuclear BCL10 expression while t(14;18)(q32;q21)-positive MALT lymphomas are characterized by a perinuclear BCL10 immunohistochemical staining pattern. Recently, *FOXP1* (located at 3p13) was identified as a new translocation partner of *IGH* at low frequency, not only in MALT lymphomas, but also in DLBCL with mainly extranodal location. Remarkably, a significant number of t(3;14)(p13;q32)-negative MALT lymphomas and DLBCLs harbors strong nuclear FOXP1 expression, suggesting that mechanisms other than underlying *FOXP1* rearrangements can upregulate FOXP1 expression. The significance of this nuclear FOXP1 overexpression in MALT lymphomas is still debated; one study found strong nuclear FOXP1 expression to be confined to MALT lymphomas that are at risk of transforming into a DLBCL with poor clinical outcome. So far, it is not clear yet how FOXP1 mediates MALT lymphomagenesis.

Except for trisomy 3 and 18, cytogenetic findings in both splenic and nodal MZL are rather heterogeneous and complex, and no unique abnormalities have been documented so far. Of interest, chromosome 7 is most frequently altered in splenic MZL, with a loss of 7q31–q32 in 40% of cases. In addition, cases with 7q loss behave more aggressively and display more frequent tumoral progression.

Deregulation of NF-κB: The Unifying Concept for MALT Lymphomagenesis?

Mounting evidence links the oncogenic activity of t(11;18)(q12;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21) to aberrant activation of the canonical NF-κB pathway by API2-MALT1, BCL10 and MALT1 respectively. The NF-κB family is composed of five proteins that share a conserved REL homology domain for DNA binding: NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and ►REL. In unstimulated B-cells, the NF-κB molecules RelA and p50 are sequestered in the cytoplasm as latent complexes through binding with inhibitory κB (IκB) proteins. However, upon antigen encounter, CARMA1 interacts with the antigen-activated B-cell receptor in the lipid-rafts and induces the oligomerization of its downstream components BCL10 and MALT1 with TRAF6. The latter elicits its ubiquitin ligase activity, resulting in ►ubiquitination of IκB kinase-γ (IKKγ or NEMO), which in turn phosporylates IκB, hereby targeting IκB for phosphorylation and proteasomal degradation. This allows the RELA/p50 dimers to enter the nucleus and mediate transcription of NF-κB-responsive genes, such as the pro-proliferative gene *cyclin D2* and the anti-apoptotic genes BCL-X_L and ►*BCL-2*.

In MALT lymphomas, the overexpression of BCL10 and MALT1 induced by fusion with the *IGH*-enhancer in t(1;14)(p22;q32) and t(14;18)(q32;q32) respectively hints at a role for NF-κB deregulation in lymphomagenesis. In t(1;14)(p22;q32)-positive MALT lymphomas, in which BCL10 is overexpressed, BCL10 is believed to form oligomers without the need for upstream signaling, hereby triggering MALT1 oligomerization and aberrant NF-κB activation. In t(14;18)(q32;q21)-positive MALT lymphomas, in which MALT1 is overexpressed, the oligomerization of MALT1 with

subsequent NF-κB activation is thought to be dependent on BCL10, as MALT1 does not have a structural domain to mediate self-oligomerization, nor does its overexpression alone activate NF-κB *in vitro*. In t(11;18)(q21;q21)-positive MALT lymphomas, it is believed that the fusion-protein API2-MALT1 activates NF-κB directly by constitutively increased IKKγ polyubiquitination, as shown *in vitro* and in mice models. Also, the API2-MALT1 fusion protein was reported to reside in the lipid-rafts. This raft association facilitates oligomerization of the API2-MALT1 fusion-protein and/or its interaction with its downstream signaling components (e.g. TRAF6), in this way bypassing the normal antigen requirement for formation of MALT1-TRAF6 oligomers and resulting in constitutive NF-κB activation. In addition, it was shown that API2-MALT1 induces transactivation of the *API2*-gene through NF-κB activation, hereby creating a positive feedback-loop mechanism of self-activation by upregulating its own expression in t(11;18)(q21;q21)-positive MALT lymphomas.

Clinical Features, Prognosis and Therapy

MALT lymphomas mostly present as Ann-Arbor stage IE disease (extranodal disease limited to the site of origin) as bone marrow and peripheral lymph node involvement are rather uncommon. It is nowadays generally accepted that *H. pylori* eradication with antibiotics is the first choice of therapy for localized gastric MALT lymphoma. The use of anti-infectious treatment in non-gastric MALT lymphomas is still under investigation, although recent reports discourage the use of antibiotics in patients suffering from non-gastric MALT lymphomas. Other effective treatment approaches include radiotherapy, chemotherapy, and anti-CD20 monoclonal antibody.

Splenic MZL is characterized by an enlarged spleen and often presents with peripheral blood and bone marrow involvement. The clinical course is indolent, even with bone marrow involvement, and splenectomy may be followed by a prolonged remission. Chemotherapy is only employed for patients with progression after splenectomy or when surgery is contraindicated. Alkylating agents (chlorambucil or cyclophosphamide) or purine analogues (fludarabine) have been reported as being effective treatments.

Finally, the typical clinical presentation of nodal MZL, without extranodal involvement, is that of a middle-aged individual with peripheral and para-aortic lymphadenopathy and advanced stage disease with bone marrow involvement. This presentation is similar to other low-grade nodal ►non-Hodgkin lymphomas, such as follicular lymphocytic lymphomas. Nodal MZL represents a therapeutic dilemma, since no studies of large series have been published so far. In addition, the 5-year overall survival as well as the failure-free survival of patients with nodal MZL was found to be lower than that of patients with extranodal MZL (56% vs. 81% and 28% vs. 65%, respectively).

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Marijuana

►Cannabinoids and Cancer

MAR-syndrome

►Melanoma-Associated Retinopathy

Maspin

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Definition

Maspin is a 42 kDa clade B ►serpin. The human maspin gene has been mapped to a cluster of serpins at chromosome 18q21.3–q23.

Characteristics

Maspin is one of 13 human clade B serpins that share high homologies with chick ovalbumin (ov-serpins).

Inhibitory serpins are known for their conformational changes in which the reactive site loop (RSL) is engaged in a covalent bond with the catalytic site of the protease. Eventually the pseudo substrate p_1p_1-bond in RSL is cleaved, and the cleaved RSL is inserted into the β-pleated sheets as strand 4A. This conformation change, also known as loop insertion, is viewed as the most critical trait for inhibitory serpins. The polypeptide sequence of maspin is the most divergent from other ov-serpins. Crystallized maspin has a hydrophilic Asn (Asn^{113}) in the shutter region at the base of the β-pleated sheets. In addition, the exposed RSL may directly interact with β-pleated sheet C1 but does not have significant flexibility. These structural features may prevent the protease-induced loop insertion. The exposed G-helix has two different conformations (closed and open).

The Role of Maspin in Epithelial Pathophysiology

Maspin is differentially expressed: The human maspin gene is expressed by epithelial cells in several types of tissues including breast, prostate, placenta, testis, colon, small intestine, tongue, thymus, and skin. Mouse maspin (mMaspin) is found to have a similar tissue expression pattern as human maspin. The epithelial-specific expression of maspin in normal somatic tissues is controlled by the DNA ►methylation mechanism. The level of mMaspin mRNA is altered at different developmental stages of the mammary gland; low in virgin mouse breast and during involution, but high in late pregnancy and lactation. Maspin expression can be induced by pathophysioloigcal stress stimuli, including ►DNA-damaging agents, cytotoxic drugs, peroxisome proliferator-activated receptor-gamma, nitric oxide, and manganese superoxide dismutase (MnSOD). The Maspin transcription may be positively regulated by ►tumor suppressor, ►p53 and negatively regulated by a cis-acting ►hormone responsive element. The Maspin protein isolated from biological sources is a monomer which may be present as a secreted, a cytoplasmic, a nuclear, as well as a cell surface-associated protein. Changes of the subcellular localization pattern of maspin have been noted in tumor progression. In ►breast cancer, ►prostate cancer, ovarian, and ►lung cancer, nuclear maspin correlates with better differentiate phenotypes, whereas cytoplasmic maspin is associated with poor prognoses. Maspin is generally downregulated or lost as tumor progresses to invasive and metastatic stages.

Maspin acts as a ►tumor suppressor: Despite the evidence that systematic maspin knockout is lethal at embryogenesis, the biological function of maspin in development remains elusive. In the meantime, accumulated evidence supports a tumor suppressive role of maspin, acting at the levels of ►invasion, ►angiogenesis, and ►metastasis. Orthotopic explants of mammary carcinoma cells transfected with maspin-coding cDNA are inhibited in tumor growth and metastasis in nude mice. In a novel intraosseous SCID-Hu model that reproduces the organ and species-specific prostate–bone interaction, maspin overexpression in prostate carcinoma cells decreased tumor growth, tumor-induced osteolysis, and tumor angiogenesis. Maspin expression also led to redifferentiation of prostate tumor cells in vivo. These findings are further supported by several in vivo experiments using genetically modified mouse models. For example, in a syngeneic mammary tumor model, maspin overexpression in TM40D mammary tumor cells blocked tumor invasiveness, angiogenesis, and metastasis.

Maspin transfected breast and prostate tumor cells are significantly inhibited in in vitro invasion and ►motility assays. Similarly, induced reexpression of maspin by γ-linolenic acid has been shown to correlate with a marked reduction of the spreading and ►migration of breast tumor cell lines. Secreted maspin sequestered by extracellular matrix inhibits tumor cell invasion. Purified recombinant human maspin exhibits potent inhibitory activities on the invasion and motility of an array of human carcinoma cell lines. Recombinant maspin is also shown to directly block endothelial cell migration toward basic fibroblast growth factor and vascular endothelial growth factor, and inhibited endothelial tube formation in vitro. A current consensus suggests that the inhibitory effects of maspin on tumor cell motility and invasion is localized to the cell surface, depends on cell interaction with extracellular matrix-extracellular matrix, and depends on maspin RSL. Maspin inhibits tumor-induced extracellular matrix degradation. In addition, maspin enhances cell ►adhesion with increased focal adhesion contacts in vitro. It has been recently shown that maspin stabilizes the mature focal adhesion contacts and retard cell detachment.

Maspin enhances tumor cell apoptotic sensitivity: Maspin protein does not induce spontaneous cell death in vitro. However, overexpression of maspin in ►cancer cells always results in growth inhibition in various in vivo tumor models. These in vivo maspin effects are associated with increased tumor cell ►apoptosis. Consistently, a recent clinical study revealed a positive correlation between maspin and drug sensitivity of ►colon cancer patients. Experimental evidence indicates that intracellular, but not extracellular, maspin significantly increases the sensitivity of breast and prostate carcinoma cells to various drugs, ranging from extrinsic death ligands to endoplasmic reticulum stress. The proapoptotic effect of maspin appears to be tumor specific since normal epithelial cells that express maspin at a high level are not sensitized to drug-induced apoptosis. Thus far, maspin is the only proapoptotic clade B serpin among all serpins implicated in apoptosis regulation.

The Molecular Mechanisms of Maspin

Maspin does not directly inhibit active serine proteases. Efforts to delineate the underlying molecular mechanisms of maspin led to the identification of several candidate extracellular maspin partners/targets: tissue-type ►plasminogen activator, α2-chain collagen type I, ►uPA zymogen (pro-uPA). Interestingly, maspin prefers pro-uPA and inhibit the proteolytic activation of pro-uPA to active uPA. Furthermore, maspin has been shown to trigger robust ►endocytosis of uPA/uPAR. uPA is thought to be responsible for initiating a powerful proteolytic cascade, by converting plasminogen to plasmin. Plasmin can directly degrade nonfibrillar extracellular matrix proteins or indirectly degrade fibrillar extracellular matrix proteins by activating members in the ►matrix metalloproteinase (MMPs) family. Plasmin and active MMPs are also implicated in the proteolytic activation and/or release of growth factors. All these consequences increase ►extracellular matrix remodeling and the vicious paracrine communication between tumor cells and tumor stroma.

Yeast two-hybrid screening method pulled out ►histone deacetylase 1 (HDAC1) glutathione s-transferase (GST), ►Hsp90, and interferon regulatory factor 6 (IRF6) as candidate intracellular maspin-binding proteins. The interaction of maspin with HDAC1, GST, and IRF6 has been confirmed. Although the biochemical reactions involved are subjects of further investigation, accumulative evidence suggests that maspin may regulate stress-responsive pathways involved in epithelial homeostasis. For example, GSTs are major glutathione-based reductases. The interaction of maspin with GST correlates with increased cellular GST activity and cell resistance to ►oxidative stress-induced generation of ►reactive oxygen species.

The interaction of maspin with HDAC1 leads to specific HDAC1 inhibition. HDAC1 is a major class I ►HDAC that hydrolyzes the ε-acetyl lysine residues in histone and other proteins. HDAC1-mediated histone deacetylation leads to chromatin condensation, preventing the access of transcription factors to DNA and repressing gene expression. HDAC1 target genes such as Bax, $p21^{WAF1/CIP1}$, $p27^{KIP1}$, and cytokeratin 18 play important roles in proliferation, apoptosis, and differentiation. Consistently, maspin expression in cancer cells correlates with increased expression of Bax. Furthermore, the effect of maspin in sensitizing cells to induced apoptosis depends on the Bax-mediated mitochondrial pathway.

This inhibitory interaction of maspin with HDAC1 may depend on the novel serpin conformation of maspin as well as the serine protease-like conformation of HDAC1. HDAC1 crystallized with synthetic inhibitor trichostatin A shows a catalytic pocket similar to that of a serine protease. However, this pocket has a unique narrow "tunnel" extending into the center of the molecule. At the bottom of the "tunnel" is a Zn^{2+} ion chelated by two Asp-His charge-relay systems. In HDAC1-mediated protein deacetylation, a H_2O and a ε-acetyl-lysine side chain bind to Zn^{2+}, leading to a series of proton transfer and lysine deacetylation. Coincidentally, maspin RSL p_1 residue $Arginine^{340}$ has a similar structure as ε-acetyl-lysine. The exposed maspin RSL with $Arginine^{340}$ at the center seems a natural cap to block the entry of HDAC1 substrates.

Taken together, maspin may act as a serpin-like regulator of serine protease-like targets. Maspin is one of loneliest and the most ancient ov-serpins. From an evolution point of view, the conservation of a serine protease-like catalytic center in many molecules (such as pro-uPA and HDAC1) should require the coexistence of endogenous antagonists. It is likely that the activity of maspin to target serine protease-like molecules may not be substituted by other serpins that have evolved to acquire higher target specificities.

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Mass Recovery

►Regeneration

Mass Spectrometry

Definition

Mass spectrometry is an analytical technique in which molecules are ionized and their mass-to-charge ratio is measured in order to determine the exact mass. If the mass is measured with precision, then the composition of the molecule can be identified. In the case of proteins, the sequence can be identified. It is most generally used to find the composition of a physical sample by

generating a mass spectrum representing the masses of sample components. The mass spectrum is measured by a mass spectrometer to give the mass-to-charge ratio of all compounds in the sample.

►Oncopeptidomics
►Proteinchip

Mast Cell Growth Factor

►Kit/Stem Cell Factor Receptor in Oncogenesis

Mast Cells

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Synonyms

Mast zellen

Definition

A large cell in connective tissues with many coarse cytoplasmic granules, containing several chemical mediators, which are released during allergic responses, inflammation, and tumor growth.

Characteristics

Mammalian mast cells (MC) express common characteristics, including high affinity plasma membrane receptors (FcεRI) binding IgE antibodies and cytoplasmic granules storing biogenic amines, proteoglycans, cytokines, and neutral serine proteases. However, MC populations show marked differences in their phenotypic expression in different species as well as distinct anatomical sites, a phenomenon called "MC heterogeneity."

MC arise from multipotential precursors in the bone marrow, circulate as mononuclear agranular cells, then traverse the vascular space and enter the tissues where they complete their development. In humans, MC derive from $CD34^+$, $FcεRI^-$, c-kit^+ progenitors. Differentiation and maturation of MC are most likely regulated by local microenvironmental factors, in particular the c-kit ligand (stem cell factor, SCF) secreted by fibroblasts, stromal cells, and endothelial cells, which represents the most important cytokine involved in MC development. In humans, tissue MC survival and differentiation are also enhanced by other cytokines including interleukin-4 (IL-4) and IL-6.

MC are found in almost all of the major organs and tissues of the body, particularly in association with connective tissue structures such as blood vessels, lymphatic vessels, and nerves, and in proximity to surfaces that interface the external environment, such as those of the respiratory and gastrointestinal system and the skin. Human MC are conventionally divided into two types depending on the expression of different proteases in their granules. MC tryptase (MCT) cells contain tryptase and are predominantly located in the respiratory and intestinal mucosa, where they colocalize around T lymphocytes. MC tryptase–chymase (MCTC) cells contain both tryptase and chymase, along with other proteases such as carboxypeptidase A and cathepsin G. They are predominantly found in connective tissue areas, such as skin, submucosa of stomach and intestine, breast parenchyma, myocardium, lymph nodes, conjunctiva, and synovium. A third type of MC, MC chymase (MCC) cell has been identified: this MC expresses chymase without tryptase and resides mainly in the submucosa and mucosa of the stomach, small intestinal submucosa, and colonic mucosa.

Human MC are usually described as round or elongated cells with a diameter ranging between 8 and 20 μm, depending on the organ examined. They present a single round or oval nucleus and numerous cytoplasmic granules that stain metachromatically with thiazine dyes such as toluidine blue. Ultrastructurally, these cells exhibit a nonsegmented monolobed nucleus with peripherally condensed chromatin. The cytoplasm contains a few mitochondria, short profiles of the rough endoplasmic reticulum, and numerous free ribosomes.

The most characteristic cytoplasmic organelle in human MC is the membrane-bound, moderately electron-dense secretory granule. Secretory granules are much abundant and correspond to the metachromatic granules of the light microscopy. They have an average diameter of 1.5 μm and present different types of substructural patterns: homogeneous, crystalline, scroll, particle or thread-like or a combination of them. Granules with the chymase protease preferentially exhibit homogeneous or crystalline substructures whereas granules lacking this protease show mainly a scroll pattern. However, significant granule heterogeneity can be found in any particular tissue and even between granules of a single MC. Besides the typical secretory granules, human MC also contain nonmembrane-bound, highly osmiophilic granules, called lipid bodies. They are fewer in number and generally larger than secretory granules, and serve as a significant storage site for arachidonic acid. Release

of preformed and lipid mediators, cytokines and chemokines (histamine, tryptase, chymase, cathepsin, carboxypeptidase A, PAF, LTC-4, PGD-2, SCF, IL-3, IL-5, IL-6, IL-8, IL-13, IL-16, IL-18, IL-25, TGF-β, GM-CSF, TNF-α, VEGF, NGF, I-309/CCL-1, MCP/CCL-2, MIP-1α/CCL-3) stored in the secretory granules can be accomplished by two morphologically distinct secretory pathways, referred to as exocytosis (also called "anaphylactic degranulation") and piecemeal degranulation. The former consists of a rapid and massive secretory process, occurring during IgE-dependent hypersensitivity reactions. In exocytosis, cytoplasmic granule membranes fuse with each other and with the plasma membrane, giving rise to open secretory channels which allow the release of granule contents into the local extracellular environment. Piecemeal degranulation, conversely, represents a slow and particulate mode of MC secretion, characterized by granule content release in a "piecemeal" fashion, not accompanied by membrane fusion events and granule opening to the cell exterior. This degranulation pattern has been observed in MC infiltrating areas of chronic inflammation or tumors.

MC and Tumor Growth

Since the first recognition by Ehrlich in 1878, MC have often been observed at different sites of neoplasms. MC are commonly recognized at the margins of diverse tumors in human and rodents. MC are prominent in the tumor ►microenvironment, are thought to provide a host response of the innate immunity to neoplasia and could either promote or inhibit tumor growth depending on the local stromal conditions.

It is now well documented that neoplastic cells are influenced by their microenvironment and vice versa. The specific organ microenvironment determines the extent of ►cancer cell proliferation, ►angiogenesis, invasion, and survival. This indicates that a permissive stromal environment is important in supporting tumor progression in combination with genetic alterations. Tumor cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages, and MC, which communicate via a complex network of intercellular signaling pathways, mediated by surface adhesion molecules, cytokines and their receptors.

MC deficient mouse had an increased tumor incidence compared with normal and in a study evaluating growth of pulmonary metastases of B16 melanoma, lung metastases were significantly decreased in controls, compared to MC deficient mice. In a murine model of squamous epithelial carcinogenesis, MC in conjunction with neutrophils and other inflammatory cells contribute to tumor growth, in part through the production of the protease matrix metalloproteinase-9 (MMP-9).

MC accumulate at sites of tumor growth in response to numerous chemoattractants, such as tumor-derived peptides, RANTES, or monocyte chemotactic protein (MCP-1). Melanoma cells recruit MC in vivo by producing MC chemotactic and mitogenic factors, such as IL-3. MC secrete histamine that could induce tumor cell proliferation through H1 receptors identified in human malignant carcinoma, whereas MCC induces the accumulation of tumor-associated macrophages, neutrophils, and other inflammatory cells in vivo.

The most aggressive human cancers, namely malignant melanoma, breast carcinoma, and colorectal adenocarcinoma, are associated with a dramatic host response composed of various inflammatory cells, especially MC at the tumor periphery. MC infiltrate hyperplasias, dysplasias, and invasive fronts of carcinomas, where they degranulate in close apposition to capillaries and epithelial basement membranes.

MC increase at sites of breast cancer and in associated lymph nodes in reaction to the tumor and might participate in tumor rejection. Perivascular tumor-associated MC in mammary adenocarcinoma could secrete several cytokines and proteolytic enzymes that could be detrimental to the tumor cells. MC are present in area of intense tumor infiltration, whereas MC in an area of marginal tumor growth appear to be degranulated. Patients with longer survival have a significantly higher number of MC in their axillary lymph nodes.

MC accumulation has also been noted around melanomas, especially invasive melanoma, where MC accumulate around the margin of the malignancies. Among the mediators released by MC, fibroblast growth factor-2 (FGF-2) and IL-8 are directly mitogenic to melanoma cells, whereas certain other mediators including tumor necrosis factor alpha (TNF-α), IL-1, IL-6, and interferon gamma suppress melanoma cell growth.

Among the localized benign tumors of mesenchymal origin, increased number of MC have been encountered in Schwannomas, hemangiomas, and dermatofibromas. MC have been noted around peripheral nerves and nerve tumors, and an increased number of MC has been reported around neurofibromatosis type 1 lesions, in which c-kit and SCF have been implicated in MC proliferation.

Mastocytosis is characterized by abnormal growth and accumulation of MC in one or multiple organs. Two groups of patients can be distinguished, namely patients with cutaneous mastocytosis (CM) and those with systemic mastocytosis (SM). Usually, CM manifests in childhood and presents as urticaria pigmentosa. The clinical course of CM is benign and in many cases skin lesions disappear during puberty. SM is usually diagnosed in adulthood and is characterized by multiorgan involvement and disease persistence. Indolent

variants as well as aggressive variants (aggressive systemic mastocytosis) have been reported. MC leukemia (MCL) is a rare subtype of SM defined by circulating MC and a rapidly deteriorating clinical course in most cases.

MC may support tumor cell expansion in Waldenstrom's macroglobulinemia through constitutive CD154–CD40 signaling and therefore may provide a framework for therapeutic targeting of MC.

MC and Tumor Angiogenesis

Tumors are endowed with an angiogenic capability and their growth, invasion and metastasis are angiogenesis-dependent. After the initial transformation and growth of cells, vascularization must occur if a tumor mass is to exceed 1 mm^3 in diameter. The synthesis and secretion of several proangiogenic factors by tumor and host cells plays a key role in establishing a vascular network from the surrounding stroma.

Several MC-dependent mediators are involved in angiogenesis. Heparin stimulates endothelial cell proliferation and migration in vitro. In vivo, however, it stimulates, inhibits, or has no effect. These differences seem to be related to its molecular size and degree of sulfation. Heparin acts as a soluble form of the low-affinity FGF-2 receptor, which displaces FGF-2 in the biologically active form, and allows its rapid interaction with endothelial cells. Histamine has an angiogenic effect through both H1 and H2 receptors. It may also increase the permeability of newly formed microvessels during tumor angiogenesis, and hence increase the leakage of plasma proteins and deposition of fibrin. Degradation products of fibrin are angiogenic in vivo. MC synthesize and store large amounts of MMP-2, MMP-9, and serine-proteinases of two subclasses: tryptase and chymase. Given the ability of MMP-2 and MMP-9 to degrade type IV, V, VII, and X collagens, as well as fibronectin, namely the major components of the interstitial stroma and subendothelial basement membrane, the findings suggest that MC may contribute to the progression from in situ to invasive and metastatic solid tumors, characterized by an enhanced angiogenesis and secretion of proteolytic enzymes. Tryptase added to microvascular endothelial cells cultured on Matrigel caused a pronounced increase in capillary growth, and this was suppressed by specific tryptase inhibitors. Moreover, tryptase directly induced endothelial cell proliferation in a dose-dependent fashion. MC release several polypeptide growth factors, including FGF-2, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), TNF-α, and IL-8. These cytokines are involved both in normal as well as tumor-associated angiogenesis. The spectrum of cytokines expressed appears to vary depending on the maturity state of the MC and of the tissue of residence. Nerve growth factor (NGF), also contained in MC secretory granules, induces endothelial cell proliferation in vitro and angiogenesis in vivo in the chick embryo chorioallantoic membrane (CAM) assay.

Isolated MC and their secretory granules, but not degranulated MC, induce an angiogenic response in the CAM assay. Addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MC and their secretory granules by 50 and 30%, respectively. These data support the evidence that the angiogenic properties of MC depend on the angiogenic molecules contained in their secretory granules, and indicate that FGF-2 and VEGF are the angiogenic cytokines primarily and perhaps synergistically responsible for this vasoproliferative activity.

Tryptase-positive MC increased in number and vascularization increased in a linear fashion from dysplasia to invasive cancer of the uterine cervix. An association of VEGF and MC with angiogenesis has been demonstrated in laryngeal carcinoma, in small lung carcinoma, where most intratumoral MC express VEGF, and in melanoma, where MC express both VEGF and FGF-2. Lastly, a prognostic significance has been attributed to MC and microvascular density in squamous cell cancer of the esophagus and in melanoma.

Bone marrow angiogenesis, evaluated as microvessel area, and MC counts are highly correlated in patients with inactive and active multiple myeloma (MM) and in those with monoclonal gammopathies of undetermined significance (MGUS). Both parameters increase simultaneously in active MM. A significant correlation has been demonstrated in non Hodgkin's lymphoma (NHL) between vessel count and the number of both MC and VEGF-expressing cells. Double fluorescence staining of VEGF mRNA and MCT revealed that MC expressed VEGF mRNA. In both B-NHL and MM, MC rest near or around blood or lymphatic capillaries. Their ultrastructural picture includes a typical morphological semilunar feature, or "piecemeal" partial degranulation of their secretory granules, unlike the IgE-mediated massive degranulation that occurs during immediate hypersensitivity reactions. This morphology is typical of the slow degranulation that takes place in delayed hypersensitivity reactions and chronic inflammation. Semilunar appearance may reflect slow but progressive release of angiogenic factors favoring chronic and progressive stimulation of MC degranulation.

Bone marrow samples of patients with myelodysplastic syndromes display a high correlation between microvessel counts and both total and tryptase-positive MC, and that both parameters increase simultaneously with tumor progression. There is also a correlation between the extent of angiogenesis and the number of tryptase-positive MC in patients with early B-cell

chronic lymphocytic leukemia and tryptase-positive MC predict their clinical outcome.

Conclusions

Inhibition or destruction of tumor cells has long been the prime goal of cancer therapy. Treatment of these cells and modulation of their microenvironment may prove a better approach. The balance or imbalance of inducers and inhibitors of angiogenesis secreted by inflammatory cells may favor a tumor's progression or regression. The circumstances in which MC are a critical source of angiogenic factors in vivo, and in such cases, what signals regulate their production and secretion are all matters that need to be determined as a prelude to the elaboration of new therapeutic strategies associated with their presence and activation. Finally, a more accurate insight into the role of MC may be obtained from the use of activation markers, along with consideration of local, microenvironmental stimuli in the regulation of their functions.

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Mast Zellen

►Mast Cells

Mastectomy

Definition

Surgical procedure that removes the breast for the treatment of cancer.

►Estrogenic Hormones
►Oncoplastic Surgery

Mastocytosis

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Definition

Mastocytosis, a ►hematopoietic neoplasm, is a clonal disorder of the mast cell and its progenitor resulting in the presence of excessive numbers of mast cells in the skin, bone marrow, and internal organs such as the liver, spleen, and lymph nodes.

Characteristics

Most patients with mastocytosis present with symptoms due to the release of mediators from activated mast cells in various tissues. The activation of mast cells can be caused by triggers such as temperature changes, emotional or physical stress, exercise, ingestion of alcohol, spicy foods, or mast cell degranulating drugs, insect venom, and allergens, but may also occur without an apparent stimulus. Patients with mastocytosis display a wide range of clinical presentations, ranging from no symptoms to severe life-threatening syncopal episodes or hematologic abnormalities. The most common symptoms of mast cell degranulation are itching, flushing, gastrointestinal symptoms such as heartburn and diarrhea, lightheadedness, and palpitations. These symptoms usually occur in well-defined episodes generally lasting from a few minutes to several hours. Other mast cell degranulation symptoms, such as angioedema, hives, and wheezing, are unusual but may be occasionally encountered, especially if the patient has co-existing allergic disease or asthma. Physical examination at the time of a symptomatic mast cell degranulation event may reveal flushing of the skin, conjunctival injection, nasal turbinate edema, hypotension, and tachycardia. Between episodes, the patient may be asymptomatic or may have constitutional symptoms such as fatigue, musculoskeletal pain, and cognitive difficulties.

Approximately 10–20% of patients with mastocytosis will be diagnosed with a non-mast-cell clonal hematologic disorder, and the clinical presentation will reflect that of the associated ►hematologic disorder. Myeloid neoplasms such as chronic myeloproliferative disorders and myelodysplastic syndromes are the most common hematologic disorders encountered in the context of mastocytosis, but ►acute leukemias and ►chronic lymphoproliferative disorders have also been reported.

Although mastocytosis generally follows an indolent course, mast cell infiltration of the bone marrow and internal organs may take an aggressive turn in a small

number of patients. These patients may suffer from bone marrow failure, liver failure with ascites, severe diarrhea and malabsorption, and pathologic bone fractures.

Mastocytosis affects both children and adults. In children, the disease commonly presents in the first year of life as characteristic skin lesions of ►urticaria pigmentosa (UP). These are fixed, hyperpigmented, and vascular maculopapular lesions, generally a few centimeters in diameter, which may urticate when rubbed or irritated (►Darier's sign). Mastocytosis is generally limited to the skin in children, who commonly experience resolution or improvement of symptoms by adolescence. In contrast, pathologic mast cell accumulation occurs in the bone marrow and other extracutaneous sites in most adult patients. Mastocytosis has been observed in patients of all ethnic backgrounds.

A somatic point mutation in codon 816 of the *KIT* gene occurs in more than 90% of patients with mastocytosis. *KIT* gene encodes for KIT, a membrane receptor with intrinsic ►tyrosine kinase activity, which is critically involved in mast cell growth, differentiation, survival, and functional activation, upon binding its ligand ►stem cell factor. In mastocytosis the D816V mutation in KIT results in this tyrosine kinase being constitutively active without the need for stem cell factor. This is thought to be a critical step in the pathogenesis of mastocytosis.

Diagnosis

Cutaneous Disease: Urticaria pigmentosa (UP) is the most common finding upon physical examination in patients with mastocytosis. UP is the initial finding that leads to diagnosis in most patients with childhood-onset disease and is present in up to 80% of patients with adult-onset disease. Other less common forms of cutaneous mastocytosis are mastocytomas and diffuse cutaneous mastocytosis (DCM) in children and ►telangiectasia macularis eruptive perstans (TMEP) in adults. Mastocytomas are benign solid mast cell tumors that can be solitary or occur with UP. Rubbing or physical irritation of the mastocytoma may lead to generalized symptoms such as flushing and itching due to mast cell mediator release. Children with DCM have diffuse involvement of the skin, which may have a coarse and thickened appearance with a yellowish hue. TMEP is a rare form of cutaneous mastocytosis presenting with generalized telangiectatic macules with or without systemic bone marrow involvement. Although experienced physicians may be able to diagnose UP by its characteristic appearance, a skin biopsy is recommended if any doubts exist. If UP is present, the biopsy will show increased mast cells in the upper dermis, particularly around blood vessels, not accompanied by other inflammatory cells. Immunohistochemical staining for ►tryptase is recommended over metachromatic stains to visualize mast cells in tissue biopsy sections.

Systemic Mastocytosis. The pathologic increase of mast cells in extracutaneous sites is termed systemic mastocytosis. Bone marrow, the site of mast cell production, almost always demonstrates pathologic changes in systemic mastocytosis. Examination of the bone marrow in patients with systemic mastocytosis provides valuable information about the extent of the disease and the presence or absence of a non-mast-cell hematologic disorder. It also helps the physician to counsel the patient about prognosis. Therefore, a bone marrow biopsy and aspirate is the diagnostic method of choice in the evaluation of patients with suspected systemic mastocytosis.

The diagnosis of systemic mastocytosis should be based on the World Health Organization's criteria (Table 1). These criteria consider not only the quantitative increase in mast cells but also qualitative abnormalities such as morphologic changes and expression of aberrant surface markers. Serum or plasma tryptase levels generally correlate with mast cell burden and are elevated in most patients with systemic mastocytosis. The normal median tryptase level in the general population is approximately 5 ng/mL, and a tryptase level greater than 20 ng/mL should raise suspicion for the diagnosis of systemic mastocytosis. Tryptase levels may be transiently elevated after acute allergic and anaphylactic reactions, even in the absence of mastocytosis. Other diagnostic evaluations in cases of suspected mastocytosis generally include a complete blood count with differential and liver function tests. Bone densitometry is also recommended as a baseline test for many patients, as mastocytosis may accelerate osteoporosis. Other tests such as gastrointestinal endoscopies, skeletal X-rays, and imaging of the chest and abdomen may be necessary depending on the presenting symptoms.

Classification

Mastocytosis is a hematopoietic neoplasm classified according to the World Health Organization into seven categories on the basis of the site of involvement and aggressiveness of the disease (Table 2).

Therapy

Treatments for mastocytosis can be divided into two broad categories: (i) those intended to control symptoms due to mediators released from mast cells and (ii) those intended to reduce the mast cell burden. Treatment of mastocytosis aimed at controlling mast cell mediator symptoms is offered in all categories of mastocytosis. Mast cell cytoreductive therapies are generally reserved for patients with aggressive systemic mastocytosis (ASM), mast-cell leukemia (MCL), and mast-cell sarcoma (MCS). However, none of the current

Mastocytosis. Table 1 WHO diagnostic criteria for systemic mastocytosis*

Major criterion	Demonstration of multifocal aggregates of 15 or more mast cells in bone marrow or another extracutaneous site by a mast cell stain such as tryptase or CD117 immunohistochemistry
Minor criteria	Serum tryptase level greater than 20 ng/mL (not applicable if the patient has a non-mast-cell clonal myeloid disease)
	Surface expression of CD25 and/or CD2 on mast cells, as detected by flow cytometry or immunohistochemistry
	Morphologic aberrancy, such as hypogranulation, elongation (spindle-shape), cytoplasmic projections, and off-centered or lobulated nucleus, in greater than 25% of mast cells in tissue sections or bone marrow aspirate smears
	Presence of a codon 816 point mutation in the *KIT* gene in bone marrow, peripheral blood, or other lesional tissue specimens

* Either the major criterion + 1 minor criteria or 3 minor criteria are needed to establish the diagnosis

Mastocytosis. Table 2 Diagnostic features and prognosis of mastocytosis

Category	Diagnostic features	Prognosis
Cutaneous mastocytosis	Lack of systemic (bone marrow) involvement. Most common category in children, rare in adults	Good. Resolves or improves by adolescence in most children
Indolent systemic mastocytosis (ISM)	Benign, multifocal collections of mast cells in bone marrow. Symptoms of mast cell mediator release. Lack of organ dysfunction due to mast cell infiltration. Most common category in adult-onset disease	Good. Inadequate control of mast cell mediator symptoms may lead to poor quality of life
Systemic mastocytosis with associated clonal hematological non-mast-cell-lineage disease (SM-AHNMD)	Commonly associated with myelodysplastic or myeloproliferative disorders; occasionally seen with acute leukemias and lymphomas	Poor. Determined by the progression of non-mast-cell hematologic disorder
Aggressive systemic mastocytosis (ASM)	Findings of end-organ dysfunction due to mast-cell infiltration, such as bone marrow failure, liver dysfunction with ascites, splenomegaly, skeletal osteolytes with pathologic fractures, gastrointestinal involvement with malabsorption, and weight loss	Poor. Average survival 3–5 years
Mast-cell leukemia (MCL)	Mast cells with high-grade morphology (multilobular or multiple nuclei), >10% mast cells in peripheral blood, or >20% mast cells in bone marrow aspirate smears. Very rare	Poor. Average survival 3–9 months
Mast-cell sarcoma (MCS)	Local destructive (sarcoma-like) growth of a tumor consisting of highly atypical immature mast cells, similar in morphology to those seen in MCL. Very rare	Poor. Average survival 3–9 months
Extracutaneous mastocytoma	Benign extramedullary mast cell tumor in extracutaneous tissue such as lungs. Mast cells are mature with non-aggressive growth pattern. Very rare	Good

cytoreductive approaches has resulted in the cure of mastocytosis.

Because there is no therapy proven to cure mast cell disease, symptom control is an important aspect of the treatment strategy. Symptom control may be accomplished through the use of anti-histamines (H1 and H2 receptor blockers), leukotriene antagonists, and mast cell stabilizers such as sodium cromoglycate.

Patients with malabsorption, ascites, or recurrent anaphylactoid (syncopal) episodes are generally managed with glucocorticoids. Calcium and vitamin D supplementation and bisphosphonates are useful in the treatment of patients with osteoporosis. Self-injectable epinephrine should be prescribed to patients with life-threatening episodes of generalized mast cell mediator release resulting in cardiovascular collapse or respiratory compromise. The avoidance of known triggers of mast cell mediator release is an important non-medical management strategy. The potential of certain medications to stimulate mast cell mediator release should be considered in the overall medical management of the patient. These medications include, but are not limited to, opioid analgesics, non-steroidal anti-inflammatory drugs, muscle relaxants, general anesthetics, and medications that were not tolerated by the patient in the past. Despite symptomatic treatment with available anti-mediator drugs, some patients continue to have a poor quality of life, probably because of the drugs' inability to block all mast cell mediators.

Patients with ASM, MCL, and MCS and selected patients with indolent systemic mastocytosis (ISM) who have a high mast cell burden and/or poor quality of life may be candidates for several approaches that aim to control the mast cell burden. ►Interferon-alpha (IFN-α) has been the subject of most of the research in this area. Due to rarity of the disease, however, most of the reports on the use of IFN-α (with or without prednisolone) are limited in the number of patients reported. For example, a recent review of the literature identified 14 well-documented patients with aggressive mast cell disease treated with IFN-α (with or without prednisolone); three (21%) had a major response, with dissolution of signs and symptoms of the disease, and five patients (36%) had a partial response. ►Clinical trial experience with IFN-α is less favorable, however. The largest phase II trial of IFN-α in 20 adult patients with systemic mastocytosis resulted in seven (35%) partial responses and no major response. Although given primarily to patients with aggressive forms of mastocytosis, IFN-α may also be considered in patients with ISM when they present with severe osteoporosis or when symptomatic therapy fails to control anaphylactic episodes. Patients with ISM who have a high mast cell burden and progressive disease (i.e., involvement of the organs but with preserved organ function) may also benefit from biological cytoreductive therapy with IFN-α.

Patients with systemic mastocytosis with associated clonal hematological non-mast-cell-lineage disease (SM-AHNMD) may have an indolent or aggressive mast cell disease component independent of the AHNMD, and therapy depends on the severity of the individual disorders. In most cases, treatment of the latter usually takes precedence and should follow standard guidelines for the associated hematologic disease. When the SM component is clinically more prominent, therapy with IFN-α (with or without the addition of corticosteroids) or chemotherapy can be beneficial. Patients with ASM have poor quality of life and shortened life expectancy and are therefore treated with cytoreductive therapy (usually IFN-α ± corticosteroids as first-line therapy). Recent experience suggests that there exists a subpopulation of patients with ASM who respond to 2-chlorodeoxyadenosine (►cladribine; 2-CDA). One report of four patients with ASM treated with cladribine noted a major response in 2 patients and a partial response in a third patient. Another report documented improvement in symptoms (partial response) in 10 of 10 patients treated with cladribine, although no major responses were observed. Some patients with ASM may benefit from hematopoietic stem cell transplantation. Patients with MCL traditionally have been treated with polychemotherapy regimens similar to those employed in patients with acute myeloid leukemia. While these modalities can render significant mast cell reduction and symptom control in some patients, responses are transient and the prognosis remains very poor.

The discovery of activating point mutations near the activation loop of the KIT tyrosine kinase domain has driven recent investigative efforts to identify suitable drugs that inhibit this target. The therapeutic potential of many target-specific small molecules in systemic mastocytosis, including ►imatinib, dasatinib, and ►nilotinib, is currently being evaluated in clinical trials. The first such medication to be tried in patients with systemic mastocytosis was imatinib mesylate. Imatinib is a potent competitive inhibitor of Abl, ►platelet derived growth factor receptor (PDGFR), and KIT tyrosine kinases and is the standard first-line therapy for chronic myeloid leukemia associated with the ►Bcr-Abl oncoprotein. Because of its inhibitory effect on wild-type (non-mutated) KIT, some investigators have utilized imatinib mesylate in patients with systemic mastocytosis, and responses were seen in selected patients. Unfortunately, the mutated D816V KIT found in the great majority of systemic mastocytosis patients is not sensitive to imatinib, as shown by in vitro laboratory studies. Therefore, the initial high expectations for imatinib treatment have diminished significantly. A review of all experience with imatinib confirmed, however, its potential to improve signs and symptoms of the disease in some patients. This led the US Food and Drug Administration to approve imatinib in the USA, as therapy for adult patients with ASM without the D816V KIT mutation or with unknown KIT mutational status. Another indication for therapy with imatinib is systemic mastocytosis associated with chronic eosinophilic leukemia characterized by the presence of the FIP1L1-PDGFRα

►fusion gene. Approximately 20% of cases of systemic mastocytosis present with persistent peripheral blood eosinophilia, and clonality can be demonstrated in a significant proportion of them. Some of these cases express the imatinib-sensitive FIP1L1-PDGFRα oncogene, which results from an interstitial deletion of chromosome 4q12, leading to the constitutive activation of the PDGFRα tyrosine kinase. For this subset of patients, imatinib must be considered first-line therapy, as it eliminates the disease in the great majority of cases. Other targets for therapy that are involved in neoplastic mast cell survival and activation are currently being investigated.

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Matricellular Proteins

Definition

Introduced in 1995 by Paul Bornstein, the term refers to a group of soluble, non-structural ►extracellular matrix (ECM) proteins, that regulate the interaction of cells with their environment by binding and modulating the function of matrix proteins, growth factors and cell surface receptors.

►SPARC

Matrigel

Definition

Is a basement membrane extract that includes many ►extracellular matrix components such as laminin, type IV collagen, proteoglygans and growth factors. It promotes tissue like differentiation in many cell types. Matrigel is a mixture of proteins of the extracellular matrix extracted from a mouse sarcoma cell line. Matrigel provides a physiological environment to cells grown in vitro.

►Three-Dimensional Tissue Cultures

Matriptase

Definition

Is an epithelial-derived, type 2, plasma membrane spanning serine protease. Is an endopeptidase that hydrolyses peptide bonds after arginine or lysine residues. These cleavage reactions, which take place on the cell surface, activate specific growth factors and ►G-protein-coupled receptors. ►Serine proteases (Type II) spanning the plasma membrane; ►TMPRSS2/ERG Fusions.

►TMPRSS2

Matriptase: Epithin, MT-SP1, suppression of tumorigenicity 14

►Serine Proteases (Type II) Spanning the Plasma Membrane

Matrix-Assisted Laser Desorption/Ionization

Definition

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (such as proteins, peptides, and carbohydrates) and large organic molecules (such as polymer, dendrimer, and other macromolecules). It is based on the usage of matrix complex with a given sample molecule that is bombarded with a laser in order for the sample molecule to form a sample

ionization. A matrix is used to protect the biomolecules from being destroyed by direct laser beam and to facilitate vaporization and ionization. Most commercially available MALDI mass spectrometers are pulsed nitrogen laser. This technique is normally coupled with a time-of-flight detector in order to obtain proper mass-to-charge ratios and generate a mass spectrum.

►Proteinchip

Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF)

Definition

One of the "soft ionization" methods used in mass spectrometry, leading to the generation of singly charged ions formed by protonation of the basic residues such as the side chains of arginine, lysine, histidine and the free α-amino group. MALDI-TOF technique is useful for the analysis of peptides, proteins, or polymers. This technique is not suitable for the measurement of small molecules (<500 Da), however is extraordinarily tolerant to the presence of non-volatile buffer components and detergents.

►Surface Plasmon Resonance

Matrix Metalloproteinase

A zinc-dependent endopeptidase that cleaves extracellular matrix proteins, cell surface receptors and other biomolecules.

►Genistein

Matrix Metalloproteinase 2

Definition

MMP2 is a metalloproteinase (►Matrix metalloproteinases) that cleaves type IV collagen, a component of the basement membrane, and has been implicated in tumor cell invasion and ►metastasis.

►Gelatinase
►Collagenase Type IV
►Securin

Matrix Metalloproteinase 3

►Stromelysin-1

Matrix Metalloproteinase-7, -9

Definition

MMP-7, or -9; Proteolytic activity is important for tumor invasion, tissue remodeling, and angiogenesis. Latent precursors have to be proteolytically cleaved to produce the active form. Can exist in soluble or bound form (e.g., to ►CD44). Potent tissue inhibitors of MMPs (TIMP) can block their activity.

►CD44

Matrix Metalloproteinase Inhibitor

►RECK Glycoprotein

Matrix Metalloproteinases

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Synonyms

Matrixins; MMPs

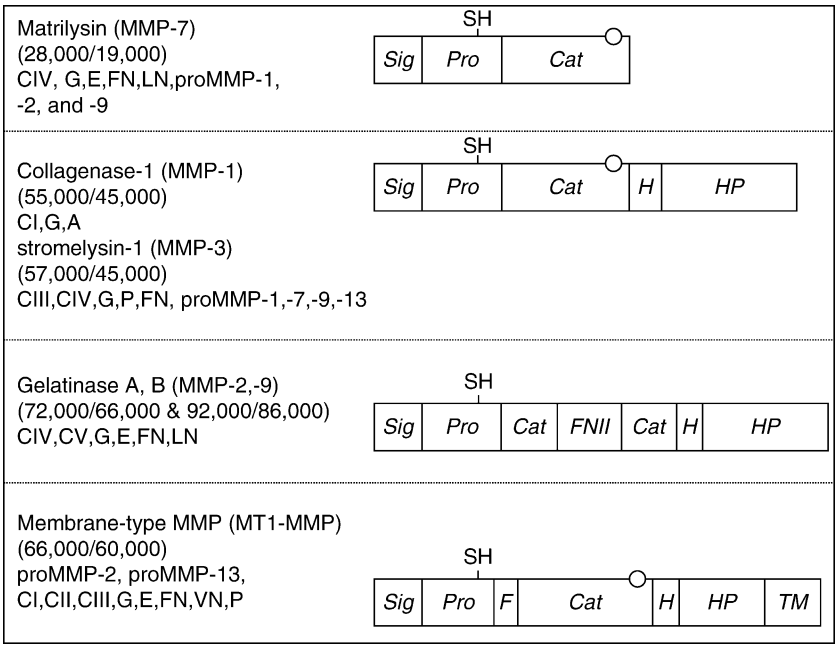
Definition

The matrix metalloproteinases (MMPs), or matrixins, are a family of zinc-dependent metalloendopeptidases that are widely expressed. They cleave a variety of extracellular substrates including, but not limited to, protein and proteoglycan components of the extracellular matrix (ECM). MMPs belong to the metzincin family of endopeptidases, characterized by the presence of three zinc-binding histidine residues within the active site (HexxHxxGxxH). MMPs are believed to catalyze localized hydrolysis of extracellular matrix proteins including collagens, fibronectin, laminins and proteoglycans, thereby modifying the integrity of the connective tissue.

Characteristics

Domain Structure

MMPs are synthesized as prepro-enzymes and, following removal of the signal peptide, are generally secreted in proenzyme or zymogen form. The latency of the zymogen is maintained by the presence of an unpaired cysteine residue in the propeptide domain (Fig. 1), contained within the conserved sequence PRCG(V/N)PD. This cysteine residue ligates the catalytically essential zinc atom in the enzyme active site, thereby preventing enzymatic activity. Exceptions exist including stromelysin 3 (MMP-11) and membrane-type MMPs (MT-MMPs), which contain a conserved RX(K/R)R sequence within the propeptide and can be activated intracellularly by proprotein processing enzymes of the PACE family, such as furin. In addition to the catalytic zinc atom described above, an additional zinc and two calcium ions provide structural stability. Inserted into the active site of the gelatinases (MMP-2 [gelatinase A] and MMP-9 [gelatinase B]) are a series of three fibronectin type II repeats that function in substrate recognition. The catalytic domain is followed by a short connecting (hinge) region and, with the exception of MMP-7 (matrilysin), a hemopexin-like domain. The role of the hemopexin-like domain is the subject of current investigation, and recent studies have demonstrated that this region participates in cell surface association (MMP-2) and collagen recognition and cleavage (MMP-1). The MT-MMPs also contain an hydrophobic transmembrane sequence and a short (20 amino acids) cytoplasmic tail. Like the hemopexin domain, the cytoplasmic tail of MT-MMPs is also currently under active investigation to evaluate the potential for association with other cytoplasmic proteins that may regulate MT-MMP localization or



Matrix Metalloproteinases. Figure 1 Domain structure of selected tumor-associated MMPs. The common name and MMP number are listed, followed by the approximate molecular weight of the latent and active species, respectively. Reported substrates are summarized as follows: CI, CII, CIII, CIV, CV – collagen types I, II, III, IV, and V, respectively; G – gelatin; E – elastin; FN – fibronectin; LN – laminin-1; A – aggrecan; P – proteoglycan; VN – vitronectin. Structural/functional domains are denoted as follows: Sig – signal peptide; pro- propeptide domain containing unpaired Cys residue denoted by -SH; Cat – catalytic domain containing the zinc binding consensus sequence that chelates the catalytically essential zinc atom (circle); H – hinge region; HP – hemopexin-like domain; FNII – fibronectin type II-like repeats; F – furin cleavage site; TM – transmembrane and cytoplasmic domain.

function. The domain structures of the MMPs most widely expressed in tumor tissues are summarized in Fig. 1.

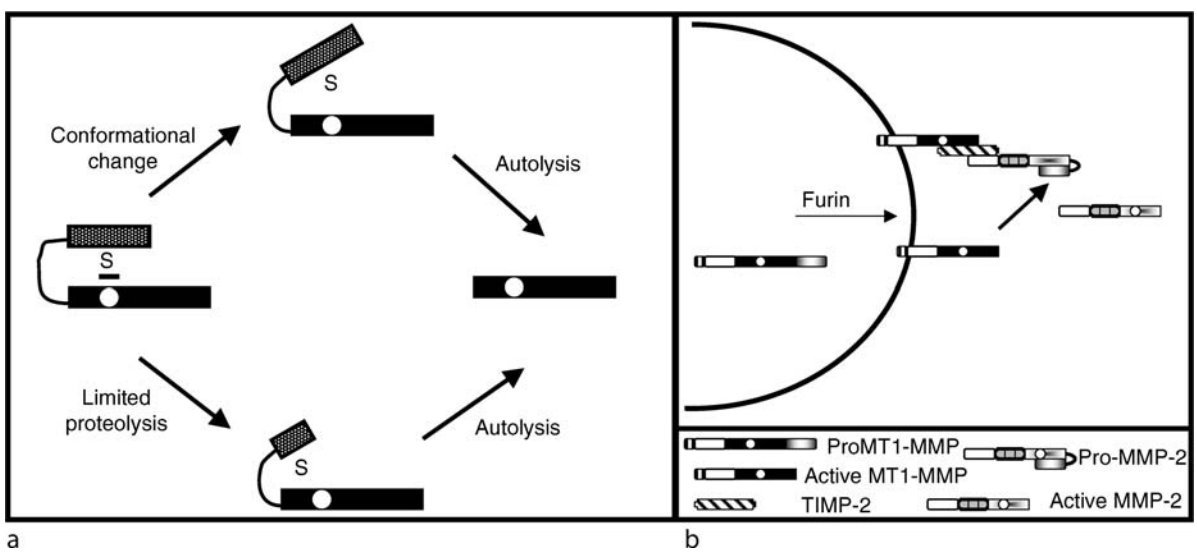
Zymogen Activation

In addition to regulation of MMP gene expression by a number of growth factors and cytokines, the activity of MMPs is stringently regulated by post-translational mechanisms, predominantly zymogen activation and enzyme/inhibitor binding. Proteolytic activation of proMMP zymogens functions primarily via a cysteine switch mechanism (Fig. 2a) and involves cleavage of the propeptide, thus destabilizing the cysteine-zinc interaction and generating catalytically active enzyme. The initial cleavage in many MMP propeptides can be carried out by proteinases of other mechanistic classes, predominantly serine proteinases such as plasmin. Following the initial proteolytic event, the partially active enzymes often undergo further inter- or intramolecular cleavages, resulting in complete removal of the prodomain and generation of fully active enzyme. Proteinase activity is frequently regulated by zymogen activation cascades, such that an initial event will generate an active proteinase that processes a downstream zymogen (Fig. 3). Many studies suggest that coupling of serine and MMP zymogen activation pathways may function to promote pericellular proteolysis during tumor invasion and metastasis.

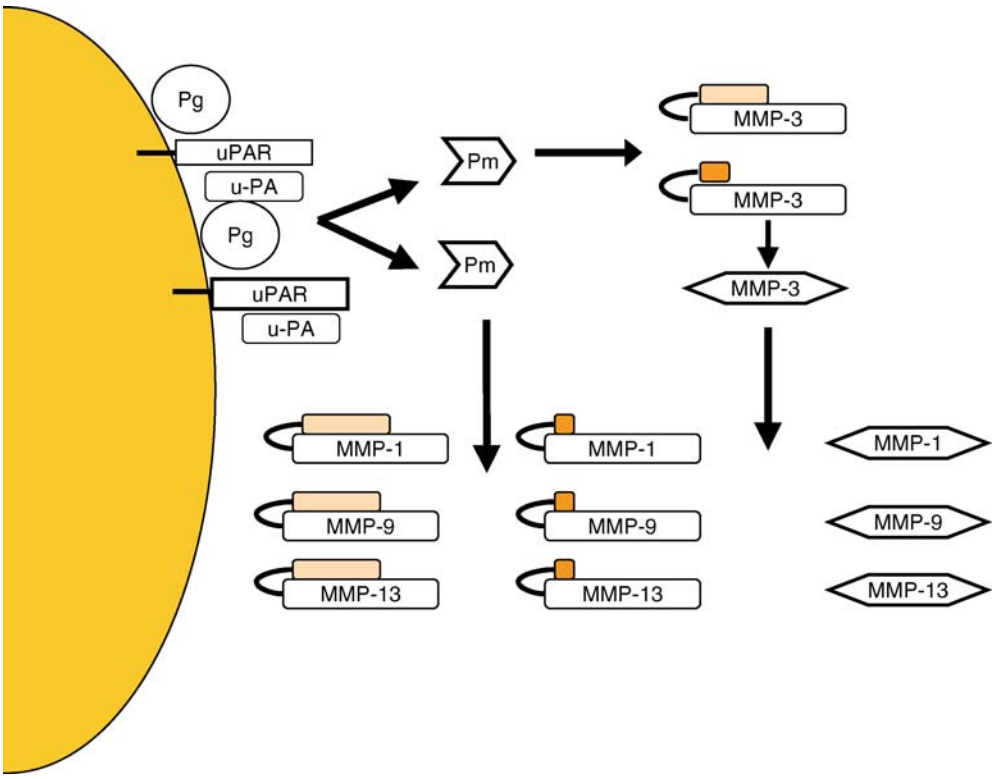
proMMP-2 (gelatinase A) is an exception to serine proteinase activation of proMMPs. Detailed biochemical studies from a number of laboratories have demonstrated that proMMP-2 is activated on the surface of many neoplastic cells following formation of a trimeric complex containing the transmembrane proteinase MT1-MMP and a molecule of tissue inhibitor of metalloproteinases 2 (TIMP-2). The MT1-MMP/TIMP-2 complex forms a binding site for proMMP-2, which is then proteolytically processed by a second MT1-MMP molecule (Fig. 2b). This is an interesting example of a reaction in which a proteinase inhibitor (TIMP-2) is also required for zymogen activation. Activation of proMT1-MMP is believed to occur intracellularly, as the zymogen contains a dibasic recognition motif (Arg108-Arg-Lys-Arg) in the propeptide region that may be cleaved by proprotein processing enzymes including the PACE family serine proteinase furin.

Inhibition

Aside from zymogen activation, a predominant mechanism for post-translational control of MMP enzymatic



Matrix Metalloproteinases. Figure 2 Activation of proMMPs. (a) Cysteine switch mechanism for proMMP activation. The latency of MMP zymogens is maintained by an interaction between an unpaired cysteine residue in the propeptide region (S) and the catalytically essential zinc atom (circle). Following conformational perturbation via the action of agents such as SDS, the Cys-Zn2+ interaction is disrupted, leading to autolytic activation characterized by propeptide cleavage. Alternatively, limited proteolysis of the propeptide initiated by serine or metalloproteinases may result in partial propeptide processing and disruption of the Cys-Zn2+ interaction, followed by autolytic processing to generate the fully active specie. (b) Cell surface activation of proMMP-2 by MT1-MMP. ProMT1-MMP is processed intracellularly by proprotein convertases, such as furin, and active enzyme is inserted in the plasma membrane. A ternary "activation complex" form, comprised of MT1-MMP/TIMP-2/proMMP-2. ProMMP-2 in the "activation complex" is proteolytically processed by a neighboring MT1-MMP molecule, followed by additional autolytic activation and release of the fully active proteinase.



Matrix Metalloproteinases. Figure 3 Cell surface-initiated zymogen activation cascade. Interaction of the serine proteinase uPA with its cell surface glycosyl phosphatidyl inositol-anchored receptor (uPAR) initiates activation of cell-associated plasminogen (Pg), forming the broad-spectrum serine proteinase plasmin (Pm). Plasmin can initiate propeptide processing of MMP-1, -3, -9, or -13. This is followed by additional autolytic cleavages or MMP-3 processing events, leading to the generation of fully activated MMPs.

activity is via interaction with protein inhibitors. Tissue inhibitors of metalloproteinases (TIMPs) inhibit MMP activity via the formation of a tight, non-covalent enzyme/inhibitor complex in a 1:1 molar ratio. Four TIMPs (TIMPs 1–4) have been identified, although the function of TIMP-1 and TIMP-2 are the most well characterized. Recent studies have demonstrated that the amino terminal cysteine residue in TIMPs is required for inhibitory activity, functioning by coordinating the catalytically essential zinc ion. Additional contact sites have been described that may interfere with substrate binding or other proteinase functions, suggesting that the inhibitor presents an extended contact surface to the enzyme. In addition to TIMPs, MMPs are also inhibited by the non-specific plasma proteinase inhibitor α-2-macroglobulin. Substantial research efforts have also been directed toward generation of synthetic MMP inhibitors to prevent pathologic proteolysis prevalent in diseases such as cancer and arthritis. These compounds contain a zinc binding functionality such as an hydroxamic acid group coupled with a peptide or peptidomimetic sequence designed to target the inhibitor to the enzyme active site. Most synthetic inhibitors are broad spectrum, blocking the activity of a wide range of MMPs. However, a current alternative strategy in inhibitor design is the generation of more specific compounds that target only a specific subset of MMPs (for example, gelatinase inhibitors that do no alter collagenase function).

Substrate Cleavage

Although it is widely believed that MMPs function in vivo to process extracellular matrix macromolecules, the precise substrates for the majority of MMPs remain unclear. In vitro experiments have demonstrated a wide range of substrates for MMPs including native and denatured collagens, adhesive glycoproteins such as fibronectin, laminins, and vitronectin, and proteoglycans. Substrate gel electrophoresis, or zymography, is a commonly utilized method to evaluate relative MMP activity levels in tissue extracts or tumor cell conditioned media. Although not quantitative, this method provides a rapid and sensitive initial evaluation of cellular MMP profiles. However, data obtained using zymography are frequently misinterpreted as

- MMP zymogens attain proteolytic activity in the presence of sodium dodecyl sulfate without propeptide cleavage;
- MMP/TIMP complexes are non-covalent and are thus dissociated during electrophoresis, leading to the potential for over-estimation of enzymatic activity.

Preliminary results obtained using zymography should be confirmed by other methods such as those based on solution interaction of the MMP and its potential target substrate such as collagen, gelatin or other matrix macromolecules. Cleavage may then be assessed by electrophoretic examination of reaction products or other methods. Alternatively, a number of quenched fluorescent peptide MMP substrates have been described that have broad utility for continuous kinetic monitoring of peptidase activity. These synthetic substrates have proven useful for comparative evaluation of synthetic MMP inhibitors. A more complex approach for elucidation of MMP substrates in vivo involves generation of mice genetically engineered for a specific MMP deficiency by targeted gene inactivation (MMP "knockouts"). The majority of these animals display relatively mild phenotypes, with the exception of animals deficient in MT1-MMP expression. These mice display severe connective tissue abnormalities characterized by dwarfism, osteopenia and arthritis, suggesting that MT1-MMP is required for pericellular collagenolysis in vivo.

Control of MMP Gene Expression

MMP gene expression is tightly regulated and transcription of many MMP genes is inducible by a wide variety of effectors including hormones, growth factors, cytokines and tumor promoters. In addition to soluble mediators, recent studies have demonstrated changes in MMP expression or activity associated with physical factors such as alterations in cell/cell or cell/matrix contact, or changes in cell shape associated with conditions such as physical stress that induce cytoskeletal rearrangement.

Clinical Relevance

MMPs have been implicated in a wide variety of pathologic processes including arthritis, cardiovascular disease, periodontal disease, emphysema and cancer. In tumor tissues, enhanced or de novo expression of MMPs is often correlated with disease progression. Conversely, experimental manipulation of TIMP levels has been shown to decrease tumor invasion and metastasis in many experimental models. MMP production in malignant tissues is not necessarily limited to neoplastic cells, as several studies have demonstrated that tumor cells can "recruit" stromally produced enzymes to promote pericellular proteolysis. Furthermore, MMPs have also been implicated in tumor-associated angiogenesis, suggesting an additional mechanism whereby MMPs can contribute to tumor progression. Therapeutic administration of MMP inhibitors is currently under investigation as a potential strategy to prevent tumor invasion and metastasis, and several synthetic MMP inhibitors have demonstrated efficacy in preclinical models of human cancers including colon, breast, and lung carcinoma and melanoma. The observed inhibition of tumor growth may be due in part to inhibition of tumor angiogenesis. In human trials, a decrease in the rate of rise in cancer antigens was observed in patients with prostate, ovarian, colorectal and pancreatic cancer. However treatment was associated with musculoskeletal pain and inflammation. Additional studies are necessary using MMP inhibitors alone or in combination with cytotoxic chemotherapies to assess the efficacy of these compounds in inhibition of tumor growth, metastasis and angiogenesis.

►Tissue Inhibitors of Metalloproteinases (Timps)

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Matrixins

►Matrix Metalloproteinases

Maturation Promoting Factor

►Cyclin Dependent Kinases

Mature B-cell Tumors

Definition

In the scheme of Revised European-American Classification of Lymphoid Neoplasm (REAL), ►B-cell tumors are divided into those arising from immature cells and those representing more mature B-cell phenotype. The latter group consists of 11 subcategories, most of which are included in the more widely used clinical term, non-Hodgkin lymphoma (NHL). Immunoglobulin genes of the neoplastic cells are rearranged, and the cells express 'pan-B' markers as well as immunoglobulin molecules on their cell surface. Oncogene rearrangement, clinical features and response to treatment vary among the subcategories.

►BCL6 Translocations in B-cell Tumors
►Hodgkin Disease

Max

Definition

Myc-associated protein X; Is a transcription factor of the Myc/Max/Mad(Mxd) network of basic region-helix-loop-helix-leucine zipper proteins. Max is ubiquitously expressed throughout the cell cycle and is required for the DNA binding and activity of additional network members. In addition to heterodimer formation with Myc, Mnt, and Mxd, Max can also form homodimers with an unclear physiological role, as well as heterodimers with the related protein Mga.

►*Myc* Oncogene

Maximum Tolerable Dose

Definition

MTD; The highest quantity of a drug, typically determined in a phase I clinical trial, that can be administered without causing unacceptable harm to patients. The MTD has traditionally been used to design phase II and phase III clinical cancer trials based on the assumption that the adverse toxicities arising from the effect of the drug(s) on normal rapidly dividing cells are related to the cytotoxic effect of the drug(s) on cancerous cells.

►Drug Design
►Preclinical Testing

Maximum Tolerated Dose (MTD)

Definition

This is defined as the maximum dose that does not cause mortality or significant loss of homeostasis in the test species.

MCA

Definition

3-Methylcholanthrene; Polycyclic aromatic carcinogen compound often used to study the effects of anticancer compounds in experimental cancer studies.

►Lunasin

MCC

Definition

Mutated in colon cancer.

Mch5

►Caspase-8

MCJ

►Methylation-Controlled J Protein

MCL

►Mantle Cell Lymphoma

Mcl-1

Definition

Myeloid cell leukemia-1 (Mcl-1) protein is an anti-apoptotic member of the ►BCL-2 family.

►Inflammation
►PUMA
►Signal Transducers and Activators of Transcription in Oncogenesis
►Mcl Family

Mcl Family

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Synonyms

Mcl-1, Myeloid cell leukemia sequence 1

Definition

Mcl family is a subfamily of the ►Bcl-2 (B-cell leukemia/lymphoma 2) family proteins involved in the regulation of ►apoptosis (programmed cell death), as represented by Mcl-1 (homo sapience myeloid cell leukemia sequence 1).

Characteristics

Mcl-1, a member of the Bcl-2 family proteins (where Bcl-2-family proteins include both anti-apoptotic and pro-apoptotic members) was originally cloned as an early response gene up-regulated in the ML-1 human myeloblastic leukemia cell line following addition of cytokines. It is a mitochondrial protein of 350 amino acids (its molecular weight is 42/40 kDa), and the gene maps to chromosome 1, q21.2.

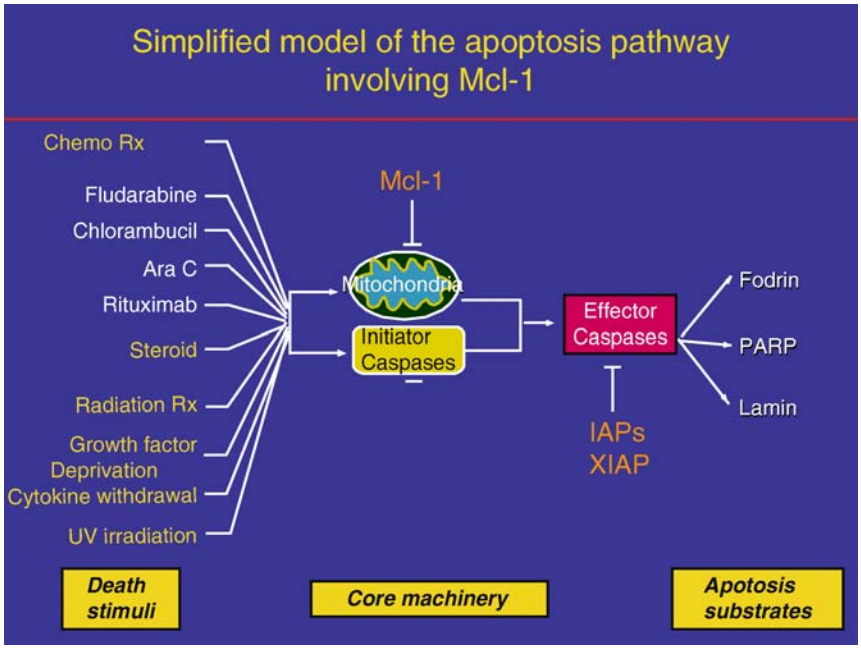
Mcl-1 contains BH-1 (Bcl-2 homology domain-1), BH-2, ►BH-3 (one helix only) but not BH-4. Mcl-1 preferentially ►heterodimerizes with Bak (a pro-apoptotic member of Bcl-2 family proteins) via ►BH-3 binding pocket which represents hydrophobic grooves, in contrast to Bcl-2 which preferentially heterodimerizes with Bax. The BH3 binding pocket is a crucial domain for functions of Mcl-1 and Bcl-2.

Imbalances in the levels or activities of these proteins are commonly associated with malignancy, including lymphocytic malignancies such as non-Hodgkin's lymphomas where the founding member of the family, Bcl-2, was first discovered nearly two decades ago by virtue of involvement of its encoding gene in chromosomal translocations, including ►t(14:18) translocations. Analogously, transgenic mice over-expressing Mcl-1 protein in lymphocytes frequently develop lymphomas, attesting to the in vivo importance of this anti-apoptotic protein for B-cell neoplasia.

The carboxyl regions of Mcl-1 and Bcl-2 share significant sequence homology, as is the case for other members of the Bcl-2 family. Like Bcl-2, Mcl-1 elicits and enhances cell viability under apoptotic conditions. Unlike Bcl-2, Mcl-1 is very labile (its half-life = 2 h), presumably because of its ►PEST sequences that target it for rapid degradation. In addition, Mcl-1 resembles Bcl-2 in that both proteins are found in the outer-membrane of mitochondria. Differentiated cells lose their proliferative capacity, but remain viable and capable of carrying out normal physiological functions. Physiologically, Mcl-1 is important in keeping cells viable during the early induction of differentiation.

Regulation

A simplified model of cell survival and cell death pathways, involving Mcl-1, is presented in Fig. 1. Cell numbers in the body are governed not only by cell division, which determines the rate of cell production, but also by cell death, which sets the rate of cell loss. Normally, these two processes of cell division and cell death are tightly coupled so that no net increase in cell numbers occurs. However, alterations in the expression or function of genes that control programmed cell death (PCD) can upset this delicate balance, contributing to the expansion of neoplastic cells. As shown in Fig. 1, Mcl-1 is located in the outer membrane of mitochondria and inhibits apoptotic process in core machinery of PCD, involving mitochondria. ►CLL (chronic lymphocytic leukemia) (B-CLL) is one of representative diseases caused by defect in programmed cell death primarily as a result of over-expression of Bcl-2. As shown in Fig. 1, chemotherapeutic agents, such as ►fludarabine, chlorambucil, cytosine arabinoside (ara C), ►Rituximab (chimeric anti-CD20 monoclonal antibody), ►Campath-1H, ►Hu1D10 and steroid as well as radiation therapy are believed to induce programmed cell death via mitochondrial pathway, and Mcl-1 inhibits this ►core machinery of programmed cell death. Therefore,



Mcl Family. Figure 1 Simplified model of the apoptosis pathway involving Mcl-1.

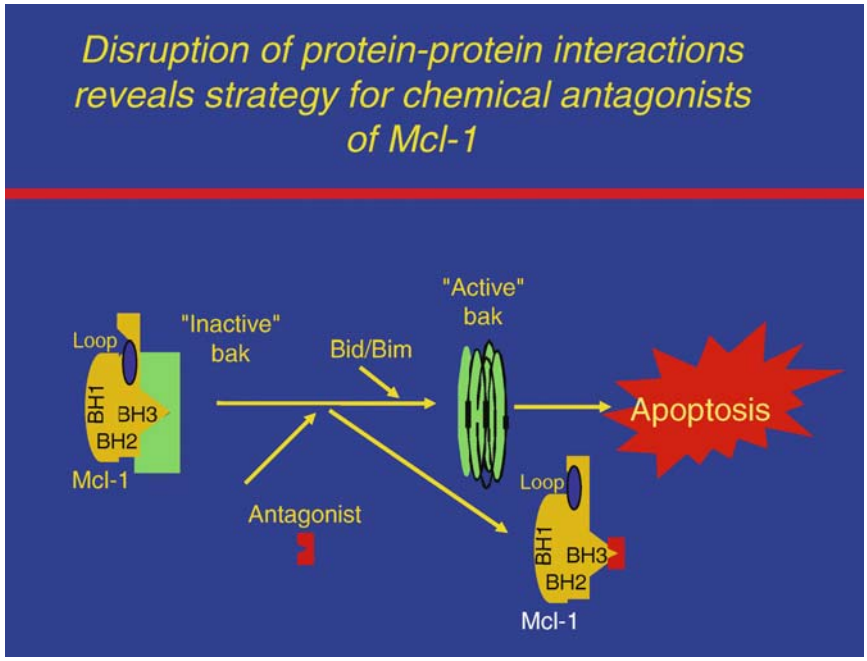
over-expression of Mcl-1 accounts for chemoresistance commonly seen in patients with CLL and AML (acute myeloblastic leukemia), at least in part. Under physiological conditions, Mcl-1 is regulated at multiple levels. In many hematopoietic cells, growth factors promote cell survival by triggering Mcl-1 transcription and stabilizing Mcl-1 protein. Conversely, cytokine withdrawal and other stress signals such as UV-irradiation initiate cell death by promoting Mcl-1 degradation.

Clinical Relevance

Previous studies documented high levels of anti-apoptotic Bcl-2 relative to pro-apoptotic Bax protein in CLL, with higher Bcl-2:Bax ratios often correlating with aggressive disease or poor response to therapy. The mechanisms responsible for the high levels of Bcl-2 in CLL are poorly understood, but gene hypomethylation has been reported. Occasionally chromosomal translocations activate the Bcl-2 gene in CLL, but these are relevant to only a few percent of cases. Thus, the reasons for elevated Bcl-2 expression in CLL are incompletely resolved. Also, Bcl-2:Bax ratios are insufficient to explain differences among CLL patients with respect to progression and chemoresponses.

Searches for other prognostic determinants of outcome and response have uncovered Mcl-1 as a candidate predictor of more aggressive disease. Roughly one-half of CLLs contain higher levels of Mcl-1 protein, as measured by immunoblotting using peripheral blood B-cells. Though cohort sizes are small, correlations have been noted for higher Mcl-1 and failure to achieve complete remission (CR) following single agent chemotherapy (either fludarabine or chlorambucil). In fact, in two independent clinical studies conducted in North America, not a single patient with higher Mcl-1 protein achieved complete remission. Thus, while larger cohorts of patients must be analyzed before firm conclusions are reached, higher Mcl-1 protein may represent an indicator of adverse outcome for patients with CLL. Interestingly, comparisons of matched pairs of untreated and relapsed specimens from acute leukemia patients have revealed elevations in Mcl-1 during progression to chemorefractory disease, further supporting a role for this anti-apoptotic protein in chemoresistance.

The unique requirement for Mcl-1 for survival of lymphoid cells in vivo suggests it might make a good target for treatment of lymphoid malignancies such as CLL and B-lymphoma. Strategies for targeting Mcl-1 include reducing its expression using antisense oligonucleotides recognizing its mRNA, kinase inhibitors such as flavopiridol and therapeutic monoclonal antibodies such as anti-CD20 (Rituximab), which have been shown to interfere with signaling events necessary for maintaining expression of Mcl-1 protein in CLL, and small-molecule compounds that mimic the inhibitory BH3 domain of endogenous Mcl-1 antagonists. Currently, a number of investigators in academia and industries are making efforts to develop small molecule inhibitors targeted on the BH3 binding pocket of Mcl-1 and other anti-apoptotic Bcl-2 family proteins, and strategies for development of chemical antagonists of Mcl-1 are illustrated in Fig. 2. As shown in this figure, disruption of protein-protein interactions, in



Mcl Family. Figure 2 Disruption of protein–protein interactions reveals strategy for chemical antagonists of Mcl-1.

particular, disruption of Mcl-1-Bak interactions has been employed as strategies for development of chemical antagonists of Mcl-1. Mcl-1 consists of three major domains, BH-1, BH-2 and BH-3, and Mcl-1 hetero-dimerizes preferentially with Bak, a pro-apoptotic member of Bcl-2 family proteins through the BH3 binding pocket, thereby neutralizing pro-apoptotic activity of Bak. Small molecule antagonist of Mcl-1 binds to BH3 binding pocket of Mcl-1 and displaces Bak. Initially, Bak is inactive, but Bid or Bim binds to inactive Bak subsequently and induces conformational changes in Bak protein, resulting in activation of Bak, leading to apoptosis. At present, a number of investigators in academia and industries have been screening natural compounds, semi-synthetic compounds and fully synthetic compounds to identify chemical antagonists of Mcl-1. Among them, GX15–070 (Gemin X Pharmaceuticals Inc. Montreal, Canada) is the first small molecule antagonist of Mcl-1 that has been under clinical development (►small molecule drugs). At present, GX15–070 has entered phase I clinical trials, using patients with CLL (chronic lymphocytic leukemia) and solid tumors, although no results are yet available. Gossypol (from cotton seeds) has been reported to occupy the BH3-binding site on Bcl-2 and Bcl-XL, and tested clinically and shown to have bioactivities against refractory cancers. An enantiomer with superior affinity for Bcl-2, (−)-gossypol, termed "AT-101," is currently being developed by Ascenta Pharmaceuticals Inc. (San Diego, CA) in collaboration with the DTP (Developmental Therapeutics Programs) at the NCI (National Cancer Institute). AT-101 has been shown to be a broad-spectrum inhibitor of Bcl-2 family proteins, including Mcl-1, Bcl-2, Bcl-xl, and Bcl-w. Clinical trials of AT-101 have been currently conducted in patients with CLL and B-lymphoma, and anti-tumor activities of AT-101 have been reported. Selective inhibition of Mcl-1 and Bcl-2 may represent a useful strategy for leukemia and cancers (►chemosensitization), including CLL (chronic lymphocytic leukemia), AML (acute myeloblastic leukemia) and small cell lung cancer. However, conditional gene ablation studies in mice indicate that Mcl-1 is also required for survival of hematopoietic progenitor cells, raising the specter of toxicities that might limit utility.

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MCM/P1 Proteins

Definition

These proteins are a family of 6 closely related minichromosome maintenance proteins (Mcm2–7) that form the RLF-M component of the replication licensing system.

►Replication Licensing System

MCP-1

Definition

Is one of the most potent macrophage chemotactic factors. MCP-1 initiates ►macrophages recruitment into inflammatory lesions, thereby being involved in various disorders including tumor ►angiogenesis.

►Minodronate

MCPH1

Definition

Microcephalin, a putative disease gene implicated in primary microcephaly; ►BRIT1 Gene.

►BRIT1 Gene

MCT-1 Oncogene

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Synonyms

MCTS1

Definition

MCT-1 is an oncogene initially identified in a human T-cell lymphoma and has been shown to induce cell proliferation as well as activate survival-related pathways. The MCT-1 protein MCT-1 contains a PUA domain, which is a recently described RNA binding domain. MCT-1 interacts with the ►cap-binding complex and modulates the translation profiles of a subset of mRNAs.

Characteristics

The MCT-1 oncogene is mapped to chromosome Xq22–24. MCT-1 was shown to interact with the density-regulated protein (DENR/DRP), a protein whose expression is increased in cultured cells grown at high density. DENR has a ►SUI1 domain which is involved in recognition of the ►initiation codon by the eIF2-GTP-Met-tRNAi ternary complex. Together MCT-1 and DENR form a functional unit which binds to the cap binding complex either directly or indirectly through interaction with ►eIF4E.

Regulation

Expression analysis of a variety of normal human tissues revealed low level ubiquitous expression of MCT-1 message. Levels of MCT-1 protein were shown to be increased after exposure to DNA damaging agents; this increase did not require new protein synthesis. Phosphorylation of MCT-1 protein by ►p44/p42 MAPK is critical for stabilization of MCT-1 protein and for its ability to promote cell proliferation.

Clinical Relevance

A subset of primary diffuse large B-cell lymphomas exhibited significantly elevated levels of MCT-1 protein compared with normal lymphoid tissue. The region of chromosome Xq22–24 has been shown to contain amplified DNA in a variety of primary B-cell lymphoid neoplasms using ►comparative genomic hybridization, suggesting that increased copy number of a gene(s) located in this region confers a growth advantage. The signaling pathways that regulate the translational machinery are abnormally active in many human cancers and are causally related to tumor formation in mouse models. The MCT-1 protein interacts with the cap-binding complex and modulates mRNA ►translational profiles through its binding to DENR. Using a ►comparative genomics approach, a homolog of MCT-1 was identified in the ►archaea *Pyrococcus abyssi*. MCT-1 is the only known oncogene homolog in archaea and highlights that MCT-1 is a highly conserved gene with critical biological function. Further underscoring the functional relevance of MCT-1 is the observation that the human MCT-1 gene can complement in yeast the translation defects

observed by the loss of the yeast gene TMA20, having significant homology (48%) to MCT-1. It is clear that MCT-1 plays a role in translational regulation, and further supports the linkage between translational control and oncogenesis. Many human malignancies gain a selective advantage through the abnormal activation of this pathway, and its selective inhibition may provide a potential therapeutic approach in malignancies that exhibit high levels of MCT-1 protein.

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MCTS1

►MCT-1 Oncogene

MDC

►ADAM Molecules

MDC1

Definition

Mediator of DNA damage checkpoint 1, a ►checkpoint protein which is required for the intra-S checkpoint and γ ►H2AX foci formation.

►BRIT1 Gene

MDM2

Definition

Human homolog of mouse double minute 2 (MDM2); the protein was first identified as the product of the mdm2 gene amplified in transformed murine cells. It is a p53-binding nuclear protein that antagonizes and down-regulates p53 activity. The gene maps to 12q14–q15 and is amplified in certain human tumors, including sarcomas, glioblastomas and astrocytomas.

►MDM Genes

MDM Genes

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Synonyms

Mouse double minute gene; HDM

Definition

The *MDM* are two genes, *MDM2* and *MDM4*, found ►amplified in different human tumors. Their main activity is the binding and regulation of the oncosuppressor p53.

Characteristics

The *MDM* gene family is comprised of *MDM2* and *MDM4* (also called *MDMX*), identified in 1992 and 1997 respectively. The first identified gene, *MDM2*, derives its name from having been isolated from small, extrachromosomal bodies present in a mouse cell line and called mouse double minute. On the same double minute, *MDM1* and *MDM3* were also isolated but they resulted completely unrelated to *MDM2*.

MDM2 and *MDM4* show high similarity at the level of gene sequence and structure and may be considered ►paralogs. Orthologs of *MDM2* and *MDM4* have been described in different animal species across evolution, from Homo sapiens to the zebra fish, Danio Rerio, and the frog Xenopus Laevis, suggesting an ancient origin of these genes. *MDM2* and *MDM4* are indeed essential genes for mouse development and viability, as demonstrated by the embryonic lethal

phenotype of the mice lacking both alleles of each gene (*MDM2* or *MDM4* ►knock-out mouse, usually indicated as $MDM2^{-/-}$ or $MDM4^{-/-}$ KO mouse).

MDM2 and MDM4 are mostly studied for their ability to bind and regulate the ►oncosuppressor p53 protein. Their structure, depicted in Fig. 1 is composed of a p53-binding domain at their amino-terminus, with a structure similarity of 54% between the two proteins; by a ►Zinc finger motif with 42% of structural similarity whose activity has not been defined, and a ►Ring finger domain at their carboxy-terminus, with a structure similarity of 53%. Through this last domain MDM2 and MDM4 interact with each other forming a functional heterodimer. The central part of the two proteins is the most divergent region. Despite their similarity, the two proteins are not redundant and play different roles in the cell and during mouse development. Indeed, their loss in knock-out mice causes a different lethal phenotype, regardless of the presence of the other gene. Notably, both mice are viable when p53 is simultaneously deleted, indicating that MDM2 and MDM4 lethality is closely related to deregulation of p53 activity.

MDM2

The human ortholog of the *MDM2* gene (Also indicated as *Hdm2* or *hMDM2*) is located on chromosome 12q13-14. It codes for a protein composed of 491 aminoacids, indicated also as $MDM2^{p90}$. In addition, a short protein, called $MDM2^{p76}$ (or $MDM2^{p75}$), derived from the initiation of translation at two internal ATG, has been detected. $MDM2^{p90}$ is present in all tissues with varying levels in response to cellular behavior.

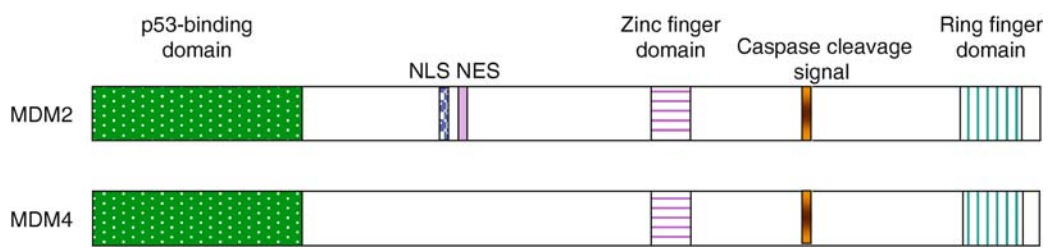
MDM2 is an E3-type ►ubiquitin ligase, able to covalently bind ubiquitin moieties to specific targets through its Ring finger domain. Multiubiquitin chains are a signal that brings proteins to degradation through the proteasome system. The main target of MDM2 is p53 but other targets have been described, many of which are►proapoptotic molecules. Degradative activity of MDM2 independent of its ubiquitination function has been also reported (i.e., toward the oncosuppressor ►Rb).

Biologically, the MDM2 most relevant activities are those exerted toward p53. To date, all studies targeting inactivation of MDM2 in different mouse tissues yielded a severe phenotype, recovered by simultaneous knocking down of p53, thus pointing to an essential role of MDM2 in the regulation of p53. MDM2 function on p53 can be summarized in two kinds of actions:

1. MDM2 controls p53 protein levels through its degradative function.
2. MDM2 inhibits p53 transcriptional activity. This function takes place through different mechanisms: by binding p53 in the same region through which p53 binds basal transcription factors, thereby preventing such interaction; by promoting a modification of p53 (►NEDDylation) that inhibits its transcriptional activity; by ubiquitinating histones surrounding the DNA p53-binding regions and inducing gene silencing.

It is still a matter of debate if and which activity prevails over the other. MDM2 activities not related to p53 regulation have also been described. Many of them confer on MDM2 a further prooncogenic role: degradation of the oncosuppressor Rb, of ►E-cadherins (a component of cell–cell junction), and the activation of the ►E2F1 transcription factor are representatives of these activities. Although these MDM2 activities do not seem to be essential for mouse development and viability, they might play a relevant role in tumor promotion. Notably, two independent ►transgenic studies targeting MDM overexpression in all tissues or specifically in mammary gland have evidenced MDM2 tumorigenic properties independent of p53. Conversely, MDM2 growth inhibitory activity in normal cells has also been described. In agreement with this function, MDM2 is expressed at high levels in terminally differentiated "growth-arrested" tissues such as brain, muscle, and skin.

Several studies have investigated the regulation of MDM2 function. What is particularly interesting is the transcriptional regulation of its levels by p53. This establishes a negative feedback loop between MDM2 and its target p53. When p53 activity raises, levels of MDM2 raise too and this in turn ensures shut down



MDM Genes. Figure 1 Scheme of MDM proteins. The main regions are indicated. NLS indicates nuclear localization signal, NES indicates nuclear export signal, Caspase cleavage signal indicates a tetrapeptide that mediates cleavage of the entire protein at this site.

M

of p53 activity, thus limiting the duration of the ►stress response. Other proteins that regulate MDM2 activity include: (i) ►kinases and ►phosphatases that modify its interactions by phosphorylating or dephosphorylating different MDM2 residues; (ii) proteins that delocalize MDM2 in different cell compartments; (iii) proteins that inhibit MDM2 degradative activity. Interestingly, some posttranslational modifications trigger MDM2 degradative activity toward itself, thereby favoring an autocontrol of its levels.

MDM2 and Human Tumors

Direct mutation of the *p53* gene that results in abrogation of the p53 oncosuppressive function is present in ~50% of human cancers. Deregulation of p53 is supposed to exist in the remaining 50% of cancers. Alterations of p53-regulators, particularly of *MDM* genes, represent a good way to achieve it.

Indeed, *MDM2* is amplified in many different human tumors that harbor wild-type *p53*, with the highest frequency in human sarcomas. Analysis of data obtained from 3,889 tumor samples from 28 tumor histotypes revealed an overall *MDM2* ►amplification frequency of 7%. In addition to gene amplification, overexpression of the protein caused by different molecular mechanisms has also been described in human tumors, indicating that the previous frequency underestimates the incidence of *MDM2* deregulation in human cancer. Moreover, a ►polymorphism in the promoter region of *MDM2* gene (SNP309) has been identified. This polymorphism causes an increased MDM2 expression that in turn should lead to attenuation of p53 oncosuppressive function. Indeed, a survey of the presence of this polymorphism in different human tumors has shown a significant earlier age of onset of several tumors, both hereditary and sporadic. It has to be mentioned that the genetic background is a strong determinant for MDM2-SNP309 activity, as it accelerates tumor formation in a gender-specific and hormonal-dependent manner.

Some studies have pointed to the worse prognosis of the tumors harboring p53 mutation and MDM2 overexpression, in respect to the tumors harboring only one of these alterations. These data confirm that MDM2 possesses tumorigenic properties also independently of p53.

Despite wide characterization of MDM2 molecular functions, the predictive value of MDM2 overexpression on cancer features is still controversial. It probably relies on additional factors as tumor type and genetic alterations that need to be comparatively evaluated to obtain significant predictive ►biomarkers. The relevance of the genetic background in the activity of MDM2-SNP309 is a good example in this respect.

In addition to full-length protein, different forms of MDM2 have been found in human tumors. Some of these forms arise from ►alternative splicing, others from ►aberrant splicing of full-length mRNA. Some of these forms are considered ►oncogenic and their activity has been evaluated by in vitro studies. However, the genetic background in which they work seems to strongly affect the biological outcome they cause. Therefore, further studies are necessary to define their function and tumorigenic potential.

In agreement with its protooncogenic function, different strategies aimed at inhibiting MDM2 have been set up. They can be essentially summarized in two types:

1. Downregulation of its levels by ►antisense nucleotides and ►siRNA.
2. Molecules that prevent its activity, mainly the binding to p53. To this group belong synthetic peptides that compete for MDM2–p53 binding. More recently, new tools represented by "low molecular weight compound" have come to light. This new group consists of: (i) Nutlin-3a (a *cis*-imidazoline analog) that disrupts p53–Mdm2 interaction by binding to the p53-binding pocket of MDM2; (ii) RITA (a furan-derived compound) that binds to p53 and alleviates its inhibition by MDM2.

Many of these approaches have shown antitumor activity in in vitro and in vivo human cancer models, used alone or in combination with chemotherapy and radiation therapy. Testing of these MDM2 antagonists in ►clinical trials is needed to ensure their effectiveness and toxicity.

MDM4

Human *MDM4* (also called *HDMX*) is located on chromosome 1q32, and codes for a protein of 490 aminoacids. Its mRNA is expressed in all human tissues, with particularly high levels in brain and thymus. Despite structure similarity with MDM2, MDM4 molecular activities are not yet completely understood. MDM4 does not function as an E3 ubiquitin ligase in vivo, in spite of the similarity with MDM2 at the level of the Ring finger domain. However, contribution of MDM4 in the regulation of MDM2-mediated p53 stability is considered. More consistently, MDM4 inhibits p53-transcriptional function especially the activation of growth arrest genes. Interaction of MDM4 with other proteins has been described too but the biological relevance is not completely defined.

Biologically, the recovery of *MDM4* KO mouse lethality by simultaneous *p53* deletion clearly indicates an essential function of MDM4 in the regulation of p53 during mouse development. In contrast to MDM2, loss of MDM4 in different cell types leads to different phenotypes, in some cases with no apparent defects, indicating that its function is more cell-type specific than that of MDM2.

Different studies have concurred to characterize regulation of MDM4. In contrast to MDM2, MDM4 transcriptional levels are not modulated by p53. At present, mainly posttranslational modifications (such as phosphorylation, ubiquitination, and ►SUMOylation) have been identified that regulate MDM4 stability and activity. Interestingly, MDM4 levels are also controlled by MDM2 during cell stress response, highlighting the complexity of the p53–MDM2–MDM4 interplay.

MDM4 and Human Tumors

To date, analysis of *MDM4* in human tumors has been performed in few studies. In analogy to *MDM2*, amplification of *MDM4* gene in human tumors characterized by wild-type *p53* has been observed in gliomas, as well as in breast carcinomas and in pediatric retinoblastoma with different percentages. Overexpression has been observed in lung and colon tumors too. These data concur to define MDM4 as a protooncogene, similar to MDM2. However, a detailed analysis of MDM4 role during tumor progression is still lacking. In fact, some studies reported down-regulation of MDM4 in some cases associated with advanced ►stage tumors, leading to the hypothesis that the presence of MDM4 might be detrimental to tumor progression. These findings need to be confirmed and further investigated.

In analogy to MDM2, ►splicing forms of MDM4 deriving from alternative or aberrant splicing events have been described and the oncogenic properties of these spliced forms are shown in vitro. Their presence coexists with that of the full-length protein, raising the question of the extent to which gene amplification contributes to their expression and of their relevance with respect to full-length protein.

Strategies aimed at counteracting MDM4 inhibitory function and reactivating p53 oncosuppression have been proposed. However, despite structural similarity between the MDM2 and MDM4 p53-binding domain, strategies that work with MDM2 appear to be not effective with MDM4, indicating the need to search for optimal MDM4 antagonists.

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MdmX

Definition

A ►p53-binding protein with structural similarity to ►mdm2.

►Daxx

MDR

Definition

Multidrug Resistance.

►Melanocytic Tumors

Mdr1 protein

►P-glycoprotein

MDS

Definition

►Myelodysplastic syndromes. Often considered a "pre-leukemic" state.

►Myelodysplastic Syndromes
►RUNX1

2ME

►Methoxyestradiol

2ME2

►Methoxyestradiol

MEA II

►Multiple Endocrine Neoplasia Type 1

Measles Virus

Definition

A member of the Paramyxoviridae family with a negative strand RNA genome, an helical nucleocapsid, and enveloped particles that is the causative agent of measles. Vaccine lineage viruses and genetically modified derivates are used in oncolytic virotherapy.

►Oncolytic Virotherapy

Mechanical Properties

Definition

Of cells are the measures of cellular deformation induced by the externally applied forces, and thus are the main parameters indicative of the mechanical balance between the external and intracellular forces. The mechanical response of the cells during deformation can be expressed in viscoelastic properties, such as stiffness or elastic modulus, and viscosity. The deformation of cells induced by external forces can be elastic if the cells deform immediately upon applying forces and fully restore to its original shape after the applied force is removed. Cellular deformation can be viscous if there is a resistance to deform in the short term which relaxes over time allowing the materials to "flow." The elastic response of the materials is measured by stiffness, which is the ratio of the applied force to the deformation or elastic modulus, which is the ratio of stress defined by force per area to strain defined by deformed length over original length. The viscosity is usually defined as a fluid's resistance to flow. Dynamic mechanical analysis measures the viscoelastic properties in which a small oscillatory strain is applied and the resulting stress is measured. Purely elastic materials will have no lag between stress and strain, so that the response of one caused by the other is immediate while viscous materials will exhibit lag. The elastic and viscous response of the material is expressed in storage and loss modulus, respectively. Storage modulus represents the stored energy in the material during elastic deformation and loss modulus corresponds to the energy dissipated as heat due to viscous flow.

►Tensional Homeostasis

Mechanical Stress

Definition

Is defined as force per area and is expressed in terms of Newtons per meter square (N/m^2) which is equal to Pascals (Pa), or dynes per square centimeter (dyn/cm^2). The types of stress include compressive or tensile stress, which is applied perpendicular to the surface, and shear stress, which is applied parallel to the surface.

►Tensional Homeostasis

Mechanosensor

Definition

The apparatus within cells which detects mechanical ►microenvironment such as applied force or extracellular matrix (ECM) stiffness.

►Tensional Homeostasis

Mechanotransducer

Definition

Mechanosensor within cells that converts mechanical stimulus into chemical activity.

►Tensional Homeostasis

Med28

►Endothelial Derived Gene-1

Mediastinum

Definition

A group of structures in the central compartment of the thoracic cavity surrounded by loose connective tissue where the heart, great vessels of the heart, trachea, thymus, and lymph nodes are located. The area between the lungs, which contains the heart and its large veins and arteries, the trachea, the esophagus, the bronchi, and lymph nodes.

Medical Foods

►Nutraceuticals

Medullary Breast Carcinoma

Definition

Accounts for approximately 5% of ►breast cancer. The tumors have unusual histologic features, they are circumscribed but anaplastic and high grade, formed by large cells with abundant cytoplasm, numerous mitoses, and a diagnostic plasmacytic infiltrate. Despite these features, patients with medullary breast carcinoma have improved survival in comparison to matched cases of infiltrating ductal carcinoma of the breast and long-term survival of individual patients correlates with the degree of tumor infiltration.

Medullary Thyroid Carcinoma

Definition

MTC; derives from the parafollicular C-cells. C-cells produce calcitonin, which can be used as a sensitive tumor marker for MTC. About 75% of all MTCs are believed to be sporadic while 25% have a hereditary background and are part of the ►Multiple Endocrine Neoplasia Type (MEN 2) syndromes. Germline mutations in the proto-oncogene *RET* are found in more than 95% of all patients with hereditary MTC. Somatic *RET* mutations are found in about 50% of sporadic MTCs but their role in the pathogenesis of MTC is unknown.

►RB1/pTP53 ►knockoutmouse shows a high incidence (about 40%) of MTC. Some of the mice acquire a somatic *RET* mutation, analogous to activating mutations seen in humans with MEN 2A and FMTC. However, ►*RB1* mutations have not yet been described in human MTC, and ►pTP53 mutations are only found in a minority of cases of this tumor.

►RET
►Thyroid Carcinogenesis

Medulloblastoma

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Synonyms

Infratentorial primitive neuroectodermal tumor; PNET

Definition

An embryonal tumor originating from the cerebellum, composed of densely packed small cells with round to oval nuclei and scanty cytoplasm. These tumors exhibit a pronounced tendency to metastasize along the subarachnoid space throughout the central nervous system (CNS).

Molecular and morphologic analyses classify medulloblastomas (MB) into five distinct histological subtypes (frequencies vary between published series):

1. Classic medulloblastoma (~65–90%)
2. Desmoplastic, nodular medulloblastoma (► Desmoplastic medulloblastoma) (~10–25%)
3. Medulloblastoma with extensive nodularity (~5%, mostly <3years)
4. ►Large cell medulloblastoma (~5%)
5. Anaplastic medulloblastoma (~25%)

Some authors suggest the presence of a defined entity of large cell, anaplastic medulloblastoma (LC/A), however, signs of anaplasia may be present in up to 24% of medulloblastomas. Thus anaplastic medulloblastoma

M

likely represents a progressed malignant variant of MB that arises on the background of other histologic subtypes. The formerly individually listed ►melanotic medulloblastoma and ►medullomyoblastoma are now viewed as extremely rare forms with markers of melanotic or myocytic differentiation but similar genetic mutations as other MB. On immunohistochemistry, MB cells predominantly express neuronal marker proteins (e.g., synaptophysin, neurofilaments). Occasionally, astrocytic differentiation (expression of GFAP) and, rarely, ependymal features are seen.

Characteristics

Clinical Significance

MB constitute the most common malignant ►brain tumors of childhood, affecting each year approximately seven in one million children (<21 years) in the Western World, amounting to an annual rate of 540 cases per year in the US. Two age peaks at 3–4 and 8–9 years and a male predominance ranging from 1.4 to 2.1 have been reported. MB have been described in neonates and in ~1% of primary adult CNS tumors. These tumors are rarely observed beyond the fifth decade.

MB commonly (≥75%) arise in the midline (the vermis) of the cerebellum, projecting and obstructing the fourth ventricle. A hemispheric location is more commonly found in the desmoplastic variant and also with increasing age (~50% of adult medulloblastomas are desmoplastic). Approximately 30% of medulloblastomas have metastasized to the cerebrospinal fluid (CSF) at the time of diagnosis; metastases outside the CNS to bone, bone marrow, peritoneum, liver, and lung have been reported in children with ventriculoperitoneal shunts but also rarely in children without any extraanatomical connections. Clinical symptoms consist of truncal ataxia, gait disturbances and, due to increased intracranial pressure, headache, lethargy, and morning vomiting. In infants, increased excitability, inadequate growth of the head, and/or lethargy may be the only clinical signs. On computed tomography (CT) and magnetic resonance imaging (MRI), MB usually present as solid, homogeneously contrast-enhancing masses. CT shows a hyperdense and T1-MRI an iso- to hypointense mass owing to the dense cellularity of the tumor (Fig. 1). Imaging modalities such as diffusion-weighted imaging, spectroscopy, and nuclear medicine techniques (e.g., ^{111}In-Pentetreotide scintigraphy) are currently restricted to selected clinical indications and clinical studies. Diagnosis and staging are established by clinical examination, imaging studies (CT and MRI), and analysis of the CSF from a lumbar puncture site. For stratification and prognostic evaluation, patients are broadly divided into two groups:

1. Standard-risk patients are ≥3 years, have a localized tumor, which can be totally or near totally resected (residual no more than 1.5 cm^2) and no CSF metastases.
2. High-risk patients exhibit metastasis on CSF examination or macrometastasis to the supratentorial or lumbar space on imaging, or a residual tumor of more than 1.5 cm^2.

Patients below the age of 3 years often exhibit a high-risk profile due to a propensity for metastasis, commonly large postoperative residuals, low tolerance of chemotherapy, and a high risk for radiotherapy-associated side effects.

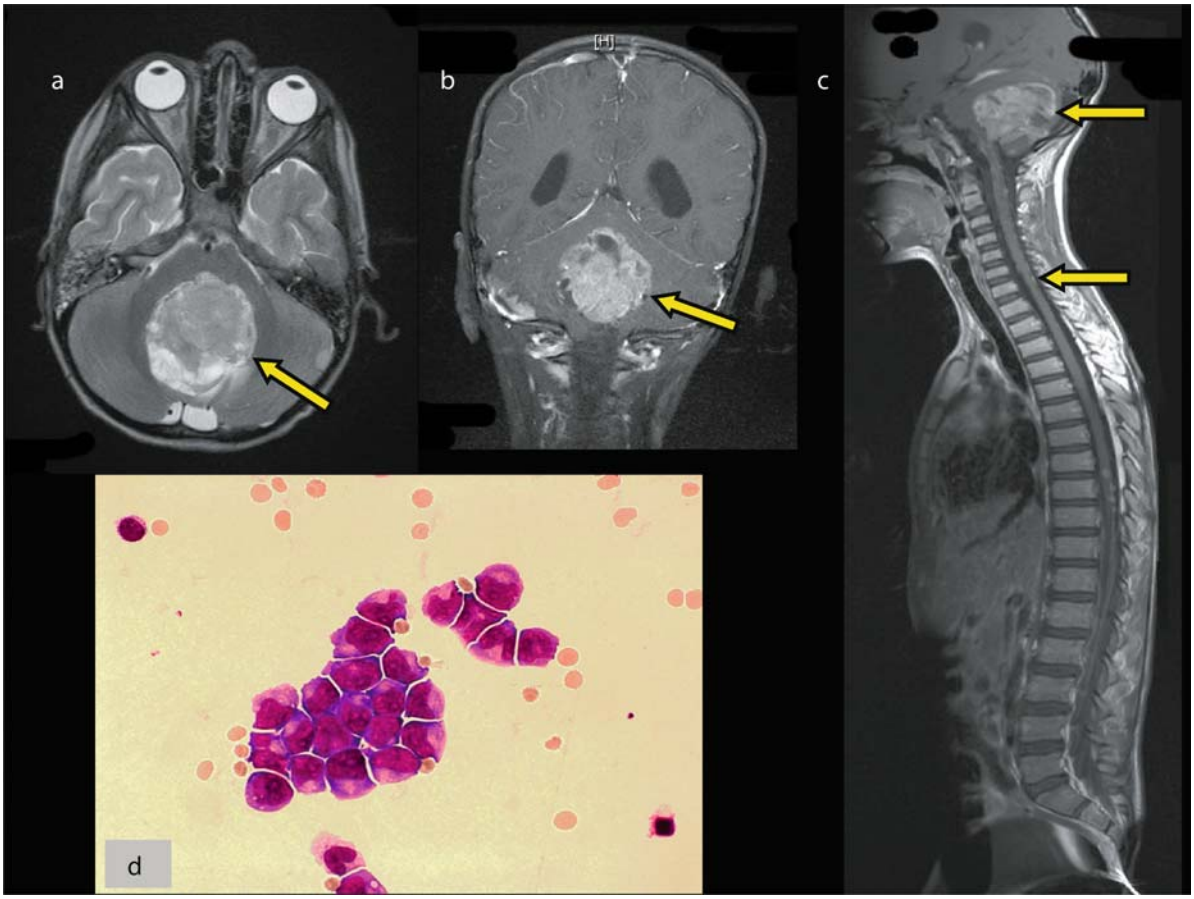
Correlational studies have demonstrated overexpression of the ►*MYC* gene (detected in ~40%) as a negative prognostic factor and high levels of the Neurotrophin 3 (►Neurotrophin) receptor ►*TRKC* (~45%) as a positive prognosticator. Furthermore stabilization of the gene *β-catenin* (~25%) seems to impose a better prognosis, while isolated studies show high expression of the *EGFR* receptor *erbB-2* (►*HER2*) (~80%) as an indicator of a bad prognosis. The prognostic value of the most common cytogenetic lesion in MB (~50%) loss of heterozygosity (►LOH) for 17p remains unclear. Some studies demonstrate a negative correlation and some deny it. Clinical trials employing molecular markers for stratification are in the planning stages. As desmoplastic, nodular histology appears to confer a rather good prognosis signs of nodular histology along with molecular markers such as *TRKC* overexpression may constitute a therapeutic stratum by itself in future trials.

Current treatment protocols consist of radio- and chemotherapy attempting to delay radiotherapy in children ≤3 years to avoid damage to the developing CNS. Using a regimen of cyclophosphamide, vincristine, etoposide high-dose, and intraventricular methotrexate, 5-year event-free survival rates of up to 82% have been achieved in children ≤3 years without using radiotherapy. Older children with standard-risk disease receive radiotherapy at doses of 23.4–36 Gy to the craniospinal axis (CSI) and a boost to the posterior fossa or tumor site up to 55.8 Gy. Further dose reductions to 18.0 Gy CSI in standard-risk patients are evaluated in ongoing studies. Current trials for high-risk patients include radiochemotherapy and high-dose chemotherapy regimens followed by autologous stem cell support.

The 5-year survival rates of 74–83% in the average-risk group have consistently been achieved. For patients with a high-risk profile, cure rates remain disappointing approaching 32–38%. The long-term survival of patients with relapse after radiotherapy remains a rare event.

Medulloblastoma Stem Cells

Experimental evidence and identification of genetic mutations restricted to individual histological subtypes



Medulloblastoma. Figure 1 (a–c) Neuroradiology of metastatic medulloblastoma. (a) Axial T2-weighted image of a typical vermal tumor with pronounced peritumoral edema; (b) coronal T1 MRI demonstrating a large midline tumor, which has caused enlargement of both ventricles (internal hydrocephalus); (c) sagittal T1 MRI of the craniospinal axis indicating the primary in the cerebellum as well as leptomeningeal metastases (yellow arrows). (d) Cytospin of a CSF specimen demonstrating a large nest of tumor cells some small erythrocytes and one lymphocyte.

suggest the presence of at least two groups of progenitor cells that fit into the paradigm of a cancer stem cell hypothesis. One progenitor cell for MB (classic histology) appears to be derived from the periventricular matrix, may demonstrate LOH of 17p, amplification of the *MYC* gene, mutations within the *WNT/WG* pathway, and epigenetic inactivation of the gene *HIC1*. Another progenitor (desmoplastic, nodular variant, and variant with extensive nodularity) is derived from the external germinal layer (►External granular layer (EGL)) of the cerebellum and is characterized by mutations in components of the *SHH* pathway and deletions of chromosome 9q22.3.

Molecular Genetics and Biology

Many clues to an improved understanding of the molecular biology of MB are derived from the study of hereditary tumor syndromes. "Turcot's syndrome," also called "brain tumor polyposis syndrome type II," is characterized by the synchronous occurrence of adenomatous polyps or carcinomas of the colon. There, as well as in the associated brain tumors, primarily glioblastomas and MB, germline mutations of the ►*APC* gene can be detected. Rarely, MB have been seen in patients with Li-Fraumeni syndrome or in neurofibromatosis. Sporadic medulloblastomas rarely carry mutations of *TP53* and *NF1* respectively, causing these syndromes. In the ►basal cell nevus syndrome (BCNS) or ►Gorlin syndrome, patients are affected by basal cell carcinomas, ovarian fibromas, keratocysts of the jaw, palmar and plantar pits, skeletal abnormalities, and MB, mainly of the desmoplastic, nodular variant. In such patients and in about 10% of the cases with sporadic MB, mutations of the human homologue of the drosophila melanogaster segment polarity gene *PTCH1* in 9q22.3 have been described. Transgenic mice, heterozygous for *PTCH1*, develop tumors intriguingly similar to MB.

Commonly detected cytogenetic abnormalities in MB include isochromosomes 17q (up to 50%), trisomy

M

7, extra copies of 1q, monosomy 8 and 22, and deletions on 10q and 4q. Comparative Genome Hybridization studies demonstrated loss of genetic material from chromosomes 10q, 11, 16q, 17p, and 8p; gains were found for 17q and 7 and amplifications were detected in 5p15.3 and 11q22.3.

On gene expression profiles, MB can be readily differentiated from other embryonal tumors of the CNS such as ►sPNET or ►AT/RT, but also desmoplastic, nodular subtypes from classic. Metastatic MB have an individual expression signature, which among others demonstrates ►*PDGFRα* as a marker for invasion and adhesion.

Analyses of pathways involved in the normal development of the cerebellum have found several targets for mutations in sporadic nonhereditary MB. Mutations have thus been detected in key protein components of the *SHH* pathway: *PTCH1* and *SUFU* are mutated in ∼10% and *SMO* in ∼5% of sporadic MB. Components of the *WNT/WG* pathway (wingless) are less commonly found mutated. β-*catenin* exhibits mutations in ∼6% and *APC* in ∼2.5% of the cases. However, stabilization of the β-catenin protein can be seen in up to 25%. Another component of the WNT pathway, the gene *AXIN1*, has also been found mutated in isolated cases.

In molecular genetic studies, LOH for the short arm of chromosome 17 (17p) was demonstrated in up to 50% of sporadic medulloblastomas and the chromosomal breakpoint has been narrowed down to a region in 17p11.2. The search for a tumor suppressor gene distal to the locus of 17p13.3 is ongoing, even though several genes are under scrutiny. Aberrant methylation of *HIC1* (17p13.3) has been described in limited series just as deletion of *REN(KCTD11)* (17p13.2), an antagonist of SHH signaling. Loss of genetic material from chromosome 10q is in some MB associated with homozygous deletions of the gene *DMBT1*. The combined incidence for amplifications of the genes *MYC*, ►*MYCN*, and *EGFR* in MB is ~10%. A multitude of other loci have been implied in the tumorigenesis of MB as they show expression differences between the tumors and normal cerebellum. Among many others are the transcription factor genes *PAX5, NEUROD3/neurogenin*, and *ZIC*, the neuropeptides somatostatin and VIP as well as the growth factor receptor genes *HER2* and *HER4*. Epigenetic changes and foremost aberrant methylation has been detected in a series of genes including *RASSF1A*, *ZIC2*, *SGNE1/7B2*, $p16^{INK4A}$, *MCJ*, *EMP3*, and *HIC1*. Many more genes are affected by epigenetic changes.

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Melanin

Definition

A number of closely related brown-black pigments produced in melanosomes from the primary substrate, tyrosin.

►Melanocytic Tumors

Melanocortin Receptor

Definition

Cell surface glycoprotein. Receptor for ►Melanocyte-Stimulating Hormone (MSH), a polypeptide with homology to other pituitary hormones. It is produced by the pituitary, and also by keratinocytes in response to ►UV radiation. A principal downstream target is tyrosinase, the rate-limiting enzyme in melanin production.

►Melanoma

Melanocyte

Definition

A nonepithelial cell type specifically dedicated to the production of melanin. Most melanocytes reside in the skin, but smaller numbers populate some mucous membranes, the eye, the meninges and inner ear.

►Melanocytic Tumors

Melanocyte-Stimulating Hormone

Definition

MSH; derives its name because of its effect on melanocyte, which are skin cells that contain the black pigment melanin. MSH is a member of the group of melanocortins, which are pituitary peptide hormones that include adrenocorticotropin (ACTH) and the alpha, beta and gamma melanocyte-stimulating hormones (MSH). In humans, melanocytes are responsible for moles, freckles, and suntan and, if they turn cancerous, melanoma. In most vertebrates, MSH is produced by an intermediate lobe of the pituitary gland.

Melanocytic Nevus

►Melanocytic Tumors

Melanocytic Tumors

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Synonyms

Nevus; Nevocellular nevus; Melanocytic nevus; Mole; Melanoma; Melanocytoma

Definition

Melanocytic tumors are neoplastic proliferations of cells featuring phenotypic characteristics of ►melanocytes. The large majority originates in the skin. Benign melanocytic tumors are melanocytic nevi (or nevocellular nevi or, briefly, nevi, or "moles"), malignant ones are ►melanomas.

Characteristics

Melanocytic tumors are neoplasms of cells showing the features of melanocytic differentiation, the most distinctive of which is the production of ►melanin. With few exceptions, this phenotype is thought to reflect their origin from a melanocyte. In mammals, most melanocytes reside in the basal epidermal cell layer and in the hair follicle. Smaller numbers are located in the dermal connective tissue, the uvea, the meninges and the inner ear. In poikilotherms such as reptiles and fish, most melanocytes reside in the dermis.

Cutaneous and extracutaneous melanocytic tumors are subdivided into melanocytic nevi (or nevocellular nevi, nevi, or "moles"), which are benign, and melanomas, which are malignant. Melanocytic nevi can be classified into congenital and acquired ones, the latter outnumbering the former by at least three orders of magnitude.

Congenital nevi manifest at birth as a macule or larger patch of increased pigmentation. The largest examples, giant congenital nevi, may cover a substantial part of the body. The congenital ►nevus grows more or less in accordance with body growth, and it becomes somewhat raised and sometimes covered with hair. Melanoma arises in some congenital nevi, but this is uncommon: the available evidence suggests that the chance of melanoma is a few percent or less, and is highest in large examples. Surgical removal of the nevus, or of its superficial part, improves cosmesis and may reduce melanoma risk, although the latter has not been established. Most surgeons prefer to operate large congenital nevi in the early infantile period.

Acquired nevi tend to arise in childhood and adolescence, but some emerge later in life. Most start out as a small pigmented flat macule, which slowly increases in size and becomes raised, producing a papule. This initial growth phase is soon followed by stabilization of size and appearance. Most nevi are less than 6 mm across. Nevi are generally symmetrical, sharply defined, are covered by an intact epidermis, and lack variations in color or degree of pigmentation. In case of doubt, an excisional biopsy (total surgical removal, with narrow margins) of the lesion allows histopathologic investigation, which usually, but not always, clinches the diagnosis.

Nevus numbers are correlated with skin type, caucasians with a fair skin generally having larger numbers than dark-haired caucasians and noncaucasians. Heavy exposure to ►UV Radiation of sunlight, especially in early childhood, is correlated with increased nevus counts. In some families with an increased melanoma risk, nevi are very numerous and more irregular, clinically as well as histologically (dysplastic nevus syndrome; familial atypical multiple mole melanoma syndrome).

Most melanocytic nevi harbor an activating ►B-RAF mutation, which results in increased signaling through the RAS-ERK pathway, which presumably drives, or contributes to, increased proliferation. Some variants, such as Spitz nevi (which are characterized by a large cell

type) instead may have an activating HRAS mutation, while large congenital nevi tend to harbor and activating NRAS mutation. These mutations are mutually exclusive. In aggregate, these findings suggest that dysregulated RAS-ERK signaling is an important factor in the pathogenesis of a nevus. The characteristic cessation of nevus growth, which is caused by loss of proliferative activity, exhibits the central characteristics of ►cellular senescence, with upregulation of $p16^{INK4A}$, activation of senescence-associated β-galactosidase, and loss of proliferative activity. Germline inactivating mutations of INK4A or of the sequence coding for the $p16^{INK4A}$ binding site of CDK4 result in the melanoma-proneness familial dysplastic nevus syndrome mentioned above, whereas somatic mutations of INK4A or amplifications of CYCLIN-D1 are common in melanoma. In aggregate, these data suggest that unscheduled pRB-inactivation and inability to mount an ►oncogene-induced senescence response play an important role in melanomagenesis.

►Melanoma is a malignant tumor of melanocytes. Epidemiologic studies indicate that exposure to sunlight is an important factor in their pathogenesis. Fair skinned and blonde or red-haired individuals are especially at risk; melanoma is uncommon in non-caucasians. Most arise in the skin and are detected when still relatively small, with a thickness of a few millimeters or less. Macroscopically, melanoma is often irregular in shape and color, and may ulcerate or produce an itching or burning sensation. Melanoma may arise in a nevus; a change in a previously stable mole is suspicious of such malignant transformation. At the earliest phase, melanoma is confined to the epithelial compartment of the skin but it subsequently invades into the dermis and underlying tissues; at this invasive phase it may disseminate through the lymphatics and blood vessels. Lymphogenous metastases manifest in the skin and subcutis of the same body region (in-transit metastases) and in regional lymph nodes. Hematogenous metastases arise in a wide variety of sites, including some that are uncommonly affected by other tumors, such as the heart, intestines and spleen. Primary melanoma is treated by wide local excision, usually with a margin of 1 cm of apparently uninvolved skin, in order to minimize the risk of local recurrence. Metastases to regional lymph nodes, manifest clinically or detected by ►sentinel node biopsy, are generally treated by regional lymph node dissection. This procedure still offers a significant chance of cure. Distant, hematogenous metastasis is not amenable to curative surgery, but a selected group of patients benefits from surgical removal of solitary metastases. The response to radiation therapy and chemotherapy is usually modest. Much clinical interest focuses on innovative immunotherapeutic strategies (►immunotherapy), which aim to capitalize on the antitumor immune response that is often evident histologically in primary melanomas, and which effaces the primary tumor (but, unfortunately, not the metastases) in some patients.

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Melanocytoma

►Melanocytic Tumors

Melanoma

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Synonyms

Malignant melanoma

Definition

Melanoma is a tumor arising from melanocytes. Melanocytes are dendritic cells that migrate from the neuroectoderm to lodge at the base of the epidermis, and in other epithelial sites, including the eye, gastrointestinal tract, and vagina. Melanoma can arise at any of these sites, but most commonly arises in the epidermis (cutaneous melanoma, CM), where the function of melanocytes is normally to produce melanin, a protective skin pigment, in response to ultraviolet radiation (UV radiation). Ocular melanoma (OM) arises from melanocytes in the uveal stroma. Melanoma may or may not develop in a preexisting ►nevus.

Characteristics

The incidence of CM is highest in fair-skinned people living in areas of high sunlight exposure. High incidence countries (>20/100,000) include Australia,

Hawaii, and New Zealand. Incidence rates are beginning to fall in younger age groups in some of these countries, attributed in part to intensive public health sun protection programs. Countries of intermediate incidence (5–15/100,000) include northern Europe, and mainland USA. The incidence continues to rise at a rate of approximately 1.8% per year in US Whites, and increased by as much as 40–80% in the UK, France, and Spain between the early 1980s and the early 1990s. A low incidence (<2/100,000) is found in Africa and South-East Asia. Five-year survival has steadily improved since the 1970s, and is now greater than 85%, but melanoma takes a high toll on younger people, such that an average of 18.6 years of potential life is lost for each melanoma death in the USA, one of the highest rates for adult-onset cancers. The world-wide burden of CM is likely to increase well into this century, due to the aging population, continued high recreational sun exposure habits, and changing climate patterns which may increase ambient UV radiation.

Although the most common form of intraocular tumor, OM incidence is less than 0.7/100,000.

Risk factors for the development of CM include the presence of multiple ►nevi, family history of CM, history of a previous primary CM, fair skin that tans rather than burns, freckling, red hair, and blue eyes. Individuals with multiple ►atypical nevi (dysplastic nevi) are at particularly high risk. The fact that CM frequently develops de novo, even in those with multiple atypical nevi, suggests that these nevi are a marker of melanocytic stability, rather than being obligatory precursor lesions. It has been estimated that approximately 65% of all CM world-wide is due to sun exposure, and as much as 90% in the USA and Oceania. Epidemiological data best fit the hypothesis that CM is related to pattern as well as amount of sun exposure, with increasing intermittency of exposure being associated with increasing risk.

CM presents most commonly as change in a nevus, the majority, but not all, of which are pigmented. Suspicion of malignant change is raised by the presence in a nevus of asymmetry, border irregularity, distinguishing color, including areas of red, blue, or white, and diameter >5 mm. None of these features is invariable. Particular difficulty is presented by amelanotic primary CM. Approximately 15% of CM presenting to Sydney Melanoma Unit presents as metastatic disease with an occult primary, presumed in many cases to have regressed.

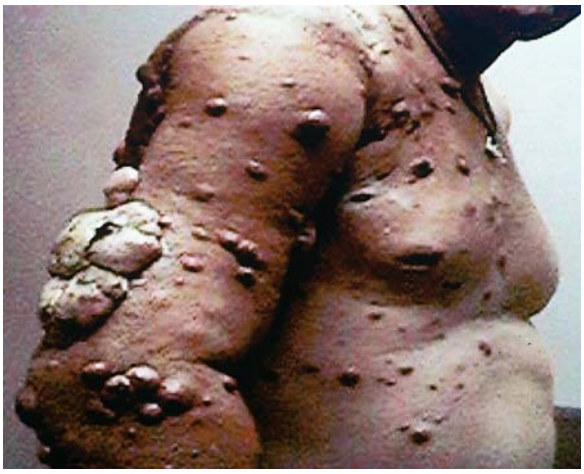
CM progresses through an in situ stage to *radial* growth phase and final *vertical* growth phase. Once cells penetrate the dermis, invasion of blood and lymphatic vessels leads to dissemination. The patterns of dissemination are highly varied and unpredictable. In typical cases, the draining lymph nodes are the first sites of apparent metastasis. In other cases, hematological dissemination occurs early. The skin, (Fig. 1), subcutis, and lungs are particularly common sites for metastases, but in later stages nearly every organ and tissue can be involved. Cerebral metastases are present in over 80% of autopsy cases.

OM metastasizes hematogenously, with a particular affinity for the liver, which may remain the only site of indolent metastatic disease until preterminal stage, when lung, brain, and distant lymph node sites are also often involved.

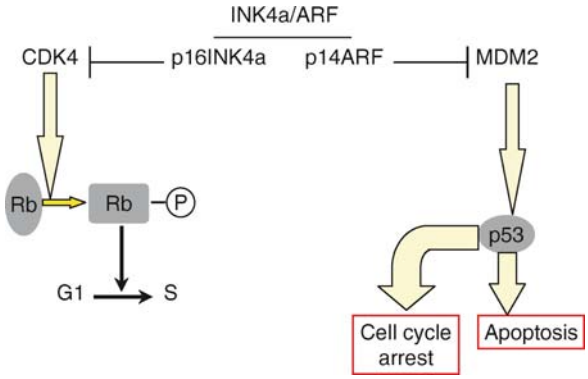
The prognosis of primary CM is linearly correlated with the thickness of the lesion (*Breslow* depth) and the presence of melanoma cells in the initial draining lymph node (sentinel lymph node). More than 85% of patients with primary CM<1.5 mm in depth survive 5 years. Fewer than 10% of patients with hematogenous metastases survive 2 years.

Genetics of Melanoma Susceptibility (Genetic Disorders Associated with Cancer Predisposition and Chromosomal Instability)

Approximately 5–10% of CM patients have a positive family history of the disease. In 20–40% of multiple (≥3 case) families the cause is an inherited mutation in the CDKN2A gene, which codes for two tumor suppressor (►tumor suppressor genes) proteins, $p16^{INK4A}$ and p14ARF (Fig. 2). Certain kindreds with CDKN2A mutations also show a propensity for pancreatic cancer. CM-associated mutations in the CDKN2A gene prevent $p16^{INK4A}$ from binding to cyclin-dependent kinase 4 (CDK4). The consequence is phosphorylation of the retinoblastoma protein, release of the E2F transcriptional regulator, and unrestricted passage of cells through the G1-S restriction point (Fig. 2). $p14^{ARF}$ stabilizes p53 levels through interaction



Melanoma. Figure 1 Multiple intracutaneous melanoma metastases.



Melanoma. Figure 2 Functions of the INK4a/ARF locus on chromosome 9p. Alternate splicing produces two proteins. p16INK4A inhibits CDK4, preventing phosphorylation of Rb, and restricting the cell cycle at the G1-S interface. p14ARF binds to MDM2, preventing degradation of p53, and permitting p53 to carry out its functions of cell cycle arrest or promotion of apoptosis.

with MDM2 (Fig. 2). The location of other CM susceptibility genes is the subject of intense research by GenoMel, the international Melanoma Genetics Consortium, and genome screening has revealed several promising new melanoma susceptibility loci, including one on chromosome 1p22. Potential modifier genes include those that determine skin color, including the melanocortin-1 receptor (►melanocortin receptor), MC1R. The ►penetrance of CDKN2A mutations is higher at earlier ages in areas of high sun exposure, demonstrating a clear synergy between environment and genetic susceptibility in the induction of a human cancer. The use of genotyping to influence clinical management of those at high risk of CM is currently discouraged because of the lack of clarity concerning penetrance, the absence of functional data on many CDKN2A mutations, and the demonstrated high risk of CM in nongene carriers in hereditary CM families. The current best strategy is for all at high risk to be subject to the same high standards of sun protection and skin surveillance.

Twin studies have shown a strong genetic basis for nevus number, although sun exposure also clearly has a strong influence. Neither nevus count nor presence of atypical nevi correlates well with genotype in CM families with CDKN2A mutations, suggesting that other genes may be involved. However, segregation studies suggest that CDKN2A, or another gene close to it, are important regulators of nevus number.

Molecular Abnormalities in Melanoma

Activating mutations in the BRAF and NRAS oncogenes are the most common oncogene mutations in CM indicating the importance of this pathway in the deregulation of melanocyte growth. There is complementarity between the presence of NRAS and BRAF mutations in any individual melanoma as each has the same effect of causing unrestrained cell proliferation. cKit mutations occur in approximately 30% of melanomas arising in chronic sun-damaged skin.

BRAF mutations are also seen in 60–80% of benign melanocytic nevi. This suggests that the complex molecular machinery providing checks and balances in the cell normally protects against the unrestrained growth stimuli propagated through such abnormalities in the Ras/RAF pathways, possibly preventing the vast majority of benign nevi from undergoing malignant change.

Immunology

There continues to be keen interest in the potential for immunological strategies in the treatment of CM (►immunotherapy). This is based on the following observations: (i) Regression is common in primary CMs, and a predominantly CD4 T-cell lymphocytic infiltration is commonly seen. (ii) Well-documented instances of spontaneous regression have been reported in metastatic melanoma. (iii) In vitro and animal evidence for a cell-mediated immune response to melanoma cell surface antigens. (iv) There is an increased incidence of CM in immunosuppressed patients.

Immunotherapies under investigation include vaccination against melanoma cell-surface membranes or purified peptide components of common melanoma cell-surface antigens. These antigens are derived either from differentiation antigens in melanoma, such as tyrosinase, MART-1, gp100, or from tumor specific gene products, such as MAGE, BAGE, and GAGE proteins. The latter are expressed normally only in the testes but become expressed in melanoma and other tumors because of demethylation of the genes. The third group of antigens is specific to individual tumors and is usually due to mutations in the gene concerned, such as CDK4 or β-catenin. Recent advances on these strategies include the use of peptide-primed autologous dendritic cells as antigen-presenting cells, the use of improved adjuvants, and the addition of cytokines, such as GM-CSF, IL2, or interferon-α. Cytokines may be administered systemically by subcutaneous infusion, or locally in the site of vaccination utilizing autologous melanoma cells or tumor-infiltrating lymphocytes (TILs) transduced with a transgene constructs containing the cytokine gene. Recent immunotherapy approaches include the use of immunotoxins to eliminate regulatory T-cells (thereby allowing tumor-specific T-cells to be activated), and the use of monoclonal antibodies such as Mipilimumab and tremelimumab against the T-cell surface protein CTLA4, to inhibit immunosuppressive cell signaling.

Rational Therapies

Surgery remains the mainstay of treatment for melanoma, and the screening and early detection of high risk groups remains a principle of melanoma control. Metastatic melanoma is relatively resistant to treatment with existing cytotoxic drugs, and there is no evidence that any form of chemotherapy prolongs survival. The reason for this is probably the evolution within the tumor of robust and redundant mechanisms of inhibition of the apoptotic pathway. Partial responses to single agent chemotherapy occur only 10% of treated patients in prospective Phase III clinical trials and most of these last only months. The single agent with the highest reproducible response rate is the alkylating agent dacarbazine (DTIC).

Biochemical pathways of particular interest in the development of future rational and specific therapies for CM include receptor signaling pathways, cell cycle regulation, angiogenesis, integrin expression and regulation of apoptosis. For example, the use of the antisense molecule to bcl-2, oblimersen, increases clinical sensitivity to DTIC in a subset of patients.

►Melanocytic Tumors

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Melanoma Antigens

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Synonyms

Melanoma associated antigens

Definition

►Melanoma antigens are a group of tumor antigens. ►Proteasomal cleavage of these intracellular proteins generates small antigenic peptides. The presentation of these peptides in conjunction with ►Major Histocompatibility Complex-MHC molecules facilitates an immune response promoting the destruction of the melanoma antigen expressing cancer cells.

Characteristics

Background

Over the years several reports have documented cancer patients mounting immune responses against their malignancies. Most of these cases are characterized by massive lymphocytic infiltration of the tumor tissue, which leads to the destruction of the malignant cells and eventually to either partial or complete disease remission. Compared to other human malignancies these immunologic phenomena are most frequently observed in malignant ►melanomas. This observation led researchers to focus their investigations on this particular tumor. Following experiments documenting the presence of tumor specific cytotoxic T-lymphocytes among the mononuclear cells infiltrating the tumor, investigators identified the first group of melanoma antigens as possible target antigens of these immune responses. Shortly thereafter it became clear that most of these proteins are not specific for melanomas but are also present in a broad range of other malignancies. ►Proteasomal degradation of these proteins produces several small unique 8–10 amino acid long peptides. Before being displayed on the cell surface in association with class I ►MHC molecules, these peptides are processed in the endoplasmic reticulum. Their subsequent presentation on the cell membrane of cancer cells results in the activation and proliferation of tumor specific cytotoxic T-lymphocytes ($CD8^+$), mediating the destruction of these melanoma antigen expressing cells.

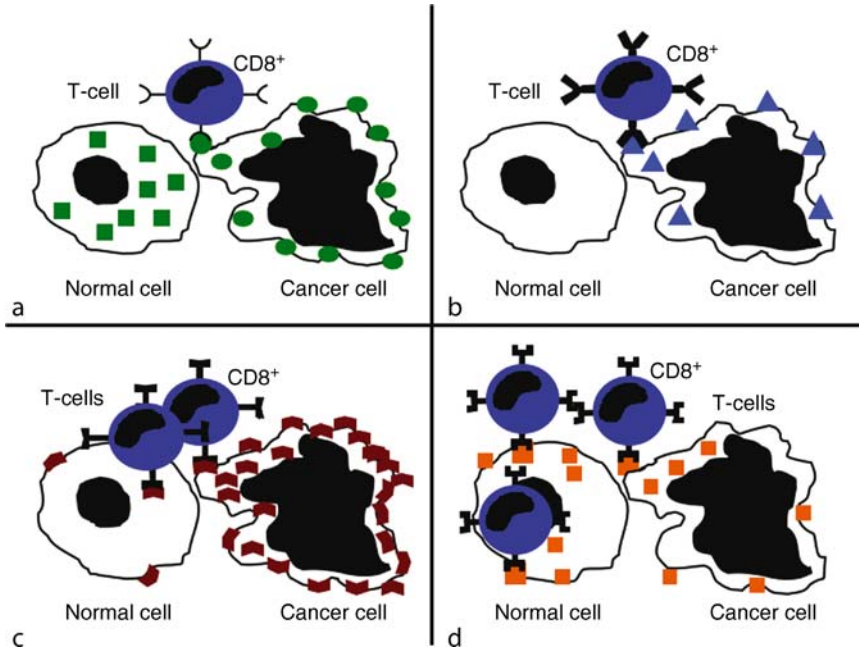
Classification

Based on how these antigens are generated, the presence of tumor cell specific gene expression, the gene expression level and whether the protein encoding the antigen represents a cell type specific differentiation gene, melanoma antigens are classified into Fig. 1:

- Tumor specific antigens
- Tumor specific shared antigens (►Cancer/►Testis antigens, ►CTA)
- Antigens encoded by genes overexpressed in tumor cells
- Antigens encoded by differentiation genes

Tumor Specific Antigens

Tumor specific melanoma antigens are the result of genetic alterations during carcinogenesis. Modifications of the structure and/or function of various physiologically expressed intracellular proteins due to



Melanoma Antigens. Figure 1 Melanoma antigens: (a) Tumor specific antigens: carcinogenesis generates genetically altered proteins which are recognized by cytotoxic T-cells; (b) Tumor specific shared antigens (Cancer/Testis antigens) represent self-antigens with restricted gene expression in cancer cells and immunologically silent male germ cells; (c) Antigens encoded by genes overexpressed in tumor cells; and (d) Antigens encoded by differentiation genes. Immune responses targeting antigens encoded by genes overexpressed in tumor cell and differentiation genes may lead to serious autoimmune reactions.

these mutations can generate novel antigenic epitopes and/or new MHC binding sites. Presentation of these new tumor specific antigens on the cell surface together with the appropriate MHC molecules can trigger an anti-tumor immune response. This immune response typically begins with the binding of T-cell receptors to the antigen and is followed by the clonal expansion of tumor specific cytotoxic ($CD8^+$) T-lymphocytes, which ultimately eliminate the melanoma antigen expressing tumor cells. Rather than being universally expressed in all patients with certain malignancies, most of these novel tumor antigens are the result of specific mutations restricted to the tumor cells of individual patients. Therefore, most of these tumor specific antigens do not represent unanimously applicable targets for cancer immunotherapy. However, some are present in a wide variety of patients. A good example for this phenomenon is the oncogenic ►tyrosine kinase, ►BCR-ABL1 which is generated as the result of a ►translocation between chromosomes 9 and 22, t(9;22)(q34;q11), also known as the "►Philadelphia Chromosome." This genetic abnormality is found in 95% of patients with ►chronic myelogenous leukemia (CML). Beside its role as an oncogenic tyrosine kinase, BCR-ABL1 also represents a tumor specific antigen which is recognized by tumor specific cytotoxic ($CD8^+$) T-lymphocytes.

Tumor Specific Shared Antigens (CTA)

The second category of melanoma antigens are genes commonly found in a wide variety of malignancies, including melanomas, but absent in most adult tissues other than male germ cells and placental tissue. The genes are also known as ►CTA. Gene expression of CTA is almost exclusively restricted to tumor cells and male germ cells. The absence of gene expression and the lack of human leukocyte antigens in CTA expressing germ cells preclude recognition of these antigens by cytotoxic T-lymphocytes outside of CTA expressing malignancies. Therefore, even though they represent self-antigens, CTA are considered to be tumor specific.

The prototype of the CTA is the Melanoma AntiGEn (►MAGE) gene family. The first members of this gene family were characterized in 1991 during efforts to identify the immunological targets of cytotoxic T-lymphocytes attacking the cells of the melanoma cell line MZ-2. Subsequently, many more members of this gene family have been identified. Up to date three subfamilies with numerous genes (MAGE-A (1–15), -B (1–17) and C (1–7)) are known. Interestingly, all these genes are localized on the X chromosome. Their messenger RNA is assembled of three exons, and the entire open reading frame is localized on the third exon. A large area (65–85%) of the gene sequence is

preserved between the different members of the MAGE gene family. This area, the MAGE-homology domain, encodes ~170 amino acids and is positioned at the carboxyl-terminal end of the MAGE proteins.

Based on the remarkable structural resemblance and the frequent co-expression of various members of the MAGE family within the same cell, it has been speculated that these proteins are involved in similar cellular pathways. However, the exact physiologic function of MAGE remains not fully understood. Several MAGE proteins have been shown to be involved in the regulation of the cell cycle and control of apoptotic pathways. In addition the unique gene expression pattern of MAGE suggests that the physiologic role of the MAGE proteins is limited to pathways active during germ cell development and carcinogenesis.

The silencing of MAGE gene expression in the majority of tissues is not attributable to a lack of the required transcription factors (Sp1 and ►Ets). These transcription factors are universally present in most cells, however methylation of their binding sites at the gene promoter inhibits gene transcription. In contrast, DNA hypomethylation as frequently seen during carcinogenesis, induces gene transcription by allowing transcription factor binding to the gene promoter. Alternatively, gene transcription can also be induced by inhibition of the ►DNA-methyl-transferase caused for example by ►5-Azacytidine. Other mechanisms potentially involved in the regulation of MAGE gene expression are genetic ►imprinting, suggested by the X chromosomal gene localization, and the presence of alternatively spliced promoters with variable transcription efficiency for some MAGE genes.

Besides MAGE-A, -B and -C (also called Type I MAGE genes) several additional MAGE gene subfamilies (MAGE-D to L) have been described (Type II MAGE genes). In contrast to Type I MAGE genes, Type II genes are almost universally expressed in normal tissues. However, due to a lack of similarity between Type I and Type II MAGE genes none of the antigenic peptides encoded by the Type I genes is present in Type II genes. This preserves the tumor specificity of the Type I MAGE genes.

Other members of the ►Cancer/Testis antigens include the B melanoma antigens (BAGE), G antigens (►GAGE), Cancer/Testis antigen 2 (LAGE-1), the New York esophageal squamous cell carcinoma 1 (NY-ESO-1) gene and the synovial sarcoma X (SSX) breakpoint genes.

Antigens Encoded by Genes Overexpressed in Tumor Cells

During carcinogenesis there are numerous modifications to the cellular gene expression profile. Besides the activation of oncogenes and the silencing or mutation of tumor suppressor genes, cancer cells frequently overexpress genes that provide them with a selection advantage. These genes commonly enhance cell proliferation or inhibit apoptosis. Furthermore, these genes can also encode antigens, which are capable of triggering a cytotoxic T-lymphocyte mediated immune response against the tumor. Examples for these antigens include ►survivin (inhibitor of apoptosis), ►telomerase (stabilizes chromosomes) and ►HER2/neu (Epidermal growth factor receptor, cell membrane bound tyrosine kinase involved in cell growth and differentiation). HER2/neu is overexpressed in 20–40% of all breast cancers. Given that these antigens represent self-proteins, the resulting immune response represents a breach of self-tolerance and is not tumor cell specific.

Antigens Encoded by Differentiation Genes

The last group of melanoma antigens includes proteins encoded by cell type specific differentiation genes. For melanoma cells this includes tyrosinase, Melan-A, gp100 and tyrosinase-related proteins 1 and 2. An effective immune response targeting these antigens also results in the elimination of normal melanocytes and may present as ►vitiligo. Other tumor antigens encoded by differentiation genes include the ►prostate specific antigen (PSA) and the ►carcinoembryonic antigen (CEA).

Clinical Implications

The elimination of cancer cells by the immune system represents an important defense mechanism against the development of malignancies. It has been well documented that immunocompromised individuals and patients receiving immunosuppressive medications are at an increased risk to develop certain types of cancer. In patients with established malignancies the presence of tumor infiltrating lymphocytes has been associated with better outcomes for several malignancies. Peptides derived from Melanoma antigens represent some of the immunological targets of these anti-tumor T-lymphocytes. Occasionally, this vigorous immune response results in spontaneous tumor regression and remission. Unfortunately, these dramatic responses are only seen in very few individuals; more commonly disease progression occurs despite the presence of anti-tumor T-lymphocytes. The mechanisms by which the majority of malignancies escape the immune system are incompletely understood. It is possible that the loss of antigen or MHC class I expression by tumor cells and/or alterations in the local milieu of inflammatory cytokines leads to local anergy and allows tumor cells to proliferate despite the presence of anti-tumor T-lymphocytes.

Not surprisingly, melanoma antigens have been of great interest as targets for the development of anti-cancer vaccines. Prior to the characterization of the melanoma antigens it has been shown that the adoptive

transfer of anti-tumor lymphocytes results in a response of the tumor in ~30% of recipients. In addition, there was some success using either allogenic or autologous tumor cell vaccines. Following the characterization of the antigenic peptides contained in various melanoma antigens investigators developed various strategies to improve the immunogenic effects of these vaccines. These approaches include the use of peptide vaccines with or without immunogenic adjuvants, genetically altered antigenic peptides to increase MHC class I binding, transfer of antigen transfected dentritic cells as well as virus and DNA vaccines. Despite these efforts, clinical response rates remain suboptimal (20–30%), and further innovations are needed. Some of the problems to be resolved include the heterogeneous expression of melanoma antigens, local anergy and the development of autoimmune phenomena.

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Melanoma Associated Antigens

►Melanoma Antigens

Melanoma-Associated Retinopathy

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Synonyms

MAR; MAR-syndrome

Definition

Paraneoplastic syndrome in ►melanoma patients characterized by different visual signs and symptoms, a reduced b-wave in the electroretinogram, and serum antibodies against retinal proteins.

Characteristics

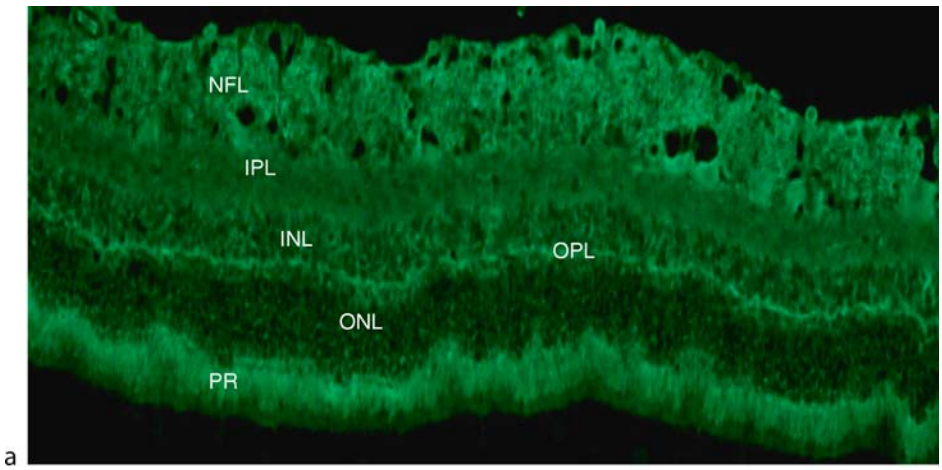
Pathogenesis

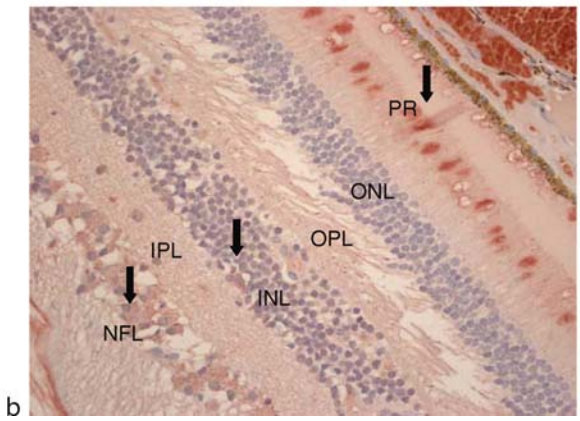
Formerly, melanoma-associated retinopathy (MAR) was supposed to result exclusively from antibody production against ►melanoma antigens that are also expressed in retinal tissue, leading to the destruction of retinal cells and resulting in defective signal transduction. Indirect immunohistochemical investigation of serum samples from MAR patients on retinal tissue lead to the detection of these antibodies (Fig. 1). In some cases, a diffuse antibody involvement with a variety of different cell populations such as optic nerve, nerve fiber layers, photoreceptors, and bipolar cells could be observed. In single cases, a 35 kDa protein in Müller glial cells, a 22 kDa neuronal antigen, and retinal transducin could be identified as antigens possibly involved in the development of MAR. Recently performed experimental investigations of serum samples from patients with MAR using ►SEREX were able to identify mitofilin and titin as further target antigens. The fact that none of these MAR-associated antigens detected to date by their capacity to elicit a humoral immune response is located on the cell surface questions a major pathogenetic role of the respective antibodies. Rather, it is probable that cellular, T cell-mediated processes are operative in the primary immune attack against the retina.

Clinical Aspects

Patients with MAR develop, often within a very short time period or even overnight, a multiplicity of visual disturbances. Patients describe frequently sudden onset shimmering, flickering, or pulsating photopsias in combination with difficulty seeing in the dark or night blindness. These first signs are often followed by progressive visual loss over months. Further, some patients develop color vision deficiencies. The electroretinogram in MAR patients reveals a characteristic pattern of a markedly reduced b-wave in the presence of a normal dark-adapted a-wave, resembling that seen in patients with stationary night blindness.

In most cases, patients have an established diagnosis of previous cutaneous melanoma and develop vision problems years later. Only in single cases, MAR symptoms preceded the diagnosis of melanoma in terms of a monitoric paraneoplastic syndrome. More often than not, the onset of MAR symptoms is associated with progression of disease and occurrence of metastases.





Melanoma-Associated Retinopathy. Figure 1 Immunofluorescence (a) and immunohistochemistry (b) using serum from different MAR patients on retinal tissue. Antibodies bind to photoreceptor cells (PR), cells of the inner and outer plexiform layer (IPL and OPL), as well as on cells of the inner nuclear layer (INL) and the nerve fiber layer (NFL).

The MAR syndrome with all its clinically apparent symptoms is very rare and to date only about 100 cases have been reported in the literature. However, recently performed clinical investigations including melanoma patients without visual disturbances could prove that subclinical MAR signs and symptoms appear more common than previously suspected. These subclinical symptoms are stage-dependent and are predominantly found in patients with advanced disease. These clinical observations are confirmed by further experimental investigations. Antibody activity similar to that ascribed to the MAR syndrome appears frequently in patients with melanoma who have no MAR-like retinopathy, but are potentially patients with a subclinical form of MAR.

Diagnostic Procedure

If clinical symptoms in a melanoma patient arise suspicion of MAR, the following ophthalmological examinations should be performed:

- Slit lamp and fundus examination
- Scotopic electroretinography
- Static and kinetic perimetry
- Nyctometry
- Color vision test (optional)

Serum samples from the individual patient should be investigated using indirect ▶immunohistochemistry or immunofluorescence on retinal tissue. However, one should be aware that the presence of antiretinal antibodies alone is not sufficient to diagnose MAR, as antiretinal antibodies are also found in sera derived from healthy human subjects or patients with different ocular or nonocular inflammatory diseases.

Therapy

Reports about successful MAR therapy are very rare. The use of immunosuppressive agents was ineffective in most cases. However, in single cases the administra-

tion of corticosteroids, corticosteroids in combination with azathioprine and plasmapheresis or the intravenous application of immunoglobulins could achieve improvement of vision disturbances. In addition, operative debulking of tumor masses or their irradiation could positively influence on MAR symptoms.

Prognostic Relevance

The prognostic relevance ►(autoimmunity and prognosis) of MAR symptoms remains unclear. Although the appearance of MAR symptoms is mostly associated with progression of disease, retrospective analyses did not find significant differences between the overall survival rates of melanoma patients with or without MAR. Further, it is still unclear if the antiretinal antibodies found in the serum from MAR patients have a protective effect for the individual patient. Background of this speculation is the observation of patients with non-small cell lung cancer or breast cancer who show extended overall survival if antitumoral antibodies are detected. However, it must be speculated that immunosuppressive therapy of MAR symptoms is associated with increased mortality. The comparison of melanoma patients with or without MAR concerning 5-year survival rates is not possible, as the number of MAR patients worldwide is too small for such analyses.

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Melanoma Vaccines

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Synonyms

Active; Specific immunotherapy for melanoma

Definition

Melanoma vaccines aim at inducing or enhancing cellular and humoral immune responses specifically against melanoma cells.

Characteristics

Historical Background

The earliest concepts for immunotherapy of malignant tumors were developed at the beginning of the twentieth century, when Paul Ehrlich suggested that the newly discovered antibodies be directed as "magic bullets" specifically against cancer cells. Various approaches to stimulate tumor immune defence have been experimentally developed in the last few decades. Patients with malignant melanoma have been of particular interest in the field of tumor immunology because clinical observations such as spontaneous regressions of primary tumors in the skin supported the idea that the immune system is capable of recognizing and eliminating melanoma cells. Immunological approaches were believed to be particularly suited to prevent the early metastatic spread of melanoma cells, which is associated with high mortality. Initial clinical studies performed with bacterial extracts derived from Corynebacterium parvum or Bacillus Calmette-Guérin as an adjuvant immunotherapy were initiated in the 1970s but were largely unsuccessful. Recombinant cytokines such as interferon-alpha (IFNα) and interleukin-2 (IL-2) were introduced for immunotherapy of melanoma in the 1980s. Encouraging results were noted in some patients with inoperable metastatic melanoma, which showed complete and long-lasting remissions following treatment with high-dose IL-2. The occurrence of vitiligo-like depigmentation and other autoimmune phenomena in patients treated with IFNα or IL-2 appear to correlate with a favorable clinical course, suggesting that an activation of the immune system against pigment cells is involved.

Tumor Immune Defence against Transplantable Tumors in Mouse Models

MacFarlane Burnet proposed in the 1950s that the immune system is principally able to recognize and eliminate cancer cells. This "immunosurveillance hypothesis" was controversially discussed for many years. The biologic understanding of immune defence against malignant tumor cells was widely studied in experimental mouse models involving the transplantation of chemically or virally transformed tumor cells. The analysis of humoral immunity against transplanted carcinogen-induced sarcomas revealed that the immune system is in principle capable of detecting molecular events associated with transformation such as the aberrant expression of p53. Adoptive transfer experiments showed that cellular components of the immune system were responsible for the rejection of transplanted tumor cells. In the 1980s it was discovered that cytotoxic T cells (CTL) recognize peptide fragments derived from intracellularly synthesized proteins bound to major histocompatibility (MHC) class I molecules on the cell surface and are able to specifically detect and destroy not only virus-infected

but also tumor cells. Various experimental studies using well-characterized model antigens showed that recombinant vaccines were able to induce immunity against viral infection and transplanted tumor cells, provided that the tumor cells express the model antigen themselves.

Culture of Melanoma-Specific Human T Cells and Identification of Melanoma Antigens

In the 1980s melanoma-specific CTL could be isolated from peripheral blood or tumor tissue of melanoma patients and propagated in vitro with the help of recombinant IL-2, a T cell growth factor. In the 1990s a number of melanoma-antigens recognized by these melanoma-specific CTL could be identified on the molecular level. These belonged to three principal categories: (i) tumor-specific antigens such as the MAGE family of proteins which were termed "►cancer-testis-antigens" because they are expressed by tumor cells as well as by germ cells in the testes; (ii) ►lineage differentiation tumor antigens such as the tyrosinase family of enzymes, gp100 or MART/MelanA which are expressed by melanoma cells as well as melanocytes; and (iii) ►individually mutated tumor antigens which in some cases even participated in malignant transformation. The identification of melanoma antigens recognized by T cells provided the scientific basis for the development of T cell-directed melanoma vaccines.

Rational Development of Melanoma Vaccine Strategies in Animal Models

Strategies for melanoma vaccination were experimentally developed over many years using the transplantable mouse B16 melanoma cell line in the syngeneic, inbred C57BL/6 mouse strain. Unlike experiments with carcinogen-induced sarcomas, vaccinations with irradiated B16 melanoma cells were not able to protect mice against a subsequent challenge with viable B16 melanoma cells showing that these melanoma cells were poorly immunogenic. Cytokine gene transfer such as the transduction of B16 melanoma cells with GM-CSF significantly improved their immunogenicity. This was later shown to be due to the ability of GM-CSF to recruit and activate antigen-presenting dendritic cells (DC) which are critical for the induction of an effective cellular anti-tumor immune response. The establishment of protocols for the ex vivo culture of DC from precursors in bone marrow or peripheral blood with the help of recombinant GM-CSF enabled their use as a biologic vaccine adjuvant. Injections with GM-CSF-derived DC pulsed with B16 tumor cell lysates were also able to promote protective immunity against a subsequent challenge with viable B16 melanoma cells. With the discovery of MHC class I-restricted antigen-recognition by T cells and the molecular identification of different melanoma antigens, active specific approaches for melanoma vaccination were developed using well-characterized model antigens. Vaccines employing defined synthetic MHC class I-binding peptides with appropriate adjuvants, recombinant plasmid DNA or recombinant viral vectors were able to successfully protect against a subsequent challenge with antigen-transduced B16 melanoma cells. Cultured DC and stimulation of Toll-like receptors (TLR) with synthetic oligonucleotides, which activate the innate immune system, were required for the induction of an effective tumor-immune response against lineage-specific differentiation antigens in a therapeutic experimental setting.

Efficacy of Melanoma Vaccines in Clinical Trials

Different strategies for therapeutic melanoma vaccination have been tested in clinical trials in the last 20 years. They can be grouped into vaccines on the basis of

1. Autologous tumor cells
2. Allogeneic tumor cells
3. Synthetic or recombinant molecules

The use of autologous tumor cells has the principal advantage that immune responses can potentially be stimulated against a broad spectrum of (unknown) tumor-antigens. This includes the mutated proteins of the individual tumor. Both the generation of adequate amounts of autologous tumor cell lysates as well as the in vitro establishment of autologous melanoma cell lines requires the acquisition of sufficient tumor material. This is only possible for patients with disseminated disease where tumor metastases can be easily excised. Clinical vaccine trials using autologous GM-CSF-transduced melanoma cells, cultured DC pulsed with autologous melanoma cell lysate or purified heat-shock protein-preparations have all shown that this strategy can successfully induce objective tumor regressions and long-lasting remissions in a small subset of patients. Unfortunately, most patients did not profit from the vaccine but it needs to be considered that these trials were largely performed in patients with advanced disease.

The idea of inducing a tumor-specific immune response using a vaccine approach appears to be particularly attractive in patients with clinical stage III melanoma after complete operative elimination of all detectable locoregional metastases. Autologous melanoma is usually not available in this situation. Therefore, well-characterized allogeneic melanoma cell lines derived from other patients have been used for vaccine purposes. This approach is potentially able to induce immune responses against cancer-testis antigens and lineage-specific differentiation antigens. Clinical trials involving large numbers of patients have been performed over many years. The results of these trials, reported in the last couple of years, claimed that this type of melanoma vaccine can be as effective as adjuvant treatment with recombinant IFNα.

The molecular identification of melanoma antigens and the fact that many melanoma patients spontaneously show ►tumor antigen-specific cellular immunity have also promoted the development of vaccine strategies with synthetic peptides and recombinant viral vectors. Peptide-based vaccines could be made increasingly efficient with the help of recombinant cytokines (such as GM-CSF, IFNα or IL-12), cultured DC or synthetic CpG oligonucleotides as a TLR9 agonist. Various recombinant viral vectors including adenovirus, vaccinia virus or canarypox virus carrying cDNA for defined melanoma antigens were also successfully used in clinical trials. Optimized melanoma vaccine strategies could effectively induce or enhance specific immune responses against the MAGE-family of cancer-testis antigens or against melanocyte differentiation antigens like tyrosinase, gp100 or MART/Melan-A *in vivo*. However, despite objectively measurable vaccine effects, only very few patients responded clinically with tumor regression and a longer-lasting remission.

Barriers for the Successful Implementation of Therapeutic Melanoma Vaccines

Current research is directed towards understanding the reasons why melanoma vaccines are not as effective as expected in the clinical situation. Sensitive and reproducible procedures for the measurement of spontaneous and vaccine-induced immune responses against defined as well as unknown melanoma antigens were developed in the last decade. These include, for example, flow cytometric enumeration of specific CTL with recombinant MHC class I-peptide tetramers and evaluation of their ability to produce IFNγ using intracellular cytokine staining or the Enzyme-Linked-Immuno-Spot (ELISPOT) technique. The detection of melanoma-specific CTL present in very low numbers in blood or tumor tissue in vivo could be achieved by in vitro expansion in limiting dilution assays.

Insights into the molecular and cellular mechanisms underlying the control of immune reactions and the maintenance of peripheral tolerance in recent years have also advanced the development of vaccines. It has become clear that the induction of CD8 + CTL is tightly controlled by regulatory lymphocyte populations via specific cell surface molecules and transcription factors such as CTLA-4 and FoxP3, respectively. In mice, selective elimination of regulatory T cells or blockade of CTLA-4 is able to circumvent peripheral tolerance mechanisms and enhance the induction of melanoma-specific cellular immunity in vivo. Stimulation of TLR was also shown to transiently suppress regulatory control and enhance vaccine responses.

Further important insights could be gained with a detailed analysis of cellular immune responses in blood and tumor tissue of individual melanoma patients, which showed remarkable clinical regressions following melanoma vaccination. Injections of autologous tumor cells together with cultured DC as an adjuvant could enhance existing spontaneous CTL-responses against various melanoma antigens. Vaccinations against the MAGE family of antigens were able to induce MAGE-specific CTL, which appeared to support the functional reactivation of preexisting CTL in the tumor tissue with other specificities. This observation demonstrated the importance of mechanisms regulating immune cell function within the tumor microenvironment, which is still not completely understood.

Tumor cells need to communicate with endothelial cells, fibroblasts and immune cells to support progressive tumor growth, invasive spread and angiogenesis. The interaction between tumor growth control, inflammatory responses and cytotoxic immunity in the pathogenesis of cancer is actively studied by many groups in the field. For example, the production of cytokines such as IL-6 and TGFβ in tumor tissue is able to stimulate cell proliferation and simultaneously suppress the function of CTL. Various mechanisms contribute to the escape of melanoma cells from recognition and destruction by the cellular immune system. For example, melanoma cells are able to impair the function of DC and T cells and can down-regulate MHC class I-restricted processing and presentation of antigens. Novel experimental mouse models have been generated in the last few years where melanomas can be induced in the skin by UV and spontaneously metastasize in lymph nodes and visceral organs on the basis of defined genetic alterations in the germline. These genetically engineered mouse melanoma models, which portray the clinical situation in patients much more closely than the transplantion of melanoma cell lines, may help to understand the role of molecular and cellular mechanisms regulating immune cell function in the pathogenesis of melanoma and find ways to improve the therapeutic efficacy of melanoma vaccines.

Summary and Future Perspective

Various strategies for melanoma vaccination have been evaluated in clinical trials in the last 10–20 years. Several approaches, which had been rationally developed in experimental animal models, were clearly able to induce melanoma antigen-specific cellular and humoral immunity in patients. A favorable influence on the clinical course of the disease could only be achieved in a small minority of patients. Melanoma vaccines have disappointed the expectations. Most vaccine approaches have been evaluated in patients with advanced metastatic disease that had failed standard treatment options. Conceptually, melanoma vaccines should be much more effective in the adjuvant setting where the immune system can specifically detect and eradicate melanoma micrometastases in patients at high risk of recurrent disease. Melanoma

vaccines may also become part of future treatment regimens for inoperable metastases which combine novel targeted anti-proliferative, anti-angiogenic and immunostimulatory measures in order to attack tumors simultaneously from several sides. These approaches will have to aim at supporting the efficacy of melanoma-specific CTL in tumor tissue, for example by creating the cytokine milieu associated with viral infections using appropriate synthetic oligonucleotides and by blocking regulatory mechanisms at the molecular or cellular level. Futuristic concepts include the adoptive transfer of ex vivo genetically engineered DC and CTL which are optimized for tumor cell destruction and may represent the "magic bullets" of the twentieth century.

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Melanosomes

Definition

A membrane-bound cytoplasmic organelle in which melanin is produced. Melanosomes are found in cutaneous and extracutaneous melanocytes, and in the pigment epithelium of the eye.

►Melanocytic Tumors
►Melanoma

Melanotic Medulloblastoma

Definition

Rare variant of ►medulloblastoma containing focal accumulations of melanin-containing epithelial next to typical medulloblastoma cells.

Melanotic Schwannoma

Definition

Is a rare pigmented nerve cell tumor most commonly occurring in the paraspinal region, but may also be located in the skin and express local aggressivity. This tumor may occur singly, but it may also appear as part of the ►Carney complex, which consists of various but specific tumors.

Melatonin

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Definition

►Melatonin is an ►indoleamine synthesized from tryptophan and secreted from the ►pineal gland during the night. Peak concentrations in blood reach 60–80 pg/mL at around 2–3 a.m. in humans, whereas daytime levels are barely detectable. The half-life of melatonin in the blood is ~50–60 min. As a result of first-pass metabolism in the liver, 90% of melatonin is catabolized to ►6-sulfatoxymelatonin, which is excreted in the urine. Most of the melatonin (e.g., 70%) circulating in the bloodstream is bound to albumin. The absolute oral bioavailability of melatonin (2–4 mg) is 15% with peak levels (2–4 ng/mL) being reached within ~1 h of ingestion. The bioavailability of lower amounts (i.e., micrograms) of oral melatonin varies widely. Melatonin is regularly consumed from commercially available nutritional supplements by millions of people throughout the world primarily for sleep problems and/or jet lag. The long term health implications of this are unknown.

Characteristics

Melatonin is among the most phylogenetically old (e.g., 3–4 billion years) and fundamental biological signaling molecules (►Hormones and Cancer) found in a wide range of organisms as simple as bacteria and as complex as man. This compound is often present in high concentrations in many flowering and edible plants as well as in medicinal herbs; in this context it is referred to as phytomelatonin. In humans, melatonin is produced during the night primarily by the pineal gland, a pea-sized ►neuroendocrine gland located deep within the center of the brain. Nighttime melatonin

production follows a circadian (e.g., daily) rhythm that is synchronized by the light/dark cycle. Light reaching specialized photoreceptors in the retina of the eye stimulates nerve impulses that are transmitted to a central ►biological clock mechanism residing in a small cluster of cells called the ►suprachiasmatic nucleus (SCN). The SCN is located in the brainstem, below the cerebral cortex, in a structure called the hypothalamus. The SCN is a central pacemaker or biological clock driving the ►circadian rhythm of nocturnal pineal melatonin production. Daylight resets the central biological clock in the SCN, whereas light, of sufficient intensity, wavelength, and duration, present during the night suppresses melatonin production. Although darkness is absolutely necessary for melatonin production to occur during the night, it is not sufficient to stimulate melatonin production during the day.

Melatonin is soluble in both lipid and water compartments of living cells and thus has the ability to easily pass through all morphophysiological barriers including cell membranes. This pleiotropic molecule is involved in myriad functions including phase-shifting of circadian rhythms, sleep/activity cycles, body temperature, seasonal reproductive rhythms, retinal physiology, immune activity, vascular tone, intermediary metabolism, free radical/antioxidant mechanisms, mitochondrial activity, and cancer development and growth. Melatonin mechanism of action in the regulation of many of these processes is thought to involve membrane-associated, inhibitory G protein-coupled ►melatonin receptors (MT_1 and MT_2) the activation of which lead to the suppression of production of the second messenger molecule, 3′, 5′ - cyclic adenosine monophosphate (cAMP). As the neurohormonal expression of darkness, the timing and duration of circadian melatonin signal in essence "tells" all the cells, tissues and organs of the body not only that it is dark, but the length of the dark period as well. In this way, melatonin acts as one of the hands of the central circadian pacemaker to help coordinate physiological and metabolic activities in synchronization with the 24-h solar day.

Melatonin Anticancer Action and Mechanisms

Both the physiological melatonin signal as well as the administration of exogenous melatonin near the beginning of or during the dark phase suppress the initiation phase of tumorigenesis in experimental rat models of chemical ►carcinogenesis. This may be accomplished via melatonin ability to suppress the accumulation of DNA adducts (the resulting complex when chemicals bind to DNA) formed by carcinogens that cause damage to and permanent alterations in DNA (i.e., mutations and amplifications), which lead to neoplastic transformation. The inhibition of DNA adduct formation appears to relate to melatonin's potent and direct free radical scavenging action (►Oxidative Stress). It may also indirectly detoxify carcinogens via activation of the glutathione and related antioxidative pathways. In addition to protecting cells from DNA damage, melatonin may also promote the repair of DNA once damage has occurred.

Melatonin inhibits the proliferation of a number of human cancer cell lines *in vitro* such as breast, prostate, ovarian, liver, endometrial, choriocarcinoma, melanoma, colon, and bladder cancer cells. At pharmacological levels, melatonin exerts cytotoxic effects on cancer cells whereas nocturnal, physiological levels induce oncostatic effects. One of melatonin important antiproliferative mechanisms is to delay the progression of cells from the $G_{1/0}$ to the S phase of the cell cycle (►Cell-cycle Targets for Cancer Therapy). Either alone or in combination with other agents, melatonin induces apoptotic cancer cell death (►Apoptosis). In some neoplastic cells, this indoleamine acts as a differentiating agent and diminishes their invasive/metastatic potential via alterations in adhesion molecule expression and the support of mechanisms responsible for gap junctional intercellular communication. A wide array of biochemical and molecular mechanisms of melatonin's oncostatic action *in vitro* have been reported including the regulation of estrogen receptor expression and transactivation, calcium/calmodulin activity, protein kinase C activity, cytoskeletal architecture and function, intracellular redox status, melatonin receptor-mediated signal transduction cascades, aromatase and telomerase activity, and fatty acid transport and metabolism.

A key to melatonin cancer inhibitory action on tumor growth *in vivo* involves the essential omega-6 ►polyunsaturated fatty acid (PUFA), ►linoleic acid (LA). As the most prevalent PUFA in the Western diet, LA levels greatly exceed those required to prevent essential FA deficiency (i.e., 1% of total calories). As a potent promoter of both murine and human tumorigenesis, LA exerts actions on cancer cells that are diametrically opposed to many of the oncostatic actions of melatonin listed above. Its oncogenic effects are related to its ability to upregulate the expression of genes involved in estrogen receptor (ERα) expression, cell cycle progression, G protein signaling, and the mitogen-activated protein kinase (MAPK) growth cascade. In both tissue-isolated ERα (+and−) human ►breast cancer xenografts, melatonin acts via melatonin receptors to suppress tumor cAMP formation leading to a suppression of LA uptake and its metabolism to the mitogenic signaling molecule ►13-hydroxyoctadecadienoic acid (13-HODE). Down-regulation of LA uptake and metabolism reduces the activation of the epidermal growth factor receptor/MAPK pathway, culminating in tumor growth inhibition. The fact that LA up-regulates whereas melatonin down-regulates transcriptional regulation of ERα in human breast cancer cells via a melatonin receptor-mediated inhibition of cAMP in these cells would potentially

provide ample opportunity for cross-talk among these pathways. Like rat hepatomas, human breast cancer xenografts exhibit a circadian rhythm of tumor proliferative activity, LA uptake, and metabolism and signal transduction activity that is driven by the nocturnal, circadian melatonin signal.

Nocturnal Melatonin Suppresson by Light at Night and Human Breast Cancer Risk

The risk of developing breast cancer is up to five times higher in industrialized nations than in underdeveloped countries. Overall, nearly 50% of breast cancers cannot be accounted for by conventional risk factors. Westernized nations have increasingly become 24-h per day societies with greater numbers of people being exposed to more artificial light during the night both at home and particularly in the workplace. It has been postulated that light exposure at night may represent a unique risk factor for breast cancer in industrialized societies via its ability to suppress the nocturnal production of melatonin by the pineal gland. This hypothesis is based on studies showing that melatonin inhibits the development and growth of experimental models of breast cancer whereas either surgical removal of the pineal gland or exposure to constant light stimulates mammary tumorigenesis in rodents. This postulate is further strengthened by recent epidemiological studies demonstrating that women working night shifts have a significantly elevated risk of breast cancer presumably due to their increased exposure to light at night (►Cancer Epidemiology). A similar observation has been made for prostate cancer in men (►Prostate Cancer – Basic Characteristics and Experimental Models).

Experimental studies have uncovered important new relationships between circadian biology, the endogenous nocturnal melatonin signal, and its suppression by light at night, relative to human breast cancer risk. The proliferative and metabolic activity of human breast cancer tissue, growing in a nude rat, perfused *in situ* with blood collected during completely dark nights from premenopausal female subjects (i.e., high melatonin), is markedly reduced as compared to when the tissue is perfused with daytime blood (i.e., low melatonin). Subsequent exposure of the subjects to bright, fluorescent light during the night completely extinguishes the tumor inhibitory activity of their blood by lowering melatonin levels. Therefore, melatonin is the first soluble, nocturnal anticancer signal to be identified in humans that directly links the central circadian clock with some of the important mechanisms regulating breast carcinogenesis. These findings also provide the first definitive nexus between the exposure of healthy premenopausal female human subjects to bright, white light at night and the enhancement of human breast oncogenesis via ►circadian disruption (i.e., suppression) of the nocturnal, oncostatic melatonin signal. The suppression of circadian melatonin production by ocular exposure to bright white light at night, leading to augmented nocturnal tumor uptake of dietary LA and its conversion to mitogenically active 13-HODE, can now be afforded serious consideration as a new risk factor for human breast cancer.

In non-shift working women, evidence suggests a decreased breast cancer risk associated with higher, first morning urine levels of 6-sulfatoxymelatonin or sleep duration ≥9 h per night (longer melatonin duration?). In some cancer patients, the nocturnal amplitude of circulating melatonin levels is reduced to various degrees. In breast cancer patients in particular, nocturnal circulating levels of melatonin are negatively correlated with breast cancer ERα content while tissue levels of melatonin correlate positively with tumor ERα status and negatively with the nuclear grade and proliferative index. These findings suggest that cancer cells elaborate soluble factors that negatively feedback on the mechanisms regulating nocturnal melatonin production.

Clinical Cancer Trials with Melatonin

Over the past three decades, nearly 50 small clinical trials have been performed by a single group in Italy to test melatonin clinical efficacy in cancer patients. In a minority of these studies, melatonin was given as a single agent while in most cases it was administered in combination with other standard therapies, including chemotherapy, immunotherapy or radiation therapy, exclusively to patients with advanced stage solid tumors that had become refractory to standard therapy alone. More than half of these trials consisted primarily of non-randomized broad phase II trials in which melatonin therapy was administered to patients with a wide range of malignancies; the remainder were randomized, controlled phase II trials that were disease-specific for lung cancer, colorectal cancer, breast cancer, glioblastoma, and brain metastases from solid tumors. In no case were the trials double-blind and/or placebo-controlled. An oral dose of anywhere from 10 to 50 mg of melatonin (usually 20 mg) was administered to cancer patients in the early evening either alone or concurrently with chemo- or radiation therapy. Although an objective partial tumor response was observed in a small percentage of patients receiving melatonin, the majority of tumor responses consisted of disease stabilization. Probably the most dramatic effect of melatonin treatment was a markedly improved 1-year survival in these patients compared with those receiving supportive care, chemotherapy, or radiotherapy alone.

Other benefits that accrue from melatonin therapy as reported in these clinical trials are a reduction in the toxicities associated with chemotherapy including myelotoxicity, nephrotoxicity, thrombocytopenia, lymphocytopenia, stomatitis, neuropathy, and cancer

cachexia. Probably the most clinically important aspect of these trials is that melatonin-treated cancer patients apparently achieve and maintain better performance status and experience less anxiety than those individuals not receiving the indoleamine. Performance status represents the general well being and quality of life of the patient. In fact, patients who have a better performance status at the outset usually respond more favorably to melatonin alone or in combination with other more conventional therapies.

The most common reported side effects of therapeutic (milligram) doses of melatonin include sedation, drowsiness, and mild hypothermia. In a recent randomized, double-blind, placebo-controlled trial, oral melatonin (10 mg) administered prior to sleep for 1 month was found to exert no toxic effects in on a wide range of physiological and neurological parameters in healthy individuals. In the clinical cancer trials, in which up to 50 mg of melatonin was administered daily for 1–3 years, no adverse side effects warranting its discontinuation were reported.

Summary and Conclusions

The nocturnal, circadian melatonin signal is a newly identified inhibitory link between the central circadian pacemaker in the brain and the processes governing cancer development and growth in both experimental animals and humans. This anticancer signal in essence organizes the processes controlling oncogenesis within biological time structure and in doing so, provides the body with a degree of protection from the development and growth of cancer cells during each night. Melatonin blocks the ability of cancer cells to take-up linoleic acid and convert it to the mitogenic signaling molecule 13-HODE. Bright light at night, which suppresses this onocostatic signal, represents a newly identified risk factor for breast cancer in night-shift workers. Clinical trials indicate that nighttime melatonin supplementation may offer a promising new approach in the treatment of advanced stage malignancies and reduction in the toxicity of chemotherapy, immunotherapy and/or radiation. Pharmacological doses of melatonin appear to be safe and generally well tolerated in cancer patients. The role of melatonin in human cancer prevention remains to be explored.

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Melatonin Receptors

Definition

A superfamily of membrane associated ►G-protein coupled receptor proteins containing seven transmembrane domains that recognize and mediate major actions of ►melatonin.

Melphalan

Definition

Also known as L-PAM, L-Sarcolysin, Phenylalanine Mustard, or Phenylalanine Nitrogen Mustard, and by the brand name Alkeran. Melphalan is classified as a nitrogen mustard-derivative alkylating agent and a cytotoxic for use in chemotherapy. Melphalan inhibits DNA and RNA synthesis through the formation of interstrand DNA cross-links. Melphalan is non-specific for cell cycle phase and also exhibits immunosuppressive properties.

►Alkylating Agents
►Hyperthermia

Melting Point Analysis

Definition

Identification of molecular mutations based on melting of mutated alleles at lower temperatures compared to unmutated alleles.

►Leukemia Diagnostics

Membrane Fluidity

Definition

This property of membranes is associated with lipid mobility, necessary for the correct distribution and

function of certain proteins. Viscosity is an antonymous term often used to refer to the same physical property.

►Membrane-Lipid Therapy

Membrane-Linked Docking Protein

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Definition

Membrane-linked docking protein (MLDP) is a signal-transducing molecule, and consists of the following family members: (i) FRS2/SNT (fibroblast growth factor receptor substrate 2/suc1-associated neurotrophic factor target) (ii) Dok (docking protein), (iii) TRAP (transmembrane adaptor protein), (iv) Gab (Grb2-associated binder), and (v) IRS (insulin receptor substrate). The protein has the following structural motifs: (i) membrane-linking function and (ii) scaffolding or adaptor function, and (iii) multiple tyrosine residues capable of being phosphorylated by tyrosine kinases. However, the protein itself has no enzymatic activity, as well as no or very short (less than 40 amino acid residues) extracellular domain.

Although MLDP works as a mediator of signal transduction, the protein families represent groups of adaptor proteins N-termini of which are anchored in the membrane and the rest of which in the cytoplasm.

Characteristics

Mechanism of Signal Transduction by MLDP

MLDP is localized on the inner side of membrane, since the protein contains (i) ►PH (Pleckstrin homology) domain, (ii) membrane-anchor domain of a stretch of hydrophobic amino acid residues, or (iii) a palmitylation/myristylation site at or close to N-terminus of MLDP. The upstream signals are received by MLDP with two steps (Fig. 1). The first step is the docking at the inner side with transmembrane receptors. Growth factors, cytokines, or antigens bind to transmembrane receptors, resulting in dimerization or oligomerization. The receptors are phosphorylated by elevated tyrosine kinase activity themselves or by activated cytoplasmic tyrosine kinases. MLDP binds to the phosphorylated tyrosine site on the receptors using ►PTB (Phosphotyrosine binding) domain. Once docked, several tyrosine residues of MLDP are phosphorylated by the activated ►receptor tyrosine kinase. In turn, MLDP provides with Tyr-phosphorylation sites as a platform to downstream effector proteins for their binding, such as to SHP2 (phosphotyrosine phosphatase = PTP) containing two ►SH2 domains. When inactive SHP2 is recruited to MLDP for its binding, conformational changes of SHP2 take place, thus resulting in the activation of phosphatase of SHP2. SHP2 subsequently activates ERK (one of the ►MAP kinases) pathway, resulting in cell proliferation. Another MLDP-binding protein, p85, regulatory subunit of ►phosphatidyl inositol (PI) 3-kinase, is also recruited after tyrosyl phosphorylation of MLDP, leading to activation of ►AKT survival pathway.

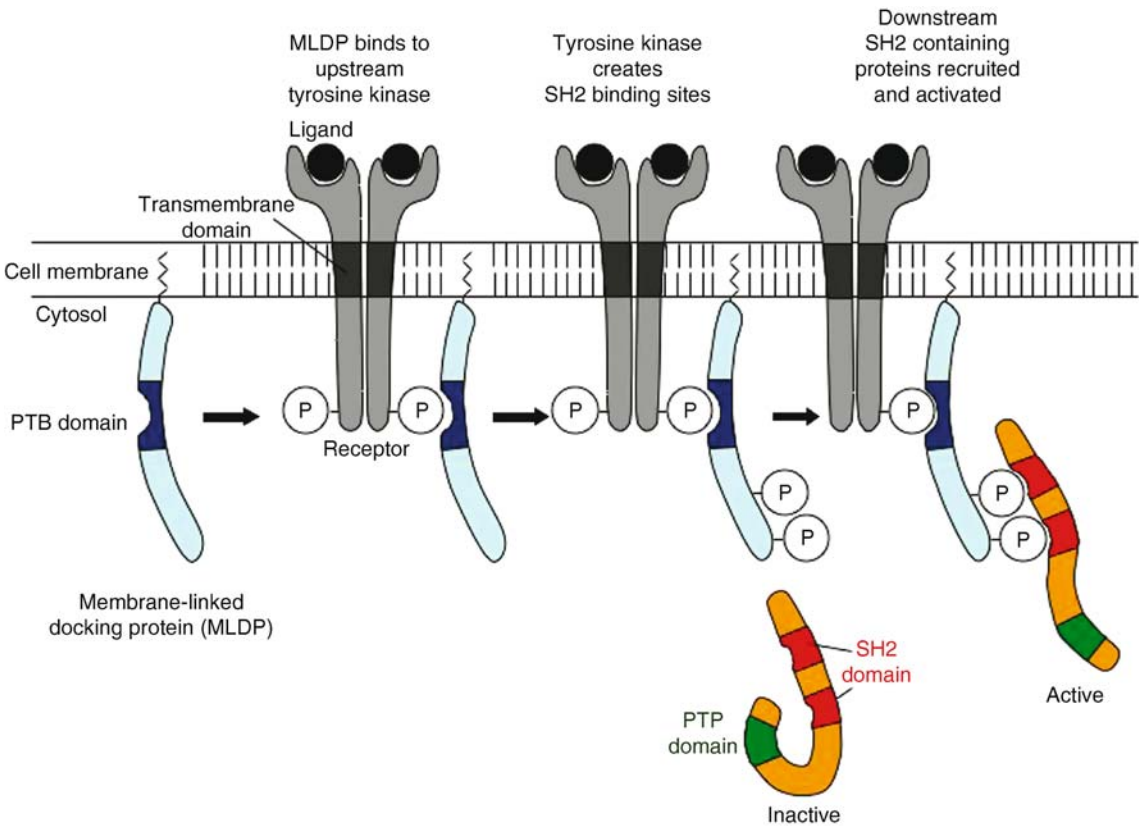
Roles of Individual MLDP Family in Normal and Cancer Cells

Five families of MLDPs, binding proteins to the ►PTB domain, and the binding molecules to the phosphorylated tyrosine residues exist (Fig. 2).

FRS2/SNT

FRS2/SNT family consists of two members: FRS2α/SNT-1 and FRS2β/SNT-2/FRS3. Each protein consists of a membrane-anchoring N-myristylation signal, a PTB domain, and a C-terminal side region containing tyrosine residues which serve as the binding sites, when phosphorylated, for the SH2 domains of ►Grb2 adaptor protein and SHP2 tyrosine phosphatase. Although both FRS2 family members are highly homologous, their different expression patterns point to the presence of specific roles for each member. *Frs2α/Snt-1* is ubiquitously expressed at every developmental stage of the mouse, whereas high levels of expression of *Frs2β/Snt-2* are confined to several tissues of neuronal or epithelial origin.

Tyrosine phosphorylated FRS2/SNT proteins serve as a platform for multiprotein complexes induced by activation of several receptor tyrosine kinases with their corresponding ligands, such as ►fibroblast growth factor (FGF), nerve growth factor (NGF), brain-derived growth factor (BDNF), and glial cell-derived growth factor (GDNF). The PTB domain of FRS2/SNT binds to phosphorylated tyrosine residues on ►TrkA, TrkB, and ►RET, although in the case of ►FGF, it binds to both phosphorylated and unphosphorylated forms of FGF receptor. The SHP2 binding sites on FRS2α/SNT-1 play a primary role for ►Ras-ERK activation and the Grb2 binding sites on FRS2α/SNT-1 recruit Gab1 and ubiquitin ligase Cbl; tyrosine phosphorylated Gab1 recruits and activates ►PI3-kinase, whereas Cbl activates the ubiquitination, leading to degradation pathway.



Membrane-Linked Docking Protein. Figure 1 Model of signaling through membrane-linked docking protein (MLDP).

RET gene rearrangements lead to generation of chimeric oncoprotein, RET-PTC, in papillary thyroid carcinomas. Point mutations on *RET* gene result in the expression of constitutively active RET in inherited ►multiple endocrine neoplasia types 2A and 2B (MEN 2A and MEN 2B) and familial medullary thyroid carcinoma. FRS2α/SNT-1 couples these oncogenic forms of RET with Ras-ERK activation and is implicated in oncogenesis. FRS2/SNT protein is also implicated in oncogenesis of another chimeric oncoprotein, nucleophosmin (NPM)-anaplastic lymphoma kinase (►ALK), which was identified in anaplastic large-cell lymphoma with the t(2:5) ►chromosomal translocation.

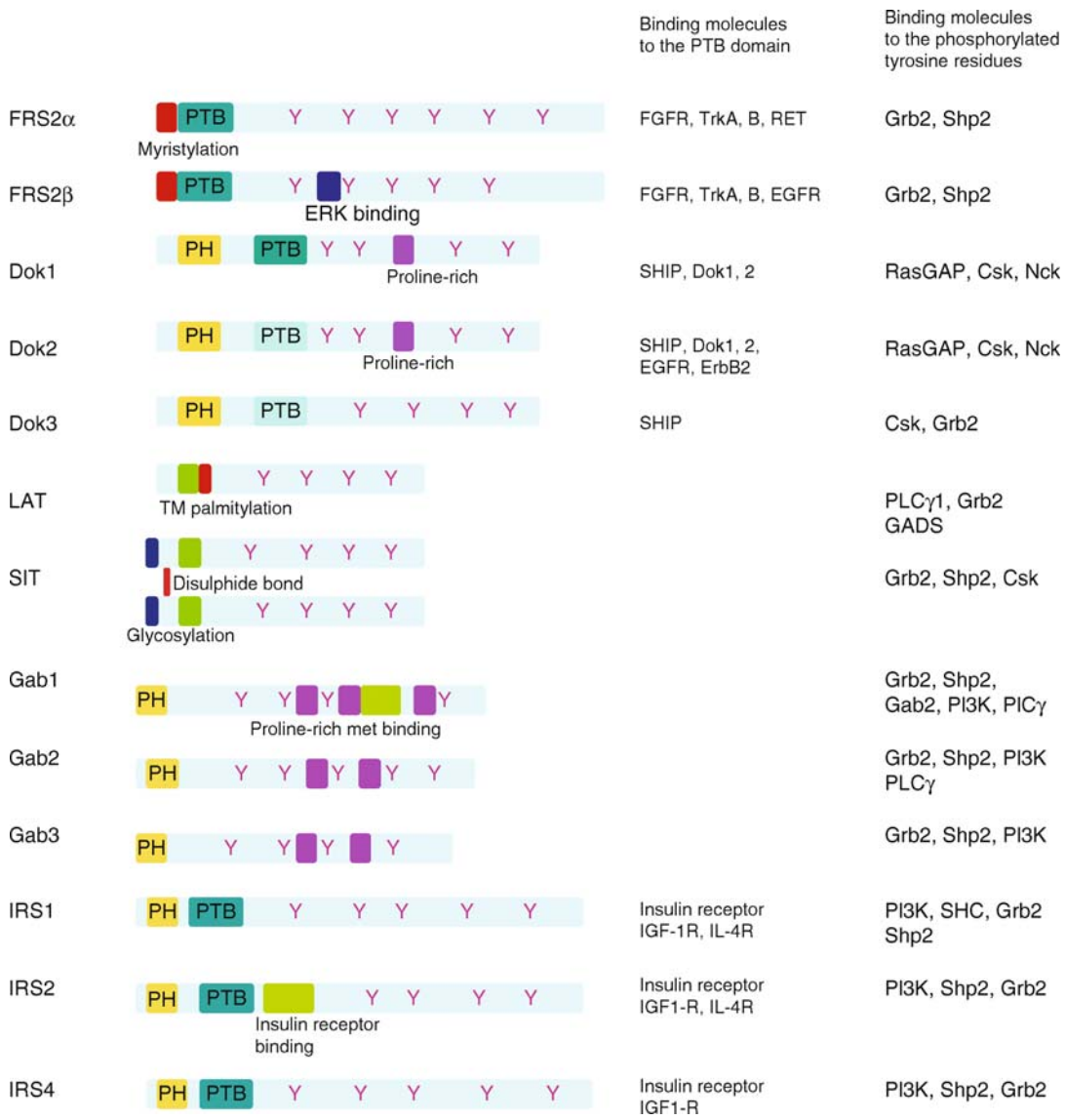
Contrary to the case of FRS2α/SNT-1, FRS2β/SNT-2 is not tyrosine-phosphorylated significantly in response to epidermal growth factor (EGF) (one of the ►epidermal growth factor receptor ligands) but it inhibits EGF signaling and cell transformation via forming a complex with ERK2. FRS2β thus acts as an adaptor protein for negative regulation of EGF receptor tyrosine kinase signaling pathways.

Dok

Dok family consists of seven members: Dok1/p62^{dok},Dok2/Dok-R/FRIP/p56^{dok}, Dok3/Dok-L, Dok4/IRS5,Dok5/IRS6, Dok6, and Dok7. Among them, Dok1–3 or Dok4 and 5 comprise a subfamily based on their structural similarity. They are typical docking proteins with an N-terminal module composed of tandem PH-PTB domains followed by a region with binding motifs for SH2 domain. Dok1 and 2 also possess proline-rich sequences within their C-terminal tails that are for binding to ►SH3 domain. The PH domain of Dok1 binds to both PI-4,5-biphosphate (PIP_2) and PI-3,4,5-triphosphate (PIP_3) in the plasma membrane. Phospholipase C (PLC) γ and PI3-kinase are also recruited to the plasma membrane where PIP_3 is synthesized from PIP_2.

Dok1 is first identified as a tyrosine-phosphorylated protein of 62 kDa (pp62) in leukemic cells isolated from patients of chronic myelogenous leukemia (CML) carrying a t(9;22) chromosomal translocation as a complex with a chimeric p210 (►Bcr-Abl) protein with elevated tyrosine kinase activity. Since *Dok1* and *Dok2* double knockout mice display CML-like disease and lymphoma, Dok proteins are considered essential for homeostatic control of activation and proliferation of hematopoietic cells.

Dok 1, 2, and 3 proteins are key negative regulators to nonreceptor tyrosine-kinases, forming multiprotein



Membrane-Linked Docking Protein. Figure 2 Schematic structures of several members of MLDPs. The phosphotyrosine binding domain (PTB) binds to phosphorylated tyrosine residues on receptors or signaling proteins. The PH domain binds to phospholipids such as phosphatidyl inositol (PI)-3 phosphate. The proline-rich domains are important for binding to SH3 containing proteins such as Src family tyrosine kinases or Grb2. Y designates potential tyrosine (Y) phosphorylation sites. The ERK binding domain in FRS2β, Met binding domain in Gab1, or insulin receptor binding domain in IRS1 contain a unique sequence for binding to ERK, Met, or insulin receptor.

complexes in response to cytokines, growth factors, and immunogens, thus controlling the delicate balance between positive and negative signals. Dok1 and 2 constitutively bind to SH3 domains of ►Src family tyrosine kinases or c-Abl. Coaggregation of B cell receptor with FcγRIIB1 or T cell receptor stimulation induces tyrosine-phosphorylation on Dok. The PTB domain of Dok binds to tyrosine-phosphorylated SHIP1 (the SH2 domain-containing inositol polyphosphate 5′-phosphatase), a negative regulator for B cell and ►T cell receptor signaling. The phosphorylated Dok1 and 2 bind to SH2 domains of Ras-GAP (►GTPase activating protein for Ras) and Csk, negative regulators for Ras and ►Src family kinases, respectively. Tyrosine-phosphorylated Dok1 and 2 also bind to SH2 domain of Nck adaptor protein, whereas Dok3 binds to Csk and Grb2 but not Ras-GAP.

Dok also regulates signaling through ►receptor type tyrosine kinases. Dok1 and 2 negatively regulate platelet-derived growth factor (PDGF), EGF, or insulin signaling. Upon EGF treatment, the PTB domain of Dok2 binds to tyrosine phosphorylated EGF receptor, and subsequently

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Dok2 is phosphorylated, and then the phosphorylated Dok2 inhibits activation of downstream effectors. On the other hand, Dok4, 5, and 6 have positive effects on neurite outgrowth stimulated by activation of RET, TrkB, or TrkC receptor tyrosine kinases. In addition, Dok7 binds to muscle-specific receptor kinase (MuSK) via the PTB domain and induces activation of MuSK for neuromuscular synaptogenesis. Mutations in *DOK7* cause a congenital limb-girdle myasthenic syndrome with a characteristic pattern of weakness, particularly affecting the proximal muscle groups.

Transmembrane Adaptor Proteins (TRAPs)

TRAPs are membrane-associated molecules that are capable of binding with different SH2 domains after the tyrosyl phosphorylation and are key mediators of immunoreceptor-mediated signaling. They are divided into two groups: raft-associated TRAPs such as LAT (linker for activation of T cells), PAG (phosphoprotein associated with glycolipid-enriched membranes)/CBP (Csk-binding protein) and LAB (linker for activation of B cells)/LAT2 and nonraft associated TRAPs such as SIT (SHP2-interacting transmembrane adaptor protein). Lipid rafts are submicroscopic regions enriched in sphingolipids and cholesterol in the plasma membrane and contain several signaling proteins including Src family tyrosine kinases. The raft-associated TRAPs but not nonraft associated TRAPs have CXXC palmitylation motif in the juxtamembrane region for targeting the lipid raft. Each TRAP has a short extracellular domain, typical transmembrane domain, and a long cytoplasmic tail that has up to nine potential tyrosyl phosphorylation sites.

LAT can be considered the master switch of T cell and mast cell activation. Stimulation of T cell receptor or high-affinity receptor for IgE (FcεRI) on mast cells leads to tyrosine phosphorylation on LAT. Then LAT binds to SH2 domains of PLCγ1, Grb2, and GADS, the Grb2-related adaptor protein. PLCγ then hydrolyses PIP_2, producing the second messengers IP_3 and diacylglycerol. Grb2 binds to Ras activator SOS. GADS binds to SLP76, another adaptor protein, leading to activation of Itk cytoplasmic tyrosine kinase and Vav, an activator for Rac.

NTAL is a LAT-like protein in non-T cells and tyrosine phosphorylated PAG inhibits Src family tyrosine kinases via binding to SH2 domain of Csk. Tyrosine phosphorylated SIT binds to Grb2, an SH2 domain-containing phosphatase Shp2 and Csk and negatively regulate T cell signaling.

Gab

Gab family consists of Gab1, Gab2, and Gab3, has PH domain at N-terminus, and phosphotyrosine sites which, upon stimulation with growth factors, cytokines, or antigens, provide binding sites for ►SH2-containing proteins (SHP2, p85 subunit of PI3-kinase, Grb2, PLCγ, or Crk). For tyrosine-phosphorylation, Gab1 directly or indirectly binds to growth factor receptors for hepatocyte growth factor (HGF)(►MET), EGF, vascular endothelial cell growth factor (VEGF), and PDGF, in addition to Src family and JAK, though tyrosine kinases involved depend on cell types and ligands. Exact mechanisms are still to be clarified for signaling from activated tyrosine-kinase to Gab proteins which contain no PTB domain, though Gab1 has direct HGF-receptor (MET)-binding domain. It has been suggested that Grb2 and/or Cbl is involved in coupling tyrosine kinase with Gab family. Gab proteins contain several proline-rich motifs that can mediate constitutive binding to the SH3 domain of Grb2. The Grb2–Gab complex targets the receptors containing Grb2 SH2 domain binding sites. In addition, tyrosine phosphorylated FRS2α/SNT-1 docking protein binds to SH2 domain of Grb2, serving as another intermediate protein for coupling Gab to the receptors. Gab1 and Gab 2 are tyrosine-phosphorylated by antigen receptors of B and T cells upon stimulation with various cytokines and antigens, whereas M-CSF stimulates Gab2 and Gab3. SHP2 activated by Gab family leads to cell proliferation by activating ERK, whereas PI3-kinase activation to cell survival by activating AKT.

Overexpression of tyrosine kinase receptors that are often detected in cancer cells leads to enhanced tyrosine phosphorylation of Gab1, which stimulates proliferation and survival through SHP2-ERK, PI3-kinase-AKT, or Crk-Rac signal transduction pathway. Constitutive activation by in-frame fusion of RET tyrosine kinase, RET-TPC phosphorylate Gab1, leading to BRAF and ERK kinase stimulation. Trp-MET oncoprotein also stimulates tyrosyl phosphorylation of Gab1.

Roles of Gab 2 in tumorigenicity have been reported: (i) frequent *Gab2* amplification was reported in ►breast cancer and (ii) Gab2-signaling pathway is used upon transformation by Bcr-Abl. In the former case, receptor activation of ERB2/►HER2/neu enhanced further Gab2 signaling.

IRS

The protein family of IRS1, IRS2, IRS3, and IRS4 has been identified originally as proteins transducing insulin signals to intracellular effectors for the glucose homeostasis. This family commonly contain PH domain near N-terminus and PTB at the following position. IRS3 may not be expressed in human. Activated insulin receptor (IR) or IGF-1 receptor binds to IRSs by its PTB domain and subsequently induces phosphorylation on multiple tyrosine sites of each IRS, which are binding sites for PI3-kinase, SHC, an adaptor protein, and also for Grb2. IRS2 is unique in that it carries insulin receptor binding domain. IRS1 has a higher binding affinity to Grb2 when compared to IRS2, thus segregating IRS1 with mitogenic and IRS2 with metabolic outcomes.

Oncogenic SV40 T antigen gene transforms human cells through IRS1 signaling, as the transforming ability was suppressed in several human cancer cell lines which do not express IRS1or at low levels. Constitutive phosphorylation of IRS1 and IRS2 appears to stimulate proliferation and motility in mammary cancer cells. IRS4 binds to nonreceptor tyrosine kinase, Brk (breast tumor kinase) which is overexpressed in a high percentage of breast cancers.

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Membrane Lipid Structure

Definition

Term used to describe the types of organizations adopted by lipids in membranes, either *in vivo* or *in vitro*, and that usually refers to the secondary structure formed by lipids.

►Membrane-Lipid Therapy

Membrane-Lipid Therapy

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Synonyms

Lipid therapy

Definition

Clinical drugs that interact with membrane lipids and that modify the composition and structure of cell membranes can change the localization and/or activity of membrane proteins. Several drugs used to combat cancer and other pathologies, both in cell and animal models or in humans, regulate ►membrane lipid structure and they produce a concomitant alteration in the localization and activity of signaling proteins. The net result of these effects is the modulation of certain signaling pathways that reverse the pathological state. Indeed, ►G proteins, ►protein kinase C (PKC) and heat shock proteins (HSPs) are among the proteins regulated by ►membrane-lipid therapy and the therapeutic agents used can inhibit cell proliferation or induce ►apoptosis and cell differentiation.

Characteristics

Heterogeneity of Membrane Lipids and Lipid Structures

Membranes are composed of thousands of different lipid molecules that interact dynamically to form the transient or stable structures used by many proteins as platforms for their activity and for their interactions with other proteins. Usually, integral (transmembrane) (►Integral membrane protein) and peripheral (amphitropic) (►Peripheral membrane proteins) proteins show important specificities in their interactions with lipids. These preferences may be associated either with a defined type of membrane lipid or a given membrane lipid structure. Most proteins are sensitive to their lipid environment, so that their activity can be modified by changes in membrane lipid composition and structure. These changes in membrane lipid composition and structure may have a physiological basis or they may be a response to external stimuli. An example of the former is the change in cell membrane lipids in response to important changes in water temperature in cold-adapted fishes, a topic widely studied by Tibor Farkas and colleagues. Alternatively, one example of the latter is the variation observed in membrane lipids after the intake of a given substance (drug, food, toxin, etc.).

Membrane lipids can organize into many more secondary structures than proteins and nucleic acids *in vitro*. Moreover, the number of lipid species exceeds the number of different amino acids and nucleic acid bases by various orders of magnitude. In contrast with proteins and nucleic acids, the spatial relationship between the lipids that form membranes is not defined by covalent bonds, they usually move freely in this environment. Furthermore, since some structural aspects related to membranes still remain unclear, the behavior of lipids is less predictable. However, this does not mean that structure-function relationships are not established in membranes and that bulk thermodynamics can explain all physico-chemical properties of membranes. In fact, changes in the structure of lipids influence not only the physical behavior of membrane lipids (membrane structure) but also the activity of

certain associated proteins (membrane function). In general, modifications in membrane lipid structure are reflected in changes in membrane lipid function. Thus, oleic acid (18:1 cisΔ9) induces a large increase in the non-lamellar (H_{II}) phase propensity of membranes and it alters the interaction and activity of G proteins. In contrast, neither the trans analogue of oleic acid (elaidic acid) nor stearic acid (18:0) markedly influence the ►lamellar phase of membranes and accordingly, they do not influence G protein activity.

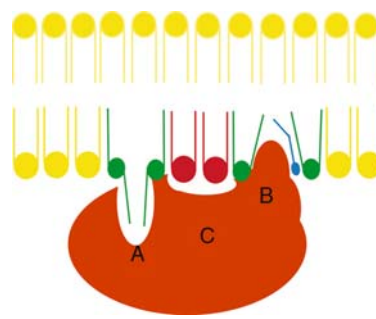
During recent years it has become accepted that the fluid mosaic model of membranes described more than 30 years ago by Singer and Nicolson is a somewhat simplified model which fails to take into account the presence of large membrane domains (e.g. the basal, lateral and apical membrane regions of polarized cells, such as glandular, endothelial and epithelial cells), as well as the smaller yet highly abundant membrane structures (lipid rafts, synaptosomes, ►caveolin domains (caveolae), coated pits, receptor clusters, etc.). These domains and structures are characterized by their characteristic lipid and protein composition. In this sense, while the membrane lipid composition most likely defines the presence of specific proteins, proteins may also influence the lipid composition of these domains.

Lipid-Protein Interactions in Membranes

Membrane proteins are classified as integral (transmembrane, intrinsic) and peripheral (amphitropic, extrinsic) proteins and both these types of proteins are very sensitive to changes in their lipid environment. For example, the exchange of sodium ions through the nicotinic acetylcholine receptor (►Integral protein) is modified by changes in ►membrane fluidity that can be achieved by altering the membrane lipid composition. On the other hand, PKCα (a ►peripheral protein) translocates from the cytosol to the membrane upon different types of stimuli. One such stimulus is an increase in the ►non-lamellar phase propensity of membranes. Thus, a high non-lamellar H_{II}-phase propensity (i.e. elevated negative curvature strain) favors the binding of PKC to membranes and its subsequent activation. Diacylglycerol induces PKC activation not only by binding to the enzyme but also by promoting the H_{II}-phase. In line with this, phorbol esters, which induce a marked activation of PKC enzyme and that are tumor promoters, also induce these non-lamellar phases. For this reason, it has been suggested that inhibitors of PKC could potentially behave as anticancer compounds. However, the anticancer drug ►minerval (►2-Hydroxyoleic acid) also increases the non-lamellar propensity of membrane lipids, activating PKC. An explanation for this apparently paradoxical behavior is that phorbol esters induce massive activation of PKC, followed by rapid enzyme depletion within minutes of the treatment. In contrast, minerval produces a mild (about two-fold) and sustained activation and overexpression of PKC. Likewise, some signaling pathways regulated by G proteins are also involved in the control of cell proliferation. In this context, the localization and activity of G proteins is also regulated by the non-lamellar propensity of membranes.

One important question regarding the regulation of protein-lipid interactions is how lipid structure can control the membrane translocation, cellular localization and the membrane sorting of these and other ►peripheral signaling proteins. Different mechanisms could be involved in these phenomena based on the formation of membrane "defects". Non-lamellar prone lipids (e.g. phosphatidylethanolamine and minerval, Fig. 1) reduce phospholipid surface packing (i.e. the lateral pressure) at the interface and the polar regions of the lipid bilayer. This allows hydrophobic domains of peripheral proteins to interact with deep hydrophobic regions of the membrane and/or fatty acid moieties of phospholipids that exit out of the bilayer plane (Fig. 1). In addition, electrostatic interactions with phospholipid headgroups and interactions with other proteins also participate in the binding of peripheral proteins to membranes. The effect of lipids in regulating the interaction of these proteins with membranes has been demonstrated using synthetic lipids and purified proteins (G proteins, PKC).

Changes in membrane lipid composition have been reported in several pathologies, including cancer. These alterations may be associated with the etiology of the



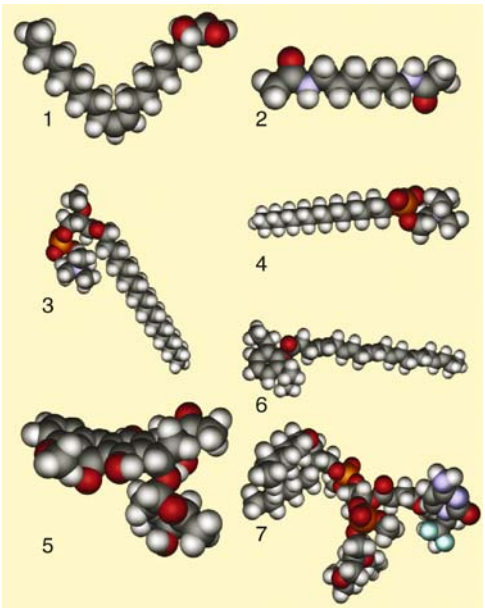
Membrane-Lipid Therapy. Figure 1 Lipids with bulky polar head, like phosphatidylcholine (yellow) and phosphatidylserine (red), have a ►molecular shape similar to a cylinder. In turn, phosphatidylethanolamine (green) has a small polar head and increases the negative curvature strain (hexagonal phase propensity) of membranes. Minerval (blue) also favors non-lamellar phases. The scheme also shows a peripheral (amphitropic) protein interacting with fatty acid moieties of phospholipids (A) or deep hydrophobic regions of the membrane (B) in non-lamellar prone regions. Electrostatic interactions with the polar head of charged phospholipids (C) also participate in the binding of this protein to membrane.

disease or they may reflect an adaptive response. Therefore, lipid interventions could be effective in reversing pathological processes. Despite the potential use of this new therapeutic approach, three important issues will require further study during the following years. First, an in-depth study of the molecular basis underlying the interaction of numerous relevant proteins with membranes will be necessary. Second, the influence of lipid changes on protein activity, pathophysiological processes and therapeutic actions must be defined. Finally, the specificity of clinical strategies based on this principle will have to be assessed. Conventional chemotherapy is based on the design of drugs to target specific proteins and the elucidation of their structure. From the molecular point of view, conventional chemotherapy and membrane-lipid therapy are quite different, although the final result is the regulation of protein activity. The fact that all membranes contain lipids could question the potential specificity of this therapy. However, there is a huge diversity of membrane lipids, a wide variety of membrane lipid compositions and structures, and tremendous variety among the protein-lipid interactions, which establishes a suitable context to design specific therapies (see above). Obviously, drugs acting through membrane-lipid therapy not only reach pathological cells but also healthy cells, as do drugs acting on a given protein through conventional chemotherapy. However, compounds used in conventional chemotherapy often interact with proteins other than their original target, causing side effects of diverse importance. Thus, the degree of specificity of both approaches could be similar. In this context, the specificity of membrane-lipid therapy is more directly associated with the effects promoted in cancer and other types of pathological cells than with its interaction with a given cell. An example of this is the activity of minerval in tumor cells where its inhibitory effects display an IC_{50} in the range of 50–100 μM in comparison with the IC_{50} value of >5,000 μM in non-tumor human fibroblasts (IMR90 cells). In addition, it has been found that minerval strongly induces the expression or repression of fewer genes than most drugs, further supporting the specificity of membrane-lipid therapy.

Development

It was proposed that the cytotoxic effects of anthracyclines on cancer cells may be exclusively exerted through their interaction with membranes. Indeed, it was later demonstrated that they acted by regulating membrane lipid structure which altered the interaction between peripheral signaling proteins and the plasma membrane. Subsequently, a potential anticancer drug under study, hexamethylene bisacetamide, was found to have a very similar effect on cell membranes, which was associated with the regulation of gene expression. In the knowledge that the mechanism of action was based on the regulation of membrane lipid structure, a number of lipid-interacting molecules were subsequently studied and accordingly, it was demonstrated that oleic acid and its derivatives are potent regulators of the membrane structure. Subsequently, minerval (the α-hydroxyl-derivative of oleic acid) was found to be a potent antitumor agent without displaying any relevant side effects.

Minerval is not the only drug used against cancer that interacts with membranes. As mentioned above, anthracyclines and hexamethylene bisacetamide also regulate membrane structure and peripheral protein-associated signaling. In addition, certain molecules that readily bind to membranes have been shown to have important anticancer effects. For instance, edelfosine (Et-18-OCH_3 (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine)) and miltefosine (HePC (hexadecyl phosphocholine)) have an important hydrophobic moiety with long hydrocarbon chains (18 and 16 C atoms, respectively), and a polar region comprised of a phosphate group and a choline moiety (Fig. 2). This polar-apolar hybrid structure appears to be a common feature of these anticancer drugs that appear to be active in membrane-lipid therapy. This physico-chemical property of these drugs may allow them to interact with both the surface-interface of the membrane and with the hydrophobic core, facilitating more stable and long-lasting



Membrane-Lipid Therapy. Figure 2 Structures of the semi-empirical RHF calculations for minerval (1), HMBA (2), edelfosine (3), miltefosine (4), ►daunorubicin (5), propofol-DHA (6) and NEO6002 in gas phase. Carbon atoms are shown in gray, hydrogen in light gray, phosphorus in orange, oxygen in red, nitrogen in light blue and fluorine.

interactions, as well as inducing the relevant effects on membrane lipid structure. Thus, an interesting class of new anticancer drugs are those compounds known to bind to lipid molecules, such as the molecule NEO6002 (Fig. 2). This drug results from the combination of ►gemcitabine with cardiolipin, a phospholipid typical of mitochondrial membranes, and it appears to be less toxic and more effective than gemcitabine alone. The lipid modification of gemcitabine induces the membrane-mediated internalization of the compound, which is not blocked by nucleoside transporter inhibitors. Another type of lipid-interacting compound is propofol-DHA (Fig. 2), which combines a well-known anesthetic (propofol) with a polyunsaturated fatty acid (docosahexaenoic acid, DHA) that is present in membranes. The resulting compound has been shown to induce apoptosis in MDA-MB-231 breast cancer cells.

Most cell functions are localized in or around membranes, and lipids control the interaction and activity of many proteins. The relevance of lipids in the treatment of cancer is also highlighted by the lipid abnormalities identified in the membranes of patients with cancer. Thus, changes in the type or abundance of lipids and other types of membrane components may produce either positive or negative effects on health. Hence, membrane-lipid therapy is a new therapeutic approach that could be used in the treatment of cancer and other pathologies.

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Membrane Permeability Transition

Definition

MPT; Increase of permeability of the inner mitochondrial membrane to solutes with a molecular mass of less than 1,500 Da. The rapid increase of permeability quickly causes depolarization, uncoupling of oxidative phosphorylation, and large-amplitude mitochondrial swelling. Onset of the MPT is caused by the opening of a very large conductance channel or pore in the inner mitochondrial membrane and can trigger the intrinsic proapoptotic pathway.

►Amine Oxidases

Membrane Potential

Definition

Is the voltage difference between the internal and the external part of the cell, in most cases ranging from −30 to −70 millivolts at rest. Membrane potential influences ion movement, for example, promotes calcium influx needed for several functions and favors potassium loss required for ►apoptosis.

►Ether à-go-go Potassium Channels

Membrane Transport Proteins

►Membrane Transporters

Membrane Transporters

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Synonyms

Membrane transport proteins

Definition

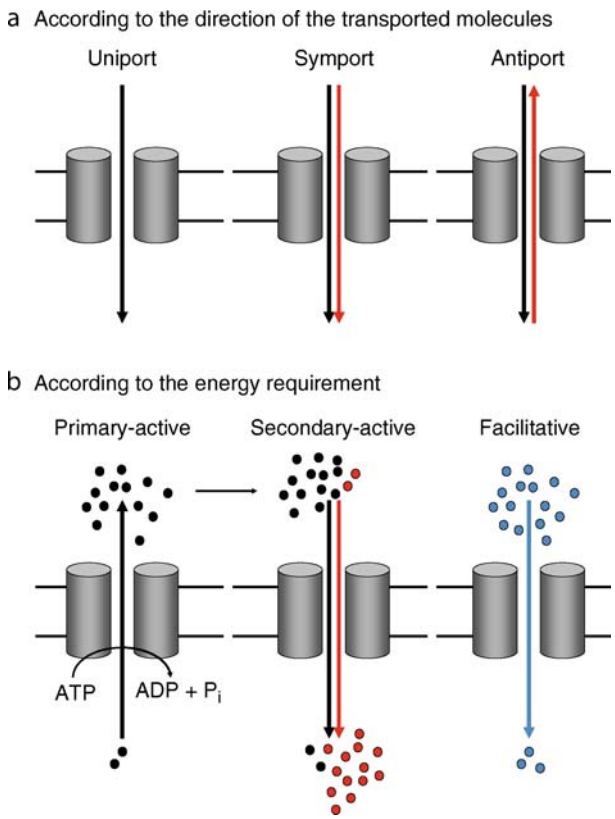
Membrane transport proteins are embedded in the lipid bilayer of biological membranes and transfer ions and small molecules across these biological membranes. Several membrane transporters contribute to the resistance of tumor cells against anticancer drugs.

Characteristics

General Features

Membrane transporters mediate the movement of ions and small molecules across the plasma membrane and the membranes of intracellular compartments such as mitochondria, lysosomes, and vesicles. The human genome comprises at least 530 genes for plasma membrane transporters (1.7% of total genes) and 350 genes for intracellular membrane transporters (1.1% of total genes). Membrane transporters typically have several transmembrane segments, each consisting of a stretch of 20–25 hydrophobic amino acids that spans the lipid bilayer of a biological membrane.

According to the direction of the transported molecules, membrane transport can be classified as uniport (one type of molecules is transported in one direction), symport (two different types of molecules are transported in the same direction), and antiport (two different types of molecules are transported in exchange with each other) (Fig. 1). Membrane transporters can also be classified as active (energy-dependent) or passive (energy-independent) (Fig. 1). Primary-active transporters are able to build up ion or solute gradients across membranes by directly utilizing the energy released during adenosine triphosphate (ATP) hydrolysis. They either belong to the ►ATP-binding cassette (ABC) transporter family or they are ion pumps (ATPases). ABC family members transport a large variety of small molecules including peptides, lipids, endogenous and ►xenobiotic organic anions and organic cations, including drugs. ATPases pump ions such as Na^+, K^+, H^+(protons), Ca^{2+}, and Cu^{2+} and contribute to maintain ion gradients across biological membranes. The Na^+ gradient is preferentially used by the so-called secondary-active transporters that can couple the flow of Na^+ down its concentration gradient with the transport of small molecules against their concentration gradients. One example is the Na^+/glucose symporter in the ►brush border membrane of enterocytes in the small intestine that mediates the uptake of glucose into the body. Passive (facilitative) transporters mediate the movement of molecules down their concentration gradients. Most of the currently known secondary-active and passive transporters are subsumed into the ►solute



Membrane Transporters. Figure 1 Classification of membrane transporters. (a) According to the direction of the transported molecules one can differentiate between uniport, symport (coupled transport), and antiport (exchanger). (b) Transporters can also be classified as primary-active, secondary-active, and facilitative. See text for details.

M

carrier (SLC) group that comprises more than 360 members in 46 families.

Similar to passive transporters, channels also mediate the movement of ions and small molecules down their concentration gradients. However, several features clearly discriminate channels from membrane transporters. Channels are pores in biological membranes that have a rather limited specificity. Substance flow through channels is controlled by the channels' open probability so that high transport rates of 10^7–10^9 molecules per second can be achieved. In contrast, transporters are able to specifically bind a substrate, but this high precision is traded in for slow transport rates of about 10^3–10^5 molecules per second. Evidently, no sugar channels have evolved, but different sugar transporters exist that can discriminate between sugars of the same size and selectively transport e.g., glucose, but not fructose.

Pharmacological Relevance of Membrane Transporters

Especially the plasma membrane transporters decisively determine which substances enter or leave a cell at sufficient rates, because it is virtually impossible for ions and most small molecules to cross the lipid bilayer of a biological membrane at a sufficient rate by simple diffusion. A number of ►SLC transporters serve as uptake transporters for nutrients and vitamins. ABC efflux pumps transport a wide variety of endogenous compounds out of cells and are localized in gastrointestinal and renal epithelia, hepatocytes, and blood–tissue barriers. The localization patterns underline that membrane transporters are essential for many physiological processes such as intestinal absorption, renal and hepatobiliary elimination and detoxification, and protection against toxins. Many SLC transporters and ABC efflux pumps transport ►xenobiotics so that they also decisively determine absorption, distribution, and elimination of drugs. They are, therefore, of important pharmacological relevance and can either be used as drug delivery systems or serve as drug targets.

Role of Membrane Transporters in Drug Resistance

Cancer cells are often resistant against a large variety of structurally diverse anticancer drugs. Two major mechanisms have been recognized that may cause this phenomenon of ►multidrug resistance, i.e., (i) an insufficient accumulation of anticancer drugs in the cancer cells and (ii) intracellular changes that impair the capability of drugs to kill the cancer cells, e.g., alterations in ►cell-cycle targets for cancer therapy, an increased repair of ►DNA damage, an inhibition of ►apoptosis, and an altered drug metabolism.

The intracellular accumulation of anticancer drugs is decisively controlled by plasma membrane transporters. Cellular drug resistance can occur either by decreased drug uptake, typically via uptake transporters, or by enhanced drug efflux, typically via primary-active transporters. Several uptake transporters that transport nutrients as physiological substrates have been implicated in the sensitivity of cells to certain drugs. For example, the presence of nucleoside transporters (SLC28 and SLC29 families) and folate transporters (SLC19 family) sensitizes cells to nucleoside analogs (e.g., ►cladribine, ►fludarabine, ►gemcitabine and ►methotrexate, respectively. Sensitivity of cells to ►platinum drugs may be due to the presence of copper transporters (SLC31 family) and organic cation transporters of the SLC22 family in the plasma membrane. The relevance of uptake transporters for the sensitivity of cells to other anticancer drugs is incompletely understood and currently intensively investigated. In addition to plasma membrane transporters, some drugs may enter cells via ►endocytosis. This pathway is e.g., utilized by some novel anticancer agents, the ►immunotoxins.

Cellular resistance resulting from enhanced drug efflux is most often mediated by members of the ABC transporter family, namely MDR1 ►P-glycoprotein (ABCB1), the ►multidrug resistance proteins (MRPs) of the ABCC subfamily, and ►breast cancer resistance protein (►BCRP; ►MXR; ABCG2). Drug substrates include methotrexate, ►vinca alkaloids, ►anthracyclines (e.g., ►adriamycin), ►podophyllotoxins, ►taxanes (e.g., ►paclitaxel), inhibitors of ►receptor tyrosine kinases (e.g., ►imatinib), and ►camptothecins (e.g., ►irinotecan). These drug efflux pumps have been identified in many human cancers so that they may contribute to drug resistance, although their precise role in establishing clinical drug resistance is still under investigation. Strategies to overcome multidrug resistance include its reversal by small molecules that function as inhibitors of ABC efflux pumps. Although already in their third generation, these reversal agents have so far not been beneficial in reversing drug resistance. Reasons for failure include the overlapping substrate specificities of several ABC drug efflux pumps and toxicity which is often caused because important physiological functions of the ABC efflux pumps are blocked by the reversal agents.

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MEN 1

►Multiple Endocrine Neoplasia Type 1

MEN 2

►Multiple Endocrine Neoplasia Type 2

MEN II

►Multiple Endocrine Neoplasia Type 2

Mendelian Distribution

Definition

The expected ratio of offspring with specific genotypes at a specific allele, as predicted by Mendel's rules of inheritance.

Meningeoma

Definition

Benign brain tumor.

Meningioma

Definition

Typically a benign tumor of the meninges

►Brain Tumors

Menopausal Symptoms After Breast Cancer Therapy

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Definition

►Menopause is the permanent cessation of menstruation resulting from loss of ovarian activity. Natural menopause is diagnosed following 12 months of amenorrhea, which is not due to a pathological cause. Menopause Secondary amenorrhea (►Early menopause, ►surgical menopause) can also be induced by surgery, chemotherapy, and radiotherapy.

Characteristics

Introduction

►Breast cancer is one of the most common causes of morbidity and mortality in women of middle years in western world. There has been a reduction in annual breast cancer death rates in almost all western countries, which may be due to the increasing use of breast screening and adjuvant systemic therapy (►Adjuvant treatment). As a result, more women are becoming long-term breast cancer survivors.

A common side effect of breast cancer treatment is the earlier onset of menopause or menopausal symptoms. Many women who are premenopausal at diagnosis will develop ►premature menopause resulting from chemotherapy, endocrine therapy, bilateral oophorectomy, or ovarian radiation, and may experience severe and long lasting menopausal symptoms. All endocrine therapy will generally induce menopausal symptoms, often for the

duration of its use. This can occur in women at any age, regardless of menopausal status. Premenopausal women who are older at the time of chemotherapy are more likely to experience chemotherapy-induced ovarian failure leading to permanent menopause. Those who do not have ovarian failure may subsequently go through menopause at a younger age. Younger patients require higher cumulative dosage of chemotherapy to induce ovarian failure and may have temporary menopausal symptoms. However, it is not possible to predict reliably whether an individual woman will go through menopause as a result of her chemotherapy treatment.

A significant number of peri- and postmenopausal women are taking hormone replacement therapy (HRT) when they are diagnosed with breast cancer. Cessation of HRT commonly leads to a return of menopausal symptoms.

Recurrence of menopausal symptoms and premature menopause in breast cancer patients can have significant negative impact on quality of life, body image, sexual function, and self-esteem. The clinical challenge lies in providing safe and effective therapy to these patients. The safety of conventional hormonal treatments for menopausal symptoms has not been established following breast cancer. Furthermore, many women will have hormone receptor positive tumors and endocrine therapy aims to reduce circulating estrogen and/or suppress ovarian activity. HRT may undermine the efficacy of these treatments. Hence, there is an urgent need to provide some alternative form of treatment to alleviate menopausal symptoms in breast cancer patients.

Treatment Options

Menopausal symptoms are variable in nature and severity, and each woman requires careful individual assessment. This should include an assessment of the likely cause, nature, frequency, severity of symptoms, and impact on quality of life of these symptoms. For some women, reassurance that her symptoms are normal and are likely to reduce over time, combined with practical suggestions for minimizing their impact is sufficient management. Available treatment interventions fall into two main groups:

Synthetic Hormonal Compound

Tibolone has weak estrogenic, progestogenic, and androgenic actions. It shows similar efficacy to HRT in reducing hot flushes and vaginal dryness, and may improve sexual function more effectively than HRT. It improves bone density but its impact on fracture rates is not known. Unwanted side effects of tibolone include a reduction in circulating high-density lipoproteins, uterine bleeding, body pain, and headache. The safety of tibolone in women with a prior history of breast cancer is subject of a large randomized controlled trial (the LIBERATE study) due to be reported at the end of 2007.

Nonhormonal Therapies

A variety of nonhormonal therapies aimed at reducing vasomotor symptoms or urogenital atrophy have been subjected to randomized controlled trials. The most common vasomotor symptom is hot flushes. However, the basic physiology underlying hot flushes is poorly understood. It is hypothesized that reduced estrogen levels cause an induction of noradrenergic activity leading to the feeling of warmth, heat loss, and sweating. Because the ►placebo effect is profound and prolonged in treatments for hot flushes, it is essential that new therapies should be tested for adequate durations (at least 12 weeks) in randomized placebo controlled trials.

Phytoestrogens. Phytoestrogens are plant derivatives either in the form of ►isoflavones found in soy products or lignans found in flax seeds. They exhibit both estrogenic and antiestrogenic effects, depending on their concentration. Although some studies have shown small benefits in hot flushes from using phytoestrogens, a recent metanalysis failed to demonstrate any benefit over placebo. Their safety in women with a history of breast cancer is unknown.

Black Cohosh (*Cimicifuga Racemosa*). It was traditionally used by native North Americans for treating range of menstrual problems. It appears to act by competing for the ►estrogen receptor, and binds to Gamma amino butyric acid (GABA), serotonin, and dopamine receptors. Again, results have been conflicting with some studies finding black cohosh mildly effective in decreasing severity and frequency of hot flushes, genitourinary symptoms, and mood disturbances in few studies. However, a metanalysis of randomized controlled trials has shown no overall benefit of black cohosh over placebo. Black cohosh has recently been associated with several episodes of hepatotoxicity and liver failure.

Neuroendocrine Agents. (i) Clonidine – It is a central alpha-adrenergic agonist, found to be moderately more effective than placebo in treating hot flushes. Some studies have also shown improved quality of life in breast cancer patients treated with clonidine for hot flushes. Clonidine is one of the very few nonhormonal therapies licensed for the treatment of hot flushes. There are no safety data regarding clonidine and breast cancer. (ii) Selective serotonin and noradrenaline reuptake inhibitors (SSRI/SNRI) – Over the menopause transition, serum serotonin levels fall and monoamine oxidase activity increases. Venlafaxine is an SNRI, which has demonstrated short-term (up to 6 weeks) superiority over placebo in reducing hot flushes. Side effects of venlafaxine are largely dose related and include nausea, constipation, dry mouth, and decreased

appetite. Other SSRI including paroxetine, fluoxetine, and citalopram have also demonstrated short-term efficacy in reducing hot flushes although their safety in some breast cancer patients has been questioned due to their interactions with the metabolism of Tamoxifen.

The GABA analogue gabapentin may be more effective than SSRI/SNRI and is well tolerated by most women, though side effects may include somnolence, dry mouth, heart palpitations, and dizziness. Gabapentin effectively reduced hot flushes for at least 12 weeks in randomized placebo controlled trials of women some of whom have had a personal history of breast cancer.

Treatment of Urogenital Atrophy and Related Symptoms

Lack of estrogen results in vaginal atrophy, urinary frequency, dysuria, and incontinence. In breast cancer patients, urogenital atrophy is relatively uncommon in tamoxifen users, due to the estrogenic effects of tamoxifen in the vagina. Urogenital atrophy is more common in those using ►aromatase inhibitors. Topical estrogens are more effective than nonhormonal preparations in the treatment of urogenital atrophy. They have been widely used following breast cancer but recent reports, which state that topical estradiol may increase circulating estrogen levels in women using aromatase inhibitors, have raised some concern. Topical estradiol is also available and may be a safer choice following breast cancer.

Sexual dysfunction is often multifactorial, and the management of urogenital atrophy should include a discussion of relationship problems, loss of libido, and depression. Physiotherapy and lubricants such as olive or almond oil may also be helpful for some women.

Treatment of Bone Loss

►Osteoporosis is a common condition seen in older postmenopausal women. Endocrine therapy for breast cancer may increase the risk of osteoporosis. Abrupt withdrawal of circulating estrogens occurs following chemotherapy-induced ovarian failure, oophorectomy, or gonadotrophin-releasing hormone (GnRH), or following treatment with an aromatase inhibitor (AI) in the postmenopausal setting. Chemotherapy alone does not cause osteoporosis if not associated with ovarian failure.

Bone loss, increased fracture risk (especially nonhip fractures) are major concerns for long-term breast cancer survivors. Tamoxifen shows agonistic estrogenic activity in bone and reduces risk of fracture in postmenopausal but not in premenopausal women.

In postmenopausal women, AIs have a deleterious effect on bone density and may increase the risk of fracture. A base line bone mineral density to be repeated up to annually is advised. A bisphosphonate in conjunction with AI may mitigate the deleterious effect of AI on bone.

Lifestyle modifications to minimize bone loss including diet, supplementary calcium weight bearing exercise, stopping smoking, and minimizing alcohol consumption should be encouraged. Calcium and Vitamin D supplements should also be advised to any women at risk.

Cardiovascular Complications

In women with early breast cancer, one of major reported causes of deaths are cardiovascular diseases. The American Heart Association guidelines stratify women in three groups based on their 10-year probability of having a coronary event. Universal recommendations for all groups are moderate exercise, cessation of smoking, body weight aimed at less than 25 BMI (body mass index), and waist circumference less than 35 in., limited intake of saturated fat and cholesterol, and dietary modification to include grains, fruits and vegetables, and fish.

Some drugs used in the treatment of breast cancer can have adverse cardiovascular effects. Tamoxifen increases the incidence of thromboembolic events and may increase triglyceride levels and lower low-density lipoproteins (LDLs). AIs have less impact on lipid profile, but their long-term effects on cardiovascular disease are not yet known. Tibolone may also have an adverse effect on LDLs in some women.

Conclusion

Menopausal symptoms are common in women treated for breast cancer. Estrogen containing HRT is the most effective treatment for menopausal symptoms, but is not recommended following breast cancer, even for those with estrogen receptor negative disease. There is an urgent need for safe and effective nonhormonal treatments. Several nonhormonal treatments have been studied with varied results and outcomes. Life style modifications are an integral part of treatment especially for osteoporosis and cardiovascular diseases. Younger women who develop premature menopause often need psychological assessment and support. Topical estrogens are effective for vaginal atrophy but their safety in women using aromatase inhibitors is not established. Gabapentin and clonidine appear effective for hot flushes but have significant side effects. Several short studies support the efficacy of SNRI and SSRI but their medium to long-term efficacy is not established. Very few studies have addressed effective nonhormonal treatments for symptoms other than hot flushes. Because of the complexity of these cases, management of menopausal symptoms following breast cancer is best conducted within a multidisciplinary environment and with individualized care.

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Menopause

Definition

The final menstrual period.

►Menopausal Symptoms After Breast Cancer Therapy

Merlin

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Synonyms

Schwannomin; NF2 gene product

Definition

Merlin is the protein product of the *NF2* ►tumor suppressor gene. Germline mutations of the *NF2* gene predispose individuals to the development of ►neurofibromatosis 2, an autosomal dominant disorder affecting one in 33,000–40,000 people. Biallelic inactivation of the *NF2* gene has been reported in nearly all sporadic vestibular schwannomas and in 50–70% of sporadic meningiomas. In addition, somatic genetic changes resulting in biallelic inactivation of *NF2* occur in about 50% of ►mesothelioma tumors, which are mesodermally derived, primarily pleural tumors unrelated to the NF2 syndrome. Development of bilateral vestibular schwannomas is a hallmark of the NF2 syndrome. Because the NF2 gene product exhibits significant homology to the highly conserved ezrin-radixin-moesin (ERM) family proteins, it was dubbed merlin (moesin-ezrin-radixin like protein). It has also been referred to as schwannomin for its suppressive effect against schwannoma formation.

Characteristics

Merlin is a 595-amino-acid protein consisting of three structural domains: an N-terminal domain that exhibits highest homology to ►ERM proteins, an α-helical region, and a unique C-terminal domain. Intramolecular self-association of the N- and C-termini causes merlin to adopt a closed conformation, which is functionally active.

Like ERM proteins, but distinct from all known tumor suppressors, merlin is cytoskeleton-associated and localizes at the cell membrane. Immunofluorescence studies have revealed that merlin predominates at membrane ruffling edges. Merlin binds directly to the actin cytoskeleton via two actin-binding sites located in its N-terminal domain. Merlin also interacts with the cytoskeleton indirectly through other cytoskeleton proteins such as ERM, βII-spectrin, and paxillin.

Merlin plays a critical role in regulating cytoskeletal organization and cell motility. In schwannoma cells, loss of merlin function results in increased membrane ruffling, disorganized stress fibers, and altered spreading. Restoration of merlin expression in these *NF2*-deficient cells has been shown to impair cell motility and rescue the aberrant cytoskeletal morphology. Cell motility is critical for tumor invasiveness and metastasis, and merlin loss-of-function has been implicated in these stages of tumor progression in a mouse model. Primary mouse embryonic fibroblasts from $Nf2^{+/-}$ mice are very motile, and tumors arising in these mice are highly metastatic. However, this feature is distinct from the situation observed in benign tumors of NF2 patients.

Consistent with its role as a tumor suppressor, merlin has been shown to play an important role in regulating cell proliferation in a variety of cell types. Overexpression of merlin in both *NF2*-negative and -positive cells led to growth suppression, G0/G1 arrest, pRB phosphorylation, and decreased cyclin D1 expression. On the other hand, knockdown experiments with *NF2* siRNA accelerated S-phase entry and cyclin D1 accumulation of *NF2*-positive cells. The tumor suppressor function of merlin has also been demonstrated in targeted mutant mouse strains. Heterozygous $Nf2^{+/-}$ mice develop osteosarcomas, fibrosarcomas and hepatocellular carcinomas, and biallelic inactivation of *Nf2* occurred in all of the tumors. In a conditional homozygous knockout mouse model, however, Cre-mediated biallelic excision of *Nf2* exon 2 specifically in Schwann cells resulted in a high incidence of benign and malignant schwannomas. Intriguingly, two mesotheliomas were also found in this mouse strain.

The growth-suppressing function of merlin has also been connected to its role in contact-dependent inhibition of cell proliferation. The primary phenotypic consequence of Nf2 deficiency in mouse primary cells is loss of contact-dependent inhibition, which is accompanied by a lack of ►adherens junction formation. In agreement with this conclusion, phosphorylation and expression levels of merlin are regulated by cell density.

Merlin also plays a role in regulating signaling by ►Rho family proteins known as Rho GTPases that participate in actin cytoskeletal remodeling. Merlin physically localizes at membrane ruffles, which can be induced by Rac activity. Extensive membrane ruffling and aberrant stress fibers characteristic of schwannoma cells were similar to those observed in other cells with active Rac or Rho A, and could be reversed by inhibition of Rac1 or RhoA signaling, respectively. Biochemical experiments have connected merlin mechanistically to Rho GTPase signaling. In response to active Rac or Cdc42, but not active Rho, merlin is phosphorylated on S518, and this phosphorylation is mediated by p21-activated kinase (Pak), a common downstream target of both Rac and Cdc42. Phosphorylation of merlin by Rac/Pak signaling weakens its intramolecular binding, resulting in an open, functionally inactive protein. Indeed, a phosphomimic mutant merlin S518D impairs merlin's ability to suppress cell cycle progression and motility, whereas a S518A phosphorylation-defective form of merlin can act as a constitutively active form of the tumor suppressor in modulating cell proliferation. Intriguingly, wild-type merlin and Pak appear to operate in a negative feedback loop, because unphosphorylated merlin can directly bind Pak and inhibit its activity.

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Mesalazine

Definition

5-Aminosalicylic acid (5-ASA) has long-term clinical experience in the treatment of patients with ►inflammatory bowel disease (IBD), is well tolerated, has limited systemic side effects, and has no gastrointestinal toxicity. In a rodent model of colorectal cancer, mesalazine inhibits tumor growth and reduces the number of aberrant crypt foci whereas in patients with sporadic polyps or cancer of the large bowel mesalazine induces apoptosis and decreases proliferation in the colorectal mucosa. Epidemiological data strongly supports a chemopreventive role for mesalazine in ►ulcerative colitis associated colorectal cancer.

Mesenchymal Stem Cells

Definition

MSCs; A type of stem cell found in bone marrow (marrow stromal cells) and many other locations e.g. adipose tissue. Defined by their ability to adhere to plastic when cultured, and of trilineage differentiation into fat, cartilage and bone. Found to be highly malleable, and can be switched to many other different cell types. Possess an extensive proliferative potential and ability to differentiate into various cell types, including: osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes and neurons.

►Stem Cell Plasticity
►Stem Cell Telomeres

Mesenchymal Tumors

Definition

Tumors derived from mesenchymal cells such as muscle, connective tissue, blood vessels, and lymphatic tissue.

►Uterine Leiomyoma
►Gastrointestinal Stromal Tumor

Mesenchyme

Definition

Is a tissue constituting of undifferentiated mostly mesodermal cells that give rise to such parts as

connective tissue, cartilage, bone, lymphatics, and blood.

►Mesoblastic Nephroma

Mesenteric Fibromatosis

►Desmoid Tumor

Mesna

Definition

A sulfhydryl compound that is used to reduce the incidence of hemorrhagic cystitis associated with certain chemotherapeutic agents. Mesna is converted to a free thiol compound in the kidney, where it binds to and inactivates acrolein and other urotoxic metabolites of ifosfamide and cyclophosphamide, thereby reducing their toxic effects on the urinary tract during urinary excretion.

►Chemoprotectants

Mesoblastic Nephroma

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Synonyms

Congenital mesoblastic nephroma; Atypical congenital mesoblastic nephroma; Leiomymatous harmatoma of the kidney; Fetal hamartoma of the kidney; Malignant mesenchymal nephroma of infancy

Definition

Mesoblastic nephroma is a rare mesenchymal tumor of the kidney, which typically occurs in neonates and young infants and constitutes the most frequent renal tumor in the first three months of life.

Characteristics

Mesoblastic nephroma (MN) is a rare malignant tumor of the kidney, which originates from the mesenchymal anlage ►(metanephrogenic blastema). The typical patient is a neonate or young infant. In the first 3 months of life MN is the most frequent renal tumor, hence preoperative chemotherapy is not recommended for renal tumors in this age group. It accounts for 5% of all childhood renal tumors. According to the ►German Childhood Cancer Registry the cumulative incidence of MN is estimated at 1 in 150,000 children (0–14 years) in Germany. Contrary to ►nephroblastoma, MN has a 2 to 1 male preponderance. Metastatic disease at diagnosis is an extreme rarity.

Diagnosis

The clinical course of the MN even in the connatal or neonatal period is mainly indolent. Incidental diagnosis because of a palpable abdominal mass or a routine ultrasound scan is frequent. Abdominal protrusion, pain, emesis, hypertension, and hematuria can be clinical symptoms of MN forcing parents to contact a pediatrician. A growing number of MNs are diagnosed even antenatally, as prenatal ultrasound scans become more and more standard care. Nevertheless MN can be the reason for preterm deliveries and in reports throughout the seventies and eighties a remarkable number of MNs had been found in stillbirths.

Ultrasound scan and MRI scan are the gold standard in the diagnosis of childhood renal tumors. Differential diagnosis includes ►neuroblastoma, ►nephroblastoma, ►nephroblastomatosis, ►cystic nephroma, ►rhabdoid tumor (RT), ►clear cell sarcoma of the kidney (CCSK), and other rarer renal tumors. In ultrasound scan MN usually appears as a solid, intrarenal heterogeneous structure of mixed echotexture with circumferential echopoor areas. Furthermore a so-called "vascular ring sign" can be seen in color-coded duplexsonography. The latter corresponds to a heterogeneous circumferential contrast enhancement in MRI scan. A pseudocapsule, often seen in case of nephroblastoma, is normally not visualized in MN. However nephroblastoma can not be distinguished from MN by imaging alone, contrary to the typical neuroblastoma (retroperitoneal and extrarenal mass with calcifications that often involves vena cava and/or aorta and displaces the kidney). Unfortunately there is no specific MN-marker yet, but catecholamines in urine and blood, NSE, mIBG scan and fine needle aspiration can be of additional use in distinguishing neuroblastoma from MN. Final diagnosis, especially differing MN from other renal tumors, can only be made by histology, therefore primary surgery is the next step in diagnosis.

Histology and Genetics

MN histology can be polymorphic and challenging. This has been the reason for a variety of synonyms for MN over the years. However classical mesoblastic nephroma is actually distinguished from a cellular and a

mixed type mesoblastic nephroma. The classical type MN is characterized by leiomyomatous histology with spindle cells in bundles, absence of necrosis, and rare mitoses. It grows in a rather displacing than infiltrative manner, but tends to develop finger-shaped projections thereby trapping normal renal tissue. The cellular type MN shows contrary to the classic type MN a high cellularity and mitotic index and an atypical growth pattern (invasion of other adjacent structures other than connective tissue, hemorrhage, necrosis, and fleshy areas), thus it can infiltrate the adipose capsule of the kidney and beyond. Earlier, this MN subtype had also been called atypical congenital MN. The mixed type MN has a predominantly classic histological pattern but with some islets of cellular type MN within.

The local tumor extension is staged according to the Children's Oncology Group (COG) or ►Society of Pediatric Oncology (SIOP) working classification of renal tumors of childhood. Macroscopic residual tumor, rupture, and positive resection margins are reasons for a local stage III.

Recent studies have shown that cellular and occasionally mixed type MN differs genetically from classic MN. The translocation t(12;15) (p13;q25), detectable in most cellular and occasionally in mixed type MN, gives rise to an ETV6-NTRK3 gene fusion. Moreover trisomy 11 is found in those MN too. The translocation t(12;15) (p13;q25) can be of use to differ cellular MN from the sometimes histologically similar clear cell sarcoma of the kidney. This translocation has also been proven in congenital fibrosarcoma, suggesting a genetical link between those two histologically similar lesions.

Treatment

Primary surgery is the gold standard in MN treatment, leading to high cure rates by complete resection. Complete nephrectomy including the adipose capsule is recommended, since MN's finger-shaped projections easily can be inadvertently left behind in case of partial nephrectomy. Furthermore, sometimes cystic growth pattern, especially in cellular type MN, makes the tumor vulnerable and can cause rupture during surgery.

MN is susceptible to chemotherapy which is indicated in case of nonresectable tumor or relapse. Adjuvant chemotherapy is discussed controversially in patients with stage III cellular type MN and older age. Standard nephroblastoma and sarcoma treatment regimens are effective in the treatment of MN (dactinomycin, vincristine, cyclophosphamide, doxorubicin, ifosfamide, etoposide and carboplatin). As MN can be treated by surgery and chemotherapy, radiation therapy is not indicated in these very young patients.

Prognosis

Though MN, if completely resected, has an excellent prognosis (overall and event-free survival rate >95%) metastatic and local relapses occur, but almost exclusively in cellular type MN. Contrary classical and mixed type MN may be followed even in case of positive surgical margins or local spillage. Second look surgery can be indicated to achieve downstaging. Relapsed patients can develop metastasis to the lung, more seldom to the liver or the brain. Incomplete resection, cellular type MN, and older age correlate with relapse but are probably not independent risk factors.

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Mesoderm

Definition

The embryonic germ cell layer from which bone, cartilage, muscle, and connective tissues are derived.

►Bone Tumors

Mesothelial Cells

Definition

Flattened epithelial cells of mesenchymal origin that line the peritoneal cavity, the chest cavity and the cavity around the heart.

►Omental Immune Aggregates

Mesothelin

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Synonyms

CAK1 antigen; SMR (soluble mesothelin-related proteins); MPF

Definition

The name Mesothelin was given by K. Chang and I. Pastan to a 40 kDa, GPI-anchored ►glycoprotein ►(GPI-anchored protein) that is physiologically expressed at the cell surface of mesothelial cells lining the pleura, pericardium and peritoneum [1]. Mesothelin is an epithelial marker highly expressed by ►cancer cells from diverse origins, including ►ovarian adenocarcinomas, pancreatic adenocarcinomas, and ►mesotheliomas. Soluble forms of mesothelin can be found in fluids from patients affected by these cancers.

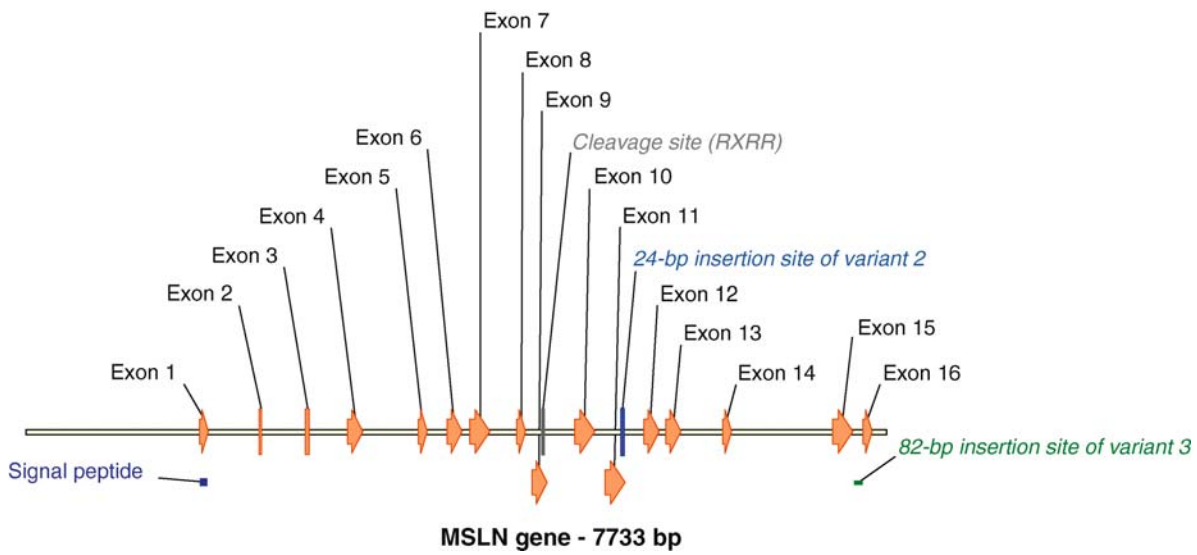
Characteristics

Mesothelin results from the cleavage of a 69 kDa preproprotein encoded by the human MSLN gene (NC_000016) that spans over 16 exons and occupies about 8 kb of human chromosome 16 (Fig. 1). The alternative splicing of MSLN gene results in at least two mesothelin transcript variants, variant 1 encoded by MSLN1 (NM_005823) and variant 2 encoded by MLSN2 (NM_013404) (Fig. 2). The variant 1 is predominant and variant 2 differs from variant 1 by 24 bp inserted in exon 11. The cleavage of MSLN-encoded preproprotein at the cationic motif TILRPRFRREVE in exon 9 releases the ►megakaryocyte potentiating factor (MPF), a 31 kDa soluble protein, while mesothelin remains membrane-bound.

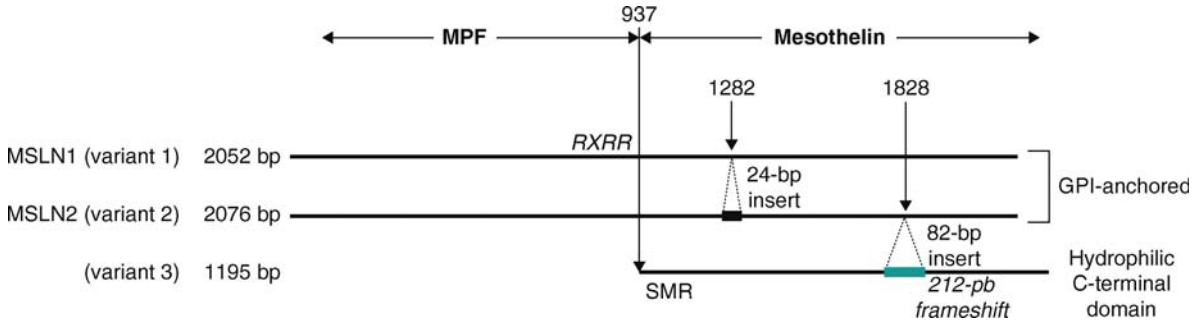
MSLN gene is composed of 16 exons spanned over 7733 bp. In human, MSLN gene is located in 16p13.3. MSLN1 and 2 gene variants encode a 69 kDa preproprotein that is cleaved in position 937 after the cationic motif *RXRR,* into two mature proteins, MPF and mesothelin. MSLN1 encodes mesothelin variant 1 and MSLN2 encodes variant 2, a less abundant, alternate splice with a 24-bp insertion in position 1282. Soluble mesothelins arise through a cleavage of GPI-anchored variants 1 or 2, or less frequently, an 82-bp insertion in position 1828 of MSLN1 resulting in a 212-bp ►frameshift mutation that transforms the GPI anchor motif into a hydrophilic domain (SMR or variant 3). The insertion of an 82-bp fragment results probably from a lack of splicing of the last intron (Figs. 1 and 2).

The core of a GPI anchor is composed of a hydrophobic phosphatidyl-inositol group and a carbohydrate-containing linker made of glucosamines and mannoses linked to a phosphoryl-ethanolamine residue (Fig. 3). The GPI anchor is linked to the C-terminal amino acid of a mature protein *via* the phosphoryl-ethanolamine residue. R1 and R2 fatty acids anchor the protein to cell membranes.

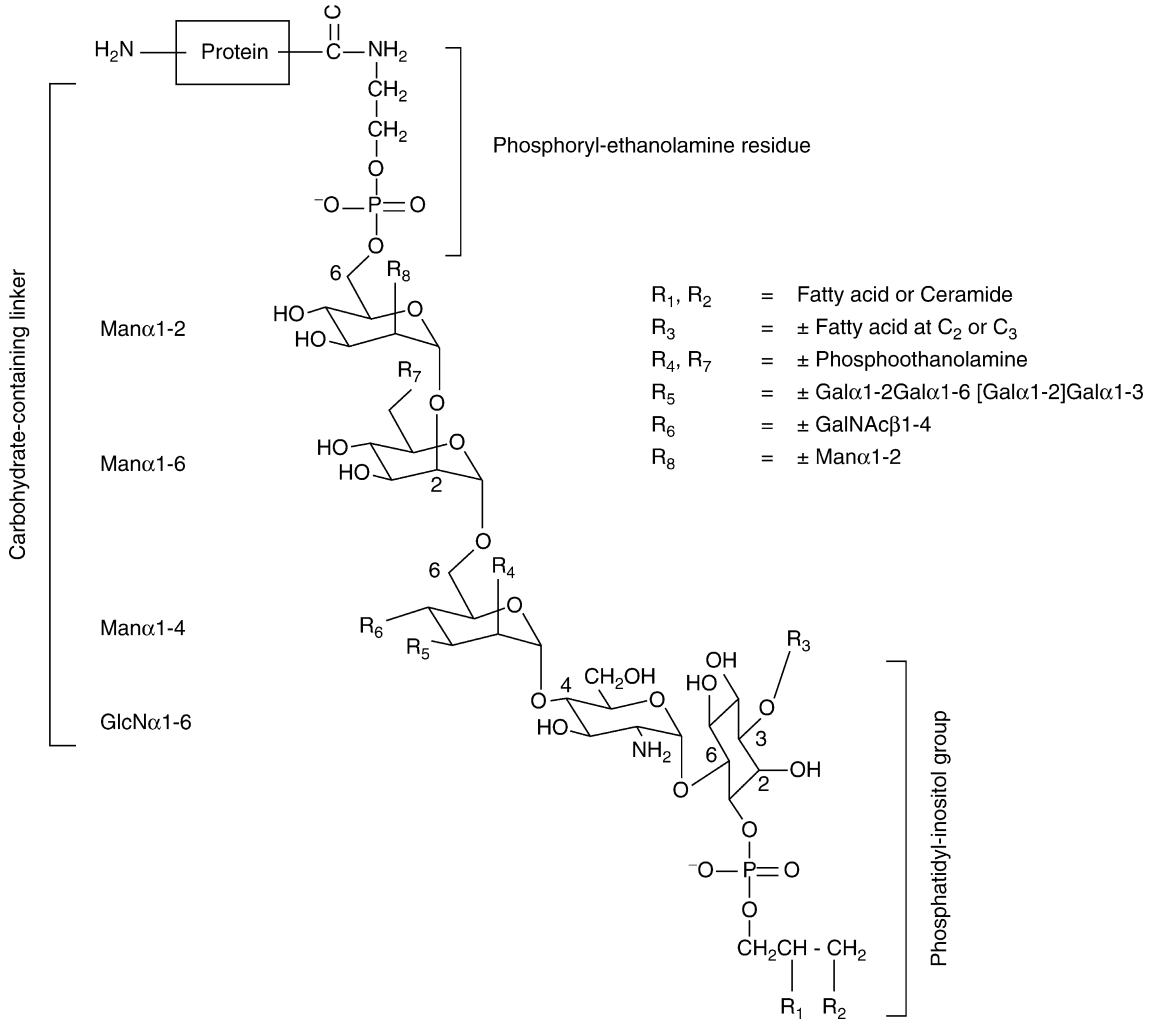
Mesothelin homologues were described for chimpanzees (MSLN gene, *Pan troglodytes*, accession DQ052446), macaque (LOC698095 gene, *Macaca mulatta,* accession XM_001087333), bovine (LOC516237 gene, *Bos Taurus,* accession XM_594389), dog (LOC611363 gene, *Canis familiaris*, accession XM_849019), rat (Msln gene, *Rattus norvegicus*,



Mesothelin. Figure 1 Exon map of MSLN gene.



Mesothelin. Figure 2 MSLN variants.



Mesothelin. Figure 3 Glycosylphosphatidylinositol (GPI) anchor.

accession NM_031658), mouse (Msln gene, *Mus musculus*, accession NM_018857) and chicken (LOC416534 gene, *Gallus gallus*, accession XM_414835). Mouse mesothelin is 55% homologous to its human counterpart and its protease target sequence TVIHPRFRRDAE is conserved.

Tissue Expression

Serial analysis of gene expression (SAGE), oligonucleotide and cDNA arrays have been used by independent laboratories to identify large sets of genes expressed at higher levels in cancer tissues compared with normal tissues. MSLN transcripts were found consistently highly

over-expressed in non-mucinous ovarian carcinomas and invasive ductal adenocarcinoma (►Pancreatic ductal adenocarcinoma (DA)) and significantly over-expressed in mesotheliomas and pulmonary, gastric/esophageal and colorectal adenocarcinomas. By real time PCR-based analysis, normal transcript levels of MSLN and CCL23, GAGED2, SPAG6, ST18, WT1 and PRAME genes were found to be associated with continuous complete remission of pediatric acute myeloid leukemia, while elevated levels of at least one of these genes were found prior to relapse in most patients.

The up-regulation of MSLN transcripts correlates well with mesothelin cell-surface expression. Studies by immuno-histochemistry (IHC) confirm that mesothelin is significantly more expressed by pancreatic cancer cells as compared to very weak or no expression in chronic pancreatitis or normal pancreatic ducts; mesothelin is found with a higher frequency in invasive intraductal papillary mucinous neoplasms (IPMN) than in non-invasive. Comparative analyses with tissue microarrays showed that the mesothelin protein expression in ovarian cancer depends on the histological type (75% of expression in serous papillary tumors, 30% in endometrioid and less than 20% in mucinous). Diffuse mesothelin staining in primary high grade ovarian serous carcinomas may be correlated with prolonged survival. Finally, mesothelin was also found to be expressed at a low level by a variety of adenocarcinomas, including endometrium, stomach/esophagus, pulmonary, ►breast and colorectal. None or very rare mesothelin expressions (less than 5%) were reported for carcinomas of ►prostate, bladder/ureter, liver, kidney and ►thyroid.

Regulation

Mechanisms that regulate MSLN transcription levels and mesothelin cell-surface expression or release as a soluble form in patient fluids are not well understood. Several pathways have been explored. MSLN gene was found ►hypomethylated in pancreatic ductal adenocarcinoma, consistently with the inverse correlation between mRNA expression and ►DNA methylation described in numerous cancers. Also, mesothelin up-regulation in carcinomas has been associated with a misregulation of ►Wnt signal transduction pathway. In mouse mammary epithelial cells, Wnt-5a down-regulates mesothelin expression, perhaps through antagonism of the ►Wnt/beta-catenin pathway, while in human ►colon cancer cells the forced expression of an N-terminal β-catenin binding site-deficient high mobility group (HMG)-box T-cell factor 1 is associated with the up-regulation of several GPI-anchored adhesion molecules, including mesothelin. Furthermore, the overexpression of mesothelin in exon 9 ►GISTs suggests that mesothelin regulation could be linked to the intracellular signaling cascade triggered by ligand-independent activation of ►KIT receptor tyrosine kinase. Finally, the presence of soluble mesothelin derived from GPI-anchored forms in ovarian and mesothelioma patient fluids could also be related to the over-expression of ►GPI PLD observed in some cancer cells.

Function

Mesothelin ►knock-out mice have no obvious phenotype and produce normal offspring without anatomical or histological abnormalities, which suggests that mesothelin is a non-essential protein. However, mesothelin binds to ►CA125 in a specific and N-linked glycan-dependent manner, thus CA125-expressing tumor cells could bind specifically to mesothelin-expressing peritoneal lining. Consequently, CA125/mesothelin-dependent cell attachment may play an important role in the peritoneal implantation of ovarian tumor cells.

Some indirect experimental evidences suggest that mesothelin could also have a role in neoplastic progression. First mesothelin up-regulation in pancreatic cancer corresponds to the transition from carcinoma *in situ* to DA. Second, mesothelin expression was found to be up-regulated after carcinogenic treatment in rats and its expression correlated with the risk of cancer development. Virgin and parous rats were compared for breast cancer incidence and mesothelin expression, before and after carcinogen exposure. Mesothelin was down-regulated before and after treatment in parous rats but only before treatment in virgin rats. It was increased after treatment in virgin rats, as were their cancer development risks.

Finally mesothelin is an immunogenic molecule. This observation is consistent with mesothelin GPI-anchor but puzzling considering its overexpression by cancer cells. In fact, most tumor antigens are weak immunogens and this has been a burden for the development of ►cancer vaccines. Nevertheless, anti-mesothelin autoantibodies have been found in about 40% of mesothelioma and epithelial ovarian cancer patients, and in some patients with pharynx/larynx ►squamous cell carcinoma. In addition, mesothelin-specific CD8-T cell responses are identifiable with a ►HLA-A2 mesothelin epitope in both normal and cancer patients, and have been reported to be increased after vaccination with pancreatic cancer lines in presence of GM-CSF.

Diagnostic Marker

The differential expression of mesothelin at the cell surface of some cancer cells and in patient fluids makes it suitable as a cancer marker. Mouse monoclonal antibodies (mAb) are commercially available for immunohistochemistry (IHC) staining, for example K1 mAb from Abcam and 5B2 mAb from BioGenex, and for ►ELISA assay (Mesomark™ kit from Fujirebio

Diagnostics, Inc., corresponding to the assay described by Scholler et al. [2]).

Anti-mesothelin mAbs stain mesotheliomas with a high sensitivity and a specificity of 75%. Several studies suggest than mesothelin can be useful for differential diagnostics, alone or combined with a biomarker panel. For example, mesothelin staining can help to identify the origin of mucinous tumors or metastatic adenocarcinomas. Mesothelin staining is much less frequent (less than 20%) in primary ovarian mucinous tumors than in metastatic pancreatic mucinous carcinomas (more than 70%). Used with PSCA, mesothelin appears highly specific for pancreatic adenocarcinoma in fine needle aspirate (FNA) specimens thus useful in categorizing suspicious lesions. In combination with p53, TAG-72, mCEA and loss of Dpc4, mesothelin staining distinguishes well differentiated liver metastasis of DA from bile duct adenomas (BDA) or hamartoma of the liver. Mesothelin, calretinin and cytokeratin 5/6 were reported to be the best positive mesothelioma markers for differentiating epithelioid mesotheliomas from renal cell carcinomas; mesothelin, WT1, p63 and MOC31 distinguish between epithelioid mesotheliomas and squamous carcinomas of the ►lung; mesothelin, calretinin, BG8 and MOC 31 distinguish between epithelioid mesothelioma and adenocarcinoma. Finally, a larger panel including mesothelin, CA125, CDX2, cytokeratins 7/20, ►estrogen receptor, gross cystic disease fluid protein 15, lysozyme, prostate specific antigen and thyroid transcription factor 1 was reported to correctly classify 88% of breast, colon, lung, ovary, ►pancreas, prostate and stomach adenocarcinomas.

Mesothelin measured in fluids is a promising marker for ovarian carcinomas and mesotheliomas. Mesothelin serum levels are elevated in most late stage ovarian cancer patients and in most patients with malignant mesotheliomas (MM) at diagnosis, and serum levels correlate with tumor size and increase during tumor progression. This suggests that mesothelin serum levels could be helpful to monitor disease progression and to screen asbestos-exposed individuals for early MM. In addition, the presence of mesothelin in MM pleural fluid can help to better discriminate mesothelioma from pleural metastasis. Recent results, taken together with mesothelin cell surface expression on pancreatic tumor, suggest that mesothelin could also be a serum marker for some pancreas cancers. A pancreatic tumor cell line was reported to release soluble mesothelin in culture supernatant using an acoustic wave device immunosensor, and mesothelin mRNA was isolated from pure pancreatic juice of pancreatic tumors and was found more abundantly in DA than in IPMN. Finally, various studies combined mesothelin with other biomarkers to form a composite marker (CM) and demonstrated that the use of CM can improve diagnostic test sensitivity. For ovarian carcinoma diagnostic, mesothelin titers have been evaluated in combination with CA125, HE4, M-CSF, ►kallikrein and/or soluble EGF receptor; for mesothelioma diagnostic, mesothelin has been combined with ►osteopontin.

Interestingly, mesothelin tumor cell expression and serum levels do not strictly correlate. Although mesothelin serum levels are more frequently increased in both MM and ovarian cancer patients whose tumor expressed mesothelin (≥30% expression by tumor cells), some patients without detectable mesothelin expression on tumor cells have elevated titers of serum mesothelin. The absence of detectable mesothelin by IHC could be due to technical artifact, or alternatively, in some cases soluble mesothelin might be mainly released from normal mesothelial cells that are in contact with the tumor microenvironment, such as in ►pleural effusion or peritoneal fluid.

Therapeutic Applications

Because of its high expression in mesothelioma, ovarian and pancreatic carcinomas, its immunogenicity and its non-essential function, mesothelin is an antigen of choice for targeted therapies and cancer vaccines. Anti-mesothelin natural and recombinant antibodies have been generated and conjugated to Pseudomonas exotoxin A (SS1P), 125I or 111In, and used alone or in combination with ►Taxol *in vitro* and *in vivo* in mouse model systems. Recent results demonstrated that SS1P synergizes with Taxol *in vivo* but not *in vitro* which underlines the importance of the tumor microenvironment for therapeutic strategies. Phase I trials with recombinant anti-mesothelin immunotoxin were completed in patients with mesothelin-expressing tumors without evidence of non-manageable side effects; about a fourth of the treated patients developed anti-SS1P antibodies. In addition, a DNA vaccine with a single-chain trimer of HLA-A2 linked to human mesothelin peptides has been successfully used to prevent the growth *in vivo* of HLA-A2 positive human mesothelin-expressing tumor cell lines in HLA-A2 transgenic mice. These results suggest possible clinical translation of mesothelin-targeted therapy and ►DNA vaccines for immunotherapy of gynecologic cancers against mesothelin.

Finally, the specific binding of mesothelin to CA125 suggests that agents able to compete with or to block CA125/mesothelin-dependent cell attachment could prevent or delay the development of peritoneal metastasis.

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Mesothelioma

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Synonyms

Malignant mesothelioma

Definition

Mesotheliomas are tumors derived from mesothelial cells that form the membranes surrounding the lungs, pericardium and peritoneum. Mesotheliomas are highly aggressive malignancies, with a median survival of 9 months from diagnosis. The incidence of mesothelioma is higher in men than in women (8:1) and these tumors usually occur during the seventh and eighth decades of life. Mesothelioma is usually associated with occupational ►asbestos exposure, or to exposure to erionite, a mineral fiber that shares some physical characteristics with crocidolite asbestos. Studies have also linked this disease with a DNA tumor virus known as ►SV40, although this remains controversial. Moreover, genetic predisposition to mineral fiber carcinogenesis may contribute to multiple cases of mesothelioma within the same family. There are 2,000–3,000 cases of mesothelioma per year in the USA and more than 1,000 in England and Italy. The incidence of mesothelioma continues to increase in spite of measures adopted in the 1970s and 1980s to eliminate (Italy) or reduce (USA) the use of products containing asbestos.

Characteristics

Pathogenesis

Asbestos

►Cancer epidemiology studies and experiments performed in vitro and in animals have clearly linked exposure to crocidolite asbestos, a form of amphibole asbestos, and to ►erionite, a type of ►zeolite, to the development of mesothelioma. Other forms of amphibole asbestos, such as ►tremolite have also been linked to mesothelioma, but some studies suggest that the risk may be lower compared to ►crocidolite. Whether other forms of asbestos, such as antophyllite or chrysotile (the latter is also known as serpentine asbestos) cause mesothelioma is controversial. The mechanisms responsible for asbestos carcinogenicity have been recently linked to the secretion of ►TNF-alpha by mesothelial cells and ►macrophages exposed to asbestos, which in turn leads to ►NFκB activation. The activation of the NFκB pathway in mesothelial cells appears to allow these cells to survive the cytotoxicity and genetic damage caused by asbestos, and these damaged cells may proliferate into a mesothelioma.

Asbestos has been shown to induce the proto-oncogenes ►Fos and ►Jun, which encode ►AP-1 transcription factors that activate various genes critical in the initiation of DNA synthesis. The persistent induction of these transcription activators following asbestos exposure may enhance cell division and favor malignant growth. AP-1 activation is enhanced in mesothelial cells infected experimentally with SV40 and exposed to asbestos, and animal studies have confirmed that asbestos and SV40 can act as co-carcinogens in causing mesothelioma. Asbestos is a ubiquitous carcinogen. Therefore, virtually everyone may have some level of exposure. It is hypothesized that there is a threshold level of exposure above which the risk of developing mesothelioma increases. But the threshold is unknown, and individual genetic susceptibility and/or infection with SV40 may influence this threshold.

SV40

100% of hamsters injected in the pleural space develop and die of mesothelioma within 6 months, compared to 20% of those injected with asbestos, which develop mesotheliomas after about 2 years. SV40 can transform human cells in tissue culture, and mesothelial cells are transformed at a rate that is much higher than in other cell types tested. This effect appears to be related to the unusually high levels of the ►p53 tumor suppressor normally present in mesothelial cells. p53 binds and inhibits the SV40 oncoprotein large T antigen (Tag), thereby limiting viral replication. Thus, in these cells infection is not lytic, as observed in other human cell types. Instead, mesothelial cells are infected, and SV40 establishes a "parasitic" relationship within mesothelial cells, i.e., replicating without lysing the cells. SV40 has been detected in 6–60% of human mesotheliomas, and Tag has been shown to bind and inhibit the tumor suppressors p53 and the ►retinoblastoma protein, pRb. Microdissection, ►immunohistochemistry and in situ hybridization experiments have shown that SV40 is present in tumor cells and not in nearby stromal cells. Because different groups have reported different percentages of mesotheliomas containing SV40, and

some have failed to detect it at all, the overall contribution of SV40 to the incidence of mesothelioma remains unknown. In vitro and animal experiments have shown that asbestos and SV40 can be co-carcinogens and that SV40 infection may lower the threshold level of asbestos required for oncogenesis.

Although asbestos fibers induce cytotoxicity in mesothelial cells, cell survival activated by key signaling pathways may promote transformation. Activation of the ►AKT signal transduction pathway is one of the major ways in which cell survival is promoted, and SV40 has been shown to inhibit programmed cell death via AKT kinase activation in mesothelial cells exposed to asbestos. Activation of AKT has been observed in about 65% of mesotheliomas, which has therapeutic implications, because AKT signaling contributes to tumor aggressiveness, in part by mediating cell survival and reducing sensitivity to chemo- or radiotherapy. Thus, the AKT signaling pathway has been proposed as a novel therapeutic target to overcome mesothelioma resistance to conventional therapies.

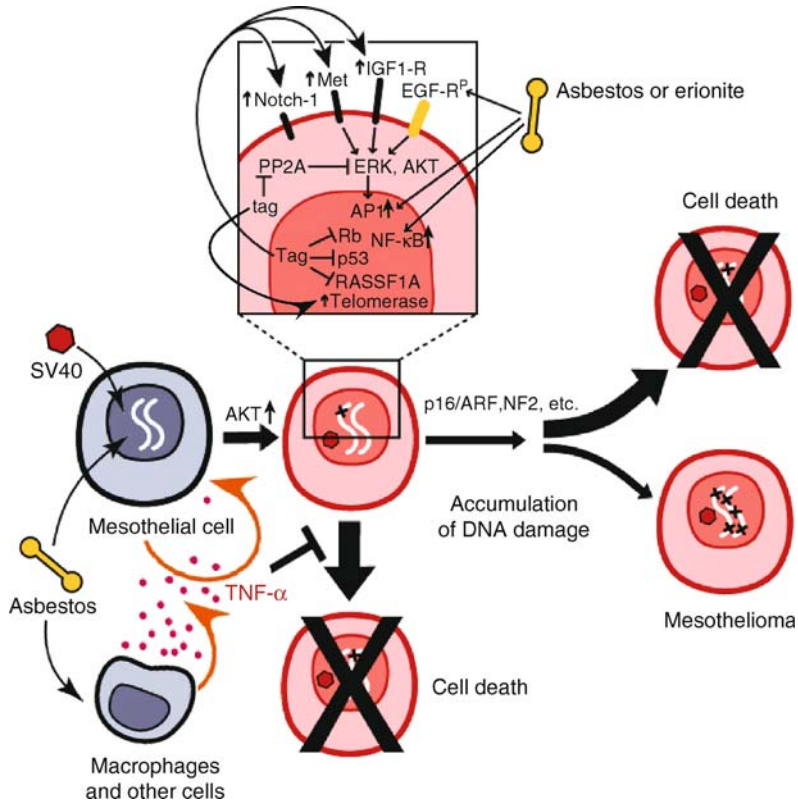
Chromosome Changes

In vitro studies have shown that human mesothelial cells acquire extensive numerical and structural chromosomal abnormalities shortly after exposure to low concentrations of asbestos fibers. SV40 can also cause genetic damage. SV40 Tag is known to be mutagenic and can cause point mutations, chromosome rearrangements and ►aneuploidy. While many of the DNA alterations caused by asbestos or SV40 will either be of no significance or lead to cell death, a few cells may develop perturbations of key cell cycle regulatory genes, leading to tumor formation and/or progression. Cytogenetic analysis has revealed multiple chromosome alterations in most human mesotheliomas. Although a specific chromosomal change is not shared by all mesotheliomas, several prominent sites of chromosomal loss have been identified. Deletions of specific regions in the short (p) arms of chromosomes 1, 3 and 9 and long (q) arm of 6, 13 and 15 are repeatedly observed. Loss of a copy of chromosome 22 is the single most consistent numerical change seen in mesotheliomas, and monosomy 4 and monosomy 14 are also common. These recurrent losses frequently occur in combination in a given tumor. Loss and/or inactivation of ►tumor suppressor genes residing in recurrent sites of chromosomal deletion are thought to contribute to the development of mesothelioma. ►Loss of heterozygosity (LOH) analysis has demonstrated high frequencies of allelic loss from 1p22, 3p21, 4q, 6q, 9p21, 13q13–14 and 15q11–15 (Fig. 1).

Tumor Suppressor Genes

Most mesothelioma cell lines exhibit ►homozygous deletion of the 9p21 chromosome region. ►*CDKN2A*, which encodes the alternative tumor suppressor gene products p16INK4a and p14ARF, appears to be the critically affected locus in this region. Genes encoding p15INK4b (CDKN2B) and methylthioadenosine phosphorylase (MTAP) are also frequently co-deleted with CDKN2A/ARF in these tumors, although whether CDKN2B and MTAP play a significant role in mesothelioma pathogenesis is uncertain at this time. The p16 ►INK4 protein binds to the cyclin dependent kinase CDK4 to inhibit the catalytic activity of the CDK4/cyclin D enzymes. Therefore, loss/inactivation of p16INK4a leads to cell cycle deregulation through the loss of a key inhibitor of G1/S progression. More than 80% of mesothelioma cell lines have homozygous deletions of one or more p16INK4a exons, and most of the remainder have greatly down-regulated expression of p16INK4a due to ►promoter hypermethylation. Immunohistochemistry studies suggest that loss of p16INK4a expression is a universal finding in mesothelioma specimens, and fluorescence in situ hybridization analysis has revealed absence or reduced copy numbers of the p16INK4a gene in most mesothelioma specimens. Collectively, these data suggest that loss of at least one copy of p16INK4a occurs in most of these tumors, with the remaining allele silenced by promoter hypermethylation. In a given tumor, all or a subset of cells may contain homozygous loss of p16INK4a, with these cells presumably having a proliferative advantage when placed into long-term culture. Importantly, alteration of p16INK4a appears to play a critical role in mesothelial cell tumorigenesis, since re-expression of p16INK4a in mesothelioma cells has been shown to result in cell-cycle arrest and programmed cell death, as well as inhibition of tumor formation or diminished tumor size.

Homozygous deletion of p16INK4a also leads, in most cases, to inactivation of p14ARF, because these two genes share exons 2 and 3, although their reading frames differ. The product of the p14ARF tumor suppressor gene is required for activation of p53 in response to the action of oncogenic proteins such as ►RAS. Unlike p14ARF, mutation of the p53 gene, ►TP53, is uncommon in mesothelioma and has been observed in a subset of tumors that retain the p14ARF locus. Since the product of the p16INK4a gene induces a G1 cell-cycle arrest by inhibiting the phosphorylation of ►pRb, homozygous loss of p14ARF and p16INK4a would collectively affect both the p53- and pRb-dependent cell regulatory pathways, respectively. In vitro studies have shown that adenovirus-mediated transfer of p14ARF in mesothelioma cell lines induces G1-phase arrest and apoptosis. Together, the available evidence suggests that alteration of either product of the CDKN2A locus, i.e., p14ARF or p16INK4a, contributes to the pathogenesis of mesothelioma. In a recent study, homozygous deletion of p16INK4a /CDKN2A was



Mesothelioma. Figure 1 Mesothelioma pathogenesis. Arrowheads indicate a stimulatory effect. Crossed bars indicate an inhibitory effect. The presence of asbestos or erionite leads to the release of TNF-α by mesothelial cells and macrophages. TNF-alpha causes NFκB activity that in turns protects mesothelial cells from the toxic effects of asbestos. This mechanism prevents asbestos-induced mesothelial cell death. Mesothelial cells that have developed genetic damage because of exposure to mineral fibers can now divide and occasionally progress to become a mesothelioma. In cells that survived asbestos exposure, asbestos induces autophosphorylation of the epidermal growth factor receptor (EGF-R), which activates the ERK kinases and leads to AP-1 induction and cell division. If the mesothelial cell is infected with SV40, large T antigen (Tag)-mediated activation and inactivation of the indicated oncogenic or tumor suppressor proteins, respectively, cause cell mitosis and genetic alterations. SV40 small t antigen (tag) inhibits phosphatase 2A (PP2A), which normally dephosphorylates and inactivates cellular kinases. This increases the stimulatory effect of asbestos on AP-1. Also, tag-induced telomerase activity promotes continuous cell growth. In addition, AKT, a critical regulator of cell survival, is also activated. Most cells exposed to SV40 and asbestos die due to apoptosis or cell lysis because of the effects of these carcinogens and because of the DNA damage they accumulate (X symbols in the nuclei). Occasionally, a cell that has accumulated numerous chromosomal changes and other genetic alterations escapes cell death to form a malignancy. Individuals exposed to both asbestos and SV40 may be at higher risk of mesothelioma when compared to individuals exposed to only one of these carcinogens.

shown to be a significant independent adverse prognostic factor in mesothelioma. Overexpression of aurora kinases was also found to be a significant independent adverse prognostic factor.

Although the ►neurofibromatosis 2 tumor suppressor gene, NF2, predisposes affected individuals primarily to tumors of neuroectodermal origin, somatic mutations of NF2 have occasionally been identified in seemingly unrelated malignancies. NF2 somatic mutations predicting either interstitial in-frame deletions or truncation of the NF2 gene product (►merlin) have been reported in 40–55% of mesothelioma cell lines. In many cases, it was possible to confirm the mutation in matched primary tumor DNA. Western blot analyses revealed complete absence of merlin expression in cell lines that exhibited alterations of the NF2 gene, suggesting that truncated forms of the protein are unstable. LOH analyses documented allelic losses of the NF2 locus in more than 70% of mesothelioma cases. All cases exhibiting mutation and/or aberrant expression of NF2 showed allelic losses, implying that inactivation of NF2 in mesothelioma occurs via a "two-hit" mechanism. One mechanism by which merlin acts as a tumor suppressor is by inhibiting the activity of p21-activated kinase (Pak), a downstream target of Rho GTPases Rac and Cdc42. Merlin has been found to

exert an antiproliferative effect, in part, via repression of Pak-induced cyclin D1 expression. Moreover, Pak is known to regulate motility in mammalian cells, which raised the intriguing possibility that merlin loss-of-function, due to biallelic inactivation of NF2, may contribute to invasiveness and/or metastasis in mesothelioma. Re-expression of merlin in NF2-deficient mesothelioma cells markedly inhibited cell motility, spreading and invasiveness. Expression of merlin attenuated the phosphorylation of ►focal adgesion Kinase, a key component of cellular pathways affecting migration and invasion, at a critical phosphorylation site and disrupted the interaction of FAK with oncogenic binding partners. Altogether, these findings suggest that merlin inactivation is a critical step in mesothelioma pathogenesis and is related, at least in part, with up regulation of FAK activity. Recently, merlin has also been shown to suppress cellular proliferation by inhibiting the activation of RAS. Merlin was found to counteract the ►ERM (ezrin, radixin, and moesin)-dependent activation of RAS, which correlated with the formation of a complex comprising RAS, ERM proteins, Grb2, SOS, and filamentous actin. Thus, part of the tumor suppressor function of merlin appears to be its interference with Ras- and Rac-dependent signal transfer.

Recent work has implicated the same set of tumor suppressor genes described above in mouse models of mesothelioma. Asbestos-exposed Nf2 heterozygous (+/−) ►knockout mice exhibited markedly accelerated mesothelioma formation compared with asbestos-treated wild-type littermates, and loss of the wild-type Nf2 allele, leading to biallelic inactivation, was observed in all of the asbestos-induced mesotheliomas from Nf2 knockout mice. As in the human disease counterpart, mesotheliomas from Nf2 knockout mice showed frequent homologous deletions of the *Cdkn2a* (*p16Ink4a/p19Arf*) locus and adjacent *Cdkn2b* (*p15Ink4b*) tumor suppressor gene, as well as reciprocal inactivation of the p53 gene, Tp53, in a subset of tumors that retain Arf. As in the human disease, mesotheliomas from Nf2 knockout mice also showed frequent activation of Akt kinase. Thus, this mouse model of environmental carcinogenesis faithfully recapitulated many of the molecular features of human mesothelioma and thus has significant implications for preclinical testing of novel therapeutic drugs. The involvement of similar somatic genetic and cell signaling perturbations in human MM and asbestos-exposed Nf2 knockout mice suggests that a specific set of molecular events cooperate in mesothelioma pathogenesis.

Diagnosis

Chest pain and accumulation of fluid in the pleura or abdomen are often the first symptoms. Radiological tests and/or cytology often reveal that the patient has a tumor and may raise the suspicion of mesothelioma, but the final diagnosis relies on thoracoscopy or laparoscopy and histological evaluation of the tumor biopsy. Because of their undifferentiated state, mesothelial cells can evolve along either an epithelial type or a fibroblastic type of differentiation. Thus, morphologically mesotheliomas are distinguished as epithelial and sarcomatous, the latter also known as fibrous or spindle cell mesothelioma. Sufficient sampling, however, will often reveal both components, thus the terminology of biphasic or "mixed type" mesothelioma. Epithelial mesotheliomas, especially when well differentiated have median survivals of 2 years or more; fibrous mesotheliomas have a median survival of 6 months from diagnosis and are resistant to therapy. Patients with biphasic mesotheliomas have a median survival slightly more favorable than patients with fibrous mesothelioma. Histologically, epithelial mesotheliomas must be differentiated from carcinomas of the lung and breast, biphasic mesotheliomas from synovial sarcoma and carcinosarcoma, and sarcomatous mesotheliomas, from other types of sarcomas. Electron microscopy (EM) reveals long branching microvilli in epithelial mesotheliomas and is very useful to distinguish these tumors from carcinomas. Immunohistochemistry also helps to distinguish mesotheliomas from carcinomas. All epithelial mesotheliomas stain diffusively and strongly positive for calretinin and ►WT1 (nuclear staining is specific, membranous staining is not), for keratin 7, Cam5.2, CK5/6 and pankeratin AE1/AE3 (membranous staining). All of these markers can also be found in various other tumors, including carcinomas, therefore the diagnosis of mesothelioma requires that the epithelial markers are negative. Mesotheliomas are negative for CEA, LeuM1 (CD15), BerEp4 (up to 5% of mesothelioma may show focal positivity with this marker), Moc31, TTF-1 and B72.3. Carcinomas are positive for some of these epithelial markers and are usually negative for calretinin and WT1. Sarcomatous mesotheliomas stain positive for ►cytokeratin, which distinguish them from other sarcomatous tumors of the pleura, and may also stain positive for WT1 and calretinin. All mesotheliomas are malignant. However, histologically malignant mesotheliomas have occasionally been associated with long survivals of years or even decades. Whether the latter should be called mesotheliomas is debatable. There are also some unrelated tumors called "benign mesotheliomas," which are histologically different from mesotheliomas and are clinically benign. It is unfortunate that the term mesothelioma is often used to identify these lesions, because it generates confusion and patient anxiety. Multicystic mesothelioma, also called multilocular peritoneal inclusion cyst, is a totally benign mesothelial lesion characteristically formed by multiple cysts arranged in grape-like clusters.

Adenomatoid mesotheliomas are benign mesothelial lesions of the genital system. Mesothelioma of the atrioventricular node is neither a mesothelioma nor a tumor. This lesion represents congenital heterotopia of the endodermal sinus in the atrioventricular node. Well-differentiated papillary mesothelioma is found more often in the abdominal cavity of young women. Histologically, it is formed by multiple papillary structures covered by cytologically benign mesothelial cells. The lesion is benign but there are cases in which several years after diagnosis the patient developed a true mesothelioma.

Clinical Approaches

To date, no therapy has been definitively shown to significantly influence the natural course of this tumor. Standard therapy with Alimta and ►cisplatin is associated with a prolonged survival of about three months. Patients with early stage disease are good candidates for surgical resection. Surgery should be performed by surgeons experienced with this type of procedure to reduce the risk of operative mortality, which in experienced hands is about 2%. Either pleurocotomy or extrapleural pleurectomy has the potential to cure very early disease. However, mesotheliomas are usually diagnosed at an advanced stage when curative resection is not possible. Chemotherapy has been disappointing to date, although new drugs (►Onconase for example) are presently being tested alone or in combination with conventional chemotherapeutic agents. The discovery of two serological markers, ►mesothelin and ►osteopontin, that are elevated in patients with mesothelioma has raised the possibility of screening individuals at high-risk of mesothelioma (e.g., asbestos workers) for early detection, which might be associated with a prolonged survival and possibly a cure. Prospective clinical trials are ongoing in Cappadocia Turkey, where there is a mesothelioma epidemic associated with ►erionite exposure and genetic predisposition, to validate these ►serum biomarkers. Moreover, clinical trials are planned to test among high-risk mesothelioma cohorts, the possible benefit of chemoprevention using Onconase, an RNAse inhibitor that has had beneficial effects in some mesothelioma patients in the absence of significant adverse side effects.

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MET

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Definition

Met is a member of the ►receptor tyrosine kinase family. Like other members of this protein family, Met possesses a highly glycosylated extracellular ligand binding region, a hydrophobic membrane spanning region, an intracellular region that contains a tyrosine kinase domain, and a C-terminal multisubstrate binding site that mediates interactions with several signal transduction pathways upon receptor activation. Activation of receptor tyrosine kinases results from binding a protein growth factor. The growth factor that activates Met is hepatocyte growth factor/scatter factor, referred to as HGF/SF, as it was identified independently as both a growth factor for hepatocytes (HGF) and as a fibroblast-derived cell motility factor, scatter factor (SF). It was later discovered that HGF and SF were identical proteins and HGF/SF-Met signaling can induce different biological affects depending on the cell context. Since then, HGF/SF-Met signaling has been implicated in a variety of cellular responses including proliferation, motility, ►invasion, chemotaxis, and ►morphogenic differentiation. Through these actions, Met regulates a diverse series of biological processes ranging from lumen formation to neuronal development to ►cancer cell invasion and ►metastasis.

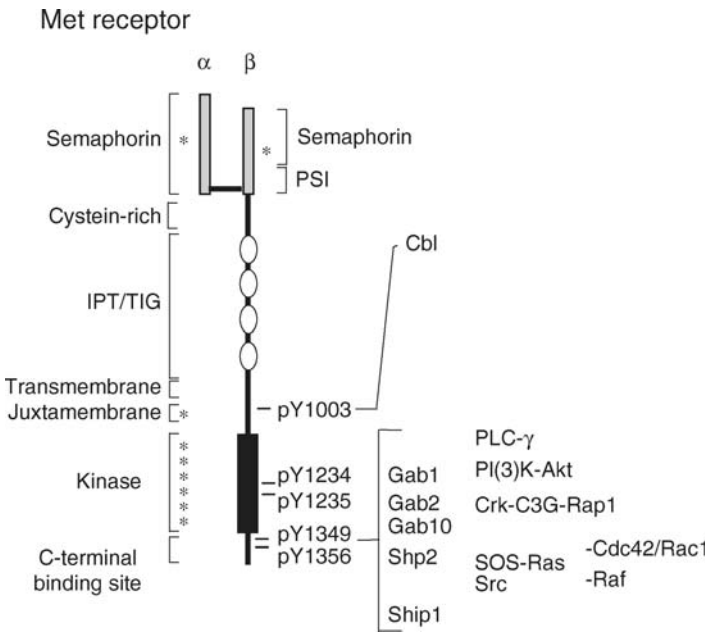
Characteristics

Met was identified in the early 1980s as an ►oncogene. A chromosome rearrangement resulted in the fusion of the N-terminal protein-protein dimerization motif of ►*tpr* (translocated promoter region) to the C-terminal tyrosine kinase domain of ►*met*. The resulting chimeric protein, Tpr-Met, has high constitutive tyrosine kinase activity and can potently transform

cells *in vitro*. Isolation of the ►*tpr-met* cDNA led to the identification of the full length Met receptor.

Human *met* spans over 120 kb of genomic sequence located on chromosome 7q31. The gene consists of 21 exons that when spliced together produce a primary mRNA transcript that encodes the complete receptor. Several *met* splice variants have been identified, but the physiologic role of these variants is not well understood. Mature, full-length, human Met is produced by proteolytic cleavage of a single 1408 amino acid, partially glycosylated precursor protein into an N-terminal α-chain, which is entirely extracellular, and a larger C-terminal β-chain (Fig. 1). The β-chain consists of an extracellular portion, a membrane spanning segment and an intracellular region that contains the tyrosine kinase domain and the C-terminal binding site. Similar to other receptor tyrosine kinases, such as the insulin receptor, the α and β-chains of Met are joined via a disulfide linkage and further glycosylated during transport to the cell surface. While at the cell surface, Met clusters together and may interact with cell ►adhesion molecules, such as ►cadherin, to help regulate cell-cell interactions.

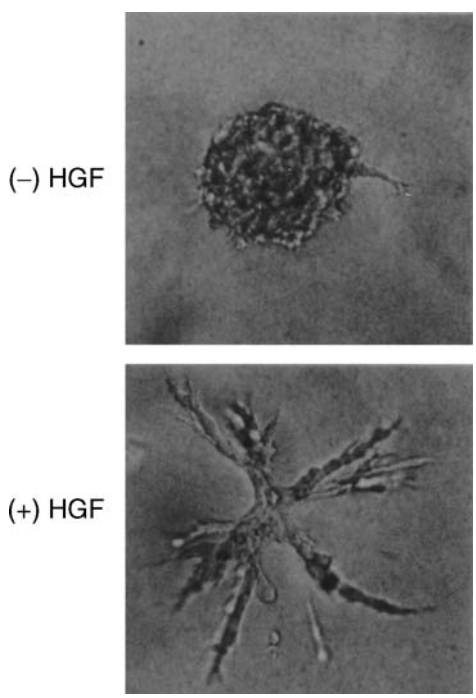
Met is normally expressed in the epithelial cells of several embryonic and adult tissues (kidney, liver, lung, skin, stomach and placenta). In contrast, HGF/SF expression is usually restricted to the surrounding mesenchyme (fibroblast stroma and other mesenchymal cells). Several aspects of organogenesis, such as tissue growth and morphogenic differentiation, are regulated by interactions between the organ epithelia and the surrounding mesenchyme. Endocrine mediated signaling between HGF/SF and Met is believed play an important role in regulating normal epithelial-mesenchyme interactions. If HGF/SF-Met signaling is inhibited by gene disruption in mice, the mouse embryos die in early gestation with several defects in liver and placental organogenesis. Furthermore, while not directly observed in the mouse mutants (partly due to the early embryonic lethality and partly due to compensation by other factors), HGF/SF-Met signaling has been implicated in regulating ►epithelial-mesenchymal transitions and other epithelial-mesenchyme interactions such as mammary gland duct formation, lung tubule formation and kidney development in organ culture models. In addition, several epithelial cell types undergo significant morphogenic



MET. Figure 1 The Met receptor tyrosine kinase stimulates multiple signal transduction pathways. The Met receptor is a membrane spanning α/β heterodimeric protein that binds the HGF/SF growth factor. The extracellular region of Met contains a ►SEMA domain (grey boxes), a ►PSI domain, and four tandem ►IPT/TIG domains (white ovals). The intracellular region of Met contains a tyrosine kinase domain (black box). Phosphorylation of tyrosine residues (-pY) within the kinase domain, following growth factor binding and receptor dimerization, activates the tyrosine kinase domain. Phosphorylation of the tyrosine near the membrane spanning region serves to inhibit the kinase activity of this receptor, though Cbl binding and subsequent receptor degradation. Phosphorylation of tyrosine residues in the C-terminal end of Met activates a multisubstrate binding site that mediates interactions with several adapter and signal transduction proteins to stimulate growth, motility, and morphogenic differentiation. Asterisks (*) indicate where missense mutations have been identified in various human tumors.

differentiation to form branched tubule structures complete with interior lumens when grown in a three-dimensional matrix and stimulated with HGF/SF (Fig. 2). While other growth factors such as ►epidermal growth factor receptor ligands (EGF) may induce proliferation and/or movement, HGF/SF is somewhat unique in its ability to induce all of the cellular responses required for ►branching morphogenesis.

However, HGF/SF-Met signaling is not exclusively involved in regulating epithelial morphogenesis. Met is also expressed in skeletal muscle and in the developing nervous system, and HGF/SF-Met signaling is required for the ►migration of myogenic precursors in limb bud formation and has been implicated in neuronal development. HGF-Met signaling also stimulates the proliferation of hepatocytes, renal epithelial cells and vascular endothelial cells, and increases the motility of both epithelial and vascular endothelial cells and may play a significant role in wound repair and tissue regeneration. The ability of HGF/SF to induce increased growth, motility and capillary-like tubule formation in vascular endothelial cells in vitro and to promote blood vessel formation in vivo suggests that Met may stimulate ►angiogenic processes as well.



MET. Figure 2 Met induced branching morphogenesis of cells grown in a three-dimensional matrix. Several epithelial cell lines (cell line C127 is shown) that express Met undergo significant changes in cellular morphology and three-dimensional organization following stimulation with HGF/SF.

Cellular and Molecular Features

Normal Met activation by HGF/SF is believed to occur through receptor dimerization and induction of ►transphosphorylation of tyrosine residues, which are critical for growth factor mediated signal transduction (Fig. 1). Much work has been done to determine which of the phosphorylated tyrosine residues in the intracellular region of Met (there are 16 of them) are important for stimulating cellular responses. Phosphorylation of two tyrosines located within the activation loop of the tyrosine kinase domain (amino acid positions 1234 and 1235 in human Met) greatly enhances the intrinsic kinase activity of the receptor and are critical for potent receptor activation. Phosphorylation of two closely spaced tyrosines at the C-terminal of Met (amino acid position 1349 and 1356 in human Met) generates a multisubstrate docking site that several adaptor proteins, including Gab1, Grb2, Shp2, and Shc, can bind. In turn, these adaptors recruit numerous signal transduction proteins including phosphotidylinositol-3-OH kinase (►PI3K), phospholipase C-γ (PLC-γ), the ►GTPases Ras, Rac1/Cdc42, ►Rap1, and others signaling molecules. Several assays that monitor HGF/SF-Met meditated cell proliferation, ►cell scattering and branching morphogenesis have been instrumental in identifying the signaling pathways that are responsive to Met activation. However, detailed analysis of which protein pathway mediates each of these cellular responses is complicated by prevalent pathway and ►receptor cross-talk [1, 2]. Generally, activation of the Ras and ►B-Raf signaling pathway via the Grb2/SOS complex is important for both cell proliferation and motility. Activation of PI3K is important for cell survival and prevention of ►apoptosis while activation of the Rac1/Cdc42 pathways and disassembly of ►E-cadherin/catenin complexes at the cell surface promotes loss of intercellular adhesion and gain of cell motility. Induction of branching morphogenesis likely requires all of these factors and additional factors downstream of Gab1, as overexpression of Gab1 can stimulate both cell scattering and branching morphogenesis in the absence of HGF/SF.

As several of the proteins activated by Met have also been implicated in tumor generation and progression, down-regulation of the activated Met signal is important to prevent hyperactivation of these protein pathways and subsequent cellular transformation. One important means of down-regulating growth factor receptors is degradation of the active receptors. ►Endocytosis can clear activated growth factor receptors either transiently, if they are recycled back to the cell surface, or permanently if they are sorted to the lysosome and degraded. Alternatively, activated growth factor receptors can undergo ►ubiquitination and subsequent degradation by the proteosome. For Met, ubiquitination is mediated in part by phosphorylation of a serine residue located

in the receptor juxtamembrane region (serine 1003 in human Met). Phosphorylation of this serine allows the binding of the Cbl E3 ubiquitin ligase. Binding of Cbl results in ubiquitination and degradation of the receptor.

Clinical Relevance

While Met plays several important roles in normal growth and development, abnormal Met signaling likely contributes to generation and progression of human tumors. As previously mentioned, Met was originally isolated as the constitutively activated oncoprotein Tpr-Met. However, Met missense mutations have been implicated in the cause of hereditary and sporadic human papillary ►renal carcinoma (HPRC). The missense mutations are located in the tyrosine kinase domain of Met and produce a constitutively active receptor that promotes tumor formation and/or progression (Fig. 1). Other missense mutation, in the kinase and other domains, have also been identified in ►gastric, hepatocellular, and other cancers. In the absence of an activation mutation, abnormal expression of Met and HGF/SF has now been identified in most solid tumors. A comprehensive table describing the different Met expression and sequence abnormalities in a wide spectrum of human cancers has been collected and can be found at http://www.vai.org/met/.

During tumorigenesis, Met can become constitutively activated by co-expression of HGF/SF in the same cell, forming an autocrine stimulatory loop. Numerous tumor types, including several different carcinomas (►lung, breast and others), sarcomas (osteosarcoma, ►Kaposi sarcoma, and others) and ►brain tumors (►glioblastoma multiforme), express Met and/or HGF/SF. Moreover, in model systems Met-HGF/SF-expressing cells are not only invasive *in vitro*, but also rapidly ►metastasize when injected into mice. Part of the mechanism behind the invasive phenotype of HGF/SF-Met expressing cells is upregulation of the urokinase proteolysis network. Induction of the proteolysis network following Met activation can degrade the surrounding extracellular basement membrane and facilitate invasion and metastasis.

Overexpression of Met is also observed in several gastric carcinoma cell lines as well as carcinomas of the lung, pancreas, thyroid, ►colon and stomach and is thought to participate in cell transformation and tumorigenicity. As HGF/SF has been found in tumor stroma and normal human serum it is possible that activation of Met in some of these cells may result from paracrine or endocrine stimulation. The paracrine model may be responsible for some ►breast cancers. Identification of HGF/SF and Met in breast cancer tumors is a strong negative prognostic indicator of reoccurrence and survival. The effects of HGF/SF in tumor progression may in part result from an increase in tumor ►angiogenesis. Invasive breast cancers that contain high levels of HGF/SF or brain tumors that are engineered to overexpress HGF/SF have increased microvessel density and increased levels of other vascular markers. Other effects, such as sustained proliferation and/or misregulated differentiation, may also contribute significantly to HGF/SF-Met mediated tumor growth/progression. As such, treatments that inhibit HGF/SF-Met signaling may be therapeutic in preventing the onset and progression of many cancer types and may also play significant roles in preventing cell metastasis and angiogenesis in these cancers.

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MET, ^{11}C-Methionine

Definition

Amino acid (methionine) labeled with the positron emitter ^{11}C enabling detection of tumor manifestation sites (especially brain tumors) due to increased transport of amino acids.

►Positron Emission Tomography

Metaanalysis

Definition

A statistical technique where all data from all available studies of something are combined, regardless of data quality. The results of several case-control studies, or cohort studies or both, are combined to generate a summary measure of the odds ratio. The individual odds ratios are combined, but are weighted by some function of the variance of each odds ratio. The technique is used by researchers to get a maximum of statistical information without worrying about distortion in the results.

►Allergy
►Adjuvant Chemoendocrine Therapy
►Cancer Epidemiology
►Coffee Consumption

Metabolic Activation

Definition
Bioconversion of unreactive procarcinogens to reactive electrophiles through the action of Phase I and Phase II drug metabolizing enzymes.

►Carcinogen Macromolecular Adducts
►Tobacco Carcinogenesis

Metabolic Capability

Definition
An individual's ability to activate or detoxify absorbed chemicals.

►Biomonitoring

Metabolic Cooperation

Definition
Is the correction of a defect in a cell by the transport of the correct molecules from neighbor cells.

►Gap Junctions

Metabolic Detoxification

Definition
The process by which a carcinogen is converted to a form that is excreted without reacting with DNA.

►Detoxification
►Tobacco Carcinogenesis

Metabolic Polymorphisms

Definition
Are ►polymorphisms of genes encoding enzymes involved in the metabolism of carcinogens or anticarcinogens. These are predisposing gene variations with relatively low penetrance that may result in a moderate increase in the risk of specific cancers. The scientific interest in such genes (e.g. sequence alterations in genes encoding ►cytochrome p450, null-polymorphisms for genes encoding ►glutathione S-transferase, or phenotypic alterations of the activities of N-acetyltransferases) is based on the possibility of identifying population subgroups that are at elevated risk of developing environmentally induced cancer.

►(NAT)
►Biomarkers

Metabolic Polymorphisms and Cancer Susceptibility

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Definition
The genetic basis for inter-individual differences in cancer susceptibility.

Characteristics
History
The term ►pharmacogenetics (synonym ►pharmacogenomics) has been used for approximately the last 40 years to describe genetic or inherited factors that determine individuality in response to drugs and other therapeutic agents. In studies starting in the mid-1950s, it became apparent that some individuals were hypersensitive to certain drug treatments. This sensitivity was also observed within other family members and was subsequently shown to be an inherited trait. Early epidemiological studies were focused on the drugs used in the treatment of malaria and tuberculosis. Inter-individual variation in the ability to metabolize these drugs was subsequently attributed to inherited differences in the enzymes glucose 6-phosphate dehydrogenase, which generated sensitivity to the anti-malarial drug primaquine, and in the N-acetyl transferases, which generated sensitivity to antitubercular drugs such as isoniazid. The term pharmacogenetics was first coined by Vogel in the late-1950s. In the 1960s and 1970s there was increased interest in this research area and many genetic variants in a range of genes involved in determining drug response were identified.

Important Genetic Factors

In order to elicit a therapeutic response, a drug first needs to enter the circulation, i.e. to cross the gastrointestinal (GI) tract. We now know that the entry of many drugs into the body is regulated by a multi-gene family of drug transporters (sugar ABC transporters) that have the capacity to pump drugs in and out of cells. In addition, the GI tract also expresses enzymes (Sugar ABC transporters) that can metabolize and inactivate drugs. Variability in the level of expression or activity of these drug metabolizing enzymes is therefore an important determinant of circulating drug concentrations. Once a drug enters the body it is transported to the liver, which is the main organ involved in drug metabolism. Nearly all therapeutic agents are subject to hepatic metabolism before they are eliminated from the body. The rate of metabolism will determine the biological half-life of the drug. Hepatic drug metabolism is carried out by many multi-gene families of proteins. Of key importance are the ►cytochrome P450-dependent monooxygenases that convert lipophilic drugs into more water-soluble products predominantly through hydroxylation reactions. Many anti-cancer drugs are substrates for one or more of the P450 monooxygenases (Table 1). The products of these P450-catalyzed reactions are further conjugated to co-factors such as glucuronic acid or sulfate, further increasing their water solubility and facilitating elimination in the bile or via the kidneys in the urine. The rate of uptake of drugs into liver cells and their rate of metabolism is therefore of central importance in determining therapeutic outcome. Finally, the capacity of a drug to exert its therapeutic effect is determined by its concentration at the target cell and the level of expression and activity of specific receptors and enzymes with which it interacts (Fig. 1).

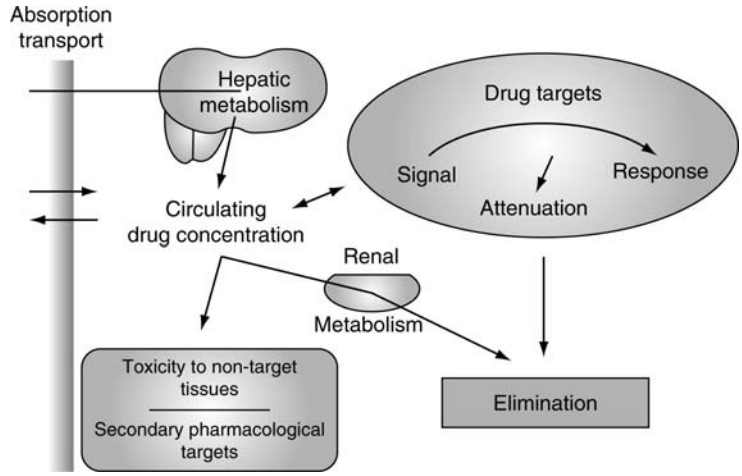
Pharmacogenetic variability resulting in altered drug response can involve any or all of these pathways, and allelic forms (►alleles) of many drug metabolizing enzymes, drug transporters and drug receptors are now known to exist within the population. In addition to individual genetic variability, for certain types of therapy genetic variability of the target cell will also be a key determinant of therapeutic efficacy. This is particularly the case in cancer chemotherapy where the genetics of the tumor cell is often a major additional determinant of the effectiveness of drug therapy. This is also of central importance in the chemotherapy of infectious agents and in the use of antibiotics, antiviral agents, etc. In these cases, drug resistance is well characterized and has been ascribed to altered patterns of gene expression in the target organism many of the factors that determine drug response in these organisms are the same as those that determine circulating drug levels in man, i.e. drug transporters and drug metabolizing enzymes.

Relevance of Pharmacogenetics

The importance of pharmacogenetics is well illustrated by studies of genetic variability in the cytochrome P450 system, in particular in cytochrome P450 CYP2D6. In the 1970s, clinical trials identified a group of people who were hypersensitive to certain types of drug including sparteine, an anti-hypertensive agent which is prescribed for the treatment of arrhythmia and

Metabolic Polymorphisms and Cancer Susceptibility. Table 1 Anticancer P450 drugs

6-Aminochrysene	Flutamide	Retinoic acid
Anastrozole	Genistein	*All-trans*-retinoic acid
Artemisinin	Halomon	Senecionine
BCNU	Ifosfamide	Tamoxifen
O_6-Benzylguanine	Irinotecan	Tangeretin
Bropirimine	Irsogladine	Tauromustine
Coumarin	Liarozole	Taxol
Cyclophosphamide	Lomustine	Teniposide
Dexamethasone	Lovastatin	Tirapazamine
Docetaxel	Mofarotene	Toremifene
Ellipticine	17α-Methyltestosterone	Trofosfamide
(–)-Epicatechin-3-gallate	Nilutamide	Vesnarinone
Ethyl carbamate	Onapristone	Vinblastine
Ethynylestradiol	Oncostatin M	Vincristine
Ecoside	Prednisolone	Vindesine
Flavone acetic acid	Prednisone	Vinorelbine



Metabolic Polymorphisms and Cancer Susceptibility. Figure 1 Factors which can limit the therapeutic effectiveness of drugs.

debrisoquine. It was subsequently shown that this variability in response was inherited and that between 5 and 10% of the white population, "poor metabolizers," do not express a functional form of CYP2D6. In later studies, the molecular basis of this polymorphism was elucidated and shown to be due to gene-inactivating changes in CYP2D6 DNA. This subsequently allowed the development of DNA-based tests to identify individuals with this metabolic defect. Cytochrome P450 CYP2D6 is responsible for the metabolism of up to 25% of therapeutic drugs, including a large number of the drugs used in the treatment of psychiatric illness (Table 2). This polymorphism has now been attributed to aberrant metabolism, and disposition of many of the drugs has been associated with adverse drug reactions induced by many of these agents.

In addition to gene inactivating alleles of CYP2D6, certain individuals contain multiple copies of this gene, which generates an "ultra-rapid metabolizer" phenotype. These individuals metabolize and eliminate drugs at a much faster rate than the rest of the population and as a result the desired therapeutic effect is not achieved. Therefore, pharmacogenetic variability can result in adverse drug reactions, exacerbated drug toxicity or lack of therapeutic effect.

Metabolic Polymorphisms and Cancer Susceptibility. Table 2 CYP2D6 drugs used in the treatment of psychiatric, neurological and cardiovascular disease

Psychiatric disease	Amitriptyline, clomipramine, clozapine, desipramine, fluvoxamine, fluoxetine, haloperidol, imipramine, levomepromazine, nortriptyline, olanzapine, paroxetine, perphenazine, thioridazine, tranylcypromine, zuclopenthixol
Cardiovascular disease	Alprenolol, amiodorine, flecainide, indoramine, mexiletine, nimodipine, oxprenolol, propranolol, timolol

Ethnic Differences

The genes and proteins that determine therapeutic outcome following drug treatment evolved to protect us against the effects of toxic environmental chemicals. Part of the genetic variability in response to these agents may be explained by different populations needing a different spectrum of enzymes to cope with their particular environment and diet. This is exemplified by the large variability in the distribution of pharmacogenetic polymorphisms between different ethnic groups. For example, there is an allele of CYP2D6 that is present in 40% of the Chinese population but is not found in the white population. This allele generates a slower metabolizer phenotype and explains why a high percentage of the Chinese population cannot tolerate doses of drugs prescribed routinely in Europe and the Western world.

Future

The current aim of research in pharmacogenetics is to exploit the information from the Human Genome Project to identify novel variant forms of genes, to establish their functional significance and to apply these usefully in the clinical environment. The ability to identify rapidly and test for variant sequences of genes will allow pharmacogenetic testing to be generally applied in medical practice so that optimal drug doses can be used. This is particularly the case in the treatment

of diseases such as cancer where inappropriate drug dosing is often life threatening and the success or failure of treatment may depend on the genetics of the tumor cell. It is anticipated that with the identification of pharmacologically important genetic variations within the population, rapid tests will be available to hospitals that will allow clinicians to prescribe drugs at optimal dose regimens or to decide whether specific drug therapies will be effective for individual patients.

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Metabolic Regulation of Cancer During Cellular Oxygen Deprivation

►Adenosine and Tumor Microenvironment

Metabolic Trapping

Definition

Intracellular accumulation of drugs or radioactive probes depending on metabolic activity of respective tissue (e.g., intracellular accumulation of the glucose analog ^{18}F-fluorodeoxyglucose after phosphorylation by the enzyme hexokinase).

►Positron Emission Tomography

Metabolism

Definition

Metabolism comprises the chemical reactions that occur in the cell; individuals can be:

- Extensive metabolizer: an individual with the ability to metabolize drugs at a rate that falls within the normal population distribution.
- Poor metabolizer: an individual with a genetically determined deficiency in their ability to metabolize certain drugs.
- Ultra-rapid metabolizer: an individual with increased expression of a particular gene. Drugs which are substrates for these genes are metabolized very rapidly, often requiring increased doses to achieve any therapeutic benefit.

►Metabolic Polymorphisms and Cancer Susceptibility
►Arachidonic Acid Pathway

Metabolite

Definition

Chemical product derived from ►metabolism of another chemical; may be biologically active or inactive.

►Biomonitoring

Metal Chelating

Definition

Capacity to bind metal ions.

►Grape Seed Extract

Metalloenzymes

Definition

Contain a mineral nutrient atom(s) as a requisite part of the enzyme active site. These are distinguished from

metal-associated enzymes where the association with the metal atom is less rigorous.

►Mineral Nutrients

Metalloprotease

Definition

Synonym: Metalloproteinase; An enzyme containing a tightly bound metal ion cofactor, such as Zn^{2+} or Fe^{2+}, that hydrolyzes peptide bonds.

►ADAM17 Metalloprotease
►Matrix Metalloproteinases

Metalloprotease Disintegrin Cysteine-Rich

►ADAM Molecules

Metalloproteases

Definition

A very large group of protein-degrading enzymes implicated in numerous physiological and pathological processes. Metalloproteases bind a metal ion such as Zn2 + in the active site. Exopeptidases catalyzes the stepwise removal of single residues from the carboxyl terminus of a polypeptide substrate, endopeptidases catalyze the hydrolysis of non-terminal peptide bonds, especially those with hydrophobic residues. Among the endopeptidases are ►ADAM molecules (►ADAM17 metalloprotease) and ►matrix metalloproteases.

Metallothionein Enzymes

Definition

Are family of enzymes that are rich in cysteine, a toxic metal-binding amino acid. These enzymes aid in scavenging toxic substances including heavy metal ions.

►Alkylating Agents

Metanephrogenic Blastema

Definition

Is a tissue constituting of undifferentiated cells (blastema) that gives rise to metanephros, i.e., the final kidney in human organogenesis.

►Mesoblastic Nephroma

Metaphysis

Definition

The widened end of the tubular bone shaft

►Cryosurgery in Bone Tumors

Metaplasia

Definition

Is a reversible change in which a given differentiated epithelial or mesenchymal cell type is replaced by another differentiated cell type. However, persistent metaplastic lesions sometimes evolve to malignancy, and represent preneoplastic lesions. Thought to be an adaptive response to chronic irritation, achieved by a reprogramming of stem cells. Some dysplasias and cancers arise in metaplasias, hence the metaplasia – dysplasia – carcinoma sequence.

►Preneoplastic lesions
►Stem Cell Plasticity

Metastasis

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Synonyms

Tumor spread; Secondary tumor

Definition

Metastasis is the growth of a tumor at a site not physically connected to the primary tumor.

Characteristics

The presence of metastasis is a key feature in determining the prognosis and the treatment strategy for any cancer patient. The probability that a patient will have a metastasis varies widely depending upon the type of cancer. Squamous cell carcinoma of the skin rarely metastasizes, but squamous cell carcinoma of the lung frequently does. An important part of the clinical workup for every patient who is diagnosed with cancer is an evaluation for metastasis. The presence and extent of metastases are a critical component of clinical Staging systems that clinicians use to choose appropriate therapy and to judge prognosis.

Metastases follow different routes that can be broadly classified into three. One is metastasis to the regional lymph nodes or lymphatic metastasis. This involves colonization of draining lymph nodes. The second is travel via open cavities such as the pleural cavity or abdominal cavity (peritoneal metastases). The third is travel though the blood stream or hematogenous metastasis.

Metastasis also has organ specificity. Different types of cancer have characteristic tendencies to metastasize to particular sites. For example adenocarcinoma of the proximal colon tends to metastasize to the regional lymph nodes within the colon and distantly to the liver. In contrast prostatic adenocarcinoma tends to metastasize to its regional lymph nodes and to the bone. Other cancer types are more promiscuous – for example breast cancer spreading frequently to the regional lymph nodes, lung, liver, brain, bone, ovaries and adrenals. Or carcinomas of the lung metastasizing, to the lung liver, brain, bone, skin and adrenals. While metastases are often identified at the time of initial diagnosis, they can also become manifest many years after therapy. This feature of tumor dormancy is particularly notable in breast cancer where metastases can emerge after 10 or 20 years. In many cancer types this long dormancy is less likely.

Mechanisms

Cancer is now recognized to be a disease not only consisting of the tumor, but involving the whole organism. Metastasis depends upon properties of the host as well as alterations in the tumor cells. The notion that either the host or the tumor might play a role was first expressed as the soil and seed hypothesis. This frequently cited expression posits that metastasis might be due to alterations in the tumor cell or alternatively to adaptations of the host that now allow the tumor cell to migrate and colonize a distant site. Many years of research now reveal that both mechanisms operate. The study of the molecular changes underlying metastasis is only in its beginnings so that much of the information lacks certainly and detail.

In keeping with the seed hypothesis however are findings showing that the patterns of gene expression in metastatic tumor cells differ from those in non-metastatic cells. The expression of these genes may help explain organ specificity and may potentially be targets for therapeutic intervention. One question that has been addressed in recent years is to ask whether the gene expression profile of a tumor might predict whether that tumor was likely to have formed metastases. If so the genes involved might also be identified from such a screen. In fact in many studies such gene profiles could be identified, but in general the actual genes identified were not the same in different studies. These profiles are now being refined and additional components such as hypoxia and wound healing profiles are being integrated into the original findings. In some cases the genes found were those made by the host stromal response to the tumor rather than by the tumor itself. Furthermore in comparing metastatic to non-metastatic tumors in experimental models, the acquisition of new gene expression patterns were clearly seen. Thus the actual situation is more complicated than the original hypothesis that the tumor might express all of the genes needed for metastasis very early in its development. It seems most likely that some of the necessary genes are present early on, but that others are acquired during progression. Finally some of the identified gene products appear to have been synthesized by host responses. Thus host responses may have several roles some of which impede tumor growth, but others that stimulate metastasis.

Invasion is a necessary precursor to metastasis. For the tumor cell reach a distant lymph node or other organ or cavity, it must first separate from the primary tumor mass itself, either as a single cell or as a clump. *In vivo* imaging in mice has revealed such tumor cell migration through tissue adjacent to the tumor itself. Tumor cells in the blood or lymph then can disseminate. In some systems host cells such as macrophages, fibroblasts and the coagulation system are required for efficient invasion and metastasis, findings in keeping with the soil concept of tumor metastasis.

The microenvironment of a tumor also appears to play a role in metastatic potential. For example hypoxic tumors are more likely to metastasize than tumors with more normal oxygen levels. The infiltrating cells in a tumor may also affect its metastatic potential including fibroblasts, macrophages, and NK cells. For metastasis to the lymph nodes there is some debate about the importance of new host lymphatic channels within the tumor. Recent data raise the exciting hypothesis that the new channels are generated in the lymph nodes themselves.

The contribution by host cells is perhaps most well understood in the formation of bone metastasis in which

tumor cells that are successful in establishing themselves in bone synthesize factors such as parathyroid hormone related protein that activate osteoblasts to produce factors such as RANKL that stimulate osteoclasts that release tumor growth factors setting up a complex positive feedback loop. The elements of this loop then form targets for bone metastasis specific therapy.

Thus potential targets for therapy are being identified, many of which will be organ specific. Less clear are the factors that cause tumors to become metastatic in the first place. While genetic mutations clearly drive tumor progression and malignancy, which changes affect metastasis are less clear. Both tumor cell factors and external factors appear to lay a role.

►Gastrointestinal Stromal Tumor
►Metastatic Colonization

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Metastasis Signaling

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Definition

Molecular pathways that cancer cells use to break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and develop into in a distant cell colony (►metastasis) in normal tissues elsewhere in the body.

Characteristics

Metastatic tumor cells interact with a variety of cell and tissue types in their journey that leads them from being a neoplastic cell within a primary tumor to developing into a clinically detectable secondary carcinoma. These cells are therefore required to modulate a multiplicity of signal transduction pathways in order to respond to the ►microenvironment to successfully colonize a distant site. Many metastasis-associated genes, both those that promote and those that inhibit the process of tumor progression, have been found to be components of important signal transduction pathways involved in controlling normal cellular responses.

The Process of Metastasis

Metastasis is the process by which cancer cells escape from a primary tumor and establish discontinuous secondary lesions elsewhere within an organism. It is an extremely complex process that likely involves interactions with many tissues and cell types. To successfully form a metastatic lesion, primary tumor cells must complete a series of sequential steps. First, the cells in the primary tumor must activate pathways that enhance cellular ►motility to enable them to escape from the primary tumor mass. The metastatic cells must be able to invade through the ►extracellular matrix that surrounds the primary tumor. In addition, they must escape from their tissue of origin into vasculature or lymphatic compartments, or in some cases penetrate neighboring body cavities (e.g. peritoneum, pleura). During and after infiltration into these structures, tumor cells must be able to survive hematogenous sheer forces and exist in an anchorage-independent manner and avoid ►anoikis, the apoptotic program that is activated when epithelial cells become detached from the underlying tissue architecture. Once the cells have successfully transited throughout the body, they must arrest in a secondary site, either by receptor-mediated adhesion to specific vascular endothelial cells or by mechanical trapping of the large cancer cells in narrow capillary beds. After the cells have taken up residence in the secondary site they must adapt to the novel microenvironment and avoid local immunological responses, and must develop the ability to proliferate into a clinically relevant secondary tumor. Failure to successfully navigate any of these steps results in failure to complete the metastatic cascade.

The TGF-β Pathway and Other Well Established Metastasis Signaling Networks

One of the most extensively characterized signaling pathways demonstrated to be involved in metastasis is that involving transforming growth factor-β (►TGF-β). TGF-β, rather remarkably, appears to play apparently opposing roles in tumor progression, and its effect upon tumor cells appears to be related to the phase of tumorigenesis. In early stage lesions, it represses tumor growth by inducing cell-cycle arrest and programmed cell death (►apoptosis). However, in the latter stages of tumorigenesis it actually enhances tumor cell invasiveness and metastatic dissemination. An important process induced by TGF-β with regards to tumor cell invasiveness and metastatic capacity is ►epithelial-mesenchymal transition (EMT). EMT involves the progressive lose of epithelial molecular characteristics

by tumor cells of epithelial origin, and the development of more mesenchymal molecular expression patterns. These changes ultimately lead to tumor cells developing more invasive characteristics including loss of the necessity for cell-cell contacts and enhanced motility.

The origins of the effects that TGF-β has upon tumor progression and EMT are currently subject to much debate. However, characterization of the complex signaling networks induced upon TGF-β activation has greatly improved our understanding of the role that this molecule plays in the process of metastasis. It is becoming clear that dysregulation of components of the TGF-β signaling pathway, and the functional relationships between this pathway and other signaling networks, are crucial in regards to tumor progression. TGF-β signaling in the latter stages of tumor progression involves TGF-β binding to its receptors TGF-βRI and TGF-βRII, followed by subsequent activation of a class of transcriptional co-regulators known as ►SMAD, which in turn activate transcription and influence cell behavior. Furthermore, the EMT effects induced by late-stage TGF-β signaling appears, at least in part, to be a result of interaction between TGF-β receptors with the regulator of epithelial ►cell polarity and ►cell junction assembly, Par6, a member of the ►Par gene family. Specifically, it seems that TGF-β-induced EMT is a result of phosphorylated Par6 causing the ►ubiquitination of the small ►GTPase RhoA by E3 ligase SMURF1, and the subsequent proteosomal degradation of RhoA, which results in loss of tight junctions and stress fibers. Other proffered mechanisms for the role of TGF-β in tumor progression include the observation that TGF-β appears to regulate other metastasis-associated genes including those encoding PTHrP, CTGF and IL-11, all of which are factors shown to induce bone metastasis by inducing osteoclast differentiation and subsequent ►osteolysis. Additionally, TGF-β induces angiogenesis by up-regulating angiogenic factors including ►vascular endothelial growth factor-A (VEGF-A) and ►angiopoietin-1.

Another relatively well described metastasis signaling pathway is that involving ►hepatocyte growth factor (HGF) and its associated tumor cell receptor, the ►receptor tyrosine kinase (RTK) ►Met, a pathway that has been shown to be a mediator of tumor invasiveness. The critical molecule in this respect appears to be the product of the *Met* gene, which is frequently up-regulated in a wide variety of cancers through germline and somatic mutation. Activation of Met induces cell scattering and invasive growth, and in addition to being activated by its "classical" ligand HGF, it is also appears to selectively interact with a number of other molecules. For example, Met can interact with and phosphorylate the cell adhesion molecule $\alpha_6\beta_4$ integrin, which creates docking sites for additional adaptor molecules and signaling effectors. This in turn can enhance the invasiveness and ability of tumor cells to metastasize induced by HGF in a manner that is independent of the integrin's adhesion activity. In addition, Met also cooperates with the ►hyaluronic acid receptor ►CD44 to enhance pro-metastatic signaling.

Signaling Pathways Involved in Cytoskeletal Remodeling

The cytoskeleton and its associated signaling molecules have also been demonstrated to be important components in the process of metastasis. The cytoskeleton is responsible for the underlying architecture of the cell, and dynamic restructuring of the cytoskeletal elements is one of a number of cellular mechanisms that must be manipulated by a neoplastic cell residing within a primary tumor to enable it to become capable of not only surviving but replicating within a distant secondary organ to become a clinically relevant metastatic lesion. For example, ►cytoskeletal remodeling is essential for cell motility, a process that is integral to metastasis. Tumor cell motility is facilitated through a number of cellular mechanisms, but typically involves the formation of cytoskeletal actin projections on the mobile edge of the cell known as ►lamellipodia and ►invadopodia. Also, morphological reshaping of a tumor cell during its travels is an integral aspect of the metastasis, and this again necessitates remodeling of the cytoskeleton.

Due to the apparent importance of these functions in tumor dissemination, it is not surprising that a number of metastasis-associated genes are involved in cytoskeleton biology. A significant number of signaling pathways are induced by interaction with adjacent cells and contact with the extracellular matrix during tumor cell migration. These signals are transmitted through the plasma membrane by a variety of integral membrane proteins, including adhesion factors such as the ►integrin family, the hyaluronic acid receptor CD44, cadherins and growth factor receptors. Signals resulting from these interactions are passed through the interior of the cell through linker molecules, such as ►ezrin and ►merlin, the products of the *Vil2* and *Nf2* genes, respectively. Examination of the known cellular processes that involve ezrin reveals that it appears to be an integral component of signaling networks whose constituents include a variety of previously identified metastasis-associated molecules. Therefore, unlike many other previously identified metastasis-associated molecules, dysregulation of ezrin functionality might well be a common point of origin for many of the challenges that a proto-metastatic cell must overcome in order to progress towards completion of the metastatic cascade. Ezrin directly interacts with the cytoplasmic tail of CD44, a highly alternatively spliced molecule implicated in metastasis. CD44 is thought to have a role in cell migration in invasive tumors and is known to induce a number of intracellular signaling pathways. Increased expression of CD44 correlates with increased

invasiveness in ►osteosarcoma. In addition, ezrin associates with Met, and since both of these molecules have been implicated in modulation of cell motility, ezrin might play an important role in mediating invasion. Stimulation of the CD44 receptor also results in both up-regulation and activation of c-Met. Recent evidence has also demonstrated that these proteins cooperate in signaling through the ►MEK/ERK pathway through the ezrin/radixin/moesin (ERM)-binding domain of CD44. ►ERM proteins link adhesion molecules to filamentous actin and participate in membrane and cytoskeletal remodeling during cell migration. Ezrin is likely to play an important role in signals integration during the development of the invasive phenotype.

Ezrin functions as a conduit and linker connecting metastasis-associated cell surface proteins to other molecules in the interior of the cell. ERM activity is regulated by a combination of phosphorylation and phospholipid binding, which in turn are thought to be mediated by the small GTPase Rho. ERM proteins bind to RhoGDI, a negative regulator of Rho activity, and induce the dissociation of RhoGDI and inactive Rho, which permits Rho to become active by exchanging its bound GDP for GTP. Over-expression of ezrin might therefore result in amplification of metastasis-associated signaling originating from plasma membrane molecules through the Rho-associated signal transduction pathways by sequestering negative regulatory molecules. The importance of activation of the Rho signal transduction pathway in metastasis has been proven in at least two tumor types. Over-expression of RhoC activity enhances the metastatic capacity of ►melanoma cells while dominant negative mutants suppress metastases. Dominant negative expression of RhoA reduces the metastatic capacity of highly metastatic cells. In addition, loss of the Rho regulatory gene RhoGDI2 has been implicated in metastatic progression in ►bladder cancer.

Additional evidence of the importance of signaling through cytoskeletal-based pathways comes from recent studies on ►germline susceptibility to metastasis. Mouse genetics and human epidemiological studies have identified the small GTPase molecule ►SIPA1 as an important metastasis molecule. SIPA1 has been shown to negatively regulate ►Rap1, which has been suggested to act at the crossroads between cadherin and integrin signaling. Rap1 is involved in a variety of integrin-related biological processes, including ►immunological synapse formation, ►macrophage ►phagocystosis, ►chemokine-induced adhesion and trans-migration of leukocytes, lymphocyte and dendritic cell homing to peripheral organs, ►platelet adhesion and aggregation as well as adhesion of cell lines to extracellular matrix components. These studies suggest that Rap1 plays an important role in the transmission of signals from the interior of the cell to the integrin signaling machinery. The role of Rap1 in the internalization of signals through the integrin-dependent signaling pathway is still unclear at this time.

Adhesion Molecules and Metastasis Signaling

►E-cadherin is an important adhesion molecule that is known to negatively regulate CD44 and suppress CD44-mediated invasion and branching morphogenesis. Loss of E-cadherin or deregulation of its function by disruption of its downstream signaling is thought to promote metastatic dissemination. Recent studies have demonstrated that disruption of ezrin result in suppression of branching morphogenesis and vasculogenic mimicry, suggesting that imbalance of signals from CD44 and E-cadherin due to ezrin over-expression might substitute for E-cadherin loss.

In addition to E-cadherin associated cell-cell interactions, interactions between tumor cells and the extracellular matrix (ECM), which are usually mediated by integrins, are also thought to be important in metastatic progression. The role of integrins in metastasis is more complex, with some integrin complexes promoting dissemination and others having potentially inhibitory roles. It has been postulated, however, that integrins are mediators in anchorage-independent cell survival and that disruption of integrin signaling may significantly contribute to tumor cell survival in the circulation and in the unfamiliar microenvironment that disseminated tumor cells will encounter at secondary sites. Consistent with this, studies have demonstrated that suppression of ezrin expression correlates with decreased survival of osteosarcoma cells. The origins of this differential survival was then investigated by determining the relative importance of the ►Akt and ►MAP kinase signaling pathways in this process since these have previously been implicated in ezrin-mediated inhibition of anoikis and in the early steps of metastases, respectively. Intriguingly, survival was partially rescued by over-expression of activated p44/42 MAP kinase, but not Akt, suggesting that ezrin-mediated cell survival either bypasses Akt or operates through an alternative anti-apoptotic pathway. Importantly, apoptosis has been demonstrated to be an important mediator of metastatic inefficiency.

Rap1 also plays an important role in cadherin signaling and function, including intercellular contacts mediated by adherens junctions. Loss of Rap1 function results in loss of adherens junctions and may play an important role in the disruption of normal epithelial contacts that accompany tumor cell migration and invasion. The importance of these cell-cell contacts in the metastatic process is further highlighted by the fact that re-expression of the ►metastasis suppressor ►Brms1 results in restoration of adherens junction formation in tumors and a decrease in metastatic

capacity, while leaving tumorigenicity intact. The mechanism by which Rap1 functions in adhesion is likely complex. Rap1 primarily exerts its effects in ►endosomal compartments and has been implicated in vesicular trafficking. As a result, it may function in the intracellular recycling machinery and/or plasma membrane targeting of recycled or newly generated cadherins or other adhesion molecules to sites of cell-cell or cell-matrix attachment.

Perspectives and Summary

Metastasis signaling pathways are diverse in their origins and extremely complex in their natures and their intricacies, which reflect the highly multifaceted character of metastasis. The fact remains that approximately 90% of cancer deaths are a direct consequence of metastatic dissemination of cancer cells, and most currently available therapies are still directed towards treating the primary tumor. One of the key elements that could lead to major improvements in the currently unacceptably high levels of cancer-related morbidity and mortality will therefore be to discern how and why a neoplastic cell within an isolated primary lesion is able to disseminate to a distant organ and grow into a clinically-relevant secondary tumor.

At the core of this problem are the mechanisms by which tumor cells, the stroma both at the primary and secondary site, and the immunological system interact to produce the observed pathophysiological phenomena. The manner in which each of these cell/tissue types behave and interact with each other is ultimately a consequence of the intra- and inter-cellular signaling pathways that are induced through these interactions. If researchers are able to more fully appreciate these processes then in is likely that ►anti-metastatic therapies might arise as a consequence, and examples of this knowledge can be applied to the development of clinically-relevant treatments is are being seen with increasing frequency. For instance, initial animal-based studies using soluble Met ►decoy receptors or a soluble version of TGF-βRII have proven encouraging and it is hopeful that these may be developed further to become clinically-applicable treatments. However, the fact remains that to better address the problem of metastasis, we will be required to develop a more comprehensive understanding of all biological aspects of this complex process.

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Metastasis Suppressor Gene

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Definition

A metastasis suppressor gene blocks metastasis without blocking tumorigenicity. The suppressing activity could be the result of alterations at any of the steps in the multi-step metastatic cascade.

Characteristics

Although the definition of a metastasis suppressor gene would at first appear straightforward, it is important to emphasize the distinctions between tumorigenesis, tumor progression, invasion and metastasis. This section will briefly highlight the key aspects of those differences, but readers are encouraged to refer to individual entries for a more complete understanding of the terms.

Tumorigenesis refers to the ability of cells to proliferate continuously in the absence of persistent stimulation by triggering carcinogenic agent(s). Tumor progression is the evolution of already tumorigenic cells (populations) towards increasing malignancy. Invasion is the migration of tumor cells away from the primary tumor mass. This process can involve the breakdown of physical barriers (e.g., basement membranes) by secretion of proteinases (i.e., protein-degrading enzymes). Metastasis is the process by which a tumor cell(s) spreads to other sites in the body and establishes a secondary tumor colony. The process is complex and involves many steps (i.e., the metastatic cascade).

The genetics of metastasis can be conceptualized as consisting of two components; positive and negative regulators. The positive regulators (i.e., metastasis promoting genes) drive metastasis formation. These genes, when expressed, enhance the ability of cells to complete one or more steps in the metastatic cascade. An example is the MMPs (matrix metalloproteinases) that are involved in enzymatic breakdown of basement membrane matrices. It is important to note that most metastasis

promoting genes are neither necessary (because of redundancy) nor sufficient (because of the multiple steps in the metastatic cascade) to confer metastatic competency upon cells. Two genes, ►RAS and ►MEK1, however, do confer both tumorigenic and metastatic potential upon NIH3T3 cells, which are murine cells established in culture and are often used for analyzing growth parameters of tumor cells.

In contrast, metastasis suppressor genes inhibit metastasis. Since metastasis requires cells to complete every step in the metastatic cascade, these genes can block any step(s) in the process. The first metastasis suppressor gene was discovered in 1989. Since that time several candidate genes have been identified. To date, more than 20 have been shown to suppress metastasis in vivo – ►BRMS1, cadherin-11, caspase-8, CD44, claudin-1, claudin-4, CRSP3, connective tissue growth factor (CTGF), gelsolin, ►E-cadherin, N-cadherin, KAI1 (►KAI1/CD82 metastasis suppressor), ►KiSS1, RECK, RhoGDI2, Src-suppressed C kinase substrate (SSeCKs), JNKK1/MKK4, MKK6, MKK7, ►NME1/Nm23-H1, RKIP, Drg-1 and TXNIP.

The role of metastasis suppressors is context dependent: the ability to suppress varies by cancer type and mode of action. They vary in the step(s) of the metastatic cascade inhibited. Each metastasis suppressor regulates by manipulating specific biochemical functions. Several affect signaling cascades that are involved in cell survival, growth, and motility. JNKK1/MMK4, MKK6, and MKK7 are involved in stress-activated signaling pathways while both Nm23 and RKIP inhibit the mitogenic ERK pathway. RhoGD12, SSeCKs, and gelsolin regulate cell motility through the Rho/Rac signaling pathway. Others modulate cell-cell or cell-matrix interactions. Claudins-1 and -4 are structural components of tight junctions. Cadherins E, N and -11 mediate cell-cell adhesion and may play a role in inhibiting cell migration. Proteins that modulate cell-matrix are important in regulating the interaction of cells with the surrounding tissue. The secreted protein CTGF and the tetraspanin KAI1 are both involved in integrin signaling. RECK negatively regulates some MMPs that are responsible for remodeling stroma while CD44 binds hyaluronic acid, a key component of extracellular matrix.

There is cross-talk among many of the intra- and inter-cellular signaling pathways. BRMS1 is involved in restoring gap junctional communication between cells, down-regulating the PI3K-Akt survival pathway, and remodeling chromatin to regulate transcription. Caspase-8 is a proapoptotic enzyme that links integrin function to apoptosis by effecting intergrin-mediated cell death. The role of some metastasis suppressors have yet to be defined in terms of function but are rather defined in terms of regulation. Drg-1 is known to be regulated by PTEN, an inhibitor of the Akt-cell survival pathway. CRSP3, TXNIP, and KISS1 form a metastasis suppressor pathway where CRSP3 regulates TXNIP which in turn regulates expression of KISS1, a secreted neuropeptide.

Metastasis suppressor genes and metastasis promoting genes are analogous to tumor suppressor genes and oncogenes, but there are important distinctions. Tumor suppressor genes dominantly inhibit tumor formation when wild-type expression is restored in neoplastic cells. By definition then, metastasis would also be suppressed (since the cells are non-tumorigenic). Metastasis suppressor genes, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor yields cells that are still tumorigenic, but are no longer metastatic.

How are Metastasis Suppressor Genes Identified?

Two general approaches have been used to identify metastasis-controlling genes. The first involves comparison of gene expression in poorly or nonmetastatic cells with matched metastasis-competent cells. The specific techniques employed are differential display and subtractive hybridization. The second takes advantage of clinical observations that identified nonrandom chromosomal changes that occur during tumor progression. This information localized the gene(s) from which cloning could commence. Based upon karyotypic patterns observed in human cancers, additional metastasis suppressor genes to those listed above are hypothesized to exist. However, the identities of the specific genes have yet to be determined.

Clinical Relevance

The existence of genes and gene products that block metastasis implies that the metastasis could be theoretically controlled by agents that regulate these genes or mimic their behavior. Differential expression also implies that measurements of metastasis suppressor expression might predict prognosis. In general, expression of metastasis suppressors predicts good prognosis, increased survival and decreased likelihood of developing metastasis. There are exceptions however. The therapeutic potential of metastasis suppressors has not yet been realized.

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Metastasis Suppressor KAI1/CD82

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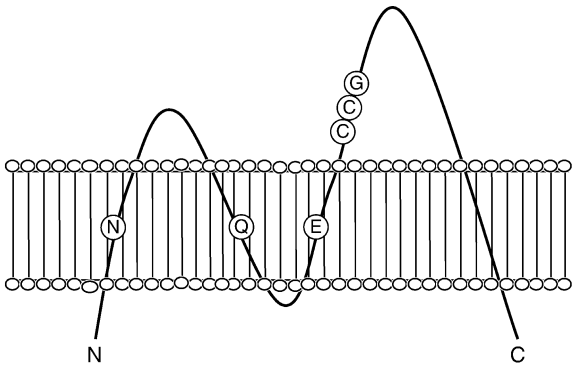
Synonyms

Tetraspanin; Transmembrane 4 superfamily protein; KAI1; CD82; 4F9; C33; IA4; R2

Definition

KAI1/CD82 is an ubiquitously expressed type III transmembrane protein or tetraspanin that functions as a ►metastasis suppressor for a variety of solid malignant tumors, an inhibitor of cell movement, and a costimulator in immune cells.



Metastasis Suppressor KAI1/CD82. Figure 1
Schematic presentation of KAI1/CD82. KAI1/CD82 protein contains a N- and a C-cytopasmic domain, two extracellular domains, one cytoplasmic intercellular loop, and four transmembrane domains. As depicted, the highly conserved CCG motif is located in the large extracellular domain, and three highly conserved polar residues are embedded in the transmembrane regions.

Characteristics

KAI1/CD82 glycoprotein contains 267 amino acid residues and belongs to tetraspanin superfamily. As a member of the tetraspanin family, it has a 11-residue cytoplasmic tail at the N-terminus and a 12-residue tail at the C-terminus region consisting of 11 and 12 amino acids, respectively, and a 4-residue intercellular loop between the second and third transmembrane domains (Fig. 1). The tails and loop forms the intracellular face of KAI1/CD82. It also contains two extracellular loop, known as large extracellular loop (LEL) and small extracellular loop (SEL), and four transmembrane domains (Fig. 1). Based on the sequence homology, the LEL is divided into two regions: variable and constant. The variable region usually mediates the heterogenous protein–protein interaction, while the constant region is predicted to be responsible for the dimerization of tetraspanins. The variable region of LEL of KAI/CD82 contains six cysteine residues that form three pair disulphide bond crucial for the correct folding of the protein. This variable region also contains three N-glycosylation sites, and the glycosylation was reported to be important for KAI1/CD82's ►motility-inhibitory activity. The constant region of KAI1/CD82 LEL likely contains the structural elements needed for the function of KAI1/CD82, since lack of this region KAI1/CD82 can no longer suppress cell ►migration. Little is known about the function of SEL of KAI1/CD82. Evidence from the studies of other members of tetraspanin superfamily suggests that the SEL may be important for the optimal folding of LEL. KAI1/CD82 contains four highly conserved transmembrane domains. The transmembrane domains are needed for the proper posttranslational maturation and the cell surface targeting of KAI1/CD82. For example, it was reported that a truncated KAI1/CD82 which lacks transmembrane domain 1 cannot reach to the cell surface and remains in endoplasmic reticulum. In addition, studies on other tetraspanins suggest that the transmembrane domains are needed for the intramolecular hydrophobic interactions and are likely to be important for the intermolecular associations between tetraspanins in the tetraspanin web or tetraspanin-enriched microdomain. In addition, KAI/CD82 contains three polar residues in its transmembrane domains. The exact biochemical role of these polar residues remains unknown. There are multiple cysteine residues located at or near the intracellular portions of KAI1/CD82. These intracellular cysteine residues are constitutively palmitoylated, and the palmitoylation of KAI1/CD82 are needed for the motility- and invasiveness-inhibitory activity of KAI1/CD82. The YRSV sequence at the C-terminal tail of KAI1/CD82 falls into the category of tyrosine-based endocytosis and sorting motif. The functional relevance of this motif remains to be determined though KAI1/CD82 is found to be internalized and trafficking between different endosomal and lysosomal compartments.

KAI1/CD82 is a component of tetraspanin web or tetraspanin-enriched microdomain, in which tetraspanins associate with other transmembrane proteins such as integrins and immunoglobulin superfamily proteins and intracellular signaling proteins such as kinases and G-proteins. Recent studies also demonstrated that KAI1/CD82 interplays with lipid rafts or ganglioside/cholesterol-enriched membrane microdomains, probably

through the gangliosides enriched in lipid rafts or caveolae.

KAI1/CD82 was initially identified as a metastasis suppressor of prostate ►cancer based on the fact that it only suppresses the formation of metastatic lesion but not the growth of primary tumors. Later studies indicated that KAI1/CD82 actually suppresses the ►progression of a variety of solid tumors. In normal tissues, KAI1/CD82 is ubiquitously expressed, while in invasive or metastatic cancer lesions, KAI1/CD82 expression is constantly reduced or lost. In prostate, gastric, colon, cervix, breast, skin, bladder, lung, pancreas, liver, and thyroid cancers, an inverse correlation between KAI1/CD82 expression and the invasive and metastatic potentials of cancer has been frequently observed. In cancer patients, the presence of KAI1/CD82 expression predicts a better prognosis, whereas the diminution or loss of KAI1/CD82 expression is constantly found in the clinically advanced cancers. Consistent with these observations, Reintroduction of KAI1/CD82 into cancer cells inhibits cell migration and ►cancer invasion in vitro and suppresses ►cancer metastasis in animal model. In addition to the suppression of ►cancer progression, it has been reported that the overexpression of KAI1/CD82 in cancer cells causes ►apoptosis.

Mechanism

The exact mechanism responsible for KAI1/CD82 as a tumor metastasis suppressor is unknown. Several lines of evidence support the notion that KAI1/CD82 may suppress cancer metastasis by primarily inhibiting cancer cell migration and ►invasion. There are several hypotheses regarding the mechanism by which KAI1/CD82 inhibits cell movement. Firstly, KAI1/CD82 may inhibit cell movement by regulating the functions of its associated proteins. The associated proteins of KAI1/CD82 include tetraspanins, integrins, immunoglobulin superfamily proteins, growth factor receptor, intracellular signaling proteins, etc. KAI1/CD82 negatively regulates integrin-dependent cell signaling and cell extracellular matrix ►adhesion. For example, KAI1/CD82 overexpression has been demonstrated to diminish the cell adhesion primarily mediated by laminin-binding integrins. KAI1/CD82 also downregulates the activity of Src family kinases and the formation of $p130^{CAS}$–Crk complex, both are important for cell motility. By associating with epidermal growth factor receptor (EGFR), KAI1/CD82 desensitizes the EGF signaling possibly by accelerating the endocytosis of EGFR and recompartmentalizing the plasma membrane EGFR. KAI1/CD82 expression also reduced the activation of c-Met. Second possible mechanism for suppression of cell movement by KAI1/CD82 is that it directly initiates signal to inhibit cell motility. This hypothesis is based on the observation that the engagement of KAI1/CD82 with its antibody triggers intracellular signaling in immune cells.

In contrast to the notion that KAI1/CD82 suppresses metastasis by directly inhibiting cell motility, a recent discovery opens a completely different avenue to understand KAI1/CD82's metastasis-suppression mechanism. In that study, the extracellular domains of KAI1/CD82 were found to directly bind to ►Duffy antigen receptor for chemokines (DARC), a cell surface protein expressed on the vascular endothelium. The receptor–counter receptor engagement leads to the inhibition of tumor cell proliferation and the induction of cellular ►senescence. In DARC knockout mice or in the absence of DARC, the metastasis suppression activity of KAI1/CD82 was compromised, whereas in DARC wild type and heterozygous littermate mice or in the presence of DARC, KAI1/CD82 inhibits the pulmonary metastasis of the implanted tumor. The proposed mechanism was that, when tumor cells enter into blood vessel after dislodge from primary tumors, only the cells that expressed KAI1/CD82 interact with DARC expressed on the surface of endothelium. This KAI1/CD82–DARC interaction transmits a senescence signal to the tumor cells, resulting in growth arrest and subsequently the suppression of metastasis. The cells that do not express KAI1/CD82 will escape and enter the circulation and subsequently establish metastasis.

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Metastatic Cancer

►Metastatic Colonization

Metastatic Colonization

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Synonyms

Metastatic cancer; Metastasis; Systemic spread of cancer

Definition

The term metastatic colonization refers to the final biological events required for cancer cells to form a clinically relevant ►metastasis at a ►secondary cancer site(s). It is a distinct process in which disseminated cells survive and subsequently proliferate to form overt metastases within this site.

Characteristics

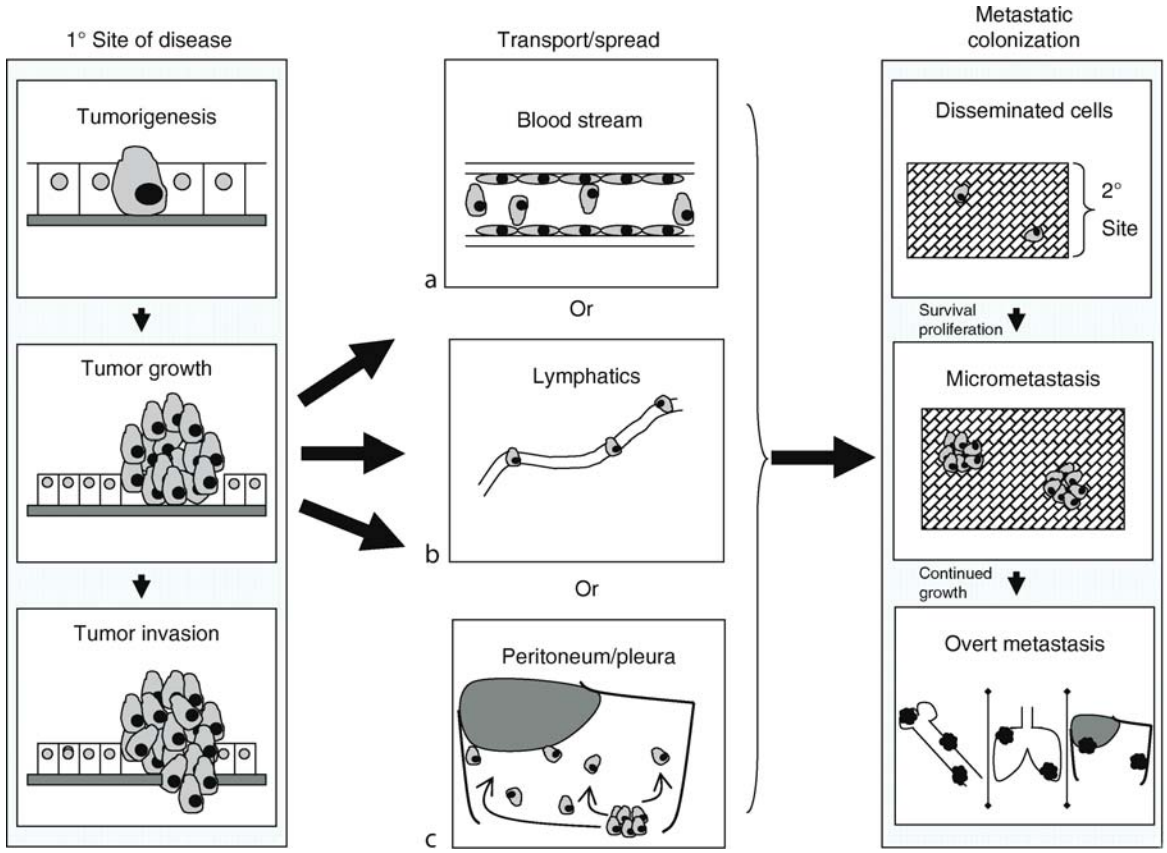
In order to successfully form overt metastases in a secondary organ/site, cancer cells must successfully complete all the steps of the metastatic process, as summarized in Fig. 1. A cancer cells must first survive, grow, and eventually break free from its ►primary cancer site. Figure 1 shows a single layer of cells upon a ►basement membrane (thick grey line), with a single tumorigenic cell. During progressive growth, tumor cells can acquire malignant properties which enable them to invade through the basement membrane and escape the primary site. Cells must then move to a discontinuous secondary site, often via blood vessels or lymphatics in order to travel to other places within the body. After surviving this process, cancer cells arriving at favorable secondary sites can survive and grow into overt metastases. In some instances, such as in the lining of the abdominal cavity and the chest cavity (peritoneum and pleura, respectively), cancer cells can be shed into those spaces and spread to other sites within those spaces without blood vessel or lymphatic transport (Fig. 1).

Once cancer cells disseminate to and lodge within an end organ, they must survive and proliferate within their new environment, often referred to as ►microenvironment. Historically, earlier events in the metastatic process, such as invasion, were considered the more critical steps in cancer metastases, setting the timing and efficiency of metastasis formation. However, work over the last decade has identified metastatic colonization as a biological phenomenon that plays a key role in the regulation and clinical progression of metastatic disease. Cancer cells can be detected in secondary sites as single disseminated cells or as small clusters of cancer cells, termed ►micrometastases, throughout the course of clinical disease. However, in many instances, these cells either remain functionally dormant (►Dormancy) at their secondary sites or are unable to survive and complete the process of metastatic colonization to become clinically apparent metastases.

Metastatic colonization therefore often begins well before symptom presentation or radiographic evidence (by CT scan, MRI, bone scan, etc.) of metastasis is apparent. A clear clinical example of this latency is seen in prostate cancer. Many men with surgically removed prostate cancer are found to have an elevated ►prostate specific antigen (PSA) levels, which is a protein only made by prostate tissue that can be monitored by a blood test, sometimes years after their surgery, despite having no symptoms or evidence of metastatic disease. In most of these instances, the elevated PSA is a marker of microscopic prostate cancer metastases colonizing the bone that have yet to present as clinically significant metastatic disease.

Distinct molecular, cellular, and biological mechanisms regulating metastatic colonization are only now being examined experimentally. There is mounting evidence that interactions between the colonizing cancer cells and their new microenvironment play a key role in regulating the process of metastatic colonization. In the 1880s the physician Stephen Paget, used the analogy of a seed growing in favorable soil, to propose a role for interactions between dispersed cancer cells (the seeds) within the microenvironment of the target organ (the soil) to explain why cells from certain types of cancers have a predilection for particular secondary organ; for example, why prostate cancer cells often metastasize to bone. Molecular insights into the process of metastatic colonization are being elaborated by studies of ►metastasis suppressor proteins. These proteins can specifically inhibit cancer metastasis, while having no effect on primary tumor formation or growth. Functional studies of several of these regulatory proteins, including Map Kinase Kinase 4 (MKK4), demonstrate that they can regulate and inhibit cancer metastasis by directly targeting the process of metastatic colonization.

Metastatic colonization is clearly a clinically relevant phenomenon. In the United States alone, there are projected to be ~1.4 million new cancer diagnoses, and 559,000 cancer deaths in 2007. Cancer is second to heart disease in overall mortality in the U.S. and, in fact, is the primary cause of death in persons under 85 years of age. The majority of patients who die from cancer succumb to metastatic disease. In addition to mortality,



Metastatic Colonization. Figure 1 Simplified schematic of the metastatic process. Metastatic disease begins with the formation of a primary tumor and its subsequent growth and invasion into the surrounding environment. In this example a single cell in becomes cancerous and subsequently replicates and is not subject to normal growth control mechanisms. To successfully metastasize, a cancer cell (or group of cells) must disassociate from the primary tumor, then travel to a discontinuous site via: (a) blood supply, (b) regional lymphatics, or, (c) through the peritoneal/pleural space (in cases such as intraperitoneal/pleural metastasis). In the final process of metastatic disease, termed *metastatic colonization*, the cancer enters its secondary site, where it must survive and proliferate to eventually form overt metastatic disease (such as in the bone, lung, and peritoneum).

cancer metastasis, and metastatic colonization specifically, is a major cause of cancer related morbidity, including pain, fatigue, shortness of breath, etc. Frequently, as is the case in prostate cancer, metastatic colonization is suspected often well before the detection of overt metastatic disease. Keeping disseminated cancer cells dormant or quiescent as long as possible is a key goal of cancer control strategies. As the biology of metastatic colonization becomes clearer, it will likely become a major target for cancer therapeutics.

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Metastatic Tumors

Definition

Tumors that arise in one organ and spread to other organs, adjacent or distant.

►Cardiac Tumors
►Metastasis

Methazolastone

►Temozolomide

Methemoglobinemia

Definition

Is a blood disorder in which the body cannot recycle hemoglobin after it is damaged. It is a disorder characterized by the presence of a higher than normal level of methemoglobin in the blood. Hemoglobin is the oxygen-carrying molecule found in red blood cells. Methemoglobin is a form of hemoglobin that does not bind oxygen.

►Polycythemia

Methotrexate

Definition

Is a structural analog of folic acid and, thus, also referred to as an antimetabolite. It strongly inhibits dihydrofolate reductase resulting in inhibition of synthesis of thymine nucleotides and subsequently of DNA synthesis. Methotrexate is used as an anticancer drug. ►Amplification of the gene encoding dihydrofolate reductase results in drug ►resistance.

►Membrane Transporters

Methoxyestradiol

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Synonyms

2-Methoxyestradiol; 2ME2; 2ME; Panzem

Definition

2-Methoxyestradiol (2ME2) is a naturally occurring substance that is produced when the hormone ►estradiol is processed (metabolized) in the body. 2ME2 prevents both tumor growth (►antiproliferative) and the formation of new blood vessels that tumors require to grow (►antiangiogenic). Although 2ME2 is derived from estradiol, it binds poorly to ►estrogen receptors. On the contrary, it binds directly to a protein called tubulin, which is involved in cell division. This binding not only interferes with cell division (cell growth), but also inhibits hypoxia inducible factor-1 (HIF-1), an important factor for tumor cell survival. Additionally, 2ME2 has been reported to preferentially kill tumor cells, sparing normal cells, by causing ►reactive oxygen species (ROS) accumulation in cancer cells.

2ME2 under the trademark Panzem® (EntreMed Inc., Rockville, MD) is currently in Phase I/II clinical studies to investigate its safety and efficacy and evaluate its drug-like properties in patients with advanced cancers.

Characteristics

Mechanisms

2-Methoxyestradiol (2ME2) is a naturally occurring estradiol metabolite that has a low binding affinity for estrogen receptors (ER) α and β, consistent with its low estrogenic activity. In addition, the antiproliferative and ►proapoptotic effects of 2ME2 have been shown to be independent of ERs, since cell lines devoid of ER were sensitive to 2ME2 and ER antagonists did not attenuate 2ME2's inhibitory effects. The biological activity of 2ME2 was first reported by Seegers et al who found it to cause ►mitotic accumulation and the formation of abnormal mitotic spindles in cells, regardless of ER status. These effects were confirmed by others in diverse cancer cell types and in ►endothelial cells.

The antiangiogenic activity of 2ME2 was reported over a decade ago. 2ME2 inhibits the ►proliferation, ►migration and ►invasion of endothelial cells *in vitro* and has antiangiogenic effects in several *in vivo* models. Unlike most antiangiogenic agents, 2ME2 targets both proliferating endothelial cells and tumor cells, leading ultimately to the initiation of ►apoptosis. 2ME2 also has indirect antiangiogenesis effects mediated through inhibition of ►HIF-1 expression in tumor and endothelial cells. HIF-1 is a proangiogenic transcription factor that interacts with ►hypoxia response elements (HREs) to stimulate transcription of multiple proangiogenic genes including, most notably, ►vascular endothelial growth factor (VEGF). In addition, HIF-1-targeted genes also include genes involved in cell proliferation, survival, invasion and drug resistance. Due to its role in several key events of cancer progression, HIF-1 is an attractive molecular target for cancer therapy. 2ME2 inhibits the expression of HIF-1 in cancer cells lines. The effects of 2ME2 are dose-dependent and occur in cells exposed to

both normoxic and hypoxic conditions. The 2ME2-mediated decrease in cellular HIF-1 levels leads to inhibition of its nuclear translocation, inhibition of VEGF transcription and a decrease in VEGF secretion. The activity of 2ME2 on HIF-1 is expected to inhibit the transcription of over 60 genes that have been identified as HIF-1 target genes. 2ME2 does not inhibit HIF-1 transcription or protein stability but rather appears to act by inhibition at the level of translation. The effects of 2ME2 on HIF-1 are not limited to cancer cells: under hypoxic conditions, 2ME2 also downregulates HIF-1 protein levels in ►human umbilical vein endothelial cells (HUVEC). Thus, 2ME2 not only directly inhibits endothelial and tumor cell proliferation leading to ►apoptosis; it also inhibits HIF-1-mediated HREs that are directly tied to ►angiogenesis, proliferation, survival and ►metastasis. Important studies link the ability of 2ME2 to inhibit HIF-1 with its ►microtubule-depolymerizing effects. The effects of 2ME2 on microtubule disruption and inhibition of HIF-1 are not unique. Other microtubule-disrupting drugs, such as ►paclitaxel and ►vincristine, also cause similar effects and these data are consistent with the reported antiangiogenic effects of microtubule disruption, either stabilization or depolymerization. Cell lines with mutations in the paclitaxel-binding site remained sensitive to 2ME2, demonstrating that the two agents do not bind to the same site on tubulin. Rather, 2ME2 binds directly to the ►colchicine binding site of the ►tubulin protein, thereby ►inhibiting polymerization. This disruption not only interferes with the ►mitotic spindle apparatus, but also inhibits HIF-1 translation and its nuclear translocation.

The inhibitory effects of 2ME2 on both cancer cells and endothelial cells involve the activation of apoptotic cascades. In multiple cancer cell lines and in endothelial cells, 2ME2 increases the expression of death receptor 5 (DR5), a member of the tumor necrosis factor (►TNF) death receptor family. Activation of the extracellular domain of DR5 by its ligand, TNF-related apoptosis inducing ligand (►TRAIL), initiates signals through the death adapter protein ►FADD in the intracellular death domain to activate ►caspase-8, an effector caspase. The effects of 2ME2 on DR5 upregulation were confirmed *in vivo* in an ►orthotopic ►xenograft model using therapeutic doses of 2ME2. The expression of dominant-negative FADD significantly inhibited 2ME2-induced apoptosis, suggesting that this apoptotic pathway plays an important role in 2ME2-mediated apoptosis. In addition to the DR5 pathway of apoptosis, 2ME2 has been reported to mediate apoptosis through activation of the c-Jun NH_2-terminal kinase (►JNK). In myeloma cells, 2ME2 causes JNK phosphorylation and translocation to mitochondria, where it initiates a decrease in mitochondrial membrane potential and the release of two key apoptotic modulators, ►cytochrome c and second mitochondria-derived activator of caspase (Smac). The ability of 2ME2 to activate JNK is not limited to myeloma cells. Breast, prostate, hepatic and colorectal cancer cell lines respond to 2ME2 by rapid JNK activation. In 2ME2-sensitive ►pancreatic cancer cell lines, 2ME2 causes cytochrome c release and Bax translocation from cytosol to mitochondria. Thus, in diverse cell types, 2ME2-mediated cytotoxicity involves activation of the mitochondrial apoptotic pathway. Furthermore, the effects of 2ME2 have been shown to be independent of ►p53 status because 2ME2 was equally effective against cell lines with wild-type or mutant p53.

2ME2 was also shown to preferentially kill tumor cells, while sparing normal cells, by causing the accumulation of ►reactive oxygen species (ROS) in cancer cells. Inhibition of the mitochondrial electron transport chain by arsenic trioxide, an antineoplastic medicine, enhanced ROS generation and increased its anticancer effect when combined with 2ME2.

Clinical Aspects

EntreMed, Inc. (Rockville, MD) has formulated 2ME2 as an orally-administered liquid suspension (Panzem® NCD) using NanoCrystal® Colloidal Dispersion (NCD) technology to enhance the bioavailability of 2ME2 in patients. NCD is a proprietary technology of Elan Drug Delivery (Elan) that is currently used in multiple marketed pharmaceuticals. The NCD technology produces nanometer-sized particles, which are up to 500 times smaller than particles manufactured by conventional milling techniques. While ►Phase I and II clinical trials in 171 patients with a prior capsule formulation showed evidence of biological activity, the newer formulation increased bioavailability 5–10-fold to levels at which optimum antitumor activity was observed in preclinical studies.

Studies with ►xenograft and metastatic cancer animal models indicate that 2ME2 targets both the tumor and the tumor vasculature in sarcomas and melanomas. In an orthotopic breast tumor model, 2ME2 caused microtubule depolymerization and the formation of aberrant mitotic spindles in both tumor cells and endothelial cells. Using non-invasive imaging and histological evaluation in a preclinical orthotopic glioma tumor model, 2ME2 treatment resulted in a dose-dependent reduction in tumor size. Tumor burden in the brain was significantly reduced following 2ME2 treatment, as determined by magnetic resonance imaging (MRI). Further histological examination of the 2ME2-treated tumors demonstrated a dose-dependent decrease in acetylated tubulin, a marker of microtubule disruption, consistent with 2ME2 causing tumor inhibition through interfering with microtubules. In this preclinical study, 2ME2 also led to a significant improvement in tissue oxygenation, which may result in improved responsiveness to therapy, and a decrease in HIF-1.

This drug is potentially well suited to ►glioma treatment because of the abundance of neovasculature, and the positive association between high expression of HIF-1 and tumor grade in glioma. The ability of 2ME2 to initiate microtubule changes *in vivo* at therapeutic concentrations suggests that these effects may play critical roles in its antiangiogenic and antitumor activities. Additionally, preclinical models show that 2ME2 has potential therapeutic applications in inflammatory diseases such as rheumatoid arthritis.

Panzem® is currently in Phase I/II clinical studies to investigate its safety, efficacy and ►pharmacokinetics. The results from two Phase Ib studies of Panzem® NCD in patients with advanced cancer conducted at the University of Wisconsin Comprehensive Cancer Center and the Indiana University Cancer Center identified a maximum tolerated dose at one site with fatigue as the dose limiting toxicity and determined a recommended Phase II dose. Since January 2006, the Company has commenced five additional clinical studies with Panzem® NCD. These include: (i) a Phase II clinical trial in ►glioblastoma multiforme (GBM) patients at the Duke University Medical Center Brain Tumor Center; (ii) a Phase Ib study of Panzem® NCD in combination with paclitaxel (Taxol®) in patients with metastatic breast cancer, also being conducted at the Duke University Medical Center; (iii) a Phase II multi-site study in combination with Avastin® in metastatic carcinoid tumor patients; (iv) a multi-center Phase II study in patients with hormone refractory ►prostate cancer; and (v) a multi-center Phase II study in patients with recurrent or resistant epithelial ►ovarian cancer. Additional Phase II studies under consideration include breast cancer, renal cell cancers, and ►multiple myeloma.

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2-Methoxyestradiol

►Methoxyestradiol

Methyl Groups

Definition

Are necessary for DNA ►methylation and the generation of phospholipids. DNA methylation in general silences tumor oncogens and thus inhibits carinogenesis. The active methyl donor is S-adenosyl-methionine, which is reduced after chronic alcohol consumption.

►Alcohol Consumption
►Epigenetic Gene Silencing

Methylation

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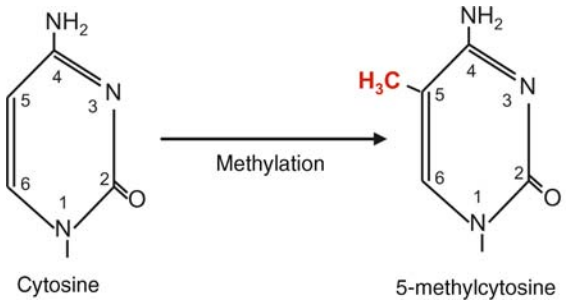
Definition

An epigenetic modification of DNA is the addition of a methyl (CH3) group to position 5 of a cytosine residue. The majority of methylation events in humans occur on cytosines that are located next to a guanine (5′-CpG-3′ dinucleotides).

Characteristics

DNA methylation results from the addition of a methyl (CH3) group to position 5 of a cytosine (Fig. 1). The addition of a methyl group by DNA methyltransferases is an ►epigenetic modification to DNA that is maintained after cell division and does not change the DNA sequence. 5′-CpG-3′ dinucleotides are not uniformly distributed in the human genome. ►CpG islands are short stretches of DNA, usually located in promoter regions of genes, with an unusually high GC content and a significantly higher frequency of 5′-CpG-3′ dinucleotides compared to the rest of the genome. It is well accepted that DNA methylation is involved in gene regulation. Inhibition of transcription factor binding by methylation of the target sequence was the first mechanism identified. This mechanism is limited to a subset of transcription factors that contain



Methylation. Figure 1 Cytosine and 5-methylcytosine.

5′-CpG-3′ dinucleotides in their recognition sequence. A second mechanism is the binding of methyl-CpG-binding protein complexes on proteins (MeCP1 and MeCP2) to methylated DNA. Transcription can then be repressed by either the inhibition of transcription factor binding or the recruitment of histone deacetylases (HDACs). HDACs mediate the deacetylation of lysine residues in the N-terminal tails of histones and thus cause an increase in compaction of the chromatin that makes the DNA less accessible for the transcriptional machinery.

The establishment of normal DNA methylation patterns is a tightly regulated process of significant importance in many developmentally regulated pathways.

- Early embryonic development in mice is characterized by a genome wide demethylation in the early cell divisions (8-cell stage) to the blastocyst stage. The methylation pattern is re-established during implantation by a wave of de novo methylation.
- ►X-chromosome inactivation is a developmentally regulated process in which DNA methylation plays an active role. In females most genes on one of the X-chromosomes are silenced. This mechanism assures the same expression levels in male and female cells. Dense methylation of only the inactive X-chromosome is correlated with transcriptional silencing of the genes located on this X-chromosome.
- While most genes are expressed from both the maternal and the paternal alleles, a small number of imprinted genes are expressed in a parent-of-origin dependent manner.

The underlying regulatory mechanisms of genomic ►imprinting are unclear but allele-specific methylation in CpG islands associated with imprinted genes seems to be involved. Since methylation patterns are established in selected sequences of the genome, a sequence specificity for DNA methyltransferases has been postulated. A first indication how this could be achieved came from a recent report describing a protein complex of DNMT1 with ►RB, ►E2F1 and HDAC1. This complex has the ability to target specifically those genes involved in growth regulation that contain E2F1 binding sites.

Cellular and Molecular Aspects

Several steps are required for the establishment of DNA methylation patterns. The methylation of an unmethylated sequence requires an enzyme with de novo methylase activity. This enzyme recognizes a potential target sequence and methylates both DNA strands. During replication of the genome a new DNA strand is synthesized that is unmethylated creating a hemimethylated state. A different enzyme that can detect and methylate the hemimethylated DNA is required for the maintenance of the methylation pattern (DNA maintenance methyltransferase). Since certain developmental processes require the erasure of the methylation pattern, an enzyme with demethylating activity was postulated. However, several rounds of DNA replication without maintaining the original methylation pattern could also accomplish demethylation. Recently, two methyltransferases, Dnmt3a and Dnmt3b, were shown to have de novo methyltransferase activity *in vivo*. The DNA maintenance methyltransferase, Dnmt1, was identified several years ago and fulfills the expected criteria. This enzyme targets hemimethylated DNA, is ubiquitously expressed in somatic tissue and interacts with the replication machinery at the replication fork. Furthermore, recent data suggests that DNMT1 can establish a repressive transcription complex that includes HDAC2, DMAP1 and the transcriptional co-repressor TSG101.

Aberrant DNA Methylation in Cancer

Hypomethylation

Both extensive aberrant hypo- and hypermethylation have been described for several human cancers. Global hypomethylation in human cancers was one of the earliest changes described to be associated with tumor progression. For example, some reports describe the activation of the ►MYC oncogene in correlation with decreased methylation of CpG dinucleotides in the third exon of the gene. The underlying mechanisms however are unclear. In addition, there is convincing evidence that links hypomethylation with chromosomal breakage. Patients with ►ICF syndrome are characterized by instability of the pericentromeric heterochromatin and decondensation in chromosomes 1, 9 and 16, resulting in multibranched chromosomes. The chromosomes involved show hypomethylation of satellite DNA, and recently several groups reported mutations in the de novo methyltransferase DNMT3B in ICF patients, consistent with the idea of a defect in the methylation pathway.

Hypermethylation

Silencing of tumor suppressor genes either by mutations of both alleles, homozygous deletion or deletion of one allele (LOH) combined with mutation of the remaining allele, are well-documented mechanisms. More recently, homozygous DNA hypermethylation of promoter sequences has been identified as an additional

mechanism of inactivation of tumor suppressor genes. The development of a methylation sensitive ►PCR (MSP; PCR) allowed the rapid identification of methylated promoter sequences in various tumor samples. The list of genes that become hypermethylated in human cancers is growing rapidly and includes genes involved in growth/development, repair and apoptosis. Hypermethylation of CpG islands located in promoter region of genes is correlated with the transcriptional silencing of the adjacent genes. Using ►restriction landmark genome scanning (RLGS) for genome wide assessment of methylation patterns in CpG islands it was shown that up to 10% of the total 29,000 CpG islands in a tumor genome could be methylated. The methylation patterns are not random, suggesting a selective pressure for either the methylation of certain susceptible CpG islands or the selection of cells with a certain methylation pattern and a growth promoting transcriptional profile. In addition, by comparing different tumor types it was shown that some targets of methylation are shared between tumor types and others are specific for one tumor type.

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Methylation-Controlled J Protein

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Synonyms

MCJ; DnaJ (Hsp40) homolog subfamily C member 15; *DNAJC15*; *DNAJD1*; *HSD18*; *MCJ*

Definition

MCJ is a member of the DNAJ family of co-chaperone proteins, whose expression is controlled by methylation of its associated CpG island. Reduced *MCJ* expression increases resistance to several commonly used cancer therapeutics by inducing expression of the ABCB1 drug-transport pump.

Characteristics

The *MCJ* gene maps at 13q14.1 and codes for a protein of 150 amino acids and 16–17 kDa. *MCJ* contains a highly conserved 70 amino acid DNAJ domain (or J-domain) located at the C terminus. J-domain proteins interact with the ►heat shock protein 70 (Hsp70) family of ►chaperone proteins, and act as ►co-chaperones recruiting Hsp70 members to specific substrate proteins. MCJ has been shown to be a type II transmembrane protein localized in the Golgi network, with a cytoplasmic N terminus and a C terminus lying within the Golgi lumen.

The expression of *MCJ* is ►epigenetically (►Epigenetics) regulated by ►methylation of a ►CpG island located in the 5′ transcribed region of the gene. Methylation is present in some normal cell types in a tissue-specific manner and changes in methylation patterns occur during cancer development. In ovarian tissue *MCJ* is methylated and not expressed in normal ovarian cells of epithelial origin, but is unmethylated and expressed in cells of lymphocyte or fibroblast origin. *MCJ* is methylated in approximately 93% of primary ►ovarian tumors (derived from the ovarian surface epithelium) but only approximately 17% show levels of methylation comparable to the normal tissue of origin, suggesting that 83% of tumors have become hypomethylated (►Hypomethylation). In contrast, *MCJ* is unmethylated in normal kidney and brain tissues, but becomes ►hypermethylated in a proportion of ►Wilms tumors (90%) and several different malignant brain tumor types, ►medulloblastomas (7%), supratentorial PNETs (30%) and ependymomas (10%). In addition, 13q14 is a common region of genetic loss in a wide range of human cancers. The disruption of *MCJ* expression in tumorigenesis, suggests it may play a role in tumor development, however the mechanisms underlying this role are not understood at present; studies have failed to demonstrate a difference in the rate of proliferation of *MCJ* ± cells in culture. To date, the major significance of *MCJ* in cancer appears to lie in its role in resistance to chemotherapy.

Initial studies from *MCJ*-►transfected ovarian cancer cell lines implicated loss of *MCJ* expression in chemotherapeutic resistance to a diversity of agents commonly used in cancer chemotherapy including ►paclitaxel, ►topotecan and ►cisplatin. Complementary studies showed that silencing of MCJ expression by ►RNA interference in a drug sensitive breast cancer cell line conferred resistance to doxorubicin and paclitaxel.

Furthermore, high levels of *MCJ* methylation in ovarian cancer patients are associated with both a poor

response to chemotherapy (cisplatin/carboplatin ± a taxoid) and poor overall survival.

Recently insights into the functional mechanism whereby loss of *MCJ* expression confers drug resistance have been made. MCJ associates with c-Jun, a component of the transcription factor complex ►AP-1, leading to its degradation. In the absence of MCJ, levels of c-Jun rise resulting in increased AP-1 mediated transcription of ABCB1 (►P-Glycoprotein), one of a family of drug exporters characterized by an ATP-binding cassette motif. ABCB1 is a large transmembrane protein which functions as an ATP-dependent pump resulting in the efflux of a variety of drugs from the cell, preventing their intracellular accumulation and cytotoxic effects. The development of simultaneous resistance to multiple structurally unrelated drugs is a major problem in cancer chemotherapy. Over expression of *ABCB1* has been associated with drug resistance in tumors. Previously it was known that this up-regulation could occur in cell lines models by amplification of its DNA locus. The discovery that it can also be upregulated by loss of *MCJ* expression has important therapeutic implications as *MCJ* silencing by DNA ►hypermethylation is a feature of certain tumor types and is reversible by DNA methyltransferase inhibitors or demethylating agents. This means that tumors proven to be resistant to chemotherapy as a result of *MCJ* methylation could potentially be re-sensitized by pharmacological re-activation of *MCJ* prior to chemotherapy.

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Methylation-Specific PCR

Definition

Polymerase chain reaction amplification of a small region of DNA using primer pairs that match endogenous sequences containing either methylated or unmethylated cytosine residues.

Methylation Status

Definition

The extent of ►methylation of cytosine residues in cytosine-guanine pairs in regulatory regions of DNA of specific genes or of global DNA within a cell or tissue.

Methylator Phenotype

Definition

A phenotype that is frequently observed in cancer tissues in which gene expression profiles are significantly affected by DNA ►methylation. DNA methylation is a critical mean in inducing epigenetic modification of genes, which are responsible for suppressing the expressions of physiologically important sets of genes in cancer by dense methylation of ►CpG islands on promoter region of a certain gene.

►Epigenetic Gene Silencing

Metrorrhagia

Definition

Any pathological discharge from the uterus.

►Granulosa Cell Tumors

Mevalonate

Definition

Mevalonic acid is a precursor in the cholesterol biosynthetic pathway, known as the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase

pathway that produces terpenes and steroids such as cholesterol.

►Zoledronic Acid

Mga

Definition

Max giant associated protein; is a basic region-helix-loop-helix-leucine zipper protein that represses transcription in complex with Max. It belongs to the Myc/Max/Mad(Mxd) network but also contains a T-box, a second DNA-binding domain found in a T-box-binding family of proteins.

►*Myc* Oncogene

MGC34538

►Aurora Kinases

MGC126245

►Daxx

MGC126246

►Daxx

MGC126806

►B-Raf Signaling

MGC138284

►B-Raf Signaling

MGF

►Kit/Stem Cell Factor Receptor in Oncogenesis

MGP-40

►Serum Biomarkers

MGUS

Definition

►Monoclonal gammopathy of undetermined significance.

►Plasmacytoma

MHC

Definition

Major histocompatibility complex HLA class I.

MHC I or MHC II

Definition

►MHC stands for major histocompatibility complex. Class I MHC molecules are present on almost all nucleated cells and are encoded by HLA-A, B, and C in humans, where as class II MHC molecules are

expressed on antigen-presenting cells, macrophages, B-cells, and dendritic cells and are encoded by HLA-DR, DQ, and DP in humans.

MHC-Restricted Antigen Recognition

Definition

Synonym: ►MHC restriction; refers to the fact that a given T cell will recognize a peptide antigen only when it is bound to a particular MHC molecule. Normally, as T cells are stimulated only in the presence of self-MHC molecules, antigen is recognized only as peptides bound to self-MHC molecules.

MHF

Definition

Methylhydrogenfumarate dimethylfumarate.

►Dimethylfumarate

MIC-1

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Synonyms

PTGF-β; PLAB; GDF15; PDF; NAG-1; PL74

Definition

Macrophage inhibitory cytokine (MIC-1) is a divergent member of the ►human TGF-β superfamily (Transforming growth factor β), that was originally cloned on the basis of increased expression with macrophage activation, but has subsequently been found to be strongly linked to cancer.

Characteristics

The MIC-1 gene, localized on chromosome 19p12-13.1, consists of two exons separated by an intronic sequence of about 1,800 bp. Exon I is 309 bp in length and contains 71 bp of 5′ untranslated sequence. Exon II is 891 bp in length with a 3′ untranslated region of 244 bp. A ►single nucleotide polymorphism in the MIC-1 coding region, results in the change of a histadine (H) to an aspartic acid (D) residue at position 6 of the mature MIC-1 protein (Fig. 1). While other polymorphisms have been documented, this polymorphism significantly impacts on predisposition to cancer and patient survival.

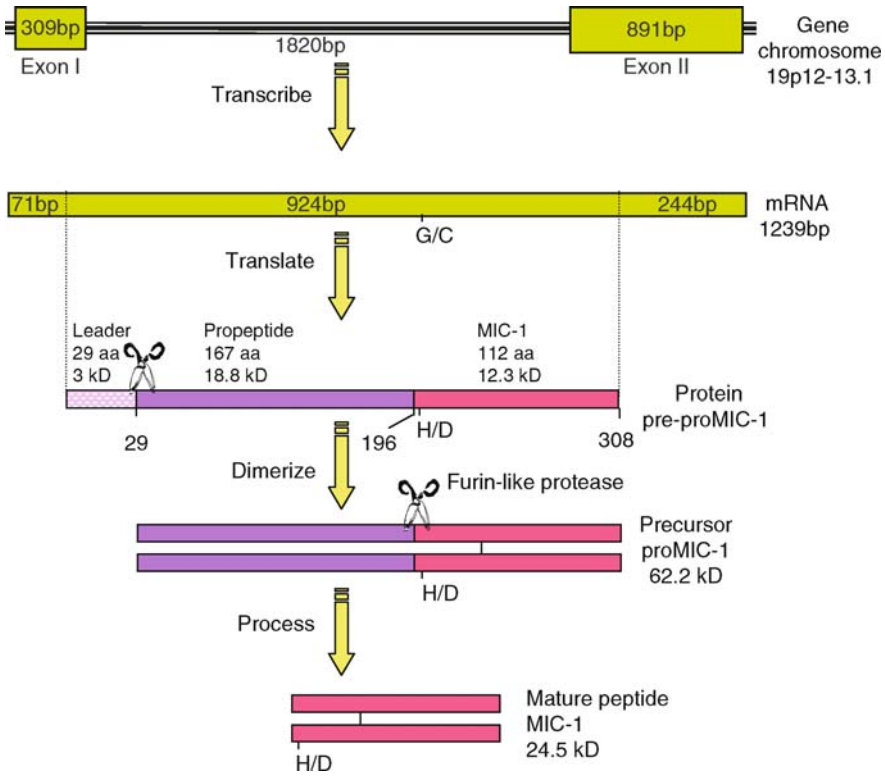
The transcribed MIC-1 mRNA is 1,239 bp, with a coding sequence of 924 bp. MIC-1 is synthesized as a 62 kD intracellular protein, which then undergoes disulphide linked dimerization. This dimeric precursor is cleaved by a furin-like protease (►Furin), separating the propeptide domain from the mature MIC-1 peptide, which is secreted as a 24.5 kD disulphide-linked dimeric protein (Fig. 1).

The receptor for MIC-1 is unknown and its signaling pathways are as yet to be delineated. However, as a TGF-β superfamily cytokine, it probably utilizes elements of the conserved TGF-β superfamily receptors and Smad signaling pathway (►Smad proteins in TGF-β signaling). There is suggestion that MIC-1 also induces activation of Akt (►Akt signal transduction pathway in oncogenesis) and ►ERK pathways.

Regulation of MIC-1 Gene Expression

Under normal physiological conditions, the placenta is the only tissue that expresses large amounts of MIC-1. Low levels of MIC-1 mRNA is found in a wide variety of epithelial cells, but little protein has been detected. However, MIC-1 mRNA and protein is usually dramatically increased in injury, inflammation and cancer in all cells and tissues.

Anti-tumorigenic Stimuli Induce MIC-1 Gene Expression. MIC-1 is one of the major secreted proteins induced by the p53 transcription factor (►p53 Protein, biological and clinical aspects), and may mediate some of the p53 tumor suppressor activity. Indeed, several studies show MIC-1 induction being associated with cell cycle arrest and ►apoptosis. However, other transcription factors such as ►Egr-1 (Early growth response-1) and NFkB are also likely to play a role in inducing MIC-1 expression. A variety of anti-tumorigenic agents, that activate p53 and/or Egr-1 related pathways can induce MIC-1 expression in cancer cell lines. These include many cytotoxic drugs, non-steroidal anti-inflammatory drugs (►Non steroidal anti-inflammatory drugs) as well as dietary compounds such as cruciferous vegetable indole-3-carbinol and green tea catechins (Fig. 2).



MIC-1. Figure 1 Genomic organization, mRNA and protein synthesis and post-translational processing of MIC-1.

M

In normal epithelial cells, there is little or no detectable expression of MIC-1. However, neoplastic transformation causes a major increase in MIC-1 expression, and this is further increased by the tumor response to a variety of anti-tumorigenic stimuli, such as gamma irradiation, cytotoxic drugs, NSAIDs etc. In early stage cancer this could lead to tumor cell apoptosis, inhibition of blood vessel formation and tumor cell cycle arrest. However, in later stage cancers, MIC-1 may help tumor spread and high serum levels of MIC-1 may lead to other systemic effects such as cancer associated anorexia and weight loss. The level of active MIC-1 in the tumor microenvironment is further regulated by creation of stromal stores of unprocessed proMIC-1, which is often secreted in varying degrees from different tumors. Only unprocessed proMIC-1 binds ►extracellular matrix (ECM) via the propeptide, whilst processed mature MIC-1 is freely soluble in the tumor stroma and can move into the blood vessel. Therefore, both intra and extracellular processing will determine levels of available active MIC-1, (Fig. 2). Indeed Stromal MIC-1 storage varies in ►prostate cancer patients and those having low stromal stores are more susceptible to relapse following removal of the affected prostate gland.

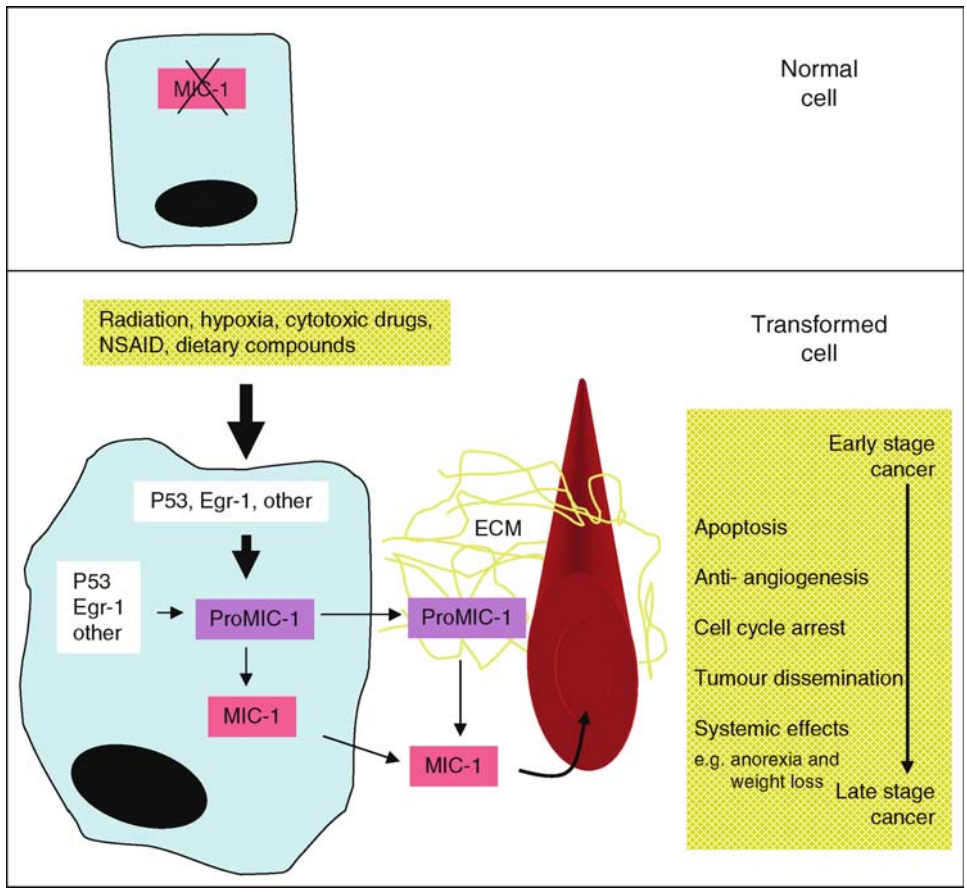
MIC-1 serum levels in advanced prostate cancer patients show a direct relationship between MIC-1 abundance and cancer associated weight loss. Furthermore normal mice given systemic MIC-1 and transgenic mice overexpressing MIC-1, both showed markedly reduced body weight due to decreased food intake. These studies define MIC-1 as a new central regulator of appetite and a potential target for the treatment of cancer associated weight loss.

Serum MIC-1 Measurement as a Clinical Tool

Increased tumor tissue expression of MIC-1 is often associated with serum levels outside the normal range of about 200–1,200 pg/ml. Serum MIC-1 levels in several cohorts of patients have revealed potential clinical utility in the diagnosis and/or monitoring of prostate, breast, pancreatic and colon cancers.

Colon Cancer. MIC-1 serum levels are increased in colonic polyps. Levels increase further with ►colon cancer development in proportion to its stage and extent. MIC-1 serum level at presentation is an independent predictor of metastasis and overall survival. Furthermore, the MIC-1 D allele is associated with significantly better survival in colon cancer.

Pancreatic Cancer. Pancreatic cancer (►Pancreas cancer, clinical oncology, basic and clinical parameters) is often diagnosed late and there is poor survival. Tumor markers have largely been unhelpful. However, measuring serum levels of MIC-1 in



MIC-1. Figure 2 Model for the proposed effects of MIC-1 on tumor biology.

combination with the known tumor marker (►Biomarkers), CA19-9, significantly improved the diagnosis of pancreatic cancer providing a sensitivity of 70% and specificity of 85%. Patients with pancreatic ductal adenocarcinoma had significantly higher levels of serum MIC-1 than those with chronic pancreatitis or healthy controls, while patients with early disease had the highest serum MIC-1 levels. This could help in monitoring patients at higher risk of pancreatic cancer development.

Prostate Cancer. In prostate cancer (►Prostate cancer, clinical oncology), when serum MIC-1 levels are combined with total and free ►PSA (Prostate specific antigen PSA) measurement, diagnosis is significantly improved and is potentially useful for the monitoring of disease progression. Furthermore, the presence of the MIC-1 H allele leads to an increased risk of developing prostate cancer. In established prostate cancer, serum MIC-1 level increases with the development of bone metastasis and measurement early in disease is potentially predictive of future metastatic disease. Additionally, the level of MIC-1 protein expression in prostate tumors is the single best predictor of disease progression in early stage tumors with a Gleason score less than 7 (►Gleason grading).

Role of MIC-1 in Tumor Biology

Whilst a lot is known about MIC-1 expression in cancer, there is less known of its effect on tumor growth and spread. Most studies suggest an anti-tumorigenic role: Firstly, reports suggest MIC-1 induces apoptosis of tumor cells via both p53 dependent and independent mechanisms. Secondly, several in vivo studies using colon and glioblastoma cell lines genetically engineered to express very high levels of MIC-1, inhibited tumor growth when transplanted into ►immunodeficient nude mice (Mouse models). Thirdly, MIC-1 is thought to inhibit blood vessel formation (►Angiogenesis), a process essential to feeding the growing tumor. Finally, transgenic mice that overexpress human MIC-1 in all cells, are resistant to the development of intestinal tumors.

However, one study suggests MIC-1 can promote tumorigenesis. High MIC-1 expression levels in a gastric tumor cell line have been to shown to correlate

with a higher invasive potential. Such contradictory effects can be due to a variety of factors including the nature of the tumor and its environment (Fig. 2).

A lot still needs to be discovered on the precise role of MIC-1 in cancer development. However, there is very strong data to support the measurement of MIC-1 levels in tumor tissue and blood as a means to detect and monitor cancer.

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MIC Molecules

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Synonyms

Major histocompatibility complex (MHC) class I-related chain molecules

Definition

MICA and MICB are human inducible MHC class I-related molecules expressed by stressed and malignant cells and serve as ligands for the NKG2D receptor (►NKG2D receptor and cancer) on natural killer (►Natural killer cells) (NK) and T cells thereby stimulating innate and adaptive immune responses.

Characteristics

Human MIC molecules are encoded within the MHC on the short arm of chromosome 6. The *MIC* gene family comprises six genes (*MICA* to *MICF*) of which only *MICA* and *MICB* are functional, whereas *MICC* to *MICF* constitute pseudogenes. *MICA* and *MICB* are tandem genes located between the *HLA-B* gene and the BAT1 locus at the transition from the class I to the class III region. The MICA locus is highly polymorphic with more than 50 alleles known to date. MICA*08 is by far the most frequent allele encoding for a MICA protein with a truncated cytoplasmic domain due to a frameshift mutation. MICA and MICB molecules are type I transmembrane glycoproteins of ~70 and ~58 kDa, respectively, consisting of three extracellular domains (α1, α2, α3), a hydrophobic membrane-spanning domain, and a cytoplasmic domain. The α1 and α2 domains form an MHC class I-like fold followed by an immunoglobulin-like α3 domain much alike an MHC class I heavy chain. However, at difference to classical MHC class I molecules, MIC molecules neither present antigenic peptides nor associate with β2-microglobulin and are not ubiquitously or constitutively expressed. Rather, expression of MIC molecules is inducible by various forms of cellular stress (►Stress response), including oxidative and genotoxic stress which is associated with malignant transformation. Accordingly, MICA and MICB are broadly expressed by malignant cell lines, epithelial tumors and leukemias. In healthy tissue, MICA has solely been detected on epithelial cells of the gastrointestinal tract which has been attributed to stimulation by the intestinal flora.

MICA and MICB are engaged by the activating NKG2D (natural killer group 2 member D) receptor expressed by almost all cytotoxic lymphocytes including NK cells, CD8 T cells, and γδ T cells. The C-type lectin-like, homodimeric NKG2D interacts with the MHC class I-like α1α2 superdomain of MIC molecules in a manner reminiscent of the interaction of αβ T-cell receptors with MHC class I molecules. NKG2D is associated with the adaptor DAP10 that transduces activating signals upon NKG2D engagement leading to stimulation of NK and T cell effector functions. Besides MIC molecules, there is a second family of NKG2D ligands (NKG2DL) in humans, the UL16-binding proteins (ULBP). ULBP are encoded by a gene cluster on the long arm of chromosome 6 comprising six functional members (ULBP1–4, RAET1G, RAET1L). The MHC class I-like α1α2 superdomain of the ULBP is only distantly related to MIC molecules (amino acid sequence identity (~25%)). A hallmark of ULBP is the lack of an immunoglobulin-like α3 domain and the GPI-anchorage of some family members (ULBP1–3) Altogether, there are eight human NKG2DL known to date, including both MIC and ULBP molecules, raising questions about the selective force behind this redundancy. There is emerging evidence that NKG2DL differ in their affinity for NKG2D or in their expression pattern, and are differentially targeted

by viral immunoevasins. For example, there are reports that MICA allelic variants vary broadly in their binding to NKG2D, that MIC molecules are primarily expressed by epithelial tumors whereas ULBP preferentially are expressed on hematopoietic cells, and that MICA and MICB are differentially targeted by immunoevasins (UL16, UL142) of human cytomegalovirus (HCMV).

Expression of MIC molecules is upregulated by cells infected with viruses (e.g., HCMV or adenovirus) or in the course of a ►DNA damage response. The latter has been linked to the frequent expression of MIC molecules on tumor cells. In fact, NKG2DL expression was upregulated during tumorigenesis (►Carcinogenesis) in mice and led to the rejection of tumor cells by stimulating NK and CD8 T cells. Further, NKG2D was shown to protect the host from spontaneous tumors in chemically induced mouse tumor models. These findings led to the hypothesis that the NKG2D/NKG2DL system acts as a novel immunosurveillance mechanism aiding the elimination of transformed, infected or other potentially harmful cells. A strong expression of NKG2DL can render tumor cells susceptible to NK cell cytotoxicity, even in the presence of MHC class I, and thus NKG2D-mediated tumor immunosurveillance may counteract the development of neoplasms already at an early stage. In this context it is noteworthy that patients with the rare autosomal-recessive Chediak-Higashi syndrome characterized by abnormal cytotoxic function of NK cells have a 200-fold higher risk of developing cancer than other individuals supporting the important role of NK cells in antitumor immunity.

During carcinogenesis, however, antitumor immunity also results in selection of cancer cells capable of evading the immune response (►Escape mechanism). Escape from NKG2D-mediated tumor immunosurveillance can be achieved either by silencing NKG2D or abrogation/reduction of NKG2DL expression. Proteolytic shedding of MIC molecules from tumor cells has been shown to impair the NKG2D-mediated immune response in both ways by locally reducing MIC expression levels on the tumor cells and by systemically downregulating NKG2D receptors on NK cells and CD8 T cells through soluble MIC (sMIC) molecules. Similarly, TGFβ which is frequently produced by tumors has been reported to downregulate both MICA and NKG2D surface expression.

Clinical Aspects of MIC Expression by Tumors

Enhanced expression of MIC molecules has been observed in many epithelial tumors as well as in hematological neoplasias. Several studies have shown a beneficial role of MIC expression on tumor cells such as in colorectal cancer or ►multiple myeloma for patient survival. In tumors with low MIC molecule expression or pronounced MICA shedding, novel agents such as ►imatinib mesylate and histone deacetylation (►Histone deacetylases) (►HDAC) inhibitors were found to induce the expression of MIC molecules and subsequently to enhance the tumoricidal activity of natural killer cells. It remains to be shown to what extent stimulation of the NKG2D/NKG2DL-system contributes to the recent clinical success of these agents in antitumor therapy.

Clinical Aspects of Soluble MIC Molecules in Blood

The process of shedding MIC molecules from tumor cell surfaces during ►progression of cancer disease opens the possibility to measure concentrations of sMIC molecules in blood for diagnostic and prognostic purposes. If benign diseases potentially interfering with diagnosis can be excluded by clinical and laboratory means, MIC levels in peripheral blood may represent valuable indicators of tumor development. Recently, sandwich enzyme linked immunosorbent assays (ELISAs) were developed with specific antibodies against MICA and MICB which allow the quantification of such sMIC molecules in serum, plasma, and other body fluids. Several studies have addressed these clinical questions, particularly the potential of MIC molecules for diagnostic, staging, and prognostic purposes.

Soluble MIC in Diagnosis of Cancer Disease

Most studies found higher sMIC levels in individuals with cancer diseases, particularly in those with advanced stages of cancer as compared to healthy controls. However, the potential impact of most studies on the clinical practice was limited due to the small patient numbers investigated. The currently most comprehensive studies analyzed sMICA and sMICB levels in sera of more than 500 individuals with cancer disease (including colorectal (►Colon cancer) and various other gastrointestinal cancers (►Gastrointestinal tumors), ►lung cancer, ►breast cancer, ►ovarian cancer and other gynecologic cancers, renal and ►prostate cancer), with benign diseases and in healthy individuals. Levels of sMICA and sMICB in sera of healthy donors were generally fairly low. In contrast, pretherapeutic serum levels in various malignancies were significantly higher. However, various benign diseases tended to have elevated serum levels of sMIC molecules limiting the diagnostic capacity for cancer disease. Particularly infectious diseases as well as hepatic and renal diseases potently affecting the marker metabolism were associated with increased levels of MIC molecules.

Nevertheless sMICA levels significantly distinguished between benign and malignant diseases in general, but also in subgroups of lung and gynecological cancers. In contrast, soluble MICB molecules failed to discriminate effectively between cancers and the relevant organ-specific benign diseases. This failure was also due to

the considerable number of individuals with "MICB-negative" cancer disease. In about 70% of cancer patients, there were no sMICB levels detectable while only about 20% of cancer patients were "MICA-negative." These data correspond with immunohistochemical analyses which revealed a significant number of tumors lacking MIC molecule expression. Further, there was only a weak correlation found between sMICA and sMICB levels suggesting differences in expression, shedding, or metabolism of MICA versus MICB. No substantial addition in clinical sensitivity was observed by the combined use of both biomarkers (►Serum biomarkers).

Soluble MIC in Staging of Cancer Disease

After diagnosis, pretherapeutic staging (►Tumor staging) of cancer disease is essential for therapy stratification and estimation of prognosis. Interestingly, elevated levels of both sMICA and sMICB significantly correlated with the extent of disease reflected by UICC classification: While there was no association between sMICA and sMICB levels and tumor size, cell differentiation (►Tumor grading), or lymph node involvement, both markers showed a clear correlation with the presence of distant metastasis. Detailed subgroup analysis confirmed the correlation of both markers with the presence of distant metastasis for various cancer entities, particularly for gastrointestinal tumors. In contrast, high levels of sMICA and sMICB were already observed in early stages of lung cancer, and there was no association with tumor stage. Because sMICA and sMICB levels were particularly elevated in patients with distant metastases, the presence of sMICA and sMICB in serum appears to be an indicator for systemic manifestation of malignancy.

Soluble MIC in Cancer Prognosis and Monitoring of Cancer Disease

As sMICA and sMICB correlate with cancer stage and as staging systems are developed to enable an efficient stratification of patients for alternative therapy options and to estimate prognosis, it can be expected that sMICA and sMICB will provide prognostic information in various tumor entities. A first study revealed a correlation between sMICA serum levels and disease progression in 23 patients with prostate cancer. In addition, a further study on 97 patients with multiple myeloma showed a clear correlation between elevated levels of sMICA and poor overall and progression-free survival in univariate and multivariate analyses.

Analysis of sMICA and sMICB in sera enables longitudinal measurements, e.g., for the estimation of therapy response of cancer disease and for the early detection of cancer recurrence. This may turn out to be valuable in advanced cancers with elevated sMICA levels and in therapies affecting the NKG2DL expression such as treatments with imatinib mesylate and HDAC inhibitors.

Altogether, levels of sMICA and sMICB are elevated in sera of cancer patients when compared to healthy controls. However, due to elevations of sMIC levels in patients with certain benign diseases and due to the considerable number of MIC-negative cancer patients, both markers seem not to be suitable for cancer diagnosis. Because MIC levels are particularly elevated in advanced cancer stages, they may add information in cancer staging and estimation of prognosis. Future studies may address the prognostic relevance of soluble MIC molecules as well as their value in longitudinal observations for the estimation of systemic therapy response and the early detection of cancer recurrence.

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MICA, MICB

Definition

►MHC class I-chain-related A and B, two human NKG2D ligands

►NKG2D Receptor

Micelles

Definition

Are the simplest form of amphiphilic assembly, being an aggregate of surfactant molecules dispersed in a liquid colloid. In aqueous solution, typical micelles

form an aggregate with the hydrophilic "head" regions on the outside and the hydrophobic tail regions sequestered in the center. Block copolymers with amphipathic characteristics can be assembled to form micelles that incorporate anticancer drugs or DNA. Such micelles are small enough (50–100 nm in diameter) to reach tumors by crossing the walls of tumor vessels.

►Non-viral Vector for Cancer Therapy

Michaelis-Type Addition

Definition

Sulfhydryl groups of nucleophilic organic thiols can add to double bonds of electrophilic proteins.

►Dimethylfumarate

Microadenomas

►Colorectal Premalignant Lesions

Microarray (cDNA) Technology

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Synonyms

cDNA chips; Parallel gene expression analysis

Definition

cDNA microarray technology is one of several developing functional genomics approaches to comparatively analyze genome-wide patterns of mRNA expression. Parallel gene expression profiling of cancer genomes with cDNA microarrays has revolutionized may aspects of cancer research. Gene expression "fingerprinting" has been used for tumor classification, prediction of therapeutic response and resistance, and for elucidating genetic pathways associated with particular cancer phenotypes.

Characteristics

cDNA microarray technology entails the development and standardization of various hardware, analytical software, statistical methodology, biological resources and biochemical methodologies. The diagram in the Fig. 1 demonstrates the various stages of cDNA microarray analysis.

cDNA Microarray Construction

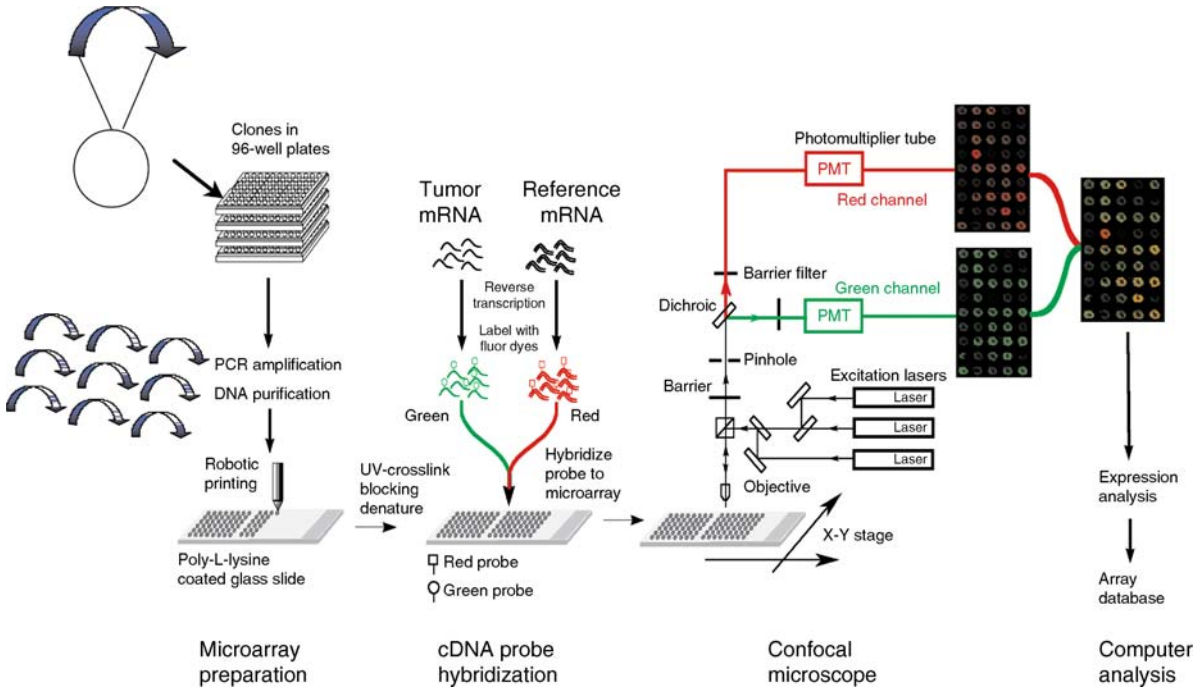
Construction of very high density cDNA microarrays of specific and distinct DNA hybridization "targets," each one representing a single gene, are spotted in an arrayed format on a poly-L-lysine coated glass microscope slide. The printing process involves the sequential transfer of individual purified PCR amplified fragments (200 bp to 2 kb) from a 96 well microtiter tray to an exact predefined location on glass slides. The "arrayer" encompasses a robotic arm that moves (x,y,z axis) the "quill pen probes" into position to either pick up or to spot down DNA (SDS solution), a manifold to hold the slides, a wash station to rinse the "quill pens" when a different DNA is to be picked up, a place to house the microtiter plates, an air flow cabinet to keep everything in and a computer which orchestrates the operation of the components. Multiple glass slides, each containing thousands of spots of DNA, can be synthesized and used in subsequent experiments.

Hybridization with Labeled cDNA

For each experiment, complex "probes" are made that consist of a pool of fluorescently labeled cDNAs. This step begins with the extraction and preparation of mRNAs from two populations of cells. Then each of the two mRNA is reverse transcribed separately with the incorporation of different fluorescently tagged nucleotides, producing two populations of differentially labeled cDNA probes. Typically Cy3 and Cy5 dyes (which emit light at distinct wavelengths after laser excitation) are used to differentially label cDNA pools from different sources. The two complex labeled probes are combined and then simultaneously hybridized to the cDNA targets on the microarray.

Scanning the cDNA Microarray Using a "Reader"

To quantitatively measure the fluorescence of the hybridized probes, the cDNA microarray is scanned by a "reader." This device is a computer controlled inverted scanning fluorescent confocal microscope which detects the emitted fluorescence of the dyes after excitation with a double laser illumination system (a 532 nm, 100 mw NdYag laser is used for Cy3 and a 633 nm, 35 mw HeNe laser for Cy5). Digital images of



Microarray (cDNA) Technology. Figure 1 Schema of microarray technology [adapted from Duggan et al. (1999) Nat Genet (1 Suppl):10–14].

fluorescence intensity data is generated by the "readers" confocal laser scanning microscope.

Image Analysis and Generation of cDNA Microarray Data

Analysis of the image data generated by the "reader" is done with a software for array target segmentation, target detection, background intensity extraction, target intensity extraction, ratio calculation and ratio normalization. Typically, cDNA microarray data is transferred to a relational database so they are available for analysis by query based data mining.

Analysis of Large Scale cDNA Microarray Data

Management and analysis of the large volume of data that results from multiple cDNA microarray experiments represents a formidable challenge. Fortunately, new and effective bioinformatic methods have been developed to effectively analyze large scale expression data in ways conducive to extracting biologically relevant conclusions. Bioinformatic approaches using statistical tools can compare gene expression profiles from multiple experiments. One bioinformatic approach involves clustering, whereby the genes with similar expression profiles across multiple experiments are statistically clustered together. In some cases, the samples can also be clustered based on how similar the global gene expression patterns are. There are a wide variety of statistical methods to cluster microarray data. The most commonly used is unsupervised, hierarchical clustering that, unlike supervised clustering, is not based on fitting the data to a model. Plotting of hierarchical gene clustering results is aided by color representations of the relative gene expression ratios and branching dendograms. Other visualization methods, such as multi-dimensional scaling (MDS), have been developed to visually illustrate (by distance in a three-dimensional graph) the degree of correlation amongst multiple samples based on global scale gene expression profiles. In other words, MDS can cluster samples together in 3-D space based on the similarity of their gene expression profiles. More detailed information on cDNA microarray technology and related links is available in http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/.

Application

DNA microarrays facilitate highly parallel analysis of gene expression. Analysis of cDNA microarray data using statistical clustering has revealed groups of genes that have very similar gene expression profiles associated with a phenotype or a response to a physiological condition. Also, genes sometimes cluster together tightly across a large number of conditions revealing co-regulated genes. Furthermore, investigation of genes that cluster together often reveals that they have related structures and functions. As a consequence, clues about the function and regulation of unknown genes can be hypothesized based on other genes that cluster with it. An added advantage is that cellular phenotypes such

as migration of cancer cells can be correlated with specific clusters and gene expression fingerprints.

Clinical Relevance

Gene clusters from cDNA microarray data analysis have been used as cancer bio-markers for diagnosis, progression and prognosis. For example, the use of global gene expression profiles has successfully identified a gene cluster that distinguishes various classifications of malignancies. This type of classification by gene expression fingerprinting is useful in identifying sub-types of hematological malignancies that were histopathologically not distinguishable but had different clinical outcomes. These profiles can also identify genes that are associated with therapy response and resistance. For example, a gene expression program was identified in prostate cancer that is associated with androgen ablation therapy and resistance to hormonal therapy. As a consequence, such genes can be used to predict individual responses to therapy. Furthermore, identification of genes that mediate therapy response also permit rational drug design to target these genes and gene products.

cDNA microarray analysis of a small set of cancers can identify hundreds of gene expression alterations. Conventional molecular pathology techniques are relatively slow, thus, creating a "bottle-neck effect" in the validation and translation of such alterations to a large population of clinical specimens. To alleviate this problem, tissue microarray technology has been developed for parallel high-throughput molecular pathology (immunohistochemistry, mRNA in situ, and fluorescence in situ hybridization) on hundreds of clinical tissue sections on a single glass microscope slide. The prevalence of candidate gene expression alterations can therefore be assayed in hundreds to thousands of clinical samples in a single experiment. These two types of microarray technology are highly complementary, allowing for rapid identification and translation of genes associated with cancer.

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Microautophagy

Definition

Uptake of cytosol into the lysosome by invagination of the lysosomal membrane in an ►endocytosis-like process.

►Autophagy

Microbes

Definition

Minute organisms, including bacteria, viruses, fungi, and protozoa.

Microbubbles

Definition

Ultrasound contrast agents consisting of micron-sized bubbles of air or inert perfluorocarbon gas encapsulated in an albumin or phospholipid shell. Gas bubbles reflect ultrasound very strongly and so greatly increase the echo signal received from the blood pool. The bubbles are small enough to circulate through capillary beds without causing occlusions, and are gradually cleared from circulation by the liver or lungs, so their use poses a minimal risk to the imaging subject.

►Ultrasound Micro-Imaging

Microcell

Definition

Single micronucleus containing a portion of the genetic material of a cell surrounded by a thin layer of

cytoplasm and covered with an intact plasma membrane.

►Microcell-Mediated Chromosome Transfer

Microcell Hybrid

Definition

Fused stable cell line produced from recipient cell containing exogenously transferred chromosome from microcell.

►Microcell-Mediated Chromosome Transfer

Microcell–Mediated Chromosome Transfer

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Definition

►Microcell-mediated chromosome transfer (MMCT) is a method of transfer of intact or truncated chromosomes from a donor cell to a recipient cell through microcell fusion and hybrid cell selection. A ►microcell hybrid stably maintains the additional genetic material as functioning units within the cells. This technique is primarily used for transfer of a single donor chromosome (monochromosome transfer) of interest into a recipient cell.

Characteristics

A method of mammalian gene transfer, termed microcell-mediated chromosome transfer (MMCT), allows the stable introduction of exogenous chromosomal material from a donor cell into a recipient cell. MMCT is generally used to transfer a portion of the genetic information (generally one chromosome) from one cell to another. This procedure is used for genetics, gene mapping, and gene expression/regulation studies in mammalian cells. One or more intact or truncated chromosomes from the donor cell can be transferred into the recipient cell. Transferred genes are under the control of their endogenous regulatory elements, ensuring their physiological expression in the recipient cell line.

In ►cancer studies, the transferred donor chromosome is generally a normal human chromosome. Complete libraries of mouse microcell hybrid cell lines containing each individual normal human chromosome, tagged with a dominant selectable antibiotic resistance gene, originating from a diploid fibroblast cell, have been generated. These cell line resources have been invaluable for gene function and mapping studies. Panels of selected donor chromosome cell lines, containing random truncations induced by irradiation to narrow down genomic regions containing tumor suppressive activity, have also been established for MMCT studies. The recipient cells are often human cancer cell lines or murine cell lines.

Using MMCT approaches, functional complementation of genetic defects present in the original recipient cancer cell line by the introduction of a normal exogenous chromosome, provides evidence for the existence of a ►tumor suppressor gene having a role in the cancer cell line studied. This approach has been used in specific tumor cell lines to detect candidate tumor suppressor genes on individual chromosomes and to aid in their identification and mapping to critical regions associated with tumorigenesis. This allowed both validation of the tumor suppressive [►tumor suppression] function of known tumor suppressor genes, such as *RB* [►retinoblastoma] and *WT1* [►Wilms Tumor], for example, and identification of yet other previously unidentified candidate tumor suppressor genes. A summary of some of these MMCT tumor suppressive studies of human cancers is shown in Table 1. Using MMCT approaches, researchers were able to localize tumor suppressor genes to specific chromosomes to provide direct functional evidence for their role in tumor suppression and to demonstrate that restoring a normal copy of a single tumor suppressor gene in a cancer cell line reversed its tumorigenic potential, despite multiple defects on several chromosomes contributing to a malignancy. MMCT approaches are also useful for identifying genes responsible for growth suppression, ►senescence, and ►metastasis.

The MMCT procedure consists of the following key steps, as illustrated schematically in Fig. 1. Exponentially growing donor cells containing the stable exogenous donor human chromosome of interest, which is tagged with a selectable marker such *neo* for G418 selection, are subjected to prolonged ►colcemid (mitotic inhibitor) treatment. Micronuclei (►Micronucleus) are formed, when the nuclear envelope reforms around one or more of the chromosomes. Micronuclei are then ►enucleated by treatment with ►cytochalasin B and centrifugation. Microcells are then formed. They are filtered through membranes to eliminate

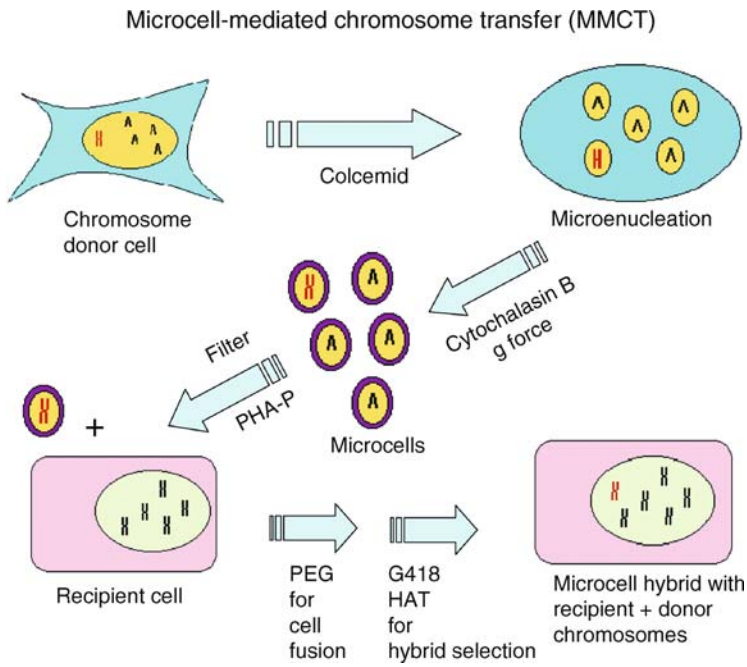
Microcell–Mediated Chromosome Transfer. Table 1 MMCT tumor suppression studies in human cancers

Chromosomes studied	Cancers	References
11	Bladder	Kugoh H et al., Cancer Genet Cytogenet 116:158, 2000
13		Banerjee A et al., Cancer Res 52:6297, 1992
6	Breast	Theile M et al., Oncogene 13:677, 1996
8		Wilson P et al., Cancer Genet Cytogenet 143:100, 2003; Seitz S et al., Oncogene 24:869, 2005; Genes Chromosomes Cancer 45:612, 2006
6, 11		Negrini M et al., Cancer Res 54:1331, 1994
11		Phillips KK et al., Cancer Res 56:1222, 1996; Negrini M et al., Oncogene 7:2013, 1992
11,17		Yang X et al., Int J Oncol 15:629, 1999
16		Reddy DE et al., Oncogene 18:5100, 1999
17		Casey G et al., Hum Mol Genet 2:1921, 1993; Plummer SJ et al., Oncogene 14:2339, 1997
2	Cervix	Uejima H et al., Genes Chromosomes Cancer 14:120, 1995
4		Backsch C et al., Genes Chromosomes Cancer 31:196, 2001; Genes Chromosomes Cancer 43:260, 2005; Forsythe NR et al., Oncogene 21:5135, 2002; Bryce SD et al., Neoplasia 4:544, 2002
6		Steenbergen RD et al., J Natl Cancer Inst 93:865, 2001
11		Saxon PJ et al., EMBO J 5:3461, 1986; Srivatsan ES et al., Cancer Res 46:6174, 1986; Koi M et al., Mol Carcinog 2:12, 1989; Misra BC and Srivatsan ES, Am J Hum Genet 45:565, 1989
7	Choriocarcinoma	Miyamoto S et al., Hum Cell 4:38, 1991
5,18	Colon	Tanaka K et al., Nature 349:340, 1991
5,17,18		Goyette MF et al., Mol Cell Biol 12:1387, 1992
8		Gustafson CE et al., Cancer Res 56:5238, 1996
17		Flanagan JM et al., Int J Cancer 106:505, 2003; Genes Chromosomes Cancer 40:247, 2004
3	Esophagus	Lo PH et al., Oncogene 26:148-157, 2007
9		Yang L et al., Oncogene 24:697, 2005
14		Ko JMY et al., Genes Chromosomes Cancer 43:284, 2005
1	Fibrosarcoma	Klein KG and Bouck NP, Cancer Genet Cytogenet 73:109, 1994
11		Benedict WF et al., Cancer Res 44:3471, 1984
1, 11,17		Anderson MJ et al., Genes Chromosomes Cancer 9:266, 1994
10	Glioblastoma	Pershouse MA et al., Cancer Res 53:5043, 1993; Kon H et al., Oncogene 16:257, 1998
8	Liver	Liu H et al., Zhonghua Yi Xue Yi Chuan Xue Za Zhi 23:540, 2006
3,11	Lung	Satoh H et al., Mol Carcinog 7:157, 1993
11		Murakami Y et al., Proc Natl Acad Sci U S A 95:8153, 1998; Oncogene 21:6930, 2002; Kuramochi M et al., Nat Genet 27:427, 2001; O'Briant K et al., Anticancer Res 17:3243, 1997
1, 6	Melanoma	Miele ME et al., Mol Carcinog 15:284, 1996
6		Trent JM et al., Science 247:568, 1990; Ray ME et al., Oncogene 12:2527, 1996; Welch DR et al., Oncogene 9:255, 1994; Int J Cancer 71:1035, 1997; Miele ME et al., Clin Exp Metastasis 15:259, 1997; Int J Cancer 86:524, 2000
10		Robertson GP et al., Cancer Res 56:3596, 1999
11		Robertson G et al., Cancer Res 56:4487, 1996; Oncogene 18:3173, 1999
7, 11	Myeloid leukemia	Wilding J et al., Genes Chromosomes Cancer 34:390, 2002
3	Nasopharynx	Cheng Y et al., Proc Natl Acad Sci USA 95:3042, 1998

Microcell–Mediated Chromosome Transfer. Table 1 MMCT tumor suppression studies in human cancers (Continued)

Chromosomes studied	Cancers	References
6, 11, 17		Cheng Y et al., Genes Chromosomes Cancer 28:82, 2000
11		Cheng Y et al., Genes Chromosomes Cancer 34:97, 2002; Lung HL et al., Int J Cancer 112:628, 2004; Oncogene 24:6525, 2005; Cancer Res 66:9385, 2006
13		Cheng Y et al., Int J Cancer 109:357, 2004
14		Cheng Y et al., Genes Chromosomes Cancer 37:359, 2003
1	Neuroblastoma	Martinsson T et al., Genes Chromosomes Cancer 1:67, 1989
11		De Preter K et al., BMC Genomics 6:97, 2005
17		Bader SA et al., Cell Growth Differ 2:245, 1991
11	Neuroepithelioma	Chen P et al., Oncogene 10:577, 1995
3	Oral cavity	Uzawa N et al., Oncogene 11:1997, 1995; Cancer Genet Cytogenet 107:125, 1998
3	Ovary	Rimessi P et al., Oncogene 9:3467, 1994
6		Wan M et al., Oncogene 18:1545, 1999
11		Cao Q et al., Cancer Genet Cytogenet 129:131, 2001; Stronach EA et al., Cancer Res 63:8648, 2003; Abeysinghe HR et al., Cancer Genet Cytogenet 143:125, 2003
22		Kruzelock RP et al., Oncogene 19:6277, 2000
18	Pancreas	Lefter LP et al., Genes Chromosomes Cancer 34:234, 2002; Asian J Surg 27:85, 2004
5	Prostate	Ewing CM et al., Cancer Res 55:4813, 1995
10		Fukuhara H et al., Oncogene 20:314, 2001; Ichikawa T et al., Cancer Res 52:3486, 1992
11		Murakami Y et al., Cancer Res 55:3389, 1996; Sanchez Y et al., Proc Natl Acad Sci USA 93:2551, 1996; Kawana Y et al., Prostate 32:205, 1997
12		Berube NG et al., Cancer Res 53:3077, 1994
13		Banerjee A et al., Cancer Res 52:6297, 1992
17		Murakami YS et al., Cancer Res 55:3389, 1995 Chekmareva MA et al., Prostate 33:271, 1997
18		Lefter LP, Clin Cancer Res 9:5044, 2003; Padalecki SS et al., Genes Chromosomes Cancer 30:221, 2001; Urol Oncol 21:366, 2003; Gagnon A et al., Genes Chromosomes Cancer 45:220, 2006
19		Gao AC et al., Prostate 38:46, 1999; Astbury C et al., Genes Chromosomes Cancer 31:143, 2001; Padalecki SS et al., Genes Chromosomes Cancer 30:221, 2001
Y		Vijayakumar S et al., Genes Chromosomes Cancer 44:365, 2005
3	Renal cell	Shimizu M et al., Oncogene 5:185, 1990
11	Rhabdomyosarcoma	Loh WE et al., Proc Natl Acad Sci U S A 89:1755, 1992
1, 6, 9, 18	Uterine endometrium	Yamada H et al., Oncogene 5:1141, 1990; Genes Chromosomes Cancer 13:18, 1995
11	Wilms tumor	Weissman BE et al., Science 236:175, 1987; Dowdy SF et al., Science 254, 293, 1991; Reid LH et al., Hum Mol Genet 5:239, 1996

contaminating whole cells and ►karyoplasts in order to obtain the fraction containing only the smaller microcells harboring single chromosomes. These microcells are then added to recipient cells, agglutinated with phytohemagglutinin-P, and then induced to fuse in the presence of ►PEG. The fusion of microcells to the recipient cell results in hybrid ►heterokaryon cells. The heterokaryons, containing the exogenous chromosome of interest, are selected with ►HAT to eliminate mouse ►HPRT-negative donor cells from the culture and with G418 to eliminate human recipient cells that do not receive the donor chromosome of interest. Microcell hybrids contain the transferred chromosome in a new



Microcell–Mediated Chromosome Transfer. Figure 1 Schematic diagram of MMCT procedure.

recipient cell environment. Generally the successful transfer of the donor chromosome is monitored by ►FISH analysis.

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Microcephalin

►BRIT1 Gene

Microcirculation

Definition

Is a term describing structure and function of blood (and lymph) vessels with diameters ≤250 μm (microvessels).

►Oxygenation of Tumors

Microcytoma

►Extrapulmonary Small Cell Cancer

Microenvironment

►Three-Dimensional Tissue Cultures

Microenvironmental Niche

Definition

Any microscopic cellular grouping that functions to provide particular environmental conditions for the maintenance of stem cells or progenitor cells, but particularly in the bone marrow.

►Chemokine Receptor CXCR4

Microfold Cells

Definition

M Cells; Antigens and pathogens enter the body from the intestines through cells called M cells, which are specialized for this function. They are found over the gut-associated lymphoid tissue (GALT). They may provide a route of infection for human immunodeficiency virus (HIV).

Microglia

Definition

Brain macrophages.

►Macrophages

Micrometastasis

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Definition

Micrometastases were originally defined by pathologists as small occult metastases (<0.2 cm in greatest dimension). With the recent development of more sensitive diagnostic tools, such as immunocytochemistry and polymerase chain reaction (PCR), the term has been used more liberally in the literature. It now includes isolated disseminated tumor cells (DTC) present in the peripheral blood or in a secondary organ (in particular bone marrow) or in a lymph node, classified as "tumor-free" by conventional histopathological analysis. Considering the different biology of true micrometastases and isolated DTC, both types of findings should be distinguished.

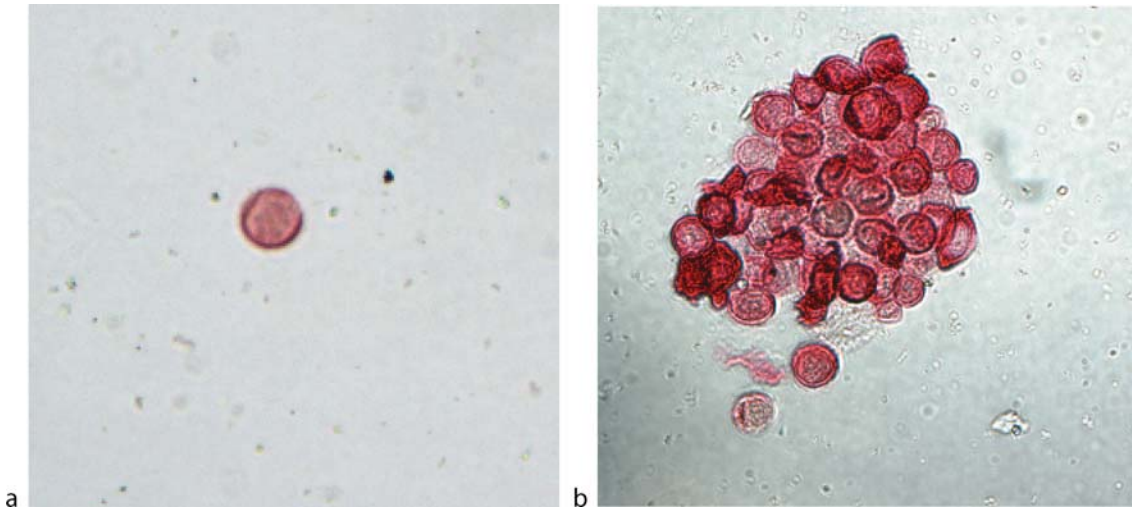
Characteristics

Detection Techniques

During the past decade sensitive techniques have been developed that allow now the detection of DTC in the blood and bone marrow (BM) at the single cell level. DTC must be separated from billions of erythrocytes and millions of leucocytes present in the blood and bone marrow sample. For this enrichment of DTC over several logarithmic units several methods have been successfully used, including density gradient separation, antibody-charged magnetic particles, filtration, and tetrameric antibody complexes. These technical advances provide an excellent opportunity to detect and analyze DTC in clinical studies in order to (i) provide helpful information for the management of patients with ►cancer and (ii) unravel the biology of the metastatic cascade in solid tumors. Solid tumors account for the majority of cancers in the European Union and USA, and the majority of solid tumors are derived from epithelial tissue. The present review will therefore focus on DTC detection in patients with solid epithelial tumors.

Immunocytochemistry

Cytokeratins (CK), as integral components of the cytoskeleton of epithelial cells, are stably expressed characteristics of epithelial tumor cells that can be clearly identified in individual carcinoma cells by means of specific monoclonal antibodies (Fig. 1). Mucin-like tumor-associated cell membrane proteins are less well suited for analysis on account of their expression on hematopoietic cells. Immunohistochemical examinations of BM biopsies have shown that CK-positive tumor cells are mostly situated in interstitial tissue outside the sinusoidal vessels, which indicates that extravasation, one of the last processes in the metastasis cascade, has been successfully completed. Although ectopic or illegitimate mRNA expression of cytokeratins in mesenchymal cells cannot be ruled out, numerous negative findings in patients without identifiable malignant processes show that ectopic expression of CK proteins in BM is very rarely identifiable by immunocytochemistry. The time-consuming microscopic screening of large amounts of



Micrometastasis. Figure 1 Isolated disseminated carcinoma cells (a) and tumor cell cluster (b) on a bone marrow cytospin stained with anti-cytokeratin antibody A45-B/B3.

cytological samples has been facilitated through automated analysis of stained preparations using sophisticated image analysis systems.

Molecular Techniques

Besides immunocytochemistry molecular detection procedures have been increasingly used to identify DTC. The major limiting factor for detection of single DTC by RT-PCR is illegitimate low-level transcription of tumor-associated or epithelial-specific genes in normal cells. Quantitative real-time RT-PCR may increase discrimination between mRNA expression of normal cells and tumor cells, thereby increasing the specificity of the RT-PCR approach. However, in view of the heterogeneity of tumor cells it might be difficult to define one cut-point that is able to discriminate between normal and tumor cells in a wide range of clinical samples.

More recently, a molecular characterization of single DTC has become possible. Using immunocytochemistry or immunofluorescence in combination with FISH or CGH, several groups have reported chromosomal aberrations of DTC in blood and bone marrow indicating the malignant origin of these DTC and their genetic heterogeneity.

EPISPOT Assay

This recent technique is an adaptation of the enzyme-linked immunospot assay. EPISPOT enumerates only viable epithelial tumor cells and can detect secretion of specific proteins at an individual cell level, allowing the direct determination of protein-secreting cell (SC) frequencies. Immunospots are the protein fingerprint left only by the viable SC. Cell culture is needed for a sufficient amount of secreted marker proteins to accumulate to form immunospots; dying tumor cells do not secrete adequate amounts of protein and thus are not detected. MUC-1, ►cathepsin D, and CK19 were studied as relevant released proteins to detect tumor cells in blood and BM from patients with ►breast cancer. In prostate cancer patients, PSA-SCs ►(prostate specific antigen, PSA) were enumerated in blood and have been considered as DTC.

Cellular and Molecular Biology

Immunocytochemical double staining methods have been developed for more precise characterization of DTC. In view of the malignant potential of CK-positive cells, a number of tumor-associated characteristics have been identified. This includes the frequent overexpression of the erbB2 ►HER-2/neu oncogene and deficient expression of MHC class I molecules, which as restrictive elements help T lymphocyte-mediated tumor cell recognition. The malignant nature of CK-positive cells in the BM has been further confirmed through genomic analysis. Extensive cell culture experiments have shown that cells disseminating into BM have a time-limited proliferative potential at the time of primary diagnosis of the tumor. It may therefore be assumed that at the primary stage these cells do not proliferate autonomously, but are in a latent state known as ►"dormancy". This assumption is also corroborated by double staining studies, demonstrating that the fraction of DTC in BM with proliferation marker expression (Ki-67, p120) is small. The low-proliferative activity of DTC is also consistent with the putative stem-like phenotype of DTC ►cancer stem-like cells in BM of early breast cancer patients.

Most DTC have a ►CD44^{+} CD24$^{-/low}$ phenotype, previously shown to represent a minor population in primary breast cancer with a high self-renewal and tumorigenic potential. Moreover, using the EPISPOT assay we showed that a subpopulation of DTC in BM had the CK19^{+}/MUC1^{-} phenotype, previously also suggested as breast stem cell marker ►(stem cell markers). The putative stem cell nature of DTC could explain the relative resistance of these cells to chemotherapy and points to the development of therapeutic strategies that are independent of the proliferative state of cancer cells (e.g., immunotherapy). Nevertheless, it is still debated whether DTC in BM might indeed have cancer stem cell properties.

Some of the DTC circulating in the blood are apoptotic. However, using the EPISPOT assay we could clearly demonstrate the presence of viable DTC circulating in the blood. Tumor cells also express CXCR4 ►(CXCR4 chemokine receptor) at their cell membrane, and it has been shown that metastatic cells may use this chemokine-mediated mechanism to home at specific distant sites (i.e., lung, liver, and bone in breast cancer). After homing, DTC need to secrete relevant growth factors to form a solid ►metastasis, and we could demonstrate that a subset of DTC in the blood of prostate cancer patients secretes FGF-2, a known stem cell growth factor (Fig. 2).

Micrometastasis. Figure 2 Dual fluorescent EPISPOT assay. Fingerprints of single disseminated breast cancer cells from bone marrow. MUC1^{Alexa555}-immunospot (top), CK19^{Alexa488}-immunospot (middle), and MUC1/CK19^{yellow}-immunospot (bottom).

Clinical Relevance

Bone Marrow

Several studies on patients with different types of epithelial tumors have confirmed that BM is a common homing organ for DTC and the presence of these cells is a prognostic factor indicating an unfavorable clinical outcome. The largest data base exists now for breast cancer; analysis of 4,703 patients with primary disease (i.e., no signs of overt metastases) clearly demonstrated an independent prognostic value of immunocytochemical DTC testing. As a result of an international collaboration, the critical variables of the immunocytochemical cytokeratin BM assays have been recently defined, which allows now a reproducible and more precise determination of the DTC count. More recently, encouraging data have also been derived from the use of real time quantitative RT-PCR assays on smaller cohorts of breast cancer patients using CK19 mRNA as DTC-marker. These data need to be sustained in a multi-center setting and standardization of the methods is urgently needed.

The observed correlation to the total metastatic relapse rate is of particular interest in colon carcinoma where clinically manifested skeletal metastases are rare. The presence of DTC in BM is therefore more likely to be an indicator of early systemic tumor cell dissemination, whereas the growth in BM or other organs is being determined by the milieu in question. BM might be an important reservoir that allows DTC to home for an extended period of time and/or recirculate into other organs where they find better growth conditions. In this context it is interesting that the repeat identification of DTC in BM over years postsurgery has shown to be a risk factor for metastatic relapse in breast and gastric cancer.

Peripheral Blood

Peripheral blood is an ideal source for the detection of DTC ►circulating tumor cells because of an easy sampling procedure. It would be therefore excellent for a "real time" monitoring of ►minimal residual disease in cancer patients, in particular during and after adjuvant systemic therapy. However, the prognostic significance of DTC in blood is at present much less clear than for DTC in BM. For tumor cells, blood is only a temporary compartment, and it is not known how many cells survive the blood passage and are capable to home in a secondary organ and form detectable metastases.

Thus far, some promising studies could show that the presence of DTC in blood is correlated with stage and course of the disease in patients with metastatic breast cancer. Furthermore, a significant correlation between the detection of DTC in blood and in BM of patients with primary breast cancer has been shown

with a higher sensitivity of the BM test. Thus far, the prognostic role of DTC detection in blood in primary breast cancer is still unclear and it seems possible that the information derived from BM and blood screening could be complementary and not redundant.

Lymph Nodes

Tumor cell dissemination via the lymphatic vessels to the regional lymph nodes is the second main route of tumor cell dissemination in cancer patients, and the presence of lymph node metastases is a very important prognosticator. The clinical importance of DTC in lymph nodes has been shown by several groups in a variety of solid tumor types, including breast, prostate, lung, and esophageal cancer. However, DTC analyses of all of the resected nodes a cumbersome and time-consuming procedure and has therefore not gained access to clinical pathology.

An important advance in the evaluation of regional lymph nodes has been therefore the development of a more limited dissection, the sentinel lymph node dissection. This is based on the identification, with dyes or radioactivity, of the specific lymph node that drains the tumor and the removal of this lymph node for analysis. This approach has been extensively evaluated in patients with melanoma and breast cancer. Detection of DTC in well selected sentinel lymph nodes may become an important alternative to an extensive lymph node dissection and histopathological search for metastases, which eventually might reduce the significant morbidity associated with tumor staging in cancer patients (e.g., lymph edema in breast cancer patients after axillary node dissection).

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Microminerals

Definition

►Trace Elements

Micronucleus

Definition

Is a small, extranuclear chromatin-containing structure in the cell cytoplasm surrounded by a membrane without any detectable link to the main nucleus.

►Micronucleus Assay

Micronucleus Assay

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Definition

Measurement of ►micronucleus (MN) frequency in peripheral blood lymphocytes is extensively used in molecular epidemiology and cytogenetics to evaluate the presence and the extent of chromosomal damage in human populations exposed to genotoxic agents or bearing a susceptible genetic profile. MN is a small, extranuclear chromatin-containing structure in the cell cytoplasm surrounded by a membrane without any detectable link to the main nucleus.

Characteristics

Micronuclei (MNi) are expressed in dividing cells and they can occur spontaneously or as a result of chemical or radiation treatment. Additional parameters that can influence MN frequencies are inherited or acquired genetic polymorphisms in genes responsible for the metabolic activation of ►clastogens (agents causing chromosomal breaks), for the fidelity of DNA repair/misrepair, DNA replication and/or chromosome segregation. Concerning the induction mechanism two types of MNi exist:

1. MNi that contain chromosome fragments mostly without a ►centromere so called acentric fragments. They may result from direct DNA double-strand breakage, from conversion of DNA single-strand breakage into DNA double-strand breaks after cell replication, or from inhibition of DNA synthesis. Misrepair of two chromosome breaks may lead to an asymmetrical chromosome rearrangement producing a dicentric chromosome and an acentric fragment. Commonly, the centromeres of the dicentric chromosomes are pulled to the opposite poles of the anaphasic cell

resulting in the formation of a nucleoplasmic bridge between the daughter nuclei and an acentric fragment that lags behind to form a MN.

2. MNi that contain whole chromosomes which were unable to migrate with the rest of the chromosomes to the spindle poles during cell division. These are primarily formed from the defects in the chromosome segregation machinery such as deficiencies in the genes controlling cell cycle, failure of the mitotic spindle, ►kinetochore, or other parts of the mitotic or meiotic apparatus, or by mechanical disruption.

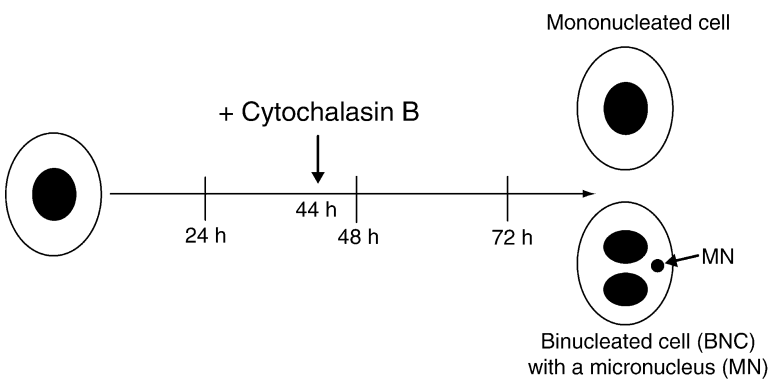
MNi can be detected using different nonspecific (Giemsa, May–Grunwald–Giemsa) or DNA-specific (4′,6-diamidino-2-phenylindole, Feulgen, acridine orange, Hoechst) stains. The frequency of MN can be quantified microscopically or by a flow cytometry. MNi are morphologically similar to the cell nucleus and have the following main features:

- The diameter of MN in human lymphocytes usually varies between 1/16th and 1/3rd of the mean diameter of the main nucleus.
- MNi are not connected to the main nucleus.
- MNi exhibit the same intensity as the main nuclei.

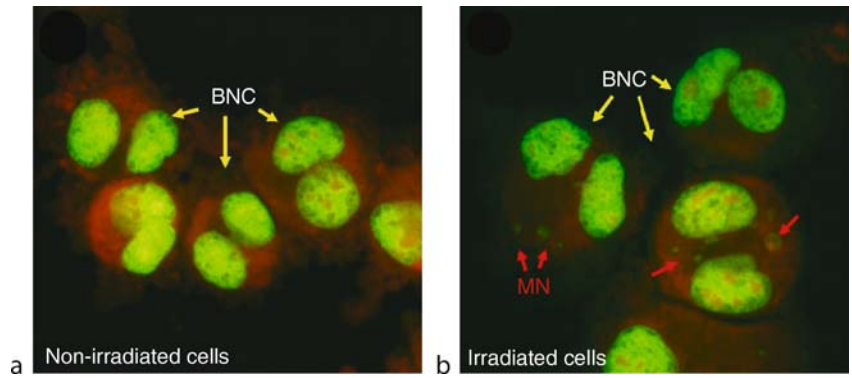
The postmitotic fates of MN are not clearly understood and include: (i) elimination of the micronucleated cells as a consequence of ►apoptosis; (ii) reincorporation into the main nucleus; (iii) retention within the cell's cytoplasm as an extranuclear entity; and/or (iv) exclusion from the cells.

MNi can only be expressed in dividing eukaryotic cells. In order to distinguish between those cells which did not divide and those cells which completed one nuclear division during in vitro culture, Fenech and Morley [1] provided an efficient approach, a ►cytokinesis-block MN (CBMN) assay using ►cytochalasin B as an inhibitor of cell division. Cytochalasin B is a cell-permeable mycotoxin that inhibits ►actin polymerization which is required for the formation of the microfilament ring and the constriction of the cytoplasm between the daughter nuclei during cellular division. Cells treated with cytochalasin B are easily recognized due to their binucleated appearance (Figs. 1 and 2), also termed as binucleated cells (BNCs). The use of cytochalasin B enables the accumulation of almost all dividing cells at the binucleate stage in growing cell population. In a classical CBMN test (Fig. 1), human lymphocytes are cultured in the presence of phytohaemagglutinin to stimulate mitosis. After 24 h of stimulation, the cells are treated with a genotoxic agent (chemicals or ionizing/ultraviolet radiation). At 44 h of culture, cytochalasin B is added and 24 h later the cells are harvested onto microscopic slides, fixed, stained, and analyzed. MNi are scored only in BNCs, which allows reliable comparison of the extent of chromosome damage between cell populations differing in their division kinetics. Although the method was initially developed for cultured human lymphocytes now it has been adapted to various cell types, such as primary fibroblasts, solid tumor and bone marrow cells, and exfoliated epithelial cells.

The advantages of the CBMN assay are simplicity, sensitivity, reliable identification of cells that have completed only one nuclear division, as well as the detection of other endpoints such as dicentric bridges and mitotic indices. Moreover, the combination of the CBMN test with the chromosome- and centromeric/telomeric-specific hybridization techniques allowed the identification of the mechanisms responsible for the induction of MNi. These mechanisms are the



Micronucleus Assay. Figure 1 Schematic diagram showing a cytokinesis-block micronucleus (CBMN) assay established by Fenech and Morley for an in vitro analysis of cells for the presence of micronucleus/micronuclei (MN/MNi). Cytochalasin B, an inhibitor of actin polymerization is added 44 h after initiation of the cell culture in order to prevent cell division thus allowing to discriminate easily between mononucleated cells that did not divide and binucleated cells (BNCs) that completed one nuclear division during cultivation in vitro. [Reprinted from [2] Mateuca et al (2006) Chromosomal changes: induction, detection methods and applicability in human biomonitoring. Biochimie 88:1515–1531, with permission from Elsevier].



Micronucleus Assay. Figure 2 Radiation-induced MNi in cultured human tumor cells. Nonirradiated (a) and irradiated in vitro (b) human melanoma cells were treated with cytochalasin B. Twenty hours later cells were fixed and stained with acridine orange, nuclear material appears green, cytoplasm in red-brown. Red arrows show MNi, yellow arrows show binuclear cells (BNC). Magnification: 1000×.

chromosome breakage leading to MNi with acentric fragments and the failure of the mitotic spindle resulting in MNi containing whole chromosomes. For these reasons the CBMN test has also become an important and useful tool to discriminate ►clastogenic (causing chromosomal breaks) and ►aneugenic (causing loss or gain of whole chromosomes) agents leading to structural and numerical chromosomal aberrations, respectively.

Clinical Aspects

Measurement of MN frequency in peripheral blood lymphocytes is extensively used in molecular epidemiology and cytogenetics to evaluate the presence and the extent of chromosomal damage in human populations exposed to genotoxic agents or bearing a susceptible genetic profile.

In populations not exposed to occupational or environmental toxins, the major contributors to differences in MN frequencies have been shown to be age and gender. Several studies have reported that MN frequencies increase with age in adults, and women have higher MN frequencies than men.

The counts of mononucleated cells containing MN provide an indication of the background level of chromosome/genome mutations accumulated in vivo, whereas the frequencies of BNCs bearing MN reflect the damage accumulated before cultivation as well as mutations expressed during the first in vitro mitosis.

Elevated frequencies of MNi could also be related to genomic instability, as seen in tumor cells, for example. A link between MN induction and ►cancer development was proposed by several studies and it can be supported by a number of findings. First, an increase in MN frequency is observed in lymphocytes of cancer patients and in patients with cancer prone diseases such as ►Bloom syndrome and ataxia telangiectasia. Additional support can be shown from the positive correlation between the induction of MN and chromosomal aberrations, whose frequency in lymphocytes of healthy subjects, in turn, was supposed to be a predictor of cancer risk. Indeed, the hypothesis of a predictive association between MN induction and cancer risk is supported by the study of El-Zein et al. [3] who found that the CBMN test may serve as a strong predictor of lung cancer risk. Another recent study by Olaharski et al. [4] has shown that MNi can be used as an additional criterion to establish cancer risk in cervical malignant transformation. Peripheral blood lymphocytes from ►breast cancer patients with an adverse early skin reaction to radiotherapy also display increased frequencies of both spontaneous and radiation-induced MN as compared with cells from apparently healthy donors [5], so the CBMN assay might be used as a ►radiation sensitivity test for optimization of radiotherapy schedules.

The mechanism of MN induction and its association with tumorigenesis is currently under study in a number of laboratories. Further important questions include the investigation of the mechanisms of genomic instability and the genetic and environmental factors influencing this instability, particularly in cancer cells.

►Toxicological Carcinogenesis

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Microprocessor Complex

Definition

Is a Protein complex consisting of at least two proteins: Drosha and DGCR8. Drosha is an RNaseIII type enzyme that cleaves RNA molecules with double-stranded regions. The function of the complex is to recognise and release the hairpin structure precursor of ►microRNAs from the primary miRNA transcript. The complex is active in the nucleus and generates the pre-miRNA molecules.

►MicroRNA

MicroRNA

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Definition

MicroRNAs are short RNA molecules that regulate the expression of protein-coding genes.

Characteristics

The expression of protein-coding genes is regulated at several levels including transcriptional and post-transcriptional mechanisms. A recently discovered layer of regulation involves short RNA molecules called microRNAs (miRNAs). These tiny RNA molecules are only 20–24 nucleotides long but are produced from a longer primary transcript (pri-miRNA). The pri-miRNA is either part of an intron of a mRNA or is derived from intergenic regions. Often miRNA genes are very close to each other forming a cluster and the pri-miRNA covers the whole cluster. The pri-miRNA is processed in the nucleus by the ►microprocessor complex. The resulting precursor miRNA molecules (pre-miRNAs) have a characteristic stem-loop secondary structure where the stem is partially double-stranded. Pre-miRNAs are exported to the cytoplasm where they are further processed by the ►dicer that releases an miRNA duplex from the pre-miRNA. The two RNA strands of the miRNA duplex are separated from each other and one of them is incorporated into a protein complex called ►RISC. This strand is called the mature miRNA because this strand has the ability to recognize mRNAs based on sequence complementarity. The mature miRNA guides ►RISC to mRNAs that contain an miRNA target site and the translation of the targeted mRNA is suppressed.

miRNAs and Cancer

At the time of writing this article more than 450 human miRNAs have been identified but it is expected that many more will be found. Some predictions estimate that there are up to 25,000 miRNA genes in the human genome. The current number of miRNAs can be found in miRBASE that is a comprehensive database of miRNAs and is regularly updated (http://microrna.sanger.ac.uk/). The function of most miRNA is unknown but there is an increasing body of evidence indicating a link between miRNAs and cancer. First, miRNAs are involved in cellular growth, differentiation and ►apoptosis, processes that are disturbed in tumor cells. The second evidence came from analyzing the genomic location of miRNAs. Genes involved in cancer often accumulate genetic mutations in patient tumors. These mutations can be point mutations or larger changes including ►amplifications or deletions. It was shown that about half of the miRNA genes are located at regions that are deleted or amplified in human cancers or at ►fragile sites. The third line of evidence was obtained from ►microarray experiments. MiRNA expression was profiled in a range of human tumors and a change in expression was detected in all investigated tumor types. Moreover, miRNA target sites were predicted in genes involved in other processes that play important roles in cancer (cell adhesion, angiogenesis, proteolysis and cell signaling) and some of these miRNAs were found to be up- or down-regulated in tumor samples. These suggest that miRNAs are likely to regulate many genes involved in several stages of tumor genesis.

miRNAs as Tumor Suppressors and Oncogenes

Not all miRNAs are ►tumor suppressor genes that show a down-regulation in tumor samples. Much of the abnormal miRNA expression in tumor samples is not a cause but a consequence, due to the loss of tissue identity that accompanies tumor genesis. However, at least three miRNAs (the miR-15/16 cluster and the let-7 family) are ►tumor suppressor genes. These miRNAs are in regions that are deleted with high frequency in cancer patients or mutations that can be found in the pri-miRNA in other patients. These miRNAs are

also shown to target anti-apoptotic proteins, such as ►BCL-2, and ►oncogenes, such as the ►RAS family, in in-vitro assays.

Other miRNAs, however, promote tumor formation and can therefore be classified as ►oncogenes. The best studied oncogenic miRNAs are members of the miR-17 cluster. This cluster contains six miRNA genes and is amplified and over-expressed in a range of tumors. It also accelerates ►MYC dependent lymphomagenesis and increases cell proliferation. Several members of the cluster target tumor suppressor genes such as ►PTEN, TGFBR2 and ►E2F1. The expression of another cluster (miR-372/373) makes cells insensitive to a normally functioning ►p53 tumor suppressor gene. Another strong oncogene candidate is miR-155 that is in fact located on a known oncogenic locus called the B-cell integration cluster (BIC) non-coding RNA.

In addition to miRNAs encoded by the human genome, other miRNAs encoded by viruses (such as the ►Epstein-Barr virus, ►Kaposi Sarcoma Associated Herpesvirus, etc.) can contribute to tumor formation. Several tumor suppressor genes are predicted targets of these viral encoded miRNAs but at the moment the exact role of viral encoded miRNAs in cancer is not known.

miRNA Profiling and Cancer Diagnosis

►Microarrays are used to profile expression of genes involved in a variety of processes including cancer. Developments in expression technologies have made possible the high-throughput analysis of miRNAs in clinical samples. These experiments show that miRNA expression profiles correlate very well with the type of cancer and the stage of cancer. In fact, it was suggested that the miRNA profile of a tumor sample is a better diagnostic and prognostic marker of human cancer than the mRNA profile. An miRNA based classifier can establish the correct diagnosis of poorly differentiated samples better than the mRNA based classifier. As the number of miRNAs increases and our understanding of their role in cancer grows, the use of miRNA profile in diagnosis and prognosis will be extremely valuable.

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Microsatellite

Definition

A microsatellite is a locus specific DNA sequence and are present throughout the genome in high copy number. They are short nucleotide sequences (1–6 base pairs) that are tandemly repeated several times and flanked by unique sequences.

►Colon Cancer
►Microsatellite Instability
►Mismatch Repair in Genome Stability
►Mutator Phenotype

Microsatellite Instability

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Definition

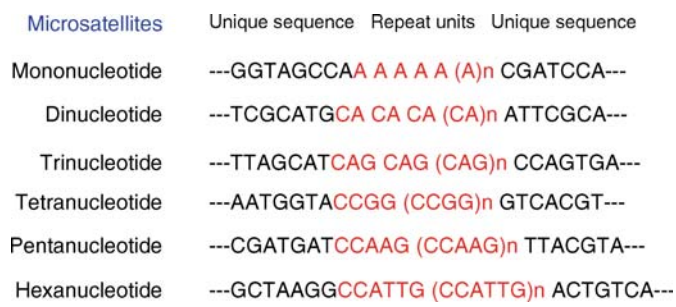
►Microsatellite instability (MSI) is the accumulation of novel microsatellite alleles in genomes (from bacteria to human) due to defect ►mismatch repair.

►Microsatellites are site specific DNA markers with high abundance throughout the genome. They consist of short repetitive motifs of 1–6 nucleotides in length that are tandemly repeated 10–60 times and flanked by unique sequences (Fig. 1).

Characteristics

Multiple different sized alleles at multiple loci in the genome accumulate during tumor development if the mismatch repair system is defect. This phenomenon is referred to as MSI. Tumors revealing such instability are said to have a ►mutator phenotype. Most microsatellites are usually located in noncoding parts of the genome, and mutations in them are selectively neutral. Occasionally the microsatellites are found in expressed regions of a functionally cancer critical gene and mutations may thereby result in a nonfunctional or altered protein with impact on tumorigenesis.

The high abundance and their polymorphic nature make microsatellites to a valuable tool for a variety of applications. Microsatellites were used to identify the chromosomal map position of genes that in a mutated condition predispose to hereditary colorectal nonpolyposis syndrome (HNPCC), also known as ►Lynch



Microsatellite Instability. Figure 1 Microsatellites consist of short nucleotide units tandemly repeated and flanked by unique sequences. The different types of 1–6 base pairs per unit are illustrated. The last unit within each type is in parenthesis followed by a"n" indicating additional units.

syndrome. Several studies of tumors from HNPCC patients revealed the characteristic pattern of novel alleles at simple repetitive sequences. About 90% of ►colorectal carcinomas from HNPCC patients and a subgroup (15%) among sporadic cases exhibit MSI. Other tumor types occurring in the HNPCC syndrome and a subgroup among sporadic cases of the same type also show MSI. This includes ►endometrial cancer, ►ovarian cancer, and ►gastric cancer. Sporadic tumors of types not characteristic to the HNPCC tumor spectrum generally show MSI at lower frequencies and the pattern is characterized by one or only a few new alleles, in contrast to the ladder of alleles often observed in the HNPCC tumor types (Fig. 2).

Sporadic tumors with the MSI phenotype, usually caused by methylation of *MLH1*, typically show promoter hypermethylation of a number of cancer critical genes and are associated with mutations in the *BRAF* gene (v-raf murine sarcoma viral oncogene homologue B1), a protooncogene serine/threonine-protein kinase involved in MAPK signaling pathway.

The HNPCC tumors and the sporadic cases of the same types share clinicopathological characteristics and are different from those with a microsatellite stable phenotype (see later: clinical relevance). In comparison with microsatellite stable colorectal carcinomas the ones which show MSI are more often located in the proximal colon, are characterized by high lymphocyte infiltration, mucinous and undifferentiated growth pattern, exhibit diploid DNA content, and normal genotype/phenotype of the tumor suppressor gene ►*TP53*. Sporadic colorectal cancer patient with tumors that reveal MSI have a prolonged postoperative survival.

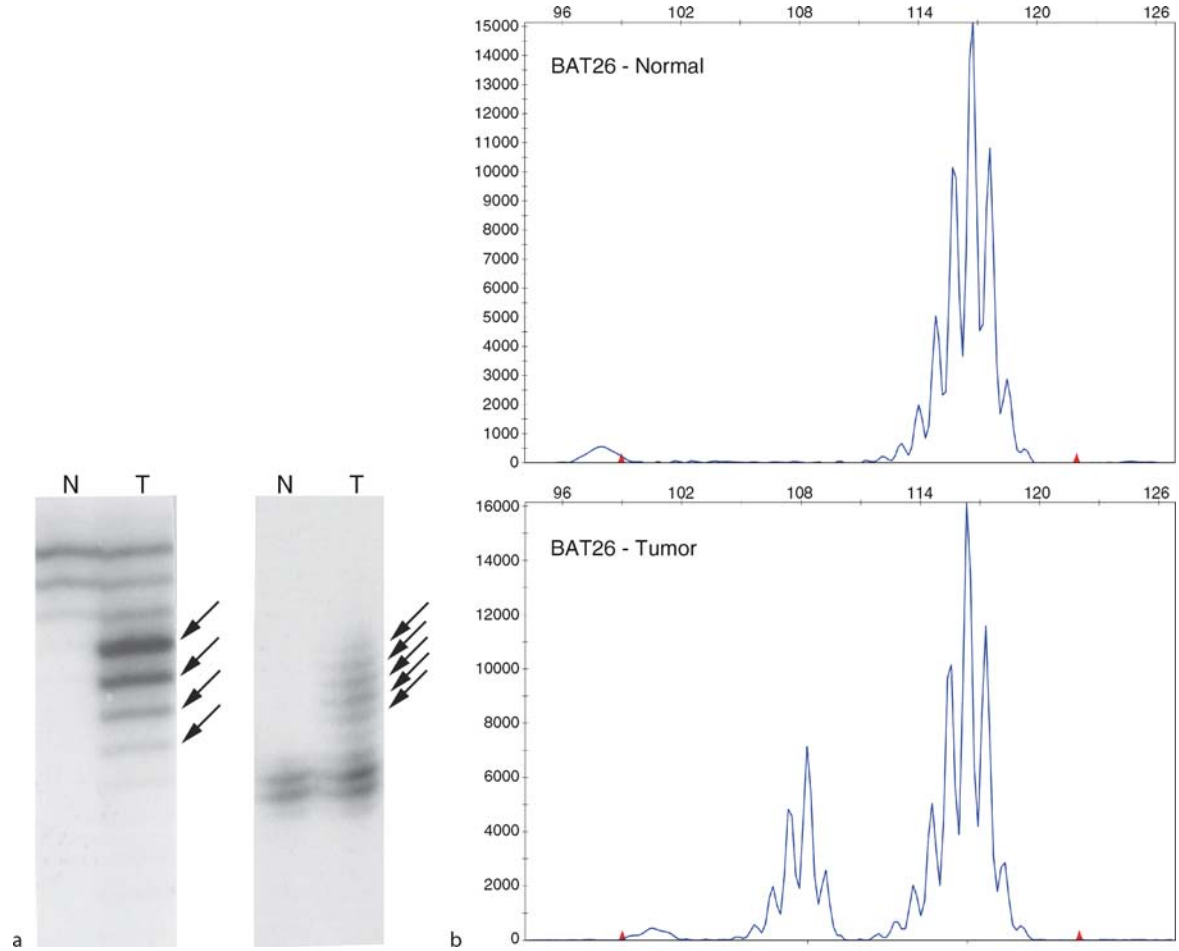
Cellular and Molecular Regulation

The genome-wide MSI pattern seen in human tumors resembles a mutational fingerprint found in the bacteria *Escherichia coli* known to be caused by failure of the mismatch repair system, ►*mutHLS*, in this organism. Several human homologues to the prokaryotic genes have been identified, and germline mutations in some of these components predispose to the HNPCC syndrome. Mutations in ►*MSH2* and ►*MLH1* account for 60% of the identified familial mutations. ►*MSH6* germline mutations are found in HNPCC with increased occurrence of endometrial cancer. Germline mutations are found in ►*PMS1* and ►*PMS2* but occur rarely.

A shared requirement between mismatch repair genes and ►tumor suppressor gene is the need for homozygous inactivation on the cellular level. In addition to the germline mutation in an HNPCC patient a somatic mutation of the second allele are necessary for tumor development. Mutations of both alleles of a mismatch repair gene have been reported in tumor studies as well as mutation of one allele and inactivation of the other by promoter hypermethylation. Among sporadic tumors, MSI is usually caused by promoter hypermethylation of *MLH1*. Biochemical analyses revealed that on the cellular level one mismatch repair gene mutation (heterozygous condition) as opposed to homozygous inactivation do not severely affect mismatch repair function. Comparable interpretation can be drawn from studies with mice.

Mismatched nucleotides arise in the DNA by polymerase misincorporation during replication, by physical damage to existing nucleotides, or by replication slippage in microsatellites with small repetitive units. Replication slippage can occur at repetitive sequences when, following a transient, local dissociation of the nascent and parental DNA strand, reassociation occurs between misaligned repeat units and thereby lengthening or shortening the newly synthesized strand. DNA polymerases are the enzymes that make mistakes especially in DNA with tandemly repeated sequences. Normally these errors are corrected by the mismatch repair system. Due to defects in one or more of these components, insertion of inappropriate bases or the making of loops are stabilized, and the following rounds of replication creates alleles of new sizes.

The genome-wide MSI phenotype does not itself provide tumor cells with a proliferative advantage, let

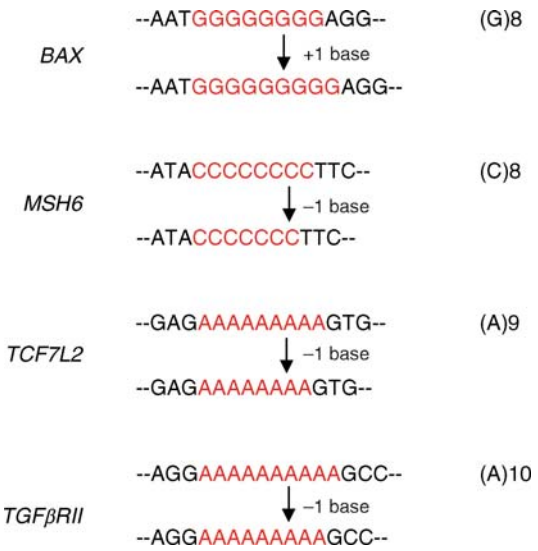


Microsatellite Instability. Figure 2 Microsatellite instability in colon carcinomas. (a) The constitutional alleles are shown in the lanes with normal (N) DNA from the patient, and instability, a ladder of novel alleles, is seen in the patient's tumor DNA (T). The DNA sequences of two microsatellite loci (with dinucleotide repeat sequences) were each amplified by polymerase chain reaction and the fragments were separated by gel electrophoresis. At one locus (to the left) all novel alleles are of smaller size than the normal alleles whereas at the other locus (to the right) the new alleles are all larger (It should be noted that at individual loci one or more novel fragments of smaller, larger, and/or intermediate sizes may be seen in microsatellite unstable tumors). (b) One of the consensus markers for MSI detection, the BAT26, is shown. This marker is monomorphic and provides a typical pattern in the normal sample. The same allele is seen in the corresponding tumor in addition to a smaller sized novel allele with the same pattern. The amplified DNA is here separated by capillary electrophoresis using a DNA sequencer instrument.

alone to transform them malignantly. Nor do mutations in defective mutator genes, known to cause the phenotypic instability, necessarily provide a neoplastic growth advantage. Instead, the genotypic and/or the phenotypic instability increase the likelihood of additional mutations in other mutator genes and in more directly cancer critical genes. Mutations in mononucleotide repeat stretches in coding regions of different genes are characteristic of tumors with MSI. Insertions or deletions in a polymononucleotide sequence may cause frameshift mutations which lead to a shortened protein with no or abnormal function. Known target genes for defect mismatch repair include *TGFβRII*, important in transforming growth factor signal transduction; *BAX*, encoding a protein related to ►apoptosis; ►*PTEN*, an important negative regulator of the ►PI3K – AKT signaling cascade; CASP5, a positive regulator of apoptosis; *E2F4*, a cell cycle regulator and *TCF7L2*, a downstream transcription factor in the ►WNT-signaling pathway (Fig. 3).

Clinical Relevance

Several clinicopathological variables and the family history of HNPCC related cancers decide if further analyses should be performed to identify the disease associated susceptibility gene mutation in a family. First



Microsatellite Instability. Figure 3 Genes revealing frameshift mutations downstream of defect mismatch repair. Examples of genes with mononucleotide repeat sequences within their coding regions prone for replication errors. If a gain (+) or loss (−) of one base(s) is not repaired, this will cause a frameshift mutation leading to a new coding sequence for the protein (each amino acid is read in base-triplets). *BAX*: encodes a protein that accelerates apoptosis, *TCF7L2*: T cell factor, a downstream transcription factor in the WNT signaling pathway, *TGF-β RII*: transforming growth factor *β* receptor II, *MSH6*: encodes a component of the mismatch repair system.

the tumor samples of affected persons should be analyzed for MSI and for altered expression of the mismatch repair proteins, MSH2 and MLH1. These analyses can be performed on serial sections of the archival samples. The criteria for diagnosis of MSI have been standardized and include the analyses of five markers, two mononucleotide repeats (BAT25 and BAT26), and three dinucleotide repeats (D2S123, D5S356, and D17S250). If two (40%) or more markers show instability the tumor is classified as high microsatellite instability (MSI-H) and if only one of the marker display instability, the tumor is classified as low microsatellite instability (MSI-L). Immunohistochemical analyses of the MSH2 and MLH1 proteins add diagnostic information, since loss of expression of either one of them correlates highly with MSI-H and thus specifies which one is most likely to be mutated in the germline. Finally, constitutional DNA from family members should be submitted for germline mutation analyses of the relevant mismatch repair gene(s) to identify the genetic predisposition pattern in the family in question.

Sporadic colorectal cancer patient with tumors that reveal MSI have a prolonged postoperative survival. This was initially shown more than a decade ago, and although many small studies have reported conflicting results the initial observation was recently confirmed in a large meta-analysis study including thousands of samples. However, the use of MSI as a predictive marker of chemotherapy benefit remains debated.

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M

Microsomes

Definition

Are artificially formed cell structures that can be formed from chopped up liver. They form some of the chemical changes to a drug that are performed by a whole liver in an animal or human.

►ADMET Screen

Microspikes

Definition

Highly motile protrusions of the cell membrane. They are characterized by a submembraneous accumulation of ezrin (an ERM-protein).

►ERM Proteins
►Podoplanin

Microtubule

Definition

A tubule in the cytoplasm that is composed of the protein tubulin, and which is an important component of the cytoskeleton, mitotic spindle, and cilia.

►Taxotere

Microtubule Associated Proteins

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Synonyms

Microtubule binding proteins; Tubulin interacting proteins; Microtubule stabilizing/destabilizing proteins; MAPs

Definition

MAPs are multifunctional proteins that bind to ►tubulins and alter the dynamics of their polymerization and depolymerization.

Characteristics

►Microtubules are hollow cylindrical structures consisting of polymers of αβ-►tubulin heterodimers. They are essential components of the cytoskeleton and play a critical role in many cellular processes including cell division, cell motility, intracellular trafficking, signal transduction and cell shape maintenance. ►Dynamic instability is an important property of microtubules and is indispensable for their function. This property is evident most notably during assembly and disassembly of ►mitotic spindle and segregation of duplicated chromosomes in mitosis. Intracellular dynamic behavior (polymerization/depolymerization) of microtubules is affected by post-translational modifications of tubulins as well as microtubule-stabilizing and microtubule-destabilizing proteins, collectively known as MAPs. Changes in phosphorylation of MAPs are known to influence ►cell cycle–specific dynamics of the microtubule network. MAPs of interest in ►cancer include (i) a family of specialized microtubule associated proteins (MAPs) and Tau, which are found most abundantly in neuronal cells, (ii) oncoprotein ►stathmin/Op18, (iii) ►tumor suppressors ►BRCA1 and pVHL (►Von Hippel-Lindau syndrome) protein and (iv) inhibitor of ►apoptosis protein ►survivin. This diverse group of proteins (ranging in MW 16–300 kDa) is characterized by a common feature, namely, association with microtubules. A microtubule binding domain is present in the N-terminus of MAP1A and MAP1B, while this domain in MAP2 and Tau is present at the C-terminus interspersed with18-amino acid repeats. Stathmin contains a microtubule destabilizing region within its alpha helical region, whereas pVHL contains a microtubule stabilizing domain within its helical domain. A γ-tubulin binding domain and baculoviral inhibitor of apoptosis repeat domain (►BIR domain) mediate the microtubule altering functions of ►BRCA1 and survivin, respectively.

MAPs and Cancer

By virtue of their importance in mitosis and cell division, MAPs are prime targets for anticancer therapy. The discovery of the first microtubule targeting drug, a *Vinca* alkaloid, was made serendipitously even before the term "microtubules" was coined in 1963. Discovery of other natural and synthetic microtubule-targeting compounds and understanding of cellular factors that influence their action was motivated by the observation that certain ►cancers are inherently resistant and others develop resistance to these microtubule targeting drugs. Although MAPs have received attention because of their role in resistance of cancers to microtubule targeting ►chemotherapeutic agents, more recently MAPs have also been implicated in other aspects of cancer including genetic susceptibility to certain cancer syndromes, development and aggressive behavior of a wide variety of cancers.

Cancer Predisposition

►Breast cancer 1 gene (►BRCA1) is a ►tumor suppressor gene implicated in predisposition to early onset of breast and ovarian cancer. ►Germline mutations in BRCA1 account for nearly half of ►hereditary breast cancer cases. A role for BRCA1 in ►microtubule dynamics and ►mitosis is indicated by its centrosomal localization during mitosis. Loss of ►BRCA1 ►ubiquitin ligase activity is thought to cause ►centrosome hypertrophy and subsequent ►aneuploidy typically found in ►breast cancers. A definitive role for BRCA1 in microtubule nucleation is supported by the observation that (i) BRCA1-dependent ►ubiquitination of γ-tubulin inhibits microtubule nucleation, and (ii) a mutant BRCA1 protein that lacks ubiquitin ligase activity, fails to inhibit microtubule nucleation. Another example of involvement of a microtubule binding protein in cancer predisposition is ►Von Hippel-Lindau protein (pVHL). VHL disease is a dominantly inherited cancer syndrome that predisposes people to tumours of the kidney (►renal cell carcinoma), retina and central

nervous system (►hemangioblastoma), the adrenal gland (►phaeochromocytoma) and various lesions affecting the kidney and the pancreas. pVHL is a microtubule-associated protein that can protect microtubules from depolymerization in vivo. Both microtubule binding and stabilization functions of pVHL depend on a region of pVHL that is also a mutational 'hot-spot' in VHL disease. Thus, inherited mutations in genes encoding multifunctional microtubule-binding proteins seem to contribute to a susceptibility to develop some types of cancers.

Cancer Development

The potential role of microtubule binding proteins in tumor development is exemplified by ►stathmin, which was originally identified as an 18 kDa oncoprotein (Op18). Stathmin is a member of a family of microtubule-destabilizing proteins. Stathmin is thought to act as tubulin-sequestering protein and also as a ►catastrophe promoter. High levels of stathmin expression are found in a variety of human malignancies. Increased expression of stathmin has been shown to precede tumor development in the induced rat mammary gland carcinogenesis model, raising the possibility that disruption of microtubule dynamics and abnormal mitosis may be critical events in breast cancer development. In human breast cancers, stathmin/Op18 levels correlate with a high fraction of aneuploid proliferative cells, larger tumor size and poor histopathologic grade. Stathmin has also been implicated in leukemogenesis and prostate tumorigenesis. Mutations in stathmin that affect its phosphorylation may also contribute to tumorigenesis.

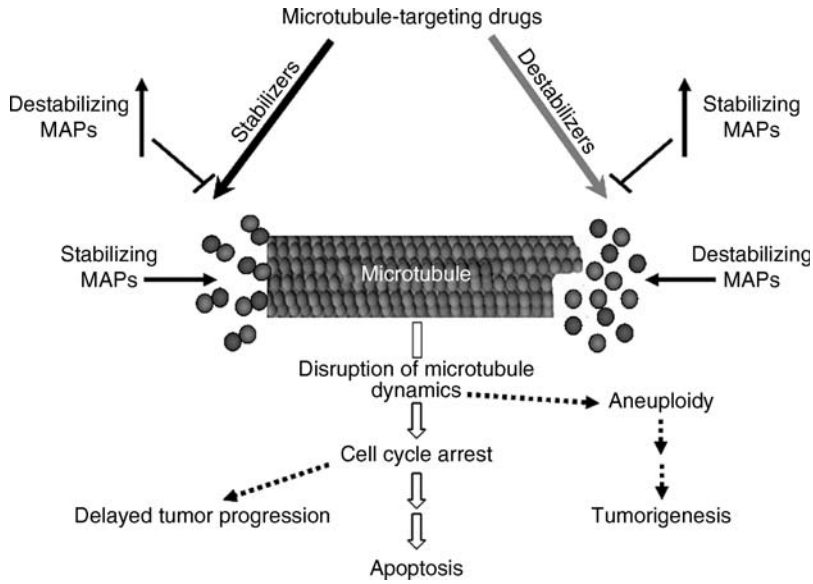
Cancer Diagnosis and Prognosis

The concept that MAPs, specifically neuronal-selective MAPs, could be cancer diagnostic markers originated from the observation that cancers of non-neural origin tend to exhibit neural or neuroendocrine differentiation. Among these MAPs, MAP2 is found most frequently in non-neural cancers. It is expressed primarily in the dendritic extensions of post-mitotic, terminally differentiated neurons. MAP2 plays a critical role in neurite outgrowth and dendrite development and its expression is considered a hallmark of neuronal differentiation. Variable MAP2 immunoreactivity is found in most pulmonary ►neuroendocrine carcinomas and in some non-small cell carcinomas. MAP2 is thought to be a valuable ancillary marker in skin tumors suspicious of neuroendocrine origin. An inducible, high molecular weight isoform of MAP2 is reported to be a diagnostic marker in a subset of oral ►squamous cell carcinomas.

In addition to being useful diagnostic markers, MAPs could also serve as valuable prognostic markers. In a variety of human malignancies, high levels of expression of stathmin is associated with poor prognosis. In contrast, there seems to be an inverse correlation between expression of microtubule stabilizing protein MAP2 and ►melanoma aggressiveness. Accordingly, while a majority of benign melanocytic nevi and primary malignant► melanomas are MAP2-positive, few ►metastatic lesions have MAP2 immunoreactivity. Expression of a neuronal differentiation-specific gene in neural crest-derived abnormal melanocytes is not entirely surprising. However, expression of a microtubule stabilizing protein in cells poised to proliferate rapidly could potentially have significant consequences on tumor growth and ►progression. In support of this notion, it was found that patients diagnosed with $MAP2^{+}$ primary melanomas had significantly better metastatic disease-free survival rates than those with $MAP2^{-}$ disease. Thus, activation of microtubule-stabilizing proteins in primary cancer cells may inhibit their proliferation and correlate with a delay or inhibition of ►metastasis. Conversely, dysregulated expression of microtubule destabilizing proteins may accelerate cell proliferation and tumor progression (Fig. 1).

Cancer Sensitivity/Resistance to Microtubule Targeting Drugs

Several natural and synthetic compounds that disrupt microtubule dynamics, and hence block mitosis, are currently in use as cancer chemotherapeutic agents. Two classes of compounds that are in wide use for chemotherapy of a variety of cancers are: ►Vinca alkaloids originally isolated from periwinkle *Vinca rosea* (►vinblastine, ►vincristine, vindesine and vinorelbine) and ►taxanes (►paclitaxel and ►docetaxel), which are diterpenes produced by the Pacific yew tree of the genus *Taxus*. Although these compounds are quite effective in killing cancer cells in vitro, variable sensitivity of different cancers in vivo and frequent acquisition of drug resistance is a major challenge in the use of these agents for cancer chemotherapy. Investigations to understand cellular factors and mechanisms involved in determining the sensitivity of cancer cells to microtubule-targeting agents have revealed MAPs as important factors in the drug-resistance mechanism. Studies on resistance of breast cancers to taxanes have provided much of our understanding of the role of MAPs in this phenotype. Gene expression analysis has identified Tau, a microtubule stabilizing protein expressed primarily in neurons, as a marker for sensitivity of breast cancer cells to paclitaxel. A low level or loss of Tau expression sensitizes breast cancer cells to the action of paclitaxel, presumably due to increased paclitaxel binding to microtubules when microtubules are assembled in the presence of low concentrations (or absence) of Tau, compared to microtubules that are formed in the presence of physiological (or higher) levels of Tau. Thus, breast cancer patients may be selected for paclitaxel therapy based on low Tau expression, and



Microtubule Associated Proteins. Figure 1 Schematic representation of role of MAPs in microtubule polymerization-depolymerization dynamics. (a) reciprocal relationship of microtubule stabilizing and destabilizing proteins to steady state dynamics as well as influence of MAPs on the effects of microtubule targeting chemotherapeutic agents is shown. Potential consequences of disruption of microtubule dynamics on cancer cells are also shown (*open and dashed arrows*).

inhibition of Tau could be a strategy to make tumors sensitive to paclitaxel. Similarly, overexpression of stathmin decreases polymerization of microtubules and consequently decreases sensitivity to paclitaxel and, to a lesser extent, to vinblastine. In contrast, stathmin content has no significant effect on the sensitivity to chemotherapeutic agents that do not target microtubules. Stathmin can affect the action of microtubule-targeting agents by both altering drug binding to microtubules and growth arrest at ►G_2/M transition of the cell cycle. Similarly, consistent with its role in microtubule nucleation during mitosis, BRCA1 also seems to mediate sensitivity of breast cancer cells to apoptosis induced by microtubule-targeting agents. The exact role of BRCA1 in determining the sensitivity to taxanes, however, is not clear.

In ductal adenocarcinomas and ►pancreas cancer, expression of MAP2 is known to be associated with sensitivity to microtubule-targeting drugs. Increased expression of ubiquitously expressed MAP4 has been observed in ovarian cancer cells and leukemia cells resistant to microtubule-targeting drugs. In ovarian cancer cells, MAP4 phosphorylation and dissociation from microtubules correlated with a decrease in ►Taxol sensitivity. Resistance of ►ovarian carcinoma cells to microtubule-targeting drugs has also been reported to be associated with altered regulatory pathways for stathmin expression and function. Additionally, survivin, a member of the inhibitor of apoptosis protein (IAP) family, also plays a role in the mitotic response in the context of Taxol resistance. Survivin functions at cell division to control microtubule stability and assembly of a normal mitotic spindle. This pathway may facilitate checkpoint evasion and promote resistance to chemotherapy in cancer. In contrast to their parental drug-sensitive counterparts, Taxol-resistant ovarian cancer cells exhibit defective mitotic response to the drug and fail to upregulate survivin levels and survivin phosphorylation.

Thus, a better understanding of factors and mechanisms and their role in conferring resistance to microtubule-targeting agents is essential for rational use of highly effective, often toxic, microtubule-targeting agents for cancer chemotherapy.

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Microtubule-Associated Proteins

Definition

MAPs are the proteins that directly bind to the tubulin subunits and stabilize the ►microtubules from depolymerization.

►Doublecortin

Microtubule Binding Proteins

►Microtubule Associated Proteins

Microtubule Network

Definition

Microtubules are polymers of α- and β-tubulin dimers. The tubulin dimers polymerize end to end in protofilaments. The protofilaments then bundle in hollow cylindrical filaments. Typically, the protofilaments arrange themselves in an imperfect helix with one turn of the helix containing 13 tubulin dimers each from a different protofilament.

►Vascular Disrupting Agents

Microtubule Stabilizing/Destabilizing Proteins

►Microtubule Associated Proteins

Microtubules

Definition

Hollow, cylindrical tubules ~30 nm in diameter, composed of dimers of alpha- and beta-tubulin. Microtubules are an important part of the cytoskeleton and mitotic spindles. Proteinaceous cylindrical hollow structures that are distributed throughout the cytoplasm of eukaryotic cells, providing structural support and assisting in cellular locomotion and transport.

►Doublecortin
►Microtubule Associated Proteins
►Paclitaxel

Micro-ultrasound Imaging

►Ultrasound Micro-Imaging

Midgut

Definition

The section of the gastrointestinal system between the ►ligament of Treitz and the distal third of the transverse colon, including the liver, pancreas, and gall bladder.

►Carcinoid Tumors

Midkine

Definition

MK; is a basic, low molecular weight protein that belongs to growth factors or cytokines and plays important roles in development. Its expression is induced during tissue repair, ►inflammation, and carcinogenesis and is involved in cancer progression, the onset of inflammatory diseases and the repair of injured tissues. Therefore, it is studied as a possible molecular target for the treatment of cancer and inflammatory diseases.

►Pleiotrophin

MIG-6

►Mitogen-Inducible Gene 6 in Cancer

Migfilin

Definition

Synonym Cal; a member of the zyxin family of proteins.

►Lipoma Preferred Partner

Migration

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Synonyms

Cell motility; Cell locomotion

Definition

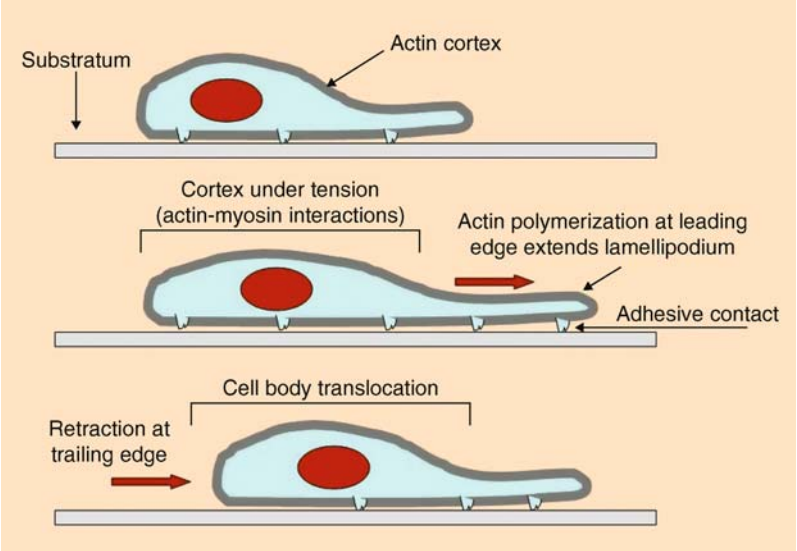
Cell migration can be defined as the movement of cells from one site to another and is a central process in the development and homeostasis of multicellular organisms. The orchestrated movement of cells in a particular direction to a specific location is essential for tissue formation during embryonic development, wound healing and immune responses. Deregulation of cell migration during any of these processes has serious consequences and can contribute to mental retardation, vascular disease, rheumatoid arthritis, tumor formation and metastasis.

Characteristics

Cell migration (the movement of cells from one site to another) is an essential process for normal development and homeostasis that can also contribute to important pathologies such as neoplasia. For example, one of the major mechanisms involved in tumor cell ►invasion is cell migration, a process in which cells demonstrating higher invasive capacity typically show higher migratory ability. Cell migration is a complex and highly coordinated processes which involves changes in the expression of several genes. Cell migration requires an intricate balance between extracellular cues and responsive intracellular signals that lead to dynamic regulation of the interactions between ►actin microfilaments, microtubules, intermediate filaments and associated ►cell adhesion molecules. The driving force for cell movement, however, is normally provided by dynamic reorganization of the actin ►cytoskeleton, directing protrusion at the front of the cell and retraction at the rear. In general, cell migration can be conceptualized as a cyclic process (Fig. 1). For a cell to move, it must establish morphological polarity resulting in leading and trailing edges with directionalized forces. To accomplish this, the initial step requires extension of protrusions in the direction of movement. These protrusions can be large, broad ►lamellipodia or spike-like ►filopodia that are typically formed by active actin polymerization. Frequently, lamellipodia evolve into ruffles either *de novo*, or when the membrane protrusions fail to adhere and are swept backward on the dorsal surface.

Protrusions are then stabilized by adhering to the ►extracellular matrix (ECM) or to adjacent cells via transmembrane receptors which are in turn linked to the actin cytoskeleton. Different forms of ►focal contacts exist that serve as adhesive structures to connect the cell with the ECM. They consist of two main elements: i) A transmembrane component comprised of clusters of ►integrin molecules linked to the ECM; and ii) A submembranous component comprised of a complex of proteins such as vinculin, talin, ►FAK and other proteins. Although, focal contacts react to actin-myosin tension, they also transmit their own tension upon the ECM to which they are linked. Since migrating cells must be able to detach, yet exert traction on the substratum, the velocity of migration is a biphasic function dependent on the strength of cell attachment. Finally, a contractile force, derived by the interaction of actin with myosin II to form a functional actin-myosin motor unit, propels the cell forward and contributes to the release of adhesive contacts in the rear of the cell and cell body translocation.

Considerable research with *in vitro* model systems has led to an increased understanding of cancer cell motility and invasion. The difficulty with many of these systems, however, is their relevance to the *in vivo* situation. Cell movement on a glass surface in a two dimensional plane is clearly different than invasion in a three dimensional environment. To improve our understanding of tumor cell migration, scientists have used *in vivo* imaging by intravital multiphoton technology, three dimensional in vitro models, and histological analyses as techniques to further characterize migration in a more physiological and representative context. The best understood system at present relates to mesenchymal cell motility which is characterized by an elongated morphology with established cell polarity. This polarity is dependent upon proteolysis to degrade the ECM. It is estimated that cancer cells which undergo ►epithelial to mesenchymal transition (EMT), a phenomenon which takes place in ~10% of all solid tumors, use this form of motility. Intravital imaging has



Migration. Figure 1 The Steps of Cell Migration. The migration of cells over substrata is a fundamental and critical function that requires the co-ordination of several cellular processes which operate in a cycle. Cell migration across two-dimensional (2-D) surfaces consists of at least three steps: (i) the protrusion of the leading edge for adhesive cell-substratum interactions is followed by (ii) contraction of the cell body and (iii) detachment of the trailing edge.

demonstrated that some cancer cells move at very high speeds with amoeboid morphology, very similar to the rounded form of migration of leukocytes and *Dictyostelium*. Another form of cell motility is called collective cell motility which involves the movement of whole clusters of sheets of tumor cells. Collective cell motility has been observed in infiltrating ►breast carcinoma and some ►ovarian carcinomas. In several other cancers, including ►gliomas, the type of motility employed has not been clearly defined and will require intravital imaging techniques to help in this assessment in the future.

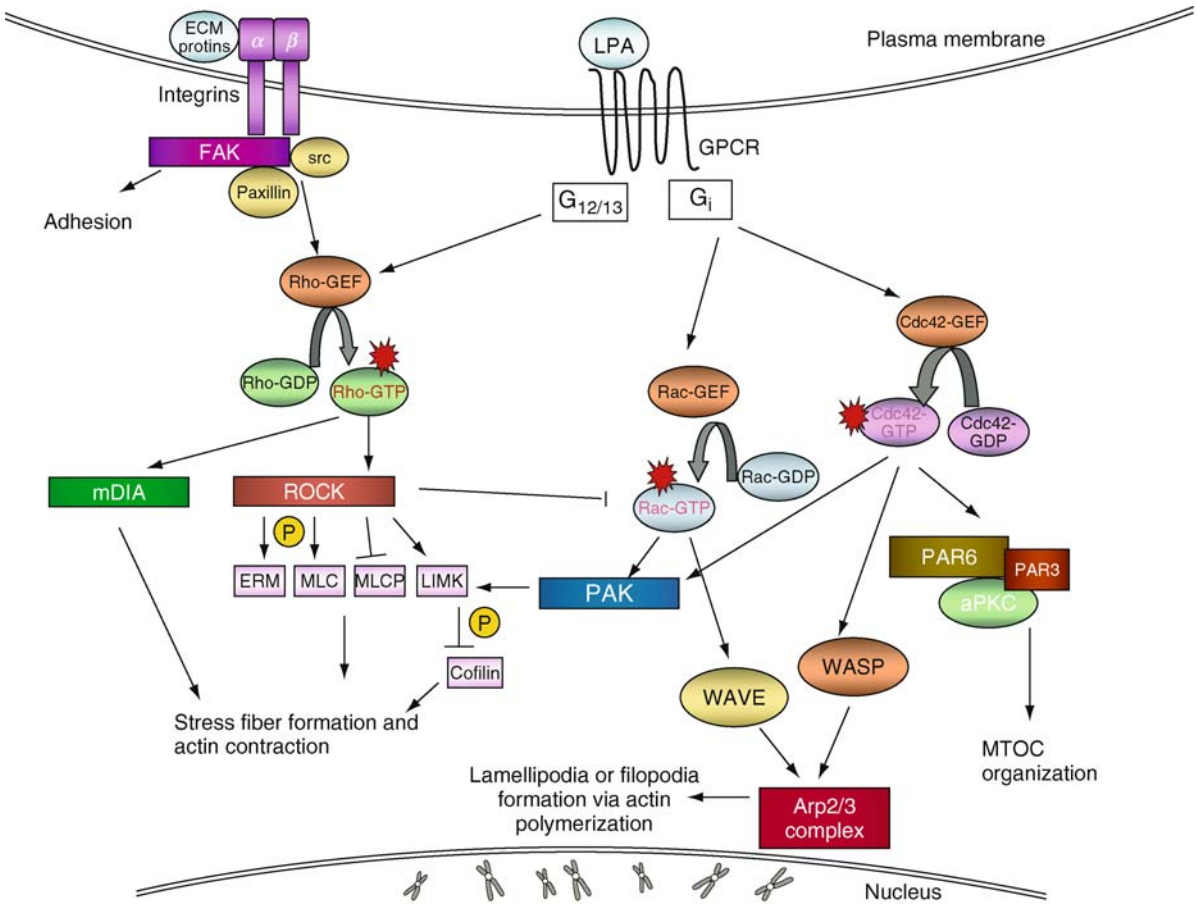
Mechanisms of Cell Migration: Rho-GTPases are Key Players

In the past decade, the analysis of a model cell line, Swiss 3T3 fibroblasts, has led to the identification of three ►signal transduction pathways responsible for controlling actin remodeling leading to directional cell migration. Each of these pathways is controlled by a member of the ►Rho family of small GTPases. Rho-►GTPases are members of the ►Ras superfamily of proteins. They are essentially molecular switches that become activated when they are bound to GTP and inactivated by hydrolysis of GTP to GDP. Rac, Cdc42, and Rho are the best characterized members of this family (Fig. 2).

Rac

Rac is required to regulate actin polymerization and formation of lamellipodia at the leading edge of cells and is thought to be the driving force for cell movement. Formation of small ►adhesions known as focal complexes which help stabilize the lamellipodium to the ECM also depends on Rac. To accomplish this, Rac recruits high affinity integrins to the area where new membrane protrusions are occurring. Members of the WASP/SCAR/WAVE family of scaffold proteins are key regulators of actin polymerization. In their stimulated state, each of these proteins is able to stimulate Arp2/3 complex which can initiate actin polymerization. Rac stimulates lamellipodia extension by activating SCAR/WAVE proteins indirectly and involves the Nck-adaptor complex.

One of the best characterized effectors that transmits the Rac signal is the Ser/Thr kinase, PAK. PAK plays an important role in regulating actin remodeling and cell adhesion during cell migration. The effects of PAK are partially controlled by its subcellular localization and interaction with binding proteins. In quiescent cells, PAK is distributed throughout the cytoplasm; however, when cells are stimulated, PAK is targeted to focal adhesions and membrane ruffles through binding of PIX. PAK has also been shown to phosphorylate and activate LIM kinase (LIMK), which in turn phosphorylates cofilin. Cofilin facilitates subunit dissociation from the pointed end of actin filaments and induces filament severing. It is also essential for promoting filament treadmilling (assembly/disassembly of free filament ends) at the front of migrating cells. PAK can also alter the phosphorylation state of myosin II. However, there are conflicting data on the role of PAK in myosin phosphorylation.



Migration. Figure 2 A schematic overview of Rho-GTPase signaling as it pertains to cell migration. Rho, Rac and Cdc42 are the best characterized members of this family of monomeric GTPases. They exert their action when extracellular stimuli transduce signals to upstream Rho-GTPase activators known as guanine nucleotide exchange factors (GEFs). Activated Rho, Rac or Cdc42 bind to downstream effector kinases which then phosphorylate a plethora of substrates leading to dynamic reorganization of the actin cytoskeleton essential to the efficacy of directed cell migration.

Expression of constitutively activated PAK resulted in decreased phosphorylation of the regulatory light chain of myosin in Hela cells which is in contrast to activated PAK in NIH3T3 cells, where activated PAK promoted MLC phosphorylation.

Cdc42

Cdc42 is the major regulator of cell polarity and plays a crucial role in controlling the direction of migration in eukaryotic organisms ranging from yeast to humans. One way in which Cdc42 influences polarity is by restricting where lamellipodia form. Cdc42 can also affect polarity by localizing the microtubule-organizing center (MTOC) and Golgi apparatus in the front of the nucleus, oriented toward the leading edge. The effects on MTOC position appear to be exerted mainly through a pathway involving the Cdc42 effector protein PAR6 which exists in a complex with PAR3 and an atypical protein kinase C (aPKC). However, the exact molecular mechanism by which PAR6/PAR3/aPKC complex orients the MTOC is still not well defined. PAK, also a downstream target of Cdc42, can itself mediate Cdc42 activation downstream of heterotrimeric G-protein coupled receptors, which are activated by many chemoattractants. These signaling events mediate a positive feedback loop between Cdc42 and PAK.

In response to bradykinin and interleukin 1, Cdc42 also induces parallel actin filament assembly to form filopodia. Filopodia are particularly well designed to serve as sensors and to explore the local environment, although they are not essential for chemotaxis. Cdc42 binds to WASP proteins and in vitro stimulates the Arp2/3 complex to induce dendritic actin polymerization and subsequent filopodia formation. WAVE/WASP proteins may themselves regulate the activity of Rac and Cdc42 by binding to GAPs and GEFs and could therefore generate positive or negative feedback loops to regulate the extent of actin polymerization.

Rho

Rho activity in migrating cells is associated with focal adhesion assembly and stress fiber formation and is responsible for cell body contraction and rear end retraction in response to a variety of extracellular stimuli such as ►lysophosphatidic acid administration or integrin engagement. The inhibition of Rho in cells like fibroblasts decreases adhesion causing a retraction of lamellae and rounding of the cell body. Rho can stimulate the clustering of integrins and thereby increases the strength of adhesion. The clustering is very pronounced and results from tension aggregating dispersed integrins, such that they align through their attachment with the ends of stress fibers in focal adhesions. Contractile actin-myosin filaments are found in all mammalian cells. In cultured fibroblasts, these are readily seen as well organized bundles known as stress fibers that are tethered to the plasma membrane at focal adhesions. The ability of Rho to induce the assembly of actin-myosin filaments and therefore contractility appears to depend on the activation of two distinct targets, Ser/Thr kinase p160ROCK (also known as ROCK, Rho-kinase) and the formin family protein, mDia.

ROCK has many targets but a key one is the myosin binding subunit of myosin light chain phosphatase. Phosphorylation leads to inactivation of the phosphatase which leads to an increase in the levels of myosin light chain (MLC) phosphorylation and cross-linking of myosin II into actin filaments and generation of cell tension. ROCK can also take the place of myosin light chain kinase (MLCK) and directly phosphorylate MLC. In its active state, ROCK, like PAK, can also phosphorylate and activate LIMK, which in turn phosphorylates and inactivates cofilin leading to stabilization of actin filaments within actin:myosin filament bundles. ►Ezrin/radixin/moesin (ERM) family proteins, adducin, and intermediate filaments (IFs) are also substrates of ROCK and the phosphorylation states of these targets are implicated in specific cell functions. mDia is another major player for promoting actin polymerization in eukaryotic cells. Upon binding Rho-GTP, mDia adopts an open conformation and binds to the barbed ends of actin filaments. It has the property of allowing addition of actin monomers to filaments while preventing the binding of capping proteins which would otherwise block elongation. mDia also co-operates with ROCK to assemble properly aligned stress fibers.

Summary

Migration is an extremely important cellular process that is important in both health and disease. For wound healing, nerve regeneration, infection control, and thrombosis, cellular migration plays a pivotal role in returning the host to a repaired and normal basal state. In the context of cancer, tumor cells have subverted the normal molecular pathways controlling migration. It is no small wonder then that the inhibition of tumor cell migration remains an important area of research with great therapeutic potential.

Acknowledgement

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Milieu

Definition

The environment or surroundings.

►Connexins
►Microenvironment

Milky Spots

►Omental Immune Aggregates

Mimetic Peptide

Definition

Refers to a synthetic drug that has the activity of a native peptide. Peptide sequences, synthesized both

chemically and biologically, are constituents of larger proteins where map to particular domains that are responsible for molecular recognition and biological responses. Inhibition of protein-protein interactions of cancer-related proteins by synthetic peptides has been one of the approaches towards cancer therapies.Peptides have been considered to be ideal drug leads. However, the use of peptides is limited as they are metabolically unstable due to protease cleavage and have poor bioavailability, which is in part due to a low membrane transport characteristics. Synthesis of mimetics follows approaches of rational drug design employing technology platforms of molecular design and structure-activity analysis. Following these approaches, mimetics have been designed that retain biological activities of the native peptide, but due to molecular modifications are metabolically stable, have good membrane transport characteristics and bioavailability, and therefore have drug-like pharmaceutical properties.

►Anti-HER2/Neu Peptide Mimetic.

Min

Definition

Multiple intestinal neoplasia; Refers to the first mouse model used to study the involvement of the Apc gene in ►colon cancer. Apc $^{Min/-}$ mice are heterozygous for an Apc mutation that leads to a truncated protein, they develop numerous intestinal adenomatous polyps therefore providing a good model for human familial adenomatous polyposis (FAP). As the number of polyps varies considerably in different mouse strains haboring mutated Apc, a genetic locus modifying this number has been proposed. Genetic linkage studies located such a modifying locus, Mom1, on mouse chromosome 4. At least two additional such loci appear to exist on other chromosomes. In humans, the existence of these modifier loci could not be confirmed.

►APC Gene in Familial Adenomatous Polyposis
►Modifier Loci

Mind Bomb Homolog 2 (Mib-2)

►Skeletrophin

Mineral Nutrients

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Synonyms

Dietary essential minerals; Macro- and microminerals; Electrolytes; Trace elements

Definition

Mineral nutrients are inorganic substances that must be ingested and absorbed in adequate amounts to satisfy a wide variety of essential metabolic and/or structural functions in the body. Mineral nutrients are sometimes categorized according to the amount required in the human diet to maintain good nutrition. Macrominerals is a general term encompassing both ►bulk minerals (calcium, phosphorus, magnesium) and ►electrolytes (sodium, potassium, chloride), which are required to be ingested by humans in amounts of hundreds of milligrams to several grams per day. Microminerals or ►trace elements (including iron, zinc, copper, manganese, ►selenium, iodine, chromium, molybdenum) are required in amounts of a few milligrams or less per day. The latter members of this group, which are required only in amounts of micrograms per day, are sometimes referred to as ultratrace elements.

Characteristics

For mineral nutrients, as for other classes of nutrients, nutrition experts have reviewed the available scientific evidence and defined the levels of intake required for nutritional adequacy and the maintenance of good health. Thus we have, for example, the Dietary Reference Intakes in North America and Dietary Reference Values in the United Kingdom. The Dietary Reference Intakes include the Recommended Dietary Allowance (RDA) for nutrients where there is sufficient scientific evidence to allow accurate assessment of the requirement level for nutritional adequacy and the Adequate Intake (AI) for nutrients where there is still uncertainty in the available evidence. In many cases a Tolerable Upper Level of Intake (UL) has also been determined, above which chronic intakes may be expected to yield increased risk of adverse health effects.

Requirements for mineral nutrients in healthy individuals can differ between males and females, at different ages, and in pregnancy and lactation. Genetic variation between individuals can have important influences on individual requirements for mineral nutrients. Mineral nutrient requirements and metabolism can also be altered by chronic diseases such as cancer.

Mineral nutrients are required to meet a wide variety of essential metabolic and structural functions in the body. Mineral nutrients fulfill structural roles in the body on different scales, from whole body to molecular levels. For example, calcium and phosphorus are deposited as hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] in an organic matrix to form bones and teeth which make up the skeleton. Zinc has a structural coordination role through specific amino acids in ►zinc-finger proteins, which interact with DNA in the cell nucleus. Magnesium is required to maintain the optimal orientation of ATP at the active site of kinase enzymes.

The metabolic functions of mineral nutrients are far too numerous to catalogue here, but include such diverse roles as iron complexed in the heme nucleus in hemoglobin and myoglobin for oxygen transport in the blood and muscle tissues, electrolyte flux in nerve transmission, calcium as an intracellular second messenger, phosphorylation and dephosphorylation in control of key enzyme activities through the actions of kinases and dephosphorylases, and the involvement of specific mineral nutrients at the active sites of ►metalloenzymes and metal-associated enzymes. Thus, mineral nutrients are involved in virtually all aspects of normal cellular metabolism. It is not surprising, therefore, that mineral nutrients should also be involved in abnormal cellular metabolism in chronic diseases, including cancer.

Concentrations of mineral nutrients in foods or biological tissues can be measured in a number of ways. As a first step it is often necessary to destroy all of the organic components of the sample, either with high temperature or concentrated acids. The residual minerals can be dissolved in mild acid solution and quantified using technologies such as atomic absorption spectrophotometry or mass spectrometry, to name just two. The total level of a mineral nutrient in a food is often not as revealing, however, as determination of its ►bioavailability, which represents the proportion of the ingested nutrient that can be released from the food matrix by digestion, absorbed across the intestinal wall and utilized by the body to meet the specific functions of that mineral nutrient in the body.

To determine the mineral nutrient status of an individual, in some cases the concentration of the mineral nutrient may be measured in a suitable sample such as whole blood, serum or urine. Blood or serum levels of some mineral nutrients are under close homeostatic control and depart from normal ranges only under extreme medical conditions. Where available, ►functional assays can sometimes provide a clearer picture of adequacy. Iron status, for example, can be measured as hemoglobin concentration (showing adequacy of red blood cells in circulation) or serum ferritin concentration (showing adequacy of body iron stores), among a number of other available indices. Activity of specific metalloenzymes may also be used, such as glutathione peroxidase for selenium or copper, zinc-superoxide dismutase for copper.

Mineral Nutrients and Cancer

The mechanisms of action of mineral nutrients in normal metabolism and in cancer are complex, varied and not yet completely understood. Researching a single component is like picking up a single piece of a complex jigsaw puzzle – you may be able to discover where it fits, but it alone can not provide the whole picture. While a reductionist approach, with the investigation of a single nutrient at a time, has been successful in the discovery and characterization of nutrient-specific deficiency diseases, an integrative approach may be more appropriate to investigation of the complex issues of nutrition, metabolism and a chronic disease having multiple etiological factors, such as cancer. This will continue to be a rich area of research for many years to come.

Epidemiological evidence of inverse relationships between intake of fruits and vegetables or whole grains and cancer incidence has been observed for many types of cancers. While antioxidant vitamins and ►phytochemicals have been implicated as potential active components contributing to this relationship, these foods also can be rich sources of mineral nutrients. Metabolic interactions between mineral nutrients, vitamins and phytochemicals add to the complexity of trying to attribute the benefits associated with these foods to a specific causative agent. The results of several large-scale trials conducted to date to investigate the effects of specific vitamin, phytochemical and/or mineral nutrient supplements on cancer rates have yielded mixed results. Some of these trials may not have used the optimal doses, or were of insufficient duration to allow the discovery of significant effects. It may also be that the interactive effects of several compounds acting together are necessary for the observed effects. Obtaining mineral nutrients through consumption of a well-balanced diet helps to ensure intake of additional dietary components which have beneficial health effects.

With the diversity of different types of cancers at different tissue sites, combined with the complexities of food consumption and combinations, the challenge of evaluating associations discovered in epidemiology studies is formidable. Clear distinction should be made between mineral nutrients used as therapeutic agents (at levels well beyond the defined requirements) and the effects of nutritionally adequate levels in preventing events associated with deficient levels of intake. Dose is also important in reconciling the evidence that excessive amounts of some mineral nutrients can have adverse metabolic effects that may contribute to cancer. Careful distinction should be maintained between epidemiological evidence of an association between a nutrient (or nutritional profile) and a cancer, versus

experimental evidence of plausible mechanisms by which a mineral nutrient may contribute to or aid in prevention of a cancer. Experimental mechanistic investigations should be done to support or refute epidemiological associations. Ultimately, evidence from human intervention trials is required to establish the effectiveness of specific approaches.

There is at present a relative paucity of human clinical intervention trials of efficacy of mineral nutrients in cancer prevention. In many of these studies relatively few doses or even just a single dose have been tested, so that limited dose-response information is provided. Also, the very long time course for development of many tumor types makes it difficult to study interventions. Baseline ►nutritional status may be critically important, as effects might only be observed in individuals with pre-existing deficiency or marginal status of a mineral nutrient.

Mineral nutrients (as with other nutrients) can influence many aspects of cancer, including effects on tissue susceptibility to carcinogens, effects on intestinal microfloral metabolism that can, in turn, influence the activation or inactivation of carcinogens in the intestinal tract, and effects on endogenous activation or inactivation of carcinogens in body tissues. Mineral nutrients may be involved in cellular metabolism in ►DNA repair, as antioxidants, in immune system modulation or in many other ways.

Either deficiencies or excesses of mineral nutrients can lead to disturbances of normal metabolism that can contribute to carcinogenesis, of which a few examples are considered here. Excessive accumulation of iron has been linked to the development of ►hepatocellular carcinoma in individuals afflicted with hereditary hemochromatosis and other iron loading syndromes. This may be related to the potential for ►oxidative stress damage when a transition metal like iron exceeds the capacities of the tissue to properly handle it, but deficiency of iron has also been associated with oxidative ►DNA damage. Adequate tissue levels of iron are also needed for dNTP synthesis as the ribonucleotide reductase enzyme contains iron.

Adequate levels of zinc are required to maintain the structure of zinc finger proteins involved in transcriptional regulation through intercalation with DNA. Deficiency of zinc also leads to loss of activity of zinc-containing DNA repair enzymes such as formamidopyrimidine glycosylase (involved in repair of oxidized guanine), compromising the ability of the cell to repair damage, hence contributing to tumorigenesis.

Several mineral nutrients are found in antioxidant metalloenzymes, including selenium in glutathione peroxidase, copper, zinc and manganese in the different isoforms of superoxide dismutase, and iron in catalase. These enzymes are part of a complex metabolic network controlling cellular redox status. Oxidative stress mechanisms have been implicated in carcinogenesis and also in certain chemotherapeutic strategies.

Inverse relationships have been reported between prediagnostic serum selenium levels and cancers at several sites including ►lung, ►colorectal and ►prostate cancers in men. Supportive evidence of anti-cancer effects of selenium, at levels above those required for maximal expression of known selenoenzymes, have been found in some animal and cell culture models, though the mechanisms of action are not fully known. Selenium is an element with a rich organic chemistry, and further investigation is needed of the effects of specific chemical forms and metabolites.

Low calcium intakes have been weakly associated in several studies with increased risk of colorectal cancer. High intakes (above nutritional requirements) have also been associated with increased risk of prostate cancer in some epidemiological studies indicating that in cancer metabolism, as with normal metabolic functions, there is an optimal range of intakes. One possible mechanism for the effect of calcium in reducing ►colon cancer risk is through binding with free fatty acids and ►bile acids or bile salts in the intestinal lumen, thereby decreasing the influence of these compounds on colonic epithelial cell proliferation.

High consumption of salt-cured or salt-pickled foods has been associated with increased risk of ►gastric cancer, leading to recommendations to minimize consumption of such foods. Excessive ►salt intake (sodium chloride) has been implicated in the development of gastritis leading to carcinogenesis, and may contribute to promoting effects at later stages.

In addition to mineral nutrients having effects on cancer, cancer can have significant effects on mineral nutrient metabolism. Tumor tissues may differ in mineral nutrient content from adjacent normal tissue, suggesting differences in metabolic requirements. Cancer patients can experience a variety of nutritional deficiencies including deficiencies of mineral nutrients, because of altered metabolism, altered dietary intakes, or drug interactions. These may sometimes be overlooked in the course of therapeutic monitoring.

Serum selenium and zinc concentrations have been shown to be significantly lower in patients with digestive tract cancers prior to commencing any therapy, than in control patients, while serum copper concentrations have been shown to be higher. Supplementation with selenium and zinc was found to minimize or prevent further worsening of nutritional status. Serum iron, too, has been shown to be lower in cancer patients in several studies. ►Chemotherapy with ►cisplatin can cause significant alterations in serum levels of mineral nutrients including copper, zinc and magnesium. Adjunctive therapies such as antacids or diuretics can also significantly influence mineral nutrient metabolism. It has

been suggested that at least some alterations in serum mineral nutrient concentrations may reflect the general changes in tissue distribution seen with ►inflammation. Such changes can also complicate status assessment in cancer patients. In all cases it is important to consider the question of whether altered status predisposes to altered metabolism, or results from it.

Restoration of altered mineral nutrient status may benefit patients (as with selenium and zinc, above) either by helping to maintain metabolism in normal tissues or by enhancing metabolic effects of therapy on tumor tissue. However, mineral nutrient supplementation could, in some cases, interfere with therapeutic effects of treatments. Specific situations call for specific understanding of the metabolic interactions involved.

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Mineralocorticoid

Definition

Member of the group of ►corticosteroids, primarily involved in the regulation of electrolyte and water balance through their effect on ion transport in epithelial cells of the renal tubules, resulting in retention of sodium and loss of potassium. The other group of corticosteroids are ►glucocorticoids.

Minichromosome Maintenance

Definition

MCM; A complex of proteins originally discovered in yeast because of their ability to increase plasmid retention by cells. The MCM complex is thought to be critical for both initiation and elongation of DNA synthesis in yeast and mammalian cells.

►S-Phase Damage-Sensing Checkpoints

Minimal Residual Disease

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Synonyms

MRD; Residual disease

Definition

The presence of disease detected in a patient who by conventional clinical and pathologic measurements is in remission.

Characteristics

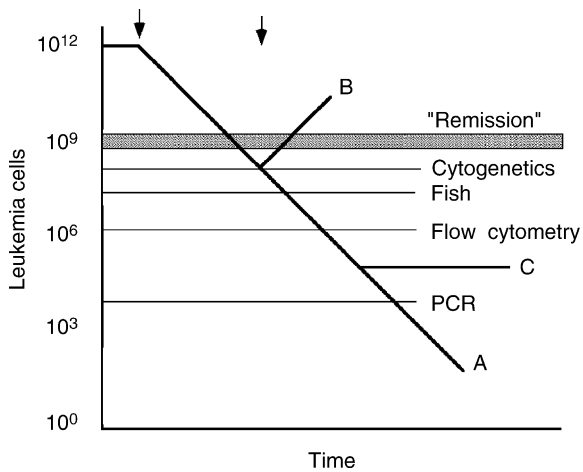
At diagnosis a patient with cancer may have billions of malignant cells. In ►leukemia, these cancer cells circulate in the bloodstream. After chemotherapy many cancer patients achieve "►remission," meaning that their disease cannot be detected by conventional clinical, radiological, and pathological examinations. Nonetheless, the major cause of failure in cancer therapy is the recurrence of disease, usually after the induction of a remission. The problem of defining remission and relapse is illustrated in (Fig. 1). In this essay we will use leukemia, a malignancy of the white blood cells, as a disease example, though the principle of remission, minimal residual disease (MRD) detection, and relapse applies to all malignancies.

At diagnosis leukemia patients may have a disease burden up to 10^{12} leukemia cells (Fig. 1). Thus, even if chemotherapy kills 99.9% of the leukemia population, up to 10^{9} cancer cells may remain despite the microscopic appearance of remission. The study of MRD aims to redefine the concept of remission, and differentiate patients with leukemia destined for relapse from those with a stable or declining leukemia load. Once identified, patients at a high risk of relapse could undergo additional or alternative therapy while the disease burden is several orders of magnitude less than at frank hematological relapse.

Methods of Detecting MRD

The limitation of the conventional definition of remission is apparent, since many leukemia patients who attain remission nevertheless relapse. Other

methods to define residual disease include "classic" metaphase and molecular ►cytogenetics, cell ►cytometry studies, and molecular genetic studies, such as the polymerase chain reaction (►PCR) detection of specific genetic targets. Each method takes advantage of differences in the characteristics of the tumor cell compared to the normal cell, and each has relative advantages, disadvantages, and sensitivity of detecting MRD (Table 1). Cytogenetics can detect approximately one leukemia cell in 10–100 normal cells (denoted as 10^{-1} sensitivity). Limitations of cytogenetics include a small sampling size (since typically only 20–50 cell metaphases can be examined), and the examination of only cells that grow and divide in culture. To increase sensitivity and facilitate genetic screening in non-dividing cells, ►fluorescence *in situ* hybridization (FISH) using chromosome-specific or locus-specific probes has been developed to identify genetic targets in both metaphase chromosomes and interphase nuclei. FISH is most useful in detecting simple losses or gains of chromosomes and ►chromosomal translocations. The sensitivity of FISH is between 10^{-1} and 10^{-2}, and detection of numerical losses or gains of chromosomes is generally more sensitive than for detecting translocations.



Minimal Residual Disease. Figure 1 The detection of MRD. At diagnosis patients may have up to 10^{12} leukemia cells. Treatment (arrows) decreases the load of leukemia below the threshold of microscopic detection ("Remission"). Further treatment further decreases the burden of leukemia. The sensitivity thresholds of other detection methods are shown as horizontal, labeled lines. Studies of MRD attempt to distinguish patients with decreasing disease (1A) versus those with disease in remission, yet bound towards relapse (1B). Lastly, there is a poorly understood phenomenon of "dormancy," whereby patients may have steady, very low disease burdens persisting for years (1C).

Normal cells display a variety of proteins on their outside surfaces, known as cell surface antigens. The expression of cell surface antigens can distinguish malignant from normal cells. Cells labeled with fluorescent antibodies can be detected and quantified by a fluorescence-activated cell sorter (FACS). While truly tumor-specific antigens are rare, studies have shown that malignant cells often express cell surface antigens in subtly different patterns than normal cells. By using combinations of multiple antibodies, flow cytometric assays can use the patterns of aberrant expression to "fingerprint" the malignant clone. The sensitivity of flow cytometry is 10^{-1} to 10^{-3}. The advantage of flow cytometry is that it is rapid, with results within hours of sample collection. However, to fully optimize the sensitivity of detecting the leukemia clone, combinations of several antibodies are needed to define the aberrant antigen expression, requiring considerable technical expertise.

The most sensitive approach to detect MRD involves nucleic acid amplification using the PCR. The power of the technique is the exponential amplification of the target genetic sequence. Each round of the PCR produces an exponential amplification from the initial starting copy number, following the equation N = No (1 + E) C (where N = PCR product, No = initial gene target number, E = efficiency of the PCR reaction, and C = number of PCR cycles). Thus, 30 cycles of amplification will yield over a million-fold amplification of the target. However, the PCR must have a specific genetic lesion as the "fingerprint" of the malignancy in order for PCR-driven reactions to have the desired sensitivity and specificity.

PCR based assays need specific genetic targets that are found in the cancer cells, but not in normal cells.

Minimal Residual Disease. Table 1 Methods to detect minimal residual disease

Technique	Target	Sensitivity
Pathologic examination	Cellular morphology	5%
Cytogenetics	Chromosome structure	1–5%
FISH	Specific genetic marker(s)	0.08–5%
Flow cytometry	Surface antigen expression	0.1–1%
PCR	DNA or RNA sequence	0.0001–0.1%

Chromosomal translocations are the most straightforward leukemia-specific markers for the detection of MRD. The prototypical translocation is the t(9;22) in ►chronic myeloid leukemia (CML), which causes the reciprocal translocation of the ABL gene on chromosome 9 to the BCR gene on chromosome 22. The juxtaposition of BCR-ABL forces the expression of the chimeric BCR-ABL ►mRNA, which can be specifically amplified by reverse transcription (RT)-PCR. Other examples of leukemia-specific translocations include the t(15:17), t(8;21), and inv. (16) found in ~30%, collectively, of ►acute myeloid leukemia (AML) cases, and the t(14;18) rearrangement detected in follicular cell ►non-Hodgkins lymphoma. However, genetic research of leukemia has discovered a rapidly expanding list of disease-specific translocations, and now genetically characterized translocations can be detected and amplified in 40–50% of AML and ►acute lymphoblastic leukemia (ALL).

In lymphoid malignancies, detection strategies take advantage of the rearrangement of the immunoglobulin heavy chain and T-cell receptor genes that occur normally in B and T-cell lymphoid development. During the development of a B-cell, for example, immunoglobulin heavy chain variable, junction, and diversity genes are merged from their germ line configuration to produce a shortened IgH V-D-J rearrangement. Each B cell has a unique IgH V-D-J sequence which determines the unique antibody that it produces. When a B-lineage cell transformed into a malignant clone, all leukemia cells will have the same IgH V-D-J rearrangement. This V-D-J gene rearrangement can serve as the leukemia-specific "fingerprint" of the leukemia. PCR methods can be used to then track the presence or absence of this clonal marker after therapy.

Clinical Relevance

The study of minimal residual disease (MRD) aims to understand the biology and clinical significance of leukemia that persists in patients who are in complete remission by conventional pathological examination. To recap, treatment of cancer during the level of disease burden below the threshold of detection by conventional pathology. This defines conventional "remission" (Fig. 1). Further therapy may drive disease levels further downward (1A). Alternately, the cancer may acquire ►resistance and begin to grow (1B). Lastly, some patients remain with MRD for years without relapse (1C).

The clinical significance of MRD has been most solidly determined in leukemia and lymphomas. The frequency of MRD detection varies with disease, type of treatment, and time in the treatment course. In CML, the detection of BCR-ABL mRNA (arising from the t(9;22) translocation) is associated with a >tenfold increase in relapse after ►hematopoietic stem cell transplant (HSCT). For CML taking tyrosine kinase inhibitors (such as Gleevec), the BCR-ABL level predicts long-term outcome. In AML and ALL, detection of MRD by PCR or flow cytometry is highly associated with subsequent relapse. The same holds for ►chronic lymphocytic leukemia (CLL) and ►multiple myeloma, both B-cell malignancies where MRD can be detected by PCR assays directed at clonal immunoglobulin gene rearrangements.

Not all patients with detectable MRD progress to relapse. The persistence of MRD without subsequent relapse has been referred to as ►dormancy (Fig. 1). Dormancy has been seen in t(8;21) AML following conventional chemotherapy, where most long-term survivors remain PCR-positive. Dormancy has also been seen in CML following HSCT. Furthermore, late relapses in pediatric ALL (who relapse >10 years after the establishment of remission) have the same clonal IgH VDJ gene rearrangement as at diagnosis, suggesting a very long-term dormancy of highly malignant cells. The mechanism of dormancy is unknown. It is likely that the clinical definition of "cure" in cancer does not mean the elimination of *all* cells involved in the leukemia process. The further study of dormancy may clarify what it takes to "cure" leukemia.

►Circulating Tumor Cells

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Minisatellite

Definition

Synonym Variable Number Tandem Repeats (VNTRs); Minisatellites are composed of blocks of longer blocks of repetitive DNA approximately 10–100 base pairs in length, dispersed throughout the genome. There is no known function for satellite DNA. Microsatellite DNA is composed of very short tandem repeats ranging from 1 to few nucleotide pairs that are dispersed throughout the genome.

►Microsatellite Instability
►Homologous Recombination Repair
►LINE Element

Minodronate

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Synonyms

YM-529; YM-529/ONO-5920; [1-hydroxy-2-(imidazo [1,2-a] pyridin-3-yl)ethylidene]-bisphosphonate; minodronic acid; nitrogen-containing bisphosphonate; amino-bisphosphonate; third-generation bisphosphonate; third-generation nitrogen-containing bisphosphonate

Definition

Bisphosphonates are potent inhibitors of bone resorption and are widely used drugs for the treatment of osteoporosis, osteolytic bone metastasis and tumor-associated hypercalcemia. These compounds have high affinity for calcium ions and therefore target bone mineral, where they are internalized by bone-resorbing osteoclasts and inhibit osteoclast function. Minodronate, a nitrogen-containing bisphosphonate, acts intracellularly by inhibiting farnesyl pyrophosphate synthase, thus leading to the inhibition of post-translational prenylation of small molecular weight G proteins, which could also contribute to its anti-resorptive activity on osteoclasts and its anti-tumor effects *in vivo*.

Characteristics

Minodronate is one of the most potent nitrogen-containing bisphosphonates. Farnesyl pyrophosphate synthase is a molecular target of nitrogen-containing bisphosphonates, and inhibition of post-translational ►prenylation of small molecular weight G proteins is likely involved in their anti-tumor effects both *in vitro* and *in vivo*. Further, since nitrogen-containing bisphosphonates accumulate in human vessels as well, it is also probable that they might have pleiotropic effects on vascular cells by blocking the protein prenylation of small G proteins, which serve as lipid attachments for a variety of intracellular signaling molecules.

Effects of Minodronate on Endothelial Cells (ECs)

►Angiogenesis, a process by which new vascular networks are formed from pre-existing capillaries, is required for tumors to grow, invade and metastasize. It is generally considered that a major event in tumor growth and expansion is the '►angiogenic switch'. ►Vascular endothelial growth factor (VEGF), a specific mitogen to endothelial cells, is a crucial factor for tumor angiogenesis. VEGF also acts as a pro-inflammatory cytokine in tumorigenesis. Several lines of evidence implicate VEGF as the key factor involved in tumor growth, expansion and metastasis. Indeed, VEGF expression levels are associated with ►angiogenesis and macrophage infiltration, the extent of which being correlated with the prognosis in various types of tumors. These observations suggest that inhibition of the VEGF signaling in tumor endothelium is a therapeutic target for preventing the development and progression of tumors.

Minodronate inhibits the VEGF-induced increase in DNA synthesis and tube formation in ECs by suppressing NADPH oxidase-mediated reactive oxygen species generation and consequent Ras activation via suppression of Rac-1 geranylgeranylation. In addition, minodronate blocks the VEGF-induced up-regulation of intracellular adhesion molecule-1 (ICAM-1) and ►monocyte chemoattractant protein-1 (MCP-1) in ECs, subsequently suppressing the VEGF-mediated T cell adhesion to ECs. Since small G proteins such as Rac-1 and Ras is involved in the VEGF signaling to ►inflammation as well, minodronate may exert anti-tumor effects *in vivo* by blocking the VEGF-signaling in tumor endothelium.

Reducing sugars, including glucose, fructose and trioses can react non-enzymatically with the amino groups of proteins to form reversible Schiff bases, and then Amadori products. These early glycation products undergo further complex reactions such as rearrangement, dehydration and condensation to become irreversibly cross-linked, heterogeneous fluorescent derivatives termed "►advanced glycation end-products (AGEs)". The formation and accumulation of AGEs in various tissues are known to progress during normal aging, and at an extremely accelerated rate in diabetes mellitus. AGE-their receptor (►RAGE) interaction elicits oxidative stress generation in vascular wall cells, thereby being involved in the pathogenesis of various types of disorders including melanoma growth, expansion and metastasis. Minodronate inhibits the AGE–induced reactive oxygen species generation and ►nuclear factor-κB activation and consequently suppresses ►vascular cell adhesion molecule-1 (VCAM-1) expression in ECs. This also suggests that minodronate may have therapeutic potentials for the treatment of AGE-related tumor growth and expansion. Since prostaglandin F2α (PGF2α) is involved in tumor ►angiogenesis and that minodronate suppresses PGF2α-induced VEGF overexpression in cultured osteoblasts, minodronate may exert anti-tumor effects by suppressing tumor VEGF expression.

Effects of Minodronate on Tumor Cells in vitro

Minodronate induces S-phase cell cycle arrest and ►apoptosis in myeloma cells by inhibiting protein geranylgeranylation and subsequently decreasing the levels of phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2), which could act as a survival

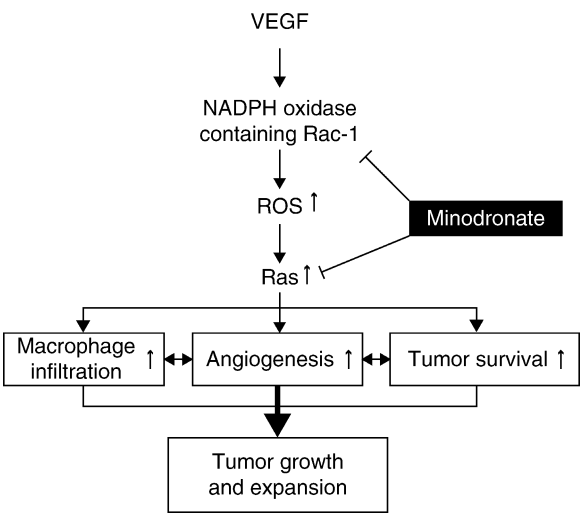
signal in myeloma cells. Minodronate also induces apoptosis of other types of tumor cells; it decreases bcl-2 expression and induces bax expression as well as ►caspase-3 activation, promoting apoptotic cell death of prostate cancer cells. In addition, minodronate inhibits proliferation of cultured murine osteosarcoma cells with induction of apoptosis. Moreover, minodronate decreases DNA synthesis and increase apoptotic cell death in cultured human melanoma cells via suppression of Ras farnesylation.

Effects of Minodronate on Tumor Cells in vivo

Minodronate inhibits ►melanoma growth and improves survival in nude mice by two independent mechanisms; one is by suppressing the tumor-associated ►angiogenesis and macrophage infiltration via inhibition of the VEGF signaling in ECs, and the other is by inducing directly apoptotic cell death of ►melanoma. These anti-tumor effects of minodronate observed *in vivo* could partly be ascribed to its suppressive properties on protein prenylation in melanoma cells themselves and tumor-associated endothelium (Fig. 1).

Minodronate is reported to exert anti-tumor effects in other animal models. It inhibits prostate cancer cell invasion into the bone matrix of an intratibial tumor injection murine model by suppressing CXC-R-4 expression in the lesions. Minodronate also suppresses osteoclast-mediated bone invasion by oral squamous cell carcinoma in mice by inhibiting cytokine expressions (tumor necrosis factor, interleukin-6, etc.). In addition, minodronate is reported to augment the anti-cancer effects of interferon on renal cell carcinoma in mice by further reducing serum levels of VEGF. Furthermore, in vivo-combination therapy with docetaxel and minodronate is found to significantly to reduce the tumor incidence compared with the control and also growth of intraossal transitional cell carcinoma of urinary tract in athymic nude mice compared with the control. Therapy with minodronate significantly enhances the inhibition of proliferation by docetaxel in osteoclasts of bone tumors compared with single therapy with docetaxel. These observations suggest that combination therapy with minodronate and docetaxel or minodronate plus interferon may also be beneficial for the treatment of tumors.

In conclusion, recent *in vitro*- and *in vivo*-data with minodronate support the concept that minodronate is a novel potential therapeutic agent for preventing the development and progression of various types of tumors. Further clinical investigation is needed to evaluate the efficacy of this new pharmacological approach as an anti-tumor therapy.



Minodronate. Figure 1 Possible mechanisms for anti-tumor effects of minodronate.

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Minodronic Acid

►Minodronate

Minor Histocompatibility Antigens

Definition

Minor Hag; are a group of immunogeneic proteins that are genetically polymorphic between HLA-identical

donors and recipients in allogeneic transplantation. The minor Hag are target structures of donor-derived T cells that recognize them on recipient cells in association with HLA molecules.

►Allogeneic Cell Therapy
►Graft Acceptance and Rejection

Mismatch Repair in Genetic Instability

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Definition

Mismatch repair acts after replication to correct mismatches which have escaped proofreading by the replication apparatus. Thus mismatch repair corrects DNA bases in non-Watson–Crick pairings or small distortions generated by incorrect strand alignment in repetitive DNA sequences. Structural abnormalities such as these can also be generated during recombination between DNA molecules which are not perfectly homologous (►Repair of DNA).

Characteristics

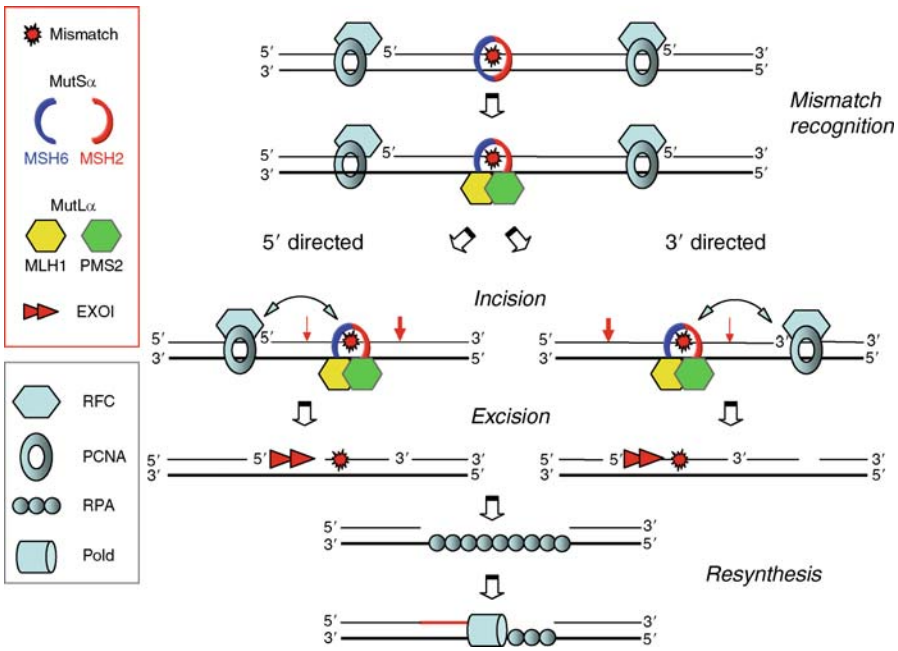
The first step in human mismatch repair is the binding of DNA mispairs by either the MutSα or the MutSβ heterodimer. These comprise of the MSH2/MSH6 and hMSH2/MSH3 proteins, respectively. Biochemical evidence obtained with purified complexes indicates that MutSα selectively recognizes single base mispairs and one base loop, whereas MutSβ prefers loops of 2–10 unpaired bases. Both bind to the two base loops that are the most common abnormalities in repetitive DNA sequences (Fig. 1). MutSα most likely forms a sliding clamp, which encircles DNA in the vicinity of the mismatch. The next step usually involves formation of a ternary complex with the MutLα heterodimer which is composed of the MLH1 and PMS2 proteins. Two minor heteroduplexes of undefined function with PMS1 (MutLβ or MLH3) MutLγ together account for <10% of cellular MLH1. Removal of the mismatch requires an exonuclease targeted to the newly synthesized strand presumably by the presence of nicks. These strand interruptions can be on either side of the mispair requiring mismatch removal in either 5′→3′ or 3′→>5′ mode. EXO1 is an ATP- and mismatch-dependent 5′→3′ exonuclease that paradoxically appears to be sufficient to direct correction in both directions. In the presence of MutSα, EXO1-mediated excision is highly processive and results in removal of >1,000 nucleotides. MutSα, the replication processivity factors PCNA and RFC activate a latent endonuclease of MutLα which yields new 5′ termini for EXO1 entry to permit 3′→5′ correction. PCNA, in its role as a processivity factor for DNA polymerase δ, is also involved together with the single stranded binding protein, replication protein A (RPA) in the subsequent repair synthesis step. It is not known which of the several DNA ligases catalyzes the ligation of the newly synthesized DNA stretch to complete repair.

Biological Consequences

Loss of mismatch repair leads to the accumulation of replication errors particularly in repetitive sequences such as ►microsatellites (long stretches of mono- and dinucleotide repeats scattered throughout the genome). During replication, template and daughter DNA strands in microsatellites are particularly prone to transient dissociation followed by realignment with the repeat units out of phase. This generates extrahelical loops that if uncorrected produce frameshift mutations. Thus loss of mismatch repair results in profound ►microsatellite instability (MSI) and accumulation of frameshifts in mono- or dinucleotide repeats. Thus inactivation of this pathway in human cell lines and in knock-out mice is associated with large increases in spontaneous mutation rates at functional genes (even by two or three order of magnitude) (►Mutator phenotype). Molecular analysis of spontaneous mutations occurring in mismatch repair-defective cells shows that both frameshifts and base substitutions are strongly increased in the absence of a functional mismatch repair.

It is acknowledged that mismatch repair proteins process some altered or damaged DNA bases and loss of mismatch repair can modify sensitivity to some therapeutic DNA-damaging agents. Mismatch repair deficiency is invariably associated with high level of resistance (tolerant phenotype) to methylating agents (►Alkylating agents), to the base analog 6-thioguanine and, to a minor extent, to ►cisplatin. Although the route of killing is not completely understood, it is clear that it involves mismatch repair processing of lesion-containing DNA mismatches. Thus hMutSα can indeed bind duplex oligonucleotides containing single O^6-methylguanine, 6-methylthioguanine, or 1,2 GpG cisplatin adducts. Following recognition, aberrant processing by the MutLα complex ("futile cycles" of repair attempts) might transform these lesions into strand interruptions, which at the next round of replication leads to the formation of lethal lesions, probably double strand breaks.

Mismatch repair is also required for triggering a G2 arrest, occurring in the second cycle after treatment, via the ATR/CHK1-dependent pathway. The late timing in



Mismatch Repair in Genetic Instability. Figure 1 The mismatch bound by MutSα recruits MutLα. The ternary complex forms a sliding clamp which can move in both 5′ or 3′ direction. MutSα, PCNA, and RFC activate a latent MutLα endonuclease, in a mismatch- and ATP-dependent manner. This activity provides incisions that give a 5′ terminus that serves as entry sites for the MutSα-activated EXOI, which removes the mismatch via its 5′→3′ exonucleolytic activity. These strand interruptions can be on either side of the mispair and EXOI is able to direct corrections in both directions. The single strand gap is stabilized by RPA. Once the mismatch is removed and the EXOI activity is inhibited by MutLα and RPA, the gap is filled by polymerase δ.

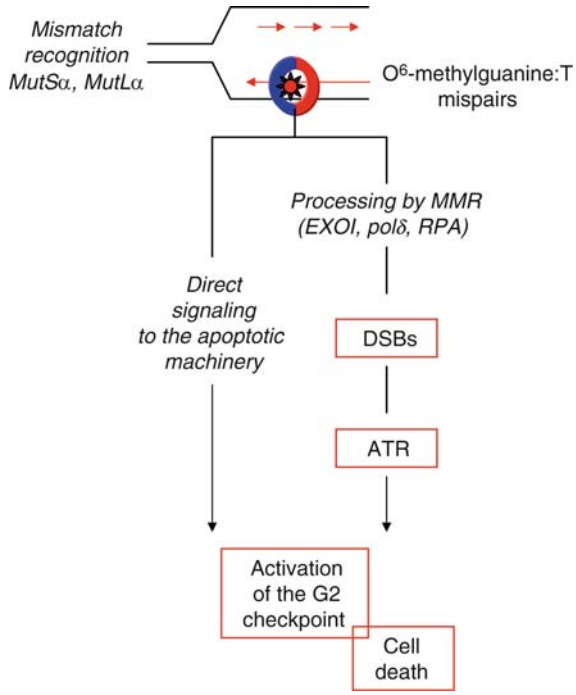
checkpoint activation suggests that the signal might be abnormal DNA structures produced by mismatch repair processing of lesion-containing mismatches. As an alternative to the "futile cycles" hypothesis, it has been proposed that mismatch repair proteins might signal directly to the apoptotic machinery (Fig. 2).

Finally mismatch repair also contributes to the control of endogenous levels of DNA oxidation – probably by removing incorporated oxidized bases from the nascent DNA strand. Thus, embryo stem cells, mouse embryo fibroblasts, and several organs of mice defective in the *msh2* mismatch repair gene show higher steady-state levels of DNA 8-hydroxyguanine, than wild-type controls. The attenuation of the mutator phenotype of mismatch repair-defective mammalian cells by efficient exclusion of 8-hydroxyguanine at replication implicates oxidized bases in the genetic instability, including ►MSI, associated with loss of mismatch repair.

Clinical Relevance

Mutations in genes encoding mismatch repair proteins underlie the predisposition to colorectal tumors in the familial syndrome ►hereditary nonpolyposis colon cancer (HNPCC) (►Lynch syndrome). The inherited germline mutation in at least one of four mismatch repair genes (*hMSH2*, *hMLH1*, *hMSH6*, *hPMS2*) and the subsequent functional inactivation of the second allele predisposes to colon cancer at an early age. It is proposed that the mutator phenotype, following loss of mismatch repair, facilitate the occurrence of mutations in genes controlling proliferation and/or apoptosis thus leading to an increased cancer risk. The majority of the mutations in the HNPCC families occur in the *hMSH2* and *hMLH1* genes. A peculiar characteristic of the *hMLH1* gene is that this gene is often silenced through methylation of its promoter. Some HNPCC families with unusual tumor spectra have mutations in the *hMSH6* gene.

At variance with the classical "tumor suppressor" pathway that usually display gross genome instability (large chromosomal changes and aneuploidy), mismatch repair defective tumors are usually pseudo-diploid. It has been proposed that the multiple genetic changes needed for malignancy can be obtained in two alternative and mutually exclusive ways: in a minority of cases instability at gene level because of mismatch repair deficiency and gross chromosomal changes in the majority of cases. Indeed, colorectal cancer occurring in HNPCC patients show a specific pattern of genetic changes compared with sporadic tumors with chromosomal instability. Runs of



Mismatch Repair in Genetic Instability. Figure 2 Treatment with methylating drugs introduce O^6-methylguanine into DNA. Error-prone bypass of the methylated base will generate O^6-methylguanine:T mispairs, which are recognized by MutSα. Processing by MutSα/MutLα complexes might remain incomplete and result in "futile cycles" of attempted repair leading to strand interruptions, probably DSBs, which become lethal. Lethal lesions trigger a G2 arrest mediated by the ATR/CHK1-dependent pathway. Alternatively, mismatch repair might signal DNA damage directly to the apoptotic machinery.

mononucleotide repeats in coding regions of genes become a preferential target of mutagenic events as a consequence of mismatch repair loss. For example, tumors with ►MSI preferentially accumulate frameshift mutations in genes involved in the control of proliferation and death. Examples of frameshifts that are likely to confer direct or indirect proliferative advantages and which are particularly prevalent in mismatch repair defective tumors include transforming growth factor β receptor (*TGFβRII*), insulin-like growth factor type II (*IGF-II*), *β*2-microglobulin, *Bax*, and *caspase 5* as well as the mismatch repair genes *hMSH6* and *hMSH3*.

►MSI is also quite common in some apparently sporadic tumors suggesting that loss of mismatch repair can be a relatively common event in human cancer. In particular 15% of sporadic colorectal tumors and, to a varying degree, tumors of several other organs (gastrointestinal tract, bladder, endometrium, and ovary) display the mutator phenotype characteristic of mismatch repair defects.

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Missense Mutation

Definition

A type of mutation where a base pair change results in a different amino acid being present in the mature protein, for example a lysine to a tyrosine at codon 12 of a given protein.

Mitochondria

Definition

►Mitochondrium

Mitochondria Apoptogenic Factors

Definition

These factors are normally located within the mitochondrial inter-membrane space and are released in response to ►apoptosis stimulation. The release is usually mediated by the pro-death ►Bcl-2 family proteins, Bax or Bak, but could be mediated by other mechanisms. The apoptogenic factors include cytochrome c, which binds to Apaf-1 and activates casapase-9; Smac and HtrA2, which bind to ►XIAP to release its inhibition on caspase-9 or caspase-3; and AIF and Endonuclease G, which move to the nucleus to cause DNA fragmentation.

►Bid
►Mitochondrium

Mitochondria Apoptosis Pathway

Definition

Synonym: intrinsic death pathway; bridges multiple death signals to the mitochondria. It is mediated by the ►Bcl-2 family proteins. The BH3-only pro-death members are sentinels to different death stimuli. For example, Bid is activated by proteases and Bad is activated by growth factor deprivation. While ►PUMA and Noxa are activated by DNA damages, Bim can be activated by cytokine deprivation, UV and calcium flux. The activated BH3-only members are translocated to the mitochondria, where they either directly activate the multi-domain pro-death molecule, Bax or Bak, or indirectly by binding to the anti-death Bcl-2 family members. Activated Bax or Bak mediates the release of mitochondria apoptogenic factors, which in turn activates caspases or inactivates the pro-survival IAP family proteins.

►Bid

Mitochondrial DNA

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Definition

Mitochondria are double membrane bound organelles, 1–2 μm in length and 0.5–1 μm in diameter and provide energy to the cell. Human cells contain two types of DNA; nuclear DNA (nDNA) and ►mitochondrial DNA (mtDNA). Before the term "mitochondria" was used, these oval subcellular granules, similar in size and shape to bacteria, were called "bioblasts" and were considered the basic unit of cellular activity.

Characteristics

Mitochondria now are considered as centers of energy metabolism and produce up to 80% of the energy needs of a cell. The inner membrane forms a series of folds called cristae and harbors enzymes involved in ►oxidative phosphorylation. Oxidative phosphorylation enzymes are located in the inner membrane and depending on the metabolic activity of a cell, the size of the inner membrane increases or decreases.

Human cells contain two types of DNA; nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Before the term "mitochondria" was used, these oval subcellular granules, similar in size and shape to bacteria, were called "bioblasts" and were considered the basic unit of cellular activity. Mitochondria now are considered as centers of energy metabolism and produce up to 80% of the energy needs of a cell. The inner membrane forms a series of folds called cristae and harbors enzymes involved in oxidative phosphorylation. Oxidative phosphorylation enzymes are located in the inner membrane and depending on the metabolic activity of a cell, the size of the inner membrane increases or decreases.

A typical mammalian cell contains about 1,000–10,000 copies of mitochondrial DNA. The mitochondrial genome is 16.5 kb which is closed circular double helical molecule encoding 2 rRNAs, 22 tRNAs, and 13 polypeptides and does not contain introns and ►histones. mtDNA represents about 1% of the cellular DNA. mtDNA is double stranded except for the D-loop which is triple stranded (contains extra 7S DNA). Promoter of mtDNA is located in the D-loop, which is the only non-coding region of mtDNA. The D-loop contains cis elements involved in mtDNA replication and transcription. All 13 peptides of mitochondria are located in a mitochondrial respiratory chain complex.

Mitochondria are an important biological source and target of ►reactive oxygen species (►ROS), free radicals and sensitive to environmental mutagens. Normal assembly and operation of the respiratory chain requires an intact and functional mitochondrial genome. Depending on the energy demand of a cell, the number of mitochondria per cell varies. Under physiologic conditions, as many as 2% of electrons leak from the mitochondrial electron transport chain and reduce oxygen into superoxide anion, triggering the formation of free radicals that indescribably damage biological molecules. Damage due to ROS and radiation is severe for mtDNA and unlike nDNA, mtDNA have a limited repair capability. The accumulation of mutations in mtDNA is about tenfold greater than in the nDNA. A continuing cycle of worsening mitochondrial dysfunction with increasing ROS production might be expected from such damage to mtDNA. Mutations in mtDNA can vary widely among tissues in an individual and mutation load may change over time. Tissues are differentially sensitive to levels of mtDNA mutations.

Genetic and/or metabolic alterations in mitochondria may lead to different diseases. The mitochondrial genome has drawn increasing attention in ►cancer research due to its unique genetics, such as maternal

inheritance, and lack of ►recombination as well as functional importance in oxidative phosphorylation. mtDNA is a critical target for cellular reactive oxygen species and the level of oxidative damage is more severe and persistent in mtDNA than in nDNA. MITOMAP (www.MITOMAP.org) is a comprehensive database of human mtDNA variation and its relation with human diseases.

In humans, 99.99% of mtDNA is inherited from the mother because sperm mtDNA is actively degraded. Sperm carries its mitochondria around a portion of its tail and has only about 100 mitochondria compared to 100,000 in the oocyte. As the cells develop, more and more of the mtDNA from males is diluted out and less than 0.01% of the mtDNA is paternal. Thus, mutations of mtDNA can be passed from mother to child. Its implication is in cloning of mammals using somatic cells. For example, when the sheep "Dolly" was cloned, the nDNA was from the donor cells, but the mtDNA was from the host cells.

Abnormality in mtDNA may lead to different diseases such as optic neuropathy (degeneration of the optic nerve accompanied by increasing blindness), myopathy, exercise intolerance, diabetes, deafness, MELAS, Pearson's syndrome, ataxia, and cancer. As an example, cancer and mtDNA has been described in details.

mtDNA has been used in ►epidemiology. ►Admixture populations have been identified based on mtDNA characteristics. To understand the utility of mtDNA in cancer epidemiology, one approach is to evaluate in patients with cancer and matching controls for somatic mutations in mitochondria. Another approach is to look for disease-associated haplotypes. The inheritance pattern of mitochondria in patients with cancer has been studied by haplotype analysis. Polymerase chain reaction (PCR) of key polymorphic sites in the mitochondrial genome was performed in samples from cancer patients and controls to determine presence of an association between mitochondrial genotype and cancer. Such analysis has been accomplished in a few studies of ►prostate and renal cancer. For example, haplogroup U, one of the nine major haplotype groups, was associated with an odds ratio of 1.95 in a prostate cancer case control study and with an odds ratio of 2.65 in a renal cancer study. There are very large differences across racial and ethnic groups in the distributions of the major haplotypes. Haplotyping is performed by restriction analysis whereas for mutation analysis whole mitochondrial genome is sequenced.

Microdissected samples have been successfully used to identify mtDNA mutations from clinical samples. Thus, a pure population of cells could be isolated from heterogeneous cells of the surgically isolated samples. To understand the etiology of the disease and to do mitochondrial genotyping, a pure population of cells is needed.

A variety of clinical samples have been utilized for mtDNA mutation detection. For example, nipple aspirate and paraffin embedded specimens for breast cancer, urine for bladder cancer, buccal cells for head and neck cancer, cerebrospinal fluid (CSF) for meduloblastoma, and sputum for ►lung cancer have been used. High-throughput technology has been developed for somatic mutation detection. Since each copy may be independently replicated, mutations can accumulate in various proportions of the genomes. The presence of a mutation in 100% of the genomes is termed ►homoplasmy, while ►heteroplasmy is a mixture of mutated and wild-type sequences for a given locus.

About 50 years ago Nobel-Laureate Otto ►Warburg reported the fundamental difference between normal and cancerous cells to be the ratio of glycolysis to respiration. Now we see a linking impaired mitochondrial function as well as impaired respiration to growth, division and expansion of cancer cells. Recent work has confirmed that this is, indeed, a promising approach in the treatment of cancer.

Cancer cells accumulate defects in the mitochondrial genome, leading to deficient mitochondrial respiration and ATP generation. In some cases, mitochondrial germline mutations have been shown to provide predisposition to cancer development. Mostly, such mutations are acquired during or after oncogenesis. Tumors rely heavily on glycolysis to meet their metabolic demands. Compared to normal cells, cancer cells exhibit metabolic imbalances and enhanced resistance to mitochondrial ►apoptosis.

Mitochondrial mutations have been reported in preneoplastic lesions suggesting that mutations occur early in multi-step tumor progression and hence, may be used as a tool for ►early detection of cancer in clinical samples. Alterations in mitochondrial structure and function might provide clinical information either for early detection of cancer or as unique molecular sites against which chemotherapeutic agents might be targeted.

The field of mitochondria has seen a recent surge of interest among cancer researchers as alterations in mtDNA have been reported in various neoplasms. Population-based studies involving environmental and occupational exposure, infectious agents, personal susceptibility factors, and acquired genetic factors may identify high-risk populations that are likely to develop cancer; additionally, such studies are very informative and significant in designing future community-based health initiatives. mtDNA biomarkers can be used to follow disease prevalence by determining their level in cohort studies with the potential for identifying high-risk populations.

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Mitochondrial Intrinsic Pathway

Definition

One of the two major routes of ►apoptosis induction. This pathway requires the participation of the mitochondria through mitochondrial membrane permeabilization which leads to the release of several apoptosis-promoting factors.

►Cannabinoids
►Mitochondrium

Mitochondrial Membrane Permeabilization in Apoptosis

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Synonyms

MMP

Definition

During ►apoptosis, many signals can converge to the mitochondrion to trigger the so-called mitochondrial membrane permeabilization (MMP), a rate-limiting step in the execution of the death process. These signals are mainly endogenous proteins, which translocate from an intracellular compartment (e.g. nucleus, cytosol, lysosomes, etc) to the mitochondrial outer membrane (OM). Accumulation of modified lipids (e.g. oxidized cardiolipin, ceramide, etc) and ions (e.g. Ca^{2+}) by the mitochondrion can also influence MMP. Moreover, the intracellular milieu, such as pH, ►reactive oxygen species (ROS), and ATP levels can contribute to define a permissive environment for MMP execution. Once initiated, MMP leads to the release of intermembranespace factors into the cytosol, caspase-dependent proteins, such as cytochrome *c*, Smac/DIABLO, procaspases and caspase-independent proteins such as apoptosis-inducing factor, AIF, and EndoG. The major consequence of these protein movements is the coordination of the final cellular degradation phase of apoptosis. Concomitantly, MMP provokes a mitochondrial failure, which manifests by an arrest of oxidative phosphorylation and ATP synthesis, an increased ROS level, and dissipation of the inner membrane potential ($\Delta\Psi m$). Therefore, MMP constitutes a point of no return of the activation cascade of apoptosis.

Characteristics

Mitochondria are intracellular organelles involved in the cell energy metabolism and also in the control of the intrinsic pathway of apoptosis. They are surrounded by two different membranes that differ in terms of composition, surface, permeability and function. The OM is permeable to solutes of MM ≈ 6kDa due to the presence of channels, such as the voltage-dependent anion channel (VDAC), which belongs to the porin subfamily. However, with an estimated pore diameter about 2.6–3nm, VDAC would not allow the passage of a folded protein like cytochrome *c*. In contrast, the inner membrane (IM) is almost totally impermeable. Specialized membrane proteins, namely the mitochondrial carriers, carry out the transport of ions and solutes across this membrane. Most mitochondrial proteins, which have been implicated in apoptosis, exhibit dual functions, a vital metabolic function and a lethal pro-apoptotic function. This applies to various channels (VDAC, adenine nucleotide translocase (ANT), Bax, t-Bid, Bak), receptors (e.g. TOM22), chaperones (cyclophilin D, CypD), as well as oxido-reductases (AIF).

Mechanisms of MMP

Mechanisms mediating MMP may be multiple depending on the cell type and the death stimuli. They can affect (only) the OM, or both mitochondrial membranes (OM+IM). Among numerous hypothetic models, experimental evidence support models involving members of the conserved ►Bcl-2 family, which can be divided into pro-apoptotic members (Bax, Bak, Bid, Bik, Bnip3, etc) and anti-apoptotic members (Bcl-2, Bcl-X_L, Mcl-1, etc), members of the permeability transition pore

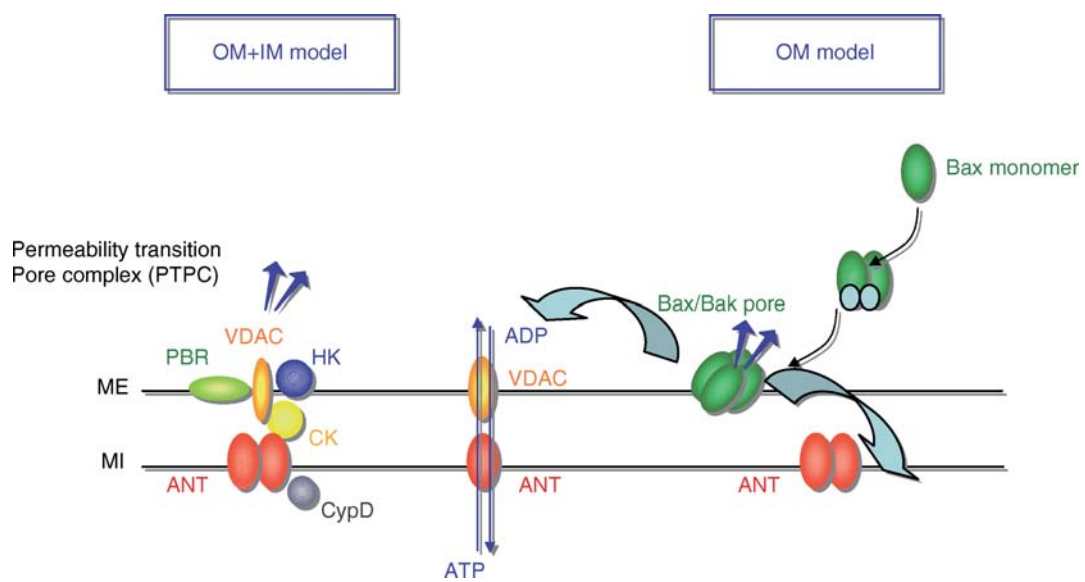
complex (VDAC, ANT, CypD) and lipids. At the onset of MMP, cytosolic and nuclear proteins such as Bax, truncated-Bid (t-Bid), p53, Nurr77, can undergo conformational changes and/or post-translational modifications favoring their translocation to the mitochondrial membranes. Subsequently, protein-protein interaction changes operate and novel homo- or hetero-oligomers form to promote the release of pro-apoptotic factors into the cytosol. In OM models, Bax would oligomerize with Bak and/or VDAC to form megachannels. In OM+IM models, the permeability transition pore would undergo long-lasting openings of high conductance (≈1.5nS) allowing a massive entry of water and solutes (MM<1.5 kDa) that is responsible of matrix swelling and OM ruptures due to the difference of size of both membranes. Thus, in the OM model, intermembrane space proteins are release into the cytosol by passage through large proteic/lipidic channels while in the OM+IM model, intermembrane space proteins are freely released into the cytosol through the OM ruptures. Nevertheless, these two models can be linked in conditions involving in the one hand tBid translocation, and in the other hand, mitochondrial calcium accumulation and ROS increase, as observed in conditions of endoplasmic reticulum stress. Fig. 1 illustrates both models of MMP.

Regulation of MMP

MMP can be regulated positively or negatively by endogenous oncoproteins (e.g. Bcl-2, Bcl-X_L) and tumor suppressors proteins (e.g. Bax, Bak, p53), viral proteins (e.g. Vpr, M11L and vMIA), bacterial proteins (e.g. porB), small molecules (e.g. MT21) and also chemotherapeutic agents (arsenite, lonidamine, nelfinavir, betulinic acid, verteporfin). Pro-apoptotic proteins, which translocate to the OM, can act directly by forming pores or indirectly by modifying proteins. Thus, some kinases and phosphatases can act by modification of constitutive mitochondrial proteins to stabilize or destabilize mitochondrial membranes. In addition, caspases have been shown to cleave NDUFS1, the 75 kDa subunit of respiratory complex I, and in turn to disrupt mitochondrial function and induce apoptosis. In contrast, some proteins (e.g. Bcl-2, gluthathion-S-transferases (GST), superoxide dismutase (SOD)) stabilize the mitochondrial membrane barrier. For instance, a mitochondrial GST can block ANT in its translocase function and thus prevent its lethal pore conversion, in vitro as well as in cellula. Finally, natural ligands (ATP, glucose, creatine) of several metabolic enzymes also favor the metabolic function of proteins rather their apoptotic function.

MMP and Cancer

Apoptosis, as well as senescence, is considered as a barrier against neoplasia. Under pathological conditions, a MMP failure can result in an inhibition of apoptosis and enhanced resistance to chemotherapy. Several non-mutually exclusive mechanisms may account for a defect in the execution or regulation of MMP. These include (i) alterations in gene transcription, (ii) gene mutations resulting in protein inactivation, and (iii) defects of intracellular localization. This may concern structural proteins of PTPC, as well as MMP regulatory proteins, such as Bax/Bcl-2 family members, p53, caspases, and cyclophilin D.



Mitochondrial Membrane Permeabilization in Apoptosis. Figure 1

Thus, a decrease in the ratio Bax/Bcl-2 is frequently observed in human cancers and appears to determine the survival or death of cells following an apoptotic stimulus. Moreover, some shifts in the isoforms expression have been reported for proteins such as ANT in human hormone-dependent cancers.

Therapeutic Implications

The targeting of MMP might be instrumental for the treatment of cancer in the future. Several strategies, inspired by basic research advances, have been designed to neutralize anti-apoptotic targets and to re-activate the death program. These strategies are based on the use of pharmacological agents and/or on selective genetic modulation. Thus, small molecules, peptides, ►antisense, ►siRNA and adeno-associated virus-mediated gene transfer constitute the majority of current preclinical studies and clinical trials in order to fight tumorigenesis. However, the major difficulty in defining clinically efficient molecules arises from the fact that many apoptosis-related proteins harbor vital necessary functions, meaning that their extinction can be deleterious for correct cell function, in particular for immune cell functions.

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Mitochondrium

Definition

Plural mitochondria; Is a double membrane-surrounded organelle, in which cellular respiration takes place. The enzymes of the mitochondrial respiratory chain are located in the inner mitochondrial membrane and synthesize most of the cell's energy equivalents (ATP). Each mitochondrium contains a 16569 Bp circular DNA molecule coding for ribosomal and mitochondrial transfer RNA molecules and for several mitochondrial proteins. The inner membrane is folded to cristae comprising the mitochondrial matrix space. Depending on the cell type, mitochondria vary in shape, size, and number but have generally longish/tubular structural characteristics.

►Photodynamic Therapy

Mitogen

Definition

An agent, usually a protein, that triggers the cell to reenter the cell cycle triggering mitosis and can cause cellular transformation.

►Protease Activated Recept Family

Mitogen-Activated Protein Kinase

Definition

MAP Kinase; MAPK; Extracellular-signal regulated protein kinases, N terminal kinases/stress-activated protein kinases, and p38 kinases. The activation of these kinases may occur by translocation to the nucleus, where these kinases phosphorylate target transcription factors.

Mitogen-Inducible Gene 6 in Cancer

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Synonyms

MIG-6

Definition

Mitogen-inducible gene-6 (*MIG-6*) – also known as gene-33,receptor-associated late transducer (*RALT*), or ErbB receptor feedback inhibitor 1 (*Errfi1*) – is an immediate early response gene whose expression can be rapidly induced by growth factors, serum, and many stress stimuli. The *MIG-6* gene locus is mapped to

human chromosome 1p36.12–33 and in the mouse to the distal region of chromosome 4. Human *MIG-6* encodes a 58-kDa protein consisting of 462 amino acids (461 amino acids for its mouse counterpart).

Characteristics

Mig-6 is expressed in many organs and tissues, with high-level expression in the liver and kidney and moderate levels in the lung and brain. The expression of Mig-6 is robustly regulated at the transcriptional level, and it peaks after serum induction at mid-G1 during cell cycle progression. Many growth factors such as ►EGF, ►FGF, and ►HGF/SF rapidly induce its expression through the activation of the RAS-MEK-►ERK pathway (►MAP kinase).

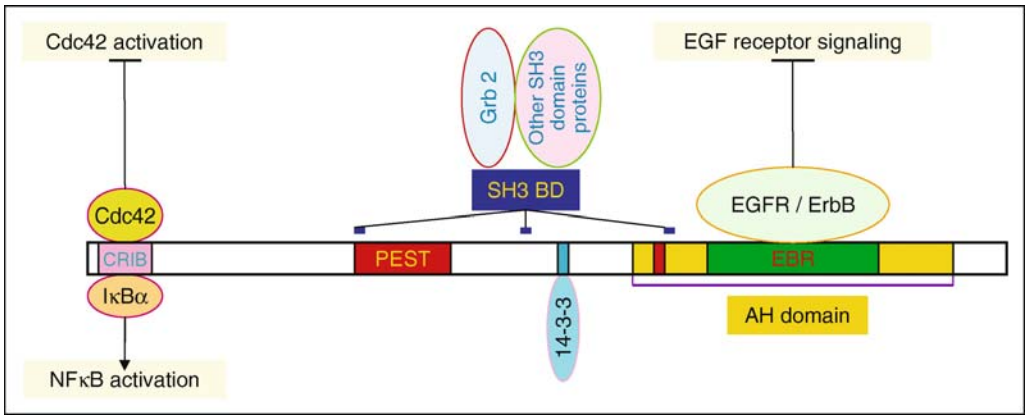
Mig-6 is a nonkinase scaffolding ►adaptor protein localized in the cytoplasm. It contains several well-known protein-protein interaction motifs/domains that allow it to interact with other signaling molecules in regulating ►signal transduction (Fig. 1). Mig-6 can interact with the small GTPase ►Cdc42 through its N-terminal Cdc42/Rac-interaction and binding (CRIB) domain. The Src-homology-3 (SH3) (►SH2/SH3 domains) binding motifs in Mig-6 mediate its interaction with ►Grb2, a bridging molecule between receptor tyrosine kinase (RTK) and the downstream RAS-MAPK pathway. It also contains a 14–3–3 binding motif for interacting with the ►14–3–3 protein. In addition, the AH domain in its C-terminal region shares a striking homology with the activated Cdc42-associated kinase-1 (Ack-1). Both Mig-6 and Ack-1 can physically interact with ►EGFR, and the region required for EGFR interaction is within the AH domain. In addition, Mig-6 contains two ►PEST sequences, and it is a labile protein subjected to ubiquitinylation and proteasome degradation.

In vitro functional analyses and characterization of *Mig-6* knockout mice reveal that Mig-6 may play a crucial role in regulating signal transduction, homeostasis, and stress response, and may act as a tumor suppressor in cancer development.

Signaling Regulation

Mig-6 integrates signal transduction by interacting with other signaling molecules to fine tune signaling output. Most notably, Mig-6 functions as a ►negative feedback regulator of EGF receptor signaling through a mechanism that involves direct interactions with members of the EGF receptor ErbB family (►epidermal growth factor receptor ligands). The up-regulation of Mig-6 by EGF results in down-modulation of the EGFR tyrosine phosphorylation and inhibits the EGF-induced activation of the downstream ERK, ►JNK, and Akt pathways. This inhibition might also involve direct interactions between Mig-6 and other factors like Grb2 or other SH3-domain-containing proteins. In addition, Mig-6 has also been shown to be capable of either activating or inactivating JNK (►JNK subfamily and cancer) in different cell types.

Mig-6 functions as a negative feedback regulator of HGF/SF-►Met signaling as well, although unlike with EGFR, no physical interaction between Mig-6 and Met has been observed. Mig-6 can inhibit HGF/SF-induced Cdc42 activation and cell ►migration in a CRIB-domain-dependent manner, likely through its ability to interact with active GTP-bound Cdc42. The importance of the CRIB domain in signaling regulation also stems from the fact that full-length Mig-6, but not its CRIB domain-deleted version, can sequester



Mitogen-Inducible Gene 6 in Cancer. Figure 1 The functional domains/motifs present in the Mig-6 protein. The CRIB domain is required for the binding of Cdc42 and IκBα. Mig-6 contains three proline-rich SH3-domain binding motifs (SH3 BD) that are capable of Grb2 or other SH3 domain protein binding. The 14–3–3 binding motif is present in the middle region. The ErbB binding region (EBR) resides in the Ack1 homology (AH) domain. Two PEST sequences (shown in red boxes) are also present.

the inhibitor of κBα (IκBα), thereby activating the nuclear factor κB (NFκB). It is possible that other domains/motifs (such as SH3-domain binding motif) of Mig-6 also play a role in regulating HGF/SF-Met pathway, as overexpression of Mig-6 can inhibit both HGF/SF-induced cell proliferation and cell migration.

The role of Mig-6 in regulating the signaling of other RTKs (such as FGFR or PDGFR) remains to be determined, even though its expression can also be induced by FGF or PDGF.

Clinical Relevance

The *MIG-6* gene resides at the human chromosome 1p36, a locus that is one of the most commonly deleted regions in many human cancers. Loss of heterozygosity (LOH) on 1p36 is frequently observed in human lung cancer, implying that putative tumor suppressor gene(s) in this locus might be responsible for lung carcinogenesis. This is supported by the fact that LOH on the distal region of mouse chromosome 4, the homologous region of human chromosome 1p36, is strongly associated with mouse lung carcinogenesis. The emergence of *MIG-6* as a tumor suppressor gene arises from data in both mouse and human cancers. In mice, targeted disruption of the *Mig-6* gene leads to the development of bronchi/bronchiole epithelial ►hyperplasia, adenoma, or adenocarcinoma in the lung, as well as the gallbladder, bile duct, gastrointestinal (GI) track, and skin cancers. Sequence analyses of the *MIG-6* gene in human non-small-cell lung cancer (NSCLC) cell lines and primary lung cancers have led to the identification of several mutations: a missense mutation at codon Asp109 to Asn in the NCI-H226 squamous cell carcinoma cell line, a nonsense mutation at codon Glu83 causing premature truncation in the NCI-H322 adenocarcinoma cell line, and a heterozygous germline mutation at codon Ala373 to Val in a primary squamous cell carcinoma patient. Mutation of *MIG-6* in the lung seems to be a rare event, as only one mutation was identified among 41 primary lung cancer samples screened. However, given that LOH on 1p36 is associated with smoking, squamous cell carcinoma, and late-stage lung cancer patients, there is a strong possibility that certain lung cancers may have a higher incidence of mutations in *MIG-6*.

LOH on 1p36 is also frequently observed in breast cancer. However, only a single nucleotide polymorphism (SNP) at codon Asp109 in *MIG-6* has been identified in breast carcinomas. Notably, this SNP is identical to the mutation identified in the NCI-H226 lung cancer cell line, and it has also been identified in several normal control populations. Although the importance of this SNP is not clear, it is possible that it might lead to predisposition to breast or other cancers. Other SNPs in the *MIG-6* gene have also been identified in lung cancer patients.

Even though genetic alteration in the *MIG-6* coding region seems to be a rare event, loss/reduction of MIG-6 expression has been observed in human breast, skin, pancreatic, and ovarian cancers. Breast cancer patients with decreased MIG-6 expression have poor prognosis. Moreover, MIG-6 expression in NCI-H226 human lung cancer cells, as well as in BT474 and SKBr-3 breast cancer cells, displays either no or low response to the activation of the MAPK pathway by either HGF/SF or EGF. The mechanism underlying MIG-6 deregulation is unclear, although loss/reduction of tumor suppressor gene expression is usually caused either by genetic changes such as mutation or deletion in its promoter regulatory region or by epigenetic modification associated with promoter hypermethylation (►epigenetic gene silencing).

MIG-6 is a negative feedback inhibitor of EGFR and the Met pathway, and the consequence of losing Mig-6 function can be a prolonged or inappropriate activation of these RTKs that may lead to a malignant phenotype. As revealed in *Mig-6*-deficient mice, losing Mig-6 function causes hyperproliferation of cells in several tissues including skin, gallbladder, and joints. Mig-6 deficiency causes prolonged activation of the EGFR-MAPK pathway in skin keratinocytes and results in skin hyperplasia. This hyperplasia can be reversed to normal appearance through the inhibition of the EGFR activity either using the EGFR inhibitor Gefitinib (Iressa) or by replacement of wild-type EGFR with a hypomorphic allele. The carcinogens 7,12-dimethylbenz[*a*]anthracene (DMBA) or 12-*O*-tetradecanoylphorbol-13 acetate (TPA) increase the incidence of skin melanomas and papilomas in *Mig-6*-deficient mice, which can be prevented by Gefitinib treatment. These data suggest that prolonged activation of the EGFR pathway is responsible for skin carcinogenesis in *Mig-6*-deficient mice. The loss of MIG-6 expression in ErbB2-amplified human breast carcinoma cells increases resistance of the cells to ►Herceptin, the antibody directed against the ErbB2 receptor. *In vitro*, overexpression of Mig-6 can inhibit EGFR- or ErbB2-mediated cell transformation, as well as cell cycle progression induced by EGF.

Further study will be required to determine if and how other functional domains/motifs in Mig-6 besides the EBR (Fig. 1) play a role in regulating the RTK signaling and in the tumorigenesis caused by Mig-6 deficiency.

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Mitogenic Carcinogen

Definition

A carcinogen that induces mitosis and cell proliferation.

►Toxicological Carcinogenesis

Mitomycin C

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Synonyms

MMC6-Amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methyl-azirino[2′, 3′ :3, 4] pyrrolo[1, 2-a]indole-4,7-dione carbamate (ester)

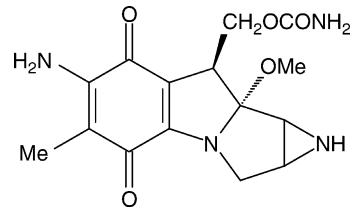
Definition

MMC is a naturally synthesized antibiotic ►alkylating agent used in ►cancer chemotherapy. Once metabolically activated, it functions as a cytotoxic drug by covalently binding DNA on both strands of the double helix, thus preventing them from separating when necessary for transcription prior to gene expression or replication prior to cell division. The binding of MMC to DNA is not amenable to repair and the affected cell subsequently dies.

Characteristics

Form

MMC was first isolated from *Streptomyces caespitosus* in 1958 and has subsequently been isolated from a number of other *Streptomyces sp*. Synthesis of MMC is coded for by a number of genes in a distinct locus of the microbial chromosome that also contains genes which confer resistance.



Mitomycin C. Figure 1 Chemical structure of mitomycin C.

The mitomycins are unique molecules being the only naturally synthesized compounds known to contain an ►aziridine ring. MMC (Fig. 1) has ►quinone, aziridine, carbamate and oxymethyl groups surrounding a mitosene (pyrrolo[1, 2-a]indole) nucleus. It is soluble in both aqueous and organic solvents. While MMC is readily soluble in water it is also unstable and is therefore shipped and stored as lyophilized powder. It is a light purple powder and immediately prior to use is dissolved in water.

Bioreductive Activation

MMC is relatively unreactive and therefore non-toxic when compared to metabolites formed following the two-electron reduction of the quinone group to the ►hydroquinone. It is considered as the prototype ►bioreductive drug, a term that is generally defined in two ways;

- That it is preferentially activated in ►hypoxic cells
- That it needs reductive activation via enzymatic catalysis

Reductive activation of MMC occurs following the two-electron reduction of the quinone to the hydroquinone, either as sequential one-electron or simultaneous two-electron reductions. Following reduction, a spontaneous rearrangement occurs resulting initially in the expulsion of oxymethyl group and opening of the aziridine ring to yield an electrophilic carbon capable of alkylating DNA. A subsequent expulsion of the carbamate group yields a 2nd reactive carbon and the MMC metabolite is capable of forming the highly toxic interstrand DNA crosslink.

In a hypoxic intracellular environment, both one-electron and obligate two-electron quinone reductase enzymes can catalyse the reduction of MMC to the hydroquinone. In contrast, in an oxygen rich environment, while the two-electron reductase activities still generate the hydroquinone which is a prerequisite for DNA alkylation, the semiquinone product of one-electron reduction is unstable in the presence of molecular oxygen and prone to oxidation back to MMC with superoxide free radical generation. Therefore four general situations can be envisaged dependent on the presence/absence of oxygen or of two electron-reductase activities. In a hypoxic environment alkylating MMC

metabolites will be generated in the presence of either or both one-electron and two-electron reducing enzymes. Alkylating MMC metabolites will also be generated in an aerobic environment in the presence of a two-electron reducing enzyme. In contrast in an aerobic intracellular environment, in the absence of two-electron reducing activity, DNA alkylation is less likely to occur and the less-damaging free radical generation is more likely.

The dependence of MMC cytotoxic activity on two-electron reduction suggested that MMC therapy could be specifically directed towards tumors that express relatively high levels of two-electron reducing enzymes or, in the absence of appropriate enzyme activities, hypoxic areas of tumors. The selective targeting of hypoxic tissues provides the rationale behind the use of MMC together with ►radiotherapy in the treatment of solid tumors. Radiotherapy preferentially targets tissues with a high oxygen content and combined with the use of MMC all regions of a tumor are attacked by one of the two treatments.

Enzymatic Bioreductive Activation of MMC

Many enzymes have been shown to reduce MMC, these include both one- and two-electron reductases, and the most widely studied are described below.

NQO1

►NAD(P)H:quinone oxidoreductase *1* (DTD, DT-diaphorase, NQO1, EC1.6.99.2) activity was first identified in 1958 on the basis that it catalyzed the oxidation of NADH and NADPH at the same rate. The human NQO1 gene has been sequenced and mapped to chromosome 16q22, comprising 6 exons and 5 introns the NQO1 gene is around 20 kb long, with the initial methionine codon at the 3′ end of exon 1 and the stop codon occurring ~300bp into the 6th codon. NQO1 is a 274 amino acid protein that exists as a homodimer and has 1 flavin adenine dinucleotide (FAD) prosthetic group per subunit non-covalently bonded to loop residues in the N-terminal domain. Both the NAD(P)H nicotinamide group and the substrate bind non covalently to the enzyme proximal to the flavin ring of the FAD. A C to T polymorphism at position 609 of the NQO1 gene results in a proline to serine substitution in the protein primary structure and loss of enzyme activity without a loss of cDNA expression. The 609C-T polymorphism results in negligible if any NQO1 activity in homozygous individuals and cell lines. Small amounts of NQO1 protein have been detected in homozygous mutant cell lines, but none in samples from mutant homozygote individuals as assayed by immunodetection methods. The protein half life of the mutant allele product is 1.2 h compared to 18 h for the wild type protein. ►Ubiquitin mediated proteosomal degradation has been implicated in the rapid breakdown of the mutant form. The 609TT genotype occurs at a frequency of between 5 and 30%, depending on the population being studied.

In contrast to the other reductase enzymes described, NQO1 activity has been observed to be higher in tumors matched to tissues that normally express low NQO1 levels (►breast cancer, ►colon cancer, ►liver cancer and ►lung cancer).

Despite some skepticism, MMC has been shown to be a substrate for NQO1 catalyzed reduction at physiological pH, although maximal reaction rates are seen at more acidic pH. It has been demonstrated that the decrease in NQO1 catalyzed reduction of MMC with increasing pH is due to the irreversible inhibition of NQO1 by MMC in a concentration- and time-dependent manner. Preincubation of purified NQO1 with MMC at pH 7.8 for 1 h results in total loss of NQO1 activity as assayed by the reduction of 2,6-dichlorophenol-indophenol. Cross-linking of NQO1 protein by MMC has been observed, and NQO1 activity is not restored following gel filtration to separate MMC and NQO1. In contrast, NQO1 activity is not lost following incubation with MMC at pH 5.8. Nevertheless, some NQO1 activity at physiological pH occurs and DNA:MMC adducts and crosslinks following NQO1 reduction have been observed. Controversy still exists concerning the putative role of NQO1 in the bioreductive activation of MMC in the clinical setting. However a large volume of research has been undertaken, resulting in a clarification of the role of NQO1.

NQO1 and Mitomycin C In vitro

Several studies have demonstrated NQO1 activation of MMC to a cytotoxic species in aerobic environments *in vitro*. These include heterogenic and isogenic cell line models comparing MMC toxicity in high NQO1 expressing cell lines with cell lines with no NQO1 activity, correlation of MMC toxicity with NQO1 activity over a cell line panel, comparing NQO1 activity in cell lines conditioned to be MMC resistant with parental cell lines, and increases in MMC sensitivity following induction or inhibition of NQO1 activity.

NQO1 and Mitomycin C in Anaerobic Conditions

The picture is somewhat different when sensitivity to MMC under hypoxic conditions is investigated. For example, the NQO1 inhibitor dicumarol potentiates MMC-induced cytotoxicity in hypoxic conditions. An indication that reductases other than NQO1 are more effective at reducing MMC to a cytotoxic species under hypoxic conditions came from studying a CHO cell line resistant to MMC under aerobic conditions. While it expressed significantly less NQO1 than the parental cell line, it was no more resistant to MMC under hypoxic conditions. While NQO1 may be important for

the efficacy of MMC under aerobic conditions, it is not a requirement for hypoxic toxicity.

However, evidence that NQO1 activates MMC under hypoxic conditions was provided by CHO cells transformed to overexpress NQO1. MMC was equally toxic to the transformed cells under hypoxic and aerobic conditions. In both conditions MMC toxicity was potentiated when compared to the parental cell line. However other reports have suggested that high NQO1 activity under hypoxic conditions will decrease MMC-induced cytotoxicity by out competing one electron reductase enzymes, believed to be more efficient at reducing MMC to cytotoxic species.

NQO1 and Mitomycin C In vivo

The role of NQO1 ►bioreductive activation of MMC *in vivo* is less clear than that seen *in vitro*. Four human tumors expressing a range of NQO1 activities implanted in nude mice showed an inverse correlation between tumor growth and NQO1 activity following treatment with MMC. NQO1-transformed BE cells respond no differently to MMC compared to the parental cell line when implanted as xenografts in nude mice. In contrast, xenografts of NSCLC cell lines with high NQO1 activity were more sensitive to MMC than ►SCLC cell lines with low NQO1 activity.

Clinical evidence that NQO1 activity potentiates MMC sensitivity *in vivo* was seen in a recent clinical study. The NQO1 genotype of patients with disseminated peritoneal cancer was assessed prior to surgical removal of cancer tissue followed by intraperitoneal administration of mitomycin C. Patients with the wild type NQO1 genotype whose disease was of colonic origin survived significantly longer than those who were heterozygotes or homozygous for the 609C-T mutation (18.2 vs. 11.5 months median survival). Significantly longer survival times were also seen in patients with the wild type genotype following complete removal of all visible tumor when compared to those who were heterozygotes or homozygous for the 609C-T mutation (43.6 vs. 23.0 months median survival). Other clinical studies have failed to identify a relationship between NQO1 expression or genotype and response to MMC.

Xanthine Oxidase/Dehydrogenase

Xanthine dehydrogenase (XDH) and ►xanthine oxidase (XO) both catalyse the conversion of xanthine to uric acid. Both enzymes can use NAD+ as an electron acceptor, but XO can also use O_2. Both enzymes are the product of the same gene with XO being a proteolytically cleaved or oxidized modification of XDH.

The reductive activation of MMC by purified XO has been described and MMC metabolites formed following this activation identified under anaerobic, but not aerobic conditions. Under aerobic conditions the generation of reactive oxygen species was identified. In contrast, XDH is capable of directly reducing MMC to its hydroquinone and, as such, can form alkylating metabolites under aerobic conditions. Neither XO or XDH are present at elevated levels in tumor cells compared to healthy tissue and it is not envisaged that either enzyme confer specificity for MMC against tumors.

NADPH-Cytochrome C P450 Reductase

As with XO, ►cytochrome P450 reductase (cytochrome C reductase) reductively activates MMC to the semiquinone in anaerobic conditions, and generates free radicals under aerobic conditions. ►CHO cells transfected with human P450 reductase show slightly greater sensitivity to MMC under oxic and much greater sensitivity under hypoxic conditions than the parental cell line. MMC sensitivity does not correlate with P450 reductase activity in the NCI 60 cell line panel under aerobic conditions. Cytochrome p450 reductase activity may contribute to the toxicity of MMC in hypoxic regions of tumors.

Other Reductases

Additional reductase enzymes have been shown to reduce MMC and include NADH:Cytochrome b5 reductase, neuronal nitric oxide synthase, and NADPH-ferredoxin reductase. All these enzymes appear to reduce MMC to the semiquinone and as such are believed to be more effective under hypoxic conditions. Two-electron activities implicated as activators of MMC include ►NRH:Quinone oxidoreductase 2 and GRP58.

Analogues of MMC

Attempts have been made to enhance the anti tumor activity of MMC by the synthesis of compounds that do not require enzymatic reduction or are improved substrates for NQO1. Compounds investigated that did not require enzymatic activation showed pulmonary toxicity in phase 1 clinical trials. One compound synthesized as an improve NQO1 substrate, EO9, was more toxic to high NQO1 expressing cells than low NQO1 expressing cells in preclinical models. However phase II trials revealed that IV EO9 was ineffective as an antitumor agent with rapid elimination from the plasma and dose-limiting renal toxicities. Intravesical administration of EO9 may be effective against superficial ►bladder cancer following ►transurethral resection.

Pharmacokinetics

Following dissolution, MMC is administered either systemically by intravenous injection, or locally by ►intravesicular instillation in the bladder or intraperitonealy in the peritoneal cavity.

Following systemic administration MMC is eliminated with a half-life of approximately 1 h and only between 1 and 20% of the unchanged drug is recovered

in the urine. The remainder is subject to both activating and deactivating metabolism, carried out predominantly in the liver but also in the spleen, kidney, heart and brain as well as tumor tissue.

Clinical Use of MMC

MMC is active against numerous solid tumors including those off the breast, ►non-small cell lung carcinoma, head & neck and anus. However, concerns about MMC toxicity mean that use of the drug is largely restricted to treatment of low grade superficial bladder cancer. Treatment of superficial bladder cancer is by the intravesical administration of 40 mg/ml in 100ml saline following transurethral resection of the tumor.

Side Effects of MMC

Systemic administration is associated with a severe dose-limiting hematological toxicity. This effect is cumulative resulting in treatment regimens of MMC involving iv administration every 6–8 weeks. MMC can also cause severe ►extravasation reactions at the site of intravenous administration. Other reported toxicities associated with MMC include nausea, vomiting and ►anorexia as well as pulmonary and cardiac toxicity.

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Mitosis

Definition

Is a process of cell division, which results in the production of two identical daughter cells from a single parent cell.

Mitosis Promoting Factor

Definition

MPF; A cyclin-dependent kinase complex composed of cyclin B1 and CDK1 that is required for progression of G2 cells into ►mitosis. The activity and intracellular location of mitosis-promoting factor are regulated by kinases and phosphatases that respectively phosphorylate and dephosphorylate the subunits.

►Decatenation G2 Checkpoint

Mitotane

Definition

[1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane, or *o*,*p*′DDD], an insecticide derivative that produces adrenocortical necrosis, has been used extensively in adults with adrenocortinal tumors (ACT), but its cytotoxic effect on adrenocortical cells produces focal degeneration of the zona fasciculata and in particular the zona reticularis; the zona glomerulosa is relatively unaffected. Moreover, mitotane impairs adrenal steroidogenesis and has a direct inhibitory effect on steroidogenic enzymes.

►Childhood Adrenocortical Carcinoma

Mitotic Accumulation & Inhibition of Microtubule Polymerization

Definition

►Mitosis is a cell division process by which the mother cell generates two identical daughter cells. Microtubules are one of the components of the cytoskeleton, which serve as structural components and are involved in mitosis. They are polymers of α- and β-►tubulin dimers. The dynamic polymerization and depolymerization of the tubulin dimers are critical for mitotic process. There are chemical or biological agents that interfere with this dynamic process and result in the accumulation of cells in mitosis.

►Methoxyestradiol

Mitotic Arrest-Deficient Protein 1

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Synonyms

TXBP181; Tax binding protein-181; MAD1L1; homo sapiens mitotic arrest deficient-like 1 protein; MAD1

Definition

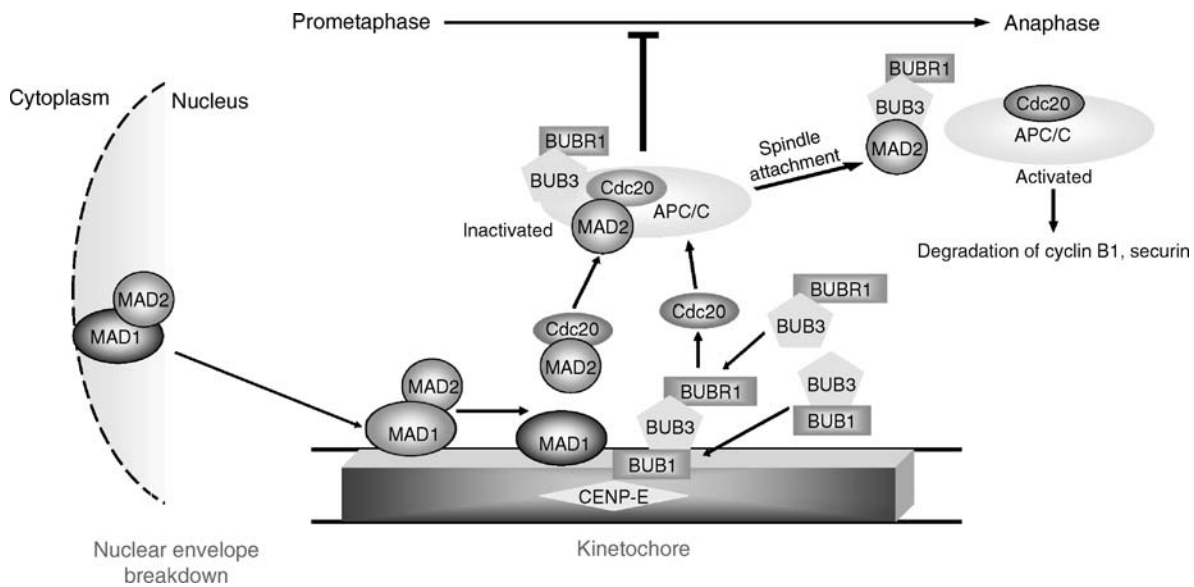
Human *mad1* gene is located on chromosome 7p22. MAD1 is a protein constituent of the mitotic ►spindle assembly checkpoint (SAC). Human MAD1 was first identified as a cellular factor targeted by ►Tax, an oncoprotein encoded by the ►human T-cell leukemia virus type 1 (HTLV-1). Cellular insufficiency of MAD1 induces gain or loss of whole chromosomes (i.e. ►aneuploidy) during mitosis. In mice, haploinsufficiency of MAD1 provokes increased tumor incidence.

Characteristics

In mitosis, duplicated chromosomes are intended to be distributed equally to two daughter nuclei. Loss of fidelity in this process can cause aneuploidy. Eukaryotic cells have evolved a checkpoint (SAC) to monitor fidelity of chromosome segregation. The SAC is a complex composed of many proteins, several located at kinetochore, which include the mitotic-arrest deficient (MAD) proteins (MAD1, ►MAD2 and MAD3), the budding uninhibited by benzimidazole (BUB) proteins (►BUB1, BUB2 and BUB3), the monopolar spindle 1 protein (►MPS1), the ►ROD-ZW10-Zwilch complex, and the microtubule motor centromere protein E (►CENP-E). Aberrant expression of SAC proteins is seen in many ►cancers, suggesting a link between SAC function and neoplasia. MAD1 is a cellular target for the HTLV-1 Tax oncoprotein. Overexpression of either Tax or a dominant-negative MAD1 results in multinucleated cells, a phenotype that is also found in some cancer cells. For ATL development, an attractive model suggests that Tax targets MAD1 abrogating SAC, creating aneuploidy which provides an initial impetus for cellular transformation (Fig. 1).

Mechanisms

In interphase cells, MAD1 binds MAD2 as a heterodimer at the nuclear pore complex (NPC). As the cell enters prophase and the NPC dissolves, MAD1 is thought to ferry MAD2 to kinetochores, and MAD1 + MAD2 compose a part of the SAC. During chromosomal partitioning, each face of replicated kinetochores is tethered by microtubules to opposing spindle poles, placing the duplicated chromatids under equal bilateral tension. If microtubule attachment is missing or improper, mis-segregation of chromatids could result. The current view suggests that a mitotic kinetochore which is not bilaterally attached by microtubules triggers MAD2 to dissociate from MAD1 to shuttle onto ►CDC20-anaphase promoting complex/ cyclosome (►APC/C). MAD2-CDC20 binding halts APC/C's proteolytic activity, prohibiting degradation of cyclin



Mitotic Arrest-Deficient Protein 1. Figure 1 A schematic representation of the role of MAD1 in spindle assembly checkpoint during the prometaphase to anaphase period of mitosis.

B1, ►securin and other factors, thus arresting the cell's progression from metaphase to anaphase. This arrest is alleviated by the proper bipolar (re-)attachment of microtubules to the kinetochore. In this paradigm, MAD1 is needed for the kinetochore localization of MAD2 and for converting kinetochore-bound MAD2 to a CDC20-receptive form. Thus, a MAD1-mediated SAC-enforced mitotic arrest offers an opportunity for aberrancies to be corrected before progression is allowed.

Although human and rodent MAD1 share 81–84% identify, they differ in the stringency of their respective SAC. Rodent cells are less responsive to spindle toxins induced mitotic arrest. It has been found that ectopic expression of human MAD1 in mouse and hamster cells corrected a relaxed rodent SAC to a more stringent human phenotype. This finding suggests that MAD1 is a species-specific determinant which influences the stringency of cellular response to microtubule depolymerization and spindle damage.

Clinical Aspects

Although the direct link between SAC and carcinogenesis in human remains to be established, up to 40% of human lung cancer cells have been found to carry mitotic checkpoint defects, including MAD1. In a study that examined for MAD1 alterations by RT-PCR-SSCP and nucleotide sequencing from a total of 44 cell lines (►hematopoietic, ►prostate, ►osteosarcoma, ►breast, ►glioblastoma and ►lung) and 133 fresh cancer cells (hematopoietic, prostate, breast and glioblastoma), eight missense, nonsense and frameshift mutations were found, along with a number of nucleotide polymorphisms. All the alterations found in these cell lines were heterozygous. Frequency of mutations was relatively high in ►prostate cancer (2/7 cell lines and 2/33 tumor specimens).

In mice, homozygous deletion of MAD1 causes embryonic lethality, suggesting an essential role for MAD1 in mouse development. Heterozygous knock out of MAD1 in mice weakens the SAC, and over an 18 months course, created a twofold constitutive increase in tumor incidence in $Mad1^{+/-}$ mice compared to wild type siblings. Tumors in $Mad1^{+/-}$ mice developed in a wide range of tissues, including ►lung adenocarcinoma, ►hepatocellular carcinoma, ►rhabdomyosarcoma, osteosarcoma, hemangiosarcoma, and uterine sarcoma. Moreover, $Mad1^{+/-}$ mice treated with spindle toxin, vincristine, showed a heightened propensity for developing ►lung tumors (42%), similar to the tumor tissue type observed in $Mad2^{+/-}$ mice (25%). It is intriguing that mouse lung tissue seems to be relatively more sensitive to deregulation in the MAD components of the SAC. Future studies are needed to establish correlations between lung tissue development and MAD-SAC functions.

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Mitotic Catastrophe

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Synonyms

Mitotic cell death

Definition

Mitotic catastrophe is a process of cell death induced by radiation, chemotherapeutic drugs or hyperthermia. Mitotic catastrophe results from aberrant mitosis, which is followed either by cell death through apoptosis or necrosis or by partial or complete fragmentation of interphase nuclei with eventual cell death or seneseence. Mitotic catastrophe is identifiable from ►apoptosis by morphologic criteria.

Characteristics

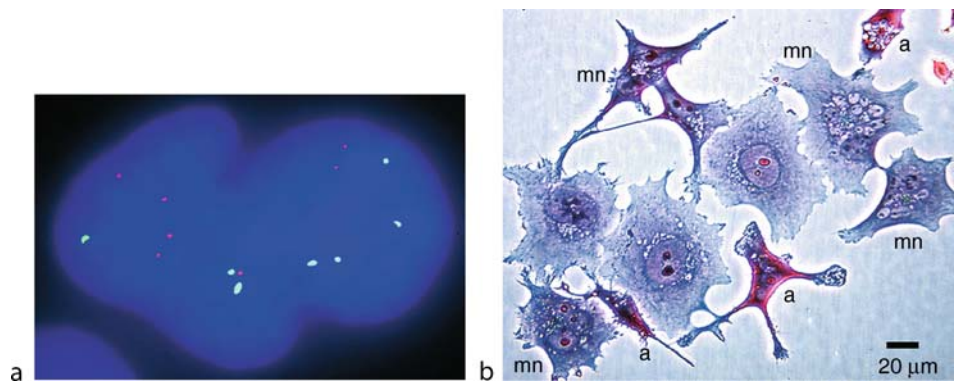
Interphase cells that suffer lethal damage from radiation or cytotoxic drugs may die before reaching mitosis. This rapid cell death usually occurs through the process of apoptosis. Alternatively, damaged cells may proceed into aberrant mitosis, which does not produce proper chromosome segregation and cell division, but results either in cell death or in the formation of large nonviable cells with two or more micronuclei that are completely or partially separated from each other. In the case of partial separation, the cell displays a large

lobulated nucleus. The micronuclei arise through the formation of multiple nuclear envelopes around chromosome clusters at the end of abnormal mitosis. Chromosomes are randomly distributed among the micronuclei of a multilobulated nucleus of a drug-treated tumor cell (Fig. 1a). The described process is called mitotic cell death or mitotic catastrophe.

Micronucleated (mn) cells, indicative of mitotic catastrophe, can be easily distinguished by their morphology from apoptotic (a) cells (Fig. 1b). While apoptotic cells may have fragmented nuclei, they are characterized by shrunken cytoplasm and condensed chromatin, whereas micronucleated cells are large and contain uncondensed chromosomes micronucleated. Cells that undergo mitotic catastrophe do not usually show DNA ladder formation or DNA breaks that are detectable by ►TUNEL staining in apoptotic cells. Mitotic catastrophe and premitotic apoptosis often arise in the same population of cells treated with a cytotoxic agent. Furthermore, the onset of mitotic catastrophe may be followed in the same cells by the activation of the apoptotic program. For this reason, mitotic catastrophe has been sometimes described as an early stage of apoptosis. However, the importance of mitotic catastrophe as an independent determinant of radiation- or drug-induced cell death has been demonstrated by the suppression of apoptosis by ectopic overexpression of apoptosis-inhibiting proteins, such as Bcl-2 or ►P-glycoprotein (the latter inhibits apoptosis through a presently unknown mechanism that is distinct from its function as a multidrug transporter). In the presence of these inhibitors, the number of apoptotic cells induced by drugs or radiation is greatly diminished, but the number of cells undergoing mitotic catastrophe (as well as terminal growth arrest in the form of ►accelerated senescence) is correspondingly increased. As a result, inhibition of apoptosis was found in several studies to have little or no effect on the survival and regrowth of treated cells. In other words, cells that have initiated the process of mitotic catastrophe may be dying through apoptosis but not because of it. This functional distinction underscores the importance of mitotic catastrophe in cellular response to anticancer agents.

Cellular and Molecular Aspects

Some insights into the mechanism of mitotic catastrophe come from the studies where this response was induced in mammalian cells or in fission yeast by genetic manipulations. In particular, mitotic catastrophe was observed in cells that were forced into mitosis through untimely activation of the mitosis-initiating complex, comprised of Cyclin B and Cdc2 kinase. This complex is regulated by a number of proteins that control the G2/M checkpoint (►G2/M transition) of the cell-cycle. Ectopic coexpression of cyclin B1 and Cdc2, cyclin A and Cdc2, and cyclin B1 and Cdc25C (an enzyme that dephosphorylates and activates Cdc2) was shown to induce premature mitosis and subsequent mitotic catastrophe in mammalian cells. The extent of mitotic catastrophe has been further increased through the use of a Cdc2 mutant, which is resistant to inhibitory phosphorylation. Mitotic catastrophe was also shown to result from mutations or inactivation of other genes that act in mitosis, such as RCC1 involved in chromosome condensation, the nuclear-mitotic apparatus (NuMA) protein and the centrosome-associated protein ►survivin. Survivin is best known as an inhibitor of apoptosis, and the loss or displacement of survivin during abnormal mitosis may potentially account for the link between mitotic catastrophe and apoptosis.



Mitotic Catastrophe. Figure 1 Morphology and chromosome distribution in micronucleated cells arising after mitotic catastrophe. (a) Random distribution of chromosomes among the micronuclei of a partially fragmented nucleus of human HT1080 fibrosarcoma cells treated with 20 nMol doxorubicin for 3 days. Fluorescence in situ hybridization was carried out with probes specific for chromosomes 18 (*green*) and 21 (*red*); nuclei were stained with DAPI [reproduced with permission from Chang et al (1999) Cancer Res 59:3761–3767]. (b) Micronucleated (mn) and apoptotic (a) cells in a population of HCT116 colon carcinoma cells treated with 50 nMol doxorubicin for 4 days [reproduced with permission from Chang et al (1999) Oncogene 18:4808–4818].

Induction of mitotic catastrophe by heat or ionizing radiation has been associated with increased cellular levels of cyclin B1 and Cdc2 kinase activity, suggesting that cellular damage may induce premature mitosis. The entry of damaged cells into mitosis is delayed by growth arrest at the G2/M checkpoint. Agents that abrogate this checkpoint, such as caffeine, okadaic acid or staurosporine, were shown to promote mitotic death in irradiated or drug-treated cells. Mitotic catastrophe is also enhanced by genetic inactivation of G2/M checkpoint control genes, such as the cyclin-dependent kinase inhibitor ►p21 (Waf1/Cip1/Sdi1) or 14–3–3-σ, a protein that maintains cytoplasmic sequestration of cyclin B1 and Cdc2. Damage-induced p21 induction is mediated largely (but not exclusively) by p53 protein, and p53 was found in several studies to be a negative regulator of mitotic catastrophe (in contrast to the usually positive effect of p53 on apoptosis). While the key role of the G2/M checkpoint in preventing mitotic catastrophe is well documented, the events resulting in the aberrant mitosis of damaged cells remain to be elucidated.

Clinical Relevance

Mitotic catastrophe has been characterized as the main pathway of cell death induced by ionizing radiation and is recognized as a prominent response to different anticancer drugs, such as doxorubicin, etoposide, taxol, cisplatin or bleomycin. This response has been documented in many cell lines derived from different tumor types, including apoptosis-resistant lines. As a general trend, mitotic catastrophe is more frequently induced by lower doses and apoptosis by higher doses of cytotoxins. Mitotic abnormalities and micronucleation have been observed both in vitro and in vivo; in fact, micronuclei formation has long been used as a common indicator of genotoxic damage.

The importance of mitotic catastrophe as a major response of tumor cells to chemotherapy and radiation has been obscured over the past decade by the emphasis on apoptotic cell death and the view that mitotic catastrophe is merely an early stage of apoptosis. However, the interest in this topic has been recently revived by the demonstration of the importance of the G2/M checkpoint as a determinant of cytotoxicity of anticancer agents and by the demonstration that the inhibition of apoptosis increases the fraction of cells that die by mitotic catastrophe. Mitotic catastrophe is potentiated by p53 deficiency and a weakened G2/M checkpoint. These are distinguishing characteristics of many tumors, suggesting that tumor cells may be more susceptible to mitotic catastrophe than normal cells. The elucidation of the molecular determinants of mitotic catastrophe may help to exploit this preferential mode of tumor cell death in a therapeutic setting.

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Mitotic Cell Death

►Mitotic Catastrophe

Mitotic Checkpoint

Definition

►Spindle Assembly Checkpoint

Mitotic Spindle

Definition

Is a structure of the eukaryotic cytoskeleton involved in ►mitosis and meiosis and helps to segregate chromosomes during cell division to the daughter cells. Consists of a bundle of microtubules joined at the ends but spread out in the middle, vaguely ellipsoid in shape during mitosis and meiosis.

►Doublecortin
►Microtubule–Associated Proteins

Mixed Gliomas

►Oligoastrocytomas

Mixed Lineage Leukemia Protein

Definition

MLL protein; is a nuclear protein that binds DNA through its three AT-hooks. It is required for the regulation of HOX gene expression during development and hematopoiesis. The MLL gene is located at human chromosome region 11q23 and is a common target for chromosomal translocations in human acute lymphoid and myeloid leukemias. Interacts with the promoters of HOXA7-10 to promote transcription. Chromosome translocations involving the MLL gene are found in 4% of adult acute myeloid leukemia and involve up to 40 different fusion partner genes.

►Lipoma Preferred Partner
►NUP98-HOXA9 Fusion

Miz-1

Definition

Myc-interacting zinc finger proten-1; is a transcription factor that promotes transcription when bound to initiator (Inr) elements in the DNA. However, when Myc binds to Miz-1, the Miz-1 activity is blocked, leading to transcriptional repression.

►*Myc* Oncogene

MJ

Definition

Methyl jasmonate, a plant stress hormone exhibiting anticancer activities.

►Jasmonates in Cancer Therapy

MK-1

►EpCAM

MK-0683, SKI390

►Vorinostat

MLH1/3

Definition

Human mut L homologue 1/3. These genes encode proteins that are components of the human ►mismatch repair (MMR) system. Defect MMR may cause microsatellite instability in tumors.

►Microsatellite Instability

MLL-ELL Leukemia

Definition

►Acute Myeloid Leukemia associated with the translocation t(11;19)(q23;p13.1). This translocation in the fusion of MLL to the ELL gene. The MLL gene encodes a large nuclear protein that is required for the maintenance of HOX gene expression while ELL was shown to encode an elongation factor that can increase the catalytic rate of RNA polymerase II.

►Cajal Bodies

MLPLI

►Leukemia Inhibitory Factor

MLS/RCLS

►Myxoid Liposarcoma

Mlx

Definition

Is a basic region-helix-loop-helix-leucine zipper protein that has been reported to form heterodimers with the transcriptional repressors Mxd1 and Mxd4 of the Myc/Max/Mad(Mxd) network.

►*Myc* Oncogene

MM

Definition

Multiple myeloma.

►Plasmacytomas

MMAC1

Definition

Mutated in Multiple Advanced Cancers; Alternative, less frequently used name for ►PTEN.

►Cowden Syndrome
►PTEN

MMP

Definition

►Matrix metalloproteinase.

►Mitochondrial Membrane Permeabilization in Apoptosis

MMP-3

►Stromelysin-1

MMPs

►Matrix Metalloproteinases

MNA

Definition

Mini nutritional assessment.

►Nutrition Status

Mnt

Definition

Max binding protein; Is a ubiquitously expressed transcription factor of the Myc/Max/Mad(Mxd) network of basic region-helix-loop-helix-leucine zipper proteins. Through its Sin3-interacting domain (SID), Mnt recruits a repressor complex containing histone deacetylases and represses gene transcription. In contrast to Myc and Mad/Mxd proteins, Mnt is expressed throughout the cell cycle and is considered to be master regulator of the proto-oncoprotein Myc.

►*Myc* Oncogene

Model Selection

Definition

Subprocess of supervised classification in which a predictive model is derived from a set of real-world observations (samples).

►Supervised Classification

Model Testing

Definition

Subprocess of supervised classification in which the value of a predictive model is determined by its ability to correctly predict the class of samples that have not been used for the construction of this model.

►Supervised Classification

Modifier Loci

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Synonyms

Genetic polymorphisms; Genetic predisposition; Quantitative trait loci; Susceptibility loci

Definition

Cancer modifier loci are genetic loci characterized by allele-specific effects on cancer development, e.g., stimulation or inhibition of tumor development and/or tumor size/progression. Cancer modifier loci may comprise a "null" allele, with no effects on tumor phenotypes, and a "susceptibility" or "resistance" allele, which affect tumor phenotype in a dominant, codominant, or recessive way.

Characteristics

Evidence for the existence of cancer modifier loci came first from studies in rodent models. Indeed, several studies showed that susceptibility to spontaneous and induced tumorigenesis are under genetic control. Crosses between cancer-susceptible and -resistant strains may result in F1 hybrids either susceptible or resistant to tumor formation, depending on which of the two alleles (susceptibility/resistance) is dominant in the cross.

In humans, evidence for the existence of cancer modifier loci derives from epidemiological studies reporting segregation of disease severity in families with cancer that cannot be explained by the type of germ-line mutations. Moreover, several epidemiological studies report an increased risk of the same type of cancer in first-degree relatives of cancer patients, i.e., a family risk ratio >1.0, consistent with a ►polygenic model of inherited predisposition to cancer. Such a model predicts that genetic susceptibility to tumorigenesis may be conferred by multiple genes with small effects and a powerful approach to identify the genetic loci involved may lie in linkage/association analyses of the entire genome in large samples.

Rodent Models

In mice, genome-wide analyses require only a few hundred genetic markers due to the genetic homogeneity of inbred strains, the limited number of genetic recombinations in crosses, and the consequent tight linkage of genetic markers over wide chromosomal regions.

The availability of a large number of genetic markers easy to genotype (►microsatellites) allowed the first genome-wide linkage studies in mouse models and lead to the mapping of hepatocellular cancer susceptibility loci (Hcs), the pulmonary adenoma susceptibility 1 locus (Pas1), the Mom1 locus as a modifier of the germ-line Apc gene mutation (►APC gene in familial adenomatous polyposis), which induces intestinal tumorigenesis, and loci affecting plasmacytomagenesis (►plasmacytomas). The hepatocellular cancer model provided the first formal demonstration of the polygenic nature of inherited cancer predisposition in mice. Since then, cancer modifier loci affecting almost all types of tumors have been mapped in the mouse. Loci affecting different types of rat carcinogenesis have also been mapped; among them, loci affecting susceptibility to hepatocarcinogenesis have been chracterized in more details and showed to control multiplicity and size of neoplastic lesions. Therefore, in liver, as well as in other models, cancer modifier loci may affect either single or multiple stages of carcinogenesis.

Humans

In humans, some authors proposed a polygenic model of inherited predisposition to cancer, whereas others propose models based on rare dominant alleles or additive gene effects, or on common alleles of low penetrance. Although all these models agree in part with epidemiological evidence, no combination of genetic polymorphisms that convincingly defines an additive or polygenic inheritance of predisposition to sporadic cancer has yet been described in humans. Despite many reports indicating a higher or lower risk of cancer associated with metabolic polymorphisms, several meta-analyses showed that the relative risks associated with most of the metabolic polymorphisms are low. The exceptions include arylamine *N*-acetyltransferase 2 (NAT2) polymorphism [►arylamine *N*-acetyltransferases (NAT)] and bladder cancer risk, where mechanistic and epidemiological evidence is strong.

The discovery of millions of single-nucleotide polymorphisms (SNPs) in human and in the genome of other mammalian species now make feasible large scale

genome-wide association studies, also thanks to the recent introduction of ►SNP array systems that enable screening of thousands of SNPs. Because of the genetic heterogeneity of the human population, complete analysis of the human genome by association studies might require a huge number of genetic markers and large sample collections. However, the presence of haplotype blocks in the human genome suggests that even a less dense marker coverage might be sufficient for a whole-genome analysis. Although the characterization of these haplotype blocks awaits completion, population-based association studies are complicated by complex ►linkage disequilibrium (LD) patterns, possible population admixture in the selection of cases or controls, differences in environmental factors, and other factors.

However, as these studies test hundred thousands polymorphisms in cases and controls, the need for robust replication in one, or preferably, several independent studies is paramount to distinguish the true positive results from the large number of expected false positives. Genome-wide association studies and family-based linkage studies mapped several modifier loci affecting different types of tumors, including breast, colon, lung, or prostate cancer.

Mechanisms

Few studies to date have addressed the site of action of cancer modifier genes, i.e., whether cancer modifier genes act directly within the tumor cells or instead in a cell-autonomous way, to determine the host response to the tumor. This issue likely bears on the mechanism of action of cancer modifier genes and on the targeting of these gene products. Experiments using chimeric mice generated by aggregation of embryos derived from genetically susceptible and resistant strains indicate that at least for hepatocarcinogenesis, intestinal tumorigenesis, and plasmacytomagenesis, most of the tumors in these mice derive from the susceptible strain. Thus, the cancer modifier loci in these models appear to act in the target cells, with no apparent systemic effects.

In humans, most inherited major germ-line defects appear to confer predisposition to one major tumor type, with several others exhibiting a minor increased incidence in families. For example, carriers of the BRCA1 mutations (►BRCA1/BRCA2 germ-line mutations and breast cancer risk) are at high risk of breast and ovarian cancer, and may have an increased risk of colon, pancreas, and stomach cancers. BRCA2 mutation carriers show a high risk of breast cancer and an increased risk of pancreatic cancer. Compared with the general population, carriers of the mutations causing hereditary-nonpolyposis-colorectal-cancer (HNPCC) syndrome (►Lynch syndrome) show, in addition to colorectal cancer, an increased incidence of endometrial, ovarian, gastric, biliary tract, and kidney cancers. On the other hand, ►Li–Fraumeni syndrome is characterized by a wide spectrum of neoplasms in children and young adults. Thus, both specificity and pleiotropy, i.e., single-gene allelism that influences two or more types of tumorigenesis, in genetic susceptibility to cancer in humans have been described.

Perspectives

Genetic loci affecting risk of several types of tumors have been identified in mouse models but the relationships with the risk of corresponding human cancer have not yet been established. The major locus affecting inherited susceptibility to lung cancer in mouse (Pas1) seems either lacking its human counterpart or playing a much weaker effect on lung cancer phenotype, whereas a human homology has been found for the rat Mcs5a locus affecting breast cancer risk. The difficulties in the identification of human homologs of mouse cancer modifier genes is obvious considering that some mouse alleles may not be carried in humans and that the human population is much more genetically complex than the relatively few mouse inbred strains used so far for mapping cancer modifier loci.

Overall, it seems highly unlikely that the enormous genetic variability underlying susceptibility and resistance to tumorigenesis in mice evolved only in rodents and not in all mammals, including humans. In fact, quantitative trait loci affecting several noncancer phenotypes have been mapped in cattle, pigs, and in humans, and it seems reasonable to expect that cancer modifier QTLs are present in humans. Even in cases where the human homologous gene does not display allelism and cancer modifier effects, identification of cancer modifier genes in any given species would provide a step toward understanding the biochemical mechanisms of inherited resistance/susceptibility to cancer. Eventually, it may become possible to devise new chemoprevention and therapeutic strategies for cancer based on the biochemical effects of such genes.

Also, new diagnostic markers might be devised to identify groups of individuals at higher risk of sporadic cancer as compared with the general population. This is now possible for members of families with ►monogenic cancer syndromes, which represent a minority of cancer cases. Identification of individuals at high risk of cancer raises the possibility of chemoprevention. For example, FAP carriers (APC gene in familial adenomatous polyposis) benefit from long-term use of nonsteroidal ►anti-inflammatory drugs.

In conclusion, the identification of cancer modifier genes may allow understanding of the genetic mechanisms responsible for the variability in the individual risk to develop a cancer and it may also provide new opportunities for diagnosis and cure.

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Modular Recombinant Transporters

►Modular Transporters

Modular Transporters

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Synonyms

Modular recombinant transporters; Multi-domain transporters

Definition

Modular transporters are engineered ►polypeptides consisting of several interchangeable parts, or ►modules designed for delivery of anti-cancer drugs to the target cancer cell and its specific subcellular compartment.

Modular transporters can also be considered as nanomedical drug vehicles (►nanotechnology), which recognize the cancer cells of choice, and once in those cells, are transported to the most sensitive compartment of the cell (e.g. nucleus).

In order to reach the desired compartment of the cancer cell, the modular transporters are first passively delivered to the surface of the cell in the blood stream. Once within the cell, depending upon the nature of the ►polypeptide modules, they are transported to a particular ►subcellular compartment utilizing the cell's intrinsic transport machinery.

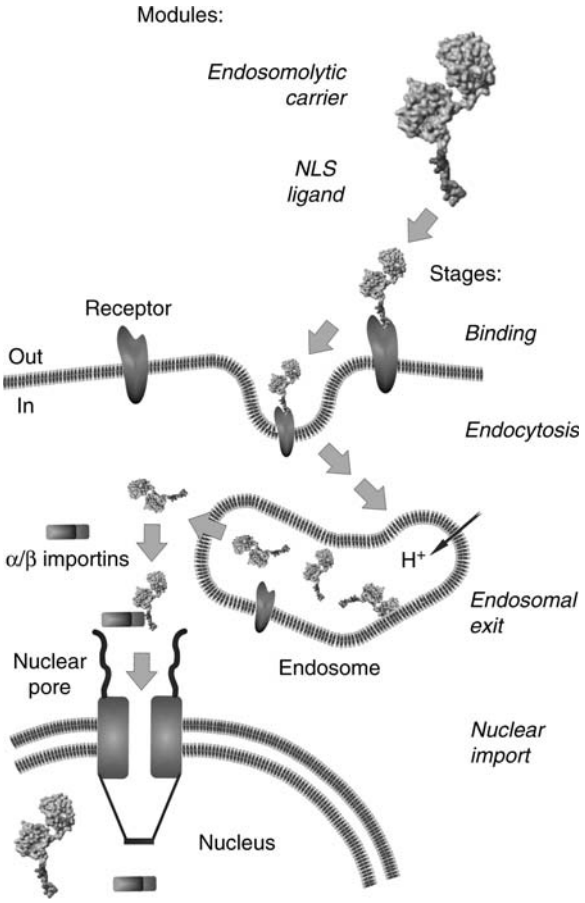
Characteristics

Objectives

To minimize side effects, many anti-tumor agents need to be delivered (►drug delivery) not only to the target cancer cell but also into a specific subcellular compartment, usually into the most sensitive/vulnerable site of the cancer cell. Examples of such anti-tumor agents are: (i) foreign DNA for cancer gene therapy, (ii) ►photosensitizers for ►photodynamic therapy or (iii) ►radionuclides emitting ►alpha-particles for endoradiotherapy (►radioimmunotherapy). All of the above should be delivered into the nuclei where they can perform their specific function. On the other hand, (iv) toxins, most of which are active in the cytosol, require a different modular transport strategy to retain in the cytoplasm.

Principles

This goal can be achieved with the use of modular transporters with preset properties, which would ensure recognition of the desired target cell and subsequent directed transport to the subcellular compartment of choice. The necessity of different modules is determined by the following considerations. First, cell type specificity together with internalization into the target cell can be achieved if the engineered transporter possesses a ►ligand module, which has high binding affinity to the ►receptor overexpressed on the target cancer cell but not on non-cancer cells. This highly specific ►ligand-receptor binding will ensure recognition of the target cell as well as a subsequent receptor-mediated ►endocytosis. The internalized transporter will then be delivered to endocytotic vesicles, or endosomes, localized in the cytoplasm (►endosomal compartments). Second, because the internalized transporter moves along the endocytotic pathway, it is necessary to provide the transporter with an endosomolytic module enabling the transporter's escape from the endosome. Third, a specific subcellular delivery can be achieved if the transporter has a specific localization amino-acid sequence, e.g. a nuclear localization sequence to target the cell nucleus. Finally, the modules as well as the anti-tumor agent should be integrated into one moiety; this goal can be achieved by inclusion of the fourth module, a carrier module. Therefore, modular transporters for nuclear drug delivery should include the following parts: (i) an internalizable ligand module providing for target cell recognition and subsequent receptor-mediated endocytosis; (ii) an endosomolytic module ensuring escape of the transporter from endosomes; (iii) a module containing a nuclear localization sequence (a sequence of amino acids that is recognized by ►importins needed for the active translocation into the nucleus); and (iv) a carrier module for attachment of an anti-tumor agent (Fig. 1).



Modular Transporters. Figure 1 Scheme of a modular transporter, and stages of its transportation within the target cell. NLS, nuclear localization sequence. *Arrows* indicate successive steps of the transporter binding to over-expressed internalizable receptors on the target cancer cell, internalization, endosomal localization within an acidifying milieu, escape from endosomes, binding to importins, and transport through the nuclear pores into the cell nucleus. (From Rosenkranz et al (2003) Recombinant modular transporters for cell-specific nuclear delivery of locally acting drugs enhance photosensitizer activity. FASEB J 17:1121–1123, with permission.)

Features

Fundamental to the success of this strategy is that the modules are functional within the transporter, i.e. they retain their activities within the chimeric molecule. Depending on the type of target cancer cells, the ligand module can be replaced; the module with subcellular localization signal can be replaced or omitted (e.g. omission of the nuclear localizing signal will leave the transporter in the cytoplasm of the target cell).

Several types of modular transporters have been created that can deliver photosensitizers into the nuclei of ►melanoma cells; photosensitizers and radionuclides into the nuclei of glioma and epidermoid carcinoma cells and toxins into the cytoplasm of ►acute myeloid leukemia cells. In all these cases, cell specificity was achieved by inclusion of a specific ligand module into the transporter that bound to a corresponding internalizable receptor overexpressed on the surface of the target cancer cell: melanocortin-1 receptor, epidermal growth factor receptor (►epidermal growth factor receptor inhibitors), (►tyrosine kinase receptors), and interleukin-3 receptor (►cytokine receptor as target for immunotherapy and immunotoxin therapy), respectively. Anti-tumor agents carried by these modular transporters acquired a significantly higher efficacy aside from cell specificity. In cases when they are delivered into the most sensitive sites of the target cancer cells, the agents become 10–3,000 times more effective.

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Module

Definition

A standardized, often interchangeable component of a system or construction that is designed for easy assembly or flexible use.

►Modular Transporters

Mohs Micrographic Surgery

Definition

(MMS) is a surgical technique for the removal of certain cutaneous carcinomas that allows precise microscopic marginal control by using horizontal frozen sections. For example, MMS has become the treatment of choice for basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) at high risk for local recurrence.

Mole

►Melanocytic Tumors

Molecular Cancer Epidemiology

Definition

Is a novel research approach in which advanced laboratory methods are used in combination with analytic epidemiology to identify specific exogenous and/or host factors that play a role in human cancer causation at the biochemical and molecular level

►Biomarkers

Molecular Chaperones

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Definition

Proteins that transiently interact with nascent polypeptide substrates to protect them from misfolding and aggregation. They also have an important role in helping to achieve the native conformation of the newly synthesized protein without forming part of the final folded product. The molecular chaperone ►HSP90 is responsible for the stability and activity of a range of oncogenic client proteins and is a target for cancer drugs.

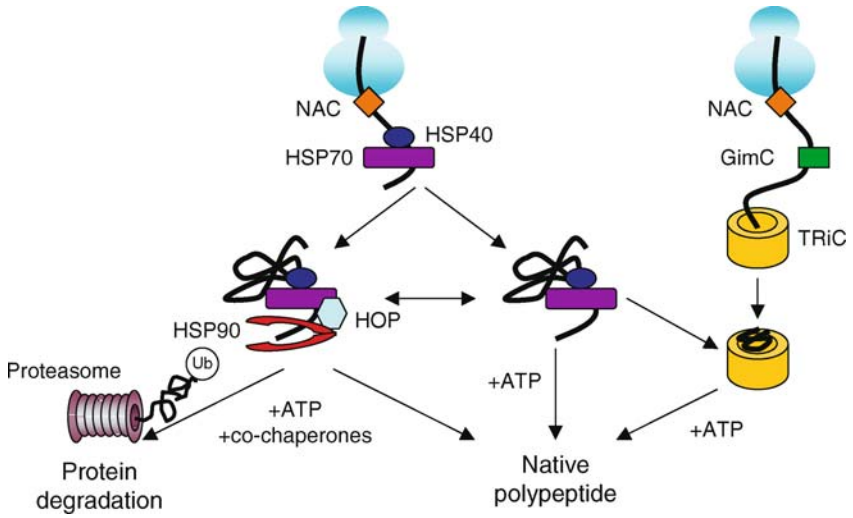
Characteristics

Proteins are mediators of a vast array of biological processes and their activity is dependent on obtaining their correct three-dimensional conformation. It has been widely accepted from *in vitro* folding experiments that the formation of the native state is a spontaneous process which is dictated by the amino acid sequence of the protein. However, protein folding *in vivo* is complicated by the crowded cellular environment which naturally favors protein mis-folding and aggregation. Under certain conditions aggregation may lead to the production of amyloid fibrillar aggregates that are associated with pathological conditions including Alzheimer's or Huntington's disease. To prevent this, nascent or partially folded proteins interact with various chaperone proteins which encourage the formation of the correct three-dimensional structure. Generally, chaperones interact with non-native proteins by recognizing hydrophobic residues and regions of the polypeptide backbone which are not normally exposed to solvent when the correct conformation is achieved. Chaperones may promote *de novo* protein folding by binding directly to the non-nascent protein to shield regions which may be involved in intermolecular aggregation and intramolecular mis-folding.

In addition to promoting productive *de novo* protein folding and preventing non-specific aggregation under normal conditions, molecular chaperones also have an important role in the cellular response to stress. This is because conditions such as elevated temperatures, ionizing radiation, heavy metals and oxidative stress may cause some proteins in their native state to unfold. Many molecular chaperones demonstrate increased expression under such conditions and are referred to as heat shock proteins (HSPs).

In the mammalian cytosol a number of chaperones are involved in native protein folding and the ►stress response (Fig. 1). Chaperone involvement begins as newly synthesized proteins exit the ribosome. This is particularly important because the tendency to aggregate at this stage is increased due to exposure of non-native features and the close proximity of nascent polypeptides of the same type at the polyribosome complex. Nascent polypeptide chains emerge from the peptide exit tunnel of the ribosome and interact with the nascent-chain-associated complex (NAC). It has been hypothesized that the NAC exerts its chaperone function passively by simply binding to and protecting the exposed hydrophobic regions of the newly synthesized polypeptides. This is because it lacks an ►ATPase domain which is characteristic of most molecular chaperones (see below). After release from the ribosome, most small proteins do not require additional chaperones. Longer polypeptide chains may require additional help by association with the HSP70 chaperones which can act in higher eukaryotes both co- and post-translationally. The HSP70 family consists of both constitutively expressed (HSC70) and stress-inducible forms (HSP72) which promote the correct folding of nascent proteins through ATP-dependent cycles of binding and release. HSP70 stably interacts with substrates in its ADP-bound form which is encouraged by its co-chaperone HSP40, a ►J-domain protein which significantly stimulates the ATPase activity of HSP70 to prolong its association with substrate. The substrate binding sites for HSP70 are typically extended hydrophobic stretches of seven residues which statistically occur every 40 residues.

Nascent polypeptides leave the ribosome via the NAC. The majority of small polypeptides require no further assistance to achieve the correct conformation



Molecular Chaperones. Figure 1 Chaperone-mediated protein quality control in the eukaryotic cytosol.

following release from the ribosome. For longer polypeptides the native conformation may be achieved via an interaction with HSP70 and its co-chaperone HSP40. The chaperone function of HSP70 may be extended through its interaction with the HSP90 molecular chaperone via the adaptor protein p60/HOP. According to the compliment of co-chaperones associated with HSP90, its function can be altered from assisting protein folding to promoting protein degradation via the ubiquitin (Ub)-►proteasome pathway. A limited number of proteins have been shown to be reliant on transfer to the GimC/TRiC pathway; these include the abundant proteins actin and tubulin.

In eukaryotes, cytosolic HSP70 can further promote the folding of a particular group of cellular proteins by interacting with the ATP-dependent HSP90 chaperone family. Proteins which are reliant on HSP90 for their correct folding, stability and functional regulation include many ►kinases and other ►signal transduction proteins such as steroid hormone receptors. Substrate transfer from HSP70 to HSP90 is mediated by the adapter protein p60/HOP which binds to the carboxy-terminus of the two chaperones via its ►tetracopeptide repeat (TPR) domains. Similar to HSP70, HSP90 chaperone function is reliant on the orchestrated interaction of a number of co-chaperones which collectively control substrate binding, ATPase activity and substrate release. In addition, co-chaperone interaction may alter HSP90 function from polypeptide folding to protein sorting either into intracellular organelles or to the ubiquitin-proteasome system for degradation. For example, in mammals the HSP70-HSP90 chaperone system can interact with the ubiquitin ligase CHIP (carboxyl-terminus of HSP70 interacting protein) via its TPR domain, promoting the ►ubiquitination of non-native or mis-folded proteins and their degradation by the proteasome. In addition, HSP70 and CHIP can interact with BAG-1 which contains an ubiquitin-like domain that can interact with the proteasome. Therefore, the chaperone function of the HSP70-HSP90 system can be modified by co-chaperone interaction to control protein quality in the cell by mediating a balance between protein folding versus proteasomal degradation.

A restricted group of proteins that are slow-folding and sensitive to aggregation are dependent on the activity of ►chaperonins. These molecular chaperones are a conserved class of large double ring complexes which are exemplified in the eukaryotic cytosol by the Group II chaperonin TRiC (TCP-1 ring complex). As with HSP70 and HSP90, chaperonins mediate folding by undergoing a conformational cycle which is driven by ATP binding and hydrolysis. The most abundant substrates for folding by TRiC are the cytoskeletal proteins actin and tubulin. However, additional proteins which have been shown to co-precipitate with TRiC include von Hippel-Lindau tumor suppressor and ►WD40-repeat 7-blade propeller proteins. Substrate transfer to the TRiC complex has been suggested to be mediated by the cytosolic chaperone Gim1-6 complex (GimC)/prefoldin. This ATP-independent chaperone is made up of six coiled-coil domains each of which contains a hydrophobic extension which is responsible for substrate recognition and interaction with TRiC. GimC/prefoldin interacts with the TRiC substrates actin and tubulin during their translation. It has also been shown to interact with TRiC during the post-translational folding of actin. However, GimC/prefoldin does not contribute to the TRiC-mediated folding of the WD40-repeat proteins.

HSP90 as an Anticancer Drug Target

At first glance molecular chaperones may not appear as obvious candidates for selection as anticancer drug targets because, unlike many oncogenic targets, they are not subject to mutation or amplification. However, the expression of HSP70 and HSP90 is frequently increased in a number of different human ►cancers. In addition, the client proteins which are reliant on HSP90 function for their stability and function includes a wide range of oncoproteins including ERRB2, BCR-ABL, ►C-RAF, mutant B-RAF, AKT/PKB, CDK4, mutant p53, PLK-1, ►HIF-1α, estrogen receptor and ►androgen receptor (for a an up to date compilation see http://www.picard.ch/downloads/Hsp90interactors.pdf). Inhibition of HSP90 function has been shown to cause the depletion of client proteins via the ubiquitin-proteasome pathway, suggesting that modulation of HSP90 function may offer a novel mechanism to simultaneously inhibit multiple oncogenic pathways, all of which may contribute to the hallmark traits of malignancy. These combinatorial effects will be valuable not only in treating cancers which are driven by multiple molecular abnormalities but should also reduce the opportunity for resistance developing.

The increased expression of HSP90 in malignant cells may be a reflection of the stress imposed on cancer cells by the effects of deregulated ►oncogenes and ►tumor suppressor genes. These molecular stresses may be coupled with the micro-environmental stress of solid tumors caused by hypoxia, acidosis and nutrient deprivation. Collectively these factors may lead to greater dependence on HSP90 and other chaperones in cancer versus normal cells, offering the prospect of therapeutic selectivity.

As described above HSP90 (along with many of the other molecular chaperones) are ATPase enzymes. The natural products radicicol and geldanamycin bind to the intrinsic N-terminal ATPase domain of HSP90 to inhibit its function and cause client protein degradation and growth arrest. Since the original discovery of these two natural products there has been an explosion of interest in the development of novel HSP90 inhibitors. Proof-of-concept for the clinical use of HSP90 inhibitors was provided by the completion of several phase I ►clinical trials on the first-in-class HSP90 inhibitor 17-AAG (tanespimycin), an analogue of the natural product geldanamycin. ►Biomarker evidence demonstrated client protein depletion and heat shock protein expression in the tumor tissue and peripheral blood lymphocytes of treated patients at doses which were well tolerated. As a result 17-AAG is now in phase II clinical trial. In addition, a more soluble analogue of 17-AAG, 17-DMAG (alvespimycin) has recently entered phase I clinical trial. Several other inhibitors are in development.

The exciting observations made *in vitro* and *in vivo* with HSP90 inhibitors highlight the possibility of modulating chaperone function which may have benefits not only in the treatment of cancer but also in the management of other protein folding diseases.

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Molecular Epidemiology

Definition

Application of biochemical and/or molecular biomarkers within an epidemiological study design to identify events directly or indirectly associated with the pathway between exposure and disease. A long-term goal of molecular epidemiology is attenuation of disease by manipulation of biomarkers early in the disease process.

►Cancer Epidemiology
►Molecular Cancer Epidemiology

Molecular Genetics

Definition

Group of methods applied for the detection of molecular genetic alterations, includes fragment analysis (Genescan), heteroduplex analysis, melting point analysis, nested PCR, PCR, real time PCR, sequence analysis, single stranded conformation analysis (SSCP).

►Leukemia Diagnostics

Molecular Imaging

Definition

Imaging of contrast agents or tracers targeted to a specific cell-surface receptor to noninvasively assess the extent to which a tissue or organ expresses that receptor. The targeted receptor is usually a marker of a disease-related process, such as angiogenesis or inflammation, or a potential target for drug activity. Image-based quantification of the binding of targeted agents yields information about molecular-scale events that would not otherwise be detectable *in vivo* with preclinical or clinical imaging. Molecular imaging can be performed with any imaging technology given a suitable choice of targeted contrast agent or tracer.

►Ultrasound Micro-Imaging

Molecular Memory

Definition

The inheritance of specific pattern of gene expression, which persists in daughter cells from generation to generation.

Molecular Mimicry

Definition

It has been proposed that infectious agents could provoke autoimmunity by molecular mimicry, the induction of antibodies, and T cells that react against the pathogen but also cross-react with self antigens.

Molecular Morphology

►Immunohistochemistry
►Molecular Pathology

Molecular Pathology

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Synonyms

Molecular morphology

Definition

Molecular pathology is the study of the molecular genetic causes of abnormal cell and tissue functioning with the goal of improved disease diagnosis and treatment. As a medical discipline, molecular pathology is a specialty training that incorporates the subject matter of genetics, inherited cancers, solid tumors, neoplastic hematopathology, infectious diseases, identity testing, HLA typing, and laboratory management, and impacts both anatomic and ►clinical pathology practice.

Characteristics

Historical and Clinical Background

►Pathology, the study of the origin, nature, and courses of diseases, has its foundation in the studies of Giovanni Battista Morgagni of Padua (1682–1771), (the "father of ►anatomic pathology") and Rudolf Virchow of Berlin (1821–1902), (the "father of cellular pathology"). Dr. Morgagni documented the relationship between diseases and the gross changes observed in autopsy specimens, and Dr. Virchow established the correlation of cellular changes with disease having formulated the cellular theory of life, *omnis cellula e cellula* (all cells come from cells). Modern anatomic pathology retains gross and microscopic tissue examination as the basis of clinical diagnostics via standardized classifications; tumor specimens are described in terms of organ of origin (e.g. breast, colon etc), cell type (e.g. carcinoma, describing cells of epithelial type; adenocarcinoma, referring to a cancer of glandular origin), and in terms of ►tumor staging and ►tumor grade. The "TNM Staging" characterizes solid tumors by the extent to which a tumor (T) has spread locally, the amount of regional lymph node (N) involvement, and whether or not there are metastases (M) to distant organs or lymph nodes. Tumor grade refers to the degree of cellular divergence from the normal condition. These criteria are increasingly supplemented by tests for additional ►biomarkers to improve disease diagnosis. The discoveries of molecular cell biology and the human genome project together with technological advancements resulting in methodologies that are reliable, rapid and cost effective have set the stage for molecular pathology as an essential and routine discipline

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for improved patient care. Potentially, molecular pathology will yield a comprehensive molecular classification of cancer improving early tumor diagnosis, resolution of equivocal diagnoses including tumors of uncertain grade or unknown organ of origin, tumor chemoresistance/drug therapy investigation, and individualized medicine. Numerous putative markers are reported in the scientific literature; the challenge is to identify those individual or sets of biomarkers that have high ►sensitivity, ►specificity, and predictive value (►Negative predictive value, ►positive predictive value) for vital clinical parameters. The following illustrates by way of current common techniques how molecular pathology can support cancer diagnosis.

Immunohistochemistry

Immunohistochemistry (IHC) allows by microscopic inspection the correlation of protein staining patterns with ►cytology or ►histology and is the most routinely used adjunct technique of anatomic pathology. Aberrant molecular genetic changes frequently result in an altered immunophenotye (nuclear, cytoplasmic, or membrane protein expression staining patterns). Typically, IHC involves application of a primary antibody to the antigen of investigation to tissue sections followed by secondary antibodies labeled to allow chromogenic or fluorescent detection. IHC has been a routine part of pathology practice since the mid-1970s, often to identify cell or tissue origin. For example, the lineage of an undifferentiated tumor or the organ of origin of a metastasis can be identified after IHC with panel of antibodies for cytokeratins as the expression fingerprint of these cytoskeletal markers is not compromised by malignant transformation. IHC is also used to differentiate lymphomas and sarcomas. Panels of markers (e.g. CD5, CD23, CD43, Cyclin D1 and bcl-6) are used to help distinguish different categories of lymphomas such as follicular, mantle cell, marginal zone and chronic lymphocytic leukemias (►Leukemia diagnostics). Similarly, IHC markers including desmin, myogenin, CD99, CD43 and FLI-1 may be used to distinguish ►rhabdomyosarcomas, ►Ewing sarcoma, ►desmoplastic small round cell tumor (DSRCT), ►neuroblastoma, and lymphoblastic lymphoma.

"Genogenic" IHC supports the demonstration of genomic translocations that result in novel chimeric proteins; for example ~90% of Ewing sarcoma cases harbor a t(11;22)(q24;q12) translocation resulting in the EWS-FLI-1 chimeric product; over-expression of the FLI-1 protein can be used to distinguish Ewing's sarcoma from histologically similar tumors. IHC can also be used as a marker of gene mutations that result in protein under- or overexpression; hereditary nonpolyposis colon cancer (HNPCC) is characterized by ►microsatellite instability (MSI) due to mutations in genes such as *hMLH1* and *hMSH2*. These mutations translate as under-expression of MLH1 and MSH2 proteins detected by IHC.

Routinely, IHC markers are employed to assess tumor aggressiveness and the appropriate course of treatment; breast tumor diagnostics requires IHC for ►estrogen receptor (ER), progesterone receptor (PR), and ►HER-2 status. ER and PR positive tumors are generally less aggressive and are more responsive to hormone suppression treatments such as tamoxifen; HER-2 positive tumors tend to be aggressive but are candidates for treatment with ►herceptin.

Defective cell-cycle checkpoint control may be critical for tumor development and there is a variety of potential IHC markers. For example, $p57^{Kip2}$ expression may be associated with poor prognosis, whereas $p21^{WAF1}$ and $p27^{Kip1}$ overexpression may signify a favorable prognosis; $p16^{INK4a}$ is widely used as a surrogate marker of ►human papillomavirus (HPV) infections and pre-invasive cervical lesion grade. High-risk HPV type E7s open-reading frame product inactivates pRb (►Retinoblastoma protein), thereby disrupting pRb negative feedback of $p16^{INK4a}$ with consequent overexpression of the latter and increased staining the more severe the lesion (Fig. 1).

IHC can also be used in the direct demonstration of oncogenic viruses. Detection of ►Epstein-Barr virus (EBV) latent membrane proteins (LMPs) and/or nuclear antigens can be used in the diagnosis of ►nasopharyngeal carcinoma, Burkitt lymphoma and ►Hodgkin lymphoma. Detection of human herpesvirus 8 (HHV-8) may assist in the diagnosis of ►Kaposi sarcoma, and detection of the HPV *L1* capsid protein of infective virions may be a marker of low-grade cervical lesions.

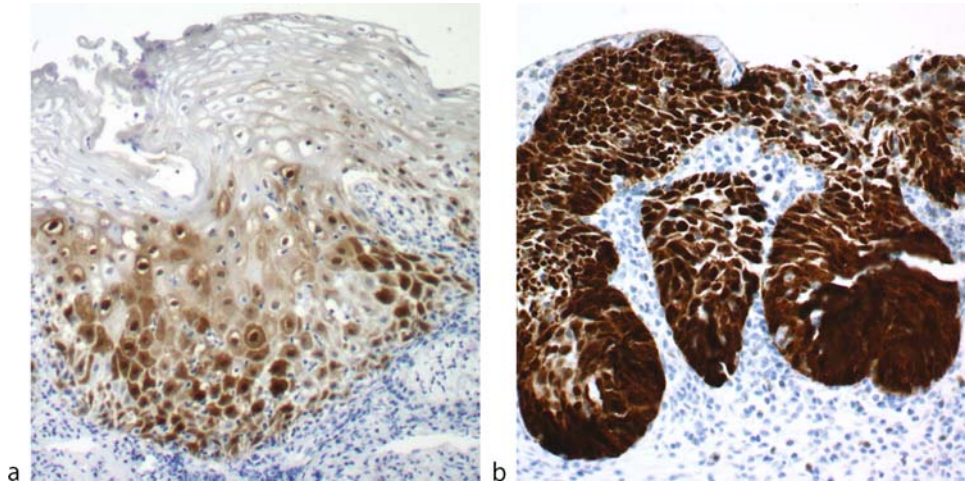
In Situ Hybridization

Fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) allow the demonstration of DNA or RNA in tissue samples by hybridization with labeled nucleic acid probes or synthetic analogs; like IHC, in situ hybridization (ISH) supports the direct correlation of test data with specimen morphology. FISH techniques represent an efficient and easier option than classical metaphase cytogenetic techniques for the detection of chromosomal rearrangements; additionally, ISH supports interphase cytogenetic applications.

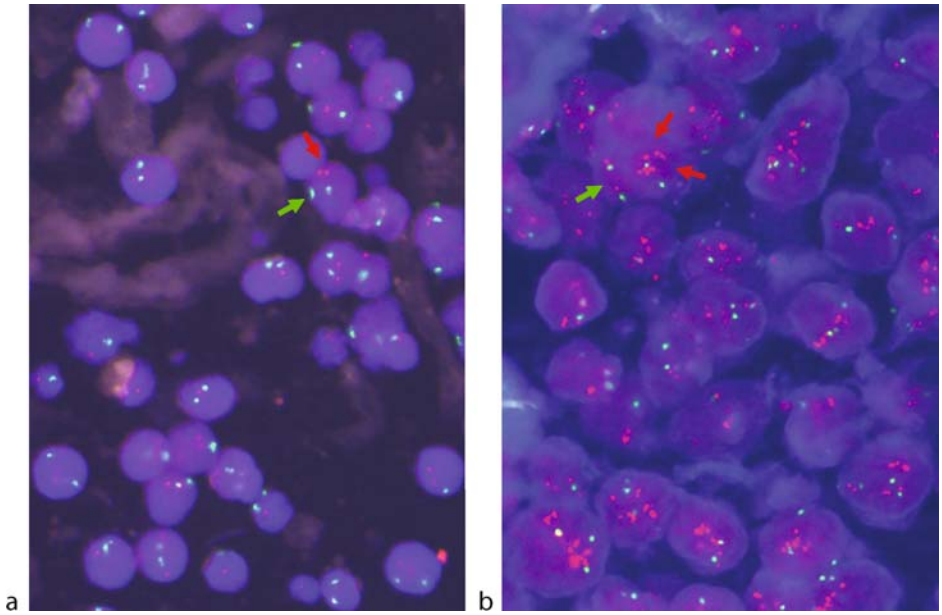
FISH is utilized in the diagnosis of hematological malignancies. For example, chronic myelogenous leukemia (CML) is characterized by the ►*BCR-ABL* reciprocal translocation involving fusion of the *BCR* region of chromosome 22 with the *ABL* region of chromosome 9. The fusion results in a shortened chromosome 22 (the Philadelphia chromosome). Predictable FISH signal patterns can be observed in interphase cells depending on whether the probes span or flank the translocation breakpoint. Other

hematological diagnoses by FISH include ►acute promyelocytic leukemia (APL) (t(15;17)(q22;q12)), and follicular lymphoma (t(14;18)(q32;q21)). Soft tissue tumors such as synovial sarcomas can also be diagnosed by FISH; the t(X;18)(p11;q11) translocation is present in ~90% of these tumors and results in the juxtaposition of the *SYT* gene (18q11) and the *SSX* gene (Xp11).

Among solid tumors, FISH is routinely used to confirm HER-2 amplification indicated after IHC assay. The PathVysion™(Vysis Inc. IL. USA) FISH assay involves dual hybridization with a probe for the centromeric region of chromosome 17 together with a probe to the HER-2 locus at 17q11.2-12; comparison of the ratio of signals is used to score amplification (Fig. 2). The UroVysion™(Vysis Inc. IL. USA) FISH assay is a non-invasive test for ►bladder cancer. The test is applied to urinary cytology specimens and uses a panel of four probes to targets frequently altered in bladder cancer (centromeres 3, 7, and 17, and 9q21 (site of the p16 gene)); if ≥4/25 cells show multiple chromosome gains, or there is loss of both copies 9q21 in ≥12/25 cells, this is taken as predictive of cancer.



Molecular Pathology. Figure 1 Differential nuclear and cytoplasmic $p16^{INK4a}$ staining patterns in low-grade (a) and high-grade (b) cervical lesions.



Molecular Pathology. Figure 2 (a) Non-amplification of HER-2 indicated by a 1:1 ratio of chromosome 17 centromeric signals (green) to HER-2 FISH signals (red). (b) Amplification indicated by abundant HER-2 signals relative to centromere 17 signals.

ISH is the preferred choice for the demonstration of EBV by the detection of EBV encoded RNAs (EBER) in circulating B lymphocytes of patients with suspected lymphoma. ISH supports the detection of low- or high-risk HPV types and HPV signal types may be useful in determining (cervical) lesion grade. "Diffuse" signals are associated with episomal HPV, whereas "punctate" signals may be demonstrative of HPV integrated into the cell genome (Fig. 3). In addition to cervical tissues, HPV ISH is applicable to head and neck, esophageal, and other tumors with a suspected HPV etiology.

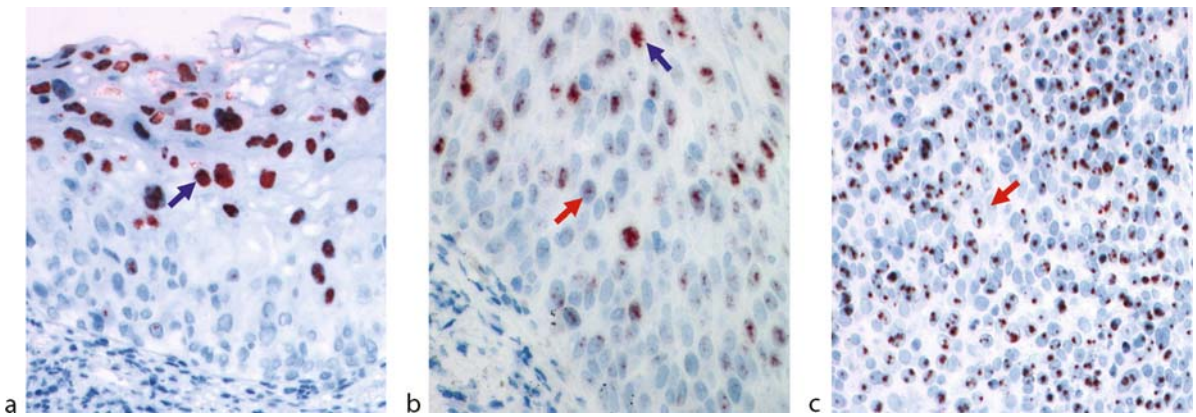
Other ISH applications include investigation of chromosome instability/aneusomy in tumors or cell lines using panels of centromeric probes. Locus specific probes can be used to investigate the association of defined abnormalities with tissue morphology. Centromeric and locus specific probes can also be used to examine intra-tumoral heterogeneity and to investigate the relationship between tumors and putative precursor lesions. Combining ISH with IHC allows observation of the correlation between nucleic acid detection and protein expression patterns. Specialized FISH techniques such as spectral karyotyping (SKY) or multiplex (M) FISH utilize chromosome paints specific for each of the 24 human chromosomes and detectable by fluorescent microscopy and computer imaging. Application of the paints to metaphase spreads from tumors allows the identification of chromosomal rearrangements with a resolution down to ~2–3 Mb.

Polymerase Chain Reaction

Pathology specimens are frequently highly limiting with respect to the amounts of nucleic acids that can be recovered for diagnostic or research applications. The polymerase chain reaction (PCR) utilizes a thermostable DNA polymerase, DNA primers, and deoxyribonucleic acid building blocks to amplify a DNA template; theoretically, after 30 cycles of denaturation, annealing, and extension, the starting template DNA sequences are amplified 1 billion-fold. Consequently, PCR techniques have been at the vanguard of molecular pathology investigations. Using fluorescent-labeled primers, PCR can be adapted for real-time quantitative (Q) PCR to allow an estimation of relative DNA load or mRNA expression. By combining PCR with microdissection of specific tissues or cells off a slide, it is possible to correlate PCR data directly with tissue morphology.

PCR is used diagnostically to screen for gene mutations (by PCR product sequencing or by techniques such as single strand conformation polymorphism (SSCP)). Mutation screening of all 19 *hMLH1* exons, all 16 *hMSH2* exons, or all 10 exons of the *hMSH6* is possible in the diagnosis of HNPCC; or MSI can be investigated directly by PCR for a panel of 5–12 standardized microsatellite loci that includes mono- and dinucleotide repeat sites (►Colon cancer). PCR mutation screening is also applicable in the diagnosis of other familial tumors such as the ►APC gene in familial adenomatous polyposis, which can lead to an autosomal dominant condition resulting in the development of myriad colon polyps some of which may have the potential progress to colon carcinoma. Mutation screening is also performed for ►BRCA1/BRCA2 germline mutations and breast cancer risk.

Numerous candidate genes associated with sporadic tumors have been described; however, uncertainty about the relationship of mutations in these genes to tumor diagnosis and prognosis has stalled translation to routine screening tests. For example, mutations leading to aberrant expression of the ►tumor suppressor



Molecular Pathology. Figure 3 HPV detection by CISH in cervical tissues. Diffuse signals (blue arrows) detected mainly in upper epithelial layers characterize low-grade lesions (a). In high-grade lesions (b) diffuse and punctate signals (red arrows) may be detected throughout the epithelial thickness. Punctate signals alone in an invasive cervical carcinoma (c).

TP53 protein ("guardian of the genome") are estimated to be present in up to 50% of sporadic tumors. Despite the undoubted contribution of *p53* mutations to tumor pathology, *p53* mutation screening is of uncertain clinical utility.

PCR is used in the diagnosis of oncogenic viruses; in particular, several PCR strategies are available for detecting HPV and determining which of more than forty HPV types associated with cervical lesions are present in a patient (cervical smear) sample. In the reverse-line blot assay, labeled PCR product is hybridized to a blot spotted with HPV type specific probes. Patients positive for high-risk HPV types may be at an increased risk for high-grade lesions/cancer. An elevated HPV DNA load as determined by Q-PCR may also correlate with increased tumor risk.

Reverse-transcriptase (RT) PCR converts RNA to DNA for PCR amplification. RT-PCR allows the detection of RNA fusion transcripts and is widely used in soft tissue tumor diagnostics. For example, alveolar rhabdomyosarcoma diagnosis is aided by the detection of mRNA transcripts created after the t(2:13)(q35;q14) reciprocal translocation that juxtaposes part of the *PAX3* gene on chromosome 2 with part of the *FKHR* gene on chromosome 13. The *API2/MALT1* RT-PCR test can be applied to stomach or other gastrointestinal tissues for the diagnosis of ►B-cell tumors of mucosa-associated lymphoid tissue (MALT lymphoma). This condition commonly develops against a background of chronic inflammation caused by ►*Helicobacter pylori* infection. Diagnosis of DSRCT can be aided by RT-PCR for transcripts from the t(11:22)(p13;q12) translocation associated with DSRCT and involving the *EWS* and *WT1* genes. RT-PCR is also used in the diagnosis of Ewing sarcoma, synovial sarcomas, and *BCL-ABL*/CML. Q-RT-PCR is used in the diagnosis of APL to detect transcripts of the fusion of the retinoic acid receptor alpha gene (*RARA*) with the promyelocytic leukemia (*PML*) gene; monitoring the tumor burden may be helpful in predicting disease relapse. The PCR based methods have greater sensitivity than their FISH counterparts for detecting tumor specific rearrangements, nevertheless "false-negative" diagnoses may occur because of variant translocation points not detected by PCR primer sets. RT-PCR also supports the assessment of HPV integration. The amplified papillomavirus oncogenic transcript (APOT) assay confirms integration by the detection of HPV transcripts that are contiguous with human sequence transcripts.

Future Developments

Improved techniques and new findings are a constant in molecular pathology. For example, application of a sophisticated high-throughput PCR mutation screening strategy of more than 13,000 genes in 11 breast and 11 colorectal cancers has shown that individual tumors typically accumulate ~90 mutant genes and that only a subset of these contribute to the neoplastic process; an average of 11 genes per tumor were mutated at significant frequency. Follow-up of such studies in course of time may reveal (sets of) mutations that have high utility as sporadic tumor diagnostic markers. Similarly, ►microarray cDNA technology and the findings of ►proteomics studies are expected to greatly increase and refine knowledge of the molecular basis of cancer, translating into effective clinical tests including improved IHC, ISH, or PCR, or the development of cost effective microarray or proteomic tests for limited sets of biomarkers. Additionally, there remains much to be learned about genome regulation; for example, the significance of ►microRNAs in the control of gene expression and contribution to tumor biology has only recently been recognized, and there is increasing awareness of the importance of ►epigenetics in tumor etiology. Advances in the conceptual frameworks within which cancer is understood will also direct the interpretation of biomarkers. Whilst ►oncogenes and ►tumor suppressor genes remain key concepts, there is a growing appreciation of cancer as a process involving a multi-interaction of cellular systems.

►Pathology

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Molecular Shape

Definition

This approximation of lipid structure is a simple but convenient way to explain the volume occupied by lipid molecules. The model explains the three dimensional structure of lipids in a simple manner and the membrane structure as a consequence of the "shape" of the lipid molecules. Thus, a phospholipid with a bulky polar head, such as phosphatidylcholine, has a molecular shape that resembles a cylinder. In contrast, the

molecular shape of phosphatidylethanolamine, with a small polar head, is similar to a truncated cone, while the fatty acids oleic and elaidic acid, are similar to a boomerang and a rod, respectively.

►Membrane-Lipid Therapy

Molecular Therapy

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Definition

Therapy with compounds which are rationally targeted against certain specific molecular structures.

Characteristics

There is yet no generally accepted definition of the term molecular therapy. A frequent opinion is that molecular therapeutics target diseases on an early molecular level and thus modulate the disease at its roots. This would comprise the classic ►gene therapy as well as other DNA- or RNA-based strategies. However, also other non-molecular therapeutics such as ►chemotherapy interact with nucleic acids. Additionally, nucleic acid based drugs may also interact with proteins. Therefore it seems more useful to define molecular therapy as a strategy which specifically targets certain molecular structures although all therapies that work have a target with some kind of molecular structure. In some cases the target is discovered first while in others the drug is discovered before the target. In contrast to other therapeutic concepts such as chemotherapy or immunotherapy molecular therapy is usually directed against a biologically important process with a measurable target in the clinic. Based on this definition certain compounds which are frequently designated as "targeted therapy" such as monoclonal antibodies or ►small molecule drugs should in addition to DNA- or RNA-based strategies also belong to the class of molecular therapeutics.

Gene Therapy

Classic gene therapy is the direct use of genetic material in the treatment of diseases. The various strategies for inhibiting cancer growth by gene therapy approaches comprise the reversion of the malignant phenotype by correcting abberrant gene expression, induction of genes that inhibit tumor growth, immune gene concepts, cytoreductive or oncolytic approaches.

Additive or corrective gene transfer involves on the one hand the introduction of foreign genes such as suicide genes or antiangiogenesis genes. On the other hand physiological but mutated and malfunctioning genes which are associated with tumor development and progression such as tumor suppressor genes may be restored. Since the cellular uptake of naked longer nucleic acid molecules is inefficient, gene therapy is dependent on effective delivery. The clinically relevant gene transfer techniques can be classified into viral (vector) delivery systems and nonviral delivery sytems. DNA molecules may be packed in viral vectors. Viruses can transfer these molecules into cells and the transgene of interest enters the nucleus and is integrated into the host gene pool, and eventually expressed. Common clinically used viruses are adenoviruses, retroviruses, lentiviruses, adeno-associated viruses, the herpes simplex virus and parvoviruses. The gene transfer by viral vectors in general is characterised by a high effectivity but considerable toxicities such as for instance immune responses. Common non viral delivery systems are polymeric systems and liposomal systems. These systems are characterised by lack of immune response as well as ease of industrial production but also by significantly lower transfection efficiency compared to viral vectors.

Clinically several gene transfer phase I-III studies in different tumours have been performed or are in progress. Earlier trials suffered especially from inefficient expression of the transgene. One modified adenovirus that delivers the p53 gene to cancer cells has been approved by the FDA.

Oncolytic gene therapy viruses are designed to infect and destroy cancer cells by expression of cytotoxic proteins while remaining innocuous to the rest of the body. Several different viruses such as adenoviruses and herpes virus have been used clinically. However, only few trials with oncolytic therapy have been performed. Most advanced (Phase-III) is the approach with an adenovirus that replicates and mediates cell lysis only in cancerous cells with p53 mutation (ONYX-015).

Since cancer cells tend to develop mechanisms to evade immune detection traditional immunotherapy is often characterised by limited effectivity. In order to overcome these problems immune gene therapy concepts have been advocated. One popular strategy are recombinant cancer vaccines. Autologous or allogeneic cancer cells are engineered to be more recognisable to the immune system by the addition of one or more genes. These genes are are mostly cytokine genes or highly antigenic genes. Another approach is the direct delivery of immunostimulatory genes such as cytokines to the tumor. The goal is to unmask the cells from immune evasion and enhance an antitumor response. A third strategy is the direct alteration of the patients

immune system by adding tumor antigens or stimulatory genes to the patients immune cells. Although early clinical trial were quit successful, long lasting immune responses were the exception rather than the rule. Several large phase-III-studies are currently underway.

Nucleic Acid Constructs

The various nucleic acid therapeutics include ►antisense oligonucleotides, ►small interfering RNAs (siRNA), aptamers, ribozymes and DNAzymes. Nucleic acid based drugs have emerged in recent years to yield extremely positive candidates for therapy of various diseases including cancer. In opposite to gene therapy the major goal of nucleic acid drugs is the inhibition and silencing of specific cancer-associated genes such as oncogenes.

The most extensive applications have been performed with antisense oligonucleotides. These are short chemically modified DNA sequences (10-25 nucleotides), designed to modulate the information transfer from gene to protein. Sequence-related hybridisation with the mRNA of a specific protein results via different mechanisms such as RNAseH-dependent mRNA clavage in selective inhibition of gene expression and downregulation of protein expression. Antisense oligonucleotide inhibitors can be designed directly from genomic sequence information by simply making the reversed complement of the desired sequence. However, active compounds are more effectively generated by computer-based approaches. One antiviral antisense drug (fomivirsene) has already been approved by the FDA. Phase-III studies with several antisense constructs in oncological patients are underway or have been finished. Some results have been particularly encouraging although antitumoral effects have to a certain extent been attributed to unspecific immunostimulatory mechanisms. In clinical studies antisense oligonucleotides have mostly been applied without any delivery system. In the majority of the current trials, oligonucleotides are combined with one or more traditional chemotherapeutic agent or another targeted agent.

Ribozymes are RNA molecules which may selectively bind to a target mRNA and form a duplex which results in cleaving of the target mRNA. Two types of ribozymes, the hammerhead and hairpin ribozymes have been extensively studied for therapeutical purpose. In DNAzymes the RNA backbone chemistry is replaced by DNA motifs which confers greater biological stability. DNAzymes have high catalytic activity. Aptamers are small single-stranded or double-stranded nucleic acid segments that directly interact with proteins. In comparison to protein inhibition by antibodies aptamers are highly specific, non immunogeneic and stable. In some cases and as opposed to antisense oligonucleotides, effects can be mediated against extracellular targets, thereby preventing a need for intracellular transportation. The three latter classes of nucleic acid drugs have been evaluated in preclinical oncological studies or have entered early clinical trials.

Small interfering RNAs are short double-stranded RNA segments with a length of typically 21-23 nucleotides. SiRNA are also complementary to a target mRNA sequence. They are upon application incorporated into RNA-induced silencing complexes (RISCs) which bind to the mRNA of interest and stimulate mRNA degradation via different mechanisms such as nuclease activation. These compounds are, compared to other gene silencing instruments, substantially more effective and nonimmunogenic. Initial non oncological phase-I studies have been initiated. A new class of non-coding small RNA molecules are the microRNAs (miRNAs). Many miRNAs are known to be up- or downregulated in a variety of cancers, suggesting a role for miRNAs in tumorigenesis. These compounds are beginning to be used therapeutically.

Nucleic acid drugs offer in comparison to viral vectors or plasmids greater safety since they do note integrate into the genome. Furthermore, delivery is mostly easier since most of these compounds do not have to enter the nucleus for their activity and because of the small size (especially antisense oligonucleotides and siRNA). However, for most medical applications several obstacles related to toxicity, stability, affinity, cellular delivery and specificity remain to be clarified and may be overcome by new chemical modifications.

Small Molecule Drugs

The small molecules of highest interest in cancer therapy are protein tyrosine kinases (PTK). They comprise over 100 ►oncogenes and more than 40 ►tumor-suppressor genes. Overexpression of PTK receptors is correlated with poor prognosis in some malignancies. Tyrosine kinase inhibitors (TKIs) are orally available, synthetic chemicals with a small molecular weight. They reversibly bind to the ATP-binding site on the receptor and prevent its activation as well as signal transduction. Small-molecule agents can translocate through plasma membranes and interact with the cytoplasmic domain of cell-surface receptors and intracellular signalling molecules. TKIs have shown objective tumor responses in chronic myeloid leukemia (CML), gastrointestinal stromal tumors and non-small-cell lung cancer. The TKIs gefitinib and erlotinib are specific for the ►epidermal growth factor receptor (EGFR). In contrast, Imatinib inactivates the kinase activity of the BCR–ABL fusion protein in CML. Sorafenib is a dual kinase inhibitor inhibiting different isoforms of Raf serine kinase as well as various receptor tyrosine kinases such as VEGFR, EGFR and PDGFR. This results in an inhibition of tumor proliferation and angiogenesis. Sunitinib is another multi-targeted tyrosine kinase inhibitor of VEGFR,

PDGFR, KIT and Fms-like tyrosine kinase 3 (FLT3). Both drugs are approved for the treatment of metastatic kidney cancer. Bortezomib, which was first developed as a selective, reversible inhibitor of the chymotryptic protease in the 26S proteasome, has been reported to be effective against hematological malignancies.

Antibody Therapy

In 1975 Köhler and Milstein first described the generation of murine monoclonal antibodies (mAbs). The typical antibody consists of two antigen-binding fragments (Fabs) which are linked via a flexible region to a constant (Fc) region. This structure comprises two pairs of polypeptide chains, each pair containing a heavy and a light chain of different sizes. Antibodies bind to antigens on the surface of cells or pathogens. The first therapeutic antibody for cancer therapy (retuximab) was approved in 1987 for the treatment of non-Hogkin's lymphoma. mAbs are relatively large proteins with a high molecular weight. They are generally administered via an intravenous infusion and often have a half-life of several days.

Disadvantages of mouse antibodies are a short half-life in serum, the inability to trigger human effector function and the induction of human antimouse antibodies (HAMA) which in turn inactivate murine mAbs. Chimeric antibodies such as retuximab combine human constant regions and mouse variable antigen-binding regions to reduce their immunogenicity. However, they can also induce human anti-chimeric antibody response (HACA). Thus, human antibodies seem ideal to overcome the latter problems.

Today about 200 antibodies are in clinical trials for cancer therapy, chronic inflammation, transplantation, infectious and cardiovascular diseases. For cancer therapy more than 10 mAbs are approved by the FDA, predominantly for the treatment of non-Hogkin's lymphoma, breast cancer and colorectal cancer.

Antibody cancer therapy aims to target tumor-associated antigens and/or tumor-specific antigens (e.g. CEA, EGFR, HER2, MUC1, CD20) to alter their signalling. They can function by three principal modes of action: blocking the action of specific molecules such as growth factors or cytokines, targeting specific cells or functioning as signalling molecules. Also, mAb therapy may result in an elimination of tumor cells by immune-effector cells via complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC).

Limitations beside immunogenicity of murine mAbs and chimeric mAbs are a potential lack of selectivity and poor penetration into solid tumors. The latter problem is due the size of mAbs and a poor permeability of the tumor surface. Monoclonal antibodies are relative specific therapies associated with low toxicity.

Clinically antibodies are often safe well tolerated with some fever and chills at first infusion. A possible side effect of antibody therapy is the cytokine-release syndrome that is probably mediated through recruitment of the immune effector cells. However, treatment-associated deaths have been described in few patients.

An other limitation is the restriction of targets to those on the surface of host cells. This problem could be solved by the development of functional antibodies within cells, so called intrabodies. Beside the development of unconjugated or naked mAbs, a conjugation with cytotoxic dugs, cytokines, toxins, radionuclides and DNA molecules have been tested in order to optimise delivery and enhance specificity and effectivity.

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Mom1

Definition

Modifier of ►Min1.

►Modifier Loci

MOMP

Definition

Mitochondrial outer membrane permeabilization is the event that triggers the mitochondrial apoptotic cascade. MOMP is regulated by proapoptotic members of the BCL-2 family. These proteins induce disruptions in the outer mitochondrial membrane and subsequent release of death-promoting proteins like cytochrome-c.

►APAF-1 Signaling

Monoclonal Antibodies

Definition

Monoclonal antibodies are antibodies that are identical because they are all clones of a single parent cell. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell.

►CD Antigens
►Monoclonal Antibody Therapy
►Prostate-Specific Membrane Antigen (PSMA)

Monoclonal Antibody Therapy

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Definition

►Monoclonal antibody therapy is a passive ►immunotherapy consisting of in vitro-generated identical immunoglobulin clones which specifically bind to a defined membrane-bound surface antigen and induce tumor cell-lysis or -►apoptosis via different pathways.

Characteristics

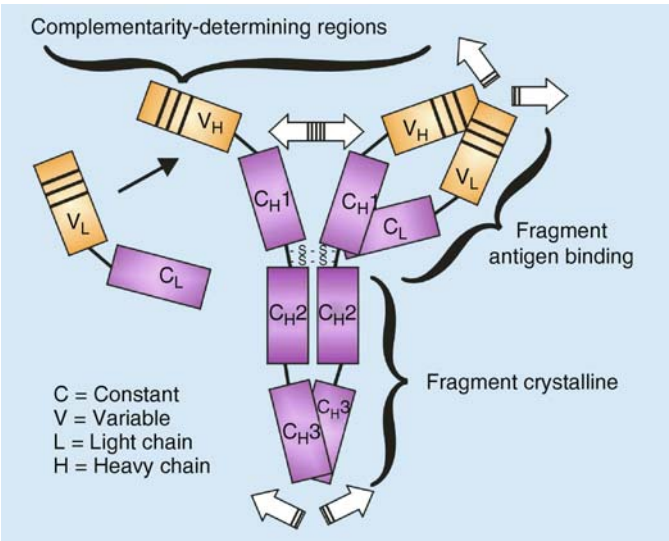
The idea of a monoclonal antibody based cancer therapy was first proposed by Paul Ehrlich at the beginning of the twentieth century. Development of monoclonal antibody therapy required characterization of defined target antigens and the establishment of monoclonal antibody production.

Antibody Structure

For generation of ►monoclonal antibodies (mAbs) the exact knowledge of antibody-structure is indispensable. As shown in Fig. 1 antibodies are Y-shaped glycoproteins composed of two heavy and two light chains connected by disulfide bonds. Heavy and light chains consist of two domains, a constant and a variable domain. Antigen specificity is mediated by ►complementarity-determing regions (CDRs) of the variable domains. In addition to the CDRs, the framework regions of the variable domains and the neighboring constant regions (CH1 and CL) contribute to the conformation required for antigen binding. The effector function of antibodies is defined by the C-terminal structure of the heavy chain; there are five main heavy-chain isotypes, IgM, IgG, IgA, IgD and IgE. The physiologically most abundant immunoglobulin isotype is IgG subtype 1. IgG subtype 1 and IgG subtype 3 are the immunoglobulins with the highest binding affinity to the Fc-receptor expressed on ►macrophages.

Monoclonal Antibody Generation

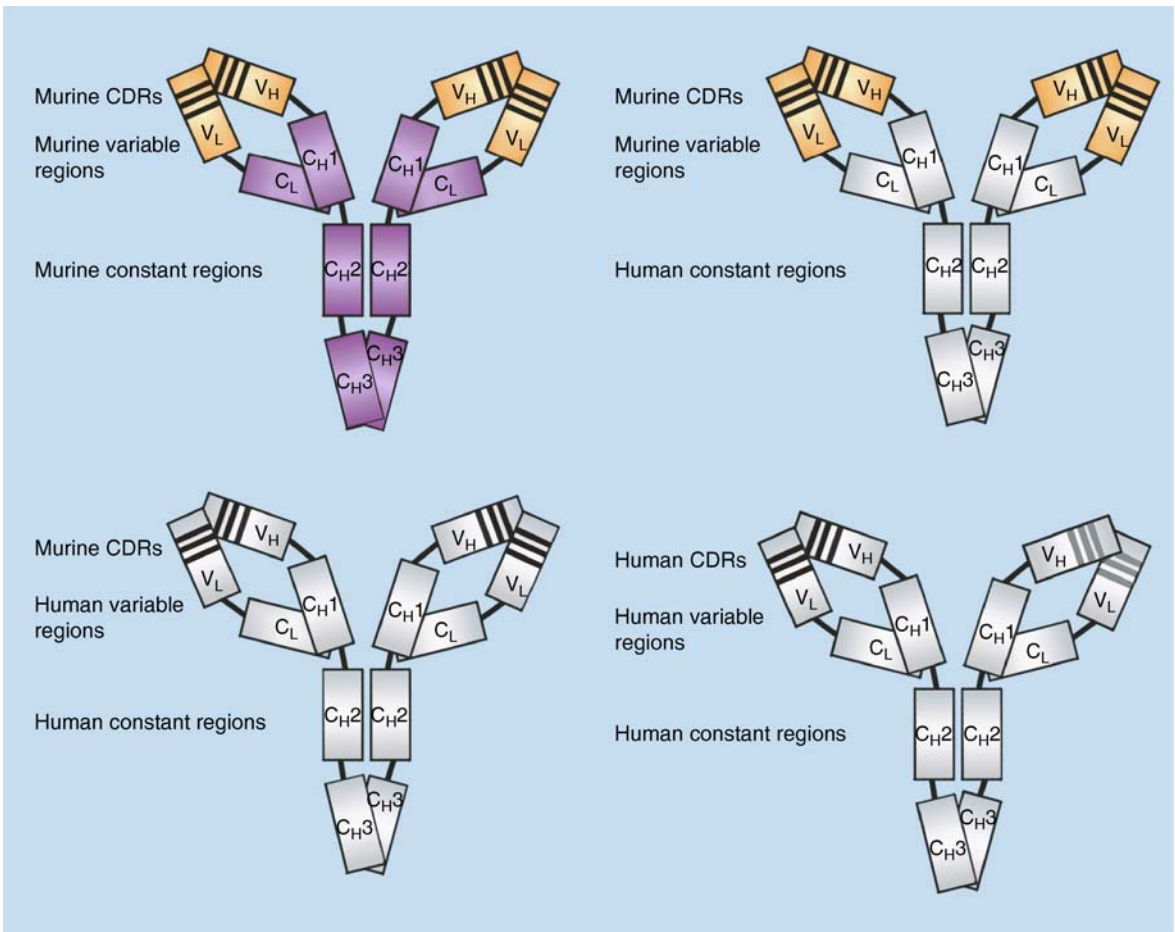
The first monoclonal antibodies were generated after the invention of ►hybridoma technology by Georges Köhler, César Milstein and Niels Jerne in 1975.



Monoclonal Antibody Therapy. Figure 1 Structure of an antibody molecule. Reprinted with permission from "UNI-MED Verlag, Bremen."

The key idea of hybridoma technology was to fuse cells of a non-secreting myeloma cell line with antibody-producing murine B-cells from animals which had been immunized with the antigen of interest. The application of mouse-derived ►hybridomas enabled the production of large amounts of murine monoclonal antibodies. Murine monoclonal antibodies are highly effective diagnostic tools; however, the application as cancer therapeutics is limited by the development of human anti-mouse antibodies. The production of human anti-mouse antibodies results in neutralization of murine antibodies and in shortening of their circulation half-lives; an anaphylactic reaction is a rare, but quite serious complication. Therefore, only two murine monoclonal antibodies have been approved to date, both serving as targeting agents for isotopes (Y90-Ibritumomab; I-131 Tositumomab).

Through advances in genetic engineering in the 1980s and 1990s less immunogenic and more efficient monoclonal antibodies have been designed. Depending on the ratio of human and mouse cDNA sequences chimeric, humanized and human monoclonal antibodies are differentiated; the difference in structure of these genetically engineered monoclonal antibodies is illustrated in Fig. 2. Replacement of murine constant regions by human DNA sequences resulted in the production of chimeric antibodies. Examples of approved chimeric antibodies are the anti-►CD20 antibody ►Rituximab and the anti-►EGF receptor antibody ►Cetuximab. Further improvement of monoclonal antibody engineering led to the generation of ►humanized antibody or completely human antibodies. Humanized monoclonal antibodies are created by insertion of murine complementarity-determing regions into the variable framework regions of a human antibody. Examples of humanized monoclonal antibodies approved for cancer therapy are the anti-HER2 antibody ►Trastuzumab and the anti-►CD52 antibody



Monoclonal Antibody Therapy. Figure 2 The graphs show a fully murine antibody on the upper left, a chimeric antibody consisting of human constant and mouse variable regions on the upper right, a humanized mouse antibody, where the murine CDR are grafted on a human antibody scaffold, on the lower left, and a fully human antibody on the lower right. Reprinted with permission from "UNI-MED Verlag, Bremen."

►Alemtuzumab. Completely human monoclonal antibodies could be obtained by development of transgenic mice or by use of ►phage display technology. The anti-EGF receptor antibody Panitumumab is the first completely human monoclonal antibody approved for treatment of cancer (Table 1).

Clinically Relevant Anticancer Monoclonal Antibodies

Approved Anticancer Monoclonal Antibodies

Since 1980 over 200 monoclonal antibodies have been evaluated in clinical trials for their efficacy and safety in the treatment of certain cancer entities. To date, 12 of these anticancer monoclonal antibodies have been approved for marketing in at least one country. The currently approved monoclonal antibodies including their target antigens, the antibody-structure and the first approved indication are described in Table 1. So far, one of the approved monoclonal anticancer antibodies has been withdrawn from the market: in a post-approval study it was shown that Edrecolomab, a murine anti-EpCAM antibody, is inferior to standard adjuvant treatment of colorectal cancer.

Unmodified Monoclonal Antibodies

As listed in Table 1 seven unmodified anticancer monoclonal antibodies have been approved to date. Two unmodified monoclonal antibodies which have significantly improved the treatment of certain cancer entities are exemplarily characterized in more detail:

Rituximab is a chimeric monoclonal antibody that binds to ►CD20, a cell surface protein located on mature B-lymphocytes and expressed on most B-cell ►non-Hodgkin lymphoma (NHLs) and on certain subtypes of B-cell differentiated ►acute lymphoblastic leukemia. Rituximab was initially approved as a single-agent therapy for relapsed or refractory low-grade, follicular ►B-cell NHL. The role of Rituximab for treatment of non-Hodgkin lymphoma has evolved

Monoclonal Antibody Therapy. Table 1 Currently approved monoclonal anticancer therapeutics

Generic name	Trade name	Description of antibody structure	Targeted Antigen	First approved indication	Year of first approval
Rituximab	Rituxan	Chimeric, IgG1κ	CD20	Single-agent therapy of relapsed or refractory low-grade or follicular B-cell NHL	1997
Trastuzumab	Herceptin	Humanized, IgG1κ	HER-2	Metastatic breast cancer expressing HER2	1998
Gemtuzumab ozogamicin	Myelotarg	Humanized, IgG4κ anti-CD33 linked to calicheamicin	CD33	Relapsed acute myeloid leukemia	2000
Alemtuzumab	Campath	Humanized, IgG1κ	CD52	Relapsed or refractory CLL	2001
Ibritumomab tiuxetan	Zevalin	Murine, IgG1κ radiolabeled (Y-90)	CD20	Relapsed or refractory low-grade, follicular B-cell NHL (refractory to Rituximab)	2002
I-131 ch-TNT		Chimeric, IgG1κ anti-DNA-associated antigens, radiolabeled (I-131)	DNA-associated antigens	Advanced lung cancer	2003
I-131 Tositumomab	Bexxar	Murine, IgG2aλ, anti-CD20, radiolabeled (I-131)	CD20	Follicular B-cell NHL, relapsed after chemotherapy, refractory to Rituximab	2003
Cetuximab	Erbitux	Chimeric, IgG1κ	Epidermal growth factor receptor (EGFR)	EGFR-positive, irinotecan-refractory metastatic colorectal carcinoma (colon cancer)	2003
Bevacizumab	Avastin	Humanized, IgG1	Vascular endothelial growth factor (VEGF)	Metastatic colorectal cancer	2004
Nimotuzumab	TheraCIM	Humanized, IgG1	EGF-receptor	Advanced head/neck epithelial cancer	2005
Panitumumab	Vectibix	Human, IgG2κ	EGF-receptor	Metastatic colorectal cancer	2006

significantly since its introduction. Current recommendations for application of Rituximab include the initial treatment of follicular and diffuse large B-cell non Hodgkin lymphoma in combination with polychemotherapy. Further clinical trials indicate that Rituximab is also beneficial in the treatment of ►CLL, ►Burkitt lymphoma and of mature and precursor B-ALL in combination with polychemotherapy. In addition to its relevance in anticancer therapy Rituximab is gaining increasing importance in the treatment of B-cell mediated autoimmune diseases such as rheumatoid arthritis. Most patients tolerate Rituximab well; the most common side effects are fever, chills, and nausea.

Trastuzumab is a humanized anti-HER2 monoclonal antibody, which has major impact on the treatment of breast cancer. Human epidermal growth factor receptor 2 (►HER-2) is overexpressed in 25–30% of ►breast cancer patients and is associated with a more aggressive form of malignancy. The addition of Trastuzumab to first-line chemotherapy in patients with ►HER2-overexpressing metastatic breast cancer significantly improved response rate, time to progression and overall survival. The first approved indication for trastuzumab therefore included the treatment of HER2-overexpressing metastatic breast cancer in combination with chemotherapy. In addition, Trastuzumab received approval as single-agent therapy in those patients with metastatic HER2-overexpressing breast cancer who do not respond to chemotherapy. Further clinical trials revealed a significant improvement of adjuvant chemotherapy results through addition of Trastuzumab. Thus, Trastuzumab has also become an important component of ►adjuvant therapy for those patients with HER2-overexpressing breast cancer. The most important adverse reaction of ►Herceptin is cardiac dysfunction; the incidence of cardiac dysfunction is highly dependent on prior ►anthracycline exposure.

The anti-cancer activity of unmodified monoclonal antibodies can be mediated by different mechanisms. An important, but not necessarily the most relevant mechanism is the destruction of targeted cells through activation of cell- and complement-mediated cytotoxicity, also called antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Only for two of the currently approved monoclonal antibodies, Rituximab and Alemtuzumab, ADCC and CDC have been identified as primary mode of action. Other modes of anti-tumor cell action of unmodified monoclonal antibodies include inhibition of growth factor and growth factor receptor interaction, downregulation of growth factor receptors and induction of ►apoptosis by influencing intracellular signaling. Three approved monoclonal antibodies, ►Cetuximab, Nimotuzumab and Panituzumab, are believed to function primarily by blocking epidermal growth factor receptor (►EGF-R inhibitors). One approved unmodified monoclonal antibody, ►Bevacizumab, inhibits tumor ►angiogenesis by binding to the ►vascular endothelial growth factor (VEGF). The in vivo mechanisms of the anti-HER2 antibody Trastuzumab have not been clearly resolved yet; downregulation of HER2-mediated signaling and induction of tumor-cell apoptosis have been proposed as possible modes of action in addition to activation of ADCC. However, functional assays with the anti-CD20 antibody Rituximab have shown that the target specific cytotoxic effect of unmodified monoclonal antibodies might be mediated by several pathways. Rituximab does not only activate cellular and complement mediated cytotoxicity; it additionally induces tumor-cell apoptosis by an increase of the intracellular calcium concentration.

Immunoconjugates

Immunoconjugates are antibodies or antibody fragments linked to cytotoxic substances such as radioisotopes, drugs or toxins. Through coupling with these cytotoxic substances the efficacy of immunotherapy with monoclonal antibodies might be improved.

Radioimmunoconjugates

Rationale of the application of ►radioimmunoconjugates in cancer therapy is a tumor-specific delivery of beta- or gamma-radiation emitting isotopes with minimal involvement of surrounding healthy tissue (synonym ►radioimmunotherapy). Additionally, tumor-resistance to unmodified monoclonal antibodies due to inhomogeneous target antigen expression and due to development of escape mechanisms might be overcome by radioimmunoconjugates.

The most commonly conjugated and clinically best studied radioisotopes are iodine-131 (^{131}I) and yttrium-90 (^{90}Y). Iodine-131 and yttrium-90 differ markedly in their physical properties including half-life, path-length, type of energy emissions, intracellular stability, and the organs targeted by the free radionuclide. ^{131}I emits beta- and gamma-radiation. Beta- and gamma-radiation of ^{131}I can be applied therapeutically; the highly energetic gamma-radiation can be additionally used for calculation of the individually required therapeutic dose of the applied radioimmunoconjugate. On the other hand, application of ^{131}I-conjugated immunoconjugates requires defined security procedures to protect the environment from gamma-radiation. Besides, gamma-radiation might be associated with severe myelotoxicity. The other radioisotope, ^{90}Y solely emits beta-radiation. Advantages of ^{90}Y might be its safety regarding environment exposure, its longer path length and its superior intracellular stability compared to ^{131}I.

The three radioimmunoconjugates currently approved for cancer therapy are the anti-CD20 immunoconjugates ^{131}I Tositumomab and ^{90}Y Ibritumomab

tiuxetan and the ^{131}I tumor necrosis treatment (TNT) antibody. The anti-CD20 immunoconjugates ^{131}I Tositumomab and ^{90}Y Ibritumomab tiuxetan received approvement for treatment of relapsed follicular or transformed B-cell non-Hodgkin Lymphoma. The ^{131}I anti-TNT immunoconjugate is approved for treatment of advanced ►lung cancer.

Toxin Immunoconjugates

Toxin immunoconjugates or ►immunotoxins are protein toxins that specifically target tumor cells via tumor-selective antibodies or antibody-fragments coupled to the toxin. Killing of tumor cells by immunotoxins requires internalization of the immunotoxin and subsequent translocation of the toxin domain to the cytosol. Various plant, bacterial or fungal toxins have been genetically fused or chemically conjugated to tumor-specific ligands. The most common fusion partners are Diphtheria toxin (DT), Pseudomonas exotoxin (PE), ricin and gelonin; these extremely potent catalytic toxins induce ►apoptosis of tumor cells by inhibition of protein synthesis.

More than twenty different immunotoxins have been evaluated concerning efficacy and safety, so far. Some of these immunotoxins including recombinant anti-CD22 Pseudomonas exotoxin have shown promising results in clinical phase I and II trials, but further clinical application has been limited through development of neutralizing antibodies, through immunotoxin-associated toxicities such as "vascular leak syndrome" and through the short half-life of recombinant, especially single chain-based immunotoxins.

Drug Immunoconjugates

Drug immunoconjugates are antibody-targeted cytotoxic drugs. One drug immunoconjugate, ►Gemtuzumab ozogamicin, has been approved for cancer treatment to date. Gemtuzumab ozogamicin is a humanized anti-►CD33 antibody that is linked to the anti-tumor antibiotic calicheamicin. In vitro studies have shown that Gemtuzumab ozogamicin induces apoptosis of ►CD33 expressing leukemic blasts. After binding to CD33, Gemtuzumab ozogamicin is internalized and calicheamicin, which is released inside the lysomomes, causes DNA double-strand breaks and cell death. Gemtuzumab ozogamicin is approved for treatment of relapsed ►acute myeloid leukemia in patients older than 60 years.

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Monoclonal Gammopathy of Undetermined Significance

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Definition

Monoclonal gammopathy of undetermined significance (MGUS) is defined by a monoclonal immunoglobulin in serum that amounts to 30 g/L or less, the presence of 10% or fewer plasma cells in the bone marrow, the absence of anemia and lytic bone lesions, and the absence of hypercalcemia and renal insufficiency related to the aberrant clonal proliferation of plasma cells. MGUS is the most common disorder among a group of diseases referred to as plasma cell dyscrasia. Because of the high prevalence in elderly patients (~5%), MGUS is a very common finding in the doctor's office. MGUS does not require treatment, but the need for monitoring is life long. This is due to the intrinsic propensity of MGUS cell clones to complete the malignant transformation process and, thus, undergo progression to multiple myeloma (MM). The progression from MGUS to MM, an invariably fatal neoplasm, occurs at a slow but remarkably steady rate of 1% per year.

Characteristics

Epidemiology

A comprehensive population-based study on the prevalence of MGUS in persons 50 years of age or older used serum samples from more than 21,000 residents of Olmsted County, Minnesota (corresponding to 77% of all county residents in this age bracket!) to find MGUS in 694 (3.2%) individuals. The age-adjusted rates were greater in men (4.0%) than in women (2.7%). The prevalence of MGUS was 5.3% among persons 70 years or older and 7.5% among persons 85 years or older. The concentration of M-protein was less than 10 g/L in the majority (64%) of cases. Of 79 patients tested, 21% had monoclonal urinary light

chain. The prevalence of MGUS was 8.6% in black individuals compared with 3.6% in white individuals. This race-related difference was confirmed in an independent study of inpatients from the U.S. Veteran Affairs hospitals, which showed that the age-adjusted prevalence rate for MGUS was 3.0-fold higher in African Americans than in Americans of Caucasian ancestry.

Etiology

The etiology of MGUS is not known. Epidemiological evidence points to age, gender and race as genetic risk factors for MGUS.

Pathogenesis

The pathogenesis of MGUS, including the reason why MGUS is a stable non-malignant disorder in the great majority of affected individuals but undergoes malignant progression in a subset of cases, is poorly understood. Approximately 50% of MGUS cell clones carry chromosomal translocations that rearrange the immunoglobulin heavy-chain gene cluster, *IGH*, at 14q32 with loci on one of five partner chromosomes: *CCND1* (cyclin D1 gene) at 11q13; *FGFR3* and *MMSET* (WHSC1) at 4p16.3; *CCND3* (cyclin D3) at 6p21; *MAF* (c-MAF) at 16q23; and *MAFB* at 20q11. Translocations of this sort are thought to play an important role in the initiation of MGUS and, in some cases, its subsequent progression to MM.

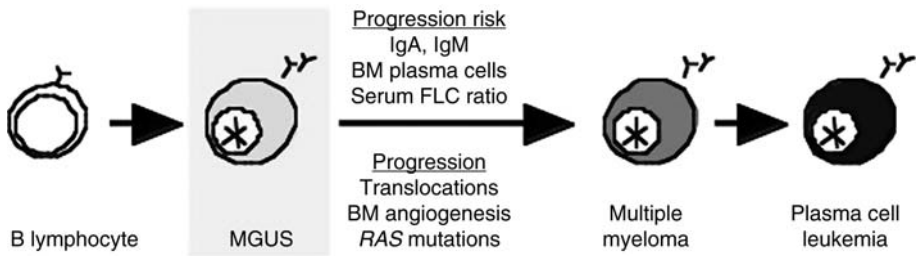
Approximately 40% of the plasma cell clones in MGUS, smoldering MM (SMM) and frank MM are hyperdiploid, suggesting that hyperdiploid MM originates from hyperdiploid MGUS. Likewise, deletions of chromosome 13q, which have an adverse prognostic association in MM, are found in similar frequencies in MGUS and MM, indicating a direct precursor-and-product relationship between the $13q^-$ pre-neoplastic and neoplastic state. Empirical observations of this kind suggest that MGUS occurs as distinct molecular subtypes, which lead, in turn, to different forms of MM.

The mechanism of progression of MGUS to MM appears to be multi-factorial, possibly involving (i) the breakdown of immune surveillance mechanisms that normally limit MGUS to the pre-malignant disease stage, (ii) the acquisition of *RAS*-activating point mutations in certain MGUS cell clones, and (iii) the shift in the balance of pro- and anti-angiogenic factors that results in increased angiogenesis in the bone marrow.

Considering that the most important features that differentiate MM from MGUS are the occurrence of lytic bone lesions, osteopenia, hypercalcemia, and pathological fractures, it is interesting to note that a recent study of 488 patients with MGUS revealed a 2.7-fold increase in axial fractures. The implication is that MGUS is associated with increased focal bone loss, albeit less severe than that seen in myeloma bone disease. This blurs the line between distinct stages of plasma cell neoplasia (MGUS, SMM, MM, plasma cell leukemia) and suggests, instead, that these disorders comprise an overlapping continuum characterized by increasing autonomy, malignancy, and clinical aggressiveness (Fig. 1).

Diagnosis

Serum protein electrophoresis in agarose gels provides a good screening method to detect the presence of a monoclonal immunoglobulin (mIg, M-protein, M-spike, paraprotein). Electrophoretic analysis is usually followed by immunofixation, which confirms the monoclonal immunoglobulin and determines the



Monoclonal Gammopathy of Undetermined Significance. Figure 1 MGUS is caused by a pre-malignant plasma cell clone that resides in the hematopoietic bone marrow and secretes monoclonal immunoglobulin (M-protein, paraprotein). MGUS is derived from a mature post-germinal center B lymphocyte that expresses immunoglobulin on the cell surface (*left*). MGUS clones that secrete IgA or IgM, lead to elevated numbers of plasma cells in the bone marrow, and/or distort the normal ratio of immunoglobulin κ and λ light-chains in the serum (FLC) exhibit increased propensity to undergo progression to multiple myeloma (MM). The molecular mechanism of this progression has been shown to include oncogene-activating *IGH* translocations and *RAS* mutations that occur in the MGUS clone and elevated blood vessel formation (neoangiogenesis) in the bone marrow. MM may be preceded by a less aggressive form of neoplasia, smoldering MM (SMM). MM may evolve into a more aggressive, terminal form of neoplasia, plasma cell leukemia (PLC). MGUS, SMM, MM and PLC thus comprise a continuum of plasma cell dyscrasias with increasing neoplastic potential.

underlying isotype of the Ig heavy-chain (γ1–4, μ, α, ε, δ) and Ig light-chain (κ, λ). The concentration of the M-protein is then measured with a rate nephelometer. This results in an estimate of the size of the plasma cell clone, which is useful for disease monitoring. Nephelometry is also used to measure the serum level of free light-chain (FLC). Deviations from the normal FLC kappa to lambda ratio (0.26–1.65) are a strong indication of monoclonal plasma cell disorder including MGUS. Serum M-protein higher than15 g/L should lead to electrophoresis and immunofixation of a concentrated aliquot from a 24-h urine specimen.

Predictors of Progression from MGUS to MM

At the time of recognition of MGUS, one cannot distinguish a patient whose condition will remain stable from one in whom progression to a plasma cell malignancy, particularly MM, will occur. Parameters not predictive of progression include age and sex; hepatosplenomegaly; levels of hemoglobin, serum creatinine and serum albumin; presence, type and amount of urinary light chain; and reduction of uninvolved immunoglobulins. Parameters that can predict progression include the concentration of serum M-protein; the type of M-protein, with IgA and IgM carrying an increased risk of progression compared to IgG; the abundance of bone marrow plasma cells; and an abnormal serum FLC ratio. Even in individuals at high risk of progression, death from unrelated diseases is greater than death from plasma cell neoplasia.

Distinguishing MGUS, SMM, and MM

Differentiating MGUS from MM can be difficult at initial presentation. It requires a complete blood count and radiographic bone survey as well as serum creatinine and calcium. A bone marrow aspirate and biopsy are indicated if the M-protein is higher than 15 g/L and the MGUS clone expresses IgA or IgM. Abnormal FLC ratio, radiographic bone survey and lab parameters are additional indications. The diagnosis of SMM is considered to be present in patients that do not have anemia, renal insufficiency, hypercalcemia or lytic bone lesions, but exhibit serum M-protein levels higher than 30 g/L and/or bone marrow plasma cell contents exceeding 10%. Elevated numbers of plasma cells undergoing cell division (plasma cell labeling index) and occurrence of plasma cells in the peripheral blood are also indicative of MM versus MGUS/SMM.

MGUS Variants

- IgM MGUS: Occurs in approximately 20% of patients with MGUS and deserves special attention because of IgM-related autoimmune disorders
- Biclonal gammopathies: Characterized by the presence of two different M-proteins; occurs in 2–6% of patients with monoclonal gammopathies including MGUS; rare cases of triclonal gammopathies have also been reported
- Idiopathic Bence Jones proteinuria: Urinary excretion of sometimes large amounts of monoclonal immunoglobulin light chain (Bence Jones protein) in the absence of "end-organ" damage, particularly kidney damage; rare, because Bence Jones proteinuria is usually associated with B cell and plasma cell neoplasms
- IgD MGUS: Almost always indicates MM, amyloidosis, or plasma cell leukemia; nonetheless, 6–8 year follow-ups in rare patients for without evidence of progression to malignancy have been reported

Association of MGUS with Other Diseases

- Lymphoproliferative disorders, such as Waldenström's macroglobulinemia, chronic lymphocytic leukemia/small lymphocytic lymphoma and marginal zone lymphoma; rarely in cases of leukemia, such as acute leukemia, hairy cell leukemia and T cell and chronic myelocytic leukemias
- Other hematological diseases including acquired von Willebrand's disease, pernicious anemia, refractory anemia, polycythemia vera, idiopathic myelofibrosis and Gaucher's disease
- Connective tissue disorders, such as rheumatoid arthritis, lupus erythematosis, scleroderma, polymyositis and ankylosing spondylitis
- Neurological disorders, particularly IgM MGUS neuropathy, in which the M-protein exhibits binding activity to myelin-associated glycoprotein (MAG).
- Motor neuron and other neurological diseases including amyotrophic lateral sclerosis and spinal muscular atrophy
- Rare dermatological diseases, such as *lichen myxedematosus*, *pyoderma gangrenosum*, necrobiotic xanthogranuloma; Schnitzler syndrome (chronic urticaria plus IgM monoclonal gammopathy)
- Liver disease associated with hepatitis C virus (HCV)
- Immunosuppression, frequently after transplantation
- Miscellaneous conditions; e.g., acquired angioedema, systemic capillary leak syndrome and idiopathic focal and segmental glomerulosclerosis

Antibody Activity of Mig and Associated Diseases

M-proteins, particularly IgM, can exhibit immunologic specificity (antibody activity) to certain self antigens and/or foreign antigens, possibly leading to full blown autoimmune disorders, such as chronic cold agglutinin disease, type II mixed cryoglobulinemia, and peripheral neuropathy. The self/foreign antigens implicated in these disorders are I/i red cell antigens/bacterial lipopolysaccharides, IgG Fc/hepatitis C virus, and neural carbohydrates/bacterial lipopolysaccharides, respectively. M-proteins with specificity for actin, von Willebrand's factor, thyroglobulin, insulin,

riboflavin, dextran, antistreptolysin-O, double-stranded DNA, apolipoprotein, thyroxin, lactate dehydrogenase, and several antibiotics have also been detected in rare cases.

Aggregation and Deposition of Mig and Associated Diseases

Independent of their immunologic specificity but dependent upon their concentration and physicochemical properties, M-proteins can become insoluble in the aqueous phase (serum, urine), resulting in homotypic aggregation (precipitation) and formation of a variety of amorphous (crystals) or structured (fibrils) protein deposits in various tissue sites. Diseases associated with protein depositions of this sort include AL amyloidosis, light chain-deposition disease, light chain-cast nephropathy, adult Fanconi syndrome due to crystal storing histiocytosis, and cryoglobulinemia type I.

Management of MGUS

Although patients with MGUS do not require treatment, they do need indefinite follow-up. Serum M-protein electrophoresis should be repeated 6 months after initial presentation and, if stable, annually thereafter. Low-risk MGUS (serum M-protein <15 g/L; IgG subtype; normal FLC ratio) can be followed less frequently. MGUS with increased risk for malignant conversion to MM (M-protein ≥15 g/L; non-IgG subtype; abnormal FLC ratio) should prompt a bone marrow examination and radiographic bone survey. Determination of the plasma cell labeling index, search for circulating plasma cells in the peripheral blood, and cytogenetic studies should also be done in these cases. Patients in the high-risk group should also be encouraged to participate in clinical trials of chemo-preventive agents, such as dehydroepiandrosterone (DHEA), biphosphonates and celecoxib, that may slow or abrogate the MGUS-to-MM progression.

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Monocyte

Definition

A large phagocytic white blood cell which, when it enters tissue, develops into a ►macrophage.

►Macrophages

Monogenic

Definition

A mode of inheritance characterized by familial segregation of the phenotype. It is due to the segregation of susceptibility/resistance alleles at one gene.

►Modifier Loci

Monokines

Definition

Powerful biomolecules secreted by ►monocytes and ►macrophages. These soluble molecules help direct and regulate the immune responses.

Monolayer Culture

Definition

Culture of cells in one cell thick sheets, usually in multiwell plates, Petri dishes or culture flasks, on flat surfaces coated with substances to promote cell attachment. Their advantage lies in high-throughput and the ability to grow cells from xenograft and patient tumors. They do not however incorporate the tumor cell microenvironment or tumor physiology.

►Three-Dimensional Tissue Cultures

Monomeric GTPases

Definition

Are a large superfamily of GTP-binding proteins that function in various cellular processes such as growth control (►Ras family members), organization of the cytoskeleton (►Rho family proteins), or intracellular transport between different compartments of the cell (Rab, ARF, Sar1, or Ran family members). Analogous

to the heterotrimeric ►G-proteins, they function as molecular switches by cycling between a GDP- and a GTP-bound state.

►G-Proteins

Mononeuropathy

Definition

Malfunction of a single peripheral nerve.

►Peripheral Neuropathy

Monosomy

Definition

A form of aneuploidy with the presence of only one chromosome (instead of the typical two in humans) from a pair.

►Gastrointestinal Stromal Tumor

Monoubiquitination

Definition

Addition of a single ubiquitin moiety to a protein.

►Ubiquitination

Morpheaform

Definition

Refers to ►basal cell carcinoma (BCC);Is an insidious tumor possessing innocuous surface characteristics that can mask its potential for deep, wide extension. The tumor is waxy, firm, flat-to-slightly raised, either pale white or yellowish, and resembles localized scleroderma, thus the designation morpheaform. Localization of this tumor by inspection or biopsy is impossible. The average subclinical extension beyond clinically delineated borders was 7.2 mm in one study. Treatment consists of wide excision or, preferably, ►Mohs micrographic surgery.

Morphogenesis

Definition

Is the development of body shape and organization during the embryonic development of an organism. It refers to a process in which cells organize into well defined three-dimensional structures that resemble the tissue of origin. For example, scatter factor has been shown to induce kidney epithelial cells (MDCK) to organize into renal tubular-like structures, breast epithelial cells to organize into mammary duct-like structures, lung epithelium to organize into alveolar-like structures, and microvessel endothelial cells to organize into capillary-like tubes. Apparently, scatter factor, acting through the c-Met receptor, activates a cell type-specific program of differentiation.

►Scatter Factor

Morphogenic Differentiation

Definition

The process in which cells convert from a less specialized structure to a more specialized structure. For example, individual cells aggregating to form a fluid filled lumen. Often, this involves significant cell movement, complex interactions to regulate cell proliferation and ►apoptosis, significant remodeling of the cytoskeleton, and differentiation into more specialized cell types.

►MET

Morphological Cell Transformation

Definition

The process by which carcinogenic chemicals, oncogenic viruses, or radiations, change the genotype and phenotype of the cell, such that the cells' morphology or

shape is changed, and the cells grow and pile up on top of one another, leading to the formation of foci.

►Chemically Induced Cell Transformation

Mortality Stage

Definition

Immortalization is the consequence of the inactivation or bypass of two mortality stage mechanisms, M1 and M2. Abrogation of M1 controls can be obtained through the activity of DNA tumor virus genes such as early genes E6 and E7 of human papillomavirus 16 (HPV16). In human fibroblast, retinoblastoma (Rb) and p53 proteins may be key factors regulating the mortality stage 1 mechanism. On the other hand, the inactivation of a second event, M2, was required to achieve immortalization by telomere stabilization due to telomerase activation or ALT.

►Early Genes of Human Papillomaviruses
►Human Papillomaviruses
►Stem Cell Telomeres

Mosaic

Definition

Mosaic tissue is composed of patches of cells from at least two sources, e.g. two sources of normal cells or a source of tumor and a source of normal cells, which, if part of the mouse skin, might result in striped or patchy patterns.

►Stromagenesis

Mosaic Vessels

Definition

Is another microcirculation pattern in tumors and the wall of mosaic blood vessels contains both tumor cells and endothelial cells. It may be a transition between Vasculogenic mimicry (VM) and endothelium-dependent vessels.

►Vasculogenic Mimicry

Mother Against Decapentaplegic, Drosophila, Homolog of 4

►Deleted in Pancreatic Carcinoma Locus 4

Motif

Definition

A distinctive sequence of residues (nucleotides or amino acids).

►Chromosomal Translocations

Motility

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Synonyms

Cell movement; Chemotaxis; Chemokinesis

Definition

Motility is a cellular function that leads to the translocation of the cell body through a coordinated sequence of distinct biological processes, including membrane protrusion at a leading edge, the formation of cell-substrate adhesion, the development of contractile force in the cell body, and detachment at the tail of the cell.

Characteristics

Cell motility is a critical feature of various physiological and pathological processes, such as development, wound healing, immunity, ►angiogenesis, ►invasion, and ►metastasis. It includes chemokinesis

(non-directional movement) and chemotaxis (directional movement). Cell motility is generally triggered by extracellular stimuli such as gradients of chemotactic factors. In the presence of chemoattractants, extracellular signals are transduced through cell-surface receptors which thus induce various intracellular responses, such as polarization and the reorganization of the actin cytoskeleton.

Cell motility has multiple processes: (i) membrane protrusion at the leading edge (filopodia and lamellipodia) (Fig. 1), (ii) ►adhesion of the protrusion to the substrate (focal complexes followed by focal adhesions), (iii) the development of contractile force (stress fibers and actin-myosin filaments) (Fig. 1), (iv) de-adhesion at the tail of the cell (rear detachment) and contraction causing a tail retraction toward the leading lamella.

Cell motility can be evaluated by various assays in experimental studies using cell lines. A gold colloid method can be used to evaluate chemokinesis. Transwell plates (Boyden Chamber method) can evaluate chemotaxis of cell penetration through the micropore membrane in such experiments; the membrane is uncoated in motility assays, with the membrane coated by matrigel in invasion assays. A wound healing assay evaluates the restoration rate of the monolayer of cultured cells after they are scratched by tips. Fluorescence imaging using green fluorescent protein (GFP) is a useful modality to elucidate the actin remodeling and focal adhesion dynamics in live cells.

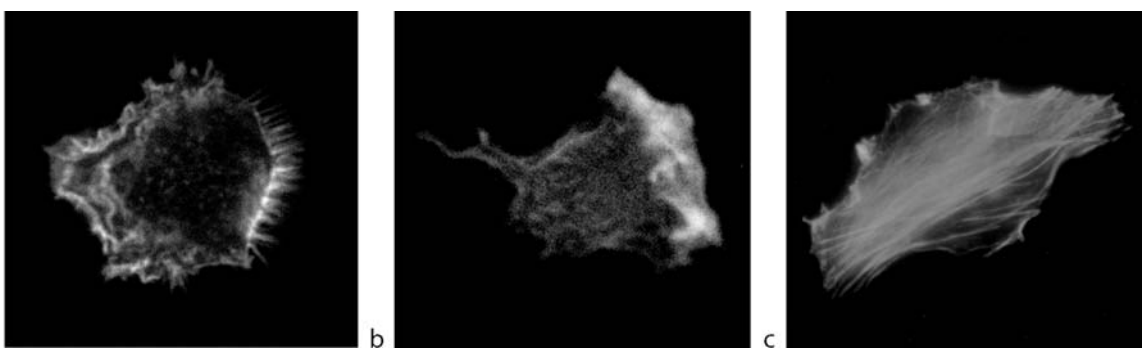
Mechanisms

The reorganization of the actin cytoskeleton and cell-substrate adhesion and de-adhesion are involved in the multiple processes of cell motility. Therefore, many molecules, including signaling molecules (►Signal Transduction), actin binding proteins, and ►cell adhesion molecules, thus play a role in cell motility.

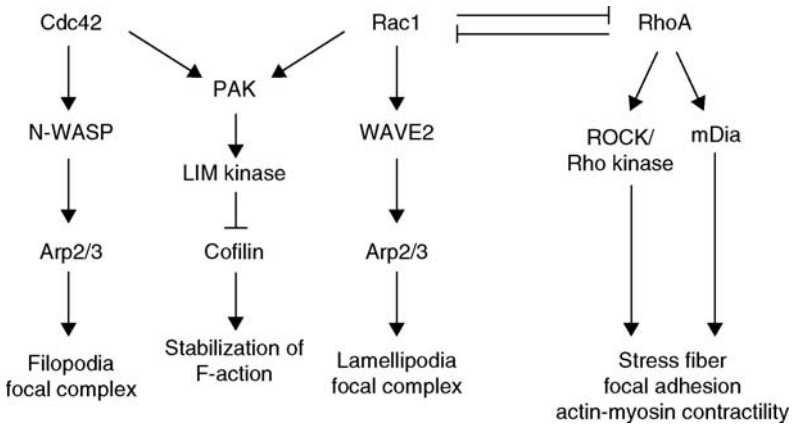
Cell motility is generally triggered by extracellular stimuli, and ►PI3K signaling plays a central role in the amplification of internal signaling asymmetry, thus helping to establish cell polarity and define the leading edge of the cells. PI3K activation in response to chemoattractants leads to the accumulation of phosphatidylinositol 3,4,5 triphosphate (PIP_3) which stimulates the ►Rho family proteins, such as Cdc42 and Rac1, and thereby inducing the formation of a leading edge.

Cell motility requires a cycle of actin polymerization and depolymerization (reorganization of actin filaments). The cytoskeleton of fibrous actin (F-actin) is formed by globular actin (G-actin), through the dynamic reorganization of actin filaments. Regarding the reorganization of actin filaments, important downstream targets of Rho family proteins are actin-related protein (Arp) 2/3 complex and actin depolymerizing factor (ADF)/cofilin (Fig. 2).

The Arp2/3 complex is composed of two Arps (Arp2 and Arp3) which act as templates for new actin filaments, and five additional subunits called ARPC1–5. The principal function of the Arp2/3 complex is to create branch points by nucleating the assembly of actin filaments near the leading edge. The activation of the Arp2/3 complex is governed by the Rho family and the Wiscott-Aldrich syndrome protein (WASP) family. Among the Rho family, Cdc42, Rac1 and RhoA play important roles in the reorganization of the actin networks. Through the activation of members of the Rho family, the WASP family can activate the Arp2/3 complex. Subsequently, the activated Arp2/3 complex can nucleate the assembly of new actin filaments, while also assembling a daughter filament at an angle of 70° to the mother filaments. As a consequence, Arp2/3 is localized at branch points in the cortical filament network, binding the pointed end of a new daughter filament to the mother filament thus leaving the barbed (growing) end available for filament elongation. This Arp2/3 complex activation is thus considered to play a critical role in the rapid formation of the actin networks at the leading edge.



Motility. Figure 1 Actin filaments in a human fibrosarcoma cell line HT1080. (a) filopodia, (b) lamellipodia, (c) stress fibers.



Motility. Figure 2 Signal pathways associated with cell motility.

The WASP family proteins, including the WASP subfamily and the WAVE/Scar subfamily, are physiological activators of the Arp2/3 complex. The WASP family proteins have a common C-terminal VCA domain (Verprolin-homology, cofilin-homology and acidic) which is responsible for the Arp2/3 complex activation. Among the WASP family members, neural-WASP (N-WASP) and WAVE2/Scar2 are widely expressed. N-WASP plays a key role in the formation of filopodia and microspike, composed of straight-bundled actin filaments. Cdc42 controls the extension of filopodia through the activation of N-WASP. In contrast, WAVE2 plays a key role in the formation of lamellipodia (membrane ruffle), branched actin filament networks. Rac1 regulates the formation of lamellipodia through the activation of WAVE2. Unlike Cdc42 and Rac1, RhoA regulates the assembly of stress fibers and actin-myosin filaments. The targets of RhoA include ROCK/Rho kinase and mDia. RhoA regulates myosin-mediated contractility through these downstream effectors. Therefore, during directional cell movement, Cdc42 and Rac1 stimulate formation of protrusions at the leading edge, and RhoA induces a retraction at the tail end of the cells.

ADF/cofilin is also involved in the reorganization of actin filaments. Cofilin increases the rate of depolymerization of the actin filaments by interacting with both G-actin and F-actin. In addition, cofilin can also increase the number of free barbed ends by severing the actin filaments and thereby increasing the rate of actin polymerization. The cofilin's actin binding and severing activities are regulated by its phosphorylation. The stimulation of cofilin's activities requires dephosphorylation of cofilin. In contrast, the inhibition of cofilin's activity requires its phosphorylation by activated LIM-kinase. The activation of LIM-kinase occurs through the stimulation of PI3K-induced activated Cdc42 and activated Rac1, which activate PAK (p21-activated kinase). PAK in turn phosphorylates LIM-kinase thereby activating it.

Regarding cell-substrate adhesion, focal contacts are the main adhesive structures connecting the cell with the extracellular matrix (ECM). Focal contacts have several stages of development such as focal complexes and focal adhesions. Focal complexes are usually formed at the surface of filopodia and lamellipodia. RhoA changes focal complexes into mature focal adhesions. They consist of two components: (i) transmembrane component made by clusters of integrins linked to ECM and (ii) submembranous component made by a complex of specific proteins such as vinculin, talin, and focal adhesion kinase (FAK), which is linked to the ends of actin microfilaments. Integrins are transmembrane receptors that function as heterodimers, thus consisting of distinct α and β subunits which are linked to form binding sites for specific ECM components such as fibronectin and laminin. The integrin-linked focal adhesions not only support cell adhesion to ECM but also transmit extracellular signals to intracellular signal transduction pathways. As a result, the dynamic regulation of focal adhesion assembly and disassembly regulates integrin-dependent cell ►migration.

The actin-myosin contractility in the cell body is directly controlled by modulating the myosin light chain (MLC). The activation of myosin contractility is achieved by the action of MLC kinase and the opposite action of MLC phosphatase. The RhoA-mediated activation of downstream effectors such as ROCK/Rho kinase and mDia promote the polymerization of actin bundles and phosphorylation of MLC. The resulting actin-myosin contractile forces contribute to the formation of stress fibers and mature focal adhesions. Therefore, the RhoA-mediated actin-myosin contractile force promotes the locomotion of the cell body and the trailing edge.

The final stage of the cell motility involves the disruption of ECM-integrin interactions, cell-substrate

detachment, and tail retraction toward the leading lamella. The cell detachment also depends on the coordinated interaction of actin and actin-binding proteins, integrins, signaling molecules and effecter enzymes including proteases, kinases, and phosphatase. However, its mechanisms are still not well understood.

Clinical Aspects

Metastasis is a critical biological behavior of cancer. The metastatic spread of a primary tumor to distant organs involves a series of predicable processes; (i) tumor cell detachment from the primary tumor, (ii) migration and invasion through the basement membranes of blood and lymph vessels, (iii) embolization, arrest and binding to vascular endothelium at secondary organs, and (iv) extravasion, and invasion to the secondary organ. During these processes, cell motility is an essential cellular function. Clinical studies in human cancers have revealed that several molecules associated with cell motility affect tumor metastasis. For example, a reduced expression of integrin α3 is associated with a poor prognosis in lung cancer patients. In addition, tetraspanins, also known as transmembrane 4 superfamily (TM4SF), are glycoproteins which have been shown to form functional complexes with integrins (tetraspan network). Among the members of tetraspanins, motility related protein-1 (MRP-1/CD9) and KAI1/CD82 (►KAI1/CD82 Metastasis Suppressor) suppress cell motility. Clinical studies among human cancers have demonstrated that a reduced expression of MRP-1/CD9 and KAI1/CD82 frequently occurs in human cancers and that such a reduced expression of MRP-1/CD9 and KAI1/CD82 is associated with tumor metastasis and a poor prognosis in cancer patients. As a result, these molecules play an important role as ►metastatic suppressor genes. Therefore, elucidating the regulation of motility in tumor cells would greatly help in the development of new treatment strategies for cancer patients (►Anti-Metastatic Therapies).

►Adhesion

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Mouse

►ARF Tumor Suppressor Protein

Mouse Double Minute 2

Definition

►Mdm2.

Mouse Double Minute Gene

►MDM Genes

Mouse Mammary Tumor Virus

Definition

MMTV; is a ►retrovirus that causes mammary tumors in mice.

►Retroviral Insertional Mutagenesis

Mouse Models

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Synonyms

Knock-out mice; Transgenics; Inbred lines; Congenics; Genetically engineered mice (GEM); Xenograft models

M

Definition

Mouse models of human cancer are mice that are used experimentally in a laboratory to understand genetic susceptibility to cancer, unravel the mechanisms of particular cancer pathways, aid in drug development, identify carcinogens, test chemopreventative and therapeutic agents, study the effect of environmental exposures on cancer risk, and understand tumor biology.

Characteristics

History

The use of mice to study cancer began in 1916 when Clarence Cook Little noted that different strains of inbred "fancy" mice had different susceptibilities to transplanted ►sarcomas. In 1928 at Cold Spring Harbor, Edwin Carleton MacDowell discovered that particular inbred strains of mice developed spontaneous leukemia. One year later, Little founded what is now known as the Jackson Laboratory for the purpose of developing pure inbred strains of mice to study biology and in particular, genetics. The breeding of mice to generate models for understanding cancer continues today, as does the Jackson Laboratory, but a wealth of sophisticated tools for manipulating and tracking genes and cancer cells using the mouse have since been developed.

Biological Relevance

Why are mice good models for human cancer? From comparative biology, it is clear that the pathology of many mouse tumors are similar to their human counterparts. Tumors in mice often begin as benign lesions, progress to an invasive stage and then may metastasize as in human cancer. Environmental factors that predispose humans to cancer including ►UV light, radiation, and ►tobacco smoke also induce cancers in mice. The same tumor suppressor genes and ►oncogenes that are important in human tumorigenesis such as ►*TP53*, ►*CMYC (MYC)*, ►*KRAS2* (RAS) and ►*PTEN* are mutated, amplified or activated in mouse tumors. There are some aspects of human tumorigenesis that are not mimicked in the mouse. For example, the size of telomeres is different between mice and humans, and the site of ►metastasis from a primary tumor varies between humans and mice. Nonetheless, mouse models have contributed greatly to the understanding of human cancer and the development of drugs for cancer therapy.

Genetically Engineered Mice

Genetically engineered mice, also known as transgenics and knock-out mice, are the work horse of mouse models. The use of GEM has led researchers to a better understanding of the mechanism of hundreds of cancer-related genes. GEM containing different genetic alterations can also be crossed to each other in order to understand the effects of perturbing both genes.

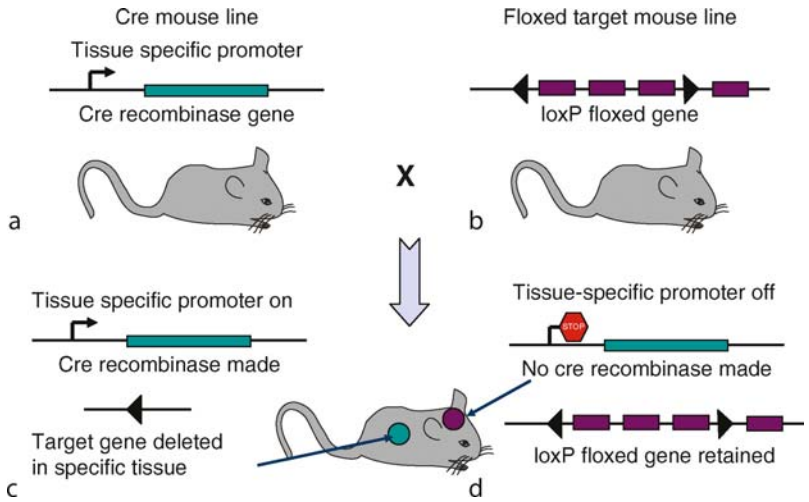
Transgenic Animals

The breakthrough in the use of mouse models to study cancer was the ability to introduce specific genetic alterations into target endogenous genes in mouse embryonic stem cells by homologous recombination. To do this, a transgene consisting of DNA of interest is injected into a newly fertilized mouse egg by pronuclear injection. Embryos carrying the inserted DNA are then implanted into females and the pups that are delivered are chimeric (carry the newly inserted sequence in some tissues but not others). Mice that have the transgene inserted into germline cells (cells that produce eggs or sperm) are bred to other mice. The resulting offspring carry the transgene in every cell of the body. Different types of mutations including null, point mutations, insertions, deletions or inversions can all be introduced into cancer genes in the mouse. Knock-out mice are those that have disruption of a target gene by insertion of a null allele. Knock-in mice are those that carry an insertion of a different form of a working target gene.

Conditional Transgenics

Since total disruption of many genes is lethal during mouse embryonic development, more sophisticated strategies for conditionally knocking-out genes have been developed. One added benefit of these conditional genetic alterations is that they more closely mimic sporadic human cancer since the gene perturbation is limited to a certain cell type or a certain phase in life. A commonly used strategy for creating conditional transgenic mice is the Cre/LoxP site-specific recombination system (Fig. 1). In this strategy, the target gene is flanked by loxP recombinase recognition sites in such a way that normal gene function is not altered in mice lacking Cre recombinase. When Cre recombinase is present, the sequence between the LoxP sites is deleted somatically. Cre expression can be driven by tissue or developmental specific promoters introduced by a second gene construct or by treating of the mice with hormones such as estrogen. Other systems that work in a similar manner include the Flp/FRT recombination system.

Two transgenic mouse lines are created. The first mouse line has the Cre recombinase gene driven by a tissue specific promoter. The second mouse line carries a construct with loxP recombination sites flanking the gene or exons to be deleted. The two lines of mice are crossed. In offspring, Cre recombinase will be expressed only in the specific tissues in which the



Mouse Models. Figure 1 Strategy for creating a tissue-specific knock-out mouse.

promoter driving cre is normally active. In those tissues the target gene will be deleted by recombination driven by Cre recombinase. All other tissues will retain a normal working copy of the target gene.

Inducible Systems

Alternative models for conditional-transgenic mice are inducible systems. These are transgenic systems in which the gene of interest is under the control of a promoter, such as the Tet promoter, and is expressed only in the presence of an antibiotic such as Doxycycline. Some inducible systems are "leaky" in that gene expression can occur in the absence of antibiotic.

Spontaneous Recombination

To more closely mimic the timing of mutations in oncogenes that occur in human tumorigenesis, a "hit and run" method of introducing oncogene mutations was developed. Transgenic mice carrying a non-activated form of an oncogene are created. After spontaneous recombination, the latent allele becomes active, and the cell in which this occurs becomes predisposed to cancer. This method leads to the creation of mice that develop activated mutations in oncogenes in somatic cells at a low frequency similar to the somatic rate of mutations that occur in humans.

Genetic Susceptibility

Inbred Strains

Inbred mice are mice in which sister and brother pairs have been mated together for many generations to produce mice that are genetically homogenous. Different inbred lines show large genetic and phenotypic differences. There are multiple inbred strains of mice used for the identification of cancer susceptibility genes due to large differences in predisposition to cancer. Some strains used for cancer susceptibility gene mapping include *FVB/NJ, C57Bl/6, 129/Sv, Cast/EiJ, Spret/EiJ, A/J, AKR/J, C3H,* and *Balb/C*.

Mapping of Susceptibility Genes

To map susceptibility genes, researchers create mice that have different combinations of genes from susceptible and resistant parents and grandparents. In brief, mice from strains that are susceptible to cancer are crossed to mice from strains that are resistant to cancer. The resulting F1 mice are a 50/50 mix of genes from susceptible and resistant strains of mice. F1 mice are then either crossed to other F1 mice (F2 intercross) or are crossed to one of the parental strains (F1 backcross). The resulting F2 or F1 backcross mice are phenotyped for cancer susceptibility and are genotyped for polymorphisms that exist between the strains of mice. Statistical correlations, called linkage or quantitative trait locus analysis, between phenotypes and genotypes are performed to identify genomic regions housing putative susceptibility or resistance genes. In addition to identification of single genes contributing to cancer susceptibility, mouse models allow the detection of genetic interactions, or specific combinations of genetic variants that determine cancer risk.

Congenics

Congenic lines are used to refine regions of linkage. Congenic mice are mice that carry a little bit of the chromosomal region housing a susceptibility gene and the rest of their DNA is from the resistant strain

of mice. To create congenic mice, susceptible F2 or F1 backcross mice that carry a particular susceptible locus are crossed to mice from the susceptible parental strain. Offspring are genotyped to determine which still carry DNA from the susceptible parent at the general region of interest. These mice are phenotyped to determine which are still susceptible to cancer. Mice carrying smaller pieces of the susceptibility region, due to recombination events in meiosis, and are still cancer prone are crossed back to the resistant parent until the locus is small enough to identify potential candidate genes.

Recombinant Inbred-Lines and Other Types of Inbred Mice

A variety of specialized inbred lines have been used for mapping cancer susceptibility genes. Recombinant inbred-lines are the result of using F2 sister/brother pairs from a mating between two different strains to establish a new set of inbred lines. A variation on classic recombinant inbred lines is recombinant congenic lines in which one parent contributes 1/8 of the genome and the other parent 7/8. Advanced intercross lines are those in which multiple generations of random mating precede interbreeding. Consomic lines are lines in which just one chromosome is replaced by a donor strain.

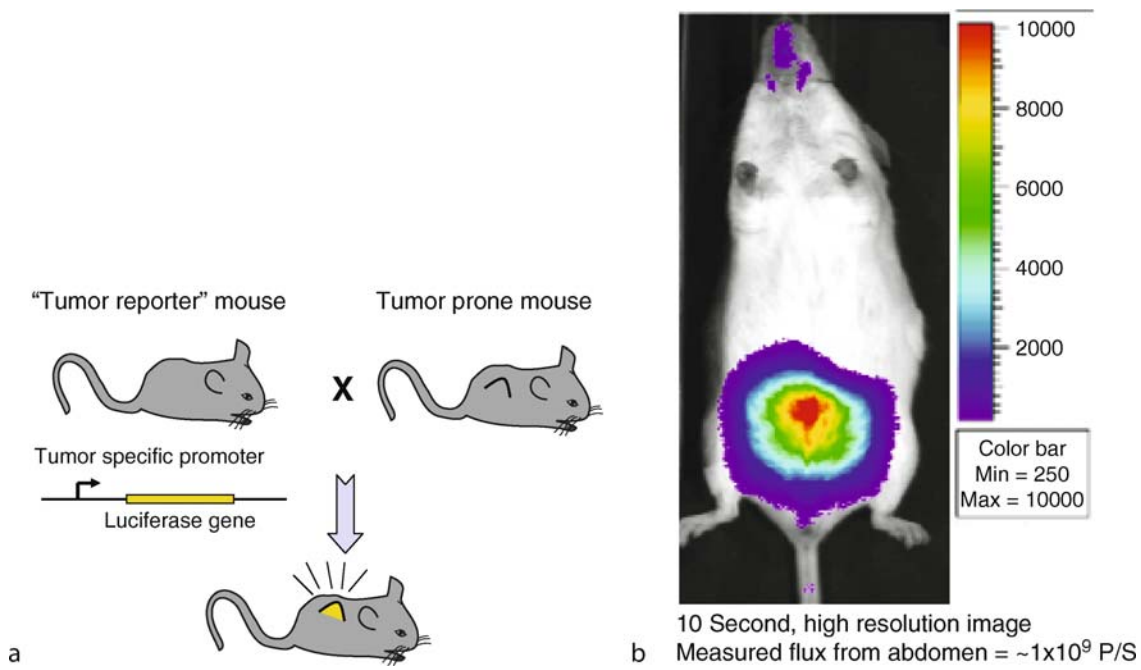
Outbred Strains

Outbred strains of mice are lines of mice which have not been bred to homogeneity. These mice retain a small number of polymorphisms which can contribute to phenotypic differences. The strength of using outbred strains for gene mapping is that the phenotypic differences and small genetic differences can be used to refine regions of linkage.

Therapeutics

Xenografts

A xenograft is the surgical transplantation of tissue from one species to another. Xenograft mouse models have been an integral part of the oncology drug discovery and development process. To make a xenograft for cancer studies, human tumor cell lines or tumor cells are injected into the flanks of nude mice. Since these mice lack a functioning immune response, most tumor cells are able to grow unchecked. Mice with tumors can be treated with potential therapeutic agents and the response of the cells or cell lines to the drugs monitored by assessing the change in tumor size.



Mouse Models. Figure 2 Bioluminescence Imaging of Tumors in Mice. (a) To create mice in which tumors can be studied and followed using bioluminescence, mice that are predisposed to developing tumors are crossed to transgenic mice that have a copy of the luciferase gene under the control of a tumor-specific promoter. The resulting offspring have tumors that can be visualized using detection systems. (Figure complements of Byron Hann). (b) A mouse predisposed to prostate cancer has been crossed to a mouse with the PSA (prostate specific antigen) promoter driving the luciferase gene. The mouse was treated with luciferin and anesthetized. The growth of the tumor can be followed over time. (Figure complements of Bryon Hann and Scott Lyon).

Several weaknesses of using xenographs for the study of human cancer have been identified; these include the inability to study premalignant states, the inability to study stromal interactions, and the lack of an intact immune response in the host animals.

Molecular Imaging

The use of the mouse for therapeutic development is enhanced by the recent ability to image tumors *in vivo*. *In vivo* molecular imaging allows researchers to continually monitor the size, growth and response rate of tumors to chemotherapeutic agents without sacrificing the mouse. Methods for imaging include magnetic resonance imaging (MRI), position emission tomographic imaging (PET) and fluorescence-based imaging with green fluorescent protein (GFP) tagged proteins or bioluminescence. Bioluminescence imaging utilizes mice that both carry a transgene with the firefly gene luciferase under a tumor-specific promoter and are tumor prone due to direct injection of tumor cells, treatment with carcinogens, or genetic causes (Fig. 2a). Following development of a tumor, these mice are injected with luciferin, a substrate for luciferase, and imaged (Fig. 2b). Imaging of the same mouse can be done on a repeated basis.

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Mouse Podoplanin

►Podoplanin

MPF

►Cyclin Dependent Kinases
►Mesothelin

MPO

Definition

Myeloperoxidase is a peroxidase enzyme most of all present in neutrophil granulocytes. It is a lysosomal protein stored in azurophilic granules of the neutrophil.

►Benzene and Leukemia

MPP2

►Forkhead Box M1

MPS1

Definition

Monopolar spindle 1; Is a kinetochore-associated protein kinase that is required to establish spindle assembly checkpoint. MPS1 acts upstream of MAD2-mediated inhibition of the APC/C. In Saccharomyces cerevisiae (yeast), MPS1 is also required for spindle pole body duplication.

►Mitotic Arrest-Deficient Protein 1 (MAD1)

MRE11

Definition

Binds to Rad50 and Nbs1 [►Nijmegen breakage syndrome], having nuclease activity on both single- and double-stranded DNA. It is involved in a number of roles in DNA maintenance. ►Repair of DNA.

►Homologous Recombination Repair

MRI

Definition

Synonym: Magnetic Resonance Imaging; NMR (Nuclear magnetic resonance). A versatile non-invasive

imaging approach that relies upon strong magnetic fields and radiowaves to produce anatomical images of the body with exquisite resolution (25–100 μm). In addition, MRI can be used to non-invasively image reporter gene function and the accumulation of contrast agents/probes *in vivo*. MRI is an important tool for cancer imaging, especially in the central nervous system, the head and neck region, liver and soft tissues.

►Bioluminescence Imaging
►Dynamic Contrast-Enhanced Magnetic Resonance Imaging
►Positron Emission Tomography

MRIT

►FLICE Inhibitory Protein

mRNA

Definition

Messenger RNA, the type of RNA that transports the genetic information coded in DNA residing in the nucleus to the cytoplasm where it is translated into protein.

mRNA Profiling

Definition

Corresponds to specific patterns of gene expression in a particular cell type of an organism, at a particular time, under particular conditions. DNA-based microarrays enable us to analyze the mRNA present in a tissue, and to quantify the levels of expression of tens of thousands of genes simultaneously in a single sample and to compare with the levels in normal control tissue. Changes in gene expression may underlie many biological phenomena, such as the tumoral process. These profiles can help to establish diagnosis and often correlate with different prognoses and different responses to therapy.

►Microarray (cDNA) Technology

MRP

Definition

►Multidrug Resistance Protein; A protein associated to resistance of cancer to most drugs.

►Mutidrug Resistance

MSC

Definition

Mesenchymal stem cell. MSCs are multipotent stem cells found in the bone marrow.

►Adult Stem Cells

MSH2–6

Definition

Human mut S homologues 2–6. These genes encode proteins that are components of the human ►mismatch repair (MMR) system. Defect MMR may cause microsatellite instability in tumors.

►Microsatellite Instability

MSP

►Macrophage-Stimulating Protein

MST

Definition

Malnutrition screening tool.

►Nutrition Status

MST1

►Macrophage-Stimulating Protein

MST1R

►Ron Receptor

MSTS Functional Evaluation System

Definition

System, produced by the Musculoskeletal Tumor Society, which rates the functional result of musculoskeletal tumor surgery and which enables global comparability of results.

►Cryosurgery in Bone Tumors

MTOC

►Centrosome

mTOR

Definition

Mammalian target of rapamycin; Is a serine/threonine kinase of ~289 kDa, contains a catalytic kinase domain and a FKBP12-rapamycin binding (FRB) domain near the C-terminus. The C-terminus of mTOR shares strong homology to the catalytic domain of PI3K. mTOR is considered a member of the PIK-related kinase (PIKK) family, which includes MEC1, TEL1, DNA-PK, ATM, and ATR.

►PI3K Signaling
►Rapamycin
►Mammalian Target of Rapamycin

MTS1

►CDKN2A

MTT Assay

Definition

(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a standard colorimetric assay for measuring cellular proliferation (e.g., cell growth). MTT is reduced to formazan by the mitochondria of living cells. It is used to determine drug cytotoxicity.

MUC1

Definition

Transmembrane glycoprotein tumor antigen that is overexpressed on cells of different tumors; can be found in large amounts in soluble form in serum and ►ascites fluid. MUC1 represents a target of human antitumor antibody and ►cytotoxic T-lymphocyte responses that are generated in the absence of ►helper T cells.

MUC1–20

Definitions

Mucins1–20.

Mucin

Definition

Large polymers of glycosylated proteins that participate in the physiological activity of the gastrointestinal tract. Often detected in glandular or adenocarcinoma tumor samples.

►Mucoepidermoid Cancer

Mucinous Cystadenocarcinoma

Definition

Type of Pancreas Cancer.

►Appendiceal Epithelial Neoplasms

Mucinous Neoplasms

Definition

Cancerous cells that produce mucus that may progress to develop gross mucinous ascites as part of the ►pseudomyxoma peritonei syndrome.

►Appendiceal Epithelial Neoplasms

Mucins

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Definition

Mucins, a family of heavily glycosylated high-molecular-weight proteins built by tandemly arranged repeat domains, establish a selective molecular barrier at the epithelial surface. Alterations in mucin expression or in their ►glycosylation patterns influence cellular growth, differentiation, transformation, adhesion, invasion, and immune surveillance. Cancer-associated mucin antigens can be exploited for the diagnosis and prognosis, as well as for the development of cancer vaccines.

Characteristics

Classically, mucins are known to play a central role in the protection and lubrication of luminal epithelial surfaces. Several lines of evidence also suggest that mucins might serve as cell-surface receptors and sensors, transducing signals in response to external stimuli leading to coordinated cellular responses that include proliferation, differentiation, apoptosis, and secretion of specialized cellular products. The basic structure of a mucin molecule includes a protein backbone, termed "apomucin," decorated with a large number of ►*O*-linked oligosaccharides and a few ►*N*-glycan chains. Additional posttranslational modifications, including sialylation and sulfation, have also been commonly reported in mature mucin glycoproteins. Structural features common to all known mucins are: (i) A large extracellular region composed of a variable number of tandem repeats (►VNTRs) rich in serine, threonine (which constitute the potential *O*-glycosylation sites), and proline (so-called STP-rich regions) residues. In humans, several distinct genes (*MUC1–MUC20*) coding for mucin core polypeptides, containing 5–500 tandem repeats, have been thoroughly characterized. Some mucins, like MUC5B and MUC7 exhibit a constant number of repeats, whereas in the majority of mucins the amino acid length of their repeated domains is subjected to genetic polymorphism. (ii) The high content of *O*-linked oligosaccharide side chains attached to a serine and threonine-rich peptide core. ►*O*-glycosylation with complex oligosaccharides plays a crucial role in determining mucin structure and function. Indeed, the sugar moieties constitute up to 80% of the mucin's total mass, confer an elongated and rigid structure to the molecule, and contribute to the rheological properties of mucus secretions. Under normal physiological conditions, mucins exhibit a well-defined tissue-, time-, and developmental state-specific expression pattern. Deregulated mucin expression has been regarded as one of the most prominent characteristics of many types of cancers.

Considering the cellular expression pattern, mucins can be grouped into two main classes:

1. Membrane-bound mucins (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, and MUC20) comprising three distinct regions: a large extracellular domain, a hydrophobic transmembrane region, and a short cytoplasmic tail that associates with cytoskeletal elements and participate in signal transduction. The extracellular region of membrane-bound mucins can be released from the cell surface through proteolytic processing. In addition, many secreted forms result from alternative mRNA splicing that eliminates the transmembrane domain. Among the cell-surface expressed mucins, MUC1 and MUC4 are the best characterized with respect to signal transduction.
2. Secreted mucins, which lack a transmembrane domain and are thus secreted into the extracellular space. Two subfamilies of secreted mucins can be distinguished, the "gel-forming" mucins (MUC2, MUC5AC, MUC5B, MUC6, and MUC19), and the "nongel-forming" mucins (MUC7, MUC8, and MUC9). Some genes encoding gel-forming mucins (MUC2, MUC5AC, MUC5B, and MUC6) share some degree of sequence similarity and appear to be clustered

on chromosome 11p15. A distinct feature of secreted gel-forming mucins is their ability to form a network of multimeric complexes as a result of intra- and intermolecular associations through cysteine-rich domains.

In epithelial cancers, many of the markers for premalignant and malignant cells have been found on the carbohydrate and peptide moieties of mucins, and these structures greatly contribute to the phenotype and biology of cancer cells. Upregulation, downregulation, and de novo expression of apomucins have been reported in cancer cells. Alterations differentiating cancer mucins from normal mucins include both, mucin protein expression and distinct patterns of *O*-glycosylation. These abnormalities are frequently associated with altered adhesion and invasion and could modulate the immune recognition of cancer. To date, it is not clear whether deregulation of mucin expression is a cause or a consequence of the transforming phenotype. An increasing number of reports point to mucins as key players in the development and onset of malignancy. The ectopic and/or overexpression of membrane-bound mucins in tumor cells have been generally associated with an augmented malignant potential and a poorer prognosis for the patient. In contrast, the expression of secreted mucins in certain types of tumors appears to correlate with a better prognosis. Muc2 ►knock out mice have been shown to be significantly more prone to the development of gastrointestinal tumors compared to their wild-type counterparts, suggesting a tumor suppressive effect for this mucin.

Several mucins appear to contribute to the malignant phenotype of tumor cells. Hence, membrane-bound MUC1 probably represents the best characterized mucin, and a number of studies converge to suggest a direct involvement of MUC1 in the cellular processes underlying the molecular pathogenesis of various neoplastic diseases. Overexpression of this mucin has been shown to induce cellular transformation and to inhibit stress-induced apoptosis. MUC1 is known to transduce signals through the MAP kinase and β-catenin pathways. Signals are transmitted to the nucleus through association of the MUC1 cytoplasmic tail (MUC1-CT) with signal transduction molecules, including ►β-catenin, p120 ►catenin, ►Ras, ►p53, and the ►estrogen receptor α (ER-α). The intermolecular association of MUC1 and β-catenin is increased upon phosphorylation of MUC1-CT by activated EGF receptor and c-Src. The increased MUC1-CT/β-catenin interaction is likely to reduce the pool of cytoplasmic free β-catenin, thereby weakening intercellular adherence. MUC1-CT has also been proposed to localize to mitochondrial membranes under conditions of genotoxic stress, where it attenuates the apoptotic pathway and confers resistance to apoptosis-inducing drugs. Another important aspect of MUC1 biology is its role in tumor immune evasion. Thus, cells overexpressing MUC1 adhere deficiently to natural killer cells and cytotoxic T-cells, and are therefore resistant to killing by these cells. The impact of MUC1 overexpression in cancer biology correlates with clinical observations; a high level of MUC1 expression and secretion has been shown to be associated with poor prognosis and high metastatic potential.

Mucins are the main carriers of altered oligosaccharide patterns in cancer cells. They are normally highly glycosylated, and thus, the antigenic peptide core is physically inaccessible to the immune system, particularly to antibodies. However, the deregulation of key enzymes (►glycosyltransferases) of the *O*-glycosylation pathway leads to the generation of new glycan antigens at the mucin surface. The resulting aberrant glycosylation patterns include short mono-, di- and trisaccharide chains, and result in both the exposure of cryptic peptide-core epitopes, and in the expression of cancer-associated *O*-linked carbohydrate chains (i.e., TF, Tn, and sialyl-Tn antigens) discontinuously distributed along the peptide backbone. Therefore, these neopeptide and neocarbohydrate epitopes become accessible for recognition by components of the immune system. The expression of distinct oligosaccharide structures, alone with the differential glycosylation of mucin core proteins, endows tumor cells with an enormous range of potential ligands for interacting with a number of cell surface receptors.

Immunohistochemical studies have identified several ►tumor-associated antigens (TAAs) on mucins. Antibodies against TAAs on mucins are widely used in the clinic as diagnostic tools (e.g., serum assays) for different types of cancers. Increased concentration of mucin-type glycoproteins in serum has been correlated with enhanced tumor burden and poor prognosis. Several assays detecting TAAs in serum samples are directed against the circulating MUC1 antigen (e.g., CA15-3, CASA, MCA, CA549, and BCM), which are used in the postoperative monitoring of breast and ovarian carcinoma patients. Another tumor marker of clinical significance is DU-PAN2, which is exposed by MUC1 and is being used for the diagnosis of pancreatic adenocarcinoma. The cancer antigen 125 (CA125), the most widely used biomarker for ovarian cancer, was identified in 2001 as mucin MUC16. MUC16/CA125 represents the only FDA-approved ovarian cancer relapse marker. Several other serum tumor assays which are mainly applied for gastrointestinal cancer diagnosis, involve mucin-associated carbohydrate antigens, like CA72-4 (sialyl-Tn) and CA19-9 and CA50 (sialyl-Lewis^a). It was established that sera from normal individuals as well as from tumor patients contain circulating antibodies directed towards the MUC1 protein core. High anti-MUC1 antibodies levels have

been associated with a significantly better survival of patients with breast, lung, or pancreatic cancer. Therefore, the determination of MUC1-specific antibodies in sera could be of clinical value.

Cancer-associated mucins show antigenic differences from normal mucins. They are highly immunogenic, and as such, represent potential targets for immunotherapy. Particularly, MUC1 is the most intensively investigated mucin protein with regard to cancer immunotherapy, and this mucin is the target of several anticancer vaccine clinical trials. Underglycosylated MUC1, the tumor-associated mucin ►glycoform, leads to enhanced accessibility of the peptide core by many MUC1-specific antibodies and to increased humoral and cellular immune responses, as shown for breast, colon, pancreatic, and ovarian cancer patients. Studies on vaccination using synthetic peptides (and glycopeptides) derived from MUC1 show promising activities including induction of the proliferation of T-cells and cytotoxic T-cell responses.

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Muclin (Mouse)

►Deleted in Malignant Brain Tumours 1

Mucocellular Layer

Definition

Layer of mucus predominantly produced by goblet cells of colonic epithelium and overlaying the surface of this epithelium. Exfoliated colonocytes and occasional free cells (leukocytes, lymphocytes, macrophages) as well as cell debris are often found in this layer.

►Exfoliation of Cells

Mucoepidermoid Cancer

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Synonyms

Malignant salivary gland cancer; Low grade/well differentiated MEC; High grade/undifferentiated MEC

Definition

Muco ►epidermoid carcinoma (MEC) is the most common malignant salivary gland tumor that can arise from both major and minor salivary gland tissues.

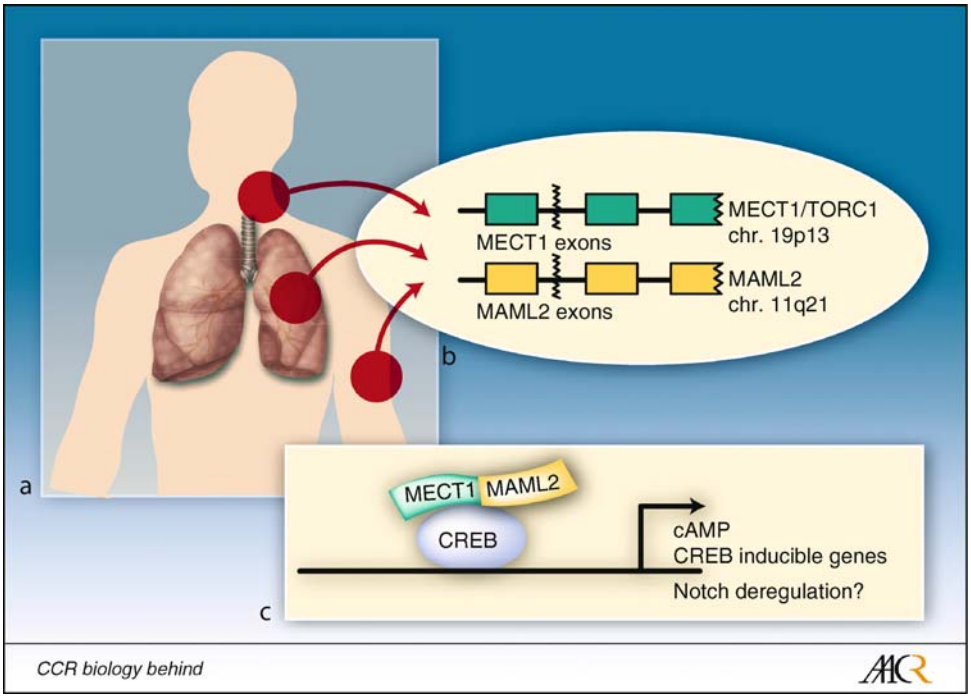
Characteristics

Historical Data

Volkmann is credited with first describing this clinical entity in 1895. Stewart (1945) first used the term "mucoepidermoid" and Foote and Frazell (1953) defined the histological features and malignant nature of this disease.

Histological Features

Mucoepidermoid carcinoma is a unique epithelial tumor that arises from salivary gland tissues and is characterized by varying proportions of three different cell types represented by epidermoid cells, ►mucin producing cells, and "intermediate cells." These tumors are predominantly detected within major salivary glands (such as the parotid and submandibular glands), as well as from minor salivary glands that are scattered throughout the oral cavity and upper aerodigestive tract. In addition, tumors with mucoepidermoid-like histological features are occasionally seen as primary lesions within the bronchopulmonary tree, the skin, as well as other organ sites. Importantly, there is cytogenetic and molecular genetic data that unifies the biology of these mucoepidermoid-like tumors irrespective of the primary tissue of origin (Fig. 1). To address the wide range of morphological differentiation and the corresponding differing degree of clinical aggressiveness, histological grading schemas have been proposed which score tumors as high grade/undifferentiated, as intermediate grade, or as low grade/well-differentiated. These systems employ either qualitative or quantitative parameters



Mucoepidermoid Cancer. Figure 1(a) MEC-like tumors arising from major or minor salivary glands of the head and neck, from the bronchopulmonary tree, or from a cutaneous clear cell hidradenoma which have been reported to express the identical Mect1-Maml2 product. (b) The t(11;19) chromosomal breakpoint maps within intron 1 of both the Mect1/Torc1 and Maml2 genes in all cases tested and results in exon1 swapping between the genes. (c) Preliminary model depicting the ability of Mect1-Maml2 to activate a group of cAMP/CREB inducible genes which are hypothesized to participate in tumorigenesis. (Figure is reproduced from Ref. [1]).

based on the relative amounts of epidermoid and mucin-type cells and the degree of cellular differentiation and cyst formation detected.

Epidemiology

Although mucoepidermoid cancer is the most common malignant salivary gland tumor, it is, nonetheless a rarely diagnosed entity that can be estimated at approximately 1,000–2,000 new cases/year in the United States. In the majority of cases, no known environmental or inherited factors can be elicited. Prior radiation exposure, however, is a known susceptibility factor and an exceptionally strong radiation dose risk has been noted for this disease. In addition, mucoepidermoid cancer frequently presents as a second malignancy in children.

Etiology

Although several oncogenes had been previously implicated in a subset of these tumors, a major advance in understanding the biology of mucoepidermoid cancer was the detection of a reciprocal t(11;19) ►chromosomal translocation in several cases of salivary gland or pulmonary mucoepidermoid cancer. This led to the eventual mapping and identification of the chromosomal breakpoints which revealed the fusion of two new genes called Mect1 (also known as Torc1 and CRTC1) on chromosome 19p13 and Maml2 gene on chromosome 11q21. This oncogenic event fused 42 amino acids from exon 1 of the Mect1 gene with 981 amino acids from exons 2–5 of the Maml2 gene. Further evidence proving that this genetic event was pathogenic for cancer development was (i) the demonstration that Mect1-Maml2 could neoplastically transform rat epithelial cells and (ii) that blocking Mect1-Maml2 protein expression resulted in the selective killing of mucoepidermoid cancer cells but had no effect on non-mucoepidermoid salivary cells. The subsequent application of ►fluorescent in situ hybridization (►FISH) techniques on tumor biopsy sections or the application of ►RT-PCR techniques on RNA extracted from frozen or paraffin embedded material has demonstrated that 70–90% of well-differentiated mucoepidermoid salivary tumors are Mect1-Maml2 fusion positive while 0% of undifferentiated cancer were fusion-positive. This is the first evidence that high grade/undifferentiated mucoepidermoid cancer did not arise from well-differentiated tumors and, therefore, is biologically distinct should be classified as a separate disease entity. Although the biology underlying Mect1-Maml2 induced tumorigenicity is still unknown, a working model has been developed based on recent data showing that Mect1 is an essential co-activator of the ►CREB/cAMP

signaling pathway while the Maml2 gene is a human homologue of the drosophila Mastermind gene which regulates ►notch signaling pathways.

Staging Parameters

►Salivary gland tumors largely use the same TNM (tumor size-nodal status-metastasis status) system from the America Joint Committee on Cancer that is applied for other head and neck cancers. One important exception, however, is the T staging for tumors located within the head using both size criteria (T1-T3) as well as evidence for infiltration into local tissues and bone (T4a; T4b). In addition, as mentioned above, histological grading is an important parameter for assessing patient prognosis and treatment planning.

Treatment

Surgical resection with clear surgical margins is the primary treatment of choice for all subtypes of malignant ►salivary gland cancer, including mucoepidermoid cancer. In addition, cervical neck dissection is undertaken for cases where there is clinical, radiological, or pathological evidence demonstrating or suggesting cervical node involvement. Due to the small number of cases, the variability in tumor differentiation, the unpredictability of selected clinical cases, and the lack of randomized trials for this disease, the role of adjuvant treatments (►Adjuvant therapy) such as radiation and chemotherapy with cytotoxic or biological agents is unknown. Many clinicians include adjuvant radiotherapy for cases with positive or close tumor margins after resection, for large or locally infiltrating tumors (stages T3-T4), and for tumors that are scored as high grade or undifferentiated. The benefit of adjuvant chemotherapy is unknown. Similarly, the optimal management of recurrent disease is undefined and these cases are best managed in referral centers with access to surgical expertise and clinical trials.

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Mucosa Associated Lymphatic Tissue Lymphoma

Definition

►MALT Lymphoma.

Mucosal-Associated Lymphoid Tissue

Definition

MALT; Comprises all lymphoid cells in epithelia and in the lamina propria lying below the body's mucosal surfaces. The main sites of mucosal-associated lymphoid tissues are the gut-associated lymphoid tissues (►GALT) and the bronchial-associated lymphoid tissues (►BALT).

Mucositis

Definition

Inflammation of the lining of the upper digestive tract.

►Temsirolimus

Mullerian Inhibiting Hormone

Definition

Synonym: MIH; Mullerian inhibiting substance (MIS) or antimullerian hormone (AMH). A homodimeric growth factor belonging to the transforming growth factor beta (TGFβ) superfamily originally identified to inhibit development of the mullerian ducts in the embryonic male.

►Granulosa Cell Tumors

Multi-domain Transporters

►Modular Transporters

Multi-Marker Panels

Definition

Simultaneous use of multiple diagnostic ►biomarkers when each biomarker is specific for some pathological

condition, but is known to be present only in a limited proportion of cases. The use of multiple overlapping biomarkers increases the probability of the successful detection of the disease, but does not guarantee it.

►Exfoliation of Cells

Multicellular Layers

Definition

MCL, multilayered cell cultures, MCC; are planar aggregates of tumor cells grown on porous support membranes in culture medium which are a model for the extravascular compartment of solid tumors considered to more accurately reflect the tumor microenvironment than monolayer cultures. They have a very similar structure to multicellular spheroids often with regions of ►hypoxia and central necrosis due to diffusion limitations of oxygen, glucose and other nutrients. Their well-defined planar structure makes them very useful for drug transport studies. Some cell lines that do not grow as spheroids will grow as multilayers. They are more expensive than spheroids with lower throughput and are used mainly for drug transport and binding and drug effect studies.

►Hypoxia and tumor microenvironment
►Three-Dimensional Tissue Cultures

Multicellular Spheroids

Definition

MS, Spheroids, Multicellular tumor spheroids, MTS, MCTS; Spherical aggregates of cells grown in culture medium in liquid overlay of stirred suspensions. They are an in vitro model of avascular tumor nodules or micrometastases or the extravascular space of solid tumors and generally grow to a diameter of 100–220 μm similar to the maximum intercapillary region. They develop many features related to diffusion limitations of oxygen, glucose and other nutrients, including hypoxia and central necrosis and three dimensional interactions typical of tumors. They are simple and cost effective with a well defined spherical geometry and provide consistent results with many cell lines available. Not all cell lines grow as spheroids and they lack of cellular stroma and hence their extracellular matrix is not entirely typical of tumors. Spheroids do not incorporate tissue elements such as functional capillaries and do not evaluate therapeutic index in therapeutic studies. They have been used to study the effect of 3D structure, cell-cell and cell-extracellular matrix (ECM) interactions on tumor cell biology, gene expression, invasion, response to therapy, drug penetration, tumor markers, nutrient gradients, and tumor cell metabolism.

►Hypoxia and tumor microenvironment
►Three-Dimensional Tissue Cultures

Multidrug Resistance

Definition

MDR; Describes the phenomenon that cancer cells are simultaneously resistant against several structurally unrelated drugs, i.e., that these drugs are not effective in killing the cells. Several mechanisms have been recognized that cause multidrug resistance, including the presence of membrane transporters that affect accumulation of anticancer drugs in the cancer cells.

►ABC-Transporters
►Membrane Transporters
►P-Glycoprotein
►Fluoxetine

Multidrug Resistance Proteins

Definition

MRP; Are plasma membrane transporters and belong to subfamily C of ATP-binding cassette transporters. In terms of amino acid sequence similarity and substrate specificity, they are very distinct from ►P-glycoprotein, which is member 1 of subfamily B. Substrates of multidrug resistance proteins are organic anions or anionic conjugates of hydrophobic substances with glutathione, glucuronate, sulfate, or phosphate. Multidrug resistance proteins are efflux pumps localized in many cells and tissues including gastrointestinal and renal epithelia, hepatocytes, and blood–tissue barriers. They are essential for absorption, elimination, and detoxification of endogenous and ►xenobiotics. Because multidrug resistance proteins also function as anticancer drug efflux pumps, they are involved in conferring multidrug resistance to cells.

►Membrane Transporters

Multidrug Resistance Transporters

►ABC-Transporters

Multidrug Transporter

►P-glycoprotein

Multilayered Post-Confluent Cultures

Definition

MPCC; Cell cultures grown in V-bottomed multi-well plates which develop a three dimensional structure and plateau phase growth considered to more accurately reflect the tumor microenvironment than monolayer cultures or stirred cell suspensions.

►Three-Dimensional Tissue Cultures

Multimeric Antibody Fragments

►Diabody

Multiple Cutaneous and Uterine Leiomyomatosis

Definition

MCUL; A dominantly inherited tumor predisposition syndrome caused by mutations in the *fumarate hydratase* gene.

►Fumarate Hydratase

Multiple Endocrine Adenopathy Type 1

►Multiple Endocrine Neoplasia Type 1

Multiple Endocrine Neoplasia Type 1

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Synonyms

Multiple endocrine adenopathy type 1; MEN1; Wermer syndrome; WMEN1

Definition

►Multiple endocrine neoplasia type 1 (MEN1) is characterized by the combined occurrence of tumors of the parathyroids, pancreas, and pituitary, and is inherited as an autosomal dominant trait.

Characteristics

Multiple Endocrine Neoplasia type 1 is characterized by the combined occurrence of tumors (Table 1) of the ►parathyroid glands, ►pancreatic islet cells and ►anterior pituitary gland. Some patients may also develop adrenal cortical tumors, carcinoid tumors, facial angiofibromas, collagenomas and lipomas. MEN1 is inherited as an autosomal dominant disorder with a high degree of ►penetrance, such that >95% of patients develop clinical manifestations of the disorder by the fifth decade. The earliest age at which manifestations of MEN1 may occur has been reported to be 5 years. Parathyroid tumors, which lead to hypercalcemia (Table 1), are the most common feature of MEN1 and occur in about 95% of patients. Pancreatic islet cell tumors, which consist of gastrinomas, insulinomas, pancreatic polypeptidomas (PPomas), glucagonomas and vasoactive intestinal polypeptidomas (VIPomas) occur in about 40% of patients; and anterior pituitary tumors, which consist of prolactinomas, somatotrophinomas, corticotrophinomas or non-functioning adenomas,

Multiple Endocrine Neoplasia Type 1. Table 1 Multiple endocrine neoplasia type 1 (MEN1), its characteristic tumors and associated biochemical abnormalities

Tumors	Biochemical features
Parathyroids	Hypercalcemia and ↑ PTH
Pancreatic islets	
Gastrinoma	↑ Gastrin and ↑ basal gastric acid output
Insulinoma	Hypoglycemia and ↑ insulin
Glucagonoma	Glucose intolerance and ↑ glucagon
VIPoma	↑ VIP and WDHA
PPoma	↑ PP
Pituitary (anterior)	
Prolactinoma	Hyperprolactinemia
GH-secreting	↑ GH
ACTH-secreting	Hypercortisolemia nad ↑ ATCH
Non-functioning	Nil or α subunit
Associated tumors	
Adrenal cortical	Hypercortisolemia or primary hyperaldosteronism
Carcinoid	↑ 5-HIAA
Lipoma	Nil
Facial	
Angiofibromas	Nil
Collagenomas	Nil

Autosomal dominant inheritance of MEN1 has been established. Key: ↑, increased; PTH, parathyroid hormone; VIP, vasoactive intestinal peptide; WDHA, watery diarrhea, hypokalemia and achlorhydria; PP, pancreatic polypeptide; GH, growth hormone; ACTH, adrenocortcotrophin; 5-HIAA, 5-hydroxyindoleacetic acid.

occur in about 30% of patients. The clinical manifestations of MEN1 are generally related to their products of secretion and less frequently to their primary sites or metastasis. In the absence of treatment, MEN1 tumors result in an earlier mortality in patients.

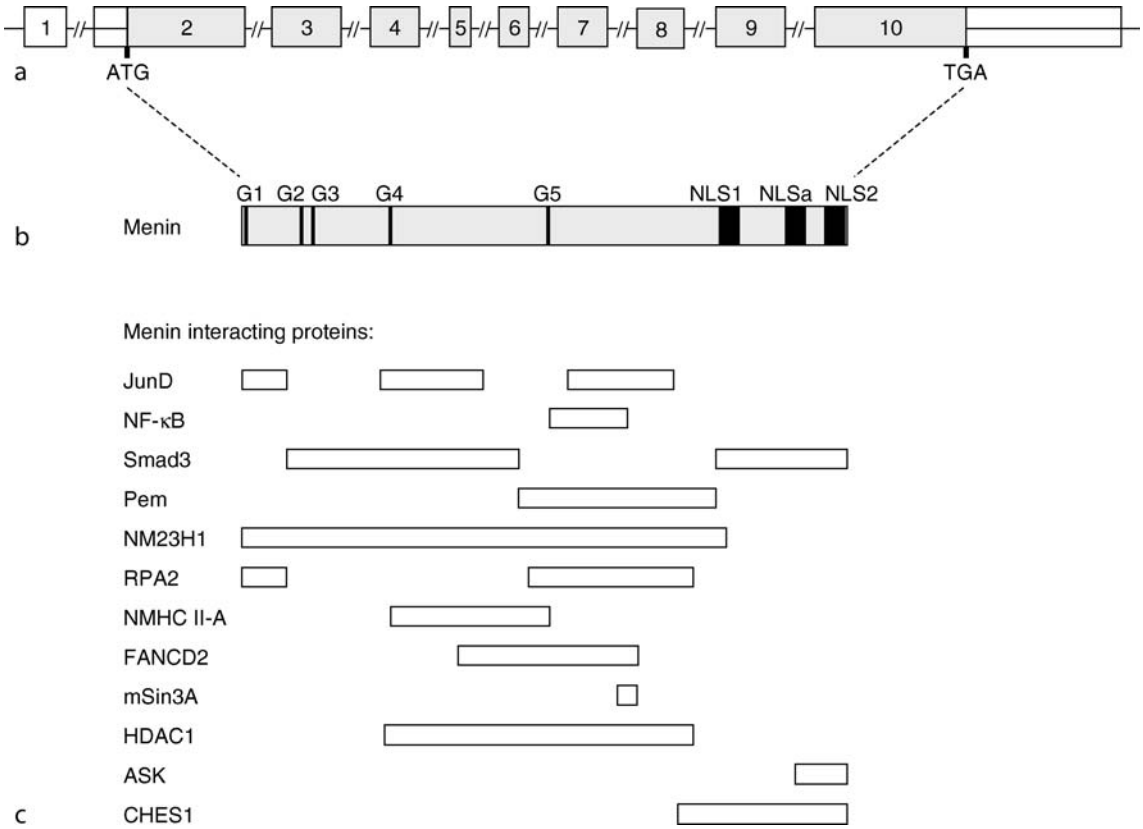
Genetics

The *MEN1* gene, which is a ►tumor-suppressor gene located on chromosome 11q13 was identified in 1997. It consists of ten ►exons (Fig. 1) that span ~>9 kb of genomic DNA and encodes a 610-amino acid protein referred to as menin. Mutations of the *MEN1* gene have been identified and the total number of mutations identified between 1997 and 2007 is 1,336. These 1,336 mutations comprise of 1,133 germline and 203 somatic mutations. The 1,133 independent *MEN1* germline mutations consist of 459 different germline mutations and the 203 somatic mutations, consist of 167 different mutations. Sixty-one of the germline mutations are also found to occur as somatic mutations, thus yielding 565 different *MEN1* mutations.

The 1,133 germline mutations are scattered throughout the entire 1,830 bp ►coding region and splice sites of the *MEN1* gene, and consist of 23% ►nonsense mutations, 9% splice site mutations, 41% ►frameshift deletions or insertions, 6% in-frame deletions or insertions, 20% ►missense mutations, and 1% whole or partial gene deletions. Several mutations recur in apparently unrelated kindreds, thereby indicating potential mutational hot spots. Mutations at four sites account for 12.3% of all mutations. These consist of a deletion at ►codons 83–84, a deletion at codons 210–211 and an insertion at ►codon 516, and a ►nonsense mutation Arg460Stop. The three deletional or insertional ►frameshift mutations occur in repetitive DNA sequences and this is consistent with a replication-slippage model of mutagenesis. Between 5% and 10% of MEN1 patients do not harbor mutations in the coding region or adjacent splice sites. Such MEN1 patients may harbor mutations in the ►promoter or ►untranslated regions, or ►introns, or they may represent phenocopies.

MEN1 Mutations in Hereditary Endocrine Disorders

The role of the *MEN1* gene in the etiology of other inherited endocrine disorders, in which either parathyroid or pituitary tumors occur as an isolated endocrinopathy, has been investigated by mutational analysis. *MEN1* mutations have been reported in 42 families with isolated hyperparathyroidism (FIHP) and 38% of these are missense mutations that are less likely to result in an inactivated protein. This contrasts significantly



Multiple Endocrine Neoplasia Type 1. Figure 1 Schematic representation of the genomic organization of the *MEN1* gene, its encoded protein (menin) and regions that interact with other proteins. (a) The human *MEN1* gene consists of ten exons that span more than 9 kb of genomic DNA and encodes a 610-amino acid protein. The start (ATG) and stop (TGA) codons in exons 2 and 10, respectively, are indicated. (b) Menin has three nuclear localization signals (NLSs) at codons 479–497 (NLS1), 546–572 (NLSa) and 588–608 (NLS2), indicated by closed boxes, and five putative guanosine triphosphatase (GTPase) sites (G1-G5) indicated by closed bars. (c) Menin regions that have been implicated in the binding to different interacting proteins (Table 2) are indicated by open boxes, below. The regions of menin that interact with GFAP, vimentin, Smad 1/5, Runx2, MLL-histone methyltransferase complex and estrogen receptor-alpha remain to be determined.

($p < 0.01$) with the situation in MEN1 patients in whom 20% are missense mutations. These observations are consistent with a more likely association between missense mutations and the milder FIHP variant, but it is important to note that the mutations associated with FIHP are also scattered throughout the coding region and not clustered, a situation that is similar to that found for germline *MEN1* mutations. Furthermore, the occurrence of protein-truncating mutations in FIHP patients and particularly deletions, such as the 4 bp, involving codons 83–84 which are identical to those observed in MEN1 patients, makes it difficult to establish an unequivocal phenotype/genotype correlation. However, the sole occurrence of parathyroid tumors is remarkable in FIHP families that harbor similar *MEN1* mutations as MEN1 families, and the mechanisms that determine the altered phenotypic expressions of these mutations remain to be elucidated. In addition, nonsense mutations (Tyr312Stop and Arg460Stop) have been detected in MEN1 families with the Burin or prolactinoma variant which is characterized by a high occurrence of prolactinomas and a low occurrence of gastrinomas. Furthermore, a splice site mutation (c.446–3C > G) has been detected in an MEN1 kindred from Tasmania, in whom there is an absence of somatotrophinomas. However, some other families with isolated acromegaly do not have abnormalities of the *MEN1* gene, even though segregation analysis and tumor deletion mapping have indicated that the gene is likely to be located on chromosome 11q13. Interestingly, mutations of the aryl hydrocarbon receptor interacting protein (*AIP*) gene, which is also located on chromosome 11q13 and 2.7 Mb telomeric to the

MEN1 gene, have been identified in some families with isolated acromegaly.

MEN1 Mutations in Sporadic Non-MEN1 Endocrine Tumors

Parathyroid, pancreatic islet cell, and anterior pituitary tumors may occur either as part of MEN1 or more commonly as sporadic, nonfamilial tumors. Tumors from patients with MEN1 have been observed to harbor the germline mutation together with a somatic loss of heterozygosity (LOH) involving chromosome 11q13 or point mutations, as expected from Knudson's model and the proposed role of the *MEN1* gene as a tumor suppressor. However, LOH involving chromosome 11q13, which is the location of *MEN1*, has also been observed in 5–50% of sporadic endocrine tumors, thus implicating the *MEN1* gene in the etiology of these tumors. Somatic *MEN1* mutations have been detected in ~20% of sporadic parathyroid tumors, ~40% of gastrinomas, ~15% of insulinomas, ~60% of VIPomas, ~15% of non-functioning pancreatic tumors, ~60% of glucagonomas, <5% of adrenal cortical tumors, ~35% of bronchial carcinoid tumors, <5% of anterior pituitary adenomas, ~10% of angiofibromas, and ~30% of lipomas. The 203 somatic mutations are scattered throughout the 1,830 bp coding region, and 18% are nonsense mutations, 40% are frameshift deletions or insertions, 6% are in-frame deletions or insertions, 7% are splice site mutations, and 29% are missense mutations. The tumors harboring a somatic *MEN1* mutation had chromosome 11q13 LOH as the other genetic abnormality, or "hit," consistent with Knudson's hypothesis. These findings indicate that inactivation of the *MEN1* gene has a role in the etiology of some sporadic endocrine tumors.

Function of the MEN1 Protein (Menin)

Menin, which is ubiquitously expresses, has three nuclear localization signals (NLSs) in its C-terminal segment. Subcellular localization studies have shown that menin is predominantly a nuclear protein in non-dividing cells, but in dividing cells it is found mainly in the cytoplasm. Studies of protein–protein interactions have revealed that menin interacts with several proteins involved in transcriptional regulation, genome stability, cell division and proliferation (Fig. 1, Table 2).

Mouse Models for MEN1

Mouse models for MEN1 have been generated through homologous recombination (i.e. knockout) of the mouse *Men1* gene. In all of these adult heterozygous *Men1* +/− mice, develop parathyroid tumors, pancreatic islet cell tumors, anterior pituitary tumors, and adrenal cortical tumors. Some *Men1* +/− mice have also developed tumors of the thyroid, testis, ovary, and mammary glands. Homozygous *Men1*−/− mice have been reported to die in utero at embryonic days 11.5–13.5. The *Men1*−/− mice are often developmentally delayed and smaller, develop craniofacial abnormalities that involve the BPM-2 signaling pathway, suffer from extensive hemorrhage and edema, and have abnormalities of the neural tube, heart, and liver. These results from the *Men1*−/− mice reveal an important role for the *MEN1* gene in the embryonic development of multiple organs.

Clinical Application

MEN1 is an uncommon disorder, but because of its autosomal dominant inheritance, the finding of MEN1 in a patient has important implications for other family members; first-degree relatives have about a 50% risk of development of the disease. Occasionally, MEN1 may arise sporadically (i.e. without a family history), although it may be difficult to make the distinction between sporadic and familial forms. In some cases, a family history may be absent because the parent with MEN1 is not available and may have already died before any manifestations developed, and other cases

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Multiple Endocrine Neoplasia Type 1. Table 2 Functions of menin indicated by direct interactions with proteins

Function	Interacting partner
Transcription regulation	JunD NFkB (p50, p52, p65) Pem Sin3A HAD Smad1 Smad3 Smad5 Runx2 MLL histone methyl-transferase complex ER-alpha CHES1 Double-stranded DNA
Genome stability	RPA2 FANCD2 NMMHC II-A
Cell division	GFAP Vimentin
Cell cycle control	nm23[a] ASK

[a]Functions reported include involvement in cell cycle withdrawal, decrease of cell motility, cell differentiation, apoptosis and DNA repair.

may be due to de novo mutations, which will be transmitted in an autosomal dominant manner in future generations.

Screening for MEN1 in patients involves the detection of tumors and ascertainment of the germline genetic state, that is, normal or mutant gene carrier. Detection of tumors entails clinical, biochemical, and radiologic investigations for MEN1-associated tumors in patients. The characterization of the *MEN1* gene has facilitated identification of individuals who have mutations and hence a high risk of acquiring the disease.

Molecular genetic analysis for MEN1 either by detecting mutations or by performing segregation studies using linked markers is useful in identifying individuals who are mutant carriers and thus have a high risk of tumor development. The advantages of DNA analysis are that it requires a single blood sample and does not in theory need to be repeated because the analysis is independent of the age of the individual and provides an objective result. Such mutational analysis may be undertaken in children within the first decade because tumors have developed in some children by the age of 5 years, and appropriate intervention in the form of biochemical testing, or treatment, or both has been considered. An integrated program of both mutational analysis, to identify mutant gene carriers, and biochemical screening, to detect the development of tumors, has been shown to be of advantage and is used by many centers. Thus, a DNA test identifying an individual as a mutant gene carrier is likely to lead not to immediate medical or surgical treatment but to earlier and more frequent biochemical and radiologic screening, whereas a DNA result indicating that an individual is not at risk will lead to a decision for no further clinical investigations. Thus, the identification of *MEN1* mutations is of help in the clinical management of patients and their families with this disorder.

Acknowledgements

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Multiple Endocrine Neoplasia Type 2

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Synonyms

MEN 2; MEN II; MEA II; Sipple syndrome

Definition

MEN 2 is a syndrome of inherited susceptibility to tumors of endocrine cell types including the thyroid "C" cells, the adrenal medulla and the parathyroid glands. In some disease subtypes, developmental abnormalities may also be present. MEN 2 is caused by inherited mutations of the gene that encodes the RET receptor tyrosine kinase which lead to its unregulated activation. Subtypes of MEN 2 are: MEN 2A, MEN 2B, FMTC (familial medullary thyroid carcinoma).

Characteristics

The principal tumor in MEN 2 is medullary thyroid carcinoma (MTC), arising from the "C" cells of the thyroid (thyroid carcinogenesis), the normal function of which is to produce the hormone calcitonin. MEN 2 is an early onset tumor syndrome and MTC may first be detected in early childhood (in the MEN 2B subtype as early as the first weeks of life) but the age of appearance of symptoms varies. While nearly 100% of individuals who inherit the susceptibility mutation will have thyroid involvement, only about 70% will have clinically significant disease by the age of 70.

Tumors of the adrenal medulla (pheochromocytoma), and the parathyroid are less frequent, affecting roughly 50% and 10–30% of individuals, respectively. Pheochromocytomas are usually (but not always) benign, and cause their effects through overproduction of adrenaline and related substances, leading to high blood pressure. The parathyroid glands may be affected by diffuse enlargement (hyperplasia) or by benign tumors (adenoma). These are commonly silent but may have

clinical effects through overproduction of parathyroid hormone, leading to elevated blood calcium levels. In addition, some individuals with MEN 2 have associated developmental abnormalities that are characteristic of the clinical subtype (Table 1). Rarely, MEN 2 may also be associated with another rare presentation, Hirschsprung disease, a developmental abnormality in which nerve cells in the wall of the lower intestine are absent, resulting in impaired function of the gut.

Cellular and Molecular Disease Aspects

All forms of MEN 2 are caused by mutations that inappropriately activate the RET receptor tyrosine kinase. RET is a cell surface protein expressed primarily in cells that originate in the neural ectoderm and in genitourinary tissues. A number of distinct developmental functions have been identified to date including roles in development of the kidney, the autonomic nerves (►Autonomic Nervous System) in the gut, and endocrine tissues in the thyroid, parathyroid and adrenal glands, as well as in maturation of spermatogonia and maintenance of dopaminergic neurons. At various developmental times, and in different cell types, RET may promote cell proliferation, survival or migration. In normal cells, RET receives specific signals at the cell surface and conducts this information, along multiple signaling pathways, into the cell where it leads to changes in gene expression and thus cellular behavior. The signals are provided by binding of members of each of a family of cell surface receptors and a family of ligands, permitting tight control on the ability of RET to signal.

MEN 2 is caused primarily by single amino acid changes (point mutations) in RET that alter the properties of the protein and cause it to be constantly active without any ligand. The majority of mutations that activate RET affect one of a small number of amino acid hot spots and there is a general correlation between the specific mutation identified and the disease subtype (Fig. 1). The molecular effects of specific RET mutations vary and the differences in the relative enhancement of RET activity, coupled to differences in sensitivities of each tissue type to increased activity may in part explain the variation between MEN 2 subtypes. For example, the mutations that cause the strongest increase in RET signals are also associated with more aggressive disease and wider range of disease features. In rare cases, a group of RET mutations in codons 609, 611, 618 and 620 with relatively lower signaling ability (JANUS mutations) may be associated with both MEN 2A and with Hirschsprung disease.

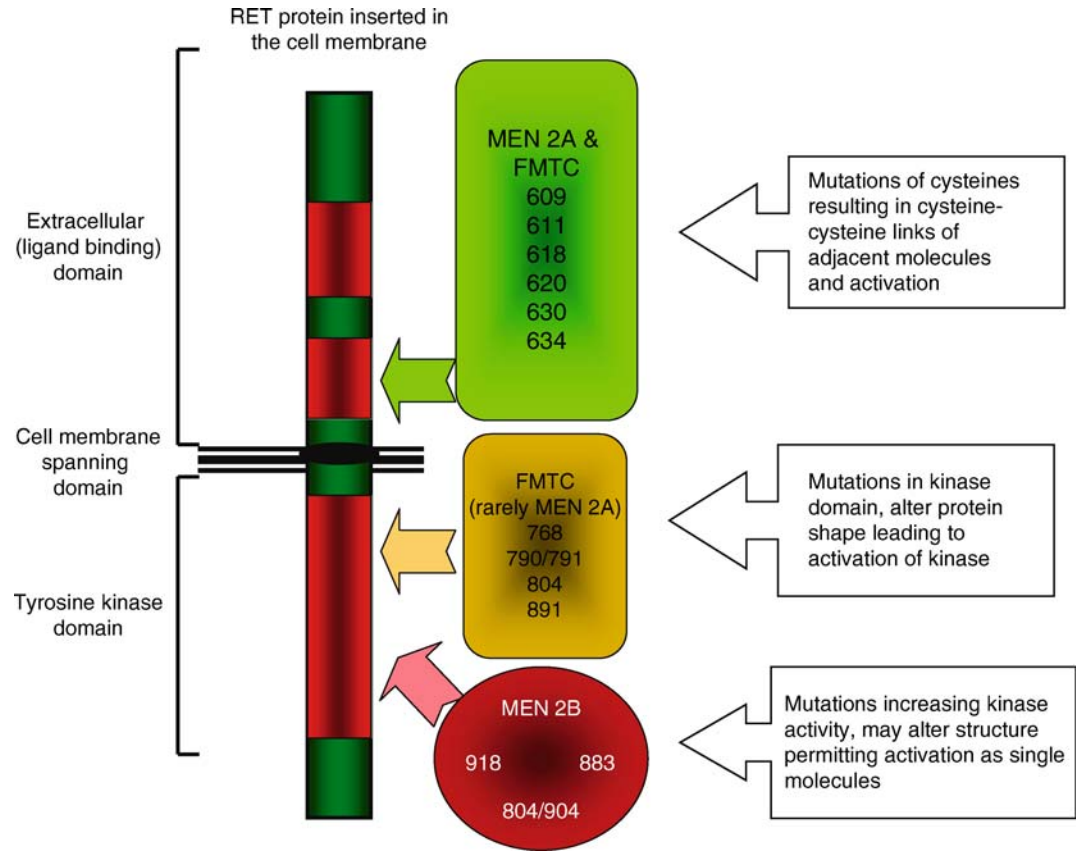
Clinical Aspects

MEN 2 is rare, with an estimated 20–25 new cases detected per year in the UK (population 55 million). However, it is important because early recognition and surgery is effective in preventing both deaths and unpleasant symptoms.

The tumors that are characteristic of MEN 2 can also occur, and more commonly, in a non-inherited form (sporadic tumors). A very young age at diagnosis, a family history of the disease, or pathology findings of multiple tumors or hyperplasias may suggest that a new patient has an inherited form of the disease (i.e. MEN 2). DNA testing for RET mutations is the accepted first investigation in suspected cases and confirms familial disease. In families with a history of MEN 2, mutation testing should be performed in early childhood to allow presymptomatic disease management. In general, prophylactic thyroidectomy is a straightforward and acceptable operation that is recommended in childhood to those who have an MEN 2 mutation. Adrenalectomy carries higher morbidity and is only recommended when there is evidence of developing adrenal disease.

Multiple Endocrine Neoplasia Type 2. Table 1 Clinical subtypes of MEN 2 and tissues

	MEN 2A	MEN 2B	FMTC
Thyroid C cells	Tumors	Tumors	Tumors
Adrenal medulla	Tumors	Tumors	Not involved
Parathyroid	Hyperplasia or tumor	Not involved	Not involved
Other	–	Skeletal abnormalities; overgrowth of nerve tissue in lips and conjunctiva leads to abnormal facial appearance, characteristic overgrowth with impaired function	–
Comments	Most common form (~85%)	Least common (~5%); earlier onset; impaired reproductive success; high proportion of isolated cases which represent “new” families	Intermediate frequency (~10%); later onset; generally less aggressive



Multiple Endocrine Neoplasia Type 2. Figure 1 MEN 2 associated mutations in the RET protein.

Although only a minority of tumors are part of the inherited syndrome, the identification of previously unrecognized RET mutations in supposedly "sporadic" cases of MTC and pheochromocytoma have led to recommendations for direct RET mutation testing for all individuals with these tumors. The presence of a mutation confirms heritable disease and has implications for further tumors in that individual or in family members. Absence of a mutation is strong evidence against inherited disease as more than 99% of cases have detectable RET mutations. If a mutation is found, other family members can be offered testing and those who test positive can be offered prophylactic surgery.

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Multiple Hamartoma Syndrome

►Cowden Syndrome

Multiple Myeloma

Definition

A malignancy of circulating plasma cells.

►Minimal Residual Disease

Multiple Osteochondromas

Definition

MO; Autosomal dominant disorder characterized by the occurrence of multiple osteochondromas causing disproportionate short stature and bowing of the forearm. MO is genetically heterogeneous and is caused by mutations in the ►EXT-genes.

►Chondrosarcoma

Multiplex Profiling Technologies

Definition

Parallel measurement of numerous entities of given biomolecules (e.g. protein or DNA microarrays)

►Oncopeptidomics

Multipolar Spindles

Definition

Mitotic spindles having more than two poles. This causes chromosomes to segregate asymmetrically, and often chaotically, leaving daughter cells with abnormal numbers of chromosomes.

►Aneuploidy
►Genomic Imbalance

Multipotent

Definition

Able to give rise to more than one differentiated cell type.

Multipotent Stem Cells

Definition

Can only produce cells of a closely related family of cells (e.g., mesenchymal stem cells, hematopoietic stem cells).

►Adult Stem Cells

Multistep Development

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Definition

Multistep development is the stepwise development of malignant tumors by successive changes in multiple characteristics (Fig. 1).

Characteristics

Tumor development can be regarded as a Darwinian process, based on variation and selection. In the neoplastic microevolution, "survival of the fittest" means the successive emergence of increasingly emancipated subclones that are less and less restrained by the multifactorial growth controls of the organism.

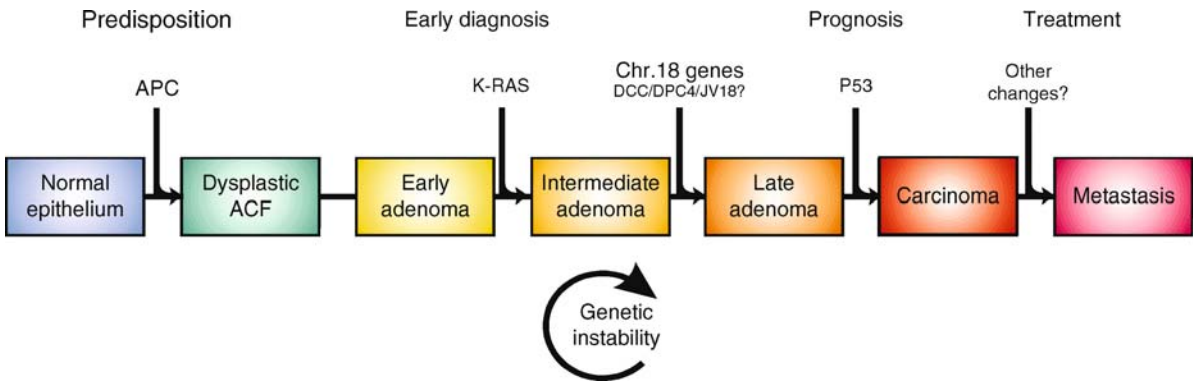
History

Epidemiological studies have shown that the incidence of cancer increases exponentially with age. The analysis of age incidence curves indicated that between three and seven mutations are required for full development of cancer. This is consistent with the modern molecular analysis. Carcinomas and sarcomas appear to require a larger number of changes than leukemias. In addition to changes in the DNA sequence that can be referred to as mutations, epigenetic changes, affecting gene expression, without any change in the DNA sequence, provide valid alternatives that may have the same or similar phenotypic consequences. MicroRNAs can also participate in the modification of gene expression.

Participating Genes

The major known categories are:

- Oncogenes that drive the cell towards an S-phase when activated



Multistep Development. Figure 1 Multistep pathway from normal cell to metastatic colon carcinoma. The rate-limiting step is postulated to be the loss of the APC gene on chromosome 5. Individuals with familial adenomatous polyposis (FAP) inherit the first mutation (APC gene) and only need to acquire a second mutation. Other individuals need to acquire both mutations in the same cell lineage. Additional genetic damage is accumulated, including loss of genes on 17p, exemplified p53 and 18q as well as other changes. These can occur in any order and result in progressively abnormal cells, eventually capable of metastasis (figure provided by Bert Vogelstein).

- Suppressor genes that can arrest the growth of illegitimately driven cells and contribute to tumor development when inactivated or deleted
- Genes that raise the apoptotic threshold when activated or genes required for the apoptotic process that contribute by their loss
- DNA repair genes whose dysfunction may create genetic instability

Genes that provide the ability for an interminable sequence of cell divisions (immortalization). Genes that influence invasion and metastasis.

Less well known genes may contribute to angiogenesis, hormone insensitivity and escape from immune rejection.

Minimum Requirements for Tumor Development

Genetic analysis of tumor development in many different tissues has created a scenario where transformation of a normal cell into a cancer cell requires the activation of at least one oncogene (e.g. myc, ras), the loss of one or several suppressor genes (e.g. ►RB, ►p53, ►p16, ►APC), activation of a gene that raises the apoptotic threshold (►Bcl-2) or, alternatively, the loss of genes required for apoptosis (e.g ►ARF or p53, ►Bax). It is a general rule, with few if any exceptions, that both the Rb and the p53 pathway is inactivated. They can be inactivated at different points, however. For instance, the p53 pathway can be crippled by p53 mutation, ARF deletion or MdM2 ►amplification. The Rb pathway can be inactivated by Rb mutation, p16 inactivation, usually by methylation (an epigenetic change), or the amplification of CDK4 or 6.

Highly immunogenic, DNA virus-associated tumors may escape rejection in immunodefective hosts. Cellular escapes may be due to the down regulation of rejection inducing tumor antigens or of presenting MHC class I proteins.

Successive steps in tumor development have now been defined as specific changes in DNA sequences or epigenetic changes affecting gene expression. The inactivation of tumor suppressor genes by either mutation or genetic loss, or by the dense hypermethylation of a CpG island at the 5′ end of the gene, an epigenetic change, may be responsible.

Distinct molecular events have been linked to progression related phenotypic changes in carcinomas of the colon and rectum, prostate, lung, breast, skin, in gliomas and tumors of the hemopoietic system. The loss of tumor antagonizing (growth arresting and/or apoptosis promoting) genes appears to be more frequent than the activation of proliferation-driving genes but this may be due, at least in part, to their easier detectability, e.g. by loss of heterozygosis (LOH). Microarray techniques that show gains or losses at the DNA level, changes in expression at the RNA level or specific methylation of the DNA in regulatory regions, provide different "signatures" that have diagnostic and prognostic significance and may give guidelines for therapy.

Colorectal tumors (►Colon cancer) progress through a series of well defined phenotypic changes, from hyperplasia, through different stages of adenomas, to malignant cancers. Two familial forms, hereditary non polyposis colon cancer (►HNPCC) and familial

adenomatous polyposis (FAP (►APC gene in familial adenomatous polyposis)) are initiated by germline mutations in mismatch repair genes in the former and the APC gene in the latter condition. The multifunctional APC protein is involved in cellular adhesion, signal transduction and apoptosis. The development of the hereditary forms of colorectal cancer is associated with the inactivation of the second allele and a series of sequential changes during somatic development. The latter are also found in the sporadic form. They include activation of Ki-ras, or N-ras (more: ►RAS), inactivation of both copies of p53 and loss of a gene on chromosome 18 that is identical with or closely linked to ►DCC. Later stages of progression are associated with extensive aneuploidy, particularly in the FAP, but less in the HNPCC based tumors. The changes do not occur in the same order in different tumors, confirming the notion of alternative pathways.

About 15–20% of ►breast cancers have a family history. About 5% can be attributed to dominant susceptibility genes. Two of them ►BRCA1 and ►BRCA2 are responsible for 40–50% and 30–40% of inherited breast cancers. The subsequent development and the biology of sporadic breast cancers is very diverse, indicating the existence of different progressional pathways. A gamut of growth factors and receptors (e.g. EGFR, ►HER-2/neu), signaling molecules (Ras, Src) and other intracellular growth regulators (e.g. Myc, Cyclin D) can be activated. Tumor suppressor genes (e.g. p53, RB) and adhesion molecules (e.g. cadherin) may be set out of function. Many as yet unidentified genes may be involved in addition, as indicated by the numerous LOHs. Amplification of HER-2/neu is a bad prognostic sign. However, the protein product of the amplified gene can be used as therapeutic target.

►Prostatic carcinoma provides a similarly diverse panorama. In addition to mutations of Ras, p53, ►RB1, CDKN2 and other known oncogenes and suppressor genes, cadherin down-regulation and LOHs affecting chromosomes 5, 6, 8, 10 and 16 have been detected. No single gene or chromosome region is affected in all prostatic carcinomas. Progression from hormone dependence to independence is often associated with the amplification of the androgen receptor gene on the X chromosome.

Lung cancer in heavy smokers (►Tobacco carcinogenesis) is often a multifocal disease. Deletions affecting the short arm of chromosome 3 are found already at the hyperplastic or dysplastic precursor stage. Subsequent progression may feature 5q deletions, K-ras mutations, p16 inactivation, ►FHIT, p53 or RB1 mutations. The deletions and rearrangements on chromosome 3 may become more extensive, affecting several genes within a cluster. During late stages of small cell lung cancer (SCLC) progression, amplification of the myc-family genes (c-, N-, or L-myc) is a bad prognostic sign.

►Astrocytomas show an increasing number of changes with ascending grade. The least aggressive forms show loss of wild type p53 and a number of LOHs. ►Glioblastoma, the most malignant form, has a large number of genetic abnormalities. They include losses from chromosomes 6, 10, 13 and Y, gains on chromosomes 7 and 19, and structural abnormalities of chromosomes 1 and 9. Frequent double minutes indicate gene amplification. Homozygous deletions on 9p, affecting the CDKN2A, CDKN2B (p15/16) and p14 ARF loci, are found in 40–50%. The inactivation of p15/16 deranges RB dependent cell-cycle control, whereas the deletion of the alternatively spliced p14 product of the same gene cripples the p53 dependent apoptotic pathway. Understandably, homozygous deletions of the p15-16-14 cluster is found in tumors that show no abnormalities of CDK4, RB1, MDM2 or p53 and vice versa. This illustrates the existence both of alternative pathways of progression, or more specifically alternative modes of inactivating the cell-cycle regulating and apoptosis controlling genetic systems. This can be further exemplified by the fact that 90% of the glioblastomas have either no wild type p16/p15 or wild type pRB1 or overexpress CDK4. In 75% of the glioblastomas, the apoptotic pathway is either inactivated by the lack of wild type p53, the inactivation of p14 ARF or over-expression of MDM2. Tumors with amplified MDM2 have two wild type p53 alleles. These examples illustrate how genetic aberrations may target different members in the same cellular control pathway and create similar phenotypes. Additional changes in glioblastomas include EGFR amplification and over-expression (35%) and allelic loss from chromosome 10 (90%), probably acting through the inactivation of PTEN/MMAC1 (>40%), a putative tumor suppressor that encodes a dual specificity phosphatase.

It is noteworthy that phenotypically different (angiogenic, non-invasive, and nongangiogenic, invasive) subclones could be selected from the same clonal glioblastoma that showed the same multiple DNA changes but expressed different genes on RNA arrays. This is another example to show that epigenetic differences may have a decisive impact on the phenotype of tumor cells with similar or identical DNA changes.

The hereditary form of ►renal carcinoma is often initiated by a mutation in the VHL gene at 3p26. The ►VHL gene is also frequently mutated in sporadic clear cell RCC. A cluster of genes at 3p21.3 are frequently deleted during tumor progression, both in the hereditary and the sporadic form. When an intact human

chromosome 3 is introduced into mouse tumor cells and the monochromosomal hybrids are grown in immunodefective mice, a corresponding region, designated as CER1 (commonly eliminated region 1) is regularly eliminated, indicating the presence of one or several tumor antagonizing genes. In contrast, a gene cluster at the telomeric end of the long arm (3q) is frequently retained and may be amplified.. Hereditary papillary renal cell carcinoma (HPRC) shows no association with chromosome 3 losses or VHL mutations.

In skin cancer (►Basal cell carcinoma), allelic losses in basal cell carcinomas are almost entirely confined to the Gorlin locus at 9q (►Gorlin syndrome). Squamous cell carcinomas show allelic losses in 25–30%, affecting chromosomes 3, 9, 13 and 17.

►Pancreas carcinoma shows a distinct profile. K-ras is activated in 90% of the tumors by point mutations in codon 12. Among the suppressor genes, p16, p53 and DPC4 are frequently inactivated, RB1 less frequently. DPC4 is biallelically inactivated in almost 50% of all pancreatic cancers, but only rarely affected in other tumors.

In endometrial cancer, microsatellite instability, K-ras, PTEN and p53 mutations are most common. p53 overexpression is correlated with poor prognosis.

In carcinoma of the uterine cervix (►Cervical cancer), the HPV (►Human papillomaviruses) encoded transforming proteins E6 and E7 play an important early role (early HPV proteins). This is at least partly due to their inactivating effect on p53 and RB1, respectively. This promotes genetic instability, counteracts apoptosis and abolishes part of the cell-cycle regulatory controls. In addition, amplification of myc and HER-2/neu, as well as loss of genes from chromosomes 1, 3, 5 and 11 may play important roles. 3p losses are particularly interesting, since the same regions are deleted in renal, lung and nasopharyngeal carcinomas.

In ►bladder cancer, chromosome losses are frequent. Monosomy of chromosome 9 is the most common abnormality. In addition, deletions are common on 9p, 17p and 13q. p53 and RB mutations are correlated with poor prognosis. Multiple primary tumors may be generated by the spread and further progression of a single progenitor clone.

Hepatocellular carcinoma (►Liver cancer, molecular biology) is initiated by the DNA virus hepatitis B virus (HBV). Sequential changes include p53 losses. The virally encoded transactivator HBV-X is believed to induce the overexpression of several oncogenes.

Hemopoietic tumors (►Hematological malignancies) are often initiated by chromosomal translocations, leading to gene fusions (in CML and a wide variety of childhood leukemias), juxtaposition of an oncogene to an Ig locus (B-cell lymphomas) or to a TCR locus (T-ALL). Sequential events affect mainly the apoptosis regulating systems, with up-regulation of bcl-2 or inactivation of p53, p19 ARF or BAX. In T-ALL, p16 may remain unaffected, whereas ARF is disrupted or deleted. In the absence of ARF, p53 usually remains in wild type configuration. A similar alternative inactivation has been found between p16 and RB, as in other tumors.

Clinical Relevance

For diagnosis, oncogene activation by chromosomal translocation, with or without gene fusion, is diagnostic for certain leukemias, lymphomas and sarcomas. For prognosis, the identity of the activated oncogene may be decisive for the biological behavior of the tumor. Oncogene amplification, a well defined step in the progression of carcinomas and gliomas, may be a bad prognostic sign; it is being used clinically to determine prognosis in ►neuroblastoma and is a parameter for therapy design.

Microarray techniques that can assess the DNA, RNA, methylation and protein profiles in tumors generate characteristic signatures that permit the division of diagnostic categories into subgroups, differing in prognosis and therapeutic sensitivity. They also provide targets for therapeutic approaches.

►Micrometastasis detection by antibodies and/or by molecular techniques, and the identification of residual, translocation carrying leukemia or sarcoma cells by polymerase chain reaction (PCR) are useful for the diagnosis of residual disease and for predicting the probability of recurrence. These parameters may also provide surrogate endpoints for the evaluation of new therapies.

Therapeutic choices are decisively influenced by the molecular findings. This can be exemplified by the identification of the genes involved in the translocations found in ALL and APML. Current therapeutic experimentation aims at the inhibition of pathologically activated signal transduction chains with small peptides, antibodies or DNA constructs. Faulty protein-protein interactions provide other important targets. It is not necessary to correct all genetic aberrations in tumors that have evolved by multiple steps. Inhibition of a dominating driving mechanism or, alternatively, induction of apoptosis may be sufficient.

►Toxicological Carcinogenesis

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Multivariate Statistics

Definition

Statistical analysis of more than one variable at a time. Examples are principal components analyses (PCA) or artificial neural networks.

►Oncopeptidomics

Murine Double Minute 2

Definition

mdm2; A major negative regulator of ►p53 tumor suppressor. The protein acts as an E3 ubiquitin ligase that recognizes the N-terminal trans-activation domain of p53 and an inhibitor of p53 transcriptional activation.

►MDM Genes
►Ubiquitination

Mutagen

Definition

Chemical or physical agent capable of effecting a change in the DNA sequence of an organism at the cellular level. Generally, a mutagenic carcinogen that forms adducts in DNA brings about a mutation when DNA-replication occurs on a damaged template. The mutation may be the first step in the sequence of events that eventually leads to the development of cancer.

►Adducts to DNA
►Carcinogenesis
►Carcinogen Macromolecular Adducts
►Chemical Carcinogenesis
►Chemically Induced Cell Transformation
►Coffee Consumption

Mutagen Sensitivity

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Definition

Mutagen sensitivity is a phenotypic measurement that indirectly reflects an individual's ►DNA repair capacity (DRC). The typical test for mutagen sensitivity is measuring the number of ►chromatid breaks in short-term cultures of peripheral blood lymphocytes (PBLs) after exposure to ►bleomycin or other types of mutagens. The unit of measurement is the mean breaks per cell (b/c): the higher the number of b/c, the higher the mutagen sensitivity ►phenotype. High mutagen sensitivity has been consistently shown to be a ►genetic susceptibility factor for a variety of cancers.

Characteristics

Overview of Human DNA Repair System

All living organisms are equipped with DNA repair systems that deal with a wide variety of DNA lesions. In mammalians, the DNA repair system can be classified into four major pathways based on the specific DNA lesions that each pathway repairs: ►nucleotide excision repair (NER), ►base excision repair (BER), ►double strand break (DSB) repair, and mismatch repair (MMR). The NER mainly removes bulky DNA adducts, which are typically generated after exposure to environmental genotoxic agents such as ultraviolet (UV) light and benzo(*a*)pyrene (a tobacco carcinogen). The BER mainly copes with damaged DNA bases arising from endogenous oxidative and hydrolytic decay of DNA. The DSB repair pathway fixes double-strand breaks caused by a plethora of pathologic stimuli, including ionizing radiation, chemotherapeutic drugs, and free radicals. The MMR pathway eliminates base-base mismatches and insertion-deletion loops occurring as a consequence of DNA polymerase slippage during

DNA replication. Deficient DNA repair system has been convincingly associated with increased cancer risks in laboratory and epidemiologic studies.

Inter-Individual Variations in DNA Repair Capacity Within General Population

It has long been recognized that there is a wide spectrum of DRC within the general population. The majority of people have normal levels of DRC. At the extreme low end are individuals with rare genomic instability syndromes who exhibit severely compromised DNA repair system due to mutations in critical DNA repair genes. Two classic examples of such DNA repair deficiency syndromes are ►Xeroderma pigmentosum (XP) and Ataxia-telangiectasia (A-T). XP patients have mutations in XP genes, which are critical components of NER. Therefore, XP patients are extremely sensitive to UV-light due to defects in NER, which is the pathway responsible for repairing UV light-induced ►DNA damage. XP patients show abnormal pigmentation and freckling in sun-exposed skin, and they usually develop skin cancer at very young age. A-T patients, on the other hand, have mutations in ATM gene. One of the major functions of ►ATM protein is in DSB repair. Therefore, A-T patients exhibit hypersensitivity to ionizing radiation due to their inability to repair radiation-induced DSB and they have increased risk of developing cancer, such as lymphoma, leukemia, and breast cancer.

In addition to these rare individuals with genetic syndromes who are at the extreme low end of DRC, there are some individuals who are at the less extreme end with suboptimal DRC. Under normal physiological conditions, the suboptimal capability of DNA repair in these individuals may not be manifested, i.e., these individuals possess latent DNA repair deficiency and latent genetic instability. However, if exposed to high level of carcinogen, these individuals may reveal higher level of DNA damage and hence greater risk of developing cancer than general population who are similarly exposed but possess normal DRC. It would be of great public health and clinical importance to be able to identifying individuals with suboptimal DRC, who may belong to high-risk groups for cancer if they are exposed to high levels of environmental carcinogens, and may therefore be candidates for intensive monitoring and cancer prevention. Mutagen sensitivity is such a quantitative assay that indirectly measures DRC and the degree of genetic instability.

Development of Mutagen Sensitivity Assay

The mutagen sensitivity assay was developed about two decades ago by Hsu et al The theoretical basis for mutagen sensitivity assay is that latent genetic instability could be unmasked by mutagen challenge. Hsu et al hypothesized that in response to mutagen exposure, higher levels of genetic damage would accumulate in people with suboptimal DRC than in normal individuals. Therefore, the level of chromatid breaks induced by a mutagen challenge would reflect an individual's ability to repair DNA damage. The basic procedures of mutagen sensitivity assay include: culture PBLs or other somatic cells in vitro, add specific mutagen to cell culture and incubate for a few hours to a few days, block cells in metaphase, fix cells to slides, stain slides with Giemsa to visualize metaphase chromosomes, and count the number of chromatid breaks in 50 metaphases per slide under a microscope. The mean number of breaks/cell (b/c) is the quantitative measurement of mutagen sensitivity. Because genetic susceptibility factor should be present in all normal somatic cells, PBL is the most convenient material and have been the predominant choice of cell source for the assay. The reading of chromatid breaks under a microscope requires a certain level of technical expertise; however, the assay is reproducible within and between different experienced observers and different laboratories. The original mutagen sensitivity assay was developed using bleomycin as the mutagen challenge. The assay has since been expanded to employ other etiologically relevant mutagens to assess genetic susceptibility to different cancers, such as ►benzo[*a*]pyrene diol epoxide (BPDE), a metabolic product of benzo[*a*]pyrene (a tobacco mutagen), for smoking-related cancers; ►4-NQO, a UV-mimetic mutagen, and ►UV radiation, for skin cancer; and γ-radiation for breast cancer. By using different mutagens to induce different types of DNA damage, specific information can also be acquired about host susceptibility with regard to a specific DNA repair pathway. For example, BPDE forms bulky DNA adducts that are repaired by the NER pathway. By using BPDE as a mutagen challenge, relative DNA repair efficiency by NER pathway can be measured. On the other hand, γ-radiation is capable of inducing single- and double-stranded breaks and initiating BER and particularly DSB repair pathways. Furthermore, if using BPDE and γ-radiation in parallel, one can examine the activity of several DNA repair pathways and increase the power of predicting cancer risk compared to using only one variable.

Heritability of Mutagen Sensitivity

The mutagen sensitivity assay measures the cumulative effect of DNA damage and repair, which is a complex phenotype affected by many factors, including genetic, environmental, or pathologic factors. To use such a phenotype as a predictor of cancer risk in ►cancer epidemiology studies, one has to determine the degree

to which mutagen sensitivity phenotype is genetically inherited. A high genetic heritability would give more confidence that this phenotype is more likely to be the cause of the disease rather than the result of the disease. Several lines of evidences have strongly supported high genetic heritability of mutagen sensitivity phenotype. A few ►case control association studies showed that mutagen sensitivity remained a risk factor for cancer independent of demographic and environmental factors, suggesting genetic contributions. Several family studies found that the first-degree relatives of mutagen sensitive individuals were also mutagen sensitive. In one study of measuring radiation sensitivity in PBLs from families of breast cancer patients, 62% of the first-degree relatives of radiation sensitive patients exhibit sensitive phenotype; in contrast, only 7% of the first-degree relatives of patients with normal sensitivity exhibit sensitive phenotype. This observation strongly suggests high genetic inheritability of mutagen sensitivity phenotype. However, the most powerful method for assessing the genetic heritability of complex traits is the classical twin study design. The twin model, comparing similarities between monozygotic (MZ) twins (who are genetically identical) and dizygotic (DZ) twins (who share half their genes), can partition inter-individual variability into components reflecting genetic and environmental factors. The simplest and most straightforward estimation of genetic contribution in twin study is to compare the intra-class correlation among MZ twins and DZ twins. If mutagen sensitivity has high genetic heritability, it is expected that the intra-class correlation of mutagen sensitivity data within MZ pairs are significantly higher than within DZ pairs, because MZ pairs are genetically identical and DZ pairs only share half of their genes. Otherwise, if environmental factors play a dominant role in determining mutagen sensitivity, the intra-class correlation should be similar within MZ pairs and within DZ pairs. Indeed, three independent twin studies have shown that mutagen sensitivity have high genetic heritability. Furthermore, by applying sophisticated genetic modeling, the percentages of genetic contribution and environmental contribution have also been estimated. The genetic heritability of mutagen sensitivity ranges from 45 to 65%, depending on mutagen types. In addition, the contribution of non-shared environment is also substantial, accounting for 30–40% of the variations, whereas the contribution of shared environment was relatively small (generally <25%). It is apparent that while heritability varies to different mutagens, it plays the most prominent role in determining mutagen sensitivity for any mutagen evaluated. The non-shared and shared environments also contribute to mutagen sensitivity but to a lesser degree. It is understandable that different mutagens exhibit varying degrees of genetic heritability, since different mutagens elicit different DNA repair pathways that involve distinct gene sets.

High Mutagen Sensitivity is a Genetic Susceptibility Factor for Cancer Development

Almost a hundred epidemiologic studies have been published evaluating the association between mutagen sensitivity and cancer risk and there has been at least one positive study showing high mutagen sensitivity confers high risks for most of the major cancers, including cancers of lung, breast, prostate, bladder, colorectum, brain, skin, head and neck, and soft tissue. Most of these studies were ►retrospective studies with modest sample sizes. The largest retrospective case-control study evaluated almost 1,000 lung cancer patients and 1,000 healthy controls. The risk estimates conferred by higher mutagen sensitivity are in the range of 1.5- to 2.5-fold depending on what mutagen is used. In addition, high mutagen sensitivity has also been shown to confer increased risk of developing ►preneoplastic lesions, progression from preneoplastic lesions to cancer, and developing recurrence and/or secondary primary tumors. Moreover, high mutagen sensitivity can greatly increase the efficiency of predicting cancer risk when combined with epidemiologic risk factors, such as tobacco smoking and alcohol consumption in the case of head and neck cancer, and smoking and occupational exposure in the case of bladder cancer. High mutagen sensitivity can also increase prediction power of cancer development in the presence of other somatic genetic events. A recent ►prospective study measured baseline bleomycin sensitivity in PBLs from patients with Barrett's esophagus (BE), a precursor of esophageal adenocarcinoma (EAC), and found that higher bleomycin sensitivity was associated with a greater risk of developing ►aneuploidy and EAC, particularly in BE tissues with loss of heterogeneity of ►TP53 locus. It is promising that mutagen sensitivity measurement may find clinical application in identifying high-risk groups of cancer development in the context of a comprehensive risk-assessment model incorporating demographic, environmental, and other genetic predictors.

Mutagen sensitivity has emerged as one of the best-established phenotypic susceptibility markers for cancer risk over the past two decades. The epidemiologic evidence supporting its association with cancer risk is strong and consistent. The classical twin study has demonstrated its high heritability. The question of which genes is responsible for the mutagen sensitivity trait for each specific mutagen is currently under study. The identification of such genes will not only improve our understanding of the mechanism of mutagen sensitivity phenotype, but also provide novel candidate genetic markers for cancer risk assessment and cancer prevention.

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Mutagenic

Definition

Mutagenic agents such as ionizing radiation or chemicals are able to induce genetic alterations, thereby, contributing to/inducing malignant transformation of cells and cancer development.

►Mutagen

Mutated in Multiple Advanced Cancers 1

Definition

MMAC1; sysnonym for ►PTEN

Mutation

Definition

Is a permanent, heritable change in the genetic information of a cell or organism that can be acquired or hereditary. It may involve an altered base sequence in DNA or a deletion or rearrangement of chromosomes. Mutations in genes are often associated with a different phenotype.

►Mutagen

Mutation Rate

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Definition

The mutation rate is defined as the probability of a cell acquiring a mutation in a gene. This probability, abbreviated by the symbol "μ," depends on specific parameters, such as the cell type and the gene, the effect of the mutation (e.g. gain of function versus inactivation) and the nature of the event (e.g. point mutation versus translocation). Factors that influence μ include the fidelity of DNA polymerases, activity of repair pathways, and exposure to mutagens. μ can be expressed as the probability of a mutation occurring in a gene per cell division, as the probability of a mutation in a nucleotide pair per cell division, or as mutations per unit of time. A related parameter, the mutant frequency (f), represents the proportion of cells in a population harboring a mutation in a gene at any one point in time, whereas μ represents the rate of increase in f over time. Because μ is typically a low number, it can be measured only in a large number of cells, for which any measurement represents a population average.

Characteristics

One can consider the *somatic* mutation rate, but it is instructive to consider how this parallels the *germline* mutation rate. By determining the incidence of individuals born with mutations that are not inherited from either parent, the probability of a new mutation in a parental germ cell (or in the zygote) can be estimated. Germline mutation rates vary markedly, and can be as high as 10^{-4} per generation for the ►Neurofibromatosis 1 gene NF1, or orders of magnitude lower for other genes. Apart from genetic diseases, the germline mutation rate will influence the generation of genetic diversity within a population, a potentially advantageous phenomenon. In contrast, somatic mutations do not appear to have a physiologic role, apart from the generation of diversity in antigen receptors.

In a culture of microorganisms, there is no distinction between the germline and somatic mutation rates, and it was in bacteria that Luria and Delbruck provided the first measurement of μ, based on the rare spontaneous acquisition of resistance to the phage T1 (a consequence of *TonB* mutations). They set up multiple replicate liquid cultures, starting from a small number of phage-sensitive cells. After expansion to N cells per tube, the cultures were plated on a petri dish containing the phage, such that only phage resistant cells could form

colonies. They made the critical observation that the variance in the number of phage resistant colonies on the plates was very high, and they concluded that mutations occurring early in culture resulted in many resistant colonies, whereas later mutations produced fewer colonies. Some tubes gave rise to plates with no colonies, presumably because no mutations had occurred. They calculated the proportion of such tubes (P_o) and from the Poisson distribution, they derived a formula for the mutation rate, $\mu = -\text{Ln}\ (P_o)/N$, which they estimated to be 4.6×10^{-9} mutations per cell division (Table 1). They also derived an alternative formula for μ, $r = \mu N \ln(NC\mu)$, where r is the mean number of resistant colonies per tube and C is the number of replicate tubes. (This formula can not be directly solved, but μ can be estimated easily by computer using iterations).

An implication of this landmark work is that if μ is constant and there is no selection in favor or against mutants, over time, the frequency (*f*) of phage resistant mutants will increase linearly as a function of cell divisions (*d*), with a slope of μ (i.e. $\Delta f = \mu\ \Delta d$). This linear relationship was demonstrated experimentally by Novick and Szilard in 1950 using a method called a chemostat, which keeps bacteria growing in a bulk culture at a constant rate over many cell divisions. In 1954 Ryan and Wainwright used the chemostat to calculate μ, starting with a histidine-requiring (auxotrophic) strain. Over time, they sampled the bulk culture, measuring the frequency of cells that spontaneously regained the ability to grow on plates lacking histidine, due to a second (reversion) mutation. (For example, if the initial strain had a single base pair insertion in a gene required for histidine biosynthesis, a single base pair deletion in the same vicinity could restore the reading frame). They plotted *f* as a function of *d* and calculated μ from the slope of the curve, with an estimate of 8.5×10^{-9} mutations per cell division (Fig. 1).

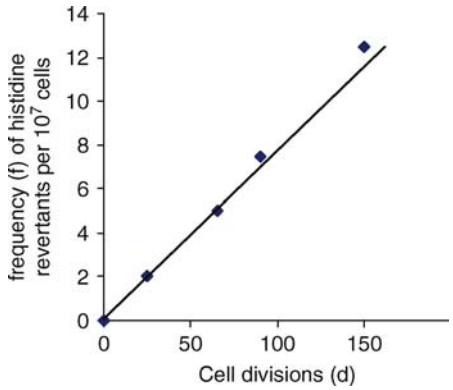
Other genes in microorganisms were subsequently used as sentinels for spontaneous mutations. Inactivating mutations in the *LacI* repressor gene result in induction of β-galactosidase, producing blue bacterial colonies in the presence of X-gal. It is estimated that for *LacI*, $\mu = 4.5 \times 10^{-7}$ mutations per cell division. Similarly, in *S. cerevesiae*, inactivation of the *CAN1* gene (encoding arginine permease) confers resistance to canavanine, and it is estimated that $\mu = 3.5 \times 10^{-7}$ mutations per cell division in this gene. These values are notably higher than in the previous studies, probably because a broader spectrum of mutations can produce the phenotype. (As an illustration, consider that a broad spectrum of mutations can inactivate ►p53, whereas only one missense mutation can result in constitutive *Jak2* activation.) In 1991 Drake used this and other data to demonstrate that in microorganisms, μ seems to be inversely related to the size of the genome.

As in microorganisms, any potential human sentinel gene must not be essential for growth or survival, but, when mutated, must produce a readily detectable phenotype in a cell, which must be identified amongst a vastly larger population of normal cells. A further limitation in humans, though, is the diploid nature of the genome: a loss of function mutation is likely to be complemented by the remaining normal allele on the homologous chromosome, whereas the probability of both copies being mutated in the cell is so low as to preclude detection. Although a single *gain of function* mutation might produce a detectable phenotype in a dominant manner, as in the case of *Jak2*, these mutations are probably much rarer than inactivating mutations and thus may also elude detection.

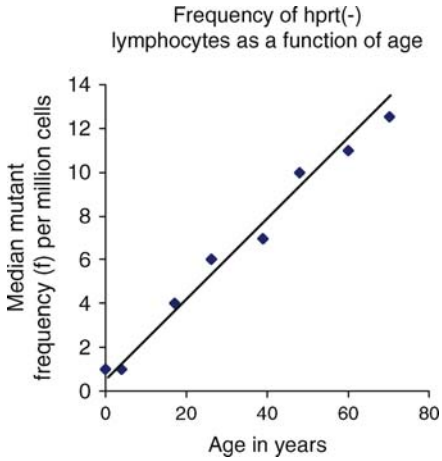
One solution is to study inactivation mutations in a gene on the X-chromosome. Due to hemizygosity in males and X-inactivation in females, a *single* inactivating mutation can cause a loss of function in X-linked genes. Germline mutations in the *hprt* gene

Mutation Rate. Table 1 From Luria, Delbruck (1943) Genetics 28:49

× = # resistant colonies per culture	# Cultures with × resistant colonies	Po (proportion of cultures with zero mutants)	*N* = number of bacteria per culture	ln (Po)	μ = −ln(Po)/*N*
0	29	29/87 = 0.333	2.4×10^8	ln(0.333) = −1.09862	4.6×10^{-9}
1	17				
2	4				
3	3				
4	3				
5	2				
6	5				
>10	24				
Total number cultures	87				



Mutation Rate. Figure 1 Mutant frequency as a function of cell divisions. Ryan and Wainwright grew histidine requiring bacteris in a single bulk culture using the chemostat technique, and the culture was periodically sampled to determine the frequency of reversion mutants that could grow on plates in the absence of histidine. The mutation rate is calculated as the slope of the linear regression curve, in this case 8.5×10^{-9} per cell division. Unlike fluctuation analysis, this method requires one bulk culture, rather than multiple cultures.



Mutation Rate. Figure 2 Median population f values for HPRT as a function of age. Green estimated that the slope of this curve is 1.95×10^{-7} mutations per year, and given estimates of the rate of cell divisions of circulating lymphocytes, estimated the mutation rate to be 8.4×10^{-7} mutations per cell division.

(Xq26–q27.2) result in Lesch-Nyhan syndrome and disrupt the purine salvage pathway, conferring resistance to 6-thioguanine (6-TG). Pioneering work by groups in Vermont and Australia demonstrated rare 6-TG-resistant mutants among the circulating lymphocytes of normal individuals, which harbor acquired mutations in *hprt* that are similar to those inherited by patients with Lesch-Nyhan syndrome. *f* can be calculated by limiting dilution cloning and dividing the cloning efficiency in the presence of 6-TG by the cloning efficiency in the absence of 6-TG. Using *hprt* as a sentinel, a wealth of information has been generated on the frequency and spectrum of spontaneous somatic mutations.

Since *hprt* mutations are growth-neutral, it is reasonable to expect that the mutant frequency in humans would increase with age, by a process reminiscent of the earlier experiments with the chemostat. By studying the mean *f* values for large populations, this has been shown to be the case (Fig. 2). The slope of the regression curve, 1.95×10^{-7}, represents the rate of new spontaneous inactivating mutations per year in *hprt*. Resting human lymphocytes are thought divide once every 4.3 years, so the mutation rate in the general population would be $\approx 8.4 \times 10^{-7}$ mutations per cell division. Fluctuation analysis has also been used to measure μ in *hprt*, with a somewhat lower estimate. Another approach applied to malignant cell lines has been to use HAT media to deplete *hprt* mutants, followed by growth of cells in bulk culture, and measuring *f* periodically by limiting dilution cloning. μ can then be calculated using a curve similar to Fig. 1.

Another X-linked gene, *PIG-A* (Xp22.1), has been used as a sentinel for spontaneous mutations. A broad spectrum of mutations in *PIG-A* can inactivate the function of the gene, resulting a loss of all glycosylphosphatidylinositol (GPI)-linked membrane proteins. This phenotype can be detected by flow cytometry, which can screen large number of cells for rare mutants and can also eliminate pre-existing mutants from a population. In bulk culture experiments similar to that of Ryan and Wainwright, μ in B-cell lines from normal individuals ranged from 3 to 29×10^{-7} mutations per cell division. In patients with the conditions ataxia-telangiectasia and ►Fanconi anemia, μ was shown to be elevated, as expected, given the predisposition to cancer and chromosomal abnormalities in these disorders. The factors that contribute to the variance in μ values in normal individuals has not been established, but may be due to differences in the activity of various repair pathways, involving not only ►ATM and the Fanconi anemia proteins, but also mdm and p53, base excision repair, nucleotide excision repair, and mismatch repair pathways, mechanisms for the inactivation of mutagens (such as endogenous anti-oxidants and glutathione S-transferase enzymes), and the fidelity of DNA polymerases.

Once a mutation has occurred in a given gene in one allele, the remaining allele can be inactivated by a second independent mutation (which will be determined by μ), or it can be lost by events that result in a loss heterozygosity (LOH), such as mitotic recombination,

gene conversion, and mitotic non-disjunction. Based on studies of heterozygotes for germline mutations in the *aprt* and Rb genes, and compound heterozygotes for HLA-A alleles, the rate of LOH events seems to be at least as high as μ itself – and in some situations considerably higher. Though the factors that influence LOH probably differ from those influencing μ, there may be an interaction between LOH events and μ. For example, in an individual with a heterozygous germline mutation in MLH1 (which is essential for mismatch repair and stability of microsatellite repeats), μ may be initially normal but then increase in the subset of cells that have undergone an LOH event that causes the normal allele to be lost. Conversely, CIN genes – which maintain chromosomal stability – could be inactivated by somatic mutations, the likelihood of which is determined by μ. The consequent increase in LOH events could then unmask the effects of previous mutations in genes – like MLH1 – that influence μ.

In 1991 L.A. Loeb proposed that an increase in μ is *indispensable* for malignant transformation. To paraphrase this reasoning, if f in the normal population is 10^{-5}, and the number of mutations required for transformation (n) = 5, then the probability of these five mutations all being present in the same cell would be $(10^{-5})^5 = 10^{-25}$. However, the number of cells in the body is only $\sim 10^{13}$, so cancer should be an exceedingly rare event. Since cancer instead is common, hypermutability must occur at some point in the generation of a malignancy. (Hypermutability could well explain the emergence of chemotherapy-resistance in clinical oncology.) To extend this reasoning, considering that the frequency of mutations in any gene would equal μd, where d represents the number of cell divisions since embryogenesis, we can estimate the probability (P) of n mutations being present in the same cell as = $(\mu d)^n$, where μ is the geometric mean mutation rate among different genes. This formula predicts that the higher the value for n, the greater the elevation in μ that would be required for malignant transformation.

The formula $P = (\mu d)^n$ also predicts that there would be a greater than linear increase in cancer risk with age (since d must increase with age) – very much in accordance with epidemiologic data. This formula also predicts that among individuals with different μ values, the probability of cancer will increase more than linearly as a function of μ. For example if $n = 5$, then an individual with an increase in μ that is twice normal will have a 32-fold increase in cancer risk. Since Knudsen originally proposed the ►two-hit hypothesis to explain the inheritance of retinoblastoma, common estimates of the value of n have increased. Hahn and Weinberg recently estimated n to be at least five, and preliminary data from the cancer genome project suggest that n may be as high as 15. Therefore, small variations in the mutation rate may be pivotal in determining an individuals' cancer risk. While this seems to be the case in special situations (e.g. Fanconi Anemia, ►Ataxia telangiectasia, inherited abnormalities in the mismatch repair and nucleotide excision pathways) the role of μ in cancer risk in the general population awaits epidemiologic confirmation.

Summary

The mutation rate is likely to be a critical parameter in carcinogenesis. μ may increase during the process of malignant transformation, a process where a related parameter, the rate of LOH events, may play a critical role as well. There are estimates of μ in humans (about 8×10^{-7} in *hprt* and $3–29 \times 10^{-7}$ in *PIG-A*), though most of the data on somatic mutagenesis in humans relates to f rather than μ. In contrast to bacteria and yeast, measuring f or μ is challenging in mammalian cells. Technical advances in the measurement of these parameters would facilitate investigations on genomic instability as a risk factor for malignancies.

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Mutator Phenotype

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Definition

Mutator phenotype refers to the increase in mutation rate of cancer cells. The Mutator Phenotype Hypothesis was formulated to account for the disparity between the infrequency of spontaneous mutations in normal cells and the large numbers of mutations observed in human tumors. The hypothesis states that an increase in mutation rate is an early event in tumorigenesis. Some of the random mutations produced throughout

the genome are located in genes that normally function to guarantee the accurate transfer of genetic information with each cell division. The expression of this "mutator phenotype" leads to a cascade of mutations throughout the genome including mutations in other genes required for the maintenance of genetic stability. Among the many mutations produced are ones that promote growth, invasion and metastasis, the hallmarks of cancer.

Characteristics

Mutations and Cancer

The ability of cancer cells to continually mutate may be central to how tumors evolve. In the case of solid tumors, there is approximately a 20-year interval from the exposure of an individual to a carcinogen until the clinical detection of a tumor. During this interval, cancer cells acquire properties that allow them to flourish in a changing environment. By the time a tumor is detectable, the cancer cells are able to divide where normal cells do not, to invade adjacent cellular architectures, to metastasize and eventually to kill the host. In addition, tumors have the capacity to rapidly develop resistance to a variety of chemotherapeutic agents. Each of these phenotypes can be mimicked by mutations in normal cells.

Chromosomal Alterations in Human Cancers

Mutations can be defined as a change in the nucleotide sequence in DNA. Mutations in cancer cells cover a wide spectrum from chromosomal alterations that encompasses millions of nucleotides to point mutations that involve only a few nucleotide substitutions in single genes. Multiple somatic chromosomal alterations are diagnostically associated with cancer cells and involve translocations, deletions, amplifications and aneuploidy (a change in the number of chromosomes in individual cells). Unique chromosomal changes occur at high frequencies in certain tumors and are of diagnostic significance. However, there is a striking heterogeneity of chromosomal alterations in cancer cells within individual tumors. In general, there is a positive correlation between the number of chromosomal changes within a tumor and the malignant potential of that tumor. As molecular techniques are becoming more sensitive, more and more chromosomal abnormalities are being reported in different tumors. In some tumors there is evidence for a sequential order in chromosomal mutations during ►tumor progressions. Measurements of the number of copies of segments of the genome in tumor cells (DNA copy number) and the loss of pieces of DNA (►loss of heterozygosity) have established that many tumors harbor as many as 40 chromosomal alterations. It should be emphasized that these methodologies only score a very small fraction of the genome and as a result may greatly underestimate the number of small chromosomal rearrangements within the genome.

Point Mutations in Human Cancer

Only recently has evidence accumulated indicating that cancer cells not only contain multiple chromosomal alterations, but also contain thousands of smaller changes in nucleotide sequence. These studies have provided strong support of the mutator phenotype hypothesis involving a reduction in the fidelity of DNA replication and/or a decrease in the efficiency ►repair of DNA. An early clue to the large numbers of mutations in cancer cells was the observations that the resistance of cancer cell lines to divergent chemotherapies was mediated by gene ►amplification. The first direct evidence in support of a mutator phenotype in cancer was provided by the demonstration that cells from patients with ►HNPCC (hereditary non-polyposis colon cancer) exhibit ►microsatellite instability in association with mutations in DNA repair genes. Microsatellites are short repetitive sequences of nucleotides in DNA that are subjected to slippage during copying by DNA polymerases. In normal individuals, mutations in microsatellites are corrected by the DNA ►mismatch repair system. In HNPCC tumors, the deficits in mismatch repair result in expansions and contractions in the length of repetitive nucleotide sequences. Based on the enormous number of microsatellites in the human genome, it has been calculated that each tumor could harbor more than 100,000 mutations in these sequences alone. Repetitive sequences located within genes are also mutated at high frequencies in these tumors. Changes in the lengths of repetitive sequences have also been reported in a variety of tumors that are not known to be associated with mutations or deficiencies in mismatch repair, but may be associated with mutations in other genetic stability genes. Thus, repetitive sequences in DNA may be a "hot-spot" for mutagenesis and serve as a sentinel for the detection of a mutator phenotype in cancer.

Rarity of Spontaneous Mutations in Normal Cells

In normal cells, DNA replication and chromosomal segregation are accurate processes. Measurements of mutagenesis of cells grown in culture yield values of $\sim 2.0 \times 10–7$ mutations/haploid gene/cell division. Taking into account this very low frequency of mutations, it seems improbable that the spontaneous mutation rate is sufficient to generate the large numbers of genetic alterations that are observed in cancer cells. If one assumes that ►stem cells, which give rise to a cancer, have a similarly low rate of mutagenesis, then it can be calculated that the average stem cell would accumulate only one or two mutations during tumorigenesis. A few stem cells could accumulate as many as 12 mutations and thus account for the inactivation of ►tumor suppressor genes in ►retinoblastomas and other tumors. However, the

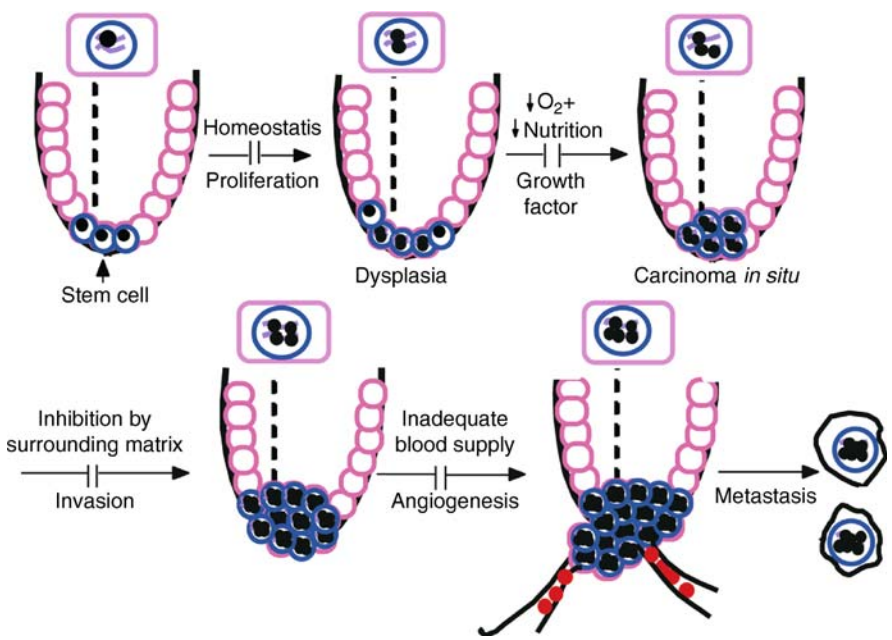
normal spontaneous mutation rate is inadequate to account for thousands of mutations observed in most tumors.

Historical Perspective

The hypothesis that cancer cells exhibit a mutator phenotype was proposed more than 25 years ago and was set forth on the postulate that there is an decrease in the accuracy of DNA synthesis during tumorigenesis. These random mutations could arise by mutations in DNA polymerases that render them error-prone during DNA synthesis or by mutations in DNA repair enzymes making them less efficient in correcting DNA damage or mistakes in incorporation by DNA polymerases. The production of more errors and/or their lack of repair could result in an increase in point mutations and, indirectly, in chromosomal aberrations in cancer cells. Further mutations in these genes would result in cascading numbers of mutations as tumor cells multiplied. Independently, Nowell postulated that cancer cells accumulate multiple mutations by successive rounds of clonal selection. Analysis was based on chromosomal changes in the evolution of human tumors. Leukemias with minimal chromosome changes were considered to be early in clonal evolution, while highly ►aneuploid solid tumors were considered to have undergone multiple rounds of clonal selection. Increases in both mutation rate and clonal selection could contribute to an increase in number of mutations in tumors. Studies in bacteria suggest that these mechanisms may reinforce one another. Sequential rounds of selection for different mutants yielded populations of bacteria that invariably contained mutations in genes that normally function to maintain genetic stability. The conclusion is that selection for mutant clones simultaneously enriches for mutations in genes that can produce the mutant clones. With successive rounds of selection there is increasing enrichment for cells that express a mutator phenotype.

Tumor Evolution

The ability of cancer cells to express a mutator phenotype provides a mechanism for tumor cells to circumvent the host's mechanisms that determine when cells divide and their position within tissues. As tumors expand, they encounter a sequence of restrictive blockages that curtail further expansion. As indicated in Fig. 1 impediments to expansion include the architecture of surrounding tissues, reduced nutrition, decreased oxygen, need for growth factors and inadequate blood supply. Each of these impediments can be overcome by the selective clonal expansion of cancer cells with mutations in genes that impart the required phenotypes. Some of these mutations could be selected from a population of cells harboring multiple mutations. Others could result from new mutations induced by additional mutations that



Mutator Phenotype. Figure 1 Accumulation of mutations during tumor proliferation. As each cancer enlarges it encounters barriers to further proliferation. Rare mutants within the tumor have the properties to escape these barriers and to permit clonal expansion. These mutants also contain mutations in DNA stability genes. Thus with repetitive selection for mutant cancer cells exhibiting specific properties there is simultaneous selection for mutators. This repetitive selection could be an important driving force in the establishment of a mutator phenotypein cancer.

render genetic stability genes error-prone. Thus, with each round of selection there would be a "piggy-backing" of mutations in genes that increase the overall mutation frequency in cancer cells. In order for a mutator phenotype to account for the many mutations in a cancer cells, it would have to be present early during the course of tumor progression. The continued expression of a mutator phenotype might not be compatible with the enhanced growth of cancer and thus may not be exhibited by cancer cells by the time a tumor is clinically apparent. Nevertheless, the footprints of its presence, multiple mutations, would persist in all cell descendents throughout the course of tumorigenesis (►multistep development).

Implications of a Mutator Phenotype

The multiplicity of mutations in cancer cells resulting from a mutator phenotype has implications with respect to pathogenesis, diagnosis, treatment and prevention of human cancer. The presence of large number of mutations in cancer cells implies that the malignant process is irreversible. Once a mutation occurs in a gene it is highly unlikely that a second mutation would occur at the same position to change the mutation back to the wildtype sequence. Also unlikely is the possibility that a second mutation would occur at a different loci and suppress the effects of the first mutation, particularly if the first mutation occurs at a tumor suppressor gene and inactivates that gene. Thus the concept of a mutator phenotype is not compatible with the hypotheses that cancer cells have the potential to differentiate into normal cells. The reports that certain tumors spontaneously regress does not necessarily indicate that these tumor cells have been transformed into normal cells. It is more likely to reflect the selective killing of tumor cells by normal host mechanisms that reject foreign cells.

The heterogeneity of mutations within a tumor provides a population of cancer cells harboring mutants that are resistant to a many chemotherapeutic agents. In addition, the mutator phenotype exhibited by many cancer cells allows them to continuously generate new mutant clones that are resistant to and proliferate in the presence of chemotherapy, immunotherapy and even radiation therapy. While such a scenario allows cancer cells to thwart many therapeutic protocols, it might offer new approaches to cancer therapy. It might be feasible to develop drugs that target the genetic instability of tumor cells. For example, nucleotide analogs can be designed that are preferentially incorporated into DNA by mutant error-prone DNA polymerases.

The large number of mutations in microsatellite sequences in cancer cells may facilitate the early detection of certain human malignancies. Rare tumor cells circulate, can be detected in the blood and could account for the hematological spread of tumor cells to distant sites. In addition, cancer cells in tumors frequently undergo cell death (apoptosis) and shed their DNA into the blood. Thus, methods can be developed for the detection of altered microsatellite sequences in DNA and cells in blood for the early detection of human malignancy and for calibrating the potential of tumors for metastatic dissemination.

If a mutator phenotype is rate-limiting for tumor progression, it is important to identify the agents and genes involved in its expression. Sources for a mutator phenotype such as mutant DNA polymerases, which are error-prone, or mutations in DNA repair enzymes are not easily corrected. Other sources might be more easily attenuated. Recent evidence indicates that cells contain a number of error-prone DNA polymerases that are induced by DNA damage, and it might be feasible to develop drugs to selectively inhibit these enzymes. More immediately open to experimental analysis is increased DNA damage by normal cellular metabolites, such as oxygen free radicals. These radicals can be scavenged by the administration of specific drugs and vitamins.

If a mutator phenotype constitutes a rate-limiting step for the development of a cancer it might be possible to prevent cancers by delay. Even a twofold reduction in the rate of accumulation of mutations in cancer cells might have profound effects on the age at which patients succumb to certain adult cancers. Consider primary hepatoma resulting from hepatitis virus B infection that occurs in early infancy and persists chronically (►Hepatitis Virus Associated Hepatocellulor Carcinoma). The persistence of chronic hepatitis in individuals increases the risk of subsequent hepatoma by more than 200-fold. It usually takes 40 additional years for the tumor to be clinically manifested. A reduction in the rate of mutation accumulation by only twofold could delay the clinical appearance of the tumor from age 50 to 90. It has been postulated that the persistence of hepatitis results in an inflammatory reaction with generation of oxygen free radicals. Thus, drugs that scavenge ►reactive oxygen species (ROS) might have a role in the prevention of primary hepatoma. It should be emphasized that prevention by delay does not affect the rate of initiation of the malignant process, but rather is directed at slowing down the rate of its progression. This approach may offer new direction to reducing the number of cancer-associated deaths.

►Microsatellite Instability

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mutHLS

Definition

The ►mismatch repair system in the bacteria *Escherichia coli*. Five human proteins (MSH2–6) with homology to the mut S component and four homologues (MLH1, MLH3, PMS1, PMS2) to mut L have been identified. Germline mutations in some of these genes predispose to hereditary nonpolyposis colorectal cancer (HNPCC) syndrome.

►Microsatellite Instability
►Lynch Syndrome

MUTYH-Associated Colorectal Polyposis

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Definition

MUTYH-associated polyposis; MAP: An autosomal recessive disorder caused by germline mutations in the base excision repair gene *MUTYH* and characterized by multiple colorectal adenomas and carcinomas.

Characteristics

Inherited Predisposition to Colorectal Cancer

Inherited factors are thought to play a major role in up to one third of colorectal cancers (CRCs), but only a minority of these can be accounted for by established CRC predisposition genes. Familial adenomatous polyposis (FAP) (MIM 175100) is an autosomal dominant disorder characterized by the development of hundreds or thousands of colorectal adenomas (CRAs), some of which progress to cancer. FAP is caused by inherited mutations in the adenomatous polyposis coli (*APC*) gene that acts as a gatekeeper regulating proliferation of colonic cells. A milder form of this disorder termed attenuated FAP (AFAP) is associated with smaller numbers of adenomas and is caused by germline mutations in the extreme 5′ or 3′ ends of *APC* or in the alternatively spliced region of exon 9. Hereditary nonpolyposis CRC (HNPCC; MIM 114500) is an autosomal dominant disorder characterized by early-onset CRC (in the absence of florid polyposis) and other extracolonic cancers and is caused by inherited deficiencies in the mismatch repair pathway.

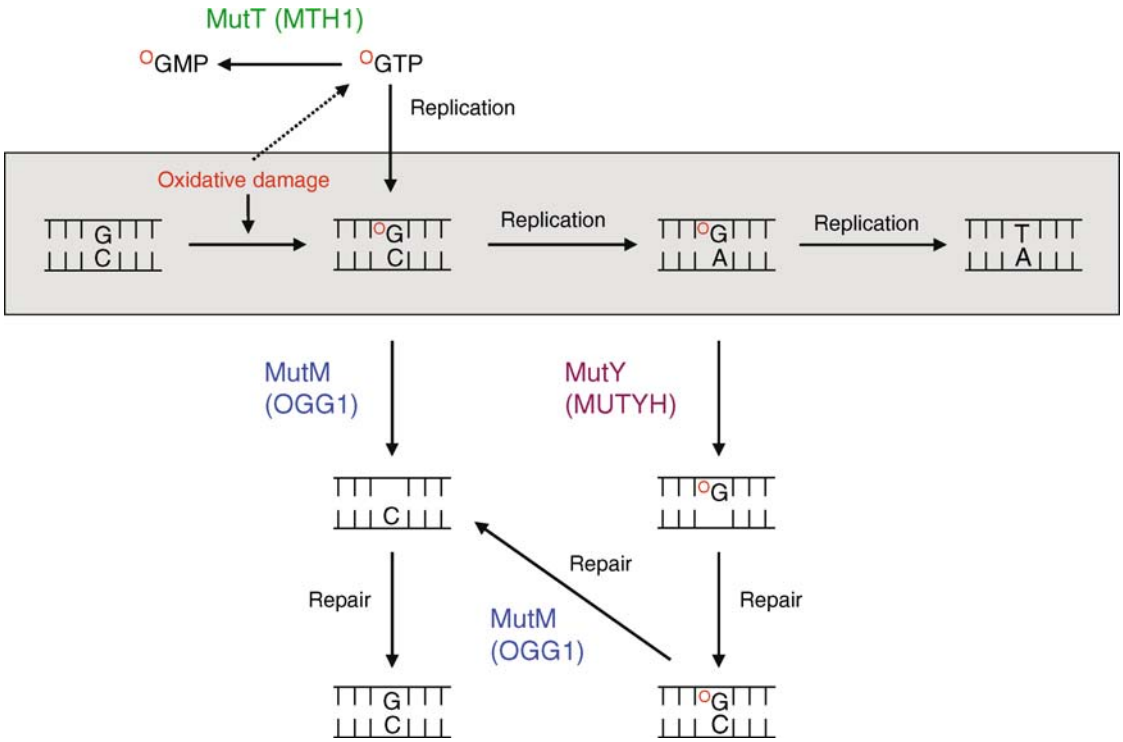
Identification of an Unusual (G:C→T:A) Mutator Phenotype in Family N

In 2002, Al-Tassan et al. [1] studied a British family (Family N) with three affected siblings with multiple CRAs and carcinoma. Sequencing of the entire *APC* gene open reading frame (ORF) in germline DNA samples from two of the affected siblings, together with haplotype and expression analyses, excluded an inherited *APC* gene defect. To provide a clue as to the underlying genetic defect, the investigators examined colorectal tumors from Family N. Sequencing of the *APC* ORF in each of the eleven tumors revealed eighteen somatic mutations, 15 of which were G:C→T:A transversions. This class of mutations accounts for only ~10% of reported somatic *APC* mutations which normally lead to inactivation of *APC* in colorectal tumors. Comparison of the findings in Family N with a database of somatic *APC* mutations from sporadic and FAP-associated colorectal tumors, confirmed that the excess of G:C→T:A transversions in Family N ("the mutator phenotype") was highly significant.

Reactive oxygen species generated during aerobic metabolism represent a potent source of DNA damage. 8-oxo-7,8-dihydro2′deoxyguanosine (8-oxoG) is the most stable product of oxidative DNA damage and readily mispairs with adenines, leading to G:C→T:A mutations in repair-deficient bacteria and yeast. In *Escherichia coli*, three enzymes help protect cells against the mutagenic effects of guanine oxidation. The base excision repair (BER) DNA glycosylase MutM removes the oxidized base from 8-oxoG:C base pairs in duplex DNA, the BER DNA glycosylase MutY excises adenines misincorporated opposite unrepaired 8-oxoG during replication, and the 8-oxo-dGTPase MutT prevents the incorporation of 8-oxo-dGMP into nascent DNA (Fig. 1). Homologues of *mutM*, *mutY* and *mutT* have been identified in human cells and termed *OGG1*, *MUTYH* (*MYH*) and *MTH1*, respectively.

Inherited Mutations in *MUTYH* Predispose to Colorectal Tumors

To determine whether an inherited defect in the 8-oxoG repair pathway was responsible for the pattern of



***MUTYH*-Associated Colorectal Polyposis. Figure 1** The 8-oxoG repair system in *E.coli*. MutT (human orthologue MTH1), an 8-oxo-dGTPase, prevents the incorporation of 8-oxo-dGMP into nascent DNA, MutM (human orthologue OGG1) DNA glycosylase removes the oxidized base from 8-oxoG:C base pairs in duplex DNA, and MutY (human orthologue *MUTYH*) DNA glycosylase excises A residues misincorporated opposite unrepaired 8-oxoG during replication. 8-oxoG readily mispairs with A residues, leading to G:C→T:A mutations in MutM and MutY-deficient bacteria (gray box). 8-oxoG is denoted by °G.

somatic G:C→T:A mutations in Family N, Al-Tassan et al. [1] sequenced the ORFs of *OGG1*, *MUTYH* and *MTH1* in a blood DNA sample from an affected sibling. Two non-conservative amino acid variants were identified in *MUTYH* (Y165C and G382D), but no likely pathogenic changes were identified in *OGG1* or *MTH1*. All three affected siblings from Family N were found to be compound heterozygotes for Y165C and G382D, suggesting transmission as an autosomal recessive trait.

In a follow up study, the investigators identified seven further unrelated patients with multiple CRAs (six with CRC) and biallelic germline *MUTYH* mutations, including four cases homozygous for truncating mutations. Again, colorectal tumors from the affected individuals displayed a highly significant excess of somatic G:C→T:A mutations in *APC*, as compared to sporadic or FAP-associated colorectal tumors. These findings supported the role of biallelic inherited mutations in *MUTYH* in causing an AFAP-like syndrome characterized by multiple CRA and CRC. This disorder has been termed *MUTYH*-associated polyposis (MAP).

The Pathway of MAP Tumorigenesis

Consistent with the G:C→T:A mutator phenotype observed in *APC*, a proportion of MAP adenomas show a specific, activating missense mutation of *K-ras* (G12C) that also results from a G:C→T:A transversion. Established genetic pathways of CRC development include those associated with microsatellite instability (MSI) and chromosomal instability (CIN). MSI is not a feature of MAP tumors. The role of CIN in MAP-associated tumors is unclear; some investigators have reported that MAP tumors appear to be near diploid whereas others have shown that up to 80% of MAP polyps are aneuploid.

The Phenotype of MAP

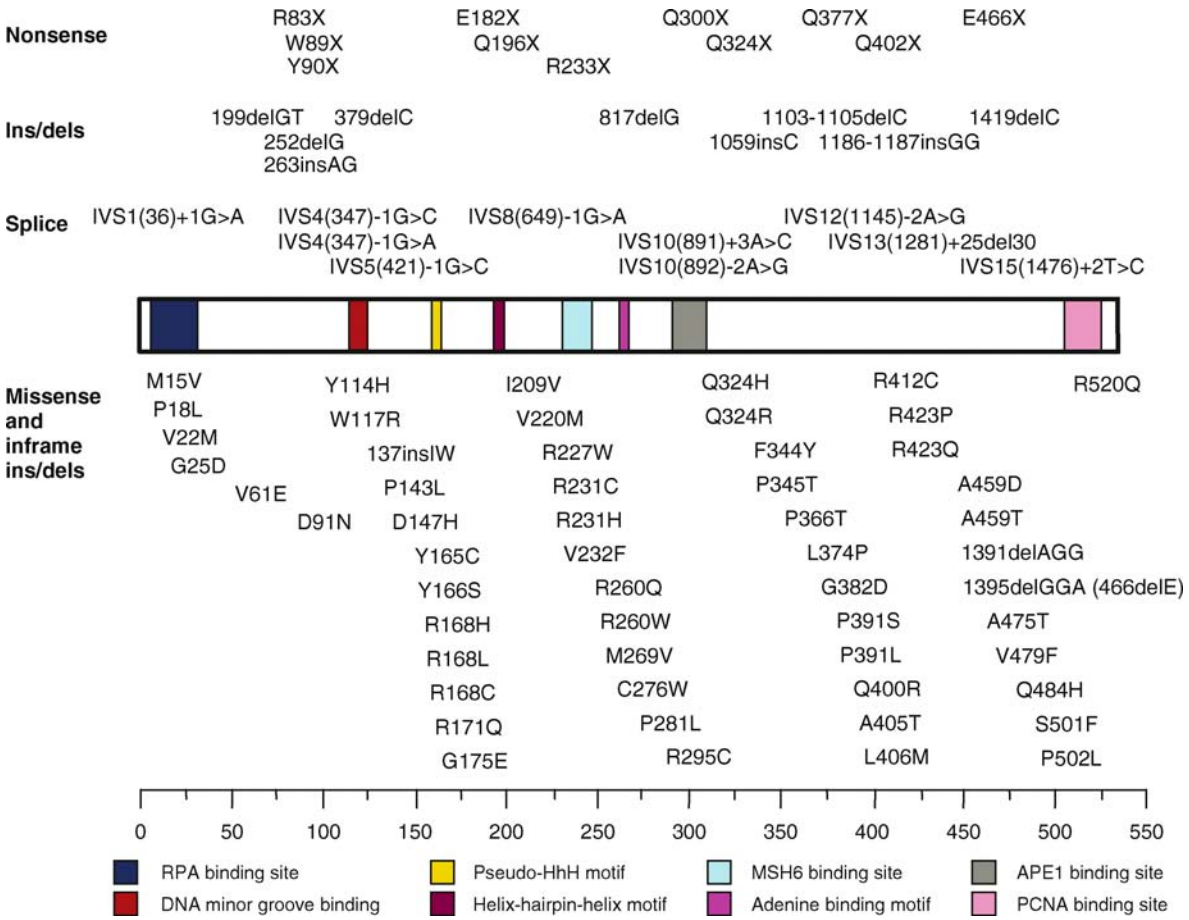
Mutation analysis of *MUTYH* has now been undertaken in several series of patients with FAP-like and AFAP-like phenotypes and in whom no inherited *APC* mutation could be identified. Biallelic *MUTYH* mutations have been identified in a quarter of such cases and, in general, segregation has been consistent with transmission of MAP as an autosomal recessive trait with high/complete penetrance. The colorectal

phenotype of MAP closely resembles AFAP (10–100 adenomas), or FAP (100–1,000 adenomas), but not severe FAP (>1,000 adenomas). In addition, some cases appear to develop fewer than ten macroscopic adenomas by middle age and to have developed CRC in the absence of obvious polyposis. As expected for a recessive trait, many cases appear to be sporadic and hence present symptomatically.

A possible explanation for the predominantly colorectal phenotype in MAP is the high level of oxidative damage that occurs in the large bowel. An alternative or additional factor was proposed after examination of the target sequence surrounding the somatic G:C→T:A mutations in MAP tumors – the two bases immediately 3′ to the mutated G are almost always AA and this preponderance of G:C→T:A mutations at GAA sequences is highly significant. *APC*, the key gatekeeper in colorectal tumorigenesis, has a total of 216 GAA sites in which G:C→T:A mutations could lead to termination codons. By comparison, gatekeeper genes for other tissues have significantly fewer target sites and therefore *APC* may be a particularly vulnerable target for mutagenesis in MAP.

MUTYH Mutation Spectrum

As of November 2006, 30 mutations that are predicted to truncate the protein product have been reported in *MUTYH*, comprising eleven nonsense, nine small insertion/deletions and ten splice site variants (Fig. 2). In addition, 52 missense variants and 3 small inframe insertion/deletions have been reported that are distributed throughout the gene. Although there is



***MUTYH*-Associated Colorectal Polyposis. Figure 2** Spectrum and distribution of truncating mutations and missense/inframe insertion/deletion variants identified in *MUTYH* (as of November 2006). Approximate positions of putative functional domains are indicated in relation to the *MUTYH* coding region (The N-terminal domain of *MUTYH* contains the catalytic region and shares several motifs with other BER glycosylases, including the helix-hairpin-helix (HhH), pseudo HhH and the iron–sulfur cluster loop motif and the C-terminal domain plays a role in 8-oxoG recognition. *MUTYH* has also been shown to interact with AP endonuclease, PCNA, RPA and the MMR heterodimer MSH2/MSH6 via MSH6).

some reporting bias, the missense variants Y165C and G382D together account for ~73% of all *MUTYH* mutations reported to-date, and have been identified commonly in the British, Italian, American, Portuguese and Dutch populations. In addition, specific mutations in *MUTYH* have been identified in different populations and diagnostic screening strategies will have to be optimized accordingly. Apart from Y165C and G382D, most missense variants are rare; however, their collective frequency and the lack of functional data for the vast majority pose major difficulties for molecular diagnostics since many will be benign polymorphisms.

Genetic Testing and Clinical Management of MAP

Genetic testing of *MUTYH* in patients with phenotypic features suggestive of MAP is essential in planning for the surveillance needs in the extended family. MAP must be distinguished from FAP/AFAP as it is the siblings, rather than offspring of MAP cases who are most likely to require further investigation. Genetic testing can be used to identify those siblings of MAP cases who are at risk and also to clarify the genetic status of spouses of those with biallelic mutations so that their offspring can be counseled accurately. Since polyp number may be very low or even zero in cases with CRC and biallelic *MUTYH* mutations, some researchers have suggested wider testing for *MUTYH* among incident CRC cases.

We recommend annual or biennial colonoscopic surveillance for individuals with biallelic *MUTYH* mutations, commencing by 20 years of age. Although duodenal adenomas have been reported in some MAP patients, the case for upper gastrointestinal tract surveillance is unclear and there is currently no evidence for screening of other organs. Surgical options for colorectal disease need to be tailored to the individual patient, since tumor burden can apparently vary from a count of one to many hundred.

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MVP

Definition

►Major Vault Protein

Myalgia

Definition

Means muscle pain.

►Bryostatin-1

MYB

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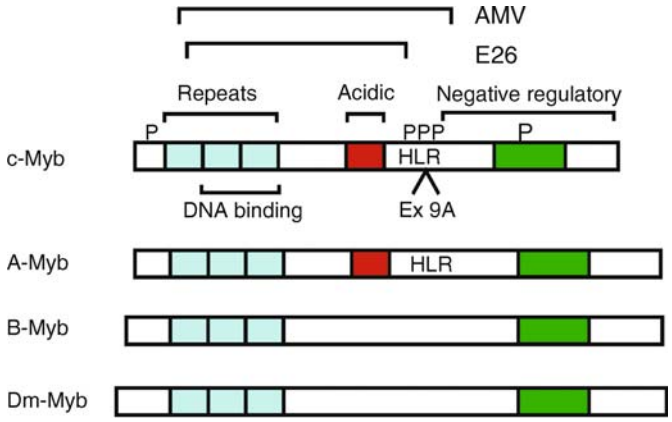
Definition

MYB is a family of genes related to the C-MYB proto-oncogene. The name of this gene comes from myeloblastosis, the type of leukemia caused by the V-MYB viral oncogene in chickens. All vertebrates contain three related MYB genes, C-MYB (MYB), A-MYB (MYBL1), and B-MYB (MYBL2). The fruit fly and sea urchin have only one closely related MYB gene, whereas the nematode *C. elegans* appears to have lost the MYB gene during evolution. Other more distantly related MYB-like genes are present in animals, plants, and fungi (Fig. 1).

Characteristics

The MYB gene family was first discovered because the avian myeloblastosis virus, a ►retrovirus that causes acute myelogenous leukemia in chickens, contains V-MYB. Like most other retroviral ►oncogenes, V-MYB represents an altered form of a normal cellular ►proto-oncogene, namely C-MYB. A second leukemia virus in chickens, the E26 erythroleukemia virus, contains both V-MYB and another oncogene of cellular origin, V-ETS (►ETS transcription factors). Furthermore, retroviruses without oncogenes can cause leukemias and lymphomas in chickens and in mice by insertion of the virus into the C-MYB proto-oncogene. Consistent with the role of V-MYB and



MYB. Figure 1 Animal Myb Proteins. The brackets next to AMV and E26 indicate the portion of C-MYB encoded by the V-MYB oncogenes of these retroviruses. The shaded boxes indicate the most highly conserved domains. HLR indicates a heptad leucine repeat. Ex 9A indicates the position of an alternatively spliced exon in C-MYB. P indicates a known phosphorylation site. Dm-Myb indicates Drosophila MYB.

altered forms of C-MYB in causing cancers of blood cells (leukemia and lymphoma), mice that lack a functional C-MYB gene die as embryos because they fail to produce blood cells. The normal C-MYB gene is turned on (expressed) in immature blood cells and then turns off as these blood cells mature (differentiate). C-MYB is also expressed in immature cells in other tissues including skin, gut and lung, but these tissues appear to develop normally in mouse embryos that lack a functional C-MYB gene. Recent studies have shown that C-MYB is required for the maintenance of normal of gut epithelium in the mouse.

Like C-MYB, the expression of A-MYB is limited to particular tissues and cell types including testes, brain, mammary gland, and germinal center B lymphocytes. Mice that lack a functional A-MYB gene survive to adulthood. However, the males do not produce functional sperm. The females are fertile but their mammary glands do not proliferate in response to pregnancy. Mice that express too much A-MYB develop an abnormal proliferation of germinal center B lymphocytes.

The B-MYB gene appears to be expressed in all dividing cells in vertebrates. Mice that lack a functional B-MYB gene die very early in embryogenesis, with a severe defect in cell proliferation. The single MYB gene of sea urchins and fruit flies most closely resembles the B-MYB gene of vertebrates. This observation suggests that the more specialized A-MYB and C-MYB genes arose more recently by gene duplication and divergence during the evolution of vertebrates.

All the proteins encoded by these MYB genes contain a very similar domain near the amino terminus that binds directly to a specific DNA sequence (AACNG). This DNA-binding domain itself is composed of three tandem repeats of a ~50 amino acid protein sequence. Sequences similar to these Myb protein repeats are also present in a number of proteins that regulate the structure of chromatin and chromosomes including SWI3, ADA2, and the major telomere (►Telomerase) binding proteins of animals, plants and fungi.

All the Myb proteins of animals also contain a conserved domain near the carboxyl terminus that appears to act as a regulatory switch to control protein function. The A-Myb and C-Myb proteins contain an additional central domain that includes an acidic region and a heptad leucine repeat. This central domain causes other genes to be activated when these Myb proteins bind to them. A similar central domain is not present in B-Myb or the Myb proteins of invertebrates, although these proteins have nevertheless been reported to activate or repress the expression of various genes.

Cellular and Molecular Regulation

The regulation of B-MYB gene expression is fairly well understood. B-MYB is off when cells are not engaged in the division cycle and is turned on just before actively dividing cells begin to synthesize new DNA (S phase). The expression of the B-MYB gene is directly regulated by a critical cellular pathway that includes the p16/Ink4a tumor suppressor gene product, the ►cyclin D oncogene product, the p107/p130 relatives of the RB (►RB1) tumor suppressor gene and the ►E2F transcription factor. This pathway keeps the B-MYB gene off until late in the G1 phase of the cell cycle. B-MYB is then turned on at the same time as many genes that encode proteins essential for DNA replication. The regulation of the C-MYB gene appears to be similar to that of B-MYB, but its expression is limited to particular cell types. In contrast,

the expression of A-MYB does not appear to be similarly regulated during the cell cycle.

The amino and carboxyl termini of C-Myb protein are critical for its normal regulation. Both the V-Myb protein of the avian myeloblastosis virus (AMV) and the V-Myb-Ets fusion protein of the avian E26 leukemia virus (E26) have truncations of both termini of C-Myb. Experiments with recombinant retroviruses have shown that these truncations are important for activating the oncogenic potential of the C-Myb protein. In addition, retroviral insertions into C-MYB that cause leukemias and lymphomas in mice and birds also result in the production of truncated versions of the C-Myb protein. Although both C-Myb and V-Myb can regulate the expression of a number of other genes, in most cases the role of these "target" genes in oncogenesis remains unclear. One exception is the abnormal indirect activation of a growth factor gene by V-Myb that clearly promotes the growth of leukemic cells.

The C-Myb protein can be phosphorylated by a number of protein kinases including casein kinase II, glycogen synthase kinase III and MAP kinase. An indirect regulation of the C-Myb protein via the pim-1 protein kinase has also been proposed, as has a direct regulation of the Myb DNA-binding domain by prolyl isomerases. The B-Myb protein can be phosphorylated by the cyclin A-CDK2 protein kinase during S phase. However, the physiologic relevance of these suggested modes of regulation remain unclear, pending appropriate genetic analyses.

Recent studies have shown that Drosophila Myb protein, the ortholog of vertebrate B-Myb, is present in a large multiprotein complex that includes homologues of Rb, a repressor E2F, DP, a histone deacetylase, and a number of other nuclear proteins. Previous studies in C elegans showed that the genes encoding many of these Myb-associated proteins (synMuvB genes) inhibit signaling by activated tyrosine kinase receptors via the RAS pathway.

Interestingly, the loss of Drosophila Myb has been shown to result in alterations in chromosome condensation, spindle assembly, and ploidy. Because vertebrate B-Myb can complement Drosophila Myb null mutants in some cell types, it seems likely that B-Myb may play a similar role in regulating mitosis, a hypothesis supported by the recent characterization of a zebrafish mutant of B-Myb. The role of other Myb-associated proteins in these processes remains to be investigated.

Clinical Aspects

Until recently, the MYB genes had not been identified as targets of frequent and consistent chromosomal translocations, gene amplifications or point mutations in human cancers. However, recent studies have identified consistent chromosomal translocations and gene duplications of C-MYB in human T cell acute lymphocytic leukemias. Other reports suggest that C-MYB may be amplified in as many as 10% of all pancreatic carcinomas. Even in the absence of obvious genetic aberrations, the levels of expression of the MYB genes have been shown to correlate with clinical prognosis for a number of different types of cancer. In particular, B-MYB is one of a small number of genes, the increased expression of which has been reported to be sufficient to predict the clinical behavior of human breast cancer. As the tools for the analysis of altered gene expression and copy number in human cancers become more refined and widely available, our knowledge of the involvement of the MYB genes in a variety of human cancers is certain to become clearer.

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Myc-associated Protein X

Definition

►Max.

N-myc Downstream-Regulated Gene

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Synonyms

N-myc downstream-regulated gene, *NDRG1*; *RTP*, *cap43*, *rit42*, *TDD5* (mouse), *Ndr1* (mouse), *Bdm1* (rat)

Definition

NDRG1 stands for N-myc downstream-regulated gene/differentiation-related gene, which was originally identified as being strongly upregulated on induction of differentiation in colon carcinoma cell lines. *NDRG1* encodes a 3.0 kb mRNA that translates to a 43-kDa cytoplasmic protein with an isoelectric point of 5.7. *NDRG1* is expressed almost ubiquitously in most of the organs, and the level of expression is particularly high in prostate, ovary, intestine, and kidney. Amino acid sequence of the NDRG1 protein reveals three serine phosphorylation sites, five calmodulin kinase 2 phosphorylation sites, five myristoylation sites, three protein kinase C phosphorylation sites, one tyrosine phosphorylation site, one thioesterase site, and one phosphopantotheine attachment site. It has been shown that protein kinase A and calmodulin kinase 2 are also involved in the phosphorylation of this protein in vitro. It has also been shown that phosphorylation of NDRG1 is negatively correlated to the cell density thereby suggesting a possible role in cell proliferation.

Characteristics

NDRG1 is a ►metastasis suppressor gene and has been shown to suppress metastasis in colon, prostate, and breast cancer. In the case of prostate cancer, the reduced NDRG1 expression correlated significantly with the ►Gleason grade as well as the ethnic origin of the cancer patients. In both prostate and breast cancers a significant level of differential expression of NDRG1 between patients with organ-confined disease and those with lymph node or bone metastasis were observed. Similar results were also observed in pancreatic adenocarcinoma thus suggesting the reduced expression of NDRG1 to be a strong predicator of lymph node and bone metastasis. In clinical specimens of prostate and breast cancer, it has been shown that phosphatase and tensin homolog deleted on chromosome 10 (►*PTEN*) gene expression in normal and poorly differentiated tumor cells has a positive correlation with NDRG1 expression. Apart from PTEN, ►p53 and N-myc have also been demonstrated to regulate the NDRG1 expression. Treatment of tumor cells with inhibitors of DNA methylation and histone deacetylation induced the expression of *NDRG1* gene indicating that histone deacetylation and ►CpG island hypermethylation as possible mechanism of suppression of NDRG1 in tumor cells. In a recent study, it has been shown that NDRG1 downregulates the expression of activating transcription factor 3 (*ATF3*) gene that is capable of promoting tumor metastasis. NDRG1 also plays a role in cell adhesion as it upregulates the expression of cell adhesion molecule, ►E-cadherin, suggesting one of the mechanisms via which NDRG1 blocks metastasis of tumor cells. In relation to angiogenesis, overexpression of NDRG1 suppressed the expression of two angiogenic factors, ►vascular endothelial growth factor-1 (VEGF-1) and interleukin-8 (IL-8). NDRG1 is also regulated by the ►hypoxia-inducible factor 1 (HIF-1) transcription factor that is activated in hypoxia thus indicating the role of *NDRG1* as a stress responsive gene. From all the above investigations it is evident that *NDRG1* has a critical role in tumor progression and a higher expression level is an indicator of less aggressive phenotype thus acting as a reliable prognostic marker in several forms of cancer.

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N-myc Downstream-regulated Gene, NDRG1

►N-myc downstream-regulated gene

MYC Family

Definition

The MYC family is a group of helix-loop-helix proteins comprising members ►MYC, ►MYCN, ►MYCL and MYCL2. They are involved in cell-cycle regulation, the control of proliferation and development.

►Helix-Loop-Helix (HLH) domain
►MYC Oncogene

Myc Oncogene

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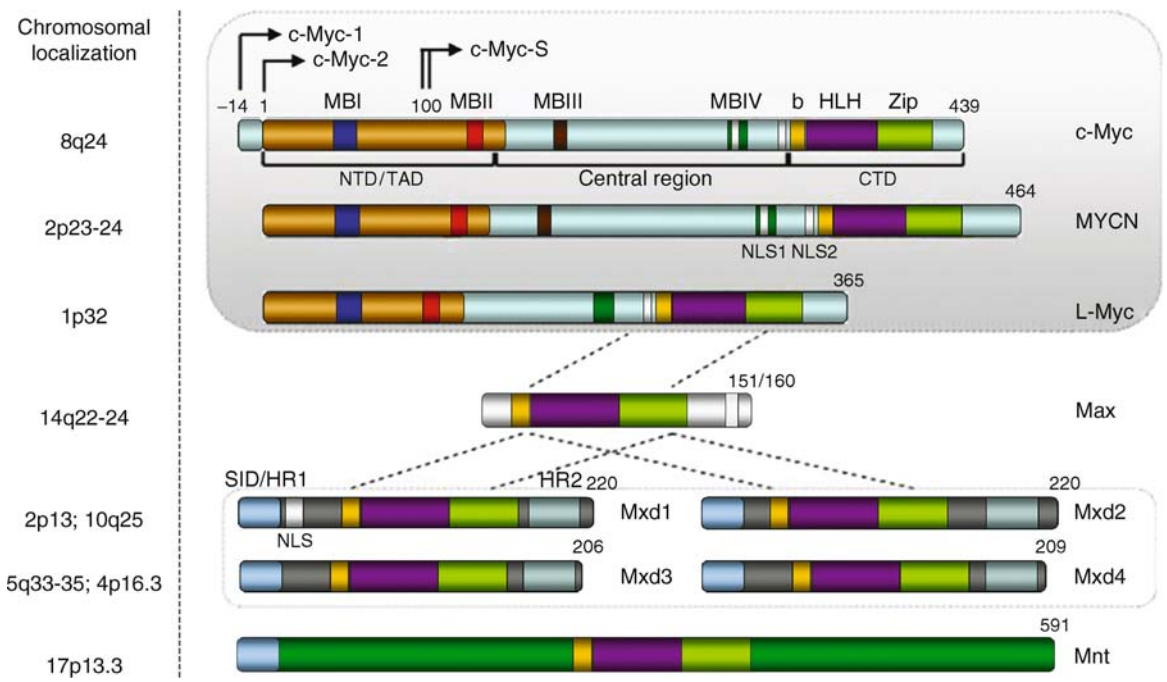
Definition

The *myc* gene was originally identified in the avian ►*myelocytomatosis* retrovirus as the ►oncogene responsible for inducing myeloid leukemia in birds. The cellular homologue, c-*MYC*, was discovered as a promoter insertion in chicken and the human gene was later identified in the ►B-cell tumor Burkitt lymphoma. The related *MYCN* and *MYCL* genes were found ►amplified in ►neuroblastoma and in small cell ►lung cancer, respectively. The *myc* genes are highly conserved through evolution and are found in all animals examined with the exception of the nematode *caenorhabditis elegans* that seems to lack a *myc* ortholog. In vertebrates, c-*myc* and *MYCN* are essential during development. Two additional *myc* genes: S-*myc* and B-*myc* are expressed exclusively in rodents and are not as well characterized. The gene product Myc is a multifunctional protein with the ability to regulate the cell cycle, cell growth and metabolism, differentiation, ►apoptosis, ►angiogenesis, and transformation.

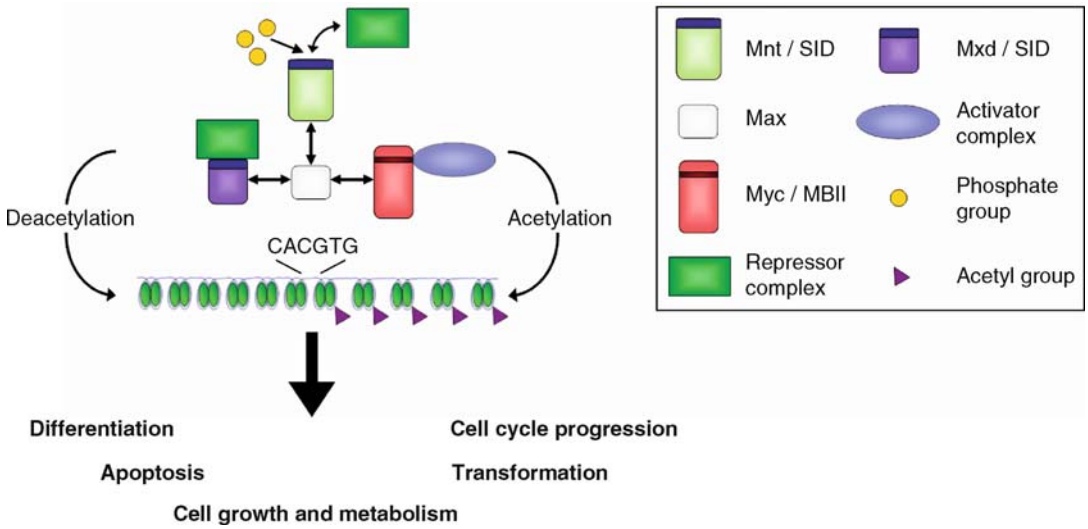
Characteristics

The *MYC* proto-oncogene encodes a nuclear ►transcription factor of the basic region-helix-loop-helix-leucine zipper (bHLH-Zip) family, belonging to the Myc►/Max/►Mad(Mxd) network (Fig. 1). The expression of *MYC* is regulated by external signals such as growth factors and extracellular matrix contacts, and through internal cell cycle control. Myc has an important role in driving proliferation and is expressed during all stages of the cell cycle during division. When quiescent cells are stimulated to re-enter the cell cycle, Myc levels increase dramatically and then return to steady-state levels once cells start to cycle. In contrast, Myc is normally downregulated during differentiation while ectopic *myc* expression inhibits this process.

Myc forms a complex with Max, and these heterodimers bind specifically to 5′-CACGTG-3′ or non-canonical ►E-box sequences and regulate transcription through recruitment of ►histone acetyltransferases (HATs) and ►chromatin remodeling proteins (Fig. 2). These proteins will acetylate Myc target gene promoters and open up the chromatin to permit access



Myc Oncogene. Figure 1 Schematic presentation of the Myc network proteins, including chromosomal localization of the human genes encoding these proteins. In the c-Myc protein, the transcriptional initiation sites for the three different isoforms are indicated. MB, Myc Homology Box; NTD, N-terminal domain; TAD, transcriptional activation domain; CTD, C-terminal domain; b, basic region; HLH, helix-loop-helix; Zip, leucine zipper; NLS, nuclear localization signal; SID, Sin3-interacting domain; HR, homology region.



Myc Oncogene. Figure 2 Transcriptional regulation by Myc network proteins upon binding to the 5′-CACGTG-3′ E-box sequence. During quiescence or differentiation, Mxd/Max represses transcription by recruiting the repressor complex containing histone deacetylases to promote a closed chromatin conformation. In proliferating cells, Myc/Max promote transcription through activator complexes containing histone acetyltransferases and co-factors resulting in an open and accessible chromatin configuration. Mnt is expressed at all stages of the cell cycle and confers transcriptional repression in a similar manner as the Mxd proteins. Indicated below are some of the biological effects of these transcription factors. SID, Sin3-interacting domain; MBII, Myc Homology Box II.

for transcription factors and co-activators. Complexes containing the HATs GCN5 or TIP60 are recruited to the N-terminal of the Myc protein via the adaptor TRansformation/tRranscription domain-Associated Protein (TRRAP). Increasing evidence suggest that Myc regulates general acetylation of the genome and not only of specific targets. Another important role of Myc is the transcriptional activation of ribosomal DNA (rDNA), promoted through interaction with Max in nucleoli.

In addition, Myc possesses the capacity to repress transcription through interaction with transcriptional activators such as ►Miz-1. Miz-1 promotes transcription when bound to initiator elements (Inr) in promoters of target genes. By forming a ternary complex with Max and Miz-1 at the Inr, Myc quenches gene activation.

To date, several thousand Myc targets have been identified, some of which are activated and some which are repressed. ►*cyclin D*2, ►*cyclin-dependent kinase* 4 *(CDK4), carbamoyltransferase-dihydroorotase* (*cad*), *ornithine decarboxylase (ODC*), and ►*telomerase* reverse transcriptase *(hTERT)* are examples of target genes activated by c-Myc, and genes repressed by Myc include those encoding the CDK inhibitors $p15^{INK4b}$ and ►$p21^{CIP1}$, and the transcriptional repressor *Mxd4*.

Myc Structure and Function

The Myc protein can be divided into three major parts: the C-terminal domain harboring the basic region and the HLH-Zip motifs, the central region, and the N-terminal domain that is also referred to as the transcriptional activation domain (TAD) (Fig. 1). Myc forms a complex with Max through the HLH-Zip motif and the basic regions of the Myc/Max heterodimer confer DNA binding to the E-box. Most of the functions of Myc have been ascribed to four highly ►conserved regions within the protein called Myc homology boxes (MB). MBI and MBII within the TAD are the best characterized ones while MBIII and MBIV in the central region were more recently identified. Collectively, the Myc boxes are important for proliferation, oncogene cooperation to induce transformation, Myc-induced apoptosis, and blockage of differentiation.

Post-Translational Modifications and Regulation of Myc Turnover

Myc is a highly unstable protein with a short half life (15–30 min). The protein is degraded by the ►ubiquitin/proteasome system where ubiquitin tagged molecules are recognized and degraded by the proteasome. To date, three ubiquitin ligases that regulate Myc protein turnover and/or activity have been identified. Ubiquitination by the F-box proteins Skp2 and Fbw7 targets the protein for degradation. In addition, Skp2-mediated ubiquitination seems to be required for Myc-induced transriptional activation. Similarly, the HectH9 ligase facilitates Myc target gene activation but forms a ubiquitin chain that does not induce protein degradation. Post-translational modifications, such as phosphorylation

M

and de-phosphorylation of highly conserved residues within the TAD of the protein, dictate whether Myc will be ►ubiquitinated for degradation or stabilized. These events are in large monitored by the ►Ras pathway, controlling both stabilization and de-stabilization of the Myc protein. Acetylation of Myc will also influence its stability by affecting its subcellular localization and turnover.

Other Members of the Max-Interacting Network

The small phosphoprotein Max is in the center of the network (Fig. 1), and a prerequisite for transcriptional activity of the other network proteins. Myc as well as the transcriptional repressors ►Mad(Mxd) and ►Mnt needs to dimerize with Max in order to bind E-box DNA (Fig. 2). Max is ubiquitously expressed at levels that remain constant throughout the cell cycle. Transcriptional repression by Mxd and Mnt is mediated through association with the adaptor protein Sin3 that in turn recruits ►histone deacetylase complexes (HDACs) to target genes (Fig. 2). Removal of acetyl groups results in a closed chromatin conformation, inaccessible for the transcription machinery. The expression pattern of Mxd proteins is mainly the opposite to that of Myc, being upregulated during differentiation and barely detectable in proliferating cells. The exception is Mxd3 that is expressed primarily during S-phase of the cell cycle. Of the four Mxd proteins identified Mxd1 and Mxd2 are the best characterized (Fig. 1). Mnt is ubiquitously expressed throughout the cell cycle and has been suggested to be a modulator of Myc function. This notion is supported by the finding that *mnt* deficient mouse fibroblasts show a disrupted cell cycle control and can be transformed by the oncoprotein Ras alone, traits resembling Myc over-expressing cells. The network also includes the larger transcriptional repressor ►Mga as well as ►Mlx that heterodimerizes with Mxd1 and Mxd4.

Effects of Myc Activation

The cellular response to activated Myc will mainly depend on the immediate cellular environment and include cell cycle progression, blockage of differentiation, apoptosis, cell growth and division as well as transformation.

Myc is required to drive cellular proliferation, a function that is carried out primarily during the ►G1/S transition of the cell cycle by directly upregulating cyclin D2 and CDK4. When in complex, these proteins will both initiate phosphorylation of the ►retinoblastoma protein (pRb) to enable release of ►E2F transcription factors and sequester the CDK2 inhibitor $p27^{KIP1}$ to release the cyclin E-CDK2 complex for continued pRb phosphorylation. In addition, Myc regulates expression of CDK inhibitors, either by repressing the expression $p15^{INK4b}$ and $p21^{CIP1}$ through inhibition of Miz-1-mediated transcription, or by promoting ubiquitin-mediated degradation of $p27^{KIP1}$. The fact that Myc also induces expression of E2Fs reveals that Myc is acting at several different levels to promote proliferation.

Another important function of Myc is potentiation of apoptosis in response to different cellular stress signals such as activation of the Fas death receptor and growth factor deprivation. The ability of Myc to enhance the apoptotic effects of many mechanistically distinct inducers indicates that Myc acts in a common control and/or execution pathway of apoptosis. It has been suggested that proliferation of cells is essentially tied to apoptosis as a built-in safety mechanism to defend against inappropriate proliferation. Thus sensitization to apoptosis is a normal function of Myc and the suicide signal can be rescued by specific survival factors such as insulin-like growth factor (IGF) or ►platelet-derived growth factor (PDGF). According to this model, the availability of apoptosis inhibitors determines whether cells divide or die.

In vitro experiments in rat cell lines have shown that cyclin A and ODC are potential mediators of Myc-induced apoptosis. Blockage of ODC inhibited apoptosis in Myc-overexpressing cells and forced expression of cyclin A was sufficient to induce apoptosis under low serum conditions. Ectopic expression of cyclin A could also restore drug-induced apoptosis in *c-myc* null cells. In addition to the Fas receptor and its ligand, induction of apoptosis by Myc has also been correlated to proteins in the ►Bcl-2 family, normally active at or in proximity to mitochondria. The anti-apoptotic proteins Bcl-2 and Bcl-X_L are repressed by Myc while expression and/or activity of pro-apoptotic members such as Bim or Bax is induced. In particular, the pro-apoptotic Bax molecule appears to be essential for triggering c-Myc-induced apoptosis by permeabilizing the mitochondrial membrane to release additional pro-apoptotic factors such as cytochrome c that will initiate the apoptosis cascade.

The tumor suppressor protein ►p53 acts as a sensor for intrinsic cellular damage signals by inducing cell cycle arrest to enable DNA-repair or, if this fails, to induce apoptosis. Myc has been reported to potentiate apoptosis both through p53-dependent and -independent mechanisms.

Consequences of Myc Deregulation

Myc activation triggers apoptosis in normal cells. However, if the apoptotic pathway is affected by mutations there will be an imbalance between proliferation and cell death with predominance for the former. This results in uncontrolled cell proliferation and, as the pool of proliferating cells has an increased risk of secondary mutations contributing to tumor development, thereby facilitates Myc driven tumor

development. Oncogenic activation of *MYC* is induced by events such as point mutations, gene ►amplification, translocation, overexpression, enhanced translation, or increased protein stability. This in turn results in immortalization, induction of genomic destabilization, and angiogenesis.

Deregulation of the *myc* gene alone is not sufficient for induction of cellular transformation of mouse cells *in vitro*, but requires co-operation with other oncogenes, such as *ras* or *bcl-2*. This is also manifested in human malignancies since many tumors with deregulated c-*MYC* overexpress *BCL-2* or harbor *RAS* mutations.

Tumors Associated with Myc

MYC is activated in a number of different tumors such as small cell lung cancer, breast ►carcinoma, osteosarcoma, glioblastoma, cervix carcinoma, myeloid myeloma, ►acute lymphoblastic leukemia, Burkitt lymphoma, and neuroblastoma. ►Chromosomal translocation is the most common aberration in ►hematological malignancies. In Burkitt lymphoma, the c-*MYC* gene is translocated to one of the immunoglobulin loci, resulting in constitutively high Myc levels. In contrast, the most common *MYC* aberration in solid tumors is gene amplification. In childhood neuroblastoma, amplification of the *MYCN* oncogene, occurring in 40–50% of high-risk neuroblastoma cases, is one of the key predictors of poor outcome.

Targeting Myc as an Approach to Treat ►Cancer

The high frequency of tumors with deregulated *MYC* expression is the main reason why Myc is an attractive target for cancer therapy. Thus, a Myc-targeting drug has the potential to provide an efficient treatment of a broad range of human cancers. However, since the protein is expressed in all proliferating cells it will be important to consider the potential damage inflicted on the gastrointestinal tract, the reproductive system, and the hematopoietic system if such a drug is administered systemically.

It has been shown that Myc-induced tumorigenesis can be reversible in conditional *myc*-driven transgenic models. In most of the cases, inactivation of *myc* resulted in induction of proliferation arrest or differentiation and/or apoptosis depending on the type of cancer as well as on the tumor-specific genetic events. However, tumor cells can also develop compensatory mechanisms to evade the dependence on Myc. In addition, some tumors may contain ►cancer stem cells that remain ►dormant when *myc* is inactivated but recover their tumorigenic properties upon *myc* reactivation. In other cases, even brief inactivation of Myc is sufficient for induction of sustained tumor regression.

Given the fact that *MYC* is one of the most frequently deregulated oncogenes in human cancer, much would be gained from specifically targeting Myc or the Myc pathway in tumor cells. For this purpose, one strategy would be to identify molecules that can reactivate the intrinsic response to Myc overexpression by reversing the acquired resistance to Myc-driven apoptosis. It is also possible to design or identify drugs directly targeting the Myc protein, stimulating its degradation or preventing its interaction with Max. With these approaches, the efficacy of conventional ►chemotherapy would be enhanced and enable the use of lower drug doses to reduce adverse effects.

Conclusion

Myc is a key regulator of cell cycle, cell growth and metabolism, differentiation, apoptosis, angiogenesis, and transformation. In spite of the efforts and progress that has been made in understanding Myc function much remains to be resolved. Unraveling the complexity of the Myc protein and its many roles may provide insights for design and development of novel therapies for treatment of human cancer.

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MYCL

Definition

Is a protein of 364 amino acids that dimerizes wih ►MAX to bind DNA in a sequence specific manner. It belongs to the family of basic region helix-loop-helix proteins, most of which are transcription factors. The gene maps to position 1p34. In a subset of small cell lung cancers, an increase in gene copy number (by ►amplification) has been reported.

►Amplification
►Helix-Loop-Helix (HLH) domain
►LMYC
►L-myc

MYCN

Definition

The MYCN gene encodes a nuclear protein of 464 aa, 49 kD Differential phosphorylation of the protein will result in two major components with the relative molecular mass of 62 and 64 kD. MYCN is a member of the basic helix-loop-helix family of transcription factors that binds DNA as a dimer with MAX to activate transcription. The gene maps to 2p24; an increase in gene copy number (by amplification) in is seen in neuroblastoma (here associated with poor prognosis) and occasionally in small cell lung carcinoma and retinoblastoma; amplified MYCN is expressed at an elevated level.

►Amplification
►Neuroblastoma
►NMYC
►N-myc

Mycobacterium

Definition

Acid-fast bacteria with a rather prolonged doubling time under laboratory conditions. Mycobacteria share a characteristic thick and biochemically complex cell-wall. Include pathogens which cause serious disease in humans such as leprosy and tuberculosis. An attenuated vaccine strain termed BCG is available for prevention of tuberculosis and for ►immunotherapy of ►bladder cancer.

►Bacillus Calmette-Guérin

Mycobacterium bovis BCG

►Bacillus Calmette-Guérin

Mycoplasma

Definition

Are a very large group of bacteria. There are more than 70 types. They are very simple one-celled organisms without an outer membrane. They penetrate and infect individual cells. *Mycoplasma hominis* is among the dozen types of mycoplasma that occur in humans. *Mycoplasma arginini* has high occurrence in cats and a wide distribution in nature.

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

MyD88

Definition

Is a protein component of the signaling pathways mediated by toll-like receptors. In response to microbial ligands MyD88 reacts with the toll receptors and also the interleukin-1 receptor to initiate a signaling cascade that results in activation of the transcription factor NFκB.

►Kupffer Cells

Myeloablative Megatherapy

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Synonyms

Autologous bone marrow transplantation; Autologous (hematopoietic) stem cell transplantation; High-dose (myeloablative) chemotherapy

Definition

The term "Myeloablative megatherapy" describes a strategy of anticancer chemotherapy, in which the dose of drugs is increased to the extent that myelosuppression as a side effect will not recover by itself. To restore the patient's hematopoiesis, his own blood stem cells are harvested and frozen before the high-dose chemotherapy and thawed and reinfused thereafter.

The alternative to autologous stem cell transplantation is allogenic stem cell transplantation; this means that a healthy donor provides the cells to restore the patient's hematopoiesis.

Characteristics

Therapeutic Concept

Anticancer drugs are not dosed according to maximal damage to the cancer cell, but to keep their toxicity within limits tolerable for the patient. The amount which can safely be given is usually less than that which would exhaust the drug's potential against cancer cells. This undertreatment – from the view point of the malignant cell – favors the emergence of resistant cells against a specific drug.

Two major strategies have been developed to overcome such drug resistance: combination of drugs and dose escalation.

Dose escalation means, if a certain drug is active against a specific type of cancer cell, more of it will – within a certain range – kill more tumor cells. If this dose range cannot be exploited because lethal bone marrow suppression would follow, autologous stem cell transplantation can circumvent this danger. With a myeloablative megatherapy regimen, more effective cancer therapy can be delivered for once (in cases even twice) because the hematopoiesis is restored afterwards by the patient's own blood stem cells.

Thus "myeloablative megatherapy" names the treatment, and "autologous stem cell transplantation" denotes the rescue from potentially lethal myelosuppression.

Timing of Megatherapy within a Treatment Strategy

At the time of diagnosis, the cumulative mass of cancer in the patient's body is highest. Each treatment course reduces the malignant mass, at best to a minimum which cannot be detected any more by imaging, histology or cytology, or by biologic markers. This state of disease is called "complete remission." At this state, the undetectable minimal residual disease (MRD) consists of the few cells which have escaped all drugs given so far; the danger that they develop resistance is high. This is the moment to escalate the dose of those drugs to which the disease has been sensitive so far.

That is why myeloablative megatherapy must always be part of a complete therapeutic protocol and should not be used as an isolated treatment.

Which Drugs Can be Used for Megatherapy?

Drugs must qualify in two ways to be useful for myeloablative megatherapy:

1. The tumor cells must be sensitive to conventional doses of the drug. Once resistance to a drug has emerged, even a higher dose of this drug will not cure the cancer.
2. The major side effect of the drug must be myelosuppression, since only this side effect is rescued by the stem cell transplantation. Negative influences upon other organs, e.g. liver, renal function or brain, are not alleviated; they can only be put up with to an extent from which the organs will recover by themselves.

Myeloablative Megatherapy. Table 1 Drugs frequently used in megatherapy regimens

Drug	Diseases
Total body irradiation (TBI)	Acute leukemia
	Neuroblastoma
	Myeloma
Cyclophosphamide	Leukemias, lymphomas
	Breast cancer
Etoposide	Acute lymphoblastic leukemia
	Many solid tumors breast cancer
Carboplatin	Solid tumors
	Breast cancer
Busulfan	Leukemias, lymphoma
Treosulfan	Ewing tumor
Cytosin-Arabinoside	Acute leukemia
	Lymphomas
Melphalan	Multiple myeloma
	Childhood solid tumors
BCNU	Lymphomas, brain tumors

Table 1 lists those drugs which are most frequently used for megatherapy.

Treatments with dose escalation carry additional risks for the patient and are expensive. Such concepts must therefore be evaluated in clinical studies in order to prove their benefit for the patient and for the society which provides the resources. In some diseases, e.g., breast cancer, the initial enthusiasm in view of a megatherapy concept did not stand large clinical trials which tested its benefit for the patients.

More is not necessarily better.

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Myelocytomatosis

Definition

Is a cancer type in birds, induced by a viral oncogene that was later defined as *v-myc* (*my*elo*c*ytomatosis).

►*Myc* Oncogene

Myelodysplasia

Definition

Abnormal bone marrow cells that may lead to myeloid leukemia.

►Myelodysplastic Syndromes.

Myelodysplastic Syndromes

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Synonyms

MDS; Preleukemia; Dysmyelopoietic syndrome

Definition

Myelodysplastic syndromes (MDS) are a group of stem cell disorders, mainly of the elderly, characterized by defects in maturation of bone marrow cells, resulting in cytopenia in peripheral blood, possibly associated with increased medullary and peripheral blasts and an increased risk of evolution to ►acute myeloid leukemia.

Characteristics

Epidemiology: MDS are diseases mainly of the elderly. The median age at the time of diagnosis is about 70 years. Less than 10% of the patients are younger than 50 years. MDS patients in Asia are 10 years younger in median. The male-female ratio shows a slight preponderance of males. The incidence of MDS is about 4–5/100,000/year and more than 30/100,000/years in patients older than 70 years (►Cancer epidemiology). This means that MDS are one of the most frequent bone marrow disorders and at least as frequent as CLL, Myeloma and acute Leukemias. There is no evidence of an age-adjusted increase of incidence, whereas the number of newly diagnosed MDS patients rises with the graying of the population.

Causative Factors: The vast majority of the patients develop MDS without known reasons. About 10% of the patients develop a treatment-related MDS after being treated with chemotherapy, radiation or radioiodine therapy. Topoisomerase II inhibitors as well as azathioprine can also cause MDS. A very small proportion of the patients bear inherited disorders that may promote MDS such as ►Fanconi anemia and paroxysmal nocturnal hemoglobinuria (PNH), as well as the presence of a family history of hematopoietic cancers. Environmental and occupational noxes may also play a role in the development of MDS. Smoking, exposure to agriculture chemicals, benzene and other solvents (►Benzene and Leukemia) significantly increase the risk of MDS. Polymorphisms and mutations of detoxifying enzymes such as ►glutathione s-transferases may also possibly play a role in the risk of MDS development.

Clinical Presentation: Almost all MDS patients present with a hemoglobin level of less than 10 g/dL. Bicytopenia is found less frequently, and about 30% of the patients are pancytopenic. The resulting clinical features are signs of anemia, infections and, less frequently, bleeding (►Neutropenia).

Diagnosis and Classification: MDS is always a diagnosis of exclusion; there is no pathognomonic finding. Morphologic assessment of blood and bone marrow cytology is the backbone of diagnostic measures. The presence of dysplastic signs in the peripheral blood in case of unexplained cytopenias leads to bone marrow examination by cytologic as well as histologic means. Major morphologic signs in blood and marrow are dysplastic features in at least one cell lineage as well as the presence of blasts in up to 19% of the nucleated cells. Typical signs of dysplasia of the erythropoiesis are megaloblastoid changes, nuclear abnormalities such as bridging, karyorrhexis, basophilic stippling, ring sideroblasts and impaired maturation. Dysplastic features of the granulopoiesis are the presence of Pseudo-Pelger cells, hypogranulation of promyelocytes and myelocytes, an elevated blast count and peroxidase insufficiency. Micromegakaryocytes, mononuclear megakaryocytes and megakaryocytes with multiple nuclear fragments are typical features of dysmegakaryopoiesis. None of these criteria alone is sufficient for diagnosis, but the combination of different dysplastic features allows confirmation of the diagnosis of MDS. Almost all MDS patients show dyserythropoiesis in more than 10% of all erythroid cells. The terms "dysmegakaryopoiesis" and "dysgranulopoiesis" are used if more than 10% of the cell lines are dysplastic.

It is necessary to assess the amount of dysplasia within a marrow because the number of affected cell lines play a major role in the classification of MDS and provide valuable prognostic information. The present classification of MDS has been proposed by the WHO. Table 1 presents the different WHO types. As long as the blast count is not elevated in blood and marrow, the number of dysplastic cell lines is the only relevant parameter for diagnosis. Patients with exclusive dyserythropoiesis are called refractory anemia (RA) or RA with ringsideroblasts if they harbor more than 15% ring sideroblasts in marrow. As soon as an additional cell line is dysplastic, the patient is diagnosed refractory cytopenia with multilineage dysplasia (RCMD) or RCMD with ringsideroblasts. If one of the above mentioned MDS types present with a deletion of 5q, they are classified as a different subtype called 5q– syndrome. Patients presenting with elevated blasts are called refractory anemia with excess of blasts (RAEB). Medullary blasts counts of less than 10% and peripheral blast counts of less than 5% defines the diagnosis of RAEB I. If the blast count is higher than 5% in blood or 9% in marrow, the disease is called RAEB II (►Leukemia Diagnostics).

Cytogenetic Findings: About 50% of all MDS patients show chromosomal aberrations in bone marrow cells at the time of diagnosis. There is a great variability of numerical and structural cytogenetic findings in MDS. Although there are some typical findings, there is no aberration that is restricted to MDS. The most frequent aberrations are del(5q) (~30% of abnormal cases), complex abnormalities (~25%), –7/7q– (~15%), trisomy 8 (~15%), 20q– (~5%) and – Y (~5). Some of the aberrations are associated with a distinct type of MDS and have prognostic implications. In general, patients with a normal karyotype, del(5q), del(20q) or – Y have a relatively good prognosis and patients with an aberration of chromosome 7 as well as those with a complex karyotype (more than 2 chromosomal aberrations) have a poor prognosis. In case of an unclear diagnosis, i.e. mild cytopenia, mild dysplasia, no blasts in marrow and blood, the karyotype has a diagnostic impact when a chromosomal aberration can be found. Chromosomal aberrations also play a role with regards to treatment, as some drugs show higher response rates in the presence of distinct cytogenetic findings (►Chromosomal translocations).

Prognosis: The natural course of the disease is extremely heterogeneous. Twenty percentage of the patients die within 6 months whereas another 20% of the patients survive 6 years and longer. The most important adverse prognostic parameters are elevated medullary blasts in blood and marrow, adverse cytogenetic findings, low cell counts and elevated LDH values in the blood. Several prognostic scoring systems have been established. The most important of them is the International Prognostic Scoring System (IPSS), which relies on medullary blast count, type of cytogenetic aberration and number of cytopenias in the blood (Table 2). This Scoring System serves as a tool for assessing the expected natural course of the disease. Low risk patients have a median survival time of about 6 years, intermediate I risk patients of about 3 years, Intermediate II risk patients of about 2 years and high risk patients of about 1 year. There are also differences regarding the risk of AML evolution. Low risk patients bear a minimal risk of AML whereas high risk patients face a cumulative risk of AML of about 80%.

Treatment Options

Supportive Care: The standard of care includes supportive care measures including red cell transfusions in case of low hemoglobin levels, and transfusion of platelets in case of bleeding or severe thrombocytopenia. Due to frequent red cell transfusions patients develop iron overload, resulting in severe end organ damage like heart failure including arrhythmias, liver cirrhosis, disorders of the endocrine organs and bronze diabetes. Patients who show ferritin levels of over 1,000 ng/mL should receive iron chelation, preferably with the oral iron chelator Desferasirox. Low risk MDS patients who received a total of 50–100 red cell transfusions are at risk of becoming iron overloaded and therefore are candidates for iron chelation therapy. Other treatment approaches are not approved by the

Myelodysplastic Syndromes. Table 1 WHO-classification of myelodysplastic syndromes

MDS subtype	Medullary blast count	Peripheral blast count	Additional criteria
RA with unilineage dysplasia	<5%	<1%	
RA with multilineage dysplasia (RCMD)	<5%	≤1%	
RARS with unilineage dysplasia	<5%	<1%	>15% ringsideroblasts
RARS with multilineage dysplasia (RSCMD)	<5%	≤1%	>15% ringsideroblasts
RA with excess blasts I (RAEB I)	5–9%	<5%	
RA with excess blasts II (RAEB II)	10–19%	<20%	Auer rods possible
5q–Syndrome	<5%	<5%	Isolated del(5q)

Myelodysplastic Syndromes. Table 2 IPSS (International Prognostic Scoring System)

	Scoring value				
	0	0.5	1	1.5	2
Blasts (%)	<5	5–10	–	11–20	21–30
Karyotype	Good	Intermediate	Poor	–	–
Blood cytopenias[a]	0/1	2/3	–	–	–
Risk group	*Score*				
Low risk	0				
Intermediate I risk	0.5–1				
Intermediate II risk	1.5–2				
High risk	2.5 or more				

Good: normal, –Y, del(5q), del(20q). *Poor*: complex (≥3 abnormalities) or abnormal chromosome 7. *Intermediate*: all other abnormalitiesz
[a]Hemoglobin level below 100 g/L, absolute neutrophil count below 1.8 × 10^9/L, thrombocytes below 100 × 10^9/L.

EMEA. Therapeutic interventions should be discussed with the patient very carefully as we do not know yet if treatment will result in a prolongation of survival, with the exception of the epigenetic drugs and ATG. Patients who present with low EPO levels should receive an ►erythropoietin subcutaneously in order to avoid red cell transfusions. The lower the erythropoietin level in the blood, the better the response with regard to the hemoglobin level. In case of non-responders, G-CSF can be added.

Immunosuppressive Agents (►*Immunotherapy*): As inhibitory cytokines in the marrow may also play a role in the development of cytopenia, immunoinhibitory therapy can be used to restore the hematopoiesis. Antithymocyte globulin (ATG) and cyclosporine A (CSA) can produce long lasting remissions by normalizing the peripheral cell counts. This treatment is appropriate for patients with RA and RCMD, possibly in particular for those with a hypocellular marrow.

Immunomodulatory Agents: Lenalidomide, a derivative of Thalidomide (►Antiangiogenesis) (►Thalidomide and its Analogues), is an immunomodulatory compound that shows astonishing erythroid responses in patients with del(5q) anomalies. Within large studies, its efficacy and an acceptable side effect profile could be shown impressively. About 2/3 of anemic patients with del(5q) achieve long lasting normalization of the cell counts and, in addition, the majority of the patients achieve cytogenetic responses as well. The median increase of the hemoglobin level is about 4–5 g/dL and the time to response averages about 1 month. The drug is approved by the FDA in the United States. There is no evidence of teratogenic effects or neuropathy. The main side effects are transitional drops of neutrophils and platelets.

Epigenetic Agents (►*Epigenetic Therapy*): Besides genetic aberration, epigenetic changes may also play a role in the pathophysiology of MDS. Gene silencing by variable states of DNA ►methylation and histone modification (►Histone Modification in Cancer biology) (►Histone Deacetylation) can lead to chromatin remodeling. Genes involved in the cell cycle, (►Cell-cycle targets for Cancer Therapy) differentiation and ►apoptosis may be inactivated via methylation status. DNA methyltransferases inhibitors like 5-azacytidine (Vidaza, s.c.) and 5-aza-2deoxyctidine (Dacogen, i.v.) (►5-aza-2′ deoxycytidine and Cancer) have been shown to prolong survival in high risk MDS patients. In up to 50% of the patients a remission or at least an improvement of cell counts can be achieved. Both drugs are approved by the FDA. A combination of these drugs with histone deacetylase inhibitors may be even more powerful. Histone deacetylating agents like ►Valproic acid are effective in a subset of low – risk MDS patients, either alone or in combination with methylating agents in high risk patients.

New investigational agents like ►Arsenic, Farnesyl transferase inhibitors, (►Farnesyl Transferases) are used in clinical trials.

Intensive Chemotherapy and Allogeneic Hematopoietic Stem Cell Transplantation: (►*Chemotherapy of Cancer*) High risk MDS patients can be administered induction chemotherapy based on cytarabine and an antracycline, followed by consolidation therapy. About 60% achieve a complete remission, but the vast majority relapse within a year. Long term remissions can be seen in about 15–20% of the patients. Only those high risk MDS patients that show a normal karyotype benefit from induction chemotherapy. Patients with a high risk karyotype, especially those with a complex karyotype, should not be treated with an induction as long as they are not going to receive allogeneic stem cell transplantation. Allogeneic hematopoietic stem cell transplantation is a potentially curative treatment approach for younger patients with MDS. The long-term success of this therapy

is primarily influenced by disease, as well as patient related factors such as medullary blast count at the time of transplantation, karyotype, age and comorbidities. Allogeneic hematopoietic stem cell transplantation should be taken into consideration for high risk patients and intermediate II risk patients according to the IPSS. Low and intermediate I risk patients may be candidates for allogeneic hematopoietic stem cell transplantation in case of disease progression. It is still unclear if there is a major difference in the outcome between those patients who receive a "standard" myeloablative conditioning and those who only receive non-myeloablative conditioning. The latter one may be useful for elderly patients with MDS.

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Myelofibrosis with Myeloid Metaplasia

►Primary Myelofibrosis

Myeloid

Definition

Means that a cell is determined to belong to a subgroup of white blood cells. There are two main types of white blood cells, lymphoid and myeloid, which are produced from different bone marrow populations. All nonlymphoid cells are grouped together as myeloid.

►Acute Myeloid Leukemia

Myeloid Leukemia of Down Syndrome (DS-ML)

►Acute Megakaryoblastic Leukemia

Myeloid Suppressor Cells

Definition

A sub-set of ►macrophages that inhibit T cell ►response.

►Macrophages

Myeloperoxidases

Definition

Is a peroxidase enzyme most abundantly present in neutrophils.

►Lead Optimization

Myeloproliferative Disease

Definition

MPD; These are clonal hemopathies associated with excessive development of one or more hematopoietic lineages e.g. erythroid in human polycythemia vera, platelet in essential thrombocytosis. These disorders are frequently associated with activating mutations in Jak2. Expression of leukemogenic genes in mouse bone marrow frequently leads to oligo- or poly-clonal MPD, with neutrophil leukocytosis and extramedullary hematopoiesis that may progress to acute myeloid leukemia (AML).

►NUP98-HOXA9 Fusion

Myelosuppression

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Definition

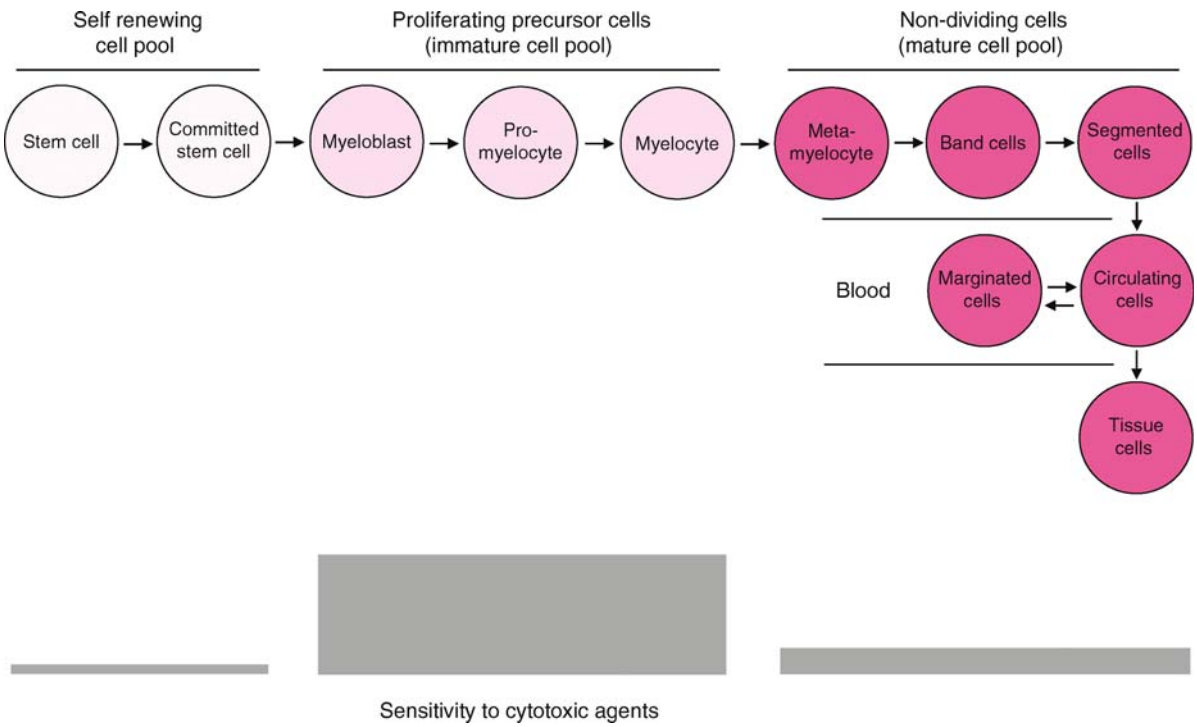
Myelosuppression (acute suppression of the bone marrow) is the most common adverse side effect of cytotoxic anti►cancer therapy. It describes the decrease in the production of blood cells in the bone marrow. The bone marrow produces three types of blood cells: red blood cells (erythrocytes) and white blood cells (leukocytes) (which include lymphocytes, monocytes, granulocytes) and platelets (thrombocytes). Since these cell types serve distinct and important functions, myelosuppression can be associated with moderate to severe life-threatening complications, such as anemia, increased risk of infection, and bleeding.

Characteristics

The bone marrow contains ►stem cells able to reproduce and differentiate into red blood cells, white blood cells, and platelets, depending on the body's need for replacing these cell pools. Because the proliferating precursor cells produced by stem cells are almost always in mitosis and reproduce rapidly, they are highly susceptible to cytotoxic damage. The stem cells themselves are usually not affected by a single administration of cytotoxic agents, but they may resume cell division to become vulnerable to subsequent treatments. Nonproliferating cells like metamyelocytes, band cells, segmented cells, and the pool of circulating mature cells in the blood (Fig. 1) are believed to be rather resistant. The decrease in blood cell counts does not occur immediately after cytotoxic therapy, however, treatment may temporarily prevent the formation of new blood cells by bone marrow stem cells and the progenitor cell pool composed of proliferating precursor cells.

Myelosuppression is the most common toxicity associated with the administration of systemic ►chemotherapy or therapeutic ►x-rays that reach the bone marrow, and infections remain a common cause of death in these patients. The cell types which are most strongly affected during treatment and the drop of which causes most of the clinical complications are platelets and ►neutrophils (neutrophil granulocytes).

The normal range for platelet counts is between 150,000 and 450,000 per milliliter of blood.



Myelosuppression. Figure 1 Neutrophil development from its birth in the bone marrow to circulation in the blood and migration in peripheral compartments.

Thrombocytopenia (low platelet count) may cause bleeding due to the inability to form blood clots at the site of injury. Drugs containing acetylsalicylic acid (aspirin) or other ►nonsteroidal antiinflammatory drugs (NSAIDS) can increase the risk of thrombocytopenia under cytotoxic therapy.

Neutrophils are the most numerous type of leukocytes normally ranging between 2,500 and 6,000 cells per milliliter, which is about 60–70% of total leukocytes. Neutropenia (low neutrophil count) itself is not associated with symptoms and does not affect quality of life. However, it can confer a substantial risk of life-threatening infections accompanied by increased morbidity and mortality, and the magnitude of this risk is closely correlated with the extent and duration of neutrophil loss. Moreover, neutropenia often prevents patients from receiving their chemotherapy at the prescribed times and dose. Neutrophils migrate to the site of infection to fight bacterial and fungal infections, thereby initiating the body's first line defense. They are constantly renewed by the pool of stem cells and immature neutrophils in the bone marrow, and their short life-span makes this cell population so sensitive to cytotoxic treatment. The lowest count that blood cell levels fall to is called the nadir. It is at this point in time when patients with a neutrophil count below 500 are at high risk of infection. Upon a bolus administration of chemotherapy, neutrophils reach the nadir within 7–14 days and suppression may continue for several days until day 21–28 before recovering to normal levels. Most combination chemotherapy regimens have therefore been designed to accommodate the kinetics of bone marrow recovery and are given in 21–28-day cycles. Although high levels of the soluble ►tumor necrosis factor ligand-receptor (p75-R-TNF) seem to be associated with an increased risk of chemotherapy-induced myelosuppression and thus may assist in risk assessment, a reliable marker for the prediction of neutropenia and its symptoms does not exist. Therefore, risk assessment relies on empirical data based on the type of anticancer agent used and its dose which can roughly predict how far the cell count drops and for how long the nadir may last. Due to their reduced responsiveness, neutropenic patients may not show the typical signs of infection, such as redness, pus and swelling, and the most reliable and often only sign of neutropenia is fever above 38°C. Febrile neutropenia is the most serious stage of this complication, and fever always indicates the presence of an infection which requires immediate antibiotic treatment.

Hematopoietic Growth Factor Support

For patients suffering from a malignant tumor, the aim of cytotoxic anticancer therapy is to destroy the malignant cells to reduce disease symptoms, prolong survival or increase the chance of cure. Usually, combinations of several anticancer agents are used in a treatment regimen. The agents are given at a defined dose and a specific time schedule. These parameters have been derived from a large number of preclinical and clinical data to produce the highest efficacy for a given cancer type. In case neutropenia occurs, the dose of the cytotoxic agents needs to be reduced or the treatment must be delayed or even discontinued. A way to avoid the symptoms of neutropenia and also thrombocytopenia is prophylactic support with hematopoietic growth factors (HGFs) as biological agents that help in maintaining hematopoiesis by regulating the proliferation, differentiation, and maturation of the hematopoietic stem cell pool. Interleukin-11 has been used to increase platelet numbers in thrombocytopenic patients, but side effects are frequent and include fluid retention, tachycardia, and dispnoe. ►Myeloid HGFs such as granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) have been extensively investigated and found useful in the management of neutropenia related to cytotoxic therapy and its clinical symptoms. These growth factors, which are generally well tolerated, can speed the recovery of neutrophil counts to enable continuation of standard therapy regimens or maintain a higher level of cellular resistance and viability to allow the use of high-dose therapy, which can be given less frequently with often better efficacy. G-CSF primarily stimulates an increase in circulating neutrophils. GM-CSF has a wider effect by promoting the expansion of granulocytes and macrophages, but obviously for the sake of more troublesome side effects like fever, myalgia, malaise, and rash.

In recombinant form these colony stimulating factors (CSF) have been available for the prevention or duration of neutropenia upon cytotoxic chemotherapy for over 15 years. Prophylactic CSF treatment thereby not only reduces the risk of neutropenia, but also helps to ensure that patients receive their planned doses of anticancer agents on schedule. On the other hand, there are indications where the use of CSFs has no clinical benefit regarding morbidity and mortality from infectious complications, frequency of antibiotic use or rate of hospitalization. Thus, CSF application should be limited to indications with proven benefits or evidence of cost-effectiveness. Patients, who most likely profit from prophylactic CSF support and for whom international guidelines have been generated, have an expected incidence of life-threatening febrile neutropenia of more than 40%. Secondary prophylaxis is recommended in cases of previous febrile neutropenia during earlier cycles of chemotherapy or when the symptoms from neutropenia require dose reduction or delay, which may thwart curative treatment intents.

Specific guidelines for the evidence-based use of CSF have also been elaborated for elderly patients, who are at greater risk of myelosuppression. Even when the chemotherapy regimen is relatively mild and myelotoxicity is limited, elderly patients tend to be more vulnerable than younger patients. As a consequence of the higher risk of neutropenia, chemotherapy delays and dose reductions are significantly more frequent in this patient population, for which factors such as treatment intent (cure vs. palliation), type, and dose of drug combinations have to be taken into more serious consideration.

Regulation of Neutrophil Cell Death

As a major part of modern biomedical research, intensive efforts in the field of molecular oncology and hematology have focused on ►apoptosis regulation in cancer and hematopoietic cells as a determinant of chemotherapy outcome and myelotoxicity. DNA damage is clearly a crucial event, which in most normal cell types results in the activation of a ►p53 tumor suppressor gene-regulated pro►apoptotic pathway. On the other hand, as cytotoxicity induced by anticancer agents not always correlates with the expression and activation status of p53, other roads to death are believed to exist in parallel. Recent research has indeed identified multiple stress-activated intracellular molecules, including Jun kinase, mitogen-activated protein kinase (MAPK), ►nuclear factor (NF)-κB and ►ceramide, which are implicated in the proapoptotic decision too. In addition, there is evidence for a role of ►phosphatidylinositol-3-OH kinase (PI3K) and protein kinase B/Akt (PKB/Akt). More downstream of this survival pathway, ►survivin, a member of the ►inhibitor of apoptosis protein (IAP) family was recently reported as a further mechanism of antiapoptosis in hematopoietic stem cells, immature neutrophils, and in certain cancer cell types. Of clinical importance is that treatment with cytotoxic anticancer agents, which reduces survivin specifically in immature neutrophils, a mechanism which likely contributes to myelosuppression in patients. The molecular basis of this adverse effect seems to be a neutrophil-typical PKB/Akt-independent reduction of PI3K activity, which is not observed in cancer cells. This may provide the rationale for the reduction of survivin levels by targeting a pathway distal to PKB/Akt as a strategy to attenuate myelosuppression without jeopardizing potential therapeutic success. As expected from their clinical activity, HGFs can also enhance the resistance of neutrophils to apoptosis induction, and antiapoptotic proteins can be activated by G-CSF.

Conclusion

Myelosuppression, in particular neutropenia, is a serious complication of cytotoxic anticancer therapy, which can make patients vulnerable to potentially life-threatening infections. This often results in dose reduction and delay of planned treatment regimens with the consequence of therapy failure and reduced survival. Neutropenia can in principle be reduced by the use of G-CSF during the first and subsequent cycles of cytotoxic therapy. However, since not all patients benefit from this effect, the use of CSF should be limited to indications recently determined by international guidelines. It is expected that the lack of appropriate risk models for severe myelosuppression motivates further efforts in this direction as they may help facilitating cost-effective strategies to reduce life-threatening neutropenia. Extensive investigation of apoptosis regulation in malignant and normal cell types including hematopoietic cells has significantly improved our understanding of the molecular basis of this clinical complication. This knowledge must be employed for the design of treatment regimens which offer reduced myelotoxicity, and may assist in the design of clinically relevant models valid for neutropenia risk assessment.

►Paclitaxel
►Taxotere

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Myocardium

Definition

Heart muscle.

►Hepatic Epithelioid Hemangioendothelioma

MyoD

Definition
Is a protein with a key role in regulating muscle differentiation, which acts as one of the earliest markers of myogenic commitment.

►Cachexia

Myoepithelial Cells

Definition
"Basal" Epithelial Cells; One of the two major epithelial cell types in the breast that are found in a basal or outer cell layer in contact with the basement membrane.

►Basal-like Breast Cancer

Myofibroblasts

Definition
Synonym activated fibroblasts or tumor-associated fibroblasts; Are fibroblasts that have been induced to undergo a phenotypic change that results in altered secretion of growth factors and the formation of alpha-smooth muscle actin (α-SMA) stress fibers. These activated cells have increased contractile abilities, produce abundant amounts of growth factors, and are important for wound healing. In the tumor microenvironment, myofibroblasts are referred to as "tumor-associated fibroblasts (TAFs)" and can dramatically influence tumor cell behavior and growth.

►Desmoplasia
►Stem Cell Plasticity

Myofibromas

►Uterine Leiomyoma

Myoma

Definition
A benign fibroid tumor of the uterus.

►Uterine Leiomyoma, Clinical Oncology
►Uterine Leiomya

Myomectomy

Definition
Refers to the surgical removal of just the ►fibroid, with reconstruction and repair of the uterus. Uterine ►leiomyoma.

►Uterine Leiomyoma, Clinical Oncology

Myometrium

Definition
The muscular outer layer of the uterus.

►Uterine Leiomyoma, Clinical Oncology

Myopodin

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Synonyms
Homologue of synaptopodin

Definition
►Myopodin, a ►zyxin binding protein, possesses capabilities to regulate cell growth and motility. The myopodin gene, deleted in many advanced ►prostate cancers, is considered a tumor suppressor.

M

Characteristics

Myopodin is a homologue of ►synaptopodin. Its sequence shares six stretches of degenerative homology with synaptopodin, an actin binding protein in glomerular podocytes and dendritic spine in nervous system. The myopodin gene is mapped to chromosome 4q25. Its mRNA sequence of 4,208 bases is translated into a protein of 80 kDa in prostate and skeletal muscle, and 95 kDa in heart muscle.

Myopodin is abundantly expressed in skeletal muscle, but also expressed in organs such as prostate, heart, bladder, kidney, liver, spleen, small and large intestines. However, it is not detected in lung, placenta, brain, leukocytes, testes, colon, ovary, thymus, stomach, thyroid, spinal cord, lymph, trachea, adrenal gland, and bone marrow.

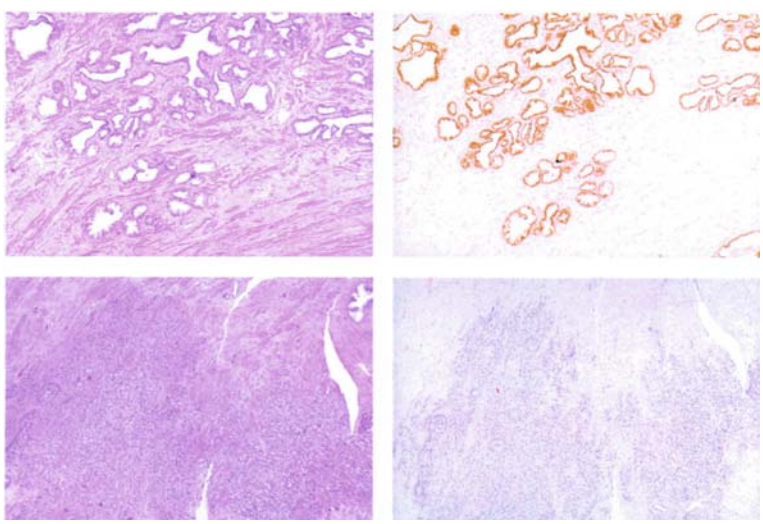
Myopodin Gene Expressed in Prostate but Deleted or Down Regulated in Prostate Cancer

In prostate, myopodin is primarily expressed in prostate epithelium with the strongest expression in the acinar cell layer (Fig. 1).

Myopodin was first recognized as a gene deleted in an advanced prostate cancer case by the analysis of genome wide differentiation subtractive chain (►differential subtraction chain) reaction. In chromosome 4q25, a 54-kb minimal common deletion region that contains the sequence encoding myopodin was identified in >50% of prostate cancers in a study. Frequent complete or partial deletions of the myopodin gene most often occur among invasive prostate cancer cases and result in decreased myopodin mRNA levels (Fig. 1). Hemizygous deletion and down-regulation of myopodin expression also occur in some aggressive prostate cancer cell lines (PC3, DU145 and LNCaP). These prostate cancer cell lines were utilized to carry out studies to investigate the function of myopodin. To study the effects of deletion or down regulation of myopodin in prostate cancer, over-expression of mypodin was achieved by transfecting myopodin gene into PC3 and LNCaP cell lines. The cancer cells with over-expression of myopodin showed decreased cell growth in proliferation and colony formation analyses. *In vitro* matrigel transmigration analysis suggested that myopodin suppressed prostate cancer cell invasion. These studies support a role of myopodin as a tumor suppressor in prostate cancer. When a truncated myopodin gene was introduced into prostate cancer cells, the cells with C-terminus deletion mutant were found to lose all suppression activities of cell growth and invasion, suggesting the critical role of this domain in regulation of cell growth and migration. In a rodent model, over-expression of mypodin in PC3 and DU145 cells suppressed the growth and metastasis of their xenografted tumors in SCID mice, and decreased xenografted tumor induced mortality.

Myopodin Expression and Relapses of Prostate Cancer

A large-scale analysis of myopodin expression in prostate cancer samples was conducted to examine the relationship between myopodin expression and several pathological and clinical outcomes. Immunostaining using anti-myopodin antibodies was performed on formalin-fixed paraffin-embedded tissue samples to



Myopodin. Figure 1 Expression of myopodin in prostate tissues. Upper left panel – H&E staining of normal prostate; upper right panel – *In situ* hybridization of myopodin mRNA of adjacent section of left panel; lower left panel – H&E staining of prostate cancer; lower right panel – *In situ* hybridization of myopodin mRNA of adjacent section of left panel.

evaluate the level of myopodin expression in prostatic tissue. Lower myopodin expression was found to associate with samples having positive surgical margins. Complete inactivation of myopodin expression correlated with a greater than 86% rate of clinical relapse. However, there was no correlation between myopodin expression and Gleason grading. As a result, myopodin is an important predictor of prostate cancer metastasis, independent of Gleason score, preoperative prostate-specific antigen level, and pathological stages.

Mechanism of Myopodin Mediated Tumor Suppression

Myopodin is localized in the cytosol of prostate cancer cells. How does myopodin regulate cell growth? Through protein binding screening in a yeast two hybrid system, a target protein, zyxin, a two compartmental protein was found in direct interaction with the C-terminus of myopodin. Zyxin is a major regulatory protein for cytoskeleton reorganization and forming plague adhesion where the cell contacts the extracellular matrix. It regulates actin filament assembly to the plasma membrane at the focal contact. Interaction of myopodin with zyxin results in slower random migration, and lower rate of matrigel penetration by cancer cells.

Zyxin was first identified as a protein involving in focal adhesion. It was later found that zyxin plays a pivotal role in regulating actin assembly through interacting with its partners such as α-actinin and VASP. Zyxin is essential for cell motility since interference of zyxin function halts cell migration and alters cell spreading. Similarly, blocking the interaction of zyxin with h-warts generates an aberrant mitosis in Hela cells. Since zyxin plays such a crucial role in cytoskeleton reorganization related activities, the interaction of myopodin with zyxin may hold the key for understanding the invasion inhibition mechanism of myopodin in prostate cancer.

Myopodin Expressed in Muscles

Myopodin is found in the Z-disc of skeletal muscle and is colocalized with α-actinin. Its function in the skeletal muscle can be purely a structural protein for bundling actin protein and anchoring of thin filaments to the Z-disc. However, much of myopodin function is still not known, since it is found to translocate to the nucleus in stress myocytes. Cytosolic myopodin is considered a differentiation marker of muscle fiber. The role of nuclear redistribution of myopodin in the stress muscle remains unclear. But, the existence of nuclear myopodin has prompted the speculation of an involvement in stress related signaling pathways.

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Myotendinous Antigen

►Tenascin-C

Myrosinase

Definition

The enzyme found in cruciferous vegetables that is released upon disruption of the cell membranes of these vegetables by chopping or chewing. This enzyme is activated by contact with water.

►Sulforaphane

Myxoid Liposarcoma

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Synonyms

MLS/RCLS

Definition

Myxoid liposarcoma (MLS) is a malignant soft tissue tumor composed of mesenchymal tumor cells and a variable number of lipoblasts. Round cell liposarcoma (RCLS) is a morphologically distinct and more

aggressive variant of MLS. In many cases the two forms are mixed within the same tumor.

Characteristics

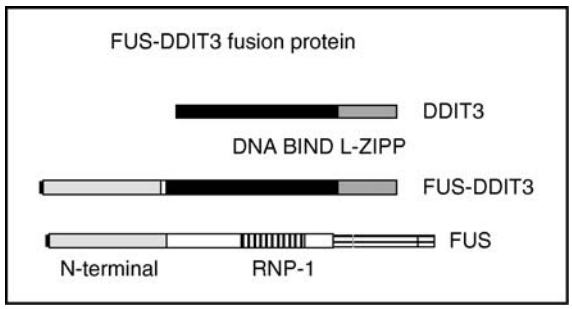
MLS/RCLS is one of the most common types of liposarcoma and accounts for more than a third of all the cases. MLS/RCLS is uncommon in children and young adults. The incidence increases with age and peaks at the middle ages. In ~70% of the cases, the tumors are found in the large muscles of the thigh but they may occur in deep seated locations in any part of the body. MLS/RCLS most often presents as a large painless mass. The tumor is multinodular and the tissue surface is pale and myxoid. The RCLS variant is more cell rich and contains less of the myxoid components.

MLS tissue is composed of round- to oval-shaped mesenchymal cells and a variable number of lipoblasts in a myxoid matrix. The tumor tissue contains a rich capillary network with a typical branched morphology.

A subset of the tumors shows progression to hypercellular round cell morphology (RCLS), associated with a more aggressive disease. Hypercellular areas with an undifferentiated round cell morphology are ranging from 5 to 80% in the mixed variant to more than 80% in the pure round cell type.

Genetics

MLS/RCLS tumor cells carry with few exceptions a t(12;16)►chromosome translocation or, more rarely, a t(12;22). In the majority of cases, these are the only chromosome aberrations found at diagnosis. Normal tissues of the patients show no chromosomal aberrations and there are no indications of heredity. The translocations result in fusions of FUS (a.k.a. TLS) on chromosome 16 or EWSR1 on chromosome 22 with DDIT3 (a.k.a. CHOP) on chromosome 12. The resulting ►fusion oncogenes typically consist of exons 1–5 or 1–7 of FUS, or exons 1–7 of EWSR1 juxtaposed to exon 2 of DDIT3. Other, rare fusion variants are also reported. In some cases, alternative splicing gives rise to two isoforms of the fusion transcripts and proteins within the same tumor. All fusions result in the formation of fusion proteins containing the N-terminal parts of FUS linked to the entire DDIT3 protein (Fig. 1). The localization of the chromosomal breakpoints within DDIT3, FUS, and EWSR1 varies and results in several variant fusion transcripts and fusion proteins but the variant breakpoints have not been shown to associate with prognosis or tumor subtype. The FUS–DDIT3 and EWSR1–DDIT3 belongs to a family of fusion ►oncogenes in which all members have one of the three closely related genes FUS, EWSR1, or TAF15 as 5′ fusion partners and one of many different transcription factor encoding genes as 3′ partners (Fig. 2). Each of the fusion oncogenes are, with exception for FUS–ERG, found in only one tumor entity.



Myxoid Liposarcoma. Figure 1 The *FUS–DDIT3* encoded fusion protein consists of N-terminal parts of the FUS protein juxtaposed to the entire DDIT3 protein. In the junction, 26 amino acids from normally nontranslated parts of *DDIT3* are added to the fusion protein. L-ZIP, leucine zipper dimer forming domain; RNP, RNA-binding domain.

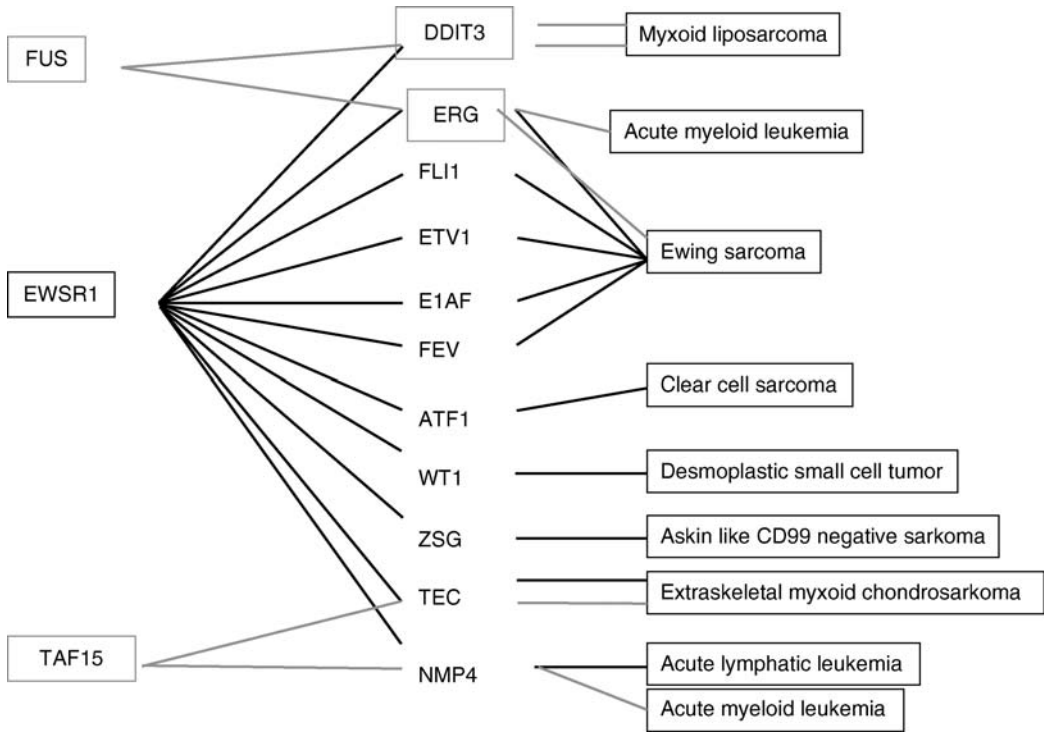
Etiology

The mechanism causing the chromosome translocations in MLS/RCLS is not well understood. No specific sequence motifs are reported in or around the breakpoint regions and the translocations are probably the result of random events.

The FUS–DDIT3 fusion gene has been shown to cause liposarcomas in transgenic mice and in mice inoculated with mesenchymal stemcells carrying FUS–DDIT3. FUS–DDIT3 also transforms NIH3T3 fibroblasts. Based on these observations, it is concluded that FUS–DDIT3 is a causing factor in development of MLS/RCLS.

FUS–DDIT3 and the normal DDIT3 can induce an MLS/RCLS phenotype when expressed in human low differentiated sarcoma cells. This shows that the tumor morphology in MLS/RCLS depends on the DDIT3 part of the fusion. The "instructive" effect of FUS–DDIT3 may explain why this lipoblast containing tumor occurs most often in muscle and rarely in adipose tissue. Similar results have been reported for EWSR1–FLI1 in ►Ewing sarcoma, indicating that at least two members of this family of fusion oncogenes have an instructive effect on cell and tissue morphology.

DDIT3 encodes a DNA-binding transcription factor of basic leucine zipper type (C/EBP family) that binds to DNA as a dimer. DDIT3 can not form homodimers, but other C/EBP family members are considered major dimer partners to DDIT3. The dimer forming function is maintained in the FUS–DDIT3 fusion protein. DDIT3 and FUS–DDIT3 may therefore interfere with C/EBP family transcription factors and their binding to transcription regulatory C/EBP sites. The DDIT3 protein has been implicated in adipocyte differentiation as a dimer partner with C/EBPα and C/EBPβ. C/EBPα is a critical factor for the development of adipose tissue and blocks proliferation as preadipocytes enter terminal



Myxoid Liposarcoma. Figure 2 The *FUS*, *EWSR1*, *TAF15* group of fusion oncogenes (only some of the members are shown). All fusion oncogenes of this group contain the 5′ parts of *FUS*, *EWSR1*, or *TAF15* juxtaposed to the 5′ or central parts of transcription factor genes. With exception for *FUS–ERG*, which is found in myeloid leukemia and in Ewing sarcoma, the fusion oncogenes are specific for one tumor entity.

differentiation. FUS–DDIT3 has been shown to interfere with this function by complex formation with C/EBPα.

The FUS–DDIT3 and EWSR1–DDIT3 encoded chimerical proteins are believed to function as abnormal transcription factors that probably act as heterodimers with other C/EBP family proteins. Several affected target genes such as IL6 and IL8 have been identified. Consistent with a role in transcriptional regulation, the fusion proteins have been found to localize predominantly to the nuclei and in a minority of the cells to multiple nuclear structures that also contain transcription and splicing associated factors.

FUS, EWSR1, and TAF15 encode RNA-binding proteins with extensive similarities. Their proteins are found within protein complexes that participate in transcription, splicing, and mRNA transport. Some reports suggest functions in translational control and in stress response. FUS-deficient mice exhibit increased radiation sensitivity, deficient DNA repair, and male sterility. These observations suggest that the FUS protein participates in DNA repair and homologous recombination.

The natural function of the N-terminal parts of FUS, EWSR1, and TAF15 that are present in the chimerical oncoproteins is not known. However, when expressed as fusion proteins with DNA-binding transcription factors, they have been shown to act as transactivating domains. They are also critical for the transforming activity of the fusion oncogenes.

Immunohistochemistry analysis of growth and cell cycle controlling factors in MLS/RCLS, revealed a strong expression of cyclin D1, cyclin E1, CDK4, and CDK6 in the majority of the tumor cells. The tumor suppressor protein P16 (a.k.a. CDKN2a or INK4a) is also strongly expressed in most tumor cells.

Diagnostics

The diagnosis of MLS/RCLS is based on microscopic examination of tissue specimens. MLS/RCLS tissue is characterized by presence of lipoblasts, myxoid matrix, and the typical plexiform capillary network. The FUS–DDIT3 or EWSR1–DDIT3 fusion oncogenes can be detected by RT-PCR and tests for the fusion genes may be helpful when the morphology is unclear.

Treatment

Surgery remains the main treatment of MLS/RCLS. MLS/RCLS tumors are sensitive to radiation therapy. Surgery is therefore often combined with pre- or postoperative radiotherapy. In cases where surgery is out of option, radiation and chemotherapy are employed.

Prognosis

The main prognostic factor is the histological grade with an RCLS component of more than 5% as an unfavorable predictor. Local recurrence is common in MLS but the pure MLS variant rarely metastasize. The risk of metastasis increases with the presence of RCLS components and common sites are retroperitoneum, subcutaneous soft tissue, bone, and lungs. Overexpression of TP53 (p53) is associated with a poor prognosis.

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Myxoid Tumors

Definition

A variety of tumors with myxoid stroma: intramuscular myxoma, juxta-articular myxoma, deep "aggressive" angiomyxoma and ossifying fibromyxoid tumor are the most common. They are locally recurrent, but not metastatic.

►Uncertain or Unknown Histogenesis Tumors

Myxoma

Definition

Is a rare, usually benign, primary tumor (a new growth of tissue) of the heart. It is the most common of all benign heart tumors (►cardiac tumors).

MZCL

Definition

►Marginal Zone B-Cell Lymphoma.

MZL

Definition

►MZCL; ►Marginal Zone B-Cell Lymphoma; synonym Marginal Zone Lymphoma.

NAALADase

►Prostate-Specific Membrane Antigen

NAD(P)H-Quinone Oxidoreductase

Definition

NAD(P)H-quinone oxidoreductase, synonym quinone reductase or DT-diaphorase, is a flavoprotein that catalyses the two-electron reduction of quinones to hydroquinones

►Detoxification

NADPH Oxidase

Definition

Nicotinamide adenine dinucleotide phosphate-oxidase; is an enzyme complex. The complex generates superoxide by transferring electrons from NADPH inside the cell across the membrane. The electrons are coupled to molecular oxygen, which becomes a superoxide, a highly reactive ►free radical.

►Oxidative Stress
►Particle-induced cancer

Naevoid Basal Cell Carcinoma Syndrome

Definition

NBCCS; Synonym ►Gorlin Syndrome; Autosomal dominant cancer predisposition syndrome which is also characterized by multiple developmental defects. Most patients with NBCCS have a heterozygous mutation in the gene encoding the Hedgehog receptor ►Patched. Tumors isolated from Gorlin patients generally show homozygous inactivation of ►*Patched.*

►Hedgehog Signaling

NAG-1

►MIC-1

NAME

►Carney Complex

Nanometer

Definition

One nanometer equals 1×10^{-9}m.

►Nanotechnology

Nanoparticles

Definition

Are engineered particles in which one of the dimensions is smaller than 100 nm. Structures that are in the 1–100 nm scale in at least one dimension that may be in

any form including spherical, cylindrical or pancake shape.

►Aptamer Bioconjugates for Cancer Therapy
►Liposomal Chemotherapy
►Nanotechnology
►Particle-induced cancer
►Nanoparticles in Diagnosis and Treatment

Nanoparticles in Diagnosis and Treatment

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Synonyms

Nano-typing; Nano-treatment

Definition

Nanoparticle (NP)-based tumor diagnostics – methods of detection of tumor cells and/or quantitative analysis of tumor biomarkers using NP.

Nanoparticle-based anti-tumor treatment – methods of anti-cancer therapy which use NP as anti-tumor agents or as delivery systems for anti-tumor drugs.

Characteristics

Types of Nanoparticles

Nanoparticles (NP) are objects that are different in shape with sizes varying from 1 to 1,000 nm. NP can be classified into two major types: particles that contain organic molecules as a major building material (organic NP) and those that use inorganic elements, usually metals as core (inorganic NP).

Among the organic NP, liposomes, dendrimers and carbon NP are commonly used for tumor diagnostics and treatment.

- Liposomes are self-assembled organic nanoparticles with closed colloid structures composed of lipid layers;
- Dendrimers are globular macromolecules in which all bonds emerge radially from a central focal point with regular branching pattern and repeated units;
- Carbon NP are carbon cylinders composed of benzene rings (nanotubes), used for gene therapy and tumor imaging.

Inorganic NP share the same basic structure with a central core that defines the fluorescent, optical, magnetic, or electronic properties of the particle and a protective, usually organic, coating on the surface that protect NP from chemical degradation. Additionally, the outer layer of NP can be conjugated with different biomolecules such as peptides, proteins, oligonucleotides. Most commonly used inorganic NPs are:

- Supramagnetic NP – contain a metal core (iron oxide, cobalt or nickel) which is magnetically active and are used as contrast enhancement agents to improve the sensitivity of magnetic resonance (MR) imaging of the tumor; as well as for targeted tumor imaging and/or drug delivery;
- Nanoshells – contain dielectric core (usually silica) coated with thin metal (usually gold) shell; used for tumor imaging and therapeutics;
- Colloidal gold NP – contain gold as a core and used mostly for tumor imaging;
- Quantum dots – usually contain cadmium selenide (or other chemical elements of II and IV groups) in the core, and zinc sulfide shell surrounded by coating of coordinating ligand and amphiphilic polymer and are used for fluorescent imaging of tumors and for profiling tumor biomarkers;
- Raman probes – typically contain a metal core of silver or gold for optical enhancement, a reporter molecule for spectroscopic detection and silica shell for biomolecules conjugation; used for tumor biomolecular profiling;
- Near infrared (NIR) NP – fluorescent NP emitting on near infra red diapason of spectrum, used for tumor cells in vivo imaging (sentinel lymph nodes mapping).

Nanoparticles in Tumor Diagnostics

Tumor Detection

Supramagnetic NPs are commonly used as contrast agents for magnetic resonance (MR) imaging. They have greater magnetic susceptibility than conventional MR contrast (►gadolinium). Because of their small size (5–10 nm), these NP demonstrate better distribution in tissues including bone marrow and lymph nodes and are more efficient in the detection of regional and peripheral metastases for tumor staging, than other contrast agents. Supramagnetic NP can be conjugated to tumor-recognizing ligands and used for selective tumor imaging. For example, iron oxide NP conjugated to antibodies recognizing receptor tyrosine kinases (HER2/neu) and luteinizing hormone-releasing hormone (►LHRH) are used for specific breast cancer detection; conjugated with an antibody specific for $\beta_v\beta_3$ (endothelial surface antigen) these NP can be used for imaging the tumor vascular system. Quantum dots (QDs) can be used for noninvasive fluorescent *in vivo*

tumor imaging. Conjugated with an antibody targeted for ►prostate-specific membrane antigen (PSMA), QDs can specifically bind human ►prostate cancer cell implanted into animals. Because of the exceptional brightness and the stable emission fluorescence from QDs tumor cells can easily be visualized by mercury lamp excitation. Because of multicolor nature of QDs several colored particles can be conjugated with specific ligands and used for imaging multiple tumor structures, for example the angiovascular system, tumor tissue and lymphatic vessels at the same time. NIR particles emit in 800–1,000 nm range which is far from tissue autofluorescence range (400–600 nm) and thus are recognizable under NIR light even in the tissues with high autofluorescence. These NP are used for the intra-operative detection of sentinel lymph nodes in breast cancer and melanoma. Because of the composition of heavy metals and potential cytotoxicity, the widespread use of QDs and NIR NP for in vivo tumor imaging may be limited.

Profiling of Tumor Biomarkers

QDs are commonly used for quantitative analysis of biomarkers in tumor cells. QDs have unique optical properties such a multicolor nature due to tunable fluorescent emissions e.g. by changing the size of NP from small to large fluorescent emission and thus visible color can be manipulated. QDs demonstrate exceptional photostability, larger Stoke's shift (distance [in nm] from excitation to emission peaks) and the ability for simultaneous visualization (only single excitation source can be used for various QDs). Multicolor QDs can be directly conjugated with antibodies to form a QD-antibody complex which can be targeted to several surface and intracellular (including nuclear) proteins of the cancer cell. A mixture of QD-antibody conjugates are incubated with preliminary processed diagnostic samples such as formaldehyde fixed paraffin embedded cancer surgical biopsies (FFPE), diagnostic fine needle aspirates (FNA), frozen tumor sections and formaldehyde fixed *in vitro* cultured tumor cells. After staining samples can be analyzed on waive-resolving spectrometry, where individual fluorescent QD emission peaks representing targeted antigens can be simultaneously visualized. The fluorescent intensity of these QD peaks represents the level of expression of labeled biomarkers. Such an approach can be used to simultaneously detect and quantify five different breast cancer biomarkers. Similarly, the expression of cellular biomolecules can be assessed using Raman probes with surface enhanced Raman scattering (SERS). These NP have a metal (usually gold) core surrounding by a coating that contains reporter molecules and is linked to a targeting antibody with a unique spectroscopic signature which represents information about the reporter molecule's vibrational quantum state. While the golden core emits fluorescence, reporters create individual spectral curves at a range from 400 to 1,600 nm. Thus biomarkers labeled with Raman probe NP can be recognized on microscopy and detected by spectroscopy. This approach needs to be further optimized, since the large size of the particle (~100 nm) can limit access to the intracellular and nuclear cancer antigens.

FISH

Multicolor QDs can be conjugated to different specific oligonucleotide probes targeted for RNA or DNA of tumor cells and used for multiplex fluorescent in situ hybridization (►FISH) of cancer cells and tissues to detect the level of matrix RNA or gene activity of the biomarker of interest.

Nanoparticles in Anti-tumor Therapy

Liposomal Based Drug Delivery

Liposomes, micelles, and polymersomes are nanoscale lipid-based vehicles. This class of delivery vehicles is used for delivery of ►anthracyclines, ►taxanes, ►vinca alkaloids, and other chemotherapy agents. Major drawbacks include nonspecific uptake and toxicity, which can be improved by additional coating of these particles with PEG and conjugation with a targeted antibody (Ab). Dendrimers can be used to deliver fluorouracil, methotrexate, doxorubicin.

Nanoparticle-albumin bound ►paclitaxel (Abraxane)–NP has a core containing paclitaxel surrounded by albumin, which significantly improves tumor penetration. This nano-drug has a more potent anti-tumor effect than conventional paclitaxel. Additionally, the albumin covering protects paclitaxel from interaction normal tissues which results in a significant decrease in chemotherapy-associated side effects such as hypersensitivity reactions and neutropenia.

Gene Delivery

NP-based DNA and RNA delivery systems offer several potential advantages for gene delivery to various tumors. A DNA plasmid can be encapsulated into a lipid containing NP and thus be protected from *in vivo* degradation. In addition, such NP can be conjugated with tumor-targeted antibodies and used for the delivery of genes with anti-tumor activity. Short interfering RNA (►siRNA) can be linked to NP for the *in vivo* delivery of siRNA. When encapsulated into NP siRNA are protected from degradation in aggressive *in vivo* environment. When conjugated with siRNA targeted for tumors ►oncogene siRNA-NP complexes demonstrated the ability to decrease tumor growth by inhibiting expression of the targeted oncogenes.

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Nanoshell

Definition

►Nanoparticles with a silica core surrounded by a thin metal shell usually gold that is optically tunable by varying their size and composition.

►Nanotechnology

Nanotechnology

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Definition

Is the rapidly progressing interdisciplinary field that is focused on the engineering and development of man-made structures between 1 and 100 nm. One ►nanometer equals 1×10^{-9} m. Cancer nanotechnology is focused specifically on the application of nanotechnology to the diagnosis and treatment of cancer.

Characteristics

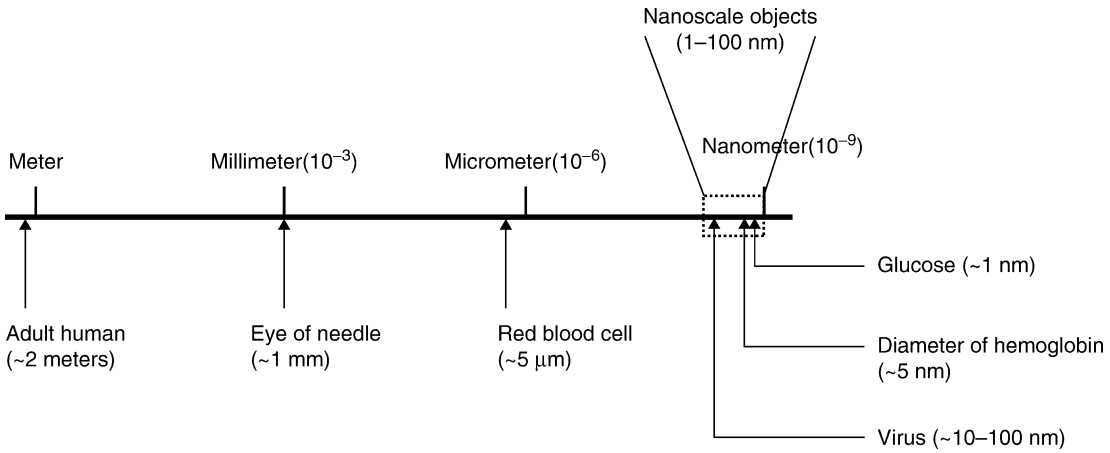
Nanotechnology involves the engineering of *man-made* structures ranging between 1 and 100 nm (Fig. 1). Nanotechnology is an interdisciplinary field that includes chemical engineering, physics, chemistry, biomedical engineering, material and particle science. Cancer nanotechnology specifically also incorporates the expertise of cancer biologists and oncologists. The goal of cancer nanotechnology is to utilize nanotechnology to improve early diagnosis and treatment of cancer. Nanotechnology has the potential to capitalize on advances in cancer biology, genomics, and proteomics and convert advances into clinical benefit for patients.

Nanoscale materials and particles have unique and useful optical, structural, and electronic properties that are not associated with either individual molecules or larger materials. Nanoscale materials and particles can be engineered to specifically function at the subcellular level. The ability to specifically engineer and functionalize particles and materials on the nanoscale represents a tremendous opportunity to design particles with no toxicity to normal cells and maximal toxicity to cancer cells. Nanoscale materials and constructs may be designed to allow ultra-sensitive detection of cancer in the laboratory.

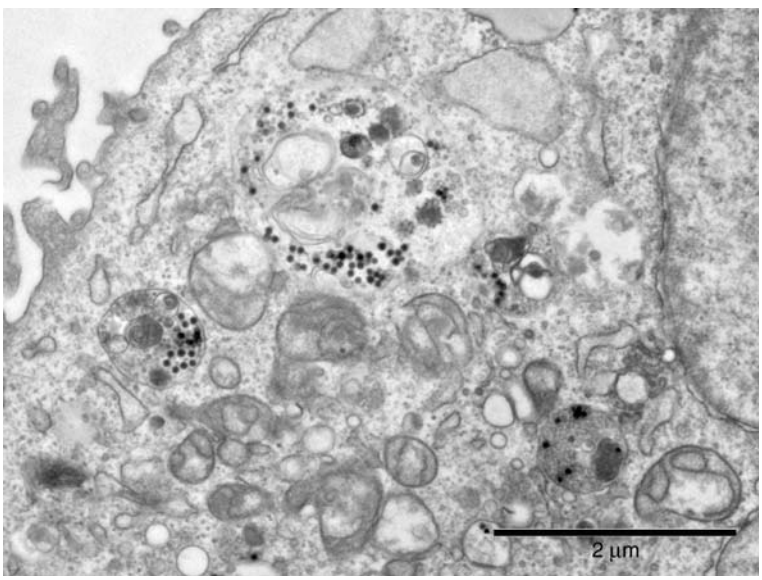
Nanoparticles

►Nanoparticles are particles designed on the nanoscale. In cancer nanotechnology, nanoparticles are used as contrast agents for imaging and as therapeutic agents. Unique properties of specific nanoparticles and the ability to specifically engineer the chemical and physical properties of nanoparticles as well as the ability to attach one or more cancer cell specific ligands to the surface of nanoparticle represent a tremendous opportunity to develop novel strategies for cancer detection and treatment. New nanoparticles with novel properties are continually under development. Some particles that are under current investigation and development include the following.

1. ►Quantum Dots are composed of an inorganic elemental core (e.g. cadmium or mercury) with a surrounding metal shell. Quantum dots absorb white light and then redirect light at a different wavelength. The emission spectra may be altered by varying the size and composition of the quantum dot. Quantum dots have been primarily studied for experimental imaging applications and ►in vitro cancer diagnostics. The use of quantum dots ►in vivo may be limited by toxicity of elemental core.
2. ►Nanoshells are composed of a silica core surrounded by a thin metal shell, usually gold. Nanoshells are optically tunable. Nanoshells have been studied for experimental imaging, tumor ablation, and drug delivery. Figure 2 demonstrates nanoshells (50 nm) delivered inside human breast cancer cells.
3. Gold Nanoparticles are biocompatible nanoparticles composed of colloidal gold. They have been utilized for experimental imaging, tumor ablation, drug delivery, and as a radiation sensitizer.
4. Silica Nanoparticles are biocompatible nanoparticles that have been used for experimental imaging and drug delivery.
5. ►Liposomes are biocompatible phospholipid based vehicles. Drug molecules can be entrapped into liposomes or inside their lipid bilayer. Liposomes



Nanotechnology. Figure 1 The nanoscale perspective demonstrating the relative sizes of conventional objects and nanoscale objects.



Nanotechnology. Figure 2 Transmision electron microscopy demonstrating intracellular accumulation of nanoshells in human breast cancer cells in culture (MCF-7). In this figure, nanoshells (50 nm) appear as black dots.

are biocompatible and have been used for drug delivery and imaging.

6. ►Dendrimers are highly branched polymeric molecules that are synthesized from monomers in a reproducible fashion. Dendrimers with highly charged surfaces allow conjugation of targeting molecules. Dendrimers have potential applications in drug delivery and imaging.
7. ►Supraparamagnetic nanoparticles are composed of iron oxide. There are used as a contrast agent for magnetic resonance imaging (MRI). These particles are attractive as MRI contrast agents because they have greater magnetic susceptibility than traditional MRI contrast agents. Iron oxide crystals have also been used for magnetic field drug targeting. In magnetic field targeting, externally applied magnets are used to preferentially direct iron oxide crystals to know sites of cancer.

Two different strategies have been utilized to deliver nanoparticles to tumors in living organisms, passive targeting (►Therapeutic passive targeting) and active targeting (►Therapeutic active targeting). Passive targeting takes advantage of fenestrations present in tumor blood vessels. Circulating nanoscale particles travel through the fenestrations allowing nanoparticles

to accumulate in tumors. Active targeting involves linking nanoparticles to tumor specific ligands such as antibodies or peptides allowing nanoparticles to accumulate preferentially on or inside tumor cells.

The introduction of man-made nanoscale particles into living organisms may be associated with toxicity to cells or organs. An understanding of toxicity related issues will be required before new nanoscale platforms can be adopted for clinical use. Research into understanding toxicity related issues with nanoscale particles is an important area of active research and development.

Nanoscale Cancer Imaging Contrast Agents

Nanoscale contrast agents are have been and are currently under development. The goal of this work is to allow cell specific imaging and functional imaging as well as to allow improved resolution of imaging with ultra-sensitive contrast agents. Nanoscale contrast agents for conventional imaging modalities including Magnetic Resonance Imaging and Computed Tomography as well as for newer imaging modalities such as Photoacoustic Tomography, which uses sound waves induced by pulsed laser light to generate images, have been and are under development. Quantum dots have been used for experimental lymphatic mapping and sentinel lymph node biopsy.

Biologically targeted contrast agents have allowed imaging of specific cell types such as tumor vascular endothelial cells as well to monitor cellular processes such as ►apoptosis. Nanoscale particles have also been developed which allow simultaneous imaging with multiple modalities. The development of ultra-sensitive contrast agents has allowed the development of high resolution in vivo imaging on the molecular level.

Nanoscale Cancer Therapeutics

Nanoparticles have been and are currently under development as cancer therapeutics. Nanoparticles may be biologically targeted to cancer specific ligands for tumor targeting. Targeted nanoparticles have also been developed as simultaneous carriers of antitumor agents. Inherent properties of specific nanoparticles also make them attractive as cancer therapeutics. For example, tumor specific nanoshells may be engineered to generate heat in response to near infrared light. This is referred to as ►photothermal ablation. Gold nanoparticles delivered to cancer cells have been demonstrated to serve as a radiation sensitizer in the experimental setting.

Laboratory Nanoscale Cancer Diagnostics

Nanoscale engineering and material development is allowing the development of new in vitro cancer diagnostic tests. The application of nanotechnology to laboratory cancer diagnostic tests seeks to lower the limits of the detection of cancer related molecules and to allow the simultaneous detection of multiple molecules. Examples of highly sensitive nanoscale diagnostics include:

1. Nanowires are made of conductive material that can be engineered to bind specific molecules. Binding of small amounts of molecules to the wires alters flow of current in the wires that can be detected as a change in electrical current.
2. Nanocantilevers are flexible nanoscale beams that can be engineered to bind small amounts of specific molecules. Binding of small amounts of molecules to cantilevers induces bending of cantilevers which can be detected by highly sensitive laser imaging systems.
3. Surface Enhanced ►Raman Scattering (SERS) Nanoparticles are nanoparticles with high scattering efficiency that can be detected by Raman instrumentation. These particles can be used in assays for high sensitivity cancer detection.

Biologically tagged nanoparticles such as quantum dots and gold nanoparticles have also been demonstrated to have application for in vitro cancer diagnostics. Nanoparticles offer improved stability, tunability, and sensitivity compared to conventional in vitro diagnostic approaches which, for instance, utilize antibodies.

Current Applications of Nanotechnology in Clinical Medicine

Several nanoscale drug formulations are in clinical use. This includes liposomal encapsulation of drugs to increase efficacy and to reduce toxicity (e.g. liposomal encapsulated Doxorubicin (►Adriamycin). Other drugs (e.g. ►Paclitaxel) linked to nanoscale proteins have demonstrated increased efficacy and reduced toxicity and are currently in clinical use.

With regard to imaging, MRI contrast agents have made the largest impact on current clinical cancer practice. Many further applications as discussed above are under development that likely will revolutionize cancer diagnosis and treatment.

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Nano-treatment

►Nanoparticles in Diagnosis and Treatment

Nano-typing

►Nanoparticles in Diagnosis and Treatment

Nasopharyngeal Carcinoma

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Synonyms

Lymphoepithelioma; Rigaud and Schmincke types of lymphoepithelioma; Transitional carcinoma

Definition

Nasopharyngeal carcinoma (NPC) is an epithelial cancer that has a worldwide distribution. It is also known as lymphoepithelioma, but this term is misleading and should not be used because this tumor derives entirely from epithelial origin. The benign lymphoid component is secondarily associated. It is endemic in southeast China, particularly in the Cantonese population of the Guangzhou area (up to 80 cases per 100,000 people per year). An intermediate incidence is found in other parts of Southern Asia, North Africa and among Eskimos in Alaska and Greenland (8–12 cases per 100,000 people per year). The incidence of NPC is extremely low in the rest of the world.

The World Health Organization (WHO) has proposed a classification of NPC based on the degree of differentiation:

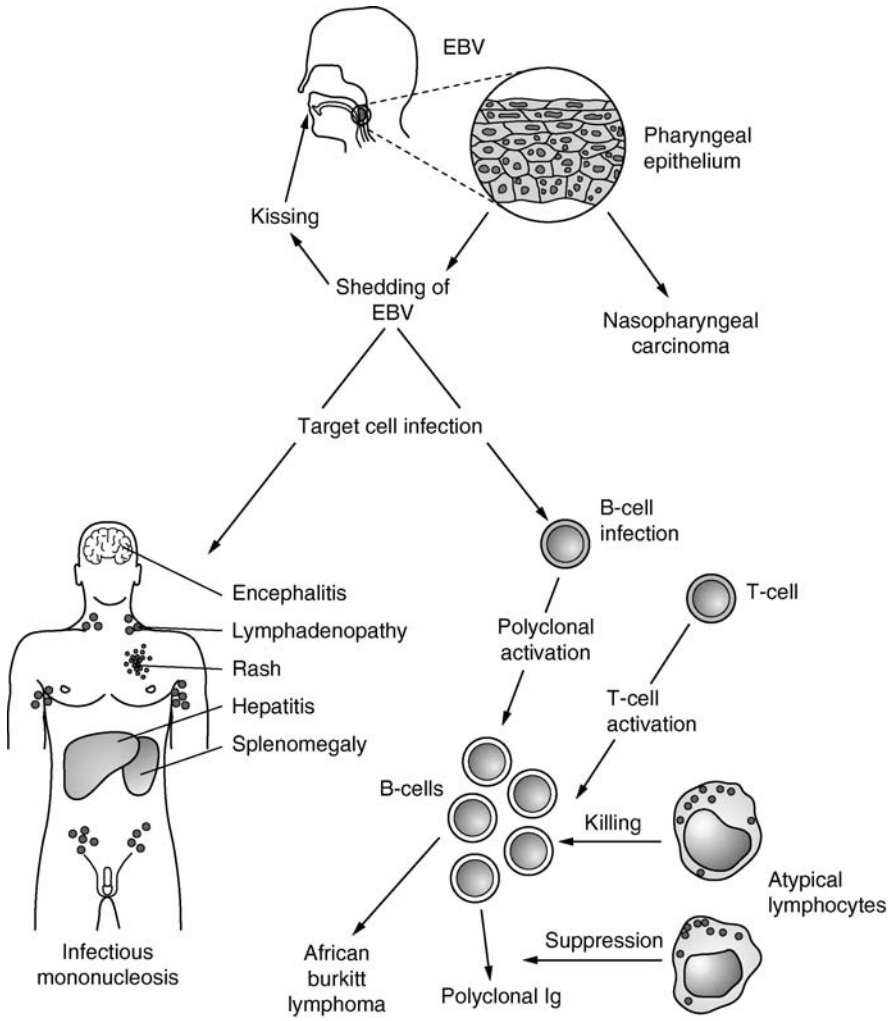
- WHO type 1: keratinizing squamous cell carcinoma (SCC)
- WHO type 2: nonkeratinizing epidermoid carcinoma
- WHO type 3: nonkeratinizing undifferentiated carcinoma (UNCT)

Characteristics

NPC is a variant of squamous cell carcinoma that has been serologically linked to the presence of ►Epstein-Barr virus (EBV) since 1966. EBV is a member of the human herpesvirus family and is the etiologic agent of infectious mononucleosis (Fig. 1). EBV consistently associates with the less differentiated types of NPC such as the nonkeratinizing and undifferentiated types of nasopharyngeal squamous carcinomas (WHO types 2 and 3). The ►human papillomavirus (HPV), a double stranded DNA member of the papovavirus family, causes proliferative lesions of squamous epithelium such as common warts, flat warts, plantar warts, anogenital warts (condyloma acuminatum) and laryngeal papillomatosis, and has been found in some keratinizing squamous carcinomas (WHO type 1).

Genetic and geographic factors play an important role in the genesis of NPC. Although the incidence of NPC among Chinese people decreases after immigration to low incidence areas such as the United States, the risk profile of the Chinese immigrants remains higher than that of the indigenous population. HLA-A2, HLA-Aw19, HLA-B17, HLA-Bw46 and HLA-Bw58 ►major histocompatibility loci have all been associated with an increased risk of developing NPC, whereas the HLA-A11 locus is associated with a decreased risk of NPC in the Chinese population. Conversely, the haplotype HLA-A2 has been correlated with a lower incidence of NPC in whites living in the United States, especially if homozygous. This suggests that different environmental insults may combine with specific genetic predispositions making the pathogenesis of NPC geographically distinct.

Additionally, deletions of the short arm of chromosome 3 as well as of 9p21-22 have been associated with NPC, which suggests that inactivation or deletion of a putative ►tumor suppressor gene located in these loci may be implicated in the pathogenesis of this disease. One of the candidates for this genetic implication is the ►p16 gene, which encodes for an inhibitor of the cyclin-dependant kinase that directly regulates the retinoblastoma tumor suppressor gene (►Retinoblastoma, cancer genetics) (RB). Homozygous deletions of the p16 gene that maps to chromosome 9p21 have been reported in 35% of the cases of primary UCNT (WHO type 3). Additionally, several reports showed that mutations of the "guardian of the genome," p53, which occur frequently in SCC, are present in only 10% of patients affected with NPC. Recently, point mutations in the retinoblastoma-related gene ►RB2/p130 have been described in 30% of NPC patients. RB2/p130 is a novel tumor suppressor gene that maps to chromosome 16q.12.2, an area that is frequently altered in several human neoplasias including breast, ovarian, hepatic, prostatic and endometrial carcinomas. Interestingly, ►amplification of the ►Bcl-2 oncoprotein, which inhibits ►apoptosis (programmed cell death), has been found mostly in NPC WHO types 2 and 3 and less frequently in the differentiated type 1.



Nasopharyngeal Carcinoma. Figure 1 Role of the Epstein-Barr virus (EBV) in infectious mononucleosis, nasopharyngeal carcinoma, and Burkitt lymphoma. EBV first invades and replicates within the salivary glands and the pharyngeal epithelium, and is shed into the saliva and respiratory secretions. In some people, the virus can transform the pharyngeal epithelium, leading to nasopharyngeal carcinoma. In people that are not immune from childhood exposure, EBV causes infectious mononucleosis. EBV infects B lymphocytes which undergo polyclonal activation. These B cells stimulate the production of atypical lymphocytes which kill virally infected B cells and suppress the production of immunoglobulins. Some infected B cells are transformed into malignant lymphocytes of Burkitt lymphoma.

Keratinizing NPC accounts for approximately 25% of all NPC and is more frequent after the age of 40 years. The nonkeratinizing nasopharyngeal carcinoma is the least common, representing only 12% of all NPC. The undifferentiated carcinoma is the most common, representing almost 60% of all NPC, and being the most frequent in younger age.

NPC has been linked by epidemiological and experimental evidence to certain environmental factors such as dietary and life style habits. In fact, a series of studies have suggested that continuous exposure to salted fish and other preserved food containing volatile nitrosamines, which are well-known carcinogens, constitutes a significant cause of NPC in the Chinese population. Tobacco use and alcohol consumption have been linked to an increased incidence of WHO type 1 NPC in white North Americans in proportion to the intake amount.

Because of their location, most NPC remain asymptomatic for a prolonged period and present at an advanced stage. Palpable cervical node metastases are most often (75–90%) the first sign of the disease, even if there are no specific local complaints from the patients. This is due to the growth pattern of this neoplasia, which is not a large space occupying mass,

but instead is an infiltrating and organ-substituting tumor. This growth pattern results in a variety of neurological symptoms such as hearing loss, tinnitus, ►ansomia, epistaxis, a feeling of an obstruction, ►dysphagia, ►odynophagia, ►dysphonia, ►hoarseness and ►diplopia.

Because NPC remain asymptomatic for a long time, an early diagnosis is difficult. Molecular biology and cytogenetic techniques have improved the detection of EBV. Southern blot analysis distinguishes between latent and infectious EBV involvement, whereas in situ hybridization allows the localization of single virally infected NPC cells. PCR of the Zebra EBV gene on fine-needle biopsies in patients with neck nodal involvement and unknown primary lesions may help in the identification of the nasopharyngeal origin of the disease. Serological analysis of different EBV ►antigens, such as screening for anti-EBNA IgG (►IGs), anti-EA IgG and IgA, anti-VCA IgG and IgA and anti-Zebra IgG, may be used to identify early cases of NPC as well as relapses. Increased anti-VCA, anti-EA IgA and IgG are used as markers to monitor the disease, the response to therapy and may suggest recurrence, but are neither highly predictive nor accurate. In fact, many long-term disease-free patients show persistently high levels of these markers.

Diagnosis

Diagnosis and staging of NPC is reserved to imaging techniques such as ►CT scanning and ►magnetic resonance imaging (MRI), which are essential for accurate evaluation of tumor extension and spreading and for guiding radiation treatment planning and evaluation of possible recurrences. Other tests and procedure commonly used to diagnose this disease are:

Physical exam of the throat: An exam used by the doctor to feel for swollen lymph nodes of the neck. It is completed by inspecting down the throat with a small, long-handled mirror to check for lesions.

Neurological exam: A series of tests to check the patient's nerve function. The exam checks a person's coordination, and how well the muscles, senses, and reflexes work.

Nasoscopy: It consists in the inspection inside the nose with a nasoscope. A nasoscope is a thin, tube-like instrument with a light and a lens for viewing and a tool for removing tissue samples (biopsy).

Head and Chest X-Ray, and PET Scan (positron emission tomography scan).

Biopsy: Removal of tissue sample that will be viewed under a microscope by a pathologist to verify the presence of cancer.

Treatment

Treatment of local NPC is based essentially on external ►radiation therapy, because of its radiosensitivity and because of its anatomic location. In fact, surgical resection of the tumor adjacent to the skull base with tumor-free margins is often impossible. Surgery, when feasible, is usually reserved for nodes that fail to regress after radiation therapy or for nodes that reappear following clinical complete response. The overall control rate of localized lesions with radiotherapy is generally about 70–90%. Although most tumors are treated with external-beam radiation therapy exclusively, in some tumors radiation therapy may be enhanced with intracavitary or interstitial implants or by the use of stereotactic radiosurgery when clinical expertise is available, and the anatomy allows for it. At 5 years, local relapse ranging between 15 and 54% has been reported. Tumor control rate of more advanced lesions is instead dramatically decreased. Future improvement of NPC treatment may result from advances in radiation therapy techniques such as three-dimensional conformal therapy, proton-beam therapy or intracavitary ►brachytherapy, which allows the delivery of higher radiation doses to the tumor. Combined radiation therapy and ►chemotherapy have been suggested to improve both local control and metastatic spreading of the disease. Novel approaches and future directions may include testing the appropriateness of adjunctive ►gene therapy oriented to either improve local control, by reducing the tumor volume prior to radiation therapy, and/or to decrease the probability of distant metastasis.

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NAT

►Arylamine *N*-Acetyltransferases

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Synonyms

NICE

Definition

►NICE is part of the of the United Kingdom's National Health Service (►NHS) which provides medical care, free at the point of delivery, to the entire population. Its overarching purpose is to provide national guidance on the promotion of good health and the treatment of ill health.

Characteristics

The Overall Purpose of NICE

According to its website NICE is "the independent organization responsible for providing national guidance on the promotion of good health and the prevention and treatment of ill health." It does this by commissioning and publishing guidance documents. These fall into one of five categories and are the responsibility of three "virtual" centers as follows:-

1. Centre for Public Health Excellence
   - Public health intervention
   - Public health programs
2. Centre for Health Technology Evaluation
   - Technology Appraisals
   - Interventional procedures
3. Centre for Clinical Practice
   - Clinical Guidelines

Although in its guidance NICE may make recommendations for future research it does not conduct or fund such research. As such its purpose is best described as ►health technology appraisal (HTA).

HTA is a rapidly expanding field NICE and is still unusual (but not unique – the Canadian Coordinating Office for Health Technology Assessment [CCOHTA] predates it by a decade) in the breadth of its remit and in its efforts to assess cost-effectiveness alongside clinical-effectiveness in many of its appraisals.

Why Was NICE Established?

It may seem evident that the provision of guidance to improve healthcare was sufficient reason for its establishment. In fact there are particular characteristics of the NHS and the way that technology has advanced that provided a strong impetus for the foundation of NICE in 1999.

Although the NHS is a national service it is delivered at local level and local managers have long struggled to balance the resources made available to them by national government with local demand. In many cases they have achieved this by restricting the availability of certain technologies and services in their areas resulting in regional inequality of access to particular treatments. These inequalities became more obvious in the early 1990's when an NHS reorganization devolved much budgetary responsibility to local NHS Trusts (►Primary Care Trusts (PCTs)) who sometimes made different decisions about whether or not treatments should be paid for, with the consequence that patients living in different PCT areas and being treated in adjacent beds in the local cancer centre sometimes had different access to certain treatments.

The problem of "►postcode prescribing" was acknowledged even by government ministers responsible for the NHS and became a source of great dissatisfaction amongst clinicians and patients whose stories regularly appeared in the media. The problem was nowhere more apparent than in oncology where the 1990's represented a period of rapid therapeutic advance as taxanes, camptothecins and monoclonal antibodies replaced or were used in conjunction with older and much cheaper drugs for the first time.

Dissatisfaction with inequalities of access to treatment was such that the government announced a number of initiatives to promote a consistent approach to NHS provision. These included the establishment of the National Institute for Clinical Excellence (the inclusion of Health in the organization's name was a later addition) in 1999. They also included, in the same year, the appointment of Professor Mike Richards was appointed as the UK's first National Cancer Director with responsibility for working with national policy makers, ►Cancer Networks and service providers to implement the first National Cancer Plan which aimed to improve the quality of all aspects of cancer services in the UK and reduce inequalities of care.

Professor Richards realized that equity and quality care required uniform access to innovative treatments and pressed for those new cancer treatments that were the subject of reported postcode prescribing to be appraised by NICE. Guidance notes on the use of taxanes in the treatment of ovarian and breast cancer were amongst the first ►Health Technology Appraisals (HTAs) completed by NICE – the third and sixth, respectively. Since then cancer drugs have formed the largest single subject group for HTAs, and are the subject of 24 of 97 completed and Appraisals currently listed on the NICE intranet site (this list excludes those now deemed obsolete).

What Sort of Guidance Does NICE Produce?

HTAs give guidance to the NHS on whether specific health technologies should be available to the NHS based on whether there is sufficient evidence that they are both clinically and cost-effective. The HTA program is not restricted to drugs, but the majority of HTAs have concerned drugs.

As well as HTAs NICE has also produced two Clinical Guidelines (CGs), about 20 pieces of interventional procedures guidance (IPG) pertinent to oncology and 10 Cancer Service Guidelines (CSGs). CGs set out to provide more comprehensive guidance on the appropriate treatment and care of people with specific diseases within the NHS, IGs provides guidance on whether interventional procedures used for diagnosis or treatment are safe enough and work well enough for routine application and CSGs, which were inherited from other bodies – there are no new ones in preparation- deal with the organization of cancer services within the NHS in England and Wales.

Why Has NICE Been Controversial?

However, it is NICE's HTA program that has attracted most attention and caused most controversy. There are several characteristics of HTA's which make them controversial:

- *HTAs are directive in their recommendations*. They always state explicitly whether or not the technology under review should be available under the NHS either as an option or as the treatment of choice. Other types of guidance weigh the evidence and give an opinion on the value of interventions but seldom direct that something must or must not be employed.
- *HTAs usually cover well-tried treatments*. Unlike most of the interventions considered in IPGs and many of the topics covered by CGs, drugs reviewed in HTAs have already been the subject of a review of their safety and efficacy for licensing purposes leading to the criticism that NICE review is duplicating work already done and thus unnecessarily delaying patient access.
- *Cost-effectiveness is an explicit part of HTAs*. Assessment of cost-effectiveness is not part of the preparation of IPGs and although CGs may make reference to the cost and cost-effectiveness of treatments they do not include a rigorous cost-effectiveness analysis of all recommendations made. However, all HTAs include such an analysis. Therefore, it is almost inevitable that the HTA process sometimes leads NICE to recommend that the NHS denies patient access to treatments which both NICE and NHS clinicians agree are highly efficacious, but which NICE deem to be not good value for money. The preferred currency for appraisal of cost-effectiveness is the cost per ►quality adjusted life year (QALY). NICE states that at a cost per QALY gained of below £20,000 cost-effectiveness is unlikely to be a barrier to positive guidance from NICE, whereas above £30,000 per QALY very compelling arguments will need to be made with regard to the certainty of the benefits associated with a new treatment and the needs of the patient population who are expected to receive it.
- Provision of treatments recommended in HTAs is mandatory, but recommendations do not trigger any increase in the funding of health services at local level. Consequently, health service managers are often unhappy about HTAs that make new treatments available and complain that they will result in cutbacks in healthcare provision in other areas.

The other controversial element of the NICE HTA process has been its lack of timeliness with appraisals carried out under the original "Multiple Technology Appraisal" (MTA) following a fixed timetable which runs over a period of one year from the invitation to the product manufacturer and other interested groups to submit evidence to the publication of final guidance (or longer if an appeal is launched against the final guidance). The delay from a product being granted a Marketing Authorization to NHS guidance being made available can also be extended – sometimes indefinitely – if an intervention is not selected promptly by the UK Department of Health as being one which satisfies its criteria for NICE review i.e. interventions:

- Which may have an impact on a clinical priority area for the NHS?
- Which may have a major impact on NHS budgets?
- Where guidance is likely to alter current practice?

One of the reported consequences of delay is "►NICE blight" where NHS organizations decide at local level not to make use of new technologies because NICE guidance has not yet been published. Despite advice to the NHS that in the absence of NICE guidance local decisions should be made about the suitability of new interventions for NHS use, there is a suspicion, fuelled by the rapid increase in NHS uptake once NICE endorsement has been received, that NICE blight is a real phenomenon.

In an attempt to reduce delays NICE has, in addition to reviewing the way appraisal topics are selected, introduced a streamlined Single Technology Appraisal (STA) process, for novel stand-alone interventions. Provided the resulting guidance is not appealed, STA process can be completed in 6 months from the point where submission of evidence is invited. If the STA process is started during the period when regulatory approval is being sought, NHS guidance can be issued within weeks of Marketing Authorization, as was the case in the recent appraisal of adjuvant trastuzumab for early breast cancer.

The main difference between the STA and MTA processes is in the role of the academic Evidence Review Group (ERG) which is involved in both processes. In the MTA process, the ERG produce their own detailed analysis of the cost- and clinical-effectiveness of the intervention. This is a time consuming step which is omitted from the STA process where the ERG role is restricted to providing a critical review of the submission received from the manufacturer.

How Does NICE Reach Its Decisions?

All evidence submitted to NICE is reviewed by an Appraisal Committee, which formulates draft guidance to the NHS in the form of an ►Appraisal Consultation Document (►ACD) which is then distributed to stakeholders for comment. These comments are then considered at a second Appraisal Committee meeting when the ACD can be either ratified as it is or with minor modifications, or subjected to major revision and circulated, once again, for stakeholder comments. Once the Appraisal Committee has agreed final guidance, in the form of a "Final Appraisal Document" (►FAD), this is issued to the NHS unless an appeal is launched against it by one of the consultees on the guidance. Appeals are allowed only on three specific grounds:

- That NICE has failed to act fairly and in accordance with its appraisal procedure.
- That NICE has prepared guidance which is perverse in the light of the evidence submitted.
- That NICE has exceeded its powers.

What Impact Has NICE Had in Oncology?

There is no definitive answer to this question. However, a recent review suggests that NICE HTA's of oncology products have had an impact, though not necessarily that expected. From a positive perspective most new oncology drugs have been recommended by NICE for use within their licensed indications with such endorsement resulting in increased usage within the NHS. On the other hand, NICE guidance has not abolished local inequality of access with large local differences in the utilization of drugs remaining many months after the publication of NICE guidance.

Additionally, it is widely accepted that it has led to the phenomenon of NICE blight described above. This has the potential to slow down the introduction of any new anticancer drug, but is likely to have a disproportionate impact on patients with less common cancers. This is because few products have regulatory approval for these tumors and NICE, generally, restricts itself to guidance on products used within their licensed indications.

Consequently, if PCTs are reluctant to pay for new drugs that have not been endorsed by NICE, patients with rarer malignancies are, inevitably, disadvantaged when it comes to accessing new drug treatments.

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Native Conformation

Definition

Most of proteins is the energetically favorable state adopted by a protein, which usually corresponds to the conformation with the lowest free energy.

►Endoplasmic Reticulum Stress

Natural Chemoprotectants

Definition

Chemoprotectants that are derived from natural sources.

►Chemoprotectants

Natural Killer Cell Activation

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Synonyms

NK cell induction; NK cell stimulation; Induction or stimulation of NK activity; Induction or stimulation of a NK cell response

Definition

Induction of ►NK cells by double-stranded RNA, soluble factors (►cytokines, antibodies, e.g.) or ligands

expressed by pathogen infected or malignant cells, to exert or enhance NK mediated immune effector functions, which include cytotoxicity, cytokine release and mobilization of the adaptive immune system.

Characteristics

Cytokine Activation of NK cells

Cytokines are important regulators of the immune system and as such have crucial functions in regulating NK activity. Several cytokines produced by immune cells, mainly by macrophages, dendritic cells and activated T cells, enhance NK cell cytotoxicity or stimulate NK cells to secrete cytokines. Important functions for the cytokines ►interleukin (IL)-2 and IL-15, which both significantly increase the killing potential of NK cells, are well established. NK stimulatory functions have been described for IL-21, a cytokine with significant sequence homology to IL-2 and IL-15. IL-21 is released by activated $CD4^+$ T-helper cells and stimulates NK cytotoxicity and cytokine release interferon (IFN)-γ e.g.). Furthermore, synergies among cytokines play important roles. For example, costimulation with IL-18 and IL-12 triggers NK cells to release high amounts of IFN-γ, whereas stimulation of NK cells with IL-15 and IL-12 induces pronounced secretion of tumor necrosis factor (TNF)-α, granulocyte-macrophage CSF (GM-CSF), macrophage inflammatory protein-(MIP)-1α), and MIP-1β. Notably, engagement of the activation receptor NKp46 in the presence of IL-12 triggers NK cells to release IFN-γ, TNF-α, and GM-CSF. Such enhancing effects of cytokines on NK effector functions are exploited in NK mediated cancer cellular therapies against malignancies.

NK cells are generally characterized by the expression of the ►adhesion marker CD56 (neural cell adhesion molecule, NCAM) and the lack of CD3 (T cell marker). However, they do not represent a uniform population of cells, but constitute different subsets with divergent functions. Most studied are $CD56^{dim}CD16^+$ and $CD56^{bright}CD16^{dim/-}$ subsets, distinguished by different expression levels of CD56 and the low affinity Fc receptor CD16 (Fc-γRIII). $CD56^{dim}CD16^+$ NK cells constitute more than 95% of peripheral blood NK cells. They are cytotoxic effectors, which express the cytolytic granule content perforin, an array of activating and inhibitory receptors and are hypothesized to be terminally differentiated NK cells. Furthermore, this subset comprises NK cells that are poor cytokine secretors, however release a unique cytokine profile, including IFN-γ, TNF-α, and IL-6, upon engagement of the immunoglobulin (Ig)-like CD160 activation receptor with its ligand ►human lymphocyte antigen (HLA)-C. NK cells of the $CD56^{bright}CD16^{dim/-}$ subset, on the other hand, constitute less than 5% of peripheral blood NK cells and reside primarily in secondary lymphoid tissues, in particular lymph nodes and tonsils, where they form the predominant NK population. The $CD56^{bright}CD16^{dim/-}$ subset embraces cytokine producers, which are generally incapable of mediating cytolysis due to the lack of perforin expression and a diminished receptor repertoire. However, activation with IL-2 for 3–7 days induces perforin expression and upregulation of receptors in these NK cells and converts them into cytolytic cells.

The study of different NK subsets for their effectiveness in tumor eradication is of particular clinical relevance. Recently, a role for a NK subset, with a $CD56^+$ $CD16^+$ phenotype, that could not be clearly delineated into $CD56^{bright}CD16^{dim/-}$ and $CD56^{dim}$-$CD16^+$ subpopulations has been implicated in survival of cancer patients, after fully HLA matched ►hematopoietic stem cell transplants and NK reconstitution. Crucial functions in autologous tumor eradication (►acute myeloid leukemia, (AML) patients) have been demonstrated for another NK subset, which is defined by the expression of the CD8 antigen. CD8 is expressed by NK cells as the CD8α/α homodimer (NK phenotype $CD3^-CD56^+CD8^+$), different from T cells, which express the CD8α/β heterodimer. The CD8α/α positive NK subset is highly cytotoxic and CD8 expression seems to remain stable after IL-2 activation and long-term culture, which argues strongly for an important role of CD8 in NK cell cytotoxicity. In fact, superior cytotoxicity for eample against K562 and autologous AML blasts has been demonstrated for NK cells of the $CD56^+CD8^+$ subtype compared to the $CD8^-$ subset. Signaling through CD8α/α triggers upregulation of the activation marker CD69 and seems to protect NK cells from "cytotoxicity induced cell death" (CICD). Consequently, $CD56^+CD8^+$ NK cells could be more powerful killers by transmitting multiple "lethal hits." Further insight into cytotoxic mechanisms of highly cytotoxic NK subsets in conjunction with cytokine activation, is a prerequisite for potential translation to effective cancer treatment.

Activation of Natural NK Cytotoxicity by Tumor Cells

In recent years significant progress has been made in elucidating the complex scenario of events involved in NK mediated target cell killing. Apart from characterization of the signaling pathways that constitute NK activation, assigning distinct functions to the numerous inhibitory and activating NK receptors remains a challenging task. A milestone was the conception of the "missing self-hypothesis" soon after the discovery of NK cells. According to this model, lack of ►MHC class I (self) molecule expression renders cells susceptible to NK lysis, since NK inhibitory killer cell immunoglobulin-like receptors (KIR) do not encounter their ligands. For example, tumor cells frequently downregulate MHC class I as a camouflage mechanism

to escape the adaptive T cell response, which in turn renders them sensitive to NK killing. An autologous NK attack, on the other hand, is prevented on account of MHC class I expression by healthy cells. Although this elegant model for NK cytotoxicity seems to define mechanisms for tumor specific cytolysis as well as self-tolerance, major roles for NK activating receptors, including ►NKG2D, and the natural killer cell receptors (NCRs) NKp30, NKp44, NKp46 e.g. are increasingly apparent. However, tumor ligands for several activation receptors remain to be identified, a prerequisite for further insights into NK cytolytic mechanisms.

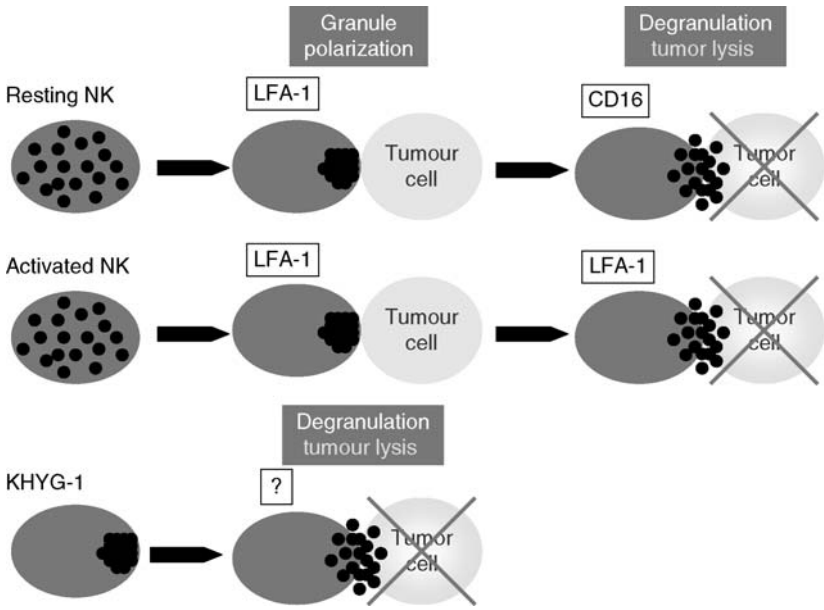
Activation of NK cells upon sensitive target cell contact triggers formation of the ►cytolytic synapse and initiates signaling pathways that include ►tyrosine kinase activation pathways, which are ►immunoreceptor tyrosine-based activation motif (ITAM)-dependent or ITAM-independent (e.g. NKG2D-DAP10), a scenario that eventually results in granule polarization and target cell lysis. The most downstream kinase known so far is the ►mitogen-activated protein kinase-extracellular signal-regulated kinase (►ERK), which is transiently induced by sensitive target cell contact. A specific signaling pathway for ERK activation has been identified, which involves ►phosphatidylinositol 3-kinase (PI3K), the small GTP-binding protein Rac1, the p21-activated kinase (PAK) 1 and the mitogen-activated protein kinase kinase (MEK) 1. Furthermore, Src and ►Syk family kinases induce ►lipid raft polarization toward the target cell. These signaling scaffolds recruit activation receptors, which aggregate and form complexes with signaling molecules, for example linker for activation of T cells (LAT), lipid kinase and PI3K. Activation of NK cytotoxicity is furthermore dependent on the activation of phospholipase C (PLC)-γ2, ►protein kinase C (PKC), the rise of intracellular calcium levels by depletion of intracellular calcium stores and the subsequent influx of calcium through membrane pores.

In contrast, inhibitory receptor engagement with cognate ligands on resistant target cells involves ►immunoreceptor tyrosine-based inhibition motif (ITIM)-dependent pathways, blockage of raft redistribution, inhibition of activation signaling pathways and recruitment of tyrosine phosphatases Src homology 2 (SH2) domain tyrosine phosphatase (SHP)-1 or SHP-2 or the lipid phosphatase SH2-containing inositol 5′-phosphatase 1 (SHIP)-1 to the contact side. It is conceivable that future advances in our understanding of NK cytotoxic mechanisms and in particular, identification of tumor ligands that engage NK activation receptors, will enable more specific targeting of different tumor types with NK cells.

Refined models are imperative to unravel specific NK receptor functions, since target cells generally express ligands for several NK receptors. Recently, insect cells (Drosophila Schneider cells (SC2) that are otherwise resistant to human NK lysis have been introduced as valuable models, since transfection of SC2 cells with cognate NK receptor ligands allows the analysis of downstream mechanisms triggered by distinct receptor-ligand interactions. In ►resting NK cells, the two major steps, granule polarization and degranulation are triggered by different receptors, for example by the ►integrin leukocyte functional antigen (LFA)-1 (polarization) and CD16 (degranulation), respectively. In contrast, in IL-2 stimulated NK cells LFA-1 alone seems sufficient to activate the entire cytolytic process. Interestingly, in the highly cytolytic IL-2 dependent NK cell line KHYG-1 granules are constitutively polarized, but target cell contact is still required to induce degranulation. KHYG-1, which was established from a patient with NK leukemia, appears to be primed for action. This is notably different from other NK cell lines (IL-2 dependent or independent) yet perhaps is also found in a subset of primary NK cells, from which KHYG-1 might have originated. KHYG-1 does not express CD16 and is an intriguing model system to identify the roles for other activation receptors in NK tumor cell killing (Fig. 1) In fact, a potential cooperative function for NKG2D and NKp44 in the induction of degranulation, based on this model, has been discussed. Future mechanistic analyses promise to elucidate additional receptor functions and signaling molecules in the granule exocytosis signaling pathways.

Activated NK Cells in Cancer Therapy

NK cell cytolytic action is immediate with the potential to target a wide range of malignancies, features that renders these innate immune cells promising candidates for ►adoptive immunotherapy (AIT) against cancer. Hematological malignancies are the most promising targets for NK cell therapy, since obstacles due to inefficient trafficking do not apply. However, treatment of solid tumors by administration of NK cells directly to the tumor site could also increase therapeutic effectiveness. Although autologous lymphokine-activated killer (LAK) cells have been employed in AIT for more than 20 years, as well as cytokine infusions to activate endogenous NK cells the overall clinical success rate was low. Obstacles included toxic side effects caused by high-dose systemic cytokine treatments, like IL-2. Moreover, the cytotoxic potential of endogenous NK cells against tumors may be insufficient. Several novel approaches aim to employ activated NK cells with enhanced cytotoxicity, which are the focus of this paragraph, to overcome limitations of traditional NK therapies. For example clinical application of stimulated, Hsp70-specific autologous NK cells to target Hsp70 expressing tumors holds promise. Safety and a potential therapeutic effect for the procedure were demonstrated in a Phase I ►clinical



Natural Killer Cell Activation. Figure 1 Simplified schematic representation of resting NK cells, IL-2 activated NK cells, and KHYG-1 encountering a sensitive tumor target cell. Cytotoxic granules are illustrated as black dots. LFA-1 (granule polarization) and CD16 (degranulation) are examples for receptors that are known to be involved. Primed KHYG-1 with constitutively polarized cytotoxic granules is shown. Target cell contact is required in NK cells for granule polarization and degranulation, however only for degranulation in KHYG-1 (receptor involvement unknown (?)).

trial reported from Germany, which involved patients with metastatic ►colorectal cancer and a patient with non-small cell ►lung cancer.

Another strategy exploits the highly cytotoxic, IL-2 activated NK cell line NK-92, which has been established from a patient with ►non-Hodgkin lymphoma. NK-92 can be easily expanded in culture and is an efficient anti tumor agent for example against hematological malignancies, including AML, T-cell ►acute lymphocytic leukemia (ALL), B-lineage-ALL, and ►chronic myeloid leukemia (CML). Clinical Phase I trials in USA and Germany demonstrated safety for the application of irradiated NK-92 and a Phase I trial in patients with refractory hematological malignancies is in progress in Canada. Clinical application of other highly cytotoxic NK cell lines, like KHYG-1, in the future, or genetically altered NK-92 variants to efficiently target specific tumors could further enhance this kind of AIT. However, feasibility and success of clinical applications of NK cell lines requires further evaluation.

Recently, a novel activation mode for resting NK cells, using the tumor cell line CTV-1 has been discovered. Interestingly, resting NK cells downregulate CD16 and upregulate the C-type lectin activation receptor CD69 upon CTV-1 priming. CD69 is in fact the predominant trigger signal for such primed tumor activated NK cells (T-aNKs) and its unknown ligand, CD69L, has been implicated as a tumor marker. T-aNK cells are capable to effectively and specifically lyse a broad range of tumor targets and seem to overcome NK resistances. These features render T-aNK cells potential novel candidates for AIT (AML e.g.).

Cellular therapies are emerging in clinical medicine and NK cells are potentially powerful tools against cancer. However, a better mechanistic understanding of specific receptor functions and signaling pathways involved in enhanced NK cytotoxicity and different activation modes are essential to significantly enhance AIT approaches in the future.

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Natural Killer Group 2D

►NKG2D Receptor

Natural Products

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Synonyms

Compounds from organisms; Secondary metabolites

Definition

"Natural products" are generally recognized as small-molecular-weight organic compounds with no known role in the primary metabolism of their terrestrial or marine prokaryotic or eukaryotic organism of origin. Natural products currently used as either cancer chemotherapeutic drugs or anticancer drug candidates are produced by higher plants, terrestrial microbes, and marine organisms.

Characteristics

Since natural products are "secondary metabolites" of organisms, such compounds of a specified structural class are biosynthesized only by certain organisms, and tend to have a limited taxonomic distribution in nature. It is generally agreed that secondary metabolites are produced by mainly sessile (immobile) organisms for ecological reasons as survival tools, and, for example, may help repel predators and locally competitive organisms, or serve as pollination attractants. These natural products are biosynthesized in the correct chiral form to elicit biological effects in mammalian systems. Natural products are produced through the interaction of a variety of proteins in living organisms, and consequently have conformational and configurational attributes that may lead them to modulate enzymes or receptors with considerable binding selectivity and high affinity.

When compared with "libraries" or collections of synthetic compounds, produced by synthesis or by "combinatorial chemistry," natural products may be observed as having more bridgehead atoms, rotatable bonds, ring fusion, and stereogenic centers, among other features in their molecules. The elemental components of natural products are reported to contain lower numbers of halogen, nitrogen, or sulfur atoms than synthetic compounds, while incorporating more oxygen-containing functional groups. Overall, natural products as perceived as having inherently more "drug-likeness" than synthetic molecules.

Natural Products Drug Discovery

There are many examples of structurally defined secondary metabolites from organisms serving as drugs in orthodox medicine either in their naturally occurring form or when subjected to synthetic modification. Of some 210 small-molecules listed in the 13th Edition of the World Health Organization Model List of Essential Medicines, there were included 44 structurally unmodified natural products, 25 semi-synthetic derivatives of natural products, and over 70 synthetic drugs based on the ►pharmacophore of a natural product or synthetic mimics of natural products. The term "pharmacophore" refers to the molecular region of a drug or other bioactive molecule that acts at the site of a receptor, and thereby confers the biological activity observed. In terms of anticancer drugs, 66% of those currently approved worldwide between 1940 and 2006 were either natural products or drugs based on a prototype natural product molecule.

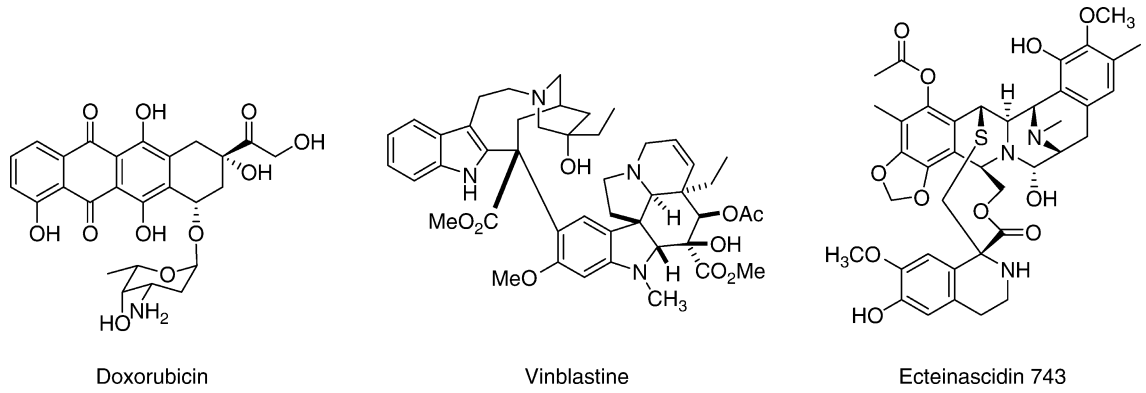
There is an active interest in the discovery of new natural product drugs in countries all over the world. A fundamental tenet of such work is that the wider the range of "biodiversity" afforded by studying as broad a taxonomic range of organisms as possible, the greater the structural diversity represented by the resultant bioactive molecules to be discovered. Major taxonomic groups of organisms include an estimated 1.5 million fungi, 300,000–500,000 flowering plants, and 200,000 invertebrate animals and algal species of marine origin. Only a small proportion of these natural resources has been analyzed for their possible medicinal uses. There is great potential for the future evaluation of marine actinomycetes and cyanobacteria (blue-green algae), as well as endophytic fungi and other microorganisms occurring on terrestrial plants. Many new chemical entities of natural origin are under preclinical development and clinical trial as anticancer drug candidates, and these have been discovered from terrestrial microorganisms, marine organisms, and plants, largely as a result of advances made in screening techniques using contemporary biological molecular targets.

Natural products drug discovery is dependent on access to diverse genetic natural resources, mainly from developing countries. According to the United Nations Convention on Biological Diversity, passed in Rio de Janeiro in 1992, genetic materials such as organisms are owned by their country of origin. Therefore, it is necessary to formulate formal benefit-sharing agreements between agencies in source countries holding genetic resources of interest and institutions in countries seeking to investigate these materials. Such "collection agreements" must be in force before the collection of any organisms commences, and they provide for an equitable distribution of any future income that may accrue from licensing agreements and patent royalties.

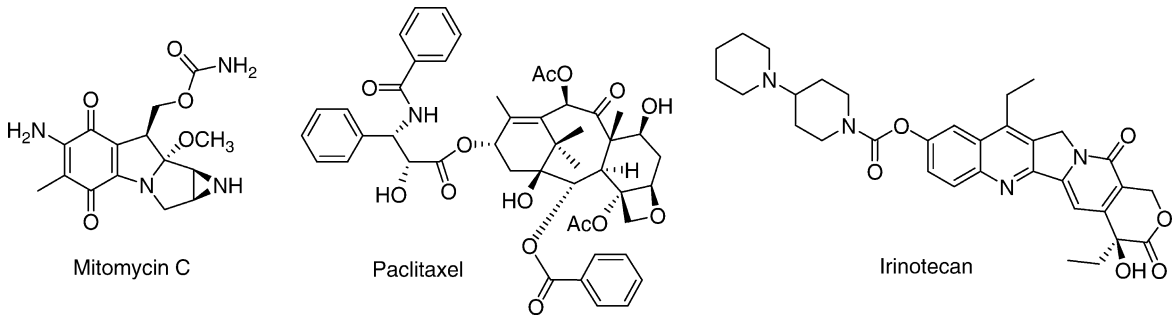
Natural Product Anticancer Drugs

In the discovery and development of cancer chemotherapeutic agents, natural products have served as drugs, drug precursors, and drug templates. Well-established representative examples have been isolated from both microorganisms and higher plants, such as actinomycin-D and vinblastine, respectively. Other examples of natural anticancer drugs used in unmodified forms are the ►anthracycline, ►doxorubicin, and the ►taxane, ►paclitaxel, obtained from a terrestrial microbe and a plant, respectively (Fig. 1). Currently, no anticancer drugs of marine origin are yet approved, but several such compounds are under development, with ►ecteinascidin-743 (ET-743) from a tunicate in late clinical trials as an anti-sarcoma agent. Several natural products have played the role of "lead" compounds, to afford currently used antineoplastic agents (Fig. 1). For example, the plant constituents ►camptothecin and podophyllotoxin, have both afforded two drugs each, namely, ►irinotecan and topotecan, and ►etoposide and teniposide, respectively.

Natural product anticancer agents have efficacy by virtue of interacting with a variety of targets in the cancer cell (Fig. 2). For example, they intercalate, cleave, or alkylate DNA and RNA (►actinomycin D, ►bleomycin, ►mitomycin C, ►ecteinascidin 743); interact with the protein tubulin (►vinblastine, ►vincristine, ►paclitaxel, ►docetaxel); and inhibit the enzymes topoisomerase I (►irinotecan, ►topotecan) and ►topoisomerase II (►etoposide, teniposide, ►daunorubicin, ►doxorubicin). Natural product-derived compounds under development are of interest by having high affinity and selectivity toward new molecular targets, such as the inhibition of ►protein kinase C (►staurosporine), ►heat shock protein 90



Natural Products. Figure 1 Examples of natural product anticancer agents from a microbe, a plant, and a marine organism.



Natural Products. Figure 2 Natural product anticancer agent with diverse mechanisms of action.

(17-allylamino-geldanamycin), and 20S proteasome (salinosporamide A). Combretastatin A4 phosphate is plant-derived stilbenoid that both inhibits tubulin assembly and can cause a selective shut down of the blood flow to tumor tissue. A number of analogues based structurally on the epothilone macrocyclic lactones, from a terrestrial sliding bacterium, are under development as potential cancer chemotherapeutic agents, and these have a similar mechanism to paclitaxel, in stabilizing microtubules and preventing their disassembly.

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Natural Yellow 3

►Curcumin

Naturally Disordered Proteins

►Intrinsically Unstructured Proteins and Cancer

Naturally Occurring Antibodies

Definition

Serum antibodies directed against antigens of ABO (or other) blood group systems in individuals not exposed to relevant antigens through transfusion or pregnancy.

Naturally Unfolded Proteins

►Intrinsically Unstructured Proteins and Cancer

Naturally Unstructured Proteins

►Intrinsically Unstructured Proteins and Cancer

NBCCS

Definition

Nevoid Basal Cell Carcinoma Syndrome; synonym ►Gorlin Syndrome, Basal Cell Nevus Syndrome (BCNS).

►Navoid basal cell carcinoma syndrome
►Hedgehog Signaling

NBS1

Definition

Nijmegen breakage syndrome 1; ►Nibrin protein is encoded by the NBS1 gene, which is mutated in patients with ►Nijmegen Breakage Syndrome. Nibrin plays critical role in both DNA damage ►checkpoint control and ►DNA repair.

NBT

Definition

Nitroblue tetrazolium. Its reduction is a marker of myelomonocytic differentiation.

►Jasmonates in Cancer Therapy

NCA-160

Definition
Nonspecific cross-reacting antigen with a Mw of 160kD.

►CEACAM1 Adhesion Molecule

NCI 60 Cell Line Screen

Definition
The National Cancer Institute (NCI) uses the in vitro human tumor cell line assay, which consists of 60 different cancer cell lines. With the use of this screen, agents are selected on the basis of potency, selective activity against a particular disease category, and/or differential activity. Drugs can then be evaluated against a small number of sensitive human tumors with the HFA (►Hollow fiber assay) and the nude mouse ►xenograft model as a basis for selecting compounds for further preclinical development. The model has been characterized extensively for sensitivity to many drugs allowing to determine the potential mechanism of action using the COMPARE program; the cell line panel has also been characterized extensively for molecular properties using several microarray platforms and quantitative ►PCR analyzes (http://dtp.nci.nih.gov/branches/btb/ivclsp.html).

NCoA3

►Amplified in Breast Cancer 1

Ndks

►NM23 Metastasis Suppressor Gene

NDP Kinases

►NM23 Metastasis Suppressor Gene

NDPKs

►NDP Kinases
►NM23 Metastasis Suppressor Gene

NEBD

Definition
Nuclear Envelope Breakdown.

Neck Dissection

Definition
Removal of specified levels of lymphatic tissue from the neck.

►Salivary Gland Malignancies

Necrosis

Definition
Is non-reversible death of tissue in the body, often due to injury, radiation, chemicals, or lack of adequate blood supply. Nonphysiological, accidental, and rapid cell death mode characterized by loss of the cell membrane integrity and unregulated leakage of intracellular material followed by inflammatory immune reactions. Necrosis may be induced by various high-dose substances or chemical/physical conditions (such as

extreme pH, pressure, osmolarity). In contrast to the regulated and controlled process of ►apoptosis (active cell death), necrosis is referred to as passive cell death which does not involve active cellular regulation or control.

►Photodynamic Therapy
►Hyperthermia
►Anoxia

Neddylation

Definition

Describes the linkage of Nedd8 – a small 76-residue protein with sequence similarity to ►ubiquitin – to a lysine residue on the substrate. Is the process by which the ubiquitin-like protein Nedd8 is conjugated to target proteins.

►Ubiquitin Ligase SCF-Skp2

Needle Aspiration Biopsy

►Fine Needle Aspiration Biopsy

Needle Biopsy

►Fine Needle Aspiration Biopsy

Negative Feedback Mechanism

Definition

Biological mechanism that prevents hyperactivation or disregulation of a specific signaling pathway.

►Suppressors of Cytokine Signaling

Negative Predictive Value

Definition

Of a test indicates the proportion of patients with a negative test that do not have the target condition.

►Molecular Pathology

Negative Resection Margins

Definition

Synonym Free Microscopically Margins; No tumor cells present in the resection margins using the microscope.

Nek2

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Definition

NIMA - related kinase 2; Nek2 is a cell cycle regulated, intracellular protein kinase that contributes to passage of cells through mitosis. It's upregulation in a number of cancer types has raised the possibility that its expression level is important to normal cell division and that it might make a novel ►cell cycle target for cancer therapy.

Characteristics

Nek2 is a ubiquitously expressed, serine/threonine protein kinase. It localizes to the centrosome and functions in cell cycle control contributing to the correct assembly of the bipolar mitotic spindle upon which chromosomes are segregated. As such, Nek2 belongs to a small group of important protein kinases, including ►Cdk1, Plk1 and ►Aurora A, that are centrosomal and regulate structural aspects of mitosis. Elevated levels of Nek2 have been detected in a number of human cancer types and cancer-derived cell lines raising the possibility that deregulated Nek2 may contribute to aspects of the disease such as ►aneuploidy and ►chromosomal instability.

By sequence, Nek2 shares similarity with the NIMA protein of the filamentous fungus *Aspergillus nidulans*.

NIMA is an essential protein for mitotic entry in *Aspergillus*, although homologues are not required for mitotic entry in budding or fission yeast. Proteins from higher eukaryotes that share homology with NIMA are called Neks, for *N*IMA-*r*elated *k*inase*s*. The human genome encodes eleven different Neks, named Nek1 to Nek11, and while studies on Neks from lower eukaryotes generally support a function in cell cycle regulation, the individual roles and relevance to cancer of the different human Neks remain to be determined.

Nek2 is subject to alternative splicing with the predominant splice variant in adult cells, Nek2A, being 445 amino acids in length (~48 kDa). Nek2B lacks the extreme C-terminus and has only 389 amino acids (~44 kDa), while Nek2C is identical to Nek2A except lacking 8 amino acids in the C-terminal region. The catalytic kinase domain is located at the N-terminal end of the protein with a number of important regulatory motifs, including dimerization and destruction signals, present in the C-terminus. The extreme C-terminal dipeptide of Nek2A, a methionine-arginine pair, is an unusual destruction motif, allowing direct interaction with the E3 ►ubiquitin ligase, the anaphase-promoting complex. The C-terminus of Nek2A also contains a binding site for the catalytic subunit of protein phosphatase 1 (PP1), which acts as a physiological inhibitor of Nek2. Interestingly, in frogs, Nek2B, which is more stable than Nek2A and does not bind PP1, is the only variant expressed during early embryogenesis implying a developmental stage-specific role for this splice variant.

The localization of Nek2 at the centrosome, the primary site of microtubule nucleation in animal cells, is consistent with a role in bipolar spindle formation. ►Centrosomes are organelles that form the poles of the microtubule-based mitotic spindle and therefore play a critical role in ensuring even chromosome segregation. During interphase of the cell cycle, the centrosome undergoes a duplication event in preparation for mitosis. However, prior to mitosis, the centrosomes are maintained in close proximity to each other due to the presence of a physical tether that links the duplicated centrosomes. It has been proposed that Nek2 facilitates the separation of the duplicated centrosomes to the opposite ends of the spindle at the ►G2/M transition by triggering the dissolution of this tether. It does this by phosphorylating centrosomal linker proteins, such as the large coiled-coil domain proteins C-Nap1 and rootletin, displacing them from the centrosome. However, Nek2 is also localized in the cytoplasm and nucleus, and possibly at the kinetochores and has been reported to phosphorylate chromatin-associated, e.g. HMGA2 and ►spindle assembly checkpoint, e.g. HEC1, proteins. Phosphorylation of HMGA2 promotes chromatin condensation, at least in mouse meiotic spermatocytes. Hence, it is plausible that Nek2 contributes to multiple aspects of mitotic and meiotic cell division.

How increased amounts of Nek2 interfere with ►mitosis is an open question. In cultured cell models, Nek2 overexpression leads to premature centrosome separation during interphase of the cell cycle, as well as to centrosome fragmentation. These consequences might be detrimental for cells that subsequently enter mitosis and attempt to establish a bipolar spindle. Indeed, Nek2 overexpression can cause mitotic failure with accumulation of multinucleated cells that have failed to divide properly. Moreover, as tumour cells frequently possess multiple centrosomes, elevated Nek2 activity may encourage the extra centrosomes to separate forming multipolar spindles, rather than allowing them to cluster and assemble a bipolar spindle. Expression of kinase-inactive mutants also cause mitotic defects, however, such mutations have not been detected in tumours. The underlying basis for the abnormal expression of Nek2 in tumours and cancer-derived cell lines is also unclear. The Nek2 gene locus, 1q32, undergoes ►amplification in ►breast cancer and ►gastric cancer which may explain some overexpression, but the Nek2 gene is also subject to transcriptional repression in normal cells which may be lost in tumours.

Further research is required to determine how abnormal Nek2 expression contributes to the cancer phenotype and whether targeting Nek2 with small molecule inhibitors will be useful in treatment of particular cancers.

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Nemosis

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Definition

Nemosis (from Nemesis, Goddess in Greek mythology who brought justified revenge or compensation) is a newly discovered type of ►fibroblast activation which

is thought to operate in ►inflammation and cancer. Experimentally, nemosis is induced when normal fibroblasts are not allowed to adhere and spread on a solid substratum but are instead forming spheroids and making cell–cell contacts. This may be achieved by placing fibroblasts e.g., on top of agarose-coated culture wells or growing them in hanging drops.

Characteristics

Nemosis is characterized by a massive induction of ►cyclooxygenase-2 (COX-2 producing prostanoids), growth factors and especially ►hepatocyte growth factor/scatter factor (HGF/SF), proteolytic enzymes (►plasminogen activation, ►matrix metalloproteinases MMP-1, MMP-10, and MT1-MMP), and proinflammatory chemokines (e.g., MIP-1α, RANTES, and IL-8) promoting leukocyte migration. In cell culture conditions the fibroblast ►spheroids terminate in programmed ►necrosis with typical ultrastructural changes in electron microscopy but no induction of ►apoptosis-related markers. Under the same conditions malignant cells grow to enlarging spheroids, used as a model of primary avascular tumors without any early signs of necrosis.

The massive amounts of ►HGF/►SF produced by the fibroblast spheroids (>200-fold in monolayer cultures of fibroblasts) promote human carcinoma cell spreading and invasion to collagen provided a functional, properly processed c-Met receptor is present on the tumor cells which was then rapidly phosphorylated. Interestingly, not only function-blocking antibodies to HGF/SF inhibited the tumor cell invasion but also ►nonsteroidal anti-inflammatory drugs (COX-2 inhibitors) which have been shown to be protective against a variety of cancers in vivo. Nemosis is initiated by fibronectin–integrin interaction which leads to over 100-fold induction of 30 genes and 10–100-fold upregulation of over 300 genes.

Possible Significance of Nemosis

While activation of fibroblasts in spheroids thus far only represents primarily an in vitro model, it is of interest that fibroblasts activated to myofibroblasts form direct cell–cell contacts in wound healing and that such myofibroblasts are found in association with cancer cells (cancer-associated fibroblasts, CAFs). CAFs are considered a prerequisite for effective tumor progression. Similarly, in chronic ►inflammation, which is often associated with tumor ►progression, sustained fibroblast activation seems to play a crucial role. The results on nemosis present a novel role for ►fibronectin as the mediator of fibroblast clustering and as the initiator of a massive induction of inflammatory genes and growth factors suggesting that this kind of fibroblast activation may be involved in pathological conditions and specifically in chronic inflammation and cancer.

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Neoadjuvant

Definition

Treatment given before the main treatment, e.g. radiotherapy before surgery.

►Ionizing Radiation Therapy
►Neoadjuvant Therapy
►Adjuvant Therapy

Neoadjuvant Chemotherapy

►Neoadjuvant Therapy
►Induction Chemotherapy

Neoadjuvant Therapy

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Synonyms

Primary chemotherapy; Primary systemic therapy; Preoperative chemotherapy; systemic therapy; Protoadjuvant therapy

Definition

Neoadjuvant therapy is an auxiliary therapy (e.g. androgen ►ablation in ►prostate cancer) administered prior to another therapy (e.g. surgery or radiation). It is given prior to surgery in an attempt to decrease the size of the tumor. It is performed in an attempt to make

the tumor easier to remove and to increase the chances that removal of the tumor is curative.

Characteristics

In most solid tumors, surgery remains the initial primary modality of treatment. With the increasing effectiveness of systemic therapies (►chemotherapy and ►hormonal therapy) in advanced disease stages and also in the post-operative "adjuvant" setting, studies were instituted in many solid tumor types using systemic therapies prior to surgery. Advantages of this approach may include:

- Downstaging of the tumor allowing for less extensive surgery to be performed
- Ability to quantitatively measure in vivo sensitivity to the systemic agent that would not be possible in the post operative "adjuvant" model
- The ability to measure pre- and post-systemic therapy biologicmarkers (e.g. by fine needle aspirates or core biopsies) providing valuable insights into the mechanisms behind systemic therapeutic effects
- Earlier initiation of systemic therapy to eradicate systemic metastases.

If neoadjuvant chemotherapy could clearly be shown to produce an overall survival benefit compared with postoperative systemic therapy, it could produce a major paradigm shift in multidisciplinary cancer care. Thus, if systemic therapy, used primarily, increased cure rates and/or produced convincing and consistent increases in overall survival compared with post-operative use, then local therapeutic modalities such as surgery and/or radiation therapy would become "adjuvants" to primary systemic therapy.

Primary (neoadjuvant) systemic therapy is being studied in a number of tumor types, and its use in breast cancer serves as an example. Neoadjuvant chemotherapy has been used effectively in women with locally advanced ►breast cancer, frequently converting cases where mastectomy was the only realistic option to a situation where breast conservation surgery could be accomplished. In one study series, women with tumors greater than 5 cm in size were able to undergo breast conservation surgery instead of mastectomy 62% of the time. However, most published series indicate a breast preservation rate of about 30% for such large tumors. Despite the ability to induce significant antitumor responses in 75–80% of patients, in general three quarters of women continue to have viable tumor within the breast. In addition, most series have not yielded a survival advantage compared with the use of the conventional therapeutic sequence of surgery, chemotherapy and then possibly radiation therapy. If (and when) more effective systemic therapies emerge, more widespread use of primary systemic therapy is likely to become a reality.

Neoangiogenesis

Definition

This is the formation of new capillaries and blood vessels by growth and differentiation from existing ones. It occurs mainly during embryonic development, but also at wound sites, and is an important feature of tumor growth.

►Angiogenesis

Neobladder

Definition

Construction of new bladder after ►cystectomy using patient's own intestine to ensure voiding from the urethra.

►Urothelial Carcinoma, Clinical Oncology

Neocarcinostatin

Definition

Member of a group of naturally occurring ►enediyne antibiotics, including esperamicin, neocarcinostatin, kedarcidin and dynemicin constituting a unique class of reactive compounds that can undergo aromatization to produce biradicals.

Neocentromere

Definition

A functional ►centromere, which lacks alpha-satellite sequences usually present in a human centromere.

►Adipose Tumors

Neohormone

Definition
Is a new term used to describe hormones that have evolved recently to address specifically mammalian traits, such as viviparity (live birth), lactation, a scrotal testis, or post-reproductive survival.

►Relaxin

Neoplasia

Definition
Is an abnormal cell growth in a tissue or organ results in the formation of a neoplasm. Synonym for cancer or tumor. Neoplasms can be benign or malignant lesions.

Neoplasms with Perivascular Epithelioid Cell Differentiation

Definition
PEComas; A family of tumors composed of perivascular epithelioid cells, including angiomyolipoma, clear cell "sugar" tumor of the lung, lymphangioleiomyomatosis, clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres and clear cell tumors of the pancreas, rectum, abdominal serosa, uterus, vulva, thigh and heart. The cells express melanocytic markers.

►Uncertain or Unknown Histogenesis Tumors

Neoplastic Cell Transformation

Definition
The process by which carcinogenic chemicals, oncogenic viruses, or radiations change the genotype and phenotype of the cell, such that the cells are able to form progressively growing tumors.

►Chemically Induced Cell Transformation

Neoplastic Development

Definition
Term used as equivalent to formation of cancerous tissue or any kind of tumor formation in which unchecked cell proliferation of the tissue affected is involved.

Neoplastic Meningitis

►Leptomeningeal Dissemination

Neovascularization

Definition
The generic name for the process of new vessel formation, which encompasses ►angiogenesis (from pre-existing vessels) and ►vasculogenesis (from endothelial precursor cells). Vasculature in cancer is newly formed to support tumor cells to grow.

►Antiangiogenesis
►Drug Delivery Systems for Cancer Treatment
►Neoangiogenesis

Nephroblastoma

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Synonym
Wilms tumor

Definition
Nephroblastoma is the most common form of renal cancer in children, representing 95% of total renal cancers. Others include clear cell sarcoma of the kidney, rhabdoid tumor of the kidney, congenital mesoblastic nephroma, and renal cell carcinoma. Nephroblastoma is synonymously called Wilms tumor after Dr. Max Wilms'

detailed description in 1899. Due to its morphological resemblance, nephroblastoma is believed to arise from metanephrogenic blastema that comprises primitive renal tissue in the early stage of embryonic development. Nephroblastoma represents ~6% of total malignant tumors among children younger than 15 years of age. The annual incidence is 8 per million children younger than 15 years of age and is slightly higher in black populations, while it is lower in children of Asian origin. Nephroblastoma occurs most commonly among children younger than 5 years of age, and 75% of the tumors are diagnosed in infants younger than 3 years of age. Both kidneys are affected in 7% of patients and familial cases are seen in less than 2% of patients. Although the majority of tumors occur in children with no other abnormalities, 10% of patients with nephroblastoma have malformation syndrome or congenital anomalies such as ►aniridia, ►hemihypertrophy, undescended testis, and ►hypospadias.

Characteristics

Genetics

Although the causes of most sporadic nephroblastomas still remain unknown, genetic abnormalities are thought to be responsible for the development of certain nephroblastomas (Table 1). The association of nephroblastoma, aniridia, genitourinary malformation, and mental retardation is called WAGR syndrome. The syndrome is associated with germline deletions at a certain region of the short arm of chromosome 11 (11p13), where the first nephroblastoma gene, *WT1*, and the *PAX6* gene that causes aniridia are located. *WT1* is a ►tumor suppressor gene and its coding WT1 protein functions as a transcriptional factor to regulate the expression of other genes involved in cell proliferation, differentiation, and cell death. *WT1* plays a role in normal genitourinary development and is important for the differentiation of renal blastema. Germline point mutations of the gene were shown in patients with ►Denys-Drash syndrome that is characterized by pseudohermaphroditism, degenerative renal disease, and nephroblastoma.

There is an association between nephroblastoma and congenital overgrowth syndromes such as ►Beckwith-Wiedemann syndrome and ►Perlman syndrome. Beckwith-Wiedemann syndrome predisposes to several embryonal neoplasms including nephroblastoma, hepatoblastoma, rhabdomyosarcoma, and adrenal cortical tumor. In Beckwith-Wiedemann syndrome, chromosomal abnormalities are frequently observed at a region of the short arm of chromosome 11 (11p15.5), where the presence of the second nephroblastoma gene, *WT2*, is assumed. One of the most probable candidates of *WT2* is the insulin-like growth factor II gene, *IGF2*, which encodes an embryonal growth factor. Because overexpression of the *IGF2* gene is sometimes observed in nephroblastomas, upregulation of the *IGF2* gene is likely to cause overgrowth in patients and tumor development. In humans, the maternal allele of the *IGF2* gene is imprinted and only the paternal allele is expressed, and loss of ►imprinting and paternal disomy is thought to be a mechanism of *IGF2* overexpression. However, whether Beckwith-Wiedemann syndrome and nephroblastoma result from alterations (mutations or deregulations) of the same gene or whether a single or more genes are involved in their pathogenesis are unknown. In association with the familial occurrence of nephroblastomas, there is some evidence showing that responsible genes are located on the long arm of chromosomes 17 (17q) and 19 (19q).

Clinical Aspects

Children with a predisposition to develop nephroblastoma should be screened until the age when nephroblastomas are unlikely to develop. Usually, nephroblastomas are solitary, spherical masses sharply demarcated from the adjacent renal tissue. Tumor extension into the renal vein is sometimes seen and it may extend up to the vena cava or the right atrium. Histopathologically, there are three different cell types: blastemal, stromal, and epithelial cells. Anaplasia is defined as the presence of cells with markedly enlarged hyperchromatic nuclei exhibiting multiple mitotic figures, which may be found focally or diffusely in

N

Nephroblastoma. Table 1 Congenital anomalies (abnormalities) and responsible genes for nephroblastoma

Congenital anomalies (abnormalities)		Responsible genes for nephroblastoma	Gene locus	Genetic function
Overgrowth (−)	WAGR syndrome	*WT1*	11p13	Genitourinary development
	Denys-Drash syndrome			
	Intralobar nephrogenic rests			
Overgrowth (+)	Beckwith-Wiedemann syndrome	*WT2* (*IGF2*, *H19*)?	11p15.5	
	Hemihypertrophy			
	Perlman syndrome			
	Perilobar nephrogenic rests			

the tumor. It is present in ~10% of tumors and is more frequent in children older than 5 years of age. Anaplasia is thought to represent a resistance to chemotherapy. When it is present, patients generally have a poor prognosis and tumors are histologically termed "unfavorable." On the other hand, nephroblastomas with no anaplasia are histologically "favorable" and patients have a good prognosis.

Nephrogenic rests, abnormally persistent embryonal kidney precursor cells arranged in clusters, are frequently seen in normally-appearing renal tissues in patients with nephroblastoma. They may be found even in children without neoplastic renal disease. The presence of multiple nephrogenic rests is designated as nephroblastomatosis. Nephrogenic rests are composed of small clusters of blastemal cells, tubules, or stromal cells, and are classified into intralobar and perilobar ones according to their location within the kidney. Intralobar nephrogenic rests are associated with WAGR and Denys-Drash syndrome, while perilobar nephrogenic rests are found in the kidneys of patients with hemihypertrophy and Beckwith-Wiedemann syndrome. Although the majority of nephrogenic rests regress spontaneously, it is considered that most nephroblastomas arise from these nephrogenic rests. However, a study regarding ethnic differences in nephroblastoma development suggested that patients in Asian children seem to be unrelated to nephrogenic rests.

Common manifestations of nephroblastoma include an abdominal mass, abdominal pain, macroscopic or microscopic hematuria, fever, and anorexia. Severe acute abdominal pain is sometimes caused by spontaneous or traumatic rupture of the tumor. Abdominal ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) are useful in evaluating the tumor extent and detecting metastases. The most common sites of metastases are the lungs, abdominal lymph nodes, and liver. Small lesions in the contralateral kidney, if present, can be demonstrated by the above examinations.

Staging of the tumor extent is essential to predict each patient's prognosis and to determine his or her treatment. The staging systems of the two major study groups, the National Wilms Tumor Study Group (NWTS) in North America and the International Society of Pediatric Oncology (SIOP) in Europe, are essentially the same and are based on the anatomical extent and surgical respectability of the tumor. The difference is that the former classifies tumors after surgery while the latter classifies those after preoperative chemotherapy and surgery. Stage I is defined as a tumor confined to the kidney and is completely resected. When a tumor extends beyond the kidney but is completely resected, it is classified into Stage II. When there are gross or microscopic residual tumors after surgical resection of the tumor, it is classified into Stage III. Stage IV is defined as a tumor with distant metastases, and Stage V as bilateral nephroblastomas. Tumor stage, along with histology, is an extremely important prognostic factor.

Treatment

Overall survival of total nephroblastoma patients is more than 90% according to recent clinical and epidemiological investigations. This has been achieved by clinical trials which were mainly performed by NWTS and SIOP. Currently, the main objectives of the trials performed in the group studies are focused on achieving higher cure rates, reducing the acute toxicities of treatment, and decreasing the incidence of late adverse effects. Generally, patients are treated according to their risk groups, and the modalities include surgery, radiotherapy, and chemotherapy.

While ►SIOP has been advocating preoperative chemotherapy, surgery is usually the initial treatment for most patients in NWTS. The procedure is radical nephrectomy and lymph node sampling through a transabdominal incision. In NWTS, preoperative therapy is recommended in patients with unresectable tumors, bilateral tumors, or such tumors as those extending deeply into the inferior vena cava. It makes tumor removal easier by reducing tumor burden and decreasing the incidence of surgical complications. Regarding the surgical management of bilateral nephroblastomas, nephrectomy of the more involved side and wedge resection or partial nephrectomy in the contralateral kidney have been performed. However, because renal insufficiency develops in many of these patients, bilateral renal salvage procedures are currently recommended. Initial biopsy of bilateral tumors followed by chemotherapy and delayed resection of the tumors are effective in controlling tumors without deteriorating renal function and adversely affecting survival. The renal salvage procedure in unilateral nephroblastoma has been a matter of controversy. It is sometimes recommended in tumors limited to a small portion of the kidney to preserve the function of the involved kidney. At the same time, however, the procedure may increase the risks for incomplete tumor resection and following local recurrence.

►Actinomycin D was the first agent demonstrated to be effective in the treatment of nephroblastoma. Multiagent chemotherapy including actinomycin D and other agents such as vincristine, adriamycin, and cyclophosphamide is given to patients according to the risk classification based on stage and histology. A two-drug regimen with actinomycin D and ►vincristine is effective against nephroblastomas of early stages (Stages I, II), while a three-drug regimen with actinomycin D, vincristine, and adriamycin is given to patients with tumors of advanced stages (Stages III, IV). In patients with tumors of unfavorable histology, more intensified chemotherapy is usually necessary.

Radiotherapy is another useful modality in the treatment of patients with advanced nephroblastoma due to the high radiosensitivity of the tumor. It is also indicated in patients with tumors of unfavorable histology that are resistant to chemotherapy. Lung or liver metastases can be controlled by irradiation, although some adverse effects such as ►radiation pneumonitis might be caused. In patients with bilateral nephroblastomas, radiotherapy is usually reserved for tumors unresponsive to chemotherapy or residual tumors after surgical resection, since it may cause damage to the remaining renal function.

Recurrences, most of which occur during the first 2 years after diagnosis, are experienced in a definite number of patients with nephroblastoma, particularly in patients with unfavorable histology. The most common sites of recurrences are the lungs, intraabdominal primary site, and the liver. Modern multidisciplinary treatment with salvage regimens has improved the survival of patients with recurrent nephroblastoma of favorable histology. The outcome in those with unfavorable histology is still poor.

Survivors of nephroblastoma are at high risk for second malignant tumors due to cytotoxic chemotherapy and irradiation as well as patients' inherited predisposition to malignant tumors. Renal function should be monitored because many of these survivors are still young and the risk of renal dysfunction continues into adult life for more than several decades.

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NER

Definition

►Nucleotide excision repair is the most versatile DNA repair pathway among the four major DNA repair pathways in mammalian cells. It operates primarily on bulky lesions caused by environmental mutagens, such as ►UV Radiation and ►polycyclic aromatic hydrocarbons.

►Mutagen Sensitivity

Nerve Growth Factor

Definition

NGF; A naturally occurring ►neurotrophin in the body that stimulates the growth and differentiation of the sympathetic and certain sensory nerves. NGF consists of three types of polypeptide chains – alpha, beta and gamma – that interact to form the protein. The NGF beta chain (NGFB) is solely responsible for the nerve growth stimulating activity of NGF. The NGFB gene is in chromosome band 1p22.

Nested PCR

Definition

Highly sensitive and specific two-step PCR using a fraction of a finished PCR as template for a second PCR.

►Leukemia Diagnostics

NET

►Carcinoid Tumors

Neurabin II

Definition

►Spinophilin

Neural Stem Cells

Definition

Cell responsible for the different lineages unique to the nervous system.

►Brain Tumors

Neuro-Cardio-Facial-Cutaneous Syndromes

Definition

Compose the congenital disorders ►Noonan syndrome, ►Costello syndrome, LEOPARD syndrome and cardio-facious-cutaneous syndrome. These syndromes share a constellation of similar phenotypic features (neural, cardial, skeletal and ectodermal defects) and a pathogenesis caused de-regulation of Ras/ERK pathway by germ-line mutations in genes encoding its core elements (►*RAS*, *BRAF*, *MEK1,2*) or important modulators of Ras activation such as *NF*1, *SOS* or *PTPN11/SHP2*. Some of the syndromes, e.g. ►neurofibromatosis, Noonan and Costello syndrome, are associated with several neoplastic diseases.

Neuro-Oncology: Primary CNS Tumors

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Synonyms

Brain tumors; Gliomas

Definition

Primary CNS tumors are those that arise from transformation of one of the cellular elements that collectively give rise to the brain and spinal cord, plus their coverings, the meninges brain tumors, cancer, ►GBM.

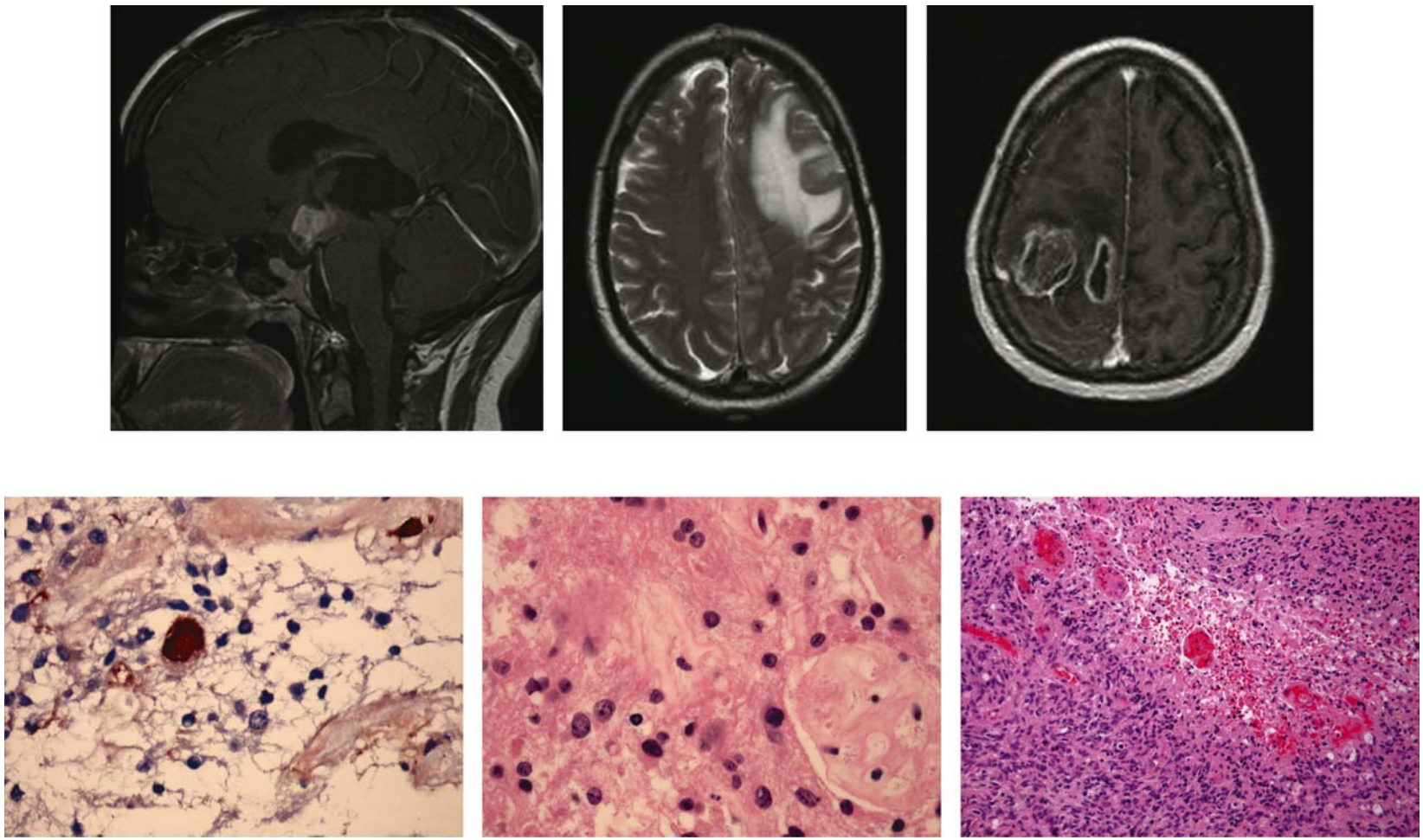
Characteristics

Epidemiology

Primary CNS tumors constitute a relatively small percentage of the total cancer burden in the human population, with an approximate incidence of 13/100,000 population and accounting for ~4% of all cancer related deaths. The incidence of CNS tumors is highest in the fourth to sixth decade of life, although as a percentage of total cancer burden, it is greater in children. Pediatric nervous system tumors however differ significantly in their clinical presentation, location, treatment, prognosis and molecular pathogenesis from those in adults. Our discussions here are limited to adult primary CNS tumors.

Classification

Primary CNS tumors can arise from any of the cellular constituents or their progenitors that collectively form the brain and spinal cord. Clues about the tumor subtype can be obtained from the age of the patient, location (supra- vs. infratentorial; intraxial vs. extraaxial), specialized regions (sella, pineal region, intraventricular), MRI signal characteristics, mode of presentation etc. However, a differential diagnosis usually exists, often requiring surgery for obtaining tissue and definitive classification. The current classification adopted by the WHO (World Health Organization) is based on immunohistopathological characterization of putative cells of origin. However, the recent postulate of the existence of different brain tumor stem cells giving rise to different tumor subtypes, based on differential molecular expression, may require revisiting this classification schema in the future. Tumors arising from neuroepithelial origin are common, with those arising from glial cells, especially the astrocytomas, being the most common primary CNS tumors in adults. Grade1 astrocytomas are present usually in children, associated with glioma pre-disposition syndromes such as Neurofibromatosis1,2 (NF1,2) ►Neurofibromatosis and rarely progress to higher grades. Grade2 or low-grade ►astrocytoma (LGA) is usually found in young adults and usually progresses to higher grades over a variable interval of time. Grade3 or anaplastic astrocytoma (AA) and Grade4 or ►glioblastoma multiforme (GBM) GBM, Glioblastoma Multiforme collectively termed as malignant astrocytomas, are unfortunately the most common and lethal of all primary CNS tumors in adults (Fig. 1). The next common glioma subtype is ►oligodendroglioma (low and malignant grade), followed by ►ependymomas (low and malignant grade) and choroids plexus papillomas. In addition to the glial derived tumors, tumors originating from neurona or neural-glial cells, such as ganglioglioma, ►medulloblastoma,



Neuro-Oncology: Primary CNS Tumors. Figure 1 (*Top*): MRI scans demonstrating various grades of astrocytomas: *Left*: Grade 1: MRI + gadolinium contrast scan of an Optic Nerve Glioma, a pilocytic astrocytoma associated with Neurofibromatosis1 patients which usually remains indolent and does not progress; *Middle*- Grade2: MRI T2 weighted scan of a non-enhancing low-grade astrocytoma (LGA) in a young adult, which usually progresses to higher grade malignant astrocytomas; *Right:* Grade4: MRI + gadolinium contrast scan of a ring enhancing GBM. (*Bottom*): Corresponding H&E sections of various grades of astrocytomas: *Left:* Pilocytic astyrocytoma with Rosenthal fibers; *Middle:* Grade2 LGA with increased cellularity and slight nuclear pleomorphism; *Right:* GBM with regional necrosis surrounded by pseudopalisading tumor cells and increased vascularity.

esthesioneuroblastoma or pineal region tumors also exist, but are rare.

After glioma, ►meningioma (benign, atypical and malignant) arising from the arachnoid cap cell is the most common. These extraaxial tumors are followed by tumors of the anterior pituitary gland (pituitary adenomas) and those arising from cranial nerves, especially the vestibular schwannomas. Hence in summary, there exists a large differential of primary CNS tumors, extending from benign tumors that are potentially amenable to surgical cure to frankly malignant and locally invasive tumors, typified by GBMs that will be the focus of the rest of this essay.

Clinical and Radiological Presentation

Patients with CNS tumors present with essentially a constellation of symptoms which can be broadly classified into those that reflect: (i) Increased intracranial pressure (headaches); (ii) Gradual global deficit in cognition and memory; (iii) Gradual and progressive focal deficit, such as those involving motor power, variety of sensory functions, speech etc.; (iv) Acute neurological dysfunction such as in a seizure or a stroke. The clinical presentation will depend on the location of the tumor and peri-tumoral edema and associated normal neurological function of that region. After excluding a secondary source of a CNS tumor, the incidence of which is increasing, CT or MRI imaging is helpful, but not always pathognomic in giving a definitive diagnosis of tumor subtype. Current imaging modalities include administration of contrast, visualization of which is dependent on breakdown of the blood-brain-barrier, however, this often fails to distinguish between CNS tumor subtypes and grade. The development of biological imaging modalities such as MR Spectroscopy, which is dependent on the metabolic state of the tumor and in the future imaging based on the tumor genetic profile, will be of tremendous benefit. Until these imaging modalities are developed and demonstrated to be robust, current requirement of surgery to obtain tissue for pathological and molecular classification will still be required.

Etiology

The majority of adult primary brain tumors occur sporadically, in an increasing age-dependent manner. Environmental links remain speculative, with radiation being the most tightly linked, usually resulting in tumors of the meninges and/or cranial nerves. A variety of chemicals, viruses and more recently electromagnetic waves from powerlines and cell phones have been implicated; however, confirmatory studies are lacking. Certain patient demographics have also been linked to certain tumor subtypes. The incidence of gliomas is slightly higher in males, while meningiomas (especially those in the spine) are far more common in females, suggestive of hormonal influences. Primary CNS lymphomas are associated with chronic immunosupression (transplant or HIV patients), though the recent increase in these tumors is mainly in the immunocompetent patient, for reasons that remain unclear.

A few CNS tumor pre-disposing germline tumor suppressor gene syndromes do exist, but account for <5% of cases. childhood cancer. Amongst these are the ►Phakomatosis Syndromes, with NF1 being the most common, associated with Grade1 astrocytomas involving but not exclusive to the optic nerve pathway. Next is NF2, associated with multiple cranial nerve schwannomas, but also meningiomas and gliomas (astrocytomas and ependymomas) of the brain and spinal cord. ►Tuberous Sclerosis (TSc) is associated with CNS developmental anomalies (tubers) and hence seizures, plus usually an indolent subtype of astrocytoma termed subependymal giant cell astrocytomas (SEGA). The next is ►von Hippel Lindau disease with multi-system vascular tumors including retinal and CNS ►hemangioblastoma and hemangiopericytomas. Among non-Phakomatosis is the ►Li-Fraumeni Syndrome, where young adult onset low- and high-grade astrocytomas occur as part of the multi-tumor types in these patients. In addition to ocular tumors, ►Retinoblastoma Syndrome patients can have other CNS tumors involving the pineal and olfactory bulb. Last are several syndromes that are rarely associated with tumors in specific regions such as the pituitary gland (►Multiple Endocrine Neoplasia: MEN) or cerebellum (Medulloblastomas associated with ►Turcot Syndrome and ►Gorlin Syndrome).

In addition to the genetically defined cohorts of predisposition syndromes cursorily discussed above, familial clustering of primary brain tumors have been noted, with likely a yet-to-be-identified multi-genetic basis. However, of importance, these syndromes share many of the molecular pathogenetic pathways found in the larger cohort of sporadic CNS tumors, thereby facilitating and focusing research which may be applicable to all patients and not just the minority of those linked with a pre-disposition syndrome.

Molecular Pathogenesis

The multi-genetic aberrations that lead to primary brain tumors are being slowly deciphered, as reviewed in the WHO primer. The pathological subtype of glioma that arise (astrocytoma vs. oligodendroglioma etc.) are a function of the specific molecular alterations and the developmental stage of the glial or glial precursor cell they occur in. Furthermore, some of these molecular alterations act as initiation factors, while others are involved in progression. Experimental proof for the above thesis for example comes from our transgenic glioma models. Mutant EGFRvIII, usually associated with adult malignant astrocytomas, by itself does not

lead to gliomas; however, it enhances tumors in transgenic glioma prone mice. Of interest, embryonic expression of EGFRvIII in these glioma pre-disposed mice leads to oligodendrogliomas, with adult expression in these mice leading to the predicted malignant astrocytoma, demonstrating the importance of the developmental stage of the transformed cell. The hypothesis of the brain tumor cancer stem cell has made us further aware of the importance of the interplay of neuro-glial development and oncogenesis. This hypothesis, extrapolated from the longstanding leukemia literature and still awaiting conclusive verification in solid tumor systems such as gliomas, has implications beyond pathological subtype. Targeting cancer stem cells may play a role in therapeutic sensitivity and/or resistance.

The Cancer Genome initiative, with a part focused on GBMs, has already added to the complexity of the required understanding of the molecular pathogenesis of astrocytomas. Clinical-pathological-molecular studies to date pointto at least two molecular pathogenic pathways to pathologically indistinguishable GBMs. A smaller and slightly younger age group of "Secondary GBMs," progressed from a documented LGA and harbored ►p53 mutations. In contrast, a larger number of older adults presented with a "Primary GBM," which did not harbor p53 mutations, but had alterations in ►EGFR (►amplification and mutation) and mutations plus loss of the tumor suppressor gene PTRN. However, this paradigm has recently been complemented by the high throughput detailed analysis of the Cancer Genome Project, suggesting not one but two relevant molecular pathways of "Secondary GBMs." Efforts such as this will likely lead to several molecular pathogenic pathways, which need to be tested for their oncogenic and therapeutic relevance.

The last overview point about molecular pathogenesis of gliomas is the influence of the tumor microenvironment on the molecular profile through genetic or ►epigenetic mechanisms. As the name "Multiforme" implies in GBMs, these are pathologically heterogennous tumors. Regional variations in the tumor ►microenvironment, notably ►hypoxia, may lead to profound alterations in the regional molecular profile, which is reflected in the heterogenous pathology. Molecular profiling of isolated regional glioma cells attests to this hypothesis of regional molecular heterogeneity. Treatment failure in gliomas is due to mainly local recurrence around the treated region. Hence, understanding the regional molecular heterogeneity will facilitate our ability to target different regions of gliomas more effectively.

Current Management

The majority of gliomas, except for the lower-grade ependymomas and choroid plexus papillomas, for the most part are not curable, with definitive optimal management pending. Some gliomas, such as Grade1 pilocytic gliomas in NF1 patients, are best served by observation. The treatment of LGA in adults is another area of controversy, due to their long and unpredictable time course of progression. GBMs, representing the largest and most lethal cohort of gliomas, are where most of the clinical trials to date have been undertaken, though with very little positive progress. What we have learned is that whenever possible with reasonable morbidity, aggressive surgery should be attempted, followed by external beam radiation of 5,500–6,000 rads. Despite a variety of chemotherapy trials, the median survival curves were not further altered and remained at a dismal 9–12 months for several decades. However, recently this median survival has been extended to 14–16 months with the use of the orally well-tolerated chemotherapeutic agent, Temodar, Temozolomide given concomitantly with radiation therapy. This regime, although far from a cure, is now the backdrop against which all novel therapies will be judged.

Future Directions

Molecular Therapy: Molecular Based Diagnostics-Prognosis-Targeted Therapy

In glioma management, the implications of molecular diagnostics in augmenting classification, predicting prognosis and in several instances tailoring therapy, have already commenced. In classification, several similar or difficult diagnostic entities which were subject to inter-pathological evaluation variability, are now clarified by genetic characterization such as ►FISH or ►PCR based diagnostics. Such examples are the diagnostic difficulties with oligodendrogliomas and mixed oligo-astrocytomas, which are now complemented with evaluation of molecular signatures associated with these tumors. Amongst the two most important independent prognosticators within a grade of astrocytoma such as GBMs, are the patient's age and neurological function at presentation. The availability of certain molecular profiles of the tumor (such as the presence or absence of mutant EGFR, methylation and thereby silencing of DNA repair pathways) are also in many cases independent significant prognostic variables. In the realm of therapeutics, molecular diagnostics has definitely altered the current management of oligodendrogliomas, based on the seminal observation of the role of 1p and to a lesser extent19q loss in dictating a more indolent biology and inherent chemosensitivity. Although the exact genetic basis of this favorable outcome is not yet known, stratification of several other glioma subtypes based on this molecular signature is under study. Less definitive but evolving are therapeutic decisions based on the knowledge of the status of DNA repair mechanisms, such as methylation of MGMT and influence on chemo- and radiation sensitivity.

The importance of this and other potential repair pathways was demonstrated by the initial molecular-translational report accompanying the favorable results of concomitant Temodar and radiation in GBMs. This observation needs further verification before it becomes part of our standard of practice and influences definitely therapeutic choices. Another example where molecular diagnostics may influence glioma therapeutics is the initial observation that small molecule EGFR inhibitors were effective in the small subset of GBMs which harbored amplified and mutant EGFRs, but not loss of PTEN expression, a receptor independent negative regulator of the PI3Kinase pathway. Simplistically, this deduction is logical and would suggest that in the larger set of GBMs, with both EGFR alterations and PTEN loss, multi-targeted therapy directed at EGFR or its downstream signaling pathway mediated by activated p21Ras plus inhibitors targeting the hyperactive ►PI3-Kinase pathway (i.e. ►mTOR inhibitors) may be required. This specific postulate is undergoing verification in larger trials, but does attest to the likely scenario that multi-modal biological therapies will be required to make perhaps small but significant alterations in the prognosis of patients with malignant gliomas.

Adjuvant Therapy: Novel Biologicals and Therapeutic Delivery

The invasive nature of gliomas likely will render focal therapies such as surgery or radiation limited in providing sustained cure. Systemic therapeutic modalities or delivery modalities that are capable of widespread delivery, hold promise though they remain unproven to date. Non-specific systemic chemotherapy was of limited use until Temodar, mainly due to toxicity and resistance of the tumor cells. Biologically targeted systemic therapies, targeting aberrant relevant signaling biological pathways in gliomas such as EGFR, Epidermal Growth Factor Receptor PDGFR, Platelet Derived Growth Factor Receptor VEGF/VEGFR, Vascular Endothelial Growth Factor/Receptor p21Ras and PI3-Kinase, are under current examination. However efficacy if demonstrated will likely be in the context of recurrence after failure from conventional therapy and in combinatorial use with other biological therapies. The immune system has long been a desirable target in gliomas. However, lack of specific glioma antigens, maintenance of an intact blood-brain barrier in the infiltrating and recurrent tumor edge, are only some of the reasons why immune modulation either passively with immune stimulants and vaccinations or passively with expansion and re-infusion of tumor cell lymphocytes, have not shown any clinical efficacy to date.

Delivery of biologicals specifically to the invading tumor cells is another challenge that has been and is currently being pursued. Notable amongst these was the replication incompetent Herpes simplex virus type 1 mediated TK (thymidine kinase) gene transfer trial, which failed mainly due to limitation in spreading beyond the injection sites to the infiltrating glioma cells. In recognition of this limitation, replication competent viruses are being brought to clinical trials in GBMs, however, with cautious optimism. ►Immunotoxin therapy, based on differential expression of antigens in glioma cells such as Transferrin or IL13receptors, conjugated to modified diphtheria or pseudomonas toxins are another promising strategy. Macromolecule strategies such as immunotoxins are theoretically limited due to their inability to penetrate and kill the invading glioma cells in regions where the blood-brain barrier is still intact. To overcome this hurdle, novel delivery modalities such as ►Convection Enhanced Delivery (CED), dependent on bulk flow induced by slow infusion rather than diffusion, holds promise, but again requires careful clinical verification.

Biological Imaging

Among the many differences in the management of primary CNS tumors from neoplasms in many other organs is the eloquence of the brain and its limitation to be sampled repeatedly. In addition, as mentioned, considerable tumor heterogeneity at a pathological and molecular level exists and evolves with a changing tumor microenvironment, especially in malignant gliomas. To overcome this limitation biological imaging beyond current conventional anatomic CT/MRI imaging will be required. MR Spectroscopy, MR based evaluation of tumor cellularity and angiogenesis, and PET scanning for determination of tumor metabolism are already under clinical evaluation, with some promise. Gene-expression and protein activation dependent imaging is under current development and experimental evaluation. These biological imaging modalities will hopefully be invaluable in the diagnosis of subtype and grade of the primary brain tumor and how the biology alters with tumor growth, thereby providing rational targeted therapies without risks of repeated biopsy.

Conclusions

Primary CNS tumors, especially malignant gliomas, have been synonymous with a terminal human cancer. Although this remains factual in many instances, there is much activity toward the understanding of the molecular biology of these tumors. The Cancer Genome initiative involving gliomas is but one important example of this heightened interest. Molecular diagnostics, imaging and therapy have started to make small positive alterations in the management and prognosis of CNS tumor patients and holds much future promise. Instead of being relegated as a lost cause, CNS tumors are being actively studied by the scientific community and are of high interest to biopharmaceutical companies towards development of novel therapeutic strategies.

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Neuroblastoma

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Definition

Neuroblastomas are childhood embryonal tumors of migrating neuroectodermal cells, derived from the neural crest and destined for the adrenal medulla and sympathetic nervous system.

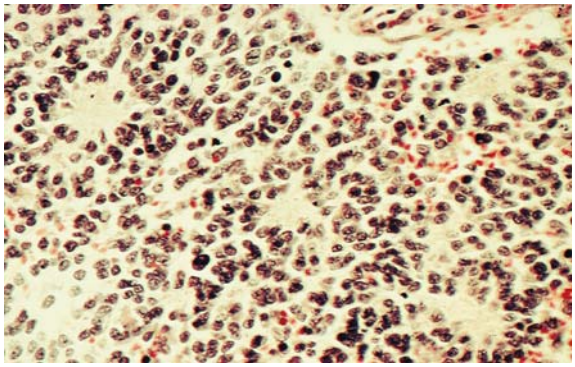
The term neuroblastoma is commonly used for all types of neuroblastic tumors. The International Neuroblastoma Pathology Committee distinguishes between four types of neuroblastic tumors:

- Neuroblastoma (Schwannian stroma-poor)
- ►Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)
- ►Ganglioneuroma (Schwannian stroma-dominant)
- Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant, and stroma-poor

Characteristics

Neuroblastic tumors are the most common solid extracranial malignant tumors during the first 2 years of life. In the United States of America there are seven new cases per million population per year are detected in black children and 9.6 per million in white children. More than 90% of cases occur in the first decade of life. Neuroblastoma has been detected in the fetus in utero by prenatal ultrasound examination. There is no sex predilection.

The primary sites include the adrenals (40% of neuroblastic tumors), followed by the abdominal (25%), thoracic (15%), cervical (5%), and pelvic



Neuroblastoma. Figure 1 Schwannian stroma-poor. A tumor composed of neuroblastic cells forming groups or nests.

sympathetic ganglia (5%). These structures are derivatives of migrating neuroectodermal cells originating from the neural crest. It has been suggested that in ganglioneuroblastoma (Schwannian stroma-rich) the diploid Schwann cells are reactive, in contrast to triploid ganglion cells. All ganglioneuromas (Schwannian stroma-dominant), the fully mature form of neuroblastic tumors, were once neuroblastomas (Schwannian stroma-poor) (Fig. 1).

Neuroblastic tumors have favorable prognosis in infants less than 1 year of age. Adrenal neuroblastic tumors have a worse prognosis than extraadrenal tumors, particularly thoracic tumors. Children with stage 1 and 2 neuroblastic tumors survive longer than those with stage 3 and 4 tumors. Spontaneous regression is most apparent in patients with stage 4S tumors, i.e., infants with a local stage 1 neuroblastoma or unknown primary with involvement of the liver, skin, and/or bone marrow with <10% tumor cells. However, even within stage 4S is a subset of tumors with poor prognosis, which is characterized by unfavorable histology or amplified ►*MYCN*. Regression also occurs in a subset of patients with other stages of neuroblastoma, but predominantly in infants. It is not clear whether spontaneous maturation or an immunological phenomenon is related to regression.

Prognostic evaluation is based on an age-linked histopathologic classification distinguishing two prognostic groups; the favorable and the unfavorable histology group (►Shimada system).

Tumors in the favorable histology group include:

- Age up to 1.5 years: neuroblastoma poorly differentiated subtype with low (<2% or <100/5,000 cells) or intermediate (2–4% or 100–200/5,000 cells) mitosis-►karyorrhexis index (►MKI)
- Age between 1.5 and 5 years: neuroblastoma, differentiating subtype with low ►MKI
- Ganglioneuroblastoma, intermixed, usually seen in older children

- Ganglioneuroma, maturing and mature subtypes, usually seen in older children

Tumors in the unfavorable group include:

- Any age: neuroblastoma, undifferentiated subtype
- Age between 1.5 and 5 years: neuroblastoma, poorly differentiated subtype
- Any age: neuroblastoma with high (>4% or >299/5,000 cells) MKI
- Age between 1.5 and 5 years: neuroblastoma with intermediate MKI
- Age 5 years or older: all neuroblastoma subtypes
- Ganglioneuroblastoma, nodular

The etiology of neuroblastoma is unknown, it appears unlikely that environmental exposure plays a major role. However, cases of neuroblastoma have been described that were associated with the fetal hydantoin, phenobarbitol or alcohol syndromes, suggesting that prenatal exposure to these substances may increase the risk of neuroblastoma. Other studies have been suggesting a weak association between neuroblastoma and paternal occupational exposure to electromagnetic fields, or maternal use of hair coloring products, but none of these associations has been confirmed. Moreover, no prenatal or postnatal exposure to drugs, chemicals, or radiation has been strongly or consistently associated with an increased incidence of neuroblastoma.

Although most neuroblastomas appear to be sporadic, a number of reports have been describing familial neuroblastoma, as well as bilateral or multifocal disease, that are consistent with hereditary predisposition.

Constitutional chromosomal abnormalities have been described in lymphocytes of patients with neuroblastoma, even though there is no apparent pattern. An interstitial ►deletion and a reciprocal ►translocation t(1;17), both affecting 1p36, were observed in patients with neuroblastoma. However, it is unclear if these 1p36 rearrangements were contributing to a neuroblastoma predisposition. Somatic genetic changes associated with neuroblastoma progression include ►amplification of ►MYCN and ►allelic loss at 1p, 11q, 14q, and gain of 17q. ►Amplification is a highly informative ►biomarker for clinical management of neuroblastoma. Different signaling pathways are under clinical investigation in clinical studies.

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Neurocytoma

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Synonyms

Central neurocytoma; Typical neurocytoma; Well-differentiated neurocytoma; Atypical neurocytoma

Definition

The term "neurocytoma" was established by Hassoun et al. in 1982 in order to distinguish these lesions, which occur mainly in adults, from ►neuroblastoma, which occurs mainly in children. Neurocytomas are rare tumors of the central nervous system, mostly located in the ventricular system, which are usually considered benign. However, in 1989, a more aggressive variant of this tumor called "atypical central neurocytoma" was reported.

Characteristics

Central neurocytomas account for 0.25% to 0.5% of all central nervous system tumors. Less than 600 cases have been reported in the literature. Because neurocytomas are very rare, no prospective studies are available. The ratio of male to female is approximately 1.25:1. In the majority of patients, the age at diagnosis is between 20 and 35 years. Most lesions are located in the ventricular system. Spinal tumors are extremely rare.

Neurocytomas can be divided in two major subgroups, typical (well-differentiated) neurocytomas and atypical neurocytomas. About 75% of the neurocytomas are typical lesions, which represent a less aggressive variant considered benign. Typical neurocytomas are characterized by a ►MIB-1 labeling index ≤3% and absence of atypical histologic features such as focal ►necrosis, increased mitotic activity, and vascular

proliferation. Intracerebral hemorrhage and transformation to a more aggressive malignant lesion (atypical neurocytoma) are considered serious complications. Atypical neurocytomas are characterized by a MIB-1 labeling index >3% and presence of atypical histologic features. Children with a neurocytoma appear to have a marginally better prognosis than adults.

Diagnosis

The diagnosis of a neurocytoma is based on histopathological findings and imaging.

Macroscopically, a neurocytoma appears as a gray, partly calcified mass. On light microscopy, the lesion is composed of small round cells with intercellular fibrillar zones and rosette-like structures. The nucleus is round or oval with a finely specked chromatin and an occasionally prominent nucleolus. Clear cells are common and result in a honeycomb appearance. Mitoses and necrosis are extremely rare. High mitotic activity and necrosis must be considered as indicators for a more aggressive behavior (atypical neurocytoma). Calcifications and well developed vascularization are common.

On electron microscopy, tumor cells have regular round nuclei with finely dispersed chromatin and sometimes a neat small nucleolus. The cytoplasm contains parallel ergastoplasmic channels, a prominent Golgi apparatus, and lysosome-like inclusions. Numerous thin and intermingled cell processes were found which contain mitochondria and microtubules.

Neurocytomas may show immunoreactivity for a variety of neuronal marker proteins such as neuron-specific enolase (NSE), synaptophysin, synapsin, neurofilament protein, neuron-associated class III beta-tubulin, microtubule-associated proteins MAP2 and tau, neuron-specific antigene L1, S-100 protein, or Leu-7. An occasional staining for glial fibrillary acidic protein (GFAP) demonstrates a potential for astrocytic differentiation. However, most of these markers are non-specific. The most reliable one is synaptophysin.

On computed tomography, a neurocytoma appears as a well circumscribed round or poly-lobed hypodense mass which may show homogeneous enhancement with contrast medium. The lesion may contain cystic areas or calcifications. Magnetic resonance imaging shows an isointense or slightly hyperintense signal in comparison to the cortex both on T1-weighed and on T2-weighed images. Administration of ►Gadolinium-DTPA usually leads to a slight homogeneous enhancement. The enhancement may be inhomogeneous if cysts or calcifications are present.

Therapy

Gross tumor resection (GTR) is associated with significantly better survival and local control than subtotal tumor resection (STR). Therefore, GTR should be performed whenever safely possible. After STR, the treatment outcome may be improved with post-operative radiotherapy. However, the role of radiotherapy and the recommended total dose vary between the different neurocytoma types (typical, atypical, neurocytoma in children). Both conventional ►radiotherapy and ►stereotactic radiosurgery may improve the outcome after STR. However, there is only little data available regarding the role of stereotactic radiosurgery in the treatment of neurocytomas.

Typical lesions were associated with better outcomes than atypical lesions. In a retrospective series of 351 patients with a typical neurocytoma, the 10-year survival rates were 100% after GTR alone ($N = 137$), 100% after GTR followed by conventional radiotherapy ($N = 29$), 90% after STR alone ($N = 90$), and 97% after STR followed by conventional radiotherapy ($N = 97$). The 10-year local control rates were 76%, 100%, 43%, and 82%, respectively. The outcome was not significantly improved by radiotherapy following GTR in terms of survival ($P = 1.0$) and local control ($P = 0.08$). After STR, radiotherapy resulted in significantly better survival ($P = 0.03$) and local control ($P<0.001$). Thus, if only STR can be performed, post-operative radiotherapy can be recommended. A radiation dose of 50 Gray (Gy) given in 2-Gy-fractions appears sufficient.

Atypical neurocytomas are more aggressive than typical lesions. In a retrospective series of 87 patients, the 5-year survival rates were 91% after GTR alone ($N = 15$), 93% after GTR plus conventional radiotherapy ($N = 14$), 46% after STR alone $N = 19$), and 69% after STR plus conventional radiotherapy ($N = 39$). The 5-year local control rates were 59%, 63%, 5%, and 65%, respectively. After GTR, the outcome was not significantly improved with post-operative radiotherapy in terms of survival ($P = 0.87$) and local control ($P = 0.30$). After STR, radiotherapy resulted in both better survival ($P = 0.05$) and better local control $P<0.001$). Thus, post-operative radiotherapy and can be recommended after STR. In contrast to patients with typical lesions, those with atypical lesions appeared to benefit from higher radiation doses. A dose of >54 Gy was associated with better local control ($P = 0.05$) than a dose of ≤54 Gy. Thus, the radiation dose after STR of atypical neurocytomas should be 56–60 Gy given in 2-Gy-fractions.

Of a retrospective series of 438 patients, 73 patients (17%) were children (age ≤18 years). The median age was 16 years (range 1–18 years). Sixty-two children had typical and 11 children atypical lesions. The prognosis for children appears quite good. In that series, the 5-year survival rates were 100% after GTR alone ($N = 25$), 100% after GTR plus conventional radiotherapy ($N = 12$), 82% after STR alone ($N = 19$), and 100% after STR plus conventional radiotherapy ($N = 17$). The 5-year local control rates were 89%, 100%, 52%, and 94%, respectively. After GTR, the

outcome was not significantly improved by radiotherapy in terms of survival (P = 1.0) and local control (P = 0.30). After STR, radiotherapy significantly improved local control (P = 0.002), but not survival (P = 0.16). The difference in survival of 18% appears reasonably large. The fact that significance was not achieved may be explained by the small sample size. A dose of 50 Gy given in 2-Gy-fractions appears sufficient to achieve long-term local control for both typical and atypical neurocytomas in children. However, caution should be used in the interpretation of these data because of the few children in the radiotherapy groups.

Although local control can be improved by post-operative radiotherapy, one should be more reserved recommending radiotherapy for children. ►Psychomotor retardation was reported in one child treated with STR followed by 40 Gy. The rate of children with psychomotor retardation may be underestimated, because neuropsychologic testing was not always performed. Until now, ►secondary tumors of the brain have not been reported after radiotherapy of a neurocytoma. However, according to the literature, the development of a radiation induced malignancy is an important issue in children. Thus, in children with a neurocytoma, it appears reasonable to administer post-operative radiotherapy only in limited situations such as incompletely resected neurocytomas, especially atypical lesions, and recurrent tumors.

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Neuroectodermal Tumor

►Carcinoid Tumors

Neuroendocrine Carcinoma

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Synonyms

APUDOMAS; Carcinoid tumors; Islet cell tumors; Neuroendocrine tumors of the pancreas; Malignant endocrine tumors

Definition

Neuroendocrine carcinomas are tumors derived from the ►diffuse neuroendocrine system.

Characteristics

Neuroendocrine carcinomas will include a heterogeneous group of tumors that can arise wherever the diffuse neuroendocrine system is found throughout the body.

Classification

It is widely accepted to divide these malignancies in two major groups: ►carcinoids tumors and ►neuroendocrine tumors of the pancreas. There are other, rare malignancies that also are classified as neuroendocrine carcinomas, including ►pheochromocytomas, ►medullary thyroid carcinoma, ►merkel cell tumors.

Other subclassifications will consider these tumors according to: site of origin (lung, gastrointestinal tract, etc.), capability or producing certain hormones (functioning and nonfunctioning tumors), degree of cellular differentiation as an indicator of biological aggressiveness (well, moderate, or poorly differentiated tumors), and type of presentation (sporadic tumors or within hereditary cancer syndromes).

Epidemiology

Neuroendocrine carcinomas are rather rare with estimated incidences of 1–2 cases per 100,000 for ►carcinoid tumors and 4 cases per million for neuroendocrine tumors of the pancreas.

The incidence increases with age, with the highest observed in the fifth decade and upwards, except for carcinoid of the appendix that occurs mostly in patients below 30 years of age. The ratio of gender distribution is about one to one for males to females.

Natural History

Neuroendocrine carcinomas usually present with indolent behavior. However, once they have spread to distant organs, the cure rates are low and the 5-year survival rate is around 30–40%.

The diagnosis of neuroendocrine carcinomas is challenging, as they can be asymptomatic for long periods of time. Even if there are symptoms, they usually are vague and nonspecific, thus resulting in difficulties and delays in the diagnostic process.

These tumors may present as a sporadic disease or within an inherited syndrome such as the ►multiple endocrine neoplasia type 1, ►multiple endocrine neoplasia type 2, ►neurofibromatosis type 1 or ►Von Hippel–Lindau disease.

Carcinoids

This term was first introduced by Oberndorfer in 1907 to describe a type of tumor in the gut that was similar to carcinomas (therefore "carcinoid") but with "slower growth and less malignant behavior."

Carcinoid tumors have been traditionally classified into foregut carcinoids (arising from lung, thymus, esophagus, stomach, duodenum, pancreas); midgut carcinoids (arising from jejunum, ileum, appendix, ascending colon); and hindgut carcinoids (arising from transverse and distal colon, rectum). The midgut carcinoids are the most frequent representing ~40–60% of all, while 25 and 15% correspond to foregut and hindgut tumors respectively.

Carcinoid tumors have cells that contain neurosecretory granules capable of synthesizing, storing, and releasing a number of substances such as serotonin, histamine, tachykinins, bradykinins, and ►prostaglandins. Some patients may present with a specific clinical syndrome related to the release of the above-mentioned substances to the systemic circulation, called the ►carcinoid syndrome.

Neuroendocrine Tumors of the Pancreas (Islet Cell Tumors)

The pancreas is a small organ of the digestive system that lies behind the stomach inside a loop formed by part of the small intestine. Its functions include: (i) Collaborating with the digestion of food through production of digestive juices and (ii) Producing hormones (such as insulin) that regulate food storage and use.

The region of the pancreatic gland that produces digestive juices is the exocrine pancreas while the one producing hormones, comprised by the so-called islet cells, is the endocrine pancreas. The neuroendocrine tumors of the pancreas derive from the islet cells and are generally denominated ►islet cell tumors.

When islet cells become cancerous, they may or may not overproduce hormones. These entities are considered as functioning and nonfunctioning endocrine tumors, respectively.

Functioning endocrine tumors constitute ~60% of all islet cell tumors, and are subclassified according to the predominant hormone produced as follows: ►gastrinoma, ►insulinoma, ►VIPoma, ►glucagonoma, and ►somatostatinoma.

Diagnosis of Neuroendocrine Carcinomas

The diagnosis of neuroendocrine carcinomas comprises three different aspects: pathological, biochemical, and imaging diagnosis.

Pathological Diagnosis

Generally, tumor tissues are obtained through a biopsy procedure whereby a small piece of tumor is removed from the body with a needle using sterile techniques. Sometimes, the diagnosis of neuroendocrine carcinomas is made incidentally, when the tissue specimens were removed for other purposes.

The first step in the diagnosis of these tumors is an accurate analysis of their appearance when examined under the microscope by a pathologist (histopathological analysis). Through specific staining techniques (►Immunohistochemistry), typical neuroendocrine markers such as ►chromogranin A, neuron specific enolase, and synaptophysin should be evaluated in the tumor tissue specimens.

Biochemical Diagnosis

Determination of certain substances in the blood of patients being worked-up for a neuroendocrine tumor is a critical component of the diagnostic process.

Chromogranin A. Serum analysis of chromogranin A seems to be the most promising biochemical test with high specificity and sensitivity values. This serum marker correlates with tumor burden and is useful in both functioning and nonfunctioning tumors.

►*5-Hydroxyindoleacetic Acid (5-HIAA)*. Measurement of 24-h urine levels of ►5-HIAA is also accepted as an important diagnostic tool. It is very helpful in midgut carcinoids while it is rarely useful in other neuroendocrine carcinoma varieties that do not secrete serotonin frequently.

Other Markers. Depending on the specific hormone(s) secreted by functioning neuroendocrine tumors, such as gastrin in gastrinoma or insulin in insulinoma, monitoring of these hormone(s) may be considered.

Other useful serum markers proposed are: plasma pancreatic polypeptide, α-HCG, chromogranin B, and secretogranin II. Their use in clinical practice is limited at present.

Imaging Diagnosis

Conventional imaging tests, such as computerized tomography, ultrasound, and magnetic resonance, are useful in the initial work-up of these tumors to demonstrate the size and extent of the primary tumor, especially for those with pancreatic neuroendocrine tumors. These imaging tests can also evaluate for any distant metastases such as liver, chest, or pelvic involvement.

►Somatostatin receptor scintigraphy (SRS) (also known as OctreoScan) is an important imaging

investigation in patients with suspected neuroendocrine carcinomas. ►SRS utilizes a radiolabeled form of the ►somatostatin analogue octreotide (^{111}Indium-Penetreotide), based on the fact that a majority of neuroendocrine carcinomas contain a high concentration of somatostatin receptors. The uptake of radiolabeled octreotide will help, not only in the staging, but also in predicting the clinical response to somatostatin analogues.

Meta-iodobenzyl-guanidine (MIBG) is a radiopharmaceutical agent that concentrates in cells of selected neuroendocrine tumors, though not as often as octreotide. I-131-labeled MIBG scintigraphy is sometimes used in addition to SRS to complete the diagnosis of neuroendocrine tumors.

►Positron emission tomography using ^{11}C-labeled 5-HTP (hydroxytryptophan) has been tested with encouraging results and has proven to be very effective in localizing small tumors.

Echocardiogram. Patients with carcinoid tumors are at risk for developing right-sided valvular heart disease. Hence, baseline and annual echocardiograms should be considered especially in those with abnormal 24-h urine ►5-HIAA levels. Patients with carcinoid heart disease should be followed by a cardiologist.

Treatment of Neuroendocrine Carcinomas

Treatment of neuroendocrine tumors requires a multidisciplinary approach. Surgeons, interventional radiologists, medical and radiation oncologists should coordinate efforts to achieve better therapeutic results.

Surgery

Surgery is the only curative treatment in patients with localized neuroendocrine tumors. Resection of local or locoregional disease can cure some patients.

Neuroendocrine tumors most frequently spread to the liver, but they can also spread to other organs such as lungs, pelvis, bones, etc. In patients with metastatic disease that has spread beyond their primary site and/or nearby lymph nodes, more aggressive treatment strategies have recently emerged, including cytoreductive procedures (partial resection of tumor), radiofrequency ablation (RFA), laser treatment, or ►embolization of liver metastases.

In selected cases, metastatic disease in the liver from neuroendocrine carcinomas can be surgically removed, along with the primary tumor, to provide long-term symptomatic relief and improve survival. Generally, this approach should be taken in patients who are medically fit and have a limited number of liver metastases, and by an experienced surgeon.

Liver transplant may be considered in young patients with no evidence of disease outside of the liver, even though the long-term outcomes of this therapy remain unclear.

Radiofrequency Ablation

RFA is a procedure whereby a special needle electrode is placed in the tumor under the guidance of computerized tomography or ultrasound, and then a radiofrequency current is transmitted through the electrode to heat the tumor tissue near the needle tip to destroy it. This technique is sometimes used in cases of liver metastases arising from neuroendocrine tumors that cannot be surgically removed. It is less invasive than surgical resection but its benefit in this disease has not been well established.

Liver Embolization

Embolization. Embolization (or temporary occlusion) of selective arteries alone (Bland embolization) or combined with intra-arterial chemotherapy (►Chemoembolization) is a procedure indicated to reduce clinical symptoms from liver tumor deposits that are not suitable for resection.

This technique is based on the principle that tumors in the liver derive most of their blood supply from the hepatic artery, while healthy liver tissue receives most of their blood supply from a different blood vessel, the portal vein.

Success rates are measured either by a decrease in hormonal secretion or by radiographic regression and vary from 30 to 70%. However, the duration of the response can be brief, ranging from 4 to 51 months in the different published series with unclear survival benefit.

Chemoembolization. Chemoembolization refers to the embolization of hepatic artery in combination with chemotherapy using agents such as dacarbazine, ►adriamycin, ►5-Fu, etc. Its role and relevance in the management of patients with neuroendocrine carcinomas remain undefined as the data are limited and it is a highly toxic procedure.

Irradiation Therapy

External Beam Radiotherapy. External radiation therapy is not very effective in neuroendocrine carcinomas, and it is only recommended as a ►palliative measure to improve symptoms in some specific cases such as bone, skin, or brain metastases.

Targeted Radiotherapy. Some studies have utilized different radiolabeled compounds as Indium-DTPA-Phe-Octreotide or 90Y-DOTA-Lanreotide, based on the principle that these substances will bind to the somatostatin receptors in the tumor and thus will deliver a high amount of radioactivity locally. Some preliminary results seem encouraging, mostly in tumors with very high uptakes in the SRS.

Systemic Treatment

The medical management of neuroendocrine carcinomas includes somatostatin analogues, alpha ►interferon and cytotoxic drugs.

Somatostatin Analogues. Based on the observation that ►somatostatin inhibits the release of different hormones, some derivatives of this substance (somatostatin analogues) have been developed for the symptomatic treatment of neuroendocrine carcinomas. ►Octreotide (Sandostatin®) is the most extensively used drug in the clinical setting. It is used either as a short acting drug or as a long acting formulation. It produces subjective symptomatic improvement in more than 70% of the patients. It is normally well tolerated with minor side effects such as gastrointestinal symptoms, gallbladder stones, hyperglycemia, and hypocalcaemia. In addition to symptom control, these compounds have shown biochemical responses (decrease in the production of hormones) in 30–70% of patients. Its cytostatic effect (delay on tumor growth) and impact on survival are still controversial. The role of somatostatin analogues in suppressing 5-HIAA levels to protect against heart damage is also unclear.

Interferons. ►Interferon (IFNs) comprises a group of proteins produced and released by white blood cells as part of the immune response to a viral infection or other immune triggers. There are different types of IFNs such as alpha, beta, and gamma. IFN-α has been demonstrated to inhibit proliferation of malignant cells, increase the activity of the immune system, and control hormonal secretion. IFN-α has been used for the treatment of carcinoid tumors either alone or in combination with somatostatin analogues.

Chemotherapy. Standard cytotoxic treatments have shown limited efficacy in the treatment of advanced neuroendocrine carcinomas, and their use for disease palliation remains suboptimal. Regimens using the drugs streptozocin and dacarbazine have been tested with only modest activity and may also be associated with significant toxicity. Other agents such as ►docetaxel, ►paclitaxel, and ►gemcitabine have demonstrated poor efficacy in neuroendocrine carcinomas.

New Agents

The limited efficacy of conventional treatments for metastatic neuroendocrine carcinomas and the emerging knowledge on the molecular biology of these tumors have facilitated the development of new targeted therapies.

Promising results have been reported with ►antiangiogenic agents such as ►bevacizumab (Avastin®), ►sunitinib (Sutent®); and ►mTOR inhibitors such as ►temsirolimus and ►everolimus. Randomized clinical trials with these promising agents are underway, to confirm their efficacy in neuroendocrine carcinomas. Other compounds are in development pending definitive results.

Confirmatory studies about the efficacy and tolerability of these drugs are needed and the future may be sought by combining targeted therapies with each other or with the more classical cytotoxic chemotherapy agents, or alternatively with different approaches such as gene transfers or immunotherapy.

►Carcinoid Tumors
►Neuroendocrine Tumors

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Neuroendocrine Cells

Definition

Cells that release a hormone initiated in some manner by the nervous system.

►Carcinoid Tumors
►Neuroendocrine Tumors

Neuroendocrine Gland

Definition

A gland usually located in the brain that acts at the interface of the nervous and endocrine systems by transforming a neural signal input into a hormonal output.

►Melatonin

Neuroendocrine Tumors

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Definition

Neuroendocrine tumors (NETs) are neoplasms with a broad range of morphologic patterns, grade of differentiation and biological behavior that share common features of neuroendocrine (NE) programming. NETs contain various amounts of molecules involved in the regulated release of neuropeptides, neurotransmitters and ►hormones, and may be associated with hypersecretory syndromes or be non-functioning.

Most NETs originate from the disseminated/diffuse NE system (DES) and are of endodermal (Foregut, midgut and hindgut) derivation. The gastroenteropancreatic (GEP) DES comprises numerous NE cell types producing different hormones and biogenic amines. NETs occur in a variety of topographic locations, with a predilection of the gut, the pancreas and the lung. Other primary organs include the skin, salivary glands, prostate and various sites in the urinary, genital and biliary tracts. A second group of NETs develops in NE glands such as adenohypophysis, parathyroid, adrenal medulla or paraganglia. Examples include catecholamine-releasing ►pheochromocytoma or endocrinologically silent paragangliomas.

Here, only NETs related to the DES are considered.

Characteristics

Diagnostic Criteria for Entering the NET Category

General marker molecules of NE differentiation which reside in different cellular compartments are currently used for the diagnosis of NETs. The cytoplasm of NE cells contains neuron-specific enolase (NSE) and protein gene product 9.5. Members of the chromogranin family, cytochrome b561, ►somatostatin and various hormones are localized in large dense-core vesicles (LDCVs). Synaptophysin is one of the most abundant proteins of synaptic vesicle-like microvesicles (SLMVs) and conserved even in poorly differentiated NETs. The SLMV membranes contain abundant trafficking proteins including SNAREs and Rabs. SNAREs of the ►synaptobrevin/VAMP family are highly expressed in normal and neoplastic NE cells. Together with the SNAREs syntaxin I and SNAP-25 on the plasma membrane they are implicated in the fusion events of regulated exocytosis. The neural cell adhesion molecule NCAM (CD56) is another membrane molecule widely used in the diagnosis of NETs but also expressed in non-NE tumors.

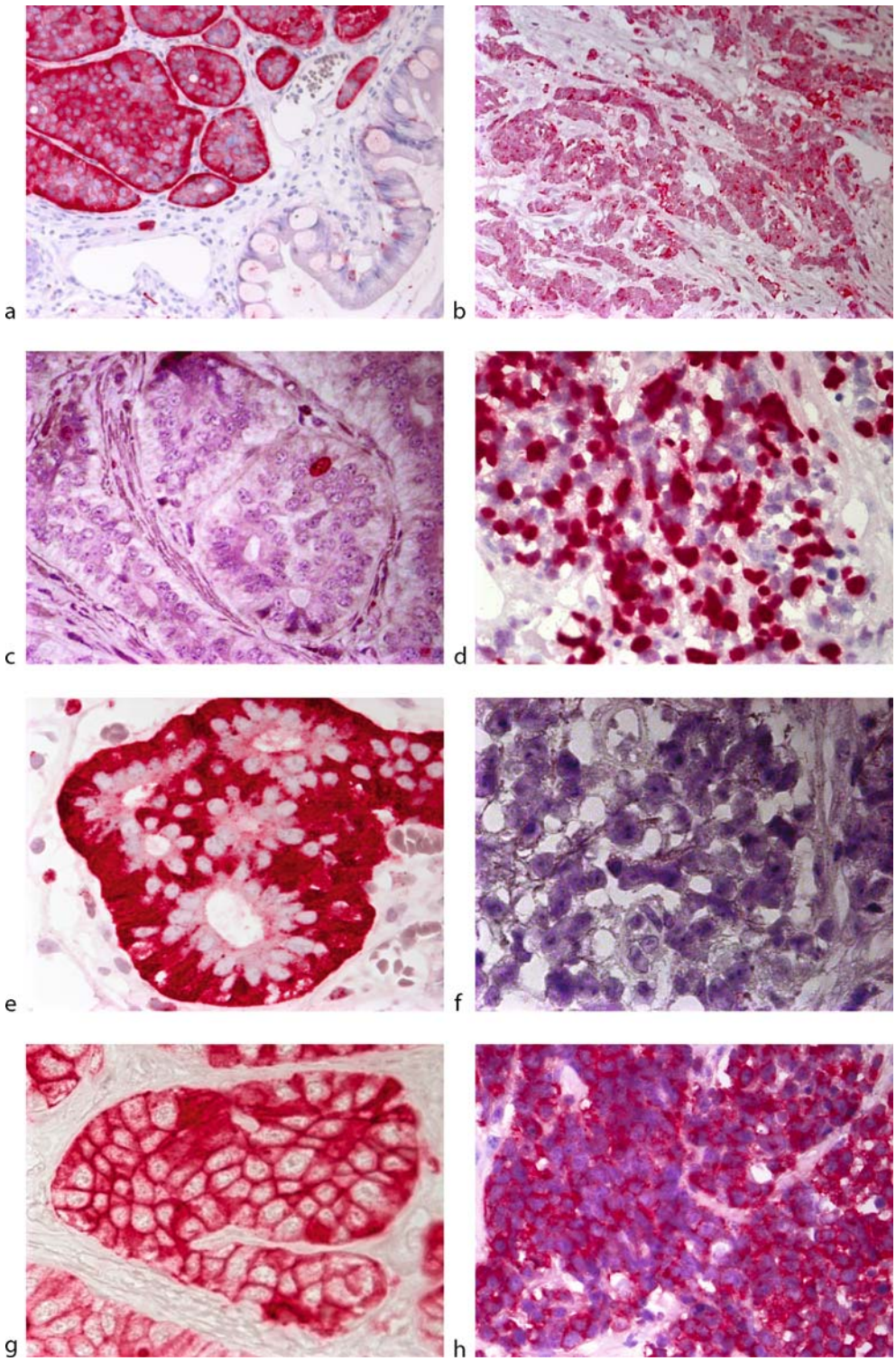
Expression of somatostatin receptors, which belong to the superfamily of G protein-coupled receptors, is found in the majority of NETs. The effective binding of somatostatin-analogues (►octreotide, lantreotide) is of therapeutic relevance. ►Serotonin and its metabolites are considered to cause the carcinoid syndrome. Traditional histochemical techniques based on silver reduction and electron microscopy, showing LDCVs and SLMVs, have now given way to immunolabeling techniques. Immunohistochemically, the positivity for synaptophysin, synaptobrevin1,2/VAMP1,2 and ►chromogranin A is generally the best way to provide evidence of NE differentiation. The synaptophysin, synaptobrevin/VAMP or SNAP-25 immunostains are the most reliable since they are positive even in poorly differentiated NETs, which may be practically devoid of LDCV constituents (Fig. 1). In mixed exocrine-neuroendocrine tumors, the neuroendocrine population should represent >30%. Tumors with exocrine and endocrine features present in identical cells are designated as amphicrine. A minority of NE cells, which may have prognostic implications, may be identified in tumors that do not belong to the NET category.

Prognostic Classification Concepts of NETs

Among various classification systems using different terminologies, the new World Health Organization (WHO) classification reflects the present state of knowledge. The WHO classification applies the term "endocrine" instead of "neuroendocrine" and defines major categories:

- Well-differentiated endocrine tumor, functioning vs. non-functioning;
- Well-differentiated endocrine carcinoma, functioning vs. non-functioning;
- Poorly-differentiated endocrine carcinoma;
- Mixed endocrine-exocrine tumor.

The term well-differentiated endocrine tumor/carcinoma now largely replaces ►carcinoid tumor (CT) or islet cell tumor. Functioning tumors may still be designated according to the hormonal syndromes, such as ►insulinoma or ►gastrinoma. Organoid growth patterns, forming islands, uniform nests, ribbons of tumor cells, are characteristic features of well-differentiated NETs. High-grade cellular atypia, high mitotic index/proliferative activity, focal necroses and an aggressive course are indicative of poorly-differentiated (large cell-form and small cell-form) NETs of category 3. The greatest difficulty concerns the distinction between lesions of benign or uncertain behavior included in category 1 and low-grade endocrine carcinoma (category 2). Features associated with malignancy are local extension, angioinvasion, cellular atypia, increased



Neuroendocrine Tumors. Figure 1 Expression patterns of synaptophysin (a, b), Ki-67 (c, d) chromogranin (e, f) and SNAP-25 (g, h) in well-differentiated (a, c, e, g) and poorly differentiated (b, d, f, h) neuroendocrine tumors (NET) of the small intestine. Synaptophysin and SNAP 25 are detected in both tumor categories while assessment of the Ki-67 index reveals a low (<2%) versus a high (>50%) proliferation. Chromogranin A is absent in the majority of tumor cells in poorly differentiated NET which shows a prevalent cytoplasmic rather than membranous pattern of SNAP 25 (labeled streptavidin–biotin immunostaining).

mitotic index/proliferative activity, ►Ki-67 labeling index and the presence of ►metastases. Tumor size and tissue-appropriate or -inappropriate hormonal products in conjunction with the primary tumor site have to be taken into consideration, since different degrees of aggressiveness may occur within each tumor category. In general, well-differentiated NETs measuring 1 cm or less rarely metastasize, while lesions >2 cm frequently metastasize, but are usually rather indolent with a median survival of ≥2 years. A mitotic count of >2 per 10 high power field (HPF) or a Ki-67 index >2–5% are correlated with a malignant behavior. The majority of poorly-differentiated endocrine carcinomas are metastatic at the time of diagnosis and have a poor prognosis with a medial survival <1 year. A correct clinicopathological evaluation may predict the clinical behavior and is essential for the management of patients.

In addition, a new consensus includes proposals for the TNM ►staging and ►grading of GEP NETs thus providing criteria for a better prognostic assessment and standardization in the management of patients.

Genetics

The understanding of the genetic background of induction and progression of NETs obtained by cytogenetic, CGH and refined molecular analyzes is rapidly increasing but still incomplete. The typical mutations of GEP adenocarcinomas are not identified in NETs. Genomic changes are highly variable according to localization, differentiation, functional activity, size and disease stage of individual tumor types. Losses of ►tumor suppressor gene function by loss of heterozygosity (►LOH) or mutations are probably more important than amplifications of oncogenes. Most NETs are sporadic neoplasms, but subsets occur as a part of heriditary cancer syndromes including ►multiple endocrine neoplasia type 1 (MEN-1), ►von Hippel-Lindau disease (VHL) or ►neurofibromatosis type 1 (NF1). Allelic deletions and mutations at 11q13 have also been documented in about 40% of sporadic GEP NETs and in up to 70% of atypical pulmonary carcinoids indicating that the MEN-1 gene, the tumor suppressor protein menin and perhaps other genes at 11q13 may play a role in their tumorigenesis. Somatic VHL mutations are extremely rare in sporadic NETs. In pancreatic NETs, losses at chromosome 1 and 11q and gains of 9q are early events. Losses at 18q are frequently found in pancreatic gastrimomas and mid-/hindgut NETs. DNA sequence copy number changes include losses of various chromosomal regions at 3pq, 6pq, 10pq, 11pq, Xq and the Y chromosome and gains at 5q, 7pq, 9q, 12q, 17pq, 20q and Xp in different tumors. Furthermore, promoter hypermethylation is a common event in GEP-NET. In the lung, small cell carcinoma (SCLC) often show ►p53 mutations, ►amplification of ►MYC, ►methylation of ►caspase-8 and inactivation of the ►retinoblastoma (RB) gene.

NETs of the Pancreas

Pancreatic NETs which have traditionally been referred to as islet cell tumors are accompanied by hormonal activity in about 50–60%. Distinct clinical syndromes are related to the inappropriate secretion of insulin (hyperinsulinemic hypoglycemia), glucagon (glucagonoma syndrome), gastrin (►Zollinger-Ellison syndrome, ZES), somatostatin (hypersomatostatinemia), vasoactive intestinal peptide (watery diarrhea syndrome), growth hormone releasing hormone (acromegaly), adrenocorticotropic hormone (►Cushing syndrome), serotonin (carcinoid syndrome) and others. The terms used to designate various subtypes reflects the specific hypersecretory syndromes (insulinoma, gastrinoma). Nonfunctioning NETs may contain certain hormones but are silent (nonsyndromic).

Pancratic NETs with a diameter of ≤1 cm, a <2% Ki-67 index and absence of angioinvasion are generally benign. When the size is >2 cm and the Ki-67 index <2%, NETs may have a benign or low-grade malignant behavior. Tumors with a size of >4 cm and a Ki-67 index between 2 and 20% fit in the low-grade malignant category. Moreover, even small tumors with evidence of angioinvasion or metastases are considered as malignant. Neoplasms of the first three categories are well differentiated. They are composed of uniform cells with no, mild or moderate atypia and show a trabecular or a solid-medullar (insular) growth pattern. Small-sized insulinomas (1–2 cm in diameter) with a benign behavior account for the majority of functioning NETs. Most other functioning and non-functioning tumors are of uncertain behavior or are low-grade malignant carcinomas. When the diameter is >2 cm, metastasis are often present at initial diagnosis, especially in gastrinomas. Pancreatic NETs measuring <0.5 cm are classified as microadenomas and may be multiple in MEN1.

High-grade malignant NETs are poorly differentiated. This category encompasses tumors with a high Ki-67 index of >20%. Small to intermediate or large-sized cells grow in solid aggregates, often with central necrosis. True mixed endocrine-exocrine (amphicrine) neoplasms, with at least one-third endocrine cells, are rare. Their prognosis depends on the exocrine component.

NETs of the Stomach and the Oesophagus

NETs of the esophagus are rare neoplasms of the middle and lower thirds. They either belong to the categories of well-differentiated carcinoid tumors (CT) or of poorly-differentiated small to intermediate-sized carcinomas with a poor prognosis and frequent systemic dissemination. A combination of small cell with ►squamous cell carcinoma or adenocarcinoma may occur. Poorly-differentiated NETs of the esophagus have been associated with smoking and ►Barrett esophagus.

Most well-differentiated gastric endocrine tumors are histamine-producing VMAT-1 positive ECL cell tumors and are composed of trabecular, adenoid or solid nests of endocrine cells. The classification depends on the underlying gastric pathology. Hypergastrinemia is involved in the pathogenesis of ECL cell tumors type 1 and 2. They often arise as multiple lesions on a background of endocrine-cell hyperplasia in chronic atrophic gastritis (type 1) or with MEN-1 or the ZES syndrome (type 2), and are benign or low-grade malignant. Sporadic (type 3) tumors develop in the absence of hypergastrinemia and are solitary. The prognosis of type 1 NETs is excellent. About one third of type 2 and 3 NETs present with a diameter is >2 cm, infiltration of the submucosa or angioinvasion and are likely to have metastasized at the time of diagnosis. Type 4 NETs of the stomach are poorly-differentiated carcinomas showing tightly packed small- to intermediate-sized cells with frequent mitoses. They are high-grade malignant carcinomas with a poor outcome.

NETs of the Small Intestine, Colon and Rectum

The majority of intestinal NETs are well-differentiated slow-growing neoplasms (carcinoids), which may metastasize to mesenteric lymph nodes and liver. Poorly-differentiated carcinomas are rare. Intestinal NETs arising in the different parts of the intestine are heterogeneous with regard to clinical symptoms and prognosis. They may be associated with production of ectopic hormones including serotonin, pancreatic polypeptide, glucagon, gastrin, somatostatin, cholecystokinin, ►calcitonin or bombesin. Secretion of may lead to the carcinoid syndrome results from the secretion of vasoactive amines especially serotonin by NETs that are metastatic to the liver NETs arising in the duodenum have a lower metastasizing rate than those of the jejunum and ileum. About 40% of duodenal NETs produce gastrin and may be associated with MEN1 and/or ZES. Lymph node metastases which are larger than the primary tumor may occur even in small gastrinomas. Most non-functioning tumors express serotonin and have a more favorable prognosis than gastrinomas and somatostatin-producing tumors. The latter tumors tend to involve the papilla, and typically include psammoma bodies and frequently develop in neurofibromatosis type I. Gangliocytic paragangliomas, composed of epithelial and neuromatous elements, may arise in the duodenal wall and are generally benign.

Serotonin- producing EC cells are the predominant component of most NETs arising in the jejunum, ileum, appendix and proximal colon (midgut), while enteroglucagon-producing cells are often present in NETs of the distal colon and rectum (hindgut). Most intestinal NETs are well differentiated, are located in the ileum, measure frequently >2 cm and have already lymph node or liver metastases at diagnosis. A carcinoid syndrome may develop if liver metastases are present. About 40% of ileal NETs are multiple. NETs of the appendix are usually benign and rarely metastasize. The majority resemble its counterparts in the ileum but their prognosis is more favorable. Appendical NETs are composed of serotonin- and substance P-producing EC cells arranged in solid nests. The so-called goblet-cell carcinoids of the appendix contain clusters of ►mucin-producing cells with an endocrine component and are now considered as mixed endocrine/exocrine neoplasms. They may involve lymph nodes and liver or spread to peritoneal surfaces.

NETs of the colon and rectum which are related to EC-cells are similar to NETs of the jejunum. Tumors of the hind-gut type are mainly derived from GLI/PYY positive L-cells. Well-differentiated NETs occur more frequently in the rectum than in the colon where they may be detected as small mucosal nodules. Poorly-differentiated NETs or composite adenocarcinoma-small cell carcinoma (category 4) arise more frequently in the colon and may present with an extensive local and metastatic tumor growth. High-grade NETs of the rectum are rare. Small well-differentiated rectal NETs which express glucacon or pancreatic polypeptide are generally confined to the submocosa.

The histologic appearance of well differentiated NETs of small intestine, colon and rectum is characterized by solid nests, trabeculae or tubules with peripheral palisading or a ribbon-like pattern. Tumor cell nests tend to be surrounded by a dense highly vascularized fibrous stroma. Poorly-differentiated carcinomas (category 3) may resemble its small cell counterparts in other organs and show clusters of anaplastic cells with high mitotic activity, necrosis and lymphatic and vascular invasion, indicating an aggressive behavior with a poor outcome.

NETs of the Lung

The actual WHO classification recognizes four main categories of bronchopulmonary NETs which share certain morphological and immunophenotypic patterns but are distinct with regard to prognosis. They may be derived from disseminated neuroendocrine cells of the airways (carcinoids) or from pluripotent bronchial precursor cells (small and large cell neuroendocrine carcinomas).

Carcinoid Tumors

Carcinoid tumors (CT) comprise two types of NETs which are not related to smoking. CTs form well circumscribed peripherally or centrally localized masses which may cause bronchial obstruction. The architecture is characterized by nests, cords, strands, microacinar or papillary structures in a fibrovascular stroma. CTs are separated into low grade typical carcinoids (TC) and intermediate grade atypical carcinoids (AC). In TCs mitoses are rare (<2 per 2 mm^2 or 10 HPF) and necroses

are absent, while ACs present 2–10 mitoses per 2 mm^2 (10 HPF) and/or focal necroses. Cellular atypia or prominent nucleoli do not reliably discriminate between TC and AC. Both categories are composed of polygonal, ovoid or spindle cells with a finely dispersed chromatin, small nucleoli and an eosinophilic cytoplasm. Immunohistochemical staining (IHS) reveals coexpression of cytokeratins (CK) (in about 80%) with chromogranin A, synaptophysin and CD56.

TCs often present with a diameter of <3 cm and have a favorable prognosis with a cure obtained by surgical resection in most cases (90–98% 5-year survival). However, since lymph node metastases may develop in a minority of patients in keeping with a low-grade malignancy. The prognosis of ACs depends on stage and criteria such as tumor size (>3.5 cm) or vascular invasion. Five-year survival rates of about 60% after radical surgery. A carcinoid syndrome is only observed in widespread metastatic disease. Most CT are sporadic. Rare cases rarely occur in the setting of MEN1 syndrome.

Small Cell Lung Carcinoma

Small cell lung carcinoma (SCLC) constitutes about 20% of ►lung cancer. This high-grade NET is strongly associated with smoking. Small-sized tumor cells with scanty cytoplasm contain ovoid to spindle shaped nuclei with dispersed chromatin and often show nuclear molding. Pseudoreosettes may be present. Mitoses (at least >11, average 70–80 per 2 mm^2 or 10 HPF), necroses and crushing artifacts are frequent. IHS generally shows reactivity for synaptophysin, CD56 and thyroid transcription factor (TTF-1) in about 90%. SCLCs with a subpopulation of at least 10% larger cells are classified as combined small and large cell carcinoma. Other combined types contain subpopulations of squamous or adenocarcinoma. Patients with SCLC may present endocrine symptoms (►Cushing syndrome inappropriate anti-diuretic hormone production, ►Eaton-Lambert syndrome). ►SCLC is a highly aggressive disease with early and rapid dissemination to lymph node and extrathoracic organ such as bone, liver or brain. Multiagent chemotherapy may obtain a favorable response.

Large Cell Neuroendocrine Carcinoma

In the actual WHO classification, large cell ►neuroendocrine carcinoma (LCNEC) are listed as a subtype of showing a large cell morphology. LCNEC cells show a high mitotic activity (at least >11, average 70–20 per 2 mm^2 or 10 HPF) but are of larger size, contain more abundant cytoplasm and prominent nucleoli than SCLC. Neuroendocrine differentiation may only be detected by IHS (synaptophysin, CD56) in carcinomas with a large cell morphology. About 3% of all lung cancers correspond to LCNEC which develop predominantly in smokers.

Thymic NETs

The classification system of pulmonary NETs is widely applied to thymic neoplasms ranging from TCs and ACs to poorly-differentiated small cell or large cell carcinomas. With regard to the tendency to local invasion and metastasis in about 50% of CTs, all NETs are actually designated as carcinomas. About 25% of thymic CTs are MEN-1 associated and more aggressive than sporadic tumors.

Medullary Thyroid Carcinoma (MTC)

This tumor of parafollicular C-cell differentiation may be sporadic or in about 25% hereditary (MEN 2A or B, familial ►Medullary thyroid carcinoma). Hereditary tumors are often multicentric and bilateral and develop on a background of C-cell hyperplasia. Tumor cells express calcitonin in addition to neuroendocrine marker proteins, TTF1and are stained by ►CEA-antibodies.

The histology may be classic, with sheets of polygonal tumor cells, or display various patterns (follicular, anaplastic, small cell). Amyloid deposits are present in about 80%. Lymph node metastases in the neck and the upper mediastinum may be found at initial presentation. Gain of function germline mutations in the ►*RET* proto-oncogene are found in MEN-2A and -2B or in familial MTC. A variety of chromosomal losses and somatic point mutations in the RET gene (RET M918T) occur in sporadic cases.

NET (Merkel Cell Carcinoma) of the Skin

This cutaneous type of NET is an aggressive neoplasm composed of small basophilic cells that are arranged in nests within the dermis. The tumor cells contain NE markers in addition to ►cytokeratin 20 (CK20). About 60% of small cell carcinomas of the salivary gland express CK20, indicating a close relationship to ►Merkel cell carcinoma.

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Neuroendocrine Tumors of the Pancreas

►Neuroendocrine Carcinoma
►Neuroendocrine Tumors

Neuroepithelioma

Definition

►Ewing sarcoma related tumor with limited neuroectodermal differentiation. Term primarily used in the US synonymously to pPNET.

Neurofibroma

Definition

A benign tumor that arises from the cells of connective tissue sheath of a peripheral nerve. Common in neurofibromatosis Type 1.

►Neurofibromatosis 1

Neurofibromatosis 1

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Synonyms

NF1; von Recklinghausen disease; peripheral neurofibromatosis

Definition

Neurofibromatosis 1 (NF1) is an autosomal dominant disorder that affects about 1 in ~3,500 persons worldwide. Half of the cases are caused by a new mutation. NF1 is a highly variable disease, the most prominent hallmarks being visible on skin: ►cafe-au-lait macules, and cutaneous ►neurofibroma. NF1 is the most common cancer predisposition syndrome.

Characteristics

Diagnostic Criteria and Clinical Aspects

The diagnosis of NF1 is based on the presence of two or more of the following findings:

Six or more cafe-au-lait macules with diameter more than 5 mm in prepubertal patients and more than 15 mm in postpubertal patients; two or more neurofibromas of any type, or one ►plexiform neurofibroma; axillary or inguinal freckling; ►optic glioma; two or more ►Lisch nodules of the iris; a distinctive osseous lesion such as sphenoid dysplasia or ►pseudarthrosis; first-degree relative with NF1 according to the preceding criteria. Based on these criteria, the penetrance of the NF1 mutation is virtually 100% by the age of 10 years. Indeed, NF1 can in most cases be diagnosed by the age of six years on routine clinical examination.

Cafe-au-lait macules are sharply demarcated spots that are slightly darker than the apparently normal skin of the patient. Cafe-au-lait macules are the most common first sign of neurofibromatosis. It is of interest to note that cafe-au-lait macules never undergo malignant transformation to ►melanoma. The appearance of the spots is followed by freckles in the flexural areas and Lisch nodules of the iris by the age of six. ►Lisch nodules are asymptomatic and non-precancerous hamartomas of the iris, the diagnosis of which requires slit lamp examination.

Cutaneous neurofibromas are benign tumors which arise from the cells of the connective tissue sheath of cutaneous nerve tributaries. Cutaneous neurofibromas usually appear in or after puberty. The number and size of these tumors varies between adult individuals with NF1: there may be only a limited number of tumors with a diameter of few millimeters, while the tumor burden in some individuals may reach thousands, some of which with a diameter of centimeters. Cutaneous neurofibromas may project out of the skin surface, or reside within the skin. It is worth to note that cutaneous neurofibromas do not undergo malignant transformation and that cutaneous neurofibromas are usually painless. Neurofibromas may also grow within the nerve forming knot-like thickenings in deeper nerves. These intraneural tumors may be painful. Subcutaneous tumors are single nodules situated underneath the skin. Neurofibromas may also grow along peripheral nerve trunks and extend to smaller nerve braches and surrounding tissue. These tumors are called plexiform neurofibromas. Plexiform neurofibromas infiltrate surrounding tissues and are poorly demarcated. About one third of NF1 patients have plexiform neurofibromas which may eventually form large and disfiguring tumor

masses in the craniofacial region, trunk, and extremities. Plexiform, subcutaneous and intraneural neurofibromas are associated with an increased risk of transformation to ►malignant peripheral nerve sheath tumor (MPNST). NF1 patients have an estimated lifetime risk of about 10% to develop an MPNST. Patients with a plexiform neurofibroma have a higher risk since MPNSTs often arise from preexisting plexiform neurofibromas. It has been estimated that approximately 2–5% of plexiform neurofibromas undergo malignant change. About half of all MPNSTs are NF1-associated. MPNSTs in NF1 patients usually occur in young age, between 20 and 40 years, in contrast to about 60 years in patients without NF1. The MPNST is an aggressive tumor with 5-year life expectancy of 20–40%, or the median survival of 17 months. Prognosis of MPNST in NF1 patients has been reported to be less favorable than in patients without NF1.

In the central nervous system, the most common NF1-related tumors are optic tract gliomas which histologically are pilocytic astrocytomas. MRI scan reveals optic gliomas in ~15% of NF1 patients. Most of these tumors however remain indolent. Gliomas in other central nervous system locations, such as cerebellum and brain stem, occur in less than 5% of patients. The majority of the central nervous system tumors are diagnosed in childhood. NF1 shortens the life expectancy by approximately 15 years, mainly due to malignancies of the central and peripheral nervous system. In addition to nervous system tumors, NF1 patients are also predisposed to ►phaeochromocytoma, ►carcinoid tumor, or ►gastrointestinal stromal tumors.

The spectrum of NF1 related manifestations include skeletal dysplasias such as pseudarthrosis of tibia, macrocephaly, short stature and sphenoid wing dysplasia. Learning disabilities are present in more than half of the NF1 patients and mild cognitive impairment also in adulthood is common. Vascular stenosis caused by the proliferation of the intimal cells most commonly manifests itself as high blood pressure, or strokes.

Genetics

NF1 results from mutations in the *NF1* gene located in chromosome 17, band q11.2. The gene is complex and large, spanning more than 350 kb of genomic DNA and divided into 61 exons. The mRNA of the *NF1* gene is 11–13 kb and it is ubiquitously expressed in tissues. All affected individuals are heterozygous for an *NF1* mutation and the mutation can be found in all cells of the individual with NF1. NF1 is inherited in autosomal dominant manner which implicates that a person with NF1 has a 50% risk of transferring the disease to the next generation. About 50% of the NF1 cases are caused by a new, sporadic germline mutation in the *NF1* gene. Approximately 80% of new *NF1* mutations are paternal in origin. About 300 mutations and several different mutation types have been reported in the *NF1* gene. The mutation types include point mutations that may affect the splicing of the *NF1* gene or create premature stop codon; small deletions or large deletions, e.g. microdeletions that cover the entire *NF1*, and additionally affect the flanking regions of the *NF1* gene; insertions and mutations in introns. Majority of the mutations cause truncation of the neurofibromin protein. Mutations have been identified throughout the gene, and with few exceptions, genotype-phenotype correlations have not been established. However, microdeletion results in a phenotype with dysmorphic facial features, heavier tumor burden, lower IQ and increased risk of ►MPNST. In opposite to most other *NF1* mutations, microdeletions are maternal in origin.

Neurofibromin

The *NF1* gene product is called ►neurofibromin with an estimated molecular mass of 280 kDa. Neurofibromin has a central domain (the Nf1-GAP-related domain or Nf1-GRD) that shares extensive sequence homology with other ►GTPase activating proteins (GAPs) that regulate ►Ras. The GAP domain accelerates the switch of active Ras-GTP to inactive Ras-GDP in various cell types. Neurofibromin is considered as a ►tumor suppressor protein since selected malignant tumors display inactivation of both *NF1* alleles, either by ►loss of heterozygosity (LOH), or by a new somatic mutation in the originally intact *NF1* allele.

Gene Mutations in NF1 Related Tumors

In NF1, all cells harbor a mutation in one *NF1* allele. This, however, is not sufficient to result in the development of a neurofibroma. According to the two-hit hypothesis, neurofibroma develops when a somatic mutation in Schwann cell lineage disrupts the remaining functional copy of the *NF1* allele leading to biallelic inactivation of the gene. One mechanism that leads to inactivation of both *NF1* alleles is ►LOH, i.e. loss of the wild type allele at a heterozygous locus. The mechanisms leading to LOH are mitotic recombination, chromosomal microdeletion, and chromosome loss with reduplication. LOH only occurs in the chromosome that does not carry the germline mutated allele. Biallelic inactivation of *NF1* has been demonstrated in neurofibromas, plexiform neurofibromas, MPNSTs, pheochromocytomas, astrocytomas and juvenile myelomonocytic ►leukemia in NF1 patients. However, since biallelic inactivation of the *NF1* occurs in benign neurofibromas, mutations in *NF1* gene alone are not sufficient for the progression from a plexiform neurofibroma to an MPNST. Additional mutations in other regulatory genes have been described in MPNSTs. These additional mutations include loss-of-function mutations of the

►p53 tumor suppressor; deletions of the region of chromosome 22 encoding ►merlin; and mutations of genes that regulate cell cycle progression. Approximately half of MPNSTs in NF1 patients contain mutation of the ►CDKN2A (also known as *INK4A* or INK4A/ARF) gene, which encodes two distinct tumor suppressors, ►p16INK4A and ►p14ARF, while this deletion is not found in benign neurofibromas. Decreased or altered expression has been reported for p16INK4A, a protein that arrests cells in the G1 phase of the cell cycle by inhibiting cyclin-dependent kinases 4 (CDK4) and 6 (CDK6). Decreased levels of p27kip1, a ►cyclin-dependent kinase inhibitor that controls G1/S progression, and ►p19ARF, a polypeptide that binds to ►Mdm2 and prevents it from degrading ►p53 have also been reported. Mutations in the ►Retinoblastoma (Rb) gene ►RBA a tumor suppressor that impedes cell cycle progression and becomes inactivated when phosphorylated by CDK4/6 kinases may also contribute to peripheral nerve sheath tumorigenesis in some cases. These findings show that tumorigenesis in NF1 is a complex multistep process involving a variety of different types of genetic defects at multiple genes.

NF1 Gene Mutations in Cancer

NF1 gene mutations and alterations in neurofibromin expression have been found in malignant tissues unrelated to NF1, which further supports its status as a tumor suppressor. Specifically, NF1 gene mutations have been found in colon ►adenocarcinoma, ►myelodysplastic syndrome, ►anaplastic astrocytoma, ►neuroblastoma, juvenile ►myelomonocytic leukemia, ►small cell lung carcinoma, ►urinary bladder carcinoma, ►uveal melanoma, and in cell lines cultured from ►malignant melanoma of patients who do not have NF1. Futhermore, the amount of neurofibromin has been reported to be altered in certain proliferative diseases, such as urinary bladder carcinoma, ►basal cell carcinoma, ►pheochromocytoma, ►meningioma and psoriasis. Additionally, changes in NF1 mRNA isoforms expression ratio have been reported in ►ovarian cancer, ►small cell lung carcinoma and ►colon cancer.

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Neurofibromatosis 2

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Synonyms

NF2; Bilateral acoustic neurofibromatosis; Central neurofibromatosis

Definition

Neurofibromatosis 2 (NF2) is an ►autosomal dominant disorder characterized by the bilateral development of ►vestibular schwannoma, along with the occurrence multiple other benign intracranial, spinal cord and peripheral nerve tumors. Cataracts are nontumorous manifestations of NF2.

Characteristics

Diagnostic Criteria and Clinical Aspects

The diagnostic criteria for NF2 are the observance of bilateral eight-nerve masses (vestibular schwannomas) with appropriate imaging techniques (e.g., ►CT or ►MRI), or the existence of a first-degree relative with NF2 and either a unilateral vestibular schwannoma or two of the following: meningioma, neurofibroma, glioma, schwannoma or juvenile posterior subcapsular lenticular opacity.

Vestibular schwannomas, formerly termed acoustic neuromas, are a universal feature of NF2. They are slow growing, benign tumors that form in the internal auditory canal where the eighth nerve and facial nerve are close together. Initial symptoms of NF2 usually begin subtly but progress as the slow-growing tumor expands, causing tinnitus, hearing loss, and balance problems. Schwannomas sometimes occur on other cranial nerves, more often on sensory than motor nerves, and on peripheral nerves, with skin tumors that can be particularly painful. The schwannomas of NF2 rarely, if ever, become malignant. About half of all NF2 patients also develop intracranial, and occasionally spinal ►meningioma, although neither shows a preferred location of occurrence. Spinal schwannomas arise in about two-thirds of NF2 patients and can be extremely debilitating. ►Astrocytoma and ►ependymoma, occur in a small minority (5–10%) of patients. Decreased visual acuity due to cataracts, which may occur even before tumors are evident, affects at least one-third of NF2 patients.

The mean onset age for NF2 is ~21 years but the disorder has been seen as early as age 2 and as late as age 70. The first manifestations of disease are usually related to vestibular schwannomas causing hearing loss,

tinnitus, or loss of balance but may include facial weakness, visual impairment, and painful skin schwannomas. The progression of NF2 shows extreme variability as it depends on tumor number, location and growth rate. The mean survival from diagnosis is currently 15 years, leading to an average age of death of 36 years for NF2 patients, although this life expectancy is likely to increase with advances in diagnostic and surgical techniques.

Genetics

NF2 affects in 25-40,000 individuals due to germline mutation in a gene on chromosome 22q that encodes a protein named ►merlin (schwannomin). About half of cases are new mutations and half show a family history. The disorder is inherited in an autosomal dominant pattern, but has a recessive mechanism of action at the level of individual tumor cells, making the NF2 gene a classic tumor suppressor locus. NF2 involves an inherited inactivating mutation that only leads to tumor formation when a somatic mutation inactivates the remaining copy of the NF2 gene in an appropriate target cell, leading to absence of functional merlin. Almost all NF2 mutations, both germline and somatic, involve evident inactivation of the gene due to deletion, chromosomal loss or rearrangement, or truncating mutations. ►Missense mutations or small in-frame deletions have been described, which presumably impact critical functional domains of merlin. Somatic mutations that inactivate both NF2 copies in target cells also account for essentially all cases of sporadic unilateral vestibular schwannoma and more than half of all sporadic meningiomas in the general non-NF2 population.

The NF2 gene spans 110 kb with 16 constitutive exons and one alternatively spliced exon. It encodes two major merlin isoforms that differ only at the extreme carboxy terminus. Isoform 1, encoded by exons 1–15 and 17, comprises 595 amino acids. Alternative splicing of exon 16 eliminates the final 16 amino acids and replaces them with 11 novel residues to produce merlin isoform 2. Germline NF2 mutations have been found in all exons except exons 16 and 17, suggesting that both major isoforms may have tumor suppressor activity. Nonsense mutations due to C to T transitions in CGA codons are particularly frequent. There is some degree of correlation between mutation type and disease severity as missense mutations in exons 7, 11, and 15 as well as large deletions are generally associated with mild disease manifestations. By contrast, nonsense mutations throughout the gene, and especially in exon 6, cause more severe disease. Somatic mutations also occur throughout the gene except in exons 16 and 17, and deletions are more frequent than in germline. Complete loss of chromosome 22 leading to readily detectable loss of heterozygosity in the tumors is an especially frequent mechanism of somatic alteration.

Merlin Protein

Merlin was named for its similarity with three known proteins, moesin, ezrin and radixin (merlin = moesin, ezrin, radixin-like protein). These closest relatives of merlin are known collectively as ►ERM proteins, and all four are members of the protein 4.1 family, which also includes erythrocyte protein 4.1, talin, certain unconventional myosins, several protein tyrosine phosphatases and several anonymous proteins. The protein 4.1 family is defined by the presence of a conserved ►FERM domain, spanning ~270 amino acids, that is believed to mediate interactions with integral membrane proteins. In merlin and the ERMs, this domain is located in the NH2-terminal half of the protein, and is followed by a long alpha helical segment and a charged COOH-terminal domain. Protein 4.1, the prototype for this family, helps to maintain membrane stability and cell shape in the erythrocyte where it connects the spectrin-actin cytoskeletal lattice with the integral membrane proteins, glycophorin, and the anion channel. The ERMs, closer relatives to merlin, also bind integral membranes proteins, such as ►CD44, via the FERM domain and to actin via a COOH-terminal binding site. The ERM proteins all show intramolecular interaction whereby the COOH-terminal region binds to a more NH2-terminal segment, effectively blocking the binding site for actin and certain other interactors. This "closed" conformation can be altered to an "open conformation" by specific phosphorylation or phosphoinositides, permitting binding to the actin cytoskeleton. Each of the ERMs is ~78% identical to the others and they may have overlapping functions.

Merlin is ~63% identical to the ERM proteins across the FERM domain and the immediate downstream region but is much more divergent across the long alpha helical domain, which has a notable interruption by a cluster of proline residues. Merlin is the only one of these four proteins that displays alternate COOH-termini, creating two major isoforms. Merlin ►isoform 1 undergoes the same "closed conformation/open conformation" regulation as the ERMs due to intramolecular interaction, although no conserved actin binding site is present near its COOH-terminus. By contrast, merlin isoform 2 does not exhibit "head to tail" intramolecular binding and remains constitutively in the "open conformation."

The precise normal function of merlin remains to be defined, although it is likely to involve linking cytoskeletal and membrane elements like protein 4.1 and the ERMs. Indeed, in cultured cells, where merlin is expressed as a protein with an apparent size of ~66 kDa in SDS-polyacrylamide gel electrophoresis, the protein

is found in areas of membrane remodeling, particularly membrane ruffles, where it co-localizes with actin. However, merlin is not associated with cytoplasmic actin stress fibers. To date, a number of proteins capable of interacting with merlin have been described, including among others F-actin, β2 spectrin, CD44, ►hepatocyte growth factor-regulated tyrosine kinase substrate, β1 integrin and ERM proteins. Perhaps the most intriguing interactor to date is NHERF, which also binds to ERMs in the "open conformation." NHERF is a 358 amino acid protein with two ►PDZ domains upstream from a C-terminal region that binds to the unmasked N-terminal half of merlin and the ERMs. It was originally described as an adaptor protein required for protein kinase A regulation of the kidney sodium-hydrogen exchanger (NHE3). NHERF binds to NHE3 via the second of the PDZ domains and to several other membrane proteins, such as the β2 adrenergic receptor, the purinergic P2Y1 receptor and the ►cystic fibrosis transmembrane conductance regulator, via the first PDZ domain. potentially tying these proteins, and merlin/ERMs to sodium-hydrogen exchange or to protein kinase A regulation.

That growth control in Schwann cells and meningeal cells is disrupted by the loss of merlin function, implies that lack of merlin subtly alters some aspect of intracellular signaling, resulting in a slow proliferation. Despite having an overall structure similar to the ERMs, merlin is the only one of these four proteins demonstrated to have a specific tumor suppressor function, suggesting that it affects one or more signaling pathways different from the ERMs. Although the tumors of NF2 are benign, merlin mutations can also be involved in progression of non-NF2 tumors, most notably malignant meningiomas, suggesting that loss of the protein can have different effects in different circumstances. For example, in the mouse, merlin deficiency is associated with the development of highly malignant tumors such as osteosarcomas and rhabdomyosarcomas. The NH2-terminal half of merlin has been implicated in growth control in Drosophila, where inactivation of the NF2 homolog leads to cellular overproliferation. Thus, it seems likely that merlin deficiency can affect different aspects of the web of signal transduction that connects the cell membrane, actin-cytoskeleton and the nucleus. Indeed, any signaling pathway in which NHERF can act as a cofactor and any of the growing list of proteins with which NHERF physically interacts are already candidates for disruption in the absence of merlin. The delineation of merlin's full complement of interactions and their effects in the target cells, Schwann cells and arachnoidal cells of the meninges, will probably be required to understand merlin's tumor suppressor activity in NF2.

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Neurofibromin

Definition

Is a tumor suppressor protein encoded by the ►NF1 gene on human chromosome 17. Neurofibromin serving as a GTPase Activating Protein (GAP) that inactivates Ras in certain glia cells. People with mutations in the NF1 gene develop ►neurofibromatosis I (NF1), a neurological disorder that affects 1 in 3,500 people world-wide. NF1 patients develop benign tumors along their peripheral and optic nerves, as well as ►café-au-lait spots on the skin.

►RAS Activation

Neurologically Eloquent

Definition

Literally means the expression of neurologic function. The phrase is applied to those regions of the central nervous system that mediate vital unduplicated neurological actions, the destruction of which would result in a functional disability to the patient.

►Oligodendroglioma

Neuromedin

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Definition

A family of peptides that comprises the neuromedins A, B and C (bombesin-like), K (neurokinin B) and L (neurokinin A), N (neurotensin-like), S, and U.

Characteristics

This family of peptides termed "neuromedins" that were initially isolated from porcine spinal cord on account of their ability to contract smooth-muscle. This family of peptides currently includes the neuromedins A (NMA), B (NMB), C (NMC), K (NMK), L (NML), N (NMN), S (NMS), and U (NMU), however their structural and functional characteristics vary greatly. These neuromedins are found in many tissues including the central nervous system as well as in the gastrointestinal tract, and have been implicated in a wide variety of physiological functions as neuropeptides. Neuropeptides function peripherally as paracrine and autocrine factors to regulate diverse physiological processes and act as neurotransmitters or neuromodulators in the nervous system. In general, the receptors which mediate signaling by binding neuropeptides are members of the superfamily of G protein-coupled receptors (GPCRs). Some of the neuromedins expressed in several types of human tumors including neuroendocrine tumors. NMK and NML abundantly express in human carcinoid tumors. NMN have growth-stimulatory effects on human pancreatic cancer cells. The ►autocrine and ►paracrine involvement of NMB or NMU as well as some of their receptors have biological significance in human cancers.

NMB in Human Cancer

The bombesin-like peptides, NMB and ►gastrin-releasing peptide (GRP), are among the first neuropeptides to be implicated as autocrine growth factors in ►lung cancer cells. Two receptors for bombesin-like peptide in mammals, NMBR and GRPR, can interact with both NMB and GRP to relay extracellular signals to cells, as measured by intracellular Ca2+ mobilization in response to applied NMB or GRP. GRPR and NMBR can also be detected in various human lung carcinoma cells. GRP and NMB have mitogenic activity as autocrine growth factors in small cell lung carcinoma (►SCLC). NMB stimulates arachidonic acid release, c-fos gene expression, and the growth of ►glioma cells.

NMU in Human Cancer

NMU is abundantly expressed in the majority of ►acute myeloid leukemia (AML), ►ovarian cancer, ►bladder cancer, and ►lung cancer. NMU expression is related to MYB and the NMU/NMU1R axis constitutes a growth-promoting autocrine loop in myeloid leukemia cells. NMU is regulated by the ►metastasis suppressor Rho GDP Dissociation Inhibitor 2 (RhoGDI2). NMU overexpression in bladder cancer cells significantly enhanced their lung metastatic ability and cancer cachexia in mice. NMU and hetero-dimerization of two GPCRs (GHSR1b and NTSR1) are likely to play an essential role in creating an autocrine growth-promoting pathway in lung cancers by modulating transcription of down stream target genes. NMU is epigenetically silenced in esophageal squamous cell carcinoma (►ESCC) and head and neck squamous cell carcinoma (►HNSCC).

Clinical Relevance

Interruption of autocrine and paracrine neuropeptide signaling with specific antagonists are considered to be new therapeutic approaches to the treatment of human cancers. Therapeutically, the ability of antagonists, radiolabeled analogs, or neutralizing antibodies acting against neuromedin peptides and/or their receptors so as to reduce the growth of some cultured cancer cells have been demonstrated *in vitro* and in animal models.

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Neuronectin

►Tenascin-C

Neuropathy

Definition

A disease caused by the damage of the peripheral nervous system. Damage resulting in dysfunction of nerves. Malfunction of a single or multiple peripheral nerves.

►Peripheral Neuropathy

Neuropeptide Y

Definition
Is a 36-amino acid peptide found in the brain and autonomic nervous system, is known to be an extremely potent stimulator of feeding behavior.

►Cachexia

Neuropeptides

►Gut Peptides

Neuropilin

Definition
Class of membrane proteins with a very short intracellular domain, and without signaling motifs. Coreceptor for ►Vascular endothelial growth factor (VEGF)-A165 and for ►semaphorins.

►Plexins

Neuropilin Ligand

►Semaphorin

Neurotensin

Definition
Gut peptide hormone found in the brain and small bowel, its main gastrointestinal functions are to aid in the digestion of fats and stimulate cell growth of the intestinal mucosa.

►Gut Peptides

Neurotoxicity

Definition
Toxic or adverse effects in the brain tissue.

►Chemoprotectants

Neurotransmitters

Definition
Chemical compounds that are used to transmit, magnify and alter electrical signals between a nerve cell and another cell.

►Fluoxetine
►5-Hydroxytryptamine
►Serotonin

Neurotrophic factors

►Neurotrophins

Neurotrophins

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Synonyms
NGF family; Neurotrophic factors

Definition
In normal neurons, neurotrophins and their receptors regulate growth, differentiation and cell survival during neural development and maintaining the adult nervous tissues. Neurotrophins are composed of ►nerve growth factor (NGF), ►brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). In cancers, neurotrophins and their receptors

are often aberrantly expressed or rearranged, and modulate biological characteristics of cancer cells or are involved in carcinogenesis.

Characteristics

Neurotrophin signaling through activation of the ►TRK family of tyrosine kinase receptors as well as $p75^{NTR}$ is important in regulating growth, differentiation and survival of neurons in both central and peripheral nervous systems. In cancers, however, aberrant expression of neurotrophins and TRK family receptors is often associated with abnormal growth, invasion and metastasis of cancer cells, resulting in acquisition of resistance to chemotherapy and radiation therapy. In addition, in certain types of cancer, the *TRK* gene is rearranged to be oncogenic.

Gene targeting: ►TRK-A signaling supports the survival of sympathetic neurons and sensory neurons responsive to temperature and pain, TRK-B signaling supports the survival of sensory neurons responsive to tactile stimuli, and TRK-C signaling supports the survival of sensory neurons responsive to limb movement and position.

Neurotrophin signaling in cells: The binding of ligands triggers activation of TRK receptor(s) by phosphorylating the tyrosine residues at the intracellular tyrosine kinase domain. Like other receptor tyrosine kinases, the main signaling pathways are mediated by Ras/MEK/ERK1,2 as well as ►PI3K/AKT, and induce cellular differentiation and survival. The depletion or inactivation of TRK often induces cell death. The $p75^{NTR}$ receptor enhances TRK signaling by heterodimering with TRK. However, $p75^{NTR}$ itself has an intracellular death domain and often sends death signals mediated by ceramide or inhibiting ►NFκB pathway. The developmentally regulated neuronal programmed cell death is caused by depletion of neurotrophins when the cells are dependent on the particular ligand(s) for survival.

TRK oncogenes in cancers: The proto-oncogene *TRK* was originally identified as an oncogene from colon cancer. The gene was fused with the tropomyosin gene at the *TRK-A* extracellular juxtamembrane region, and the chimeric receptor was constitutively activated. The similarly oncogenic TRKs have been reported in ►papillary thyroid carcinoma and ►acute myeloid leukemia. Mutation of the *TRK* genes is rare in human cancers. Rearrangement of the *TRK* gene is rarely observed in cancers with neuronal origin.

Aberrant expression of neurotrophins and their receptors in cancers: In more than several cancers, abnormal expression of neurotrophins and TRK receptors have been reported. In general, neurotrophins are supplied from the target cells to neurons by retrograde transport. In tumor tissue, however, the neurotrophin-mediated cross-talk between stromal cells and tumor cells or in tumor cells themselves may be regulated abnormally in autocrine and/or paracrine manner, resulting in aggressive growth, enhanced invasion and metastasis.

Clinical Relevance

►Neuroblastoma is originated from sympathetic precursor cells derived from neural crest. Both TRK-A and $p75^{NTR}$ are highly expressed in favorable neuroblastoma occurred in patients under one year of age, that might lead to spontaneous regression of tumor cells under relative deficiency of the stromal NGF, while they are strongly down-regulated in aggressive tumors in patients over one year of age. TRK-A alone or $p75^{NTR}$ alone may cause apoptosis in neuroblastoma cell, but their cooperative function may prevent the cell death and induce cellular differentiation and survival. The TRK-A signal also inhibits ►angiogenesis in the tumor. In neuroblastoma with ►*MYCN* amplification, the autocrine/paracrine loop of BDNF or NT-4/5 and TRK-B is functional and enhances invasion and metastasis. The reagents to inhibit BDNF/TRK-B pathway could be potential therapeutic tools against high-risk neuroblastoma. The downstream signaling molecule ShcC is expressed at high levels in advanced neuroblastoma and is significantly correlated with poor prognosis. Interestingly, in ►medulloblastoma and ►Wilms tumor, high expression of TRK-C is associated with favorable prognosis.

In ►papillary thyroid carcinoma, there are somatic rearrangements of the *TRK-A* gene, producing ►chimeric oncogenes with constitutive tyrosine kinase activity. The *TRK* oncogene with replacement of ligand-binding domain by heterologous sequences including tropomyosin or *TFG* gene is found in about 25% of thyroid papillary carcinoma.

In ►prostate cancer, expression of TRK-A and TRK-B is associated with invasive capacity and aggressiveness of the tumor. The TRK inhibitors suppress tumor growth *in vivo*. Human ►lung cancer also expresses both TRK-A and TRK-B, but not TRK-C. There is no expression of $p75^{NTR}$ receptor protein in the cancer tissue. In human astrocytoma and ►glioblastoma, expression of TRK-A and TRK-B may contribute to progression towards malignancy.

In ►pancreas cancer, TRK-A and TRK-C are expressed in duct, islet and cancer cells, while TRK-B is expressed only in alpha-cells in islet. The neurotrophins are also expressed in both normal and cancer cells in a different manner. The expression and distribution of neurotrophins and their receptors may play a role in regulating neural invasion of the pancreatic cancer cells.

Both NGF and TRK-A are abundantly expressed in epithelial cells of epithelial ►ovarian cancer, and enhance the cell growth in an autocrine manner.

TRK-C mRNA is widely expressed in human soft tissue tumors. The expression of *TRK-C* may be involved

in the progression of early stages of melanoma. The ►*ETV6-TRK-C* fusion gene, whose tyrosine kinase is constitutively activated, is found in infantile fibrosarcoma, acute myeloid leukemia and secretary ►breast cancer.

Neurotrophins and their receptors are down-regulated in human ►gastric cancer.

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Neutropenia

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Synonyms

Leukopenia; Granulocytopenia; Agranulocytosis

Definition

Is an inherited or acquired condition manifested by low numbers of circulating white blood cells known as neutrophils. The absolute neutrophil count (ANC) may be estimated directly or as the product of the total white blood cell count and the fraction of polymorphonuclear cells and band forms in the blood count differential and is generally expressed as the number of cells per mm^3. The Common Toxicity Criteria established by the US National Cancer Institute grades neutropenia as: Grade 0: ≥2,000/mm^3; Grade 1: ≥1,500–<2,000/mm^3; Grade 2: ≥1,000–<1,500/mm^3; Grade 3: ≥500–<1,000/mm^3; Grade 4: <500/mm^3. However, the term neutropenia clinically is generally reserved for ANC <1,500/mm^3 (grades 2 or greater).

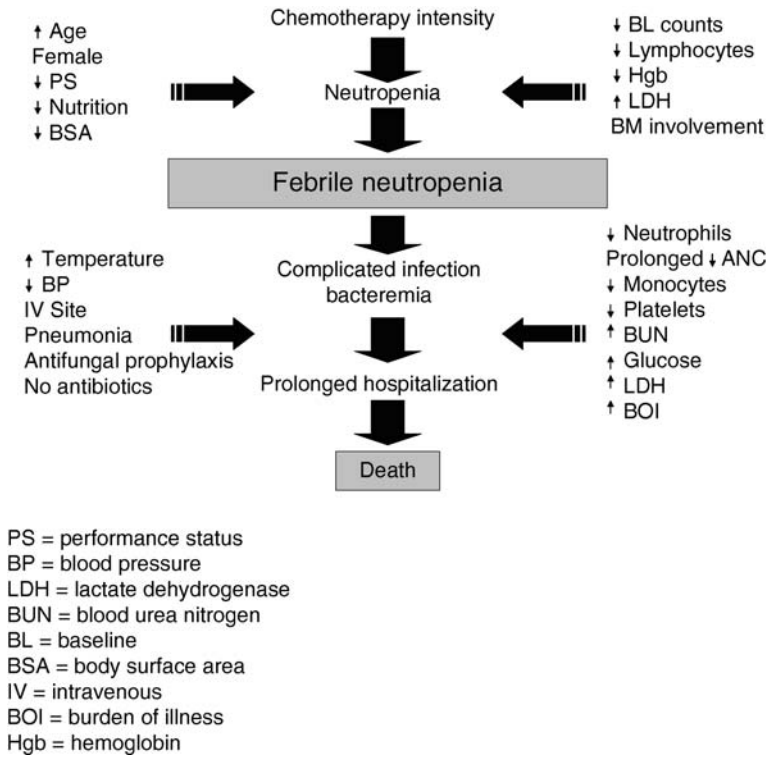
Characteristics

Hematopoiesis

Normal hematopoiesis is characterized by the controlled proliferation and maturation of multiple cell lines critical to the development of the healthy individual. The maturation and functional integrity of these cell lines are controlled by an array of hematopoietic cytokines which regulate the rate and direction of maturation to the myeloid cell line from a multipotential stem cell through cells committed to the granulocyte and monocyte lineage and then eventual maturation into functional end cells including ►monocytes, ►basophils, ►eosinophils, and ►neutrophils. ►Cytokines involved in the early maturation of the myeloid cell line include ►interleukin 3 (IL-3) and granulocyte-macrophage (GM-) ►colony-stimulating factor (CSF) while granulocyte (G-) CSF facilitates the terminal morphologic and functional maturation of the neutrophil. In healthy adults, this process occurs almost entirely in the bone marrow and extends over a period of 10–14 days, following which mature neutrophils migrate into the circulation where they persist, on average, for 5–10 h. In the setting of active infection, circulating neutrophils move quickly into infected tissues to fight infection by ingesting and destroying infectious organisms. Neutrophils clearly represent the principle white blood cell lineage responding to bacterial infection.

Neutrophil Disorders

Neutropenia may be either inherited (congenital, cyclic) or acquired from disease or exposure to certain toxic agents. Severe Congenital Neutropenia (►Kostman syndrome) is rare but often associated with severe recurrent infections. A specific syndrome of cycle neutropenia is characterized by regular oscillations in the numbers of cells of the various myeloid cell lines. Acquired neutropenia may result from either decreased production or increased destruction of neutrophils. Common causes of the later include various infections and primary immune disorders and hypersplenism. Antibodies to the ►G-CSF receptor may result in ►Felty syndrome. Decreased production of neutrophils may result from bone marrow disorders including conditions such as certain nutritional deficiencies, alcoholism, aplastic anemia and ►myelodysplasia and ►acquired immunodeficiency syndrome (AIDS) as well as in association with neoplasms including the leukemias and any malignancy infiltrating the bone marrow inhibiting normal myelopoiesis. Drug-induced neutropenia is most often idiosyncratic and may result from a wide variety of agents including the thionamide antithyroid agents, several ►nonsteroidal anti-inflammatory drugs, several antibiotics including chloramphenicol and the sulfonamides, the quinine antimalarials and a range of cardiovascular, diuretic,



Neutropenia. Figure 1 Clinical management of neutropenia. Lyman GH, Kuderer NM, Djulbegovic B (2002) Prophylactic granulocyte colony-stimulating factor in patients receiving dose intensive cancer chemotherapy: a meta-analysis. Am J Med 112:406–411.

psychotropic and anticonvulsant agents among many others. The most common cause of neutropenia in the cancer population is the direct result of the ►myelosuppression associated with cytotoxic chemotherapy and radiation therapy. Myelosuppression and ensuing neutropenia as well as anemia and thrombocytopenia represent the leading cause of dose-limiting toxicity associated with systemic cancer chemotherapy. The risk of myelosuppression and neutropenia correlate directly with the dose intensity of cancer chemotherapy and are most profound in association with marrow ablative regimens in preparation for hematopoietic stem cell transplantation. Fever in the setting of neutropenia (febrile neutropenia) represents a potentially serious infection requiring immediate evaluation, cultures and broad-spectrum antibiotics until recovery. As a result, episodes of febrile neutropenia are associated with considerable morbidity, mortality as well as cost and often compromising effective cancer treatment. The risk of febrile neutropenia correlates directly with both the severity and duration of myelosuppression and neutropenia. Febrile neutropenia often occurs early in the course of cancer chemotherapy and is associated with a number of demographic and clinical factors (Fig. 1). Of importance, hematopoietic reserves in the bone marrow diminish with age such that older individuals are more prone to neutropenia. In particular, older patients experience a greater risk of neutropenia and febrile neutropenia in the setting of myelosuppressive chemotherapy (Table 1).

Neutropenia. Table 1 Febrile neutropenia

Fever is defined as:
• A single oral temperature of ≥38.3°C (101.0°F)
• A temperature of ≥38.0°C (100.4°F) for a least 1 h
Neutropenia is defined as:
• An absolute neutrophil count of <500 cells/mm^3 (grade 4)
• An absolute neutrophil count of <1,000 cells/mm^3 (grade 3 or 4), with a predicted decrease to <500 cells/mm^3

Neutrophil Growth Factors

Neutrophil growth factors are a specific class of cytokines termed the CSF. The term CSF has been applied to certain glycoproteins that are capable of promoting and supporting the formation of *in vitro*

Neutropenia. Table 2 Pharmacokinetics of the colony-stimulating factors

CSF	Generic name	Brand name	Vector	$t_{1/2}$ life (h)	Clearance (mL/min/kg)
G-CSF	Filgrastim	Neupogen	E. coli	SC 2.5–5.8 IV 1.3–5.1	SC 19–56 IV 4–21
	Lenograstim	Neutrogin	CHO		
GM-CSF	Sargramostim	Leukine	Yeast	SC 1.6–5.8 IV 1.1–2.4	SC 249–312 IV 9.9–178
	Molgramostim	Leucomax	E. coli		
	Regramostim		CHO		
Pegylated G-CSF	Pegfilgrastim	Neulasta	E. coli	15–80	

CSF, colony-stimulating factor, CHO, chinese hamster ovary cells; SC, subcutaneous; IV, intravenous.

colonies of hematopoietic elements when present in bone marrow cell cultures. Endogenous CSF is produced by various sources including monocytes, macrophages, endothelial cells, fibroblasts and bone marrow stromal cells. The ►G-CSF receptor is expressed primarily on mature neutrophils and their precursors while the GM-CSF receptor is expressed on monocytes, eosinophils, myeloid progenitors and T-lymphocytes as well as neutrophils. Following binding and dimerization of the receptor, the biologic action of the cytokine is orchestrated by a number of signaling pathways, the most important of which is the ►JAK/►STAT pathway, followed by transcriptional activation in the nucleus. Defects in the receptor or its down stream signaling elements are incriminated in the pathogenesis of severe congenital neutropenia or Kostman syndrome. Endogenous G-CSF is normally present in very low concentrations but is necessary to sustain neutrophil production and is highly stimulated by inflammatory cytokines and bacterial cell wall products. Endogenous G-CSF levels rise in neutropenic states and most dramatically with febrile neutropenia. G-CSF acts on late myeloid progenitors leading to increase cell division and shortens the transit time through the bone marrow to the peripheral blood and tissues. It also increases differentiation and functional maturation of neutrophils including chemotaxis, phagocytosis, and antibody dependent cellular toxicity. GM-CSF stimulates ►macrophages and monocytes as well as granulocytes and stimulates the functional activity of neutrophils but appears to have little role in steady state white blood cell production. Following identification and cloning of the genes associated with both G-CSF and GM-CSF, recombinant forms of these cytokines were produced and are commercially available. The pharmacology and ►pharmacokinetics of the recombinant CSFs have been studied in both healthy individuals as well as those with chronic or episodic neutropenia due to chemotherapy (Table 2). Most CSFs are eliminated by renal clearance and metabolized by receptor binding and internalization. ►Pegylation of recombinant G-CSF virtually eliminates glomerular filtration, significantly prolonging systemic circulation and action, most notably in the setting of neutropenia. When administered to healthy volunteers, the CSFs result in a prompt increase in circulating neutrophils. Clinical trials have convincingly demonstrated that these agents are effective in reducing the risk of neutropenia and its complications in both congenital and acquired neutropenia, most notably in the prevention of infection in patients receiving myelosuppressive chemotherapy.

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Neutrophil

Definition

A white blood cell that is an abundant and important ►phagocyte.

Neutrophil Elastase

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Synonyms

Neutrophil Elastase 2 (*ELA2* as the official gene symbol); Leukocyte elastase; Serine elastase; Polymorphonuclear leukocyte elastase; Granulocyte elastase

Definition

NE; is a single-chain glycoprotein with 218 amino acid residues and belongs to a member of the chymotrypsin superfamily of ►serine protease, which includes chymotrypsin, trypsin, and elastase. With three residues in the active site, His^{41}-Asp^{88}-Ser^{173} comprises a "charge-relay" system to carry out hydrolysis. All serine proteases have their own internal inhibitors to prevent any overdose effects of cell lysis. A natural inhibitor of NE is α_1-antitrypsin (α_1AT). The major physiological function of NE is host-defense, i.e., to degrade foreign microorganisms or organic molecules that were phagocytosed by neutrophils. NE also degrades a variety of substrates including elastin, the most important of all, and other ►extracellular matrix proteins (►collagen, ►proteoglycan, ►fibronectin, and ►cadherins), ►platelet receptors, ►complement receptors, thrombomodulin, lung surfactant, and certain growth factors (►granulocyte-colony stimulating factor (G-CSF), stromal cell-derived factor-1, and their respective receptors, G-CSFR and CXCR4). NE mostly releases and acts locally but can be detected in serum.

Characteristics

The most commonly used assay method is enzyme-linked immunosorbent assay (ELISA), which measures the level of NE and α_1AT complex. The reported normal range is 56–136 ng/ml but fluctuates greatly under ►inflammation, ►Behcet disease, interstitial lung disease, and pulmonary fibrosis.

NE Gene and Its Variations

The gene for NE is officially named by HGNC (HUGO Gene Nomenclature Committee) as Neutrophil Elastase 2 with the gene symbol *ELA2* to differentiate from similar enzymes elastase1 and elastase 3 (*ELA1* and *ELA3A* or *B*) produced in the pancreas. The gene is located on chromosome 19p13.3 with five exons and four introns. During the past few years, up to 30 different mutations have been identified on the gene and these mutations are linked to two rare inherited diseases, cyclic neutropenia and congenital neutropenia. There are over 47 ►SNPs deposited in the NCBI SNP database within the 5 kb region of the gene, with one validated from the HapMap project, but their functional significance is largely unknown.

In the 5′ flanking region of ELA2, there are six tandem repeats of a 53-bp nucleotide sequence, which contains a potential binding site for a basic helix-loop-helix protein located at −1032 to −716. The structure has the enhancer function of *ELA2* activity. Two SNPs and one tandem repeat variation in the promoter region and their significance with lung cancer have been reported in the literature: T or G SNP at −903 bp, A or G at −741 bp from the transcription initiation site, and an extra 52-bp repetitive sequence between the fourth and fifth repetitive motifs.

NE and Cancer Development

The role of NE in cancer development is mostly inferred from cell line and animal models. In ultraviolet B or chemically (Benzopyrene) induced skin tumors, elastase-deficient mice develop significantly less tumors than normal mice, and administration of elastase inhibitors to normal mice generates the similar reduction of induced tumors. Inhibition of NE can significantly suppress the proliferation, motility, and chemotaxis of a ►pancreatic cancer cell line and completely stall the tumor growth or metastasis of human lung and ►colon cancer cell lines.

The polymorphisms at the promoter region of *ELA2* likely affect the gene expression activity and have been implicated in lung cancer risk from epidemiologic studies in humans. Individuals with −903TT or −741GG or certain haplotype (T-G) at the two loci have a significantly increased risk (two- to four-fold) of developing lung cancer than those with −903TG, −741AA/AG alleles or a haplotype of T-A; the elevated risk seems related to increased functional activity of the gene.

Long-term follow-up of patients with severe congenital neutropenia caused by mutations in one or several genes including *ELA2* reveals that ~1 in 10 patients develops ►myelodysplastic syndrome or acute ►leukemia; Proteinase 3 and neutrophil elastase are overexpressed in many myeloid ►leukemia. These suggest that NE dysfunction may lead to tumor development.

NE and Cancer Progression and Prognosis

Elevated NE has been detected in many different types of cancer, and the level of NE is associated with cancer stage, grade, and survival. Higher levels of NE are observed more often in later stage, larger or more invasive tumors than in earlier stage, and smaller or less invasive tumors. The patients with a high level of NE within tumors have shorter survival than those with lower levels of NE. Higher levels of NE in tumors are also associated with rapid relapse or recurrence of ►breast cancer and a higher grade of ►brain tumors.

Prognostic significance of NE in hematological tumors seems different from that in solid tumors. For example, low expression of CD7 in combination with high expression of NE or proteinase 3 in ►chronic myelogenous leukemia (CML) correlates with longer patient survival. This may be related to the fact that instead of working as an enzyme to degrade surrounding tissues, NE may be a tumor specific antigen to induce cytotoxic T lymphocyte immune response to kill tumor cells.

NE as Cancer Therapeutic Target

The association between elevated NE levels and tumor progression and metastasis has prompted researchers to explore the possibility of targeting NE as a treatment (Therapeutic target). A NE specific inhibitor such as ONO-5046_Na has shown a strong tumor inhibitory effect. In laboratory studies, when the inhibitor is administered into the tumor-bearing animals, it can completely suppress the tumor growth and appearance of metastatic foci for some solid tumors (breast and ►gastric carcinoma). Additionally, NE levels may be a marker in predicting a treatment response of breast cancer to an anti-HER2 antibody like Trastuzumab: a higher NE level was correlated with a better response. Using NE in ►CML as a specific antigen to stimulate CD8 induced immune response or developing an anti-NE vaccine to treat the tumor has also been proposed and is under investigation.

Possible Mechanisms of NE in Cancer Development and Progression

In spite of observed roles of NE in tumor development, progression, and metastasis, the mechanisms are unclear. Several potential mechanisms have been postulated based on laboratory experimental data. Multiple mechanisms more likely act together to give rise to the observed clinical presentations, acting and interacting differently among different cell types.

Carcinogen Exposure Enhancer

Individuals with varying degrees of lung tissue damage from NE as seen in ►emphysema may have longer exposures due to air trapping, thereby increasing absorption and enhancing the action of ►carcinogens present in ►tobacco smoke. This may be part of the reason emphysema patients have a much higher risk for developing ►lung cancer.

Apoptosis Inhibitor

NE may be directly involved in ►carcinogenesis through the ►tumor necrosis factor receptor (TNFR) signaling pathway. NE is capable of removing an active fragment of the TNFR-2 from the cell surface, and the removal of the TNFR-2 lowers the affinity of ►TNF for TNFR-1. The shed TNF receptors consume extra circulating TNF. All these cause significantly reduced response to TNF signaling and continued cell growth. TNFα signaling is a very complicated cell process, and TNF plays an important role in the pathway as a mediator of ►apoptosis, ►inflammation, and immunity. In its initial step, TNF, produced primarily by activated ►macrophages, binds to the outer domain of TNF-R1 located on the surface of the cell membrane. The binding triggers a series of intracellular events that ultimately result in the activation of ►caspase-8, leading to an ►apoptosis cascade and two other major transcription factors, ►nuclear factor κβ and ►c-Jun. c-Jun overexpression, an indicator of oncogenesis, was observed in lung cancer and its surrounding atypical areas. c-Jun overexpression was associated with increased breast cancer cell motility and invasiveness, and tumor formation in nude mice. All these may lead to uncontrolled tumor cell growth.

Invasion and Metastasis Promoter

NE released by inflammatory cells or produced by tumor cells breaks down the structural proteins of the extracellular matrix surrounding the tumor and makes it easier to invade and migrate. NE can activate matrix metalloproteinases such as ►MMP2 through membrane-type 1 matrix metalloproteinase (MT1-MMP) and ►MMP3. The MMPs are a group of Zn-dependent enzymes that have been extensively documented for their roles in tumor invasion and metastasis, particularly MMP2 and MMP9. Furthermore, NE released from activated leukocytes can injure endothelial cells and cause microcirculatory disturbance. These changes may in turn result in the expression of ►adhesion molecules and the formation of microthrombi in the capillaries, the conditions that promote cancer ►metastasis.

Cancer Promoting Gene Derepressor

Protease may remove the repression of a variety of genes via proteolytic cleavage of protein repressors and promote carcinogenesis. In prokaryotic and eukaryotic cells, the SOS system (►DNA repair system) plays a key role in fixing DNA damage caused by carcinogens and preventing cell transformation. However, the existence of proteases may induce an error-prone SOS system by hydrolyzing the protein repressor and render cells prone to transform.

NE Suppresses Granulopoiesis of Hematopoietic Cells

Patients with hematological disorders, such as severe chronic neutropenia and myelogenous leukemia, may have mutations in *ELA2* or alterations in the expression, localization, or activity of the proteins. These changes may lead to the deregulation of stem cell proliferation and differentiation in bone marrow through the ►G-CSF. NE generally has a counteractive effect (negative feedback) on the G-CSF and suppresses normal granulopoiesis. NE production, combined with

a relative independence from the G-CSF for proliferation, allows ►CML clonal dominance and accounts for the early loss of Philadelphia negative granulocytes during the course of leukemia.

In summary, excess neutrophil elastase may facilitate cancer development, but may also serve as a marker in predicting a treatment response. NE inhibitor may deter cancer progression and prolong patient survival therefore would deserve further evaluation for cancer prevention at multiple levels.

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Neutrophil Granulocytes

Definition

Phagocytic cells of the innate immune system with a characteristic segmented, multilobed nucleus. Active in the early phases of ►inflammation and equipped with multiple antimicrobial effector mechanisms.

►Bacillus Calmette-Guérin

Neutrophil Polymorphonuclear Granulocytes

Definition

PMNs; Synonym ►neutrophils; Are the most abundant type of white blood cells in humans important for initiation of the inflammatory process and in the protective immune response against infection.

►BORIS

Neutrophils

Definition

White blood cells, contain tiny sacs of enzymes that help the cell to kill and digest microorganisms which it has engulfed by ►phagocytosis. The neutrophil has a lifespan of about 3 days. Decrease of neutrophils is referred o as ►neutropenia.

Nevi

Definition

Plural of ►Nevus.

►Melanocytic Tumors

Nevocellular Nevus

►Melanocytic Tumors

Nevoid Basal Cell Carcinoma Syndrome

Definition

Synonym ►Naevoid Basal Cell Carcinoma Syndrome.

Nevus

Definition

Plural: nevi. UK: naevus; naevi); Usually brief for: ►melanocytic nevus, a benign neoplasm of pigment-producing cells (melanocytes) in the skin. Originally, a

nevus has been defined as a circumscribed developmental lesion of skin or mucous membrane involving excess or deficiency of any one of the normal structures. This broader definition explains the use of the term in some nonmelanocytic lesions (i.e. epidermal nevus, or sebaceous nevus, which are not melanocytic lesions). Clonal proliferation of melanocytes in the skin. The precise location of the nevus cells determines a histological classification into dermal, junctional or compound nevi. The majority of nevi are acquired. Nevi are more common in sun-exposed areas of the body. The distribution and number of nevi are strongly determined by heredity, interacting with sun-exposure. Numbers and size typically increase during puberty.

►Basal Cell Carcinoma
►Melanoma
►Melanocytic Tumors

Nevus of Ota

Definition

►Oculodermal Melanocytosis.

Newcastle Disease Virus

Definition

NDV; A poultry pathogen of the Paramyxoviridae family with a negative strand RNA genome, an helical nucleocapsid, and enveloped particles used in ►oncolytic virotherapy.

NFκB

Definition

Nuclear Factor kappa B.

►Nuclear Factor-κB

NF1

►Neurofibromatosis 1

NF2

►Neurofibromatosis 2

NF2 Gene Product

►Merlin

NGF

Definition

►Nerve Growth Factor.

NGF Family

►Neurotrophins

NHL

Definition

Non Hodgkin lymphoma.

►Childhood Cancer
►Hodgkin Disease
►Hodgkin and Non-Hodgkin Lymphomas

NHS

Definition

The National Health Service – the United Kingdom's social health care system which provides healthcare free at the point of delivery to the whole population which is funded from taxation.

►National Institute for Health and Clinical Excellence
►NICE

Nibrin

Definition

►NBS1.

NICE

Definition

Abbreviation for the ►National Institute for Health and Clinical Excellence. The organization that describes itself as "the independent organization responsible for providing national guidance on the promotion of good health and the prevention and treatment of ill health" in England and Wales.

NICE Blight

Definition

Refusal of local managers within the United Kingdom's National Health Service to allow the use of a new treatment because it has not yet been the subject of a review by the ►National Institute for Health and Clinical Excellence.

Nickel Carcinogenesis

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Synonyms

Nickel-induced cell transformation; Nickel tumorigenesis

Definition

Is the process of the formation of respiratory tumors (carcinomas) induced by inhalation of specific insoluble nickel compounds, and the series of sequential steps that occur when animals or humans are exposed to specific nickel compounds that leads to tumor development. After all these steps are accomplished, the physiological mechanisms regulating control of growth in the normal cells are degraded. Hence, the normal cells are degraded and converted into tumor cells. The tumor cells then grow autonomously in an unregulated fashion and evade the host immune system, leading to development of visible tumors.

Characteristics

Nickel, the Chemical Element, and its Ionic Species and Chemical Compounds

Nickel and iron form alloys that are found in many meteors. Nickel is also found in deposits in central regions of the Earth. Nickel is commonly found in nature. In nature, nickel is found in combination with ►arsenic, antimony, and sulfur. An example of a nickel compound found in nature is millerite, NiS.

Pentlandite, a sulfidic ore, and garnierite and nickeliferous limonite, which are lateritic ores, are some of the nickel-containing minerals of economic importance. Garnierite is commonly used as a generic name for a series of mixed nickeliferous silicates with a wide range of nickel contents. Nickeliferrous limonite is the term used to describe poorly crystalline nickel-bearing ferric oxide. The main constituent of nickeliferrous limonite is goethite (α-FeO.OH).

Some of the processes used in the refining of sulfidic ores include the carbonyl process and the chloride electrowinning process. The carbonyl process produces high purity nickel metal. In the carbonyl process, carbon monoxide is reacted with impure ►nickel oxide feed at 50°C at atmospheric pressure, or with nickel-copper matte at higher pressures and temperatures, to yield nickel carbonyl [$Ni(CO)_4$]. When nickel carbonyl is heated to 200°C and thereby thermally decomposed,

nickel metal of purity >99.9% can be obtained. Lateritic ores are usually smelted with or without the addition of sulfur to form ferronickel mate. This matte can be further refined by a variety of processes.

Elemental nickel is a solid metal with a silver-white color. It has a high thermal conductivity, a high electrical conductivity, and a high melting point (1452°C). Nickel metal can be drawn, rolled or forged, and polished. It is highly resistant to attack by air or water at ordinary temperatures when compact. Because of this, nickel is often electroplated and used as a protective coating.

The oxidation state of +2 is the most common state for most ordinary nickel chemistry. Higher oxidation states, such as Ni^{+3} and Ni^{+4} can occur in certain oxide systems, but these are rare. Ni^{+2} complexes exist as octahedral complexes, tetrahedral complexes, planar complexes, and trigonal complexes.

In specific biological systems, particularly in bacteria and in plants, nickel is a necessary component of four enzymes: (i) urease, (ii) carbon monoxide dehydrogenase, (iii) hydrogenase, and (iv) methyl-S-coenzyme M reductase. To date, nickel has been shown to be an essential element for certain animals (e.g. rats and goats). However, nickel has not been shown to be an essential element in humans, and no enzymes from mammalian cells have been shown to contain nickel.

Important Commercial Uses of Nickel and Nickel Compounds

Nickel is a very important and useful metal commercially. Nickel is used in large quantities in many important alloys, such as stainless steels. Nickel is also used as a catalyst in the hydrogenation of fats and oils, as a pigment in paints, and in ceramic glazes. In addition, nickel is used in nickel-cadmium batteries, in nickel plating, and in the manufacture of coins.

Exposure of Humans to Nickel Compounds

Humans are commonly exposed through the skin to nickel in the form of coins and jewelry without any harmful effects, except for rare cases of allergies to nickel in jewelry. In the past, exposures of refinery workers, who processed sulfidic nickel ores, to mixtures of insoluble and soluble nickel compounds as dusts and aerosols, in combination with simultaneous exposures to arsenic, in workers who also smoked cigarettes, correlated with an increased incidence of ►lung cancer and nasal cancer in the workers. These incidences of induction of cancer of the respiratory tract were believed to be due to exposure to a combination of mixtures of nickel compounds, sulfuric acid mists, and arsenic in workers who often smoked cigarettes. By contrast, exposures of workers to dusts containing metallic nickel, or to dusts produced during the refining of lateritic ores, have not been associated with increased respiratory cancer risks.

Genotoxicity of Nickel Compounds

Nickel compounds come in the soluble and insoluble forms. Nickel chloride and nickel sulfate hexahydrate are examples of water-soluble compounds of nickel. Soluble nickel compounds are not taken up into mammalian cells to any significant extent, since there is no known biological receptor for soluble nickel ions in mammalian cells. Hence, when humans or animals are exposed to soluble nickel compounds, these soluble nickel compounds are largely excreted unchanged into the urine.

There are many insoluble nickel compounds, such as ►nickel subsulfide (Ni_3S_2), green (high temperature) NiO, black (low temperature) NiO, and crystallline nickel monosulfide (NiS). If these insoluble nickel compounds are present in particle sizes of <10 μm, they are phagocytosed by mammalian cells. ►Phagocytosis involves an invagination of the plasma membrane around the particles, to form a phagocytic vesicle. The phagocytic vesicle then is internalized into the cell with its entrapped particle of insoluble nickel compound. Hence, by the process of phagocytosis, particles containing insoluble nickel compounds can be taken up into mammalian cells. The phagocytosed insoluble nickel compounds then enter the lysosomal network, as shown by fluorescent microscopy studies. The resultant intracellular nickel compounds then dissolve into soluble nickel ions and counterions. The nickel ions then migrate through the cell in an attempt to establish chemical equilibrium. Eventually, some Ni^{+2} ion may travel into the nucleus.

Following phagocytosis of insoluble nickel compounds by mammalian cells, the resultant intracellular Ni^{+2} ions cause cytotoxicity to the mammalian cells. In cell culture studies, the Ni^{+2} ions also induce ►chromosomal aberrations, in the forms of gaps, breaks, fragments, dicentrics, and satellite associations. In addition, Ni^{+2} ions have been shown to induce ►gene amplification in mammalian cells. This has been demonstrated for ►amplification of the ect-2 gene, a guanosine triphosphate (GTP)-guanosine diphosphate (GDP) exchange factor for the rho A protein (►rho family proteins). Hence, insoluble nickel compounds of particle size <10 μm can be phagocytosed by mammalian cells, liberate Ni^{+2} ions inside the cells, and as a result of this are ►genotoxic to mammalian cells. This genotoxicity is manifested in the form of chromosomal aberrations and gene ►amplification in the cells. Whether these effects occur in vivo remains to be confirmed. To date, there have been no mutation studies conducted in lung epithelial cells of animals inhaling insoluble nickel compounds.

There is also some evidence that nickel compounds can generate ►reactive oxygen species (ROS) in mammalian cells. In mammalian cells, ►superoxide radicals arise from normal cellular oxidative metabolism.

Following the dismutation of two superoxide radicals by superoxide dismutase, ►hydrogen peroxide is formed, particularly in the mitochondria. There is some evidence that intracellular Ni^{+2} ions can catalyze pseudo-Fenton reactions, in which Ni^{+2} ions can catalyze the reaction of superoxide radical and hydrogen peroxide to generate hydroxyl radicals and hydroxyl ions. The resultant hydroxyl radicals are able to cause formation of 8-hydroxy-deoxyguanosine, and therefore mutations, in DNA. However, this latter pathway has not been demonstrated conclusively with rigorous experimentation.

Nickel-Induced Cell Transformation

As noted above, nickel compounds can be phagocytosed into mammalian cells, leading to the accumulation of intranuclear Ni^{+2} ions. These intranuclear Ni^{+2} ions can induce chromosomal aberrations, which are genotoxic events. If these aberrations lead to loss of regions of chromosomes bearing ►tumor suppressor genes, or disrupt areas of chromosomes bearing tumor suppressor genes, this can lead to loss of tumor suppressor genes from cells, contributing to morphological, anchorage-independent, and neoplastic cell transformation.

Specific insoluble nickel compounds, such as green NiO and crystalline NiS, can also cause gene amplification, in particular, amplification of the ect-2 proto-►oncogene in cultured C3H/10T1/2 Cl 8 mouse embryo fibroblastic cells. This is also a genotoxic event. This leads to over-expression of the ect-2 proto-oncogene at the mRNA and protein levels. This event also contributes to nickel compound-induced morphological, anchorage-independent, and neoplastic cell transformation.

In addition, Ni^{+2} ions also bind strongly to histidine, imidazole, and cysteine, with association constants of approximately 100,000. Ni^{+2} ions can also bind strongly to histidine, imidazole, and cysteine groups of proteins, particularly to histones. When intracellular Ni^{+2} ions bind to histones inside the nucleus of mammalian cells, this causes condensation of chromatin. The resultant condensation of chromatin has been shown to lead to ►methylation of the promoters of genes, leading to their transcriptional repression. When this happens to actively transcribed tumor suppressor genes, they will become transcriptionally quiescent. This is an ►epigenetic event, or a non-genotoxic event. Transcriptional silencing of tumor suppressor genes by Ni^{+2} ions therefore could also be part of the mechanism leading to carcinogenesis by nickel compounds. Therefore, induction of cell transformation by specific, insoluble, carcinogenic nickel compounds occurs by a combination of genotoxic events (chromosomal aberrations and gene amplifications) and non-genotoxic/epigenetic events (►DNA methylation and transcriptional silencing of tumor suppressor genes).

Nickel Carcinogenesis

Some water insoluble compounds of nickel are respiratory carcinogens in rats. These include nickel subsulfide and green nickel oxide (NiO), when administered by inhalation. Mice and hamsters do not appear to be susceptible to the carcinogenic effects of nickel compounds. Interestingly, administration of soluble nickel compounds, such as nickel sulfate, to rats by inhalation does not induce lung tumors in these animals, even at the maximum tolerated dose. In humans, exposure of workers in nickel refineries in the past, to aerosols generated during the refining of sulfidic ores of nickel-containing mixtures of insoluble and soluble nickel compounds, was associated with increased incidences of nasal and lung cancer. In many cases, these workers were simultaneously exposed to arsenic and mists of sulfuric acid, as well as to cigarette smoke.

Mechanisms of Nickel Carcinogenesis

Mechanisms of nickel carcinogenesis appear to be due to a combination of genotoxic effects and non-genotoxic effects. The genotoxic effects include the ability of intracellular Ni^{+2} ions to induce ►amplification of ►proto-oncogenes, such as the ect-2 gene, and to cause chromosomal aberrations, which are both genotoxic events. There is also some thinking that nickel compounds can act as redox catalysts to cause pseudo-Fenton reactions, generating hydrogen peroxide and hydroxyl radicals. This could lead to generation of 8-hydroxy-deoxyguanosine in the DNA of mammalian cells, and hence to mutations in specific genes, such as proto-oncogenes and tumor suppressor genes. The evidence for this latter mutational pathway is less clear. In addition, intracellular nickel ions can also bind to histones, leading to chromosomal condensation. This can then lead to methylation of the promoters of various actively transcribed tumor suppressor genes, silencing their transcription. It is currently thought that a combination of genotoxic and epigenetic events leads to nickel carcinogenesis. These genotoxic events include induction of mutations in proto-oncogenes, activating them to oncogenes, through oxygen radical-induced mutations, or chromosomal aberrations in regions containing proto-oncogenes, activating them to oncogenes, or amplification of proto-oncogenes. Additional types of genotoxic events include mutational inactivation of tumor suppressor genes, possibly through nickel-generated oxygen radicals, or loss of chromosomes bearing tumor suppressor genes. An example of an epigenetic event induced by intracellular ions would be nickel-induced chromosomal condensation, leading to methylation and hence transcriptional silencing of tumor suppressor genes. All these intracellular nickel ion-induced events together, both genotoxic and epigenetic, activate proto-oncogenes and inactivate

specific tumor suppressor genes, leading to global deregulation of gene expression, the emergence of the first tumor cell, and hence, carcinogenesis.

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Nickel-induced Cell Transformation

►Nickel Carcinogenesis

Nickel Oxide

Definition

A nickel compound containing one atom of nickel and one atom of oxygen. During nickel refining, the nickel in nickel-containing ores is usually converted to nickel subsulfide, then roasted to convert it to nickel oxide. There are two prominent types of nickel oxides – green (high temperature) NiO, which is formed at calcining temperatures above 1,100°C, and black (low temperature) NiO, which is formed at calcining temperatures below 1,100°C.

►Nickel Carcinogenesis

Nickel Subsulfide (Ni_3S_2)

Definition

A nickel-containing compound consisting of nickel and sulfur, that is an insoluble nickel compound. This compound is one that nickel in sulfidic nickel-containing ores is usually converted to during the process of nickel refining. This nickel compound is phagocytosed by mammalian cells and can cause cytotoxicity, chromosomal aberrations, and morphological transformation of mammalian cells and lung tumors when lower animals inhale it.

►Nickel Carcinogenesis

Nickel Tumorigenesis

►Nickel Carcinogenesis

Nicotine

Definition

A compound present in tobacco and tobacco smoke which is responsible for the addictive properties of these products.

►Tobacco Carcinogenesis
►Nicotine Addiction

Nicotine Addiction

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Synonyms

Nicotine dependence; Tobacco addiction; Tobacco dependence

Definition

Some authors define nicotine addiction as a loss of autonomy over the use of tobacco resulting from craving

N

or other drug withdrawal symptoms that make it difficult or unpleasant to discontinue use. Others define addiction in terms of various combinations of symptoms that include nicotine withdrawal symptoms, loss of control over use, and continued use despite impairment to the person's health or social functioning.

Characteristics

People use tobacco to obtain nicotine. However, when tobacco is smoked or chewed, chemical and radioactive carcinogens are absorbed along with the nicotine. It is estimated that tobacco is responsible for about one third of all cancer deaths in the US, (►tobacco carcinogenesis and ►tobacco related cancers). The vast majority of tobacco users have made multiple attempts to discontinue tobacco use, but over 90% of quit attempts end in failure.

The Nicotine Withdrawal Syndrome

The ►nicotine withdrawal syndrome presents a formidable obstacle to maintaining abstinence. Symptoms include strong craving, urges or a need to use tobacco, restlessness, anxiety, bad mood, irritability, difficulty concentrating, and disturbed sleep. The nicotine withdrawal syndrome is the patient's experience of neurophysiologic events. The nicotine from a single cigarette occupies 88% of the nicotinic receptors in the human brain, and in rats, a single dose of nicotine triggers a number of enduring modifications in brain physiology. Some youths report nicotine withdrawal symptoms after smoking their first cigarette. Most smokers have experienced nicotine withdrawal before they have progressed to daily smoking. Indeed, nicotine withdrawal appears to be the primary factor leading to daily smoking. The time elapsed between the last cigarette and the onset of withdrawal symptoms is termed the ►drug latency to withdrawal. When symptoms of nicotine withdrawal first develop, the latency to withdrawal may be measured in days or weeks. In other words, a single cigarette can completely relieve withdrawal symptoms and postpone their reappearance for long stretches of time.

Drug Tolerance

With repeated use, ►drug tolerance to nicotine develops and the latency to withdrawal ►progressively shortens as cigarettes become less effective at staving off withdrawal. Over time, smokers who could initially manage their withdrawal by smoking one cigarette per week, find they must smoke several days per week, and then every day. Unless a constraint is placed on the frequency of smoking, most will progress to smoking every hour. As nicotine has a half-life of about 2 h, this maintains the presence of nicotine in the brain throughout the day. In some smokers, the latency to withdrawal can be as short as 30 min and withdrawal begins even while nicotine is present in high concentrations in the brain. Behaviors such as smoking immediately upon awakening, smoking when sick in bed, and giving up other activities in order to smoke, are manifestations of severe tolerance and the correspondingly short latency to withdrawal.

Early Onset of Addiction

All of the symptoms of nicotine addiction that are not manifestations of tolerance are present in individuals who smoke only one cigarette per week or less. Many adolescent smokers report symptoms of nicotine addiction after smoking only a few cigarettes. Failed attempts at smoking cessation are very common among smokers who have never smoked as much as one cigarette per day. If tolerance did not develop, nicotine addicted smokers might forever be content with smoking one cigarette per week, and tobacco use would be a negligible contributor to cancer mortality.

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Nicotine Dependence

►Nicotine Addiction

Nicotine Withdrawal Syndrome

Definition

Dysphoric symptoms that appear upon the discontinuation of even brief or intermittent use of nicotine.

►Nicotine addiction

NIH-3T3 Cells

Definition

Murine fibroblast permanent in vitro cultured cell line, often used in experimental analyses, like ►NIH3T3 transformation assay, or in experimental drug tests.

NIH3T3 Transformation Assay

Definition

This assay, which is essentially based on the focus-forming assay developed by Howard Temin for titrating particles of transforming ►retroviruses in chicken embryonic fibroblasts, makes use of the contact inhibition response of the murine embryonic fibroblast cell line NIH3T3. Originally, this assay was developed to discover novel oncogenes by transfecting NIH3T3 cells (host) with sheared genomic tumor cell DNA (donor DNA). Host cell clones, which had acquired a active oncogene from the donor DNA, loose the characteristic contact inhibition response, continue to proliferate in the environment of a confluent monolayer and will eventually give rise to a clonal colony of transformed cells (focus). In later years, cDNA libraries were employed using classical transfection techniques or retroviral vectors. The focus can then be isolated and the acquired oncogene from the donor DNA can then be identified using various molecular biological techniques. Several ►oncogenes, e.g. ►*RAS*, were identified using this assay.

►B-Raf Somatic Alterations
►RAS Activation

Nijmegen Breakage Syndrome

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Synonyms

Ataxia-telangiectasia variant 1 and variant 2; Berlin breakage syndrome; Semanová II syndrome

Definition

A genetic disease characterized by an extreme sensitivity towards ionizing radiation and a high risk for the development of lymphatic tumors.

Characteristics

Nijmegen breakage syndrome (NBS) is a rare genetic disease; inheritance follows an autosomal recessive modus with complete penetrance. Although birth weight and size of homozygotes are normal, microcephaly and growth retardation soon become apparent and are major symptoms of the disorder. Progressive mental retardation is typical, however, IQ is usually within the normal range during early childhood. A sloping forehead and receding mandible give NBS patients a characteristic facial appearance which becomes more pronounced with age. NBS patients are immunodeficient in both cellular and humoral immune systems leading to frequent infections particularly of the respiratory tract. Approximately 40% of NBS patients develop cancer before the age of 21. B- and ►T-cell lymphomas are the most common malignancies seen, although ►rhabdomyosarcoma, hemoblastoma, and ►neuroblastoma have all also been observed.

NBS is caused by biallelic mutations in the *NBN* gene which codes for ►nibrin, a protein involved in the repair of DNA damage. The majority of patients are homozygous for a 5 bp deletion in the *NBN* gene, a ►founder mutation. However, ten further mutations, all of which also lead to a truncated protein, have also been described.

The involvement of nibrin, in the repair of DNA damage, is demonstrated by the chromosomal instability observed in patient cells. The cytogenetic aberrations seen are chromatid breaks and recombination figures, frequently involving chromosomes 7 and 14 where immunoglobulin and T-cell receptor genes are located. The frequency of chromosome breaks is greatly increased in patient cells after irradiation, or treatment with radiomimetic drugs. Indeed, their radiosensitivity makes treatment of malignancy in these patients problematical.

Cellular and Molecular Regulation

The DNA lesion caused by ionizing radiation, to which NBS patients and their cells are most sensitive, is the double-strand break (DSB). Unrepaired DSBs increase the mutation rate in NBS cells and thus contribute towards accelerated tumorigenesis.

Three distinct repair systems are available for the repair of DSBs in eukaryotic cells: nonhomologous end joining (NHEJ), single-strand annealing (SSA), and homologous recombination (HR). NHEJ/SSA systems are active particularly before DNA replication while repair after or during DNA replication can exploit the potentially error-free process of homologous recombination.

Nibrin is a component of the RAD50/MRE11/nibrin complex which is probably involved in all three repair pathways. DSB repair by the RAD50/MRE11/nibrin complex after ionizing radiation can be visualized microscopically in cells by immunostaining with antibodies to MRE11, RAD50, or nibrin. Discrete nuclear foci, the sites of DSB repair, can be seen in normal cells but are completely lacking in cells from NBS patients: nibrin is apparently responsible for the recruitment of the complex to the sites of DSB repair. Electron microscopy and other techniques suggest that the trimeric complex forms a bridge at the site of a DSB to hold the DNA ends together, clearly a requisite for repair by any of the three mechanisms mentioned above. Furthermore, the MRE11 component of the complex has exonuclease activities which may be required for general preparation of DNA ends before repair by one of the specialized processes.

Mutations in *MRE11* and *RAD50* lead to genetic diseases with a similar cellular and clinical phenotype to NBS as do mutations in the gene ►*ATM*, responsible for ►Ataxia telangiectasia. ATM is a central player in the response to DNA damage and it seems that a major function of the RAD50/MRE11/nibrin complex is to recruit ATM to the sites of DNA-DSBs where it is activated by monomerization and autophosphorylation. Activated ATM then leads to arrest at cell cycle checkpoints to prevent entry into S-phase or mitosis with damaged DNA. Indeed both AT and NBS patient cells fail to stop semiconservative DNA synthesis or prevent entry into S-phase in response to a mutagenic challenge. Thus in addition to its repair functions, nibrin is also clearly important for the correct functioning of cell cycle checkpoints.

Clinical Relevance

Reduced cell proliferation and increased apoptosis due to the accumulation of DNA damage explain the growth retardation and microcephaly of NBS patients. Similarly, their immunodeficiency stems from the involvement of nibrin in the processing of DSBs which are formed as a natural intermediate during immunoglobulin gene-rearrangement and class switching. In terms of somatic mutation rate, the NBS cell, which due to the loss of nibrin function is unable to effectively repair DNA-DSBs by any pathway, and to arrest the cell cycle appropriately, is particularly endangered. Null mutation of *NBN* in mice is embryonically lethal and survival of NBS patients is attributable to the fact that the known *NBN* mutations are hypomorphic and partially active nibrin fragments are found in patient cells. The occurrence of cancer in NBS patients is probably modulated by the amount of these nibrin fragments.

Cancer incidence amongst NBS heterozygotes is increased and the impact of NBN mutations within the general population is therefore of some concern. In several studies, sequence variations in NBN have been found in patients with various malignancies suggesting that in some populations, *NBN* mutations may be responsible for a significant proportion of cancers.

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Nilotinib

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Synonyms

Tasigna™; AMN107; 4-Methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide

Definition

Nilotinib (Novartis Pharma AG) is a novel and selective inhibitor of the ►BCR-ABL tyrosine kinase, which was developed to override resistance to the BCR-ABL inhibitor, ►imatinib (Glivec®, Gleevec™, STI571; Novartis Pharma AG).

Characteristics

Implications of Nilotinib in Treatment of Imatinib-Resistant CML

►Chronic myelogenous leukemia (CML) comprises approximately 15% of adult leukemias. The *BCR-ABL* ►oncogene, which is the end product of a reciprocal ►t(9;22) chromosomal translocation, encodes a protein that has constitutively activated ABL tyrosine kinase activity, and which is the underlying cause of CML. CML patients express a 210 kDa BCR-ABL protein,

and ►Philadelphia chromosome positive (Ph±) ►acute lymphoblastic leukemia (ALL) patients express a smaller 190 kDa BCR-ABL protein.

The essential role that BCR-ABL plays in causing CML prompted the design and development of the small molecule kinase inhibitor (►small molecule drugs), imatinib, which is an effective frontline therapy for CML. In addition to ABL kinases, imatinib also inhibits the PDGFR and c-KIT kinases. Imatinib induces ►complete hematologic remissions in the majority of newly diagnosed patients in the ►chronic phase of the disease, and complete ►cytogenetic responses in a high percentage of patients. However, patients in the ►accelerated phase or ►blast phase/►blast crisis advanced of CML, as well as Ph + ALL patients, have substantially poorer response rates and shorter remissions in response to treatment with imatinib as a single agent.

Resistance to imatinib often results from the development of point mutations in BCR-ABL that impedes binding of imatinib to its target, although resistance has also been attributed to amplification of the *BCR-ABL* gene with overexpression of the BCR-ABL protein. In some patients, BCR-ABL mutations may exist as a rare event prior to imatinib therapy and result in expansion of that clone due to the selective pressure of imatinib. However, imatinib-resistant BCR-ABL point mutations can also be acquired, or arise during imatinib treatment, or may be transient in some cases. The majority of the more than 50 BCR-ABL point mutations that have been described are found in the kinase domain of BCR-ABL, however those conferring the most resistance are found at or near residues that are in direct contact with imatinib. For example, an isoleucine substitution at residue 315 in the BCR-ABL kinase domain (T315I mutation) is highly resistant to imatinib. This residue has been found to be critical for the interaction of imatinib with ABL, and is located at the so-called ►gatekeeper position at the periphery of the nucleotide binding site of the protein. This mutation sterically hinders imatinib binding to ABL, which leads to imatinib resistance.

Development and Preclinical Studies with Nilotinib

Researchers at Novartis Pharmaceuticals carried out rational design of novel kinase inhibitors displaying efficacy against imatinib-resistant BCR-ABL point mutants, leading to the discovery of the ►phenylaminopyrimidine-based ATP-competitive inhibitor, nilotinib. Nilotinib was designed to fit into the ATP-binding site of BCR-ABL with a higher affinity than imatinib. In addition to displaying a higher potency than imatinib against BCR-ABL, nilotinib also has the ability to inhibit many forms of BCR-ABL that are resistant to imatinib due to the presence of kinase domain point mutations. An exception is the T315I mutation, which in addition to being highly resistant to imatinib, is also resistant to nilotinib, as well as ►dasatinib (Sprycell™; BMS-354825; Bristol Myers Squibb), another second generation inhibitor of BCR-ABL. Dasatinib has been granted US Food and Drug Administration (FDA) approval for the treatment of adults in all phases of CML with resistance or intolerance to imatinib therapy, as well as for treatment of adults with Ph + ALL with resistance or intolerance to prior imatinib therapy.

Crystallographic analysis of nilotinib in complex with ABL, shows that the drug binds, like imatinib, to the inactive conformation of ABL, and suggests that the better overall topographical fit to ABL and subtle differences in the nature of binding to ABL are responsible for the higher potency of nilotinib (versus imatinib) (►drug design).

Nilotinib is ~20- to 30-fold potent than imatinib in inducing programmed cell death of BCR-ABL positive cells. Nilotinib was also shown to prolong the survival of mice harboring BCR-ABL positive leukemia, as well as mice harboring imatinib-resistant disease. There is growing interest in testing the notion that administration of multiple ABL kinase inhibitors simultaneously or sequentially can prolong the onset of emergence of imatinib-resistant BCR-ABL point mutants. Indeed, additive to ►synergistic toxicity against BCR-ABL positive cells that are either sensitive or resistant to imatinib has been observed when combining nilotinib and imatinib compared to treatment with a single inhibitor. Preliminary reports suggest that the different mechanisms by which nilotinib and imatinib are taken up by cells may contribute to the ability of these agents to synergize with one another.

Nilotinib Resistance

To assist in the selection of patients who are most likely to benefit from nilotinib, as well as to assess those combinations of ABL inhibitors that are likely to be the most effective, researchers have appreciated the importance in being able to establish the resistance profiles of imatinib, nilotinib, dasatinib and other BCR-ABL inhibitors that are expected to be used alone as therapeutics, or in combination with other BCR-ABL inhibitors. As is the case with imatinib, mutations in the kinase domain of BCR-ABL are likely to be associated with resistance to second generation inhibitors such as nilotinib. *In vitro* random mutagenesis assays have been designed and developed to predict such mutations, and some overlap has been observed between the pattern of mutations arising that confer nilotinib or dasatinib resistance to the pattern of mutations arising that confer imatinib resistance. The T315I mutant is one common mutation recovered in these assays that confers resistance to imatinib, nilotinib, and dasatinib. These *in vitro* findings are consistent with clinical findings in imatinib-treated CML and ALL patients. In addition to

random mutagenesis of BCR-ABL *in vitro* serving as a tool to help predict mechanisms of ►drug resistance, highly sensitive, ►PCR-based screening assays and denaturing high performance liquid chromatography (D-HPLC)-based assays have also greatly facilitated the identification of BCR-ABL point mutations in drug-resistant patients. These strategies could be used to establish the best course of treatment for patients resistant to imatinib or second generation BCR-ABL inhibitors like nilotinib and dasatinib.

Clinical Assessment of Nilotinib

Early phase clinical findings indicate that nilotinib may have significant clinical utility in treating patients who have developed resistance to imatinib treatment due to the emergence of point mutations. The clinical trial data also support the notion that nilotinib could potentially be used as a single agent in patients who are at risk for disease progression. The promise of nilotinib in the treatment of imatinib-refractory CML is due to its strong binding affinity to ABL, its efficacy against imatinib-resistant BCR-ABL point mutants, and its effectiveness and tolerability in clinical trials.

Significant clinical responses have been obtained in phase I and phase II trials in CML patients with advanced (accelerated or blast crisis) disease and CML patients in the early, chronic phase of the disease.

Future Prospects

While second generation BCR-ABL inhibitors, such as nilotinib and dasatinib, are predicted to substantially slow down the progression of BCR-ABL-positive disease in imatinib-resistant patients, the development of ►drug resistance is a general challenge in treating cancer patients. Even with the current push toward design of more "global" pan-BCR-ABL inhibitors, which have the ability to override resistance to highly imatinib-resistant BCR-ABL point mutants like T315I, there will still be the potential for the emergence of new drug-resistant BCR-ABL point mutations in positions that interfere with drug-target binding or that influence the expression and/or activity of the BCR-ABL protein. This warrants the continuation of identification and development of novel inhibitors of BCR-ABL, as well as the combined therapeutic use of more than one BCR-ABL inhibitor, especially those that bind at non-overlapping sites, or inhibitors of different signaling pathways.

►Signal Transduction

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Nipple Construction

Definition

Surgery to reproduce the appearance of the nipple after it has been removed for cancer.

►Oncoplastic Surgery

Nitric Oxide

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Definition

NO; is a combination of two of the most common gasses in the atmosphere; nitrogen and oxygen, and is produced in mammals by various cell types where it has a physiological role and also plays a role in some pathophysiological states.

Characteristics

NO was previously considered to only be an atmospheric pollutant until it was first demonstrated in 1987 that mammalian cells generate NO and that it accounted for the bio-activity of a substance produced by the endothelial cells initially named as endothelium-derived relaxing factor (EDRF). Once produced by the endothelial cells, this substance diffuses rapidly to the surrounding vascular smooth muscle and causes relaxation and, hence, the initial name. This discovery that NO is produced in mammals led to an explosion in NO research to identify its role in both normal and diseased states in mammals. NO produced by endothelial cells plays a physiologic role in maintaining blood pressure and blood flow to various organs. NO is also produced by numerous other cell types, including the central nervous system where it serves as a neurotransmitter. In the peripheral nervous system, NO serves as a neurotransmitter in a network of nerves termed the nonadrenergic and noncholinergic nerves and mediates some forms of neurogenic vasodilation regulating various gastro-intestinal, respiratory, and genito-urinary functions. NO modulates platelet aggregation, cardiac contractility, and is also produced in large quantities during host defense as it is generated by ►macrophages following their activation and

probably has a role in non-specific immunity. It is a mediator in various pathophysiologic states, such as inflammation, septic shock, and in the hyperdynamic state of cirrhosis. More recently, its role as an intracellular messenger, in modulation of cellular respiration, and in tumor biology affecting, both, growth and death of cancer cells has received increased attention.

Chemistry and Biological Synthesis of NO

NO is a colorless gas that is moderately soluble in water (up to 2 mmol/l) but is highly soluble in organic solvents. It has a biological half-life of 3–30 s. In view of its lipophilic nature it can diffuse through cell membranes very easily and because of its short half-life, acts locally at the site of production. NO is biosynthesized in mammalian systems via the enzymatic oxidation of a terminal guanidinium nitrogen on the amino acid l-arginine. *N*-Hydroxy-l-arginine is formed as an intermediate in the conversion of l-arginine to NO. The other product of this reaction is the amino acid, citrulline (Fig. 1).

NO biosynthesis from arginine is carried out by a class of enzymes known collectively as ►nitric oxide synthases (NOS). NOS can be immediately classified into two distinct classes: an inducible class (►iNOS), and that which is constitutively present in cells, the constitutive class (►cNOS). Within each class, subclasses exist. For example, ►cNOSs have been isolated and characterized from endothelial cells and brain and are sometimes referred to as ►eNOS (also known as type III) and nNOS (also known as type I), respectively. Inducible NOS has been found in a variety of cells, although most of the enzymological work has been performed on ►iNOS (also known as type II) from macrophages. The eNOS and nNOS are regulated by calcium ions via the calcium binding protein calmodulin and produce low concentrations of NO, usually in the nanomolar range, and are responsible for physiological functions such as smooth muscle relaxation. iNOS is translationally regulated and does not require calcium for activity. iNOS induction leads to production of high concentrations of NO, usually in the micromolar range, and is usually produced during pathophysiological states.

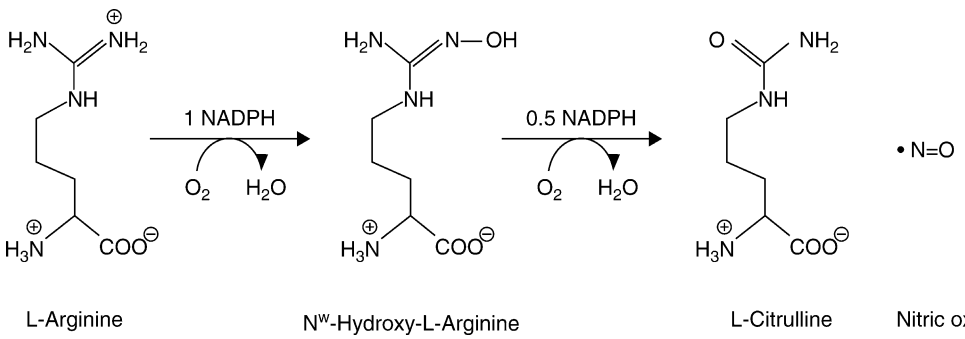
Biochemical Basis of NO Action

NO has an unpaired electron and is, therefore, a free radical. It can be either oxidized or reduced to form the nitrosonium cation (NO^+) or nitroxyl (HNO), respectively. NO and derived species can react with numerous substances, including metals and thiols. As such, it can cause cysteine modification to form *s*-nitrosothiols and many proteins can be modified by nitrosation after their synthesis (post-translational modification), which can affect various cellular functions.

NO can also react with the superoxide anion O_2^- to form peroxynitrite ($ONOO^-$), which, although less stable and short lived than NO, is much more cytotoxic and can cause DNA damage. Another method of NO signaling involves the activation of cytosolic soluble guanylate cyclase where NO acts as a ligand for the enzymes heme group and, following binding, alters the coordination state of the heme iron leading to an increase in the rate of ►cGMP formation. In general, low concentrations of NO, as produced in physiologic states, act by activation of ►guanylate cyclase (GC), whereas, higher concentrations of NO act through a GC-independent manner.

Cancer and NO

The precise mechanism by which cancer develops is not exactly known. It is characterized by uncontrolled cell division with the ability to spread, either by direct growth into adjacent tissue through invasion or by implantation into distant sites by ►metastasis. The unregulated growth that characterizes cancer can be caused by damage to DNA. This can result in mutations to genes that encode for proteins controlling cell division.



Nitric Oxide. Figure 1 Nitric oxide biosynthesis from L-arginine is carried out by a class of enzymes known collectively as the nitric oxide synthases (NOS). The terminal guanidinium nitrogen on the amino acid L-arginine is initially N-hydroxylated by NOS to give as a biosynthetic intermediate, N-hydroxy-L-arginine, which then undergoes further oxidation to NO and citrulline, which is formed in stochiometric amounts.

Role of NO in Initiating Cancer

NO can cause mutation of genes and, therefore, long term exposure of cells to high concentrations of NO following induction of iNOS as a result of chronic inflammation could play an active role in carcinogenesis. This can occur by various mechanisms, including DNA damage or by inactivating a tumor suppressor oncoprotein ►p53. There is a strong association between iNOS activity and p53 mutation in many forms of ►lung cancer, in human ►colon cancer, and also some cases of carcinoma of the head and neck. However, a cause and effect is yet to be established, although peak iNOS activity has been reported in colonic adenomas before transition to carcinoma, thereby, supporting a carcinogenic role. Increased NOS activity has also been suggested for some forms of ►breast cancer.

Role of NO in Cancer Cell Proliferation and Tumor Growth

Nitric oxide has both tumor promoting properties and it can also cause ►cytostasis and ►apoptosis of cancer cells and this is usually concentration dependent. Low concentrations of NO are usually tumor promoting, whereas, high concentrations are usually tumoricidal. Low concentrations of NO increase the translation of cyclin D_1 and ornithine decarboxylase proteins, and lead to the proliferation of some breast cancer cell lines. However, this effect is cGMP-independent indicating that low concentrations of NO can act by both a cGMP-dependent and independent manner. This effect is mediated by ►ras, a protein which is attached to the cell membrane following its posttranslational modification. NO activates ras, which acts as a molecular switch that links the activation of cell surface receptors with signaling cascades by activating ►MAP kinase, certain key proteins downstream by attaching a phosphate group, which ultimately leads to translation of some key proteins involved in cell proliferation. Low concentration of NO may also cause cancer cell proliferation by a cGMP-mediated mechanism.

Various other factors modulated by NO, in addition to tumor cell proliferation, like cell survival, migration invasiveness, function of immune cells infiltrating tumors, as well as blood supply to the tumor, can affect tumor growth. Increase in blood supply to the tumor is due to the development of new blood vessels from preexisting vessels, a process known as ►angiogenesis. NO stimulates proliferation and migration of endothelial cells in vitro. ►Vascular endothelial growth factor (VEGF) is the major factor implicated in angiogenesis of many human tumors and NO is its final mediator. Administration of NOS inhibitors has been shown to reduce neovascularization in gastric ulcers induced by acetic acid in rats and human squamous cell carcinoma xenografts in rabbit cornea. The source of NO in tumors could be from the tumor cells themselves or from macrophages infiltrating the tumors. In breast cancer, iNOS expression by macrophages, stromal cells, and tumor cells accounted for most of NOS activity and the greater the iNOS activity, the more the undifferentiated form and the worse the prognosis of the tumor. Although iNOS produces high concentrations of NO and should therefore slow tumor growth, in breast cancer it is associated with a worse prognosis. This paradox has been explained on the basis that, although iNOS is expressed in breast cancer, the activity is at least one to two orders of magnitude lower than the enzyme activity associated with cytotoxicity and apoptosis.

NO and Tumoricidal Activity

NO was first shown to be an essential component of the macrophage antitumor activity against leukemia cells in vitro. Macrophage-produced NO has also been shown to have tumoricidal activity in vivo. When cultured in vitro, peritoneal macrophages obtained from mice previously inoculated with ►bacillus calmette guerin (BCG) release NO, which is cytostatic and/or cytolytic for tumor cells. ►Interferon-γ (IFN-γ) is important for the priming of macrophages and ►tumor necrosis factor-α (TNF-α) or some other cytokine or bacterial lipopolysaccharide, is necessary for full induction of activated macrophage toxicity. NO plays an important role in vivo in mediating both the host resistance to a ►syngeneic ovarian tumor in BCG inoculated mice and host resistance to a ►xenogeneic ovarian tumor graft. Inhibiting NO synthesis obliterates this action of BCG by inhibiting the NO-mediated immune response in both syngeneic and xenogeneic tumor grafts. Similarly, transfection of human tumor cells with iNOS resulted in enhanced iNOS activity, as well as complete inhibition of tumor growth and regional lymph node metastasis in nude (immunocompromised) mice. Moreover, the ablated tumor growth was associated with NO-mediated cytostasis and enhanced apoptosis of the transfected cells, as well as the bystander cells. Various sites of action are involved in the cytostatic and apoptotic actions of high concentrations of NO. These actions are cGMP-independent and involve various signaling cascades with the ►BCL-2 pro-apoptotic proteins ultimately converging to the mitochondria leading to cytochrome *c* release and activation of caspases. The differences in the mechanism of apoptosis of cancer cells, when compounds which release NO are utilized, when compared with cells transfected with NOS, is that when utilizing the NO donor, only the specific action of NO is observed. However, when the effects of NO produced by activated macrophages or cells transfected with NOS are utilized, in addition to NO production, the superoxide radical can be produced leading potentially to peroxynitrite formation, which is cytotoxic and will have a different mechanism of apoptosis when compared with NO.

Furthermore, during biosynthesis of NO from l-arginine, the intermediate compound formed, i.e. *N*-hydroxy-l-arginine, has apoptotic action on human breast cancer cells, which is mediated by an entirely different mechanism than that of NO.

Action of NO on Cellular Respiration

Mitochondria, which are organelles inside the cell, have been considered to be the powerhouse of the living cell, generating energy in the form of the molecule ATP via the reduction of oxygen. They play a role in apoptosis (►apoptosis signaling), or programmed cell death At physiological concentrations, NO, in the nanomolar concentration range that activates soluble GC, can also bind to ►cytochrome *c* oxidase (complex IV) and inhibit it in a manner that is reversible and in competition with oxygen. Cytochrome *c* oxidase is the terminal enzyme of the mitochondrial respiratory chain and is responsible for 90% of cellular oxygen consumption in mammals. NO competes with oxygen at cytochrome *c* oxidase and, therefore, any increase in NO concentration can prevent the enzyme from using any available oxygen, thus, causing a kind of metabolic hypoxia. Such "metabolic hypoxia" may also be responsible, in part, for the well known increase in glucose uptake in tumors.

Clinical Relevance

At present, the potential for treatment of cancer with compounds that release NO are in the experimental stage (►apoptosis-induction for cancer therapy). As endogenous NO increases blood flow to tumors, and also mediates the action of ►VEGF in inducing ►angiogenesis, inhibitors of NOS have been used in animal models with tumor to asses their effects. Inhibitors of NO synthesis reduced blood flow in both animal models of tumors as well as in humans with ►lung cancer, ►prostate cancer and ►cervical cancer. All patients had sustained reduction in tumor vascular blood volume following administration of an inhibitor of NO synthesis. However, the long term effects of inhibiting NOS on tumor and their side-effects on other organs needs to be assessed. Use of NO donors to increase tumor sensitivity to ►radiotherapy is also being tried. Elevated oxygen tension in tumor cells increases their sensitivity to radiation. Tumor sensitivity to radiation is compromised by low oxygen tension, especially at the center of the tumors, which is usually necrotic. It has been suggested that use of hyperbaric oxygen along with NO donors would permit blood vessels to dilate, thereby increasing the blood flow and oxygen tension in the tumor. This in turn would increase its sensitivity to radiation. Targeted delivery of NO by combining ►nonsteroidal anti-inflammatory drugs (NSAID) to an NO donor (NO)-NSAIDs, which were initially conceived for rheumatologic and cardiovascular applications, are now under intense study for their anticancer properties, especially as a safe and effective chemopreventive agent against ►colon cancer. Similarly, targeted delivery of NO by combining an NO donor with tamoxifen may be potentially useful for the treatment of some cases of breast cancer. Intraluminal installation of ►BCG has been utilized for treatment of some forms of ►bladder cancer, and this is likely due to increased activation and production of NO by immune cells. Further studies need to be carried out to assess whether intraperitoneal installation of BCG may be effective for treating minimal residual disease following cytoreductive surgery for ovarian cancer.

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Nitric Oxide Synthases

Definition

NOS; Nitric Oxide biosynthesis from arginine is carried out by a class of enzymes known collectively as nitric oxide synthases.

►Nitric Oxide

Nitrogen-containing Bisphosphonate

►Minodronate

Nitrogen Mustards

Definition

The nitrogen mustards and their derivatives are a class of ►alkylating agents used in ►chemotherapy. Common nitrogen mustards and nitrogen mustard-derivatives include chlorambucil, chlormethine, cyclophosphamide, ifosfamide, mechlorethamine, mustine, mustargen and melphalan. These agents are most often used to treat ►Hodgkin disease, ►non-Hodgkin lymphoma, chronic leukemias, ►cutaneous T-cell lymphoma, and ►lung cancer and ►breast cancer.

►Chemotherapy of Cancer
►Hyperthermia

N-Nitrosamine

Definition

Compounds having a nitroso group bound to the nitrogen of a secondary amine.

►Tobacco Carcinogenesis

Nitrosamines

Definition

Are carcinogenic chemical compounds produced when nitrite, a preservative typically added to certain foods (especially beer, fish, fish byproducts, and certain types of meat and cheese products), combines with amino acids in the stomach. High temperatures, as in frying, can also enhance the formation of nitrosamines. Nitrosamines are also found in tobacco smoke and latex products.

►Carcinogen Metabolism

Nitrosoureas

Definition

Are a class of ►alkylating agents used in ►chemotherapy. Common nitrosoureas include carmustine, fotemustine, lomustine, and streptozocin. These agents act in a non-cell cycle phase specific manner to interfere with enzymes that are involved in the copying and repair of DNA. Are also strong ►carcinogens.

NK

Definition

Natural killer cells are part of the host defense against foreign invasion including infection.

►Metastasis

NK Cell Induction

►Natural Killer Cell Activation

NK Cell Stimulation

►Natural Killer Cell Activation

NKG2D

►NKG2D Receptor

NKG2D Receptor

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Synonyms

NKG2D; natural killer group 2D; KLRK1; killer cell lectin-like receptor subfamily K member 1; CD314

Definition

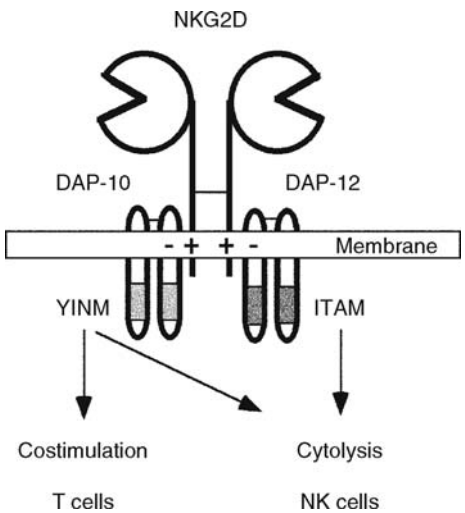
The NKG2D receptor complex mediates the elimination of distressed host cells via the activation of innate immune effector cells such as ►natural killer cells.

Characteristics

The NKG2D Receptor

NKG2D is a homodimeric, type II integral membrane protein, which belongs to a sub-family of C type lectin-like receptors. The NKG2D receptor is constitutively expressed on natural killer (NK) cells, most TCRγδ T cells, and a large fraction of NK T cells where it serves as a primary activation receptor. The *NKG2D* gene is located in the NK gene complex on mouse chromosome 6 or human chromosome 12. This locus harbors a significant number of C-type lectin like receptors that are preferentially expressed in NK cells.

NKG2D is a multi-subunit receptor complex, whereby the NKG2D homodimer mediates ligand binding. Specialized adaptor molecules, which are associated non-covalently, transduce signals into the cell (Fig. 1). Mouse NKG2D is linked to the adaptors ►DAP-10 and ►DAP-12, while human NKG2D exclusively uses DAP-10. DAP-12 contains a single immunoreceptor tyrosine-based activation motif (►ITAM). ITAM's are defined by the consensus sequence YxxL/I x_6 YxxL/I (whereby x denotes any amino acid). Upon receptor engagement, the tyrosines in the ITAM are phosphorylated by ►src family tyrosine kinases, which generate docking sites for Syk family kinases. The recruitment of Syk family kinases is needed to induce calcium flux and the activation of extracellular signal-regulated kinase (►ERK). Both events are required for the exocytosis of cytotoxic granules, which mediates target cell lysis. DAP-10 lacks an ITAM but instead contains a YINM sequence, which closely resembles a motif present in co-stimulatory molecules such as CD28. Nevertheless, also the NKG2D/DAP-10 complex is able to mediate granule release in human NK cells. In addition to cell-mediated cytotoxicity, NKG2D cross-linking results in the production of cytokines such as interferon-γ (IFN-γ).



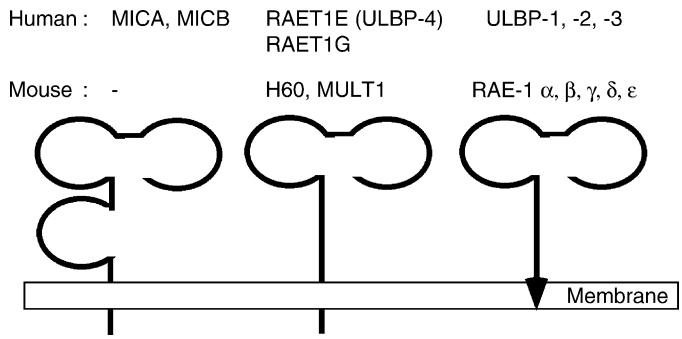
NKG2D Receptor. Figure 1 Schematic representation of the NKG2D receptor complex. The NKG2D receptor homodimer is non-covalently associated with the signaling adapters DAP-10 and/or DAP-12 (only in the mouse). DAP-12 signals via a single ITAM and mediates primary activation function in mouse NK cells. DAP-10 signaling is mediated via the YINM motif. In T cells, NKG2D/DAP-10 is costimulatory while in NK cells it mediates primary activation function.

Besides NK cells, NKG2D is constitutively expressed on human $CD8^+$ T cells and, upon activation, on mouse $CD8^+$ T cells. Since T cells lack DAP-12, NKG2D signaling is mediated exclusively via DAP-10. Signaling via the NKG2D/DAP-10 complex serves a co-stimulatory role in the T cell receptor (TCR) mediated induction of a T cell response. There are, however, instances where NKG2D can act as a primary activation receptor also in T cells. The prolonged exposure of ►T cells to ►interleukin (IL)-15 confers NKG2D-mediated cytolytic function to CD8 T cells independent of the specificity of their TCR. Thus the consequences of NKG2D receptor engagement are influenced by additional signaling inputs.

NKG2D Ligands

The NKG2D receptor recognizes a significant number of distinct ligands that are all distantly related to major histocompatibility complex (MHC) class I molecules (Fig. 2). In the human these ligands include the two ►MIC (MHC class I-chain-related) ►MICA, MIC proteins, and five ►RAET1 (►retinoic acid early transcripts)/ULBP (UL-16 binding proteins) family proteins. Murine NKG2D ligands correspond to the latter protein family and comprise MULT-1 (Murine UL-16-binding protein like transcript 1), ►H60 and the five ►Rae-1 (retinoic acid early 1 α to ε) molecules. The *RAET1*/*ULBP* genes are clustered in the telomeric region of human chromosome 6; a corresponding region with NKG2D ligands is found on mouse chromosome 10. The *MICA* and *MICB* genes localize to the human HLA locus on chromosome 6. They are not present in the mouse genome.

NKG2D ligand expression is not detected on differentiated cells in healthy adult humans or mice. Some expression occurs on immature precursor cells in the thymus and the bone marrow. In mature cells, surface expression of NKG2D ligands is induced upon various types of cellular stress. For example, heat shock induces MICA expression on colon epithelial cells. Retinoic acid, an agent used to differentiate cells, rapidly induces Rae-1 expression in embryocarcinoma



NKG2D Receptor. Figure 2 Schematic representation of NKG2D ligands. All NKG2D ligands are distantly related to MHC class I-molecules and contain MHC class I-like α1 and α2 domains, which mediate the interaction with NKG2D. MICA/B also have an α3 domain. None of these ligands is associated with β2m. Several murine and human NKG2D ligands have transmembrane and cytoplasmic domains. Others (ULBP1–3 and Rae-1s) are inserted into the membrane via glycosylphosphatidylinositol (GPI) anchors.

cells. NKG2D ligand expression is also detected in the context of bacterial and viral infection, which is based in part on signaling via ►Toll-like receptors (TLR). The importance of the NKG2D pathway to protect against infection is highlighted by viral mechanisms, which prevent the cell surface expression of NKG2D ligands on infected cells.

Normal cells express NKG2D ligands transiently due to various types of stress. In contrast, established tumor cell lines of various tissue origins constitutively over-express NKG2D ligands. This is linked to the process of malignant transformation, with a role for certain oncogenes (such as BCR-ABL or adenovirus E1A) or ►tumor suppressor genes (such as JunB). The ►DNA damage response plays an important role for the induction of NKG2D ligands in primary, untransfromed cells. DNA damage induced by ionizing radiation or alkylating agents results in double strand breaks and/or stalled DNA replication, which induces the ►ATM and ATR protein kinases. These mediate cell cycle arrest and DNA repair. ►Apoptosis via ►p53 is induced when the genomic damage is too extensive. p53 is not required for the induction of NKG2D ligand expression. Thus, cells sense genotoxic stress and rely this information to the cell surface in order to alert the immune system of a potential threat.

NKG2D and Cancer Immunosurveillance

The over-expression of NKG2D ligand on tumor cell lines confers the rejection of tumor cell grafts *in vivo*. Depending on the tumor cell line used, rejection is mediated by NK cells or the combined action of NK cells and $CD8^+$ T cells. The recognition of NKG2D ligand-expressing tumor cells can induce T cell memory, which is capable of controlling a subsequent challenge with corresponding NKG2D ligand-negative tumor cells.

Many primary human tumors express substantial levels of NKG2D ligand, whereas more advanced tumors and metastases express lower levels, if any, ligand. This is consistent with a role of NKG2D in tumor immunoediting: The immune system is thought to eliminate tumor cells that it recognizes as "transformed". Transformed cells, which progressively lack these specific tumor antigens, are spared. These give rise to tumors that can spread without eliciting an anti tumor immune response.

Consistent with this model, blocking of NKG2D receptor exacerbates the development of chemically induced skin tumors. Cell lines derived from primary tumors express high levels of NKG2D ligand and fail to grow in wild type mice. In contrast, when NKG2D function is left intact, growing tumors express lower levels of NKG2D ligand and grow upon transfer into wild type mice. The protective role of NKG2D during cutaneous carcinogenesis is mediated at least in part by resident TCRγδ cells, while NK cells reject transplanted tumors. Despite the role of NKG2D to protect against grafted or chemically induced tumors, there is currently no evidence that NKG2D protects from spontaneously arising tumors.

Tumor Escape

NKG2D ligands are induced at some point during the development of most types of cancers. Nevertheless, many NKG2D-ligand positive tumors progress, suggesting that NKG2D function is impaired at some stage during cancer progression. Indeed, tumor-derived ►matrix metalloproteinases can cleave MICs from the surface human tumors. The binding of soluble MIC results in NKG2D down-regulation and a reduction of NKG2D function. Similarly, prolonged engagement of NKG2D with cell-bound NKG2D ligands greatly impairs NKG2D function. Finally, tumor-derived immunosuppressive cytokines such as TGF-β (►transforming growth factor β) also reduce NKG2D cell surface expression and function.

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NM23 Metastasis Suppressor Gene

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Synonyms

Nme nonmetastatic in the human; Awd (abnormal wing discs) in Drosophila; Nucleoside diphosphate kinases; NDPKs; Ndks; NDP kinases

Definition

Nm23 is a ►metastasis suppressor gene. Metastasis suppressors are typically identified by their reduced expression in primary tumors or tumor cell lines of high metastatic potential as compared to equally tumorigenic but less metastatic cell lines. Validation of a gene as a metastasis suppressor involves its experimental introduction into a metastatic tumor cell line, resulting in expression at physiologic levels. Upon in vivo injection, MSGs significantly reduced metastasis with no significant effect on primary tumor size. Where studied, most of the MSGs impacted the late steps in the metastatic process, extravasation from the circulatory system (Kai-1), or colonization at the metastatic site (►Kiss1, Mkk4, ►Nm23-H1), consistent with their lack of effect on primary tumor size. Because of their novel mechanisms of action and impact on metastatic colonization, the MSGs may represent important therapeutic targets in cancer.

Characteristics

The ►metastatic process, in which cells spread from a primary tumor to distant sites of the body, is a significant contributor to cancer patient morbidity and mortality. Defining the key molecular pathways that contribute to metastasis will fuel the development of therapeutic approaches.

The concept of metastasis suppressive pathways arose from studies in which nonmetastatic and metastatic cells were fused, resulting in tumorigenic but nonmetastatic hybrids. Introduction of chromosomes into metastatic cells reduced metastatic capacity but not tumorigenicity. The list of validated metastasis suppressor genes (MSGs) is growing. Several, including ►E-cadherin, ►maspin, and the ►TIMPs, have predictable mechanisms of action in adherence, proteolysis and invasion. Others including ►*nm23*, ►*kiss1*, ►*KAI1*, and *Brms1*, have unknown mechanisms of action and may identify new pathways important to metastasis.

NM23-H1 and Family Members

The *nm23* gene was discovered using differential colony hybridization between low and high tumor metastatic potential K-1735 murine ►melanoma cell lines. Nm23 mRNA and protein levels were quantitatively higher in two cell lines of low metastatic potential than five related, more highly metastatic cell lines. A correlation of reduced *nm23* expression and high tumor metastatic potential was observed in many, but not all other model systems examined, consistent with the complexity and heterogeneity of the metastatic process. Since its discovery, eight human homologs of Nm23-H1 have been identified. These conserved (DR+HS larger). 16–20 kDa proteins localize to all subcellular compartments and are expressed by many cell types.

Several lines of investigation indicate a role for Nm23 in normal development and differentiation. Reduced expression or mutation of Drosophila awd resulted in defective differentiation of the presumptive epithelial cells in the imaginal discs postmetamorphosis, resulting in lethality. Introduction of *nm23* into rat PC12 ►phaeochromocytoma cells induced the extension of neurite processes. Similarly, *nm23* introduction into human MDA-MB-435 breast carcinoma cells induced morphological and biochemical aspects of mammary ductal differentiation. The function of Nm23 in "normal" physiology may be in the acquisition or stabilization of the differentiated phenotype.

A number of experiments have been reported in which *nm23* homologs were introduced into metastatic cancer cells. A significant reduction in experimental or spontaneous metastatic potential was consistently observed, with no effect on tumorigenicity, confirming *nm23* as a suppressor gene. Nm23 re-expression reduced metastatic propensity of multiple cancer histologies exerting suppressive effects on lymph node, lung, and liver metastases.

In vitro assays of Nm23 transfectants have pointed, consistently, to reduced ►motility, ►invasion and ►metastatic colonization. The latter may be clinically

relevant, as colonization of metastatic cells in a distant organ has not been completed in many cancer patients at the time of diagnosis and therapy, and would therefore remain open for therapeutic intervention. ►Motility in ►Boyden chamber assays was significantly reduced in the *nm23* transfectants to a wide variety of ►chemoattractants, including serum and growth factors, suggesting that Nm23 blunts signal transduction responses downstream of specific receptors. Whenever tested, the proliferative capacity of the control- and *nm23*-transfectants in tissue culture was comparable.

The *in vivo* impact of Nm23 on the metastatic process was recently bolstered by the characterization of mice lacking the Nm23-M1 gene, the murine homolog of Nm23-H1. Nm23-M1$^{-/-}$ mice developed normally, but were smaller in size than wild-type littermates and exhibited a mammary defect. The incidence of lung metastases was significantly higher for the Nm23-M1 knockout mice as compared to Nm23-M1$^{+/+}$ mice, when the mice were genetically induced to develop ►hepatocellular carcinoma (HCC) by simian virus 40 large T antigen expression, despite similar primary tumor sizes.

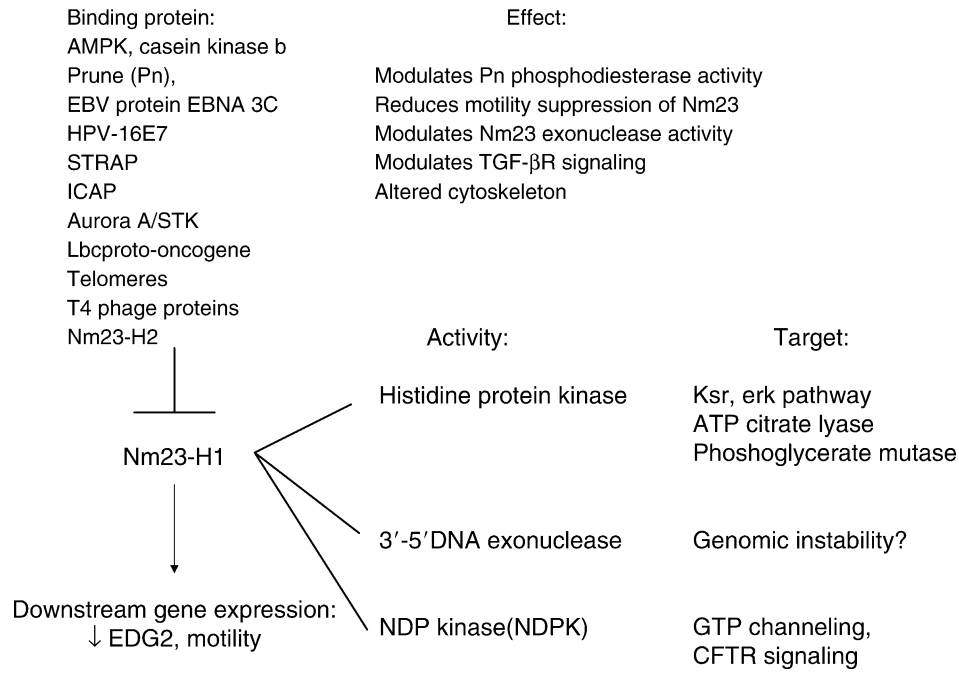
Mechanism of Metastasis Suppression

Nm23 proteins are abundant and exist as monomers and homo- and hetero-oligomers. The proteins are found in all cell compartments and are generally regarded as "sticky," resulting in a number of nonspecific interactions. Tremendous advances have been made in identifying biochemical pathways involving Nm23. Pinpointing those activities involved in the biology of differentiation and/or tumor metastasis is a major ongoing effort. The emerging picture of Nm23 action is tripartite, including Nm23 biochemical activities, Nm23 binding proteins, and gene expression changes downstream of Nm23 (Fig. 1).

Biochemical Activities

The first described activity for Nm23 was that of a NDP kinase, which transfers a terminal phosphate from a nucleoside tri- to a diphosphate via a high energy Nm23-phosphohistidine intermediate. The reaction is reversible. In model systems, NDP kinase activity failed to correlate with metastasis suppression, casting doubt on its participation in biology. However, interest in this activity was recently heightened with the report of a complex between AMP-activated protein kinaseal (AMPk), Nm23-H1, and fatty acid synthetase (ACC) in lung epithelial cells, resulting in the "channeling" of ATP. The CFTR story is separate from the ACC story.

The same Nm23 phosphohistidine has been demonstrated to transfer a phosphate to protein substrates constituting a histidine kinase activity. Demonstrated substrates include the Kinase suppressor of ras (Ksr) and ATP citrate lyase. Ksr is a scaffold protein for the



NM23 Metastasis Suppressor Gene. Figure 1 The mechanism of metastasis suppression by Nm23 likely involves three sets of molecular pathways. Nm23 functions as both a histidine kinase and a nucleoside diphosphate kinase. The function of Nm23 may be limited by sequestration upon binding to an assortment of proteins. Finally, alterations in gene expression downstream of Nm23 may influence the motility phenotype.

Erk ►Map kinase pathway, assembling component proteins near the cell membrane. Altered Nm23-H1 levels were shown to affect the stoichiometry of client protein binding to Ksr and Erk activation. Using in vitro motility as a readout, the histidine kinase activity of Nm23-H1 correlated with its inhibition of tumor cell motility among a panel of wild-type and substitution mutants, supporting a role for this activity in metastasis. Nm23-H1 was also reported to complex with GAPDH, and the complex to phosphorylate phosphoglycerate mutase with resultant effects on glycolytic flux.

Other activities described for Nm23 include a DNA exonuclease activity in vitro. Exonucleases play roles in DNA repair and ultimately genomic stability which may be important in cancer aggressiveness.

Binding Proteins

Nm23 has been reported to bind many proteins, of which a partial list is shown on Fig. 1. Protein-protein complexes involving Nm23-H1 serve at least two purposes: in some cases, protein complexes serve to remove free Nm23-H1, thus enhancing the aggressiveness of tumor cells. In other cases, the association of Nm23-H1 and another protein changes the function of either partner. The ►Prune (Pn) protein is a representative example of the latter case. Pn, a cAMP phosphodiesterase, can bind Nm23-H1 and overcome its inhibition of motility. Binding is mediated by Nm23 phosphorylation at serines 122 and 125 via casein kinase I CKI mediates this intraction.

The same serine 122 of Nm23-H1 is involved in another protein complex with AMPK. AMPK bound and phosphorylated Nm23-H1 on serine 122, resulting in "substrate channeling," formation of ATP by the NDPK activity of Nm23 which is shielded from the surrounding medium and preferentially activates AMPK activity.

Nm23-H1 also binds serine-threonine kinase receptor-associated protein (STRAP), a ►TGF-β receptor binding protein. As a result of this interaction, TGF-β signaling is reduced, with enhanced association between the TGF-β receptor and Smad7, and a prevention of Smad3 nuclear translocation.

Finally, Nm23-H1 was reported to bind the ►Epstein-Barr virus nuclear antigen 1 (EBNA1) protein, which is involved in the maintenance of viral episomes in infected cells. The complex abrogated the motility suppressive capacity of Nm23-H1 in lymphoblastoid cell lines. These and related HPV-16E7 data suggest that viral proteins may induce a more aggressive disease via Nm23-H1 binding.

Downstream Gene Expression Alterations

Given the effects of Nm23-H1 on tumor cell motility, invasion, and colonization, it is likely that multiple signaling pathways are involved. We recently reported a microarray gene expression analysis of MDA-MB-435 tumor cells expressing wild type Nm23-H1 or substitution mutants that could not suppress motility. The analysis identified eight cell surface or secreted proteins whose expression was down-regulated by wild-type Nm23-H1, of which only EDG2, the LPA receptor, was able to restore motility to Nm23-H1 suppressed tumor cells. EDG2 was upregulated in liver tumors from Nm23-M1$^{-/-}$ mice, demonstrating the generality of this effect.

Viral proteins can also serve to down-regulate Nm23-H1 expression. The human papillomavirus-16E7 down-regulated Nm23-H1 in vitro, resulting in increased invasion.

Translational Development

Nm23 mRNA and protein levels were reported in many human cancer types and inversely correlated with indicators of metastatic propensity, such as patient disease-free and overall survival, the presence of lymph node metastases, tumor grade, etc. These studies indicate that reduced Nm23 expression is relevant to human cancer progression. Generally, they do not indicate that Nm23 is a independent prognostic factor on multi-variate analysis. This is consistent with the presence and activity of many binding proteins, where Nm23 can be present but taken out of the active pool by protein-protein sequestration.

Based on the observations that (i) many aggressive human tumors exhibit relatively low levels of Nm23 expression and (ii) introduction of Nm23 significantly decreased tumor metastatic potential, it can be hypothesized that elevation of Nm23 expression in overt occult metastases may be of therapeutic benefit to the patient. High dose medroxyprogesterone acetate (MPA) was identified to elevate Nm23-H1 expression in two metastatic cell lines, MDA-MB-435 and -231, via an unconventional interaction with the glucocorticoid receptor. In preclinical experiments MPA reduced the outgrowth of breast cancer micrometastases in the lung. A Phase II trial of MPA for hormone receptor negative, aggressive breast cancer is being conducted at Indiana University.

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NMP22

Definition
A nuclear matrix protein liberated from necrotic cancer cells.

►Urothelial Carcinoma

NMR

Definition
Nuclear Magnetic Resonance Spectroscopy.

►Structural Biology
►MRI

NNK

Definition
4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone; A tobacco-specific carcinogen which induces lung tumors in laboratory animals. A powerful lung carcinogen that is formed specifically from nitrosation of nicotine during tobacco curing and smoking. It belongs to the nitrosamine class and is believed to be involved in lung cancer induction by smoking.

►Tobacco Carcinogenesis
►Sulforaphane

Nodal Involvement

Definition
►Lymph Node Metastasis

NOD-SCID

Definition
Non-obese diabetic severe combined immune deficient mice. Breed of immune incompetent mice that lacks both humoral (B-cell antibody production) and cell-mediated adaptive (T-cell) immunity.

Non-adaptive Immunity

Definition
Immunity from various exogenous molecules and microorganisms afforded by physical barriers, leukocytes and soluble molecules. A locution used in antithesis with ►adaptive immunity and implying the absence of antigen-specific receptors, clonal diversity and immune memory.

►Innate Immunity
►Natural Immunity
►Non-specific Immunity
►Immunoprevention

Non-essential Amino Acid

Definition
Is an amino acid that can be synthesized *de novo* by the organism (usually referring to humans), and therefore does not need to be supplied in the diet.

►Arginine-Depleting Enzyme Arginine Deiminase

Non-genotoxic Carcinogen

Definition
A carcinogen that is unable to cause a change to the structure of the genome.

►Toxicological Carcinogenesis

Non-Hodgkin Lymphoma

Definition

A wide group of hematological malignant diseases with differing histology, clinical course, treatment, and prognosis, distinct from ►Hodgkin lymphoma. A type of cancer involving lymphocytes. In lymphoma these malignant cells are predominately in lymph node tissues.

►Anaplastic Large Cell Lymphoma
►Malignant Lymphoma, Hallmarks and Concepts
►Hodgkin and Non-Hodgkin lymphomas

Non-homologous End Joining

Definition

NHEJ is the joining of broken DNA directly at its ends, mediated by DNA-dependent protein kinase. It is the ligation of double-stranded DNA ends with no need for sequence homology between the ends joined. Several proteins are known to be involved in this process, which is considered the major double-strand break (DSB)-repair pathway in mammalian cells. ►Nijmegen breakage syndrome

►Double-Strand Break Repair
►Homologous Recombination Repair

Non-Insulin Dependent Diabetes

Definition

►Diabetes Type 2.

Non-invasive Breast Cancer

►Ductal Carcinoma In Situ

Non-ionizing Radiation

Definition

Any type of electromagnetic radiation that does not carry enough energy to ionize living material – that is, to remove completely an electron from an atom or molecule.

►UV Radiation

Non-Lamellar Phase

Definition

Refers to secondary lipid structures other than the lipid bilayer and includes hexagonal, cubic, rhombohedral phases, etc.

►Membrane-Lipid Therapy

Non-myeloablative

Definition

Refers to ►stem cell transplant; synonym mini-transplant, transplant-lite, or reduced intensity transplant. Is a stem cell transplant from a donor (►allogeneic) that uses a less aggressive combination of chemotherapy and/or radiation to prepare the patient for the transplant. In the "conventional" allogeneic transplant the goals of the preparative chemotherapy/radiation are to kill as many cancer cells as possible and to suppress the immune system of the patient to allow the donor cells to grow. The non-myeloablative transplant aims just to suppress the patient's immune system sufficiently to allow engraftment of the donor cells.

►Chronic Lymphocytic Leukemia

Non-receptor Tyrosine Kinases

Definition

Are intracellular ►tyrosine kinases present in the cytoplasm, nucleus or attached to the inner leaflet of

the plasma membrane. Multiple families of these kinases exist, with Src being the prototypical member for which ►Src homology domains (SH2, SH3 and SH1) are named.

►Receptor Cross-Talk

Non-Rhabdomyosarcoma Soft Tissue Sarcomas

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Definition

NRSTS; Soft tissue sarcomas (STSs) are a heterogeneous group of mesenchymal extraskeletal malignant tumors, classified on the basis of their differentiation according to the adult tissue they resemble.

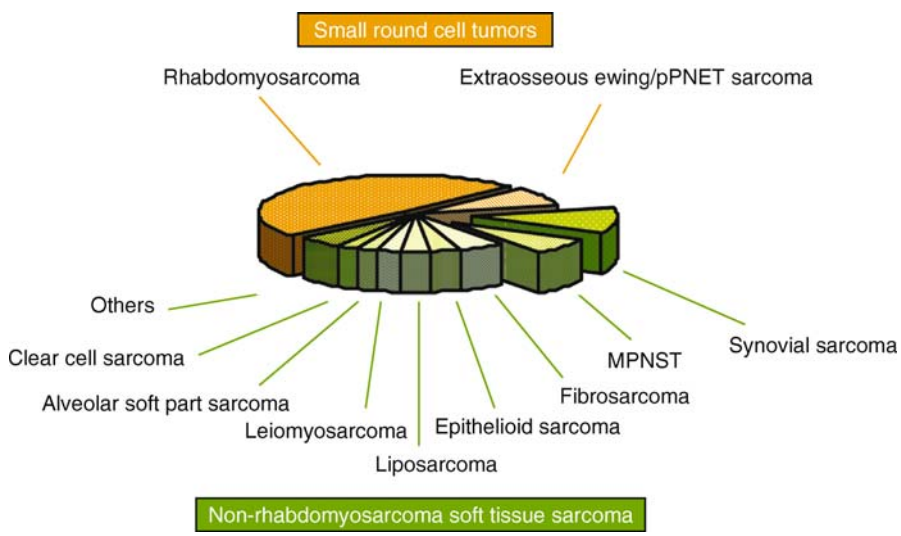
While benign neoplasms of soft tissues (i.e., ►lipoma, ►fibroma, ►leiomyoma, ►hemangioma) are relatively frequent and outnumber by 100 times malignant cases (incidence of about 300 new cases per 100,000), malignant tumors are rare, with an incidence of around 2–3/100,000/year, accounting for less than 1% of all malignant tumors and 2% of all cancer-related deaths. However, in pediatric age STSs represent about 8% of all malignancies, though their absolute number is lower than in adult age.

Characteristics

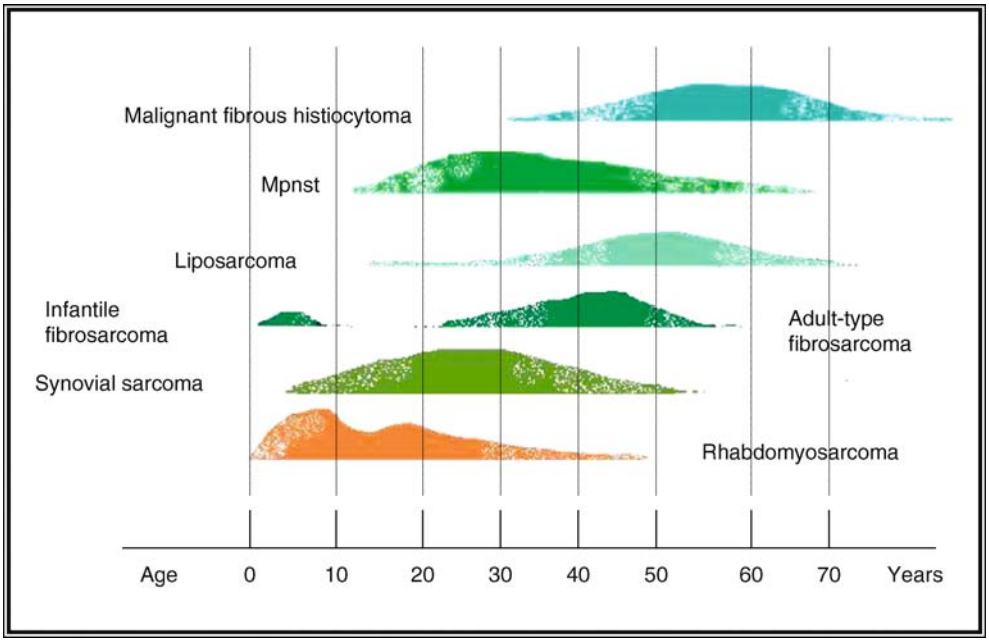
In childhood and adolescence, about half of STSs are represented by ►rhabdomyosarcomas (RMSs), one of the most typical ►childhood cancer, characterized by a peak incidence between 3 and 5 years (and a second, smaller peak in adolescence). The remainder 50% of pediatric STSs are the so-called "non-rhabdomyosarcoma" soft tissue sarcomas (NRSTSs).

Different entities with a different biology and clinical behavior are included in the heterogeneous group of pediatric NRSTSs: some histotypes are more common in adults and very rare in children (i.e., ►liposarcoma), others are peculiar of young children (i.e., infantile fibrosarcoma), while others are typical of adolescents [i.e., ►synovial sarcoma, ►malignant peripheral nerve sheath tumors (MPNST)] (Figs. 1 and 2).

STSs are malignant tumors by definition, with an aggressive and destructive local behavior together with high propensity to local relapse and potential tendency to distant metastases. Though, they can show a different grade of malignancy along histotype and grading. The tumor aggressiveness and the propensity to give metastases (and thus, at the end, the outcome of patients) are usually correlated with the grade of malignancy, and different histotypes with the same grade of malignancy often display the same clinical behavior. Low-grade tumors are often locally aggressive, but unlikely to metastasize (about 2–10% of risk). High-grade tumors are more aggressive and have a strong propensity to metastasize, particularly to the lung, in 20–100% of cases. However, the clinical course



Non-Rhabdomyosarcoma Soft Tissue Sarcomas. Figure 1 Percentage of different histotypes of soft tissue sarcomas in pediatric age.



Non-Rhabdomyosarcoma Soft Tissue Sarcomas. Figure 2 Incidence of soft tissue sarcomas in the different age groups.

can widely vary, not only according to the grading but also along the histotype. NRSTS can grow rapidly and present at diagnosis with metastatic spread, but can also have an indolent growth rate (they are sometimes diagnosed after removing a small swelling that has existed for several years and relapses can occur many years after the first diagnosis).

As for most pediatric malignancies, the pathogenesis of NRSTS is still unknown and there are no well-established risk factors. Ionizing radiations, chemical carcinogens and oncogenic viruses have been variously associated to the development of some type of sarcomas, but the aetiological relation is yet unclear. The clinical evidence of a genetic predisposition is rare: ►Li–Fraumeni syndrome for STSs and bone sarcomas, ►neurofibromatosis type 1 (particularly associated with ►MPNST), ►Costello syndrome, and other genetic diseases may be rarely associated with pediatric STSs.

Clinical Diagnosis and Staging

NRSTSs can virtually develop anywhere in the soft part of the body, generally as a progressively expanding mass. The most common clinical presentation is that of a mass in the soft tissues of the lower extremities; less frequent sites are the trunk or the head and neck region. A painless mass is the most usual presentation, but pain, functional impairment, and other specific symptoms correlated to the anatomic location can be present.

Ultrasonogram is often the first instrumental assessment utilized in case of suspected STS. More careful imaging studies, such as computed tomography (CT) scan or magnetic resonance imaging (MRI), are however required for the determination of the size and invasiveness of the tumor. MRI, in particular, is currently considered superior to CT scan in defining soft tissue extension, and should be required in all cases of STS.

After the clinico-radiologic accurate description of the local extent of the tumor, pathological assessment is necessary to define the histological diagnosis and grading when appropriate. This assessment should be performed before any surgery or other therapy, since the surgical and therapeutical approach varies in dependence of histotype and grading. The initial biopsy is aimed not only to define the diagnosis, but should also provide enough material to allow cytogenetic and molecular studies. A central pathological review is also required for patients that could be included in multicentric trials. In case of large and deep soft tissue mass, biopsy should be always the initial surgical procedure, in order to avoid inadequate surgery (i.e., tumorectomy). Open biopsy (incisional biopsy) or core needle biopsy (tru-cut, guided by ultrasound or CT scan) should be preferred to fine needle aspirates (FNAC). In fact, a cytological approach could establish the presence of malignancy, but exceptionally identifies the histotype and grade and provides enough tissue required for additional studies. In any case, the surgical procedure should be carefully planned by experienced surgeons, taking into account the definitive surgery that must include the scar and the biopsy tract.

Diagnostic procedures are completed by staging investigation, aimed to detect regional and distant

metastases: chest CT scan, technetium bone scan, and abdominal ultrasound are required to identify lung, bone, and abdominal metastases, respectively.

All these examinations are fundamental to stratify patients in stage-groups and then define a risk-adapted treatment strategy. NRSTS patients are usually staged according to the clinical TNM classification (based on local invasiveness and tumor size, T1 and T2, A or B, i.e., less or more than 5 cm; absence or presence of nodal and distant involvement, N0 and N1, M0 and M1, respectively) and the Intergroup Rhabdomyosarcoma Study (IRS) postsurgical grouping system (based on the quality of surgical resection: group I – completely excised tumors with negative microscopic margins; group II – grossly resected tumors with microscopic residual disease and/or regional lymph nodal spread; group III – gross residual disease after incomplete resection or biopsy; and group IV – metastases at onset).

Pathology and Biology

The definition of histotype and grading is of paramount importance not only for prognostic implications but also in the planning of therapy. The most updated classification of STSs is the WHO classification of 2002, which is based on pathology and genetics of the tumors and gives a stratification of histotypes along their degree of malignancy. Sarcomas are defined as malignant tumors with a locally destructive behavior, tendency to local relapse and a percentage of distant metastases above 2%. They are subgrouped on the basis of their differentiation in adipocytic tumors, fibroblastic/myofibroblastic tumors, so-called fibrohistiocytic tumors, smooth muscle tumors, pericytic/perivascular tumors, skeletal muscle tumors, vascular tumors, and tumors of uncertain differentiation. Today in the major part of cases the diagnosis of histotype requires the application of immunocytochemistry and cytogenetics/molecular techniques. The definition of histotype has prognostic and predictive implications.

Once the histotype is defined, the grade of malignancy is estimated. The grade of malignancy usually indicates how aggressive a tumor is and its natural history; however, some histotypes (i.e., synovial sarcoma, alveolar sarcoma, angiosarcoma) should be considered high-grade regardless of their morphological parameters, whereas in some cases (i.e., clear cell sarcoma, extraskeletal myxoid chondrosarcoma) the biological course seems impossible to predict from any histology. Tumor grade is usually established from a combined assessment of histological features: degree of cellularity, cellular pleomorphism or anaplasia, mitotic activity, degree of necrosis. Over the years, different grading systems have been developed, in particular the Pediatric Oncology Group (POG) system for pediatric sarcomas and the French system (FFCLCC – French Federation Nationale des Centres de Lutte Contre le Cancer), a three-tiered system tested on adult series (but used in Europe also for pediatric ►NRSTSs). Another task of pathologist is to assess the quality of margins on the surgical specimen, always in strict collaboration with the operating surgeon.

In particular, an aspect emerging in recent years, with the application of preoperatory therapies, is the evaluation of treated tumors, to assess the effect of therapies on the tumor mass. In these cases, the diameters of the tumor mass should be registered, along with the percentage of viable tumor, its mitotic index and the presence of differentiation induced by therapy, and the percentage and description of post-treatment changes (i.e., ►necrosis, hemorrhage, fibro-histiocytic reaction with hemosiderin, sclerosis, hyalinosis, calcification, myxoid changes).

Treatment and Outcome

The first concept is that the treatment of pediatric patients with STS is complex and demands a multidisciplinary approach: the rarity and the heterogeneity of these tumors suggest that children and adolescents with STS should be referred to selected institutions with adequate experience in treating patients with STS, and with multidisciplinary skills in enrolling patients in clinical trials. It is a fact that in the past 30 years the cure rate for pediatric STSs has significantly improved, and currently approximately 70% of cases are cured. As for other pediatric malignancies, the improvement in therapeutic results is largely due to the development of treatment approaches that have been: (i) *multidisciplinary* (including surgery, radiotherapy, and chemotherapy), (ii) *risk-adapted* (different prognostic factors have been used to stratify treatment intensity in the different subsets of patients), (iii) *cooperative multi-institutional* (within trials able to enroll a large number of patients). However, it is true that pediatric ►STS researches addressed their main attentions on ►RMS studies: the published studies on pediatric NRSTS are far less numerous than RMS and it is noteworthy that the largest two reported series of pediatric NRSTS are single-institution experiences (published by two highly qualified centers, the St Jude Children's Research Hospital and the Istituto Nazionale Tumori of Milan). Also the term "non-rhabdomyosarcoma" ►STS reflects the fact that most of the experience gained on the treatment of pediatric NRSTS has been based on principles deriving from the management of RMS, which is a clearly distinct entity. But times have changed, and both the North-American Children's Oncology Group (COG) and the newly created European pediatric Soft Tissue Sarcoma Study Group (EpSSG) are currently carrying out clinical trials specifically tailored to NRSTS (also drawing insight from the numerous reports on adult sarcomas).

Another important issue to consider in establishing the treatment strategy for NRSTS concerns the heterogeneity of these tumors, that are a very mixed group of different histotypes, with very different clinical history: i.e., MPNST occur most frequently at axial sites, are often associated with neurofibromatosis type 1 and are characterized by high local aggressiveness and poor prognosis; epithelioid sarcomas present typical features such as peculiar superficial distal location (i.e., hand, fingers), indolent growth, and tendency for lymph node involvement; infantile fibrosarcomas may have initial rapid growth, but also indolent evolution, can give metastases, but also spontaneous regressions have been described, and the overall prognosis is good; ►desmoplastic small round cell tumors (DSRCT) usually present as a large abdominal mass already widely disseminated at the time of diagnosis, with extensive spread to regional lymph nodes, peritoneal seeding, and distant metastases, and the outcome is extremely poor outcome despite intensive multimodality treatment approaches.

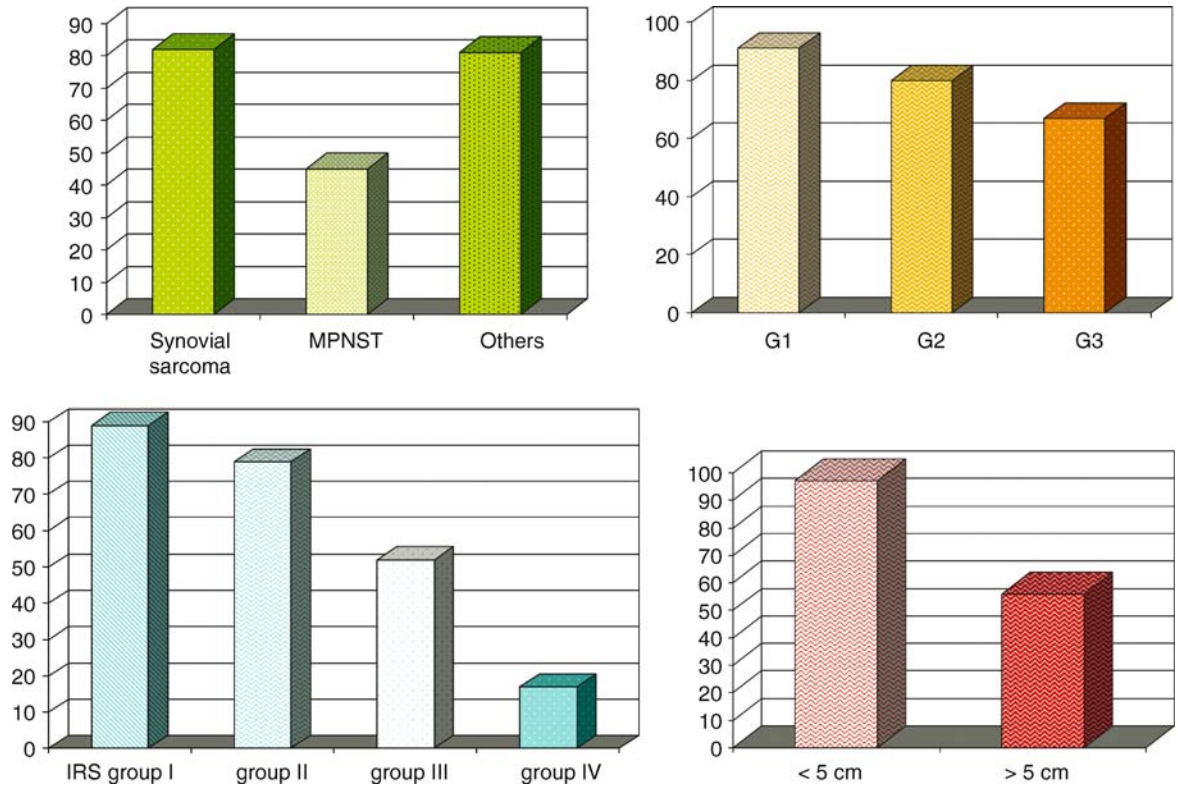
Despite this heterogeneity, NRSTS have to be analyzed as a group because the rarity of each histotype makes it impossible to perform clinical trials on single tumor types. However, it is important for investigators to identify and select diagnostic subgroups that are as specific (and homogeneous) as possible. The definition of "adult-type" NRSTS focuses on a subgroup of NRSTS histotypes that are typical of adulthood, definitely malignant, with morphological features resembling differentiated/mature tissues, thus ruling out infantile fibrosarcoma, for instance, intermediate malignancy tumors and small round cell tumors (i.e., RMS, extraosseous ►Ewing sarcoma and ►DSRCT), which are biologically and clinically different entities that are sometimes studied together with adult-type NRSTS, giving rise to misleading results. Therefore, the definition of "adult-type" NRSTS includes adult-type fibrosarcoma, MPNST, epithelioid sarcoma, leiomyosarcoma, clear cell sarcoma of soft part, liposarcoma, alveolar soft part sarcoma, undifferentiated polymorphous sarcomas, malignant solitary fibrous tumor/hemangiopericytoma, angiosarcoma, dermatofibrosarcoma.

In the direction of selecting specific subgroups of NRSTS histotypes it is also the decision to analyze *synovial sarcoma* separately, not only because it is the most common NRSTS in childhood and adolescents, but also because its chemosensitivity would probably stand midway between that of the most typical adult STS (less than 40% of patients respond to chemotherapy) and that of pediatric small round cell tumors, such as RMS (up to 80% respond to chemotherapy).

In order to plan a risk-adapted treatment strategy, basically, it is of fundamental importance to identify the prognostic variables capable of influencing the outcome. Various findings have suggested that the quality of initial surgery (namely the IRS group), the initial tumor size, and the grade of the tumor are the most important prognostic factors Fig. 3.

Surgery. Surgery is unquestionably the keystone of treatment of NRSTS. The aim of surgery is that of obtaining adequate surgical margins with limited or no long-term sequelae. The quality of the surgical operation is crucial and the chances of adjuvant therapies being able to compensate for inadequate surgery are still debatable. For this reason, patients should be referred to specialist centers for local treatment, preferably before undergoing biopsy. A critical issue concerns the definition of "adequate margins," that means the quality and the quantity of healthy tissues surrounding the tumor (the quality of the resection is defined by its worst margin). A surgical resection could be defined as complete when histological margins were microscopically free, including the so-called *compartment resections* (when the tumor is removed en bloc with the entire muscular or anatomical compartment of origin, where tumor was entirely anatomically confined) and the *wide excisions* (en bloc excisions through normal tissue, beyond the reactive zone but within the muscular compartment, removing the tumor with its pseudocapsule; the tumor is covered at every point by healthy tissue, i.e., muscle, subcutaneous tissue, thick fascia, or intermuscular septum). *Marginal resection*, or microscopically incomplete resection, is any resection coming just outside the pseudocapsule, with suspected microscopic residual disease (the tumor surface emerges macroscopically at the resection surface), while *intralesional resection* means that macroscopic tumor residue is left in situ. A crucial question remains, besides, whether and how to give a "metric" definition of a "safety distance" between tumor and resection margins: "adequate" margins could be considered those >1 cm of healthy tissue around the tumor, in all directions, when the tissue is a muscle or adipose tissue, while when the tissue is periostium, vessel sheath, epineurium, or muscular fascia (that act as barriers) healthy tissue >1 mm may be considered sufficient.

Radiotherapy. Radiotherapy plays a well-defined role in local control in ►STSs. In particular, in adult STSs irradiation is recommended not only after incomplete resection, but also after wide excision, especially in case of large tumors. Certainly, the indication for radiotherapy has to be stricter in children and young adolescents with NRSTSs than in adults, given the higher risk of severe late effects of radiotherapy (in particular, the risk of retardation or arrest of irradiated bone growth, the risk of functional impairment and that of second postirradiation tumor): the patient's age must always be borne in mind when planning radiotherapy in order to keep its sequelae to a minimum. In patients who received initial wide resection, in fact, the indication for radiotherapy is still debated due to the problem of sequelae, though reported



Non-Rhabdomyosarcoma Soft Tissue Sarcomas. Figure 3 Overall survival of NRSTSs according to histotypes, tumor grade, surgical margins (IRS group), tumor size. (Ferrari et al (2005) J Clin Oncol 23:4021–4030).

series showed a favorable trend for the addition of radiotherapy and therefore seemed to suggest the use of postoperative irradiation in those cases with tumor larger than 5 cm. The indication for postsurgical radiotherapy is clearer, instead, after initial marginal resection (the so-called IRS group II patients): when suspected microscopical residual tumor is left in situ (and when a re-excision is considered unfeasible), radiotherapy should be given, since the risk of local recurrence appears very high. The local treatment strategy is more complicated in patients whose tumors are judged unresectable at diagnosis, and thus receive initial chemotherapy (IRS group III patients). For these patients, delayed surgery is the treatment of choice, but surgery and radiotherapy should be discussed in a multidisciplinary setting and combined in order to define (and customize) the best local treatment for each patient, considering again that radiotherapy should be administered not only considering the need to maximize the chances of local control, but also containing the radiation-related sequelae and preserve function: for instance, irradiation after delayed surgery could be avoided in younger patients, while it might be recommended in the case of large tumors; postoperative radiotherapy may be easier to plan and carries a lower risk of complications, but preoperative irradiation can improve the chances of achieving free margins at the secondary resection, may reduce the risk of intraoperative contamination, and smaller radiotherapy fields and lower doses can often be used.

Chemotherapy. While conservative surgical resection unquestionably remains the mainstay of treatment for NRSTSs, and the effectiveness of radiotherapy is widely appreciated, oncologists are still wondering about systemic chemotherapy and NRSTSs, as adult ►STSs, continue to be considered scarcely chemosensitive (unlike the case of ►RMS, which is a highly chemosensitive tumor). However, it is generally agreed that the outcome is reasonably good in patients with resected, small, noninvasive NRSTSs (survival rate up to 90%), whereas even after initial gross resection, the prognosis for patients with high-grade and large invasive tumors would be unsatisfactory if the treatment were limited to surgery alone (with or with out radiotherapy). The risk of developing distant metastases, particularly to the lung, is higher for high malignant NRSTSs, and the role of different chemotherapy regimens has been variously investigated. Several findings emerging from adult trials have suggested that the combination of full-dose ifosfamide and ►doxorubicin (►adriamycin) was the regimen achieving the highest response rate in STSs.

Of course, chemotherapy has to be recommended in frontline treatment in patients with metastatic and advanced disease at diagnosis, and also in all those

cases where the surgeon is unsure of being able to achieve a complete resection at the first attempt. Neoadjuvant chemotherapy might convert such cases into conservative complete resections, as well as treating any micrometastases promptly, since these patients have a high risk of distant dissemination whatever the local control measures adopted. Overall, the response rate (complete response plus partial response >50%) of NRSTSs to frontline chemotherapy is considered around 40%, though more recent findings would suggest better percentage of response when minor responses were also included (and some cases that had initially been considered unresectable reportedly underwent complete delayed surgery after minor tumor shrinkage induced by chemotherapy).

Even more doubts surround the role of ►adjuvant chemotherapy, though recent evidence seems to suggest that it may have a more significant beneficial impact in selected cases than is generally believed. Despite the relatively good prognosis for patients who received initial resection, a particular group of high-risk patients can be in fact earmarked: the combination of two variables – *high tumor grade* plus *large tumor size* – gives rise to a very high risk of metastases, irrespective of the results of initial surgery, suggesting that systemic chemotherapy should, in principle, be used to improve survival. In patients with these characteristics, in fact, the metastases-free survival is around 30–40%, but it seems clearly better in those patients who received adjuvant chemotherapy, as compared with those who have not been given. Of course, the debate on whether or not to provide adjuvant chemotherapy for STS remains wide open. The role of adjuvant chemotherapy in preventing distant recurrences after initial surgery has long been a point of controversy in the field of clinical studies on adult STS. Most randomized trials performed by North American and European groups showed neither statistically significant benefit for patients given adjuvant chemotherapy, but some of the negative results recorded in those studies need to be reconsider since these trials did not use the combination of drugs currently recognized as the most effective in STS (ifosfamide, in particular, was not included in most of these studies), nor had they selected patients most likely to respond to chemotherapy (tumors of diverse histology, grade, and size were grouped together).

A completely separate discussion should be dedicated to the role of chemotherapy in synovial sarcoma. Over the years, different strategies have been developed for pediatric and adult oncology protocols dealing with synovial sarcoma. High rates of response to chemotherapy were recorded in pediatric series, so synovial sarcoma came to be considered an "RMS-like" tumor and most pediatric patients were consequently included in RMS protocols, receiving adjuvant chemotherapy regardless of the risk factors (i.e., even after the complete excision of very small tumors), whereas adjuvant chemotherapy was generally used only in adult patients as part of trials with a no-therapy control arm and including all soft tissue sarcoma histotypes.

The various reported experiences with this tumor in the field of pediatric oncology reported a 5-year overall survival rate around 80%, i.e, higher than the one usually reported in adult series, and an approximately 60% rate of response to chemotherapy in patients with measurable disease, i.e., higher than the response rate usually reported for other adult STS. Data from adult literature, instead, reported less satisfactory overall results: this finding may have to do with a different incidence of adverse prognostic factors in different age groups (i.e., large tumors were more frequent in older patients), but the different results might also be related, to some degree at least, to the different treatment strategies adopted, and particularly to the different use made of chemotherapy. A formal demonstration of the efficacy of adjuvant chemotherapy in synovial sarcoma is not available, but various data would nonetheless suggest that it does have a part to play. It may be that adjuvant chemotherapy for all synovial sarcoma patients (as pediatric oncologists did) is tantamount to overtreatment: it is probable that a subset of low-risk patients – i.e., completely resected, with tumor smaller than 5 cm – may be identified for which adjuvant chemotherapy can be omitted without jeopardizing the results.

Particular Histotypes

The above discussed considerations are suitable for most of the NRSTS histotypes, and in particular for the so-called "adult-type" NRSTS. However, there are some particular entities that are characterized by peculiar biology and clinical course, and deserve to be considered separately.

Extra-osseous primitive peripheral neuroectodermal tumor (pPNET)/Ewing sarcoma is less frequent than the skeletal ►Ewing sarcoma, but it is likely that there are no biological differences between Ewing sarcomas arising at different sites, so the clinical considerations should be the same: it is a high malignant tumor, with a strong propensity to give metastases, that should be treated with a multimodality strategy including, in all cases, multi-agent chemotherapy.

►DSRCT is a very aggressive neoplasm, usually arising in the abdominal cavity, often characterized by a poor outcome despite the various intensive multimodality treatment approaches attempted over the years (i.e., aggressive surgery, radiotherapy, intensive chemotherapy including high-dose myeloablative chemotherapy (►myeloablative megatherapy) with stem cell rescue).

Soft part ►*rhabdoid tumors* represent the soft tissue counterpart of the intracranial and renal entities: they are very rare and very aggressive disease, for which

improvement in genetic studies are strongly needed to cast light on their common biology in order to think to new treatment approaches.

The heterogeneous assortment of NRSTS includes tumors as ►*epithelioid hemangioendothelioma*, a neoplasm that sometimes arises at soft part, as a single lesion located in the extremities or cervical district, with very little propensity to metastasize or become fatal, but sometimes develop at bone, lung, and liver, often multifocal or metastatic, with indolent course but also a not negligible risk of death, for which treatment with alpha-interferon may have a significant role, probably due to an antiangiogenic effect.

Finally, *infantile fibrosarcoma* is a peculiar tumor of infants (it is the most common soft tissue sarcoma under 1 year of age): its clinical behavior may widely vary (i.e., it can rapidly grow and also give metastatic spread, but some cases of spontaneous regression have been described). However, chemotherapy is fairly effective in this tumor, also utilizing mild alkylating/anthracyclines-free regimens, and, consequently, the overall prognosis is very good.

Future Issues

Despite their heterogeneous nature, NRSTS currently have to be analyzed as a group because of their rarity and the availability of few therapeutic options (i.e., surgery remains the keystone of treatment, and few drugs are effective). However, this situation is changing and the next steps may go in the direction of histology-driven therapies: drugs other than ifosfamide–doxorubicin combination have proved fairly active against particular histotypes (i.e., ►taxanes for ►angiosarcoma, ►gemcitabine for ►leiomyosarcoma, ►ET-743 for ►myxoid liposarcoma). But what physicians are waiting is to really improve the understanding of the biology of these tumors, paving the way toward novel molecular therapeutic approaches. The specific chromosomal translocations occurring in ►STS may become the targets of new molecular agents specifically designed to influence the tumor's biology. Of course, if the near future sees STS studied no longer as a mixed bunch, but focusing separately on each histotype, this will only be feasible (especially for investigators working on pediatric NRSTS) if there is close cooperation between international groups, and between pediatric and medical oncology groups.

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Non-small Cell Lung Cancer

Definition

NSCLC; About 80–85% of all cases of ►lung cancer are of the non-small cell type. There are three sub-types of NSCLC. The cells in these sub-types differ in size, shape, and chemical make-up.

Non-syntenic

Definition

Refers to genes or genetic loci that lie on different chromosomes, i.e. are not genetically linked.

Non-Viral Vector for Cancer Therapy

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Definition

Non-viral vectors can deliver therapeutic molecules into cells for the treatment of cancer. The molecules used for this purpose are usually anticancer drugs, short interfering RNA (►siRNA), and DNA (Table 1). Non-viral vectors can also be employed for the delivery

of proteins, peptides, and messenger RNA, but such agents are not so frequently used for cancer therapy. Anti-sense oligonucleotides (►Antisense DNA therapy) were previously employed to suppress gene expression, but siRNA has recently taken their place.

Characteristics

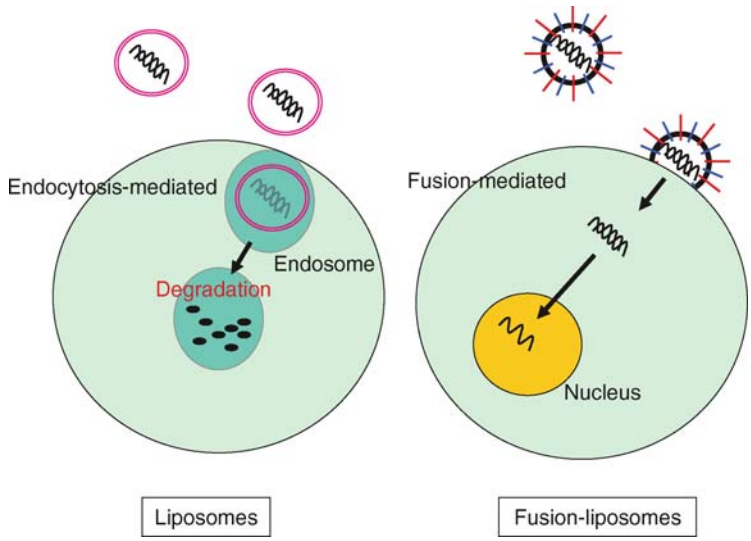
Viral vectors are generally constructed by inserting a therapeutic gene into an engineered viral genome. In contrast, construction of non-viral vectors does not require viral genome engineering and these vectors are synthesized from chemical products or native materials, after which they associate with therapeutic molecules to promote more efficient delivery. Non-viral vectors are generally less efficient at promoting gene delivery and gene expression than viral vectors (►Viral vector-mediated gene transfer), but pose less risk and can also be employed for drug delivery. The most representative non-viral vectors are ►liposomes and ►polymers. Physical methods, such as naked DNA injection, electroporation, and sonoporation, also allow non-viral delivery of molecules, but no carrier or vector is associated with the therapeutic molecules.

Non-Viral Vector for Cancer Therapy. Table 1 Therapeutic molecules for cancer treatment delivered using non-viral vectors

Anticancer drugs	siRNA	DNA
Bleomycin	Rad51	p53
Cisplatin	VEGF	B7–1
Doxorubicin	HIF-1α	IL-12
Methotrexate	β-catenin	HSV-tk

Liposomes

Liposomes are artificial phospholipid bilayer vesicles formed from an aqueous suspension of phospholipid molecules that can be employed for the targeted delivery of macromolecules. Cationic liposomes can bind DNA more tightly and show improved transfection efficiency. However, DNA is taken up into cells by ►endocytosis during lipoplex-mediated transfection. The main problem with endocytosis-mediated delivery is that therapeutic molecules are often subject to degradation within endosomes or lysosomes, as shown in Fig. 1. To solve this problem, several methods have been tried. One method is to employ a neutral lipid (DOPE) that facilitates the endosomal release of DNA. Its discovery has led to the use of a mixture of cationic lipids and DOPE for lipofection. Further analysis of various lipids has revealed that a 1:1 mixture of *N*-[1-(2,3-dimyristyloxy) propyl]-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl) ammonium bromide and cholesterol is capable of destabilizing the endosome membrane more effectively than DOPE. To further protect therapeutic molecules delivered by liposomes, DNA is now conjugated with cationic molecules. For example, protamine sulfate or adenovirus mμ protein is conjugated with DNA, after which the newly formed complexes are incorporated into or mixed with cationic liposomes. Investigations of these methods have shown that cationic liposomes containing the HLA-B7 and β-2 microglobulin genes can induce antitumor immunity in HLA-B7-negative



Non-Viral Vector for Cancer Therapy. Figure 1 Pathways by which therapeutic molecules can be introduced into cells using liposomes or fusion-liposomes. Molecules delivered in liposomes escape into the cytoplasm from the endocytotic pathway, while molecules delivered by fusion-liposomes directly reach the cytoplasm by membrane fusion.

►melanoma patients, and a number of centers have performed clinical trials of a liposomal drug (Allovectin-7) for the treatment of metastatic melanoma. Delivery of the β-interferon gene by cationic liposomes has also been evaluated for the treatment of patients with ►glioblastoma. In addition, several other clinical trials have tested the delivery of various anticancer agents using liposomes.

Tissue targeting (►Targeted drug delivery) is important for the efficient delivery of therapeutic molecules with minimum toxicity.

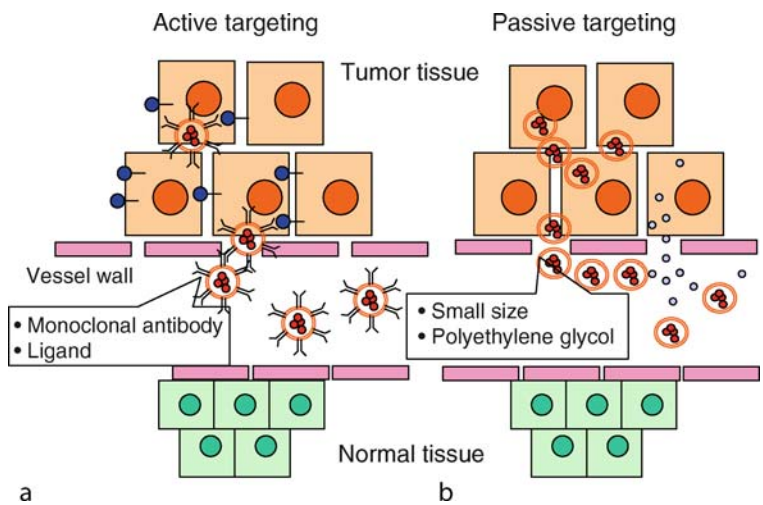
Especially in cancer therapy, tumor-specific delivery is essential for treating metastases. Generally, there are two approaches to tissue targeting, which are known as ►therapeutic active targeting and ►therapeutic passive targeting (Fig. 2). Passive targeting is the method used for most cancer-targeting vectors, because tumor vasculature has an abnormally high permeability due to its incomplete architecture. If a vector remains stable in the circulation and is not trapped by the reticuloendothelial system, it can cross the walls of tumor vessels and accumulate in tumor tissues. To achieve this, the diameter of a vector should be less than 300 nm (preferably less than 150 nm), and the vector should be coated with polyethylene glycol. In addition, the vector should associate effectively with tumor cells. In order to promote the internalization of therapeutic DNA by tumor cells, various cell receptor ligands (such as folate and transferrin) have been used to take advantage of receptor-mediated endocytosis.

Polymers

Polymers are also used as DNA carriers and can be divided into two categories based on their biodegradability. Various cationic non-biodegradable polymers have been widely investigated for enhancing the internalization of therapeutic molecules. The most common linear cationic polymers are poly (ethyleneimine) and poly-L-(lysine). Other polymers include poly (*N*-ethyl-4-vinylpyridinium bromide), poly (dimethylaminoethyl methacrylate), chitosan, and dimethylaminodextran, or branched polymers such as poly (amidoamine) ►dendrimer and branched poly (ethyleneimine). Since DNA is a large and negatively charged molecule, it is difficult to attach it directly to the cell membrane (also negatively charged) and achieve internalization into cells. It is well known that cationic polymers can easily form complexes with negatively charged DNA via electrostatic interactions. Such complex formation condenses the DNA molecule and converts the net electrical charge to positive under appropriate conditions. This condensation and the positive charge of a DNA-cationic polymer complex facilitate attachment to cells and subsequent internalization via the normal endocytosis pathway.

In addition to electrostatic interactions, hydrophilicity and hydrophobicity can be utilized to incorporate therapeutic molecules. Block copolymers with both hydrophilic and hydrophobic characteristics can be assembled to form a micelle structure that incorporates anticancer drugs or DNA. Such ►micelles are small enough (50–100 nm in diameter) to reach tumors by extravasation from their vessels. Targeting molecules can be also bound to particles of the nanometer order in size for tissue targeting.

Biodegradable polymers have been used to achieve controlled release of DNA, thus enhancing and prolonging gene expression. Controlled-release technology increases the concentration of DNA and



Non-Viral Vector for Cancer Therapy. Figure 2 Two different approaches for tumor-specific delivery. (a) Vectors that possess tumor-specific molecules such as monoclonal antibodies and ligands recognize tumor cells by molecular interactions. (b) Vectors with a small size and polyethylene glycol coating passively accumulate in tumor tissues, which are characterized by a high interstitial pressure, enhanced vascular permeability, and the lack of functional lymphatic drainage.

prolongs its persistence at an injection site. By using this method, the biological activity of an antitumor DNA plasmid (NK4) is enhanced.

Virosomes

In order to enhance the efficiency of delivery, trials of viral envelopes or other proteins have been performed. Empty viral envelopes without the viral genome that are used to incorporate drugs and non-viral vectors decorated with viral components are called ►virosomes. The representative virosomal vectors are described below.

Fusion-Liposomes

To avoid degradation prior to reaching the cytoplasm, fusion-mediated delivery systems have been developed (Fig. 1). A fusigenic viral liposome with a fusigenic envelope derived from ►HVJ (►Sendai virus) was constructed first. Primitive ►HVJ-liposomes are made by fusion of liposomes with UV-inactivated HVJ. Reconstituted fusion liposomes can also be created. Use of HVJ-liposomes to deliver anticancer treatment has already been investigated in animal models. A melanoma-associated antigen gene (►Melanoma antigen) or RNA injected into skeletal muscle or the spleen successfully evokes antitumor immunity and prevents the growth of melanoma. A radiation-inducible HSV-TK gene driven by the Egr-1 promoter enhances the efficacy of radiotherapy for ►hepatocellular carcinoma when delivered by HVJ-anionic liposomes. Using an ►EBV replicon plasmid containing the HSV-TK gene, ►suicide gene therapy (►HSV-TK/Ganciclovir mediated toxicity) is more effective against melanoma in tumor-bearing mice. A similar approach to enhance the efficiency of gene transfer uses fusion peptides derived from influenza virus hemagglutinin for receptor-mediated gene delivery. Combining transferrin/poly-L-lysine/DNA complexes with the hemagglutinin peptide increases the efficiency of gene transfer into cultured cancer cells by more than 1,000-fold compared with transfer in the absence of this peptide.

HVJ-Envelope Vector

To simplify the vector system and to increase the efficiency of gene delivery, plasmid DNA has been incorporated into inactivated HVJ particles without liposomes by detergent treatment and centrifugation. The HVJ-envelope (inactivated Sendai virus) vector can deliver DNA, siRNA, proteins, and anticancer drugs to cells both in vitro and in vivo. Rad51 siRNA has been delivered to tumors in mice with approximately 50% efficiency by this HVJ envelope vector. After Rad51 siRNA was delivered to tumors using this envelope vector, sensitivity to ►cisplatin was enhanced more than tenfold compared with use of cisplatin alone. The HVJ envelope vector can also enhanced transfection efficiency after conjugation with a biocompatible polymer or magnetic beads. The vector itself is an effective anticancer agent because it can induce T cell-mediated antitumor immunity through activation of dendritic cells and inhibition of regulatory T cells.

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Nonparametric

Definition

Nonparametric analysis refers to empirical estimation of a distribution without relying on an underlying population distribution typically performed by using some form of ranking methods.

►Kaplan–Meier Survival Analysis

Nonseminomatous Germ Cell Tumor

►Testicular Cancer

Nonsense Mutation

Definition

A mutation that occurs within a codon and changes it to a stop codon.

Nonspecific Cross Reacting Antigen

Definition

The second member of the ►CEA gene family to be discovered. NCA contains the characteristic IgV domain at the N-terminus but has only two IgC2 like domains. It is linked to the cell membrane via a glycosyl phosphatidyl inositol moiety.

Nonsteroidal Anti-inflammatory Drugs

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Definition

NSAIDs; Are a structurally diverse group of similarly acting compounds that prevent symptoms of pain, fever, and inflammation without steroid chemistry.

Characteristics

NSAIDs are the most commonly prescribed drugs worldwide for the treatment of pain and ►inflammation. They are effective as ►antipyretic and ►analgesic and are also effective in prevention of cardiovascular complications. In recent years, evidence from animal as well as prospective and retrospective clinical studies indicate that NSAIDs may lower the risk of cancer development and more importantly progression of cancer, especially colorectal and breast cancers.

Types of NSAIDs

Depending on their strength, duration of action, and elimination from the body NSAIDs have been classified as

- *Salycylic acids:* Aspirin, Salsalate, and Diflunisal
- *Acetic acids:* Sulindac, Indomethacin, Diclofenac, and Tolmetin
- *Propionic acids:* Ibuprofen, Naproxen, Flurbiprofen, Oxaprozin, Ketoprofen, and Fenoprofen
- *Enolic acids:* Meloxicam and Piroxicam
- *Fenamic acids:* Meclofenamate and Mefenamic acid
- *Pyranocarboxylic acids:* Etodalac
- *Napthylalkanones:* Nabumetone
- *Pyrroles:* Ketorolac
- ►*COX-2 inhibitors*: Celecoxib, Valdecoxib (Bextra), and Rofecoxib (Vioxx). Bextra and Vioxx were withdrawn from market.

Mechanism of Action

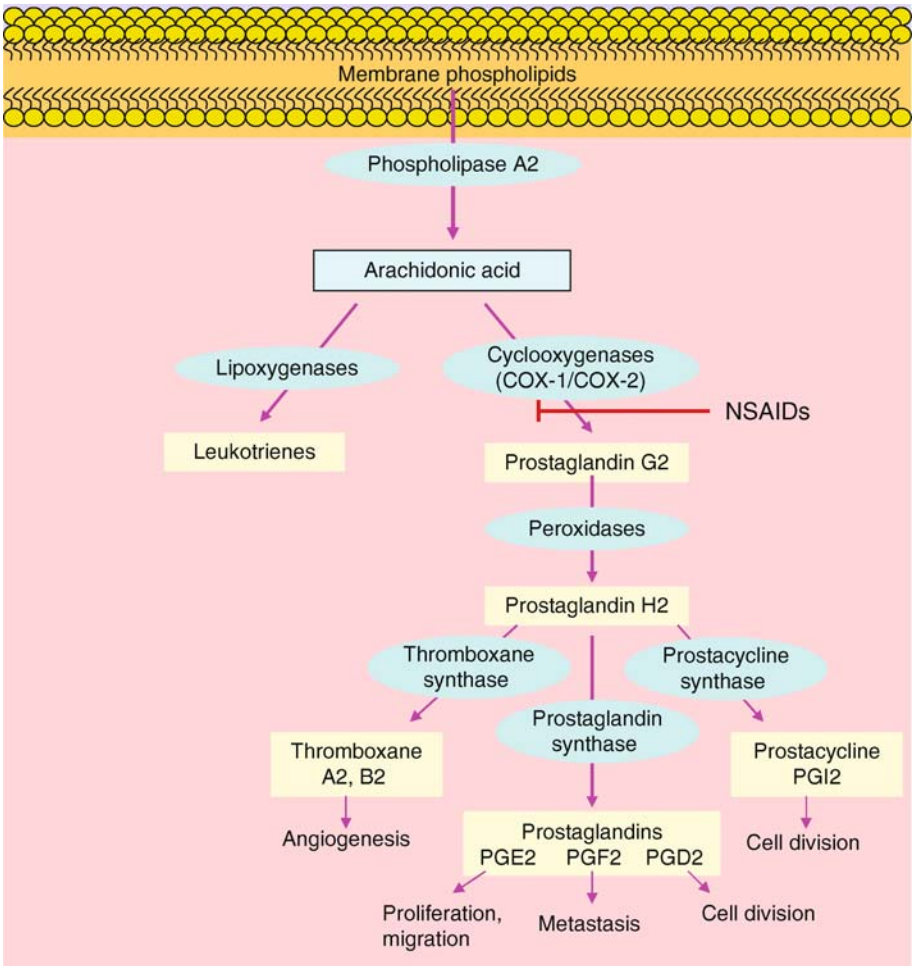
Most NSAIDs act as nonselective inhibitors of the enzyme ►cyclooxygenase – they inhibit both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. Cyclooxygenases catalyze the formation of ►prostaglandins and ►thromboxane from ►arachidonic acid (Fig. 1). Prostaglandins act as messenger molecules in the process of inflammation and neoplasia. Overexpression of COX-2 stimulates angiogenesis, formation of new blood vessels, in tumors. Angiogenesis is required for the transition of normal cancers to invasive cancer leading to metastasis. COX-2 can stimulate ►angiogenesis by promoting production of ►vascular epithelial growth factor (VEGF), ►matrix metalloproteinases, and prostaglandins. Further, COX-2 increases production of antiapoptotic protein ►Bcl-2 and thus causes resistance to death of cancer cells.

Cancer Chemoprevention

►Inflammation originating from infections, chronic injury, or ►autoimmune diseases can cause immune response, which subsequently forms ►free radicals and ►reactive oxygen species (ROS) through a cascade of reactions. These interact with cellular DNA, proteins, and lipids and cause cellular and genomic damage. In addition, the signals initiated by ROS release ►eicosanoids which trigger cell proliferation, cause resistance to ►apoptosis and facilitate carcinogenesis [2]. Although NSAIDs have been used for many years to treat rheumatic diseases and inflammatory symptoms, in recent years a large number of studies suggest the important chemopreventive properties of NSAIDs against various forms of cancer such as ►colorectal cancer, ►breast cancer, ►bladder cancer, ►prostate cancer, and ►lung cancer.

Colorectal Cancer

Adenomatous polyps are the initial risk for most of the colon cancer tumors. ►Cox-2 overexpression and ►PGE2 production has been observed in various colorectal ►adenomatous polyps and tumors but not in normal. Treatment of patients with familial adenomatous polyps with sulindac for 9 months resulted in a 44% and 35% decrease in the number and size of colonic polyps. Similarly, ►celecoxib resulted in ~30% decrease in the number and size of colonic polyps. In another study, aspirin treatment also significantly reduced the risk of colon polyps. Thus, these and other ongoing clinical studies suggest the significance of NSAIDs in chemoprevention of colorectal cancer.



Nonsteroidal Anti-inflammatory Drugs. Figure 1 COX-2 inhibition by NSAIDs. Cyclooxygenases catalyze arachidonic acid to prostaglandin H2, which is further catalyzed to prostaglandin E2, D2, F2, and I2 and thromboxane A2. The prostaglandins cause cell growth, angiogenesis, and metastasis, leading to cancer development.

Breast Cancer

Increased levels of ►PGE2 production and COX-2 expression along with ►aromatase have been observed in the breast cancer biopsies. Subsequently, in a number of studies NSAIDs such as ►aspirin have been used to prevent breast cancer-related complications. One study has reported a 21% reduction in the incidence of breast cancer in women taking NSAIDs at least twice a week for a period of 5–9 years and a 28% reduction after 10 or more years of use. Further, COX-2 overexpressing transgenic mice developed mammary tumors after several cycles of pregnancy and lactation while wild type animals remained tumor free. In another study, a 35-day course of ibuprofen administered to rats with carcinogen-induced mammary tumors, led to a significant reduction in tumor volume. In animal models, celecoxib was found to be effective in preventing tumors associated with breast cancer. The combination of standard chemotherapy drugs with COX-2 inhibitors has been shown to increase the efficacy of chemoprevention to breast cancer in a number of studies.

Prostate Cancer

Several investigations suggest that chronic ►inflammation leads to ►prostate cancer. Even though overexpression of COX-2 in malignant prostate tissues is not well noticed, the use of NSAIDs such as sulindac and flubiprofen has been shown to decrease risk of prostate cancer in animal models.

Bladder Cancer

The expression of COX-2 has been shown to increase in bladder transient cell carcinoma. Most of the studies performed using NSAIDs in animal models of ►bladder cancer suggest that regular use of NSAIDs

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could prevent the risk of bladder cancer and is more effective with combinational therapy. COX-2 inhibitors are in ongoing clinical trials in preventing bladder cancer recurrence after transurethral resection.

Lung Cancer

COX-2 overexpression has been observed in the biopsies of ►non-small cell lung cancer. In patients with asbestosis, idiopathic fibrosing alveolitis, and in heavy tobacco users the increased incidence of lung cancer is associated with overexpression of COX-2. Clinical studies have shown that NSAIDs are effective drugs in reducing risk of lung cancer, especially in heavy smokers.

Conclusions

A number of clinical, epidemiological, and animal studies suggest that NSAIDs could be promising anticancer agents. Targeted inhibition of cyclooxygenases alone or in combination with standard chemotherapy could be effective in the treatment of colorectal, bladder, and breast cancers. NSAIDs could also be effective in preventing recurrence of various types of tumors. To discover novel and most potent inhibitors of COX enzymes requires extensive investigations of molecular mechanism(s) by which COX and PGE2 promote angiogenesis and tumorigenesis. An exciting area for future investigations will be to test the nonNSAIDs such as ►aldose reductase inhibitors alone or in combination with standard chemotherapy for the prevention of colon cancer as well as other oncogenic pathways.

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Noonan Syndrome

Definition

►Autosomal dominant disorder presenting with characteristic facies, short stature, skeletal anomalies, congenital heart defects, and predisposition to tumors.

►Neuro-Cardio-Facial-Cutaneous Syndromes

Nootropic

Definition

Improvement in functions of brain.

►Grape Seed Extract

Normoxia

Definition

Is the physiological (normal) O_2 partial pressure distribution in a defined tissue, allowing unrestricted function and activity of cells making up the tissue (or organ). Normoxic refers to a physiologically adequate supply of oxygen.

►Oxygenation of Tumors

Norton-Simon Hypothesis

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Definition

The Norton-Simon hypothesis states that the rate of cancer cell death in response to treatment is directly proportional to the tumor growth rate at the time of treatment.

Characteristics

Several experimental models have attempted to describe the fundamentals of tumor cell growth and kinetics. From these systems arose an improved understanding of tumor growth characteristics, a foundation for the key principles of chemotherapy and eventually, recognition of the importance of dose scheduling. Specifically, the Norton-Simon hypothesis of tumor kinetics was an important advance in the history of oncology. The concept of ►dose-density is derived from its principles and ultimately led to improved survival in patients with cancer as a result of optimal dose scheduling.

The Log-kill Model

The Norton-Simon hypothesis builds upon the ►log-kill hypothesis established by Skipper, Schabel and Wilcox. These investigators used a murine leukemia model to describe tumor growth and cell death. A unique feature of this L1210 mouse system is its rapid and exponential growth pattern. The log-kill concept of tumor cell growth suggests that tumor growth is exponential and that growth rate is a constant. In response to treatment with a dose of drug, a constant fraction of cells are killed regardless of the size of the tumor at the start of therapy. Therefore, one dose of drug leads to cytoreduction from 10^6 cells to 10^4 cells in the same way that one dose at 10^4 cells reduces tumor volume to 10^2 cells. Theoretically, enough doses of enough drugs over time could cytoreduce a tumor to <1 cell level, thereby achieving cure. Unfortunately, the observation that cure is rare in the majority of advanced cancers and that even patients with presumably early stage cancer relapse despite treatment suggests that the log-kill model is not entirely representative of clinical reality.

Gompertzian Growth Kinetics

An alternative model of population growth was proposed by Benjamin Gompertz, a nineteenth century British actuary who devised an equation to describe biologic growth curves which occur in nature (e.g. herds of animals). The application of Gompertzian growth to tumor kinetics improved upon our understanding of tumorigenesis. According to the ►Gompertzian growth curve, the rate of cancer growth is not uniform. Cell numbers increase over time, but the relative rate of increase falls exponentially until the tumor reaches a plateau phase of very slow absolute growth.

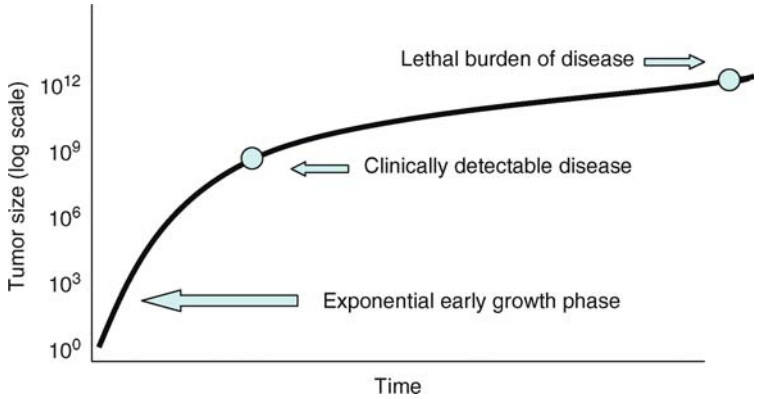
The difference between the log-kill model and Gompertzian growth is most easily visualized using a semilogarithmic plot of the logarithm of cell number versus an arithmetic time scale. In Fig. 1, early exponential growth appears as a straight line, whereas the Gompertzian curve turns continuously downward reaching a plateau that represents a stable population size. Although all tumors seem to observe the characteristic Gompertzian curve, the plateau phase for a particular type of cancer may be theoretically greater than the lethal tumor. Rapidly growing malignancies such as leukemia may appear to grow by nearly exponential growth because the host organism succumbs to the burden of disease well before the plateau phase is attained.

Norton-Simon Hypothesis

Inspired by clinical observation and mathematical modeling, Norton and Simon refined the Skipper-Schabel log-kill model, taking into consideration the kinetic, non-constant nature of population growth described by the Gompertzian equation. The underlying philosophy of the Norton-Simon hypothesis recognizes that a changing process such as cancer growth requires a treatment approach that considers the trajectory of tumor kinetics.

The hallmark of the Norton-Simon hypothesis is that the rate of cytoreduction is proportional to the rate of tumor volume growth. According to this model, log-kill is not constant, but instead it is proportional to the relative growth rate. Therefore, this model predicts that smaller tumors experience greater log-kill in response to treatment because of their rapid growth rate in comparison to larger tumors. Extrapolating further, there is a kinetic advantage to treatment when tumor volume is small such as in the post-operative setting.

The Norton-Simon hypothesis recognized that unless all cancer cells are eradicated, the eventual outcome is similar because of the rapid regrowth anticipated according to the Gompertzian curve. This led Norton and Simon to postulate that delivering treatments at a greater rate ("dose density") could optimize chemotherapy efficacy. Minimizing the regrowth of cancer between



Norton-Simon Hypothesis. Figure 1 A Gompertzian growth curve is shown here, demonstrating the early exponential growth rate at small tumor volumes and a plateau phase as the relative rate of increase diminishes with increasing tumor size.

doses of therapy would increase the cumulative cell kill, thereby achieving greater clinical benefit. The superiority of dose density in chemotherapy scheduling was first supported in a large, randomized ►clinical trial in patients with early stage breast cancer receiving ►adjuvant therapy every 2 weeks rather than the conventional every three week schedule (►Doxorubicin and cyclophosphamide (AC) followed by ►paclitaxel (T)). Since that time, several trials have supported dose dense administration of chemotherapy, leading to the improved survival of patients with cancer.

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Notch Signaling

Definition

Activation and regulation of Notch receptors are critical elements for the growth and differentiation of many different cell lineages.

►Notch/Jagged Signaling in Neoplasia

Notch/Jagged Signaling

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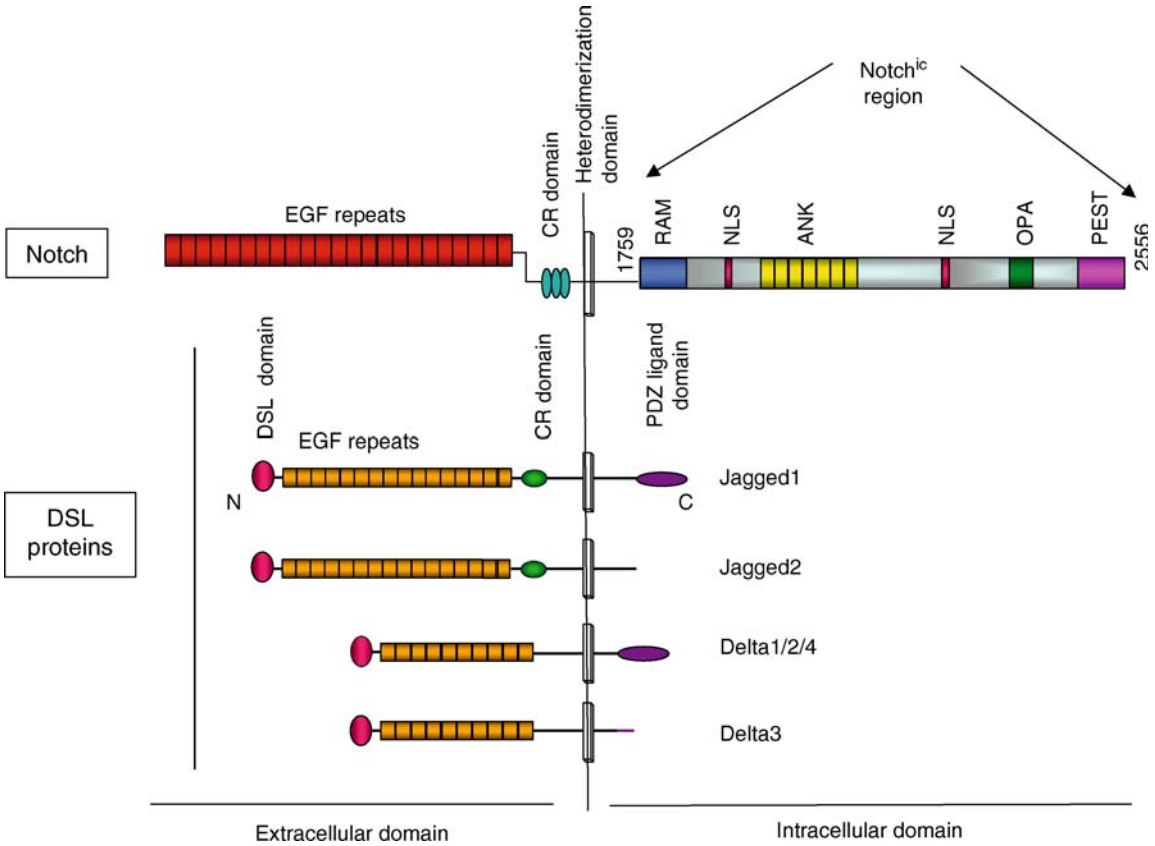
Definition

Notch and Jagged proteins define an evolutionarily conserved signal transduction pathway that is critically important for cell fate decisions in metazoan development. Notch and Jagged are single pass membrane-spanning proteins that share a receptor/ligand relationship, respectively. Jagged is the mammalian orthologue of the *Drosophila Serrate* gene. Collectively, the Notch ligands are referred to as the DSL proteins (Delta/Serrate/Lag). Constitutive activation of the Notch signaling pathway by chromosomal translocation, point mutation or gene amplification plays a role in tumor initiation and/or progression. Aberrant expression of the intracellular portion of Notch proteins ($Notch^{IC}$) is associated with the oncogenic transformation of cells. Notch was linked to cancer through its frequent mutation in T-cell acute lymphoblastic leukemia (T-ALL).

Characteristics

DSL proteins are encoded by the *Serrate* and *Delta* genes. *Drosophila melanogaster* possess only a single *Serrate*, *Delta* and *Notch* gene. However, mammalian genomes possess at least two *Serrate* and four *Delta* genes. Serrate/Jagged and Delta are large (>100 kDa) single pass transmembrane-spanning proteins (Fig. 1). The primary amino acid sequence of the extracellular domain of Serrate/Jagged and Delta reveals a striking similarity to the extracellular domain of Notch proteins. Their extracellular domains are composed of between 1 and 16 tandem copies of an EGF-like repeat. One common feature shared among the ligand proteins is a 45 amino acid sequence rich in cysteine. It is termed the DSL domain and it is located in the N-terminal of the EGF-like repeats. This domain is thought to play a role in ligand-receptor interactions. Unlike Notch proteins, the intracellular sequences of Serrate/Jagged and Delta are short (ca. 100–150 amino acids) and contain no sequence homology to any other proteins in the database. The function of the intracellular sequences of Serrate/Jagged and Delta is not understood. However, the integrity of these sequences are important since deletion of the intracellular domain from either Delta or Serrate/Jagged leads to a phenotype similar to that exhibited by loss of Notch signaling in *Drosophila*. In addition, Serrate/Jagged proteins contain a cysteine-rich domain (CR) located between the EGF-like repeats and the outer face of the plasma-membrane. The presence of this domain is used to classify ligand proteins as either Serrate- or Delta-like.

►Endocytosis plays an important role in Notch activation and inhibition. It was shown that endocytosis of DSL ligands are required for Notch activation in a neighboring cell. It has been determined that Liquid facet, Neuralized and Mindbomb are required for ubiquitination and endocytosis of DSL ligands. However, the mechanism by which endocytosis of DSL ligands activates Notch pathway in the neighboring cell has not been determined. Additionally, it has been shown that ubiquitination and endocytosis of Notch by Numb,



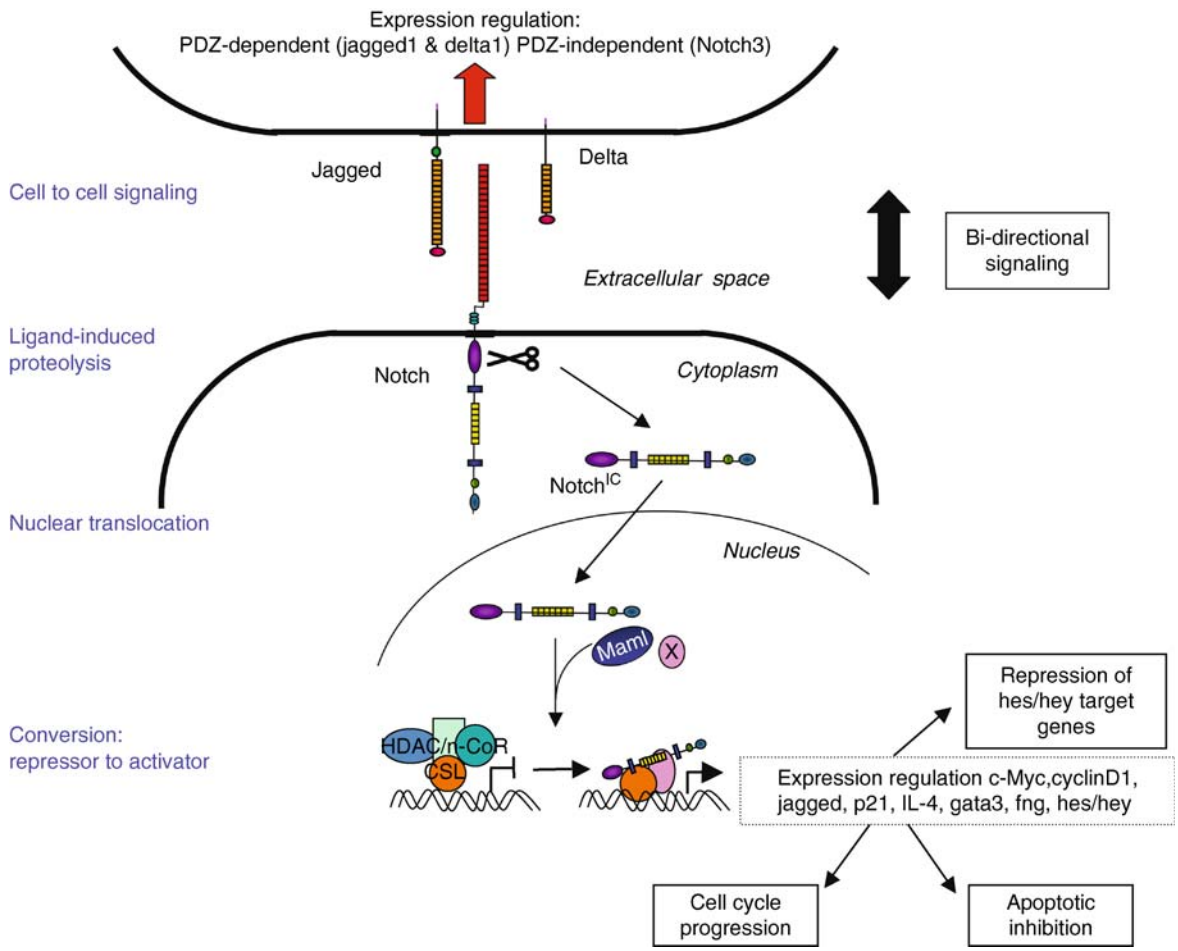
Notch/Jagged Signaling. Figure 1 Structure of Notch and DSL proteins. Notch proteins are transmembrane-spanning receptors for DSL proteins. Notch^{ic} is thought represent an activated Notch molecule. In T-cell Leukemia, the chromosomal translocation t(7;9)(q34;q34.3) fuses the T-cell receptor β locus to the Notch1 locus at the indicated break point (BP). The result of this translocation is the constitutive production of Notch^{IC}-like molecules. RAM, binding domain for CSL transcription factors; ANK, 7-tandem copies of ankyrin repeats; OPA, Glutamine-rich region; PEST, a domain thought to be involved in protein turnover. Putative nuclear localization sequences are indicated by small boxes and flank the ANK domain. Notch ligands are encoded by the Serrate/Jagged and Delta genes. DSL, a 45 amino acid sequence unique to DSL proteins thought to be involved in interactions with Notch. CR, a cysteine-rich domain of unknown function used to classify DSL proteins as either Serrate/Jagged or Delta. The intracellular sequences of the DSL proteins are of variable length and contain no recognizable motifs.

α-adaptin and AIP4/Itch leads to degradation and, therefore, inhibition of Notch.

There are four known mammalian Notch proteins, termed Notch1–4. These proteins are related through a high degree of sequence identity and structural organization. Notch proteins are membrane-spanning receptors with molecular weights of approximately 300 kDa (Fig. 1). The extracellular domain of Notch1 is composed of approximately 1,750 amino acids, which include 36 tandem repeats of a sequence resembling ►Epidermal Growth Factor (EGF) and three repeats of a motif designated as lin-12 repeats. The cytoplasmic domain comprises a sequence of approximately 750 amino acids with no apparent enzymatic activity, but containing seven tandem copies of an ankyrin-like repeat (CDC10/ANK), a region rich in glutamine (OPA), and a region rich in proline, glutamate, serine and threonine (PEST). The CDC10 and OPA repeats are thought to mediate protein-protein interactions, whereas the PEST domain might target the proteins for degradation. The RAM domain is located between the ANK repeats and the interface of the plasma-membrane. This domain is the primary binding site for the transcription factor CSL (CBF1/Supressor of Hairless/Lag1) (Fig. 1).

Notch proteins are synthesized as precursor proteins of approximately 300 kDa. During trafficking through the Golgi network, Notch undergoes a proteolytic processing event that produces the mature receptor. This processing step appears to be carried out by a furin-like protease and the resulting mature Notch receptor is composed of two fragments, the extracellular fragment and an intracellular fragment that remains embedded in the plasma-membrane. One of the major questions in Notch signaling is what is the mechanism of ligand

activation of Notch? One proposed mechanism is that the association of Notch with a DSL ligand on a neighboring cell induces a proteolytic event that releases the intracellular domain of Notch from its membrane tether (Fig. 2). Ligand-induced processing involves at least two distinct cleavage events. The first cleavage event linked to ligand binding is carried out by an ►ADAM-type ►metalloprotease that cleaves Notch in the extracellular domain close to the plasma-membrane. This cleavage is thought to induce a conformational change that allows the intracellular domain of Notch ($Notch^{IC}$) to be released from the plasma membrane following cleavage by a presenilin-dependent γ-secretase-like protease. Currently, it is not understood how ligand engagement of the receptor induces these processing events or if activation of Notch can occur through interactions with ligands present in the same cell. Moreover, there is still debate over the possibility of signaling from membrane-tethered forms of Notch that do not require additional proteolytic processing. Then, $Notch^{IC}$ translocates to the nucleus to form a transcriptional activation complex with the DNA-binding factor CSL and co-activators belonging to the Mastermind-like family of proteins (Maml). Recently, two CSL-$Notch^{IC}$-Mastermind ternary complex structures bound to DNA were determined for *C-elegans* and human orthologous proteins. These studies showed that CSL simultaneously mediates interactions with $Notch^{IC}$, Mastermind and DNA. The structures revealed that the N-terminal helical region of Mastermind forms a tripartite complex with ankyrin repeats 3–7 of $Notch^{IC}$ and the C-terminal domain of CSL. Additionally, the C-terminal helix of Mastermind interacts with the N-terminal domain of CSL. The activation complex exhibits a rapid turn-over rate, in part as a consequence to phosphorylation events triggered by cyclinC-CDK8 and the activity of E3-ligases of the Sel-10 family.



Notch/Jagged Signaling. Figure 2 Model for Notch signaling. Notch signaling is thought to occur following the interaction of DSL proteins and Notch proteins on neighboring cells. This interaction triggers a series of proteolytic processing events (indicated by scissors). Once Notch is cleaved, it is released from its membrane tether and translocates to the nucleus. In the nucleus $Notch^{IC}$ displaces the HDAC/co-Repressor complex and interacts with the transcription factors CSL and Maml and thereby activates gene expression.

The Notch transcriptional activation complex participates in the regulation of gene expression that, in part, serves to govern cellular processes such as differentiation, proliferation and apoptosis. Numerous Notch target genes have been identified, including ►c-myc, ►cyclin D1, Jagged, p21, IL-4 and Fringe. Notch directly upregulates c-myc, a potent oncogene, in T-cells and mammary epithelial cells. Notch has also been found to directly induce the expression of cyclin D1, a positive cell cycle regulator, as well as indirectly suppress the pro-apoptotic protein, ►p53, via downregulation of ►ARF by a yet unidentified mechanism. Additionally, Notch activates two families of basic helix-loop-helix proteins, HES and HEY, which homo- and heterodimerize to actively repress target genes (Fig. 2).

Bi–Directional, Jagged1-Mediated Signaling

The presence of a cysteine-rich region (CR) is used to classify Notch ligands into Delta-like or Serrate-like. Delta1, 2 and 4, not Jagged2 or Delta 3, also contain a putative PDZ-ligand domain (RMEYIV). The significance of the PDZ-ligand domain and the downstream events is not well understood in the context of Notch-DSL signaling. However, it has been shown that overexpression of Jagged1 can induce cellular transformation in RKE cells, which is dependent on its PDZ-ligand domain (PSD-96/DLG/Zo-1).Both the intracellular and extracellular domains are required for Jagged1-mediated transformation. To date, a number of Jagged1 target genes have been identified, including Notch3, Delta1 and Jagged1, itself, although whether they are direct targets has not been determined. Deletion of the PDZ-ligand domain of Jagged1 prevents upregulation of Delta1 and Jagged1, but not Notch3, suggesting that there are at least two signaling pathways downstream of Jagged1.

Fringe proteins provide another level of specificity between the two classes of DSL proteins. Fringe modifies Notch by adding O-fucose glycan to the EGF repeats which results in a decreased affinity for Jagged proteins and a higher affinity for Delta proteins. Therefore, upon expression of Fringe, Delta proteins are able to initiate Notch signaling, while Jagged proteins cannot (Fig. 3). A possible model for bi-directional signaling in the Notch pathway may involve a PDZ mechanism mediated by both Delta and Jagged proteins. In this model, Jagged1 could signal in both directions in the absence of Fringe, whereas the Delta signal would always be bidirectional. In the case of Jagged2, signaling would only be in the Notch direction and this signal could be attenuated by Fringe. In contrast, Delta3 is insensitive to Fringe and would always allow signaling in the Notch direction. This model provides exquisite flexibility for signal specificity and accounts for the multiple distinct DSL proteins.

C-terminal residues		PDZ ligand	Inhibited by fringe
R M E Y I V	Jagged1	+	+
R Y A G K E	Jagged2	–	+
V I A T E V	Delta1/2/4	+	–
I Y A R E A	Delta3	–	–

Notch/Jagged Signaling. Figure 3 Effects of Fringe on Notch signaling. Fringe modifies Notch by adding O-fucose glycan to the EGF repeat region which modulates Notch affinity for its ligands. Upon expression of Fringe, Delta proteins are able to initiate Notch signaling, while Jagged proteins cannot.

The major question is: what is the mechanism by which the Notch activation complex exerts the pleiotropic effects that are observed in distinct cellular contexts?

Over the last several years many proteins have been identified that can physically interact with Notch^{IC}. However, a clear picture has not emerged to describe the mechanism of Notch signaling.

Deltex, an ►E3 ubiquitin ligase, is an exclusively cytoplasmic proline-rich protein localized to endosomal vesicles. It genetically acts as an enhancer of Notch signaling and has been shown to physically associate with Notch through the ankyrin repeat domain. As previously explained, data showed that Deltex ubiquitinates Notch preventing its degradation by sequestering it to endosomal vesicles.

Mastermind like-1 (Maml1) is an integral component of the Notch pathway whose function is poorly understood. Maml1 encodes a nuclear co-activator protein that binds to the ankyrin repeat domain of Notch proteins. It forms a trimeric complex with the intracellular domain of Notch and the DNA binding protein, CSL. It is thought that Mastermind functions, at least in part, by recruiting histone acetyltransferases such as p300.

CSL is the collective term for the mammalian counterparts to Su(H) and Lag1. CSL proteins are transcriptional regulatory proteins that repress transcription under non-induced conditions. Repression by CSL is thought to be mediated by a histone deacetylase (HDAC) complex containing n-CoR/Smrt. Upon ligand activation of Notch it is thought that Notch^{IC} translocates to the nucleus and interacts with Skip to displace the HDAC complex from CSL which leads to the formation of a transcriptional activation complex (Fig. 2).

Additional proteins have been identified that appear to genetically inhibit Notch signaling. These proteins are Dishevelled (Dsh), Numb and Ikaros. Dsh is a protein

with unknown biochemical function that is a component of the Wnt signal transduction system. Dsh has been shown to physically interact with the carboxy-terminal portion of Notch and appears to influence Notch signaling. Numb is a cytoplasmic protein that has homology to phosphotyrosine binding (PTB) domains and may provide a link to protein tyrosine kinase signaling cascades. The molecular basis of these interactions and the mechanism of inhibition of Notch signaling are not known. Additionally, it has been shown that Notch activation of target genes is perturbed by Ikaros, a transcriptional regulator required for development of all lympoid derived cells. This inhibition is the consequence of the ability of Ikaros to bind CSL consensus sequences. Proviral insertion mutagenesis experiments performed in $Notch^{IC}$ transgenic mice using Moloney murine leukemia virus showed an insertion into the Ikaros locus in 40% of tumors generated. These proviral insertions result in truncated Ikaros proteins, and those lacking DNA binding domains may then function as dominant negative inhibitors to full-length Ikaros by forming dimers. These dimers prevent Ikaros-mediated inhibition of Notch transactivation of target genes.

Clinical Relevance

T-Cell Leukemia

The human homologue of Notch1 (TAN1) was cloned from a T-cell acute lymphoblastic leukemia (T-ALL) that harbored the chromosomal translocation, t(7;9)(q34;q34.3). This translocation joins a portion of *NOTCH1/TAN1* to the *T-cell receptor β* locus. This translocation generates aberrant Notch proteins that lack most of the extracellular domain and are not tethered to the plasma membrane. As described above, these forms of Notch are thought to be constitutively active. This translocation can be identified in approximately 10% of all T-ALL cases. Additionally, 50% of human T-ALL exhibit activating Notch1 mutations in the heterodimerization domain and/or the C-terminal PEST region.

Cervical Carcinoma

In situ expression studies comparing normal and neoplastic cervical epithelium, showed an aberrant Notch expression in the development of ►cervical carcinoma. In normal cervical tissue, expression of Notch is limited to the basal layer of the stratified epithelium. However, in dysplastic tissues both Notch and its ligands' expression is increased compared to normal tissues, indicating that inappropriate activation of Notch signaling might play a role in the generation of cervical neoplasia. Approximately 99% of cervical neoplasms are positive for ►Human Papillomavirus (HPV) E6 and E7. HPV infection of cervical epithelial cells frequently leads to cellular transformation. HPV viral proteins, E6 and E7, transform cells, in part by targeting the tumor suppressors p53 and Rb for degradation. It has been demonstrated that E6 and E7 upregulate Notch and presenelin expression, and that growth of CaSki cells (cervical carcinoma cells expressing HPV16) are Notch-dependent. Additionally, the *Notch1* gene is an integration site for HPV16. Paradoxically, data indicate that Notch1 must be downregulated in invasive cervical cancers expressing E6 and E7, suggesting that Notch1 plays a positive role in early cervical carcinogenesis, but suppresses E6 and E7-mediated transformation in later stages of tumorigenesis.

B-Cell Leukemia

►Epstein-Barr Virus (EBV) is the etiologic agent of ►Burkitt lymphoma. The latent viral protein EBNA-2 is a transcriptional activator that functions by binding to host cell CSL proteins and displacing the HDAC repressor complex in an analogous manner to the model for $Notch^{IC}$. Therefore, in transformation of B-cells by EBV infection, EBNA2 may provide a function similar to $Notch^{IC}$ by usurping CSL proteins. Notch can induce the same phenotypic changes as EBNA2 in Burkitt lymphoma cells.

Mammary Carcinoma

Infection of mice with MMTV has been used as an insertional mutagen to identify genes that contribute to the generation of mammary carcinoma. Among the genes affected by such mutations is *Int-3*, a Notch gene family member now termed *Notch4*. The mutations result in aberrant expression of truncated Notch4 proteins comprising only the intracellular portion of the molecule. Additionally, in MMTV-neu/ErbB2 insertional mutagenesis studies, Notch1 insertions were found in 8% of tumors screened. Insertions were found in the genomic region between the transmembrane domain and lin-12 repeats. Furthermore, it has been demonstrated that the expression of constitutively active Notch1, Notch3 or Notch4 is sufficient to induce breast tumors in mouse models. Moreover, it was determined that loss of myc prevents the formation of MMTV-$Notch1^{IC}$ nonregressing mammary tumors. Additionally, it was recently shown that human breast tumor samples expressing high levels of Jagged1 have poor clinical outcome compared to low Jagged1 expressing samples.

Prostate Carcinoma

Jagged1 expression correlates with ►prostate cancer progression, metastasis and recurrence. Additionally, downregulation of Jagged1 expression results in growth inhibition of prostate cell lines, PC3, Du145, LNCaP and C4-2B. Taken together, these data suggest that deregulated Jagged1 expression may contribute to prostate cancer cell growth either by changing the microenvironment and increasing Notch activation in neighboring cells, via its downstream, PDZ-dependent

Notch/Jagged Signaling. Table 1 Evidence of Notch's function as an oncogene and tumor suppressor in human and mouse models

Evidence of the oncogenic potential of Notch	
Human cancers	*Genetic alteration*
Non-small cell lung carcinoma	Increased Notch3 expression via translocation (15;19)(15, 25)
T-ALL	Notch1 activating mutations (25, 72)
Ovarian serous carcinoma	Notch3 gene amplification (49)
Cervical carcinoma	Increased expression of activated Notch1 and Notch2 (75)
Mouse models of cancer	*Genetic alteration*
Brain cancer (choroid plexus)	Constitutively active Notch3 expression (14)
Breast cancer	Constitutively active Notch1, Notch3 or Notch4 expression (24)
T-ALL	Constitutively active Notch1 expression (3, 25)
Evidence of a tumor suppressor role for Notch	
Mouse models of cancer	*Genetic alteration*
Squamous cell carcinoma	dn MAML1 (52)
Basal cell carcinoma	Notch1 knockout (45)

signaling events in the cell in which it is expressed or bi-directionally.

Feline Leukemia Virus (FeLV) Induced T-Cell Leukemia

Infection of cats with replication competent Feline Leukemia Virus (FeLV) yielded T-cell leukemia that harbored recombinant FeLV that had transduced a portion of the feline *Notch2* gene. The transduced gene encodes a Notch2 protein analogous to those expressed in human T-ALL.

Additionally, aberrant expression and/or activation of the various Notch isoforms have also been recently implicated in contributing to non-small lung carcinoma, ovarian serous carcinoma, brain cancer, squamous cell carcinoma and basal cell carcinoma (Table 1).

Cross-Talk Between Signaling Pathways

Notch linked to cancer through its frequent mutation in T-ALL, can integrate with other pathways to accelerate oncogenesis. Like Wingless (Wnt) and ►Sonic Hedgehog (Shh), the Notch signaling pathway is essential in controlling both developmental processes and tumorigenesis. For example, in human and murine medulloblastoma, Notch and Shh synergize to promote tumor proliferation and survival. Also in ►medulloblastoma, Shh dependent tumor growth involves synergy with Notch and Wnt. Notch/Wnt crosstalk has been suggested in Melanoma, activation of Notch1 enhances primary melanoma cell growth and the potential for metastasis through β-catenin upregulation. An important positive oncogenic collaboration was also shown in breast cancer cells between Notch and Wnt.

Notch as a Potential Therapeutic Target

Due to the important role of Notch signaling in cancer development, it has been proposed that targeting the Notch signaling steps including receptor/ligand binding, release of $Notch^{IC}$ and downstream targets could have antitumor effects. One of the approaches used to inhibit Notch signaling is to suppress the γ-secretase-proteolytic step leading to the release of active $Notch^{IC}$. Inhibitors for γ-secretase have been studied for decades for their potential to block the formation of beta-amyloid peptide (Aβ) associated with Alzheimer disease. Aβ peptide is synthesized much in the same way that active $Notch^{IC}$ is generated, via a series of proteolytic cleavages, the final of which is cleavage of the precursor by γ-secretase. It has been demonstrated that γ-secretase inhibitors are capable of preventing Notch receptor activation and are currently being tested for their anti-tumor effects. Encouragingly, various γ-secretase inhibitors have been successfully used in mouse cancer models to inhibit tumorigenesis. Inhibition of tumor growth was demonstrated in xenografts of the ►Kaposi sarcoma cell line, SLK, upon injection of the tumor with a γ-secretase inhibitor, GSI-I. Additionally, intraperitoneal injection of a γ-secretase inhibitor, DBZ, inhibited epithelial cell proliferation in intestinal adenomas from $Apc^{-/-}$ mice and induced goblet cell differentiation. Currently, Merck is recruiting or has completed recruitment of patients for phase I clinical trial to test safety/tolerability and efficacy of a Notch inhibitor, MK0752, in relapsed or refractory T-ALL patients and advanced breast cancers (ClinicalTrials.gov Identifier: NCT00100152 & NCT00106145, respectively).

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NPM

Definition

Nucleophosmin; a ubiquitous protein that is normally involved in maturation of ribosomes and nucleocytoplasmic protein trafficking.

►Anaplastic Large Cell Lymphoma

NPM–ALK

Definition

A protein derived from the fusion gene NPM-ALK that is characteristically expressed in ►anaplastic large cell lymphoma. It contains a portion of the wild-type ALK and NPM proteins and, as a dimer, maintains the phosphorylation activity of ALK (ALK protein).

NQO1

Definition

NAD(P)H:quinone oxidoreductase-1, synonym quinone reductase (QR) or DT-diaphorase, catalyzes the obligatory two-electron reduction and ►detoxification of quinones, stabilizes ►p53 and strengthens cellular antioxidant defense.

►Benzene and Leukemia
►Phase 2 Enzymes

4-NQO

Definition

4-Nitroquinoline 1-oxide 4-nitroquinoline 1-oxide (4-NQO) is a UV-mimetic mutagen that causes DNA bulky lesions and can induce tumors in laboratory animals.

►Mutagen Sensitivity

NR1C

►Peroxisome Proliferator-Activated Receptor and Cancer

NR3C4

►Androgen Receptor

Nrf2

Definition

Nuclear factor erythroid 2-related factor 2, is a master transcription regulator of cytoprotective genes. It forms hetrodimer with partners such as small Maf and binds to ►ARE (a cis-acting DNA regulatory element) and stimulates transcription of the downstream gene.

►Phase 2 Enzymes

NSC-638850

►UCN-01 Anticancer Drug

NSC-684766

►Trabectedin

NSC-710428

►Epothilone B Analogue

NSC-312887

►Fludarabine

NSC-362856

►Temozolomide

NSC-10514-F

►Cladribine

NSCLC

Definition

►Non-small cell lung cancer.

Nuclear Atypia

Definition

Abnormality of the cell nucleus that may be associated with a precancerous condition.

Nuclear Envelope Breakdown

Definition

NEBD; The nuclear envelope consists of two concentric lipid layers fused to one another that are punctured by nuclear pore complexes, which allow passive and regulated transport of proteins and RNA between the nucleus and cytoplasm. Underlying the inner lipid bilayer is the nuclear lamina, composed predominantly of polymerized intermediate filaments known as nuclear lamins. Nuclear envelope breakdown (NEBD) requires vesicularization of the lipid bilayers, depolymerization of the nuclear lamins to disassemble the nuclear lamina, and the falling apart of nuclear pore complexes. The best understood of these three processes, nuclear lamina disassembly, is caused by a multisite phosphorylation of the nuclear lamins. The number of kinases directly involved in depolymerization of nuclear lamins is unclear, however at least cyclin B1/CDK1 and ►protein kinase C are likely to be involved.

►G_2/M Transition

Nuclear Export Signal

Definition

NES; Amino acid sequence that when present in a protein in appropriate location confers the ability to be recognized by the nuclear export machinery to permit its translocation to the cytoplasm. Several amino acid sequences can act as NES. This domain targets its protein for export from the cell nucleus to the cytoplasm through the nuclear pore complex. The NES is recognized and bound by the transporting molecule family exportins.

►Snail Transcription Factors

Nuclear Factor-κB

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Definition

Nuclear Factor-kappaB (NF-kappaB, NF-κB) represents a family of transcription factor proteins that regulate expression of multiple genes important in cell survival, host responses to injury and infection, and pathogenesis of various diseases, including cancer. Originally, the transcription factor was discovered as a bacterial lipopolysaccharaide (LPS) induced nuclear transcription factor regulating expression of kappa light chains in B lymphocytes. Currently, it is considered one of the major links between immunity, inflammation, and cancer. Aberrant activation of NF-κB has been linked with a variety of malignancies including head and neck squamous cell carcinoma, ►lung cancer, ►esophageal cancer, ►breast cancer, ►prostate cancer, ►pancreas cancer, ►colon cancer, ►cervical cancer, ►melanoma, and ►hematological malignancies, ►leukemias and lymphomas.

Characteristics

The original link between pathogens, NF-κB activation, and development of cancer was identification of REV-T, a transforming oncogene contained in avian ►retroviruses, which leads to reticuloendothelial lymphomatosis. Like its mammalian homologues (►REL A, REL B, cREL), it shares a C-terminal transactivation domain. In mammalian cells, the NF-κB family includes five known subunits: NF-κB1 (p105/p50), NF-κB2 (p100/p52), REL A (p65), cREL, and RELB (Fig. 1). Each subunit contains a 300 amino acid sequence in its N-terminus known as the REL homology domain. In addition, cREL, REL A, and REL B each contain a transactivation domain in their C-termini. NF-κB1 p105 and NF-κB2 p100 are initially expressed containing contiguous ankyrin repeats. Processing of NF-κB1 and NF-κB2 by the proteasome results in degradation of the ankyrin domains to produce the NF-κB p50 and p52 subunits. The NF-κB and REL subunits form homo- and heterodimers, excluding RELB, which appears to form only heterodimers. The most ubiquitous of these combinations is the p50/REL A heterodimer. In most cells in the resting state, these homo- and heterodimers remain predominantly in the cytoplasm in dormant form, complexed with inhibitor-κBs (IκBα, β, γ), which mask the nuclear localization sequence and DNA binding pocket.

Regulation

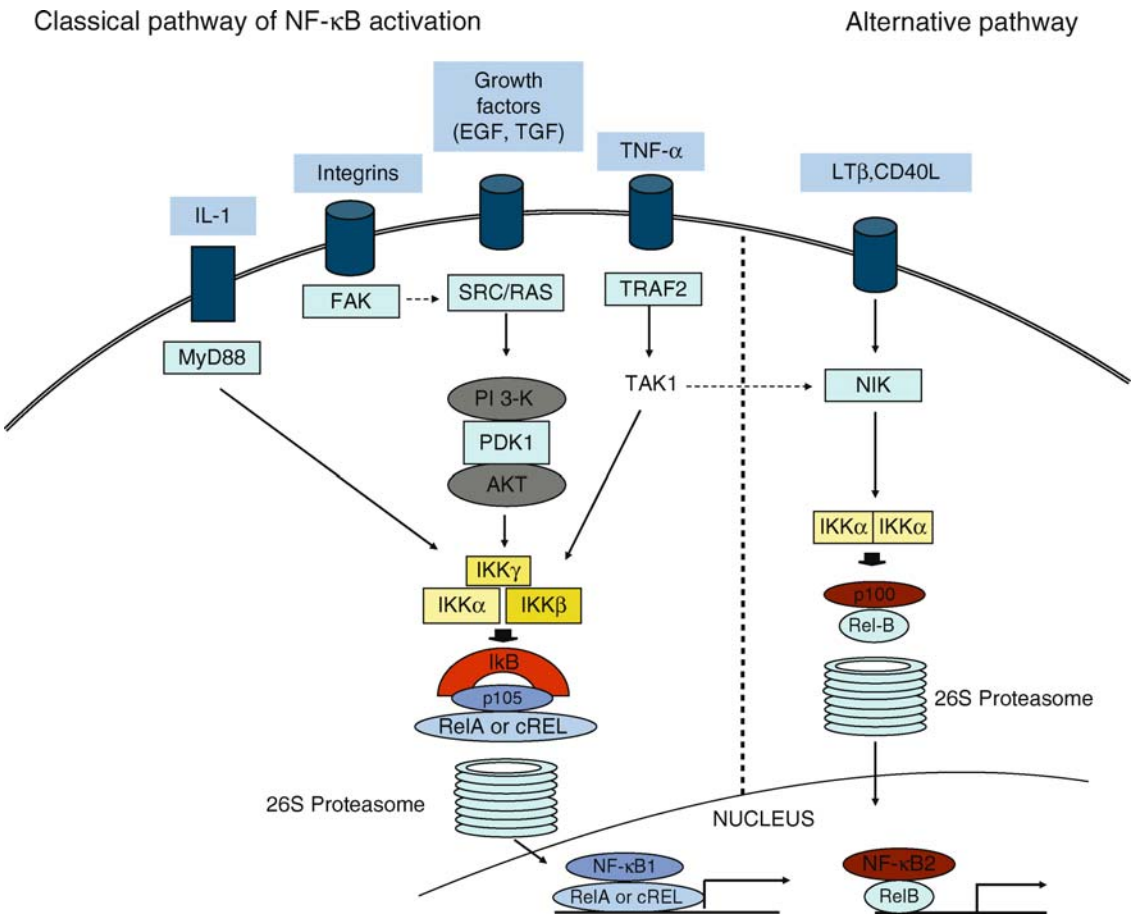
Activation of inhibitor-κB kinases (IKKs) by various cell stimuli leads to phosphorylation and subsequent ubiquitinylation of IκB molecules (Fig. 1). Ankyrin/IκB degradation by the ►proteasome results in exposure of the nuclear localization sequence and DNA binding sites of NF-κB and REL dimers, thus allowing nuclear translocation and DNA binding to 9–10 bp κB binding sequences in DNA of the promoter of target genes. Additional phosphorylations of some of the subunits or cofactors by IKK and other kinases, regulate whether bound NF-κB/REL complexes repress or transactivate target genes.

In a current paradigm, IKK and NF-κB activation is separated into the "classical" (canonical) and "alternative" (noncanonical) pathways (Fig. 1). The classical pathway is typically induced by inflammation, injury, infection, and cytokines such as tumor necrosis factor (TNF), or interleukin-1 (IL-1). It appears to be important for cell survival as well as for innate and adaptive immunity. The alternative pathway, which is activated by other TNF family members, has been implicated in regulation of survival of premature B lymphocytes and development of peripheral lymphoid tissue. In disease states such as cancer, infection, and inflammation, the canonical pathway is often aberrantly activated and NF-κB1/REL A are localized to the nucleus.

Clinical Relevance

Several viral genes which activate the IKK/NF-κB pathway have been identified in ►Epstein–Barr virus, human T cell leukemia virus, ►hepatitis B virus, ►hepatitis C virus, and human papillomaviruses, and have been implicated in their role in pathogenesis of ►Hodgkin disease, leukemias, ►hepatocellular carcinoma, and ►cervical cancer and head and neck ►squamous cell carcinomas. Chronic bacterial-induced ►inflammation by ►*Helicobacter pylori* and colonic flora in inflammatory bowel disease has been implicated in gastric and colon carcinomas.

NF-κB activation by chemical promoters and DNA damage is more widely involved in cancer development and ►progression. Examples of this include nicotine and other carcinogens in tobacco and the betel nut (areca) ►betel Quid, that have been linked to NF-κB activation in pathogenesis of malignancies of the lung as well as head and neck. In addition to such inducible effects, aberrant activation may result from mutation or overexpression of a variety of growth factor receptors (►EGFR, ►ErbB2, etc.), cytokines (►TNF-α, IL-1), cell adhesion molecules (►integrins), and signaling kinases (►RAS, ►BCR-ABL1), which can activate NF-κB via IKK, and promote molecular pathogenesis of epithelial and lymphoid malignancies. In addition to such endogenous stimuli, NF-κB may also be activated by exogenous stimuli, secondary to stress, hypoxia, chemotherapy, and radiation. Inducible activation by these factors



Nuclear Factor-κB. Figure 1 NF-κB activation. In the classical pathway, stimulation by IL-1, integrins, ErbB Family growth factors, and TNF-α leads to activation of an inhibitor-κB kinase (IKK) α, β, γ complex, which in turn leads to phosphorylation, and subsequent ubiquitinylation of IκB molecules. Following proteasome processing, the liberated complex NF-κB1/RELA or NF-κB1/cREL) translocates to the nucleus to promote target genes. In the alternative pathway, stimulation via ligands including CD40L and LTβ leads to activation of the intermediary NF-κB inducing kinase (NIK), an IKKα dimeric complex, and proteasome processing of P100/RELB to p52/RELB. TNF-α may also activate NIK in the alternative pathway. The liberated NF-κB2/RELB complex translocates to the nucleus to promote target genes.

can promote therapeutic resistance to chemotherapeutic agents, immune toxins (i.e., TNF), and radiation therapy.

NF-κB promotes expression of a diversity of genes involved in proliferation, cell survival, invasion, ►inflammation, and ►angiogenesis that have been implicated in cancer. These include genes crucial to cell proliferation and survival such as ►cyclin D (key in progression of cell cycle from G0 to G1 and S phase), and ►BCL-2 and ►IAP gene families (inhibit mitochondria-mediated ►apoptosis). Expression of NF-κB modulated angiogenesis factors such as GRO1, IL-8, and ►VEGF allow tumors to obtain sufficient neovasculaization for tumorigenesis. NF-κB-mediated alterations in expression or interaction of cell adhesion molecules (integrins, CAMs) and ►matrix metalloproteinases (MMPs) with matrix or other cells have been implicated cell migration, invasion, and metastasis.

With all of the above mechanisms by which NF-κB plays a role in cancer progression, inhibiting the transcription factor has become an important candidate for therapeutic investigation. As a result of its central role in inflammation, the first drugs to be used for cancer therapy and prevention and found to inhibit NF-κB were corticosteroids and ►nonsteroidal anti-inflammatory drugs (NSAIDs). Corticosteroids in combination with cytotoxic chemotherapy are now a mainstay of therapy in certain leukemias, lymphomas, and myelomas. NSAIDS, several of which inhibit IKK as well as arachidonic acid synthesis, have been shown to reduce development of inflammatory and heritable colon carcinomas in experimental models and clinical studies. Natural compounds such as ►curcumin, which comes from tumeric (a common Indian spice), have been shown to effectively inhibit NF-κB activation, and have been implicated in the

reduced incidence of colon cancer in India. Standard ►chemotherapy agents, such as ►cisplatin, have been shown to have cytotoxic activity as a result of inhibition of NF-κB activation, in addition to their DNA damaging capability. Recently, agents more specifically targeting the proteasome, IKKs, and other upstream kinases have been investigated. The 26S ►proteasome inhibitor ►bortezomib (VELCADE, Millennium pharmaceuticals) when used alone or in combination with other therapies has been shown to have preclinical and phase I/II clinical activity in ►multiple myeloma, mantle cell lymphomas, ►Waldenstrom macroglobulinemia, ►lung cancer, and head and neck ►squamous cell carcinoma. It is currently approved for treatment of patients with therapy-resistant multiple myeloma. Because of remaining concern regarding the broader effects of proteasome or other NF-κB inhibitors on its physiologic roles in immunity, inflammation, and cellular homeostasis, the role of these agents remains investigational.

Aberrant and inducible activation of NF-κB via multiple signaling pathways has been observed in multiple human malignancies. While the specific role it plays in the progression of each of these diseases is yet to be fully elucidated, its activation in most forms of cancer points to its overall importance in cancer. As more is learned about the molecular regulation of NF-κB and its target oncogenes, novel targeted therapies may allow improvement in current treatment outcomes.

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Nuclear Hormone Receptors

Definition

Are ligand-activated transcription factors that regulate gene expression by interacting with specific DNA sequences upstream of their target genes. Upon ligand binding, these cytosolic proteins translocate to the nucleus to bind as dimers to response elements in the promoter regions of target genes and stimulate or suppress gene transcription (e.g. retinoids bind to retinoic acid receptors and retinoid X receptors, which bind as dimers to ►retinoic acid response elements and retinoid X response elements in the promoters of retinoid-responsive genes).

►Carotenoids

Nuclear Imaging

Definition

The use of photon-emitting radionuclides to visualize tumor boundaries.

Nuclear Localization Signal

Definition

NLS; Is an amino acid sequence which acts like a “tag” on the exposed surface of a protein. This sequence is used to confine the protein to the cell nucleus through the nuclear pore complex and to direct a newly synthesized protein into the nucleus via its recognition by cytosolic nuclear transport receptors.

Nuclear Magnetic Resonance

Definition

NMR.

►Magnetic Resonance Imaging

Nuclear Magnetic Resonance Spectroscopy

Definition

A method that uses the nuclear isotopes of atoms to determine the tertiary structure of a molecule, generally in aqueous solution.

►Structural Biology

Nuclear Pore Complex

Definition

NPC; A large multiprotein complex, embedded in the double membrane nuclear envelope, which provides the sole channel for nucleocytoplasmic trafficking of ions, small molecules, proteins, RNAs, and ribonucleoprotein particles in eukaryotic cells through both a passive diffusion and an energy-dependent signal-mediated active transport.

►Major Vault Protein

Nuclear Receptor

Definition

Nuclear receptors are ligand-inducible transcription factors, such as receptors for ⇒ thyroid hormones and ⇒ steroid hormones, retinoids (⇒ ►retinoic acid) and ⇒ ►vitamin D. They typically form homo- or heterodimers that bind to specific DNA binding elements.

►Estrogen Receptor
►Orphan Nuclear Receptors and Cancer
►Parathyroid Hormone-Related Protein
►Thyroid Hormone Receptors
►Steroid Hormone Receptors

Nuclear Receptor Coactivator 3

►Amplified in Breast Cancer 1

Nuclear Translocation

Definition

Transport of proteins through nuclear pores (usually by a specific transport system) into the nucleus, enables transcription factor proteins to get access to the nucleus.

Nucleolin

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Definition

Is a ubiquitous, nonhistone nucleolar phosphoprotein of eukaryotic cells and is present in abundance at the sense fibrillar and granular regions of the nucleolus. Nucleolin is also able to localize in the nucleus, in the cytoplasm, and at the cell surface. Nucleolin is an RNA-binding protein and multifunctionally involved in many cellular processes, including ribosome biogenesis, the processing of ribosomal RNA (rRNA), mRNA stability, transcriptional regulation, and cell proliferation, and it is also a downstream target of several signal transduction pathways.

Characteristics

Regulation of Nucleolin Function as a Phosphoprotein

The multiple activities of nucleolin are regulated by covalent modifications, most notably phosphorylation, methylation, proteolysis, and ADP-ribosylation. Nucleolin function is coupled to growth control by its phosphorylation. Active rRNA transcription is positively correlated with highly phosphorylated nucleolin. In growing cells, casein kinase II (CKII) phosphorylates nucleolin. Phosphorylation by CKII enhances nucleolin (~110 kDa molecular size) as a substrates for protease to produce smaller fragments 30 and 72 kDa proteins, which trigger rDNA transcription by RNA polymerase I. Both CKII activity and nucleolin phosphorylation are enhanced after stimulation with mitogens, in regenerating rat liver after partial hepatectomy and in tumor cells. In addition, nucleolin phosphorylation by CKII and rRNA synthesis is dependent on hormones, such as dexamethasone and androgen, and growth factors. Major phosphorylation sites of nucleolin are serine and threonine. Serine phosphorylation is mediated by CKII and related to nucleolar function in the control of rDNA transcription, and threonine phosphorylation is linked to cdc2 kinase during mitosis through condensation of nucleolar chromatin, leading to increased rDNA transcription by RNA polymerase I in the G1 to S phase of cell cycle. Thus, sequential CKII and cdc2 phosphorylation modulates nucleolin function in regulating nucleolar structure and activities between interphase and the mitotic phase during cell growth.

Nucleolin Function of Cell Cycle and Ribosomal Biogenesis

Nucleolin levels are highest in tumors or other rapidly dividing cells. Nucleolin is necessary for cell

proliferation and nucleogenesis and is downregulated during differentiation. In nondividing cells, nucleolin level is low and is preferentially associated with chromatin. In G1 phase, nucleolin decondenses the chromatin by replacing histones. Hormones such as ►androgen and growth factors regulate the expression and phosphorylation of nucleolin by CKII, resulting in increased DNA transcription by RNA polymerase I in the G1 to S phase of cell cycle. Nucleolin binds to the 5′ end of the external transcribed spacer region of preRNA and participates in the preRNA processing along with fibrillarin and small nucleolar RNA. The 48S RNA is rapidly cleaved to yield the mature 18S, 28S, and 5.8S rRNA species during the transcription of rDNA by RNA polymerase I. During ►mitosis, nucleolin is phosphorylated by cdc2 kinase and condensates the chromatin. The nucleolus may be involved in functions other than ribosome biogenesis. The functions of nucleolin are the result of assemblies of nucleolin with other factors to form large complexes which induce or regulate cell growth and cancer cells.

The Role for Nucleolin in Cancer

Role of Nucleolin in B-cell Lymphomas

Nucleolin is a subunit of the ►transcription factor LR1 (switch region binding protein), which activates expression of the c-myc gene and the EBNA-1 (►Epstein-Barr virus nuclear antigen 1) gene, which promote cells to malignancy. LR-1 is a B-cell-specific DNA-binding protein that contains two polypeptides of 106 kDa and 45 kDa. The 106-kDa component of LR-1 is nucleolin. Although nucleolin is ubiquitous, LR1–DNA binding activity is ►B cell specific. LR-1 is present only in activated B cells, not resting B cells. LR-1 regulates the promoter of Epstein-Barr virus, which produces EBNA-1 protein. Nucleolin that is component of LR-1 may contribute to cell-type specific transformation by Epstein-Barr virus.

Nucleolin's Interaction with Cellular Protein, p53, and Rb

Nucleolin is directly involved in two major cellular tumor suppressors, ►Rb and ►p53. The p53 gene is commonly mutated in human cancer cells. The p53 protein has a critical role in cellular responses to DNA damage and other stresses by inhibiting proliferation or inducing G1 arrest, resulting in program cell death. Heat shock induces nucleolin to relocalize from nucleus to nucleoplasm. This nucleolin relocalization is dependent on p53 and cellular stress, including formation of p53–nucelolin complex. Nucleolin relocalization and complex formation are required the p53 C-terminal regulatory domain. Nucleolin–p53 interaction indicates the p53-dependent mechanism in which cell stress mobilizes nucleolin to cause the transient replication inhibition and DNA repair. In addition, ribosomal protein L26 (RPL26), nucleolin, and p53 protein itself bind to the 5′ UTR of p53 mRNA. p53 translation and induction after DNA damage is controlled by these factors. Increased levels of RPL26 enhance DNA-damage-induced p53 translation and its cellular function, such as G1 arrest and ►apoptosis. In contrast, nucleolin suppresses the translation and induction of p53 after DNA damage. Nucleolin has an opposite effect of p53 and RPL26.

High risk ►human papillomaviruses (HPVs) play a central etiologic role in the development of ►cervical cancer, and in this process the deregulated expression of high-risk HPV oncogenes is a critical event. Cervical cancer represents the second most common malignancy in women, with an annual incidence of ~500,000 new cases, and nucleolin is involved in HPV18-induced cervical carcinogenesis. Nucleolin controls the chromatin structure of the HPV18 enhancer in vivo and is directly linked to HPV18-induced cervical cancer formation. Nucleolin binds to the HPV18 enhancer in a sequence-specific manner. Nucleolin is a phosphoprotein whose synthesis positively correlates with cell division rates. When cells are synchronized to the G0, G1, S, and G2 phases of the cell cycle to detect nucleolin DNA binding to HPV18 enhancer, nucleolin binding is detected predominantly in S phase of cell cycle. The E6 and E7 oncoproteins of HPV18+ exert their carcinogenic potential by inactivation of the tumor suppressors pRB and p53. E6 and E7 oncogene transcription are blocked by nucleolin downregulation. Nucleolin expression is altered in HPV18+ precancerous and cancerous tissue from the cervix uteri. Nucleolin distributes mostly diffuse in the nuclei of normal squamous and glandular epithelial cells, however, it is speckled in the nuclei of HPV18+ squamous cell carcinoma.

Rb is a prototypical ►tumor suppressor, frequently inactivated in certain types of human cancer. There are many molecular mechanisms of Rb-mediated tumor suppression to understand the cancer cell proliferation. Nucleolin is associated with Rb in intact cells in the G1 phase of the cell cycle, and the complex formation is mediated by the growth inhibitory domain of Rb. Interaction with Rb inhibits the DNA binding activity of nucleolin for the HPV18 enhancer, resulting in Rb-mediated repression of HPV18 oncogenes. Rb controls the interaction of nucleolin with the HPV18 enhancer and the nucleolin-dependent activation of transcription of E6 and E7, oncoprotein of HPV18. Nucleolin could be a target molecule for the therapy of HPV18+ carcinomas of the cervix uteri.

Nucleolin's Interaction with Other Factors

Nucleolin has been found to interact with several RNA/DNA/protein targets within the nucleus, cytoplasm, and on the cell surface of several cell lines. From these different interactions, a function for nucleolin in different processes was deduced. Nucleolin also binds to Acharan sulfate (AS), isolated from the giant African snail *Achatina fulica*, responsible for inhibitory effect

of tumor growth. AS inhibits tumor growth by binding the nucleolin protein on the surface of cancer cells.

Nucleolin interacts with transcription factors and regulates negatively and positively. The mammalian ►Myb family of oncoproteins is composed of three transcription factors, A-Myb, B-Myb, and c-Myb. c-Myb is expressed mostly in hematopoietic cells and regulates their proliferation and differentiation. Nucleolin binds DNA-binding domain of c-Myb and A-Myb and downregulates Myb-mediated transcriptional activity. Interferon regulatory factor-2 (IRF-2) acts as a positive modulator for interferon-stimulated response element (ISRE)-like sequences such as the promoter H4. The nucleolin acts as a positive modulator of IRF-2-dependent transcriptional activation through an association with IRF-2. IRF-2 plays an important role in cell growth regulation through H4 gene activation and has been shown to be a potential oncogene. IRF-2 is acetylated by histone acetylase PCAF or p300 and acetylation of IRF-2 regulates cell growth by activation of H4 promoter in NIH3T3 cells. Nucleolin associates with an acetylated IRF-2, but its binding activity with nonacetylated IRF-2 is very low. For H4 gene regulation by IRF-2, nucleolin acts as an oncogenic activator via transcriptional activation, suggesting the cooperation of IRF-2 and nucleolin in cell growth regulation. IRF-2 binds H4 promoter with PCAF and nucleolin in growing NIH3T3 cells; however, in growth-arrested cells, nucleolin binding to H4 promoter is much lower compared to growing cells. Nucleolin binds to acetylated IRF-2 and IRF-2/PCAF/nuclelin complexes in turn stimulate the activation of gene transcription, which drives cell growth.

From proteomic analysis, many proteins are identified from the isolated nucleolin-binding complex. Nucleolin binds to the ribonucleoprotein (RNP) complex mainly through the sequence-specific protein–RNA interaction. As nonribosomal protein which binds nucleolin, B23, hnRNP U, DNA helicase (Ku), eukaryotic initiation factor 2(eIF-2), and nuclear factors 90 are identified.

Nucleolin is also induced by ►Myc (c-myc protein product), which is a transcription factor of basic helix-loop-helix zipper (bHLH-Zip) family. c-myc is an immediate early response gene following mitogen stimulation and regulated by tumor suppressor genes, tyrosine kinase receptors, and growth factors and known to be induced in S phase of cell cycle. Myc activates the nucleolin promoter via E-boxes located in intron I, resulting in increase of nucleolin. Nucleolin is implicated in the maturation of ribosomal RNAs with other c-myc-target factors such as a cofactor of RNA polymerase III BN51, indicating of the association of the ribosome biogenesis with Myc.

Nucleolin is a multifunctional protein which located in the nucleolus, nucleus, cytoplasm, and at the cell surface and is abundant in proliferating and cancer cells. Nucleolin is a cell cycle regulated transcriptional activator. There is much information about nucleolin that correlates with normal and cancer cells. Further, function of nucleolin should be clarified in future because of its abundance in the nucleolus.

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Nucleolus

Definition

Plural nucleoli; Is a sub-organelle of the cell nucleus. A main function of the nucleolus is the production and assembly of ribosome components (RNA, proteins). Within the nucleus are one or more nucleoli. The nucleolus is roughly spherical, and appears as a mass of densely stained granules and fibers under an electron microscope. It consists of nucleolar organizers. They are specialized regions of some chromosomes with multiple copies of genes for ribosome synthesis, along with a considerable amount of RNA and proteins representing ribosomes in various stages of production. An average, healthy cell can produce up to 10,000 ribosomes per minute.

►G2/M Transition

Nucleophile

Definition

Is a reagent that forms a chemical bond to its reaction partner (the electrophile).

►Xenobiotics

Nucleoporin

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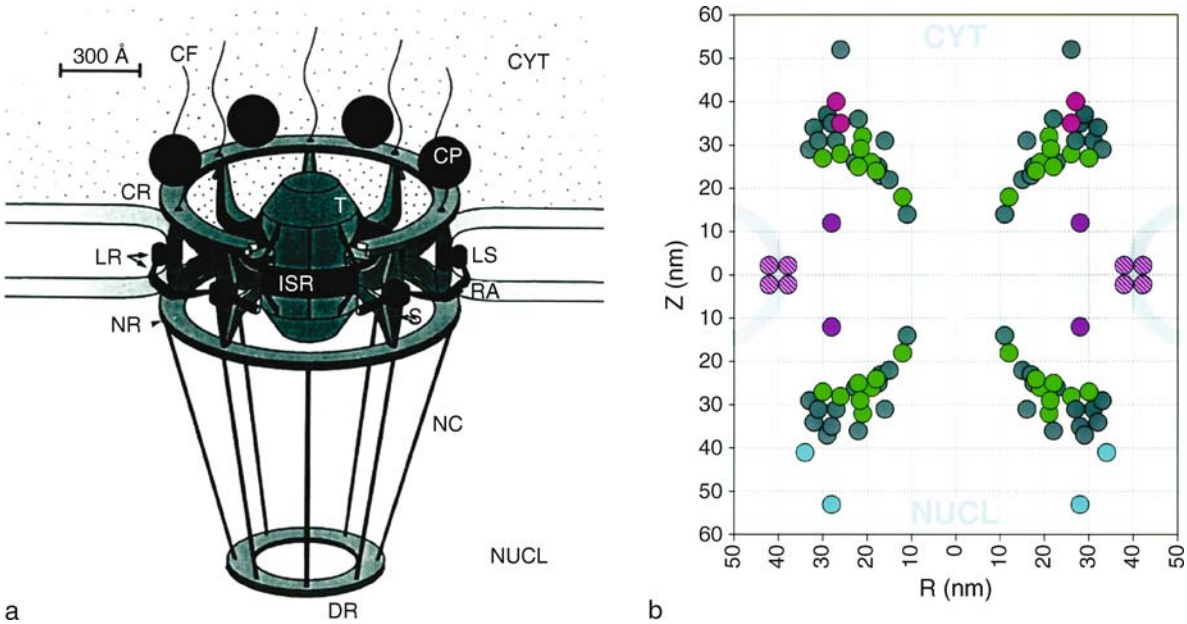
Definition

Nucleoporins are the main components of the nuclear pore complex in eukaryotic cells, and mediate bidirectional nucleocytoplasmic transport, especially of mRNA and proteins. Defects in nucleoporins, e.g., overproduced or involved in ►chromosomal translocation, may affect nucleocytoplasmic transport and cell function, leading to cell transformation and cancer.

Characteristics

The Nucleocytoplasmic Transport

The nucleus is the defining feature of the eukaryotic cell. Unlike their prokaryotic counterparts, eukaryotic cells separate the nuclear synthesis of DNA and RNA from cytoplasmic protein synthesis with a barrier termed the nuclear envelope (NE). The NE is perforated by large proteinaceous assemblies, called nuclear pore complexes (NPCs), which are multiprotein channels that span the double lipid bilayer of the NE and act as the sole gatekeepers controlling the exchange of material between the two locales (Fig. 1a). In yeast, half of the NPC is made up of a core scaffold, which is structurally analogous to vesicle-coating complexes. NPCs are freely permeable to small molecules (such as water and ions), but they restrict the movement of larger molecules (such as proteins and RNAs) across the NE. The selective barrier for transport is formed by large numbers of proteins with disordered regions that line the inner face of the scaffold. To overcome this barrier, macromolecules carry specific signals that allow them to access the nucleocytoplasmic transport machinery of the cell. In this way the cell ensures that only selected macromolecules can travel between the nucleus and cytoplasm. Factors that are important for nuclear transport can be divided into four categories: the proteins of the NPC (nucleoporins), the Ran►GTPase, transport receptors called karyopherins (or importins/exportins) that recognize cargoes for transport, and specialized factors that promote transport of some



Nucleoporin. Figure 1 The NPC and nucleoporins in the NPC. (a) Three-dimensional reconstruction of NPCs (Akey, Radermacher (1993) J Cell Biol 122:1). CF, cytoplasmic filaments; CP, cytoplasmic particles; CR, cytoplasmic ring; CYT, cytoplasm; NR, nucleoplasmic ring; NUCL, nucleus; LR, lumenal ring; LS, lumenal spoke; RA, radial arms; ISR, inner spoke ring; S, spokes; T, transporter; NC, nuclear cage (basket); DR, distal ring. (b) The position of the Nups relative to the NPC cylindrical axis (R) and mirror plane of pseudosymmetry (Z). Each circle represents a Nup position with green for symmetrical FG Nups, blue for strictly nuclear FG Nups, red for strictly cytoplasmic Nups, gray for non-FG Nups, and purple and purple stripes for different integral membrane proteins associated with the NPC (Rout MP et al. (2000) J Cell Biol 148:635).

protein/RNA complexes. Macromolecular transport in or out of the nucleus generally begins with recognition of the transported cargo by a receptor, followed by docking and movement through the NPC. Karyopherins can generally be thought of as superhelices with an inherent flexibility. The RanGTPase and its regulators control the rate at which cargo is moved in and out of the nuclear pore, as well as the loading and unloading of cargoes.

Nucleoporins

Nucleoporins (Nups) are the individual protein components of the NPC (Fig. 1b). Proteomic analyses of the mammalian NPC have estimated 30–50 Nups, many of which are functionally conserved from yeast to mammals. Nups can be divided into two families: pore membrane proteins (Poms) and FG nucleoporins. Poms are membrane-spanning integral proteins specific to the pore membrane. All of the FG nucleoporins share domains consisting of repeated phenylalanine/glycine (FG), which are separated by spacers that are either highly charged or rich in polar residues. The repetitive motifs are probably distantly related to one another and are predicted to assume a β-sheet conformation. Two classes of FG repeats, GLFG and FXFG subtypes, have been identified. A number of nucleoporins contain one or the other or a combination of these repeats. In addition to the shared repeats, each of the FG nucleoporins has distinct domains that appear to mediate localization and function, such as the coiled-coil domains found in Nup49, Nup84, the leucine zipper found in Nup214, the zinc finger domain found in Nup153, the RNA binding domain included in Nup98 or Nup145. The Nup98 and Nup96 proteins are encoded by a single gene. Much evidence showed that nucleoporins play important roles in docking, translocation and releasing of the cargo. Except for roles in transport, several studies in yeast have demonstrated that nucleoporins are essential for mediating ►epigenetic control of transcription. For example, Nup60 and Nup145 are found to be required for full repression of HMR locus in yeast.

Nucleoporins and Cancer

To date, Nups are reported to be involved in several types of cancers through different mechanisms. Firstly, Nups are shown to be overexpressed in cancer cells. For example, Nup88 was shown to be upregulated in a broad spectrum of tumors. Nup88 was similarly enhanced in severe dysplasias and in situ carcinomas of organs such as colon, stomach, breast, and prostate. Conversely, Nup88 was either sporadic or not detectable in most benign tumors and hyperplasias. In normal adult tissues, Nup88 was occasionally noted in sites such as colonic crypts, bronchial mucosa, and fallopian tubes. However,

Nucleoporin. Table 1 Chromosomal translocations and nucleoporins fusion genes associated with cancers

Chromosomal translocations	Fusion genes	Associated cancers
t(7;11)(p15;p15.5)	Nup98–HoxA9	AML, MDS, t-MDS/AML, CML
t(11;17)(p15;p15)	Nup98–HoxA13	AML, MDS
t(11;17)(p15;p15)	Nup98–HoxA11	CML
t(11;12)(p15;q13)	Nup98–HoxC11	AML
t(11;12)(p15;q13)	Nup98–HoxC13	AML
t(2;11)(q31;p15)	Nup98–HoxD11	AML
t(2;11)(q31;p15.5)	Nup98–HoxD13	AML, t-MDS/AML
t(1;11)(q23;p15.5)	Nup98–PMX1	t-MDS/AML
t(3;11)(q29q13;p15)	NUP98–IQCG	T-ALL/AML or mixed lineage leukemia
inv11(p15.5;q22)	Nup98–DDX10	AML, t-MDS/AML
t(11;20)(p15.5;q11)	Nup98–TOP1	AML, t-MDS/AML
t(9;11)(p22;p15.5)	Nup98–LEDGF	AML
t(4;11)(q21;p15.5)	Nup98–RAP1GDS1	T-ALL
t(5;11)(q35;p15.5)	Nup98–NSD1	AML
t(8;11)(p11.2;p15)	Nup98–NSD3	AML
t(10;11)(q25;p15)	Nup98–ADD3	T-ALL
t(6;9)(p23;q34)	DEK–Nup214/CAN	AML, MDS
t(9;9)(q32;q34)	SET–Nup214/CAN	AML
Amplified episomes	Nup214–ABL1	T-ALL

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; T-ALL, T-cell acute lymphoblastic leukemia; t-MDS/AML, therapy-related acute myeloid leukemia.

whether Nup88 overexpression was simply the result of increased nucleocytoplasmic transport required to meet the increased demand of proteins by transformed cells, or Nup88 might play a role in ►carcinogenesis, remained obscure. Secondary, Nups are involved in ►chromosomal translocations which are predominantly found in ►leukemia (Table 1). The t(7;11)(p15;p15.5) was the first rearrangement discovered in patients with ►acute myeloid leukemia (AML), while the resultant Nup98–HoxA9 was the first fusion protein that was found to involve a nuclear-pore protein. So far the Nup98 has been reported to fuse with at least 20 partner genes in leukemia having 11p15 translocation. An interesting finding is that, about 50% of Nup98 fusion partners are ►homeobox domain (HD)-containing transcription factors. In addition, the non-homeobox fusion partners do not have DNA binding domain, but all bear a putative protein-protein interaction motif, a feature also shared by HD and suggestive of a possible common mechanism. Nup214 has also been reported to be fused to several partners, e.g., DEK, SET in AML and ABL1 in acute lymphoid leukemia (ALL). Interestingly, both Nup98 and Nup214 fusion proteins retain the FXFG repeat region that is characteristic of several nucleoporins. Normally, the FXFG repeats help to regulate the nucleocytoplasmic transport of proteins and RNAs as well as function as docking sites for the karyopherins. One model for the mechanism by which Nup98 and Nup214 fusion proteins promote leukemogenesis is that the FXFG repeats act as protein–protein interaction domains that allow the fusion protein to interact with other transcription factors and act as transcriptional activators.

Changes in nuclear transport machinery can markedly alter cellular function and potentially promote tumorigenesis. For example, nuclear mislocalization of ►nuclear factor κB as a result of IκB degradation and improper acetylation by ►p300, mislocalization by activated AKT, and nuclear localization and activation of the transcriptional activator ►β-catenin, can facilitate tumorigenesis.

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Nucleoside Analogs

Definition

Molecules that are similar to the building blocks of DNA and RNA.

►Epigenetic Therapy

Nucleoside Diphosphate Kinases

►NM23 Metastasis Suppressor Gene

Nucleoside Transporters

Definition

Proteins that traverse the plasma membrane and permit the passage of nucleosides and structurally-related drugs into and out of the cell cytoplasm.

►Adenosine and Tumor Microenvironment

Nucleosomal DNA Fragments

►Nucleosomes

Nucleosome Remodeling

►Necleos omes
►Chromatin Remodeling

Nucleosomes

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Definition

Are, complexes formed by a core particle of eight histone proteins which are surrounded by 147 bp of double-stranded DNA, are the basic elements of the chromatin in eukaryotic cells.

Characteristics

Structure and Function of Nucleosomes

About 99% of human DNA is localized in the nucleus of the cells where it is organized in a multistep manner. In its secondary structure, chromatin is arranged as a chain of nucleosomes. These consist of a central protein component formed by an octamer of the double-represented histone aggregates H2A-H2B and H3-H4. Hundred and forty seven base pairs of double-stranded DNA are twisted around this complex with 14 defined fixing sites and build the disk-like nucleosomes with a molecular weight of ~206 kDa. Thereof nearly half is part of the histones and half of the DNA component. Neighboring nucleosomes are connected by so-called linker DNA, which varies between 10 and 100 bp. A further histone H1 is located at these linking sites outside of the nucleosomes and stabilizes the chromatin chain in its tertiary structure as chromatin fiber.

Beyond the organization and stabilization of the DNA, the arrangement in multinucleosomal order plays an essential role for the regulation of the transcription of genetic information, for the replication and for repair processes. The access of transcription factors to relevant DNA sequences is mainly coordinated by nucleosomal histones which can be modified at their tails by acetyl-, methyl-, phosphor-, ubiquitin-, and ADP-ribose groups. Histone acetylation leads to decondensation of the chromatin, unfixing of intra- and internucleosomal connections, and promotes the transcription process, whereas deacetylation (►Histone deacetylases) and condensation suppress it. Further, genetic and ►epigenetic modifications of the DNA may contribute to the regulation of the interaction between transcription factors and specific promoter regions, too.

The active involvement of DNA in transcription processes requires a flexible and dynamic structure of the nucleosomal organization which is enabled by ATP-dependent chromatin-remodeling factors. These promote the liberation of the DNA from its close connection to the histones by disrupting the contacts, transferring the histone octamer to another DNA molecule, or sliding the core particle along the DNA.

Origin of Circulating Nucleosomes

As small amounts of nucleosomes are also found in blood of healthy adults, ►Apoptosis during physiological cell regeneration is thought to be a major source of nucleosome release. Although most of liberated nucleosomes are engulfed and digested by ►macrophages and neighboring cells, a part of them reaches the blood circulation (►Circulating nucleic acid). In situations of enhanced cell death such as during degenerative, autoimmune, inflammatory, ischemic, traumatic and toxin-mediated diseases, or in malignant tumors, this elimination system seems to be overloaded or impaired resulting in higher levels of circulating nucleosomes in blood. Depending on the type and intensity of the cell damaging stimulus as well as on the type and energy level of the affected cells, various cell death modes can lead to the cellular demise such as apoptosis, oncosis, or mixed forms between these extreme forms. Because most of the DNA is present in blood as small mono- and oligonucleosomal fragments of 200, 400, 600, and 800 bp, apoptosis, which is associated with internucleosomal cleavage of the chromatin by activated endonucleases, is assumed as a major mechanism of nucleosome liberation. In contrary, oncosis produces high molecular weight DNA fragments released into blood after acute damaging events. As a further mechanism, active secretion of nucleosomes by lymphocytes is in discussion.

Metabolism of Circulating Nucleosomes

Generally, cell-free DNA (►Circulating nucleic acids) can circulate in blood as naked DNA, associated with histones in nucleosomes, bound to other plasma proteins, or packed in apoptotic bodies. Although the exact contribution of each form may vary inter- and intraindividually, the main part of the circulating DNA was shown to be organized in multimeric complexes as mono- and oligonucleosomes.

Hemodialysis experiments which produce a considerable release of nucleosomes from lymphocytes have revealed that nucleosomes are removed in vivo from circulation in a biphasic, saturable, and concentration-dependent manner with an initial half-life of 4 min. The degradation and elimination of nucleosomes is thought to be exerted by various systems:

- Intraplasmatic degradation by circulating endonucleases
- Intraplasmatic immunological complexing by anti-nucleosome-antibodies
- Phagocytosis and lysosomal degradation by cells of the reticulo-endothelial system
- Metabolization of nucleosomes in the liver
- Direct renal elimination in form of liposomes

During acute ►inflammation, nucleosomes may bind to acute phase proteins which delay the elimination process.

Relevance of Circulating Nucleosomes

The role played by nucleosomes in the pathogenesis of diseases is only partially known. In systemic lupus erythematodes, the high antigenic potential of circulating nucleosomes in blood or on the surface of antigen-presenting cells leads to the early production of antinucleosome-antibodies. Complexes of nucleosomes and these antibodies were found to aggregate at the glomerular basal membrane in the kidneys and to promote the disease ►progression.

In tumors, circulating nucleosomes are suspected to carry metastatic information as the transfection of DNA to mice resulted in the generation of new tumors. Further reports suggest an important role of nucleosomes for the evasion of tumor cells from immunosurveillance by inhibition of ►natural killer cell-mediated tumor cell lysis. Finally, nucleosomes liberated from tumor cells are reported to stimulate the expression of interleukin-8 in endothelial cells which promotes the local ►angiogenesis in the tumor tissue and supports tumor progression.

Clinical Aspects of Circulating Nucleosomes in Blood

In recent years, several enzyme linked immunosorbent assays (►ELISAs) were developed to quantify circulating nucleosomes in serum, plasma, and other body fluids using mainly monoclonal mouse antibodies directed specifically against the DNA and histone component, respectively. It is noteworthy to point out, that a good correlation between a nucleosome assay and the current "golden standard" for DNA quantification by real time ►PCR was found, suggesting that clinical results obtained by nucleosome or DNA measurements are quite comparable. Although most clinical studies focus on the quantification of DNA amount in plasma and serum, several studies have analyzed the relevance of circulating nucleosomes for diagnostic, staging, and prognostic purposes in cancer diseases as well as in noncancer diseases. As nucleosomes are nonspecific cell death products, they are supposed to appear in circulation in various acute and chronic diseases.

Circulating Nucleosomes in Noncancerous Diseases

Levels of nucleosomes in plasma and serum were found to be elevated in patients with acute infections as compared with healthy individuals and correlated with ►C-reactive protein values. Another study reported elevated nucleosomal levels in sera of septic patients and a strong correlation to the severeness of the disease.

Further, nucleosome levels were elevated in sera of patients after cerebral stroke, particularly in patients with extended volumes of stroke lesions. During the first week after stroke, the levels increased faster and stronger in patients with severe functional deficits than in those with only slight deficits. In addition, nucleosomes showed independent prognostic value for the 1-year recovery after acute stroke.

High nucleosome levels were observed in patients with various autoimmune diseases, too. Because anti-nucleosome-antibodies are found only in patients with systemic lupus erythematodes, it was assumed that nucleosomes may undergo specific qualitative processing, e.g., in antigen-presenting cells to achieve its high antigenicity in this disease.

Similarly, elevated concentrations cell-free DNA were reported in sera of patients with trauma, stroke, burns, graft versus host reaction (graft-versus-host disease) after transplantation, and exhaustive exercise.

Circulating Nucleosomes in Diagnosis and Staging of Cancer Disease

Several studies found higher nucleosome levels in individuals with cancer diseases, particularly in those with advanced stages. A comprehensive study analyzed sera levels of 590 patients with cancer disease including colorectal (►Colon cancer) and various other gastrointestinal cancers (►Gastrointestinal tumors), ►lung cancer, ►breast cancer, ►ovarian cancer and other gynecologic cancers, renal and prostate cancer (►Prostate cancer, clinical oncology), and lymphoma, as well as of the relevant organ-specific benign diseases and of healthy individuals. Nucleosome concentrations in sera of healthy donors were generally fairly low. In contrast, pretherapeutic serum levels in various malignancies were significantly higher. However, various benign diseases, particularly infectious diseases, tended to have elevated serum levels of nucleosomes, too, limiting the diagnostic capacity for cancer disease. While nucleosome levels significantly distinguished between healthy donors and malignant diseases, the difference between benign and malignant diseases did not reach the level of significance in general, but only in subgroup of lung cancer. Among the various cancer types, medians, percentiles, and ranges of nucleosome values were comparable, but lung cancer was associated with significantly higher levels and prostate cancer with lower ones. Regarding tumor ►staging, nucleosome values were found to correlate with tumor stage and the presence of distant metastases only in patients with ►gastrointestinal cancers, in other subtypes there was only a tendency or no correlation at all. In the contrary, in patients with lung cancer and breast cancer, high nucleosome values often were observed already in early stages. No association was found between nucleosome levels and lymph node involvement, cell differentiation (Tumor ►grading), age or gender.

Similar results concerning the diagnostic capacity of nucleosomes or cell-free DNA were obtained

by other studies which, however, mainly analyzed only the difference between cancer patients and healthy donors. Heterogeneous results were obtained concerning the correlation of nucleosomes or cell-free DNA with tumor stage.

Circulating Nucleosomes in Prognosis of Cancer Disease

The prognostic relevance of circulating nucleosomes or cell-free DNA is still in discussion. While some studies reported a prognostic value of nucleosomes or DNA in patients with lung cancer and breast cancer, others could not confirm these findings in the same tumor types. Reasons for the discrepant results may be found in the low number and the heterogeneity of the patients investigated as well as in differences concerning the statistical analysis and the consideration of other relevant prognostic factors. In a large study on 300 patients with advanced ►non-small cell lung cancer, pretherapeutic serum levels of nucleosomes had prognostic impact in the univariate analysis, while it did not show independent prognostic information when other clinical and biochemical markers were included in multivariate analysis.

Circulating Nucleosomes in Therapy Monitoring of Cancer Disease

During systemic cytotoxic therapies such as chemotherapy (►Chemotherapy of cancer, progress and perspectives) and radiotherapy (►Ionizing radiation therapy), the changes in the courses of circulating nucleosomes indicate the response to the therapy. Several studies have shown in various cancer types that strongly decreasing levels were mainly found in patients with remission of tumor disease whereas constantly high or even increasing values were associated with progressive disease. Independent from these general observations, nucleosome levels showed an immediate increase during the first days after start of therapy reaching a maximum after 2–5 days followed by a rapid decrease. These kinetics were observed in various cancers during chemotherapy, radiotherapy and ►immunotherapy. The courses were influenced by the spontaneous, and the therapy-induced release of nucleosomes, and the elimination capacity from circulation of the individuals. Although nucleosomes are not specifically liberated only by tumor cells but may also be the result of the cell death of other cells with high proliferation rates, lung tumor cells have shown to be more susceptible to systemic therapy in vitro than physiological bronchoepithelial cells.

The early nucleosomal course showed significant differences between patients with the favorable and nonfavorable outcome after therapy. A comprehensive study on more than 300 patients with advanced non-small cell lung cancer patients revealed that patients with clinical remission during chemotherapy initially started from lower nucleosome values, had lower maximum values and a more complete elimination of nucleosomes from circulation at the end of the first week of therapy than patients with progressive disease. When nucleosomes were combined with the more specific lung cancer biomarker (►Serum biomarkers) CYFRA 21-1, the nonresponse could be anticipated in about 30% of progressive patients with a specificity of 100% already after the first course of therapy. This information would help to change the therapy earlier than by currently available imaging techniques in 30% of nonresponding patients and may contribute to increase the therapeutic efficacy. Similar results were obtained in smaller studies on colorectal and pancreatic cancer (►Pancreas cancer, clinical oncology) during chemotherapy and radiotherapy.

Altogether, levels of nucleosomes are elevated in sera of cancer patients when compared to healthy controls. However, due to elevations of nucleosome levels in patients with benign diseases relevant for differential diagnosis, they seem not to be suitable for cancer diagnosis. Although nucleosomes showed prognostic relevance in some univariate analyses, their independent value in multivariate models will have to be elucidated further.

Most informative are circulating nucleosomes for the monitoring of anticancer therapy, particularly for the early estimation of therapy efficacy.

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Nucleotide Base

Definition

One of four compounds residing between the two strands of DNA which makes up the genetic code by sequence arrangement.

►DNA Damage

Nucleotide Excision Repair

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Definition

NER; Is a complex biochemical mechanism that recognizes alterations in the chemical structure of DNA due to base modification by physical agents (most notably ►ultraviolet radiation) or endogenous or exogenous chemicals. After recognition of the alteration in the structure of DNA, the damage is removed by excision of the oligonucleotide that contains the damage. The resulting gap is filled in by DNA polymerase using the complementary (undamaged) strand as template and finally ligated. The process is essentially error-free. There are at least 27 polypeptides required to complete the recognition, excision and gap-filling phases.

Characteristics

NER was initially characterized in *E. coli* in the 1960s and was identified by the ability of wild type bacteria to remove ultraviolet light (UV)-induced photoproducts from large molecular weight DNA. The kinetics of this process correlated well with the resumption of DNA replication and led to the idea that removal of such damage was directly related to the recovery of DNA synthesis and improved survival. Since that time, NER processes in mammalian cells have been documented by a great number of cellular and molecular biology studies. The dissection of the pathway in eukaryotic cells has been greatly facilitated by the availability of mutant cell lines, many of which are derived from individuals who are defective in some aspect of NER and are consequently cancer-prone. During the 1990s, all of the central factors involved in NER were cloned, and the basic "cut and refill" reactions have been reconstituted in vitro from purified components. Despite these advances, however, many important aspects of the regulation of NER and its integration with other basic cell biology processes remain to be elucidated. For review, see [1,2].

Cellular Regulation

NER is a versatile and sophisticated pathway for the removal of DNA damage induced by a variety of environmental and endogenous factors. One of the most relevant, and best studied, DNA damaging agents is UV light, which induces dimerization of adjacent pyrimidine bases. The major products of this photochemical reaction (hence the term "photoproducts") are cyclobutane pyrimidine dimers (CPD) and 6–4 pyrimidine-pyrimidones (6–4s). Both lesions induce structural distortions in DNA, and if left unrepaired, can cause errors in replication that can lead to mutations. In addition to photoproducts, a wide variety of bulky chemical adducts are removed by NER, and the common denominator amongst these diverse lesions is distortion of the DNA helix, which in turn interferes with basic nuclear transactions such as transcription and DNA replication. These lesions present such a potential threat to the integrity of the cell that the basic mechanism for removing them has diverged surprisingly little throughout evolution. In all eukaryotic cells that have been studied, two modes of NER have been identified. These are global genomic repair (GGR) and transcription-coupled repair (TCR). These mechanisms are distinguished primarily by the way in which damage is detected. After the damage is detected, the pathway for excision, removal and gap filling is common to both GGR and TCR.

Many of the proteins were identified by cloning the genes responsible for the NER-defective syndrome ►xeroderma pigmentosum (XP). There are seven distinct groups of XP termed XP-A through XP-G, and the general molecular defect associated with each group is outlined in the Table 1.

Global Genome Repair

The global genome is defined as that part of the genome that is not transcribed, which is estimated to be 95% of the human genome. The XPC protein is specifically required for repair of lesions in the global genome, and cells with nonfunctional XPC are completely deficient in such repair. XP-C cells, however, are capable of removing lesions from the transcribed strand of active genes. The exact role of XPC in GGR has been obscure, but recent advances have elucidated its function more fully. XPC is a 125 kDa protein that acts as a heterodimer with one of two homologs of the yeast protein Rad23 (these homologs are termed hHR23A and hHR23B). It is now thought that the XPC-hHR23B (or A) complex acts at the very earliest stages of NER in the global genome in a damage recognition fashion. In ways that are not yet understood, this complex senses and binds to the damaged DNA. In this process, it locally distorts the double helix and then recruits the core repair apparatus. The rate of GGR is strongly dependent on the type of lesion, which presumably reflects the degree of helical distortion and the affinity of the XPC heterodimer. The XPC complex has been shown to have high affinity for DNA that contains a 6–4 photoproduct, and these lesions are repaired very rapidly. Recognition of CPD in the global genome probably requires the XPE protein; the XPE-CPD complex is then recognized by the XPC complex.

Nucleotide Excision Repair. Table 1 Molecular defects in XP complementation groups

Proteina	Defect
XPAb	No lesion recognition
XPBc	Reduced helicase activity that unwinds DNA 3′–5′ of the lesion
XPC	Reduced recognition of lesions in global genome, normal transcription-coupled repair
XPDc	Reduced helicase activity that unwinds DNA 5′–3′ of the lesion
XPE	Reduced recognition of cyclobutane pyrmidine dimers in the global genome
XPF/ERCC1	Mutant endonuclease that cuts 5′ to the lesion
XPGd	Mutant endonuclease that cuts 3′ to the lesion

[a]Cells from complementation groups signified with a hyphen (XP-A); the corresponding proteins are not hyphenated (XPA).
[b]Patients are very severely affected and may have developmental defects.
[c]Proteins also found in transcription factor TFIIH. Patients are likely to have developmental abnormalities.
[d]Defective transcription-coupled repair of oxidative damage suggests a second, non-endonucleolytic function for this protein. These patients may also have developmental defects, possibly due to reduced repair of oxidative damage.

Due to this two-stage mechanism, repair of CPD in the global genome is much slower than that of 6–4s.

Transcription-Coupled Repair

Elongating RNA polymerase II stalls at a wide variety of lesions and must act as a damage sensor, although the mechanism by which it does so remains obscure. In addition to the stalled polymerase, at least two other proteins are required for TCR. These are termed CSA and CSB, and are thought to act in a structural rather than in a catalytic fashion. Depending on the type of lesion, damage in the transcribed strand of an active gene can be repaired either by TCR or GGR. For instance, UV-induced 6–4s in active genes is primarily repaired by GGR because of the very high affinity of the XPC complex for these lesions. UV-induced CPD on the other hand, is repaired by TCR. TCR is highly conserved in both prokaryotes and eukaryotes, presumably because blocked RNA polymerases seriously interfere with cellular metabolism. TCR has also been shown to specifically reduce the frequency of mutations in active genes; mutations in such genes would also be expected to have a deleterious effect on cellular biochemistry and cell-cycle control.

Excision and Gap Filling

After the damage is initially sensed, the XPA protein plays a critical early role in NER. XPA is a DNA binding protein with high affinity for damaged DNA, and nonfunctional XPA leads to a complete loss of NER capacity. The protein was long thought to be the initial sensor of DNA damage, but more recent data implicates the XPC complex in this process. Current thinking is that XPA verifies that the DNA is damaged and acts to position the repair machinery correctly around the lesion. The human single strand binding protein, RPA, acts in concert with XPA and probably helps to maintain the open conformation. Through its extensive network of protein binding sites, XPA recruits the basal transcription factor TFIIH to the lesion. TFIIH is composed of five polypeptides, including the XPB and XPD helicases, which unwind the DNA on both sides of the lesion. Two structure-specific endonucleases, XPF-ERCC1 and XPG cut the DNA on either side of the lesion to release an oligonucleotide 24–32 bases in length that contains the damage. The 3′ terminus acts as a primer, and the gap is filled in by either DNA polymerase d or e, together with cofactors; the relative contribution of each polymerase remains to be determined. The final step in the process is ligation of the 5′ terminus, which is probably carried out by DNA ligase I. Since the gap to be filled is short, and the fidelity of the polymerase complex is very high, NER is considered to be an error-free process.

Clinical Relevance

The central role played by DNA repair in the prevention of cancer was first appreciated in 1968 when Cleaver recognized that patients with the skin cancer-prone disorder xeroderma pigmentosum (XP) had a defect in the repair of UV-induced damage (3). XP patients have a greater than 1,000-fold increase in the incidence of sunlight-associated skin cancer, and if they are exposed to any sunlight they usually succumb to metastatic skin cancer. The cells of XP patients are also defective in the repair of bulky chemical adducts, such as those induced by cigarette smoke and endogenous metabolic processes. Evidence is emerging that XP patients who live longer, because they have compulsively avoided the sun, have an increased risk of internal cancers, particularly if they smoke. Many XP patients also undergo progressive neurological deterioration, and this is thought to be due to the accumulation of damage induced by reactive oxygen species. Repair of such

damage is thought to require some of the NER proteins acting in pathways that are independent of NER. In addition, patients in XP groups that have a molecular defect that affects transcription in addition to NER (groups B and D), are likely to have developmental abnormalities that are presumably related to subtle defects in transcription.

There are two additional syndromes that manifest defects in excision repair but are curiously not cancer-prone. These are ►Cockayne syndrome (CS) and ►trichothiodystrophy (TTD). CS patients exhibit severe mental retardation and varying degrees of photosensitivity. Cells from these patients are defective in TCR because they lack a protein that couples the blocked RNA polymerase to the repair machinery. It has been postulated that the reason these patients do not develop skin cancer is the increased level of apoptosis induced in these cells following UV. The exaggerated apoptotic response may be due to the persistently blocked RNA polymerase II molecules, which cause accumulation of p53. TTD patients exhibit many of the same symptoms and signs of CS patients, but the molecular defect affects the same protein responsible for the XPD phenotype, namely the XPD helicase in TFIIH. The reason that one syndrome is cancer-prone while the other is not is puzzling, but recent studies have shown that mutations in the XPD gene occur in different regions in TTD and XP-D cells. It has been postulated that the mutations in TTD patients differentially affect transcription to a greater degree, and that subtle defects in transcription prevent cellular transformation. It has also been shown that TTD cells are quite proficient in repairing 6–4s while XP-D cells are not. The development of cancer in the latter cells may be related to the greater mutagenic potential of 6–4 photoproducts.

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Nude Mice

Definition

Lack a thymus, they are highly immunocompromised and tolerant for engrafted cells or tissues of other species.

NUP98-HOXA9 Fusion

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Definition

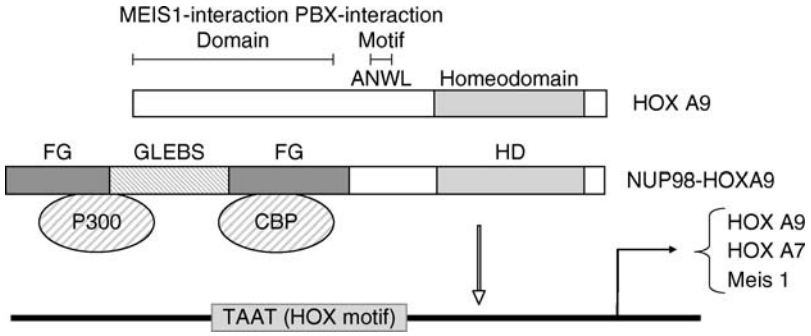
The t(7;11)(p15;p15) chromosome translocation is found in human leukemic bone marrow cells in a subpopulation (1–2%) of patients with acute myeloblastic leukemia (AML), ►myelodysplastic syndrome (MDS) and blastic crisis of ►chronic myeloid leukemia (CML-BC). The translocation involves the association of the C-terminal GLEBS and FG (phenylalanine-leucine) regions of the ►nucleoporin protein NUP98 with the N-terminal region of the ►HOXA9 protein containing the ►PBX heterodimerization domain and the DNA interacting homeodomain (Fig. 1). The clinical course of this subset of leukemias is aggressive, and prognosis is poor.

Characteristics

NUP98 is a component of the ►nuclear pore complex (NPC), an evolutionarily conserved structure made up of multiple copies of ~30 different NPC proteins (nucleoproteins) embedded in the nuclear envelope. The NPC mediates transport of proteins (>40 kDa) and RNA species between the cytoplasm and nucleus and helps organize nuclear architecture. The 98 kDa NUP98 protein is generated by an unusual biogenesis pathway that involves synthesis and autocleavage of precursor proteins yielding NUP98 and NUP96, another stable component of the NPC. The mRNA export factors ►TAP and ►Rae-1 associate in a mutually exclusive manner with different binding sites on NUP98 to form stable ternary compounds. The Rae-1–Nup98 complex functions in both the export of mRNA and import (recycling) of mRNA export factors into the nucleus indicating that this complex is an active player in the gene expression pathway.

The human NUP98 gene, located on chromosome 11p15.5, has been found fused to 21 different genes as a consequence of chromosomal translocations in acute leukemia. The resulting chimeric transcripts encode fusion proteins that juxtapose a common N-terminal region of NUP98 to the C-terminus of the partner gene. The most frequent partner genes are members of the ►Homeobox (HOX) and HOX-related family, particularly HOXA9.

HOXA9 plays an important role in normal hematopoiesis, being expressed in early ►hematopoietic stem cells (HSC) together with Meis1, downregulating with differentiation. Meis1 is a HOX cofactor that alters HOX-DNA binding specificity and affinity, and increases



NUP98-HOXA9 Fusion. Figure 1 Comparison of the structure of HOXA9 and the NUP98-HOXA9 fusion protein. The transcriptional regulatory region of HOXA9 is replaced by the C-terminal FG region of NUP98. Note the retention of the N-terminal DNA binding homeodomain and the PBX-interaction domains of HOXA9 in the chimeric protein

HOX-transcriptional activity. ►Thrombopoietin (Tpo), a key regulator of HSC proliferation, enhanced HOXA9 nuclear import and interaction with Meis1 in HSC in a MAPKinase-dependent fashion. Targeted disruption of HOXA9 in mice leads to reduced numbers of progenitor cells, and a profound defect in HSC. Conversely, enforced expression of HOXA9 promotes enhanced proliferation of HSC with impaired differentiation. These data highlight the importance of precise control of HOXA9 protein levels during hematopoiesis. The ubiquitin ligase ►CUL4A (►Cullin4A) regulates HOXA9 protein levels by ►ubiquitination and subsequent proteosome degradation of the protein. Knockdown of CUL4A by siRNA in myeloid cell lines extended the HOXA9 protein half-life 6–10-fold and enhanced proliferation and blocked differentiation in response to ►granulocyte colony stimulating factor (G-CSF). HOXA9 is one of the most upregulated genes in AML, particularly in the ►CD34+, CD38-leukemic stem cell fraction. HOXA9 behaves as an oncogene in leukemia following mutations that induce its persistent expression or that convert it to a persistent transcriptional activator.

Mouse Models of Leukemogenesis

Expression of NUP98-HOXA9 in murine bone marrow by retroviral gene transfer results in enhanced proliferation *in vitro* resulting in development of an oligo- or poly-clonal ►myeloproliferative disease (MPD) upon transplantation into mice, with neutrophil leukocytosis and extramedullary hematopoiesis, progressing to a mono or bi-clonal AML by 7–8 months. In a transgenic NUP98-HOXA9 model created with a myeloid restricted cathepsin G promoter, ~20% of mice developed MPD and eventually AML by 2 years. Retroviral insertional mutagenesis identified a number of co-factors that interacted with NUP98-HOXA9 in leukemia progression, the most frequent being Meis1. Collaboration of Meis1 with NUP98-HOXA9 reduced the median latency of AML development to 4–5 months. NUP98-HOXA9 has been detected in blastic crisis of ►BCR-ABL positive ►CML indicating that cooperation between NUP98-HOXA9 and the BCR-ABL signaling pathway via receptor tyrosine kinase may be important in progression from chronic disease to ►blastic crisis. Oncogenic interaction is seen between NUP98-HOXA9 and BCR-ABL chimeras in retroviral mediated gene transfer experiments in mice leading to rapid development of AML.

Human Models of NUP98-HOXA9 Leukemogenesis

Retroviral transduction of NUP98-HOXA9 into human HSC and progenitor cells from umbilical cord blood or G-CSF mobilized adult peripheral blood confers a proliferative advantage *in vitro* in long term cytokine stimulated or stromal co-culture assays, and *in vivo* following transplantation in irradiated immunodeficient NOD/SCID mice. The mechanism involves acquisition of serial myeloid progenitor colony recloning capacity and enhanced HSC self-renewal as confirmed by quantitative in vitro limiting dilution assay. Granulocytic and erythroid differentiation is also impaired.

Proposed Mechanism of Leukemic Transformation by the NUP98-NUP98 Fusion

Aberrant Transcriptional Activity

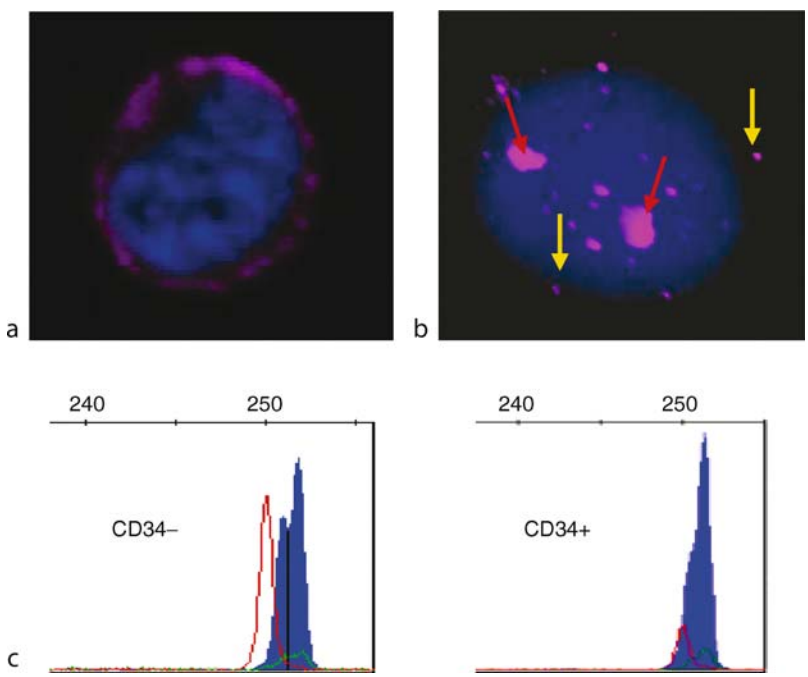
Unlike HOXA9, NUP98-HOXA9 has a strong but aberrant transcriptional activating potential mediated through NUP98FG binding of the transcriptional co-activators ►CBP/p300. Transduction of NUP98-HOXA9 into a human myeloid leukemic cells line significantly altered expression of over a hundred cytoplasmic mRNAs, and in studies with human CD34+ stem and progenitor cells 50–60 genes were upregulated and 4–15 downregulated. The transcriptosome of NUP98-HOXA9 showed little overlap with that of HOXA9. The most striking feature was upregulation of homeobox genes (HOXA5, HOXA6, HOXA7, endogenous HOXA9 HOXB2, HOXC6, HOXB5,

and TALE homeodomain genes *Meis1* and *PBX3*). The "HOX code", minimally defined by expression of the HOXA5-A9 cluster, is reported to be central to leukemogenesis associated with ►mixed lineage leukemia protein (MLL) fusion genes and is probably a significant mechanism in NUP98-HOXA9 transformation. Overexpression of these HOX genes favors self-renewal and blocks differentiation. Downstream pathways involving the HOXA9 component of the fusion protein involve binding to the promoter region of the threonine kinase ►Pim-1 promoter. Pim-1 is a proto-oncogene overexpressed in the transcriptosome of HOXA9 and NUP98-HOXA9 transduced human hematopoietic cells. Pim-1 increases phosphorylation and inactivation of pro-apoptotic ►BAD protein and since unphosphorylated BAD normally binds to and inactivates anti-apoptotic proteins such as ►Bcl-X_L and ►Bcl-2, this would be an anti-apoptotic event. NUP98-HOXA9 upregulation of ►hepatic leukemia factor (HLF) is consistent with upregulation of HLF reported in normal CD34+ and CD133+ and leukemic HSC. HLF is a basic leucine zipper protein defined by a PAR domain that plays a critical role in hematopoietic specific expression of the LMO2 gene. A role for HLF in HSC self-renewal is supported by studies showing that ectopic expression of HLF enhanced HSC engraftment and inhibited apotosis.

The transcription factor CCAAT enhancer binding protein-α (►C/EBPα), a tumor suppressor gene and a crucial regulator of granulopoiesis through inhibition of c-JUN, is down modulated by NUP98-HOXA9. Disruption of C/EBPα, including dominant negative mutations of CEBPα, are found in AML and conditional C/EBPα knockout in mice blocked the differentiation of the common myeloid progenitor with myeloblast accumulation in marrow, absence of neutrophils and enhancement of HSC competitive repopulating capacity and self-renewal. Co-expression of an estrogen-inducible C/EBPα-ER protein together with NUP98-HOXA9 in CD34+ cells showed that re-expression of C/EBPα counteracted the stem cell proliferation and cell expansion associated with C/EBPα downregulation.

Stabilization of HOXA9 Protein Expression

Fusion to NUP98 to HOXA9 results in persistent expression of the homeobox protein due to its reduced sensitivity to CUL4A mediated ubiqitination.



NUP98-HOXA9 Fusion. Figure 2 NUP98 protein localization in normal and NUP98-HOXA9 leukemic cells. (a) NUP98 immunostaining of normal CD34+ cells. Note localization of the protein (purple) in the nuclear membrane and absence of nuclear aggregates. (b) NUP98 immunostaining of a leukemic cell from the bone marrow of a patient with the NUP98-HOXA9 translocation. Note the presence of numerous NUP98+ nuclear aggregates (purple) and impairment in NUP98 association with the NPC. (c) LOH analysis performed by PCR for NUP98 allelic markers on CD34- non-leukemic and CD34+ leukemic cells from the AML patient shown in 2B. Note NUP98 LOH is restricted to the CD34+ leukemic population.

This may be achieved by steric hindrance, dimerization or by the NUP98 FG domains binding CBP/p300 transcriptional co-activators leading to acetylation of the HOX lysine residues required for ubiquitination. The prolongation of the HOXA9 protein half-life in turn leads to persistence of aberrant transcriptional activation.

Depletion of NUP98 from the NPC in Cells Expressing NUP98-HOXA9

NUP98 protein normally moves between the NPC and the nuclear interior where it associates with novel 0.2 μm diameter nuclear bodies termed ►GLFG bodies because the GLFG domain of NUP98 is required for targeting of these structures. Immunostaining for Nup98 and HOXA in normal CD34+ cells transduced with NUP98-HOXA9 also reveals localization of staining in nuclear aggregates with loss of NUP98 from the NPC. In patients with t(7;11)(p15;p15) AML large NUP98+ nuclear aggregates are observed with absence of NUP98 associated with the nuclear membrane (Fig. 2). The chimeric NUP98-HOXA9 may be irreversibly associating with GLFG bodies and may have a dominant negative effect by sequestrating the remaining wild type NUP98 in heterodymeric co-localization bodies. The loss or depletion of NUP98 likely mediates a leukemogenic action by disrupting nuclear export of mRNAs that are critical to a normal balance of HSC proliferation and differentiation. While disruption of NUP98 by NUP98 translocations is a relatively rare event in normal human leukemogenesis there is growing evidence for a wider role for NUP98 disruption in oncogenesis, supported by the observation that there is ►loss of heterozygosity (LOH) at the p11.5 NUP98 locus in nearly a third of human AML (Fig. 2) and this is associated with adverse prognosis.

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NUPR1

►p8 Protein

Nur77

Definition

An orphan member of the ►nuclear receptor superfamily that is expressed in various types of cells and mediates diverse biological processes. It regulates gene transcription by binding to the Nur77-binding response element (NBRE) as a monomer. It is rapidly induced by various stimuli, including growth factors and ►phorbol ester- and ►cAMP synthesis-dependent pathways.

►Retinoid Receptor Cross-talk

Nutraceuticals

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Synonyms

Functional foods; Designer foods; Medical foods; Pharmafoods; Food for specified health use

Definition

Nutraceutical is a food (or part of food) that provides medical and health benefits, including the prevention and/or treatment of a disease beyond providing nutrition. It has also been defined as a product produced from foods but sold in powders, pills, and other medicinal forms not generally associated with food and demonstrated to have physiological benefits or to provide protection against chronic disease.

Characteristics

The term Nutraceuticals was created by Stephen DeFelice in 1989 by combining the words “nutrition” and “pharmaceuticals.” The name nutrition defines the important difference between nutraceuticals and pharmaceuticals. While the pharmaceuticals do not come

from nature and they are chemically created, nutraceuticals come from food naturally. Food provides calories needed to perform daily functions and maintain normal metabolic processes. Moreover, food contains nutrients and other components that are essential to maintain health and reduce the risk of disease. Some examples of relationships between nutraceuticals and health benefits include the importance of calcium in preventing osteoporosis, folate in the prevention of neural tube defects in infants, and vitamin C in the prevention of the disease scurvy. Mounting evidence from epidemiological studies, animal research, clinical trials, and research in nutritional biochemistry suggests that many nutraceuticals may be beneficial in diabetes, osteoporosis, and other chronic and degenerative diseases such as Parkinson and Alzheimer diseases, coronary heart disease, and cancer.

Nutraceuticals and Cancer

It has been estimated that 30–40% of all tumors can be prevented with a correct lifestyle and diet. It has been demonstrated that ►carcinogenesis is inhibited by factors such as ►retinoids, vitamins E, D, C, polyphenols, fibers, calcium, selenium, and polyunsaturated fatty acids such as ►Omega-3 fatty acids. Other factors, such as lipids, sodium chloride, nitrite, and nitrate tend to favor it.

Numerous epidemiological studies have shown a strong correlation between frequent consumption of fresh fruits and vegetables and a decreased cancer risk. These foods are low in fat, rich in vitamins and minerals, and often contain significant amounts of fiber. The benefits of these are well known, but fruits and vegetables contain a variety of naturally occurring compounds, called phytochemicals that have biological activity in humans. It is the combination of nutrients and phytochemicals working together that benefits health, and helps in reducing the risk of cancer, as well as other important disease including heart disease. Scientists have identified thousands of phytochemicals, although only a small fraction has been studied closely. It has been demonstrated that they may exert physiological effects by different mechanisms of action. They may incite the immune system, contribute to reduce the toxicity of adverse chemical products, influence hormone levels, and control cell growth. They may also involve the activation of antioxidant defenses, signal transduction pathways, cell survival associated gene expression, cell proliferation and differentiation, and preservation of mitochondrial integrity. Furthermore, many of these substances exert antiinflammatory actions through inhibition of oxidative stress-induced transcription factors, cytotoxic ►cytokines, and ►cyclooxygenase-2.

Four main types of phytochemicals are found in fruits and vegetables, terpenoids, phenolics (including polyphenolics), nitrogen-containing alkaloids, and sulfur compounds. The terpenoids include ►carotenoids, many of which are precursors to retinol (►vitamin A). Carotenoids are organic pigments naturally occurring in plants and some other photosynthetic organisms like algae, some types of fungus, and some bacteria. Carotenoids are important factors in human health. Protective effects of carotenoids against serious disorders such as cancer, heart disease, and degenerative eye disease have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune response system.

Of the phenolic compounds, the ►flavonoids are the most numerous. They are found in a wide variety of fruits and vegetables, tea, and coffee. In addition to their antiallergic, antiinflammatory, and antimicrobial properties, flavonoids have an important role in the prevention of cardiovascular disease and cancer. They are potent antioxidants and also can influence the expression of ►phase I enzymes and ►phase II enzymes. These enzymes metabolize potentially carcinogenic substances to make them more water soluble and hence readily excreted. This ►detoxification process has a potential protective effect against cancer.

►Isoflavones are a class of organic compounds and biochemicals related to the flavonoids. They can be found in many foods but the best known source is the soybean. Due to their similar structure to the hormone ►estrogen, isoflavones act as ►phytoestrogens in mammals with potent hormonal activity. Other beneficial effects on human health such as antioxidant, antiatherogenic, hypolipidemic, antiosteoporotic, and anticarcinogenic effects have been well demonstrated. Several mechanisms have been proposed for the anticarcinogenic activity of isoflavones. These include upregulation of ►apoptosis, inhibition of angiogenesis, inhibition of ►DNA topoisomerases II, and protein ►tyrosine kinase. Their weak estrogenic activity may be involved in its putative activity against some kinds of cancer, such as ►prostate cancer and ►breast cancer. Isoflavones appear to work in conjunction with the ►soy proteins to protect against cancer, as well as against cardiovascular disease and osteoporosis.

Consumer interest in the relationship between diet and health has increased the demand for information about nutraceuticals and functional foods. Found in a mosaic of products emerging from the food and pharmaceutical industries, and the newly merged herbal and dietary supplement market, nutraceuticals are in high demand among consumers looking for specific health benefits. Credible scientific research now makes it fairly evident that diet can help in reducing the risk of chronic diseases and promotes general good health.

►Phytochemicals in Cancer Prevention

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Nutrition Assessment

Definition

Thorough evaluation of medical history, dietary history, physical examination, anthropometric measurements, and laboratory data with the intent of developing an individualized nutrition care plan.

►Nutrition Status

Nutrition Screening

Definition

Use of objective and subjective data in a easy to use, cost effective, valid, quick tool to identify the presence or risk of malnutrition.

►Nutrition Status

Nutrition Status

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Definition

The definition of nutrition status varies by discipline. In general it refers to the presence or absence of malnutrition. The term "malnutrition" applies to both underweight and overweight populations. Malnutrition is defined as "any disorder of nutrition status including disorders resulting from deficiency of nutrient intake, impaired nutrient metabolism, or overnutrition."

Although no singe index can accurately indicate poor nutrition status, weight and weight history are the parameters most commonly used. This method has limitations. Weight alone does not indicate the nature and extent of tissue loss in patients with cachexia. It also does not indicate specific metabolic or biochemical nutritional issues.

Characteristics

Weight loss is common in patients with cancer, with 31–100% of oncology patients experiencing weight loss depending upon tumor site, stage, and treatment. As little as 5% weight loss is associated with increased mortality and poor prognosis. Unfortunately the weight loss is multifactorial and commonly requires multiple interventions by a variety of disciplines.

Loss of body weight in patients with solid tumors is attributed to losses of fat, water, and ►lean body mass (LBM). Patients with lung, gastrointestinal (GI), and head and neck tumors experience weight loss >10%, which manifests as a loss of both muscle and fat. Individuals with GI malignancies seem to experience the largest decreases (>50%) in muscle mass and protein content, as well as 30–40% loss in body fat. Despite this, even in severe wasting patients retain some body fat. Visceral mass is also preserved to an extent and skeletal muscle loss is the primary form of lean body mass loss.

►Cancer cachexia syndrome (CCS) manifests as the characteristic wasting seen in some cancer patients. Symptoms associated with CCS include weight loss, anorexia (loss of appetite), fatigue, early satiety, and asthenia. CCS is a complex metabolic state which results from a intricate, and yet to be fully elucidated, interaction of pro-inflammatory cytokines such as tumor necrosis factor, interferon-γ, and interleukins 1 and 6 and tumor byproducts such as proteolysis inducing factor, lipid mobilizing factor, and mitochondria uncoupling proteins 1, 2, and 3. CCS results in depletion of energy and muscle stores unresponsive to aggressive nutrition support (i.e. enteral nutrition or parenteral nutrition). Provision of nutrients in excess of estimated needs does not reverse this weight loss. Treatment of the underlying disease is the only current effective therapy for CCS.

Mechanisms

Nutritional decline is often accepted as part of the cancer course and its treatment. Anti-cancer treatment modality choice (i.e. surgery, ►chemotherapy, radiation therapy) can dictate this decline in nutrition status.

The metabolic stress caused by surgery can lead to a hypercatabolic state. This hypermetabolism contributes to muscle and fat breakdown leading to weight loss

post-operatively. Diet restriction (i.e. liquid diet, nothing per oral) and poor appetite lead to decreased dietary intake further adding to this weight loss. Patients with ►GI malignancies that have lost LBM experience increasing rates of severe complications associated with surgical intervention. Surgically induced anatomical changes to the ►GI tract may present mechanical barriers to food ingestion or digestion. Bowel, esophageal, gastric, hepatic, and pancreatic resections can lead to diarrhea, malabsorption, and ultimately dehydration.

Side effects related to chemotherapy administration vary greatly. Chemotherapy is highly toxic to rapidly dividing cells such as those that line the GI tract. Chemotherapeutic agents may directly impair food intake via stomatoxic reactions such as mucositis, diarrhea, and ►emesis, or indirectly via fatigue, pain, food aversions, and taste changes. Nausea and vomiting are frequently reported as the most distressing side effects associated with chemotherapy. Chemotherapy induced symptoms can occur prior to treatment, during treatment or several weeks later and can last from several hours to days. Aggressive ►supportive care is essential to minimize nutrition related side effects.

Radiation to any portion of the GI tract can cause extreme susceptibility to malnutrition. Radiation to the pelvic and abdominal area causes an acute inflammatory response in bowel tissue. This ►inflammation produces diarrhea, abdominal pain and nausea. Chronic radiation enteritis develops in certain cases necessitating lifelong medication and diet changes. Radiation to the head and neck can also lead to nutrition related side effects, such as mucositis, xerostomia, taste change, and dysphagia. These side effects peak two-thirds of the way through treatment and can become chronic. Radiation therapy in the area of the thyroid can cause permanent thyroid damage leading to changes in metabolism.

Gastrointestinal symptoms induced bay any of the above therapies, such as nausea, vomiting, diarrhea, and constipation; and oral symptoms, such as xerostomia and mucositis may lead to weight loss early in the course of cancer. Fatigue and psychological symptoms such as, depression and anxiety, also influence weight loss.

Assessment of Nutrition Status

►Nutrition screening of all oncology patients facilitates the early recognition of malnutrition. Screening is intended to quickly identify individuals at nutritional risk. ►Nutrition screening tools should be easy to use, cost effective, valid, reliable, and sensitive. Screening tools incorporate objective and subjective data to identify the presence or risk of malnutrition. Objective measures commonly included in nutritional screening tools include height, weight, weight change, primary diagnosis, disease stage and the presence of co-morbidities. Individual objective measures, such as a laboratory value or current weight, alone are not specific enough to indicate nutrition risk so multiple objective measures are combined with subjective measures that may impact nutrition. The American Society for Parenteral and Enteral Nutrition (ASPEN) and the American Dietetic Association (ADA) recommend that all patients receive nutrition screening as a component of their initial evaluation.

Many nutrition screening tools have been developed, with several specifically validated in an oncology population. The ►patient generated subjective global assessment (PG-SGA) and the mini nutritional assessment (MNA) are commonly used in the outpatient oncology setting. Many acute care facilities have designed facility specific screening forms to be completed by the nursing staff upon admission. These acute care forms commonly combine nutrition screening with other disciplines.

The PG-SGA is a modification of an earlier screening tool called the Subjective Global Assessment (SGA) developed by Detsky. Faith Ottery, a surgical oncologist, modified the SGA specifically for the oncology population. The PG-SGA is comprised of two sections; a patient completed section and a section completed by the healthcare professional. Patients provide data regarding weight history, symptoms, dietary intake, and activity level. Healthcare professionals evaluate metabolic demand, disease in relation to nutritional requirements, and perform a physical assessment. The nursing staff, a nurse practitioner, a registered dietitian, or a physician can complete this section. A numeric score is calculated by adding the points obtained in sections one and two. The numeric scores can be used as a triage system to initiate nutrition intervention and guide follow-up. The PG-SGA numeric score, when repeated at subsequent time points, is useful in illustrating small improvements or deteriorations in nutrition status.

The Nestle Mini Nutritional Assessment (MNA) is a commonly used nutritional screening tool in the elderly population. The 18-item MNA, developed by Guigoz with Nestle Nutritional Corporation, is comprised of two main components; screening and assessment. The six-item screening takes approximately three minutes to complete and includes questions related to decline in food intake, weight loss, mobility, stress and body mass index. The healthcare practitioner is directed to complete the assessment section of the MNA if the screening provided a score of 11 or less. The assessment component includes specific medical history and eating habits as well as some anthropometric measurements.

The Malnutrition Screening Tool (MST), although not commonly used in the United States, is a short nutritional screening tool. This three-item tool utilizes data on weight history and appetite to predict nutrition risk The MST, developed by Maree Ferguson, has been validated in both acute care patients and oncology patients receiving radiation therapy.

Nutrition screening should lead to a more in-depth ►nutrition assessment. Nutrition assessment differs from screening in that it is a thorough evaluation which assimilates data obtained from the medical history, dietary history, physical examination, anthropometric measurements, and laboratory data. The nutritional assessment integrates the review of anthropometrics with data on disease and clinical status to evaluate impact on metabolism and nutrient need. Appraisal of disease and treatment related symptoms is also necessary to plan nutrition interventions. This process leads to the identification and diagnosis of nutritional issues. The nutritional assessment is usually completed by a registered dietitian (RD) or nutrition professional, however other members of the healthcare team can complete it as well.

Nutrition Intervention

Nutrition intervention refers to the specific activities required to address and correct the nutrition diagnosis. The nutrition intervention is selected, planned, and implemented with the intent of improving nutrition status. Planning of the nutrition intervention requires the input of all disciplines involved in patient care. Reflection must be taken on the causes of weight loss or weight gain. In taking into consideration these factors the patient should remain at the center of the intervention, with patient preferences of paramount importance.

The goals of the intervention must be documented and reevaluated frequently. The intervention must be individualized to the patient and consideration should be taken for patient comfort and wishes. Although variable between and among patients, common nutritional goals include symptom management, weight maintenance, and preservation of functional status. Attaining these goals may require modified diets, the addition of oral nutritional supplements, or the initiation of enteral or parenteral nutrition. Supportive care measures should be employed as needed These modifications to dietary intake can be costly and flexibility is required on the side of the individual designing the intervention with respect to specific formulas and administration. The services of financial planners and social workers are very beneficial in this situation.

Benefit of Maintenance of Nutrition Status

Health-related ►quality of life (QOL) refers to the physical, psychological and social domains of health that are influenced by an individual's experiences, beliefs, expectations and perceptions. Cancer treatments influence the social, psychological and emotional aspects of patients' lives. The presence of malnutrition can also impact a patient's QOL. Difficulty with eating resulting from the side effects of treatment or disease may cause patients to pass up experiences of social interactions with family and friends. This in turn leads to further depression of appetite. Poorer overall QOL has been observed in patients experiencing symptoms that impact socializing such as odynophagia, weak voice, and unclear speech.

A relationship has been established between weight loss and QOL in oncology patients. Changes in body composition affect symptom control and complication rates. Increased incidence of nutritional symptoms is correlated with decreases in quality of life. To date, there is little information relating changes in specific body compartments to QOL. Preliminary research relating to the use of an anabolic steroid indicate that increases in body cell mass are associated with improved QOL and ►ECOG performance status scores in head and neck and lung cancer patients. However, there has been little quantification of the impact of loss of LBM on QOL.

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NWTSG

Definition

National Wilms Tumor Study Group (NWTSG) is an organization that has co-ordinated clinical trials for the treatment of Wilms tumor in North America since the early 1970s. They have data on epidemiological, genetic and clinical features together with treatment outcome on over 6,000 children treated for ►Wilms tumor.

O/E

►Early B-cell Factors

O6-Alkylguanine-Alkyltransferase

Definition

Is a member of the family of alkyltransferase enzymes that catalyzes alkylation of O6 position of the guanine residue in DNA.

►Alkylating Agents

Oat Cell Carcinoma

►Extrapulmonary Small Cell Cancer

Oat Cell Lung Cancer

Definition

►Small Cell Lung Cancer

Obesity

Definition

Is a status of excess fat accumulation in the body, to a degree that is much greater than what is considered healthy. It is a major risk factor for type 2 diabetes, cardiovascular disease, and some types of cancers.

►Obesity and Cancer Risk

Obesity and Cancer Risk

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Definition

Obesity is a condition in which excess fat has accumulated in the body. The only widely accepted criteria for obesity are based on body mass index (BMI, also called Quetelet index), which is defined as the weight in kilograms divided by the square of the height in meters. According to the World Health Organization (WHO) classification, 'normal weight' is defined as BMI between 18.5 and 25 kg/m^2, 'overweight' is BMI between 25 and 30 kg/m^2, and obesity is defined as a BMI of 30 kg/m^2 or greater.

Characteristics

Trends in Obesity

Over the past few decades, the proportion of people with excess body weight has been increasing in most developed and developing countries along with an adoption of a westernized lifestyle characterized by excessive energy intake and lack of physical activity. There are large between-country and within-country differences in the ►prevalence of obesity. The prevalence of adult obesity is high in North America (particularly in the United States), the Eastern Mediterranean, Central and South America (particularly in Mexico, Paraguay, Argentina, and Chile), Eastern Europe, and some Western European countries (Finland, Spain, Germany, and the United Kingdom). In the United States, the prevalence of obesity doubled over the past two decades, and currently about one-third of the

US adult population is obese and an additional one-third is overweight. Smaller increases in the prevalence of obesity have been observed in many European countries. The MONICA project – a large international study of the monitoring risk factors for cardiovascular disease, led by the WHO – showed a prevalence of obesity above 20% in many parts of Western Europe in the 1990s, and it was has high as 35% in some Eastern European countries. Obesity is still uncommon in China and Japan and most parts of Africa.

Obesity and Cancer Risk

Being overweight or obese is a well-known risk factor for ►type 2 diabetes mellitus and cardiovascular disease, but ►epidemiologic studies are also providing mounting evidence for a link between body weight and cancer risk. In 2002, the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Cancer Preventive Strategies published an extensive review of the literature on body weight and cancer. The Working Group concluded that, based on data from epidemiologic studies, there is sufficient evidence of a cancer-preventive effect of avoidance of weight gain for cancers of the colon, breast (in postmenopausal women), endometrium, kidney (renal cell), and esophagus (adenocarcinoma). Many studies have been published since the IARC report, and the accumulated evidence indicates that obesity may be a risk factor also for pancreatic, liver, gallbladder, and hematopoietic cancers, and possibly for aggressive prostate cancer. Obesity is not associated with risk of lung cancer, and is probably not a risk factor for ►bladder cancer or noncardia ►gastric cancer. Findings for cancer at other sites are sparse or inconsistent.

Obesity-Related Cancers

There is an extensive body of epidemiologic data showing a relation between obesity and ►colon cancer risk. In general, obesity has been found to be associated with an approximately 30–90% increased risk of colon cancer in men and with a 10–50% increased risk in women. The association between obesity and risk of rectal cancer is less clear, with a generally weak positive association observed in men but no association in women.

The first evidence of an association between excess body weight and increased risk of breast cancer came in 1974 from a Dutch ►cohort study of postmenopausal women. A large number of subsequent studies have found that obesity increases the risk of breast cancer in postmenopausal women by about 30%. Conversely, there is consistent evidence that obesity is associated with a decreased risk of breast cancer in premenopausal women. This reduction in risk may be related to the increased tendency of young obese women to have anovulatory menstrual cycles and reduced circulating concentrations of progesterone and ►estradiol. Several studies have shown a stronger association between BMI and postmenopausal breast cancer risk in women who have never used postmenopausal hormones. This observation is consistent with the hypothesis that the effect of obesity on postmenopausal breast cancer risk may be mediated through increased endogenous estrogen production.

Obesity has been associated consistently with an increased risk of ►endometrial cancer in both pre- and postmenopausal women. The risk increased about linearly with increasing BMI in most studies. In different studies, the risk of endometrial cancer was approximately 2–3 times higher in overweight and obese women than in normal weight women.

An association between excess body weight and risk of kidney (mainly renal cell) cancer has been consistently observed in epidemiologic studies. The majority of studies have reported a linear increase in risk with increasing BMI. The increase in risk generally ranges from 1.5- to 3-fold in overweight and obese individuals.

The ►incidence rates of adenocarcinoma of the esophagus and gastric cardia have been rapidly increasing in developed countries over the last three decades. In contrast, the rates for squamous cell carcinoma of the esophagus and noncardia gastric adenocarcinoma have remained stable or decreased slightly during the same period. Compelling evidence indicates that overweight and obesity increase the risk of adenocarcinoma of the esophagus by about 2–3-fold and of gastric cardia by about 1.5–2-fold. However, excess body weight is not associated with an increased risk of esophageal squamous cell carcinoma or noncardia gastric adenocarcinoma.

Cancers Likely Related to Obesity

Several recent cohort studies have observed a 1.2–2-fold increased risk of pancreatic cancer in obese men and women. While some studies found a dose-response relationship with increasing BMI, other studies showed an increase in pancreatic cancer risk only among obese individuals.

Seven cohort studies in the United States and Europe have observed a 1.5–3.5-fold increased risk of liver cancer in obese individuals, whereas a ►case–control study in Canada reported no association. Obesity may contribute to the risk of liver cancer by promoting the development of non-alcoholic fatty liver disease.

To date, eight cohort studies and three case–control studies have examined the association between obesity and risk of gallbladder cancer, with most studies showing an increased risk in obese men and women. The increase in risk generally ranges from about 1.5–2-fold in obese individuals. Obesity may increase

gallbladder cancer risk indirectly by increasing the risk of gallstones, which, in turn increase the risk of gallbladder cancer.

Several studies published during the last 5 years have found that obesity is associated with an increased risk of hematopoetic cancers, including ►non-Hodgkin lymphoma, ►leukemia, and ►multiple myeloma. Overall, obesity has been associated with an approximately 20–40% excess risk of these cancers.

Other Cancers

Results from a large number of studies of the association between obesity and incidence of overall prostate cancer have been largely null. However, obesity has been related to increased risks of more advanced prostate cancer and prostate cancer mortality, and with higher recurrence rates after radical prostatectomy or radiotherapy treatment. These findings suggest that obesity may influence prostate cancer aggressiveness and progression.

Obesity has been found to be inversely related to risk of lung cancer in several studies, but this association appears to be due to ►confounding by smoking (smoking is the main cause of lung cancer and is inversely associated with body weight). There is no association between obesity and lung cancer in nonsmoking populations.

Studies of the relation between obesity and ►ovarian cancer risk are inconclusive. Although some studies found an increased risk (1.5–2-fold) of ovarian cancer in obese women, several studies observed no association. Results on obesity in relation to cervical cancer risk are limited and inconsistent.

Mechanisms Linking Obesity to Cancer Risk

Several biological mechanisms have been postulated to explain the relation between obesity and cancer risk, including alterations in the metabolism of insulin, ►insulin-like growth factor-I (IGF-I), and sex steroids (►estrogens, androgen, and progesterone). Obesity leads to a state of relative insulin resistance, chronically increased insulin concentrations, and an increase in bioavailable IGF-I. Both insulin and IGF-I act as growth factors that stimulate cell proliferation and inhibit ►apoptosis. Epidemiologic studies have shown that increased circulating concentrations of insulin, C-peptide (a marker of pancreatic insulin secretion), and IGF-I as well as type 2 diabetes mellitus are associated with an increased risk of several cancer types, especially cancers of the colon, endometrium, and pancreas. Sex steroids are involved in the control of cell differentiation, proliferation, and apoptosis, and may also favor the selective growth of preneoplastic and neoplastic cells. There is considerably evidence that the increase in breast and ►endometrial cancer risk with increasing body weight in postmenopausal women is largely the result of the associated increase in circulating concentrations of estrogens, particularly bioavailable estradiol. The relationship between obesity and endometrial cancer in premenopausal women may in part be related to chronic anovulation and decreased progesterone concentrations in young obese women.

Adipocytes (fat cells) secrete a number of fat cell hormones/cytokines or adipokines, such as leptin, ►adiponectin, resistin, tumor-necrosis factor-α, and ►interleukin-6. These ►adipokines have important roles in the regulation of energy balance, lipid metabolism, and insulin resistance as well as in ►inflammation and immune response. It has been proposed that in addition to growth factors and sex hormones, obesity-related alterations in the concentrations of adipokines may be linked with cancer risk.

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Observational Study

Definition

A study of changes in distribution of exposures, cancer, and other factors, without the intervention of the investigator.

►Cancer Epidemiology
►Epidemiology of Cancer

Occult Cancer

►Dormancy

OCP

Definition
Oral Contraceptive Pill.

Octreotide

Definition
Is a chemical compound used in nuclear medicine for localization of gastrinoma. Analog of ►somatostatin.

►Gastrinoma

Oculodermal Melanocytosis

Definition
Nevus of Ota; ►Hamartoma of melanocytes giving a characteristics blue-gray appearance to the periocular skin and sclera. In Caucasians and Asians, there is an increased incidence of ►uveal melanoma as well as ►glaucoma. In African Americans, there is only an increased risk of glaucoma.

ODC

Definition
Ornithine Decarboxylase.

Odontoblasts

Definition
Primary: Single cell line of highly specialized cells which are located inside the dental pulp, but whose processes reach from the pulp chamber through the dentin tubules to the dentinoenamel junction (primary odontoblasts). Their competence lies in secretion of (physiologic) secondary dentin and reparative dentine due to caries, trauma or tooth preparation.
Secondary: Derive from mesenchymal precursor cells when primary odontoblasts become destroyed. Secondary odontoblasts (or "odontoblast-like" cells) form irregular masses of tertiary dentine. This capability to produce such calcifications plays an important role as a barrier mechanism after a carious, traumatic or iatrogenic insult to the tooth.

►Dental Pulp Neoplasms

Odynophagia

Definition
Is pain during swallowing.

►Nasopharyngeal Carcinoma

8-OHdG

Definition
8-Hydroxydeoxyguanosine.

►UV Radiation

Okadaic Acid

Definition
Is a toxin isolated from the marine sponge *Halichondria okadai*. Okadaic acid accumulates in bivalves and causes diarrhetic shellfish poisoning. The molecular formula of okadaic acid is $C_{44}H_{68}O_{13}$. Okadaic acid strongly inhibits protein serine/threonine phosphatase 1, 2A, and 2B. The inhibitory effect of okadaic acid is strongest for 2A, followed by 1, and then 2B.

►Doublecortin

OKT3

Definition
A monoclonal antibody that targets mature ►T cells.

Oleanolic Acid

Definition
A triterpenoid from plant species.

►Betulinic Acid

Olefins

Definition
Chemicals with a vinyl bond.

►Carcinogen Metabolism

Olf

►Early B-cell Factors

Olfactory Neuronal Transcription Factor

►Early B-cell Factors

Olfactory/Early B-cell Factors

►Early B-cell Factors

Oligoastrocytomas

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Synonyms
Mixed gliomas

Definition
Are tumors located mainly in supratentorial subcortical brain areas showing composite histological features and including cells similar to oligodendrocytes or astrocytes.

Characteristics
Oligoastrocytomas and pure ►oligodendrogliomas constitute a subgroup of ►brain tumors receiving increased attention because of reports of chemosensitivity and of favorable survival rate when compared with ►astrocytomas. This is also reflected in the increasing rate of diagnosis of tumors with an oligodendroglial component raising from about 5% in the past decade to approximately 15% in present years.

The morphological borderlines between astrocytomas, oligoastrocytomas, and oligodendrogliomas are difficult and controversial issues. However the accurate distinction between these tumors has important prognostic and therapeutic implications. Patients with pure oligodendrogliomas are more likely to respond to chemotherapy and have a longer survival than patients with diffuse astrocytomas or oligoastrocytomas of the same grade.

Oligoastrocytomas show a conspicuous mixture of two distinct neoplastic cell types, resembling the tumor cells in oligodendrogliomas and diffuse astrocytomas; the two components may be separated into distinct areas or be diffusely admixed.

A pathological diagnosis based on the assessment of the individual component is difficult, since in most instances the true extent of such component can be hardly determined because of incomplete tumor sampling. Furthermore, precise diagnostic criteria are still lacking, the proposed minimum astroglial component ranging from 1 to approximately 50%. A subclassification of oligoastrocytomas, based on histological features, into oligodendroglioma-predominant, astrocytoma-predominant, or equivalent oligodendroglial and astroglial component has been proposed. However, prognosis and response to therapy were not found to be significantly different for such subtypes.

Oligoastrocytomas are graded, based on the WHO classification, as either low-grade (Grade II WHO classification) or high-grade tumors (anaplastic, grade III WHO classification). The diagnosis of anaplastic oligoastrocytoma requires the presence of histological features of malignancy such as increased cellularity, nuclear atypia, pleomorphism, and increased mitotic activity. Signs of histological malignancy may be present in the oligodendroglial component, the astroglial component, or in both cell types. The histological grading is both of prognostic and therapeutic use.

Glioma genotyping can provide important information complementing the histological analysis. Until now no specific abnormalities associated with oligoastrocytomas have been found. Genetic alterations of these tumors include loss of heterozygosity (LOH) on chromosome 1p and/or 19q, typically found in oligodendrogliomas, but also LOH on chromosome 17p or 10q, frequently found in astrocytomas and associated with the progression to glioblastoma.

►LOH on 1p and/or 19q are present in approximately 50% of oligoastrocytomas, independently of their histological grade; LOH on 17p is seen in 20% and LOH on 10q in 15% and is significantly associated with anaplasia; the observation of 1p loss with intact 19q (or vice versa) is more frequent in oligoastrocytomas than in oligodendrogliomas. Similar to oligodendrogliomas, LOH on 1p and/or 19q has been associated with chemosensitivity and prolonged survival, while LOH on 10q has an unfavorable impact on prognosis.

Therefore, genomic changes may permit the division of tumors into more homogenous groups: oligoastrocytomas with LOH on 1p behave like WHO grade II or III oligodendrogliomas with 1p LOH and they are associated with longer survival; on the other hand, oligoastrocytomas without LOH on 1p behave like grade II or III astrocytomas and have shorter survival.

Despite the clinical relevance of 1p and 19 q losses, the genes targeted on these two chromosomes are unknown. Some very recent progress, however, may help pinpointing the genetics underlying these alterations. Combined loss of on 1p and 19q is mediated by the ►chromosomal traslocation (t(1,19)(q10;p10). The high sequence homology of chromosome 1 and 19 centromeric regions could be the mechanisms favoring translocation, facilitated by tumor-related alterations of normal DNA methylation or histone modification affecting the centromeric region. Chromosome engineering was used to generate mouse models with gain and loss of a region corresponding to human 1p36. By this strategy chromodomain helicase DNA binding domain 5 (Chd5) was identified as a tumor suppressor located on 1p36 that controls proliferation, apoptosis, and senescence via the p19(Arf)/p53 pathway. Future studies will clarify the relevance of Chd5 in the formation of different gliomas, including oligoastrocytomas.

Due to the high variability of morphological criteria used for their classification, oligoastrcytomas incidence rate is highly variable, accounting from 1.8 to 10% of brain tumors. Males are affected slightly more frequently than females.

Oligoastrocytomas typically occur in young adults (mean age at diagnosis, 35–45 years) and epilepsy is the most common initial symptom. Less commonly, patients may present with headaches, progressive paresis, cognitive impairment, signs of increased intracranial pressure, or have no symptoms at all.

Supratentorial subcortical areas are the most frequent locations of oligoastrocytomas. The frontal lobes are most commonly affected. Lesions are usually single, but multiple lesions have been reported. There may be a correlation between the profile of molecular alterations and tumor location. Also, oligoastrocytomas and oligodendrogliomas with LOH on 1p appear preferentially located in the frontal regions.

The issues concerning the diagnosis are similar to those for other low-grade tumors. At neuroimaging oligoastrocytomas do not show features allowing a reliable distinction from oligodendrogliomas. Computed tomography (CT) scans reveal a well-demarcated hypodense subcortical mass. Calcifications may be present, but they are less common than in pure oligodendrogliomas. In about half of low-grade oligoastrocytomas CT scans show contrast enhancement.

Magnetic resonance (MR) shows a hypointense mass in T1 weighted images and a hyperintense mass in T2 weighted images. The abnormal T2 signal represents both vasogenic edema and the infiltrating tumor. Enhancement is associated with anaplasia, but especially in older people, the absence of enhancement does not exclude the diagnosis of anaplasia; when present, enhancement is irregular and nodular.

In most studies patients with oligoastrocytomas are combined with patients with oligodendrogliomas or astrocytomas. However in cohorts of WHO grade II oligoastrocytomas a median survival time of 6.3 years and survival rate of 70% at 5 years and 49% at 10 years have been reported. In WHO grade III oligoastrocytomas the median survival time of 2.8 years and survival rates of 36% at 5 years and 9% at 10 years have been referred. Clinical parameters associated with prolonged survival are young age (<40 years), long duration of symptoms, excellent postoperative neurological status.

Surgery is an important diagnostic and therapeutic modality for patients with oligoastrocytomas. First, it can relieve global symptoms caused by raised intracranial pressure; furthermore, it provides adequate tissue for a pathological diagnosis and grading. The role of gross total surgical excision remains unresolved, although most retrospective studies, in which the extent of surgery is often defined by the surgeon rather than by

postoperative imaging, suggest a survival benefit from aggressive surgical resection.

Postsurgical treatments of oligoastrocytomas are similar to those of oligodendrogliomas and are different in grade II and grade III tumors; nevertheless the optimal therapy at diagnosis and recurrence remains controversial. Generally, in WHO grade II oligoastrocytoma treatment is deferred until there is a clinical or radiological evidence of progression, unless patients have disabling symptoms or signs at presentation. In fact the disease can be indolent for years and neurotoxic effects of radiotherapy must be considered. Indeed, the optimal timing of radiotherapy is not yet established; a conventional treatment with focal radiotherapy up to a total dose of 54 Gy is needed for WHO grade III oligoastrocytomas.

The role of chemotherapy is evolving and some form of chemotherapy may ultimately replace radiotherapy as the initial treatment. PVC chemotherapy (Procarbazine, CCNU, and ►vincristine) is the best studied chemotherapy regimen and can cause measurable tumor regression. However, because of PCV toxicity, temozolomide may be a reasonable alternative.

Most oligoastrocytomas, as well as astrocytomas and oligodendrogliomas, will progress to malignancy. Patients with worsening clinical symptoms and the appearance of contrast enhancement at MRI or hypermetabolism at PET warrant reevaluation. Resection or biopsy is often performed, and further therapy is chosen on the basis of histological features and prior treatment.

Like anaplastic oligodendroglimas, WHO grade III oligoastrocytomas require immediate treatment after diagnosis. A conventional focal radiation dose of 60 Gy is appropriate for WHO III grade tumors. It is unclear how to best incorporate chemotherapy into the treatment strategy. According to many physicians treatment can be initiated with chemotherapy using PCV or temozolomide, followed by radiotherapy; this treatment schedule allows to measure treatment response, when there is a residual tumor. A recently published, large, randomized EORTC trial showed that adjuvant PCV chemotherapy does not prolong overall survival but increases progression free survival. Similarly, a RTOG trial showed that despite significant toxicity, PCV plus radiotherapy is associated with prolonged progression-free survival without changing overall survival.

The deletion of particular genomic regions could have direct consequences for therapeutic intervention. Losses on 1p and 19q correlate with sensitivity for treatment with nitrosureas or temozolomide and with longer survival. However, both in EORTC and RTOG trials, patients with LOH on 1p and 19 q did not achieve a benefit from early chemotherapy and had a longer disease course irrespective of treatment modality, suggesting that this genetic signature is rather a prognostic marker than a predictor of chemotherapy response.

Therefore, at this time it is unclear how to incorporate molecular genetic information into treatment decision for patient with oligoastrocytomas.

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Oligodendrocyte

Definition

A cell type found in the central nervous system, the function of which is to wrap neuronal processes with myelin in order to facilitate the transmission of action potentials.

►Oligodendroglioma

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Oligodendroglioma

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Definition

Oligodendrogliomas are a diagnostic classification of ►brain tumors formed of cells that cytologically resemble the ►oligodendrocyte and predominately arise in the frontal lobes of adults but may also be found in other regions of the brain and spinal cord and affect all ages.

Characteristics

Clinically, oligodendrogliomas are considered to be aggressive cancer and infiltrative cancer, earning World Health Organization grades of II and III. Recent

therapeutic trials have indicated that the tumor is responsive to certain therapies and that genetic ►biomarkers seem to predict good therapeutic responses to these therapies.

History

Historically, the tumors were first described by Bailey and Bucy (1929) in a classic publication that also described their calcifications, their tendency to occur in the cerebral hemispheres of adults and their slow clinical ►progression. They also noted the presence of astrocytic-like cells in some tumors with transitional cells also present in some tumors.

Epidemiology

Oligodendrogliomas represent ~3–4% of all primary tumors arising in the brain. The incidence is ~0.3 per 100,000 populations. There are no known familial syndromes of which these tumors are a component and there is no known inherited tendency to acquire the tumors. No environmental or occupational hazards are implicated in causation. The majority of these tumors are diagnosed in the fourth and fifth decades though they affect all ages. Infratentorial examples seem to be more common in children than adults. There is a modest male predominance.

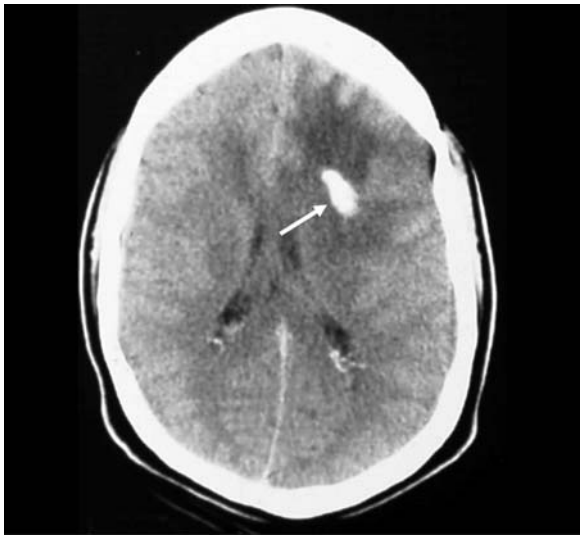
Clinical Presentation

The tumors are commonly associated with ►epilepsy and/or headache. Not uncommonly, a pre-diagnostic period of symptoms related to the tumor of 5 years or more is noted. Examples of adult patients with seizures beginning in childhood are well documented.

Until recently, the post operative survival was relatively uniform despite the variable presentation. Reports indicate a post-diagnostic ►Kaplan Meier survival of 81% at 2 years. Age greater than 65 years has been associated with a significantly shorter survival. Other clinical features associated with poor prognosis include poor post-operative ►Karnofsky performance score, tumoral biopsy rather than gross total removal, and no ►radiation therapy. Of course many clinical decisions are reflected in the choices of biopsy and therapy that may reflect independent features of poor prognosis rather than represent factors themselves. For example, a tumor arising in a ►neurologically eloquent location may preclude gross total excision.

Radiologic Appearance

Radiographic studies reveal that the tumors tend to affect both the cortex and white matter with a proclivity to appear in the frontal lobes (Fig. 1). They are the most common primary intraparenchymal tumor to exhibit calcification which may be seen on plain skull films but



Oligodendroglioma. Figure 1 Noncontrast ►CT scan demonstrates focal (*white arrow*) calcification in a frontal lobe oligodendroglioma.

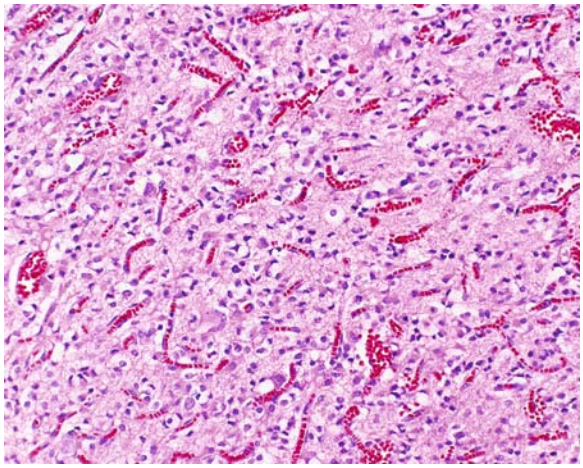
are very commonly encountered on CT scans. While the calcifications may be seen in low grade tumors, they persist after the tumor has progressed to a higher grade. The tumors appear well circumscribed and do not manifest significant edema on CT scanning.

The MR features are variable. Most tumors exhibit regions that are iso- to hypointense on T1-weighted images and hyperintense on T2-weighted images. Microcysts may also be visible on T1- and T2-weighted imaging. Enhancement with gadolinium is rarely significant and typically manifests fine linear or dot-like patterns, if present.

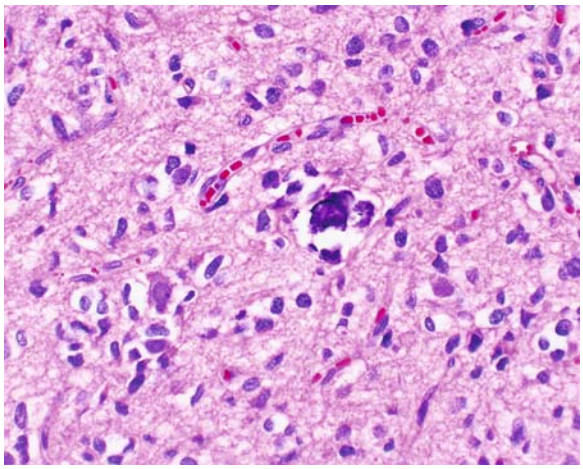
Pathologic Features

Upon gross inspection, oligodendrogliomas often exhibit infiltration into the leptomeninges, resulting in a white, gelatinous, "toothpaste" appearance to the operative surgeon. Other tumors, more deeply situated, may infiltrate the ventricular system or appear to cross the corpus callosum. The gelatinous tumors have a pink appearance in more solid regions with a relatively sharply demarcated border.

Microscopic examination reveals a relatively monotonous cytologic appearance of oval cells of similar size and shape (Figs. 2–4). The tumor cells have a proclivity to accumulate about blood vessels and, in the cortex, about neurons in a process called "satellitosis." The tumor cells exhibit nuclear features that are monotonous with round, regular nuclei that seem to be made from the same mold. The nuclei exhibit filamentous or speckled chromatin that is often associated with a single punctate ►chromocenter, or karyosome. Mitotic activity

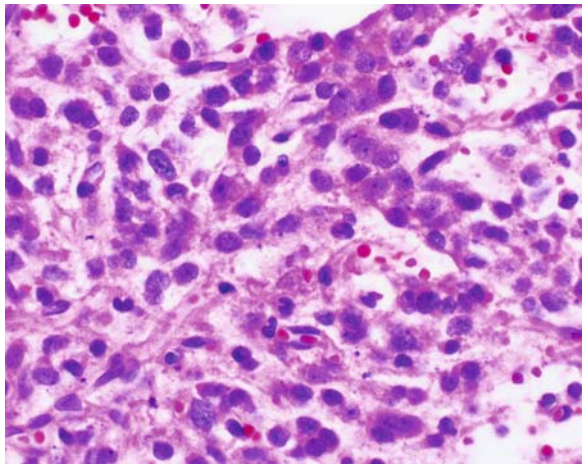


Oligodendroglioma. Figure 2 This microscopic image of an oligodendroglioma shows numerous branching vessels filled with red blood cells.



Oligodendroglioma. Figure 3 This microscopic image of a well differentiated oligodendroglioma shows the round regular blue stained nuclei surrounded by clear zones that are typical of the histologic appearance of the tumor. The clump of blue indicates a small focus of calcium.

is not uncommon, even among low grade tumors. While the cytoplasm of oligodendroglial cells is often clear, resulting in a "fried egg" appearance, the finding itself is artifactual and can be lost with rapid fixation. The vasculature is often described as net-like with small tubular vessels often seen to be branching at various angles (Fig. 2), however, this vascular pattern is not consistently found and may represent the dilated indigenous vasculature of ischemic cortex where it is most commonly found. The calcifications noted by radiology consist of scattered punctate mineral deposits



Oligodendroglioma. Figure 4 This image of an anaplastic oligodendroglioma shows more abundant pink staining cytoplasm in contrast to the image of Fig. 3 and now shows small blue particles indicative of dead nuclei. Cell death and high cell density are indicative of high cell turnover.

along blood vessels and dead cells (Fig. 3). Phenotypically, the tumor cells are glial and show ►immunohistochemical reactivity for Glial Fibrillary Acidic Protein.

Tumor Grading

Spontaneous geographic necrosis is considered an ominous finding indicative of high grade tumor. High grade tumors also frequently demonstrate eosinophilic-cytoplasm maintaining the oval outlines (Fig. 4). Mitotic activity becomes more pronounced and vascular proliferation is manifest. However, in contrast to the ►glioblastoma with its varied histologic appearance, anaplastic oligodendrogliomas maintain a uniform cytologic appearance and often preserve areas of low grade morphology.

Mixed Gliomas

The so-called mixed glioma, or oligo-astrocytoma, is a controversial entity with regards to its definition. Some pathologists insist on two distinctive histologic populations of oligodendroglioma and ►astrocytoma while others indicate tumors with transitional cells exhibiting round regular, "oligodendroglial" nuclei but exhibiting fibrillary eosinophilic "astrocytic" cytoplasm represent these tumors. Therapy often renders once well accepted oligodendrogliomas with clear cytoplasm and round to cuboidal cell outlines into transitional forms on subsequent biopsies.

Genetics

In the early 1990s, researchers using ►loss of heterozygosity analyses found that tumors with oligodendroglial

morphologies commonly exhibited allelic losses of 1p and 19q in up to 85% of these tumors. Subsequent researchers have noted that this change occurs after mitotic division as a result of a t(1,19)(q10,p10) ►chromosomal translocation which results in an apparent centromere to telomere loss of a 1p and a 19q arm. Earlier researchers had noted that tumors with these genetic markers had significantly better responses to chemotherapy and radiation therapy versus gliomas that lacked these markers. However, even so-called mixed gliomas that lost only the distal 1p36 locus had better therapeutic responses compared with those exhibiting intact loci. Oligodendrogliomas of childhood tend not to exhibit the 1p/19q LOH marker and the therapeutic implications of these intact markers in these children are not established. ►*p53* mutations, common in astrocytomas, are not found in oligodendrogliomas.

Mixed gliomas (►oligoastrocytomas) have been studied by 1p/19q analysis but the results are varied due to definitional problems. However, it is clear that this set of tumors holds the highest variation in genetic marker loss than seen in typical oligodendrogliomas. Among these tumors, 1p and 19q loss often do not occur in tandem with only 1p or only 19q exhibiting LOH or only a partial 1p loss is manifested suggesting that the microscopic ambiguity is related in someway to the incomplete genetic picture.

Therapy

Oligodendrogliomas are not cured by current therapeutic regimens. They appear to respond to chemotherapeutic protocols that include external beam radiation and/or ►chemotherapy with ►alkylating agents (such as ►Temozolomide). As noted previously, the response to therapy seems to be related in some unknown way to 1p/19 LOH status.

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Oligomerization Domain

Definition

OD; Refer to a region in a protein that mediates the assembly of higher order protein complexes. A major distinction between ODs and other protein-binding modules is that ODs are generally responsible for homotypic interactions or interactions between proteins in the same family.

►p53 Family

Oligonucleotide

Definition

Short sequence of single stand DNA.

►ArrayCGH

Oligosaccharides

Definition

One of the most important posttranslational modifications on proteins. They contain both *N*-glycans and *O*-glycans. There are three kinds of major oligosaccharide molecules, namely, glycoproteins, glycolipids and proteoglycans.

►Fucosylation

Ollier Disease

Definition

Non-hereditary disorder characterized by the occurrence of multiple ►enchondromas preferentially located in the short and long tubular bones of the limbs causing deformity and limb asymmetry, with one side of the body more severely affected. The genetic deficit is unknown so far.

►Chondrosarcoma

OM

Definition
Oligomycin, an inhibitor of oxidative phosphorylation.

►Jasmonates in Cancer Therapy

Omega-3 Fatty Acids

Definition
Omega-3 fatty acids are a family of polyunsaturated fatty acids that have in common a carbon–carbon double bond in the ω-3 position. They are essential to human health but cannot be manufactured by the body. They must be obtained from food being present in fish and certain plant oils. Also known as polyunsaturated fatty acids (PUFAs), omega-3 fatty acids play a crucial role in brain function as well as in normal growth and development.

►Nutraceuticals

Omental Immune Aggregates

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Synonyms
Milky spots; Omentum-associated lymphoid tissue

Definition
Omental immune aggregates are organized clusters of leukocytes (white blood cells), surrounded by an extensive vascular network and partially covered by a layer of ►mesothelial cells. They are found on the ►omentum in a wide variety of mammalian species as well as in frogs and chickens. Similar structures are also found within the mesentery, and interspersed along adipose tissue along the reproductive tract.

Characteristics
Structure and Cellular Composition
These immune aggregates were first described by Recklinghausen in 1863 and in 1874 Ranvier named them "taches laiteuses" or milky spots due to their translucent appearance on the more transparent omentum of rabbits. They have been identified on the omentum from a large number of other mammalian species including man, mouse, rat, guinea pig, sheep, goat, bat, mole, pig, cat, dog as well as frog and chicken. The original studies primarily employed electron microscopic techniques to study tissues from human and rat. More recent studies with mouse tissues utilized immunofluorescence techniques as well as flow cytometric assays to delineate the cell types present. The omentum itself is a thin tissue composed of a double layer of mesothelial cells with blood vessels, a central lymphatic vessel and numerous adipocytes between the layers. The immune aggregates are visible macroscopically on this transparent sheet as opaque spots composed of a variety of immune cells, whose relative amounts appear to vary somewhat among species. In humans and rats macrophages have been reported to be the major cell type present, whereas more recent studies in mice indicated that lymphocytes account for the majority of cells present. In humans the cellular composition of the immune aggregates have been reported to be 48% macrophages, 29% B cells and 11% T cells with small numbers of mast cells. In recent studies with mice, the composition has been found to be 15% macrophages, 45% B cells, 15% T cells, 5% dendritic cells (CD11c+) as well as small numbers of ►NK cells and NKT cells and gamma/delta T cells. It is not clear if these differences are due to the different techniques employed or real differences among species. In humans milky spots have first been observed at 26 weeks gestational age with increasing numbers and size that appear to reach a maximum during the first year of life. Extensive ontogeny studies have not been done in mice or rats.

Structurally, these immune aggregates have a distinct architecture, resembling lymph nodes. There is a central area of T cells surrounding by a B cell area. ►Germinal centers are generally not observed in human or rodent omental immune aggregates. These lymphoid areas are punctuated by small numbers of dispersed ►dendritic cells (found predominately within the T-cell zone) and smaller numbers of NKT cells. Most peripheral are the ►macrophages, which form a diffuse ring around the more central lymphoid areas. The mesothelial cells, which comprise the entire omentum forming an almost continuous lining on both sides of the omentum, have been reported to be discontinuous over the immune aggregates. Basement membrane is also lacking in these areas. In addition to the immune cells, there are also limited numbers of reticular cells, which

electron microscopy studies have shown to have long slender cell processes, which are often connected with neighboring cells. Further structure is provided to the immune aggregates by fine fibers comprised of type I collagen, which form a mesh throughout the aggregates. This mesh is confined to or much denser in the areas of the immune aggregates compared to the rest of the omentum.

Vascularization

A striking feature of the immune aggregates is the extensive and well-formed vasculature network that underlies the immune cells. The omentum is served by a central artery and vein that diverge into a mass of smaller vessels and capillaries at each immune aggregate. These vessels encircle each aggregate forming a dense vascular network that commonly extends beyond the immune cells. Segments of these vessels have been shown to stain positively for the ►peripheral lymph node addressin molecule (PNAd), which would facilitate homing of immune cells from the blood stream. Interestingly, vessels in these areas also stain for the marker CD105, a proliferation-associated antigen also known as endoglin, which is localized primarily to endothelial cells undergoing active ►angiogenesis. Furthermore, vascular sprouting has been observed within the vessels populating the immune aggregates. A percentage of specialized (VCAM+) mesothelial cells located above the immune cells of the MS have been shown to constitutively produce ►vascular endothelial growth factor A(VEGF-A), a potent angiogenic factor involved in new vessel formation. These results are all consistent with the vessels within the immune aggregates being in a pro-angiogenic state. This finding may be indicative of the function (see below) of these cellular aggregates in immune protection within the peritoneal cavity, as the aggregates have been shown to increase in number and in size (in terms of cells/aggregate) in response to the introduction of immune stimuli within the peritoneal cavity. The proangiogenic state of the vessels may be in preparation for the rapid influx of immune cells that can occur in response to inflammatory stimuli.

Function

The function of the ommental immune aggregates and the relationship of the cells present there compared to the cells free in the peritoneal fluid has been an object of considerable study and controversy. It is generally believed that these cells serve as sentinels to provide protection against bacteria that enter the peritoneal cavity due to gut perforation or during operations. It is well-documented that particles within the cavity end up ingested by macrophages found in the milky spots. It is less clear whether the particles are ingested by macrophages within the peritoneal fluid, which then traffic to the milky spots or directly ingested by macrophages within the milky spots. Labeled immune cells injected into the peritoneal cavity have been found to attach to milky spots thus indicating that there is a cellular homing mechanism to these sites. The incomplete coverage of mesothelial cells over milky spots implies that foreign particles might have direct access to omental macrophages. Regardless of the mechanism, the omental immune aggregates appear to play an important role in immune defense of the peritoneal cavity as surgical removal of the omentum results in an impaired anti-bacterial response in the peritoneal cavity.

The milky spots also serve as a site for proliferation and maturation of cells by providing a microenvironment with the appropriate growth factors and vascularization needed for these processes. Several studies have indicated that monocytes from the blood stream can serve as progenitors for macrophages in the milky spots and the peritoneal fluid. The immune aggregates are also the site of large numbers of B1 cells, a unique B cell population with a limited repertoire that can respond in a T independent manner to bacteria. These cells produce large amounts of IgM, which have been termed natural antibodies. In the mouse B1 cells comprise approximately half of the B cells present on the omentum. Recent evidence has demonstrated that B1 cells can home from the blood stream to the peritoneal cavity via the blood vessels in the milky spots on the omentum. They appear to be attracted to these areas by the ►chemokine CXCL13, which is thought to be produced by the mesothelial cells of the omentum. Once established, this population is thought to be self-replenishing within the immune aggregates. Thus, these cell populations play an important role in intercepting infectious agents that enter the peritoneal cavity.

Tumor Cell Adherence and Metastases

Another characteristic of the immune aggregates that is well-documented is the preferential adherence of metastatic tumor cells to these sites. This has been observed in a variety of experimental models in which intraperitoneal injection of tumor cells results in localization to the milky spots as soon as one hour after injection. The omentum is also a primary site of metastatic tumor formation in humans with primary intra-abdominal tumors such as ovarian, gastrointestinal and pancreatic carcinomas. These appear to form from cells that directly seed from the primary tumor spontaneously or are released during surgery, and are perhaps carried by the natural flow of peritoneal fluid. The experimental systems have uniformly indicated that the omentum is the primary site of tumor cell

attachment, although cells subsequently seed to other structures including the mesentery, the peritoneal wall and the diaphragm. The mechanism of this preferential attachment is completely unclear. Early reports based on electron microscopy have indicated that the mesothelial layer overlying the immune cells is incomplete allowing access to the immune cells below. However, very recent studies with confocal microscopy indicate that the tumor cells initially attach to the mesothelial cells. It has been suggested that this attachment is via specific cell adhesion molecules such as integrins, but the mechanism remains unsubstantiated. This is an important question because prevention of attachment might have a substantial effect on slowing or eliminating metastases formation. In addition to the preferential attachment, the immune aggregates appear to be sites particularly well-suited for tumor growth due to the well-formed vasculature present in these locations. Furthermore, the vessels in these areas appear to be in a pro-angiogenic state, presumably to provide the needed oxygen and nourishment when the numbers of immune cells increase during infections. However, this also provides the ideal environment for rapid tumor growth. The immune cells appear to have little if any effect on the growing mass of tumor cells, presumably due to the lack of specifically reactive cells. As the metastases increase in size the structure of the immune aggregates are disrupted. Because the omentum is such a favorable site for metastases, it is often removed during surgery to resect the primary tumor. However, the propensity of cells to attach to the immune aggregates suggests that adoptive immunotherapy might be targeted to these sites of ►metastasis formation.

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Omentum

Definition

From Latin, meaning apron; A sheet of tissue composed of a double layer of mesothelial cells encompassing a layer of fat that is well-vascularized and contains aggregates of immune cells. Two are present in the human abdomen. The greater omentum hangs down from the stomach, and extends to the transverse colon. The lesser omentum extends from the liver to the lesser curvature of the stomach and the first part of the duodenum.

►Omental Immune Aggregates
►Transcoelomic Metastasis

Omentum-associated Lymphoid Tissue

►Omental Immune Aggregates

Omeprazole

Definition

Is an antisecretory agent, which is a proton pump inhibitor that is commonly used for suppression of gastric acid output in patients with ►Zollinger-Ellison syndrome; ►gastrinoma.

►Gastrinoma

Oncoantigen

Definition

Tumor antigen expressed on the membrane of tumor cells and playing a causal role in neoplastic transformation. Membrane expression allows recognition by antibodies

even if tumor cells evade T cell recognition through downmodulation of antigen processing machinery and of major histocompatibility complex molecules. Involvement of the antigen in neoplastic transformation precludes the emergence of antigen-negative tumor variants because the proliferation and tumorigenicity of such variants would be abolished or greatly impaired. The prototypic oncoantigen is ►HER-2/neu.

►Immunoprevention of Cancer
►Persistent Tumor Antigen

Oncocyte

Definition

Epithelial cell characterized by an eosinophilic granular cytoplasm usually packed with mitochondria. Can be present in many organs including kidney and thyroid.

►Hurthle Cell Adenoma and Carcinoma

Oncodevelopmental Proteins

►Alpha-Fetoprotein – Historical

Oncofetal Antigen

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Definition

Oncofetal genes are those that are expressed in developing (fetal) as well as cancer (onco) cells. This expression can reflect shared functions important during development and which are re-activated, during neoplasia. Sometimes the latter can involve functional changes which may be delivered through structural modifications (e.g. amino acid sequence change) or by the levels or context of the expression (altered or different gene promoters of transcription). The term antigen relates to the property of the gene product and/or its added post translational modifications (e.g. ►glycosylation) which can be recognized by the immune system, (e.g. antibodies). Most self molecules are non-immunogenic and not able to induce antibodies in the same organism which are said to be tolerant. Often oncofetal antigens are immunogenic since they are not expressed widely in the adult animals and the immune system is not tolerant and they can elicit self immunity under some circumstances.

Characteristics

Cancers are the mostly the result of the accumulation of successive alterations in gene and cellular function which influence the tumors cells with respect to their replicative potential, self sufficiency in growth signals, evasion of negative growth factors, avoidance of cell death (►apoptosis), acquisition of the ability to spread (►metastasis) and provision of a suitable blood supply (►angiogenesis). The latter properties are all obligatorily shared by embryonic cells and so it is not surprising that during ►carcinogenesis, such embryonic pathways are reactivated to hijack these hall marks of cancer. In doing so the tumor cells express oncofetal antigens which are potential targets for the mature immune system which of course was not present during the time when their expression related to development. Although the overall similarity is striking between cancer and embryonic development, ultimately a cancer is the product of the deranged control of the multiple targets not all of which involve embryonic pathways.

The idea that the human immune system is capable of eliminating cancer was suggested as early as the late 19th century. Paul Ehrlich speculated that an anticancer vaccine or Zauberkugel (magic bullet) in which serum-derived Antikorper (antibodies) would be capable of eliminating cancer cells. We now know that any useful antitumor immunity is likely to involve both the humoral (antibody-mediated) and the cellular mediated arms of the immune response. Oncofetal antigens can be recognized in patients by specific antibodies and T cells. Such natural immunosurveillance may indeed control many nascent tumors but in many advanced cancers multiple tumor immune escape mechanisms including an impaired cellular mediated immunity can lead to progressive disease. Nevertheless the potential for the immune system to be able to recognize oncofetal antigens has driven research seeking to boost such immunity for therapy. Alternatively the ability to recognize the oncofetal antigen in cancer patients compared to normal subjects and been exploited for disease diagnosis and monitoring. Three examples of

oncofetal antigens with some research developments are given.

Alpha-Fetoprotein: Role as an Oncofetal Antigen

►Alpha-fetoprotein (AFP) was first described in the 1960s as a marker for ►hepatocarcinoma. Normally expressed during fetal development, high levels of transcript are present within the hepatocytes and the endodermal cells of the yolk sac with the level of expression being repressed after birth. Measured by immunoassay, elevation of AFP in the serum has been observed concurrent with aberrant growth manifestations such as ►hepatocellular carcinomas and embryonic carcinomas. Although AFP may not be the direct cause of the altered growth, it is conceivable that some shock/stress-induced conformational variant form of this fetal protein may influence the progression of this growth as the 70 kDa glycoprotein is known to bind and transport a number of ligands such as bilirubin, fatty acids, steroids and various drugs. This wide spectrum of interactions has however, made the measurement of AFP, and its ligands, a useful ►biomarker for the differential diagnosis of cancer.

The targeting of the receptor for AFP has therefore been suggested as a potential therapeutic modal for the treatment of cancer. This is based on the observation of the overexpression of AFP receptors on the surface of malignant cells versus the negligible expression on normal cells. Targeting of these receptors would therefore allow the specific delivery of anticancer drug conjugates to cancer cells *in vivo*. With many tumors displaying multi-drug resistance, possibly through the overexpression of membrane receptors/and/or transporters, the cancer cells evade conventional chemotherapeutic approaches. Successful conjugation of AFP to different compounds such as doxorubicin, ►cisplatin or methotrexate has been shown to allow the selective entry into these tumor cells via the AFP receptor mediated endocytic pathway. These tumor cells subsequently have raised drug sensitivity and a greater tumor ablation using these AFP conjugates compared to the unconjugated chemotherapeutic has been demonstrated *in vivo*.

Targeting the immune system against the AFP expressing cells could provide an alternate therapeutic approach. Breaking the natural immune-tolerance towards AFP is possible and DNA-based immunization has been shown to induce ►cytotoxic Tlymphocytes and ►CD4-T-cell mediated growth inhibition of AFP-expressing subcutaneous tumors in mice. Their potential in achieving an effective tumor regression is, however, to date, limited. In another approach patient-derived dendritic cells have been infected with recombinant adenoviruses expressing AFP. Co-culturing of these cells with autologous cytotoxic lymphocytes induced an AFP-specific strong immune response against hepatocarcinoma cells *in vitro* but this is yet to be tested in animal models of cancer. It is likely therefore, that a combination of different treatment modalities such as selective targeting and immunotherapy will need to be utilized for the treatment of AFP positive tumors in patients.

Carcinoembryonic Antigen: Role as an Oncofetal Antigen

►Carcinoembryonic antigen (CEA) was first described nearly 40 years ago as an oncofetal antigen and remains one of the most widely studied human tumor-associated antigens. First characterized in ►gastrointestinal tumors its expression within tumors and levels of circulating CEA is recognized as a valuable adjunct to post-operative surveillance and preoperative prognosis of patients diagnosed with colorectal and other types of carcinoma. The antigen has a molecular weight of between 180–200 kDa and is part of a sub-group of the immunoglobulin super family. It is the structural similarity of CEA with immunoglobulins which mediates a functional role for CEA both as an ►adhesion molecule and as a regulatory protein controlling the movement across the gut of fimbriated bacteria. The regular shedding of CEA thereby influences lymphocyte homing and the translocation of microorganisms.

The anchoring of CEA to the plasma membrane and the overexpression of CEA in a wide range of epithelial cancers has made it a popular target for novel cancer therapies, including cancer vaccines such as those against AFP. In these studies a variety of viral vaccine vectors containing the CEA gene alone or in combination with other molecules that enhance the immune response have been evaluated in clinical trials. These trials are aimed at testing whether generating CEA specific killer T-cells is related to clinical responses and increased survival rates Alternate strategies center around antibody based therapies. One example is ►radioimmunotherapy where antibodies against CEA are labeled with radioisotopes such as yttrium 90 or iodine 131. The antibody when delivered into the circulation binds to the bound CEA on the cell surface of the cancer cells delivering the therapeutic radioisotope to the site of the cancer. Another strategy has been the generation of genetically engineered T-cells where a ►chimeric T cell receptors has been inserted that are composed of a single chain antibody against CEA fused to the activating domain of the T-cell receptor. T-cells naturally recognize processed antigens only when they are presented on ►major histocompatibilty complex (MHC) class I or II molecules This antigen presentation pathway is complex and there are often defects in cancer cells that can result in failure of T-cell recognition, leading to tumor evasion. By engineering T-cells with chimeric receptors, activation occurs on binding to the CEA target on the tumor bypassing the need for MHC presentation. This therapeutic

strategy has proven successful in preclinical animal models and is currently being assessed in Phase I clinical trials.

5T4 Oncofetal Antigen

5T4 oncofetal antigen was discovered in 1988 by looking for molecules that might share functional attributes common to both invasive trophoblast and carcinomas. The name was derived from the monoclonal antibody "5T4" raised against solubilized human syncytiotrophoblast plasma membrane glycoproteins. This antibody was selected by screening for low reactivity with normal tissues but high reactivity with different tumor cell types. Isolation of the human gene established it as a unique member of the leucine rich repeat family which are often associated with molecules that function in protein-protein interactions. The subsequent isolation of the murine gene and mouse specific monoclonal antibodies has allowed the determination of 5T4 expression throughout mouse development. As in humans, there is little expression of the antigen in adult tissues such as the liver, lung, bronchus, heart, testis, ovary, brain, or muscle whilst several specialized epithelia retain low level expression into adulthood; there is expression by all types of trophoblast in the placenta. Embryonic expression of mouse 5T4 antigen is first detected on trophectoderm at implantation and is restricted to extra-embryonic tissues until about day 11. After this the 5T4 expression profile is widely associated with actively cycling, undifferentiated epithelial progenitor cells which may contribute to their migration. In the brain, expression is observed in roof plate, ependymal layers, choroid plexus and subventricular zones of lateral ventricles until about day 14 but this then decreases in the subventricular zone with further restriction to the choroid plexus in adult brain.

5T4 oncofetal molecules function in the process of ►epithelial to mesenchymal transition which facilitates movement of cells in development and metastasis in malignancy. This is consistent with the association of 5T4 tumor expression with poorer clinical outcome in gastric or colorectal or ovarian cancer since this mostly the result of spread (metastasis).

The restricted adult tissue but high frequency and cell surface expression by multiple carcinoma types makes 5T4 antigen an attractive target for both antibody and cell mediated immunotherapies. One approach now in clinical trial takes advantage of the potential of superantigens to activate T-cells by redirecting the specificity to the tumor by using antibodies to 5T4 antigen. A 5T4 specific antibody-superantigen fusion protein interacts with 5T4 positive tumor cells and nearby T cells, activating them to destroy the cancer directly or indirectly through the release of cytokines like gamma interferon. The treatment can safely used and does stimulate the desired immunity in patients and a recent trial has documented very encouraging clinical responses in patients with renal cell carcinoma.

Alternatively the human 5T4 gene has been engineered into a modified version of the vaccinia virus (smallpox vaccine) and this has then been used as a cancer vaccine in patients. This virus is unable to replicate or spread but infected cells produce the 5T4 molecules and this can generate both antibodies and killer T cells which can then target the tumors. Several clinical trials of this vaccine called Trovax® have been performed in patients with colorectal cancer and these showed the treatment to be safe and capable of induced both antibodies and cellular immunity.

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Oncofetal Antigens

►Alpha-Fetoprotein – Historical

Oncogene

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Synonyms

Transforming gene

Definition

An oncogene is any gene that has the ability to stimulate cellular growth. Oncogene products can transform eukaryotic cells such that they grow in a way analogous to tumor cells. The definition was originally applied to the transforming genes acquired by RNA tumorviruses

through ►transduction of oncogenes; today the term is used rather broadly.

Characteristics

Oncogenes were originally isolated from ►RNA tumor viruses, where they are responsible for the ability of rapid tumor induction after infection of an animal host. In the viral genome the oncogene was referred to as a viral oncogene or v-onc.

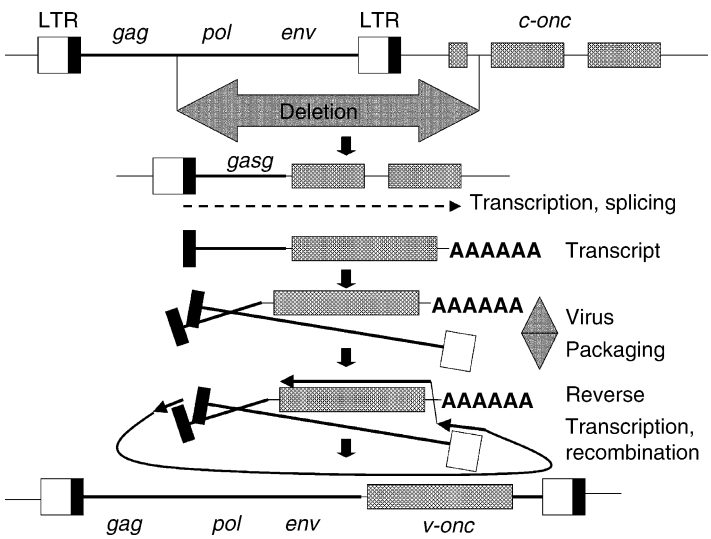
It was soon established that the v-oncs are actually derived from the genome of the host cell. They are captured by the virus after infection of the cell by a process called transduction (Fig. 1; transduction of oncogenes). Transduction appears in a wide range of animal species from chickens to monkeys (Table 1), it has not been observed in humans. The cellular counterparts, from which the v-oncs are derived, are referred to as proto-oncogene, or c-onc. Proto-oncogenes are normal constituents of the cellular genome and are highly conserved among all eukaryotic organisms.

This original rigid definition has softened in subsequent years. Broadly speaking, the term oncogene now includes any gene that has a growth stimulatory effect on cells, with the result of:

- Conferring sustained cellular multiplication
- Advancement of ►cell cycle progression
- Decreased requirement for ►growth factors
- Focus formation under conditions of cell culture
- Ability of cells to grow under more restricted experimental conditions, such as in soft agar
- Tumorigenic conversion, such as in experimental animals
- Conversion of cells to form tumors that undergo ►metastasis
- Escape from ►apoptosis

The precursors of oncogenes (proto-oncogenes) are present in their normal structure and expression activity in all eukaryotic cells, they represent potential "enemies within," but perform normal, usually vital, functions. Their oncogenic potential can be activated by any one of the following mechanisms (Fig. 2)

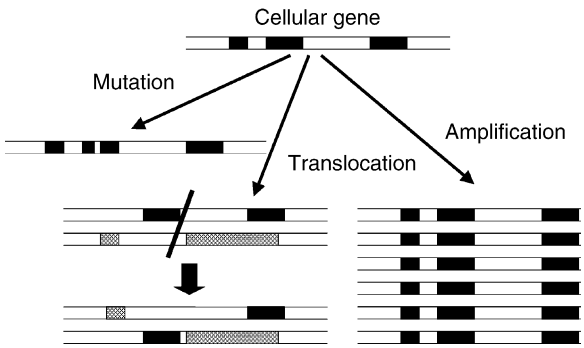
- Expression deregulation, by which the normal expression pattern is altered by a variety of mechanisms
- Mutation within the gene, which results in an abnormal protein that has a different biological activity (e.g. ►RAS)
- Translocation, by which DNA of two genes on two different chromosomes can recombine with the result of a fusion gene and a fusion protein (e.g. BCR/ABL [►BCR-ABL1]) or a deregulated gene expression, when gene expression signals are replaced by other DNA sequences



Oncogene. Figure 1 Model for the transduction by retroviruses. The process begins when the provirus of a retrovirus integrates into the vicinity of a cellular oncogene. The characteristic "long terminal repeats" (LTR) are drawn as black and white boxes. A deletion enables the c-onc and the provirus to fuse into a single genetic unit, one of the proviral LTR plus regions of both the provirus and the c-onc, are deleted. Transcription from the hybrid unit generates a hybrid RNA, introns have been removed by splicing. This RNA can be packaged, together with a wild type retroviral RNA, to form a virion (retroviruses have two RNA molecules). Infection of a cell initiates reverse transcription of viral RNA. Beginning at the 5′-end, the reverse transcriptase can jump to the 3′-end to continue chain propagation along the retroviral genome. Reverse transcriptase can also jump to the hybrid RNA and generate a new retrovirus genome, containing the c-onc.

Oncogene. Table 1 Oncogene. Retroviral genes in different animal species and oncogenes

Virus	Name	Species	Tumor	Oncogene
Rous sarcoma	RSV	Chicken	Sarcoma	*src*
Harvey murine sarcoma	Ha-MuSV	Rat	Sarcoma	*H-ras*
Kirsten murine sarcoma	Ki-MuSV	Rat	Erythroleukemia	*K-ras*
Moloney murine sarcoma	Mo-MuSV	mouse	Sarcoma	*mos*
FBJ murine osteosarcoma	FBJ-MuSV	Mouse	Chondrosarcoma	*fos*
Simian sarcoma	SSV	Monkey	Sarcoma	*sis*
Feline sarcoma	Pi-FeSV	Cat	Sarcoma	*sis*
Feline sarcoma	SM-FeSV	Cat	Fibrosarcoma	*fms*
Feline sarcoma	ST-FeSV	Cat	Fibrosarcoma	*fes*
Avian sarcoma	ASV-17	Chicken	Fibrosarcoma	*jun*
Fujinami sarcoma	FuSV	Chicken	Sarcoma	*fps*
Avian myelocytomatosis	MC29	Chicken	Carcinoma, sarcoma and myelocytoma	*myc*
Abelson leukemia	MuLV	Mouse	B cell lymphoma	*abl*
Reticuloendotheliosis	REV-T	Turkey	Lymphatic leukemia	rel
Avian erythroblastosis	AEV	Chicken	Erythroleukemia and fibrosarcoma	*erbB* (*erbA*?)
Avian myeloblastosis	AMV	Chicken	Myeloblastic leukemia	*myb*



Oncogene. Figure 2 Molecular pathways for activating the oncogenic potential of cellular oncogenes. Translocation defines exchange of genetic material between two non-homologous chromosomes.

- ►Amplification, where the number of gene copies multiplies, with the consequence of enhanced gene expression (e.g. ►MYCN)

Oncogene Cooperation

Experimental approaches have shown that alteration of a single oncogene is insufficient to achieve full tumorigenic conversion of a normal cell. Only when at least two altered oncogenes are introduced can a normal cell assume a tumorigenic phenotype. Oncogene cooperation is well in line with the multiple genetic changes that a tumor acquires during its evolution to metastatic disease (►Multistep development). Oncogenes can also cooperate with ►tumor suppressor genes.

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Oncogene Addiction

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Synonyms

Pathway addiction

Definition

►Oncogene addiction describes the phenomenon in which some cancers that contain multiple genetic, epigenetic, and chromosomal abnormalities remain dependent on (addicted to) one or a few genes for both maintenance of the malignant phenotype and cell survival.

Characteristics

Multistage Carcinogenesis and Oncogene Addiction

There has been considerable progress in the systemic treatment of cancer due to the rapid development and

clinical application of molecular targeted agents (►Molecular Therapy). Although patients with a particular type and stage of cancer are often treated as a single group, more specific therapy is being considered, as subsets of patients who are more likely to benefit from treatment with particular agents are being identified. It is now an axiom in oncology that human cancers often evolve through a multistage process that extends over a period of decades. The marked increase in molecular biology studies has revealed that this process is driven by the progressive accumulation of mutations, and epigenetic abnormalities in expression (►Epigenetics), of multiple genes that have highly diverse biochemical functions. Malignant carcinomas of the lung (►Lung Cancer), colon, breast, and other organ sites often display mutations in multiple oncogenes (►Oncogene) and ►tumor suppressor genes, harbor epigenetic abnormalities that result in increased or decreased expression of hundreds of genes, and contain chromosomal abnormalities that include ►aneuploidy and ►loss of heterozygosity at numerous loci. Therefore, it is surprising that despite this extensive disruption in the genomes of cancer cells there are multiple examples in both experimental systems and in patients with cancer whereby the reversal of only one or a few of these abnormalities can profoundly inhibit the growth of cancer cells and in some cases lead to improvements in patient survival (Table 1). We previously introduced the concept of "oncogene addiction," to emphasize the apparent dependency of some cancers on one or a few genes for maintenance of the malignant phenotype.

Evidence for Oncogene Addiction

Evidence that supports the concept of oncogene addiction has been obtained in three diverse systems: genetically engineered mouse models of human cancer, mechanistic studies in human cancer cell lines, and ►clinical trials involving specific molecular targeted agents (Table 1). Several investigators have generated transgenic mice that overexpress an oncogene in a specific target tissue under conditions in which the oncogene can be switched on or off. Switching on the ►c-*myc* oncogene in the hematopoietic cells of mice led to the development of T-cell and myeloid leukemias (►Acute Myeloid Leukemia); however, when this gene was subsequently switched off the leukemia cells stopped dividing and displayed differentiation and ►apoptosis. Dependence on continued expression of a single oncogene for maintenance of the neoplastic state has also been seen in similar murine models of other tissues, including: myelocytic leukemia induced by the ►*Bcr-Abl* oncogene; ►melanoma induced by the *H-ras* oncogene (►RAS); lung tumors induced by the *K-ras* oncogene; pancreatic β-cell tumors and osteogenic sarcoma (►Osteosarcoma) induced by the *c-myc* oncogene; and breast (mammary) tumors induced by the ►Her-2/neu, *c-myc*, and Wnt (►Wnt Signaling) oncogenes. In the *c-myc* ►breast cancer model when the *c-myc* oncogene was switched off although 50% of the breast tumors regressed, the remaining 50% showed only partial regression. Furthermore, breast tumors that recurred were found to be *c-myc* independent and some of these displayed an activated *K-ras* oncogene. Similarly, in the Her-2/neu breast tumor model tumors that recurred were found to be Her-2/neu independent, and this was recently found to be due to increased expression of the transcription factor *Snail* (►Snail Transcriptional Factor). In the Wnt-1 murine model even though downregulation of Wnt-1 resulted in rapid and extensive regression of aneuploid and invasive breast tumors and pulmonary metastases, a number of breast tumors recurred that were Wnt-independent. Apparently this was due to acquisition of mutations in the ►*p53* tumor suppressor gene. The relevance of these examples of "escape from oncogene addiction" will be discussed later with respect to human cancers and combination therapy.

A variety of studies using human cancer cell lines also indicate that although these cells are aneuploid and carry numerous genetic and epigenetic abnormalities, they can also be highly dependent on the activity of a single oncogene for maintaining the malignant phenotype. Blocking expression of the oncogenes *Her-2*, *cyclin D1* [►Cyclin D], *K-ras*, β-catenin, *cyclin E*, β-raf, or microphthalmia transcription factor (*MITF*) using either antisense DNA (►Antisense DNA Therapy) or RNA interference (RNAi) strategies (►RNA Interference) can markedly inhibit the *in vitro* growth of various types of human cancer cells. In some cases blocking oncogene expression also increases the sensitivity of these cells to specific chemotherapy agents and inhibits their tumorigenicity in mice. Because of the efficacy of the RNAi method for inhibiting the expression of specific genes the list of such examples of oncogene addiction is now rapidly expanding.

The most convincing and clinically relevant evidence for the concept of oncogene addiction comes from the increasing number of examples (i.e. prospective randomized trials) of the therapeutic efficacy of antibodies or drugs that target specific oncogenes in human cancers. One of the earliest examples is the use of the antibody ►trastuzumab (►Herceptin), which targets the receptor tyrosine kinase ►Her-2/neu (►Receptor Tyrosine Kinases). This membrane associated receptor is overexpressed in 20–30% of breast cancers, and it is now established that use of trastuzumab in these patients can markedly inhibit tumor growth and prolong patient survival in both the adjuvant (►Adjuvant Therapy) and metastatic settings. We should, however, emphasize that the therapeutic effects of trastuzumab may be

Oncogene Addiction. Table 1 Oncogene addiction: studies in mice, studies in human cancer cell lines, and clinical evidence

Studies in mice[a]		
Targeted oncogene	Cancer cell line	
c-myc	T cell and acute myeloid leukemia	
Bcr-Abl	Leukemia	
H-ras	Melanoma	
K-ras	Lung	
c-myc	Pancreatic βcell	
c-myc	Osteogenic sarcoma	
Her-2/neu	Breast	
c-myc	Breast	
Wnt-1	Breast	
Studies in human cancer cell lines[b]		
Targeted oncogene	Cancer cell line	
Her-2	Breast	
Cyclin D1	Esophagus, colon, pancreatic, squamous, nasopharyngeal	
$K\text{-}ras^{mut}$	Pancreatic	
$K\text{-}rasv^{12}$	Pancreatic	
β-Catenin	Colon	
Cyclin E	Liver	
Mutant β-Raf	Melanoma	
MITF	Melanoma	
Clinical evidence		
Targeted oncogene (s)	Disease	Agent[c]
HER-2	Breast[d, e]	Trastuzumab (combination)
BCR/ABL	Chronic myeloid leukemia[e]	Imatinib (monotherapy)
C-KIT	Gastrointestinal stromal tumor[e]	Imatinib (monotherapy)
EGFR	NSCLC[e]	Gefitinib, erlotinib[d] (monotherapy)
EGFR	Head and neck, colorectal[e]	Cetuximab (combination)
EGFR	Pancreas[e]	Erlotinib (combination)
VEGF	Breast, colorectal[d, e], Kidney	Bevacizumab (combination)
VEGFR, RAF	Kidney[e]	Sorafenib (monotherapy)

[a]Switching off the indicated oncogene led to growth inhibition, differentiation, apoptosis and/or tumor regression.
[b]Treatment of these cell lines with an antisense oligonucleotide or an RNAi directed to the respective oncogene caused growth inhibition, and in some cases decreased tumorigenicity and increased chemosensitivity.
[c]Treatment regimen indicates agent alone (monotherapy) or in combination with cytotoxic agents (combination).
[d]Phase III evidence demonstrates improved disease-free or overall survival rates.
[e]FDA-approved.

mediated at least in part via immune mechanisms. Within the past few years several low molecular weight drugs have been developed that target and inhibit the activity of other specific protein kinases that have key roles in the growth and survival of human leukemia and carcinoma cells. The remarkable therapeutic efficacy of some of these compounds provides direct evidence for the concept of oncogene addiction. Examples include ►imatinib that targets the oncogenic ►BCR/ABL protein in ►chronic myeloid leukemia (CML) and also targets the product of the oncogene *c-kit* in gastrointestinal stromal tumors (GIST) (►Gastrointestinal Tumors), and the EGFR targeted drugs ►gefitinib (►Epidermal Growth Factor Receptor Inhibitors) and ►erlotinib in ►non-small cell lung carcinoma (NSCLC), ►pancreatic cancer, and glioblastoma (►Glioblastoma Multiforme). ►Cetuximab, a monoclonal antibody (►Monoclonal Antibody Therapy) that targets the EGFR, may have significant antitumor activity in head and neck and colorectal carcinomas;

and ►bevacizumab, a monoclonal antibody to ►VEGF, may have significant antitumor activity in carcinomas of the breast, colon and kidney. These clinical studies also provide insights into the phenomenon of oncogene addiction. For example, in a subset of patients with CML who initially responded to imatinib but later suffered a relapse, examination of the leukemic cells showed a *de novo* mutation in the kinase domain of the BCR/ABL protein, which blocked the inhibitory activity of imatinib. Likewise, it was recently found that the tumor from a patient with NSCLC who relapsed 2 years after an initial dramatic response to gefitinib, had acquired a second point mutation in the kinase domain of the EGFR, which blocked the binding of gefitinib. The strong selective pressure for emergence of cells that carry *de novo* mutations in the respective oncogenes indicates the remarkable dependence of these neoplastic cells on these oncogenes, and provides further evidence for the concept of oncogene addiction. At the same time these findings reveal the emergence of resistance mechanisms to molecular targeting agents. Studies in progress indicate that in the case of the *Bcr/Abl* oncogene there are other drugs that can inhibit the kinase activity of the mutant Bcr/Abl protein, and it may be possible to develop similar drugs that act on resistant mutants of the EGFR and resistant forms of other protein kinases. Furthermore, it might be possible to suppress the emergence of these types of resistant cells by combining a specific protein kinase inhibitor with an agent that inhibits cell proliferation via a different mechanism; this would limit the likelihood of emergence of mutant clones. This aspect is further discussed later in the section on combination therapy.

The concept of oncogene addiction is also relevant to both ►tumor suppressor genes and tumor-stromal interactions. We have emphasized the roles of dominant acting oncogenes that enhance cell proliferation and cell survival. The disordered circuitry of cancer cells, however, is also a consequence of inactivation or loss of expression of tumor-suppressor genes, which normally inhibit proliferation or enhance apoptosis. Experimental studies indicate that reintroducing a wild-type tumor-suppressor gene (e.g. those encoding ►p53, ►Rb, or ►APC) into human cancer cells where the respective endogenous gene was inactive usually caused marked inhibition of growth, induction of apoptosis and/or inhibition of tumorigenesis in mice. These results would not be expected if cancer cells evolved simply through the stepwise addition of genetic abnormalities, because in this setting correction of just one mutagenic event should have only a modest inhibitory effect. Thus, some cancer cells seem to be "hypersensitive" to the growth-inhibitory effects of specific tumor suppressor genes. We postulate that, like oncogene addiction, this effect also reflects the bizarre circuitry of cancer cells, and have termed this phenomenon "tumor suppressor gene hypersensitivity". This phenomenon can also be exploited in cancer therapy by ►gene therapy, for example the use of an adenovirus that encodes a normal p53 protein or by targeting a downstream signaling pathway that was activated as a result of loss of activation of a tumor suppressor gene. Alternatively, if a tumor suppressor gene is inactivated by histone deacetylation (►Histone Deacetylases) or DNA ►methylation (which frequently occurs in cancer cells; e.g. ►$p16^{INK4}$ gene), drugs that inhibit histone deacetylase enzymes (e.g. depsipeptide, suberoylanilide hydroxamic acid) or cause demethylation of cellular DNA (e.g. 5-azacytidine, zebularine) may reactivate silenced tumor suppressor genes and thereby inhibit tumor growth. Synergistic or additive effects on tumor growth inhibition might be obtained by combining drugs that exploit both oncogene addiction and tumor suppressor gene hypersensitivity.

Activated oncogenes like *EGFR* or *ras* stimulate signaling pathways that can also affect tumor-stromal interactions by increasing expression of matrix metalloproteinases (enhancing tumor ►invasion and ►metastasis) and of the angiogenic factor VEGF. Inactivation of these genes in cancer cells can inhibit tumor invasion, ►angiogenesis, and even recurrence. The neovasculature associated with tumor-induced angiogenesis is abnormal compared with normal vasculature, and the circuitry that regulates the growth and function of endothelial cells in this neovasculature may differ from that of normal vasculature. Bevacizumab provides a promising example of preferentially targeting abnormal tumor vasculature, and its use has led to improved disease-free and overall survival rates in patients with ►colon cancer.

Mechanisms of Oncogene Addiction

The phenomenon of oncogene addiction was a consequence of the fact that the ►multistep development of ►carcinogenesis may not simply a summation of the individual effects of activation of multiple oncogenes and inactivation of multiple tumor suppressor genes. This is consistent with the fact that the proteins encoded by these genes often have multiple roles in complex and interacting networks, which display both positive and negative feedback control. The functions of these proteins are also influenced by their levels of activity and the context in which they are expressed. Thus, a given oncogene can enhance cell proliferation but it can also enhance apoptosis. Furthermore, throughout the multistage carcinogenic process, the evolving cancer cell must maintain a state of homeostasis between positive- and negative-acting factors in order to maintain structural integrity, viability, and the capacity to replicate. For these reasons, the intracellular circuitry

or "wiring diagram" that regulates ►signal transduction and gene expression in cancer cells is very different, i.e. "bizarre," when compared to that of normal cells. Therefore, in cancer cells a given oncogene may play a more essential and qualitatively different role in a given pathway or "module" compared with its role in normal cells. Thus, cancer cells may be much more dependent on the activity of a specific oncogene than normal cells.

Within the context of disordered cell circuitry, specific mechanisms have been proposed to explain why inactivation of an oncogene might lead to selective growth inhibition, differentiation and/or apoptosis in cancer cells but not in normal cells that express the same oncogene. One explanation is that in order to maintain homeostasis, the proliferation enhancing effects of a specific ►oncogene in cancer cells may be partially buffered through negative feedback mechanisms, through increased expression of proliferation inhibitory factors. If this oncogene is then inactivated the cancer cells might suffer a relative excess of the latter inhibitory factors and thus undergo apoptosis, before a new level of homeostasis can be achieved. The apparent propensity of some cancer cells to undergo apoptosis when stressed could enhance this process.

A second mechanism is based on the concept of "synthetic lethality" originally derived from studies in lower organisms. According to this concept two genes are said to be synthetic lethal if mutation of one of the two genes is compatible with survival but mutation of both genes causes cell death. For example, certain cancer cells might be highly dependent on a given oncogene because during their development they lost the function of another gene that normally performs a similar function. Therefore, a drug that inhibits the activity of the oncogene would selectively target the cancer cells and spare the normal cells. Furthermore, because of the bizarre circuitry of cancer cells, pairs of genes in cancer cells that have a synthetic lethal relationship may differ from those in normal cells, thus, increasing the dependence of tumor cells on a specific oncogene. A related explanation for oncogene addiction is that during the multistage carcinogenesis process, cancer cells become highly dependent on specific oncogenes and their related pathways because of the large numbers of mutated and inactivated genes that function in other pathways. This could render cancer cells less adaptable than normal cells.

Only a subset of patients with NSCLC (about 10–20%) display favorable and often impressive clinical responses to the EGFR inhibitor gefitinib, and this is often associated with tumors that have specific activating mutations in the kinase domain of EGFR. For reasons that are not understood patients with these activating mutations are also more likely to have adenocarcinomas, be female, non-cigarette smokers, and of Japanese origin. Thus, addiction to a specific oncogene might occur only in a subset of specific types of cancers with a distinct etiology, and only when that oncogene is mutated and not simply activated. Normal EGFR activation results in induction of multiple downstream signaling pathways some of which enhance cell proliferation and others that enhance cell survival (i.e. inhibit apoptosis). An experimental study indicated that mutations in the *EGFR* can preferentially enhance activation of the survival, Akt-associated pathway (►AKT Signal Transduction Pathway). This may explain why NSCLC cells that harbor this mutation in EGFR are highly dependent on this activated oncogene for survival. Similarly, the presence of specific deletion mutations in the EGFR gene in glioblastoma was recently shown to correlate with clinical responses to an EGFR inhibitor. These data fit the paradigm that oncogene addiction can be due to the establishment of distorted pathways of signal transduction (i.e. bizarre circuitry) during tumor development. Recent studies suggest that in addition to point mutations in the gene encoding the EGFR other factors may influence the sensitivity of NSCLC tumors to EGFR inhibitors, including *EGFR* gene ►amplification, the activation state of the EGFR protein, specific downstream signaling pathways, and pharmacologic factors.

Future Directions and Clinical Applications

Identification of the Critical Oncogene or "Achilles' Heel" in Specific Human Cancers

Human cancers display multiple genetic and epigenetic abnormalities. Furthermore, it is now apparent that these abnormalities frequently differ between different types of cancer, and also between subsets of the same type of cancer. In view of this complexity how can we identify the specific oncogene or oncogenes that have a critical role in maintaining the malignant phenotype in these different cancer types, or within individual cases? In other words how do we identify the "Achilles' heel" in specific cancers so that each patient can be treated with the appropriate molecular targeted agent? At the present time there are no methods to fully assess the total circuitry that controls cell proliferation, differentiation and apoptosis in normal or cancer cells. Advances in network theory, systems biology, and computer modeling, may eventually make this possible.

Currently, several empiric approaches can be used to help identify the Achilles' heel of specific types of human cancer. One is to use high throughput screening of thousands of compounds in chemical libraries to identify specific compounds that preferentially inhibit *in vitro* growth or induce apoptosis in specific types of human cancer cells. A related approach that is being rapidly expanded is to use a library of ►siRNAs, which are low-molecular weight RNAs that are taken up by

cells and inhibit the expression of specific genes, to identify which genes are required to maintain the proliferation and/or survival of specific types of cancer cells. Once such genes are identified drugs can be designed to target the related protein(s). A recent study in mice suggests that it might become feasible to administer to patients a specific siRNA preparation that knocks down the expression of a critical oncogene in the tumor, thus providing a novel approach for delivering cancer therapy.

In addition, specific criteria might be used to assist in identifying genes that are most likely to be critical for maintaining the malignant phenotype. For example, oncogenes that are mutated early in the multistage process of tumor development might be favored candidates because they played a critical role in determining subsequent aspects of the abnormal circuitry in the evolving cancer cells. Oncogenes that are mutated and not simply overexpressed might also be more likely targets for therapy since they reflect the "hard-wiring" of cancer cells, rather then epigenetic abnormalities. Mutated oncogenes may therefore be more likely to be present in the stem-cell population of tumors rather than just in the progeny cells. In addition, mutated oncogenes might be more likely to have qualitatively different roles than oncogenes that are only overexpressed. This aspect is exemplified by the properties of a mutated EGFR receptor in NSCLC cells. Specific cancers display increased expression of genes that normally have critical roles in ►stem cells, or during normal tissue development and differentiation. These include the genes that encode proteins involved in the Wnt, Hedgehog (Hh) (►Hedgehog Signaling), TGFβ/BMP, Notch, Snail, Slug, and MITF signaling pathways, and inhibition of the Hh and MITF pathways have led to growth inhibition in murine ►medulloblastoma and human ►melanoma models, respectively. It is possible that dependence of a cell on a specific oncogene may be different in the stem cells than in the progeny cells in a given tumor, because of differences in their intracellular circuitry. Optimal therapy would then require developing molecular targeted agents that target only the critical oncogene in the stem cells of a specific cancer. Further characterization of stem cells in specific tumors should clarify this aspect of oncogene addiction, and the potential limitations of specific molecular targeted agents.

Advances in microarray (►Microarray (cDNA) Technology) and proteomic (►Proteomics) methods have compared expression profiles of thousands of genes and proteins between normal tissues, cancers, and subtypes of specific cancers. "Network theory" and the emerging field of systems biology may eventually provide methods for conceptualizing and analyzing the entire circuitry of specific types of normal and cancer cells, and thus facilitate identification of specific pathways of oncogene addiction. Networks in mammalian cells share similar principles with networks that are relevant to engineering and other disciplines. Thus, by viewing the cancer cell as a "complex" or "complicated" system, genes and proteins as "nodes", and groups of interacting proteins in a signal transduction pathway as "modules", engineering tools and concepts can be used to analyze large datasets to generate an holistic view of the regulatory network of normal and cancer cells. Furthermore, a concept called Boolean genetic network theory can be used to study the cancer cell as a dynamic system. By analyzing the myriad of individual and interacting genes in a cancer cell in a Boolean fashion (i.e. active = "on", inactive = "off"), the overall activity state ("attractor state") of specific cancer cells (e.g. proliferation, apoptosis, differentiation) might be determined or predicted. Thus, in the future it could be possible to use network theory to describe and predict the existence of an "addicted" attractor state, and to also predict the specific oncogene responsible for this addicted state. A more detailed understanding of these networks might also provide insights into tumor resistance and the appropriate choice of combination therapy for specific patients.

Combination Therapy

Although the concept of oncogene addiction may apply to a given cancer at a particular time or stage, it is apparent from some of the mouse model experiments and from clinical experience with molecular targeted agents, that cancers can "escape" from a given state of oncogene addiction. Presumably, this reflects the genomic instability of cancers. It could also reflect epigenetic changes in gene expression that lead to an altered state of cell circuitry; for example, ongoing changes in DNA methylation and chromatin structure in a cancer cell population. It is not known whether this escape leads to secondary addiction to another oncogene or to the growth of a population of "non-addicted" cancer cells. For these reasons, as well as the likelihood of heterogeneity within tumors, it is unlikely that the use of a single molecular targeted agent will achieve long lasting remissions or cures in human cancers, especially for late-stage disease. Combination therapy will, therefore, be required, which raises several unresolved questions. Can such combinations be rationally designed? Should the individual agents act on the same molecular target but by different mechanisms, or on different targets in the same pathway; or should each agent target a different pathway or cellular mechanism? The first approach may prevent emergence of the types of resistance recently seen with imatinib and gefitinib. The second approach also seems rational because it is likely that cancer cells may be addicted to

specific signaling pathways rather than a single oncogene. A drug combination that targets proteins that function at different stages in the same pathway may, therefore, be more likely to inactivate that pathway, than a single drug. The third approach can also be justified because of the disturbance of multiple pathways in cancer cells. It is becoming increasingly apparent that certain molecular targeted agents are actually promiscuous, i.e. they target more than one molecule, and this may enhance their therapeutic efficacy. For example the drug sorafenib appears to have activity against ►renal carcinomas by targeting both a mutant *Raf* oncogene and the VEGF receptor, thus inhibiting both cancer-cell proliferation and angiogenesis. Clinical studies indicate that the efficacy of certain molecular targeted agents can be enhanced by combining them with cytotoxic agents, i.e. agents that often act by inhibiting DNA or chromosomal replication. Trastuzumab that targets HER2 can improve response and survival rates if given in combination with ►paclitaxel to patients with metastatic breast cancer. The combination of bevacizumab or cetuximab with cytotoxic chemotherapy agents can also improve response rates in patients with metastatic breast and colon cancer, respectively (►Chemotherapy of Cancer, Progress and Perspectives). Furthermore, when bevacizumab was added to a combination chemotherapy regimen it improved overall survival rates in patients with metastatic colon cancer. As with chemotherapy, the efficacy of targeted therapy will likely be greater in patients with minimum residual disease. Thus, treatment with trastuzumab after adjuvant chemotherapy significantly improves disease-free survival in patients with early-stage breast cancer.

Conclusion

At the present time the choice of the best molecular targeted agent and the appropriate combination therapy for a specific patient with cancer is largely empirical. Nevertheless, the rapid development of diverse molecular targeted agents coupled with further mechanistic studies, and advances in profiling the molecular circuitry of specific subsets of human cancer, should make it possible to further exploit the concept of oncogene addiction to achieve more effective and selective therapies for several types of human cancer.

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Oncogene-induced Senescence

Definition

Cellular senescence response, featuring irreversible cessation of proliferative potential, senescence-associated acid β-galactosidase (SA-β-GAL) activity, activation of $p16^{INK4A}$ and/or $p14^{ARF}$, resulting from unscheduled mitogenic signaling.

►Melanocytic Tumors
►Senescence and Immortalization

Oncogene Transduction

►Transduction of Oncogenes
►Oncogene

Oncogenic Viruses

Definition

Viruses, also known as oncoviruses, capable of transforming cells into a malignant phenotype. These viruses encode ►oncogenes that can be inserted into the mammalian genome causing transformation. Examples of oncoviruses are ►human papillomavirus (HPV) and ►Epstein–Barr virus (EBV), in animals ►retrovirus.

►Transduction of Oncogenes

Oncologic Surgical Pathology

►Pathology

Oncolytic Adenovirus

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Synonyms

Conditionally replicating adenovirus; Conditionally replicative adenovirus; Replication-competent adenovirus; Replication-selective adenovirus; Replication-activated adenovirus

Definition

Oncolytic ►adenoviruses are recombinant adenoviruses that can replicate preferentially in tumor cells and consequently destroy the tumors. ►Virotherapy is a term primarily used for cancer therapy with oncolytic viruses.

Characteristics

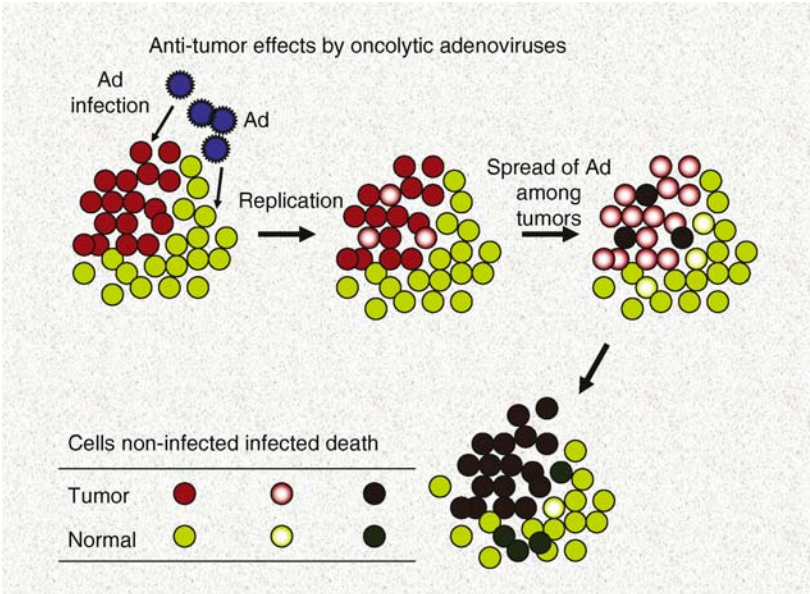
General Description

Adenoviruses, nonenveloped double-stranded DNA viruses with about a 38-kb genome, cause respiratory infection, pharyngitis and keratoconjunctivitis depending on their ►serotypes. Adenoviruses are not oncogenic to human but cytotoxic to human cells. The viral replication induces cell death; consequently, adenoviruses are released from the burst cells and spread to other target cells in the vicinity. Recombinant adenoviruses are widely used as a vehicle to express an exogenous gene in target cells. These adenoviruses used for gene transfer cannot replicate in target cells (replication-defective) because they are devoid of the genes encoded in the E1 region, which are required for viral replication. HEK 293 cells, which are frequently used for production of replication-defective adenoviruses, constitutively express the genes mapped in the E1 region and thereby can support the viral replication. In contrast, oncolytic adenoviruses are replication-competent in target tumors and killed tumor cells but scarcely or to less extent are cytotoxic to nontumorous cells (Fig. 1). Historically speaking, clinicians experienced tumor regression after infection of a certain type of viruses and later viruses with cytotoxic activities were demonstrated to destroy rodent and human tumors. The wild-type viruses used for cancer treatments in fact produced a limited benefit in clinical settings due to antiviral immune responses and the therapy with such viral agents was abandoned. Advent of recombinant DNA technology however enables us to modify viral genomes and to produce viruses as a tool for gene transfer and therapy. Oncolytic adenoviruses are renewed and preclinical and clinical developments have been primarily pioneered by bioventure companies. In 2005, Chinese Food and Drug Administration firstly approved ONYX-015-type oncolytic adenoviruses for cancer treatment.

Mechanism

Understanding of tumor-selective replication of adenoviruses needs to know the functions of the *E1A* and *E1B* genes mapped in the E1 regions. The *E1A* gene is firstly transcribed after the viral infection (immediate early gene) and the product bind to the ►RB1 ►tumor suppressor gene product which binds to transcription factor ►E2F. Binding of E1A to ►pRB releases E2F and facilitates S-phase entry of the infected cells to transactivate both viral and cellular genes that are necessary to produce viral progenies. The *E1B* gene encodes 19 and 55 kDa molecules. The E1B-55kDa molecules bind to the ►p53 ►tumor suppressor gene and accelerate ►p53 degradation; subsequently, the E1B-55kDa inhibits transactivation of the downstream genes and ►apoptosis mediated by p53.

Currently there are two major strategies to produce oncolytic adenoviruses. One is to utilize molecular defects of tumor cells, which render tumors more permissive for the viral replication, and the other is to activate the *E1A* gene with a transcriptional regulatory region that has a tumor-specificity. The typical type to use the molecular lesion is adenoviruses defective of E1B-55kDa molecules, which are also known as *dl*1520, ONYX-015, and Cl-1042. The viruses take advantage of p53 defects in tumors that are frequently found in various types of human cancer. Since adenoviruses induce p53 expression in infected cells, the viruses need to inactivate p53 to avoid p53-mediated cell cycle arrest and apoptosis for continuous viral replication. ONYX-015 fails to degrade p53 because of E1B-55kDa deficit; thereby, the viral replication is not halted. In normal cells, replication of ONYX-015 is immaturely terminated due to p53-mediated apoptosis. The initial idea that ONYX-015 could replicate in p53-mutated or -defective tumor cells but not in cells with wild-type 53 was evidenced experimentally but subsequent studies showed that replication of ONYX-015 was not related with the p53 status of infected cells. The results of clinical trials of ONYX-015 also suggested that the outcomes were not linked with the p53 status of tumors. The discrepancy could be due to the complexity of p53



Oncolytic Adenovirus. Figure 1 Concept of oncolytic adenoviruses. Adenoviruses (Ad) infect tumors as well as normal cells but the viral replication occurs preferentially in tumors. The progenies released from burst cells subsequently infect tumor masses in the vicinity.

regulation. The overall p53 functions are influenced by a number of molecules including p14 and ►Mdm2. The genetic information of the p53 therefore cannot predict whether the p53 pathways are intact or not. Nonetheless, ONYX-015 shows cytotoxic activities to tumors and produces antitumor effects in clinical trials. Precise mechanisms of preferential cytotoxicity of ONYX-015 to tumor cells remain uncharacterized. Recent studies suggest that selective cytotoxicity to tumor cells of ONYX-015 is attributable to defective export of late viral RNAs to cytoplasm in tumors but not in normal tissues. Tumor cells through unidentified mechanism support the viral RNA migration that is a novel function of E1B-55kDa whereas normal cells cannot activate transport of the viral RNAs and consequently cannot induce lytic viral replication.

Another type of oncolytic adenoviruses with transcriptional regulation of the *E1A* gene was developed by incorporating so-called tumor-specific promoters. The expression of E1A and E1B is controlled by the viral E1A promoters located in the upstream of the *E1A* gene and these oncolytic adenoviruses are produced by replacing the viral E1A promoter with an exogenous transcriptional regulatory region of the gene that is highly expressed in tumors but scarcely in nontumorous cells. In the adenoviruses, expression of the *E1A* and *E1B* gene is subjected to the specificity of the putative tumor promoter such as ►carcinoembryonic antigen, ►ERB82 and ►alpha-fetoprotein. It could use a tissue-specific promoter and subsequently replication-competent adenoviruses can destroy the target cells depending on the specificity of the promoter activity. The initial clinical studies were performed for ►prostate cancer using the ►PSA gene promoter to activate both *E1A* and *E1B* genes. Subsequent studies showed that control of the respective *E1A* and *E1B* genes with different promoters bearing similar specificity such as probasin could enhance the cytotoxicity for prostate cancer with tumor-specificity. Further investigations demonstrated that integration of such exogenous promoter regions could sometimes suppress viral replication although the adenoviruses kept the specificity for their cytotoxicity.

The concept of oncolytic adenoviruses is intriguing but several points have to be considered to achieve better clinical outcomes. Tumor masses consist of a number of normal cells such as ►stroma cells and these cells hamper efficient viral spread into tumors in the vicinity. The virotherapy is essentially a local therapy and may not be a systemic therapy although systemic administration is under investigation. Antibody against adenoviruses produced by repeated injections decreases the efficacy of virus infection. ►Humoral immunity could rather increase the safety by inhibiting wide spread of the viruses to whole body. Interestingly, many clinical studies suggested that humoral immune responses did not suppress the infectivity of adenoviruses to target cells when locally administered.

Clinical Applications

Although oncolytic adenoviruses have been yet commercially unavailable except in China, a number of clinical studies have been conducted in particular in combination with conventional ►chemotherapy. ONYX-015 has been examined for the clinical feasibility especially head and neck cancer because it is relatively easy to approach to the tumor sites

and to estimate the tumor growth. Most of the patients could tolerate ONYX-015 despite adverse effects such as flu-like symptoms. These primary data of clinical studies need further investigations and large-scale randomized studies are required to evaluate the clinical efficacy. Recent reports demonstrated that H101, ONYX-015-type adenoviruses approved in China, produced antitumor effects for head and neck cancer in combination of an anticancer agent ►cisplatin. The clinical data obtained in China are similar to those conducted in USA, suggesting potentiality of the viral agent for cancer therapy.

The tumor promoter-mediated oncolytic types have been also clinically tested but most of them did not proceed to further trials. The clinical reports currently obtained showed the evidences of viral replication in human tumor tissues and limited adverse effects.

Future Directions

There are about 50 kinds of serotypes of human adenoviruses discovered and the type 5 is most frequently used as a vector for gene transduction and oncolytic adenoviruses. The type 5 adenoviruses use the coxsackie and adenovirus receptor and ►integrin (αvβ3, αvβ5) as cellular receptors and bind to them with their fiber and penton regions, respectively. In human cancer, however, the expression of their cellular receptors is often down-regulated and consequently the infectivity of type 5 adenoviruses often decreases. In order to circumvent the lower infectivity, several strategies have been examined. One of them is to replace the fiber region with that of other types such as type 3 and type 35, which use different receptor molecules with a higher expression level in tumors. Incorporation of a binding motif such as ►RGD sequences, which bind to integrin, enhanced the infectivity. Antibody-mediated "retargeting" is also examined. Preparation of adenoviruses covered with antibody against cell surface molecules which are expressed highly on tumor cells facilitates the infectivity.

Enhancement of cytotoxic activity by oncolytic adenoviruses is important from a clinical standpoint. Since oncolytic adenoviruses replicate within the infected cells, an exogenous gene incorporated in the viruses is amplified together with the viral replication and thereby the gene product increases. For example, increased expression level of a ►suicide gene enhances the sensitivity of infected cells to a ►prodrug. Enhanced systemic immunity can be achieved by integrating the ►cytokine gene such as interleukin-12, which belongs to T helper type 1 cytokines. These modifications are currently being examined preclinically.

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Oncolytic Therapy with Viruses

►Oncolytic Virotherapy

Oncolytic Virotherapy

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Synonyms

Virus therapy of cancer; Oncolytic therapy with viruses

Definition

Oncolytic virotherapy is the experimental treatment of cancer patients based on the administration of replication-competent viruses that selectively destroy tumor cells but leave healthy tissue unaffected. Oncolytic viruses currently being characterized include vaccine strains of human viruses and apathogenic human and animal viruses. Moreover, viruses are being genetically modified to increase their tumor specificity and oncolytic efficacy.

Characteristics

Viruses as Therapeutics: Clinical Trials of Oncolysis

Although infections can be a serious threat for cancer patients, it was observed at the end of the nineteenth century that some patients experience remissions of their disease after natural infections. Starting in the 1920s, many experiments involving the treatment of tumor-bearing animals with viruses like vaccinia virus, herpes virus, ectromelia virus and fowl plague virus were carried out. Between 1940 and the early 1970s, many types of viruses were inoculated into cancer patients, including rabies vaccine virus, West Nile virus, ►adenoviruses, mumps virus, Sendai virus, ►Newcastle

disease virus, influenza virus, Semliki Forest virus and Sindbis virus. Although some encouraging results were reported, oncolytic virotherapy never became a standard treatment. Reasons for this include lack of predictable efficacy, regrowth of tumors with appearance of neutralizing antibodies against the viruses, limited availability of standardized virus preparations for the administration to patients, and the great enthusiasm that initially accompanied the development of ►radiotherapy and ►chemotherapy.

Since the beginning of the 1990s, there has been a renewed interest in using viruses for cancer treatment. The renewed enthusiasm was based on a better understanding of the mechanisms of virus replication and oncolytic specificity, and on the availability of genetic methods to modify viruses.

Viruses currently tested in clinical trials include the DNA viruses ►herpes simplex virus, ►adenovirus, and vaccinia virus as well as the RNA viruses ►reovirus, ►Newcastle disease virus, ►measles virus and Coxsackievirus.

Herpes simplex virus type 1 (HSV-1) was the first virus to be genetically engineered for selective replication in tumor cells in 1991. Different genetic modifications that selectively disable viral replication in non-dividing cells have been described, among them mutations in the thymidine kinase, the ribonucleotide reductase and the γ34.5 gene. Additionally, up to 30 kbp of foreign DNA can be inserted into the HSV-1 genome. Several antiherpetic drugs are available and can be used to treat possible unwanted side-effects of infection. On the other hand, the majority of the population has neutralizing antibodies against HSV-1, interfering with the delivery of the virus.

Since HSV-1 has a natural ►tropism for neural cells, the initial phase I clinical trials focused on the treatment of patients with malignant glioma. Two vectors, HSV 1716 and G207 have been stereotactically injected into the tumors of glioma patients. Up to 3×10^9 plaque forming units (pfu) were well tolerated without major side effects. Additionally, two small pilot studies with ►melanoma and liver ►metastasis in colorectal carcinoma were performed that also showed no significant toxicity. Further studies focus on the combination of virotherapy with radiation and the insertion of therapeutic transgenes into viral vectors.

Adenoviruses (Ads) have been tested in several phase I and II trials since 1996 (►Virotherapy). The most widely tested virus, ►ONYX-015, has a deletion in the E1B region that confers tumor selectivity. Adenoviruses can easily be genetically manipulated, have the capacity to carry up to 10 kb of foreign genetic material while maintaining replication competence, and can be grown to very high titers (up to 10^{12} pfu/ml). While the first clinical studies involved direct intratumoral injections of Ads in patients with head and neck ►squamous cell carcinoma, Ads were later also applied intraperitoneally, intravenously, and by hepatic artery infusion. Many tumors have been treated, including ►pancreatic cancer, ►glioblastoma, ►colorectal cancer, hepatobiliar cancer, sarcomas and ►prostate cancer. One phase III study executed in China that used a virus slightly different from ONYX-015, H101, showed a 79% response rate for virotherapy in combination with chemotherapy as opposed to 40% for chemotherapy alone. This trial led to the approval of H101 for the treatment of late-stage refractory ►nasopharyngeal cancer by China's regulatory authorities.

Reovirus is a double-stranded human RNA virus that does not appear to cause any serious disease. It can not yet be genetically modified but has a natural tropism for transformed cells. A phase I study investigating intratumoral injections of the type III Dearing strain (Reolysin®) into cutaneous lesions showed some promising responses and no dose-limiting toxicity for up to 10^{10} pfu per injection. Studies involving patients with glioma, prostate cancer and other malignancies are ongoing. The virus will also be applied intravenously, and virotherapy will be combined with radiation.

Newcastle disease virus (NDV) is a negative strand RNA virus of the Paramyxovirus family that naturally infects poultry. It is apathogenic for humans and had been investigated previously for the generation of so called oncolysates, a suspension of tumor cells that are explanted, infected with NDV, inactivated, and given back to the patient to induce an immunological response. Oncolysates do not contain replicating viruses and thus are not discussed in more detail. In another line of research, the replicating NDV strain PV701 was administered to patients with various solid tumors. When very high doses (up to 1.2×10^{11} pfu) were administered, the patients experienced flu-like side effects. Extensive studies showed that these side-effects are well tolerated when the patient is treated with non-steroidal anti-inflammatory drugs and when dosing starts low ("desensitization"). In one study, a patient with previously compromised lung function and pulmonary metastases died from respiratory failure after the treatment with PV701, the only treatment-related death in the field of oncolytic therapy so far. Some patients experienced stabilization of their disease for several months, suggesting that therapy with PV701 might be efficacious for some cancer patients. Phase II studies with the virus are in course.

Infection with another Paramyxovirus, measles virus (MV) was sporadically associated with cancer regression in lymphoma and leukemia patients, and the live attenuated MV vaccine is under development as an oncolytic agent. A small phase I study of intratumoral injections in patients with cutaneous T-cell lymphomas has demonstrated the safety of this approach as well as some clinical responses. Another phase I study involving intraperitoneal injection of a genetically

modified virus that secretes a marker peptide into patients with ►ovarian cancer is under way. Further studies involving a virus expressing the sodium-iodine-symporter as a therapeutic gene in combination with radioactive iodine for the treatment of myeloma patients and a study with glioma patients are planned, and several genetic modifications to improve viral efficacy and safety are currently tested in preclinical studies.

Targeting Viruses to Tumor Cells

The molecular basis for the preferential replication of many viruses in tumor cells is currently active focus of research, and ►targeted viruses are created by genetic modifications. There is increasing evidence that many genetic changes in tumor cells that result in malignant transformation also undermine cellular antiviral defense mechanisms. For example, the susceptibility of cell lines to human reovirus infection correlates with activation of the ►RAS-pathway, and the process of malignant transformation of murine embryonic fibroblasts leads to an impaired cellular response to interferon that results in susceptibility to vesicular stomatitis virus.

Restriction of viral ►tropism to tumor cells can occur, or be engineered, at different stages in viral replication. Efficient targeting of the first infection stage, cell entry, has been achieved for MV. Small ligands, single-chain antibodies or T-cell receptors displayed on the viral attachment protein can efficiently mediate entry through targeted receptors; entry through natural receptors can also be avoided by modifications of the attachment protein. Proteins on tumor cells that have been successfully used as a receptor by retargeted MV include carcinoembryonic antigen, CD20 and CD38. Alternatively, a targeting system based on selective activation of the viral fusion protein by tumor-specific proteases has been described. Genetic modification of the receptor-binding proteins has also been attempted with other viruses such as adeno-associated viruses, adenoviruses and retroviruses, but the efficiency of these targeting systems is not yet optimal.

Selective replication of naturally oncolytic viruses in tumor cells is often based on post-entry events, especially defects in innate defenses of cancer cells. Many viruses encode accessory proteins that are not strictly required for viral replication but counteract innate defenses, and their genes have been deleted to engineer viruses selectively replicating in tumor cells, e.g. E1A and E1B deleted adenoviruses and NS1-deleted influenza viruses. The VSV M protein counteracts cellular innate immunity. This essential gene cannot be deleted, but M protein mutations confer to viruses greater selectivity for tumor cells because the mutant viruses cannot block interferon production in infected cells.

For viruses that rely on cellular polymerases for transcription, promoters driving essential genes can be replaced by promoters that are only active in tumors, e.g. the carcinoembryonic antigen promoter for ►colon cancer, the alpha-fetoprotein promoter for ►liver cancer and the ►mucin-1 promoter for breast cancer. This approach has been widely used with adenoviruses but also with herpesviruses and retroviruses.

"Armed" Oncolytic Viruses

Even with targeted oncolytic viruses, it might not be possible to infect and thereby kill all tumor cells of a patient. Strategies to destroy uninfected transformed cells are therefore required, and attempts have been made to increase efficacy of tumor cell killing by insertion of therapeutic genes into the genome of oncolytic viruses, generating "►armed viruses." Arming can be based on ►suicide genes, which encode proteins that toxify systemically administered prodrugs, resulting in killing of not only the transduced cell but also neighboring cells. Local production of toxic substances in tumors is expected to result in fewer side effects than systemic administration. Similarly, transduction with the sodium-iodine symporter leads to the uptake of radioactive iodine that kills the tumor cell and some surrounding cells.

Another important approach to improve the efficacy of oncolytic virotherapy are strategies that enhance the reactivity of the immune system against cancer cells. In oncolytic virotherapy, the patient's immune is a double-edged sword: on the one hand, it will fight the oncolytic viruses like any other infection, thereby protecting malignant cells. On the other hand, viral replication in cancer cells might cross-prime the immune system and favor elimination of cancer cells. A variety of transgenes improving the activation of the immune system have been used, including interleukin (IL)-2, IL-4, IL-12 and granulocyte-macrophage colony-stimulating factor.

Perspectives

The concept of ►virotherapy has been revived by the availability of reverse genetics systems for many virus types and the better understanding of the molecular mechanisms of carcinogenesis. Many oncolytic viruses are currently developed and tested in clinical trials, and the results of phase I and II studies indicate that this form of treatment can be well tolerated and safe. Although these trials were not designed to demonstrate efficacy, efficacy appears to be low. However, a great variety of new approaches, including genetic modification of viruses to carry transgenes, new methods to monitor viral replication, improved dosing schemes, combinations with other treatment modalities like chemotherapy and radiation, and application of immunosuppressive drugs during virotherapy are currently tested in preclinical models and clinical studies. Genetic modifications and treatment combinations have to be tailored to the different forms of cancer, taking into account the natural tropism of different viruses. The

recent approval of the oncolytic adenovirus H101 for the treatment of late-stage refractory nasopharyngeal cancer in China may be the prelude of a new, much needed treatment modality for cancer.

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Oncolytic Virus

Definition

Refers to genetically modified viruses that replicate selectively or conditionally within tumor cells, leading to their lysis. Some oncolytic viruses are also vectors since they encode one or more non-viral genes of interest.

►Oncolytic Virotherapy
►Viral Vector-mediated Gene Transfer
►Oncolytic Adenovirus
►Oncolytic Therapy with Viruses

Oncolytic Virus

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Synonyms

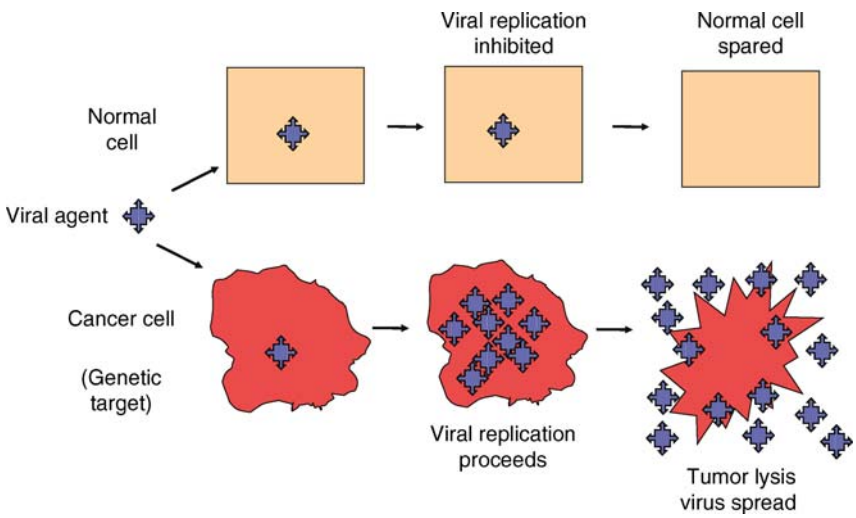
Therapeutic viruses; Virotherapy; Replication-selective viruses; Targeted viruses

Definition

Oncolytic viruses are viruses that replicate (multiply themselves) selectively in cancer cells, and are attenuated (crippled) in normal cells, and as a result destroy cancer cells ("onco" cancer, "lysis" cell destruction through necrosis) and tumors with minimal toxicity to normal cells and tissues.

Characteristics

Therapeutic oncolytic viruses (virotherapeutics) constitute a platform of targeted anti-cancer agents that have unique mechanisms of action compared with other cancer therapeutics, such as chemotherapy or radiotherapy. Oncolytic viruses are viruses that replicate (multiply themselves) selectively in cancer cells, and are attenuated (crippled) in normal cells, and as a result destroy cancer cells and tumors with minimal toxicity to normal cells and tissues. The development of virotherapeutics has evolved from the use of *in vitro*-passaged strains (first generation), to genetically-engineered



Oncolytic Virus. Figure 1 Schematic of oncolytic virotherapy mechanism-of-action: viral replication, cell killing, virus release and spread within cancer tissue but not normal tissues. (Reproduced with permission from REF. 28. © (2001) Nature Publishing Group).

Oncolytic Virus. Table 1 Oncolytic viruses tested in clinical trials

Virus	Genetic target in cancers	Genetic modifications to virus	Transgene encoded (rationale)
Non-engineered viruses			
NDV	Unknown (boosts immune response)	None	None
Reovirus	Defects in tumor PKR/interferon pathways	None	None
Mumps	Unknown	None	None
West Nile	NA	None	None
Adenovirus	NA	None	None
Vaccinia	NA	None	None
Engineered, non-armed viruses			
Adenovirus			
*dl*1520 (Onyx-015)	p53 pathway defects; late RNA transport defects	E1B-55K (–), E3B (–)	None
H101	Same	E1B-55K (–), E3 (–)	None
CV7060	None (prostate-specific)	PSE-driven E1A, E3B (–)	None
CG7870	None (prostate-specific)	Probasin-driven E1A, PSE-driven E1B	None
HSV			
1716	Defects in tumor PKR/interferon pathways (ICP34.5 –); attenuated neurotoxicity	ICP34.5 (–)	None
G207	Tumor cell complementation of ribonucleotide reductase (ICP6 –); Defects in tumor PKR/interferon pathways (ICP34.5 –); attenuated neurotoxicity	ICP34.5 (–), ICP6 (–)	None
NV1020	Attenuated toxicity	ICP34.5 (–), U_L24 (–), U_L56 (–); replaced with a fragment of HSV-2 U_S DNA (U_S2, U_S3, gJ, and gG)	None
Engineered, armed viruses			
Vaccinia			
JX-594	Tumor cell complementation of thymidine kinase deletion; expression E2F pathway-driven	Thymidine kinase (–)	GM-CSF (immunostimulatory; cancer-specific CTL)
Adenovirus			
Ad5-CD/TKrep	p53 pathway defects; late RNA transport defects	E1B-55K (–), E3B (–), CD/TK expression	CD/TK (prodrug-activation in tumor)
HSV			
Oncovex^{GM-CSF}	Defects in tumor PKR/interferon pathways (ICP34.5 –)	ICP34.5 (–), ICP47 (–), Us11 upregulation	GM-CSF (immunostimulatory; cancer-specific CTL)
Measles			
MV-CEA	Tumor-selective binding through CD46	None (vaccine strain)	CEA (blood marker for virus monitoring in patients)

Abbreviations: PKR, double-stranded RNA activated inhibitor of translation (known as PKR); PSE, Probasin-driven E gene; CEA, carcinoembryonic antigen; CD/TK, cytosine deaminase and herpes virus thymidine kinase fusion gene; GM-CSF, Granulocyte-macrophage colony-stimulating factor; CTL; cytotoxic T lymphocytes; MV; Measles virus vaccine strain; NDV; Newcastle disease virus; HSV, herpes simplex virus; NA, not applicable

selectivity-enhanced viruses (second generation) and finally to genetically-engineered transgene expressing "armed" oncolytic viruses (third generation). Descriptions of cancer remissions following virus infections date back to a century ago. Initial patient treatment publications, written up to 50 years ago, consisted of case reports or case series of treatment with first-generation, non-engineered viruses. Over the past decade, hundreds

of cancer patients have been treated on prospectively designed clinical trials (including phase III), evaluating over ten different engineered second- and third-generation viruses (Fig. 1).

Selectivity and Cancer-Targeting Mechanisms

Oncolytic viruses are by definition cancer-selective. Cancer selectivity is based on genetic differences between cancer cells and normal cells. Many genetic targets in cancer can activate the replication of oncolytic viruses and their cancer-specific cytolysis capacity. Examples include the loss of anti-viral mechanisms in cancer cells such as type-I interferon induction and anti-viral activity. Deletion of the interferon genes can block induction of interferon expression from the infected cancer cell. The interferon response pathway can be blocked by ras pathway (►RAS activation) activation or by loss of p53 (encyclopedia link) function. Other genetic abnormalities in cancer that can be targeted include cell cycle abnormalities (►Cell-cycle targets for cancer therapy), cell surface virus receptors and tissue-specific promoters.

Viruses can target these cancer genetic abnormalities naturally (or inherently) without genetic engineering. Once example is the vesicular stomatitis virus (VSV) that is hyper-sensitive to type-I interferon-mediated clearance in normal cells, but can replicate and lyse cells in tumors that have inactivated interferon responses. Other oncolytic viruses must be engineered genetically to make their replication and cytolysis dependent on genetic targets in cancer. Examples include the deletion of viral genes that are necessary for replication of the virus, but whose function can be supplied in *trans* by the cancer cell. For example, deletion of the vaccinia viral thymidine kinase (TK) gene markedly attenuates (weakens) the vaccinia virus in normal cells, whereas in cancer cells the ►E2F transcription factor is activated and drives high-level expression of the cellular TK. Cellular TK in cancer cells supports and activates vaccinia replication and cytolysis. JX-594 (Jennerex Biotherapeutics Inc, SF, CA, USA) is an oncolytic vaccinia virus with a TK deletion that also is "armed" (engineered to express) with human GM-CSF; the virus is in Phase II clinical trials. Other examples of gene deleted viruses are in Table 1. Viruses that replicate in the nucleus of infected cells can also be targeted by expressing critical viral genes under control of a cancer tissue-specific promoter (e.g. E2F promoter-enhancer and telomerase promoter).

Efficacy and Anti-Cancer Mechanisms

The primary mechanism-of-action (MOA) for oncolytic viruses is direct cell lysis as a result of viral replication cycle completion. However, this primary MOA can be complemented and augmented by additional MOA. These secondary MOA can be a direct result of the direct virus replication-dependent oncolysis or can be engineered into the virus therapeutic. Direct oncolysis can also lead to induction of cancer-specific antitumoral cell-mediated immunity. In addition, oncolysis can result in the blockage of tumor-associated blood vessels (also known as "vascular shutdown"). In addition, these agents can be engineered to express therapeutic transgenes to express proteins selectively in tumors that have anti-cancer MOA. Examples include prodrug-activating enzymes that activate chemotherapy locally in tumor tissue, and immune response activators such as GM-CSF (see JX-594 above).

Virus Species

Oncolytic viruses can be made from a diverse array of viral species. Each species has distinct characteristics that give them specific spectra of activity and behaviors in cancer patients. Examples include enveloped viruses such as vaccinia, herpes simplex virus (HSV) and measles, as well as non-enveloped viruses such as adenovirus and reovirus. These viruses represent the equivalent of the chemical "pharmacophore" (starting material for a therapeutic agent) that can then be engineered to optimize efficacy and safety. Species-dependent differences can be profound. For example, whereas vaccinia virus is a blood-borne virus that can naturally spread via blood to disseminated tumor sites in the body, other viruses such as ►adenovirus (Ad5) or HSV that are not naturally blood-borne are limited to local-regional applications in cancer patiens.

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Onconase

Definition

Is a ribonuclease-type enzyme inhibitor found in the oocytes of the frog *Rana pipiens*. It is being studied in the treatment of ►mesothelioma.

►Ranpirnase

Oncopeptidomics

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Definition

Oncopeptidomics is defined as application of peptidomics technologies to the field of oncology. Peptidomic technologies encompass the systematic, comprehensive multiplex analysis of native or endogenous peptides in a biological sample with respect to temporal and spatial resolution.

Characteristics

Peptides

The proteome is the set of all proteins encoded by the genome and present within an organism for a specific time frame. While the genome remains relatively stable, the proteome is remarkable dynamic with multiple mechanisms of up and down regulation as well as different mechanisms of post-translation modifications. One important, irreversible proteomic control mechanism is the proteolytic processing of proteins by proteases that initiate, modulate and terminate many important cellular functions through highly specific and limited substrate cleavages. One important result of proteolytic activity is the generation of peptides. The Greek origin of the term "peptide" (from the Greek terms "peptos," meaning digestible, and "poly-saccharide," referring to its composition of two or more amino acids) reflects the fact that peptides are the products of proteolytic cleavage events. It is estimated that there are 500 genes that encode proteases and approximately 150 protease inhibitors have been identified in the human genome.

The role of bioactive peptides in biology and their importance for therapeutic applications has been known for several decades. Insulin, for example was the first peptide used to treat a disabling and life-threatening disease, Type 1 diabetes. In general, the function (or bioactivity) of peptides is linked to some defined molecular interaction that invokes a change of physicochemical state (e.g. a signal transduction). But peptides are also often produced during metabolic processes and, as such, represent or mirror that process as an objective ►biomarker. Due to their involvement in almost all biological processes, peptides are highly relevant as biomarkers.

Peptide Biomarker

In the field of clinical diagnostics, there is increasing interest in native peptides as potentially useful biomarkers. The discovery of tumor biomarkers useful for early detection of cancer is one main goal in cancer research, since the 5-year survival rate of cancer patients shows a high correlation to the tumor stage. Consequently the axiom of "the earlier the diagnosis, the better the chance of effective treatment" is still regarded to be true.

Since the peptidome (or the low-molecular weight proteome) reflects proteolytic processing of precursor molecules, a close connection to tumor pathophysiology exists. Increased or altered expression and secretion of proteases (e.g. ►matrix metalloproteases (MMP) and ►cathepsins) exerting proteolytic activity inside the cells or extracellularly in the tumor tissue is associated with tumor cell growth, invasion, angiogenesis and metastasis. Peptides, as proteolytic reaction products of these altered expression are capable to reflect these changes in enzymatic activity. In contrast to cell-associated proteases or precursor proteins, peptides exhibit better permeability between tissue compartments due to the relative low molecular weight. Therefore, the probability of detecting proteolytic fragments from tissue-born proteins in body fluids is significantly higher than that of the protein precursor. Taken together, endogenous peptides can be regarded as versatile biomarkers that are capable of revealing the different pathologies and heterogeneity of diseases by reflecting changes in the expression of their precursor proteins or activity of the corresponding enzymes.

For example, ►fibrinogen degradation products (FDP) have long been known to be increased in plasma of cancer patients. It is likely that FDPs are produced by plasmin, which is generated from plasminogen around neoplastic cells due to enhanced release of plasminogen-activator by these cells.

Peptide Biomarker Discovery

►Multiplex profiling technologies for peptides are most often based on ►mass spectrometry which offers the analytical comprehensiveness required to monitor peptides, and thus proteolytic events, in an efficient way. Differences can be linked to altered regulation of protein synthesis and degradation which, in turn, are representative of altered cellular mechanisms associated with the advent and growth of tumors. There are two basic approaches for peptide biomarker discovery: broad-shallow and narrow-deep.

The broad-shallow approach uses the entire information content (peptide pattern) present in a given dataset for stratification of healthy individuals from cancer patients by use of ►multivariate statistics. One prominent example of this approach was published in 2002. In the study, the authors describe the application of bioinformatics tools (e.g. genetic algorithms) to mass spectrometric data patterns acquired from serum samples of patients with ►ovarian cancer or healthy individuals. The results were used as a basis for stratifying cancer patients from controls with exceptionally high specificity and sensitivity. Although the study

initialized a controversial debate around the lack of ►sequence identification of the discriminatory peaks and the utility of abstract patterns with the risk of data over-fitting, it initiated a conceptual breakthrough for mass spectrometry in biomarker research.

The narrow-deep approach aims at the discovery of a very limited set of marker candidates for a given problem but with extensive biological and analytical characterization of these candidates. The identification of the protein or peptide sequence not only allows the generation of antigen specific antibodies as the part of the major prevailing form of in vitro diagnostic (IVD) assays, but also the validation of these biomarkers with independent approaches. The differences in peptide expression levels can be analyzed in different biological sources such as tissue and plasma, the test validation can be performed on various other detection systems (e.g. gene expression, immunohistochemistry, immunoassays), and a biological hypothesis can be generated and tested. Even more important, the biomarker can be transferred to multiple sites and approved clinical validation procedures can be used.

Challenges and Limitations in Oncopeptidomics

Currently most of the applied multiplex technologies for discovery of biomarkers do not allow detecting concentrations of endogenous peptides in the low picomolar to the high femtomolar range – a sensitivity level required for the measurement of classical tumor markers in blood. Most published results describe fragments of liver proteins as biomarker candidates. These highly abundant (micromolar concentrations) plasma proteins (e.g. apolipoprotein A-I, serum-amyloid protein, hemoglobin, transthyretin) or their fragments have been proposed to discriminate between controls and cancer patients. Since many of these proteins originate from the liver and not from the diseased tissue itself, it is a matter of the current debate whether markers reflecting a tumor host response (e.g. acute phase proteins) will eventually have sufficient sensitivity and specificity to bring them from discovery through the rigorous clinical validation process. Nevertheless, concepts have been proposed, that fragments derived from high abundant plasma proteins are tailored by tumor exopeptidases during serum generation. Until now a definitive assessment has not been made, since direct measurements of these postulated peptidases has not been carried out yet. As a result, current peptidomics discovery strategies should integrate the analysis of model systems, tumor and control tissue, and the consequent use of active controls as well as body fluids to enhance sensitivity. The challenge of the next years is to further promote such integrative approaches and to improve the sensitivity of mass spectrometric based multiplex systems to extend the analytical range beyond highly abundant components.

Conclusion

Peptide biomarker bear the potential of adding high value for the diagnosis and stratification of cancer and different peptidomics technologies in the field of oncology are currently under broad investigation. At present, a definitive assessment on the hitherto obtained results cannot be made, since most candidates are not validated yet. The complexity of clinical validation of proteomic biomarkers is a challenge and the time requirement has typically been underestimated. The objective to establish more reliable biomarkers for the stratification of patients, the determination of clinical or therapeutic outcomes (►prognostic biomarkers or ►predictive biomarkers), or the early detection of cancer necessitate a more holistic viewpoint and will presumably require a duration comparable to the drug development process.

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Oncoplastic Surgery

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Definition

Oncoplastic surgery is an evolving surgical subspecialty that combines the principles and techniques of surgical oncology (removal of cancer) with aesthetic and reconstructive surgery.

Characteristics

Historical Background

►Breast cancer is not a new disease. Historical documents as early as the Egyptian Papyrus writings have noted its existence. Since that time, women have lived in fear of this dreaded disease, the lethal nature of its course and the often disfiguring surgery that accompanies treatment.

Today, many of those fears can be alleviated. A broad range of new and innovative techniques are now used in

the diagnosis and treatment of breast cancer which has changed the stage of disease that physicians are treating to a much earlier stage. In turn, this has allowed for new surgical modalities such as minimally invasive biopsy, ►breast conservation. The advances in surgical care and the development of new and successful reconstructive techniques have all stimulated the development of oncoplastic surgery as a new surgical subspecialty.

The surgical history of breast cancer treatment dates back to the late 1800s when Dr. William Halsted and others realized that removal of the breast and surrounding tissues resulted in an improved quality of life for breast cancer patients. Unfortunately, since the majority of these women were diagnosed at a late stage of disease, even this dramatic and extensive surgery did not necessarily extend their lifespan. This type of surgery (the ►Halsted radical mastectomy) required complete removal of the tissues surrounding the breast, including the entire breast itself, all of the overlying muscle, the skin of the chest wall and most of the auxiliary lymph nodes and tissue. While the advent of anesthesia and antibiotics made this surgical procedure possible for the removal of cancer, the "radical mastectomy" resulted in a horribly disfiguring appearance (Fig. 1a) and left many women feeling ashamed.

As mammographic screening programs became more available, and as women became more familiar with the signs and symptoms of breast cancer, the medical community began to see smaller, earlier tumors. Ultimately, research proved that with breast cancers diagnosed at an earlier stage, women have improved survival rates even when less radical surgery is performed. This led to promotion of the "modified radical mastectomy" which surgically removed the breast and auxiliary contents, but preserved the underlying muscle and more of the skin (Fig. 1b). This procedure was much less invasive than the radical mastectomy, and resulted in a better contour and appearance of the chest wall allowing women to wear a breast prosthesis more easily and feel less deformed. It also enabled surgeons to develop methods for reconstructing the breast, and by the mid to late 1980s many women were able to have immediate ►breast reconstruction at the time of ►mastectomy (Fig. 2a–d) or undergo delayed reconstruction using tissue expansion with ►breast implants (Fig. 3a, b). Thus, they were saved the physical and psychological discomfort of living without their breast.

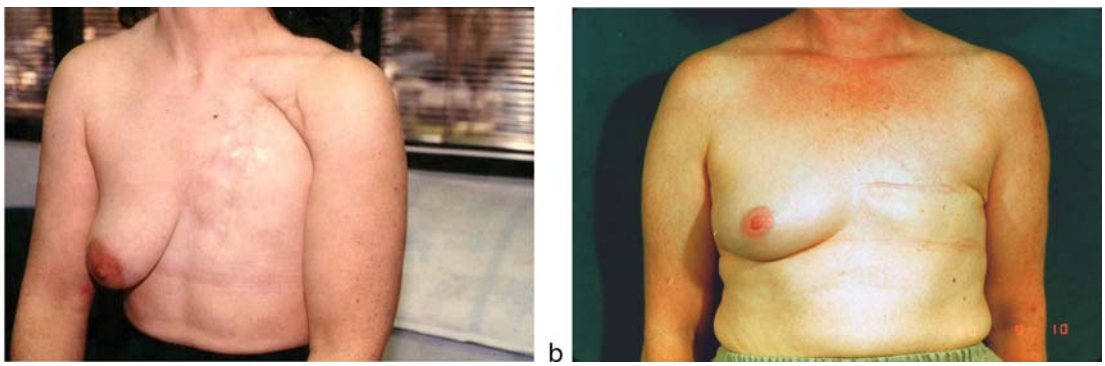
Additional intensive research studies in the United States and Europe ultimately revealed that small tumors in the breast could effectively be removed while preserving the surrounding tissue and saving the nipple and areola. This became known as "breast conservation" surgery and now is used to treat over 50% of breast cancers that are diagnosed. Depending upon the type, size and location of the tumor, breast conservation surgery is often followed by additional treatments such as ►radiation therapy and/or chemotherapy. These changes in the surgical management of breast cancer have provided the impetus for continued improvement and have led to the recent expansion of oncoplastic surgery.

Clinical Impact of Oncoplastic Surgery

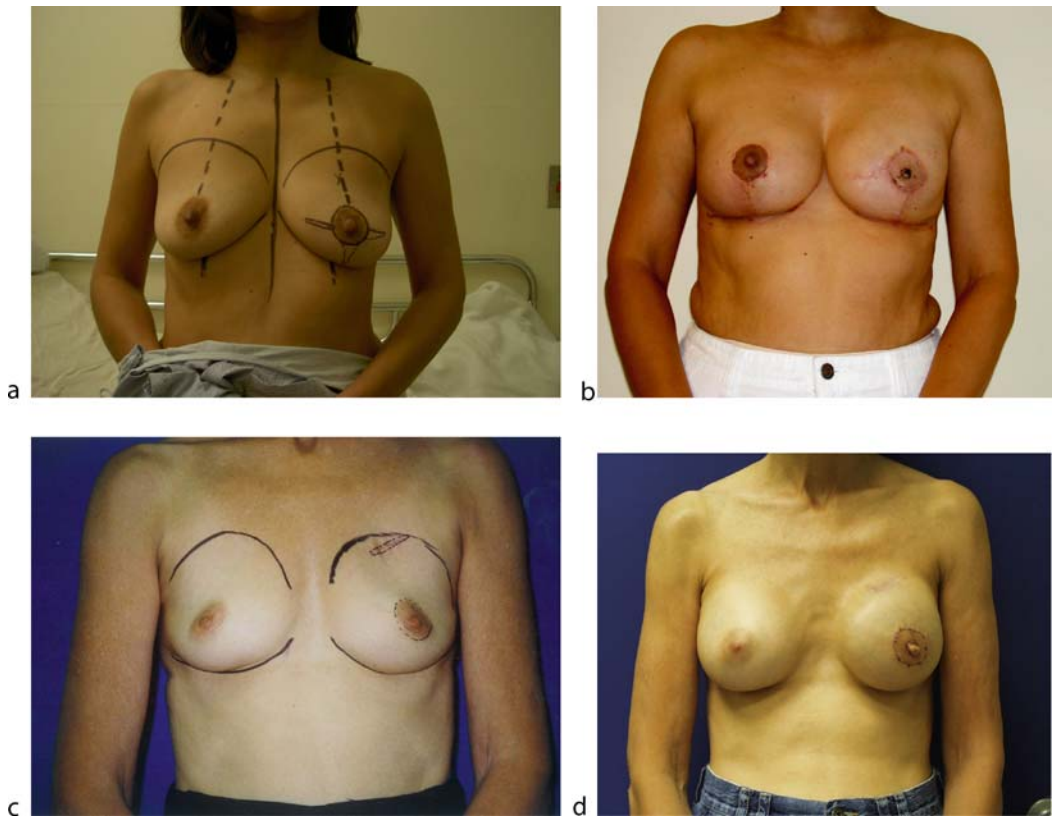
The term "oncoplastic surgery" was first used by Dr. Werner Audretsch to describe a new surgical approach – one in which the breast surgeon considers cancer treatment in conjunction with aesthetics. Thus, the clinical objectives of oncoplastic surgery are to optimize patient outcomes by combining the principles of surgical oncology with those of aesthetic and reconstructive surgery. First and foremost, there must be no compromise in the primary cancer treatment. Aesthetic outcome of the surgery is critically important, but follows as "secondary" after all consideration for cancer treatment. For example, ►breast reduction can be performed to remove the tumor and improve the overall appearance of the breast (Fig. 4a, b).

Pre-operative assessment of the patient begins with the following:

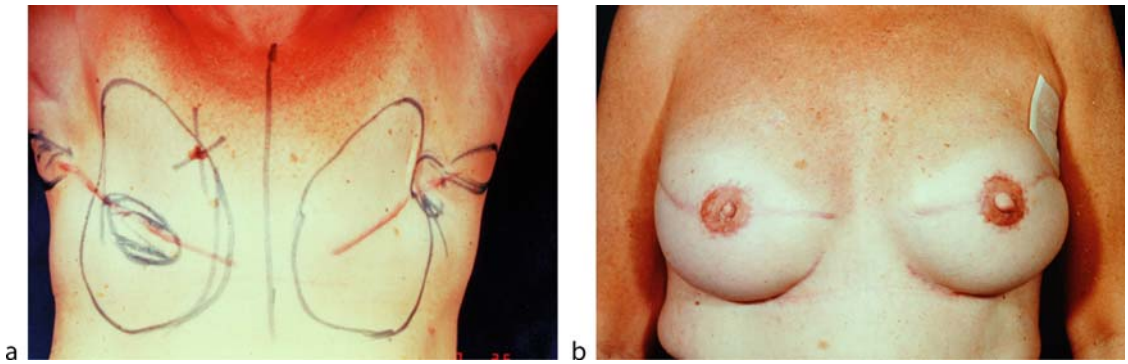
Complete and thorough history and physical examination



Oncoplastic Surgery. Figure 1 (a) Radical mastectomy and (b) modified radical mastectomy.



Oncoplastic Surgery. Figure 2 (a) Breast cancer patient prior to removal of the left breast, (b) same patient following removal of the left breast and immediate reconstruction, (c) breast cancer patient prior to removal of the left breast, and (d) same patient following removal of the left breast, immediate reconstruction, ►nipple construction and augmentation of the right breast.



Oncoplastic Surgery. Figure 3 (a) Breast cancer patient following removal of both right and left breasts and (b) same patient following reconstruction of both right and left breasts with nipple reconstructions.

Diagnostic imaging studies with tissue biopsies as needed

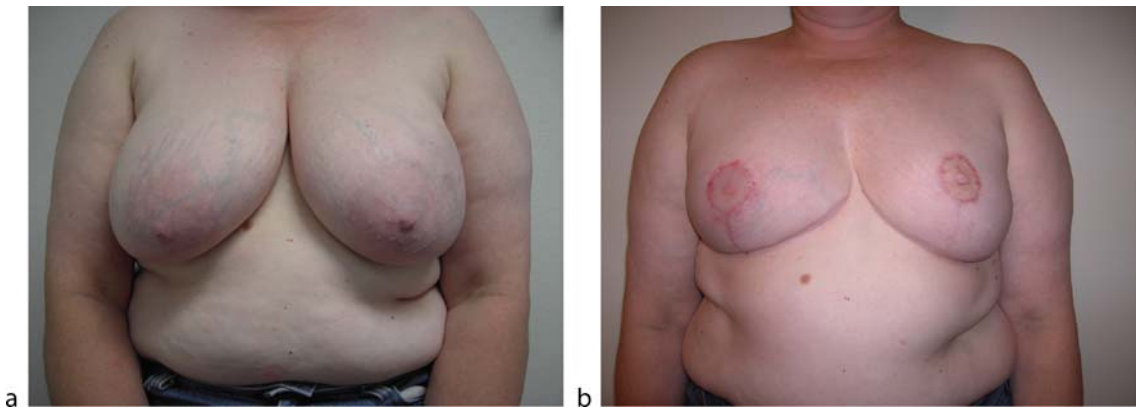
Risk assessment – including risk of recurrence and risk of a new cancer occurring in the opposite breast

Surgical objectives are then determined and must take into consideration:

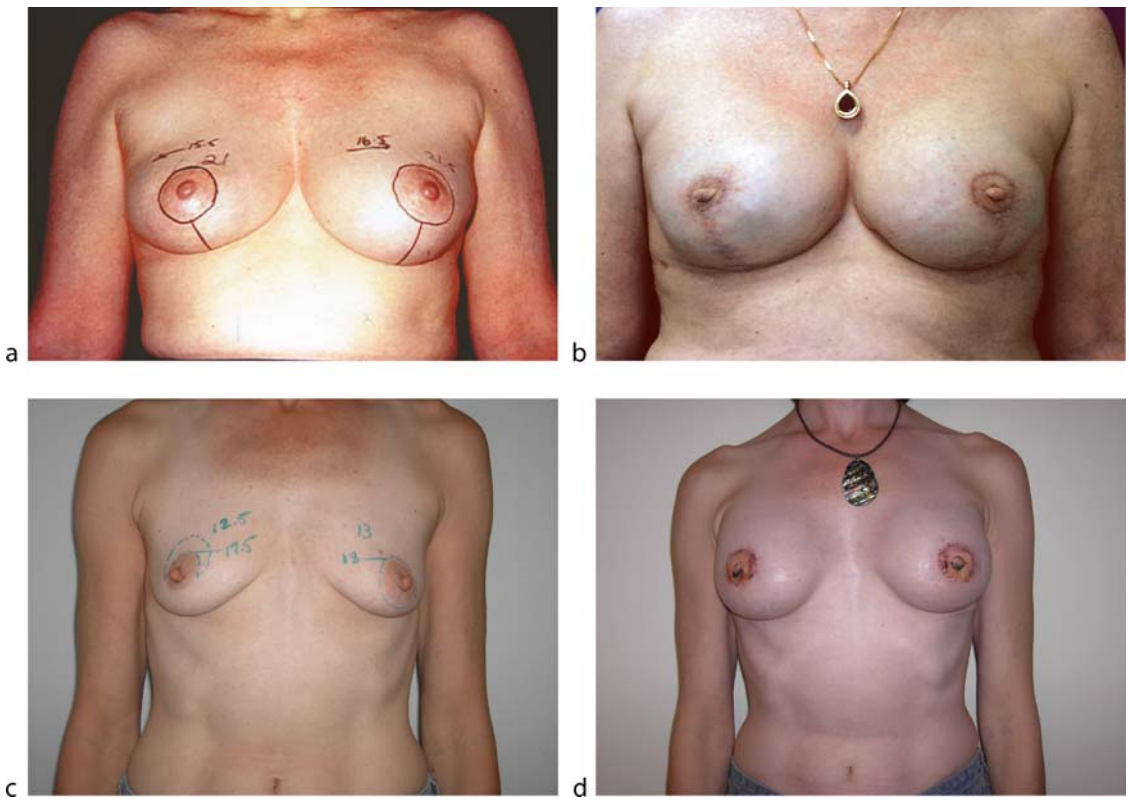
Optimizing removal of involved tissue with clean margins and appropriate staging

Preservation of non-affected tissues (i.e. skin, muscle, etc.)

Consideration of additional therapy that may be necessary (i.e. radiation)



Oncoplastic Surgery. Figure 4 (a) Breast cancer patient with large breasts and (b) same patient following removal of left breast, immediate reconstruction, left nipple reconstruction and reduction of the right breast.



Oncoplastic Surgery. Figure 5 (a & c) Patients with strong family history of breast cancer and genetic predisposition to the disease. (b & d) Same patients following prophylactic removal of the breasts with immediate reconstruction of the breasts and nipples.

Optimizing aesthetic appearance of the breasts (reconstruction, reduction, etc.)

In order to achieve these objectives, the pre-op testing of patients at risk or diagnosed with breast cancer can be extensive. Various imaging modalities are commonly employed for diagnosis, such as mammography, ultrasound and magnetic resonance imaging (MRI), and should include thorough assessment of both breasts using minimally invasive tissue sampling for any suspicious lesions.

In addition, patients with a family history of breast cancer should undergo genetic testing for BRCA mutations (►BRCA1/BRCA2 Germline Mutations and Breast Cancer Risk). These genetic mutations are

predictors of patients at high risk for developing breast cancer during the course of their lifetime. Often patients with this known predisposition to breast cancer will choose to have the breasts surgically removed as a precautionary measure since surgery will markedly decrease and/or prevent their lifetime risk of breast cancer. This procedure is termed a "►prophylactic mastectomy". Oncoplastic surgery is particularly beneficial for these patients since the elective removal of the breasts can be done in an anesthetically pleasing fashion, preserving almost all of the skin and in some cases even preserving the nipple and areola. This procedure is combined with immediate breast reconstruction providing a nice appearance to the new breasts with a markedly decreased risk of development cancer (Fig. 5a–d).

After evaluation and consideration of all aspects of each patients' cancer care, the surgeon explores the desires and needs of the patients' aesthetic/reconstructive desires and applies them to the surgical plan. This provides for a comprehensive surgical approach and often requires fewer surgeries to achieve the desired surgical outcome.

Summary

Although at present the field of Oncoplastic surgery is in its' embryonic growth phase, breast surgeons around the world have recognized the benefits of providing this type of care to breast cancer patients and hopefully soon these techniques will be available to women throughout the world.

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Oncoprotein 18

Definition

Stathmin.

Oncostatin M

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Synonyms

OSM; Oncostatin M Precursor

Definition

Oncostatin M (OSM) is a member of the hemopoietic ►interleukin-6 (IL-6) cytokine subfamily and is most closely related structurally and functionally to ►leukemia inhibitory factor (LIF).

Characteristics

Human Oncostatin M is a multifunctional, 28-kDa glycoprotein which was originally isolated from histiocytic lymphoma cells treated with phorbol 12-myristate 13-acetate (►PMA). Oncostatin M was identified and cloned on the basis of its ability to inhibit the growth of ►melanoma cell lines but is now known to exhibit unique biological functions in hemopoiesis, neuropoiesis, ►inflammation, bone remodeling, muscle proliferation and embryonic development.

OSM is produced by activated T-lymphocytes, ►macrophages, monocytes and neutrophils and is a member of the glycoprotein 130 (gp130) cytokine family which exert their actions via a shared signal-transducing receptor, gp130. Members of this family include ►interleukin-6 (IL-6), interleukin-11 (IL-11), ►leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin-like cytokine (CLC), cardiotrophin-1 (CT-1) and neuropoietin (NP). The genes for oncostatin M and LIF occur in tandem on human chromosome 22q12 and share similar gene structures thus probably arose through gene duplication. The human OSM gene is approximately 4 kb in length and contains three exons encoding a 2 kb transcript. Oncostatin M is most closely related structurally and functionally to LIF, exhibiting 22% sequence identity to LIF.

Oncostatin M Protein Structure

Oncostatin M and other IL-6 cytokines belong to the 'long chain four helix bundle' subgroup of cytokines together with ►granulocyte colony stimulating-factor (G-CSF), growth hormone and ►erythropoietin. This subgroup exhibits four helices (A-D) joined together by polypeptide loops in an UP-UP-DOWN-DOWN topology. The IL-6 cytokine family can be further subdivided into straight chain and kinked chain cytokines (IL-6 and IL-11 belong to the straight chain subgroup while OSM, LIF and CNTF have a kink in

helix A). This structural divergence reflects differences in receptor recruitment.

Oncostatin M has an N-terminal secretory signal sequence of 25 amino acids and a basic hydrophilic C-terminus of 31 amino acids. Both are cleaved off during protein processing but there may be slight differences in post-translational modification depending upon the cell line hence generating differently sized mature Oncostatin M protein. The unprocessed OSM precursor is 28 kDa (252 amino acids) while the N-and C-terminal modifications make the mature and fully active form of OSM approximately 22 kDa (196 amino acids).

Oncostatin M Ligand-Receptor Interactions

Different members of the gp130 family signal either by the formation of homodimeric gp130 complexes (facilitated in some cases by non-signaling co-receptors) or via heterodimeric receptor complexes containing gp130 and a second signaling receptor. Human OSM is unusual in that it can form two types of heterodimeric signaling complexes. The type I OSM receptor complex consists of the gp130 receptor and the LIF receptor α (gp130/LIFRα). This configuration is also utilized by LIF. As a consequence, human OSM and LIF can exhibit similarities in biological activity. The type II OSM receptor complex comprises gp130 and the OSM receptor β (gp130/OSMRβ) and this is activated by OSM only. The type II receptor complex may activate signaling pathways distinct from the OSM type I complex thus conferring OSM–specific signaling functions.

OSM ligand has two receptor binding sites; II and III. Site II (as with all the IL-6 cytokines) binds to gp130 via conserved residues within the A and C helices. Site III binds to either the LIFRα or OSMRβ via conserved residues at the N-terminus of the D-helix (two conserved amino acids, phenylalanine-160 and lysine-163, referred to as the FK motif). All the other LIFR binding ligands contain this FK motif.

Oncostatin M Signaling

The generic features of gp130 cytokine signaling are well defined. The formation of a high affinity transmembrane signaling complex results in activation of the receptor associated Janus kinase family (Jak1, ►Jak2 and Tyk2) leading to phosphorylation of gp130, OSMRβ and LIFRα. Phosphorylated tyrosine residues in the receptor C-terminal regions recruit phosphotyrosine binding (PTB) and Src homology-2 (►SH2 domain) domain containing proteins such as the adaptor protein Shc to OSMRβ and phosphatase Shp2 to gp130 and LIFRα. Both these associations result in activation of the mitogen-activated protein kinase (MAPK) cascade. The second target of receptor activated Jak kinases are the ►signal transducers and activators of transcription (STAT) family of transcription factors (STATs-1, -3 and -5) which, upon Jak-mediated phosphorylation, migrate to the nucleus and activate the transcription of downstream gene targets.

Clinical Relevance of Oncostatin M in Cancer

The clinical manifestations of oncostatin M within the tumor ►microenvironment are poorly understood and are further complicated by virtue of its multiple roles in ►inflammation, tissue remodeling and wound healing processes. In normal physiological processes OSM is implicated in a variety of cellular functions in immune, hemopoietic, neural, bone and hepatic systems. Many of these functions are shared by other members of the interleukin-6 cytokine family while some are restricted to oncostatin M. The role of OSM in cancerous tissues is controversial as some actions are considered anti-tumorigenic while others may potentially contribute to tumor progression, ►angiogenesis and increased metastatic potential. OSM-induced growth inhibition of tumor cells is clearly beneficial while some of the pro-inflammatory actions (e.g. increased ►cyclo-oxygenase-2 expression) coupled with stimulation of proteolytic pathways involved in extracellular matrix (ECM) degradation and associated localized tissue damage could be detrimental. The biological activities of oncostatin M most likely to have implications in tumor biology are outlined below.

OSM inhibits the growth of normal primary mammary epithelial cells and stimulates proliferation of dermal fibroblasts, endothelial cells, vascular smooth muscle cells and T-cells. In addition OSM modulates the growth of a number of solid tumor-derived cell lines exhibiting antiproliferative effects on breast tumor-derived cell lines, lung carcinoma cells, melanoma cells, ►neuroblastoma cells, glioma cells and ovarian carcinoma cells while stimulating the proliferation of primary ►plasmacytoma and myeloma cells, prostate carcinoma cells and cells derived from acquired immunodeficiency syndrome-related ►Kaposi sarcoma (AIDS-KS). Oncostatin M has also been shown to inhibit *in-vivo* glioma formation after intracerebral inoculation of mice with ►glioblastoma cells. In many of these tumor cell lines (e.g. melanoma, breast tumor) the specific OSMRβ is a prerequisite for the effects on growth. However, some cell lines derived from advanced-stage cancers can become refractory to OSM growth inhibition and may undergo OSM autocrine growth stimulation (e.g. melanoma cells).

OSM treatment of breast-cancer cell lines and normal breast epithelial cells reduces the proportion of cells in the S-phase of the cell cycle while increasing the proportion of cells in G0/G1 phase. Both MAPK and STAT signaling are implicated in the anti-proliferative effects (and gene regulatory anomalies) of OSM on most of these tumor cells. OSM-mediated growth responses involve alterations in protein expression and

may include gene targets such as ►c-myc, p53, p21, cyclins A and D and c/EBPδ.

OSM stimulates differentiation of hemopoietic cells (T-cells, megakaryocytes) and vascular endothelial cells. OSM also induces morphological changes in several tumor cell lines which, in some reports (breast cancer, glioma, lung cancer and myeloblastic M1 murine leukemia) are associated with cell differentiation processes. In the MCF-7 breast tumor cell line, OSM stimulation results in increased expression of epithelial membrane antigen, a component of milk fat-globule proteins and differentiation marker. However, morphological changes involving loss of cell-cell and cell-matrix interactions (breast tumor cells and lung cancer cells) coupled with modulation of adhesion receptor expression and increased cell motility have also been reported and associated with tumor cell detachment from the substratum and enhanced invasive capacity.

OSM secretion by immune cells leads to a range of effects involved in both pro- and anti-inflammatory processes. OSM is known to be expressed during initiation of immune responses along with other cytokines such as interleukin-2 (IL-2) and ►tumor necrosis factor-α (TNFα). OSM mediates the expression and/or activity of other cytokines, for example, in ►Kaposi sarcoma cells OSM induces expression of IL-6 while in endothelial cells OSM stimulation results in increased IL-6, GM-CSF and G-CSF expression. OSM also has the capacity to act synergistically with other inflammatory cytokines (e.g. IL-1, TNFα) for example in the stimulation of ►prostaglandin E_2 synthesis in astrocytes and astroglioma cells. OSM induces synthesis of CXC and CC subfamilies of ►chemokines such as growth regulated protein (GRO-α, GRO-β) production by endothelial cells, stromal cell-derived factor 1 (SDF-1) production by mesenchymal stem cells or monocyte chemoattractant protein 1 (MCP-1) expression by synovial fibroblasts. In addition, OSM protein acts as a chemoattractant for leukocytes, neutrophils and capillary endothelial cells thus may act to recruit immune cells to sites of inflammation and help revascularization during the wound healing process. OSM can also inhibit expression of some cytokines and chemokines.

OSM stimulation of mammary adenocarcinoma cells, endothelial cells, astroglioma cells and ►neuroblastoma cells results in increased ►prostaglandin (PG) synthesis due to upregulation of cyclooxygenase-2 expression. PGE_2 acts as an inflammatory mediator and stimulates further expression of cytokines and growth factors by immune cells localized to the area of inflammation.

Vascular endothelial cells are one of the main targets of oncostatin M due to their high expression of gp130 family receptors including OSMRβ. OSM induces endothelial cell surface expression of adhesion receptors (e.g. P- and ►E-selectin, ICAM-1, VCAM-1) which aid in the recruitment, adhesion and transmigration of leukocytes and neutrophils across the endothelium. In addition, OSM also alters fluid (and cell) movement in and out of the vasculature by inducing expression of vasodilators (vasoactive intestinal peptide) or vasoconstrictors (►endothelins).

One of the earliest defined characteristics of OSM and all gp130 family cytokines is stimulation of acute phase protein (e.g. serum amyloid A, α1-antichymotrypsin, complement factors) production both in local tissues at sites of inflammation and by liver hepatocytes. Thus, as well as exhibiting pro-inflammatory properties, OSM is a prominent cytokine during immune attenuation and probably aids in the re-establishment of tissue homeostasis.

OSM is involved in ►extracellular matrix remodeling and contributes to the degradation and reformation of stroma in wound healing processes. This is achieved by mediating the expression of proteolytic enzymes, their associated receptors and inhibitors. For example, OSM modulates expression of components of the ►plasminogen activating system (►urokinase-type plasminogen activator (uPa), uPa-receptor, tissue-type plasminogen activator (tPa) and plasminogen activator inhibitor (PAI-1)) in endothelial cells, fibroblasts, astrocytes and lung carcinoma cells. OSM also regulates the balance between ►matrix metalloproteinases (e.g. MMP1, MMP2, MMP3 and MMP9) and ►tissue inhibitors of matrix metalloproteinase (TIMP) in a variety of normal and tumor cell lines. OSM induces expression of additional proteolytic enzymes (e.g. ►cathepsins) and stimulates production of ►ECM components such as ►fibronectin, laminin and vitronectin presumably during formation of new stroma. OSM can also stimulate expression of angiogenic regulators including ►vascular endothelial growth factor (VEGF) and ►basic fibroblast growth factor (bFGF) by several different cell types so can potentially promote ►angiogenesis.

Lastly, OSM has been implicated in modulation of tumor cell ►drug resistance. On exposure to oncostatin M, ►prostate cancer-derived (PC-3) cells acquire enhanced resistance to both ►etoposide and ►cisplatin. OSM is also known to mediate downregulation of ►cytochrome P450 enzyme expression in hepatocytes thus may impair drug metabolism in both normal and cancerous tissues during inflammatory processes. OSM may also override growth responses of tumor cells to drug treatment. For example, myeloma cells normally undergo growth inhibition in response to dexamethasone but this response is reversed by OSM.

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Oncostatin M Precursor

►Oncostatin M

ONYX Vector

Definition

Refers to a human gene therapy vector based on a modified adenovirus, with deletion of the E1B 55-kDa gene, that renders the virus incapable of replication in cells with normal p53 genes. By contrast, in cancer cells with mutated p53 genes, ONYX vector can replicate and lyse the host cell, perhaps contributing to effective tumor control. As many human tumors have a mutated p53 genes, they are potentially vulnerable to lysis by ONYX-vector approach.

►Adenovirotherapy
►Oncolytic Adenovirus

Oocyte

Definition

Female germ cell in the process of development.

►Granulosa Cell Tumors
►Germ Cell Tumors

Open Biopsy

Fine Needle Biopsy

►Fine Needle Aspiration
►Surgical Biopsy

Open Reading Frame

Definition

Orf; Region of a gene that is translated into a protein; typically flanked by a start and stop codon.

Opportunistic Infections

Definition

Infection induced by a microorganism that normally does not lead to a disease, but becomes pathogenic when the body's immune system is compromised.

►Inflammation

Opsonin

Definition

Is any molecule that acts as a binding enhancer for the process of ►phagocytosis, for example, by coating the negatively-charged molecules on the membrane. ►Opsonization.

Opsonization

Definition

Refers to a process that makes bacteria or other cells more susceptible to the action of phagocytosis. Is the process where particles such as microorganisms become coated with molecules that allow them to bind

to receptors on phagocytes. Antibodies (especially IgG) and complement proteins like C3b can opsonize and are therefore referred to as ►opsonin.

Opsonize

Definition

To coat an organism with antibodies or a complement protein so as to make it palatable to phagocytes.

►Opsonin
►Opsonization

OptiBerry

Definition

A synergistic combination of six edible berry extracts including wild blueberry, wild bilberry, cranberry, elderberry, raspberry seed, and strawberry.

►Chemoprotectants

Optic Glioma

Definition

Grade 1 pilocytic ►astrocytoma, a tumor that arises from glial cells and grows in and along the optic nerve or optic tract.

►Neurofibromatosis 1

Optical Mammography

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Synonyms

Light tomography; Breast transillumination

Characteristics

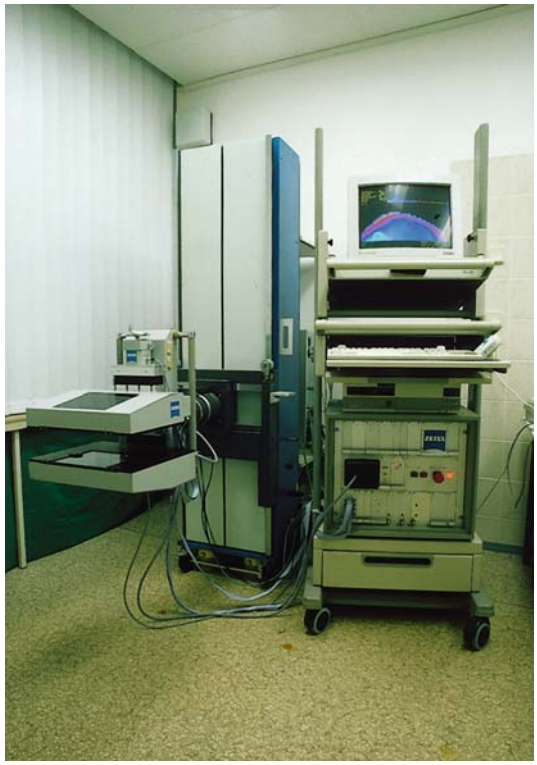
Optical mammography is an imaging modality using transilluminated light in the visible and near infrared wavelength range, which is used to detect or to differentiate neoplastic lesions of the breast.

Description

Many attempts to detect ►breast cancer by transillumination have failed in the past due to the strongly diffusive nature of light transport in tissues. Lasers might initiate a technical revolution in this field. The use of several wavelengths may enable differential spectroscopy, leading to functional and tissue composition information. High frequency intensity modulation or pulsed laser systems provide additional information of the length of the photon path in tissue, eventually enabling differentiation between absorption and scattering properties of breast tissue or even three-dimensional reconstruction of optical properties.

Application

Two-frequency domain instruments have been clinically evaluated in Germany. The Fig. 1 demonstrates the Carl Zeiss instrument "LIMA" that uses two laser diodes



Optical Mammography. Figure 1 Prototype of a Frequency Domain optical mammograph. The instrument uses two intensity modulated (110 MHz) laser beams at wavelengths of 690 and 810 nm to scan a breast. Images are calculated from amplitude and phase data.

at 690 nm and 815 nm, and a modulation frequency of 110 MHz. A clear improvement in image quality compared to conventional imaging was demonstrated. Furthermore, the approach enabled the characterization of individual tumors with regard to size, position and tissue-optical properties. However, no significant improvement in sensitivity appears to result from the frequency domain approach.

Actual research focuses on time domain instruments using laser pulses in the picosecond range. Entire flight time distributions are registered. By using only the first photons leaving the tissue, contrasted pictures of differentially absorbing tissue formations may become possible. However, the probability for a photon to pass a 4–7 cm layer of breast tissue without any scattering (ballistic photon) is zero. Thus, image contrast will always be inferior to X-ray images. On the other hand, additional and quantitative information can be obtained by the use of different wavelengths such as tissue oxygenation parameters or tissue composition information, which may compensate for the lack of contrast and detail resolution. Such time domain instruments are entering initial clinical testing. Furthermore, for the purpose of enhanced specificity in detectable lesions, contrast agents may prove useful in the future.

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Oral Cancer

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Synonyms

Lesion on tongue, lip and other areas in the mouth

Definition

Oral cancer is cancers of the mouth. It is a common form of head and neck cancer.

Characteristics

Etiology

Oral cancer is amongst the most commonly diagnosed cancers worldwide and its incidence high in developing countries. The main etiological factors for oral cancer are tobacco and alcohol use, with 75% of those diagnosed with disease described as current or ex-smokers. Regular combined use of tobacco and alcohol significantly increases the chance of developing this type of tumor; those who both smoke and drink have a 15 times greater risk of developing oral cancer than the overall population. While the majority of people are over the age of 40 at the time of diagnosis, oral cancer may also occur in younger individuals. Exact causes for those affected at a younger age have been linked to young men and women who use "smokeless" chewing or spit tobacco. It is also a possibility that those in this younger age group have a causal link which is viral-based, as their exposure to tobacco and other known causative agents is short in time. The ►human papillomaviruses, particularly versions 16 and 18, is implicated in the increasing incidence of young non-smoking oral cancer patients. This is the same virus that is the causative agent in more than 90% of all ►cervical cancers.

Oral cancer carries a poor prognosis and a high rate of recurrence; second primary tumors are estimated to occur at an annual rate of 3–10% and their presence is known to greatly impact survival. Despite the development of new therapeutic options, the overall survival rate for oral cancer has not changed in the last two decades.

Progression

Oral cancer progresses through various histopathological stages – from hyperplasia, various stages of dysplasia, and carcinoma *in situ* (►CIS) to invasive disease. There are two types of precancerous oral lesions: ►leukoplakia and ►erythroplakia. Both show varying degrees of dysplastic change. In general, erythroplakia contains more severe ►dysplasia and is more likely to progress, while incidence of leukoplakia is much higher.

Oral leukoplakia is a white-plaque mucosal lesion that confers increased risk for the development of ►oral squamous cell carcinoma (OSCC). Five to 15 percent of oral white patches are classified as dysplasia. Of these, only 15–20% develop into invasive carcinoma. Treatment for oral leukoplakia is removal via surgery or laser. Because histology of low grade dysplastic lesions is of limited prognostic value, therapeutic intervention is considered only where there is evidence of transition to CIS or invasion. Hence there is a need to identify

molecular markers that predict progression for individual pre-invasive lesions.

Genetics

Oral tumors have been proposed to arise through "►field cancerization", a process where whole tissue exposure to a carcinogen leads to increased risk of multiple pre-malignant lesions or invasive tumors. The frequent occurrence of tumors with dissimilar histology in the same individual, as well as distinct genetic signatures in these tissues are evidence for this phenomenon. With field cancerization, each tumor is thought to arise by clonal evolution of a cell population that is accumulating cancer-causing genetic and epigenetic changes.

Oral cancer generally arises through dysregulation of ►oncogenes and ►tumor suppressor genes (TSGs) that drive cell proliferation. Oncogenes are altered growth-promoting regulatory genes that govern cell signal transduction pathways. Mutation of these genes leads to either overproduction or increased function of the resulting protein. TSGs, on the other hand, are "gatekeepers" of cellular proliferation. They can either inhibit cell growth or promote cell death. Mutations in a number of TSGs and oncogenes are commonly observed in oral cancer. These include ►*RAS*, ►*MYC*, ►*CCND1 (CyclinD1)*, ►*EGFR* (*Epidermal Growth Factor Receptor)*, ►*TP53, CDKN2A* (*Cyclin Dependent Kinase Inhibitor 2A*), and ►*FHIT* (*Fragile Histidine Triad*).

►Allelic loss involving chromosome arm 3p is one of the most frequent and earliest genetic events in the development of ►HNSCC. This loss is significant as several potential TSGs fall in this region. For oral cancer, multiple discontinuous regions of allelic loss support the idea that there are multiple TSGs on this chromosome arm. There is evidence that several genes residing on chromosome 3p are important for tumorigenesis, however the exact role each of these genes plays in the development of disease is still being determined. One gene residing on chromosome 3p that has been implicated in oral cancer is *FHIT*. This large gene, located at 3p14.2, also spans one of the most widely characterized ►fragile sites in the human genome, FRA3B. The *FHIT* gene, a member of the histidine triad gene family, is a target of tyrosine phosphorylation by the SRC protein kinase.

Treatment

Treatment for oral cancer is usually surgery and ►radiotherapy. Chemotherapy may be added to sensitize the malignant cells to radiation, to decrease the possibility of metastasis, or to treat patients with confirmed distant metastases. The stage of the disease at diagnosis dictates which treatment modality is used. Patients who are treated for early-stage cancer may have little in the way of post treatment disfigurement. For those diagnosed with later stage cancer, the results of surgical removal of the disease may require reconstruction of portions of their oral cavity or facial features.

►Oral Squamous Cell Carcinoma

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Oral Squamous Cell Carcinoma

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Synonyms

Oral cancer

Definition

Over 90% of malignant tumors affecting the mouth (including lips, mouth and oropharynx) are ►squamous cell carcinomas (SCC) arising from the lining mucosa. Other types of malignant tumor also develop within the mouth (e.g. salivary gland adenocarcinomas, melanoma, lymphoma); however, the term 'oral cancer' tends to be used synonymously with SCC.

Characteristics

Epidemiology

Oral cancer is estimated by the WHO to be the eighth most common cancer worldwide, with approximately 400,000 new cases each year. It accounts for around 4% of all cancers (5% of cancers in men, and 2% of cancers in women) and 2% of all cancer deaths. Oral cancer

most commonly occurs in middle-aged and older patients, and over 95% of patients are older than 40 years at time of diagnosis (mean age of diagnosis of around 60 years).

The incidence of oral cancer shows striking geographical variation, with around two thirds of all cases arising in the developing world, particularly the Indian subcontinent, which accounts for about a third of all cases. Oral cancer is less common in the western world (USA 30,000 new cases each year, Europe 66,500 new cases each year). Males are affected more commonly than females (worldwide male:female ratio 2:1), although this also varies geographically.

The incidence and mortality from oral cancer is rising in several regions of Europe, Australia, Japan and Taiwan. For example, the oral cancer incidence in the United Kingdom has risen by 19% in the last decade, with an increasing number of young people affected, and 25% of cases having no known risk factors. Nonsmokers are more likely to present with oral cancer at relatively young or relatively old age compared with smokers.

Etiology

It is estimated that around 80–90% of oral cancer cases are due to tobacco use (►tobacco-related cancers) and alcohol abuse (►alcohol-mediated cancers), which act both separately and synergistically. Epidemiological studies suggest that the risk for developing mouth cancer is five to nine times greater for smokers than non-smokers, and may be even higher in heavy smokers. Patients who are both heavy smokers and drinkers can have over a hundred times greater risk for developing a malignancy. Such patients often have a poor diet, and a low consumption of fruits and vegetables is also regarded as a risk factor for oral cancer development.

In India and South East Asia, tobacco may be chewed in the form of ►betel quid (paan). This habit is associated with a vastly increased risk for developing oral cancer, and accounts for the much higher incidence of the disease in these countries. The quid typically, is chewed over a period of many hours and is held in the buccal pouch within the mouth. Typically, tumors arising in betel chewers tend to arise on the buccal mucosa or retromolar area.

►Human papillomavirus (HPV) also has been postulated to play a role in oral cancer pathogenesis. A subset of mouth cancers (particularly oropharyngeal tumors) may result from infection with the oncogenic papillomaviruses, HPV16 and HPV18.

Long-term exposure to ►UV radiation is a major risk factor for lip cancer, due to actinic radiation damage. It is more common therefore in outdoor workers, people who regularly holiday abroad, and who live in sunny climates.

Several conditions are associated with increased risk of developing oral cancer: Submucous fibrosis has a malignant transformation rate of around 7–13%. Whether ►lichen planus is premalignant is more controversial, but it appears that oral cancer may arise in 0.5–2% of patients with this disease. Other rare precancerous conditions, such as syphilitic glossitis and sideropenic dysphagia, are now rare in developed countries.

Genetics

Like most epithelial cancers, oral cancer develops in a multistep process, through the accumulation of genetic and ►epigenetic alterations. Studies have demonstrated multiple genetic abnormalities including amplification or over-expression of various ►oncogenes (*CCND1* (►cyclin D1), ►*MYC*, ►*RAS*, and epidermal growth factor receptor), and deletion, mutation or promoter ►methylation of ►tumor suppressor genes (►*FHIT*, ►*P53*, ►*INK4A* [p16], *RAR-β*). No one change is found in 100% of tumors.

►Loss of heterozygosity (loss of genomic material from one of a pair of chromosomes) occurs at numerous chromosomal loci. Common sites of loss of heterozygosity include 3p, 9p and 17p, sites of tumor suppressor genes encoding proteins involved in the regulation of cell cycle and/or apoptosis (*FHIT, INK4A* and *P53* respectively). The most common genetic changes involve loss of chromosomal region 3p21. This results in inactivation of the p16 gene and occurs early in the development of oral cancer.

Approximately half of oral cancers also contain mutations of the *P53* gene, and the prevalence of mutations is greater in patients who smoke and drink alcohol compared with non-smokers/drinkers. Mutations in ►*RAS* are observed more frequently in patients from India whose cancer is linked to quid chewing.

Genes may also be silenced by promoter methylation. This occurs through epigenetic mechanisms rather than direct loss or mutation of DNA. Studies indicate that the genes most commonly methylated in oral cancer are *INK4A, CDH1* (►E-cadherin), *MGMT* (methylguanine-DNA methyltransferase) and *DAPK1* (death-associated protein kinase 1).

Several rare inherited cancer syndromes predispose to the development of oral (and other) cancers. Cancer predisposition may be caused by alterations in one of three groups of genes: tumor suppressor genes, oncogenes and DNA stability genes. Generally, oral cancer arises in syndromes resulting from defects in DNA stability genes. These include ►Fanconi anemia, ►Bloom syndrome, ►ataxia telangiectasia and ►xeroderma pigmentosum. With the exception of ►Li–Fraumeni syndrome (P53 mutation) oral cancer does not develop in syndromes caused by defective tumor suppressor genes.

►Polymorphisms in genes involved in the metabolism of carcinogens found within tobacco and alcohol have been linked to individual susceptibility to oral cancer. ► It is suggested that people with a null genotype for the ►gluthione *S*-transferase enzymes (*GSTM1* and *GSTT1*) have an increased risk of oral cancer. Other enzymes are implicated in oral cancer susceptibility, including ►cytochrome p450, the N-acetyltranferases and alcohol dehydrogenase, but their role has not been clearly determined. First-degree relatives of people with oral cancer have been reported to be at greater risk of developing the disease, and it is suggested that this possibly is associated with a greater susceptibility to genetic damage by environmental mutagens.

Premalignancy

Oral cancer develops in a multi-step process requiring a step-wise transition from normal mucosa through pre-malignant lesions to overt malignancy. This transition occurs as cells accumulate genetic damage and may alter the appearance of the oral mucosa both clinically and histologically.

Pre-malignant lesions usually present as a white patch (leukoplakia), less commonly as a red patch (erythroplakia) or a mixed red/white patch (speckled leukoplakia). Microscopically, the oral mucosa may show pre-malignant dysplastic changes, graded as mild, moderate or severe dysplasia. The risk of a cancer developing from such lesions is though to be around 10–15%. However, ability to predict the malignant potential of these lesions based on pathologic findings is very limited; transformation does not always correlate with the degree of dysplastic change and, additionally, tumors can develop from histologically normal mucosa. Several studies have found that early genetic changes do not result in observable changes in tissue morphology.

In an effort to predict malignant transformation more effectively, attempts have been made to use molecular biological markers to assess cancer risk. These have included biomarkers of genomic instability such as ►aneuploidy (abnormal number of chromosomes) and allelic imbalance (loss of heterozygosity). Although studies suggest that these approaches may be better than current histological practice, no single method is yet regarded as powerful enough to predict the risk of oral cancer development.

Treatment of premalignancy usually involves excision of lesions, which are graded as moderately or severely dysplastic (although studies suggest that this does not significantly alter the risk of future malignancy). However, patients may have wide fields of genetically altered mucosa, which are too extensive to be treated surgically. Researchers have investigated chemopreventive agents in an attempt to prevent both malignant progression and recurrence of cancer after surgery. Synthetic ►vitamin A derivatives (►retinoids), such as isoretinoin and fenretinide, have been tested, sometimes in combination with interferon-α and vitamin E. Results have been variable; there has been some success in reversing clinically and histologically evident dysplasia, however, genetic abnormalities persist. Compounds such as the PPARγ inhibitor, Rosiglitazone, and ►non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit the cyclooxygenase enzymes (COX) currently are being investigated. The conditionally replicating ►adenovirus, ►ONYX-15, which destroys cells containing defective p53 protein has also been tested.

Clinical Features

The majority of oral cancers present as a non-healing ulcer or growth. The sites most commonly affected are the tongue and floor of mouth. Patients with posteriorly-located tumors have a decreased 5-year survival, mostly because of increased risk of ►metastasis to cervical lymph nodes.

Symptoms vary according to site and include pain, bleeding, paraesthesia, anaesthesia, restriction of tongue movement, limitation of mouth opening, loosening of the teeth and enlarged lymph nodes in the neck. However, patients may experience few symptoms, possibly explaining why around two thirds of cancers are diagnosed when the disease is at an advanced stage.

Clinically tumors are staged using the TNM classification. Tumor size and status of lymph nodes are the most reliable clinical prognostic indicators. A large tumor size at presentation (greatest tumor surface diameter) is associated with increased risk of lymph node metastasis, local recurrence, and poor survival.

Multiple cancers may develop synchronously or metachronously in the upper aerodigestive tract, and the reported frequency of second (or subsequent) primary tumors in patients with oral cancer lies between 10–30%. Evidence suggests that secondary tumors may develop through two different mechanisms; either arising independently in a field of defects (►field cancerization) or via clonal spreading of transformed cells from the primary site.

Pathology

Although various ►biomarkers have been suggested as being useful for predicting prognosis and determining treatment, post-operative management of the patient still, to a large extent, is determined through detailed pathological examination of the tumor resection specimen. Most importantly, this determines whether the tumor has been completely removed; positive excision margins are regarded as one of the main causes of local recurrence and poor outcome. Other pathological parameters of the primary tumor, which are discussed

below, also have a bearing on patient prognosis. Additionally, a ►neck dissection involving the removal of cervical lymph nodes often is performed, and these nodes are examined for evidence of metastatic spread.

There are different pathological subtypes of oral cancer. Most cancers are of conventional type; other less common variants include verrucous, basaloid, papillary, spindle, acantholytic, adenosquamous and carcinoma cuniculatum. Prognosis may vary according to subtype. For example verrucous carcinomas rarely metastasize and have a better prognosis than conventional SCC. On the other hand, basaloid squamous cell carcinomas are aggressive tumors that metastasize widely.

Conventional oral cancers are graded as well, moderately or poorly differentiated depending on the degree of keratin production and cell/nuclear pleomorphism. It is now recognized that histological grading of the whole tumor is a poor predictor of patient outcome, probably because around 90% of oral cancers are designated moderately differentiated. However, several studies have shown that the grade and pattern of invasion of the deep advancing edge of the tumor has prognostic value. This 'invasive front' grading has been shown to be useful in predicting local recurrence, lymph node metastasis and survival. Spread of tumor along nerves or into vessels (perineural and lymphovascular invasion respectively) may also have roles in predicting patient outcome.

Although clinically the tumor diameter is used to TNM stage the patient, tumor thickness (depth of invasion) is a more accurate predictor of overall survival. Critical tumor thickness appears to vary according to site affected within the mouth. However on average, tumors greater than 4 mm in thickness have a fourfold increased risk of metastasis compared with thinner tumors.

The number of positive lymph nodes containing tumor and the size of the metastatic deposit are used in TNM staging, and are the most important factors in determining prognosis. Generally, prognosis is halved if metastases are present, and halved again if extracapsular spread is present (spread of metastatic tumor beyond the lymph node into adjacent tissues).

Treatment and Management

A number of different imaging techniques may be used to determine the extent of the primary disease and the possible involvement of neck nodes. These include plain radiography, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound and positron emission tomography (PRT). At present, patients with oral cancer usually have a staging CT or MRI scan. There is debate about whether the chest should be included (CT chest) since the incidence of chest metastasis is quite low.

Despite many advances in imaging technology, surgical techniques, radiotherapy, and chemotherapy, oral cancer remains associated with marked morbidity and a 5-year survival rate of less than 50%, which has not improved for decades. This may possibly reflect the fact that most patients are diagnosed when the disease is in an advanced stage (III and IV). Surgery and radiotherapy either alone or in combination are the main primary treatment modalities. The role that chemotherapy has to play is more controversial.

Treatment typically is stage related and the most important factors in determining the type of treatment is the site and size of the primary tumor, and the status of the cervical lymph nodes. Early disease may be treated either surgically or with definitive radiotherapy with similar efficacy. Radiotherapy is avoided if the tumor lies close to, or involves, bone because of the risk of osteoradionecrosis. In advanced disease and for patients at high risk of local recurrence combined therapy is used with surgery combined with postoperative radiotherapy. Chemotherapy may also be used and several studies have shown that the addition of ►cisplatin to postoperative radiotherapy improves the outcome among high-risk patients. Treatment-related morbidity, particularly of advanced tumors, is a significant problem and includes problems with swallowing, and speech as well as cosmetic deformities.

Patients with metastatic disease in cervical lymph nodes require a neck dissection. If multiple nodes are involved and/or there is evidence of extracapsular spread this usually is followed by radiotherapy. The management of patients that do not have clinically detectable disease in the neck is more debatable; the choices being an elective neck dissection, radiotherapy or close monitoring of the patient.

Future Directions

It is clear that the current treatments available for oral cancer are, to a certain degree, inadequate. The overall survival rate of oral cancer patients has not improved for decades and surgery, radiotherapy and chemotherapy all are associated with significant morbidity, damaging normal tissues, as well as tumor cells. A number of different molecules are over-expressed in oral cancer and have been suggested as potential therapeutic targets. These include the epidermal growth factor receptor (EGFR), COX-2 and the integrin αvβ6. Reagents developed to target these proteins include NSAIDS, monoclonal antibodies and chemical tyrosine kinase inhibitors. Adenoviral gene therapy and immunotherapy using autologous dendritic cells also are being considered.

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Orchiectomy

Definition

A surgical procedure to remove one or both testicles, the main source of male hormones, to decrease hormone production. It has the same efficacy and same side effects as LHRH analogs therapy.

►Adjuvant Chemoendocrine Therapy
►LHRH Agonists

ORG 2766

Definition

A neuroprotective chemoprotectant that slows down ►cisplatin- and ►taxol-induced neuropathy, neurotoxicity, and ototoxicity.

►Chemoprotectants

Organ Cultures

Definition

Tissues generally cultured, often within a network of perfused artificial capillaries or other bioreactor, so that functional systems may be studied. They have been used to study tumor invasion and have potential for studying tumor cell and normal tissue drug metabolism and toxicity and complex microenvironmental interactions involved in tumor physiology.

►Organotypic cultures
►Three-Dimensional Tissue Cultures

Organotypic Cultures

Definition

Aggregates of cells usually grown as cultures embedded in a porous extracellular matrix that promotes tissue like architecture and cell-cell interactions. They are considered to more accurately reflect the tumor microenvironment than monolayer cultures. They give consistent results, are more easily manipulated than organ culture, and many human cell lines may be used. Cancer cells are generally co-cultured with syngeneic stromal immune and endothelial cells to provide further approximation to the in vivo physiology. They do not fully integrate physiological function such as pressure gradients or blood flow and do not model therapeutic index in therapeutic studies. They are used to study cancer cell biology, cell interactions, migration and invasion. They have potential for studying drug mechanism of action and drug screening.

►Three-Dimensional Tissue Cultures

Origin of Replication

Definition

Is the site where the DNA helix is melted and initiation of DNA synthesis occurs. Mammalian cells are estimated to use between 50,000 and 100,000 origins of replication per cell cycle.

►S-Phase Damage-Sensing Checkpoints

Origin Replication Complex

Definition

Orc; A complex of six proteins originally discovered in yeast. Genetic and biochemical data strongly suggests Orc is required for initiation of replication from chromosomal origins.

►S-Phase Damage-Sensing Checkpoints

Orlistat

Definition
A potent natural inhibitor of pancreatic lipases. It was isolated from the bacterium Streptomyces toxytricini and has been used to treat obesity. Orlistat is also a selective inhibitor of fatty acid synthase by binding the thioesterase domain of fatty acid synthase.

►Fatty Acid Synthase

Ornithine Cycle

Definition
►Urea Cycle

Ornithine Decarboxylase

Definition
ODC; The rate limiting enzyme of polyamine biosynthesis. ODC converts ornithine (itself derived from arginine) to putrescine and the other polyamines. This enzyme is irreversibly inhibited by the chemopreventive agent, difluoromethylornithine (DFMO).

►Arginine

Ornithine Transcarbamylase

Definition
OTC; synonym ornithine carbamoyltransferase; Is an enzyme that catalyzes the reaction between carbamoyl phosphate and ornithine to form citrulline and phosphate. In mammals it is located in the mitochondria and is part of the urea cycle.

►Arginine-Depleting Enzyme Arginine Deiminase

Orphan GPCR

Definition
Orphan G-protein coupled receptors are members of the GPCR family of transmembrane receptors based on their sequence homology, but have no known ligand.

►Orphan Nuclear Receptors

Orphan Nuclear Receptors

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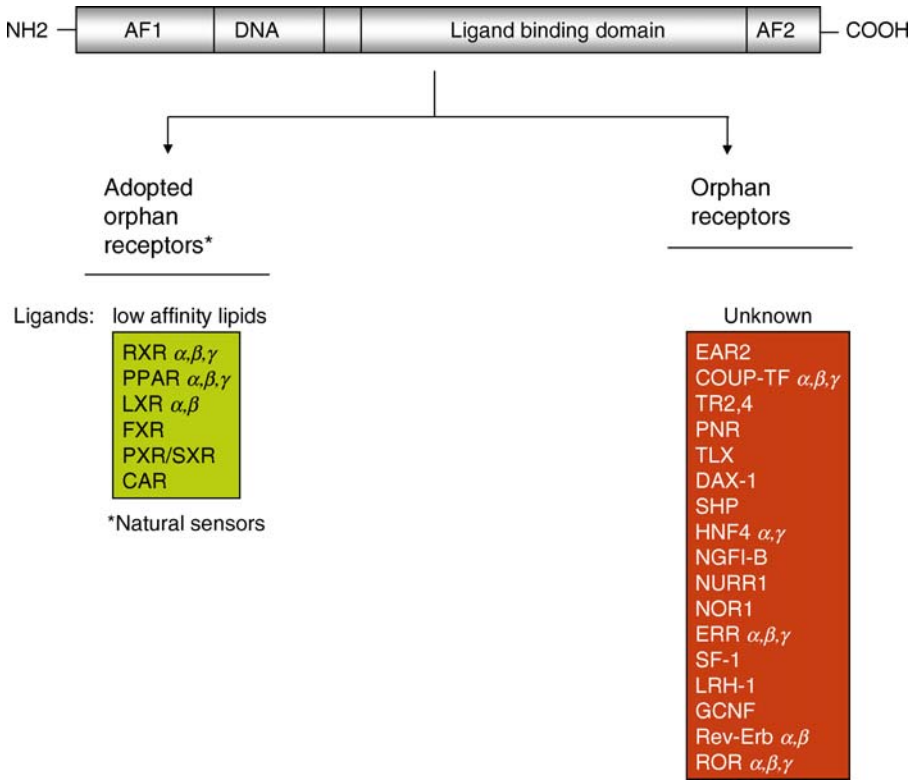
Synonyms
Adopted orphan nuclear receptors; Receptors with no ligand-binding pocket; Receptors with empty ligand-binding pockets; Receptors with structural ligands; Receptors regulated by ligands

Definition
The word "orphan" means lacking "support, supervision or care" (The American Heritage® dictionaries) and when applied as an adjective to ►nuclear receptors implies that these receptors do not have a defined ►ligand (for which no ligand is known). This definition is paradoxic in the sense that a "receptor" implies that there is a physiologic ligand even though that ligand has not been discovered. At present, there is no consensus in the field with regard to the presence or absence of ligands for all orphan receptors. Once a physiologic ligand is discovered for a particular receptor, then that receptor no longer belongs in the "orphan" category (adopted orphan receptors); however, there are examples of liganded receptors (e.g., RXR, PPARs) where the controversy surrounds the identity of the true physiologic ligand (Fig. 1).

Characteristics
Orphan nuclear receptors are a diverse group of nuclear receptors characterized by one or more of the following features:

1. Lacks a ligand that defines a physiologic or pathophysiologic phenomenon. Compounds have been found inside the ligand binding pocket of some



Orphan Nuclear Receptors. Figure 1 Schematic structure of orphan nuclear receptor superfamily present in human, mouse and rat. A schematic structure of a typical nuclear receptor is shown describing the activation factor domains (AF 1 and 2), the DNA binding domain (DNA) and the ligand binding domain (which incorporates AF-2). Low affinity dietary lipids serve as natural ligands to receptors classified under the adopted orphan status ▶in *green* (these receptors are natural sensors of the environment) and receptors whose ligands have not yet been characterized as orphan receptors ▶in *red* (figure is adapted and modified from Chawla A et al Science 30 November 2001:vol 294, no 5548, pp 1866–1870).

receptors (e.g., SF-1, LRH-1) but its physiologic relevance is unknown.

2. The ligand binding pocket is larger than that observed for classic nuclear receptors (e.g., steroid receptors) and this feature allows them to bind to a diverse group of molecules with lower affinity (usually in the micromolar range).
3. Domain variations are the largest amongst those seen with nuclear receptors. For example, in vertebrates, DAX-1 and SHP do not have a classic DNA binding domain (DBD) but only a ligand binding domain (LBD).
4. There is extreme diversity of these receptors in terms of their mode of binding to DNA. Most bind as homo dimers on direct repeat (DR) elements (e.g., HNF-4, TR2/4), others interact with RXR (e.g., NGFI-B, NURR1) while others like GCNF oligomerize upon binding to hexameric repeat AGGTCAAAGGTCAA. Monomeric binding to half-site DNA sequences defines orphan nuclear receptors (e.g., SF-1, LRH-1, ERRs bind to TCAA/GGGTCA elements). In all monomeric interactions with DNA, the interaction between DBD of the receptor and DNA is in the A/GGGTCA motif. There is a recognition helix in the C-terminus of the first zinc finger interacting with the major groove of DNA. The second helix in the second zinc finger stabilizes the interaction with the DNA, which then allows for dimerization with its partner proteins. The C-terminal portion of the receptor is able to interact with the extended 5′ element (e.g., in the structural analysis of Rev-erb DBD associated with DNA, there is a core DBD and an A box beyond this core which forms a third helix of the DBD and is implicated as a recognition site for 5′ extension DNA element).
5. The biological functions of orphan receptors are diverse and yet not fully characterized. However, two principles have emerged. One, these receptors have an important function that is unique to each receptor. Two, these receptors play an important role in modulating the function of classic liganded receptors.

6. The biological function of orphan receptors is clear and unambiguous. A summary of this with regard to individual orphan receptors is shown in Table 1.

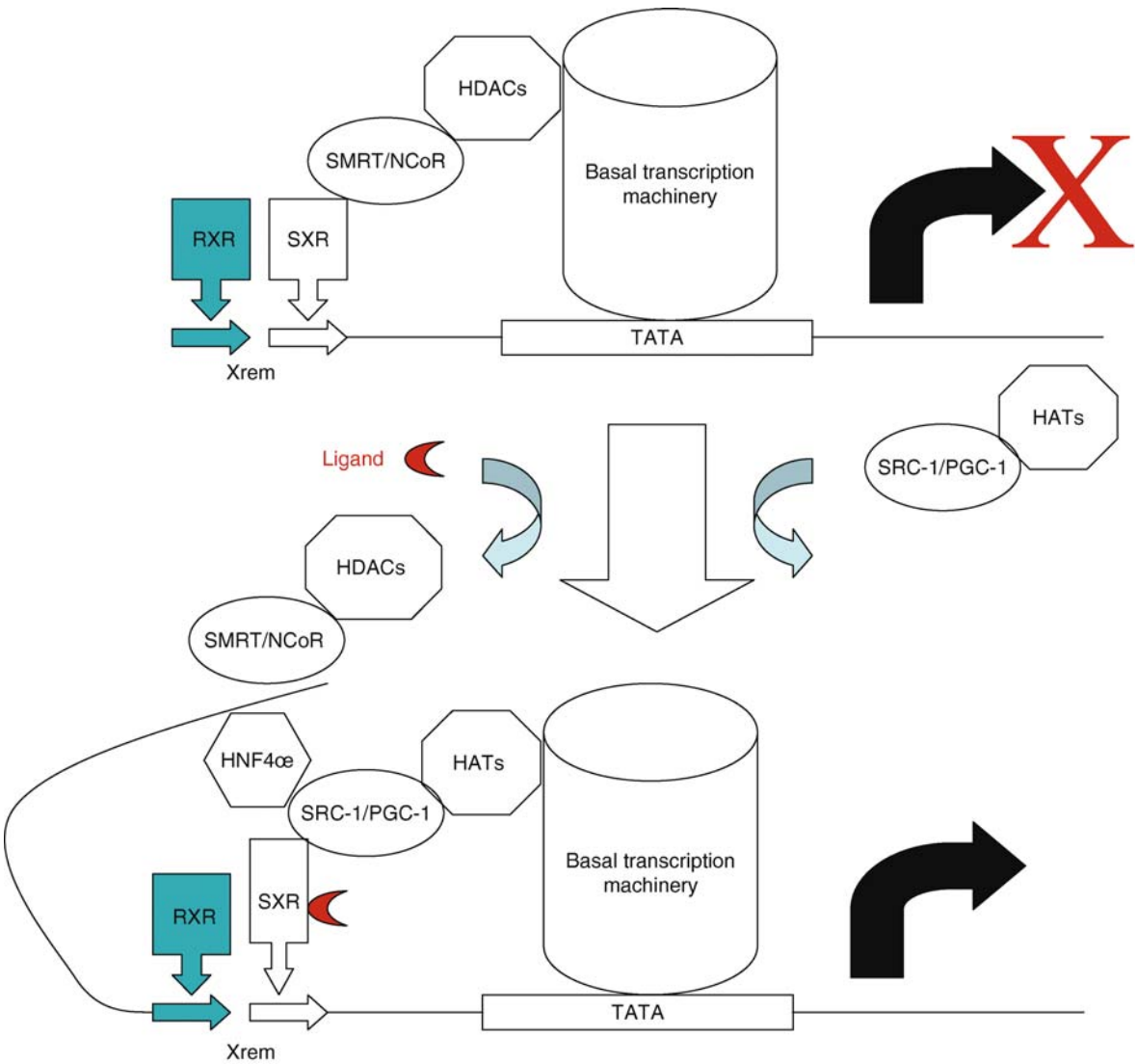
Mechanism

Orphan receptors, like their classical receptor counterparts, reside or translocate to the nucleus upon ligand binding. They are either basally repressed or more commonly constitutively active and their activity may be enhanced upon binding to their cognate ligand. A classical model for nuclear receptor action implies that these receptors also bind to co-repressors and other ►co-regulators (►transcriptional coregulators). In this model, co-repressor complexes that can recruit and alter histone deacetylases mediate basal repression. Several known co-repressors that are involved include silencing mediator of retinoid and thyroid receptor, (SMRT), and nuclear receptor co-repressor (NCoR), and their associations are tissue specific. N-CoR and SMRT are paralogs of each other and are encoded by distinct genetic loci and exert overlapping biological functions. Both N-CoR and SMRT operate by tethering to their ►transcription factor partners and nucleating the assembly of a larger, functional co-repressor complex. SMRT and N-CoR tether to nuclear receptors through a set of C-terminal "CoRNR box" motifs. A secondary

Orphan Nuclear Receptors. Table 1 Orphan nuclear receptor phenotype and its biologic implications to human disease. The mutant phenotype is almost always characterized in mice either as a knock-out phenotype or a Cre/Lox knock-out (*Mouse Models)*

EAR2 (NR2F6)	Circadian gene expression/behavior	Human disease
COUP-IF1 (NR2F1)	Non-viable at birth	?
COUP-TFII (NR2F2)	Embryonic lethal (day	?
TR2 (NR2C1)	Fertile	?
TR4 (NR2C2)	Reduced spermatogenesis	?
	Defective reproduction	
	Growth retardation	
PNR (NR2E3)	Retinal degeneration	Enhanced S-cone syndrome
		Retinitis Pigmentosa
		Goldmann-Favre syndrome
TLX (NR2E1)	Reduced forbrain size, increased aggression	?
DAX- 1 (NR0B1)	Delayed testis development, DS S syndrome	Hypogonadotropic hypogonadism
SHP (NR0B2)	Increased bile acid synthesis	Obesity
HNF-4(NR2A 1) α, β	Embryonic lethal; adults increased hepatic lipids decreased serum cholesterol and triglyceride	Early-onset type II diabetes
NGFI-B (NR4A1)	No clear phenotype	?
NURR1 (NR4A2)	Loss of ventral dopaminergic neurons; death at birth	Parkinson's disease
NOR1 (NR4A3)	Inner ear defects; bidirectional circling behavior	Extraskeletal myxoid chondrosarcoma
ERRα (NR3B 1)	Reduced body weight and peripheral fat; resistant to high-fat diet obesity	Osteoporosis
SF-1 (NR5A 1)	Lack adrenal glands and gonads	Adrenocortical insufficiency
LRH- 1 (NR5A2)	Embryonic lethal; adults (+/-) are hypercholesterolemic	?
GCNF (NR6A 1)	Cardiovascular abnormalities, defective trunck development, embryonic lethal; aberrant estrous cycle (crc-Lox knockouts)	?
Rcv-Erb α	Cerebellar abnormalities; cicadian rhythm changes	?
Rcv-Erb β	?	?
ROR α	Stagger phenotype (cerebellum)	?
ROR β	Duck-like gait, disrupted reproduction in males, blindness, abnormal circadian rhythm	?
RORγ	Lack peripheral and mesenteric lymph nodes and Pcyer's patches	?

hierarchy of co-repressor subunits, including histone deacetylases, TBL-1, TBLR-1, GPS-2, and a number of other modulatory and effector proteins are recruited through docking surfaces (repression domains, RDs) located principally in the N-terminal and central regions of SMRT and N-CoR. Co-activator recruitment after ligand binding is complex and tissue-specific and includes, but may not be limited to, SRC-1, PGC-1 and other regulators like SHP and HNF-4. There is cross talk between distal CAR/PXR sites and HNF4α binding sites in the CYP2C9 promoter and the HNF4α sites are required for maximal induction of the CYP2C9 promoter. Ligand-activated PXR interferes with HNF-4 signaling by targeting the common co-activator PGC-1, which underlies physiologically relevant inhibitory cross talk between drug metabolism and cholesterol/glucose metabolism (►Receptor cross-talk). SHP is defined as a negative regulator of PGC-1-hNF-4 interactions on PXR. This observation suggests that perhaps HNF-4 directly interacts as a co-activator of the SXR-PGC-1 complex (Fig. 2). There are other modes of gene transcription mediated by orphan receptors and involved in trans-repression or activation (e.g., SHP), and these modes vary from receptor to receptor.



Orphan Nuclear Receptors. Figure 2 Cartoon schema of transcriptional repression and activation by SXR and its co-regulators. In the absence of ligands, denoted as a red half moon, SXR, steroid and xenobiotic receptor (also known as human (h) PXR) is repressed. In the presence of a ligand, significant structural changes alter SXR-co-regulator interface, resulting in the recruitment of co-activators and the histone acetyltransferase (HAT) complex. The net result is transcriptional activation).

Orphan receptors are critical in development and ►cell differentiation. For example, HNF-4 is critical in liver development but in adults alterations lead to significant changes in hepatic lipid accumulation. TFs are fundamental to the development of the nervous system, NURR members for brain development, GCNF for silencing of pluripotency gene expression during gastrulation and ES cell differentiation, and TLX and PNR in retina formation. Orphan receptors are key regulators of cellular metabolism, adipogenesis and energy metabolism. For example, LRH-1 regulates cholesterol metabolism and SF-1, steroidogenesis. A more recently explored physiologic phenomenon is that of regulating ►circadian rhythm. In this context, Rev-erbs and RORs are key members of the circadian pacemaker system within peripheral tissues and supra-chiasmatic nucleus. Furthermore, orphan receptors modulate liganded receptor activity. Receptors such as COUP-TFs, TR2, and TR4 repress activation mediated by liganded receptors like RAR, TR and PPAR. DAX-1 and SHP regulate the activity of several orphan and non-orphan receptors like PXR and steroid receptors.

Clinical Aspects

The role of orphan receptors in cancer is still evolving. A clear example of the role orphan receptors may play with regard to cancer therapeutics is their effect on controlling drug metabolism. For example, paclitaxel metabolism is significantly impacted by the activation of PXR and such paclitaxel serves as an agonist of PXR activity (►Xenobiotics). Paclitaxel mediated activation of PXR may accelerate its own metabolism through induction of CYP3A4 and CYP2C8 genes, lowering blood levels and the activity of the drug. A recurrent t (9; 22) (q22; q12) ►chromosome translocation has been described in extraskeletal myxoid chondrosarcoma (EMC) and both NURR1 and NOR1 have been implicated as an example of the oncogenic conversion of a nuclear receptor and the first involving the orphan subfamily. Chimeric fusions of NOR-1 with ►EWS, TCF12 and TAF2N have been linked to ►extraskeletal myxoid chondrosarcoma. The oncogenic activity of EWS/FLI1 in ►Ewing sarcoma may be mediated in part by upregulation of DAX-1. Furthermore, the role of the NR4A subfamily members in cancer is also largely defined by the implication of the subfamily in the regulation of ►apoptosis. Apoptosis in many tumor types, including colon, breast, prostate, lung, and gastric cancers, have been shown to involve the NR4A family members. In another example, RORs have been implicated in mitigating the proliferative effects of fatty acids on androgen dependent and independent ►prostate cancer cells. DAX-1 may inhibit endometrial carcinomatosis through its interactions with ER. In liver carcinogenesis, the orphan receptor, CAR, is essential for liver tumor promotion by phenobarbital. Thus orphan receptors can regulate the process of carcinogenesis. Cellular translocation of nuclear receptors may underlie the pathophysiology of apoptosis inducing agents in cancer. For example, the orphan receptor NURR77 translocates to mitochondria in the early phases of apoptosis induced by chenodeoxycholic acid in stomach cancer cells. All-trans ►retinoic acid (ATRA), a primary mode of therapy for ►acute promyelocytic leukemia, is a ligand for the orphan receptor RORβ. TR3 orphan receptor may play an important role in modulating drug-induced prostate cancer apoptosis. COUP-TFII is required for ►angiogenesis and may play an important role in cancer-mediated angiogenesis. ERRs play a role in human breast cancer, as its expression is associated with certain biomarkers predicting outcome. LRH-1 has been implicated in breast carcinogenesis and regulates the activity of P450 aromatases in surrounding adipose tissue and hence serves as an important target for therapy in breast cancer. More recently, LRH-1 has been implicated in intestinal tumorigenesis through effects on cell cycle and intestinal inflammation, both linked to carcinogenesis. The PPARs have been widely known to be associated with a variety of tumor types, in which it may represent a link between diet and increased risk of cancer. Furthermore, PPARs may serve as targets for cancer therapy as agonists are known to induce apoptosis in a variety of cell lines (e.g., breast, colon).

In patients with breast cancer, ERR and ERR status may be predictive of sensitivity to hormonal blockade therapy, and ERR status may also be predictive of ErbB2-based therapy such as ►Herceptin. Moreover, ERR and ERR status are candidate targets for therapeutic development. Synthetic agents blocking RXRs (e.g., bexarotene) have been approved for treatment of ►cutaneous T-cell lymphoma. They serve as examples of translational capabilities, as we begin to find out more about the biologic function of orphan receptors.

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Orthologue

Definition

Synonym ortholog; Describes the same gene in different species, e.g. c-R*mil* in chicken *versus* *BRAF* in mammals. Genes in different organisms that are direct evolutionary counterparts, i.e., they are related by descent from a common ancestor. As orthologues often retain their biological function during evolutions they are subject to less sequence variation than a ►paralogue.

Orthotopic

Definition

Refers to the implantation of ►xenografts of cancer cells in the organ where the primary colony of cells were originated.

Orthotopic Model

Definition

Tumor models produced by transplanting cancer cells into an anatomically appropriate location in the host organism, which is usually a mouse. The transplanted cells can be xenografts (from a different species) or allografts (from the same species).

►Ultrasound Micro-Imaging

OSF-1

►Pleiotrophin

OSM

►Oncostatin M

Osmotic Pump

Definition

A small, implantable and non-mechanical device that regulates the continuous dosing of a chemical into the body via the process of osmotic displacement.

►Bioluminescence Imaging

Osteoblast

Definition

Mononucleated cell that produces bone matrix proteins and take charge of bone mineralization. Osteoblasts share a common progenitor, the undifferentiated mesenchymal cell, with chondrocytes, myocytes and adipocytes.

►Bisphosphonates

Osteoblast Specific Factor

►Pleiotrophin

Osteoblast-specific Factor-2

►Periostin

Osteoblastic Bone Disease

►Bone Loss, Cancer Mediated

Osteoblastic Bone Lesion

Definition

Are characterized by mineralized or calcified deposition into the lesional tissues. The most current malignant osteoblastic lesions are bone metastases from ►prostate cancer. These lesions are associated with a deregulation of osteoblast activities, the specialized bone forming cells.

►Zoledronic Acid
►Bone Loss, Cancer Mediated

Osteoblastic Metastasis

Definition

►Metastasis that is characterised by the ability to promote the formation of bone.

►Bone Loss, Cancer Mediated

Osteochondroma

Definition

Benign peripheral cartilaginous tumor. Osteochondromas arise at the surface of bone and consist of a reactive bony stalk containing a marrow cavity that is continuous with that of the underlying bone, covered by a neoplastic cartilaginous cap. A exophytic growth composed of a cap of proliferating hyaline cartilage that transforms to bone by the process of endochondral ossification during the time of active growth.

►Bone Tumors
►Chondrosarcoma

Osteochondromyxoma

Definition

►Osteochondroma containing myxoid elements.

Osteoclast

Definition

Are multinucleated cells specialized in the bone degradation process. Differentiated cells of the mononuclear phagocytic lineage that resorb bone. Secretes acid and hydrolytic enzymes which dissolve the mineralized and organic components of the bone matrix. Osteoclasts are closely to the ►macrophage lineage, are located in the bone marrow and are formed by fusion of mononuclear precursors.

►Bone Loss, Cancer Mediated
►Zoledronic Acid

Osteogenic Sarcoma

►Osteosarcoma

Osteolysis

Definition

Is a bone disease originate from various aetiologies (tumor, infection, trauma) characterized by focal or multi-focal bone destruction associated with an excessive ►osteoclast activity.

►Bone Loss, Cancer Mediated
►Zoledronic Acid

Osteolytic Bone Disease

►Bone Loss, Cancer Mediated

Osteolytic Metastasis

Definition
Metastasis that is characterised by the ability to promote ►osteolysis.

►Bone Loss, Cancer Mediated

Osteoma

Definition
Is a benign, local growth usually confined to the facial skeleton of patients with ►familial adenomatous polyposis (FAP), in particular in mandibular and maxillary bones. It is often visible by X-ray, more seldom as external swellings, and is a good diagnostic predictors for FAP.

►APC Gene in Familial Adenomatous Polyposis

Osteomimicry

Definition
The acquisition of bone cells' genotypic and phenotypic properties by cancer cells with ►bone tropism.

Osteonectin

Definition
Is a glycoprotein in the bone that binds calcium.

►Doublecortin
►Secreted Protein Acidic and Rich in Cysteine

Osteopetrosis

Definition
Skeletal abnormality characterized by increased bone mass.

►RANK–RANKL Signaling

Osteopontin

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Synonyms
2ar; Eta-1; Bone sialoprotein; Secreted phosphoprotein 1

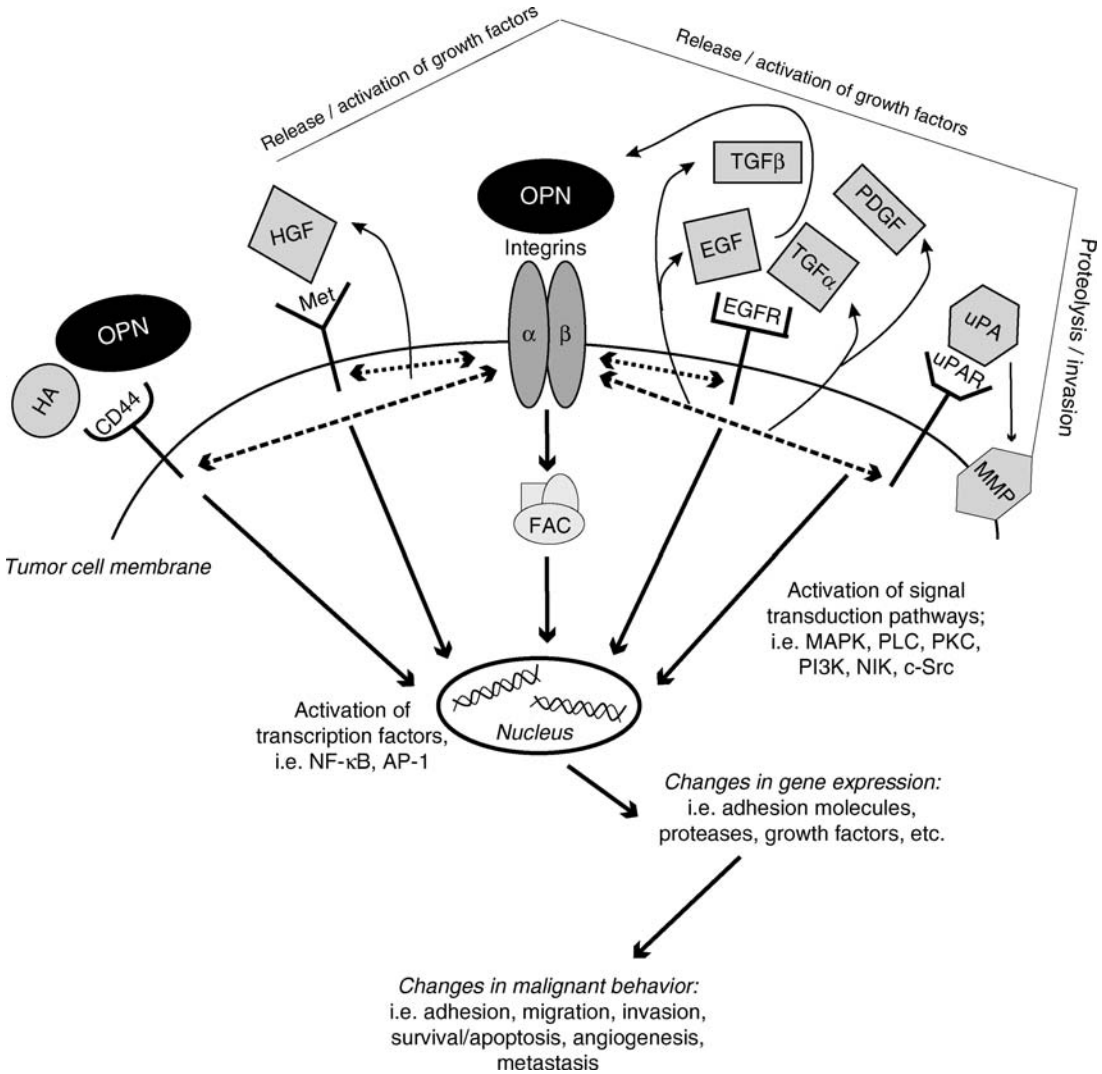
Definition
Osteopontin (OPN) is an acid-rich, phosphorylated mammalian ►glycoprotein that is a member of the small ►integrin-binding N-linked glycoprotein (SIBLING) family of proteins. Osteopontin is synthesized and secreted by tumor cells and by many normal cell types, including cells in the mammary gland, the vasculature, the bone, the immune system, and the kidney.

Characteristics
OPN Gene Structure and Regulation of Gene Expression
The OPN gene has been identified in many different species, including human, mouse, rat, rabbit, cow, and pig, with approximately 40% homology of nucleic acid sequence between species. The human OPN gene is located on the long arm of chromosome 4 (4q21–4q25) and contains seven exons spanning approximately 11.1 kb. The OPN promoter contains several potential regulatory sequences that can mediate transcription of OPN in response to a wide variety of stimuli in different tissue types. These sequences include GATA-1, TATA-like and CCAAT-like motifs, as well as a ►Ras-activated enhancer sequence and ►vitamin-D-responsive elements (VDREs). Many other potential binding sites for ►transcription factors such as ►AP-1, PEA3, ►Ets-1, TCF-1, ►E2A, E2BP, ►Myb, AP2, SP1, and Oct-1 have also been characterized. The

number and diversity of regulatory elements present in the OPN promoter are reflective of the fact that OPN expression is controlled by complex regulatory pathways that influence both normal and malignant cell behavior. In particular, the expression of OPN can be upregulated by a number of cancer-related ►growth factors, including ►epidermal growth factor (EGF), ►scatter factor/►hepatocyte growth factor (HGF), ►platelet-derived growth factor (PDGF), ►vascular endothelial growth factor (VEGF), and ►transforming growth factor β (TGFβ), and this upregulation often results in increased OPN-mediated malignancy of tumor cells (Fig. 1).

OPN Protein Structure and Interaction with Other Proteins

The human OPN protein contains approximately 314 amino acid residues, and is particularly rich in aspartate, glutamate, and serine residues. The molecular weight of OPN and associated isoforms has been reported to vary between 41 and 98 kDa depending on the cellular source. Extensive post-translational modifications in the form of ►sulfation, ►phosphorylation, and/or ►glycosylation can lead to cell type and condition-specific differences, and likely accounts for variability in molecular weights. The OPN protein contains several conserved structural elements including heparin- and



Osteopontin. Figure 1 Functional contribution of OPN to malignant cell behavior. This schematic diagram is a representation of how OPN can help tumor cells to both respond to and influence the tumor microenvironment. As discussed in the text, OPN-mediated molecular cross-talk between itself and other tumor-derived and host-derived factors such as integrins, proteases, and growth factor/receptor pathways has the potential to influence tumor and host cell behavior through multiple different signaling pathways.

calcium-binding domains, a thrombin-cleavage site, a CD44 binding site, and an ►RGD (Arg-Gly-Asp) integrin-binding sequence. Based on the presence of these domains, it is not surprising that the functional importance of OPN lies in its ability to interact with a diverse range of factors, including cell surface receptors (►integrins, ►CD44), ►growth factor/receptor pathways (►VEGF, ►HGF/Met, TGFα/EGFR), and secreted ►proteases (►urokinase-type plasminogen activator (uPA), ►matrix metalloproteinases (MMP)). These complex signaling interactions can result in changes in gene expression which ultimately lead to increased malignant cell behavior and cancer progression (Fig 1).

Functions of OPN in the Context of Cancer

OPN is involved in a number of normal and pathologic conditions, including mammary gland development, lactation, bone remodeling, vascular remodeling, immune reactivity/inflammation, renal disease, and cancer. OPN can be produced by many cell types, such as bone cells (osteoblasts, osteocytes, osteoclasts), fibroblasts, inflammatory cells (►T-cells, ►natural killer (NK) cells, ►macrophages), endothelial cells, epithelial cells, and tumor cells. In the context of cancer, OPN can functionally contribute to several types of malignant cell behavior which are discussed below. OPN-mediated tumor cell malignancy is largely dependent on its interaction with other factors such as integrins, proteases, and growth factor/receptor pathways (Fig 1). Additionally, highly aggressive tumor cells tend to not only express high levels of endogenous OPN, but also seem to be more responsive to exogenous OPN produced by other cells in the tumor ►microenvironment.

OPN-Mediated Cell Adhesion

OPN plays an important role in cell ►adhesion through direct interaction with cell surface integrins such as αvβ1, αvβ5, αvβ3, α4β1, α8β1, and α9β1. These interactions serve to activate downstream integrin signaling and subsequently increase cell adhesion and malignancy of tumor cells. In addition, OPN can also directly interact with another cell surface receptor, CD44, in order to mediate cell adhesion.

OPN-Mediated Cell Migration and Invasion

OPN can promote cell ►migration and ►invasion, two important components of the ►metastatic process. Experimental studies have demonstrated that OPN-mediated migration is dependent on its interaction with cell surface integrin receptors (as described above), in particular the αvβ3 integrin. In addition, OPN can upregulate ►uPA and members of the MMP family, and these proteases provide cells with the ability to generate localized proteolytic activity at the tumor-host interface in order to mediate cell invasion and migration through the ►extracellular matrix. The growth factor/receptor pathways ►EGF/►EGFR and ►HGF/►Met are upstream and downstream effectors of OPN and have also been shown to influence OPN-mediated migration and invasion.

OPN-Mediated Cell Survival/Resistance to Apoptosis

OPN can confer resistance to ►apoptosis in cancer cells through a variety of mechanisms related to evasion of ►immune surveillance. Direct interaction between OPN and the αvβ3 integrin or CD44 can protect tumor cells in the circulation through inhibition of ►complement-mediated cell death. Furthermore, OPN can inhibit induction of inducible ►nitric oxide synthase (iNOS) by immune cells, thus protecting the tumor cell against nitric oxide-mediated cell death and allowing tumor progression.

OPN and Angiogenesis

OPN can contribute to neovascularization and ►angiogenesis in various animal models of cancer. The mechanisms by which this occurs are largely hypothetical, but are believed to involve molecular cross-talk between OPN, the αvβ3 integrin, and/or VEGF. In particular, signaling through αvβ3 is critical for endothelial cell survival, and OPN can enhance this survival.

OPN and Metastasis

Given the involvement of OPN in all of the malignant behaviors described above, it is clear that OPN plays a central role in cancer as a ►metastasis-promoting factor. OPN has been shown to contribute to experimental metastasis in a number of animal models of cancer, in particular to ►breast cancer metastasis to the lymph nodes and the bone via interactions with integrins and/or growth factors. Furthermore, OPN-deficient mice injected with ►melanoma tumor cells show reduced experimental metastasis compared to wild-type controls. Taken together with clinical observations (described below), these experimental studies indicate that OPN is not merely associated with cancer and metastasis, but that it actually plays a multifaceted functional role in malignancy.

OPN Expression in Human Tumors and Prognostic Significance

OPN has been shown to be overexpressed by a number of human tumors, including ►breast cancer, ►prostate cancer, ►colon cancer, ► lung cancer, ►liver cancer, ►gastric cancer, ►esophageal cancer, ►ovarian cancer, ►cervical cancer, renal and ►brain cancer. In many cases, OPN has also been correlated with poor prognosis, reduced survival, and/or increased metastatic burden in cancer patients. OPN can be measured in tumor samples by ►immunohistochemistry or measured as a secreted protein in the blood by ►ELISA. In tumor samples, it has been observed that OPN can be expressed by both tumor cells and ►tumor associated macrophages, although only tumor cell-expressed OPN has been correlated with prognosis. In blood samples,

the cellular source of OPN is not known, since multiple cell types including tumor cells, activated immune cells, and remodeling vasculature may be secreting OPN in the tumor microenvironment. However, a growing number of clinical studies have demonstrated a value for OPN as a blood marker in oncology, suggesting that regardless of the cellular source, OPN can provide prognostic information and potentially provide indications of treatment response in cancer patients.

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Osteoporosis

Definition

Is a bone disorder, characterized by a lowered bone mineral density, due to which the bone is more susceptible to fractures. An important factor in osteoporosis etiology lies in ►estrogen deficiency following menopause. Imbalance between bone resorption by ►osteoclasts and bone formation by ►osteoblasts is the underlying mechanism.

►Bisphosphonates
►Isoflavones
►Menopausal Symptoms After Breast Cancer Therapy
►Zoledronic Acid
►RANK–RANKL Signaling

Osteoprotegerin

Definition

OPG; Is a RANK homolog belonging to a member of the ►TNF receptor super-family. OPG is secreted from osteoblast/stromal cells and regulates bone resorption by blocking ►RANK-RANKL signaling through binding to RANKL.

►Langerhans Cell Histiocytosis

Osteosarcoma

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Synonyms

Osteogenic sarcoma

Definition

Is a rare malignant tumor of the bone. It arises in the bone (osteo) from mesenchymal cells (sarcoma).

Characteristics

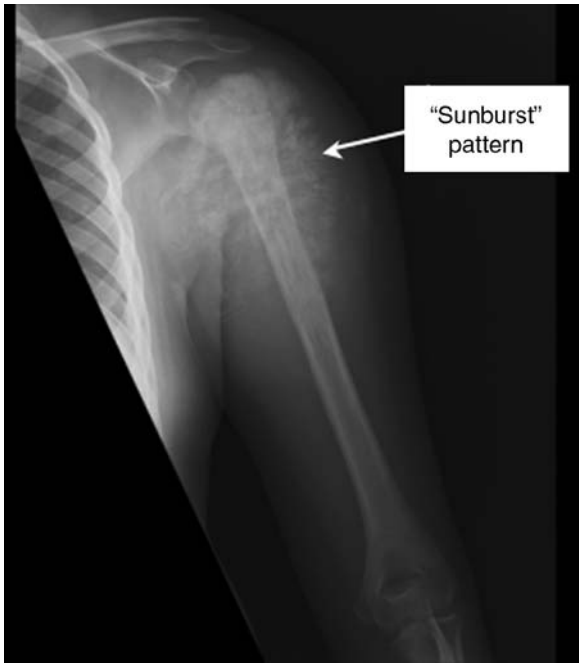
Historically, osteosarcoma was once a fatal diagnosis. Patients had only a 15% chance of cure despite the use of aggressive surgical techniques to remove the primary tumor. With the increasing use of chemotherapy in the 1970s, survival rates increased dramatically. Currently, patients have a 65% chance of survival.

Epidemiology

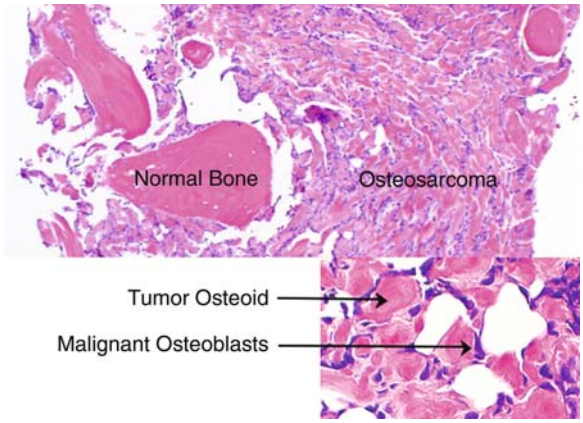
Osteosarcoma is the most common primary ►bone tumor. World-wide incidence rates are not known; however, ~800 patients are diagnosed with osteosarcoma in the United States annually. Half of these patients are teenagers. Men are affected more than women (60% vs. 40%) and African-Americans slightly more than Caucasians. Osteosarcoma has a propensity to affect rapidly growing bones. Half of all tumors occur around the knee and 10% occur in the shoulder. In addition, almost all tumors affect the metaphysis (the distal growing end) of the bone. Teenagers with osteosarcoma also tend to be slightly taller than their peers, suggesting the involvement of growth factors in this tumor.

Clinical Course

The first symptom that patients notice is usually pain. Due to the active nature of teenagers and the lack of systemic signs (such as fever, weight loss, or fatigue) in the early course of disease, most patients are treated with pain medications. The average time from the initial symptom to diagnosis is three months. The presence of a mass or progressive signs of discomfort usually leads



Osteosarcoma. Figure 1 An x-ray of the shoulder reveals the presence of ossification in the soft tissue that surrounds the cortex of the bone. This results in a classic "sunburst" pattern suggestive of osteosarcoma.



Osteosarcoma. Figure 2 Areas of normal bone (left) are being infiltrated by osteosarcoma cells on the right. The inset, at higher magnification, shows malignant osteoblasts that produce tumor osteoid, the defining feature of osteosarcoma.

to obtaining x-rays. These may reveal soft tissue ossification, resulting in the classical "sunburst" pattern (Fig. 1). An MRI of the affected area is required to delineate the extent of the mass and the amount of bone involvement. A definite diagnosis requires a biopsy, preferably obtained by an orthopedic surgeon who specializes in oncology. The ►biopsy should be performed in such a way that future limb salvage surgery will not be adversely affected. The biopsy must show frankly malignant sarcomatous stroma, with production of tumor osteoid, as shown in Fig. 2. Once the diagnosis of osteosarcoma is established, workup to determine whether there has been spread of the tumor (►Metastasis) include a chest cat scan (75% of metastases are to the lungs) and a bone scan (10% of metastases are to the bone, an additional 10% are to both sites). Other tests that are useful to monitor toxicity of chemotherapy agents include a hearing test, an echocardiogram and liver and kidney function tests. Serum alkaline phosphatase and LDH levels are elevated in 40% of patients, high levels of the former correlate with poor prognosis.

Treatment

Following the histological diagnosis of osteosarcoma and workup for metastatic disease, treatment is initiated. Current regimens utilize a neo-adjuvant (►chemotherapy given prior to surgery) (►Neoadjuvant therapy) approach. Four drugs with demonstrated activity against osteosarcoma include, ►methotrexate (M), ►adriamycin (A), ►cisplatin (P) and ifosfamide (I). A common current treatment regimen employs AP followed by M, for two cycles, lasting 8–10 weeks. There are many side effects associated with the use of chemotherapy, included nausea and vomiting, loss of hair and bone marrow suppression, which leads to increased chances of infection, bleeding and fatigue. Side effects that are specific for the agents named above include liver and kidney toxicity (M), decrease in heart function (A), hearing loss (P) and bladder damage (I). The severity of side effects cannot be predicted for any one patient. Protective agents may be considered to ameliorate some of these side effects.

Following the initial course of chemotherapy, definitive surgery is performed. It is imperative that the entire tumor is removed. Patients who have residual tumor have only a 10% chance of long-term survival. Prior to 1970, all patients with osteosarcoma underwent amputation of the affected extremity if possible. Thanks to advances in orthopedic surgery, the vast majority of patients have the option of undergoing limb salvage. This entails removal of the area involved with tumor. The bone that is removed is then replaced with modular metallic rods. These are adjustable, thus reducing the chance of leg length discrepancy. The only instances when amputation is absolutely required are when the blood and nerve supply to the distal extremity is compromised. The rare occurrence of osteosarcoma in the hand or foot also requires amputation due to the lack of prostheses that function well in these small bones.

Following surgery, patients continue with chemotherapy to complete a total of 30–40 weeks of treatment. These patients are then monitored for five years after the

completion of therapy. Patients with localized disease (those who did not have signs of spread) have a 70% chance of cure.

Osteosarcoma has been referred to as a "radioresistant" tumor. This is not quite accurate in that radiation can destroy osteosarcoma cells. However, the doses required to do so are so large (>50 Gy) that conventional radiation approaches would lead to too much damage to the surrounding normal cells. Radiation is used in cases of osteosarcoma of the pelvis when surgery has not been able to remove the entire tumor. Recent advances in radiation delivery techniques such as intensity-modulated and proton-based therapy may increase survival in these patients. Radiation is also used in palliation, in cases to relieve pain due to impingement of the tumor against vital sites.

Metastases

Twenty percent of patients have detectable spread of disease at the time of diagnosis. Unfortunately, these patients have only a 25% chance of cure. The management of these patients is similar to those of patients with non-metastatic osteosarcoma, removal of the primary tumor and all sites of tumor spread, in conjunction with chemotherapy. There have been many efforts to increase the intensity of chemotherapy drugs given to these patients in an attempt to increase the survival rate. As expected, the number of side effects increased greatly, but there has not been an increase in survival.

Recurrence

Another group of patients who do poorly are those who have recurrence of the tumor following treatment. These patients have only a 20% chance of cure. Over 90% of recurrences are in the lung. Patients who do not achieve a second surgical remission have greatly diminished prognosis. Those who are able to have all of their tumor nodules removed have a 40% chance of cure. Again, attempts to increase survival by giving more chemotherapy or radiation to the lung have not been very successful. If any of the four chemotherapy drugs that have activity against osteosarcoma have not been used in the original treatment, those agents should be given in conjunction with surgery. Unfortunately there is still no effective salvage regimen for these patients.

Genetics

The cause of osteosarcoma remains unknown with most patients having no defined genetic risk factors. The risk of developing osteosarcoma increases two-fold over the general population for patients who receive radiation or chemotherapy (especially alkylating agents and anthracyclines) for treatment of other cancers. Two groups of patients have a much higher risk of osteosarcoma however, those with hereditary retinoblastoma (►Retinoblastoma, cancer genetics) and those with ►Li-Fraumeni syndrome. These syndromes occur due to mutations in the tumor suppressor genes *Rb* (►Retinoblastoma protein, biological and clinical functions) and *p53* (►p53 Protein, biological and clinical aspects), respectively. Analysis of patient tumor samples reveals that over 50% of samples have mutations in either *Rb* or *p53*. Other populations that have increased risk of developing osteosarcoma are geriatric patients with ►Paget disease and children with ►Rothmund-Thomson syndrome who have truncating mutations in the *RecQL4* gene. When karyotypes of patient samples are prepared, all samples show ►aneuploidy (more than the normal chromosome number of 46), suggesting that a defect in DNA replication may prove important in the etiology of osteosarcoma.

Future

Research in osteosarcoma has focused on metastatic disease due to the poor prognosis associated with both metastases and recurrence. The futility of efforts aimed at targeting the tumor through intensification of chemotherapy has led to efforts aimed at other targets. In particular, agents that decrease the blood supply to the tumor (angiogenesis inhibitors) (►Antiangiogenesis) and agents that target the reactive cells that surround the tumor (stromal inhibitors) are receiving increased focus, both in the laboratory and in clinical trials.

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Osteosynthesis

Definition

The surgical fixation of fractured bone fragments, or the prophylactic reinforcement (internal fixation) of bone (to prevent for fracture) by plates, screws or intramedullary nails.

►Cryosurgery in Bone Tumors

Ototoxicity

Definition
Ear poisoning.

►Chemoprotectants

Outer Edge of the Tumor Mass

Definition
Consists of the path-leading cells that interact with the surrounding tissue and form the invasive front of the tumor. The molecular expression profile of cells at the outer edge of the tumor can differ from that of cells in the interior. Nuclear localization of ►β-catenin, and upregulation of β1-integrin, the L1 ►adhesion molecule and ►podoplanin were specifically reported in cells of the tumor rim.

►Podoplanin

Ovarian Ablation

Definition
►Ovarian Function Suppression.

Ovarian Adenocarcinomas

Definition
Are malignant ovarian neoplasms classified according to the histology of the tumor. Surface epithelial-stromal tumors are the most common and prototypic ovarian cancers, and include serous cystadenocarcinoma and mucinous cystadenocarcinoma. ►Ovarian cancer staging is by the ►FIGO staging system.

Ovarian Cancer

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Definition
A heterogeneous group of malignant tumors derived from the ovary (there are also a wide assortment of benign tumors derived from the ovary). Approximately 90% of malignant ovarian tumors originate from the ovarian surface celomic epithelium and are therefore designated epithelial ovarian cancer (EOC). Germ cell tumors and stromal tumors account for the remaining cases.

EOC can be divided according to histological appearance:

- Serous, the most common type (composed of epithelium resembling that of fallopian tubes)
- Endometrioid (composed of epithelium resembling that of the endometrium)
- Clear cell (composed of clear cell epithelium and resembling gestational endometrium)
- Transitional cell tumors (composed of epithelium resembling urothelial cells)
- Mucinous (composed of epithelium resembling that of the endocervix)
- Squamous cell (squamous epithelial cells)
- Mixed epithelial tumor (mixture of two or more of the above subtypes)
- Undifferentiated (no recognizable differentiation features)

Ovarian cancer is staged according to the following International Federation of Gynecology and Obstetrics (►FIGO) guidelines. Grading of EOC is based on degree of differentiation, cytologic ►atypia and mitotic index.

- Stage I: restricted to the ovaries
- Stage II: involvement of ovaries and pelvic extension
- Stage III: involvement of ovaries with peritoneal implants outside the pelvis
- Stage IV: involvement of ovaries with distant ►metastasis, such as liver, lung or brain

Characteristics
In westernized countries, ovarian cancer is the sixth most common cancer in women. Over half of the women diagnosed with ovarian cancer are over 65 years of age. The age-adjusted incidence for ovarian cancer is ~15 per 100,000 and the total number of cases is expected to increase as the overall population ages. Overall, ovarian cancer prognosis is poor. In spite of the

recent introduction of aggressive treatments, five year survival rates for patients with advanced ovarian cancer (stage III and IV) has remained low and ranges between 5 and 30%. However, the outcome is much more favorable for stage I patients where 5-year survival can reach 90–95%. Unfortunately, because of lack of symptoms, only 25% of the women with ovarian cancer are diagnosed with stage I disease. These facts make ovarian cancer a disease for which early detection represents an intervention of choice in reducing morbidity. Several putative ovarian cancer serological markers have been identified, including ►CA125, ►TATI, ►CEA and ►CA19-9. Of those, CA125 has proven to be the most clinically useful. Unfortunately, studies have exhibited mixed results, with some studies detecting only 23% of stage I ovarian carcinoma with CA125. CA125 may be useful when used in combination with pelvic ultrasound assessment, and CA125 has been used in monitoring recurrence in patients with CA125-positive tumors. Overall, CA125 lacks the specificity and sensitivity required for the screening of the general population.

The etiology of ovarian cancer is not well understood but the following risk factors have been identified:

- Age: half the cases occur in women of 65 years or older
- Menstrual history: ovarian cancer risk increases with increasing numbers of menstrual cycles
- Birth control pills: the use of which, for at least five years, lowers the risk of ovarian cancer
- Pregnancy and breastfeeding: lowers the risk of ovarian cancer
- Family history

Typical treatment for ovarian cancer is surgery followed by chemotherapy. The exact details of treatment depend on the tumor type, grade and stage, the age, as well as the general health of the patient.

In spite of recent advances in the field of cancer genetics and molecular biology, little is known about the mechanisms of ovarian tumorigenesis. Chromosome abnormalities are frequent in ovarian cancer and allelic losses have been observed in chromosomes 4p, 6p, 6q, 7p, 7q, 8p, 8q, 9p, 11q, 12p, 12q, 13q, 16p, 16q, 17p, 17q, 19p, 19q, 22q and Xq. These observations suggest the presence of several tumor suppressor genes important in ovarian cancer, but very few have been unambiguously identified. The tumor suppressor ►p53 is inactivated in a large number of ovarian cancers. Although the tumor suppressor genes ►BRCA1 and ►BRCA2 have been implicated in familial breast and ovarian cancer syndromes, the vast majority of ovarian cancers are sporadic and may have a different natural history.

The K-►Ras, ►Her-2/neu and c-►myc ►oncogenes have all been implicated in EOC. The frequencies of alteration of these different oncogenes varies according to the subtype of ovarian cancer. No clear association has been reliably observed between the activation of these oncogenes and survival. Indeed, stage and grade have been the only factors consistently shown to predict outcome of ovarian cancer.

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Ovarian Function Suppression

Definition

Synonym Ovarian ablation; Includes the use of surgery, radiation therapy, or a drug treatment to stop the functioning of the ovaries.

►Adjuvant Chemoendocrine Therapy

Ovarian Steroid Hormones

Definition

Refer to the female sex steroid hormones 17β-►estradiol and ►progesterone, secreted by the ovary and by the placenta during pregnancy. Steroid hormones are lipid molecules derived from a common cholesterol precursor (cholestane, C27). Based on the distance between the site of hormone synthesis and secretion and the target tissue, their mechanism of action can be classified as ►endocrine (distant

target), ►paracrine (neighboring cells), or ►autocrine (same cell).

►Progestin

Ovarian Stroma

Definition

Solid inner part of the ovary consisting of various secretory cells.

►Granulosa Cell Tumors

Ovarian Surface Epithelium

Definition

The tissue covering the outer surface of the ovary, from which epithelial ovarian tumors arise.

►Endometriosis

Ovarian Teratoma

Definition

Synonym dermoid cyst; most frequent ovarian germ cell tumor. Mature ovarian teratomata develop from postmeiotic germ cells and present as cystic tumors limited to the ovary with no metastatic spread. In contrast, immature teratomata may show considerable infiltration into neighboring organs and may be a component of mixed malignant ►germ cell tumors.

►Ovarian Tumors During Childhood and Adolescence
►Teratoma

Ovarian Tumors During Childhood and Adolescence

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Synonyms

Dysgerminomas – Dysgerminoma, Embryonal carcinoma, Yolk sac tumor, Choriocarcinoma, Teratoma, mature/immature (cystic teratoma, syn.: dermoid cyst); Sex cord stromal tumors – Granulosa cell tumor, juvenile type, adult type, Sertoli-Leydig cell tumor (syn. gynandroblastoma), Sclerosing stromal cell tumor, Sex cord stromal tumor with annular tubules, Steroid tumor, Brenner tumor; Ovarian small cell carcinoma, hypercalcemic type; Cystadenoma; Ovarian carcinoma

Definition

Primary ovarian neoplasms derived from the germinative, stromal or epithelial component of the ovary during infancy, childhood and adolescence.

Characteristics

Ovarian tumors of childhood and adolescence include a broad variety of clinically, histologically and biologically different entities. The epidemiological patterns differ significantly from those in adult patients. The classical epithelial ►ovarian carcinoma of adults constitutes a rarity in childhood and adolescence. In contrast, ►germ cell tumors (GCT) prevail in young patients and represent the most frequent ovarian tumor during childhood and adolescence.

The histologic variability of ovarian tumors is related to the varying histogenesis of the different entities, because ovarian tumors may develop from all different histologic components of the ovary. Among the largest group of ovarian tumors, the GCTs, ovarian cystic ►teratomas are distinguished from malignant GCTs, which may sometimes include mature or immature teratomatous components. Less frequent histologic entities during childhood and adolescence include ►sex cord stromal tumors (SCST), ►granulosa cell tumors, ovarian small cell carcinoma of the hypercalcemic type (OSCC) as well as epithelial tumors such as cystadenomas, borderline tumors and classical ovarian carcinomas (►Ovarian cancer).

Epidemiology

Among ovarian tumors of childhood and adolescence, GCTs constitute the largest group, with a peak incidence during adolescence and young adulthood. Ovarian tumors account for ~1% of all registered childhood cancers. In addition, an unknown number of unregistered ►ovarian teratomas and rare ovarian tumors must be considered.

In contrast to ►testicular GCTs, which show a bimodal incidence distribution according to age with a first peak during infancy and a second during young adulthood, ovarian GCTs show their peak incidence during adolescence and young adulthood, with no distinct cohort during infancy.

►OSCSTs represent the second largest groups of ovarian tumors during childhood and adolescence. During infancy, the almost exclusive histology is juvenile granulosa cell tumor. With the onset of puberty, ►Sertoli-Leydig cell tumors become more prevalent. The other histologic subentities are too rare to allow for the evaluation of epidemiological patterns.

Epithelial cancers such as OSCC and borderline lesions and the classical carcinoma are diagnosed almost exclusively during adolescence and are rarely reported to pediatric registries.

Histology and Biology

Ovarian tumors may develop from any histologic component of the ovary. The histologic appearance of ►GCTs is interpreted in the light of the holistic concept first proposed by Teilum. Accordingly, GCTs may show pure germ cell differentiation on one hand (►dysgerminoma, in analogy to the seminoma and germinoma of the testis and CNS, respectively) or somatic differentiation on the other. Morphologically, dysgerminomas resemble immature germ cells and express immature stem cell markers such as c-kit, human placenta like alkaline phophatase and OCT3/4. They may develop in the presence of gonadoblastomas; thus indicating underlying disorders of gonadal development, e.g. related to Ullrich-Turner syndrome of testicular feminization with streak gonads.

Among the non-seminomatous GCTs, immature embryonal structures may be observed in embryonal carcinomas (EC) that morphologically remind of the embryoid bodies of embryonal stem cell cultures. The distinct histologic subentities of nonseminomatous GCTs can be distinguished by clinical, histopathologic and immunohistochemic analysis. Embryonal carcinoma may be CD30 and OCT3/4 positive. In contrast, yolk sac tumors express alpha fetoprotein (AFP) and may stain positive for CD34, while choriocarcinoma is detected by staining for ß-human chorionic gonadotropin (ß-HCG). These two markers may be measured in the patient's serum and may therefore be valuable for diagnosis and follow-up of such secreting GCTs.

Teratomas show a higher degree of organoid differentiation with presence of mature or immature structures that may be derived from all three germ layers such as hair, squamous and mucous epithelium teeth, cartilage, bone, glial or thyroid tissue. Characteristically, such mature teratomas contain large tumors cysts and have therefore been named dermoid cyst. These tumors are biologically distinct in that they show an isodisomic chromosome count, related to their development from rather mature germ cells that have already undergone meiotic recombination. Immature teratomas present with varying components of immature tissue that mostly resembles immature neural tissue with neurotubular structures. In immature teratomas, the grade of immaturity can be determined according to the relative amount of immature tissue.

In conventional cytogenetic analysis, differences between GCTs in children and in adults can be detected: The ►isochromosome 12 p (designation: i(12p)), which is the result of a centromeric junction of two short arms of chromosome 12 and loss of the long arms, constitutes the pathognomic cytogenetic abnormality in adult GCTs and can be seen in more than 80% of tumors. The remaining "i(12p)-negative" GCTs show gain of regions from 12p, either as double minutes or homogeneously staining regions. In contrast, the i(12p) can not – or only rarely – be detected in GCTs that arise before the onset of puberty. During childhood, deletions (resulting in ►LOH) of the short arm of chromosome 1 and the long arm of chromosome 6 can be found frequently. According to the unimodal age distribution of ovarian GCTs, no such childhood genetic pattern can be detected in ovarian GCTs of young girls, but the characteristic pattern of adult GCTs including i(12p) may be detected in girls as young as four years of age.

GCTs may arise in the context of constitutional sex chromosomal abnormalities. Ovarian GCTs can be associated with Ullrich-Turner syndrome (45,X0), gonadal dysgenesis (Swyer Syndrome) and/or testicular feminization. Characteristically, these tumors arise in the presence of gonadoblastomas, which has therefore been proposed as the ovarian equivalent of the testicular intratubular neoplasia (TIN, syn. carcinoma in situ).

Sex cord stromal tumors (SCSTs) may include granulosa-, Sertoli-, Leydig- and theka-cells as well as their respective immature progenitor cells and fibroblast that are derived from the specialized gonadal stroma Granulosa Cell Tumors. In the developing gonads, granulosa and Sertoli cells show a sex-specific differentiation. As a morphologic correlate of their hormonal activity, a positive immunohistochemical staining constitutes the diagnostic hallmark of SCSTs.

SCSTs may develop in association with several defined hereditary disorders. In juvenile granulosa cell tumors, there is an association with multiple enchondromatosis, syn. Ollier disease. In addition, there is a pronounced association of ►Peutz-Jeghers syndrome with sex cord tumors with annular tubules. These tumors usually develop at a younger age than in otherwise healthy patients, and they may develop bilaterally. In contrast, testicular large cell calcifying Sertoli Cell tumors can be found in boys with Peutz-Jeghers syndrome.

Genetic analysis of sporadic juvenile ovarian granulosa cell tumors with ►comparative genomic hybridization has not revealed frequent or characteristic chromosomal imbalances. The majority of tumors show balanced karyotypes, and in approx. 25% of patients chromosomal imbalances such as gain of the whole chromosome 12 can be found.

The ovarian small cell carcinoma of the hypercalcemic type (OSCC) represents a rare ovarian neoplasm with enigmatic histogenesis that most commonly develops after the second decade of life. The histologic diagnosis is complicated by the pronounced morphologic similarity between JGCT and OSCC; however, the lack to demonstrate Inhibin by immunohistochemistry strongly argues for the diagnosis of OSCC, if additional characteristic morphologic features are present. The clinical presence of hypercalcaemia in approximately two thirds of patients does not substitute the histologic diagnosis, since a variety of other ovarian neoplasms such as clear cell carcinoma or serous carcinoma may also be associated with hypercalcemia. Clinically, OSCC are characterized by a usually very aggressive clinical behavior associated with fatal outcome in the vast majority of patients.

Adolescent girls may rarely present with large cystic serous tumors, histologically resembling either a simple or papillary cystadenoma. These tumors show a clinically benign behavior after complete resection, but serous adenocarcinoma, which represents the malignant form of papillary serous cystadenoma, must be distinguished histologically. In analogy, mucinous adenocarcinoma constitutes the malignant counterpart of the mostly huge but clinically benign mucinous cystadenomas. The majority of Brenner tumors are benign and occur as small solid and well-circumscribed nodules. Again, a malignant variant (malignant Brenner tumor, pure transitional cell carcinoma) has to be distinguished.

Ovarian adenocarcinomas rarely develop during adolescents; however these tumors often take a malignant clinical course. Particularly in young patients and in patients with frank familial history, these tumors are associated with inherited BRCA mutations. CA125 can be used as tumor marker following tumor resection, but the vast majority of patients will require adjuvant chemotherapy.

Clinical Diagnosis

Ovarian tumors are commonly detected as large tumors which may have led to a visible swelling of the abdomen or fatigue. In some patients, tumor related torsion of the ovary or tumor rupture may result in severe acute abdominal pain and require emergency laparatomy.

Yolk sac tumors secrete alpha1-fetoprotein (AFP) and choriocarcinoma human chorionic gonadotropin (HCG), which can be detected in the serum. In

Ovarian Tumors During Childhood and Adolescence. Table 1 Clinical and biologic characteristic of ovarian tumors correlated with histology

Histology	Clinical behavior	Tumor markers in Serum				Sensitivity to	
		AFP	HCG	Inhibin	Ca++	Chemo	Irradiation Gy
Germinoma	Malignant	–	(+)	–	–	+++	24
Embryonal CA	Malignant	–	–	–	–	+++	45
Yolk sac tumor	Malignant	+++	–	–	–	+++	45
Choriocarcinoma	Malignant	–	+++	–	–	+++	45
Teratoma	Benign (potentially malignant)	–/(+)	–	–	–	>54[b]	>54[b]
SCSTs	Malignant	–/(+)[a]	–	(+)[b]	–	++	n.d.[b]
Ovarian small cell carcinoma, hypercalcemic type	Malignant (presumably fatal)	–	–	–	++	++*	n.d.[b]
Cystadenoma	Benign (potentially malignant)	–	–	–	–	–	–

[a]Some poorly differentiated Sertoli-Leydig cell tumors may secrete AFP.
[b]Under evaluation.
Abbreviations: n.d., no data.

combination with characteristic radiographic findings such as calcifications in mixed tumors with teratomatous elements, these markers can be indicative of malignant GCTs, after other rare diagnoses that may be associated with elevated tumor markers have been excluded. Pure embryonal carcinomas and teratomas are usually not associated with specific serum tumor markers. In ~20% of dysgerminomas, serum levels of placenta like alkaline phosphatase may be elevated. Syncytiotrophoblastic cells in germinoma may produce as well human chorionic gonadotropin (ß-HCG), which therefore can also be elevated (►Serum biomarkers).

SCSTs often induce clinical symptoms that are related to the production of sex hormones by the tumor. Characteristically, infants and children present with signs of isosexual precocity, including breast enlargement, pubarche and vaginal bleeding. In (post-)pubertal girls, tumors may lead to primary or secondary amenorrhea and unspecific signs of virilization. As other steroid hormone producing cells SCSTs also produce Inhibin. Free Inhibin can be measured in the serum and may serve as a serological tumor marker during follow-up.

In rare but well documented cases of poorly differentiated ovarian Sertoli-Leydig cell tumors, ►AFP production has been reported, which may interfere with clinical diagnosis. Histologically, most of these tumors resemble SLCT with retiform, often hepatoid differentiation and heterologous elements.

►CA125 constitutes a rather unspecific marker of ovarian tumors that may be elevated in different histologic entities. CA125 may provide useful information regarding the response to treatment and for follow-up in tumors that are otherwise tumor marker negative.

Ovarian small cell carcinoma may present with significant hypercalcemia, which is not related to skeletal metastasis. However, hypercalcemia has also been reported in some patients with ovarian GCTs, SCSTs and cystadenoma or other epithelial tumors.

The staging system of the World Health Organization and the International Federation of Gynecologic Oncology (►FIGO) can be applied. Tumors confined to the ovary (stage I) are distinguished from tumors with locoregional spread in the small pelvis (stage II) and the abdominal cavity (stage III) and distant metastases (stage IV). Microscopic spread may have occurred even in stage I tumors, if malignant ascites is noted or if pre- or intraoperative violation of the tumor pseudocapsule occurred (stage Ic). Tumors with no microscopic spread on complete staging are categorized as stage Ia (unilateral tumor) or stage Ib (bilateral tumors).

Peritoneal gliomatosis constitutes a specific phenomenon in teratomas and mixed malignant GCTs that refers to glial nodules in the peritoneal surface. Although gliomatosis imposes like wide-spread metastases, it represents a reactive and non-neoplastic disorder so that upstaging is inadequate.

Surgical Therapy

All ovarian tumors require surgical resection. In most patients, a primary tumor resection constitutes the standard approach and will result in complete tumor resection, because most tumors are encapsulated. In metastatic tumors with peritoneal spread, an up-front chemotherapy followed by delayed tumor resection is recommended as it may significantly facilitate complete tumor resection. Therefore, diagnosis must be made on the basis of imaging and tumor markers, and only in tumor marker negative tumors, an initial biopsy will be required.

A median ►laparatomy is considered the classical standard approach to ovarian tumors in childhood and adolescence, as it will allow for preparation and exstirpation of the tumor in one piece. With the modern advances in laparascopy, more patients will undergo laparoscopic surgery. However, the safety of this approach has not been prospectively evaluated in ovarian tumors of childhood. By no means, tumors should be punctured or resected in separate pieces in order to facilitate removal through the laparoscope, because this would not comply with the oncological criteria of complete resection and may thus be associated with an increased risk of relapse.

For the same reasons, organ sparing surgery (e.g. enucleation of a cystic tumor) should be avoided, and might only be reserved to those rare patients with bilateral tumors, which are additionally treated with consolidating chemotherapy.

Tumor resection may be restricted to ovarectomy, if surgical staging indicates a stage I tumor. Only in stage II or III tumors, unilateral adnectomy is indicated. In general, hysterectomy is considered unnecessary.

Surgical staging limited to resection of suspicious lymph nodes only does not adversely affect outcome in the presence of cisplatin containing combination chemotherapy. Thus, routine sampling of unsuspicious nodes is not recommended.

In non-metastatic tumors, omentectomy and appendectomy are not required for oncological reasons, but in specific situations may be performed on the basis of individual judging by the surgeon.

Last, resection of glial nodules in gliomatosis peritonei is not generally recommended due to the non-malignant nature of this disorder. However, resection of large nodules may become necessary if they result in local complications such as mechanic ileus.

Adjuvant Therapy

After resection, a choice regarding the indication for adjuvant therapy has to be made. ►Adjuvant therapy may be chosen from a broad and risk-stratified panel of chemotherapeutic strategies, which may include two to four cycles of a two- or three-agent chemotherapy

as well as strategies with locoregional or systemic treatment intensification. The adequate choice of treatment does not only require the individual skill and experience but must always consider a careful clinical and histopathologic assessment, preferably by a central reference pathologist. Therefore, all children and adolescents with ovarian GCTs should be enrolled into cooperative protocols, which will provide the treating physician with the required infrastructure for diagnosis and risk-assessment.

While previously, adjuvant chemotherapy has been widely used even in completely resected stage I tumors, recent experience has shown that a careful watch-and-wait strategy may be justified in stage I tumors. In analogy to testicular GCTs, it is advisable to administer adjuvant chemotherapy to those patients with histological evidence of vessel-invasion. A watch-and-wait strategy must always include a careful and close follow-up schedule, which specifically includes the assessment of the paraaortal lymph nodes, the most frequent site of relapse. In addition, the patients should be informed that there is a general risk of relapse under a watch-and-wait strategy that is ~20–30%. However, with delayed chemotherapy, there is an excellent salvage rate, so that the overall survival exceeds 90%. Nevertheless, although ►chemotherapy can be avoided in two thirds of patients, those who relapse will have to bear a higher therapeutic burden.

Adjuvant therapy generally consists of platin-based combination chemotherapy. Previously, irradiation has also been administered in dysgerminomas, however, compared to chemotherapy, it will result in infertility of the contralateral ovary without better cure rates. Therefore, irradiation is reserved to salvage treatment only.

Current modern platin-based chemotherapy usually consists of a three-agent combination including ►cisplatin (100 mg/m^2/cycle) and ►etoposide (300–500 mg/m^2/cycle) in combination with ►bleomycin (15–30 U/m^2/cycle) or ifosfamide (7500 mg/m^2/cycle) ►platinum drugs. The comparably effective ►vinblastin is less commonly used because of vascular complications, in particular in combination with bleomycin.

Cisplatin can be substituted by ►carboplatin (600 mg/m^2/cycle) with similar cure rates. However, carboplatin at lower doses (400 mg/m^2/cycle) results in unacceptably high relapse rates. In general, carboplatin is assumed to have a better toxicity profile than cisplatin with lower nephro- and ototoxicity but higher hematotoxicity. Intensification of cisplatin with 200 mg/m^2/cycle) results in significant ototoxicity, however also yields a survival advantage in high risk patients.

The other drug consistently administered in GCTs is ►etoposide. In general, etoposide is associated with an increased risk of secondary malignancies, in particular acute myelogeneous leukemia. However, at the doses usually administered for GCTs and in the absence of concomitant radiotherapy, the risk is acceptably low and does not exceed 1%.

In the different national protocols, different choices of the third drug have been made. In the UK and the USA, platin-compounds and etoposide are combined with bleomycin, thus resembling the traditional ►BEP chemotherapy for testicular GCTs. In contrast, the national groups in France, Brazil and Germany are administering ifosfamide. The toxicity profiles of these two drugs differ significantly. Bleomycin in combination with cisplatin may result in pneumopathy, which in rare patients may be lethal. In contrast, ifosfamide has a more pronounced hematotoxicity and may result in chronic renal tubulopathy.

As for both drugs, the risk is highest in neonates and infants, both have not been administered in this age group. Thus, the two-agent regimen has been extended to a larger group of patients with a moderate risk profile, with promising treatment response.

Some tumors may show an only insufficient response to conventional three agent chemotherapy and should therefore be selected for treatment intensification. In tumors with locoregional (i.e. peritoneal) spread, treatment intensification may be achieved by combination of chemotherapy with locoregional hyperthermia ►Hyperthermia. Metastatic tumors however may be selected for dose intensified chemotherapeutic strategies (►Platinum-refractory testicular germ cell tumors).

In contrast to the more frequent GCTs, the experience regarding the adjuvant chemotherapy in the rare tumor entities such as SCSTs and OSCCs is much more limited and awaits prospective evaluation. The overall prognosis of SCSTs is favorable and cure rates exceed 80%. Patients with stage I tumors are followed expectantly, while patients with peritoneal spread are selected for adjuvant chemotherapy (e.g. cisplatin, etoposide, ifosfamide). Patients with stage Ic tumors and malignant ascites of preoperative tumor rupture should also be selected for chemotherapy, since the outcome of this specific group was comparable to that of stage II and III tumors.

Experience regarding the optimal treatment of OSCC is extremely small and mostly derived from retrospective clinico-pathological surveys and single case reports, indicating for an extremely aggressive natural course of this disease with an almost invariable fatal outcome. Regardless of stage, all patients with ►ovarian small cell carcinoma, hypercalcemic type require multi-agent chemotherapy during first-line treatment. High-dose chemotherapy can be used to consolidate the therapeutic success.

The guidelines for the treatment of other epithelial tumors such as cystadenoma and adenocarcinomas follow those for the corresponding tumors in adults. If necessary, a combination of carboplatin and ►taxol is considered the current standard chemotherapy ►ovarian cancer.

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Overexpression

Definition

Gene overexpression refers to the increased expression of a gene. This can occur either endogenously by regulotary pathways, by genomic ►amplification of a gene or exogenously by experimentally introducing DNA-constructs (plasmids) encoding the respective gene into cells.

Ovulation

Definition

The process by which an ovum is released from within the ovary in a cyclical manner. The fluid-filled follicle containing the ovum distends the surface of the ovary until it breaks down, and the ovum is released.

►Endometriosis

Oxaliplatin

Definition

Chemotherapeutic agent; platinum-containing; causes DNA disruption.

►Peripheral Neuropathy

Oxidation

Definition

The chemical process involving removal of electrons. If dithiols are oxidized with two electrons, they may form disulfides.

►Thioredoxin System
►Reactive Oxygen Species

Oxidative DNA Damage

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Definition

Represents free radical damage to DNA. Oxidation essentially involves the addition of oxygen or removal of hydrogen atoms from a molecule. Oxidation of DNA may simply result in a small change to one of the bases, or a deoxyribose in the backbone of the molecule may be altered to such an extent that the continuity of the backbone is broken. Single-strand breaks are more common than double-strand breaks.

Characteristics

DNA is thought of as a very stable molecule and yet it readily undergoes damage, by a variety of agents that can be either endogenous or exogenous in origin. Ionizing radiation (e.g. X-rays), ►ultraviolet radiation and various chemicals, including some present in tobacco smoke (►Tobacco carcinogenesis), cause the release of free radicals, and if DNA is not protected, oxidative damage can occur. Free radicals (►Reactive oxygen species) also occur within the cells of the body, arising as a minor product during the cycle of oxidation of carbohydrates in the mitochondria. The hydroxyl radical, *OH, is particularly reactive with DNA.

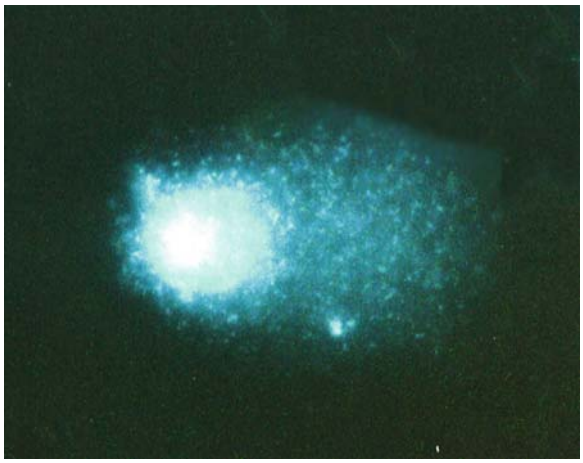
In addition to single- and double-strand breaks, many different oxidation products of the four bases (►Adducts to DNA) have been identified in DNA treated with radiation or other free radical-generating chemicals. Some of these modified bases are potentially capable of giving rise to a ►mutation. For instance, ►8-oxoguanine, if present in the DNA when it is replicating, may lead to the incorporation of adenine rather than cytosine into the newly synthesized complementary strand, thus changing the DNA sequence.

Some oxidation is detectable in the DNA of normal human cells. It is usually measured by gas

chromatography (►GC-MS) or high performance liquid chromatography (►HPLC). Normally for GC-MS, the DNA is acid-hydrolyzed to bases (guanine etc.), while for HPLC the DNA is enzymically hydrolyzed to nucleosides (deoxyguanosine (dG) etc.). Both of these methods have given relatively high values for the extent of conversion of guanine to 8-oxoguanine, with up to 300 or more 8-oxo-guanines for every 10^6 unaltered guanines. However, recently it has been recognized that guanine tends to oxidize during preparation of samples for analysis and so the early estimates of 8-oxoguanine are considered to be excessive. Values as low as 3 per 10^6 have been reported from HPLC analysis of anaerobically prepared samples.

There is another approach to measuring oxidized bases using bacterial repair endonucleases, which recognize the damage and make a corresponding break in the DNA. The enzyme FPG (formamidopyrimidine DNA glycosylase) recognizes 8-oxoguanine. DNA breaks can then be measured in various ways, including the ►comet assay (Fig. 1). This approach gives values for 8-oxoguanine that are even lower, with around 0.5 per 10^6 guanines.

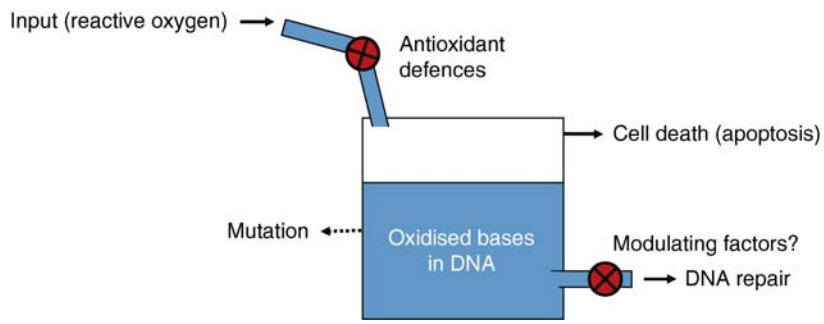
The extent of background DNA oxidation in normal cells remains an important question. Methodological problems must be solved before a consensus can be reached.



Oxidative DNA Damage. Figure 1 The comet assay. Cells are embedded in agarose on a microscope slide, lysed, and electrophoresed at high pH. This view is of the DNA from one cell, stained with DAPI and visualized by fluorescence microscopy. The percentage of DNA fluorescence in the tail of the "comet" is proportional to the frequency of breaks – such as breaks introduced at 8-oxoguanine sites by the enzyme FPG.

Mechanisms

Measurement is made of the steady state level of DNA damage, which is a dynamic equilibrium between input of damage and its repair (Fig. 2). In the case of oxidative damage, input is controlled by ►antioxidant defenses. The tripeptide glutathione is present at high concentration in the nucleus and "mops up" free radicals before they can cause damage. Superoxide dismutase and catalase are enzymes that convert superoxide and hydrogen peroxide (two reactive forms of oxygen) ultimately to non-harmful products. Other enzymes combine various organic free radicals with glutathione, thus inactivating them. Fruits, vegetables and grains in the diet are a source of ►antioxidants, including ►vitamin C, ►vitamin E, ►carotenoids and flavonoids. These ►natural products have the ability to quench or scavenge free radicals; whether they act as antioxidants in vivo depends on whether they are taken up from the gut in sufficient amounts, and has been the subject of recent human intervention trials. In general, it is possible to detect a significant decrease in the steady state level of base oxidation (and/or an increased resistance to in vitro oxidation of DNA) in white blood cells of volunteers taking individual antioxidant



Oxidative DNA Damage. Figure 2 DNA damage: a steady state. There is a constant inflow of damage, caused by free radical attack and attenuated by antioxidants. This is normally balanced by cellular DNA repair processes, which remove the damage and restore the DNA sequence. Little is known about what modulates repair activity.

supplements or antioxidant-rich foods, ranging from fried onions to kiwifruit.

However effective the antioxidant defenses, some DNA oxidation does occur. The turnover of this damage is achieved by ►repair of DNA. Small base damage, which includes base oxidation, is repaired primarily by base excision repair. Here, the damaged base is removed, followed by the base-less sugar-phosphate residue and perhaps a few neighboring nucleotides. A small repair patch of new nucleotide(s) is inserted and ligated.

As well as being present in cellular DNA, 8-oxodG is detectable in urine as free nucleoside. The idea that urinary 8-oxodG represents the accumulated product of DNA repair in all the cells of the body is attractive but flawed; base excision repair releases the base not the nucleoside. Even if this 8-oxodG originates in the oxidation of broken down DNA from dead cells passing through the kidneys, it still reflects ►oxidative stress, and it has given useful information about oxidative stress related to exercise, smoking and nutrition. Most impressively, consumption of 300 g a day of brussels sprouts led to a decrease of 28% in urinary 8-oxodG concentration.

Clinical Aspects

It is commonly stated that oxidative DNA damage is a significant cause of cancer, and that fruits and vegetables protect against cancer because the antioxidants they contain decrease the amount of base oxidation in the cellular DNA. However, there is little evidence that this is true. In two large scale human intervention trials, smokers and/or ►asbestos workers were given β-carotene (a carotenoid antioxidant) daily for several years. The ►lung cancer incidence was actually higher in these subjects compared with those taking placebo (or other supplement). Other intervention trials have shown no beneficial or harmful effect of supplementation with antioxidant micronutrients in terms of cancer risk – in spite of their ability to decrease oxidative damage.

In an experimental animal system, a high level of oxidative DNA damage is not necessarily marked by an elevated cancer risk. In a ►knockout mouse model, which is defective in the murine equivalent of FPG, there is a slight increase in the steady state level of 8-oxoguanine, but no increase in cancer incidence. It seems that there is a back-up repair pathway that deals (more slowly, but adequately) with oxidative damage.

Oxidative stress is a feature of many other diseases, including heart disease, diabetes, cataract, and rheumatoid and arthritic conditions. It may be a cause of the clinical condition, or an effect. A common theory of ageing argues that the accumulation of free radical-induced damage to biomolecules – lipids, proteins and nucleic acids – is responsible for the general cellular dysfunction and deterioration of body processes in later life, but the evidence for accumulation of oxidative DNA damage is weak.

The importance of fruits and vegetables in a healthy diet is not in doubt. But it is clear that antioxidants are not their only feature, and we should be looking at other effects that phytochemicals might have on metabolism to account for their capacity to prevent disease.

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Oxidative Metabolism

Definition

Consists of replacing a carbon hydrogen bond in a drug by a carbon oxygen bond or the breaking of a carbon nitrogen bond (N-dealkylation).

►ADMET Screen

Oxidative Phosphorylation

Definition

Biochemical reactions in the mitochondria that generate ATP. Is an oxygen-dependent process by which aerobic cells obtain energy (ATP) in the mitochondria. In this process, electrons from NADH and $FADH_2$ are passed along the electron-transport chain to oxygen. This electron transport generates an electrochemical proton gradient across the inner mitochondrial membrane that is used by ATP synthase to phosphorylate ADP and form ATP.

►Hydrogen Peroxide

Oxidative Stress

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Definition

Is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. It is defined as a disturbance in the prooxidant–►antioxidant balance in favor of the former leading to potential damage. Oxidative stress is believed to contribute to the development of several diseases, including cancer.Corresponding author.

Characteristics

The complex of oxidative stress involves a number of cell-chemical elements:

►Free radicals include any species that contain one or more unpaired electrons, generating electrons singly occupying an atomic or molecular orbital. The presence of unpaired electrons confers a considerable degree of reactivity upon a free radical. Dioxygen is the main source of intracellular free radicals.

►Reactive oxygen species (ROS) is a collective term that includes both oxygen free radicals such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) and other reactive molecules as ►hydrogen peroxide (H_2O_2). H_2O_2 is not a free radical but is also reactive and easily converted into radicals.

ROS are physiological byproducts of normal aerobic metabolism. 90% of intracellular superoxide anion comes from the mitochondrial electron transport chain. Other significant sources of superoxide anion include ►NADPH oxidase (NOX), ►xanthine oxidase, and NADPH-►cytochrome P450 reductase. NOX are a family of multimeric membrane oxidases tightly regulated by various stimuli including growth factors.

Superoxide anion is considered as the "primary" ROS. The chemical reactivity of superoxide anion is weak in aqueous medium. Moreover, its negative electronic charge makes it unable to diffuse through lipid membranes. That's why superoxide anion by itself has little cellular effects.

The conversion of $O_2^{\bullet -}$ into H_2O_2 is catalyzed by enzymes of the ►superoxide dismutase (SOD) family. Three isoforms of SOD have been identified in humans: CuZn-SOD, which is located in the cytosol, Mn-SOD, located in the mitochondria, and extracellular (EC)-SOD. H_2O_2 is able to go trough cellular membranes and can diffuse relatively far from its production origin. At physiological concentrations, H_2O_2 reacts mainly with sulfydril radicals, inducing reversible alterations of cysteines. Thus, H_2O_2 is able to specifically alter activity of proteins containing cysteine residues in their enzymatic sites, especially tyrosine phosphatases.

The hydroxyl radicals are formed through the Fenton or Haber-Weiss reaction that converts $O_2^{\bullet -}$ and H_2O_2 into $OH^{\bullet}$ in the presence of Fe^{2+} or Cu^{2+}. $OH^{\bullet}$ is extremely reactive and induces severe cellular oxidative damages as DNA alterations, being thus highly mutagenic. It leads to polyunsaturated fatty acids peroxidation, increasing cellular membrane permeability. $OH^{\bullet}$ is also able to react with proteins, inducing irreversible inactivation.

Several antioxidant enzymes can detoxify the whole cascade of ROS and protect normal cells from the potential damages implicated by oxidative stress. SOD catalyses the conversion of $O_2^{\bullet -}$ into H_2O_2, which is further detoxified by catalase or by enzymes of the glutathione peroxidase family using reduced glutathione. This complex enzymatic antioxidant system is completed by exogenous compounds such as arginine, vitamins A, C, E, β-carotene, glutathione, polyphenols, and minerals (selenium and zinc).

Oxidative Stress is Involved in Oncogenesis

There are several evidences that ROS play a key role in the oncogenesis process. In vitro exposure to chronic oxidative stress is associated with oncogenic ►transformation and tumor growth. Overexpression of NOX1 (the catalytic subunit of NOX) stimulates the generation of O_2^- and induces the transformation of mouse ►NIH 3T3 cells. Patients carrying specific polymorphisms on GSH-peroxidase 1 gene associated with decreased antioxidant activity present an increased risk of lung and breast cancers. An excessive production or a defective detoxification of ROS may favor the promotion and the development of cancer during chronic infection or ►inflammation which is considered to cause one third of world cancers.

The tumoral transformation induced by ROS, especially by $OH^{\bullet}$, is at least in part consecutive to their ►mutagenic effects. They can act directly on DNA structures leading to single- or double-strand breaks, to point and frame-shift mutations, and to chromosome abnormalities. ROS can also promote proliferation and survival of cancer cells and tumor angiogenesis by means of ►epigenetic effects. By altering activity of several tyrosine phosphatases, including ►PTEN and ►MAPK phosphatases, H_2O_2 activates mitogenic and survival pathways. This molecule has also been reported to stimulate ►VEGF production by tumor cells.

Increased Oxidative Stress in Cancer Cells

Under basal conditions of culture, various cancer cell lines produce more $O_2^{\bullet -}$ and H_2O_2 than nontransformed

cells. The presence of oxidative modifications of DNA in primary human cancer cells has also been demonstrated. The oxidative stress observed in cancer cells mostly results from the increase production of superoxide anion. The overexpression of ►Myc oncogene has been associated with increased production of superoxide by mitochondrial respiratory chain, while ►RAS oncogene mutations induce NOX activation. A decrease activity of antioxidant enzymes could also contribute to oxidative stress since tumor cells, compared with normal cells, often express lower levels of catalase, glutathione peroxidase, reductase and their respective cofactors.

The overproduction of ROS in cancer cells contributes to the genetic instability, leading to nuclear and mitochondrial DNA mutations and more aggressive phenotype. Thus, oxidative stress is part of a positive amplification loop where ROS induce malignant transformation and transformation is itself associated with more ROS production.

Therapeutic Perspectives of Oxidative Stress Modulation

Differential Effects of Oxidative Stress in Normal and Cancer Cells

Growing evidence suggests that ROS can induce a wide-type of cellular responses from proliferation to senescence and cell death. Adding increasing amounts of exogenous H_2O_2 or increasing its intracellular levels by overexpression of SOD leads to a dose-dependent decrease in proliferation and tumor cell death. H_2O_2 can stimulate proapoptotic signal molecules such as apoptosis signal regulating kinase 1, c-Jun-NH2-kinase, and p38; activation of the p53 protein pathway; startup of the mitochondrial apoptotic cascade. When adding ROS to cells culture media, differential cellular effects have been observed in normal and cancer cells. In normal cells, persistent ROS production leads to activation of the ►JNK pathway and apoptosis, whereas low concentrations or transient high levels of ROS induce the proliferation of those cells, through the activation of the ERK pathway. In sharp contrast, similar expositions to ROS cause tumor cell growth arrest and apoptotic death because of their high basal level of ROS that is close to the threshold of cytotoxicity. Thus, the fate of tumor cells exposed to oxidative stress is tightly correlated with the duration of ROS stimulation and depends on the basal redox status of the cell.

Use of Antioxidant Agents in Cancer Patients

Clinical data regarding the effects of antioxidant molecules in cancer patients have been controversial. Because oxidative stress induced DNA damages and is involved in malignant transformation, the hypothesis was made that antioxidant agents could have preventive effect against cancer. Numerous clinical trials were performed using various antioxidants (N-acetylcysteine (NAC), β-carotene, vitamin A, vitamin C, vitamin E, …), but failed to show any protective effect. Furthermore, a doubt has been cast on the possibility that some of these molecules, especially NAC might trigger tumor growth. These results should be viewed in light of the biological effects of antioxidants in cancer cells. Indeed, we previously observed that NAC, which is endowed with catalase and glutathione-like activities, decreases intracellular H_2O_2 concentration and increases the proliferation of tumor cells in vitro and in vivo, probably because tumor cells are submitted to detrimental oxidative stress.

Antioxidant molecules probably play a protective role against cancer in healthy individuals by preventing DNA damages linked to the oxidative stress. However, once cancer cells have emerged, a cancer-promoting effect could result from the administration of agents that decrease intracellular H_2O_2 level. Therefore, antioxidant should be used with caution in cancer patients.

Anticancer Agents Increase Oxidative Stress in Cancer Cells

The most commonly used anticancer agents, such as ►cisplatin, ►adriamycin, ►fluorouracil, ►irinotecan, or ►paclitaxel, are able to induce ROS production in cancer cells. Hydrogen peroxide and superoxide accumulation are observed within few hours of drug exposure and occur before the commitment of the cells into apoptosis. Moreover, antioxidants such as NAC and catalase are able to decrease their cytotoxicity. These results strongly suggest that oxidative stress is involved in the cytotoxicity of most anticancer agents. However, the underlining mechanisms seem to differ from an agent to the other. For example, ►5-FU and ►anthracyclins induce ROS production by a p53-dependent pathway involving the activation of several mitochondrial oxidoreductases such as proline oxoreductase and ferrodoxine reductase. On the other hand, membrane NOX activation is induced by ►paclitaxel and ►arsenic trioxide.

Tumor cells have higher levels of ROS than normal cells and could therefore be more sensitive to the additional oxidative stress generated by anticancer agents. This hypothesis offers a new explanation to the observation that anticancer agents are usually more toxic to cancer cells than to normal cells.

Finally, cellular antioxidant enzymes may influence the sensitivity of tumor cells to anticancer agents. Thus, high levels of reduced glutathione, the cofactor of glutathione peroxidase, in tumor cells have been associated with a ►multidrug resistance phenotype. A correlation was found between resistance of breast cancers to docetaxel and the overexpression of several genes controlling the cellular redox environment.

Using Oxidative Stress Modulators as Anticancer Agents

The fundamental differences between normal and tumor cells in terms of responses to H_2O_2 overproduction

provide new possibilities in the treatment of cancer, taking into account the tumor cell susceptibility to ROS-induced apoptosis. Overexpression of SOD in cancer cell lines induces an increase in H_2O_2 production and reduces tumor growth. However, the SODs are polypeptides of high molecular weight that are not able to cross the cellular membranes and therefore have a limited interest for clinical use. Several nonpeptidyl SOD mimics with lower molecular weight have been developed, such as (Cu(II)(3,5-diisopropylsalicylic acid)2) (CuDIPS), Mn(III) tetrakis(4-benzoic acid) porphyrin (MnTBAP), or mangafodipir. The SOD mimics increase the concentration of H_2O_2, resulting in the abrogation of tumor cells proliferation in vitro. Similar results have been observed in vivo.

Increasing the Therapeutic Index of Anticancer Agents by SOD Mimics

Several reports have suggested that compounds that increase intracellular hydrogen peroxide concentration could enhance the activity of anticancer agents. For example, buthionine sulfoximine (BSO), a glutathione synthesis inhibitor, can increase the cytotoxicity of melphalan by inhibiting glutathione peroxidase activity and increasing H_2O_2 level. Similarly, we previously showed that the antitumor activity of oxaliplatin and paclitaxel is enhanced by SOD mimics. A potential limitation to the clinical development of such compound is that they could also increase the toxicity of anticancer agents on normal cells. As an example, BSO depletes glutathione in both normal and cancer cells, increasing melphalan's hematologic toxicity and thus abrogating any enhancement of the therapeutic index of this anticancer agent. Mangafodipir has SOD-, catalase- and glutathione reductase-like properties, allowing it to act at multiple steps of the ROS cascade. We showed that mangafodipir protects mice treated with paclitaxel from developing leucopenia and also amplifies the antitumoral effect of paclitaxel on implanted tumor, increasing its therapeutic index. These opposite effects of mangafodipir in normal and cancer cells may be related to the differences in the redox status of these cells, as described above.

Clearly, oxidative stress modulators, especially SOD mimics, warrant further development in anticancer treatment.

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Oxidized

Definition

The product of a compound that has gone through a reaction involving an ►oxidation.

►Thioredoxin System

Oxidized Phospholipids

Definition

Are formed by a non-enzymatic (radical attack) breakdown of polyunsaturated fatty acids (arachidonic, linoleic) leading to phospholipids with short (only few carbons) oxidized acyl chains at the sn-2 position of glycerol.

►Lipid Mediators

Oxidoreductase

Definition

An enzyme having the capacity to catalyze reactions involving reduction or oxidation, in the case of thioredoxin (Trx) or TrxR usually converting disulfide motifs into dithiol motifs.

►Thioredoxin System

8-Oxoguanine

Definition

Is one of many products of the oxidation of DNA. It differs from guanine by replacement of a hydrogen

atom with a hydroxyl group, 8-oxodeoxyguanosine occurs when combined with deoxyribose.

►Oxidative DNA Damage

Oxygen Free Radicals

Definition

Reactive Oxygen Species (ROS).

Oxygen Partial Pressure Distribution in Tumor

►Oxygenation of Tumors

Oxygen Tension Distribution in Tumor

►Oxygenation of Tumors

Oxygenation of Tumors

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Synonyms

Oxygen partial pressure distribution in tumor; Oxygen tension distribution in tumor; Oxygenation status and tumor oxygen level

Definition

Tumor oxygenation, which reflects the distribution of oxygen (O_2) partial pressures (pO_2 values) or O_2 concentrations, results from O_2 availability (O_2 supply) to the tumor tissue, the ►diffusional flux of O_2 from the microvessels to the cells and the ►respiration rate (O_2 consumption rate) of the parenchymal and stromal cells making up the tissue. O_2 supply is mainly influenced by the efficacy of blood flow, diffusional O_2 flux and blood hemoglobin (Hb) level.

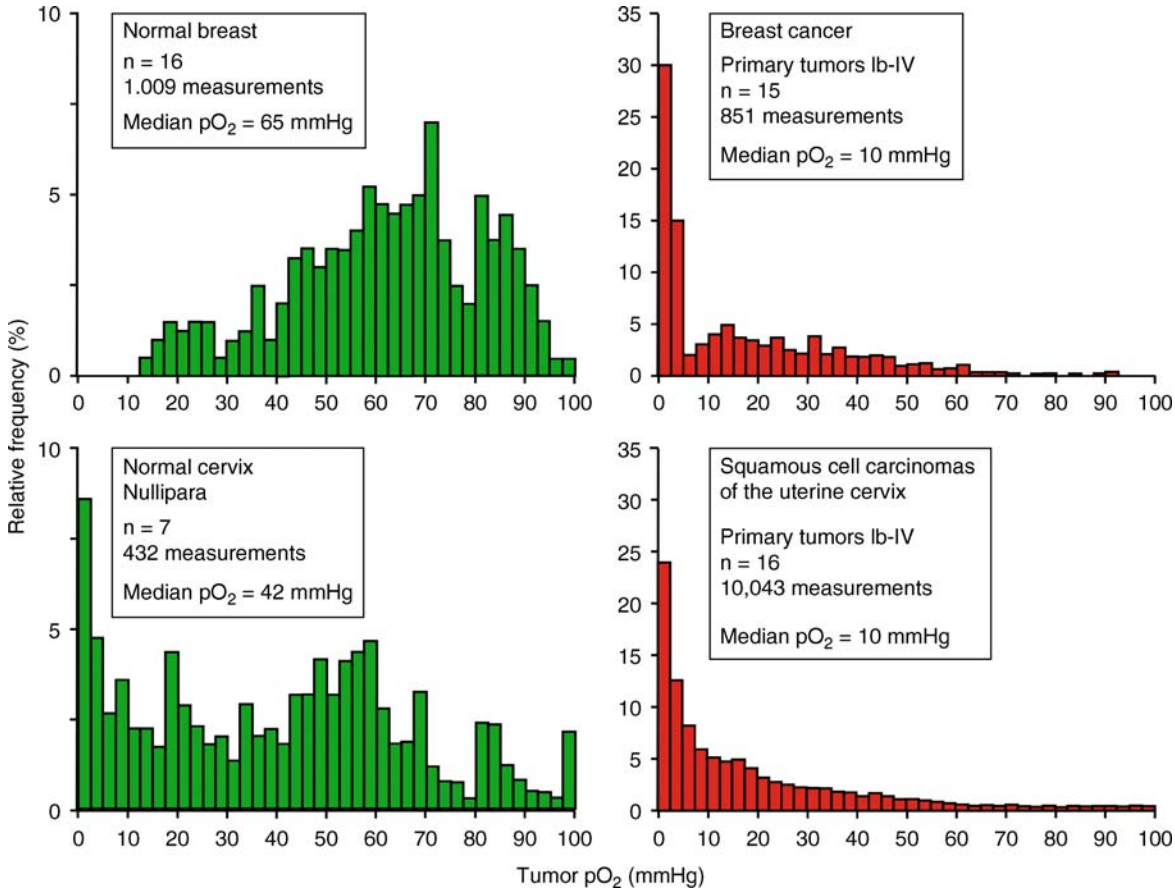
Characteristics

Whereas in normal tissues the O_2 supply meets the metabolic demands, in many solid tumors the respiration rate may outweigh an insufficient O_2 supply and result in the development of tissue areas with very low O_2 levels (►hypoxia, O_2 depleted tissue areas) or even areas completely lacking O_2 (►anoxia).

Considerable evidence demonstrates that in most human tumors oxygenation is compromised as compared to normal tissues, which are characterized by "normal" O_2 partial pressure distributions (►normoxia). Oxygenation is extremely heterogeneous within an individual tumor (intra-tumor heterogeneity). Furthermore, considerable heterogeneity of oxygen-depleted areas (hypoxic areas) has been shown between tumors of the same clinical size, stage, grade or histological type (inter-tumor heterogeneity). Tumor oxygen supply is not regulated according to the metabolic demand, as is the case in the physiological situation. On average, the median pO_2 values in tumors are substantially lower than in normal tissues at the site of tumor growth (Fig. 1). Tumor oxygenation is independent of clinical size, stage, grade, histology and various oncologic parameters or patient demographics. In some cancer entities, the oxygenation status significantly deteriorates in ►anemia (as a result of a decreased O_2 capacity of the blood) and at Hb concentrations above the median Hb level (most probably due to rheological problems within the chaotic tumor microvessels). Metastatic lesions seem to have an oxygenation status comparable to that of the primaries, whereas local recurrences have a poorer oxygenation status than the primary tumors.

Pathomechanisms of Tumor Hypoxia

Tumor hypoxia results from an inadequate O_2 delivery to the respiring neoplastic as well as stromal cells. Limited and even abolished O_2 supply is due to severe structural and functional abnormalities of the ►microcirculation, as well as due to a deterioration of diffusion geometry. In addition, cancer-related and/or therapy-induced anemia and carboxyhemoglobin formation (in heavy smokers) can lead to a reduced O_2 content of the blood. As a result, areas with very low (down to zero) O_2 partial pressures exist in solid tumors. These very low pO_2 microregions are heterogeneously distributed within the tumor mass and may be located next to regions with pO_2 values corresponding to the normal tissue from which the tumor has been derived. In



Oxygenation of Tumors. Figure 1 Oxygenation status of normal tissues (*left panels*) and solid tumors (*right panels*). Frequency distributions of measured pO_2 values (pO_2 histograms) for normal breast tissue and uterine cervix are compared with the respective cancer tissues (clinical stages Ib – IV). Oxygen partial pressures (pO_2) in the tissue were measured with a computerized polarographic microsensor technique which enables direct assessment of the pO_2 data with an O_2 sensitive needle-electrode. Oxygen partial pressures were measured along several electrode tracks in each individual tumor. Pooled data presented here are derived from pre-treatment measurements in conscious patients.

addition, significant temporal variations in the oxygenation status have been observed in tumors.

Methods of Measurement

Assessment of the tumor oxygenation status in experimental tumors and in the clinical setting has been performed using various techniques. So far, the most direct and often used method for description of oxygenation is the polarographic measurement of O_2 partial pressures. With this invasive microtechnique, frequency distributions (►histograms) of measured intratumor pO_2 values can be obtained with a relatively high spatial resolution. Other direct procedures include fiber-optic O_2 sensors and electron paramagnetic resonance oximetry. This latter technique is minimally invasive requiring only application of the paramagnetic material.

Measurement of intravascular oxyhemoglobin (HbO_2) saturation is another method that has the potential to allow a characterization of the oxygenation status of human tumors. However, it only provides information related to the vascular compartment and thus the situation in the extravascular space can only be inferred (e.g., BOLD-MRI).

Tumor oxygenation can be measured in tomographic images in the clinical setting upon inhalation of radiolabeled ^{15}O-gas in ►positron emission tomography (PET) studies. However, as with magnetic resonance imaging (MRI) procedures, limitations include poor quantification and sparse spatial resolution. The parameter measured with these non-invasive techniques is not directly interpretable as a tumor pO_2 value or O_2 concentration.

Non-invasive methods for detection of tumor hypoxia include the binding and the retaining of radiolabeled ►bioreductive drugs, such as fluoromisonidazole (labeled with ^{18}F and detected by positron emission

tomography) and iodoazomycin-arabinoside (labeled with ^{123}I and detected with single photon emission computerized tomography, SPECT).

Several techniques for assessment of tumor oxygenation require the analysis of tumor biopsy specimens. Using immunohistochemistry with exogenous hypoxia markers (such as misonidazole, pimonidazole, etanidazole or nitroimidazole-theophylline), detailed information concerning hypoxia at the cellular level can be obtained. Disadvantages include the need for injection of a hypoxia marker and possible sampling errors. The role of HIF-1α, GLUT-1 or CA IX as endogenous markers of tumor hypoxia is questionable.

Clinical Relevance

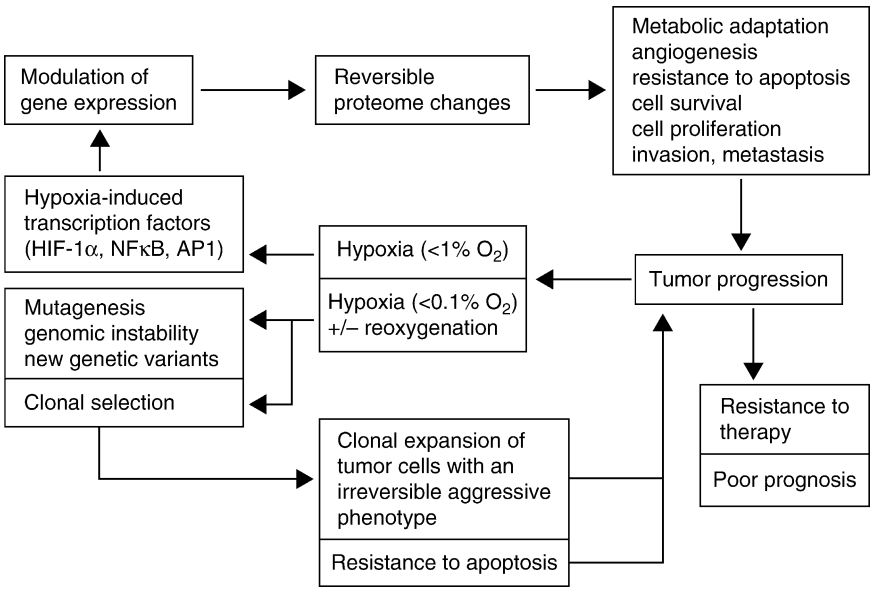
Tumor hypoxia has been considered to be a therapeutic problem since it renders solid tumors resistant to sparsely ionizing radiation (X- and γ-rays), some forms of chemotherapy (e.g., cyclophosphamide, carboplatin) and photodynamic therapy. Oxygen levels may furthermore influence a series of biological parameters, which in turn may markedly increase the malignant potential of a tumor irrespective of tumor treatment modalities.

Hypoxia is a common characteristic of locally advanced solid tumors that has been associated with malignant ►progression, that is, an increasing probability of recurrence, locoregional spread, and distant ►metastasis. Emerging evidence indicates that the effect of hypoxia on malignant progression is mediated by a series of hypoxia-induced proteomic (<1% O_2) and genomic changes (<0.1% O_2) leading to an activated ►angiogenesis, anaerobic metabolism, local invasive growth, metastasis, loss of apoptotic potential, and other processes that enable tumor cells to survive or escape their oxygen-deficient environment. The transcription factor ►hypoxia-inducible factor 1 (HIF-1) is a key regulator of tumor cell adaptation to hypoxic stress. Tumor cells with proteomic and genomic changes favoring survival under hypoxic conditions will proliferate, thereby further aggravating the hypoxia. Mutagenesis, genetic instability, the selection and expansion of new (and more aggressive) clones which eventually become the dominant tumor cell type, lead to the establishment of a vicious circle of hypoxia and malignant progression (Fig. 2). According to recent observations, malignant progression is most probably promoted by iterative cycles of hypoxia and reoxygenation episodes which have emerged as pivotal factors of the tumor pathophysiome.

Hypoxia-associated resistance to radio- and chemotherapy is multifactorial. Besides direct effects (e.g., DNA damage) and hypoxia-induced genome and proteome changes, indirect effects may also play a pivotal role. For example, inhibition of cell proliferation or tissue ►acidosis which often coincides with hypoxia in tumors that show a high glycolytic rate.

Tumor Oxygenation as a Prognostic Parameter

Clinical trials have been conducted in more than 700 patients with locally advanced cervical cancer, in squamous cell carcinomas of the head and neck and in soft tissue sarcomas. In these studies, pretreatment



Oxygenation of Tumors. Figure 2 Schematic representation of the mechanisms causing hypoxia-induced proteomic and genomic changes leading to tumor aggressiveness, malignant progression, treatment resistance, and poor prognosis.

tumor oxygenation has turned out to be a very powerful prognostic factor. Tumor hypoxia adversely affects locoregional control, disease-free survival and overall survival (cancers of the uterine cervix and head & neck tumors). This effect is independent of other known prognostic variables. Hypoxia also diminishes disease-free and overall survival in soft tissue sarcoma.

Modulation of Tumor Oxygenation

Manipulations of the tumor oxygenation status for therapeutic benefit have been performed for many years. A number of strategies to improve tumor oxygenation and to increase its uniformity have been considered. These include enhancing O_2 availability and/or reducing cellular respiration rate. The former can be achieved either by increasing the O_2 content in the tumor microvessels or by improving tumor blood flow. A reduction in the O_2 consumption rate would permit further diffusion of O_2 from blood vessels into distant cell layers to meet the needs of those tumor cells far away from the "O_2 source," i.e., from microvessels perfused with red blood cells.

Considering the data accumulated so far on strategies to improve tumor oxygenation, it seems unlikely that there is a single solution to overcoming tumor hypoxia. A more rational approach may be to combine a number of these means since many of the factors contributing to tumor hypoxia work in parallel rather than in series with one another.

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Oxygenation Status and Tumor Oxygen Level

►Oxygenation of Tumors

P6 Protocol

Definition

Seven courses of high-dose multiagent chemotherapy consisting of four cycles with cyclophosphamide, doxorubicin, and vincristine, and three cycles with ifosfamide and etoposide.

►Desmoplastic Small Round Cell Tumor

p8

►p8 Protein

p8 Protein

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Synonyms

p8; Candidat of Metastasis 1; NUPR1; *Homo sapiens* nuclear protein 1

Definition

The *p8* gene, first described as overexpressed in the ►pancreas during the acute phase of pancreatitis, encodes a ubiquitous nuclear and cytoplasmic ►stress protein. Expression of the p8 mRNA is rapid, strong and transient in response to several stresses. The human p8 gene was assigned to chromosome 16, at position p11.2, and the gene is organized in three exons interrupted by two introns. The sizes of exons I, II and III are 214, 150, and 329 nucleotides, respectively, and the complete mRNA sequence comprises 693 nucleotides (exclusive of the poly A tail) and has only one open reading frame.

Characteristics

The *p8* gene was cloned from human, rat, mouse, and *Xenopus laevis*, conceptually translated from the *Drosophila melanogaster* genome or deduced from EST libraries (*Bos taurus*, *Xenopus tropicalis*, *Zebrafish*, *Oryzias latipes*, *Bombyx mori* and *Paralichthys olivaceous*). p8 is a highly basic 82-aa polypeptide, with a theoretical molecular mass of about 8 kDa, containing a canonical bipartite domain of positively charged amino acids typical of ►nuclear localization ►signals (NLS) and a nuclear/cytoplasmic location has been demonstrated for human p8. Importantly, the nuclear or cytoplasmic localization of p8 depends on growth conditions. When cells are growing p8 is nuclear whereas it is at the cytoplasm when cell growth is arrested. The transport to the nucleus is ►ATP-dependant and localization is also regulated by its p300-dependent acetylation.

The p8 protein contains an N-terminal ►PEST region (Pro/Glu/Ser/Thr-rich), suggesting a regulation of p8 expression by the ubiquitin (Ub)/proteasome system. Homology searching in databases yielded no homology of p8 with other proteins of known function. Biochemical properties of the mammalian p8 proteins are a high isoelectric point (9.6–10.4), 14% of acidic amino acids, 20–24% of basic amino acids, 14–17% of phosphorylable amino acids (serine, threonine, and tyrosine), and a high abundance of proline (6–9%) and glycine (5–6%). The negatively charged residues appear located at the amino-terminal region, and all the positive residues accumulate in the carboxy-terminal portion of the molecule. All these features are shared by some of the ►high mobility group proteins (HMG), particularly by the HMG-I/Y, family. The overall identity of human p8 with human HMG-I/Y is only around 35%, but the molecular mass, isoelectric point, the percentage of Arg + Lys, Glu + Asp, Ser + Thr, Gly + Pro, hydrophilicity plot, and charge separation (despite a reverse orientation) are very similar. A characteristic property of these HMG proteins, also shared by p8, is that they are neither denatured by heating at 100°C, nor precipitated by 2% trichloroacetic acid. NMR and CD

analyses of p8 showed absence of stable secondary structure. The protein binds DNA weakly and is a substrate for protein kinase A (PKA). The phosphorylated p8 (PKAp8) has a higher content of secondary structure than the nonphosphorylated protein and PKAp8 binds DNA strongly. Moreover, secondary structure prediction methods indicated that within the high homology region of others proteins, there is a basic helix-loop-helix secondary structure motif, characteristic of some classes of ►transcription factors. An architectural role in transcription is proposed and several apparently unrelated functions have been ascribed to p8.

p8 is described as a stage-specific component of the gonadotrope transcriptome that may play a functional role in the initiation of LHβ gene expression during embryonic cellular differentiation. p8 is also a transcriptional regulator critical to two key cellular events in heart failure: cardiomyocyte hypertrophy and cardiac fibroblast matrix metalloproteases (MMP) expression. Furthermore, p8 is an important component of the defense program. For example, inactivation of the p8 gene increases liver sensitivity to CCl4 or increases sensitivity to systemic LPS treatment. Thus, p8 is an important element in the ►stress response. Finally, p8 and p53 are involved in an autoregulatory loop, p8 regulating p53 transactivation activity and p53 acting as a strong repressor of p8 expression. Also, p8 is involved in two major mechanisms, ►cell cycle regulation and ►apoptosis. To account for these various functions, it is suggested that the small size of the protein, its lack of ►specific tri-dimensional structure, and its nuclear-cytoplasm localization allow its interaction with several partners to target different signaling pathways. Several partners have been identified by yeast two-hybrid screening cDNA libraries.

Tumor Establishment and Progression

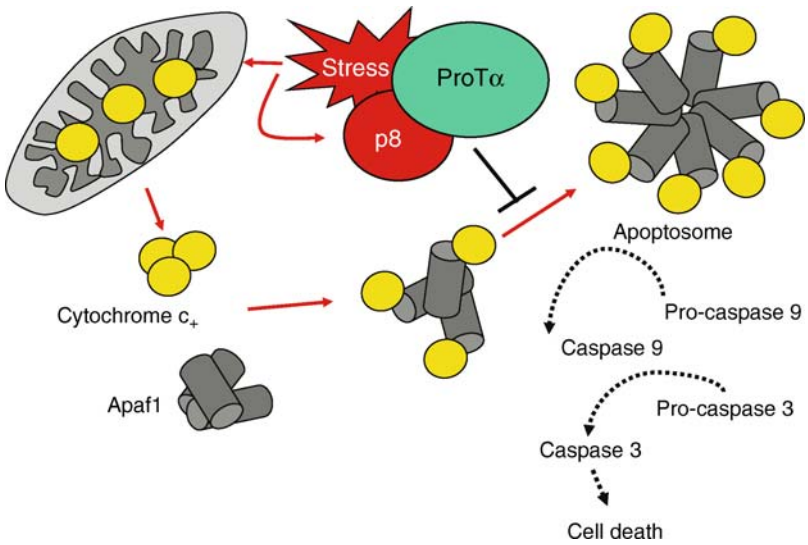
Tumor cells form ►metastasis to distant organs in a selective manner, and the organ specificity of the metastatic process is assumed to be governed, at least in part, by interactions between the malignant cells and local microenvironmental factors. It is currently admitted that development of metastases is primarily the result of the ability of disseminated tumor cells to initiate and continue growth in the target organ. In fact, cancer progression occurs in several steps; during transformation some cancer cells are positively selected within the tumor on the basis of their growth capacity, low response to apoptotic signals, and ability to escape the immunological survey of the host. After leaving the primary tumor, transformed cells migrate through the body. Yet, metastasis will not develop in all tissues. Capacity for invading the target organ is a first limitation. But once within the organ, metastasis will develop only if transformed cells can cope with their new microenvironment. Therefore, invading cells are exposed to the stress induced by the new microenvironment and their capacity to react by activating stress-associated genes should be determinant in metastasis formation. Supporting this hypothesis, several stress-associated genes were found overexpressed in tumors and their expression level often correlated with aggressiveness. Therefore, the stress-associated genes might facilitate tumor progression and metastasis formation by helping cell adaptation to the microenvironment of the host tissue. p8 is overexpressed in many human ►cancers. Its expression is crucial for tumor development and the stress-response mechanisms governed by p8 are required for tumor establishment and progression. Impossibility for p8-deficient cells to form colonies in soft-agar, to develop as subcutaneous tumors, or to generate intraperitoneal spreading strongly suggest that expression of p8 is required for the organization and development of tumors. Furthermore, p8 mediates the growth of tumor cells after metastatic establishment in a secondary organ, indicating that activated expression of p8 in metastatic cells is required for tumor progression. Some clinical data indicate that p8 expression in breast and pancreatic cancers correlates with aggressiveness and that metastatic cells express high levels of p8. However, the molecular mechanisms by which p8 allows tumor progression is still unknown but its contributions to cell cycle regulation and apoptosis are probably involved.

p8 and Cell Cycle Regulation

In response to stress agents, cells activate various intracytoplasmic pathways, depending on cell type and on the nature of the agent, that eventually reach the nucleus to modulate gene expression. By regulating genomic response, these stress-associated pathways will determine whether a cell re-enters the cell cycle, undergoes cell cycle arrest, or enters a cell-death program. *p8* is a stress gene, regulating cell cycle progression. It can act as a growth-promoting factor when it is overexpressed in pancreatic or HeLa cells or as a growth inhibitor when expressed in MEF (murine embryonic fibroblasts) or breast cancer-derived cells. These functions seem to involve regulation of the cyclin-dependant kinase inhibitor p27Kip1, in part through its interaction with JAB1.

p8 and Apoptosis

p8 expression has been inversely correlated to apoptosis in samples obtained from human pancreatic and breast cancers. According to this antiapoptotic effect, the interaction p8/Prothymosin alpha (ProTα), one of the molecular partners of p8, is very exciting. This ►natively unstructured protein was originally considered as a thymic hormone but, like p8, it was eventually attributed several other functions. p8 and ProTα are two



p8 Protein. Figure 1 p8 and prothymosin alpha (ProTα) are required for caspase inhibition in stressed cells. In response to apoptogenic stimuli, Apaf1 oligomerizes with cytochrome c to form the apoptosome that recruits and activates caspase 9 that, in turn, activates effector caspase 3. Following a stress p8 and (ProTα) complex impedes apoptosome formation, which prevents caspase 9 activation and blocks the apoptotic cascade.

small proteins without stable secondary structure in solution, showing opposite electrostatic charges at neutral pH. They interact and promote mutual stabilization of their structures in a particular conformation, the resulting p8/ProTα complex becoming able to block staurosporine-induced apoptosis. In other words, two natively unfolded proteins, p8 and ProTα, which had been attributed an antiapoptotic function, are in fact inactive if alone and require interacting with each other to exert that function, probably because the active complex has acquired stable secondary structure. Moreover, p8 is involved in the effect of ►gemcitabine, the only chemotherapeutic treatment presently available for pancreatic cancer. It was demonstrated that in pancreatic cancer cells, a large part of gemcitabine-induced apoptosis results from the inhibition of the constitutive antiapoptotic activity of p8 (Fig. 1).

p8 can also act as a proapoptotic protein. In fibroblast, the presence of p8 sensitizes cells to apoptosis induced by DNA damage. Moreover, recent works demonstrate a link between p8 and the antitumoral effects of ►cannabinoids. 9-tetrahydrocannabinol (THC) is the most abundant compound of *Cannabis sativa*, with potential therapeutic applications in patients with cancer. This antitumoral action of THC relies, at least in part, on its ability to induce p8 expression and subsequent p8-mediated apoptosis of tumor cells.

Conclusion

In conclusion, p8 is a small, highly basic and natively unfolded protein whose expression is induced by several stresses. p8 interacts with numerous partners to regulate transcription, cell cycle, and apoptosis. Functions of p8 depend on its molecular partner, its cellular location, the cell-type, and its level of expression. Of particular interest is its role on tumor development. Finally, p8 might be a new drug-targetable gene whose blockade would prevent cancer progression and metastasis development.

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Acknowledgments

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p14ARF

Definition

p14ARF is an alternate reading frame (ARF) product of the ►CDKN2A locus (14 kDa polypeptide in humans, 19 kDa in mouse). p14ARF inhibits a E3 ubiquitin ligase, mdm2 that degrades p53, a potent tumor suppressor via ►ubiquitination.

►Doublecortin
►ARF Tumor Suppressor Protein

p16

►CDKN2A

$p16^{INK4}$

►CDKN2A

$p16^{INK4A}$

►CDKN2A

p19ARF

Definition

A polypeptide that binds to ►Mdm2 and prevents it from degrading ►p53.

►Neurofibromatosis 1
►MDM Genes

p21 (Waf1/Cip1/Sdi1)

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Synonyms

Waf1; Cip1; Sdi1; CAP20; mda-6; CDKN1A; cyclin-dependent kinase inhibitor 1A

Definition

The p21 protein has been defined as an inhibitor of ►cyclin-dependent kinases (CDKs). Inhibition of this group of enzymes by p21 leads to the cessation of cell proliferation. Additionally, p21 also binds to and inhibits the activity of ►PCNA (proliferating cell nuclear antigen, which is a subunit of DNA polymerase), which results in the termination of DNA replication. Because both of these functions lead to cell cycle arrest, p21 is also called an inhibitor of the ►cell cycle.

Additional functions of p21 are known that are unrelated to its ability to inhibit cell proliferation. For example, p21 can regulate transcription, restrain ►apoptosis, impose on cell motility, interfere with cellular differentiation and ►senescence, act as cytoplasmic regulator of nuclear import, and may affect DNA methylation status. These multiple functions rely on different cellular localizations and different targets that are present either in the cytoplasm, in the nucleus, or on DNA. Because of this pleiotropic nature of p21 function, its traditional definition as a "CDK inhibitor" or "cell cycle inhibitor" is incomplete; the well-informed investigator should not consider p21 merely as an inhibitor of the cell cycle.

Characteristics

Because p21 has been discovered by diverse experimental approaches, it has received different suffixes, including wildtype p53-activated fragment 1 (Waf1), senescent-derived inhibitor-1 (Sdi1), CDK2-interacting protein (Cip1), CDK2-associated 20K protein (CAP20) or melanoma differentiation-associated protein (mda-6). Most commonly, it is called p21Waf1 ($p21^{Waf1}$) or p21Cip1 ($p21^{Cip1}$).

The p21 gene is called CDKN1A (cyclin-dependent kinase inhibitor 1A). It is composed of three exons that are located on human chromosome 6 (6p21.2). Its 2.1 kb transcript encodes a 21 kD polypeptide (hence its name), which consists of 164 amino acids. The p21 protein is primarily located in the nucleus, although it may relocate to the cytosol where it exerts important

functions as well. ►Orthologues of this gene have been cloned from mouse, rat, cat, and *Xenopus*, and are likely to exist in organisms as diverse as insects, plants and yeast.

Cell Cycle Inhibition by p21

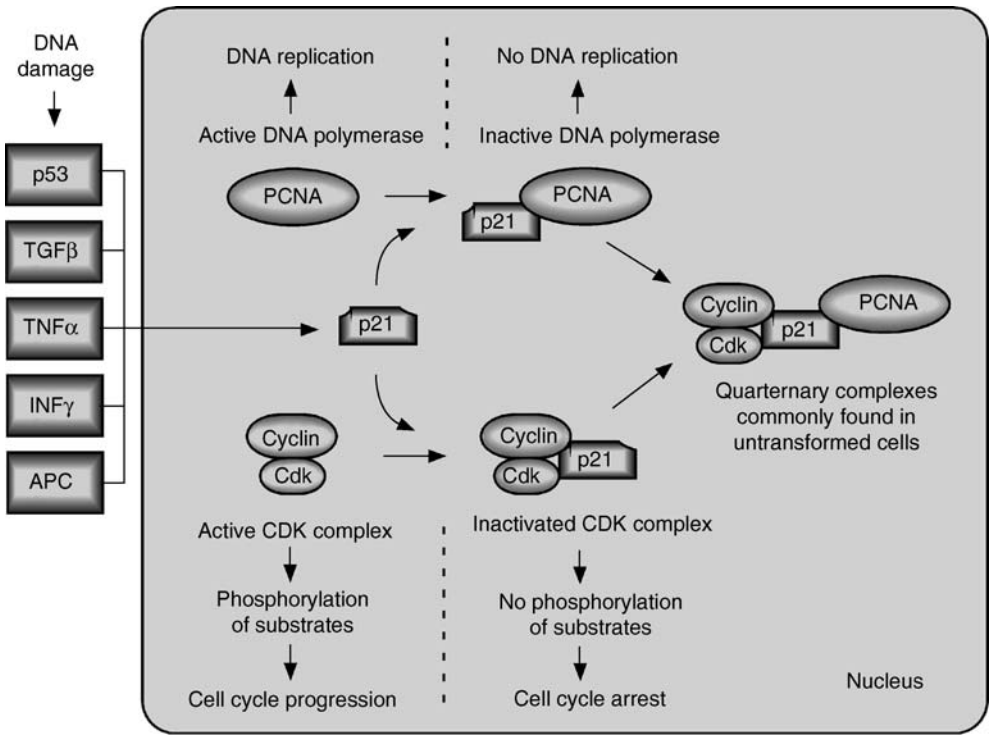
The activity of CDKs is required for cell cycle progression, while PCNA is essential for the execution of DNA replication. Therefore, the inhibition of CDK and PCNA activity by elevated levels of p21 causes cell cycle arrest, in that way defining p21 as a negative regulator of cell proliferation (Fig. 1). In extension, p21 has been implicated in several other processes that are related to cell growth, such as differentiation, senescence, and carcinogenesis.

The major targets of p21, the cyclin-dependent kinases, are considered the "engine of the cell cycle," and they direct the events required for cellular proliferation. Each CDK consists of two parts: one part is a cyclin protein (which acts as a regulatory subunit) and the other part is the actual cyclin-dependent kinase (cdk, which constitutes the catalytic subunit). The complex, composed of one cyclin subunit plus one cdk subunit, is called CDK. Several different ►cyclin proteins (cyclin A, cyclin B, etc.) as well as several different ►cdk (►cyclin-dependent kinases) subunits (cdk1, cdk2, etc.) exist and can combine to form different combinations of CDK heterodimers. For cell cycle progression and cell division to occur, the following CDK complexes are absolutely required: cyclin D together with cdk4 or cdk6 (cyclin D/cdk4 and cyclin D/cdk6), cyclin E/cdk2, cyclin A/cdk2, cyclin A/cdk1, and cyclin B/cdk1. The enzymatic activity of all CDK complexes, and thus their ability to drive cells through the cell cycle, is strictly regulated by post-translational modifications (phosphorylation and dephosphorylation) and by their interaction with inhibitory proteins, the cyclin-dependent kinase inhibitors (abbreviated CKI or CDI).

There are two families of ►CKIs:

- one is the Cip/Kip (CDK inhibitory protein) family that is constituted of p21 itself, p27Kip1, and p57Kip2
- the other is the INK4 (inhibitor of kinase 4) family, of which the tumor suppressor p16INK4a is the best-studied member.

While the INK4 proteins bind to and inhibit the activity of only two CDKs, namely cdk4 and cdk6, members of the ►Cip/Kip family are rather indiscriminatory and affect all of the further above mentioned CDK complexes. p21 in particular has been labeled as a universal inhibitor of CDKs.



p21 (Waf1/Cip1/Sdi1). Figure 1 Cell cycle-inhibitory function of p21.

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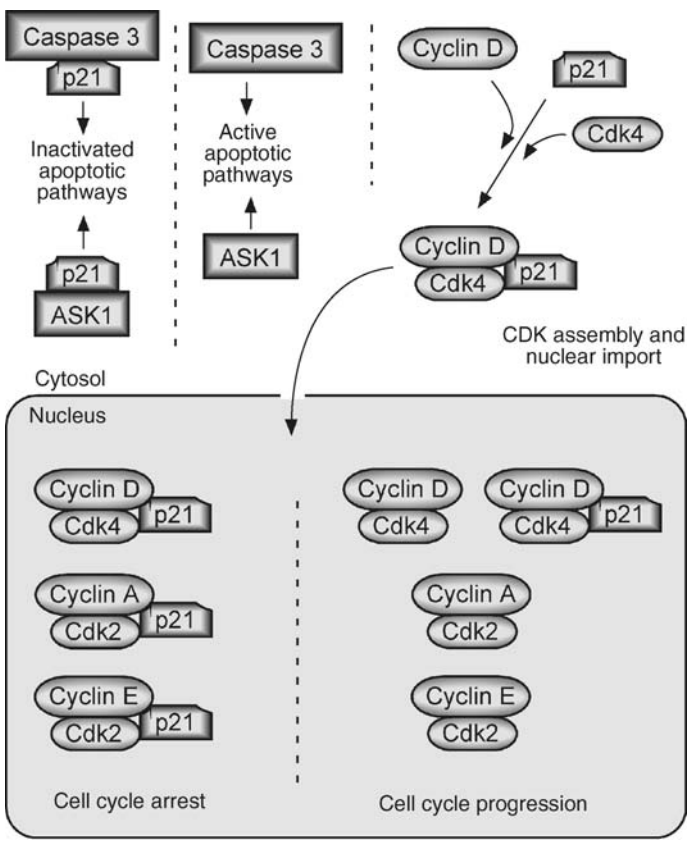
Positive Regulation of Proliferation by p21

The major critical function of p21, in particular at elevated expression levels, is the inhibition of CDK activity, which subsequently causes cell cycle arrest and inhibition of cell division. However, at rather low concentrations, p21 does not inhibit all CDKs with the same efficiency. In the case of cyclin E/cdk2 and cyclin A/cdk2, a single molecule of p21 is sufficient to completely inhibit the respective catalytic activity. Thus, in this instance, p21 fully conforms to its reputation as a potent CDK inhibitor. However, in the case of cyclin D/cdk4 and cyclin D/cdk6, two molecules of p21 are required for inhibition; at equimolar concentrations (one molecule of p21 plus one cyclin/cdk complex) p21 does not inhibit cyclin D/cdk4 or cyclin D/cdk6 activity. Rather, under these conditions, p21 stimulates the assembly and nuclear targeting of these complexes (Fig. 2). Moreover, the binding of p21 by cyclin D/cdk4 and cyclin D/cdk6 sequesters this inhibitor away from cyclin E/cdk2 and cyclin A/cdk2, thus indirectly promoting activation of these latter CDK complexes. As a consequence, the relatively low concentrations of p21, which are present in most cells, do not act cell growth inhibitory. For these reasons, the widely accepted definition of p21 as a general CDK inhibitor may have to be modified to accommodate its recognized role as a differential regulator of individual cyclin/cdk combinations.

p21's activity as an assembly factor for cyclin D/cdk4/6 is counter-intuitive and in contrast to its function as a CDK inhibitor. In this context, the complete repression of p21 repression of p21 leads to impairment of cell cycle progression due to decreased cyclin D/cdk4/6 complex formation. This outcome clearly does not support the simplified expectation that repression of p21 should result in growth promotion, as predicted from its traditional role as a CDK inhibitor. The biological rationale behind this aspect of p21 function is not yet completely understood, and further studies are required to illuminate this conundrum.

The p21 gene is a transcriptional target for multiple proliferation-inhibitory signaling cascades, including p53 (which can be activated in response to DNA damage), TGFβ (transforming growth factor beta), TNFα (tumor necrosis factor alpha), IFNγ (interferon gamma), or APC (adenomatosis polyposis coli) (Fig. 1).



p21 (Waf1/Cip1/Sdi1). Figure 2 Inhibitory and stimulatory functions of p21 protein.

p21 binds to the DNA polymerase subunit of ►PCNA and inactivates its DNA-replicative function. As a consequence, DNA replication, but not DNA repair, is blocked. p21 also binds to cyclin/cdk complexes and inhibits their enzymatic activity. As a result, the substrates of the cyclin/cdk complexes are not being phosphorylated, and the cells become arrested in the cell cycle. Thus, through the increase of p21 levels, the cells are able to stop proliferation. This process can be reversed: a reduction in the amount of p21 protein will release the inhibition of PCNA and of cyclin/cdk, and the cells resume growth.

Binding of p21 to Target Proteins

The CDK- and PCNA-inhibitory activities of p21 are functionally independent and reside in separate parts of the protein. The amino-terminal region, which is strongly conserved among the ►Cip/Kip family members contains distinct cyclin- and cdk-interacting domains; amino acids 17–24 (the Cy1 site) constitute the binding site for cyclin, whereas residues 53–58 (K site) in conjunction with amino acids 74 to 79 (the 3_{10} helix) contact the cdk. Thus, p21 simultaneously binds to both subunits of the CDK complex, whereby the interaction with the cyclin subunit serves to stabilize the ternary complex. As deduced from the crystal structure of the cyclinA/cdk2/p27Kip1 complex, the mechanism through which Cip/Kip proteins inhibit the kinase is through blocking the binding of ATP. This is achieved by a two-fold process: first, the inhibitor inserts a small helix inside the catalytic cleft of the cdk subunit that directly blocks ATP binding; second, the inhibitor causes a conformational change within the amino-terminal region of the cdk subunit that results in the loss of many ATP-interacting elements. Together, these processes result in the efficient, yet reversible, inhibition of kinase activity. It remains to be established why binding of one molecule of p21 to complexes containing cdk4 or cdk6 does not result in a similar inhibition of kinase activity.

Cytoplasmic p21 can bind to caspase 3 and ASK1 (apoptosis signal-regulating kinase 1) and thereby inhibit apoptosis (Fig. 2). Moreover, cytoplasmic p21 functions as an assembly factor that promotes the association of cyclin D with cdk4 (or cdk6) and supports nuclear entry of this complex, thereby stimulating cell cycle progression at low concentrations (Fig. 2). Increased levels of nuclear p21 inhibit cyclin A/cdk2 and cyclin E/cdk2 complexes, leading to cell cycle arrest.

In addition to modulating CDK activity, p21 protein is able to inhibit DNA synthesis through its ability to bind to and block the activity of ►PCNA, which is an accessory protein of the major replication enzymes DNA polymerase δ and ε. The interaction with PCNA is achieved via a high-affinity binding site that stretches from amino acids 143–160 in the carboxy-terminal domain of p21. As a result, p21 masks contact sites on PCNA that are required for interaction with other proteins of the polymerase assembly. The interaction of PCNA with DNA, however, is not impeded by p21 binding. It is interesting to note that by binding PCNA, p21 inhibits DNA replication but not DNA repair. Rather, it appears that p21 actually stimulates PCNA-dependent nucleotide excision repair. Thus, by interacting with PCNA, p21 executes a two-fold task: it blocks replicative DNA synthesis, which allows time for DNA repair to take place before the errors are duplicated, and at the same time it helps the repair to be executed.

Regulation of p21 Expression and Activity

Low amounts of p21 protein are present in most cell types. However, the expression levels can be increased significantly in response to a wide spectrum of diverse external stimuli and through the activation of different intracellular signal transduction pathways. In general, p21 expression can be regulated at multiple levels. The promoter of the p21 gene contains numerous binding sites for various transcription factors; most prominent among these is the tumor suppressor ►p53. In addition, enhanced expression of p21 can be mediated post-transcriptionally through the increased stability of its mRNA, and post-translationally through alterations in p21 protein half-life, degradation through the ubiquitin/proteasome pathway and cleavage by caspases. Moreover, the ability of p21 to interact with its respective targets can be modified by phosphorylation of p21, as well as by alterations in its subcellular localization.

The p21 protein contains a variety of phosphorylation sites that are targeted by several distinct kinases. The best characterized of these sites is threonine-145 (T145), which is phosphorylated by the pro-survival kinase ►Akt (also known as protein kinase B, PKB) and possibly also by protein kinase A (►PKA). As a result of T145 phosphorylation, p21 loses its ability to interact with PCNA and furthermore relocalizes from the nucleus to the cytoplasm. Restriction of p21 to the cytosol would be expected to severely limit its access to nuclear CDK complexes and thereby interfere with its ability to inhibit the cell cycle machinery; on the other hand, cytosolic p21 would be available to support the assembly of cyclin D/cdk4/6 complexes, which takes place in the cytosol. Although these consequences could not be demonstrated unequivocally in all cell types, they are entirely consistent with the growth-stimulatory and pro-survival role of Akt/PKB. In this context, it has been noted that p21 functions that take place in the cytosol are generally growth-stimulatory, whereas nuclear p21 dictates cell

cycle inhibitory outcomes, primarily via the inhibition of PCNA and CDK complexes, and the direct transcriptional repression of the cyclin B gene.

Besides T145, at least six additional phosphorylation sites have been described in the p21 protein: T57, S998, S130, S146, S153, and S160. Their precise functions are less well understood, but might play a role in regulating the interaction of p21 with other proteins and/or might impinge on its role as a transcriptional cofactor. Besides interacting with CDK complexes and PCNA, more than a dozen other proteins have been identified as binding partners for p21. Although many details remain to be established, it is evident that phosphorylation of p21 has profound consequences for its function. For instance, phosphorylation of serine-130 (S130) prevents the interaction of p21 with cdk2, which in effect neutralizes its function as a CDK and cell cycle inhibitor.

Role of p21 in p53 Pathway

The best understood regulation of p21 is in response to stress conditions that generate DNA damage, such as during cellular exposure to certain chemotherapeutic drugs, oxidants or irradiation. These insults activate the tumor suppressor ►p53 (►p53 Protein, Biological and Clinical Aspects), which binds to the p21 promoter and increases p21 expression. Elevated levels of p21 cause cell-cycle arrest and provide the cells with the opportunity to repair the DNA damage. In this sense, p21 exerts a protective function against stress, which relies on its ability to suppress cell proliferation. Cells that were engineered to lack the p21 gene have been found to inefficiently arrest in the cell cycle after DNA-damaging insults and are much more prone to cell death (apoptosis). Thus, p21 is a major executioner of p53 functions and contributes to cellular survival after exposure to harmful stimuli. In cells that are deficient in p53 function, as is the case in more than 50% of all human cancers, the transcriptional induction of p21 is not observed in response to many DNA-damaging agents.

Role of p21 in Differentiation and Senescence

The expression of p21 is highly modulated during the course of cellular differentiation and development. At least in some tissues, the exit from the cell cycle during terminal differentiation is mediated by p21, and this function of p21 is regulated by pathways that are independent of p53. Thus, these differentiation-inducing signals act through pathways that target transcription factors other than p53. One such factor is MyoD, which is a central regulator of myogenesis that induces myocytes to exit the cell cycle and fuse to form myotubes. A binding site for MyoD has been identified in the p21 promoter and shown to mediate p21 induction during myogenesis. Furthermore, the ability of p21 to be induced in a p53-independent manner has been established for various differentiation models in vitro as well as during development in vivo. From these studies it has been determined that the induction of p21 is a primary mediator of differentiation. Moreover, in the case of hematopoietic stem cells, p21 has been shown to be the molecular switch that keeps these cells in relative quiescence; proliferative restriction is critical to the survival of these cells as increased cycling causes their premature depletion.

Most normal cells that are cultured in vitro cease to proliferate after a finite number of doublings, a process called ►senescence. It has been shown that p21 accumulates as cell cultures age and approach senescence. In parallel with the increase in p21, the enzymatic activity of cyclinE/cdk2 complexes is decreased and PCNA activity is inhibited. In addition, probably through indirect mechanisms, p21 has been implicated in the negative regulation of ►telomerase, which is an enzyme that is required for the extension of cellular life span. In this regard, it has been shown that the inactivation of the p21 gene delayed cellular senescence, i.e., the cells underwent more doublings before they reached senescence. In other studies, it was shown that the experimental inhibition of p21 function in early senescent cells stimulated the cells to re-enter S-phase and replicate their DNA, although the cells did not undergo cell division. This finding is consistent with the fact that in early senescent cells, cyclinE/cdk2 complexes and PCNA are inhibited by p21. Upon removal of p21, both enzymes resume their function and drive cells through S-phase. Overall, p21 appears to be a critical initiator of the senescent process. However, once cells are fully senescent, other proteins, such as the CKI p16INK4a, take over for the long-term maintenance of this arrest.

Role of p21 in Apoptosis

The p21 protein protects cells from genotoxic and other cellular stresses. It was described above that after DNA damage, p53 transcriptionally activates the p21 gene, and elevated levels of p21 protein protect cells from apoptosis by arresting the cell cycle and permitting DNA repair. Moreover, cytoplasmic p21 can act as an anti-apoptotic component in a more direct manner (Fig. 2). It can bind to pro-caspase 3 and prevent its activation, thereby providing resistance to ►Fas-mediated apoptosis. Furthermore, p21 interacts with and inhibits the pro-apoptotic kinase ►ASK1 (apoptosis signal-regulating kinase 1), which phosphorylates p21 at serine-98 (S98). Both of these events are enhanced by the pro-survival kinase Akt/PKB (and possibly PKA), which phosphorylate p21 on T145,

thereby stabilizing the protein and maintaining its location outside the nucleus.

The pro-survival and anti-apoptotic function of p21, which is executed in the cytoplasm, seems to be in contrast to its primary nuclear function, which is inhibition of CDK and PCNA activity with ensuing cell cycle arrest and inhibition of cell growth. Thus, the tumor suppressor-like characteristics of nuclear p21 appear to conflict with its cytoplasmic purpose, which rather resembles typical oncoprotein function.

Tumor-Suppressing Function of p21

Clinical Relevance

In theory, every cyclin-dependent kinase inhibitor should be a tumor suppressor. By virtue of their ability to arrest cells in the cell cycle and prevent their proliferation, CKIs are crucial negative regulators of cell growth. The elimination of CKI activity, for example by mutation or deletion, should release CDK complexes from this form of inhibition and promote unrestricted, inappropriate cellular growth. Conversely, the artificially increased expression of CKIs in tumor cells, for example through a gene therapy approach, should lead to the cessation of tumor cell proliferation.

In reality, a tumor suppressor function of p21 has been difficult to prove. Unlike p16INK4a, which is a *bona fide* tumor suppressor and frequently found inactivated in human cancers, p21 has not yet convincingly revealed such a function. Efforts to find tumors that harbor mutations in the p21 gene yielded such events only at a very low rate. However, polymorphisms of p21 have been observed in various cancers and one of them (at position 149) was found to be associated with human esophageal cancer.

Initially, the generation of mice with a homozygous deletion of the p21 gene yielded disappointing results; these mice did not exhibit an increased rate of spontaneous tumor formation. This was surprising because p21 is one of the major executioners of the tumor suppressor p53, and mice without the gene for p53 have a rapid rate of early tumor formation. Nonetheless, keratinocytes that were established from the p21-deficient mice exhibited increased susceptibility to transformation by the ►ras oncogene, and the resulting tumors grew much more aggressively as compared to ras-transformed keratinocytes from mice that contained the normal p21 gene. Thus, it appears that the lack of p21 function contributes to the promotion of malignant tumorigenesis, and that the absence of p21 may become obvious and detrimental to cellular function only under conditions of stress, such as in response to genotoxins or aggravated growth-stimuli by oncogenes.

When artificially introduced and overexpressed in either normal or tumor cells, all CKIs tested to date cause cell cycle arrest. This effect could be useful in gene therapy-based treatments of human cancers in the future, after the numerous difficulties associated with gene therapy approaches have been solved. Two different approaches can be envisioned: First, CKIs could be used to complement defects in the tumor cells and either restore normal growth control or completely suppress tumor cell growth. Second, CKIs could be used to selectively, and reversibly, block the growth of only normal cells; this would permit the use of high doses of chemotherapeutic drugs to efficiently kill and eliminate the rapidly dividing tumor cells. The normal cells, due to their transiently arrested state, would be protected from the cytotoxic drugs. After termination of the chemotherapy, the elevated amount of CKI activity would have to return to pre-treatment levels in order to allow resumption of normal cell growth. Although still far from reality, such CKI-based gene therapy approaches could one day supplement our arsenal of anti-cancer weapons.

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p28

►Gankyrin

p40AIS

►p53 Family

p51

►p53 Family

p53

Definition

Synonym: TP53; A protein encoded by a tumor suppressor gene that is frequently mutated in many types of cancer. This 53-kilodalton phosphoprotein mediates a key cell cycle regulatory checkpoint in response to DNA damage or activation of certain oncogenes and, thereby, is able to cause potentially cancerous cells to repair or destroy themselves.

►p53 Family
►TP53

p53 Family

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Synonyms

p63 – TP63; Trp63; p51; ket; TP73L; p40AIS; p73 – TP73; Trp73

Definition

The p53 family consists of ►p53 and its two homologues, p63 and p73. All three proteins are transcription factors that bind specific DNA sequences to mediate gene expression involved in cell cycle arrest, apoptosis, and differentiation. *p63* and *p73* map to chromosomes 3q27 and 1p36, respectively. The three p53 family genes and their protein products share many characteristics, including some "p53-like activities." However, some differences in structure and function suggest that each protein has unique roles in various biological and pathological processes from development to oncogenesis.

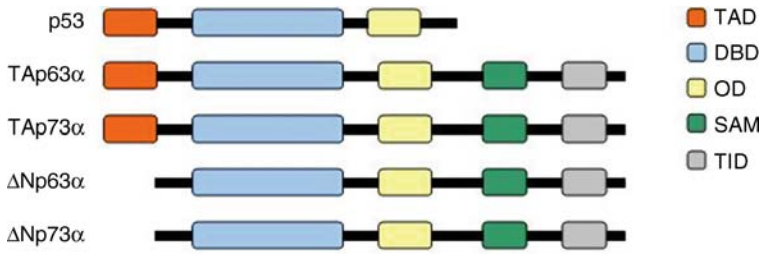
Characteristics

Molecular Structure and Function

In the late 1990s, two decades after the initial discovery of the ►p53 tumor suppressor protein, the p53 homologues p63 and p73 were identified. The *p63* and *p73* genes give rise to multiple mRNA, which are translated into several distinct isoforms (Fig. 1). Different isoforms result from the utilization of two promoters as well as alternative splicing. The three p53-family proteins have a common modular architecture with an N-terminal ►transactivation domain (TAD or TA), a ►DNA-binding domain (DBD), and an ►oligomerization domain (OD). The full-length p63 and p73 proteins that are translated from the first promoter are most similar to p53 and are referred to as TAp63 and TAp73, respectively. Like p53, TAp63 and TAp73 form oligomers and can induce apoptosis and cell cycle arrest. TAp63 and TAp73 regulate many of the same downstream target genes as p53 by binding to p53-responsive elements in genes such as ►*p21*, ►*PUMA* and *BAX*. In addition, some unique target genes have been identified for p63 and p73, likely reflecting different promoter binding sequence preferences as well as unique tissue patterns of expression. Known TAp63 and p73 target genes are summarized in Table 1.

p63 and *p73* also encode N-terminally truncated isoforms, ΔNp63 and ΔNp73, that lack the TAD. These ΔN variants are transcribed from the second promoter located within the third intron of both genes. Although the lack of TAD renders ΔN proteins transcriptionally inactive, they retain the ability to bind DNA and thus compete with transactivation-competent p53-family proteins for promoter-binding sites. They can also form ►hetero-oligomers with TA proteins, preventing the formation of functional tetramers. Therefore, ΔNp63 and ΔNp73 are not only unable to induce downstream target genes, but they can act as dominant-negative proteins that block TAp63, TAp73, and p53 activities and thus, have anti-apoptotic properties.

Alternative splicing at the C-terminus adds further to the complexity of p63 and p73, resulting in unique coding sequences (Fig. 1). The longest p63 and p73 proteins (p63 and p73 alpha) have a ►sterile-α-motif (SAM) domain not found in p53. SAM domains are known to serve as protein-protein interaction modules, raising the possibility that p63 and p73 splice variants with SAM domains are capable of recruiting isoform-specific binding proteins. A transcriptional inhibitory domain (TID) is also found in a subset of the different C-terminal isoforms. Thus, differential splicing of the C-termini influences the ability of these proteins to



p53 Family. Figure 1 The structure of the p53-family of proteins. Shown are the transactivation (TAD, orange), DNA-binding (DBD, blue) and oligomerization (OD, yellow) domains for p53, p63 and p73. These p63 and p73 domains show significant homology to p53 (TAD, ~25%, DBD ~60%, and OD ~40%). Alternative splicing at the C-terminus of p63 and p73 generates multiple isoforms (p63 α, β, γ and p73 α, β, γ, δ, ε, Φ, ζ, η) that are identical through the OD. The longest p63 and p73 isoforms (alpha) are shown in the figure and contain a SAM (green) and transinhibitory domain (grey) not found in most other C-terminal isoforms.

p53 Family. Table 1 Target genes of the p53-family

Common p53-responsive p63 and p73 target genes	Puma
	Noxa
	p21
	Mdm2
	Bax
	CD95
	Apaf-1
	TNF
	TNF-R1
	Perp
	AIP
	Redd1
Unique p63 and p73 downstream genes	Jagged 1/2
	β4-integrin
	Dlx3 (p63)
	Ets-1
	IKKa
	Gata-3
	CyclinD1
	Aquaporin (p73)
	P57 kip2

p63 and p73 can induce many p53-target genes that are involved in cell-cycle arrest and apoptosis (upper panel). However, both proteins can also upregulate novel target genes that cannot be activated by p53 (lower panel). Noted in parentheses are genes only regulated by p63 or p73, but not both.

transactivate target genes. For example, in comparison to TAp63α, TAp63γ more strongly induces downstream pro-apoptotic genes. Thus, the differences in coding sequences and isoform specific binding partners result in different activities of the various isoforms.

Various upstream pathways and stimuli, including DNA damaging agents and oncogenes, such as ►E2F1 and ►Ras, regulate the expression and stability of p63 and p73 isoforms (summarized in Table 2). In addition, like p53, p63 and p73 are regulated by post-translational modifications including phosphorylation, acetylation, and ubiquitination. Interactions with binding partners, such as ►p300 and ►MDM2, as well as heterotypic interactions with each other, further modulate the activities of the p53-family proteins.

Roles in Development

Unlike p53, which is ubiquitously expressed, p63 and p73 are expressed in a tissue-specific fashion and each play roles in critical developmental processes. In contrast to p53 null mice in which only a small percentage have developmental defects in neural tube closure, p63 and p73 mutant mice have significant developmental abnormalities. *p63*−/− mice that lack all TA and ΔN isoforms have significant limb and craniofacial malformations as well as failed development of epithelial tissues including the skin. In humans germline *p63* mutations have been detected in patients with characteristics reminiscent of the *p63* knockout mice. Heterozygous *p63* mutations have been found in 6 different human ►ectodermal dysplasia syndromes characterized by combinations of skin, hair, mammary gland, craniofacial and limb abnormalities. Studies into these disorders have provided important clues as to which tissues express p63 and have revealed that p63 is essential in epithelial morphogenesis and involved in senescence. Studies of *p73*−/− mice have demonstrated that ΔNp73 is crucial in the development of the nervous system. *p73* knockout mice have significant neurologic abnormalities due to the absence and/or loss of specific populations of neurons. ΔNp73 promotes cell survival by the inactivation of the full-length p53 family proteins and thus, the loss of ΔNp73 leads to enhanced apoptosis in ►cortical and ►sympathetic ganglia neurons. The relative balance between the TA and ΔN forms of p63 and p73, as well as p53, are important in these developmental processes and similarly, in cancers that arise from these tissues.

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p53 Family. Table 2 Regulators of the p53-family

Upstream regulators	Oncogenes:
	• E2F-1
	• Ras
	DNA damage:
	• chemotherapeutic agents*
	• γ-radiation*
	BMP signaling
	Notch signaling
Kinases	c-Abl
	Chk
	Akt
Binding proteins	Itch
	Mdm2*
	MdmX*
	p300
	Aspp family of proteins
	PML
	Yap
	Cyclins
	Pin1
	PIAS
	p53 family proteins (hetero-oligomerization)

The p53-family is regulated by a number of proteins and signaling pathways. These upstream regulators can affect the p63 and/or p73 at the transcriptional or the post-translational level. Some regulators have been shown to affect all three p53-family members (denoted with *).

Role in Cancer: Tumor Suppressor or Oncogene?

The high incidence of p53 mutations in human tumors coupled with the chromosomal locations of *p63* and *p73* led to the prediction that p63 and p73 were also tumor suppressor genes that are inactivated by mutations in certain human malignancies. Surprisingly, despite the functional similarities among the p53 family proteins, and specifically their ability to induce p53-responsive pro-apoptotic genes, only rare *p63* and *p73* mutations have been detected in both cell lines and primary tumors. Although the lack of mutations initially raised doubts as to whether these two genes have roles in human cancers, many studies have reported high levels of p73 and p63 expression in human cancers. However, many of these studies did not distinguish between the various isoforms. More recent evidence from mouse models and human tumors suggests that the relative expression levels of the different TA and ΔNp63 and p73 isoforms are important in tumor development and progression, as well as the response to treatment.

Knockout mice lacking one or both copies of p53, as well as mice that express specific p53 point mutations, develop a wide range of tumors at a young age. In contrast, *p63*+/− and *p73*+/− mice develop pre-malignant and cancerous lesions only when aged. Furthermore, in *p53* mutant mice, the additional loss of *p63* or *p73* leads to different tumor types, and higher tumor burden with more metastatses. Importantly, tumors from p63 or p73 heterozygous mice were shown to have ►loss of heterozygosity (►LOH) of the remaining allele, suggesting that p63 and p73 have recessive properties of a classical tumor suppressor gene. However, the relative contribution of the different TA and ΔN isoforms is difficult to determine since these knockout mice lack the expression of all p63 or p73 isoforms.

Loss of p63 and p73 has been described in several human tumor types. Loss of p63 expression is associated with bladder cancer progression and correlates with poor prognosis. TAp73 silencing by methylation in leukemias and lymphomas has also been described. Furthermore, low expression of p63 and p73 has been reported in breast cancer due to a number of mechanisms including LOH and allele silencing.

In addition to missense mutations, p53 is inactivated by binding to inhibitory proteins such as the papilloma virus E6 oncoprotein in cervical cancer and MDM2 in sarcomas. While TAp63 and p73 can likewise bind to MDM2, as well as other modulatory proteins such as MDMx, iASPP, YAP and PML (see Table 2), the relative role of these complexes in human cancers has not been elucidated. However, there is growing evidence that inactivation of the putative tumor suppressor TA forms of p63 and p73 is mediated by hetero-oligomerization with a subset of p53 mutant proteins found commonly in tumors. These mutants include the p53 conformational mutants with "►gain-of-function" properties. These mutant p53 proteins bind to and inhibit TAp63-and TAp73-dependent transactivation and apoptosis, leading to enhanced survival and growth. Furthermore, this binding affinity of mutant p53 for TAp73 is influenced by the status of a p53 polymorphism at codon 72. In certain tumors, such as head and neck squamous cell carcinoma presence of arginine, instead of proline, at p53 codon 72 is associated with worse prognosis due, at least in part, to more potent binding to TAp73. Therefore, although cancer-associated missense changes are almost never found in the new p53-family members, other mechanisms exist to inactivate the tumor suppressor like forms, TAp63 and TAp73.

Ironically, despite the focus on p63 and p73 as tumor suppressors, there is perhaps more evidence supporting roles for ΔNp63 and p73 isoforms as oncogenes. Amplification of the genomic region encompassing *p63* and overexpression of the ΔNp63 protein have been detected in several epithelial-derived tumors. In head and neck squamous cell carcinomas (HNSCCs)

and breast cancers, ΔNp63 promotes the survival of transformed cells by blocking TAp73-dependent apoptosis. Thus, ΔNp63 behaves as a potent oncogene and highlights how perturbations in the equilibrium between ΔN and TA p63 and p73 proteins are important in tumorigenesis. Whether this holds true for other tumors that express high p63 levels including cervix, nasopharynx and bladder, remains to be determined.

Much like p63, p73 is but highly expressed in several types of human malignancies. Overexpression of p73 has been reported in numerous cancer types including neuroblastoma, breast, lung, esophagus, bladder, ovarian, liver, colon and certain types of leukemias. There is emerging evidence that when carefully studied, like p63, the upregulation of the ΔNp73 forms are relevant in the pathogenesis of these cancers. However, to date specific overexpression of ΔNp73 transcripts and/or protein has only been shown in a few tumor types including mesenchymal or precursor-derived tumors such as rhabdomyosarcoma and neuroblastoma as well as breast, colon and hepatocellular carcinomas. The tissue specificity of these findings is likely due to the fact that p73 has a restricted pattern of expression throughout development. The mechanisms responsible for p73 overexpression and specific mechanism(s) by which ΔNp73 promotes cancer development remain unknown. However, there is some evidence that ΔNp73 promotes immortalization of primary cells and can cooperate with oncogenes such as Ras to enhance transformation. Furthermore, the binding of ΔNp73 to p53 andTAp73 and TAp63 has been proposed to modulate differentiation in neuroblastoma and rhabdomyosarcoma.

Clinical Relevance p63 and p73-Prognosis and Chemosensitivity

Evidence in cell and animal systems supporting a role for the different isoforms in tumorigenesis has led to the assessment of p73 and p63 expression as a marker for clinical prognosis. The relative ratio of TA versus ΔN isoforms as measured at the transcript level is linked to prognosis in several tumor types, including breast and colon cancers. High levels of ΔNp73 protein have also been correlated with poor prognosis in small series of patients with neuroblastoma and breast cancers. Immunohistochemistry for p63 has also begun to be used as a marker in several tumor types including prostate and breast. However, large analyses of primary tumor samples will be required to determine if the relative expression of TA and ΔN isoforms of p63 and p73 can be linked to clinical prognosis. Nevertheless, roles for these two p53 family proteins in the response to DNA damaging agents such as chemotherapies suggest that their expression has important therapeutic implications.

Currently, the two major treatments for cancer, radiation and ►chemotherapy, both exert their cytotoxic effects by stabilizing and activating p53. However, these therapies are also capable of killing cancer cells harboring p53 mutations, suggesting that p53-independent mechanisms exist to initiate apoptosis. Many studies support a role for the p53 family proteins in chemosensitivity. TAp73 is induced by ►γ-irradiation and many chemotherapeutic drugs and in a variety of cancer cell types, including those with mutant and wild-type p53. Studies using cells in which p73 or p63 is inactivated by genetic deletion, use of dominant-negative proteins, and short interfering RNA (►siRNA) demonstrate that loss of p63 or p73 leads to chemoresistance. Similar to their effect on p53 many chemotherapies also induce p73 and p63 post-translational modifications that stabilize and enhance their ability to induce apoptosis. These include c-abl mediated phosphorylation in response to cisplatin and irradiation. p73 is also upregulated at the transcriptional level by some drugs. For example, doxorubicin treatment leads to E2F1 mediated induction of TAp73. Similar to p53, TAp73 and TAp63 mediate the expression of genes that control cell-cycle arrest and apoptosis (Table 1). Thus, in response to anti-cancer treatments, TAp73, and perhaps TAp63, can substitute for and in certain cases, cooperate with other p53 family proteins to eradicate transformed cells and promote tumor regression.

The interactions among the p53 family proteins also modulate their ability to induce chemotherapy induced apoptosis. The expression of various tumor derived p53 mutant proteins leads to chemoresistance via the ability of these mutant proteins to bind to and inactivate TAp73 and p63. Experiments in cell lines demonstrate that manipulation of the levels of these p53 mutant proteins by overexpression or siRNA leads to chemoresistance or chemosensitivity, respectively. Some chemotherapies also affect the levels of ΔNp73 and p63. Furthermore, overproduction of ΔNp73 or ΔNp63 in cell lines also leads to chemoresistance, and this is likely due to hetero-oligomerization and inactivation of the full length p53 family proteins. This paradigm has been best studied in head and neck squamous cancers where downregulation of ΔNp63 in response to cisplatin results in diminished ΔNp63-TAp73 complex formation and enhanced apoptosis. Given the importance of ΔN proteins in cancer cell survival, this represents a second potential mechanism through which chemotherapeutic drugs induce apoptosis. Although the mechanisms by which chemotherapies downregulate ΔNp63 and p73 are not known, it is clear the balance between ΔN and TA proteins, as well as mutant forms of p53, are important determinants of chemosensitivity of tumor cells.

Given the importance of p53 in tumor suppression, ►small-molecule inhibitors are actively being designed

to activate p53. However, since p53 is non-functional in approximately half of all human cancers recently there has been interest in developing therapeutic strategies to activate p73 and p63 to induce tumor cell death. Several different approaches might lead to enhanced p73 or p63 activity. Panels of drugs and small molecules can be screened for agents that either directly increase TAp73 or p63, or enhance their activation by upstream pathways (e.g. via E2F1 activation). Furthermore, in tumors with high levels of ΔNp73 or ΔNp63 strategies might be aimed at donwregulating these anti-apoptotic isoforms. Drugs that interfere with proteins that inactivate TAp73 and p63 can also be targeted. To this end, small peptides have been designed to modulate the interactions between the iASPP proteins and p63 and p73. Furthermore, therapeutic strategies using drugs and siRNA designed to interfere with the interaction between "gain of function" mutants of p53 and TAp73 have been successful in vitro. Finally, many drugs have been designed to interfere with p53 binding to its negative regulator MDM2. It is predicted that some of these drugs might also interfere with MDM2 binding to TAp73 and TAp63, raising the possibility that these small-molecule inhibitors may be useful for a broad spectrum of cancers.

Concluding Remarks

Since their discovery, studies of p63 and p73 have been focused on comparisons to p53. While the prospect of TAp63 and TAp73 compensating for the loss of p53 in tumor suppression and treatment is exciting, it is equally important to recognize very significant differences in their roles in both cancer and development. Not all tissues and cancers exhibit the same expression pattern of p63 and p73. The levels and balance between the various pro and anti-apoptotic p53 family proteins appear to play important roles in both development and cancer. Given the importance of p63 and p73 in physiological apoptosis as well as in tumors and their response to chemotherapies, understanding how they function will likely lead to the development of better cancer treatments.

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p63 – TP63

►p53 Family

p73

Definition

Tumor protein p73, a homolog of the tumor suppressor p53. p73 can function as a transcription factor to regulate p53 target genes.

►p53 Family
►PUMA

p73 – TP73

►p53 Family

p94

►B-Raf Signaling

p185neu

►HER-2/neu

p300

Definition

p300/CBP Co-Activators.

►Retinoblastoma Protein, Biological and Clinical Functions

p300/CBP-interacting Protein

►Amplified in Breast Cancer 1

p/CIP

►Amplified in Breast Cancer 1

P-glycoprotein

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Synonyms

Multidrug transporter; Mdr1 protein; gp170

Definition

P-glycoprotein (Pgp), the product of the human ►MDR1 gene responsible for a major form of ►multidrug resistance in tumor cells, is a transmembrane protein that carries out ATP-dependent efflux of various lipophilic compounds, including many anticancer drugs. Pgp renders tumor cells resistant to such drugs, and Pgp expression has been shown to correlate with clinical drug resistance or negative prognosis in several types of cancer.

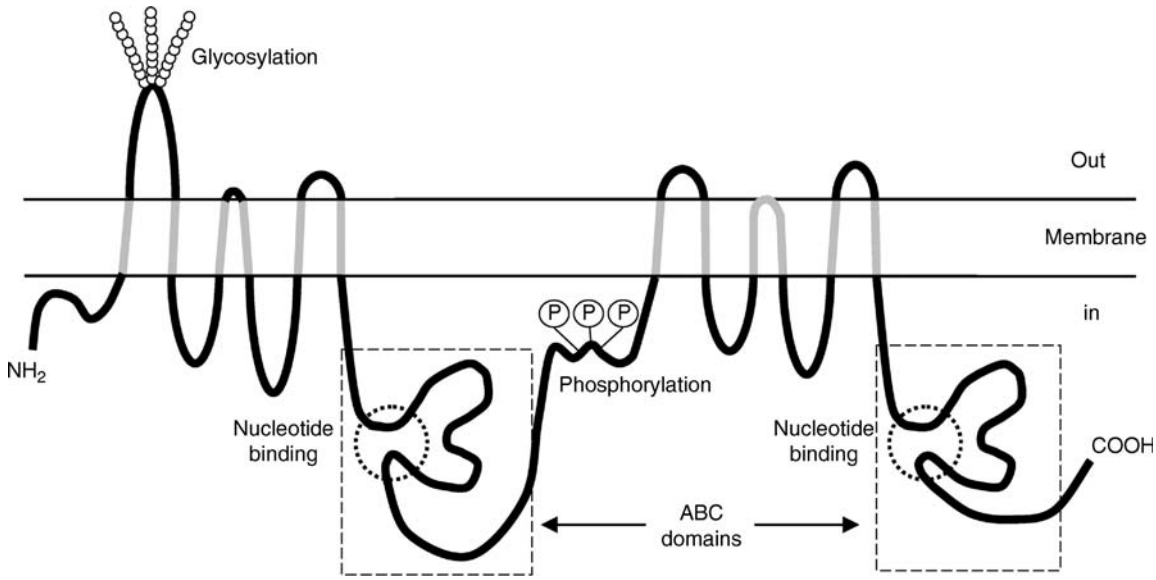
Characteristics

The best known form of multidrug resistance in mammalian cells involves cross-resistance to a large group of lipophilic drugs with different structures and mechanisms of action. Cellular targets of such drugs include microtubules (►vinblastine, ►vincristine, ►taxol, ►colchicine), ►topoisomerase II (►doxorubicin, ►etoposide), RNA polymerase (►actinomycin D), ribosomes (puromycin), plasma membrane (gramicidin D) and mitochondria (rhodamine 6G). This multidrug resistant phenotype is due to increased expression of the gene termed MDR1 (multidrug resistance 1). Expression of the MDR1 gene in drug-sensitive cells is sufficient to produce the full pattern of multidrug resistance. Rodent cells carry two homologs of the human MDR1 gene, both of which confer multidrug resistance. Another member of the MDR gene family, which is alternatively called MDR2 or MDR3 in humans, is closely related to MDR1 but does not confer detectable multidrug resistance (the product of the gene functions as a phosphatidylcholine translocase).

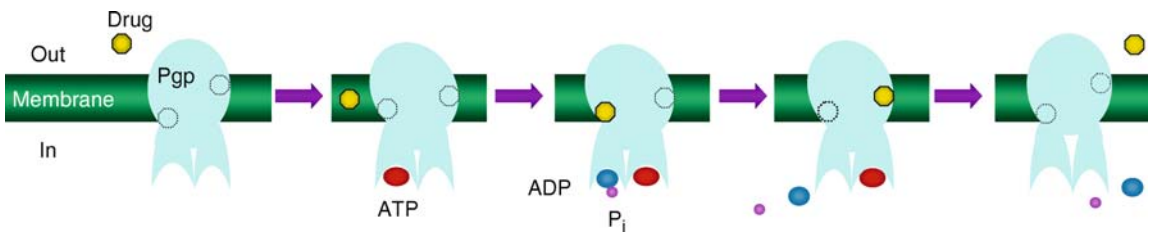
The resistance of MDR1-expressing cells was shown to result from decreased intracellular accumulation of the drugs, associated with an increased rate of drug efflux. Many lipophilic fluorescent dyes (e.g. Rhodamine 123, Hoechst 33342, calcein AM) also show decreased accumulation in MDR1-expressing cells. The exclusion of such dyes provides a convenient approach to the detection of multidrug resistant cells. The MDR1 gene encodes a glycoprotein with a mobility of ~170 kD. This protein was originally found to be elevated in the membranes of multidrug resistant cell lines and was termed P- (for permeability) glycoprotein (Pgp). Pgp, encoded by the human MDR1 gene, contains 1,280 amino acids and consists of two similar halves separated by a short linker region containing multiple charged residues and phosphorylation sites (Fig. 1). Each of the two halves of Pgp contains a N-terminal hydrophobic and a C-terminal hydrophilic region. Each hydrophobic region includes six membrane spanning α-helices. The transmembrane segments were originally deduced through hydrophobicity analysis and subsequently confirmed by epitope mapping studies. Some studies suggest, however, the existence of one or more alternative transmembrane orientations for Pgp, which may coexist with the orientation shown in Fig. 1. Pgp conformation can be altered through the binding of different ligands, as indicated by differential immunoreactivity, altered patterns of proteolytic digestion and some other assays. However, it is unknown whether these conformational transitions involve changes in the transmembrane orientation of Pgp.

The hydrophilic regions of Pgp contain nucleotide binding sites and display the characteristic sequences of the ATP-binding cassette (ABC), a conserved domain shared by a large superfamily of proteins known as ►ABC transporters. Some other ABC transporters are also involved in multidrug resistance, including the ►multidrug-resistance protein (MRP) family and the MXR/BCRP/ABCP1 protein. The ABC domains of Pgp are responsible for its ATPase activity, which enables the pumping of Pgp transport substrates against the concentration gradient (Fig. 2). The ATPase activity of Pgp is strongly stimulated by many of its transport substrates. The presence of functional nucleotide binding sites in both ABC domains is required for both the ATPase activity and the drug efflux by Pgp.

All the documented Pgp transport substrates are lipophilic or amphiphilic, but not all the lipophilic compounds are transported by Pgp. A common functional feature of Pgp substrates is their entry into a cell by passive diffusion through the lipid bilayer and not through a specialized carrier. In agreement with this



P-glycoprotein. Figure 1 Scheme of the two-dimensional structure and transmembrane orientation of Pgp.



P-glycoprotein. Figure 2 A simplified hypothetical scheme of drug efflux by Pgp.

mode of uptake, there is substantial evidence that the initial binding of many substrates to Pgp occurs within the lipid bilayer rather than on the cytoplasmic surface of the plasma membrane. Photoaffinity labeling assays have implicated the transmembrane domains of Pgp as the primary sites for substrate binding. The ability of Pgp to transport different substrates can be increased or decreased by point mutations of specific amino acid residues. Most (but not all) of such residues localize to transmembrane domains. Mutations that affect the specificity of Pgp-mediated transport were found to affect substrate binding or release and the ability of individual substrates to stimulate the ATPase activity of Pgp.

In addition to its function as an efflux pump for different lipophilic compounds, Pgp was shown to act as a short chain lipid translocase in the plasma membrane. Pgp also confers resistance toapoptosis induced by different agents (which need not be transported by Pgp), through an unknown mechanism. Other changes associated with Pgp expression in some cell lines include stimulation of volume regulated chloride channels, alteration of intracellular pH and increased ATP release. However, the putative role of Pgp in these phenomena requires further documentation.

Expression

Pgp is normally expressed in a variety of human tissues, including the adrenal cortex, lumenal surfaces of the kidney, liver, colon and jejunum, and endothelial capillaries of the brain, testes and papillary derma. It is also expressed in placental trophoblasts, in certain types of lymphocytes (particularly CD56-positive ►natural killer cells and CD8-positive T-cells) and in early hematopoietic progenitor cells of the bone marrow, where Pgp expression is positively correlated with the stem cell marker CD34. Analysis of mice with a knockout of one or both of their MDR1 homologs revealed some of the physiological functions of Pgp. Pgp ►knockout mice show a large increase in the accumulation of different drugs in the brain, indicating that Pgp in brain endothelial capillaries plays an important role in the blood-brain barrier. The knockout mice also show an increase in drug accumulation in the intestine and cardiac tissue, indicating that Pgp plays a physiological barrier role in several types of tissues.

Cellular and Molecular Regulation

Overexpression of Pgp in multidrug resistant cell lines, isolated after multistep selection with chemotherapeutic drugs, is frequently associated with the ►amplification of the MDR1 gene. Such amplification, however, appears to be specific for in vitro selection, since it occurs rarely if at all in Pgp expressing tumors. Similarly, point mutations of the MDR1 gene that increase its ability to transport specific drugs have been found in vitro but not in vivo. Another mechanism of MDR1 gene activation in vitro involves genetic rearrangements that bring the previously silent MDR1 gene into proximity with a strongly transcribed gene, sometimes providing MDR1 with an alternative transcription initiation site. There is evidence that such rearrangements may also occur in certain lymphoid malignancies. In most cases, however, increased MDR1 mRNA expression does not involve any detectable genetic changes. Several in vitro studies and in vivo observations with tumors undergoing chemotherapy indicate that the MDR1 gene can be activated epigenetically in response to different forms of cytotoxic stress or after treatment with ►protein kinase C-inducing agents. This activation may occur both at the level of transcription and of RNA stability. In particular, two CCAAT box-binding proteins, NF-Y and YB-1, were reported to mediate the induction of the MDR1 promoter by genotoxic stress, but it is still unknown to what extent these transcription factors are responsible for MDR1 activation during cancer chemotherapy.

At the protein level, Pgp undergoes post-translational modification through N-linked ►glycosylation in the first extracellular loop and ►phosphorylation by protein kinase C and protein kinase A in the linker region (Fig. 1). Neither of these Pgp modifications appear to be essential for the drug efflux function, as indicated by the activity of glycosylation- or phosphorylation-deficient mutants of Pgp in drug resistance assays. The phosphorylation mutants, however, show some quantitative changes in the stimulation of their ATPase activity by Pgp transport substrates. Another potential level of Pgp regulation is suggested by the observed variability in the percentage of Pgp that localizes to the plasma membrane (as opposed to Golgi or endoplasmic reticulum) in different cells. Modulation of the Pgp transport to the plasma membrane may represent another mechanism of its regulation.

Clinical Relevance

Pgp expression in clinical cancers has been extensively analyzed both at the RNA and protein level. These studies showed that, with regard to Pgp expression, most tumors can be divided into two classes. The first class is represented by those tumors that arise from Pgp expressing normal tissues, such as renal cell carcinomas, hepatomas, adrenocortical tumors or colorectal cancers. Pgp is commonly expressed in such tumors whether treated or untreated, and Pgp levels in some of these cancers show a positive correlation with morphological differentiation. ►Acute myeloid leukemias (AML) may also belong to the same category. As stated above, Pgp expression in normal hematopoietic progenitor cells correlates with the expression of the ►stem cell marker ►CD34, and similar correlations for CD34 and Pgp were also reported in AML. Higher Pgp expression in more primitive hematopoietic cells might also account for the high levels of Pgp observed in the blast crisis of chronic myelogenous leukemia, in contrast to low or undetectable expression in the chronic phase.

The second class of tumors arise from normal tissues with low Pgp expression (carcinomas of the lung, ovary or breast, sarcomas, some types of leukemias). Pgp expression in untreated tumors of this class is usually low and it is often undetectable even by the most sensitive assays. Pgp expression in such tumors is frequently increased after chemotherapeutic treatment. It is unknown, however, whether this increase represents the selection of drug-resistant cells by anticancer drugs, results from ►epigenetic MDR1 induction by cellular damage or perhaps even represents a treatment-unrelated parameter of tumor progression. A number of studies showed a strong correlation between MDR1 expression, on one side, and clinical drug resistance or the overall negative prognosis, on the other side. The strongest correlations of this type were reported in pediatric solid tumors (►neuroblastoma, ►sarcoma, ►retinoblastoma). There are also many reports that Pgp is a negative prognostic factor in various adult cancers, such as ovarian, breast, esophageal and small cell lung cancers, as well as myelomas, leukemias and lymphomas. Not all investigators, however, have observed such correlations for Pgp, a conundrum which probably reflects the variability and poor standardization of diagnostic methods for Pgp detection.

Given the clinical significance of Pgp expression, considerable efforts have been placed into developing and testing agents that reverse Pgp-mediated multidrug resistance, as non-toxic supplements to chemotherapeutic drugs. Many agents (some of which are themselves transported by Pgp) were found to inhibit drug binding or transport by Pgp and to sensitize Pgp-expressing cells to anticancer drugs in vitro and in animal models. The first generation of agents to undergo clinical trials included Pgp inhibitors that had already been used in clinical practice, such as verapamil or cyclosporin A. The second generation comprises much more potent compounds developed specifically for Pgp inhibition, such as Valspodar (PSC-833), a non-immunosuppressive ►cyclosporin analog. Clinical trials of Pgp inhibitors showed a moderate but significant improvement in the treatment response of several ►hematological malignancies, but solid tumor trials have not yet shown unambiguous

benefits. Pgp inhibitors in clinical trials were found to increase the plasma drug concentrations, apparently through the inhibition of Pgp function in secretory organs. There are also reports of increased systemic toxicity in the presence of Pgp inhibitors, but such side effects appear to be clinically manageable. It remains to be determined whether the relatively disappointing results of the Pgp inhibitor trials may be improved through the use of inhibitors with different pharmacokinetic properties (such as inhibitory anti-Pgp antibodies) or different clinical protocols. It is also possible that the inhibition of only one of several mechanisms of drug resistance that exist in tumor cells may be insufficient to achieve clinical benefit.

Another Pgp-based therapeutic approach that has entered phase I clinical trials involves the use of the MDR1 gene for gene therapy aimed at chemoprotection of hematopoietic stem cells. Several animal studies with MDR1-containing retroviral vectors have shown that this approach can provide chemoprotection against Pgp-transported drugs (principally ►taxol). It is conceivable, however, that some of the observed effects may not only be due to the effects of Pgp on taxol but to a general effect of MDR1 on stem cell maintenance, possibly due to the antiapoptotic effect of Pgp. While the animal studies have been promising, substantial technical improvements in the methodology of gene transfer into human hematopoietic stem cells are needed to elucidate the clinical potential of this approach.

Other Pgp-oriented therapeutic approaches are being explored in preclinical studies. One such strategy is aiming Pgp inhibitors not at the multidrug-resistant tumor cells but rather at the blood-brain barrier, which may be permeabilized by inhibiting Pgp in brain endothelial cells. Such permeabilization would allow Pgp-transported drugs to be delivered into the brain, a known pharmacological "sanctuary" of tumor metastases. Another potential strategy aims to prevent the conversion of Pgp-negative tumors to Pgp-positive. MDR1 expression is induced epigenetically in response to cellular damage with chemotherapeutic drugs. This induction may be prevented in vitro by combining cytotoxic drugs with the inhibitors of protein kinase C and several other signal transduction pathways. The potential in vivo utility of these novel approaches remains to be explored.

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PAC

Definition

Acronym of P1-derived artificial chromosomes. A vector system used to clone large DNA fragment.

►ArrayCGH

PACE

►Furin

Paclitaxel

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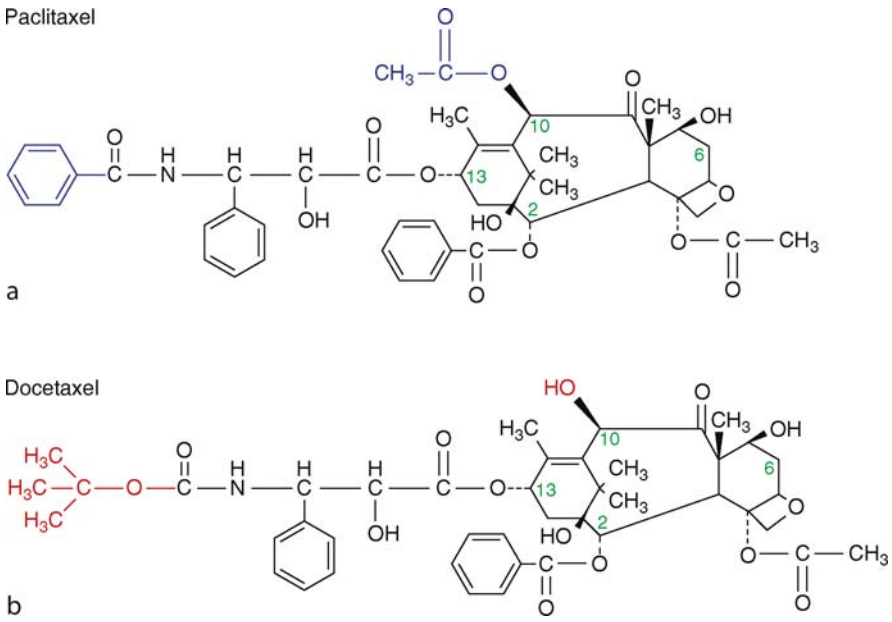
Definition

Paclitaxel is an anti-cancer agent derived from the North America Pacific yew tree (Taxus brevifolia) that is used in the treatment of a number of common malignancies including ►lung, ►ovarian, and ►breast cancer.

Characteristics

Paclitaxel is a cancer chemotherapeutic agent that is widely used for several types of malignancies (Fig. 1a and b). It is a natural product produced from the pacific yew tree. While first isolated from the bark of the Pacific yew, it is now commonly synthesized from a compound obtained from yew needles or cell culture systems.

Paclitaxel is a member of the ►taxane class of drugs, and was the first taxane to enter clinical trials and receive FDA approval. Another commonly used taxane



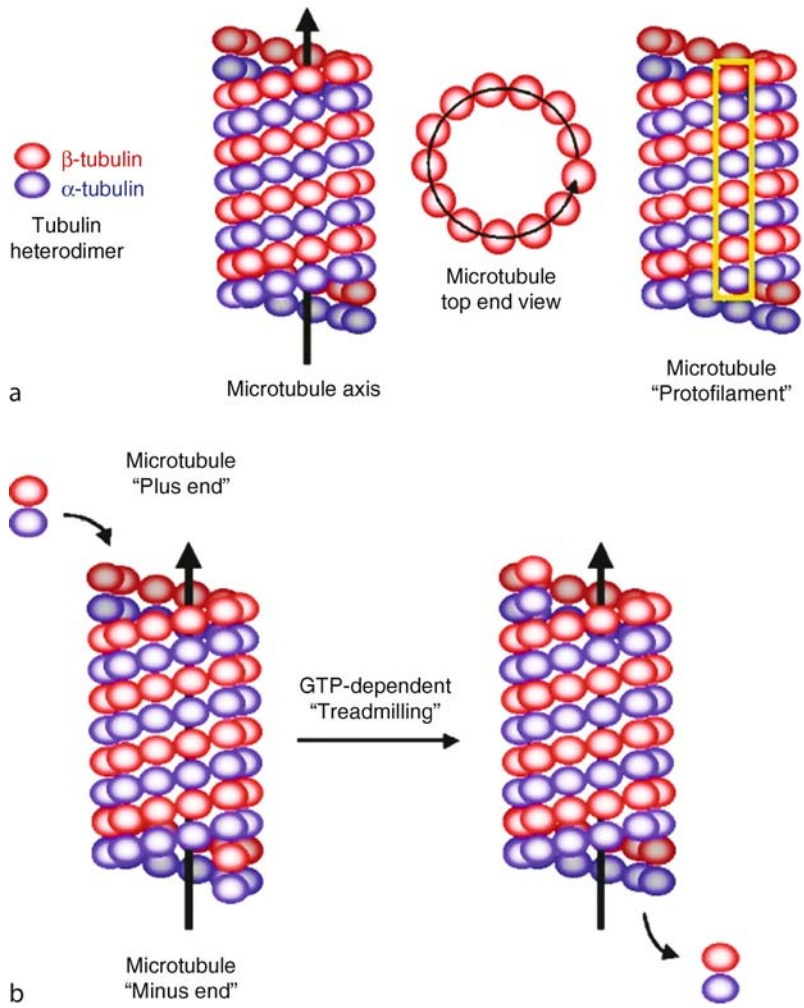
Paclitaxel. Figure 1 Structure of paclitaxel and docetaxel. Molecular structure of paclitaxel (a) and docetaxel (b). Dissimilarities are marked in blue (a) and red (b). Reproduced from Andersen et al (2006) BMC Clin Pharmacol 6(1):2, with permission per BMC Central policy.

is taxotere, which is derived from the English yew tree. Although paclitaxel was identified in the 1970s, its development was limited by ►hypersensitivity reactions. It is poorly soluble in water, and it is therefore commonly administered in a vehicle known as ►Cremophor EL, which may contribute to the hypersensitivity reactions observed when administered. Premedication with drugs such as anti-histamines and glucocorticoids, and slower administration of the drug, allowed the practical use of paclitaxel in patients, but in some people this reaction prevents its use. It is administered by intravenous infusion over times ranging from a few hours to several days.

Paclitaxel is commonly used as first line therapy in many common malignancies, including lung cancer, breast cancer, ovarian cancer, and head and neck cancer. It also has high activity in some specific uncommon malignancies such as angiosarcoma and kaposi sarcoma. Paclitaxel is also used as a component of drug-eluting vascular ►stents, where slow local release of the drug inhibits ►restenosis.

Mechanism of Action

Paclitaxel binds the beta-subunit of ►tubulin and stabilizes tubulin polymerization (Fig. 2a and b). ►Microtubules are composed of polymerized alpha- and beta-tubulin monomers, and play important roles in cell function. Tubulins are guanosine triphosphate (GTP) binding proteins. Beta-tubulin has ►GTPase activity while alpha-tubulin does not. During the formation of microtubules, dimers of alpha- and beta-tubulin bound to GTP attach to the growing (+) end of the microtubule. After incorporation into the microtubule, the GTP is cleaved to GDP. The stability of the tubulin dimer in the microtublules is determined by whether it is bound to GDP or GTP, with GTP favoring assembly while GDP favors disassembly. Thus, microtubules are in a dynamic state. One important role of microtubules is the formation of the ►mitotic spindles, which pull the chromosomes apart during mitosis. Microtubules also contribute to cell shape as a critical component of the ►cytoskeleton. The cytoskeleton is composed of microtubules, actin, and intermediate filaments, and plays an important role in the control of cell shape and locomotion. Microtubules are also involved in the movement of intracellular organelles. Paclitaxel binds polymerized tubulin and inhibits the dissociation rate of the tubulin subunits from the tubule to free monomer, thus stabilizing the microtubule. Paclitaxel administration to cells at micromolar concentrations results in the formation of microtubule bundles and ►asters, and arrests cells in mitosis. However, much lower concentrations (1–20 nM) can be ►cytotoxic or ►cytostatic. In vitro studies suggest that the time a cell is exposed to paclitaxel above a threshold concentration may be an important determinant of biologic effect. While other mechanisms may also be involved, it is generally believed that the stabilization of tubulin polymerization is the major mechanism by which paclitaxel exerts its anti-tumor activity.



Paclitaxel. Figure 2 Schematic structure of a microtubule. The tubulin dimers associate to form a hollow tube known as a microtubule. The tubulin dimers are arranged in a helical manner with 13 dimers of tubulin per turn. When viewed from the side, the tubulin dimers form microtubule "protofilaments." The arrangement of the dimers results in the exposure of an alpha-tubulin surface on one end of the microtubule, and a beta-tubulin surface on the other. The microtubules are dynamic structures, with tubulin dimers being added to the "+" end and dissociating from the "−" end. (Courtesy of Dr. Michael Blaber, Department of Biomedical Sciences, Florida State University, Tallahassee, Florida).

Mechanism of Resistance

More than one mechanism of resistance to paclitaxel occurs. One clearly defined mechanism is mutations in the tubulin gene that result in altered paclitaxel binding. Other less well-defined mechanisms exist, at least one of which may include over-expression of transmembrane pumps, such as ►P-glycoprotein family members, that actively remove drug from the cell.

Pharmacokinetics

Paclitaxel is highly protein bound. Pharmacokinetic studies suggest a very large volume of distribution (~50–180 l/m^{2}of body surface area), possibly reflecting binding to tubulin or another intracellular compartment. While renal elimination does not appear to be an important route of paclitaxel clearance, the liver plays a more important role in drug removal, and drug doses should be modified when liver function is abnormal.

Common toxicities of paclitaxel include, ►myelosuppression, hair loss, myalgias, fatigue and peripheral ►neuropathy. The toxicities of paclitaxel depend on dose and time of administration. Paclitaxel is commonly given as a 1- or 3-h intravenous infusion, although a variety of infusion times ranging up to several weeks have been described. Different infusion times are associated with different maximally tolerated doses. Longer infusions may be associated with less hair loss, neuropathy, myalgia, and hypersensitivity reaction.

Newer formulations of paclitaxel have been developed that do not require cremophor for solubility, and have additional desirable properties. One such formulation is nanoparticle paclitaxel, in which paclitaxel is bound to albumin; this formulation has been FDA approved and is in clinical use. A second formulation is a polymer form in which paclitaxel is linked to poly-L-glutamic acid; this formulation is in late-stage clinical trials. Other formulations and analogs are also in development.

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Paget Disease

Definition

Localized disorder of bone remodeling, characterized by increased bone resorption followed by increased in new bone formation, with alteration of bone architecture. Osteoclasts are generally increased in number, and larger than normal.

►Bisphosphonates

PAH

Definition

►Polycyclic Aromatic Hydrocarbons; A group of compounds consisting of more than two condensed benzene rings, generally formed in the incomplete combustion of organic matter.

►Tobacco Carcinogenesis

Paired Basic Amino Acid Cleaving Enzyme

►Furin

PAK1

Definition

p21 activated kinase 1 is a serine/threonine kinase that is activated when GTP-bound Rac or Cdc42 interacts with it. PAK1 functions to regulate the actin cytoskeleton in ►lamellipodia and is important in cancer cell ►invasion.

►Cortactin

PALB2

Definition

Partner and localizer of ►BRCA2.

Palliative

Definition

A treatment approach, usually employed in the care of terminally ill patients, aimed at relieving symptoms caused by disease rather than actual treatment of the disease itself. Treatment of tumor to only minimize tumor symptoms or to increase quality of life, without trying to cure the disease.

Palliative Care

►Supportive Care

Palliative Therapy

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Definition

The term "Palliative" (from the Latin palliare, to cloak) is often applied to treatment that is given with the intention of reducing the severity of the symptoms of cancer, or is intended to slow the progress of the disease. Palliative treatment is given without the intention of providing a cure. Much of the care that is provided to cancer patients is effectively palliative in nature and represents an enormous expenditure of resources by health agencies in developed countries.

Characteristics

A decision to use palliative therapy in cancer implies that the patient has a malignant disease process that has been recognized as being impossible to cure. The use of "radical" or aggressive therapies with curative intent would be futile for such patients. Palliative therapies for cancer are therefore given without any real expectation of permanently eradicating the disease process causing the symptoms, although very rarely, long-term survival and even cure can be an unintended but welcome consequence. Palliative therapies for cancer work by inducing regression of all or part of the malignant disease process. This is in distinction to the disciplines of "Palliative Care" and ►"Supportive Care," which stress the skilful relief of pain and other physical and psychological symptoms in cancer and other chronic diseases, usually without direct treatment of the chronic illness responsible for the symptoms.

In the course of the illness of a patient with cancer, the character of the treatment may become progressively more palliative in nature when initial treatment fails and the disease is gradually recognized as being incurable. In some diseases, such as ►non-small cell lung carcinoma, the majority of patients present with disease that is already too-advanced for cure and all therapy is palliative in nature at the outset. Palliative therapies are often, but not always, less intense or toxic than treatments given with curative intent. Palliative therapies often require that the patient experiences a temporary worsening of quality of life due to transient toxicity from the treatment, in exchange for the hope or expectation of a later improvement in the symptoms caused by the cancer. Alternatively the patient with incurable malignancy may have few symptoms at the outset but may still be offered palliative treatment in an effort to prevent an impending catastrophe, such as obstruction of a major airway, or to achieve longer survival due to a temporary reduction in the burden of disease.

The range of symptoms that can be relived by palliative therapies is wide. This is a reflection of the enormous differences between different types of malignant disease and the endless possibilities for variation of patterns of disease progression between different patients with the same type of cancer. The best method of palliation varies widely between the different malignant disease types and the site of the disease responsible for the symptoms. The symptoms may reflect local anatomical problems, such as pain from bone destruction, or be systemic in nature, such as fevers or anemia.

The most commonly used palliative therapies for cancer patients are radiotherapy (►Ionizing radiation therapy), ►chemotherapy, hormonal therapy and surgery, but a wide range of additional palliative anticancer therapies can have important roles in specific clinical situations. Examples of these other therapies include radionuclide therapy for bone metastases or thyroid cancer, immunotherapy and ►radioimmunotherapy for B-cell lymphoma, laser coagulation of bleeding bronchial carcinomas, radiofrequency ablation for liver tumors, and psoralen combined with ultraviolet light treatment (PUVA) in the treatment of some skin lymphomas. Surgical interventions can play a major role in the palliation of symptoms, such as placement of an esophageal stent to allow swallowing to resume in esophageal cancer, relief of intestinal obstruction by bowel diversion procedures, relief of spinal cord compression or resection of a solitary or dominant brain metastasis. The most widely used palliative therapy modalities will be discussed in a little more detail below.

Palliative Radiotherapy

Palliative radiotherapy (also often known as Radiation Therapy) is a widely used and highly effective treatment for localized tumor lesions that are causing symptoms or are about to do so. Most palliative radiotherapy is now given using high energy photons from linear accelerators or telecobalt machines. For more superficial tumors, electron beams or orthovoltage of superficial X-ray machines may be used. For some accessible tumors, radioactive sources may be placed directly into the tumor or adjacent to the tumor to provide local symptom-control with a very low radiation dose to normal tissues more that a few centimeters from the source. Palliative radiotherapy differs from potentially curative radical or "definitive" radiotherapy, not only in the intent of the treatment but also in some technical aspects. Because cure is not the intention, lower total doses are often used than in radical radiotherapy, fraction sizes are usually larger and fewer treatments are given so that overall treatment

time is as short as possible to achieve the goal of timely palliation. Simpler and less costly beam arrangements may be used. It is not always necessary to include all of the disease in the treatment field to obtain a benefit and the treatment intensity should ideally be just enough to provide relief of the symptoms for the duration of the patient's remaining lifespan. For very ill patients with a very short anticipated lifespan, a single fraction of radiotherapy may be sufficient to relieve symptoms. However, most treatments are fractionated (that is given in separate small increments) on a daily basis. Most of the widely used fractionation schedules in common cancers involve treatment of consecutive week days for approximately one or two weeks. There is a wide spectrum of radiosensitivity between different cancers and this is considered when planning treatment. Some malignancies, such as follicular lymphoma, an often indolent type of ►malignant lymphoma, can be exquisitely radiosensitive and respond completely to doses as low as 4 Gy, without any toxicity. Others, such as recurrent rectal cancer may be relatively radioresistant and require doses of radiation in excess of 40 Gy to obtain durable relief of pain, often with significant treatment related side effects.

Palliative radiotherapy is used across virtually the complete spectrum of cancers and plays a large role in all of the most common malignancies, including lung, breast, prostate and bowel cancers, brain tumors and ►multiple myeloma. Many of the most common consequences of these diseases are effectively treated with palliative radiotherapy, including pain caused by bone metastasis, breathlessness and hemoptysis caused by bronchial tumors, brain impairment caused by central nervous system metastases or malignant spinal cord compression. Radiotherapy can reach any part of the body and is useful in situations where other treatments cannot gain access; for example brain metastases may be relatively protected from chemotherapy by the blood brain barrier but are often effectively treated with radiation.

The toxicity of radiotherapy varies with the dose and fractionation and the region of the body treated. Because palliative doses are usually lower than radical doses, side-effects are usually relative mild and transient, but commonly include fatigue, some skin redness, inflammation of mucous membranes in the treatment field and localized hair loss.

Palliative Chemotherapy

The term chemotherapy in oncology usually means the delivery of cytotoxic drugs to kill cancer cells. Chemotherapy drugs are often very powerful agents targeted at proliferating cells. Most, but not all, have a relatively narrow therapeutic window, meaning that the difference between an effective dose and a dangerously toxic dose is relatively small. For this reason drugs with overlapping toxicities are often given together in combinations of two or more agents. Many of these drugs suppress bone marrow function and therefore need to be given in "cycles" some weeks apart, allowing bone marrow recovery to occur before the next cycle can be given.

Malignant diseases show a very wide range of sensitivities to chemotherapy. In those diseases with the highest sensitivity to chemotherapy, including malignant lymphomas and ►germ cell tumors, chemotherapy is often given with curative intent at the outset. After relapse or after failure of salvage therapy, the patient may be recognized to have disease that is no longer curable. Chemotherapy may then play an important role in palliation. Sometimes the distinction between curable and incurable disease may not be clearly made and chemotherapy may serve both a palliative role, to relieve symptoms, but also provide a small hope for long-term survival. In advanced follicular lymphoma, which is highly chemo-sensitive but is usually considered incurable, patients may survive for many years following palliative chemotherapy. Relapse or progression after initial palliative chemotherapy is usual and second line chemotherapy may be considered. Subsequent courses of chemotherapy are usually more difficult to tolerate than early courses. In the late stages of an initially chemo-sensitive illness the patient may be faced with the dilemma of choosing further chemotherapy associated with a high risk of toxicity and a low probability of response, or concentrating solely on supportive care.

There is a larger group of moderately chemotherapy-sensitive cancers where chemotherapy can play a significant role in palliation when the patient is known to have incurable disease. These diseases are not generally considered to be curable with chemotherapy alone. They include multiple myeloma and many epithelial cancers such as advanced non-small cell ►lung cancer, extensive stage small cell lung cancer, ►ovarian cancer, ►breast cancer and advanced head and neck cancers. Patients most likely to benefit from palliative chemotherapy are those with a good performance status and without serious concomitant illnesses. In advanced non-small ►cell lung cancer, for example, chemotherapy provides such patients with a significant but relatively short improvement in overall survival, perhaps 6 weeks on average. Some patients may consider the risk of toxicity excessive for a relatively short prolongation of their lives in this situation. However quality of life is a major consideration when deciding whether or not to use palliative chemotherapy and well conducted studies have shown that quality of life is actually better in lung patients who receive chemotherapy than in those who do not. In other cancers such as breast cancer where response rates are usually

higher and patients are often younger and fitter when palliative chemotherapy is needed, greater benefits may be obtained with chemotherapy and initial decision making about whether or not to have chemotherapy is easier.

There is a further group of diseases that are relatively unresponsive to chemotherapy, in that the majority of patients do not have a response and randomized trials have shown that overall survival is not improved compared to patients who do not receive chemotherapy. Nevertheless, even in this group excellent responses to chemotherapy are sometimes seen. This group includes diseases like malignant ►melanoma, renal cell carcinoma and some soft tissue sarcomas.

Like radiotherapy, chemotherapy can be a double-edged sword. In cancers with low chemo-sensitivity it can often produce toxicity without any benefits whatsoever, unlike radiotherapy, which at least causes a response in most tumors that are treated even if they are chemo-resistant. However, unlike radiotherapy chemotherapy is a whole body treatment and often is the only available means for modifying the overall course of a widely disseminated cancer and improving overall survival.

Hormonal Therapy

Breast cancer and prostate cancer are very common malignancies that are often responsive to palliative hormonal therapies when patients are known to have incurable advanced disease. In both diseases, hormonal therapy can also play a role as adjuvant therapy for patients with potentially curable localized disease. Breast cancer cells commonly have receptors on the cell surface for ►estrogen and progesterone that indicate that the cells may behave more aggressively in the presence of estrogen in the bloodstream. In patients with advanced, receptor-positive breast cancer, the disease can often be made to regress by manipulating the patient's hormonal environment. In the past this was commonly accomplished by inducing an artificial menopause by oophorectomy or radiation to the ovaries. In modern practice, oestrogen receptor antagonists such as ►tamoxifen or ►aromatase inhibitors such as letrozole are most commonly used, and are effective in both pre- and postmenopausal women. Compared to chemotherapy, these drugs have low toxicity. Response rates are high in receptor-positive cancers and may last several years, with regression of painful bony lesions or symptomatic soft tissue tumors.

In advanced prostate cancer, often associated with widespread painful bone metastasis, tumor cells are usually dependent on presence of circulating androgens which stimulate tumor growth by binding to ►androgen receptors on the cell surface. By inducing a reduction in the circulating level of androgens, as in bilateral orchidectomy, or by blocking the pituitary secretion of hormones that stimulate androgen production by the testis using LH-RH agonist drugs such as Zoladex or by using drugs that block androgen receptors on the tumor cells such as Bicalutamide, advanced prostate cancer can be made to regress in many cases. Such regressions last on average a little less than two years but in individual cases may last for many years and may be achieved with acceptable side effects.

Conclusion

The palliative treatment of cancer is complex and should ideally be coordinated in a multidisciplinary team setting where a range of palliative therapies is available together with access to appropriate supportive care. Patients may benefit from the concurrent or sequential use of a range of different palliative treatments to achieve the best quality of life for the longest possible time. Treatments must be tailored both to the disease extent and to the wishes and personal philosophy of the patient, always bearing in mind that the dignity and autonomy of the suffering person should be paramount.

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Palmitoylated

Definition

A molecule that has a posttranslational modification that adds a sixteen carbon fatty acid residue.

►RAS

PAMPA

Definition

Is an acronym for Parallel Artificial Membrane Permeability Assay. In PAMPA a measurement is made

of the speed with which a chemical crosses a porous material like fritted glass or plastic soaked with a fat or a mimic of the gastrointestinal tract wall.

►ADMET Screen

Pancreas

Definition

Is involved in the digestive and endocrine system. It is both exocrine (secreting pancreatic juice containing digestive enzymes) and endocrine (producing several important hormones, including insulin, glucagon, and somatostatin).

Pancreas Cancer, Clinical Oncology

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Definition

Pancreatic cancer is an adenocarcinoma of the pancreas (carcinoma of the pancreas). Cancer of the pancreas is usually lethal. The pancreas is a gland that secretes both digestive enzymes and insulin (from special cells called islet cells).

Characteristics

Anatomy

The pancreas is located on the upper abdomen located in the midst of many vital organs, including the liver, spleen, stomach, small bowel and large bowel. Because of its central location, it is very problematic when cancer spreads from the pancreas directly into the adjacent organs. In addition, the head of the pancreas (the portion on the patients' right side) covers the common bile duct. The common bile duct is the duct through which bile runs from the liver and gall bladder, is mixed with pancreatic juices and then emptied into the small bowel. Blockage of this common bile duct can lead to one of the early symptoms of pancreatic cancer.

In addition, the location of the pancreas near the back (in an area called the retroperitoneum) is problematic as that is the area which contains important vessels, such as the superior mesenteric artery and veins and the celiac plexus. This means that a cancer in the pancreas will frequently (and very early in the course of the disease) invade these vessels, which enables the tumor to spread to distant sites by these vessels (such as to the liver or lung). Vessel invasion also makes the pancreas cancer inoperable (see below). There are also many nerves that are located behind the pancreas and those nerves frequently affected by cancer of the pancreas.

Etiology (Cause of the Disease)

The cause of pancreatic cancer is unknown. In about 8% of patients, the disease appears to be inherited (familial pancreatic cancer). The primary risk factor for pancreatic cancer that is not inherited is ►smoking. Other risk factors include ►alcohol consumption, a history of surgical procedures for peptic ulcer disease and a history of ►inflammation of the pancreas (pancreatitis). Diabetes is frequently associated with cancer of the pancreas. However, it is unclear if diabetes is a risk factor for the development of pancreatic cancer or whether it is just a result of the pancreatic cancer damaging the insulin-producing (islet cells) cells of the pancreas.

Pathology

It is very important to determine what type of cancer of the pancreas one is talking about. The types of cancer involving the pancreas are as follows:

- Ductal adenocarcinoma of the pancreas: This is the most common (accounts for more than 90%) and the most lethal form of the disease. It is believed that this type of cancer arises in the cells that line the ducts of the pancreas. Ductal adenocarcinoma rapidly invades blood vessels, nerves and other organs. It is frequently at an advanced stage when it is diagnosed and has a bad prognosis.
- Mucinous cystadenocarcinoma: This pathologic type is an uncommon form of pancreas cancer. It tends to be less invasive and mainly causes problems because of its size. The usual treatment is surgical removal, unless other vital organs, blood vessels or nerves are involved. It is not a benign condition.
- Islet cell tumor: These are cancers that appear to arise from the small clusters of cells called islets, which are scattered throughout the normal pancreas. Islets have cells capable of making many different hormones including insulin and glucagon. Islet cell tumors can cause symptoms as the excessive hormones that they make (such as insulin) can cause severe physiologic problems (such as hypoglycemia or low blood sugar). In general, islet cell tumors have a far better prognosis than other types of cancer of the pancreas.

The sections below will only deal with the most common type of pancreas cancers – ductal adenocarcinoma of the pancreas.

Symptoms

Unfortunately, the symptoms associated with ductal adenocarcinoma of the pancreas are fairly non-specific

and appear late. They include pain in the midepigastric (stomach area) or the back (usually due to nerve invasion by the tumor), nausea and/or vomiting, fatigue, loss of appetite and weight loss. A change in bowel habits with light colored stools is also a sign of the disease, as is a severe darkening of the urine. One rather drastic symptom is the appearance of jaundice (a yellowness of the whites of the eyes and skin), which is caused by the tumor closing off the bile drainage such that the bile (containing the pigment bilirubin) cannot be secreted, builds up in the blood and is deposited in the skin.

Making the Diagnosis

The most effective way to determine whether or not there is a mass in the pancreas is via a special X-ray known as a spiral CT scan. Another, perhaps more sensitive method, is called an endoscopic ultrasound (or EUS) in which a tube (endoscope) is passed through the mouth into the stomach and an ultrasound device at the end of the tube sends out signals that are used to detect a mass in the pancreas. If a mass is detected, it is critical to obtain a histologic diagnosis to determine if the mass is cancer (or just a benign inflammation). A histologic diagnosis is obtained by inserting a needle into the mass to look for tumor cells, or the histologic diagnosis is obtained by performing an open surgical procedure and biopsy of the pancreas.

Staging of the Disease

Pancreatic cancer is staged as being localized, locally advanced or metastatic (with distant spread).

- Localized: This stage means the tumor is confined within the pancreas with no major blood vessel involvement or involvement of areas outside of the pancreas.
- Locally advanced: Tumor involves major blood vessels or regional lymph nodes, but no cancer in other organs. In general, the tumor can be encompassed by a radiotherapy port. There can be no spread to distant organs.
- Metastatic: The pancreatic cancer has spread beyond the pancreas (usually into the liver, other surrounding organs or lung).

Prognosis

Pancreatic cancer has the worst survival of any cancer. The overall 1-year survival for all patients is about 18% with fewer than 2% of patients living 5 years. For patients with localized pancreatic cancer who have surgical resections of their disease and no evidence of tumor spread beyond the pancreas in their pathology resections there may be as many as 20% who survive 2 years. However, it is rare to find that the pancreatic cancer is truly localized. For patients with locally advanced pancreatic cancer, the average survival is about 10 months if the patient is treated with radiation to the area in addition to chemotherapy. For patients with advanced metastatic pancreatic cancer, the average survival (with treatment) is about 6 months.

Thus, the diagnosis of pancreatic cancer comes with a terrible prognosis. In order to make progress against the disease, it is important that new therapies be developed.

Treatment

- For patients with localized disease, the treatment is surgical resection. This gives the patient their only chance for prolonged survival (if the patient is fortunate to have truly localized disease). The surgical resection of the pancreas with surrounding organs and bowel reconstruction is frequently referred to as the Whipple procedure. Even with localized disease with no invasion noted on scans at the time of surgery, the pancreatic cancer frequently is found to have spread beyond the pancreas. Therefore, an important area of research is to use a ►neoadjuvant therapy approach. The idea with neoadjuvant therapy is to give therapy, such as radiation therapy, chemotherapy, or both, before surgery to try to 'down stage' the disease. Hopefully, this approach will help make more patients truly operable with complete removal of their tumor.
- For patients with locally advanced disease, there is controversy in terms of what constitutes the best treatment. The standard treatment is considered to be radiation plus chemotherapy (usually the drug, 5-fluorouracil) to sensitize the tumor to the radiation. This treatment has been reported to increase the average survival for a patient with locally advanced disease from 5 months up to 10 months. However, the radiation plus chemotherapy regimen is associated with substantial side effects. Therefore, a better approach (perhaps chemotherapy alone) for patients with locally advanced pancreatic cancer is being investigated.
- For patients with metastatic pancreatic cancer, treatment is usually chemotherapy or supportive care (pain control and treatment of other medical problems) only. Until recently there has been no chemotherapy that has improved patient survival. The anticancer agent, ►gemcitabine, has been shown to improve the survival of patients with advanced pancreatic cancer, plus improve the quality of life of the patient (decreased pain, improvement of performance status). When patients with advanced pancreatic cancer were treated with gemcitabine, their survival was improved from 2% (in the control arm treated with 5-fluorouracil) up to 18% for patients receiving gemcitabine. Currently, investigators are building on this modest advance against advanced disease by combining gemcitabine with other new cancer therapy approaches.

Familial Pancreatic Cancer

Only about 3–8% of pancreatic cancers is thought to be familial. It is an area of intense study to determine if you have a parent or brother and/or sister with the disease, what type of monitoring and/or treatment is necessary. It is clear that some type of monitoring, such as endoscopic ultrasound, should be performed at special centers.

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Pancreatic Cancer, Basic and Clinical Parameters

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Definition

Epithelial malignancies of the pancreatic gland that originate in more than 85% from ductal cells. In rare cases tumors may also arise from acinar or endocrine cells.

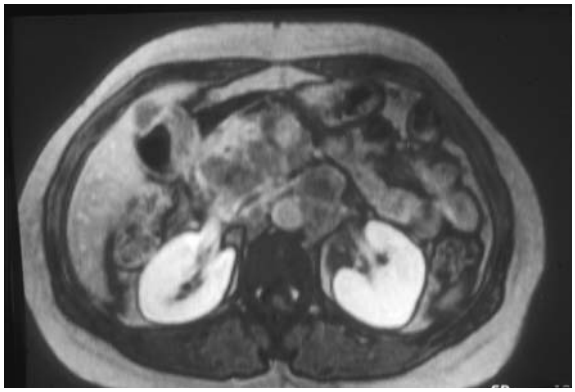
Characteristics

Pancreatic adenocarcinoma is one of the most aggressive human malignancies reflected by a mortality rate which closely follows that of the incidence. Its incidence is steadily increasing and today it is the fifth leading cause of cancer related deaths in the western hemisphere. Most patients are diagnosed with pancreatic cancer in the late course of the disease with unspecific symptoms such as fatigue, weight loss, jaundice and upper abdominal pain. At this stage pancreatic cancer frequently has invaded surrounding organs such as the duodenum and stomach, and the retroperitoneal tissue and blood vessels (Fig. 1). Lymph node metastases occur in most cases and reflect a high metastatic potential.

Most patients present between the fifth and eighth decade of life with a male/female ratio of ~1.5:1. The etiology of pancreatic cancer remains unclear. However, environmental factors such as cigarette smoking and a high fat diet may predispose patients to the development of pancreatic malignancies. Chronic inflammatory diseases such as chronic pancreatitis might also increase the risk for pancreatic adenocarcinoma. Genetic factors, such as p16 germline mutations and mutations in the mismatch-repair system may also increase the probability to develop pancreatic adenocarcinoma. However, less then 10% of patients belong to a hereditary pancreatic cancer syndrome.

Pathology

The most frequent site of pancreatic cancer is the head of the pancreatic gland (~60%). The remainder of cases arise in the body (15%), the tail (5%) or disseminated throughout the pancreas (20%). The vast majority of pancreatic cancers are of ductal origin. Rarely, they originate from acinar or endocrine cells. Ductal adenocarcinoma of the pancreas are desmoplastic malignancies composed of mucin-producing glandular cells infiltrating a non-neoplastic stroma which accounts for more than 50% of the tumor tissue. In addition to the fibrocytic stroma, cancer cells are also admixed with inflammatory cells, including lymphocytes. The histological progression from benign to malignant pancreatic disease starts from flat mucinous lesions, to papillary lesions without atypia to lesions with atypia, to in-situ carcinoma and finally to infiltrating adenocarcinoma.



Pancreatic Cancer, Basic and Clinical Parameters. Figure 1 Pancreas cancer, basic and clinical parameters. Abdominal magnetic resonance imaging (MRI) picture. The MRI depicts a huge mass in the upper abdomen that originates from the corpus of the pancreas.

At the time of diagnosis, pancreatic cancer generally has invaded the peripancreatic fat tissue and lymph nodes or adjacent organs such as the duodenum, stomach, peritoneum and vessels. Cancers restricted to the pancreatic gland are rare. In particular, carcinomas located in the body and tail are diagnosed at a more advanced stage due to unspecific symptoms. Sites of hematogenous metastases formation are primarily the liver, rarely the lung. However, local tumor recurrence due to remaining microscopic tumor foci even in smaller tumors seems to be the determining factor for patients survival.

Staging

The UICC staging system is based on the size of the primary tumor (T), the extent of regional lymph node involvement (N) and the presence of metastases (M).

Primary tumor (T):

- TX Primary tumor cannot be assessed
- T0 No evidence of primary tumor
- Tis Carcinoma in situ
- T1 Tumor <2 cm
- T2 Tumor >2 cm
- T3 Tumor extends directly to the duodenum, bile duct or peripancreatic tissues
- T4 Tumor extends directly to the stomach, spleen, colon, or adjacent vessels.

Regional lymph nodes (N):

- NX Regional lymph nodes cannot be assessed
- N0 No regional lymph node metastasis
- N1 Regional lymph node metastasis

Distant metastasis (M):

- MX Presence of distant metastasis cannot be assessed
- M0 No distant metastasis
- M1 Distant metastasis

Genetics

An array of technologies has been applied to investigate relevant chromosomal and genetic changes in pancreatic cancer over the last couple of years. The detection of specific chromosomal changes and altered tumor suppressor genes and oncogenes has significantly improved our understanding of the development and progression of this dismal disease (Fig. 2).

Cytogenetics

The application of modern fluorescence-in-situ technologies (FISH) such as comparative genomic hybridization (CGH) or spectral karyotyping (SKY) for the study of numerical and structural chromosomal aberrations have revealed new insights into pancreatic tumorigenesis. Pancreatic carcinoma cells have a surprisingly high degree of chromosomal instability but also have recurrent pattern of chromosomal alterations. Genetic losses usually involve chromosome arms and chromosomes 8p, 9p, 17p, 18q, 19p and 21, whereas gains can be mapped to 3q, 5p, 7p, 8q, 12p and 20q. These chromosomal regions are affected in up to 90% of pancreatic adenocarcinomas and correlate very well with those regions harboring oncogenes and tumor suppressor genes such as *DPC4* at 18q, *p16* at 9p, *p53* at 17p and *Kras* at 12p.

Oncogenes

The K-ras oncogenes codes for a GTP-binding protein and plays a major role in pancreatic cancer. Activating point mutations of the K-ras oncogene occur in 70–90% of all ductal adenocarcinomas and are largely restricted to codon 12 and 13 of the K-ras gene at 12p12. Due to these mutations, the K-ras oncogene cannot be inactivated and the signal transduction pathway remains active stimulating proliferation and cellular transformation. K-ras mutations have been found in proliferative, non-invasive ductal lesions indicating that K-ras might play a role during early carcinogenesis. However, K-ras mutations have also been found in inflammatory and normal pancreatic tissue without neoplastic potential. This finding might limit the development of a gene based test system using K-ras mutations as an indicator for neoplastic or malignant cells in pancreatic juice, blood or stool of patients with pancreatic disease.

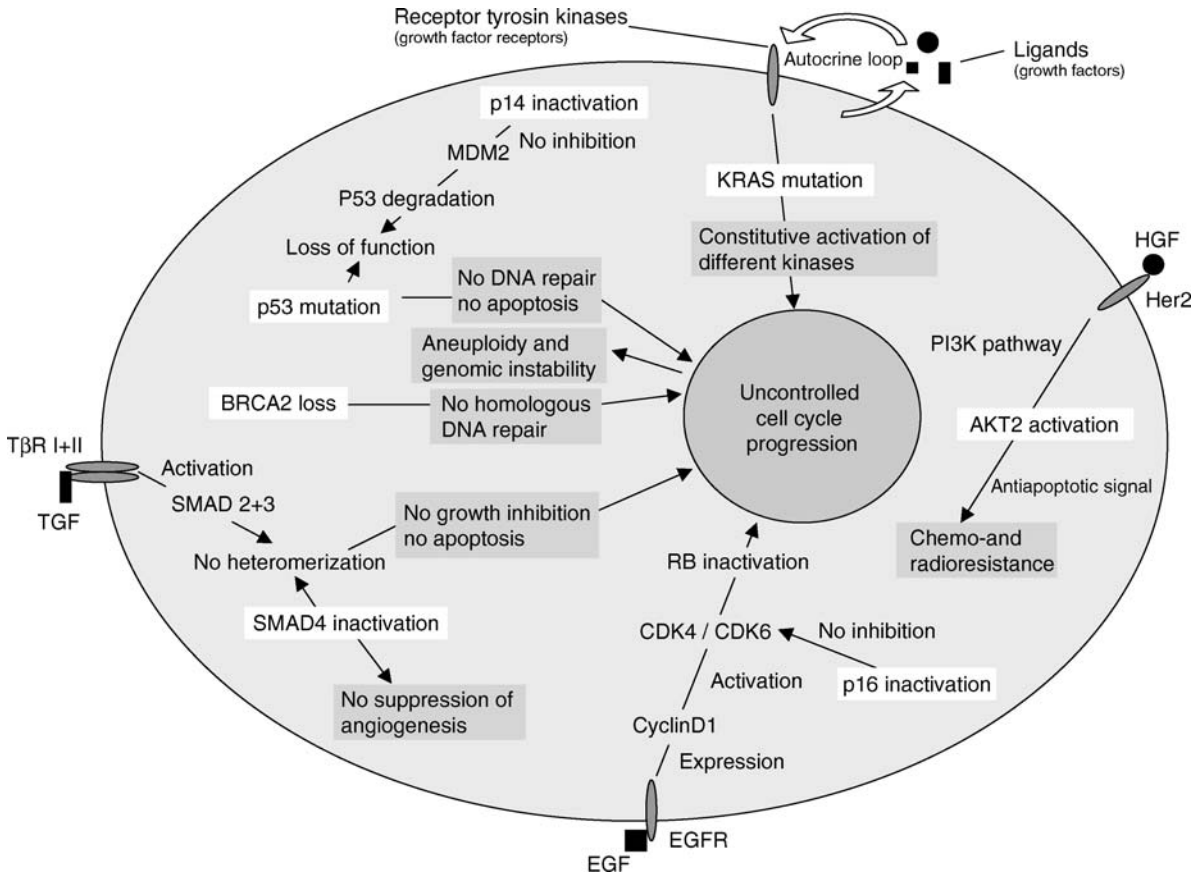
EGFR (epidermal growth factor receptor) and Her-2/neu (heregulin-, neuregulin- or glial growth factor-receptor) belong to the *ERBB*-family. In normal pancreatic tissue and chronic pancreatitis HER-2 expression remains unaffected, whereas in patients with pancreatic cancer over-expression of HER-2 can be found in early morphologic duct lesions. Using immunochemistry, over-expression of HER-2 ranges from 21% to 80%, and only 27% of the immunohistochemical positive cases showed amplification of *HER-2* as detected by FISH.

The *EGFR* gene is located on chromosome 7p12. Expression of EGFR can be detected in about 50% of pancreatic carcinomas. EGFR expression seems to play an important role in the metastatic potential.

Tumor Suppressor Genes

The relevant tumor suppressor genes in pancreatic cancer are p53, p16 and DPC4.

p53 is a nuclear binding protein that arrests cells at the G1/S checkpoint and also plays an important role in the induction of apoptosis after DNA damage. P53 resides on chromosome 17p13. It is inactivated by allelic loss and inactivating mutations in about 50% of pancreatic cancers. Therefore, loss of p53 function results in a disturbed cell cycle and loss of programmed cell death.



Pancreatic Cancer, Basic and Clinical Parameters. Figure 2 Pancreas, basic and clinical parameters. Schematic diagram of major pathways in the development of pancreatic adenocarcinomas which finally lead to the loss of cell cycle control.

p16 has been identified at 9p21 and is inactivated in about 90% of pancreatic cancers via allelic loss, inactivating mutations and/or hypermethylation of the promoter have been found. p16 inhibits the promotion of the cell cycle by binding to the cyclin-CDK4 complex and preventing CDK4 activation of RB protein. Therefore, inactivation of p16 in pancreatic cancer disregulates another relevant cell cycle checkpoint.

The tumor suppressor gene DPC4 is biallelically inactivated in about 50% of pancreatic cancers. Located on chromosome 18q, DPC4 codes for a peptide which is closely related to the MAD family of proteins (SMAD). These molecules play an integral part in the signal transduction from TGF-β superfamily cell surface receptors. Since it is known that TGF-β inhibits cell growth and proliferation, inactivation of DPC4 and loss of its inhibitory function may bestow a growth advantage upon cancer cells.

Epigenetic Changes

Another cause of gene expression changes in the development of pancreatic cancer is DNA-methylation. The methylation of the promoter regions of different genes (e.g., *p16, RB, VHL, hMLH1, hMSH2*) was also found in pancreatic carcinomas. Beside *p16,* the gene Preproenkephalin (*ppENK*), which has growth inhibiting function, was found methylated in 90% of the pancreatic cancers. Methylation of genes with a significant role in carcinogenesis occur early in cancer development. The methylation of *p16* can be observed in up to 50% of such cancers. The number of methylated loci increases with the size of the tumor and the age of the patients. Non-neoplastic epithelium is not methylated.

Hereditary Pancreatic Cancer

Several family studies have suggested that between 5 and 10% of pancreatic cancer may have a hereditary basis. A predisposition to the development of pancreatic cancer has been shown for several genetic syndromes including hereditary pancreatitis, hereditary non-polyposis colorectal cancer (HNPCC) and the familial atypical mole-multiple melanoma (FAMMM) syndrome.

Hereditary pancreatitis is an autosomal-dominant disorder which is characterized by an early onset of age.

P

Patients suffer from recurrent acute pancreatitis which leads subsequently to chronic pancreatitis carrying its significant risk factor for the development of pancreatic cancer. In the disorder, a mutation in the trypsinogen gene at 7q35 results in the inability to deactivate trypsin which results in autodigestion of pancreatic tissue.

Hereditary non-polyposis colorectal cancer (HNPCC) syndrome is another syndrome that predisposes individuals to pancreatic cancer. It is an autosomal transmitted disease, caused by germline mutations in the mismatch-repair system. Besides pancreatic cancer, patients also inherit a predisposition to other cancers, including colonic, breast, ovarian and endometrial carcinomas.

Patients with the FAMMM syndrome have an elevated risk for the development of multiple atypical nevi, malignant melanomas and pancreatic cancer. In a subset of patients, a germline mutation in the p16 tumor suppressor gene is implicated.

Diagnosis of Pancreatic Cancer

There are unfortunately no early signs or symptoms to identify pancreatic cancer as gastrointestinal obstruction and other compromising sequellae occur. Patients that are worked up for abdominal pain or jaundice suspicious for pancreatic cancer typically undergo the following diagnostic procedures.

Abdominal Ultrasound

Transabdominal pancreatic ultrasonography (US) is performed using high-resolution real-time linear array or sector scanners combined with Doppler examination. The sensitivity of US in pancreatic cancer is as high as ~85%. Lesions in the head are more visible than lesions in the body or tail of the gland, due to intestinal gas. Computer tomography (CT) is clearly superior in terms of sensitivity and specificity compared to ultrasonography. In summary, US is a reliable screening method for the detection of pancreatic masses and liver metastases but is not recommended for examining patients if a malignant disease is strongly suspected.

Computed Tomography (CT)

Current techniques including high resolution spiral CT provide detailed images of the pancreas, the pancreatic and biliary duct system, the peripancreatic vessels and surrounding organs. Several studies have reported a sensitivity of 92%, a specificity of ~100% with spiral CT. However, only 65% of all tumors which were staged as resectable with spiral CT scans were actually candidates for resection. Therefore, laparotomy or surgical laparoscopy remain the only specific approaches to determine resectability.

Magnetic Resonance Imaging (MRI)

The importance of MRI in pancreatic cancer diagnostics, despite the advantage of avoiding ionizing radiation, is still to be determined. In most centers MRI is used to differentiate between malignant and benign disease when US or CT are equivocal or the administration of intravenous contrast agents is contraindicated.

Magnetic Resonance Cholangiopancreatography (MRCP)

Although the exact role of MRCP has not yet been determined it has demonstrated its potential to display changes in the pancreatic duct pathology. MRCP and ERCP findings correlate with pathology in 80–90% of the cases.

Endoscopic Retrograde Cholangiopancreatography (ERCP)

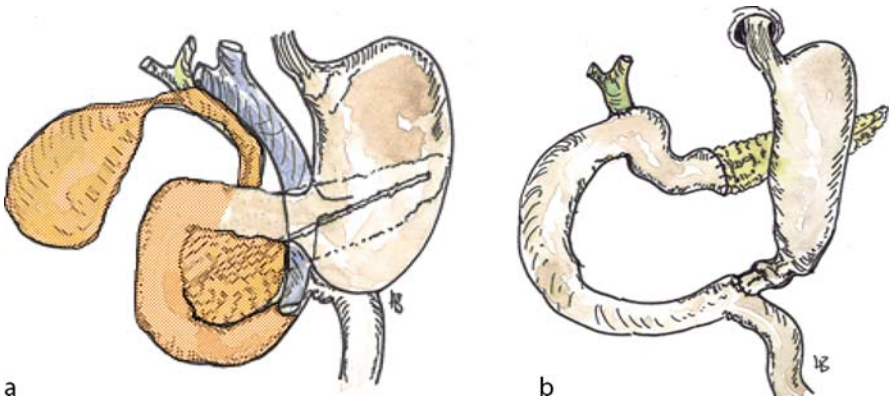
Endoscopic techniques, such as ERCP, can be applied for diagnosis and interventional management for patients with pancreatic disorders with a sensitivity and specificity of 90%. Endoscopic pancreatographic features of malignant disease include stenosis or displacement of the pancreatic and bile duct (e.g. double duct sign), alteration of secondary branches and extravasation of contrast dyes due to necrosis. An additional major role of ERCP is the opportunity to provide drainage of an obstructed common bile duct by facilitating the insertion of stents into the biliary system. Also tissue sampling can be performed during ERCP, using brush cytology, direct biopsy, endoscopic needle aspiration and aspiration of pancreatic and bile fluid. The opportunity to obtain tumor material during endoscopic procedures might become an important step since molecular markers can be applied to these samples and might assist in the differential diagnosis of pancreatic disease.

Therapy of Pancreatic Cancer

Although there has been considerable progress in the biological understanding and diagnostic tools of pancreatic cancer, the neoplasms continues to have one of the poorest prognoses of all human cancers. Surgical resection of small tumors is the only option for curative treatment. The 5-year survival is below 5% and due to advanced tumor stage at time of diagnosis, therapies remain palliative for the vast majority of patients. Five-year survival rates increase to 30% when tumors smaller than 2 cm are resected.

Surgical technique is dependent on location of the tumor and includes local excision in papillary tumors and left hemipancreatectomy in tumors of the pancreatic tail.

However, the majority of pancreatic cancers are located in the pancreatic head on which two methods of resection are most commonly employed. The classical method, the Whipple-Kausch pancreatectomy, involves en-bloc removal of (i) the distal third of the stomach and the right half of the greater omentum, (ii) the gall bladder including the distal bile duct system, (iii) the



Pancreatic Cancer, Basic and Clinical Parameters. Figure 3 Pancreas, basic and clinical parameters. Pylorus-preserving pancreatectomy (PPPD) (a) Intraabdominal Situs before surgical resection – areas in orange color mark the organs which will be removed. (b) Intraabdominal Situs after resection according to PPPD.

duodenum and the proximal 10 cm of the jejunum, (iv) the head and parts of the body of the pancreas and (v) the peripancreatic and hepatoduodenal lymph nodes. The surgical therapy of choice and modern technique is the so called the pylorus-preserving pancreatectomy (PPPD). Instead of resection of the distal stomach, the pylorus is attached directly to a jejunal loop in PPPD (see Fig. 3). It remains unclear if either method is superior but advocates of PPPD claim better nutritional outcomes and shorter operative times compared to standard Whipple-Kausch pancreatectomy. Therefore, the PPPD is considered as state-of-the-art in the major surgical centers in Europe.

In patients with advanced disease, multidisciplinary approaches are needed involving the surgeon, endoscopist and radiologist to optimize palliative therapy in the setting of a limited life expectancy. Operative bypasses including choledochojejunostomies and gastroenterostomies have many advantages and remain as highly acceptable choices in the management of terminal disease. Non-operative stenting should be reserved for elderly patients or patients with very advanced disease who are poor operative candidates.

The resistance of pancreatic cancer to adjuvant, neoadjuvant and palliative chemo- and/or radiotherapy has remained a consistent disappointment over the last decades. Trials with gemcitabine, a cytidin analogue, have shown a decrease of disease-related symptoms thus benefiting patient's quality of life and have resulted in very modest prolongation in survival. Recently, oral 5-Fu (Capecitabine) has been added to gemcitabine and some oncologist recommend additionally EGFR-blockers. Overall, there are no convincing data about any drugs significantly increasing patient's survival. Such results emphasize the importance of developing new diagnostic methods of pancreatic cancer to allow surgical resection at an earlier tumor stage.

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Pancreatic Cancer: Molecular Targets for Therapy

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Definition

Pancreatic cancer is a virulent disease with no effective therapy besides surgical resection. The possibility for

surgical tumor removal is limited by early metastatic spread of tumor to sites outside the pancreas. The survival for patients with metastatic pancreatic cancer is less than 1 year following diagnosis. Clinicians must translate the available knowledge of the molecular basis of this disease into rationale and effective therapeutic strategies for treatment.

Pancreatic cancer is one of the tumors with the highest number of genetic mutations of any solid malignancy. These include oncogenes, tumor-suppressor genes and DNA-stability genes. The stability genes include mismatch repair (MMR) and base-excision repair (BER) genes, which control the mutation rate of other genes. A number of genetic alterations have been, and still is being, tested as molecular target for antipancreatic cancer therapy. It is clear that novel molecular targets and strategies need to be developed for the treatment of pancreatic cancer.

Characteristics

Pancreatic cancer ranks thirteenth in incidence but eighth as a cause of cancer death worldwide. In the United States and Europe, pancreatic cancer is the fourth leading cause of cancer death in both men and women. Chemotherapy and radiation therapy have had little impact on survival, prompting the National Cancer Institute to declare that survival for pancreatic cancer has remained unchanged for three decades and its treatment has consistently been identified as an area of unmet medical need.

Oncogenes

K-Ras Oncogene

The *K-Ras* proto-oncogene is the most commonly mutated gene in pancreatic cancer. K-ras is a member of the RAS family GTP-binding proteins and has intrinsic GTPase activity. The Ras proteins mediate a wide variety of cellular functions including proliferation, differentiation, and survival by transduction of growth-promoting signals from the cell surface tyrosine kinase receptors to intracellular pathways. Activating K-Ras point mutations at codon 12 results in substitution of amino acids and are early genetic alterations, occurring in 30% of early pancreatic neoplasms and with a frequency approaching 100% in advanced pancreatic adenocarcinoma.

Ras has been considered an important therapeutic target in pancreatic cancer due to its frequency of mutation and possible role in the initiation of this malignancy. Unfortunately, Ras inhibitors to date have not been clinically effective. Most studies have focused on the efficacy of farnysltransferase inhibitors (FTIs) which inihibit posttranslational modification of Ras by interfering with lipid modification of the C terminus of Ras. Despite showing promise in vitro and in xenograft models, FTIs have failed to significantly improve survival of patients with pancreatic cancer, possibly due to compensatory geranyltransferase activity preserving Ras function. A phase II trial of FTI alone on 53 patients with locally advanced or metastatic pancreatic cancer showed a median survival of 2.6 months and a 6-month survival rate of 19%. Another phase II trial on 20 patients with metastatic disease showed a median survival of 19.7 weeks and a 6-month survival rate of 25%. A phase III trial combining the FTI tipifarnib with gemcitabine chemotherapy did not lead to any improvement in median survival compared with gemcitabine chemotherapy alone. Therefore, at the present time, the role of FTIs in the treatment of pancreatic cancer remains uncertain.

Activated K-Ras signals to multiple critical pathways including the RAF-mitogen-activated kinase (MAPK), phosphoinositide-3-kinase, and Ral GDS pathways. These downstream targets are logical alternative points of study for therapeutic intervention.

Signaling Pathways of Ras

Raf-MAPK

The Raf serine/threonine kinases lead to MAP kinase activation resulting in proliferative behavior in a variety of cell types. Activating B-Raf mutations have been demonstrated in a variety of malignancies including melanoma, colorectal, thyroid, and ovarian cancers. However, B-Raf and Ras mutations appear to be mutually exclusive in these cancers. Pancreatic cancers rarely have B-Raf mutations, except for a low incidence in the histologic subtype of pancreatic medullary carcinoma. Nevertheless, inhibition of MAP kinase leads to pancreatic cancer cell cycle arrest and decreased proliferation. This arrest may be mediated through increased expression of p27KIP1.

Phosphoinositide 3-Kinase (PI3K) Pathway

The PI3K pathway regulates cell size, survival, and proliferation via several downstream effectors including AKT. Activating mutations in the catalytic subunit of PI3K and loss-of-function mutants of the *PTEN* tumor suppressor have been frequently seen in some cancers but not in human pancreatic adenocarcinoma cells. However, the PI3K pathway appears to have general importance in pancreatic adenocarcinoma. Decreased PTEN expression in pancreatic adenocarcinoma, possibly due to promoter hypermethylation, has been reported. In addition, activation of the PI3K pathways appears necessary to maintain Ras-transformed tumors growth in nude mice after Ras expression is abolished. Finally, inhibition of PI3K appears to increase the sensitivity of pancreatic adenocarcinoma cells to chemotherapy. These observations have made the PI3K pathway an interesting potential target for drug development, but no successful clinical trials have been reported to date.

Activation of the serine/threonine kinase AKT is common in pancreatic cancer; inhibition of which sensitizes cells to the apoptotic effect of chemotherapy. Inhibition of either phosphatidylinositol-3 kinase or AKT led to a decreased protein level of the anti-apoptotic gene *BCL-2* and an increased protein level of the proapoptotic gene *BAX*. Furthermore, inhibition of AKT decreased the function of nuclear factor κB (NF-κB), which is capable of transcriptional regulation of the *BCL-2* gene. Inhibiting this pathway had little effect on the basal level of apoptosis in pancreatic cancer cells, but increased the apoptotic effect of chemotherapy. The antiapoptotic effect of AKT activation in pancreatic cancer cells may involve transcriptional induction of a profile of BCL-2 proteins that confer resistance to apoptosis; alteration of this balance allows sensitization to the apoptotic effect of chemotherapy.

Nuclear Factor κB

The NF-κB transcription factor is an important mediator of mutated K-Ras signaling in pancreatic adenocarcinoma cells. This pathway is activated by a variety of cellular stresses through stimulation by cytokines and growth factors and subsequently aids in the control of apoptosis and immune responses. Most primary pancreatic cancers and cell lines demonstrate constitutive NF-κB activity. NF-κB induction has been shown to be necessary for Ras transformation of several cell types. NF-κB helps to regulate cell survival genes, VEGF, urokinase, and other proinvasive and angiogenic factors. The NF-κB pathway may also contribute to chemoresistance which is widespread amongst pancreatic adenocarcinomas possibly due to the ability of NF-κB to upregulate BCL-2 and BCL-XL. The diverse roles of NF-κB have led to the development of inhibitors that have been studied in patients with advanced pancreatic cancer.

The phosphorylated form of I-κB is degraded by the 26S proteasome and inhibition of the proteasome prevents I-κB degradation, thereby preventing the activation of NF-κB. Bortezomib (PS-341, Velcade) is a 26S proteasome inhibitor that causes cell cycle arrest of pancreatic cancer cells via accumulation of cyclin-dependent kinase inhibitor $p21^{cip1/waf1}$ and apoptosis via downregulation of BCL-2. In vivo experiments have shown that PS-341 increases the cytotoxicity of gemcitabine. However, despite encouraging preclinical data, neither PS-341 alone nor combined with gemcitabine is considered of any benefit to patients with metastatic pancreatic cancer. This is due to findings from a randomized phase II trial involving 87 patients with metastatic pancreatic adenocarcinoma, where the use of PS-341 alone or in combination with gemcitabine, resulted in a median survival of only 2.5 and 4.8 months, respectively.

Ras Family GTPases

The Ras family of GTPases has more than 150 members and is divided into five groups: Ras, Rab, Arf, Rho, and Ran. Some of the best characterized proteins include the Rho family. Activating mutations of Rho family members have not been demonstrated in human pancreatic tumors; however, overexpression of Rho has been shown to correlate with pancreatic cancer metastasis.

Ral GTPases are downstream of Ras and are members of the Ras subfamily. Ral A has been shown to be activated in several pancreatic cancer cell lines and RAL A inhibition suppressed tumorigenicity of Ras-transformed human cells. Much evidence supports K-Ras and many of its downstream effectors in both the initiation and maintenance of pancreatic cancer. While agents that target FTIs have not shown to be of benefit in pancreatic cancer, ongoing studies are investigating the possibility of targeting Ras-directed downstream signaling mediators.

Tumor Suppressor Genes

CDKN2A/INK4A/p16

Chromosomal alterations in the 9p21 region that contains the gene for *CDKN2A/INK4A/p16* are frequently observed in pancreatic cancer. These alterations typically result in cellular inactivation through deletion or mutation, although gene silencing by hypermethylation does also occur. The *CDKNA2A* gene encodes a cyclin-dependent kinase inhibitor that induces cell cycle arrest at G1 in cooperation with normal retinoblastoma function. In animal models, loss of CDKNA is an early event in pancreatic cancer that functions to enhance the oncogenic effect of K-Ras.

SMAD4 and TGF-β

The *SMAD4* gene plays an important role in signaling pathways involving transforming growth factor beta (TGF-β), bone morphogenetic protein, and activin. Inactivation of SMAD4 is noted in approximately 50% of pancreatic cancers. SMAD4 has been termed a progression allele for pancreatic adenocarcinoma as it is lost in later stages of pancreatic intraductal neoplasms. Recent reports suggest that wild-type SMAD4 restricts activated K-Ras initiated neoplasms that would otherwise progress to frank invasive disease in the context of SMAD4 deficiency.

TGF-β is the prototypic member of a superfamily of proteins that upon ligand binding phosphorylate receptor-regulated SMAD proteins regulating a variety of cellular functions including proliferation, migration, differentiation, and apoptosis. In numerous cell lines, TGF-β exerts a growth inhibitory effect that involves cell cycle regulators, repression of c-Myc, and induction of apoptosis. Elevation in TGF-β signaling inhibits epithelial cancer initiation in vivo and defects in

this pathway promote pancreatic tumorigenesis. Recent evidence suggests that SMAD4 deficiency inhibits TGF-β-induced cell cycle arrest and cell migration, thereby, altering TGF-β signaling from one of tumor suppression to tumor promotion.

p53

Loss of activity of the transcription factor p53, which plays a critical role in cell cycle arrest and apoptosis, has been reported in pancreatic tumors. Missense mutations in part of the *p53* gene encoding the DNA-binding domain of this tumor suppressor protein result in the production of a protein that is able to translocate to the nucleus but is unable to function in DNA binding and therefore accumulates in the nucleus. This accumulation has been described in high grade tumor and late intraepithelial lesions, suggesting that these mutations contribute to the later stages of pancreatic tumorigenesis. As with SMAD4 deletions, loss of p53 activity induces further chromosomal instability in the context of mutated, activated K-ras.

Growth Factors

Epidermal Growth Factor Receptor (EGFR)

The EGFR is a family of transmembrane glycoproteins that has four members. Upon phosphorylation, the activated receptor subsequently signals to a variety of intracellular adaptor molecules and mediators. These allow EGFR to play a role in a range of cellular functions including proliferation, survival, and motility. Two major downstream pathways in normal EGFR signaling are the Ras–Raf–MAPK and the PI3K/Akt pathways. Constitutive activation of EGFR promotes oncogenic cellular processes including uncontrolled proliferation with migration, invasion, angiogenesis, and resistance to apoptosis.

Overexpression of EGFR has been observed in many pancreatic tumors and appears to correlate with poor prognosis and disease progression. Preclinical studies have shown that antagonizing EGFR signaling inhibits growth and metastasis of pancreatic tumors in xenograft animal studies. Two therapeutic approaches have been utilized to interfere with EGFR signaling. The first involves blocking ligand binding with monoclonal antibodies specific to epitopes within the ligand-binding domain. Once bound to the domain, these antibodies prevent receptor dimerization and subsequent tyrosine phosphorylation. The second approach to inhibit EGFR signaling uses small-molecule tyrosine kinase inhibitors (TKIs) that function inside the cell. These TKIs inhibit the kinase activity of the receptor independent of ligand binding through competition with ATP binding, thereby preventing receptor activation. The advantage of kinase inhibitors over receptor antibodies is that cross reactivity with the ATP-binding domain of other EGFR family members as well as the ability to inhibit truncated receptor mutants that lack a functional extracellular domain may allow for more thorough receptor inhibition with TKIs. The disadvantage of TKIs is that other kinases can be inhibited leading to increased toxicity or death of normal cells.

A small molecule EGFR TKI, erlotinib, has been evaluated in randomized studies of patients with advanced pancreatic cancer. Patients were randomized to gemcitabine chemotherapy with placebo or gemcitabine with erlotinib. The latter treatment was well tolerated and resulted in a small but significant improvement in survival. This study validated EGFR as a target in pancreatic cancer and provides further rationale for ongoing studies of targeted molecular agents in this disease.

Vascular Endothelial Growth Factor Receptor (VEGFR)

Angiogenesis refers to the process by which new blood vessels develop from existing vasculature. Tumor blood vessels are highly disorganized and structurally abnormal resulting in irregular blood flow, increased permeability, poor nutrient and drug delivery, and areas of hypoxia. VEGFR is one of the central mediators of angiogenesis and is overexpressed in pancreatic cancer. Recent preclinical data suggest that this growth factor has mitogenic activity on endothelial as well as tumor cells, making it a target for dual cancer therapy intended to produce both antiangiogenic and antitumor effects.

Angiogenesis has prognostic importance in pancreatic cancer, a disease in which VEGF expression correlates positively with local recurrence, metastatic potential, and overall survival. The VEGF pathway has been most commonly targeted with monoclonal antibodies that bind the ligand (e.g., bevacizumab) or small molecule VEGFR TKIs. Bevacizumab has been shown to suppress pancreatic tumor growth in preclinical models. Subsequently, a phase II study evaluated the combination treatment of bevacizumab with gemcitabine in 52 patients with metastatic pancreatic cancer. Results showed a 21% partial response rate with a median survival of 8.8 months. Overall, the treatment was well tolerated but plasma VEGF levels did not correlate with outcomes. The response rates and survival results from this phase II study were considered encouraging and this treatment regimen was evaluated in a phase III study. In this double-blinded randomized trial in 602 patients with pancreatic cancer, bevacizumab plus gemcitabine treatment was compared with gemcitabine plus placebo therapy. Unfortunately, bevacizumab plus gemcitabine treatment failed to show a survival benefit compared with gemcitabine monotherapy.

An alternative approach to targeting the VEGF pathway involves the use of TKIs that operate on an intracellular level. Such agents have already been approved for use in other cancers and include sunitinib

in renal cell and gastrointestinal stromal tumors and sorafenib in advanced renal cell carcinoma. The knowledge obtained with the use of targeted anticancer molecular agents suggests that less selective inhibitors with multiple targets may be more efficacious than inhibitors of single targets. This is likely due to the crosstalk and redundancy in molecular pathways that promote essential functions such as proliferation and survival. In addition, a single inhibitor with multiple targets may promote fewer drug interactions, potentially inducing less toxicity than combinations of more specifically targeted agents.

Sorafenib is a multitargeted TKI of Raf-1, VEGR-2, and the platelet-derived growth factor receptor. Proposed antitumor activity of sorafenib targets both antiangiogenic and antiproliferative mechanisms. A phase I study in 23 patients with advanced pancreatic cancer demonstrated encouraging results with a 57% incidence of disease stability. Subsequently, a phase II study of gemcitabine with sorafenib was performed and preliminary results have been reported. Unfortunately, there were no objective responses to this combination with a median survival of 4 months.

Cytoplasmic Tyrosine Kinases

Several agents targeting a variety of pathways are in early clinical testing in pancreatic cancer and include those directed at src and focal adhesion kinase (FAK). *Src* was the first reported proto-oncogene and encodes a nonreceptor tyrosine kinase with involvement in the regulation of proliferation, differentiation, survival, motility, angiogenesis, and cell–cell interactions. Malignant Src activity is related to the overexpression of the wild-type protein rather than expression of a mutated genotype. Src activity may contribute to both constitutive and EGF-induced VEGF expression and, therefore, to angiogenic potential. This has been shown in pancreatic cancer cells in vitro and in animal models growing human pancreatic tumors. Src inhibitors are in phase I and II clinical testing, and clinical trials combining an oral Src inhibitor with gemcitabine are in process.

FAK is a cytoplasmic protein tyrosine kinase that, as its name suggests, is localized to focal adhesions, which are contact points between a cell and its extracellular matrix (ECM). Tyrosine phosphorylation of FAK occurs in response to clustering of integrins, during formation of focal adhesions and cell spreading, and upon adhesion to fibronectin. FAK does not function as a classic oncogene, affecting cell transformation. Rather, it promotes a more invasive and metastatic phenotype of an established malignancy. FAK appears to have many functions in cells, linking integrin signaling to downstream targets, acting as part of a survival signal pathway and having a connection with cell motility. FAK is phosphorylated following activation of a number of transmembrane receptors. FAK functions not only as a kinase, but also as a scaffolding protein for the assembly of a number of cellular signaling molecules, suggesting that FAK is a critical mediator of cell-ECM signaling events.

Our laboratory group has played an important role in defining the biology of FAK in human tumors, including pancreatic cancer. We have demonstrated in pancreatic cancer cells, as well as in vivo models, that FAK inhibition is an effective antineoplastic strategy by inducing apoptosis and sensitizing tumor cells to chemotherapy. FAK inhibitors are currently in phase I testing.

Matrix Metalloproteinases

Tissue invasion and the ability to metastasize are among the hallmarks of cancer. Disruption of cellular junctions and the interactions between cells and the ECM are essential prerequisites for cell detachment from the primary tumor, invasion of the blood stream, and growth at distant sites. Several families of proteases are implicated in these processes. The largest and best characterized is the family of matrix metalloproteinases (MMPs), which are zinc-dependent proteolytic enzymes with different substrate specificities for ECM molecules (eg, collagen, fibronectin, laminin, and elastin). Among the various family members, MMP-2 and MMP-9 are most commonly implicated in tumor angiogenesis. The activity of MMPs may be inhibited by members of the family of proteins known as tissue inhibitors of metalloproteinases (TIMPs). In pancreatic cancer, overexpression of several MMPs has been demonstrated in several studies. It has been suggested that an imbalance between MMPs and TIMPs plays a role in pancreatic tumor progression and that MMP-2 activation correlates with more-advanced pathologic stages and with early recurrence after resection.

Synthetic MMP inhibitors (MMPIs) have been developed and tested against a variety of tumors. One such MMPI is marimastat, an orally bioavailable drug that is active against several MMPs. In preclinical models, marimastat and other MMPIs delay tumor growth and prolong animal survival, but no cytotoxic effect is observed. Phase I studies found an inflammatory polyarthritis to be the dose-limiting toxicity of marimastat. Pharmacokinetic analyses showed that marimastat is rapidly absorbed and eliminated, with time to maximum concentration of 1–2 h and an elimination half-life of 4–5 h.

In a dose-finding study among patients with pancreatic cancer, marimastat doses of 5 mg, 10 mg, and 25 mg twice daily were considered adequate. CA19-9 changes in that study suggested evidence of activity, which was also noted in a phase II trial in which patients who had CA19-9 stabilization or responses (30% of cases) lived longer than patients with a rising tumor marker. Based on these findings, randomized trials of marimastat were performed.

Bramhall et al. randomized 414 patients with advanced pancreatic cancer to receive either gemcitabine or marimastat. Although the median survival in the 25-mg marimastat group was lower than that in the gemcitabine group (125 days vs. 167 days), the log-rank analysis showed no difference between the survival curves of the two groups. Survival of patients treated with the other doses of marimastat (5 mg b.i.d. and 10 mg b.i.d.) was much lower than that of patients given gemcitabine. A subgroup analysis of patients with metastatic disease (approximately 65% of the cases) suggested an interaction between marimastat and disease stage; the median survival of patients with nonmetastatic disease was longer than that of patients with metastatic disease. In the gemcitabine group, there was no difference in median survival between patients with and without metastases. Consistent with the mode of action of these two drugs, the median progression-free survival among patients treated with marimastat, which has no cytotoxic effect, was approximately half that of patients treated with gemcitabine. Likewise, the reported response rates were lower in the marimastat arms of the trial (3% vs. 26% for gemcitabine). Musculoskeletal toxicity was reported in 39–55% of patients across different dose levels of marimastat, but this toxicity was grade 3 or 4 in only 7–12% of cases.

A different approach was taken in a second phase III trial, in which 239 patients were treated with gemcitabine and were concurrently randomized to receive marimastat (10 mg b.i.d.) or placebo. The median and 1-year survival was nearly identical in the two groups. Likewise, the log-rank comparison between the two survival curves yielded no differences in overall or progression-free survival. In this trial, there was no interaction between marimastat and disease stage.

The results of these trials indicate that marimastat, alone or in combination with gemcitabine, has no role in the treatment of advanced pancreatic cancer. Furthermore, another MMPI, BAY12-9566 proved inferior to gemcitabine in a randomized trial of 277 patients with advanced pancreatic cancer. Therefore, the currently available studies of MMPIs show that these agents have no role in the treatment of advanced pancreatic cancer. However, due to their cytostatic effect, it is conceivable that the administration of marimastat or other MMPIs to patients with tumors in earlier stages could improve on the existing therapies. The suggested interaction between marimastat and disease stage in one of the trials is in line with this hypothesis.

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Pancreatic Cancer Stem Cells

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Definition

A subpopulation of highly tumorigenic cells that possess the stem cell-like properties of the ability to self-renew and generate differentiated tumor cell types found in pancreatic cancer.

Characteristics

Pancreatic cancer is an aggressive malignancy arising from the exocrine pancreas. Greater than 30,000 people per year are affected by the disease in the United States alone where it is the fourth leading cause of cancer death. Pancreatic cancer portends the worst prognosis of any solid tumor malignancy, with a 5-year survival rate of less than 3%. Conventional therapies, such as chemotherapy and ionizing radiation often produce minimal clinical response in tumor shrinkage and cancer regression. While our understanding of the molecular mechanisms underlying the development and progression has significantly improved by examining genomic and proteomic profiling of human pancreatic cancers, these studies do not account for the heterogeneity of cancer cells within a particular tumor.

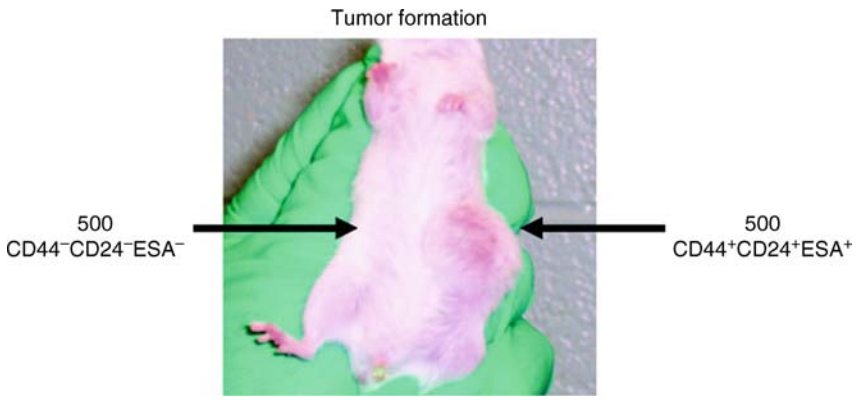
Recently, there has been a paradigm shift in our understanding of how cancers develop and propagate termed the ►cancer stem cell theory. In the traditional model of how cancer develops and propagates, it is though that all cells within the cancer are able to

proliferate extensively and form new tumors. In the cancer stem cell theory, tumors arise from a small population of cancer cells, usually less than 5% of cells, that have properties of adult stem cells; particularly the abilities to self-renew and differentiate into the multiple cell types found in the tumor. Of clinical relevance, this model may explain why standard chemotherapy and ionizing radiation therapy may result in tumor shrinkage, but do not prevent cancer recurrence, since cancer stem cells are resistant to standard types of therapies. Conventional treatments may be able to target the bulk of the tumor, but not necessarily these tumor-initiating cells, subsequently leading to recurrence or disease progression following standard treatment.

The existence of cancer stem cells was originally demonstrated in acute myelogenous leukemia and now has been well established in a several types of solid tumors, including breast cancer, brain cancer, prostate cancer, colon cancer, head and neck cancer, lung cancer, and melanoma. Identification of cancer stem cells within human tumors has been facilitated by the development of ►xenografts (or implants) of human tumors into immunodeficient mice for study. Using this model system, either primary tumors derived directly from patients or implanted and expanded in mice can be dissociated into single cell suspensions. These cells then undergo ►flow cytometry and are isolated based on differential cell surface marker expression and implanted into mice with monitoring of tumor growth (Fig. 1).

Study of human pancreatic cancers have identified a subpopulation of cells that express cell surface markers ►$CD44^{\pm}$ ►$CD24^{\pm}$ ►$ESA^{\pm}$ as putative pancreatic cancer stem cells. In pancreatic cancer, less than 1% of the cancer cells are $CD44^{+}$ $CD24^{+}$ ESA^{+}. As few as 100 $CD44^{+}$ $CD24^{+}$ ESA^{+} cells injected into immune incompetent ►NOD-SCID mice form tumors (Table 1) while 10,000 unsorted primary bulk pancreatic cancer cells were required to initiate tumor growth, revealing a greater than 100-fold enhanced tumorigenic potential in the triple surface marker positive cell population compared to bulk pancreatic cancer cells.

The $CD44^{+}$ $CD24^{+}$ ESA^{+} pancreatic cancer cells possess the stem cell properties of self-renewal and the ability to produce differentiated progeny that recapitulate the phenotype of the parent tumor. Tumors formed in NOD-SCID mice as xenografts derived from pancreatic cancer stem cells are histologically identical to patient's primary tumor from which the cells were derived (Fig. 2). While some different types of tumors may have common cell surface makers that best define the cancer stem cell population, it is likely that different tumor types each have their own "best" set of makers that identify cancer stem cells. In addition, the secondary tumors that form from implantation of the primary tumor's $CD44^{+}$ $CD24^{+}$ ESA^{+} cells reproduce the surface expression pattern of



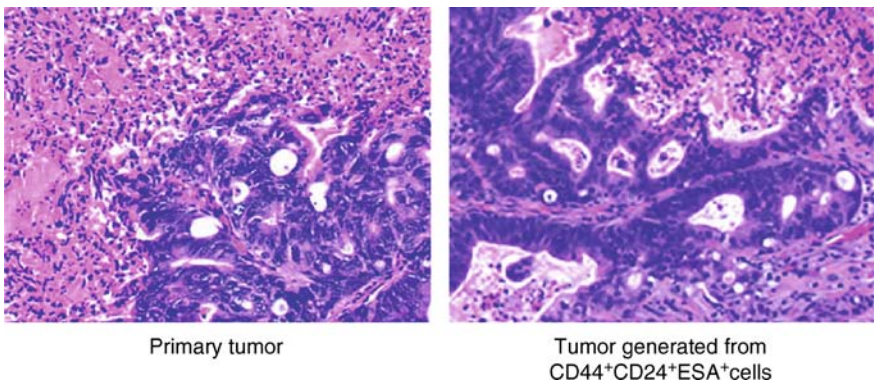
Pancreatic Cancer Stem Cells. Figure 1 Tumor formation in NOD/SCID mice injected with 500 $CD44^{+}$ $CD24^{+}$ ESA^{+} cells, with no tumor formation seen at the injection site of 500 marker negative $CD44^{-}$ $CD24^{-}$ ESA^{-} cells.

Pancreatic Cancer Stem Cells. Table 1 Tumor formation ability of sorted pancreatic cancer cells using cell surface markers

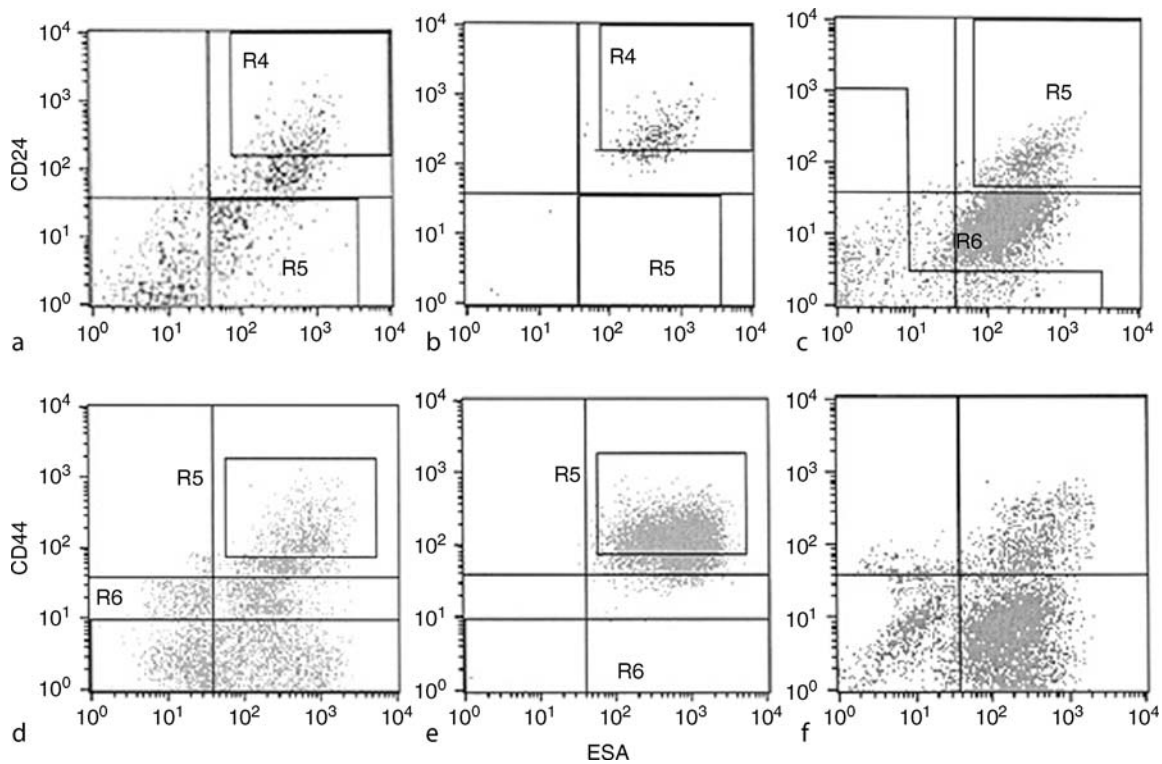
Cell number	10K	1K	500	100
Unsorted	4/6	0/6	0/3	0/3
$CD24^{+}$ ESA^{+}	6/8	5/8	5/8	2/8
$CD24^{-}$ ESA^{-}	2/8	1/8	0/8	0/8
$CD44^{+}/CD24^{+}/ESA^{+}$	10/12	10/12	7/12	6/12
$CD44^{-}/CD24^{-}/ESA^{-}$	1/12	0/12	0/12	0/12

commonly expressed proteins seen in the original tumor (Fig. 3). The capacity of these highly, tumorigenic $CD44^+$ $CD24^+$ ESA^+ pancreatic cancer cells to both self-renew and produce differentiated progeny highlight their stem cell properties.

It is not known at present whether these pancreatic cancer stem cells arise from a mutated stem cell, or a differentiated cell that has regained stem cell-like properties because of genetic alterations. Several studies examining other cancers have supported the



Pancreatic Cancer Stem Cells. Figure 2 H&E staining of the tumor generated from $CD44^+$ $CD24^+$ ESA^+ cells (*right*) has similar histologic features to the corresponding patient's primary pancreatic tumor (*left*). Magnification, 200×.



Pancreatic Cancer Stem Cells. Figure 3 Highly tumorigenic pancreatic cancer cells display stem cell-like properties. (a–f) Phenotypic diversity in tumors arising from $CD44^+$ $CD24^+$ ESA^+ cells. Plots are representative of the CD44, CD24, and ESA staining patterns of human pancreatic cancer cells. (a and d) Staining pattern from a patient tumor. $CD44^+$ $CD24^+$ ESA^+ tumorigenic cells from the tumor were then isolated (b and e) and injected into the flank of NOD/SCID mice. (c and f) The staining pattern of the resultant tumor that arose from the $CD44^+$ $CD24^+$ ESA^+ cells. The tumorigenic cells formed tumors that contained phenotypically diverse cells (c and f) similar to those seen in the original tumor (a and d).

concept that cancer stem cells likely arise from self-renewing adult stem cells which are transformed by dysregulation of a self-renewal pathway. Characterizing normal pancreatic stem cells will greatly enhance our understanding of pancreatic cancer stem cells. Unfortunately, despite extensive effort, isolation of normal pancreatic stem cells has not yet been achieved to date.

A critical consequence of the identification of pancreatic stem cells is the potential impact this may have on approaches to treatment of pancreatic cancer. It is likely that therapies specifically targeting this stem cell population will be needed to improve the likelihood of long-term cure for patients with pancreatic cancer. Approaches targeting markers expressed specifically on the cell surface of pancreatic cancer stem cells (i.e. CD44) or drugs that target signaling pathways that are specifically altered in these pancreatic cancer stem cells, such as ►sonic hedgehog, are likely to lead to significant improvements in outcome for pancreatic cancer patients. In short, a better understanding of pancreatic cancer stem cells will aid in refining current anti-cancer therapies, and ultimately help to identify novel diagnostic markers and therapeutic targets.

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Pancreatic Ductal Adenocarcinoma

Definition

DA; Is the most important of the ductal neoplasia and constitutes the vast majority of pancreatic tumors. DA is one of the most aggressive cancers and is produced by progressive mutations in cancer-related genes that can appear early or late in the evolution of the disease.

►Pancreatic Cancer

Pancreatic Islet Cells

Definition

The pancreas is organized into the exocrine and endocrine tissues. The endocrine pancreas consist of islands i.e. islets, of cells that secrete a number of hormones that include, insulin, gastrin, glucagon, pancreatic polypeptide, VIP, and somatostatin.

Panitumamab

Definition

In September of 2006, FDA approved panitumumab, a recombinant human monoclonal antibody that binds the human epidermal growth factor receptor (EGFR), for the treatment of patients of patients with EGFR-expressing metastatic adenocarcinoma of the colon or rectum (MCRC) after disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. The efficacy of panitumumab was demonstrated in a phase III randomized, open-label, controlled trial comparing panitumumab plus best supportive care (BSC) versus BSC alone in a total 463 patients with 5-FU, irinotecan and oxaliplatin refractory disease. Nineteen of 231 (8%) subjects in the panitumumab arm had a partial response, and there was significant prolongation of progression free survival in the panitumumab arm.

►Colon Cancer
►Epidermal Growth Factor Receptors Inhibitors

Pannexins

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Definition

Pannexins are a second family of ►gap junction genes, distinct from ►connexins. However, in function they are similar to connexins, forming ►hemichannels,

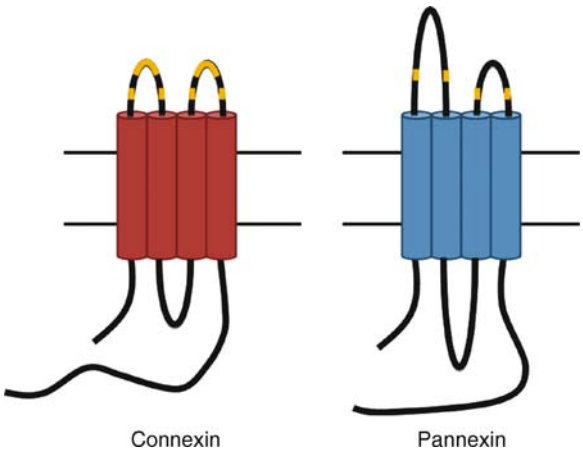
proteinaceous cylinders spanning the plasma membrane and encompassing a hydrophilic channel.

Characteristics

Overview. Until quite recently, only one mammalian family of gap junction genes had been identified, namely the connexins. However, a novel family of mammalian gap junction genes with low sequence similarity to the invertebrate gap junctions, ►innexins (Inxs), has been discovered in chordates and termed pannexins (Panxs). Currently, three pannexin members (►Panx1, ►Panx2 and ►Panx3) have been identified in vertebrates. Previous studies on Inx mutants in *Drosophila* have demonstrated Inx-specific functions including synaptogenesis in the giant-fiber system, epithelial organization and morphogenesis, and germ cell differentiation processes. The role of pannexins in mammalian cells and tissues has yet to be fully explored.

Similarities Between Connexins and Pannexins. Despite sequence dissimilarity between connexins and pannexins, the two protein families share structural resemblance. Similar to connexins, pannexins have a predicted topology of four membrane-spanning domains, two extracellular loops, a cytoplasmic loop, and cytoplasmic amino- and carboxy-termini. Intriguingly, whereas connexins contain three regularly spaced cysteine residues in the two extracellular loops, pannexins, like innexins, only have two such residues. Numerous studies have suggested the importance of the cysteine residues in facilitating functional connexin-based gap junctions and hemichannels, and therefore the variation in the number of cysteine residues may underlie functional differences between pannexin and connexin functions. Analogous to connexins, pannexin hemichannel and intercellular channel formation are pannexin-specific. Although the two channels have similar characteristics, their differences imply that they could exhibit unique functions via different mechanisms (Fig. 1).

Pannexins as tumor suppressors. Gap junctions, and their constituent connexin proteins, have long been considered to play a role in the control of cell proliferation, with disruptions in expression and gap junctional coupling correlating with cell transformation. However pannexins have just been recently identified and their implication in cancer is only now being examined. Indeed, like connexins, pannexins are now emerging as being ►tumor suppressors. Panx1 is normally expressed in brain ►astrocytes, and has been shown to be reduced in brain tumors, specifically ►gliomas. Panx2 has also been suggested as a tumor-suppressor gene in glial cells. Thus transfection of either Panx1 or Panx2 suppresses the tumorigenic phenotype of glioma cells. High-throughput microarray analysis of human brain tumor samples has also shown an overall reduction of Panx2 gene expression in gliomas. Furthermore, a correlation between Panx2 up-regulation and post diagnosis survival in patients with glial tumors was found using a brain cancer gene expression database (REMBRANDT). In addition, the Panx2 gene is located within chromosomal region 22q13.3 where deletion was often found in human astrocytomas and ependymomas.



Pannexins. Figure 1 Comparison of the topology of connexin versus pannexin proteins. They are both tetraspan transmembrane proteins with two extracellular loops, one intracellular loop and intracellular amino and carboxy termini. While connexins possess three cysteine residues (*yellow regions*) in each extracellular loop, pannexins only have two.

Summary. Targeting some of the cellular mechanisms controlling proliferation, migration and invasion would constitute effective therapeutic strategies. One such mechanism involves intercellular communication through gap junctions. Gap junctions and their constituent proteins, connexins and pannexins, have been shown to play a role in controlling proliferation in many cell types. This has been exploited for possible therapeutic potential by directly targeting cell proliferation, or by utilizing properties of connexins/pannexins and/or gap junction channels to augment ►suicide gene therapy. While the clinical application of therapies targeting Cx/Panx pathways has not yet been realized, valuable approaches to such therapy remain fertile territory.

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Panzem

►Methoxyestradiol

PAP Smear

Definition

Cytological screening method for cervical cancer invented by Georgios Papanikolaou.

►Exfoliation of Cells
►Papanicolaau Test

Papain

Definition

Papain is a cysteine protease originally derived from papaya (*Carica papaya*). The tertiary structure of papain consists of two distinct structural domains with a cleft between them. This cleft contains the active site, which contains a catalytic triad made up of three amino acids – cysteine-25 (from which it gets its classification), histidine-159, and asparagine-158.

►Calpain
►Stefins

Papanicolaou Test

Definition

A polychrome staining method originally developed by George N. Papanicolaou. Routinely used in all cytology labs for staining cells so that they can be seen microscopically. Is excellent for evaluating nuclear and cytoplasmic structure. Is the microscopic examination of exfoliated cells obtained by swabs from the uterine cervix (cytology). Abnormal cells showing morphologic signs of papillomavirus infection and/or malignant changes can be identified, in which case usually a direct inspection of the cervix (colposcopy) is performed, eventually followed by biopsy and histological diagnosis. Cytology is currently used as the method in cervical cancer screening programs. Because of the relatively high false negative rate of cytology, it is being evaluated if detection of papillomavirus infection may become a supplementary method in cancer screening.

►Fine Needle Aspiration
►Human Papillomaviruses

Papillary Carcinoma

►Follicular Thyroid Tumors

Papillary Carcinoma, Oncocytic Variant

Definition

Papillary carcinoma of the thyroid characterized by an oncocytic cytoplasm that is not large. The cell has the characteristic nuclear features of papillary carcinoma.

►Hurthle Cell Adenoma and Carcinoma

Papillary Thyroid Carcinoma

Definition

Papillary thyroid carcinoma (PTC) derives from the follicular thyroid cells. It is the most common type of thyroid cancer. PTC usually occurs sporadically. However, familial occurrence has been noted. Patients with familial ►adenomatosis polyposis (FAP), which is caused by mutations in the ►*APC* gene, seem to have an

increased risk developing PTC. Patients with ►Cowden syndrome, an inherited hamartoma syndrome caused by mutations in the tumor-suppressor gene ►*PTEN*, also have an increased risk developing thyroid cancer, specifically follicular thyroid carcinoma but PTC has also been reported. Somatic rearrangements in *RET* have most often been found in about 10–40% of PTC. In transgenic mice, these rearrangements are capable causing PTC. Recently, a somatic point mutation in *BRAF* (V600E; previously designated as V599F) has been identified as the most common (35–70%) genetic change is PTCs.

►RET
►Thyroid Carcinogenesis

Papilloma

Definition
A benign tumor arising from both cutaneous or mucosal epithelium. It is associated with papillomavirus infection.

►Bovine Papillomavirus
►Human Papillomaviruses

PAR

Definition
Proteinase-activated receptor.

►Proteinase-Activated Receptors

PAR1

►Protease Activated Receptors

PAR_1

►Proteinase-Activated Receptor-1

PAR_2

►Proteinase-Activated Receptor 2

PAR_3

►Proteinase-Activated Receptor-3(PAR_3)

PAR_4

►Proteinase-Activated Receptor-4

Paracentesis

Definition
Insertion of a thin needle or tube into the abdomen to remove accumulated fluid from the peritoneal cavity.

►Acites

Paracetamol

Definition
Synonym acetaminophen; has analgesic and antipyretic properties, but, unlike aspirin, it is not a very effective anti-inflammatory agent. It is well tolerated, lacks many of the side-effects of aspirin, and is commonly used for the relief of fever, headaches and other minor aches and pains. Paracetamol has not been shown to be carcinogenic in any way.

Paracrine

Definition
Is a factor acting on the immediate neighbors of the cells that produce it. Following interaction with the related

receptor(s), a paracrine activation is obtained (autocrine; receptor tyrosine kinases). Refers to a short-range biologic action mediated by a soluble factor produced by one cell type that acts on another cell type. For example, it was initially thought that scatter factor was produced only by mesenchymal cells such as fibroblasts and acted solely in a paracrine fashion on nearby epithelial cells through their c-Met receptors. However, scatter factor may promote tumorigenesis by autocrine as well as paracrine loops. An example of the latter is the ability of scatter factor produced by primary tumor cells (e.g., glioma or breast cancer cells) to stimulate tumor growth by inducing angiogenesis in the host stroma in experimental mouse and rat tumor models.

►Gut Peptides
►Scatter Factor

Paracrine Stimulation

Definition

production of a factor that stimulate cells in the immediate environment of the producer cell.

Paraesthesia

Definition

Unpleasant sensation of tingling, pricking or numbness.

►Peripheral Neuropathy

Parallel Gene Expression Analysis

►Microarray (cDNA) Technology

Paralogue

Definition

Synonym: Paralog; Describes a set of closely related genes within one species, e.g. the human MDM, RAS and MYC genes. Paralogues share a common evolutionary origin and have been generated by gene or genome duplication events followed by sequence diversification. The products of paralogues often fulfill similar or redundant functions, however, they also display isoform-specific functions with increasing sequence diversification.

►BORIS
►CCCTC-Binding Factor (CTCF)

Parametric

Definition

Parametric, as a statistical term, refers to analyses that assume an underlying mathematical model for the population of interest, like the normal distribution, that is fully characterized by typically unknown constants.

►Kaplan–Meier Survival Analysis

Paraneoplastic Neuropathy

Definition

Malfunction of peripheral nerves associated with an underlying malignant disease.

►Peripheral Neuropathy

Paraneoplastic Syndromes

Definition

The term paraneoplasic syndrome refers to a clinical picture produced by organ damage in areas remote from the tumor and not produced as a direct consequence of the tumor itself. There are many types of paraneoplastic syndromes. A group of paraneoplastic syndromes have an immunologic basis. As an example, several neurologic syndromes have been described in association with small cell lung cancer, including paraneoplastic encephalomyelitis and sensory neuropa-

thy, paraneoplastic cerebellar degeneration, cancer-associated retinopathy, opsoclonus-myoclonus, and Lambert–Eaton myasthenic syndrome. Nearly all these paraneoplastic syndromes are associated with the presence of autoantibodies in the sera. Anti-Hu antibodies are associated with paraneoplastic encephalomyelitis, sensory neuronopathy, cerebellar degeneration, and autonomic neuropathy. These antibodies are generated against the Hu antigen found in neurons. Anti-Hu antibodies may be found in the sera of patients with small cell lung cancer, but they are not useful diagnostic biomarkers of this malignancy, because although most small cell lung cancers express the Hu antigen, <20% of all patients with small cell lung cancer have detectable levels of anti-Hu antibodies. Two thirds of patients with anti-Hu antibodies have other autoantibodies not yet characterized. Although anti-Hu antibodies have been found in ovarian, breast, prostate, and colon cancer, the presence of anti-Hu antibodies is almost always indicative of an underlying small cell lung cancer. Autoantibodies that bind to ganglionic acetylcholine receptors have been reported in patients with various forms of autoimmune autonomic neuropathy. The correlation between high levels of ganglionic-receptor antibodies and the severity of autonomic dysfunction suggested that the antibodies may have a pathogenic role. Autoantibodies may be directly responsible for tissue injury. Examples of autoantibodies that have been thought to be associated with tissue injury are those of cancer-associated retinopathy. Also, anti-Hu, and anti-Yo antibodies which have been shown to react with tumor antigens, and bind proteins of the endoplasmic reticulum in Purkinje cells in patients with paraneoplastic cerebellar degeneration in breast and ovarian carcinomas. In addition, although these paraneoplastic syndromes are rare, they provide clear examples of autoantibodies preceding the clinical diagnosis of breast cancer and ovarian cancer.

►Autoantibodies

Parathyroid Glands

Definition

Consist of usually two pairs of endocrine glands, situated near the thyroid gland in the neck. The parathyroid glands secrete a hormone – parathyroid hormone (PTH) – which acts on the kidneys and bone to regulate calcium in the extracellular fluid.

►Multiple Endocrine Neoplasia Type 1

Parathyroid Hormone-Related Protein

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Synonyms

PTHrP; PTHLH; PLP

Definition

PTHrP was discovered as a humoral factor that induces hypercalcaemia of malignancy. PTHrP shares with parathyroid hormone (PTH), a protein that regulates calcium homeostasis, the ability to interact with the parathyroid hormone receptor 1 (PTH1R), a ►G-protein coupled receptor. Activation of PTH1R in bone, kidney and intestine leads to increased calcium blood levels.

Characteristics

Protein Structure

Three isoforms of the PTHrP protein exist, containing 139, 141 or 173 amino acid, each of which is coupled to a prepropeptide of 36 amino acid on its N-terminus. The mature PTHrP consists of a least three functional domains, the N-terminus, the mid-region domain and the C-terminal domain. In the 173 amino acid isoform, the last 34 amino acids may form a fourth functional domain. Following post-transcriptional cleavage of PTHrP, these domains are able to act separately. The N-terminus shows similarities to the N-terminus of PTH and is a secretory peptide that interacts with PTH1R. This interaction gives rise to an increase in cellular cAMP levels, but can also lead to activation of calcium (►Calcium Binding Proteins and Cancer)- or ►protein kinase C-dependent pathways. The mid-region domain translocates into the nucleus and is able to accumulate in the nucleolus. Nuclear translocation is blocked upon phosphorylation of this domain by CDK1/2 (►Cyclin-Dependent Kinases). The import of the mid-region domain into the nucleus is therefore thought to be regulated in a cell cycle dependent manner. The C-terminal domain (osteostatin) antagonizes the bone-absorbing action of the N-terminal domain of PTHrP, probably by interfering with PTH1R internalization. The C-terminal domain contains four serine phosphorylation sites that are required for its mitogenic activity on vascular smooth muscle cells. The last 34 amino acid of the 173 amino acid isoform has been shown to interfere with nuclear localization of PTHrP and to raise the cellular cAMP level. PTHrP mRNA undergoes extensive splicing. N-terminal splicing of PTHrP mRNA varies depending on the promoter that initiated

transcription. C-terminal splicing of the PTHrP mRNA that give rise to the different protein isoforms seems to be independent of promoter usage. All PTHrP transcripts contain the coding exons for the three functional domains (Fig. 1).

Regulation of PTHrP Expression

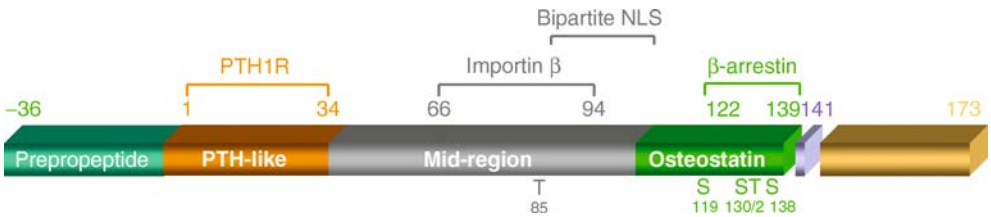
PTHrP expression is mainly regulated on the transcriptional level. In humans, three different PTHrP promoters (P1, P2 and P3) have been identified. The best characterized promoter is the proximal P3-promoter. It contains functional binding sites for ►Ets transcription factors, Sp1 and for the TGFβ (►transforming growth factor β)-regulated Smads. The Ets binding site is required for the activation of PTHrP by the ►Ras/Raf/MEK-1/ERK1/2 pathway. This signaling cascade is used by tyrosine receptor kinases, such as EGFR (►Epidermal Growth Factor Receptor Ligands), and ►G-protein coupled receptors, such as the calcium sensing receptor, to activate PTHrP transcription. TGFβ upregulates PTHrP gene transcription by recruiting Smad proteins to the Smad site of the PTHrP P3 promoter where the Smad proteins cooperate with Ets and Sp-like proteins. A number of cytokines, including IL-1β, IL-2, IL-4 and IL-6, also activate PTHrP expression on the transcriptional level. In addition, the interaction of cells with particular ►extracellular matrix proteins can trigger PTHrP expression. Inhibition of PTHrP transcription is achieved by treatment with nuclear receptor ligands, such as vitamin D, retinoic acid and glucocorticoids. Vitamin D represses PTHrP transcription through a vitamin D response element in the P1 promoter.

Normal Functions

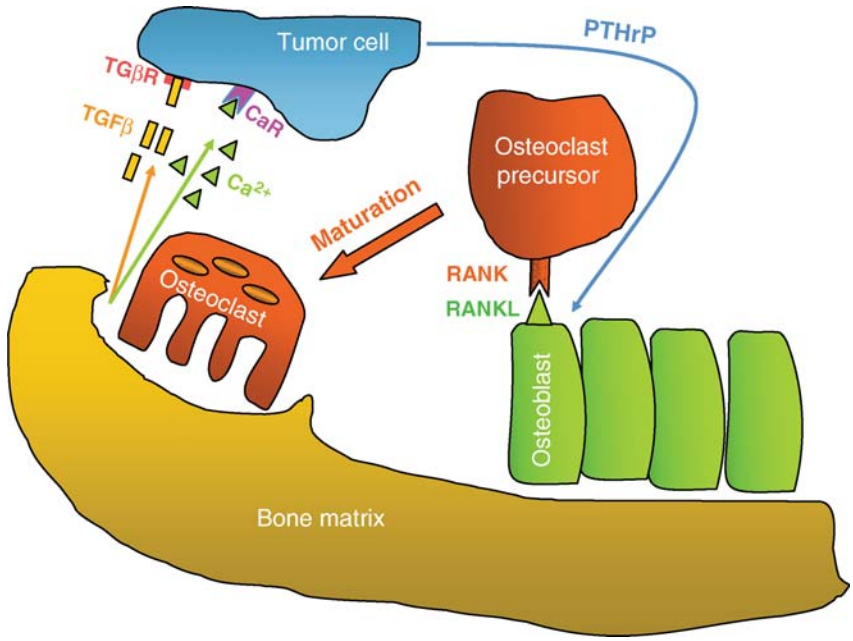
The normal function of PTHrP is to regulate many biological processes critical in embryonal development and adult physiology. PTHrP usually acts locally as a paracrine or autocrine factor. As a paracrine factor, PTHrP plays a crucial role in bone development where it is expressed by the periarticular perichondrium to activate proliferation of PTH1R-expressing condrocytes, thereby preventing premature ossification. PTHrP knockout mice die shortly after birth because of fatal skeletal dysplasia. Of similar importance is PTHrP for mammary gland development. PTHrP is required for ductal morphogenesis and sexual dimorphism as it triggers formation of mammary-specific mesenchyme. A number of other processes have been linked to PTHrP function, such as lactation and tooth development. In tooth development, PTHrP stimulates bone resorption of the alveolar bone, a process needed for tooth eruption. By interfering with keratinocyte differentiation, PTHrP likely plays a role in skin development. Proliferative or antiproliferative effects on regeneration processes, such as wound healing, have been attributed to PTHrP. An important role of PTHrP in controlling the vascular tone is emerging. As a vasorelaxant PTHrP regulates the contractility of many different vascular beds, including kidney microvessels and the arteries and veins of the mammary gland. During pregnancy, PTHrP is essential for the fetal–placental calcium transport, an activity that seems to be dependent upon the mid-regional part of PTHrP. Furthermore, a role of PTHrP in the survival of neurons is discussed (Fig. 2).

PTHrP and Cancer

PTHrP is expressed by many tumors, including carcinomas of breast, kidney, prostate, colon, stomach and lung. In hematopoietic malignancy, it is predominantly found in HTLV-I (►Human T-cell Leukemia Virus) induced adult T-cell leukemia, probably as a result of the ability of the HTLV-1 protein Tax to strongly induce PTHrP transcription. Cancers can secrete PTHrP at levels that are sufficient to raise PTHrP blood levels and to induce hypercalcaemia of malignancy, a disease that complicates cancer. Besides this endocrine cancer-related function, PTHrP displays autocrine and paracrine activities that



Parathyroid Hormone-Related Protein. Figure 1 The human parathyroid hormone-related protein. The three initial translational products (-36/139, -36/141 and -36/173) of human PTHrP contain a prepropeptide of 36 amino acid at their N-termini. The mature protein consists of three functional domains, the PTH-like N-terminal domain, the mid-region domain and the C-terminal domain (osteostatin). The PTH-like domain interacts with the PTH1 receptor. The mid-region domain harbors two sequences that mediate its translocation into the nucleus. The first sequence interacts with importin β, the other is a bipartite nuclear localization sequence (NLS). T and S denotes threonine- and serine-based phosphorylation sites, respectively. Thr^{85} can be phosphorylated by CDK1/2. The β-arrestin interaction domain of osteostatin is indicated. Arrestins are involved in internalization and inactivation of ligand-stimulated G-protein coupled receptors, such as PTH1R.



Parathyroid Hormone-Related Protein. Figure 2 Involvement of PTHrP in skeletal metastasis. PTHrP produced by breast cancer cells that metastasized to bone may fuel a vicious cycle by stimulating osteoblasts to interact with osteoclast precursors via RANK/RANKL (►RANK-RANKL Signaling). As a result, the osteoclast precursors will mature to osteolytic osteoclasts. Following its destruction, the bone matrix releases TGFβ and calcium which then activate their corresponding receptors on the tumor cells (TGβR and CaR). The activated receptors further stimulate PTHrP production and more osteoclasts are recruited for bone degradation. This positive feedback loop is thought to facilitate metastasis growth by providing space for tumor expansion.

seem to influence tumor progression and metastasis. One of the best characterized examples for the importance of PTHrP in cancer is its involvement in breast cancer growth in the bone. By secreting PTHrP, breast cancer cells that metastasized to bone induce osteoclastic bone-absorption leading to bone destruction and release of TGFβ which then acts back on the cancer cells and further boosts PTHrP expression in these cells. This positive feedback fuels a vicious cycle that accelerates osteolysis and facilitates expansion of the metastatic cells in the bone environment. It is likely that calcium is supporting this process. Calcium is also released upon bone degradation and stimulates PTHrP expression in the tumor cells by activating the calcium sensing receptor CaR. PTHrP secreted into the bone may also interfere with hematopoiesis by stimulating the activity of hematopoietic stem cells. Other studies show that PTHrP protects cancer cells from apoptosis, stimulate their proliferation and triggers their ability to migrate and invade extracellular matrix. A number of genes that are targeted by PTHrP in cancer cells have been identified. Among them are genes encoding integrin α6 (►Integrin Signaling and Cancer), PAI-1 (►Plasminogen Activating System), CDK1 (►Cyclin-Dependent Kinases) and ►KiSS-1. Activation of the PI3K/AKT pathway (►AKT Signal Transduction Pathway in Oncogenesis) by PTHrP has also been shown. The PTHrP domain that mediates these effects varies with the targeted gene. For example, integrin α6 is activated via the mid-region domain, whereas the N-terminal domain seems to be important for the repression of CDK1. Supporting the notion that PTHrP is a tumor-promoting factor, many survival analyses of cancer patients revealed that PTHrP expression in the tumor increases the risk of a relapse und is linked with unfavorable prognosis. In breast cancer, coexpression of PTH1R along with PTHrP further reduced disease-free survival. It should be noted, however, that the value of PTHrP as a prognostic factor of breast cancer is still a matter of debate, as also data have been published that show that PTHrP expression in breast cancer correlates with a more favorable prognosis.

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Paresthesia

Definition

An abnormal sensation in the skin, including tingling, prickling, or burning, that has no objective cause, and is usually due to injury to a sensory nerve.

►Taxotere

Parity

Definition

The number of pregnancies a woman has had which have gone beyond 24 weeks gestation.

►Endometriosis

Parkinson Disease

Definition

A progressive neurodegenerative disorder characterized the loss of dopamine neurons, resulting in tremor, rigidity, slowness of movement, and postural instability.

►Synuclein

Parosteal Lipoma

Definition

Is a rare benign neoplasm of adipose tissue that exhibits a contiguous relationship with the periosteum. These lipomas of the bone share some histopathologic features with their commonly occurring soft tissue counterparts. While the latter ones are one of the most common mesenchymal tumors, only slightly over 100 cases of parosteal lipoma have been reported in the literature.

►Lipoma Preferred Partner

PARP

Definition

►Poly(ADP-ribose) polymerase.

PARP Inhibitors

Definition

Poly(ADP-ribose) polymerase inhibitors are a large number of low molecular weight compounds (NAD+ analogs) that competitively inhibit PARP activity. Amongst the first to be described were nicotinamide, benzamide and 3-aminobenzamide. However, their potency and specificity is rather low. Subsequently, more sophisticated new compounds have been developed such as 4-amino-1,8-naphthalimide, 3,4-dihydro-5-methoxyisoquinolin-1(2H)-one (PD 128763), 8-hydroxy-2-methylquinazolin-4(3H)-one (NU1025) or 2-methylbenzimidazole-4-carboxamide (NU1064), to name but a few. These are much more potent than the first generation inhibitors and possess an improved pharmacokinetic profile.

With respect to specificity, while all inhibitors inhibit PARP-1 (by definition), at least some of the first generation inhibitors also interfered with other ADP-ribosyl transfer reactions, such as mono-ADP-ribosylation of proteins or NAD+ glycohydrolases, albeit at different IC50 levels. Furthermore, the novel poly (ADP-ribose) polymerases recently identified are likely to be inhibited as well, perhaps at similar IC50 levels as PARP-1.

►Poly(ADP-Ribosyl)ation

Particle-induced Cancer

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Definition

Particle-induced cancer refers to cancer arising after exposure to particles.

P

Introduction

Particles are here defined as solid spherical or fibrous materials suspended in air. It has been documented that particle-induced cancer occurs in animals and humans after inhalation of solid insoluble particles. The ►International Agency for Research on Cancer (IARC) has classified a number of particles according to their carcinogenicity. Group 1 agents are carcinogenic to humans. Group 2A agents are probably carcinogenic to humans, while group 2B agents are possibly carcinogenic to humans.

In the outdoor environment ►ultrafine particles are formed by the combustion processes e.g. of diesel (group 2A) and biomass (group 2A). Indoor sources of particle pollution include smoke from tobacco (group 1), cooking fires, wood stoves, fireplaces and candles (smoky coal is classified as group 1 carcinogen). Some occupational exposures to aerosols of solid particles have been associated with ►cancer in the respiratory tract. Examples are quartz (group 1), asbestos (group 1), soot (group 1), welding fumes (group 2B) and wood dust (group 1).

The effects of particles deposited in the lung may be related to carcinogenic effects that have been noted in animals after surgical implantation of some stable solid materials such as thin films of polymeric or metallic materials or solid metallic bodies of specific compositions.

In the hitherto largest study, in which 500,000 persons from 51 urban areas in USA were followed for over 16 years, the incidence of lung cancer correlated with long-term exposure to fine particulate matter: For every 10 $\mu g/m^3$ increase in fine particles (PM2.5) lung cancer mortality increased by approximately 8% each year. The study provides the strongest evidence yet for linking ►lung cancer to air pollution in urban areas.

Particle Size and Form

Particles in urban air tend to have a multimodal distribution, partly reflecting the sources. Particles are traditionally divided in three different size fractions: the ultrafine (<0.1 μm), fine (<2.5 μm) and coarse (>2.5 μm). In relation to traffic sources the fine and ultrafine fraction are generated by combustion from motor vehicles, whereas the coarse fraction consists of particles generated by mechanical processes. The ultrafine particles (UFP) aggregate fast, forming larger accumulation particles. For equivalent masses of inhaled particles, ultra-fine particles provide a greater surface area per mass unit for adsorption of potentially toxic agents, and present a greater capacity for the formation of ►free radicals and oxidants on the particle surface than do the larger sized particles. This means that they have a greater potential of reacting with the lung cells.

Another group of particles are the engineered ►nanoparticles which have been produced in a multitude of forms and sizes, ranging from spherical fullerenes to nanotubes.

Fibers, such as asbestos, exist in different lengths and diameters. The size and shape of the asbestos fibers are important determinants for their toxicity. Fibers with a diameter of less than 3 μm and a length of more than 5 μm are considered to be most hazardous to health (diameter: length = 1:3).

Deposition and Clearance of Particles in the Lungs

The deposition of particles in the lungs depends on particle size. Particles with a diameter greater than 10 μm, are deposited primarily in the upper airways (nose, nasopharynx), whereas smaller particles are able to deposit in greater number and deeper down in the lungs, in the bronchia. The ultrafine particles are able to reach the alveoli. The retention of particles depends on many factors, including particle characteristics (such as size, shape, solubility and surface chemistry) and anatomical factors.

There are a number of defence mechanisms in the respiratory tract that manage to keep the mucosal surfaces free from foreign materials deposited after inhalation. In the ciliated airways in the nose, trachea, the bronchia and bronchioli, deposited materials, such as insoluble particles, are effectively removed by transport of the mucus blanket by the beating of cilia towards the pharynx, where the foreign material is swallowed. Mucociliary clearance is fast; however, if the insoluble materials are deposited in the alveoli, where there is no mucociliary transport, the clearance is much slower. Moreover, ultrafine particles in bronchia and bronchioli have a slow mucociliary clearance. Usually particles deposited in the alveoli are cleared by phagocytosis by alveolar macrophages. The macrophages are capable of crossing the lung epithelium or phagocytized material may be dissolved by the macrophage. It is unclear if particles are able to pass over the lung epithelial cells by other mechanisms. Sometimes particles are trapped in the lung for a very long time and may be concentrated in foci at which interstitial fibrosis may develop.

Mechanisms

Different kinds of particles have been shown to induce genotoxic damage after deposition in the lung. This may result in cancer if the ►DNA damage is not repaired or if selective ►apoptosis of the damaged cells fails.

1. A primary mechanism of particle-induced ►genotoxicity has been attributed to the surface characteristics. Urban air pollution particles and diesel exhaust particles consist of a carbon core onto which ►polycyclic aromatic hydrocarbons (PAHs), ►quinones,

and ►transition metals are adsorbed. Transition metals, quinones and PAHs on the surface or released from the particles are able to generate ►reactive oxygen species (ROS) thereby causing DNA damage.
2. Another direct way of action is due to the surface activity of the particles to generate ROS extracellularly or intracellularly via activation of mitochondria and ►NADPH oxidase.
3. A decade ago another indirect pathway for genotoxicity was proposed on the basis of the finding that carbon black exposed rats developed tumours similar to DEP exposed rats, despite the fact that carbon black is almost devoid of PAH's on the surface. The fact that tumour formation in rats has been found to be paralleled by the degree of chronic neutrophilic ►inflammation led to the idea about a relationship between particle-induced inflammation and genotoxicity. Likewise, most human cancers are accompanied by the infiltration of inflammatory cells and a wide range of chronic inflammatory diseases predispose to cancer in the affected organ. The genotoxic effect of particle-induced inflammation is related to the release of reactive oxygen species by the inflammatory process. It has been demonstrated that the inflammatory response depends on the particle size. Exposure studies have shown that ultrafine particles cause more inflammation in the lungs of rodents than exposure to the same mass concentration of fine particles. The greater surface area has been suggested to be responsible for the greater inflammatory response of the ultrafine particles. This is based on a linear correlation between surface area of relatively inert particles and inflammatory response measured by the total number of neutrophils in rats.
4. Chronic inflammation and fibrosis are pathological changes in the cellular and ultrastructural composition of tissues. Fibrosis is a type of scarring reaction that involves excess fibrous connective tissue. Such changes in the tissue environment may promote the growth of cellular growth and carcinogenetic transformation. There is a clear association between the occurrence of cancer and fibrosis in the lung and other tissues. However, it is unclear if the tissue changes are a part of carcinogenic transformation or if it is a parallel process.

Thus, the genotoxic effects of PM can either be directly due to surface properties of the particles or due to ROS released by the inflammatory process.

The mechanisms behind the carcinogenic effects of particles at present are still poorly understood. The importance of obtaining more knowledge about the mechanisms behind the carcinogenic effect of particles is underlined by the emerging nanotechnology. Nanotechnology may lead to the release of persistent nanosized particles, and must be considered as an important contributor to the exposure of particles at the work place and the environment in the future.

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Particulate Matter

Definition

Is suspension of tiny solid (a smoke) or liquid (an aerosol) particles suspended in a gas.

►Xenobiotics

Parvovirus

Definition

Eukaryotic virus family characterized by unusually small size of viral capsid (~20 nm) and viral DNA genome (~5,000 nucleotides).

►AAV

Passive Cell Death

Definition

►Necrosis.

Passive Immunity

Definition

Immunity resulting from the transfer of antibodies or antiserum produced by another individual.

Passive Targeting

Definition

A mechanism by which drugs and drug delivery vectors can be preferentially delivered to target cells *in vivo*, especially cells in tumor tissue. Based on their physicochemical properties (small size and/or surface modification with polyethylene glycol), drugs or drug delivery vectors can escape from nonspecific trapping by the reticuloendothelial system and accumulate in target tissues after circulating in the blood. Because cancer tissues are characterized by a high interstitial pressure, enhanced vascular permeability, and the lack of functional lymphatic drainage, passive targeting has become a popular approach for drug delivery.

►Non-viral Vector for Cancer Therapy
►Targeted Drug Delivery

Patch-Clamp Technique

Definition

Is an electrophysiological method in which a seal is formed between the tip of a glass pipette and a cell membrane. The technique allows the recording of ion channels activity and is widely used in pharmacology, for example studying chemotherapeutic drugs and ion channels involved in cancer.

►Ether à-go-go Potassium Channels

Patched

Definition

PCTH; Is a member of the patched gene family. The protein is the receptor for sonic hedgehog, a secreted molecule implicated in the formation of embryonic structures and in tumorigenesis. This gene functions as a tumor suppressor. Mutations of this gene have been associated with ►naevoid basal cell carcinoma syndrome, esophageal squamous cell carcinoma, trichoepitheliomas, and transitional cell carcinomas of the bladder.

►Hedgehog Signaling

Pathogen-Associated Molecular Pattern

Definition

PAMP; Collective term for biochemically diverse substructures of pathogenic origin recognized by the target cells of the respective hosts. Usually bind to so-called pattern recognition receptors (PRR) on the cell surface or in the cytoplasm of the target cell and initiate an immediate (antimicrobial) cellular response.

►Bacillus Calmette-Guérin

Pathogenesis

Definition

Pathogenesis is the mechanism by which a certain etiological factor causes disease.

►Gastrointestinal Stromal Tumor

Pathological Tumor Cell-Platelet Interaction

►Tumor Cell-Induced Platelet Aggregation

Pathology

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Synonyms

Surgical pathology; Diagnostic pathology; Oncologic surgical pathology; Tumor pathology; Tumor typing; Molecular pathology

Definition

The study of human tissues to establish a diagnosis and determine the nature, extent, optimal therapy and possible future behavior of a disease process.

Characteristics

The traditional role of the surgical pathologist has been to analyze tissues obtained from various biopsy procedures in human subjects to establish the type of disease. Due to advances in modern science, the role of the surgical pathologist has been considerably expanded, and we currently act as highly specialized consultants who not only are responsible for rendering a diagnosis, but also for mapping the extent of the disease, determining its degree of aggressiveness, predicting response to therapy and advising the clinician as to the possible biologic behavior of the lesion.

The most basic method used by the pathologist for the study of tumors is conventional histology. This technique involves processing representative tissue samples in a standard fashion to produce thin tissue sections that are stained with special chemical dyes (hematoxylin and eosin) and then examined under transmitted light in a microscope. The information obtained from this simple and basic technique will permit answering most routine questions in 70–80% of cases in most pathology laboratories. A series of other ancillary techniques has been traditionally employed to expand our ability to understand and identify the processes being studied, including ►histochemistry, ►electron microscopy, ►immunohistochemistry, ►cytogenetics, ►flow cytometry, and ►molecular pathology. In recent years, immunohistochemistry and molecular pathology have largely superseded histochemistry and electron microscopy in the routine practice of surgical pathology, although there still remain many valid indications for their use.

The most central role of diagnostic pathologists remains tumor typing. Another important role of the pathologist is to provide proper ►staging and ►grading information for malignant neoplasms. Proper staging and classification requires determining the type of tissue and anatomic site of origin of the tumor (i.e. breast, colon, etc). In the past, human neoplasms were traditionally classified on the basis of their purported cells of origin (histogenesis). In recent years there has been a shift towards classifications based on the realized lines of differentiation of the tumor cells rather than on a hypothetical cell of origin. Based on a given tumor's realized lines of differentiation, several broad categories of tumor types or tumor families are identified including epithelial, mesoderm-derived (i.e. ►mesenchymal tumors), ►neural tumors, ►neuroectodermal tumors, ►neuroendocrine tumors, ►germ cell tumors, ►blastemal tumors, ►hematolymphoid tumors, ►melanocytic tumors and mixed tumors showing combinations of the above. Finally, tumors of unknown or uncertain histogenesis constitute another important category (Table 1).

Pathologic Factors Involved in the Prognostication of Human Neoplasms

Factors that provide important prognostic information for the management of human neoplasms include the histologic type of the tumor, the grading, the staging, and the status of resection of the tumor. The grading of a tumor is generally established by determining the degree of cytologic atypia (or deviation from the normal) of the tumor cells, which is usually manifested by increasing nuclear size and aberrations of nuclear components (i.e. enlarged nuclei, hyperchromatism, prominent nucleoli, mitotic figures, etc). Alternatively, grading can be also done by determining the degree of differentiation of the tumor cells (i.e. the extent to which the tumor cells resemble their normal counterparts). As such, tumors may be categorized as well, moderately or poorly-differentiated, with progressive loss of differentiation (i.e. higher grade) generally implying a more aggressive biologic potential.

Staging of malignant neoplasms has been found to directly correlate with prognosis in the majority of human cancers. The staging for most tumor systems utilizes a combination of clinical and pathologic parameters to determine how advanced or localized a disease process is. A variety of staging systems has been proposed over the years; the most commonly employed is that based on the assessment of the size of the tumor, the presence or absence of lymph node metastases, and the presence or absence of distant metastases (so-called T-N-M staging). The most widely used ►TNM staging system is the one proposed by the American Joint Commission on Cancer (AJCC), although other systems also are employed. Staging in combination with grading has historically been the most reliable prognostic parameter for the assessment of biologic behavior in human neoplasms.

Determination of the status of resection for a given neoplasm is another important piece of information provided by the surgical pathologist that plays an important role in the management of cancer patients. This is done by histologically examining the surgical resection margins of a specimen. Another important function of the surgical pathologist is to offer guidance to the surgeon during the operative act to determine the extent of resection and the status of the margins. This intraoperative consultation is done utilizing a frozen section technique which consists of rapid freezing of the tissues which are immediately cut, stained and read by the pathologist to provide a rapid interpretation of the findings. This method is often used during surgery to determine the status of the surgical margins. Newer

Pathology. Table 1 Classification of solid tumors

Epithelial neoplasms
General
Adenoma/adenocarcinoma
Squamous cell carcinoma
Papilloma/papillary carcinoma
Transitional cell carcinoma
Clear cell carcinoma
Large cell carcinoma/giant cell carcinoma
Spindle cell (sarcomatoid) carcinoma
Anaplastic carcinoma
Organ/tissue specific
Adenoid cystic carcinoma (salivary glands and other organs)
Adenomatoid tumor (serosal surfaces and other organs)
Acinar cell carcinoma (pancreas)
Acinic cell carcinoma (salivary glands and other organs)
Ameloblastoma/ameloblastic carcinoma (mandible)
Adrenal cortical adenoma/Adrenal cortical carcinoma
►Basal cell carcinoma (skin and other organs)
Brenner tumor (ovary)
Calcifying epithelial odontogenic tumor (Pindborg tumor) (mandible)
Ceruminous gland carcinoma (ear)
Cholangiocarcinoma (liver)
Choroid plexus papilloma/Choroid plexus carcinoma (brain)
Collecting duct carcinoma (kidney)
Craniopharyngioma (brain)
Epi-myoepithelial carcinoma (salivary gland and other organs)
Follicular adenoma/Follicular carcinoma (thyroid)
Glassy cell carcinoma (cervix, uterus)
Hepatoma (liver)
Hepatoid adenocarcinoma (endometrium, stomach and other organs)
Hurthle cell adenoma/Hurthle cell carcinoma (thyroid)
Metanephric adenoma (kidney)
Mucoepidermoid carcinoma (salivary glands and other organs)
►Mesothelioma (serosal surfaces)
Myoepithelial carcinoma (salivary glands and other organs)
Polymorphous low-grade adenocarcinoma (salivary gland and skin)
Serous papillary carcinoma (ovaries, uterus, peritoneum)
Thymoma, atypical thymoma, thymic carcinoma (thymus)
Mesenchymal neoplasms
Fibroblastic/myofibroblastic/fibrohistiocytic
Fibroma/fibrosarcoma
Fibromyxoid sarcoma/myxofibrosarcoma
Myofibroma/myofibroblastic sarcoma
Myofibroblastoma
Dermatofibrosarcoma protuberans
Malignant fibrous histiocytoma
Cartilage
Chondoma/chondrosarcoma
Chondroblastoma
Extraskeletal myxoid chondrosarcoma
Mesenchymal chondrosarcoma
Bone
Osteoma
Osteoid osteoma
Osteoblastoma
Osteosarcoma
Osteoclastoma (giant cell tumor)
Chondromyxoid fibroma
Smooth muscle
Leiomyoma/leiomyosarcoma
Glomus tumor/glomangiosarcoma
Skeletal muscle
Rhabdomyoma/rhabdomyosarcoma
Adipose tissue
Lipoma/liposarcoma
Lipoblastoma
Vascular endothelium
Hemangioma
Hemangioendothelioma
Angiosarcoma
►Kaposi sarcoma
Organ specific
Meningioma/meningeal sarcoma (meninges)
Meningeal hemangiopericytoma (meninges)
Sex cord/stromal tumors (gonads)
Solitary fibrous tumor (serosal surfaces and other organs)
Cystosarcoma phyllodes (breast)
Gastrointestinal stromal tumor/sarcoma (gastrointestinal tract)
Cementoma/cementoblastoma (mandible)
Embryonal sarcoma (liver)
Endometrial stromal sarcoma (uterus)
Hemangiopericytoma-like intranasal tumor (nasal cavity)
Juxtaglomerular cell tumor (kidney)
Papillary fibroelastoma (heart)
Neural neoplasms
Schwannoma
Neurofibroma
Perineurioma

Pathology. Table 1 Classification of solid tumors (Continued)

Granular cell peripheral nerve sheath tumors
Malignant peripheral nerve sheath tumors
Neuroblastoma
Esthesioneuroblastoma
►Medulloblastoma
►Retinoblastoma
Ganglioneuroma
Ganglioneuroblastoma
Gliomas
Glioblastoma multiforme
Ganglioglioma
Oligodendroglioma
Astrocytoma
Ependymoma
Hemangioblastoma
Neurocytoma
Hematopoietic neoplasms
►Malignant lymphoma
►Hodgkin disease
Granulocytic sarcoma (myelosarcoma)
Plasmacytoma/anaplastic plasmacytoma
True histiocytic sarcoma
Dendritic reticulum cell tumors
Malignant mastocytosis
Langerhans cell histiocytosis/sarcoma
Neuroendocrine neoplasms
Neuroendocrine carcinomas (carcinoid, atypical carcinoid, small cell carcinoma, large cell neuroendocrine carcinoma)
Paraganglioma
Tissue/organ specific
Medullary carcinoma (thyroid)
Pheochromocytoma (adrenal)
Pituitary adenoma/Pituitary carcinoma
Parathyroid adenoma/Parathyroid carcinoma
Merkel cell carcinoma (skin)
Glomus jugulare tumor (ear)
Goblet cell carcinoid
Islet cell tumors (pancreas)
Struma ovarii (ovaries)
Neuroectodermal neoplasms
Ewing sarcoma
Primitive neuroectodermal tumor (PNET)
Melanotic progonoma (melanotic neuroectodermal tumor of infancy)
Blastemal neoplasms
Pulmonary blastoma
Mesoblastic nephroma (kidney)
Wilms tumor (kidney)

Pathology. Table 1 Classification of solid tumors (Continued)

►Hepatoblastoma (liver)
Gonadoblastoma (testis and ovaries)
Pancreatoblastoma (pancreas)
Polar spongioblastoma (brain)
Melanocytic neoplasms
Melanocytic nevi
►Malignant melanoma
Clear cell sarcoma of soft parts
Germ cell tumors
Teratoma (benign, immature and malignant)
Seminoma/dysgerminoma
Yolk sac tumor
Choriocarcioma
Embryonal carcinoma
Combined germ cell tumors
Tumors of mixed histogenesis
Benign mixed tumor (skin, salivary gland and other organs)
Fibroadenoma (breast)
Lipoadenoma (parathyroid and other organs)
Myelolipoma (adrenal and other organs)
Myolipoma/lipoleiomyosarcoma
Dendritic fibromyxolipoma
Lipoleiomyoma (uterus and other organs)
Adenofibroma/adenosarcoma (uterus, ovaries and other organs)
Malignant mixed Mullerian tumors (uterus, ovaries and other organs)
Mixed epithelial and stromal tumor (kidney)
Carcinosarcoma (various organs)
Germ cell tumors with malignant hematopoietic elements
Myomelanocytic tumors (PECOmas)
Angiomyolipoma
Mesenchymoma/malignant mesenchymoma
Ectomesenchymoma
Gangliocytic paraganglioma
Gliosarcoma
Malignant “triton” tumor (malignant schwannoma with rhabdomyosarcoma)
Tumors of uncertain or unknown histogenesis
Clear cell “sugar” tumor (pulmonary and extrapulmonary)
Small round cell desmoplastic tumor
Synovial sarcoma
Epithelioid sarcoma
Rhabdoid tumor
Alveolar soft part sarcoma
Chordoma/parachordoma
Solid pseudopapillary tumor of pancreas

molecular methods have more recently been applied to the determination of residual disease in certain tumor systems, thus enhancing the capability of the oncologic team to detect and treat such lesions at an early stage prior to recurrence.

Techniques in Diagnostic Pathology

Immunohistochemistry is the most widely used ancillary technique for the diagnosis of human tumors. A large variety of tumor-associated surface, cytoplasmic and nuclear antigens have been developed that are amenable to detection in routinely processed human tissue samples. Specific immunophenotypes of neoplasms help characterize their properties and identify their realized lines of differentiation. In addition to serving to establish the correct diagnosis, immunohistochemistry can also be used to determine prognosis and response to therapy. Although a few fairly specific markers have been developed over the years, no truly specific marker exists and it is therefore better to talk of tumor-associated markers for diagnosis. Because of this lack of absolute specificity, antibodies are best used in panels rather than singly. Immunohistochemistry is merely an ancillary technique and the selection of markers must be always guided by the histologic features of the tumor in concert with the clinical background; tumor diagnosis should never be primarily based on the results of immunohistochemical stains independent of the morphologic findings and clinical history.

There is a large variety of tumor-associated or differentiation antigens employed in routine practice for diagnosis. These include ►epithelial-associated antigens (epithelial membrane antigen, ►carcinoembryonic antigen, etc); ►intermediate filaments (keratins, vimentin, desmin, glial fibrillary acidic protein, neurofilaments); ►muscle-associated antigens (actin, myosin, myogenin, etc); ►neural associated markers (►S-100 protein, nerve growth factor receptor, etc); ►endothelium-associated markers (►von Willebrand factor, CD31, etc); ►melanocyte-associated antigens (HMB45, Melan-A, tyrosinase, etc); ►hematolymphoid markers (►cluster-designation [CD] lymphoid markers such as CD3, CD5, CD10, CD20, CD30, CD45, etc.; plasma cell and myeloid-associated markers, etc.); ►germ cell markers (alpha-fetoprotein, placental-like alkaline phosphatase, human chorionic gonadotropin hormone, etc); neuropeptides and hormones (calcitonin, somatostatin, thyroglobulin, etc); ►neuroendocrine markers (chromogranin-A, neuron specific enolase, etc); and others.

Immunohistochemical markers that identify specific oncogenes, growth factors and receptors have also been utilized both for diagnosis and prognosis of tumors. These include ►Her-2-neu (c-erb-b2 proto-oncogene), ►estrogen receptor and ►progesterone receptor, ►bcl-2, ►c-mic^2 (CD99), ►kit (CD117), p16, ►p53, p63, ►epidermal growth factor receptor (EGFR) and others. Overexpression of some of these markers has been associated with certain tumor types, or can correlate with a particular behavior for such tumors. Detection of overexpression of some of these markers is now also being utilized to guide targeted therapy, for example with ►cyclin-dependent kinase inhibitors, such as CD117, whose expression is linked to favorable response to imatinib mesylate, a tyrosine-kinase inhibitor drug with potent antitumor properties that has been applied with success in the treatment of gastrointestinal stromal tumors.

Immunohistochemical markers can also be applied for predicting response to therapy. A variety of tumors are known to be under the regulation of steroid sex hormones (estrogen, progesterone, etc). Response to therapy in such tumors can be monitored and predicted from the status of hormone receptors by immunohistochemistry. Expression of p53 oncoprotein has also been linked to drug resistance and can be utilized to assess chemosensitivity in certain types of tumors. Detection of ►MDR-1 (►P-glycoprotein) has been associated with a multidrug resistant phenotype, whereby tumor cells are able to reduce intracellular accumulation of chemotherapeutic drugs. Her-2-neu expression in ►breast cancer has been shown to predict resistance to hormone therapy in estrogen-receptor-positive tumors and resistance to some types of chemotherapy. Another valuable application of immunohistochemistry is for the detection of proliferation markers, such as ►MIB-1, ►Ki-67, etc. Such markers can provide prognostic information about a variety of tumor processes and have been associated with good predictive value in several tumor systems.

Flow cytometry is a technique that has been proven valuable for the establishment of cell clonality and for aiding specific cell typing and classification in hematopoietic malignancies. This technique can be utilized with body fluids, tissues, peripheral blood and bone marrow. ►Hematological malignancies, lymphomas and leukemias can be immunophenotyped as to their cell lineage (lymphoid, myeloid, plasma cell) and their degree of differentiation. Flow cytometric analysis can also be used for the evaluation of minimal residual disease in hematopoietic neoplasms. DNA flow cytometric analysis of tissues has also been successfully applied to obtain prognostic information for certain types of tumors, such as breast cancer and ►colon cancer.

Molecular pathology and genetic analysis has had an important impact in the management of cancer patients. The application of molecular cytogenetic analysis of human tumors has acquired an important role in recent years not only for diagnosis but also as a tool for determining the extent of disease, provide prognostic

information, and monitor patients for recurrence. Molecular cytogenetic analysis has elucidated specific chromosomal abnormalities associated with hematopoietic and non-hematopoietic neoplasms. A variety of molecular techniques are applied by pathologists for the study of tumors, including ►polymerase chain reaction (PCR), reverse-transcriptase polymerase chain reaction (RT-PCR), ►Southern blot and ►in-situ hybridization. These techniques allow the detection of gene rearrangements, gene fusion products for chromosomal deletions and oncogene analysis. Traditional cytogenetic metaphase studies using cell culture is being slowly replaced in modern surgical pathology laboratories by interphase cytogenetic analysis by fluorescence in-situ hybridization (FISH).

One of the most common applications of molecular pathology is in the diagnosis of hematolymphoid neoplasms. Diagnosis of T cell lymphomas and B cell ►lymphomas can be aided by the demonstration of clonal ►immunoglobulin gene rearrangement or ►T-cell receptor gene rearrangements. Similarly, demonstration of specific ►chromosomal translocations by a variety of molecular techniques are used for the diagnosis of ►follicular lymphoma [t(14;18)], ►chronic myeloid leukemia [t(9;22)], ►mantle cell lymphoma [t(11;14)], ►anaplastic large cell lymphoma [t(2;5)] and others.

Another area in which molecular pathology has made significant advances has been in the molecular genetic characterization of soft tissue sarcomas. In contrast with most epithelial cancers, many soft tissue sarcomas have shown consistent molecular alterations, particularly chromosomal translocations. Identification of such translocations can be of aid in the diagnosis of these tumors. The majority of such translocations often affect genes involved in coding of transcription factors and results in the creation of a unique fusion product that can be detected by PCR or FISH techniques. These fusion proteins can be assayed in small formalin-fixed paraffin-embedded tissue samples, making molecular diagnosis a viable and useful adjunct for pathologic diagnosis. An example of this is the demonstration of the t(X;18)(p11;q11) in ►synovial sarcoma, which involves the fusion of the SYT gene from chromosome 18 to one or two homologous genes on the X chromosome, SSX1 or SSX2. Identification of the SST/SSX chimeric transcript will help establish the diagnosis of synovial sarcoma. This characteristic translocation does not appear to occur in histologically similar soft tissue sarcomas that enter in the differential diagnosis.

Another family of lesions for which molecular analysis has proved helpful is ►Ewing sarcoma and related tumors, such as primitive neuroectodermal tumor (PNET), peripheral neuroepithelioma and the ►Askin tumor. These tumors share a genetic alteration in which the EWS gene fuses with at least five different ETS genes, most commonly Fli-1 on chromosome 11, producing an ►EWS/Fli-1 fusion protein. A similar pattern has also been detected in several other unrelated soft tissue tumors, such as clear cell sarcoma, desmoplastic small round blue cell tumor, and extraskeletal myxoid chondrosarcoma. In clear cell sarcoma, the EWS gene combines with another transcription factor unrelated to the ETS family, ATF1. In desmoplastic small round cell tumor, the EWS gene recombines with the ►Wilms tumor suppressor gene WT1 resulting in a t(11;22)(p13;q12) with the creation of a characteristic ►EWS fusion protein, EWS/WT1. In extraskeletal myxoid chondrosarcoma, a consistent translocation is identified, t(9;22), resulting in the EWS/CHN fusion protein. Some of these fusion products, however, have also been detected in other unrelated neoplasms, somewhat reducing their specificity. Other types of sarcomas associated with distinctive genetic alterations include alveolar ►rhabdomyosarcoma, which is associated with t(13;2) resulting in a ►Pax/FKHR fusion product; dermatofibrosarcoma protuberans, characterized by t(17;22)(q22;q13); and myxoid and round cell liposarcomas, characterized by t(12;16)(q13;p11).

Another area in which molecular diagnosis has been useful is in the detection of minimal or residual disease, and for the assessment of metastases or recurrence. For example, in Ewing sarcoma, the extent of disease at diagnosis represents one of the most powerful predictors of prognosis. In patients with otherwise localized disease, RT-PCR can identify occult tumor cells in the bone marrow. Detection of the Pax/FKHR fusion product in the bone marrow by RT-PCR has also been used for the staging and monitoring of therapy in patients with alveolar rhabdomyosarcoma. PCR has also been used for the detection of occult epithelial metastatic cancer cells in lymph nodes that were negative for malignant cells by routine histologic examination. The clinical significance of such findings, however, has not yet been determined.

Finally, molecular pathology can also play a role in the assessment of prognosis of malignant tumors. For example, determination of ►mismatch repair in colon cancer, typing of ►human papillomavirus in cervical cancer, ►amplification of *MYCN* in ►neuroblastoma, and p53 overexpression in Wilms tumor are but a few examples of this application to tumor pathology.

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Pathway Addiction

►Oncogene Addiction

Patient Generated Subjective Global Assessment

Definition

PG-SGA; A nutrition screening tool that incorporates patient solicited data with an evaluation by the healthcare team to identify and quantify nutrition risk.

►Nutrition Status

PAX

Definition

Refers to Paired box genes; PAX genes encode nuclear transcription factors that are regarded as major controllers of developmental processes in both vertebrates and invertebrates. Nine members of the family have been described in mammals, with orthologues present in worms, flies, frogs, fish and birds. PAX genes have been implicated in several human cancers on the basis of their expression level, although the mechanistic implications for expression levels in tumorigenesis are not clear. In alveolar rhabdomyosarcoma (ARMS), chromosome translocations involving juxtaposition of *PAX3* or *PAX7* and the forkhead transcription factor gene, *FKHR* (forkhead box M1) are often seen. These translocations are associated with t(2;13) in 55–73% of cases or t(1;13) in 22% of cases, respectively, and yield chimeric *PAX3-FKHR* or *PAX7-FKHR* genes.

PAX5

Definition

Is a member of the paired box (PAX) family of transcription factors, encodes the B-cell lineage specific activator protein (BSAP) that is expressed at early stages of B-cell differentiation. It is also expressed in developing central nervous system and testes. Alterations of the gene contribute to the neoplastic transformation of B-cell progenitors.

►Acute Lymphoblastic Leukemia

Paxillin

Definition

Is an adaptor protein that is found mainly in sites of integrin-based cell substrate contacts (termed focal adhesions). It plays a role in the turnover of cell-substratum adhesions during motility. It is also present in invadopodia.

►Cortactin

PBPC

Definition

Peripheral blood progenitor cells obtained from peripheral blood by apheresis after mobilizing stem cells by administration of hematopoietic growth factors.

►Ewing Sarcoma

PBX

Definition

The Pre-B cell leukemia transcription factor family (PBX1-3) belongs to the TALE (three amino acid loop extension)-class of homeodomain protein and has a wide functional diversity during development. PBX is

required for hematopoiesis as well as for multiple developmental processes such as skeletal patterning and organogenesis. A conserved YPWM motif in the HOX protein is necessary for cooperative binding with PBX. It has furthermore been shown that PBX1 functions as a HOX cofactor during development. A and B-like HOX proteins form trimeric DNA-binding complexes with PBX and TALE superclass proteins MEIS1A and MEIS1B. The DNA binding affinity and sequence selectivity of HOX homeodomain proteins are increased by the formation of cooperative complexes with the PBX homeodomain protein. A conserved YPWM motif in the HOX protein is necessary for cooperative binding with PBX. More recent data suggest that PBX1 may act even more broadly by modulating the activity of non-homeodomain transcription factors.

►NUP98-HOXA9 Fusion

PCNA

Definition

Proliferating cell nuclear antigen. An accessory protein of the major replication enzymes DNA polymerase δ and γ that is required for DNA synthesis. Commonly used as an indicator of active cell proliferation.

►p21(Waf1/Cip1/Sdi1)

PCP

Definition

Planar cell polarity; the organization of cells within the plane of a single layered sheet of cells.

►Wnt Signaling

PCR

Definition

Polymerase chain reaction; Molecular biological enzymatic technique to amplify DNA sequences from few copies to easily detectable quantities by repeated cycles of melting the DNA double helix, annealing synthetic oligonucleotides complementary to the ends of the DNA sequence to be amplified, and de-novo DNA synthesis by a heat-stable polymerase using the annealed oligonucleotides as anchors.

PCSK3

►Furin

PCT

Definition

►Plasmacytomas.

PD-ECGF

►Thymidine Phosphorylase

PDCD4/Pdcd4

►Programmed Cell Death 4

PDD

Definition

Photodynamic diagnosis; Synonym ►fluorescence diagnostics; Is a technique that uses photosensitizers to produce visible fluorescence as blue, green, or red depending on the photosensitizer. The visual contrast between normal and malignant tissues under examination is enhanced, allowing for easy detection of flat

neoplastic lesions such as carcinoma in situ which would otherwise not be picked up using conventional white-light endoscopy.

►Hypericin

PDF

►MIC-1

PDGF

Definition

►Platelet-derived growth factor.

PDGFR

Definition

►Platelet-derived growth factor receptor
►PDGF.

PDK1

Definition

phosphoinositide-dependent kinase-1; Is a master protein kinase that activates other kinases (e.g., PKB/Akt, SGK) by phosphorylation thereby controlling multiple signaling pathways.

►BCL3

PDMP

Definition

D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, a cationic ceramide analog that inhibits the ►glycosylation of ►ceramide (to form glucosylceramide), thereby forcing accumulation of ceramide and sphingomyelin. It also inhibits the conversion of glucosylceramide to GalGlcCer and the conversion of the latter to α-GalGalGlcCer (globotriaosyl ceramide) and ganglioside GM3 (NeuAcGalGlcCer). The enzyme that cleaves the ceramide–glucose linkage in GSLs (ceramide glycanase) and the enzyme that transfers fatty acid from phosphatidylcholine and phosphatidylethanolamine to form fatty acyl-1-O-ceramide (lysosomal phospholipase A_2) are also inhibited by PDMP. All of these effects add up to increased levels of ceramide.

►Sphingolipid Metabolism
►Glycobiology

PDZ Domain

Definition

Domain named after the three proteins PSD-95, Dlg and ZO-1. Is a modular protein interaction domain that often occurs in scaffolding proteins that bind in a sequence-specific fashion to the C-terminal peptide sequence or at times the internal protein sequences of target proteins. These domains of approximately 90 amino acids are known by an acronym of the first three PDZ-containing proteins identified including the postsynaptic protein PSD-95/SAP90, the Drosophila septate junction protein Discs-large, and tight junction protein ZO-1. In addition to their interaction with membrane proteins and receptors, the PDZ domain-containing proteins also interact with F-actin cytoskeleton through ezrin–radixin–moesin (ERM) proteins

►Calcitonin

PDZ Ligand

Definition

A small peptide sequence (4–6 amino acids) that binds to ►PDZ domain, which are peptide-binding modules, and are usually embedded in large scaffolding proteins along with additional PDZ domains and/or peptide-binding modules. PDZ domains function to assemble their ligands into large complexes with other scaffold-associated molecules

►Calcitonin

PEA3

Definition

PEA3, ER81 and ERM form the PEA3 subfamily of ►ETS transcription factors. PEA3 activates gene expression and is thought to play an important role in promoting tumor metastasis.

►Lipoma Preferred Partner

PEBP2αB

►Runx1

PEComas

Definition

►Neoplasms with Perivascular Epithelioid Cell Differentiation.

PEG

Definition

Polyethylene glycol.

►Microcell-Mediated Chromosome Transfer

Pegylated Interferons

Definition

Are interferons that are chemically modified by the covalent attachment of poly-ethylene glycol (►PEG), which are used in antiviral therapies.

►Hepatitis C Virus

Pegylation

Definition

Is the act of covalently coupling a polyethylene glycol (►PEG) structure to another larger molecule, for example, a therapeutic protein (which is then referred to as Pegylated).

►Arginine-Depleting Enzyme Arginine Deiminase (ADI)

Pemphigus

Definition

A rare, grave skin disease of unknown cause characterized by the development of bullae on the skin and mucous membrane.

►Rituximab

Penetrance

Definition

Is a genetic term that describes the proportion of individuals with a specific genotype who express that character in the phenotype; the frequency with which a genetic trait is expressed phenotypically. The penetrance is often reduced for example, if by age 70, a disease had occurred in 60% of carriers of a particular mutation in gene, the penetrance would be 60% at age 70.

►APC Gene in Familial Adenomatous Polyposis

Pentakisphosphate

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Definition

Inositol pentakisphosphate (abbreviated IP_5) is a molecule derived from inositol containing five phosphate groups and possessing anti-cancer properties.

Characteristics

Inositol Phosphates Family

Inositol is a polycyclic polyalcohol whose structure forms the backbone for all ►inositol polyphosphates (IPs) and ►phosphatidylinositol lipids (phospholipids). Over 30 different inositol polyphosphates have been identified so far in mammalian cells yet, despite their importance, the majority of these remain completely uncharacterized.

Many inositol derivatives play a key role in eukaryotic cells and are involved in a large number of biochemical processes mostly acting as second messengers in intracellular signaling. The best example is represented by ►inositol 1,4,5-trisphosphate (IP_3), which plays a very important role in many signaling pathways and also serves as a precursor for the synthesis of several highly phosphorylated inositol polyphosphates such as ►inositol tetrakisphosphate (IP_4), inositol pentakisphosphate (IP_5), and ►inositol hexakisphosphate (IP_6), also known as phytic acid.

Inositol Pentakisphosphate (IP_5)

IP_5 is a derivative of IP_3 possessing two additional phosphate groups. It is present in nearly all eukaryotic cells at levels of 15–50 μM and can be generated through phosphorylation of IP_4 or dephosphorylation of IP_6. The intracellular distribution of IP_5 is, to date, unknown although the fact that the inositol polyphosphate kinases (the enzymes responsible for the synthesis of IP_5 and IP_6) are localized in the nucleus may suggest that these inositol polyphosphates accumulate in the nuclear compartment. IP_5 is present at a very high concentration in several foods, including cereals, beans and nuts. For example IP_5 concentrations range from 0.4 g/kg in corn to 1.6 g/kg in millet and from 0.1 to 1.3 g/kg in beans. High levels of IP_5 have also been found in nuts, in particular in cashews (1.5 g/kg) and peanuts (0.6 g/kg).

IP_5 Intracellular Functions

The intracellular functions of IP_5 are just beginning to be appreciated. It has been shown that IP_5 can modulate the human immunodeficiency virus (HIV) type 1 and Gag protein assembly and therefore IP_5 may contribute to the pathogenicity of HIV-1 infection.

IP_5 has also been identified as essential in the regulation of chromatin remodeling and for transcription of some phosphate-responsive genes, possibly by affecting the ability of these complexes to interact with specific molecules and/or chromatin. One report illustrates a novel pathway of modulation of vascular L-type voltage-gated Ca^{2+} channels, in which IPs (mainly IP_5 and IP_6) help in their activation. IP_5 is also known to be involved in vascular L-type Ca^{2+} channel activation via stimulation of the protein kinase C pathway. These results indicate that IP_5 may act as intracellular messengers in modulating L-type Ca^{2+} channel activity and so could be implicated in mediator-induced contractions of vascular smooth muscle cells.

More evidence for the role of IP_5 as second messenger derives from genetic studies in mice. Deletion of *Ipk2*, a gene that encodes an inositol polyphosphate kinase enzyme, whose function includes the conversion of IP_3 and IP_4 to IP_5 and IP_6, is required for proper development of the mammalian embryo.

IP_5 Anti-Cancer Properties

The use of IPs as potential anti-cancer agents has been supported by several studies indicating that IP_6 possesses anti-tumor activity *in vitro* and *in vivo*. However the very high concentrations required for IP_6 to be active (1–5 mM) suggest a lack of selectivity of this compound, although it is noteworthy that, even at these concentrations, IPs do not appear to have any toxic effects. Little is known about the mechanisms by which IP_6 exerts its anti-cancer actions. The recent observation that IP_6, when taken up into cancer cells, becomes dephosphorylated to lower IP forms (mainly IP_5) and that in these cells IP_5 is more active than IP_6, strongly suggests that the anti-cancer activity of IP_6 is due to its dephosphorylation to IP_5. Indeed, increasing evidence indicates that IP_5 itself possesses anti-tumor properties. It has been clearly shown that IP_5 significantly reduces growth of ovarian cancer cells implanted in mice. Strikingly, the efficiency of IP_5 in these experiments was comparable to cisplatin, the most commonly used drug for ovarian cancer treatment. Data indicates that the anti-tumor activity of IP_5 is mostly due to its pro-apoptotic properties (i.e. it promotes death of cancer cells) combined with an anti-angiogenic activity (i.e. it inhibits formation of new blood vessels therefore blocking the supply of nutrients and oxygen to cancer cells).

Mechanism of Action of IP_5

It is now well established that the enzyme ►phosphoinositide 3-kinase (PI3K) is a key mediator of tumor development and progression. This enzyme catalyses the phosphorylation of position 3 in the inositol ring of phospholipids leading to formation of 3-phosphorylated compounds, among which the trisphosphate ►phosphatidylinositol-3,4,5-trisphosphate (PIP_3) is the most studied. PIP_3 activates several proteins that in turn regulate many intracellular processes crucial for cell survival, proliferation, migration etc. Key targets of PIP_3 action are the enzymes ►phosphoinositide-dependent kinase-1 (PDK1) and ►protein kinase B (PKB) also known as ►Akt. In normal conditions the PIP_3-dependent activation of such enzymes is time-restricted and specific regulators turn off the signal therefore processes like proliferation and migration are tightly regulated and

limited in time. Dysregulation of this signal is often observed in cancer cells, mostly due to a constitutive activation of PI3K/generation of PIP_3 or loss of the enzymes responsible for "switching off" PIP_3. As a consequence there is a permanent activation of Akt (amongst other enzymes) and processes like proliferation and migration remain activated.

The mechanism of PDK1/Akt activation downstream of PI3K activation is based on a physical interaction between a region in both PDK1 and Akt known as the ►pleckstrin homology domain (PH domain) and PIP_3. PH-domain containing proteins often bind both inositol phosphates and inositol lipids, and the subcellular distribution of these proteins can be affected by changes in the degree of competition between the two classes of ligands.

Being a phospholipid, PIP_3 possesses a lipid structure enabling it to be inserted into the cellular membrane. As a consequence its interaction with the PH domains of PDK1 and Akt triggers the relocation of both enzymes to the cell surface where Akt can be activated. IP_5 possesses the core structure necessary for binding PDK1 and Akt PH domains but it lacks the membrane anchor. Because of this, IP_5 remains in the cytosol and, by interacting with PDK1 and Akt, inhibits their relocation to the membrane and therefore Akt activation.

Such a mechanism of action is supported by data indicating that IP_5 is able to selectively inhibit Akt activity in cancer cell lines whereas other inositol phosphates tested have no effect. Data confirm a strict correlation between the IP_5-dependent pro-apoptotic rate and the degree of inhibition of Akt signaling. In fact, the specificity of IP_5 activity is confirmed by evidence indicating that this compound is active in human cancer cell lines characterized by an elevated PI3K/Akt activity, whereas it is inactive in other cell lines that do not possess such increases in Akt activation. More importantly, a complete inhibition of Akt activation has been observed in IP_5-treated mice demonstrating that IP_5 can inhibit Akt activation *in vivo* and block tumor growth. These data therefore indicate that IP_5 is a very specific inhibitor of this pathway both *in vitro* and *in vivo*.

It is of note that the derangement of this pathway is believed to be involved in the molecular mechanisms underlying *Salmonella* pathogenicity. When *Salmonella*, an important enteric pathogen of humans, invades cells, this leads to the rapid depletion of the cellular IP_5 pool. It has been proposed that the loss of cellular IP_5 may underlie the ability of *Salmonella* to activate Akt and promote cell survival during infection.

Clinical Perspective of IP_5

As discussed above, dysregulation of PI3K-dependent function (mostly the PI3K/Akt pathway) is common in cancer therefore PI3K is a key target for development of novel anti-cancer drugs. Being such a critical enzyme in many different cellular functions it should not be surprising that strategies based on a blockade of PI3K itself have resulted in many toxic effects. At the moment therefore, there is an urgent need to find specific and selective inhibitors of PI3K signaling that block specific downstream targets without interfering with other PI3K-mediated signals.

In this respect, the specific action on Akt of IP_5 might prove beneficial in reducing toxic side effects. Indeed, even at concentrations ten times higher than those used in the *in vivo* experiments (50 mg/kg) IP_5 did not appear to have any toxic effects. These properties, together with the fact that IP_5 is a water-soluble, natural compound, suggest that IP_5 may overcome problems concerning solubility, chemical stability and toxicity that limit the use of other potential inhibitors of the PI3K/Akt pathway.

IP_5 has also been shown to sensitize breast, ovarian and lung cancer cell lines to other, commonly used anti-cancer drugs. For example, IP_5 enhances the pro-apoptotic effect of cisplatin in ovarian cancer cells and etoposide in lung cancer cells. These data suggest that combination therapy of IP_5 with other commonly used drugs might enhance their activity. This would ultimately prove beneficial in terms of lowering the toxic effects of these drugs and the cost of their use.

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PEP Syndrome

Definition

►POEMS Syndrome.

Peptide

Definition

A compound that is made up of two or more amino acids joined by covalent bonds.

►Modular Transporters

Peptide Vaccines for Cancer

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Synonyms

Active immunization using synthetic peptides corresponding to T cell epitopes.

Definition

An immunogen composed of synthetic peptides (small protein sequences) administered with the intent of eliciting antitumor immune responses for treating cancer.

Characteristics

Principles of Antitumor Immunity

The immune system has the capacity to recognize tumor cells and in many instances this recognition results in antitumor effects such as slowing the tumor growth or tumor eradication. In view of this, many approaches have been made to develop ►cancer vaccines, and other modes of ►immunotherapy. ►Cytotoxic T lymphocytes (CTL) are considered the most effective elements of the immune system that can kill tumor cells. CTL recognize small fragments (peptides of 8–10 residues) derived from the proteolytic processing of a tumor antigen. These peptides are presented to the CTL antigen receptor in the context of products of the class I ►major histocompatibility gene complex (►MHC) known as ►HLA class I in humans. Recognition of peptide/MHC class I complexes that are expressed on the surface of antigen-presenting cells leads to CTL activation and subsequently to an effector antitumor function such as cytotoxicity or the production of ►lymphokines that have antitumor effects. Because the MHC is highly polymorphic (i.e., there are many alleles within a species), a particular peptide may only bind to one MHC allele (i.e., MHC restriction). Effective CTL responses require the participation of ►T helper lymphocytes that recognize slightly larger peptides (12–15 residues), which associate with MHC class II molecules. Helper T lymphocytes can augment CTL activity by increasing their capacity to proliferate and by facilitating the generation of memory CTL responses that will prevent tumor recurrences. In view of this, there is a general consensus that vaccination strategies against cancer should attempt to concurrently stimulate tumor-reactive CTL and T helper cells.

Tumor Antigens for T Lymphocytes

Almost any protein expressed by a tumor cell (or by a normal cell) will generate MHC-binding peptides, but only some peptides can be recognized by tumor-reactive CTL and T helper lymphocytes. Multiple mechanisms involved in the development of immune tolerance operate to prevent the induction of T cell responses against normal cells, which would lead to autoimmune pathology. However, the immune system has the capacity to detect alterations that take place on cancer cells. There are many types of alterations that take place in cancer cells that lead to the generation of peptides that can be recognized as tumor antigens by T lymphocytes. Proteins derived from products of ►oncogenic viruses such as ►human papillomaviruses, ►Epstein–Barr virus, ►human T cell leukemia virus or products of mutated genes could generate MHC-binding peptides that would function as tumor-specific antigens (TSA). In these circumstances, no immune tolerance or potential autoimmunity will exist because these peptides are absent in normal cells. However, in many instances tumor-reactive T lymphocytes can recognize peptides that are derived from proteins that are also present in normal cells. These tumor-associated antigens (TAA) can be the products of overexpressed genes (e.g., ►HER-2/neu, ►CEA, ►p53 protein, ►biological and clinical aspects), developmental genes such as ►oncofetal antigen, members of the cancer-testes gene family such as ►MAGE, ►GAGE proteins, NY-ESO-1, which have been identified using techniques such as ►SEREX, or tissue differentiation proteins such as ►prostate specific antigen (PSA) and ►prostate-specific membrane antigen (PSMA) both applicable for prostate cancer and various ►melanoma antigens (gp100. MelanA/MART1, tyrosinase), many of which have already been used in developing a ►melanoma vaccine. Both TSA and TAA are considered as candidates for developing antitumor vaccines and at the present time there is no final word of which one will generate more effective antitumor responses.

Selection of Peptides for Vaccination

CTL and T helper peptide epitopes have been identified through two main strategies. The first approach relies in the isolation of tumor reactive T cells from cancer patients that are used to screen cDNA expression libraries or peptide fractions eluted from MHC

molecules both prepared from tumor cells recognized by the T cells. The second strategy is a predictive approach commonly known as "reverse immunology" where a putative TSA or TAA of known amino acid sequence is analyzed for the presence of peptides capable of binding to specific MHC molecules. Several peptide/MHC binding algorithms have been constructed to predict the likelihood of a peptide to bind to a particular MHC allele based on the presence of conserved MHC binding anchors situated at specific positions within the peptide sequence (motifs). These algorithms are readily available to investigators through the Internet, where the amino acid sequence of a potential TAA/TSA can be analyzed for the existence of peptides with high MHC binding predictive scores to one or several alleles. Performing peptide-MHC binding assays can help confirm the results from these algorithms and narrow down the number of potential T cell epitopes. The last step of the predictive approach is to assess whether the predicted MHC binding peptides function as epitopes for tumor-reactive T cells. Synthetic peptides representing the predicted epitopes are prepared by organic chemistry methodologies and are tested for their capacity to stimulate T cell responses *in vitro* using lymphocytes from peripheral blood of normal individuals. In this process, the naïve T lymphocytes are "vaccinated" *in vitro* with peptide-pulsed autologous ►antigen-presenting cells (APCs) to generate CTL (or T helper) antigen-specific cell lines that can be propagated in tissue culture. These peptide-reactive T cells are then tested for their ability to recognize tumor cells that express the corresponding TAA/TSA (from where the peptide sequence was first generated). Recognition of tumor cells by the peptide-reactive T cell lines validates that the tumor cells through natural antigen- processing pathways generate the MHC binding peptide, and this serves as the final and most significant selection criteria for a peptide to be used as a vaccine. This information can also be valuable for the design of ►DNA vaccines.

Immunization Strategies Using Synthetic Peptides

Antigen stimulation of naïve T cells can lead to either activation (proliferation and differentiation into effector cells) or tolerance (lack of function and cell death). The outcome of the T cell response is dictated in great part by the type of APCs and its activation status. The most potent type of APCs are ►dendritic cells (DCs), which have the unique functions of harvesting antigens in peripheral organs (skin, gut, lungs), processing the antigens into MHC-binding peptides and migrating into secondary lymphoid organs (lymph nodes, spleen) where they present the antigen to naïve T lymphocytes. When DCs receive proinflammatory signals they become stimulatory APCs, while DCs not receiving these signals will behave as a toleragenic APCs. The proinflammatory signals that activate DCs can be in the form of cytokines (interleukin-1 and tumor necrosis factor) or molecules that function as ligands for any member of the ►toll-like receptors (TLR) family. The most common type of TLR ligands are pathogen-associated molecular patterns (PAMPS) commonly found in infectious agents, such as double stranded ribonucleic acid (dsRNA), lipopolysaccharide (LPS) and prokaryotic DNA containing unmethylated cytosine-guanine motifs (CpG). Many immune cells including DCs express TLRs and through their interaction with TLR ligands, the cells will become activated and functional. Thus, to generate an effective immune response against a TAA, peptides must be combined with a proinflammatory signal such as a TLR ligand. For example, synthetic polynucleotides such as deoxyoligonucleotides containing CpG motifs, or double strand polyinosinic-polycytidylic acid (poly-I:C), which stimulate TLR-9 and TLR-3, respectively are ideal candidates to be used as ►immune adjuvants for antitumor vaccines based on peptide T cell epitopes. Immunization using synthetic peptides with the aim of stimulating antitumor T cell responses can be achieved through two different methods. For the first approach, a cell-based vaccine is generated using autologous APCs such as DCs or macrophages that are pulsed *ex vivo* with synthetic peptides. In some instances, the APCs can be stimulated (or matured) with proinflammatory factors before they are administered into the patients. The second approach is to directly administer the synthetic peptide (together with an adjuvant) to the patient via an injection, which most frequently is done through a subcutaneous or an intradermal route. Direct administration of peptide vaccines can also be accomplished in a "needleless" fashion, through a transcutaneous route using a topical vaccine formulation that may include immune adjuvants.

The main advantage of APC-based peptide vaccines is that the T cell epitope is efficiently delivered onto the APCs, presumably decreasing the possibility of peptides being presented to naïve T cells by nonactivated APCs, which could generate T cell tolerance. The main disadvantage of the cell-based vaccines is their high cost associated to amount of labor required to produce the autologous APCs suitable for human use. On the other hand, the direct administration of peptides via an injection or topically, may not be an efficient way to deliver the epitopes to APCs but it is certainly more cost effective than cell-based vaccines. Using mouse tumor model systems both vaccination approaches have shown to induce strong T cell responses that translate into antitumor effects. However, at the present time neither peptide-pulsed cell-based vaccines nor direct peptide vaccination have yielded conclusive positive results in the clinic. Among the possible explanations for these divergent observations are that many of the mouse tumor models are not truly representative of human disease and that the vaccines are usually

tested in mice at earlier disease stages as compared to the clinical studies done in humans, where most of the patients are in advanced stages of cancer.

Remaining Challenges for Developing Effective Antitumor Peptide Vaccines

Without any doubt the overall clinical performance of most therapeutic cancer vaccines including those composed of synthetic peptides remains far from optimal. In first place, the strength of immune responses, measured by the percentage or total numbers of CTL induced by most antitumor peptide vaccines is in reality meager as compared to the intensity of T cell responses observed during the organism's successful battle against infections. Many factors will contribute towards an effective expansion of T cells during an immune response such as the duration of the antigenic stimuli. For instance, peptides that are rapidly cleared by the organism may not be able to sustain the T cell activation level leading to successful expansion, while peptides that remain for extended periods of time may generate T cell exhaustion or tolerance. Successful T cell expansion by peptide vaccines will also depend on presence of APC-derived costimulatory signals that are either expressed on the cell surface (CD80, CD86, CD70, CD137L, CD252) or are in the form of ►lymphokines (interleukin-12, type I interferon). As mentioned above, concurrent activation of helper T cells will also contribute towards the expansion of CTL and will enhance the generation of memory CTL responses. A successful peptide vaccination strategy must also deal with the inhibitory signals that the immune system utilizes to prevent or terminate CTL responses. For example, ►T regulatory cells that normally function to prevent autoimmune diseases are also known to inhibit antitumor CTL responses. In addition, several molecular interactions such as those derived from the stimulation of CTLA-4 and PD-1 on CTLs will lead to inhibitory signals that will arrest CTL expansion and effector function. The successful design of a peptide vaccination strategy leading to the generation of large numbers of effector T lymphocytes may not be sufficient by itself to ensure a clinical benefit against cancer. Unfortunately, tumors count on many defensive mechanisms against the immune system. In some instances tumors may cease to express the TSA/TAA or MHC molecules and will no longer be recognized by the CTLs. Tumors may also generate an inhospitable microenvironment by producing T-cell inhibitory substances such as transforming growth factor-beta (TGF-β) and inteleukin-10, or by recruiting immune suppressor cells like regulatory T lymphocytes and myeloid derived suppressor cells. Thus, the likelihood of an immunogenic peptide vaccine to deliver the desired antitumor effectiveness will depend in great part in the patient's disease stage, since advanced tumor burden will be accompanied by more immune suppressive activity. In view of these issues, it seems logical that peptide vaccines should be used in patients with minimal residual disease and perhaps in the adjuvant setting, combined with other therapies that would reduce tumor burden. Even if these issues are solved, one major limitation for the use of peptide vaccines is due to fact that each peptide epitope will only offer coverage to subset of patients that have the corresponding MHC allele and have tumors that express appropriate TSA/TAA. Future polyepitope peptide vaccination strategies either using peptide cocktails or multiepitope peptide constructs will lead to broadening patient population coverage.

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Peptidomimetic

Definition

Is a compound that mimics the biological action of a peptid. It is a molecule containing non-peptidic structural elements and is capable of mimicking or antagonizing the biological action(s) of a natural parent peptide. These modifications involve changes to the peptide that will not occur naturally, such as altered backbones and the incorporation of nonnatural amino acids that are not recognized by cellular degradation systems. A peptidomimetic does not longer have classical peptide characteristics such as peptidic bonds that can be degraded by cellular enzymatic activities. This approach is important in ►drug design aiming to develop anticancer drugs that are not rapidly degraded by the organism and that specifically bind to target in the cell.

►Anti-HER2/Neu Peptide Mimetic (AHNP)

Perforin

Definition
Perforin is a protein that can polymerize to form the membrane pores that are an important part of the killing mechanism in cell-mediated cytotoxicity. Perforin is produced by cytotoxic T cells and natural killer cells and is stored in granules that are released by the cell when it contacts a specific target cell.

►Natural Killer Cell Activation
►Immunoediting
►Cystatins

Performance Status

Definition
An index that measures the overall health status of a patient as defined by his/her ability to perform ordinary tasks and activities of daily living.

(Peri-) Hilar Cholangiocarcinoma

►Klatskin Tumors

Pericardium

Definition
Membranous sac surrounding the heart.

Pericellular Matrix

Definition
The zone immediately surrounding a cell, and the interface between the plasma membrane and extracellular matrix. The site where integral membrane proteins can interact with the meshwork of proteoglycans, glycolipids and hyaluronan immediately surrounding a cell.

►Hyaluronan Synthases

Pericyte

Definition
A mesenchymal-like cell, associated with the walls of small blood vessels. As a relatively undifferentiated cell, it serves to support these vessels, but it can differentiate into a fibroblast, smooth muscle cell, or macrophage as well if required. Specialized support cells that are precursors to smooth muscle cells and coat blood vessels and some portions of lymphatic vessels.

►Lymphangiogenesis
►Lymphatic Vessels
►Vascular Disrupting Agents

Periodontium

Definition
Tooth-supporting tissue consisting of bone, periodontal ligament, cementum and gingiva.

►Dental Pulp Neoplasms

Periostin

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Synonyms
Osteoblast-specific factor-2 (Osf2)

Definition

Periostin, originally named as osteoblast-specific factor-2 (Osf2) (genebank D13664), first identified in bone, is implicated in regulating ►adhesion and differentiation of osteoblasts. Periostin is a secreted protein that shares a structural homology to the axon guidance protein ►fasciclin I (FAS1) in insects. Proteins that share homology with FAS1 include βig-h3, stablin I and II, MBP-70, Algal-CAM, Periostin, and Periostin-like factor (PLF). An approximately 90 kDa periostin has an NH_2-terminal secretory signal peptide, followed by a cysteine-rich domain, four internal homologous repeats, and a COOH-terminal hydrophilic domain. In N-terminus, periostin has an ►EMI domain, which is a small cysteine-rich module of approximately 75 amino acids. Periostin binds to αvβ3, αvβ5, and α6β4 ►integrins, ►fibronection, ►tenacin-C, collagen V, and periostin itself. Mouse and human *periostin* gene (POSTN) share 89.2% amino acid identity overall and 90.1% identity in their mature forms. The human periostin gene is located on chromosome13q.

Characteristics

Periostin expression is observed in a wide range of normal adult tissues, including aorta, thyroid, lung, breast, stomach, colon, placenta, uterus, vagina, ovary, testis, and prostate. Periostin overexpression is found in various types of human ►cancer including ►neuroblastoma, head and neck cancer, ►nasopharygeal carcinoma, thyroid carcinoma, ►non-small cell lung carcinoma, ►breast cancer, ►colon cancer, pancreatic ductal adenocarcinoma, and ►ovarian cancer. In addition, elevated levels of periostin are detected in sera of patients with thymoma, non-small cell lung carcinoma, breast cancer, and pancreatic ductal adenocarcinoma, and in ►ascites from ovarian cancer patients. Overexpression or elevated serum levels of periostin appear to be associated with poorer prognosis of various cancers. Although the detailed function of periostin is still unclear, it plays important roles for tumor progression including ►invasion, ►angiogenesis, cellular survival, and ►metastasis. In vivo studies revealed that periostin is associated with a phenotype of greatly accelerated tumor metastatic growth through invasiveness, cancer cell survival, and angiogenesis by using the animal model system of metastasis.

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Peripheral Lymph Node Addressin Molecule

Definition

PNAd; A family of sialomucins expressed on high endothelial venules and serving as a receptor for L-selectin mediated homing of lymphocytes to peripheral lymph nodes.

►Omental Immune Aggregates

Peripheral Lymphoid Organs

Definition

►Secondary Lymphoid Organs.

Peripheral Membrane Proteins

Definition

Synonym: extrinsic membrane proteins or amphitropic proteins. They bind reversibly to membranes through lipid co/post-translational modifications or through specific amino acid regions that provide the right context for electrostatic and/or hydrophobic interactions.

They are relevant in the context of signal transduction and cellular physiology as they can propagate messages from the plasma membrane to intracellular membranous or aqueous compartments.

►Membrane-Lipid Therapy

Peripheral Nervous System

Definition

The part of the nervous system that lies outside of the brain and spinal cord, and serves the limbs and organs.

►Synuclein
►Neuroblastoma

Peripheral Neurofibromatosis

Definition

►Neurofibromatosis 1
►Neurofibromatosis Type 2
►Neurodibromatosis 2

Peripheral Neuropathy

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Synonyms

Neuropathy; Mononeuropathy; Plexopathy; Polyneuropathy

Definition

Peripheral ►neuropathy is a general term indicating the malfunction of peripheral nerves due to various causes. Peripheral neuropathies can be categorized by location (distal, proximal), distribution pattern (unilateral, bilateral, symmetrical), underlying cause (toxic, metabolic, vascular, autoimmune, paraneoplastic), impaired neuronal quality (motor, sensory, autonomic), dynamics of manifestation (acute, subacute, chronic) and underlying histopathology (axono-, myelino- and ganglionopathies).

Characteristics

Depending on the type of peripheral neuropathy and the impaired neuronal qualities, sensory, motor and autonomic symptoms may appear. Sensory symptoms may include ►paraesthesia, numbness, tingling, burning and pain while motor symptoms may range from mild muscular weakness to paralysis. Appearance of orthostatic hypotension, cardiac arrhythmia and paralytic ileus may reflect autonomic involvement. Diagnosis is usually made based on clinical examination. This includes evaluation of the vibration sense by the tuning-fork test, deep tendon reflexes, muscle strength and sensory symptoms. Additional neurophysiologic evaluations such as measurements of distal latencies, conduction velocities and amplitudes improve diagnostic sensitivity. Nerve biopsies, usually obtained from the sural nerve, provide a further but rather invasive diagnostic tool. Novel approaches include the examination of intraepidermal fibers in skin biopsies and magnetic resonance imaging (MRI) of the spinal cord dorsal columns to detect ganglionopathies.

In cancer patients ►mononeuropathies and ►plexopathies are often caused by local tumor-associated compression while ►polyneuropathies are frequently caused by the systemic anti-tumor treatment itself. These treatment-related, toxic peripheral neuropathies need to be distinguished from other forms such as pre-existing neuropathy or ►paraneoplastic neuropathy. Furthermore, overlaps between different forms of peripheral neuropathy can be observed frequently, especially in elderly cancer patients with additional metabolic disorders.

Mononeuropathies and Plexopathies

Local tumor or metastatic growth infiltrating or compressing peripheral nerves can cause neuronal malfunctions and pain in a circumscribed area. Diagnosis is usually established clinically but may be supplemented by computed tomography (CT) and magnetic resonance imaging (MRI). Treatment options include systemic anti-tumor therapy and from case to case local surgical or radiotherapeutical intervention.

Paraneoplastic Neuropathies

Mild forms of mixed sensory and motor peripheral neuropathies can be observed among many patients suffering from cancer and are often attributable to weight loss, nutrition deficits, organ failure or disease progression. True paraneoplastic syndromes of the peripheral nervous system are rather rare but represent an important differential diagnosis as neurological symptoms my precede those of the tumor. Paraneoplastic neuropathies

Peripheral Neuropathy. Table 1 Typical neuropathic symptoms associated with the application of selected, broadly applied chemo therapentic agents

►Cisplatin, Carboplatin	Predominantly sensory neuropathy; large sensory fibers are affected; impaired vibration and proprioception; carboplatin is considered less neurotoxic than cisplatin
►Oxaliplatin	Two forms of peripheral neurotoxicity:
	►1. *Acute*: occurs in a majority of the patients; sensory neuropathy; causes paraesthesia; may involve mouth and throat; appears within minutes to days; usually self-limiting; often triggered by cold temperatures
	►2. *Chronic*: cumulative, dose-limiting sensory neuropathy
►Paclitaxel, ►Docetaxel	Cumulative, dose-limiting sensory neuropathy; usually causes numbness, paraesthesia, tingling and burning in a symmetrical stocking-and-glove distribution; rarely causes motor neuropathy with mild distal weakness; autonomic involvement possible; incidence increased with dose-dense schedules; presumably combined axono-, myelino- and ganglionopathy; docetaxel is considered less neurotoxic than paclitaxel
►Thalidomide	Sensory peripheral neuropathy; distal paraesthesia with or without sensory loss; may be dose-related; usually late onset
►Vincristine, ►Vinorelbine	Predominantly cumulative sensory neuropathy; dose-limiting for vincristine; impaired or lost pain and temperature sensation; stocking-and-glove distribution; may cause motor and autonomic neuropathy (paralytic ileus, prophylaxis with stool softeners suggested)

can appear in various cancer types including lung cancer, ►malignant ►lymphoma and ►multiple myeloma. The majority of cases have been described in patients with small cell lung cancer presenting a severe sensory loss attributable to a dorsal root ganglionitis. In some of these patients' sera anti-onconeuronal (►anti-Hu) antibodies can be detected. A paraneoplastic brachial neuritis and an acute rapidly progressive sensorimotor ►polyneuropathy have been described in patients with ►Hodgkin disease. Treatment of paraneoplastic neuropathies requires systemic anti-tumor therapy of the underlying disease. However, rapidly progressive sensorimotor ►polyneuropathies may require plasma exchange and immunoglobulin infusions.

Toxic, Treatment-Related Neuropathies

Development of peripheral neuropathy following systemic chemotherapy can be frequently observed in cancer patients. The majority of patients receiving neurotoxic chemotherapeutics are prone to developing cumulative ►polyneuropathies. The exact incidence is presumably underestimated and varies significantly depending on the used chemotherapeutic regimens. The specific characteristics of broadly applied chemotherapeutic agents known to cause peripheral neuropathies are listed in Table 1.

Therapy for peripheral neuropathy is limited und includes symptomatic treatment with tricyclic antidepressants and anticonvulsants to control the neuropathic pain. The tricyclic antidepressant nortryptiline, the norepinephrine-and-serotonin-reuptake-inhibitor venlafaxine and the anticonvulsant gabapentin, have shown moderate clinical activity. Unfortunately, dose reduction of the chemotherapeutic agent and treatment interruptions are associated with the risk of losing therapeutic efficacy. Several neuroprotective agents such as acetyl-L-carnitine and ►amifostine are under evaluation. Their clinical value needs to be determined in further trials. Taken together, a close monitoring of all patients receiving neurotoxic substances is suggested, especially in the palliative setting where quality-of-life can be significantly compromised by severe peripheral neuropathy.

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Peripheral Primitive Neuroectodermal Tumor

►Ewing Sarcoma

Peritoneal Carcinomatosis

►Carcinomatosis

Peritoneal Debulking Surgery

Definition

Synonym: Cytoreductive Surgery; Refers to an operation to remove as much tumour or tumour cells as possible from the peritoneal cavity. This may involve removing all or a portion of tumour-infiltrated peritoneal organs as well e.g. omentum, uterus, ovaries, appendix and portions of bowel.

►Transcoelomic Metastasis

Peritoneal Dissemination

Definition

The spread of cancer from the primary site within the appendix or other intraabdominal organ to the peritoneal surfaces. Intraabdominal and intrapelvic dissemination may follow a characteristic pattern.

►Appendiceal Epithelial Neoplasms

Peritoneal Malignancy

►Carcinomatosis

Peritoneal Metastasis/Dissemination

►Transcoelomic Metastasis

Peritoneal Mucinous Carcinoma

Definition

PMCA; A mucinous appendiceal neoplasm in which the cancer cells have an invasive component resembling a mucinous adenocarcinoma from the colon or other site.

►Appendiceal Epithelial Neoplasms

Peritoneal Tumor

►Carcinomatosis

Peritonectomy

Definition

Removal of peritoneal surface using electrosurgical dissection in order to resect ►carcinomatosis.

►Appendiceal Epithelial Neoplasms

Peritoneovenous Shunt

Definition

Surgical insertion of a shunting tube to achieve the continuous emptying of ascitic fluid into the venous system.

►Acites

Peritoneum

Definition

The peritoneum (derived from Greek meaning "stretched over") is the serous membrane of the

abdominal and pelvic cavity which covers and supports the abdominal and pelvic organs, and serves as a conduit for their blood and lymphatic vessels and nerves.

►Transcoelomic Metastasis

Peritoneum as the First Line of Defense

Definition

The ►peritoneum is an anatomic barrier to further dissemination of ►carcinomatosis. If surgical procedures remove peritoneum with free cancer cells remaining within the abdomen or pelvis, the first line of defense against carcinomatosis has been violated.

►Appendiceal Epithelial Neoplasms

PERK

Definition

An endoplasmic reticulum (ER) transmembrane protein kinase that phosphorylates the α subunit of translation initiation factor 2 (eIF2α) in response to ER stress and anoxia. PERK is maintained in an inactive state by the binding of the ER chaperone BiP, that under conditions of ER stress dissociates from PERK.

►Anoxia
►Endoplasmic Reticulum Stress

Perlman Syndrome

Definition

Characterized by fetal gigantism, renal dysplasia, nephroblastoma, islet cell hypertrophy, multiple congenital anomalies, and mental retardation.

►Nephroblastoma

Permeases (for Import Systems)

►ABC-Transporters

Peroxisome

Definition

Dynamic ubiquitous cellular organelle involved in the metabolism of hydrogen peroxide (generation and elimination) and lipids (fatty acid α- and β-oxidation).

►Autophagy

Peroxisome Proliferator-Activated Receptor

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Synonyms

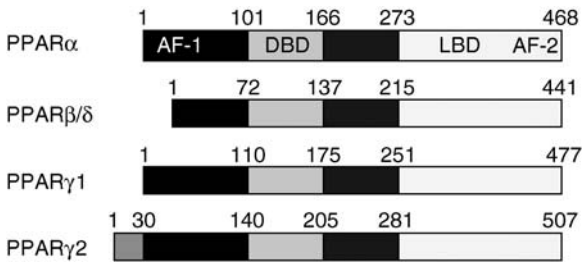
PPAR; NR1C

Definition

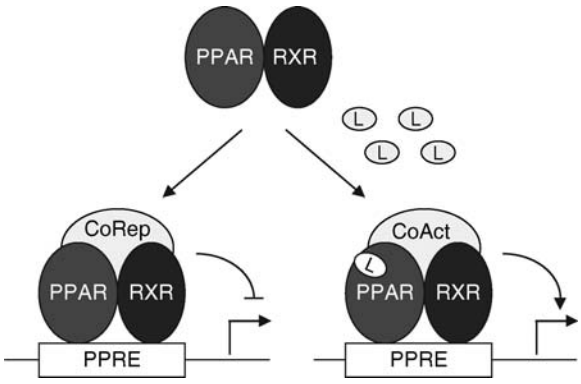
Peroxisome proliferator-activated receptors (►PPARs) are nuclear hormone receptors originally identified as molecular targets of synthetic compounds inducing peroxisome proliferation. As ligand-dependent transcription factors PPARs mediate the effects of fatty acids and their derivatives, and thereby regulate proliferation, differentiation and cell survival.

Characteristics

The three peroxisome proliferator-activated receptor isoforms PPARα, ►PPARβ/δ; and PPARγ are encoded by different genes and belong to the largest family of transcription factors, the nuclear hormone receptors (NHRs). PPARs possess a modular structure composed of a central DNA binding domain (DBD) with two zinc fingers and a ligand binding domain (LBD), including the ligand binding pocket and the ligand-dependent activation domain AF-2 in its C-terminus (Fig. 1). A ligand-independent, poorly characterized transactivation function at the N-terminus is known as AF-1.



Peroxisome Proliferator-Activated Receptor.
Figure 1 The PPAR receptor family. Human PPARs share a common modular structure typical for the nuclear receptor superfamily. The highly conserved central DNA binding domain (DBD) contains two variant zinc fingers. By each of them one zinc ion is bound by a tetrahedral arrangement of cysteine ligands. The C-terminal ligand binding domain (LBD), as well as the ligand-independent and ligand-dependent activation domains (AF-1 and AF-2, respectively) are less conserved and confer transcription regulation via their interaction with coactivator and corepressor complexes. Amino acid numbers are indicated for each receptor isoform.



Peroxisome Proliferator-Activated Receptor.
Figure 2 Transcriptional activity of PPAR-RXR heterodimers. *Left*, in the unliganded state, PPAR-RXR heterodimers bind to PPREs in the promoter region of target genes and inhibit their transcription by recruiting co-repressors (Co-Rep). *Right*, upon ligand (L) binding, co-repressors are released and co-activators (Co-Act) such as p300/CBP are recruited to cooperatively activate transcription of PPAR target genes.

PPARs control gene expression by binding to specific PPAR response elements (PPREs) corresponding to the consensus motif 5′-AACT AGGNCA A AGGTCA-3′ typically located in the promoters of their target genes. Like many other NHRs, PPARs bind DNA as obligatory heterodimers by interacting with one of the ►retinoid acid receptors (RXRs). In the unliganded form, PPAR-RXR dimers can actively silence gene expression by recruiting co-repressor complexes. Conformational changes upon ligand binding promote interaction with coactivators such as ►p300/CBP, leading to transcriptional activation of PPAR target genes (Fig. 2). Endogenous ligands are fatty acids and their derivatives, for which PPARs act as intracellular sensors and thereby regulate pathways involved in lipid and glucose metabolism. Correspondingly, synthetic PPAR ligands such as fibrates and ►thiazolidinediones (TZDs) are used for the treatment of dyslipidemia and type II diabetes, respectively. Beside their role in metabolism, PPAR functions are also associated with other processes of clinical importance, such as arteriosclerosis, ►inflammation and especially cancerogenesis.

PPARα

PPARα is expressed in the liver, kidney, heart, intestine, skeletal muscle, brown fat tissue, adrenal gland and pancreas. It was the first PPAR family member identified in a screen for molecular targets of peroxisome proliferators (PPs) using a mouse liver cDNA library. PPs are liver-targeting chemically unrelated compounds, including natural lipids and steroids as well as ►xenobiotics, industrial plasticizers, pesticides and solvents. Chronic PP administration in rodents leads to liver ►hypertrophy, ►hyperplasia and eventually to ►hepatocellular carcinoma. The exclusive role of PPARα in mediating these processes was deduced from PPARα-null mice that are resistant to the effects of PP treatment, including the development of hepatocellular carcinomas. The exact mechanisms underlying the tumorigenic PPARα function are unclear but several explanations have been proposed: PP-activated PPARα triggers DNA replication and proliferation of hepatocytes, accompanied by an induction of cell cycle regulators such as ►cyclin dependent kinases CDK-1 and -2 as well as c-►myc. Further, PPs attenuate hepatocytic ►apoptosis in vitro and in vivo. PPARα appears to be critical to this effect since inhibition of apoptosis can be prevented by a dominant-negative PPARα protein. Another PPARα-mediated mechanism reported is the production of reactive oxygen species as by-products of β-oxidation, possibly leading to ►DNA damage and increased proliferation of hepatocytes.

Despite the clear evidence for PPARα-mediated PP action in rodent hepatocarcinogenesis, the situation in humans appears to be different. Studies did not reveal activated peroxisome proliferation in the liver or any increased incidence of hepatocellular carcinoma in patients treated with hypolipidemic fibrate drugs. Consistently, in vitro experiments with human cells showed a reduced transcriptional response to activated PPARα compared with rodents. A lower level of hepatocytic PPARα expression (1–10%), different cofactor availabilities and PPREs of target genes, as well as a reduced activation efficiency and bioavailability of certain PPs are discussed as possible explanations for

these species differences. Nevertheless, as many humans are constantly exposed to PPs (e.g. hypolipidemic drugs, pesticides, plasticizers) it is very important to constantly resurvey the cancerogenic potential of these compounds.

PPARβ/δ (NUC1, FAAR)

PPARβ/δ is expressed in a wide range of tissues with high levels in brain, skin, adipose tissue and colon. Although the role of PPARβ/δ in cancer is controversial, its involvement in processes related to tumorigenesis such as proliferation, differentiation and survival is well accepted. For example, a role for PPARβ/δ in the differentiation of adipose tissue and oligodendrocytes was concluded from the phenotype of PPARβ/δ-null mice, exhibiting a decreased fat mass and myelination defects in the corpus callosum, respectively. In keratinocytes, PPARβ/δ inhibits proliferation but promotes cell migration and survival via activation of ILK, PDK1 and AKT1. A role for PPARβ/δ as a mediator of proliferation in hepatic stellate-cells and vascular smooth-muscle cells has been reported. The strongest link to cancerogenesis has been demonstrated in murine colorectal cancer cells where PPARβ/δ expression is repressed by the ►APC/β-catenin tumor suppressor pathway. Aside from the APC pathway, PPARβ/δ is also targeted by the ►Ras pathway, as it becomes activated in response to an overexpression of oncogenic Kras. For both pathways, regulation of cyclooxygenase 2 (COX2) levels might be the underlying mechanism, as COX2 is the rate-limiting factor in the generation of some natural PPARβ/δ ligands (►prostaglandins). PPARβ/δ activation in human hepatocellular carcinoma cells increases proliferation and COX2 expression, suggesting a growth-promoting positive feedback loop. Consistent with a direct role in colorectal tumorigenesis, PPARβ/$\delta^{-/-}$ ►colon cancer cells show a significantly decreased tumorigenic potential in xenograft mouse models. Moreover, PPARβ/δ upregulates VEGF in colon carcinoma cells, which directly promotes colon tumor epithelial cell survival through activation of ►PI3K-Akt signaling. The repression of PPARβ/δ activity by the ►non-steroidal anti-inflammatory drug (NSAID) sulindac might explain NSAID-mediated chemoprevention of colon cancer.

The role of PPARβ/δ in tumors other than colon cancer is far from being clear. Significant upregulation of PPARβ/δ has been observed in head and neck ►squamous cell carcinomas, endometrial adenocarcinomas and breast cancer cell lines. A growth inhibiting activity of PPARβ/δ has been suggested in lung cancer cells. In summary, pro- and anti-cancerogenic PPARβ/δ functions seem to exist, depending on the cell- and tissue-specific context.

PPARγ

PPARγ exists as two protein isoforms, expressed from different promoters and alternatively spliced at the 5′ end of the gene, resulting in 30 additional amino acids at the NH_2 terminus of PPARγ2 compared with PPARγ1. Whereas expression of PPARγ2 is mainly restricted to adipose tissue, PPARγ1 has also been detected in other tissues, including heart, skeletal muscle, pituitary, brain, kidney, liver and colon.

The concept of PPARγ playing a role in tumorigenesis and therefore being a potential target in cancer therapy was developed from its antiproliferative activity in fibroblasts during adipogenesis. Also in other cell types PPARγ activation induces programs of gene expression that reflect the differentiation potential of progenitor cells. For instance, its expression in epithelium-derived cells stimulates the production of markers of epithelial differentiation/maturation, such as keratin 20 and krüppel-like factor 4. Additionally, PPARγ inhibits cell proliferation through induction of cyclin-dependent kinase inhibitors (i.e. ►$p21^{Cip1}$ and $p18^{Ink4c}$) and attenuates ►E2F/DP DNA-binding activity via down-regulation of PP2A protein phosphatase. Consistent with an integrating role coupling cell differentiation and growth arrest, loss of one allele of PPARγ predisposes to carcinogenesis in wild-type mice, while loss-of function mutations in PPARγ associate with human colon cancer. Additional evidence for the impact of PPARγ on tumorigenic processes comes from the identification of a chromosomal translocation in a subset of ►follicular thyroid carcinomas, resulting in a PAX8-PPARγ fusion protein which abolishes the ligand activation of the wild type PPARγ protein. Building up on these findings, intense clinical interest has been raised in the possible application of synthetic PPARγ ligands as effective chemotherapeutic agents. Indeed, PPARγ ligands have been shown *in vitro* to induce cell cycle arrest and/or apoptosis in a variety of cancer cell types, including liposarcoma, breast adenocarcinoma, prostate carcinoma, ►neuroblastoma, pancreas and colon carcinoma cells. However, the role of PPARγ ligands – which have also PPARγ-independent effects – in the regulation of neoplastic transformation *in vivo* remains controversial: In contrast to the reduction of aberrant crypt foci and the inhibition of colon cancer growth in wild-type mice, PPARγ ligands in APC^{Min} mice not only failed to suppress polyp formation but also led to a small although significant increase in colon tumors. Moreover, epidemiological data emphasize that the development of colorectal cancer is promoted by prostaglandins, which are potential ligands of PPARγ. In mice with mutations in the cyclooxygenase gene or in animals and humans treated with COX inhibitors, decreased production of prostaglandins prevents or attenuates colon cancer development. Furthermore, the intake of fatty acids from animal origins strongly correlates with colon tumors, suggesting that PPARγ possibly links high-fat diet and colon cancer. Studies aimed to find an explanation for the contradictory results with PPARγ

in colon cancer revealed that the tumor-suppressor function of PPARγ entirely depends on the presence of an intact adenomatous polyposis coli (APC) gene. The cross-regulation of ►Wnt/β-catenin/Tcf ligands, kinases, and transcription factors with members of the nuclear receptor family has emerged as clinically and developmentally important area of endocrine cell biology. Wnt/β-catenin can modulate NRs activity, and NRs ligand dependently inhibit the Wnt/β-catenin/Tcf cascade. As a case in point, PPARγ can suppress β-catenin levels in wild-type mice whereas in the presence of a mutant APC the ability of PPARγ to regulate β-catenin and colon tumorogenesis is completely lost. In normal cells, PPARγ targets β-catenin to the proteasome partly through a process possibly involving PPARγ induction of ►PTEN and activation of the downstream effector GSK3-β. β-Catenin and PPARγ interact with each other through the TCF/LEF binding domain of β-catenin and the putative coactivator binding sites of PPARγ to influence each other's activity. As a result, in normal untransformed cells, PPARγ additionally induces the proteasomal degradation of β-catenin through complex formation independent from GSK3-β, while in transformed cells, oncogenic β-catenin escapes its PPARγ-associated degradation and inhibits in turn PPARγ activity. In this model, the oncogenic form of β-catenin is dominant over PPARγ activity, thus explaining why PPARγ is capable of suppressing tumorigenesis only in cells that have a functional APC to facilitate the GSK3-β-mediated inactivation of β-catenin. As a further indication for a role of PPARγ in suppressing the oncogenic activity of β–catenin, NSAIDs have been reported to antagonize β-catenin function via high levels of PPARγ and its co-receptor RXR-α, which is independent from the presumed role of NSAIDs in COX inhibition. Finally, epidemiological studies demonstrate long-term ingestion of various NSAIDs to associate with a reduced incidence of colorectal cancer, and probably of prostate and breast tumors. Taken together, the multilayered and cell type specific activity of PPARγ as well as its interconnection with the Wnt/β-catenin/Tcf pathway argue against the indiscriminately and broad use of PPARγ ligands in cancer treatment. Thus, despite the substantial progress being made in the understanding of the role of PPARγ in cancerogenesis during the last decade, further investigations appear necessary to elucidate and refine indications for a PPARγ-targeted therapy.

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Peroxisome Proliferator-Activated Receptors

Definition

PPARS; A subfamily of non-steroid nuclear receptors that includes various subtypes (α, β/δ, γ) encoded by separate genes that display different tissue distribution and distinct ligand selectivity.

►Retinoid Receptor Cross-talk

Personalized Cancer Medicine

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P

Synonyms

Individualized treatment

Definition

►Personalized medicine is defined as the application of the right treatment for the right patient, at the right dose, and at the right time. Personalized medicine is therefore a model of the way medicine will evolve through the use of specific treatments and therapies best suited to an individual's genotype and is driven by patient demand for safer and more effective medicines and therapies.

Characteristics

The concept of individualizing treatment is not new and the study of hereditary influences on the response to drugs (►pharmacogenetics) is reflected in the scientific literature from the 1960s. The number of articles published remained constant until 1996 when technical

developments and new insights into the highly complex nature of many diseases resulted in the advent of ►pharmacogenomics. Pharmacogenomics is closely related to pharmacogenetics but the emphasis is on the influence of a whole set or complement of genes on the response to drugs, with the aim of improving drug efficacy rather than simply being concerned with drug safety. Subsequent growth in the new field of pharmacogenomics-based personalized medicine has been exponential.

The term "personalized medicine" is mainly used in the context of pharmacogenomics and although pharmacogenomics is one of the main drivers of this new area, it is important to realize that this view can be rather narrow, and personalized medicine also includes areas such as disease susceptibility in addition to the molecular traits and mechanisms underlying individual characteristics. Applications range from personal risk profiles leading to personal preventative measures, such as vaccination and personalized health planning leading to early diagnosis, to genetic counseling, databases and decision support tools. This essay focuses on the role of personalized medicine in cancer and explores its use in current clinical practice and its likely application in the future.

Why is Personalized Medicine Important in Cancer?

Personalized medicine is more important for cancer patients than for those with other diseases for several reasons. Firstly, cancer is a highly heterogeneous disease with significant molecular differences in the expression and distribution of tumor cell makers among patients with the same type and grade of tumor. Secondly, cellular mutations tend to accumulate as cancer progresses, further increasing tumor heterogeneity. Thirdly, currently used cancer therapies are often toxic to normal cells resulting in severe side effects rarely seen in other diseases. Finally, cancer patients have limited time to try one kind of treatment and, if it does not work, to try others until the right medicine is found. Therefore, cancer therapy presents a unique example of personalized medicine in which an individual's genotype not only determines the therapeutic and toxic responses to a drug (the ►pharmacogenotype), but also that of the tumor.

To what Extent is Cancer Medicine Already Personalized?

Over the past 20 years, a large amount of the fine detail of the basic biological processes that become disturbed in cancer has been amassed. We now know the key elements of growth factor binding, signal transduction, gene transcription control, cell cycle checkpoints, apoptosis, angiogenesis and metastasis. The new paradigm is to develop agents that target the precise molecular pathology driving the progression of individual cancers. Investment in ►genomics, genetics and automation has already resulted in a large number of rationally designed anti-cancer drugs with a record number of novel compounds currently in clinical trials. High profile drugs such as Gleevec (►imatinib), ►Herceptin (trastuzumab), Tarceva (►erlotinib) and Avastin (bevacizumab) specifically target underlying molecular lesions and are in widespread clinical use. Patients are selected to receive treatment based on the detection of a precise molecular lesion and the likelihood of benefit with subsequent drug treatment. These agents have provided important proof-of-principle evidence for the benefits of targeted therapy in cancer and represent a significant advance towards achieving personalized medicine.

One of the consequences of using targeted therapy against a defined molecular lesion is that a move to target rather than disease based drug clinical development is required as a defined molecular lesion may occur in more than one type of cancer. An example among the new approved agents for targeted therapy in cancer is the use of imatinib (Gleevec). Imatinib was originally developed to target the ►BCR/ABL receptor tyrosine kinase, formed by the fusion of the head of the *bcr* gene with the body of the *abl* gene, in chronic myelogenous leukemia cells. The chimeric ►BCR/ABL molecule transforms primary myeloid cells to leukemic cells via its ABL receptor tyrosine kinase activity. Imatinib is a competitive inhibitor of the ABL tyrosine kinase activity resulting in 89% overall survival of patients after 5 years. Subsequent analysis has also revealed activity against the tyrosine kinases associated with the ►KIT protein (stem cell factor receptor) and ►platelet-derived growth factor receptor. The KIT tyrosine kinase is abnormally expressed in gastrointestinal stromal tumor (GIST), a rare neoplasm for which there had been no effective systemic therapy. Clinical trials subsequently demonstrated imatinib to be an effective therapy in GIST with 1 year survival rates of 88% and imatinib is now licensed for treatment of GIST.

To achieve the goal of personalized medicine, in addition to developing drugs with defined molecular specificities, it is essential to have ►biomarker and imaging tests to identify the molecular targets in a patient's tumor and the patient's pharmacogenotype. ►Biomarkers act as molecular signposts of the physiological state of a cell and are determined by the genes, proteins and other organic chemicals made by the cell. A related objective of the same tests is to enable monitoring of the effect of a defined drug on its molecular target and to assess early response so that a non-responding patient can be spared unnecessary treatment and be offered alternative therapies. Biomarkers can also provide essential information regarding how much drug is needed to inhibit a particular

molecular target in a tumor and whether there is a benefit to giving more drug. Ideally these tests should be non-invasive as biopsy of solid tumors is expensive and subjects patients to additional invasive procedures with associated risks. Although recent advances in non-invasive techniques, such as buccal cell isolates offer potential as viable sources of biomarkers, traditional sources such as serum or plasma are currently in routine use.

The advantage of using biomarkers to identify patients likely to benefit from a particular treatment is demonstrated by the success of the monoclonal antibody Herceptin (trastuzumab) in breast cancer. Herceptin targets the receptor tyrosine kinase ►HER2/neu which leads to increased cell growth, decreased cell death and increased metastasis to distant sites when mutated. Patients likely to benefit from Herceptin are selected using ►immunohistochemistry test kits or fluorescence in-situ hybridization assays which detect the presence of the protein. Patients whose tumors do not express HER2/neu are spared futile treatment and potential treatment-related side-effects and offered alternative therapy saving precious time and resources.

The newly emerging area of cancer ►phenomics can be used as a practical illustration of how genetic studies can be successfully integrated with phenotypic data to improve the specificity of personalized medicine. Cancer phenomics refers to the systematic and detailed collection, objective documentation and cataloguing of phenotypic data at many levels, including clinical, molecular and cellular phenotype. Data is most mature for germline mutations in the receptor tyrosine kinase "rearranged during transfection," ►*RET*. *RET* plays a crucial role in transducing growth and differentiation signals in tissues derived from the neural crest. Germline mutations in *RET* which lead to a constitutively active receptor tyrosine kinase or decreased specificity for its substrate predispose to the cancer-associated syndrome ►multiple endocrine neoplasia-2 (MEN2). MEN2 is a clinical syndrome involving predisposition to medullary thyroid cancer and phaeochromocytoma in addition to other endocrine, mucocutaneous and skeletal features. MEN2-asociated tumors are more often bilateral and multifocal, and occur at a much younger age than their sporadic counterparts. Meticulous characterization of *RET* phenomics at the clinical and biochemical levels has revolutionized management of families with MEN2. When a mutation is identified in an individual, predictive testing can be performed on family members and only those who carry the mutation require further MEN2 management. The correlation between genotype and phenotype in MEN2 has lead experts to suggest individualized recommendations for the timing of prophylactic thyroidectomy (surgical removal of the thyroid gland) on the basis of the *RET* mutation genotype. For other mutations in *RET,* phenomics has suggested a specific routine surveillance program for early detection is a more appropriate management route. Knowledge of the molecular pathways that are deregulated as a result of *RET* mutations will enable targeted therapies to be explored that might prove useful in treating tumors arising as a result of *RET* mutations.

The Future of Personalized Medicine in Cancer

The Human Genome Project, and its successor the Cancer Genome Project and related activities are expected to identify the majority of genes in most of the common human cancers over the next 5 years. Together these projects will provide a vast repository of comparative information about normal and malignant cells. Instead of licensing drugs for empirical use in different types of cancer with relatively poor response, new therapies will require the identification of specific molecular lesions resulting in improved response rates. Reduced toxicity due to increased selectivity will allow new therapies to be given over prolonged periods of time, in some cases for the rest of the patients' life, a concept unthinkable with current cytotoxic therapy. More complete knowledge of tumor biology and the role of target pathways in normal physiology will reduce the likelihood of unexpected toxicities that can limit or even curtail a drug's usefulness. The recent example of unexpected cardiotoxicity in some patients treated with imatinib is an example.

Detailed analysis of the differences between normal and malignant cells also allows the possibility of individual cancer risk assessment leading to tailored prevention programs and specific screening programs to detect early cancer. Although cancer prevention currently absorbs only 2% of the total current funding of cancer care and research, this percentage is likely to increase in the post-genomic era. Currently it is accepted that approximately 100 genes are associated with the development of a range of cancers. However, the detection of ►polymorphisms in low-penetrance cancer-related genes – or a combination of changed genes – will allow identification of people at increased risk and demand for prevention strategies will increase. It is predicted that within 20 years most people will be genetically mapped with the information easily stored on a smart card. Thus, predisposition to some cancers will be found in early adult life with potential for correction well before manifestation of the disease.

Multiple technologies exist to achieve the goal of personalized treatment in cancer and molecular diagnosis can be established at multiple levels where molecular differences can be found between individuals with identical clinical manifestations. Currently available diagnostic procedures are insufficient to determine

which patients will primarily benefit from rational tumor therapy. In the future, gaining information on the morphology, genetic, ►proteomic, and ►epigenetic alterations will be essential to provide clinicians with relevant information for individualized medicine to be prescribed. DNA ►microarrays are the first approach close to being used in routine diagnosis. These consist of thousands of DNA fragments specific for individual genes which are used to ultimately obtain a genetic expression profile indicating over-expression, under-expression, no change, or complete absence for each gene in the tissue sample. Patterns of gene changes or novel genes associated with disease development or clinical outcome of an individual tumor can then be identified by specialist ►bioinformatic programs which use statistical methods to evaluate the complex information, and translate molecular information into clinically relevant data. Similar analysis of proteins as proteomic patterns also appears on the horizon. The development of high throughput methods for investigation of the methylation status of DNA provides a further level of information. It is likely that individually none of these technologies will provide all the information required for an efficient individualized approach, therefore a "multiplex" approach combining the different biological levels of DNA, RNA, and protein, may be necessary to functionally classify malignant tumors.

The Challenges for Achieving Personalized Medicine

One of the major limitations to achieving personalized medicine is our incomplete knowledge of tumor biology. Our understanding will continue to be supplemented by data from The Human Genome Project and The Cancer Genome Project and their related activities. However, continued investment in technical developments and ►bioinformatics is necessary to develop special methods capable of detecting the entire spectrum of rate-limiting oncogenic pathways in tumors before and after therapy to identify those patients who will benefit from novel therapies. These include the broad profiling of human tissue for morphology, genetic, proteomic, and epigenetic alterations as described above. Before establishment of such an integrated approach into daily routine can occur, extensive research needs to be performed to allow a firm prediction on the activation of a certain pathway in clinical material.

More insidious challenges stem from the way cancer drugs are currently developed. The simplest being that currently drugs frequently enter clinical trials without a "validated" biomarker or imaging test to select patients most likely to respond or for identifying response to treatment. "Validated" means that the test is standardized and has been shown to be reproducible across institutions, if not in patients, then at least in animal models. Instead of being an after-thought, these tests need to be developed early in the development of a drug and be ready for use in clinical trials. Additionally, current requirements for disease-specific drug trials mean that responding patients must be identified by separate trials for each disease group. A move to target rather than disease-based drug clinical development will require a paradigm shift in the way cancer drugs are developed.

A further challenge for drug development results from the likely need to give the newer types of cytostatic cancer drugs (which arrest cell growth but do not shrink tumors) in combination. A rational way to develop molecularly targeted cancer drugs is to use combinations that attack different points in the same or parallel signaling pathways. However, the reluctance of pharmaceutical companies to test their experimental agents in combination with a competitor's agent is a frustration for many clinical investigators. A framework for providing intellectual property protection to companies that test drugs in combination has recently been provided by the US National Cancer Institute Cancer Therapy Evaluation Program (CTEP) with a number of CTEP-sponsored drug combination studies underway. This type of model will need to be extended and made more readily available in the future.

Other challenges for drug development include the widespread frustration with the cost and time that it takes to bring a new cancer drug to trial. Initiatives are in place to help to speed up the drug development process both in the drug discovery pipeline and in the design of clinical trials. The implementation of chemical biology, which provides experimental techniques for linking together elements from all stages of what was previously viewed as a linear pathway from gene to drug, may help to speed up the drug development process. To encourage the more rapid introduction of drugs into clinical trial, the Federal Drug Agency recently introduced the concept of phase 0 or micro-dose trials at less than one-one hundredth of the dose calculated to yield a pharmacologic effect, thus allow the collection of human ►pharmacokinetic and ►bioavailability data earlier in the drug development process. These human data can then be combined with preclinical data to select the best candidates to advance to further, more expensive and extensive clinical development. However, much work needs to be done in this area to ensure personalized medicine becomes a reality.

Finally, there has been much discussion of the economic, social, and ethical considerations of personalized medicine. Although these discussions are outside the scope of this essay, it is important to note that although the post-genomic era provides the molecular tools needed to provide personalized medicine, it may not provide for the risks involved. These include privacy, protection of data, and prevention of

discrimination. Information and communication management is also required to handle the wealth of personal information and link to global medical knowledge.

Summary

We are at one of the most exciting times in modern cancer therapy with the development of rational therapy and the move towards ►personalized treatment for cancer. The concepts to be tested are well established, the drugs are becoming available, and new emerging technologies in target identification, drug discovery, molecular markers, and imaging can finally make the goal a reality: personalized health planning, early diagnosis, the right drugs for the right patient, with predictable side effects.

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Personalized Medicine

Definition

Personalized medicine is the application of the right treatment for the right patient, at the right dose, and at the right time and is therefore a model of the way medicine will evolve through the use of specific treatments and therapies best suited to an individual's genotype.

►Personalized Cancer Medicine
►Pharmacogenomics in Multidrug Resistance

Pertussis Toxin

Definition

Toxin produced by **Bordetella pertussis** that catalyzes the ADP-ribosylation of some G-proteins at a cysteine residue near the C-terminus resulting in uncoupling of receptor and G-protein

►G-Proteins

PEST Sequence

Definition

Is a region rich in the amino acids proline (P); glutamic acid (E); serine (S); or threonine (T), and is a target proteins for rapid turnover through proteosomal degradation.

►Mcl Family
►Mitogen-Inducible Gene 6 (MIG-6) in Cancer

PET

Definition

►Positron emission tomography; A nuclear medicine imaging technique that produces a three-dimensional image of functional processes in the body upon decay of a short-lived isotope conjugated to a metabolically active molecule (e.g., fluorodeoxyglucose) administered systemically to patients.

PET/CT, Integrated Positron Emission Tomography/Computed Tomography

Definition

Combination of two different imaging modalities (hybrid imaging) enabling simultaneous morphologic and functional imaging.

►Positron Emission Tomography

Petechiae

Definition

Petechiae are small red or purple spots on the body, caused by a minor hemorrhage as a sign of

thrombocytopenia (low platelet counts) or other disorders of coagulation.

►Acute Myeloid Leukemia

Peutz-Jeghers-Syndrome

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Synonyms

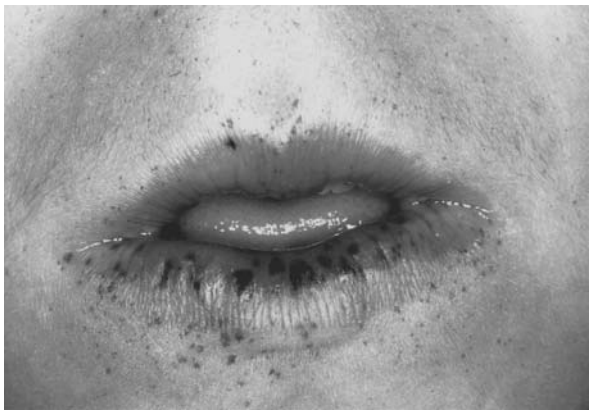
Peutz-Touraine-syndrome; Hereditary hamartosis Peutz-Jeghers; Hutchinson-Weber-Peutz-syndrome; Lentiginosis polyposa Peutz; PJS

Definition

Peutz-Jeghers-syndrome is an autosomal dominantly inherited disorder that is characterized by the combination of:

1. Lentiginosis, i.e., typical pigmented lesions.
2. Hamartomatous polyposis that occurs mainly in the small intestine but also in the colon and the stomach. Extraintestinal hamartomas are rare; possible localizations include the gallbladder, the urinary bladder, the heart, and the respiratory tract.
3. Increased risk for various types of cancer (e.g., pancreas, gastrointestinal tract, bilateral breast cancer, rare gynecological tumors).

The clinical diagnosis is considered established when either two or more hamartomas or at least one hamartoma together with pigment spots or one hamartoma and positive familial history are found (Fig. 1).



Peutz-Jeghers-Syndrome. Figure 1 Typical pigmented lesions perioral and on the lips.

Characteristics

Although Peutz-Jeghers-syndrome is autosomal dominantly inherited, a large number of sporadic cases (possibly new mutations) occur. The disease is equally distributed between the sexes and the races. The clinical problems are bleeding, pain, and intussusception caused by the intestinal hamartosis and the increased risk for various tumors. Initial studies showed mutations in the coding region or splice sites of the *LKB1/STK11* gene in 35–70% of PJS cases. A second (yet unknown) gene that also causes PJS was therefore discussed. Since then, the ability to identify large deletions provided evidence suggesting that all PJS cases may be due to STK11 germline mutations.

Lentiginosis (Pigment Spots)

The typical pigmented spots occur mainly around the mouth, on the lips, and in the buccal mucosa but may also be found on the hands, the fingers, the feet, and around nose and eyes. In size the spots range from few millimeters up to up to ~1 cm in diameter. They are not elevated above skin level. Their color may vary from light brown to nearly black. They may be present at birth or can emerge during the first years of life, although in some cases first spots appear in higher age. In most patients the spots change during life, loosing intensity with increasing age. Histopathologically they show an increase of melanin and melanocytes in the basal layer of the epidermal epithelium. No malignant transformation has ever been reported. If they represent a cosmetical problem, laser treatment is possible. Of the PJS-patients 5–50% have no pigmented spots.

Hamartomatous Polyposis

The hamartomatous polyps are mainly localized in the jejunum followed by the duodenum, the ileum, the colon, and the stomach. Other localizations like the esophagus, the gall bladder, the urinary bladder, the heart, and the respiratory tract have been described but remain extremely rare.

The main problems caused by the intestinal polyps are intussusception, bleeding, and pain. Intussusception leading to bowel obstruction and necrosis is the main cause for emergency surgery in those patients. The age range where symptoms are initiated reaches from newborn babies to old age. Initial clinical symptoms first occur in 33% of patients in the first decade of life, further 33% in the second.

The polyps in the intestine show a typical histopathological picture: intact intestinal goblet cell rich mucosa covers branching bundles of smooth muscle from the lamina muscularis mucosa. These polyps are usually benign but in some cases adenomatous and carcinomatous changes have been reported, leading to the suggestion of a hamartoma-adenoma-carcinoma sequence.

Increased Risk for Malignant Tumors

An increased risk for a wide variety of malignant tumors in PJS-patients has been reported by several authors. In a Meta analysis, the risk ratio for all cancers was 15.2 with a cumulative risk of 93% from age 15 to 64 years old. The most frequent tumor sites include the gastrointestinal tract (stomach, small intestine, colon), the pancreas, the lung, and the female breast (often bilateral). Furthermore, many patients develop tumors of the urogenital tract. In males Sertoli-cell tumors are found. In females rare tumors, such as the benign bilateral form of the sex cord tumor with annular tubes (SCTAT) and the adenoma malignum of the uterine cervix, are found next to the normal carcinomata of the ovaries, the cervix, and the myometrium. Furthermore, malignant tumors in PJS-patients have been found in almost all organ systems. It is therefore impossible to exclude any tumor from the spectrum found in PJ-syndrome.

Pathogenesis

In most cases of PJS an inactivating mutation in the *STK11* or *LKB1* gene can be shown. Initial studies showed mutations in the coding region or splice sites of the *LKB1*/*STK11* gene in 35–70% of PJS cases. A second (yet unknown) gene that also causes PJS was therefore discussed. Since then, the ability to identify large deletions provided evidence suggesting that all PJS cases may be due to *STK11* germline mutations. But still, a second gene responsible for PJS remains possible.

The gene product has a strong homology to the serine–threonine protein kinase XEEK1 from Xenopus. The STK11/LKB1 protein is nuclear as well as cytoplasmic. Furthermore, LKB1 is involved in other human disorders like diabetes and lung adenocarcinoma. The gene has been also investigated in many other sporadic tumors like colorectal, gastric, testicular, pancreatic, ovarian, and breast cancer, in malignant melanoma as well as in different tumor cell lines. Mutations of the *STK11*/*LKB1* gene were rare in these tumors, suggesting a minor role in the evolvement of sporadic tumors besides lung adenocarcinoma.

Loss of heterozygosity of the wild-type allele has been demonstrated in the hamartomatous polyps and in 70–100% of the malignant tumors investigated from PJS-patients.

Therapy

Besides surgical or endoscopic removal of polyps and tumors, there is no established therapy for PJS. However, recent results give reason for hope that in the future medications will be able to favorably influence the course of disease. One possible target for medication could be the cyclooxygenase-2 (COX-2), an enzyme which is frequently elevated in PJS hamartomas. Pharmacological suppression of COX-2 is possible and leads to encouraging results in mouse model and a small series of patients by reducing polyp burden in some cases. Due to adverse events reported from patients treated with COX-2 inhibitors, further studies are on hold.

Screening Recommendations

Regular examinations of PJS-patients have two different aims: (i) to avoid complications caused by intestinal polyps such as bleeding, pain, intussusception, and bowel obstruction and (ii) the early diagnosis of cancer or precursor lesions.

For the first aim, regular screening of the gastrointestinal tract is recommended. The second one is more difficult to achieve since malignant tumors have been reported in virtually all organ systems of PJS-patients. To date, there is no consensus what is the most adequate screening program for people at risk.

The "Deutsche Krebshilfe" (German Cancer Aid Society) recommends annual physical examinations, starting at age 12. Endoscopy of colon and stomach and imaging of the small intestine by either capsule endoscopy, MR-Sellink procedure, or double balloon enteroscopy should start at age 12 and be repeated every 2 years.

However, in a case report, first symptoms of PJS have been described as early as within the first days of life. It has also been shown that one third of patients become symptomatic during the first decade of life. The 12-year of age threshold as a starting point for regular examinations is therefore debatable.

Should symptoms occur in people at a younger age that carry the gene or that are, with an otherwise unknown genetic status, at risk, immediate clarification is warranted.

In female patients, regular gynecological examination including vaginal ultrasound should start at the age of 18 and be repeated on an annual basis. In male patients, regular examination of the genital organs is required in order to detect Sertoli-cell tumors.

At the age of 25, bilateral breast sonography in females should commence on an annual basis. At age 35, bilateral mammography or MRT of the female breast and pancreaticobiliary MRT (MRCP) is recommended to be carried out every other year.

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Peutz-Touraine-Syndrome

►Peutz-Jeghers-Syndrome

Peyer Patches

Definition

Are secondary lymphoid organs identified by the Swiss anatomist Johann Conrad Peyer in the seventeenth century. They are aggregations of lymphoid tissue that are usually found in the lowest portion of the small intestine (ileum) in humans.

►DNA Vaccination

pgp-1

►Adipose Tumors

PH Domain

Definition

Pleckstrin Homology; A protein region of ~100–120 amino acid residues found in intracellular signaling proteins which bind to phospholipids in the plasma membrane.

►Membrane-Linked Docking Protein

Phaeochromocytoma

Definition

A ►neuroendocrine tumor of the medulla of the adrenal glands originating in the chromaffin cells. The tumor secretes catecholamines, most commonly adrenaline and noradrenaline.

►Neurofibromatosis 1

Phage

Definition

Phage is another (short) name of bacteriophage, a virus of bacteria.

►Phage Display

Phage Display

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Synonyms

Bacteriophage display

Definition

►Phage display is a molecular technique used to select molecules with specific binding characteristics out of a large pool of genetically engineered ►bacteriophages

displaying combinatorial libraries of proteins via fusion to outer coat proteins of viruses that contain the genes encoding the displayed proteins.

Characteristics

Phage display is very effective way for producing large numbers of diverse peptides and proteins and isolating molecules with specific binding and functional characteristics. Filamentous phage was the first to be used, and currently remains the most commonly employed bacterial viruses for phage display. Phage display involves the expression of polypeptides on the surface of ►bacteriophage. Typically foreign proteins are incorporated into the amino-terminus of pIII, pVIII and, less frequently, pVI coat proteins in filamentous phage display. The lytic bacteriophage vectors such as lambda, T4, and T7 have also been used, especially for displaying ►cDNA expression libraries. Corboxy-terminal display is more common when expressing cDNA library and other proteins for studying protein–protein interactions. Polypeptide libraries in phage display system are encoded by a degenerate oligonucleotide that has been introduced into a location in a phage or ►phagemid genome such that the encoded proteins are displayed or expressed as a fusion to one of the proteins on the outer surface of the bacteriophage. The source of DNA coding for the expressed protein may either be synthetic random or biased sequences, especially for short peptides, or may come from pooled genetic sequences that code for the relevant proteins. During phage assembly process, the fusion proteins are incorporated into the phage particle, and the DNA encoding the displayed fusion protein is packaged inside the same particle. This provides a physical linkage of each polypeptide in the library to its encoding nucleic acid sequence, which can be amplified and decoded. Each phage clone displays a single protein, thus billions of phage particles created from batch-cloned gene libraries express large numbers of diverse proteins constituting phage-displayed polypeptide library. The most popular molecules that are displayed on phage combinatorial libraries today are based on the framework of constrained peptide loop or an antibody fragment. The antibody libraries may be classified as "naïve" or "synthetic" repertoire. A naïve library contains combinatorially assembled ►V-gene from unimmunized animals or human donors to create arrays of antibodies. In "synthetic" repertoire, the combinatorial diversity is generally introduced by appending degenerate complimentary determining regions to a fixed antibody sequence. Single chain variable fragments (scFv), in which variable heavy (V_H) and light (V_L) chain domains of an antibody are sequentially joined by an interdomain peptidic linker, have been the most commonly displayed format for phage antibody libraries. The affinity selection of peptides and antibodies for diagnostic and therapeutic purposes are today the most widely used and commercially most successful applications of phage display libraries. In addition, phage-displayed enzymes, receptors, protein domains, and high complexity cDNA expression libraries have been used for identifying interacting partners.

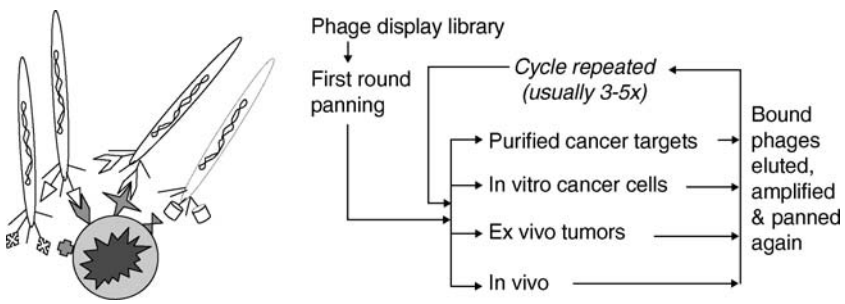
The rapid enrichment and identification of target-binding phages is achieved by affinity selection of phage library on the immobilized target, through a process known as panning. In this process, binding phages are captured and nonbinding clones are washed away. The target-bound phages, following elution with acids, bases, denaturants, or proteases, are amplified in the appropriate host bacterial cells and exposed to the target again for binding. This panning cycle of phage binding, elution, and amplification is usually repeated 3–5 rounds for an optimal enrichment of binding phages. The phages that show considerable binding on individual screening are analyzed for their DNA sequences for identification of the polypeptides they represent. Using similar approach, in vivo panning of injected phage display libraries identified several target-specific binders in human and animal studies also. An ability to select affinity-based ►ligands from a diverse library of polypeptides against any target in a relatively easy way is the key for an extensive utilization of this technology in the cancer research.

Development of Therapeutic and Imaging Agents for Cancer

Developing medications that can target cancer cells without harming the rest of the body is one of the most important challenges for cancer researchers today. It is believed that an ability to home in on cancer cells makes targeted therapy both more effective and less likely to cause side effects. A variety of strategies are being evaluated to achieve this goal. The development of targeted therapeutic methodologies relies in most cases on the availability of binding molecules specific for cancer cell-associated targets. Phage display technology offers a potential tool for developing anticancer agents for both therapeutic and diagnostic applications. Phage display technology has been used to identify peptides and antibodies against a diverse population of known tumor targets. In the cases where ligands isolated from phage-displayed libraries do not show enough affinity and specificity for efficient tumor targeting, phage display techniques are usually combined with affinity maturation methods for optimizing such ligands. In a majority of the cases, ligands have been identified by in vitro panning a phage-displayed library against purified proteins of known cancer-specific targets. Using this approach, ligands have been obtained to an incredibly wide variety of cancer-specific targets

including cell membrane receptors, tumor-associated antigens, ►cellular messengers, matrix-related elements, tumor vasculature targets, etc. However, the approach of developing ligands only to known targets leaves out a variety of other possible targets including novel ones that may uniquely be present in cancer cells. Screening intact cells, tissues, or whole organisms without prior information of the specific molecular targets is a promising strategy to identify cell-specific ligands against an array of targets. Phage-displayed random libraries can be defined as "all-purpose" ►ligand libraries, since expressed polypeptides that have different shapes and properties can interact with linear peptides or folded protein domains, and even with nonprotein molecules. An exposure of the entire library to tumor should identify out of the millions of different shapes at least a few polypeptides that fit specifically to attachment spots on the cancer-specific targets (Fig. 1). In recent years, in vitro and ex vivo selection of phage-displayed ligands on highly complex and diverse target as whole cancer cells, endothelial cell surface of the tumor neovasculature, and whole tumors has been successfully accomplished. In vivo approaches, where phage library has been injected intravenously, have been reported to identify promising ligands against tumor targets in several animal studies with established xenograft tumors. In few studies, the administration of these ligands in conjugation with a cytotoxic agent to tumor-bearing animals resulted in considerable reduction in tumors, lower systemic toxicity, and increased survival time. The benefits of performing in vivo procedure include the presence of a vast number of possible targets in their native configuration, subtraction of the pools of ligands binding to normal tissue, identification of ligands that are stable in blood, and the possibility of identifying unique targets for individual tumors. Recently, the first series of cancer patients who received phage library for serial panning of tumor-targeting ligands have been reported. Patients with Stage IV cancer including breast, melanoma, and pancreas had phage-displayed random peptide or scFv library administered intravenously, tumors excised and tumor-homing phages recovered. In a subset of patients, repeat panning was possible using phage recovered and amplified from that same patient's tumor. No serious side effects, including allergic reactions, were observed with up to three infusions. Several tumor-homing clones from a subset of the patients were identified that specifically bound to a tumor from the same patient in whom clones were recovered. The lack of toxicity and the ability to recover clones with favorable characteristics is a first step for further research with this technology in cancer patients.

A wide variety of options are available to utilize tumor-binding ligands for cancer treatment. Whichever strategy is subsequently chosen, it will vastly benefit if tumor-binding ligands for any patient are reliably available. The application of ligands derived from a phage-displayed library panning is believed to be an important component of a modularized drug. One module is the antiproliferative component and the other is the tumor-binding element. The possibilities of the antiproliferative module include virtually every current anticancer drug category in which increased accumulation of drug at the cancer site would be advantageous. Thus there are several therapeutic options for advancing this technology to the clinic. Cancer-specific ligands identified by phage display technology have been successfully exploited for the ►targeted drug delivery in ►gene therapy, ►radioimmunotherapy, ►photodynamic therapy, prodrug therapy, etc. In general, these ligands act as a delivery vehicle in different forms of therapies to transport therapeutic agents, including genes, ►radionucleotides, ►cytotoxins, ►photosensitizers, ►immunotoxins, ►apoptotic, ►antimetabolic, ►antiangiogenic, enzymes, ►liposomes, ►nanoparticles, etc to cancer site. Cancer cell-targeting ligands



Phage Display. Figure 1 Left side figure shows schematic representation of phages from a filamentous phage library displaying amino-terminal polypeptides fusion to phage outer coat protein pIII. When phage-displayed library is exposed to targets, e.g., cancer cell in this presentation, certain phages depending on their affinity bind to cell surface molecules (targets) and rest are washed away. Right side figure shows the process of panning with cancer targets in different forms, where phages bound to targets are eluted, amplified, and exposed to targets again. The repetition of this process leads to an enrichment of phages that bind to targets with considerable affinity. DNA analysis of target-binding phages decodes the sequences of polypeptides they represent.

isolated from phage display technology also find use in imaging, diagnosis, and monitoring of cancer. The ligands against cancer-specific targets including those associated with ►angiogenic tumor vasculatures can be attached to an imaging agent for localizing tumors. Possible agents for imaging applications are radioisotopes such as ^{99m}TC for scintography, fluorophores for infrared photodetection, gadolinium-loaded liposomes for magnetic resonance imaging, etc.

Identification Cancer-Specific Antigen, Interacting Partners and Immune Response

Affinity-based selection by phage display is also used for tumor profiling, identification of cancer ►biomarkers, to probe protein–protein interactions, and to identify interacting partners and binding domains. This leads to a better understanding of cancer at molecular level and provide information on novel targets proteins, their pathways and networks, and their role in carcinogenesis. Identifying valid cancer cell-specific targets is a difficult challenge as the genetic and biochemical changes that cause disease are often quite subtle, perhaps involving many minor modifications in several genes. Therefore, the success of targeted therapy heavily depends on the identification of appropriate targets under such a complex situation. cDNA expression libraries of cancer cells in filamentous and T7 bacteriophage have been used for identifying partners in protein–protein interactions. Such libraries are also exploited for the analysis of humoral immune responses in cancer patients. Serological analysis of cDNA expression libraries of human tumors with autologous serum (►SEREX) has been used for identifying relevant tumor antigens. The panning of tumor cDNA expression library on serum antibodies from cancer patients can select IgG-binding phages, which subsequent to phage DNA analysis helps in identifying cancer-specific proteins. Alternatively, the use of a phage-displayed peptide library for panning serum antibodies of cancer patients leads to the identification cancer-related ►epitopes that may have diagnostic and/or prognostic value. Phage display technology has also been used to isolate human antitumor antibodies. It is a powerful strategy in which phage-displayed scFv libraries constructed from the peripheral blood lymphocytes of cancer patients are panned against tumor cells. Such a screening of antibody repertoire of cancer patients helps in identifying the human monoclonal antitumor antibodies with clinical and research potential.

The knowledge of cancer-specific cell surface markers is very useful in targeted delivery of therapeutic and diagnostic agents to cancer cells; however, the heterogeneity in the expression and distribution of biomarkers among different cancer patients results into variable patient responses to targeted anticancer therapies. Phage display repertoire may be useful in identifying a panel of very specific cancer cell-binding ligands in high throughput manner without any prior knowledge of the cell surface. Such a panel of binding molecules reflects the expression of whole cell membrane markers and thus helps to generate functional profile of a tumor. Future technological advances may allow functional profiling of individual tumors rapidly in a cast-effective manner, which may lead to the development of ►personalized cancer therapy.

►Monoclonal Antibody Therapy

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Phagemid

Definition

Is a plasmid vector that carries within its ►sequence a ►bacteriophage replication origin (ori), desired phage capsid protein gene with appropriate cloning sites for fusion polypeptide, and a phage packaging signal. The phagemid encoding the polypeptide-capsid protein fusion is packaged into phage particles following infection of a host bacterium with a helper phage that supplies all the structural proteins.

►Phage Display

Phagocyte

Definition

A white blood cell that can engulf particles such as bacteria, other microorganisms, aged red blood cells,

foreign matter. Executes phagocytosis. The principal phagocytes include the neutrophils and monocytes. The prefix "phago" comes from the Greek "phagein," meaning "to eat."

Phagocytic Glycoprotein-1

►Adipose Tumors

Phagocytosis

Definition

A process by which cells, such as immune cells, bind to and internalize foreign objects, for example, bacteria by macrophages. Tissue macrophages are typical scavenger cells, which have the capacity to engulf other cells, cell debris, extracellular matrix, or foreign bodies by a mechanism termed phagocytosis. The intracellular phagosomes formed this way fuse with lysosomes leading to complete digestion of the engulfed material. Some aggressive tumor cells have the capacity to phagocytose neighboring normal cells and extracellular matrix, leading to tumor invasion and local spread. Refers to a journal to the process by which a particle contacts a cell, and the cell then invaginates its membrane around the particle and internalizes the phagocytic vesicle containing the nickel particle. This results in delivery of a large amount of particles inside the cell.

►Cystatins

Phakomatoses

Definition

Familial cancer syndromes that have the characteristic of benign hamartomatous tumors in common. Includes ►neurofibromatosis Type 1 and ►neurofibromatosis Type 2 (NF1, NF2), ►tuberous sclerosis complex (TSC), von Hippel–Lindau disease (VHL), ►nevoid basal cell carcinoma syndrome (NBCCS), ►Cowden syndrome, ►Peutz–Jeghers syndrome, juvenile polyposis (JP), and ►familial adenomatous polyposis (FAP).

Pharmacodynamics

Definition

Is the study of the biochemical and physiological effects of drugs and the mechanisms of drug action, and the relationship between drug concentration and effect.

►Hollow Fiber Assay
►Pharmacokinetics/Pharmacodynamics

Pharmacogenetics

Definition

Discipline in which DNA analysis is used to predict or explain the pharmacokinetic behavior of drugs. Used to indicate the analysis of a limited number of selected genes, known to be involved in drug metabolism. The field in which multiple genes and genetic variants are investigated in relation to drug metabolism or efficacy of drugs is termed ►pharmacogenomics.

Pharmacogenomics in Multidrug Resistance

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Definition

►Multidrug resistance in cancer is the major obstacle to long-term, sustained patient response to ►chemotherapy. It has been long recognized that the effectiveness of anticancer drugs can vary significantly among individual patients. It is obvious that the susceptibility of cancer cells to particular anticancer drugs cannot be predicted by a single factor but is determined by many factors that influence overall sensitivity. Cancer cells appear to have the capacity to generate variants resistant to anticancer drugs, as part of biological responses to external challenges. Tumors, and even individual cancer cells, can exhibit multiple mechanisms of resistance simultaneously. High-speed ►genotyping and the acquisition of clinical pharmacogenomic data will

be of importance to improve the efficacy and safety of pharmacotherapy of cancer.

Characteristics

Cancer is one of the gene-associated diseases, involving multiple factors in its cause and development. Despite enormous efforts spent on the development of cancer chemotherapies, these therapies are often effective only in a relatively small proportion of cancer patients. Acquired and intrinsic drug resistance in cancer is the major obstacle to long-term, sustained patient response to chemotherapy. It has been convincingly documented that several ATP-dependent drug transporters can cause drug resistance in cancer cells by actively extruding the clinically administered chemotherapeutic drugs. By far the best known major drug transporters, i.e., ABCB1 (P-glycoprotein/MDR1), ABCC1 (MRP1/GS-X pump), ABCC2 (MRP2/cMOAT), and ABCG2 (BCRP/MXR1/ABCP), have been characterized in detail with respect to their structure and function. These drug transporters belong to the ATP-binding cassette (ABC) transporter gene family.

To realize the promise of ►personalized medicine in cancer chemotherapy, it is critically important to understand the molecular mechanisms underlying inter-individual differences in drug response, namely, the pharmacological effect vs. side effect. The differences in response to medications are often greater among members of a population than they are within the same person or between monozygotic twins at different times. The existence of large population differences with small intra-patient variability is consistent with the idea of heredity inheritance as a determinant of drug response. It is estimated that genetic factors can account for 20–95% of variability in drug disposition and effects. ►Genetic polymorphisms in drug metabolizing enzymes, transporters, receptors, and other drug targets (*e.g.*, toxicity targets) are linked to inter-individual differences in the efficacy and toxicity of many medications.

The pharmacological effect of a drug depends on both pharmacodynamics (interaction with the target or the site of action) and pharmacokinetics (absorption, distribution, metabolism, and elimination). While drug metabolism has long been recognized as one of the major determinants of drug clearance and the factor that is responsible for individual differences in pharmacokinetics, it is now widely accepted that the drug transport process plays a major role in drug absorption, distribution, and elimination.

Pharmacogenomics has significantly contributed to our understanding of genetic causes underlying differences in drug metabolism (*e.g.*, cytochrome P-450 mediated drug metabolism). In fact, recent technological advances allowing massive DNA sequencing have in turn allowed us to identify single nucleotide polymorphisms (SNPs) as one possible cause of variable drug response among individuals. In light of such advances, it is important to carefully examine the clinical significance of polymorphisms in drug response genes.

Single Nucleotide Polymorphism (SNP)

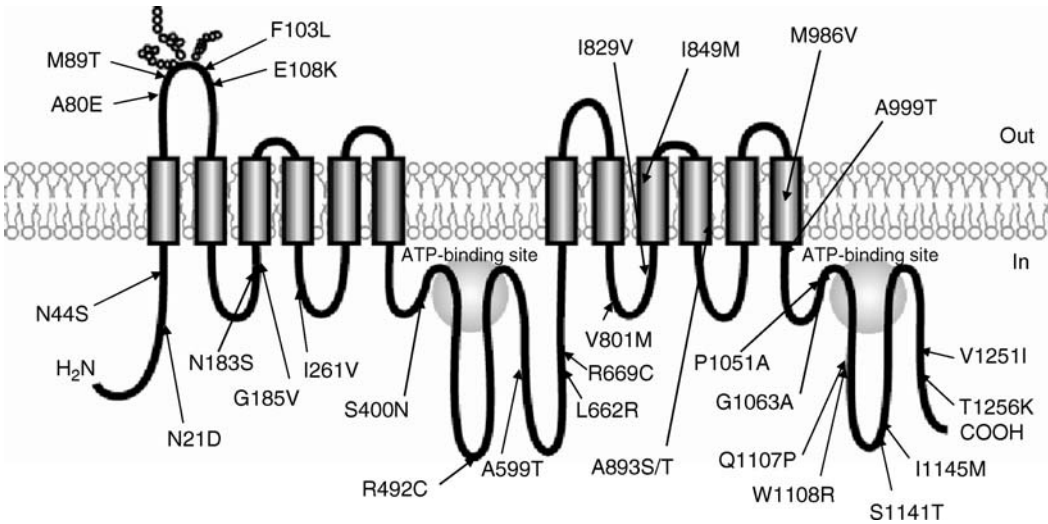
Genetic polymorphisms relevant in a population aspect are usually defined as variants present in more than 1%. It was originally estimated that single nucleotide polymorphisms (SNPs) in the human genome occur at a rate of 1 in every approximately 1,000 base pairs, a recent report indicates a much higher rate of polymorphism that averages one SNP in every 185 nucleotides. The rate of polymorphism within gene encoding regions was estimated at 3.4 per kilobase. Therefore, it is likely that a large number of coding region SNPs exist among drug transporter genes. Many of those SNPs are expected to cause amino acid changes that directly alter protein function. Furthermore, the rate of SNPs in non-coding regions such as promoter, introns, splice junctions, and 3′-untranslated regions is greater than within exons, suggesting that genetic polymorphisms invoking alterations in transcriptional activation and splicing may also prove to be relevant in determining the function of proteins encoded by the corresponding genes.

ABC Transporters

The ABC transporter gene family form one of the largest protein families encoded in human genome. Hitherto more than 48 human ABC protein genes have been identified and sequenced. It has been reported that mutations of ABC protein genes are causative in several genetic disorders in humans. Many of human ABC proteins are involved in membrane transport of drugs, xenobiotics, endogenous substances or ions, thereby exhibiting a wide spectrum of biological functions. Based on the arrangement of molecular structure components, i.e. nucleotide binding domains and topologies of transmembrane domains, hitherto reported human ABC proteins are classified into seven different sub-families (A to G). The HUGO Human Gene Nomenclature Committee developed a new system of nomenclature for the human ABC-transporter family. The new nomenclature scheme was implemented in 1999, and detailed information is available on the web site at http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html.

ABCB1 (P-glycoprotein/MDR1)

Human ABCB1 (P-glycoprotein/MDR1) was identified because of its overexpression in cultured cancer cells associated with an acquired cross-resistance to multiple anticancer drugs (Fig. 1). ABCB1 is expressed not only in cancer cells but also in many normal tissues. For



Pharmacogenomics in Multidrug Resistance. Figure 1 Schematic representation of ABCB1 and nonsynonymous polymorphism. The positions of amino acid substitutions are indicated by arrows. G185V is an acquired mutation.

example, it is located in the apical domain of the enterocytes of the gastrointestinal tract (jejunum and duodenum) and limits the uptake and absorption of drugs and other substrates from the intestine into the systemic circulation by excreting substrates into the gastrointestinal tract. In addition, ABCB1 is expressed in the endothelial cells lining the small vessels of the human cortex, in which the transporter appears to be concentrated within the luminal cellular compartment. The expression of ABCB1 on the luminal membrane of capillary endothelial cells of the brain restricts drug distribution into the central nervous system. This function of ABCB1 appears to be very important for protecting the central nervous system from attack by toxic compounds. However, such function becomes critical in the case of chemotherapy of brain tumors, since ABCB1 expressed in the ►blood–brain-barrier limits the penetration of anticancer drugs into the central nervous system.

To date, genetic variations of the human *ABCB1* gene have been most extensively studied. Hitherto, more than 50 SNPs and several insertion/deletion polymorphisms in the *ABCB1* gene have been reported. Preclinical and clinical studies have provided evidence for naturally occurring polymorphisms in ABCB1 and their effects on drug absorption, distribution, and elimination. It has been reported that the 2677G>T/3435C>T haplotype is of clinical importance. Recent reports as well as the NCBI dbSNP database show a tri-allelic polymorphism of ABCB1 (2677G>T/A) and one rare mutation (2677G>C). Functionally evaluation showed that nonsynonymous polymorphisms (2677G>T, A, or C) at amino acid position 893 (Ala>Ser, Thr, or Pro) have a great impact on both the activity and the substrate specificity of ABCB1. The polymorphisms of 2677G>T (893 Ala>Ser) is reportedly associated with high risk of lung cancer.

ABCC1 (MRP1/GS-X pump) and ABCC2 (MRP2/cMOAT)

Human ABCC1 (MRP1) gene is located on chromosome 16p13.1 and spans at least 200 kb consisting of 31 exons. The ABCC1 gene encodes a 1531 amino acid protein which has a molecular weight of 190 kDa in its mature glycosylated form. In the promoter region of the ABCC1 gene, there are a number of putative transcription factor motifs, such as the activator proteins AP1 and AP2 (Sp1), glucocorticoid response element (GRE), and also estrogen response element (ERE) and cAMP response element (CRE). Human ABCC2 (MRP2 or cMOAT) is a 1545 amino acid protein whose exon gene is located in chromosomal region 10q23–24. Although there is limited sequence similarity between ABCC1 and ABCC2 (49%), the primary structure and membrane topology of the two proteins are similar. In addition, the two transporters also have similar substrate characteristics. Both ABCC1 and ABCC2 transport a wide range of organic anions, including glutathione disulfide (GSSG), glutathione-metal complexes, glutathione conjugates, as well as glucuronate and sulfate conjugates.

Elevated expression of ABCC1 mRNA and/or protein levels have been observed in many multidrug resistant cancer cells. Transfection of ABCC1 cDNA in cultured cells resulted in enhanced resistance to many cytotoxic agents including doxorubicin, vincristine and VP-16. ATP-dependent transport of these

anticancer drugs can be enhanced by the presence of ►glutathione (GSH) in membrane vesicles prepared from ABCC1-overexpressing cells, suggesting that ABCC1 co-transports anticancer drugs and GSH. In patients, human colorectal cancers frequently overexpress ABCC1 and ►γ-glutamylcystein synthetase (γ-GCS), a rate-limiting enzyme of GSH biosynthesis, as compared to the surrounding normal tissue. The frequency of ABCC1 expression in carcinoma was higher than that in adenoma ($p<0.0001$). The ABCC1 up-regulation and the *p53* status were significantly correlated.

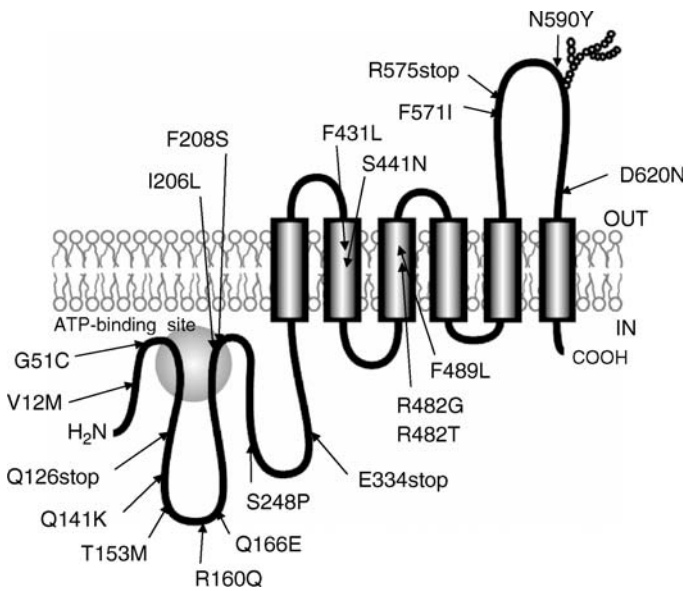
ABCG2 (BCRP/MXR1/ABCP)

Human ABCG2 (BCRP/MXR1/ABCP) is another member of the ABC transporter gene family, ant its overexpression in cancer cells is related with cellular resistance to anticancer drugs, such as mitoxantron, topotecan, and ►SN-38, an active metabolite of irinotecan (Fig. 2). SNPs of ABCG2 have been documented to be a significant factor in the patients' responses to medication and/or the risk of diseases. Sequencing of the ABCG2 gene from human samples has revealed over 80 different, naturally occurring sequence variations. Drug resistance profiles of ►Flp-In-293 cells expressing two major SNP variants, i.e., V12M, and Q141K, toward SN-38 demonstrated that the IC_{50} value for Q141K was about 50% of that for the wild-type (WT). The contributions of the minor SNP variants, i.e., F208S, S248P, F431L, S441N, and F489L, to drug resistance toward SN-38, mitoxantrone, doxorubicin, daunorubicin, or eroposide were significantly lower than that of WT.

The Q141K polymorphism located in exon 5 (c.421C>A) leads to the replacement of the negatively charged glutamic acid residue with a positively charged lysine residue. This polymorphism affects the ATP-binding domain, between the ►Walker A motif (amino acid residues 83–89) and the signature region (amino acid residues 186–189). The Q141K variant was also detected in all ethnic groups tested: the allele frequency ranged between 0% and 35%, (the Africans in North of Sahara, the Africans sub-Saharan, and African-American subjects with low; the Japanese and Chinese populations with high allele frequencies). The SNP (Q141K) was postulated to cause increased sensitivity of normal cells to anticancer agents that are ABCG2 substrates such as topotecan, diflomotecan, and SN-38.

Perspectives

Drug transporters as well as drug-metabolism play pivotal roles in determining the pharmacokinetic profiles of drugs and, by extension, their overall pharmacological effects. There are an increasing number of reports addressing genetic polymorphisms of drug transporters. Information is still limited, however, regarding the functional impact of genetic polymorphisms in drug transporter genes. Detailed functional analysis *in vitro* is critically important to provide clear insight into the biochemical and therapeutic significance of genetic polymorphisms. Functional validation of SNPs and



Pharmacogenomics in Multidrug Resistance. Figure 2 Nonsynonymous polymorphisms of human ABCG2. Schematic illustration of the structure of ABCG2 protein and the locations of amino acid changes. R482G and R482T are acquired mutations.

their linkage with clinical data would provide a new approach to individualized pharmacotherapy in the 21st century.

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Pharmacogenotype

Definition

Is the influence of genotype on the therapeutic and toxic responses to a drug.

►Personalized Cancer Medicine

Pharmacokinetic Profile

Definition

The study of the fate of a drug in the body as a function of time, in terms of absorption, metabolism, and excretion.

►Liposomal Chemotherapy

Pharmacokinetics

Definition

PK; Is the study of how a drug is absorbed, distributed, metabolised, and excreted over time.

►Pharmacokinetics/Pharmacodynamics

Pharmacokinetics and Pharmacodynamics in Drug Development

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Definition

Pharmacokinetics

►Pharmacokinetics (PK) is the study of how a drug is absorbed, distributed, metabolized, and excreted over time.

Pharmacodynamics

►Pharmacodynamics (PD) is the study of how a drug affects its target(s) in a dose- and time-dependent fashion.

Characteristics

To maximize the chance of successful drug development, a comprehensive knowledge of the compound under investigation is required. Assuming the drug target has been correctly selected, drug efficacy requires delivery into the patient such that adequate drug concentration is achieved within the plasma and tumor (measured by PK studies) to effect target modulation (measured by PD studies) resulting in anticancer effect(s). These principles have been incorporated into a pharmacological audit trail consisting of a series of questions that should be addressed during drug design and development. These can be summarized as:

- Is the drug target expressed in the tumor of interest?
- Are adequate plasma and tumor drug levels achieved?
- Does this level of drug exposure result in target modulation?
- Can effects be demonstrated on the biochemical pathway downstream of the target?
- Are the desired biological effects achieved, e.g., cell cycle arrest, induction of apoptosis or inhibition of angiogenesis?
- What are the therapeutic consequences of drug exposure and target modulation?

Related to these questions, it is valuable to determine if biomarkers of sensitivity or resistance can be identified.

From these questions it is obvious that PK and PD studies form an important part of the knowledge base required for modern drug development. The advantage

of using PK/PD biomarkers, and of implementing the pharmacological audit trail, is that decisions in drug development can be made on a rational basis, therefore making the process more effective.

Pharmacokinetics

It is usual to assume that the therapeutic and toxic effects of a drug are related to its concentration at the site of action. Previously it was estimated that around 40% of drugs failed clinical development due to poor PK properties. Inappropriate PK can lead to inadequate or variable drug exposure, and hence to lack of therapeutic activity or undesirable toxicity. This figure has fallen to around one in ten, due mainly to more thorough preclinical PK modeling.

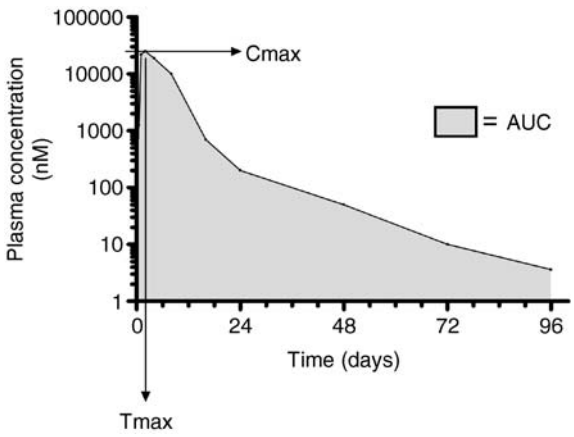
Early in drug discovery, physicochemical properties need to be addressed, in order to maximize the chance of the final molecule possessing appropriate drug-like properties. The Lipinski rule of five provides a useful guide. This is based on the fact that most marketed drugs have a molecular weight of less than 500 Da, partition coefficient cLog P < 5 (a measure of lipophilicity), <5 hydrogen bond donors, and <10 hydrogen bond acceptors. Algorithms can be applied and experiments performed to predict oral bioavailablity or metabolic liability as well as unwanted toxic effects.

Additional related studies that are carried out alongside preclinical PK include the potential for adverse interactions with cytochrome P450 enzymes, potentially leading to variable drug levels and drug-drug interactions, and with hERG channels, potentially predictive of side effects on the heart.

During preclinical testing it is valuable to determine PK in tumor-bearing animals where drug concentrations in plasma and tumor tissue can be determined after both single and multiple doses. These data can be used to predict human exposures and to guide initial clinical trials. The drug concentration measured over time is a function of:

- Drug entry into the body; for an oral drug this relates to absorption across the gastrointestinal tract or for intravenous delivery whether a bolus is given or an infusion over prolonged period
- Distribution into body tissues, including tumor
- Metabolism, most frequently by liver enzyme systems
- Excretion of drug, usually by the liver or kidneys

In patients, plasma samples are usually collected and analyzed over a time-course following drug administration. From these data it is possible to calculate maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}) and the area under the plasma drug concentration curve (AUC); Fig. 1 shows a hypothetical example. The time taken for half the drug to be eliminated from plasma can also be determined (t½).



Pharmacokinetics and Pharmacodynamics in Drug Development. Figure 1 Hypothetical graph plotting the concentration of drug in plasma versus time from start of an intravenous infusion. Key measured parameters are indicated, namely maximum concentration of drug (Cmax), time to reach Cmax (Tmax) and area under the curve (AUC).

Other PK variables can be derived from the data such as steady state volume of drug distribution (V_{SS}), total body clearance (CL) and oral bioavailability (F_{PO}) if applicable. V_{SS} is the amount of drug in the body divided by the drug concentration in plasma. Clearance of a drug is the volume of plasma from which the drug is completely removed per unit time. F_{PO} represents the fraction of the administered dose that reaches the systemic circulation.

Determination of a drug's PK properties facilitates the selection of the correct drug dose and administration schedule in patients. This requires consideration of:

- Preclinical information on the drug exposures (both concentrations and exposure times) required for the desired anticancer effect
- Concentrations (C_{max} and AUC) of drug and any metabolites in the circulation and ideally the tumor if data are available
- Protein binding, which may limit tissue uptake
- If applicable, oral bioavailability

An important objective is to determine if it is possible to achieve levels of exposure that would be required for a significant therapeutic effect.

The extent of PK studies performed in early clinical trials varies. In phase I testing where safety is paramount and few prior data are available, the relationship between drug dose administered and patient exposure (C_{max} and AUC) is examined and PK parameters may be linked to toxic and therapeutic events. Once the dose of drug for future study has been determined, it may be useful to perform additional studies to address specific

issues. Examples might be to determine possible effects of food on oral drug absorption or the impact on organ (e.g., liver or kidney) dysfunctions on the PK profile. Thorough absorption, distribution, metabolism and excretion (ADME) studies using radiolabeled drug in small groups of patients are sometimes beneficial but these are time-consuming and resource intensive as they require measurement of all input into, and output from, the patient.

In modern drug development, PK measurements are usually made by mass spectrometry coupled to liquid chromatography.

Pharmacodynamics

In association with PK data, PD biomarkers can be valuable in answering further questions in the pharmacological audit trail.

In the development of a modern molecularly targeted cancer drug, PK and PD biomarker assays will usually be developed during the preclinical discovery phase. These biomarkers are used to demonstrate proof of mechanism in model systems, i.e. target modulation, for example in human tumor cell lines and human tumor xenografts in immunosuppressed mice. Careful studies must be carried out to demonstrate that any PD assay is able to measure changes in the predicted endpoint, for example kinase inhibition. If sufficiently robust and reproducible, the assay can be applied to measure PD changes in human trials.

PD endpoints may be measured in surrogate normal tissue, such as buccal mucosa, skin, peripheral blood mononuclear cells (PBMCs) or hair follicles, which have the advantage of being readily available and safe to obtain. For example, in the development of the epidermal growth factor receptor (EGFR) inhibitors gefitinib and erlotinib, skin biopsies were used to provide surrogate tissue to confirm inhibition of phosphorylation of EGFR itself and of downstream markers such as ERK 1/2, as measured by immunohistochemistry.

Although PD data from surrogate normal tissue can be valuable, the gold standard remains to measure the effects of the drug in the tumor. However, the acquisition of tumor tissue is complicated by logistical and ethical issues. Hence there is increasingly a need for minimally invasive techniques such as functional imaging, e.g., using positron emission tomography (PET) scanning. Use of serum markers or circulating tumor cells is also attractive.

Demonstrating the PK-PK-Clinical Relationship

Based on the premise that to obtain a beneficial clinical effect (e.g., tumor stabilization or shrinkage) a drug must be effective on its target (assuming the target is a bona fide anticancer target), it is valuable to define a relationship between PK, PD and clinical effect. As set out in the pharmacological audit trail, adequate drug exposure must occur in plasma and tumor which is measured by PK studies (using plasma). Both concentration and time of exposure may be important.

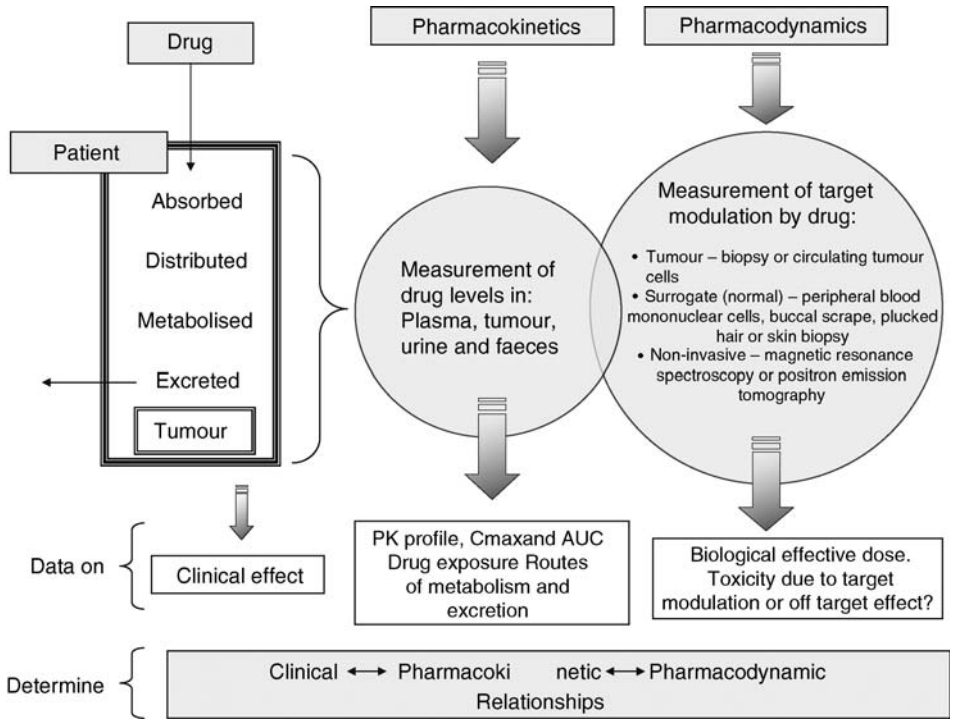
Drug effects on the target are determined using PD endpoints. Fig. 2 summarizes the integration of PK and PD studies with examples of substrates for analysis. The approach is exemplified in the development of heat shock protein 90 (HSP90) inhibitors such as tanespimycin 17-Allylamino, 17-Demethoxygeldanamycin (17-AAG).

A series of orchestrated interactions between the molecular chaperone HSP90 and other proteins (client proteins) occur within a cell to maintain normal cellular functions. Inhibition of HSP90 function disrupts these interactions and targets client proteins, many of which are oncogenic (e.g., CDK4, ERBB2, CRAF or BRAF) for destruction by the proteasome. Other heat shock proteins, such as heat shock protein 70 (HSP70), are induced in response. These changes can be detected by methods including western blotting or enzyme linked immunosorbant assay (ELISA). To follow the PD effects of HSP90 inhibition by tanespimycin, a panel of relevant proteins was chosen, including CDK4, CRAF, ERBB2 and HSP70. These were based on candidate markers derived from early mechanistic work, cDNA microarray gene expression and proteomic profiling studies. Preclinical determination of PK-PD-tumor response relationships were undertaken in a human tumor xenograft model. PK studies in these models confirmed that tanespimycin concentrations were achievable, in both plasma and tumor, that had been shown to inhibit proliferation of tumor cells in culture. PD measurements proved HSP90 inhibition by demonstrating that the client proteins CRAF, CDK4, and ERBB2 were depleted and that HSP70 was induced, as predicted. These changes in protein expression can be regarded as a pharmacological signature of HSP90 inhibition after being reproduced in multiple models and by several independent investigators.

PBMCs were investigated as a surrogate normal tissue in which to measure HSP90 inhibition. Previously, reversible occurrence of the PD signature of HSP90 inhibition was confirmed in PBMCs from mice with human tumor xenografts, as well as ex-vivo human PBMCs exposed to tanespimycin. Similar HSP0 inhibition was seen in the xenograft tumor tissue, and then subsequently in tumor biopsies from treated patients.

Building on the preclinical data, multiple phase I trials using a variety of dosing schedules with tanespimycin demonstrated that it was possible in patients to:

- Achieve adequate plasma concentrations of tanespimycin and metabolites compared to preclinical models



Pharmacokinetics and Pharmacodynamics in Drug Development. Figure 2 Summary of the role of PK and PD in drug development.

- Inhibit HSP90, based on the PD signature
- Observe early indicators of antitumor activity, e.g., tumor stabilization
- Demonstrate that toxicity was manageable

HSP90 inhibitors, such as tanespimycin, are now in Phase II and III clinical trials and future studies will aim to complete unanswered questions, such as how to determine which patients will respond and how tumors might develop resistance, in order to guide clinical use of these agents.

These studies of HSP90 inhibitors provide scientific validation of PK and PD biomarker assays. In addition, it is increasingly important that validation is performed to the requisite, technical level to ensure compliance with regulatory requirements. This should be done in a phased, fit for purpose fashion.

Although current PD studies mainly involve measurement of a single marker or a small group of them, more complex measurements can also be made, including global mRNA, proteomic and metabonomic profiling.

Conclusions

A comprehensive understanding of a drug's properties involves study of both PK and PD. Both the safety and success of clinical trials are reliant to a large extent on such information. Use of PK and PD biomarkers provides a rational basis for drug development and increases the likelihood of a successful outcome.

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Pharmacophore

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Definition

The precise arrangement of atoms, groups, or functionalities in a small molecule required for specific interactions with its biological target and its activity.

Characteristics

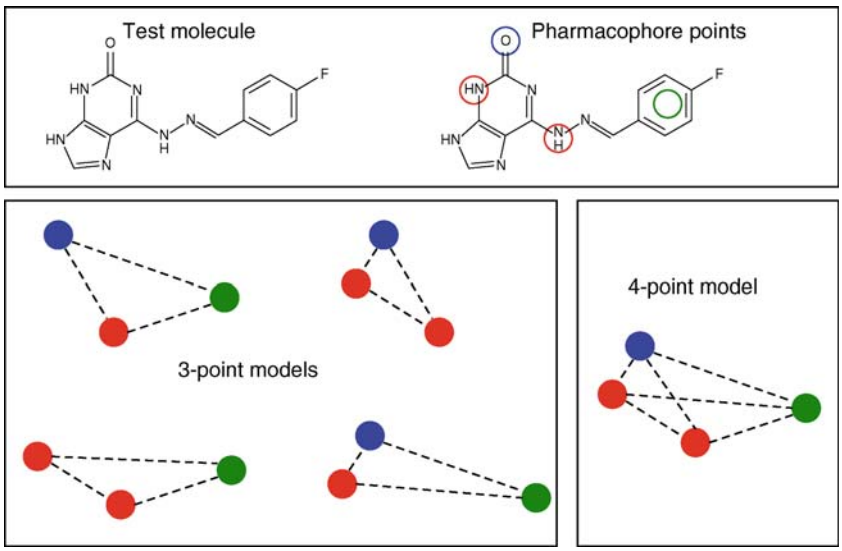
Specific binding to biological targets, mostly proteins, is a necessary but not sufficient requirement for the biological activity of small molecules and their ability to ultimately become drugs (►Small molecular drugs). Pharmacological parameters (►Pharmacokinetics/pharmacodynamics) and ADMET properties (►Absorption, ►distribution, ►metabolism, ►excretion, ►toxicity, ►ADMET screen) are equally important determinants for synthetic compounds or natural molecules to become drug candidates. However, specific target-ligand interactions (►Druggable target) are a pre-requisite of inhibitory, antagonistic, or agonistic effects of small molecules. The discovery of such interactions and their exploration present an essential starting point for drug development. The pharmacophore concept, formulated in the 1970s, aims to define critical interactions at the small molecule level, often without detailed structural knowledge of the underlying receptor-ligand interactions. Together with ►QSAR (►Quantitative structure activity relationship) and molecular similarity analysis, the pharmacophore concept is one of the major paradigms in ligand-based drug design (►Drug design).

In general terms, a pharmacophore is best defined as the arrangement of those substructures or functional groups in an active compound that are crucial for its activity. Pharmacophores can be derived from two- or three-dimensional (2D, 3D) molecular representations, but 3D-pharmacophores are most popular (considering the fact that molecules are active in three dimensions). Accordingly, a 3D-pharmacophore refers to the precise spatial arrangement of activity-determining parts of a molecule. This geometric arrangement is a consequence of the conformation and orientation of a ligand within its receptor binding site. Although experimentally determined structures (►X-ray crystallogaphy, ►nuclear magnetic resonance) of receptor-ligand complexes would provide the best possible basis to derive pharmacophore models, they are often not available. In fact, as a ligand-based approach, pharmacophores are most often deduced from active compounds. This is typically accomplished by comparing the structures of series of analogs or more distantly related compounds and predicting their binding conformations. As a consequence, accurate prediction of the bioactive conformation of a small molecule is critically important for the validity of a pharmacophore model and often presents a major bottleneck.

An important advancement of the pharmacophore concept has been the transition from an atom-centric representation to pharmacophoric functions. This means that important atoms or groups are replaced with different "features," which represent functionalities important for binding. The most relevant pharmacophore features include hydrogen bond acceptors (e.g. hydroxyl or carbonyl groups), hydrogen bond donors (e.g. hydroxyl or amide groups), positively charged (e.g. guanidines) or negatively charged groups (e.g. carboxylates), and hydrophobic (e.g. cyclohexyl or isopropyl groups) or aromatic moieties (e.g. phenyl ring or other aromatic ring systems). In pharmacophore models, features are represented as points; for example, for five- or six-membered rings, the centroid position is used as a point. Feature points are separated by inter-feature distances, which represent the second major component of pharmacophore models. The combination of features and inter-feature distances captures the chemical nature of a pharmacophore and its geometric arrangement.

As any predictive approach, pharmacophore modeling has intrinsic limitations. First, pharmacophores implicitly account only for direct binding interactions and not other parameters that determine protein-ligand interactions such as, for example, de-solvation or entropic effects. Furthermore, pharmacophore models are by design qualitative in nature; they do not take the relative strengths of binding contributions into account and do not include other energetic parameters. Therefore, pharmacophores are best rationalized as a ligand-centric approximation of short-range interactions and molecular complementarity.

Originally, most pharmacophore models were three-point pharmacophores but during the late 1990s, they were extended to 4-point pharmacophores. Thus, the three or four most important centers for binding are extracted from a molecule and their pairwise intramolecular distances are measured. Accordingly, a three-point pharmacophore is determined by three feature points and three inter-feature distances and a four-point pharmacophore by four features and six inter-feature distances. Distances between feature points are usually divided into different ranges or bins, for example, six to ten distinct intervals per inter-feature distance. This is done to permit a certain degree of flexibility in evaluating pharmacophore patterns, taking into account, for example, that corresponding hydrogen bonds might have slightly different lengths or that differently sized rings or groups might engage in similar interactions (a concept termed "bioisosterism") (Fig. 1).



Pharmacophore. Figure 1 Model pharmacophore. For an arbitrary test molecule, pharmacophore points and features are assigned (red, hydrogen bond donor; blue, hydrogen bond acceptor; green, aromatic center) and potential three-point pharmacophores combining these features and the corresponding four-point pharmacophore model are shown. Dashed lines are inter-feature distances.

Pharmacopohore models containing more than four features can, in principle, be easily defined, but there are both conceptual and practical reasons to limit the number of feature points. First, only a few parts or functional groups of a small molecule and the interactions they form determine binding and biological specificity. This suggests that one should be conservative in assigning features and not artificially complicate models. Second, from a practical point of view, a systematic evaluation of potential pharmacophore patterns is currently computationally infeasible for pharmacophores containing more than four feature points and increasing numbers of inter-feature distances, as further discussed below.

Major applications of pharmacophore models include 3D database searching, the design of active compounds and target-focused libraries (►Combinatorial libraries), and the derivation of models for 3D-QSAR. Searching databases of 3D structures of small molecules has been one of the original applications of pharmacophores and continues to be a major focal point. For database search calculations, pharmacophore queries consist of specific features and distance constraints. The goal of 3D database searching is the identification of molecules that meet the pharmacophore constraints but are chemically distinct from the query compounds. For these applications, it is important that pharmacophore queries have some degree of flexibility in terms of features and, in particular, inter-feature distances. This rationalizes the introduction of distance binning schemes, as described above. A pharmacophore search using single values for inter-feature distances would be overly rigid and likely fail to produce hits because no database compound might exactly fit all the distance constraints, although some molecules might share the pharmacophore arrangement. In addition to database searching, another original application of pharmacophores has been the design of analogs of active compounds. Once a pharmacophore has been derived from a series of active molecules, medicinal chemists often utilize 2D pharmacophore representations and apply chemical knowledge to predict compound modifications that are consistent with the pharmacophore (e.g. bioisosteric replacements) but perhaps lead to an improvement in the biological activity of lead compounds or their ADMET properties.

Pharmacophore models have increasingly been used to design combinatorial libraries that are focused on biological target families. Among others, important target families for pharmacophore-based library design include G-protein-coupled receptors (►G-proteins, ►GPCRs) and protein kinases (►Tyrosine kinase). In both cases, so-called "privileged" structural motifs have been identified that are highly recurrent in active compounds. For GPCRs, which represent approximately 50% of current drug targets, abundant information about active small molecules is available, but very little target structure information, which is a typical application scenario for pharmacophore modeling. Combinatorial or other compound libraries can be focused on target families such as GPCRs by using pharmacophore models derived from known ligands as filters for the evaluation of candidate compounds. By filtering out compounds that do not fit pharmacophore constraints and accepting those that do, libraries can be significantly enriched with molecules having an increased probability to be active. In

addition to pharmacophore filters, specialized pharmacophore models have been introduced for the purpose of compound design. These models are used to orient substituents relative to core structures of molecules in order to fulfill pre-defined pharmacophore requirements and then select suitable building blocks (scaffold and R-groups) for combinatorial synthesis. Finally, pharmacophore models also serve as starting points for the generation of 3D-QSAR models and the calculation of descriptors that form steric or electrostatic fields surrounding compounds having similar activity. In 3D-QSAR, pharmacophores serve as anchor points for the generation of compound conformations and their spatial alignment.

As stated above, a principal limitation of pharmacophore modeling is the difficulty to accurately predict bioactive conformations of small molecules from first principles. A prediction is typically attempted on the basis of systematic conformational analysis of active compounds and classification and energetic evaluation of sets of similar conformations. Given these uncertainties, modifications of the pharmacophore concept have been introduced that alleviate the need to predict single bioactive conformations. This has been accomplished by a systematic exploration and comparison of potential pharmacophore patterns in molecules, which is called "pharmacophore fingerprinting" and requires the generalization of pharmacophore models. Generalization involves the assignment of alternative features to each point in a three- or four-point pharmacophore (i.e. the six features described above) and the division of inter-feature distances into different intervals. Alternative features and distance ranges are systematically combined and the presence or absence of each unique combination in a molecule is examined using a conformational ensemble and recorded in a bit string format (where "1" indicates its presence and "0" its absence). The overlap between the resulting pharmacophore fingerprints of different molecules is quantified using a similarity metric. The more pharmacophore patterns two molecules share, the more similar they are, at least in "pharmacophore space."

Pharmacophore fingerprinting is computationally by far the most expensive pharmacophore calculation because distance and feature combinations can quickly become very large. For example, given six alternative features at each point and ten distance intervals for each inter-feature distance, a three-point pharmacophore model would produce 33,000 distinct pharmacophore arrangements, whereas a corresponding four-point model would produce 9.7 million potential pharmacophores. This explains why systematic exploration of possible pharmacophores is currently limited to four-point models. Furthermore, for each test molecule in a database a complete conformational search must be carried out as a pre-requisite for pharmacophore fingerprinting, which adds to the computational expense of large-scale database searching. Other recent extensions of the pharmacophore concept include the introduction of "fuzzy" features that further generalize or simplify pharmacophore functions. The goal of such efforts is to enable pharmacophore searching to identify molecules having increasingly diverse structures but similar binding characteristics and activities.

Since their introduction, pharmacophores have played an important role in medicinal chemistry and small molecule-based drug design. Pharmacophore models of very different complexity have been successfully applied on a case-by-case basis, ranging from simple "paper drawn" 2D pharmacophores to elaborate 3D pharmacophore fingerprints that literally capture millions of potential pharmacophore patterns. Pharmacophore modeling continues to be a highly active area in computer-aided pharmaceutical research, as exemplified by recent advancements such as the introduction of fuzzy pharmacophores or the derivation of complementary pharmacophores from binding site features of target proteins.

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Pharmafoods

►Nutraceuticals

Phase I and II Clinical Trials

Definition

The clinical development of a new drug or treatment is along a series of clinical trials called Phase I, II, and III. In Phase I clinical trials, a new drug or treatment is tested in a small group of people (20–80) for the first time to evaluate its safety, determine a safe dosage

range, and identify side effects. In Phase II clinical trials, the study drug or treatment is given to a larger group of people (100–300) to see if it is effective, i.e. if it works against a particular disease like cancer, and to further evaluate its safety. Phase III trials include a large group of patients (often more than 1000) comparing a new treatment to standard treatment.

►Clinical Trial

Phase 1 Enzymes

Definition

A class of endogenous enzymes, able to activate or deactivate carcinogens.

►Resveratrol

Phase I Metabolism

Definition

Enzymatic reactions, including oxidations, reductions and hydrolyses, that create sites for future conjugations to facilitate excretion of a drug.

►Lead Optimization

Phase II Biotransformation Enzymes

Definition

Catalyze the chemical conversion of compounds into metabolites that are generally more water soluble.

►Arylamine *N*-Acetyltransferases (NAT)

Phase 2 Detoxification Enzymes

Definition

Phase 2 Enzymes

►Phase 2 Enzymes

Phase 2 Drug-Metabolizing Enzymes

Definition

Phase 2 Enzymes

►Phase 2 Enzymes

Phase 2 Enzymes

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Synonyms

Phase 2 detoxification enzymes; Phase 2 drug-metabolizing enzymes; Phase 2 proteins

Definition

Phase 2 enzymes in principle are part of the cellular ►biotransformation machinery. Cellular biotransformation of ►xenobiotics and endobiotics, including ►carcinogen metabolism, may be divided into two sequential phases: phase 1 (oxidation, reduction and hydrolysis reactions) and phase 2 (conjugation reactions). Phase 2 enzymes traditionally refer to the enzymes catalyzing the conjugation reactions, such as ►glutathione *S*-transferase (GST), UDP-glucuronosyltransferase (►UGT), *N*-acetyltransferase (NAT) and ►sulfotransferase (SULT). However, the term has gradually become broader in scope as it has also been used by an increasing number of investigators for a number of enzymes that catalyze phase 1 reactions, such as epoxide hydrolase (►EH), heme oxygenase-1 (►HO-1), NAD(P)H:quinone oxidoreductase type 1 (►NQO1), and enzymes that are not directly involved in the biotransformation process, such as glutamate cysteine lygase (►GCL) which is critical in glutathione biosynthesis. The term "phase 2 proteins" is more inclusive, as it also refers to certain antioxidant proteins that have no known catalytic activities such as ►ferritin, which is the main intracellular iron storage protein. A major reason for this seemingly ambiguous classification is that many of these enzymes/proteins are often coordinately induced by a variety of chemical agents through the Keap1-Nrf2-ARE signaling pathway, as will be briefly described later, and that all of them play important roles in cytoprotection and cancer prevention. However, some "classic" phase 2 enzymes such as NAT and SULT are not known to be regulated by this signaling pathway. Examples of Keap1-Nrf2-ARE-regulated phase 2 genes include EH, ferritin, GST, GCL, HO-1, NQO1 and UGT.

Characteristics

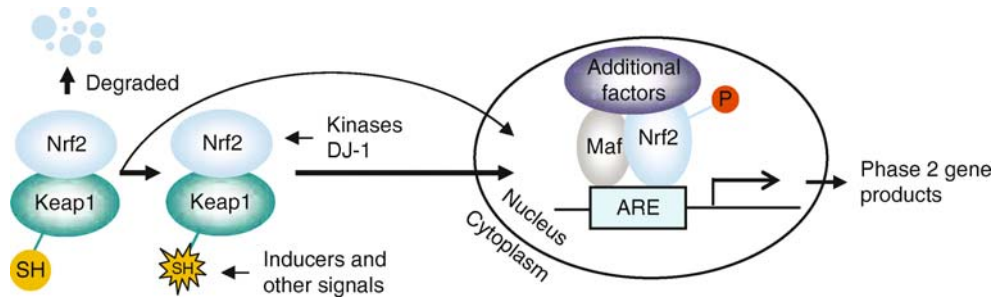
Many phase 2 enzymes/proteins have multiple isoforms. For example, GST is a super family of enzymes comprised of cytosolic (five known classes: alpha, mu, pi, theta and zeta), mitochondrial (kappa) and membrane-bound members; UGT is divided into two families: UGT1 and UGT2, each of which comprises a long list of isoforms; NAT has two isoforms (NAT1 and NAT2), and eleven human SULT isoforms are known; there are three known isoforms of heme oxygenase (HO-1, HO-2 and HO-3) and two isoforms of NAD(P)H:quinone oxidoreductase (NQO1 and NQO2). Moreover, a number of phase 2 genes are polymorphic in humans, and the genetic variants may yield either a null or sub-optimal phenotype. The best-known examples of genetic variants regarding this family of enzymes are GST mu1-null, GST theta1-null, NQO1-null, and NAT1/NAT2 slow acetylators.

Phase 2 enzymes are major ►detoxification enzymes and an important part of cellular defense against carcinogens, oxidants and other toxic chemicals. The cancer-preventive role of many phase 2 enzymes has been well documented. For example, epidemiological studies have shown that individuals deficient in a phase 2 enzyme (e.g. GST, NAT or NQO1) are at considerably higher risk of developing certain cancers; likewise, susceptibility to cancer increases in rodents if a phase 2 gene (e.g. GST or NQO1) is knocked out; moreover, forced over-expression of a phase 2 enzyme through gene transfection in cultured cells protects cells against carcinogen-induced DNA damage and/or cytotoxicity. However, it is also known, perhaps not surprising in view of their cytoprotective activities, that some phase 2 enzymes (e.g. GST pi-1) have been shown to contribute to drug resistance in cancer cells. Moreover, it has also been reported that catalysis of certain substrates by a phase 2 enzyme may actually result in bioactivation of the compounds. For example, several anticancer quinones (e.g. ►mitomycin C and streptonigrin) depend on NQO1 for bioactivation and anticancer activity.

Although the mechanisms by which each phase 2 enzyme prevents cancer are complex and not fully understood, it is clear that cellular protection offered by these enzymes as a group is versatile and far-reaching. For example, enzymes such as GST and UGT have low substrate specificities, each of which is capable of catalyzing the detoxification of a diverse group of carcinogenic chemicals; GCL is the major biosynthetic enzyme of GSH, which serves as an important cellular antioxidant and is also critical for the normal function of GST and other enzymes; NQO1 not only detoxifies carcinogenic quinones but also stabilizes tumor suppressor p53 and strengthens cellular antioxidant capacity. It is important to consider phase 2 enzymes as a group, because many of them not only are readily inducible but also are often coordinately induced in cells and tissues both in vitro and in vivo by chemical inducers. Many cancer chemopreventive agents (or ►chemoprotectants), including dietary ►phytochemicals (e.g. certain isothiocyanates and polyphenols), are inducers of these enzymes, and a number of phase 2 inducers (e.g. oltipraz and ►sulforaphane) when tested in animal models inhibited tumor development. It is now a widely held view that dietary or pharmacological induction of phase 2 enzymes is an important strategy for prevention of chemical carcinogenesis.

Regulation

While each individual phase 2 gene may potentially be subjected to regulation through multiple mechanisms, it is the Keap1-Nrf2-ARE signaling system (Fig. 1) that unifies many phase 2 genes and is the most relevant to cancer prevention. ►Nrf2, nuclear factor erythroid 2-related factor 2, is a transcription factor that is normally sequestered by its repressor ►Keap1, Kelch-like ECH-associated protein 1. Binding to Keap1 promotes proteasomal degradation of Nrf2. Dissociation of Nrf2



Phase 2 Enzymes. Figure 1 A brief scheme for the induction of phase 2 genes through the Keap1-Nrf2-ARE pathway. Binding of Keap1 to Nrf2 in the cytoplasm targets Nrf2 for proteasomal degradation, but some Keap1-Nrf2 complexes may translocate to the nucleus, where Nrf2 may be released. Disruption of the association of Keap1 with Nrf2 by phase 2 inducers or other stress signals, through interaction with critical Keap1 cysteine residues, Nrf2 phosphorylation by kinases, and Nrf2 stabilization by DJ-1, causes increased nuclear accumulation and ARE binding of Nrf2, leading to increased transcription of phase 2 genes and increased cellular protection.

from Keap1 allows the former to heterodimerize with partners such as small muscle aponeurotic fibrosarcoma (Maf) protein, bind to a *cis*-acting DNA regulatory element, and promote transcription of the downstream gene. The DNA regulatory element to which the Nrf2 hetrodimer binds and activates is termed the antioxidant response element (►ARE) or electrophile response element (►EpRE). This element was so named because it was initially discovered to respond to phenolic antioxidants but subsequently found to respond broadly to agents that contain an electrophilic center. One or more copies of the typical ARE sequence (5′TGACnnnGC-3′, where n represents any nucleotide) is known to exist in the 5′-flanking region of phase 2 genes including GCL (both catalytic and regulator subunits), several GST isozymes, HO-1, NQO1, and ferritin heavy subunit. A functional ARE may also operate in the following phase 2 genes: EH, ferritin light subunit and UGT (1A1, 1A6 and 1A10), as it was shown that Nrf2 deletion or silencing inhibited both basal and inducible response of EH and UGT genes and that the genes for both ferritin heavy and light subunit were induced by phase 2 inducer 1,2-dithiole-3-thione. However, to the knowledge of this author, there has been no report that Nrf2 may up regulate HO-2, HO-3, NAT1, NAT2, NQO2 or SULT isoforms. Interestingly, there seems to be a certain degree of degeneracy in the ARE sequence, as a recent study of mouse NQO1 gene showed that the ARE spans 24 bp and comprises 5′-GAGTCACAGTGAGTCGGCAAAATT-3′, where the underlined nucleotides are those with mutation resulting in a complete loss of its function.

While the traditional view about Nrf2 activation has been that Nrf2 is repressed by Keap1 in the cytoplasm and that inducers cause dissociation of the complex leading to Nrf2 nuclear translocation and ARE activation, more recent studies have revealed the presence of a cytoplasmic-nuclear shuttling mechanism of Keap1-Nrf2 complex and indicated that dissociation of the complex within the nucleus might contribute to Nrf2/ARE activation. Studies show that Nrf2 dissociation (activation) from Keap1 occurs upon alkylation or oxidation of critical cysteine residues on Keap1 by chemical activators or other signals. Two cysteine residues in mouse Keap1 (C273 and C288) were shown to be critical for the inducer-sensing function of Keap1 and for its Nrf2-repressing activity. Electrophilic inducers are believed to directly interact with these cysteine residues, causing conformational change in Keap1 and subsequent release of Nrf2. However, other studies show that modifying specific cysteines of Keap1 might be insufficient for Nrf2 activation, and additional mechanisms contribute to Nrf2 activation. It was shown that phosphorylation at Ser40 in Nrf2 by protein kinase C promoted Nrf2 dissociation from Keap1. Additional factors, including p160 family coactivators and cyclic AMP-responsive element binding protein/p300 factors, appear to bind to Nrf2-Maf-ARE complex and enhance transcription. Kinase such as mitogen activated protein kinases and phosphatidylinositol-3-kinase also were shown to play a role in Nrf2 activation, although detailed information is not yet available. Moreover, DJ-1/PAMK7, a cancer- and Parkinson's disease-associated protein was recently reported to stabilize Nrf2.

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Phase II Metabolism

Definition

A conjugation reaction that links a endogenous moiety to a Phase I metabolite or polar functionality on an unmetabolized drug to facilitate its excretion.

►Lead Optimization

Phase 2 Proteins

Definition

Phase 2 Enzymes.

►Phase 2 Enzymes

Phenomics

Definition

Is the systematic and detailed collection, objective documentation and cataloguing of phenotypic data at many levels, including clinical, molecular and cellular phenotype.

►Personalized Cancer Medicine

Phenotype

Definition

The physical properties (e.g., eye color and height) of an organism or the detectable manifestation of a specific trait (e.g., disease and DNA repair), as distinguished from genotype (the genetic makeup of a cell, either of the entire cell or more commonly for a certain gene or a set of genes). The entire physical and biochemical constitution of an organism as determined both genetically and environmentally.

►Mutagen Sensitivity

Phenotype-Orientated Screens

Definition

A procedure in which molecules are systematically tested for their ability to produce or inhibit a biological process or characteristic of interest.

►Small Molecule Screens

Phenylalanine Mustard

Definition

►Melphalan.

Phenylalanine Nitrogen Mustard

Definition

►Melphalan.

Phenylaminopyrimidine

Definition

Chemical structure of ►imatinib and ►nilotinib.

Pheocromocytoma

Definition

Synonym: Pheocromocytoma; Is a rare tumor in the adrenal glands that causes excess secretion of catecholamines. This leads mainly to hypertension but can also lead to hyperglycemia and diabetes-like symptoms. Only about 1 in a 1000 people with hypertension have a pheochromocytoma

►Adrenomedullin

Philadelphia Chromosome

Definition

Refers to the chromosome 22 lacking approximately half of its long arm due to a reciprocal translocation between the long arms of chromosomes 9 and 22 [t(9;22)(q34;q11)], resulting in a chimeric gene (►BCR ABL1). The fusion protein product is a constitutive tyrosine kinase that alters signaling pathways that control the proliferation, survival, and self-renewal of hematopoietic stem cells. The Philadelphia chromosome is found in chronic myeloid leukemia (CML) cells in nearly every patient with this disease, and a subset of patients with acute lymphoblastic leukemia (ALL), where it connotes a worse prognosis.

►Chronic myeloid leukemia

Phlegm Dampness

Definition

Is a term in Chinese medicine. It refers to phlegm produced from the accumulation of turbid fluid retained in the body. Different from the concept of Western medicine, spleen represents a macroscopic concept of digestion, absorption, and nutrition metabolism in Chinese medicine. Phlegm dampness often results from the dysfunction of spleen in transporting and distributing nutrients and water, as well as the accumulation and stagnation of dampness due to deficient spleen. It is manifested as profuse frothy sputum, nausea, or fullness in the chest, cough with dyspnea, and plump tongue with slippery or greasy coat.

►Chinese versus Western Medicine

Phorbol Ester

Definition

Belongs to a class of tumor promoters that stimulate protein kinase C by acting as analogs of diacylglycerol. They can be used to stimulate T lymphocytes.

►TPA

Phorbol 12-Myristate 13-Acetate

Definition

PMA; synonym: 12-O-Tetradecanoylphorbol-13-acetate (TPA). A very potent tumor promoter and activator of protein kinase C.

►Protein Kinase C Family

Phosphatase and Tensin Homolog Deleted on Chromosome 10

Definition

PTEN; A tumor suppressor which acts as both a lipid and a protein phosphatase. PTEN gene is deleted or mutated in diverse human cancers including glioblastoma, endometrial, breast, and prostate cancers and dominantly inherited hamartoma syndromes such as Cowden and ►Bannayan–Riley–Ruvalcaba syndrome.

Phosphatase

Definition

Is an enzyme that removes a phosphate group from its substrate by hydrolyzing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group. This action is directly opposite to that of a ►kinase, which attach phosphate groups to their substrates by using energetic molecules like ATP. A common phosphatase in many organisms is alkaline phosphatase. Serine and threonine protein phosphates are stable under physiological conditions, so a phosphatase has to remove the phosphate to reverse the regulation. There are four known groups: PP1 (α, β, γ1, γ2), PP2A, PP2B (AKA calcineurin), PP2C, PP4, and PP5. The first three have sequence homology in the catalytic domain, but differ in substrate specificity. Ser/Thr-specific protein phosphatases are regulated by their location within the cell and by specific inhibitor proteins.

Phosphatidic Acid

Definition

An intracellular second messenger generated by the phospholipase D enzyme.

►Laminin Signaling

Phosphatidyl Inositol 3-Kinase

Definition

PI3k; PI3 Kinase; A kinase activated by a variety of receptor associated protein tyrosine kinases that phosphorylates inositol lipids in the plasma membrane.

Such phosphatidyl inositols can then serve as docking sites for proteins with pleckstrin homology domains.

►PI3k Signaling

Phosphatidylinositol-3,4,5-trisphosphate

Definition

Second messenger produced by the enzyme phosphoinositide-3 kinase through the addition of a phosphate group to the 3 position in the inositol ring of phosphatidylinositol-4,5-bisphosphate (PIP_2).

►Pentakisphosphate

Phosphatidylinositol 3-kinase

►PI3K Signaling

Phosphatidylinositol Lipids

Definition

A family of molecules present in cellular membranes. They contain a polar phosphate head group attached to an inositol sugar, a glycerol backbone and two hydrophobic fatty acid tails. The conversion between phosphatidylinositol lipids forms an important step in several signaling cascades.

►Pentakisphosphate
►Inositol Lipids

Phosphatidylserine

Definition

An abundant acidic phospholipid at cell membranes, capable of activating ►protein kinase C. Phosphatidylserine transfer to the external surface of the plasma membrane is a key signal of apoptotic cell recognition for phagocytes.

►Protein Kinase C Family

Phosphodiesterase

Definition

Group of enzymes which hydrolyze the phosphodiester bond in cyclic nucleotides like cAMP and cGMP to form AMP or GMP. Main inactivation mechanism for cyclic nucleotides involved in signal transduction

►G-Proteins

Phosphoinositide-Dependent Kinase-1

Definition

PDK-1; Enzyme possessing a ►pleckstrin homology domain able to target it to intracellular domains rich in PIP_3 where it can activate Akt by phosphorylating it on a threonine amino acid residue. By its activation of ►Akt, PDK-1 can be involved in carcinogenesis.

►Pentakisphosphate

Phosphoinositide 3-kinase

►PI3K Signaling

Phosphoinositides

Definition

Are glycerophospholipids containing a phosphorylated inositol polyalcohol chain at the sn-3 position of glycerol. They are source either of inositoltrisphosphate and diacylglycerol upon hydrolysis by phospholipase C or phosphoinositide-3,4,5-trisphosphate (PIP3) upon phosphorylation by PI3kinase.

►Lipid Mediators

Phospholipase

Definition

PL; Is an enzyme that converts phospholipids into fatty acids and lysophospholipids. There are four major families, termed A1, A2, C and D that specifically hydrolyse the ester linkage at a defined position of glycerol; PLA1 clives the chain at the sn-1, PLA2 at the sn-2 and PLC (before phosphate), PLD (after phosphate) at the sn-3 position of glycerol. Lysophospholipase D, called autotaxin specifically cleaves the phosphocholine moiety of lysophosphatidylcholine leading to lysophospatidic acid (LPA).

PLA2 cleaves arachidonic acid (also called arachidonate) a substrate for eicosanoids. PLC cleaves inositolbisphosphate from phosphatidylinositol and generates inositoltrisphosphate (IP3), instrumental in Ca^{2+} mobilization and diacylglycerol (DAG) involved in selective activation of isoforms of ►protein kinase C (PKC).

►Lipid Mediators
►Phospholipase A2

Phospholipase A1

Definition

PLA1; Is an enzyme that hydrolyzes the *sn*-1 fatty acids from phospholipids resulting in the formation of 2-acyl-lysophospholipids.

►Lysophosphatidylcholine

Phospholipase A_2

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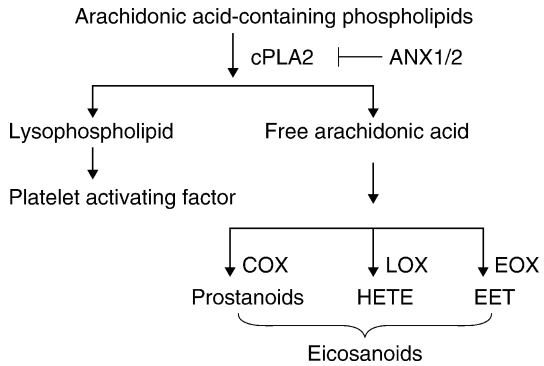
Definition

Phospholipase A_2 (PLA_2) enzymes are a family of proteins and to date at least 20 members have been identified in mammals. The family can be classified into four classes on the basis of their nucleotide and amino acid sequence homology. First, there are at present ten secreted phospholipase A_2 enzymes ($sPLA_2$-IB, -IIA, -IIC, -IID, -IIE, -IIF, -III, -V, -X, and -XII), which are of low molecular weight (13–18 kDa) with a catalytic histidine in their active site and a requirement for calcium for enzyme activity. Second, there are three characterized human cytosolic PLA_2 enzymes ($cPLA_2$-α, -β, and -γ, also known as Group IVA, IVB, and IVC PLA_2) that use a catalytic serine in their active site. $cPLA_2$-α and -β contain a C2 calcium binding domain and enzyme activity is calcium-dependent while $cPLA_2$-γ lacks this domain and is thus a calcium-independent PLA_2. Recently, a comprehensive homology search against the murine genome and EST databases using conserved sequences of $cPLA_2$ as the query, led to the identification of $cPLA_2$-δ, $cPLA_2$-ε, and $cPLA_2$-ξ (also known as Group IVD, IVE, and IVF PLA_2), all of which are calcium-dependent enzymes. Third, three calcium-independent cytosolic PLA_2 enzymes ($iPLA_2$-α, -β, and -γ also known as Group VIA-1, VIA-2, and VIB) with an active-site serine, and fourth, four platelet-activating factor acetylhydrolase (PAF-AH) enzymes (Group VIIA, VIIB, VIIIA, and VIIIB) also involve a catalytic serine. Many of the different forms of PLA_2 are differentially expressed in a tissue-, species-, and/or genotype-specific manner.

Characteristics

Function

Much research has focused on $cPLA_2$-α because of its central role in the initiation of arachidonic acid metabolism. $cPLA_2$-α enzymes catalyze the hydrolysis of the *sn*-2 position of membrane glycerophospholipids, leading to the production of free fatty acids and lysophospholipids (Fig. 1). If the esterified fatty acid is arachidonic acid (AA), this is converted to ►prostaglandins (PGs) by ►cyclooxygenases (COX), hydroxyeicosatetraenoic acids (HETEs) by lipoxygenases (LOX), or epoxyeicosatrienoic acids (EETs)



Phospholipase A_2. Figure 1 Diagram depicting the role of cytosolic $cPLA_2$-α.

P

by P450-dependent epoxygenase (EOX). By binding to ►G-protein-coupled receptors, these eicosanoids are central mediators of ►inflammation and could be mitogenic. The other reaction products of $cPLA_2$ action, lysophospholipids, are also biologically active via binding to ►G-protein-coupled receptors and, in some settings, are the precursors of platelet-activating factor.

A direct role for $sPLA_2$ in supplying arachidonic acid to downstream enzymes for eicosanoid synthesis has now been demonstrated in vivo. $sPLA_2$ enzymes have been implicated in a number of biological processes, such as ►inflammation and host defence. $sPLA_2$-IIA found in inflammatory fluids, tissue exudates, or serum, and is believed to propagate inflammation in response to proinflammatory cytokines including interleukin-1, ►interleukin-6, and tumor necrosis factor. A gene deletion experiment in mice shows that knockout of the *sPLA*$_2$-V gene reduces eicosanoid production in response to inflammatory stimuli. There are also reports describing the necessity of *sPLA*$_2$-V in eicosanoid production in murine mast cells.

Biological roles for the other $cPLA_2$ enzymes including the newly identified $cPLA_2$-δ, $cPLA_2$-ε, and $cPLA_2$-ξ have not yet been clearly defined. The Ca^{2+}-independent iPLA2 appears to play a role in phospholipid remodeling, regulation of store-operated calcium channels, ►apoptosis, and release of arachidonic acid.

Regulation

For cPLA2-α, Ca^{2+} binding to the N-terminal C2 domain is required for translocation of the enzyme from the cytosol to the endoplasmic reticulum, golgi apparatus, and perinuclear membranes – where $cPLA_2$-α associates with the preferred substrate, arachidonoyl phospholipids, resulting in eicosanoid biosynthesis, as well as brings close proximity to eicosanoid-producing enzymes such as COX and LOX. $cPLA_2$-α activity is also regulated by phosphorylation on serine residues with protein kinases including ►MAPK. The main site of cPLA2 phosphorylation is Ser-505 and replacement of Ser-505 with Ala abolishes agonist-stimulated arachidonate release.

Annexin 1 (ANX1, also known as lipocortin I and calpactin II) and annexin 2 (ANX2, also known as lipocortin II and calpactin I) are naturally inhibitors of $cPLA_2$-αin various cell types. There are 13 members in annexin family and inhibition of $cPLA_2$-α appears to be specific and not a general phenomenon for all ANXs. Although the exact mechanism for the inhibition of $cPLA_2$ is not clear, it is unlikely due to competition with cPLA2 for substrate. Some in vitro studies have shown that secreted phospholipase A_2 can also be inhibited by annexins due to substrate sequestration by annexins.

A homeodomain-interacting protein kinase-2 (HIPK2), also a corepressor for homeodomain transcriptional factors, is capable to retrain $cPLA_2$-α gene expression through interacting with histone deacetylase-1.

Some studies suggest that $sPLA_2$ can mediate indirect activation of $cPLA_2$-α via mobilization of calcium and/or ►MAP kinase-mediated phosphorylation. There are two possible mechanisms underlying this mediation. It is possible that $sPLA_2$ binds to the external cell surface resulting in the release of fatty acid and lysophospholipid. Arachidonic acid released in this manner will be metabolized to HETE and/or PGE_2. These metabolites and/or lysophospholipid, may in turn activate $cPLA_2$-α by mobilization of calcium intracellularly and/or by activation of the ►MAP kinase pathway. Alternatively, $sPLA_2$ could exert its action on $cPLA_2$ by binding to heparan sulphate proteoglycans (e.g., glypican) leading to internalization.

Cancer Relevance

Eicosanoid pathway is activated in many types of cancers, and contributes to disease progression by promoting cell proliferation, ►motility, ►invasion, and ►angiogenesis. As the predominant source of intracellular AA for eicosanoid synthesis, $cPLA_2$-α is induced and/or activated in a range of human tumor types, such as colon and lungs. In ►prostate cancer, ANX1 and ANX2, the negative regulator of $cPLA_2$-α, are lost and secreted PLA_2-IIA, the potential positive regulators of $cPLA_2$-α, is increased in prostate cancer. Thus, in addition to ►COX and LOX enzymes, PLA_2 enzymes are dysregulated and could contribute to the pathogenesis of cancer. Considering the fact that (i) COX and LOX inhibitors suppress the production of PGs or HETEs only, (ii) platelet activating factors produced from the remaining lysophosphoplipid after AA cleavage by $cPLA_2$-α are oncogenic in the breast and colon, and can stimulate angiogenesis and ►NF-kB, and (iii) blockade of PLA_2 enzymes is expected to block AA supply to both COX and LOX pathways as well as the production of platelet-activating factors, PLA2s represent potential targets for the treatment of cancer.

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Phospholipase C

Definition

PLC; Ubiquitous enzyme which catalyses the cleavage of phosphatidyl inositol 4,5-bisphosphate in inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases intracellularly stored Ca^{2+} resulting in an increase in the free cytosolic Ca^{2+} concentration, while DAG regulates various effectors like some isoforms of protein kinase C. While β-isoform of phospholipase C are regulated by G-proteins, γ-isoforms are primarily regulated through receptor tyrosine kinases.

►G-Proteins

Phospholipase D

Definition

PLD; Represents a family of enzymes responsible for the hydrolysis of phosphatidylcholine (PC) into phosphatidic acid (PA) and choline. It is regulated by growth factors and cytokines receptors, as well as GTPases of the Ras superfamily, mostly Rho, Ral and Arf proteins. At least two members in mammalians have been identified that are involved in secretion and cell growth control. At least PLD1 has been shown to have oncogenic potential.

►GTPase

Phospholipid Bilayer

Definition

Plasma membrane.

Phospholipids

Definition

Are a major component of all biological membranes and are a class of lipids contaning: fatty acids, a negatively-charged phosphate group, nitrogen containing alcohol and a backbone. Phospholipids with a glycerol backbone are known as glycerophospholipids or phosphoglycerides. The unique phospholipid with a sphingosine backbone is named sphingomyelin. Lipids containing one or more phosphate groups, particularly those derived from either glycerol or sphingosine (sphingolipids). They are polar lipids that are of great importance for the structure and function of cell membranes and are the most abundant of membrane lipids.

►Fatty Acid Synthase
►Lipid Mediators

Phosphoprotein Enriched in Diabetes/ Phosphoprotein Enriched in Astrocytes-15kDa

Definition

PED/PEA-15 is a protein of 131 amino acids and 15 kDa that consists of one N-terminal death-effector-domain (DED) and two phosphorylation sites for PKC (Ser 104) and calcium calmodulin kinase II (Ser 116). PED/PEA-15 inhibits apoptosis by interfering with the recruitment of caspase-8 to activated death receptors and also promotes MAPK signaling pathways.PED/PEA-15

►Caspase-8

Phosphorylation

Definition

Is the addition of a phosphate (PO_4) group to a protein molecule or a small molecule. The process of phosphorylating a compound (e.g., chemical, protein) either by a reaction with an inorganic phosphate or by transferring a phosphate from another organic phosphate. This process can be used to regulate biological processes in cells. Another way to define it would be the introduction of a phosphate group into an organic molecule. In eukaryotes, protein phosphorylation is probably the most important regulatory event. Many enzymes and receptors are switched "on" or "off" by

phosphorylation and dephosphorylation. Phosphorylation is catalyzed by various specific protein kinases, whereas phosphatases *de*phosphorylate. Phosphorylation is observed on serine, threonine, tyrosine and histidine residues.

Photoactivation

Definition

A chemical process that is induced by a photosenstizer upon light irradiation.

►Hypericin

Photocarcinogenesis

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Synonyms

Solar light induced cancer; Ultraviolet radiation induced cancer; Sun light induced cancer

Definition

Photocarcinogenesis is a complex multistage process of tumor growth and development involving three distinct stages exemplified by initiation, promotion and progression.

Characteristics

Ultraviolet Radiation and Skin Cancer

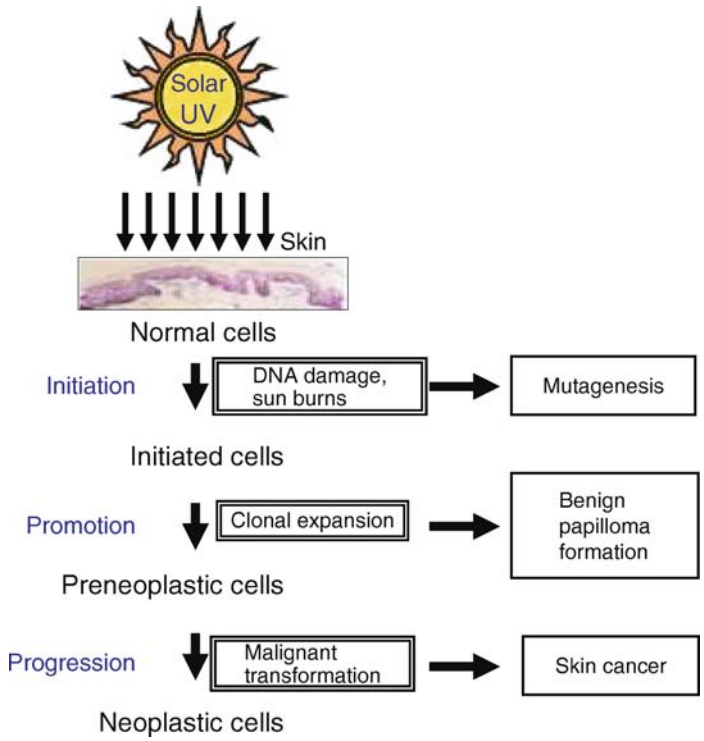
Skin, the largest body organ, situated at the interface between the body and its environment, directly suffers from the deleterious effects of xenobiotics and genotoxic agents including solar ultraviolet (UV) radiation. Solar UV radiation has been implicated as the main cause for skin cancer. The prevalence of UV-induced skin cancer is rapidly increasing, accounting for more than 40% of all human cancers in the United States, with about 1.2 million new cases being diagnosed each year.

Solar UV radiation is divided into three regions depending on wavelength, short wave UVC (200–280 nm), mid wave UVB (280–320 nm) and long wave UVA (320–400 nm). UVC has the highest energy and, hence, is the most biologically damaging region of UV radiation. However, it is effectively blocked from reaching the Earth's surface by the stratospheric ozone layer and therefore its role in human pathogenesis is minimal. It is unknown how its physiological significance will change with depletion of the ozone layer and continuous influx of UVR at the earth's surface. The only wavelengths that reach the earth surface are comprised of UVB (1–10%) and UVA (90–99%). Currently UVB and to a much lesser extent, UVA radiation are considered to be responsible for inducing various skin disorders including skin cancer. UVA radiation constitutes the majority of solar radiation at the earth's surface but only accounts for 10% of the carcinogenic dose of sunlight. Conversely, UVB radiation is a minor but active constituent of solar light and has direct and indirect adverse biological effects on the skin. In UV skin tumorigenesis approximately 90% of the carcinogenic dose of sunlight is derived from UVB.

Exposure to UVB (mid wave) plays a major role in the development of non-melanoma skin cancers (NMSCs) comprising of basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs), the most frequently diagnosed cutaneous malignancies and account for approximately 80% and 16% of all skin cancers, respectively. Both BCCs and SCCs are derived from the basal layer of the epidermis of the skin. SCCs are invasive and more than 10% of these cancers metastasize. On the other hand, BCCs do not metastasize but can be locally invasive and destructive. Malignant melanoma, the deadliest form of skin cancer has reached epidemic levels and continues to increase at the rate of 4% annually. In the year 2006, 62,190 new cases of melanoma were diagnosed, resulting in 7,910 deaths while 2,800 deaths were expected to occur from non-epithelial skin cancers.

Ultraviolet Radiation-induced Skin Cancer or Photocarcinogenesis

Solar UV radiation-induced skin cancer or photocarcinogenesis is a complex multistage phenomenon involving three distinct stages exemplified by initiation, promotion and progression (Fig. 1). Each of these stages is mediated via alterations in various cellular, biochemical, and molecular changes. Initiation, the first step in the carcinogenesis process is essentially an irreversible step in which genetic alterations occur in genes that ultimately leads to DNA mutation in normal cells. Tumor promotion is the essential process in cancer development involving clonal expansion of initiated cells giving rise to premalignant and then to



Photocarcinogenesis. Figure 1 Stage of solar UV radiation induced skin carcinogenesis.

malignant lesions, essentially by alterations in signal transduction pathways. Tumor progression involves the conversion of premalignant and malignant lesions into an invasive and potentially metastatic malignant tumor. To name a few, the processes of skin cancer development involve stimulation of DNA synthesis, DNA damage and proliferation, ►inflammation, ►immunosuppression, epidermal hyperplasia, cell cycle dysregulation, depletion of antioxidant defenses, impairment of signal transduction pathways, increase in prostaglandin synthesis, and induction of ornithine decarboxylase and cyclooxygenase.

Oxidative Stress and Photocarcinogenesis

The major role of skin is to afford a protective covering at the crucial interface between the body and its environment. The skin directly suffers from the deleterious effects of UV radiation and exogenous chemicals. Environmental factors may stimulate the skin responsive system through their oxidizing properties. UV radiation jeopardizes the integrity of the skin that is critical for cellular homeostasis. UV exposure results in an increased generation of reactive oxygen species (ROS) that overwhelms the antioxidant defense mechanisms of the cells. This condition of prooxidant/antioxidant disequilibrium is defined as "►*oxidative stress*."

The epidermis is composed mainly of keratinocytes, which are rich in ROS detoxifying enzymes such as superoxide dismutase, catalase, thioredoxin reductase and glutathione peroxidase, and in low-molecular-mass antioxidant molecules such as tocopherol, glutathione and ascorbic acid, and thus provides some natural protection against ROS. Skin spontaneously responds to increased ROS levels; however the capacity of these detoxifying enzymes is not unlimited and can be overwhelmed by excessive generation of ROS. Studies have shown that UV radiation to the skin results in the formation of ROS and can act both as an initiator and promoter of tumors by damaging critical cellular molecules such as proteins, lipids and DNA, and by acting as a stimulator or inducer of cell-signaling molecules. Studies have demonstrated that oxidative stress elicited by UV irradiation activates redox-sensitive transcription factors, including nuclear factor kappa B (NF-κB), mitogen activated protein kinases (MAPK), and members of the activator protein-1 (AP-1) complex, such as c-Fos and c-Jun. The induction of oxidative stress and imbalance of antioxidant defense system have been associated with the onset of several disease states including inflammation, rheumatoid arthritis, immunosuppression, photoaging and skin cancer. Accumulating body of evidence suggests that ROS are involved in all three stages of

photocarcinogenesis process. The cause of these events is contingent upon the UV dose, time of exposure and the wavelength.

DNA Damage and Photocarcinogenesis

DNA contains purine and pyrimidine bases and their absorption maxima lies between 230 and 300 nm. Therefore, DNA is a major UVB-absorbing cellular chromophore in the skin. The most common and frequent photoproducts that are formed in the skin after UVB exposure are cyclobutane pyrimidine dimmers (CPDs) and pyrimidine (6–4) pyrimidone photodimers. UVB irradiation to the skin also results in photoisomerization of trans- to cis-urocanic acid, DNA strand break, DNA crosslinks and DNA-protein crosslinks. Formation of CPDs, if not repaired through nucleotide excision repair, replication leads to signature mutations. These mutations are in the form of C to T and CC to TT transition and have been observed in tumor suppressor gene p53 in large population of human SCCs, BCCs and actinic keratoses. Studies suggest that most of the UVB-induced CPDs are found in the epidermis but a significant amount is also found in the dermis. Pharmacokinetic studies reveal that UVB-induced DNA damage in the form CPDs in human skin declines over the period of time and this may occur because cells with damage DNA undergo apoptosis or has been repaired. CPDs are primarily produced in keratinocytes and Langerhans cells following UVB exposure, and also in dendritic cells in lymph nodes draining the irradiated sites. CPDs have been implicated in UVB-induced immunosuppression and initiation of photocarcinogenesis.

p53 and Photocarcinogenesis

Apoptosis is regarded as an ideal way of elimination of damaged cells. It is now well established that uncontrolled cellular growth that may be a result of defects in cell cycle and apoptotic machinery, is responsible for the development of skin cancer. Therefore, to maintain the integrity of the cells after DNA damage, several cellular responses are activated that include mechanisms for removal of DNA damage, delay in cell cycle and DNA repair or apoptosis by transcriptional activation of *p53*-related genes. The *p53* tumor suppressor gene plays a crucial role in protecting cells from DNA-damage as a consequence of UVB exposure. It has also been suggested that p53 can directly or indirect regulate UVB-induced, transcription-coupled DNA repair. An important function of p53 protein is to act as a transcription factor by binding to a p53-specific DNA consensus sequence in responsive genes. The increased level of p53 protein after DNA damage is associated with enhanced apoptosis, presumably in cells that are too damaged for adequate DNA repair. High doses of UVB have been shown to be associated with the formation of sunburn cells, initiated via a p53-dependent pathway, and cause the removal of damaged cells, thus minimizing the risk of skin cancer. Lower doses of UVB allow cell survival and repair of genetic damage, but the cell survival response is a state of local and systemic immunosuppression, which in itself may be deleterious and contribute to the process of formation of skin cancers.

Mutations in the p53 gene have been detected in 50% of all human cancers and in almost all skin carcinomas. Studies have shown that early responses in the skin of mice after an acute UVB exposure included an increased number of cells expressing both p21 and p53. Chronic UVB exposure to these mice resulted in modulation of the expression of cell cycle markers, with increased expression of p53 and cyclin D_1 correlating with the development of skin tumors. It has been suggested that increased expression of cyclin D_1 in SCCs contributes to the tumor phenotype even in the presence of elevated p53 levels. Studies have also demonstrated that elevated cyclin D_1 expression occurs in squamous carcinoma and papillomas, while decreased expression leads to reduce skin carcinogenesis. SCCs developed in SKH-1 mice after chronic exposure to UVB showed increase in cyclin D_1 protein levels.

Studies have shown that overexpression of $P16^{INK4A}$ reduces the proliferation of human and murine squamous epithelial cells. Transcriptional upregulation of $p16^{INK4A}$ has also been shown in melanoma cells following UVB irradiation. Induction of $P16^{INK4A}$ in human keratinocytes was reported in human epidermis and in cultured keratinocytes after UVB irradiation. UV-induced mutations of $p16^{INK4A}$ and $p14^{ARF}$ genes have been reported in epithelial skin tumors from sporadic patients and from Xeroderma Pigmentosum patients who suffer from strong UV hypersensitivity. There is considerable evidence that p53, $p16^{INK4A}$, $p21^{waf1/cip1}$ and $p14^{ARF}$ proteins are part of parallel pathways that control cell cycle and responses to UV-DNA damage.

Inflammation, Immunosuppresion and Photocarcinogenesis

Skin inflammation that includes the release of growth factors, proinflammatory cytokines, erythema, edema, hyperplasia, infiltration of inflammatory cells and ROS production in response to UVB plays an important role in skin cancer development. Chronic UVB-mediated inflammation causes the induction of the COX-2 enzyme resulting in increased prostaglandin E_2 (PGE_2) levels in the skin. Increased PGE2 levels have been associated with skin cancer development and spread. Increased myeloperoxidase activity is associated with neutrophils infiltration that plays an important role in inflammation.

UVB exposure initiates a cascade of events that modify gene expression profiles and alters the immune system of the skin. There is ample experimental and clinical evidence to suggest that immune factors contribute to the pathogenesis of solar UV-induced skin cancer. Chronically immunosuppressed patients living in regions of intense sun exposure experience and exceptionally high rate of skin cancer. Exposure to UVB radiation results in inhibition of contact hypersensitivity (CHS) induced by contact allergens that is a prototypic T-cell mediated immune response. UVB-induced infiltration of $CD11b^+$ cells (cell surface markers of macrophages and neutrophils) upregulates interleukin (IL)-10 and downregulates IL-12 in the skin and/or draining lymph nodes. IL-10 possesses immunosuppressive activity, whereas IL-12 is immunoregulatory cytokine. In UVB exposed skin, IL-10 is primarily secreted by activated macrophage and inhibits antigen presentation, thereby down regulating CHS responses. IL-12 by stimulating the production of interferon-γ regulates the growth of T-cells and enhances T helper cell type 1 functions.

Defects in Signaling Pathways and Photocarcinogenesis

UVB radiation to mammalian skin results in alterations in a variety of signaling pathways that are responsible for maintaining a balance between cell proliferation and death. NF-κB is a ubiquitously expressed transcription factor that belongs to the Rel family and regulates genes involved in inflammation, immunity, cell cycle progression, tumor promotion, angiogenesis and metastasis. NF-κB is present in the cytosol as a heterodimer usually consisting of its p50 and p65 subunits bound to its inhibitory proteins IκB. UVB activates IκB kinases (IKK) either directly or indirectly that are central to NF-κB activation. The activated IKK phosphorylates the inhibitory κB proteins on either serine residue 32 or 36 of IκBα. The released NF-κB then moves from the cytoplasm to the nucleus and activates transcription of target genes. Phosphorylation and activation of IκB kinase is controlled by an NF-κB-inducing kinase.

MAPK encompasses a large number of serine/threonine kinases involved in regulating a wide array of cellular processes including proliferation, differentiation, stress adaptation, and apoptosis. The MAPKs are divided into three multimember subfamilies: the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNK), and the p38 kinases and are activated in response to oxidant injury and therefore could potentially contribute to influence cell survival. Studies have shown that UVB activates ERK1/2 and p38 signaling pathways via ROS generation. Low-dose UVB irradiation of normal human skin induces rapid and reversible phosphorylation of JNK and p38. Studies have demonstrated that both p38 and ERK were required for UVB induced c-*fos* expression, a member of the AP-1 transcription factor complex. AP-1 transcription factor is a protein dimer that consists of either heterodimers between *fos* and *jun* family gene products or homodimers of *jun* family gene products. There are three Jun proteins (c-Jun, Jun B and Jun D) and four Fos proteins (c-Fos, Fos B, Fra-1 and Fra-2). There is growing evidence that the AP-1 family of proteins is involved in cell proliferation and survival by regulating the expression and function of a number of cell cycle regulatory proteins. Sublethal doses of UVB produce strong induction of c-jun and c-fos transcripts in primary human keratinocytes. Studies have demonstrated that both p38 and ERK are required for UVB induced c-*fos* expression. Inhibition of both p38 and ERK simultaneously completely abrogated UVB induced c-*fos* transcription and down regulated basal transcription of the c-*fos* gene.

Conclusions and Recommendations

In recent year there is a significant increase in the incidence of skin cancer due to greater amount of UV radiation reaching the surface of the earth because of depletion of ozone layer, out door recreational activities and extensive use of sun tanning devices for cosmetic purposes. For primary prevention of photodamage and cutaneous disease, education about the harmful effects of UV radiation present in the sunlight, the need to avoid its excessive exposure by wearing protective clothing and the use of sunscreen has been persistently emphasized, but for many reasons these primary prevention approaches have had limited success. Therefore, additional efforts are needed to prevent skin cancers that result as a consequence of UVB exposure. UVB radiation to mammalian skin is known to alter cellular function via DNA damage, oxidative stress, p53 mutation, generation of ROS, inflammation, immunosuppression and skin cancer. Many signaling molecules and pathways have been found to play a role in UVB induced skin damages.

Accumulating data consistently support the view that a wide variety of botanical agents (such as green tea polyhenols, resveratrol, pomegranate, curcumin, genistein, apigenin and silymarin) possess substantial anti-photocarcinogenic and anti-mutagenic activities because of their antioxidant and anti-inflammatory properties. The use of these botanical agents alone or in the form of a formulation could be developed for chemoprevention. The concept of chemoprevention is to control the occurrence of cancer by slowing, blocking, or reversing the development of the disease by the administration of naturally occurring or synthetic compounds. One of the excitements of chemoprevention is that agents can be targeted for intervention at

the initiation, promotion, or progression stage of the multistage photocarcinogenesis process. Since multiple pathways are involved in photocarcinogenic response, a mixture of several botanical agents working through different mechanisms could be an effective strategy. Skin care products supplemented with botanicals, in conjunction with the use of sunscreens and educational efforts may be an effective approach for reducing UVB-generated ROS-mediated photodamage, inflammatory responses, immunosuppression and skin cancer in humans.

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Photochemical Reaction Type-I

Definition

Of an activated photosensitizer after intersystem crossing to the triplet state. The photosensitizer transfers electrons to a target molecule, often molecular oxygen. Photochemical reactions type-I and type-II cause the production of reactive oxygen (ROS), the cytotoxic product employed in photodynamic therapy for destruction of tumor cells.

►Photodynamic Therapy

Photochemical Reaction Type-II

Definition

Of an activated photosensitizer after intersystem crossing to the triplet state. The photosensitizer transfers energy to triplet oxygen, which is activated to the reactive singlet state. Photochemical reactions type-I and type-II cause the production of reactive oxygen (ROS), the cytotoxic product employed in photodynamic therapy for destruction of tumor cells.

►Photodynamic Therapy
►Reactive Oxygen Species

Photochemoprevention

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Definition

"Photochemoprevention" has 2 very different meanings. The term was first coined in 2001 to describe ►chemoprevention of skin damage, including skin ►carcinogenesis (►Photocarcinogenesis), due to ►ultraviolet (UV) light (►Solar Ultraviolet light, ►UV radiation). A second use of the word "photo-chemoprevention," as defined in 2003, is to refer to the combination therapy of ►pulsed dye laser (PDL) to inhibit ►angiogenesis and chemoprevention to block both proliferation of cancer cells and tumor revascularization (►vascular targeting agents in cancer). The comments below will describe the term "photochemoprevention" as originally used.

Characteristics

Photochemoprevention can refer to the use of any chemopreventive agent, either ingested or topically applied, to block the damaging effects of UV light. UV light, in particular UVB light, is directly responsible for much of the damage leading to skin cancer development, especially basal cell (►Basal Cell Carcinoma) and ►squamous cell carcinomas, both of which are considered to be non-melanoma skin cancers. Preventive agents block the action of UV light in one or more ways, including (i) acting as a sunscreen, (ii) acting as an anti-oxidant, and/or (iii) inhibiting signaling and ►signal transduction pathways activated by UVB light. Through these activities, chemopreventive agents can prevent or slow the emergence of mutated cells (►Repair of DNA) and their ►progression to malignant tumors. Several targets for chemopreventive agents have been identified, including inhibition of: (i) polyamine or ►prostaglandin synthesis, (ii) oxidation (►Oxidative DNA damage), (iii) specific retinoid receptors (►Retinoid Receptor crosstalk in Cancer), and (iv) components of the ►NFkB (Nuclear Factor *f* ÛB), ►Ras (►Rat Sarcoma) (►RAS, ►RAS Activation) and ►MAP kinase signaling pathways.

Although photochemoprevention can refer to the use of any agent to prevent, ameliorate or repair

►photodamage associated with skin cancer, it has generally come to mean the use of natural products, especially botanicals, as skin cancer chemopreventive agents. There are numerous reports of natural products reducing UV damage and preventing skin carcinogenesis. A few of the commonly studied natural products are green tea (►Epogallocatechin), grapes (►Grape Seed Extract), berries and brown algae (►Polyphenols and Cancer). The majority of these entities have both sunscreen and anti-oxidant properties, depending on how they are used (►Phytochemicals and Cancer Prevention).

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Photodamage

Definition

Damage to the skin from ►UV radiation.

►Photochemoprevention

Photodynamic Diagnostic

Definition

PDD; Uses similar principles as ►photodynamic therapy, especially to detect and delineate flat neoplastic lesions.

►Urothelial Carcinoma
►Fluorescence Diagnostics

Photodynamic Laser Therapy

►Photodynamic Therapy

Photodynamic Therapy

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Synonyms

Photodynamic laser therapy; Photodynamic tumor therapy

Definition

Photodynamic Therapy (PDT) uses the action of visible, low energetic light on a ►photosensitizer to activate its destructive potential in a target tissue. PDT is a selective treatment modality since the photosensitizer shows preferential accumulation in target (e.g., tumor) cells and the light application can be restricted to the target area. By light activation the photosensitizer forms cytotoxic compounds, mainly ►reactive oxygen species (ROS), destroying the target. The emission of ►fluorescence light is employed in ►Fluorescence Diagnosis for tumor diagnosis. PDT is applied for malignant and several nonmalignant diseases.

Characteristics

Historical Perspective

Photodynamic effects have been known for a long time from substances enhancing the healing effect of sunlight. It lasted until 1904 when the term "photodynamic" was used for light-induced cytotoxic action and fluorescence of a photosensitizer. Modern PDT started in the 1970s with the investigation and usage of ►hematoporphyrin derivative (HPD) as a photosensitizer. ►Photofrin (a HPD) became the first drug approved for clinical oncologic treatment in 1993. In recent years, also nononcologic diseases such as bacterial or fungal infections or nonmalignant skin diseases became a potential application of PDT. The following sections refer to PDT as a tumor therapy, although the basics also apply to the nononcologic indications.

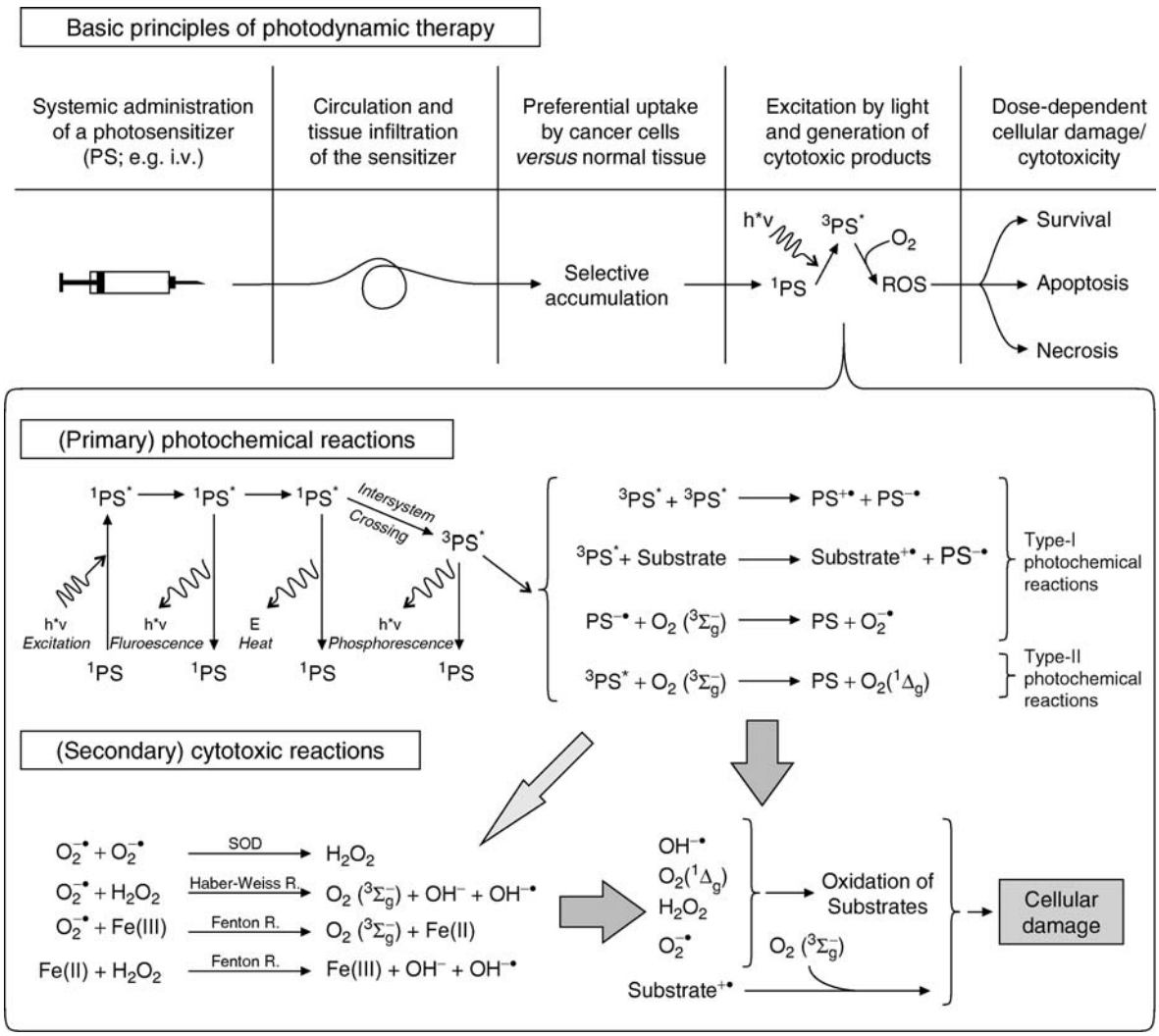
Principle of PDT

The principle of PDT is a two-step procedure with accumulation of a nontoxic photosensitizer in a target and activation of the molecule by harmless visible irradiation according to the photosensitizer's absorption spectrum. The photosensitizer is excited by the light from its electronic ground state, S_0, to the first excited

state (referred to as singlet state), S_1. This is followed by fluorescence emission, heat dissipation or a radiation-free conversion to the electronic triplet state, T_1. The triplet state is relatively long lasting (about 10^{-3} s) and allows the excited photosensitizer to interact with surrounding molecules (molecular oxygen or cellular constituents) and transfer energy or electrons, resulting in generation of cytotoxic products. Reactions involving transfer of electrons or energy are referred to as ►photochemical reactions type-I or ►photochemical reactions type-II, respectively (see Fig. 1).

Available Photosensitizers

An ideal photosensitizer for PDT should (i) be nontoxic in the absence of light, (ii) have a high quantum efficiency (i.e., a high efficiency in absorbing light energy and transferring it to other molecules), (iii) be able to absorb light above 600 nm for deep penetration



Photodynamic Therapy. Figure 1 Basic Principle of Photodynamic Therapy. Following administration the ►photosensitizer (PS) accumulates preferentially in tumor tissue and causes cellular damage and cell death subsequent to irradiation with light of the appropriate wavelength (h*v). Excitation of the photosensitizer from its ground state (^{1}PS) to its first activated singlet state (^{1}PS*) is followed by either ►fluorescence emission or a transition into the activated triplet state of the photosensitizer (^{3}PS*). ►Photochemical reaction type-I of ^{3}PS* with other photosensitizer molecules, substrates or molecular oxygen $\left(O_2\left(^3\Sigma_g^-\right)\right)$ via hydrogen atom abstraction or electron transfer or ►photochemical reaction type-II with oxygen via energy transfer yields radical molecules or – following further (secondary) reactions – various types of ►reactive oxygen species (ROS). These reactive species oxidate a number of cellular substrates causing overall oxidative stress and damage. H_2O_2, ►hydrogen peroxide; SOD, ►superoxide dismutase; *, activated state; •, radical species.

of tissue, (iv) accumulate preferentially within the target, and (v) clear rapidly from normal tissue and the whole body. As sensitizers serve several ►porphyrin-like substances, artificial photosensitizers like different ►phthalocyanines or plant-extracted dyes like ►hypericin (from St. John's wort, *Hypericum perforatum*). A special endogenously formed substance is the strong photosensitizer and fluorescence dye ►protoporphyrin IX (PpIX). PpIX is a precursor of ►heme, which represents the prosthetic group of several proteins, such as ►hemoglobin. PpIX forms in the heme pathway in ►mitochondria starting with the precursor ►δ-aminolevulinic acid (ALA). If ALA is externally given in excess to a cell, PpIX is accumulates due to its slow conversion to heme.

For tumor eradication, excitation of the photosensitizer would be most beneficial at high wavelengths since light penetration into tissues increases with the wavelength due to absorption of $\lambda<600$ nm by tissue chromophores (oxy- and deoxyhemoglobin and melanin). Light absorbance by water becomes significant at a wavelength $\lambda>1{,}300$ nm; this gives an "optical window" for optimal photosensitizer excitation with respect to minimal absorbance and maximal tissue penetration.

Up till now, several countries have approved the use of PDT for various types of tumors and precancers (see Table 1).

Principles of Clinical Application

PDT is minimally invasive, prevents scarring, exhibits excellent cosmetic outcome, and is only minimally ►mutagenic. It can be applied repeatedly and in combination with other therapies. Side effects are light sensitivity of the skin after systemic application, vertigo, and nausea, and also local pain during PDT with ALA-induced PpIX. The mechanisms of photosensitizer excretion and its elimination rate via liver to bile, kidney to urine, or/and via intestine to feces represent important factors in determining the grade and duration of light sensitivity.

PDT is applied after approval or in the case of failure of a conservative therapy or if resistance toward conventional tumor treatments is present. Since its damaging mechanisms differ from those of ►chemotherapy and ►radiotherapy, PDT does not interfere with those therapies. Finally, from an economical point of view, PDT is simple, useful, effective, and relatively cheap.

In clinical applications, the photosensitizer is applied either systemically (e.g., by intravenous injection or orally) or topically (e.g., as an ointment) and accumulates in the target tumor tissue. Irradiation is usually performed at a time, when the sensitizer shows the optimum accumulation and the greatest ratio between diseased and normal surrounding tissues. For many photosensitizers, a differential (tumor-selective) accumulation has been proven. Selective enrichment may be supported by delivery of a photosensitizer coupled to suitable carriers such as liposomes or antibodies.

As light sources for PDT serve different kinds of lasers, various lamps (e.g., tungsten filament or xenon arc lamps) equipped with filter systems, semiconductor diode lasers, and conventional light-emitting diodes. Irradiation in inner organs is generally carried out by fiber optics connected with an organ-specific shaped applicator coupled to a light source and at the skin by a conventional lamp, equipped with a cut-off filter to mask out ultraviolet and infrared wavelength portions.

Three general mechanisms contribute to the tumoricidal action of PDT: (i) direct tumor cell killing by light-induced production of cytotoxic compound (ROS), (ii) effects on the tumor vasculature (attachment of the photosensitizer to the tumor endothelial walls and PDT-mediated vascular shutdown, vessel constriction, blood flow stasis, and ►hypoxia), and (iii) immunological effects such as the stimulation of the immune system, which could even be employed to generate a PDT vaccine based on the induction of a cytotoxic T-cell response. Especially in the case of vascular targeting by PDT the pharmacokinetics of the photosensitizer are of utmost importance in defining a drug-to-light interval optimal for induction of vascular damage.

Molecular Mechanisms

Photosensitizers or the prodrug ALA enter the cell or vessel by several mechanisms, ranging from passive diffusion to active uptake via receptors. There is evidence that some photosensitizers (e.g., HPD) are taken up by ►low-density lipoprotein receptors (LDL). The fact that several types of tumor cells bear extensive amounts of LDL receptors could partly explain the tumor selectivity of PDT. ALA seems to enter the cell via the ►Gamma-aminobutyric acid (GABA) receptors. In general, the uptake of photosensitizers into tumor cells is influenced by several factors such as the administration protocol and vehicle, the chemical properties of the photosensitizer, pH, tumor architecture, and cellular parameters (e.g., cell cycle).

As mentioned above, molecular oxygen represents an important reaction partner as the activated photosensitizer reacts with the singlet state oxygen resulting in different forms of ROS. Therefore, the grade of tumor vascularization and the oxygenation of tumors affects the efficiency of the photodynamic process; as a consequence, in some cases, lowering the irradiation intensities and prolonging the irradiation times or fractionating the light dose increases the effectiveness of PDT. However, the benefits of these protocols depend on the tumor type and architecture since fractionated irradiation may also allow recovery of the

Photodynamic Therapy. Table 1 Current Indications for Photodynamic Therapy

Generic name	Chemical name	Activation wavelength	Approval	(Potential) indications
Benzvix	Benzyl δ-aminolevulinic acid	635 nm	No[1]	►Gastrointestinal Tumors
BOPP	Boronated protoporphyrin	630 nm	No[1]	►Brain tumors
Levulan	δ-aminolevulinic acid, ALA	635 nm	Yes[2]	►Actinic keratosis
			No[1]	Head and neck tumors, gynecological tumors
Lu-Tex	Lutetium texaphyrin	732 nm	No[1]	►Cervical Cancers, Prostate Cancer and ►Brain tumors
Metvix	δ-aminolevulinic acid methyl ester	635 nm	Yes[3]	►Basal cell carcinoma
Pc-4	Phthalocyanine-4	670 nm	No[1]	Cutaneous/subcutaneous lesions from diverse solid tumor origins
Photochlor	2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-alpha	665 nm	No[1]	Basal cell carcinoma
Photofrin, Porfimer sodium,	Hematoporphyrin derivative, polyhematoporphyrin	630 nm	Yes[4]	►Barrett's high-grade dysplasia
			Yes[5]	Cervical dysplasia and Cervical Cancers
			Yes[6]	Endobroncheal cancer
			Yes[7]	►Esophageal Cancer
			Yes[5]	►Gastric cancer
			Yes[8]	Papillary ►Bladder Cancer
			No[1]	Brain tumors cholangiocarcinoma
Photosense	Tetrasulfonated Aluminium Phthalocyanine	672 nm	Yes[9]	Head and neck cancer
SnET2	Tin ethyl etiopurpurin	664 nm	No[1]	►Breast cancer, Basal cell carcinoma, ►Kaposi Sarcoma, prostate cancer
Foscan, Temoporfin	Meso-tetrahydroxyphenyl chlorine	652 nm	Yes[10]	►Palliative Therapy of head and neck cancer
			No[1]	Prostate cancer, ►Pancreas Cancer, cholangiocarcinoma
Visudyne, Verteporfin	Benzoporphyrin derivative-monoacid ring A	689 nm	No[1]	Basal cell carcinoma

[1] Not yet approved, [2] EU, U.S., [3] EU, [4] Canada, EU, UK, U.S., [5] Japan, [6] Canada, Denmark, Finland, France, Germany, Ireland, Japan, The Netherlands, UK, U.S., [7] Canada, Denmark, Finland, France, Ireland, Japan, The Netherlands, UK, U.S., [8] Canada, [9] Russia, [10] EU, Iceland, Norway.

tumor cell's repair systems causing reduced PDT effects.

The intracellular localization and redistribution of the photosensitizer in the cell or vessel determines the primary damage, since ROS have short half-lives, i.e. typical diffusion lengths are 10–20 nm. Consequently, the localization of the photosensitizer determines the cellular target such as specific organelles. The intracellular localization of the photosensitizers depends on their chemical characteristics (e.g., lipophilic dyes accumulate in various membrane systems), the route of internalization, and the time. Except a few photosensitizers used in specialized clinical fields (such as toluidine blue for caries treatment), the dyes do not localize in the nucleus, and therefore PDT does not seem to have a mutagenic potential.

PDT induces damage to target cells via generation of ►oxidative stress and oxidative damage to cellular constituents such as proteins and lipids; this is reflected by the frequently observed increased expression of stress genes coding for proteins such as ►JUN and ►FOS. The resulting cellular response is essentially dose dependent, that is, with increasing PDT dose a transition of survival and repair over induction of ►apoptosis to necrotic cell death (►necrosis) can be observed. The cytotoxic action of PDT is antagonized by the cellular antioxidative defense mechanisms such as antioxidative enzymes and low molecular weight

scavengers. Since PDT does not specifically affect single proteins or cellular mechanisms like most chemotherapeutics, no resistance of tumor cells is induced, even following repeated applications of PDT.

Severe damage caused by massive production of ROS by PDT, results in necrotic cell death, which is characterized by plasma membrane disruption, leak of intracellular material, and immediate and passive cell death. Cell death by apoptosis is induced and executed by a complex cell death machinery, which is reflected by active regulation and complete reorganization of the cellular material. Several models of ►Apoptosis Signaling describe the events and regulation points of apoptosis; the mitochondrial pathway of apoptosis induction and execution is very common following PDT-induced cellular damage. This pathway often involves ►Mitochondrial Membrane Permeabilization (MMP) caused by transformation of the adenine nucleotide translocator into an unspecific pore in the mitochondrial membranes accompanied by reduction of the mitochondrial membrane potential, mitochondrial swelling, and release of proapoptotic factors such as ►cytochrome c, apoptosis-inducing factor (AIF), and ►procaspases. Association of cytochrome c, APAF-1 (apoptosis protease activating factor-1), ATP (adenosine triphosphate), and procaspase-9 results in activation of ►caspase-9. Caspases are endoproteases involved in apoptosis executing the apoptotic programme by cleavage of cellular proteins. The active initiator caspase-9 itself activates caspase-3 (an effector caspase) and triggers the execution of apoptosis. Beside the mitochondrial pathway, also the receptor-mediated, and the stress-induced, ►endoplasmic reticulum (ER)-mediated pathway of apoptosis induction was found. In those cases, different subsets of initiator caspases are induced, all activating the executing caspase-3, -6, or -7. An important class of regulatory proteins is represented by the ►Bcl-2 protein family members (named after ►B-cell lymphoma), which interact with components of the apoptotic pathways at several stages (e.g., mitochondrial pore opening, activation of caspases). Eventually, activation of caspases generates a reaction chain leading to the induction of a cascade of caspase activity, which finally deconstructs the cell and the nucleus, packs it to vesicles (apoptotic bodies), and offers it to immune and neighboring cells for uptake and recycling. Several other (signaling) systems such as intracellular calcium, cathepsins, ceramides, arachidonic acid metabolites, or cyclic nucleotides have been reported to be involved in the cytotoxic action of PDT depending on the model system and photosensitizer used. These data may be further exploited for the design of more efficient combined therapeutic approaches.

In general, the induction and execution of apoptotic cell death depends on several factors such as the damage quantity and quality (dose and intracellular targets), susceptibility of the cell (e.g., antioxidative status), and the availability of cellular energy for execution of apoptosis.

Beside the above mentioned acute stress response, several other effects of PDT on the gene expression of treated tumor cells could be found. Following detailed functional studies and in vivo experimentation, these observations help providing therapeutic strategies that could further enhance the efficiency of PDT.

PDT is known to cause acute inflammation (expression of inflammatory cytokines ►interleukin-1 and ►interleukin-6), invasion of inflammatory cells (neutrophils, mast cells, and macrophages), attraction of antigen presenting cells, and possible development of a systemic immune response with generation of an immunological antitumor memory. Due to induction of a cytotoxic ►T-cell response involving expression of ►interleukin-12, PDT could have potential as a systemic immune therapy or as an in situ ►cancer vaccine. The effects on the immune system represent a potentially important side action of PDT, however, intensive detailed trials have to prove whether this is a useful phenomenon or in which cases it is practical to pay special attention to PDT-mediated antitumor immune effects.

Nononcological indications of PDT include condyloma, warts, psoriasis, acne, skin rejuvenation, sterilization of blood cells, treatment of local bacterial, fungal, viral and parasitic infections, rheumatic arthritis, age-related macular degeneration, caries, dermatophytic fungi, malaria, vascular diseases such as intimal hyperplasia, arteriosclerosis, and prophylaxis of restenosis after ►angioplasty.

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Photodynamic Tumor Therapy

►Photodynamic Therapy

Photofrin

Definition

Synonym porfimer sodium; Is the trade name of hematoporphyrin derivatives. It is used as a photosensitizer in ►photodynamic therapy and fluorescent diagnosis due to its photochemical and fluorescent properties.

►Photodynamic Therapy

Photolithography

Definition

Is the process of transferring geometric shapes on a photomask to the surface of a light-sensitive chemical on the substrate. It is used in microfabrication to selectively remove parts of a thin film or the bulk of a substrate. The steps involved in the photolithographic process include wafer cleaning, barrier layer formation, photoresist application, soft baking, mask alignment, exposure, and development, as well as hard-baking.

►Proteinchip

6,4-Photoproduct

Definition

A ►DNA photoproduct that is generated upon covalent linkage between the C-6 position of one pyrimidine and the C-4 position of the 3υ adjacent pyrimidine.

►Solar Ultraviolet Light

Photosensitivity

Definition

Is the increased sensitivity to the development of edema and/or erythema by ultraviolet (UV) light; ►xeroderma pigmentosum.

►Fluorescence Diagnostics
►UV Radiation

Photosensitizer

Definition

A substance that induces photosensitivity normally to a light insensitive chemical or physical process. This may be an adverse effect of many drugs or a desired effect in ►photodynamic therapy. Upon light irradiation in the presence of oxygen found in living tissues, a photosensitizer is excited to produce fluorescence that can be used in ►photodynamic diagnostic of cancer.

►Hypericin
►Photodynamic Therapy

Photothermal Ablation

Definition

The process of destroying tumor cells by activating nanoparticles inside tumors with light which results in heating of the particles and subsequent destruction of the tumor.

►Nanotechnology

Phthalocyanines

Definition

Are artificial photosensitizers consisting of an extensive ring system based on pyrrole groups. Phthalocyanines are used as photosensitizers in ►photodynamic therapy and fluorescent diagnosis due to their photochemical and fluorescent properties.

►Photodynamic Therapy

Phytoalexin

Definition

Plant-derived natural antibiotic.

►Chemoprotectants

Phyto-Cannabinoids

►Cannabinoids

Phytochemicals in Cancer Prevention

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Synonyms

Plant-derived agents; Nutraceuticals

Definition

Phytochemicals are molecules which occur in the plant kingdom; the prefix phyto- is used in words related to botany. Strictly speaking, the term phytochemical encompasses compounds belonging to a large range of chemical classes, from essential minerals such as selenium to large organic molecules such as plant proteins, fats and carbohydrates, which serve as nutrients. Most people would probably associate the term phytochemical with small non-nutrient organic molecules contained in edible fruits and vegetables. Representative examples of potentially cancer chemopreventive phytochemicals (with their dietary precursors in brackets) are genistein (soya), vitamin c (fruits, vegetables), β-carotene (fruits, vegetables), curcumin (curry spice turmeric), lycopene (tomatoes), tea catechins such as epigallocatechin gallate (green tea), resveratrol (red grapes, other fruits) and apigenin (leafy vegetables) (see Fig. 1).

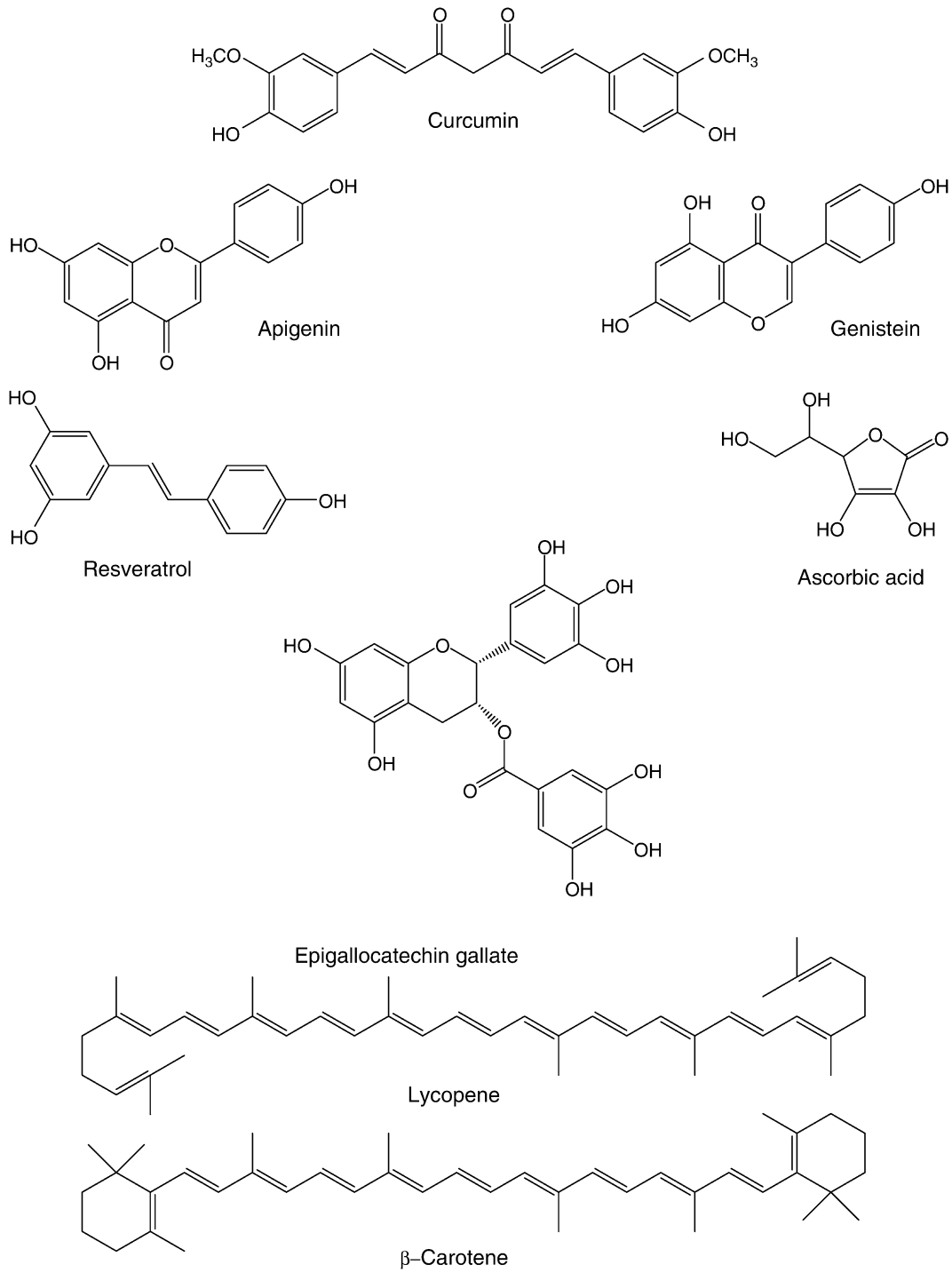
Characteristics

Epidemiology. There is epidemiological evidence which links the incidence of cancer in people in certain areas of the world to the geography of their origin. Often emigrants from areas characterized by a low incidence of a particular cancer, who move to an area with a high prevalence of that cancer, rapidly adopt the high risk of the indigenous population of their chosen immigrant country. For example, Japanese women, who traditionally have a low risk of developing breast cancer, adopt the high breast cancer risk pattern prevalent in the USA within a generation. The low risk of breast cancer in Japan has been tentatively linked to the ubiquitous consumption of plentiful amounts of soya. During the last two decades many attempts have focused on the chemical isolation and pharmacological characterization of dietary constituents, exemplified by those shown in Fig. 1, which may be responsible for the cancer-preventive properties of the parent foodstuff. In the case of soya, constituent chemicals which have been hypothesized to cause, or contribute to, the cancer-preventive properties, are the isoflavones genistein and diadzein.

Cancer Chemopreventive Mechanisms

Research on chemopreventive mechanisms of phytochemicals has primarily focused on their ability to modulate the following biochemical processes: activation/detoxification of chemical carcinogens by xenobiotic enzymes, DNA repair, cell cycle progression, cell proliferation, differentiation, ►apoptosis, expression and functional activation of ►oncogenes or tumor suppressor genes, ►angiogenesis, ►metastasis and hormonal and growth-factor activity. It is important to note that phytochemicals engage pharmacological mechanisms in a species- and tissue-specific manner. Table 1 shows the spectrum of mechanisms in cells *in vitro* activated by curcumin as a paradigm of polyphenolic phytochemicals.

Cancer Chemopreventive Activity in Rodents. Phytochemicals have been shown to possess preclinical cancer chemopreventive efficacy in two types of rodent model. Within weeks of continued exposure to carcinogens such as azoxymethane or benzanthracene, rodents develop cancer in a variety of tissues depending on dose and route of administration. These are the so-called "carcinogen-induced models of carcinogenesis." They contrast with "genetic models," rodents (usually mice) which harbor a gene defect, deletion or mutation, which predisposes them to the formation of malignancies. Particularly interesting for cancer chemoprevention research are rodent models with the propensity to develop cancer caused by a genetic defect, which resembles the genetic fault underlying the corresponding disease in humans. Two examples of genetic mouse models are the Apc^{Min} and the TRAMP (transgenic adenocarcinoma of the mouse prostate) mice. Apc^{Min} mice harbor a functionally inactive mutated *Apc* tumor suppressor gene. All mice with the min genotype develop tumors in the small intestine. The Apc^{Min} mouse is a model of an inherited human disease called Familial Adenomatous Polyposis Coli (FAP) characterized by an ►*APC* gene mutation not unlike that seen in Apc^{Min} mice. FAP patients present with colorectal adenomas early in life, which ultimately progress to colorectal carcinomas. In TRAMP mice expression of the SV40 transforming sequences is targeted to the prostate, and all male TRAMP mice develop prostate tumors. In chemoprevention efficacy testing experiments, carcinogen-induced or genetic models receive the phytochemical under study usually



Phytochemicals in Cancer Prevention. Figure 1 Examples of chemopreventive phytochemicals.

added to the diet, and the number and size of tumors which develop are compared with those in animals which received control diet only. The chemopreventive phytochemicals shown in Fig. 1 have been demonstrated to possess cancer chemopreventive activity in a variety of carcinogen-induced and genetic preclinical models.

Clinical Trails. Definitive phase III clinical trials which show without doubt that a specific isolated phytochemical (rather than a dietary mixture) can prevent cancer in humans have yet to be published. Two randomized trials with a lung cancer endpoint, the α-tocopherol, β-carotene (ATBC) prevention study

Phytochemicals in Cancer Prevention. Table 1 Chemopreventive mechanisms of curcumin in cells *in vitro*

Pharmacological effect	Molecular target	Model system
Inhibition of carcinogen activation/DNA binding	↓CYP1A1 activity	Human breast cancer cells
	↓Carcinogen-DNA adduct formation	
Stimulation of carcinogen detoxification	↑Detoxifying enzymes (e.g. GST, QR)	Rodent liver; human hepatoma cells
Control of cell cycle and proliferation	↓Cyclin D1 expression	Prostate/breast/squamous carcinoma cells, lymphoma cells, murine epidermal cells
	↓Rb phosphorylation	
	↓NF-κB activation	
	↓c-Jun, c-Fos expression	
Inhibition of oncogen product activity	↓c-Myc, c-Jun, c-Fos expression	Mouse epidermis
Induction of apoptosis/ differentiation	↓NF-kB, IKK activation	Human myeloma/breast cancer/ leukemia cells
	↓BCl-2; ↑p53 and BAX expression; ↑caspase activity; ↑BID cleavage; ↑Cytochrome C release	
Inhibition of angiogenesis, metastasis, invasion	↓VEGF; ↓MMP-2 mRNA expression; ↑TIMP-1 mRNA expression	Human breast carcinoma cells

Abbreviations: CYP: cytochrome P450; GST: glutathione S-transferase; QR: quinone reductase; Rb: retinoblastoma tumor suppressor; NFκB: nuclear factor κB transcription factor; IKK: I κB kinase; VEGF: vascular endothelial growth factor; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase.

and the β-carotene and retinol efficacy trial (CARET) were conducted in the early 1980s. Their outcome suggests that β-carotene was not only devoid of imparting any health benefit, but that it might have even exacerbated the risk of lung cancer. A meta-analysis of all randomized trials conducted up to 2004, in which antioxidant supplements including vitamins A, C and E, carotenes and selenium were compared with placebo for prevention of gastrointestinal cancers failed to find evidence for efficacy. Results like these intimate that ultimate incontrovertible clinical proof of the ability of isolated phytochemicals to prevent a particular malignancy will be exceedingly difficult to obtain. In order to gather indirect evidence for anticarcinogenic efficacy of phytochemicals, ►pharmacodynamic markers reflecting chemopreventive efficacy are under intense exploration.

Compelling evidence for testing specific phytochemicals in the prevention of certain malignancies comes from secondary findings of clinical trials. For example, the Nutritional Prevention of Cancer study, a randomized controlled trial of selenium for prevention of non-melanoma skin cancer conducted in the 1990s in regions of the USA where selenium intake is low, showed a 63% reduction in risk of prostate cancer. This finding provided the rational for the SELECT trial of selenium and vitamin E for prostate cancer prevention, which is currently ongoing in the USA. There have also been a few intriguing small pilot studies in humans, which hint at the ability of certain phytochemicals to delay cancer onset, exemplified by the finding that consumption of green tea catechins delayed the progression of high-grade prostate intraepithelial neoplasia to prostate cancer. Studies like this hint at the prospect that certain phytochemicals may turn out to be really useful in clinical cancer chemoprevention.

P

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Phytoestrogens

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Definition

Phytoestrogens are natural chemicals of plant origin which have similar action to the mammalian ►hormone estrogen.

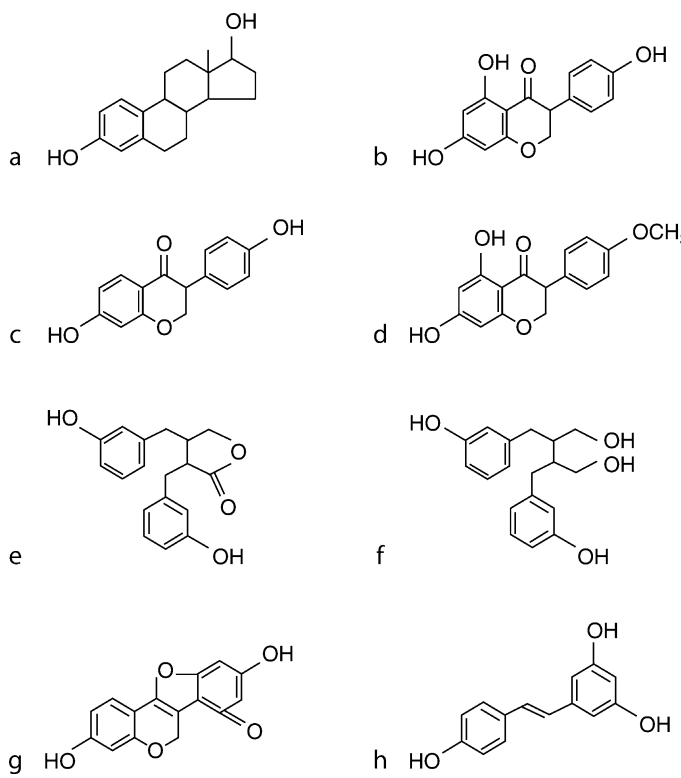
Characteristics

Phytoestrogens are non-steroidal ►polyphenolic compounds. They are found naturally in many edible plants and have structural similarity to the mammalian steroid hormone ►17β-estradiol (Fig. 1). This permits binding to ►estrogen receptors (ERs), but they have a much higher affinity for ERβ than ERα. Based on their chemical structure, phytoestrogens can be broadly divided into four distinct categories: the ►isoflavones (►genistein, daidzein, biochanin A), the ►lignans (enterolactone, enterodiol), the ►coumestans (coumestrol) and the ►stilbenes (►resveratrol).

Phytoestrogens are found in a range of plant products consumed in the diet although their concentrations depend largely on the food type. Isoflavones are found mainly in soy-based products including soybeans, tofu, soy milk, textured soy protein and miso. Of these, the best studied are genistein and daidzein. The coumestans are found in plants less frequently consumed in the human diet e.g. alfalfa sprouts, clover and pinto beans. Lignans are present in flaxseed and are ingested as plant lignan precursors from most fruits and vegetables. These are then converted by gut microflora and into mammalian lignans. Stilbenes are found mainly in peanuts but also in the skin of grapes and as a component of red wine.

Potential Benefits of Phytoestrogens

Many putative health benefits of phytoestrogens have been proposed, the most interesting of which include their potential role as anti-cancer agents (►Phytochemicals and cancer prevention), especially in hormonally-regulated ►cancer (►Estrogenic hormones and cancer). This stems from ►epidemiological (►Epidemiology of cancer) evidence revealing a much higher incidence of breast cancer in Asian populations who consume a soy-rich diet and where the average daily intake of phytoestrogens is 30mg/day compared to



Phytoestrogens. Figure 1 Structural comparison of 17β-estradiol (a) with the principal classes of phytoestrogen, the isoflavones genistein (b), daidzein (c) and biochanin A (d), the lignans enterolactone (e) and enterodiol (f), the coumestan coumestrol (g) and the stilbene resveratrol (h).

<10mg/day in Western countries. Increasing epidemiological evidence suggests that the perceived ►chemoprotectant effects of phytoestrogens are dependent upon a critical period of exposure to these agents pre-puberty and that consumption later in life will have little chemopreventative benefit. This has also been borne out in animal studies which showed neonatal but not postnatal administration with phytoestrogens afforded protection against ►breast cancer development.

Although much of the work on phytoestrogens has been done on breast cancer, these agents have also been implicated in the prevention of prostate (►Prostate cancer, clinical oncology) and colon (►Colon cancer) cancer through a dose-dependent reduction of circulating hormones but data remain inconclusive.

Phytoestrogens bind to and activate ERs but are weak estrogens compared to natural hormones (►Hormones and cancer). It is believed that by ER binding, phytoestrogens prevent the biologically more potent systemic estrogens binding to and activating the receptors. Other more indirect modes of action of phytoestrogens have been reported and include: suppression and/or inhibition of enzymes involved in steroid hormone biosynthesis, inhibition of ►neovascularization required for tumor growth and the inhibition of growth factor signaling. There are additional reports of their action as ►antioxidants by preventing formation of free radicals that can cause cell damage.

Potential Drawbacks of Phytoestrogens

As well as potential protective effects of phytoestrogens, other studies suggest that these agents may actually promote cancer with studies on breast cancer cells showing growth stimulatory effects. This seems to be concentration dependent with low levels exerting estrogenic effects while high levels seem to be more protective. However levels of the latter are non-physiological and considerably higher than plasma levels achieved after a soy-rich meal. Overall further experimental studies, especially prospective epidemiological, are required to advance our understanding of beneficial effects (or otherwise) of phytoestrogens in ►carcinogenesis.

►Genistein

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PIAS

Definition

Protein inhibitor of activated STAT; proteins interact with and modulate the activities of various transcription factors. PIAS1 and PIAS3 bind to STAT1 and STAT3, respectively, and inhibit their action.

►Signal Transducers and Activators of Transcription in Oncogenesis

PIBIDS

Definition

►Trichothiodystrophy.

PI3-K

Definition

Phosphoinositide 3-kinase.

►PI3K signaling

PI3K Signaling

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Synonyms

Phosphoinositide 3-kinase; Phosphatidylinositol 3-kinase; PI-3-kinase; PI3-kinase; PtdIns 3-kinase

P

Definition

PI3Ks are members of a unique and conserved family of intracellular lipid kinases that catalyze the phosphorylation of phosphatidylinositol and phosphoinositides at the D3 position of the inositol ring, generating new intracellular second messengers such as phosphatidylinositol-3-phosphate (PI-3-P), phosphatidylinositol-3,4-bisphosphate (PI-3,4-P2), and phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P3 or PIP3) (►inositol lipids). This reaction leads to the activation of many intracellular signaling pathways through the binding of these proteins to these lipid products. As a consequence PI3K regulates functions as diverse as cell metabolism, survival and polarity, and vesicle trafficking.

Characteristics

PI3K Classification and Signaling Pathways

PI3Ks are grouped in three classes (I–III) according to their substrate preference and sequence homology (Fig. 1). The Different classes of PI3K have distinct roles in cellular signal transduction.

Class IA PI3K

p85 regulatory isoforms have a common core structure consisting of a p110-binding domain flanked by two Src-homology 2 (SH2) domains. p85 isoforms also have an extended N-terminal region containing an Src-homology 3 (SH3) domain and a BCR homology (BH) domain flanked by two proline-rich (P) regions. p110 catalytic subunit isoforms possess an N-terminal p85-binding domain, a Ras-binding domain that mediates a C2 domain, a phosphatidylinositol kinase (PIK) homology domain and a C-terminal catalytic domain. The PIK and catalytic domains of p110 are homologous to domains found in a family of protein kinases that includes ►mTOR (mammalian target of rapamycin), ataxia telangiectasia-mutated, ataxia telangiectasia Rad3-related, and DNA-dependent serine/threonine protein kinase.

Class IB PI3K

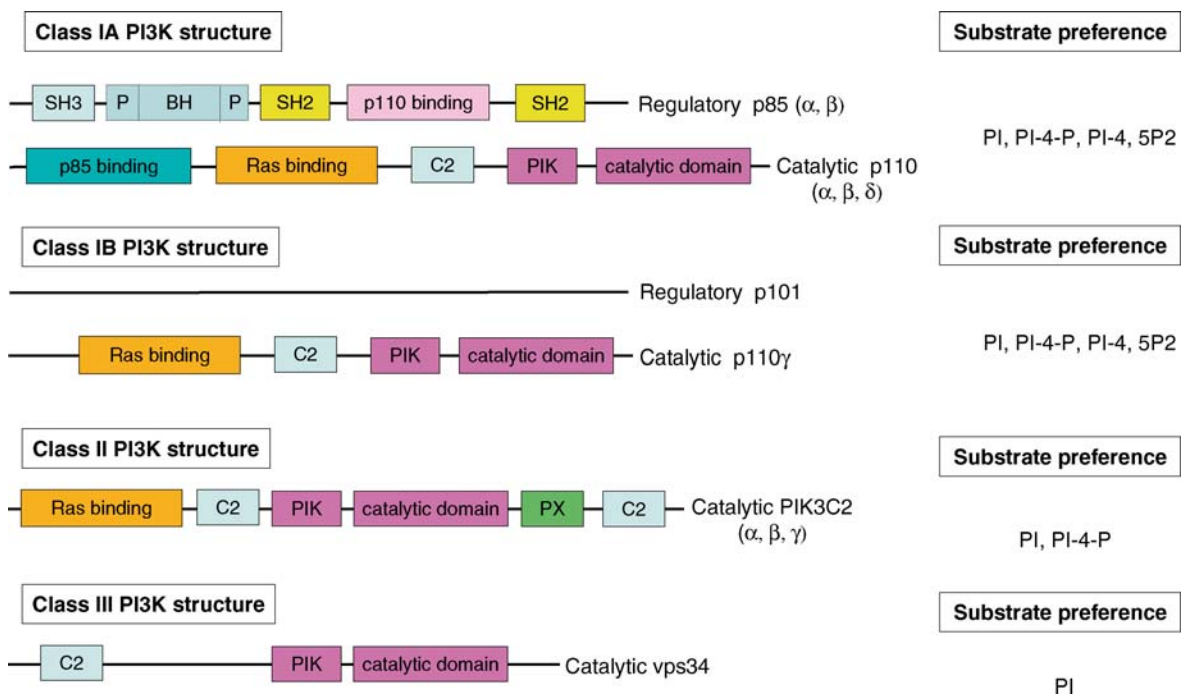
Although p110γ shares extensive homology with the class IA p110 proteins, p101 is distinct from p85 proteins.

Class II PI3K

All three p110-like catalytic subunit isoforms share significant sequence homology with the class I p110 subunits. In addition, class II PI3Ks have an extended divergent N-terminus, and additional PX and C2 domains at the C-terminus.

Class III PI3Ks

This class only displays an N–terminal. C2 domain a PIK domain and a C-terminal catalytic domain.



PI3K Signaling. Figure 1 Classification of PI3K members and substrate preference.

Class I PI3Ks

This class is divided into two subfamilies depending on the receptors to which they couple. Class IA PI3Ks are heterodimers of a p85 regulatory subunit (α or β) and a p110 catalytic subunit (α, β, or δ) and are activated by growth factor receptor tyrosine kinase (RTKs), whereas class IB PI3Ks are heterodimers of a regulatory subunit p101 and a p110 catalytic subunit (γ) and are activated by G-protein-coupled receptors. This binding both relieves the basal inhibition of p110 by p85 and recruits the p85-p110 heterodimers to its substrate (PI-4,5-P2) at the plama membrane. Class I PI3K are involved in many important physiological mechanisms including metabolism and longevity such as glucose homeostasis, cell migration, growth and proliferation.

Class II PI3Ks

This class consists of a single p110-like catalytic subunit (α, β, or γ). Class II PI3Ks bind to clathrin and localize to coated pits, indicating a function in membrane trafficking regulation and receptor internalization.

Class III PI3Ks

This class consists of a single member, vps34 (vacuolar protein defective 34).

In budding yeast, vps34 was originally found to have a role from trafficking vesicles from the golgi apparatus to the vacuole. In mammals, this class seems to be crucial for controlling cell growth.

Two commonly used PI3K inhibitors, wortmannin and LY294002, inhibit class I and II and at a lesser extent class II as well as other PI3K-like protein kinases.

PI3K Signaling Pathways

PIP3 is a lipid second messenger that activates many downstream molecules by binding to their pleckstrin-homology (PH) domains. The protein serine/threonine kinase AKT (also known as PKB) (►AKT signal transduction pathway in oncogenesis) is a principal target of PIP3. Binding of PIP3 to AKT leads to the membrane recruitment of AKT and subsequent phosphorylation by the mTOR–rictor kinase complex and by PDK1 (3-phosphoinositide-dependent kinase). This leads to the full activation of AKT, which in turn phosphorylates many target proteins, thereby regulating a range of cellular functions. An important AKT target is the forkhead (FOXO) family of transcription factors. AKT-mediated phosphorylation of FOXO proteins leads to their inactivation through cytoplasmic sequestration by 14-3-3 proteins. The main biological functions of these signaling pathways are described later (Fig. 2) [2, 3].

Cell metabolism: In muscle and fat, AKT promotes glucose uptake by stimulating the membrane translocation of the glucose transporter GLUT4. In addition, AKT activates glycogen synthase through the inhibition of glycogen synthase kinase 3 (GSK3) and regulates fatty acid synthesis by activating ATP citrate lyase (ATP-CL). In the liver, AKT inhibits gluconeogenesis by blocking FOXO-mediated transcription of gluconeogenic enzymes, such as phosphenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase).

Cell cycle and cell survival: AKT promotes G1–S cell-cycle transition by blocking FOXO-mediated transcription of cell-cycle inhibitors, such as p27Kip1 and RBL2 (retinoblastoma-like 2). AKT might also directly phosphorylate and inactivate p27Kip1. In addition, AKT indirectly stabilizes the cell-cycle protein c-Myc (►Myc oncogene) and cyclin D1 (►cyclin D) through the inhibition of GSK3. AKT promotes cell survival by blocking FOXO-mediated transcription of proapoptotic proteins such as FasL (Fas-ligand) and Bim (BCL2-like 11). AKT can also directly phosphorylate the proapoptotic protein BAD (BCL2-antagonist of cell death), causing its inactivation by 14-3-3 binding. Furthermore, AKT can phosphorylate MDM2 (transformed 3T3 cell double-minute 2 p53-binding protein), leading to p53 degradation (►p53 protein, biological and clinical aspects). Finally, AKT can phosphorylate some PKC isoforms (PKCζ) (►protein kinase C family) which activate IKK leading to IκB degradation and NF-κB activation (►nuclear factor κB).

Protein synthesis: AKT phosphorylates the tuberous sclerosis complex 2 (TSC2) protein tuberin, and therefore inhibits the GTPase-activating protein (GAP) activity of the TSC1–TSC2 complex toward Rheb (small G-protein Ras homologue enriched in brain). This allows GTP-bound Rheb to accumulate and activate the mTOR–raptor kinase complex, which in turn mediates phosphorylation of 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1) and p70S6Kinase, ultimately leading to increased protein synthesis.

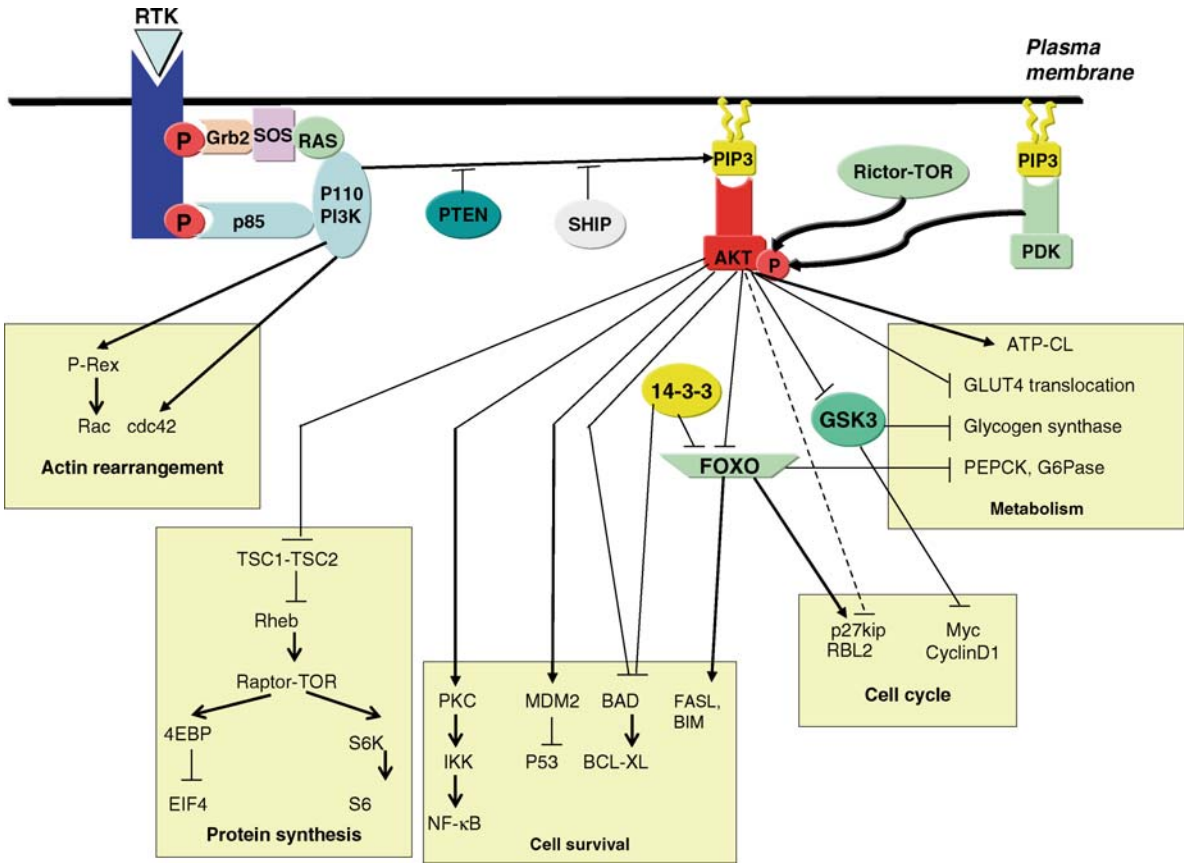
Cell polarity and motility: PI3K, together with the small GTPase Rac and Cdc42, regulates cell polarity and motility by controlling actin dynamics in motile cells.

Vesicle sorting: Class III PI3K regulates proper intracellular vesicle trafficking and may mediate aminoacid-induced mTOR activation.

Negative Regulation of PI3K Signaling Pathways

Two main phosphatases are involved in PIP3 dephosphorylation: PTEN (phosphatase and tensin homologue deleted on chromosome ten) and SHIP (SH2-domain, containing inositol 5-phosphatase).

►PTEN dephosphorylates phosphoinositides at the 3′ position of inositol leading to PI-4,5-P2. As a consequence, PTEN negatively regulates all signaling pathways induced by PI3K activation. Germline mutations in the *PTEN* gene result in Cowden disease and Bannayan–Riley–Ruvalcaba syndrome (also known as macrocephaly, multiple lipomas, and haemangiomata), which are two familial diseases characterized by benign



PI3K Signaling. Figure 2 Class I PI3K signaling pathways activated by RTK. Many pathways are activated leading to regulation of metabolism, cell cycle, cell survival, protein synthesis, and actin rearrangement. Figure derived from Engelman et al (2006).

tumors and high risk of cancer. Somatic mutations, gene deletion or gene inactivation of *PTEN* occurs in a sizeable fraction of glioblastomas, prostate cancers, breast cancers, and melanomas.

SHIP contains a centrally located phosphoinositide phosphatase domain that selectively hydrolyzes the 5′-phosphate from PIP3 leading to PI-3,4-P2 which interact at a lesser extent with AKT. SHIP may act as a tumor suppressor in hematopoietic progenitors, since inactivating mutation in the catalytic domain was found in acute myelogenous leukemia.

Main Phenotypes Induced by PI3K Signaling Pathways

The *in vivo* functions of the various isoforms of PI3K in mammals have begun to be addressed by gene deletion studies in mice. These studies highlight the importance of class IA PI3K signaling in regulating growth and metabolism. Furthermore, dysregulation of this pathway is crucial for the pathophysiology of several human diseases: mutations that lead to the amplification of PI3K signaling are among the most common mutations in human cancers, whereas attenuated PI3K signaling downstream of the insulin receptor is a major contributor toward type-2 diabetes (also known as noninsulin-dependent diabetes mellitus). Most of our understanding of PI3K signal transduction is based on studies of class I PI3Ks.

PI3K Signaling and Cell Growth

In myocytes, PI3K signaling controls cell growth (i.e., cell size). For example, overexpression of either a constitutively active form of p110α or a constitutively active form of AKT in the heart results in increased organ and cell size, whereas overexpression of a dominant-negative form of p110α reduces heart size. Furthermore, the expression of a dominant-negative form of p110α suppresses the growth induced by IGF1 receptor in the heart. Accordingly, tissue-specific deletion of PTEN leads to increased cell size, whereas mice with germline deletion of AKT1 or AKT3 show growth retardation.

PI3K Signaling, Tumorigenesis, and Cancers

In the 1980s, PI3K was discovered because of its association with onco proteins. Mutants of polyoma middle T that failed to bind PI3K were compromised in their ability to transform fibroblasts and polyoma middle T-transformed cells had elevated levels of PIP3. A few years later, the p110α catalytic subunit of PI3K was identified as an avian retrovirus-encoded oncogene that could transform chick embryo fibroblasts *in vitro*. Genetic analyses of human tumors performed in the 1990s showed that a locus on chromosome 10q23 was frequently deleted in advanced cancers. PTEN was located in this region indicating that it functioned as a tumor suppressor because of its ability to turn off the PI3K pathway. This idea has been further strengthened by the recent discovery of several genetic alterations in the pathway in human cancers, including p85, p110α, PTEN, and AKT. The *PIK3CA* (phosphatidylinositol 3-kinase, catalytic, α-polypeptide) (►PIK3CA oncogene) gene that encodes p110α is frequently amplified in several human cancers, such as head and neck cancers, cervical cancers, gastric cancers, and lung cancers. Recently, point mutations in p110α were identified in significant fractions of brain cancers, colon cancers, breast cancers, and hepatocellular cancers. Interestingly, most point mutations in p110α cluster around two hotspots: E545 in the helical PIK homology domain and H1047 near the end of the catalytic domain. These mutations increase *in vitro* PI3K activity of the holoenzyme. The expression of these p110α mutants in cells confers AKT activation in the absence of growth factor stimulation. Significantly, these mutants can transform fibroblasts and mammary epithelial cells, and drive tumor formation in mouse xenografts. Interestingly, mutations in other p110 isoforms have not been identified in cancers, indicating that p110α harbors more oncogenic potential. Amplification of AKT2 has also been found in human cancers, indicating that the tumorigenic effect of aberrant PI3K signaling is at least partly mediated through AKT. Recently, AKT2 mutations and IRS2 amplifications were also observed in colon cancers.

PI3K, Insulin Signaling, and Diabetes

In mammals, the PI3K–AKT signaling pathway has a central role in the regulation of downstream metabolism of the ►insulin receptor and IRS adaptor molecules. Indeed, insulin can mediate glucose uptake and membrane translocation of the glucose transporter GLUT4 in adipocytes and myocytes. In hepatocytes, PI3K–AKT signaling inhibits the FOXO-mediated transcription of gluconeogenic enzymes and therefore suppresses hepatic glucose production. Knockout and transgenic mice have ascertained the role of class IA PI3K in mediating insulin signaling *in vivo*. Mice with germline deletion of either p110α or p110β die early during embryogenesis, whereas mice that are doubly heterozygous for p110α and p110β, and mice that are heterozygous for a kinase-dead knock-in allele of p110α, exhibit glucose intolerance. In addition, tissue-specific deletion of PTEN in muscle, fat, or liver leads to enhanced insulin sensitivity in these tissues. Besides, mice deficient in all p85 isoforms in either muscle or the liver exhibit severely impaired insulin signaling in these tissues. These findings show that class IA PI3K is essential in mediating insulin action *in vivo*. Interestingly, the loss of PI3K signaling in the muscle results in impaired muscle glucose uptake, whole-body glucose intolerance, hyperlipidaemia, and adiposity, whereas the loss of PI3K signaling in the liver leads to unregulated hepatic gluconeogenesis, hyperglycaemia, hyperinsulinaemia, and reduced circulating lipids. Moreover, several polymorphisms in the human gene coding for p85α are associated with increased risk of type-2 diabetes. Elevated levels of p85, but not p110, were observed in muscles of type-2 diabetic individuals. The importance of the PI3K pathway in the regulation of metabolism has been confirmed by mutations of other components of the pathway. For example, germline deletion of AKT2, which is an AKT isoform that is abundant in muscle and liver, results in a diabetic phenotype in the mouse. The relevance of this model for human disease is supported by the identification of a point mutation in AKT2 in a familial form of severe insulin resistance.

Taken together, PI3K signaling mediates different cellular responses depending on the tissue context, and defective PI3K signaling in many tissues contributes collectively to the complex metabolic defects associated with type-2 diabetes.

Future Directions

Our understanding of the diverse roles of PI3K signaling puts us in a position to manipulate this pathway therapeutically for the treatment of cancers and diabetes.

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PIK3CA Oncogene

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Synonyms

Phosphatidylinositol 3-kinase (PI3-Kinase)

Definition

PIK3CA is a human gene that regulates various cellular functions including proliferation and invasion. Because it is an ►oncogene, its activation by either gene amplification or mutation results in a cellular growth advantage contributing toward cancer formation and progression.

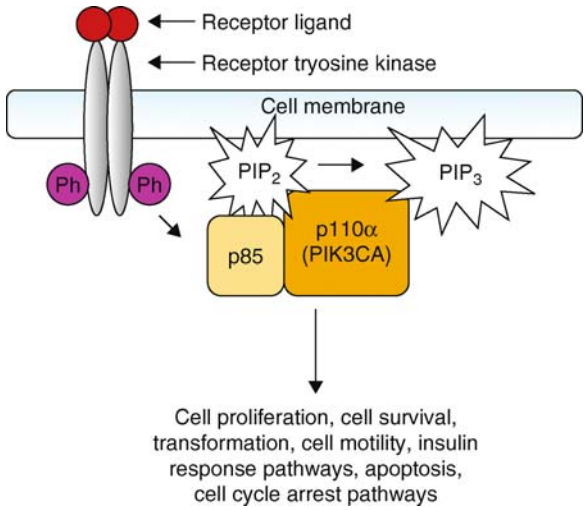
Characteristics

The PI3-Kinase pathway is one of many signaling pathways that are important for cell growth, ►transformation, adhesion, apoptosis, survival and invasion. PI3-►Kinases themselves are heterodimeric lipid kinases composed of catalytic and adaptor/regulatory subunits encoded by separate genes and alternative splicing. The PI3-Kinase enzyme family is organized under three main classes (class I, II and III) and various subgroups have been categorized based on their primary structure, substrate specificity and regulation.

The *PIK3CA* oncogene encodes for the alpha catalytic subunit and along with the p85 regulatory subunit, catalyzes the generation of the second messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP_3) from phosphatidylinositol 4,5-bisphosphate (PIP_2) (Fig. 1). This action is often initiated by ligand binding and subsequent activation of receptor tyrosine kinases, with the end result of increased cell signaling as measured by phosphorylation and activation of many downstream proteins. Signaling pathways and molecules affected by PI3-Kinase activity include the ►Ras/Raf/MAP kinase pathways as well as the ►AKT pathway, all known to be involved with cellular growth and proliferation. Normally these signaling pathways are tightly regulated to ensure that proper cell growth is controlled and maintained. In the case of oncogenes, including *PIK3CA*, genetic changes such as a mutation can result in a cellular state whereby the *PIK3CA* gene product is constantly activated. Ultimately, this contributes toward uncontrolled cellular growth and other features that are the hallmarks of cancer.

Genetics

The human *PIK3CA* gene is located on chromosome 3q26.32 and is ~34 kb consisting of 20 coding exons.



PIK3CA Oncogene. Figure 1 PI3-Kinase activity. PI3-Kinase is activated upon ligand binding to a receptor tyrosine kinase (RTK) which then activates the regulatory subunit (p85) to bind the catalytic p110α subunit. This catalyzes the reaction phosphatidylinositol (PI) 4,5-bisphosphate (PIP_2) to phosphatidylinositol (PI) 3,4,5-triphosphate (PIP_3) and ultimately initiates various downstream signaling cascades affecting cell survival, apoptosis, transformation, metastasis, and invasion.

The protein contains 1068 amino acids yielding a 124 kDa size protein. The PIK3CA protein contains five known functional domains including a p85 binding domain, a Ras binding domain, a protein kinase C homology 2 domain, a helical domain and a kinase domain.

In general, two main genetic alterations can lead to oncogene activation in human malignancies. The first mechanism is gene amplification, in which a ►wild type gene is duplicated, usually involving multiple duplications, leading to increased protein production of the amplified gene. This increase in oncogenic protein continually activates downstream signaling molecules and pathways, thus driving cellular growth and transformation. For *PIK3CA*, gene amplification has been described in human cancers and ►precancerous lesions including breast cancers, ovarian cancers and thyroid adenomas. However, *PIK3CA* gene amplification appears to be a relatively uncommon event in most human malignancies. Rather, a second mechanism, somatic ►missense mutation, is the predominant method of *PIK3CA* gene activation found in most human cancers. Accordingly, the majority of *PIK3CA* mutations found in human malignancies occur in one of two domains, the helical and kinase domains. These mutations are predicted to render the kinase function of the protein as being constantly activated and experimental data has supported this supposition. *PIK3CA* mutations occur at a relatively high frequency in breast

and colorectal cancers and at lower frequency in other solid malignancies. Strikingly, in most studies of activating *PIK3CA* missense mutations, three common "hotspot" mutations have been described, E542K, E545K, H1047R, with the first two mutations occurring in exon 9 (helical domain) and the third mutation affecting exon 20 (kinase domain). These frequently occurring mutations account for 80–90% of all *PIK3CA* mutations, and lend themselves as potential targets for diagnostic and therapeutic exploitation.

Clinical Relevance

Frequently recurring "hotspot" mutations have many implications for clinical oncology. First and foremost, because these mutations are somatic, meaning they are present in the cancer cell but not in normal tissues, these *PIK3CA* mutations become obvious targets for therapeutic intervention since the "holy grail" of all cancer therapy relies upon isolating medicines capable of specifically killing cancer cells while leaving non-cancerous cells relatively unharmed. The somatic activating nature of these mutations therefore makes them perfect for this type of targeted therapy and indeed small molecule PIK3CA inhibitors are beginning to enter early phase clinical trials. Additionally, because activating "hotspot" mutations are so common in a number of different human malignancies, targeting these mutations for therapy could have a tremendous impact on the morbidity and mortality of human cancers. Second, these mutations could also be potentially useful as a more sensitive and specific means of cancer detection and/or diagnosis. For example, given newer technologies that enable the detection of single mutant molecules in blood and stool, it is tempting to speculate that genetic testing could be developed to either detect cancer cells with mutant *PIK3CA* as an adjunct to an initial screening test, or to use mutant *PIK3CA* as a molecular marker to detect metastatic disease and/or recurrent disease after treatment. Along those lines, the detection of mutant *PIK3CA* in human cancer may also lend itself as a prognostic indicator of disease recurrence, and clinical studies in a number of cancers are currently addressing this important question. Ultimately, the discovery of the *PIK3CA* oncogene as one the most mutated oncogenes in human cancers makes it one of the most important cancer causing genes to date, and places it at the forefront for targeted drug development and cancer diagnostics.

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PI3-Kinase

►PI3K Signaling

PIM-1

Definition

Proto-oncogene that is a potent collaborator of ►Myconcogene in inducing lymphomagenesis in the mouse, encodes a serine/threonine protein kinase. While expression of PIM-1 is restricted primarily to the hematopoietic lineage, inducible PIM-1 expression has been observed in response to a wide variety of mitogens and cytokines, including PMA, IL-2, GM-CSF, G-CSF, IL-6, IL-3 and interferon γ.

►BCL6 Translocations in B-cell Tumors

PIN

Definition

Premalignant Intraepithelial Neoplasia

►Calcitonin

Pineal Gland

Definition

A small, pea-sized and pine cone-shaped gland located deep within the middle of the human brain between the

two halves of the cerebral cortex; produces the neurohormone melatonin.

►Melatonin

p16INK4A

Definition

Is a tumor suppressor gene that encodes a protein of 16 kDa. p16INK4a is a member of the ►INK4 family of cyclin-dependent kinase (CDK) inhibitors (CKIs); ►CDKN2A.

►Malignant Lymphoma: Hallmarks and Concepts

Pinocytosis

►Endocytosis

PI-PLC

Definition

Phosphoinositide-specific ►Phospholipase C; ►Inositol Lipids.

Pirarubicin

Definition

Is an analogue of the anthracycline antineoplastic antibiotic adriamycin.

►Adriamycin

Pituitary Tumor-Transforming Gene 1

►Securin

PJS

►Peutz-Jeghers-Syndrome

PKA

Definition

Protein kinase A is a serine/threonine kinase that depends on cAMP for its activity. The inactive holoenzyme consist of two catalytic and two regulatory subunits. Upon binding to cAMP, the tetramer dissociates into the dimer of the regulatory subunits and two active monomeric catalytic subunits. Cellular cAMP levels increases upon stimulation of adenylyl cyclase by a ►G-protein coupled receptor. PKA phosphorylates and activates certain transcription factors, such as CREB.

►CREB-binding Protein (CBP)/p300
►ETS Transcription Factors
►p300/CBP Co-Activators

PKB

Definition

Protein kinase B.

►Akt

PKC

Definition

►Protein kinase C.

►Protein Kinase C Family

PL74

►MIC-1

PLA2

Definition
Phospholipase A2.

PLAB

►MIC-1

Placebo

Definition
Tablet without medication.

►Menopausal Symptoms After Breast Cancer Therapy

Placenta Growth Factor

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Synonyms
Placental growth factor; PlGF

Definition
Placenta growth factor (►PlGF) is a member of the ►vascular endothelial growth factor (VEGF) family. PlGF is a secreted, homodimeric glycoprotein, originally isolated from human placenta.

Characteristics
PlGF shares 53% of similarity in its amino acid residues with VEGF. PlGF is highly expressed in placenta, but it is also present in other normal tissues and organs, such as thyroid, trophoblasts, heart, and lung, and it is upregulated in the skin, under physiological and pathological conditions.

Human PlGF gene maps to chromosome 14q24 and encodes four isoforms generated by alternative splicing. These isoforms differ in the ability to interact with other proteins, due to the presence or not of a heparin-binding domain and of a ►neuropilin-binding motif.

It is now widely accepted that PlGF isoforms function as angiogenic growth factors through direct binding and activation of the tyrosine kinase receptor vascular endothelial growth factor receptor-1 (►VEGFR-1). PlGF uses this receptor as well as VEGF-B and VEGF, the latter showing higher affinity in VEGFR-1 binding. Even so, PlGF transmits through VEGFR-1 its own specific signal that differs from that of VEGF both in the amino acidic tyrosine residues that are phosphorylated in the receptor molecule and in the downstream effectors.

PlGF is also able to sustain VEGF actions when a small amount of VEGF is present. Different mechanisms have been proposed to explain this event. One states that binding of PlGF to VEGFR-1 leads to an intermolecular trans-phosphorylation of VEGFR-2, the main transducer of VEGF proliferation signal. Another one indicates that PlGF forms heterodimers with VEGF and these dimeric proteins directly bind and activate VEGFR-2.

Animal models give evidence that PlGF plays important functions in adult pathological ►angiogenesis, while it is redundant for the development of the embryonic vasculature. PlGF-overexpression results in significant vessel growth, with formation of enlarged, mature vessels, covered with ►mural cells. PlGF has been often associated with ►arteriogenesis. Besides acting on ►endothelial cells, PlGF is chemotactic for monocytes, and can restore early and late phases of hematopoiesis following bone marrow suppression.

The role of PlGF in cancer has been debated for a long time and its significance is not completely clear. Nevertheless, scientific data have been continuously produced, indicating that PlGF is a major factor in tumor progression. PlGF expression has been detected in various human tumors, such as meningiomas, melanoma, cervical squamous cell carcinoma, gastric, breast and lung cancer. In some cases, PlGF expression is significantly associated with tumor stage, showing constant up-regulation during tumor progression. PlGF expression is down regulated in thyroid carcinoma and cervical adeno-carcinoma. Chromosomal translocations involving the PlGF locus with consequent overexpression of PlGF have been reported in a case of hemangioendothelioma, pointing to a direct involvement of PlGF in the pathogenesis of this tumor.

PlGF produced by tumor cells contributes to tumor angiogenesis by acting in a paracrine way on the surrounding microvessels, sustaining endothelial cell proliferation, migration and differentiation. The resulting augmented vascularization promotes tumor growth.

Consistently, in experimental animal models, PlGF-deficient mice show markedly reduced angiogenesis and tumor growth, whereas mice transplanted with PlGF-overexpressing glioma cells have enhanced endothelial cell survival and tumor progression. Moreover, transgenic mice over-expressing PlGF exhibit a rapid and important enlargement of inoculated tumors due to augmented vessel area. Altogether, these data assign to PlGF a significant role in supporting tumor angiogenesis. PlGF-induced tumor vessels show a reduced cover of mural cells, indicating that PlGF is no more able to sustain maturation of the newly formed vessels in the tumor environment.

It is commonly known that after cytotoxic therapy survived tumor cells rapidly expand again. A mechanism that permits recurrence consists of re-vascularization of the tumor lesion supported by tumor-produced angiogenic factors. By stimulation of vessel growth and survival, PlGF takes part to tumor recurrence. In fact, both PlGF and its receptor VEGFR-1 are up regulated in those cancer cells that survive cytotoxic treatment.

Tumor vascularization does not exclusively rely on sprouting and enrolment of pre-existing vessels, but also depends on mobilization from the bone marrow into the blood flood and recruitment at the tumor site of ►bone marrow-derived precursor cells (BMPC). PlGF is able to augment the amount of circulating BMPC. It is not presently clear the mechanism by which PlGF mediates BMPC mobilization from the bone marrow. It has been proposed that PlGF acts through activation of ►matrix metalloproteinase-9-dependent pathways. Preliminary evidences indicate that PlGF does not recruit BMPC to the tumor site but locally up-regulates the expression of other cytokines, such as the stromal cell-derived factor-1α, able to function as BMPC chemoattractants.

The presence of a microenvironment that fosters recruitment of BMPC has been revealed also at sites of future metastasis localization. VEGFR-1-expressing BMPC are mobilized from the bone marrow and recruited to distal organs to contribute in the formation of a ►premetastatic niche. Since PlGF is a specific activator of VEGFR-1 greatly involved in BMPC mobilization, it may take part to this early step of metastasis spreading, but no definitive proof has been given to support a direct contribution of PlGF in the formation of the premetastatic niche.

Augmented vessel area nearby the primary tumor facilitates cancer cells entering into circulation and dissemination of metastases. In a transgenic mouse model over-expressing PlGF, inoculation of tumor cells gives rise to increased metastasis formation. Metastases can spread to distant organs through invasion of the vascular and/or the lymphatic system. However, PlGF has no effect on ►lymphangiogenesis, thus PlGF-induced metastatic spreading mainly depends on angiogenesis and blood vessel invasion.

Besides its role in tumor angiogenesis, the most important feature of PlGF in cancer is that PlGF can directly promote invasiveness of VEGFR-1-expressing tumor cells. Melanoma cells stimulated *in vitro* with PlGF show a higher mobility rate that is dependent on VEGFR-1 activation. In fact, cell treatment with a function-blocking antibody against VEGFR-1 inhibits melanoma cell migration. PlGF also induces leukemia cell invasion in a VEGFR-1-dependent mode. The molecular mechanisms whereby PlGF stimulates tumor-cell migration and invasion have not been fully investigated, but cell treatment with PlGF increases the secretion of active matrix metallo-proteinase-2 and -9 that can contribute to ►extracellular matrix remodeling and facilitate cell movements.

Furthermore, VEGFR-1 expression on subsets of human tumor cells raises the possibility that PlGF promotes tumor growth both by inducing angiogenesis and by directly activating an autocrine signaling on tumor cells. Under cell culture conditions, PlGF induces proliferation of human melanoma and breast carcinoma cells.

Clinical Relevance

VEGF is widely recognized as an important regulator of tumor-induced angiogenesis, and a VEGF antagonist has been approved for clinical use in cancer treatment. Nevertheless, a number of recent data indicates that inhibition of VEGF signaling alone may not be sufficient to obtain regression of the disease and that PlGF is an additional factor that should be targeted as well. At present, the role of PlGF in cancer is supported only by preclinical data and specific compounds suitable for clinical trials have not been developed. However, it should be taken into account that inhibition of PlGF activity would significantly affects not only tumor angiogenesis but also metastasis formation. As PlGF is not significantly involved in physiological angiogenesis, targeting of this growth factor would not cause severe collateral effects. Therefore, a great effort has been asked to scientific community and pharmaceutical industries to push the development of specific anti-PlGF inhibitors.

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Placental-derived Growth Factor

Definition

PIGF; A pro-angiogenic cytokine that belongs to the vascular endothelial growth factor (VEGF) family of growth factors; PlGF is expressed in many cancers and specifically binds VEGF receptor 1.

►Angiogenesis

Placental Growth Factor

►Placenta Growth Factor

Placental Site Trophoblastic Tumor

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Synonyms

PSTT

Definition

Placental site trophoblastic tumor (PSTT) is a relatively uncommon type of gestational trophoblastic neoplasm that primarily occurs in the uterus. PSTT develops from neoplastic transformation of presumable trophoblastic stem cell and assumes a differentiation toward a specific type of trophoblastic cells, called intermediate (extravillous or interstitial) trophoblastic cells that are normally located in the placental site of the endometrium during a pregnancy. Thus, the "PSTT" is named for the tumor that arises from the placental site trophoblastic cells. As compared to other human cancers, PSTT is unique because the tumor is related to fetal tissue (i.e., trophoblastic cells from a placenta) but not to the patient's tissue. Like other gestational trophoblastic tumors (epithelioid trophoblastic tumor and choriocarcinoma), PSTT is considered as a "semiallograft" tumor in patients.

Characteristics

Clinical Features

- Patients with PSTT are usually of reproductive ages (mainly from 17 to 50 years) and present with either amenorrhea or abnormal vaginal bleeding, often accompanied by uterine enlargement.
- The preceding pregnancy history can be remote and the tumor can follow an abortion, a hydatidiform moles, or a term pregnancy.
- The serum β-hCG levels in PSTT patients usually elevate but are much lower than those in choriocarcinoma (100–2,000 μ/ml in PSTT vs. >10,000 μ/ml in choriocarcinoma).
- Most (70–80%) of the PSTTs behave in a benign fashion in which the tumor is confined to the uterus and simple hysterectomy or tumor resection is the treatment of choice. For the malignant PSTTs in which the tumor has locally invaded or distantly metastasized, chemotherapy may be required in conjunction with surgery. Metastases may develop several years after the initial diagnosis.
- The behavior of PSTT is difficult to predict since it has been demonstrated that no correlation exists between clinical outcome and molecular or morphological markers.
- With the use of dose-intensive chemotherapy, imaging techniques to define disease spread, surgery for localized disease and close surveillance with serologic measurement of β-hCG levels, most patients with PSTT are curable.

Pathological Features

On gross inspection most PSTTs are well circumscribed or polypoid, projecting into the uterine cavity or predominantly involving the myometrium. Invasion frequently extends to the uterine serosa but rarely to the adnexal structures. Under a microscope, PSTT is composed of neoplastic intermediate (extravillous) trophoblastic cells with morphology similar to trophoblastic cells in a normal placental site. The large and polygonal tumor cells infiltrate deep into the myometrium and insinuate themselves between smooth muscle fibers. The cells may be present singly, in nests or masses. PSTT is characterized by extensive deposition of fibrinoid material and a unique pattern of vascular invasion – replacement of the muscular wall of an artery by trophoblastic cells and fibrinoid material deposition.

Molecular Etiology

The molecular etiology of PSTT is largely unknown because of its rarity and lack of animal models for studies.

- The trophoblastic nature (fetal origin) of PSTT has been demonstrated by molecular genetic studies showing that the tumor contains new (paternal) alleles not present in adjacent normal uterine tissue.
- PSTT is associated with abnormal expression of cell cycle regulatory genes including cyclins, cyclin-dependent kinases, and p53.
- As compared to the normal extravillous (intermediate) trophoblastic cells in the implantation site, the majority of PSTTs overexpress the activated (phosphorylated) form of mitogen activated protein kinase (MAPK). The RAS/RAF/MEK/MAPK signaling pathway participates in various cellular activities including proliferation, differentiation, apoptosis, angiogenesis, and migration. It is likely that activation of MAPK contributes to the local invasion and distant metastasis in PSTTs.

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Plakin Family

Definition

A family of proteins that crosslink cytoskeletal filaments and attach them to cell junctions at the membrane. Includes desmoplakin, a constituent of ►desmosomes.

Plakoglobin

Definition

A protein belonging to the armadillo family of structural proteins and signaling molecules. Plakoglobin is a cytoplasmic constituent of ►adherens junctions and ►desmosomes. In adherens junctions it is interchangeable with another armadillo family member, β-catenin. Plakoglobin has an essential structural role in desmosomes and may act as a signaling molecule in the ►APC/β-catenin pathway.

Plakophilin

Definition

PKP; a protein belonging to the plakophilin subfamily of the armadillo family of structural proteins and signaling molecules. In humans three plakophilins are known (PKP1–3). They exhibit dual localization in ►desmosomes and in the nucleus.

Plant-derived Agents

►Phytochemicals in Cancer Prevention

Plaques

Definition

Are the spots seen in cellular plasma membranes by optical microscopy in immunofluorescence or immunohistochemistry using antibodies to ►connexins.

►Gap Junctions

Plasma Cell Neoplasm

►Plasmacytoma

Plasma Cells

Definition

Are terminally differentiated B lymphocytes and are the main ►antibody-secreting cells of the body. They are

found in the medulla of the lymph nodes, in splenic red pulp, and in bone marrow.

Plasma Membrane

Definition

Is a semipermeable bilayer found in all cells. It consists of a wide variety of biological molecules, primarily proteins and lipids, which are involved in a vast array of cellular processes such as cell adhesion, ion channel conductance and cell signaling. The plasma membrane also serves as the attachment point for the intracellular cytoskeleton.

►Cell Membrane
►Plasmalemma
►Phospholipid Bilayer

Plasma Thromboplastin Antecedent

Definition

►Factor XI.

Plasmacytoma

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Synonyms

Plasma cell neoplasm; Solitary plasma cell myeloma; Solitary bone plasmacytoma; SBP; Solitary extramedullary/extraosseous plasmacytoma; SEP

Definition

►Plasmacytoma (►PCT) refers to a solitary, localized, malignant, monoclonal plasma cell neoplasm that grows either in bone (►solitary bone plasmacytoma: SBP) or soft tissue (►solitary extraosseous or extramedullary plasmacytoma: SEP). PCT is a rare, curable cancer. Most patients are treated with moderate-dose radiotherapy. A subset of patients requires surgical intervention. Adjuvant chemotherapy is not indicated. The likelihood of local control after treatment is high. The most common pattern of relapse is systemic, indicating progression of PCT to ►multiple myeloma (MM). This is more likely to occur after treatment for SBP compared to SEP. The importance of the diagnosis PCT rests in large measure with the potential for this malignancy to progress to MM. An excellent current review of PCT recently updated with useful clinical observations is available [1,2].

Characteristics

Epidemiology: PCT is a rare form of cancer that affects fewer than 10% of patients with plasma cell neoplasms. Plasma cell neoplasms account for 1–2% of malignancies and occur at an overall rate of ~4.5 per 100,000 people per year. PCT is more common in males, with a male-to-female ratio of ~2:1 in case of SBP and ~3:1 in case of SEP. The median age of patients with PCT is 55 years, ~15 years younger than patients with MM. SBP develops into MM in 50–60% of patients, resulting in a median overall survival time of ~10 years. Progression of SEP to MM is less frequent (~20% at 10 years after diagnosis), leading to a higher survival rate (70% at 10 years) compared to SBP.

Etiology: Although no definitive cause for PCT has been identified, it is likely that the genetic and environmental factors associated with increased risk of MM also play a role in the etiology of PCT. Genetic risk factors for MM include gender (male preponderance), race (e.g., African Americans in the U.S. exhibit twice the incidence of U.S. whites) and age (median age at diagnosis is ~70 years). Clustering of MM in certain families points to a hereditary predisposition consistent with an autosomal dominant mode of inheritance. However, the underlying tumor susceptibility alleles or "MM genes" have not yet been identified. Ionizing radiation, a well-recognized leukemogen, has long been considered the strongest environmental risk factor for MM, yet it has not been possible, thus far, to confirm radiation as a definitive cause of MM. Associations of MM with (i) occupational exposure to various metals (nickel), chemical compounds (aromatic hydrocarbons, silicone, petrochemical industries), pesticides and animal viruses (farming), (ii) protracted infections that can lead to sustained immune stimulation of B lymphocytes with antigen (*Helicobacter pylori*, human herpesvirus-8), (iii) acquired immunodeficiency syndromes that can result in reduced immune surveillance by T lymphocytes (HIV/AIDS), and (iv) autoimmune diseases, such as rheumatoid arthritis, have also been proposed, but the evidence continues to be weak.

Pathogenesis: The natural history of PCT is complex and poorly defined. SBP and SEP share many

histopathologic, genetic and biologic features, including sheets of neoplastic plasma cells that produce monoclonal immunoglobulin; recurrent chromosomal translocations that involve the immunoglobulin heavy-chain locus, *IGH*, at 14q32; dependence on the plasma cell growth, differentiation and survival factor, interleukin 6 (IL-6); and a common global gene expression profile. Nevertheless, there are also major differences of SBP and SEP that point to a different tumor precursor (cell of origin) and pathway of neoplastic development. The above-mentioned epidemiologic differences of SBP and SEP in terms of tumor progression and overall survival support this view.

Just like MM, SBP is thought to be derived from an antigen-experienced isotype-switched post-germinal center B-lymphocyte that has undergone somatic hypermutation of the expressed immunoglobulin heavy and light chain genes. The clinical observation that SBP can be an intermediate step in the evolution from ►monoclonal gammopathy of undetermined significance (MGUS) to MM underlines the view that SBP and MM share a common pathway of neoplastic cell transformation. Pathogenetic factors implicated in MM are thus likely to be also important for SBP. Factors of this sort include cytogenetic and molecular genetic alterations that result in the deregulated expression of oncogenes, such as *CCND1* (encoding cyclin D1), *FGFR3* (fibroblast growth factor receptor 3) and *WHSC1* (Wolf-Hirschhorn syndrome candidate 1; also known as *MMSET* or multiple myeloma SET domain containing protein type III). The interaction of tumor cells with the bone marrow microenvironment, which leads to the production of cytokines, such as IL-6, insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), stromal cell-derived factor 1α (SDF-1α), tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β), is also of crucial importance.

Unlike MM, SEP is believed to arise from a B lymphocyte or plasma cell that resides in the mucosa-associated lymphoid tissue of the aerodigestive tract and has performed isotype switching. Most SEP express IgA. It is possible that isotype switching occurred outside the germinal center, without T-cell help, under the influence of microbial factors from the aerodigestive tract. But this has not been demonstrated. The molecular genetics of SEP including the cellular signaling pathways that govern growth, proliferation and survival of tumor cells is less well defined than the molecular genetics of its counterpart in the bone. Likewise, the interaction of SEP with the special microenvironment of the subepithelial mucosa is less well understood than the interaction of SBP/MM with the bone marrow. As mentioned above, SEP can progress to MM, albeit less frequently than SBP. The genetic and/or epigenetic changes underlying the switch from bone-avoiding SEP to bone-seeking MM are unknown.

Clinical Features: PCT can arise in any body part. Tissue biopsy shows monoclonal plasma cell histology. Bone marrow plasma cell infiltration does not exceed 5% of nucleated cells. Peripheral blood cell count, renal function and calcium are within the reference range. Serum or urine monoclonal protein (►M protein) is absent in more than half of patients or minimal in the majority of patients in which it can be detected. Levels of immunoglobulins not involved in the M spike are preserved. A rare occurrence is multiple solitary PCT, which, at first glance, sounds like an oxymoron. Nonetheless, this form of PCT exists, without evidence of MM, in up to 5% of patients with apparent SBP or SEP.

- SBP: The most common symptom is pain at the site of the skeletal lesion, which shows predisposition for the red marrow-containing axial skeleton. Compression fractures of the thoracic and lumbar vertebral bodies (spinal disease, observed in ~50% of cases) result in severe spasms, back pain, and nerve root or spinal cord compression. The latter represents an emergency requiring immediate attention to avoid permanent neurologic damage, including paraplegia, bowel and bladder dysfunction, and chronic pain. Occasionally, patients may present with peripheral polyneuropathy or features consistent with the syndrome of ►POEMS: polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes.
- SEP: Although extraosseous tumors occur in any tissue site, 80% of SEPs develop as head-and-neck tumors in the upper aerodigestive tract, especially in the paranasal sinuses, pharynx, nasal cavity, gums and oral mucosa. Patients with tumors involving the base of the skull may present with cranial nerve palsies. In one third of cases, local lymph nodes are involved at presentation.

Imaging Studies: Skeletal survey is preferred over bone scan, which is less sensitive for detecting bone lesions. Approximately 25–50% of bone trabeculae must be destroyed for a bone defect to be visible on a plain radiograph, where SBP classically exhibits a lytic appearance with clear margins and a narrow zone of transition to healthy surrounding bone. Rare occurrences of a cyst, a trabeculated lesion resembling a giant cell tumor or an aneurysmal bone cyst, and sclerotic lesions associated with ►POEMS syndrome have been described. Computed tomography (CT) is more sensitive for evaluating the extent of bone destruction. For SPB, magnetic resonance imaging (MRI) is helpful to monitor the treatment response. In most cases of SEP with nasal cavity or maxillary sinus involvement, radiographic assessment shows local bone destruction.

Treatment: The treatment of choice for SBP is moderate-dose radiotherapy (40 Gy for spinal lesions

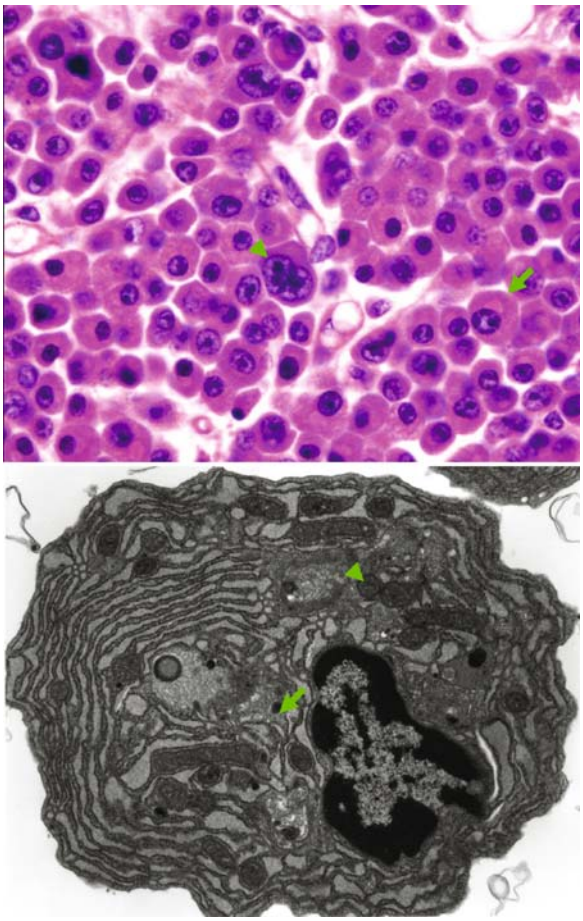
and 45–50 Gy for other bone lesions) administered once daily at 1.8–2.0 Gy per fraction in a continuous course. Virtually all patients have major symptom relief. Local tumor recurrence is ~10%. Surgery is contraindicated in the absence of structural instability or neurologic compromise. Chemotherapy using regimens proven in MM may be considered for patients not responding to radiation therapy. No role exists for adjuvant chemotherapy. In case of SEP, the accepted treatment is radiotherapy, surgery when a lesion can be completely resected, or combined surgery and radiotherapy, which may provide the best results. Chemotherapy may be considered for patients with refractory or relapsed disease. As an exception to the general rule that adjuvant chemotherapy is not indicated in PCT, this therapy may be considered for the subset of patients with positive surgical margins after resection or tumors larger than 5 cm and exhibiting high-grade histology.

Follow-up Outpatient Care: For both SBP and SEP, periodic evaluation for progression and development of MM is recommended every 6 weeks for the first 6 months, with extension of clinic appointments thereafter. Besides a complete physical examination, the following tests are recommended: complete blood cell count; complete metabolic panel with lactic dehydrogenase, calcium, phosphorus, ►C-reactive protein (CRP), and ►β2 microglobulin; sedimentation rate; serum ►protein electrophoresis with immunofixation; serum immunoglobulin quantification; urinary protein electrophoresis with immunofixation; and skeletal bone survey. Orthopedic and/or ear–nose–throat follow-up is recommended for SBP and head-and-neck SEP, respectively.

Prognosis: SBP progresses to MM in the majority of patients, with the median onset of conversion ranging from 2 to 5 years. Ten-year disease-free survival has been reported to range from 15 to 45%. Overall median survival is 10 years. In SEP, the rate of progression to MM is lower compared to SBP, ranging from 10 to 30% at 10 years after diagnosis. The 10-year overall survival rate is 70%. Thus, the prognosis of SEP is better than that of SBP.

Medical and Legal Pitfalls: Failure to diagnose spinal cord compression syndrome or impending pathologic fractures can cause irreversible neurologic damage. Inappropriate follow-up or failure to evaluate progression to MM delays institution of appropriate therapy to control the well-known systemic complications of this invariably fatal malignancy. These complications include generalized bone destruction, hypercalcemia, anemia, hyperviscosity, infections, and amyloid.

Basic Research: PCT occurs not only in human beings but also in mammals including mice (Fig. 1). The laboratory mouse is the ideal model organism for research studies on the biology and pathogenesis of PCT, because mice are prolific, easy to maintain and genetically defined. Importantly, mice are genetically manipulable using gene targeting and other methods, which afford an opportunity to accurately recapitulate genetic changes of human PCT in transgenic mice. Indeed, mouse models of human PCT are enhancing our understanding of the role of oncogenes, tumor suppressors, cytokines and environmental co-factors of oncogenesis provided by the bone marrow stroma,



Plasmacytoma. Figure 1 Histo- and cytomorphology of plasmacytoma (PCT). Shown at the top is a photomicrograph of a histological section of a mouse PCT that developed in the gut-associated lymphoid tissue of a BALB/c mouse harboring a widely expressed human interleukin-6 (IL-6) transgene. Neoplastic plasma cells are rich in cytoplasm and have a single, eccentric nucleus that contains clumped chromatin, forming in some cases the characteristic wheel spoke-like pattern (*arrow*). A large, atypical, binucleated tumor cell is located in the center (*arrowhead*). Depicted at the bottom is an electron micrograph of a neoplastic plasma cell from the same tumor. The cell nucleus (*arrow*) contains euchromatin in the center (*light grey*) and heterochromatin attached to the nuclear membrane (*black*). The abundance of rough endoplasmic reticulum (*arrowhead*) in the cytoplasm indicates that PCT cells produce copious amounts of monoclonal immunoglobulin.

inflammatory cells, and the innate and adaptive immune system. Two widely used mouse models of SBP and SEP have been developed in strains C57BL/6 and BALB/c, respectively.

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Plasmalemma

Definition

Plasma membrane.

Plasmamembrane Microdomains

Definition

Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains and lipid rafts. Clustering of signaling complexes such as the T-cell receptor complex (TCR) and CD44, associated with other molecules.

►CD44

Plasmin

Definition

Serine proteinase circulating in the blood stream which degrades many plasma proteins. Its most important function is the degradation of ►fibrin clots. It is secreted in its pro-form, plasminogen, which is activated by ►tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), thrombin, plasmin, and ►factor XII. Elevated levels of uPA are found in advanced breast cancer tissues.

►Proteinase-Activated Receptors

Plasminogen Activating System

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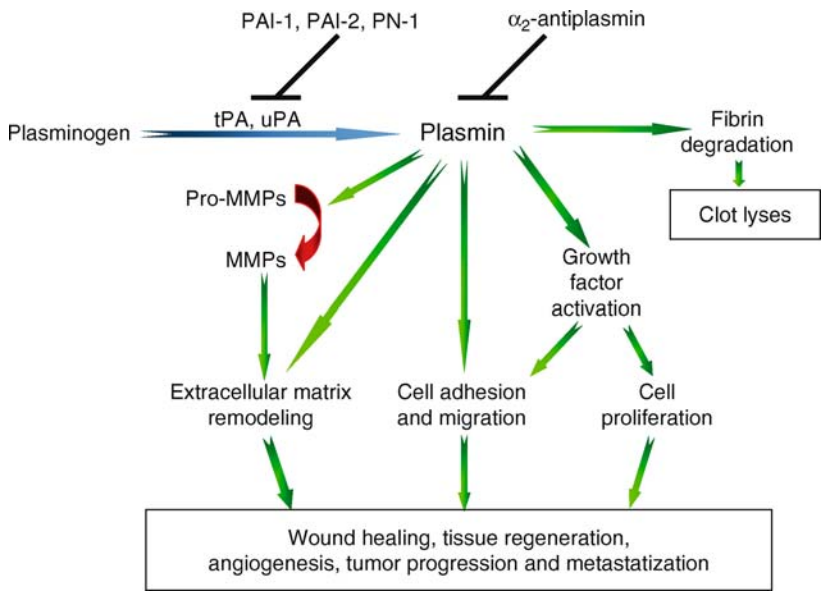
Definition

The plasminogen activating system (PAS) consists of two immunologically distinct serine proteases, the urokinase plasminogen activator (uPA) and the tissue-type plasminogen activator (tPA), their specific inhibitors, the plasminogen activator inhibitor 1 (PAI-1) and 2 (PAI-2) and the protease nexin-1 (PN-1), which belong to the serine protease inhibitor superfamily of serpins, and the glycolipid-anchored cell membrane receptor for the uPA. The two plasminogen activators convert, in the extracellular environment, the proenzyme plasminogen into the serine protease plasmin. The latter is involved in fibrin degradation during clot lysis and in a number of physiological and pathological processes requiring basement membrane (BM) and/or ►extracellular matrix (ECM) ►remodeling, such as wound healing, mammary gland development and its postlactational involution, tissue regeneration, ►angiogenesis, tumor ►progression, and metastatization (Fig. 1). Beside fibrin, in fact, plasmin may directly degrade several ECM and BM components, including laminin, vitronectin, type IV collagen, and proteoglycans, and may also activate latent ►matrix metalloproteinases (MMPs). Physiological inhibition of the plasminogen activating system may occur either at the level of plasmin by α_2-antiplasmin, or at the level of the two plasminogen activators, tPA and uPA, by PAI-1, PAI-2, and PN-1 (Fig. 1).

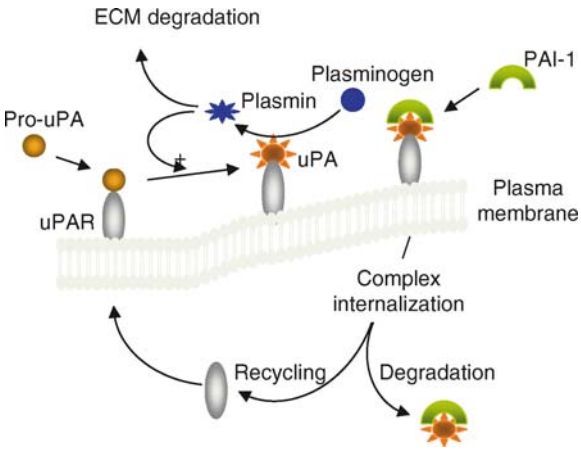
Characteristics

Plasminogen conversion to plasmin into the blood circulation during clot lysis is mostly due to the action of tPA, while uPA plays a major role in ECM remodeling at the tissue level. The uPA is secreted from the cells as a single chain proenzyme (pro-uPA or sc-uPA) able to bind to a specific cell membrane glycolipid-anchored receptor, the uPA receptor (uPAR) (Fig. 2). The membrane-bound pro-uPA may be converted into the active uPA, a two-chain molecule held together by a single disulfide bridge (tc-uPA), by the



Plasminogen Activating System. Figure 1 Schematic representation of the major biological processes involving the plasminogen activating system. MMP, matrix metalloproteinases; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; PAI, plasminogen activator inhibitor; PN-1 protease nexin-1.

action of different enzymes, including plasmin, cathepsin B, and kallikrein. In view of the fact that also plasminogen may bind to plasma membrane receptors, the occurrence in the same cells of both uPAR and plasminogen receptors may result in the formation of cell membrane-associated plasmin, leading to a localized pericellular ECM degradation. As mentioned, uPA activity is inhibited by PAI-1, PAI-2, and PN-1, which interact with active uPA either in solution or bound to the uPAR. In the latter case, the complex is rapidly internalized by the cells, leading to the degradation of both uPA and its inhibitor, while the uPAR is recycled to the cell membrane (Fig. 2).



Plasminogen Activating System. Figure 2 Schematic representation of the urokinase plasminogen activator (uPA), its cognate receptor (uPAR) and the plasminogen activator inhibitor (PAI-1) interaction and function. ECM, extracellular matrix.

The Plasminogen Activating System in Cancer Progression

An efficient ECM and BM degradation, achieved through the action of different proteolytic enzymes, is required for cancer invasion and dissemination. Several evidences, obtained using different experimental models, indicate that PAS, especially the uPA/uPAR complexes, plays a relevant role in this process. In fact, it has been demonstrated that lung ►metastasis of different ►melanoma-derived cell lines inoculated in nude mice, positively correlated with cancer cell uPA expression, and that overexpression of uPA in ►prostate cancer cells, following transfection with uPA cDNA, increased skeletal metastasis in vivo. Also, inhibition of uPA activity by low molecular weight inhibitors or antibodies, as well as inhibition of uPA or uPAR expression by antisense oligonucleotides, induced a decreased tumor growth and metastasis.

The main function of PAS, along with members of the MMPs family, was originally thought to be limited to the degradation of ECM and BM required for local diffusion and spread to distant sites of malignant cells as well as for tumor angiogenesis. However, it has become increasingly clear that PAS affects also multiple aspects of the neoplastic evolution, including tumor cell proliferation, ►adhesion and ►migration, intravasation and growth at the metastatic site (Fig. 1). In particular, uPA, directly or through the generation of plasmin,

P

promotes cell proliferation and tumor neoangiogenesis by activation or release of several growth factors, including epidermal growth factor (EGF), ►insulin-like growth factor (IGF), ►transforming growth factor-β (TGF-β), basic ►fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and ►vascular endothelial growth factor (VEGF). Moreover, uPAR, following uPA binding, may affect cell proliferation and other cellular functions by triggering intracellular signaling, leading to the activation of tyrosine and serine protein kinases (i.e., lymphocyte protein tyrosine kinase, lck; hematopoietic cell kinase, Hck; focal adhesion kinase, FAK, and mitogen-activated protein kinase, ►MAPK). Since uPAR lacks transmembrane and cytosolic domains, the activation of the intracellular signaling is realized through its interaction with at least three different transmembrane proteins, which include members of the integrins adhesion receptor superfamily, G-protein coupled receptor (GPCR), and caveolin.

The occurrence of metastasis implies the ability of malignant cells to migrate from the primary site to distant places. Cell migration initiates with the extension of the plasma membrane at the leading edge of the cell, to which corresponds an intracellular reorganization and polymerization of new actin filaments. The latter are then stabilized by the formation of new adhesions to ECM components. At the same time, at the rear or trailing edge of the cells, plasma membrane releases its binding to ECM. Thus, consecutive cycles of attachment and detachment from ECM substrates take place during cancer cell migration. Several reports demonstrated the ability of PAS to affect this process and the involved molecular mechanisms include: (i) ECM proteolysis at the leading edge of the cell allowing plasma membrane extension; (ii) ECM-cell adhesion receptor degradation causing the release of the trailing edge of the cell; (iii) activation of motogenic growth factors such as HGF, bFGF and TGF-β; and (iv) modulation of new cell adhesion to ECM. Regarding the latter, experimental evidences suggest that binding of uPA to its cognate receptor induces conformational changes in uPAR increasing its affinity for the ECM component vitronectin. The uPAR/vitronectin interaction, however, occurs only in presence of low levels of PAI-1. In fact, if the concentration of PAI-1 exceeds that of uPA, then the inhibitor will displace uPAR binding to vitronectin causing the release of the cell from the ECM. In addition, PAI-1 inhibits the binding of vitronectin to integrins receptors and reduces cell migration.

Clinical Significance of PAS Components Expression in Cancer

Consistent with their role in cancer progression and metastasis, a marked increase in the expression of uPA, uPAR, and PAI-1, with respect to normal counterpart tissues, has been observed in malignant tumors. For example, in breast cancers, the expression of uPA and uPAR has been shown to be upregulated approximately of 5- and 19-fold, respectively, compared with normal or benign tumor tissues. Similarly, in thyroid papillary carcinoma the upregulation of uPA and uPAR expression by more than fourfold, with respect to normal matched thyroid tissues, has been documented.

Different clinical studies demonstrated that high tumor tissue levels of uPA and its cognate receptor uPAR correlate with a poor prognosis in several malignancies including leukemia, breast, lung, brain, esophageal, gastric, pancreatic, colorectal, hepatocellular, endometrial, ovarian, kidney, and bladder cancers. Paradoxically, high levels of PAI-1 expression, considered the primary inhibitor of uPA, are also associated with more aggressive diseases and poor prognosis in several types of cancer. Different hypotheses have been proposed to explain these observations, including an essential role of PAI-1 in tumor angiogenesis, in cancer cells adhesion, migration, and inhibition of apoptosis.

The clinical relevance of PAS component expression is particularly evident in ►breast cancer, in which the prognostic value of uPA, uPAR, and PAI-1 cancer tissue expression has been exhaustively investigated. Indeed, the prognostic value of breast tumor tissue uPA expression was first described in 1988, when it was demonstrated that patients with breast tumors containing a high level of uPA activity had a significantly shorter disease-free interval, compared to patients with low level of tumor-associated enzyme activity. This initial observation was then confirmed in several large case studies, demonstrating that uPA is the most potent independent prognostic factor, in terms of overall survival and/or disease-free interval, described to date, with a predictive value stronger than those of patient age, tumor size, estrogen and progesterone receptors, ►HER-2/neu or ►p53 expression. High levels of PAI-1 are also associated with adverse outcome in breast cancer patients and it has been demonstrated that it represents an independent prognostic factor. Moreover, the concomitant assay of both uPA and PAI-1 possess a greater prognostic value with respect to that of each marker alone. Therefore, both uPA and PAI-1 are now candidate molecular markers for routine clinical use in patients with breast cancer.

PAS as Target for Anticancer Therapy

The involvement of PAS in cancer progression and the observation that its inhibition is devoid of toxicity, as demonstrated in uPA or uPAR-deficient mice, identify the PAS inhibition as a suitable target for anticancer therapies. The first evidence showing that uPA inhibition could be effective in preventing cancer metastasis was reported in 1983. In this study, it was demonstrated that antibodies which inhibit uPA

activity prevent metastasis from the human epidermoid carcinoma cell line Hep3 in the chick chorioallantoic membrane system. Since then, different therapeutical strategies have been designed to inhibit the uPA/uPAR function, including selective inhibitors of uPA activity, antagonist peptides and monoclonal antibodies able to prevent uPA binding to uPAR, antisense or gene therapy reducing uPAR expression and peptides that inhibit uPAR/integrins interaction, thus preventing uPAR-mediated cell signaling and adhesion. These approaches have demonstrated, in xenographic animal models, antitumor effects, including a reduction in local tumor growth and cancer dissemination. However, these new anticancer therapeutical strategies, although promising, need definitive confirmation in humans and, up-to-date, only one uPA inhibitor, the WX-UK1 (Wilex AG, Munich, Germany) entered clinical trial, the results of which are still awaited.

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Plasminogen-Related Growth Factors

Definition

Are considered a family of proteins that activate a unique biological signaling cascade leading to invasive growth.

►Ron Receptor

Plasticity

Definition

The ability to cross cell-lineage boundaries; ►stem cell.

Platelet

Definition

Synonym: Thrombocyte; Cells without nuclei which circulate in the blood. They are involved in thrombus (blood clot) formation. They contain fibrinogen, factor V, vitronectin, thrombospondin and ►von Willebrand factor, which are released upon platelet activation. Platelets are produced in the bone marrow. The progenitor cell for platelets is the megakaryocyte which sheds platelets into the circulation.

►Proteinase-Activated Receptors

Platelet-Activating-Factor

Definition

PAF; Is a potent inflammatory mediator; it is an ether-linked analogue of phosphatidylcholine, bearing an ester linked acetyl moiety at the sn-2 position of glycerol.

►Lipid Mediators

Platelet-derived Endothelial Cell Growth Factor

►Thymidine Phosphorylase

Platelet-Derived Growth Factor

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Definition

Platelet-derived growth factor (PDGF) is a family of growth-factors with mitogenic activity for connective

tissue cells, such as fibroblasts and smooth muscle cells, as well as for certain other cell types.

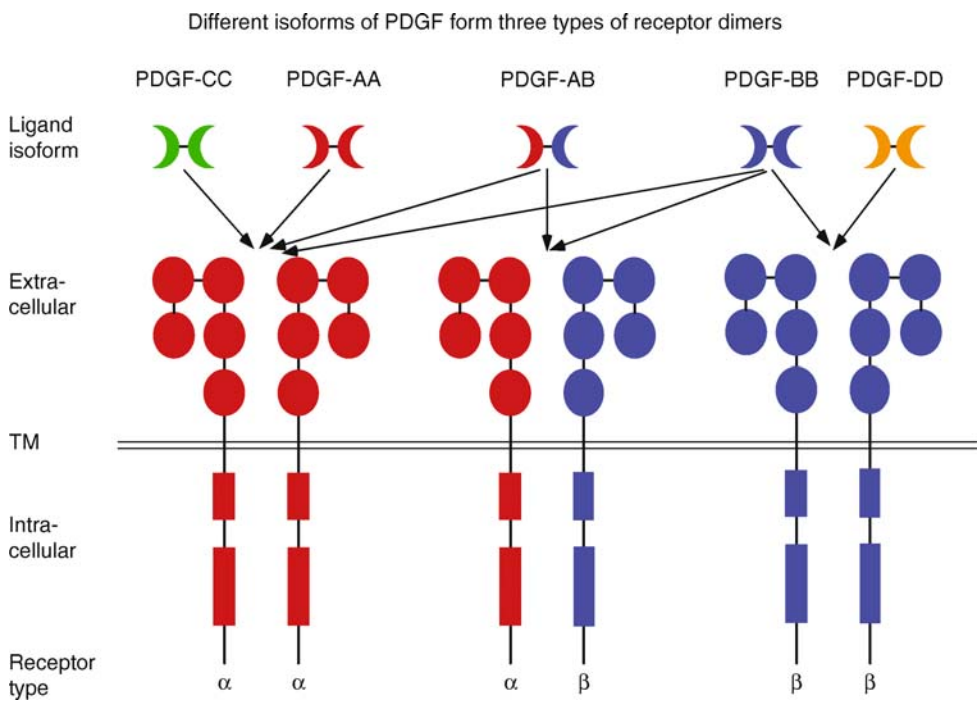
Characteristics

PDGF isoforms are disulfide-bonded homodimers of homologous A-, B-, C- and D-polypeptide chains, as well as a heterodimer PDGF-AB. PDGF A- and B-chains are proteolytically processed during secretion from the producer cell, whereas PDGF C- and D-chains are secreted as inactive precursors containing N-terminal ►CUB domains that need to be cleaved off before these isoforms can bind to receptors. The mature growth factor domain has about 100 amino acid residues with a perfect conservation of 8 cysteine residues. The two subunits in the dimers are arranged antiparallel. In addition to the two interchain disulfide bonds, each subunit contains six additional cysteine residues that are arranged in a characteristic cystine knot structure. The amino acid sequences of PDGF chains are homologous to those of vascular endothelial cell growth factors (►vascular endothelial growth factor).

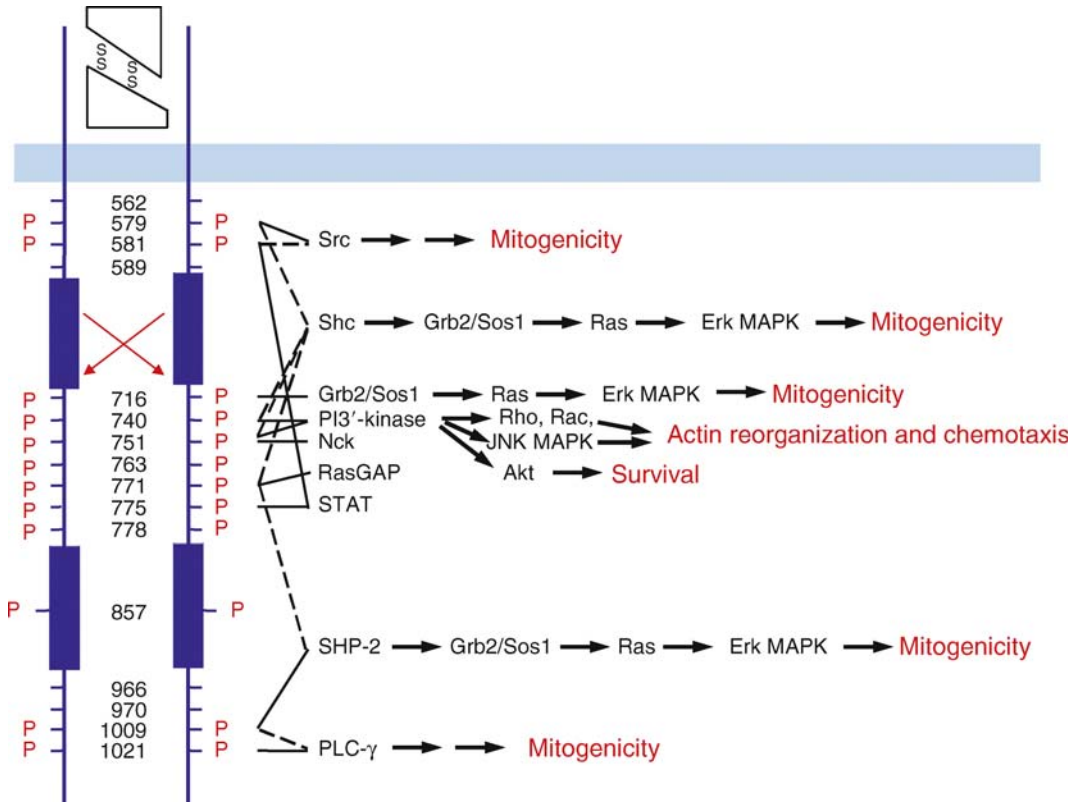
PDGF isoforms exert their cellular effects via binding to two structurally related protein tyrosine kinase receptors (►receptor tyrosine kinase), denoted α- and β-receptors. The A-, B- and C-polypeptide chains binds with high affinity to α-receptors, whereas B- and D-polypeptide chains bind to β-receptors (Fig. 1). The dimeric PDGF isoforms bind two receptor molecules simultaneously; receptor dimerization leads to juxtaposition of the kinase domains of the receptors, whereby they phosphorylate each other in *trans*. The "autophosphorylation" activates the receptor kinases and initiates intracellular ►signal transduction by recruitment of ►SH domain (SH2/SH3 domain) containing signal transduction molecules. Thereby several signal transduction pathways are initiated, ultimately leading to cell proliferation, cell migration, changes in cell morphology and inhibition of cell death (Fig. 2). Intracellular cell signaling is characterized by extensive cross-talk between different signaling pathways. Moreover, stimulatory and inhibitory pathways are often induced in parallel.

Genes

The PDGF A-, B-, C- and D-chain genes are localized on chromosomes 7, 22, 4 and 11, respectively. In all genes, the first exon encodes untranslated sequences and the signal sequence. In the A- and B-chain genes, the second and third exons encode N-terminal sequences that are removed during proteolytic processing, the fourth and fifth exons encode most of the mature parts of the proteins, and exon 7 is mainly noncoding. Exon 6 encodes C-terminal sequences that in the case of the B-chain may be removed during processing. The A-chain occurs as two different splice forms, with and without the exon 6 sequence. Since the exon 6 sequence contains a stretch of basic amino acid



Platelet-Derived Growth Factor. Figure 1 Binding of PDGF isoforms to α- and β-tyrosine kinase receptors.



Platelet-Derived Growth Factor. Figure 2 Ligand-induced dimerization of the PDGF β-receptor, followed by autophosphorylation and docking of SH2-domain-containing signaling molecules. The importance of individual signaling pathways for the effects of PDGF in cell growth, survival, migration and actin reorganization, is depicted.

residues that bind to extracellular matrix molecules, the absence or presence of the exon 6 sequence affects the localization of PDGF. In the C- and D-genes, exons 2 and 3 encode the CUB domain, exon 4 (exon 4 and 5 for the D-chain gene) encodes a linker region, and exon 5 and 6 (6 and 7 for the D-chain gene) encode the growth factor domain.

Bioactivity

Sequencing of PDGF revealed that the B-chain is almost identical to the v-Sis ►oncogene product, the transforming protein of simian sarcoma virus. Subsequent studies have shown that transformation of cells by the simian sarcoma virus occurs via autocrine stimulation involving a PDGF-like growth factor.

The demonstration that overactivity of PDGF had transforming effects prompted studies on the expression of PDGF in human tumors. PDGF was found to be commonly expressed in glioblastomas and sarcomas, tumors derived from cell types normally expressing receptors, suggesting ►autocrine stimulation of growth.

PDGF is expressed also by several cancers that are derived from cell types that do not express PDGF receptors. Such tumor-derived PDGF has been shown to be involved in a ►paracrine manner in the formation of supporting connective tissue cells that surrounds the tumor cells. PDGF stimulation of connective tissue cells in the stroma also contributes to the increased interstitial fluid pressure of tumors, which causes a decreased uptake of drugs into the tumor and precludes efficient chemotherapy of tumors. PDGF has also been shown to have an angiogenic activity (►angiogenesis), through direct effects on capillary endothelial cells, as well as indirectly through the recruitment of supporting pericytes that reinforce the walls of the newly formed vessels.

Clinical Relevance

The normal function of PDGF is to promote the development of different kinds of connective tissue cells during embryonal development. Often PDGF is produced by different epithelial cell types and acts on neighboring connective tissue cells expressing PDGF receptors. In the adult PDGF stimulates wound healing, and topically applied PDGF-BB (becaplermin) has been shown in clinical trials to increase the rate of healing of different types of wounds. PDGF also

regulates the interstitial fluid pressure in connective tissue. PDGF released from platelets may also exert a negative feed back control of platelet aggregation; binding of PDGF to α-receptors on platelets inhibits platelet aggregation.

The important function of PDGF in the development and homeostasis of connective tissue suggests that overactivity of PDGF may result in fibrotic reactions. In support of this notion, overexpression of PDGF has been shown to be involved in glomerulonephritis, liver cirrhosis, myelofibrosis and lung fibrosis. The stimulating effect of PDGF, released from platelets or secreted from macrophages, on smooth muscle cells at sites of injury to the endothelial cell layer of arteries, also contributes to the intimal hyperplasia seen in atherosclerotic reactions. In malignancies, PDGF may be involved in autocrine stimulation of tumor cell growth as well as in ►paracrine stimulation of cells in blood vessels and fibroblasts in the stromal compartment. Certain tumor types show specific perturbations in PDGF signaling pathways through mutations of genes for PDGF isoforms or PDGF receptors, including dermatofibrosarcoma protuberans (fusion of the genes for PDGF B-chain and collagen 1α1), chronic myelomonocytic leukemia and hypereosinophilic syndrome (fusion of genes for PDGF α- or β-receptor with different other genes), gastrointestinal stromal tumor (►gastrointenstinal tumor) (point mutation in the PDGF α-receptor gene), and glioblastoma (amplification of the PDGF α-receptor gene) (►brain tumor).

The involvement of PDGF in several serious disorders including malignancies makes PDGF antagonists highly warranted. Several types of such antagonists have been developed, including molecules that bind PDGF and prevent it from binding to its receptors, e.g. antibodies, soluble extracellular domains of receptors (e.g. ►STI571 or ►imatinib) or ►DNA ►aptamers, as well as low molecular weight inhibitors of the receptor kinase.

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Platelet Derived Growth Factor Receptor

Definition

PDGFR; Two known PDGF receptors exist, that bind different platelet-derived growth factor (PDGF) isoforms; both have tyrosine kinase activity.

►Mastocytosis

Platinating Agents

►Platinum Complexes

Platinum Antitumor Agents

►Platinum Complexes

Platinum Antitumor Compounds

►Platinum Complexes

Platinum Complexes

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Synonyms

Platinum drugs; Platinum antitumor agents; Platinum antitumor compounds; Platinating agents

Definition

In 1844 Michele Peyrone synthesized cisplatin (*cis*-platinum(II)-diammine-dichloride, CDDP) without

being aware of its tumor-inhibiting effect. Not until the mid-1960s Barnett Rosenberg discovered the inhibition of cell division in the presence of cisplatin. Due to its outstanding effectiveness in treating numerous tumors, cisplatin became the prototype for a new class of antineoplastic substances. Meanwhile it has become one of the most frequently used cytotoxic drugs in tumor therapy. However, its use is limited due to its side effects and the development of resistance which has led to the search for new platinum complexes.

Most of the platinum complexes are uncharged *cis* configurated square-planar platinum(II) complexes that can be described by the general formula *cis*-[PtA_2 X_2], with A_2 as either two monodentate or one bidentate stable amine ligand(s) and X_2 as two monodentate or one bidentate anionic leaving ligand(s). Octahedral platinum(IV) complexes, which are now under development, can be described by the general formula *cis*-[PtA_2 X_2 Y_2] with Y_2 as monodentate anionic leaving ligands. The advantage of platinum(IV) complexes is the possibility of oral administration due to their increased stability and solubility in the gastrointestinal tract.

Characteristics

In the following paragraphs the processes taking place after the administration of ►cisplatin are described. Different characteristics of other platinum complexes are outlined in the section "Approved platinum complexes."

Mode of Action

Bioactivation

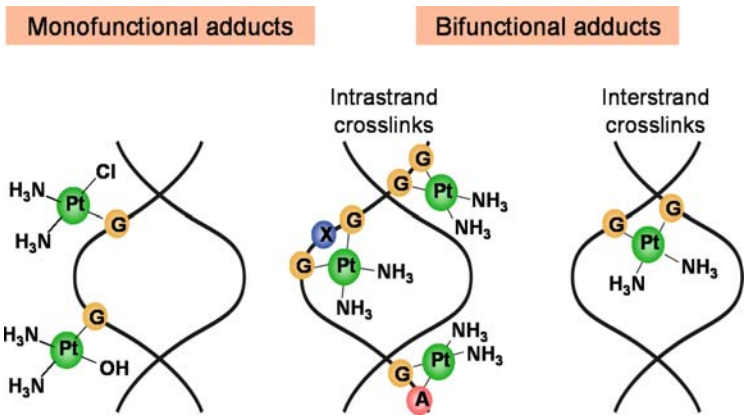
After intravenous administration a high amount of cisplatin is bound to plasma proteins and thereby inactivated. Because of the high extracellular chloride ion concentration (~100 mM), free cisplatin exhibits relatively low reactivity in the plasma. After entering the cell containing low chloride ion concentrations (~4 mM) reactive monoaqua and diaqua complexes are formed by exchange of the chloride ligands. Upon activation, cisplatin is able to form complexes with a variety of macromolecules present in the cell.

Formation of Platinum-DNA Adducts

The cytotoxic effect is mainly a consequence of the formation of platinum–DNA adducts. The primary target is the N^7 position of the purine bases due to the high nucleophilicity of the imidazole ring. Beside monoadducts, cisplatin forms bifunctional 1,2- or 1,3-intrastrand crosslinks and interstrand crosslinks (Fig. 1).

Cellular Response

1. Inhibition of DNA synthesis
   As a result of the structural change of the DNA helix binding of DNA polymerases is hampered inhibiting replication and transcription.
2. DNA repair mechanisms
   Platinum–DNA adducts are mainly repaired by ►nucleotide excision repair (NER). Due to the small substrate specificity of this repair system, it is unlikely that platinum–DNA adducts of structurally different platinum complexes are differentiated. A complete removal of the DNA damage is not possible in this way because of the limited capacity of the NER. Furthermore, adducts can be bypassed by some DNA polymerases. This translesion DNA synthesis – the so-called replicative bypass (►post replicative repair) – allows the cells to progress through the S-phase of the cell cycle.
   The ►mismatch repair (MMR) system is an important prerequisite for the cytotoxic activity of cisplatin. Base-pair mismatches are recognized and repaired by MMR proteins. The DNA strand previously synthesized is cut out and the DNA is again synthesized beyond the damaged sites entering



Platinum Complexes. Figure 1 Formation of platinum–DNA adducts.

in a vicious cycle. In the long term, these futile repair attempts lead to an induction of apoptosis. Thus, deficiency of MMR is associated with cisplatin resistance (see below).

3. Binding of HMG-proteins and transcription factors
   The disturbance of the DNA structure results in the binding of different proteins. Among them ►high mobility group box proteins– such as HMGB1 – protect platinum–DNA adducts against repair proteins by building stable bonds. Many HMG box proteins are transcription factors and partly exhibit a higher affinity for the platinated DNA than for their natural substrate. The transcription factors are intercepted by the platinum–DNA adducts, therefore the transcription is inhibited. This phenomenon is called "transcription factor hijacking."
4. Induction of ►apoptosis and necrosis
   The DNA damage caused by cisplatin triggers ►apoptosis and necrosis (Fig. 2).
   Among others, the following ways of apoptosis are being discussed:
   - The ►p53-dependent mitochondrial apoptosis with subsequent activation of ►caspase 9 and ►caspase 3
   - The ►Fas/Fas ligand (FasL)- and ►caspase-8-mediated apoptotic cascade
   - Activation of c-Abl tyrosine kinase and subsequent activation of p73 (►p53 genes family)
   - Mitogen-activated protein kinase (►MAPK) pathways

   Necrosis occurs following excessive DNA damage, which induces hyperactivation of poly(ADP-ribose) polymerase (PARP). PARP causes ATP/NAD^+ depletion leading to necrotic cell death.

Mechanisms of Resistance

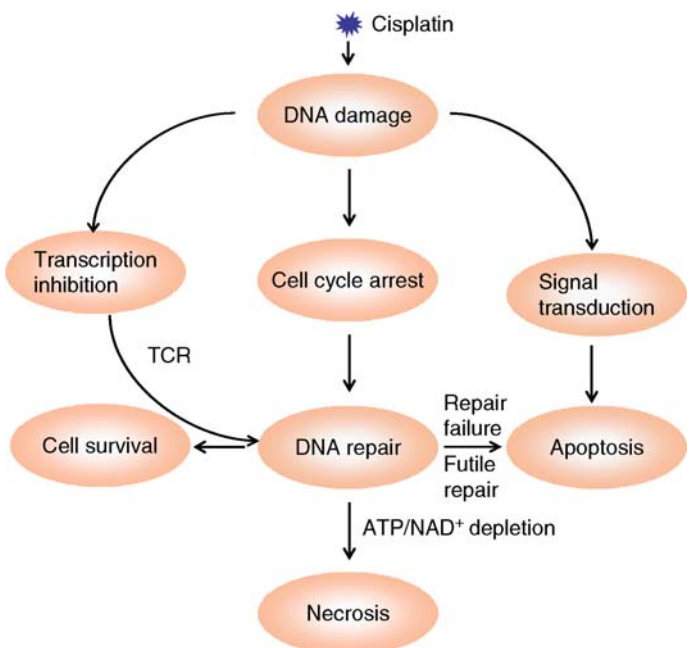
Resistance can be intrinsic or acquired by chronic drug exposure. The resistance to cisplatin is multifactorial, different resistance mechanisms may develop in parallel. Four principles of resistance mechanisms can be distinguished.

Reduced Accumulation

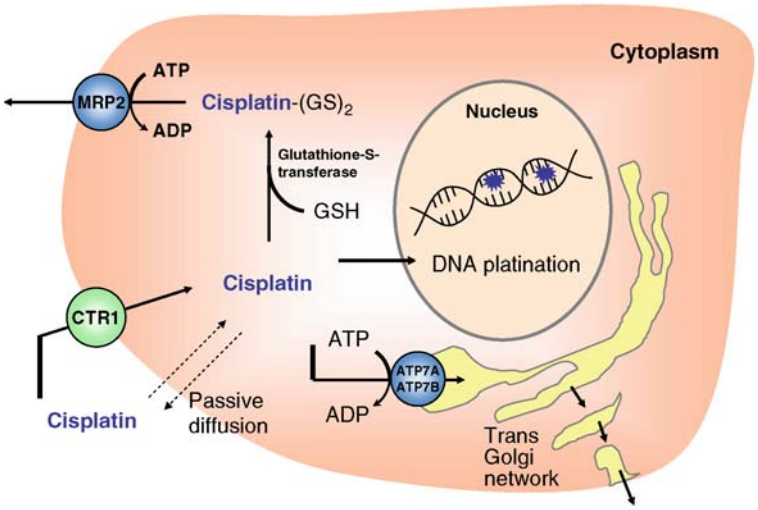
After exposure to platinum complexes, a decreased influx, an increased efflux, or a combination of both phenomena was observed. Previously, it has been assumed that platinum complexes enter the cell primarily by passive diffusion (Fig. 3). During the last decade a link between copper transporters (CTR1, ATP7A, ATP7B) and platinum influx and efflux has been discovered in numerous studies. Recent results furthermore indicate an involvement of organic cation transporters (OCT). Platinum resistance may develop by upregulation of efflux or downregulation of influx transporters.

Increased Inactivation

Platinum complexes react to a great extent with intracellular molecules that contain thiol groups, e.g.,



Platinum Complexes. Figure 2 Cell death pathways activated in response to cisplatin (modified from [5]). TCR, transcription-coupled repair.



Platinum Complexes. Figure 3 Mechanisms of cisplatin influx, efflux, and detoxification. CTR1, copper transporter 1; ATP7A, adenosine triphosphate alpha-polypeptide; ATP7B, adenosine triphosphate beta-polypeptide; MRP2, multidrug resistance protein 2.

glutathione (GSH) or metallothionein. Only a relatively small fraction of the intracellular cisplatin binds to the genomic DNA, however, more than 60% is bound to GSH. The conjugate is biologically inactive. The platinum–GSH complex can be removed from the cell by the transporter MRP2 (Fig. 3). In addition, the formation of crosslinks between platinum–DNA monoadducts and the opposite DNA strand can be prevented by reaction of the thiol-containing molecules and the monoadducts. An increased intracellular GSH production can contribute to the development of resistance.

Increased Adduct Tolerance and Failure of Apoptotic Pathways

An increase of the replicative bypass leads to an increased adduct tolerance as well as to a high mutation rate, whereby changes, which lead to subsequent development of resistance, are favored. Defects in the MMR system prevent the beginning of futile repair cycles, hence contributing to resistance.

The complex cascades of apoptosis offer a further starting point for the development of resistance. Many factors can be changed: Examples are the loss of p53 function, reduced activity of caspases, or a deregulated MAPK pathway.

Increased Repair

A general resistance mechanism is an increased repair by the NER system. The capacity of this kind of resistance mechanism seems, however, to be limited.

Approved Platinum Complexes

The chemical structures of approved platinum complexes are shown in Fig. 4.

Cisplatin

Indications. FDA labeled indications: Metastatic malignant tumor of testis, metastatic ovarian tumor and advanced transitional cell carcinoma of bladder. Furthermore one finds a broad range of non-FDA labeled indications.

Adverse Effects. Relevant and dose-limiting side effects are nephrotoxicity, ototoxicity, emesis, and neurotoxicity.

Carboplatin

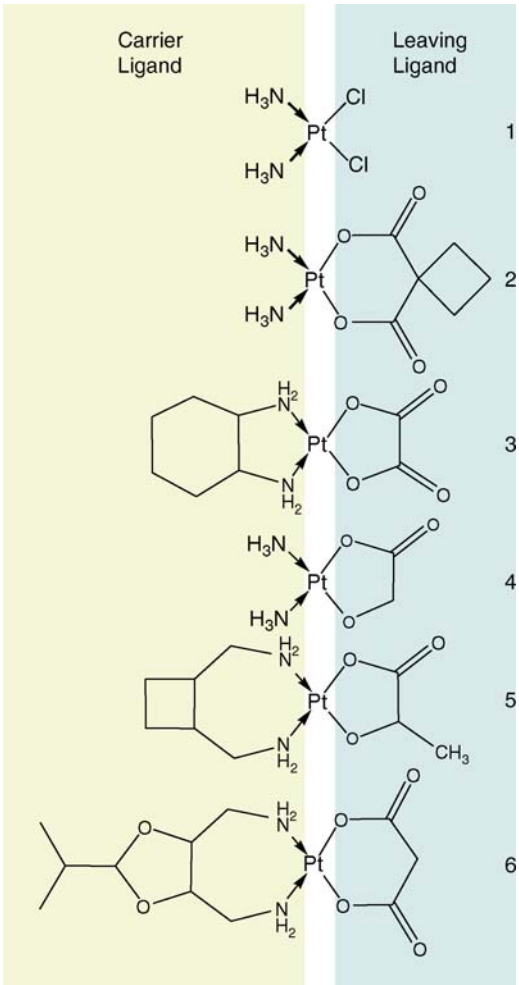
Characteristics. By the exchange of the labile chloro ligands for cyclobutan-1,1-dicarboxylate, water solubility and stability were increased compared to cisplatin, the toxicity was decreased. However, cisplatin and carboplatin show crossresistance, which points to a similar mechanism of action.

Indications. FDA labeled indication: Advanced ovarian cancer. Carboplatin is approved worldwide for the treatment of many other tumors.

Adverse Effects. The only dose-limiting side effect is myelosuppression. Among the serious side effects are an imbalance of electrolytes, a peripheral neuropathy and a less-common visual disturbance.

Oxaliplatin

Characteristics. Oxaliplatin shows another spectrum of efficacy and resistance. The oxalate ligand as the leaving group leads to a slightly reduced reactivity compared to cisplatin. The bulky 1,2-diaminocyclohexane (DACH) ligand is probably responsible for the improved water solubility as well as the changed profile of activity. Using equimolar and equitoxic concentra-



Platinum Complexes. Figure 4 Approved platinum complexes: cisplatin (1), carboplatin (2), oxaliplatin (3), nedaplatin (4), lobaplatin (5), heptaplatin (6).

tions of cisplatin and oxaliplatin, it was noted that the extent of DNA platination is substantially lower after treatment with oxaliplatin. However, the rate of single strand breaks is higher. The replicative bypass is probably disturbed by the bulky DACH ligand leading to an increased single strand break rate and enhanced cytotoxicity. An intact MMR system does not seem to be essential for the cytotoxic effect of oxaliplatin, because the DACH ligand prevents the attachment of the MMR proteins. These changes in the repair mechanisms are a possible explanation for the absence of crossresistance to cisplatin. Oxaliplatin is effective in the treatment of metastatic colon carcinoma, which exhibits intrinsic resistance to cisplatin and carboplatin.

Indications. FDA labeled indications: Colon cancer and advanced colorectal cancer.

Adverse Effects. The crucial and dose-limiting toxicity of oxaliplatin is peripheral sensory neuropathy. Myelosuppression is low. Neither ototoxicity nor nephrotoxicity has been observed.

Nedaplatin

Characteristics. The complex contains glycolic acid as leaving group. Nedaplatin exhibits a lower reactivity than cisplatin, but a higher one than carboplatin. It has a better side effect profile than cisplatin, but does not, however, show genuine advantages regarding treatment response and survival rate. Studies comparing nedaplatin with carboplatin are missing.

Indications. No FDA approval. Approved in Japan for the treatment of several tumors.

Adverse Effects. Myelosuppression, including severe thrombocytopenia, is dose-limiting. Nonhematological toxicity is reduced compared to cisplatin – except ototoxicity.

Lobaplatin

Characteristics. Lobaplatin was developed to extend the spectrum of activity of the platinum complexes. It is as active as cisplatin with absence of crossresistance. Its efficacy in cisplatin resistant tumors has to be proven in larger phase III studies.

Indications. No FDA approval. The complex has received approval in China for the treatment of different kinds of tumors.

Adverse Effects. Thrombocytopenia represents the dose-limiting toxicity.

Heptaplatin

Characteristics. Heptaplatin exhibits a more favorable adverse effect profile and a slightly reduced resistance compared to cisplatin.

Indications. No FDA approval. Heptaplatin gained approval in South Korea for the treatment of advanced gastrointestinal tumors.

Adverse Effects. Nephrotoxicity is dose-limiting.

New platinum Complexes Under Development

Platinum(IV) Complexes

These octahedral complexes exhibit some advantages compared to the square-planar Pt(II) complexes. They can be administered orally due to their increased stability and solubility in the gastrointestinal tract. Adverse effects are reduced due to their lower reactivity. New opportunities of synthesis and more possibilities of structural variations are opened. In the body they are reduced to more reactive Pt(II) complexes which form DNA adducts. In addition, there is evidence that the Pt(IV) complexes interact directly with the DNA.

Satraplatin is furthest progressed in clinical evaluation. Antitumor activity was partly determined in tumors with intrinsic and acquired resistance to cisplatin.

Sterically Hindered Platinum Complexes

The introduction of bulky substituents may lead to the reduction of detoxification and repair mechanisms.

NX473 (formerly AMD473, ZD0473), *cis*-amminedichloro(2-methylpyridine) platinum(II), was designed in order to reduce the inactivation of the complex by glutathione and other cellular thiols. In vitro activity against different human tumor cell lines, partly with intrinsic or acquired resistance to cisplatin, has been demonstrated.

Multinuclear Platinum Complexes

Two to four platinum centers with *cis*- or *trans*-configuration are connected by polyamine chains of different lengths. This approach has led to the development of highly antitumor active complex BBR3464. In clinical trials, BBR3464 was able to overcome resistance through formation of long chain interstrand crosslinks, because this type of crosslinks is not recognized by the NER system and HMG proteins.

Platinum Complexes with Bioreactive Ligands

An accumulation of the complexes in the target tissue can be achieved by the use of selective transporters or retention in the tumor tissue. Water-soluble porphyrin platinum complexes represent an example. Their accumulation in the tumor tissue is probably mediated by carrier-mediated transport with low-density lipoproteins (LDL) followed by internalization into the cell by endocytosis. The enhanced permeability and retention (EPR) effect can be used when platinum complexes are coupled to macromolecules. Synthetic (e.g., polyethylene glycol) and physiological (e.g., albumin) macromolecules are suitable for this approach.

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Platinum Drugs

►Platinum Complexes

Platinum-Refractory Testicular Germ Cell Tumors

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Synonyms

Cisplatin-refractory germ cell tumors; Cisplatin resistant germ cell tumors; Treatment-refractory germ cell tumors

Definition

Platinum-refractory germ cell tumors (GCTs) are GCTs which no longer respond to cisplatin-based chemotherapy. *"Cisplatinum-refractory" disease* is defined as response or disease stabilization during, but disease progression within 4 weeks after cisplatin-based chemotherapy, whereas "*absolutely cisplatin-refractory" disease* refers to patients with progression during cisplatin-based treatment.

In a broader sense, multiply relapsed (≥3 recurrences) GCTs are also often referred to as treatment-refractory GCTs.

Characteristics

Today, approximately 70–80% of all patients with metastatic germ cell cancer will achieve long-term cure after standard-dose, cisplatin-based combination chemotherapy, such as PEB (cisplatin, etoposide, bleomycin) or VIP (etoposide, ifosfamide, cisplatin). Patients relapsing after cisplatin-based first-line chemotherapy have a less favorable prognosis, although 20–25% of patients are still cured with conventional second-line cisplatin-based chemotherapy. However, patients relapsing again after conventional or even high-dose salvage chemotherapy have a very poor prognosis, particularly those with cisplatin-refractory or absolutely cisplatin-refractory disease and less than 5% of these patients are long-term survivors.

The molecular basis for the exquisite chemotherapy-sensitivity and mechanisms of cisplatin resistance of malignant GCTs is poorly understood.

Mechanisms of Platinum Resistance

Despite various studies, no uniform hypothesis has been developed to explain the exquisite chemosensitivity of most GCTs as well as the chemoresistance of the minority of malignant GCT. Multiple factors on different cellular levels seem to play a role in the induction of cell death following cisplatin-based

chemotherapy, but the exact mechanisms of cisplatin resistance are still unknown.

Cisplatin is believed to kill cells through interaction with the DNA, mainly by the formation of various DNA adducts, which lead to the initiation of apoptosis. The tumour cell can escape the initiation of apoptosis on several levels: First, cisplatin can be inactivated by changes in the level of thiol-containing cell compounds such as glutathione or metallothionein or can be exported out of the cell by several export pumps, even before it reaches the DNA. Second, cisplatin-induced DNA damage can be repaired, predominantly by the so-called "nuclear excision repair (NER) pathway" prior to the activation of the apoptotic cascade; Third, the recognition of the critical DNA damage by mechanisms, which initiate apoptosis, can fail; and fourth, the execution of apoptotic cell death may be prevented by anti-apoptotic signals or by defects of apoptosis effectors.

A number of studies predominantly performed in cell lines and xenograft models, have suggested a correlation between glutathione and metallothionein levels and cisplatin resistance as well as between various export pumps, such as the ABC transporters and the lung resistance protein, and cisplatin resistance. However, validation on clinical tumour samples is generally lacking. Investigations of the role of the NER, which is thought to be the most important DNA repair mechanism for cisplatin induced damage, indicate a low capacity of GCT cells for NER. The low intrinsic capacity of the NER demonstrated in GCT cell lines has been attributed to low levels of xeroderma pigmentosum complementation group A protein (XPA) and the NER protein ERCC1. Alternatively, it has been proposed that the DNA adducts could be concealed by testis-specific high mobility group (HMG)-box proteins preventing damage detection and repair by NER factors. The finding of a low NER capacity itself and its potential clinical relevance have not been confirmed in samples from patients with GCTs, yet it is conceivable that a low NER activity contributes to the overall chemosensitivity of GCTs. A high level of wild-type p53 in GCTs has commonly been regarded as the biological explanation for GCT chemosensitivity. Two recent studies investigating the role of p53 in refractory GCT, have demonstrated that p53 mutations are rare even after treatment, that these mutations are unlikely to be the cause for chemotherapy resistance, and that the inactivation of p53 does not lead to a sensitivity change to chemotherapy in vitro. These findings suggest that induction of apoptosis in testis cancer cells can be executed even independent from p53, which questions the previously suggested important role of p53 in cisplatin resistance. A high incidence of microsatellite instability (MSI) was found in samples of patients with refractory GCT. In most refractory cases MSI was found in several loci whereas in unselected GCT only very few patients had a MSI and, most of them just in one locus. MSI in refractory GCT indicates that defects in DNA mismatch repair pathway may represent a clinically relevant resistance mechanism. Other proteins involved in the regulation of apoptosis, such as BAX, BCL-2, BCL-X_L, and others have also been investigated, but no single factor seemed to correlate with treatment response. More research is necessary to conclusively define the role for antiapoptotic regulators downstream of the initiation of apoptosis in chemotherapy resistance of GCT.

Unfortunately, most models described earlier are based predominantly on in vitro analyses of cell lines and largely lack confirmation of their relevance in clinical material.

Treatment of Patients with Cisplatin-Refractory GCTs

The treatment of patients with cisplatin-refractory disease remains a challenge due to the limited number of effective treatment options. Treatment for these patients remains largely noncurative and palliative.

Drugs with No or Minor Activity in Refractory GCTs (Table 1)

A large number of different agents have been evaluated in patients with refractory testicular cancer, mostly based on a promising preclinical activity in cell lines or xenograft models. However, the vast majority of these agents could not demonstrate any meaningful clinical activity, despite a well-received preclinical rationale and results (Table 1).

Active Agents (Table 2)

Only four chemotherapy agents, oral etoposide, paclitaxel, gemcitabine and oxaliplatin, have thus far demonstrated clinical activity in these patients. These agents were tested based on preclinical results indicating activity in germ cell cancer cell lines or tumor models as well as based on the lack of cross-resistance with cisplatin. Toxicity with all these single agent therapies is acceptable. Given as single agent therapy, all of these treatments yield response rates of approximately 20%. In all these studies single patients were long-term survivors.

Over the past years, these agents have been combined in combination chemotherapy regimens. The main objective of these studies was proof of feasibility of combination chemotherapy in these intensively pretreated patients as well as to improve the outcome for this particular patient population. Response rate and survival served as outcome measures in these trials. Achieving a high response rate in refractory patients is important since the induction of a remission may subsequently allow the resection of residual masses

Platinum-Refractory Testicular Germ Cell Tumors. Table 1 Agents without or minor clinical activity in patients with refractory germ cell cancer (modified according to Kollmannsberger et al. 2006)

Author, year	Agent	No. Patients	Responses
Williams et al. (1983)	Amsacrine	6	0/6
Williams et al. (1985)	Mitoxantrone	14	0/14
Drasga et al. (1987)	Iproplatin	14	1/14
Harstrick et al. (1990)	Epirubicine	16	1/16
Hoskins et al. (1990)	Mitomycin C	7	2/7
Murphy et al. (1992)	Iproplatin	15	0/14
Stoter et al. (1992)	Epirubicine	18	0/18
Bokemeyer et al. (1993)	Vinorelbine	7	0/7
Motzer et al. (1993)	Suramin	14	0/14
Moasser et al. (1995)	All-*trans* Retinoic acid	16	0/14
Puc et al. (1995)	Topotecan	15	0/14
Kollmannsberger et al. (2000)	Bendamustine	19	1/19
Kollmannsberger et al. (2002)	Irinotecan	15	0/15
Kondagunta et al. (2004)	Temozolomide	14	0/14
Rick et al. (2006)	Thalidomide	15	0/15

Platinum-Refractory Testicular Germ Cell Tumors. Table 2 Results of chemotherapy in selected studies for patients with refractory or multiply relapsed GCTS (modified according to Kollmannsberger et al. 2006)

Author, year	Treatment	No. Patients	Patients pretreated with HD-CT	Cisplatin-refractory disease	Response rate (confidence interval)
Motzer (1994)	Paclitaxel	31	16%	76%	26% [95%-CI: 3–29]
Bokemeyer (1996)	Paclitaxel	24	50%	75%	25% [95%-CI: 10–47%]
Bokemeyer (1999)	Gemcitabine	31	71%	55%	19% [95%-CI: 13–45%]
Einhorn (1999)	Gemcitabine	20	55%	65%	15% [95%-CI: 3–38%]
Kollmannsberger (2002)	Oxaliplatin	32	78%	85%	13% [95%-CI: 1–24%]
Hinton (2002)	Paclitaxel/ gemcitabine	28	36%	36%	21% [95%-CI: 12–49%]
Miki (2002)	Irinotecan/ cisplatin	18	22%	n.s.*	50% [95%-CI: 27–73%]
Kollmannsberger (2004)	Gemcitabine/ oxaliplatin	35	89%	63%	46% [95%-CI: 30–64%]
Pectasides (2004)	Gemcitabine/ oxaliplatin	28	14%	100%	32% [95%-CI: 16–53%]
Theodore (2004)	Paclitaxel/ oxaliplatin	26	n.s.	61%**	30% [95%-CI: 16–55%]
Pectasides (2004)	Oxaliplatin/ irinotecan	18	0%	All patients resistant*	40% [95% CI: 17–64%]
Einhorn et al. (2007)	Paclitaxel/ gemcitabine	32	100%	n.s.	31% [95% CI: n.s.]

*All patients “resistant” defined as failure to achieve a durable complete remission to a cisplatin-based regimen and one or more of the following unfavourable prognostic features: relapse after CR or progression within 4 weeks after first-line therapy; poor or no response to prior conventional dose cisplatin/ifosfamide therapy; extragonadal primary site

**Refractory being defined as a disease progression during or within 2 months after cisplatin-based chemotherapy

n.s. = Not stated

and may thus be a chance to still achieve long-term survival in selected patients.

The first study which gave proof of the feasibility and activity of combination chemotherapy in these heavily pretreated patients was a phase II study investigating the combination of paclitaxel and gemcitabine. Twenty-one percent of patients responded and single long-term survivors were observed. A subsequent study which used paclitaxel/gemcitabine as treatment regimen after failure of salvage high-dose chemotherapy with autologous stem cell support found a response rate of 31%. Again, some long-term survivors were seen.

Two phase II studies examined the activity and toxicity of a combination consisting of oxaliplatin and gemcitabine in refractory germ cell cancer patients. These studies demonstrated again the feasibility of combination of chemotherapy in this heavily pretreated patient population. Both studies reported high response rates of 46% and 32% with a number of patients surviving long-term. These response rates are the highest published to date. Other tested combinations include paclitaxel/oxaliplatin and irinotecan-based combinations such as irinotecan/oxaliplatin or irinotecan/cisplatin. Hematological toxicity is usually the main side effect in these extensively pretreated patients.

Some trials have investigated the use of biological agents for the treatment of refractory or relapsed GCTs. None of the tested agents including suramin, an antitrypanosomal drug, all-*trans*-retinoic acid (ATRA) or thalidomide have shown activity. Novel molecular targets are also being explored now in germ cell cancer, including agents targeting the VEGF and EGF pathway. Preclinical experiments have suggested an important role for these pathways in germ cell cancers.

Salvage Surgery

Salvage surgery remains an important treatment option for chemotherapy-refractory patients, in particular for patients with limited and potentially respectable metastases.

A number of studies have investigated the role of surgery for patients with persistently elevated markers after cisplatin-based chemotherapy or patients relapsing after salvage chemotherapy (sometimes referred to as "desperation surgery"). Approximately 20–25% of patients may remain disease-free after a complete resection of all tumor manifestations. The complete resection of residual masses is the most crucial factor for success in this setting.

The complete resection of residual masses also appears to be an important step toward long-term disease free survival in cisplatin refractory patients who achieve a remission on salvage chemotherapy. Most long-term survivors in recent trials investigating novel agents had surgery subsequent to a chemotherapy response as part of their salvage treatment for refractory disease. Surgery alone or surgery after salvage chemotherapy should therefore always be considered for selected patients with cisplatin refractory disease, particularly in patients presenting with localized and potentially completely resectable recurrences or with completely resectable masses after response to salvage chemotherapy.

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Platyfish-Swordtail Melanoma

►Xiphophorus

Pleckstrin Homology Domains

Definition

A region of a protein whose tertiary structure allows it to specifically bind to phosphorylated lipids. This often results in the recruitment of the proteins to the plasma membrane where they become activated. An example of such a protein is the kinase Akt. A protein region comprised of ~120 amino acids through which proteins bind to phosphoinsositides, resulting in their recruitment to the plasma membrane for participation in signal transduction pathways.

►Signal Transduction

Pleiotrophin

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Synonyms

Heparin binding growth associated molecule; HB-GAM; Heparin affin regulatory peptide; HARP; Heparin binding brain mitogen; HBBM; Heparin binding neurite promoting factor; HBNPF; Heparin binding growth factor 8; HBGF-8; Heparin binding neurotropic factor; HBNF; Osteoblast specific factor; OSF-1

Definition

Pleiotrophin (PTN) is an 18 kDa growth factor that has high affinity for heparin and together with ►midkine forms a family of structurally related heparin-binding growth factors. The two proteins share 45% homology in their amino acid sequence and ten perfectly conserved cysteine residues. They also use the same receptors and share many biological activities; the best characterized being neural development and tumor growth.

Characteristics

PTN consists of 168 amino acids that are highly conserved across different species, such as human, mouse, rat, bovine, fish, chicken, frog, and insects. The cleavage of the 32 amino acids signal peptide leads to a secreted protein, which consists of 24% of cationic residues, mainly lysines, and contains ten conserved cysteines. The 28 lysines are organized in two clusters at the NH_2- and COOH-terminal regions and the cysteines form five intrachain disulfide bonds. The tertiary structure of PTN is arranged in two β-sheet domains connected with a flexible linker. Each of the domains contains three antiparallel β-strands and possesses one ►thrombospondin type 1 (TSR-1) homology motif, implicated in its binding to heparin. The NH_2- and COOH-terminal lysine-rich regions lack a detectable structure and appear to form random coils.

PTN interacts with ►heparan sulphate ►proteoglycans (HSPGs) and other ►glycosaminoglycans (GAGs), such as ►dermatan sulphate and ►chondroitin sulphate A and can thus be found located onto the extracellular matrix and the surface of different cell types. GAGs induce PTN dimerization and enhance the mitogenic but not the neurite outgrowth activity of PTN. PTN binds ►N-syndecan, a HSPG implicated in the neurite outgrowth activity of PTN. It also binds to the ►receptor protein-tyrosine phosphatase beta/zeta (RPTPβ/ζ), which seems to be responsible for the migratory responses of neuronal, endothelial, and ►cancer cells to PTN. The more recently identified receptor for PTN is ►anaplastic lymphoma kinase (ALK), which is a transmembrane ►tyrosine kinase receptor of a 200 kDa molecular mass, expressed by many types of cancer cells. The ►signal transduction pathways used by PTN are not clearly established. It has been reported that PTN activates ►phosphatidylinositol 3-kinase (►PI3K), ►Akt, and extracellular signal-regulated kinases ½ (ERK½), although the signaling from the receptors to PI3K is not well understood. In the case of RPTPβ/ζ, it is likely that c-src is involved, which activates ►focal adhesion kinase that associates with ►PI3K and leads to activation of ERK½.

Little is still known on the regulation of PTN expression. It has been shown that PTN transcription is directly stimulated by the transcription factors ►HOXA5 and ►activator protein-1. PTN protein levels are also increased by cAMP, ►platelet derived growth factor BB and ►hypoxia.

Numerous biological activities have been attributed to PTN. The first and most prominent is stimulation of neurite outgrowth and a role in the growth and maturation of brain. PTN also induces proliferation of several types of cells, is involved in a variety of processes in bone formation, seems to play a critical role in chondrogenesis, and participates in normal spermatogenesis.

A role of PTN in human ►cancers was first suggested after purification of the growth factor from conditioned media of the highly malignant ►breast cancer cell line MDA-MB-231. Screening of various human tumor cell lines and tumor specimens of different origin revealed that PTN is expressed in many types of cancer, such as glioma, ►melanoma, meningioma, ►neuroblastoma, choriocarcinoma, ►leukemia, and cancers of ►pancreas, ►prostate, stomach, ►colon, ►breast, ►ovary, and lung. PTN receptors are also up-regulated in a plethora of tumors and are being tested as targets for anti-cancer therapy.

Regarding the biological activity of PTN in cancer, there is ample evidence that it is a tumor-promoting factor. This is supported by data showing that overexpression of PTN in human embryonic kidney cell line 293 and human adrenal carcinoma cell line SW-13 leads to autonomous growth in soft agar and formation of tumors in athymic nude mice. Moreover, the secreted PTN stimulates proliferation of fibroblasts, endothelial, and epithelial cells. PTN purified from lung cancer cell lines also stimulates the growth of fibroblasts, endothelial and SW-13 epithelial cells. In the same line, overexpression of the bovine PTN cDNA in NIH 3T3 cells results in a transformed phenotype, as judged by increased cell numbers at confluence, anchorage-independent growth, and tumor formation in nude mice.

More recently it has been shown that deletion of the tumor suppressor gene ►*PTEN* leads to up-regulation of PTN, which seems to participate in tumorigenesis caused by ►PTEN loss.

Inhibition of PTN expression in several types of cancer cells inhibits colony formation of the cells, decreases angiogenicity, and prevents tumor growth in mice. For example, human breast cancer MDA-MB-231 cells transfected with a mutant cDNA that encodes a truncated form of PTN, fail to form plaques or colonies in soft agar and are unable to form tumors in athymic nude mice. Stable transfection of human melanoma WM852 cells that express high levels of PTN mRNA, with PTN targeted ribozymes, quenches production of PTN, inhibits colony formation of the cells, and prevents tumor growth in mice. In another highly metastatic human melanoma cell line 1205Lu, transfection with PTN-targeted ribozymes decreases tumor growth and ►angiogenesis in nude mice. A replication-deficient recombinant adenovirus generated to express antisense PTN at high efficiency, induces transcripts that completely inhibit PTN protein production and decrease melanoma cell growth in soft agar and SCID mice. The down-regulation of PTN in these cells coincides with down-regulation of the cell cycle regulator cyclin E and up-regulation of the cell cycle inhibitor $p21_{WAF1/Cip1}$. PTN-targeted ribozymes have been used to deplete PTN mRNA from Colo357 ►pancreatic cancer, choriocarcinoma, and glioma cells. Reduction of PTN results in a decrease in the proliferation rate, soft agar colony formation, and tumor growth in nude mice. In human prostate LNCaP cells, antisense PTN expression decreases cell ►migration, as well as anchorage-dependent and independent growth and abrogates the stimulatory effects of ►hydrogen peroxide and ►fibroblast growth factor 2 on LNCaP cell proliferation and migration.

In contrast to a stimulating effect of PTN on tumor growth, it has also been suggested that overexpression of PTN in NIH 3T3 cells may be implicated in cellular quiescence rather than an oncogenic phenotype. In several cases, PTN has been identified as a confluence specific protein, secreted by normal cells but not cells transformed by ►ras or other ►oncogenes. High PTN expression is associated with poor vasculature in neuroblastomas, and HOXA5 that induces apoptosis of breast cancer cells and inhibits angiogenesis, directly activates PTN transcription. It has also been shown that PTN directly binds the 165-amino acid form of ►vascular endothelial growth factor ($VEGF_{165}$) leading to inhibition of $VEGF_{165}$-induced endothelial cell proliferation, migration, and tube formation on matrigel. This negative regulatory effect is attributed to both thrombospondin type I repeats of PTN, which are located in the β-sheet domains of the molecule and are responsible for the binding to $VEGF_{165}$.

Besides a direct effect of PTN on tumor cells, there is also a plethora of reports indicating a positive correlation between PTN and in vivo or in vitro angiogenesis, a key step in the progress of many tumors. Numerous in vitro studies demonstrate that PTN is involved in the control of endothelial cell migration, proliferation, and differentiation into tube-like structures. The angiogenic potential of PTN in vivo has been shown in the chicken embryo chorioallantoic membrane assay and in mice using matrigel implants. Moreover, PTN expression by monocytes/macrophages leads to downregulation of their monocytic cell markers and upregulation of endothelial cell characteristics, thus inducing the transdifferentiation of monocytes into functional endothelial cells. PTN may also serve to recruit stromal tissue and blood supply to the expanding tumor. Culture supernatants derived from a PTN transfected human adrenal carcinoma cell line (SW-13) were shown to possess mitogenic activities for fetal bovine heart endothelial cells and human umbilical vein endothelial cells (HUVEC), making PTN a candidate tumor angiogenesis factor. In the same line, PTN purified from lung cancer cell lines stimulates the proliferation of HUVEC in vitro, and ribozyme targeting of PTN mRNA, which is constitutively expressed in the human melanoma cell line 1205Lu, reduces the number of vessels in the primary tumor and the metastatic spread of these cells in athymic nude mice. PTN-transfected MCF-7 human breast carcinoma cells are mitogenic for HUVEC in vitro and strongly angiogenic in vivo, in mice xenografts and in the rabbit corneal assay. Endothelial cell functions in vitro and angiogenesis in the chicken embryo chorioallantoic membrane in vivo induced by culture medium of human ►prostate cancer LNCaP cells were also inhibited when PTN expression was diminished. Finally, in MCH66 murine mammary tumor cells, which metastasize depending only on tumor angiogenesis, PTN could be regarded as the only plausible candidate for this activity.

The identification of PTN domains responsible for its angiogenic and transforming activities is considered important and the data existing so far suggest distinct or even opposite effects for different PTN regions. The last 25 amino acids of the C-terminal region are considered important for the binding to ALK and the angiogenic effect of PTN and the last 43 amino acids of the same region enhance plasminogen activator activity and decrease the plasminogen activator inhibitor levels. An angiogenic role for the residues 65–136, as well as peptides that correspond to both the NH_2 and COOH domains of the molecule have also been suggested. Proteolysis of PTN by plasmin results in the production of five peptides with distinct activities on endothelial cell activation in vitro or angiogenesis in vivo. PTN is also a substrate for trypsin or chymotrypsin and the

hypothesis that PTN is a possible substrate for several proteolytic enzymes, which may control its angiogenic or/and tumorigenic potential, is reinforced by the presence of proteolytic forms of PTN in media from endothelial or tumor cells.

In addition to being a therapeutic target, PTN may also be an attractive circulating growth factor for prognostic monitoring. In mice, serum PTN levels increase as a function of tumor size. In humans, elevated serum PTN levels are measured in patients with pancreatic or colon cancer, but not in patients with stomach cancer. In both mice and humans, serum PTN levels drop after successful tumor removal. Elevated PTN serum levels are also measured in 30% of patients with chronic pancreatitis, a disease that frequently precedes pancreatic cancer. Moreover, PTN expression increases from 7% observed in normal tissue to 34% in inflammatory and 67% in pancreatic cancer tissues. Finally, PTN may represent a promising new diagnostic marker for ►testicular cancer with high sensitivity in early-stage disease.

In summary, PTN exhibits important biological activities in cancer and its expression or/and expression of PTN receptors in of PTN receptors at high levels may play a crucial regulatory role in many tumors of diverse origin. Clarifying the exact role of PTN in diverse tumor types could lead to the development of therapeutic tools that can be used of therapeutic tools to control tumor growth and metastasis.

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Pleiotropy

Definition

Ability of certain genes or proteins to concomitantly evoke a series of different apparently unrelated downstream responses within a cell or organism; most proteins related to cellular growth have a multiplicity of activities that are determined by the individual cell type in which they act.

Pleura

Definition

Serous membrane that covers the lung.

►Hepatic Epithelioid Hemangioendothelioma

Pleural Biopsy

Definition

The removal of a sample of pleural tissue followed by microscopic examination to see whether cancer cells are present.

►Pleural Effusion

Pleural Effusion

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P

Synonyms

Malignant pleural effusion; Thoracentesis; Pleurodesis

Definition

Pleural effusion is the presence of excessive fluid in the pleural cavity. The pleura is a two-sheet, serous membrane that covers chest wall, mediastinum (parietal pleura) and lungs (visceral pleura). Between parietal and visceral pleura there is a virtual space filled with a small liquid pellicle with a high daily turnover which permits sliding. Daily fluid production is about 0.01–0.02 mL kg^{-1} h^{-1} being continuously absorbed in a way that remaining the pleural fluid is about 0.1–0.2 mL kg^{-1}. Fluid movement and absorption are supported by a balance between plasmatic and pleural pressures (hydrostatic and oncotic pressures) and thoracic lymphatic drainage. Pleural effusions occur because of an upset in these mechanisms that reabsorb the fluid normally present in the pleural space.

Characteristics

Pleural effusion is a common clinical problem seen at the advanced stage of several malignant diseases and is called ►Malignant Pleural Effusion (MPE).

The physiopathology of a malignant pleural effusion includes vascular and lymphatic alterations due to a direct invasion of the pleura by a primary tumor or a metastasis. These pleural changes affect the normal reabsorptive flow of fluid from parietal to visceral pleura and can even cause an increased capillary leaking and increased fluid production.

A malignant effusion can be present whatever the direct neoplastic involvement of the pleura as a clinical consequence of a malignancy or its treatment (radio or chemotherapy, caquexia, tromboembolism, pneumonia, and others) and in these cases it is called paramalignant effusion.

An indirect increased capillary permeability, increased hydrostatic pressure, a decreased oncotic pressure, increased negative intrapleural pressure (as in cases of atelectasis), and decreased or blocked lymphatic drainage are the final organic alterations responsible by fluid overload.

The main causes of MPE are reported with different incidence rates between the literature series.

Widely, lung cancer is the most common cause of malignant pleural effusion in men. In women, breast cancer seems to be the first cause, followed by lung cancer and genital tract cancer.

Other related causes of MPE are lymphoma, gastric cancer, and mesothelioma.

In about 10% of malignant effusions no primary tumor is identified.

Table 1 shows main neoplasms related to pleural effusion.

Clinical Manifestations and Diagnosis

The most common related symptom is dyspnea. Other symptoms are cough, chest discomfort, and pain. Many patients with pleural effusions are asymptomatic, especially if a small pleural effusion is present.

Pleural Effusion. Table 1 Causes of malignant pleural effusion

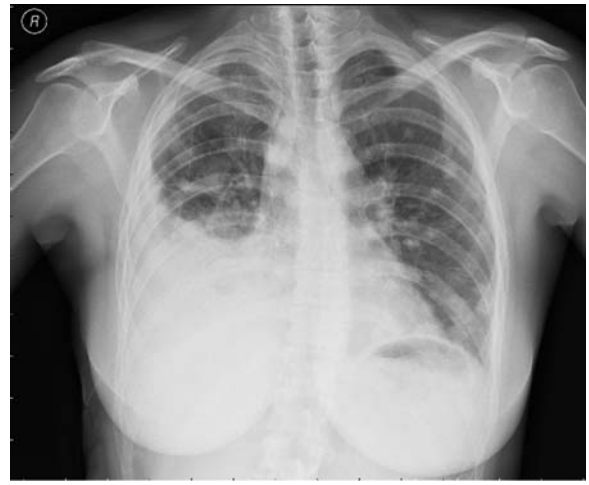
Male	Female
Lung cancer	Breast cancer
Lymphoma	Female genital tract
Gastrointestinal tract	Lung cancer
Genitourinary tract	Lymphoma
Melanoma	Gastrointestinal tract
Less common tumors	Melanoma
Primary unknown	Less common tumors

Physical examination is poor in cases of small effusions or should reveal decreased chest expansion, dullness to percussion, and reduced breath sounds in cases of a larger effusion.

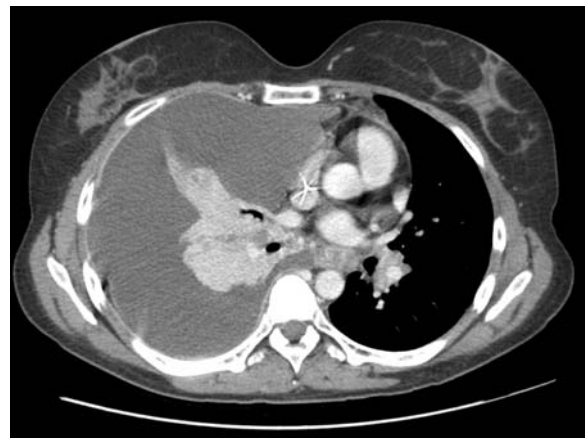
A simple chest radiography is the first diagnostic approach. It can confirm the presence of an effusion and other parenchymal alterations.

Small pleural effusions could not be seen in a simple chest radiogram but larger pleural effusions can cause a complete opacification of the hemithorax. Fig. 1 shows a chest radiogram with a large right pleural effusion.

When a massive pleural effusion is present there are symptoms of a tension hydrothorax with mediastinal shift, severe dyspnea, and hemodynamic instability (Fig. 2).



Pleural Effusion. Figure 1 Chest radiogram showing a large right pleural effusion due to breast cancer.



Pleural Effusion. Figure 2 CT scan disclosing massive pleural effusion with mediastinal shift and lung atelectasis.

Loculated effusions are difficult to diagnose in a simple chest radiogram because it is difficult to distinguish from other pulmonary parenchymal process such as atelectasis and consolidation.

Ultrasound and CT scan are useful diagnostic means, especially in cases of small or loculated effusions and can determine the precise site for thoracentesis.

Thoracentesis

►Thoracentesis is useful as a preliminary "looking for" diagnosis and could play a therapeutic role.

The fluid analysis permits distinguishing an exudative from a transudative effusion.

Transudative effusion is a plasma filtrate with the same characteristics and results of an elevated hydrostatic pressure or increased capillary permeability.

Exudative effusion is a rich protein fluid resulting from a local pleural inflammation or obstructed lymphatic drainage or both and it is common in neoplasm.

The fluid analysis includes the cell count (total and differential), total protein count, lactate dehydrogenase (LDH), glucose level, pH, amylase, and cytology.

Usually MPE is an exudative effusion that is characterized by serum ratio of protein greater than 0.5 (relation between pleural and plasmatic protein level); LDH ratio greater than 0.6 (relation between pleural and plasmatic LDH level), or the absolute LDH pleural level greater than 2/3 of the upper normal limit for serum level and low glucose level.

Other analyses include cholesterol, triglycerides (when a quilothorax is suspected), and ADA (adenosine deaminase), helpful to differentiate neoplastic from tuberculous effusion.

A hemorrhagic fluid is highly correlated to a malignant effusion.

The fluid analysis also permits to identify neoplastic cells and some direct and indirect tumor markers.

Some tumor markers such as ►CEA and CA 15-3 may improve the diagnostic value of cytology.

High fluid levels of CEA are seen in pulmonary squamous cell carcinoma and pulmonary adenocarcinoma. Differently, high levels of CA 15-3 are observed in breast cancer.

Chromosomal and tumor growth factors analyses are still being investigated as a worth diagnostic mean.

When an exudative effusion is present and the etiology is not clear, thoracoscopy and pleural biopsy have been described as the most rational diagnostic approaches.

Treatment

The goal of the treatment is the resolution of the effusion and its recurrence prevention.

Secondary treatment endpoints are minimizing patient's symptoms and reducing hospital stay.

Chemo and radiotherapy play a role in the treatment if the primary tumor is responsive to them and may spontaneously resolve the effusion.

Therapeutic Thoracentesis

Therapeutic thoracentesis can immediately provide relief from dyspnea and other acute symptoms, but it has an elevated recurrence rate (as high as 98–100% Fig. 3).

There is no absolute contraindication for thoracentesis. Relative contraindications are bleeding disorders, anticoagulation, and mechanical ventilation.

Complications related to thoracentesis include ►pneumothorax, hemothorax, pain, and vasovagal reaction (with bradicardia, low systemic blood pressure, and reduced conscious level) during the procedure.

Repeated thoracentesis can result in infection, tumor implantation at the site of the puncture, and adhesions between lung and chest wall.

Chest Tube Drainage

Chronic ►chest tubes have a poor indication in controlling MPE.

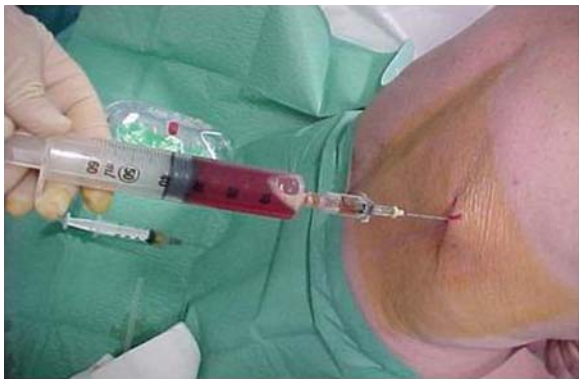
A low number of patients have some benefit with this technique and it is only indicated in cases of no choice of ►pleurodesis.

Patients with suspected or proven mesothelioma should receive prophylactic radiotherapy at the site of chest drainage or biopsy because about 40% of patients with malignant mesothelioma may develop tumor implant at the site of the pleural procedures.

Pleurodesis

Instillation of sclerosing agents in the pleural space causes an inflammatory process with a fusion between parietal and visceral pleura.

A successful pleurodesis provides complete lung expansion and stops the fluid collection.



Pleural Effusion. Figure 3 Thoracentesis of hemorrhagic pleural effusion.

Pleurodesis can be performed with chemical agents or mechanical abrasion of the parietal pleura.

Recently, asbestos free talc has been the most established and diffused method for the management of MPE.

It can be directly instilled in the pleural space with a chest tube or via a thoracoscopic procedure.

Pleurodesis with ►video-assisted thoracoscopic surgery (VATS) has a very high efficacy in terms of effusion control (Fig. 4).

The advantage of this method is that it offers a possibility of a uniform distribution of the talc in a direct view and high accuracy ►pleural biopsy.

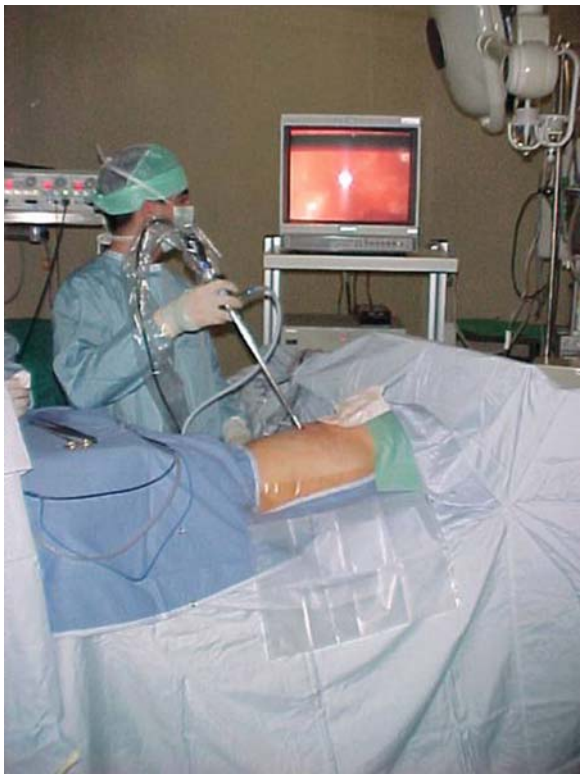
Talc is usually well tolerated with few side effects. Most common ones are fever and pleuritic pain. Other described complications are acute respiratory distress syndrome (ARDS) and talc pneumonitis. These complications are rare and appear to be related with larger doses of talc (>5 g).

Other chemical agents have been described as sclerosing agents.

Bleomycin sulfate is the most used antineoplastic drug for pleurodesis. Described side effects are fever, chest pain, and nausea. The drug has a limited systemic absorption and the efficacy is about 70–80% with a high cost.

Tetracycline was frequently used in the past as a sclerosing agent with widely different results between patients' series.

Other agents as Interferons (alpha and beta) and Interleukins (IL2) have showed controversial results in the literature.



Pleural Effusion. Figure 4 One-port video-assisted thoracoscopy (VATS) for pleural biopsy and talc poudrage.

Mechanical Pleurodesis

Thoracoscopic mechanical pleurodesis has a similar effusion control rate when compared with talc pleurodesis. It has, however, more complications. The most common complications are prolonged lung deflation, bleeding, subcutaneous emphysema, and wound inflammation.

Other Treatment Options

Pleurocath is the insertion of a long-term, tunneled pleural catheter in patients with recurrent and symptomatic effusions particularly in cases of trapped lung. Pleurocath can allow the patient to lead an independent life outside the hospital. The most common complications are reaccumulation of effusion, loculations within the pleural space, empyema, and cellulitis at the catheter insertion site.

►*Surgical Pleurectomy* and ►*Decortication* are options with very high morbidity and mortality. It requires thoracotomy with frequent complications and prolonged hospital stay.

►Pleuroperitoneal shunting is an alternative for patients who have intractable symptomatic pleural effusions that have failed chemical pleurodesis or are not candidates for surgery. Shunting insertion is well tolerated in minithoracotomy or thoracoscopic procedure.

Complications related to this technique are shunt occlusion, infection, and tumor implant.

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Pleurectomy

Definition

Surgical procedure to remove parietal pleura.

►Pleural Effusion

Pleurodesis

Definition

The use of chemicals or drugs to cause inflamation and adhesion between the parietal and visceral pleura. This prevents the build up of fluid in the pleural cavity.

►Pleural Effusion

Pleuroperitoneal Shunting

Definition

Catheter placed in the pleural space and in the abdominal cavity tunneled under the skin. The catheter is an one-way valve and an area that can be compressed to pump fluid from the pleural space into the peritoneal space.

►Pleural effusion

Plexiform Neurofibroma

Definition

A benign tumor mass which arises from the cells of connective tissue sheath of a peripheral nerve. It grows along nerve trunks, may involve smaller nerve branches and infiltrates the surrounding tissues without clear demarcation. Typical feature of neurofibromatosis 1

►Neurofibromatosis Type 1

Plexin Ligand

►Semaphorin

Plexins

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Synonyms

PLXN; Semaphorin receptors

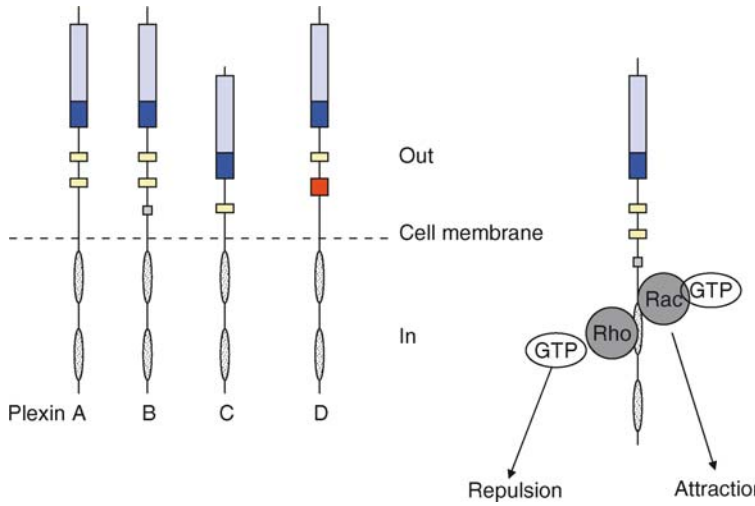
Definition

Plexins are large, transmembrane receptors with structural homology in their extracellular domains to the ►scatter factor receptor, ►c-MET. The family of plexins consists of four groups, plexin A, B, C and D, each with several subgroups. The plexins differ mostly from each other in their extracellular domains.

These ectodomains are typically composed of a ►semaphorin-like or sema domain, followed by two or three Met-related sequences (MRS). The Met related sequences of class B plexins show the highest similarity to c-Met. The intracellular parts of the plexins are highly conserved during evolution and are characterized by two conserved regions, known as the SEX-plexin (SP) domain (Fig. 1).

Characteristics

Plexin-associated molecules: Plexins are receptors for the membrane-bound ►neuropilins and semaphorins, which exist both as secreted and membrane-bound forms. Identified receptor-ligand pairs are: PLXNA1-Sema3A; PLXNA1-Sema6D, PLXNB1-Sema4D; PLXNB3-Sema5A; PLXNC1-Sema7A, PLXND1-Sema3C, and PLXND1-Sema3E. Interactions of plexins with the class 3 semaphorins are mostly mediated by the neuropilins, but exceptions to this rule exist. For example, Sema3E binds directly to PLXND1 in the absence of neuropilins. The neuropilins are also receptors for the larger variants of the angiogenic factor ►vascular endothelial growth factor-A (►VEGF-A) and binding of class 3 semaphorins to neuropilin/plexin complexes can be competed for by VEGF-A, resulting in antagonistic effects toward each other. Neuropilins are non-signaling receptors that occur in complex with VEGF receptor 2 (VEGFR2) on angiogenic ►endothelial cells and enhance binding of VEGF-A to this receptor. Since several classes of



Plexins. Figure 1 Structural domains of plexin family members: four subfamilies have been identified, plexin A–D. Blue boxes indicate sema-domains, yellow boxes represent Met-related sequence (MRS) motifs. The red box indicates an atypical MRS motif of plexin D1 which distinguishes it from the other plexin family members. Plexin B subfamily members have a potential ►furin-like proteolytic site, marked by a grey ribbon. Two intracellular conserved domains are shown as ovals. In the family of B plexins, ►RhoA and ►Rac small GTPase binding sites are activated upon semaphorin binding, resulting in repulsion (growth cone collapse) or attraction (►filopodia formation and extension) respectively.

plexins are also expressed on endothelial cells during ►angiogenesis, it is likely that multicomponent complexes of neuropilins, plexins and VEGF receptors exist. This is further supported by the finding that an association between PLXNA1 and VEGFR2 (and Off-track) is required for Sema6D signaling. Thus, it is likely that a delicate balance between semaphorins and VEGF-A provides a fine tuning mechanism to regulate angiogenesis.

Class B plexins have been found in a complex with c-Met, the receptor for hepatocyte growth factor (HGF) and Ron, the receptor for ►macrophage stimulating protein (MSP). These proteins are also known as the scatter factor receptors, and c-Met is critically involved in invasive growth (cell scattering) during development but also during tumor cell ►invasion and ►metastasis. Except for direct phosphorylation upon binding of scatter factor/HGF, c-Met is also directly activated upon binding of sema4D to a PLXNB1/c-Met complex, resulting in an invasive cell phenotype.

Taken together, most plexins exist in multicomponent receptor complexes and the outcome of activation of these complexes is determined by a concerted action of different growth factors.

Plexin Function

Initially, neuropilins and semaphorins were discovered as determinants of patterning of the central nervous system. Most work has been done on the class 3 semaphorins which act via the plexin A family as ►chemorepulsive cues during axonal growth. There are distinct similarities between development of nerves and vessels. Both display directed growth and branching morphogenesis in an orderly fashion. In this respect it is perhaps not surprising that factors that are involved in axon guidance also play a role in angiogenesis. A first indication for this came from studies, in which expression of plexin D1 was demonstrated in developing vasculature during mouse embryogenesis. A later study showed that plxnd1 was essential for angiogenesis: plxnd1 knock out mice die within one day after birth due to cardiovascular defects. Maldevelopment of the vasculature was also observed in plxnd1-loss of function mutants of zebrafish.

Evidence is emerging now that plexins play distinct roles not only during developmental angiogenesis, but also during tumor angiogenesis. At the moment an involvement in tumor biology of the B and D plexins is most clear. Therefore these two family members will be dealt with in more detail.

Plexins in Tumor Biology

Plexin D1

The occurrence of PLXND1 on developing vasculature has resulted in research towards a potential role of this family member during angiogenesis in tumor development: angiogenesis is critically involved in growth and metastasis of solid tumors, and plexin D1 is prominently expressed in angiogenic tumor blood vessels. Plexin D1 is also expressed on tumor cells in a wide variety of human tumors.

Semaphorins 3C and 3E are ligands for PLXND1. Whereas Sema3C binding to PLXND1 requires the

involvement of neuropilins, this interaction is not needed for Sema3E binding. Ssema3C seems to be involved in chemoresistance of tumor cells whereas sema3E has been identified from array analyses as a protein, implicated in tumor progression and metastasis. Because functional studies on plexin D1 during tumor development are lacking so far, it is not clear whether plexin D1 mediates these specific semaphorin effects. However, whatever its potential function in tumor biology, the presence of plexin D1 on tumor vessels and tumor cells makes it a potentially attractive target for tumor diagnosis and ►targeted drug delivery as it allows the simultaneous targeting of multiple tumor compartments.

The ►signal transduction pathways activated by plexin D1 have not yet been elucidated, but studies on other plexins suggest that active cytoskeletal rearrangements are induced upon plexin D1 activation via the small RhoGTPases. Whereas activation of the Rac RhoGTPase leads to ►lamellipodia formation and extension of cellular processes, the RhoA GTPase results in the retraction of cell processes via depolymerization of F-actin. Plexin D1 contains a consensus Rac-binding site as well as two conserved arginines that in PLXNA1 are crucial for sempahorin-mediated cellular collapse. It is not known which conditions favor Rac or RhoA activation.

B-Plexins

Another plexin that is expressed on both endothelial cells and tumor cells is plexin B1, a receptor for semaphorin 4D. It has been demonstrated that plexinB1 is present in a complex with the scatter factor receptors Met and Ron. These two tyrosine kinase receptors are implicated in the invasive growth programme of tumors. C-Met is the prototype receptor for Hepatocyte Growth Factor (HGF) and is, upon activation by this ligand, phosphorylated on two tyrosines that are docking sites for a number of signal transduction molecules such as phosphatidyl inositol 3-kinase (PI3K), SRC, GRB2, GAB1 and ►signal transducer and activator of transcription 3 (STAT3). C-Met is also associated with ►integrins, adhesion molecules that modulate cellular interactions with extracellular matrix components. C-Met is a prototype oncogene that upon constitutive activation results in malignant tumors. Constitutive activation may be the result of activating mutations, but overexpression of c-Met in itself may cause receptor oligomerization and phosphorylation.

Sema4D binding to plexin B1 in the complex also results in tyrosine phosphorylation of c-Met. Furthermore, endogenous overexpression of plexin B1 in epithelial cancers results in constitutive activation of c-Met. Thus, the B-plexins may play an important role in c-Met activation and tumorigenesis.

Unlike plexin D1, intracellular signaling from a PLXNB1/Sema4D complex has been elucidated to a large extent. Upon ligand binding, PlexinB1 interacts at its carboxyterminal domain with leukemia associated rho guanine exchange factor (LARG), an exchange factor for the small GTPase Rho. Also binding of PDZ-Rho-GEF has been demonstrated. Plexins have also been shown to activate r-RAS, resulting in down-regulation of integrin-mediated adhesion to the extracellular matrix. The resulting loss of cell adhesion is implicated in invasive growth.

In summary, plexins constitute a family of receptor proteins which occur in multicomponent complexes on the cell membrane. Evidence for an important role in tumor biology is emerging in the last years. Multiple ligands for the plexins have been identified, and intracellular signaling pathways initiated by plexin activation, results in cytoskeletal rearrangements which may lead to extension but also collapse of cellular processes, events that are indispensable for cellular migration.

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Plexopathy

Definition

Malfunction of multiple peripheral nerves descending from a plexus.

►Peripheral Neuropathy
►Plexins

PIGF

►Placenta Growth Factor

PLK

Definition
Polo-like Kinase.

Ploidy

Definition
Degree of repetition of the basic number (23) of chromosomes, e.g. diploidy refers to a set of 46 chromosomes.

►Flow Cytometry and Cancer

PLP

►Parathyroid Hormone-Related Protein

Pluripotency

Definition
Is the capacity of a cell to differentiate into any mature cell type.

►Stem Cell Markers

Pluripotent

Definition
Able to give rise to all cell types found in the embryo and adult animal.

►Adult Stem Cells

Pluripotent Stem Cells

Definition
Are the descendants of totipotent cells and can differentiate into cells derived from the three germ layers.

►Adult Stem Cells

PLXN

►Plexins

PMA

Definition
►Phorbol 12 myristate 13-acetate. The most common phorbol ester which acts as a tumor promoter and used as biomedical research tool in models of carcinogenesis.

►Skin Carcinogenesis

PML

Definition
Promyelocytic Leukemia gene. Is an onco-suppressor gene initially identified in the 15;17 translocation typically found in ►Acute Promyelocytic Leukemia. Is the major component of PML bodies.

►Cajal Bodies
►Acute Promyclocytic Leukemia (PML)

PML Bodies

Definition
Refers to a class of nuclear bodies; they react against SP100 auto-antibodies (PML, promyelocytic leukemia);

cells typically contain 10–30 PML bodies per nucleus; alterations in the localization of PML bodies occurs after viral infection.

►Cajal Bodies

PML Nuclear Bodies

Definition

Are nuclear structures that are disrupted in ►acute promyelocytic leukemia and by certain viruses, which are the repository for several proteins.

►Senescence and Immortalization

PML-RARα

Definition

The ►acute promyelocytic leukemia-specific PML–RARα fusion protein is generated by the t(15;17) translocation. RAR αencodes one of the retinoic acid receptors (RAR), PML encodes a protein of unknown function localized in nuclear structures called nuclear bodies or PML oncogenic domains. PML–RARα is a dominant-negative transcriptional repressor of retinoic acid receptor (RAR) target genes.

►Epigenetic Therapy
►Retinoic Receptor Cross talk

PMS1/2

Definition

Human homologues of mut L that, if inactivated in yeast, cause a high frequency of postmeiotic segregation. These genes encode proteins that are components of the human ►mismatch repair (MMR) system. Defect MMR may cause microsatellite instability in tumors.

►Microsatellite Instability

PNAd

Definition

Peripheral Lymph Node Addressin Molecule.

PNET

Definition

Primitive neuroectodermal tumor

►Brain Tumors
►Medulloblastoma

Pneumothorax

Definition

An abnormal collection of air outside the lining of the lung, between the lung and the chest wall, often a consequence of pressure injuries (barotraumas). It may even be caused by accidental lung parenchyma puncture during thoracentesis.

►Pleural Effusion

Podophyllotoxins

Definition

Is a toxin isolated from the American Mayapple (*Podophyllum peltatum*) and used for the synthesis of ►etoposide and teniposide. These anticancer drugs are DNA ►topoisomerase II inhibitors and inhibit DNA synthesis.

►Membrane Transporters

Podoplanin

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Synonyms

gp36; Human podoplanin; OTS-8, gp38, T1α2, PA2.26, RANDAM-2, Aggrus; Mouse podoplanin; E11 antigen, RTI40; Rat podoplanin

Definition

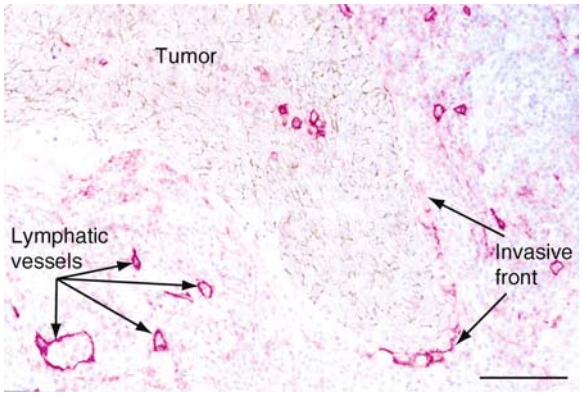
Human podoplanin is a type-1 transmembrane glycoprotein consisting of 162 amino acids, nine of which form the intracellular domain. The extracellular domain is extensively O-glycosylated. Depending on glycosylation, the molecular mass is between 36 and 45 kDa. Podoplanin is physiologically expressed in kidney podocytes, skeletal muscle, placenta, lung, heart, myofibroblasts of the breast and salivary glands, osteoblasts and mesothelial cells, and on the apical surface of alveolar type I cells. Occasionally, focal expression of podoplanin can be found in circumscribed areas of the basal layer of the human epidermis. Podoplanin is also expressed in lymphatic endothelium, but not in blood vessels. Pathological expression of podoplanin is observed in many human cancers, in particular squamous cell ►carcinomas.

Characteristics

In tumors, podoplanin is expressed by the cancer cells themselves, where it is involved in ►tumor progression. In addition, it is expressed in lymphatic endothelial cells and serves as an immunohistochemical marker for ►lymphatic vessels.

Podoplanin Expression in Human Tumors

The expression of podoplanin is upregulated in testicular carcinoma *in situ* and in many invasive human cancers, in particular squamous cell carcinomas of the skin, larynx, lung, cervix, mouth and esophagus, as well as invasive tumors of the germinal cells and the central nervous system. Podoplanin expression has also been reported in mesothelioma and several human ►sarcomas, but not in adenocarcinomas such as colorectal or prostate cancer. Podoplanin can either be selectively expressed on the ►outer edge of the tumor mass, or diffusely throughout the cancerous tissue (Fig. 1). The expression of podoplanin in a single cell layer at the tumor surface is most often observed in squamous cell carcinoma, whereas sarcomas tend to express podoplanin more diffusely. Clinical studies indicate that the expression of podoplanin in human cancer may positively correlate with tumor progression and a poor prognosis (example: malignant astrocytoma of the brain).



Podoplanin. Figure 1 Histological section of a human squamous cell carcinoma. The cells of the tumor bulk express E-cadherin (brown staining) and form an invading conus which protrudes into the surrounding tissue. Podoplanin (red staining) is expressed by cells of the invasive front and by the lymphatic endothelium (as indicated by the arrows). Size bar = 100 µm. Microphotograph courtesy of D. Kerjaschki, MUW, Vienna.

The physiological role of podoplanin remains in great parts unknown. Podoplanin-deficient mice die at birth owing to respiratory failure and exhibit a phenotype of alveolar hypoplasia, dilated malfunctioning lymphatic vessels, and lymphoedema. In addition, podoplanin has an extracellular platelet aggregation-stimulating domain and is therefore able to promote hemostasis.

Regulation

The podoplanin gene promoter is characterized by the absence of a consensus TATA and CAAT box, the presence of multiple Sp1 binding sites and a high GC-content. This promoter structure is mostly found in ubiquitously expressed or growth-related genes. In human sarcoma cell lines, the basal transcription of podoplanin is regulated by the transcription factors Sp1 and Sp3, and presumably by other, not yet identified factors. In lymphatic endothelium, podoplanin is an early responder to Prox-1, a master regulator of lymphatic vessel formation. In human carcinoma cells, upregulated expression of podoplanin is observed upon treatment of cells with EGF, FGF-2, TNF-α, or bradykinin. The expression of podoplanin is increased in mouse skin during tissue regeneration after wounding or by treatment with the carcinogen phorbol-12-myristate-13-acetate. Podoplanin expression is also induced by 12-*O*-tetradecanoylphorbol-13-acetate in

mouse osteoblastic cells and is it constitutively expressed in oncogenic Ras-transformed cells.

Podoplanin and Tumor Invasion

Podoplanin induces tumor invasion *in vitro* and *in vivo*. Transfection of podoplanin into human cancer cells usually results in increased spreading of the cells on ►fibronectin, a component of the extracellular matrix. Podoplanin also enhances cell migration and invasion through a collagen IV containing, basal membrane-like substrate. The enhanced migration is accompanied by a strong polarization of the cells. Depending on the cell of origin, podoplanin promotes collective or ►single cell invasion. During ►collective cell invasion, epithelial cancer cells remain attached to each other, since they continue to express ►E-cadherin, a cell adhesion molecule required for the formation of epithelial adherens junctions. In a transgenic mouse model of ►insulinoma, podoplanin was shown to shift the invasion pattern from single to collective cell invasion. Podoplanin also mediates single cell invasion upon loss of E-cadherin (e.g. in MDCK cells, an epithelial canine kidney cell line). Single cell invasion and loss of E-cadherin are often associated with ►epithelial-mesenchymal transition (EMT), a phenomenon that also occurs during embryogenesis, for example during neurulation or gastrulation. During both collective and single cell invasion, podoplanin promotes phosphorylation of ►ERM-proteins, in particular ezrin. Upon phosphorylation, ezrin associates with the cell membrane and re-organizes the actin cytoskeleton.

The expression of podoplanin also affects cell morphology. Stress fibers, which are often found in quiescent cells, are lost and filopodia are formed. Both cell migration and membrane motility are increased, and the formation of multiple ►microspikes of the cell membrane is induced.

Podoplanin modulates the activity of the family of ►Rho GTPases, in particular RhoA, Cdc42 and Rac. The modulation of RhoA signaling can directly translate into an increased cell movement. However, depending on the cell type, migration and invasion are induced by either up- or downregulation of distinct Rho GTPases.

Podoplanin and Metastasis

The extracellular portion of podoplanin contains a platelet-aggregation domain. Indeed, the expression of podoplanin on circulating tumor cells increases the formation of ►thrombotic cancer cell emboli, thereby increasing the efficiency of metastasis formation. In a mouse model, acetylsalicylic acid inhibits platelet aggregation, and reduces the incidence of metastasis after intravenous injection of podoplanin-expressing Chinese hamster ovary (CHO) cells. Thus, podoplanin may be involved in the transport of tumor cell-platelet clusters in the bloodstream.

Podoplanin as a Marker for Tumor-Associated Lymphatic Vessels

Podoplanin is expressed in lymphatic endothelial cells, but not in blood endothelium. Therefore, podoplanin is frequently used as a selective marker for lymphatic vessels. Together with ►Lyve-1, another lymphatic endothelium specific marker, it is employed to visualize and quantify lymphatic vessels. Tumor-associated lymphatic endothelial cells are thought to be involved in intralymphatic transport of cancer cells, and the lymphatic microvessel density is important for prognosis. For example, the density of lymphatic vessels is an independent prognostic factor for the prediction of melanoma metastasis and patient survival.

Conclusions

Podoplanin promotes tumor invasion and metastasis formation in both carcinoma and sarcoma. However, in carcinoma *in situ* and in regenerating epithelium its role has remained elusive. Alone or together with Lyve-1, podoplanin is an important marker for the assessment of lymphatic microvessel density in tumors.

►Lymphangiogenesis

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POEMS Syndrome

Definition

Synonym: Crow-Fukase syndrome, Takatsuki disease or PEP syndrome; Is a rare medical syndrome named

for its main clinically recognizable features: Polyneuropathy (peripheral nerve damage), Organomegaly (abnormal enlargement of organs), Endocrinopathy (damage to hormone-producing glands)/Edema, M-protein (a monoclonal immunoglobulin produced by plasma cells) and Skin abnormalities, such as hyperpigmentation and hypertrichosis.

►Plasmacytoma

Point Mutation

Definition

Is a change on a single base of a sequence of DNA. If the mutation is placed within a sequence that codifies for a protein it may have three different outcomes. Point mutations may be silent when the substitution of a base has no effect on the sequence of the protein producing no change of the amino acid residue codified due to redundancy of the genetic code. It may also induce an alteration of the sequence of the protein. In this case, a point mutation may render either a substitution of an amino acid for a different one or generate and stop codon with a truncated protein.

Polo-Like Kinase

Definition

PLK; Are important regulators of cell cycle progression during M-phase. Named after the polo gene of Drosophila melanogaster, Plks are involved in the assembly and dynamics of the mitotic spindle apparatus and in the activation and inactivation of CDK/cyclin complexes. In mammalian cells, Plk1 protein levels increase as cells approach M phase, with the peak of phosphorylation activity reached during mitosis.

►G2/M Transition

Poly (ADP-Ribose) Polymerase

Definition

PARP; A zinc-finger DNA-binding protein which detects and signals DNA strand breaks. This protein/enzyme catalyses the synthesis of poly (ADP-ribose), from its substrate and the polymer is attached to several nuclear proteins and PARP itself. As a result, PARP converts DNA breaks into intracellular signals which activate DNA repair programs or cell death machinery.

►Inflammation
►Poly(ADP-Ribosyl)ation

Poly(ADP-Ribosyl)ation

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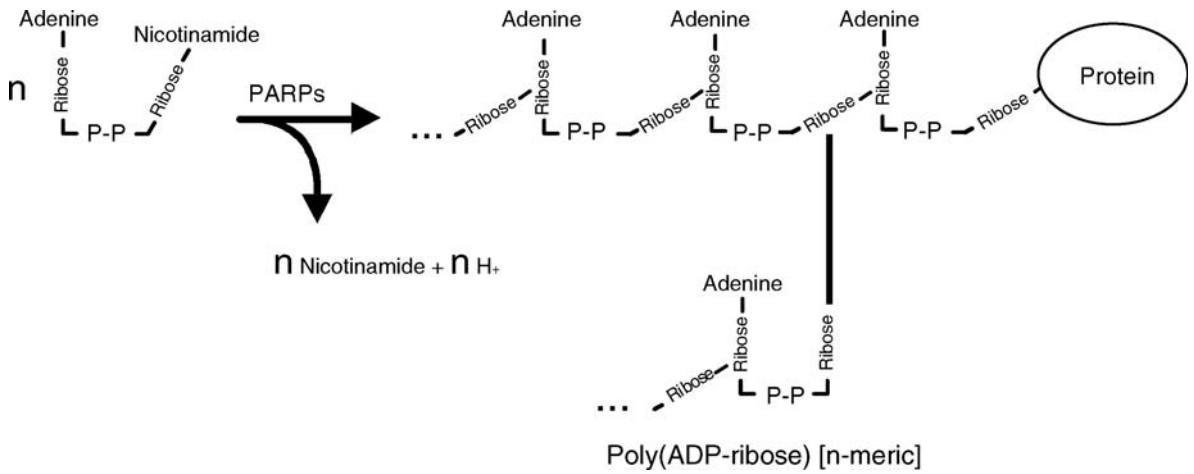
Definition

Poly(ADP-ribosyl)ation is a post-translational modification of glutamate or aspartate residues of proteins, with NAD^+ serving as precursor (Fig. 1), and represents an immediate eukaryotic cellular response to DNA damage as induced, e.g. by ionizing radiation, alkylating agents or oxidants [1–5]. Poly(ADP-ribosyl)ation is catalyzed mostly by the 113-kD enzyme ►poly (ADP-ribose) polymerase-1 (PARP-1). The *PARP1* gene (previously termed *ADPRT*) has been mapped to human chromosome 1q42. A number of additional polypeptides that are able to catalyze poly(ADP-ribosyl)ation have been identified in recent years, and sequence homology searches have revealed over a dozen different gene loci in the human genome that comprise the so-called PARP signature sequence and are now termed the *PARP* gene superfamily. The members of the PARP protein family include PARP-2, PARP-3, vPARP and tankyrases, and they collectively seem to account for up to 25% of the total cellular poly(ADP-ribose) production. The major enzyme catalyzing the catabolism of poly(ADP-ribose), by hydrolyzing the ribose-ribose linkages in the polymer, is poly(ADP-ribose) glycohydrolase (PARG). The single *PARG* locus mapped to human chromosome 10q11.23 has been shown to encode several polypeptides arising from differential splicing and displaying differential subcellular localization.

Characteristics

Catalytic Function of PARP-1 and Life Cycle of Poly(ADP-Ribose)

PARP-1 has been detected in most eukaryotes and displays a characteristic and highly conserved domain structure (which can be further broken down into subdomains). While the protein is constitutively and



Poly(ADP-Ribosyl)ation. Figure 1 Schematic representation of the structure of poly(ADP-ribose).

abundantly expressed in all proliferating and some non-proliferating cells, its catalytic activity is stimulated dramatically by DNA single-strand or double-strand breaks. Its DNA-binding domain, which is located at the amino-terminus, binds to single- or double-strand breaks in DNA via two zinc fingers. Binding of PARP-1 to broken DNA induces an immediate and dramatic activation of the catalytic centre residing in the carboxy-terminal NAD^+ -binding domain of the enzyme. PARP-1 is catalytically active as a dimer. In intact cells the enzyme itself is the major target protein ("acceptor") for covalent modification with poly(ADP-ribose). This "automodification" reaction is thought to occur mostly on a specific domain located between the DNA-binding and the NAD^+ -binding domain, respectively. However, in living cells a number of additional acceptor proteins have been identified, such as histones and ▶topoisomerases, and *in vitro* many more proteins can be modified with this polymer. The half-life of poly(ADP-ribose) is very short under conditions of DNA breakage due to its rapid degradation by PARG and other catabolic enzymes. Hence, the existence of poly(ADP-ribose) in intact cells is transient, and is restricted to the presence of DNA strand breaks. The life cycle of poly(ADP-ribose) is depicted schematically in Fig. 2.

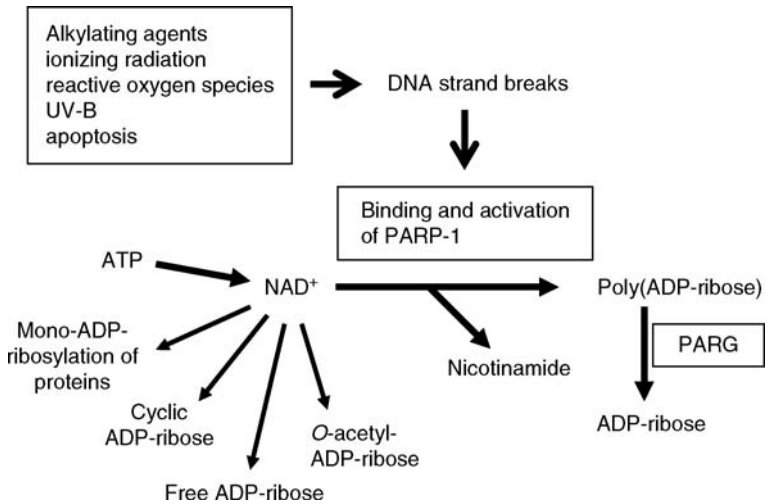
A number of molecular functions for PARP-1 and/or poly(ADP-ribose) have been proposed, and the following list is by no means exhaustive. It must be emphasized that many of the claims are based on work on subcellular systems, and the relevance for the *in vivo* situation is not known.

Molecular Functions Related with Regulation of DNA Strand Breaks and of DNA Repair

- Direct control of the activity of DNA-processing enzymes (DNA polymerases, ligases, topoisomerases etc) by their covalent modification with poly(ADP-ribose).
- Poly(ADP-ribosyl)ation of histones as a mechanism to induce localized chromatin relaxation, thus allowing access of DNA repair enzymes to the damaged site.
- Poly(ADP-ribose) as a "histone shuttle"; high-affinity non-covalent binding of (unmodified) histones to automodified PARP-1 should lead to localized chromatin relaxation, thus allowing access of DNA repair enzymes to the damaged site.
- PARP-1 as a component of a multi-protein complex carrying out DNA base-excision repair; such a protein complex would also comprise XRCC1, DNA polymerase-β and DNA ligase III. PARP-1 would detect DNA strand breaks at the original lesion site and could recruit the other partners. PARP-1 automodification might serve to regulate formation or function of such a complex.
- Physical and functional interaction of PARP-1 and poly(ADP-ribose) with the double-strand break-associated proteins DNA-dependent protein kinase (DNA-PK) and "ataxia telangiectasia mutated" (ATM).
- Locally restricted generation of ATP from poly (ADP-ribose) by pyrophosphorylytic cleavage, to be used for the DNA ligation step in DNA base excision repair.
- Signaling of DNA damage through non-covalent binding of poly(ADP-ribose) with p53, $p21^{WAF}$ etc.

Molecular Functions Related with the Maintenance of Genomic Stability

- Protection of "open" DNA strand breaks by PARP-1 binding, in order to prevent nonspecific DNA degradation and/or undesirable recombination. PARP-1 automodification might then serve to weaken the interaction with DNA, thus allowing repair activities



Poly(ADP-Ribosyl)ation. Figure 2 Life cycle of poly(ADP-ribose). PARP-1, poly(ADP-ribose) polymerase-1; PARG, poly(APD-ribose) glycohydrolase.

to proceed. It should be noted that a primary role of PARP-1 and/or poly(ADP-ribose) in DNA repair (see above) would of course indirectly contribute to the maintenance of genomic stability under conditions of genotoxic stress.

- Regulatory function of PARP-1 at the centrosome, thus preserving cellular euploidy.
- Stabilizing function of poly(ADP-ribose) at the mitotic spindle.
- Involvement of PARP family members in ►telomeres function.
- Physical interaction and functional cooperation of PARP-1 with the Werner syndrome protein (WRN).

Molecular Functions Related with DNA Replication

- PARP-1 as a component of a multi-protein DNA replication complex comprising enzymes for leading and lagging-strand DNA synthesis, some of which are potential targets for poly(ADP-ribosyl)ation (DNA polymerase-α, RPA, topoisomerase I, PCNA, DNA ligase I).
- Direct interaction between the PARP-1 DNA-binding domain and the catalytic subunit (p180) of the DNA polymerase-α-primase tetramer, independent of DNA. Thus PARP-1 might participate in a DNA damage-monitoring system, with its nick-sensor function perhaps playing a regulatory role when the progressing replication fork encounters strand breaks in the template.

Molecular Functions Related with Gene Expression

- Poly(ADP-ribosyl)ation as a regulator of chromatin compaction and DNA methylation status.
- Participation of PARP-1 in the activation of ►E2F-1 gene promoter after mitogenic stimulation of cells, leading to increased expression of both E2F-1 and DNA polymerase-α and entry into S-phase (see above, "Molecular functions related with DNA replication").
- PARP-1 as a transcriptional co-factor for B-MYB and for ►AP2 (independent of the catalytic function of PARP-1).
- PARP-1 as a cofactor for NFκB-mediated transactivation; in *Parp1*-deficient mice, the typical lipopolysaccharide-induced increases in serum levels of TNF-α, in inducible nitric oxide synthase (iNOS) expression and in nitric oxide production are lacking, and endotoxic shock does not develop.
- Silencing of RNA polymerase II-dependent transcription by PARP-1 activity in a cell-free system. Inhibition of TATA-binding protein (TBP) and the transcription factor YY1 by poly(ADP-ribosyl)ation (possible only prior to their binding to DNA).
- PARP-1 as interaction partner of transcription factors such as with retinoid X receptors (RXR), p53, Oct-1, PC1, E47, TEF-1 and DF1–4.

Molecular Functions Related with Energy Metabolism and Mitochondrial Changes

- Massive poly(ADP-ribose) synthesis leading to NAD^+ depletion and/or release of apoptosis-inducing factor (AIF) from mitochondria and consequently cell death.

Cellular Functions

Depending on the intensity of DNA damage inflicted to the cells and the cellular proliferation/differentiation status, PARP-1 has two contrasting functions:

- Cytoprotection and maintenance of genomic stability

To study the cellular function of poly(ADP-ribosyl)ation various strategies have been employed to abrogate poly(ADP-ribosyl)ation. These include competitive low-molecular weight ►PARP inhibitors, expression of a dominant negative PARP-1 version, PARP-1 antisense RNA expression, PARP-1 siRNA, or *Parp1* gene disruption in the mouse germ line. The results have consistently revealed that poly(ADP-ribosyl)ation significantly contributes to the recovery of proliferating normal and malignant cells from low-level DNA damage as induced, e.g. by alkylating agents or ionizing radiation, both *in vivo* and in cell culture. This effect has been linked mechanistically with an involvement of PARP-1 in DNA base-excision repair. Furthermore, there is clear evidence that poly(ADP-ribosyl)ation counteracts the induction of genomic instability by DNA damage, as assessed by several biological markers such as chromosomal aberrations, sister-chromatid exchange, gene amplification or mutagenesis. In addition, fibroblasts from *Parp1*-deficient mice display morphological abnormalities and reduced growth rate. These protective functions of PARP-1, as well as its role in maintaining telomere length in mice, are in line with correlative data showing an association of mammalian and human longevity with high cellular poly(ADP-ribosyl)ation capacity.

- Cell death induction

In stark contrast to the cytoprotective function mentioned above, PARP-1 overactivation may lead to cell suicide due to severe and irreversible depletion of $NAD^+$and consequently of ATP pools (Fig. 2). Whether the type of cell death induced is always necrotic or may be of the apoptotic type has not yet been clearly resolved. By comparing *Parp1*-deficient and wild-type mice and derived cells and by using competitive PARP inhibitors, such a cytotoxicity mechanism has been identified to be operative in several nonproliferating cell types. These include (i) pancreatic islet cells exposed to relevant DNA-damaging compounds (reactive oxygen species, nitric oxide metabolites, streptozotocin), (ii) neurons after regional ischemia-reperfusion damage of the brain (known to induce widespread release of reactive oxygen species and nitric oxide in the affected area, thus leading to the loss of many neurons and to brain infarct), (iii) dopaminergic neurons exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a drug known to induce release of reactive oxygen species only in the dopaminergic neurons of the *substantia nigra*, thus leading to selective neuronal death and Parkinson syndrome). In each case it was shown that the cells from *Parp1*-deficient mice were highly protected from cell death and the animals displayed increased resistance to clinical disease. Likewise, administration of PARP inhibitors *in vivo* led to prevention of cell death in post-ischemic heart and skeletal muscle and renal tubular cells.

Recent data reveal that, apart from NAD^+ depletion, cell death by active PARP-1 can also be mediated by poly(ADP-ribose) itself. It was shown that the polymer can leave the nucleus and then trigger the release of apoptosis-inducing factor (AIF) from mitochondria. AIF, in turn, translocates to the nucleus and induces high-molecular weight DNA fragmentation and caspase-independent cell death.

It should be noted that classical ►apoptosis is associated with dramatic changes concerning the poly(ADP-ribosyl)ation system. After an initial burst of cellular poly(ADP-ribose) formation early in apoptosis, there is a well-documented proteolytic cleavage of PARP-1 into two fragments by activated caspase-3/7 during the execution phase of apoptosis, representing one of the most frequently used biochemical markers of apoptosis. This cleavage is thought to abrogate the responsiveness of PARP-1 to DNA strand breaks and thus to limit NAD^+ consumption. PARP-2 also undergoes caspase-mediated cleavage during apoptosis.

Clinical Relevance

Genetic Cancer Risk Assessment

Genetic polymorphisms and any resulting functional polymorphisms in cellular proteins involved in recognition and processing of DNA damage can be expected to contribute to the genetic risk profile of an individual with regard to the development of organ-specific cancers or cancer in general.

The active human *PARP1* gene locus on chromosome 1q42 displays several polymorphisms, such as a polymorphic dinucleotide repeat located in the promoter region and base substitutions in the coding region. Interestingly, recent publications report that the V762A polymorphism in PARP-1 leads to reduced enzymatic activity of PARP-1 and is associated with increased risk of lung, prostate and esophageal cancer. These initial observations are perfectly in line with the positive role PARP-1 activity plays in DNA repair and maintenance of genomic stability. According to this, a diminished enzyme activity based on a less active genetic variant can be expected to be limiting for the efficiency of the clearance of DNA damage and thus increase the risk of damage accumulation and genomic instability, which will act as a driving force for carcinogenesis.

Furthermore, a two-allele polymorphism in *PARP1P1*, a PARP-1-related pseudogene at 13q34, has been described. The B allele, arising from a 193-bp conserved duplicated region within the more frequent A allele, has been studied in several ethnic subpopulations, and the association of each allele with different types of cancer has been investigated. In endemic Burkitt lymphoma there was a strong association with the presence of at

least one copy of the B allele. An increased frequency of the B allele was also found in cases of multiple myeloma and prostate and colon cancer, but only among black patients. By contrast, comparisons of lung cancer cases with controls revealed significant enrichment of the B allele in Mexican-American patients only (an ethnic group with very low B allele frequency in general) and not in African Americans. Taken together this *PARP1P1* polymorphism may prove useful as a predictive factor for the risk for specific cancer types in persons from specific ethnic backgrounds. Whether or not this polymorphism has any impact on the cellular poly(ADP-ribosyl)ation status or represents just a marker for a relevant neighboring gene is unknown.

Therapy

For several decades cytotoxic tumor therapy, as delivered by the application of cytostatic drugs or ionizing radiation, has been the most frequently administered forms of cancer therapy. The sensitizing effect of PARP inhibitors in proliferating cells undergoing sublethal DNA damage has long been considered as a potentially useful mechanism to render conventional cytotoxic treatment regimens more effective at eradicating tumor cells in the body. Available data from cell culture experiments suggest that the most suitable cytotoxic agents to be combined with PARP inhibitors would be methylating agents, bleomycin and ionizing radiation, but not anti-metabolites. The first-generation inhibitors (benzamide and its derivatives), however, had significant drawbacks, particularly for the *in vivo* application, such as poor solubility, lack of potency and limited specificity of the compounds. Therefore, in recent years large numbers of novel PARP-inhibitory compounds have been synthesized and characterized, some of which proved to be at least 50-fold more effective as chemopotentiators than the reference inhibitor 3-aminobenzamide, and act in submicromolar (rather than millimolar) concentrations. Animal work revealed that combination treatment of tumors with the alkylating agent temozolomide and PARP inhibitors increases the efficacy of temozolomide. It will be interesting to see the effects of these novel compounds in clinical studies of cytotoxic tumor therapy.

Intriguingly, it was reported that tumor cells with non-functional ►BRCA1 or ►BRCA2 proteins can be sensitized to cell death by mere PARP inhibition in the absence of any DNA-damaging treatment [5]. Apparently DNA single-strand breaks arising spontaneously at rather low frequency will accumulate in under these conditions and are converted into double-strand breaks during DNA replication. As BRCA proteins are crucial for the signaling and proper repair of double-strand breaks by homologous recombination, repair is blocked and cells die as a result of the accumulating double-strand breaks. This scenario could form the basis for a highly selective future cancer chemotherapy protocol in patients with BRCA1 or BRCA2-deficient tumors, as the PARP inhibitor would kill the tumor cells, yet spare non-malignant cells as those would retain at least one functional copy of these genes.

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Poly-Drug Chemotherapy

Definition

An approach to cancer therapy that utilizes the peculiar properties of the sphingolipids. Forcing a tumor to elevate its concentration of ►ceramide and gangliosides GM3 and GD3 induces their ►apoptogenic death. Simultaneously forcing the tumor to lower its concentration of proliferative sphingolipids (sphingosine-1-phosphate, ceramide-1-phosphate, glucosylceramide, and lactosylceramide) synergizes with the other procedure. Since these lipids are enzymatically interconvertible, therapy that controls only one or two of the lipids is likely to fail.

►Sphingolipid Metabolism

Polyadenylation

Definition

Step in the protein synthesis pathway in which a polyadenosine stretch is added to the 3′-end of a messenger RNA.

Polyamines

Definition

Are organic compounds, such as putrescine, spermidine, and spermine, that are growth factors in both eukaryotic and prokaryotic cells. They are synthesized in cells in pathways that are very highly regulated. The polyamines are essential for life, however in excess they can promote tumorigenesis. Biosynthesis is regulated by ►ornithine decarboxylase, and cellular export is through spermidine:spermine acetyl transferase (SSAT).

►Arginine and Cancer
►Transglutaminase-2

Polyaromatic Compounds

►Polycyclic Aromatic Hydrocarbons

Polyaromatic Hydrocarbons

Definition

Polycyclic Aromatic Hydrocarbons; PAH.

Polychlorinated Biphenyls

Definition

Are a class of xenobiotics with several chlorine atoms attached to biphenyl.

►Xenobiotics
►Polycyclic Aromatic Hydrocarbons

Polycomb

Definition

Polycomb complex modulates histone H3 by ►methylation of lysine residues, ►ubiquitination of histone H2A, and ►deacetylation of histones, and binds to the modified histones to inactivate the expression of the adjacent genes. Polycomb protein complex works as a molecular lock to suppress the gene expression to maintain the cellular homeostasis in adult cells.

Polycomb Group

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Definition

The Polycomb group (PcG) is a family of proteins required for proper eukaryotic gene expression that determines ►cell fate (►Epigenetics, ►epigenetic gene silencing). They exert their role mainly by repressing gene transcription through ►epigenetic modifications on specific histone tails that are inherited through cell divisions, thus functioning as the cellular memory. They are involved in the maintenance of cell type and differentiation, and play a role in tumor development.

Characteristics

Cell differentiation depends upon keeping certain genes "on" and other genes "off" through generations. The differential gene expression patterns that define a cell type are maintained during DNA replication and at mitosis so that the daughter cells maintain the differentiated cell type of the parental cell. This is possible because cells have a ►transcriptional memory. Perturbance of the transcriptional memory can lead to severe developmental defects and to malignancy. The molecular basis of the cellular memory is controlled mainly by two groups of proteins: the Polycomb group (PcG) and the Trithorax group (TrxG).

The PcG and TrxG are chromatin associated proteins which function by activating and repressing transcription, respectively. While PcG proteins act mainly by repressing gene expression, TrxG proteins, operationally defined as antagonistic of PcG proteins, activate gene expression. Some proteins possess functions of both PcG and TrxG proteins. PcG and TrxG proteins have been well conserved throughout evolution. There are at least twenty PcG and TrxG proteins in *Drosophila*, and many have mammalian counterparts. Biochemically, they function in large protein complexes. In the following discussion we will concentrate on the PcG proteins.

Studies in *Drosophila melanogaster* have been instrumental in our understanding of proteins involved in transcriptional maintenance. The formation of *Drosophila melanogaster* relies on the expression pattern of a cluster of genes (►Homeotic genes) along the anterior-posterior axis during development. The PcG proteins maintain the correct spatial and temporal expression pattern of the homeotic genes through transcriptional repression. PcG mutants in *Drosophila* express homeotic genes outside the body segments they are normally expressed, causing marked transformations in body plan.

PcG Protein Complexes

PcG proteins function by forming two large multimeric complexes, termed Polycomb repressive complexes (PRCs). The PRC2, composed of EED, EZH1, EZH2, and SUZ12, is involved in the initiation of gene repression. The PRC1 contains multiple proteins including BMI1, HPC proteins (CBX2, CBX4, CBX7, CBX8), and RING proteins (Table 1). One of the hallmarks of transcriptionally active genes is that their nucleosomes contain acetylated histone H3. When genes are repressed, the ►histones are generally deacetylated and H3 is trimethylated on specific lysine residues (especially K9 and K27). The functional conservation between *Drosophila* and human PcG proteins in methylating histone H3 on lysine 27 has resulted in the development of a model for PcG-mediated gene silencing. In this model, at the initiation of gene repression, PRC2 associated ►histone deacetylases remove the ►acetylation of H3, and allow EZH2 to trimethylates lysine 27, and to a lesser extent lysine 9 of histone H3 ($H3K27me^3$ and $H3K9me^3$, respectively). The PRC1 can recognize the trimethylation mark through the chromo domain of the Polycomb protein, leading to the recruitment of the PRC1 maintenance complex. This interaction is proposed to target the PRC1 to specific repression sites. It should be noted that this model has not been yet confirmed in mammalian cells. The precise mechanisms by which the PcG proteins inhibit transcription are still unclear. One suggested mechanism by which the PcG proteins can block the transcription initiation machinery is though PRC1 ubiquitin E3 ligase activity of histone H2A lysine 119 (H2AK119), a modification associated with gene repression. Another suggested mechanism of PcG mediated repression is through PRC2 methyltransferase activity on lysine 26 of the linker histone H1 (H1K26). Methylated H1K26 can influence the chromatin structure. Furthermore, EZH2, a member of the PRC2 has been found to recruit DNA methyltransferases (DNMTs) to specific target genes. DNA ►hypermethylation of gene promoter regions results in transcriptional repression. By repressing specific genes, PcG proteins are involved in regulating a wide variety of fundamental cellular processes such as stem cell maintenance, cell fate, cell division, and neoplastic cell transformation.

Polycomb Group. Table 1 PcG proteins and their altered expression in human malignancies

Drosophila	Human	Human cancer type
Initiation complex-PRC2		
Extra sex combs, Esc	EED	
Enhancer of zeste, E (z)	EZH1	
	EZH2	Lymphoma, melanoma, carcinomas of the breast, prostate, bladder, colon, liver
Suppressor of zeste, Su (z) 12	SUZ12	Carcinomas of the breast, colon, liver
Maintenance complex-PRC1		
Polycomb, Pc	CBX2/ Pc1/M33	
	CBX4/ Pc2	
	CBX6	
	CBX8/ Pc3	
Polyhomeiotic, Ph	Rae28/ EDR1	Acute lymphoblastic leukemia
	EDR2	
	EDR3	
Sex combs extra, Sce	RING1A/ RNF1	
	RING1B/ RNF2	
Posterior sex combs, Psc	Bmi1/ RNF51	Lymphoma, leukemia, medullobastoma, neuroblastoma, non-small cell lung cancer
	RNF110/ Mel18	
	RNF134	
	NSPC1	
	RNF3	
	RNF159	
Pleiohomeotic, PHO	YY1	

PcG Proteins and Stem Cell Maintenance

An intriguing function of the PcG proteins is their emerging role in the maintenance of embryonic and

adult ►stem cells (►Stem cells). Stem cells have the ability to self-replicate and give rise to specialized cells, processes that require a transcriptional memory system that maintains the cell type identity from one generation of cells to another. This function is underscored by studies showing that targeted disruption of EZH2 or EED results in early embryonic lethality. Consistent with a critical role in the maintenance of stem cells, PcG proteins are able to silence genes with roles in embryonic development and genes responsible for cellular differentiation. Among these targets are homeodomain-containing transcription factors of the Dlx, Irx, Lhx and Pax gene families with functions in the early development of the neural and hematopoietic systems, and members of the Fox, Sox, Gata, and Tbx transcription factor families which not only have roles in development, but also in disease. For example, Gata3 has been found to play a role in breast cancer, with strong association with estrogen receptor expression. Furthermore, PcGs target genes in signal transduction pathways required for embryonic differentiation as well as for maintenance and proliferation of stem cells in tissues, such as transforming growth factor and fibroblast growth factor families, Hox, Notch (►NOTCH/JAGGED signaling in neoplasia), Hedgehog, and ►Wnt signaling pathways.

PcG Proteins in Human Malignancies and Their Clinical Relevance

Lack of differentiation, or anaplasia, is a hallmark of cancer which results from normal cells "forgetting" their cellular identity. Thus, it is not surprising that dysregulation of the transcriptional maintenance system can lead to malignancy. Current data suggest that altered expression of the PcG proteins may influence tumor development mainly by transcriptional repression of tumor suppressor genes and by maintaining the cells in a stem cell state. This is particularly intriguing in light of the stem cell model of carcinogenesis which proposes that human tumors contain and arise from transformed stem cells and early progenitor cells that have sustained specific genetic alterations. At least three mammalian PcG proteins, Bmi1, Rae28, and Mel18, are already implicated in the self-renewal of hematopoietic and neuronal stem cells. Although this theory is under extensive investigation and many questions need to be addressed, PcG proteins play a central role in the process of neoplastic transformation.

The first connection between PcG proteins and cancer development came from studies on Bmi1, which was found to cooperate with the proto-oncogene c-myc (►Myc oncongene) to promote the generation of B- and T-cell lymphomas and later shown to inhibit c-myc induced apoptosis through repression of the Cdkn2a locus. This tumor suppressor locus is frequently inhibited in human tumors. Further studies showed that other PcG proteins are also strongly associated with neoplastic transformation and progression. Although in human malignancies PcG proteins have primarily been found to be dysregulated in cells of hematologic origin, they play an important role in the development of carcinoma of different organs, melanoma, and neural tumors. EZH2 is overexpressed in multiple types of lymphoma and in solid tumors including carcinomas of the breast, prostate, colon, bladder, liver, and melanoma. In adenocarcinomas of the prostate gland, elevated levels of EZH2 protein are able to distinguish patients with localized prostate cancer that are more likely to experience tumor recurrence after prostatectomy. In breast tissues, overexpression of EZH2 occurs before histological evidence of malignancy and its overexpression in breast cancer is associated with larger tumors, the presence of lymph node and distant metastasis, and a worse clinical outcome. Furthermore, in breast cancers increased levels of EZH2 are associated with absent estrogen (►Estrogen receptor, estrogenic hormones and cancer) and progesterone receptors. Another PcG protein, SUZ12, is upregulated in both breast and colon cancers. The specific mechanisms leading to the overexpression of PcG genes and the events resulting in the localization of PcG proteins to different target sites await further investigations.

It is clear that the PcG proteins are overexpressed in several human malignancies, and that in carcinomas of the breast and prostate their up regulation is associated with an aggressive and metastasizing clinical course. For these reasons the use of PcG proteins as potential tissue ►biomarkers of cancer diagnosis, prognosis, and prediction to treatment response holds promise. Because PcG proteins appear to be expressed in certain cancers such as breast cancers before histological atypia, they may mark epithelium at higher risk for development of carcinoma. Importantly, given their role in malignant transformation of hematopoietic and solid human tumors, components of the PcG family may provide attractive opportunities for therapeutic intervention (►Epigenetic Therapy).

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PolyComb Group Genes

Definition

Code for proteins that remodel chromatin as a way to prevent transcription factors from binding to DNA promoter sequences during development.

►Stem Cell Markers

Polycyclic Aromatic Hydrocarbons

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Synonyms

Polyaromatic compounds

Definition

Polycyclic aromatic hydrocarbons (PAHs) belong to the more general class of polycyclic aromatic compounds that contain two or more aromatic rings fused together in a linear or angular configuration. PAHs consist of carbon and hydrogen only. Hence, polycyclic aromatic compounds containing heteroatoms (e.g., N, S, and O) in their molecular structure would not belong to the group of PAHs in a narrow sense of the chemical nomenclature.

Characteristics

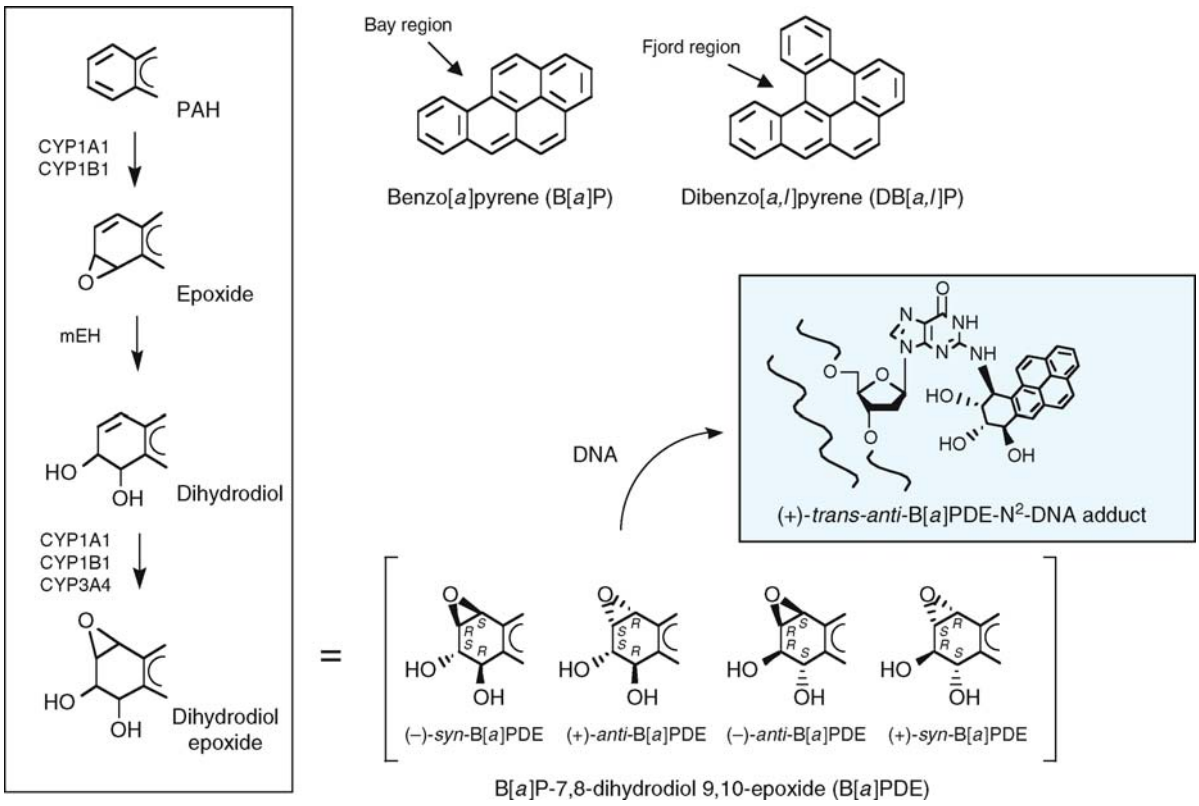
Anthropogenic Origin and Carcinogenicity

PAHs are formed during incomplete combustion processes of organic matter. Due to the abundant use of fossil energy sources, PAHs are readily detectable as ubiquitous contaminants in the environment. Humans are exposed to complex mixtures of PAHs in the atmosphere from combustion sources such as cigarette smoking, vehicle emissions, and fossil fuels, in foodstuffs from pyrolysis products such as over barbequed meat or charbroiled food. In addition, industrial and work place exposures occur through coal gasification, aluminum production, steel founding, and coal tar production. The great interest in this group of chemicals originated on the observation in animal tumor models that some member compounds possessed strong carcinogenic activity in skin, lung, breast, and other organs. Early observations of individual cancer cases occurring from occupational exposures to mixtures highly contaminated with PAHs lend support to the notion that these compounds can also act as carcinogens in man. Epidemiological data identified the occupational exposure to complex PAH-containing mixtures such as coal tar or coal tar pitches (consisting of up to 50% of PAHs with 1–2% benzo[*a*]pyrene, B[*a*]P; see Figure) as being carcinogenic to humans (IARC classification Group 1). Recent epidemiological meta-analyses confirmed that heavy exposures to mixtures of PAHs entails a substantial risk to develop cancer in lung and skin, and possibly also in the bladder (less consistent evidence), larynx, kidney, and pancreas (uncertain evidence).

No matter what kind of PAH source, humans are always exposed to mixtures of aromatic hydrocarbons with different degrees of biological activity. For instance, more than a hundred different PAHs can be detected in the air. The composition of these PAH profiles vary depending on the environmental compartment. Since there is no international agreement on which panel of individual PAHs should be analyzed and reported in order to characterize distinct emission sources, PAH lists released from different organizations may contain different compounds. Sixteen different PAHs such as pyrene, B[*a*]P, benz[*a*]anthracene, benzo-[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, and others are prioritized by the US Environmental Protection Agency. Lower molecular weight PAHs such as naphthalene, phenanthrene, anthracene, or fluoranthene are also listed due to their occurrence in environmental samples. From all hydrocarbons detectable in the human environment the most intensively studied example, B[*a*]P, has been traditionally used as an indicator for carcinogenic PAHs.

Molecular Mode of Action

PAHs can be readily absorbed from lung, skin, and gut. Rapid absorption of B[*a*]P and other PAHs from the gastrointestinal tract or from skin has been widely demonstrated in animal models, and sufficient evidence is also available for humans. On the other hand, absorption through the lungs may be affected by the size of particles at which most of the airborne PAH fraction is adsorbed. It has been shown that a considerable fraction of B[*a*]P adsorbed on soot, diesel exhaust, or carbon black particulate matter can be retained for up to weeks in the respiratory tract. The slow passage through bronchial mucosa can therefore result in very high local concentrations of these compounds directly within one of their main target tissues, the lungs. To react with cellular macromolecules, however, lipophilic and chemically inert compounds such as PAHs require enzymatic biotransformation into electrophilically reactive descendants (metabolites).



In a landmark paper from 1964, Brookes and Lawley reported on the tissue binding levels of six PAHs, i.e., naphthalene, dibenz[*a,c*]anthracene, dibenz[*a,h*]-anthracene, B[*a*]P, 3-methylcholanthrene, and 7,12-dimethylbenz[*a*]anthracene in mouse skin in vivo. The authors concluded that there is a significant positive correlation between the binding to DNA and the carcinogenic potency of these compounds. On the contrary, this correlation was not found for the binding to proteins or RNA. In more recent years it became clear that vicinal dihydrodiol epoxides of PAHs that contain the epoxy moiety in a sterically crowded bay or fjord region are the actual DNA-binding metabolites that mediate the biological effects associated with their parent structures (Figure). By direct application of these reactive descendants it has been demonstrated that their mutagenic potency correlates to the level of PAH–DNA adduct formation. Moreover, the tumor-inducing potencies of different PAHs in the lungs of strain A/J mice, another experimental tumor model, were found to correlate to the time-integrated DNA adduct levels (TIDAL) calculated as area under the curves of total dihydrodiol epoxide–DNA adduct levels during a time course of 30 days after injection. This parameter represents the total effective molecular dose delivered to target lung DNA, and it linearly correlated to the PAH doses administered. The intimate relationships between DNA binding level and mutagenicity and the TIDAL and carcinogenicity observed in mice in vivo support the notion that the DNA binding level can serve as an important biomarker for the tumor threat that may result from exposures of humans to carcinogenic PAHs. Hence PAH-DNA damage is considered to be causative and directly related to tumor formation by representing the first important step during the multistage process of PAH-induced carcinogenesis.

The PAH–DNA adduct level at a given time point is an integrated product of compound's toxicokinetic and toxicodynamic behavior, including metabolic activation and detoxification prior to covalent binding, as well as the effectiveness of the repair of those DNA lesions that have been formed. PAHs would not be carcinogenic if they were not metabolized by cytochrome P450-dependent monooxygenases (CYP enzymes) and microsomal epoxide hydrolase (mEH) through subsequent steps of epoxidation and hydrolysis (Figure). Deletion of the genes encoding the enzymes involved in this activation route (e.g., CYP1B1, mEH), or of the gene encoding the regulator protein involved in the induction of these enzymes (arylhydrocarbon receptor protein), renders mice resistant to the biological effects of potent PAHs such as B[*a*]P, 7,12-dimethylbenz[*a*]anthracene, dibenzo[*a,l*]pyrene, and others.

Metabolic activation of PAHs has been shown to be highly stereoselective. As demonstrated for a wide range of carcinogenic PAHs the initial epoxidation hydrolysis sequence produces a dihydrodiol with (*R,R*) configuration in high enantiomeric excess. Subsequent epoxidation at the vicinal double bond then predominantly generates the (*R,S*) dihydrodiol (*S,R*) epoxide with the epoxide moiety *trans* to the benzylic hydroxy group and spanning into a bay or fjord region. For B[*a*]P, all four possible stereoisomeric bay region 7,8-dihydrodiol 9,10-epoxides (B[*a*]PDE) are depicted in Figure, with (+)-*anti*-B[*a*]PDE as the major species formed during bioactivation. Depending on the activation system, very small amounts of the other isomers may also be generated. However, B[*a*]P-induced DNA damage in vitro or in vivo predominantly results from covalent interaction of (+)-*anti*-B[*a*]PDE, most of which is trapped by 2′-deoxyguanosine residues via *trans* opening of the epoxide moiety [(+)-*trans*-*anti*-B[*a*]PDE-N^2–DNA adduct; Figure]. If not repaired, this adduct causes nucleotide misincorporation at the opposite DNA strand during the next round of DNA replication, thus leading to base substitutions or frameshift mutations. In the case of B[*a*]P, recent data also suggest that poor enzymatic repair of its predominant (+)-*trans*-*anti*-B[*a*]PDE-N^2–DNA adduct is actually preceded by an insufficiently activated DNA damage checkpoint. At nontoxic doses of the dihydrodiol epoxide, a significant number of synchronized cells in vitro were found to enter the synthesis (S) phase of the cell cycle. The failure to induce a proper DNA damage arrest along with insufficient enzymatic repair increases the likelihood of transforming mutations because DNA replication continues on a damaged template via engagement of error-prone (Y-family) polymerases during translesional synthesis. Analysis of the cell cycle and the expression profiles of human epithelial cells or tumor cells of epithelial origin in culture revealed that the DNA damage arrest was insufficient even at a DNA damage level of about 180,000 *anti*-B[*a*]PDE–DNA adducts per cellular genome.

Bay or fjord region dihydrodiol epoxides of other carcinogenic PAHs were also identified as the actual metabolites that mainly mediate cellular binding to DNA and thus the genotoxic effects of their parent structures. Covalent PAH–DNA adducts are fixed as mutations if left to error-prone excision repair, misrepair, or replication errors during early S phase. Most importantly, if these fixed mutations occur in proto-oncogenes and/or tumor suppressor genes, they can contribute to the aggravation of neoplastic growth through the processes of tumor promotion and progression. Members of the oncogene family *RAS* have been found to be commonly mutated in human cancers and animal models for chemical carcinogenesis. Codon 12 (within exon 1) of *KRAS* is the most frequently affected codon in human cancers including human lung adenocarcinomas. Using human bronchial epithelial cells it was demonstrated that the first dG residue in codon 12 is a preferential binding site for the DNA-damaging dihydrodiol epoxide metabolite of B[*a*]P (at N^2). As compared with other sites, the "hotspot" character of codon 12 in target cells of chemical lung tumorigenesis results from a synergism between the preferential binding of the carcinogenic metabolite and a poor repair of those lesions that had been formed. On the other hand, binding studies of B[*a*]P at dG residues located in the DNA binding domain of the tumor suppressor *TP53* gene (exons 5, 7, and 8) have revealed that the codons and the positions within the codons affected matched between human bronchial epithelial cells exposed to *anti*-B[*a*]PDE and the *TP53* mutational hotspot pattern registered in the database of human lung cancer. This observation provided strong evidence for an etiological role of B[*a*]P in the causation of the disease.

Given the chemical complexity of most environmental matrices, it seems difficult, if not, impossible to uncover any causative relationship between certain forms of human cancer and the exposure to particular carcinogenic compounds. In addition to epidemiological hints, collective evidence from molecular toxicology and molecular epidemiology may nevertheless be able to point to the role of individual compounds (or single classes of compounds), and to extract their contribution from the overall biological response on environmental mixtures. One of the most well worked-out example is the crucial role of carcinogenic PAHs (i.e., B[*a*]P) in the etiology of human lung cancer based on their presence in cigarette smoke. Their important role in tumor initiation is supported by several lines of evidence such as (i) increased levels of PAH activating enzymes (CYP1A1 and CYP1B1) in lung cancer patients compared with controls, (ii) a correlation between pulmonary CYP1A1 levels and bulky *anti*-B[*a*]PDE–DNA adduct levels in human lung tissue from cancer patients, (iii) increased levels of *anti*-B[*a*]PDE-DNA adducts in lung tissue of smokers compared with nonsmokers, and (iv) the coincidence of mutational hotspots (G to T transversions) in *KRAS* (codon 12) or *TP53* (codons 157, 248, 273) and *anti*-B[*a*]PDE-N^2–DNA adduct hotspots as the preceding lesions found at the same sites.

►Toxicological Carcinogenesis
►Carcinogen Metabolism

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Polycystic Kidney Disease

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Synonyms

Inherited human polycystic kidney disease

Definition

Polycystic kidney disease (PKD) is a common genetic disease characterized by accumulation of multiple fluid-filled cysts in each kidney and other organs. The renal cysts originate from the renal tubular epithelial cells lined by a single layer of cells that have higher rates of cellular proliferation and apoptosis, and are less differentiated than the normal tubular cells. Progression of cysts in the kidneys could ultimately cause ESRD (end-stage renal disease).

Characteristics

PKD consists of two forms: ADPKD (autosomal dominant PKD) and ARPKD (autosomal recessive PKD). ADPKD is the most common genetic disease affecting 1 in 500 to 1 in 1,000 in adults of all ethnic groups worldwide. ARPKD is far less common, affecting 1 in 6,000 to 1 in 40,000 at a far younger age, including newborns, infants and children. While kidney cysts are arise from all the segments of the nephron and collecting ducts in ADPKD, they are arise from collecting ducts in ARPKD. Extrarenal systems are affected in PKD.

Clinical Features

ADPKD

It is estimated that there are over 12.5 million affected ADPKD individuals worldwide. The development of bilateral, multiple, focal renal cysts characteristics of ADPKD lead to the clinical manifestations include abdominal mass, chronic flank or back pain, gross hematuria, urinary tract infection, and urolithiasis. Affected individuals typically present in the third and fourth decade, and ESRD usually develops in the sixth to eighth decades of life. Extrarenal cysts in ADPKD can arise from liver (~70%), pancreas (~5%), ovaries, and choroid plexus (rare). Cardiovascular abnormalities include mitral valve prolapse (~25%), mitral regurgitation, aortic insufficiency, and tricuspid regurgitation. Hypertension and left ventricular hypertrophy are common. Other extrarenal manifestations include cerebral and aortic aneurysms, cerebral dolichoectasis, and colonic diverticuli. A striking feature of ADPKD is the variability of the phenotype: the severity of the disease, the age of onset of ESRD, and the spectrum of extrarenal manifestation vary widely between affected individuals.

ARPKD

ARPKD is an important childhood nephropathy. The clinical phenotype is characterized by the combination of renal cystic disease and congenital hepatic fibrosis. Affected children often present in utero with enlarged, echogenic kidneys, as well as oligohydramnios secondary to poor urine output. Approximately up to 30–50% of affected neonates die shortly after birth as a result of severe pulmonary hypoplasia and secondary respiratory insufficiency. Those who survive the perinatal period have a 56–67% probability of survival to age 15 without ESRD and prolonged survival to age 55 has been reported. Those survivors express widely variable disease phenotypes with systemic hypertension, renal insufficiency, and portal hypertension due to portal tract fibrosis as the most common clinical features. Long-term survivors often develop sequelae of portal hypertension including esophageal varices, hepatosplenomegaly, and hypersplenism. Other clinical manifestations include growth retardation, urinary tract infection, and hyponatremia.

Molecular Basis

ADPKD is caused by mutations on a single gene of either *PKD1* or *PKD2*, while typical cases of ARPKD are resulted from mutations on *PKHD1* (polycystic kidney and hepatic disease 1) gene. Mutations have been identified throughout the gene of any among those three genes in affected PKD individuals without evidence for clustering. Both ADPKD and ARPKD might be recessive at molecular level: with two germline mutations in ARPKD or one germline mutation plus one somatic mutation caused by "second-hit" in ADPKD generating PKD phenotypes.

ADPKD

1. *PKD1*, located on chromosome 16p13.3 and responsible for ~85% of affected individuals of ADPKD, is comprised of 46 exons distributed over 52 kb of genomic DNA. The first 34 exons are duplicated several times elsewhere on chromosome

16, which has made routine mutation analysis technically difficult. *PKD1* produces a ~14 kb mRNA transcript. Polycystin-1 (PC-1), gene product of *PKD1,* is an integral membrane protein of 4302 amino acid and has molecular weigh of about 500,000 D. PC-1 contains an 11 membrane spanning domain with a ~3000 amino acid extracellular amino (NH_2)-terminus and a ~220 amino acid cytosolic carboxyl COOH-terminus. The large extracellular NH2-terminus contains domains many of which are novel and of unknown function, however, unique array of distinct protein motifs, including leucine-rich repeats flanked by cysteine-rich domains, a C-type lectin domain, a WSC domain, and 16 immunoglobin-like domains named PKD repeats, suggest PC-1 may function as a receptor since many of such motifs are involved in protein–protein or protein–carbohydrate interactions. Closer to the first transmembrane domain, there is a region of homology to the sea urchin egg jelly receptor and a consensus G-protein-coupled receptor proteolytic site (GPS site). The extracellular NH_2-terminal fragment (NTF) is cleaved at GPS site where the resulting COOH-terminal fragment (CTF) contains all the membrane spans and cytosolic tail. In addition, between the first and second transmembrane spans, there is a domain homologous to lipoxygenase (PLAT domain). The intracellular COOH-terminus contains a coiled-coil domain which mediates protein–protein interaction including the interaction with polycystin-2 (PC-2). Several potential phosphorylation sites are suggested within the COOH-terminus.

PC-1 expressed widely in vivo including kidney, brain, heart, and muscle. Subcellular expression of PC-1 is somewhat controversial as it has been detected in apical and basolateral membrane, tight junctions, adherens junctions, desmosomes, focal adhesions, and primary cilia, the key player for cystogenesis (further discussion see section "cilia and PKD"). Functions of PC-1 might be exerted through signal transduction including Wnt, JAK-STAT, AP-1, NFAT, and B-Raf/ERK-dependent pathway, ion-channel regulation, cell proliferation regulation, G-protein-coupled signaling, cellular adhesion properties, and transcription, however, precise function of PC-1 demand further investigation.

2. *PKD2*, the second gene responsible for ~15% of affected individuals of ADPKD, is located on chromosome 4 q21–23. *PKD2* gene contains 15 exons covering ~ 46 kb of genomic DNA and encodes a 5.3 kb mRNA transcript that is translated into an integral membrane protein polycystin-2 (PC-2). *PKD2* is approximately 25% homologous to a region of the *PKD1* gene. Unlike *PKD1*, *PKD2* gene is not duplicated on the chromosome. PC-2 is composed of 968 amino acids and has molecular weight of about 110,000 D. PC-2 contains six transmembrane spans and both intracellular NH_2- and COOH-termini. The transmembrane segments of PC-2 are about 50% identical to the last 6 transmembrane segments of the 11 transmembrane segments of PC-1. PC-2 is a nonselect cation channel sharing structural features with the transient receptor potential (TRP) channel family. The intracellular COOH-terminus of PC-2 contains a motif known as EF hand that can bind calcium, and an ER (endoplasmic reticulum) retention domain, deletion of which cause disruption of PC-2 localization in ER.

PC-2 is expressed widely in all tissues tested so far, including kidney, heart, brain, lung, pancreas, liver, testis, ovary, vascular smooth muscle, and intestine. At the subcellular level, located primarily in the ER, immuno-reactive PC-2 is also detected in basolateral plasma membranes, lamellopodia, mitotic spindles, in addition to the primary cilia (for further discussion see section "cilia and PKD"). PC-2 channel conducts divalent cations including calcium.

ARPKD

PKHD1, the gene responsible for ARPKD, is located on chromosome 6 p21.1-p12. *PKHD1* is very large and consists of at least 86 exons extending over 469 kb of genomic DNA on chromosome 6. The gene undergoes a complex pattern of alternative splicing to generate mRNA transcripts ranging in size from 8.5 kb to 13 kb. *PKHD1* gene is expressed in kidney with higher level and in liver and pancreas with lower level. Distribution of *PKHD1* mRNA transcript includes renal tubules, the bile ducts, blood vessels, testis, and dorsal root ganglia.

Polyductin (or Fibrocystin), encoded by *PKHD1* gene, is composed of 4074 amino acids and predicted to contain a large extracellular domain, a single transmembrane span, and a short intracellular COOH-terminus. Polyductin has molecular weigh of about 500,000 D and is expressed in cilia, cilia-associated structure basal bodies, and possibly in the plasma membrane. Functions of polyductin remain elusive.

Cilia and PKD

PKD is a human cilial disease. Cilia are microtubule-based organelles that project like antennae from the surface of almost every vertebrate cell. Ultrastructurally, cilia consist of membrane that is continuous with the cell plasma membrane but is a separate domain with unique complement of proteins and a central axoneme that is composed of microtubules. Cilia originate from the basal body, an intracellular organelle containing a pair of centrioles and surrounding matrix. There are two types of cilia: motile cilia and nonmotile primary cilia in the body. The axonemes of typical motile cilia contain nine peripheral bundles of microtubules and two central microtubules (9 + 2 pattern), whereas the axonemes of primary cilia contain nine peripheral bundles of microtubules (9 + 0 pattern). While

motile cilia move fluid past cells, nonmotile primary cilia transduce a multitude of sensory stimuli, including chemical concentrations of growth factors, hormones, odorants, and developmental morphogens, as well as osmolarity, light intensity, and fluid flow. Primary cilia have been identified in all segments of the nephron from Bowman's capsule to collecting ducts.

Abnormal structure and function of the renal primary cilium and its associated structures, i.e., centrosome and basal body, play key role in the pathogenesis of PKD. PC-1, PC-2, and polyducin are localized in primary cilia of renal epithelia. While structural disruption may be important in cystogenesis of ARPKD, dysfunction of the polycystins (PC-1 and PC-2)-functional-axis, rather than structural defect in cilia, may play a key role in cystogenesis in ADPKD.

Animal Models

Animal models for PKD have arisen through spontaneous mutations (such as *cpk, bpk, jck, kat, pcy,* and *Han:SPRD-cy*), chemical-induced mutagenesis (*jckp*), and gene-targeting approach (conventional or conditional knockout models for *Pkd1*, *Pkd2, and Pkhd1*). The pathology in these models resembles that seen in human PKD with regards to the localization, progression, and morphology of cysts, as well as involvement of the liver and pancreas. Homozygous of gene-targeting mouse models of *Pkd1, Pkd2*, and *Pkhd1* are embryonic lethal, however, an unique and most relevant mouse model that recapitulates ADPKD is the $Pkd2^{ws25}$ mouse line, which develop kidney and liver cysts postnatally. The $Pkd2^{ws25}$ allele was generated fortuitously by a targeting event that duplicated the first axon of *Pkd2*. The duplicated exon is able to undergo intragenic recombination in somatic cells at a relatively high frequency to yield either a wild-type or a mutant *Pkd2* locus. The spontaneous LOH (loss of heterozygosity) in the mouse carrying both the *Pkd2* null allele and a $Pkd2^{ws25}$ allele would result in focal cysts formation, which offer experimental evidence supporting the "two-hit" hypothesis of cysts formation in ADPKD.

Proteins encoded by genes responsible for the PKD animal models, including cystin (*cpk)*, polaris ($Tg737^{orpk}$), NPHP3 (*pcy*), Nek-8 (*jck*), Pc-1 (*Pkd1*), Pc-2 (*Pkd2*), and polyductin (*Pkhd1 and pck*), are localized in primary cilia. In addition, the proteins encoded by the *NPHP* gene family (*NPHP1–4*) and *BBS* gene family (*BBS1–8*), responsible for the nephronophthisis I–IV and Bardet–Biedl syndrome respectively, are localized in cilia or cilia-associated structure.

Nonmammalian PKD models have provided significant insights into cyst development. Most notable among these are the *Caenorhabditis elegans*, the *chlamydomonas,* and zebrafish. PKD-related genes in *C. elegans* including *lov-1* (*PKD1*), *pkd-2* (*PKD2*), *osm-5* ($Tg737^{orpk}$), *Nphp1*, and *Nphp4* are identified. Mutations in *lov-1* and *pkd-2* cause mating behavior defects associated with cilia-mediated sensory reception, whereas mutations in *osm-5* result in defects in cilia formation as seen in $Tg737^{orpk}$ mice. *Chlamydomonas* expresses many proteins that are mutated in murine and human PKD, including PC-2, polaris (IFT88), Kif3A (FLA10), and BBS proteins. Those proteins localize in flagella or cilia and their function is required for both assembly machinery such as intraflagella transport (IFT) and normal signaling activity of this organelle. In zebrafish, knock-down of zebrafish homologues such as *inv* and *Nek8* produce a distinctive renal cystic phenotype.

Therapy and Perspective

No specific treatment for PKD currently exists. Dialysis and kidney transplantation are the only effective treatments for the ESRD of PKD. Currently, therapeutic tests of V2R (Vesopressin-2 receptor) and mTOR (mammalian targets of rapamycin) inhibitors on animal models of PKD result in promising outcomes. Cyst fluid contains many hormonal activities, including antidiuretic hormone (ADH) and epidermal growth factor (EGF), as well as lipophilic substance, capable of stimulating the accumulation of cAMP (cyclic adenosine monophosphate). In the epithelial cells of distal tubules and collecting ducts, ADH activates the V2R, a $G\alpha_s$-coupled receptor linked to adenylate cyclase. PC-32160, a V2R antagonist, could prevent renal accumulation of cAMP and influences disease course in animal models of PKD including *PCK* rat (ARPKD), *pcy* mouse (adolescent nephronophthisis), $Pkd2^{ws25/-}$ (*Pkd2* gene-targeting mouse). OPC-41061, a structural analog of OPC-31260, also inhibits development of PKD in the *PCK* rats. Like OPC-31260, OPC-41061 significantly decreased renal cAMP levels, kidney weights, cyst and fibrosis volumes, mitotic and apoptotic indices. It also inhibits activation of Ras and ERK. None of those antagonists prevent development of fibropolycystic liver disease, consisting with the absence of V2R in liver.

mTOR, a key player in proliferation of activated T-lymphocytes, is a member of the phosphoinositide kinase-related kinases, and phosphorylates target protein such as S6 kinase and 4E binding protein on serine/threonine residue. Inhibition of mTOR by treatment of rapamycin was effective in reducing cyst and kidney volume in two models of recessive PKD, the $Tg737^{orpk}$ and the *bpk* mice. In addition, rapamycin was also effective in *pcy* mouse model even when started at a later time point. Reagents which have been successfully tested in different model systems are the EGFR tyrosine kinase inhibitor (Han:SPRD rat), caspase inhibitor (Han:SPRD rat), taxol (cpk mouse), methylprednisolon

(Han:SPRD rat), bicarbonate (Han:SPRD rat), and lovastin (Han:SPRD rat).

Development of renal cysts is the key phenotype of PKD. Maintenance of an intact, normal functional signaling pathway, mediated by cilia and its resident proteins polycystins, and polyductin, play a key role in preventing cyst formation. Enhanced understanding of the molecular mechanism regulating the polycysins and polyductin signaling would facilitate the intervention of effective targets which could restore the normal functional axis of polycystins and polyductin.

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Polycythemia

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Synonyms

Erythrocytosis

Definition

A condition in which the hemoglobin amount and the erythrocytes count are increased over the normal values.

Characteristics

The Greek term, polycythemia (literally, many cells in the blood) corresponds, in the clinical setting, to the word erythrocytosis. This condition exists when hemoglobin amount and the erythrocytes count are increased as the consequence of a build-up of the total volume of red cells.

It is possible to distinguish primary and secondary forms as well as congenital or acquired polycythemias.

Primary polycythemias show an enhanced capability of the erythroid progenitors to respond to normal levels of circulating cytokines as the consequence of genetic mutations (both germline inherited or somatically acquired) that cause an increased proliferation or a decreased apotoptosis of erythrocytes precursors. From a functional point of view, in these forms there is a dissociation between the total oxygen carrying ability (i.e. the total hemoglobin content) and the oxygen tissutal requirement or the status of oxygens-sensing pathways.

It is possible to identify forms of primary polycythemias both congenital and acquired.

The primary congenital and familial polycythemia is an autosomal dominant disorder characterized by low serum erythropoietin (Epo) levels, in vitro hypersensitivity of erythroid progenitors to Epo, normal leukocyte and platelet counts, vitamin B12 level, and bone marrow cytogenetics. A number of cases of primary congenital and familial polycythemias are due to mutations of the ►erythropoietin receptor gene. Particularly, the mutated receptors lacks C-terminal domains and show ineffectiveness of the mechanism(s) by which the receptor activation is shutted-down by inhibitory loop(s). This results in an excess of Epo stimulatory activity on erythroid precursors.

The commonest acquired form of primary erythrocytosis is the polycythemia vera (PV) also known as polycythemia rubra vera. In a frequent number of cases of PV, an excess of white blood cells and platelets, a low Epo level, and splenomegaly are also evidentiable. Thus, PV is classified as a myeloproliferative disease. This erythrocytosis rarely occurs in people younger than 20 years since it usually develops very slowly. The frequency is about 1–2 individuals affected over 100,000.

PV has been ascribed to a mutation of the gene encoding the kinase ►JAK2 (i.e. the change of the nucleotide G at the 1849 position into T that corresponds to the substitution of valine to phenylalanine at the residue 617 of the protein). About 90% of patients with PV has this mutation, but the precise percentage of them that shows the mutation in homozygosity or heterozygosity has not been definitely determined.

The JAK2 mutation results in a non synonymous amino acid substitution located in the JH2 pseudokinase auto-inhibitory domain. The change renders the enzyme constitutively active and leads to cytokine hypersensitivity, Epo-independence of erythroid precursor growth and erythrocytosis in a mouse model. Moreover, the constitutive JAK2 activation is accompanied by the enhancement of growth activating pathways (ERK) and antiapoptotic mechanisms (Akt).

Although the *JAK2*V617F mutation seems to explain some features of PV, it is becoming increasingly evident that the genetic change is not be the initial clonogenic event in PV. Indeed, the analysis of single colonies of erythroid precursors from PV patients suggests the existence of molecular changes distinct (but yet unknown) from *JAK2*V617F mutation.

In its early stages, PV usually doesn't cause any signs while as the disease progresses several symptoms might occur.

These include: headaches, weakness, dizziness (vertigo), and/or a ringing noise in the ear (tinnitus). In some cases, individuals with polycythemia vera experience itching (pruritis), especially after a hot bath. Affected individuals often have an abnormally enlarged spleen (splenomegaly) and/or liver (hepatomegaly). In some cases, patients may have associated conditions including high blood pressure (hypertension), the formation of blood clots (thrombosis), rupturing of and loss of blood (hemorrhaging) from certain blood vessels, and/or Budd-Chiari syndrome, a rare disorder characterized by obstruction (occlusion) of veins of the liver (hepatic veins).

Secondary polycythemias are characterized by a physiological responsiveness of bone marrow erythroid progenitors to cytokines and by an increased serum levels of factors driving erythropoiesis, including Epo, Insulin Growth Factor 1, cobalt, etc. Therefore, secondary polycythemias must be regarded both (i) as a normal response of bone marrow to oxygen requirements of tissues and (ii) as a mutation (germline or somatic) affecting the oxygen sensing pathway and its target (see above). In other words, in secondary polycythemias normal erythroid progenitors are stimulated by alteration in external factors.

Like primary, also secondary polycythemias might be distinguished in congenital and acquired forms.

Several congenital forms have been identified including high affinity hemoglobins due to globin gene mutations or ►bisphosphoglycerate (BPG) deficiency, congenital ►methemoglobinemia and cyanotic congenital heart or lung diseases. Particularly, cyanotic congenital heart disease represents an important cause of polycythemia in young children worldwide. In all these hereditary conditions exist a pathological low tissue oxygen tension that represents the initial event to activate mechanisms stimulating an increased erythropoietic activity.

So far, there are more than 100 mutations of hemoglobin leading to an increase in oxygen affinity and a reduction in oxygen delivery resulting in compensatory polycythemia. These mutations affect the cooperative binding between the globin subunits (α1/β2 interface) or interfere with the binding of BPG. These mutations are dominantly inherited and cause a benign polycythemia. *P*50 (partial pressure of oxygen where hemoglobin is 50% oxygenated) offers the best initial screening to detect high oxygen affinity accurately and can be estimated mathematically from venous blood gas measurements. BPG deficiency is a rare congenital red cell disorder and results from a deficiency of biphosphoglyceromutase (BPGM). BPG is present in very high concentrations in red blood cells. It binds hemoglobin and allosterically changes its configuration thereby modulating its ability to bind oxygen. A decreased BPG level shifts the oxygen dissociation curve of hemoglobin to the left (increases hemoglobin affinity for oxygen), resulting in decreased delivery of oxygen to the peripheral tissues and compensatory polycythemia. BPGM mutation should be considered in the setting of a low *P*50 and after globin gene mutations have been definitely excluded.

An important form of secondary congenital erythrocytosis, due to peculiar mutations of the von-Hippel Lindau (VHL) gene has also been recently identified.

The normal response to acute hypoxia (low oxygen pressure) includes hyperventilation, pulmonary vasoconstriction, systemic peripheral vasodilation, and tachycardia. The mechanisms by which these complex systemic responses are activated and modulated are still limited explained and, in some cases, remain elusive. Conversely, it is now clear that intracellular responses to hypoxia are coordinated by the hypoxia-inducible factor (HIF) family of transcription factors, which regulate (both directly or indirectly) the expression of numerous genes in any given cell type.

About these downstream target HIF genes, it appears important to cite *Epo*, *SLC2A1* (also known as ►*Glut-1*, encoding facilitated glucose transporter member 1 of solute carrier family 2), transferrin, transferrin receptor, vascular endothelial growth factor (VEGF) and a number of genes coding for enzymes of the anaerobic glucose metabolism. The VHL tumor suppressor protein is an essential component of the degradation pathway through which some members of HIF family are primarily regulated by oxygen pressure. Indeed, the HIF-alpha is synthesized continuously but is rapidly destroyed in the presence of O_2. Oxygen-dependent prolyl hydroxylases hydroxylate specific residues in HIF-1, increasing its affinity for VHL. The binding of VHL to hydroxylated HIF-alpha then targets the protein for destruction by the ubiquitin–proteasome pathway. Under hypoxic conditions, HIF-alpha hydroxylation is inhibited and thus there is rapid accumulation of the transcription factor and the up regulation of hypoxia-responsive genes, including Epo gene.

In the classical VHL-associated cancer syndrome, affected individuals are heterozygous for a germline VHL mutation that predisposes to specific tumors. Known clinical manifestations are confined to cancers and discrete benign lesions that arise following somatic inactivation of the second allele. However, alterations

of hypoxia-dependent pathways are limited to cancer cells and only rarely result in polycythemias.

Chuvash polycythaemia (CP) is a form of erythocytosis endemic in Chuvashia (a republic of the Russian Federation) where approximately several hundred cases are recognized among a population of about 1.5 million people. An additional cluster has been subsequently identified in Ischia island (Italy) and a number of cases in other region of world. CP patients are homozygous for 598C > T mutation in VHL gene that reduces but does not abolish HIF-alpha activity. The VHL mutation diminishes HIF-alpha degradation by pathologically upregulating HIF-alpha target genes, including Epo. CP is characterized by congenital erythrocytosis, but has yet to be extensively phenotyped. The CP is a non-benign hematological disease associated with lower peripheral blood pressures, higher estimated pulmonary artery pressures, varicose veins, vertebral hemangiomas, arterial and venous thrombosis, major bleeding episodes, cerebral vascular events and premature mortality due in part to cardiovascular and thrombotic events. Moreover, erythroid progenitors of CP patients are hypersensitive to Epo by a molecular mechanism totally obscure. This last property is, however, reminiscent of primary hereditary polycythemias, thus rendering, at least in part, uncertain the classification of CP as a primary or a secondary erythrocytosis.

Because CP is characterized by a germline mutation in the VHL gene, it has been hypothesized that homozygotes for this mutation might develop certain vascular tumors similar to those associated with the classic VHL syndrome. However, in no case, spinocerebellar hemangioblastomas, renal carcinomas, and pheochromocytomas typical of classical VHL tumor predisposition syndrome have been found, and no increased risk of cancer has been demonstrated. Through experiments conducted on CP patients, it has been demonstrated the potential role of the VHL pathway in cardiopulmonary physiology. In particular, patients with CP have an elevated basal ventilation and pulmonary vascular tone, with extremely high ventilatory, pulmonary vasoconstrictive, and heart rate responses to acute hypoxia. The abnormalities they displayed mimicked those caused by acclimatization to hypoxia at high altitude.

It has been estimated that the VHLC598T mutation arose in a single ancestor between 12,000 and 51,000 years ago. It is possible that the wide dissemination from the original founder may be associated with some survival advantages for heterozygotes carrying this mutation. Such an advantage might be related to a subtle improvement of iron metabolism, erythropoiesis, embryonic development, energy metabolism or some other yet unknown effect. An intriguing possibility is raised by the recent demonstration of a protective role for HIF-alpha in protecting against pre-eclampsia, the leading cause of maternal and fetal mortality worldwide. Another positive role of a mildly augmented hypoxic response is an improvement in the bactericidal action of neutrophils, as recently observed in HIF1alpha knock-in mice. Finally, other VHL mutations have been detected in either homozygotes or compound heterozygotes affected by secondary congenital polycythemias. It is tentative to classify these cases and Chuvash erythrocytosis as VHL-dependent polycythemias.

Although several genetic alterations responsible for secondary polycythemias have been identified, more than 50% of these erythrocytoses with normal or increased serum levels of Epo (and other cytokines), do not have a definite molecular basis. These cases include both diseases inherited with a recessive or a dominant fashion.

Secondary polycythemias due to acquired conditions include a large array of causes, all resulting in low peripheral pressure of oxygen. Among these, high altitude dwelling, chronic obstructive pulmonary disease, sleep apnea, cyanotic heart disease result in secondary polycythemia as a physiological adaptation.

In addition, kidney transplantation has polycythemia as a complication. The cause of this acquired secondary erythocytosis might be correlated to specific pharmacological treatments required during the post-transplantation period.

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Polycythemia Vera

Definition

PV, synonym primary polycythemia, a condition in which there is an overproduction of red blood cells in the body as a result of an abnormality of the bone marrow.

►Erythropoietin

Polygenic

Definition
A mode of inheritance characterized by a sporadic occurrence and variable intensity of manifestation of the phenotype. It is due to the segregation in the same individual of susceptibility/resistance alleles at multiple unlinked genetic loci.

►Modifier Loci

Polygenic Diseases

Definition
Are caused by a combination of environmental factors and mutations in multiple genes; each mutation has only a small contribution to the disease susceptibility. These include most of the common chronic diseases like obesity, hypertension, Alzheimer, diabetes, arthritis, and many cancers.

►Case–Control Association Study

Polymers

Definition
Are high molecular weight molecules consisting of a repeating chain of identical base units (called monomers) connected by covalent chemical bonds. Cationic non-biodegradable polymers enhance the internalization of therapeutic molecules by cells. Biodegradable polymers are used to achieve the controlled release of therapeutic molecules.

►Non-viral Vector for Cancer Therapy

Polymorphism

Definition
Literally means existing in a variety of different shapes. Genetic polymorphism is variability at a gene locus in which the variants occur at a frequency of greater than 1%. The major histocompatibility complex is (►MHC) the most polymorphic gene cluster known in humans.

►Linkage Disequilibrium
►Personalized Cancer Medicine
►Pharmacogenomics
►MDM Genes

Polymorphonuclear Leukocyte Elastase

►Neutrophil Elastase

Polyneuropathy

Definition
Malfunction of multiple peripheral nerves.

►Peripheral Neuropathy

Polypeptide

Definition
A chain of amino acids linked by peptide bonds, in the range of 10–100 amino acids; proteins are composed of much longer sequences of amino acids and their functions are determined additionally by the 3-dimensional structure.

►Gut Peptides
►Modular Transporters

Polyphenols

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Definition
Polyphenols are plant substances possessing more than one aromatic ring bearing one or more hydroxyl

P

groups, including their ►functional derivatives, and may occur as unconjugated aglycones or as conjugated with sugars, organic acids, amino acids or lipids. Examples of polyphenols are ►epigallocatechingallate, ►genistein, ►resveratrol, quercetin, rutin. Common dietary sources rich in polyphenols include tea, soybean, berries, chocolate, wine, apple and orange juices, black beans, tomato, sweet peppers, broccoli, onion.

Characteristics

Polyphenols consist of a family of diverse compounds, which comprises chalcones (butein), dihydrochalcones (tephropurpurin), flavanones (naringenin), flavones (apigenin), dihydroflavonols (engeletin), flavonols (quercetin), flavanols (catechins), ►isoflavones (genistein), proanthocyanidins (propelargonidins), and anthocyanidins (delphinidin). Their main physiological function is as antioxidants. Therefore, a long-term consumption of a diet rich in plant foods containing polyphenols may offer some protection against chronic diseases, including ►cancer. Thus fruits, vegetables, tea, red wine and cocoa consumption have been suggested to have the capacity to reduce cancer development. ►Flavonoids may also exert other effects unrelated to their ►antioxidant capacity, for example, ►anti-inflammatory effects and inhibition of tumorigenesis. Cancer has been associated with ►oxidative stress and mechanisms involving inflammation, aberrant signaling pathways and ►gap junction intercellular communication. It is possible that the presence in the diet of compounds with the capacity to ►scavenge free radicals like polyphenols may play a role in oncogenesis. While the free radical scavenging and antioxidant properties of phenolics are well established, emerging literature reports suggest that their chemopreventive effects may also be attributed to their ability to modulate components of cell signaling pathways.

Different polyphenols have different degrees of absorption in humans, however, it is believed that isoflavones are the best absorbed even though this parameter is influenced by the matrix of the diet and enhanced by a high fat diet. Studies in humans and animals have indicated that some polyphenols can be absorbed in the small intestine (5–10%), and most of them enter the circulation as methyl, sulfate and glucuronide conjugates; of these, only a very small amount (5–10%) enter the plasma as unchanged plant polyphenols. The 90–95% ingested total polyphenols are fermented in the colon and a variable portion of these (5–50%) is absorbed mainly as conjugates of microbial metabolites. It is clear that the major part of polyphenols consumed never reach the plasma and systemic circulation so the tissues most exposed are those of the oro-gastrointestinal tract. Animal studies have shown that diets rich in polyphenols that reach the colon may protect rodents from carcinogenesis. Inside the human body, flavonoids themselves are of little or no direct antioxidant value; however, inducing phase II enzymes can help in the elimination of mutagens and ►carcinogens and may be of value in cancer prevention. Polyphenols could also induce mechanisms that help kill cancer cells and inhibit tumor invasion.

Cellular and Molecular Studies

The biological mechanisms related to the chemopreventive activities of polyphenols are believed to occur by the regulation of signaling pathways such as ►nuclear factor-kappa B, ►activator protein-1 or mitogen-activated ►protein kinases. By modulating cell signaling pathways, polyphenols activate cell death signals and induce ►apoptosis in precancerous or malignant cells resulting in the inhibition of cancer development or ►progression. However, regulation of cell signaling pathways by dietary polyphenols can also lead to cell proliferation/survival or inflammatory responses due to increased expression of several genes. Dietary polyphenols can exert their effects on these pathways separately or sequentially and in addition the occurrence of crosstalk between these pathways can also take place. Polyphenols can also behave as detoxifying enzyme inducers, modulating gene expression including induction of phase II enzymes, such as glutathione S-transferases and quinone reductase, which usually leads to protection of cells/tissues against exogenous and/or endogenous carcinogenic intermediates. Phase II gene inducers also activate ►MAPK kinases that are involved in the transcription activation of antioxidant response element-mediated reporter gene.

Genistein, an isoflavonoid with ►phytoestrogenic properties, in animal models has shown to have a very complex effect on carcinogen-induced mammary cancer and great care is required in extrapolation of this information to human ►breast cancer. Some unconjugated isoflavones from fermented soybean and ►tamoxifen promoted an additive reduction in the number of mammary tumors in rats. Isoflavonoids have biphasic effects on the proliferation of breast cancer in culture; genistein at low concentrations can stimulate the growth of estrogen receptor-positive breast cancer cells but it does not stimulate the growth of ►estrogen receptor-negative breast cancer cells. Phytoestrogen responsive genes characterized from these cells can be used to clarify the role of isoflavones in cancer prevention. Of course many other mechanisms of action have been suggested for isoflavones and in particular for genistein. An important aspect of cancer risk is the involvement of the inflammatory response thus soy isoflavones may have potentially protective benefits at sites of inflammation due to their antioxidant action and could contribute to anticancer ability because ►reactive oxygen species could initiate signal transduction through the ►mitogen activated protein kinases.

Clinical Studies

Human studies are still contradictory and not final conclusions can be drawn on the effect of polyphenols and cancer. Flavonoids may be capable of exerting antioxidant effects in humans with the possibility of direct radical scavenging, down regulation of radical production, elimination of radical precursors such as hydrogen peroxide, metal chelation, inhibition of ►xanthine oxidase and of course elevation of endogenous antioxidants. Dietary polyphenols as health promoting dietary antioxidants have a broader mechanism of action than simple radical scavenging and radical suppression.

Studies with pre and postmenopausal women administered beverages and supplements with isoflavones gave no conclusive results on breast cancer risk factors and more studies are needed to clarify the effect of isoflavones and breast cancer in women. In cultures where the intake of soy is high and consequently dietary isoflavones, breast fed infants are exposed to high levels without adverse effects and it has been observed that early exposure may even protect against cancer. Safety and efficacy of isoflavones in humans is a topic that needs further investigation.

While experimental models have suggested that flavonoids attenuated cancer risk, epidemiological studies have failed to demonstrate a clear effect for tea, although there is moderate evidence for a slightly positive or no effect of black tea consumption on colorectal cancer. Studies on cancer have been limited by sample sizes and insufficient control of confounder factors.

In addition, caution must be exerted when polyphenols bypass the gastrointestinal tract (intravenous injections) or mega doses of these compounds (purified and presented in the form of tablets or capsules) are taken due to possible adverse effects such as nephrotoxicity.

The available evidence for tea polyphenols tentatively supports their advancement into phase III clinical intervention trials aimed at the prevention of progression of prostate intraepithelial neoplasia, ►leukoplakia or premalignant cervical disease. In the case of curcumin and soya isoflavones more studies in premalignacies seem appropriate to optimize the nature and design of suitable phase III trials. There is insufficient evidence from human research as yet to claim benefits of polyphenols in relation to cancer prevention. Epidemiological data that suggest tea consumption contributes to cancer prevention do exist, however, these failed to differentiate between green, black or oolong tea. Studies of colorectal cancer suggested either a slightly positive effect or null effect.

In conclusion, further mechanistic insights are needed as well as an accurate knowledge of the concentrations of the chemopreventive agents and their metabolites occurring in humans. Only small amounts of flavonoids may be necessary to see medical benefits. In terms of safety, consistent with the expectation that dietary constituents are harmless and well tolerated, unexpected cases of severe toxicity associated with the consumption of polyphenols have been rare. Severe adverse effects have only been reported for a very limited number of cases when consuming daily gram amounts of green tea polyphenols in the form of green tea extracts or when administering high doses of quercetin intravenously in cancer patients.

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Polyubiquitination

Definition

Is a process in which a chain of at least four ubiquitin peptides are attached to a lysine residue on a protein substrate, resulting in the degradation of the protein via proteasome.

►Ubiquitiuation

Polyunsaturated Fatty Acid

Definition

PUFA; An unsaturated fatty acid whose carbon chain contains more than one double bond; found chiefly in fish, corn, soybean oil and safflower oil.

►Melatonin
►Lipid Peroxidation
►Fatty Acid Transport

Population-based Cancer Research

►Cancer Epidemiology

Population Candidate Gene Association Study

►Case Control Association Study

Porfimer Sodium

Definition

►Photofrin

Porphyrin

Definition

Porphyrins (uroporphyrin I and III, coproporphyrkn I and III, protoporphyrin IX, and heme) are colored compounds within the heme biosynthetic pathway and mainly consist of four pyrrole rings with conjugated double bonds. Derivatives of porphyrins are used as photosensitizers in photodynamic therapy and ►fluorescence diagnostics.

►Photodynamic Therapy
►Diagnosis

Portal Hypertension

Definition

Portal venous pressure elevation resulting from intrahepatic or extrahepatic venous compression or occlusion.

►Acites

Positional Cloning

Definition

Cytogenetic and molecular approach to identifying a disease gene based on an identified abnormality in sequence or structure in a specific region of the genome.

Positive Nodes

Definition

►Lymph Node Metastasis.

Positive Predictive Value

Definition

The positive predictive value of a test indicates the proportion of target condition patients that test positive.

►Molecular Pathology

Positron Emission Tomography

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Synonyms

Hybrid positron emission tomography/computed tomography; Integrated positron emission tomography/computed tomography; Small animal positron emission tomography

Definition

Positron emission tomography (PET) is a non-invasive nuclear medical imaging modality enabling the visualization and quantification of biological processes. PET provides integral information regarding metabolic activity of the primary tumor and potential lymph node or distant organ metastases. PET can be used for cancer detection (►early detection), ►tumor staging and

restaging, assessment of response to treatment and anticancer drug development.

Characteristics

Principle of Positron Emission Tomography (PET)

PET allows non-invasive assessment of the three-dimensional distribution of a positron labeled compound within the living body. Positrons are anti-particles of electrons and originate from β+ decays of radioactive isotopes such as ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{68}Ga, ^{86}Y, or ^{124}I. During β+ decay a positron and a neutrino are emitted, both sharing a certain amount of kinetic energy. Once the positron is slowed down, a positronium consisting of a positron and an electron is created. The positronium has a very short half-life of 10^{-10} s and the masses of the positron and the electron are finally transferred into energy. This annihilation results in two gamma quants with an energy of 511 keV each. Decay events are detected by coincidence registration enabling the measurement of activity distribution in a specific transaxial section of the body. Activity distribution can be calculated from respective projections after correction for scatter, attenuation, dead time and random coincidences. Attenuation correction can be performed using a radioactive transmission source rotating around the patient. Emission and transmission scanning from scull to mid thigh usually takes 30–45 min, whole body-scans 60–90 min. The radiation dose of a standard PET examination is low with approximately 7.4 mSv and similar to spiral CT of the thorax.

Radiolabeled Biomarkers for PET Imaging Specifically Addressing Metabolic Pathways or Target Molecules

Depending on the clinical situation, various radiolabeled pharmaceuticals can be utilized for tumor imaging (Table 1). The most important biomarker for functional diagnosis of tumors is the glucose analog 2′-[^{18}F]-fluoro-2′-deoxy-D-glucose (FDG, ^{18}F-Fluorodeoxyglucose) (Fig. 1). Since conventional imaging modalities such as ►computed tomography (CT), ►magnetic resonance imaging or ultrasound detect malignant lesions because of characteristic morphological alterations, FDG-PET enables the diagnosis of malignant tumors due to an increased glucose metabolism in malignant cells. After intravenous administration, FDG is predominantly taken up by tumor cells. After enzymatic conversion of FDG to FDG-6-monophosphate by hexokinase, the metabolite can not be further metabolized resulting in an intracellular "trapping" of FDG (►metabolic trapping).

There are many other radiopharmaceuticals capable of assessing distinct pathophysiological processes (Table 1). As an example, radiolabeled nucleoside analogs such as 3′-deoxy-3′-[^{18}F]-fluorothymidine (►FLT, ^{18}F-fluorothymidine) can be used to non-invasively assess the proliferative activity of tumors. With the positron emitter ^{15}O, $H_2{}^{15}O$ can be synthetized and used for assessment of tumor blood flow. A variety of radiolabeled amino acids such as [^{11}C]-methionine (►MET, ^{11}C-methionine), [^{11}C]-leucine (LEU) or [^{18}F]-fluoro-ethyl-tyrosine (►FET, ^{18}F-fluoroethyltyrosine) can be used to evaluate transport rates of amino acids and/or protein biosynthesis. Imidazole derivatives such as [^{18}F]-misonidazole (FMISO) can be used to delineate hypoxic tissue areas of the tumor which is particularly useful for radiation treatment planning. Synthesis of phospholipids is increased in many neoplasms leading to increased uptake of [^{18}F]-choline and [^{11}C]-choline (►CHO, ^{11}C-choline). [^{68}Ga]-DOTATOC (►DOTATOC, [^{68}Ga]DOTATOC) specifically binds to somatostatin receptors and is therefore highly sensitive for detection of neuroendocrine tumors. [^{18}F]-galacto-RGD (►RGD, ^{18}F-Galacto-RGD) has a high affinity to the vitronectin receptor αvβ3 and can be used as potential surrogate marker of neoangiogenesis. These and many other radiopharmaceuticals specifically address metabolic pathways or bind to specific target structures and therefore enable molecular imaging of cancer. Specific radiotracers are especially helpful for evaluation of new drugs and early response assessment in cancer.

Clinical Applications of PET and PET/CT

The introduction of PET to clinical medicine has influenced the management of patients with cancer. In most industrialized countries, PET is now accepted as a both useful and economic diagnostic tool for characterization of indeterminate lesions, initial staging, restaging and assessment of response to therapy in a variety of cancers. Combination of a PET scanner with spiral computed tomography in a single examination (►PET/CT, Integrated positron emission tomography/computed tomography) allows integrated functional (PET) and morphologic (CT) imaging. Additionally to the results returned by individual modalities, coregistration of CT allows precise localization of PET lesions. The addition of PET to CT leads to an increase of sensitivity as well as specificity for tumor imaging. Moreover, CT data can be used for attenuation correction which leads to a significant reduction of scanning time making PET/CT more comfortable for the patient. A standard examination including the head, thorax, abdomen and pelvis can be performed within 25–30 min. Since it's introduction to clinical medicine in 2001, PET/CT represents the fastest growing imaging modality. The Centers of Medicare and Medicaid Services (CMS) approved a variety of clinical indications including staging and restaging of non-small cell ►lung cancer, esophageal (►esophageal cancer), colorectal (►colorectal cancer), breast (►breast cancer) and head and neck cancers (►oral squamous cell

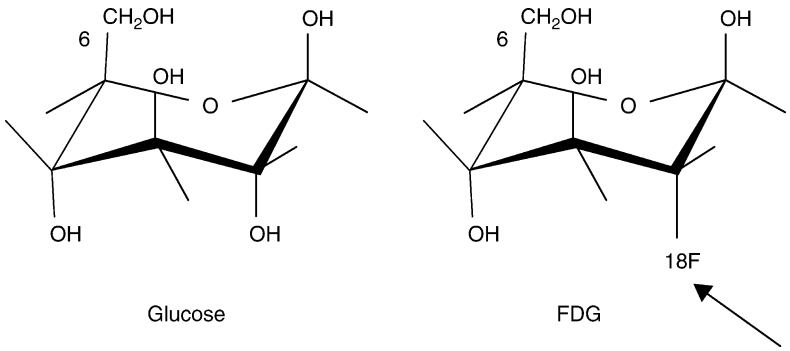
Positron Emission Tomography. Table 1 Radiopharmaceuticals (tracer) used for PET imaging (CUP, cancer of unknown primary)

Radiopharmaceutical (*under investigation)	Native molecule	Uptake mechanism in cancer	Clinical applications
18F-fluorodeoxy-glucose (FDG)	Glucose	Glucose transport/phosphorylation by hexokinase	Diagnosis, staging, restaging (e.g., cancer of the lung, breast, colon, rectum, thyroid, lymphoma melanoma, sarcoma); CUP monitoring of response to therapy (e.g., lymphoma, various cancer types including breast, GI tract, lung*)
11C-choline (CHO) 18F-fluorethylcholine (FEC) 18F-fluorocholine	Choline	Uptake by active transport and phosphorylation by choline kinase; incorporation into phospholipids (cellular membrane)	Staging, restaging (prostate cancer, bladder cancer*); therapy monitoring (prostate cancer*, bladder cancer*)
11C-acetate	Acetate	Lipid synthesis (key enzyme, fatty acid synthase, FASE)	Staging, restaging, monitoring response to therapy in a variety of cancers
68Ga-DOTATOC 68Ga-DOTATATE	Octreotide (somatostatin analog)	Binding to somatostatin receptors (predominantly SSTR-2)	Diagnosis, staging, restaging of neuroendocrine tumors, CUP (neuroendocrine)
18F-DOPA	Dihydroxy-phenylanaline	Uptake in tumors capable of DOPA decarboxylation	Diagnosis, staging, restaging of neuroendocrine and brain tumors, CUP (neuroendocrine)
18F-fluoro-ethyl-tyrosine (FET) 11C-methionine (MET)	Tyrosine Methionine	Amino acid transport, protein biosynthesis	Diagnosis, staging, restaging of brain tumors; differentiation of scar/recurrence, monitoring response in various tumor types
18F-galacto-RGD (RGD*)	Peptide containing the sequence RGD (arginine, glycine, aspartate)	Binding to integrin αvβ3 (vitronectin receptor), expressed on activated endothelial cells	Assessment of tumor angiogenesis (e.g., melanoma, sarcoma, head and neck cancer, breast cancer), monitoring response to anti-angiogenic treatment
18F-fluorothymidine (FLT*) 11C-thymidine (THY*)	Thymidine	DNA synthesis, tumor cell proliferation	Assessment of tumor proliferation (monitoring response to cytotoxic treatment in lymphoma, sarcoma, breast cancer)
18F-fluoroazamycine arabinoside (FAZA*) 18F-fluoromiso-nidazole (FMISO*)	Hypoxia markers (no biologic analog)	Passive diffusion into hypoxic cells; reactive intermediates are formed by intracellular nitroreductase and trapped within the cell	Assessment of tumor hypoxia (especially for use in tumors of the head and neck, radiation treatment planning)
11F-fluoro-17-β-estradiol (FES*)	Estradiol	Binding to estrogen receptors	Monitoring of response to antihormone treatment in breast cancer
18F-fluoride	Fluoride	Bone mineralization	Screening for bone metastases at staging or restaging

carcinoma, oral cancer), ►malignant lymphoma and ►melanoma. Monitoring response to treatment in breast cancer is also covered. Just recently, the CMS announced to provide widespread coverage of PET when respective examinations are part of prospective clinical trials.

Differentiation of Benign from Malignant Tumors and Detection of the Primary Tumor (Cancer of Unknown Primary)

Due to different glucose consumption of benign and malignant lesions, FDG-PET allows assessment of



Positron Emission Tomography. Figure 1 The glucose analog FDG (2′-[^{18}F]-fluoro-2′-deoxy-D-glucose) is the most widely used radiopharmaceutical for PET imaging. After i.v. injection, FDG is taken up by glucose transporters Glut-1 (cancer tissue, brain) and Glut-4 (skeletal muscle, heart) and is phosphorylated by hexokinase II. FDG-6-P is not a substrate for hexokinase phosphate isomerase which is the next enzyme of the glycolytic pathway. Consecutively, FDG accumulates in the cytoplasm (metabolic trapping). The figure shows the structure of the native glucose molecule (left) and the [^{18}F]-labeled analog (right).

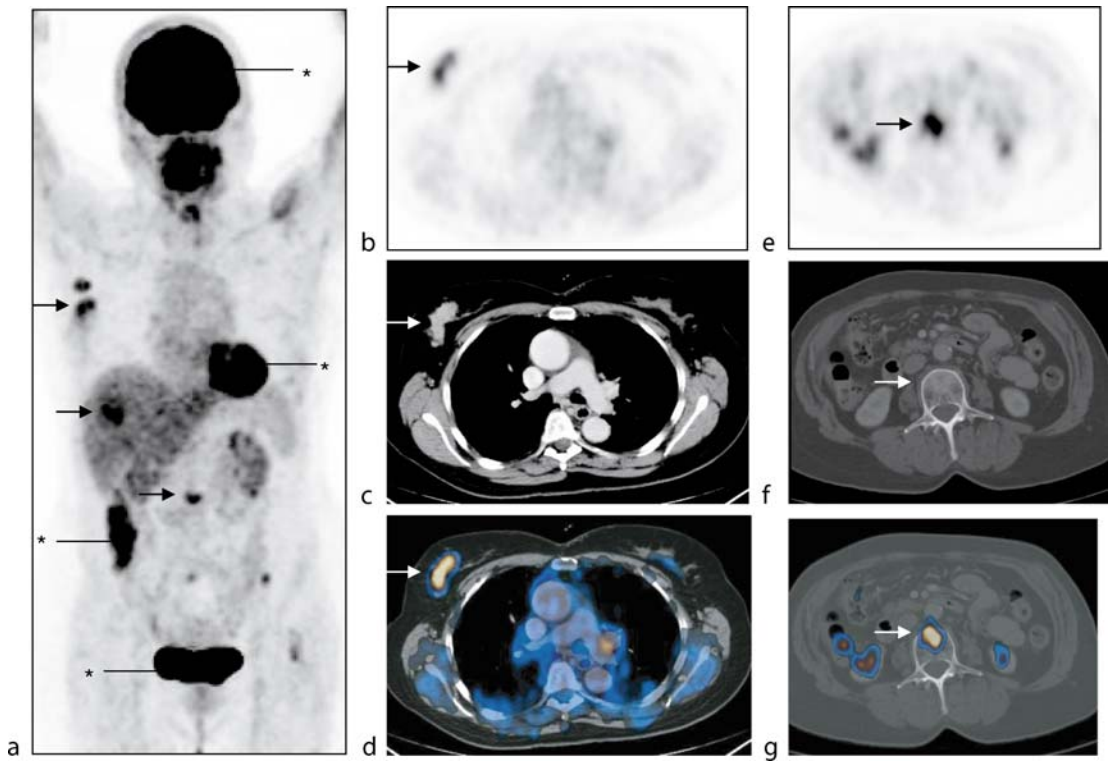
undefined tumors detected by conventional imaging modalities such as CT or MRI. Furthermore, PET sometimes allows detection of malignant lesions even when no or only minimal morphologic alterations are present. Regarding evaluation of indeterminate pulmonary nodules, prospective studies reported sensitivity values for FDG-PET between 89 and 100%, a specificity of 69–100% and an overall accuracy of 89–96%. FDG is not tumor specific leading also to non-specific tracer accumulation in benign, predominantly inflammatory lesions. However, surgery may be circumvented in patients with increased perioperative risk if the PET scan is negative. Dynamic data acquisition can further enhance the accuracy of PET imaging. In malignant lesions, a continuous increase of glucose uptake has been described, whereas benign lesions showed an increase of FDG-uptake followed by rapid efflux of FDG. Dual time point imaging or delayed PET imaging after 1 and 2 h contributes to better differentiate between benign and malignant tumors.

PET can also be used for detection of the malignant primary (►cancer of unknown primary, CUP). PET is especially useful in detecting primary tumors in the head and neck region. In case of increased cancer biomarkers (►clinical cancer biomarkers) or paraneoplastic syndromes, PET can aid in localizing the primary tumor manifestation site.

Staging of Cancer, Prognostic Potential of PET

For optimal treatment of patients with cancer, precise knowledge of the extent of the disease is crucial (tumor staging). If cancer is detected at a stage in which uncontrolled growth of tumor cells takes place but no tumor manifestations are present in distant organs, surgery is usually performed to obtain ultimate cure. However, if the tumor has already spread to distant organs, cure can usually not be achieved by surgery alone (Fig. 2). In this situation, surgery has to be replaced or supported by systemic chemo- and/or radiotherapy to entirely destroy the primary tumor and metastatic sites or to induce growth arrest in the tumor. In this context, PET has several advantages compared to conventional imaging modalities. Small tumor manifestation sites such as ►metastases in the bone, liver, lung, adrenal gland or in rare locations such as soft tissues, thyroid or (sub-) cutaneous lesions can be detected. However, micrometastases or single tumor cells can also not be detected with PET. Also, small lung metastases may appear negative at FDG-PET. In principal, staging of all tumors is possible. With the standard radiotracer FDG, PET is highly accurate for staging of non-small cell ►lung cancer, thyroid cancer, tumors of the head and neck region (►oral squamous cell carcinoma, ►oral cancer), ►colon cancer and ►esophageal cancer, ►malignant lymphoma, sarcoma (►osteosarcoma, ►Ewing sarcoma, ►chondrosarcoma), and ►melanoma. PET has been demonstrated to cause a change in patient management in 15–40% depending on the type of cancer. Some tumors present without increased glucose consumption such as prostate or neuroendocrine cancer. ^{11}C-Choline PET and ^{11}C-Choline PET/CT have been demonstrated to be highly accurate for staging and especially restaging of prostate cancer. ^{68}Ga-DOTATOC is a new PET-tracer for imaging neuroendocrine tumors. A variety of molecular probes have been evaluated to address biologic targets or metabolic pathways in vivo (Table 1). In the majority of these compounds, clinical utility remains to be determined.

The most important prognostic factor (►Prognosis, prognostic factor) is the tumor stage at initial presentation. However, risk stratification according to the



Positron Emission Tomography. Figure 2 (a) FDG-PET/CT in beast cancer (maximum intensity projection); malignoma-associated intense FDG-uptake in primary breast cancer, lymph node metastases in the axilla, liver metastases and bone metastases (arrows). (b) Transaxial PET section showing intense FDG-uptake of the primary tumor. (c) Transaxial section of helical CT indicates the anatomic correlate of primary breast cancer. (d) Fused image. (e) Transaxial PET section, high FDG-uptake in a bone metastasis of the vertebral column. (f) Transaxial section of helical CT indicates a sclerotic lesion corresponding to the PET lesion. (g) Fused image. Intense physiologic uptake of FDG in the brain, the heart, and intestines (*).

TNM-system is also subject to error, because patients with limited disease undergoing definite therapy may also develop recurrent disease. Other factors such as tumor aggressiveness or metabolic activity of tumors may aid in individual risk assessment. Several studies have correlated the intensity of FDG-uptake in the primary tumor to progression free and overall survival in various cancers. In lung cancer, intensity of FDG-uptake turned out to be an independent prognostic marker. The prognostic potential of PET has also been described for colorectal cancer, breast cancer and malignant lymphoma.

Assessment of Response to Therapy

Therapeutic efficiency of chemo- and radiotherapeutic strategies varies significantly between individual patients. Therefore, non-invasive assessment of the performance of a therapeutic protocol in an individual patient is highly desirable. With conventional imaging modalities such as CT or MRI, response (►radiological response criteria) to therapy can be detected as early as a reduction in tumor size occurs. On the contrary, PET allows assessment of response to treatment at an earlier time point before tumor shrinking can be detected by conventional imaging. In responding tumors, metabolism of tumor cells is markedly decreased due to the cytotoxic effect of the respective therapeutic regimen. Concomitantly, accumulation of FDG is reduced. This is a sign of an efficient treatment and has a high prognostic value regarding the success of further treatment. In case of a non-responding tumor, the therapeutic regimen can be altered by changing the combination of cytotoxic drugs or the radiation dose. In ►breast cancer, rapid decline of FDG-uptake already after one cycle of chemotherapy was demonstrated, whereas in non-responding tumors increasing or unchanged FDG-uptake was described. A variety of other neoplasms including ►malignant lymphoma, ►gastric cancer and ►esophageal cancer, head and neck (►oral squamous cell carcinoma, ►oral cancer) or non-small cell ►lung cancer showed rapid reduction of FDG-uptake in responding tumors. Significantly better disease-free and overall survival was described in responders compared to tumors without

significant reduction of tumoral FDG-uptake (Fig. 3). Clinical studies are needed reporting on the clinical benefit of a PET-guided change of patient management.

Restaging of Cancer, Detection of Recurrence

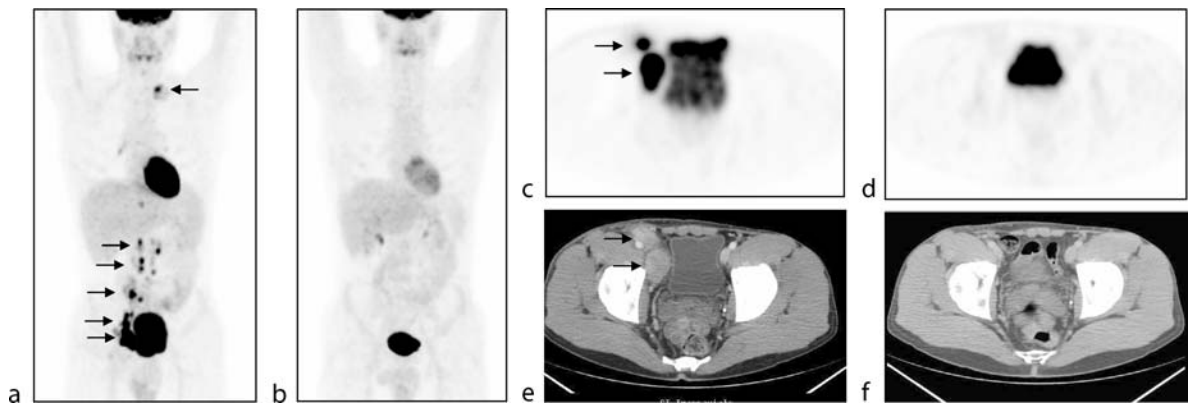
After definite surgery or chemo/radiotherapy, examinations and imaging at follow-up is important to early detect disease recurrence originating from residual tumor cells. In daily clinical practice, differentiation between scar tissue and vital tumor tissue is a frequent problem. At anatomically based imaging modalities, both are present as indeterminate tissue formation and, frequently, biopsy is needed for further clarification. Differentiation of scar tissue from vital tumor tissue is a prerequisite of PET imaging. While new onset of cancer tissue is associated with increased metabolism causing increased uptake of, e.g. FDG, scar tissue is frequently associated with reduced metabolism compared to surrounding normal tissue. PET is especially useful in the follow-up of tumor entities such as colorectal (►colorectal cancer) and ►esophageal cancer, non-small cell ►lung cancer, ►breast cancer, tumors of the head and neck (►oral squamous cell carcinoma, oral cancer), brain tumors (►malignant brain tumors), ►melanoma and ►malignant lymphoma. Restaging with PET is also approved for differentiated thyroid carcinoma with a negative ^{131}I whole-body scan and elevated tumor marker thyroglobulin.

Radiation Treatment Planning

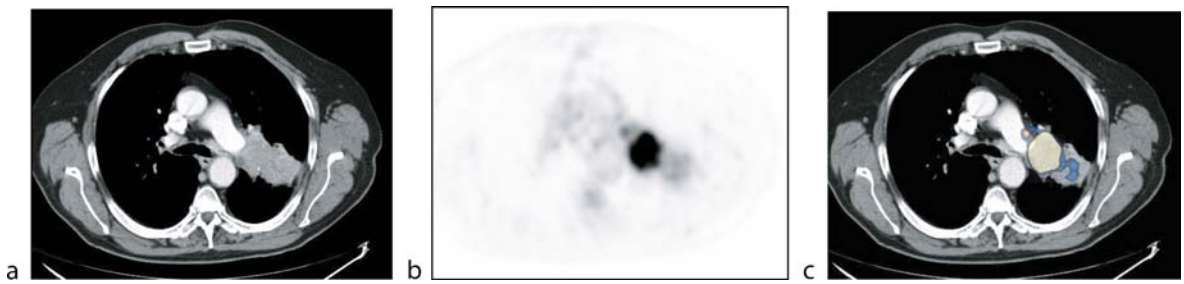
The use of metabolic information leads to biological target volumes which can have substantial impact on radiation treatment planning (►radiation oncology) by increasing or reducing the target volume. The additional identification of tumor manifestation sites which are not visible at conventional staging causes an enlargement of respective target volume. On the other hand, the radiation field can be reduced when non-malignant lesions such as atelectatic tissue can be reliably characterized as benign (Fig. 4). Consecutively, radiation dose to surrounding normal tissue can be reduced. The use of PET for radiation treatment planning leads to a change of the target volume in up to 60% of patients. This is in part related to pretherapeutic detection of distant metastases, previously unknown metastases in locoregional lymph nodes or characterization of suspicious lesions as benign. However, PET-based radiotherapy planning is not trivial. Especially the delineation of the primary tumor is subject to a relevant interobserver variability. There is a need for standardized evaluation criteria of PET allowing also the quantification of metabolic changes. The recent introduction of PET/CT hybrid scanners has lead to a reduction of errors concerning image coregistration. In several prospective studies it was shown that overall survival of patients receiving PET-guided radiation therapy was significantly longer compared to patients receiving standard treatment. Prospective randomized studies have to be performed demonstrating that the use of PET positively affects patient outcome and overall survival.

PET for Anticancer Drug Development

PET imaging has unique properties for use in anticancer drug development. Therapeutic efficiency of a novel drug can be evaluated non-invasively by assessment of specific biologic endpoints such as changes in cellular proliferation (e.g., by the use of [^{18}F]FLT), glucose utilization ([^{18}F]FDG), tissue perfusion ([^{15}O]H_2O), metabolism of amino acids ([^{18}F]FET, [^{11}C]MET),



Positron Emission Tomography. Figure 3 (a) FDG-PET for response assessment in malignant lymphoma (maximum intensity projection), intense FDG-uptake of multiple lesions of high-grade non-Hodgkin's B-cell lymphoma (arrows indicate lymphoma manifestations in the left supraclavicular region, in paraaortic, parailiac and inguinal lymph nodes. (b) Corresponding PET image of the same patient 3 weeks after completion of 8 cycles of chemotherapy with R-CHOP. No pathologic FDG-uptake in residual lymph nodes indicates complete remission of the disease and favorable outcome. (c–f) Corresponding transaxial sections of PET and CT.



Positron Emission Tomography. Figure 4 FDG-PET/CT for radiation treatment planning of nonsmall cell lung cancer. (a) Transaxial section of spiral CT shows a central tumor in the left lung which can not be discriminated from adjacent atelectatic tissue. (b) Corresponding section of FDG-PET indicates high metabolic activity of the malignant primary but anatomic landmarks are missing preventing precise tumor localization. (c) Fused PET/CT image allows exact delineation of the tumor which can be distinguished from adjacent atelectatic tissue.

or inhibition of ►angiogenesis ([^{18}F]galacto-RGD). (Over-) expression of the therapeutic target such as thymidylate synthase, VEGF receptor, ErbB2 or ►estrogen receptor status can be quantified with [^{11}C] thymidine, radiolabeled antibodies specifically binding to VEGF or ErbB2, or ^{18}F-fluoro-17-β-estradiol, respectively. Assessing biologic endpoints further provides proof of principle of the proposed mechanism of action. PET can also be utilized for in vivo-evaluation of gene expression, e.g. by the use of the substrate [^{124}I] fluoro-5-iodo-1-β-D-arabinofuranosyluracil (FIAU) for detection of Herpes simplex virus thymidine kinase type 1- or Na[^{124}I] for detection of sodium iodide symporter expression.

Generic endpoints can also be studied by PET. Drugs or biochemical probes can be labeled with positron emitters such as small molecules, proteins, or antibodies. Drugs which have been evaluated so far include ^{18}F-fluorouracil, ^{18}F-tamoxifen or ^{13}N-cisplatin. Pharmacokinetics of a drug can be investigated in tumors and normal tissues, in animal models or as part of clinical phase I (or phase II) studies. In the future, PET will be increasingly used to assess the efficiency of novel anticancer drugs.

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Post Surgical Systemic Therapy

►Adjuvant Chemoendocrine Therapy

Postcode Prescribing

Definition

A consequence of local decision making within the United Kingdom's National Health Service that results in patients having differential access to treatments according to their place of residence.

►National Institute for Health and Clinical Excellence

Postirradiation Sarcoma

►Radiation-Induced Sarcomas After Radiotherapy

Postischemic Reperfusion

Definition

The restoration of blood flow to an organ or tissue. After a heart attack, an immediate goal is to quickly open blocked arteries and reperfuse the heart muscles.

►Amine Oxidases

Postlabeling

Definition

32_P-postlabeling is a sensitive method for the detection and quantification of adducts to DNA. DNA is extracted in microgram quantities from tissue samples and enzymatically digested to mononucleotides. These are labeled with radioactive phosphorus (32_P) and the resulting 32_P-labeled adducts are separated from normal nucleotides by two-directional thin layer chromatography. Quantification can proceed by autoradiography, and identification is possible by co-chromatography with authentic samples.

►Biomarkers

Postnatal Stem Cells

►Adult Stem Cells

Postreplication Repair

►DNA-Damage Tolerance

Posttranslational Modification

Definition

PTM is the chemical modifications of proteins following their translations. After syntheses, the proteins are subjected to reversible or permanent modifications. They are one of the later steps in protein biosyntheses for many proteins. To identify the specific sites of modifications in the analysis of enzymatically digested proteins, tandem mass spectrometry with collision-induced dissociation of peptides can be used. More than 300 PTMs have been identified, such as phosphorylation, ►glycosylation, ►ubiquitination, deamidation, proteolytic processing, fatty acylation, and glycosylphosphatidylinositol lipid anchor attachment. Analysis of a protein for PTMs is very important for understanding issues such as activity, stability, interaction, and turnover.

►Proteinchip
►Histone Modification

Pou Transcription Factors

Definition

(Acronym for Pit, Oct and Unc); Pou transcription factors are a family of proteins with a conserved region of 150 amino acids that comprise a DNA binding/regulatory region. This DNA binding domain recognizes the octamer ATGCAAAT in specific target genes for the purpose of regulating transcription during development.

►Stem Cell Markers

Pox Viruses

Definition

Enveloped DNA viruses that can cause pox diseases in vertebrates.

►Semaphorin

pp60c-Src

►Src

pp60$^{v\text{-}Src}$

►Src

PPARγ

Definition
Peroxisome proliferator-activated receptor-γ that is associated with cancer expression.

►Conjugated Linolenic Acids
►Fucoxanthin

PPARs

Definition
Peroxisome proliferator-activated receptors; A group of nuclear receptor isoforms that exist across biology. Originally identified in *Xenopus* frogs as receptors that induce the proliferation of peroxisomes in cells, they are intimately connected to cellular metabolism (carbohydrate, lipid, and protein) and cell differentiation. They function as transcription factors. PPAR-γ forms heterodimers with retinoid X receptors and is believed to be involved in adipocyte differentiation.

►Arachidonic Acid Pathway
►Fatty Acid Synthase
►Peroxisome Proliferator-Activated Receptor

ppb

Definition
Parts per billion, ratio to determine the molecular presence of a particular substance per billion parts in relation to others.

►Benzene and Leukemia

ppm

Definition
Parts per million, ratio to determine the molecular presence of a particular substance per million parts in relation to others.

►Benzene and Leukemia

PPNAD

Definition
Primary pigmented nodular adrenocortical disease.

pPNET

Definition
Peripheral primitive neuroectodermal tumor as opposed to the unrelated central ►primitive neuroectodermal tumor (PNET) mainly comprised by ►brain tumors; synonym for ►Ewing sarcoma with at least two neural markers expressed on the cell surface or with presence of ►Homer-Wright rossettes in the tumor.

PR

Definition
►Progestin receptor.

►Adjuvant Chemoendocrine Therapy

pRb

Definition
►Retinoblastoma Protein; Is the product of a tumor suppressor gene, which is inactivated in ►retinoblastoma

and various other tumor types. pRb inhibits G1/S cell cycle progression by interacting with transcription factors, such as ►E2F, to block transcription of growth regulating genes; ►retinoblastoma protein, biological and clinical functions, retinoblastoma protein, cellular biochemistry.

Pre-invasive Breast Cancer

►Ductal Carcinoma In Situ

Pre-mRNA

Definition

Precursor mRNA, an RNA that has not been processed and contains all of its introns and exons.

►pre-mRNA Splicing

Pre-mRNA Splicing

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Definition

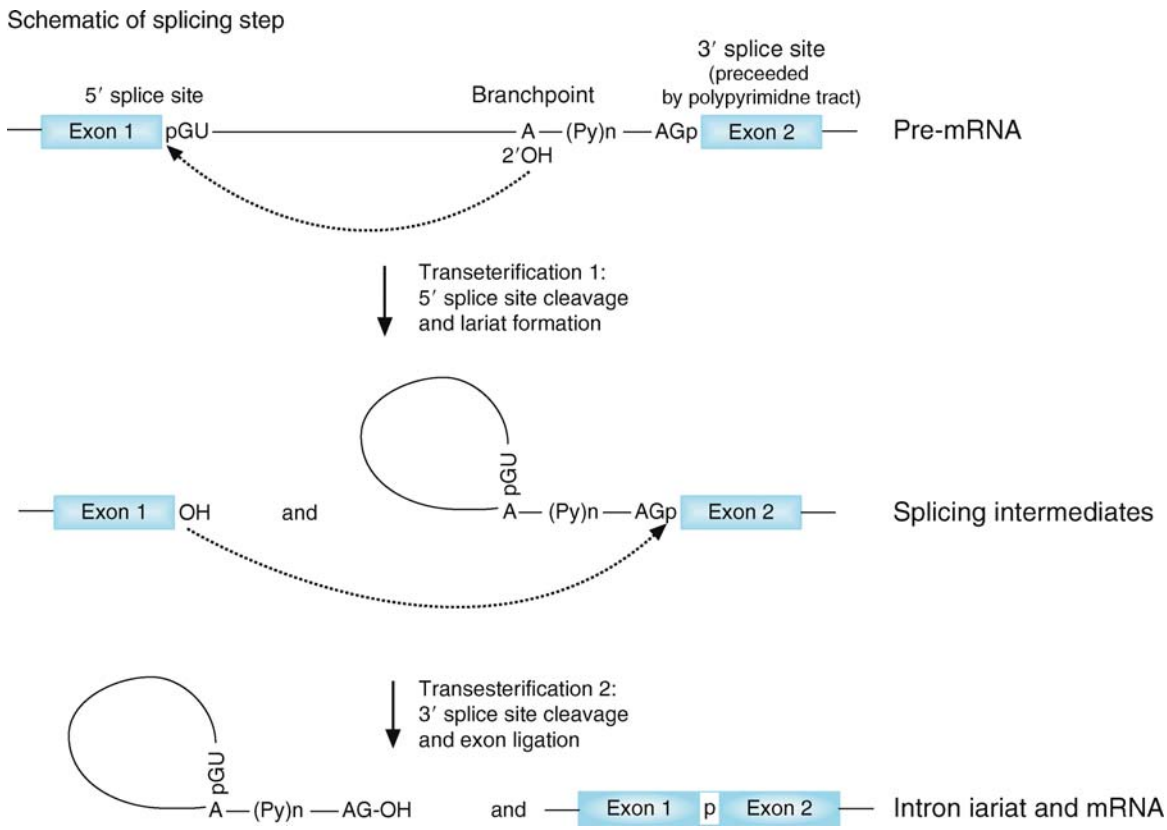
Splicing is a tightly regulated mechanism for the control of gene expression that involves the precise removal of ►introns from the precursor mRNA molecule and the subsequent ligation of the remaining ►exons.

Characteristics

Premature RNAs that are transcribed within the cell nucleus contain both coding sequences (contained within functional units called exons) and non-coding sequences that are eventually excised. Splicing is the mechanism by which the non-coding portions of the RNA (also known as introns) are removed from ►pre-mRNAs, via two cleavage-ligation reactions, each involving transesterification at a splice site phosphate (see Fig. 1). Ultimately, the two exons are ligated to generate the spliced mRNA and the excised intron is released in a lariat configuration that is eventually degraded. The splicing reaction has been well-defined and is mediated by the dynamic ordered assembly of numerous ►spliceosome components directly on the pre-mRNA. To avoid the production of nonfunctional proteins, it is essential that splicing occurs precisely and consistently as introns that are removed incorrectly or not at all can cause prematurely truncated proteins resulting from in frame stop-codons located in the intron or in proteins translated out of frame. Splicing is therefore, by necessity, an extremely accurate and specific reaction.

The way in which regulation of splicing is achieved is a complex intersection of distinct recognition sequences for binding of the splicing machinery and accessory molecules with the proteins and ►snRNP complexes that bind to these elements. Since exons are separated by introns and can be literally thousands of base-pairs away, the splicing machinery acts to recognize the splice sites and to remodel the RNA such that the splice sites are juxtaposed. In the first step of splicing the splicing components U2AF and U1snRNP bind to the 3′ and 5′ splice sites respectively. These proteins, along with accessory molecules known as ►SR proteins that help to stabilize the reaction, form the splicing commitment complex. Differential splicing regulation can be achieved by the relative concentration of these accessory molecules in the nucleus and by the expression of proteins that enhance or inhibit the splicing reaction. Subsequent steps involve the binding of the U2snRNP to the branchpoint and the U4/U5/U6 tri-snRNP complex remodeling the RNA and catalyzing the splicing reaction.

Several systems have taken advantage of the precise nature of the splicing reaction and have implemented it as a mechanism for regulation of gene expression and function. Alternative RNA splicing is the process by which mRNAs encoding several distinct proteins are produced from one single pre-mRNA sequence by use of differential splice site choices. There is a large degree of diversity in the ways that cells use alternative splicing as a mechanism for gene regulation (see Fig. 2). Exons can encode discrete functional domains and thus exons that are differentially included may dramatically alter the protein function. The inclusion or exclusion of exons may also change the reading frame of the RNA and thus regulate protein function by affecting whether or not an RNA with an intact open reading frame is produced. Because of the versatility of splicing regulation alternative splicing is prevalent in several developmental processes including muscle development, neurogenesis, meiosis, and spermatogenesis.



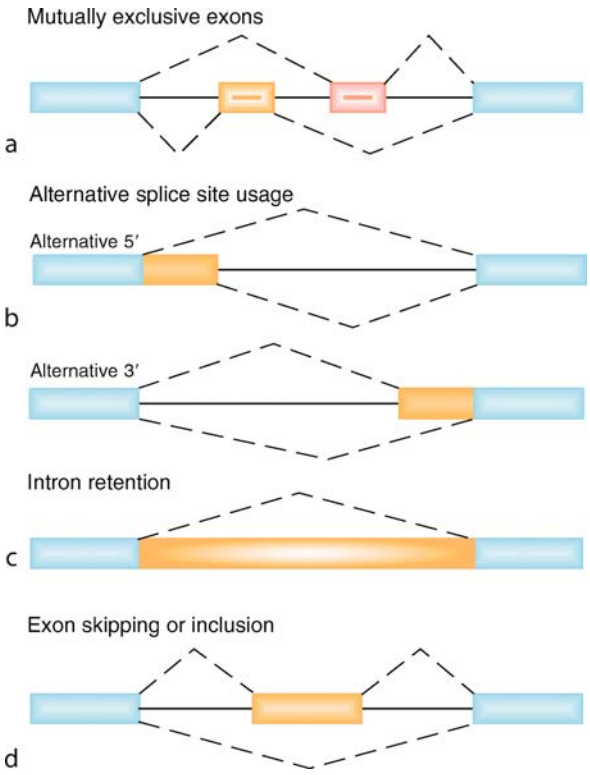
Pre-mRNA Splicing. Figure 1 The catalytic steps of pre-mRNA splicing. The required elements of the pre-mRNA are shown schematically. The exons are depicted as boxes and the intron as a line. The conserved 3′ and 5′ splice site sequences are depicted along with the relevant phosphate groups (p). The polypyrimidine tract $(Py)_n$ and the branchpoint (A) are also shown. The dashed arrows signify the hydroxl group attack of the splice site phosphate. The first transesterification reaction produces two splicing intermediates, the free 5′ exon and the intron/3′ exon in a lariat configuration. The second transesterification reaction results in the ligation of the two exons and the release of the intron lariat that is degraded. The resultant ligated exons make up the mRNA that eventually gets transcribed into protein. Figure adapted from Ref [1].

However, disruption of the developmental regulation or alteration in appropriate RNA splicing can lead to incorrect expression of alternatively or aberrantly spliced isoforms and can lead to disease.

Spliced Forms in Cancer

For each of the alternative splicing patterns there exists multiple examples that exhibit cancer specific expression. An example of each is discussed below. Mutually exclusive splicing of exons (Fig. 2a) occurs such that only one of a group of adjacent exons is included in an RNA at any one time. This type of splicing regulation is common for control of RNAs that encode proteins with defined tissue-specific functions. In the case of the c-Jun amino-terminal kinase 2 (►JNK2, ►JNK subfamily and ►cancer) that is known to phosphorylate the c-Jun protoconcgene, there is a well-defined tissue specific splicing pattern. In neuronal cells, exon 6A is included and 6B excluded, while in non-neuronal cells, 6B is included in the absence of exon 6A.

There are several examples of alternate splice site usage (Fig. 2b) for genes that are associated with cancer. The ►KLF6 tumor suppressor gene produces oncogenic variants due to alternative 5′ splice site selection. These splice variants are over-expressed in prostate cancer and seems to negatively affect the tumor suppressor activity of its normally spliced counterpart. In another example, the telomerase reverse transcriptase, TERT, utilizes alternative splicing of a 3′ splice site to generate TERT alpha. TERT is activated in most human cancers and is overexpressed in high grade astrocytic tumors. The alpha TERT isoform, that results from the use of a downstream 3′ splice site, lacks twelve amino acids and is deficient for telomerase activity. TERT alpha is furthermore suspected to cause apoptosis and thus provide cancer protection. In these



Pre-mRNA Splicing. Figure 2 Examples of possible alternative splicing patterns. Splicing choices are shown schematically with exons depicted as boxes and introns depicted as lines. A single pre-mRNA may employ multiple of these alternative choices in a combinatorial manner and thus greatly increase the diversity of proteins encoded. Figure adapted from Refs. [2] and [3].

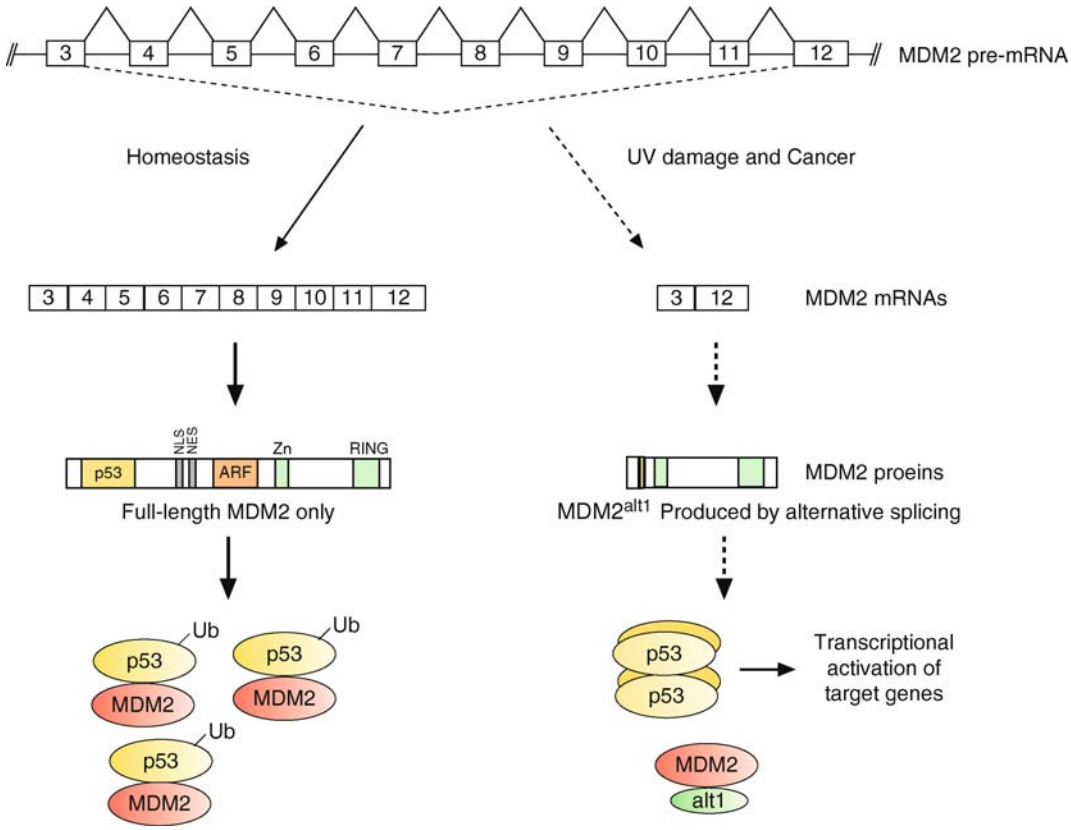
two cases of splicing regulation in cancer, the proteins that result from alternative splicing appear to have antagonistic functions to their full-length counterparts, one form promotes tumors and the other protects against cancer.

Intron retention (Fig. 2c) is yet another mechanism of regulating the splicing and thus function of genes that are involved in cancer. Intron retention occurs when the 5′ and/or 3′ splice sites are not recognized by the splicing machinery and the intron is therefore not removed from the transcript. A gene that is thought to regulate invasion and metastasis, CCK2, is altered in various cancers by retention of an intron that encodes a large intracellular loop as part of its transmembrane domain. The splice variant promotes growth via interaction with the ►Src tyrosine kinase.

The misregulation of splicing can occur via two general mechanisms. The first way is via point mutations that affect the cis-regulatory elements important for splicing control. Point mutations that create or destroy splicing signals within the RNA itself can affect splicing and are a prevalent way to destroy RNA splicing in a number of different cancers. In one example, the breast cancer susceptibility gene, ►BRCA1 (►BRCA1/BRCA2 germline mutations and ►breast cancer risk), is spliced incorrectly and the resultant truncated BRCA1 protein leads to breast cancer. In this case, a single nucleotide mutation was identified in exon 18 of BRCA1. This point mutation disrupts an ►exonic splicing enhancer (ESE) that is known to bind to the SR protein SF2/ASF. This disruption leads to the inability of exon 18 to be recognized by the splicing machinery and the exon is skipped as in Fig. 2d. The resultant mRNA is in a different reading frame than the normally spliced RNA and a truncated protein is produced. This example underscores the importance of regulatory element sequence fidelity for efficient splicing and is an important example of a cancer-causing point mutations that leads to a splicing deficiency.

The second way to express incorrect splice forms is through the altered expression or function of splicing trans-regulators. Changes in expression levels, phosphorylation state and sub-cellular localization of splicing accessory molecules are all known to be ways of regulating specific splice site choices. In the case of the ►*Mdm2* (or *Hdm2* in humans, ►MDM genes), the modulator of tumor suppressor p53 activity, splicing is regulated in normal cells as a response to stress. The resultant splice form is also an example of exon inclusion (normally) versus exon skipping (in stressed cells). As shown in Fig. 3, the internal eight exons are skipped (exons 4–11) making this an extreme example of exon skipping. Although the mechanism of MDM2 splicing regulation is still unknown, it is thought to be a result of transfactor control since this represents a normal cellular process that is reversible once the stress is removed.

The MDM2 protein derived from the alternatively spliced form has been shown to bind to the full-length MDM2 and interfere with its ability to bind to and regulate p53 (see Fig. 3). In this way, expression of MDM2alt1 facilitates upregulation of p53 activity and promotes the damage response playing an important role in tumor suppression. However, this alternatively spliced form of MDM2 is contradictorily found to be overexpressed in a number of human cancers including gliomas, rhabdomyosarcomas, breast and ovarian cancers. Although the expression of the MDM2 alternatively spliced form is predicted to suppress tumors by activating the trp53 tumor suppressor pathway, its prevalent expression in multiple tumor types has led to much speculation about a possible role of MDM2 splicing in tumor formation. One prevailing hypothesis for the role or MDM2alt in tumorigenesis is a cancer progression model in which the expression of the alternative form of MDM2



Pre-mRNA Splicing. Figure 3 Alternative splicing of MDM2. The MDM2 pre-mRNA is shown schematically; exons are depicted as boxes and introns as lines. Under normal conditions the MDM2 is spliced to include all exons and encodes the full-length protein as depicted in the left part of the figure. Under stress conditions and in certain cancers, MDM2 alternative splicing causes skipping of exons four through eleven and results in the expression of a novel MDM2 protein that lacks the p53 binding domain, the nuclear localization and export signals (NLS and NES), and the ARF binding domain (as shown in the right part of the figure). The short MDM2 protein negatively regulates its respective full-length counterpart and ultimately activates the p53 pathway. This example of regulated splicing uncovers a novel mechanism by which cellular injury can control distribution and activity of p53 within the cell and possibly lead to cancer.

initially protects the cells by activating trp53, but sustained expression of the MDM2 form and upregulated trp53 puts a selective pressure on the cells that results ultimately in mutations in the p53 gene itself or other genes in the pathway. It is these secondary mutation(s), then, that induce the cancer phenotype. This predicted model mirrors a similar situation in which perpetual ►*myc (Myc oncogene)* expression likewise induces trp53 providing cancer protection initially, but secondary mutations in genes of the trp53 pathway lead to cancer, in the end. Although experimental proof for the exact role the MDM2 alternatively spliced form plays in the initiation of cancer is lacking, the expression of alternatively spliced forms of MDM2 is clearly a marker for many different tumor types.

In summary, the expression of many alternatively spliced isoforms is associated with cancer. In many cases, the new proteins formed are ant-apoptotic or growth-promoting lending clear clues to their roles in tumor formation. In other cases, the role of the alternative forms in tumorigenesis is more elusive.

Therapeutic Intervention

Since a number of tumor specific isoforms have been identified that block apoptosis and/or promote cell growth or invasion, modification of the splicing profile in tumors is a possible therapeutic intervention point. There have been several approaches that have been successfully utilized to change splice site choices in a variety of human diseases. Low molecular weight drugs such as neomycin, aclarubicin, and sodium butyrate have been successfully utilized to promote exon inclusion. Likewise, heterologous expression of tran-acting splicing factors can alter splicing patterns of their target genes. These types of therapies must be carefully tested for non-specific effects as they have the potential

to affect the splicing of many genes in addition to the targeted disease-causing gene. This danger of non-specificity may be avoided by designing selective agents that only recognize the RNA in question. Antisense oligonucleotides and RNAi technology have been shown to be effective in recognizing specific sequences in the target RNA and successfully modulating splicing. As more becomes known about the splicing of genes and the roles of this process cancer, there is great promise for modulating gene expression by directing splice choices that encode tumor-suppressor proteins and squelch the tumor-promoting alternatively spliced isoforms.

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Pre-replicative Complex

Definition

Is a complex of proteins, originally identified by in vivo footprinting, present at replication origins only during late mitosis and G1.

►Replication Licensing System

Precancerous Lesions

Definition

During the process of transformation, cells will accumulate mutations in genes in a step wise fashion. At some point, the cell may proliferate resulting in growth, but does not yet have the capacity to invade surrounding tissues. At this point the cell(s) are considered to be a benign growth or a precancerous lesion. Adenomas in colon cancer are representative of this state.

►Colorectal Premalignant Lesions
►Preneoplastic Lesions

Preclinical Drug Safety Evaluation

►Preclinical Testing

Preclinical Imaging

Definition

Imaging of live small-animal disease models (most commonly mice or rats) for biomedical research. The term usually refers to an instrument analogous to a clinical diagnostic imaging technology such as x-ray computed tomography, magnetic resonance imaging, radionuclide imaging, or ultrasound that has been designed to resolve structures smaller than 200 μm. That resolution permits the anatomy of a mouse to be visualized at a level of detail equivalent to a clinical image of a human patient.

►Ultrasound Micro-Imaging

Preclinical Safety Testing

►Preclinical Testing

Preclinical Studies

Definition

Describe the phase of drug discovery before the first clinical studies start in man.

►ADMET Screen

Preclinical Testing

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Synonyms

Toxicity testing; Preclinical safety testing; Preclinical drug safety evaluation

Definitions

The process of testing potential new therapies in animal and cell-based test systems prior to their study in patients in order to assure their potential safety and efficacy.

Characteristics

It was recognized many years ago that potential medicines were often "poisons" so that some form of screening in animal models was required before new drugs could be tried in patients. For all types of medicines it has been generally agreed that these tests should take the form of both pharmacological and toxicological studies in animals to avoid patients being exposed to drugs that are excessively toxic or without evidence of potential efficacy. Added impetus has come from the Nuremberg Code that was formulated after the trials of the Nazi doctors convicted for the conduct of horrific medical experiments on prisoners. Whilst this code deals primarily with the consent of volunteers and patients, one of its articles places emphasis on the justification for any experiments in humans being based on prior information derived from animal experiments:

"*The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study so that the anticipated result will justify the performance of the experiment.*"

Hence, the main concept underpinning the preclinical safety testing is the protection of volunteers and patients in the testing of new drugs. The basic paradigm for testing is similar for all drug types and falls into three main phases. Firstly, toxicity testing is often used in the discovery phase to select the least toxic candidate drug from a series of chemicals. Secondly and most importantly it is used to provide the basic safety data to permit first dosing of a novel agent to volunteers. Finally, further detailed testing using repeated dosing schedules over longer periods and specialist protocols is conducted in parallel with ongoing clinical trials to complete the preclinical safety data. This permits extended dosing of patients and supports eventual marketing of the drug (Fig.1). Although cytotoxic anticancer drugs represent a special case often needing only modest preclinical testing programmes, it needs to be kept in mind that not all drugs used in the therapy of cancer are conventional cytotoxic drugs. Some of these such as those used to manage pain, nausea or vomiting are developed in a manner similar to non anticancer drugs and consequently often have preclinical testing programme analogous to novel drugs intended for long-term use.

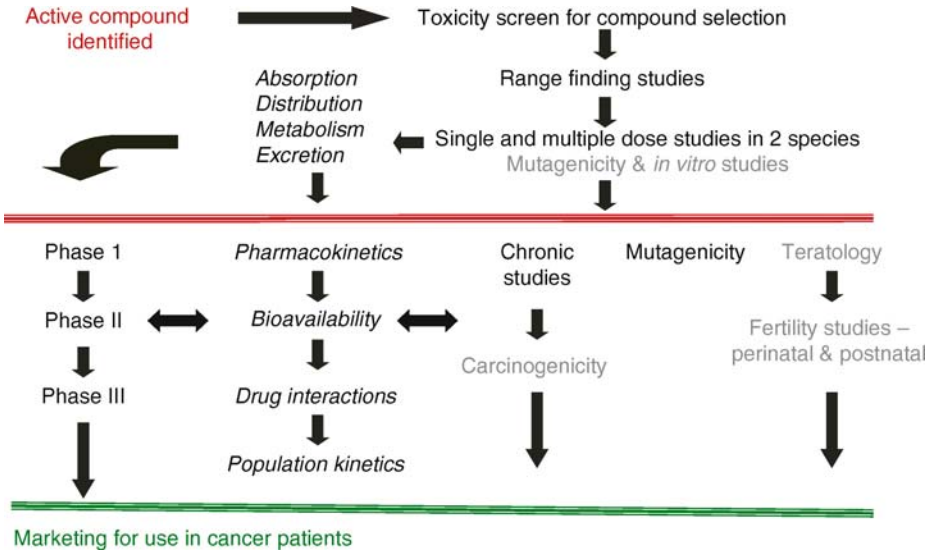
The use of preclinical safety testing is primarily screening for serious drug toxicity to permit their safe testing in humans. They are no substitute for careful study of new drugs in patients to test their ability to treat disease and for monitoring of adverse effects. Adverse effects occurring in one out of 10,000 or 100,000 patients can be devastating but would never be detected in the small number of healthy animals used in safety testing.

Discovery Phase

Basic toxicity testing has an important place in the selection of a potential new anticancer therapy, despite application of a vast array of novel approaches using molecular biology, combinatorial chemistry and bioinformatics. The activity of anticancer drugs is typically assessed using a battery of ►mouse models for different cancer types. At this stage, informal studies, so called ►screening toxicity using small numbers of rodents, often mice, might be conducted. This is often performed when series of potential drugs cause toxicity in particular organs such as the liver and kidney to enable selection of the least toxic for progression to the next phase of formal testing prior to administration to humans. Depending on the nature of the toxicity, these studies usually employ simple endpoints – clinical observation, examination of blood values and microscopic examination of important organs.

Phase I Clinical Study

Following identification of a potentially active new drug, the next major step is testing for effects in humans. However, the transition from the laboratory bench to the bedside represents a large step for which preclinical studies are crucial. At this stage, a small number of carefully planned and conducted animal toxicity and safety pharmacology experiments are performed. Typically, two laboratory animal species are used for each new drug. One of these is a rodent usually rat and the other a non rodent usually dog. These allow the precise characterization of the effects activity of the



Preclinical Testing. Figure 1 This diagram shows the place of preclinical safety studies within the context of drug discovery and development. So called screening toxicology might be conducted in rodents prior to entry into formal "single dose" and "repeat dose" toxicology studies immediately before testing in humans (red line). In parallel with the conduct of clinical phases I, II and III further testing including "chronic" (6 months or more duration) toxicity and reproductive studies are carried out prior to marketing (green line). Italics show drug kinetics and metabolism studies – performed in *both* humans and animals and enables effects in animals and humans to be correlated. Cancer drugs usually do not require the testing marked in grey print, although these are needed for drugs of other types intended for long-term use.

drug on body functions and any cellular toxicity. They provide the basis for the design, conduct and safety monitoring of clinical phase I studies of the new drug in volunteers. Whilst healthy volunteers may be studied for cancer drugs that are not cytotoxic, cytotoxic agents are usually studied in volunteer cancer patients.

The paradigm for a toxicity experiment is similar for most types of studies. Animals are dosed drug by the similar route to that intended for use in patients. This is usually done at a clinically relevant dose and usually two higher doses on a body weight or surface area basis. The minimum number of animals of each sex in each dose group is mandated in most government guidelines, notably those of the European Union, United States and Japan. These studies are conducted to an agreed international laboratory standard known as ►Good Laboratory Practice (GLP). This requires regular auditing of studies as well as inspection of laboratories by government agencies.

In all these toxicology studies the animals are monitored clinically with particular attention being paid to changes in animal behavior that could signify adverse effects on critical functions such as the nervous system. Blood pressure, heart rate and electrocardiograms are usually monitored in the dog as they are more difficult to perform in rodents that have very fast heart rates. Eyes are also carefully examined using sophisticated optical equipment similar to that employed by an ophthalmologist or optician. Blood is sampled and tested for any biochemical or hematological alterations in a similar manner to human patients, often using identical laboratory analytical apparatus. Levels of drug circulating in the body are also usually monitored in these experiments, often referred to as ►toxicokinetics.

At the end of the dosing period, or if any ill health intervenes, the animals are humanely killed using an anesthetic or barbiturate and subject to a full autopsy examination. Over 30 tissues from all the organs are taken for microscopic examination by an experienced ►toxicological pathologist. This is to check for any organ damage that could reflect danger to patients if they were to be treated with the new drug. Organs such as the liver, kidneys, ovaries and testes are particularly important, as are the lymph nodes, thymus and spleen for any deleterious effect on the immune system. Additional special studies are performed if there are findings of concern that need elucidation. As many drugs, particularly cancer drugs are locally irritant to tissues, the gastrointestinal tract is examined closely if

the oral route is employed. Likewise, injection sites are also studied microscopically if a parenteral route is used. Severe local irritancy might preclude dosing to humans or would dictate particular caution in use.

These studies are designed in a particular way for cytotoxic cancer drugs because these are liable to damage rapidly dividing cells such as those in the bone marrow and gastrointestinal tract. For these drugs it has been shown that the ►maximum tolerated dose (MTD) is often similar in mouse, rat, dog, monkey and man when compared on the basis of mg/m^2 of body surface area. Experience has shown that a safe starting dose in humans is one tenth of the MTD based on ►single dose toxicity studies. This means that a key study for cytotoxic drugs is a single dose toxicity study that has sufficient number of animals per dose to establish an MTD. These single dose studies usually have a follow up period of clinical examination of the animals for at least 14 days after dosing to exclude delayed toxic effects. Usually two animal species, one rodent and one non rodent are used to confirm the MTD. This study is required by ►government drug regulatory authorities prior to testing cytotoxic cancer drugs in cancer patient volunteers.

►Repeat dose toxicity studies are also performed with cytotoxic drugs prior to phase I clinical studies. These are designed to mirror the proposed clinical schedule with particular attention being paid to organ toxicity and reversibility of toxic effects. These are of limited duration usually 2–4 weeks or one or two cycles of treatment in two species.

However, increasingly many new anticancer drugs are not cytotoxic because they act through other mechanisms such as modulation of hormones or growth factors. These are tested in a more conventional manner similar to most other drugs intended for long-term use. Here, toxicity experiments comprise single dose studies in rodents and repeated dosing over a period of up to 1 month in two animal species. One species is a rodent, usually laboratory rat, and the other a non-rodent species, usually the beagle dog. Experiments in monkeys are avoided where possible unless the compound being studied has particular pharmacodynamic or metabolic characteristics that demand it.

Yet another variation on the basic preclinical testing scheme is used for biological products. Again the aim is to mirror the clinical treatment schedule. However, as some biological agents do not possess activity in non-primate species, monkey studies are sometimes needed to assure safety in humans. Immunogenic potential is also thoroughly investigated in specialized animal models.

At this stage determining the safety profile of a new drug is not limited to animal toxicity experiments. There are also *in vitro* ►genotoxicity studies, such as the Ames test which uses bacteria and other experiments using cells to study whether the drug can damage DNA. A standard battery of both *in vitro* and *in vivo* genotoxicity experiments is conducted prior to phase II clinical studies for conventional drugs (►Micronucleus assay).

A particularly important component of preclinical study is the investigation of absorption, distribution, metabolism and excretion of drug and their metabolites in the blood and tissues of animal species chosen for toxicity testing. This data helps interpretation of the relevance of adverse findings in animals for humans because it forms a point of comparison of drug handling between animals and humans. It also helps to validate the relevance of the data obtained in animals (►Pharmacokinetics/pharmacodynamics).

At the end of this phase of work scientists and physicians review the assembled data from all these experiments along with information from studies of drug metabolism prior to design and conduct of first studies in humans. In all cases a clear rational based on a trade-off between any potential risks with likely long-term benefit would be generated prior to the conduct of any human experiment. Moreover, there is a mandatory ethical review of the study protocol by a panel not directly involved in the human studies. The review panel would be provided with a summary of the results of the animal safety information. Government agencies in the United States and in the European Union have approval processes for all such human studies.

Phase II and III Clinical Studies

In clinical studies in patients, dosing periods are usually lengthened and more subjects are involved. These are accompanied by animal toxicology studies of longer duration and when appropriate, other specialist studies. The organization of these toxicity studies is similar to the earlier studies but the period of dosing is extended from a period of up to 1 month to period of dosing of 6 or 9 months or 1 year for most drugs. For anticancer drugs studies are usually conducted with continuous or intermittent dosing for a period equal to the duration of clinical trials although not longer than 6 months.

Further drug kinetics and metabolism studies are conducted at this stage as data from humans is available and enables comparison between species. This also serves to validate the preclinical data. Metabolites may be isolated and their toxicity studied if there is particular concern about the adverse effects of metabolites. Enzyme induction potential may also be tested in animals or *in vitro* hepatocyte test systems (Pharmacokinetics/pharmacodynamics).

Although for most drugs special experiments to examine the effects of a new drug on reproductive function and the developing foetus (►reproductive toxicity studies) are required in to be conducted in two sensitive animal species, conventionally rat and rabbit. However for most cytotoxic anticancer drugs these experiments are not usually performed.

Although *in vitro* ►genotoxicity tests are not required for anticancer drugs prior to phase I and phase II clinical trials, tests such as the Ames test which uses bacteria and other experiments using cells to study whether the drug can damage DNA are usually expected prior to clinical phase III trials and marketing applications. A full battery of *in vitro* and *in vivo* genotoxicity tests is required before phase II for more conventional drugs (Micronucleus assay).

The last preclinical experiments that are usually conducted for most drugs are the so called ►carcinogenicity studies. Although these are a requirement for most drugs that are going to be used for extended periods in patients, they are not performed for many anticancer drugs. Exceptions exist for drugs such as the selective estrogen modulating drugs such as tamoxifen that are used for extended periods and for cancer prophylaxis The studies have typically been mandated in two rodent species, mouse and rat. Again the organization of the studies is similar to those conducted previously except that the dosing period is for 2 years – most of the lifetime of a rodent (►Carcinogenesis or ►toxicological carcinogenesis). More recently, shorter studies using genetically modified, cancer-prone mice have been accepted by ►government drug regulatory agencies in place of one of these two large studies (Mouse models).

At the end of this process, the preclinical information is summarized along with information from clinical trials and manufacturing data and assembled for submission to government drug regulatory authorities to obtain marketing authorization.

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Predictive Biomarkers

Definition

Biomarkers to predict whether the drug and other therapies will be effective, or to monitor the effectiveness of treatment; ►early detection.

►Oncopeptidomics

Prednisone

Definition

Is a synthetic steroid with potent antiinflammatory and immunosuppressive activity used in treating acute graft rejection, autoimmune disease, and lymphoid tumors.

Preleukemia

►Myelodysplastic Syndromes

Premature Menopause

Definition

Menopause before the age of 40 years.

►Menopausal Symptoms After Breast Cancer Therapy

Preneoplastic Changes

Definition

Are phenotypical changes in cells that follow a malignization pathway but are not yet irreversibly malignized.

Preneoplastic Lesions

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Definition

The development of primary tumors is often preceded, both in humans and experimental animals (mainly

rodents), by the appearance of lesions referred to as preneoplastic. These consist of genetically and phenotypically altered cells exhibiting a higher risk of malignant evolution than normal cells. These lesions generally lack one of the principal characteristics of neoplastic lesions: the capacity to grow autonomously after cessation of the stimuli that induced the lesion. Nonetheless, the distinction between preneoplastic lesions and benign neoplasias is sometimes difficult, and the terms "preneoplastic" and "premalignant" are often considered synonyms. However, benign tumors, constituted by autonomously growing cells, cannot be strictly classified as preneoplastic but only as premalignant lesions, whereas, premalignant lesions can include both preneoplastic lesions and benign tumors.

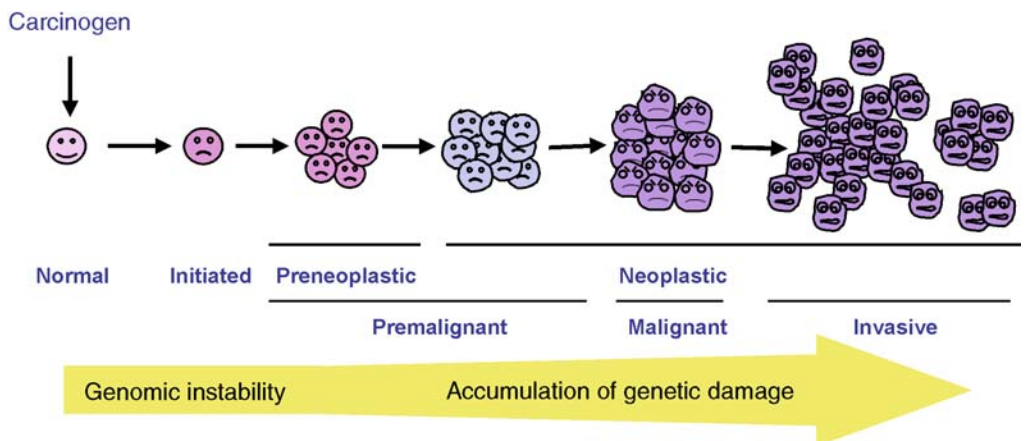
Characteristics

Tumorigenesis is considered a multistep process characterized, both in humans and rodents, by the progressive development of preneoplastic, premalignant, and malignant lesions. During this process, genomic instability occurs (►Microsatellite instability), followed by alterations in oncogenes, oncosuppressor genes, and DNA repair genes, with consequent changes in the ►signal transduction network (Fig. 1). The progressive accumulation of genetic changes generates autonomously growing premalignant and malignant lesions.

Some Experimental Models of Preneoplastic Lesions

Preneoplastic lesions have been induced in different organs, including liver, pancreas, lung, colon, skin, thyroid, mammary gland, gall bladder, prostate, in rodents treated with carcinogens and in transgenic mice. Although a close correspondence of experimental and human preneoplastic lesions does not always occur, the study of experimental lesions allowed discovery of pathogenetic mechanisms and diagnostic and prognostic markers of different tumor types. Only lesions preceding tumor development in some organs (liver, colon, lung) will be given as examples.

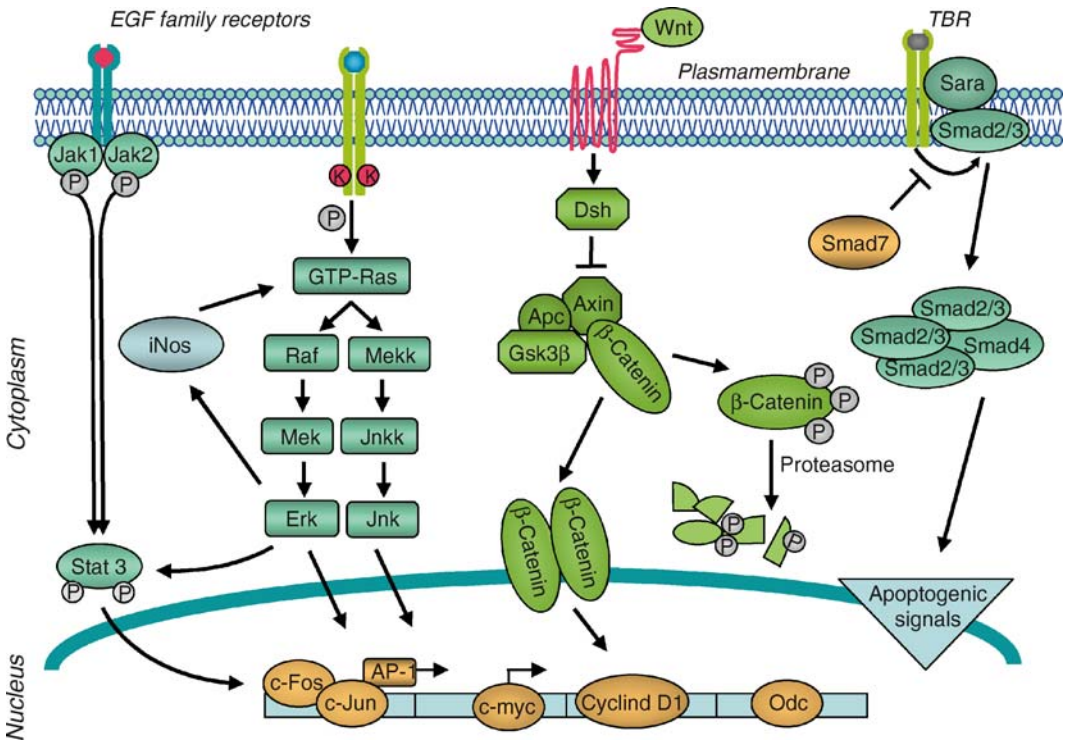
In hepatocarcinogenesis rodent models, irreversibly initiated cells by a carcinogenic stimulus can undergo clonal expansion by overresponse to the administration of promoting agents, such as phenobarbital, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, chlorinated hydrocarbons, peroxisome proliferators, for several weeks. Only few initiated cells undergo clonal expansion under promoting stimuli, probably because of irreversible change and apoptosis, giving rise to a heterogeneous population of foci of altered hepatocytes (FAH) expressing specific patterns of marker genes, such as Glutathione S-transferase (GST) 7-7, γ-Glutamyl transpeptidase, and alterations of carbohydrate metabolism defining different lineages of preneoplastic cells. When the treatment with promoters is suspended before the appearance of neoplastic lesions, early preneoplastic liver lesions partially disappear by a process called "►remodeling." GST 7-7 positive cells that acquire autonomous growth persist and further evolve to premalignant neoplastic (persistent, atypical, dysplastic) nodules and then to well-differentiated ►hepatocellular carcinomas (HCCs; ►Liver Cancer, Molecular Biology), which progress to moderately and poorly differentiated carcinomas. Other



Preneoplastic Lesions. Figure 1 Schematic representation of multistage carcinogenesis. Carcinogenesis initiation is associated with the appearance of genomic instability. Clonal expansion of initiated cells leads to the development of preneoplastic lesions carrying various genomic alterations. The accumulation of these alterations is associated with the acquisition of the capacity of autonomous growth and evolution to neoplastic lesion, which progress to moderately differentiated and poorly differentiated, invasive, carcinomas.

hepatocarcinogenesis models are based on the stable transfection of one or two cancer related genes (i.e., c-*myc*, c-*myc* plus *Tgf*-α genes) in mouse and, less frequently, rat genome, or in inactivating a gene of intact mice (knockout mice). In these models, generally the liver is dysplastic and progressively becomes adenomatous before the development of HCCs.

Alterations of several signal transduction pathways occur in rodent HCC and, at a lower extent, in preneoplastic liver lesions. They include upregulation of *EGF* receptor family, resulting in induction of the Ras-dependent activation [►Ras] of Mitogen activated protein kinases (MAPK, ►Map Kinase) and *JAK-Stat* signaling, both transducing extracellular signals resulting in phosphorylation of transcription factors targeting various genes (Fig. 2). c-Raf/MAP kinase kinase-extracellular signal-related kinases (Mek-Erk) pathway is primarily responsible for responding to cellular proliferation signals, whereas the Mitogen activated protein kinase kinase (Mekk) and c-Jun N-terminal kinases (JNK; ►JNK Subfamily and Cancer) respond to cellular stress signals and treatment with carcinogens. This leads, trough upregulation of the c-*fos* and c-*Jun* genes, to the activation of AP-1 and its targets (i.e., *Cyclin D1* gene; ►Cyclin D). As a consequence *Odc*, a c-Myc (►Myc Oncogene) target, is overexpressed and its gene product, Ornithine decarboxylase, activates polyamine synthesis required for nucleotide biosynthesis. Upregulation of c-*myc*, cyclin D1, and other Cyclins and Cyclin-dependent kinases, activates cell cycle and cell proliferation. This is favored by downregulation of cell cycle inhibitors,



Preneoplastic Lesions. Figure 2 Schematic representation of signal transduction pathways involving *Jak/Stat*, Ras/Mapk, Wnt/β-Catenin, and Tgf-β1 signaling pathways. Binding to ligands or autologous upregulation of Epidermal growth factor (EGF) family receptors causes phosphorylation of Jak1 and 2 and activation of Stat3 and formation of active GTP–Ras complex, which activates the Raf/Mek/Erk and Mekk/Jnkk/Jnk pathways. This leads to the phosphorylation of nuclear factors that, migrating into nucleus, activate numerous target genes. The inactivation of components of the complex Apc/Axin/Gsk3β/β-catenin complex, or the inhibition of Gsk3β by Dishwelled (Dsh) protein by Wnt1/Freezzled activation, suppresses β-Catenin phosphorylation and ubiquitination followed by disruption by proteasome. β-Catenin accumulation into the cytoplasm causes its nuclear translocation and activation of various target genes. Activation of Tgf-β receptor (TBR) leads to the formation of Smad2/3/Smad4 heterodimers leading to cell death by apoptosis. Overexpression of Smad7 and/or downregulation of Smad2/3 and Smad4, inhibit the TBR signaling pathway. Enhancing and inhibitory effects are indicated by pointed arrows and blunt arrows, respectively.

such as $p16^{INK4a}$ and p53, and Tgf-β/Smad signaling. Profound alteration of the Wnt/β-Catenin pathway (►Wnt Signaling), leading to inactivation of the complex Apc/Axin/Gsk3β/β-Catenin, decreases β-Catenin disruption via proteasome. Consequent nuclear translocation of β-Catenin activates various targets, including c-*myc* and cyclins. Finally, overexpression of the inducible nitric oxide synthase (iNos) contributes to activation of the MAPK cascade and the hypoxia inducible factor-1, leading to an increase in vascular endothelial growth factor (Vegf) expression and angiogenesis. The presence of these alterations in preneoplastic lesions indicates their role in early stages of hepatocarcinogenesis.

Another largely investigated model of multistep tumorigenesis is represented by chemically induced colorectal cancer (CRC; ►Colon Cancer) of rodents. Aberrant crypt foci (ACF; Colorectal Pre-malignant Lesions) identified in colonic mucosa of rodents treated with chemical carcinogens are considered preneoplastic lesions. These lesions exhibit increased expression of c-*fos*, decreased c-*myc* expression and hexosaminidase activity, and loss of transforming growth factor-α (Tgf-α), whereas mutations of *Adenomatous polyposis coli* (*Apc*; ►APC & ►β-catenin pathway) gene are absent. K-*ras* mutations occur in azoxymethane-induced ACF in rats. However, various observations strongly suggest that ACF are not preneoplastic, but only hyperplastic lesions. A subset of ACF, called "dysplastic ACF," consisting of fast-growing crypts with altered β-Catenin expression associated, in some cases, with β-Catenin mutation, are considered true CRC precursors. In colonic mucosa of rats, treated with azoxymethane, mucin depleted foci (MDF) have been described. MDF are more dysplastic and more likely to express β-Catenin than common ACF. β-Catenin accumulating crypts (BCAC) with higher cell proliferative activity than ACF significantly increase with time during colorectal carcinogenesis in rodents. The study of the molecular events in early colorectal preneoplastic lesions indicates that various mutations occur and are selected during colorectal carcinogenesis, the main selective factor being represented by those leading to a β-Catenin downregulating function.

►Lung cancer develops in a gradual and stepwise fashion. Numerous studies have addressed lung tumorigenesis in tobacco smokers. Preneoplastic lesions include focal epithelial cell ►hyperplasia, squamous metaplasia, and ►dysplasia. These lesions have been induced in rats by various carcinogens and are followed by the development of adenomas, adenocarcinomas, and squamous cell carcinomas. Exposure of rats to tobacco smoke induces dose-dependent cell proliferation and squamous metaplasia. These effects are paralleled by activation of MAPK signaling pathways and AP-1 binding to DNA (Fig. 2). This is associated with upregulation of AP-1-dependent cell cycle proteins, such as Cyclin D1 and Proliferating cell nuclear antigen (PCNA).

Human Preneoplasia

Etiologic factors of HCC include ►cirrhosis induced by hepatitis B virus (HBV) and hepatitis C virus (HCV), alcoholic cirrhosis, exposure to Aflatoxin B1, estrogenic steroids, some naturally occurring carcinogens in food, and some rare genetic syndromes (i.e., hemochromatosis, glycogenosis type I, α1 antitrypsin deficiency). About 4% of HBV infections evolve to persistent hepatitis, 30% of which can further evolve to chronic hepatitis and cirrhosis. HBV positive cirrhosis is considered a preneoplastic lesion, although the evolution to HCC has been found in only 10% of cases. Different from HBV infection, HCV-induced hepatitis becomes chronic in the majority of patients. At least 20% of them develop cirrhosis, which in most cases evolves to HCC. In these patients, as well as in individuals with alcoholic cirrhosis, the development of FAH and adenomatous nodules, correspondent to those chemically induced in rats and mice or developing in woodchucks' viral hepatitis, exhibits several molecular alterations in common with the corresponding lesions of rodent liver.

Various preneoplastic lesions have been identified in the gastroenterical tract. About 10% of patients with long-standing gastroesophageal reflux develop the Barrett esophagus, in which the distal squamous mucosal epithelium is replaced by metaplastic columnar epithelium. This lesion is preneoplastic, and the patients with Barrett esophagus develop adenocarcinomas, preceded by dysplastic lesions, with a 30–40 times increased rate over the general population. Dysplastic epithelium exhibits cell cycle deregulation, high proliferative rate, upregulation of TP53, and in more advanced stage, amplification of chromosome 4. Further evolution to carcinoma implicates deregulation of Wnt/β-Catenin pathway and c-*ERB-B2* amplification.

Preneoplastic conditions of human gastric cancer have not yet been well characterized to date. Ménétrier disease, resulting from profound hyperplasia of mucosal epithelium and glandular atrophy, rarely exhibits epithelial metaplasia, a condition that may favor the development of gastric carcinoma. Interestingly, transgenic mice overexpressing Tgf-α at gastric level, develop a syndrome similar to human Ménétrier disease. A multistep model from chronic active *Helicobacter pylori* infection through multifocal mucosal atrophy, intestinal metaplasia, dysplasia, and carcinoma has been described. During this process, complex interactions

between several bacterial, host genetic, and environmental factors determine whether *H. pylori* infected individual develop cancer. *H. pylori* infection is characterized by upregulation of various inflammation-associated genes, including chemokines, adhesion molecules, surfactant protein D and CD74 in infected stomach. Nitric oxide and reactive oxygen species, which may induce DNA damage (►DNA Damage Response; ►Repair of DNA), are overproduced. The role of these and other factors in the evolution of metaplastic and dysplastic lesions to carcinoma is still an object of study. *H. pylori* infection also predisposes to the lymphomatous transformation of the mucosa associated lymphatic tissue.

Most knowledge on multistep carcinogenesis in the gastrointestinal tract derives from colorectal tumorigenesis. Inherited syndromes, occurring in less than 10% of patients, include familial adenomatous polyposis (FAP), hamartomatous polyposis, hereditary nonpolyposis colorectal cancer (►Lynch syndrome), and common cancer family syndrome, not belonging to aforementioned syndromes, causative germline mutations of high-penetrance genes affect APC gene for familial adenomatous polyposis, LKB1, SMAD4, and *BMPR1* genes for hamartomatous polyposis, various mismatch repair genes (e.g., hMSH2, hMLH1, hPMS1, hPMS2, hMSH6) (►Mismatch Repair in Genome Stability), for Lynch syndrome I, and AXIN2, TGFβR-2, and POLD genes for Lynch-like syndromes. The homologue of human FAP, the Min mouse strain-1, carries the APC^{Min} mutation and develops intestinal neoplasms. The study of these syndromes allowed identifying early human colorectal preneoplastic lesions and the so called adenoma–carcinoma sequence, as well as the molecular alterations underlying the sequence of events leading to CRC. The morphological events include early appearance of preneoplastic ACF, followed by premalignant lesions such as adenomas and adenomatous polyps, and, finally, carcinoma development. The appearance of ACF, however, is preceded by numerous molecular events, such as germline (in the inherited syndromes) or somatic (acquired first "hit" in a "multihit" process, in sporadic cases) mutations of APC or mismatch repair genes. The mucosa harboring these mutations is at risk. Inactivation of normal alleles of tumor suppressor genes (i.e., by promoter methylation of APC, MSH2, β-Catenin) causes hyperproliferation and appearance of early preneoplastic lesions, which following K-*RAS* mutation evolve to adenomas. The loss of further suppressor genes (i.e., TP53) precedes the appearance of carcinomas whose ►progression is characterized by additional mutations of various oncogenes, chromosomal aberrations, etc.

A four to fivefold increase in the incidence of gastrointestinal tract cancer occurs in patients with Crohn disease, in which the development of carcinoma is generally preceded by dysplastic lesions of the ileum and/or colon mucosa. The pathogenesis of neoplastic transformation is unknown. Crohn disease seems to occur in predisposed individuals carrying some susceptibility loci on chromosomes 3, 7, 12 or 16. The ileum localization seems to be linked to mutations of *NOD2* and CARD15 genes. However, the relationships between these alterations and cancer development are not known. A 20–30-fold rise in CRC incidence occurs in patients with ulcerative colitis. Cancer development is preceded by premalignant multifocal dysplastic lesions exhibiting DNA instability. Genomic instability has also been documented in the colon mucosa outside of dysplastic lesions and it has been hypothesized that some deficit of DNA repair occurs in these patients.

Preneoplastic and premalignant lesions have been described for different other human tumors (Table 1). Epidemiological, clinical, and molecular characteristics of a number of these lesions are often incompletely known, but particular attention must be focused on these parameters because of their importance for prevention and early diagnosis of malignancy.

Genetic Predisposition to Neoplasia

A body of evidence shows the existence of a genetic predisposition to tumors. Strong predisposition by high penetrance mutations of oncogenes, oncosuppressor genes, and DNA repair genes, occurs for a relatively small number of the so-called hereditary tumors. Genetic predisposition to sporadic tumors depends on the inheritance of several susceptibility or resistance allelic variants, which influence (modify) the behavior of the molecular mechanisms of tumorigenesis and, hence, the phenotypic features of preneoplastic and neoplastic lesions. Several modifier genes have been mapped, but only few genes involved in genetic predisposition have been identified so far. These observations may in some way modify the definition of preneoplastic lesion. Indeed, a cell carrying polymorphic variants of cancer modifier genes responsible for increased susceptibility to malignancy, namely increased cancer risk, could be considered preneoplastic. The identification of modifier genes and the signaling pathways that they influence may lead to the discovery of early diagnostic and prognostic markers as well as therapeutic targets, which may prevent the evolution of preneoplastic cells to full malignancy.

Clinical Relevance

One of the major challenges of cancer research has been the discovery of tools for efficient prevention of malignancy. In this context, the identification of

Preneoplastic Lesions. Table 1 Preneoplastic and premalignant lesion of various human tissues

Tissue	Preneoplastic		Premalignant[a]	
	Morphology	Molecular markers	Morphology	Molecular markers
Lung	Atypical adenomatous hyperplasia; bronchial dysplasia			
Liver	FAH		Dysplastic nodules	Upregulation of MAPK, JAK-STAT *ODC*, *C-JUN*, *C-FOS*, *C-MYC*, RAS family genes, cell cycle
Pancreas	Fat or papillary mucinous hyperplasia	*HER-2*, Ki-67, $P21^{WAF1}$, *CYCLIN D1* upregulation,. *SMAD 4* underregulation	Atypical hyperplasia	*HER-2*, Ki-67, $P21^{WAF1}$, CYCLIN D1 upregulation, *SMAD 4* underregulation
Oral epithelium	Leukoplakia	LOH at 3p and 9p	Dysplastic leukoplakia	*TP53* overexpression; loss of differentiation-related keratins
Esophagus	Barrett's epithelium with metaplasia	*TP53* mutations, *CDX* mutations	Barrett's dysplasia	*TP53* mutations, *APC* LOH, $p16^{INK4a}$ hypermethylation or LOH
Stomach	Ménétrier disease with metaplasia	TGF-α overexpression		
	H. Pylori atrophic gastritis with intestinal metaplasia	Overexpression of *GASTRIN*, *COX-2*, *SURVIVIN*, *BCL-2*	Dysplastic lesions	Overexpression of Gastrin, *COX-2*, SURVIVIN, *BCL-2*
Gastrointestinal tract	Crohn disease	*NOD2* and *CARD15* mutations		
	Ulcerative colitis	*TP53* mutations, Nitric oxide overproduction	Epithelial dysplasia	*TP53* mutation, Nitric oxide overproduction
	ACF	β-catenin activation	Adenoma	β-Catenin activation, *APC* and K-*RAS* mutation, LOH at *TP53*, *SMAD2* and *4*
Urothelial	Papillary hyperplasia	LOH at 9q	Papilloma	LOH at 9q, *TP53* mutations
Vulva			VIN I and II	ei5A-1 overexpression
Uterine cervix			Dysplasia (CIN I, CIN II)	$P16^{INK4A}$ overexpression, pRb ubiquitination
Endometrium			Atypical hyperplasia	Loss of *PTEN* expression
Ovary	Metaplastic surface epithelium and inclusion glands		Atypical hyperplasia	*TP53* mutation. Loss of *BRCA1* and *2*
Breast	Hyperplastic lesions	Microsatellite instability	Atypical hyperplasia	Microsatellite instability
Thyroid	C-cell hyperplasia	Overexpression of *BCl-2* and *BCL-X*	Follicular adenoma (atypical)	Mutations of *RAS* family genes
Skin	Lentiginous melanocytic hyperplasia; Lentiginous junctional nevus		Dysplastic nevus	Overexpression of bFGF, and IL-8

[a]The definition of premalignant lesion is often based on morphologic, clinical, and epidemiologic criteria.

very early biochemical, molecular, and morphologic changes, predisposing normal cells to tumorous transformation, plays a pivotal role. In principle, tumor prevention would imply the protection of humans against tumor initiation. When this is not possible, an efficacious preventive strategy may attempt to block the evolution of initiated cells to malignancy. Of course, early identification of precancerous lesions is

a prerequisite for efficacious prevention. The recognition of these lesions (i.e., polyps, adenomas, dysplastic nevi, leukoplakias) may allow their surgical or medical treatments. The knowledge of early molecular alterations, in preneoplastic lesions, is also important to adopt chemopreventive strategies aimed at contrasting the growth and progression of early lesions to cancer and/or to block the expression of genes involved in cell transformation.

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Prentice Criteria

Definition

Named after the criteria formulated by Ross Prentice in an influential 1989 article on validation of ►surrogate endpoints, were developed to ensure that rejection of the null hypothesis under the surrogate endpoint implies rejection of the null hypothesis under the true endpoint. The main criterion, sometimes called the Prentice criterion, is that the distribution of the true endpoint conditional on the surrogate endpoint does not depend on the intervention. In other words, the Prentice criterion says that, for all treatments under consideration, there is a single pathway from treatment to true endpoint that goes through the surrogate endpoint, so once the surrogate endpoint is known, no other information is needed to determine the distribution of the true endpoint.

Prenylation

Definition

Is a biochemical reaction resulting in the transfer of a farnesyl or geranylgeranyl lipid group onto a cysteine residue in characteristic carboxy-terminal motifs, giving rise to farnesylated and geranylgeranylated proteins. Such reactions are essential to the anchorage of small GTPases to cell membranes and to protein–protein interactions.

►Zoledronic Acid

Preoperative Chemotherapy

►Neoadjuvant Therapy
►Induction Chemotherapy

Prevalence

Definition

A measure of the proportion of people in a population affected with a particular disease at a given time.

►Obesity and Cancer Risk

Prevention

►Cancer Causes and Control

Primary Biliary Cirrhosis

Definition

An autoimmune liver disease resulting in intrahepatic bile duct destruction leading to liver ►cirrhosis.

►Hepatic Epithelioid Hemangioendothelioma

Primary Cancer Prevention

Definition
Prevention of tumor onset through the elimination of risk factors, *e.g.* chemical carcinogens. ►Carcinogenesis.

►Immunoprevention of Cancer

Primary Cancer Site

Definition
The organ in which the initial cancer forms. For example, the primary site for metastatic breast cancer is the breast.

►Metastatic Colonization

Primary Care Trust

Definition
PCT; Local commissioning organizations within the United PCT Kingdom's National Health Service whose Trusts (Primary Care Trusts (PCTs)) charged with purchasing specialist services, including cancer treatments, from hospitals.

►National Institute for Health and Clinical Excellence

Primary Chemotherapy

►Neoadjuvant Therapy

Primary Cilium

Definition
Non-motile, hair-like projection on most mammalian cells involved in signal transduction and chemical sensation.

Primary Dissemination

Definition
Dissemination at diagnosis.

►Leptomeningeal Dissemination

Primary Hepatic Carcinoma

►Hepatocellular Carcinoma

Primary Liver Cancer

►Hepatocellular Carcinoma – Etiology, Risk Factors and Prevention
►Hepatocellular Carcinoma

Primary Lymphedema

Definition
A congenital deficiency in the lymphatic system in which lymphatic vessels are poorly functional, resulting in blocked drainage of fluid from tissues, skin thickening and adipose tissue accumulation. In some patients, this condition is caused by inherited mutations in the FOXC2 or VEGFR3 gene.

►Lymphangiogenesis

Primary Myelofibrosis

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Synonyms
Myelofibrosis with myeloid metaplasia; Chronic idiopathic myelofibrosis; agnogenic myeloid metaplasia; Idiopathic myelofibrosis

Definition

Primary myelofibrosis (PMF) is a stem cell-derived clonal myeloproliferative disorder (MPD) that is characterized clinically by anemia, marked enlargement of the spleen and liver, and severe constitutional symptoms. Peripheral blood findings include the presence of immature myeloid cells including nucleated red blood cells, immature granulocytes, and tear drop-shaped erythrocytes. The bone marrow histology exhibits reticulin and collagen fibrosis, osteosclerosis, and angiogenesis.

Characteristics

Background

The blood and bone marrow features associated with PMF are discovered either de novo (i.e. PMF) or in the setting of either polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF). PMF is also known by many synonyms. However, the use of the term "PMF" was recently endorsed by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT).

Historical Perspective

The first description of PMF is credited to Heuck (1879). William Dameshek classified PMF as a MPD, along with chronic myeloid leukemia (CML), ET, and PV. In 1960, the Philadelphia chromosome was described in CML, which was later shown to harbor first t(9;22)(q32;q13) and subsequently the *BCR-ABL* disease-causing mutation. Accordingly, modern classification systems list PMF, PV, and ET as *BCR-ABL*-negative classic MPDs. In 1967, the Polycythemia Vera Study Group (PVSG) provided, for the first time, formal criteria for the diagnosis of PMF. Subsequently, a WHO-sponsored committee on classification of hematological malignancies revised the PVSG diagnostic criteria for PMF and reorganized the overall classification system for myeloid neoplasms.

Disease Mechanisms

In 1978, G6PD-based clonality studies established PMF as a stem cell-derived clonal myeloproliferation. In 2005, a novel gain-of-function (GOF) mutation involving the JAK2 tyrosine kinase (*JAK2*V617F) was described in ~50% of PMF patients but also in the majority of those with PV as well as ET. In 2006, another GOF mutation involving MPL (*MPL*W515L/K) was described in ~5% of patients with PMF.

*JAK2*V617F is an exon 14 *JAK2* mutation at nucleotide position 1,849 representing a G to T somatic point mutation. The mutation results in the substitution of valine to phenylalanine at codon 617. *MPL*W515L mutation represents a G to T transition at nucleotide 1,544 resulting in a tryptophan to leucine substitution at codon 515 of the transmembrane region of the MPL receptor. Both of the above mutations induce an MPD-phenotype in mice, the former a PV-like disease and the latter a PMF-like disease.

In addition to clonal myeloproliferation, PMF is characterized by bone marrow stromal aberration including collagen fibrosis, osteosclerosis, and angiogenesis. In mice, similar histological features have been induced by either systemic over-expression of thrombopoietin (TPO^{high} mice) or by megakaryocyte lineage restricted under-expression of the transcription factor GATA-1 ($GATA\text{-}1^{low}$ mice).[17] In both human PMF and experimental myelofibrosis in mice, the bone marrow stromal changes are believed to be secondary to abnormal release fibrogenic and angiogenic cytokines including transforming growth factor-β1 (TGF-β), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), tissue inhibitors of matrix metalloproteinases, and neutrophil-derived elastase and other proteases.

Clinical and Laboratory Characteristics

The prevalence of PMF is similar in men and women (M:F = 1.6:1) and overall reported incidence figures range from 0.4 to 1.5/100,000. Median age at diagnosis is estimated between 55 and 60 years. Most, but not all, patients with PMF are symptomatic at diagnosis. The typical presentation includes anemia, marked splenomegaly, and constitutional symptoms including fatigue and night sweats. Hepatosplenomegaly in PMF is secondary to extramedullary hematopoiesis (EMH) that might also involve other organs including lymph nodes, pleura, peritoneum, and the paraspinal and epidural spaces.

The peripheral blood smear in PMF often shows leukoerythroblastosis (presence of nucleated red blood cells and immature granulocytes) and tear drop-shaped red blood cells. Other laboratory abnormalities at diagnosis include anemia, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia, and increased serum levels of lactate dehydrogenase (LDH). Bone marrow examination reveals both "cellular phase" and "overtly fibrotic" stages of the disease. In both instances, the most characteristic feature is the presence of dense megakaryocyte clusters with atypical megakaryocyte morphology (cloud-like nuclear morphology) that is accompanied by increased granulocyte proliferation and reduced erythropoiesis. Additional histological features of advanced disease include new bone formation and intra-sinusoidal hematopoiesis.

Diagnosis

Bone marrow examination is essential in the diagnosis of PMF and should be accompanied by mutation screening for *BCR-ABL* in order to exclude the diagnostic possibility of CML and *JAK2*V617F in order

exclude the possibility of bone marrow fibrosis associated with non-malignant condition, lymphoid disorder, or metastatic cancer. It should be noted, however, that *JAK2*V617F can not distinguish PMF from other myeloid disorders such as MDS, ET, PV, or other MPD. Therefore, accurate diagnosis requires careful morphological evaluation. Cytogenetic abnormalities occur in approximately half of the patients with PMF at diagnosis and include del(20)(q11;q13), del(13)(q12;q22), trisomy 8, trisomy 9, del(12)(p11;p13), monosomy or long arm deletions involving chromosome 7, and partial trisomy 1q). Although none of these abnormalities are specific to PMF, the presence of either del(13)(q12;q22) or der(6)t(1;6)(q21–23;p21–23) is strongly suggestive of the specific diagnosis.

Prognosis and Treatment

Causes of death in PMF include development of blast phase PMF, which occurs in ~10% of patients during the first decade of their disease, and infections. Survival in PMF is estimated by the use of one of several prognostic scoring systems (PSSs) that rely on the presence or absence of well-established adverse prognostic features. Among the latter, the *Mayo Clinic* PSS has been reported to be superior, compared to other PSSs, in delineating both good risk and intermediate risk disease categories. The *Mayo* PSS is based on four adverse prognostic variables: hemoglobin < 10 g/dL, platelet count $<100 \times 10^9$/L, leukocyte count either $<4 \times 10^9$/L or $>30 \times 10^9$/L, and monocyte count $\geq 1 \times 10^9$/L. In the absence of any adverse feature (low-risk disease), median survival is expected to exceed 10 years. The presence of two or more adverse features (low-risk disease) predicts a median survival of <5 years.

Current management in PMF is suboptimal; erythropoietin, androgen preparations, corticosteroids, thalidomide, lenalidomide, splenectomy, and involved field irradiation of the spleen or other sites of extramedullary hematopoiesis. Treatment with either myeloablative or reduced-intensity conditioning (RIC) allogeneic stem cell transplantation (ASCT) is used for selected patients with high-risk disease. However, because ASCT is associated with relatively high incidence of regimen-associated mortality and morbidity, it is not recommended for all patients. The aforementioned Mayo PSS for PMF helps in the selection of patients suitable for ASCT.

In the asymptomatic patient with low-risk PMF, I recommend watchful waiting. The primary reason for using drug therapy in PMF is either anemia or splenomegaly. Drug options for the former include subcutaneous erythropoietin or oral drugs including androgen preparations, corticosteroids, danazol, thalidomide, and lenalidomide. The response rates from such therapy ranges from 10% to 30%. Responses last between 6 and 12 months. Single agent therapy in unselected patients with either thalidomide or lenalidomide produces 15% and 20% response rates in anemia, respectively. The addition of corticosteroids doubles the response rate with thalidomide and the presence of del(5)(q31) is associated with complete hematologic remission in the majority of patients treated with lenalidomide. The drug of choice for symptomatic splenomegaly in PMF is hydroxyurea. The drug is also used for controlling symptomatic thrombocytosis and/or leukocytosis.

Splenectomy is a strictly palliative treatment modality in PMF and does not alter the natural history of the disease. The procedure is associated with ~10% mortality and a higher incidence of morbidity that includes thrombosis, bleeding, post-splenectomy enlargement of the liver, and exacerbation of thrombocytosis/leukocytosis. Current indications for splenectomy in PMF include complications of portal hypertension including ascites and variceal bleeding, drug-refractory symptomatic splenomegaly, or very frequent red blood cell transfusions. Involved field radiotherapy provides transient relief of mechanical discomfort from hepatosplenomegaly. However, such therapy is often complicated by protracted pancytopenia and drug therapy is instead preferred. In contrast, irradiation therapy is very useful in patients with non-hepatosplenic EMH; most frequent sites include vertebral column, lungs, pleura, and peritoneum.

Both myeloablative and RIC transplant are used in advanced PMF. The experience so far with myeloablative ASCT is encouraging in very young patients (age < 45 years) but post-transplant long-term survival in older patients is less than 20%. Furthermore, the majority of survivors after ASCT experience reduced quality of life because of chronic graft versus host disease (GVHD). To date, the advantage of RIC transplant over myeloablative ASCT has not been examined in a controlled setting although single cohort studies suggest better outcome in terms of both 1-year mortality (0–33%) and morbidity (0–50% rate of acute GVHD).

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Primary Pigmented Nodular Adrenocortical Disease

Definition

PPNAD; is a rare disease caused by excess production of cortisol by the adrenal glands. The adrenal glands are small glands located above each kidney and produce hormones. Hormones are chemical substances formed in one organ or part of the body that travel in the blood to other body parts where they influence how that body part works. Cortisol is one of the hormones made by the adrenals. Cortisol influences body metabolism (how the body converts small molecules to large and vice versa) and can decrease inflammation. People with PPNAD have adrenal glands that make too much cortisol or make it at inappropriate times (when the body does not need it).

Primary Sclerosing Cholangitis

Definition

PSC; Is an inflammatory disease of the bile ducts, which leads to cholestasis or blockage of bile flow. The ►inflammation of the bile ducts causes scarring and hardening (►fibrosis) that narrows the bile ducts. Because bile cannot exit, it accumulates in the liver causing damage to liver cells. Eventually, with chronic inflammation and long term cell damage, the liver develops ►cirrhosis (hardening or fibrosis) and can no longer function properly. PSC is considered to be an autoimmune disease. However, there is not universal agreement on the pathogenesis of the disease. Can develop in patients with ►inflammatory bowel disease.

►Cholangiocarcinoma
►Bile Duct Neoplasms

Primary Systemic Therapy

►Neoadjuvant Therapy

Primitive Neuroectodermal Tumor

Definition

PNET; Tumors composed of small densely packed hyperchromatic cells found in the central nervous system CNS (central PNET) or originating from soft tissue and bone (peripheral PNET). Peripheral PNET are genetically characterized by a translocation t(11;22)(q24;q12), are of neural crest origin and grouped with the ►Ewing sarcoma family of tumors. Central PNET do not show a t(11;22) translocation and exhibit divergent differentiation patterns along neuronal (e.g., cerebral ►neuroblastoma) or ependymal (e.g., ependymoblastoma) lines or are found in locations above the cerebellar tentorium (connective tissue covering the dorsal surface of the cerebellum) (e.g., pineoblastoma). Medulloblastomas are infratentorial (infra = below) central PNET.

►PNET
►Medulloblastoma

Proaccelerin

Definition

►Factor V.

Procarcinogen

Definition

Chemically unreactive precursor of an intermediate metabolite and/or ultimate carcinogen that undergoes biotransformation by drug metabolizing enzymes before becoming biologically active.

►Carcinogen Macromolecular Adducts
►Toxicological Carcinogenesis

Procaspase

Definition

Is the inactive precursor of a ►caspase.

►Photodynamic Therapy

Processed Pseudogene

Definition
Sequences with close similarity to paralogous functional genes that generally lack the ability to be transcribed and which are derived from cellular RNAs that have been reverse transcribed and inserted into the genome.

►LINE-1 Elements

Processing

Definition
►N- or C-Terminal Processing.

Proconvertin

Definition
►Factor VII.

Prodrug

Definition
A precursor (forerunner) of a drug. A compound that is converted within the body into its active form that has medical effects. A prodrug must undergo chemical conversion by metabolic processes before becoming an active pharmacological agent. For example, ►sulfasalazine is a prodrug. It is not active in its ingested form. It has to be broken down by bacteria in the colon into two products – 5-aminosalicylic acid (5ASA) and sulfapyridine – before becoming active as a drug. Prodrugs are useful when the active drug may be too toxic to administer systemically, the active drug is absorbed poorly by the digestive tract, or the body breaks down the active drug before it reaches its target.

►ADMET Screen
►HSV-TK/Ganciclovor-mediated Toxicity
►Xenobiotics

Progenitor Cells

Definition
These can include both stem cells and transient amplifying cells, or even cells that are well on the way to becoming differentiated.

►Adult Stem Cells

Progesterone

Definition
Is a steroid hormone involved in female menstrual cycle, pregnancy and embryogenesis. As a drug it has several indications related to its biological function.

►Fluoxetine
►Progestin

Progesterone Receptor

Definition
PR; A steroid hormone receptor that confers progesterone-responsiveness and is often co-expressed with the ►estrogen receptor (ER) in normal breast and breast cancer.

►Basal-like Breast Cancer
►Progestin

Progesterone Response Elements

Definition
PRE; Refer to a specific DNA sequence located in the promoter of ►progesterone target genes that consists on

an inverted repeat, separated by three nucleotides: AGAACAnnnTGTTCT.

►Progestin

Progestin

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Synonyms

Progesterone; Progestogen

Definition

Progesterone (4-pregnene-3,20-dione, P4) is an ►ovarian steroid hormone that plays a key role in the regulation of cell proliferation and differentiation in the female reproductive tract. Interestingly, progesterone also regulates diverse biological effects in a broad range of tissues, even of the cardiovascular and the central nervous systems, and participates in bone maintenance and regulation of thymic involution. Progesterone has also been implicated in the development and progression of ►breast cancer. Progestogen defines the category of hormone molecules (natural and synthetic) that act like progesterone in the uterus and the term progestin has been generally used to refer to both a series of synthetic hormone molecules and progesterone. We will use here the generic term progestin to refer to both progesterone and synthetic progestins.

Characteristics

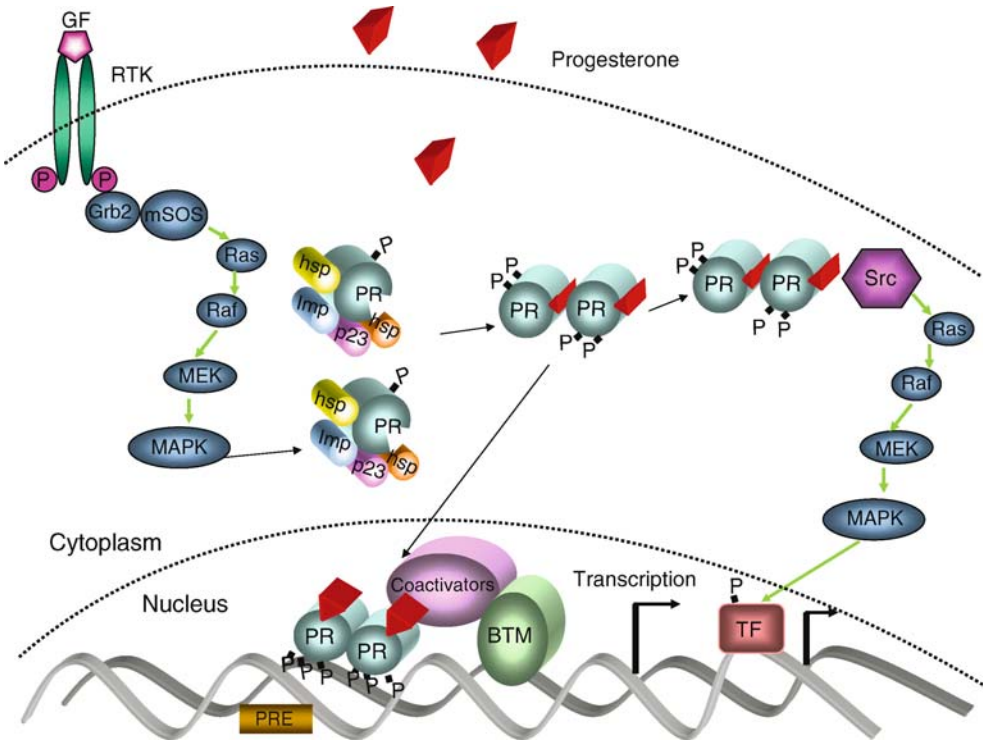
The two most studied progesterone target tissues are the uterus and the mammary gland. In the uterine epithelium progesterone antagonizes the capacity of estrogen, the other ovarian steroid, to induce cell proliferation and acts as the major differentiating hormone. These two ovarian steroid hormones are generally present jointly in physiological conditions and so are their specific receptors coexpressed in the same tissues. Notably, a series of findings have shown that estrogen and progesterone counterbalance each other's effects in target tissues. Progesterone also plays a key role in mammary gland development and function, as demonstrated, among an increasing body of evidence, by incomplete mammary gland ductal branching and failure of lobuloalveolar development in progesterone receptor (PR) knockout mice (PRKO). Interestingly, in contrast with its antagonistic effect on estrogen-driven proliferation in the uterus, in the mammary epithelium proliferation reaches its peak during the progesterone-dominant luteal phase of the human menstrual cycle, evidencing a proliferative role of progesterone in the normal breast. Accumulated findings indicate that progestins are also involved in controlling mammary gland tumorigenesis both in women and in animal models. Progestin effects in in vitro growth of breast cancer cells were found to be highly dependent on experimental culture conditions and on the presence of estrogens. Particularly, progestins were found to either support sustained growth or to induce cells to progress through one round of cell division, followed by growth arrest at the G1/S phase of the second cycle in the human breast cancer cell line T47D, the ideal model of breast cancer cell with constitutive expression of PR. A series of seminal findings evidenced that the initial pulse of progestins primes progesterone-arrested breast cancer cells for the action of secondary proliferative or differentiative ►signaling pathways. Hence, the commitment to one path or the other would be determined by cross talks between growth factor (GFs)/cytokine pathways and by progesterone/PR signaling.

Mechanisms

The biological effects of progestins are mediated by interaction with the PR, a member of the ►steroid receptor superfamily of ligand-activated nuclear transcription factors. The PR consists of two isoforms, PR-A and PR-B, expressed from a single gene by the use of alternative promoters. PR-B differs from PR-A in that PR-B contains an amino terminal extension of 128–165 aminoacids, depending on species. Both PR isoforms contain a C-terminal hormone binding domain, a DNA-binding domain, a hinge region, and two transcriptional activation function (AF) domains located at the amino terminus (AF-1) and at the ligand-binding domain (AF-2). The PR-B isoform contains a third transactivation function (AF3) within its amino terminal extension, absent in PR-A. The two PRs exhibit different transcriptional properties that depend both on the cell type and on the target gene promoter, being the PR-B a much stronger transactivator for PR target genes. On the other hand, PR-A is a more potent dominant-negative transcriptional inhibitor of PR-B and other ►steroid hormone receptors, including ►estrogen receptor (ER). Recent screens for differential regulation of gene expression by PR-A and PR-B in breast cancer revealed that although some genes are modulated by progesterone through both PR isoforms, the majority of the target genes are exclusively regulated through one or the other isoform, principally through PR-B. PR-A and PR-B ratios vary during the menstrual cycle in the uterus, indicating the physiological importance of these variations. Contrastingly, equal expression of the two PR isoforms is required for

normal development and function of the mammary gland. Different ratios of PR isoform expression have been found in several cancers. Overexpression of PR-B has been found in ovarian, endometrial, and cervical tumors. On the other hand, PR-A-rich breast tumors have poorer disease-free survival rates and exhibit resistance to ►endocrine therapy.

The classical pathway of progesterone inducible PR-mediated gene transcription has long been described. Upon progesterone binding, PR undergoes a conformational change and dissociates from a multi-protein chaperone complex, which includes ►heat shock protein (hsp) 90 and its co-chaperone p23, hsp70 and three co-chaperones: hsp40, hip (hsp70-interacting protein), and hop (hsp70-organizing protein) and ►immunophilins such as FKBP51 and FKBP52 (Fig. 1). PR then dimerizes, translocates to the nucleus, and binds to specific ►progesterone response elements (PREs) in the promoter of target genes where DNA-bound receptor recruits ►coactivators which facilitate transcription initiation through interaction with components of the basal transcription machinery (Fig. 1). Several PR coactivators have been described. Among them, well characterized has been the SRC/p160 family of proteins (NcoA/SRC1, GRIP1/SRC-2, NcoA-3/SRC3/RAC3) that binds to the AF2 region of PR. Notably, accumulating evidence indicates that coactivators not only participate in the initiation step of transcription, but also play a role in ►alternative RNA splicing, revealing another mechanism through which PR controls gene expression. Progesterone also regulates transcription of genes that lack a canonical PRE through interaction with other transcription factors, such as Sp1 and ►AP-1, at the response elements of these transcription factors. In addition to their direct transcriptional effects, rapid, extranuclear or "nongenomic" biological effects of progesterone have been described. One physiologically important process that depends on nontranscriptional effects of progesterone is the maturation of fish and amphibian oocytes, in which progesterone induces germinal vesicle breakdown, resumption of the meiotic cell cycles, and development into the mature, fertilizable egg. Assessment of the signal transduction pathways involved in this process evidenced that progesterone activates the Mos/MEK1/p42 ►mitogen-activated protein kinases (MAPK) cascade, which ultimately brings about the activation of the universal M phase trigger cdc2/►cyclin B. The receptors that mediate the nongenomic effects of progesterone remain largely unknown and have constituted a controversial issue over the years. Several findings suggested that the oocyte PR involved in nongenomic effects could be a membrane seven-pass, ►G-protein-coupled receptor, unrelated to the classical intracellular PR. Thus, progesterone can induce maturation when applied to the outer side of an oocyte. Moreover, it stimulates some aspects of maturation in enucleated oocytes and induces inhibition of adenylyl cyclase and cyclic AMP concentration. However, the recent cloning of the *Xenopus* homolog of the classical PR (X-PR) revealed that oocyte maturation could be mediated by nontranscriptional effects of the classical PR since injection of X-PR mRNA was found to accelerate progesterone-induced Mos synthesis, p42 MAPK activation, and oocyte maturation. In addition, injection of X-PR antisense oligodeoxynucleotides into oocytes inhibited progesterone-induced maturation which could be restored by overexpression of X-PR or human PR. This classical X-PR has also been found to associate with the cytosolic kinases p42 MAPK and ►phosphatidylinositol 3-kinase (PI-3K). Recently, a novel membrane progesterone receptor (mPR) gene that encodes a seven transmembrane-spaning G-protein coupled receptor was cloned from spotted sea trout ovaries and other members of this family of putative mPRs have been cloned from humans and other vertebrates. In mammalian, nongenomic effects of progestins have been described in human spermatozoa and appear to be mediated by interaction with progesterone membrane receptors. Rapid effects of progestins also include modulation of neurotransmitter receptor activity. An intense and particularly important field of investigation, for its potential therapeutic implications, lies in the nongenomic biological effects of progestins in breast cancer cells. Pioneering work demonstrated that progestin treatment of human breast cancer T47D cells activates the signal-transducing c-►Src/p21^{ras}/MAPK pathway (Fig. 1), which results in cell proliferation. This startling work has shown that progestin ability to activate c-Src/MAPK pathway depends on the presence of unliganded ERα, which is the one that activates c-Src. ERα and PR-B interaction occurs via two domains that flank a specific amino-terminal PR proline-rich sequence. On the other hand, direct interaction of the polyproline motif of PR-B with the ►SH3 domain of c-Src, which results in c-Src activation by a SH3 displacement mechanism, has been found in vitro, in human normal breast MCF-12 cells transduced with PR-B, and in T47D cells. In these two cell types, interaction is progestin-dependent. Notably, cross talk between PR/progestins and cytoplasmic signaling pathways is of a bidirectional nature, where progestins activate cytoplasmic pathways and conversely, where activation of cell signaling pathways induces ligand-independent activation of PR. It is to be noted that PR is a heavily phosphorylated protein in multiple serine residues. Though the functional role of PR phosphorylation has not been completely deciphered, increasing evidence indicates that it plays a key role in both ligand-dependent and -independent transcriptional activation of PR. Particularly, MAPK activated by ligands of the type I ►receptor tyrosine kinase family (RTKs-I/ErbBs) were



Progestin. Figure 1 Mechanisms of progesterone receptor (PR) activation. Upon progesterone binding, PR undergoes a conformational change and dissociates from a multi-protein chaperone complex, which includes heat shock protein (hsp), p23, and immunophilins (imp). PR then dimerizes and translocates to the nucleus. In it classical mechanism of action, PR binds to specific progesterone response elements (PREs) in the promoter of target genes where the DNA-bound receptor recruits coactivators. The latter facilitate transcription initiation through interaction with components of the basal transcription machinery (BTM). PR nongenomic action occurs through PR activation of cytoplasmic signaling pathways, such as c-Src/p21ras/MAPK, which results in activation of transcription factors (TFs). Shown is also a model of ligand-independet activation of PR by growth factors (GFs) that bind to receptor tyrosine kinases (RTKs), in which GF-activated MAPK induce the PR phosphorylation required for its transcriptional activity.

found to play a key role in ligand-independent activation of PR in both human and mouse breast cancer cells (Fig. 1). Convergence between growth factors/RTKs and PR signaling is directly involved in progestin-mediated proliferative effects in breast cancer cells.

Clinical Significance

Accumulating findings have clearly evidenced the involvement of progestins/PR in breast cancer development. Breast cancer is a devastating disease and is a major cause of death among women between ages of 40 and 55 in most industrialized countries. Considerable epidemiological and clinical evidence have related the cumulative and sustained exposure to ovarian steroids, estrogen and progesterone, to increased risk of developing breast cancer. Furthermore, it has been shown that PRKO mice are less susceptible to develop mammary tumors in a chemically-induced model of tumorigenesis. Strong evidence of PR implication in breast cancer etiology was also provided by the observation that postmenopausal women who undergo a combined estrogen and progestin hormone replacement therapy suffer a higher incidence of breast cancer than women who take estrogen alone. In addition, a recent study demonstrated that treatment of ►breast cancer susceptibility gene *Brca-1*-deficient mice with the ►antiprogestin RU486, prevented mammary tumorigenesis thereby highlighting the importance of a reappraisal of the therapeutic effects of targeting PR in breast cancer. At the time of diagnosis, at best only two third of tumors expressing ER and PR are responsive to endocrine therapy. Approximately 70% of all patients with ER- and PR-positive invasive breast cancers will initially benefit from ►antihormonal therapy. However, as these tumors progress, they will often acquire steroid hormone resistance. This is likely due to the fact that control over

tumor growth is undertaken by GFs that are not only able to act as mitogens for breast tumor cells, but also to transactivate steroid receptors. Clearly, understanding the molecular mechanisms of progestins action and interaction with GF signaling in breast cancer is a major question with important therapeutic implications.

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Progestogens

Definition

Group of naturally occurring or synthetic steroid ►hormones that includes ►progesterone. They are important in reproduction, particularly in maintaining the course of pregnancy. They are used in oral contraceptive pills and ►hormone replacement therapy (HRT).

►Endometriosis
►Progestin

Prognosis

Definition

Prognosis is a medical term denoting the doctor's prediction of how a patient's disease will progress, and whether there is chance of recovery. Likelihood of cure after diagnosis of cancer. Prognostic factors aid in estimation of individual patient outcome.

►Prognostic Biomarker

Prognostic Biomarker

Definition

A ►biomarker that is informative on the clinical outcome at the time of diagnosis, but independently of therapeutic intervention (e.g. markers of invasion, growth and metastatic potential). Marker to assess survival probabilities of patients or to detect the aggressive phenotype and determine how the cancer will behave.

►Oncopeptidomics

Programmed Cell Death

Definition

Synonym ►Apoptosis; Is a process in which cell plays an active role in its own execution. Programmed cell death serves to remove unwanted or damaged cells from an organism.

Programmed Cell Death 4

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Synonyms

197/15a (human PDCD4 variant 1); H731 (human PDCD4 variant 2, cancer-related); MA-3, TIS (mouse Pdcd4); DUG (rat Pdcd4); PDCD4/Pdcd4

Definition

PDCD4/Pdcd4 is a tumor suppressor. This protein contains two ►MA-3 domains homologous to the M1 domain of ►eIF4G and nuclear localization signals. PDCD4/Pdcd4 exhibits multiple functions such as the regulation of protein synthesis, the controls of transcription, and the induction of ►apoptosis. The human gene maps to 10q24.

Characteristics

Human *PDCD4* (*H731*) was first identified as a gene involved in the cell cycle. The human gene has also been isolated as a gene whose expression in lymphocytes was modulated by interleukins. The mouse gene (*MA-3*) was found to be upregulated during the induction of apoptosis, and the same gene (*TIS*) was downregulated by topoisomerase inhibitors such as etoposide and camptothecin. In chickens, Pdcd4 expression has been demonstrated to be controlled by the transcription factor ►myb. PDCD4/Pdcd4 is ubiquitously expressed and localized in the cytoplasm or in the nuclei, or in both, depending on the cell types and conditions. Since Pdcd4 was initially shown in 1999 to have a tumor suppressor function, the mechanisms and functions of PDCD4/Pdcd4 have gradually been elucidated.

Tumor Suppressor Activity

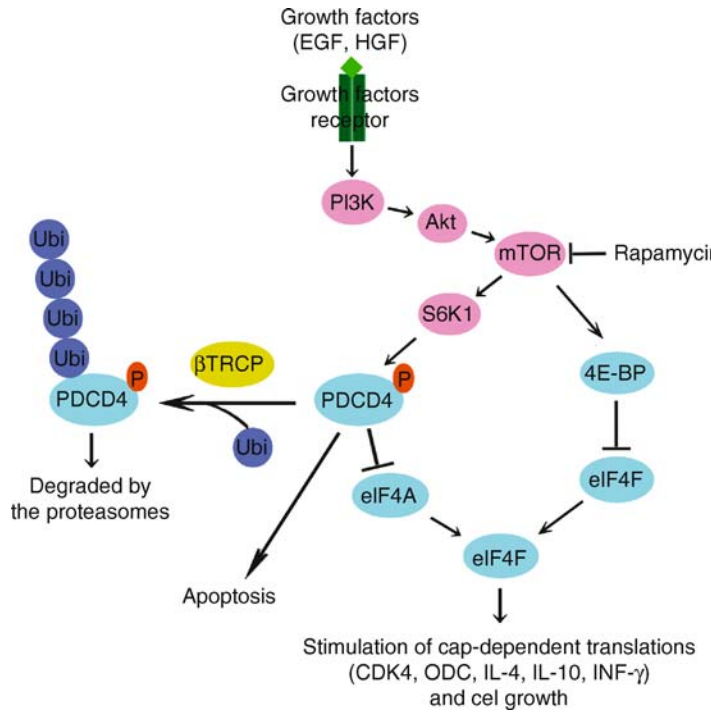
The tumor suppressor activity of Pdcd4 is considered to be well established. In the mouse JB6 epidermal cell model system, cells with high levels of Pdcd4 expression were found to be more resistant to carcinogenesis induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) than cells with low levels of Pdcd4. The ectopic expression of Pdcd4 in metastatic RKO human colon carcinoma cells suppresses the ►invasion of the carcinoma cells. Transgenic mice overexpressing Pdcd4 in the epidermis (K14-Pdcd4) are resistant to ►skin carcinogenesis in response to TPA: The ►K14-Pdcd4 mouse shows significant reduction in papilloma formation, carcinoma incidence and papilloma-to-carcinoma conversion frequency. A ►Pdcd4-deficient mouse developed spontaneous lymphomas, mostly B cell lymphomas, and has a significantly reduced life span. They developed tumors around the age of 85 weeks, in comparison to p53-deficient mice, which developed tumors around the age of 12 weeks, thus indicating that Pdcd4 is a weaker repressor than p53 (►p53 Protein, biological and clinical aspects).

Control of Translation

Pdcd4/PDCD4 protein interacts with the translation initiation factors ►eIF4A and eIF4G through the MA-3 domains and thus modulates protein synthesis. Pdcd4 was shown to inhibit ►cap-dependent translation by inhibiting both the binding of eIF4A with eIF4G and the RNA-helicase activity of eIF4A. The levels of ►cycline-dependent kinase 4 (CDK4) and ornithine decarboxylase (ODC) protein, which are the candidates for Pdcd4-regulated translation as their mRNAs contain a structured 5′ untranslated region (UTR), decreased by 40 and 46%, respectively, in the ►K14-Pdcd4 transgenic mouse in comparison to their matched wild-type siblings. The PDCD4 protein was phosphorylated by ►ribosomal protein S6 kinase 1 (S6K1) at Ser67, downstream of the growth factor-induced PI3K-Akt-►mTOR signaling pathway (►AKT signal transduction pathway in oncogenesis, ►P3IK signaling), ubiquitinated by the E3 ubiquitin ligase complex $SCF^{\beta TRCP}$ (SKP1-Cull-F-box protein) and degraded by proteasomes (►Ubiquitination). The phosphorylation at Ser67 may promote the phosphorylation of Ser71 and Ser76 in the βTRCP-binding motif ($D^{70}SGRGDS^{76}$) of PDCD4 protein, which must be phosphorylated to allow recognition by βTRCP. A loss of PDCD4/Pdcd4 resulted in the modulation and increase of protein synthesis, thereby promoting cell growth that can subsequently lead to cancer (Fig. 1). The rate of protein synthesis in splenocytes derived from the Pdcd4-deficient mouse increased more than twofold in comparison to that in the cells derived from a wild-type sibling, and ~50 proteins were affected by the *PDCD4* gene mutation. Among these, three growth factors/cytokines, ►interleukine-4 (IL-4), interleukine-10 (IL-10), and interferon-γ (IFN-γ were significantly increased in the PDCD4-deficient cells, due to a posttranscriptional regulation. The PDCD4-deficient mice were significantly resistant to two models of inflammatory diseases caused by self-reactive lymphocytes: type1 diabetes induced by streptozotocin (STZ) and the experimental autoimmune encephalomyelitis (EAE) induced by the myelin oligodendrocyte glycoprotein (MOG). The PDCD4-deficient lymphocytes preferentially produce cytokines which promote oncogenesis but inhibit ►inflammation. All of these cytokines contain structured GC-rich 5′ UTRs.

Transcriptional Controls

Pdcd4 inhibits the activation of c-Jun which is a component of activator protein 1 (►AP-1) and consequently may suppress neoplastic transformation by inhibiting AP-1-dependent transcription. Pdcd4 does not directly target either c-Jun or the Jun N-terminal kinase (JNK) which activates c-Jun, but it inhibits transcription of ►MAP4K1, an upstream kinase of JNK. Therefore, Pdcd4 may contribute to the suppression of neoplastic transformation, tumor progression and invasion by the novel mechanisms of down-regulating MAP4K1-transcription, with consequent inhibition of c-Jun activation and AP-1-dependent transcriptions.



Programmed Cell Death 4. Figure 1 Controls of protein synthesis and apoptosis by PDCD4. PDCD4 inhibits ►cap-dependent protein synthesis which may stimulate carcinogenesis. The growth factor-mediated PI3K-Akt-mTOR pathway stimulates the phosphorylation and degradation of PDCD4. The loss of PDCD4 results in the increase of protein synthesis and in the suppression of PDCD4-induced apoptosis. 4E-BP, eIF4E-binding protein; βTRCP; the E3 ubiquitin ligase $SCF^{\beta TRCP}$; Ubi, ubiquitin.

Induction of Apoptosis

The Pdcd4 expression has been found upregulated upon apoptosis induction in various apoptosis-inducible mouse cell lines of thymocytes, T cells, B cells and pheochromocytoma. *PDCD4* gene expression is also upregulated in the ►transforming growth factor-β1 (TGF-β1)-induced apoptosis of human hepatoma cell lines and in the ►retinoic acid-RAR (retinoic acid receptor) signaling system-induced apoptosis of human breast cancer cells. The PDCD4 protein induces the ►apoptosis of cancer cells when the protein was over-expressed by the transfection of a PDCD4-plasmid. Upon apoptosis induction of Huh7 hepatoma cells by PDCD4 overexpression, the caspase cascade was found activated via Bax activation and mitochondria events; caspase-9, -3, and -8 were activated (►Apoptosis signaling), as in the case of TGF-β1-induced apoptosis. TGF-β1 induced the PDCD4 mRNA expression through the TGF-β1-activated Smad signaling pathway (►Smad proteins in TGF-β1 signaling). PDCD4 accumulated in the nuclei and induced the fragmentation of the nuclei in Huh7 cells induced by TGF-β1 or transfected with the PDCD4-plasmid. The PDCD4 protein amounts in the nuclei were increased in parallel to the increase in apoptotic cells, while the amount in the cytoplasm only slightly increased during the treatment of Huh7 cells with TGF-β1, thus indicating that the control mechanism of PDCD4 localization in cells may play an important role in the induction of apoptosis in Huh7 cells. In contrast to the case of Huh7 hepatoma cells, the proapoptotic molecule Bad was upregulated and the antiapoptotic ►Bcl-2 was suppressed in the lungs of K-ras null mice with pulmonary cancer exposed to aerosol containing urocanic acid-modified chitosan (UAC)/PDCD4 complexes.

Roles in Cell Differentiation

PDCD4 protein is abundantly expressed in the differentiating keratinocyte cell layers of the epidermis and its appendages, but expressed only in low levels in their germinative cell layers, in the human skin. The protein is mostly localized in the nuclei of keratinocytes. PDCD4 might contribute to the differentiation of keratinocytes inhibiting the activation of c-Jun and consequently AP-1-dependent transcriptions which would otherwise induce the cells to proliferate. Alternatively, PDCD4 might also help to activate

the proapoptotic caspase cascade that may induce cell death, during the terminal differentiation of keratinocytes.

Clinical Aspects

PDCD4 protein levels are downregulated in the human lung-, liver-, colon-, pancreas-, epidermal cell-, and glia-derived cancer tissue specimens so far tested, in comparison to corresponding normal tissues. Among these tissue samples, relatively high PDCD4 expression has thus been associated with a slower progression and a better prognosis in some lung cancers. In pancreatic cancers, this expression has been shown to correlate with the differentiation levels; high expression was observed in relatively well-differentiated tissues. In other cases, little or no correlation between the expression levels and pathological and clinical features has so far been found. However, transgenic mice preferentially expressing Pdcd4 in the epidermis, resisted skin carcinogenesis. The Pdcd4-deficient mouse exhibited an earlier development of spontaneous lymphomas, especially those of B lymphoid origin. These results indicate that the loss of PDCD4 expression may increase the risk of carcinogenesis. PDCD4 expression is modulated by various factors such as growth factors, cytokines, and vitamins. PDCD4 modulates protein synthesis. An overexpression of PDCD4 induces apoptosis of cancer cells (►Apoptosis induction for cancer therapy). Therefore, PDCD4 is expected to be a target gene for cancer prevention and therapy. The aerosol delivery of a gene carrier-PDCD4 complex may be useful for the treatment of lung cancers. High PDCD4 expression has been shown to enhance the sensitivity of cancer cells to anticancer drugs geldanamycin and ►tamoxifen using the National Cancer Institute drug-screening panel of 60 human cancer cells (NCI60).

PDCD4-deficient mice are not only sensitive to carcinogenesis of lymphocytes, but they are also resistant to inflammatory diseases such as autoimmune encephalomyelitis and diabetes. PDCD4-deficient lymphocytes preferentially produce cytokines such as IL-4, IL-10, and IFN-γ that promote oncogenesis but inhibit inflammation. Therefore, PDCD4 may also be a target gene for the therapy of inflammatory diseases.

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Progression

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Definition

Tumor progression defines the process of invasiveness which leads to settlement and growth of a tumor cell in an organ that is distant from the site of the primary tumor. Such a "secondary" tumor is called metastasis.

Characteristics

Benign tumors are defined by loss of growth control, loss of contact inhibition, a reduced requirement for growth factors, or autocrine production of growth factors. Malignant tumors, i.e., metastasizing tumors, are defined by their capacity of invasiveness. This implies that the tumor invades the surrounding tissue, the vascular system, and distant organs. The process is called the metastatic cascade and is composed of the following elements:

1. Loss of contact between the individual tumor cell and the tissue of the primary tumor
2. Penetration through the basal lamina of the primary tumor
3. Invasion of blood and/or lymphatic vessels
4. Adaptation to the blood pressure
5. Adhesion to vessel endothelia, extravasation, embedding, and growth in organs different from the primary tumor, which includes the requirement for supply with nutrients by angiogenesis

Tumor Stem Cell and Metastasis

Metastasis formation is independent of the process of oncogenesis, which is the consequence of genetic alterations within a single cell, whereas tumor progression relies on a concerted interaction with surrounding

elements, like the extracellular matrix and host cells. It is important to note that no single step in the metastatic cascade is necessarily associated with the malignant phenotype. Corresponding processes will be seen during implantation of the fertilized egg, organogenesis, stem cell maturation, and lymphocyte migration. It is therefore hypothesized that tumor progression must not necessarily be accompanied by additional genomic alterations of the transformed tumor cell. Instead, tumor progression may be initiated by inappropriate silencing or activation of (regulatory) genes. Thus, Sell and Pierce postulated in 1994 that malignant tumors arise from a blockade in stem cell differentiation. Stem cells are characterized by immortality, self renewal, differentiation into daughter cells, and migratory potential. All these features also are characteristics of metastasizing tumor cells. The transformation of stem cells into highly specialized epithelial and mesenchymal cells is guided by the transcription of selective genes and is initiated via the stem cell environment. Notably, differentiation of stem cells, including stem cells in the adult organism, is reversible. This so called ►epithelial-mesenchymal transformation (EMT) and its reversion, MET, are the fundamental principle of reorientation of transcriptional programs. A transient EMT also is central for tumor progression. It involves tumor cells and the tumor stroma. Both embryonal and metastatic EMT are initiated by identical mediators and proceed via corresponding signal transduction cascades. The theory that metastasizing tumors arise from stem cells is not in contradiction to the contribution of genetic and epigenetic alterations as essential contributors to the evolution of a malignant tumor. It only implies that stem cells in the adult organism are the target of genetic and epigenetic alterations.

Metastatic EMT is accompanied by profound changes in gene transcription. Main components are TGFβ and autocrine growth factors. Essential components of signal transduction pathways are receptor tyrosine kinases/Ras, Wnt-, Notch-, Hedgehog-, and NF-κB. *Snail*, a ►master regulator gene in the gastrulation of the zebrafish, is also a master regulator gene in the metastatic EMT. An additional important element of EMT is the downregulation of the cell–cell adhesion molecule E-cadherin that can be achieved via posttranscriptional control, somatic mutation, suppression of gene expression by hypermethylation of the promoter region, histone deacetylation, and transcriptional suppression via special E-boxes in the proximal E-cadherin promoter. An essential transcription factor of E-cadherin repression is Twist. Both Snail and Twist are central regulators of EMT, but do not influence the growth of primary tumors. Thus, these transcription factors are bona fide metastasis genes. EMT is accompanied, concomitantly with the loss of epithelial components and loss of cell polarity, with the activation of mesenchymal gene expression, like vimentin, tenascin, and matrix metalloproteinases that are required during tumor progression. The EMT process predominantly takes place at the invasive front of the primary tumor during emigration of individual tumor cells. Without question, EMT is the central element of tumor progression that does not require genetic alterations, but depends on an intimate interaction between the tumor and the tumor stroma. The assumption that the EMT process is linked to the stem cell nature of the metastasizing tumor cell is supported by the reversibility of the process.

It should be mentioned that besides of the stem cell nature of the metastasizing tumor cells, which allows these cells the activation of genetic programs via so called master regulatory genes, like glycogen synthase kinase 3 (GSK3, Snail, Slug, Twist and others), the role of the epigenom also is coming into focus. The epigenom, chromatin, associated proteins, and the pattern of covalent modification of the DNA methylation, directs the gene expression program. Tumors are characterized by significant alterations in DNA methylation. Hypo- as well as hypermethylation is observed and likely present independent processes, whereby activation of metastasis-associated genes is mostly linked to hypomethylation that contributes to chromosomal instability. Though epigenetic modifications via DNA methylation and covalent histone modifications are not yet utilized as a therapeutic tool, these can well be considered as a future therapeutic option.

The Tumor Stroma and Metastasis

Stem cell, including tumor stem cell maintenance and differentiation essentially depends on the interaction with the surrounding tissue, that can be defined as a microecosystem, that is characterized by significant changes of the system by minimal alterations of any of the individual components, i.e., the tumor cell as well as host cells and the tumor matrix are equally important parameters for tumor progression. One example for the involvement of the surrounding tissue in tumor progression provides the organ preference of metastases, the ►seed and soil theory of Paget (1889) that frequently cannot be explained mechanistically.

Besides the process of angiogenesis, where tumor cells independent of their metastatic potential require a crosstalk with the surrounding tissue, metastasizing tumor cells essentially depend on the surrounding tissue at several steps of the metastatic cascade. These are invasion of the surrounding tissue including the vasculature and the settlement and growth in a secondary organ.

Most elements of both the extracellular matrix and the basal lamina, like collagens, hyaluronic acid, and

proteoglycans, form networks that fulfill static functions by maintaining the tissue structure. However, the extracellular matrix has pores that also allow cell migration. Besides their static function, the extracellular matrix also fulfills dynamic functions by binding to cell membrane anchored receptors that possess signal transducing capacity, and by serving as a depot for growth factors. During tumor progression, the extracellular matrix can become modified to allow for invasiveness. These modifications comprise:

1. Quantitative or qualitative alterations in the components of the extracellular matrix or their cellular receptors
2. Enhanced delivery of cytokines, growth, and motility factors; these factors are frequently bound to elements of the extracellular matrix and are inactive in this form; delivery requires the degradation of the binding element, e.g., by matrix degrading enzymes
3. Regulation of gene expression in the tumor cell by contact with elements of the extracellular matrix, a process important during organogenesis; the reverse process, namely stroma induction by the tumor cell, is of particular importance for the metastatic process

Thus, it is the stroma that is responsible for changes in the composition of the extracellular matrix, for support of cell motility via matrix degrading enzymes and the liberation of bioactive fragments of the extracellular matrix and of growth factors. The changes in the tumor as compared to the normal tissue stroma are called ►desmoplasty, where activated fibroblasts, also called myofibroblasts play a central role. They are essential for EMT and become recruited by the tumor cell via TGFβ. The tumor matrix supports migration and prevents tumor cell apoptosis. The tumor matrix provides the prerequisites for the local invasiveness of tumor cells by the provision of matrix degrading enzymes, like metalloproteinases, ADAM proteinases, bone morphogenetic protein 1 (BMP-1), and serinproteases (uPA, Thrombin, and Plasmin). Notably, too, an important factor for the organ preference of metastasis is the communication between the metastasizing tumor cell and the host tissue via chemokines and their receptors that are shared between metastasizing tumor cells, circulating leukocytes and stem cells for homing in defined organs. Thus, the tumor stroma, particularly activated fibroblasts could well provide a therapeutic target. Candidate molecules are inhibitors in TGFβ- and PDGF-mediated signal transduction.

The Metastatic Cascade: Effector and Suppressor Molecules

The process of metastasis formation commences with the dissociation of the metastasizing cell from the surrounding tumor cell. The network elements involved in this dissociation are homotypic cell–cell adhesion molecules like E-cadherin, whose expression becomes strongly reduced. Thereafter, the isolated tumor cell starts to interact with the surrounding tissue. Effector molecules of the metastasizing tumor cell, which become upregulated or de novo expressed are adhesion molecules, growth factors and their receptors, chemokines and their receptors, and matrix degrading enzymes, some of which are also provided by the tumor matrix. These molecules are required for the invasive process itself that is composed of three steps:

1. Binding
2. Local proteolysis
3. Migration

Adhesion molecules that dominate the interaction of the metastasizing tumor cell with the vascular endothelial cell and the extracellular matrix are integrins, selectins, and CD44. Integrins, particularly those that interact with receptor tyrosine kinases, support internalization of the E-cadherin/β-catenin complex and, thus, reduction in homophilic tumor cell adhesion. Other integrins, particularly the α6β4 integrin contribute to tumor cell motility by its relocalization towards the migrating front of the tumor cell. The αvβ3 integrin contributes to the degradation of the extracellular matrix by associating with membrane-bound metalloproteinases and the urokinase receptor. Selectins mainly contribute to the adhesion of tumor cells to vessel endothelium. CD44 is the main receptor for hyaluronan and for several chemokines. It is of special importance in leukocyte and metastasizing tumor cell migration, and contributes to the activation of survival signals in the isolated, metastasizing tumor cell.

Local proteolysis proceeds via metastatic tumor cell–matrix interaction. Main players are metalloproteinases, the plasminogen activator system (uPA/uPAR), ADAMs, transmembrane proteins with disintegrin and metalloproteinase domains, heparanase that is also important for the settling in the metastatic organ, and molecules that regulate matrix degrading enzymes like tissue inhibitor of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs).

The central elements for migration of the metastasizing tumor cells are cytokines, chemokines and their receptors, as well as changes in the organization of the cytoskeleton of the metastasizing tumor cell. Reorganization of the actin cytoskeleton of the metastasizing tumor cell will be regulated mainly via Rho GTPases. It is important to note that the tumor cell can shift between mesenchymal and amoeboid migration, where the latter is independent of matrix degradation. Chemokines and their receptors are particularly important for the organ-selectivity of

metastasis formation. This has, besides others, been demonstrated for lymph node, lung, and bone marrow metastases of mammary carcinoma via CXCR4 and its ligand SDF-1 and for bone metastasis via osteopontin.

Taken together and as outlined above for the metastatic MET and its reversion, which proceeds through the reactivation of master regulator genes of developmental programs, the metastatic cascade, too, is not a consequence of genetic alterations of the contributing molecules. Instead, quantity, distribution, and posttranslational modifications become modulated.

The definition of metastasis suppressor genes is functional and based on the assumption that interference with any single step in the multistep process of the metastatic cascade should suffice for interruption. Accordingly, metastasis suppressor genes should hamper metastasis formation without having an impact on growth of the primary tumor. So far 12 metastasis suppressor genes have been identified. Most of the metastasis suppressor genes act at the step of settlement in secondary organs. The molecules frequently block essential signal transduction pathways. Yet, so far, the function of none of the metastasis suppressor genes could be fully unraveled.

In conclusion, metastasis formation is the result of a reversible activation of master regulatory genes-initiated programs of embryonic and adult stem cells. Thus, it is most likely that metastases arise from tumor stem cells. Progression through the metastatic cascade requires concerted activities of the tumor cell and the surrounding tissue, where the tumor cell influences the tumor stroma and vice versa.

Clinical Relevance

Conventional therapeutic regimen, in most instances, can cope with the primary tumor. Cancer mortality, therefore, is mainly due to the formation of metastasis. Tumor progression by silencing or activation of unaltered genes via master regulatory genes of stem cell programs supported by the interplay between the tumor stem cell and the tumor matrix will allow for the elaboration of new therapeutic, physiology-based protocols. Though research in the field of metastasis progression has most significantly progressed in the last years, (tumor) stem cell programs are just beginning to become unraveled. Also, the mutual impact of tumor and tumor stroma requires further exploration. The same accounts for epigenetic alterations in metastasizing tumor cells. Thus, there is promise for metastasis-selective therapeutics, that could not have been thought about before. Although further progress is required, highly encouraging observations in individual patients support the concept of intervention with metastasis formation by biological targeting.

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Progressive Myoclonus Epilepsy

Definition

PME; Is a syndrome involving the central nervous system representing a number of different diseases, which share muscle contractions and seizures. The rate of progression of PME may be quick or slow, depending on the underlying disease.

►Stefins

Prohormone Convertase

►Furin

Prolactin

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Synonyms

Mammotropin; Mammotropic hormone; Luteotropin; Luteotropic hormone

Definition

Prolactin is a 23 kDa protein hormone secreted by the anterior pituitary gland in many species. Although it has many reported biological activities, prolactin's most noted effects pertain to mammary gland differentiation and promotion of lactation.

Characteristics

Pituitary Prolactin Secretion and Its Regulation

The pituitary gland is located at the base of the brain and is composed of two parts – anterior and posterior. The posterior pituitary is an extension of brain tissue from the ►hypothalamus and secretes hormones such as vasopressin, which are synthesized in the hypothalamus. The anterior pituitary arises from non-neural tissue and during embryology migrates to a position anterior to the posterior pituitary. The anterior pituitary is connected to the hypothalamus by a network of blood vessels that run with the neural "stalk" connecting the posterior pituitary to the hypothalamus. This hypophyseal-portal circulation bathes the anterior pituitary with hypothalamic factors that regulate secretion of a number of hormones; these anterior pituitary hormones include factors that control thyroid activity (TSH; thyroid stimulating hormone), adrenal gland activity (ACTH; adrenocorticotropic hormone), gonadal (ovarian or testicular) function (LH and FSH; leutinizing hormone and follicle stimulating hormone, respectively), and growth (GH; growth hormone). Additionally, prolactin is secreted by the lactotrophic cells of the anterior pituitary. ►Dopamine, produced in the hypothalamus and carried by the hypophyseal-portal circulation to the lactotrophs, inhibits the production of prolactin.

Prolactin Structure and Activation of Cellular Signaling

Human prolactin is secreted as a protein of 199 amino acids. Structurally, prolactin is comprised of four helical bundles with loops of varying length connecting these bundles. This general structure is similar to a large group of hormones and cytokines, including growth hormone, erythropoietin, leptin, and various interleukins and interferons. Prolactin interacts with the cell surface transmembrane prolactin receptor on target cells by virtue of two binding sites on prolactin that are formed three dimensionally by folding of the loops and bundles. Prolactin receptor binds prolactin in its extracellular domain and transmits signals to the inside of the cell by virtue of regulated interactions of the receptor intracellular domain with protein members of several signaling systems. The receptor encodes no enzymatic activity; rather, prolactin causes activation of the receptor-associated ►tyrosine kinase, ►JAK2 and thus stimulates pathways including the ►MAP kinase cascade and ►STAT (signal transducer and activator of transcription) 5 (►signal transducers and activators of transcription in oncogenesis; ►signal transduction). These pathways ultimately lead to changes in cellular behavior and expression of prolactin-specific genes related to prolactin's biological effects at breast and other target tissues.

Biological Effects of Prolactin

In mammals, prolactin's main effects relate to milk production. Prolactin stimulates mammary gland differentiation in pregnant females in concert with other hormones that are required for its optimal effects. Knockout studies in mice indicate that prolactin, via its receptor, exerts its role in mammary morphogenesis by regulating ductal side branching and terminal end bud regression and by directly causing lobuloalveolar development in pregnancy. Several milk protein genes are also regulated by prolactin. In humans, prolactin levels rise during pregnancy, thereby preparing the mammary gland for postpartum lactation. Initiation and maintenance of lactation are also dependent on prolactin and prolactin receptor. This function is also served by prolactin in some nonmammalian species; for example, nursing pigeons utilize prolactin to induce cropmilk in the cropsac.

In addition to its effects on mammary tissue, prolactin also affects the functions of other organs and systems. Gonadal functions are affected by prolactin both indirectly and directly. Prolactin elevation suppresses hypothalamic production of gonadotropin releasing hormone and thereby pituitary gonadotropin secretion, resulting in suppressed gonadal function. Although less profound than its reproductive functions, prolactin also exerts immunomodulatory, psychological, and osmoregulatory effects. In particular, modulation of water and electrolyte balance is an important effect in nonmammalian species such as fish and reptiles. Notably, secretion of prolactin from nonpituitary sites has become increasingly appreciated. Cell types known to produce prolactin include those found in reproductive tissue (gonads, uterus, prostate, and mammary gland itself), immune cells (B-cells, mononuclear cells, natural killer cells, and thymic epithelial cells), skin, kidney, and adrenal. The significance of this extrapituitary prolactin secretion is incompletely understood.

Disease States Related to Prolactin

Hyperprolactinemia and Prolactinoma

Normal levels of prolactin in adult humans vary and can be as high as 25 μg/l in nonpregnant females and 20 μg/l in males. As mentioned above, levels rise dramatically (roughly 10-fold) through normal pregnancy. Prolactin levels can be abnormally elevated (hyperprolactinemia) in a number of clinical circumstances. For example, certain medications

(e.g. antipsychotic agents, metoclopramide, and others) can cause hyperprolactinemia by virtue of reduction of dopamine delivery or antagonism to dopamine action at the lactotroph. Stress and chest wall stimulation can also raise prolactin, as can primary thyroid failure (by raising levels of hypothalamic thryotrophin releasing hormone, which can stimulate prolactin secretion by lactotrophs). These situations typically do not cause elevations of prolactin of sufficient magnitude to cause symptoms.

Greater degrees of hyperprolactinemia (usually more than 100 μg/l) may cause symptoms. In women, these include ►amenorrhea (with ►secondary hypogonadism), infertility, and ►galactorrhea. In men, the secondary hypogonadism of hyperprolactinemia manifests as impotence, decreased libido, and infertility. These greater degrees of hyperprolactinemia are typically caused by tumors at or in the region of the anterior pituitary/hypothalamus in the midline. Tumors that impinge upon the pituitary stalk (such as craniopharyngioma or non-lactotroph pituitary tumors) or infiltrative diseases that affect the stalk can cause hyperprolactinemia by inhibiting dopamine delivery to the lactotroph. Typically, the non-lactotrophic tumors (for example, growth hormone-, ACTH-, or non-secreting tumors) that cause this effect are non-malignant adenomas, but are large enough to exhibit suprasellar extension to the region of the stalk. By definition, these are usually macroadenomas (>1 cm in size) and they can be associated with symptoms related to the hypersecretion of the hormones originating from the tumor as well as symptoms of the intracranial mass and its extensions, such as headaches, visual field disturbance (from extension superiorly to the optic chiasm), and extraocular movement dysfunction (from extension laterally into the cavernous sinuses and impingement on the cranial nerves governing eye movement). In addition to these effects, growth hormone-secreting tumors may themselves cosecrete prolactin, suggesting somatotrophs (growth hormone-secreting cells) and lactotrophs may share common origins.

Lactotrophic tumors (prolactinomas) themselves account for the majority of pituitary tumors (roughly 40% of all pituitary adenomas) and have an estimated prevalence of 100/million population. The vast majority of prolactinomas are adenomas. Prior to the sixth decade of life, prolactinomas are much more common in women (roughly 10:1 female:male), but the distribution between the sexes is roughly equal thereafter. Most prolactinomas in women (therefore most prolactinomas in general) are microadenomas (<1 cm in size) and therefore do not themselves usually extend outside the ►sella turcica to cause mass effect symptoms. The same is not so in men, who often present with macroprolactinomas.

In distinction to other pituitary tumors that are primarily approached therapeutically with surgery or radiotherapy, prolactinomas are often treated medically with dopamine agonist drugs. These include bromocriptine and cabergoline, orally active ergot alkaloids that interact with dopamine receptors at the pituitary and elsewhere. Bromocriptine is shorter acting and typically administered more frequently than cabergoline, which is given only several times per week. Dopamine agonists are usually quite potent in reduction of hyperprolactinemia of both tumorous and nontumorous etiologies. Prolactinomas often also shrink in size in patients treated with these drugs. Surgery and radiotherapy are reserved as options for patients unresponsive to medical therapy, but are not typically as effective as dopamine agonist therapy.

Prolactin and Breast Cancer

While controversial, substantial literature suggests a role for prolactin, possibly via autocrine/paracrine effects, in human cancers, including ►breast cancer. Clinical trials with dopamine agonists to reduce circulating prolactin in women with breast cancer have not succeeded, yet epidemiologic data suggest increased serum prolactin levels correlate with breast cancer risk in postmenopausal women. Prolactin and its receptor are expressed in nearly all breast cancer samples and some studies reveal more prolactin receptor in cancerous tissue vs. normal surrounding tissue. Transgenic overexpression of prolactin results in mammary tumor formation and T47D human breast cancer xenografts in nude mice respond to PRL with increased tumor growth *in vivo*. This area of research is clearly important and may yield major insights into breast cancer biology and treatment. However, there is as yet no clear consensus on the role of prolactin in breast cancer etiology or behavior.

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Proliferation

Definition

An increase in the number of cells as a result of cell growth and cell division. The expansion in numbers of a cell population.

►Sprouty

Proline

Definition

One of the common amino acids that is a single building block for peptides and proteins.

Promoter

Definition

A region upstream from a gene acting as a controlling element for the expression of that gene. It is bound by an RNA polymerase and determines the transcription start site. A constitutive promoter is permanently active in cells. A conditional or inducible promoter is only active when specific inducers (e.g. hormones) are present.

►Prostate-Specific Membrane Antigen (PSMA)

Promoter Hypermethylation

Definition

Transcriptional silencing of a gene via ►methylation of ►5′ CpG islands in a gene's ►promoter region. Promoter hypermethylation represents an epigenetic mechanism that can act as an alternative to mutations to disrupt tumor-suppressor gene function and can predispose to genetic alterations through the inactivation of DNA-repair genes.

►Epigenetic Gene Silencing

Promotion

Definition

In ►carcinogenesis, ►Tumor Promotion.

►Initiation and Promotion

β Propeller

Definition

A protein domain characterized by 4–8 blade-shaped beta sheets arranged toroidally around a central axis. Each sheet has four antiparallel β-strands.

►APAF-1 Signaling

Prophylactic Mastectomy

Definition

Removal of the non-diseased breast to reduce the risk of cancer.

►Oncoplastic Surgery

Prophylactic Vaccine Therapy

Definition

Is a form of active ►immunotherapy mediated predominantly by the humoral immune system, which causes the destruction of disease agents residing in the

body of the immunized host thereby preventing the onset of disease. Once stimulated, the immune system maintains surveillance against subsequent infection or disease development involving the targeted antigen. Prophylactic vaccines can limit the development of existing diseases as well as preventing their recurrence.

Proproteins

Definition

Are latent precursors that require proteolytic cleavage for the removal of the pro-part and for generating mature, functionally active, proteins.

2-Propylpentanoic Acid

►Valproic Acid

Prosome

Definition

►Proteasome

Prospective Study

Definition

Study type in which one or more groups (cohorts) of subjects who have not yet had the outcome event in question (e.g. cancer diagnosis) are identified and then followed forward in time. A study or clinical trial that looks forward in time, in which participants are identified and then followed over a period of time to find associations between certain factors and a particular outcome.

►Mutagen Sensitivity

Prostaglandins

Definition

Are a group of hormone-like molecules derived from arachidonic acid and mediate a wide range of physiological functions, such as control of blood pressure, contraction of smooth muscle, gastrointestinal function, pain, and inflammation. The prostaglandins together with the thromboxanes form the prostanoid class of fatty acid derivatives. ►Cyclooxygenases are responsible for prostanoid formation. The prostanoid class is a subclass of ►eicosanoids.

►Anti-Inflammatory Drugs
►Arachidonic Acid Pathway

Prostaglandins

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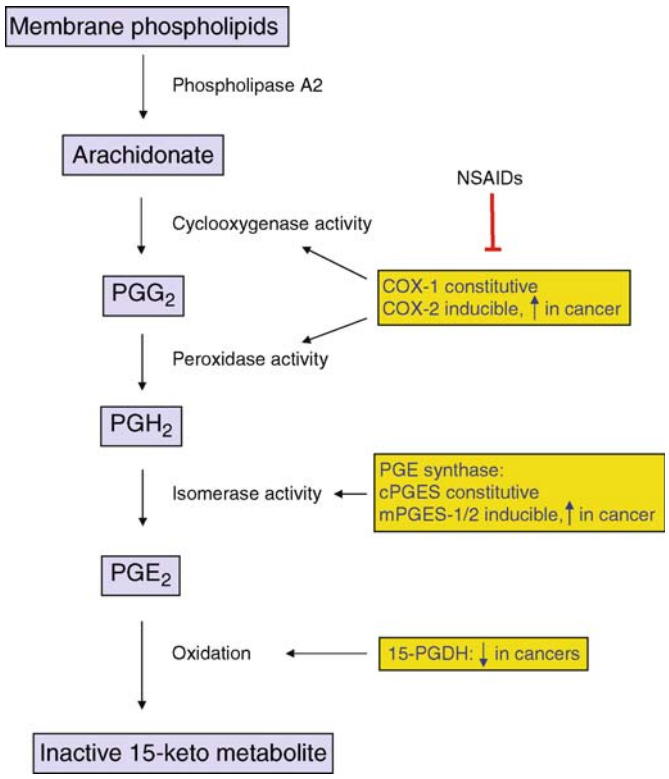
Definition

Prostaglandins are lipid compounds, which are synthesized from arachidonic acid. They are expressed in most tissues and participate in a wide array of physiologic activities, but are also involved in the pathogenesis of various malignancies. Prostaglandins levels are regulated by the balance between their metabolism, which is mainly regulated by the ►cyclooxigenase (COX) enzyme, and their degradation, which is mainly regulated by the activity of the enzyme 15-hydroxyprostaglandine dehydrogenase (15-PGDH). Following their synthesis, the prostaglandins interact with specific ►G protein coupled receptors and activate signaling pathways.

Characteristics

Biosynthesis

The first step in prostaglandin synthesis is the hydrolysis of ►arachidonic acid, by the enzyme ►phospholipase A2, from cell membrane phospholipids and the formation of free arachidonate (Fig. 1). The next two steps of prostaglandins synthesis are mediated by the COX enzymes. The cyclooxygenase activity of COX mediates the insertion of oxygen to arachidonate and the formation of prostaglandin G_2 (PGG_2), which in turn is



Prostaglandins. Figure 1 Schematic representation of PGE_2 biosynthesis.

converted to prostaglandin H_2 (PGH_2) by the peroxidase activity of COX. These COX activities are considered to be the main regulatory steps in prostaglandin synthesis. The final step of prostaglandins synthesis is the isomerization of PGH_2 into various prostaglandins, such as prostacyclin and thromboxane. This process is mediated by specific prostaglandin synthases. For example, the conversion of PGH_2 to prostaglandin E_2 (PGE_2), the major prostaglandin implicated in carcinogenesis, is mediated by PGE synthase. The prostaglandins, prostacyclin and thromboxane, are rapidly metabolized by initial oxidation into nonactive 15-keto metabolites. The first and rate-limiting step in this inactivation process is mediated by the enzyme 15-PGDH.

Regulation of Prostaglandins Synthesis and Degradation

Prostaglandin levels are determined by the balance between the expression of the rate limiting enzymes involved in their synthesis (COX) and in their degradation (15-PDGH). Two COX isoforms are currently known: COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and mediates various physiologic activities, including platelets aggregation, protection of gastric mucosa, and regulation of renal blood flow. On the other hand, COX-2 is an inducible enzyme, which is undetectable in most normal tissues but is expressed in activated ►macrophages, as well as in other cells, in response to ►inflammatory cytokines, (includes tumor necrosis factor α (TNF α) and ►interleukin 1β (IL-1β)), hormones, and growth factors. The COX enzymes are the targets of the ►nonsteroidal anti-inflammatory drugs (NSAIDs). While some of these drugs inhibit both COX-1 and COX-2, others can selectively inhibit the inducible COX-2. PG synthases may also play a regulatory role in prostaglandins synthesis. For example, three PGE synthases are currently known, and while one of them, cytosolic PGE synthase (cPGES) is not inducible and is associated with COX-1 activities, another, microsomal PGE synthase-1 (mPGES-1), is inducible in response to inflammatory cytokines and mitogenic stimuli and is coupled to COX-2 activity. 15-PGDH, is a major regulator of prostaglandins levels and, similar to COX, its levels can be regulated by various stimuli, often in the opposite direction to that of COX. For example, TNF α and IL-1β simultaneously decrease 15-PGDH levels and increase COX-2 levels.

Prostaglandin Signaling Pathway

The prostaglandins have a relatively short half-life and are thought to act over short distances in either an autocrine (the same cell) or paracrine (adjacent cells)

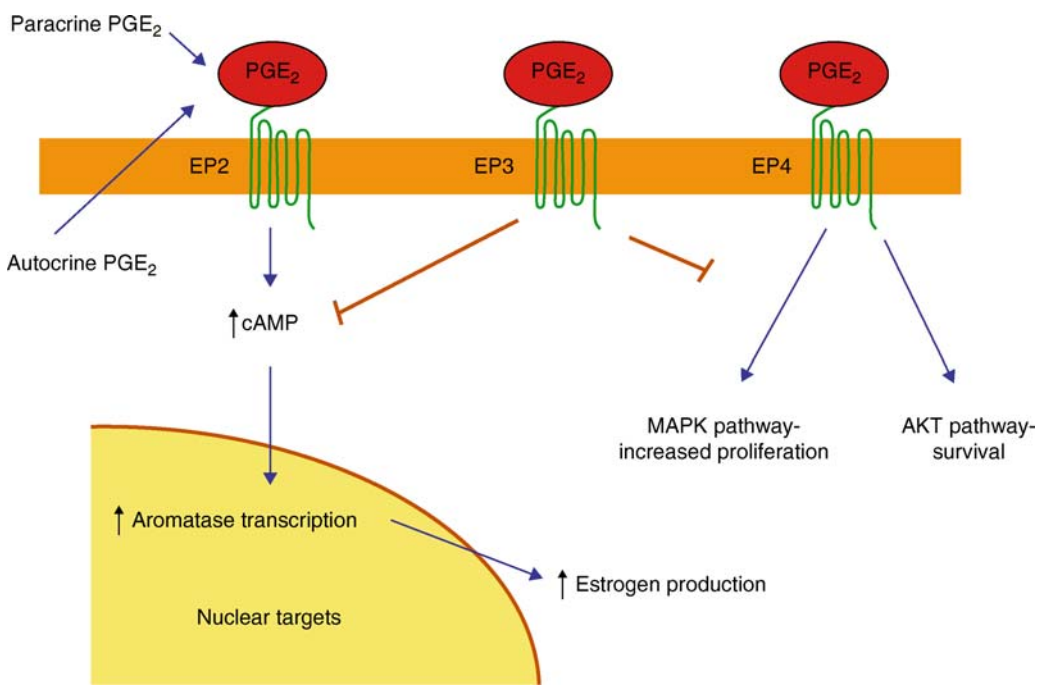
manner. Prostaglandin signaling is initiated by their binding to specific receptors, which belong to the family of seven transmembrane G protein coupled receptors. These receptors are designated according to their ligand. Thus, EP is the PGE_2 receptor and TP is the thromboxane (TXA_2) receptor. Four PGE_2 receptors, EP1, 2, 3, and 4, are currently known and differ in their downstream targets. EP1 signaling is associated with phospholipase C/inositol triphosphate pathway and elevation of intracellular calcium, EP2 and EP4 activity is associated increased cyclic AMP (cAMP), and EP3 is considered to be inhibitory and its activity is associated with decreased cAMP levels. Several downstream targets of EP activity have been identified and include the ►mitogen activated protein kinase (MAPK), ►AKT, and the ►PI3K/β-catenin pathways. A schematic representation of some of the EP activities is shown in Fig. 2.

Prostaglandin Signaling Pathway in Cancer

While early studies focused on prostaglandin levels in tumors, the cloning of the COX-1 and COX-2 enzymes during the late 1980s have shifted the interest to studies regarding their expression and activity in tumors. Recent studies investigated the role of additional components of the prostaglandin pathway (e.g., 15-PGDH, the EP receptors) in carcinogenesis.

The major prostaglandin implicated in tumorigenesis is PGE_2, which is synthesized either by the tumor or by adjacent cells. Elevated PGE_2 levels were identified in various tumors, including ►colon and ►breast cancers. PGE_2 upregulation is associated with a vast array of promalignant activities. Several signaling pathway mechanisms can be activated by PGE_2 in cancer. For example, in colorectal cancer the PGE_2 pathway can interact with the ►epidermal growth factor (EGF) pathway and activate the EGF receptor, thus leading to activation of its downstream targets, the MAPK and the AKT pathway. Activation of these pathways increases proliferation and ►invasion, inhibits ►apoptosis and promotes ►angiogenesis. In breast cancer, PGE_2 has also been shown to induce expression of tumor aromatase, which catalyzes estrogen synthesis, thus leading to upregulation of estrogen levels. As PGE_2 is a small molecule, which is rapidly metabolized, measuring its level in tumors is complicated and is not done routinely.

Ample data implicate COX-2 in carcinogenesis. COX-2 levels can be evaluated relatively easily in tumors, using either immunohistochemical staining or mRNA measurements by PCR and, importantly, COX-2 levels in tumors often correlate with PGE_2 levels. Increased amounts of COX-2 are commonly found in premalignant, as well as malignant tumors, including colorectal polyps and cancer, ►ductal carcinoma in situ and breast cancer, ►glioblastoma, ►endometrial cancer, and ►prostate cancer. Several mouse models further support the major role of COX-2



Prostaglandins. Figure 2 Schematic representation of various PGE_2 downstream pathways.

in tumor development. For example, COX-2 expression in the mammary gland was sufficient to induce cancer, while COX-2 knock-down in a mouse model of familial adenomatous polyposis reduced the number of polyps. Laboratory studies revealed that COX-2 inhibition by NSAIDs slows the growth of various cancer cells, and prevent cancer development both in vitro and in vivo. Finally, epidemiological data, as well as controlled trials, demonstrated the ability of NSAIDs, and especially of selective COX-2 inhibitors, to prevent the formation of colorectal adenomas. However, recent concerns regarding the safety of COX-2 inhibitors and the association between their use and increased incidence of cardiovascular events reduced the enthusiasm for their use as chemopreventive agents.

EP expression pattern and activities in cancer is complex and despite their pivotal role in the prostaglandin signaling, data regarding their function in carcinogenesis is limited. Different cancer models suggest a tumor promoting role for EP1, EP2, and EP4, but a tumor suppressor role for EP3. For example, in breast cancer EP1 and EP2 induce, while EP3 inhibits aromatase expression; and in different colon cancer models EP1, EP2, or EP4 has been associated with carcinogenesis. Higher EP1, EP2, and EP4 expression was noted in prostate cancer compared to normal prostate tissue, and EP1 expression was also associated with more aggressive tumor features. As the EP isoforms show specific activities and may have unique expression pattern in tumors, their targeted inhibition may serve as a novel therapeutic modality for malignant diseases. Several specific inhibitors of the EP isoforms have been developed and are now being tested in preclinical studies.

mPGES-1, the inducible isoform of PGES, has also been implicated in tumorigenesis. In several cancer models, high mPGES-1 expression was associated with increased PGE_2 levels. Moreover, high mPGES-1 levels were noted in colon, breast, lung, and ovarian cancers; and in some of these tumors were associated with more aggressive features. Laboratory data suggest that mPGES-1 inhibition downregulates PGE_2 levels and may be associated with growth inhibition. Thus, mPGES-1 may be a selective target for blocking the PGE_2 pathway. No selective mPGES-1 inhibitors are currently available.

Recent data indicate 15-PGDH, the key PGE_2 catabolic enzyme, as a tumor suppressor gene in various malignancies, including colorectal, breast, and lung cancers. Low 15-PGDH levels were identified in these tumors and upregulation of its levels in cancer cells reduced PGE_2 levels and inhibited their growth. Moreover, low 15-PGDH expression may also be associated with adverse prognostic factors. DNA methylation of the 15-PGDH promoter may play a role in its silencing in cancer.

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Prostanoid

Definition

Refers to a subclass of ►eicosanoids consisting of: the ►prostaglandins, the thromboxanes, and the prostacyclins.

►Arachidonic Acid Pathway

P

Prostate Cancer, Clinical Oncology

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Definition

Prostate cancer is a malignant neoplasm that arises in the male prostate gland. Most prostate cancers are adenocarcinomas (95%). About 4% have transitional cell morphology and are thought to arise from the lining of the prostatic urethra. Few have neuroendocrine morphology and are believed to arise from the neuroendocrine stem cells normally present in the prostate or from aberrant differentiation programs during cell transformation. Of the adenocarcinomas, 70% arise in the peripheral zone, 15–20% in the central

zone and 10–15% in the transition zone. These zones can sometimes be identified by transrectal ultrasonography (TRUS). Most adenocarcinomas are multifocal with synchronous involvement of multiple zones of the prostate and this may be due to clonal tumors that have spread intraprostatically or and mutiple tumor initation sites intraprostatically. In addition, multifocality may indicate a more aggressive tumor biology. The most commonly used system of classifying histiologic characteristics of prostate adenocarcinomas is the Gleason score, which is determined by the glandular architecture within the tumor at low magnification.

The predominant pattern, as well as the second most common pattern is given a grade from 1 to 5 (less malignant to more malignant). Grades are based on the extent to which the epithelium assumes a normal glandular structure. Grade 1 indicates a near normal pattern and grade 5 the absence of any glandular pattern. The sum of these two grades is referred to as the Gleason score. This scoring method was found to be superior in predicting disease outcomes when compared to using the individual grades alone. A score of 2–4 is considered low grade, 5–7 is considered moderate grade and 8–10 is considered high grade. A Gleason grade 7 can consist of 3 + 4 or 4 + 3 disease; however, a predominat grade 4 pattern tumor has a significantly worse prognosis than a predominant grade 3 pattern. High grade prostatic intraepithelial neoplasia (PIN) represents the putative precancerous end of the morphologic continum of cellular proliferations within prostatic ducts, ductules and acini. Two grades of PIN are identified (low grade and high grade), and high grade PIN may be a precursor to invasive adenocarcinoma. The continum which culminates in high grade PIN and early invasive cancer is characterized by basal cell layer disruption, basement membrane disruption, progressive loss of secretory differentiation markers, increasing nuclear and nucleolar abnormalities, increasing proliferative potential and increasing variation in DNA content (aneuploidy). Clinical studies suggest that PIN may predate carcinoma by up to 10 years or more; however, PIN need not be present for carcinoma to arise. The clinical importance of recognizing PIN is based on its strong association with carcinoma and hence its identification in prostate biopsy specimens warrants further search for concurrent invasive carcinoma. In patients with high grade PIN, the risk of cancer on subsequent biopsy is 30%. Low grade PIN should not be commented on in diagnostic pathology reports because of variability distinguishing low grade PIN and benign prostate tissue and patients diagnosed with low grade PIN on needle biopsy are at no greater risk of having carcinoma on repeated biopsy that are men with a benign biopsy finding. Atypical small acinar proliferation (ASAP) represents findings suggestive of but not diagnostic of carcinoma. The incidence of atypical needle biopsy specimens ranges from 5% to 8% and may be termed "atypical hyperplasia" or "atypical small acinar proliferation." The risk of cancer on subsequent biopsy after atypical diagnosis is 42–49%, therefore, all patients with an initial atypical diagnosis on needle biopsy should all undergo a repeat biopsy.

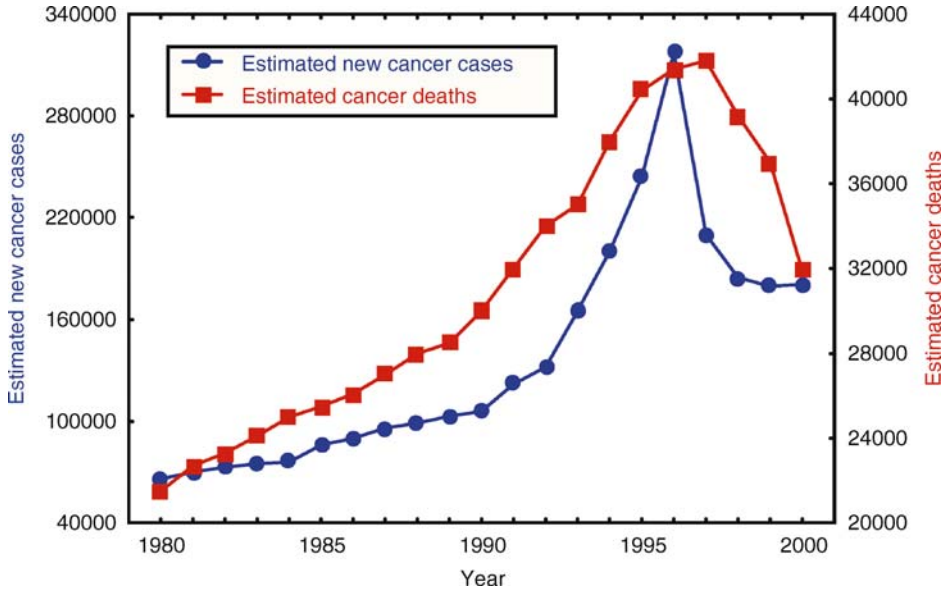
Characteristics

Clinical Epidemiology and Risk Factors

Prostate cancer is the fourth most common male malignant neoplasm worldwide. In the United States, prostate cancer incidence rates remain significantly higher in African American men than in Caucasian men. Age adjusted prostate cancer specific mortality is 2.4 times higher for African Americans than for Caucasians. Between 1989 and 1992 prostate cancer incidence rates increased dramatically, due to earlier diagnosis in men without any symptoms through the increased use of the ►prostate-specific antigen (PSA) blood test. Prostate cancer incidence rates are currently declining among all men. During 1992–1996, prostate cancer mortality rates declined significantly by 2.5% per year (Fig. 1). Prostate cancer's potential long natural clinical history is reflected in tumors found during autopsies carried out following other causes of death. The incidence of latent or autopsy cancer is much greater than cases of clinical cancer. It may reach up to 80% by age 80. Interestingly, incidence of the latent form of the disease is similar worldwide, while the incidence of clinical cancer is high in northern Europe and North America, intermediate in southern Europe, Central and South America and low in eastern Europe and Asia. In addition to changes in prostate cancer incidence and mortality since the incorporation of the PSA serum test into routine clinical practice, there has been a significant shift to more favorable stage at presentation in men with newly diagnosed prostate cancer. Nonpalpable cancers diagnosed exclusively through elevated PSA and subsequent ultrasound-guided biopsy now account for 75% of new cases. The effective treatment of prostate cancer and biologic indolence relative to life expectancy of some prostate cancers has contributed to an overall disease specific mortality of only 16%. However, given the long natural history of prostate cancer, the treatment of low stage cancers detected during the PSA era may have an effect on mortality statistics for 10 to 15 years, if ever. The Prostate, Lung, Colorectal, and Ovary (PLCO) cancer trial and the European Randomized Screening for Prostate Cancer (ERSPC) trial are two ongoing large-scale randomized trials that are designed to evaluate the effectiveness of screening for prostate cancer.

Etiology

Genetics and environment have been implicated as etiologic factors in the origin and evolution of prostate



Prostate Cancer, Clinical Oncology. Figure 1. New cancer cases and deaths in the United States of America. Data compiled from 'Cancer Statistics' issues 1980–2000 (published by the American Cancer Society).

cancer. Alteration of genes on chromosome 1 and on the X chromosome have been found in many patients with a family history of prostate cancer. In addition, genetic studies suggest that strong familial or hereditary predisposition may be responsible for up to 15% of prostate cancers. The relative risk of prostate cancer increases according to the number of affected family members and the age at which they were affected. In the US, African Americans have a higher incidence of the cancer when compared to Caucasian men, which in turn have a higher incidence than men of Asian origin. Whether tumor aggressiveness is higher in African American men is unclear. A high fat diet may lead to increased risks while a diet rich in soy protein may be protective and may account for the low incidence of clinical forms of this cancer in Asia. Hormonal causes have also been postulated since androgen influences development and maturation of the prostate and ablation causes regression of prostate cancers. Recent evidence suggests a link with an infectious agent and multiple studies have reported a significant association of prostate cancer with a history of sexually transmitted infection (Fig. 2).

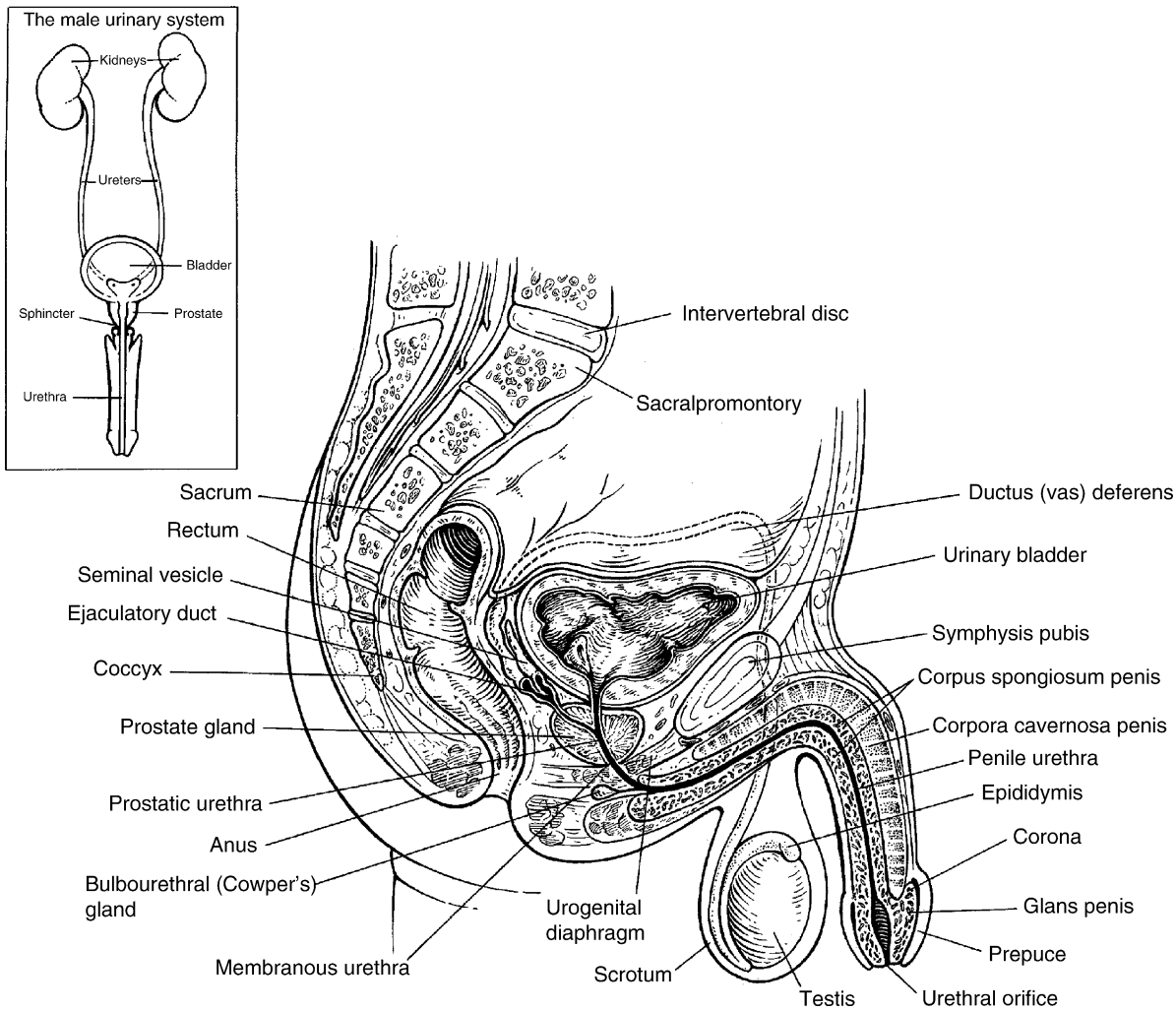
Tumor Biology and Genetics

Genetic alterations in the ►tumor suppressor genes RB1 and ►TP53 are associated with advanced metastatic or hormone refractory prostate cancer. In addition, alterations in the oncogenes ►MYC, ERBB2, and BCL2 have also been observed in advanced prostate cancer. Lack of expression of glutathione S-transferase, a protective enzyme against reactive oxygen species, and ►E-cadherin, a cell adhesion molecule, are correlated with cancer progression. Loss of p27 expression, a cell cycle regulatory inhibitor, is associated with an increased risk of biochemical failure following radical prostatectomy. Loss of PTEN, a tumor suppressor gene, is seen in advanced cancers. Vascular endothelial growth factor (VEGF), a mediator of tumor ►angiogenesis, has increased expression in metastatic tumors.

Clinical Presentation

Incidental/Screening findings:

- Elevated prostate specific antigen (PSA). The PSA threshold for that most efficiently lead to the detection of life-threatening cancer while avoiding unnecessary testing and over diagnosis is not known, but 4.0 ng/ml for men older than 50 years has been accepted by most clinicians. In addition, PSA velocity of more than 0.75 ng/ml per year is positively associated with the risk of prostate cancer over a 7 year period for men with PSA < 4.0 ng/ml. In men with a serum PSA between 4 and 10 ng/ml, using criteria of free PSA less than 25% would lead to a detection of 95% of cancers while avoiding 20% of unnecessary biopsies.
- Tissue removed at the time of transurethral resection for benign hypertrophy of the prostate (BPH).
- Abnormal digital rectal examination (DRE).
- The combination of DRE and serum PSA is the most useful first line test for assessing risk of prostate



Prostate Cancer, Clinical Oncology. Figure 2 Anatomy of the male genitourinary system.

cancer and is generally recommended for screening of populations.

Local findings:

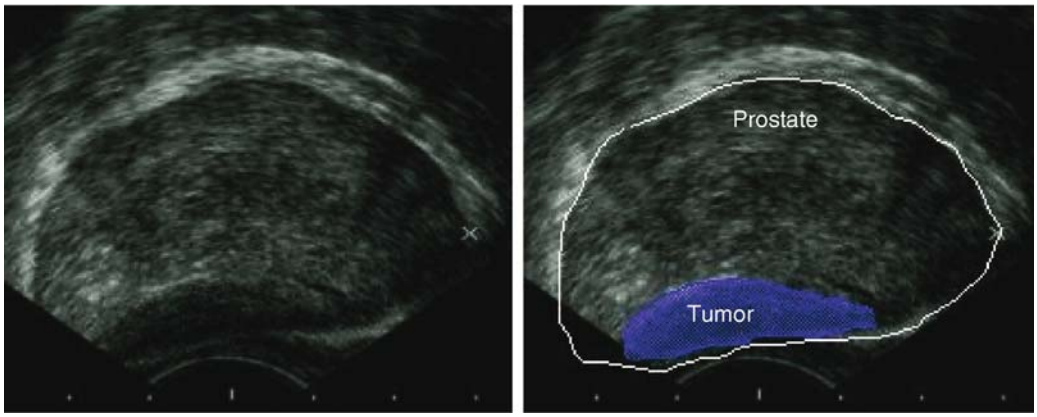
- Hematuria.
- Incontinence.
- Urinary retention due to bladder outlet obstruction with associated hydronephrosis and renal failure.

Metastatic findings:

- Weight loss and loss of appetite.
- Bone pain with or without pathologic fracture.
- Lower extremity pain and edema from nodal metastasis obstructing venous and lymphatic tributaries.
- Uremic symptoms from ureteral obstruction either due to local prostate growth or retroperitoneal adenopathy secondary to nodal metastasis.
- Neurological compromise from bony metastases to spine.
- Following in frequency after lymph nodes, bones, and lung, the next most common regions for metastatic spread include bladder, liver, and adrenal gland.

Diagnosis and Staging

Transrectal ultrasound (TRUS) is used to examine the prostate (Fig. 3) and is usually carried out in the office setting. Historically, it was believed that all cancers appear as hypoechoic (low echoes on the ultrasound, compared to normal prostate). However, more recent data indicates that such areas, even when found are not specific enough for diagnostic purposes. Therefore, a systematic approach to prostate biopsy was developed to sample most areas of the gland, irrespective of ultrasonographic abnormalities. In this approach,



Prostate Cancer, Clinical Oncology. Figure 3 Transrectal ultrasound of the prostate.

TRUS is used to guide the performance of 8–13 core needle biopsies of the prostate. The biopsies are taken from the peripheral zone and transition zone and directed laterally from the base and mid-gland bilaterally. Recently, several groups have shown extensive systematic biopsies, called "saturation biopsy," incorporating on average 22 cores improve cancer detection, especially in the setting of repeat biopsies in select patients with high suspicion of cancer. However, saturation biopsy requires local, regional or general anesthetic. In addition, the clinical signficance of these additionally detected tumors is unknown. Complications of TRUS guided biopsy include, hematospermia (9–50%), urinary tract infection (2%), and acute urinary retention (0.7%). Currently, advanced ultrasonographic techniques, including color and power Doppler TRUS using vascular contrast agents are being evaluated to selectively visualize prostate cancers based on the presence of increased tumor angiogenesis.

Once a cancer is found a staging work-up is undertaken. It includes:

- Laboratory work-up: determination of PSA (prostate specific antigen) level if not already done.
- Imaging work-up: Work-up depends on the clinical staging.

Clinical stage of primary lesion, as determined by DRE (digital rectal examination), PSA level and Gleason score of the primary lesion, as determined by TRUS guided biopsy. For example, increased clinical stage of the primary, PSA level >10 and Gleason score >7 are correlated with increased risk of extra-prostatic spread and are considered to be the key factors in determining the need for staging work-up. PSA levels <10 with low or moderate grade histology with no findings on DRE exam may proceed on to treatment. PSA levels >10 with high grade histology or DRE findings suggesting stage T3 disease should undergo staging CT scan and bone scan (Fig. 5). MRI is superior to bone scan in evaluating bone metastasis, but is so far relatively impractical for routine total body surveys. Instead it is used to determine the etiology of questionable lesions found on bone scan. Use of MRI to stage or determine the location of the local lesion is controversial. Prostate specific membrane antigen (PMSA) immunoscintigraphy with the indium-labeled immune conjugate CYT-356 (ProstaScintTM) has also been used for the detection of prostate cancer metastases, especially to the lymph nodes. The 1997 UICC/AJC TNM staging system is most commonly used (Table 1).

Management

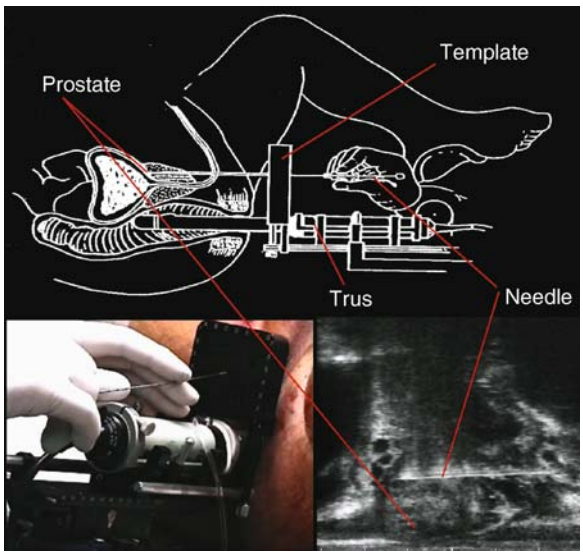
The stratification of patients to particular treatments is based on the evaluation of the patients life expectancy and the biological aggressiveness of the tumor. The biopsy grade, clinical stage PSA level and, when available, the results of imaging studies can provide such prognostic information. Treatments such as active surveillance, hormonal therapies and potentially curative therapies such as radiation and surgery are available. Treatment selection also requires a thorough analysis of the risks and benefits of each option by the patient, enabling him to have input on the therapy selection.

1. Early localized disease (T1-2N0M0): A multitude of treatment modalities are available that include active surveillance, watchful waiting, radiation therapy, and surgery.
   (a) Active surveillance is a program of regular examinations and PSA and DRE monitoring to distinguish clinically insignificant cancers from life-threatening cancers with intent to administer curative therapy. Watchful waiting is defined as delaying active treatment until patient develops symptomatic disease progression in order to limit morbidity from the disease and therapy without the goal to administer curative treatment.

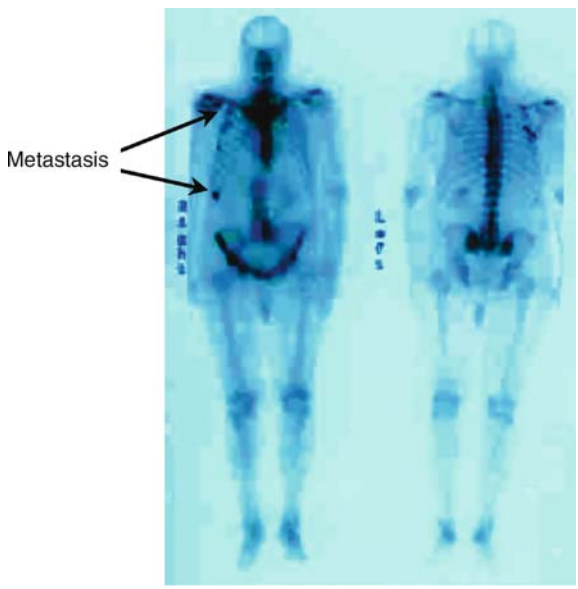
Advanced age, significant comorbidities are indications for watchful waiting. A prospective, randomized clinical trial reported that patients with clinically localized prostate cancer managed with watchful waiting have significantly higher rates of local cancer progression, metastases, and death from prostate cancer than do those treated initially with radical prostatectomy.

(b) External beam radiation treatment used with curative intent. Modalities include conventional external beam, conformal/IMRT external beam and ▶brachytherapy (Fig. 4). Randomized trials have found total androgen ablation should be combined with radiation for improved disease specific survival and increased time to recurrence in locally advanced cases. Complications from radiation include cystitis, proctitis, enteritis, impotence and urinary incontinence. Prostate cancer specific mortality can be estimated on the basis of pretreatment serum PSA, biopsy Gleason score, and clinical T category. The percentage of positive biopsies is an independent predictor of time to postoperative PSA failure after external beam radiotherapy. Nomograms have been developed that have excellent predictors for PSA failure after radiotherapy.

(c) Radical prostatectomy is removal of the prostate and seminal vesicles and pelvic lymphadenectomy. For low stage, low PSA, well-differentiated tumors the lymph node dissection may be omitted. There are currently three approaches used to remove the prostate gland: the retropubic, perineal and laparoscopic or robotic assisted retropubic approach. Laparoscopic and robotic-assisted prostatectomy are generally associated with decreased blood loss compared to the open technique. The following patient criteria are commonly used guidelines for patients to be candidates for radical prostatectomy regardless of the approach; age less than 70 years, few comorbidities, life expectancy >10 years, Gleason score $\leq$7 and PSA $\leq$10. Complications include impotence, incontinence and urethral stricture. Modifications to the classic technique to spare the neurovascular bundles has allowed improved potency and continence outcomes. Most recently, a study found that men whose PSA level increases by more than 2.0 ng/mL/year before the diagnosis of prostate cancer have an increased risk for death from prostate cancer despite undergoing radical prostatectomy. Important pathologic criteria that predict prognosis after radical prostatectomy are tumor grade, surgical margin status, presence of extracapsular disease, seminal vesicle invasion, and pelvic lymph node involvement. Nomograms have been developed that have excellent predictors for PSA failure after prostatectomy as well as adverse pathological outcomes if these tools are used pre-operatively.



Prostate Cancer, Clinical Oncology. Figure 4 Prostate brachytherapy.



Prostate Cancer, Clinical Oncology. Figure 5 Staging bone scan.

2. Locally advanced disease (T3-4N0M0): Active surveillance is an option in highly selected patients but failures usually result due to relative aggressive nature of these tumors. Radiation options as for early disease. The most common approach is external beam radiotherapy combined with androgen deprivation. If brachytherapy is used, it is often combined with external beam and androgen deprivation and

Prostate Cancer, Clinical Oncology. Table 1 The 1997 UICC/AJC TNM staging system

UICC/AJC 1997	Description
T1a	Tumor incidental histologic finding in 5% or less of tissue resected and Gleason < 7
T1b	Tumor incidental histologic finding in more than 5% of tissue resected or Gleason ≥ 7
T1c	Tumor identified by needle biopsy due to elevation of PSA; tumors found in one or both lobes by needle biopsy, but not palpable or visible by imaging
T2a	Tumor involves 1 lobe or less
T2b	Tumor involves more than 1 lobe
T3a	Unilateral extracapsular extension
T3b	Bilateral extracapsular extension
T3c	Tumor invades seminal vesicle(s)
T4a	Tumor invades bladder neck, external sphincter, and/or rectum
T4b	Tumor invades levator muscle and/or fixed to pelvic wall
N(+)	Involvement of regional lymph nodes
M(+)	Distant metastatic spread

patients receiving such multimodality radiation therapy display superior biochemical relapse free survival compared with those receiving surgery or brachytherapy alone. Surgery is usually not an option outside clinical trials and ►neoadjuvent androgen deprivation therapy before radical prostatectomy does not appear to improve cancer-specific (PSA free) or overall survival.

3. Hormonally naïve metastatic disease: Radiation therapy as described above for patients with nodal only disease. When this is accompanied by long term androgen deprivation, up to 30% of patients may have a long terms disease free interval. In patients with metastatic disease to bone, medical (►LHRH agonists ± anti-androgens) or surgical (bilateral orchiectomy) androgen ablation is the treatment of choice. Androgen ablation is associated with sexual dysfunction, osteoporosis, hot flashes, and cognitive function alterations. Serious liver toxicity is a possible side effect of all anti-androgens. Radiation therapy is used to palliate symptoms such as bone pain from metastatic deposits and neurologic symptoms secondary brain metastases or emergently in the setting of spinal cord compression (Fig. 5). Laminectomy or other orthopedic procedures can be used for pathologic fractures in the setting of bone metastases.
4. Hormonally refractory metastatic disease: Androgen ablation therapy can induce a prolonged response (several years) in most men with metastatic disease. However, in most patients the disease becomes 'androgen independent' and begins to grow despite androgen withdrawal. At this time, hormone-refractory prostate cancer is not curable. All the available forms of therapy are palliative, which means that they can be used only to slow the progression of the disease and to relieve symptoms.

Generally, for the patient who begins to fail hormonal therapy and has received combined hormonal therapy with an LHRH agonist and an antiandrogen or with an orchiectomy and an antiandrogen, antiandrogen withdrawal (stopping the antiandrogen) is undertaken. In many patients this will result in a short term decrease in serum PSA level. For patients who received LHRH agonist alone or orchiectomy alone, secondary forms of hormonal therapy may be considered, such as the addition of an antiandrogen or suppression of adrenal androgen synthesis. However, these clinical therapies are generally of limited benefit. Radiation therapy is used to help manage pain associated with the growth of bone metastases and this can be delivered via external beam therapy or osteomimetic radioisotopes (strontium-89 radionuclide) which tend to be absorbed into areas of bone remodeling such as is found in bone metastases. ►Docetaxel (Taxotere) is a cytotoxic agent and induces apoptosis of cancer cells and has become the standard treatment for hormone refractory prostate cancer. Large phase III randomized trials have demonstrated docetaxel prolongs progression free and overall survival, improves pain, and improves quality of life. Newer experimental approaches such as ►bisphosphonates, immunotherapeutics/vaccines, differentiation therapies, induction of ►apoptosis and inhibition of angiogenesis are currently in clinical trials.

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Prostate-Specific Membrane Antigen

Synonyms

Glutamate carboxypeptidase II; NAALADase; folate hydrolase; PSMA

Definition

Prostate-specific membrane antigen (EC 3.4.17.21), is a 750 amino acid, 100 kDa type II transmembrane glycoprotein that is highly expressed by normal prostate and ►prostate cancer cells and by the neovasculature of many epithelial cancers. Due to this relative cancer restricted expression, PSMA-based imaging agents and PSMA-targeted therapies are under development.

Characteristics

PSMA Expression

PSMA is a type II transmembrane protein consisting of a small intracellular segment of 18 amino acids, a transmembrane domain, and an extensive extracellular domain that contains the catalytic site (Fig. 1). The extracellular domain of PSMA is highly glycosylated and ►glycosylation is required for enzymatic activity. Human PSMA was originally isolated by the laboratory of Dr. Warren Heston from a cDNA library from the androgen responsive LNCaP human prostate cancer cell line and was later found to be located on chromosome 11p11–12. The entire sequence of PSMA contains 19 exons and has a modest degree of homology to at least four other human proteins: NLDL, NLD2, transferrin receptor (TfR) 1 and 2. Both TfR1 and PSMA are internalized into endosomes via cytoplasmic YXRF and MXXXL motifs, respectively. Whereas TfR1 delivers iron-loaded transferrin cells, it is currently not known whether PSMA may internalize ligands such as polyglutamated folate (a PSMA substrate).

Immunohistochemical studies using monoclonal antibodies have demonstrated that PSMA is expressed by normal prostate epithelium and is even more highly expressed by a large proportion of prostate cancers, including metastatic prostate cancers. Low-level detection of the PSMA protein has also been seen in the duodenal mucosa and in a subset of proximal renal tubules. PSMA enzymatic activity is also present in the brain. In all other human tissues, including normal vascular endothelium, PSMA expression was not detectable by ►immunohistochemistry. The number of PSMA molecules per 10,000 actin molecules in prostate tissue is approximately 170, which is approximately 10–15-fold higher than expression in liver, kidney, and brain. As a comparison, the ratio of PSA per 10,000 actin molecules is 18,000.

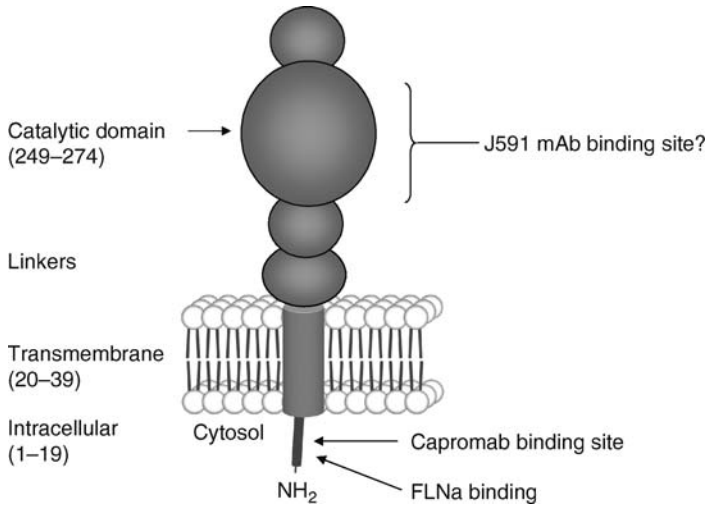
PSMA expression was also undetectable in other nonprostatic primary tumors. In multiple studies, however, PSMA expression has been detected in the neovasculature of a large number of different tumor types including breast, renal, colon, and transitional cell carcinomas. In contrast, PSMA expression was not observed in nontumor induced neovascularization associated with choroidal neovascular membrane development.

Two regulatory elements controlling PSMA expression have been characterized: the proximal 1.2 kb PSMA promoter and a PSMA enhancer located within the third intron. This PSMA enhancer is activated in a prostate specific manner and is negatively regulated by the ►androgen receptor. This negatively regulated enhancer seems to underlie findings from preclinical and clinical studies demonstrating that PSMA mRNA is upregulated upon androgen withdrawal.

The PSMA structure revealed a twofold symmetric homodimer with overall structural similarity to the transferrin receptor. Unlike the transferrin receptor, PSMA possesses an enzymatically active protease domain containing a binuclear zinc site, catalytic residues, and a proposed substrate-binding arginine patch.

Enzymatic Functions of PSMA

In addition to its role as a tumor marker and imaging target, PSMA contains a binuclear zinc site and functions as a glutamate carboxypeptidase II possessing two discrete enzymatic functions. Initially, PSMA was demonstrated to possess the hydrolytic properties of an *N*-acetylated α-linked acidic dipeptidase (NAALADase). NAALADase is a membrane hydrolase activity that is able to hydrolyze the neuropeptide *N*-acetyl-*l*-aspartyl-*l*-glutamate (NAAG) to yield the neurotransmitter glutamate and *N*-acetyl-aspartate. In addition to the NAALADase activity, PSMA also functions as a pteroyl poly-γ-glutamyl carboxypeptidase (folate hydrolase). It is able to progressively hydrolyze γ-glutamyl linkages of both poly-γ-glutamated folates and methotrexate analogs with varying length glutamate chains. Mhaka et al. demonstrated that PSMA could also hydrolyze peptides containing both α- and γ-linked acidic amino



Prostate-Specific Membrane Antigen. Figure 1 Structure of PSMA with Filamin A (FLNa), J591, and Capromab monoclonal antibody binding sites indicated.

acids. This finding has implications for exploiting the enzymatic activity of PSMA for the design of prodrugs, in which an inactive aspartyl/glutamyl peptide is selectively cleaved from the prodrug by PSMA thereby activating the drug within the vicinity of PSMA expressing cells.

The role that PSMA's NAAG or folate hydrolase activity plays in the physiology of prostate cells is presently unknown. NAAG is an inhibitor of the NMDA ionotropic receptor and an agonist of the type II metabotropic glutamate receptor subtype 3. A breakdown of the regulation of glutamatergic neurotransmission by -NAAG is implicated in schizophrenia, seizure disorders, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Thus, inhibition of PSMA potentially confers neuroprotection both by reducing glutamate and increasing –NAAG and has been shown to provide neuroprotection in cell culture and/or animal models of ischemia, diabetic neuropathy, drug abuse, chronic pain, and amyotrophic lateral sclerosis.

Does PSMA have a Signaling Function?

PSMA's structural similarities with the transferrin receptor, its homodimeric membrane form, and its selective expression on tumor endothelial cells have raised questions whether PSMA may have a ►signal transduction functionality in addition to its carboxypeptidase activity. Inhibition of PSMA activity in cell culture or in subcutaneously implanted PSMA expressing xenografts does not lead to inhibition of growth. However, Ghosh et al. demonstrated that ectopic expression of PSMA in human prostate cancer cells reduced the invasiveness of these cells while knockdown of PSMA expression or expression of mutant PSMA lacking carboxypeptidase function increased invasiveness. Thus, this study suggested that the enzymatic activity of PSMA is somehow associated with pathways involved in promoting invasiveness.

The cytoplasmic tail of PSMA interacts with the actin binding anchor protein Filamin a (FLNa) and this association appears necessary for PSMA localization to the recycling endosomal compartment. PSMA also binds to ►caveolin-1 in human microvascular endothelial cells and undergoes internalization via a caveolae-dependent mechanism. These associations suggest a link between PSMA at the cell surface, internalization, and downstream signaling processes. ►Angiogenesis is severely impaired in PSMA-null animals and this angiogenic defect occurs at the level of endothelial cell invasion through the extracellular matrix barrier. PSMA is a principal component of a regulatory loop that modulates ►laminin-specific integrin signaling and GTPase-dependent, p21-activated kinase 1 (PAK-1) activity. PSMA inhibition, knockdown, or deficiency decreased endothelial cell invasion in vitro via ►integrin and PAK whereas inactivation of PAK increased PSMA activity. This negative regulation appeared to be mediated by the cytoskeleton as the disruption of interactions between the PSMA cytoplasmic tail and FLNa decreased PSMA activity, integrin function, and PAK activation, while the inhibition of PAK activation enhances the PSMA–FLNa interaction and increased PSMA activity. These data suggest a model whereby PSMA participates in an autoregulatory loop, in which active PSMA facilitates integrin signaling and PAK activation, leading to both productive invasion and downregulation of integrin beta(1) signaling via reduced PSMA activity. While enzymatically active PSMA appeared necessary for this signaling, the role of PSMA hydrolysis of its putative ligands was not addressed in this study.

PSMA and Imaging

In contrast to other prostate-related antigens such as PSA, prostatic acid phosphatase, and prostate secretory protein, PSMA is a type II integral membrane cell surface protein that is not secreted and, therefore, is an excellent target for monoclonal antibody (mAb)-based imaging and therapy. PSMA is constitutively internalized, but internalization is also induced by antibody binding (). Initial validation of PSMA as an in vivo mAb target has been demonstrated by imaging trials with mAb 7E11/CYT-356 (marketed as ProstaScint, Cytogen, Princeton, New Jersey) an FDA approved imaging agent. 7 However, molecular mapping indicates that mAb 7E11/CYT-356 targets an intracellular portion of the PSMA molecule not exposed on the outer cell surface. Since the 7E11/CYT-356 epitope is within the cell, the mAb does not bind viable cells.

ProstaScint has met with limited success in the clinic. ProstaScint scans are considerably less sensitive than bone scans at detecting bone disease. Since >80% of prostate cancer patients have metastatic disease in the bone, ProstaScint is not used routinely in the clinical evaluation of these patients. Thus, the only patients in whom clinically useful information is derived from a ProstaScint scan may be those in whom regional and/or systemic disease is demonstrated, and where a biopsy or other modality could confirm the findings were not a false-positive result. Given these limitations, the ProstaScint scan has not been used to any significant degree by clinicians in the work up of patients with suspected recurrent prostate cancer outside of the prostatic fossa.

The recognition that ProstaScint recognizes an internal epitope of PSMA led to the development of a series of monoclonal antibodies to extracellular epitopes of PSMA that could bind to both intact and permeabilized cells. Researchers at Cornell University, led by Dr. Neil Bander, developed four IgG mAbs (J591, J415, J533 and E99) to the extracellular domain of PSMA. These antibodies demonstrated high affinity binding to prostate cancer cells in tissue culture, on tissue sections and in animal models in vivo 9,11. Moreover, once bound, PSMA antibody complexes were shown to be rapidly internalized with intracellular accumulation of Ab, resulting in the potential internalization of toxins or isotopes conjugated to the mAb. Clinical studies using humanized antibody (HuJ591) labeled with ^{111}In, ^{90}Y and ^{177}Lu confirmed that mAb HuJ591 targeted and could image PSMA expressing human prostate cancer xenografts. Subsequently, several clinical studies have been performed evaluating efficacy of labeled humanized mAb to PSMA. ^{111}In-DOTA-huJ591 was tested in phase I trial with tumor visualization seen in both soft tissue and bone metastases. Additional phase I trials have been performed with ^{90}Y and ^{177}Lu DOTA-huJ591 conjugate and demonstrated excellent imaging capabilities with detection of >95% of bone metastases. A few PSA responses were observed in these trials. With all three radiolabeled approaches, significant nonspecific accumulation of antibody uptake was observed in the liver and kidney in all studies. In each study, the dose-limiting toxicity has been myelosuppression, particularly thrombocytopenia, which was most likely due to the long plasma half-lives of these antibodies. However, despite the theoretical advantage of targeting an extracellular PSMA epitope, a report describing initial experience with a technetium-99m-labeled J591 antibody found decidedly inferior prostate cancer imaging relative to ProstaScint.

Encouraging results were reported using 11C or 125 I radiolabeled urea derivatives that have high affinity for PSMA to image in experimental models of prostate cancer in vivo. These low molecular weight compounds offer potential advantages over antibody-based imaging that include better tissue penetration, higher specificity, and shorter half-life.

PSMA as a Therapeutic Target

The demonstration of PSMA expression by tumor vasculature, high expression in prostate cancer, and upregulation in androgen ablated patients have made PSMA an attractive therapeutic target. Current targeting strategies include ►immunotoxin conjugates, ►cancer vaccines, PSMA inhibitor–drug conjugates, and PSMA-activated prodrugs/protoxins. As described, ►radioimmunotherapy using the antiPSMA mAb coupled to radionuclides like ^{90}Y and ^{177}Lu has been tested in clinical trials. PSMA-based ►monoclonal antibody therapy using the J591 antibody coupled to maytansinoid, a highly potent microtubule depolymerizing natural product (MLN2704) has been tested clinically. This agent showed antitumor response against PSMA producing human prostate cancer xenografts and has been evaluated in a phase I trial in 23 patients. Overall, MLN2704 was well tolerated in the majority of patients and two patients had a >50% decline in PSA from baseline. Further studies with this antibody–toxin conjugate are anticipated. In addition, a number of groups have described PSMA-based vaccine strategies that range from PSMA-primed dendritic cells to injection of naked human and mouse DNA.

Since PSMA is a carboxypeptidase, a large number of PSMA inhibitors have been generated. While these inhibitors show some promise in neurologic diseases, to date no antitumor effect from direct inhibition of PSMA has been reported. However, strategies involving coupling of PSMA inhibitors or PSMA-binding moieties such as ►aptamer bioconjugates or peptides to cytotoxic agents are under exploration. Prodrug and protoxin strategies attempt to take advantage of the unique enzymatic activities of PSMA to selectively

activate cytotoxic agents within the peritumoral extracellular fluid surrounding PSMA producing cells. Unlike antibody and other PSMA-binding strategies, an advantaged of this ►targeted drug delivery approach is that drug/toxin release in the extracellular fluid is amplified by PSMA's activity. In addition, since every cell does not need to produce PSMA to be killed this approach overcomes potential resistance based on heterogeneous expression of PSMA while, at the same time, producing a substantial bystander effect. Finally, PSMA targeted cytotoxins may also function as ►vascular disrupting agents and have therapeutic potential against a variety of solid tumor types expressing PSMA in the neovasculature.

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Protease Activated Receptor Family

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Definition

The Protease Activated Receptors (PARs) are a group of seven transmembrane ►G protein-coupled receptors with a unique mechanism of activation. Most receptors are activated when the ligand binds to the ligand binding domain of that receptor. However, the different ►serine protease ligands that activate PAR will cleave the N-terminus of the receptor. This results in the formation of a new N-terminus peptide which acts as a tethered ligand that will now bind to the activation site of the receptor and cause irreversible activation of PAR (Fig. 1).

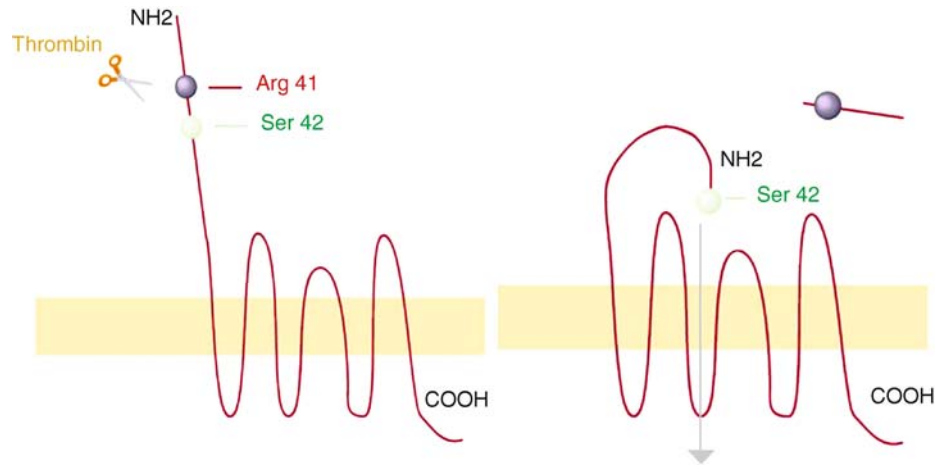
Characteristics

To date, there are four members of the PAR family that have been identified. PAR-1, PAR-3 and PAR-4 are activated by thrombin although other proteases can cleave these receptors. PAR-2 is activated by trypsin like proteases including trypsin and tryptase. Studies on PARs have been performed most extensively with PAR-1 and to a lesser degree with PAR-2. However, the role and importance of PAR-3 and PAR-4 have yet to be fully understood in cancer. Therefore, only the role of PAR-1 and PAR-2 in cancer will be discussed.

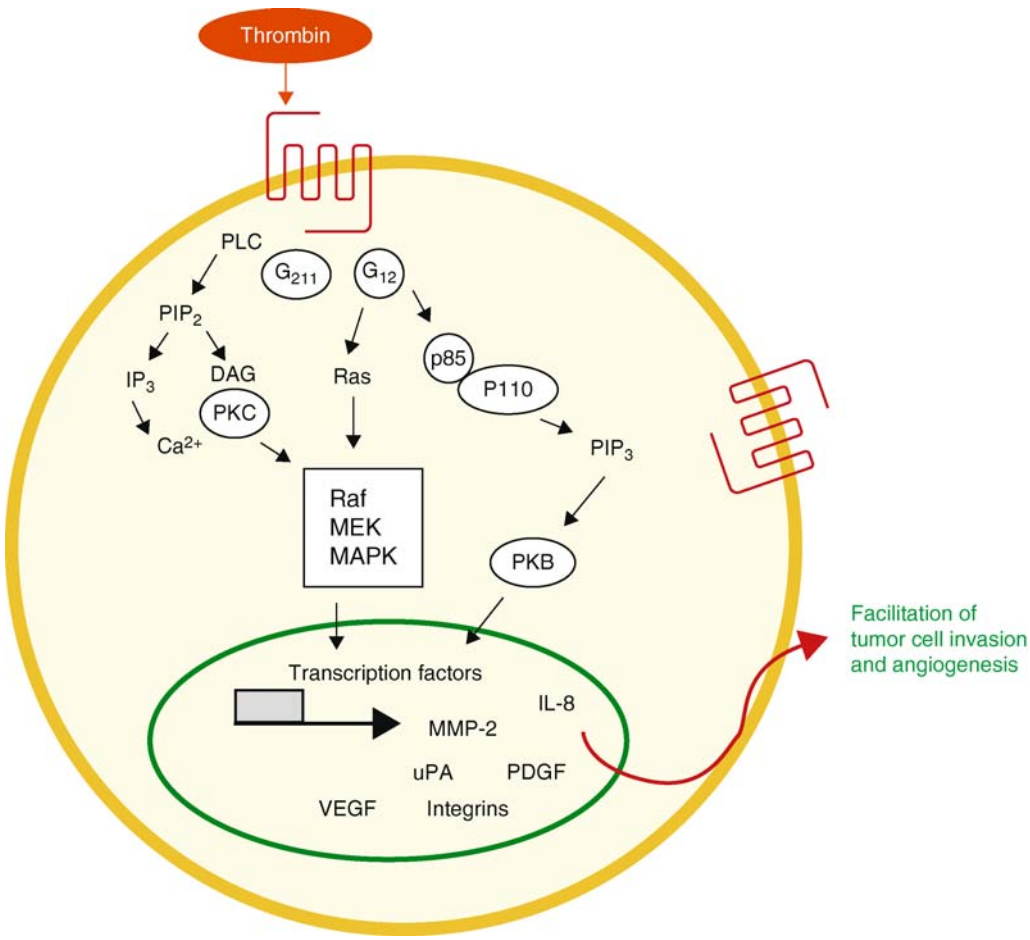
PAR-1: PAR-1, also known as the thrombin receptor, was the first family member to be discovered. It is expressed on a wide variety of cells including endothelial, epithelial, T-cells, astrocytes, neurons and platelets. It can be activated by thrombin as well as coagulation factor Xa, ►granzyme A, trypsin and ►matrix metalloprotease 1 (MMP-1) although thrombin is the most potent activator. The role of PAR-1 in platelet aggregation and degranulation has been extensively studied. In cancer, studies have shown that activation of coagulation factors, including thrombin, ►tissue factor and ►fibrinogen, is a common occurrence. In fact, activation of PAR-1 leads to induction of G proteins that trigger various downstream molecules and signal transduction pathways such as Rho kinase, phosphoinositol-3-kinase (PI3-K) and ►mitogen-activated protein kinases (MAPK), which have been shown to be involved in cell growth, tumor promotion and carcinogenesis. Activation of PAR-1 has also been implicated in secretion of many factors involved in angiogenesis and tumor cell invasion such as interleukin 8 (IL-8), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), urokinase plasminogen activator (uPA), and MMP-2 (Fig. 2).

Therefore the thrombin receptor has been implicated in tumorigenesis and metastasis of several human cancers including melanoma, breast, prostate, pancreatic, lung and colon carcinomas among others.

PAR-2 is the second member of the PAR family to be discovered. Similar to other PAR family members, activation of PAR-2 occurs through the cleavage of the extracellular domain which forms a tethered ligand followed by downstream signaling. In contrast to other PAR family members, PAR-2 is activated mostly by trypsin which is the most potent protease, as well as by other trypsin-like proteases such as tryptase and factor Xa. PAR-2 can also be activated by PAR-1 and therefore, indirectly by thrombin. Data also suggests that the tethered ligand domain from activated PAR-1 can trans-activate PAR-2 when both receptors are present on cells in close proximity to one another as occurs on endothelial cells. This trans-activation of PAR-2 might enhance the response of cells to thrombin. In addition, tissue factor (TF) which has been found to be activated in cancer, can indirectly activate PAR-2



Protease Activated Receptor Family. Figure 1 PAR-1 is cleaved by proteases such as thrombin at Ser 41 resulting in formation of a new N-terminus pepide. This tethered ligand binds to the activation site of PAR-1 causing its activation and downstream signaling.



Protease Activated Receptor Family. Figure 2 Schematic of cell invasion and angiogenic molecules involved in tumor progression via activation of PAR-1 in melanoma cells. The tumor-promoting signals transduced by PAR-1 through G proteins upregulate molecules involved in invasion and angiogenesis, including uPA, IL-8, VEGF, PDGF, MMP-2, and integrins.

through components of the coagulation cascade. TF binds factor VIIa which can either directly activate PAR-2 or cause activation of factor X thereby mediating cleavage of PAR-2. Another manner of activation of this receptor is mediated through membrane type serine protease-1(MT-SP1), an enzyme which has been found on tissues that also express PAR-2. MT-SP1 can directly cleave PAR-2 thereby causing its activation.

PAR-2 activation induces the release of IL-6 and IL-8, granulocyte macrophage-colony stimulating factor, prostaglandins and intracellular calcium mobilization. In fact, it has been suggested that trypsin and PAR-2 act in an autocrine loop to activate signal transduction pathways, promoting cell proliferation invasion and metastasis. *In-vitro* studies have shown that activation of PAR-2 causes an induction of different signaling pathways that induce proliferation, migration, invasion and metastasis. PAR-2 also contributes to reorganization of the cytoskeleton in a different manner than PAR-1 thereby elucidating different functions of these two receptors in cell migration. PAR-2 is also involved in integrin activation by promoting integrin $\alpha_5\beta_1$ dependent cell adhesion.

PAR-2 is highly expressed in different tissues such as colon, liver, pancreas, stomach and small intestine. It is also expressed in a wide variety of cells such as endothelial cells, T cells, smooth muscle cells and surface epithelial cells. Both PAR-1 and PAR-2 are expressed in tumor cells and in the tumor microenvironment and contribute to tumorigenisis and metastasis. PAR-2 is expressed in tumors such as lung, colon, pancreas, stomach and melanoma. However, the role of PAR-2 in cancer has not been completely elucidated.

Melanoma

In human melanoma cells, thrombin acts as a growth factor and is ►mitogenic, suggesting that signaling by PAR-1 is involved in the biological response of these cells. Thrombin sources not only include systemic activation of coagulation factors seen in many cancer types but also several studies have shown that tissue factor can be expressed independent of the coagulation pathway in malignant melanoma cells thereby activating thrombin. Furthermore, fibrin deposits are formed from the cleavage of fibrinogen by thrombin. These fibrin deposits in the stromal microenvironment surrounding tumor cells may store active thrombin that is released when the clots undergo degradation.

PAR-1 has been found to be differentially expressed within human melanoma cell lines. Highly metastatic melanoma cells have over-expression of PAR-1 mRNA, while non-metastatic melanoma cells have lower levels. Over-expression of the thrombin receptor has also recently been found in clinical specimens of invasive melanoma and metastases as compared to levels found in ►common melanocytic nevi and normal skin. *In-vitro* studies have shown that when PAR-1 expression is induced in non-metastatic melanoma cells that normally have low levels of PAR-1, they acquire a more invasive and aggressive phenotype. Furthermore, there is evidence that demonstrates a physiological role for PAR-1 in the melanoma metastatic cascade where PAR-1 was the rate limiting factor in an experimental murine lung metastasis assay.

It has been recently proposed that in highly metastatic melanoma cell lines, cell migration requires activation of PAR-Z, in addition to PAR-1, and results in enhanced metastasis by promoting cell motility. *In-vivo* experiments demonstrated that PAR-2 activation by trypsin or by agonist peptide in highly metastatic melanoma cell lines have more lung metastasis as compared to those cells not exposed to these agonists.

Breast Cancer

The levels of PAR-1 expression on tumor cells directly correlates with the degree of invasiveness and therefore metastatic potential in both primary breast carcinomas and established breast cancer cell lines. The more metastatic breast cancer cell lines expressed very high levels of PAR-1, while the minimally invasive lines showed minimal expression of PAR-1. In human specimens, high grade ductal carcinoma in-situ (DCIS) showed expression of the thrombin receptor while the highest levels of PAR-1 were found in infiltrating ductal carcinoma. In contrast, undetectable levels of PAR-1 were found in low grade DCIS and normal breast tissue. *In-vitro* studies revealed increased levels of migration and invasion in a null PAR-1 breast cancer cell line transfected with PAR-1. These cells expressing PAR-1 were examined in a mouse ►xenograft model for human breast cancer which showed increased tumor growth and invasion in nude mice. Recent findings have demonstrated that MMP-1, a collagenase of the MMP family, which is found in the stromal tumor microenvironment, is an additional proteolytic activator of PAR-1 promoting invasion and tumorigenesis of breast cancer cells.

A new study on breast cancer suggests that PAR-2 has a significant role in induction of migration and invasion. In this study, an invasive breast cancer cell line transfected with PAR-2 siRNA showed a decrease in both migration and invasion comparable to cells that were transfected with PAR-1 siRNA.

Prostate Cancer

PAR-1 expression has been found in prostate cancer cell lines. Over-expression in highly invasive prostate cancer cell lines correlates with increased metastatic potential. Recently clinical specimens were analyzed for expression of PAR-1. Advanced stage prostate cancer tissue specimens had significantly higher expression of PAR-1 as compared with tissue from early stage patients. PAR-1 expression of the activated

receptor in the vasculature from benign prostatic hyperplasia (BPH) tissue was also compared with early stage and advanced stage prostate cancer specimens. BPH tissue showed very low expression of PAR-1 with the most expression being found in advanced stage prostate cancer specimens. Early stage cancer also showed positive expression of the thrombin receptor but less than advanced stage cancer. Furthermore, prostate cells from bone metastases have higher expression of PAR-1 than those from soft tissue metastasis. Bone metastasis from prostate cancer is a common feature of prostate cancer and a significant cause of morbidity. These data suggest that PAR-1 plays a pivotal role in the progression of prostate cancer.

Pancreatic Cancer

In-vitro studies have shown that pancreatic cancer cell lines express PAR-1 while no expression is seen in normal pancreatic epithelial cells. Higher levels of PAR-1 mRNA were seen in pancreatic cancer tissue samples as compared to healthy pancreas which suggests that PAR-1 could play a key role in the pathogenesis of pancreatic cancer.

PAR-2 might play a unique role in pancreatic cancer since the pancreas is a major site of production of the trypsin ►zymogen (trypsinogen) by the ►acinar cells. Studies have also proposed that it is produced by pancreatic cancer cells. It has been shown that PAR-2 is highly expressed in the pancreas and in pancreatic ductal carcinoma. *In vitro* studies suggest that trypsin might act as a stimulating growth factor in pancreatic cancer cells expressing PAR-2 thereby inducing proliferation and invasion. Examination of pancreatic cancer tissues, have shown that in tissues with infiltrative growth patterns, PAR-2 expression was higher than in tissues with expansive growth patterns. Moreover, a correlation between fibrosis, which is one of the characteristics of human pancreatic tumors that is linked to poor prognosis and high expression of PAR-2, exists in pancreatic tumors. Therefore, PAR-2 might also be involved in induction of fibrosis.

Lung Cancer

PAR-1 is highly expressed in the lung alveolar wall tissue of patients with lung adenocarcinoma when compared to those tissues without neoplastic cells. In a recent study, more than half of all specimens of non-small-cell lung cancer (NSCLC) patients also showed over expression of PAR-1. A higher and more uniform expression of the receptor was detected in aggressive tumors as compared to those with a more favorable outcome. In fact, the 3 year survival for patients expressing PAR-1 was significantly shorter than those with no expression of PAR-1. These data suggest that PAR-1 might represent a new prognostic factor in NSCLC patients.

Colon Cancer

PAR-1 has also been found to be expressed in human colon cancer cell lines and clinical specimens but is absent in epithelial cells from normal human colon. It is present in dysplastic mucosa and cancer mucosal tissues but not in the adjacent normal mucosa. *In-vitro* studies revealed that thrombin can act as a growth factor for colon cancer cells. Thrombin induces cell migration and proliferation of human colon cancer cells through PAR-1 in a similar manner seen with a PAR-1 agonist. This effect in part is due to the activation of the MAPK pathway by thrombin and its receptor.

In colorectal cancers (CRC), PAR-2 has been shown to be over expressed and its activation may promote both tumor progression and metastasis. It has been shown that in a colon carcinoma cell line, PAR-2 activation results in cell proliferation. Upon proteolysis of PAR-2 by trypsin, a signal transduction pathway is activated by the phosphorylation of MEK1/2 and ERK1/2 leading to proliferative response. This might indicate a significant role of MAPK pathway activation through PAR-2 in CRC. Furthermore, activation of PAR-2 can lead to activation of MMPs which promotes invasion and metastasis. MMP activation causes release of TGF-α which triggers activation and phosphorylation of EGFR, promoting proliferation.

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Protease Activated Receptors

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Synonyms

PAR1; Thrombin-receptor

Definition

►*Protease Activated Receptors* (PARs) form a family of seven transmembrane ►G- protein coupled receptors consisting of 4 gene members. PAR1, 3 and 4 respond to the serine protease thrombin while PAR2 is activated by trypsin, tryptase and the coagulation factors VIIa and Xa, but not by thrombin. All members are uniquely activated via proteolytic cleavage that exposes an otherwise hindered ligand at their N-terminus extra cellular portion. PARs can be viewed as receptors that carry their own ligands. In addition to thrombin, PARs convey cellular responses to a wide spectrum of serine proteases as also the ►matrix metallo protease 1, MMP1. PARs1-4 are similarly organized, all encoded by two exons and separated by an intron of variable size. Exon 1 encodes a signal peptide, and exon 2 encodes the mature receptor protein. In humans, PARs1-3 are localized on chromosome 5q13.

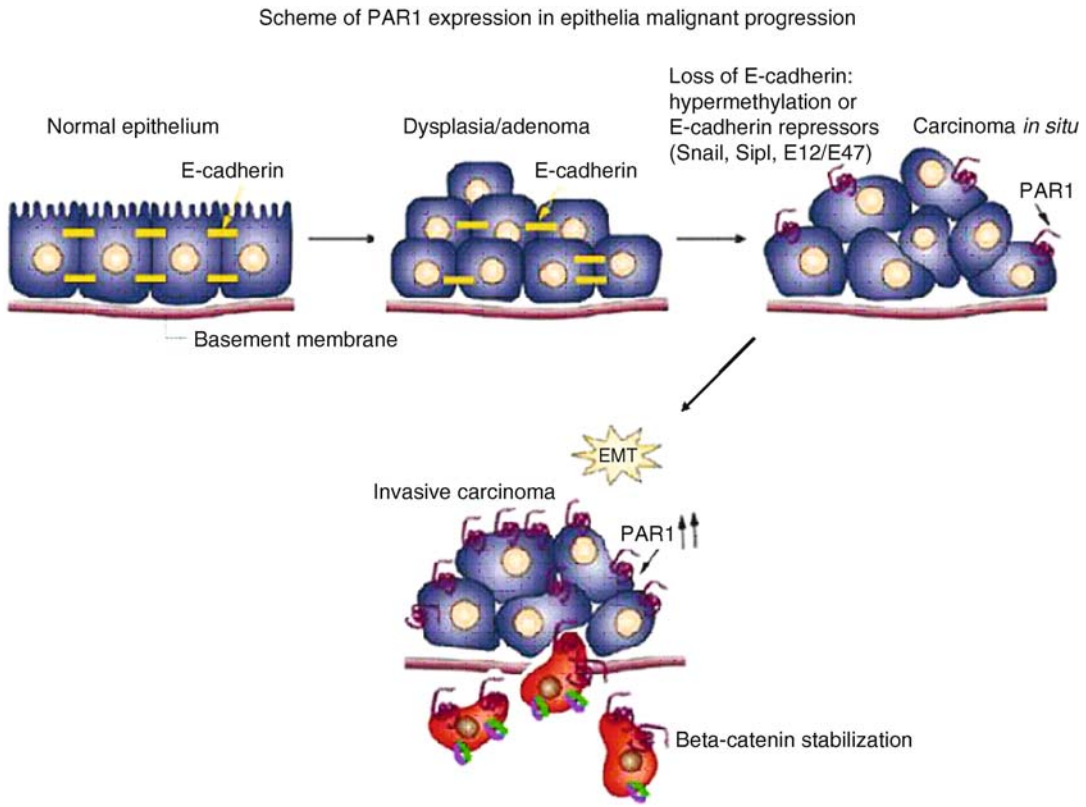
Characteristics

Protease Activated Receptors (PARs) belong to the large group of ►G-protein coupled receptors (GPCRs) of seven transmembrane domain proteins, uniquely activated by proteolytic cleavage. While originally established as thrombin receptors, they serve nonetheless as cell surface sensors that convey signaling to an array of serine-proteases and a matrix metallo protease, MMP-1. In addition to their critical roles in thrombosis, hemostasis, inflammation and vascular biology, PAR1 emerges with considerable assignments in tumor biology (►Epithelial tumors) and ►angiogenesis. PARs possess an unusual mode of activation. These receptors carry their own ligand/s, hindered in the non-activated state and exposed after proteolytic cleavage at their N-terminal, extra cellular portion. Once exposed, the ligand remains tethered and interacting with loop number two and initiates cell signaling. Alternatively, proteolytic cleavage can be bypassed by ectopic application of a synthetic peptide representing the internal ligand sequence that directly associates with loop number two for cellular signaling. The mechanism of PAR1 activation is striking also because of its shut-off mechanism. PARs are irreversibly activated and their desensitization and cell trafficking control, in part, the magnitude and duration of PARs signaling. Human PAR1, PAR3, and PAR4 are all activated by thrombin. In contrast, PAR2 is activated by trypsin and tryptase as well as by coagulation factors VIIa and Xa, but not by thrombin. PAR1 is the first and prototype member of the family, originally discovered in a search for a functional receptor that confers thrombin signaling in human platelets. PAR2 was next identified in a screen of a mouse genomic library using probes homologous to the transmembrane regions of substance K receptor. Studies in PAR1 knock-out mice showed fifty percent lethality with no effect on thrombin signaling in mice platelet, but abrogated thrombin signaling in fibroblasts. These unexpected findings initiated the exploration and identification of PAR3 and PAR4. The latest family members revealed a selective expression pattern in human and mice platelets. While PAR1 and PAR4 are the functional receptors of thrombin in human platelets, PAR3 and PAR4 are responsible for thrombin signaling in the mice platelets.

The notion that *hPar1* is one of a series of genes that are part of an invasive program stems from studies showing that *hPar1* expression correlates directly with the invasion properties of breast carcinoma (Fig. 1) and of trophoblast placenta physiological invasion into the uterine decidua. In fact, *hPar1* plays an active role in breast carcinoma invasion because antisense silencing of the gene abrogates efficiently metastatic breast carcinoma cells (►Breast cancer) from invading Matrigel-coated filters, *in vitro*. PAR1 expression is found high in a wide spectrum of ►epithelia malignancies (e.g., colon, breast, prostate, ovary, esophagus, pancreas, etc) as compared to no expression in normal epithelia shown both in a collection of tissue biopsy specimens and differentially metastatic cell lines. In prostate cancer (►Prostate cancer clinical oncology) PAR1 has been implicated in bone metastasis and the over expression of PAR1 in murine and human melanoma have yielded enhanced experimental lung metastasis in mice. In colon cancer PAR1 has been demonstrated to cross talk with EGFR, thereby promoting cell proliferation. In parallel, a cDNA expression library screen based on the loss of anchorage-dependent growth and focus-forming activity in NIH3T3 cells have identified PAR1 as a novel ►oncogene. With these observations, PAR1 joins other GPCRs, encoded by *mas* and *g2a*, that behave as oncogene. The oncogenic properties of PAR1 accompany a collection of data showing that *hPar1* is over expressed in a wide range of epithelial tumors, pointing altogether to the central role of PAR1 in carcinoma invasion.

PAR1 has been shown to promote tumor progression through several mechanisms on different levels. PAR1 has been shown to promote cell growth, invasion, and metastasis, and also contribute to tumor cell survival by preventing the apoptotic pathway.

In a xenograft nude mice model, it was demonstrated that the expression of PAR1 in non invasive MCF7 breast carcinoma is sufficient to promote growth and invasion. In contrast, when a truncated form of *hPar1*, devoid of the entire cytoplasmic tail, is introduced to MCF7 cells and injected to the mice mammary fat pads, tumor development was markedly attenuated (Cohen I and Bar-Shavit R manuscript in preparation). This outcome underscores the significance of PAR1 signaling in tumor progression and invasion.

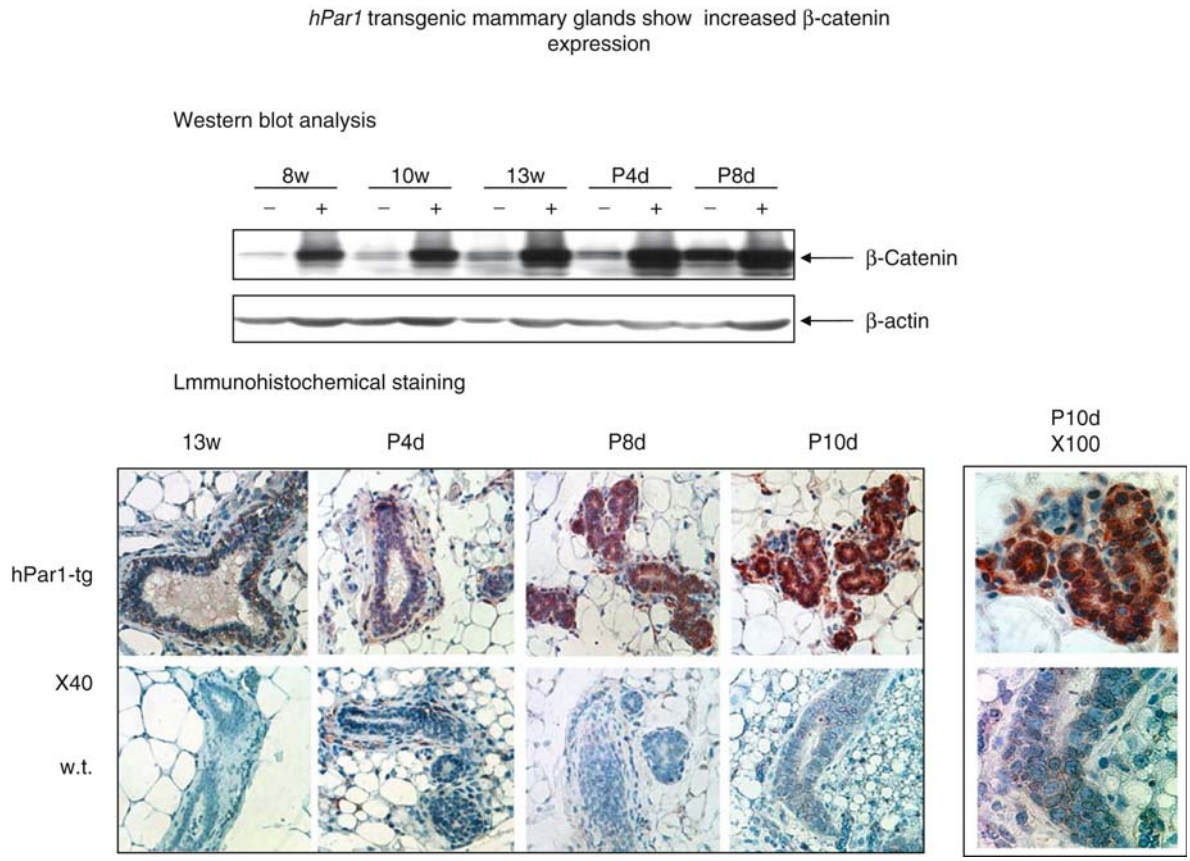


Protease Activated Receptors. Figure 1 Schematic illustration of PAR1 pattern of expression, along the progression of epithelia malignancy. While no PAR1 is seen in either normal or dysplastic tissues, PAR1 expression is high in DCIS (ductal carcinoma in situ) IDC (invasive ductal carcinoma).

Since PARs are irreversibly activated, signaling must be tightly regulated. Part of this regulation is the extent and mode of cell surface receptor internalization targeted to lysosomal degradation. Cellular trafficking of the proteolytically activated PARs leads to its desensitization, thereby controlling the magnitude, duration and spatial aspects of receptor signaling. In breast carcinoma, in addition to PAR1 over expression, these cells display aberrant PAR1 trafficking, which causes persistent signaling and cellular invasion.

Tissue factor is also up regulated in many tumor cells and supports FVIIa and FXa activity. TF bound to FVIIa can activate PAR2, whereas FXa can signal through either PAR1 or PAR2. There is increasing evidence that PAR2 is also an important mediator of tumor progression. Trypsin, a potent and physiological activator of PAR2, is elevated in gastric, colon, ovarian and lung tumors.

To gain further insight into the causal relationship between *hPar1* expression, breast tumor formation and mammary gland development, a line of mice carrying MMTV-driven *hPar1* designated to over express in the mammary glands was established. These mammary glands exhibited hyperplasia characterized by a dense network of ductal side branching and accelerated proliferation. In addition, these glands showed increased levels of *wnt-4* and *wnt-7b* and the striking stabilization of β-catenin. The canonical *wnt/wingless* signaling pathway directs cell fate in many cell types and plays a central role in development and in tumor progression. The core event of the *Wnt* pathway (►Wnt signaling) is the stability of β-catenin. Nuclear localization of β-catenin is observed in *hPar1* transgenic mice tissue sections but not in the wild-type age-matched counterparts (Fig. 2). PAR1 also induces β-catenin nuclear localization in established epithelial tumor cell lines. Once localized in the nuclei, β-catenin forms functional complexes with the lymphoid enhancer factor (Lef)/T-cell factor (Tcf) transcription factors. Introducing *hPar1 Si*RNA constructs depleting efficiently *hPar1* levels efficiently inhibited *wnt-4* expression. Therefore, it is proposed that PAR1 induced ►β-catenin stabilization is mediated in part via the generation of *Wnt/s*. The novel link between PAR1, β-catenin and *Wnt* may impinge significantly on both development and tumor progression processes.



Protease Activated Receptors. Figure 2 *hPar1- tg* mammary glands show hyperplasia and increased β-catenin expression in the nuclei**.** A. Whole mount hematoxylin staining of *wt* and *hPar1*-tg mammary glands at different developmental stages. The epithelial tissue derived from the *hPar1*-tg mammary glands displays increased lateral branching and pervasive intraductal hyperplasia in virgin (V-13w) and pregnant mammary glands (days 8 and 12 of pregnancy, respectively. B. Western blot analysis of β-catenin expression in *hPar1-tg* mammary glands at different developmental stages compared with age matched wt mammary glands. C. Immunohistochemical staining shows β-catenin localization in the mammary glands of *hPar1-tg* and wt mice. At 8 & 10 days of pregnancy, nuclear β-catenin is observed in *hPar1-tg* mice but not in *wt* mice (×40). At 10d of pregnancy a higher magnification is shown (×100).

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Protease Signaling

Definition

For example the protease-activated receptors (PARs) convey protease cellular signaling.

Proteases

Definition

Proteins with enzymatic function (or enzymes) capable of catalyzing the hydrolysis of peptide bonds in

proteins. Synonyms: proteolytic enzymes; exopeptidases (amino- or carboxypeptidases); endopeptidases (or proteinases). Depending on the amino acid or chemical group involved in the enzymatic catalysis, proteases are divided into different classes:

- serine-proteases (serine residue)
- cysteine-proteases (cysteine residue)
- aspartyl-proteases (aspartic acid residue)
- metallo-proteases (bivalent cation, e.g. Zn^{2+} or Mg^{2+})
- threonin-proteases (threonin residue)

►Cystatins

Proteasomal Cleavage

Definition

►Proteasomes are large protein complexes that degrade unneeded or damaged proteins by proteolysis. This process is initiated by the addition of an ubiquitine tag to these proteins. The ubiquinated proteins are subsequently cleaved into seven to eight amino acids long peptides, which can be degraded further into single amino acids.

►Proteasome Inhibitors

Proteasome

Definition

Synonym prosome or macropain; Is a large protein complex that functions mainly to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. Proteasomes are a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins. Proteasomal degradation yields peptides of about seven to eight amino acids long. Those peptides can be further degraded into amino acids that can subsequently be used for the synthesis of new proteins. Proteins are marked for proteasomal degradation by a small protein called ubiquitin, which is attached to the target protein by enzymes called ubiquitin ligases. Once a protein is tagged with a single ubiquitin molecule, this is a signal to other ligases to attach additional ubiquitin molecules. The result is a polyubiquitin chain that is recognized by the proteasome, whereupon the tagged protein is degraded.

►Apoptosis-Induction for Cancer Therapy
►Ubiquitination
►Proteasome Inhibitors

Proteasome Inhibitors

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Definition

►Proteasomes are multi-subunit ►protease complexes that selectively degrade ►ubiquitinated intracellular proteins. ►Proteolysis is essential for normal cell cycle progression, inflammation, transcription, DNA replication, and spontaneous ►apoptosis. The ►Ubiquitin-Proteasome System (UPS) mediates normal intracellular protein degradation; conversely, blockade of UPS with proteasome inhibitors trigger apoptosis in tumor cells. Bortezomib (Velcade™), the first in class of proteasome inhibitors, has become a standard therapy for treatment of refractory ►multiple myeloma (MM).

Characteristics

Rationale for Therapeutic Utility of Targeting Proteasome. Many cellular proteins, such as those regulating metabolism, cell cycle, growth, survival, stress and immune responses, are substrates of proteasomes. The UPS pathway is responsible for elimination of most of the misfolded, short or long-lived, proteins in the cell; conversely, blockade of proteasome-mediated protein degradation results in intracellular accumulation of unwanted/toxic proteins, induction of heat-shock response, and apoptosis. Because protein breakdown is a component of normal cellular functioning, the notion of targeting proteasomes as an anti-cancer therapy was initially viewed with cynicism, due to potential apoptotic activity against normal cells. Inhibition of proteasome activity is more cytotoxic to malignant than normal cells, thereby providing an acceptable therapeutic index. Additionally, proteasome inhibition abrogates pro-survival signaling pathways. For example, nuclear factor-kappa B (NF-κB) mediates growth and drug-resistance in cancer cells; conversely, proteasome inhibitors downregulate NF-κB, thereby enhancing

the cytotoxic effects of chemotherapy. Together, these findings support the notion of targeting proteasomes in novel therapeutics.

Proteasome and Its Functional Inhibitors. The 26S proteasome complex consists of 19S units flanking a barrel-shaped 20S proteasome core; the 19S units regulate entry of proteins marked for degradation into the 20S core chamber. Protein breakdown *via* UPS is a multi-step process: protein is first labeled with ubiquitin molecules; E1 ubiquitin enzyme then activates ubiquitin and links it to the ubiquitin-conjugating enzyme E2; E3 ubiquitin ligase then links the ubiquitin molecule to the protein; a long polypeptide chain of ubiquitin moieties is formed; and finally, proteasomes degrade the protein into small fragments and free ubiquitin for recycling.

The three primary proteolytic activities of the proteasome are chymotrypsin-like (CT-L) (beta-5), trypsin-like (beta-1) and caspase-like (beta-2) proteolytic activities; all reside within the 20S proteasome core. Natural and synthetic inhibitors of the UPS pathway include peptide epoxyketones, peptide aldehydes, non-peptide inhibitors, peptide boronates, and peptide vinyl sulfones. Peptide aldehydes (e.g. MG-132, ALLN) potently, but reversibly, block the CT-L activity; they also inhibit lysosomal cysteine and serine proteases as well as calpains, thereby limiting their clinical utility. Lactacystin is a natural, irreversible, nonpeptide inhibitor; the clasto-lactacystin beta-lactone, an analog of its active metabolite, is currently in phase-I clinical trails.

The dipeptidyl boronic acid Bortezomib/PS-341 is a reversible inhibitor of CT-L activity and exhibited remarkable anti-tumor activity against 60 NCI tumor cell lines. Preclinical studies showed that Bortezomib induces MM cell apoptosis, downregulates adhesion molecules, inhibits constitutive and MM cell-adhesion-induced cytokine secretion, and blocks angiogenesis in the BM milieu. These laboratory studies translated into Phase-I trials, which showed safety and acceptable toxicity. Phase II clinical trials showed durable responses, with associated clinical benefits, and these results led to the FDA approval of Bortezomib for the treatment of patients with MM. A randomized Phase-III trial showed higher responses as well as prolonged time to progression and survival in patients treated with Bortezomib versus Dexamethasone, providing the basis for extension of FDA approval to include relapsed MM. While Bortezomib therapy is a major advance in the treatment of MM, it is associated with toxicity and the development of drug-resistance. Ongoing studies are delineating the mechanisms mediating Bortezomib-triggered cytotoxicity and drug resistance in order to design novel therapeutic strategies to both enhance anti-MM activity of Bortezomib and overcome drug resistance.

Mechanisms Mediating Bortezomib-Induced Apoptosis in MM Cells

Blockade of Pro-Survival Signaling. Treatment of MM cells with Bortezomib is associated with inhibition of a major survival signaling pathway mediated *via* NF-κB. Specifically, adhesion of MM cells to bone marrow stromal cells (BMSCs) triggers NF-κB-mediated transcription and secretion of IL-6 and insulin like growth factor-I (IGF-I), both of which promote survival and conventional drug resistance in MM cells in the BM milieu; conversely, treatment of MM cells with Bortezomib inhibits not only NF-κB activation, but also its downstream targets and related cytokine production, thereby overcoming the survival advantage for MM cells conferred by BMSCs. Importantly, NF-κB inhibition alone does not account for the overall anti-MM activity of Bortezomib. Besides NF-κB, Bortezomib downregulates MAPK/JAK-STAT growth/survival signaling pathway, and disables DNA-repair machinery *via* inactivation of DNA-PK. Treatment of MM cells with Bortezomib is not always associated with the inhibition of pro-survival signaling, as in the case of NF-κB, but can also induce pro-survival signaling, such as Akt phosphorylation or heat shock proteins (hsp-27/70/90). These *in vitro* findings have already led to phase-I clinical trials of combined Bortezomib therapy with agents that specifically block Akt or hsp's in an attempt to enhance the anti-MM activity of Bortezomib.

Induction of Pro-Apoptotic Signaling. Gene profiling and proteomic studies show that Bortezomib-induced apoptosis is associated with induction of apoptotic signaling pathways: (i) upregulation of pro-apoptotic c-Jun-NH2-terminal kinase (JNK); (ii) induction of ER-stress response *via* GADD153, CHOP, or PERK; (iii) change of mitochondrial membrane potential and production of reactive oxygen species (ROS); (iv) the release of mitochondrial proteins cytochrome-c/Smac into cytosol, resulting in activation of caspase-9 > caspase-3 cascade; and (v) activation of the extrinsic apoptotic cascade *via* caspase-8 cleavage. Studies using dominant negative strategies and knockout cell line models have established a direct role for JNK and Bax/Bak during Bortezomib-induced apoptosis. Additionally, Bortezomib also affects signaling events upstream of mitochondria; i.e. Ca^{2+} influx into mitochondria triggers cyto-c release and caspase-9-mediated apoptosis, whereas treatment of MM cells with mitochondrial Ca^{2+} uptake inhibitor abrogates Bortezomib-triggered apoptosis. Bortezomib-induced apoptosis involves activation of ER-related stress pathways, including activation of caspase-12.

Clinical Relevance

The multiple signaling pathways associated with Bortezomib-induced cytotoxicity or the development of resistance suggest that combinations of Bortezomib with other conventional and novel agents may both

enhance its anti-tumor activity and overcome drug-resistance. Combined Bortezomib and irinotecan treatment triggers apoptosis in pancreatic tumor xenografts and enhances chemosensitivity in colorectal cancer xenograft models. Studies to date in MM show that combining Bortezomib with Dex, Doxorubicin, or Melphalan, induces additive or synergistic anti-MM activity. Treatment of MM cells with Bortezomib and novel agents Lenalidomide or triterpenoids CDDO-Imidazolide induces synergistic anti-MM activity and overcomes Bortezomib-resistance by activating both intrinsic (*via* caspase-9) and extrinsic (*via* caspase-8) apoptotic signaling, thus providing the rationale for clinical protocols using combination regimens. Microarray analysis of Bortezomib-treated MM cells shows robust induction of hsp-90 in MM cells; conversely, blockade of hsp-90 with 17-AAG enhances sensitivity and even overcomes Bortezomib-resistance. Ongoing phase-1 clinical trials show promise of combined therapy in Bortezomib refractory MM. An alternative aggresome cascade for protein catabolism in MM cells may be significant: histone deacetylase-6 (HDAC-6) is required for the chaperoning of ubiquitinated proteins for aggresomal degradation; and conversely, blockade of HDAC triggers modest anti-MM activity. However, simultaneous blockade of proteasomes and aggresomes with Bortezomib and HDAC inhibitor, respectively, triggers additive/synergistic cytotoxicity, providing the framework for clinical translation of this new class of cancer therapeutics.

New Proteasome Inhibitor NPI-0052

The novel proteasome inhibitor NPI-0052 can overcome Bortezomib-resistance in MM cells. NPI-0052 is a small molecule derived from fermentation of Salinospora, a new marine gram-positive actinomycete. NPI-0052 is a nonpeptide proteasome inhibitor with structural similarity to Omuralide, a beta-lactone derived from naturally occurring lactacystin. NPI-0052, in contrast to Omuralide, possess a uniquely methylated C3 ring juncture, chlorinated alkyl group at C2, and cyclohexene ring at C5, which accounts for its higher anti-tumor activity than omuralide. Initial screening of NPI-0052 against the NCI panel of 60 tumor cell lines showed GI50 of <10 nM in all cases. (i) NPI-0052 triggers apoptosis in MM cells sensitive and resistant not only to conventional, but also to Bortezomib, therapies; (ii) The IC^{50} of NPI-0052 for MM cells is within the low nanomolar concentration; and (iii) NPI-0052 is less toxic to normal PBMNCs than Bortezomib. Importantly, NPI-0052 similarly triggered apoptosis in purified tumor cells from several MM patients relapsing after various prior therapies, including Bortezomib and thalidomide.

The mechanism whereby NPI-0052 overcomes Bortezomib-resistance in MM cells are now been defined. For example, NPI-0052 and Bortezomib differentially affect 20S proteasomal activities. The comparative kinetics of proteasomal activities suggests that NPI-0052, in contrast to Bortezomib, triggers a sustained inhibition of CT-L, T-L and C-L activities (up to 7 days). Simultaneous inhibition of multiple proteasome activities is a prerequisite for significant (i.e. >50%) proteolysis. Therefore NPI-0052 may block more protein breakdown than Bortezomib in MM cells. Moreover, mechanisms conferring Bortezomib-resistance may not be effective against NPI-0052.

NPI-0052 is a potent inducer of apoptosis in tumor cells obtained from Bortezomib-refractory MM patients. Examination of signal transduction pathways further showed that (i) NPI-0052 is a more potent inhibitor of NF-κB and related cytokine transcription and secretion than Bortezomib; (ii) NPI-0052-induced MM cell-death is predominantly mediated by caspase-8; and (iii) Bortezomib-induced apoptosis requires both caspase-8 and caspase-9 activation. These findings confirm differential actions of NPI-0052 *versus* Bortezomib in MM cells. Animal studies using a human plasmacytoma xenograft mouse model showed that NPI-0052 inhibited MM tumor growth and prolonged survival of mice at concentrations which were well tolerated and without either significant weight loss or any neurological behavioral changes. Analysis at day 300 showed no recurrence of tumor in 57% of NPI-0052-treated mice. Together, these data provide the preclinical framework for the ongoing multi-center phase-I clinical trial of NPI-0052 in MM patients.

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Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10

►Gankyrin

26S Proteasome

Definition

A multicomponent complex within the cell responsible for processing and degrading ubiquitinated proteins that are target for degradation via the ubiquitin signal. A multicatalytic proteinase complex composed of a 20S core and a 19S regulator. The 19S regulator is composed of a base, which contains six ATPase subunits and two non-ATP subunits, and a lid, which contains up to 10 non-ATPase subunits.

►Gankyrin
►Herpesvirus-Associated Ubiquitin-Specific Protease
►(HAUSP) De-Ubiquitinase
►Ubiquitination

26S Proteasome Regulatory Subunit p28

►Gankyrin

Protein Array

►Proteinchip

Protein DATA Bank

Definition

An organization that maintains a database of experimentally determined three-dimensional structures of macromolecules (http://www.pdb.org). Tools for visualizing and manipulating these structures are also available.

►Structural Biology

Protein Disulfide Reductase

►Thioredoxin System

Protein Disulphide Isomerase

Definition

PDI; An enzyme belonging to the thiol-disulphide oxidoreductases family of proteins that catalyzes the oxidation, isomeration and reduction of disulphide bonds.

►Endoplasmic Reticulum Stress

Protein-fragment Complementation Assays

Definition

PCAs are designed to measure the physical interaction of two cellular components through the reconstitution of the enzymatic activity of a reporter gene. Each of the components (typically proteins) are genetically or chemically fused with a fragment of the reporter, and their binding reconstitutes enzymatic activity to produce a detectable signal. Most common reporter genes can be used in PCAs, with the two most common being β-lactamase and luciferase.

►Luciferase Reporter Gene Assays

Protein Kinase

Definition

Enzyme that moves phosphate groups from ATP to serine, threonine or tyrosine residues in another protein.

Protein Kinase B

Definition

PKB synonym Akt; Is an important "effector" enzyme lying downstream of ►PI3K turnover of PIP_2 to PIP_3. Active PKB acts on many target proteins the majority of which inhibit ►apoptosis (cell-death). Its hyper-activation therefore can lead to carcinogenesis.

►Akt Signal Transduction Pathway
►Pentakisphosphate

Protein Kinase C

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Definition

Protein kinase C (PKC) represents a family of serine/threonine protein kinases comprising at least 11 structurally related enzymes. PKC isozymes have been grouped into three subfamilies. The conventional or classical PKCs (cPKCs) can be activated by Ca^{2+}, diacylglycerol (DAG) or phorbol esters and include the isotypes α, β I, β II and γ. Novel PKCs (nPKCs), comprising the isoforms ó, ε, δ, η and θ are also activated by DAG or phorbol esters but are Ca^{2+}-independent. The more recently discovered "atypical" PKCs (aPKCs) ι (the mouse homologue of ι has been termed λ) and ζ are Ca^{2+}- and DAG-independent and do not respond to phorbol esters. Each PKC isozyme is encoded by a separate gene with the exception of PKC β I and β II, which represent alternative spliced variants.

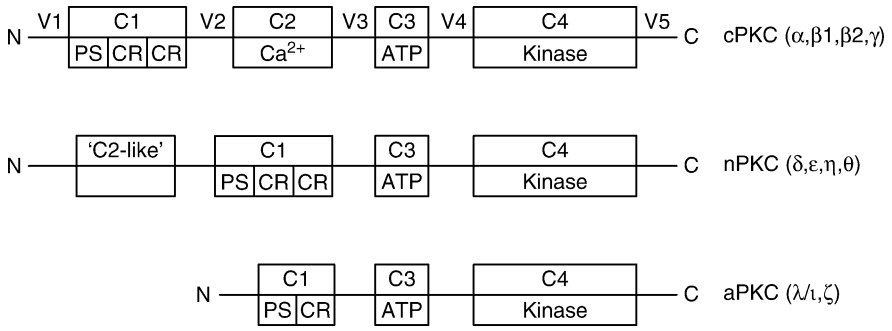
Characteristics

Each PKC isozyme consists of a single polypeptide containing an amino-terminal regulatory domain and a carboxy-terminal catalytic domain connected by a hinge region that is highly sensitive to proteolytic cleavage by cellular proteases (Fig. 1). The enzymes possess regions that are highly conserved between different PKC isoforms (termed C1–C4) and five variable regions (V1–V5).

1. The C1 region, which is present in all PKC isozymes, contains an autoinhibitory pseudosubstrate domain. The amino acid sequence of the pseudosubstrate domain resembles the phosphorylation motifs in PKC substrates but contains an alanine instead of the serine or threonine, which act as phosphate acceptors in PKC substrates. The C1 region of cPKCs and nPKC contains two cysteine-rich domains in tandem orientation, which serve as DAG- or phorbol ester binding sites. Only one copy of this cysteine-rich domain is found in DAG- and phorbol ester- non-responsive aPKCs.
2. The C2 region of the cPKCs has been identified as the Ca^{2+}-binding site. A homologous region is also found in nPKCs but the absence of a critical aspartate residue renders this C2-domain incapable of Ca^{2+}-binding. Atypical PKCs lack a C2 domain. The C3- and C4-domains are parts of the catalytic region. ATP is bound to the C3 region, whereas the C4 domain contains binding sites for substrates.

Mechanism of Activation

Activation of PKC requires an unmasking of the catalytic domain by the removal of the pseudosubstrate. The necessary conformational change is mediated by the binding of DAG or phorbol ester in the presence of a lipid cofactor, especially phosphatidyl-serine. Other lipids, like free fatty acids, phosphatidyl-choline, lysophosphatidic acid and phosphatidyl inositol 3,4,5-trisphosphate, can also act as co-factors for some PKC isotypes. There is evidence that the pseudosubstrate sequence, once removed from its binding site, may contribute to membrane binding through its basic residues; a process that is also controlled by protein-protein interaction. Membrane association or translocation to membrane compartments is often considered as a hallmark of PKC activation. Ca^{2+} has been shown to increase the affinity of cPKCs to acidic phospholipids. In addition to allosteric regulation by Ca^{2+} and lipid co-factor, phosphorylation of PKC is essential for enzyme activation. The enzyme phosphoinositide dependent kinase 1 (PDK1) has been identified as a major upstream kinase catalyzing phosphorylation probably of all PKC isotypes within the activation loop of the C4 domain of the catalytic region. In cPKCs, the PDK1-mediated phosphorylation is followed by phosphorylation on two additional sites within the V5-region of the carboxy-terminal sequence, probably due to autophosphorylation. Autophosphorylation of one site, Thr-641, is essential for the catalytic activity of nPKC δ. Novel PKC δ is the only PKC isoform that is subject to phosphorylation on tyrosine residues. However, the biological significance of the tyrosine phosphorylation of PKC δ remains controversial. Protein-protein interaction appears of particular relevance for the regulation of atypical PKCs. LIP (λ/ι interacting protein) has been identified as a PKC λ/ι



Protein Kinase C. Figure 1 Structure of PKC isozymes: C1–C4, constant regions; V1–V5, variable regions; PS, pseudosubstrate domain; CR, cysteine-rich region; ATP, ATP-binding domain; Ca^{2+}-binding domain (note that the C2 region in nPKCs is uncapable of Ca^{2+}-binding as outlined in the text).

specific activator. Binding of par-4 to aPKC λ/ι or a PKC ζ inactivates the enzyme.

PKC-Binding Proteins

PKC-interacting proteins can be classified into different subgroups according to their function:

1. PKC substrates (also termed STICKS)
2. Regulatory proteins like LIP, par-4, or syndecan-4
3. Docking proteins like RACKS, AKAPS or caveolin that may be involved in regulating the intracellular localization of PKCs
4. Scaffolding signaling proteins that cluster specific PKC isoforms together with other signaling elements in order to regulate a particular signal transduction pathway

Putative PKC scaffolds include ZIP and proteins of the 14-3-3 family. This list of PKC-binding proteins is incomplete and grows continuously. The techniques employed to detect PKC-interacting proteins, e.g. the yeast two-hybrid system or overlay assays, do not always prove that these interactions occur in vivo and do not indicate the biological significance of this interaction. Therefore, these data should be interpreted with caution.

Bioactivity

Enzymes of the PKC family are important elements of intracellular signal transduction. They are differentially involved in the regulation of a broad variety of cellular functions: These include proliferation; differentiation; ►apoptosis; immune response; release of messengers from endocrine, exocrine and neuronal tissues; modulation of ion channels, receptors, transporters; organization of the cytoskeleton; contraction and basal tone of smooth muscles. In many cases, however, the implication of PKC is based on effects obtained with phorbol esters or PKC-inhibitors. Since none of them are PKC-specific, the role of PKC in several systems needs to be re-examined. More specific techniques that are now available include depletion of defined PKC-isozymes by siRNA, antisense techniques, expression of dominant negative or constitutively active mutants of particular isoforms, and generation of "knock-out" mice lacking functional alleles for a defined PKC isoform.

PKC in Cancer

PKC first attracted the attention of oncologists when it was found that PKC acts as a highly specific receptor for the classical tumor promoting phorbol esters like ►TPA. The mechanism by which TPA exerts its tumor promoting activity is still unclear. Elucidation of the mechanism of action of phorbol esters is hampered by the fact that they act in a bimodal fashion; after an initial activation of phorbolester-responsive PKC isozymes, they cause a depletion of these proteins. Thus, it is unclear whether the phorbol ester effect is due to an activation or an inhibition of a PKC-mediated reaction. Furthermore, in addition to PKC, other highly specific non-kinase receptors for phorbol esters have been described, including the chimerins, Munc13/1 and RasGRP. Binding to these receptors is activated by the same co-factors activating c- and nPKCs and inhibited by classical PKC inhibitors. Interestingly, chimerins act as Rac-GAPs, thereby regulating the duration of the active state of the Ras-homology protein Rac. Ras-GRP functions as an guanylate nucleotide exchange factor for Ras and activates Ras by promoting the GTP-charged form of Ras. Thus, the chimerins as well as Ras-GRP should be considered as perhaps equally important as PKC in tumor promotion.

The implication of a role of the enzymes of the PKC family in the regulation of cellular proliferation is documented by a vast number of studies. However, when the effects are analyzed with respect to individual PKC isotypes, the observations are frequently contradictory and generally confusing. For instance, PKC α has been described as activated and overexpressed in several tumors, like hepatomas and breast cancer, and

to transform MCF7 cells following ectopic overexpression. However, in B16 melanoma cells or K562 cells ectopic expression of PKC α was found to inhibit cellular proliferation or to induce differentiation. Similar controversial findings have been reported for PKCs β, γ and ζ. Thus, the function of PKC isozymes is to a great extent cell type dependent. A more general picture has emerged with regard to nPKC ε, which was found to act as a growth-promoting enzyme in most systems, except in neuronal cells. Overexpression of PKC ε leads to transformation in fibroblasts and epithelial cells. Based on these findings, the PKC ε encoding gene is considered to represent an oncogene. Expression of dominant negative aPKC λ/ι reverses transformation by Ras. These and other findings (see below) suggest that PKC λ/ι acts as a positive regulator of cell growth. More uniform effects have also been observed with PKC δ, which appears to act as a general suppressor of tumor growth. A better understanding of the biological role of PKC requires elucidation of their function at the molecular level. Information describing the biochemical function of individual PKC isoforms in mitogenic signal transduction is accumulating.

An implication for the role of PKC isoforms in the regulation of the Ras > Raf > MEK > ERK pathway is well documented. This pathway, also known as the MAP kinase cascade, is a major route for the transmission of mitogenic signals from growth factor receptors or oncogenic Ras. PKCs α and η activate cRaf and PKC ζ stimulates MEK. Evidence for a co-ordinate function of aPKC λ, nPKC ε and aPKC ζ for the Ras-mediated induction of c-Fos by the Ras > Raf > ERK pathway and the transcriptional activation of cyclin D, a critical step for the progression of cells through the G1-phase of the cell cycle, has been published. Other PKC isoforms including α, δ and η have been shown to arrest cells in G1 by enhancing the cell cycle inhibitory proteins p21waf/cip and p27kip. Several PKC isozymes, notably λ/ι and ζ, are implicated in cytoskeletal functions, thereby linking cell shape alterations to the cell cycle.

Activation of survival pathways by an inhibition of apoptosis is of utmost importance for malignant transformation and maintenance of the transformed phenotype. PKC isotypes α, δ and ζ are frequently associated with these mechanisms. The function of PKC α has been reported to act pro- and anti-apoptotic, dependent on cell type. The anti-apoptotic activity of α has been correlated to a PKC α-mediated activation of Akt, an inhibitor of programmed cell death. PKC δ is involved in the execution phase of apoptosis; its function is at least in part explained by a caspase 3-mediated activation of this PKC isoform. A clear picture has emerged with regard to PKC ζ; atypical PKC ζ stimulates cell survival by activating NfκB either by mediating the release from the inhibitor I-κB or by phosphorylating the RelA subunit of NFκB. Similar effects have been reported for the structurally related aPKC λ/ι. Furthermore, par-4, a prominent mediator of apoptosis binds and inactivates PKC ζ.

Resistance to anti-tumor agents has been correlated to overexpression or activation of several PKC isotypes. This also applies to multidrug resistance mediated by an overexpression of the MDR1 gene encoded P-glycoprotein. However, the biochemical basis of this correlation remains obscure. A PKC-mediated phosphorylation of the P-glycoprotein could be excluded, as deletion of all PKC phosphorylation sites did not affect the pumping activity of the P-glycoprotein. The correlation is in part based on observations indicating a reversion of the resistant phenotype by PKC inhibitors. In some cases, however, these PKC inhibitors were found to interact directly with the P-glycoprotein and to inhibit its activity by a PKC-independent mechanism. It is intriguing to speculate that the observed correlation between intracellular PKC activity and drug resistance is due to a PKC-mediated activation of anti-apoptotic survival pathways described above. This may also explain the observed synergistic effects of combinations of PKC inhibitors with established anti-tumor agents.

Clinical Relevance

The implication of PKC isozymes in tumor cell proliferation, invasion and metastasis, apoptosis and drug resistance has led to the development of PKC inhibitors that can be used for the chemotherapy of cancer. Several of these agents have entered clinical trials. Bryostatin-1, a macrocyclic lactone derived from Bugula neritina, occupies the same binding site as phorbol esters but is not a tumor promoter and leads to a depletion of c- and n-type PKCs. Phase II trials with bryostatin including a wide range of tumor types have so far been disappointing. Phase II studies on combinations of bryostatin I with other cytotoxic agents are ongoing. Phase II studies with an antisense construct targeted against PKC α (aprinocarsen, ISIS 3521) are in progress. These studies include patients with brain tumors, breast, colon, ovarian and prostate cancer. Phase II studies in breast cancer patients and patients with high grade astrocytomas did not reveal any activity. A phase III study in NSCLC failed to show any clinical benefit from a combination of aprinocarsen with gemcitabine and cisplatin or paclitaxel and carboplatin. Safingol, a dihydrosphingosine analogue, has been employed in clinical trials as a sensitizing agent in order to potentiate chemotherapy. Many other compounds are now in pre-clinical and early clinical development. The majority of these PKC inhibitors interact with the ATP binding site of the catalytic region. Most of them are bisindolylmaleimides or indolocarbazoles and are structurally related to the

biological compound staurosporine, a product of Streptomyces staurosporeus. Clinical phase I/II trials with the indolocarbazoles midostaurin and enzastaurin (LY317615) revealed some sensitizing effects in combination with doxorubicin or vinblastine (midostaurin) or gemcitabine, 5FU, cisplatin or radiotherapy (enzastaurin). More recently, a series of very potent PKC inhibitors was described that represent derivatives of balanol, an azepine natural product. Balanol analogues also interact with the ATP binding site of c- and nPKCs.

However, PKC inhibitors are not only of interest as potential anti-tumor agents. Other areas in which PKC inhibitors are presently investigated include diabetes, cardiovascular diseases, inflammation and immunological disorders and diseases of the central nervous system.

►Toxicological Carcinogenesis

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Protein Kinase C Family

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Synonyms

Ca^{2+}-activated phospholipid dependent protein kinase; PKC

Definition

Protein kinase C was found originally in 1977 by Nishizuka and co-workers. The group named this ►serine/threonine kinase as Ca^{2+}-activated, phospholipid dependent protein kinase, or protein kinase C in short, since the kinase activity was found to be increased in the presence of phospholipids and calcium. Today, human protein kinase C (PKC) family is known to consist of at least 11 serine/threonine kinases which are classified into three major groups based on their structure and biochemical properties: classical (α, βI, βII, γ), novel (δ, ε, η, and θ), and atypical (ζ and ι). PKCμ differs structurally from all three major groups. The genes of different PKC isoenzymes are dispersed throughout the genome. Different PKCs play a role in multiple cellular processes, which are important in cancer cell behavior. PKCs exert their functions by phosphorylating their target proteins, which are numerous and largely unknown. Originally, relevance of PKC to cancer development was discovered when PKC was identified as a primary target of tumor-promoting phorbol esters.

Characteristics

Regulation

Activation of classical PKC isoenzymes (cPKC) depends on calcium, ►diacylglycerol, and acidic phospholipids, such as ►phosphatidylserine. Novel isoenzymes (nPKC) are activated by diacylglycerol and acidic phospholipids, and atypical isoenzyme (aPKC) activation takes place independent of calcium and diacylglycerol. Activation of aPKCs takes place by acidic phospholipids, ►ceramides, and protein–protein interactions, such as interaction with other PKCs.

Growth factor-mediated ►phospholipase C activation plays a central role in the activation of cPKC and nPKC. On ligand binding, growth factor receptor activates and induces phospholipase C translocation from cytosol to the plasma membrane. Activated phospholipase C then generates diacylglycerol and ►inositol trisphosphate (IP_3) from plasma membrane phospholipids. Subsequently, diacylglycerol activates both cPKC and nPKC, and IP_3 releases calcium from intracellular stores. Calcium enhances the activation of cPKC. PKC activation also depends on complex series of phosphorylations which make PKC catalytically competent. Following ligand binding on the plasma membrane, PKC acts as a target for different kinases. These kinases phosphorylate PKC at activation loop sites and hydrophobic sites. PKC is stabile and phosphatase-resistant after autophosphorylation. However, PKC activity is not determined by these phosphorylations only. If the ligand dissociates, PKC can diffuse away from plasma membrane but remain phosphorylated. In this case, PKC can be reactivated by diacylglycerol binding alone.

PKC Expression in Cancer

Members of PKC family are normally ubiquitously expressed in a wide range of tissues, isoenzymes α, β,

and δ being the most abundant. However, PKC isoenzymes have been shown to display altered expression in cancer when compared with normal tissues. The most common isoenzymes displaying alterations in expression during cancer progression are α, β, and δ, but change in expression of other isoenzymes may also take place. Immunohistochemical studies on human tumors have shown PKCα overexpression in urinary bladder, prostate, and endometrial cancers, whereas low grade tumors and normal epithelia of the respective organs show significantly lower expression. In contrast, breast, colon, hepatocellular, and basal cell cancers display downregulation of PKCα expression. PKCβ expression has been shown to be high in colon and prostate cancers, and low in high grade urinary bladder cancers. Furthermore, the expression profile of PKCs within a single tumor can be variable, as exemplified by the fact that increased PKCα- and βI expression and activity have been shown to associate with high proliferation areas of urinary bladder tumors. Even single neighboring cells may display manifold difference in the expression of these enzymes. This suggests that PKC expression level is under control even in carcinoma tissue.

Similarly to cPKC expression, PKCδ can be either upregulated or downregulated. For instance, hepatocellular cancer displays increased PKCδ expression, while in urinary bladder cancer the isoenzyme is downregulated. Studies have mainly focused on cPKC and PKCδ, while the expression of other PKC isoenzymes in cancers is less well known. The expression of PKCλ, ι, and ε is decreased in pancreatic cancers, PKCη is increased in renal cancers, and PKCλ is increased in ovarian cancer.

It is unclear, whether PKC isoenzyme expression in cancer can be associated with patient survival. Expression of PKCι has been suggested to be a negative prognostic factor in ovarian carcinoma and PKCγ as positive prognostic factor in B-cell lymphoma. Based on the variability of expression of different PKC isoenzymes in different cancers, it can be stated that no general conclusions can be drawn from the expression patterns with respect to carcinogenesis in general. In addition, the levels of isoenzyme proteins do not directly correlate with the overall enzyme activity, making conclusions difficult.

PKC Activation in Carcinogenesis

Evolution of a malignant tumor is a multistep process, which includes mutations in several genes as well as other molecular changes in cellular proteins. First, initiating agents mutate proto-oncogenes and thus initiate the malignant transformation. Second, a prolonged exposure to ►tumor promoters induces the clonal outgrowth of the mutagen-initiated cell population and increase genetic instability. The primary target of the tumor promoters, such as ►phorbol 12-myristate acetate (PMA, TPA), aplysiatoxin, and teleocidin seems to be PKC. One mechanism of expressional changes seen in human cancers may result from the tumor promoter action. Tumor promoters, such as PMA, bind to the diacylglycerol binding site of PKC and promote chronic activation of PKC. Prolonged activation of PKC leads to degradation of the enzyme by ubiquitin–proteasome pathway. In fact, changes in expression and activity of PKC are apparently not due to genetic chances, since mutations in PKC genes are found very rarely in human cancers. However, evidence has been provided for loss-of-function mutation of PKCα gene in thyroid and pituitary cancers. Furthermore, a deletion has been reported in PKCα gene in melanoma. Significance of this mutation is unknown, but several melanoma cell lines display decreased PKCα expression.

The role of PKCs in cancer seems to result from their action as targets of tumor promoters or from their action as downstream effectors of growth factor receptors. Tobacco smoke contains a number of substances considered as tumor promoters which activate PKCs, such as catechol and hydroquine. Furthermore, high consumption of dietary lipids is carcinogenic. Carcinogenicity of dietary lipids may, at least in part, be exerted through direct or indirect PKC activation, which have also been shown in animal models. PKC expression and activity seems to be modulated by a wide variety of genetic and epigenetic factors. Chemical carcinogens such as nitrosamines which are present in tobacco smoke are known to induce increased expression and activation of PKCα and β, while PKCδ is downregulated. Furthermore, oncogenic ►Ras increases PKCα, ►c-myc oncogene increases PKCβ expression, and wild type ►p53 tumor suppressor decreases PKCα expression.

Targets of PKC and its Cancer-Related Functions

PKCs are involved in multiple physiological processes of cells. Short-term activation of PKC is often associated with short-term events such as secretion and ion influx. In contrast, sustained activation has been shown to induce cellular proliferation, differentiation, ►apoptosis, ►migration, ►adhesion, tumorigenesis, and epithelial barrier function.

The role of PKC in the control, or rather in the loss of control of cancer cell growth is well established. However, different isoenzymes function differently and have variable effects on cell growth which may also vary depending on the cell type. Thus, general conclusions with respect to PKC function in cell growth may be difficult to make. PKCα has been linked to decreased and increased cellular proliferation, and to induction or inhibition of apoptotic cell death of cancer cells. However, most of the studies consider PKCα as an inducer of proliferation and suppressor of apoptosis.

Also PKCβ has been associated with increased proliferation rate of cancer cells. Of novel isoenzymes, PKCδ has been strongly associated with control of apoptosis. PKCδ activity results in many proapoptotic signals, such as increased mitochondrial ►cytochrome *c* release and ►c-Abl activation. In malignancies, downregulation of PKCδ activity has been shown to lead to inhibition of apoptosis and resistance to DNA-damaging agents.

►Telomerase activity is associated with immortalization and malignant phenotype of a cell. PKCα, βI, δ, and ζ isoenzymes phosphorylate and activate telomerase, and PKC inhibitors inhibit its activity. The target of PKC appears to be telomerase reverse transcriptase, hTERT, one of telomerase subunits.

There is strong evidence that PKCα activity is associated with increased motility and invasion of cancer cells. This has been suggested to occur through inhibition of ►adherens junctions and ►desmosomes, inhibition of hemidesmosomes, and changes in focal adhesions. Also, ε, βII, and ζ isoenzymes have been associated with cellular motility.

Other cancer-related associations for PKC include ►multidrug-resistance (mdr), since PKCα activity has been shown to phosphorylate, and PKCα and θ to increase expression of ►P-glycoprotein. Furthermore, simultaneous loss of P-glycoprotein and PKCα expression is associated with reversion of mdr, while inhibition of PKCα attenuates resistance to cytotoxic agent.

PKCs may also regulate tumor vascularization, since PKCβ has been shown to act as a mediator of vascular endothelial growth factor (VEGF) signaling and its inhibition leads to decreased endothelial cell proliferation and reduction of neovascularization of a malignant tumor. Furthermore, PKCα- and ζ isoenzymes regulate VEGF expression through posttranscriptional mechanisms.

A high number of different potentially cancer-related direct or indirect targets have been reported. Some of the most important include, in addition to the targets mentioned above, the following: PKCα, βI, and γ have been shown to phosphorylate and inactivate ►glycogen synthase kinase-3 beta (GSK-3β), leading to increased c-Jun DNA binding activity. C-Jun is an important controller of cell growth and apoptosis. PKC can directly activate ►Raf-1/MEK/MAPK pathway by phosphorylating Raf-1. Raf-1/MEK/MAPK pathway affects several targets related to cell growth. Transcription factor ►nuclear factor kappa beta (Nf-κB), which is a central regulator of several apoptosis-related genes, is also activated by PKC. PKC also regulates intercellular communication through gap junctions and PKC has been shown to be directly phosphorylate ►gap junction protein, connexin 43. Recently, it was shown that PKCα directly phosphorylates ►neurofibromin, a Ras GTPase-activating tumor suppressor, and increases its association to actin cytoskeleton.

PKC as a Target in Cancer Therapy

Since many tumor promoters act as PKC activators and as PKCs play an important role in diverse cellular functions, PKCs are potential therapeutic targets. Multiple PKC modulating approaches have been developed during the last decades. PKC inhibitors may be divided to isoenzyme selective and broad spectrum inhibitors. However, PKC agonists, such as bryostatin-1, may also be used to inactivate PKC, since long-term activation of PKC leads to its downregulation.

Many PKC inhibitors have been tested in vitro and in vivo animal models, with promising results. The most tested PKC inhibitors are bryostatin-1, PKC412 (broad spectrum inhibitor, *N*-benzoyl staurosporine, also named CGP41251 and midostaurin), UCN-01 (cPKC pointed broad spectrum inhibitor, 7-hydroxy-staurosporine), aprinocarsen (PKCα antisense oligodeoxynucleotide, also named ISIS3521, LY900003, CGP 64128A, or affinitak), enzastaurin (PKCβ inhibitor, also named LY317615), safingol (PKCα inhibitor), and Go6976 (PKCα/βI inhibitor, also named Gö6976). PKC412, aprinocarsen, and Go6976 have shown impressive anti-invasive properties. Furthermore, reduced growth of cancer cells has been shown with UCN-01, PKC412, aprinocarsen, Go6976, and enzastaurin. The antiproliferative mechanism of these agents has been suggested to be cell cycle arrest and increased apoptosis. Also bryostatin-1 inhibits proliferation, induces differentiation, and apoptosis. In addition to its direct effects on cancer cell growth, enzastaurin has been shown to reduce plasma ►VEGF levels and inhibit angiogenesis in a tumor model.

Combining different PKC inhibitors with classical ►chemotherapeutic agents or radiotherapy has resulted in additive growth inhibiting effects. It has been speculated that these additive effects result from inhibition of ►mdr or modulation of apoptosis. An interesting mechanism for PKC inhibitors in increasing cancer cell death is ►cell cycle checkpoint abrogation. When a cell faces DNA damage induced by chemotherapy or ionizing radiation, it activates its cell cycle checkpoints, resulting in cell cycle arrest. It has been demonstrated that many PKC inhibitors inhibit, either directly or through PKC, kinases that control especially G2 checkpoint of the cell cycle. Therefore, cancer cells that are arrested to G2 by chemotherapy can be forced from G2 to mitosis by using selected PKC inhibitors. This treatment kills the cancer cells very effectively by ►mitotic catastrophe or apoptosis.

Bryostatin, aprinocarsen, UCN-01, PKC412, enzastaurin, and safingol have been tested in clinical trials as single agents and in combination with various chemotherapeutic drugs. Go6976 has not been studied in

clinical trials. The clinical responses for the substances have varied considerably. For instance, aprinocarsen displayed some promising effects, but phase III studies showed that the substance did not improve response or survival when used in combination with chemotherapy in advanced non-small cell lung cancers. Additionally, major thrombocytopenia was observed. The clinical efficiency of UCN-01 and PKC412 has been poor as well. UCN-01 has shown unusually prolonged half-life and low clearance, which results from high binding to plasma proteins. Several human studies are ongoing with these substances and results are eagerly awaited. www.gancer.gov/clinicaltrials.

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Protein Microarray

Definition

Protein microarray is composed of array containing full-length functional proteins or protein domains. It is fabricated by depositing a library of proteins or antibodies immobilized in a 2-D addressable grid on a chip coated with a chemically or physically functional layer for covalent or noncovalent immobilization of proteins or other capture reagents. Quantification of microarray-based assay is achieved by capturing array images with an optical device and using compatible image analysis software to determine fluorescent, chemilluminescent, or colorimetric intensity of each spot on array images. Protein microarray is used to study the biochemical activities of an entire proteome in a single experiment, such as protein–protein, protein–DNA, protein–RNA, protein–phospholipid, and protein–small molecule interactions.

Protein Stabilization

Definition

A generalized term that represents the process of preventing protein degradation, either through de-ubiquitination or some other type of cellular process.

►Herpesvirus-Associated Ubiquitin-Specific Protease (HAUSP) De-Ubiquitinase

Protein-tyrosine Kinase Inhibitors

►Tyrosine Kinase Inhibitors

Proteinase

Definition

Synonym protease; An enzyme which catalyses hydrolysis of peptide bonds. Endopeptidase refers to proteinase activity within an intact protein.

►Proteasome Inhibitors

Proteinase-Activated Receptor-1

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Synonyms

Thrombin receptor; Coagulation factor II receptor; PAR_1

Definition

►PAR_1 is a ►G protein-coupled receptor which can be activated preferentially by ►thrombin, ►trypsin, ►factor Xa, activated ►protein C, ►plasmin and ►matrix metalloproteinase-1 (MMP-1). It consists of 425 amino acids (47 kDa) including the amino-terminal signal sequence, and is widely expressed in different tissues and overexpressed in numerous cancers. The gene maps to 5q13.3 (gene name: F2R).

Characteristics

PAR_1 is stimulated by specific cleavage at the site LDPR^{41} ↓ S^{42}FLLRN to expose the tethered ligand SFLLRN which binds to the second extracellular loop, thus activating the receptor, resulting in ►signal transduction. A synthetic peptide ("activating peptide") that mimics the tethered ligand domain (TFLLRN-NH_2) directly binds to and activates PAR_1 *in vitro* without proteolytic cleavage. Thrombin can activate PAR_1 by a two-step mechanism. First the proteinase binds to a ►hirudin-like site distal to the cleavage site, subsequently cleaving the receptor. This mechanism allows efficient activation of the receptor. Hence, PAR_1 is a high-affinity receptor for thrombin, mediating rapid and transient cellular responses. Moreover, evidence exists that uncleaved PAR_1 can also be activated intermolecularly by another activated PAR_1 receptor molecule (transactivation).

Overexpression of PAR_1 in breast cancer cells correlates with tumor progression. Activation of PAR_1 in these cells induces reorganization of the cytoskeleton and the formation of focal contact complexes. These complexes are important for cell migration and invasion. Moreover, overexpression in murine mammary glands results in accelerated cell proliferation due to persistent activation of ►β-catenin. In contrast, experimental downregulation of PAR_1 in an aggressively metastatic breast cancer cell line reduces invasion. In prostate cancer, PAR_1 has been implicated in bone metastasis. Metastatic human melanoma cell lines express PAR_1, and overexpression of PAR_1 in melanoma cells leads to enhanced invasion and metastasis. Deregulation of PAR_1 expression was found to be associated with a loss of the transcription factor activator protein-2α. PAR_1 activation also contributes to tumor growth by enhancing tumor cell proliferation, as has been shown for melanoma and colon carcinoma cell lines. Moreover, the ability of tumor cells to activate the coagulation system and to generate thrombin has been shown to enhance metastasis, while anticoagulant therapies can interfere metastasis formation. Thrombin and potentially other coagulation proteinases can modify tumor cell behavior directly through the activation of PAR_1 or other thrombin receptors.

PAR_1 is present on ►platelets, where it mediates platelet aggregation, a central event in ►thrombus formation. Thrombus formation is a crucial step during metastasis: cancer cells circulating in the blood stream produce thrombin which promotes ►fibrin (Factor Ia) and platelet deposition. This enables tumor cells to adhere to the blood clot, facilitating penetration through the vessel wall. Interestingly, factor Xa (FXa), apart from its role in the extrinsic and intrinsic ►coagulation pathways to activate ►zymogens, can directly cleave PAR_1. However, optimum cleavage capacity is only achieved when the factor is immobilized on the cell membrane via ►tissue factor (TF) as a complex together with ►factor VIIa (FVIIa). Both TF and FVIIa can be produced by the cancer cells themselves.

PAR_1 is able to couple to ►epidermal growth factor receptor (EGFR) signaling. This receptor ►transactivation was found to promote migration of renal carcinoma cells.

Metastasis requires adhesion of tumor cells to endothelial cells of the vessel wall. Melanoma cells secrete MMP-1 which is able to activate PAR_1 on endothelial cells. This leads to secretion of ►von Willebrand factor (vWF) and proangiogenic ►interleukin-8. ►Angiogenesis has also shown to be stimulated in the presence of the PAR_1 agonists thrombin and activated protein C. Tumors induce the formation of new blood vessels. These provide nutrition for the tumor cells which have a high metabolical turnover. In addition, MMP-1 that was secreted by stromal cells of the tumor microenvironment was shown to activate PAR_1 on breast cancer cells, increasing tumor cell migration and invasion.

In addition to the hypercoagulable state which is associated with advanced cancer, activation of the fibrinolytic system has also been linked to poor prognosis: plasmin can activate PAR_1 to induce the angiogenic cytokine Cyr61. In turn, Cyr61 expressed by the tumor cells induced MMP-1 secretion by the stromal host cells. Together, the role of PAR_1 in tumor progression involves not only the tumor cells themselves but also a close interaction between the cancer cells and the surrounding tumor microenvironment.

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Proteinase-Activated Receptor 2

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Synonyms

Thrombin receptor-like 1; Coagulation factor II receptor-like 1; G-protein coupled receptor 11; PAR_2

Definition

►PAR_2 is a ►G protein-coupled receptor which can be activated by ►trypsin, ►tryptase, ►factor VIIa, ►factor Xa, ►matriptase (MT-SP1), and ►proteinase 3. It consists of 397 amino acids (44 kDa) including the amino-terminal signal sequence, and is widely expressed in different tissues and upregulated in numerous cancers. The gene maps to 5q13.3 (gene name: F2RL1).

Characteristics

PAR_2 is activated by specific proteolysis at the site $SKGR^{36} \downarrow S^{37}LIGKV$ to expose the tethered ligand SLIGKV which binds to the second extracellular loop, thus activating the receptor, resulting in ►signal transduction. A synthetic peptide ("activating peptide") that mimics the tethered ligand domain (SLIGKV-NH_2) directly binds to and activates PAR_2 *in vitro* without proteolytic cleavage. *In vivo*, PAR_2 can also be stimulated by ►transactivation through PAR_1: the tethered ligand domain of PAR_1 is able to bind to and activate uncleaved PAR_2. Hence, the peptide sequence SFLLRN, derived from the tethered ligand domain of PAR_1, can stimulate both PAR_1 and PAR_2 *in vitro*. In addition, it was observed that thrombin is able to activate PAR_2 on melanoma cells indirectly and independent of receptor cleavage. The underlying mechanism, however, is yet unclear.

PAR_2 activation in cancer cells induces cytoskeletal reorganization. This results in enhanced ►migration, ►invasion and ►metastasis. Moreover, PAR_2 is able to promote proliferation of various tumor cells. This is achieved at least partly by ►transactivation of the epidermal growth factor receptor (EGFR). In ►pancreatic cancer, PAR_2 was reported to induce fibrosis.

As observed with PAR_1, the complex TF-FVIIa-FXa can also activate PAR_2. In breast cancer cells, PAR_2 is able to promote migration and invasion via TF-FVIIa-FXa and TF-FVIIa. Activation of PAR_2 in these cells leads to release of the angiogenic factor ►vascular endothelial growth factor (VEGF). PAR_2 promotes proliferation of most cells. An exception are epidermal keratinocytes of the upper skin layer, where activation of PAR_2 inhibits cell growth. Although PAR_2 is overexpressed in several tumor cell lines, the receptor is downregulated in poorly differentiated ►squamous cell carcinomas of the skin and esophagus, which are derived from keratinocytes, and in certain ►gastric carcinomas. These findings point to a tumor-suppressing role of PAR_2 in certain tissues. However, the underlying mechanisms are not elucidated yet. Another serine proteinase with trypsin-like activity is ►matriptase (MT-SP1), a transmembrane proteinase. PAR_2 can be cleaved by this enzyme, and matriptase is overexpressed in numerous cancers and has been implicated in tumor progression and ►metastasis.

Proteinase-Activated Receptor-3 (PAR_3)

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Synonyms

Thrombin receptor-like 2; Coagulation factor II receptor-like 2; PAR_3

Definition

►PAR_3 is a G protein-coupled receptor which can be activated by ►thrombin (Factor IIa). It consists of 374 amino acids (42 kDa) including the amino-terminal signal sequence, and is highly expressed in ►megakaryocytes of the bone marrow. A lower expression is found in mature ►megakayrocytes, platelets, heart and gut. The gene maps to 5q13.3 (gene name: F2RL2).

Characteristics

PAR_3 is activated by specific proteolysis at the site $LPIK^{38} \downarrow T^{39}FRGAP$ to expose the tethered ligand LPIK which binds to the second extracellular loop, thus activating the receptor. No synthetic peptides exist which are able to stimulate PAR_3 *in vitro*, as observed with the other members of this receptor family. Distal to the thrombin cleavage site, there is a ►hirudin-like binding site located. This enables binding and concentration of thrombin at the cell surface, rendering PAR_3 a high-affinity receptor for thrombin. However, it seems that PAR_3 needs the interaction with other PARs for ►signal transduction, since it was found that mouse PAR_3 is unable to signal when expressed in the absence of other PAR receptors. PAR_3 is well expressed in mouse platelets, which are deficient in PAR_1. Here, PAR_3 is a cofactor for PAR_4. Thus in mouse platelets, PAR_3 adopts the role played by PAR_1 in the human system. PAR_3 does not seem to play a major role in tumorigenesis. However, it was reported to be expressed in ►renal cell carcinomas together with PAR_1.

Proteinase-Activated Receptor-4

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Synonyms

Thrombin receptor-like 3; Coagulation factor II receptor-like 3; PAR_4

Definition

►PAR_4 is a ►G protein-coupled receptor which can be activated by ►thrombin (Factor IIa), ►plasmin, ►trypsin and ►cathepsin G. It consists of 385 amino acids (41 kDa), including the amino-terminal signal sequence, and is widely expressed in various tissues. It is not expressed in brain, kidney, spinal cord and peripheral blood leukocytes. The gene maps to 19p2 (gene name: *F2RL3*).

Characteristics

PAR_4 is activated by specific proteolysis at the site $PAPR^{47} \downarrow G^{48}YPGQV$ to expose the tethered ligand GYPGKV which binds to the second extracellular loop, thus activating the receptor. A synthetic peptide ("activating peptide") that mimics the tethered ligand domain ($GYPGQV\text{-}NH_2$) directly binds to and activates PAR_4 *in vitro* without proteolytic cleavage. PAR_4 does not carry a ►hirudin-like binding site for thrombin as observed in PAR_1 and PAR_3. Thus, PAR_4 is a low-affinity receptor for thrombin. Despite this fact, cellular responses mediated by PAR_4 are sustained, and the receptor desensitizes only slowly in contrast to the high-affinity thrombin receptor PAR_1, leading to a prolonged signal. PAR_4 is involved in ►invasion and ►angiogenesis, and it was reported to be overexpressed in adenocarcinomas and ►squamous cell carcinomas of the lung.

Proteinase-Activated Receptors

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Definition

►Proteinase-activated receptors (PARs) belong to the protein family of ►G protein-coupled receptors with seven transmembrane domains. So far, four different PARs are known. They are stimulated by specific proteolytic cleavage of the extracellular N-terminus (see Fig. 1). The newly formed N-terminus, the so-called "tethered ligand," is then able to bind intramolecularly to the receptor to initiate multiple signaling cascades. There is no known function of the removed N-terminal peptide fragment. Despite the irreversible activation mechanism, PAR signaling is efficiently terminated by desensitization (phosphorylation and uncoupling from ►G proteins) and degradation. A wide range of proteinases, most of them serine proteinases, cleave and activate PARs. They include proteinases from the ►coagulation cascade, inflammatory cells, the digestive tract, and the tumor microenvironment as well as the malignant cells themselves. PARs are ubiquitously expressed but are often found to be overexpressed in many different tumor types. In certain tumor types, downregulation of PARs has also been described.

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Proteinchip

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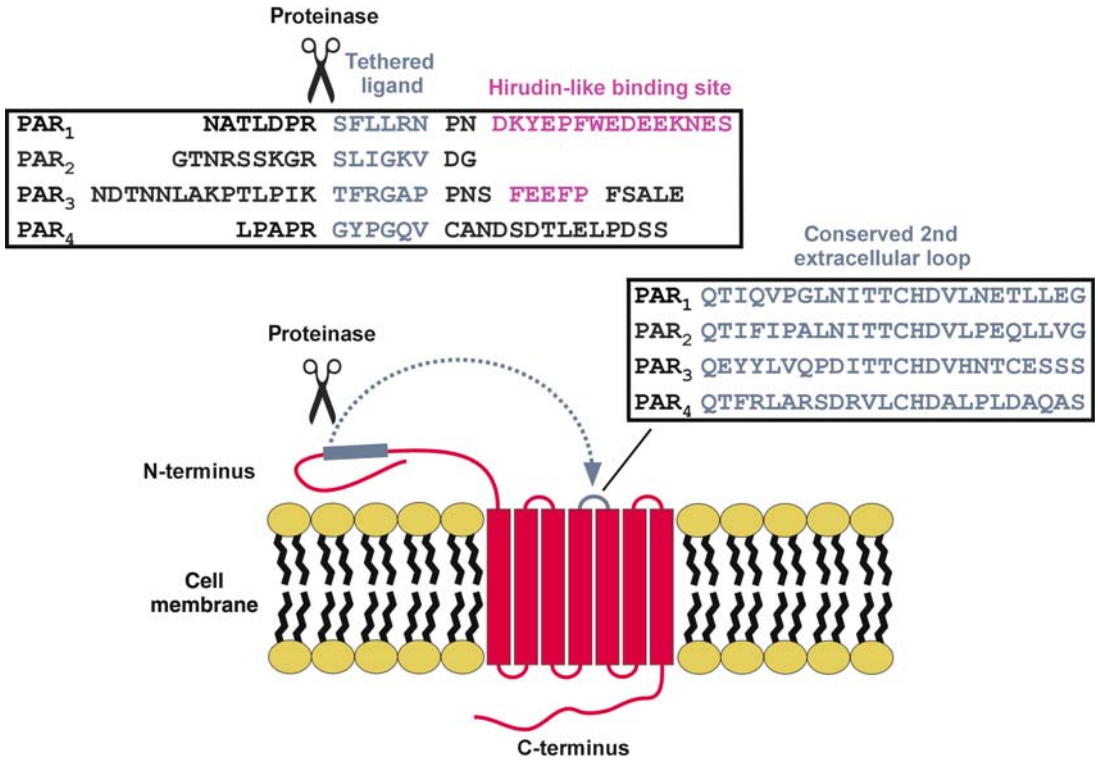
Synonyms

Protein array; Biochip

Definition

The functional proteinchip is a small size planar analytical ►protein microarray with probes arranged in high density on which different molecules of protein are affixed at separate locations in an ordered manner to form a microscopic array. The recent development of protein microarray offers the possibility to simultaneously analyze the expression of several hundred proteins. It is used to identify protein–protein interactions, the substrates of protein kinases, or the targets of biologically active small molecules. The innovative reverse-phase protein microarray has the potential to



Proteinase-Activated Receptors. Figure 1 Schematic presentation depicting the mechanism of PAR activation. The particular proteinase cleaves the PAR receptor at a specific site (scissors), unmasking a previously hidden peptide sequence, termed "tethered ligand" (*blue box*). The newly formed N-terminus then binds to the conserved second extracellular loop (*dotted arrow*). This interaction leads to conformational changes and subsequent receptor activation (a detailed description of PAR_1 and PAR_2 signal transduction pathways is given by Steinhoff et al. [1]). The human sequences of the "tethered ligand" domains and the second extracellular loops (both *printed in blue*) are presented in the boxes. The hirudin-like binding domain sequences of PAR_1 and PAR_3 are depicted in *purple color*.

provide information about the state of cellular circuitry from minute samples. Nevertheless, the most common functional proteinchip is the ►antibody microarray, where antibodies are spotted onto the proteinchip and are used as capture molecules to detect proteins from biological samples.

The analytical proteinchip is used in conjunction with a ►time-of-flight (TOF) ►mass spectrometry (MS) detector to enable protocols of protein capture and detection from complex samples without additional labels. Based on chromatographic interactions, protein–protein interactions, DNA–protein interactions, or receptor–ligand interactions, the analytical proteinchip is designed with a wide variety of capture affinities. The techniques include reverse-phase, cation exchange, and anion exchange. The most common analytical proteinchip is the ►surface-enhanced laser desorption/ionization (SELDI)-TOF MS array, where proteins are captured and enriched on a chemically or bioaffinity active solid phase surface to detect proteins in tissue, blood, urine, or other clinical samples.

Characteristics

Technologies

There are three main types of proteinchip formats. The glass slide chips are relatively inexpensive and they are usually compatible with standard microarray equipment, however, the samples are prone to evaporate and there may be cross-contamination between spots. Using well chips or 3-D matrix gels these problems can be reduced, but they are more expensive and can not be used with DNA microarray spotting equipment.

To prepare the proteinchip, coating with a substance should be bound to the slide without denaturing the proteins. There are several types of binding methods, which include the absorption, cross-linking, and hybridization. With the absorption method, the noise level of

the attachment layer is high due to nonspecific medium, such as sugary gel. In contrast, the cross-linking or hybridization methods allow the binding of specific groups of proteins covalently. However, the conformation or activity of the proteins may be affected.

Many proteinchips are spotted using automated technology, with spot densities greater than 30,000 spots per chip. Besides, synthesis of peptides can be performed on the chip by ►photolithography.

Analytical Approaches

The labeled analytical approaches involve fluorescence or radioisotope labeling techniques. The label is directly attached to the protein or capture agent and detected straight away. It is then attached to a different molecule (such as antibody, antigen, or substrate) with subsequent washing of the proteinchip to avoid altering the conformation of proteins. The most common labeled detection methods include the enzyme-linked immunosorbent assay, sandwich immunoassay, isotopic labeling, planar waveguide, and electrochemical. However, although the approaches try to prevent label from altering protein conformation, interference with protein interactions may still exist. As label influence on binding properties, along with a knack for having a lack of reproducibility, some studies thus shift to use the label-free approaches.

There are three main types of label-free analytical approaches. With the MS, proteinchips are coupled to a ►matrix-assisted laser desorption/ionization-TOF MS detector. Improved resolution can be obtained using SELDI-TOF MS. Following the sample fractionation and subsequent processed with a proteinchip reader, protein profiles are generated and analyzed by specific software. Unlike MS, ►surface plasmon resonance is an optical effect produced when polarized light is shone on a specially designed proteinchip. It can be coupled to MS for protein identification using microrecovery from the chip surface. A third method is the atomic force microscopy, which uses changes in surface topology to detect protein interactions. It is a high resolution technique and the data is collected in the form of topographical maps.

Applications in Cancer Research

The main applications of proteinchip profiling in ►cancer research include identifying cancer ►biomarkers, investigating protein–protein and protein–drug interactions, study of ►posttranslational modifications, cancer profiling, determining the amount of protein in a sample, detection of microorganisms and toxins in cancer fluid, as well as testing for the presence of antibodies in a cancer cell. Proteinchip profiling has been used successfully to discover biomarkers with potential clinical usefulness in some cancer types, several examples are illustrated underneath.

There was a development of integrated antibody microarray for simultaneous detection of multiple antigens and antibodies of five human ►hepatitis viruses (HBV, HCV, HDV, HEV, and HGV). This integrated antibody microarray can be used for clinical diagnosis and epidemiological screening of ►hepatitis virus-associated hepatocellular carcinoma.

Serum samples from the patients with malignant or benign prostatic disease and control subjects were analyzed on antibody microarray targeting multiple candidate prostate cancer biomarkers to distinguish malignant from benign prostatic diseases. With the several detected disease-associated protein alterations, thrombospondin-1 was found to be the most significantly altered protein. It was decreased in the patients with prostate cancer, but increased in the patients with benign prostatic disease.

To explore the use of combined measurements for serum sample classification, antibody microarray was also used to examine the associated proteins of pancreatic cancer. The antibodies that had reproducibly different binding levels between the patients with malignant and benign pancreatic diseases revealed different types of alterations, reflecting ►inflammation (high C-reactive protein, alpha-1-antitrypsin, and serum amyloid A (SAA)), immune response (high IgA), leakage of cell breakdown products (low plasma gelsolin), and possibly altered vitamin K usage or glucose regulation (high protein-induced vitamin K antagonist-II).

Using SELDI-TOF MS, two isoforms of SAA protein that were useful in the diagnosis of relapse in ►nasopharyngeal carcinoma were identified. Monitoring the patients longitudinally for SAA level both by proteinchip and immunoassay showed a dramatic increase in SAA correlated with relapse, and a drastic fall in SAA correlated with response to salvage chemotherapy. Subsequent tandem MS sequencing and immunoaffinity capture assay identified platelet factor-4 as a treatment-associated serum biomarker that might serve for the triage of patients with nasopharyngeal carcinoma to appropriate chemotherapy treatment.

The SELDI-TOF MS was also used to identify differentially cytosolic expressed proteins with a prognostic impact in node-negative ►breast cancer patients with no relapse versus patients with metastatic relapse. The proteinchip laser desorption ionization-tandem quadrupole-TOF MS was used for biomarkers identification. Results showed that a high level of cytosolic ubiquitin and/or a low level of ferritin light chain were associated with a good prognosis in breast cancer.

Challenges and Perspectives

There are many challenges when developing a protein microarray, such as creating an expression clone library, actual protein production that includes isolation and purification, adaptation of microarray technology, stabilizing the proteins on the array, as well as keeping the concentrations of the protein constant between slides and spots on the same slide. The analysis of proteinchips also comes with many challenges. Protein concentration is dynamic within a cell and there is trillion fold of difference between the low abundant proteins and the high abundant proteins. Unfortunately, the proteins that are usually of interest are normally in low concentration. Although purification and enrichment of proteins can address this problem, it is quite laborious and time-consuming. Besides, the application of protein microarray is limited by various fundamental problems, such as its multiplexing ability is limited by the cross-reactivity in parallel sandwich immunoassays. Therefore to determine a specific probe for each protein constitute another challenge. In addition, to integrate the proteomic profiling data with genomics and metabolomics, as well as translated them from bench to bedside applications is also a major challenge.

Nevertheless, protein microarray is among the novel classes of rapidly evolving proteomic technologies that holds great promise in biomedicine. Out of a large background of immunoglobulins including natural antibodies, patients with cancer produce a minute amount of specific ►autoantibodies against protein antigens. The ability to identify the fingerprintings of antibody signatures in malignancies can be useful in cancer diagnosis and prognosis. The great power of multiplex antibody microarray lies in its potential for rapid identification of antibody biomarkers found in blood and other biological fluids, which is sorely needed in the clinic to improve diagnosis, prediction of prognosis, and selection of ►targeted therapy. With the development of advanced technologies, such as improved quantitative ►proteomics methods, MS of ultra resolution, speed, and sensitivity, the development of functional protein assays, as well as sophisticated ►bioinformatics, further advancement of the high-throughput proteinchip technology will be achieved. After confirmation in independent proteinchip profiling studies, promising potential cancer biomarkers should be submitted to higher-throughput methods practical for large-scale multi-institutional validation studies and, ultimately, for clinical and epidemiological applications.

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Proteins

Definition

Are relatively large organic compounds made of amino acids arranged in a linear chain and joined together between the carboxyl atom of one amino acid and the amine nitrogen of another. This bond is called a peptide bond. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code.

14-3-3 Proteins

Definition

Are abundant, highly conserved adaptor proteins of ~30 kDa found in all eukaryotes. Similar to the binding of ►SH2 domains to phosphorylated tyrosine residues, 14-3-3 proteins usually bind to their client protein *via* a phosphorylated Ser- or Thr-residue in a sequence-specific context (mode I: RSXpS/TP; mode II: RXXXpS/TXP with p indicating the phosphorylated residue and X any amino acid). 14-3-3 proteins assist in the stabilization of the client protein in either an active or inactive conformation. 14-3-3 proteins regulate a multitude of biological processes ranging from metabolic control to the regulation of apoptosis, cell cycle progression and mitogenic signaling. One of the seven of human 14-3-3 genes, 14-3-3σ/Stratifin, is frequently epigenetically silenced in breast, lung and prostate cancer and is discussed as a ►tumor suppressor gene.

►B-Raf Signaling
►Mitogen-Inducible Gene 6 (MIG-6) in Cancer
►G_2/M Transition
►Kit/Stem Cell Factor Receptor in Oncogenesis

Proteoglycan

Definition

Glycosaminoglycans that are covalently linked to a core protein. Proteoglycans are complex macromolecules present primarily at the cell surface, in the extracellular matrix that surrounds most mammalian cell types, and also in body fluids. By virtue of the multiplicity of their protein binding partners (e.g., growth factors, chemokines, enzymes, and other extracellular matrix proteins), they have been shown to play major roles in regulating normal cellular processes, such as ►adhesion, proliferation, remodeling, ►migration, or ►angiogenesis, which can become dysregulated in ►inflammation and during cancer progression. Structurally, PGs consist of a core protein, of variable size and structure, and one or more associated glycosaminoglycan (GAG) chains. These GAGs can be of five types: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin or keratan sulfate (KS). Growing evidence suggests that DS, like the better studied heparin and HS, is an important cofactor in a variety of cell behaviors.

►Endocan
►Adhesion
►Cell Adhesion Molecules
►Hyaluronan Synthases

Proteoglycanase

►Stromelysin-1

Proteoglycans

Definition

Proteoglycans are large glycoproteins, consisting of long polysaccharide chains (glycosaminoglycans) attached to a relatively small protein core. They are mainly found in the extracellular matrix or attached to the cell surface by a membrane spanning region or a glycosylphosphotidylinositol anchor.

►Cell Adhesion Molecules

Proteolysis

Definition

Protein breakdown (degradation), which occurs generally as a result of hydrolysis at one or more peptide bonds.

►Proteasome Inhibitors

Proteolytic Processing

Definition

Cleavage of proteins yielding peptides with biological functions distinct from the uncleaved form.

►GLI Proteins

Proteome

Definition

Is the complete profile of proteins expressed in a given tissue, cell or biological system at a given time.

►Chromophore-Assisted Laser Inactivation

Proteomic Techniques

Definition

Methods for analysis of the large repertoire of proteins in a human cell, procedures for analysis of membrane proteins are highly specialized.

►CD Antigens
►Protein Microarray

Proteomics

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Definition

Proteomics is any systematic large scale study of a proteome or sub-proteome. The term proteome was coined in the mid-1990s as a linguistic equivalent to the concept of the genome. It refers to the complete set of proteins present in a biological specimen such as a single cell organism, a cell line, tissue, or biological fluid (serum, urine, saliva etc.). The proteomes of complex organisms are far more complex than the set of expressed genes because genes are usually alternatively spliced and expressed proteins are often extensively and heterogeneously posttranslationally modified. Unlike a genome that is largely static over the lifetime of a cell or organism, proteomes are constantly changing in response to changes in the environment of the cell, tissue or organism. Hence terms such as the "human proteome" or even the proteome of a cell line in tissue culture are meaningless because there are an essentially infinite number of proteomes.

Characteristics

Because many proteomics studies are discovery-based rather than hypothesis driven, they are not constrained by prior knowledge. Proteomics, therefore, provides opportunities for unprecedented progress in biomedical and biological research. These discovery based studies are highly complementary to hypothesis driven research, and they are quite often appropriately referred to as "hypothesis generating."

Three major types of proteome analyses are: (i) protein profile comparisons, where quantitative changes in proteins between two or more experimental conditions are compared; (ii) compositional analyses, where the goal is to identify all proteins present in a single biological sample; and (iii) analysis of protein–protein interactions. Another way of phrasing proteome studies is as an attempt to define: who, when, where and with whom. Protein profiling and compositional analyses often utilize similar separation and analysis tools, with the latter type of study simply lacking a requirement for quantitation.

The successive steps and associated critical issues for most types of proteome studies are: (i) experimental design – this includes selection of the organism and biological sample, conditions to be studied, goals of the study, and methods that will be utilized; (ii) sample preparation – method should be reproducible and care must be taken to avoid introducing artifacts that may be difficult to segregate from biologically important features; (iii) analytical separations – how will the proteins in the targeted proteome or sub-proteome be separated and how many tandem separation methods will be used; (iv) protein identification – while tandem mass spectrometry (MS/MS) is used to identify proteins in most proteomics studies, important considerations include the type of mass spectrometer to be utilized and whether intact proteins or proteolytic fragments will be analyzed in the mass spectrometer; and (v) proteome informatics – issues include the sequence database that will be searched, the computer program(s) used to compare the MS/MS data to the database, and the software tools used to manage, view and data mine search results.

Subproteomes and their Advantages

The complexity of most proteomes from multi-cellular organisms is usually much greater than the capacity of most current analytical methods both in terms of the number of unique protein forms present and the wide range of protein abundance. As a result, when one attempts to analyze entire complex proteomes, only a small portion of the total proteins present in the biological specimen are analyzed. Hence, where feasible, it is advantageous to focus on a sub-proteome that can address the biological question of interest. Key factors are to select the appropriate sub-proteome and to ensure that isolation of this sub-proteome is highly reproducible as variations in sample preparation could be misinterpreted as experimentally important changes. Sub-proteomic approaches rely on enrichment techniques for isolation of proteins with similar biochemical characteristics, with similar function or within a specific cellular compartment. In many cases, widely used methods are used for sample isolation, such as sucrose gradient centrifugation for isolating organelles or cellular machines. Affinity purification using antibodies, the tandem affinity tag system or similar methods are typically used to isolate large multi-protein complexes.

Common Protein Profiling Methods

The most common type of proteomics analysis involves the quantitative comparison of protein changes under two or more experimental conditions. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the oldest and most commonly used method for comparative proteomics, despite several well known limitations. A newer version of 2D gels is 2D differential in gel electrophoresis (DIGE) where two to three different samples can be compared in a single gel after fluorescently tagging each sample with a different cyanine dye (Cy2, Cy3 or Cy5). Even though 2D-PAGE and 2D-DIGE can provide high resolution separation of proteins with detection of many single charge changes,

some groups of proteins are poorly recovered and detected, including very basic or acidic proteins, large proteins and membrane proteins. Quantitative changes are detected on 2D gels using either the cyanine dyes or conventional protein stains. Protein spots that are observed to change in intensity in the biological samples being compared are identified by excising the spot, digesting it with trypsin and analyzing it by LC–MS/MS

An alternative strategy for quantifying protein changes in complex biological samples is to digest the entire sample with a protease, usually trypsin, and then separate the extremely complex mixture by ion exchange chromatography prior to capillary scale reverse phase chromatography interfaced directly with a mass spectrometer. This LC/LC–MS/MS approach is often referred to as "bottom up" or shotgun proteomics. It is better than 2D gels at detecting low abundance, acidic, basic, and membrane proteins but it provides very little information about changes in protein splicoforms, closely homologous proteins or posttranslational modifications because most protein identifications are inferred from identification of a few tryptic peptides. Quantitative comparisons are usually achieved in shotgun proteomics analyses by tagging two samples to be compared with a reagent that is chemically identical where one form is heavier than normal because it contains heavy stable isotopes of one or more atoms. Where feasible, the best method of introducing a heavy label is achieved by growing cells or simple organisms in media enriched in stable isotope-containing metabolites such as ^{13}C labeled essential amino acids. This stable isotope labeling in culture (SILAC) approach has the advantage that labeling occurs before any sample processing occurs, thereby avoiding the potential to interpret variable recovery of specific proteins during sample processing as biologically important changes. The alternative methods for introducing mass tags including modifying reactive groups on the proteins or chemical end labeling of peptides after proteolysis by a variety of different chemistries. An alternative method of quantitating proteins that is gaining in popularity is "label-free" quantitation, which compares the intensity of the ion current for a given peptide in a tandem MS experiment on an instrument with an electrospray interface (ESI). Label-free methods have somewhat larger errors and lower reproducibility than stable isotope tagging methods, but they may prove adequate for detecting most biologically meaningful changes. Ion currents from ESI MS analysis are substantially more reproducible than signals from matrix assisted laser desorption/ionization (MALDI) MS measurements and the related surface enhanced laser desorption/ionization (SELDI) method.

Other methods of comparing protein changes include using antibody arrays, reverse arrays where biological lysates are immobilized and probed with specific antibodies or other ligands of interest, and "top down" proteomics. Top down proteomics uses one or more modes of protein separation prior to introducing the intact protein into a mass spectrometer where the intact mass is measured prior to fragmenting the protein in the mass spectrometer in an attempt to identify the protein from the fragment information. Each method has its strengths and weaknesses and most of these methods are likely to continue to be useful for the foreseeable future.

Proteome Informatics

Computational analysis of the complex data resulting from most proteomics studies is often quite challenging. The importance of having good informatics tools for data interpretation and management has continued to increase as sequence databases become more complex, proteomes are divided into more fractions in attempts to dig more deeply into biological samples, and mass spectrometer become faster and more sensitive, thereby producing far larger datasets than older models. There is a need for standard methods to: describe proteomics experiments; store, manage and analyze MS data; and exchange data within the scientific community. The Proteome Standards Initiative of the Human Proteome Organization (HUPO), leading proteomics journals, and others are developing standards for data management, interpretation and dissemination in proteomic research across instrumentation and software platforms.

Clinical Aspects

Most diseases are expected to substantially alter the composition of the proteomes of affected cells and tissues. Such changes can include differences in protein levels, post-translational modifications, proteolytic processing, and sub-cellular location. Invariably when diseased and normal cells or tissues are compared, many protein changes are seen. The major challenge is to discern those changes that are most critical from incidental or nonspecific changes. Direct analysis of proteome changes in disease studies has great potential to identify novel diagnostic and therapeutic targets because protein activities are directly responsible for observed phenotype and most known drug targets are proteins. Logically, one focus of disease related proteomics studies is to compare diseased tissue with normal tissue, ►Biomarkers. An alternative promising approach is to analyze changes in the blood or other biological fluids that are proximal to the affected tissue, ►Serum Biomarkers. In general, potential disease biomarkers should be easiest to detect in the most proximal biological fluid but ultimately a blood test would be preferred since collection of blood is routine and minimally invasive. While proteomics has great potential for discovery of new disease diagnostic

and therapeutic targets, progress has been slowed by: the high complexity of human proteomes, especially blood; the substantial biological variability among the human population, the polygenetic and often heterogeneous nature of many diseases, and the limited detection and throughput capacity of current proteomics technologies. The discovery of cancer serum biomarkers is particularly challenging because the most specific markers are likely to be shed by only the tumor and not by other tissues. Hence, these proteins will be very low abundance in blood and very hard to detect, especially for early stage, small tumors, ►Early Detection. However, proteomics technologies continue to evolve steadily and promising advances are being made in investigation of cancer and other diseases using proteomics approaches.

►Proteomic Techniques

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Prothrombotic, Proinflammatory, and Proadhesive Endothelium

Definition

Upon stimulation, the physiologically quiescent endothelium shifts into an activated state that promotes the adhesion of leukocytes, platelets, and tumor cells.

►Tumor-Endothelial Cross-talk

Protoadjuvant Therapy

►Neoadjuvant Therapy

Proton Beam Therapy

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Synonyms

Proton therapy; Proton radiation therapy; Charged particle therapy

Definition

Proton beam therapy is a form of advanced radiation therapy that uses charged particles; it is used principally for the treatment of malignant tumors and occasionally for benign diseases.

Characteristics

Advanced radiation techniques that use photons (X-rays), such as ►three-dimensional conformal radiation therapy (3D CRT) and ►intensity-modulated radiation therapy (IMRT), have seen rapid recent development. The common goal of these treatments is to deliver a high dose to the target while minimizing the dose given to normal tissues. This is essential to achieve better local control and lower toxicity in the treatment of malignant tumors. Although advanced radiation therapies such as these enable higher-dose irradiation to a target, they produce a larger low-dose irradiated volume of normal tissues around the target due to the dispersion of radiation; this will increase the risk of radiation-induced secondary malignancies, especially for children, or unexpected late adverse events.

In contrast, proton beams can theoretically produce excellent dose localization to a target compared with photons because of the sharp distal fall-off of the Bragg peak, which gives no dose to normal tissues beyond the target. This physical characteristic is extremely useful for dose escalation to malignant tumors and is expected to yield enhanced treatment efficacy and reduced toxicity, especially in the treatment of tumors adjacent to critical organs.

Physical Properties

A proton is a positively charged elementary particle and is the nucleus of the hydrogen atom. It is 1,836 times more massive than an electron, which is the other constituent particle of a hydrogen atom. When a proton obtained by stripping a hydrogen atom of an electron is accelerated to high energy in electric fields using a particle accelerator such as a cyclotron or synchrotron, it becomes a type of ionizing radiation and can penetrate a deep area of the tissue. The charged proton decelerates

as its energy is lost due to interactions with electrons in atoms of the tissue; eventually it stops. Just before the proton stops, the interaction rapidly augments and abruptly increases ►excitation and ►ionization of the atoms. This sharp increase in dose absorption is called the "Bragg peak." Once the proton stops, the interactions no longer arise, and no dose is given beyond the Bragg peak. The penetration depth of the Bragg peak depends on the initial energy of the charged proton. Accordingly, the Bragg peak can be precisely placed according to the depth of the target; however, pristine and unmodulated monoenergetic protons are unsuitable for clinical use because the dimensions of most targets are far greater than that of the narrow Bragg peak. To overcome this problem, the Bragg peak is modulated in practical treatment, the so-called "spread-out Bragg peak (SOBP)," to cover the targets effectively (Fig. 1).

Biological Properties

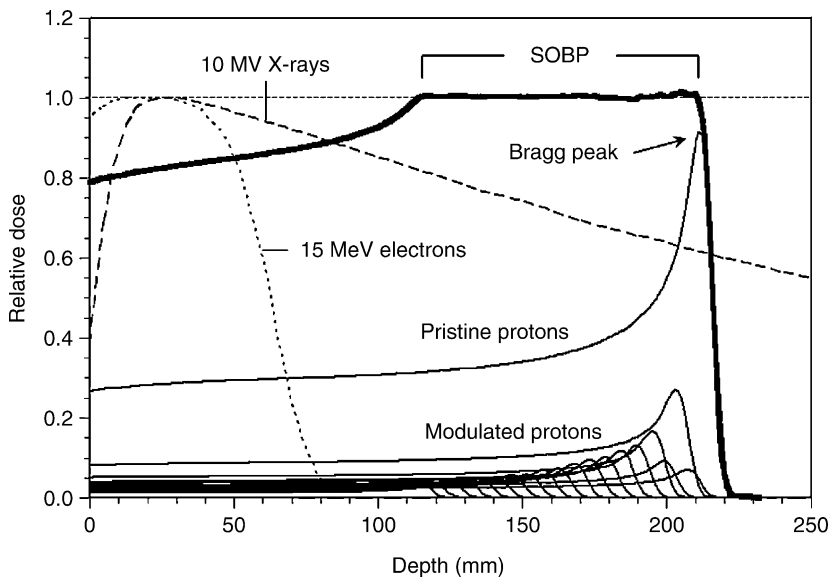
Excitation and ionization occur in tissues along a proton path, producing chemically active ►radicals that impair DNA in the chromosome of a cell. Although cells usually have the ability to self-recover from such impairment, they cannot divide normally or proliferate when the impairment exceeds a certain level. Proton beams are used for cancer treatment utilizing these actions in the same way as photons. Relative biological effectiveness (RBE) is defined as the ratio of the physical dose required to produce a specified effect using reference photons (usually ^{60}Co) relative to that of a specific radiation required to produce the same effect. RBE values in the mid-SOBP of proton beams obtained from outcomes of various experiments in vitro and in vivo range from 0.7 to 2.1. It is known that different accelerators of protons or instruments for beam delivery used at each institution have different RBE values. Furthermore, different RBE values occur even in different measured points of the SOBP, e.g., in the proximal, middle, or distal SOBP. An RBE value of 1.0 or 1.1 is usually adopted for clinical use, and a physical dose of protons multiplied by the RBE value is indicated using the unit of Gray Equivalent (GyE). An RBE value of 1.0 or 1.1 signifies that there is no great difference between protons and photons in terms of the physical doses required to produce a certain specified effect. Thus, the abundant medical data available concerning treatment efficacies for tumors and toxicities of normal tissues using photon irradiation are directly applicable to proton beam therapy.

Clinical Aspects

Compared with conventional photon irradiation, proton beam irradiation is expected to yield enhanced treatment efficacy and reduced toxicity in radiation therapy for a wide range of malignancies. Representative diseases that are widely treated with proton beam therapy are described in the following section.

Uveal (choroidal) Melanoma

►Uveal melanoma is one of the most common primary ocular tumors; it was formerly treated with enucleation of the eye. ►Brachytherapy with radioactive plaques



Proton Beam Therapy. Figure 1 Depth-dose curves for 10 MV X-rays, 15 MeV electrons, pristine protons, and the spread-out Bragg peak (SOBP) produced by a composition of variously modulated protons. For pristine protons, the dose suddenly increases and the Bragg peak is formed before the dose abruptly decreases to zero due to sharp distal fall-off; X-rays penetrate a deeper area, while electrons do not penetrate deeply.

has been used as an eye-preserving therapy in place of enucleation over the past few decades. This approach provides local control of more than 80% at 5 years. Proton beam therapy has recently been applied as a less invasive treatment, and total doses of 50–70 GyE in 4–5 fractions achieves an excellent 5-year local control rate of approximately 95%.

Sarcomas of the Skull Base and Spine

►Chondrosarcoma and chordoma are common tumors of the skull base and spine. Surgical resection is performed for these tumors with curative intent, but they are frequently resected incompletely because of their proximity to critical organs such as the brain and spinal cord. Although conventional photon irradiation is used for unresectable tumors, it is very difficult to give a sufficient tumoricidal dose due to the limited tolerable doses of the adjacent critical organs. Proton beam irradiation allows the delivery of higher doses over 70 GyE and provides 5-year local control of almost 100% for chondrosarcoma and 50–60% for chordoma.

Head and Neck Cancers

Radiation therapy alone or in combination with chemotherapy is widely used for head and neck cancers for the purpose of preserving the function and configuration of organs. Proton beams facilitate the prevention of brain and bone necrosis and the conservation of salivary function while delivering high doses to tumors. The advantages of proton beams are of particular benefit in irradiation of paranasal sinus and ►nasopharyngeal carcinomas located adjacent to the central nervous system. Although few reports describe the use of proton beams for head and neck cancers, this group is among the most suitable subjects for proton beam therapy.

Lung Cancer

Surgical resection remains the most standard and curative treatment for early stage ►non-small cell lung cancer (NSCLC); however, radiation therapy is a feasible treatment option for patients who are inoperable because of comorbidities or who refuse surgical procedures. The irradiated volume and dose given to a pulmonary tumor are frequently restricted due to the low tolerable dose of the lung, and irradiation with large radiation fields and high doses causes severe and occasionally mortal ►radiation pneumonitis. The use of proton beams for lung cancer may reduce the incidence of radiation pneumonitis and enable the safe delivery of high doses to tumors adjacent to the heart, esophagus, and spinal cord. Hypofractionated high-dose proton beam therapy, e.g., with a total dose of 60 GyE in ten fractions, provides local control of approximately 95% for Stage I NSCLC.

Hepatocellular Carcinoma

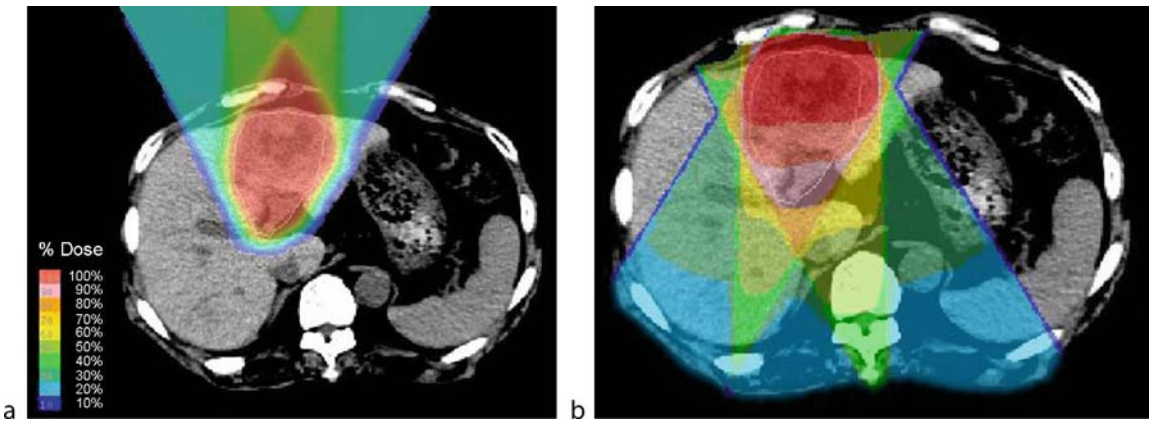
The most reliable curative treatment for ►hepatocellular carcinoma (HCC) remains surgical resection; however, approximately 80% of HCCs are unresectable due to advanced tumors and coexisting cirrhosis at diagnosis. Although nonsurgical treatments such as transcatheter arterial chemoembolization (TACE), percutaneous ethanol injection (PEI), and radiofrequency ablation (RFA) are usually applied to inoperable HCC patients, local failures are common following TACE, and PEI and RFA are unsuitable for patients with bleeding tendency due to cirrhosis, large-sized tumors, or unfavorable tumor location. Therefore, there are numerous patients who have limited treatment options and are unable to undergo effective treatments. Proton beam therapy is widely available for such patients, and provides local control of approximately 90% at 5 years. Furthermore, almost all unresectable HCCs with portal vein tumor thrombus are locally controlled with proton beam therapy. HCC patients commonly develop new intrahepatic lesions remote from the initial tumor after irradiation. In these cases, proton beams enable reirradiation of the new lesions because the irradiated volume and dose given to the normal liver was minimized at the time of previous irradiation. The difference in isodose distributions between protons and photons is shown in Fig. 2.

Prostate Cancer

►Prostate cancer is one of the diseases that are most commonly treated with proton beam therapy worldwide. Radiation therapy can almost match the treatment results of surgery in patients with localized prostate cancer. Prostate cancer is more likely to arise in the elderly; the number of prostate cancer patients is expected to increase because the average life span has been prolonged in many countries. As a less invasive treatment is generally more favorable for aged patients, the proportion of prostate cancer patients receiving radiation therapy will probably increase in the future. Because the prostate is located adjacent to the urinary bladder and rectum, high-dose irradiation to the prostate causes an increase in the incidence of radiation-induced urinary and rectal complications. Proton beam irradiation is used with the aim of decreasing these toxicities. Proton beam therapy alone or in combination with photon irradiation with total doses of 74 GyE or more in conventional fractions provides a biochemical disease-free (►prostate-specific antigen failure-free) rate of approximately 80% at 5 years for patients with early-stage prostate cancer.

Childhood Cancers

Proton beams are used for the treatment of ►childhood cancers, e.g., brain tumors such as ►medulloblastoma and optic pathway glioma, ►retinoblastoma, ►malignant



Proton Beam Therapy. Figure 2 Treatment planning with CT for a hepatocellular carcinoma patient with tumor thrombus in the left main portal vein. (a) Isodose distribution of proton beams delivered through three ports (one anterior and two oblique) to a tumor, including portal vein tumor thrombus. The entire target (contoured by the white line) is homogeneously encompassed by the 100% dose level (red color). Critical organs such as the spinal cord and digestive tract are located entirely outside the irradiated volume, due to the sharp distal fall-off of the Bragg peak of proton beams. (b) Isodose distribution shaped by 10 MV X-rays delivered through the same ports. The irradiated volume increases considerably, meaning that normal structures, including the above-mentioned critical organs and normal liver, are exposed to radiation.

lymphoma, and soft-tissue sarcomas including ►rhabdomyosarcoma. As proton beam therapy is useful in reducing the risk of radiation-induced secondary malignancies, growth failure, and neurological deficits, it is a treatment of great significance for this group.

Miscellaneous

Proton beam therapy is also attempted for other various malignant tumors such as ►glioblastoma multiforme, ►breast cancer, ►esophageal cancer, ►bladder cancer, and ►cervical cancer, as well as benign diseases such as pituitary adenoma and arteriovenous malformation.

Facilities and Costs for Proton Beam Therapy

There are more than 20 facilities for proton beam therapy in operation worldwide; more than 48,000 patients have received proton beam therapy as of January 2007. Although several new facilities are being built internationally, the number remains insufficient to meet the needs of cancer patients. The large area and great expense involved in building a new facility hamper the spread of proton beam therapy; however, construction of a new facility is becoming easier because miniaturization of the particle accelerator has reduced the size requirement of the site, and construction costs have fallen with the rationalization of production procedures. This reduction in cost will reduce the cost disparity per treatment between radiation therapies using protons and photons. Furthermore, the greater the number of patients that receive proton beam therapy, the lower the cost per treatment. The relative cost of proton beam therapy compared to IMRT with photons is currently estimated at approximately 2.4, but is predicted to decrease to 1.7–2.1 over the next few years. There is a need for a future environment in which more patients can undergo proton beam therapy at lower cost.

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Proton Radiation Therapy

►Proton Beam Therapy

Proton Therapy

►Proton Beam Therapy

Proto-oncogene

Definition

A proto-oncogene is a normal cellular gene that, when activated by mutations, acquires oncogenic function. The activating mutations can include intragenic point mutation, translocation (creating a fusion transcript or resulting in transcriptional up-regulation) or amplification with the consequence of enhanced expression; oncogene. Also referred to as cellulor Oncogene (c-onc).

►Transduction of Oncogenes

Protoporphyrin IX

Definition

PpIX; Is the precursor of heme, which is the prosthetic group of hemoglobin, myoglobin, cytochromes, and metalloproteins. PpIX is a potent fluorescent dye and photosensitizer. Its induced accumulation in malignant cells is used for fluorescence-guided resection or fluorescence diagnosis and photodynamic therapy of malignant and nonmalignant diseases.

►Photodynamic Therapy

Provirus

Definition

The double-stranded DNA form of a retroviral genome.

►Transduction of Oncogenes

Prox-1

Definition

A transcription factor expressed in lymphatic endothelial cells but not vascular endothelial cells that is required for development of the lymphatic system. This factor regulates expression of a number of lymphatic endothelial cell genes and suppresses vascular endothelial cell specific gene expression.

►Lymphangiogenesis

Proximal-type Epithelioid Sarcomas

Definition

Highly aggressive soft-tissue tumors of young adults, characterized by both epithelial and mesenchymal phenotype; they harbor a variable amount of rhabdoid cells and occasional *hSNF5/INI1* inactivation.

►Tumor Suppressor hSNF5/INI1

Proximate Carcinogen

Definition

A metabolite of a carcinogen intermediate in the conversion to an ultimate carcinogen.

►Toxicological Carcinogenesis

Prozac

►Fluoxetine

Prune

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Definition

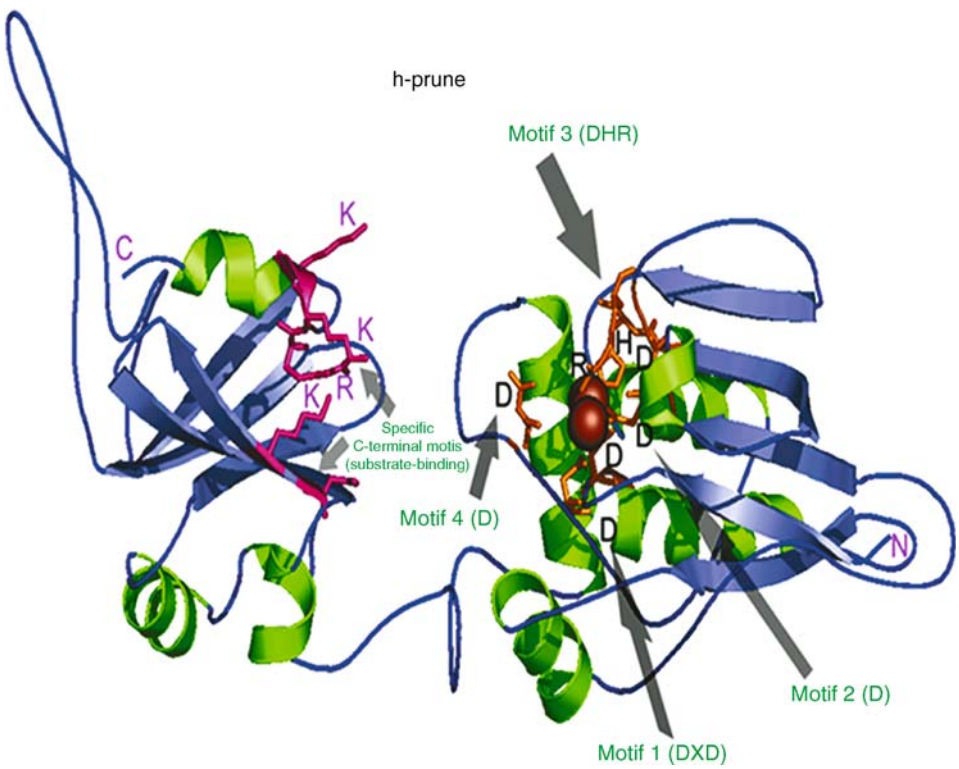
Prune stands for the human homologue of the Drosophila prune gene. Prune protein was initially identified in *Drosophila*, in which its mutation caused a brownish-purple "prune" eye color due to significant loss of drosopterins "red pigments," compared to the bright red eye of the wild-type fly, thus explaining its mutant name.

Characteristics

Prune is appertaining to the DHH family phosphoesterase proteins including RecJ DNA repair Exonucleases, Pyrophosphatases (PPASEs) and Exopolyphospatases (PPX). The DHH super-family can be divided into two main groups on the basis of a C-terminal motif that is very well conserved within each group, but not across the groups. All the members of this super-family possess four other motifs that contain highly conserved charged residues predicted to be responsible for binding ions and catalyzing the phosphoesterase reaction. The most characteristic of these is the third motif, with the signature DHH (Asp-His-His), after which this super-family was named. Prune is a cyclic nucleotides phosphodiesterase. Due to its protein similarities, Prune might possess other biochemical functionalities within the DHH family of proteins.

Human prune, a 62 kDa protein, acting as a cytoplasmic cyclic nucleotides phosphodiesterases (cNMP-PDE) is involved in both promoting cellular mobility and stimulating expression of genes involved in metastatic pathways.

In breast cancer, metastatic spread is responsible for virtually all cancer deaths. ▶Metastasis is a highly complex molecular and cellular process. To become invasive, tumor cells need to change their adhesive properties, to lose contact with other cells in the primary tumor, and make new contacts with the extracellular matrix of host cells they encounter as they invade. They also need to be able to penetrate into the surrounding host tissue, and here the modulation of protease activity in the vicinity of the tumor cells plays a critical role.



Prune. Figure 1 Ribbon structure of the h-prune protein based on the crystal structure of PPASE and the RecJ protein. Red balls indicate potential cofactor ions (Mg2+ and/or Mn2+) and the region of binding to motif III. Arrows indicate the aspartic acids (D). Aspartic acids of the four DHH motifs are represented, indicating the potential catalytic site of DHH protein family [1].

To migrate away from the primary tumor, tumor cells also need to gain motility functions. These same properties are also thought to be important when circulating tumor cells exit the circulatory system and start metastatic colonization in secondary organs.

Prune possesses a cAMP phosphodiesterase activity, which is responsible for increasing cell motility in breast carcinoma cell lines. Within the breast cancer cellular model, using gene expression analyses, several key players were identified acting as signals of cytoskeleton remodeling and cell motility, thus influencing the malignancy of Prune overexpressing breast cancer cells.

The physical interaction between Prune and nm23-H1, a suppressor of metastasis protein, enhances Prune cAMP-PDE activity, thus the interaction causes the subtraction of unbound nm23-H1 proteins into the cell. The major component of the nm23-H1 anti-metastases function is its capability to induce "low motility cellular processes" if overexpressed in aggressive breast cancer cells, although this activity was found in other solid tumors such as prostate, colon, lung, head and neck.

Amplification of Prune copy numbers induces cell proliferation and high levels of Prune expression compared to the moderate or lower nm23-H1 level of expression that is correlated to aggressiveness of sarcoma and breast carcinoma, colon and gastric carcinoma, thus postulating an inhibitory role of Prune versus the anti-metastasis nm23 function "in vivo."

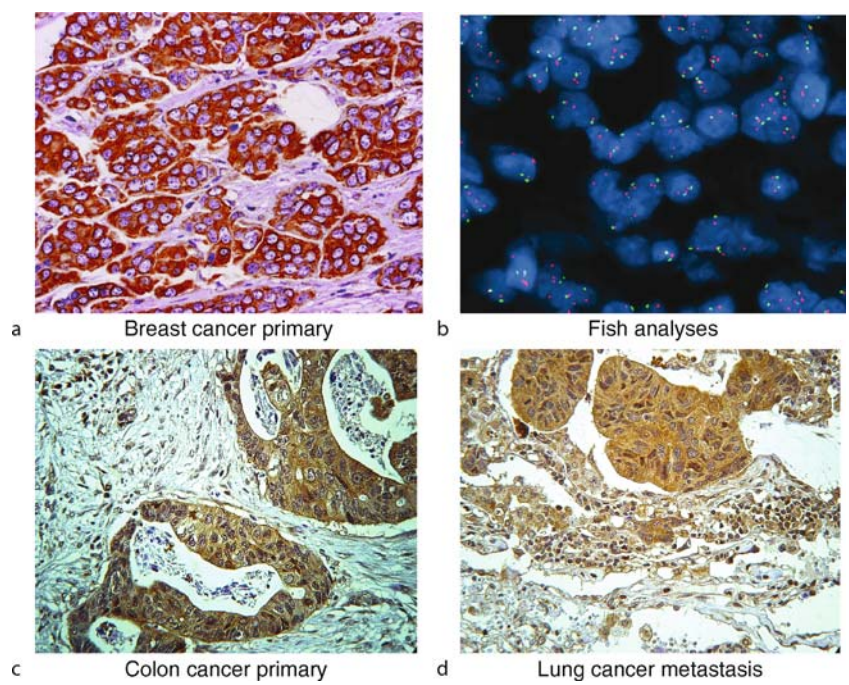
The region of the nm23–h-prune interaction lies between S120 and S125 of nm23, where missense mutants show impaired binding; this region can undergo serine phosphorylation by casein kinase I. Thus, the casein kinase I δ-ε specific inhibitor IC261 impairs the formation of the nm23–h-prune complex, which translates "in vitro" into inhibition of cellular motility in a breast cancer cellular mode.

Regulation

Prune participates in the complex network of interactions with proteins involved in cell cycle and cell motility. It is known that: (i) Prune together with glycogen synthase kinase-3 (GSK3β), a kinase involved in ►WNT signaling pathway, cooperatively regulates the disassembly of focal adhesions to promote cell migration, and (ii) Prune via interaction with Gelsolin, an ATP severing protein acting in focal adhesions, leads to invasion properties of cancer cells. Both of these pathways promote cancer metastasis. Interestingly, Prune has also been shown to be highly expressed in brain development together with nm23-H1, expression was observed in cortex, hippocampus, midbrain and during cerebellum development.

Clinical Relevance

Prune has a role in the metastatic processes through specific inhibition of the anti-metastasis function of



Prune. Figure 2 (a) Expression and cytogenetic analysis of h-prune in breast carcinomas. (b) FISH analyses on the same samples using h-prune/ PAC279-H19 (*red*) and control pUC177 (*green*) as probes [4]. (c) Expression and cytogenetic analysis of h-prune in colon cancer. (d) Expression and cytogenetic analysis of h-prune in a metastasis site in lung from primary colon cancer (see c) (data unpublished).

►nm23-H1 in vivo. Overexpression of Prune is involved in cancer progression and tumor aggressiveness, particularly by defining the lymphnode positive status and correlated to breast cancer advanced disease status. This condition is enhanced in colon carcinoma where Prune positive tumors have a bad sign of malignancy and prognosis. Significant statistical analyses of Prune immunostaining positive colon carcinoma associates the level of expression to tumor grade (T1-T2/T3-T4) (►grading of tumors), lymphnode status (N0/N1-N2) and metastases grade (M0/M1), while only to tumor grade (T1-T2/T3-T4) and lymphnode status (N0/N1), in pancreatic cancer. Two reports described h-prune involvement with tumor progression in gastric cancer and in esophageal squamous cell carcinoma as an independent prognostic marker.

Inhibition

Four ways of inhibition of Prune pro-motility activity in cancer cells (in breast, colon and pancreas) are postulated at this time. Firstly the use of Dipyridamole, which alters its cAMP-PDE function in breast cancer cells, and thus negatively influencing its pro-motility effect. Secondly, the strategy of using the inhibition of Prune-GSK3β functional interaction in focal adhesion using SB216763 (a specific GSK3β inhibitor) or a combination of dipyridamole and SB216763. Thirdly, by using a peptide mimicking the region of interaction between Prune and GSK3β including the amino-acid region position 333–443 of Prune protein. Fourthly, by using IC261, a drug that impairs the CKI function thus inhibiting nm23-H1 S125 phosphorylation and consequently impairing the binding with Prune.

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PS 341

►Bortezomib

PSA

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Synonyms

Human kallikrein 3; KLK3

Definition

Prostate-specific antigen (PSA) is a serine protease (33 kD) secreted mainly by prostatic epithelium. It is important for its role in the liquefaction of seminal coagulum.

Characteristics

After a tissue specific antigen was identified in the human prostate in 1970 and the discovery of prostatic antigens in seminal plasma, PSA was subsequently isolated and characterized in 1979. PSA was cloned in 1987. PSA mRNA encodes for (261) 244 amino acid (pre)proforms of the protein, although the mature, catalytically active, single-chain form of the protein contains 237 amino acid residues. As member of the human ►kallikrein family, PSA shares considerable structural and functional homology with all other 14 human kallikreins, together with a gene location on the long arm of chromosome 19 (19q13.2-q13.4). Kallikreins are proteases that cleave vasoactive kinin peptides from kininogen. PSA, however, functions as a chymotrypsin-like serine protease cleaving substrates such as seminogelin I, seminogelin II, fibronectin, and insulin-like growth factor/binding protein in the seminal plasma which helps maintain sperm motility. PSA is made primarily by prostatic epithelium and periurethral glands in males, but it has also been detected in endometrium, breast tissue, breast cancer, breast milk, and female serum. In normal prostate, the epithelium secretes PSA into the seminal fluid, where it reaches concentrations of 0.4–5 g/L. In the normal male, PSA is detected in the circulation at low concentrations of 0–1 (2) μg/L.

Approximately 75% of PSA found in serum is irreversibly bound to the protease inhibitor alpha-1-antichymotrypsin (ACT). Serum PSA also binds to alpha-2-macroglobulin (A2M). Measured PSA in serum comprises 5–50% unbound or free (fPSA). PSA has five immunoreactive sites. Two of these epitopes are hidden in ACT-PSA, allowing commercial immunoassays to differentiate bound from free PSA. All five epitopes are hidden in A2M-PSA. Therefore, total PSA (tPSA) measured by commercial assays corresponds to fPSA plus PSA bound to proteins other than A2M-PSA.

A small part of tPSA (0.9–1.6%) is also bound to alpha1-protease inhibitor (API-PSA). Complexed PSA (cPSA), defined on the basis of an assay utilizing a blocking antibody to fPSA, detects ACT-PSA and API-PSA, but not the A2M-PSA complex. Several uses of the cPSA test have been proposed.

Free PSA has recently been shown to exist in at least three molecular forms; "benign" PSA (bPSA), inactive "intact" PSA (iPSA), and proPSA. An overview of all PSA forms is given in the figure. The bPSA is a specifically clipped subform of fPSA, which is highly associated with the transition zone containing benign prostatic hyperplasia (BPH) nodules in prostate tissue. BPSA represents 0–60% of fPSA but it cannot discriminate between BPH and ▶prostate cancer (PCa). Based on the development of novel anti-PSA antibodies that do not recognize internally cleaved PSA at Lys145-Lys146 and thus are specific for intact, unclipped PSA, an assay has been developed for "intact" PSA (iPSA). The iPSA assay detects both proPSA and other inactive non-clipped fPSA entities and can discriminate between BPH and PCa. Recently, proPSA forms were isolated in serum and tissue from PCa patients. The proPSA in serum and prostate tissue exists as a mixture of differently designated forms including the (–7), (–5), (–4) forms and, partially, the (–2) and (–1) forms. Whereas the (–5, –7) proPSA have limited validity to improve PCa detection, the (–2) proPSA seems to better discriminate BPH and PCa and may further detect more aggressive PCa, as indicated by Gleason score 7 or greater.

The ratio of free to total PSA used in literature as f/tPSA ratio (percent free PSA, %fPSA) tends to be increased in benign disease compared to PCa. The reason for this difference is not completely understood. It is assumed that due to the loss of tissue architecture in PCa the intracellular active PSA gains quicker access to the circulation and protease inhibitors, like ACT and A2M, can bind PSA easily. If PSA reaches the circulation from normal or BPH cells, it first has to leak into the extracellular space, where it is susceptible to proteolytic degradation. After degradation, the inactive PSA can still form complexes with A2M but only to a very small degree with ACT. This may explain the decreased capability of PSA to form complexes with ACT and the higher concentration of PSA-A2M in BPH patients.

Clinical Aspects

PSA is organ-specific, but not cancer-specific, since it can by produced by normal, hyperplastic, and neoplastic prostate epithelial cells. PSA synthesis is influenced by testosterone levels.

It has limited sensitivity and specificity in appropriately detecting the earlier stages of abnormal prostate growth. The elevated circulating PSA levels can be due to PSA leakage into the bloodstream from abnormal prostate growth or inflammation, such as prostatitis.

PSA as Diagnostic Marker

Measurement of serum PSA is commonly used to aid the diagnosis of PCa. The introduction of routine PSA-based screening, together with digital rectal examination (DRE), over the past 20 years has led to a dramatic increase in the rate of disease detection and a subsequent stage shift at the time of diagnosis. For many years, a PSA value of 4 μg/L has been used as the cutoff for prostate biopsy. However, in 2004, an analysis of 2950 men, who never had a PSA level >4.0 μg/L or an abnormal DRE, PSA levels of 0–4 μg/L were associated with a positive predictive value (PPV) for PCa of 6.6% (PSA 0–0.5 μg/L) to 26.9% (PSA 3.1–4 μg/L). Overall, 15% of men with PCa had high-grade disease, with this rate reaching 25% among men with a PSA level of 3.1–4 μg/L. Thus, there is no PSA level, where PCa or even aggressive PCa can be excluded.

A further problem is evidence that PSA screening can reduce PCa mortality in large randomized prospective studies and therefore population screening is debated. In 2008/2009, results of two large randomized studies in Europe and USA will provide sufficient information whether PSA screening can reduce PCa mortality. Despite these controversies, PSA has revolutionized the management of PCa especially for the early disease detection, which has improved curative treatment. To date, the most commonly used and easy available PCa screening tool is the combined use of DRE and PSA as each can identify cancers not detected by the other. It has been shown, that screening intervals can be extended to 5–8 years for men with an initial serum PSA level of <1 μg/L, and to 2 years for men with an initial PSA level of 1–2 μg/L. In men older than 65, with PSA levels of <1 μg/L, it is presumed that follow-up may be omitted.

A principal problem with PCa screening is that only approximately a third of men with elevated serum PSA have cancer in prostate biopsies. Most false-positive PSA results are caused by BPH. False-positive results cause unnecessary prostate biopsies, generating anxiety, discomfort and costs.

To improve this dilemma, several calculated parameters, such as PSA density (PSA divided by the prostate volume), PSA transition zone density, PSA velocity (PSA change over time) or age- and race-specific PSA ranges were introduced but have only been partially successful in enhancing the specificity of PSA. Due to large biological variations in the measured PSA concentrations up to 20–30%, a repeat measurement of PSA can also avoid significant numbers of unnecessary prostate biopsies.

Percent free PSA

The use of %fPSA has been established as a clinically routine test since the mid 1990s, especially for indication of a repeat biopsy. Various studies have demonstrated a significant improvement in specificity for the 4–10 μg/L tPSA range and for lower tPSA values (<4 μg/L). Generally, with %fPSA cutoffs at 90–95% sensitivity, the number of unnecessary biopsies could be reduced by approximately 15–20%. Possible influencing factors such as prostate volume, tPSA, race, sample stability, prostate manipulation or drug treatment should all be considered. It has been proposed to use %fPSA as a priority decision tool for first time biopsy in men with unsuspicious DRE within the tPSA range 4–10 μg/L, as well as for lower PSA values. Expanding the range of additional fPSA measurements from 4–10 μg/L to 2 or 2.5–10 μg/L could be beneficial for detecting significant PCa at low tPSA values. Using %fPSA-based multivariate models like logistic regression or artificial neural networks for prostate biopsy decision can further improve PCa detection rate. These models include tPSA, %fPSA, prostate volume, age, DRE status or other clinical variables and this may decrease the overdiagnosis of PCa when using PSA alone.

PSA, %fPSA for Staging and Grading

It is well established that serum tPSA has both a diagnostic utility for the detection of PCa, and a prognostic impact. Clinical stage, ►Gleason grade and serum tPSA individually and independently correlate with pathological stage and prognosis of PCa. Subsequently, tPSA is always taken into consideration before planning PCa therapy. For example, patients with elevated levels of tPSA (>20 μg/L) are considered to be at high risk of non-localized disease, and are often contraindicated for curative treatment alone (radical prostatectomy, brachytherapy, or radiotherapy). However, the use of tPSA alone is not sufficiently sensitive or specific for staging. Although tPSA directly correlates with clinical and pathological tumor stage, studies have revealed that this marker cannot accurately predict the final pathological stage for individuals.

The ability of %fPSA to improve PCa staging has been controversial. Several centers have reported that %fPSA was useful to predict pathological stage, while others noted no significant improvement in staging, even when %fPSA was combined with Gleason score and clinical stage. The same situation exists when using %fPSA to predict grade. It is assumed that lower %fPSA values correlate with more aggressive disease. However, an individual prognosis is not possible by using tPSA or %fPSA.

PSA for Recurrence of PCa

After initial diagnosis and treatment, PSA plays a vital role in the follow-up of PCa. Serial PSA measurements demonstrate the growth characteristics of each tumor. Thus PCa control, recurrence or hormonal escape can be determined. This allows detection and treatment of skeletal metastases prior to fracturing. Second line treatments can then be introduced before the onset of symptoms.

Monitoring After Radical Surgery

The clinical use of ultrasensitive tPSA assays in recent years has increased the lead time for identifying recurrence of PCa following local therapy. The most effective method to monitor disease recurrence postoperatively is serial tPSA determinations. After radical prostatectomy, the majority of men have a rapid decline in serum tPSA to undetectable levels. As a corollary, the failure of PSA to become undetectable is highly suggestive of persistent disease. PSA elevations following the attainment of undetectable levels postoperatively provide an early warning sign of disease progression. The preoperative tPSA level and the timing of PSA recurrence after surgery can predict disease-free survival and the pattern of recurrence, respectively. Very recently it has been proposed that biochemical recurrence, defined as a tPSA of at least 0.4 μg/L followed by another increase, best identifies metastatic progression after radical prostatectomy.

Use of PSA to Monitor Radiation Therapy

PSA has been used as a surrogate end point to monitor disease activity following prostatic irradiation and this has been a major advance recently. The simplicity of serum PSA measurements, avoidance of routine radiographic imaging and cost savings are apparent. Like radical prostatectomy, PSA elevations provide early biochemical evidence of disease recurrence before clinically significant local disease or metastasis develops. The response of PSA to irradiation is more unpredictable than surgery in which PSA levels often become undetectable. After radiation therapy PSA levels decline more slowly and may not reach undetectable levels. The kinetics of a rising PSA (PSA doubling time) appear to be the best surrogate marker for disease risk, clinical progression, and ultimately cancer-specific death.

PSA monitoring After Androgen Deprivation Therapy

Androgen deprivation therapy is a commonly used treatment modality for men with advanced PCa, and includes bilateral orchiectomy, various antiandrogens, luteinizing hormone-releasing hormone agonists and 5-alpha-reductase inhibitors. Since PSA production and secretion require hormonal influence, androgen deprivation therapy diminishes the ability of benign and malignant prostatic epithelial cells to manufacture PSA. Unless the cancer comprises a critical mass of androgen independent neoplastic cells, serum PSA usually declines significantly and often to undetectable levels.

Further increases in serum PSA values, despite androgen deprivation therapy, indicate progression due to an emerging subpopulation of hormonally resistant clones. PSA levels should decline within the first 6 months after androgen deprivation therapy. However, progression of PCa may occur despite undetectable or low PSA levels. This likelihood increases in patients with high-grade, locally advanced tumors, especially when atypical histological variants are present.

Conclusion and Future

PSA has revolutionized the clinical care of PCa. It is vital to the diagnosis and subsequent follow-up of the disease, despite all the controversies with PSA screening and the urgent need for better tumor markers. One of the most important goals for PCa serum marker development is the search for a marker which can predict the Gleason grade 4/5 which to date is the only independent prognostic factor for a biochemical failure after radical prostatectomy.

►Serum Biomarkers

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Pseudarthrosis

Definition

False joint. Typically located in tibia in patients with neurofibromatosis 1.

►Neurofibromatosis Type 1

Pseudogene

Definition

DNA sequences thought to be non functional that are very similar to sequence encoding a functional gene product.

Pseudokinase

Definition

A protein that presents a conserved kinase-like domain in its structure that lacks at least one of the conserved catalytic residues. Although these proteins are predicted to be catalytically inactive they regulate important cellular processes via protein-protein interactions.

►Cannabinoids

Pseudomonas Aeruginosa

Definition

A gram-negative bacteria that is an opportunistic pathogen.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Pseudomonas Exotoxin

Definition

Toxin produced by ►Pseudomonas aeruginosa that inactivates eEF-2 and inhibit translocation during eukaryotic protein synthesis.

►Cytokine Receptor as the Target for Immunotherapy and Immunotoxin Therapy

Pseudomyxoma Peritonei

Definition

A mucinous appendiceal neoplasm rupture causing the release of mucus-producing epithelial cells into the free peritoneal cavity. These tumors accumulate and then grow in large volume beneath the hemidiaphragms, within the greater omentum, and within the pelvis.

►Appendiceal Epithelial Neoplasms

Pseudomyxoma Peritonei

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Synonym

Jelly belly

Definition

Pseudomyxoma peritonei (PMP) is a rare epithelial neoplasm, arising in the majority of cases from the ►appendix, characterized by disseminated peritoneal mucinous tumor deposition and progressive accumulation of mucinous ascites. PMP is a registered rare disease (no. 843) by the National Organization of Rare Disorders.

Characteristics

Epidemiology

In Western populations, the estimated incidence is 1–2 per million, accounting for approximately 1,500 new cases per year in the USA; 50–70 new cases per annum in the UK. Most series show a female predominance; typically a 70:30 ratio. The mean age at presentation is in the mid-sixth decade of life, ten years younger than the mean age for colorectal cancer. Although of "colorectal origin," it is not known whether PMP shares PMP aetiological risk factors such as family history, westernized diet, obesity, and physical inactivity, as there is a paucity of large-scale epidemiological studies in this area.

Historical Perspective

PMP was originally described by Rotikansky in 1842, and for many decades, was believed to arise from mucinous tumors of the ovary. It is now widely held that the majority of PMP arise from an ►appendiceal epithelial neoplasm.

Natural History

The process of tumor dissemination may start with an occult perforation of the appendix, and while the appendiceal lesion often maintains indolent histological features, if unabated, the disease disseminates aggressively throughout the abdominal cavity. Large volumes of mucinous deposits may accumulate within the greater and lesser omentum, the spaces between the liver and diaphragm and within the pelvis, but the small bowel is often free of disease (except for the jejunum that is adjacent to the ►ligament of Treitz). It has been suggested that the principles of fluid hydrodynamics and gravity control this "redistribution phenomenon" such that the movements by small bowel peristalsis spares these structures. However, this does not explain dissemination in the absence of appendiceal perforation and the tumor's biology may be an alternative explanation.

The accumulation of extra-cellular mucin is central to the pathogenesis of the disease. Longstanding disease may fistulate into retroperitoneal spaces and beyond, including crossing the diaphragm into the lungs. The disease may also "burrow" into anatomical corners such as the reflection of the diaphragmatic peritoneum onto the liver and the insertion of the Ligamentum teres on to the liver, and may invade organs such as the spleen. However, PMP seldom metastases in a conventional manner via lymphatics to the nodal system or via blood to the liver and lungs. Untreated, progressive mucinous accumulation (over years in most cases) leads to extrinsic compression of bowel, malnutrition, and eventual death.

Appendiceal Origin

The reasons why it is now widely held that the majority of PMPs arise from an appendiceal primary tumor (as opposed to ovarian origin) are as follows:

- Careful histological examination of the appendix reveals an adenoma in most cases.
- Perforated appendix is frequently observed at laparotomy and/or histological examination.
- Immunohistochemistry staining is predominantly cytokeratin (CK) 20 positive (a marker of gastrointestinal epithelium), rather than CK7 positive (a marker of ovarian origin).
- PMP in males accounts for 30–50% of cases (arguing against an ovarian origin).

- K-ras mutational analysis supports the view that PMP is clonally derived from an associated appendiceal mucinous adenoma.

A small proportion of PMP cases arise from organs other than the appendix including stomach, colon, pancreas, gallbladder, urachus, and (confusingly) ovaries. PMP has also been described in the presence of dual pathologies such as ovarian teratoma.

Pathological Classification

Pathological nomenclature and histological classification in relation to PMP is potentially confusing. If the histological description is confined to that of the appendiceal lesion, the terms low-grade appendiceal mucinous neoplasm (LAMN) or appendiceal mucinous tumor of uncertain malignant potential are used.

For disseminated disease, the Ronnett classification of three groups is widely used: disseminated peritoneal adenomucinosis (DPAM), peritoneal mucinous carcinomatosis (PMCA) and PMCA with intermediate or discordant features (PMCA-I/D). In the original description of 109 peritoneal tumors, 95% were considered appendiceal primaries. This classification correlates well with 5-year and 10-year survivals: DPAM (75% and 68%, respectively); PMCA-I/D (50% and 21%); and PMCA (14% and 3%). Recently, Bradley et al reviewed 101 patients with PMP of appendiceal origin and applied a new (simplified) classification using the term mucinous carcinoma peritonei-low grade (MCP-L) to those cases referred to as DPAM and PMCA-I/D by Ronnett, and mucinous carcinoma peritonei-high grade to those referred to as PMCA.

Presentation

The classical presentation is one of advanced disease with a distended abdomen, mucinous ascites and peripheral wasting. In a contemporary practice, it is not uncommon for PMP to present earlier as an incidental finding, for example: a cystadenoma of the appendix on CT scan; mucinous tumor deposit noted at ►herniorrhaphy; on histological examination after appendicectomy; and during gynaecological laparoscopic diagnostic examination.

Staging Systems

There is no universally held pre-operative staging system. The correlation between pre-operative assessment by imaging (usually contrast-enhanced CT scan) and tumor volume found at surgery is modest. The following serum tumor markers has been evaluated in the pre-operative setting – ►CEA, CA19.9, CA125 and CA15.3 – but their accuracy to discriminate histological types and/or the prediction of the completeness of cytoreduction is modest.

A variety of intra-operative staging systems (Gilly Peritoneal Carcinomatosis Staging; Japanese system; Peritoneal Cancer Index; and Dutch Simplified Peritoneal Cancer Index) have been described to quantify tumor volume in other peritoneal tumor diseases, but they do not readily extend to quantifying PMP and its prognosis.

Cytoreductive Surgery

Until a decade ago, the mainstay of treatment for patients with PMP was repeated interval debulking surgery for relief of symptoms but with no expectation of improved survival and/or cure. Since then there has been a major paradigm shift towards management by complete surgical cytoreduction and the application of intra-peritoneal chemotherapy. This operation is often referred to as the ►Sugarbaker procedure after the surgeon who promulgates this approach. It involves up to six different peritonectomy procedures in combination with visceral resections as required, with the aim to remove all visible tumor, or if this is not possible, to leave tumor deposits less than 2.5 mm (2.5 mm being the maximum direct penetration of locally applied chemotherapy). This is a lengthy operation (may take 8–12 h) with a list of recognized complications including haemotological toxicity, substantial blood loss, re-operation, and 30-day mortality in the order of 2–4%.

Rationale for Intra-Peritoneal Chemotherapy

Traditionally, systemic chemotherapy for PMP is ineffective and thus intra-peritoneal chemotherapy as become part of the loco-regional multimodality standard in combination with cytoreductive surgery. There are two approaches: hyperthermic intra-peritoneal chemotherapy (HIPEC) or early post-operative intra-peritoneal chemotherapy (EPIC), and in combination. A randomized trial has shown that HIPEC is associated with lower complication rates. In HIPEC, the chemotherapy agent is typically perfused within the abdominal cavity for ninety minutes at 42°C. Based on historical in vitro experiments using ovarian and colorectal cancer cell lines, mitomycin has been the most widely used chemotherapy agent. Others include: doxorubicin, cisplatin, oxaliplatin, irinotecan and 5-fluorouracil.

Prognosis

Completeness of cytoreduction is the single most important predictor of patient prognosis. The completeness of cytoreduction score (CC score) is used to assess this and complete (CC-0 or CC-1) or incomplete (CC-2 or CC-3) cytoreductions are determined. A CC-0 is apparent when there is no peritoneal seeding visualized within the operative field; CC-1 indicates nodules persisting after cytoreduction less than 2.5 mm; CC-2 indicates nodules between 2.5 mm and 2.5 cm; and CC-3 indicates nodules greater than 5 cm or a

confluence of tumor nodules at any site within the abdomen or pelvis. The 5-year survival for complete cytoreduction is 86% versus 20% for incomplete surgical excision of tumor.

Palliative Treatment Options

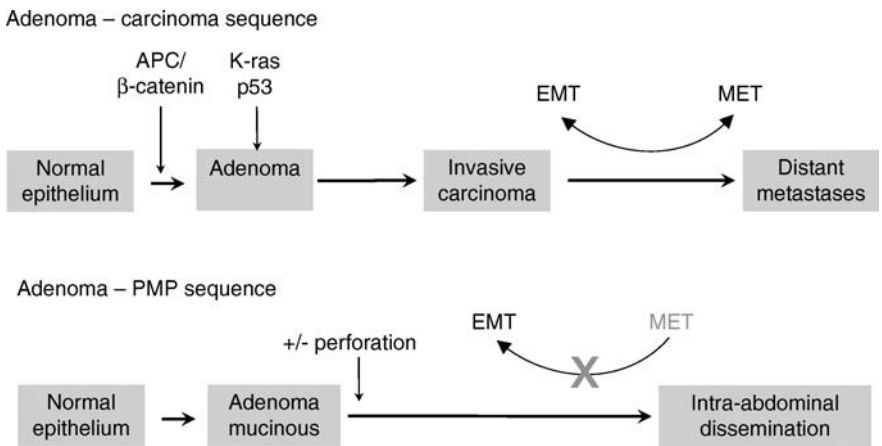
In the absence of effective systematic chemotherapy, in patients deemed unsuitable for cytoreductive surgery, the main option has been debulking surgery for symptom relief. However, oncologists are now beginning to explore the use of combinations of new chemotherapeutic agents, effective in colorectal cancer, and administer systemically. These approaches, however, stabilize disease rather than reduce tumor volume.

There have been a small number of studies on quality of life in patients with PMP, which in general support the role of intensive loco-regional multimodality therapy and debulking, though long-term depressive symptoms are common and information needs are high in these patients.

Basic Science

Given the rarity of this tumor, molecular investigation of PMP has been scarce. However, the following have been elucidated from a number of small studies:

- In common with colorectal neoplasia, ►K-ras mutations seem to be common.
- Unlike most colorectal adenomas, loss of heterozygosity of ►*APC* (adenomatous polyposis coli) is not a prequisite in PMP.
- ►P53 mutation occurs but perhaps not as common as sporadic colorectal cancer.
- Consistent with the more indolent nature of PMP, proliferation indices tend to be lower than counterparts in colorectal carcinomas.
- ►Microsatallite instability, common amongst right-sided colonic tumors, has not yet been studied.
- Given that PMP is characterized by excessive mucinous deposition, not unexpectedly, there is increased expression of MUC-2 (a mucin with gel-forming physicochemical properties similar to those exhibited by PMP), and of interleukin-9 (which induces MUC-2 and MUC-5AC gene expression).
- Recent data demonstrate that PMP has a specific pattern of adhesion-related protein expressions of increased N-cadherin, reduced ►E-cadherin, and increased vimentin; a phenotype suggesting an ►epithelial-mesenchymal transition state. During this transition, cells loose polarity and adherens junctions, and become more "fibroblast-like." These observations suggest that a specific phenotype may characterize the distinct non-metastasing behavior of PMP (Fig. 1), and form the basis for future mechanistic and therapy-targeting research.



Pseudomyxoma Peritonei. Figure 1 The "Vogelstein" adenoma-carcinoma sequence of sporadic colorectal adenocarcinoma (upper panel). The pathological transitions from normal epithelium through adenoma to invasive carcinoma are characterized by a multi-step accumulation of molecular events including APC mutation, accumulation of nuclear β-catenin, and mutations of p53 and K-ras. It is increasingly recognized that during the processes of invasion and metastases, the intestinal epithelial cells under phenotypic changes and take on the features of a mesenchymal cell – the epithelial-mesenchymal transition (EMT). Once a cancerous cell metastasizes, it establishes itself in a new micro-environment and reverts back to an epithelial phenotype – the ►mesenchymal-epithelial transition (MET). PMP arises from an appendiceal adenoma (lower panel), but unlike sporadic colorectal adenocarcinoma, it disseminates throughout the abdominal cavity rather than metastasize. In this disseminated state, PMP seems to have an "exaggerated" EMT phenotype but without an ability to undergo MET and establish metastases. It is unclear whether perforation of the appendix is a prerequisite to dissemination. This is currently a hypothesis.

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PSG1–11

Definition

Pregnancy-specific glycoproteins are secreted proteins of the ►CEA family.

►CEA Gene Family

PSI Domain

Definition

A cysteine rich repeat found in several different extracellular receptors including the N-terminal regions of plexin, semaphorin, and integrin – from which its name is derived. The conservation of cysteine residues within this domain suggest this region may contain a series of disulfide bridges.

►MET

PSMA

►Prostate-Specific Membrane Antigen

PSMD10

►Gankyrin

PSTT

►Placental Site Trophoblastic Tumor

Ptaquiloside

Definition

A norsesquiterpene glucoside from bracken fern proven to be highly carcinogenic.

►Bovine Papillomavirus

PTB Domain

Definition

Most phosphotyrosine-binding domains show the function similar to the SH2, facilitating interactions only with activated tyrosine-phosphorylated, but some of them also bind to unphosphorylated tyrosine. Examples containing PTB domain are FRS/SNT, DOK, and IRS family proteins.

►Membrane-Linked Docking Protein
►SH2/SH3 Domains

PTC

Definition

Papillary Thyroid Carcinoma.

PTCH

Definition
►Patched.

PtdIns

Definition
Phosphatidylinositol; Inositol lipids.

PtdIns 3-kinase

►PI3K Signaling

P-TEFb

Definition
Positive-acting transcription elongation factor also known as Tat-Associated Kinase (TAK), is a complex of cellular proteins involved in regulation of gene transcription.

►TAT Protein of HIV

PTEN

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Synonyms
MMAC1; TEP1

Definition
PTEN (phosphatase and Tensin homolog deleted on chromosome Ten) is a tumor suppressor gene located in human chromosome band 10q23. The protein product of the PTEN gene (PTEN) is a phosphatase, a protein that removes phosphate groups from other molecules. Specifically, PTEN is a phosphatase for both phosphorylated lipids and phosphorylated proteins. The former activity is linked to the ability of PTEN to block cellular signals that promote growth and survival.

Characteristics
Somatic Mutation of PTEN
All normal cells have two copies of the PTEN gene. However, loss of one or both copies of the PTEN gene in the tumor cells of a number of common cancer types has been increasingly recognized. These tumors types include cancers of the prostate, endometrium, brain (►glioblastoma multiforme), thyroid, ovary, kidney (►renal carcinoma) and skin (►melanoma). In cancers of the prostate and in glioblastoma, PTEN mutation is associated with the more aggressive forms of the disease. For example, astrocytic brain tumors are classified by the histological grade of the tumor. While PTEN mutations are rare in anaplastic astrocytoma (less aggressive), mutations are common in glioblastoma multiforme (the most aggressive). Similarly, loss of the PTEN protein product is associated with higher grade prostate tumors, and PTEN mutations are found more frequently in metastatic prostate tumors when compared to those that remain confined to the prostate organ.

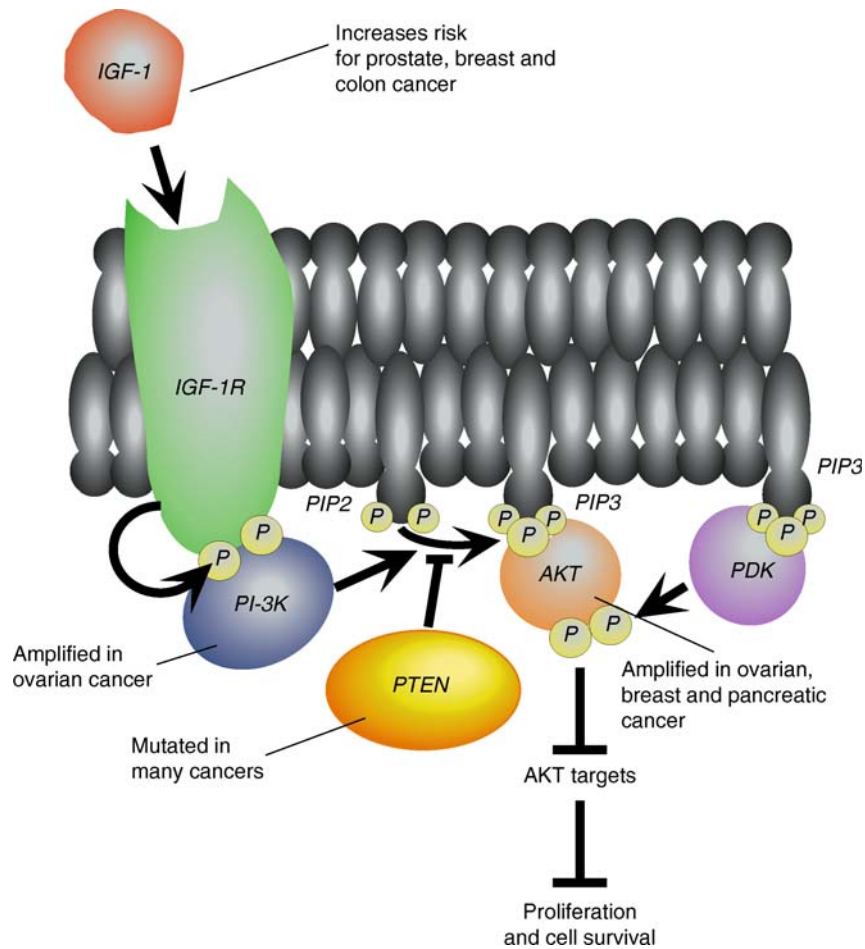
Germline Mutation of PTEN
Mutation of PTEN in the germ line DNA is associated with the development of two inherited syndromes with overlapping clinical features. Patients with these syndromes, Cowden disease and Bannayan-Zonana syndrome, develop abnormal growths (hamartomas) of the skin, hair follicle (trichilemmoma), breast, thyroid and intestinal tract, as well as neurological abnormalities. Such patients also have a higher incidence of cancers of the breast and thyroid. Thus both common cancers and certain rare inherited cancer predisposition syndromes can result from mutation of PTEN and the consequent loss of function of the gene product.

Biochemical Functions
Biochemical and structural studies of the PTEN protein have revealed that it is a member of the phosphatase family of proteins. These proteins typically remove phosphate groups (often the elements transferred during cellular signaling) from other macromolecules including proteins, lipids and nucleic acids. When assayed in vitro, PTEN can dephosphorylate (removes the phosphate from) protein substrates and in particular has a strong preference for acidic protein substrates (such as poly-glutamic acid:tyrosine co-polymers). In addition,

and more importantly with respect to its in vivo function, PTEN dephosphorylates specific membrane associated lipids. Specifically, PTEN can dephosphorylate the phosphate on the third position of phosphoinositide-3,4,5-trisphosphate (PI3,4,5P3) and phosphoinositide-3,4-bisphosphate (PI3,4P2). These lipids are produced in cells by the action of a specific kinase known as phosphoinositide-3 kinase (PI3K) (discussed further below). Of note, the vast majority of mutations in PTEN, which are derived either from tumors or from the germ line of patients with Cowden disease, alter the PTEN protein such that is can not dephosphorylate these lipids, while in some case these mutations do not alter protein phosphatase activity. These data suggest that PTEN lipid phosphatase activity is essential for preventing tumor growth. These considerations suggest that PTEN functions as a tumor suppressor, primarily by acting as a damper on the transmission of signals through the pathway governed by phosphoinositide-3 kinase.

Phosphoinositide-3 Kinase Signaling

The signals carried by this pathway typically begin at the cell surface, where growth or survival factors in the extracellular environment bind to their cognate receptors at the cell surface (Fig. 1). For example, insulin-growth factor I (IGF-I) binds to the IGF-I receptor (IGF-IR). These receptors are typically tyrosine kinases (proteins that phosphorylate other proteins on tyrosine residues), and when triggered by growth factor binding there is an activation of the kinase activity and a resulting auto-phosphorylation on the part of



PTEN. Figure 1 The Phosphoinositide-3 kinase/PTEN signaling pathway. Growth factors such as IGF-I (*red*), bind to their cognate receptors (*green*). Binding triggers receptor autophosphorylation and recruitment of phosphoinositide-3 kinase (PI3K) (*blue*). Activated PI3K phosphorylates phosphoinositide-4,5-bisphosphate (PIP2) and converts it to phosphoinositide-3,4,5-trisphosphate (PIP3). PIP3 recruits AKT (*orange*) to the membrane where it is phosphorylated and activated by PDK1 (purple). Activated AKT can then phosphorylate a number of downstream targets and thereby typically inactivating them. Usually, these proteins exert an inhibitory effect on proliferation or survival. PTEN (*yellow-orange*) inhibits signaling through this pathway by dephosphorylating PIP3 back to PIP2.

the receptor that resides on the inner surface of the plasma membrane. This auto-phosphorylation creates a docking site for PI3K, which is recruited to the receptor and activated. PI3K then phosphorylates the inositol head group of the lipid creating PI3,4,5P3 or PI3,4P2. Once these new phosphoinositides are generated they in turn serve as docking sites for other protein kinases. In particular, a kinase known as AKT is recruited to the membrane where it in turn becomes activated. AKT is then free to phosphorylate a number of other proteins. AKT phosphorylation typically acts to inhibit downstream targets such as BAD, GSK3 and the forkhead transcription factors (AFX, FKHR, and FKHRL1). As a result AKT is a potent survival factor and can induce cellular proliferation.

How is the Pathway PI3K/PTEN Relevant to Cancer?

The association between activation of the PI3K and the development of cancer is well established and can be achieved through multiple mechanisms: In animals, a number of tumor causing viruses produce proteins that activate this pathway. For example, the DNA tumor virus known as Polyoma produces an oncogenic protein known as Middle T antigen. Middle T can activate PI3K by recruiting it to membrane through a growth factor-independent mechanism. In chickens, a form of the avian sarcoma retrovirus (ASV 16) contains a copy of the chicken PI3K gene which has been modified so that it directly targets to membrane where it can activate signaling, again without growth factor induction. Finally, a murine retrovirus known as AKT8 contains a copy of the AKT gene that is activated by fusion to the retroviral gag protein.

In human cancers, activation of this pathway can be achieved through loss of PTEN, which results in constitutive phosphorylation and activation of AKT. In addition, the gene for the catalytic subunit of PI3K (PI3KCA) undergoes amplification in ovarian cancer. Finally, there are three AKT kinases in mammalian cells (AKT-1, -2, -3) and amplification of AKT-2 has been noted in breast, ovarian, and pancreatic cancers.

Conservation of the PI3K/PTEN Pathway in Caenorhabditis Elegans

The PI3K pathway is remarkably conserved through evolution. In C. elegans this pathway regulates life span and also regulates the induction of a so-called dauer state. The pathway includes homologs of the IGF-I receptor (daf-2), PI3K (AGEI), PTEN (daf-18), AKT 1 and AKT 2 (AKT1, AKT2), PDK1 an enzyme that activates AKT (PDK1) and a forkhead transcription factor (daf-16). The connection between AKT and forkhead has proved illuminating with respect to this pathway in mammalian cells, where homologues of daf-16 appear to regulate both proliferative and apoptotic signals downstream of PTEN and AKT. In the future, additional relationships and genes discovered in C. elegans are likely to have relevance to human cancers.

Loss of PTEN Deregulates PI3K Signaling

As predicted from the biochemical considerations, loss of PTEN leads to constitutive deregulation of components of the PI3K pathway. Murine cells genetically engineered to lack PTEN have elevated levels of PI(3,4,5)P3, and AKT is found in a constitutively phosphorylated and hence constitutively activated state. Tumor cells that lack PTEN, likewise, are marked by a dramatic increase in AKT activation and in the phosphorylation state of AKT substrates, including downstream targets such as FKHRL1, GSK3 and 4EBP1. Tumor cell lines that lack PTEN can be used to ask about the cellular consequence of PTEN restoration. As described below PTEN, like p53, can act as a negative regulator of proliferation and as an enhancer of apoptosis. In addition, PTEN can alter cellular motility, invasion and potentially tumor angiogenesis.

PTEN as a Cell Cycle Regulator

Reconstitution of PTEN to certain tumor cell lines (786-O renal carcinoma cells and U87MG glioblastoma cells) that lack PTEN results in the accumulation of these cells in the G1 phase of the cell cycle and a decrease in their proliferative rates in culture. When these cells are suspended in soft agar cultures, growth is arrested. The ability of PTEN to regulate cell-cycle progression requires lipid phosphatase activity and is antagonized by constitutive activation of AKT kinase. In addition, the connection to cell cycle regulation appears to be mediated through the induction of the cyclin dependent kinase inhibitor, p27, by PTEN. This latter step is probably mediated through the forkhead transcription factor family. These transcription factors, in the absence of a functional PTEN protein, are constitutively phosphorylated by AKT, and are consequently held in the cytoplasm through interactions with 14-3-3 proteins. When forkhead proteins are relocalized to the nucleus, they can induce p27 and arrest cells in G1.

The effects of PTEN reconstitution on cell cycle control are in keeping with the resulting proliferative lesions seen in tissues and cells genetically engineered to knock-out the PTEN gene. In cells that are heterozygous or homozygous null for PTEN, AKT is aberrantly phosphorylated, with evidence for a dose dependent effect of PTEN loss. In tissues, PTEN heterozygosity results in excessive proliferation (hyperplasia) in the thyroid and prostate. Labeling studies indicate that this results from an excessive number of cells entering the cell cycle. In addition, murine cells genetically engineered to lack both copies of PTEN have abnormal cell cycle kinetics. Thus, PTEN plays a necessary role in preventing unwanted cellular proliferation.

PTEN as a Regulator of Cell-Death

The PI3K pathway and in particular AKT have been previously linked to the regulation of cell survival. In keeping with this idea, PTEN reconstitution to certain PTEN null cells, results in the induction of cell death. Again, loss of function studies have been revealing. Specifically, PTEN null murine fibroblasts have a defective response to apoptotic stimuli, and PTEN heterozygous mice develop a lymphoid hyperplasia syndrome that results from a failure of these cells to undergo cell death at the appropriate time.

Mechanistically there are a number of ways that PTEN could influence apoptotic signaling. AKT kinase is known to phosphorylate a number of substrates that are particularly relevant to apoptosis, including the pro-apoptotic proteins BAD and Caspase 9. For example, AKT phosphorylation of BAD results in BAD binding to and being sequestered by 14-3-3 proteins. In addition, the Forkhead transcription factors (FKHR, FKHRL1 and AFX) that are AKT substrates can all induce apoptosis in certain cells. Furthermore, FKHRL1 appears to be capable of regulating the transcription of Fas ligand, a potent inducer of apoptosis. This is of particular interest because the lymphoid hyperplasia syndrome that develops in PTEN +/− mice phenocopies (mimics) a similar syndrome seen in mice bearing mutations in elements of the Fas signaling pathway. Thus, one possibility is that loss of PTEN leads to constitutive phosphorylation of forkhead factors, defects in Fas mediated signaling and a subsequent failure to undergo apoptosis in response to pro-apoptotic signals.

PTEN as a Regulator of Cell Motility and Adhesion

Cancer cells, especially those that metastasize, must acquire the ability to escape their local environment, and thus cancer cells are often more motile and invasive then normal cells. PTEN can regulate adhesion and motility in at least two ways: First, the PI3K pathway, in addition to regulating AKT, also regulates a set of small GTPase proteins (Rho/Rac/Cdc42) that regulate motility and invasion. Loss of PTEN can lead to deregulation of this arm of the PI3K pathway. In addition, PTEN protein phosphatase activity has been linked to the regulation of cell spreading through the dephosphorylation of focal adhesion kinase (FAK). With respect to FAK regulation, the protein phosphatase activity was sufficient, as the aforementioned tumor derived mutants lacking lipid phosphatase activity remain active in these assays.

PTEN as a Regulator of Angiogenesis

Lastly, AKT activation has been associated with the regulation of endothelial cell nitric oxide (eNOS), and PTEN loss has been associated with the loss of regulation of the hypoxia-inducible factor 1 (HIF-1) gene product. These connections suggest that PTEN null tumors may have an increased tendency to become vascularized, and in certain PTEN null prostate tumors there is evidence that this may be the case.

Clinical Relevance and Therapeutic Implications

The demonstration that PTEN, a commonly mutated tumor suppressor, functions primarily as an antagonist of signaling through the PI3K pathway has triggered considerable enthusiasm for the development of small molecule inhibitors of the protein kinases activated by PTEN loss. Drugs that inhibit certain kinases have been developed previously and thus strategies and methods are in place for doing so again. In this pathway the therapeutic targets include the IGF-I receptor, PI-3 kinase, AKT kinases and PDK kinases. The hope for the future is that loss of PTEN will serve as a molecular marker or so-called predictive factor. That is, PTEN loss would lead to the development of a tumor that is particularly dependent upon constitutive signaling through the PI3K pathway. If so, then these tumors might be exquisitely sensitive to small molecule inhibitors of the kinases in this pathway.

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PTEN Hamartoma Tumor Syndrome

►Cowden Syndrome

Pterophyllus Salisburiensis

►Ginkgo Biloba

PTGF-β

►MIC-1

PTHLH

►Parathyroid Hormone-Related Protein

PTHrP

►Parathyroid Hormone-Related Protein

PTKIs

►Tyrosine Kinase Inhibitors

PTPase

Definition

A protein tyrosine phosphatase (PTPase) is an enzyme that dephosphorylates tyrosine residues.

►Signal Transducers and Activators of Transcription in Oncogenesis

PTPC

Definition

Permeability transition pore complex, a complex of proteins situated at contact sites between the inner and outer mitochondrial membranes. Its abnormal opening promotes cell death by various agents including jasmonates.

►Jasmonates in Cancer Therapy

PTTG1

Definition

Pituitary Tumor-Transforming Gene1.

►Genomic Imbalance
►Securin

Pulsed Dye Laser

Definition

PDL; A laser with wavelength of 577–595 nm used for microvascular targeting. PDL is effective because the wavelength of the laser coincides with the peak absorbance of hemoglobin, thus blood vessels exposed to the laser beam are destroyed.

►Photochemoprevention

PUMA

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Synonyms

JFY1; bbc3; Bcl-2 binding component 3

Definition

PUMA (p53 upregulated modulator of ►apoptosis) is a p53 target and an initiator of apoptosis. PUMA is a member of the ►Bcl-2 protein family, which are evolutionarily conserved regulators of apoptosis.

Signaling by PUMA is critical for the apoptosis induced by p53 and a wide range of anticancer agents.

Characteristics

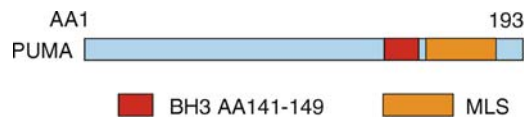
Discovery and Function

PUMA was independently identified by three groups in 2001 based on its transcriptional induction by the tumor suppressor ►p53, and interaction with the antiapoptotic protein Bcl-2. It is highly conserved between mouse and human, with over 90% of sequence identity at both DNA and protein levels. However, no PUMA ►homolog has been identified in lower eukaryotes.

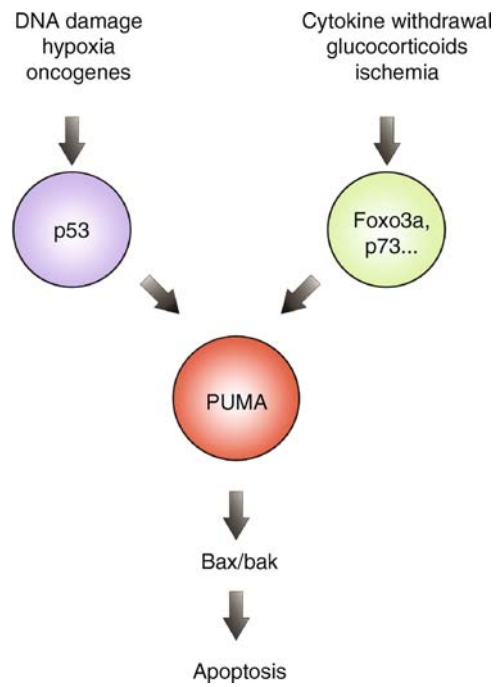
PUMA belongs to the ►BH3-only subgroup of Bcl-2 family proteins, which share sequence similarity only within the BH3 (Bcl-2 homology 3) domain, a short nine amino acid stretch. More than ten BH3-only proteins have been identified in mammalian cells. They function in a tissue- and stimulus-specific manner to initiate apoptosis. In contrast, only a single BH3-only protein EGL-1 has been found in the worm nematode *Caenorhabditis elegans*, which is responsible for all the somatic cell death during development. The BH3 domain of PUMA is required for its interactions with other Bcl-2-like proteins, such as Bcl-2, ►Bcl-xL, and ►Mcl-1 (Fig. 1). The C-terminal portion of PUMA contains a hydrophobic domain that is required for its mitochondrial localization (Fig. 1). Both domains are essential for its ability to induce apoptosis.

BH3-only proteins often display different binding affinity with the antiapoptotic Bcl-2 family members and function as their selective inhibitors. Unlike most other BH3-only proteins, PUMA potently antagonizes the antiapoptotic functions of all Bcl-2-like molecules. ►Bax and ►Bak, two Bcl-2 family members containing multiple BH domains, appear to work downstream of PUMA, and are necessary for PUMA-induced apoptosis (Fig. 2). PUMA does not physically interact with Bax or Bak, but indirectly activates Bax/Bak by dissociating them from the antiapoptotic Bcl-2-like proteins. Activation of Bax/Bak leads to the disruption of mitochondrial integrity and release of the apoptogenic proteins such as ►cytochrome *c* and ►SMAC/Diablo. Subsequently, ►caspases are activated, and irreversible cell death ensues.

Studies using gene targeted human somatic cells and mice have yielded insight into the function of PUMA. Human colorectal cancer cells deficient in PUMA are resistant to apoptosis induced by p53, hypoxia, endoplasmic reticulum stress, and DNA damaging agents. PUMA-deficient mice develop normally, but are protected from tissue damage and apoptosis induced by gamma irradiation in the thymus, hematopoietic system, small intestine, and developing neurons. Other pathological stimuli, including oncogenic stress, hypoxia, growth factor deprivation, glucocorticoids, and ischemia reperfusion, are also dependent on PUMA to induce apoptosis in mice (Fig. 2). Importantly, some of the apoptotic defects seen in *PUMA* knockout mice or cells resemble those seen in p53-deficient mice or cells. The ability of PUMA to antagonize all Bcl-2-like molecules might explain why loss-of-function mutants of PUMA display profound deficiencies in the responses to a wide range of stresses in multiple tissues.



PUMA. Figure 1 Schematic representation of PUMA protein. The major form of PUMA (alpha form) protein contains 193 amino acids and two functional domains. The amino acid residues 141–149 consist of a BH3 domain responsible for its interactions with Bcl-2-like proteins. The C terminus contains a mitochondrial localization signal (MLS) which is not well-defined.



PUMA. Figure 2 PUMA mediates p53-dependent and -independent apoptosis. Upon stress, PUMA transcription is activated to induce apoptosis via the mitochondria through a Bax/Bak-dependent manner. p53 activates PUMA to induce apoptosis following DNA damage, hypoxia, and oncogene activation. Transcription factors, such as p73, FOXO 3A, and those yet to be identified, activate PUMA to induce apoptosis following cytokine withdrawal, glucocorticoids, and ischemia/reperfusion.

Regulation

PUMA is normally expressed at a very low level but rapidly induced when cells are exposed to agents that damage DNA, such as gamma irradiation and commonly used chemotherapeutic agents (Fig. 2). Under these conditions, the tumor suppressor p53 directly binds to the two p53 responsive elements in the *PUMA* promoter to activate its transcription. This p53-dependent induction of PUMA occurs within several hours of exposure, and is required for subsequent apoptosis in many cell types. Most studies have convincingly placed PUMA downstream of p53. However, recent studies suggested that PUMA can also modulate the apoptotic function of cytoplasmic p53 in response to ultra violet irradiation, thus serving as a link between the nuclear and cytoplasmic p53. Such a mechanism still awaits additional supporting evidence.

Some stresses that do not typically damage DNA, such as growth factor deprivation and cytokine withdrawal, can activate *PUMA* transcription independent of p53. The underlying mechanism is not well understood (Fig. 2); the transcriptional factors ►p73 and ►FOXO 3A, which can induce PUMA in p53-deficient cells, might be involved.

PUMA transcripts exhibit extensive ►alternative splicing, which results in mRNA species that may encode proteins without the BH3 domain. The functional role of these spliced variants is currently unknown. The *PUMA* promoter and its first two exons contain a high percentage of guanine and cytosine nucleotides, suggesting a propensity of forming secondary structures capable of inhibiting its basal transcription. The complex regulation of PUMA expression perhaps reflects its critical role in apoptotic responses to multiple stress conditions, which are important in oncogenesis and tissue damage.

Clinical Considerations

It is increasingly apparent that apoptosis acts as a barrier against oncogenesis. Alterations of apoptosis regulators, which are common in human malignancies, contribute to tumor formation, progression, and impaired responsiveness to anticancer therapies. Several lines of evidence suggest that the function of PUMA is defective in cancer cells. First, more than 50% of human tumors contain *p53* mutations, which abrogate the induction of PUMA by radiation and many chemotherapeutic drugs, and render resistance to the killing effects of these agents. Second, PUMA function might be compromised because of altered expression of Bcl-2 family proteins. For example, overexpression of antiapoptotic Bcl-2 family proteins has been commonly observed in tumors. PUMA expression was also found to be reduced in malignant cutaneous melanoma, and reduced PUMA expression was correlated with shorter survival of patients and poorer response to therapies. Third, *PUMA* is located on 19q3.3, a region frequently lost or deleted in several tumor types, including glioma, neuroblastoma, B-cell lymphomas, head and neck squamous cell carcinoma, and lung cancer. Animal studies using a Eμ-Myc-induced mouse lymphoma model also suggested that PUMA functions as a tumor suppressor. However, to date, there is no evidence that *PUMA* is a target of mutation in human cancer.

Manipulation of PUMA expression can be explored as a therapeutic strategy. PUMA has been shown to sensitize lung and esophageal cancer cells to several commonly used anticancer agents. The rationale is that activation of PUMA via p53-independent mechanisms restores the apoptotic response in tumor cells while sparing normal cells. Compared with p53, it might be advantageous to target PUMA because it can induce rapid and extensive apoptosis in a variety of cancer cells regardless of their p53 status, and it is a much more potent killer of cancer cells than p53 itself.

On the other hand, several recent studies suggest that inhibiting PUMA function in normal tissues might be beneficial in cancer treatment. Radiation and chemotherapy often cause severe side effects on the rapidly proliferating normal tissues, such as bone marrow, hair follicles, and gastrointestinal tracts, which limit the dose that can be used to treat patients. Because PUMA is an important mediator of these side effects in these tissues, PUMA inhibitors might be clinically useful in controlling the side effects of chemo and radiation therapies.

In summary, PUMA mediates both p53-dependent and -independent apoptosis. It plays a central role in regulating mitochondria-mediated cell death induced by a wide range of stimuli, including many anticancer agents. Selective activation of PUMA in cancer cells and inhibition of PUMA in normal tissues might be useful for improving the therapeutic responses of cancer patients. These intriguing possibilities certainly need to be carefully examined in the future.

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Purging

Definition
Removal of cancer cells from bone marrow, such as before bone marrow transplantation.

Purine Analogues

A class of chemotherapeutic agents usually refering to fludarabine, cladribine and pentostatin. Fludarabine and cladribine are synthetic analogues of deoxyadenosine. Pentostatin is not an analogue of deoxyadenosine, but increases deoxyadenosine levels by inhibition of adenosine deaminase.

Putative NF-kappa-B-activating protein 002N

►Skeletrophin and Cancer

PVHL

Definition
Von Hippel Lindau Protein; protein encoded by the von ►Hippel-Lindau Tumor Suppressor Gene.

Pyelography

Definition
X-ray photography of the kidneys, renal pelvises, ureters, and urinary bladder after injection with a radiopaque contrast dye.

►Bladder cancer
►Transitional Cell Carcinoma

Pyrexia of Unknown Origin

►Fever of Unknown Origin

Pyrimidine

Definition
A heterocyclic organic compound that contributes to the structure of RNA and DNA.

►Fluorouracil

Pyrophosphate

Definition
Is a chemical compound formed by the hydrolysis of ATP into AMP in cells.

►Zoledronic Acid

Qi

Definition

Is a philosophical conception developed in ancient China; it is believed to be the basic element that constitutes the cosmos and produces everything in the world through its movement and changes. In Chinese medicine, qi refers to the basic element comprising the human body and supports its vital activities, such as food essence and fresh air. Since the existence of qi in the body can only be perceived through the resultant activities of organs and tissues, it is more frequently used with the meaning of functional activities.

►Chinese versus Western Medicine

Qigong

Definition

Refers to a method of physical and mental self-exercise created by the Chinese people to strengthen health and cure diseases, mainly by regulating postures, training respiration, concentrating, and conducting mind so as to integrate and coordinate the mind will, vital energy and vitality.

►Chinese versus Western Medicine

QSAR

►Quantitative Structure Activity Relationship

Quadriradial Chromosomes

Definition

Are four-armed, aberrant chromosomes probably formed by unresolved recombination between homologous chromosomes.

Quality Adjusted Life Year

Definition

QALY; Measure of health gain from an intervention used in pharmacoeconomic evaluation. The additional survival gained by the use of an intervention is adjusted to account for the degree of health impairment experienced by the patient. For example one year of additional survival in full health or two years of additional survival with 50% impairment both equate to a gain of one QALY.

►National Institute for Health and Clinical Excellence (NICE)

Quality of Life

Definition

QOL; Physical, psychological and social domains of health that are influenced by an individual's experiences, beliefs, expectations and perceptions

►Nutrition Status

Quantitative Structure Activity Relationship

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Synonyms

QSAR

Definition

The use of chemical descriptors and mathematical models to quantitatively describe the relationship between molecular structure and biological activity and predict structural modifications that increase compound potency.

Characteristics

Quantitative structure activity relationship (QSAR) analysis is based on the observation that the structures of active small molecules and the resulting molecular properties are directly related to biological activity. In other words, structure determines activity, similar molecules should have similar activity, and many (but not all) structural modifications cause changes in activity. This concept is intuitive and provides the basis for QSAR analysis, which is one of the major techniques in small molecule-based drug design (►Drug design). The basic ideas of QSAR analysis are also related to the ►pharmacophore concept. However, pharmacophores are designed to represent parts of a molecule that are responsible for biological activity and are qualitative in nature. By contrast, QSAR analysis attempts to capture the relationship between structure and activity in quantitative terms through the use of mathematical functions. QSAR models are applied to predict small structural modifications of active compounds that significantly increase their potency. As such, QSAR analysis has become the major computational approach to support hit-to-lead transformation and ►lead optimization studies in drug discovery (►Small molecule drugs). The traditional small molecule focus of QSAR explains why this methodology since its formal introduction during the 1960s has become the most widely used computational approach in the field of medicinal chemistry.

An important distinction between QSAR and pharmacophore modeling is that QSAR correlates structure and activity through calculation of molecular property descriptors. Such descriptors are used to represent molecular structure and properties and are an integral part of QSAR analysis. Property descriptors are mathematical models of greatly varying complexity and can be divided into different categories. Some descriptors are designed to calculate physico-chemical properties such as "logP (o/w)," the logarithm of the octanol/water partition coefficient, a measure of the hydrophobic character of a molecule. By contrast, others are purely mathematical in nature, for example, descriptors accounting for "molecular connectivity" or "topology." Another way to distinguish chemical descriptors is to consider the dimensionality of the molecular representation from which they can be calculated. For example, a one-dimensional (1D) descriptor like "molecular weight" is simply calculated from the composition formula, 2D descriptors like "atomic partial charges" or "aromatic character" can be calculated from a 2D graph representation of a molecule, and 3D descriptors such as "molecular volume" or "surface area" are derived from molecular conformations.

The fundamental premise of QSAR analysis is that differences in biological activity can be quantitatively described through mathematical functions that utilize property descriptors as variables. The classical and most widely used assumption is that activity can be expressed as a linear combination of chosen descriptors:

$$A = c_1D_1 + c_2D_2 + c_3D_3 + \ldots + c_nD_n + const$$

In this formulation, A is the predicted biological activity, D_i ($i = 1,\ldots,n$) are molecular descriptors, and c_i ($i = 1,\ldots,n$) coefficients that determine the relative weight and importance of each descriptor. This formalism establishes a linear relationship between molecular structure and activity, which is the basic assumption of traditional two-dimensional (2D-) QSAR ("2D-QSAR" means that molecules are studied using 2D-graph representations). However, generally assuming the presence of a linear relationship is clearly an approximation because many structure-activity relationships are at least in part non-linear in nature. Therefore, some advanced QSAR methods attempt to take non-linearity into account, for example, by deriving QSAR equations using machine learning techniques such as neural network simulations. Nevertheless, modeling linear structure-activity relationships represents the most widely applied form of QSAR analysis.

Building a QSAR model depends on the availability of a training set that consists of molecules having similar activity but different potency levels. QSAR modeling is generally limited to similar compounds sharing the same core structure (often called "congeneric" molecules). Otherwise, it would be very difficult to identify preferred descriptor sets and derive linear models of activity. Consequently, series of closely related structures (often called "analogs") are used for building a QSAR model. The ultimate purpose of the model is the prediction of novel analogs with improved potency, following the paradigm "small chemical changes, large

biological effects." This theme perfectly fits the challenges of chemical lead optimization efforts (and rationalizes the popularity of QSAR in medicinal chemistry).

QSAR model building begins with the assembly of the training set and the selection of an initial set of relevant descriptors. Training set compounds should have sound potency values associated with them (if possible, determined in the same assay system) and span a wide potency range so that later predictions are not attempted far outside the training set range, which typically cause inaccuracies. It is also important to avoid the inclusion of statistical outlier values in the training data. Descriptor pre-selection is attempted either on the basis of chemical experience and intuition or computational descriptor selection protocols (e.g. genetic algorithms). An experienced QSAR scientist usually has an empirical set of "successful" descriptors at hand that address effects known to be important for mediating receptor-ligand interactions (e.g., hydrophobicity or hydrogen bonding). The values of initially chosen descriptors are then calculated for the training set compounds. During descriptor selection and model building process, care must be taken to remove redundant or strongly correlated descriptors and reduce the number of descriptor variables to a required minimum. In 2D-QSAR, there should be significantly fewer descriptor variables than observations (i.e. compound potency values). Otherwise, the resulting model will be too specific for the training set compounds and will have low predictive value outside the training set, a process commonly referred to as "over-fitting" of a model. Multiple linear regression calculations are used to derive the values of the coefficients and the constant of the linear equation from predicted activity values. Statistical tests and cross validation studies are carried out in order to determine how well the model reproduces the potency values of training set compounds and, subsequently, of single compounds removed from training. In an iterative manner, descriptors are eliminated or added and coefficients are adjusted to re-build and refine the model until training set data can be reproduced with typically greater than 80% accuracy. Then the model is used to predict the activity of test set compounds. The test set consists of similar compounds with known potency not used for training. On the basis of these calculations, the prospective predictive performance of the QSAR model is statistically assessed and it is subjected to further refinement. Once the accuracy of the model is satisfactory and cannot be further improved through descriptor modification and linear regression, novel predictions can be attempted. This is done by calculating descriptor values for analogs other than training or test set compounds and predicting their potency using the model. New compounds with predicted increases in potency become candidates for synthesis and biological evaluation. These model building and (statistical) validation procedures generally apply to QSAR analysis, irrespective of the descriptors and mathematical methods used.

Classical 2D-QSAR analysis has been extended through the introduction of three-dimensional (3D-) QSAR methods. "3D-QSAR" means that QSAR equations are derived from 3D conformations of active compounds. The basic idea of 3D-QSAR is to quantitatively estimate the interaction between ligands and hypothetical receptor binding sites ("hypothetical" because binding sites are not known or taken from experimental structures, but computationally constructed around active compounds). Pharmacophore models provide popular starting points for 3D-QSAR analysis to model and orient compound conformations, thus providing a link between a qualitative and quantitative evaluation of structure-activity relationships. The prototypic approach that introduced 3D-QSAR is called Comparative Molecular Field Analysis (CoMFA). In CoMFA, superposed active compounds are placed on a computational grid and an artificial probe atom is used to calculate interaction energies with these molecules at each grid point. Different probe atoms produce steric (van der Waals) or electrostatic (Coulombic) interaction energies. These grid point energies represent the descriptors for 3D-QSAR analysis. In contrast to the principles of 2D-QSAR, CoMFA generates many more descriptor variables than the observations that are used (i.e. compound potency values). Therefore, the application of descriptor reduction techniques is required for 3D-QSAR model building. Among others, Partial Least Squares (PLS) regression is a popular statistical technique to select latent descriptor combinations that best account for training set activities. PLS generates multiple new and uncorrelated descriptors that are linear combinations of the original grid point energies. Regression analysis is carried out on these new descriptors to produce the 3D-QSAR model in "PLS space" (Fig. 1).

Since original grid point energies are retained in the PLS equations, the final PLS solution can be projected back into the original grid space and grid points with similar coefficients are displayed as contours that represent molecular interaction fields surrounding active molecules. Graphical analysis of these fields can suggest where compound modifications (e.g. by introducing bulky or charged groups) might produce more favorable interaction energies. For modified analogs, all the descriptors in the QSAR equation are calculated and used to predict compound potencies, and the best candidates are selected for further study. Over the years, many variants of the CoMFA approach have been introduced that mainly differ in the types of energy calculations. For example, to name just one,

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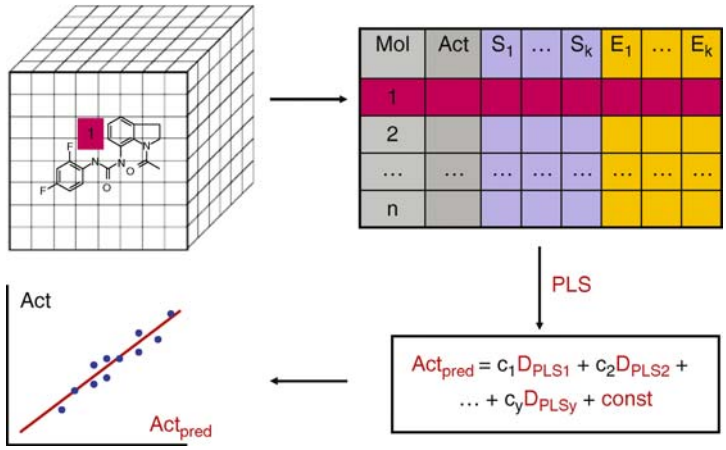

Quantitative Structure Activity Relationship. Figure 1 Foundations of 3D-QSAR. Conformations of active molecules are superposed on a three-dimensional grid and a QSAR table is generated. This table records n test molecules, their experimentally determined activity (Act), and interaction energy descriptor values. For each molecule (only compound 1 is shown), each of k grid points is associated with two descriptors, steric (S) and electrostatic (E) interaction energy. The descriptors in the QSAR table are subjected to PLS analysis, which produces a reduced number of novel descriptors (D_{PLS}) that are linear combinations of the original ones. Applying the resulting QSAR equation, compound activities are predicted (Act_{pred}) and the correlation between experimental and predicted training set (and, later on, test set) potency values is analyzed. For evaluating the performance of the QSAR model, statistical measures such as a squared correlation coefficient (R^2) are conventionally used.

Comparative molecular similarity index analysis (CoMSIA) calculates steric, hydrophobic, electrostatic, and hydrogen bonding energy terms.

Similar to pharmacophore modeling, the major principal limitation associated with 3D-QSAR analysis is the correct prediction of bioactive ligand conformations. If the binding conformation of an active molecule is incorrectly predicted, the resulting QSAR model has little, if any value. Since 3D-QSAR models are derived from multiple active compounds, there is an additional requirement: active conformations must not only be correctly predicted, but must also be correctly superposed in three dimensions (which represents the so-called "alignment problem"). In order to address the conformational problem, the 4D-QSAR formalism has been introduced. Here conformational flexibility is added as a fourth dimension to 3D-QSAR and instead of single conformations, conformational ensembles are used for each active molecule. Thus, 4D-QSAR models become largely independent of single compound conformations. However, the alignment problem must still be solved, which becomes more difficult in the presence of an increasing number of diverse compound conformations. Therefore, multiple alignment schemes are evaluated when deriving a 4D-QSAR model to select the one yielding most accurate predictions.

Traditionally, 3D-QSAR methods have not incorporated information from receptor sites. In fact, when 3D-QSAR became popular during the late 1980s, very few 3D structures of popular drug targets were available so that much emphasis had to be put on small molecule-based approaches. In recent years, this situation has changed and the increasing availability of target structures is also beginning to influence the QSAR field, giving rise to the development of "receptor-dependent" QSAR techniques. Basic ideas of receptor-dependent QSAR are that experimental structures of target-ligand complexes greatly simplify the generation of bioactive compound conformations, guide the construction of a pharmacophore model, and enable the calculation of energy descriptors directly from observed target-ligand interactions. Thus, receptor-dependent QSAR alleviates some of the uncertainties associated with conventional 3D-QSAR and is capable of utilizing more realistic (albeit still approximate) interaction energies as descriptors, which makes further development of such techniques an attractive task.

In conclusion, QSAR methods are one of the most widely applied and traditional approaches in computer-aided drug design and the dominant ligand-based approach. This is at least in part due to the high relevance of QSAR analysis for analog design and lead optimization. Future directions for research in the QSAR field include a more systematic exploration of the complementarity of QSAR and structure-based design methods and the development of multi-dimensional QSAR formalisms where additional degrees of freedom are added to 3D-QSAR analysis.

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Quantitative Trait Loci

►Modifier Loci

Quantum Dots

Definition

Are a type of ►nanoparticle composed of an inorganic elemental core (e.g. cadmium or mercury) with a surrounding metal shell. The emission spectra of quantum dots may be changed by altering their size and composition.

►Nanotechnology

Quasispecies

Definition

Are viral variants resulting from mis-incorporation of nucleotides during replication and leading to diversification of the original strain.

►Hepatitis C Virus

Quaternary Structure

Definition

The three-dimensional structure of a protein that has two or more subunits.

►Structural Biology

Quercetin

Definition

(3,3′,4,5,7-pentahydroxyflavone) flavonoid contained in bracken fern proven to be mutagenic in both prokaryotic and eukaryotic cells. It induces chromosomal aberrations.

►Flavonoids

Quinones

Definition

A group of highly aromatic compounds derived from benzene or from multiple ring hydrocarbons and contain two ketone group substitutions. A class of aromatic yellow compounds including several biologically important as coenzymes or acceptors or vitamins.

►Particle-induced Cancer
►Benzene and Leukemia

R2

►Metastasis Suppressor KAI1/CD82

Rab

Definition

Small GTPase, member of a branch of the Ras superfamily involved in vesicle transport.

►RAS

Rac

Definition

Belongs to the ►Rho family proteins small GTPases. Activated Rac (GTP-bound state) induces reorganization of the ►actin cytoskeleton in ►lamellipodia and thus controls cell protrusion and ►cell migration.

►Gelsolin

RAC3

►Amplified in Breast Cancer 1

RACE

Definition

5′-rapid amplification of cDNA ends (5′-RACE) allows the amplification of the 5′ end of a cDNA where there is incomplete knowledge of the mRNA of interest. ►PCR primers are designed for the known sequence within the mRNA and for a homopolymer tail that has been added to the 3′ end of the first strand of cDNA. The amplified fragment, obtained by ►nested PCR, contains the 5′ remainder of the cDNA that is followed by the known cDNA sequence.

RAD50

Definition

Is a protein forming a multi-function complex with Mre11 and Nbs1 (►Nijmegen breakage syndrome), and is involved in the processing of DNA damage for DNA maintenance including ►repair of DNA and recombination.

►Homologous Recombination Repair

RAD51

Definition

Is a protein found in all eukaryotic organisms from yeast to man, with a central role in homologous recombination. It has homology to the bacterial RecA protein, and similarly catalyses pairing and strand

exchange between a damaged DNA molecule and an undamaged homologous molecule.

►Homologous Recombination Repair
►BACH1

RAD54

Definition

Is a member of protein family suggested to have DNA ►helicase activity, unwinding DNA to promote recombination.

►Homologous Recombination Repair
►BACH1 Helicase

Radiation Biology

Definition

The study of the action of ►ionizing radiation on living matter.

►Radiation Oncology

Radiation Carcinogenesis

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Synonyms

Ionizing radiation induced cancer; Gamma-ray induced cancer; X-ray induced cancer; Radiation induced neoplastic transformation

Definition

Radiation ►carcinogenesis is a biological phenomenon whereby living normal cells are damaged by ionizing radiations which starts a progressive process causing the surviving cells to change their phenotype such that normal controls of cell death and apoptosis are lost and uncontrolled cancerous growth is initiated.

Characteristics

Radiation

Radiation is the deposition of energy into mass. Energy can appear as both corpuscles or electromagnetic quanta, or photons. Some of the elementary forms are protons, neutrons, electrons, x-rays, and gamma rays. X-rays originate outside of the nucleus of an atom while gamma rays originate within the atomic nucleus. Both are identical electromagnetic radiation with varying energies. The equivalence of matter expressed as energy is given by Albert Einstein's relation:

$$E = mc^2$$

Where E is energy, m is mass, and c is the velocity of light (3×10^8 m/s).

People are exposed to radiation naturally from the surrounding environment, from space radiation arriving on earth, and from man made causes such as chest and dental x-rays, and fall-out from nuclear weapons testing. Due to the nature of radiations, that is they cannot be seen, or smelled, governments, and states have taken a conservative regulatory approach to containing and minimizing exposure of both those who may be exposed occupationally and for the general public. There are strict regulations that must be followed to keep exposures very low and thus the risk from exposures is also very low.

Interaction of Radiation with Matter

Matter is composed of atoms, which in turn are composed of protons, neutrons, and electrons. High-energy x-rays, photons, used in diagnostic radiology and radiation therapy and gamma rays from natural sources are electromagnetic radiation that can interact with the orbiting electrons which consequently cause the expulsion of secondary high-energy electrons. The energy of these secondary electrons is in millions of electron volts [MeV]. The binding energy of electrons with molecules is only a few electron volts. Therefore, the energetic secondary electrons can collide with many atoms and molecules knocking out electrons as they give up energy until they no longer have enough energy to break any more chemical bonds. Each time an electron is dislodged from an atom or molecule, an ion is created that can combine with other atoms and molecules which therefore change the chemical composition of the molecules involved. It does not take very much ionizing radiation exposure to "hit" all the cells in the body. The number of times each cell will be hit will depend on the total dose and the time between hits will depend on the total dose and the rate at which dose is delivered. In general, calculations show that at total body dose of 1 cGy, each cell nucleus will be hit about 3.2 times if Cesium 137

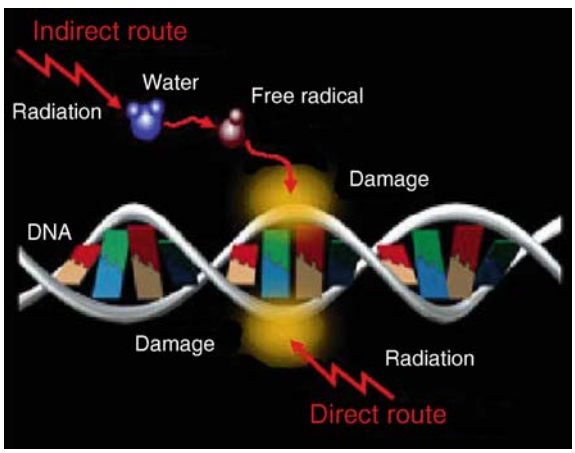
is the source of the gamma rays and these expected hits produce about 18 single strand breaks per nucleus per 1 cGy. The whole cell will record four times as many hits on average (12.5 hits).

Interaction with Living Cells

DNA in the nucleus is the most sensitive region of the cell. Damage to DNA can take place by either direct or indirect action as a result of radiation exposure. Fig. 1 shows this interaction schematically.

In direct action, the secondary electron interacts with the DNA directly. In indirect action, the secondary electron reacts with water to produce hydroxyl radicals which react with DNA. Approximately two thirds of the x-ray damage to DNA in mammalian cells is caused by the indirect action. It should also be noted that all the other types of radiation including alpha particles, neutrons, heavy ions and protons have been shown to interact with DNA and cause damage that could lead to cancerous events. The biological consequences of DNA damage can take from days to many years to become manifest. DNA is a double-stranded molecule held together by hydrogen bonds. Single strand breaks of DNA can be repaired efficiently in mammalian cells under normal physiological conditions. Double strand breaks on the other hand (some of which maybe formed from two fairly closely aligned single strand breaks) are more difficult to repair and are responsible for most of the biological consequences of radiation including cell death and genetic changes that may lead to disease, some of which manifests itself as cancer (►DNA Damage).

In the context of the a human going through life the genetic changes are mutations in the DNA that lead to changes in chromosome shape and function and ultimately changes in the genes that can be expressed as changes in the protein coded for by the gene or if a gene is lost the loss of a protein (►DNA damage responses).



Radiation Carcinogenesis. Figure 1 Schematic depiction of the interaction of radiation with DNA in cells. Reproduced with permission of Engstrom, Lipscomb and Lack LLC.

Clearly the cells that make up human bodies and the bodies of all other living organisms must be endowed with repair systems to counteract damage done both internally by the machinery of the cell and by external forces such as radiations or cells would very quickly die from the accumulating DNA damage produced by radiation exposure (►Repair of DNA). Cells would surely die much sooner than is the case. In fact best estimates suggest that of every 1,000 single strand breaks produced by gamma rays 99% are quickly and correctly repaired, however that does leave ten that are not repaired or not repaired correctly (►DNA damage-induced Apoptosis). Since there are trillions of cells in the body there are also trillions of single strand breaks that do not get repaired or do not get repaired correctly. Gamma rays also produce ►DNA double strand breaks. About 40 for every 1,000 single strand breaks. These double strand breaks are much more difficult to repair and some may also be repaired incorrectly. These incorrect repair events are the basis for mutation events in the DNA that lead to chromosome changes and or permanent genetic change in the cells affected when the cells divide and multiply. It is this damage (refered to as ►initiation) that is the first step in the process known as carcinogenesis if a cell was previously undamaged. If a cell already carried genetic damage, which many do, the radiation damage may supply the next step in the progression to a cancerous event. In both cases the direct effect of radiation exposure is causal in the eventual expression of a cell that can form a diagnosable cancer. As we will see later the consensus of radiobiological opinion at present is that carcinogenic events (or overt cancers) are induced in a linear fashion by radiation as dose is increased. That is as the dose increases so does the number of cancers in a proportional manner. A tenet of this type of response is that any dose can have an effect. There is no threshold. From what has been said above where it is clear that almost all cells in the body are hit multiple times even by a small whole body dose it is not difficult to deduce that the chance (or risk) of one of those many billions of cells eventually forming a cancer is real and thus no dose can be assumed to be free of the potential for causing a cancerous event (►Radiological Response Criteria).

What do we know about carcinogenic events induced by radiations? In brief, the present state of scientific knowledge gained from cell, animal and human studies regarding exposure to radiation at low doses or protracted low doses tells us that carcinogenic events are:

1. Linear with dose, meaning there is a straight-line proportionality between exposure size and the cancer endpoint measured.

2. There is no threshold in radiation dose for carcinogenic events. This means there is no dose below which radiation has been shown to have no effect.
3. There is a latent period between exposure and the appearance of the carcinogenic event. For example, in the human body this means that exposure may be separated by many years from the onset to the diagnosis of cancer.
4. Radiation is capable of causing most types of cancer in most tissues of the body.
5. There is no biological signature or "finger print" to tell us radiation was the cause of a cancer.
6. There is a lifetime risk of developing a cancer associated with any exposure (the size of the risk may change with time however).
7. Effects of repeated (fractionated) or low dose rate continuous exposure are cumulative. However due to the efficient repair systems present in mammalian cells, the effect of each dose when many doses are given and of continuous low dose is reduced compared to the same dose given in one exposure at a high dose rate. In other words, protracted exposures produce fewer cancers.
8. Age at exposure is important. In most cases, if you are young when exposed to radiation, the risk of a cancer occurring is higher than if you are an adult when exposed.
9. Total body risk is not a good measure of overall risk, if exposure is for some reason concentrated in one or more organs but the whole body.

Clinical Issues

Medicine owes a lot to the discovery of x-rays, which can be used to image the body. Since that discovery over 100 years ago, diagnostic radiology has become an integral part of modern medicine. There is a strong belief in the medical community as well as by the public that the small risk associated with medical exposures is far outweighed by the health benefits. Another use for radiations also started nearly 100 years ago. This is the use of much larger doses of directed radiation to treat (kill the cells of) cancers. Today, this use of radiation is called radiation oncology and has become a sophisticated part of the treatment of almost 70–80% of all human cancers (►Radiation Oncology). Patients are given doses of radiation of about 2 Gray each in fractions usually five times per week over several weeks. The radiation field is carefully planned to encompass the tumor but spare the surrounding healthy critical organs. There have been many technological advances in the delivery of radiation in radiation oncology, all of which are aimed at reducing side effects of treatment while enhancing the effect on the tumor. In other words, radiation oncologists and medical physicists have and still are striving to minimize risk to healthy surrounding critical organs in treated patients (►Ionizing Radiation Therapy, ►Radioimmunotherapy, ►Chemoradiotherapy) There is a cancer risk associated with these treatments but this risk is offset by the health benefits to the patients, many of whom might die or die sooner but for the radiotherapy treatments. As treatments for cancer have improved and many cancer patients now live for many years there are now some studies that show increases in secondary cancers due to the exposures the patients were given to treat their tumors.

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Radiation Dose

Definition

Is the mean amount of energy imparted to matter of certain mass. The special name for the absorbed dose is the Gray (Gy) after the British radiation physicist Louis Harold Gray. 1 Gy corresponds to energy absorption of 1 Joule/kg. In a routine multiple-slice CT scan dose ranges from 10 to 60 mGy (1 mGy = 10^{-3} Gy). The majority of radiation therapy patients receive 2 Gy once *per* day five days *per* week for 7 weeks for 70 Gy total dose.

►Radiation Oncology

Radiation-Induced Neoplastic Transformation

►Radiation Carcinogenesis

Radiation-Induced sarcomas

►Radiation-Induced Sarcomas After Radiotherapy

Radiation-Induced Sarcomas After Radiotherapy

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Synonyms

Radiation-induced sarcomas; RIS; Postirradiation sarcoma; Second primary cancers

Definition

Cahan's criteria for diagnosis of radiation-induced sarcomas (RIS): (i) history of radiation therapy (RT), (ii) asymptomatic latency period of several years, (iii) occurrence of sarcoma within a previously irradiated field, (iv) histological confirmation of sarcomatous nature of the postirradiation lesion.

Characteristics

►RIS are a rare but recognized complication of RT and are associated with poor prognosis. The first case of bone sarcoma, related to external irradiation was described in 1922 by Beck. Soft tissue sarcoma occurring in the treatment volume after breast cancer was described first by Warren and Sommer in 1936. In 1948, Cahan et al. defined the criteria for the diagnosis of RIS. Independent of RT, chronic ►lymphoedema is an important predisposing factor for angiosarcoma. Stewart and Treves in 1948 were the first to describe six cases of ►angiosarcoma occurring in lymphadematous extremities after radical mastectomy. This is a Stewart–Treves sarcoma and can appear in nonirradiated patients (not to confuse with RIS).

Carcinogenesis

Carcinogenesis is multifactorial. Drugs, genetics, chemotherapy agents, irradiation, and others have been shown to be risk factors.

There are still a lot of uncertainties about the biological effects of radiation. The most popular theory for the mechanism of radiation carcinogenesis is that human cancers frequently result from different irreversible DNA lesions, which are induced by the irradiation. Carcinogenesis is a long-term multistage process comprising gene mutations and gene deletions. Human cancers result from activation of dominant acting ►oncogenes or the deletion of a ►suppressor gene. Oncogenes are activated by a ►point mutation, ►chromosomal translocation, or by gene ►amplification.

Dose–Effect Relation

The biological effects that occur provide information about the dose–effect relation at high dose. The somatic effects that have been ascribed to radiation include induction of various types of cancers such as lymphomas, leukemia, solid tumors, and skin cancer. The incidence of the cancer is the function of the dose. The most commonly reported dose for RIS is 60–80 Gy with a minimal dose of 10 Gy in conventional fractionation. In some series there was a tendency that for higher total dose there was a longer latent period. On the other hand, they showed that the rate of tumor induction was induced as the dose increased. Some authors studied the risk of subsequent bone cancer among 9,170 patients, treated for cancer in childhood; have found a sharp dose–response gradient reaching a 40-fold risk after doses of more than 60 Gy to the bone. Other authors have found that the risk increased linearly with the integral dose to 150–200 J and stabilized at higher energies. In their experience the odds ratio (OR) was 2.4 (95%, CI: 1.4–4.2) for an energy of 50 J, approximately corresponding to the radiation of the breast after breast-conserving surgery.

Megavoltage or Orthovoltage Radiotherapy

Several authors have reported different characteristics of RIS according to RT modalities (megavoltage or orthovoltage). Some authors reported that megavoltage RT is associated with shorter latency period. Others indicated that bone tumors were more frequent in the megavoltage group. In the IGR series of 11 patients, 9 developed RIS after megavoltage RT and 2 after total body irradiation delivered using an orthovoltage machine. All the patients received megavoltage RT (^{60}Co or combination of ^{60}Co and electrons).

Latent Period

For RIS there are reports of a latent period from 2 years to 50 years.

Histological Findings

Robinson et al (1988) described in their series more high grade RIS. Some authors described a predominance of ►osteosarcoma and ►fibrosarcoma in RIS of the chest wall. Stout and Lattes described the light microscopic findings of malignant fibrous histiocytoma (MFH) in 1967. In the series of Laskin et al. with 53 RIS, 36 were MFH. Other papers report that postradiation

►angiosarcomas form a significant subgroup of skin and soft tissue sarcoma, especially in the series after conserving breast treatment.

Age and RIS

There is no correlation between age and onset of RIS.

Incidence

The risk to develop RIS is extremely low for the individual patient but increases as the number of long-term survivors' increase. Numerous studies reported cases of RIS after RT for different cancers. The largest series is published after breast cancer treatment (Table 1).

Radiation-Induced Sarcomas After Radiotherapy. Table 1 Incidence of RIS after breast cancer treatment

Reference	Type of retrospective study	Total patients (*N*)	Total number of sarcomas (*N*)	RIS (*N*)	Incidence [95% CI] after RT	Incidence without RT	Risk
Yap et al. (2002)	Registry data (SEER)	274,572	263	87	0.9 ± 0.2/1,000 cases at 15 years	0.1 ± 0.1/ 1,000 cases at 15 years	0.32–15 years cumulative risk[a]
Huang and Mackillop (2001)	Cohort (SEER)	194,798	135	54 soft tissue sarcomas	SIR 26.2 [16.5–41.4] (angiosarcoma) 2.5 [1.8–3.5] (other sarcomas)	2.1 1.3	RR: 15.9 RR: 2.2
Karlsson et al. (1998)	Swedish Cancer Register (case control study)	122,991	40 angiosarcomas; 76 other sarcomas including STS		SIR: 1.9 [1.5–2.2]		AR: 1.3/10^4 person-years
Cozen et al. (1999)	Los Angeles registry (case control study)		20 upper extremity; 48 chest				OR 11.6 [1.7–5.8]
Marchal et al. (1999)	French cancer centers (without Curie)	18,115	9 angiosarcomas		5 cases/10,000 (=prevalence for healthy breasts)		
Kirova et al. (2005)	Single center (Institut Curie)	16,705 (13,472 with RT; 3,233 without RT)	36 (35; 1)	27 RIS (+2 STS)	Cumulative RIS incidence: 0.07% (±0.02) at 5 years; 0.27% (±0.05) at 10 years; and 0.48% (±0.11) at 15 years. SIR 10.2 [9.03–11.59]	SIR 1.3 [0.3–3.6]	RR: 8 [4.98–12.7]
Blanchard et al. (2002)	Single center (?) (Mayo clinic)	?		34			
Taghian et al. (1991)	Single center (Institut Gustave RoussylGR)	6,919		9 in field; 2 STS	Cumulative index: 0.2% at 10 years; 0.43% at 20 years; 0.78% at 30 years		
Zucali et al. (1994)	Single center (Milan)	3,295		3 soft tissue sarcomas			
Doherty et al. (1993)	Single center (Ontario)	3,199		4			
Pierce et al. (1992)	Single center (Boston)	1,624		3			

[a]50% in field.

SIR, standardized incidence ratio; AR, absolute risk; RR, relative risk; SML, secondary malignancies; STS, Stewart–Treves Syndrome.

Genetic Findings

Molecular biology analyses of 27 RIS by genomic hybridization suggest that ►chromosomal gains at 7q or 8q are associated with a poor prognosis or large tumors.

Treatment

RIS are associated with poor prognosis because most of them are diagnosed in advanced stage and frequently they are located in areas where radical surgery cannot be performed. The conventional treatment for RIS is surgery, and Robinson et al. postulated: "…The radical surgical approach is the only chance of cure." The results of chemotherapy treatment tend to be poor. The treatment of most cases is late and ineffective.

Conclusion

RT can induce malignancy after a latent period of several years. The risk is not high for the individual patient but becomes higher as the number of long-term survivors increases. RIS are associated with poor overall prognosis. The treatment of most cases is late and ineffective; therefore careful and longer follow-up is needed for early detection. The future development of treatment modalities will be possible with advances of genetic changes.

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Radiation Oncology

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Synonyms

Radiation therapy; Radiotherapy

Definition

Radiation oncology is the use of ►ionizing radiation to treat cancer and some non-malignant conditions.

Characteristics

The goal of radiation therapy is to eradicate cancer cells without damaging surrounding normal cells. In theory, this can be accomplished by delivering a sufficiently high ►radiation dose to a tumor volume, while simultaneously sparing normal tissue. These two conflicting demands have challenged radiation oncologists, physicists and radiobiologists for more than 100 years.

Ionizing radiation is a potent agent for controlling tumor growth of locally advanced cancer, because ionizing radiation kills cells by releasing of large amounts of energy within the genetic material (DNA) in individual cells. Irradiation induces both single- and double-stranded breaks in the DNA molecule. The induction of ►DNA double strand breaks is generally considered a lethal event. In a mammalian cell 2 *Gy* of X- or γ-rays there are about 1,000 single- and about 40 double strand breaks of DNA molecules immediately following irradiation but only 20 breaks remain a few hours later. This demonstrates the important role of ►DNA repair mechanisms: only a small fraction of the initial ►DNA damage is irreparable or incorrectly repaired. However, un-repaired DNA damage is biologically important because it may result in loss of genetic information after the cell divides and, subsequently, lead to various biological outcomes including cell death.

Cell death following ionizing radiation is defined as the irreversible loss of reproductive capacity. This definition is relevant to radiotherapy because a tumor is locally controlled when all its cells have lost the ability of indefinite proliferation and hence of local invasion and distant ►metastasis. In the same way, the damage to normal tissue is caused by loss of cellular viability. The manifestation of cell death is more evident in tissues with rapidly dividing cells (e.g. tumors, crypt cells in the intestinal jejunum) of than those of slowly dividing cells (e.g. skin stem cells).

The second component of radiation damage relevant to radiation therapy is sublethal damage, a type of damage occurring after an initial dose that is not lethal by itself and can be repaired unless additional sublethal damage is added by a second dose of radiation. Post-radiation repair of sublethal damage results in a decrease of cell killing and is observed if a given radiation dose protracted, that is split into smaller amounts (called fractions) separated by a time interval, or delivered at reduced dose rate. Sublethal damage repair is one of the four known biological responses of cancer cells following irradiation and is often referred to as the first "R" of radiation therapy. The remaining three "Rs" are ►redistribution (throughout the ►cell cycle),

repopulation (or the rate of proliferation after irradiation) and ►reoxygenation (the diffusion of oxygen into a tumor as it shrinks in size). All four of these Rs have different mechanisms and occur in different rates in normal versus cancer cells. Fractionated radiation in modern radiation therapy, that is delivering a limited dose each day over several weeks rather than delivering a large amount of radiation in a single dose in a short time, takes advantage of these four Rs. Until recently, the choice of 70 Gy given in 2 Gy fractions has been considered "the gold standard" for achieving a good tumor control (that is destroying the cancerous tissue) and avoiding short and long-term side effects. Short-term effects occur within a few weeks into a fractionated therapy, whereas long-term effects become evident after the conclusion of therapy. More recently, observed differences in the Rs among tumors as well as early responding and late responding tissues led to more tumor- and tissue-specific fractionation schemes. Listed below are examples of dose fractionation schedules currently used in external beam radiation therapy. Each fractionation schedule is described in terms of the number of fractions, dose per fraction, total dose and the treatment duration (weeks, days).

- The gold standard: 35 fractions × 2 Gy/day = 70 Gy in 7 weeks
- The silver standard: 30 fractions × 2 Gy/day = 60 Gy in 6 weeks
- Hyperfractionation: 70 fractions × 1.15 Gy × 2/day = 80.5 Gy in 7 weeks
- Accelerated treatment: 45 fractions × 1.5 Gy/day = 72 Gy in 5 weeks
- Continuous hyperfractionated accelerated radiation therapy (CHART): 36 fractions × 1.5 Gy × 2/day = 54 Gy in 12 days
- Hypofractionation: 16 fractions × 2.65 Gy/fraction in 22 days

Over the past decade new imaging technologies, notably ►computed tomography (CT) have greatly improved clinicians' ability to identify tumors in three dimensions (3D). Three-dimensional (3D) CT scans replaced flat two-dimensional X-ray films for selecting the treatment targets, and are now considered the standard imagining modality for radiotherapy. This 3D modality approach represents a major advance because it takes into account cross-sectional anatomy and complex tissue contours. However, a number of recent studies demonstrated that there is a considerable inter-observer variation with CT, and that ►magnetic resonance imaging (MRI) is more precise in defining the anatomy of tissues.

CT scans are used for accurate dose calculations for complex and irregular 3D shapes of selected treatment targets. Present technologies allow simulation for radiation therapy by integrating data from the CT scanner with the 3D treatment computer planning systems. The current capability of planning and calculating doses is accurate to within millimeters. Simulation is a critical part of a treatment planning session prior to starting radiation therapy. Other procedures occurring during a planning session are measurements of the area to be treated as well placing markings on the patient's skin to help with positioning during the actual treatments. Usually during a planning session, it is necessary to make appropriate immobilization devices to prevent body movements. Examples of immobilization devices are a porous mesh mask to immobilize the head when treating patients with cancers of the head and neck, and plastic casts to immobilize the patient's pelvic area. Immobilization devices have special brackets riveted to the sides; these brackets lock into the treatment couch, allowing little body movement. Effective strategies that address involuntary target movements such as caused by respiration or digestion remain to be developed.

There are several new techniques for the accurate delivery of radiotherapy based on 3D planning. In conventional two dimensional radiation therapy, treatment planning was based on flat X-ray films and bony anatomy, and the optimization of dose distribution was achieved by manual lead blocking, that is the lead shielding to block out the radiation to certain vital structures within the treatment field. Beam setups were usually quite simple and consisted of a single intensity beam coming in from one to four directions. Treatment plans frequently included opposed lateral fields. The new "standard" in radiation therapy is 3D conformal therapy (3D-CRT) that links treatment planning and software with the actual treatment delivery devices. As a result, multiple radiation beams can be shaped exactly (conform) to the contour of the area. A medical ►linear accelerator (LINAC), the device most often used in radiation oncology to deliver treatment doses, produces high-energy X-rays in a typical clinical setting. Beams can be directed from any number of angles (typically nine). Where the beams intersect is the region of the highest intensity and is centered on the treatment target. To improve dose distributions further, the beam cross-sectional shapes are conformed to the 3D outline of the target area by passing the beam through a multi-leaf collimator (MLC). The MLC is a computer-controlled device inside the linear accelerator head with as many as 120 tungsten leaves that slide in and out of the radiation beam during treatment, thus varying the dose at a given point. This results in radiation dose distribution that can be sculpted to cover the target in three dimensions.

A more advanced form of three-dimensional conformal approach is intensity modulated radiation therapy (IMRT). IMRT uses 3D imaging and radiation delivery as described above for 3D-CRT, but in addition allows for varying intensity within beam cross-sections to produce dose distributions that conform more

accurately than those possible with standard 3D conformal therapy. The cross sectional distribution of intensity is achieved by dividing the beam shaped by MLC into "beamlets" or 1-cm^2 areas of isointensity (also called "pixels" or "blocks"). The cross-sectional intensity distributions are calculated for each direction and then programmed directly into the treatment equipment. IMRT is gaining widespread application as a curative treatment for tumors that might be have been considered untreatable in the past due to close proximity of vital organs and structures. For example, in the case of head and neck tumors, IMRT allows sparing of the spinal cord, optic nerves and salivary glands. In the case of ►prostate cancer exposure to the nearby bladder or rectum is greatly minimized. Other tumor sites suitable for IMRT include the ►brain cancer, ►breast cancer, ►liver cancer, ►pancreas cancer, ►uterus cancer and ►lung cancer.

The present limitations of conformal approaches (3D-CRT and IMRT) to external beam radiation therapy include the logistic difficulties of immobilizing a patient for the duration of an IMRT treatment, lasting 15–40 min per daily session as well as tumor motions caused by respiration or digestion (1 mm in a target movement translates into 10% deviation in dosimetry). Tumors tend to shrink as treatment progresses, but 3D dosimetry planning in real time and based on the actual changing tumor anatomy remains to be developed. In addition, the ►radiation biology of conformal therapies is poorly understood. There are considerable uncertainties concerning the impact of prolonged fraction delivery time in IMRT on cell survival, compared to conventional beam therapy that requires only 2–5 min to deliver the same fractional dose. Second, compared to conventional therapy, 3D-CRT and IMRT involve the use of more fields and thus larger volumes of normal tissue are exposed to low-levels of radiation (up to 20% of treatment fraction dose). Third, multi-leaf collimators allow more ►leakage radiation than conventional collimators. The above considerations add up to a prediction that IMRT may more than double the risk of ►secondary cancers, especially in long-term cancer survivors or young patients. IMRT is currently not recommended for treatment of childhood cancers.

Newer 3D CRT and IMRT approaches are based on charged particle radiation. This type of therapy differs from photon therapy in that it involves the use high-energy protons or accelerated light ions (mainly carbon ions) to treat localized tumors. Although particle radiation therapy was developed in the 1950s, the real advantages of particle therapy could not be realized until imaging systems (CT, MRI) for tumor localization were invented. The intrinsic advantage of proton (and light ion) radiation over photons is the well-defined range. Consequently, the maximum dose is delivered at the target depth (►Bragg (curve) peak), an intermediate dose is delivered at the entrance surface, and a minimum dose is delivered beyond the target. In contrast, photons lose energy along the track and deposit more dose at the surface (skin) than they do at depth. Because of their depth-dose distribution profile, proton (and light ion) therapy is intrinsically conformal. Dose conformity using protons (or light ions) is superior to that presently achievable with 3D planned photons or IMRT and can be realized with a substantially fewer beams. Although protons and light ions have similarly favorable physical dose distribution profiles compared to photons, light ions such as carbon ions kill cells more efficiently than do protons and photons. In addition, the lethal effects of light ions are considerably less influenced by the tumor biology (that is, the four Rs) and its microenvironment (►hypoxia). Radiotherapy with proton and carbon beams is becoming a clinically available modality at several radiation therapy centers around the world, especially in Europe and Japan. There are currently two operating ►proton beam therapy centers and three more are under development in the USA. Carbon ion therapy is now available in Japan and Germany. Clinical indications for proton or ion therapy are similar than those for photon 3D CRT or IMRT. That is, particle therapy is most frequently used to treat tumors that are near critical tissues, such as optic nerve, functional centers of the brain, spinal cord, rectum, intestine, heart, and urinary tract. Proton radiation therapy is especially important for pediatric cases, which require radiation delivery through the growing bones or bony structures. Although childhood cancers such as ►retinoblastoma can be effectively treated with photons, photon therapy may result in permanent malformations of the child's face or body from irradiating through the skull or bones.

Another technique for 3D conformal radiation therapy is internal radiotherapy. ►Brachytherapy (also called interstitial brachytherapy) and intracavitary therapy are types of internal therapy. Internal therapy is a form of radiation therapy where radioactive sources ("seeds") are implanted directly into a specific part of the body. Radiation from an implant is delivered directly to small treatment volumes. Normal tissues are largely spared, because radiation does not pass through the body from the outside before reaching a tumor. Internal therapy can be the only form of treatment or it can be combined with external beam radiation, surgery, ►hyperthermia, or ►chemotherapy. Brachytherapy refers to the technique of implanting radioactive sources into cancer tissue. Intracavitary therapy refers to the placement of implants into a body cavity in close proximity to the tumor. Radiation sources used in internal therapy could be high dose rate (HDR) (12 Gy/h) temporary sources or low dose rate (LDR) (<2 Gy/h; typically 0.4–0.6 Gy/h)

permanent sources. The present ►isotopes of choice for LDR or HDR brachytherapy are iodine-125 (►half-life 60 days) and palladium-103 (half-life 17 days) or iridium-192 (half-life 74 days), respectively. Based on the four Rs of radiation therapy, the radiation delivered at a higher dose rate is expected to produce better clinical outcomes than the radiation delivered at a lower dose rate, especially for fast-growing tumors. Other benefits of HDR versus LDR brachytherapy include a short treatment time (<1 h), greater ease and comfort for the patient, a more precise dose delivery, and less radiation exposure to medical personnel. However, because HDR therapy uses sources of very high ►radioactivity (10 ►Curie), it requires specialized dosimetry and source deployment and retrieval approaches as well as the ability to produce isotopes for HDR brachytherapy applications. The development of technology for the remote and rapid insertion and retrieval of implants, called computer controlled after loading, and industrial production of isotopes such as iridium-192, has led to widespread adoption of HDR techniques. HDR brachytherapy is presently used for cancers of the breast, uterus, cervix and lung. LDR brachytherapy with iodine-125 or palladium-103 is frequently used for prostate cancer, a slowly proliferating tumor. Iodine-125 or palladium-103 produce lower ►energy of photons (20–30 keV) than iridium-192 (380 keV) and so the dose from iodine-125 or palladium-103 falls more rapidly with distance outside the treatment volume than the dose from iridium-192. Consequently, the iodine-125 or palladium-103 seeds efficiently spare the adjacent rectum and bladder and can be left permanently in the prostate. At the same radiation dose, low energy photons kill about 50% more cells than do high-energy photons (6–10 MeV) in used in external beam therapy. These advantages are offset by cell proliferation and sublethal damage repair during LDR brachytherapy in fast growing tumors, but present less of a problem in slowly proliferating tumors, such as carcinoma of the prostate.

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Radiation Pneumonitis

Definition

Arises within a radiation field following thoracic irradiation and sometimes extends outside the field. It is an interstitial pulmonary ►inflammation following radiation injury to endothelial and epithelial cells, as well as being an immunological reaction.

►Proton Therapy
►Proton Radiation Therapy
►Charged Particle Therapy
►Proton Beam Therapy

Radiation-Resistant DNA Synthesis

Definition

RDS; When mammalian cells are exposed to ►ionizing radiation, DNA synthesis is inhibited. Failure to inhibit DNA synthesis in response to ionizing radiation, which occurs in ►ataxia telangiectasia (AT) cells, is termed radiation-resistant DNA synthesis.

►S-Phase Damage-Sensing Checkpoints

Radiation Sensitivity

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Synonyms

Radiosensitivity

Definition

Refers to an increased susceptibility to cellular damage following exposure to radiation.

Characteristics

Radiation sensitivity can be measured by testing cultured cells for various endpoints, including cytoxicity (survival assays), clastogenic responses (chromosome damage),

mutagenicity and DNA repair. Sensitivity to ionizing and ►ultraviolet radiation is associated with rare cancer-prone syndromes. Ionizing radiation sensitivity is a characteristic phenotype of patients with the distinct but closely related rare cancer-prone syndromes ►Ataxia telangiectasia (AT) and ►Nijmegen Breakage syndrome (NBS). Homozygote carriers of mutated *ATM* and *NBN* alleles showing acute radiation reactions when treated with conventional radiotherapeutic doses for cancer and cell cultures extreme sensitivity in *in vitro* assays. The ATM protein kinase is a key component in the cellular response to DNA double-strand breaks. It becomes rapidly activated in response to such damage and initiates signaling cascades in which various substrates, each of which are key factors in damage response pathways and some of which are themselves protein kinases, are phosphorylated, thus ensuring a coordinated and rapid response to this toxic DNA lesion. The NBN protein is a component of the MRE11-NBN-Rad50 complex which acts as a sensor in mammalian cells of DNA double stand breaks in addition to its DNA processing activities. Indeed ATM activation depends on the presence of this complex and the interaction of the damage sensors and activators amplifies the damage response. Ultraviolet radiation sensitivity is the hallmark of patients with ►nucleotide excision repair (NER) diseases, the major pathway for UV-DNA photoproducts, with patients with ►xeroderma pigmentosum (XP) in addition having an increased risk of developing cancer. The total number of genes directly involved in NER is estimated to be around 40 with a dozen found to be deregulated in NER-related human diseases.

Clinical Radiation Sensitivity or "Adverse Radiation Effects"

Clinical radiation sensitivity or "Adverse radiation effects" can generally be defined as undesirable clinical and physiological responses secondary to radiation treatment. Adverse radiation responses can be observed during and within several weeks after radiotherapy – acute side effects, or can develop at later times – late effects. The significant improvements in cancer treatment over the last decades have resulted in longer survival but this also means that a proportion of cancer patients are living with such late, adverse effects and growing numbers of radiation-related second cancers have been observed and in particular following treatment for childhood cancer. A number of radiation toxicity scoring systems have been developed for reporting the adverse reactions as the Radiation Therapy Oncology Group (RTOG) or SOMA-LENT scales which assign quantitative descriptive grades from 0 to 5 for the appearance of specific side effects. Acute skin side effects include erythema, oedema and dry or moist desquamation, whilst the late effects include telangiectasia and fibrosis and develop over time. Pulmonary injury manifested as sub-acute pneumonitis and late fibrosis is also found in some patients following radiotherapy.

It is known that a variety of patient, tumor, cellular and molecular factors contribute to the severity of normal tissue reactions exhibited after radiotherapy. Patient characteristics include age, nutritional status, medications, coexisting morbidities (e.g. diabetes, recent surgery) and tumor-related factors such as size. Variation in treatment-related parameters, including treated volume, field size, total dose, dose fraction and use of concomitant adjuvant chemotherapy may also contribute to response heterogeneity. In addition individual genetic variation may also influence the development of adverse radiation responses. Indeed the known ionizing radiation radiosensitivity syndromes arising from single-gene defects, such as AT and NBS, involve mutations in components of DNA double-strand break signaling and repair pathways and common sequence variants in genes implicated in the cellular responses to radiation, which may alter protein function and thus an individual's capacity to repair damaged DNA modifying the response of the normal tissue, are good candidates to influence the response to therapeutic radiation.

One major focus of research in ►radiobiology is the development of assays designed to predict individual radiosensitivity of normal and tumor tissue prior to radiotherapy. Studies correlating *in vitro* radiosensitivity using a variety of biological endpoints and clinical sensitivity in breast cancer patients have lead to contradictory results or have not been found to predict well the side effects among patients receiving radiotherapy. The *in vitro* assays that have been used to date include testing the ability of a "surrogate target" such as skin fibroblasts or lymphocytes from patients to survive and replicate after exposure to ionizing radiation, cytogenetic test systems counting the frequency of specific ►chromosomal aberrations or micronuclei (►micronucleus allay) in irradiated cells, the ability of the cells to repair radiation-induced damage or other radiation-induced endpoints such as ►apoptosis induction or ►TGF-β plasma levels. However to date no assays have proved to be consistently sensitive and accurate for the prediction of side effects amongst patients receiving radiation, and none have been incorporated into routine clinical practice.

New insights into the underlying molecular mechanisms of therapeutic radiation sensitivity are coming from radiogenomics studies and the characterization of molecular profiles that predict tissue and tumor responses to radiotherapy and chemotherapy. There have been a limited number of studies screening for abnormalities in expression of candidate proteins. For instance the level of expression of proteins involved in DNA double strand break repair in fibroblast cultures or

lymphoblastoid cell lines established from patients who showed different degrees of clinical radiosensitivity has been examined in relation to clinical radiation sensitivity. No association between abnormalities in expression levels and radiosensitivity were reported. However, western blotting was used to assess protein expression levels, and this technique cannot generally accurately detect lower than two-fold differences and may not be a sufficiently sensitive technique to detect subtle differences between the different cell lines that might still be functionally relevant. It is also feasible that certain changes may not alter protein levels but may modify the function of the protein. There are several other possible reasons why no correlation between protein expression or activity and radiation sensitivity have been reported to date. One possibility is that the activity of the proteins *in vitro* may not reproduce their *in vivo* activity. The tissue environment is clearly very different and cannot easily be simulated *in vitro*. This has also been invoked as a possible reason why some of the *in vitro* test systems investigated to date, as potential predictors of radiation sensitivity show no clear correlation between *in vitro* and *in vivo* cellular responses. A second possibility is that correlations may be masked by the simultaneous effects of other variables; that is to say the variations noted are real and relevant but are not the only determinants of the reaction to IR which is controlled by a complicated network of inter-linked pathways. One recent study in this area has shown promising results in which the high constitutive expression of DNA repair-related genes measured in peripheral blood lymphocytes seemed to protect from acute adverse reactions.

A number of large scale studies have been initiated to establish banks of biological samples linked to detailed radiation treatment outcome databases for patients treated with head-and-neck, breast, lung, rectal and prostate cancer suitable for genetic studies. Various approaches have been used to try and identify alterations in single genes that may modulate clinical radiosensitivity. This has involved identifying mutations and ►single nucleotide polymorphisms (SNPs) in candidate radiosensitivity genes. The presence of homozygote mutations in genes associated with altered responses to radiation and extreme radiation sensitivity are relatively rare events – for instance the incidence of AT is estimated to between 1 per 40,000 and 1 per 300,000. However whilst the frequency of *ATM* heterozygotes in the general population is of the order of 0.36–1% there are few studies that have screened the whole *ATM* gene, which covers over 160 kb of genomic DNA and contains 66 exons, and it remains to be established whether carriers of *ATM* mutations are over-represented in cohorts of radiation sensitive patients. Several groups have adopted a different approach and investigated whether specific SNPs or haplotypes in genes implicated in the cellular response to radiation are associated with an increased risk of an adverse response to radiotherapy. SNPs and haplotypes in the *XRCC1*, *ATM*, *hHR21, TGFβ1, XRCC3 and SOD2* genes have been found to be associated with an increased risk of developing an adverse normal tissue reaction to radiotherapy in breast cancer patients, and it is not unreasonable to assume that SNPs or specific haplotypes in other genes involved in DNA damage detection and repair, pro-fibrotic and inflammatory cytokines, endogenous anti-oxidant enzymes and general metabolism and homeostasis will also be identified that are associated with such adverse reactions. To date no genetic factors, that specifically influence the temporal occurrence of the adverse reactions, have been identified but it should be noted that many of the studies are relatively small in size and thus lacking in predictive power.

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Radiation Therapy

Definition

Synonym radiotherapy, is a kind of therapy that uses radiation energy that comes from radioactive materials to fight neoplastic disease. It is typically performed with an external beam origin.

►Radiation Oncology
►Radiological Response Criteria
►Ionizing Radiation Therapy

Radical

Definition
Radicals (such as OH) are highly unstable ions that attack and damage biomolecules such as DNA.

►DNA Damage
►Oxidative DNA Damage
►Photodamage

Radical Mastectomy

Definition
Removal of the breast tissue including removal of the underlying muscles.

►Oncoplastic Surgery

Radio-Immunodetection

Definition
Radio immunolocalization allows visualization of tumors following injection of an antibody to a tumor marker that is labeled with a radioisotope.

Radio-Isotope Therapy

►Ionizing Radiation Therapy

Radio-Labeled Tracer

Definition
A substance containing a radioactive isotope (radioisotope) that can be visualized by a gamma camera.

►Sentinel Lymph Nodes

Radioactive Seed Therapy

►Brachytherapy

Radioactivity

Definition
Is the process of releasing nuclear particles from unstable atomic nuclei, also called the radioactive decay or disintegration. All elements with atomic number greater than 82 (lead) are unstable and decay with a characteristic time, called the ►half-life, a time for half the atoms to decay. The number of disintegrations *per* unit time is called the activity. The special name for activity is the Curie (►Ci) after the Polish physicist and chemist Maria Sklodowska-Curie.1 Ci corresponds 3.7×10^{10} decays/s.

►Radiation Oncology

Radiobiology

Definition
Synonym ►Radiation Biology; The study of ionizing radiation and its relation to living cells.

Radiochemotherapy

►Chemoradiotherapy

Radioimmunoconjugates

Definition
Are antibodies linked to radioisotopes.

►Monoclonal Antibody Therapy

Radioimmunotherapy

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Synonyms

Systemic antibody-directed radionuclide therapy

Definition

Radioimmunotherapy (RIT) is the most common form of systemic targeted radionuclide therapy (STaRT): monoclonal antibodies directed against tumor antigens (►monoclonal antibody therapy) deliver therapeutic radioisotopes to sites of (disseminated) disease and induce radiolysis in both the targeted cells and neighboring cells.

Characteristics

Principle of RIT

RIT represents an evolving therapeutic approach that combines the tumor targeting attributes of monoclonal antibodies with therapeutic radioisotopes to be delivered to sites of (disseminated) disease. Radiolysis induces cellular damage in both the targeted cells and neighboring cells (the latter also called "crossfire-" or "►bystander effect"), potentially beneficial for treating bulky, poorly vascularized tumors and those with heterogeneous antigen expression. Efficacy of RIT depends largely on tumor characteristics, monoclonal antibody and tumor antigen, and radionuclide.

Tumor Characteristics

RIT may be applied in ►hematological malignancies as well as in solid tumors. The most promising response rates and response durations have been observed in advanced stage malignant lymphoma (►malignant lymphoma, hallmarks and concepts), while antitumor effects in other malignancies have been less impressive. The difference in efficacy is largely due to differences in radiosensitivity (►radiation sensitivity); however, other factors may contribute, like poor vascularization, increased interstitial pressure, and limited diffusion capacity in solid tumors impeding radiolabeled macromolecule uptake. As all these factors amplify with tumor growth, it may be expected that RIT may have its greatest impact when treating microscopic tumor burdens, i.e., in the adjuvant (►adjuvant therapy) or consolidation setting.

Monoclonal Antibody and Tumor Antigen

The ideal target antigen has not been identified yet. It should be specific for the unique tumor type and be expressed stably in high level on all malignant cells allowing formation of a firm antigen–antibody complex. The most extensively studied and, to date, the only approved radioimmunoconjugates, Yttrium-90 (^{90}Y)-ibritumomab tiuxetan (Zevalin®) and Iodine-131 (^{131}I)-tositumomab (Bexxar®) are both directed against the CD 20 antigen. This B-cell specific epitope (►B cell tumors) represents an excellent example of a target antigen, as it is expressed on virtually all B-cell lymphomas, does not internalize or shed from the surface in response to antibody binding and is absent on hematopoietic stem cells. While it can be assumed that radiolysis exhibits the predominant antitumor effect, antibody binding itself may also contribute to efficacy in terms of antibody dependent cellular cytotoxicity (ADCC), ►complement-dependent cytotoxicity (CDC) as well as antibody induced intracellular signaling, as has been shown for malignant lymphoma.

A variety of growth factors and their receptors (e.g., epidermal growth factor (►EGF) and its receptor (►EGFR)) are upregulated in many solid tumors, providing a rationale for therapeutic use of radiolabeled monoclonal antibodies directed against these molecules. Other examples include anti-►CEA antibodies that have been used for RIT in colorectal cancer (►colon cancer), and a recombinant chimeric tumor necrosis treatment (TNT) antibody that was tested in ►non-small cell lung cancer (NSCLC). ►Tenascin is an ►extracellular matrix glycoprotein that is expressed in high grade gliomas, and intracerebral delivery of radioimmunoconjugates directed against tenascin into a surgical resection cavity has been evaluated.

Radionuclides

Today, the most commonly used radionuclides are iodine-131 (^{131}I) and yttrium-90 (^{90}Y). ^{131}I and ^{90}Y differ markedly in their physical properties including type of energy emissions, half-life, path length, intracellular stability, and the organs targeted by the free radionuclide (Table 1). Potential advantages of ^{131}I for RIT include availability, stable chemistry, longer half-life, and an emission spectrum (beta and gamma radiation) that allows for dosimetric studies and therapy with the same immunoconjugate. By contrast, the pure beta emitter ^{90}Y has a longer path length and superior intracellular stability compared with ^{131}I. As ^{90}Y emits no gamma photon,

Radioimmunotherapy. Table 1 Physical properties of commonly used radionuclides

Radionuclide	Yttrium-90	Iodine-131
Gamma emission	No	Yes
Beta emission energy (mev)	2.3	0.6
Half-life (days)	2.7	8.0
Path length (mm)		
Maximum	11.0	2.9
Mean	2.5	0.4

dosimetry studies can only be performed using a surrogate radionuclide such as indium-111 (^{111}In). Both ^{131}I- and ^{90}Y-labeled anti-CD20 antibodies have demonstrated efficacy in treating relapsed/refractory ►non-Hodgkin lymphoma and published results suggest that the two reagents have somewhat similar response rates and response durations, although no comparative clinical trial has ever been performed.

Preclinical and clinical research evaluates radionuclides decaying by the emission of alpha-particles. They offer the possibility of matching the cell-specific reactivity of monoclonal antibodies with radiation with a range of only a few cell diameters. Furthermore, alpha-particles have important biological advantages including a higher biological effectiveness, which is almost independent of oxygen concentration and cell-cycle phase. The three most promising alpha-particle emitting radionuclides encompass Bismut-213 (^{213}Bi, additional beta emission), Actinium-225 (^{225}Ac), and Astatine-211 (^{211}At, additional beta emission).

RIT in Malignant Lymphoma

Although it has been over 20 years since the original publication of ►radioimmunoconjugates being used in the treatment of malignant lymphoma (►malignant lymphoma, hallmarks and concepts), clinical experience is now growing rapidly with encouraging efficacy and favorable safety profiles, exemplified by the following two landmark trials.

Kaminski et al. reported on the experience with the Bexxar® regimen (^{131}I– labeled IgG_2 κ anti-CD20 antibody tositumomab) in 76 previously untreated follicular lymphoma patients. Despite the favorable prognostic profile of this patient population, the high response rates (overall response rate (ORR) 95%, complete response (CR) rate 75%) and the nature of the durable remissions with 59% of the patients remaining in ongoing complete remission with a minimum follow-up of greater than 4 years from treatment are remarkable.

A randomized phase III trial by Witzig et al. including 143 patients with relapsed or refractory low-grade, follicular or transformed NHL compared efficacy of the Zevalin® regimen (^{90}Y-labeled IgG_1 κ anti-CD20 antibody ibritumomab) with ►rituximab (375 mg/m^2 once weekly for 4 weeks). Response rates were significantly higher in the ^{90}Y-ibritumomab tiuxetan arm with an ORR of 80% versus 56% and a CR rate of 30% versus 16%. However, the overall time to progression (TTP) was not different in both treatment groups, but patients treated with ^{90}Y ibritumomab tiuxetan showed a trend toward longer median duration of response (14.2 vs. 12.1 months) and more often achieved responses lasting longer than 6 months (64% vs. 47%).

Both the ^{90}Y-ibritumomab tiuxetan as well as ^{131}I-tositumomab induces considerable ►myelosuppression with a delayed onset 6–10 weeks posttreatment. In the case of adequate bone marrow reserve and normal platelet counts (>150,000/mm^3) the recommended (^{90}Y-ibritumomab tiuxetan dose is 0.4 mCi/kg or 14.8 MBq/kg (to a maximum of 32 mCi or 1,184 MBq), for ^{131}I-tositumomab the desired total body dose is 75 cGy. Furthermore, bone marrow infiltration by lymphoma cells should not exceed 25% because of the observed pronounced and prolonged myelosuppression in these patients. Principally, if murine antibodies are administered, a human anti-mouse antibody (HAMA) response may be seen, but is diminished in patients with prior chemotherapy. By interfering with biodistribution and targeting presence of high titers of HAMAs seem to influence outcome of radioimmunotherapy and also precludes re-administration of the foreign protein.

There are highly encouraging results from several important clinical trials evaluating RIT in other subtypes of lymphoma, earlier in the treatment algorithm, as consolidation therapy or as part of myeloablative regimens (►myeloablative mega therapy) followed by autologous or allogeneic bone marrow/stem cell support.

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Radiological Response Criteria

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Definition

Radiological response criteria attempt to provide objective measures of tumor response to therapy. They are widely used as ►surrogate endpoints in clinical trials of cytotoxic chemotherapy and novel therapeutic agents.

Characteristics

Historical Developments

The need for standardized and objective outcome criteria to measure response to anti-cancer treatment has been recognized for over fifty years. In 1960, the Eastern Co-operative Oncology Group proposed that objective measurement of tumor size should be used as an endpoint in the evaluation of ►cytotoxic chemotherapy, since killing tumor cells had been shown to reduce lesion dimensions.

These principles were first codified in a World Health Organisation (WHO) document, published in 1979. In particular four categories of response to ►chemoradiotherapy were defined – complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). While many early assessments of tumor size relied on clinical measurement and chest radiography, 2-dimensional evaluation of tumor size using ►computed tomography (CT) became widely adopted as surrogate endpoints in clinical research. However, calculation methods and reporting of the WHO criteria exhibited considerable variation between centers. This discrepancy eventually precipitated formation of new agreed standard criteria for measuring tumor response, the response evaluation criteria in solid tumors (RECIST), published in 2000. The criteria were proposed to unify existing radiological evaluation of tumor response, while at the same time acknowledging the need for subsequent development.

The RECIST criteria are founded on the same principles as those proposed by WHO. Like its predecessor, RECIST criteria were developed to evaluate response to chemotherapy agents that typically produced substantial changes in tumor size. One key difference between the RECIST and WHO criteria was the precise definition of some of the response categories; most crucially, the definition of what constitutes PD was changed, as well as adopting measurements based on one dimension. Emphasis was placed on cross-sectional imaging with CT and ►magnetic resonance imaging (MRI), although use of chest radiographs was permitted, along with some clinical and ultrasonographic measurements. The magnitude of change required to demonstrate partial response or disease ►progression was largely derived from retrospective statistical evaluation of measurements (not image data) from pharmaceutical trials. The quality control for these measurements was not known. Details of the WHO and RECIST response criteria are presented in Table 1 and are discussed thoroughly in the original report of the RECIST criteria by Therasse and colleagues.

Strengths of Radiological Response Criteria

RECIST and the WHO definitions provide an objective standardized method for reporting response to therapy. No extra equipment or analysis steps are required; hence radiological response criteria have been widely and successfully applied over the last few decades to both the trial setting and to routine clinical practice. Since many traditional chemotherapy agents produce a change in tumor size, the criteria have been adopted as an acceptable surrogate endpoint for many clinical trials.

Limitations of RECIST

Criticisms of current radiological response criteria have been widely cited in recent years. It is worth noting that many current limitations were identified by the authors of the RECIST criteria in their initial publication in 2000.

RECIST was not designed to evaluate small lesions. Any radiological definition of complete response is limited by the spatial resolution of the scanning protocol. For instance, a 1 cm breast tumor can contain

Radiological Response Criteria. Table 1 Details of the WHO and RECIST response criteria

	WHO	RECIST
Method		
Lesions	Two dimensional	One dimensional; target lesions identified
Measurement	Area calculated by product of longest diameter *x* longest perpendicular diameter	Longest diameter recorded
Response criteria		
Complete response (CR)	Disappearance of all known disease. Confirmed ≥4 weeks later	Disappearance of all known disease. Confirmed ≥4 weeks later
Partial response (PR)	≥50% decrease in size	≥30% decrease in the sum of longest diameters from baseline
Stable disease (SD)	Patient does not meet criteria for PR or PD	Patient does not meet criteria for PR or PD
Progressive disease (PD)	≥25% increase in size of at least one measurable lesion; appearance of new lesions	≥20% increase in the sum of longest diameters from baseline; appearance of new lesions

approximately 10^9 cells. Since reliable detection of sub-centimeter tumor deposits is difficult with most imaging modalities, reporting 'complete response' can, in reality, miss one or more lesions containing tens of millions of tumor cells. This is evidenced in high dose therapy for breast cancer, where usually the response rate approaches 100%, but where many patients do not achieve long term cure. While advances in CT and MRI hardware can enable increasing small lesion detection, spatial resolution will always limit the specificity of defining complete response by imaging criteria.

Radiological response criteria are difficult to apply to some tumor types. Poorly delineated lesions such as ►mesothelioma and diffuse peritoneal infiltration, typically seen in ►ovarian cancer, are difficult to accurately define in either one or two dimensions. Furthermore, bone lesions, which constitute a common and sometimes solitary site of ►metastasis, are deemed non-measurable by RECIST, as are meningeal deposits, ►pleural effusions and cystic or necrotic lesions.

Furthermore, achievement of objective response in early phase clinical trials (as assessed by radiological response criteria) does not guarantee success at phase III evaluation. The average response rate, assessed by radiological response, is over 40% in phase II studies but is around one third in phase III clinical trials. In addition, many promising early phase studies failed to have their results replicated at phase III testing – this has, in part, contributed new agent failures. Thus, while the RECIST criteria were explicitly designed as a biomarker of response in early phase trials, the response criteria are not as sensitive or specific as investigators would like.

Finally, one major limitation of current radiological response criteria is their dependency on change in tumor size as an indicator of response. while this is a reasonable basis to measure response to cytotoxic chemotherapy, it is not appropriate for many new anti-cancer agents, many of which work by cytostatic mechanisms that halt tumor growth rather than reduce lesion size. Several studies of ►antiangiogenic agents have demonstrated evidence of disease stabilization without evidence of tumor regression in both pre-clinical and human studies. For example, studies of ►sorafenib, ►bevacizumab and other ►protein-tyrosine kinase inhibitors of ►signal transduction have shown significantly improved median progression free survival and overall survival in phase II and III trials. Indeed, the improvement in clinical outcome was sufficient to lead to approval of sorafenib and bevacizumab by the Food and Drug Administration (FDA), despite modest partial response rates in many of these studies of around 5%. These drugs would have been considered ineffective if assessed by the RECIST criteria alone.

Future Developments and Possible Solutions

There is widespread consensus that while traditional radiological response criteria such as the WHO and RECIST approaches provide a useful objective codification of response to therapy, significant limitations exist. This has prompted discussion of how improvements may be made in lesion evaluation, particularly in the context of clinical trials of novel anti-cancer agents.

1. Are size-dependent radiological response criteria valid – should we use them? This question has been prompted by the issues discussed above concerning the limitations of categorizing tumor response based solely on changes in the size of lesions. Despite these concerns, radiological response evaluation based on measures of tumor size is often an appropriate for agents that act through cytotoxic mechanisms in a dose-dependent fashion. In contrast, trials of novel agents that, at best, halt tumor growth may require assessment by both size change and alternative imaging and non-imaging techniques.
2. If they are invalid, then what measure(s) should we use? Here, there are several options. First, there is an argument for keeping the current approach but applying less stringent criteria for response. For example, a decrease of tumor size of just 10% has been used in conjunction with the RECIST criteria to evaluate response in a group of patients with gastrointestinal stromal tumors (GIST), treated with the tyrosine kinase inhibitor imatinib. Here, no statistical difference was found between those defined by RECIST as responders versus non-responders; however, there was a highly significant ($p = 0.0002$) longer time to progression (TTP) in those who exhibited ≥10% reduction in tumor size. Second, new endpoints may be incorporated, in addition to change in size that predict outcome better than RECIST. Decrease in tumor density (measured by Hounsfield units) and was more sensitive than RECIST at detecting response measured by ^{18}FDG-►positron emission tomography and predicted prognosis in GIST treated with imatinib. Thus, in the context of treating GIST with tyrosine kinase inhibitors, measuring a change in size of 10% or decrease in tumor density of 15% (the proposed Choi criteria) may be more appropriate than RECIST. Similar disease specific approaches, each with differences in image modality, parameter and requisite magnitude of change for significance, may end up being formulated and tested alongside traditional response evaluation. Examples already in use include the Southwest Oncology Group lung cancer response criteria. Finally, as imaging capability develops, the measurement techniques employed to assess changes in size require modification. Three-dimensional image acquisition now enables accurate assessment

of tumor volumes. For example, in a study of patients with recurrent anaplastic astrocytoma or ►glioblastoma multiforme, volumetric measurements were predictive of overall survival. No similar correlation was found with WHO or RECIST measurements. However, three-dimensional analyses are at present not widely performed in routine clinical practice (as noted in the original report of the RECIST criteria).

3. What alternatives could be used instead?

If changes in tumor size, based on radiological response criteria, are considered inadequate measures of response, then are there any alternative viable methods that could be readily implemented? One popular option is to implement TTP or progression free survival (PFS) as surrogate endpoints in clinical trials. TTP can directly demonstrate patient benefit, but is vulnerable to significant error in calculation of the time of progression, often caused by infrequent sampling. Addition of a control group may minimize bias of early re-assessment, but necessitates modification of trial design. Also, at present, radiological evaluation is required to define progression in many cases.

Numerous imaging, histology and bio-fluid measurements are being evaluated as potential ►clinical cancer biomarkers of tumor response. Examples include simple measures of circulating tumor antigens and pro-angiogenic growth factors. In addition, more complex biomarkers – such as those produced in ►dynamic contrast-enhanced magnetic resonance imaging and positron emission tomography – are particularly amenable to non-invasive, serial tumor evaluation. Only a small minority of biomarkers have been reasonably well validated, for example, use of ►prostate-specific antigen (PSA) in phase III trials. While biomarkers of tumor function are of particular interest in assessing novel anti-cancer therapies, most biomarkers are not sufficiently validated for use in large scale phase III trials. Therefore, at present, few biomarkers have been approved as surrogate endpoints to evaluate new agents by the FDA.

Finally, symptom control (quality of life) measures offer an alternative endpoint for evaluating treatment success. However, these are labor intensive and expensive. Also, they rely on application of questionnaire-based assessment, which has been regarded as 'less objective' than radiological and other traditional response criteria.

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Radiomimetic

Definition

Imitating biological effects of radiation; A radiomimetic drug is one that imitates the effects of radiation as in the case of chemicals such as ►nitrogen mustards, which are used in cancer ►chemotherapy.

Radionuclide

Definition

A nuclide that has artificial or natural origin and that exhibits ►radioactivity.

►Radioimmunotherapy
►Radioimmunoconjugates
►Radiochemotherapy

Radioresistant DNA Synthesis

Definition

RDS; The inability to down-regulate DNA synthesis following treatment with ►ionizing radiation, result from ►checkpoint defect at the ►GI/S transition, in S and at the ►G2/M transition. This way, damaged DNA may undergo ►replication an be passed on in mutated version to next cell formations.

►BACH1 Helicase
►Radiation Sensitivity

Radiosensitivity

Definition

The relative susceptibility of cells, tissues, organs, organisms, or other substances to the injurious action of ►ionizing radiation. In general, it has been found that cell radiosensitivity is directly proportional to the rate of cell division and inversely proportional to the degree of cell differentiation.

►Radiation Sensitivity
►Radiosensitization
►Vascular Disrupting Agents

Radiosensitization

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Definition

Radiosensitization is a physical, chemical, or pharmacological intervention that increases the lethal effects of radiation when administered in conjunction with it. In experimental studies or in clinical practice, the aim of a radiosensitization approach (or the use of ►radiosensitizers) is to make tumor cells more sensitive to ►radiation therapy. A clinical benefit can be expected only if a differential effect can be demonstrated between tumors and normal tissues.

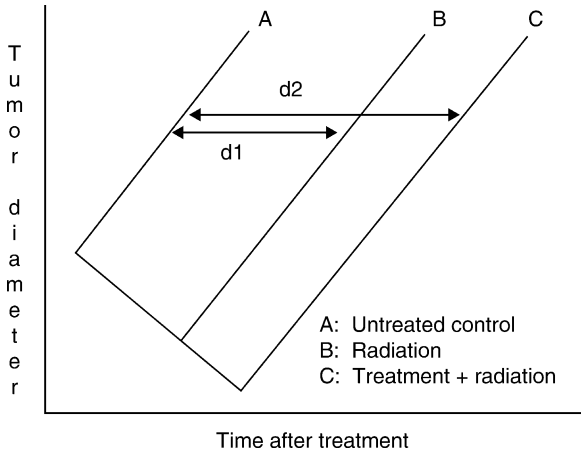
Characteristics

To understand the possible mechanisms to enhance the ►radiation sensitivity, it should first be reminded that irradiation of a biological material initiates a cascade of events that will ultimately lead to cell death, with consequent tumor shrinkage or impairment of tissue functional integrity. The first events between the irradiation beam and the irradiated material consist in transfers of energy through excitations and ionizations. It is accepted that ►DNA damage is the major event leading to clonogenic cell death. Depending on the linear energy transfer (►LET) of the radiation beam, the DNA damage could be the result of direct interaction of radiation with DNA (for high LET), or the result from free radicals created from physical interactions with intracellular water molecules (for low LET). For this latter mechanism, it is well established that the presence of molecular oxygen dramatically increases the extent of DNA damage.

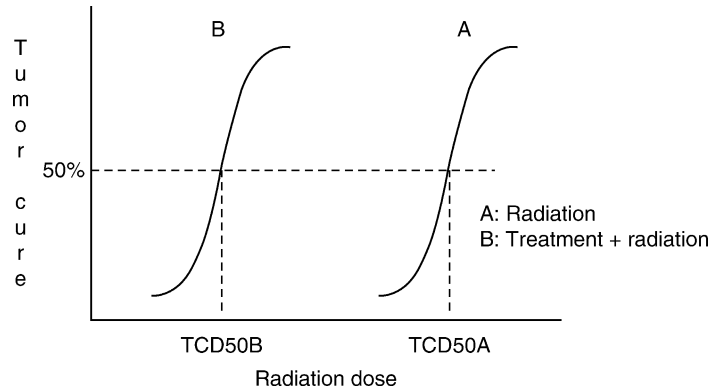
Evaluation of a Radiosensitization Effect

The effects of radiation can be increased or decreased by various interventions or chemical substances called radiosensitizers or radioprotectors. Radiosensitization is the most extensively studied approach because of its possible application in ►ionizing radiation therapy. Radiosensitization should be first considered in terms of efficacy on tumor cells, tumor growth, and effect on other tissues.

The assessment of the radiosensitization of tumor cells includes in vitro and in vivo assays. The most relevant in vitro assay consists in the clonogenic assay by measuring the number of colonies formed after irradiation of tumor cells using several irradiation doses. The efficacy as a ►radiosensitizer is established by comparing cell survival curves after irradiation alone or in the presence of the compound tested. Two tests are classically carried out in vivo to assess the value of a radiosensitizing treatment. The tumor regrowth delay assay (Fig. 1) consists in irradiating population of tumors grown in animals at suboptimal dose. The dose is chosen to avoid tumor cure but to still achieve a measurable regrowth delay. The radiosensitizing property is estimated by comparing the regrowth delay (time in days for tumors to reach a given diameter) with or without agent, compared to the time to reach the same diameter in untreated control animals. The tumor cure assay (Fig. 2) is more clinically relevant, but also is more logistically difficult to carry out. Tumors grown in



Radiosensitization. Figure 1 Tumor regrowth delay assay. The radiosensitizing property is estimated by comparing the regrowth delay (time in days for tumors to reach a given diameter) with or without agent, compared to the time to reach the same diameter in untreated control animals.



Radiosensitization. Figure 2 Tumor cure assay. The number of mice locally cured is calculated for each radiation dose group. Dose–response curves are generated, and the radiation dose yielding local tumor control in 50% of animals (TCD50) are calculated. The radiosensitizing effect is quantitatively expressed by the dose modification factor (DMF), which is calculated by the ratio of the TCD50 for radiation alone (TCD50A) over that for the combined agent and radiation treatment (TCD50B).

animals are randomly assigned to a treatment group, i.e., local irradiation alone or radiosensitizing agent combined with local irradiation. The number of mice locally cured is calculated for each radiation dose group. Dose–response curves are generated, and the radiation dose yielding local tumor control in 50% of animals (TCD50) are calculated. The radiosensitizing effect is quantitatively expressed by the dose modification factor (DMF), which is calculated by the ratio of the TCD50 for radiation alone over that for the combined agent and radiation treatment.

A potential clinical advantage of the combination of a sensitizing agent and radiation can only be foreseen if the potentiation of radiation response is higher in tumors than in normal tissues in the radiation field, indicating that the therapeutic ratio of the combined treatment is above unity. Therefore, the toxicity for normal tissues should be checked. Classically, several normal tissue models are used to determine whether an agent is responsible for toxicity on early responding tissues (e.g., intestinal regenerated crypt assay, acute skin reaction assay) or late responding tissues (e.g., leg contracture assay, rat spinal cord radiation tolerance assay).

Mechanisms of Radiosensitization

There are several ways to enhance the radiation sensitivity. The examples presented below are illustrative rather than exhaustive, and describe some strategies to improve the therapeutic efficiency of radiation therapy. Overall, two types of strategies are described. The first ones include approaches to render DNA as sensitive as possible through direct or indirect interactions. Counteracting tumor ►hypoxia, increasing the initial radiation damages, and redistributing the cell cycles are parts of this first strategy. The second set of approaches tries to modulate the biological response of irradiated cells by inhibiting cellular repair mechanisms, by overcoming the accelerated repopulation, or by targeting some molecular events associated with the radiation response. More precisely, the mechanisms of radiosensitization may include:

1. *Counteracting Tumor Hypoxia*. Many mechanisms are involved in oxygen radiosensitization including the yield and types of free radicals produced after irradiation by low LET radiations as well as by fixation of DNA radical damages. The survival curves for oxygenated and hypoxic cells display an oxygen enhancement ratio for most cells and tissues of 2.5–3. Tumor ►hypoxia is considered as one of the major source of radioresistance. Cells in tumors can become hypoxic as a result of an abnormal tumor blood supply and of an elevated metabolism and rapid tumor cell growth. A first approach to overcome this source of oxygen-dependent radioresistance is the elimination of hypoxic cells. That can be achieved by the use of bioreducible cytotoxins such as mitomycin-C or tirapazamine. These drugs are enzymatically activated in cells to form cytotoxins, which are selectively toxic to hypoxic cells. The second way is to increase the cell death by a transient reoxygenation of the tumor at the time of irradiation. This can be achieved by increasing the oxygen delivery to the tumors, or by decreasing the oxygen consumption by the tumor cells. There are several ways to increase the oxygen delivery: breathing gas enriched in oxygen (hyperbaric oxygen, carbogen), modulating the vascular tone of vessels feeding the tumors and opening the tumor vascular bed, decreasing the tumor interstitial fluid pressure, increasing the oxygen transport of blood by perfluorochemicals, or increasing the oxygen release

by allosteric modifiers of hemoglobin structure. To decrease the oxygen consumption, some drugs have been found to inhibit the mitochondrial respiration of tumor cells: for example, metaiodobenzylguanidine, insulin, antiinflammatory agents. When the therapeutic benefit is mediated by an oxygen effect, a radiosensitizing property on normal tissues is unlikely: the radiosensitizing effect will more than likely be higher for hypoxic tumor regions than for well-oxygenated tissues.

2. *Increase in Initial Radiation Damage*. Some agents, such as halogenated pyrimidines (5-bromodeoxyuridine or 5-iododeoxyuridine) make DNA more susceptible to radiation damage. These compounds are incorporated into DNA in place of the DNA precursor thymidine, and "weaken" DNA molecule, rendering it more sensitive to radiation injury.
3. *Cell Cycle Redistribution*. The cell sensitivity to irradiation depends on the position of cells in the division cycle. Cells in the G_2 and M phases of the cell cycle are more radiosensitive than cells in the S phase. Cell cycles redistribution strategies can be exploited to radiosensitize tumors. As illustrative examples, taxanes (paclitaxel, ►docetaxel) arrest cells in radiosensitive phases of the cell cycle. Nucleoside analogues, such as ►fludarabine or ►gemcitabine, specifically eliminate radioresistant S-phase cells.
4. *Inhibition of Cellular Repair*. Mammalian cells have the capacity to repair sublethal and potentially lethal lesions induced by irradiation. Any drug that interferes with cellular repair mechanisms can enhance cell response to irradiation. Many chemotherapeutic agents such as ►cisplatin, doxorubicin, and nucleoside analogues interact with cellular repair mechanisms.
5. *Overcoming Accelerated Repopulation*. Tissues with rapid cell turnover respond to radiation injury by compensatory proliferation of surviving cells. This accelerated repopulation by tumor cell clonogens can be counteracted by shortening the duration of radiotherapy treatment (i.e., hyperfractionated accelerated radiation therapy). It is also the rationale for the use of chemotherapeutic drugs combined with concurrent radiotherapy.
6. *Modulating Other Biological Determinants of the Radiation Response*. Biologic Response Modifiers (BRMs) represent a diverse group of biologic agents and molecular targets that modulate cellular responses to ionizing radiation in ways that may be exploited to sensitize tumors. BRMs may influence a range of biochemical processes including cytokine networks, DNA repair, cell cycle regulation, stress response, ►apoptosis, and metabolism. As examples, some BRMs targets include cytokines, angiogenic pathways, and cyclooxygenase (►COX) expression.
   a. *Cytokines*. Cytokines are soluble proteins that act as universal modulators of intercellular communication. Several cytokines act on tumor cells with direct cytotoxic or cytostatic effect. Others act on tumor growth by indirect mechanisms such as ►angiogenesis, metastatic potential, or antitumor immune response. One possible target is the ►Epidermal Growth Factor (EGF) and its receptor (►EGFR). In neoplastic cells, EGFR overexpression has been associated with uncontrolled proliferation and increased radioresistance. Increased radiosensitivity has been obtained by blocking the EGFR from binding to its ligands and by inhibiting tyrosine kinase phosphorylation so as to block the signal propagation. Other radiosensitization approaches by manipulation of the cytokines include the targeted delivery of TNF-α, the inhibition of ►RAS with ►farnesyltransferase inhibitors, and the inhibition of ►RAF-1.
   b. *Angiogenic Pathways*. Radiation induces VEGF expression by tumors and stimulates angiogenesis. Inhibition of ►VEGF represents a way to increase the response of tumors to irradiation. Another mechanism by which ►antiangiogenic drugs may sensitize tumors is the transient increase in tumor perfusion at the early phase of antiangiogenic treatments as a consequence of the remodeling of the tumor vascular network (process called "normalization" of the tumor vasculature network).
   c. *COX*. ►COX-2 is an inducible enzyme involved in carcinogenesis, tumor growth, metastatic spread, and resistance to cytotoxic therapy. Several COX-2 inhibitors were identified as radiosensitizers.

Radiosensitization is often obtained by more than one mechanism, a fact that significantly contributes to the increase in the tumor response to radiation therapy. As examples, the C225 anti-EGFR antibody acts by inhibiting the radiation damage repairs, by arresting cells in the radiosensitive G2/M phase of the cell cycle, and by inhibiting the tumor angiogenesis. COX-2 inhibitors radiosensitize tumors by multiple mechanisms involving inhibition of repair of sublethal radiation damage, inhibition of tumor angiogenesis, and ►reoxygenation of the tumors by inhibition of the respiratory chain.

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Radiosensitizer

Definition

A drug that makes tumor cells more sensitive to ►radiation therapy.

►Radiosensitization

Radiotherapy

Definition

The treatment of a cancer by exposure to radiation from a radioactive substance.

►Hepatic Epithelioid Hemangioendothelioma
►Ionizing Radiation Therapy
►Photodynamic Therapy
►Radiation Oncology

Radon

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Synonyms

Radon daughter products; Radon decay products; Radon progeny

Definition

Radon (Rn) is a chemically inert gas with atomic number 86 in the periodic table. The most stable ►isotope is radon-222 from the decay series of uranium-238. The half-live of radon-222 is 3.8 days. The short-lived daughter products of the noble gas radon are itself radioactive isotopes of the elements polonium, lead, bismuth, and thallium. Rn is an alpha-particle emitter. The decay products generate alpha particles, beta particles, and gamma-rays.

Characteristics

Occurrence

Radon is ubiquitous but of relative low abundance, because of the distribution of its uranium precursor in the earth's crust. The concentration of radon and its decay products depends on the geological situation. In certain areas the radon exposition is naturally high, especially in mountainous regions with some granitic soils or shales. As a decay product of uranium, the gaseous radon then seeps out of the rocks into the environment. The concentration in the ambient air is usually low, due to natural ventilation and dilution with air. Higher concentration can occur after avalanches, because freshly broken rocks emit more radon gas. Radon and radon decay products occur naturally also in ground water and during mining operations. Some hot springs contain high concentrations of radon.

Exposure Situations

If radon and radon progeny cannot disperse they accumulate over the time. The highest concentrations were found in underground ore mines. Radon concentration in homes can be higher than outdoors depending on the underground material, the building material (e.g. clay bricks), and other factors. Radon seeps out of the rocks in the basement of the buildings and can add up to high concentrations. In underground mines the exposure can be reduced by wet drilling, ventilation, and other dust-controlling measures. Ventilation and structural measures of the building decrease exposure indoors.

Population Under Risk

Due to the ubiquitous nature of radon the whole world population is under risk that varies by geological and other conditions and can be a matter of public health in certain regions. For the general population, the radon risk is higher indoors than outdoors depending on the geological situation, state of the building, climate (e.g., wind speed), ventilation habits, and other factors. In regions with low radon concentration but high population density, more persons are under risk than in regions with high radon concentration but low population density. Occupation-related exposure to radon and its decay products is highest in uranium mining and while working with uranium. Exposure can also occur in mining of other ores, spas with hot springs, and in water works.

Health Hazards

Radon is classified as human carcinogen by the International Agency for Research on Cancer (IARC). Radon gas is mostly incorporated due to inhalation. Ingestion by water uptake and incorporation through the skin are minor routes of exposure. Radon, in contrast to its short-lived decay products, is exhaled from the lungs. The radon decay products (as radioactive metal ions) attach to very small (0.1–0.3 μm diameter) particles in the ambient air like quartz dust. The particles can be deposited in the lung, where the cells will be irradiated with alpha particles. Finally, radon decay products can cause ►lung cancer. The strongest evidence came from epidemiologic studies of underground miners. Cohort studies in miners (uranium and other igneous rocks) were conducted in China, Czech Republic, United States of America, Canada, Sweden, Australia, France, and Germany. Lubin et al. analyzed 11 of these cohort studies. The ►excess relative risk (ERR) of radon-induced lung cancer ranges from 0.16 to 5.06 for 100 ►working level months (WLM) in these studies and the overall ERR was 0.005 for 1 WLM with a 95% ►confidence interval (CI) of 0.002–0.010. The risk increased with cumulative exposure and decreased with attained age and time since last exposure. Other known lung carcinogens like crystalline silica, arsenic, diesel exhaust, and tobacco smoke may modify the lung cancer risk and have to be considered as potential confounders or competing risk factors in the analyses. ERR of never-smokers is higher than among smokers although the higher ►relative risk (RR) of lung cancer of smokers compared with nonsmokers remains unchanged by radon exposure. A comprehensive overview on this topic is found in the Committee on Biological Effects of Ionizing Radiation (BEIR) VI report. The BEIR VI committee preferred a submultiplicative (more than additive) relation between radon and tobacco smoke.

Another concern is the lung cancer risk for residential radon exposure. A survey of radon concentrations in U.S. homes revealed a mean of about 50 ►Becquerel (Bq)/m^3. To assess the lung cancer risk of residential (indoor) radon, several case-control studies and ecological studies were conducted. In a pooled analysis of 13 European case-control studies with 7,148 lung cancer cases and 14,208 controls, Darby et al. estimated a smoking-adjusted lung cancer ERR of 0.08 per 100 Bq/m^3 (95% CI 0.03 – 0.16). The *p*-value of 0.0007 for this estimate gave strong evidence for a causal relationship between lung cancer and residential radon. The mean time-weighted average of residential exposure to radon was about 100 Bq/m^3 geographically varying between 50 and 500 Bq/m^3. This analysis of European studies confirmed estimates from well-conducted studies in North America and China and previously published meta-analyses.

Darby et al. as well as the BEIR VI committee concluded that the data from residential and occupational exposure to radon support a linear non-threshold hypothesis, i.e., the dose–response relationship between radon and lung cancer risk is linear with no threshold in dose. Radon levels are usually 10–100 times higher in ore mining than in residential homes. When restricting the analysis of miner studies to workers with radon exposures in the range of residential radon levels, the results are coherent with residential studies.

While there is sufficient evidence for radon-induced lung cancer, the evidence for other malignancies like ►chronic lymphocytic leukemia, myeloid leukemia, and ►Hodgkin lymphoma is limited or even not present. The reported results came mostly from ecological analyses, an epidemiological design that is not able to assess evidence in etiology research. So far, there is insufficient evidence for a carcinogenic effect of radon on other organs than the lung.

Mechanisms

The results from ►epidemiologic studies provided evidence for a causal relationship between radon and lung cancer. A complete biological model that explains the mode of action of radon is yet missing although various mechanisms have been proposed. The BEIR VI report reviewed the present knowledge of radon ►carcinogenesis obtained in experimental and human studies. ►Genomic instability, ►apoptosis as well as radiation-induced perturbation of cellular proliferation may play a major role. ►Stem cells or progenitor cells are possible targets of carcinogens. There are different possibilities for stem cells to protect their DNA from radiation-induced mutations. Cells can have strong ►DNA repair mechanisms, whereas ►apoptosis is inhibited, or stem cells can be protected by apoptotic suicide and subsequent replacement by adjacent stem cells, whereas DNA repair is down-regulated. The latter mechanism is associated with a high sensitivity to ionizing radiation. In case of a compromised apoptosis, a lack of DNA repair would allow an accumulation of carcinogenic mutations. The assumed linear non-threshold model is supported by some experimental findings as well as a bystander mutagenic effect induced by a single alpha particle. In some studies ►small-cell lung cancer was the predominant histological type of lung cancer under radon exposure. In a recent study among 3,414 lung cancer cases in German ►uranium miners, small-cell lung cancer was closer associated with high radon exposure than ►non-small cell carcinoma of the lung. It is known that small-cell lung carcinoma responds better to ►radiotherapy than non small-cell carcinomas of the lung. In cell lines from small-cell carcinoma of the lung, the DNA repair enzyme O^6-methylguanine-DNA methyltransferase was found inactivated by ►methylation. However, the hypothesis

that radon progeny induced small-cell lung carcinoma has to be confirmed yet.

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Radon Daughter Products

►Radon

Radon Decay Products

►Radon

Radon Progeny

►Radon

Rae-1

Definition

Retinoic Acid Early 1, a family of murine NKG2D ligands homologous to RAET/ULBP

►NKG2D Receptor

RAEB

Definition

Refractory anemia with excess blasts.

►ETV6

RAET

Definition

Retinoic acid early transcripts, synonym ULBP, a family of human NKG2D ligands

►NKG2D Receptor

Raf

Definition

A serine/threonine kinase, activated downstream of Ras, which in turn activates the MEK/MAPK pathway.

►Raf Kinase

Raf-1

►Raf Kinase

Raf Kinase

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Synonyms

C-Raf; Raf-1; cRaf; EC 2.7.11.1

Definition

Raf-1 (synonym C-Raf; RAF) encodes for a serine/threonine protein kinase that is activated by ►RAS and

in turn phosphorylates and activates ►Map/Erk Kinase (MEK) 1/2 which then phosphorylates ►Extracellular-regulated Kinase (ERK). This signaling pathway is a key mediator of cell proliferation, survival, differentiation and migration.

Characteristics

First discovered as mutant retroviral transforming agent, v-*raf*, Raf is a dual serine/threonine protein kinase. There are three forms of Raf, with Raf-1 being the most studied one. Other forms, A-Raf and ►B-Raf, are not as ubiquitously expressed. A-Raf is primarily in muscles and B-Raf is primarily in neurologic tissue. In contrast, Raf-1 is expressed in multiple tissues.

Raf-1 is 73 kDa and consists of 648 amino acids (aa). Four protein domains have been defined for Raf-1, including an N-terminal RBD (Ras-binding domain) (aa 56–131), a cysteine-finger region (aa 138–184) (CR1), a second cysteine-rich region (CR2) (aa 253–264) and a C-terminal Ser/Thr kinase catalytic domain (aa 354–611). CR2 negatively regulates the C-terminal Ser/Thr kinase domain.

Raf Signaling

Raf is positioned in a signaling cascade to transmit the signal of activated Ras to downstream mediators. Specifically, a number of growth factors such epidermal growth factor and transforming growth factor, activate transmembrane ►receptor tyrosine kinases. These in turn recruit and activate Ras, which then, in turn, binds to Raf and recruits it to the cell membrane. Activation of Raf-1 requires several steps including its phosphorylation by other kinases (e.g. ►Src) in combination with dephosphorylation of inhibitory sites by protein phosphatase 2A. Activated Raf phosphorylates and activates MEK which, in turn, phosphorylates and activates ERK. Once activated, ERK can enter the nucleus and phosphorylates a variety of transcription factors thus completing the signaling path from the extracellular membrane through the nuclear DNA. In addition to transcription factor activation, ERK can regulate cell cycle proteins.

Several endogenous inhibitors of Raf-1 exist. For example, 14-3-3, which binds phosphorylated phosphoserine sites of inactive Raf-1 and maintains Raf-1 in an inactive configuration. Dephosphorylation of these phosphoserine sites release 14-3-3 and its inhibitory effects. Another inhibitor of Raf-1 is Raf kinase inhibitory protein (RKIP). The role of RKIP in cell signaling was identified in a yeast two-hybrid assay for screening clones from a human T-cell library that bound to Raf-1 kinase binding domains. RKIP was shown to bind Raf-1, MEK-1 and weakly bind to ERK-2, interfering with MEK phosphorylation and activation by Raf-1. However, RKIP was not a substrate for Raf-1 or MEK. RKIP did not bind to Ras, nor possess kinase activity. RKIP sets the threshold for Raf-1 activation and subsequent activation of the MEK/ERK pathway. Raf-1 dissociates from its complex with MEK in the presence of RKIP. As a result, downstream ►mitogen-activated protein kinase (MAPK) signaling is interrupted and diminished.

Raf Function

The central position of Raf in the Ras-Raf-MEK-ERK pathway confers on it a multifunctional role. Genetic deletion of various Rafs provides clues to their function. For example, based on these studies it appears that Raf-1 has great importance during development and tissue production. Specifically, Raf-$1^{-/-}$ mice die in utero with many developmental defects. In contrast, other raf mutations have more specific defects. A-raf deficient mice survive birth with intestinal and neurological abnormalities, while mice with a targeted disruption of the B-raf gene die of vascular defects during mid-gestation.

In mature organisms, Raf-1 has been identified to have several specific activities. Raf-1 may promote anti-apoptotic activity. Interactions of Raf-1 and Bcl-2 have been observed that indicate Bcl-2 promotes localization of Raf-1 to mitochondria. Raf may also promotes ►angiogenesis through its ability to diminish endothelial cell ►apoptosis. Specifically, both activation of the ►vascular endothelial growth factor (VEGF) receptor and the basic ►fibroblast growth factor (bFGF) receptor by their respective ligands induces phosphorylation of Raf-1 which diminishes apoptosis. Evidence has also accumulated that Raf-1 alters the cells cytoskeletal architecture. Raf-1 promotes phosphorylation of vimentin, a critical component of the cytoskeleton. This results in altering the polymerization of the vimentin scaffold and thus ties Raf-1 into modulation of the cytoskeleton. This activity appears to occur independent of the MEK-ERK pathway.

Raf in Cancer

The Ras-Raf-Mek-Erk pathway has been identified as being upregulated in at least 30% of cancers. Upregulation of Raf-1 activation may occur due to constitutive activation of upstream factors such as overexpression or activating mutations of growth factor receptors or activating mutations of Ras. For example, the ►epidermal growth factor receptor (EGFR) is overexpressed in a large number of ►non-small lung cell carcinomas. The EGFR overexpression results in stimulation of the Ras-Raf pathway and, thus, is accompanied by Raf-1 overactivation that leads to unregulated cell proliferation and resistance to anti-cancer therapies. In addition to overactivation of Rafs due to upstream factors, Rafs can be mutated themselves, resulting in constitutive activation of Raf. In colon cancer, all Raf family members have been demonstrated to be mutated and associated with increased ►MAP kinase pathway activity. In another example, C-Raf has been shown to

be mutated in ►acute myeloid leukemia. The mutation was able to induce activation of ERK and transformation of cells.

Raf Pathway Inhibitors

The importance of the Raf pathway in the cancer pathophysiology has led to a variety of inhibitors targeted to Raf. Many inhibitors either prevent activation of the kinase domain or compete for co-factor, such as ATP, binding sites resulting in preventing kinase activation. L-779,450 competes with ATP for the Raf catalytic site. It has been demonstrated to prevent DNA synthesis and promote apoptosis in transformed cells. BAY 43-9006 (►Sorafenib) is a urea-based compound that has been shown to inhibit several Raf-family members and tumor growth. Sorafenib is not specific to Raf and in facts targets multiple kinase pathways; however, the inhibitory concentration needed for Raf is much lower than other kinases. A phase III trial was performed on patients with advanced renal cell carcinoma that failed first line therapy. Sorafenib prolonged progression-free survival compared to placebo.

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RAFB1

►B-Raf Signaling

Rafts

Definition

Microdomains in the cell surface membrane, enriched in cholesterol, gangliosides (especially ganglioside GM1), and specific proteins (caveolin-1, Fas, Lyn kinase, Cu/Zn superoxide dismutase, the DNA-dependent protein kinase, neuraminidase).

►Caveolins
►Sphingolipid Metabolism

RAGE

Definition

Is a cell surface receptor for AGEs that belongs to the ►immunoglobulin superfamily. RAGE is a signal-transducing receptor that mainly mediates the biological effects of AGEs *in vivo*.

►Minodronate

Ral

Definition

Small GTPase, member of the ►Ras family that is supposed to be involved in vesicular transport and in ►metastasis.

Ral Interacting Protein 76kDa

►Glutathione Conjugate Transporter RLIP76
►Ral

Raman Scattering

Definition

A laser based optical spectroscopy that generates a characteristic vibrational spectrum for materials. It has high sensitivity and is suitable for multiplexing.

►Nanotechnology

Ran

Definition

Small GTPase, member of a branch of the ►Ras gene super-family involved in nuclear cytoplasmic transport.

Randomization

Definition

Is the process of assigning patients to treatment groups by chance in clinical trials so that selection into a treatment regimen is not biased by either the investigator or the participant.

►Kaplan–Meier Survival Analysis

RANK–RANKL Signaling

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Synonyms

RANK – Receptor activator of NF-κB, TNFRSF11A, OFE, ODFR, TRANCE-R, ODAR, CD265; RANKL – Receptor activator of NF-κB ligand, TNFSF11, OPGL, ODF, TRANCE, CD254

Definition

RANKL is a member of the ►tumor necrosis factor (TNF) superfamily of cytokines that binds to its cognate receptor RANK. RANKL is a type II transmembrane glycoprotein of 317 amino acids which exists either in a membrane-bound form (40–45 kDa) or a soluble form (31 kDa) and is expressed with highest levels in skeletal and lymphoid tissues, and to lower extent in heart, skeletal muscle, lung, stomach, placenta, and thyroid gland. RANK encodes a type I transmembrane glycoprotein of 616 amino acids (80–96 kDa) and is expressed in skeletal muscle, thymus, liver, colon, small intestine, adrenal gland, osteoclast, mammary gland epithelial cells, prostate, and pancreas. Signaling through RANK provides the essential signal for osteoclast differentiation, activation, and survival. Moreover, genetically RANK(L) signals are also required for lymph node development and the development of a lactating mammary gland during pregnancy.

Characteristics

RANK(L) Function in Bone Remodeling

The most prominent function of RANKL–RANK interactions in vivo lies in the regulation of bone turnover via ►osteoclasts. For all its rigidity, bone is by no means an immutable tissue but is constantly being remodeled. One class of cells, called osteoclasts (OCs, related to macrophages), erode bone matrix, while ►osteoblasts (OBs) deposit new bone matrix, thereby assuring a continuous turnover and replacement of bone matrix.

RANKL was initially cloned as a factor that enhanced differentiation of bone marrow cells into osteoclasts in an in vitro osteoclast coculture system. From these studies, a role of RANKL in regulating osteoclast function and, indirectly, bone mass is inferred. The definite proof of the essential function of RANKL in osteoclastogenesis came by the generation of RANKL-deficient mice. Mice with a targeted deletion of RANKL (►knock-out mice) develop severe ►osteopetrosis and show a defect in tooth eruption due to the complete lack of osteoclasts. Subsequently, these findings were complemented by showing that the receptor for RANKL, RANK, is also essential for osteoclast differentiation and activation. RANK knockout mice are exact phenocopies of RANKL knockout mice, i.e. they are osteopetrotic, have a defect in tooth eruption, and lack osteoclasts.

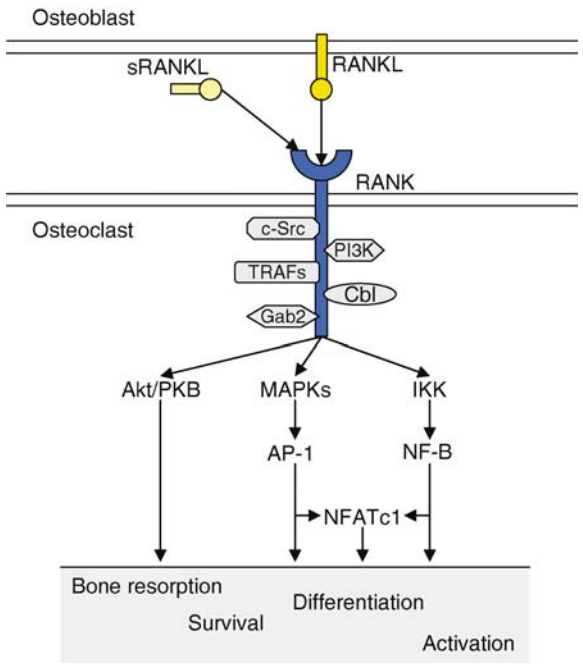
The positive effect of RANKL on osteoclastogenesis is counterbalanced by another member of the TNF superfamily, namely ►osteoprotegerin (OPG). OPG is secreted as a soluble protein and functions as a natural decoy receptor for RANKL by interfering with RANKL binding to RANK to maintain normal skeletal homeostasis. Consequently, the biological effects of OPG on osteoclastogenesis oppose those of RANKL: OPG overexpression in transgenic mice results in increased bone density and osteopetrosis, respectively, while genetic ablation of OPG in mice results in ►osteoporosis. Taken together, these findings unambiguously established the pivotal role of RANKL–RANK interactions in positively regulating osteoclastogenesis, counteracted and balanced by OPG, the natural decoy receptor for RANKL. By elucidating the regulatory interplay of the RANKL–RANK–OPG axis in osteoclastogenesis, a molecular framework emerged which could account for the bone loss observed in various bone diseases, such as osteoporosis, rheumatoid arthritis, or cancer-associated osteolytic lesions.

RANK(L) Signaling

Binding of RANKL to RANK results in the activation of signaling cascades that control lineage commitment

and activation of osteoclasts. In its 383-amino acid cytoplasmic domain, RANK contains binding sites for TNF receptor-associated factors (TRAFs; ►TRAF-1; ►TRAF-2), adaptor proteins that recruit and activate downstream signaling transducers. These TRAF binding domains were shown to be functionally important for ►NF-κB and c-Jun NH2-terminal kinase (JNK) activities in response to RANKL stimulation. RANKL also activates the anti-apoptotic serine/threonine kinase Akt/PKB through a signaling complex involving c-►Src and TRAF6. Moreover, RANK can recruit TRAF6, Cbl family scaffolding proteins, and the phospholipid kinase ►PI3-K in a ligand- and Src-dependent manner. Recently, another molecular adapter was found to be important for RANK signaling. Grb2 associated binder 2 (Gab2) associates with RANK and mediates RANK-induced NF-κB, Akt, and JNK activation. Genetic inactivation of Gab2 in mice results in osteopetrosis and decreased bone resorption due to defective osteoclast differentiation.

Stimulation of RANK eventually induces the expression of a variety of target genes via the transcription factors AP-1 and NF-κB. One important, convergent target of these two pathways is the transcription factor NFATc1, a member of the NFAT (nuclear factor of activated T cells) family of transcription factor genes. NFATc1 has been shown to be essential for RANKL-induced osteoclast differentiation both in vitro and in vivo, switching on and sustaining the transcriptional program for the terminal differentiation of osteoclasts (Fig. 1).



RANK–RANKL Signaling. Figure 1 RANKL–RANK signaling pathways. Membrane-bound RANKL or soluble RANKL (sRANKL) produced by alternative splicing or cleavage by MMPs or ADAMs binds and activates the receptor RANK. RANK activation leads to the cellular context-dependent association of adaptor molecules such as TRAFs and Gab2 or Cbl proteins, and consequently results in the activation and/or modulation of the NF-κB, MAPK, PI3 kinase, and Calcineurin/NFATc1 pathways. In turn, activation of these pathways regulates bone resorption, activation, survival, and differentiation of osteoclasts.

RANK(L) and the Vicious Cycle of Bone Metastases

Many tumors eventually invade bone tissue which causes skeletal complications since the intricate balance between bone resorption and apposition is usually shifted in favor of bone resorption. In patients, tumor-induced osteolysis causes severe bone pain, pathological fractures, nerve compression syndromes (paralysis) and profound hypercalcemia. The strong prevalence of certain tumor types, especially of breast and prostate origin, to metastasize to bone (in ~75% of all breast and prostate cancer cases) is known for a long time but the molecular mechanisms for such osteotropism have begun to unfold only recently. Importantly, the altered regulation in RANKL and OPG expression seems to be of key importance in cancer-induced bone diseases.

The tumor/bone interface is a rich source of factors that are produced either by the tumor cells or by stromal cells upon tumor infiltration and stimulate bone resorption. Amongst these factors are for example ►parathyroid hormone-related protein (PTHrP), ►TGF-β, ►IL-6, ►IL-8, IL-11, or ►prostaglandin E2, which have all been shown to increase RANKL expression in different in vitro or in vivo models. In particular, tumor-produced PTHrP is a well-known bone-resorbing factor and is expressed in ~50–60% of primary human breast tumors. The effects of PTHrP were shown to be mediated by up-regulating RANKL expression in osteoblasts thereby promoting osteoclastogenesis. In addition, several tumors such as prostate cancers seem to express RANKL and could therefore directly contribute to osteolytic lesions themselves by functioning as surrogate osteoblasts to stimulate osteoclast maturation.

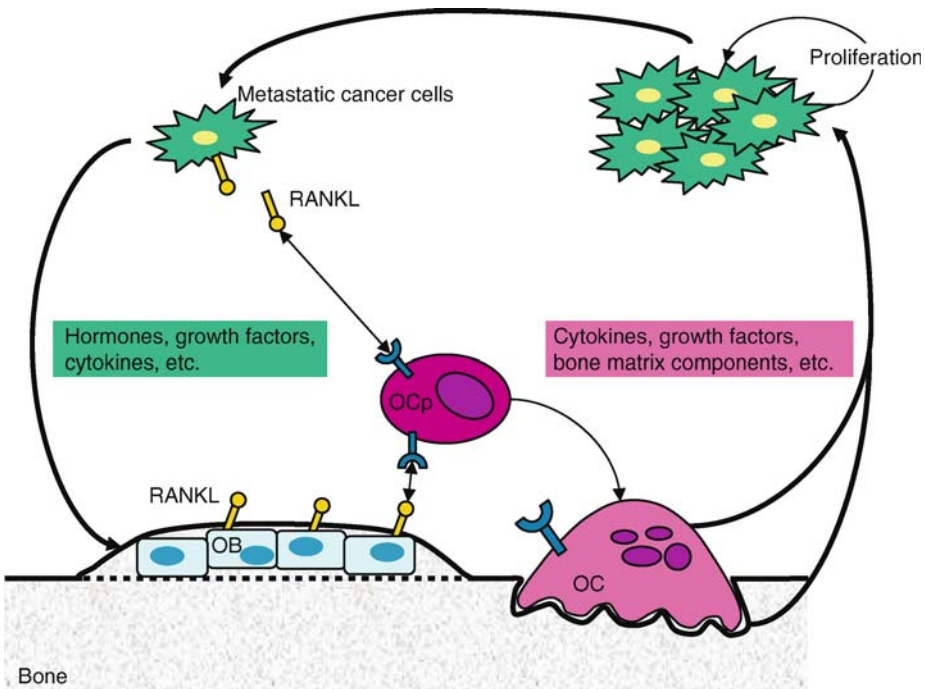
On the other hand, bone resorption by RANKL-activated osteoclasts results in the release of growth factors, such as TGF-β, from the bone matrix which in turn can stimulate the proliferation of tumor cells and thereby increases production of PTHrP in cancer cells. Besides increasing RANKL expression in osteoblastic stromal cells, PTHrP was also shown to decrease OPG levels from osteoblasts, thereby overriding another mechanism to counteract RANKL action and shifting the balance in favor of bone resorption.

Taken together, RANKL – either induced in osteoblastic stromal cells by tumor cells or provided directly by tumor cells – promotes osteoclastogenesis and moreover enhances cancer cell proliferation indirectly through the release of growth factors from the bone matrix during osteoclastic bone resorption. This reciprocal feedback loop between cancer cells and the bone microenvironment fueled by RANKL is referred to as the "vicious cycle" enhancing bone destruction and increasing tumor burden, respectively (Fig. 2).

Given the pivotal role of RANKL in fueling this vicious cycle, the prediction arises that blocking RANKL signals by pharmacological inhibitors, e.g. by OPG-Fc, should be of great benefit to patients suffering from bone metastases. Indeed, in several murine models of tumor-induced osteolytic lesions inhibition of RANKL by OPG-Fc effectively prevented the progression of osteolysis induced by cancer cell lines, such as MDA-MB-231 (breast cancer-derived) or PC3 (prostate cancer-derived). Therefore, clinical trials have been initiated to evaluate the usefulness of RANKL inhibition by a highly-specific monoclonal antibody as a therapeutic approach for treating skeletal complications associated with malignancies of breast and prostate origin, respectively. Moreover, clinical trials are also underway for assessing the clinical benefits of RANKL inhibition prior to the actual formation of bone metastases in prostate cancer patients.

Additional Functions of RANK(L) in Cancer Cells

Several reports have also shown that RANK is expressed in both primary and metastatic tumors of different origin. In particular, RANK (over)expression is frequently found in breast cancer patients and breast cancer-derived cell lines, respectively. Given the observation that both RANK and RANKL female knockout mice fail to produce milk due to impaired proliferation of mammary epithelial cells during pregnancy, it is tempting to speculate that RANK(L) might be directly required for breast cancer cell proliferation and help to explain their strong preference to metastasize to bone. Surprisingly, while RANKL treatment of various RANK-expressing breast and



RANK–RANKL Signaling. Figure 2 The vicious cycle in metastatic bone cancers. Metastatic cancer cells, e.g. of prostate or breast origin, secrete osteolytic factors (PTHrP, IL-11, etc.) that stimulate the production of RANKL in osteoblastic stromal cells (OB). RANKL expressed on osteoblasts binds to and activates RANK expressed on osteoclast precursors (OCp), thereby promoting osteoclast (OC) maturation and increasing bone resorption. Alternatively, some cancer cells express RANKL themselves or secrete soluble RANKL and might thus stimulate osteoclast development directly from osteoclast precursors. Active osteoclasts then release growth factors, cytokines, or bone matrix components that in turn activate tumor cell proliferation. The vicious cycle is closed and results in enhanced tumor burden and bone destruction by shifting the balance between apposition and resorption of bone in favor of the latter.

prostate cancer cell lines did not increase their proliferation, it positively influenced their migration. Exposure of various RANK-expressing cancer cell lines to RANKL triggered cytoskeletal changes and induced cell migration towards the source. Importantly, in vivo inhibition of RANKL with OPG could selectively abrogate metastasis and tumor burden in an in vivo melanoma model of bone metastases. Thus, RANKL may also act as a chemotactic factor for RANK-expressing tumor cells which might help to shed some light as to why certain cancers show a strong prevalence for bone metastases: RANK-expressing cancer cells sense the presence of the chemoattractant RANKL which is provided by osteoblasts and at the same time up-regulated by factors produced by them, such as PTHrP. In such a model, RANKL would serve as one of the long sought-after "soil" factors that facilitates metastasis to bone. In this context, it still needs to be determined if soluble RANKL, which is present in the plasma of humans, could play any role in the development and establishment of a metastatic phenotype, respectively. Whether RANKL inhibition indeed effects the formation of metastases now needs to be tested in human patients.

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RANKL

Definition

Receptor Activator for Nuclear Factor κ B Ligand), also known as TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and ODF (osteoclast differentiation factor) is a molecule important in bone metabolism that activates bone resorption through activation of osteoclasts.

►RANK-RANK signaling
►Metastasis

Rap1

Definition

Ras-proxymity1; Is a small molecular mass G protein (GTPase) of the ►Ras family with the highest homology to classical Ras (K-, H-, and N-Ras) among the family. There are two highly homologous Rap1 proteins, Rap1a and Rap1b, encoded by distinct genes in both human and rodents.

►Rap1/SIPA-1

Rap1 and Sipa-1

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Definition

Ras-proximity1 is a small molecular mass ►G protein (►GTPase) of the ►Ras family. There are two highly homologous Rap1 proteins, Rap1a and Rap1b, encoded by distinct genes in both human and rodents. Signal-induced proliferation-associated protein 1 is a GTPase-activating protein (GAP) specific for Rap1 and is encoded by the *Sipa-1* gene. There are several proteins with a homologous structure and function, including human ►E6TP1 (human papillomavirus E6-targeted protein 1, also called Spa-L (Spa-1 like) 1 in mouse and SPAR in rat), Spa-L2, and Spa-L3.

Characteristics

Among the Ras family ►G proteins, Rap1 shows the highest overall homology (around 50%) to classical Ras proteins (K-, H-, and N-Ras) with an identical effector region. Rap1GTP is an active form capable of binding a number of specific effector molecules to transduce the signals, while Rap1GDP dissociates from the effectors terminating the signals. Rap1 is activated by a wide variety of external stimuli, and the activation is mediated by specific ►GEFs, which facilitate the dissociation of bound GDP and the reloading of GTP. There are many GEFs that are coupled functionally with various signaling pathways, including C3G recruited by ►receptor protein tyrosine kinases, Epacs activated by binding cyclic AMP, and CalDAG GEFs activated by Ca^{2+} and/or diacylglycerol. Although Rap1 per se has weak GTPase activity, the swift inactivation of Rap1GTP to Rap1GDP can be achieved only by the aid of specific ►GAPs, which enhance the intrinsic GTPase

activity by more than 100 folds. There are two groups of Rap1GAPs, Spa-1 family and RapGA1, sharing a catalytic domain called GAP-related domain (GRD).

Biological Functions of Rap1

Rap1 was discovered by two independent approaches; cloning of the genes with high homology to Ras and search for the genes that could revert the malignant cellular contours of a fibroblastic cell line transformed by oncogenic K-Ras. Although it was thought originally that Rap1 functions as an antagonist of Ras based on the latter effect, subsequent studies have revealed that Rap1 has a number of unique functions on its own.

Interference with Ras-Mediated ERK Activation

Rap1 shares the effector region with Ras, through which it can bind to c-Raf, a MEK1, 2-activating kinase, with even higher affinity than Ras. Unlike Ras, however, Rap1 hardly induces the phosphorylation of c-Raf that is required for the activation of MEK1, 2, and is thus unable to activate MEK1, 2/ERK signal pathway. Rather, when Rap1 is activated strongly concomitantly with or preceding Ras activation, it may competitively interfere with the Ras-induced c-Raf-mediated ERK activation. While such occasions may be rather rare, it is known to occur in certain conditions such as a form of immunological unresponsiveness called anergy in T cells.

Activation of MAP Kinases and Other Signaling Pathways

Rap1 can directly activate ERK independently of Ras in selected cell types that express B-Raf. Unlike c-Raf, ►B-Raf is constitutively phosphorylated at the corresponding sites of c-Raf, and thus Rap1 can bind and recruit B-Raf to the plasma membrane to activate MEK1, 2/ERK pathway in such cell types. While Ras activation by Sos, a RasGEF, is inhibited by the activated ERK forming a tight negative feedback loop for Ras signaling, Rap1 activation is not affected by the activated ERK, and therefore Rap1-induced B-Raf-mediated ERK activation may persist as long as Rap1 activation continues. Thus, Rap1-mediated ERK activation may show distinct kinetics and biological consequences from Ras-mediated ERK activation. For instance, epidermal growth factor (EGF) induces only transient ERK activation via Ras signaling leading to the cell proliferation in PC12 cells, while nerve growth factor (NGF) induces persisted ERK activation via Rap1 causing their neuronal differentiation. Rap1 can also activate other ►MAP Kinases (MAPKs) such as p38MAPK as well as PI3-kinase in certain cell types, leading to the specific gene activation.

Regulation of Cell Adhesion and Migration

The most unique and prominent function of Rap1 is to activate ►integrins. Through the effect, Rap1 activation induces the enhanced cell-matrix ►adhesion and ►intercellular junctions as well as cell migration in many cell types. Rap1 is a major mediator of the "inside–out" activation of integrins in response to various extracellular stimuli including chemokines. Rap1GTP binds to a specific effector called RapL, which associates with the intracellular domain of α-units of integrins and induces the configurational changes to active forms of integrins. Rap1GTP also binds to RIAM (Rap1-interacting adapter molecule) that controls F-actin dynamics via interaction with profillin and Ena/VASP, thereby regulating overall actin-cytoskeletal function as well. Most recent studies further indicate that Rap1 regulates the ►cadherin function in epithelial and endothelial cells. Thus, Rap1 plays a central role in controlling the cell adhesion and migration in many cell types including cancer cells (see below).

Regulation of Rap1 Signal by Spa-1

Sipa-1 was originally isolated in mouse lymphohematopoietic cells as a gene strongly induced in association with the cell proliferation. Purified Spa-1 protein shows a potent and specific GAP activity for Rap1a, Rap1b, and Rap2 without affecting other small G proteins such as Ras, Rac, and Rho. Human and mouse Spa-1 proteins show quite high overall homology (more than 90% amino acids being identical), and are encoded by the genes at the synthetic chromosomal regions, 11q13.3 in human and near the centromere of 19 in mouse, at the close vicinity to *RRAD1* gene encoding cyclin D1. Spa-1 is expressed rather ubiquitously in most tissue cells including cancers (see below), although it is most prominently expressed in the lymphohematopoietic cells. There are several proteins with high homology to Spa-1, including human E6TP1 (called Spa-L1 in mouse and SPAR in rat). E6TP1 was identified as a protein that was specifically bound to ►human papillomavirus (HPV) oncoprotein E6 and targeted to the degradation by E6AP1 via ►ubiquitination in epithelial cells like p53 tumor suppressor protein. E6TP1 has also Rap1GAP activity, and it is reported that the E6 oncoprotein-mediated degradation of endogenous E6TP1 is correlated with the HPV-induced epithelial cell transformation in vitro. Overexpression of Spa-1 abrogates the Rap1 activation via extracellular stimuli, while reduction of the endogenous Spa-1 results in the increased basal Rap1GTP level, and as such Spa-1 functions as one of the major factors controlling Rap1 signal.

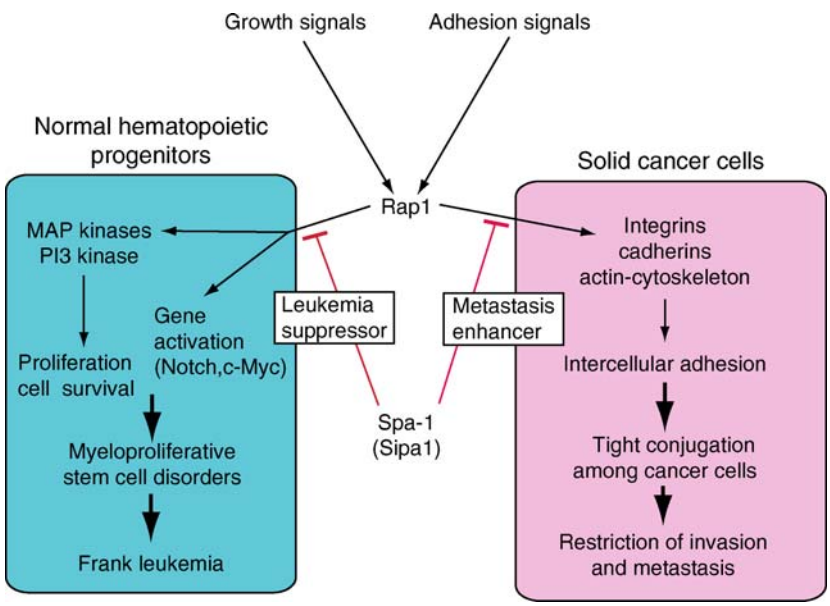
Rap1 and Spa-1 in Leukemia

Overexpression of wild-type Rap1 in Swiss 3T3 fibroblasts, which expressed B-Raf, strongly enhanced the proliferation induced by EGF or cAMP. While such cells remained to be anchorage-dependent in vitro, they formed tumors in nude mice. In contrast to the classical oncogenes such as mutant *Ras* genes that bypass the

normal growth signals, overexpressed wild-type Rap1 may function as a "conditional oncogene" in certain cell types, causing tumors by constitutively activating, rather than bypassing, the growth signals via environments.

The oncogenic effect of Rap1 in vivo was revealed in *Sipa-1* gene-targeted mice. *Sipa-1* gene-deficient (*Sipa-1*$^{-/-}$) mice showed gradual increase in the hematopoietic ►stem cells (HSC) and progenitor cells (HPC) in bone marrow as they aged in association with the constitutive activation of endogenous Rap1. The vast majority of them eventually developed overt myeloproliferative disorders (MPD) after long latency, including ►chronic myeloid leukemia (CML) in chronic phase characterized by the marked increase in granulocytic cells with ►extramedullary hematopoiesis, lethal blast crisis with leukemic invasion to vital organs, and ►myelodysplastic syndrome with severe anemia. Preleukemic *Sipa-1*$^{-/-}$ HPC show enhanced expression of ►MYC-oncogene gene, which promotes the cell cycle progression and controls the interaction of HSC with ►hematopoietic niche stroma cells. Paradoxically, they also revealed enhanced expression of basal ►p53 protein. The simultaneous overexpression of protooncogene c-myc and p53 tumor suppressor protein is reminiscent of so-called oncogene-induced ►senescence and may explain the long latency for leukemia development as well as the infrequent development of severe pancytopenia resembling human MDS in *Sipa-1*$^{-/-}$ mice. The CML and MDS were eventually followed by blast crisis in myeloid or B-lymphoid cells often associated with gross chromosomal abnormalities (Fig. 1).

In human, the majority of CML is caused by ►BCR–ABL1 fusion gene derived from a Philadelphia chromosome with t(9;22)(q34:q11) ►chromosomal translocation. Bcr–Abl oncoprotein can convert HSC, but not committed HPC, to leukemic stem cells, and thus it is suggested that Bcr–Abl oncoprotein transforms HSC by functional collaboration with the intrinsic signals in normal HSC. Rap1 is strongly activated downstream of Bcr–Abl, and Spa-1 overexpression significantly compromises the proliferation and survival of Bcr–Abl^{+} cells in vitro. Conversely in a mouse model, *Sipa-1*$^{-/-}$ HSC transduced with *Bcr–Abl* gene exhibit prolonged survival in vivo followed by the frequent blast crisis as compared with the *Bcr–Abl*-transduced control HSC. This suggests a significant role of Rap1 signal downstream of Bcr–Abl oncoprotein in the human CML genesis. Accumulating evidence reveals that human CML stem cells tend to resist a Bcl–Abl kinase inhibitor, ►imatinib, which is highly effective in reducing the peripheral leukemic cell



Rap1 and Sipa-1. Figure 1 Roles of Rap1 and Spa-1 (*Sipa-1*) in leukemia and cancer metastasis. Rap1 G protein mediates diverse signals depending on the cellular contexts, including proliferation, cell survival, gene activation, and cell adhesion, and the signals are negatively regulated by GTPase-activating protein Spa-1. In hematopoietic progenitors, dysregulated activation of Rap1 in the absence of *Sipa-1* causes myeloproliferative stem cell disorders eventually followed by frank leukemia of various types, and thus Spa-1 functions as a leukemia suppressor. In solid cancer cells such as breast and prostate cancers, the endogenous Rap1 signal contributes to the maintenance of intercellular cancer cell adhesion to restrict their spread, invasion and metastasis. Cancer cells expressing higher levels of Spa-1 show enhanced metastasis, and germline polymorphisms of *Sipa-1* gene in humans significantly affect the metastatic efficiency of cancers such as breast and prostate cancers in the individuals.

burden in the chronic phase, being consistent with the involvement of intrinsic signals in the maintenance of leukemic stem cells. Such leukemic stem cells are bona fide target for the ultimate control of human leukemia, and Rap1 signal may be an important target for controlling CML stem cells in humans.

While *Sipa-1*$^{-/-}$ mice only rarely developed T-cell leukemia probably because of the redundant expression of other Rap1GAPs in the thymic cells, Rap1 signal may be also involved in the development of thymic lymphoma/leukemia. Transduction of C3G-F, a modified C3G to promote the membrane localization, into normal and *Sipa-1*$^{-/-}$ HPC followed by the transplantation into irradiated mice caused thymic lymphoma and thymic acute T-lymphocytic leukemia (T-ALL), respectively, correlating to the extent of endogenous Rap1 activation. The leukemia cells revealed the features of pro/pre-T cells with markedly enhanced expression of ►*Notch*, and *c-Myc* genes, being reminiscent of human T-ALL caused by TAN1 (translocation-associated Notch homolog) derived from t(7;9)(q34; q34.3) chromosomal translocation. Deregulated overexpression of wild-type *Notch* genes has been reported in many types of leukemia and cancers even in the absence of frank chromosomal anomalies, and it may be an intriguing possibility that Rap1 signal plays a part in the constitutive activation of *Notch* genes.

Rap1 and Spa-1 in Cancer Metastasis

Certain human and mouse cancer cells show mutations in *Dock-4* gene. Dock-4 protein is capable of activating Rap1, and the loss-of-function mutations found in such cancer cells result in reduced ►adherence junction among the cancer cells and their enhanced invasiveness. Expression of wild-type *Dock-4* gene in such cancer cells restored the basal Rap1GTP levels as well as the intercellular adhesion and strongly suppressed their invasiveness in vivo.

Experiments using an animal model have provided a genetic evidence that Rap1 signal may control cancer ►metastasis. Transgenic mice of *polyoma middle T* gene under a mouse mammary tumor virus promoter develop mammary tumors irrespective of the genetic backgrounds of mouse strains. Intriguingly, however, such developed mammary tumors show high tendency for lung metastasis in certain strains of mice, while they rarely metastasize in other strains. Genetic analysis revealed that the efficiency of lung metastasis was controlled by a dominant genetic locus termed *Mtes-1*. By using a multiple cross mapping strategy, *Sipa-1* gene has been identified as a candidate gene for *Mtes-1* locus. In mice, there is a nonsynonimous polymorphism in the coding region of *Sipa-1* gene, either alanine (A) or threonine (T) at the amino acid position 741 in the PDZ domain, and all the strains of mice with *Sipa-1/741A* allele showed high metastatic tendency, while those with *Sipa-1/741T* allele revealed significantly less lung metastasis. Spa-1/741A showed stronger Rap1GAP activity than Spa-1/741T in cancer cells probably due to the altered binding affinity of the PDZ domain for yet unidentified proteins. Consistently,\ overexpression of wild-type *Sipa-1* in the mammary tumor cells enhanced the lung metastatic activity, while knockdown of endogenous *Sipa-1* gene significantly reduced it. Interestingly, such procedures barely affected the tumor sizes in the primary sites, indicating that the cancer cell proliferation in the primary sites was not directly correlated to their metastatic potential.

Exact mechanisms for *Sipa-1* gene polymorphisms to affect the efficiency of cancer metastasis remain to be investigated. A probable explanation would be that the basal Rap1GTP level controls the metastatic efficiency of cancer cells by regulating the intercellular adherence junctions among cancer cells in the primary sites via ►cell adhesion molecules such as cadherins and integrins. The phenomenon reinforces a provocative concept for cancer metastasis, that is, the genetic predispositions of the individuals significantly contribute to the metastatic efficiency of cancers in them (Fig. 1).

Clinical Aspects

Human ►prostate cancers with metastasis have been shown to express significantly more *Sipa-1* transcripts than those without metastasis, and it was confirmed that the primary prostate cancer cells with metastasis exhibited significantly more Spa-1 protein by ►immunohistochemistry. Consistently, while human prostate cancer cell lines with little Spa-1 expression hardly metastasized when transplanted into the testes of immunodeficient ►scid mice, those transduced with human *Sipa-1* gene showed efficient metastasis to the abdominal lymph nodes, with the primary tumor sizes being unchanged. Also, Spa-1^{high} prostate cancer cells showed enhanced invasion activity to the matrix in vitro than Spa-1^{low} ones, suggesting that the enhanced metastatic efficiency was a direct reflection of the enhanced tissue invasiveness. Thus, the expression levels of Spa-1 by the immunohistochemistry of biopsy specimens, for instance, may provide a reasonable prognostic estimation for the metastatic potential of primary cancers.

In human *Sipa-1* gene, at least three single nucleotide polymorphisms (SNPs) have been identified, one within the promoter and two exonic, and based on the combinations of *Sipa-1* gene SNPs at least five haplotypes can be discriminated. Analysis in non-Hispanic Caucasian ►breast cancer patients has revealed that a particular *Sipa-1* haplotype shows strong correlation with positive lymph node involvement and another with ►estrogen receptor and ►progesterone receptor-negative tumors. Overall, the *Sipa-1* gene germ line polymorphisms are significantly related to the

aggressiveness in human breast cancer behavior. At present, it remains to be seen whether such germline polymorphisms are indeed reflected to the *Sipa-1* gene expression levels. Nonetheless, this may be one of the first substantial evidence supporting the hypothesis that host genetic predispositions in humans significantly influence the metastatic efficiency of cancers. *Sipa-1* gene may provide not only a useful marker to enhance the staging protocols for prostate, breast, and possibly other cancers but a potential molecular target for the control of cancer metastasis.

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Rapamycin

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Synonyms

Sirolimus; Rapamune®; CCI779; temsirolimus (Torisel®); RAD001 (everolimus)

Definition

A ►macrolide antibiotic isolated from the soil bacteria *Streptomyces hygroscopicus*.

Characteristics

Discovery of Rapamycin

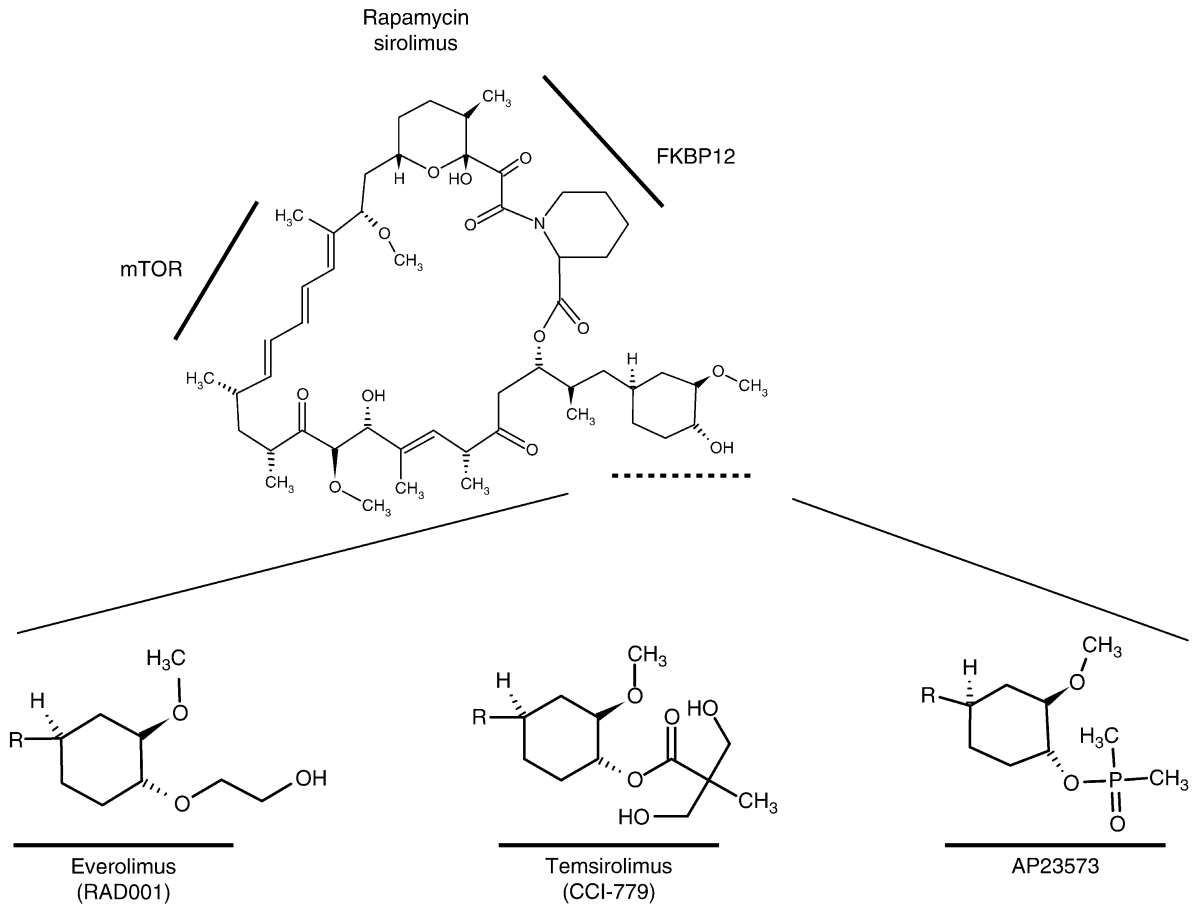
Rapamycin (sirolimus, Wyeth-Ayerst Laboratories; Fig. 1) was originally isolated as a fungicide from the soil bacteria *Streptomyces hygroscopicus*, collected from Easter Island (known as Rapa Nui to the natives) in the South Pacific in 1975. Structurally similar to the immunosuppressive drug ►FK-506 (tacrolimus; Fujisawa Pharmaceutical Co. Ltd.), rapamycin was initially developed to prevent transplant rejection.

While rapamycin was being developed as an immunosuppressant, it was also found to exert potent antitumor activity *in vitro* and *in vivo*. However, perhaps because its mechanism of action was unknown at that time, and a parenteral formulation could not be developed, rapamycin was not further considered as a cancer therapeutic. Rapamycin has emerged as a potent inhibitor of a signaling pathway that may be deregulated in many forms of cancer, leading to both increased growth, survival and malignancy of cells. Rapamycin is a lipophilic macrolide drug, that selectively inhibits ►mTOR (the *m*ammalian *t*arget *o*f *r*apamycin, also known as FRAP1, RAPT1, RAFT1 and SEP) a serine/threonine kinase, that is a member of the ►phosphatidye inositol 3-kinase (PI3K)-like kinases (PIKKs). mTOR lies downstream of PI3K in the signaling pathway. TOR proteins represent a class of evolutionarily conserved kinases in eukaryotes. In the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, there are two TOR genes, *TOR1* and *TOR2*, both sharing 67% homology and encoding proteins of approximately 280 kDa, whereas in higher eukaryotes a single TOR gene exists. The potential for rapamycin as a cancer therapeutic was refocused, in part, by studies that showed rapamycin potently blocked proliferation stimulated by exogenous insulin-like growth factors (IGF-I/II), and by the development of analogs with minor modifications that were chemically more stable than rapamycin and allowed formulation suitable for parenteral administration (CCI-779, ►temsirolimus, Wyeth Ayerst). Other analogs (RAD001, ►everolimus, Novartis and AP23573, Ariad Pharmaceuticals) are administered orally, Fig. 1.

Mechanism of Action

Rapamycin competes with FK506 for binding to an ►immunophilin (the 12 kD FK506 binding protein; FKBP12) to form an active complex that binds through a hydrophobic face of rapamycin to the FKBP-rapamycin binding domain (FRB) domain of mTOR that is part of the mTORC1 complex with raptor. The FRB domain lies outside the catalytic domain of mTOR, consequently rapamycin is not a direct inhibitor of mTOR kinase activity. Rather, the model suggested is that rapamycin-FKBP12 binds to mTOR and displaces raptor. As raptor binds substrates (4E-BP1 and S6K1) it is postulated that displacement by the rapamycin complex prevents accurate presentation of substrates to the kinase domain of mTOR.

mTOR signaling results in the phosphorylation and inactivation of 4E-BP1, the suppressor of eukaryotic ►initiation factor 4E (eIF4E), a protein that binds the ^{7}mGTPpppN structure (cap) of mRNA. mTOR phosphorylates and activates ribosomal S6K1. By inhibiting mTOR signaling rapamycin treatment results in the inhibition of ►cap-dependent translation that



Rapamycin. Figure 1 The structure of rapamycins under investigation as cancer chemotherapeutic agents. *Bold line*s show the mOR and FKBP12 binding faces of rapamycin. The *broken line* shows the position of structural modifications for the rapamycin analogs RAD001, CCI-779 and AP23573.

is required for translation of mRNA species that have highly structured 5′-untranslated regions. These mRNAs include cell cycle regulators, cyclin D1, and ornithine decarboxylase and the ►transcription factors ►c-MYC and in some cells, hypoxia inducible factor 1α. As a consequence cells accumulate in G1 phase of the cell cycle. Rapamycins have been reported to inhibit cancer stem cell renewal, block cancer cell proliferation, induce apoptosis and prevent cell motility [2]. Rapamycins, through inhibition of mTOR, may have direct actions on cancer cells, preventing secretion of angiogenic factors, or prevent vascular or bone marrow endothelial cells from responding to elicited angiogenic factors, thus inhibiting tumor ►angiogenesis.

Mechanism(s) of Sensitivity and Resistance

Mutations conferring rapamycin resistance were first identified in yeast. Mutants of RBP1, the yeast homolog of FKBP12, that prevent rapamycin binding confer recessive resistance to rapamycins. In contrast, mutations in mTOR that decrease binding of the rapamycin-FKBP12 complex result in dominant resistance. Cellular sensitivity to rapamycin and its analogs has also been related to constitutive activation of Akt (also known as protein kinase B), usually as a consequence of mutation or silencing of the dual specificity phosphatase ►PTEN. Acquired resistance to rapamycin is also associated with decreased levels of ►4E-BP1, thus decreasing the stoichiometry between the suppressor and eIF4E.

Clinical Activity of Rapamycins

Rapamycin was approved by the US Food and Drug Administration in September 1999 and the European Commission in March 2000 for use in kidney transplantation. Rapamycin, and three analogs (CCI-779, RAD001 and AP23573) are currently in various stages of development for treatment of human cancer. Daily intravenous treatment resulted in significant grade 3 toxicities, including hypocalcaemia, vomiting, ►thrombocytopenia, and increase in the level of hepatic transaminases in cancer patients. Objective responses or stable disease were noted (►non-small cell carcinoma, ►cervical carcinoma, uterine carcinoma, ►renal cell carcinoma, and soft tissue sarcoma). On a weekly treatment schedule patients experienced no

grade 3 toxicities regardless of dosage. Partial tumor regressions were noted (renal cell, neuroendocrine, and breast carcinomas). Phase II trials of CCI-779 for renal cell carcinoma yielded an objective response rate of 7%, a minor response rate of 29%, and stable disease in approximately 40% of the patients. Treatment is associated with a increase in survival time of ~4 months. The phase II trial for ►mantle cell lymphoma consisted of weekly treatment with 250 mg (fixed dose) of CCI-779 administered intravenously, resulted in an overall objective response rate of 38%, with a median time to progression of 6.9 months in responders. CCI-779 also appears to have significant activity in ►endometrial cancer, irrespective of ►PTEN status, yielding objective responses in 25% of patients, and causing disease stabilization in over half of the patients on study. For AP23573 there is currently a Phase I trial to determine the maximum tolerated dose (MTD) with oral administration in advanced cancers, and phase II evaluation against ►sarcomas. There are also trials to determine the maximum oral tolerated dose in combination with ►doxorubicin in the treatment of sarcomas, as well as a trial to determine the MTD dose in patients with multiple myelomas, and a Phase II trial of patients with recurrent endometrial cancer. Phase II results suggest significant activity against various subtypes of sarcoma. Phase III trials are in progress to test the efficacy of CCI-779 either alone or in combination with ►interferon-α as a first line treatment of renal cell carcinoma, and the use of the oral form of CCI-779 or RAD001in combination with the ►aromatase inhibitor letrozole for the treatment of locally advanced or metastatic hormone-responsive breast cancer.

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Rare Fragile Sites

Definition

Specific chromosomal loci that are especially prone to forming gaps and break on metaphase chromosomes under conditions of replication stress. These sites are found in a small percent of the population and are associated with expanded di-or trinucleotide repeats.

►Fragile Sites
►Fragile-X Syndrome
►Common Fragile Sites

RAS

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Definition

The word Ras comes from a contraction of Rat sarcoma, the tumor where the first gene of the family was identified, as part of the genome of a ►retrovirus isolated from a carcinogenesis protocol.

Characteristics

Structure

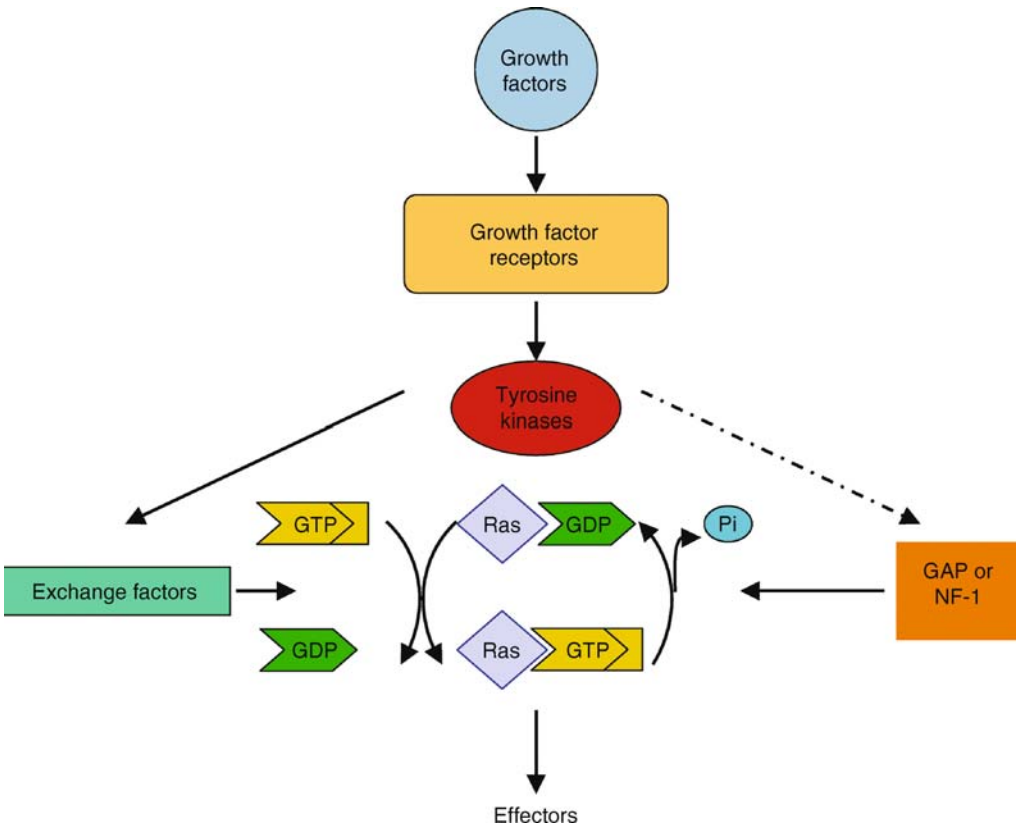
The Ras genes, strictly speaking, are only a small group of a large family of related genes (the Ras superfamily) that perform a host of important cellular functions including ►signal transduction (Ras, ►Ral, ►Rho), cytoskeletal regulation (Rho), vesicle transport (►Rab) and nuclear-cytoplasmic transport (►Ran). The Ras genes more relevant for human cancer are: H-ras, K-ras and N-ras and they will be the subject of this essay. H-ras was initially isolated from the Harvey sarcoma virus. K-ras from the Kirsten sarcoma virus and N-ras was isolated by DNA-mediated gene transfer from a human neuroblastoma cell line. In humans they are located in chromosome 11p15, H-ras; 12p12, K-ras and 1p22, N-ras. These three genes code for very similar proteins of 189 a.a. with four coding exons. Although the proteins are very similar, the genes have very different lengths due to the variable length of their introns. The expression of the three genes is essentially ubiquitous, although different tissues show differences in the levels of expression for the three genes. Analysis of the protein sequence shows that they contain a domain for ►GTP binding, which represents a common characteristic for the whole superfamily.

The H-ras protein has been crystallized with GTP and the main regions that contact the phosphates are G1 (residues 11–17) and G3 (residues 53–62). The regions contacting the guanine ring are G4 (residues 112–119) and G5 (residues 144–146). The biochemical function of ras proteins is to hydrolyze GTP (►GTPase). The

other main structural feature is at the carboxyterminal end where they have the CAAX box motif, where C is a cysteine, A are aliphatic amino acids and X is any amino acid. This box is required for the processing of the protein that is ►farnesylated at the cysteine, cleaved by a protease (eliminating AAX) and carboxymethylated. This processing is essential for the protein to reach the plasma membrane, the main place where it is functionally active. In the last few years evidence has been accumulating that Ras can also localize to the Golgi apparatus and be active there, although the exact functions that specifically elicits from there are still under study. Although the first three exons of the three proteins are quite similar, there is more divergence in the fourth exon and this has resulted in different processing for K-ras versus the other two. H- and N-ras have other cysteine(s) upstream from the one that is farnesylated and they are ►palmitoylated. K-ras lacks these cysteine(s), which are substituted by a run of positively charged amino acids. This feature appears to modify the way by which K-ras is brought to the membrane, and it appears to make it more resistant to inhibition by ►farnesyltransferase inhibitors (FTIs), a potential treatment for tumors. This failure is due to the fact that K-ras can be posttranslationally modified by the geranylgeranyl transferase enzyme in the absence of farnesyltransferase, and this modification allows the protein to localize to the plasma membrane. K-ras also has two alternative exons 4 (4A and 4B). The specificity of the function for each isoform has not been conclusively proven, but it appears to be strongly supported by the gene inactivation studies. Although N- and H-ras knockouts are born normal and only have a few alterations in signal transduction pathways, K-ras is lethal embryonic, but the double knockout between K-ras and N-ras is lethal even earlier than the K-ras knockout, indicating that N-ras is providing a function that cannot be substituted by K-ras.

Cellular and Molecular Regulation

The function of the Ras proteins is to act as signal transducers between the membrane and other cellular



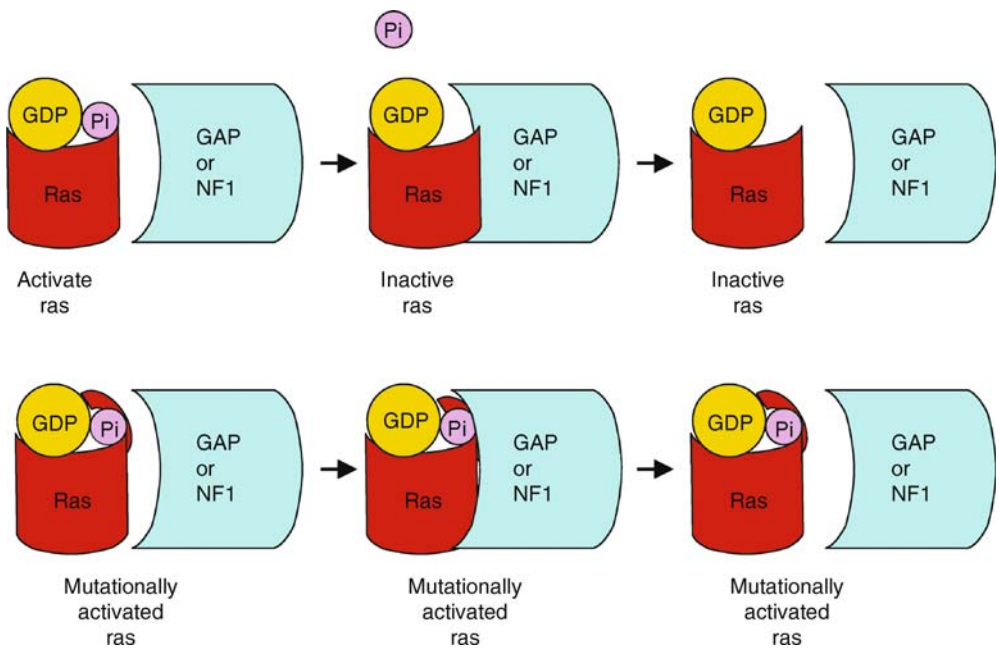
RAS. Figure 1 Biochemical Ras cycle. The Ras protein is a molecular switch that cycles between activated and inactive states. External signals, acting through receptors, elicit the interaction between the exchange factor and Ras-GDP (inactive), which produces the dissociation of GDP from Ras. Given the much higher concentration of GTP in the cytoplasm, Ras binds then to GTP and becomes activated, interacting with its effectors and activating them. To prevent constitutive activation, there are molecules that stimulate the intrinsic GTPase activity of Ras enhancing the hydrolysis and producing Ras bound to GDP, closing the cycle.

structures, most often the nucleus. Ras molecules are in the inner face of the plasma membrane and in the Golgi apparatus in an inactive state, bound to ►GDP. When a stimulus has to be transduced a Ras guanine dissociation stimulator (RasGDS or Ras ►GEF) interacts with Ras resulting in its dissociation from GDP. Given the much higher concentration of GTP in the cellular environment, the next nucleotide to bind to ras is usually GTP. This results in a conformational change that activates Ras, which is now poised to transmit a signal downstream. Ras binds now to its effectors and facilitates their activation. To prevent excessive signaling, Ras has a weak intrinsic GTPase activity. To increase this GTPase activity there are molecules that enhance this GTP hydrolysis and are called GAPs (GTPase Activating Proteins). For Ras, at least two such proteins have been identified GAP and ►NF1 (Fig. 1). Some mutations can abolish the GTPase activity of Ras and block its response to GAPs. This results in a constitutive ►Ras activation with uninterrupted signaling as it has been found in tumors (Fig. 2).

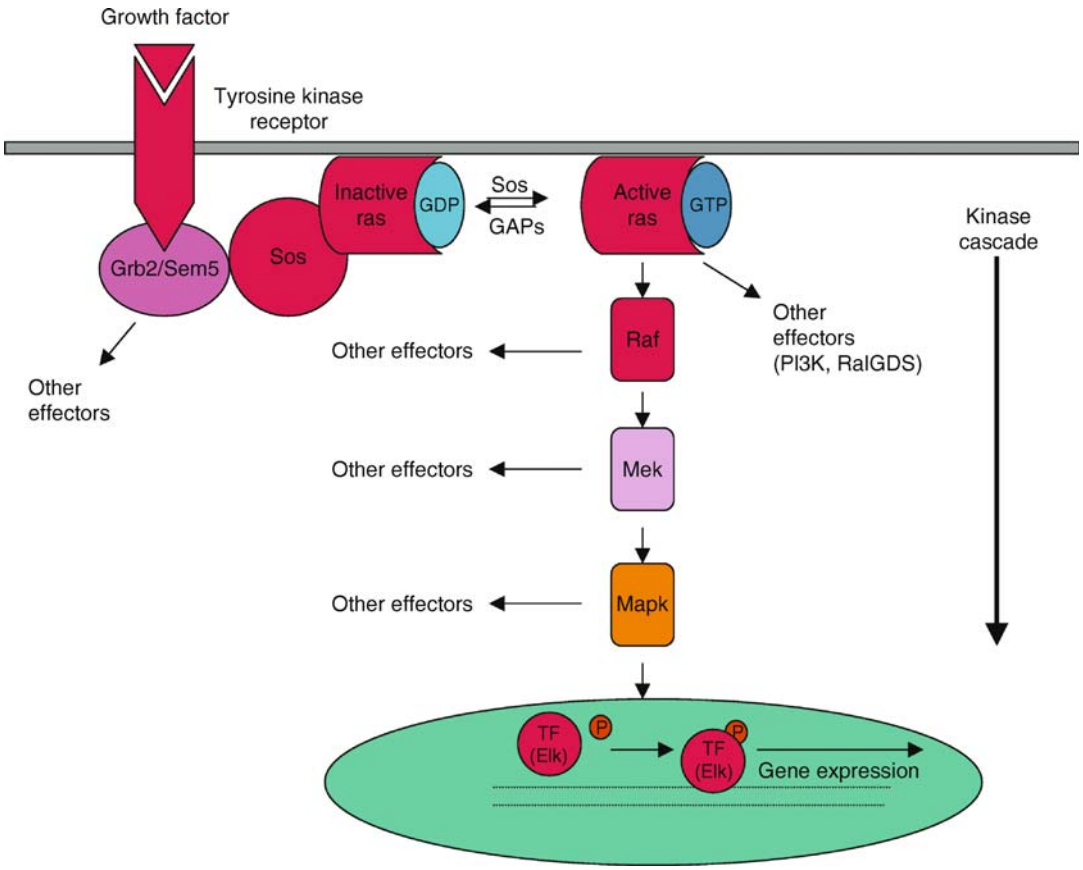
The previous description corresponds to the so-called Ras cycle that involves the steps from a Ras-GDP molecule to Ras-GTP and back to Ras-GDP. The linear ras pathway describes the route followed by an outside signal through Ras and its final destination in the nucleus (Fig. 2). As an example, the signal triggered by EGF is chosen. EGF is a growth factor that interacts with its specific receptor in the cell surface. This interaction triggers a dimerization and autophosphorylation of the cytoplasmic domains. These phosphorylated residues recruit SH2 containing molecules like the adaptor Grb2. This molecule contains also an SH3 domain that recruits to the membrane the Ras ►GEF through its polyproline domain. RasGEF activates Ras, which interacts in its GTP bound conformation with ►Raf (other important effectors are ►PI3 kinase and RalGDS). Raf is a kinase that now activates another downstream kinase called ►MEK, which in turn activates another downstream kinase called ERK. This kinase now translocates to the nucleus and activates a number of transcription factors like Elk, which are responsible for executing the action triggered by the original signal (►EGF) by inducing the appropriate genes (Fig. 3). Although Ras best-known function is to stimulate cell proliferation, signals transduced through Ras can also induce differentiation, growth arrest and ►senescence, depending on the cellular context where its action is studied.

Clinical Relevance

The great effort that has been devoted to study the Ras genes and their regulation stems from its importance in cancer. Nevertheless, recently a number of studies have been reported indicating that some versions of weakly ►activated Ras genes can be found in the germ line of patients with hereditary developmental syndromes like



RAS. Figure 2 Mutational activation of Ras makes it resistant to the inactivating effects of GAP proteins. A crucial biochemical difference between the normal Ras protein and the mutationally activated version found in many tumors is the fact that the abnormal version is unable to respond to the effects of GAP or NF1 by hydrolyzing GTP into GDP.



RAS. Figure 3 A representative signal transduction pathway where Ras is a crucial step. The signal starts from a growth factor and through its receptor activates Ras. Activated Ras activates a kinase cascade which last member translocates into the nucleus activating transcription factors that will be executors of the functions that each particular growth factor induces in a particular cell context. It is relevant to mention that the elements of the pathway in *red* have been shown to be oncogenic in different systems.

Noonan, Costello and cardio-facio-cutaneous syndrome. Only in Costello syndrome the mutations present have been occasionally reported in cancer, for the others the mutations reported in the syndromes are new and in all cases only weakly activating. In Noonan and Costello patients there are tumors associated with the syndrome, but not in the cardio-facio-cutaneous syndrome patients. The relevance of Ras genes in cancer was hypothesized when two of the genes (H- and K-ras) were identified as the ►oncogene in an acute rodent retrovirus and the other (N-ras) as a cellular oncogene in a human neuroblastoma. Using DNA-mediated gene transfer and rodent cells as recipients (usually NIH3T3) a large variety and number of human and animal tumors were tested for the presence of oncogenes in the focus formation assay, and, when positive, most of the time were shown to be one of the Ras isoforms. The ability of ►activated Ras genes to induce ►malignant transformation has its molecular base on mutations acquired in tumor development that render the molecule in a constitutively active conformation. In animal tumors induced by chemical carcinogens it has been shown that the Ras mutations correlate with the chemical reactivity of the carcinogen, suggesting that in some cases Ras mutations could be the initiating alteration in tumor development. The ability of Ras genes to induce tumors has also been approached in transgenic mice where Ras oncogenes under the control of tissue specific promoters have induced tumor development in a number of tissues.

Evidence of the clinical relevance of the Ras genes is its significant frequency of mutation in human tumors. The methods of detecting the mutations started by cloning and sequencing the transforming genes. After the advent of PCR, most of the detection strategies use that technique as part of the protocol. The overall frequency in human cancer is calculated to be between 20 and 30%. There is nevertheless, a large variation in the frequencies depending on the tumors to be considered. The highest frequency is in ►pancreatic cancer (80–90%). In ►colon carcinoma is around 50%, thyroid tumors, 50%, ►non-small cell lung cancer, 30%

and in some acute ►leukemias also around 30%. In ►bladder cancer there has been some controversy about the frequency of ras activation, depending on the techniques utilized for the detection that ranges from 10 to 60%. The frequency of Ras mutations in many other types of human tumors is lower and in particular in ►breast cancer and ►prostate cancer is around 5%. Nevertheless, it appears that in a number of tumors, the presence of a wild type Ras is substituted by activating mutations in ►B-raf, ►PTEN, ►PI3 kinase, ►AKT1 kinase or ►EGFR ►amplification, all of which result in a Ras pathway activation. Therefore, regardless of the fact that Ras genes are mutated or not in the tumors, it appears that the activation of this pathway plays a very important role in many human cancers.

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RAS Activation

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Definition

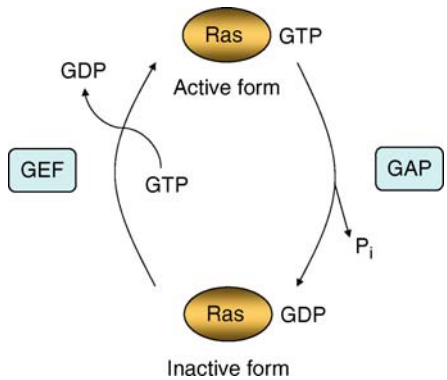
Increase of the active GTP-bound form of Ras proteins promoted by ligand-bound ►receptors or other mechanisms.

Characteristics

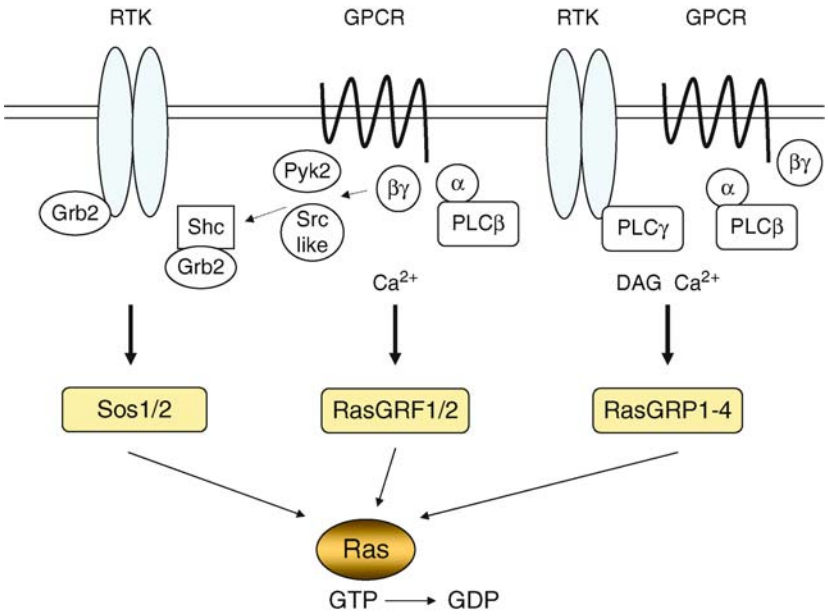
The family of ►Ras proteins (Ha-Ras, N-Ras, Ki-RasA and Ki-RasB) consists of low molecular weight guanine nucleotide-binding proteins that play essential roles in the control of cellular growth and differentiation. Ras alternates between an active GTP-bound state and an inactive GDP-bound state. The slow intrinsic rate of GTP hydrolysis on Ras is stimulated by ►GTPase-activating proteins (►GAPs), such as p120GAP and ►neurofibromin, while Ras activation requires ►guanine nucleotide exchange factors (►GEFs) that stimulate the dissociation and exchange of bound GDP for GTP on Ras in response to upstream signals. (Fig. 1).

Ras proteins are localized mainly at the inner surface of the plasma membrane, where they participate in transmitting signals from ►tyrosine kinase receptors (RTK) and some receptors coupled to ►heterotrimeric G-proteins. It is well established that ligand-bound receptor tyrosine kinases initiate Ras activation through formation of heterotrimeric complexes consisting of autophosphorylated receptors, the SH2/SH3 ►adaptor protein Grb2 and the guanine nucleotide exchange factor Sos. The ►SH3 domains of Grb2 bind to the carboxy-terminal proline-rich domain of Sos, whereas the ►SH2 domain binds to specific tyrosine phosphorylated sequences. Complex formation of Sos with autophosphorylated receptor tyrosine kinases via Grb2 results in the translocation of Sos from the cytosol to the plasma membrane, where its target, Ras, is localized. Interaction of Sos with Ras at the plasma membrane further increases its activity (Fig. 2).

The mechanism(s) whereby G protein-coupled receptors (GPCRs) regulate Ras activation and cell proliferation is not completely understood. It is clear that ligand binding to GPCRs in a variety of cellular systems leads to rapid tyrosine phosphorylation of the Shc adaptor protein, followed by the formation of Shc-Grb2 complexes. The candidate kinases responsible for Shc phosphorylation are Pyk2 and Src-like kinases, which link Gβγ subunits to ►MAP kinase activation through phosphorylation of Shc and subsequent complex formation of Shc with Grb2-Sos. Transactivation of the ►epidermal growth factor (EGF) receptor



RAS Activation. Figure 1 Cycling of the Ras protein between the inactive GDP-bound form and the active GTP-bound form. Binding of growth factors to their receptors promote formation of active, GTP-bound Ras. This is achieved through the action of guanine nucleotide exchange factor (GEF), which stimulates the dissociation of the tightly bound GDP from Ras. Hydrolysis of bound GTP is accelerated by GTPase activating protein (GAP).



RAS Activation. Figure 2 Activation of Ras following ligand-binding to receptor tyrosine kinases (RTKs) or G protein-coupled receptors (GPCRs). Three distinct GEFs specific for Ras have been identified: Sos1/2, RasGRF1/2, and RasGRP1–4. Binding of growth factor (e.g. epidermal growth factor) to its RTK initiates dimerization and autophosphorylation of the receptor and subsequent recruitment of the Grb2/Sos complex to the plasma membrane, where Ras activation occurs. GPCRs that signal through heterotrimeric G proteins can activate all three classes of GEFs. The Gβγ subunit may activate tyrosine kinases that through Shc phosphorylation recruit Grb2/Sos complex to Ras at the plasma membrane. In addition, the Gβγ subunit can also stimulate the activity of RasGRF1. Calcium signals triggered by GPCRs seem to be required for the activation of both Ras GRFs. Finally, RasGRPs, that contains calcium- and diacylglycerol-binding domains, may also contribute to Ras activation downstream of either RTKs or GPCRs.

may also contribute to Ras activation downstream of GPCRs. Two other Ras exchange factors, the brain-specific RasGRF1 and the more ubiquitously expressed RasGRF2, also participate in the signaling events from GPCRs to Ras activation. In addition to their catalytic domain, both RasGRFs contain calcium and calmodulin-binding motifs.

The third class of the Ras exchange factor family is the Ras guanine nucleotide releasing proteins (RasGRP). Although they have different tissue expression profiles, all members contain a C1 domain that binds diacylglycerol (DAG) and DAG analogs such as the tumor promoting phorbol esters. ►Protein kinase C (PKC) family members can phosphorylate RasGRPs resulting in their further activation.

Two distinct GTPase activating proteins (GAP) for Ras have been identified; p120GAP is widely expressed, while neurofibromin is present predominantly in cells of the nervous system. Both GAPs stimulate the weak endogenous rate of Ras GTP hydrolysis, thereby negatively regulating signaling via Ras.

In addition to the plasma membrane, Ras proteins can be activated on intracellular membranes such as the Golgi apparatus, endoplasmatic reticulum, and mitochondrion. Ras is directed to those membrane compartments by posttranslational modifications, including palmitoylation and farnesylation. On the Golgi membranes, Ras activation is mediated by RasGRP1 that is activated in a calcium- and diacylglycerol-dependent manner.

Clinical Aspects

Mammalian Ras proteins have been studied in great detail because mutant Ras proteins are associated with many types of human cancer. These mutant proteins are permanently in the GTP-bound, active state and can cause neoplastic transformation. The point mutations found in Ras genes fall into two functional groups: those affecting codons 12, 13, 59, 61 and 63, reduce the rate of hydrolysis of GTP on Ras protein and critically block its stimulation by GAPs, and those at codons 116, 117, 119 and 146 increase the rate of nucleotide exchange.

One of the Ras GAPs, neurofibromin, is the product of the NF1 gene. Damage to this gene has been implicated in the hereditary disease Von Recklinghausen neurofibromatosis (►neurofibromatosis type 1), which is characterized by a number of developmental defects including benign and malignant tumors of neural crest origin, such as neurofibromas and neurofibrosarcomas.

Germline activating mutation can occur in genes encoding Ras proteins, and other components of the pathway, leading to development defects. ►Costello syndrome, characterized by a complex developmental disorder involving characteristic craniofacial features, failure to thrive, developmental delay, cardiac and skeletal anomalies and a predisposition to develop neoplasia, both benign and malignant, can result from germline mutational activation of H-Ras. ►Noonan syndrome is an autosomal dominant disorder presenting with characteristic facies, short stature, skeletal anomalies, congenital heart defects, and predisposition to tumors. This syndrome is caused by activating germline mutations in K-Ras, Sos1, or a SH2 domain-containing phosphatase (PTPN11).

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Ras Association Domain Family 1

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Synonyms

RASSF1

Definition

The Ras association domain family 1 (*RASSF1*) is a member of a new family of ►RAS or RAS-like small GTPases effectors. There are five other bonafide RASSF members all harboring a C-terminus Ras-Association (RA) and Salvador/RASSF/Hippo (SARAH) domains. Genome databases have classified two proteins as RASSF7 and RASSF8 where the RA domain is located at the N-terminus. *RASSF1* also exists in at least seven isoforms generated by alternative splicing or promoter usage. Only the longest isoform, isoform A, harbors an additional cysteine-rich diacylglycerol (DAG or C1) lipid-binding domain. The RA domain shares a similar structure with the highly characterized Ras-binding protein, Raf. However, unlike Raf, RASSF1 and the other RASSF proteins lack catalytic domains and they are more likely to function as adaptors to recruit additional proteins. The SARAH domain is thought to form a coiled-coil region that mediate homo- and heterotypic interactions with other SARAH domain-containing proteins SAV1 and MST1 and MST2 (Fig. 1).

Characteristics

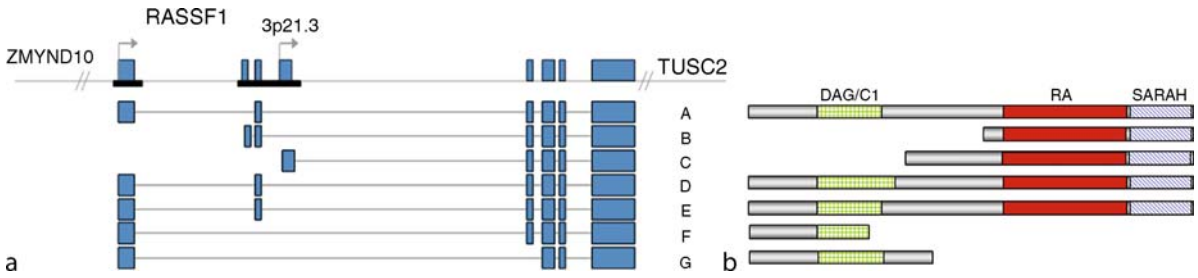
RASSF1A is a novel tumor ►suppressor gene. It is inactivated in a large subset of tumor types by ►hypermethylation of its ►promoter region. The mechanisms of action of this protein are not fully elucidated but it has been implicated in ►cell cycle control, ►apoptosis, cell ►migration, and control of ►mitosis.

RASSF1A as a Tumor Suppressor Gene

Interest in *RASSF1A* started with the consistent observation that alterations of the short arm of chromosome 3 (3p) is one of the most common and earliest events in the pathogenesis of ►lung cancer. Frequent and early loss of heterozygosity and the presence of homozygous deletions, especially at 3p21.3, suggested that at least one tumor suppressor gene may reside in this region. The detection of overlapping homozygous deletions in cancer cell lines narrowed the region of interest to 120 kb. The minimal region contained eight genes; hence mutation and expression analysis was focused on these eight genes. Despite extensive investigation, only rare tumor-associated mutations have been identified in lung, breast, kidney, and nasopharyngeal cancer. Interestingly, however, expression of *RASSF1A*, but not *RASSF1C* is lost or downregulated in a variety of tumors. The main cause for this loss of expression is promoter ►CpG island hypermethylation. *RASSF1A* inactivation by hypermethylation of its promoter region was originally described in lung and breast cancers. Since then it has emerged that *RASSF1A* is one of the most frequently switched-off genes in over 40 cancer types (Table 1).

RASSF1A Methylation as a Cancer Biomarker

Tumor-specific ►methylation of *RASSF1A* in a very broad spectrum of tumor types, in moderate-to-high frequencies makes it an ideal cancer ►biomarker suitable for diagnosis and prognosis. In addition, it is also possible to detect *RASSF1A* methylation in body fluids extracted via noninvasive procedures, such as sputum for lung cancer, nipple aspirate fluids for breast cancer, urine for bladder and kidney cancers, and even DNA collected from tampons for endometrial cancer. An example of a possible clinical application for the



Ras Association Domain Family 1. Figure 1 *RASSF1* is expressed from its locus on chromosome 3p21.3 in multiple isoforms (a) that result in the expression of several proteins (b).

Ras Association Domain Family 1. Table 1 Comparison of *RASSF1* and *TP53* inactivation frequencies in human cancers

Cancer type	*%TP53* mutation[a]	*% RASSF1A* methylation[b]
Lung	45	78 (SCLC)
Breast	48	55
Prostate	40	75
Kidney	29	55
Liver	60	92.5

[a]COSMIC, catalogue of somatic mutation in cancer.
[b]Average of percentages reported in literature.

detection of *RASSF1A* methylation is the screening of populations at risk of lung cancer, such as smokers. The lifetime risk of people who never smoked developing lung cancer is 1 in 10,000 whereas for current and former chronic smokers the yearly risk is 3 in 1,000. About 1 in 5 smokers suffering from lung cancer show *RASSF1A* methylation compared with 1 in 100 nonsmokers with lung cancer. The frequency of *RASSF1A* methylation also increases with heavy smoking. Regular screening for *RASSF1A* methylation in sputum, bronchoalveolar lavages and serum from populations at risk, particularly smokers and lung cancer survivors, may enable earlier detection of lung cancer and the reduction of lung cancer mortality.

RASSF1A methylation in benign growths or hyperplastic lesions can be used as a means of predicting breast cancer risk. *RASSF1A* methylation was found in 70% of samples of benign breast tissues from unaffected women at high-risk for breast cancer, but in only 29% of samples from women at low/intermediate-risk. Demonstrating that in benign breast epithelium, *RASSF1A* promoter methylation is associated with epidemiological markers of increased breast cancer risk. Detection of *RASSF1A* methylation probably means a bad prognosis for a lung cancer patient. Studies have shown that ►NSCLC patients with *RASSF1A* methylation suffer from significantly reduced survival rates compared with patients without *RASSF1A* methylation. This is possibly due to the fact that tumors with *RASSF1A* methylation tend to be more aggressive and metastatic, have increased vascular invasion, pleural involvement, and poor differentiation. *RASSF1A* methylation, therefore, would provide a powerful marker for patient prognosis at an early stage of lung cancer development. Other studies have also suggested that *RASSF1A* methylation may offer a marker for chemotherapeutic drug resistance (cisplatin and tamoxifen) in some tumors and could be monitored throughout the course of treatment to adjust the therapeutic regimen accordingly.

Functional Characterization of RASSF1A

Investigation of RASSF1A has revealed it to be an ►adaptor protein with diverse functions including the regulation of ►apoptosis and ►microtubule dynamics during mitotic progression. The ability of RASSF1A to suppress growth in vitro and in vivo as well as increase susceptibility of Rassf1a-negative mice indicates that it has tumor suppressive functions. Overexpression of RASSF1A results in cell cycle arrest and is accompanied by dramatic changes in gene expression. This includes changes to the expression of important cell cycle regulatory genes such as ►*cyclin D* but also to genes involved in diverse functions including transcription, cytoskeletal organization, ►angiogenesis, signaling, cell ►adhesion, cell ►migration, and apoptosis. RASSF1A regulates apoptosis via at least two pathways. RASSF1A binds the proapoptotic serine/threonine kinases ►MST1 and MST2. The MST1/2 ►ortholog in the fruit fly is called hippo. Hippo functions as a tumor suppressor in fruit fly through its activation of another

kinase, Warts. Warts blocks the cell cycle by preventing the expression of cyclin E and promotes apoptosis by downregulating the antiapoptotic agent, Diap1. The Hippo–Warts pathway is conserved in mammals where Warts have two conserved orthologs, LATS1 and LATS2. Both of these kinases are considered tumor suppressor genes. The fly RASSF ortholog, cg4656, functions in the Hippo pathway, but unlike the mammalian RASSF, it does this through inactivating Hippo. This may suggest that a member of the mammalian RASSFs may have a similar function, but that is yet to be demonstrated since RASSF1A is more likely to be an activator of MST1/2. RASSF1A may also regulate apoptosis in an MST-independent manner through modulator of apoptosis 1 (MOAP-1). MOAP-1 associates with ►Bcl-2 family members Bax and Bcl-2 and initiates caspase-dependent apoptosis when overexpressed. RASSF1A is also involved in the regulation of microtubules dynamics and colocalizes with microtubules, spindles, and centrosomes during metaphase and promotes microtubule stability and polymerization. RASSF1A microtubule association may be mediated by interaction with C19ORF5/MAP1S. MAP1S has been shown recently to function as a spindle-associated microtubule-associated protein with an activity required for correct spindle pole formation and mitosis. RASSF1A has been controversially suggested to control the spindle checkpoint through binding to ►Cdc20, which negatively regulates ►anaphase-promoting complex (APC) and subsequently causes a mitotic block prior to anaphase. Mouse embryonic fibroblasts derived from mice engineered for complete loss of Rassf1a suffer from cytokinesis defects. This aberrant stage of cell division is attributed to inaccurate control of the LATS1/2 kinases due to the loss of Rassf1a. These cells are also more motile and morphologically different than control cells.

Another aspect of RASSF1A function is its relationship with ►oncogenic RAS. Due to the presence of the RA domain in all members of the RASSF family, it has been predicted that they bind and negatively control RAS activation or mediate the proapoptotic functions of oncogenic RAS. However, a conclusive evidence for the association of RASSF1A with RAS remains to be demonstrated. Where one study showed that both proteins interact directly, another showed that this interaction is not direct and it has to be mediated by RASSF5 (NORE1). It is intriguing to postulate that RASSF1A/RAS interaction is cell type and stimulus dependent. An example of the need of stimulation for the involvement of RASSF1 in the RAS pathways comes from the observation that RASSF1C is anchored in the nucleus by binding to DAXX. When cells are exposed to DNA-damaging agents like MMS and UV irradiation, DAXX is degraded allowing RASSF1C to leave the nucleus and attach to the microtubules. RASSF1C can then associate with H-RAS and participate in the activation of the SAPK/JNK pathway.

Conclusion

There is an accumulating body of evidence for the classification of *RASSF1A* as a tumor suppressor gene. However, there is a need to fully understand how it performs its suppressive functions and what cell signaling pathways are involved upstream and downstream of this molecule. *RASSF1A* is epigenetically inactivated by hypermethylation of its promoter in a large majority of cancers of various types, including adult and children's cancers. Hence, it may be a useful methylation marker across multiple malignancies for early detection of cancer and for prognostics and diagnostic purposes. Since hypermethylation is reversible, the search for targeting strategies that can reverse this block on *RASSF1A* expression may yield a very useful therapeutic approach to several types of cancers. Until the function of the RASSF1A protein is well understood, its methylation can serve as a biomarker for diagnosis and prognosis of various cancers using noninvasive methods of screening patient populations.

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RAS Gene

Definition

Gene and gene product originally detected in the genome of RNA tumor viruses (►retrovirus) causing sarcomas in laboratory rats (Rat sarcoma).

►RAS Transformation Targets
►RAS
►RAS Activation

Ras-Homologous Proteins

►Rho Family Proteins

RAS Transformation Targets

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Synonyms

RAS-regulated genes; RAS-responsive genes

Definition

The term "RAS transformation targets" is used for genes whose normal expression is either up-regulated or down-regulated by the permanent activation of ►RAS-mediated ►signal transduction in cancer cells. Critical target genes exert functions essential for the initiation or maintenance of malignancy, while other targets may be deregulated as a consequence of the ►transformation process.

Characteristics

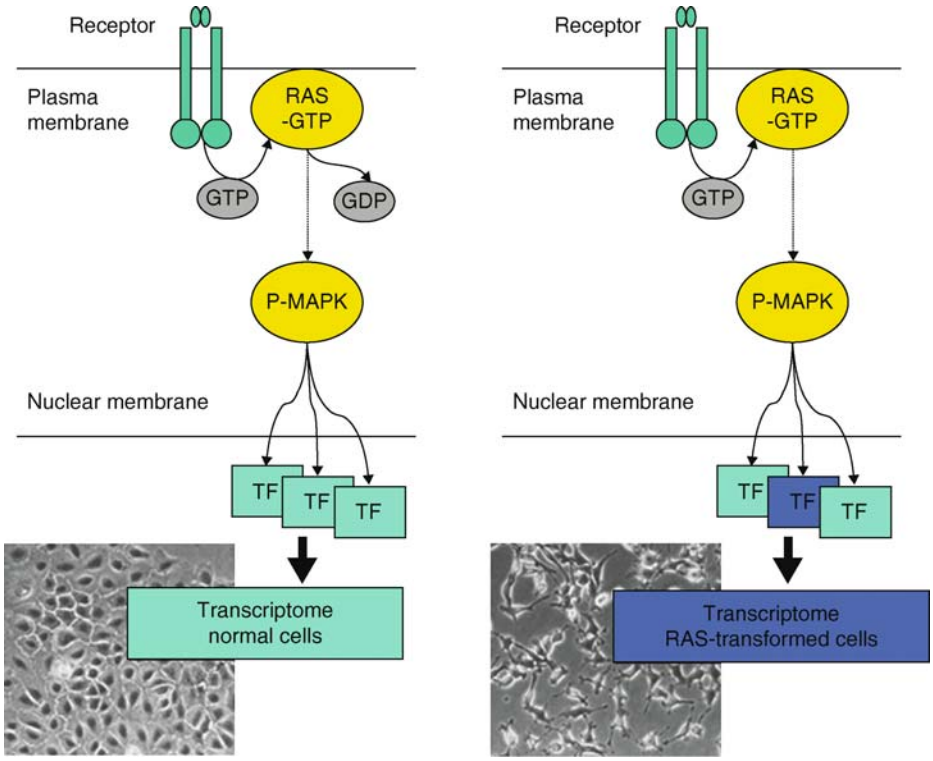
Target gene patterns specific for transformed cells may partially overlap with those obtained in normal cells stimulated with growth factors and in cells in which ►differentiation or ►senescence are induced via the RAS pathway. In a general way, the term "target" defines a gene whose ►transcription is turned on or off by a positive or negative regulator present in the nucleus. In eukaryotic genes, gene regulation is governed by binding of ►transcription factors to defined transcriptional control regions (motifs) on genomic DNA proximal to the coding sequence. RAS proteins are cytoplasmic signaling molecules attached to the inner face of the plasma membrane and thus mediate transcriptional alterations indirectly via a network of cytoplasmic effector molecules. There are short-term and long-term effects on the transcriptional response. Therefore, some authors prefer to designate only those genes as RAS targets that are immediately deregulated when a RAS ►oncogene is expressed (e.g. from a conditional ►promoter). Regardless of the timing of transcriptional alterations, there exist target genes whose transcriptional deregulation is fully or partially reversible, when the ►mitogen-activated kinase (MAPK) pathway, one of the most important RAS effector pathways, is blocked and the phenotypic aspects of cellular transformation are reversed.

Background

RAS pathway activation is a paradigmatic example for a cancer-inducing process at the cellular and molecular level. Cellular oncogenes of the RAS family were discovered in human and experimental tumors. Gene transfer studies (►transfection experiments) showed that mutationally activated RAS proteins induced characteristic morphological alterations in pre-neoplastic, non-tumorigenic cell lines. RAS transfectants showed features of cancer cells including anchorage-independent proliferation and rapid tumor formation following injection into nude mice. After the initial discovery of RAS oncogene function in ►cellular transformation assays, refined experimental approaches showed that cellular transformation requires more than a single genetic alteration. The transformation process requires cooperating oncogenes and the inactivation of ►tumor suppressor genes in normal cells. Genetic analysis of human tumors indicated that RAS activation occurs in conjunction with tumor suppressor gene loss and epigenetic changes. Cellular transformation by RAS is invariably coupled with profound alterations of gene expression (Fig. 1). For this reason, identification of the genes (targets) which respond to the expression of RAS oncogenes is of paramount importance for understanding the biological properties of cancer cells and the transformation process itself.

RAS Proteins Affect Gene Transcription via Cytoplasmic Effectors

►RAS genes encode ►small GTP-binding proteins located at the inner face of the plasma membrane. The RAS proteins affect gene transcription in a global way by acting as major switches in signal transduction processes that couple extra-cellular stimuli, e.g. elicited by growth factors, with the transcriptional machinery (Fig 1). Briefly, the signaling pathway is made of a branched chain (cascade) of interacting proteins located at the cell surface (►receptor tyrosine kinases) and in the cytoplasm (signaling kinases, small GTP-binding proteins). Shuttling of phosphorylated signaling kinases into the nucleus induces transcriptional changes by phosphorylating transcription factors. In normal cells, the RAS signal transduction mediates proliferation, developmental processes and differentiation upon external stimulation. In resting cells, RAS proteins are GDP-bound and inactive. RAS proteins are switched on (activated) by a complex set of subsequent protein interactions initiating with the binding of the ►ligand (e.g. a growth-stimulating polypeptide or hormone outside the cell) to a receptor tyrosine kinase at the cell surface and terminating with the loading of GTP catalyzed by nucleotide exchanger proteins. RAS proteins are switched off (inactivated) by hydrolysis of GTP to GDP. Inactivation is catalyzed by GTPase-activating effector proteins.



RAS Transformation Targets. Figure 1 Differential effects of RAS signaling on the transcriptome. *Left*: In normal cells, the molecular RAS switch is activated via a protein complex of tyrosine kinase receptors, adapter proteins and nucleotide exchangers (ON-state) and then deactivated by GTP hydrolysis (OFF-state). Signaling to downstream cytoplasmic effectors such as the mitogen-activated protein kinase (MAPK) and nuclear targets (multiple transcription factors, TF) is transiently activated and rapidly terminated. The cartoon is highly simplified. Most of the elements of cytoplasmic signaling have been omitted. P-MAPK indicates that the signaling kinases are activated by phosphorylation. *Insert*: rat ovarian surface epithelial cells (phase contrast). *Right*: In transformed cells, the molecular RAS switch is locked in its active, GTP-bound state, generating a permanent stimulus. By transcriptomic profiling, various researchers have discovered profound alterations of gene expression in transformed versus normal cells. Critical RAS pathway targets execute the malignant properties of cells. *Insert*: KRAS-transformed rat ovarian surface epithelial cells.

Oncogenic forms of RAS are locked in their active, GTP-bound state and transduce signals essential for cellular transformation, cell survival, ►angiogenesis, ►invasion and ►metastasis. The transformed cellular features are mediated by several branches of the signaling pathway, each triggered by a different effector protein interacting with GTP-bound RAS. These involve the mitogen-activated protein kinase ►RAF that subsequently activates MEK (MAPKK) and ►ERK (MAPK), the small GTP-binding proteins RAC and ►RHO, phosphatidyl inositol-3-kinase (►PI3K) and others. Transcription factors stimulated in RAS-transformed cells comprise among others the ►ETS-domain transcription factor ►ELK1, serum-responsive factor ►SRF, the leucin zipper protein ►JUN, activation transcription factor 2 (►ATF2), and ►nuclear factor κB (NF-κB). The diversity of factors involved explains the overall complexity of the transcriptional response to oncogenic RAS. Moreover, cell-type specific characteristics, cross-talk of individual pathways downstream of RAS and the duration of signaling stimulation modulate the transcriptional program.

Identification of RAS Transformation Targets

Cellular ►transcriptomes comprise between 5,000 and 15,000 expressed genes at a time, depending on the state of growth, development and differentiation. The common approach for RAS target isolation takes advantage of reproducible mRNA expression differences between normal and RAS-transformed cells. Differentially expressed elements were initially identified on a single gene basis and later recovered by various differential cloning techniques and by interrogating ►microarrays. The experimental strategies for establishing catalogues of RAS-responsive genes vary significantly. Some authors have contrasted expression profiles representative for mRNA steady states related to the normal state and to RAS transformation. Others have used conditional

oncogene expression systems and followed the transcriptional alterations in a time-dependent fashion. RAS pathway targets were also identified in given tumor lines without matching normal precursors by contrasting the expression profile of inhibitor-treated cells and controls. In genome-wide screens for expression differences, a few percent of the total number of expressed transcripts were found to be affected by RAS pathways. Recent progress in ►proteome analysis at high resolution and sensitivity allowed to extend expression studies to the protein level as well.

A Selected List of RAS Transformation Targets

A recent review on the global effects of Ras pathway on transcription summarizes 17 studies based on cDNA subtraction and microarray analysis. For example, a proto-typic genome-wide study lists 393 genes differentially expressed in normal rat fibroblasts versus a RAS-transformed derivative. The targets include 168 up-regulated and 225 down-regulated genes. A meta-analysis comparing all available target gene catalogues is still missing, mainly because data were retrieved on different microarray platforms, under different experimental conditions and with different types of cells. The RAS targets included in Tables 1 and 2 have been chosen for reasons of independent validation and of their specific contribution to the process of tumorigenesis. Thus, the list is highly selective rather than comprehensive. An important cautionary note is that many target genes identified in model systems have not been thoroughly analyzed in human cancers, hence, their general importance is far from being clear. The finding of differential expression in transformed cells as compared to normal cells does not prove a causal role in the transformation process. Verification experiments based on forced expression or gene silencing are required. For example, the functional role of ►matrix metalloproteinase genes was verified by determining their effects on the invasive properties of cancer cells using cell biological assays. Functional studies of differentially expressed genes retrieved in genome-wide expression surveys will be complemented by currently available genetic screens based on ►siRNA libraries.

Clinical Relevance

Permanent activation of the RAS signaling cascade due to mutation in RAS genes or caused by overexpression of cell-surface receptors is a general feature of tumorigenic cells and determines a bad prognosis of many tumors. The malignant properties of cancer cells are executed by an unknown number of RAS pathway-induced genes, some of which are common

RAS Transformation Targets. Table 1 Genes up-regulated by RAS-mediated signaling

Gene product	Gene name	Functional role of gene product
Ornithine decarboxylase	*ODC1*	Polyamine biosynthesis, essential for proliferation
Glucose transporter	*GLUT*	Glucose metabolism, energy supply
v-Jun avian sarcoma virus 17 oncogene homolog	*JUN*	Transcription factor, component of AP-1 transcriptional activator complex, gene regulation
►Cyclin D1	*CCND1*	►Cell cycle stimulation
►Transforming growth factor-β	*TGFB1*	Autocrine growth stimulation
►Matrix metalloprotease 3 (Stromelysin 1)	*MMP3*	Metalloproteases, stimulate invasive properties
Matrix metalloprotease 1 (collagenase)	*MMP1*	
Urokinase-type ►plasminogen activator	*PLAU*	Controls synthesis of the serine protease plasmin, degradation of ►extracellular matrix
►Cyclooxygenase-2	*COX2/ PTGS2*	Prostaglandin synthesis, necessary for cell survival
►Vascular endothelial growth factor	*VEGF*	Stimulates angiogenesis
Integral cell membrane glycoprotein ►CD44	*CD44*	Induces metastasis
Ras homolog gene family member C (►RhoC)	*RHOC*	Small GTPase, induces metastasis
Dual-specificity phosphatases 5 and 6	*DUSP5/ DUSP6*	Dual specificity phosphatases, involved in feedback regulation of MAPK signaling
►Heparin-binding EGF-like growth factor	*HBEGF*	Autocrine growth stimulation

RAS Transformation Targets. Table 2 Genes down-regulated by RAS-mediated signaling

Gene product	Gene name	Properties
Myoblas differentiation factor 1	*MYOD1*	Stimulates myoblast differentiation, commitment to form myotubes
►Fibronectin (large external transformation-sensitive protein)	*FN1*	Structural component of extracellular matrix
Smooth muscle α-actin	*ACTA2*	Structural component of cytoskeleton
Type I collagen α	*COL1A1*	Structural component of cytoskeleton, cytoskeletal tumor suppressor
Lysyl oxidase	*LOX*	Extra-cellular enzyme, stabilizes extra-cellular matrix, regulates NF-κB signaling
►Thrombospondin 1	*THBS1*	Inhibits angiogenesis
Growth-arrest specific protein 1	*GAS1*	Growth inhibitor
►Tissue inhibitor of metalloproteinase 2	*TIMP2*	Inhibits metalloproteases and invasive properties
Fas antigen (CD95)	*APO1/APT1/ TNFRSF6*	Inducer of ►apoptosis
►E-cadherin	*CDH1*	Cell-cell adhesion, suppresses invasion and epithelial to mesenchymal transition
►TGF-β receptor type II	*TGFBR2*	Mediates growth inhibition by TGF-β
►Connexin 43 (►Gap junction protein α-1)	*CX43/GJA1*	Controls gap junctional communication
►Wilms tumor 1	*WT1*	Wilms tumor suppressor, suppresses Ras-induced transformation
Cystein-rich acidic secreted protein 1 (Osteonectin)	*SPARC/ON*	Extracellular protein, tumor suppressor
Suppressor of tumorigenicity 5 (Hela tumor suppression 1)	*ST5/HTS1*	Suppressor of neoplastic transformation, inhibits stimulation of MAPK/ERK2 by EGF
Follistatin-related protein (Follistatin-like 1)	*FSTL1/FRP*	Activin-binding protein regulated by TGF-β, causes growth arrest and inhibits invasion in cancer cell lines
H-Ras revertant protein 107	*HRSL3/H-REV107-1*	Interferon-γ inducible protein, binds to protein phosphatase 2A, induces apoptosis

pathway targets, while others differ among various types of cancers. Thus, genome-wide identification of target genes executing the diverse repertoire of biological activities of RAS-expressing tumor cells will increase our knowledge about the systems biology of cancer cells as well as aid in defining novel tumor markers and therapeutic targets.

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Rasburicase

Definition

Is a recombinant urate oxidase enzyme that catalyses the conversion of uric acid to allantoin, a more soluble substance, which is excreted by the kidneys more effectively than uric acid. It is used for the prevention and treatment of ►tumor lysis syndrome in patients receiving ►chemotherapy for ►hematological malignancies, leukemias and lymphomas.

►Rituximab

RasGEF

Definition
Ras guanine exchange factor. A ►Ras activator.

RAS-Regulated Genes

►RAS Transformation Targets

Ras-Related Small GTPases

►Rho Family Proteins

RAS-Responsive Genes

►RAS Transformation Targets

RASSF1

►Ras Association Domain Family 1

Rat Podoplanin

►Podoplanin

RB1

Definition
Is a tumor suppressor gene. ►Homozygous functional inactivation results in childhood retinoblastoma.

►Retinoblastoma
►Senescence and Immortalization
►Tumor Suppression

RB2/p130

Definition
Is a member of the ►retinoblastoma family of tumor suppressor genes. To date, three members of the retinoblastoma family (RB/p105, p107 and RB2/p130) have been identified. One of its functions is to protect cells from uncontrolled growth.

►Retinoblastoma Protein, Biological and Clinical Functions

Reactive Oxygen Species

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Synonyms
Free radicals; *ROS*

Definition
Reactive oxygen species (ROS) are small molecule metabilites of oxygen that tend to participate in redox reactions because of their high reactivity. Redox reactions are divided into two chemical processes: oxidation and reduction. Oxidation is a chemical process to gain oxygen and lose hydrogen or electrons, whereas reduction is a chemical process to lose oxygen and gain hydrogen or electrons.

Characteristics
ROS are composed of free radicals and non-radicals, both inorganic and organic. Free radicals are a cluster of atoms that contain an unpaired electron in the outermost shell of electrons and are an extremely unstable configuration so that free radicals quickly react with other molecules or radicals to achieve the stable configuration of four pairs of electrons in their outermost shell (one pair for hydrogen). Typical free radicals include superoxide anion ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and peroxyl ($RO_2^{\bullet}$). Non-radicals do not contain an unpaired electron but are prone to exchanging electrons with other molecules. Typical non-radicals include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$) and lipid peroxides (LOOH).

Chemistry of Major *ROS*

1. $O_2^{\bullet-}$, a weak oxidant radical, is considered the "primary" *ROS*. $O_2^{\bullet-}$ is produced deleteriously by electron transfers in the mitochondrial electron transfer chain. Other enzymes capable of producing superoxide are the xanthine oxidase, NADPH oxidase and ►cytochrome P450. The life of $O_2^{\bullet-}$ is only milliseconds.
2. H_2O_2 is a more potent without an unpaired electron and has a longer life (minutes) than O_2^-. H_2O_2 is produced from $O_2^{\bullet-}$ by superoxide dismutase (SOD). H_2O_2 is also produced by a wide variety of enzymes including monoxygenases and oxidases. The properties of H_2O_2 include (i) being metabolized by catalase or glutathione peroxidase to H_2O; (ii) being permeable through the plasma membrane to extracellular sides; (iii) reacting with the reduced transition metals (i.e., Fe^{3+}) to become the highly toxic hydroxyl radical (HO^-) or a metal-peroxide complex (Me-OH) (*i.e.,* Fenny reaction).
3. Hypochlorite (HOCl) and hypobromite (HOBr) are produced in lysosome. Through complex redox reactions catalyzed by myeloperoxidase (MPO) in the presence of chloride (Cl^-) or bromide ion, HOCl or HOBr is generated by degrading H_2O_2 to O_2 and H_2O. These ions play an important role in carcinogenesis and allergic reactions.
4. Peroxynitrite anion ($ONOO^-$), a short-lived but highly oxidizing *free radical*, is a product of reaction between $O_2^{\bullet-}$ and nitric oxide (NO) and plays a role in nitrosylation of proteins.

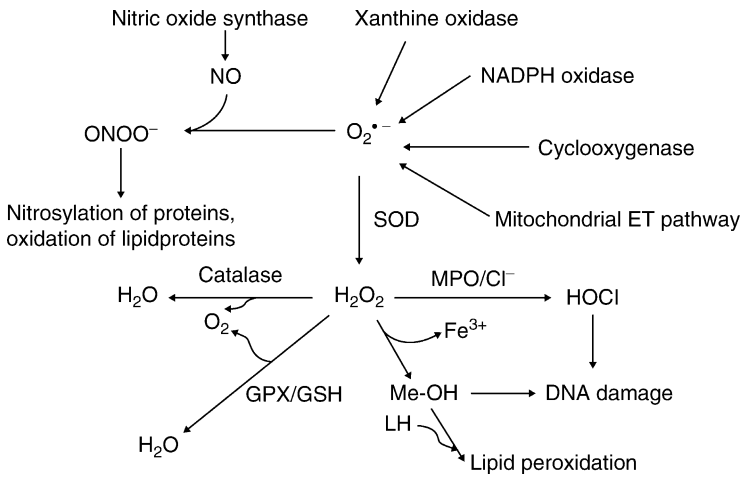
Generation and *Scavenger* of ROS

1. Endogenous generation. Mitochondria produce a large amount of $O_2^{\bullet-}$ and H_2O_2. The source of mitochondrial *ROS* appears to involve a non-heme iron protein that transfers electrons to oxygen. This occurs primarily at complex I and, to a lesser extent, following the auto-oxidation of coenzyme Q, at complex II or III. $O_2^{\bullet-}$ is produced deleteriously by 1-electron transfers in the mitochondrial electron transfer chain. In addition, the NADPH oxidase serves as another major source of ROS and generates superoxide *via* one electron transfer from NADH or NADPH to oxygen. It is comprised of membrane-bound subunits, $gp91^{phox}$ and $p22^{phox}$, and cytoplasmic subunits, $p47^{phox}$, $p40^{phox}$, $p67^{phox}$, and GTPase Rac1 or Rac2. The catalytic subunit $gp91^{phox}$, also termed Nox2, has several homologues, Nox1, Nox3 through 5, the location of which is cell-type specific. The NADPH oxidase generates ROS in a regulated manner, producing $O_2^{\bullet-}$ in response to growth factors, transduction signaling and cytokines. Moreover, the xanthine oxidase is a cytoplasmic oxidase that produces $O_2^{\bullet-}$ and H_2O_2 when converting hypoxanthine and xanthine into uric acid. This reaction may serve as an important source of ROS in a variety of pathophysiological states, including hypoxia, hypertension, atherosclerosis and heart failure. Other sources of *ROS* may include cyclooxygenases and cytochrome P450 metabolism; peroxisomes also contribute to a certain amount of ROS generation.
2. Exogenous insults. Enviromental insults leading to oxidative stress include radiation, ozone, herbicides, ►xenobiotics (i.e., ►polycyclic aromatic hydrocarbons and ►phorbol esters) and ferritin, all of which are capable of inducing steady-state increases in *ROS* production.
3. Anti-oxidant systems. Cells are normally able to defend themselves against *ROS* damage through their scavenger systems: (i) enzymes such as ►superoxide dismutase (SOD), catalases and ►glutathione peroxidase (GPX) with glutathione (GSH); (ii) transition metal chelators; (iii) polyphenol antioxidants such as small molecules tocopherol (►Vitamin E); (iv) ascorbic acid (►vitamin C), uric acid, and glutathione also play significant roles as cellular antioxidants. The most important plasma (extracellular) antioxidant in humans is probably uric acid. Clinically, there is a significant difference between cancer incidence and controls in the GSH-related enzyme function and the ratio of the reduced form of glutathione (GSH) over the oxidized form of glutathione (glutathione disulfide, GSSH).

Oxidative Stress

ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in the normal cellular signaling, which includes delivery of electrons across membranes, heme oxidation, oxidative modification of proteins and DNA. Another important beneficial effect of ROS is the physiological role in the defense against infectious pathogens. However, under environmental stress or certain pathological conditions such as hypoxia, intracellular ROS levels can increase dramatically, leading to the formation of ►oxidative stress. Oxidative stress exerts significant harmful effects on cell structures by inducing structural changes in lipids, membranes, proteins or nucleic acids. Oxidative stress also induces significant changes in signal transductions (Fig. 1).

1. Oxidative nuclear and mitochondrial ►DNA damage. The hydroxyl radical is known to react with all components of the DNA molecules in nucleus and mitochondria, damaging both the purine and pyrimidine bases. The hydroxyl radical inserts to DNA double bonds of DNA bases as well as the deoxyribose backbone. Permanent modification of genetic material resulting from these "oxidative damage"



Reactive Oxygen Species. Figure 1 Metabolism of reactive oxygen species. $O_2^{\bullet -}$, is considered the "primary" reactive oxygen species and mainly generated by mitochondria, NADPH oxidase, cyclooxygenase and xanthine oxidase. On the one hand, $O_2^{\bullet -}$ can rapidly react with NO to form highly reactive $ONOO^-$ that can modify protein targets. On the other hand, $O_2^{\bullet -}$ is dismutated by SOD to H_2O_2, which has three major fates: (i) H_2O_2 is most efficiently scavenged by catalase, as well as glutathione peroxidase (GPX), into H_2O; (ii) H_2O_2 is broken down by some transition metals to reactive hydroxyl radical (Fenton reaction), which plays an important role in DNA damage or lipid peroxidation; (iii) H_2O_2 is degraded by myeloperoxidase (MPO) to O_2 and H_2O, generating HOCl in the presence of chloride (Cl^-).

incidents represent the first step in mutagenesis, carcinogenesis and ageing. DNA damage may result in the arrest of transcription, replication errors and genomic instability, all of which are linked with carcinogenesis.

2. The hydroxyl radical is able to initiate ►lipid peroxidation. The metal-induced generation of oxygen radicals also results in the attack of the bilayer membrane lipid components, including polyunsaturated fatty acid residues of phospholipids. Hydroxyl radicals are generated *via* Fenton chemistry, to initiate lipid peroxidation. As a consequence, lipid peroxidation leads to structural changes in the membrane, formation of adducts or crosslinks with non-lipids and disruptions of membrane-dependent signaling. This deleterious process of the peroxidation of lipids is apparent in cancer, inflammation and arteriosclerosis.
3. Protein modification. Peroxynitrite anion ($ONOO^-$) is able to nitrosate the cysteine sulphydryl groups of proteins, to nitrate tyrosine and trytophan residues of proteins and to oxidise methionine residues to methionine sulphoxide. As a consequence, these protein thiol oxidations result in the oxidation of the catalytic sites of proteins, formation of sulfide bonds and increased susceptibility to proteolysis.
4. Signal transduction. Oxidative stress plays an important role in the regulation of cell growth because the cell cycle is regulated by intracellular concentrations of GSH. *ROS* can activate the cell growth transcription factors including MAP-kinase/AP-1, NF-κB and p53 pathways that have a direct effect on cell proliferation and apoptosis. *ROS* also regulates protein kinase or tyrosine kinase activities.

Clinical Aspects

1. Oxidative burst is characterised by massive production of ROS during bacterial infections and plays a key role in the defense against invading pathogens. Phagocytosis of micro-organisms is the major impetus for activation of the $gp91^{phox}$-containing NADPH oxidase in neutrophils and macrophages, leading to the production of a large amount of $O_2^{\bullet -}$ and its secondary metabolites. ►Chronic granulomatous disease, a rare genetic disorder, is caused by defects in $gp91^{phox}$, $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ and results in inactive NADPH oxidase and recurrent infections.
2. Oxidative DNA lesions and cancers. When ROS are overproduced or expression of antioxidants is altered, an imbalance between pro-oxidants and anti-oxidants prevails in cancer cells. As a consequence, *ROS* induce the formation of 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG), which is the best example of the oxidative stress-induced damage to nucleobases and sugar moieties of DNA and RNA. This damage can cause single- or double-strand breaks, to point and frameshift mutations and to chromosome abnormalities, which may be linked to initiation of cancer. This lesion is clinically important because it is a potential biomarker of carcinogenesis. In addition, the reaction of HOCl with various nucleosides yields chlorinated

nucleosides, including 8-chloro-2′deoxyqunosine, 8-chloro-2′deoxyadenosine and 5-chloro-2′-deoxy-cytidine. DNA oxidation also triggers DNA repair, imbalancing DNA repair enzymes or inducing error prone polymerases.

3. ROS-regulated signal transduction and cancers. Furthermore, oxidative stress can promote cancer cell growth and/or metastasis by activating the growth factor or protein kinase pathway where the transcription factors mitogen-activated protein (MAP)-kinase; activation protein 1 (AP-1), NF-κB and p53 are involved. As a signaling messenger, ROS are able to oxidize the critical target molecules such as protein tyrosine phosphatases (PTPs) and serine/threonine kinases such as protein kinase C (PKC). Inactivation of multiple PTPs by *ROS* may relieve the tyrosine phosphorylation-dependent signaling, triggering directly non-receptor protein kinases and eventually initiating MAP-kinase, NF-κB and p21-activated kinase-1 (PAK) pathways. PAK is an effector of Rac-mediated cytoskeletal remodeling that is responsible for cell migration and angiogenesis. All these ROS-regulated signal transductions are major pathways for driving tumor cell growth and metastasis. In addition, ROS also activate serine/threonine PKC, a member of the tumor growth factor-β (TGF-β) superfamily. MAP-kinase and AP-1 can be activated by PKC and TGF-β1, respectively, in a *ROS* dependent manner, resulting in cancer proliferation and metastasis.
4. ROS and other clinical diseases. Oxidative stress plays a role in pathogenesis of many other human disorders. The ROS-induced oxidative stress plays an important role in various cardiovascular diseases such as atherosclerosis, hypertension, ischemic heart attack, stroke, and congestive heart failure. The major sources of oxidative stress for these diseases include NADPH oxidase, xanthine oxidase and nitric oxide synthase.The ROS-induced oxidative stress in cardiac and vascular cells has been linked with cardiovascular tissue injury. In addition, the pathogenesis of rheumatoid arthritis is associated with the free radical formation at the site of inflammation. Moreover, diabetes is characterized by glucose-induced ROS production. mainly from mitochondria and NADPH oxidase.

The brain is particularly sensitive to *ROS* because of its high oxygen utilization. It has been established that the pathogenesis of several neurodegenerative diseases, such as Alzheimer disease (AD) and Parkinson disease (PD), and ageing, is linked with ROS accumulation. The brain of AD patients is charactrized with a marked accumulation of amyloid-β peptide (Aβ). Aβ and amyloid precursor protein have strong Cu-reductase activity, generating Cu^{+} from Cu^{2+}. This electron transfer process leads to the production of H_2O_2. In the PD brain, one of the earliest detectable changes is a dramatic decrease in GSH in dopaminergic neurons in substantia nigra, resulting in mitochondrial complex I inhibition.

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Reactive Species Overload Diseases

Definition

Diseases associated with chronically high levels of reactive oxygen and nitrogen species in specific tissues or the whole body.

►Reactive Oxygen Species

Reactive Stroma

Definition

Describes the altered state of ►stromal cells near tumors.

►Aging

Real-Time PCR

Definition

PCR that follows the general pattern of a polymerase chain reaction, but the DNA is quantified after each round of amplification. It is used to simultaneously

quantify and amplify a specific part of a given DNA/cDNA molecule. It determines whether a specific sequence is present in the sample and the number of copies in the sample.

►Circulating Nucleic Acids
►Leukemia Diagnostics

RecA

Definition

RecA protein is central to homologous recombination in bacteria. It forms a DNA-protein filament utilizing the energy of ATP to catalyze homologous pairing of DNA molecules and strand exchange. It is the product of the recA locus of *E. coli*; a protein with dual activities, activating proteases and also able to exchange single strands of DNA molecules. The protease-activating activity controls the SOS response, and the nucleic acid handling activity is involved in recombination-repair pathways.

►Homologous Recombination Repair

RecA/Rad51-like Proteins

Definition

Share homology to the ►RecA (bacteria) and ►Rad51 (eukaryotes) proteins. They are involved in the repair of DNA damage by homologous recombination. In humans they include the Xrcc2, Xrcc3, Rad51L1, Rad51L2 and Rad51L3 proteins.

►Homologous Recombination Repair

Recall Bias

Definition

Systematic bias in a study, which can occur when participants are asked to report something (e.g. exposure to an agent or behavioral factors) after they already have been diagnosed with the disease under study

►Stress

Receptor

Definition

A protein that binds to a specific molecule (►ligand), such as a neurotransmitter, hormone, or other substance, and initiates the cellular response to the ligand.

►Kit/Stem Cell Factor Receptor in Oncogenesis
►Modular Transporters
►Receptor Cross-Talk
►Receptor Tyrosine kinases
►Nuclear Receptor
►Prostate-Specific Membrane Antigen (PSMA)

Receptor-Associated Coactivator 3

►Amplified in Breast Cancer 1

Receptor Clustering

Definition

Stimulation of many receptors results in a re-organization of activated receptor molecules and the aggregation within a defined area named receptor clustering.

Receptor Cross-Talk

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Synonyms

Receptor interactions

Definition

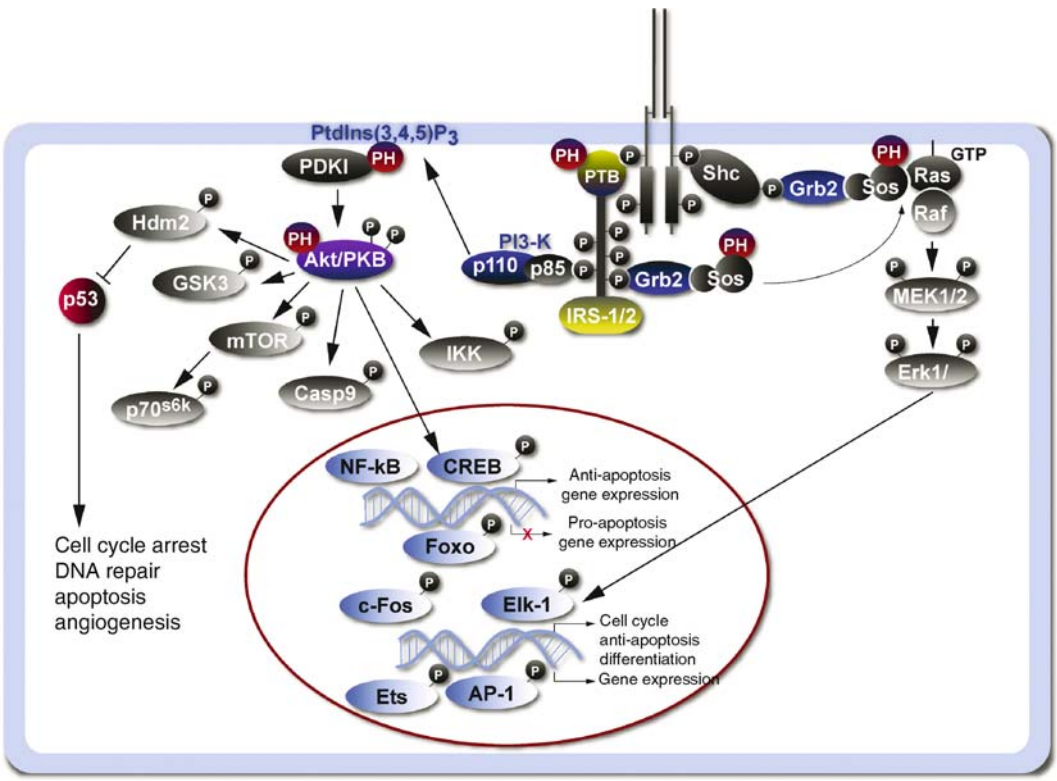
Refers to the ability of a given receptor, whether a cell surface ►receptor tyrosine kinase (►RTK), ►G-protein coupled receptor (GPCR) or ►nuclear receptor, to influence the signaling activity of a second receptor and/or its downstream cascade.

Characteristics

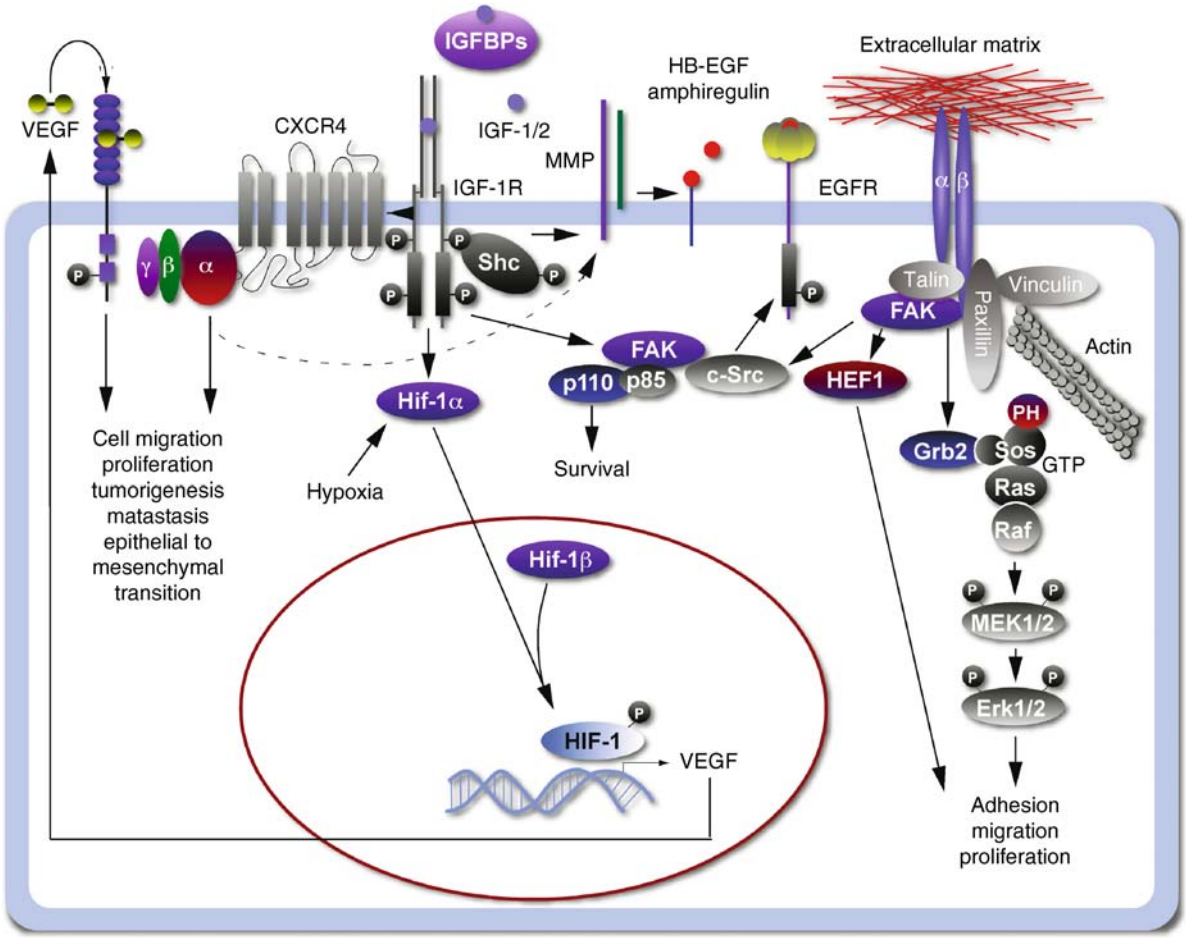
In its simplest terms, receptor cross-talk refers to the up- or down-regulation of receptors or their actions via the actions of heterologous receptors and their signaling intermediates. Over the years this has evolved to include a wide spectrum of receptor interactions both via direct protein:protein interactions and indirectly via interactions mediated by downstream effectors. Receptor cross-talk has been shown to influence cancer cell tumorigenicity, ►proliferation, ►metastasis, death and signaling to ►angiogenesis, particularly via growth factor receptor tyrosine kinases. Cell signaling pathways activated by the insulin-like growth factor 1 receptor (IGF-1R) (►Insulin-like Growth Factors) are shown in Fig. 1. The IGF-1R is an important regulator of cell survival and anti-apoptotic signaling (►Apoptosis; ►Apoptosis Signaling). Work over the last few years suggests that many actions of the IGF-1R are mediated via cross-talk to other RTKs (RTK) and G-protein-coupled receptors (GPCRs – ►G proteins) as illustrated in Fig. 2.

As shown in Fig. 2, IGF-1Rs can activate the epidermal growth factor receptor (EGFR/HER1/ErbB1 – ►HER2) by activating a cell surface proteinase (►ADAM Molecules; ►Matrix Metalloproteinases), that cleaves the membrane tethered ligand, heparin binding EGF (HB-EGF), enabling it to bind to the EGFR. This form of receptor cross-talk is an example of indirect activation, with the action of the first receptor (IGF-1R) stimulating the production of a ligand that acts in an autocrine manner to activate a second receptor (EGFR). A number of GPCRs indirectly activate the EGFR in this manner, either by proteinase release of membrane-bound ligand or by activating a Src family ►non-receptor tyrosine kinase (►SRC), which in turn phosphorylates the EGFR leading to its activation and downstream signaling.

The IGF-1R has been reported to activate the ►chemokine receptor CXCR4 by direct physical association, leading to enhanced migration of breast cancer cells (►Breast Cancer). The IGF-1R may also activate other GPCRs, such as the sphingosine-1-phosphate



Receptor Cross-Talk. Figure 1 Signaling by the IGF-1R. IGF-1 binding to the IGF-1R leads to receptor transphosphorylation and tyrosine phosphorylation of insulin receptor substrate-1 or 2 (IRS-1 or IRS-2) proteins. Phosphorylated IRS proteins serve as docking sites for SH2 domain containing effectors leading to their activation. As shown, following binding to phosphorylated IRS via its regulatory p85 subunit, the catalytic p110 subunit of PI3 kinase dissociates from the p85 subunit – IRS-1 complex. The p110 subunit generates inositol-3,4,5-phosphate at the plasma membrane, which serve as docking sites for pleckstrin homology (PH) domain containing proteins, such as Akt. Akt leaves the plasma membrane and phosphorylates a number of key cytoplasmic and nuclear effectors, leading to cell survival and anti-apoptotic signaling. IGF-1 can also activate the Erk family of mitogen activated protein kinases (MAPKs), to stimulate cell proliferation. IGF action is regulated by a number of transcription factors, some of which are shown.



Receptor Cross-Talk. Figure 2 Signal Cross-talk by the IGF-1R. In addition to directly activating the Erk leg of MAPKs, IGF-1R can also transactivate the EGFR as a result of ADAM proteinase activation. This releases membrane tethered HB-EGF from the cell surface, enabling it to bind EGFRs in an autocrine manner. In addition to indirect cross-talk, there is growing evidence that IGF-1Rs are capable of directly transactivating GPCRs such as CXCR4, to effect a chemokine receptor signaling in response to IGF-1 action. Cross-talk between integrins and growth factor receptor tyrosine kinases mediated by the non-receptor tyrosine kinase, FAK (focal adhesion kinase), that serves as a docking protein for SH2 domain containing effector proteins. In addition to autophosphorylation sites, FAK contains multiple tyrosine residues that are phosphorylated by Src. This underscores the bidirectional signaling paradigm occurring between integrins and RTKs that FAK performs.

receptor (►Lipid Mediators), a ►sphingolipid receptor that impacts cell proliferation and migration. In contrast, IGF-1R activation of the chemokine receptor CCR5, occurs indirectly as a result of IGF-1R induced synthesis and secretion of RANTES, the ligand for CCR5. A similar type of autocrine-based cross-talk occurs for prostaglandin E2 (►Prostaglandins) derived from the activation of COX-2. PGE2 acts at its cognate GPCRs, EP2 and EP4 receptors to increase cAMP levels and stimulate the expression of amphiregulin, an EGFR ligand. These events underscore the concept that IGF-1R signaling can synergize with GPCRs and other RTKs to modulate cell proliferation, tumorigenesis and metastasis.

In addition to IGF-1R cross-talk to the EGFR, EGFR activation can enhance IGF-1R signaling by modulating the expression of downstream mediators of IGF-1R action, including the ►insulin receptor substrate proteins (IRS). Similarly, estrogen and progesterone receptor signaling has also been shown to cross-talk to IGF-1R signaling by modulating IRS levels and MAPK sensitivity. Other nuclear receptors, such as peroxisome proliferator-activated receptors, may influence receptor, ligand or effector expression, resulting in defined receptor cross-talk actions.

Survival Signaling/p53 and Suppressor Cross-Talk

Growth factor RTK signaling leading to the activation of PI3 kinase (►PI3K Signaling) results in the formation of phosphatidyl-inositol-3,4,5 phosphate (►Inositol Lipids), a ►pleckstrin homology domain (PH domain) binding site in the inner leaflet of the plasma membrane.

Consequently, Akt (►Akt Signal Transduction Pathway in Oncogenesis) is recruited to the plasma membrane where its PH domain binds to PI3 enabling its phosphorylation and activation by phosphoinositide-dependent kinase 1 (PDK1). Activated Akt leaves the plasma membrane to effect anti-apoptotic signaling by phosphorylating key downstream effectors such as caspase 9, Bad and Mdm2. ►Mdm2 in turn destabilizes ►p53 by ►ubiquitination targeting it for ►proteasome degradation. In contrast, signaling activity that increases p53 activity results in cell cycle arrest, inhibition of angiogenesis and apoptosis. These actions are effected by a wide spectrum of p53 effectors, including IGFBP-3. Increased IGFBP-3 expression results in the sequestration of IGF-1 and IGF-2 leading to abrogation of IGF-1R action, further contributing to apoptotic signaling.

Signaling to Angiogenesis

A number of receptors have been shown to stimulate ►angiogenesis by regulating the production of ►vascular endothelial growth factor (VEGF), a key mediator of angiogenesis. VEGF mRNA expression and protein synthesis is tightly regulated by the ►hypoxia-inducible factor-1 (HIF-1) transcription factor, which is a heterodimeric complex comprised of Hif-1α and Hif-1β. Hif-1α is regulated by oxygen tension, with normoxia leading to Hif-1α ubiquitination and proteasomal degradation mediated by the von Hippel-Lindau ubiquitin ligase (►von Hippel-Lindau Tumor Suppressor Gene) and hypoxia stabilizing Hif-1α leading to VEGF synthesis and secretion. The IGF-1R, as well as other growth factor receptors can induce the expression of Hif-1α mRNA via Akt activation and elevate Hif-1α protein levels via $p70^{S6K}$/►mTOR activation, increasing VEGF expression. Secreted VEGF acts in an autocrine manner, to activate VEGFRs, leading to enhanced cell proliferation, cell survival or cell motility.

Integrin: Growth Factor Receptor Cross-Talk

Considerable cross-talk occurs between growth factor RTKs and ►integrins (►Cell Adhesion Molecules; ►Adhesion; ►Extracellular Matrix Remodeling). This area of signal cross-talk has significant implications in the context of cancer cell signaling as it relates to cell adhesion, cell migration and epithelial to mesenchymal transition and ►metastasis. Signals from ►extracellular matrix proteins are transduced to the cell interior as a result of integrin engagement, to influence cell adhesion, migration and proliferation. Integration of these signals with those from cell surface RTKs and GPCRs occurs at the level of the cytoplasmic non-receptor tyrosine kinase, ►FAK. FAK is localized to focal adhesions where in addition to signaling activities it fulfills an important role as a scaffolding protein. In addition to influencing cell survival and cell migration, FAK action also protects cells from suspension-induced cell death or ►anoikis. It is notable that elevated FAK activity has been linked to the progression of premalignant cells to malignant carcinoma, which coincides with an integrin switch exhibited by a number of cancer cell types. Taken together, these findings suggest a role for growth factor receptor:integrin cross-talk in this process. Altered integrin expression, particularly of β_1 species, can significantly influence IGF-1R signaling in cancer cells in which an apoptotic response is replaced by a proliferative phenotype.

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Receptor Interactions

►Receptor Cross-Talk

Receptor-Mediated Endocytosis

►Endocytosis
►Endosomal Compartments

Receptor Protein Tyrosine Phoshatase Beta/Zeta

Definition

RPTPβ/ζ; Is a member of a family of receptor-type transmembrane protein tyrosine phosphatases, known to exist in three ►splicing variants: a transmembrane

long, a truncated transmembrane short, and a secreted form termed phosphacan. It is predominantly expressed in the central nervous system as a chondroitin sulfate proteoglycan or in a non-proteoglycan form in some tissues, such as the stomach. Several different ligands can bind to the extracellular domain of RPTPβ/ζ, including the growth factors ►pleiotrophin and ►midkine, the ►extracellular matrix molecules ►tenascin-C and -R, the neuronal ►cell adhesion molecules contactin, Nr-CAM, neural cell adhesion molecule, L1/Ng-CAM, and TAG-1/axonin-1, as well as amphoterin and fibroblast growth factor-2.

Receptor for Stromal Cell-Derived Factor-1 alpha

►Chemokine Receptor CXCR4

Receptor for TNF-Related Apoptosis-Inducing Ligand

Definition

►TRAIL, is the ligand for the DR-4 and DR-5 receptor is a member of the tumor necrosis factor (►TNF) family of death signal tranduction proteins with a mechanism of cell death, similar to the Fas (►FAS/APO-1/CD95) and Fas ligand (Fas-L) system.

►Apoptosis

Receptor Tyrosine Kinases

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Synonyms

Growth factor receptors

Definition

RTKs, Are high affinity cell surface receptors for specific polypeptide ligands, which have an intrinsic tyrosine kinase activity: they can transfer a phosphate group from ATP to a tyrosine residue. In the human genome, 58 RTK genes have been identified. RTK are key components of signaling pathways, which control cell proliferation, survival, differentiation, and metabolism. Deregulation of RTKs by different mechanisms may contribute to neoplastic growth or developmental abnormalities.

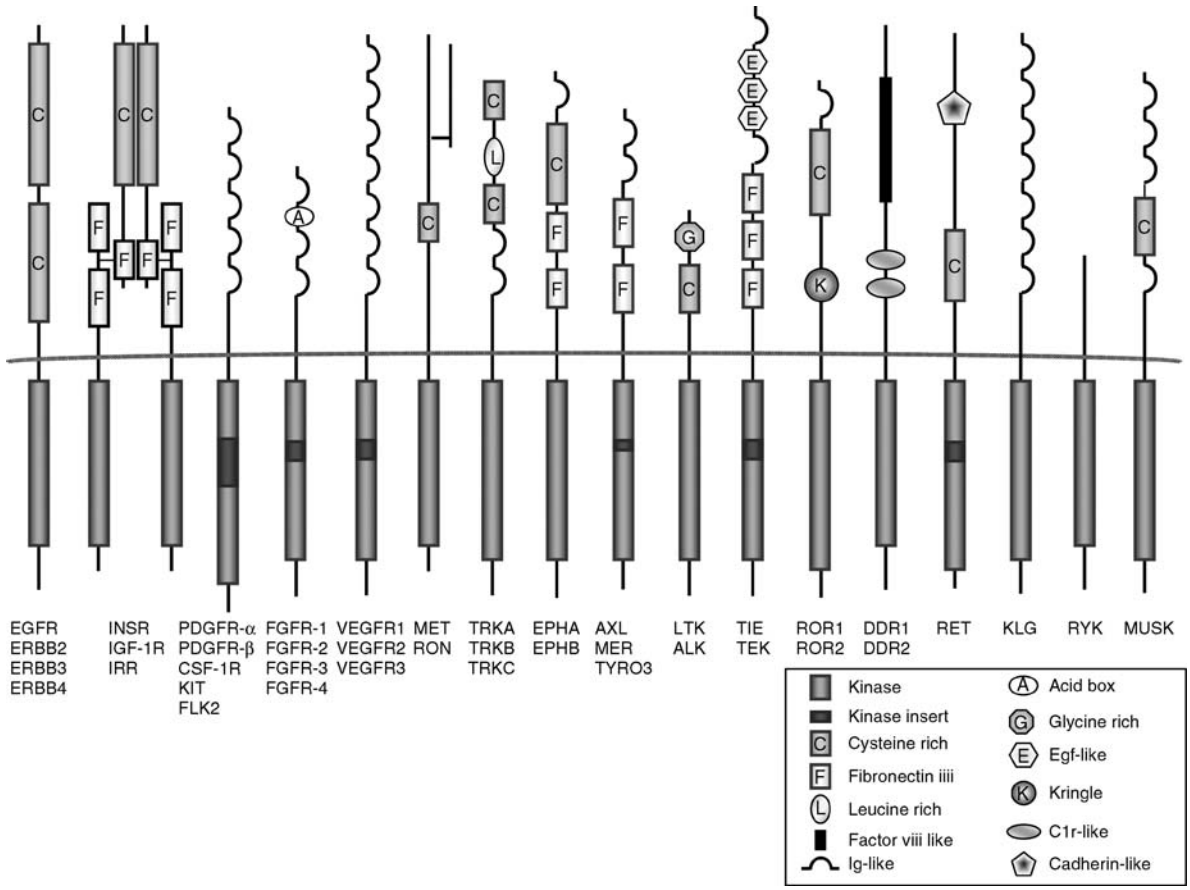
Characteristics

Receptor tyrosine kinases show a common architecture comprising an extracellular portion that binds polypeptide ligands, a transmembrane helix, and a cytoplasmic portion that displays catalytic activity (Fig. 1). Docking sites for protein-protein interactions with cytoplasmic signaling molecules are also present. The majority of RTKs are formed by a single polypeptide chain in a monomeric conformation in the absence of a ligand. ►MET and its related receptors RON and SEA, after proteolytic processing of a single chain precursor, consist of a short extracellular α-chain that is disulfide-bonded to a membrane-spanning β-chain. Members of the insulin receptor subfamily are disulfide-linked dimers of two polypeptide chains, forming a α2β2 hetero-tetramer.

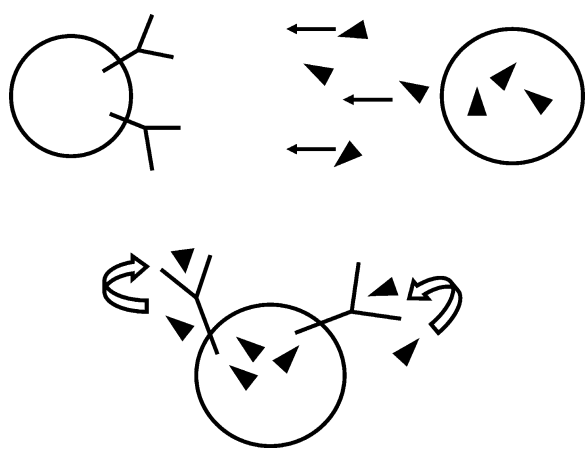
The extracytoplasmic portion, which binds specific ligands, shows considerable diversity among members of the family, usually containing a linear array of discrete folding modules such as immunoglobuline-like domains, cysteine-rich domains, fibronectin type III-like domains and EGF-like domains. The cytoplasmic portion of RTKs is more conserved. Next to the transmembrane portion is located the so-called juxtamembrane region, followed by the tyrosine kinase (TK) catalytic domain and finally by the C-terminal region.

Physiological RTKs Activation

Ligand-mediated activation of RTKs results in autophosphorylation of both the receptor catalytic TK domain and C-terminal non-catalytic region. The ligand can be provided by a cell different from those expressing the related RTK or by the same cell. The latter is defined as autocrine activation and the former as paracrine activation (Fig. 2). TK domain phosphorylation leads to activation and potentiation of receptor kinase activity. The non-catalytic regions phosphorylation creates docking sites for downstream cytoplasmic targets, which bind to receptor phosphotyrosine residues embedded in specific consensus sequences. Downstream signaling pathways are constructed in a modular fashion. In addition to ►SH2 and ►PTB (phosphotyrosine binding domains), downstream signal proteins also contain catalytic domains and/or domains that recognize other signaling molecules. The arrangement of various combinations of modular domains in



Receptor Tyrosine Kinases. Figure 1 Structure and domain organization of representative RTKs.



Receptor Tyrosine Kinases. Figure 2 Paracrine (*top*) and autocrine (*bottom*) RTK activation. The latter occurs following an inappropriate expression of a ligand for a constitutively expressed RTK.

different signaling proteins allows for the creation of complex signaling networks and pathways. These proteins, in addition to performing catalytic functions, serve as scaffolds for the assembly of multiprotein signaling complexes as adaptors, transcription factors, and pathway regulators. Understanding the physiological regulation of RTKs may help in identifying the mechanisms causing their oncogenic activation.

Deregulation of RTKs in Malignant Cells

Different mechanisms leading an RTK to a constitutively active form contributing to or driving neoplastic transformation have been identified: (i) over-expression, due to gene amplification or enhanced gene transcription, (ii) ►autocrine or ►paracrine stimulation (Fig 2); (iii) activating mutations including ►point mutation, ►deletion, insertion; (iv) ►chromosomal rearrangements resulting in the expression of ►fusion proteins containing the tyrosine kinase domain of RTK and dimerizing/activating sequences from an unrelated donor gene.

In the last two cases, RTKs undergo germ-line or somatic oncogenic transformation. The common mechanism of activation from the physiological to the neoplastic status implies structural changes that deregulate the receptor kinase activity, with the delivery of a continuous ligand-independent signal.

Gain of function mutations or deletions have been identified in many RTKs. Mutations that promote ligand-independent dimerization are a well-represented activation mechanism. The loss by point mutation of a single cysteine residue in the extracellular domain of Ret in ►MEN2A (multiple endocrine neoplasia type 2) syndrome, which includes medullary thyroid carcinomas and pheochromocytomas, results in receptor constitutive activation. This mutation most likely destroys an intramolecular disulfide bond that frees a cysteine residue that, through the formation of an intermolecular bond, enhances receptor dimerization and results in constitutive activation of the receptor catalytic activity. ►HER2/Neu, belonging to the EGFR family, can be activated by a single point mutation in the transmembrane domain. Different activating mutations of c-KIT are present in ►gastrointestinal stromal tumor (GIST) as well as in ►acute myeloid leukemia (AML), seminoma, germinoma and mast cell leukemia. In AML, seminomas, and germinomas, KIT mutations are mainly clustered in the exon 17 of the TK domain. In GIST, different mutations, occurring in justamembrane, extracellular or TK domains, comprising point mutations as well as short in frame insertions/deletions, are described. All these alterations cause receptor activation. Different RTKs as ►RET, ►TRKA, and PDGFR, are constitutively activated, following chromosomal rearrangements in different tumors. A classical example of RTK activation by chromosomal rearrangement driving neoplastic transformation is represented by RET and TRKA somatic rearrangements, that are alternatively present in about 30 and 5%, respectively, of papillary thyroid carcinoma (PTC) cases. A similar activation mechanism has been described for ►PDGF receptors in chronic myelomonocytic leukemia (CMML) through the production of the fused ►oncogene TEL/PDGFRB. The same receptor/ligand complex has been found constitutively activated in dermatofibrosarcoma protuberans (DFSP) by the activation of an autocrine loop due to the formation of a ►fusion gene COLA1α/PDGFβ that results in the constitutive production of a PDGFβ ligand product.

RTK may also become activated by gene amplification or over-expression. Over-expression leads to constitutive kinase activation by increasing the concentration of dimers. EGF receptor family members, such as EGFR and ERBB2 in breast, colorectal and non-small cell lung (►NSCLC) carcinomas, are frequently activated by these mechanisms.

The same receptor may be activated by different mechanisms in different pathologies. KIT for example is activated by over-expression and paracrine/autocrine mechanisms in SCLC, ►Ewing sarcoma, and ►synovial sarcoma and by mutations in GIST, AML, seminomas, and germinomas, as mentioned above. RET is activated by different germ-line point mutations in MEN2 cancer syndromes, MEN2A, MEN2B, and FMTC (familial medullary thyroid carcinoma), but by somatic rearrangement in PTC. PDGFR is activated by paracrine/autocrine loop, by mutations or by chromosomal rearrangement, in different neoplasias.

In Table 1 the main RTK alterations in malignant cells and the involved neoplasias are listed.

VEGF receptors also act in cancer development, even if not altered in malignant cells, because they are involved in tumor ►angiogenesis. This latter is implicated in the pathogenesis of malignancy and ►metastasis and its inhibition has clinically demonstrated improvements in outcomes in a variety of malignancies.

Clinical Relevance

- HER2/ERBB2 gene ►amplification represents an already validated negative prognostic marker for both mammary and ovary carcinomas. Moreover, a humanized anti p185-ERBB2 monoclonal antibody (►Trastuzumab), which has anti-proliferative effects, enhances the killing of human breast cancer cells, following treatment with different cytotoxic drugs.
- The detection of Ret germ-line mutations offers an invaluable diagnostic tool for the identification of asymptomatic individuals at high risk to develop a MEN2-associated neoplasia.
- Moreover, fusion oncogenic proteins derived from RET and TRKA rearrangements are distinctive diagnostic features of the papillary type of thyroid cancer. In general, this class of oncogenic products (►fusion proteins) are viewed as a potential tumor-specific target for therapeutic interventions.
- A variety of tyrosine kinase inhibitors have now been developed and much progress has been made toward

Receptor Tyrosine Kinases. Table 1 Relevant receptor tyrosine kinases involved in cancer

RTK	Mechanism activation	Neoplasia
►EGFR	Over-expression	►Breast carcinoma
	Gene ►amplification	Colorectal carcinoma
	Mutations	►Non-small cell lung carcinoma (NSCLC)
	Autocrine/paracrine loop	►Ovarian carcinoma
		►Cervical carcinoma
		►Bladder carcinoma
		►Prostate carcinoma
		Head and neck cancer
		►Glioblastoma multiforme
►HER2/ERBB2	Gene ►amplification	Breast carcinoma
	Over-expression	►NSCLC
	Mutations/truncation	►Colon cancer
		Head and neck cancer
		Prostate carcinoma
		Ovarian carcinoma
		►Glioblastoma
HER3/ERB3	Over-expression	Breast carcinoma
IGF-1R	Over-expression	Carcinomas and sarcomas
PDGFRA	Autocrine/paracrine loop	►Chondrosarcoma
PDGFRB		Chordoma
c-KIT		Ewing sarcoma
c-KIT PDGFRA	Mutations	Gastrointestinal stromal tumor (GIST)
PDGFRB	Chromosomal rearrangement	Chronic myelomonocytic leukaemia
c-KIT	Over-expression	►SCLC
	Autocrine/paracrine loop	►Ewing sarcoma
	Mutations	►Synovial sarcoma
		Acute myeloid leukemia
		Seminoma/►germinoma
		Mast cell leukaemia
RET	Point mutations	►FMTC, ►MEN2A, ►MEN2B hereditary cancer syndromes
RET	Chromosome rearrangement	Papillary thyroid carcinoma
TRKA	Chromosome rearrangement	Papillary thyroid carcinoma
HGFR/MET	Over-expression	►Hepatocellular carcinoma
	Mutation	►Papillary thyroid carcinoma
		►Renal carcinoma
EPHA2	Over-expression	Gastric, esophageal and ►colon carcinomas
EPHB2	Over-expression	►Breast carcinoma
EPHB4	Over-expression	►Acute myeloid leukemia
FLT3	Mutations	Acute myeloid leukemia
IGF-1R	Over-expression	Carcinomas and sarcomas
VEGFR1/VEGFR2/ VEGFR3	Expression	►Tumor angiogenesis

The mechanisms of activation are indicated.

obtaining specific and potent compounds that have entered clinical trials. They include, in particular:

- ►Imatinib (Gleevec) to inhibit PDGFR in the sarcoma type DFSP and in chordomas, and c-KIT in ►GIST;
- ►Gefitinib and ►Erlotinib to inhibit ►EGFR mutated in advanced ►NSCLC;
- ►Lapatinib to inhibit activated EGFR and ERBB2 in breast cancer;
- CP547632 to inhibit VEGF receptors in ►ovarian carcinoma and NSCLC.

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Receptors with Empty Ligand-Binding Pockets

►Orphan Nuclear Receptors

Receptors with no Ligand-Binding Pocket

►Orphan Nuclear Receptors

Receptors Regulated by Ligands

►Orphan Nuclear Receptors

Receptors with Structural Ligands

►Orphan Nuclear Receptors

Recessive

Definition

Referring to an ►allele of a gene that is unable to dictate a phenotype in the presence of a second allele that acts in a ►dominant way. This is particularly true for many mutated alleles.

Referring to one of several alternative traits that can be specified by a genetic locus; when the locus is ►heterozygous and carries information specifying two distinct traits, the dominant trait will be exhibited by the organism and the recessive will not dictate phenotype in the presence of a second allele that acts dominantly. Very often, wildtype alleles are dominant over mutated alleles. In this case, the disease can only become manifest when the second normal allele also suffers inactivating damage.

►Tumor Suppression
►Tumor Suppressor Genes

Recessive Oncogenes

►Tumor Suppressor Genes

Reciprocal Translocation

Definition

Chromosomal translocation whereby parts of two nonhomologous chromosomes are interchanged.

►BCR-ABL1
►Chromosome Translocations

RECK Glycoprotein

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Synonyms

Matrix metalloproteinase inhibitor; Serine protease inhibitor-like domain; Regulator of extracellular matrix integrity; Suppressor of invasion, metastasis, and angiogenesis

Definition

RECK is named after its isolation, identification, and structural characteristics as a "reversion-inducing, cysteine-rich protein with Kazal motif" protein. RECK negatively regulates matrix ►metalloproteinases (MMPs) and plays an important role in the control of development, extracellular matrix remodeling, angiogenesis and metastasis.

Characteristics

The *RECK* gene was originally isolated from a cDNA clone whose expression induces flat morphology in *v-ki-*►*RAS*-transformed ►NIH/3T3 cells. The gene product is a 110-kDa glycoprotein, which anchors to the plasma membrane via glycosylphosphatidylinositol (GPI) modification. Several specific features are found in RECK protein. First, RECK is rich in cysteine and has a number of typical cysteine knot motifs at its N-terminal region. Second, RECK is glycosylated at asparagine (Asn) 86, 200, 297, and 352 residues. Functional study indicates that the glycosylation of Asn297 residue is involved in the suppression of MMP-9 secretion and Asn352 residue is necessary to inhibit MMP-2 activation. Third, RECK contains three serine protease inhibitor-like domains. The sequence of one of these domains matches well to the consensus sequence of Kazal motif, which is usually found in protease inhibitors.

RECK is a critical regulator for development. ►Knockout mice, show embryonic lethality in due to disruption of mesenchymal tissues and organogenesis. In addition, RECK is involved in the control of myotube formation. The myoblast differentiation factor MRF4 and myoblast determination factor MyoD have been shown to positively or negatively regulate the expression of RECK to modulate myogenesis. Moreover, RECK can regulate the proliferation of endothelial cells and participate in the control of angiogenesis.

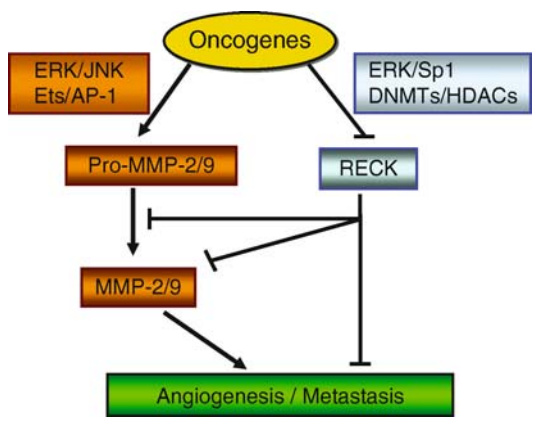
Regulation

RECK is widely expressed in most of human and mouse tissues. On the contrary, expression of RECK is significantly down-regulated or undetectable in many types of human tumor tissues and cancer cell lines. In addition, reduction of RECK is frequently associated with lymph node metastasis and poor prognosis. Cell-based studies indicate that down-regulation of RECK may be caused by oncogene activation. A number of oncogenes including ►Ras, ►Fos, ►Myc, ►HER-2/neu, and ►Src have been shown to repress RECK expression. In addition, viral oncoproteins such as ►Epstein–Barr virus latent membrane proteins may also suppress RECK to promote invasive ability of infected cells. Therefore, RECK is a common and critical target for oncogenic signaling.

The molecular mechanism by which oncogenes repress RECK has been clarified recently. The repression occurs at transcriptional level and is mainly mediated by epigenetic inactivation. For example, RAS oncogene may induce recruitment of histone deacetylases (HDACs) to RECK gene promoter, which causes deacetylation of histones and reduction of promoter activity. In addition, activation of RAS up-regulates the expression of DNA methyltransferase (DNMTs) and increases the binding of DNMTs to RECK gene promoter. This causes ►hypermethylation of ►CpG islands at the RECK promoter and results in silencing of transcription (Fig. 1).

Clinical Relevance

Down-regulation of RECK expression has been reported in lung, breast, glioma, pancreatic, prostate, colorectal, and gastric cancer. In addition, RECK reduction is usually associated with increased lymph node metastasis and poor prognosis. These results suggest that RECK is a typical tumor and metastasis suppressor gene. Indeed, ectoexpression of RECK potently suppresses the invasive ability of cultured cancer cells and inhibits tumor formation in ►nude mice. Reduction of tumor formation is caused by suppression of angiogenic sprouting, which leads to inhibition of tumor growth and invasion.



RECK Glycoprotein. Figure 1 Oncogene-induced gene silencing of RECK and promotion of tumor metastasis.

Because down-regulation of RECK is frequently caused by ►epigenetic inactivation, drugs which can restore RECK expression via reversion of DNA hypermethylation and ►histone deacetylation may effectively suppress cell invasion and tumor metastasis. Indeed, DNMT inhibitor 5-azacytidine and ►HDAC inhibitor ►trichostatin A have been shown to up-regulate RECK expression in cancer cells and effectively suppress tumor formation. Results from in vitro study and clinical investigations suggest that RECK is a rational target for the treatment and/or prevention of tumorigenesis.

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Recombinant

Definition

Typically referring to nucleic acids; describing combinations of DNA or RNA sequences in a fashion that does not occur naturally.

►V(D)J Recombination
►Recombinant Therapeatic Proteins

Recombinant Inbred

Definition

Inbred mice that are created from two (or more) inbred strains of mice and contain a mixture of the genomes.

►Mouse Models

Recombinant T Cell Receptor

►Chimeric T Cell Receptors

Recombinant Therapeutics

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Synonyms

r-DNA derived therapeutic proteins; Biotechnology derived therapeutic proteins

Definition

Recombinant therapeutics are therapeutic proteins produced by recombinant DNA technology

Characteristics

Proteins are used already for more than a century in the treatment of disease. The first generation were proteins derived from animals such as antisera used to treat infectious diseases as diphtheria and tetanus and later bovine and porcine insulin for the treatment of diabetes. The second generation were natural proteins from human source like the plasma derived clotting factors and human growth hormone. The development of the recombinant DNA and cell fusion technology in the seventies of the twentieth century opened up the possibilities to produce human proteins and ►monoclonal antibodies in unlimited amount in microbial and mammalian host cells. In 1982 human insulin was introduced as the first recombinant DNA derived biopharmaceutical and since than more than 160 have gained approval. The pipeline contains many more potential biopharmaceuticals and at present 1 in 4 new drug applications concerns a biotechnology derived product. Many of these products are being used for treatment of cancer either as supportive therapy, like ►epoetins and ►G-CSF, as general immune modulators like interleukine-2 and ►interferon alpha 2 or specific therapy in the form of an increasing number of monoclonal antibodies.

The first generation of biopharmaceuticals were produced by recombinant DNA technology and were intended to be exact copies of naturally occurring regulatory proteins. These supposed physiological proteins showed sometimes severe side effects. ►Tumor necrosis factor (TNF) and some of the interleukins proved very toxic and in some cases even fatal and never reached the market.

Other products like ►interferon were marketed, but for severe conditions with the side effects as dose limiting factors. The most common explanation offered for this type of toxicity is an exaggerated biological activity by non-physiological levels at unnatural sites.

Because many of these proteins act as ►paracrine or ►autocrine factors their ►pharmacokinetic properties are unfavorable for systemic treatment. Protein modification was introduced such as hyperglycosylation (e.g. Darbepoetin alfa, Aranesp®) and ►pegylation (e.g. Peginterferon alpha 2a, Pegasys®) which extend half-life. Also hybrid molecules were developed such as etanercept (Enbrel®), where the soluble receptor of TNF is combined with the Fc-part of IgG to reduce the clearance from the circulation which occurs after injection of the unmodified receptor.

Another major problem of therapeutic proteins appeared to be the induction of antibodies. For foreign proteins such as the murine derived monoclonal antibodies this immunogenicity was to be expected. However the humanization of monoclonal antibodies has reduced but not solved the problem of immunogenicity. And also the proteins which are homologues of endogenous factors such as GM-CSF, interferons etc. induce antibodies, sometimes even in the majority of patients.

By definition we are immune tolerant to products which are copies of endogenous proteins. The products not necessarily need to be exact copies of the natural proteins to share this immune tolerance. When human therapeutic proteins induce antibodies, they are breaking B-cell tolerance, which starts with the activation of autoreactive B-cells. Presenting the self-epitopes in an array form is very potent activator of these B-cells. This explains why aggregates of human proteins are the most important factor in induction of antibodies.

These aggregates may not be immediately present in the product, but may appear during storage making stability and formulation an important issue in predicting the immunogenicity. There are only a few studies in experimental model systems on the properties of the aggregates which break B-cell tolerance, indicating that only multiple order aggregates (>trimers) are involved. The only biological test to study the capacity of a protein product to break B-cell tolerance are mice made transgenic for the specific protein. These mice are immune tolerant and there is a good correlation of an immune response in these mice and in patients. Although these models have helped to identify the factors important for breaking B-cell tolerance and also have been useful in improving the formulation of products, there is not yet enough experience to use them as absolute predictors of immunogenicity of human proteins.

The clinical consequences of immunogenicity may be severe. The most common consequence is loss of efficacy. Sometimes this loss can be overcome by increasing the dose or change the product. However in some cases the antibodies compromise the treatment with a complete class of products. The association between antibodies and inhibition of efficacy has mainly been established at population level. Only with some products can a defined level of antibodies be translated in the treatment strategy of individual patients.

In some cases the unpredictable disease progression, the lack of good efficacy markers and the extended effect after treatment has stopped, also complicate the evaluation of the clinical effects of immunogenicity.

Because of the lack of adequately designed studies and standardized assays for many products the consequences of their immunogenicity is unclear. Some manufacturers and clinicians interpret this lack of reliable data as a lack of consequences. However, it is difficult to explain how high levels of neutralizing antibodies in the circulation could exist without any consequence.

The most dramatic consequence of these antibodies is the neutralization of the native protein with the subsequent loss of its important biological function. This has been observed in patients treated with a specific formulation of epoetin alfa in countries other than the USA. This epoetin induced antibodies in a number of patients which neutralized endogenous erythropoietin leading to the development of pure red cell aplasia (PRCA). In 1998, the formulation of epoetin alfa outside the USA was modified, which most likely led to a decrease in the protein stability, the tendency to form aggregates and thereby to an increased immunogenicity. Additional potential factors have been suggested, such as inappropriate handling of the product, polysorbate 80-epoetin interaction and leachates from uncoated rubber stoppers of the prefilled syringes with an adjuvant effect.

Now the first patents of the first wave of recombinant DNA derived pharmaceuticals have expired or will expire shortly, we are entering the new era of biopharmaceuticals, which includes both improved second generation products as well as the introduction of copy products the so-called biosimilars or follow up biologicals.

In the case of classical drugs, expiration of patents opens the possibility of the introduction of generic products. Limited documentation showing chemical similarity and bioequivalence in a small volunteer study is in general sufficient to obtain marketing authorization. However the concept of generics developed for small therapeutic molecules cannot be extrapolated to the majority of biopharmaceuticals which are large proteins. The technology is lacking to establish whether the structure of two biopharmaceuticals is completely identical. Moreover, the properties of biotechnology products are highly dependent on the production process and their biological behavior remains partly unpredictable. Therefore the European Medicine Evaluation Agency (EMEA) based on EU-legislation expects producers of biosimilars to submit comparative clinical data in addition of full documentation of quality and safety.

The Food and Drug Administration (FDA) which regulates drugs in the USA prefers the term follow up biologicals for these products, but still needs to issue their guidelines.

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Recombinase Activating Gene-2

Definition

Rag-1 and *rag-2* encode lymphocyte-specific recombinases that are required to generate B cell (BC) and T cell receptors (TCRs). BCRs and TCRs are constructed from recombined genetic elements V, D and J. These genes are flanked by Recombination Signal Sequences (RSSs) on the 3' side (downstream) of a V region and the 5' side (upstream) of the J region. These are the sides that will be involved in the joining. RAG1 and RAG2 associate with each other to recognize the RSS sequences and induce DNA cleavage at these sites. Following the activity of RAG1 and RAG2, DNA repair enzymes facilitate the joining of the segments. In this way B cells and T cells can use limited numbers of V, D and J genes to generate widely diverse receptor specificities.

►Immunoediting
►V(D)J Recombination

Recombination

Definition

The process of breakage followed by exchange and rejoining of strands between one DNA molecule and another

►BCR-ABL1
►Recombinant

Record-Linkage Study

Definition

A study based on comparisons of information on exposure, cancer and other factors collected in medical and administrative records.

►Cancer Epidemiology

Recurrence

Definition

Synonym Relapse; Return of the disease after previous complete ►remission of the disease.

Recycling

Definition

Of receptors; The process that allows receptors to be diverted from the degradation fate and to be rerouted to the plasma membrane after endocytosis. Recycling constitutes the default pathway for the majority of receptors undergoing constitutive internalization and involved in the uptake of nutrients. Kinetic measurements revealed that each receptor can recycle about every 20 min.

►Endocytosis

Redifferentiation

Definition

A process of reprogramming of the cancer cell phenotype, resulting in a state closer to its normal tissue of origin. This is a therapeutic approach to cancer.

►Jasmonates in Cancer Therapy

Redistribution

Definition

Dividing cells differ in radiation sensitivity throughout the ►cell cycle. In general, cells are most sensitive in

►mitosis (M) and pre-mitotic gap (G_2), and most resistant at the end of DNA synthesis phase (S). Cells in early S and post-mitotic gap (G_1) have intermediate sensitivities. Exposure to ►ionizing radiation eliminates radiosensitive cells causing partial synchronization, which is followed by re-assortment into radiosensitive phases of the cell cycle.

►Radiation Oncology

Redox Cycling

Definition

The reduction-oxidation cycle inherently associated with ►cytochrome p450 drug and ►xenobiotic metabolism, where Fe^{3+} is reduced to Fe^{2+} upon drug or xenobiotic oxidation, and where a second electron is donated by a co-enzyme [NADPH] so that Fe^{2+} is reoxidized and Fe^{3+} is restored.

►Carcinogen Macromolecular Adducts
►Xenobiotics

Redox Reaction

Definition

Short for reduction/oxidation, including all chemical reactions in which atoms have their oxidation state changed.

►Reactive Oxygen Species
►Reduction/Oxidation

Reduced

Definition

The product of a compound that has gone through a reaction involving a ►reduction.

►Thioredoxin System

Reduced Intensity Conditioning (RIC) Allogeneic Stem-Cell Transplantation

Definition

As compared with standard allogeneic transplantation where tumor cell eradication by high-dose ►radiochemotherapy, RIC-allogeneic transplantation is ►immunotherapy, where the donor stem cell are infused following milder, ►non-myeloablative radiochemotherapy of the recipient with immunosuppressive aim. Subsequently, in weeks-months, the donor-derived stem cells will proliferate and often completely replace (full chimerism) the recipient bone marrow. When full chimerism is achieved, the graft-versus-tumor effect may occur, which eventually may cure (or exert long-term control) the malignancy. RIC-allografting is associated with fewer side-effects and less treatment-related mortality, and allows allogeneic transplantation in patients of somewhat older age than possible with standard myeloablative conditioning.

►Mantle Cell Lymphoma

Reduced Penetrance

Definition

Variable or absent phenotypic effect of a gene known to cause a developmental abnormality or cancer.

►Rhabdoid tumor

Reductases

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Synonyms

Carbonyl reductases; Aldo-keto reductases; Hydroxysteroid dehydrogenases

Definition

Carbonyl reduction of aldehydes, ketones and quinones to their corresponding hydroxy derivatives plays an important role in the phase-I metabolism of many

endogenous (biogenic aldehydes, steroids, prostaglandins, reactive lipid peroxidation products) and xenobiotic (pharmacologic drugs, carcinogens, toxicants) compounds (►Xenobiotics).

Characteristics

From the pharmacologist's point of view, carbonyl reduction is of significance in various inactivation processes of drugs bearing a carbonyl group. The carbinols formed may retain therapeutic potency, thus prolonging the pharmacodynamic effect of the parent drug, or, in some instances, a compound gains activity through carbonyl reduction. From the toxicologist's point of view, carbonyl reduction plays an important role in the toxification of drugs such as daunorubicin and doxorubicin (►Adriamycin), whereas numerous reports corroborate the concept of carbonyl-reducing enzymes being involved in ►detoxification processes of endogenous and ►xenobiotic reactive carbonyl compounds.

Carbonyl Reduction by SDRs and AKRs

Carbonyl-reducing enzymes (►carbonyl reductase) are grouped into two large protein superfamilies, the aldo-keto reductases (AKR) and the short-chain dehydrogenases/reductases (SDR).

AKRs are monomeric $(\alpha/\beta)_8$-barrel proteins of about 320 amino acids in length. Their active site consists of a conserved tetrad of the amino acids Y-H-D-K and the hydride transfer from NADPH occurs through an acid/base catalyzed mechanism. A nomenclature system for the AKR superfamily has recently been established and is available on the AKR superfamily homepage (www.med.upenn.edu/akr). At present there are 13 human AKRs, of which, for example, AKR1A1 represents aldehyde reductase and AKR1B1 is aldose reductase. Found in almost every living organism, the AKRs metabolize steroids, sugars, prostaglandins, ►polycyclic aromatic hydrocarbons, and a great variety of endogenous and exogenous aldehydes and ketones.

The SDR superfamily comprises a wide range of NAD(P)(H) dependent oxidoreductases involved in functions as diverse as the metabolism of steroids, sugars, prostaglandins, xenobiotics, drugs and carcinogens, the fixation of nitrogen and the synthesis of antibiotics. The superfamily is made up of enzymes averaging 250–350 amino acids in length. Their three-dimensional structures have a common α/β-folding pattern characterized by a central β-sheet typical of a classical Rossmann-fold with α-helices on either side. The SDRs have a low identity (typically 20–30%), but share common sequence motifs that define the cofactor binding site (TGxxxGxG) and the catalytic tetrad (N-S-Y-K). Their catalytic mechanism resembles the acid/base catalyzed hydride transfer of the AKRs. One of the most prominent SDR enzymes involved in the detoxification of xenobiotics is human carbonyl reductase (CBR1) which has been supposed to have a role in endogenous prostaglandin and steroid metabolism. However, the best substrates for human CBR1 are K region ortho-quinones of polycyclic aromatic hydrocarbons (benzo[a]anthracene and benzo[a]pyrene) and menadione, the latter of which is often used as a model CBR1 substrate.

Among the AKRs and SDRs exists a variety of pluripotent hydroxysteroid dehydrogenases (HSDs) of both superfamilies which specifically catalyze the oxidoreduction at different positions of the steroid nucleus, and which also catalyze rather non-specifically the reductive metabolism of a great number of non-steroidal carbonyl compounds. In target tissues, HSDs convert potent steroid hormones to their cognate inactive metabolites (and *vice versa*) and thus regulate the occupancy of steroid hormone receptors. Therefore, in normal cell physiology, HSDs function as important pre-receptor regulators of signaling pathways by acting as "molecular switches" for receptor-active and receptor-inactive ►hormones. For example, 11β-HSD1 is a microsomal enzyme responsible for the interconversion of cortisone (the glucocorticoid receptor inactive ligand) to the hormonally active 11-hydroxy form ►cortisol. A variety of diseases, including insulin resistance/►diabetes type 2, dyslipidemia, and ►obesity, have been discussed in relation to 11β-HSD1.

Several HSDs exhibit other activities besides steroid oxidoreduction. In addition to being specific for their physiological steroid substrate, they can catalyze the carbonyl reduction of a great variety of non-steroidal aldehydes, ketones and quinones. Accordingly, HSDs participate in drug metabolism and play a significant role in the defense of an organism against the deleterious effects of endogenous and exogenous toxicants and carcinogens. Due to the fact that several enzymes of both superfamilies exhibit pluripotency for steroidal and non-steroidal carbonyl substrates, AKR and SDR seem to be the result of a convergent evolution. Extensively characterized HSDs with carbonyl-reducing activity are four members of the AKR superfamily (AKR1C1–AKR1C4) and 11β-HSD1 from the SDR superfamily.

The "Pro"

Reductases Protect Against Lung Cancer

Among the more than 40 different carcinogens present in tobacco and tobacco smoke (►Tobacco Carcinogenesis), 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK = nicotine-derived nitrosamine ketone) is the most potent and abundant. NNK induces lung tumors in all laboratory animal species tested and is believed to play a role in human ► tobacco-related cancers. NNK requires metabolic activation (►Carcinogen Metabolism) in order to exert its carcinogenic effect and there are competing pathways for NNK activation and

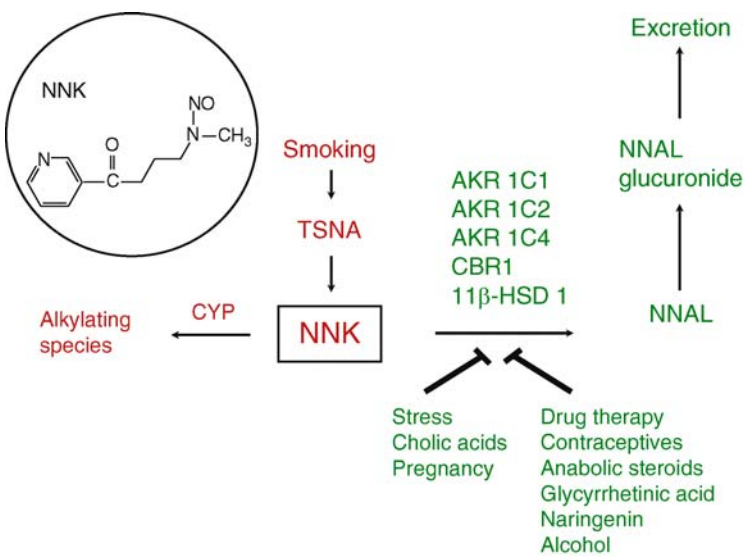
detoxification (Fig. 1). NNK activation proceeds by ►cytochrome P450 mediated α-hydroxylation of the carbon adjacent to the *N*-nitroso group which leads to a series of unstable intermediates that alkylate DNA (►Adducts to DNA) and hemoglobin.

The competing pathway for NNK activation is NNK detoxification, which proceeds via carbonyl reduction of NNK to NNAL, followed by glucuronidation of NNAL. Consequently, the susceptibility of a tissue to tumor formation may vary depending on the extent of the competing pathways α-hydroxylation versus carbonyl reduction. Carbonyl reduction to NNAL is a very efficient pathway of NNK in animal and human tissues and greatly exceeds metabolism by NNK α-hydroxylation. The predominance of carbonyl reduction has been confirmed *in vivo* by a characterization and quantification of NNK metabolites in the urine of smokers and smokeless tobacco users. Five different enzymes catalyzing NNK carbonyl reduction in man have been identified (Fig. 1). These are 11β-HSD1 and CBR1 from the SDRs, and AKR1C1, AKR1C2 and AKR1C4 from the AKRs.

Clinical Aspects

Any impact on NNK carbonyl reduction could have consequences with regard to NNK-induced cancerogenesis (►Chemical Carcinogenesis). For example, differences in tissue expression of NNK reductases may be causally related to the organospecificity of NNK-induced cancerogenesis. Moreover, genetic polymorphisms, age-dependent differential expression and/or modulation by several xenobiotics of NNK reductase activity may impair the activation-detoxification balance in favor of activation, rendering the lung vulnerable to the carcinogenic effect.

The profile of NNK metabolites in smokers' urine reveals high interindividual variations with respect to NNAL, NNAL-glucuronide and NNAL-N-oxide quantities. It is these variations that are of greatest interest, since they may not only be linked to ►lung cancer susceptibility but also to a genetic polymorphism in terms of NNK reductase expression. Only 16% of habitual smokers (►Nicotine Addiction) develop lung cancer, suggesting that individual susceptibility and environmental factors (e.g. air pollution) contribute to the risk of developing this disease. A number of studies have demonstrated a familial component to lung cancer risk. Evidence supporting familiarity suggests that the etiology of lung cancer includes shared genes, shared environments, or both. Association studies performed on polymorphic genes coding for phase-I and phase-II enzymes (►Phase II Enzymes) that play a role in the activation of carcinogens in tobacco smoke, i.e. CYP1A1, CYP2D6, CYP2E1, and GSTM1 (►Glutathion-S Transferase), have often yielded conflicting results. However, preliminary experiments on the expression of 11β-HSD1 in human lung demonstrated that the level of this NNK carbonyl-reducing



Reductases. Figure 1 Simplified scheme of the metabolic fate of the tobacco-derived carcinogen NNK. The NNK/NNAL equilibrium is determined by the level of expression and activity of enzymes that mediate NNK carbonyl reduction. Inhibition of NNK reductases would lead to a shift to NNK activation via cytochrome P450s (CYPs), thereby enhancing the carcinogenic consequences of smoking. NNK, nicotine derived nitrosamine ketone; NNAL, nicotine derived nitrosamine alcohol; TSNA, tobacco-specific N-nitrosamines.

enzyme varied by a factor of 20 in only ten patients investigated.

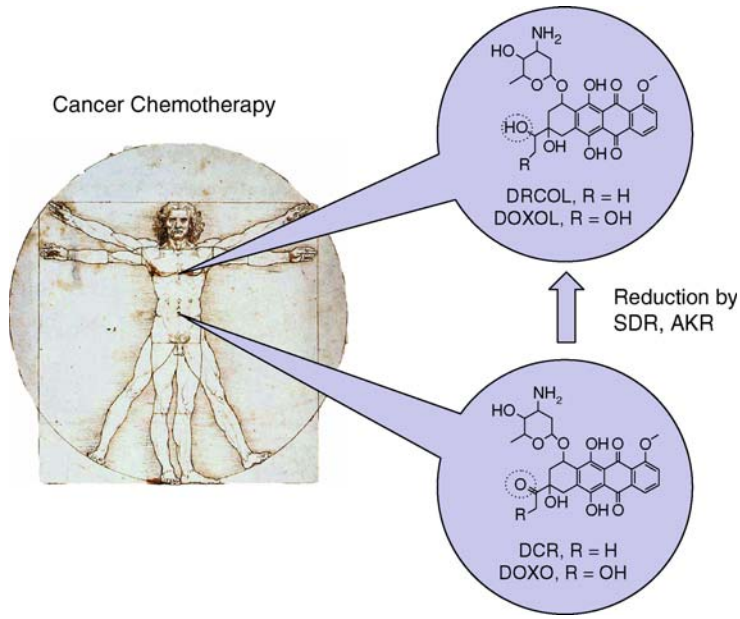
Several factors of endogenous and/or endogenous origin may influence the detoxification capacity of the NNK reductases, thereby shifting the NNK/NNAL equilibrium towards NNK and thus contributing to the interindividual variabilities observed in the response to smoking. In response to stress, elevated levels of endogenous glucocorticoids occupy the 11β-HSD1 enzyme (►Stress). High levels of cholic acids in cholestatic states, or estrogens and progesterone during pregnancy, would also affect NNK detoxification, as those steroids are substrates or inhibitors of most of these reductases. The same consequences may occur upon administration of exogenous steroids (glucocorticoid therapy), or other pharmacologic agents such as some diuretics and ►anti-inflammatory drugs which inhibit one or more of these enzymes already at therapeutic doses. A similar situation might also result upon usage of oral contraceptives, anabolic steroids, or the exposure to food constituents like glycyrrhetinic acid (GA, the principal constituent of licorice) and naringenin (a flavonoid (►Isoflavones) with high concentrations in grapefruit juice). Licorice as a tobacco additive or as a confectionery may potentiate the carcinogenic response towards cigarette smoking. Moreover, GA is known to act as an inducer of cytochromes P450. The resulting increase in NNK activation would be synergistic to NNK reductase inhibition, thereby further aggravating the toxicological consequences of smoking. The fact that chronic alcohol consumption greatly enhances the carcinogenic response to NNK might now be explained on the base that ethanol has been shown to be inhibitory for all of these enzymes.

The "Contras"

Reductases in Chemotherapy Resistance

In anti-tumor chemotherapy, the occurrence of drug resistance is a major obstacle and originates principally from pharmacodynamic or pharmacokinetic mechanisms. ►Anthracyclines like ►daunorubicin (DRC) and ►doxorubicin (DOX) are the most valuable cytostatic agents in chemotherapy, but their usefulness is limited by intrinsic or acquired resistance towards these drugs. Anthracycline resistance is not merely the result of alterations in drug uptake and retention (►Membrane Transporters) (►ABC-Transporters) (►P-glycoprotein), but also mediated by enzymatic anthracycline detoxification that is upregulated upon exposure to these drugs. The 13-hydroxy metabolites of anthracyclines, such as daunorubicinol (DRCOL) and doxorubicinol (DOXOL), are significantly less potent than the parent drug in terms of inhibiting tumor cell growth, suggesting that carbonyl reduction is an important biochemical mechanism in the detoxification of carbonyl group-bearing anthracyclines. Therefore, elevated levels of anthracycline carbonyl-reducing enzymes constitute a mechanism in the development of resistance towards DRC and DOX (Fig. 2). For example, in a variety of different cancer cell systems, including pancreas carcinoma, breast cancer, stomach cancer and ovarian carcinoma, DRC carbonyl reduction was inducible by the substrate DRC itself.

Five enzymes capable of catalyzing anthracycline carbonyl reduction have been identified in human liver: AKR1A1, AKR1B1, AKR1B10 and AKR1C2 from the AKR superfamily, and CBR1 from the SDR superfamily. Generally, increased expression levels of these reductases were observed in drug-resistant cells. In pancreas carcinoma cells, CBR1 turned out to have the highest potency. Overexpression of CBR1 lead to a sevenfold increase in DRC-resistance when IC_{50} values were taken into account. This is in accordance with DRC resistant human stomach carcinoma cells which showed strongest increase in CBR1 mRNA expression and an eightfold increase in resistance towards DRC. CBR1-deficient K562 cells gained protection towards DRC toxicity after transfection with CBR1. Therefore, CBR1 seems to be the dominant reductase in tumor cells during generation of DRC resistance by induction of phase-I metabolism. AKR1B1 was linked to DOX and ►cisplatin resistance in ►HeLa cells of ►cervical carcinoma. AKR1C2 expression levels were altered in prostate and breast tumors. In an ovarian cancer cell line, resistance to *cis*-platin chemotherapeutics correlates with the induction of AKR1C2 expression. AKR1C2 is emerging as an important cellular-stress response marker in several different cell types. Furthermore, it has been suggested that the antitumor effect of ►nonsteroidal anti-inflammatory drugs (NSAID) is mediated in part by the inhibition of certain AKR1C isoforms, in addition to inhibition of COXs. Non-MDR-resistant Chinese hamster ovary cells resistant to the calpain inhibitor (N-acetyl-leucyl-leucyl-norleucinal) overexpressed an NADPH-dependent AKR that exhibits about 70% sequence identity with AKR1B1. The enzyme was later identified as being AKR1B10 (intestinal aldose-like reductase) which is also capable of reducing DRC. AKR1B10 belongs to the aldose reductases (AKR1B subfamily) and was discovered as an enzyme being overexpressed in human lung and ►liver cancer, and even suggested as a diagnostic marker of ►non-small cell lung carcinomas. The overexpression of AKR1B10 in ►squamous cell carcinoma of the lung raises the question whether inhibition of this enzyme might be beneficial for patients, in as much as the physiological role of AKR1B10 is not yet fully understood. Recent observations suggest that its physiological substrates are retinal isomers.



Reductases. Figure 2 Carbonyl reduction is a main but undesired metabolic pathway of the anti-cancer drugs DRC and DOX. The resulting alcohol metabolites DRCOL and DOXOL, respectively, have a far less anti-tumor potency and, in addition, are responsible for the life-threatening cardiac toxicity that limits the clinical use of DRC and DOX. Elevated levels of carbonyl-reducing enzymes in cancer cells may therefore contribute to the development of anthracycline chemoresistance and affect the clinical outcome.

Cardiotoxicity

►DRC and ►DOX are highly effective antineoplastic agents, but they can produce the serious side effects of acute cardiac injury and chronic congestive heart failure. The mechanism by which they cause chronic cardiomyopathy is not fully understood. Hypotheses regarding the mechanism of cardiac toxicity include perturbation of calcium homeostasis, formation of iron complexes, mitochondrial dysfunction, and damage to cell membranes. Since the development of chronic cardiomyopathy usually coincides with an accumulation of anthracycline secondary alcohol metabolites in the heart, DOXOL and DRCOL are hypothesized to be responsible for this severe side-effect that limits the clinical use of DRC and DOX (Fig. 2).

In agreement with this, CBR1, AKR1A1 and AKR1B1 have been implicated in the development of doxorubicin-induced cardiotoxicity. For example, transgenic mice that overexpress human CBR1 exclusively in the heart show increased heart damage and decreased survival after doxorubicin treatment. On the other hand, significant cardiac protection is seen in mice heterozygous (+/−) for *Cbr1* expression with a 40–50% decrease of CBR1 protein levels. The variability of CBR1 levels in the human heart is not known. High CBR1 levels could contribute to the extreme DOX sensitivity some patients experience during treatment and also to the long-term chronic cardiotoxicity that can develop in cancer patients 10–20 years after treatment.

Clinical Aspects

The clinical consequences in chemotherapy with carbonyl group bearing anthracyclines remain to be established. Anthracycline cumulative doses upon chemotherapy of cancer patients must not exceed 500 mg/m^2, since otherwise serious side effects in cardiotoxicity can be expected. Hence, inhibition of DRC carbonyl reduction in a chemotherapeutic regimen would have a twofold benefit especially at low DRC or DOX doses: first, the preservation of the antineoplastic potency of the parent drug and second, the prevention of cardiomyopathy caused by their reduced alcohol metabolites DRCOL and DOXOL. Since several flavonoids (isoflavones) function as potent inhibitors of carbonyl-reducing enzymes, co-administration of flavonoids during anthracycline chemotherapy may therefore be cardioprotective without a loss of antineoplastic potency.

Chemical modification may lead to a decreased metabolic inactivation. In the case of the new antineoplastic agent benfluron, carbonyl reduction resulted in the rapid loss of antitumor activity. The molecule was then derivatized by the addition of two methoxy substituents, protecting the ketone from reduction, with the goal of improving the pharmacokinetic profile of

benfluron. The dimethoxy substitution indeed did protect the molecule from deactivation in incubations with human liver subcellular fractions, human hepatocytes, and purified CBR1. However, the outcome of pharmacokinetic studies with this derivative has not yet been reported.

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Reduction

Definition

The chemical process involving an addition of electrons. If disulfides are reduced with two electrons, they become dithiol motifs.

►Thioredoxin System

Reduction/Oxidation

Definition

►Redox reactions properly refer to reactions in which there is a change in oxidation number. However, they can usually be considered as an exchange of electrons between two molecules, atoms or ions. Reduction describes the gain of electrons and oxidation describes the loss of electrons. Reducing agents loss electrons and oxidizing agents (also called oxidants or oxidizers) gain electrons.

►Hydrogen Peroxide

Reed-Sternberg Cells

Definition

R-S cells; Refers to a lymphoid cell and in most cases, a ►B cell. R-S cells are very large with abundant pale cytoplasm and two or more oval lobulated nuclei containing large nucleoli. Hodgkin lymphoma (synonym Hodgkin disease, HD) is a neoplastic proliferation of lymphoid cells predominantly involving lymphoid tissues. The malignant cell is the Reed-Sternberg cell. Reed-Sternberg cells are essential to the diagnosis of Hodgkin lymphoma. However, R-S cells are not unique to HD and therefores alone are not sufficient for the diagnosis of HD.

Regeneration

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Synonyms

Mass recovery; Tissue proliferation

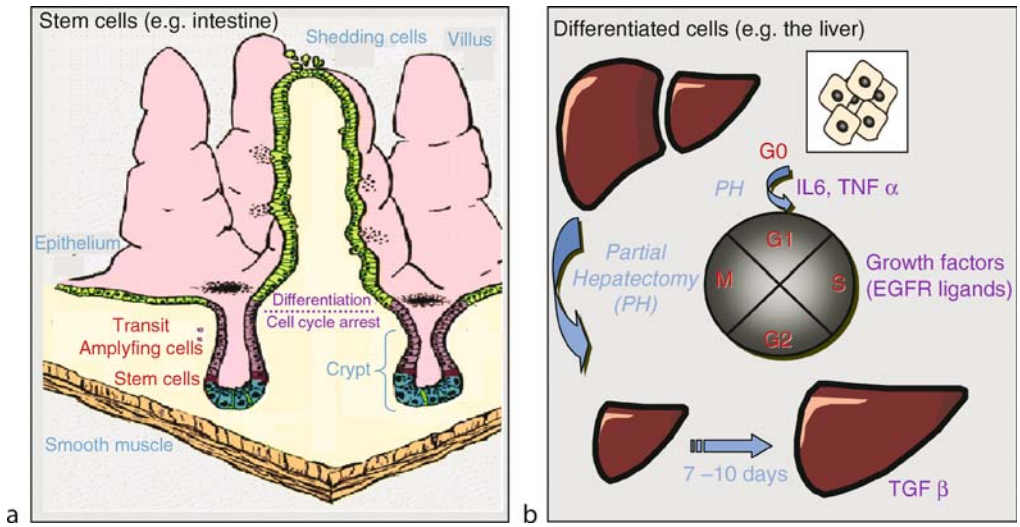
Definition

Regeneration is the recovery of cell mass of a given organ following cell loss. The balance between cell loss and cell renewal is tightly controlled. When this balance is altered, inappropriate cell growth occurs leading to the development of cancer.

Characteristics

Under normal circumstances, the processes of tissue regeneration or homeostasis are tightly regulated by several pathways to prevent excessive or inappropriate cell growth. Two kinds of cell types can participate in tissue regeneration: stem cells, which are multipotent cells that can both self-renew and give rise to more differentiated cells; and terminally differentiated cells, which are normally quiescent but are able to proliferate upon a stimulus. Some tissues regenerate from their pool of stem cells, like the intestine and the skin, and others, like the liver and the kidney, regenerate from fully differentiated cells that constitute these organs.

One classic example of tissue renewal by stem cells is the intestine (Fig. 1a). Intestinal epithelial stem cells are

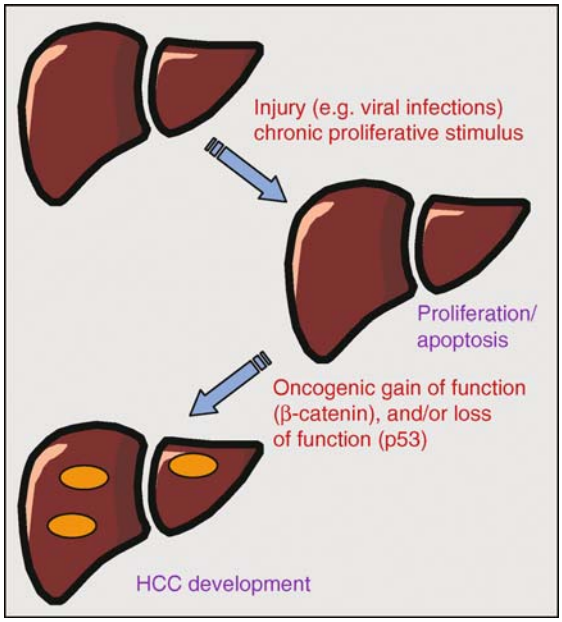


Regeneration. Figure 1 Regeneration in normal tissues. Certain tissues, such as the intestine (a), regenerate from a pool of stem cells, while others, such as the liver (b), regenerate from fully differentiated cells.

thought to be located in the crypt compartment. Gut stem cells differentiate as they migrate upwards to the surface epithelium. Before terminally differentiating into absorptive enterocytes or secretory goblet cells, progenitor cells give rise to transit amplifying cells, the immediate precursors to the effector cells. Terminally differentiated cells are eventually shed into the lumen. Wnt/ß-catenin signaling appears to be one of the major pathways controlling intestinal homeostasis. The distribution of ►APC (adenomatous polyposis coli), a gene required for degradation of ►β-catenin, is in agreement with the role of ß-catenin signaling in maintaining stem cell properties and controlling differentiation in the intestine. A gradient of APC is observed along the crypt-villus axis, where it counteracts ß-catenin signaling and allows differentiation.

The liver is the major example of fully differentiated cells that massively regenerate following injury (Fig. 1b). Following two thirds partial hepatectomy, liver regeneration is carried out by the remaining mature cellular populations composing the liver. Stem cells, although present, do not feature largely in hepatocyte proliferation. The first cells to proliferate are the hepatocytes, followed by biliary epithelial cells, ►Kupffer cells and endothelial cells. There is evidence to suggest that hepatocytes provide a certain mitogenic stimuli which initiates the other cell types to commence DNA synthesis and proliferation. The initial (priming) phase after injury/partial hepatectomy, involves the up-regulation of cytokines and pro-inflammatory proteins, like ►TNF-α and ►IL-6. This priming phase renders the liver cells receptive to the mitogenic effects of growth factors (mainly ►ERFR ►ligands). Since the size of the liver is highly regulated, once hepatocytes complete one to two rounds of division, regeneration will eventually stop. The main factors involved in the termination phase are TGF-β1 and other members of its family, such as activin.

In both systems described above, proliferation is tightly controlled, involving a careful balance between cell loss and cell renewal that keeps the normal organ homeostasis. Once this balance is disrupted, an exacerbated proliferation will occur, which can eventually lead to a tumorigenic process. Several mechanisms are involved in countering the carcinogenic process in proliferating tissues. Potentially cancerous cells can be eliminated by programmed cell death (►apoptosis) or their proliferation prevented by permanently halting their cell cycle (►senescence). However, cancer can develop if certain genes involved in these pathways are mutated or dysregulated (e.g. ►p53, ►Rb, ►p16) (Fig. 2). Different factors, both hereditary and environmental, can be responsible for the mutations leading to imbalanced growth. Environmental mutagens can vary from ultraviolet light (skin), to viral infections (liver) and dietary carcinogens (stomach and intestine). It has been demonstrated that signaling pathways involved in cancer are often the same associated with normal tissue renewal. In the kidney, for example, most genes implicated in regeneration are also involved in the tumorigenic process. Exacerbated tissue growth results indeed from dysregulation of pathways involved in regeneration/repair. Extensive evidence has pointed out two main pathways that are dysfunctional in various forms of cancer, the WNT/β-catenin and the ►Hedgehog signaling pathways.



Regeneration. Figure 2 Dysregulated regeneration during liver tumorigenesis. A proliferative stimulus triggered by injury leads to exacerbated growth. When this growth is combined with mutations inducing gain and/or loss of function of key cell cycles genes, it can lead to malignant transformation.

In normal tissues, in the absence of WNT signaling, β-catenin is phosporylated by functional interactions with glycogen syntase kinase (GSK)-3β (►GSK3), ►axin and ►APC, and subsequently targeted to the ►ubiquitin-►proteasome system. Thus, in normal adult epithelial cells, β-catenin is present in a sub-membranous location and it plays a major role in adherens junctions. Activation of Wnt signal during cell proliferation induces β-catenin stabilization and translocation to the nucleus and its association with Tcf/LEF factors causes transcriptional activation of target genes as *c*-►*myc* and ►cyclin D1. During tumorigenesis, different factors can lead to β-catenin constitutive activation. Thus, mutations in APC leading to the accumulation of β-catenin are involved in most ►colon cancers. The Wnt pathway is also constitutively activated in some types of skin and ►liver cancers, but in these cases, this activation is due to β-catenin mutations that prevent it from being degraded by the proteasome.

Three Hedgehog (Hh) genes have been isolated in humans: Sonic (SHH), Indian (IHH) and Desert (DHH) Hedgehogs. They produce secreted glycoproteins that act through several components, including the transmembrane receptors Patched 1 (►PTCH1) and Smoothened (SMOH), to initiate a complex intracellular signaling cascade that ultimately leads to the activation of the ►GLI zinc-finger transcription factors. Studies in model systems have shown that the Hh–Gli pathway controls the normal development and growth of several organs, including the skin, brain, pancreas, gut and prostate, at different stages of development. Aberrant activation of the Hh pathway in cancers is caused by mutations in the pathway (ligand independent) or through Hh overexpression (ligand dependent). This pathway has been extensively described as being dysregulated in cancers involving tissues that are renewed by their stem cells, such as pancreas, prostate and intestine. However, recent data have demonstrated that this pathway is also involved in liver cancers.

In conclusion, cancers arise when the balance between cell growth and cell loss is disrupted, and it is therefore crucial to define the precise mechanisms involved in this balance in order to develop new therapeutic tools to fight cancer.

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Regional Disease

Definition

►Lymph Node Metastasis.

Regulator of Extracellular Matrix Integrity

►RECK Glycoprotein

Regulatory T Cells

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Synonyms

Suppressive T cells; Treg

Definition

Regulatory T (Treg) cells are a specialized subpopulation of T cells that are capable of suppressing immune responses and thereby inducing immune tolerance to self and non-self. Suppressive T cells were initially described in the early 1970s and 1980s and their suppressive function was implicated in tumor-induced suppressive immunity. However, due to a lack of phenotypic markers for defining these cells, there was extensive skepticism about their existence in vivo. In 1995, Sakaguchi and colleagues showed that CD25, the ►interleukin-2 (IL-2) receptor α chain, could serve as a phenotypic marker for $CD4^+$ suppressor T cells or $CD4^+$ Treg cells. Over the past few years, several distinct subsets of Treg cells have been identified, which include (i) naturally occurring $CD4^+$ $CD25^+$ $FoxP3^+$ Treg cells; (ii) antigen-induced $CD4^+$ Treg cells such as antigen-specific $CD4^+$ $CD25^+$ $FoxP3^+$ Treg cells, IL-10-producing Tr1 cells, and TGF-β-producing Th3 cells; (iii) $CD8^+$ Treg cells; and (iv) NKT Treg cells. Recent studies demonstrate that the immunosuppressive potential of these cells can be harnessed therapeutically to treat various immune-related diseases, including autoimmune diseases, allergy, transplantation, infectious diseases as well as cancer.

Characteristics

Molecular Markers of Treg Cells

Although the expression of CD25 on T cells has been used as a useful marker of Treg cells, its expression is not necessarily associated with Treg cell function, in that it is also expressed by activated, nonregulatory effector lymphocytes. Several other molecules, including the TNF-family molecule GITR and ►cytotoxic T-lymphocyte antigen-4 (CTLA4), have been used as markers for Treg cells. However, both GITR and CTLA-4 are activation markers, they also express on activated effector T cells. Foxp3 is the best marker identified to date for $CD4^+$ Treg cells in mice and humans. Foxp3 is also expressed in $CD8^+$ Treg cells, but not in Tr1 cells. More recently, CD127 expression, the alpha chain of the interleukin-7 receptor, was found to inversely correlates with Foxp3 and the suppressive function of $CD4^+$ Treg cells. The combined use of these markers including $CD25^+$, $Foxp3^+$ and $CD127^-$ may better define Treg cell population with suppressive function.

Treg Cell in Cancer and their Antigen Specificity

Treg cells within tumor microenvironment may play a significant role in the suppression of antitumor immune responses against cancer cells. Elevated percentages of $CD4^+$ $CD25^+$ Treg cells in the total T cell population have been found in tumor tissues or peripheral blood in variety of cancers, including lung cancer, breast cancer, ovarian, melanoma, liver cancer, gastric cancer, and lymphoma. The generation and maintenance of Treg cells have long been speculated to require the presence of target antigen or tissues, but the identity of antigens recognized by Treg cells remains largely unknown. Recent efforts have led to successful identification of several ligands recognized by $CD4^+$ Treg cells, which include LAGE1 (a homolog of NY-ESO-1) and ARTC1 (*a*ntigen *r*ecognized by *T*reg *c*ells). They have potent ability to suppress naïve T cell proliferation through a cell-contact dependent mechanism. Because both LAGE1 and ARTC1 are dominantly expressed in cancer and normal testis, but not in other normal tissues tested, these Treg cells are activated only when they encounter with tumor cells, thus providing molecular basis for their specificity in the induction of immune tolerance. Since tumor cells express an array of tumor-specific antigens as well as self antigens, it is likely that Treg cells specific for tumor-specific antigens as well as Treg cells specific for nonmutated self antigens co-exist in tumor microenvironment. Overall, these tumor-infiltrating Treg cells are present at tumor sites and mediate antigen-specific, local immune suppression.

Suppressive Mechanisms of Treg Cells

$CD4^+$ Treg cells require antigen-specific activation or polyclonal ►TCR stimulation to exert their suppressive function. Once activated, they can suppress $CD4^+$ and $CD8^+$ T cells in an antigen-nonspecific manner. Several mechanisms have been proposed to explain how $CD4^+$ Treg cells inhibit $CD4^+$ effector T cells. Some investigators suggest that IL-10 and/or TGF–β are directly involved in T-cell-mediated suppression, while others contend that cell-cell contact is required for suppression. It is likely that more than one mechanism of $CD4^+$ Treg cell-mediated suppression appears to operate in vitro and in vivo. Most tumor-specific $CD4^+$ Treg cells suppress immune responses (proliferation and IL-2 secretion of naïve or effector T cells) through a cell-contact mechanism, but the precise mechanism and molecules responsible for Treg cell-mediated suppression remain to be identified.

Manipulation of the Number or Suppressive Function of Treg Cells

Successful cancer ►immunotherapy will need to manipulate the Treg cell number or their suppressive

function. One approach is to eliminate $CD25^+$ Treg cells by a specific antibody or Ontak (a IL-2-toxin fusion protein). However, because the CD25 marker is not specific for Treg cells and all activated T cells are positive for CD25 marker, this approach may not efficiently eliminate Treg cells or deplete both Treg cells and activated effector cells. Hence, more specific and effective reagents are needed to deplete Treg cells in cancer patients.

An alternative approach is to reverse the suppressive function of Treg cells. TLR signaling activation on DCs can render naïve T cells refractory to suppression mediated by Treg cells in mice, but it is not clear whether TLR activation on Treg cells can directly reverse their suppressive function. Poly-G oligonucleotides can directly reverse the suppressive function of human Treg cells in the absence of DCs. The reversal effect of Treg cell function by Poly-G oligonucleotides requires the signaling from TLR8 to MyD88 pathway in Treg cells. Future studies will focus on evaluation of the therapeutic potential of cancer vaccines in patients by reversing Treg cell function via TLR stimulation or in the combination with peptide-based vaccination.

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REL

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Synonyms

NF-κB

Definition

Rel can refer generically to a family of structurally-related eukaryotic ►transcription factors. The vertebrate Rel family includes the proteins RelA, RelB, c-Rel, p50/p105, and p52/p100, and a viral protein (v-Rel) (Table 1). Alternatively, Rel can specifically refer to v-Rel or c-Rel. The term ►NF-κB is also often used in a general fashion interchangeably with Rel, as in Rel/NF-κB transcription factors.

Characteristics

Rel family proteins are related through an N-terminal domain of ~300 amino acids, called the Rel homology domain, which contains sequences important for the formation of dimers, DNA binding, and nuclear localization. Rel proteins must form dimers to bind to DNA and these dimers bind to a related set of DNA target sites called κB sites. The consensus sequence for the κB site is GGGRNWTTCC (where R is any purine, N is any nucleotide, and W is A or T).

Vertebrate Rel proteins can be divided into two classes based on sequences C-terminal to the Rel homology domain (Fig. 1). The C-terminal half of one class of Rel protein (including p105 and p100) contains multiple copies of a 33 amino acid repeat, called the ankyrin repeat, that causes these proteins to be inactive. However, these C-terminal inhibitory sequences can be removed to yield shorter, active DNA-binding forms (p50 and p52) that consist primarily of the Rel homology domain. A second class of Rel family protein (RelA, RelB, c-Rel) contains C-terminal sequences that function as transcription activation domains. In the fruit fly *Drosophila* there are three Rel proteins – Dorsal, Dif, and Relish – which control an early step in development and a primitive immune response to bacterial and fungal infections (Fig. 1, Table 1). Recently, NF-κB- like proteins have also been identified in marine invertabrates in the phyla cnidaria (see anemones, corals) and porifera (sponges).

Almost all Rel family proteins can form homodimers and heterodimers. The most common Rel dimer in many vertebrate cells is a heterodimer consisting of p50-RelA, which is often specifically called NF-κB. The binding of Rel dimers to DNA target sites generally activates the transcription of a large variety of cellular genes, including many whose protein products are involved in immune responses (e.g. anti-bacterial peptides), ►inflammation (►cytokines and cytokine receptors), adhesion (cell surface attachment proteins), and programmed cell death (►Bcl-2 proteins). In addition, several viral genes (including those of human oncogenic viruses such as ►Human immunodeficiency virus [HIV-1] and ►Epstein-Barr virus [EBV]) are activated by Rel/NF-κB dimers.

Cellular and Molecular Regulation

Rel ►transcription factors can be regulated at several levels. First, because there are five interacting members of the Rel transcription factor family, they can combine to form a diverse set of dimers with distinct DNA

REL. Table 1 Fly and vertebrate Rel/NF-κB transcription factors

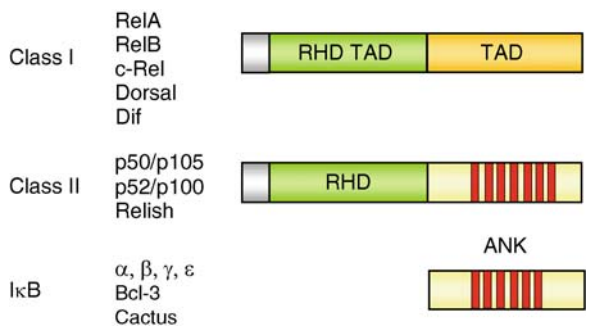
Gene	Protein	
Drosophila melanogaster		
Dorsal	Dorsal	
Dif	Dif	
Relish	Relish	
Avian Rev-T retrovirus		
v-*rel*	v-Rel	
Vertebrates		
		Human gene (chromosome)
Rela	RelA (p65)	*RELA* (11q12-q13)
Relb	RelB	*RELB* (19q13)
c-*rel*	c-Rel	*REL* (2p13-12)
nfkb1	p50, p105, or p50/p105	*NFKB1* (4q23-q24)
nfkb2	p52, p100, or p52/p100	*NFKB2* (10q24)

target site binding specificity. The composition of these dimers is determined by the affinity of specific Rel subunits for one another, by the concentration of specific subunits in a given cell, and by the post-translational modification of specific subunits. The composition of the Rel dimers in a given cell is important as it influences which genes they can control and which inhibitors (see below) can bind to the complexes.

Second, in most cell types, Rel complexes are located in the cytoplasm due to interaction with an inhibitor protein called ►IκB. IκB proteins are members of a structurally-related family of proteins (Fig. 1) that inhibit Rel complexes by masking sequences involved in nuclear localization and DNA binding. A variety of extracellular signals, including cytokines, phorbol esters, virus infection, interferon, and the bacterial outer membrane component lipopolysaccharide, can activate Rel transcription complexes by inducing their dissociation from IκB. In the best-characterized example, the p50-RelA NF-κB complex is held in the cytoplasm by interaction with IκBα (Fig. 2).

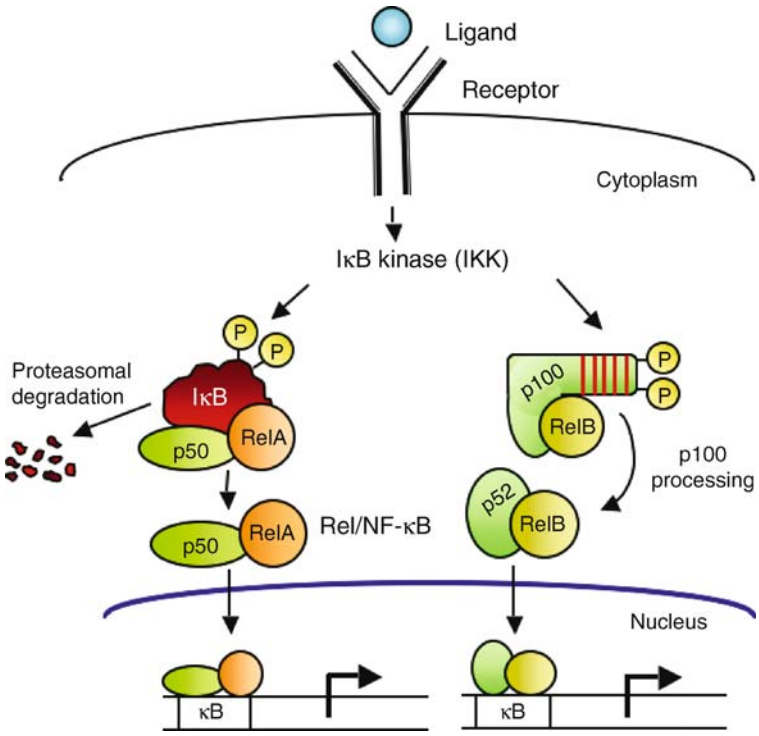
Stimulation of the cell by an appropriate signal leads to activation of an IκBα kinase (IKK), which then phosphorylates IκBα at two N-terminal serine residues. Phosphorylation of IκBα signals it for ►ubiquitination at a nearby lysine residue. Ubiquitinated IκBα is then degraded by the ►proteasome, and the NF-κB complex is free to enter the nucleus to activate transcription of specific genes. One of these genes encodes IκBα. Newly synthesized IκBα can then pull NF-κB off DNA, and export and resequester NF-κB in the cytoplasm. Thus, activation of NF-κB is a transient (~30–60 min) event in most cell types.

However, in some cell types NF-κB is continuously located in the nucleus and active. Normal cells with



REL. Figure 1 Generalized structures of Rel/NF-κB/IκB proteins. Rel/NF-κB proteins are related through the Rel homology domain (RHD), which has sequences important for the formation of dimers, DNA binding, inhibitor (IκB) binding, and nuclear localization. Dimers can form within or between Rel classes (e.g. p50-p50 or p50-RelA, etc.). Class I proteins have C-terminal sequences that are required for transcription activation (TAD). Class II proteins have C-terminal sequences with multiple copies of inhibitory ankyrin repeats (ANK, *red bars*), which can be removed by proteolysis to release the mature N-terminal DNA-binding proteins (p50 or p52). Independent IκB proteins consist largely of a series of ankyrin repeats. The protein names refer to separate proteins in each family (e.g. see Table 1 and text for details).

constitutively nuclear and active NF-κB include B cells, Sertoli cells, and some neurons. In addition, chronically active NF-κB has been documented in many cancer cell types (including ►leukemia, ►lymphoma, ►breast cancer, ►cervical cancer, ►ovarian cancer, vulva, ►prostate cancer, ►bladder cancer, ►kidney cancer, ►lung cancer, ►pancreas cancer,



REL. Figure 2 Activation of the Rel/NF-κB pathway. Many types of extracellular signals (Ligand) can initiate activation of the Rel/NF-κB signal transduction pathway by binding to a specific cell surface receptor. In most cases, these signals lead to the activation of a cytoplasmic IκB ►kinase (IKK), which phosphorylates (P) an IκB (*left*) or the C terminus of p100 (*right*). Phosphorylation of IκB targets it for degradation by the proteasome, and phosphorylation of p100 leads to removal of the C-terminal ANK repeat sequences. Free Rel/NF-κB dimers (e.g. p50-RelA or p52/RelB) then enter the nucleus, bind to specific DNA sites (κB sites), and turn on the expression of many cellular genes. Often, one of these activated genes encodes IκB; newly-synthesized IκB can remove NF-κB from DNA and resequester NF-κB in the cytoplasm. Thus, activation of NF-κB is usually a transient (30–60 min) process, which is subject to negative auto-regulation.

►oral cancer, ►larynx cancer, ►colon cancer, ►thyroid cancer, ►skin cancer, head and neck and ►neuroblastoma), and in cells infected with certain human oncogenic viruses (e.g. ►Human T-cell Lymphotropic Virus-1, ►Epstein-Barr Virus, Hepatitis B Virus). In the case of oncogenic viruses, specific viral gene products (►Tax, HTLV-1; ►LMP-1, EBV; X protein, HBV) can constitutively induce the degradation of IκB, allowing NF-κB to continuously enter the nucleus of infected cells.

Clinical Aspects

Rel transcription factors have been associated with oncogenesis in several settings. First, the avian Rev-T ►retrovirus has a single gene, v-*rel*, that encodes the v-Rel transcription factor. Young chickens infected with high titer stocks of Rev-T succumb to leukemia/lymphoma quite rapidly, often within 7–10 days, and have multiple lymphoid cell tumors, primarily in their spleens and livers. The v-*rel* ►oncogene arose by a recombination event between an avian retrovirus and the turkey c-*rel* gene, such that v-*rel* encodes a chimeric protein that has internal c-Rel amino acids and retroviral (Env) amino acids at its N and C termini. v-Rel is mutated in several ways as compared to the c-Rel proto-oncoprotein and these mutations contribute to the oncogenicity of v-Rel. It is likely that v-Rel malignantly transforms cells by forming homodimers that freely enter the nucleus, bind DNA and turn on the transcription of genes that enhance cell growth and block ►apoptosis.

Second, alterations in Rel transcription factor regulation or gene structure are found in several human cancers. As mentioned above, several cancer cell types have constitutive nuclear NF-κB activity. This chronic NF-κB activity endows the cancer cells with an unregulated proliferative capacity and an increased resistance to apoptosis. In some cases, this increased NF-κB activity is due to the production by the tumor cell of autocrine factors that continuously activate the NF-κB

pathway. In addition, genetic alterations in Rel and IκB family genes, including ►amplificational, ►point mutational, and ►chromosome translocational, have been identified in several human cancers, especially hematopoietic cell cancers. For example, the human *REL* gene is amplified in many ►Hodgkin lymphomas and ►diffuse large B-cell lymphomas. In some lymphoid cell cancers there are deletions of the 3′ region of the human *NFKB2* gene, leading to the expression of a C terminally-truncated p100 protein with altered activity. Some Hodgkin's lymphoma tumor cells have inactivating mutations in the gene encoding IκB, which enables Rel/NF-κB complexes to enter the nucleus unchecked. In addition, some ►diffuse large B-cell lymphomas and ►multiple myelomas have mutation in genes encoding upstream signaling proteins, which lead to chronic activation of nuclear Rel/NF-κB.

Pharmacologic or molecular agents that target the Rel/NF-κB signal transduction pathway have been investigated as anti-inflammatory and anti-cancer therapeutics. Many common ►anti-inflammatory drugs, including ►aspirin, ►glucocorticoids, and ►green tea (►epigallocatechin), act, at least in part, by inhibiting NF-κB. Similarly, many recent anti-cancer therapies seek to use molecular or pharmacological agents to sensitize tumor cells to chemotherapeutic agents by inhibiting the anti-apoptotic activity of NF-κB. For example, expression of a super-repressor form of IκBα, which cannot be released from NF-κB, sensitizes several tumor cell types to anti-tumor agents.

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Relapse

Definition

►Recurrence

Relative Risk

Definition

RR; Is the ratio between the disease risk of exposed persons by the disease risk of non-exposed persons (background risk).

►Uranium Miners

Relaxation

Definition

Describes the response of nuclei in a strong magnetic field that occurs due to excitation. Two major classes of relaxation exist – longitudinal and transverse.

►Dynamic Contrast-Enhanced Magnetic Resonance Imaging

Relaxin

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Definition

Is a small ►peptide ►hormone comprising two subunits, together about 6,000 Da in size. Relaxin has been identified as a locally produced factor in a number of key tissues, such as the thyroid gland, gastrointestinal tract, heart, uterus, and lung. Relaxin isknown to function in cells by activating a ►G-protein-coupled receptor on the cell surface called LGR7 or RXFP1 (relaxin family peptide 1). The main functions of relaxin are linked to uniquely mammalian traits such as lactation, viviparity, and post-reproductive survival.

Characteristics

Is a small ►peptide ►hormone belonging to the insulin family of hormones and growth factors. Its major sources in the body are in women the corpus luteum of the premenopausal ovary and the placenta, and in men the prostate gland. In humans, unlike most other mammals, there are two genes for relaxin, *RLN1* and *RLN2*. The products of these genes appear to behave identically in most bioassay systems. Since the *RLN1* gene product is

only ever expressed at very low levels in the placenta and prostate, and does not appear to contribute at all to the circulating relaxin concentration, the term relaxin is used to denote the product of the *RLN2* gene. Whereas in women relaxin can be detected in the circulation with maximum concentrations up to about 1 ng/mL during early pregnancy, in men the peptide is generally below the level of detection for most assays. Relaxin has been identified as a locally produced factor in a number of key tissues, such as the thyroid gland, gastrointestinal tract, heart, uterus, and lung. Relaxin is known to function in cells by activating a G-protein-coupled receptor on the cell surface called LGR7 or RXFP1 (relaxin family peptide 1). The principal ►second messenger system activated by relaxin involves raising the intracellular levels of cyclic AMP (cAMP), though a recent study suggests that relaxin may also be able to activate ►glucocorticoid receptors, and hence glucocorticoid responsive genes in some cells.

Relaxin is a recent development of evolution and is specific to mammals. It appears to have evolved from an ancestral peptide known as relaxin-3, which is found in the brains of all vertebrates. The main functions of relaxin are linked to uniquely mammalian traits such as lactation, viviparity, and post-reproductive survival, which are the hallmarks of a new class of regulatory factors called "►neohormones." These molecules appear to have evolved specifically to address the new needs of mammals. To achieve these novel functions, besides being expressed in specific tissues, relaxin appears to coordinate its activity via a group of specific physiological processes: most importantly ►extracellular matrix ►remodeling and ►collagen remodeling principally by the coordinate control of ►matrix metalloproteinases and their specific inhibitors; ►neoangiogenesis, involving the local induction of ►vascular endothelial growth factor (VEGF); and ►vasodilation in which both nitric oxide and endothelin appear to participate. In addition, relaxin can promote growth and/or differentiation in some cell types, for example, endometrial stromal cells.

The association of relaxin with cancer first arose as a possible link to the observation that women who have had no children have a higher risk of ►breast cancer than those who have started families at a relatively young age. The notion is that a specifically pregnancy-related factor such as relaxin might have a protective capacity in regard to breast cancer, though a recent study using a rat model appears to disallow this possibility. Nevertheless, a series of studies have identified relaxin as a product of ►breast cancer, ►endometrium cancer, ►colon cancer, ►thyroid cancer and ►prostate cancer, with increased relaxin gene expression in the neoplastic tissue compared to the healthy tissue from which the tumors arose. Secondly, a landmark study by Binder and colleagues showed that patients with ►metastatic breast cancer had significantly higher circulating relaxin levels than either a control group of healthy women, or a group of breast cancer patients of good prognosis without metastatic disease. The obvious question that then arose was whether the relaxin was causal in the context of the metastatic disease, merely an index of disease progression, or whether it was a defensive response by the body to the cancer.

To address these issues several research groups have begun to assess the role of relaxin in various parameters of tumor growth, differentiation, and the formation of ►metastases. Initially it is important to determine whether the specific relaxin receptor, RXFP1, is expressed in tumor tissues. Mostly this has made use of ►RT-PCR to detect RXFP1 gene transcripts. The difficulty is that this is a very complex gene, comprising 17 exons, with described alternative splicing giving rise to numerous variants, which may or may not have functional relevance. Very few studies have addressed this issue in the context of cancer cells and tissues, such that reported evidence of transcript expression does not necessarily imply the presence of a functional receptor. However, some studies have made use of specific and validated RXFP1 antibodies (e.g. in breast cancer), or have applied ligand-binding assays to verify the presence of potentially functional receptor protein (e.g. in ►prostate cancer). Some have assessed cell signaling and specific functional responses to relaxin using tumor-derived cell-lines.

Various cell-lines derived from ►prostate cancer, ►breast cancer, ►endometrium cancer, and ►thyroid cancer, are stimulated by relaxin to increase their invasiveness in *in vitro* ►transmigration assays. This has been shown to be due to an increased expression by the cells of metalloproteinases 2 and 9, which effectively degrade the artificial extracellular matrix in these assays. These findings would thus support the notion that relaxin could exacerbate tumor invasiveness, and assist the escape of metastatic cells.

In both ►xenograft and ►autograft mouse cancer models, over-expression of relaxin by either pure peptide delivery, by specific lentiviral or adenoviral expression, or by ►transfection of the cancer cells prior to tumor formation, in general leads to an increase in tumor size, and/or an increase in tumor differentiation, usually accompanied by an increase in tumor vascularization. The latter has been shown for a prostate carcinoma model to be due to induced expression of ►VEGF in the tumor cells. siRNA downregulation of the RXFP1 receptor blocks the effect of relaxin in the *in vitro* models. Finally, in a ►xenograft mouse model of human prostate carcinoma, application using lentiviral vectors of a relaxin receptor antagonist convincingly showed that blocking the endogenous relaxin hormone-receptor system in this way led to a reduction in tumor growth and progression. The endogenous

relaxin-RXFP1 system appears to be involved in the well-known escape of this tumor from androgen regulation. Introduction of the R273H ►p53 mutation into the LNCaP prostate cancer cell-line leads to androgen-independence by the specific induction of relaxin gene expression.

In summary, the peptide hormone relaxin appears like a key player in the growth, progression and formation of metastases for cancers of the prostate, thyroid, endometrium, and breast, and novel antagonists should soon indicate whether relaxin is an important new target for cancer therapy.

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Remission

Definition

A disease state in which the symptoms or signs of a disease are abated. In leukemia, complete remission is defined by normal bone marrow, peripheral blood characteristics, and a normal performance status. The state following therapy where no disease can be detected by convention means (i.e., microscopic or radiological exam).

►Acute Lymphoblastic Leukemia
►Minimal Residual Disease
►Complete Hematologic Remission

Remodeling

Definition

Consists of a series of phenotypic changes in preneoplastic and neoplastic lesions leading to the acquisition of a normal apparent morphology. This phenomenon may indicate a ►redifferentiation of preneoplastic and neoplastic cells which, however, does not seem to be terminal.

►Preneoplastic lesions

Renal Carcinoma

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Definition

Renal carcinoma is a cancer, arising from the epithelial cells of the kidney and must be distinguished from tumors of the renal pelvis.

Characteristics

World wide about 150,000 people develop renal carcinoma each year, and ~78,000 individuals die from the disease. Most cases of renal carcinoma are sporadic, that is most renal carcinomas occur without an inherited predisposition to develop renal cancer. About 1–5% of renal carcinomas are the consequence of an inherited tendency to develop renal cancer.

Cigarette smoke (►tobacco carcinogenesis) is a recognized environmental factor that can lead to renal cancer. Exposure to high doses of trichloroethylene, an industrial solvent, may also lead to renal cancer. Renal tumors occur more commonly in men than woman. The male/female ratio for renal cancer is about 2/1.

Early Diagnosis

Currently, there are no accepted methods for the early diagnosis of renal cancer. Although population-based screening by renal ultrasound would undoubtedly detect renal tumors in asymptomatic individuals, this procedure is not considered cost effective. No serological tests are currently available for the early detection of renal cancer.

Treatment

Surgical removal of the renal cancer, usually by nephrectomy, is so far the best treatment of renal cancer. Treatment of patients with metastatic disease is difficult; there are no treatments available that consistently eradicate metastatic renal carcinoma.

Types of Renal Cancer

Renal cancer is not one disease as such. There are at least four types of epithelial renal cancer, which can be distinguished by their histological appearance (Fig. 1):

- Clear cell renal carcinoma (75% of sporadic renal carcinomas)
- Papillary renal carcinoma (15%)
- Chromophobe renal carcinoma (5%)
- Renal oncocytoma (5%)

There is evidence, suggesting a correlation between the histological type of renal cancer and prognosis.

Renal Cancer Genes

Studies of families with inherited forms of renal cancer have led to the identification of two genes that, in their mutant form, predispose individuals to the development of renal neoplasia, the ►von Hippel-Lindau (VHL) tumor suppressor gene and the ►MET proto-oncogene. Current studies are aimed at identifying a third renal cancer gene – the BHD gene, responsible for the ►Birt Hogg Dube syndrome.

The VHL and the MET gene are mutated in sporadic renal carcinomas. The VHL gene is mutated in about 50% of sporadic clear cell renal carcinomas. It is inactivated by hypermethylation in about 15% of sporadic clear cell renal carcinomas. The MET proto-oncogene is mutated in about 15% of sporadic papillary renal carcinoma.

Types of Inherited Renal Cancer

The currently recognized forms of inherited renal carcinoma are: von Hippel-Lindau disease (VHL), inherited balanced translocations involving human chromosome 3, hereditary papillary renal carcinoma (HPRC) type 1, the Birt Hogg Dube (BHD) syndrome and familial clear cell renal carcinoma.

Clinical Features of Renal Cancer Suggesting Hereditary Renal Cancer

Several characteristics of inherited renal cancers make them distinct from sporadic renal cancers. Frequently, other family members are affected with renal tumors. In general, the renal tumors are multiple and often involve both kidneys. Individuals with renal tumors need to determine whether their tumors are single or multiple and whether other family members have had renal cancer. In cases of hereditary renal cancer, it is essential to test other, apparently healthy, family members for occult renal tumors.

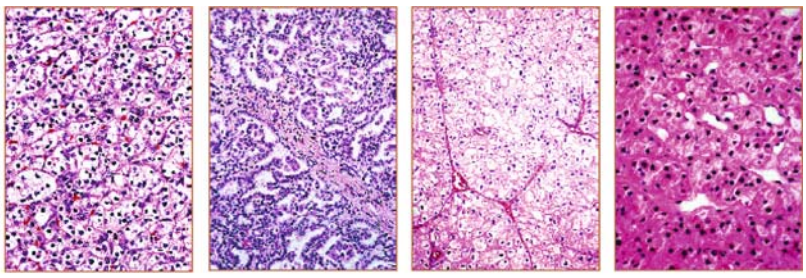
Mechanisms of Development of Inherited Renal Cancers

The VHL gene is a classic ►tumor suppressor gene. In the ►von Hippel-Lindau disease and in sporadic clear cell renal carcinoma, renal tumors develop as a consequence of the loss or inactivation of both copies of the VHL gene. The result of an inactivated VHL gene is the lack of the VHL protein in proximal renal tubular epithelial cells.

The von Hippel-Lindau disease is a rare inherited disorder characterized by a predisposition to develop tumors in multiple organs: brain, spinal cord, eye, adrenal gland, kidney, pancreas, and epididymis. There are three distinct clinical types of VHL:

- Type 1 is characterized by renal carcinoma, brain, spinal and eye tumors without tumors of the adrenal glands (►pheochromocytoma)
- Type 2A is characterized by pheochromocytoma, eye and ►brain tumors without renal cell carcinoma
- Type 2B is characterized by pheochromocytoma, renal cell carcinoma, eye and brain tumor

VHL types 2A and 2B are produced by germline missense mutations in the VHL gene; VHL type 1 is produced germline mutations that produce truncated VHL proteins.



Renal Carcinoma. Figure 1 Frequency of mutations in sporadic human renal epithelial neoplasms.

The proto-oncogene MET encodes the receptor for hepatocyte growth factor/►scatter factor. Renal tumors in patients with hereditary papillary renal carcinoma type 1 as well as a subset of sporadic papillary renal carcinomas, develop as a consequence of the constitutive activation of the MET cell surface receptor. Hereditary papillary renal carcinoma type 1 is characterized by an inherited predisposition to develop multiple, bilateral papillary renal carcinomas. Founder effects have been observed in several families with HPRC. All germline and somatic mutations, identified to date in the MET gene, were missense mutations and were located in the tyrosine kinase domain of the gene.

The Birt Hogg Dube syndrome is an inherited disorder, characterized by a predisposition to develop multiple tumors of the hair follicle (fibrofolliculomas). Usually, the fibrofolliculomas occur on the face and neck. Furthermore, a predisposition a predisposition to develop spontaneous pneumothorax and renal cancer has been described. Pathologists have had difficulty classifying the renal tumors associated with the Birt Hogg Dube syndrome, they appear to be either chromophobe renal carcinomas or renal oncocytomas.

►Von Hippel-Lindan Tumur Suppressor Gene

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Renal-Cell Carcinoma

Definition

Synonym ►Renal Carcinoma; Is a type of kidney cancer that involves cancerous changes in the cells of the renal tubule (epithelial cells) and composed of large, polygonal cells. Also known as clear-cell carcinoma, carcinoma of the renal parenchyma, or sometimes referred as hypernephroma.

►Birt–Hogg–Dubé Syndrome

Renal Excretion

Definition

Is the excretion of a drug through the kidney into the urine.

►ADMET Screen

Reovirus

Definition

Reo is an acronym for respiratory, enteric, orphan (of a disease). Reoviruses are apathogenic human viruses with a double stranded RNA genome that make naked icosahedral particles used in ►oncolytic virotherapy.

Reoxygenation

Definition

Is the process by which viable hypoxic cells (cancer cells living in the tumor ►microenvironment characterized by the oxygen tension below 10 mm Hg) become better oxygenated after irradiation. Reoxygenation of hypoxic tumor cells occurs between treatments during fractionated irradiation. This process is relevant to the therapy outcome, because fully oxygenated cells are three times more radiosensitive than are hypoxic cells.

►Hypoxia and Tumor Physiology
►Oxygenation of Tumors
►Radiation Oncology
►Radiosensitivity

Repair of DNA

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Definition

The repair of DNA comprises a group of distinct biochemical pathways by which various types of

damage to DNA, especially damage to the nitrogenous bases A, T, C and G, is repaired. Such damage can occur spontaneously in living cells as the result of the generation of products of oxidative metabolism that interact with the bases of DNA. Base damage in DNA also transpires as the result of interactions between environmental agents, especially cancer-causing chemicals, with DNA. DNA repair of base damage can be conveniently classified as follows.

Characteristics

Reversal of Base Damage

This mode of DNA repair involves the direct reversal of selected types of base damage. A good example is the restoration of thymine dimers in DNA to their native monomeric state. Thymine dimers (T↔T) are formed by the abnormal chemical joining of adjacent thymine bases (T, T) in DNA and frequently occurs in cells that are exposed to ultraviolet (UV) radiation, such as sunlight. These lesions in DNA can interfere with normal DNA functions such as DNA replication and/or transcription. One way that thymine dimers are repaired in cells is by the action of an enzyme called DNA photolyase, which directly reverses the chemistry of the thymine dimers so that they are once again in their monomeric native state (T↔T→ T, T).

Removal of Damage

This mode of DNA repair involves the enzymatic excision of damaged bases.

- One biochemical pathway of excision repair involves the excision of damaged bases as the free base and is therefore called base excision repair. Base excision repair is employed by cells to remove simple chemical modifications such as those produced by ►oxidative DNA damage or by simple chemicals, including alkylating agents that are used to treat various cancers.
- A second biochemical pathway for the removal of damage involves the excision of a relatively large piece of one strand of the DNA duplex that includes the damaged base. This is called nucleotide excision repair. ►Nucleotide excision repair operates against thymine dimers in DNA (in addition to DNA photolyase) as well as in the removal of many other types of chemical altered bases.
- A third mode of excision repair involves the excision of bases in DNA that are incorrectly paired (mispaired bases). This type of excision repair is called ►mismatch repair.

Repair of DNA and Cancer

Defective DNA repair allows the persistence of damaged bases in the genome. When such DNA is copied during ►DNA replication mistakes are made leading to permanent mutations. Mutations that affect oncogenes or tumor suppressor genes can lead to cancer. Hence, defective DNA repair is an important cancer predisposing factor. Patients who suffer from the hereditary disease ►xeroderma pigmentosum (XP) are unable to carry out nucleotide excision repair of DNA. Hence, when exposed to sunlight, the thymine dimers generated in the DNA of their skin cells accumulate and result in a very high incidence of skin cancer. Patients who are genetically prone to ►colon cancer suffer from a disease called hereditary non-polyposis colon cancer (►HNPCC) and are often defective in ►mismatch repair. Thus, they accumulate mutations that lead to cancer of the colon.

Base excision repair often removes bases that are altered by alkylating agents used for cancer treatment. In principle therefore, inhibiting base excision repair in cancer (but not in other normal) cells would be expected to improve the therapeutic efficacy of alkylating agents. For this reason DNA repair enzymes are strategic targets for rational design of anticancer agents.

Repair of DNA Strand Breaks

Strand breaks can be produced in DNA after exposure to ►ionizing radiation (X-rays) and can also occur spontaneously in cells as a result of oxidative damage. Specialized repair pathways operate to repair both single- and double-strand breaks in DNA. The proteins encoded by the breast cancer genes ►BRCA1 and ►BRCA2 are believed to be involved in the repair of strand breaks in DNA.

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Repeat Dose Toxicity Studies

Definition

Sometimes referred to as “subacute toxicity studies” or if dosing for longer periods “subchronic” for periods up to 3 months or “chronic studies” if longer, these are simply standardized studies whereby groups of animals are given daily doses of drug for different periods of time. Periods are usually 14 days or 1 month, 6, 9 and sometimes 12 months. Many parameters are usually

measured such as general clinical comportment, blood chemistry, hematology and electrocardiographic activity Full autopsies and microscopic examinations are conducted at the end of these studies. They are conducted according to ►Good Laboratory Practice.

►Preclinical Testing

Reperfusion

Definition
Restoration of blood flow.

Repetitive DNA

Definition
DNA sequences that are repeated in the genome. These sequences do not code for protein. One class consists of sequences referred to as ►minisatellites, 10–100 nucleotides, repeated thousands of times. Another class, much shorter from one to few nucleotides, is termed microsatellite (►microsatellite instability). Longer repeats, approximately 300 nucleotides in length, are ►Alu elements. A class of much longer repetitive DNA (up to 6,000 nucleotides) is represented by long interspersed nuclear elements (►LINE element).

Replication

Definition
Refers to the duplication of DNA during ►S-phase of the ►cell cycle.

Replication-Activated Adenovirus

►Oncolytic Adenovirus

Replication-Competent Adenovirus

►Oncolytic Adenovirus

Replication-Dependent Histone Genes

Definition
Are genes coding for ►histone proteins, specifically expressed in a ►cell cycle dependent manner. They differ from constitutive histone genes because they lack ►introns, and their mRNA end with a specific structure known as "stem-loop' instead of a poly-A tail. In the human genome they are located in two clusters on chromosomes 1 and 6.

►Cajal Bodies

Replication Factories and Replication Foci

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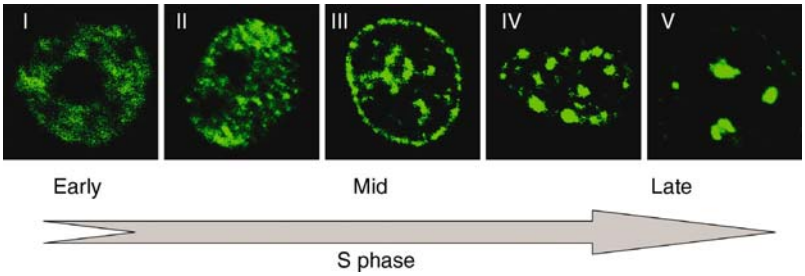
Synonyms
Replication sites

Definition
Are subnuclear functional domains where genomic DNA replication takes place.

Characteristics
In mammalian cells ►DNA replication takes place at discrete nuclear sites called replication foci where newly synthesized DNA accumulates. Replication foci can be revealed by pulse-labeling cells with thymidine analogues such as 5-bromo-2′-deoxyuridine (►BrdU) or biotinylated ►dUTP, which are subsequently detected by fluorescent probes. The distribution pattern of replication foci within the nucleus changes according to a precise program during ►S phase. The succession of these spatial patterns during the course of S phase of tumor HeLa cells is shown in Fig. 1. During the first 5 h



Replication Factories and Replication Foci. Figure 1 Subnuclear distribution of replication foci in different moments of the S phase. Exponentially growing HeLa cells were pulse labeled with BrdU and stained with FITC-conjugated anti-BrdU antibodies. Five patterns of replication foci (indicated by roman numbers) that are diagnostics of different moments of the S phase (from early to late-S) can be distinguished.

of S phase, replication takes place within many foci distributed throughout the nucleoplasm (type I/II patterns). Several sets of these early-replicating foci are activated over the course of the first half of S phase, each completing DNA synthesis in about 60 min. In mid-S phase, replication takes place at nuclear periphery and in perinucleolar regions for 2–3 h (type III). In the final few hours, replication occurs within a few relatively large foci that are present throughout the nucleus (type IV/V). Individual replication foci are only active for about 1 h before adjacent foci are activated and reflect the timing of firing of the ►origins of replication (►Replication licensing system). Notably, labeling with BrdU in vivo allows the distribution of ►replicons labeled during any interval of S phase to be visualized in any subsequent ►cell cycle phase and even over many cell generations. This suggests that replication foci are a general feature of ►chromatin organization (►Nucleosomes).

Colocalization on replication foci of replicative enzymes gave rise to the idea that replication takes place within factories. Replication factories assemble in response to demand and their appearance precedes DNA synthesis at specific nuclear sites. The number and size of the replication factories vary throughout S phase according to a program that reflects the replication of various portions of the genome. At the onset of S phase, the factories are associated with transcribed genes whereas the appearance of mid- and late-S phase factories coincides with the replication of silent ►heterochromatic regions. Replication factories are composed of groups of coordinately replicated chromosomal domains. Considering the number of factories, the length of the genome and the rate of a bi-directional ►replication fork, it has been calculated that an average factory in early S phase contains ~5–6 replicons spatially distributed in a small nuclear volume. Replication factories are stably anchored in the nucleus and their distribution changes through a gradual, coordinated assembly and disassembly process throughout S phase. Thus, the large replication factories in late S phase do not originate from the coalescence of several small factories but are assembled de novo during S phase.

Protein Composition of Replication Factories

Several studies have focused on identifying the constituents of the replication factories. The general approach consisted of the staining of the cells with antibodies against specific candidate proteins during S phase and observing whether these proteins colocalize with BrdU-labeled replication foci. Using this approach, several replication proteins, including ►PCNA, DNA ligase I, replicative DNA polymerases (DNA polymerase α and δ) and flap endonuclease 1, have been shown to be present at replication foci during S phase. In the last few years, the use of confocal microscope analysis has shown that, in addition to replicative enzymes, replication factories contain a still growing list of factors involved in DNA metabolism and cell cycle control (Table 1), underscoring the relevance of these functional domains in the integration and coordination of DNA replication with ►DNA repair (►Repair of DNA) and cell cycle progression. Therefore the factory model favors flexibility and crosstalk between these metabolic pathways.

Interestingly, early and late S phase factories differ not only in size but also in their composition. For instance, DNA polymerase ε colocalizes only with late-S replication factories, suggesting that this enzyme participates in DNA replication late in S phase. Also the ►histone deacetylase colocalizes with late S phase but not with early S phase replication factories. This seems functionally linked to the hypoacetylated status of the ►heterochromatin that is replicated in late S phase. The proteomic analysis (►Proteomics) of replication factories is under way and the identification of the protein composition of the factories will help to understand the mechanisms that control early- and mid/late-firing origins of replication in mammals.

Replication Factories and Replication Foci. Table 1 List of the proteins that colocalize with the replication factories

Proteins	Function in DNA replication
DNA polymerase α	Synthesis of Okazaki fragments
Flap endonuclease 1	Maturation of Okazaki fragments
DNA ligase I	Ligation of Okazaki fragments
Replication protein A	Single strand DNA binding
DNA polymerase δ	Synthesis of the leading strand
Proliferating cell nuclear antigen (PCNA)	DNA polymerase clamp
Replication factor C	Clamp loader
Minichromosome maintenance (MCM) 8	DNA unwinding
DNA polymerase ε	DNA replication? Checkpoint?
Kin17	DNA replication?
Proteins	Function in chromatin organization
DNA methyltransferase 1	Cytosine methylation
Histone deacetylase 2	Deacethylation of histone tails
Chromatin assembly factor 1	Nucleosomes assembly
Proteins	Function in DNA repair
Uracil-DNA glycosylase (UNG2)	Removal of uracil from DNA
DNA polymerase η	Translesion DNA synthesis
DNA polymerase j	Translesion DNA synthesis
MSH3/MSH6	DNA mismatch repair
MYH	DNA mismatch repair
MRE11	DNA double strand breaks repair
NBS1	DNA double strand breaks repair
Proteins	Function in cell cycle progression
Cyclin A/Cdk2	Phosphorylation of replicative factors
Rad17	Checkpoint protein

Extensive mutational analysis of human DNA ligase I and DNA methyltransferase 1 identified a short motif highly homologous in the two proteins as the determinant sufficient to target a protein to replication factories. This sequence, known as the replication factory targeting sequence (RFTS), corresponds to the first 20 amino acids of human DNA ligase I and consists of two boxes: box 1 contains the sequence IXXFF (I, isoleucine; X, any amino acid; F, phenylalanine) whereas box 2 is rich in positively charged amino acids (arginine and lysine). The identification of the RFTS opens up the possibility of disassembling the factories by targeting specific peptides. This perspective could be relevant in the search for new anti-proliferative drugs for cancer therapy.

The RFTS overlaps an evolutionary conserved binding site for PCNA, an essential component of human ►replisome. Several proteins are recruited to the factories through the same conserved PCNA-binding site, suggesting that PCNA plays a central role in the recruitment and stable association of DNA replication proteins at replication factories. Consistent with its role in the organization of the factories, PCNA, unlike replication protein A and DNA ligase I, shows only a little turnover at replication factories.

The cell cycle inhibitor $p21^{CIP1/WAF1}$ (►p21WAF1/CIP1/SDI1) binds PCNA via the same motif. Peptide mapping studies and the elucidation of the crystal structure of the PCNA-binding peptide of $p21^{CIP1/WAF1}$ complexed with PCNA identified the interdomain connector loop of PCNA as the binding site for $p21^{CIP1/WAF1}$. This implies that all the other DNA replication proteins that contain a similar PCNA-binding motif bind to the same or at least overlapping regions of PCNA. Consistent with this idea $p21^{CIP1/WAF1}$ inhibits the binding of flap endonuclease 1, DNA methyltransferase 1 and DNA ligase I to PCNA. Therefore it has been proposed that $p21^{CIP1/WAF1}$ inhibits DNA replication by binding PCNA within replication factories and causes the release of DNA replication proteins from these structures.

Regulation of the Formation of Replication Factories

Post-translational modifications of the replicative factors are involved in the dynamic program of

replication factories. For instance, two immunologically distinct DNA polimerase α-primase subpopulations have been described in mammalian cells. The two pools differ mainly in the phosphorylation status of the p68 regulatory subunit by Cdk2/cyclin A (►Cyclin dependent kinases) protein kinase. By using monoclonal antibodies selective for the two enzyme populations, it has been shown that only the phosphorylated enzyme revealed by 132–20 antibody colocalizes with the replication factories. Several pools of human DNA ligase I, differing in their phosphorylation level in vivo, have been described. Four serines phosphorylated during the cell cycle have been mapped by mass spectrometry. Substitution of these serines with the phosphoserin-mimetic aspartic acid hampers the recruitment of human DNA ligase I to replication factories, supporting the idea that phosphorylation is one of the mechanisms controlling the dynamics of replication factories during S phase.

The ordered assembly and disassembly of replication factories are monitored by the cell cycle ►checkpoints, signaling pathways that halt progression through the cell cycle until an earlier process, such as DNA replication, is complete. In the presence of drugs that stall replication forks, such as aphidicolin, the distribution patterns of replication factories are stabilized until the drug is removed from the culture medium and the dynamic program of genome replication is temporarily blocked. This requires the action of the ►ATR-dependent checkpoint pathway. On the contrary, activation of the intra-S phase checkpoint (►S-phase damage-sensing checkpoints) by ►topoisomerase II-DNA cleavage complexes induced by the anticancer drug ►etoposide causes the disappearance of active replication factories, strongly affecting the functional organization of S phase nuclei in tumor cells. Therefore the type of damage, its distribution relative to the moving fork and the mechanism involved in the ►DNA damage (►DNA Damage Responses) recognition, could determine the choice between stabilization and dispersal of replication factories in S phase.

Clinical Relevance

The clinical relevance of the replication factories derives essentially from the following aspects:

- Replication factories can be easily detected in immunocytochemistry with antibodies against several replicative proteins and selectively mark S phase cells. Thus cell staining with anti-replication factories antibodies is useful to estimate the fraction of replicating cells in a specimen. Moreover the expression level of several constituents of the replication factories strongly correlates with the rate of cell proliferation. This makes it possible to expand the repertoire of clinical ►biomarkers for the analysis of cell ►proliferation. Monoclonal antibodies against PCNA, flap endonuclease 1, Chromatin assembly factor 1 and DNA ligase I, are used in ►immunohistochemistry to analyze the kinetics of cell proliferation of neoplastic disorders and (►Preneoplastic lesions) in human pathology.
- Anticancer drugs belonging to the class of topoisomerase I and II poisons, such as ►camptothecin and ►etoposide induce the dispersal of replication factories in tumor cells. This effect is easily appreciable in staining cells with anti-replication factories antibodies. Therefore the effect on the replication factories can be considered to develop cell-based assays for screening of new potential anticancer drugs. Since the dynamics of the replication factories is controlled by cell cycle checkpoint pathways, this kind of assay could also be relevant to optimize strategies to selectively target checkpoint-deficient cancer cells.

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Replication Fork

Definition

Y-shaped regions of a replicating DNA molecule at which the two daughter strands are formed and separate. Replication forks are the protein machine that translocates along double-stranded DNA, replicating both strands as it moves.

►Replication Factories and Replication Foci
►Replication Licensing System

Replication Licensing System

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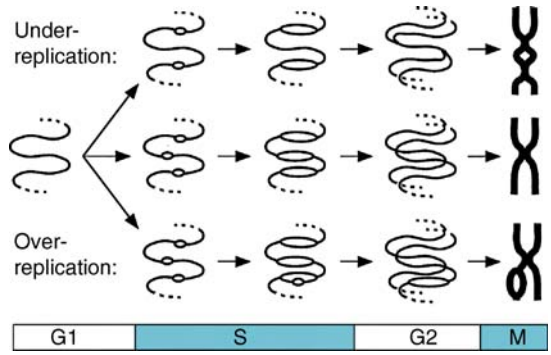
Definition

Ensures that no sections of chromosomal DNA are replicated more than once in a single ►cell cycle. It loads complexes of ►MCM/P1 proteins onto ►replication origins early in the cell cycle, which "license" the origin for a single initiation event in the subsequent S phase. As a consequence of replication, the MCM/P1 proteins are removed from the DNA, thus ensuring that re-replication of DNA does not occur in a single cell cycle.

Characteristics

In order to minimize the risk of potentially cancer-causing mutations occurring whenever a cell divides, the duplication of chromosomal DNA must be as accurate as possible. All the genome must be replicated, with nothing left unreplicated and nothing replicated more than once. This problem is made complicated by the way that eukaryotes undertake genome duplication. ►Replication forks progress along double-stranded DNA and copy both strands at a rate of 10–100 base pairs per second. However, a single replication fork would take years to duplicate a typical genome and so thousands of replication forks are used, initiated from replication origins scattered throughout the genome (Fig. 1). To ensure the complete replication of eukaryotic chromosomes, these origins must be sufficiently closely spaced so that all the intervening DNA can be replicated before entry into mitosis occurs. Failure to replicate even small sections of DNA could lead to disastrous consequences as the sister chromatids are pulled apart during mitosis (Fig. 1, "under-replication"). On the other hand, to prevent any section of DNA being replicated more than once in a single S phase, each origin must fire no more than once in each cell cycle. Over-replication of DNA is likely to be very harmful as it would represent an irreversible genetic change, potentially leading to the risk of recombination and amplification occurring in the duplicated region (Fig. 1, "over-replication"). The replication licensing system permits replication origins to be used efficiently, whilst preventing them from firing more than once in single cell cycle.

Precise chromosome duplication is achieved by separating the initiation of DNA replication into two phases (Fig. 2, left-hand panel). In the first phase, which occurs early in the cell cycle, replication origins are



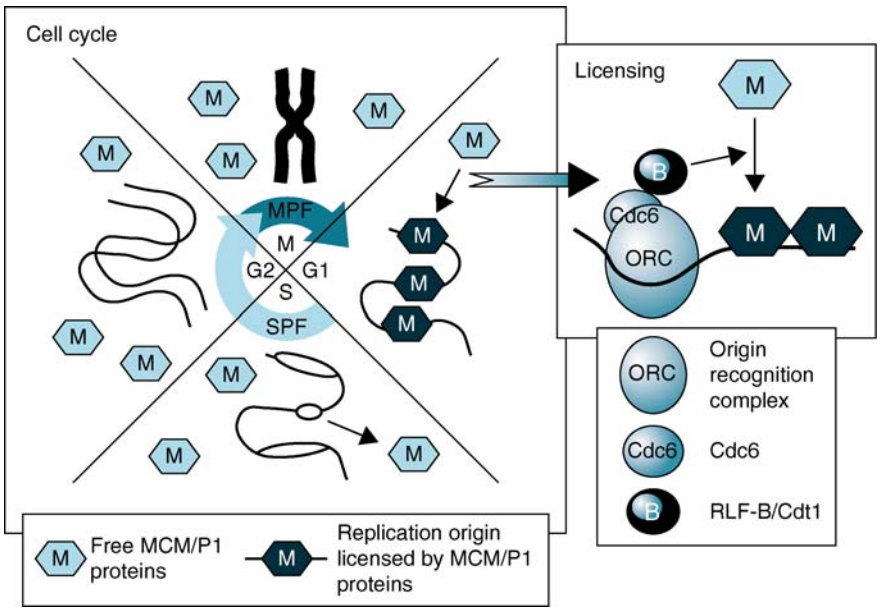
Replication Licensing System. Figure 1 Ensuring precise chromosome replication. (a) Small segment of chromosomal DNA, replicated from three origins is shown during the cell cycle. *Middle panel*: successful duplication. *Top panel*: under-replication due to the failure of one of the origins to fire. *Bottom panel*: over-replication, due to one of the origins firing a second time in S phase. The four stages of the cell cycle – G1, S (DNA synthesis), G2 and M (mitosis) – are shown below.

"licensed" by loading the RLF-M complex of MCM/P1 proteins (shown as "M" in Fig. 2). In the second phase, ►S-phase promoting factor (SPF) becomes active and promotes the initiation of a single pair of replication forks at each licensed origin. S-phase promoting factors probably consist of various ►cyclin-dependent kinases (CDKs) as well as the ►Cdc7/Dbf4 protein kinase. As each origin initiates its MCM/P1 proteins are removed, thereby resetting the origin to the unlicensed state. To ensure that no re-initiation occurs, the ability to license origins must completely cease before any replication forks are initiated; even a small amount of licensing activity present during S phase could lead to the re-replication of part of the genome. The way this regulation is achieved is currently poorly understood.

Molecular Composition

The replication licensing system consists of two components: The RLF-M complex of MCM/P1 proteins, which constitutes the license, and RLF-B/Cdt1, which is required to load RLF-M onto chromatin early in the cell cycle. Other proteins are also required on the DNA that allow it to serve as a substrate for the replication licensing system. These include the Origin Recognition Complex (ORC) and the Cdc6 protein (Fig. 2, right-hand panel). This group of proteins form the "►pre-replicative complex" present at replication origins during late mitosis and G1. All of these are potentially under cell-cycle regulation to prevent the re-licensing of replicated DNA.

There are six MCM/P1 proteins, termed Mcm2, 3, 4, 5, 6 and 7, which are in the form of a hetero-hexamer in the active RLF-M complex. However, a number of other MCM/P1 protein complexes also exist, which



Replication Licensing System. Figure 2 Left-hand panel: a small segment of genomic DNA containing three replication origins is shown during the cell cycle. The factors that drive cell cycle progress (SPF and MPF) are shown by arrows. In late mitosis and early G1, replication origins are "licensed" by binding the MCM/P1 complexes (hexagons). During S phase, S-phase-promoting factor (SPF) induces replication forks to initiate at licensed origins, and the MCM/P1 proteins are displaced. In G2 phase, all replication forks have terminated, all origins are unlicensed, and all DNA is replicated. Mitosis-promoting factor (MPF) then triggers the condensation of DNA into chromosomes and its segregation to the two daughter cells. *Right-hand panel*: the licensing reaction requires ORC, Cdc6 and RLF-B/Cdt1, and results in multiple copies of the MCM/P1 complex being assembled onto each replication origin.

are probably intermediates in an assembly/disassembly pathway. In vertebrates they are abundant nuclear proteins, and 10–20 copies of the RLF-M hetero-hexamer are loaded onto each ►replication origin. The precise role of the MCM/P1 proteins in DNA replication is currently unclear, though they have been shown to have helicase (DNA unwinding) activity. This helicase activity may be involved in allowing the replication fork proteins to load onto or move along the DNA. Various lines of evidence suggest that the MCM/P1 proteins are substrates of the Cdc7/Dbf4 kinase that is involved in activating replication origins during the cell cycle. As a consequence of MCM/P1 protein phosphorylation by Cdc7/Dbf4, other replication factors such as DNA polymerase α are then recruited to the replication origin.

RLF-B/Cdt1 acts to promote the loading of the RLF-M complex onto DNA, but thereafter does not appear to be required for the maintenance of the licensed state. Its precise molecular activity is currently unknown. RLF-B/Cdt1 is under tight cell cycle control and this regulation is likely to be important in preventing re-replication of DNA in a single cell cycle.

The Origin Recognition Complex (ORC) consists of six polypeptides termed Orc1, 2, 3, 4, 5 and 6. Binding of ORC to DNA appears necessary for that place to be defined as a replication origin. In addition to its role in establishing replication origins, ORC also appears to be involved in establishing repressive chromatin states at specific sites on DNA.

The binding of Cdc6 to chromatin is dependent on the presence of ORC. It is required for the origin to become licensed but is not for the maintenance of the licensed state once licensing has occurred. In mammalian cells, Cdc6 is exported from the nucleus at the end of G1 and does not regain access to the chromatin until the end of the subsequent mitosis. In yeast, Cdc6 is degraded at the end of G1. These features are likely to contribute to mechanisms that prevent re-licensing of DNA once S phase has started.

Regulation

Since re-replication of segments of chromosomal DNA is likely to cause very significant genetic changes to the cell, it is obviously crucial for eukaryotic cells to prevent this from happening. In order for the licensing system to operate properly and ensure that no DNA re-replicates, its activity must completely cease before S phase starts (Fig. 2). Recent work suggests that cells may possess multiple redundant mechanisms for ensuring this. One important consideration is that the components required to load the MCM/P1 proteins onto DNA (ORC, Cdc6, RLF-B/Cdt1) are not required

for the continued binding of the MCM/P1 proteins. Therefore in principle, licensing can be prevented by inhibiting the activity of either ORC, Cdc6 or RLF-B/Cdt1/Cdt1, whilst the licensed state is maintained. Although it is currently not possible to describe in molecular detail how licensing is prevented late in the cell cycle, three important themes have emerged: regulation by changes in subcellular localization, regulation by cyclin-dependent kinases and regulation by geminin.

Early experiments that characterized the replication licensing system showed that in order for replicated nuclei from the G2 phase of the cell cycle to undergo a further round of DNA replication, they had to undergo a transient permeabilization of their nuclear envelope. This suggested that re-replication of chromosomal DNA was regulated at least in part by compartmentalization of regulatory components between the nucleus and cytoplasm. Recent work has confirmed this idea, but has shown that different organisms actually regulate different components of the licensing system by this mechanism. In mammalian cells, the Cdc6 protein, which is required on the DNA for licensing to occur, is nuclear in late mitosis and G1 and is exported from the nucleus during S phase and G2. The lack of Cdc6 in the nucleus late in the cell cycle is likely to play an important role in preventing the re-licensing (and hence re-replication) of the DNA. A different version of this story is seen in the yeast *Saccharomyces cerevisiae*, where the MCM/P1 proteins, rather than Cdc6, are present in the nucleus only in late mitosis and early G1 and are exported from the nucleus at later stages of the cell cycle. Physically separating essential licensing components from their DNA substrate by nuclear export would seem to be a powerful way of preventing the inappropriate licensing of replicated DNA.

Another important aspect of the regulation of the replication licensing system involves cyclin-dependent kinases (CDK). These kinases promote the major transitions of the eukaryotic cell division cycle, including entry into S phase (SPF) and entry into mitosis (MPF) (Fig. 2). During late mitosis and early G1, however, CDK activity is low and this is the period when replication licensing can occur. Research in a number of different experimental systems have shown that CDKs, either directly or indirectly, can inhibit origin licensing. The nuclear exclusion of yeast MCM/P1 proteins and mammalian Cdc6 appears to be promoted by high CDK activity. CDKs may also have other inhibitory effects on the activity of the licensing system. For example, in S. cerevisiae, the CDK-dependent degradation of the Cdc6 protein occurs during later stages of the cell division cycle. This idea provides an elegant explanation for cell cycle regulation of chromosome replication, with an early pre-replicative phase where CDK activity is low and licensing occurs, followed by progression into S phase where CDK activity is high and licensing is inhibited. Another key licensing regulator that has recently been identified is geminin, a small protein whose abundance changes dramatically during the course of the cell cycle: it builds up in S phase and G2, but is degraded on exit from metaphase. Geminin tightly binds and inhibits the RLF-B/Cdt1 component of the licensing system, and may represent the major activity preventing re-licensing of replicated DNA late in the cell cycle of higher eukaryotes.

Clinical Relevance

When cells terminally differentiate or enter quiescence, their replication origins do not remain licensed and the MCM/P1 proteins are lost from the chromatin. The MCM/P1 proteins therefore can be used as unique markers for proliferative cells. Antibodies to the MCM/P1 proteins been shown to be useful in analyses of cervical smears and urine samples to detect abnormal proliferative cells. More speculatively, failures of the replication licensing system may underlie the ►chromosome rearrangement and gene ►amplification observed in many types of cancer. The replication licensing system as a whole also represent a potentially powerful target for anti-proliferative drugs, though this potential has yet to be explored.

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Replication Origin

Definition

Is the site on DNA where ►replication forks are initiated.

►Replication Licensing System

Replication-Selective Adenovirus

►Oncolytic Adenovirus

Replication-Selective Viruses

►Oncolytic Virus

Replication Sites

►Replication Factories and Replication Foci

Replicative DNA Lesion Bypass

►DNA-Damage Tolerance

Replicative DNA Polymerase

Definition

A group of enzymes that synthesize a new DNA strand on a template strand by adding nucleotides one at a time to the 3′-OH end of DNA.

►DNA-Damage Tolerance

Replicon

Definition

Region of DNA replicated form one origin of DNA replication, replication unit.

►Replication Factories and Replication Foci

Replisome

Definition

Multiprotein complex assembled at the replication fork that carries out the synthesis of DNA.

►Replication Factories and Replication Foci

Reporter Gene Assays

Definition

Indirectly monitor the transcriptional activity of cellular genes through the production of proteins with easily measurable activities such as luciferase (►Luciferase Reporter Gene Assays), β-galactosidase (β-Gal), chloramphenicol acetyltransferase (CAT), β-lactamase (β-lac), alkaline phosphatase (SEAP or SPAP), or green fluorescent protein (GFP). They have a wide variety of uses as surrogate markers for gene transcription in cells and *in vivo*, and are valuable tools for cancer research, detection, and treatment.

Repressor Complex

Definition

A multi-protein complex that represses ►transcription. Contains three components: a DNA-binding subunit, a ►corepressor (for example SMRT, Sin3, co-REST) and a ►histone deacetylase.

Reproductive Toxicology Studies

Definition

Comprise teratology studies at examine the potential for drugs to induce structural defects to the developing fetus when the mother is being dosed with drug and studies which investigate the effects of drugs on gonads, fertility, gestation, the fetus, lactation and general reproductive performance. Such studies are usually conducted either in rabbits or rodents or both.

►Preclinical Testing

REPSA

Definition

Restriction endonuclease protection, selection, and amplification (REPSA) is a ►combinatorial selection method used to identify consensus protein, nucleic acid, and small molecule binding sites on duplex DNA. It differs from other combinatorial selection methods in that it does not require the physical separation of ligand-bound from unbound selection templates and therefore can be applied to unknown and uncharacterized mixtures of multiple, native ligands.

Reserve Cell Carcinoma

►Extrapulmonary Small Cell Cancer

Residual Disease

►Minimal Residual Disease

Resistance

Definition

The growth of cancer cells despite therapy. There are many mechanisms, but the final endpoint is that cancer cells are unresponsive to therapy.

►Minimal Residual Disease
►Multidrug Resistance

Resistance Modulation

►Chemosensibilization

Resistance Reversion

►Chemosensibilization

Respiration Rate

Definition

Synonym O_2 consumption rate or O_2 uptake rate, is the amount of oxygen consumed by a tissue or organ per unit time.

►Oxygenation of Tumors

Respiratory Burst

Definition

Synonym oxidative burst; refers to the rapid release of ►reactive oxygen species (ROS) by immune cells, such as ►macrophages and ►neutrophils. Superoxide and hydroxyl radicals as well as ►hydrogen peroxide are the most important chemicals.

Respiratory Chain

Definition

Is a series of four mitochondrial protein complexes which transport electrons from reducing equivalents (NADH, $FADH_2$) to oxygen, to form H_2O, coupled to proton extrusion. The consequent establishment of an electrochemical proton gradient drives ATP synthesis.

►Amine Oxidases

Response Elements

Definition

Are sequences of DNA within the ►promoter region of genes that are able to bind specific proteins or protein

complexes to regulate transcription of that particular gene. A particular gene may have different types and multiple response elements. This enables well-regulated and sensitive control over ►transcription.

Restenosis

Definition

Narrowing of a conduit such as a blood vessel after a blockage has been opened.

Resting Energy Expenditure

Definition

REE; Refers to the energy expended for normal cellular and organ function during resting conditions.

►Cachexia

Resting NK Cell

Definition

Are freshly isolated primary Natural Killer (NK) cells that have not been stimulated with cytokines in in vitro cultures.

►Natural Killer Cell Activation

Restriction Landmark Genomic Scanning

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Definition

RLGS; Is a two-dimensional gel electrophoresis method that is used as a genome scanning technique to survey genomes for copy number changes as well as changes in DNA ►methylation status of promoter sequences.

Characteristics

RLGS was developed as a high speed genome scanning technique for the identification of DNA ►amplification as well as DNA methylation changes. For RLGS, genomic DNA is restriction digested with a landmark enzyme, usually a rare cutting enzyme (e.g. *Not*I). The restriction ends are endlabeled in a fill-in reaction using [α-32P] labeled nucleotides. In the following second restriction digest (e.g. *EcoR*V), the DNA fragments are cut into smaller fragments suitable for separation in an agarose disc gel electrophoresis (first dimension). The next step is an in-gel restriction digestion with a third enzyme (e.g. *Hinf*I) that generates fragments with a size range between 100 and 2,000 base pairs. DNA fragments are separated in a polyacrylamide gel (second dimension). The gel is dried and exposed to an x-ray film. The resulting RLGS profile display highly reproducible patterns of RLGS fragments in which each fragment represents an end-labeled *Not*I site. Up to 2,000 end-labeled rare cutting restriction sites are displayed in a single RLGS profile. Most of the rare cutting restriction enzyme sites with GC-rich recognition sequences are located in the promoter region of genes, resulting in a selective display of gene sequences rather than random genomic sequences. The sequence of RLGS fragments can be determined through standard ►PCR-based or direct cloning procedures. An alternative to these methods is now the used of the available genomic sequences and virtual RLGS that allows to precisely predict the sequence of an RLGS fragment. If methylation sensitive enzymes are used as restriction landmarks, the method is able to provide a search for altered DNA methylation. Methylation in a *Not*I site would inhibit the restriction digest resulting in lack of endlabeling at this particular site. As a result this fragment would be missing from the RLGS profile. Since RLGS profiles are highly reproducible, this system allows the comparison of profiles derived from different tissues (e.g. normal versus diseased tissue) or between different individuals.

Application

Identification of Low Level DNA Amplification in Human Cancers

The endlabeling step in the RLGS procedure guarantees a correlation between RLGS fragment intensity and copy number of the particular sequence in the genome. The technique is sensitive enough to distinguish diploid (full intensity fragments) and haploid (half intensity fragments) copies. The majority of fragments in a human RLGS profile (about 90%) show diploid intensity, while a much smaller fraction of fragments (about 10%) have

haploid intensity. In addition, 10–15 fragments show markedly enhanced intensities (~50–200 times the intensity of a diploid fragment). It was shown that these fragments are derived from rDNA sequences, present at higher copy numbers in the genome. Such quantitative capacity allows the use of RLGS to scan tumor genomes for regions of DNA amplification. RLGS profiles established from tumor DNA are compared to profiles of matching normal tissue or blood of the patient. The tumor profile is analyzed for fragments that show an enhancement relative to the normal profile. Using this strategy CDK6 amplification in gliomas and low level amplification of MYCN and NAG was identified in medulloblastomas for the first time.

Global Scanning for DNA Methylation Changes in Human Cancers

If methylation sensitive restriction enzymes are used for the RLGS analysis, it is possible to scan genomes for differences in methylation patterns. The restriction landmark enzyme *Not*I is methylation sensitive. If a genomic *Not*I site is methylated, the enzyme does not cut and the site will not be endlabeled, resulting in a loss of this fragment in the RLGS profile. If the *Not*I site is unmethylated, the site is cut, the restriction ends are end-labeled and the fragment is present in the profile. ►CpG islands frequently contain gene promoters and/or exons and are usually unmethylated in normal cells. Since *Not*I sites are mainly located in CpG islands, RLGS is a method that allows the determination of the methylation status in thousands of CpG islands at one time. It was shown that methylation of CpG islands is associated with delayed replication, condensed chromatin and inhibition of transcription initiation in cancer. In a recent study, aberrant CpG island methylation in multiple human cancers was studied using RLGS. In this global analysis, the methylation status of 1,184 unselected CpG islands in 98 primary human tumors was tested. An estimated average of about 600 CpG islands (range of 0–4,500) of the 45,000 in the genome were aberrantly methylated in the tumors. This study also showed patterns of CpG island methylation that were common to several types of tumors together with patterns and targets that displayed distinct tumor type specificity.

Detection of Imprinted Genes

RLGS was used in two studies to identify allele specific methylation in the mouse genome. Allele specific methylation is associated with genes that show imprinted expression (►imprinting). Imprinted genes are either expressed exclusively from either the paternal or maternal allele and possess important functions during development. The screen is based on the identification of polymorphic RLGS fragments between two mouse inbred strains. Allele-specific methylation in a polymorphic fragment results in the presence of this fragment in one cross, but its absence in the reciprocal cross. RLGS was the first method that allowed a systematic screen for loci that showed allele specific methylation. The cloning of those sequences that were methylated gave access to novel imprinted loci. So far two novel imprinted genes (U2afbprs and Grf1) were identified in an RLGS scan.

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Restriction Point

Definition

R-point; Represents the most important regulatory mechanism controlling ►cell cycle progression of mammalian cells in response to external mitogenic or growth inhibitory signals. The R-point is located in late G1 and is governed by the ►retinoblastoma protein pathway (►INK4 – ►cyclin D – ►CDK4/6 – ►Rb – ►E2F). Beyond the R-point, cell-cycle progression is largely independent of extracellular signals.

►Cell-Cycle Targets for Cancer Therapy

Resveratrol

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Synonyms

3,4′,5-trihydroxystilbene

Definition

Is a natural molecule belonging to the class of non-►flavonoid ►polyphenols. It exists as *trans* or *cis* isomers, both of which are present in nature, although the *trans* isomer is by far the most abundant, and commonly cited simply as resveratrol.

Characteristics

Resveratrol was first identified in 1940 in the roots of hellebore, then found to be present in grapevines in 1976 and in wine in 1992. Now it is known to be present in minor amounts in other nutritional sources, such as peanuts, blueberries and mulberries. In grape skin, where it is highly concentrated (50–100 mg/g), resveratrol is primarily biosynthesized in response to environmental stresses, such as ►UV radiation, ozone exposure, low temperature, injury or fungal infections (mainly by *Botrytis cinerea*). In wine, its concentration ranges from 0.7 to 7.7 mg/L, depending on cultivar, climate, and stress agents, red wines being approximately tenfold more enriched than white wines. Resveratrol is the active ingredient in the powder from the *Polygonum cuspidatum* root, which is used in traditional Japanese and Chinese medicine to treat various diseases. Many investigations in the last three decades have demonstrated a variety of biological properties of this molecule, including antioxidant activity, platelet antiaggregative effects, estrogen-like functions, immunomodulatory activity and chemoprevention. Resveratrol, as a wine ►polyphenol, has been claimed to be responsible for the so-called "French Paradox," i.e. the finding that the level of mortality for cardiovascular accidents is lower in France, where the consumption of wine is elevated, than in other highly industrialized Western countries with similar risk factor profile and a lower consumption of wine.

Mechanism of Action

In 1997, Jang et al. reported that resveratrol exerts anticancer effects by reducing tumor mass in rats. These authors demonstrated that resveratrol is effective in blocking *in vivo* the three stages of ►carcinogenesis: ►initiation, ►promotion and ►progression. Since this pioneer work was published, many studies employing human cancer cell lines have confirmed this observation and accepted resveratrol as a chemopreventive agent. Less convincing and still to be established is its chemotherapeutic potential.

The mechanisms underlying the antitumoral effects of resveratrol include a broad range of intracellular targets, whose modulation gives rise to overlapping responses that lead to growth arrest and death. Anyway, the efficacy of this molecule is still debated because of the multiplicity of affected targets and contradictory effects related to dose and time of treatment and to cellular phenotype, since often the specific cell molecular setting determines the response to treatment.

Since resveratrol mimics the structure of the synthetic estrogen ►diethylstilbestrol (for this it is defined also as a "►phytoestrogen"), it may compete with the growth promoting effect of estrogens. ►Breast cancer incidence among women in Western countries is sixfold higher than that among women in Asia who consume daily soy products rich in phytoestrogens.

Mechanisms of action include the inhibition of ►phase I enzymes, as ►Cytochrome P450, responsible for activating ►xenobiotics and the induction of ►phase II enzymes that conjugate these activated compounds to endogenous ligands (e.g., ►glutathione). Because of its lipophilic nature, resveratrol can cross the ►plasma membrane, be subjected to cellular metabolism and interact with ►phase 1 enzymes. Moreover, it reduces the insurgence of ►preneoplastic lesions in mouse mammary gland cultures and decreases the incidence of tumor formation in mice treated with ►DMBA used as tumor initiator. Since DMBA requires bioactivation by phase 1 enzymes, the antitumoral activity of resveratrol *in vivo* includes prevention of the initiation phase of carcinogenesis by inhibiting such enzymes. It additionally impairs the carcinogenic effect of aryl-hydrocarbons. Aryl-hydrocarbons are carcinogens acting *via* nuclear receptors to promote cytochrome P450 transcription for the enzymatic conversion of xenobiotics in carcinogen elements. Resveratrol, without binding the receptors, impairs their interaction with the promoter region of the CYP1A1 gene. Resveratrol has also been shown to induce phase 2 enzymes such as UDP-glucuronyltransferase and ►NAD(P)H:quinone oxidoreductase.

Resveratrol was first discovered in roots for its ability to inhibit the activity of ►cyclooxygenases (COXs), enzymes that catalyze the first committed steps of ►prostaglandin (PG) biosynthesis. It noncompetitively inhibits both the COX and hydroperoxidase activities of COX1 *in vitro*. This dual effect of resveratrol is unique, since classic ►nonsteroidal anti-inflammatory drugs (NSAIDs) affect only the activity of COXs. Additionally, it down-regulates COX2 transcription, likely by impairment of activation of the transcriptional factor ►NF-kB, upstream COX2 expression. ►Ornitine decarboxylase (ODC) is an additional target enzyme for resveratrol. Since both COXs and ODC are promoters of ►angiogenesis, the role of resveratrol as suppressor of tumor mass neovascularization is evident.

Resveratrol was found in a variety of cell models to arrest proliferation, mostly in an irreversible way, leading to ►apoptosis. This effect has been traced to its ability to modulate the activity of many key mediators of ►cell cycle and survival. One subject of debate is whether resveratrol-induced cell cycle arrest is reversible or is the first step of an irreversible

apoptotic program. Apoptosis-related proteins have been identified to be targeted by resveratrol, including ►p53. In a variety of cellular models, resveratrol strongly upregulates p53 and induces its posttranslational modification (phosphorylation and acetylation) required for regulating gene transcription. Thus, it imposes a checkpoint at G1/S transition and leads to the modulation of ►Cyclin dependent kinases and ►cyclins.

Resistance to apoptosis may depend on intracellular levels of prosurvival and proapoptotic factors such as members of the ►BCL-2 family and the inhibitors of apoptosis (IAP) proteins family. Resveratrol treatment up-regulates the expression of proapoptotic BCL-2 members and decreases the expression of ►survivin, a member of the IAP family.

Finally, resveratrol induces a ►ceramide-mediated apoptosis. Ceramide is a ►sphingolipid mediator of intracellular signals, present in low amounts in biological membranes. In conditions of stress and ►aging, there is an increased production of ceramide by its *de novo* synthesis and release by hydrolysis of complex sphingolipids. Ceramide interacts with a variety of intracellular targets, leading to differentiation, cell cycle arrest and apoptosis. Recently, resveratrol has been shown to promote intracellular accumulation of ceramide in breast and ►prostate cancer cells, elevating *de novo* synthesis by increasing the activity of the rate-limiting enzyme (serine-palmitoyl-transferase). This observation identifies a new important checkpoint in the actions of resveratrol. Although this polyphenol triggers multiple pathways, ceramide production may be a common step that drives cells toward irreversible death.

In addition to interfering with cell cycle control, resveratrol affects multiple targets that are involved directly or indirectly in chemotherapy responsiveness. An intriguing hypothesis is that resveratrol, like other flavonoids, is able to overcome the drug resistance of tumours that express multidrug resistance-associated proteins (►MRP).

As a polyphenol, resveratrol has an intrinsic antioxidant potential. Since the tumour initiation and progression may depend on ►DNA damage by ►Reactive Oxygen Species (ROS) generation, an antioxidant agent may prevent DNA damage, by ROS scavenging. Anyway, it appears restrictive to assimilate resveratrol to other common antioxidant agents. The large spectrum of molecular targets of resveratrol and the consequent wide range of biological effects contribute to the classification of resveratrol as a potential endogenous mediator, albeit it is an exogenous molecule for the human body. Tentatively, this concept could be enforced by the similarity of resveratrol structure to that of estrogens. Anyway, the literature does not provide convincing evidence to assign to resveratrol the role of an *in vivo* estrogen mimetic.

Clinical Relevance

Resveratrol is known to have minimal side-effects both *in vitro* and *in vivo* models. This plays in favor of resveratrol as a promising drug against many diseases, including cancer. Unfortunately, its bioavailability seems to be rather low, although the literature is confusing and contradictory in this regard. Modifications such as glucuronidation and sulphation occur in large amounts after intake and contribute greatly to diminish absorbtion and tissue distribution and increase excretion. To overcome this obstacle, attempts are currently on to develop novel strategies based on protection of resveratrol from conjugation. This may extend its half-life, thus promoting its biological activity.

Even considering these precautions, clinical attempts have been initiated to test the resveratrol efficacy in humans. Particularly, Phase 1 studies for ►colon cancer treatment are currently underway at the University of Michigan, University of California Irvine and at University of Leicester.

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RET

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Definition

RET is the abbreviation for a gene that was found to be rearranged during transfection studies of ►NIH-3T3 cells with human lymphoma DNA. It is a ►receptor tyrosine kinase, for which the ligand is glial cell line derived neurotrophic factors (GDNF). It was originally detected as an oncogenic version from a ►T-cell lymphoma.

Characteristics

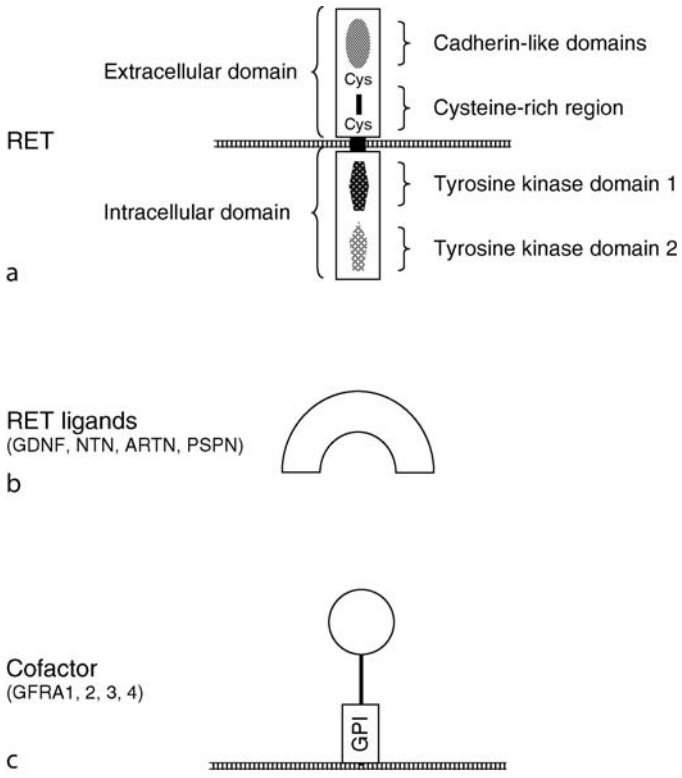
RET is located on chromosome sub-band 10q11.2 and consists of at least 21 exons. Alternative polyadenylation sites and ►alternative splicing result in different transcript sizes. RET splice variants contain the first 19 exons, followed by distinct three-prime ends. These variants produce three protein isoforms that have 9 (RET9), 51 (RET51), or 43 (RET43) distinct amino acids at their C termini. The gene encodes for a transmembrane tyrosine kinase receptor. The first ten exons, and maybe part of exon 11, encode the extracellular domain; exon 11 encodes the transmembrane domain, while the remaining exons encode the intracellular or cytoplasmic domain (Fig. 1a). The extracellular domain contains multiple cadherin-like domains and a cysteine-rich region. For the first cadherin-like domain, a surface for binding to the GDNF-GFRA (GDNF family receptor alpha)1 complex has been described. The cysteine residues are bound to each other via intramolecular disulfide bonds. Two tyrosine kinase domains have been identified in the intracellular domain.

Cellular and Molecular Regulation

RET expression is largely restricted to neural, ►neuroendocrine, and nephrogenic tissues. During embryogenesis, RET is expressed in developing kidneys, the presumptive enteric neuroblasts of the developing enteric nervous system, cranial ganglia and the presumptive motoric neurons of the spinal cord.

RET can be activated by binding to one of its ligands. Ligand binding leads to dimerization of the RET-receptor and subsequent phosphorylation of tyrosine residues (Fig. 2a). There are at least four ligands:

- Glial cell line derived neurotrophic factor (GDNF)
- Neurturin (NRTN or NTN)
- Persephin (PSPN or PSP)
- Artemin (ARTN or ART). (Fig. 1b and Fig. 3). A potentially fifth ligand, enovin (EVN), had been described which, however, turned out to be identical to artemin. All ligands belong to the GDNF family. The similarity among the various members is 40–60%. The ligands cannot bind directly to RET but rather require the presence of a membrane bound glycosyl-phosphatidylinositol (GPI)-linked cofactor. Four cofactors, named GFRA1–4 (also known as GFRα-1, -2, -3 and -4), have been identified (Fig. 1c and Fig. 3). GFRA1 is also known as GDNFR-α, RETL1 and TrnR1; GFRA2 is also known as GDNFR-β, NTNR-α, RETL2 and TrnR2. Each ligand preferentially binds to one cofactor; however, alternative binding has been observed in vitro (Fig. 3). The prevalence of these alternative interactions *in vivo* is unknown.



RET. Figure 1 Putative structure of RET, its ligands and their cofactors.

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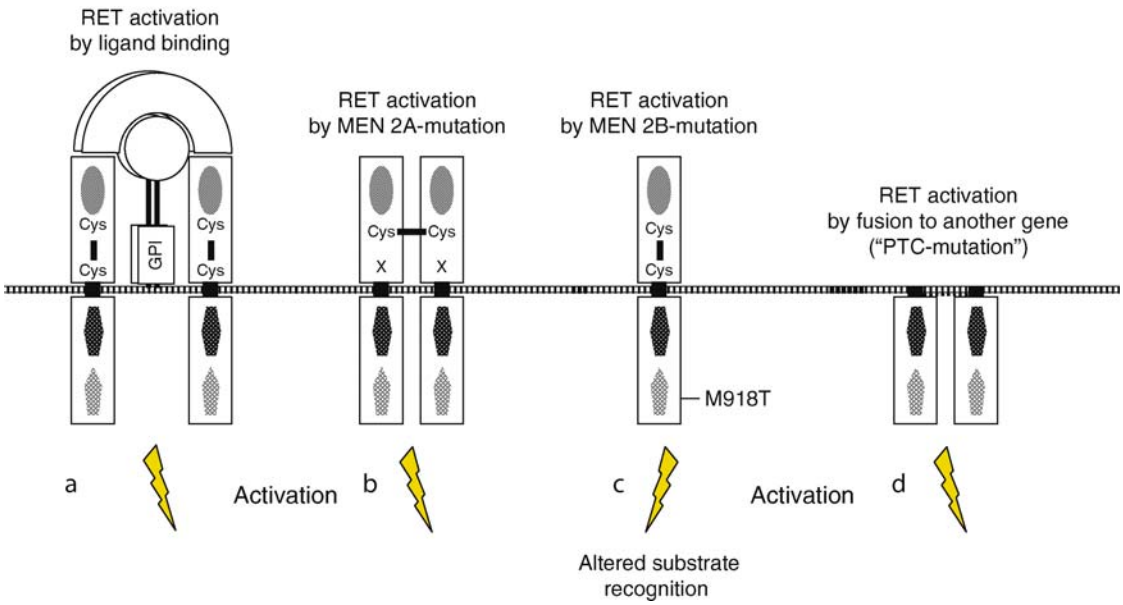
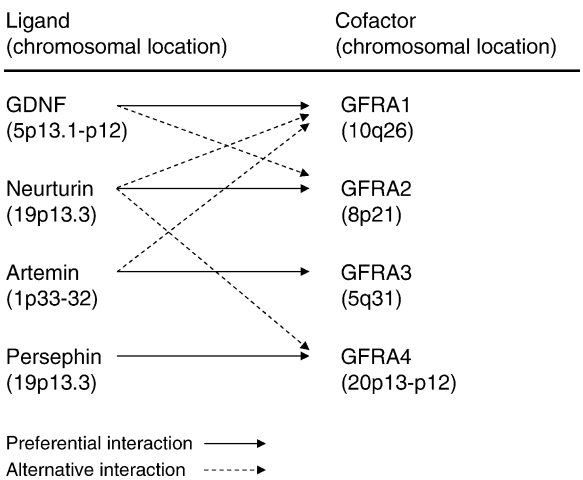


RET. Figure 2 Mechanisms of RET activation.



RET. Figure 3 Interation between various RET ligands and cofactors.

The downstream signaling pathways of RET have been investigated intensively. There is evidence that RET signals are, at least in part, transduced by intracellular serine/threonine kinases designated as ►MAPK (mitogen activated protein kinases). Other pathways involve phosphatidylinositol-3-kinase (►PI3K), Jun N-terminal kinase (►JNK), and phospholipase-C (PLC)γ.

Knockout Mice

RET −/− ►knockout mice die early after birth due to agenesis of kidneys and lack of enteric neurons distal to the stomach. In addition, RET knockout mice show depressed ventilatory response to inhaled CO2. *GDNF* −/− and *GFRA1* −/− mice also die perinatally due to the failure to develop enteric neurons and kidneys, similar to mice lacking *RET*. The GDNF-GFRA1 system has also been shown to be critical for the survival of subpopulations of sensory neurons in the dorsal root ganglion and in the nodose ganglion. Apparently the survival of neurons in the central nervous system does not depend solely on the GDNF-GFRA1 system as only a few or no deficits have been found. The critical role in kidney development emphasizes that classifying these molecules as being strictly neurotrophic is difficult. Approximately 30% of *GDNF* +/− mice lack one kidney, while *RET* +/− and *GFRA1* +/− mice have two normal kidneys. In contrast, *NTN* −/−, *GFRA2* −/− and *GFRA3* −/− mice have a relatively mild phenotype. They present with ptosis (*NTN* −/−, *GFRA2* −/− and *GFRA3* −/−), i.e. drooping of the eyelids, and deficits in the enteric nervous system, i.e. reduced density of acetylcholine esterase (AChE) fibers (*NTN* −/− and *GFRA2* −/−), parasympathetic nervous system (*NTN* −/−, *GFRA2* −/−), e.g. absence of parasympathetic innervation to the lacrimal gland, and sympathetic nervous system (*GFRA3* −/−), e.g. severe defect in superior cervical ganglion (SCG) development. *GFRA3* −/− mice and *ART* (a vascular derived neurotropic factor) −/− mice showed similar abnormalities in the migration and axonal projection pattern of the entire sympathetic nervous system. *PSP* −/− mice show normal development and behavior, but are hypersensitive to cerebral ischemia. Of note, *GFRA4* −/− mice revealed impaired thyroid calcitonin (a tumor marker produced by ►medullary thyroid carcinoma) production.

Clinical Relevance

In humans, two types of mutations have been identified in *RET*: mutations associated with ►loss-of-function and mutations associated with ►gain-of-function. The strict differentiation between mutations associated with loss-of-function and mutations associated with gain-of-function is not correct, as some mutations can cause both loss and gain-of-function depending on the time of expression, i.e. embryonic versus adult, and tissue of expression. This seemingly contradictory observation has been explained as follows. For example, the mutation C609W leads to a drastic reduction of mature RET product and subsequently reduces the level of functional receptors at the cell surface. Consequently, during formation of the enteric nervous system, the overall signal transmitted by the RET/GDNF and the RET covalent dimer complex is below the threshold required for the survival of enteric neurons. On the other hand, the ligand-independent constitutive signaling activity of the RET covalent dimer is high enough to lead to proliferation of thyroid C-cells with subsequent development of ►MTC. Biochemical analysis revealed that mutations affecting codon 618 and 620, and to a lesser extent codon 609, result in marked reduction of the level of RET on the surface.

Mutations causing loss-of-function of RET are associated with ►Hirschsprung disease (HSCR). All kinds of mutations have been found; missense mutations, non-sense mutations, frame-shift mutations, and splice-site mutations, as well as small and large insertions and deletions. Depending on the mutations, RET function may be only slightly impaired or completely lost. *RET* is highly expressed in the presumptive enteric neuroblasts of the developing enteric nervous system. Humans harboring inactivating germline *RET* mutations may lack enteric neurons and have HSCR. Interestingly, despite high levels of *RET* expression in presumptive motoric neurons of the spinal cord and developing kidneys, no clinical evidence of spinal cord involvement has been shown in patients with inactivating *RET* mutations, and renal agenesis has been reported in only a few patients with HSCR. HSCR has been reported to be associated with congenital central hypoventilation syndrome (CCHS or Ondine'e curse). Despite the fact that *RET* –/– mice show depressed ventilatory response to inhaled CO2, no *RET* mutations have been reported in patients with CCHS. Recently, mutations in *PHOX2B* have been found to be associated with HSCR and CCHS.

Mutations causing gain-of-function of *RET* have been shown to be associated with a hereditary syndrome named ►multiple endocrine neoplasia type 2 (MEN 2), with an overall life-time risk of developing MTC of about 70%, as well as with non-hereditary ►papillary thyroid carcinoma (PTC).

Mutations Found in Medullary Thyroid Carcinoma

RET has become the paradigm for molecular medicine in the management of familial cancer syndromes. Germline *RET* mutations have been found in more than 95% of all patients with hereditary MTC as part of MEN 2. The knowledge of *RET* as the disease-causing gene helps to identify individuals at risk of developing MTC.

Most mutations found in MEN 2 are almost always missense mutations. Germline *RET* mutations affecting one of six cysteines (Cys609, 611, 618, 620, 630, 634) within the cysteine-rich region (Fig. 1a) are responsible for the vast majority of two hereditary syndromes, multiple endocrine neoplasia type 2A (MEN 2A) and familial medullary thyroid carcinoma (familial MTC or FMTC), both of which are part of MEN 2. It has been shown that replacement of these cysteines by an alternate amino acid abrogates the intramolecular disulfide bonds and leads to aberrant intermolecular disulfide bonds with subsequent homodimerization and autophosphorylation (Fig. 2b).

A variety of other mutations affecting the intracellular domain have been found (e.g. E768D, L790F, Y791F, V804M, V804L, A891S). Most of them have been found in patients with FMTC but some of them have also been found in patients with MEN 2A (e.g. V804L, L790F). For some mutations (e.g. Y791F and A891S) activation of the Src/JAK/STAT3 pathway has been described.

►Missense mutations in two other codons (M918T, A883F) of *RET* affecting the tyrosine kinase domain have been found in patients with multiple endocrine neoplasia type 2B (MEN 2B), which is also part of the MEN 2 syndromes. Most often, M918T is found in most patients with MEN 2B. It has been shown that the point mutation M918T causes RET activation (Fig. 2c) but also changes the substrate specificity of the tyrosine kinase. Dimerization does not occur.

Somatic *RET* mutations have been found in sporadic MTC. Depending on the mutation analysis technique, mutations have been found in 30–70%; most often the MEN 2B-specific mutation M918T is found in exon 16. The identification of this mutation in DNA extracted from cells obtained by performing fine needle aspiration cytology can help in making the diagnosis of MTC. However, the absence of this mutation in the cytologic specimen does not exclude the presence of MTC, either hereditary or sporadic. Also, the presence of the M918T mutation does not allow determining whether the MTC is sporadic or hereditary. Additional germline mutation analysis of *RET* is required.

Mutations Found in Papillary Thyroid Carcinoma

In contrast to mutations found in MTC, specific somatic rearrangements (translocations and inversions) of *RET* have been found in PTC. The tyrosine kinase domain of

RET is fused to the 5′-terminal region of heterologous genes. The resulting chimeric molecules have been named RET/PTC. Currently, more than ten types of *RET* rearrangements have been found in PTC (RET/PTC1–8, ELKS/RET, PCM/RET, and RFP/RET). The fusion partners of *RET* are mainly *CCDC6* (coiled-coil domain containing gene 6; formerly known as H4), and *NcoA4* (nuclear receptor co-activator gene 4; formerly called *RET* fused gene (RFG)/ELE1/androgen receptor activator 70 (ARA70)) in the case of *RET/PTC1* and *RET/PTC3*, respectively. The promoter of these genes, which substitutes the *RET* promoter, is able to drive expression of *RET* in the thyroid gland. Even though the extracellular domain of RET is missing, coiled-coil domains in the fusion partners cause a constitutive dimerization (Fig. 2d). The subsequent activation of RET in this manner seems to be restricted to PTC; only one study reports RET rearrangement in one thyroid adenoma. Transgenic mice with *RET/PTC* develop PTC if driven by a thyroid-specific promoter such as the thyroglobulin promoter. The frequency of *RET* rearrangements in PTC are most often found in about 10–40% with regional/methodical differences (5–85%). Irradiation is able to cause *RET/PTC1* rearrangements in cultured cells (thyroid carcinoma cells and fibrosarcoma cells) *in vitro*. Mainly *RET/PTC1* has been found in long latency PTCs from patients following the Chernobyl nuclear accident in 1986, when a dramatic increase of PTCs was observed. *RET/PTC1* is associated with the classical variant of PTC. In contrast, *RET/PTC3* is more prevalent in short latency PTCs. *RET/PTC3* is closely associated with the solid variant of PTC.

Similar to MTC, somatic *RET* rearrangements can be diagnosed in cells obtained performing fine needle aspiration cytology in patients with PTC. However, since the frequency of *RET* rearrangements in PTC is generally low (10–40%), no conclusion can be drawn in the absence of *RET* rearrangements. The identification of *RET/PTC*, however, justifies the diagnosis of PTC. Recently, a somatic point mutation in *BRAF* (V600E; previously designated as V599F) has been identified as the most common (35–70%) genetic change is PTCs. Apparently, BRAF is required for RET/PTC-induced activation of MAPK.

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Rete Ovarii

Definition

Vestigial tubules or cords of cells near the ovarian hilus (site where blood vessels and nerves enter or leave the ovary); corresponds with the male rete testis.

►Granulosa Cell Tumors

Reticulin

►Calreticulin

Retinoblastoma

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Synonyms

Glioma retinae

Definition

Retinoblastoma (Rb, OMIM 180200) is a malignant childhood tumor of the eye that originates from neuronal cells of the developing retina. Diagnosis is based on clinical signs and symptoms and is usually made in the first 3 years of life. Development of Rb is initiated by mutations that alter both ►alleles of the ►*RB1* gene. In most patients with sporadic unilateral Rb, both mutations have occurred in somatic cells. Almost all patients with sporadic bilateral Rb are heterozygous for an oncogenic *RB1* gene mutation that was either transmitted from a parent or has occurred de novo in the germline. Germline *RB1* gene mutations

cause hereditary Rb. ►Penetrance and ►expressivity of this ►autosomal dominant trait are variable between families, which is explained in part by residual gene function of some mutant alleles.

Characteristics

Clinical Aspects

Diagnosis of Rb

The most frequent presenting sign is a white pupillary reflex (leukocoria). Strabismus is the second most common sign and may accompany or precede leukocoria. Most patients diagnosed with Rb are less than 3 years old. Age at diagnosis is earlier in children with bilateral Rb compared to children with unilateral Rb. Usually, diagnosis of Rb is established by examination of the fundus of the eye using indirect ophthalmoscopy. Magnetic resonance imaging (MRI) and ultrasonography are required for staging. Because of the high risk of tumor spread, biopsies must not be taken and, therefore, histopathology can confirm diagnosis of Rb only if therapy includes removal of the eye (enucleation).

Presentation and Family History

Most patients (60%) have Rb in one eye only (unilateral Rb). Some of these patients show multiple distinct tumor foci (unilateral multifocal Rb). The remaining 40% of patients have tumors in both eyes (bilateral Rb), which are often multifocal. More than 95% of patients with unilateral Rb and about 75% of patients with bilateral Rb have no family history of Rb (sporadic disease). Familial Rb is seen in 10–15% of patients. This includes families where retinal scars or quiescent tumors (retinomas) were identified in relatives only upon examination of the fundus of the eye.

Therapy and Prognosis

Treatment of patients with Rb depends on tumor stage, the number of tumor foci in the eye, the presence of vitreous seeding, and the age of the child. Treatment options include enucleation, external-beam radiation, cryotherapy, photocoagulation, ►brachytherapy with episcleral plaques, and ►chemotherapy combined with local therapy. Prognosis is excellent if the tumor has not invaded extraocular tissues. Metastasizing Rb often has a fatal outcome.

Second Tumors

Patients with bilateral Rb have an increased risk to develop neoplasms outside of the eye (second tumors). The spectrum of second tumors includes ►osteogenic sarcoma, ►soft tissue sarcoma, ►malignant melanoma, and ►lung cancer. The risk to develop sarcomas is significantly higher in patients with bilateral who have received external beam radiation for treatment of Rb.

Molecular Genetics

Rb is Caused by Two Mutations

Development of retinoblastoma depends on two mutations that alter both alleles of the retinoblastoma gene, *RB1* (Fig. 1). The timing of the first of these mutations is relevant for the genetic form of retinoblastoma:

- In most patients with ►sporadic bilateral Rb, the first mutation has occurred de novo in the germline of one of the parents (Fig. 1a).
- In patients with familial Rb, the first *RB1* gene mutation has occurred in an ancestor and is transmitted via the ►germ line. Family members who have inherited this mutant allele are heterozygous. Inactivation of the other, normal allele occurs in somatic cells.
- In some patients with sporadic bilateral or with sporadic unilateral Rb, the first mutation has occurred de novo during embryonal development of the patient. Consequently, these patients are mutational mosaics. Tumor development is initiated if a second mutation inactivates the normal allele in a cell that is part of the mutant sector (Fig. 1b).
- In most patients with sporadic unilateral Rb, the both mutations that are necessary to alter both copies of the *RB1* gene occur in somatic cells (Fig. 1c).

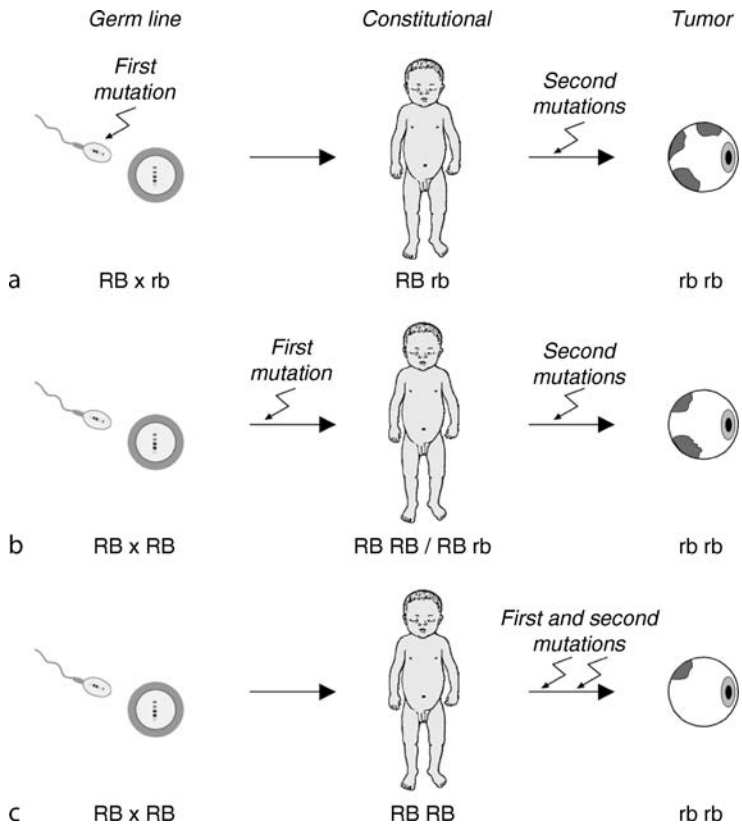
Structure of the RB1 Gene

The *RB1* gene (Acc No. L11919) spans 180 kB of genomic sequence on chromosome 13, band q14. It is composed of 27 exons and transcribed in a 4.7 kB mRNA. The 2.7 kB open reading frame starts in exon 1 and ends in exon 27 and codes for 928 amino acids. Under normal conditions there are no alternative splice forms or alternative translations start sites. The gene product is a nuclear protein which, dependent of the state of phosphorylation, enables the cell to pass to the S-phase of the cell cycle. Several other functions have been documented in addition to cell cycle regulation.

Spectrum of RB1 Gene Mutations

Mutations of the *RB1* gene are identified in constitutional DNA (e.g. from peripheral blood) of patients with hereditary Rb and in DNA from tumors (e.g. retinoblastoma):

- Large deletions: The spectrum of deletions and insertions that include all or parts of the *RB1* gene is heterogeneous. Cytogenetic deletions involving 13q14 are detected in less than 10 and 5% of patients with bilateral and sporadic unilateral retinoblastoma, respectively. Subcytogenetic gross *RB1* alterations, which can be detected by quantitative multiplex PCR or multiplex ligation-dependent probe amplification (MLPA), are present in about 10% of patients with bilateral or familial.



Retinoblastoma. Figure 1 The genetic form of retinoblastoma is determined by the timing of first RB1 gene mutation.

- ►Point mutations: More than 70% of the mutations detected in peripheral blood DNA from patients with hereditary retinoblastoma are single base substitutions and small length mutations (database of *RB1* gene mutations: http://*RB1*-LSDB.d-lohmann.de). Most point mutations result in premature termination codons as they cause of nonsense or frameshift alterations.

The following two types of mutations are identified in tumor DNA only:

- In most tumors, inactivation of one of the *RB1* alleles is accompanied by loss of constitutional heterozygosity (►LOH) at polymorphic loci located on chromosome 13. LOH can result from deletions and several chromosomal mechanisms such as mitotic recombination and nondisjunction.
- Another class of mutation only found in tumors is ►hypermethylation of the ►CpG-rich island at the 5′-end of the *RB1* gene, which is normally unmethylated. Hypermethylation is observed in about 10% of retinoblastomas.

Genotype–Phenotype Associations

The majority of *RB1* gene mutations identified constitutional DNA from patients with hereditary Rb or in DNA from tumors result in transcripts with premature termination codons (nonsense substitutions, frameshift length mutations). Available data indicate, that, at least in constitutional cells, most of these transcripts are degraded (nonsense mediated decay). With few exceptions, individuals heterozygous for mutations of this kind develop Rb (complete penetrance) and, most often, both eyes are affected.

Families with incomplete penetrance of Rb and patients hereditary unilateral Rb usually show *RB1* gene mutations that are distinct. The following types of mutation are frequently associated with incomplete penetrance and unilateral Rb:

1. Missense mutations (these account for less than 5% of known *RB1* point mutations).
2. Small deletions that keep the open reading frame intact (including skipping or deletion of in-frame exons).
3. Substitutions that affect splice signals in exons or less conserved intronic splice signals.
4. Mutations in the promoter region that inhibit binding of transcription factors and thus reduce transcription levels of the gene.
5. Deletions of the whole *RB1* with breakpoints outside of the gene.

With respect to the functional consequences it appears that mutations associated with milder phenotypic expression result in regular expression of a qualitatively altered protein (mutation types 1 and 2, see above) or in reduced levels but not loss of expression of a qualitatively normal protein (4). Within this concept, incomplete penetrance associated with some splice mutations (3) may result if some mutant pre-mRNAs are spliced normal (leaky splice mutation).

Retinoblastoma. Table 1 Risk for Rb in family members

Clinical presentation of index case	Risk to siblings	Risk to offspring
Sporadic unilateral Rb	≤1%	2–6%
Sporadic bilateral Rb	≤2%	Close to 50%
Familial bilateral Rb (one parent affected)	Close to 50%	50%
Familial Rb, incomplete penetrance type	Variable, depending on penetrance	Variable, depending on penetrance

Retinoblastoma. Table 2 Identification of the genetic cause in patients with Rb

Clinical presentation	Genetic analyses start with	Findings	Interpretation
Sporadic unilateral Rb	*RB1* gene mutation analysis in DNA from tumor. Two mutations have to be identified Testing of DNA from blood for the presence either of these mutations	In 85% of patients, neither of the two mutations is detected in DNA from blood. Sensitive methods are needed to detect patients with mutational mosaicism	Both *RB1* mutations have occurred in somatic cells (somatic mutation). The patient has not inherited a mutant *RB1* allele. Siblings have no increased risk of retinoblastoma
		In 15% of patients a predisposing *RB1* mutation is detected in DNA from blood	In patients with mutational mosaicism, both *RB1* mutations have occurred in somatic cells and siblings have no increased risk of retinoblastoma Heterozygous patients may have inherited a mutation from a carrier parent. Siblings of these patients have an increased risk of retinoblastoma unless genetic testing shows absence of the mutant allele
Sporadic bilateral Rb	*RB1* gene mutation analysis in DNA from peripheral blood of the patient or from tumor. Because of mutational mosaicism in some patients, analysis of tumor DNA is preferred	A predisposing *RB1* mutation is detected in DNA from blood	Fewer than 10% of patients are mutational mosaics. Both *RB1* mutations have occurred in somatic cells. Siblings have no increased risk of retinoblastoma
			In about 90% of patients a heterozygous predisposing mutation is detected. These patients may have inherited a mutation from a carrier parent. Siblings have an increased risk of retinoblastoma unless genetic testing shows absence of the mutant allele
Familial Rb	*RB1* gene mutation analysis in DNA from peripheral blood of family members that have inherited a mutant allele	A predisposing *RB1* mutation is detected in DNA from blood	Relatives at risk may be tested for the presence of the mutant allele

Genomic Alterations Associated with the Progression of Rb

In addition to mutational inactivation of both alleles of the *RB1* gene, retinoblastomas frequently show genomic alterations. Cytogenetic and molecular studies have shown that gains on chromosomes 1q and 6p and losses of chromosome 16q are most frequent except in tumors from very young children. It is reasonable to assume that these genomic regions contain genes that contribute to promotion and progression of this tumor. Candidate genes include the *KIF14* and *MDM4* (*MDMX*) on 1q, *DEK* and *E2F3* on 6p, and *CDH11* on 16q.

Risk Prediction and Genetic Testing

Relatives of all patients with RB are at an increased risk to carry a predisposing *RB1*-gene mutation and, consequently, tumor development (Table 1).

The genetic analyses required to identify the cause of disease in a patient depend on the clinical presentation in the index patient (Table 2).

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Retinoblastoma Protein, Biological and Clinical Functions

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Synonyms

Gene: *RB1* – retinoblastoma 1 (including Osteosarcoma), RB, OSRC, retinoblastoma tumor suppressor gene, *Rb1* (refers to mouse ortholog of human *RB1*); Protein: pRB – retinoblastoma susceptibility protein, Rb/p105

Definition

pRB is the protein product of the Retinoblastoma Tumor Suppressor Gene, *RB1*. Its biological functions include controlling the cell cycle, mediating differentiation, as well as protecting cells from ►apoptosis. Mutation of *RB1* resulting in functional inactivation of pRB leads to the childhood retinal tumor retinoblastoma and predisposes to osteosarcoma and other cancers. Defective function of pRB or its pathway also contributes to tumor progression in many other cancer types.

Characteristics

The RB1 Gene Encodes the pRB Protein

The *RB1* gene contains 27 exons and is located on human chromosome 13q14.2 (NCBI Entrez Gene ID: 5925). The product of *RB1* is the 110 kDa retinoblastoma susceptibility protein (pRB), composed of 928 amino acids. The protein consists of two conserved domains, named A and B. Domain A contributes to the proper folding of domain B, creating a "pocket domain" which is the site of many pRB protein interactions. The pocket domain contains an LXCXCE binding motif, which was initially found to bind oncoproteins ►Simian Virus 40 Large T-antigen, ►human papillomavirus E7 and ►adenovirus E1A.

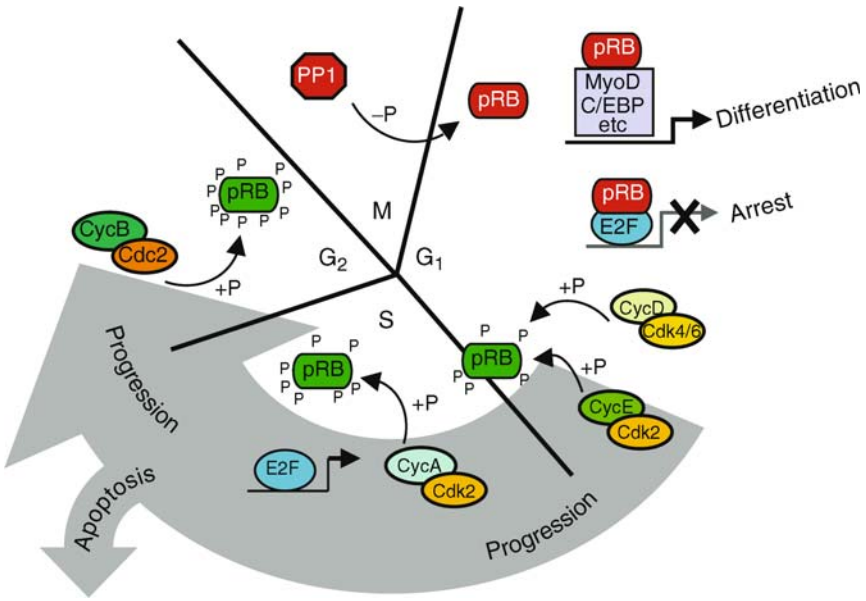
pRB is Post-Translationally Modified

Phosphorylation of pRB

The ►phosphorylation status of pRB changes with the progression of the cell cycle (Fig. 1). Key regulators of pRB phosphorylation are ►cyclin dependent kinases (Cdks), which are bound to and activated by ►cyclins; together, they phosphorylate pRB at multiple sites. The phosphorylation status of pRB affects how it interacts with its multiple binding partners. For example, hyperphosphorylated pRB is unable to bind to ►E2F transcription factors; consequently, pRB is found in its hypophosphorylated state at G_0 and G_1 stages of the cell cycle, where it effectively halts proliferation by binding to and blocking transcriptional activity of E2Fs. During the G_1 to S phase transition, Cdk4/6 and ►cyclin D or Cdk2 and cyclin E form complexes that phosphorylate pRB; as a result, pRB cannot bind E2F transcription factors. pRb continues to be phosphorylated by Cdk2-cyclin A as the cell cycle progresses from S phase through G_2, and by Cdc2-cyclin B as the cell cycle progresses into M phase, after which it is dephosphorylated by phosphoprotein phosphatase type 1 (PP1).

Acetylation of pRB

The pRB binding partners ►CBP/p300, ►histone acetyltransferases, acetylate pRB. Like phosphorylation, ►acetylation of pRB is cell cycle regulated, and can affect the protein interactions of pRB. For example, upon acetylation, pRB interacts more strongly with ►MDM2, an ►E3 ubiquitin ligase that targets proteins such as ►p53 and EID-1 for degradation. Acetylation was found to be important for the role of pRB



Retinoblastoma Protein, Biological and Clinical Functions. Figure 1 pRB Regulates cell cycle progression, differentiation and apoptosis.

in promotion of differentiation, but not in its ability to form complexes with E2F transcription factors.

Functions of pRB

The NCBI Entrez Gene database reports close to two hundred protein-protein interactions involving pRB, implicating it in a number of wide-ranging functions. The various roles of pRB likely overlap, however for simplicity we have grouped them into the following: control of the cell cycle, cellular differentiation and protection from apoptosis.

Cell Cycle Control

Unphosphorylated pRB was initially found to be essential for blocking the G_1 to S phase transition of the cell cycle. This was later attributed to its ability to bind E2F family transcription factors and effectively stop them from activating their target genes, many of which promote in G_1 to S phase transition and DNA synthesis. Transcriptional repression by pRB also involves ►chromatin remodeling, as pRB binds to and recruits ►histone deacetylase HDAC1, nucleosome modifiers BRG1 and hBRM, and ►histone methyltransferase SUV39H1, which help to maintain a repressive chromatin state, inaccessible to transcriptional machinery. In some cases, pRB can also recruit ►polycomb group proteins, a family of proteins that act as repressors.

pRB has also been implicated in E2F-independent roles for cell cycle control. In some cell types, transcription of Cdk inhibitor ►p21 can be upregulated by interaction of pRB with transcription factor Mitf. Additionally, pRB interacts and inactivates ►SKP2, part of a ubiquitin ligase complex that recognizes and marks for degradation $p27^{KIP1}$, another Cdk inhibitor. As a result of both of these potential pathways, the Cdks that normally phosphorylate and inactivate pRB are blocked, resulting in cell cycle arrest.

There is a growing body of evidence that implicates a role for pRB in the control of the ►DNA damage ►checkpoint. pRB negatively regulates a number of DNA replication factors, a function that is stimulated upon induction of DNA damage. Interestingly, even in E2F1-deficient, over-proliferating cells, presence of functional pRB prevents the accumulation of DNA ►double strand breaks, implicating a role for pRB in maintaining genomic integrity independent of its function in inhibiting E2Fs.

Differentiation and Development

Rb1 knockout mice do not develop to term, but die during gestation due to defects in many tissues including placenta, muscle, brain, blood, skin, liver and lens, suggesting that pRB is important in the cellular differentiation. It is unknown if the role of pRB in differentiation is simply an indirect result of its role as a cell cycle inhibitor, or if this reflects an additional, separate function of the protein. Certainly, only differentiated cells exhibit methylation of histone 3 at the K-9 residue (specific result of the pRB mediated function of SUV39H1) at E2F-regulated promoters, suggesting pRB contributes to differentiation by inducing cell arrest. However, study of *Rb1* mutant

mice that express pRB lacking the ability to bind E2F1-3 (and to inhibit the cell cycle) showed fewer defects in differentiation than the complete *Rb1* knockouts, indicating that the functions of pRB in cell cycle control and differentiation operate through separate pathways. Interestingly, the mutant pRB in these incompletely penetrant mice still retains the ability to bind and inhibit E2F4 and Id2, both of which act to prevent differentiation. EID-1, also a differentiation antagonist, binds to pRB in complex with CBP/p300, in an attempt to disrupt the histone acetyltransferase activity that represses chromatin. Acetylated pRB however, recruits and more securely tethers MDM2 to the complex, which then targets E1D-1 for degradation.

In addition to sequestering and inactivating inhibitors of differentiation, interaction of pRB with transcriptional activators of genes important to cell fate specification could also promote differentiation. In this respect, complexes of pRB and MyoD are thought to be essential for muscle differentiation, pRB/c-jun complexes result in differentiation of keratinocytes and adipocyte differentiation of murine embryonic fibroblasts ensues after pRB interaction with CCAAT/enhancer binding proteins (C/EBPs).

A new emerging role for pRB involves the construction and assembly of neuronal architecture, which is spatially and temporally regulated during development. It seems that the repression of E2F responsive promoters by pRB regulates not only pro-survival genes, but also those involved in laminar patterning and migration of neurons. A ►microarray study of upregulated genes in neural precursors from conditional *Rb1* knockout mice identified several genes known to be involved in interneuron migration. Of particular interest was neurogenin, an E2F-regulated gene, which regulates neuronal migration.

Apoptosis

Inappropriate entry into ►S-phase by E2F-mediated transcription can result in ►apoptosis. Thus, pRB repression of E2F factors prevents cell death; indeed, the *Rb1* knockout mice display a massive induction of cell death in several tissues. Knockout of E2F1 in conditional *Rb1* mutant mice (►knockout mice) rescues this apoptosis, indicating that E2F1 is a requirement for this cell death pathway.

Interestingly, E2F-mediated apoptosis is abolished when p53 is mutated. E2F1 can lead to the stabilization of p53 by upregulating transcription of $p14^{ARF}$, a repressor of MDM2. Now inactive, MDM2 is unable to mediate the degradation of p53, resulting in stabilization of the protein and subsequent apoptosis.

Hypophosphorylated pRB causes cell cycle arrest by inhibiting E2F-mediated transcriptional activation of genes necessary for entry into S phase, and also activates genes necessary for differentiation by binding to cell-type specific transcription factors (e.g. MyoD, C/EBP) and by recruiting ►chromatin remodeling factors (e.g. CPB/p300, not shown). Phosphorylation of pRB by Cdk4/6-cyclin D and Cdk2-cyclin E kinases in late G_1 releases E2F, allowing cell cycle progression. pRB continues to be phosphorylated in S and G_2 phases by Cdk2-cyclin A and Cdc2-cyclin B complexes, respectively. In late mitosis, pRB is activated/dephosphorylated by PP1. Inappropriate entry into S phase in the absence of pRB induces apoptosis.

Clinical Relevance

Inactivation of RB1 Initiates Retinoblastoma Development

Retinoblastoma is a childhood cancer of the retina. With the cloning of *RB1* as the first tumor suppressor gene, retinoblastoma was the first cancer to be described as a genetic disease. All retinoblastoma have mutation or loss of expression of both alleles of *RB1*. Individuals with hereditary retinoblastoma carry a constitutional mutation of one *RB1* allele (M1) and lose the second allele (M2) in the retinal cell that becomes the tumor; consequently, hereditary retinoblastoma is likely to be bilateral (affecting both eyes). Non-hereditary retinoblastoma results from somatic loss of both alleles (M1/M2) in the developing retinal cell of retinoblastoma origin, thus it is usually unilateral (affecting one eye).

Mutations in the *RB1* gene that lead to retinoblastoma are of numerous types, consisting of splice variants, missense mutations, point mutations leading to premature stop codons and deletions of all or any part of the gene. The type of *RB1* mutation generally makes no difference to the presentation of the disease, however some "low penetrance" mutations, which result in residual activity of pRB, may cause few or no tumors to be produced.

Inactivation of *RB1* is necessary, but not sufficient, for retinoblastoma tumor development. Instead, loss of *RB1* results in retinoma, a benign tumor of the retina. Subsequent genetic alterations, such as the gain of oncogenes and/or loss of additional tumor suppressor genes, lead to retinoblastoma development.

RB1 Loss in Other Cancers

Although loss of *RB1* is implicated in a wide variety of cancers, it is an initiating and rate-limiting tumorigenic factor specifically in the retina. In addition to developing retinoma and/or retinoblastoma, individuals who inherit one mutant copy of *RB1* have a 30-fold increased risk of developing osteosarcoma and other tumors. This risk is increased further by use of external beam radiotherapy to cure retinoblastoma. As a contributing factor to tumorigenesis, *RB1* mutation or pRb inactivation is observed in numerous cancers,

including but not limited to ►prostate cancer, ►breast cancer, ►lung cancer and ►bladder cancer.

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Retinoblastoma Protein, Cellular Biochemistry

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Definition

Retinoblastoma protein is a 110 kDa nuclear phosphoprotein (►tumor suppression). The gene locus maps to 13q14.1–q14.2. Retinoblastoma is an embryonic neoplasm of retinal origin. Young children with a ►germline mutation in one Rb1 ►allele have a 95% chance of developing a retinoblastoma tumor in their eyes. Mutation in the Rb1 allele also predisposes patients to develop other tumors, such as ►osteosarcomas and fibrosarcomas. Almost two-thirds of the secondary tumors arising in patients with retinoblastoma are mesenchymal in origin. Most mutations in Rb1 lead to premature termination of translation.

Characteristics

pRb and Its Family Members

The pRb, p107 and p130 proteins form the "pocket protein" family that is crucial in ►cell-cycle regulation. pRb and p107/p130 have five domains of high conservation. pRb shares between 30% and 35% sequence identity with p107/p130. Most of the conserved sequences lie within the so-called "pocket region" (amino acid 379–792), which is composed of two subdomains, known as the "A" and "B" box. Viral oncoproteins, such as ►SV40 large T antigen, ►adenovirus E1A and "high risk" ►human papillomavirus early protein E7, all target the pocket domain, displacing cellular proteins that interact with the pocket, thereby leading to loss of pocket protein function.

At least three distinct protein binding activities of pRb have been described to be important for its function: the large pocket (amino acid 395–876) binds to ►E2F, the small pocket (amino acid 379–792) binds to the LXCXE (L = leucine, C = cysteine, E = glutamic acid, X = any amino acid) peptide and the C pocket binds to tyrosine kinase ►ABL and oncoprotein ►Mdm2. Viral oncoproteins (e.g. E1A, E7, SV40 LT) and some cellular proteins (e.g. ►histone deacetylase (HDACs), ►cyclin D) possess an LXCXE motif in their protein sequence that enables them to bind to the pocket. The three-dimensional structure of the pRb A/B pocket bound to a LXCXE-containing peptide from E7 has been solved. The peptide binds a highly conserved groove on the B box portion of the pocket, and the A box is required for the stable folding of the B box. The A/B box interface is highly conserved, suggesting an additional protein binding region. Furthermore, both E2F and E7 peptides occupy distinct sites in the pocket. Indeed, HDACs that contain an LXCXE-like motif may form a trimeric complex with pRb and E2F. c-Abl was also reported to bind to pRB when the pocket region is occupied by E2F. Taken together, the evidence suggests a model for pRb as a "molecular matchmaker" in mediating protein complex formation.

Despite the close similarities among the family members, only Rb has been shown to be mutated in tumor cells. Neither p107 nor p130 have been found to be mutated in naturally occurring tumors. In order to study the specific cellular function of pRb, p107 and p130, knockout mice in Rb, p107 and p130 have been developed. The phenotypes indicated both distinct and overlapping functions among the family members. The absence of pRb in mice causes embryonic lethality, although p107−/− and p130−/− ►knockout mice survive to term, possibly as a result of functional redundancy between p107 and p130. This is consistent with the phenotype of the p107−/−: p130−/− mice which exhibit embryonic lethality. Rb+/− mice do not suffer from retinoblastoma, but develop tumors of the pituitary and thyroid origin. This difference may be attributed to the physiology of human and mice. Bilateral, multifocal retinal dysplasia is observed in Rb+/−: p107−/− mice. This suggests that pRb and p107 may share overlapping functions in controlling cellular homeostasis in the murine retina, and loss of both is required for tumor formation. p107−/−: p130+/− and p107+/−: p130−/− mice were developed to investigate if p107 and p130 possess tumor suppression function.

However, analysis of such animals did not reveal any obvious tumor phenotype. An alternative explanation is that, unlike the mutations in pRb, mutations in p107 and/or p130 may not be advantageous for tumorigenesis. Therefore mutations in other components of the pathway such as p16, which is frequently found to be mutated in human tumor(s), contribute to the inactivation of p107/p130 tumor suppression function, without abrogating other necessary functions of p107/p130.

Cell-Cycle Regulation of pRb

The pRb protein is ubiquitously expressed in most cycling and resting cells. pRb acts as a negative regulator for cellular proliferation, sequestering a variety of nuclear proteins involved in cellular growth. The most studied pRb target is the family of transcription factors known as ►E2F. E2F consists of a heterodimer of an E2F protein bound to a DP partner. Together, they regulate the timing and levels of expression of many genes involved in cell-cycle progression. E2F target genes encode proteins involved in DNA ►replication (for example, DNA polymerase a, thymidine kinase, dihydrofolate reductase and cdc6), chromosomal replication (for example, replication origin-binding protein HsOrc1, MCM proteins) and cell-cycle regulation (for example, cyclin A, E and D1, p107, cdc2, E2F1, 4 and 5 and p19ARF). During G0/G1, hypophosphorylated pRb binds to E2F, inactivating and thereby preventing cell-cycle progression. Cyclin D/cdk4/6 and cyclin E/cdk2 progressively phosphorylate pRb at late G1 to early S phase. In S phase, pRb phosphorylation is maintained by cyclin A/cdk2. Progressive phosphorylation of pRb results in reduced affinity for E2Fs, the release of free E2F and thereafter induction of E2F transcription.

Different pocket proteins have a preference for binding to different E2F family members. The E2F family now consists of six members (E2F-1 to -6). E2F-1, -2 and -3 exhibit high affinity binding towards pRb, but bind weakly to p107/p130. In contrast, E2F-4 and -5 show greater specificity for p107/p130, but also bind pRb. Complexes between the pRb family members and E2F form at different phases of the cell cycle. In general, p130/E2F complexes are mainly found in quiescent or differentiated cells and p107/E2F complexes predominate in S phase cells. pRb/E2F complexes are most evident during G1/S phase transition, but also exist in quiescent or differentiated cells.

pRb can modulate E2F transcription activity in at least two distinct ways: First, pRb binds to the transcription activation domain in E2F and directly inhibits E2F transcription activity. Using in vitro transcription and footprinting assays, it has been demonstrated that pRb blocks the recruitment of the transcription initiation complex by E2F. Second, pRb was shown to bind to ►HDACs, which are believed to deacetylate histones on the promoter, and actively repress transcription via chromatin remodeling. It has been shown that active repression of pRb/E2F complex is important in mediating G1 arrest triggered by ►transforming growth factor-β (TGFβ), p16 and contact inhibition.

There is evidence that phosphorylation of the pRb C -terminus by CDK4/6 causes successive intramolecular interactions between the C-terminus and the central pocket region. The initial interaction is thought to disrupt HDAC binding and therefore relieve active repression by pRb. This event then facilitates the phosphorylation of the pocket region by cyclin E/CDK2, thereby disrupting the pRb/E2F interaction. These intramolecular interactions provide a molecular basis of how phosphorylation of pRb progressively inactivates its growth suppression function.

Apart from regulating E2F transcription activity, pRb proteins also regulate E2F protein stability and apoptotic function. Overexpression of pRb family members can stabilize E2F-1 and E2F-4. E2F-1 was shown to be degraded by a ubiquitin-dependent proteasome pathway through an ►SCF-like complex. E2F-1 mutants which cannot be degraded by the SCF pathway drive cell-cycle progression into S phase followed by ►apoptosis. E2F-1-mediated apoptosis occurs independently of the transcription activation domain, but requires DNA binding. Furthermore, E2F-1-mediated apoptosis is both p53-dependent and p53-independent. Overexpression of pRb in tissue culture cells and analysis of the Rb−/− mouse embryos versus the E2F-1−/−:Rb−/− embryos indicated pRb can suppress E2F-1-mediated apoptosis. E2F-1-mediated apoptosis can, at least in part, occur through a death receptor-dependent mechanism by inhibiting activation of anti-apoptotic signals including NFκB.

Role in Terminal Differentiation

The role of pRb in differentiation was suggested from the phenotype of Rb−/− ►knockout mice, which show a pronounced defect in erythroid, neuronal and lens development. Although loss of pRb function allows the initiation of differentiation, the embryos fail to achieve a fully differentiated state, indicating that pRb is likely to play an important role in achieving and maintaining the post-mitotic state. Furthermore, the aberrant cell-cycle entry observed in the central nervous system (CNS) and the peripheral nervous system (PNS) of the Rb−/− embryo causes elevated levels of apoptosis. Again this implies that pRb may function in protecting cells from apoptosis. Indeed, Rb−/−: E2F-1−/− mice show reduced levels of ectopic cell-cycle entry and apoptosis in both the CNS and the lens at 13.5 d.p.c. as compared to Rb−/− embryos, suggesting that these defects are in part due to de-regulated E2F-1 activity.

However, this mechanism is tissue type specific, as loss of E2F-1 has less of an effect on cell-cycle entry and apoptosis in the PNS.

It is clear that pRb interacts with other non-E2F targets. This is best demonstrated in the analysis of pRb mutants that show reduced binding to E2Fs, but are still capable of augmenting MyoD transcriptional activity and inducing tissue specific gene transcription. Furthermore, these mutants still retain certain tumor suppressor functions. Therefore, at least part of the tumor suppression function of pRb correlates with its ability to promote tissue differentiation.

Although the pRb protein level does not change dramatically upon differentiation, pRb is found to be hypophosphorylated during cell-cycle exit. Hypophosphorylated pRb augments the transcriptional activity of various transcription factors important for tissue differentiation, including MyoD for myogenesis and C/EBPα for adipogenesis. Also, pRb was shown to directly bind to C/EBPα, NF-IL6 and ►AP-1 transcription factors and enhance their DNA binding activity.

Other Targets of pRb

Low ►penetrance pRb mutants, such as substitution of Trp for Arg 661 in the B pocket of pRb (661W) which are inactive in both E2F and LXCXE binding, still retain tumor suppressor activity. In cell based assays, the 661W mutant was shown to inhibit G1/S progression. Furthermore, C pocket mutations in full length pRb also reduce pRb function. Taken together, these suggest non-E2F targets of pRb contribute to pRb tumor suppression function.

It was shown that pRb can bind to the oncoprotein Mdm2 through a C-terminal region in pRb, and Mdm2 overcomes pRb-mediated growth suppression. Direct interaction of pRb and Mdm2 can overcome both the anti-apoptotic function of Mdm2 and the Mdm2-dependent degradation of p53.

The C-terminus of pRb also interacts with the c-Abl tyrosine kinase. The ►c-Abl kinase is ubiquitously expressed in both the cytoplasm and nucleus. The nuclear kinase activity of c-Abl is under cell-cycle control, being activated during cell-cycle progression. Interestingly, c-Abl can simultaneously interact with pRb when the pocket region is occupied by E2F.

The human homologue of yeast SNF/SWI2 proteins, hBrm/hBrg, can interact with pRb in a pocket-dependent manner. hBrm/hBrg has chromatin remodeling activity and co-operates with pRb in the transcriptional activation of the glucocorticoid receptor. Together with the observation that pRb also recruits HDACs, it is likely that pRb regulates transcription by altering chromatin structure.

pRb also interacts directly with the largest TATA-binding protein associated factor, TAFII250, through multiple regions in each protein. Apart from being part of the basal transcriptional machinery, TAFII250 possesses intrinsic histone acetyltransferase activity and kinase activity. Mutagenesis studies suggested TAFII250 is a cell-cycle regulated protein and its acetyltransferase activity is required for cell-cycle progression. pRb was reported to inhibit the kinase activity of TAFII250. These findings point to an additional mechanism of pRb regulating transcription by modulating the activity of the basal transcription apparatus.

Evidence exists that pRb can modulate transcription that is mediated by RNA polymerase I and III. During differentiation of U937 myeloid progenitor cells, pRb becomes localized to nucleoli, which are the major sites of ribosomal gene transcription by RNA polymerase I. Immunoprecipitation experiments demonstrated that pRb can associate with transcription factor UBF1 and this interaction compromised the transcriptional activity of UBF1, thus leading to the down-regulation of Pol I transcription activity.

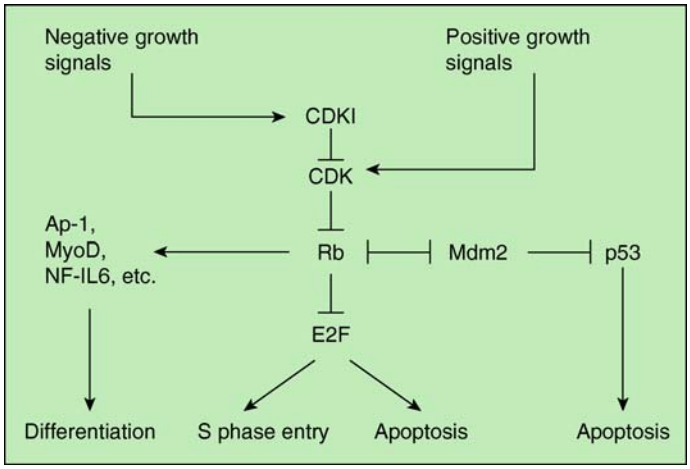
Loss of pRb function also correlated with up-regulation of Pol III transcription in tumor cells. Rb−/− fibroblasts have elevated Pol III activity compared to Rb+/+ fibroblasts. Immunoprecipitation and co-fractionation experiments demonstrated pRb interacts with TFIIIB, and TFIIIB was identified as a target of repression by pRb.

Animal Models

Rb−/− ►knockout mice fail to develop to full term, dying in utero at about 12–13 days post-coitum. The embryonic lethality is due to the failure of certain cell lineages to undergo terminal differentiation, particularly in the hemopoietic and central nervous systems (CNS). These observations suggest that pRb plays a crucial role in controlling cellular differentiation during development. Interestingly, Rb+/− mice do not develop retinoblastoma. Instead, 95% of the Rb heterozygotes die between day 300 and 400 after birth with tumors of the intermediate lobe of the pituitary (Fig. 1).

Summarizing Remarks

The RB tumor suppressor protein is an essential component of the ►cell-cycle clock, integrating both positive and negative signals for cellular growth and proliferation with the transcription machinery. The pRb protein exerts its function in tumor suppression by both antagonizing and synergizing with downstream effectors such as E2F. pRb has two modes of action: it can inactivate E2F transcription activity, and assemble an active repression complex with E2F. Apart from E2F, pRb synergies with various factors to promote cellular differentiation. The differentiation property of pRb at least contributes partly to its tumor suppressor function. The pRb-c-Abl and pRb-Mdm2 interactions also adds



Retinoblastoma Protein, Cellular Biochemistry. Figure 1 pRb integrates negative and positive signals in regulating the cell-cycle clock. Mitogenic signals activate cyclin-dependent kinase activity and inactivate pRb function by phosphorylation. In reverse, inhibitory signals, such as TGFβ and contact inhibition mobilize CDK inhibitors (CDKI) to maintain active pRb in its hypophosphorylated form. pRb has at least two distinct activities in tumor suppression: by inactivating downstream E2F activity, and by augmenting transcription activities of various transcription factors for cellular differentiation. Mdm2 acts as a mediator bridging together both the p53 and pRb pathways.

to its growth suppression function, though the mechanisms remain to be elucidated.

It is also clear that pRb is a master regulator for transcription. It can both activate and repress transcription in a context-dependent manner. pRb interacts directly with histone acetyltransferase, deacetylase and hBrm/hBrg, all of which are classes of proteins involved in chromatin remodeling. It will be important to investigate how pRb regulates transcription in a chromatin environment. Last but not the least, pRb regulates transcription from DNA polymerase Pol I, II and III, thereby integrating the cell-cycle clock with the biosynthetic capacity of the cell. pRb is indeed a bona fide master regulator of the cell.

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Retinoic Acid

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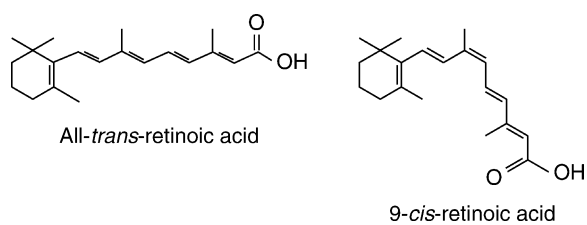
Definition

Retinoic acid, a pale yellow lipid-soluble compound, is a natural metabolite of vitamin A which plays important roles in embryonic development and in adult tissues. Retinoic acid functions by activating transcription factors termed retinoid receptors, thereby regulating the expression of multiple target genes. Two isomers of retinoic acid, all-*trans*-retinoic acid and 9-*cis*-retinoic acid (Fig. 1), are transcriptionally active. All-*trans*-retinoic acid activates retinoic acid receptors (RARα, RARβ, and RARγ), while 9-*cis*-retinoic acid can serve as a ligand for both RARs and the retinoid X receptors (RXRα, RXRβ, and RXRγ). Synthetic compounds that mimic the biological activities of retinoic acid have



Retinoic Acid. Figure 1 Transcriptionally active retinoic acids.

been developed and some of these are in current use in treatment of various diseases including cancer. All-*trans*-retinoic acid and its natural and synthetic derivatives are termed "retinoids." The term "rexinoids" has been coined to describe 9-*cis*-retinoic acid and synthetic ligands that activate RXRs.

Characteristics

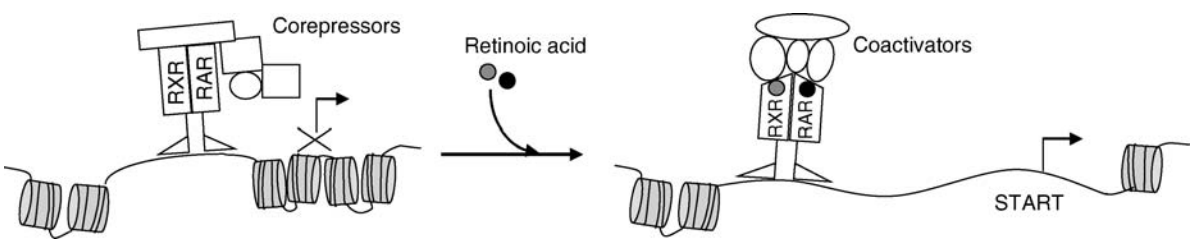
Retinoic acids regulate cellular behavior by activating RARs and RXR, ligand-activated transcription factors that are members of the super-family of nuclear hormone receptors. RAR and RXR function as a dimeric complex which binds to specific DNA recognition sequences (called response elements) in regulatory regions of target genes. In the absence of retinoic acid, the receptors are associated with accessory proteins, termed corepressors, which repress target gene expression. Binding of ligands induces the receptors to undergo a conformational change, resulting in dissociation of corepressors and recruitment of multicomponent complexes of transcriptional coactivators. In turn, coactivators modify the structure of the chromatin and facilitate the assembly of the general transcription machinery at the target gene start site (Fig. 2). Ligand-activated retinoid receptors thus regulate the expression of genes that play key roles in cellular differentiation and proliferation. Consequently, retinoic acid often inhibits cell growth and it displays antitumorigenic activities in various cancers.

Targets that mediate the antiproliferative activities of activated RAR include genes involved in differentiation, e.g. CCAAT/enhancer binding proteinε (*C/EBPε*); apoptosis, e.g. ►*TRAIL* and ►*Caspase-9*; and cell cycle regulation, as for example *Btg2* which targets ►cyclin D1. Hence, the pathways by which retinoic acid inhibits cell growth in the context of different carcinoma cell types can involve induction of ►cell cycle arrest, or differentiation, or ►apoptosis, or a combination of effects. The biological activities of 9-*cis*-retinoic acid and other rexinoids are mediated by a broad repertoire of target genes. Among nuclear receptors, RXR holds a special place because this transcription factor is an obligatory dimeric partner not only for RAR but also for other nuclear receptors. These include receptors that were reported to display anticarcinogenic activities such as the ►vitamin D receptor (VDR). The participation of RXR in numerous dimeric complexes may allow this receptor to affect carcinoma cell growth through multiple pathways that converge at the genome.

Regulation

►Vitamin A (retinol) is stored in vivo in various lipid-storing tissues, with the main pool existing in the liver. Retinol is secreted from these tissues and circulates in blood bound to retinol-binding protein (RBP). In target cells, retinol may be esterified to the storage species retinyl esters, a reaction catalyzed by lecithin:retinol acyltransferase (LRAT), or it may be converted to retinoic acid by two metabolic steps. The first, catalyzed by retinol dehydrogenase (ROLDH), leads to the formation of retinaldehyde (also called retinal). In the second step, retinal is converted to retinoic acid by a reaction catalyzed by retinal dehydrogenase (RALDH). Several isotypes of retinol- and retinal-dehydrogenases are known to exist, and these are differentially expressed in different cells and across developmental stages. Retinoic acid is degraded through the action of a member of the ►cytochrome P450 gene family termed CYP26. The several known CYP26 isotypes display a retinoic acid-4-hydroxylase activity and they catalyze the oxidation of retinoic acid to non-active metabolites. The concentration of retinoic acid in cells is thus tightly controlled by the expression levels and the activities of the enzymes that regulate its homeostasis. Cellular needs for retinoic acid are fulfilled by upregulation of expression of RALDH, and protection from excessive retinoic acid levels is provided by increases in the expression of CYP26 and LRAT.

In cells, retinoic acid is synthesized in the cytosol while its transcriptional activities are mediated by nuclear RAR. Transport of retinoic acid from the cytosol to the nucleus is mediated by a member of the family of intracellular lipid-binding proteins termed cellular retinoic acid-binding protein type II (CRABP-II).



Retinoic Acid. Figure 2 Ligand-induced activation of retinoid receptors. RXR-RAR dimers bind to response elements in regulatory regions of specific target genes. In the absence of retinoic acid, the receptors are associated with an accessory protein complex containing transcriptional corepressors. Upon binding of cognate ligands, corepressors dissociate and complexes of coactivators are recruited. These, in turn, loosen the structure of the chromatin and facilitate the assembly of the transcription initiation complex.

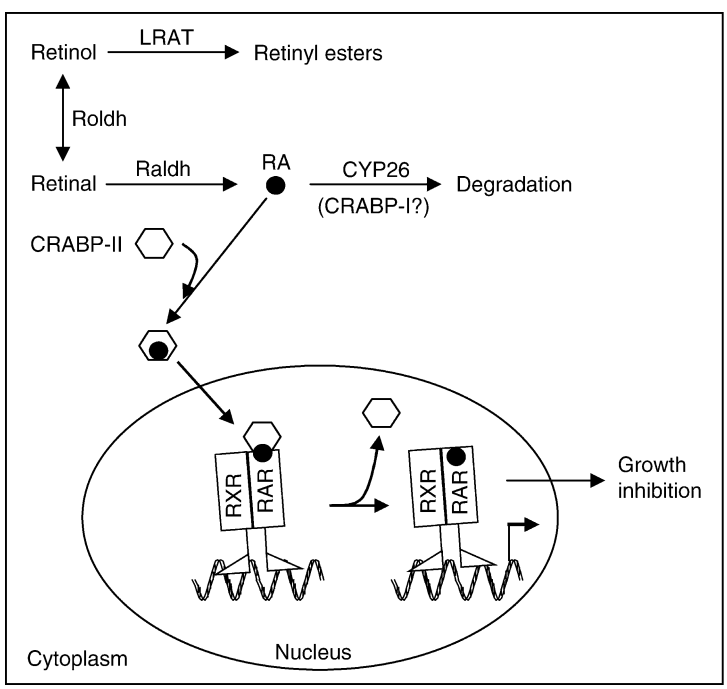
CRABP-II, which is cytosolic in the absence of retinoic acid, relocates to the nucleus upon binding of its ligand. In the nucleus, CRABP-II associates with RAR to form a complex through which retinoic acid is directly "channeled" from the binding protein to the receptor. By efficiently delivering retinoic acid from its sites of biosynthesis to RAR, CRABP-II enhances the transcriptional activity of the receptor (Fig. 3). An additional binding protein, termed CRABP-I, is involved in regulating retinoic acid biology. CRABP-I appears to dampen retinoic acid signaling, perhaps by facilitating its degradation.

Following activation by their ligands, both RAR and RXR undergo phosphorylation at specific residues, tagging the receptors for ►ubiquitination and targeting them to degradation by the ►proteasome. Receptor degradation serves as an "off-switch" for the retinoid response. Both RARs and RXRs also undergo other phosphorylation events mediated by several signaling pathways, and these modifications alter the transcriptional activities of the receptors. Various signaling cascades thus cooperate with retinoic acids in regulating the expression of retinoid target genes.

Clinical Relevance

It has been long established that vitamin A deficiency is associated with increased susceptibility to carcinogenesis both in experimental animal models and in human populations. The protective activity of the vitamin is believed to be mediated by the retinoic acid through its ability to modulate cell growth, differentiation, and apoptosis. Indeed, retinoids exhibit chemotherapeutic activities in a number of cancers. Retinoic acid is particularly efficacious in treatment of ►acute promyelocytic leukemia (APL), a malignancy caused by a ►chromosomal translocation which results in an oncogenic protein in which RARα is fused to PML. Treatment of APL patients with retinoic acid in conjunction with conventional chemotherapy results in complete remission rates as high as 90%. Retinoic acid has also been successfully used in treatment of ►neuroblastoma, ►Kaposi sarcoma, and ►cutaneous T-cell lymphoma, and it may be effective in therapy and perhaps prevention of other malignancies such as ►bladder cancer, ►liver cancer, ►lung cancer, ►pancreas cancer, head and neck, ►prostate cancer, and breast cancer. In addition to retinoic acid, several retinoids and one rexinoid are being tested for the treatment of specific cancers.

Abnormal cell growth and tumor development in various malignancies is associated with aberrations in many aspects of retinoic acid signaling. These include disregulation of the expression of RARs, most notably RARβ, deviant expression levels of enzymes and binding proteins involved in regulating retinoic acid homeostasis and action, and persistent association of



Retinoic Acid. Figure 3 Components of the retinoid signalling pathway. Retinol can be stored in the form of retinyl esters or converted to retinoic acid. Retinoic acid is mobilized to the nucleus where it activates retinoid receptors, thereby upregulating the expression of genes involved in carcinoma cell growth inhibition. Depicted are enzymes, binding proteins, and receptors involved in retinoid signalling (see text).

retinoid receptors with transcriptional corepressors. Targeting of the aberrant point(s) in RA signaling in specific cancers may allow for the improvement of current therapies and the development of novel strategies for treatment of these diseases.

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Retinoic Acid Receptors

Definition

RAR; Belong to the ►nuclear receptor superfamily. Are nuclear receptors related to the ►steroid hormone receptors and ►thyroid hormone receptors, a family of proteins that function as ligand-dependent transcription factors. Several loci encoding RAR isoforms have been identified in mammals – RAR-alpha, -beta and gamma – as well as novel nuclear receptors known as RXR (Retinoid X receptor), which are distantly related to RARs and respond to high concentrations of ►retinoic acid (RA). The RARs show spatially restricted distribution patterns during embryogenesis, which have led to speculation on a variety of roles for RA in developmental processes. As with other enhancer-binding proteins, nuclear receptors act as transcription factors by binding to specific DNA recognition sequences generally located upstream of responsive genes. Although RARs can activate gene expression through binding to thyroid hormone response elements, a much more specific and potent RA response element (RARE) has been identified recently within the promoter of the RAR-beta gene. This RARE is essential for RA induction of the RAR-beta gene and, when linked to heterologous promoters, can confer transcriptional activation via all three RARs.

►Retinoid Receptor Cross-talk

Retinoid Receptor Cross-Talk

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Synonyms

Nuclear receptors; Retinoid acid receptors – RAR; Retinoid X (Rexinoid) receptors – RXR; Retinoids

Definition

The process of intercellular retinoid receptors activation by another activated ►transcription factor or the alternate. It may also be considered as the cooperation among different signal transduction pathways in various normal and pathological states, whereby activation of retinoid receptors enhances the activity of other transcription factors in the absence of direct activation, thus leading in positive or negative regulation of specific target genes.

Characteristics

Retinoids

Retinoids (►Retinoic acid) comprise a group of structural and functional derivatives of ►vitamin A that are considered physiological regulators of a plethora of essential biological processes, including embryonic development, vision, reproduction, differentiation, proliferation and ►apoptosis. Pharmacologically, they have been shown to suppress ►carcinogenesis in a wide gamut of tissues (e.g. skin, respiratory epithelium) in animal models, while clinically they seem able to reverse premalignant lesions (►Preneoplastic lesions) and inhibit the development of ►second primary tumors. Until 1987 it was known that retinoids are implicated in gene transcriptional control, but the precise mechanism was still a mystery. This was the year that two research groups identified the retinoid receptors. Many scientists have now concluded that retinoids' molecular actions are mainly modulated through their receptors which are members of the ►nuclear receptor superfamily.

Retinoid Receptors

Retinoid receptors contain six domains, designated A to F. The N-terminal region (domains A and B) includes a ligand-independent activation function (AF-1). The C domain contains a highly conserved DNA-binding domain, which might also participate in protein–protein interactions with transcriptional co-factors. The D domain is involved in ligand-induced functional changes and is crucial for the binding of receptor to transcriptional co-factors. The E and F

domains, which are moderately conserved among receptors, are involved in ligand binding and include a ligand-dependent activation function (AF-2) and a dimerization surface. The ligand-binding domains of RARs and RXRs are distinct and can be pharmacologically targeted separately.

Two types of retinoid receptors have been identified: the RARs and the RXRs. There are three RAR isotypes and three RXR isotypes (α, β and γ), which are encoded by distinct genes. For each isotype, there are at least two isoforms, which differ in their N-terminal A regions and are generated by differential usage of promoters and/or alternative splicing. Isotretinoin, also known as 13-*cis*-retinoic acid (Retinoic acid), is converted to all-*trans*-retinoic acid, which activates the RARs, whereas 9-*cis*-retinoic acid (Retinoic acid) activates both the RARs and the non-classical RXRs. RARs can heterodimerize with RXRs, whereas RXRs have a pivotal role as heterodimerization partners for several other nuclear receptors, such as the thyroid hormone receptors (TRs), the vitamin D3 receptors (VDRs), the ►peroxisome proliferator-activated receptors (PPARs) and several orphan receptors. Because RXRs form heterodimers with nuclear receptors that affect lipid physiology, their effects are also being investigated in other than carcinogenesis medical conditions, including the metabolic syndrome, which is characterized by ►obesity, dyslipidemia, ►diabetes, and hypercoagulability.

Like other members of this family, retinoid receptors act as ligand activated DNA-binding gene specific transcription factors through binding, as RAR–RXR heterodimers, to *cis*-acting retinoic acid response elements (RAREs) present in cognate genes. In the absence of ligand, RAR–RXR heterodimers are bound in a complex with co-repressors that actively repress the transcription of targeted genes. Transcriptional repression occurs mainly through the recruitment of ►histone deacetylases (HDACs), which prevent the opening of chromatin that is associated with the deacetylation of nucleosomes. Upon ligand binding, co-repressors are released and several multi-protein co-activator complexes are recruited to activate transcription. ►CREB-binding protein (CBP)/p300 associates with nuclear receptors in a ligand-dependent manner and shares intrinsic ►histone acetyltransferase (HAT) activity. RAR–RXR dimers bind to RAREs, which are characterized by two half sites with the consensus sequence AGGTCA. These are usually arranged as direct repeats separated by two to five nucleotides. Other RXR heterodimers bind to similar half sites with different preferences for spacing and orientation.

Retinoid Acid Receptors (RARs)

The three RAR isotypes have similar, but not identical, sequence and structure to one another, and they are expressed in distinctive tissue specific and developmentally regulated patterns. Each RAR also performs unique functions in development and differentiation that cannot be replaced by the actions of the other isotypes. Thus, each RAR isotype exerts a mixture of overlapping and specific actions concerning target gene expression control.

The crystal structure of RARβ has only recently been completed and revealed significant differences of its ligand-binding domain compared with the other two isotypes, reflecting the size and activity of potential agonists and antagonists on RAR-mediated transcriptional control.

As do other members of the nuclear receptor superfamily, RARs possess bimodal transcriptional properties, mainly due to their association with transcriptional co-factors. One pivotal difference among RARs isotypes that might contribute to their distinct function properties is that RARβ and RARγ inefficiently interact with co-repressor complexes to represses target gene transcription in the absence of ligand, whereas they can mediate significant transcriptional activation. That means that RARβ–RXR heterodimers are more responsive to rexinoids than are RARα–RXR heterodimers. These different co-repressor interaction properties of RAR isotypes is thought to be mediated either by proximal or/and apical co-repressor docking site conformational changes. Another important distinct property of RARβ is the ability to trans-repress the transcription factor ►activator protein-1 (AP-1), reversing established tumor promotion, because anchorage-independent growth of transformed cells is efficiently inhibited. Although the exact mechanism of the anti-►AP-1 activity of retinoids remains elusive, the importance of this cross-talk (►Receptor cross-talk) for growth control is increasingly recognized and considered as a phylogenetically highly conserved function.

Rexinoid Receptors (RXRs)

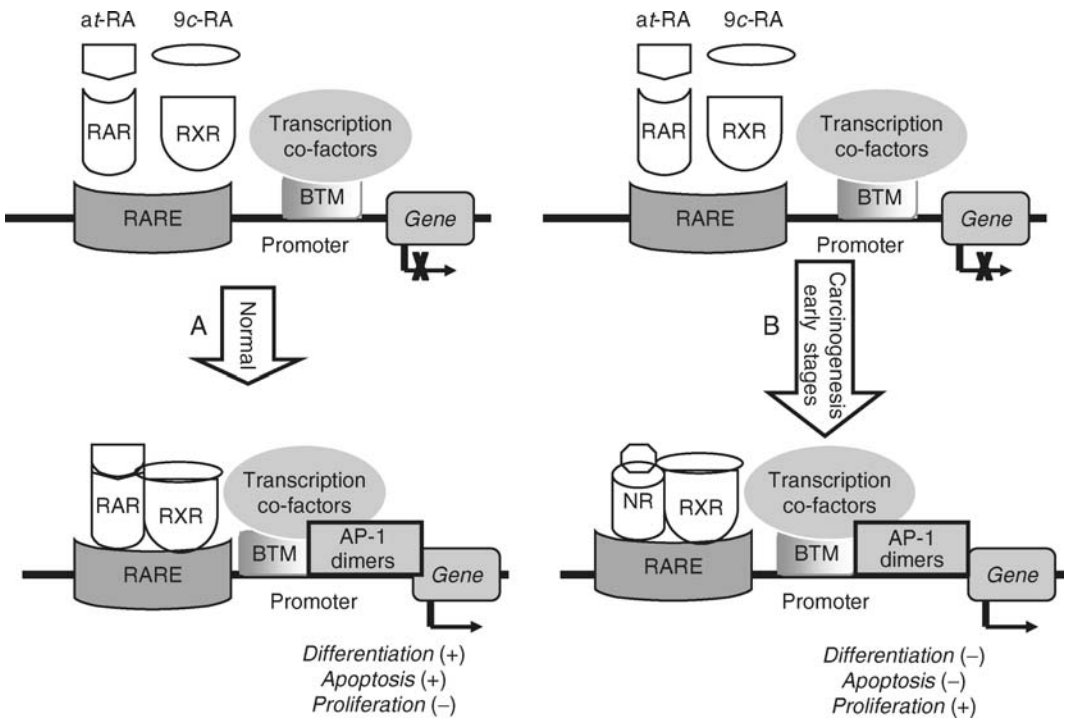
The ability of RXR to function as a ligand-dependent transcription factor is differentially regulated by its dimerization partners, which differentially control the binding of RXR ligands and the interactions of the heterodimer with transcriptional co-factors. Non-permissive heterodimers, such as those between RXRs and RARs, are characterized by the fact that the partner drastically interferes with the ability of RXR to activate transcription in response to RXR-specific ligands. However, recent studies have demonstrated that the binding of certain RXR ligands contributes to the activation of RAR–RXR heterodimers in some cell types. Heterodimers of RXRs with permissive partners have been identified and include the PPARs, Liver X receptors (LXRs) and ►Nur77 (TR-3 or NGFI-B). Recent studies suggest that nur77 is associated with trans-RA resistance in human cancer cells. The retinoic acid response element (βRARE) in the RARβ gene promoter

mediates trans-RA-induced RARβ gene expression in many different cell types and binds to both RXR–RAR and RXR–nur77 heterodimers. Gene transcriptional activation by RXR–RAR binding is mainly activated by RAR-specific ligands, whereas trans-activation by RXR–nur77 is induced by RXR-specific ligands. Thus, RARβ expression might be associated with both retinoid-induced proliferation and apoptosis processes.

Retinoid Receptors Cross-talk During Carcinogenesis

It is increasingly recognized that altered expression of RARβ protein is crucially involved in the pathogenesis of a diverse range of solid tumors, although the loss of RARα or RARγ expression has also been reported, but not consistently. Although the loss of RARβ expression is a frequent event in the early stages of carcinogenesis, the causative mechanism is still unclear and has been attributed to multiple mechanisms. However, it seems that RARβ down-regulation might be directly correlated with the sub-optimal observed clinical results of the currently used retinoids in cancer chemoprevention trials, although recent data has suggested that retinoids might also share receptor-independent anti-tumor activity. The well-recognized negative cross-talk of RARβ and AP-1 functions as a "molecular balance," thus favoring under normal conditions the RARβ mediated positive transcriptional regulatory effects regarding the induction of differentiation and apoptosis. During the very early stages of carcinogenesis this balance is disrupted by a combination of molecular aberrations, such as down-regulation of RARβ, up-regulation of AP-1 and crucial transcriptional co-factors (e.g. CBP/p300). AP-1 has the ability to enhance cellular proliferation, through cyclin D1 (►Cyclin D) enhanced expression, thus providing a developmental advantage in these gradually transformed cells in the setting of genomic instability (►Chromosomal instability).

Under normal conditions, such an aberration should trigger apoptosis intracellular feedback mechanisms to handle this imbalance. However, it seems that retinoid receptors are also implicated in the deregulation of apoptosis. The underlying mechanisms remain unknown, although the currently existing data support both the role of RARβ with its association with certain orphan receptors (e.g. Nur77) and RXRs through their complex interplay with nuclear receptors (e.g. PPARs), other transcription factors (e.g. NF-kB) as well as with important cancer-related intracellular molecules (e.g. ►Cyclooxygenase-2 (COX-2)) (►Arachidonic acid-pathway). The final outcome of these cross-coupling interactions of various retinoid receptors with other molecular pathways is differentiation inhibition, apoptosis blockade and uncontrolled cellular proliferation, namely some of the most important "hallmarks of cancer" (Fig. 1).



Retinoid Receptor Cross-Talk. Figure 1 (a) Schematic presentation of retinoid receptors activation and transcriptional activity under physiological conditions; (b) In the very earl stages of carcinogenesis the deregulated retinoid receptors cross-talk interactions with other nuclear receptors (NR) and/or other oncogenic transcriptional factors (e.g. AP-1) dictates malignant transformation of the "affected" cells.

The gradual elucidation of the paramount importance of retinoid receptors cross-talk in the carcinogenesis process has generated the notion that either more selective synthetic retinoids are warranted or/and combinatorial approaches targeting more than one crucial molecules might represent the next step in the intriguing concept of cancer ►chemoprevention.

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Retinoid X Receptor

Definition

Is a member of the ►nuclear receptor superfamily which is activated by ►retinoic acid and acts as a direct regulator of transcription by binding to specific DNA sequences.

►Retinoid Receptor Crosstalk

Retinoids

Definition

Is a collective term that refers to both naturally occurring and synthetic compounds that bear structural resemblance to ►vitamin A (all-trans retinol) and/or exhibit biological activity similar to retinols, retinals, or ►retinoic acid. The main functions of retinoids include involvement in reproduction, growth and differentiation (skin differentiation, bone remodeling), and immune system functioning. They exert many and diverse functions throughout the body including roles in vision, regulation of cell proliferation and differentiation, growth of bone tissue, immune function, and activation of tumor suppressor genes.

►Carotenoids
►Nutraceuticals
►Retinoid Receptor Cross-talk

Retinol

Definition

The animal form of ►vitamin A, is a fat-soluble vitamin important in vision and bone growth. It belongs to the family of chemical compounds known as ►retinoids. Precursor in forms of retinol animal sources (liver and eggs); plants (carrots, spinach) contain pro-vitamin A ►carotenoids.

Retromer

Definition

Multiprotein complex important in recycling transmembrane receptors from endosomes to the ►trans-Golgi network.

►Wnt Signaling

Retroperitoneum

Definition

Anatomical space behind the peritoneal cavity. Retroperitoneal organs, e.g. kidneys, are relatively fixed in their location whilst intraperitoneal organs, e.g. small bowel, are generally mobile.

►Transcoelomic Metastasis

Retrospective Study

Definition

A study design that looks backwards in time and examines events that have already taken place, for example, the typical case control study that compares people who already developed disease to people who did not. Study type in which the outcome (e.g. cancer diagnosis) has occurred to the subjects before the study commences

►Mutagen Sensitivity

Retrotransposition

Definition

A still incompletely understood "copy and paste" mechanism that involves replication by reverse transcription of an RNA intermediate, mobilization and reinsertion of new cDNA copies of repetitive elements within the human genome.

►LINE-1 Elements
►ALU Elements

Retroviral Insertional Mutagenesis

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Synonyms

Retroviral insertional tagging

Definition

Is an experimental screen performed to identify genes whose expression is linked to a ►phenotype of interest. It involves the ►infection of ►mouse models (or other model organism) with ►retrovirus, observing the mice until they develop the phenotype of interest (often a malignancy), and then identifying relevant candidate genes by determining the site of proviral insertion.

Characteristics

Retroviral ►insertional mutagenesis is an experimental approach to gene discovery that takes advantage of the life cycle characteristics of ►retroviruses.

History

Retroviruses have long been known to induce cancer. The first oncogenic retrovirus identified was avian erythroblastis virus (AEV), the existence of which has been known since around 1900. More avian and murine viruses were soon discovered. With the advent of DNA sequencing, many of these were found to contain ►oncogenes. Indeed, many of the first oncogenes were discovered in this way, including ►*ras*, ►*src* and ►*abl*. The mechanism of oncogenesis of these viruses was easily solved; expression of the oncogene contained within the virus induced the cancer. However, there were also a number of viruses that induced cancer but did not contain an oncogene. The mechanism by which these viruses induced cancer was less obvious. A study by William Hayward et al. in 1981 identified the mechanism. These authors found that insertion of the provirus in the *c*-►*myc* ►promoter led to activation of the cellular proto-oncogene *c-myc*. Even with this knowledge of the oncogenic mechanism, large-scale retroviral insertional mutagenesis projects were not feasible until a draft of the mouse genome became available. The pioneering study of this type took place in the laboratory of Neal Copeland and Nancy Jenkins in the late 1990s.

Provirus Structure

As part of their replication cycle, retroviruses are required to integrate a DNA copy of their genome into the host genome. At this stage of the replication cycle the virus is referred to as the provirus. The structure of the provirus is illustrated in Fig. 1. This represents a replication competent virus. Replication competent viruses contain genes encoding various proteins essential for viral replication, including the *gag*, *pro*, *pol* and *env* genes. At either end of the provirus are two Long Terminal Repeat regions (sometimes referred to as Long Tandem Repeats). As the name suggests, these regions contain identical, or repeated, sequence, and form the boundaries of the proviral structure. They contain sequences that are important for the transcription of the viral genes, including a promoter and an ►enhancer element, both within the U3 region. The promoter in the 5′ LTR drives expression of the proviral genes, thus facilitating viral replication. Many of the oncogene-containing viruses are replication incompetent because some or all of the required genes are replaced by the sequence of the oncogene.

Retroviral Insertional Mutagenesis. Figure 1 Elements of a retroviral provirus. The 5′ and 3′ LTRs are indicated, subdivided into the subregions U3, R and U6. E and P indicate the transcriptional enhancer and promoter respectively, with the *arrow* indicating the direction of transcription initiated at the promoter. The gag, pro, pol and env genes are indicated, along with the splice donor (SD) and splice acceptor (SA) sites.

Tumor Types

The types of tumors that can be investigated by retroviral insertional mutagenesis are limited by the types of tissues that are infected by retroviruses. Retroviral insertional mutagenesis has been used most frequently to study leukemia and lymphoma, but recent reports have used insertional mutagenesis to study ►breast cancer and ►brain tumors. A newly developed related technique, ►transposon insertional mutagenesis, offers a potential means of investigating tumors in other tissues.

LTRs Regulate Transcription of Host Genes Near the Insertion Site

The promoter in the 3′ LTR can drive expression of nearby genes in the host genome, and the enhancer element from either LTR can activate transcription of nearby host genes. It is through this mechanism that retroviral insertional mutagenesis provides an efficient cancer gene screen. Transcription of host genes that provide the cell with an oncogenic advantage lead to a clonal outgrowth and malignancy. In this way retroviral insertional mutagenesis identifies potential proto-oncogenes. There are additional mechanisms by which retroviral insertion can dysregulate host genes in such a way as to cause cancer; examples are listed below and illustrated in Fig. 2.

Promoter Insertion

Insertion of the provirus into an endogenous promoter results in replacement of the endogenous promoter with the promoter in the 3′ LTR. This results in a higher level of expression for the gene in question, as the viral promoter is very active. A frequent example of this mechanism is promoter insertion at the *c-myc* locus.

Enhancer Activation

Insertion of the provirus nearby or within the host gene can result in increased host gene expression via the endogenous promoter and proviral enhancer sequences. This is thought to be the most frequent oncogenic mechanism that occurs via retroviral insertional mutagenesis, and there are numerous examples. One common example is sp. retroviral insertion 5′ or 3′ of the *Meis1* gene.

Protein Fusion

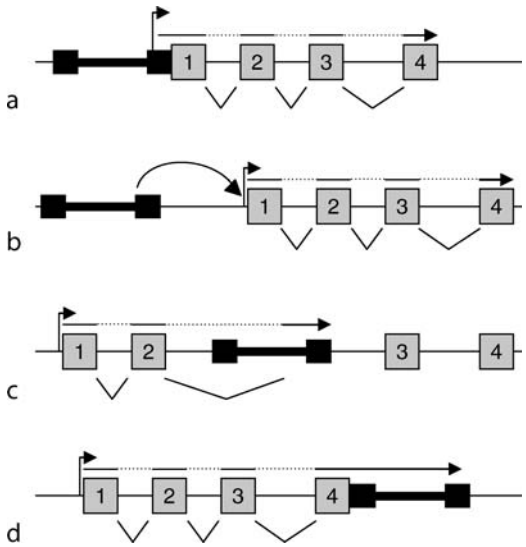
Insertion of the provirus within a gene, either within an exon or within an intron, can lead to protein truncation. In the case of an intronic insertion, this requires that the transcript splicing machinery, when splicing the endogenous transcript, take advantage of a splice acceptor site within the viral genome such that an exon of the host gene is spliced directly to the viral coding sequence. In the event of an exonic insertion, no splicing event is necessary as the coding sequence will be directly adjacent to the proviral sequence. Either of these scenarios will result in a fusion of the two proteins, which in some cases can be oncogenic. This mechanism often results in inactivation of the target gene rather than activation, and therefore occurs in ►tumor suppressor genes as well as oncogenes (as it provides a mechanism of ►dominant negative mutation). An example of a ►tumor suppressor gene inactivated by this mechanism is ►*p53*, and an example of a proto-oncogene activated by this mechanism is *c*-►*myb*.

Transcript Stabilization

Proviral integration into the 3′ UTR of a gene can result in loss of regulatory sequences that normally result in degradation of the transcript. The loss of this regulation results in an effective upregulation of the gene. An example of a proto-oncogene activated by this mechanism is *Pim1*.

Cloning of Insertion Sites

Cloning of insertion sites refers to identification of the loci into which the provirus inserted. It is important to realize that, when the organism is infected with retrovirus, many normal cells are infected and a given tissue may contain millions of insertion sites. However, even though a tissue sample may contain contaminating



Retroviral Insertional Mutagenesis.
Figure 2 Mechanisms of proviral dysregulation of ►endogenous gene transcription. Gray boxes numbered 1–4 represent exons of an endogenous gene. The black bar represents the proviral insertion. The right-angle arrow represents initiation of transcription, the solid and dashed line indicates the resulting transcript, the dashed section indicating regions that are spliced out during RNA ►splicing, also indicated by wedges below the line. (a) Promoter insertion. (b) Enhancer activation (looped arrow indicates transcriptional activation). (c) Protein fusion. (d) Transcript stabilization.

normal, non-malignant cells, because the cell containing oncogenic insertion sites has undergone numerous cell divisions, these oncogenic insertion sites are now greatly over-represented in the tumor sample.

The most common basic strategy to identify insertion loci is one of a variety of "anchored ►PCR" techniques. One of these, inverse PCR, involves digestion of the host genomic DNA with a restriction enzyme, followed by circularization of the resultant fragments. PCR is then performed using these circularized fragments as templates and primers designed to the known proviral sequence. Several additional anchored PCR techniques exist, mostly involving the ligation of some form of adaptor to the digested fragments rather than circularization of the fragments. One primer can then be designed to the adaptor, and the other to the proviral sequence. Either approach allows specific amplification of the sequences adjacent to the provirus without prior knowledge of the identity of those sequences. The sequence obtained from the PCR product can then be compared to the genome databases to identify the exact locus of integration, and to investigate the genes known to be in the region.

One complication that arises during the insertion sites cloning process is the identification of false positive "bystander" mutations. These arise when more than one proviral integration occurs within a single cell. Not every integration within the cell is essential for tumorigenesis, but if the cell is oncogenically transformed it will produce numerous ►daughter cells, and all insertion sites within it will be represented in the final tumor sample equally. Therefore, if bystander insertions exist within a tumor sample, they are just as likely to be cloned as a genuine, biologically relevant insertion site. Therefore, individual proviral insertion site results must be interpreted carefully.

Common Integration Sites

One means of distinguishing those genes likely to be important for malignant transformation in an insertional retroviral mutagenesis screen is the identification of common integration sites. A common integration site is a region in which more than one insertion, or event, is discovered in the same cohort of mice (or other organism). This is based on the simple premise that an event that occurs in multiple cancers is much more likely to be involved in malignant transformation than one that occurs in only one cancer. However, the definition of a common integration site is complicated by several factors. Firstly, proviral integration can have biological effect on host genes over a very large distance (in excess of 100 kb, for example, in the case of enhancer activation). Secondly, the number of cancers in a given cohort increases the number of insertion sites and, therefore, insertions at a given loci are more likely to occur by simple chance. Finally, as discussed below, proviral insertion is not a truly random event, and this must also be taken into account when determining whether a site is truly a common integration site. Several algorithms have been developed to address this issue.

Randomness of Insertion

One of the early attractions of retroviral insertional mutagenesis was that it allows investigation of the whole genome without bias. However, more recent studies have suggested that proviral integration is not truly random, and that different viruses have different mechanisms of insertion that affect the frequency of their insertion at particular loci. For example, viruses in general show a preference for integration into open chromatin regions, which can vary with cell type. Viruses show different preferences for integration within transcriptional units and upstream or downstream of transcriptional units. This means that retroviral insertional mutagenesis studies are probably not truly random (although they remain approximately random), and that studies performed with different viruses may generate different results.

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Retroviral Insertional Tagging

►Retroviral Insertional Mutagenesis

Retroviral Transduction

►Transduction of Oncogenes

Retrovirus

Definition

A retrovirus, formerly referred to as RNA tumor virus, belongs to the group of RNA viruses. RNA viruses have an RNA, not a DNA genome. Following the virus entry into a cell, this RNA is transcribed by the enzyme reverse transcriptase into one single strand of DNA, which in turn serves as the template for the host cell DNA polymerase to synthesize the complementary DNA strand, eventually forming double stranded (ds) DNA. The ds DNA is stably inserted into the DNA of the host cell as a provirus, providing the source for an RNA template that is identical to the initial virus RNA genome. Viral proteins can now be synthesized. The provirus therefore behaves like any of the other normal genes within the host genome and, during cell division, is regularly passed on to the daughter cells. Synthesized viral proteins, together with the newly synthesized RNA, can be packaged and to yield new virus particles. Upon their release by the cell, in a process referred to as "budding" new cells can be infected.

Usually the retrovirus infects a somatic cell and the provirus is integrated into the genome of that particular cell. If a virus infection occurs in a germ cell (a cell destined to become a sperm or egg cell) the resulting provirus can be passed on to the progeny and is inherited like a normal gene. Such genetic elements are called endogenous proviruses; they occur and are detectable in vertebrate DNA, including that of humans. Although in some species, such as mouse, thousands of endogenous proviruses exist, they are not transcribed, a fact at least partly due to heavy methylation. Endogenous proviruses can however, be induced artificially to produce virus particles.

Retroviruses were first isolated as agents that can cause cancer in chickens, the prototypical retrovirus being the Rous sarcoma virus (RSV). Some naturally occurring cancer diseases in humans are associated with retroviruses. The ►human T-cell leukemia virus I (HTLV-I) cause a relatively rare, but invariably fatal cancer of T lymphocytes. Another retrovirus, the ►human immunodeficiency virus, HIV (also known as HTLV-III or LAV) has been identified to cause acquired immunodeficiency syndrome (AIDS). The study of retroviruses has laid the basis to identify ►oncogenes.

The analysis of the ►transduction of oncogenes has directly led to the concept of cancer as the result of a malady of genes. In pursuit of defining the role of oncogenes in cancer development, fundamental insights into the molecular basis of cancer and cellular growth control have been achieved. Retroviruses that cause, within short time after infection, cancer in animals (acutely transforming retroviruses), possess an oncogene (viral oncogene, v-onc) that is responsible for cellular transformation. Such viral oncogenes are originally not viral. Instead they but have been taken up from the host cell by the provirus, followed by their incorporation into the virus genome. Viral oncogenes therefore, have once been normal cellular genes (proto-oncogenes or cellular oncogenes). They are evolutionary conserved and are present in all mammalian cells, often even in non-mammalian cells. Alteration of these cellular oncogenes (by ►point mutation, ►translocation or ►amplification) is one of the main contributions to cancer in all mammals, including humans.

►Human T-cell Leukemia Virus
►Retroviral Insertional Mutagenesis

Reverse Chemical Genetics

Definition

An approach for studying proteins of interest by first identifying a compound that perturbs the protein, and then evaluating the phenotypes induced by this compound in cells or whole organisms.

►Small Molecule Screens

Reverse Transcriptase

Definition

An RNA-dependent DNA polymerase found in retroviruses and certain organisms.

►Transduction of Oncogenes
►Retrovirus

Reverse Transcription PCR

Definition

►RT-PCR

Reverse Transcription-Polymerase Chain Reaction

Definition

RT-PCR; Analysis of RNA levels by copying RNA into complementary DNA by ►reverse transcriptase and then amplifying DNA using the polymerase chain reaction (►PCR).

Rexinoids

Definition

Has been coined as a term to describe 9-*cis*-retinoic acid and synthetic ligands that activate the ►retinoid X receptors (RXRα, RXRβ, and RXRγ).

►Retinoic Acid

RGD

Definition

A tripeptide Arg-Gly-Asp; Arganine-Glysine-Aspartic Acid is a recognition motif found in the amino acid sequence of many integrin-binding proteins. The RGD motif facilitates binding of many ►integrins, including α3β1, α4β1, α5β1, α8β1, α9β1, αvβ1, $\alpha_{IIb}\beta3$, αvβ3, αvβ5, αvβ6, and α4β7.

►Integrin Signalling
►Osteopontin
►TAT Protein of HIV

RGD, ^{18}F-Galacto-RGD

Definition

The selection of patients considered for antiangiogenic therapy may benefit from specific biomarkers defining individual angiogenetic activity in tumor tissue. ►Integrins are heterodimeric transmembrane glycoproteins consisting of different α- and β-subunits, which play an important role in cell-cell- and cell-matrix-interactions. An important member is αvβ3 which mediates the migration of endothelial cells through the ►basement membrane during blood vessel formation facilitating molecular imaging of ►angiogenesis. The pentapeptide cyclo(-Arg-Gly-Asp-dPhe-Val-) which shows high affinity and selectivity for αvβ3 can be labeled with ^{18}F. [^{18}F]Galacto-RGD represents the first PET tracer enabling imaging of αvβ3 expression.

►Positron Emission Tomography

RGS Proteins

Definition

Regulators of ►G-protein signaling inhibit G-protein-mediated signaling by activating the GTPase activity of defined G-protein α-subunits. The currently identified RGS proteins appear to predominantly interact with members of the G_i and G_q families of heterotrimeric G-proteins.

Rhabdoid Tumor

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Definition

A highly malignant tumor that primarily affects infants and young children. Rhabdoid cells contain large nuclei with prominent nucleoli and abundant eosinophilic cytoplasm that often contains filamentous cytoplasmic inclusions. In tumors of the central nervous system (CNS), areas of rhabdoid cells may be seen in juxtaposition to areas of primitive neuroepithelial cells resembling primitive neuroectodermal tumor, as well as mesenchymal tissue and/or epithelial tissue. The designation atypical teratoid/rhabdoid tumor (AT/RT) is used to describe the CNS tumor.

Characteristics

The true incidence of rhabdoid tumors in the population is not known due to the fact that many of these malignancies, especially in the brain, have been misdiagnosed. The most common sites of presentation for rhabdoid tumor are the kidney and brain. Rhabdoid tumors account for approximately 1–2% of childhood renal tumors and 2% of pediatric CNS tumors (►Brain Tumor). ►Extrarenal tumors are less common and may arise in a wide variety of sites, such as the liver, orbit, skin and other soft tissues. Most children are less than 3 years of age at diagnosis, and there is a slight male predominance (3:2). Rare reports of adult patients with rhabdoid tumors have demonstrated similar genetic alterations in these cases as compared to classic rhabdoid tumors of childhood. Rhabdoid tumors are highly aggressive malignancies and there have been few long term survivors.

The cell of origin for rhabdoid tumor is unknown. Variable histologic features and immunoreactivity to epithelial, neural, glial and/or myogenic markers may obscure the diagnosis of rhabdoid tumor. In the brain, the rhabdoid components of AT/RT usually exhibit positive staining for epithelial membrane antigen and vimentin, which helps distinguish it from ►medulloblastoma or supratentorial ►primitive neuroectodermal tumor. Approximately 40% of AT/RTs originate in the posterior fossa, notably in the cerebello-pontine angle. Forty percent of tumors are supratentorial, 5% are located in the pineal and the remainder are brain stem or multifocal. The tumors are highly metastatic, spreading rapidly throughout the cerebrospinal pathway.

To date, seven families have been reported in which a child with a rhabdoid tumor has had a first or second degree relative with a rhabdoid or CNS tumor. The small number of families with multiple affected individuals is likely due to the fact that few survivors have reached child bearing age. A genetic predisposition to rhabdoid tumor was originally supported by the finding that approximately 10–15% of infants with renal rhabdoid tumors had second primary tumors of the CNS. In past reports, the predominant histology of the brain tumors was consistent with a medulloblastoma or other primitive neuroectodermal tumor. It is now recognized that the brain tumors in these infants are AT/RT. Children may present with an AT/RT and rhabdoid tumor of the liver, lung or soft tissues, or with multiple primary tumors. The occurrence of two primary rhabdoid tumors (e.g. bilateral kidney tumors or rhabdoid tumors of both the kidney and brain) predicts the worst prognosis.

Genetics

The genetic predisposition to rhabdoid tumor and AT/RT is associated with a germline alteration in the INI1/hSNF5/BAF47/SMARCB1 gene. Cytogenetic and molecular studies of AT/RT, renal and extrarenal rhabdoid tumors demonstrated consistent deletions within chromosome band 22q11.2. ►Positional cloning strategies were employed to identify a candidate gene for rhabdoid tumor, which led to the identification of INI1 as a rhabdoid ►tumor suppressor gene. INI1 is a member of the ►SWI/SNF ►chromatin remodeling complex and may regulate transcription of specific target genes involved in both growth and differentiation.

Germline alterations of INI1 have been reported for patients with AT/RT, renal and extrarenal rhabdoid tumor. Germline deletions or mutations have been identified in at least 40 children. Loss of the wildtype allele or a mutation in the second allele has been documented in the tumor cells of these individuals, consistent with a ►two-hit model for a tumor suppressor gene. In several children with AT/RT and a renal or extrarenal rhabdoid tumor, the second hits arose

through different mechanisms in the two tumors, providing evidence that they were distinct primary malignancies and not metastases.

Two families in which a non-affected carrier mother transmitted a mutated INI1 gene to children diagnosed with AT/RT have been reported, suggesting that there may be ►reduced penetrance for the inherited form of this disease. Furthermore, several families with multiple affected siblings in which a carrier parent could not be identified were thought to be due to ►gonadal mosaicism.

►Biallelic inactivation of INI1 is also observed in the majority of sporadic rhabdoid tumors. ►Homozygous deletion has been documented in rhabdoid tumors from all anatomic sites, although the highest frequency appears to be among extrarenal rhabdoid tumors. ►Monosomy 22 appears to be more common in AT/RT than renal rhabdoid tumors, both in infants with predisposing germline INI1 mutations and in children with sporadic disease. Rarely, tumors demonstrate compound ►heterozygous somatic mutations in INI1, also consistent with the two-hit model.

In the majority of rhabdoid tumors, loss of one allele of INI1 is accompanied by a mutation in the remaining copy. Although some ►missense mutations have been reported, most of the mutations are ►point mutations or ►frameshift mutations that introduce a novel stop codon and thus predict premature truncation of the protein. The INI1 gene contains nine exons. The mutations are distributed throughout the coding sequence of INI1, although exons 3 and 8 are clearly under-represented. Identical mutations have been observed in rhabdoid tumors of the kidney and brain, demonstrating that most mutations are unlikely to be site specific.

The total number of INI1 mutations reported to date is approximately 200, and predictions regarding potential hot-spots or specificity of INI1 mutations may change as more data is accumulated. A single base pair deletion in codon 382/383 of exon 9 is the most frequently observed mutation in AT/RTs. To date, it has not been observed as a germline mutation or in a renal or extrarenal rhabdoid tumor. Three mutations, C157T in exon 2, C472T in exon 4 and C601T in exon 5, also appear to be present at an increased frequency. Each of these mutations has been observed as a constitutional change in patients with brain, kidney or brain and soft tissue tumors. These nonsense mutations have also been demonstrated as somatic alterations in sporadic renal tumors and AT/RTs.

The specificity of INI1 alterations for rhabdoid tumors is subject to debate. Mutations of INI1 have been reported in medulloblastoma, supratentorial primitive neuroectodermal tumor, choroid plexus carcinoma and epithelioid sarcoma. Each of these types of tumors may be included in the differential diagnosis of AT/RT in children with CNS tumors, or with rhabdoid tumor in children or adults with soft tissue malignancies. An immunohistochemistry assay for the INI1 protein was developed as an aid in the clinical diagnosis of rhabdoid tumor. Normal cells and non-rhabdoid malignant tumor cells demonstrate nuclear expression of INI1. Rhabdoid tumor cells show loss of nuclear expression of the protein. All genomic deletions and mutations of INI1 result in loss of nuclear expression of INI1. Furthermore, the loss of expression of INI1 can be observed in the 20% of rhabdoid tumors for which an INI1 deletion or mutation is not observed. The question of whether identification of an INI1 mutation is sufficient to make a clinical diagnosis of rhabdoid tumor or if there are several related tumors that may have overlapping molecular genetic alterations, will ultimately be addressed in large clinical and molecular genetic correlative studies. At present, the demonstration of an INI1 mutation is considered a poor prognostic feature and aggressive therapy is indicated.

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Rhabdomyosarcoma

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Definition

Sarcomas are malignant tumors thought to be derived from mesenchymal cells and contain cells that resemble those of connective tissues in the body. Rhabdomyosarcomas (RMS) are ►sarcomas resembling developing

skeletal muscle and consist of cells showing specific stages of skeletal muscle development (myogenesis). They are a heterogeneous group of tumors and although rare, they are more prevalent in childhood than later life. Historically they are broadly divided into two major histological subtypes:

- Alveolar RMS (ARMS) – cellular architecture resembling the alveolar spaces of the lungs. Based on molecular evidence, a solid variant of ARMS has been described that does not show alveolar-like spaces.
- Embryonal RMS (ERMS) – more frequent group and classical ERMS are seen predominantly in young children. Variants include botryoid and spindle cell.

In addition, a rarer pleomorphic subtype exists that is predominant in adults and most often found in the extremities and the trunk.

Characteristics

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood (►childhood cancer) and accounts for 4–8% of all childhood cancer cases. Males are more commonly affected, with an approximate ratio 1.3:1. The embryonal subtype accounts for around 70% of RMS cases and most frequently affects young children. The alveolar subtype is more prevalent in older children. Although RMS can arise anywhere in the body, they occur most commonly in three regions; (i) the head and neck (mainly ERMS), (ii) the genitourinary tract (botryoid) and retroperitoneum (ARMS), and (iii) the upper and lower extremities (ARMS).

Overall, more than 70% of RMS patients survive long term. Treatment of RMS usually involves two or more modalities (multi-modal therapy can include surgery, chemotherapy or radiotherapy). The survival rate depends significantly on tumor size, location, histology and whether metastasis has occurred. The outcome for the alveolar subtype is generally worse than for the embryonal cases. Accurate diagnosis of more undifferentiated RMS and tumors without distinct histological features may be difficult. ARMS are characterized by small round cells that may be difficult to distinguish from other so-called small round cell tumors, including ►neuroblastoma and the ►Ewing sarcoma family tumors. ARMS and especially the solid variant may also be difficult to distinguish from ERMS. Development of improved disease-specific therapeutic strategies, with concomitant improvements in outcome, have rendered accurate diagnosis of paramount importance. Distinctive cytogenetic and corresponding molecular changes associated with the various small round cell tumors can be useful diagnostic markers in addition to standard immunohistochemical and morphological features.

The expression of the muscle related factor family of transcription factors and the expression of the *PAX3* and *PAX7* genes in RMS is consistent with their morphological resemblance to developing skeletal muscle. The *PAX* genes are also involved in ARMS through specific translocations; t(2;13)(q35;q14) and variant t(1;13)(p36;q14) result in *PAX3-FKHR* and *PAX7-FKHR* ►fusion genes, respectively. The fusion genes encode chimeric transcription factors with the DNA binding domain from PAX3/PAX7 fused to the potent transactivation domain of FKHR (also called ►FOXO1a). The fusion proteins are more potent transcription factors than the wild type PAX3 or PAX7 gene products. The *PAX3-FKHR* fusion gene is associated with cases that have a poorer prognosis than those with *PAX7-FKHR*. Although the fusion proteins can transform ►NIH3T3 cells mice very rarely developed tumors in a knock-in *PAX3-FOXO1a* mouse model. The prevalence of tumors increased when crossed with a *INK4a/Arf/p53* deficient background. This indicates that the fusion product alone may not be sufficient to induce malignancy or that the animal models to date do not recapitulate the necessary conditions.

A number of downstream targets of the PAX3-FKHR fusion protein have been indicated through expression profiling analyses such as, *EN2, BVES, FLT1 Itam2A* and ►*MET*. *MET* encodes the HGF/SF receptor (hepatocyte growth factor/scatter factor) and silencing MET expression in both ARMS and ERMS cell lines impaired cell replication, survival, invasiveness and anchorage independent growth. Furthermore, RMS were induced in mice at a very high frequency, and with short latency, through simultaneous loss of INK4a/ARF function and disruption of MET. ►Amplification and expression of genes such as ►*MYCN*, ►*MDM2*, ►*CDK4*, and *GPC5* are also features of RMS. In addition, disruption of genes such as *ATR, PTC, P16* and ►*TP53* have been implicated in RMS development. In contrast to the *PAX-FKHR* fusion genes associated with alveolar subtype, the hallmark of ERMS is recurrent ►loss of heterozygosity, loss of ►imprinting or paternal disomy at the 11p15 locus. This loss leads to overexpression of the *IGFII* gene and is consistent with a role for the ►IGF pathway in ERMS development.

►Beckwith-Wiedemann syndrome is associated with fetal overgrowth and development of embryonal tumors including RMS and is also linked to the involvement of the 11p15.5 region. ►Costello syndrome is caused by mutation of the *HRAS* gene at 11p15.5 and children with Costello syndrome have a high incidence of ERMS. However, although sporadic

ERMS show uniparental disomy at 11p15.5, this is not driven by *HRAS* mutations. ►Li-Fraumeni syndrome involves germ line *TP53* mutations and is associated with increased risk of several tumor types including RMS.

Further understanding of RMS tumorigenesis, and in particular determining the key genes and molecular pathways involved, is resulting in novel targeted therapeutic strategies. This should lead to increased cure rates and reduce treatment associated toxicity for children with these tumors.

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RHAMM

Definition

Receptor for Hyaluronan Mediated Motility – A hyaluronan binding receptor that is distributed at the cell surface, cytoplasm, mitochondria and nucleus. Involved in regulation of cell locomotion and the cell cycle.

►Hyaluronan Synthases

Rheb

Definition

*R*as *h*omolog *e*nriched in *b*rain. Rheb, a member of the ►Ras family, is negatively regulated by tuberin's highly conserved GTPase-activating domain which converts Rheb-GTP to Rheb-GDP, thereby inactivating Rheb. Two roles have been identified for Rheb: activation of ►mTOR and inhibition of B-Raf.

Rheumatoid Arthritis

Definition

Is a common inflammatory joint disease that is probably due to an autoimmune response. The disease is accompanied by the production of ►rheumatoid factor, an IgM anti-IgG antibody that can also be produced in normal immune responses.

Rheumatoid Factor

Definition

An ►autoantibody found in the serum of most persons with ►rheumatoid arthritis.

Rho

Definition

Small GTPase, member of a branch of the ►Ras gene super-family which is involved in cytoskeletal regulation.

►RAS
►Rho family proteins

Rho Family Proteins

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Synonyms

Ras homologous proteins; Ras-related small GTPases

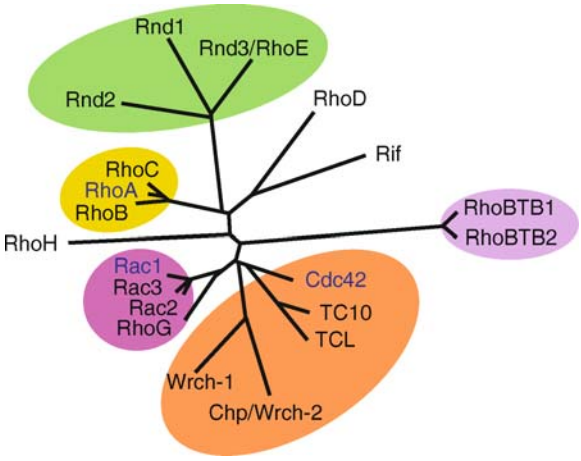
Definition

The Rho family proteins are members of a major branch of the ►Ras superfamily of small ►GTPases. Currently, 20 human members are known, with homologs present

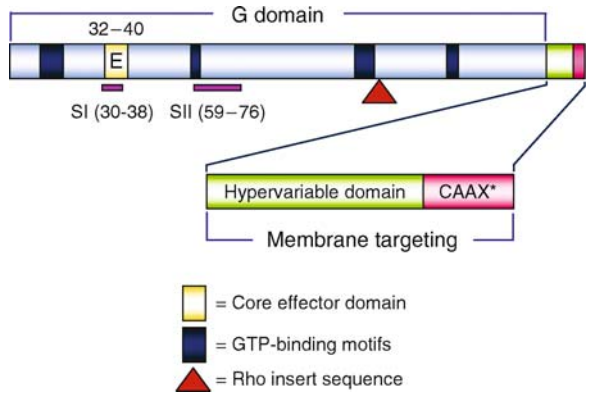
in invertebrates (S. cerevisiae (5), S. pombe (3), C. elegans (6), Drosophila (5), Dictyostelium (8), Aplysia (1), plants (2)) (Fig. 1). The best known and most widely characterized human Rho family proteins are Rac1, RhoA and Cdc42. These proteins function as GDP/GTP-regulated binary switches which regulate signal transduction pathways that control actin cytoskeletal organization, gene expression and cellular proliferation.

Characteristics

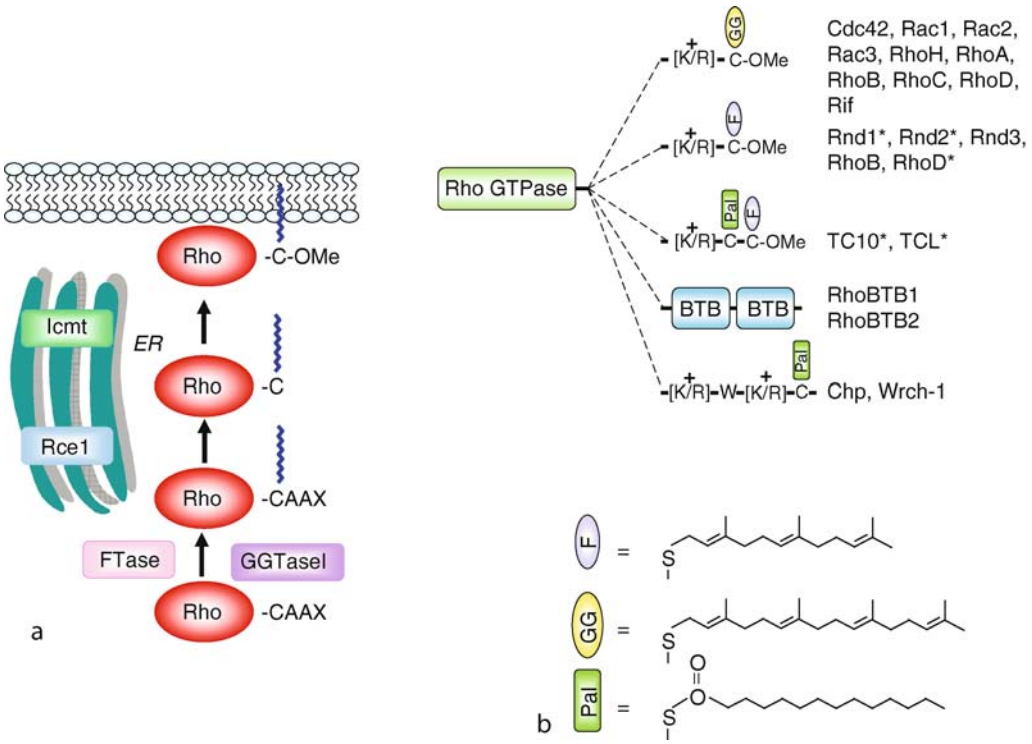
Rho family proteins are approximately 200 amino acids in length and a molecular weight of approximately 21 kDa. They share approximately 30% amino acid identity with the Ras ►oncogene proteins and between 50 and 90% identity within the family. All members share three distinct amino acid sequence elements (Fig. 2): First, they possess consensus GDP/GTP binding motifs shared with other GDP/GTP-binding proteins. Like Ras proteins, Rho family proteins possess high affinity binding for guanine nucleotides (GDP and GTP). Their biological functions are controlled by cycling between active GTP-bound and inactive GDP-bound states. Second, like Ras, 14 of 20 members terminate with a CAAX tetrapeptide sequence (C = cysteine, A = aliphatic amino acid, X = terminal amino acid). The CAAX motif signals three posttranslational modification steps; the addition of either a C15 ►farnesyl lipid (when X = M) or C20 ►geranylgeranyl (when X = L, F) ►isoprenoid lipid group to the cysteine of the CAAX motif, proteolytic removal of the AAX residues and carboxylmethylation of the now terminally prenylated cysteine residue (Fig. 3). These modifications increase the hydrophobic nature of the protein and facilitate their association with membranes. Two atypical Rho GTPases, Wrch-1 and Chp/Wrch-2, lack CAAX prenylation signals, and instead, are modified by a palmitate fatty acid essential for their membrane association. Third, sequences corresponding to Ras residues 32–40 represent the core effector domain and these sequences are involved in interaction with downstream effector targets. Sequences flanking these residues, as well as other sequences throughout Rho family proteins, are also involved in effector interactions. Finally, Rho family proteins possess a short sequence, designated the Rho



Rho Family Proteins. Figure 1 The Rho branch of the Ras superfamily. To date, 20 distinct mammalian Rho family proteins have been identified. Based on sequence and/or functional similarities, the 20 human Rho family GTPases are subdivided into the RhoA-related subfamily (RhoA, RhoB and RhoC), the Rac1-related subfamily (Rac1, Rac1b, Rac2 and Rac3), the Cdc42-related subfamily (Cdc42, TC10, TCL, Wrch-1 and Chp/Wrch-2), the Rnd subfamily (Rnd1, Rnd2 and Rnd3/RhoE) and the RhoBTB subfamily (RhoBTB1 and RhoBTB2/DBC2). The RhoD, Rif and RhoH/TTF proteins do not fall into any of these subfamilies. Generally, members of a subfamily are regulated by common RhoGEFs and RhoGAPs, and utilize overlapping effectors. However, some Rho family proteins are GTPase-deficient, constitutively GTP-bound and may not be regulated by GEFs or GAPs.



Rho Family Proteins. Figure 2 Primary structure of Rho GTPases. Rho GTPases are comprised of an amino terminal G domain that is characterized by consensus GDP/GTP-binding motifs shared with other GTP-binding proteins and a carboxyl terminal CAAX tetrapeptide sequence (C = cysteine, A = aliphatic amino acid, X = terminal amino acid). Residue numbers for the switch I (SI), switch II (SII) and core effector (E) sequences correspond to those of analogous residues of human Ras proteins. Triangle indicates Rho insert sequences positioned between Ras residues 122 and 123. The terminal X residue dictates modification by FTase (X = Ser, Met, Ala) or GGTaseI (X = Leu). While the CAAX-signaled modifications are necessary for Rho GTPase membrane association, additional sequences upstream of the CAAX motif (hypervariable region) contain sequence elements (palmitoylated cysteines or polybasic sequences) that are required for proper subcellular localization and membrane association.

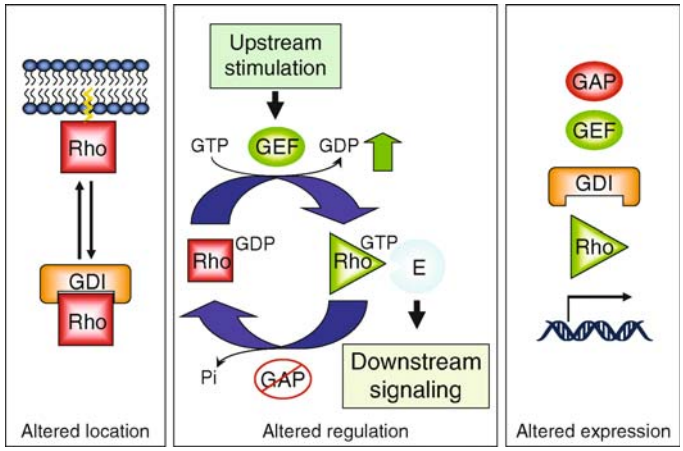


Rho Family Proteins. Figure 3 Posttranslational processing of Rho GTPases is necessary for membrane association. (a) The majority of Rho family GTPases terminate with CAAX tetrapeptide sequences that signal posttranslational processing required for membrane association and function. The first step is catalyzed by either farnesyltransferase (FTase) or geranylgeranyltransferase I (GGTaseI) stimulated covalent modification of the cysteine residue by a C15 farnesyl or C20 geranylgeranyl isoprenoid. Rce1 the catalyzes proteolytic cleavage of the AAX residues, and Icmt catalyzes carboxylmethylation of the now terminal prenylated cysteine residue. (b) Rho GTPase membrane association typically requires a second signal provided by sequences directly upstream by either palmitoylated cysteines or lysine or arginine rich sequences. RhoBTB proteins lack CAAX motifs and are not predicted to undergo posttranslational lipid modification. Shown are the verified or predicted (*) membrane-targeting and posttranslational modifications of the carboxyl terminal sequences of Rho GTPases.

insert sequence, that is not present in other Ras superfamily proteins and may also be involved in effector interaction.

Cellular and Molecular Regulation

Much of the functional information on Rho family proteins has come from studies of the three classical members, Rac1, RhoA and Cdc42. Hence, a majority of the information summarized in this article apply to the classical Rho GTPases and closely related isoforms. The GDP/GTP cycling of Rho family proteins is controlled by three distinct functional classes of regulatory proteins (Fig. 4). ►Guanine nucleotide exchange factors (GEFs) stimulate the weak intrinsic exchange activity of Rho family proteins to cause an exchange of the bound GDP for GTP to promote formation of active Rho-GTP. RhoGEFs are also called Dbl family proteins. Dbl family proteins (named after the founding member, a transforming protein identified from a human diffuse ►B-cell lymphoma) share a tandem Dbl homology (DH) domain and ►pleckstrin homology domain. The DH domain is a catalytic domain that stimulates GDP/GTP exchange. The PH domain is believed to regulate DH domain catalytic activity and can also serve to promote Dbl protein association with the plasma membrane. A number of Dbl family proteins were initially identified in gene transfer screening searches for transforming (e.g. Dbl, Vav, Ect2, Lsc) or invasion-inducing (►Tiam1) genes. Others were identified as proteins with other catalytic functions, such as the breakpoint cluster region protein (BCR). BCR is the translocation partner of the Abl tyrosine kinase present in ►Philadelphia chromosome positive human leukemias, and this genetic rearrangement causes the formation of a chimeric ►BCR-Abl fusion oncoprotein. To date, 69 distinct human Dbl family proteins have been identified. Since they function as RhoGEFs, their transforming actions are due to chronic activation of Rho GTPases. Additionally, a second structurally-distinct family of RhoGEFs, DOCK family proteins (15 human members), also serve as activators of



Rho Family Proteins. Figure 4 Rho family proteins function as GDP/GTP-regulated binary switches. In response to extracellular stimuli, RhoGEFs stimulate formation of active Rho-GTP, which in turn forms complexes with various downstream effectors (designated E) to initiate downstream signaling events. RhoGAPs serve as negative regulators of the GDP/GTP cycle and stimulate GTP hydrolysis. Deregulated Rho GTPase activity can occur by upregulated activation of RhoGEFs (e.g. fusion protein, missense mutation, phosphorylation), loss of function of RhoGAPs (e.g. deletion, promoter methylation), and altered gene expression of Rho GTPases, RhoGEFs and RhoGAPs. Deregulated expression of RhoGDIs can lead to altered membrane association of Rho GTPases.

Rho GTPases. Some atypical Rho GTPases (e.g. Rnd proteins) are GTPase-deficient and constitutively GTP-bound and active, and consequently, are not believed to be regulated by RhoGEFs.

The second class of regulators of Rho family proteins are ►GTPase activating proteins (GAPs) that stimulate the weak intrinsic GTP hydrolysis activity of Rho family proteins to promote formation of the inactive GDP-bound protein. Presently, at least 60 RhoGAPs have been identified (e.g. chimerins, BCR). The third class of regulators is the Rho guanine nucleotide dissociation inhibitory factors (GDIs). While RhoGDIs can inhibit GDP dissociation as well as GAP-stimulated GTP hydrolysis, their main cellular function involves regulation of the association of Rho family proteins with membranes. To date, three distinct Rho GDIs have been identified.

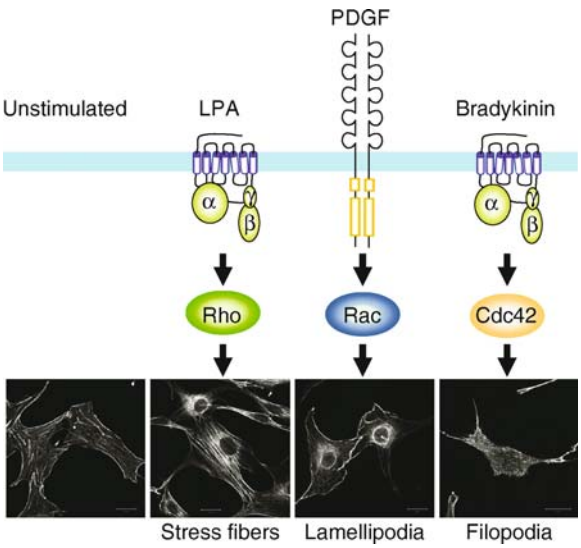
Structural analyses of Ras and Rho family proteins reveal that the GDP- and GTP-bound proteins differ in conformation in two regions, designated switch I and II (Fig. 2). The conformation of the GTP-bound protein results in increased binding affinity for downstream effector proteins. Each Rho family protein recognizes multiple effectors and some effectors are recognized by multiple Rho family proteins. Rho family protein interaction and activation of effector function leads to the stimulation of effector-mediated cytoplasmic signaling pathways that regulate the diverse functions of Rho family proteins. The effectors for RhoA, Rac1 and Cdc42 have been the most intensively studied and characterized. The multitude of effectors identified for each Rho family protein reflects the complex and diverse functional properties of these proteins.

Genetically engineered structural mutants of Rho family proteins have provided very useful research reagents to evaluate the biochemical and biological functions of Rho family proteins. The first class of mutants are ►gain-of-function mutants. Single amino acid substitutions at residues analogous to those that convert normal Ras proteins into highly oncogenic, constitutively activated proteins (at Ras residues glycine 12 or glutamine 61) also create constitutively activated mutants of Rho family proteins. These mutations render Ras and Rho proteins insensitive to GAP stimulation and thus these proteins persist in the GTP-bound state. The second class of mutants are dominant-negative mutants that contain a serine to asparagine substitution at the residue analogous to amino acid 17 of Ras proteins. These mutants can prevent activation of specific Rho family proteins, presumably by forming inactive complexes with specific Dbl family proteins. The third class of mutants are effector domain mutants that possess single amino acid substitutions in the core effector domain. These impair interaction of Rho family proteins with downstream effectors, thus leading to impairment in biological activity. Since a particular effector domain mutation leads to differential impairment of effector interaction, such mutants have been very useful reagents in establishing the specific contribution of different effector targets to Rho family protein function.

Functions

A diverse spectrum of extracellular stimuli via interaction with ►receptor tyrosine kinases (►RTKs), heterotrimeric ►G protein-coupled receptors (GPCRs) or integrins cause activation of specific Rho family proteins, most

commonly via activation of specific RhoGEFs (Fig. 5). For example, GPCRs that lead to activation of the heterotrimeric Gα13 subunit which then directly binds to the RGS (Regulator of G protein signaling) box of the p115 RhoGEF, PDZ-RhoGEF and Larg Dbl family proteins. These Dbl family proteins are specific GEFs for RhoA and related isoforms. Platelet-derived growth factor (PDGF) stimulation of the PDGF RTK causes activation of Rac1. This may be caused by activation of phosphatidylinositol 3-kinase and generation of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). PIP_3 can then bind the pleckstrin homology domain of the P-rex Rac-specific RhoGEF, leading to Rac activation. PDGF-stimulated activation of Ras can be another mechanism for Rac activation. Activated Ras binds to the Ras binding domain (RBD) of the Tiam1 RhoGEF, promoting Rac activation. Bradykinin stimulation of a GPCR causes activation of Cdc42. These various extracellular signals cause a transient increase in the GTP-complexed protein that then rapidly cycles back to the GDP-complexed form to terminate the signal.



Rho Family Proteins. Figure 5 Rho family proteins regulate extracellular stimulus-activated actin cytoskeletal organization. Extracellular stimuli that activate cell surface G protein-coupled receptors, for example, lysophosphatidic acid (LPA) and bradykinin, or receptor tyrosine kinases (e.g. platelet-derived growth factor (PDGF)) cause Rho GTPase-dependent actin reorganization. LPA activates RhoA and causes formation of actin stress fibers, whereas bradykinin activation causes formation of actin microspikes and filopodia. PDGF activates Rac and accumulation of cortical actin at the leading edge of migrating cells and formation of lamellipodia and membrane ruffling. Shown are immunofluorescence analyses of NIH 3T3 mouse fibroblasts stimulated with the indicated ligand (provided by Dr. Cercina Onesto). NIH 3T3 cells were incubated for 16 h in serum-free growth medium and the cultures were then stimulated for 15 min with the indicated ligand, then fixed and actin filaments were visualized with Alexa-phalloidin. Scale bar represents 20 μm.

Rho family proteins are regulators of diverse cellular processes. Perhaps their best characterized function involves the regulation of specific filamentous F-actin organization (Fig. 5). The ►actin cytoskeleton is a highly dynamic cytoplasmic structure that is reshaped and reformed in response to diverse extracellular stimuli. Specific Rho family proteins regulate distinct changes in actin cytoskeletal assembly and function. RhoA promotes the formation of stress fibers and ►focal adhesion, whereas RhoE/Rnd3 and Rnd1 cause the disruption of these structures. Rac1 promotes ►lamellipodia, curtain-like extensions that consist of thin protrusive actin sheets. Membrane ruffles represent lamellipodia that have lifted from the substratum at the leading edge of migrating cells. Cdc42 and related proteins (e.g. TC10, TCL, Wrch-1 and Chp) cause formation of filopodia, which are thin, finger-like cytoplasmic extensions that contain tight actin bundles and may be involved in recognition of the extracellular environment.

- Rho family proteins regulate actin cytoskeletal organization. For example, extracellular stimuli that activate cell surface GPCRs (e.g. lysophosphatidic acid, ►thrombin) or ►integrins (e.g. ►fibronectin) cause activation of cytoplasmic signaling cascades that promote the formation of actin stress fibers and focal adhesions (Fig. 3).
- A second major function of Rho family proteins involves the stimulation of cytoplasmic signaling pathways that regulate the activity of nuclear transcription factors. These transcription factors include those that regulate genes involved in the regulation of cell growth, differentiation and ►apoptosis. For example, Rac1 and Cdc42 activate the ►Jun N-terminal kinases (JNKs; also called SAPKs), and activated Jun can stimulate transcription from promoters containing ►AP-1 DNA binding motifs. RhoA, Rac1 and Cdc42 are activators of the NF-kB transcription factor. ►NF-kB regulates the expression of genes that serve an anti-apoptotic function. Rho family proteins activate the serum response factor (SRF), which forms a complex with ternary complex factors (e.g. Elk-1) at the serum response DNA element in promoter sequences of growth factor early response genes (e.g. fos).
- A third major function of Rho family proteins involves their regulation of cellular proliferation. RhoA, Rac1 and Cdc42 have been shown to be essential components required for cells to progress through the G1 phase of the ►cell cycle. Constitutively activated mutants of some Rho family proteins can promote G1 progress and DNA synthesis in quiescent cells, and

growth ►transformation of rodent fibroblasts. Extracellular signals that regulate cell proliferation cause transient activation of specific Rho family proteins. For example, ►platelet-derived growth factor is a potent growth factor for many cell types and is an activator of Rac1 function. Hence, Rho family proteins may facilitate the mitogenic actions initiated by extracellular stimuli.

Clinical Relevance

There is presently considerable experimental evidence linking Rho family proteins to cancer (Fig. 4). Unlike ►Ras, where mutational activation of Ras proteins is associated with 30% of human cancers, mutationally activated Rho GTPases are not found commonly in cancer. Instead, indirect mechanisms that include altered gene transcription or the altered function of Rho regulators are most commonly observed. For example, the Vav1 RhoGEF, normally hematopoietic cell-restricted in expression, is ectopically overexpressed and activated in pancreatic cancers. Tiam1, a Rac-specific GEF, functions as an effector of Ras and is required for Ras-mediated oncogenesis. Ras binds to and activates Tiam1, leading to persistent Rac activation. The expression of DLC-1 and related RhoGAPs are lost in a wide variety of human cancers. Since DLC-1 (►Deleted in liver cancer 1) functions as a negative regulator of RhoA, the loss of DLC-1 may promote oncogenesis by causing persistent RhoA activation. Rac1b is a splice variant of Rac1 that is preferentially expressed in breast and colon cancers. Unlike Rac1, Rac1b is persistently activated and cannot bind RhoGDI, and consequently, is a transforming variant of Rac1.

Aberrant Rho GTPase function is also associated with other human diseases. Oligophrenin-1 is a RhoGAP whose expression is lost and involved in nonspecific X-linked mental retardation. FGD1 is a Cdc42-specific RhoGEF that is mutated and inactivated in Aarskog-Scott syndrome (also called faciogenital dysplasia), which is a rare, clinically and genetically heterogeneous condition characterized by facial dysmorphic features, short stature, brachydactyly, and genital anomalies. *Salmonella typhimurium*, *Yersia pestis*, *Shigella flexneri* and other virulent bacteria have evolved mechanisms that alter the Rho GTPase function of their human host cells that facilitate their ability to promote infection and disease. *Salmonella typhimurium* causes typhoid fever, *Yersia pestis* was the causative agent for the Black Death plague which accounted for the death of approximately one-third of the European population in the fourteenth century, and *Shigella flexneri* causes dysentery. These bacteria use a type III secretion system to deliver bacterial effector proteins directly into the host cell. These function as activators or inactivators of human Rho GTPases and include proteins that act as RhoGEFs or RhoGAPs. Since bacteria do not possess Rho GTPases that are regulated by these effector proteins, these proteins exist solely for the purpose of deregulating the cellular functions of host cells.

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Ribavirin

Definition

Is a synthetic 1-beta-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide used against ►hepatitis C virus.

Ribonucleotide Reductase

Definition

Enzyme responsible for the reduction of ribonucleotides to deoxyribonucleotides for DNA synthesis. This is the rate-limiting enzyme in the biosynthesis of deoxyribonucleotides in mammalian cells.

►Chelators as Anti-cancer Drugs
►HSV-TK/Ganciclovir Mediated Toxicity

Ribosomal Protein S6 Kinase1

Definition

S6K1; A serine/threonine kinase, which is implicated in the activation of the ►mitogen-activated protein kinase cascade and the stimulation of cell proliferation.

►Programmed Cell Death 4

Ribosome

Definition
Complex structure composed of ribosomal RNAs and ribosomal proteins that associates with messenger RNA and coordinates protein synthesis.

►Funnel Factors
►Translation

Richter Syndrome

Definition
Is a rare complication of B cell ►chronic lymphocytic leukemia (CLL) or ►hairy cell leukemia (HCL). With Richter, CLL changes into a fast-growing ►diffuse large B-cell lymphoma, which is one of type of ►non-Hodgkin lymphoma. There are no obvious causes for the transformation of CLL to Richter syndrome, which can affect patients at any stage of CLL, including those in complete remission. The incidence of Richter transformation, which carries a poorer prognosis than CLL, has been reported at between three and ten percent. Richter can appear suddenly, even in patients who were in remission. The prognosis is generally poor, and aggressive treatment is usually warranted.

►Hodgkin Disease
►Richter Transformation
►non-Hodgkin lymphoma
►chronic lymphocyclic lukemia
►Rituxmab

Rigaud and Schmincke Types of Lymphoepithelioma

►Nasopharyngeal Carcinoma

RING

Definition
Really Interseting New Gene; protein motif.

►RING Finger Domain

RING Finger Domain

Definition
Really interesting new gene (RING) finger domain is a zinc-binding motif found in a variety of proteins. That of ►E3 ubiquitin ligase binds an E2 and might mediate enzymatic activity in the E2–E3 complex.

►Gankyrin

RIS

►Radiation-Induced Sarcomas After Radiotherapy

RISC

Definition
Acronym for RNA Induced Silencing Complex. RISC can assemble with short RNAs (►microRNAs or ►siRNAs) that give sequence specificity to the complex by guiding the complex to mRNAs containing a stretch of sequence complementary to the short RNAs. If the sequence complementarity is perfect or near perfect, RISC cleaves the mRNA and this is exploited by the ►RNA interference technology. If there are several mismatches between the short RNA and the target RNA, RISC does not cleave the mRNA but suppresses its translation.

►MicroRNA

Risk Factor

Definition
Is a variable (characteristic, behavior, etc.) that is associated with an increased risk of a disease. Risk factors are not necessarily causal, depending on the nature and on the way they were observed.

Rituximab

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Synonyms

Rituxan® (US), MabThera® (non-US countries)

Definition

Is a genetically engineered chimeric murine/human ►monoclonal antibody that binds specifically to the surface antigen ►CD20. CD20 is a hydrophobic transmembrane protein, which is typically expressed by pre-B, and mature B lymphocytes. It binds to lymphocytes in the thymus, white pulp of the spleen, and to most B lymphocytes in peripheral blood and lymph nodes, but not to hematopoietic stem cells, pro-B cells, or normal tissues. CD20 is involved in the initial steps of regulating the activation process for cell cycle initiation and differentiation and may also function as a calcium ion channel receptor with a key role in preventing apoptosis. CD20 is not shed from the cell surface and does not internalize upon antibody binding. Because of its human component, rituximab has low immunogenicity so that repeated administration is tolerable.

Characteristics

Rituximab is a 145-kDa monoclonal antibody, and its pH is 6.5. Its binding affinity for CD20 antigen is ~8 nanomolar. The elimination half-life of rituximab is 59.8 h (range, 11–105 h) after the first dose and 174 h (range, 26–442 h) after the fourth dose. It has been designated orphan product status for use in the treatment of ►non-Hodgkin lymphoma. Rituximab belongs to the classes of antineoplastics and immunological therapy drugs.

Mechanism of Action

The mechanism of its antineoplastic action may involve mediation of B cell lysis (seen in vitro) via binding of the Fab domain of rituximab to the CD20 antigen on B lymphocytes and by recruitment of immune effector functions by the Fc domain. The key mechanism by which rituximab exerts antitumor activity is ►antibody-dependent cell-mediated cytotoxicity (ADCC), followed by a minor role of ►complement-dependent cytotoxicity (CDC). Other mechanisms include indirect effects, such as structural changes, apoptosis, and sensitization of cancer cells to ►chemotherapy. Direct effects, including growth inhibition and apoptosis, although shown in vitro, have not been confirmed in the clinic.

Administration

Rituximab was the first monoclonal antibody approved by the United States Food and Drug Administration (FDA) in humans. The recommended dose for non-Hodgkin's lymphoma is 375 mg/m^2 via an intravenous infusion once weekly for 4 or 8 doses. The recommended dose for rheumatoid arthritis is two 1000 mg intravenous infusions separated by 2 weeks. Rituximab should not be administered by intravenous push or bolus; the initial rate of infusion should not exceed 50 mg/h. If tolerated, the infusion may gradually be increased by 50 mg/h increments every 30 min, up to a maximum of 400 mg/h.

Rituximab is an efficacious treatment option because of its non-overlapping toxicities and the ability to interrupt multiple pathogenic pathways when combined with cytotoxic agents. By virtue of its ability to target CD20, demonstrated in clinical trials initiated in 1993, rituximab conferred significant antitumor activity and minimal toxicity to patients with recurrent/relapsed indolent ►non-Hodgkin lymphoma. The ►FDA approved rituximab in 1997 based on its clinical efficacy against relapsed low-grade and follicular non-Hodgkin lymphoma demonstrated in multicenter studies.

Indications

The FDA approved rituximab for the treatment of the following CD20-positive, B-cell lymphoproliferative disorders:

1. Diffuse large B-cell non-Hodgkin lymphoma, in combination for first-line treatment
2. Follicular non-Hodgkin lymphoma, in combination with CVP (►cyclophosphamide, ►vincristine, and prednisone) chemotherapy for first-line treatment
3. Low-grade non-Hodgkin lymphoma, stable or responsive to prior CVP chemotherapy
4. Relapsed or refractory, low-grade or follicular non-Hodgkin lymphoma
5. In combination with ►methotrexate to reduce signs and symptoms in adult patients with moderate to severe ►rheumatoid arthritis, who had an inadequate response to one or more tumor-necrosis-factor antagonist therapies.

Rituximab has shown activity and is used in but not approved by FDA for the treatment of the following diseases:

1. ►Autoimmune hemolytic anemia
2. B-cell lymphoma, including ►splenic marginal zone lymphoma
3. ►Chronic lymphocytic leukemia, in combination for first-line treatment

4. Relapsed or refractory chronic lymphoid leukemia
5. ►Evans syndrome, refractory to immunosuppressive therapy
6. Chronic, ►steroid-refractory graft-versus-host disease
7. Post-transplant lymphoproliferative disorder
8. ►Systemic lupus erythematosus, refractory to immunosuppressive therapy
9. Immune or idiopathic ►thrombocytopenic purpura
10. ►Waldenstrom macroglobulinemia
11. Severe, refractory ►Wegener granulomatosis, in combination with ►corticosteroids

Adverse Events

Rituximab has a more tolerable toxicity profile than cytotoxic agents, which is particularly important for the elderly. Although dose-escalation trials were conducted, no maximum tolerated dose was established for rituximab, which is the case for various other monoclonal antibodies. The first dose of rituximab can cause an infusion reaction, which is manifested by fever, chills/rigors, nausea, urticaria, angioedema, bronchospasm, and/or hypotension. This complication can be avoided by premedicating patients with acetaminophen and an antihistamine. Infusion reactions usually respond to meperidine or hydrocortisone. Patients with a high tumor load, i.e., bulky lymphadenopathy or circulating malignant cells >25,000/mm^3 and those with renal disease are at high risk of developing ►tumor lysis syndrome (TLS). Prophylactic measures such as correction of electrolyte abnormalities, monitoring of renal function, hydration and administration of ►allopurinol or ►rasburicase usually prevent this complication, but rarely, dialysis is required.

The most common adverse events associated with rituximab are pruritus, nausea and vomiting, dizziness or headache, and pulmonary sequelae. Grade 3 or 4 lymphopenia, ►neutropenia, ►leukopenia, anemia, and ►thrombocycenia occur in the majority of patients. However, grade 3 or 4 non-hematologic toxicities are uncommon. They include angina, ►cardiac dysrhythmia, drug-induced ►pemphigus, ►lichenoid dermatitis, ►Stevens-Johnson syndrome, ►toxic epidermal necrolysis, bowel obstruction, gastrointestinal perforation, transient aplastic or hemolytic anemia, reactivation of type B viral hepatitis, immune hypersensitivity reaction, nephrotoxicity, obliterative bronchiolitis, pneumonitis, and infections. Rare cases of severe mucocutaneous reactions and progressive multifocal leukoencephalopathy have been reported.

Clinical Trials with Rituximab Monotherapy

Phase II clinical trials of rituximab therapy in relapsed or refractory, low-grade or follicular non-Hodgkin lymphoma demonstrated response rates ranging from 36% to 57% (complete response, 3–14%) and a median response duration of 7–16 months. Rituximab retreatment in patients with low-grade or follicular non-Hodgkin lymphoma who initially responded to antibody therapy and subsequently relapsed is feasible, as shown in a clinical trial, in which rituximab induced an overall response rate in 40% of patients (complete remission rate, 11%). However, in relapsed small lymphocytic lymphoma, rituximab had limited antitumor activity, probably due to a lower expression of CD20 molecules on the surface of small lymphocytic lymphoma cells. In addition, rituximab also had a shorter half-life in those patients compared with follicular lymphoma patients, raising the possibility of an "antigen sink." Subsequent studies demonstrated a soluble form of CD20 in the plasma of patients with ►chronic lymphocytic leukemia, and suggested that immune complex formation with the antigen sink may explain the shorter half-life of rituximab in that setting.

Clinical Trials with Rituximab Combination Regimens

Rituximab has been combined successfully with other active agents for the treatment of lymphoproliferative disorders (Table 1). The rationale for using rituximab in conjunction with cytotoxic agents derives from the observation that rituximab enhances the cytotoxicity of drugs, such as fludarabine and cyclophosphamide in lymphoma cell lines as well as from the principle of combining active agents with nonoverlapping toxicity profiles. The incorporation of rituximab into chemotherapy regimens for the treatment of indolent and large cell lymphoma is associated with superior clinical outcomes (response, failure-free survival and/or survival) than chemotherapy alone. For instance, the addition of rituximab to CHOP (R-CHOP; rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone) demonstrated prolonged failure-free survival in elderly patients with diffuse large B-cell lymphoma compared to CHOP alone. In addition, survival was lengthened in elderly patients abrogating drug resistance mediated by the antiapoptotic gene ►*BCL-2*. The National Comprehensive Cancer Network (NCCN) practice guidelines for the management of patients with ►diffuse large B-cell lymphoma currently recommend ►R-CHOP as a treatment option for induction therapy.

Rituximab is being investigated in several settings, including high-dose chemotherapy with ►autologous stem cell transplantation, in which in vivo purging of CD20 cells before stem cell harvesting is being investigated. Rituximab is also used as consolidation therapy after marrow recovery and in AIDS-related and central nervous system lymphomas.

The introduction of rituximab in the treatment of lymphoproliferative and immune-mediated disorders has established a new era in the treatment of hematologicmalignancies and immunologic diseases and has generated intensive research into the development of

Rituximab. Table 1 Rituximab-containing regimens in lymphoproliferative disorders

Disease	Regimen and schedule	Acronym
►Indolent lymphoma (follicular and variants)	Fludarabine, novantrone (mitoxantrone), dexamethasone, rituximab	FND-R
	Rituximab, cyclophosphamide, vincristine, prednisone	R-CVP
	Rituximab, granulocyte-macrophage colony-stimulating factor	R-GM-CSF
►Mantle cell lymphoma	Rituximab, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone	R-Hyper-CVAD
Large cell lymphoma	Rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone	R-CHOP
	Rituximab, gemcitabine and oxaliplatin	R-GemOx
►Chronic lymphocytic leukemia	Fludarabine and rituximab	FR
	Fludarabine, cyclophosphamide, rituximab	FCR
	Cyclophosphamide, fludarabine, alemtuzumab, rituximab	CFAR
	Oxaliplatin, fludarabine, cytarabine, rituximab	OFAR
	Pentostatin, cyclophosphamide, rituximab	PCR
	Rituximab, alemtuzumab	N/A
	Fludarabine, cyclophosphamide, mitoxantrone, rituximab	FCM-R
	Rituximab, granulocyte-macrophage colony-stimulating factor	R-GM-CSF
►Richter syndrome	Rituximab, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone	R-Hyper-CVAD
	Oxaliplatin, fludarabine, cytarabine, rituximab	OFAR

similar approaches for other diseases. Investigation of monoclonal antibodies comprises one of the largest growth areas in drug development.

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RLIP76

Definition

Ral-Interacting Protein 76 kDa.

►Glutathione Conjugate Transporter RLIP76

RNA Interference

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Synonyms

RNAi; RNA interference; Small interfering RNA; siRNA; Short hairpin RNA; shRNA

Definition

RNA interference (RNAi) is an evolutionarily conserved gene-silencing pathway that is triggered by double-stranded RNA (dsRNA) with a short 21–23 nucleotide-long chain. Experimentally, RNAi is widely used for knocking down the expression of cancer-related genes and also provides a systematic screen to discover more about genes that trigger or inhibit cancer.

Characteristics

RNA Interference as a Research Tool

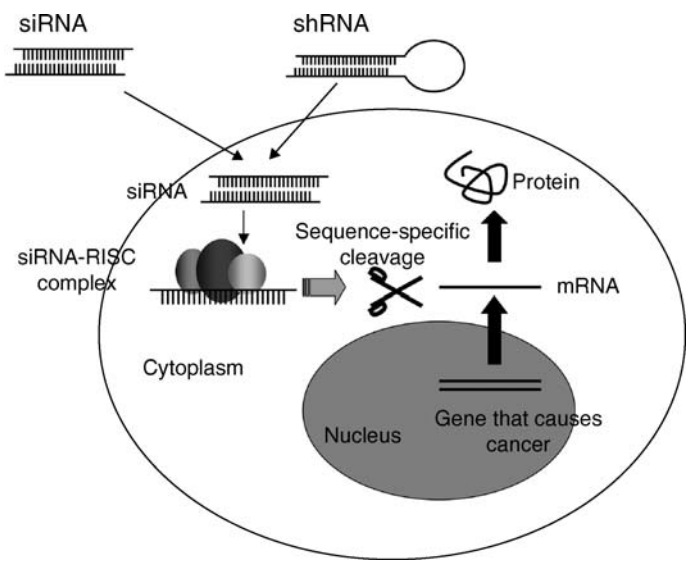
RNAi has become an extremely useful research tool because it allows genetic researchers to "knock down" specific genes, observe the consequent disruptions, and, in this way, determine exactly what a gene does. RNAi is being used as a genome-wide tool to search thousands of genes in a cell and screen for their functions. Moreover, researchers are using RNAi in

drug development, hoping that, by shutting down the genes that cause certain diseases, they will be able to cure diseases such as cancer. RNAi-mediating molecules can be chemically synthesized as a small interfering RNA (siRNA) or they can be synthesized from expression vectors as short hairpin RNA (shRNA) that can be cleaved into siRNAs by endogenous cellular dicer. They are incorporated into the RNA-induced silencing complex (►RISC), where the strands are separated. The RISC containing the guide or antisense strand seeks out and binds to complementary mRNA sequences. These mRNA sequences are then cleaved by Argonaute, the enzyme within the RISC responsible for mRNA degradation, which leads to target cancer-causing mRNA downmodulation (Fig. 1).

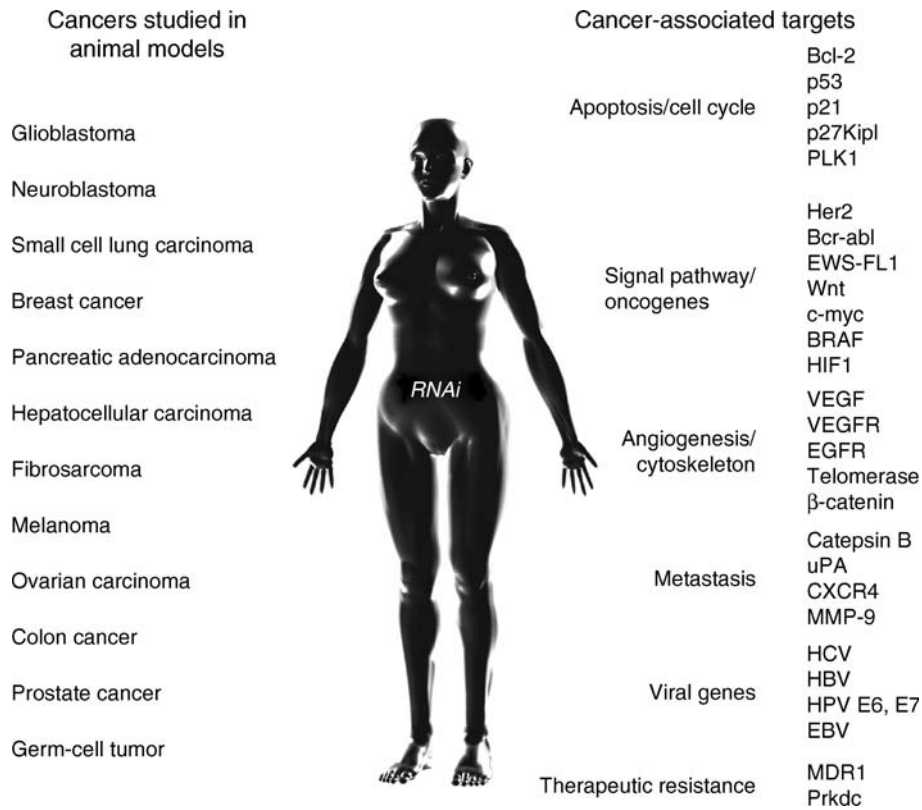
RNA Interference as a Novel Therapeutic Agent Against Cancer

The key is developing delivery strategies that are safe, specific, effective, and long lasting so that RNAi may truly become a new class of human therapeutic agent. For application to cancer therapeutics, the direct delivery of siRNAs and viral delivery of shRNAs to tumors have been successful in inhibiting ►xenograft growth in several animal models. To obtain efficient and long-lived ►gene silencing by RNAi, numerous approaches, including cationic lipid-based formulation and complexation with polyethyleneimine (PEI), cholesterol-oligoarginine, a protamine–Fab fusion protein, and biomaterials such as atelocollagen, have been shown to facilitate delivery into tumor cells. Particularly, these siRNA delivery approaches are effective with several intratumor injections of siRNA, or even a single one, at doses of 15–50 μg.

Evidence of the concept of using RNAi for cancer therapeutics in animal models is shown in Fig. 2. In mouse tumor xenograft models, the efficacy of systemic RNAi has been demonstrated using a variety of delivery strategies. Systemically delivered cationic cardiolipin liposomes containing synthetic siRNA specific for ►Raf-1 inhibited tumor growth in a xenograft model of human ►prostate cancer. ►Vascular endothelial growth factor receptor-2 (VEGF-R2)-targeting siRNAs complexed with self-assembling ►nanoparticles consisting of polyethylene glycol-conjugated (PEGylated) PEI with an Arg-Gly-Asp peptide attached at the distal end of the ►PEG accumulate in tumors and cause inhibition of VEGF-R2 expression. Intravenous administration of these complexes into tumor-bearing mice inhibits both tumor ►angiogenesis and growth rate. Simpler PEI formulations have also shown efficacy in xenograft tumor models, as have complexes of siRNA duplexes with atelocollagen. Systemic administration of atelocollagen siRNA complexes has marked effects on subcutaneous tumor xenografts as well as bone metastases of human prostate tumors. Another delivery strategy made use of a recombinant antibody fusion protein to achieve cell type-specific delivery. One attractive method is the delivery of siRNA using cancer cell-specific antibodies. In fact, the nucleic acid-binding protein protamine fused to the antibody (Fab) targeting the HIV-1 envelope protein and showed that the Fab–protamine fusion protein was able to deliver an siRNA to mouse melanoma cells expressing the envelope protein, leading to the inhibition of tumor growth in vivo.



RNA Interference. Figure 1 Targeting cancer by RNA interference. RNAi-mediating molecules can be chemically synthesized as siRNAs or they can be synthesized from expression vectors as shRNA which can be cleaved into siRNAs by endogenous cellular dicer. The strand complementary to the mRNA from target cancer-causing gene is incorporated into RNA-induced silencing complex (►RISC) then the siRNA-RISC complex degrades target mRNAs.



RNA Interference. Figure 2 Evidence of the concept of using RNAi for cancer therapeutics in animal models. In most tumor xenograft models in animals, the effectiveness of RNAi as a therapeutic tool against cancer has been demonstrated (left). An example of targeting cancer-related genes for RNAi therapy is shown (right).

The same technology was used with an antibody against ►ErbB2 fused to a protamine fragment that specifically and effectively delivered siRNAs only to ErbB2-expressing ►breast cancer cells. In another example of ligand-directed delivery, transferrin-conjugated nanoparticles were used to deliver an siRNA targeting the oncogenic EWS-FLI translocation-derived mRNA in a mouse model of metastatic ►Ewing sarcoma.

To obtain efficient and long-lived gene silencing using RNAi, several groups have used viral vectors. An ►adenovirus, despite its disadvantage, namely, that the immunogenicity of a viral vector precludes multiple administrations and results in toxicity limitations, is one of the best-known ►virus vectors for gene delivery. An intratumoral injection of an adenovirus encoding the ►HIF-1-targeted siRNA as shRNA has been shown to have a significant effect on tumor growth when combined with ►ionizing radiation. Intratumoral administration of an adenovirus siRNA vector for ►Skp-2, which is involved in the degradation of cell-cycle regulators, including p27Kip1, p21, and ►c-myc efficiently inhibited the growth of an established subcutaneous tumor of human small-cell lung carcinoma cells on NOD/SCID mice. Cre-recombinase-inducible RNAi mediated by a lentiviral vector system has been developed. As for the lentiviral vector delivery of tumor-targeting siRNAs, inhibition of ►chronic myeloid leukemia cells proliferation by a lentiviral vector-delivered anti-bcr/abl shRNA has been reported. Inhibition of the growth and invasive ability of melanoma by inactivation of mutated serine/threonine kinase ►BRAF has revealed that the lentivirus-mediated RNAi targeting for oncogenic mutations may be a promising tool for cancer therapy.

Prospects and Obstacles Regarding RNAi Therapeutics

RNAi is on a rapid pathway from its discovery to promising progress in animal studies and now preclinical and clinical trials for various types of diseases, including cancers. Despite all the enthusiasm, there are still many obstacles that need to be overcome. First, dsRNAs can stimulate innate ►cytokine responses including the ►interferon response and resulting nonspecific inhibition of ►transcription and ►translation, thereby being cytotoxic to cells. Second, although more sustained gene silencing with RNAi is achieved with the viral delivery of shRNAs, viral delivery causes virus-associated immune stimulation. Third, it is generally accepted that siRNA causes an off-target effect; therefore, when designing siRNA sequences aimed to gain insights toward improving the specificity of RNAi for potential future therapeutic applications, it is

important to minimize the sequence-specific cross reactivity by reaching a better understanding of the off-target effects of RNAi. A practical guide for siRNA selection is available elsewhere.

One possible RNAi therapy may help to defeat cancer by supporting current ►chemotherapy or ►radiotherapy. In fact, drug ►resistance is a major problem in cancer chemotherapy; it has led to the treatment failure of 30–50% of the current first-line anticancer drugs. The expression of the multidrug transporter ►P-glycoprotein (P-gp) has been reported to be one of the causes of clinical drug resistance to anticancer drugs. In this regard, targeting of P-gp by RNAi to restore the sensitivity to existing drugs is an effective strategy in RNAi-based cancer treatment.

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RNA Silencing

Definition

Is the inhibition of gene expression by small sequences of RNA. These small sequences are able to cleave messenger RNA, block protein synthesis or inhibit ►transcription.

►RNA Interference

RNA Tumor Virus

Definition

Is a member of the family of retroviruses with the ability of inducing tumor growth.

►Retrovirus
►Transduction of Oncogenes

RNAi

►RNA Interference

RNP Complex

Definition

Complexes that contain RNAs and their respective RNA-binding proteins.

►pre-mRNA Splicing

Robo

Definition

Roundabout; Is the ►Slit receptor, and four *ROBO* genes have been identified. Typically, Robo proteins, including Robo1, consist of five extracellular immunoglobulin (Ig) domains, three fibronectin repeats, and a conserved intracellular region of four cytoplasmic motifs.

ROCK

Definition

Serine–threonine Rho-kinase.

►Trefoil Factors
►Rho Family Proteins

ROD-ZW10-Zwilch Complex

Definition

RZZ; Is composed by three proteins, Rough Deal (Rod), Zeste-white 10 (Zw10) and Zwilch. The RZZ

complex is responsible for both Mad1 and Mad2 recruitment to unattached kinetochores and is involved in activation of the spindle assembly ►checkpoint.

►Mitotic Arrest-Deficient Protein 1

ROI

Definition

Reactive Oxygen Intermediate.

►Reactive Oxygen Species

RON

Definition

Recepteur d'Origine Nantaise Transmembrane receptor belonging to the ►Met-receptor tyrosine kinase family.

►Macrophage-Stimulating Protein.

Ron Receptor

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Synonyms

Macrophage stimulating 1 receptor; MST1R

Definition

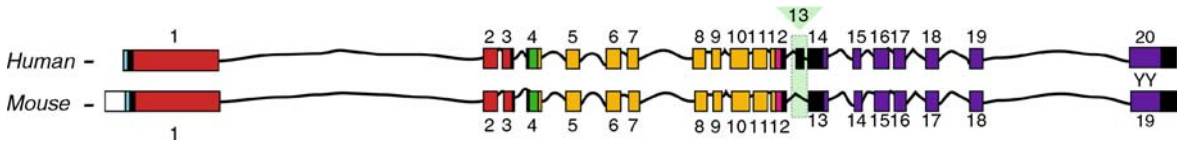
Cell surface ►receptor tyrosine kinases regulate critical signaling pathways and elicit a variety of important biological responses that contribute to tumorigenesis. The Ron receptor tyrosine kinase has been implicated in a number of human cancers. Ron belongs to a family of receptor tyrosine kinases that includes the ►*Met* proto-►oncogene. The *Ron* ►ortholog in the mouse is also referred to as *stem cell derived kinase* (*Stk*) while the chicken counterpart encodes *c-sea*. The human *Ron* gene is located on chromosome 3p21.3 and contains 20 exons with a transcript length of 4,531 basepairs (bps) and a translation length of 1,400 residues. The murine counterpart contains 75% identity to the human protein and is located on chromosome 9. The mouse *Ron* gene contains 19 exons with a transcript length 4,710 bps and 1,378 residues. A comparison of the genomic organization of the human and mouse *Ron* is depicted in Fig. 1.

Characteristics

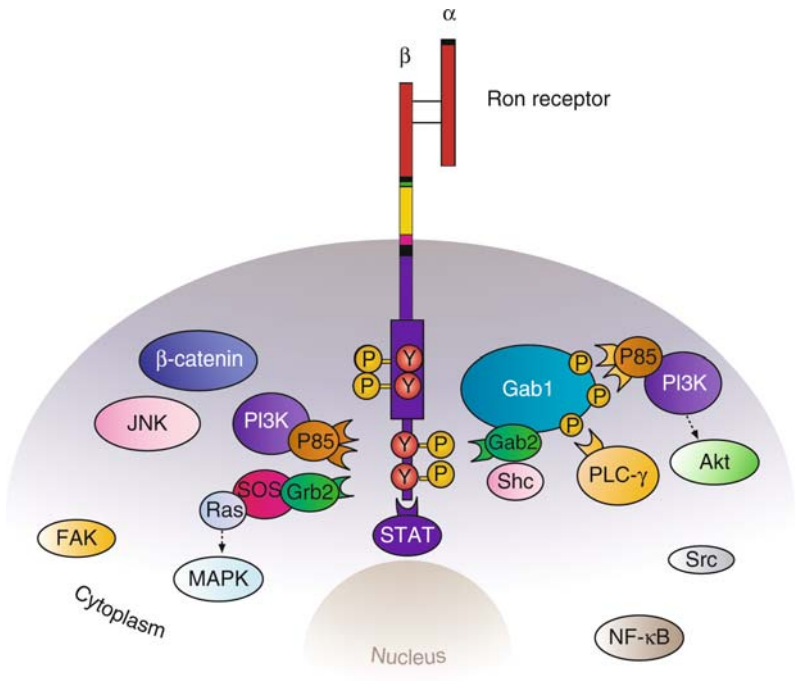
The Ron protein is a heterodimeric ►glycoprotein with disulfide linked alpha (35 kDa) and beta (150 kDa) chains. The protein is synthesized as a single chain precursor of 185 kDa that is cleaved into its heterodimeric form before exposure to the cell surface. A diagram of the domain structure of Ron is presented in Fig. 2. The protein includes a 24 amino acid signal peptide, an extracellular domain of 933 amino acids, a 25 amino acid transmembrane domain and an intracellular tyrosine kinase domain of 418 amino acids. The tyrosine kinase domain of Ron is 63% identical to the same domain in the Met receptor, while the extracellular domain is only 25% identical. The Ron alpha chain contains regions important for ligand binding while the beta chain includes an extracellular domain, a transmembrane domain and an intracellular region including the tyrosine kinase domain. Based on functional annotation of the Ron protein sequence, the extracellular domain contains a ►SEMA domain characterized by a conserved set of cysteine residues that form disulfide bonds to stabilize the protein structure. The extracellular portion of Ron also contains a plexin domain, a cysteine rich motif found in many extracellular receptors, and several IPT domains, which contain an immunoglobulin-like fold and is found in intracellular transcription factors involved in DNA binding.

Expression and Function

Ron is preferentially expressed on epithelial cells and ►macrophages. Ron expression has been detected in the central nervous system, liver, kidney, testes, bone, lung, breast, and epithelia of the digestive tract. In macrophages, Ron signaling regulates select ►cytokine and ►chemokine production in response to injury. In epithelial cells, the normal functions of Ron include a wide array of biological activities such as inducing DNA synthesis, triggering cell scattering, branching morphogenesis, and the regulation of ►apoptosis. Based on the signaling pathways elicited by Met and Ron, the activities elicited by this receptor family have been termed "invasive growth."



Ron Receptor. Figure 1 The human and mouse *Ron* gene structure. Exons are depicted by numbered boxes and introns by black lines. Red boxes code for the SEMA domain, aqua box for the signal peptide, green box for the plexin domain, yellow boxes for the immunoglobulin-like, plexins, transcription factor (IPT) domains, pink box for the transmembrane domain, and blue boxes for the tyrosine kinase domain. The Ys refer to the critical autophosphorylation sites in the kinase domain and for the docking site tyrosine residues in the C-terminal region of Ron. Note that exon 13 in human *Ron* is missing in the mouse gene.



Ron Receptor. Figure 2 Ron protein structure and signaling pathways activated by Ron. The Ron receptor is a single pass membrane spanning, disulfide-linked heterodimeric protein. The alpha chain is an extracellular glycoprotein and the beta chain spans the membrane and contains the intracellular tyrosine kinase domain. Activation of Ron leads to the phosphorylation of tyrosine residues within the kinase domain and subsequent phosphorylation of tyrosine residues that form docking sites for a number of downstream signaling proteins.

R

Ligand

The ligand for Ron is a protein termed ►hepatocyte growth factor-like protein (HGFL), and is also called ►macrophage-stimulating protein or macrophage stimulating 1 (MST1). HGFL is part of a larger family of ►plasminogen-related growth factors, which also includes hepatocyte growth factor (HGF)/►scatter factor (the ligand that activates c-Met), plasminogen and urokinase. The *HGFL* gene, similar to *Ron*, is located at chromosome 3p21.3. HGFL is predominantly produced in hepatocytes. Following synthesis, HGFL is constitutively secreted into the bloodstream, at a concentration of approximately 400 ng/ml, as a biologically inactive single chain precursor of 80 kDa. Pro-HGFL is proteolytically cleaved by membrane-associated proteins on the cell surface or by members of the kallikrein family. Proteolytic cleavage results in the formation of a disulfide-linked heterodimer containing an alpha chain of approximately 50 kDa and a beta chain of approximately 35 kDa. The beta chain of HGFL contains a serine protease-like domain and is primarily responsible for binding to Ron, while the alpha chain contains four kringle domains and appears to regulate the functional consequences of this binding.

HGFL was originally identified as a chemoattractant for macrophages, but it can also act as a motogen or mitogen for several other cell types. Depending upon cell type and context, HGFL can also induce a number

of pleiotropic effects. However, gene deletion studies in mice have shown that HGFL is not an essential gene product as HGFL ►knockout mice have limited phenotypic abnormalities.

Signaling Through Ron

Binding of HGFL to Ron leads to activation of the receptor's intrinsic tyrosine kinase activity and trans-autophosphorylation of two C-terminal tyrosine residues, creating high affinity binding sites for proteins containing Src homology 2 and phosphotyrosine-binding domains (Fig. 2). These phosphorylated tyrosine residues can bind ►phospholipase C-gamma (PLC-γ), ►phosphatidylinositol 3-kinase (PI3K), growth factor receptor-bound protein 2 (Grb2), and Shc. Ras, ►mitogen-activated protein kinase (MAPK), β-catenin, nuclear factor kappa B (NF-κB), and ►Akt mediate HGFL induced activities. Focal adhesion kinase (FAK), Src, c-Jun N-terminal protein kinase (►JNK), and ►signal transducers and activators of transcription (STAT)3 activation result from HGFL/Ron signaling. Thus, Ron activation induces pleiotropic responses through the recruitment of numerous signaling molecules.

Ron Overexpression in Multiple Tumor Types

Increased Ron expression has been documented in several tumor types including breast, colon, lung, liver, kidney, ovary, stomach, pancreas, bladder and prostate. Ron expression is also elevated in cells derived from these tumors. Clinically, high expression of Ron is positively correlated with poor prognosis in several types of human cancers including breast, prostate, colon, and pancreas. Based on gene expression analyses, in the breast, increasing Ron expression is associated with metastatic disease, and in the prostate, Ron expression is correlated with advanced, androgen-independent cancers, suggesting that Ron is important in tumor progression. Overexpression of both Ron and Met in breast and bladder cancers is associated with overall decreased patient survival.

Ron Variants

Several variants of Ron result from ►alternative splicing or alternative initiation. These variants, designated by their molecular weight, have been found in lung, colon, and breast cancers, and many are associated with increased Ron expression. Four of these variants (RonΔ165, RonΔ160, RonΔ155, RonΔ55) result in constitutive activation of the receptor and three (RonΔ160, RonΔ155, RonΔ55) have oncogenic properties. Less is known about RonΔ170 and RonΔ110.

RonΔ55, also known as Short Form (SF)-Ron, is generated from an alternative start site in intron 10 of the *Ron* gene that eliminates most of the extracellular portion of the receptor. SF-Ron is expressed in ovarian and breast cancers, and in cell lines derived from colorectal, lung and pancreatic carcinomas and several leukemias. This variant is constitutively phosphorylated, has strong kinase activity, and alters morphology, growth, motility, and anchorage-dependent growth when transduced into epithelial cells. Expression of SF-Ron can also negatively regulate E-cadherin transcription, resulting in a loss of cellular adhesion.

Mouse strains that express SF-Ron in adult bone marrow tissue are susceptible to Friend virus-induced erythroleukemia, and SF-Ron kinase activity is necessary for erythropoietin-independent expansion of erythroid progenitors in response to Friend virus. Moreover, recent studies have also suggested that SF-Ron has distinct nonredundant biological functions relative to full-length Ron in the progression of inflammatory immune responses in vivo.

Ron-Dependent Transformation

Overexpression of the full-length Ron receptor has transforming capabilities in vitro and in vivo. Mouse fibroblasts overexpressing wild-type Ron exhibit loss of contact inhibition, elongated cellular processes, and increased cellular proliferation and motility compared to fibroblasts with endogenous Ron expression. These phenotypes are augmented with HGFL. Overexpression results in tyrosine phosphorylation of the receptor, suggesting that overexpression is sufficient to elicit constitutive activation. Ron overexpression in colon epithelial cells induces cellular ►migration and invasion and is able to protect cells against apoptosis. Ron overexpression in fibroblasts and epithelial cell lines is sufficient for tumor formation in ►xenograft models.

Transgenic Mouse Models Overexpressing Ron

Important ►mouse models have been created to examine tissue-specific Ron overexpression. The mouse mammary tumor virus (MMTV) promoter was utilized to drive Ron overexpression in mammary epithelium. The female mice expressing the transgene developed *mammary tumors* with 100% incidence. The tumors were highly metastatic, with metastases observed in about 90% of the lungs and livers of all tumor bearing mice. This study demonstrates that Ron overexpression may be important in metastatic breast cancer. This data also supports the observation that Ron overexpression in human breast cancer is associated with an aggressive cancer phenotype with decreased disease free survival time and an increase in ►metastasis. In another mouse model, the surfactant protein C promoter was utilized to drive Ron overexpression in distal lung epithelial cells. Between 6 and 14 months of age, about 90–95% of the transgenic mice developed lung adenomas. These mouse models

suggest that Ron is important to tumor formation, progression, and metastasis.

Ron Loss in Murine Tumor Models

The development of a mouse model containing a targeted deletion of the Ron tyrosine kinase domain has allowed the functional significance of Ron signaling in vivo to be examined. These mice have been crossed with mice engineered to develop tumors. In a well-characterized mouse model of skin cancer, v-Ha-Ras transgenic mice were utilized in which 12-O-tetradecanoylphorbol-13-acetate (►TPA) treatment results in the formation of benign skin papillomas that progress to frank tumors. TPA-treated v-Ha-Ras mice lacking Ron signaling have a greater number of benign skin papillomas, although the papillomas are smaller in size, and have decreased cell proliferation. Importantly, the loss of Ron in this model leads to significantly reduced progression of the benign papillomas toward malignancy.

Another transgenic mouse model lacking functional Ron has been created using mice that have expression of the polyoma virus middle T antigen (pMT) under the control of the MMTV promoter. In the pMT-induced model, the mice develop mammary tumors that metastasize to the lung. The loss of Ron in this model results in a significant decrease in the rate of mammary tumor initiation, fewer tumors, a decrease in tumor size, and is associated with decreased tumor cell proliferation and increased apoptosis. Tumors lacking functional Ron also exhibit a decrease in microvessel density, suggesting that Ron may be important in regulating events impacting tumor ►angiogenesis.

Oncogenic Ron Signaling

Several signaling pathways (β-catenin, MAPK, PI3K) are activated by Ron overexpression as well as ligand binding. β-catenin is part of the ►Wnt signaling pathway and β-catenin expression has been associated with several human cancers. Mammary tumors derived from mice overexpressing Ron are associated with an increase in β-catenin and in the β-catenin target genes ►*c-myc* and *cyclin D1*. Human pancreatic cancer cells treated with HGFL also have increased nuclear β-catenin, and human colorecatal carcinoma cells lacking Ron expression have decreased β-catenin expression.

The MAPK pathway is also upregulated in a variety of cancers, and this pathway is important for many cellular processes including cellular differentiation, survival, proliferation and gene expression. In several human cancer cell lines with high levels of Ron expression, such as those derived from colon, prostate, lung, pancreas and stomach, MAPK activation can be abrogated using a neutralizing antibody against Ron. Mammary tumors from mice lacking the tyrosine kinase domain of Ron also have decreased MAPK activation. Conversely, the addition of HGFL to several cell types results in increased MAPK activation.

Similar results have been found for the regulation of the PI3K/Akt signaling pathway by Ron. Like β-catenin and MAPK, this pathway is often upregulated in various cancers. PI3K can bind directly to Ron. Akt, a serine/threonine kinase downstream of PI3K, is then activated and elicits responses decreasing apoptosis and increasing cellular survival. In colon, prostate and stomach cancer cell lines, there is a decrease in activated Akt when Ron is neutralized. In vivo, the amount of phosphorylated Akt is decreased in mammary tumors from mice lacking functional Ron. Taken together, these data suggest that Ron expression is important to the regulation of signaling pathways that are often highly associated with many human cancers.

Ron as a Therapeutic Target in Human Cancer

The mounting evidence that Ron is important in tumor initiation, progression, and metastasis suggests that Ron may be a therapeutic target for cancer treatment. Several approaches have been initiated to target Ron. Antibodies have been developed to neutralize Ron activity by preventing HGFL binding, and may be effective in treating tumors containing high Ron expression. Antibodies directed against Ron are able to inhibit MAPK and Akt activation as well as cellular migration. Moreover, in xenograft models using cancer cells that overexpress Ron, antibody neutralization is able to reduce subcutaneous tumor growth. Increased apoptosis of Ron-expressing pancreatic cancer cells was demonstrated by concurrent Ron receptor antibody blockade and ►gemcitabine treatment. In addition to neutralizing antibodies to target Ron, small molecule inhibitors have also been developed. A small peptide inhibitor containing five amino acids can inhibit HGFL- and HGF-induced cellular scattering and migration. A soluble molecule homologous to the ►SEMA domain of Ron has been shown to exhibit a ►dominant negative effect. This molecule is able to inhibit HGFL-induced phosphorylation of Ron, and has an inhibitory effect on the growth of colon cancer cells. ►Geldanamycin, a class of anti-tumor drugs that inhibit tumor cell growth by preventing proper folding and increase the degradation of oncogenic proteins, have also been identified as possible treatment modalities leading to receptor tyrosine kinase degradation, including Ron, in human cancers.

The Ron receptor participates in ►receptor cross-talk with other ►receptor tyrosine kinases and ►integrins, which may be important in regulating the therapeutic response of human tumors. Several studies have shown that Ron can interact with the epidermal growth factor

receptor (►EGFR). Moreover, treatment of one receptor can modulate the response of an interacting receptor. Targeting this interaction in cell lines results in the modulation of cellular responses including cell scattering and tumor regression. In addition to EGFR, Ron is also co-expressed with the Met receptor in bladder, breast and liver cancers, and this co-expression has been linked with poor prognosis. Combined, these studies suggest that targeting multiple receptor tyrosine kinases in these cancers might prove more effective than targeting one alone.

Although there are currently no ►clinical trials being done selectively targeting the Ron receptor, there are several ongoing clinical trials with compounds that target Met, and which cross-react with Ron. Initial data from these trials suggests that the compounds are clinically well tolerable, and show an impressive anti-tumor activity in patients with a broad range of metastatic tumor types who have failed prior treatment. In total, the data outlined suggests that Ron may be a critical factor in tumorigenesis and that inhibition of Ron, either alone or in combination therapy, may prove to be beneficial in the treatment of high-risk cancer patients.

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ROS

Definition

Reactive Oxygen Species; Chemical species formed by the chemical or biochemical reduction of molecular oxygen, usually by normal metabolic processes in the cell, including energy metabolism. These consist of the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). These oxygen species are reactive, and can react with DNA bases to cause mutations in the cells.

►Hepatic Ethanol Metabolism
►Reactive Oxygen Species

ROS Accumulation

Definition

►Reactive oxygen species (ROS) includes oxygen ions, free radicals, and peroxides. The accumulation of ROS levels can result in damage to cell structures.

►DNA Damage
►Oxidative DNA Damage

ROS Scavenger

Definition

A chemical substance added to a mixture in order to remove ►reactive oxygen species.

Rothmund-Thomson Syndrome

Definition

An autosomal ►recessive genetic disease characterized by distinctive skin abnormalities (poililoderma congenitale), juvenile cataracts, skeletal abnormalities, endocrine abnormalities, abnormal growth, and an increased propensity to develop malignant tumors.

►Bone Tumors

RT-PCR

Definition

Reverse transcriptase polymerase *c*hain *r*eaction; synonym Reverse transcription ►PCR; molecular biological technique for the in-vitro enzymatic amplification of

defined RNA sequences from as few as one copy to visually detectable levels. In a first step, RNA is transcribed into complementary DNA (cDNA) by the enzymatic activity of reverse transcriptase; the cDNA is amplified in between two oligonucleotides complementary to the ends of the region to be analyzed by the enzymatic activity of a heat stable DNA polymerase; the oligonucleotides serve as sequence specific anchors that prime the synthesis of complementary DNA strands. Serial amplification is achieved by repeated cycles of high temperature DNA denaturation separating complementary strands, annealing of oligonucleotide primers to the single stranded DNA daughter strands and de-novo DNA synthesis from precursor trinucleotides by the polymerase.

Rule of Five

Definition

Is a set of four parameters that should not be exceeded if a drug is to be orally active. The 5 refers to parameter cutoff values; MWT $\leq$ 500; Log $P \leq 5$; H bond donors $\leq$5; H bond donors $\leq$10.

►ADMET Screen

Runx1

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Synonyms

AML1; acute myeloid leukemia 1; CBFA2; Core binding factor A2; PEBP2αB; polyomavirus enhancer binding protein 2αB

Definition

The RUNX1/CBFβ heterodimeric transcription factor regulates the expression of genes required for ►hematopoiesis and is the one of the most frequent targets of chromosomal translocations associated with human leukemia.

Characteristics

Expression and Regulation

RUNX1 was originally isolated from ►acute myeloid leukemia patient samples containing the ►t(8;21) chromosomal translocation. In addition to RUNX1, mammals also express RUNX2 and RUNX3. All three RUNX proteins share homology with the *Drosophila* Runt protein and contain the 128 amino acid Runt homology domain (RHD), which mediates both DNA binding and interaction with the core binding factor β (CBFβ) protein. Through this interaction, CBFβ stabilizes RUNX-DNA binding and protects the RUNX subunit from degradation via ►ubiquitination.

RUNX1 maps to chromosome 21, spans 260 kb and contains 11 exons, with transcription proceeding from telomere to centromere along the chromosome. The expression of *RUNX* family members is highly regulated at the levels of transcription, splicing, and translation. Transcription is initiated from two distinct promoters, P1 and P2, with the resulting transcripts being processed into numerous alternatively spliced mRNAs. These transcripts can subsequently be translated either by cap-dependent or cap-independent mechanisms. RUNX activity is also tightly controlled by post-translational modifications, including phosphorylation, acetylation and ubiquitination. The multiple isoforms of RUNX1 range in size from 20 to 52 kDa. RUNX proteins localize to the nucleus and are differentially expressed in a tissue- and developmental-specific manner.

RUNX1 binds the target DNA sequence, TTG/cGGT, found in the promoters of numerous genes required for normal hematopoiesis, including myeloperoxidase, interleukin 3, the T-cell receptors (TCR), and the B cell receptors. Depending on its associated protein partners, RUNX1 can act either as a transcriptional activator or as a transcriptional repressor. Although RUNX1 binding sites alone are not sufficient for robust activation of target genes, RUNX1 can cooperate with additional factors such as ►c-Myb, ►ETS family members, and ►C/EBPα, whose binding sites lie near the consensus RUNX1 binding sites, to enhance transcription. Additionally, RUNX1 can recruit various transcriptional co-activators such as ►p300/CBP, ALY, and Yes-associated protein (YAP) to stimulate the expression of target genes.

RUNX1 also contains several domains that mediate transcriptional repression. These repression domains associate with known transcriptional co-repressors, such as mSin3A and TLE, the mammalian homologue of the *Drosophila* protein, Groucho. RUNX1 can also bind ►histone deacetylases (HDACs) and the histone methyltransferase, SUV39H1, suggesting that ►chromatin modifications are an essential feature of RUNX1-mediated transcriptional repression.

Gene knockout experiments in mice have confirmed that deletion of *Runx1* is lethal during mouse embryonic

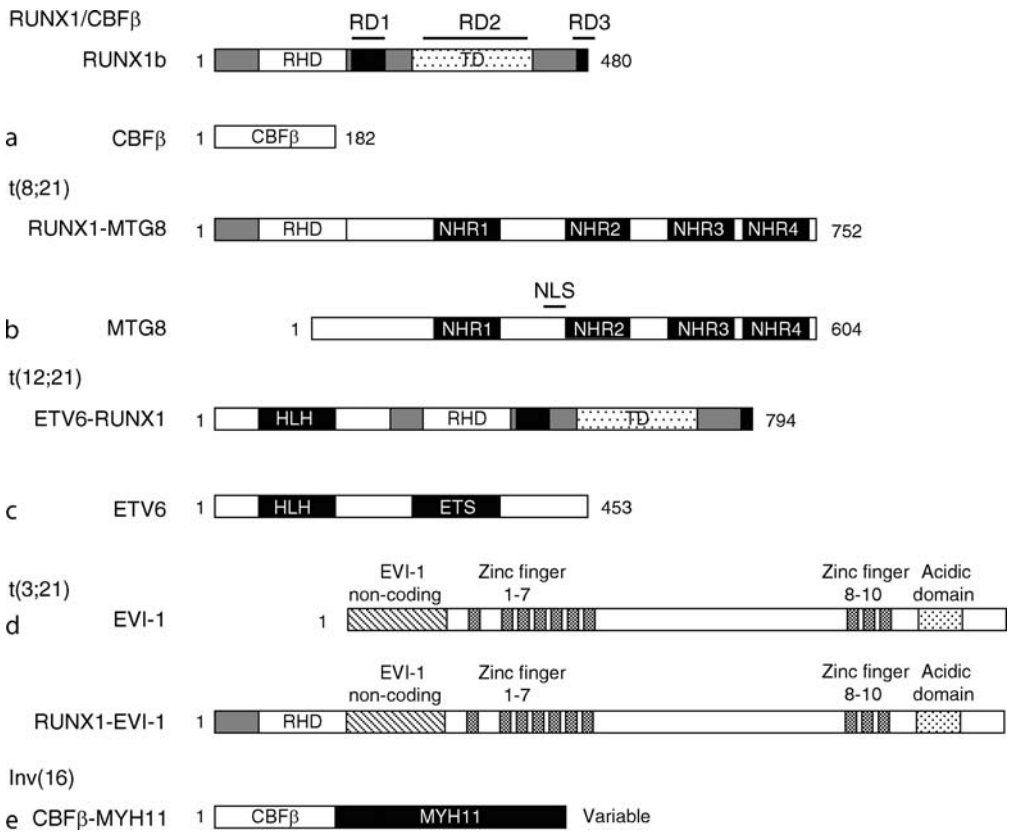
development. Although mice lacking the *Runx1* gene appear morphologically normal, they display severe central nervous hemorrhaging and die *in utero*, secondary to a complete absence of definitive hematopoiesis. A similar phenotype was observed in mice lacking *CBFβ,* suggesting that RUNX1 and CBFβ function together in the regulation of hematopoiesis.

RUNX1 in Leukemia

►Leukemia arises when the normal processes regulating the proliferation, differentiation, and survival of blood cells are disrupted. ►Chromosomal translocations have been found in up to 65% of acute leukemias. The RUNX1/CBFβ transcription factor is disrupted by at least 18 different chromosomal translocations, and is the most frequent target of translocations associated with the pathogenesis of acute leukemia. The t(8;21), t(12;21), t(3;21), and inv(16) chromosomal translocations target ►AML-1/ETO/CBFβ/TEL (Fig. 1).

The t(8;21) chromosomal translocation, which accounts for 15% of all cases of ►AML, fuses *RUNX1*, including the RHD, to a nearly full-length *Myeloid Translocation Gene on chromosome 8 (MTG8;* also known as *ETO, RUNX1T1 or CBFA2T1*). The resulting fusion protein, RUNX1-MTG8 (also known as AML1-ETO), retains the ability to bind RUNX1 target sequences on DNA to repress RUNX1-regulated genes. This ability of RUNX1-MTG8 to repress RUNX1 target genes requires the MTG8 portion of the fusion protein.

MTG8 is the founding member of a gene family that also includes *MTG16* (also known as *ETO2* or *CBFA2T3*) on chromosome 16 and *MTGR1* (also known as *CBFA2T2*) on chromosome 20. Like *MTG8, MTG16* is fused to RUNX1 by the t(16;21), a



Runx1. Figure 1 Schematic diagram of representative chromosomal translocations targeting RUNX1/CBFβ. (a) RUNX1b is shown as the predominant RUNX isoform. The Runt homology domain (RHD), transactivation domain (TD), and repression domains (RD1–3) are indicated. CBFβ is shown. (b) The RUNX1-MTG8 fusion protein, resulting from the t(8;21) translocation is shown. MTG8 contains four Nervy homology regions, NHR1–4. The MTG8 nuclear localization signal (NLS) is marked. (c) ETV6-RUNX1, generated by the t(12;21) translocation, is shown. ETV6 contains a helix-loop-helix domain and ETS DNA binding domain. (d) The RUNX1-EVI-1 fusion protein results from the t(3;21) translocation. EVI-1 contains two sets of DNA binding zinc finger domains, a proline-rich central domain, and an acidic carboxy-terminal domain. (e) The inversion 16 translocation, inv(16), is shown, in which CBFβ is fused to the smooth muscle myosin heavy chain. The length of the MYH11 portion varies in distinct fusion proteins and is thus indicated as variable.

translocation associated with therapy-related cases of AML. Though *MTGR1* is not associated with a chromosomal translocation, it is often deleted in ►myelodysplastic syndrome (MDS). Furthermore, MTGR1 was initially identified through its ability to bind the RUNX1-MTG8 protein.

The MTG proteins are homologous to the *Drosophila* Nervy protein. Like Nervy, the MTG proteins contain four highly conserved domains, called Nervy homology regions 1–4 (NHR1-4). Though the MTG proteins are unable to bind DNA directly, the NHR1 domain mediates the interaction of MTG proteins with various DNA-binding proteins, including Gfi1, Gfi1b, TAL1/SCL, TCFs, PLZF, Ldb1, and BCL6. The ability of the MTG proteins to interact with these various regulators of hematopoiesis suggests that the MTG family plays a key role in normal blood cell development. The remaining domains of the MTG proteins, including NHR domains 2–4, mediate interactions between MTG family members as well as associations with various transcriptional co-repressors, including mSin3A, N-CoR, and HDACs. Thus, the main functions of MTG proteins stem from their ability to nucleate the assembly of transcriptional co-repressor complexes.

The RUNX1-MTG8 fusion protein alters the expression of genes normally regulated by RUNX1 or MTG8. For example, by recruiting transcriptional co-repressor proteins that normally bind MTG8 to RUNX1 binding sites, RUNX1-MTG8 acts as a dominant repressor of RUNX1 target genes, including p^{14ARF} and ►Neurofibromatosis 1. RUNX1-MTG8 may also be able to recruit this transcriptional co-repressor machinery away from promoters normally regulated by MTG8, thereby indirectly stimulating expression of these genes. For example, RUNX1-MTG8 stimulates transcriptional activation of the TCF-dependent reporter plasmid, TOP-FLASH, in a manner that requires the co-repressor binding domains of the fusion protein.

When introduced into a mouse model, RUNX1-MTG8 alone is not sufficient to cause leukemia. Instead, the fusion protein causes increased ►self-renewal of adult hematopoietic progenitor cells and impairs hematopoietic differentiation. Thus, it appears that additional secondary mutations are required for development of full-blown leukemia. Consistent with this idea, clinical studies have demonstrated the presence of additional mutations affecting tyrosine kinase signal transduction pathways in patients carrying the t(8;21) chromosomal translocation. Thus, by creating an imbalance in the normal processes of hematopoietic ►stem cell self-renewal and differentiation of the various hematopoietic lineages, the main role of RUNX1-MTG8 in the pathogenesis of ►AML may be to expand the hematopoietic stem cell population and impair oncogenic checkpoints, which allows the accumulation of secondary mutations leading to the development of fully malignant disease.

Another chromosomal translocation affecting *RUNX1* is the t(12;21) translocation that occurs in 25% of pediatric cases of B cell ►acute lymphoblastic leukemia (ALL). The t(12;21) fuses the ETS family transcriptional regulator ►ETV6 (also known as TEL) with RUNX1, leading to the fusion protein ETV6-RUNX1 (also known as TEL-AML1). ETV6 contains a helix-loop-helix (HLH) oligomerization domain and Ets DNA binding domain. The ETV6-RUNX1 fusion protein contains almost all of ETV6, except for the Ets domain, fused to a nearly full-length RUNX1. Similarly to RUNX1-MTG8, ETV6-RUNX1 can still bind DNA through the RHD of RUNX1 and is able to bind transcriptional co-repressors including ►HDACs and mSin3A through the ETV6 and RUNX1 portions, respectively. Thus, ETV6-RUNX1 also acts as a dominant repressor of RUNX1 target genes. Mice expressing the ETV6-RUNX1 fusion protein show altered lymphopoiesis, but fail to develop leukemia, again suggesting that additional mutations are required for the development of fully malignant disease.

RUNX1/*CBFβ* is also involved in an inversion or chromosomal translocation event with chromosome 16. The result is a chimeric mRNA that fuses most of *CBFβ* to a smooth muscle myosin heavy chain gene, *MYH11*. The inv(16) fusion protein retains the ability to bind RUNX1 through the *C*BFβ portion, while the MYH11 portion promotes oligomerization and contains a cryptic transcriptional repression domain.

The mechanism by which the inv(16) fusion protein mediates leukemogenesis is the subject of debate. The initial model proposed that the inv(16) promotes leukemogenesis through cytoplasmic sequestration of RUNX1. This model is based on the observation that CBFβ-MYH11 co-localizes with RUNX1 in the cytoplasm upon over-expression in fibroblasts. Thus, by limiting the amount of RUNX1 in the nucleus, the fusion protein may negatively regulate RUNX1 activity. The second model proposes that the CBFβ-MYH11 fusion protein acts similarly to the t(8;21) and t(12;21) fusion proteins to dominantly repress RUNX1-regulated genes. This model is based on the observation that the fusion protein is able to bind various transcriptional co-repressor proteins, such as mSin3A and HDAC8, through its MYH11 domain and actually cooperates with RUNX1 to repress RUNX1-regulated genes. This model is consistent with the finding that RUNX1 is mostly nuclear in myeloid cells expressing the inv(16), and that expression of RUNX1 cooperates with the inv(16) to induce AML. Moreover, conditional deletion of *Runx1* does not promote leukemogenesis.

Like RUNX1-MTG8, the inv(16) fusion protein is not sufficient to cause leukemia on its own and instead requires cooperating mutations for the development of disease. For example, mice expressing inv(16) alone fail to develop leukemia after a long latency period but

readily develop disease following treatment with a mutagenic agent such as ►ENU or upon expression of the fusion protein on an *ARF*-deficient background.

RUNX1 is also involved in a translocation with chromosome 3 in therapy-related ►AML, ►MDS, and ►CML ►blast crisis. The t(3;21) results from a breakpoint between the RHD and the transactivation domain of RUNX1, causing fusion of *RUNX1* with a full-length *Evi-1* gene on chromosome 3. The Evi-1 portion of the fusion protein interacts with the transcriptional co-repressor C-terminal binding protein (CtBP). Thus, RUNX1-EVI1 may affect the expression of RUNX1 target genes through the aberrant recruitment of co-repressor complexes. Furthermore, the fusion protein also exhibits higher affinity for CBFβ, which may contribute to its dominant negative effects on RUNX1 activity. Like EVI1, the RUNX1-EVI1 fusion protein also retains the ability to induce ►AP-1 activity and repress ►TGF-β signaling, which may further contribute to its leukemogenic properties.

RUNX1 activity is also affected by point mutations or gene dosage effects. For example, point mutations in *RUNX1* have been identified in approximately 10% of sporadic cases of AML. Most of these mutations occur in the RHD of the protein and thus disrupt the ability of RUNX1 to bind DNA. Gene ►amplification of RUNX1 has also been detected in some cases of pediatric ALL, suggesting that increased dosage of RUNX1 may contribute to leukemogenesis.

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Runx-1

Definition

Synonym ►Runx1.

RUNX1/CBFA2T1

►Runx1
►Chromosomal Translocation t(8;21)

RUNX1/MDS1/EVI1

►Runx1
►AME Transcription Factor

RUNX1/RUNX1T1

►Runx1
►Chromosomal Translocation t(8;21)

S1P

Definition

Sphingosine-1-phosphate, an anti-apoptotic, angiogenic sphingolipid. A pro-proliferative sphingolipid that counteracts ceramide effects.

►Sphingolipid Metabolism

S-9

Definition

Is a cell free fraction derived from a chopped up liver that performs mostly ►phase II metabolism on a drug.

►ADMET Screen

S-Phase

Definition

Short for synthesis phase, S-phase is a period during the ►cell cycle where DNA synthesis or replication occurs. The cell cycle is divided into four phases: G1 (Gap1), S (synthesis), G2 (Gap 2) and M (mitosis). Origins of replication fire once per cell cycle allowing the entire genome to be replicated in S-phase.

►S-Phase Damage-Sensing Checkpoints
►Flow Cytometry

S-Phase Checkpoint

Definition

The ►cell cycle checkpoint that monitors cell cycle progression, and decreases the rate of DNA synthesis following DNA damage.

►BACH1 Helicase

S-Phase Damage-Sensing Checkpoints

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Definition

Cell-cycle checkpoints function by sensing DNA damage and transmitting a signal that results in arrest at defined stages of the cycle. The ►S-phase "checkpoint" halts cells during the DNA synthetic period in response to DNA damage (►DNA Damage Responses) and is somewhat different from other checkpoints in two ways. First, in the classic paradigm, cell-cycle progression is halted at various *discrete* points in the cycle (e.g., in G_1, at the G_2/M transition, and during mitosis), and cells are usually arrested for many hours prior to re-entry into the cycle. In contrast, the S-phase damage-sensing pathway arrests cell-cycle progression for only an hour or so and seemingly at any position within the S-phase, therefore, it is difficult to think of it as a checkpoint *per se* (although we will refer to it as such in this review). As more is learned about the

molecular basis of all of the checkpoints, it is becoming clear that each may have multiple targets. Thus, the underlying strategies for mobilizing these vital damage-sensing pathways may turn out to have many similarities.

A second possible difference is that the classic G_1, G_2/M, and mitotic checkpoints are thought to serve an anticipatory or surveillance function by arresting cells *prior to* critical processes such as DNA replication or mitosis, both of which are capable of converting potentially repairable damage into catastrophic lesions, such as double-strand breaks or chromosome non-disjunction. However, there is little evidence to suggest that the classic S-phase damage-sensing pathway has an anticipatory function, and it is formally possible that the arrest of DNA synthesis in response to damage results from a competition for *trans*-acting factors between multiple DNA-templated responses (repair, transcription, and replication).

Characteristics

At what point(s) in S-phase does the S-phase checkpoint operate? It has been known for decades that when exponentially growing cells are subjected to ionizing radiation, DNA synthesis is rapidly and significantly inhibited. Compelling evidence has been obtained that damage-induced inhibition of DNA synthesis represents a *bona fide* damage-sensing signal transduction pathway, as opposed to a simple arrest of replication forks by damage in the template (i.e., a *cis*-acting mechanism). For example, a dose of 5 Gy of gamma radiation results in a single-strand break every 25 replicons on average, yet inhibits overall DNA synthesis by ~50%. Therefore, it has been presumed for years that the S-phase checkpoint is a global cellular response that functions in *trans* and inhibits initiation of DNA replication as opposed to chain elongation. Indeed, there is direct experimental evidence from studies on mammalian viruses that doses of ionizing radiation too small to damage individual viral templates nevertheless down-regulate viral replication, suggesting a *trans*-regulatory mechanism. Results from three different experimental systems also provide direct evidence that the S-phase checkpoint down-regulates DNA synthesis by inhibiting initiation ►Radiation Sensitivity. Alkaline sucrose gradient analysis of pulse-labeled DNA showed that ionizing radiation inhibits the appearance and maturation of the smallest nascent fragments to a much greater extent than the maturation of larger fragments. Secondly, DNA fiber autoradiographic analysis of the changes in the pattern of replication after irradiation suggested that initiation is preferentially inhibited. Thirdly, a ►2-dimensional gel replicon mapping approach that can distinguish between initiation and elongation of DNA synthesis, showed clearly that ionizing radiation preferentially down-regulates initiation in a defined chromosomal replicon (the amplified, early-firing dihydrofolate reductase domain). To determine whether damage-induced inhibition of initiation also occurs at origins that fire later in the S period, advantage was taken of the naturally amplified ribosomal gene cluster, in which a restriction site polymorphism distinguishes between early- and late-firing rDNA replicons. The 2-D gel replicon mapping approach showed that both the early- and late-firing origins were inhibited by ionizing radiation. By extension, it is likely that all origins in mammalian chromosomes will be inhibited in response to DNA damage, regardless of when they are activated.

An interesting recent observation suggests that there may be more than one checkpoint or pathway responsible for the overall inhibition of the rate of DNA synthesis that occurs when ionizing radiation is delivered to an asynchronous culture of growing cells. When cells are synchronized and replicate cultures are irradiated at hourly intervals during S-phase, the subsequent inhibition of DNA synthesis never exceeds more than about 25% at any time in the S-period, whereas DNA damage delivered to an asynchronous population results in 50% or greater inhibition of the overall rate of DNA replication. By careful fluorescence-activated flow sorter analysis of gamma-irradiated log cultures, it was possible to demonstrate the existence of a population of late G_1 cells whose entry into S-phase was prevented for several hours. Since this potentially new checkpoint was uncovered in CHO cells, which are deficient in p53 activity ►p53 Family, it is distinct from the well-known p53-mediated G_1 checkpoint. This pathway is therefore analogous to a G_1/S checkpoint that has been described in *Saccharomyces cerevisiae*, which acts between the cell cycle steps affected by the DBF4 and CDC7 gene products.

It is possible that down-regulation of entry into the S-phase in mammalian cells (i.e., at the G_1/S transition) functions by inhibiting the earliest S-phase origins and is therefore mechanistically related to damage-induced inhibition of late-firing origins (i.e., does not differ from the S-phase checkpoint *per se*). Since irradiation delays the entire S-period for several hours (i.e., it effectively repositions the S-phase along the cell-cycle time axis), it would have to be argued that late-firing origins cannot fire until early-firing origins have done so. Interestingly, however, cells arrested near the G_1/S boundary in either mimosine or aphidicolin (both effective inhibitors of chain elongation) are resistant to radiation-induced inhibition of DNA synthesis, lending weight to the argument that there is a unique damage-sensing pathway that operates at the G/$_1$S transition *prior to* origin firing. Since the events preceding initiation of DNA synthesis in mammalian cells are only now being characterized, it will be some time before the molecular nature of this potential new checkpoint is uncovered.

What proteins are involved in the S-phase damage-sensing checkpoint? The ataxia telangiectasia mutated (►ATM) (►ATM Protein) and the ATM Rad3-related (ATR) protein kinases are critical checkpoint factors in human cells. Both are members of the phosphatidylinositol 3 kinase-related family of protein kinases (PI3K) that play a major role in sensing damage and triggering repair of DNA lesions in mammalian cells. ATM and ATR are preferentially activated by different forms of DNA damage. ATR is activated following induction of DNA adducts by UV or by chemical crosslinking agents, whereas ATM is activated in response to double strand DNA breaks induced by irradiation, etc.

The ATM gene was cloned in 1995. Cells derived from patients with ►ataxia telangiectasia (AT) fail to inhibit DNA synthesis in response to irradiation. This phenomenon was termed ►radiation-resistant DNA synthesis (RDS), which reflects a failure of intra S-phase checkpoint control. ATM is thought to play a central role in the cellular responses (including the S-phase checkpoint) to DNA double strand breaks (DSBs) and other genotoxic stresses. ATM exists as a catalytically inactive dimer in the absence of DNA damage. In response to DNA damage, especially DSBs, ATM undergoes rapid autophosphorylation on serine 1981, resulting in dissociation of the inactive dimers to yield active monomers. However, when the 1987 site was mutated (the mouse equivalent of serine 1981) and expressed in mice as the sole ATM species, the mutated ATM kinase was activated by radiation, suggesting that an alternative mode for stimulation of the ATM kinase must exist. The fact that several different serine/threonine phosphatases type 2 (PPP2) alpha isoform and type 5 (PPP5) regulate ATM activity, raises the question whether additional phosphorylation sites exist in the ATM protein that regulate its activity. In addition to DNA damage, chromatin remodeling molecules are also able to induce rapid activation of ATM/ATR in the absence of detectable DNA strand breakage. The activation of ATM/ATR by DNA strand breakage might thus be mediated, at least in part, by a change in chromatin structure.

In 2000, the ►Seckel syndrome (an autosomal recessive disorder characterized by developmental abnormalities including growth retardation and microcephaly) gene was localized to human chromosome 3q22.1-q24 (SCKL1) by homozygosity mapping. This gene has subsequently been identified as ATR. ATR is thought to be activated by both stalled replication forks and bulky adducts produced by agents such as Mitomycin C. ATR function in cell cycle checkpoint signaling pathways is dependent on an ATR-interacting protein known as ATRIP. ATRIP contains domains similar to the ATM-binding domain of NBS1, which is required for the interaction of ATRIP with ATR. ATR and ATRIP both localize to intranuclear foci after DNA damage or inhibition of replication. ATRIP is phosphorylated by and interacts with the single-stranded DNA (ssDNA) binding protein RPA. RPA coats ssDNA at replication forks to form an RPA–ssDNA complex in response to damage. Deletion of ATR in mice mediated by the Cre recombinase causes the loss of both ATR and ATRIP expression, the loss of DNA damage checkpoint responses, and cell death. Depletion of RPA inhibits ATR association with chromatin and abrogates the aphidicolin-induced DNA replication checkpoint. Moreover, RPA is also required for activation of ATR-mediated phosphorylation of Chk1 and Rad17.

►Nijmegan breakage syndrome (NBS) is a recessive genetic disorder characterized by elevated sensitivity to ionizing radiation, chromosome instability, and a high frequency of malignancies. Since cellular features partially overlap with those of ataxia-telangiectasia (A-T), NBS was long considered an A-T clinical variant. Nbs1, the product of the gene underlying the disease, contains three functional regions: a forkhead-associated (FHA) domain, a BRCA1 C-terminal (BRCT) domain at the N-terminus, an Mre11-binding region at the C-terminus, and several SQ motifs (consensus phosphorylation sites by ATM and ATR kinases) in the central region. Nbs1 forms a multimeric complex with hMre11/hRad50, which is recruited to the vicinity of DSBs sites by direct binding to histone H2AX via the FHA and BRCT domains of Nbs1. Recent studies suggest that the Mre11/Rad50/Nbs1 (MRN) complex, and possibly other proteins, play a role in the recruitment of ATM to the region of DNA strand breaks. In addition, BRCA1 (►BRCA1/BRCA2 Germline Mutations and ►Breast Cancer Risk) appears to be necessary for recruitment of ATM to damage foci. In response to IR, ATM fails to localize to damaged sites in the cells lacking full-length BRAC1 or full-length NBS1 despite the fact that ATM is activated. Once ATM is recruited to damage sites it can phosphorylate substrates at the break, including Nbs1, 53BP1, BRAC1, H2AX, and Smc1. Smc1 is a member of the ►Structural Maintenance of Chromosomes Protein (SMC) (family of proteins) and is thought to enhance the efficiency of DSB repair. Nbs1 therefore acts both as an upstream modulator of ATM as well as a downstream target of ATM.

In response to different genotoxic stresses, ATM and ATR transduce the damage signal to checkpoint control proteins to activate checkpoints. There are multiple parallel pathways underlying the intra S-phase checkpoint. The first pathway involves the ATM/ATR-Chk1/Chk2-Cdc25A-Cyclin E(A)/Cdk2-Cdc45 cascade, which links the upstream checkpoint kinases with the core cell cycle machinery in S-phase cells. DNA-damage-activated ATM and/or ATR phosphorylate Chk1 (serines 317 and 345) and Chk2 (threonine 68). Chk1 and Chk2 inhibit Cdc25A activity by phosphorylation of its serine

123 residue followed by ubiquitin-mediated degradation. Cdc25A is a dual-specificity phosphatase, removing inhibitory phosphates from threonine 14 and tyrosine 15 from Cdk2. Thus, Cdk2 is inhibited, resulting in decreased phosphorylation of Cdc45. This prevents its association with chromatin and thereby decreases initiation of DNA replication at origins.

Another S-phase pathway involves the ATM-Nbs1-Smc1 cascade. Following IR, the MRN/Smc1 complex is recruited to sites of DSBs. Once ATM is recruited to the complex, it phosphorylates Nbs1 on serine 278 and serine 343. Mutation of these residues results in an S-phase checkpoint defect following ionizing radiation. In addition, phosphorylation of the protein Smc1 on Serines 957/966 by ATM is also necessary for activation of the S-phase checkpoint. The ATM-Nbs1-Smc1 axis is clearly distinct from the ATM/Chk2/Cdc25A pathway.

►Fanconi anemia (FA) is a recessive genetic disease characterized by cellular hypersensitivity to DNA interstrand crosslinking agents, mild sensitivity to other genotoxic agents, and clinical features that overlap with NBS, A-T, and ATR-seckel syndrome. Phosphorylation of serine 222 of Fanconi anemia protein D2 (FANCD2) by ATM is dependent on Nbs1 and is necessary for the IR-activated S-phase checkpoint. Since the ATM-FANCD2 pathway apparently acts independently of SMC1 phosphorylation, its downstream effect is presently unknown.

What are the target(s) of the S-phase checkpoint? Precise regulation of the initiation of DNA synthesis is critical, since it ensures that the genome is replicated once and only once per cell cycle. Much of what we know about the regulation of initiation at origins comes from yeast. Autonomously replicating sequence (ARS) elements were first identified in a functional assay by virtue of their ability to support plasmid replication. In *Saccharomyces cerevisiae*, it is now known that a step-wise assembly of proteins onto origins precedes origin firing. In yeast, a multi-protein complex (the ►origin replication complex (ORC)) has been isolated and shown to interact with ARS elements. ORC is comprised of six proteins and remains bound to yeast origins throughout the cell cycle. During M-phase, the cdc6 protein is recruited to ORC ►Replication Licensing System, which, in turn, recruits ►minichromosome maintenance (MCM) proteins to form the pre-replicative complex (pre-RC) on origins. An S-phase cyclin is then thought to be necessary for the association of the cdc45 protein with the pre-RC just prior to the onset of DNA synthesis. Several other proteins such as Cdt1, Gins, and MCM10 play critical but poorly understood roles in effecting initiation at origins. Origin firing is likely down-regulated through MCM2 phosphorylation, which itself is regulated by checkpoint pathways signaling through cdc45.

What is the biological significance of the S-phase checkpoint? Cell-cycle checkpoints by definition provide an adaptive cellular advantage following genotoxic stress. In the case of the S-phase checkpoint, radiation sensitivity has been uncoupled from checkpoint function, arguing that the S-phase checkpoint may not always function to enhance survival following DNA damage. However, these experiments were performed on AT or AT-like cells in which it is not possible to exclude other mutations that are epistatic to the checkpoint defect. Furthermore, ATM likely directly influences DNA repair possibly *via* SMC1. Another possibility is that the S-phase checkpoint functions to maintain genomic stability. Irradiation produces single-strand DNA breaks, and replication through single-strand breaks has the potential to produce double-strand lesions that, if not correctly repaired, can lead to chromosomal rearrangements and carcinogenesis. An interesting further possibility is that the S-phase checkpoint functions in normal cell division to cope with endogenous oxidative or other genotoxic stresses. Future work will clarify these questions.

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S-Phase Kinase-associated Protein 2

►Ubiquitin Ligase SCF-Skp2

S-Phase Promoting Factor

Definition

SPF; Has cyclin-dependent kinase activity that is capable of inducing the initiation of replication.

►Replication Licensing System

S-100 Protein

Definition
Is a low molecular weight protein normally present in cells derived from the neural crest (Schwann cells, melanocytes, and glial cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells, and keratinocytes.

►Langerhans Cell Histiocytosis

SAAB

Definition
Selected and amplification binding (SAAB) is a combinatorial selection method that makes use of preliminary information regarding a ligand's binding specificity when designing a selection template. Unlike other combinatorial selection methods, SAAB does not require subcloning of selected templates but rather obtains binding specificity through direct sequencing of the selected template pool.

►Combinatorial Selection Methods

SAGE

Definition
Serial analysis of gene expression (SAGE) is a method that allows the analysis of overall gene expression patterns with digital analysis.

SAHA

Definition
Suberoylanilide hydroxamic acid, an ►inhibitor of histone deacetylases.

►Vorinostat

Salisburia Adiantifolia

►Ginkgo Biloba

Salisburia Macrophylla

►Ginkgo Biloba

Salivary Agglutinin (SAG, Human)

►Deleted in Malignant Brain Tumours 1

Salivary Gland Malignancies

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Definition
Salivary gland tumors are an uncommon and epidemiologically diverse group of tumors. Though rare, these tumors can pose a diagnostic and treatment challenge for the head and neck surgeon.

Characteristics
Epidemiology/Risk Factors
There are three major paired glands (parotid, submandibular and sublingual) and numerous minor salivary glands which line the mucosa of the upper aerodigestive tract. The normal function of these glands is to produce saliva, which serves as a lubricant, aids in early digestion, and is important to good dental hygiene.

The incidence of salivary gland tumors is ~5 per 100,000 population, which yields ~2,500 new cases in the United States per year. They comprise less than 0.5% of all malignancies and 3–5% of malignancies of the head and neck region. Most salivary gland tumors (70–80%) arise in the parotid gland and the majority of all salivary gland tumors are benign. Of the tumors originating from the parotid gland roughly 75% are benign, while only 25% are malignant. The percentage of malignant tumors increases for other locations (37–45% for submandibular glands, 75% for sublingual gland and slightly less than 50% for minor salivary glands). The most common malignant tumor of the parotid gland is mucoepidermoid ►carcinoma, while in the submandibular gland it is adenoid cystic carcinoma. Although the most common malignancy of the minor salivary glands is mucoepidermoid carcinoma as well, when polymorphous low-grade carcinoma is found, it is virtually pathognomonic for a minor salivary gland malignancy.

The etiology of salivary gland malignancies is unknown. Several studies have demonstrated an association between exposure to ionizing radiation and subsequent occurrence of salivary malignancy. Although both alcohol and tobacco use are thought to increase the risk of salivary gland malignancies, the current literature has conflicting data. Various nutritional factors and certain diets (including those high in fruits and vegetables and low in cholesterol) are thought to lower the risk of salivary gland cancer.

Staging/Classification

Salivary gland cancers constitute a heterogeneous group of tumors with distinct histology and very diverse clinical behavior.

The World Health Organization (►WHO) has described 23 different histologic types of salivary gland cancers (Table 1), most of which are exceedingly rare. Salivary gland malignancies can further be subdivided into high and low grade tumors. Low grade tumors usually exhibit a less aggressive behavior. These include acinic cell carcinoma, cystadenocarcinoma, polymorphous low-grade adenocarcinoma and other rare tumors. In contrast, high grade tumors display a significantly more aggressive behavior. The examples of high grade tumors include salivary duct carcinomas, anaplastic and undifferentiated carcinomas and carcinosarcomas. Mucoepidermoid carcinoma, adenocarcinoma and squamous cell carcinoma of salivary glands can present with various histologic grades (low, intermediate or high) and, thus, exhibit a variety of biological behaviors (Fig. 1). Certain tumors, such as adenoid cystic carcinoma, while low-grade, exhibit very aggressive behavior due to a propensity for perineural spread (Fig. 2).

Salivary Gland Malignancies. Table 1 WHO (World Health Organization) classification of salivary gland neoplasms

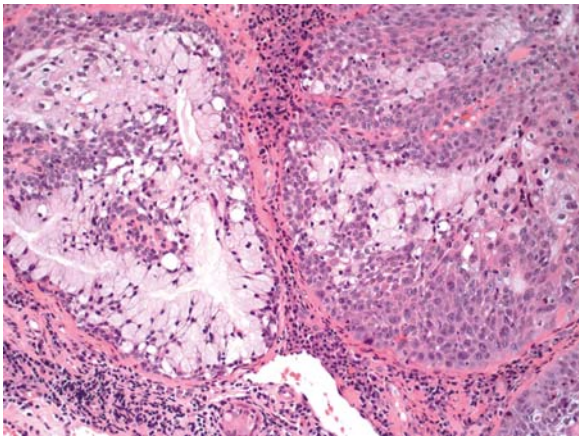
Malignant neoplasms	Benign neoplasms
Acinic cell carcinoma	Mixed tumor (pleomorphic adenoma)
Mucoepidermoid carcinoma	Warthin tumor
Adenoid cyctic carcinoma	Myoepithelioma
Polymorphous low-grade adenocarcinoma	Basal cell adenoma
Epithelial-myoepithelial carcinoma	Canalicular adenoma
Clear cell carcinoma, N.O.S	Oncocytoma
Basal cell adenocarcinoma	Cystadenoma
Malignant sebaceous tumours	Sialadenoma papilliferum
Cystadenocarcinoma	Inverted ductal papilloma
Low-grade cribriform cystadenocarcinoma	Intraductal papilloma
Mucinous adenocarcinoma	Lymphadenomas and sebaceous adenomas
Oncocytic carcinoma	
Salivary duct carcinoma	
Adenocarcinoma, N.O.S	
Myoepithelial carcinoma	
Carcinoma ex pleomorphic adenoma	
Carcinosarcoma	
Metastasizing pleomorphic adenoma	
Squamous cell carcinoma	
Small cell carcinoma	
Large cell carcinoma	
Lymphoepithelial carcinoma	
Sialoblastoma	

The current American Joint Committee on Cancer (►AJCC) staging system takes into account the extent of the primary tumor as well as regional and metastatic disease (Table 2).

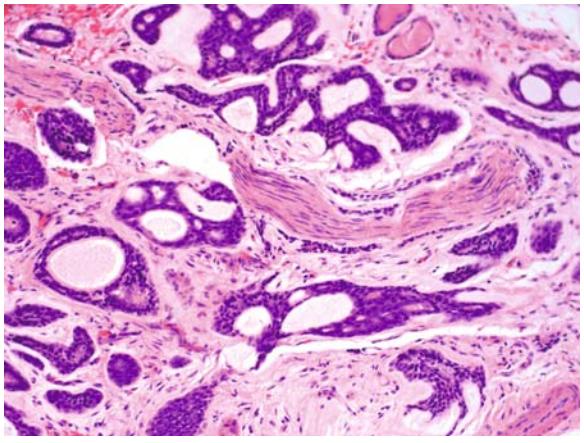
At this time, this staging system for salivary malignancies does not take into account histologic grading. Because of the wide variety of histological presentations and resultant diversity of clinical aggressiveness, the treatment of each individual cancer depends on both clinical staging and tumor histology.

Diagnosis

Detailed clinical history and comprehensive head and neck examination are critical in evaluation of salivary



Salivary Gland Malignancies. Figure 1 High grade mucoepidermoid carcinoma (hematoxylin and eosin-medium power magnification).



Salivary Gland Malignancies. Figure 2 Adenoid cystic carcinoma of parotid origin demonstrating perineural invasion (hematoxylin and eosin-medium power magnification).

gland neoplasms. Cross-sectional radiologic studies such as Computerized Tomography (►CT) or Magnetic Resonance Imaging (►MRI) are also very helpful. The real advantage of cross-sectional imaging in evaluating salivary gland masses, which primarily involve the parotid gland, is the ability to accurately reveal the location and extension of a tumor, its relationship to the facial nerve, and to assess for perineural tumor spread (Fig. 3). MRI is the modality of choice for evaluation of parotid masses and there are a few general rules that help in the differentiation of these lesions. A well-defined parotid mass of very high T2 signal is consistent with benign mixed tumor (BMT) or pleomorphic adenoma. Malignant tumors are, however, frequently also well-defined but they tend to be of lower T2 signal (Fig. 4). Positron Emission Tomography (►PET) scanning has been emerging as a new useful modality in evaluation of metastatic disease of head and neck cancers. Salivary gland tumors, however, belong to a small group of neoplasms for which PET (and PET-CT) cannot reliably distinguish malignant from benign masses, since some carcinomas do not show increased metabolic activity, while benign tumors such as BMT and especially Warthin's tumor may show very high Fluorine-18 2-Fluoro-2-Deoxy-D-Glucose (►FDG) uptake.

►Fine needle aspiration biopsy (FNAb) has been demonstrated to be a highly sensitive and specific tool in the evaluation of salivary gland neoplasms. In a review of 325 patients with salivary gland neoplasms investigated by FNAb and correlated with histologic review, the technique was found to be 85.5% sensitive and 99.5% specific. While the sensitivity and specificity may vary, especially in the smaller community hospitals, FNAb continues to help with treatment planning, providing the physician and patient adequate preparation for the necessary surgical procedure, potential for complications, and need for ►neck dissection or ►adjuvant therapy. CT-guided biopsy is the technique of choice for deep (such as in the deep parotid lobe) or poorly localized masses.

Treatment

Surgical resection has been the primary modality for treatment of salivary gland cancers. Commonly, it necessitates removal of the entire gland of origin often in conjunction with removal of involved or at risk lymph nodes (selective or modified neck dissection). In order to achieve a complete tumor extirpation without oncologic compromise, surrounding structures including cranial nerves, subcutaneous tissues, skin and muscles may require resection as well. Adjuvant radiation therapy (XRT) has long been used for achievement of better local and regional control of the disease. Frequently, it has been employed post-operatively for advanced T3 or T4 tumors. In addition, there is new limited data available in the current literature for support of adjuvant XRT even for earlier T1 and T2 cancers. In the recent retrospective review from Switzerland, the local recurrence rate was significantly lower for the group with combined treatment as compared to after surgery alone. Fast neutron-beam radiation and accelerated, hyperfractionated photon beam radiation have been reported to be more effective than conventional radiation therapy in treatment of more advanced lesions. Adjuvant XRT is typically recommended for all high grade tumors and for low grade tumors with unsatisfactory margins.

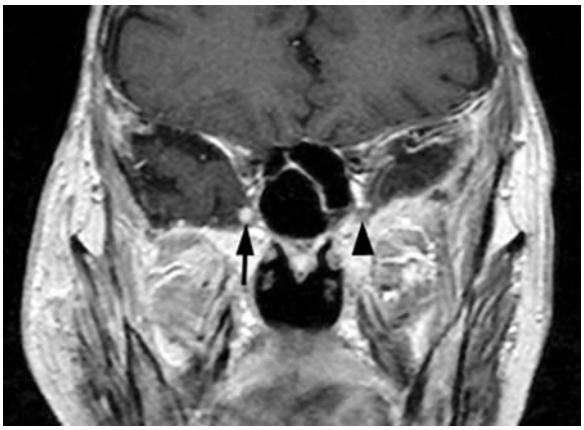
Salivary Gland Malignancies. Table 2 AJCC stage groupings for salivary gland malignancies

Stage I	$T_1N_0M_0$
Stage II	$T_2N_0M_0$
Stage III	$T_3N_0M_0$
	$T_1N_1\ M_0$
	$T_2N_1\ M_0$
	$T_3N_1\ M_0$
Stage IV	T_4 any N M_0
	Any T N_2 M_0
	Any T, any N, M_1
Primary tumor (T)	*Tx* Primary tumor cannot be assessed
	T0 No evidence of primary tumor
	T1 Tumor 2 cm or less in greatest dimension without extraparenchymal extension[a]
	T2 Tumor more than 2 cm but not more than 4 cm in greatest dimension without extraparenchymal extension[a]
	T3 Tumor more than 4 cm and tumor having extraparenchymal extension[a]
	T4a Tumor invades skin, mandible, ear canal, and facial nerve
	T4b Tumor invades skull base and pterygoid plates and encases carotid artery
Regional lymph nodes (N)	*Nx* Regional lymph nodes cannot be assessed
	N0 No regional lymph node metastasis
	N1 Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
	N2 Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension, or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
	N2a Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
	N2b Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
	N2c Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
	N3 Metastasis in a lymph node more than 6 cm in greatest dimension
Distant metastasis (M)	*Mx* Distant metastasis cannot be assessed
	M0 No distant metastasis
	M1 Distant metastasis

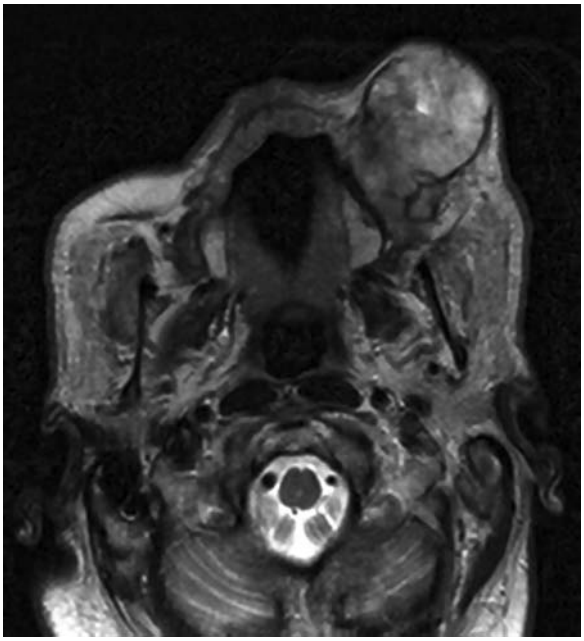
[a]Extraparenchymal extension is clinical or macroscopic evidence of invasion of soft tissues. Microscopic evidence alone does not constitute extraparenchymal extension for classification purposes.

There is also a continuing effort to identify more successful systemic therapies for salivary gland cancers. There are several current trials which employ conventional chemotherapeutic agents such as taxol, or cisplatin/carboplatin combined with gemcitabine. Other medical agents have also been tried with variable success in the treatment of salivary gland cancers, though certain histologic types, such as adenoid cystic carcinoma, have consistently demonstrated resistance to systemic therapy. However, new agents that target specific tumor molecules such as ►HER2/neu tyrosine kinase and epidermal growth factor receptor (EGFR) are under active investigation.

Overall, treatment of salivary gland cancers constitutes an important component of practice for head and neck surgeons. The wide variety of clinical behaviors usually requires a multidisciplinary team approach with cooperation between otolaryngology/head and neck surgeons, medical and radiation oncologists, oral and surgical pathologists, radiologists, oral/maxillofacial surgeons and dentists, speech pathologists, dieticians and other clinical support personnel.



Salivary Gland Malignancies. Figure 3 Post-contrast T1-weighted coronal MRI showing enhancement of the right maxillary nerve within the foramen rotundum (*arrow*), consistent with perineural tumor spread of an adenoid cystic carcinoma. There is no enhancement of the contralateral nerve (*arrowhead*).



Salivary Gland Malignancies. Figure 4 T2-weighted axial MRI showing large carcinoma ex-pleomorphic arising along Stenson's duct.

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Salmonella typhi and paratyphi

Definition

Salmonella typhi and *paratyphi* are parasites, serotypes of *Salmonella enterica*. If invasive, the infections by *Salmonella typhi* or *paratyphi* lead to the development of typhoid or paratyphoid fever. The organisms can be transmitted by the fecal-oral route: it is excreted by humans in feces and may be transmitted by contaminated water, food, or by person-to-person contact (with inadequate attention to personal hygiene).

►Gallbladder Cancer

Salt Intake

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Synonyms

Edible salt; Sodium chloride; Salting

Definition

The composition of salt is approximately 39.3% sodium and 60.7% chloride by molecular weight. Salt regulates the water content (fluid balance) of the body, and plays an essential role in homeostasis. The nutritional requirement of salt has been estimated to be 1.25 g per day for adults.

Characteristics

Salt intake varies substantially around the world, though in developed countries, typical diets include salt far in excess of requirements. In the INTERSALT study,

daily salt intake as determined by 24-h urinary sodium excretion ranged from 5 g in Trinidad and Tobago to 14 g in Tianjin, China. The average intakes were around 8–10 g per day in most western countries and 10–12 g per day in Japan and Korea.

Epidemiological studies have shown that ►nasopharyngeal carcinoma and ►gastric cancer are associated with high salt intake.

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma is a rare malignancy, with age-adjusted incidence rates of under 1 per 100,000 person-year for both men and women in most parts of the world. In certain geographical regions, however, the incidence of nasopharyngeal carcinoma is dramatically higher. The highest rates of nasopharyngeal carcinoma have been documented among Cantonese residing in the central region of Guangdong Province in Southern China and in Hong Kong (25–30 per 100,000 person-year).

John Ho first suggested in 1971 that Cantonese-style salted fish, a common item in the local diet and a popular weaning food, may be an etiological factor in nasopharyngeal carcinoma. Since then, a large number of case-control studies conducted in various populations residing in different parts of Asia [Hong Kong, Guangzhou (Canton) and Malaysia] have confirmed the association between age at first exposure to salted fish and this type of cancer. It has been estimated that >90% of nasopharyngeal carcinomas in Hong Kong, 50% of those in Guangzhou and >60% of those in Chinese people in Malaysia are due to childhood consumption of salted fish. Animal data have further strengthened the evidence for Cantonese-style salted fish as a nasopharyngeal carcinogen. For example, rats fed this human food developed cancer of the nasal cavity in a dose-dependent manner, though this cancer rarely occurs in this species. Based on these evidences, the relationship between Chinese-style salted fish and nasopharyngeal carcinoma was determined to be "convincing" by the WHO/FAO (World Health Organization/Food and Agriculture Organization) Expert Consultation on Diet.

However, it may be that salted fish has an oncogenic action not shared by salt alone. Experimental studies have shown that low levels of several nitrosamines/precursors and ►Epstein–Barr virus-activating substances exist in preserved salted fish. Some epidemiological studies have also indicated that nitrosamines and nitrates included in preserved food play a role in the development of nasopharyngeal carcinoma if such foods are consumed during childhood. On the other hand, the antibody of Epstein–Barr virus has been found in the sera of nasopharyngeal carcinoma patients, suggesting that this virus is associated with nasopharyngeal carcinoma as well as with Burkitt's lymphoma. The consumption of salted fish may lead to the activation of Epstein–Barr virus in the sera. Further investigations are needed to examine the mechanisms through which Chinese-style salted fish increases the incidence of nasopharyngeal carcinoma.

Gastric Cancer

The incidence of gastric cancer also differs by geographic location and ethnicity. In 2002, the age-standardized incidence rates (per 100,000) of gastric cancer in Japan were 62.0 in men and 26.1 in women, and these rates as well as those in Korea (69.7 in men and 26.8 in women) were among the highest in the world. In contrast, among the white population in western countries, the incidence rate was 7.4–12.8 in men and 3.4–6.6 in women, which is clearly far lower than that in Asian countries. In the INTERSALT study, median sodium levels were analyzed in relation to national gastric cancer mortality rates. For the 24 countries studied, the Pearson's correlation coefficient for gastric cancer mortality with sodium was 0.70 in men and 0.74 in women (both $P<0.001$).

Many, but not all, results of case-control studies have shown a positive association between gastric cancer and the intake of high-salt foods such as salted fish, cured meat, and salted vegetables, or the use of table salt. However, in prospective studies among Caucasian populations, the effect of high salt intake or salted foods on the incidence of gastric cancer remains controversial. Several studies have found no positive association. Only a cohort study on 120,852 Dutch subjects and 282 gastric cancer cases showed a weak significant association between salt intake and gastric cancer incidence, though there were no clear trends. In contrast, in the Japanese population, two prospective studies have identified a significant association between dietary salt intake and gastric cancer incidence: Tsugane et al. reported that in a total of 18,684 men and 20,381 women, the quintile category of salt intake and salted food was associated with a risk of gastric cancer in men during a 12-year follow-up. Another Japanese study also investigated the relationship between the amount of dietary salt intake and gastric cancer especially intestinal-type, taking into account the effects of other risk factors such as ►*Helicobacter pylori* infection and atrophic gastritis, finding that salt intake is an independent risk factor for the subsequent incidence of gastric cancer in Japanese. Several reasons for the negative findings in Caucasian studies are proposed. The amount of salt intake in Caucasians may be lower than the threshold for incident gastric cancer. Furthermore, it may also be that the salt intake in the negative studies was not estimated accurately by detailed food frequency questionnaires. Given the lack of definitive evidence in the prospective studies, the WHO/FAO Expert Consultation concluded that salt "probably" increases the risk of gastric cancer.

Several possible mechanisms of gastric carcinogenesis induced by a high-salt diet have been discussed in the literature. High dietary salt intake is believed to alter the viscosity of the protective mucous barrier, leading to mucosal damage in the stomach and making mucosal cells more susceptible to carcinogens in food. Moreover, intragastric high salt concentrations are known to cause mucosal damage and inflammation. Persistent inflammatory changes in the stomach may promote temporary cell proliferation and increase the rate of endogenous mutation. Infection with the bacterium ►*H. pylori* is an established risk factor for the development of gastric cancer. In earlier-mentioned latter Japanese ►epidemiological study, the effect of high salt intake on gastric carcinogenesis was strong in subjects who had both atrophic gastritis and *H. pylori* infection. The synergistic promoting effects of a high-salt diet and *H. pylori* infection on gastric carcinogenesis have also been observed in an experimental study using a gerbil model. On the other hand, atrophic gastritis is thought to be a precancerous lesion of gastric cancer. Thus, the synergistic promoting effects of a high-salt diet and *H. pylori* infection may appear particularly strongly when gastric atrophy is present. It is generally accepted that gastric adenocarcinoma, particularly of the intestinal type, arises through a multistep process originating with chronic gastritis, and progresses through stages of atrophy, intestinal metaplasia, and dysplasia, eventually resulting in carcinoma. Based on these results, it is more likely that a high intake of salt is involved primarily in the latter stages of multistep gastric carcinogenesis and thereby promotes gastric carcinogenicity because atrophic gastritis is one of the morphological hallmarks of these stages. These studies suggest that salt per se is associated with incident gastric cancer directly or indirectly.

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Salting

►Salt Intake

Sanctuary Site

Definition

Any location in the body that is poorly penetrated by drugs, for example, central nervous system, testes, ovary.

Sandwich ELISA

Definition

The technique of sandwich ELISA uses antibody bound to a surface to trap a protein by binding to one of its epitopes. The trapped protein is then detected by an enzyme-linked antibody specific for a different epitope on the protein's surface. This gives the assay a high degree of specificity.

SAPKα

►JNK Subfamily

SAPKβ

►JNK Subfamily

SAPKγ

►JNK Subfamily

Sarcoid

Definition
Locally aggressive fibroblastic skin tumor. It is associated to bovine papillomavirus type 2 infections in horses.

►Bovine Papillomavirus

L-Sarcolysin

Definition
►Melphalan

Sarcoma

Definition
Is a malignant tumor of the mesenchymal tissues, commonly the connective tissues, but includes muscle of all kinds and tendons and bones.

►Cardiac Tumors
►Gastrointestinal Stromal Tumor

SBP

Definition
Solitary bone plasmacytoma.

►Plasmacytoma

SCA1

Definition
Spinocerebellar ataxia type 1.

►Cajal Bodies

Scaffold Proteins

Definition
Synonym docking proteins; Coordinate the spatio-temporal activation of signaling pathways by assembling their individual components to a multi-protein complex or ►signalosome.

►B-Raf Signaling

Scatter Factor

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Synonyms
Hepatocyte growth factor; HGF

Definition
Scatter factor (SF), also known as hepatocyte growth factor (HGF), is a multifunctional cytokine that participates in various biologic processes, including: embryonic development (►morphogenesis), oncogenesis (tumor formation), ►angiogenesis (new blood vessel formation), and the regulation of ►apoptosis (programmed cell death). SF was originally characterized as a protein secreted by mesenchymal cells (e.g. fibroblasts) that disperses (or "scatters") contiguous sheets of epithelium and stimulates cell motility. HGF was identified as a serum-derived protein that stimulates the proliferation of adult rat hepatocytes. Subsequent studies revealed that SF and HGF are identical. HGF is the ligand of a ►tyrosine kinase receptor encoded by a ►proto-oncogene (c-►Met), HGF binding causes receptor activation.

Characteristics
SF is a heparin-binding glycoprotein composed of a 60 kD α-chain and a 30 kD β-chain. The α-chain is composed of an N-terminal hairpin loop, followed by four ►kringle domains (looped structures that mediate protein interactions). The β-chain resembles protein-degrading enzymes such as trypsin, but SF lacks protein-degrading activity due to two key amino acid substitutions at the catalytic center. The binding of heparin to SF modulates its biologic activity, protects

it from degradation and allows it to be stored in the extracellular matrix. Several shortened forms of SF containing only the N-terminal loop and the first ►kringle domain (NK1) or the first two kringle domains (NK2) are sufficient to bind with high affinity to the c-Met receptor. NK1 and NK2 are produced as a result of mRNA editing, and they can function as partial agonists or antagonists of SF. However, structure-function analyses indicate that the entire SF molecule, including the β-chain, is required to generate the full spectrum of SF's biologic activity. The human SF gene maps to the long arm of chromosome 7 (7q11.2–21).

SF is synthesized as a 728 amino acid precursor (preproSF) that is converted within the cell to its secreted form (proSF) by cleavage of a short segment (►signal sequence). However, the secreted proSF is not biologically active, and must undergo an internal cleavage within the linker region between the α-chain and β-chain. Cleavage of proSF occurs outside of the cell and results in the production of the mature, two-chain biologically active SF. Thus, the cleavage of proSF to SF is a potential control point for the regulation of SF activity. Several enzymes capable of cleaving and activating SF have been identified. These enzymes include the plasminogen activators (urokinase and tissue plasminogen activator), proteins that convert plasminogen – an enzyme that circulates in blood in inactive form – into its active form, plasmin. Plasmin is the major enzyme responsible for dissolving blood clots. Another enzyme capable of converting proSF into active SF is called "►HGF activator." HGF activator is a novel protein-degrading enzyme structurally related to a blood clotting factor (factor XII, or Hageman factor). HGF activator is itself produced in an inactive ("pro-enzyme") form. It may be activated by blood coagulation factors, such as thrombin. The physiologic processes that regulate SF activation have not been fully elucidated, but there is evidence to suggest that an enzymatic cascade that results in activation of HGF activator and then SF is triggered by tissue injury.

The SF Family

SF is not related to classic growth factors (e.g., ►fibroblast growth factor or ►platelet-derived growth factor), but is a member of the kringle domain protein family, which includes blood coagulation and fibrin-degrading enzymes (e.g., ►plasminogen, prothrombin, factor XII, urokinase, tissue plasminogen activator) and a ►macrophage-stimulating protein (MSP). Within this family, SF is most closely related to the plasminogen and MSP, with which it shares a similar αβ chain structure, a similar activation mechanism (i.e. cleavage between the α and β chains), and a high degree of amino acid sequence identity (38% and 50%, respectively). MSP was formerly called HGF-like protein and is the closest relative of SF. The MSP receptor, a tyrosine kinase receptor encoded by the Ron gene, is closely related to the SF receptor, but these two proteins do not cross-activate each other's receptor. MSP circulates in the bloodstream as an inactive profactor (proMSP) which, when activated, causes macrophages to become competent and to undergo chemotaxis and phagocytosis.

SF Receptor (The c-Met Proto-oncogene Product)

The MET proto-oncogene was (c-Met) originally discovered in rearranged form as a carcinogen-induced transforming oncogene (Tpr-Met) generated by a transposition between human chromosomes 1 and 7, resulting in fusion of a powerful promoter from chromosome 1 ("Transposed promoter region") to a portion of the c-Met proto-oncogene (at 7q21–31) that codes for the intracellular region of the receptor. The Tpr-met oncogene product is a membrane-bound tyrosine kinase that is constitutively active (i.e., does not require SF for activation). The full-length c-Met proto-oncogene encodes a growth factor receptor-like tyrosine kinase that consists of an extracellular SF-binding domain, a transmembrane domain, and an intracellular portion containing a kinase domain and sites that associate with various cytoplasmic signaling proteins.

The binding of SF to c-Met triggers a molecular events similar to those triggered by the interactions between classic growth factors and their receptors. The receptor undergoes a change in three-dimensional conformation, resulting in:

- Activation of the catalytic kinase domain
- Dimerization (association of two c-Met receptors)
- Cross-phosphorylation of the two receptors on multiple tyrosines
- Initiation of a signal cascade ("signal transduction") causing transfer of information from the cell surface to the nucleus. Understanding signal transduction from c-Met will provide the key to understanding SF's biologic actions.

Signal initiation involves the interaction of phosphorylated tyrosines internal to the kinase domain of the activated c-Met with regions known as ►SH2 domains (src-homology domain-2) of proteins that act as signaling intermediaries. Most c-Met signaling involves the interaction of these signaling intermediaries with a "multi-functional docking site" involving two tyrosine (Y) residues located at amino acids 1,349 and 1,356:1349YVHVXXX1356YVNV. This unique site associates with many signaling proteins, including phosphatidyl-inositol-3′-kinase (►PI3K), phospholipase C-γ, pp60c-src, c-Cbl, and the Grb2/Sos complex, which binds p21Ras. Amino acid sequences similar to the multi-functional docking site of c-Met are found in the two related receptors: Ron and c-Sea (a tyrosine kinase receptor whose ligand has not been identified). Similar sequences are not found in the receptors for

the epidermal growth factor, platelet-derived growth factor, or other factors.

The manner in which SF binding to c-Met can result in different physiologic consequences depending upon the cell type and context (see below) is just beginning to become unraveled. For example, it was recently found that SF-induced epithelial morphogenesis (i.e., the formation of a three-dimensional network of branching tubules) specifically requires association with c-Met at cell-cell junctions and phosphorylation of a protein known as Gab1 (the Grb2-associated binder). Grb2 binds to the tyrosine-1,356 site of c-Met via its SH2 domain, while another portion of the Grb2 protein (the SH3, or src-homology-3 domain) binds to Gab1. Gab1 is a member of the family of the "multi-substrate docking proteins," which includes IRS-1 (insulin-responsive substrate-1), a cytoplasmic protein that is a major mediator of the biologic effects of the insulin-like growth factor ►IGF-I.

Cellular and Molecular Regulation

SF Producer and Responder Cell Types

In vitro studies initially suggested that SF is produced predominantly by cells of mesenchymal (connective tissue) origin, including: fibroblasts, vascular smooth muscle, endothelial cells, glial cells, macrophages, activated lymphocytes, and other cell types. However, based on subsequent in vivo studies (immunohistochemistry and in situ hybridization), it is now apparent that a variety of epithelial cell types, including keratinocytes, mammary epithelial cells, and many carcinoma cells may also produce SF. For reasons not understood, cultured epithelial and carcinoma cells lose the ability to produce SF when placed in culture, although they often retain the c-Met receptor. A variety of cell types express the c-Met receptor and are biologically responsive to SF, including (but not limited to): keratinocytes, hepatocytes, mammary epithelium, vascular endothelial cells, melanocytes, glial cells, and the corresponding malignant cell types.

Regulation of SF Production

The complexity of the regulatory mechanisms for SF production is becoming increasingly apparent as the list of known and partially characterized factors that regulate SF production continues to grow. In addition to well-known pro-inflammatory (IL-1α, IL-1β, TNF-α) or anti-inflammatory (TGF-β) cytokines that enhance or inhibit production of SF by fibroblasts, a group of partially characterized scatter factor-inducing factors distinct from IL-1 and TNF stimulate SF expression in fibroblasts and other SF-producer cell types. SF-inducing factors are secreted by various carcinoma cell lines, and they appear in the serum of rats following a subtotal hepatectomy. When co-cultured with epithelial cells, fibroblasts cease to express SF mRNA and protein, again by a regulatory mechanism that has not been elucidated. Heparin and heparan sulfate proteoglycans, which are known to bind to SF, also appear to stimulate its production. However, these molecules may simply function to stabilize the SF protein and to prevent its degradation.

Biologic Responses Induced by SF and Their Regulation

The major biologic responses induced by SF fall into four broad categories:

- Motility
- Proliferation
- Morphogenesis
- Cell survival (or more properly, protection against apoptotic cell death)

The c-Met receptor can transduce each of these biologic functions. These biologic responses may overlap (e.g., morphogenesis involves a component of cell migration through extracellular matrix); and they appear to be determined by the extracellular environment and by cell-specific programs of differentiation. For example, Madin-Darby canine kidney (MDCK) epithelial cells cultured on flat surfaces are scattered, while cells cultured in collagen gels respond to SF by forming networks of branching tubules similar to those found in the kidney.

Activation of specific pathways for motility, proliferation, morphogenesis, and/or cell survival may be determined at the receptor level or more distally. The specific pathways that activate each of these processes are only now beginning to be dissected. For example, recent studies suggest that treatment of various epithelial and cancer cell types with SF induces resistance to DNA-damaging drugs and radiation by a process that involves the sequential activation of c-Met, phosphatidylinositol-3′-kinase, c-►Akt (protein kinase B). The latter is a serine/threonine kinase that functions to protect cells against apoptotic death.

The extracellular environment plays a major role in modulating the biologic responses to SF. Studies of SF-induced branching morphogenesis of MDCK epithelial cells provides clues as to how this modulation might occur. Thus, certain extracellular matrix molecules promote forward extension of tubules (collagen I, laminin), while others promote branching (heparan sulfate proteoglycans, collagen IV). TGF-β, a component of the extracellular matrix, inhibits the entire process of branching morphogenesis. The binding of matrix proteins to integrins activates intracellular signaling processes, including tyrosine phosphorylation; and SF may induce the expression of a specific set of integrins that allows the extracellular matrix to modulate intracellular signaling. There is evidence that the extracellular matrix may modulate c-Met signaling by inducing the phosphorylation and dephosphorylation of different sites on c-Met and other signaling proteins.

SF and c-Met Participate in Various Physiologic and Pathologic Processes

Development

An important role for SF in development was suggested by the finding that homozygous deletion of either SF or c-Met results in embryonic lethality in mice. Various studies implicate SF as a mediator of mesenchymal: epithelial signaling during embryogenesis. For example, during mouse development, the SF gene is expressed in mesenchymal cells, while the c-Met gene is expressed in adjacent epithelia. This pattern is observed in multiple developing organs, and appears to be regulated with great precision in space and time. Injection of SF into the developing chick embryo induces abnormalities of the neuraxis, indicating that inappropriate exposure to SF can interfere with normal development. In addition, several studies suggest that SF and c-Met can mediate the conversion of mesenchymal cells to an epithelial phenotype, as judged by its ability to induce morphologic alterations as well as the expression of epithelial specific markers (e.g., cytokeratins and epithelial-specific junctional proteins). Mesenchymal:epithelial interconversion is commonly observed during embryogenesis, further supporting a role for the SF-c-Met ligand-receptor pair in development.

Oncogenesis

Malignant cell transformation is mediated by the Tpr-Met oncogene, which encodes a truncated and constitutively active form of the c-Met receptor. This finding raises the possibility that SF-mediated over-stimulation of c-Met has similar consequences. The idea that SF could mediate tumorigenesis in vivo is suggested by several considerations. First, SF stimulates the motility, and invasiveness of a variety of carcinoma cell types in vitro. Secondly, SF is a potent inducer of angiogenesis (new blood vessel formation), a process considered to be essential for the continued growth of solid tumors. Finally, SF can overcome apoptosis (programmed cell death) of epithelial cells which is associated with detachment of cells from their substratum. Detachment of carcinoma cells from the underlying basement membrane is an early step in tumor invasion. Studies of experimental animal models and human clinical samples further support a role for SF in tumorigenesis. Overexpression of the SF and/or c-Met genes in a variety of cell types induces or further enhances the tumorigenic phenotype in vivo, by ►autocrine and/or ►paracrine mechanisms. In studies of human breast cancer, bladder cancer, gliomas, and other tumor types, significantly higher levels of SF and/or c-Met were observed in high grade invasive cancers than in low grade non-invasive cancers. And in a study of 258 primary invasive breast cancers, a high SF content in the tumor was strongly predictive of relapse and death. Finally, recent genetic-epidemiologic studies have strongly linked activating mutations of the c-Met gene to a specific type of kidney cancer: hereditary papillary renal carcinoma.

Angiogenesis

The formation of new blood vessels from pre-existing vessels, occurs extensively during normal development and tissue remodeling, but occurs only to a limited degree in normal adults. Physiologic ►angiogenesis in adults is observed transiently during wound healing, ovulation, and placental implantation. However, persistent and inappropriate angiogenesis contributes to certain pathologic processes, including chronic inflammatory diseases (e.g., rheumatoid arthritis) and cancer. SF induces an angiogenic phenotype in cultured vascular endothelial cells (i.e., stimulates endothelial cell proliferation, ►chemotactic ►migration, and capillary-like tube formation) and induces angiogenesis in vivo in several different experimental animal models. SF may contribute to angiogenesis in AIDS-related ►Kaposi sarcoma, a cytokine-dependent neoplasm associated with extensive endothelial cell proliferation and neovascularization. The observations that both SF content and tumor angiogenesis are powerful independent prognostic indicators for breast cancer suggest a role for SF as a tumor angiogenesis factor. However, a causal relationship between SF and tumor angiogenesis is not yet proven.

Clinical Relevance

The ability of SF (HGF) is to stimulate epithelial cell growth and morphogenesis, to induce angiogenesis, and to protect cells against toxins or environmental conditions that induce apoptosis suggests a variety of potential therapeutic applications for SF. In this regard there are a number of experimental animal (mouse and rat) studies suggesting that administration of the SF protein can block or ameliorate acute and chronic injury to the liver, kidney, or lung. For example, administration of SF prevents or reduces the loss of renal function caused by toxins such as HgCl2 or ►cisplatin in mice. In a rat model, infusion of SF blocked or slowed the development of liver fibrosis and cirrhosis; and SF blocked the development of pulmonary fibrosis induced by bleomycin in the mouse lung. These findings suggest that SF is potentially clinically useful as a hepatotrophic factor for repair of liver damage or as a renotrophic factor for repair of kidney damage. The use of SF in humans presents significant challenges, such as the delivery of sufficient quantities of the factor to the sites where it is needed, in view of its short biologic half-life. Nevertheless, if reliable methods of protein or gene delivery can be developed, there may be a variety of clinical applications for SF to promote organ repair and regeneration.

Several studies suggest that the administration of other angiogenic factors (►VEGF and basic ►FGF) is

potentially useful in restoring the blood supply and preventing tissue damage, in animal models in which coronary or peripheral blood vessels are ligated in order to produce acute ischemic injury. Because of its ability to induce angiogenesis as well as its ability to protect a variety of different cell types against apoptotic cell death, it is anticipated that SF may be particularly advantageous in these settings. There is also the potential for development of small molecule inhibitors of the c-Met receptor that could be used to treat pathologic processes driven by excessive production of SF, such as certain cancers. Such inhibitors have already been developed to inhibit the function of the ►EGF receptor. A criticism of this approach is that tumor growth is driven by a variety of cytokines, growth factors, and angiogenic factors, so that the specific inhibition of a single receptor type will be insufficient to halt tumor growth. Nonetheless, combinations of receptor inhibitors may be clinically useful, and there may be situations in which inhibition of a single receptor is sufficient to inhibit tumor growth due to synergistic interactions among growth factors and cytokines.

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Scavenge Free Radicals

Definition

Work by reducing the action of free radicals and thus preventing cell damage by compounds like polyphenols.

►Polyphenols

Scavenger Cells

Definition

Any of a diverse group of cells that have the capacity to engulf and destroy foreign material, dead tissues, or other cells.

SCF

Definition

Skp1/Culin/F-box protein is a multi subunit complex that ubiquitylates proteins that are phosphorylated at specific sequences known as phosphodegrons. This ►ubiquitination targets proteins to degradation.

►Anoxia and Cancer
►Kit/Stem Cell Factor Receptor in Oncogenesis

SCF-R

►Kit/Stem Cell Factor Receptor in Oncogenesis

scFv

Definition

Single chain fragment of variable region antibody composed of the V_H and V_L immunoglobulin regions joined via a flexible peptide linker, e.g., $(Gly_4Ser)_3$ peptide.

►Chimeric T Cell Receptors

SCH52365

►Temozolomide

Schirrous (archaic)

►Desmoplasia

Schistosomas Hematobium

Definition

A trematode species from freshwater snails whose cercaria imbed in human organs such as the urinary bladder, causing chronic inflammation and eventually cancer.

►Urothelial Carcinoma

Schwannoma-derived Growth Factor

►Amphiregulin

Schwannomin

►Merlin

SCID

Definition

Severe combine immunodeficiency disease.

►Childhood Cancer

Scid Mice

Definition

Severe Combined Immunodeficiency; A mutant strain of mice that has a mutation in DNA-dependent protein kinase gene. Due to the mutation, scid mice show the defective DNA rearrangement of antigen receptor genes and thus develop no functional ►T cells and ►B cells.

►Rap1 and Sipa-1

SCID Mouse Model

Definition

Severe combined immunodeficiency mice, due to a genetic autosomal recessive mutation (SCID), have no functional specific humoral and cellular immune system. Therefore, these animals serve as hosts for many xenograft tumor models.

Scintigraphy

Definition

An imaging modality that involves injection and detection of radioactive. A nuclear medicine technique based on the administration of radioactive substances that are selectively taken-up by specific cells or organs thus giving a two-dimensional imaging based on radiopharmaceutical distribution.

SCLC

Definition

►Small Cell Lung Carcinoma; Lung Cancer.

Sclerosing Angiogenic Tumor

►Hepatic Epithelioid Hemangioendothelioma

Sclerosing Endothelial Tumor

►Hepatic Epithelioid Hemangioendothelioma

Sclerosing Epithelioid Angiosarcoma

►Hepatic Epithelioid Hemangioendothelioma

Sclerosing Interstitial Vascular Sarcoma

►Hepatic Epithelioid Hemangioendothelioma

Screening Toxicity

Definition

Represent informal toxicity studies, usually in very limited numbers of small rodents, often mice, to test small amounts of novel chemical agents to provide an approximate idea of their toxicity and their appropriateness for attempting to develop as a new drug. They may also be used to screen series of chemicals with a view of selecting the least toxic.

►Preclinical Testing

SDF-1

Definition

The ►chemokine SDF-1 has been isolated from stromal fibroblasts and interacts as a ligand with the ►G-protein coupled receptor CXCR4.

►Trefoil Factors

SDF-1α

►Chemokine Receptor CXCR4

SDGF

►Amphiregulin

SEA Domain

Definition

Acronym for **S**ea urchin sperm protein, **E**nterokinase, **A**grin. Is an extracellular domain of a number of proteins and associated with ►glycosylation. The common module might regulate or assist binding to neighbouring carbohydrate moieties.

Seckel Syndrome

Definition

Is an autosomal recessive disorder characterized by dwarfism, intrauterine growth retardation, bird-like facies, microcephaly, and mental retardation. ATR-Seckel Syndrome has been found in patients with mutations in ►ATR. Cell lines from patients with Seckel syndrome, who are normal for ATR, show defective ATR signaling, suggesting that Seckel syndrome can be caused by mutations in other components of the ATR pathway.

►S-Phase Damage-Sensing Checkpoints

Second Malignant Neoplasm (SMN)

►Second Primary Tumors

Second Messenger

Definition

Refers to chemical signals created within a cell in response to a hormonal stimulus from outside. Common second messengers are Calcium ions, cyclic AMP, or inositol triphosphate. Second messengers act in a cell to orchestrate metabolic or gene expression responses to a particular stimulus.

►Relaxin

Second Primary Cancers

►Radiation-Induced Sarcomas After Radiotherapy
►Second Primary Tumors

Second Primary Malignancy (SPM)

►Second Primary Tumors

Second Primary Tumors

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Synonyms

Second primary cancer SPC; Second primary malignancy SPM; Second malignant neoplasm SMN

Definition

A ►second primary tumor, usually malignant, is defined as a histologically and/or clonally distinct tumor diagnosis that develops after the first cancer.

Introduction

Advances in the management of cancer have substantially improved the prognosis of cancer patients, including their life expectancy. Currently, more than 60% of cancer patients can survive over 5 years post-treatment; cancer survivors constitute 3.5% of those living in the West. However, advances in cancer management are not without adverse consequences, and long-term complications following successful treatment have become increasingly important. Second primary tumor is one of the most devastating issues facing long-term cancer survivors.

Second or higher order primary tumors account for ~6–10% of all cancer diagnoses, and are the fifth most commonly diagnosed cancer in Western countries. For instance, ~100,000 of the 1.4 million new cancers diagnosed in 2006 in the United States are second cancers. Although the aim of the management of second primary tumors assimilates that of the initially diagnosed malignancies, the diagnosis and treatment of SPTs are far more complex, especially when the effects resulting from previous therapy limit such management.

Causal and Risk Factors

Patients with one form of malignancy are known to have a higher risk of developing a second cancer.

The underlying causes and risk determinants are multifactorial; however, the majorities are related to shared etiology such as immunodeficiency, cigarette smoking and alcohol abuse. Secondly, cancer patients may possess a certain genetic predisposition to cancer. In other words, hereditary susceptibility can explain the development of some second primary cancers. Furthermore, cancer diagnosis and treatment modalities such as surgery, chemotherapy, and radiotherapy are all known carcinogenic factors. In addition, the incidence of second primary malignancy is significantly higher when the first cancer is diagnosed and treatment is delivered at early age. Survivors of ►childhood cancers and adulthood cancers have, when compared with the general population, an increased risk of 3–6 and 1.5–2 times, respectively of developing a second primary tumor. The reasons for this age effect are unknown, but might be related to a higher rate of cell proliferation, susceptibility of tissue to the carcinogenic effect of treatment, and a longer period of follow-up.

Shared Etiologic Exposure

Most second primary tumor cases are associated with some forms of shared etiologic exposure. Cancer inducing factors can be life-style or work-related. For example, ►asbestos exposure is associated with ►lung cancer and mesothelioma, while endocrine and dietary factors are associated with the development of breast, endometrial, and ovarian cancers. Patients with

S

acquired immunodeficiency syndrome (AIDS) or on immune suppression following an organ transplant are prone to develop ►non-Hodgkin lymphoma, cervical cancer, and certain forms for skin cancer, i.e. Kaposi sarcoma. Among all of the cancer-inducing risk factors, cigarette smoking is the most common and important. ►Tobacco related cancers include non-small cell and small cell lung cancers, ►squamous cell carcinomas of head and neck area, pancreatic cancer, ►bladder cancer, kidney cancer, and cancer of uterine cervix.

It has been well demonstrated that patients who have been successfully treated for squamous cell cancer of head and neck area have an increased risk of 20% in 5 years of developing a second primary cancer in the upper aerodigestive track. Studies have also demonstrated that lung cancer patients with an extended history of tobacco use are at increased risk of a second lung carcinoma, cancers of the larynx/hypopharynx, bladder, and pancreas. This indicates that tobacco use is a common causative factor.

Excessive alcohol consumption is related to increased incidence of ►esophageal cancer, liver cancer, and squamous cell carcinomas of head and neck areas. Recent clinical studies have shown that alcohol may serve as a risk factor for adenocarcinoma of the breast and ►colon. In addition, synergistic effects exist for second malignancies of the upper aerodigestive track for cigarette smoking and alcohol use.

The underlying mechanism of the development of second or higher order primary tumors due to exposure to a shared etiology is not well understood. It has been suggested that repeated carcinogenic exposure may cause "field cancerization," where the mucosa accumulates genetic alterations resulting in the induction of multiple, independent malignant lesions. This theory is now widely accepted and supported by research results in molecular biology.

Genetic Predisposition

Some types of cancers are related to hereditary genetic abnormalities, and cancer syndromes are most recognizable in familial settings. Numerous cancer syndromes and their related genetic alterations have been identified. Table 1 illustrates several more commonly diagnosed cancer syndromes, the related genetic alterations, and associated malignancies.

With the development of molecular testing technology, more genetic alterations associated with increased risk of cancer or familial cancer syndrome will be discovered.

Therapy-Related Risk Factors

As the survival of cancer patients improves following treatment, identification of long-term treatment-related complications becomes critical. Treatment-related second primary tumor is one of the most devastating side effects. Commonly utilized cancer treatment modalities including surgery, chemotherapy, and radiotherapy are all known to cause cancer. Hence, some second primary tumors are iatrogenic.

Although uncommon, cancer surgery is related to some kinds of second primary cancers. For example, radical mastectomy has been the standard primary treatment of locally advanced invasive ►breast cancer, one of the most commonly diagnosed cancers in female patients. Surgical manipulation of the axilla in order to access the axillary lymph node during mastectomy, a necessary surgical procedure, is related to the swelling of the ipsilateral arm due to lymph fluid accumulation (i.e. lymphedema) secondary to the damage to the lymph vessels. The incidence of this complication increases with the addition of radiotherapy. Arm lymphedema has been recognized as the causative factor of angiosarcoma of the affected arm or forearm, known as Stewart-Treves syndrome, named after two American physicians who initially described the cause–effect relation. Improvement in the surgical techniques involved in breast cancer treatment, including the use of lumpectomy with more limited axillar surgery and sentinel node dissection, has significantly reduced the occurrence of angiosarcoma. Some forms of colon and stomach cancer have also been described as

Second Primary Tumors. Table 1 Cancer syndromes and their associated malignancies

Cancer syndrome	Affected gene(s)	Associated malignancies
Li Fraumeni syndrome	►p53	Breast, soft-tissue, bone (osteosarcoma), brain, adrenal gland, hematological (leukemia), etc.
Hereditary non-polyposis colorectal cancer (HNPCC)	MLH1, MSH2, MSH6, CHEK2	Colon, small intestine, endometrium, ovary, stomach, kidney, ureter, etc.
Ataxia-telangiectasia	ATM	Breast
Fanconi anemia	FANC A-G, L	Breast, ovary, head and neck, cervix, esophagus, liver, brain, etc.
Retinoblastoma (RB)	RB-1	Eye, orbit, bone (osteosarcoma), soft-tissue, melanoma, brain, etc.

surgery-induced cancers. However, second primary cancer is not being considered as a substantial long-term clinical complication of cancer surgery.

Unlike surgery, chemotherapy and radiotherapy are more likely to induce iatrogenic malignancies. Second primary tumors caused by chemotherapy and radiotherapy usually develop after a lengthy latent post-treatment period. This delay, which can take years, can be partly due to the time needed for the ►DNA damage that is responsible for malignant transformation of cells to develop.

The carcinogenic effect of numerous chemotherapeutic agents has been repeatedly illustrated. The significance of chemotherapy in the development of second primary cancer was first studied in detail in a group of patients who were successfully treated for ►Hodgkin disease (HD). Researchers discovered that the cumulative risk of second primary cancer diagnosis following HD treatment during childhood was 7% at 15 years after diagnosis; the two common diagnoses of second cancer are leukemia (2.8%) and non-Hodgkin's lymphoma (1.1%). Further investigations revealed that certain agents used for HD chemotherapy, such as ►alkylating agents, were directly responsible for the increased incidence of leukemia in that group of patients. Other studies aimed at related chemotherapy drugs and cancer showed that many other agents such as ►cisplatin or cyclophosphamide are cancer-causing to various degrees.

A radiotherapy-induced primary second cancer is suggested when, after a long latency period of a minimum of 5 years but typically 15–20 years, a tumor with a histology type differs from the first tumor develops within (or close to) the irradiated field. Ionizing radiation can cause most types of cancer. The commonly known radiation-induced second malignancies include: bone or soft-tissue sarcoma, breast cancer, thyroid cancer, leukemia, and brain tumors. Some of the radiation-induced tumors, such as bone or soft-tissue sarcoma, can be more aggressive than their sporadic counterparts and respond poorly to conventional therapy.

The incidence of radiation-induced second primary tumor is related to the radiation dose exposure, but reversely related to the age of radiation exposure, i.e. the risk is higher among young irradiated patients. The underlying mechanism of this phenomenon is unknown, but it is suggested that younger patients have a larger number of dividing stem cells that are more susceptible to radiation-induced malignant transformation.

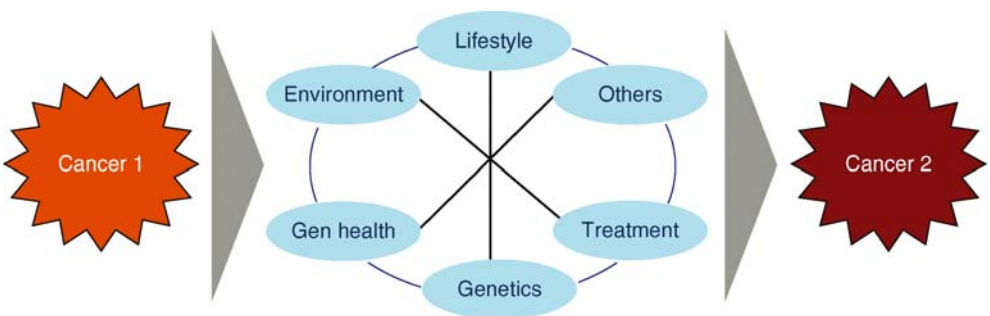
These risk factors are all associated with second or higher order primary tumors; the mechanisms of SPT development are more likely caused by more than one factor. In other words, many influences and the interactions between these influences may contribute to the development of the second primary malignancies. For example, patients with hereditary retinoblastoma, who have undergone radiation therapy, when compared to sporadic RB cases, are more susceptible to second primary cancer(s) in the irradiated field. Figure 1 illustrates the multifactorial nature of the risk factors contributing to second primary tumors.

It is important to emphasize that although surgery, chemotherapy, and radiation are related to second malignant neoplasm development, the benefits of these treatments usually surpass this complication. Therefore, second primary tumor is rarely a contraindicatory factor associated with these therapeutic modalities.

Diagnosis

Since long-term follow-up is required for most successfully treated cancer patients, the majority of second primary tumors are diagnosed during routine post-treatment follow-ups.

The diagnostic procedures utilized for second primary cancer diagnoses are no different from those used for primary malignancies. Usually, a careful history and physical examination, together with



Second Primary Tumors. Figure 1 Risk factors for second or higher order primary tumors.

pertinent laboratory and imaging tests are required for the diagnosis of any neoplasm, followed by pathological confirmation. The diagnostic process of any second primary cancer is similar; however, as local and/or distant recurrences are common following cancer treatment, especially those with more advanced disease, differentiation between a second malignant neoplasm with local relapse or distant metastasis is crucial, as the treatments of the two entities differ significantly.

The diagnosis of a second primary cancer can be confirmed if the newly discovered malignancy is histologically different from the previous diagnosis. When features of the tumor and/or cells appear similar under the microscope, a second primary malignancy is likely when premalignant changes such as carcinoma *in situ* are found within or near the specimen, as these premalignant changes indicate a *de novo* development process of a malignancy.

However, when the morphologies of the two diseases are identical under the microscope, and features that may suggest a second primary disease are lacking, further investigation with more advanced diagnostic techniques such as molecular diagnostics will be required in order to determine the difference. It is well accepted that all cancers are initiated from a single clone of transformed cell; therefore, pathological test results that can rule out monoclonal nature from the previous cancer diagnosis will be required for a diagnosis of cancer recurrence.

Treatment

The pathological diagnosis, patient's health status and preference, as well as available medical resources determine the management strategy of any type of malignancy. However, although the aim and principle of second primary tumor treatment assimilate those of the original disease, treatment of a SPT is complicated and usually restricted by previous therapy, and any associated complications.

Whenever possible, curative treatment should be considered for second primary tumors. Like their original counterparts, treatment modalities utilized for second primary cancer can include surgery, chemotherapy, radiation therapy, and/or other less commonly used methods such as immunotherapy, hormonal therapy, and molecular targeted therapy.

Usually, surgery and medical therapy can be repeatedly utilized as long as the patient's overall health condition, performance status and functionality allows. However, if the location of the SPT is within the previously irradiated field, the utilization of radiation therapy, especially external beam photon therapy, is stringently restricted by previous radiation dose, the tolerance dose of irradiated tissues and/or organs, as well as the period between the original and subsequent radiotherapy. The dose limitation of a specific tissue and organ is commonly described as TD 5/5 (the total dose equivalent that may cause severe long-term complications with 5% probability within 5 years), and any treatment dose equivalent above such limitation may cause a high incidence of substantial toxicities. Unfortunately, curative treatment for a second primary tumor usually necessitates high dose radiation that exceeds TD 5/5. Hence, patients should be provided with and fully understand the consequence of such treatment prior to re-irradiation.

The success of second primary tumor treatment depends on ►early detection. In a certain sense, early diagnosis is more critical for a second primary tumor because under many circumstances only limited treatment can be utilized due to limitations set by any previous cancer therapy. Therefore, one cannot over emphasize the importance of close follow-up after any cancer treatment.

Prevention

The development of any effective prevention methods of second primary cancers inevitably needs a thorough understanding of the mechanisms of its development. As multiple risks usually exist for the development of a second primary tumor, and the incidence of a particular type of second primary cancer is relatively low, there is currently no effective preventive approach to the development of second primary cancer. Nevertheless, some confirmed common etiologies of cancer development exist. For example, cigarette smoking has been proven as a strong risk factor of second primary cancers of aerodigestive track for patients with lung cancer or squamous cell carcinoma of the head and neck areas. Studies have shown that after successful treatment of their initial malignancies, those patients that stop smoking can reduce the relative risk of developing a second primary cancer. Therefore, while additional information and knowledge are needed in order to develop more effective prevention strategies for second primary tumors, termination of exposure to known risk factors such as tobacco and alcohol serve as the most effective preventative modality of second primary tumors.

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Second-Tier/Third-Tier Lymph Node

Definition

After passing through a sentinel node, lymph fluid moves subsequently to second-tier and third-tier nodes.

►Sentinel Lymph Nodes

Secondary Amenorrhea

Definition

Absence of menstruation for >3 months.

►Menopausal Symptoms After Breast Cancer Therapy

Secondary Cancer Prevention

Definition

Prevention of tumor progression through early diagnosis of asymptomatic tumors and/or treatments against preneoplastic or early neoplastic lesions.

►Immunoprevention of Cancer

Secondary Cancer Site

Definition

The discontinuous end location where a metastatic cancer cell grows after it spreads from its primary site. For metastatic breast cancer to the bones, the secondary site is the bones.

►Metastatic Colonization

Secondary Dissemination

Definition

Dissemination following initial treatment.

►Leptomeningeal Dissemination

Secondary Hypogonadism

Definition

The failure of testicular or ovarian function caused by decreased gonadal stimulation from the pituitary/hypothalamic unit (as opposed to primary failure of the gonad itself).

►Prolactin

Secondary Lymphedema

Definition

A condition in which the lymphatic system is physically damaged by surgery (such as to remove lymph nodes) or injury, resulting in interrupted flow of lymph and blocked drainage of fluid from tissues, skin thickening and adipose tissue accumulation.

►Lymphangiogenesis

Secondary Lymphoid Organs

Definition

Secondary (or "peripheral") lymphoid organs include the lymph nodes, spleen and mucosa-associated lymphoid tissue (MALT). The secondary lymphoid structures function to survey all entering or circulating antigen and to mobilize an immune response against antigen upon its discovery. This is in contrast to the primary (or "central") lymphoid organs. These are the sites where the cells of the immune system are produced including the bone marrow and the thymus required for T cell maturation.

►DNA Vaccination

Secondary Metabolites

►Natural Products

Secondary Structure

Definition
The linear arrangement or topology of α-helices and β-sheets with respect to the amino acid sequence.

►Structural Biology

Secondary Tumor

►Metastasis

Secondhand Tobacco Smoke

Definition
A composite of sidestream smoke and the smoke exhaled by a smoker. Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material which diffuses through the paper.

►Tobacco Carcinogenesis

Secosteroid

Definition
Steroid molecule with a broken B-ring (C9 and C10).

►Vitamin D

Secretagogues

►Gut Peptides

γ-Secretase

Definition
A protease that is responsible for the processing of the cytoplasmic domains of several type I membrane proteins, such as the amyloid beta-protein precursor (in Alzheimer disease), Notch, and CD44. The cytoplasmic regions are translocated to the nucleus where they function as transcription factors.

►CD44

Secreted Phosphoprotein 1

►Osteopontin

Secreted Protein Acidic and Rich in Cysteine

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Synonyms
Osteonectin; BM-40; SPARC

Definition
Secreted Protein Acidic and Rich in Cysteine (SPARC) belongs to a group of non-structural proteins of the extracellular matrix (ECM), termed ►matricellular proteins, which modulate interactions between cells and their environment. It is expressed in developing and remodeling tissues, and regulates cell attachment, deposition of ECM, matrix mineralization, and ►angiogenesis.

Characteristics
SPARC is a counter-adhesive protein that induces cell rounding, inhibits cell spreading and mediates focal adhesion disassembly and the reorganization of actin stress fibers. In several cell types, it delays cell cycle in G_1 phase. It is the main non-collagenous component of bone, where binding of SPARC to

collagen can induce deposition of calcium. However, inhibition of ►hydroxylapatite crystallization suggests that SPARC prevents matrix mineralization rather than inducing it. SPARC also regulates the production, assembly, and organization of ECM, and accordingly, it is expressed at high levels in developing tissues during embryogenesis and in remodeling adult tissues, such as gut, ovary, testis, mammary gland, bone and in healing wounds. SPARC is predominantly secreted by non-epithelial cells including endothelial and smooth muscle cells, osteoblasts and platelets. Fibroblasts and ►macrophages express SPARC in healing wounds, where it is also released by platelet degranulation.

Gene Organization

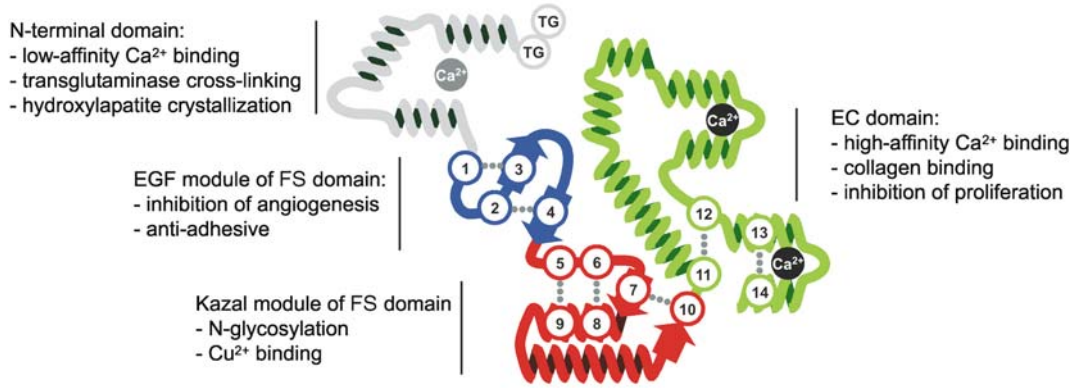
The SPARC gene spans 25.9 kb on human chromosome 5q31.3-q32. Its first non-coding exon is separated from the following coding exons by a large 10.6 kb intron. Exon 10 contains the entire 3′ non-translated region. This gene organization and the transcription start site are highly conserved in vertebrates. The major human 2.2 kb transcript contains an open reading frame from nucleotides 84 to 992, followed by 1,137 bp of 3′ non-translated region. The less abundant 3.0 kb transcript has an identical coding region, but utilizes a downstream polyadenylation signal. The SPARC promoter lacks TATA and CAAT boxes, but contains a GGA box 1 between nucleotides −51 and −120 which drives transcription. A GGA box 2 is located between nucleotides −131 and −165. Both boxes are mainly composed of a repetitive GGA motif. They are positive regulators of promoter activity, while a 10 bp spacer of low purine content has a negative impact.

Three ►Retinoid X/►Vitamin D receptor binding sites, immediately followed by an ►E-box in GGA box 1, may be responsible for stimulation of SPARC expression by ►retinoic acid and dexamethasone. Histone acetylation is essential for the activation of transcription by ►retinoid receptors. Sodium butyrate, an inhibitor of ►histone deacetylase, has been shown to stimulate SPARC expression. The activation of SPARC transcription by cAMP is consistent with several cAMP consensus sequences found in the promoter region and in the first intron. The presence of a heat shock element correlates with heat- and stress-induction of SPARC expression. Jun and ATF family members repress SPARC transcription in fibroblasts, but stimulate expression in epithelial cells, although this regulation appears to be indirect.

Protein Structure

The structure of SPARC is highly conserved among different species. All cysteine residues are found at identical positions from human to nematode, indicating that protein folding is critical for function. After the 17 amino acid signaling peptide is removed, the human protein is secreted as a polypeptide of 286 residues with a calculated mass of 32 kDa, which migrates at 40–43 kDa on SDS-PAGE due to ►glycosylation and possibly other posttranslational modifications.

The protein is divided into three distinct domains (Fig. 1). The loosely structured N-terminal acidic domain (Ala^{1}-Glu^{52}) has an α-helical character, which depends on binding several Ca^{2+} molecules with low affinity. Clusters of glutamic acid in this region resemble the γ-carboxyglutamic acid (Gla) domain of vitamin K-dependent proteins of the blood clotting system. Gln^{3} and Gln^{4} are the amine acceptor residues for tissue ►transglutaminase-catalyzed crosslinking, of which SPARC is the major substrate in maturing cartilage. This domain also mediates the interaction with hydroxylapatite, indicating that it may play role in



Secreted Protein Acidic and Rich in Cysteine. Figure 1 Domain organization of SPARC. Schematic representation of the structure of each SPARC domain. All domains and modules are shown in individual colors. Ca^{2+} ions bound with high and low affinity are respectively in black and grey. The numbers in the circles represent cysteines and TG denotes transglutaminase cross-linking acceptor residues. The N-glycosylation site is located immediately after cysteine 7.

stabilization of connective tissue. A peptide from this domain inhibits endothelial cell spreading.

The follistatin-like (FS) domain (Asn^{53}-Pro^{137}) consists of two loosely linked modules. The N-terminal epidermal growth factor-like (EGF) module is a highly twisted β-hairpin with two disulfide bonds. Peptides from the EGF module have anti-►angiogenic properties. They inhibit endothelial cell ►migration and proliferation, and promote the disassembly of focal adhesions of endothelial cells. An adjacent small hydrophobic core of mixed α/β structures stabilized by three disulfide bonds has high structural homology to the serine proteases of the Kazal family. A peptide from the Kazal module, which contains the copper-binding sequence KGHK, can stimulate angiogenesis. This module carries an N-linked carbohydrate at Asn^{99}, which causes a ~2 kDa shift in electrophoretic mobility. SPARC glycosylation is tissue-specific and has functional significance. Bone SPARC, which carries high-mannose and biantennary glycans, binds collagens with high affinity. Platelet and recombinant SPARC have bi- and triantennary structures and bind collagens with low affinity. SPARC glycosylation is apparently sensitive to neoplastic transformation, as tumor-produced SPARC has a unique hybrid pattern of glycosylation.

The C-terminal extracellular ►calcium-binding (EC) domain (Cys^{138}-Ile^{286}) folds into a compact globular and predominantly α-helical structure. It carries a canonical pair of ►EF-hand motifs, which bind Ca^{2+} with high affinity. A peptide corresponding to the second EF-hand motif inhibits endothelial cell proliferation. A long α-helix at the N-terminus of the EC domain and a short loop, which connects two EF-hand motifs, form a collagen-binding site.

This domain organization is shared by a family of proteins that have common FS/EC domain pairs, but different acidic N-termini and sometimes C-termini. Other members of the family include hevin, tsc36, testicans and SMOCs.

Functional Properties

SPARC binds and modulates the activities of structural and soluble components of the ECM, often in a Ca^{2+}-dependent manner. Ca^{2+} enhances binding to collagens, but inhibits interaction with multimeric vitronectin. In the α-granules, the major storage organelle for platelet-secreted proteins, SPARC binds to ►thrombospondin with high affinity in the presence of Ca^{2+}.

The action of various growth factors is modulated by SPARC. SPARC co-localizes with PDGF in platelet α-granules and binds with high affinity to PDGF-AB and PDGF-BB, but not PDGF-AA. This interaction results in interference of PDGF binding to its receptor on fibroblasts and inhibition of PDGF-stimulated proliferation of human vascular smooth muscle and mesangial cells. SPARC has also been shown to bind ►VEGF and antagonize its pro-angiogenic effect on endothelial cells. Although SPARC does not directly bind ►bFGF, it antagonizes its effect on the proliferation and migration of endothelial cells. SPARC can also suppress ligand-induced autophosphorylation of the bFGF receptor and inhibit both bFGF- and VEGF-induced ERK activation in endothelial cells.

►TGFβ and SPARC induce one another's expression in a reciprocal manner. In mesangial cells, SPARC binds the TGFβ/TGFβRII complex. Accordingly, in these cells, treatment with SPARC has no effect, but in combination with TGFβ it causes stimulation of ►SMAD phosphorylation, ►JNK activation, and an increase in total and phosphorylated c-jun. Treatment with SPARC stimulates SMAD phosphorylation in TGFβ-responsive epithelial and endothelial cells, but inhibits TGFβ-induced activation in fibroblasts.

Integrin-linked kinase (ILK) was identified as a SPARC binding partner. SPARC can induce Ser^{473} phosphorylation of ►AKT through ILK and ►focal adhesion kinase (FAK) in glioma cells. In contrast, in ►ovarian cancer, SPARC significantly suppresses activation of AKT and ERK signaling.

A scavenger receptor, stabilin-1, was identified as another SPARC binding partner. Binding results in receptor-mediated ►endocytosis of SPARC, followed by its targeting for degradation in macrophages.

Animal Models

In the nematode *C. elegans*, SPARC is expressed in muscle cells along the body wall and in the sex muscle, with no evidence of expression in other cell types. SPARC overexpression leads to the *Unc* phenotype consisting of a lack of coordinated movement or paralysis, accompanied by frequent disorganization of gonad and vulval protrusions. The offspring embryos are deformed and not viable.

During *Xenopus laevis* development, SPARC is expressed in the notochord and mesoderm prior to appearance of the first somites, and later in the neural tube. Disruption of normal SPARC function causes a broad spectrum of developmental abnormalities including embryonic axes deformities associated with disorganized myotomes, lack of intersomitic boundaries, and defects in eye development.

SPARC knockout mice do not have significant developmental abnormalities, but display multiple defects associated with abnormal ECM deposition. Animals develop early-onset cataracts accompanied by abnormal collagen IV deposition in the lens capsule. A reduced number of osteoblasts and osteoclasts leads to decreased bone remodeling and profound osteopenia. Animals have an enlargement of fat pads due to an increase in the number and diameter of adipocytes. The accumulation of adipose tissue compensates for

the body weight loss caused by osteopenia. The skin of adult transgenic mice has decreased tensile strength and reduced collagen content with smaller diameter collagen fibrils compared to wild-type mice. SPARC-null mice also show significant acceleration of wound healing *in vivo*.

SPARC in Cancer

The role of SPARC in tumorigenesis is complex and appears to be cell-type specific due to its diverse function in a given ►microenvironment. In some types of cancer, high levels of SPARC expression have been shown to correlate with disease progression and poor prognosis. In ►melanoma cells, high levels of SPARC expression induce ►epithelial to mesenchymal transition (EMT), and increase ►invasion and tumor progression. High levels of SPARC are also associated with invasive meningioma and ►osteosarcoma. In glioma, SPARC promotes invasion, but delays tumor growth.

In other types of cancer SPARC functions as a tumor suppressor. It inhibits the proliferation of ►breast cancer cells and induces apoptosis in ovarian cancer cells. In the majority of primary ►lung adenocarcinomas, SPARC is silenced by ►methylation, and this epigenetic aberration is associated with poor outcome. In ►non-small cell lung cancer, SPARC is not ►methylated, but its expression is frequently downregulated due to methylation of the recently identified ►tumor suppressor gene RASSF1A. Similarly, in breast and ►prostate cancers and ►neuroblastoma, the majority of neoplastic cells do not express SPARC. In neuroblastoma, high levels of SPARC are expressed in Schwannian stromal cells, which is associated with a favorable outcome.

Stroma-associated fibroblasts are commonly SPARC-positive, which in some types of cancer correlates with poor prognosis. However, other studies indicate that SPARC plays a role in creating a microenvironment that is inhibitory to tumor progression. SPARC has been characterized as a potent inhibitor of angiogenesis. It also induces the formation of tumor stroma and prevents the activation of fibroblasts. Enhanced growth of Lewis lung carcinoma and ►pancreatic cancer xenografts in SPARC-null mice is associated with altered production and organization of ECM within and surrounding the implanted tumors.

SPARC has also been found to have tumor suppressive activity in a number of animal studies. Significantly lower numbers of ►metastases are seen following injection of ►adenoviral SPARC-infected breast cancer cells compared to controls. In SPARC-null mice, enhanced tumor growth and extensive dissemination has been reported following inoculation of ovarian cancer cells. Continuous infusion of SPARC into nude mice has also been shown to potently inhibit the growth of neuroblastoma xenografts. Recent studies have demonstrated that SPARC sensitizes ►colon carcinoma cells to ►radiotherapy and ►chemotherapy. In nude mice with xenografted colon carcinoma tumors, SPARC enhances the anti-tumor effects of cytotoxic agents, resulting in improved survival.

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Secretor Enzyme

Definition

A product of the Secretor gene, also named fucosyltransferase2 (FUT2), that catalyzes addition of fucose in α1,2 position onto type 1 chains. The name of the gene and enzyme stem from the presence of the gene product in secretions, namely in saliva.

►Lewis Antigens

Securin

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Synonyms

Human pituitary tumor-transforming gene 1; hPTTG1; Human ESP1-associated protein 1; EAP1; Tumor-transforming 1; TUTR1; Pituitary tumor-transforming gene 1; PTTG1

Definition

Securin is a 22 kDa protein that is crucial for the stability of the cells' genome. By preventing premature

►sister-chromatid separation during mitosis, securin is involved in the regulation of accurate cell cycle progression.

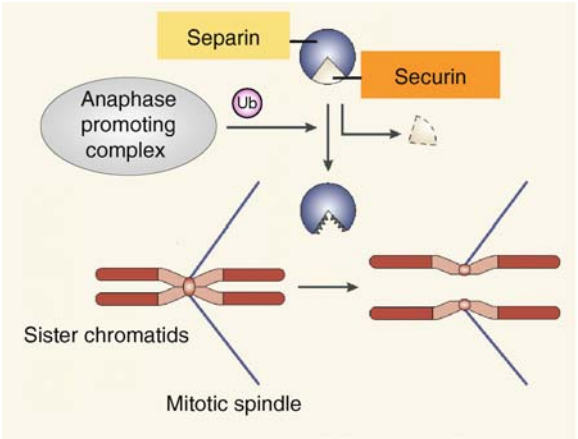
Characteristics

The protooncogene *PTTG* was first isolated from rat pituitary tumor cells. The human homolog of *PTTG*, *securin* (hPTTG1), was found to be overexpressed in Jurkat T cells (human lymphoma T cell line) and in leukocytes from patients with different types of hematopoietic neoplasms or myelodysplastic syndromes. High levels of securin protein expression have been reported for various other tumors, including tumors of the pituitary gland, adrenal gland, kidney, endometrium, uterus, and ovary as well as esophageal and colorectal cancer. Hence securin has been implicated in cell transformation and tumor development. In contrast, most normal adult tissues express little securin with the exception of tissues with highly proliferating cells, e.g., testis and thymus. Thus the level of securin expression correlates with cell proliferation in normal tissue. In fact, the securin protein is expressed in a cell cycle-dependent manner.

Securin Function

The cell division cycle (►Cell cycle targets for cancer therapy) is a tightly regulated process to ensure the correct division of the genome into the daughter cells. Low securin expression is characteristic for noncycling cells and for cells at the beginning of the cell cycle (G1-phase). As the cell cycle proceeds, the securin level rises continuously, peaking in mitosis (M-phase). During M-phase the ►sister-chromatids are connected by cohesin proteins and aligned at the metaphase plate while being attached to the mitotic spindle. In this phase of the cell cycle, securin acts as sister-chromatid separation inhibitor by binding to separin (human ESP1), which is responsible for the destruction of the cohesin complexes (Fig. 1). In late M-phase, the metaphase–anaphase transition, securin is ubiquitylated (►Ubiquitination) by the activated ►anaphase-promoting complex or cyclosome (APC/C). This triggers securin destruction by the proteolysis machinery enabling separin activation and sister-chromatid separation, followed by cell division. In addition to regulating cell cycle progression, securin is involved in processes like DNA repair, ►apoptosis, angiogenesis, and tumor development.

The multifunctional protein securin consists of 202 amino acids and contains, like many APC/C substrates (e.g., cyclins), a destruction box motif (D-box) within the N-terminus. The D-box is recognized by the APC/C complex at the end of metaphase leading to securin ubiquitylation and subsequent proteolysis. Furthermore, a central ►transactivation domain, a DNA-binding domain and proline-rich motifs representing potential Src homology 3 domain (SH3)-binding sites



Securin. Figure 1 During M-phase of the cell cycle, chromosomes are aligned at the metaphase plate with the centromers linked to the mitotic spindle. The sister-chromatids are connected in the centromeric region by cohesin proteins. Securin binds to separin, thereby inhibiting the cohesin degradation activity of the protein. In late M-phase when all chromosomes are accurately aligned, the activated anaphase-promoting complex induces securin degradation by ubiquitylation (Ub). Activated separin cleaves the cohesin proteins enabling an even distribution of the sister-chromatids to the daughter cells (Adapted from Malumbres M, Barbacid M (2001) Nat Rev Cancer 1:222–231 with permission).

(►SH2/SH3 domains) have been identified in securin. Securin (hPTTG1) is the only member of the PTTG protein family that has been studied in detail. The protein family consists of at least three members that have no significant homology to other known proteins. The different expression pattern of the *hPTTG1*, *hPTTG2*, and *hPTTG3* genes in normal and tumor tissue may suggest a tissue-specific expression and diverse functions of the encoded proteins.

Securin Function in Tumor Development

Overexpression of *securin* can transform mouse and human cells (NIH3T3, HEK293), enabling them to form tumors in ►nude mice, thus specifying securin as an oncoprotein. Mutation of the proline-residues of the SH3-binding sites abrogates the transforming (in vitro) and tumor inducing (in vivo) activity. However, securin seems to be involved in tumor development in several ways, although the precise mechanisms remain unknown. First, as securin is essential for sister-chromatid separation during mitosis, it regulates cell proliferation and chromosome stability. Loss of securin function in tumor cells increases abnormal mitosis resulting in ►aneuploidy and apoptosis. The antiapoptotic function of securin is partly mediated by the interaction with the tumor suppressor protein p53 (►p53 Protein),

blocking its DNA-binding and transactivating activity. Despite this effect, overexpression of securin can also cause aneuploidy by failure of the cells to divide the chromosomes evenly between the daughter cells. The induction of chromosomal instability in combination with the inhibition of apoptosis possibly accounts in part for the oncogenic activity of securin. Consequently securin overexpression results, depending on the degree of expression, in elevated cell proliferation and non-tumor cell transformation, which may also be mediated by the induction of *c-Myc* oncogene expression (►Myc oncogene). Recently an additional mechanism for the induction of genetic instability by securin in tumor cells has been suggested. Securin binds to DNA repair proteins (p53 and ►Ku70) and, when overexpressed, inhibits ►double-strand break DNA repair activity in colorectal cancer cells.

Second, high securin expression levels induce angiogenesis in vitro and in vivo possibly by activating the expression of basic fibroblast growth factor (►bFGF) and ►vascular endothelial growth factor (VEGF), both potent mitogenic and angiogenic factors. This activity of securin is dependent on the proline-rich domain of the protein suggesting that securin may function through SH3-signal transduction pathways.

Third, securin expression correlates with tumor cell ►invasion and has been implicated to serve as prognostic marker for poor prognosis of pituitary, thyroid, colorectal, breast, esophageal cancers, and squamous cell carcinoma of the head and neck. The proinvasive and angiogenic effect of securin may be facilitated by its induction of ►matrix metalloproteinase 2 (MMP-2) expression. Taken together, securin appears to contribute to at least three important hallmark features of cancer: cell transformation, angiogenesis, and cell invasion.

However, the regulation of securin expression in tumors is largely unknown. The growth factors ►HGF, TGFα (►Transforming growth factor), ►EGF, IGF-1 (►Insulin-like growth factors), and the hormone insulin have been implicated in securin regulation. Recently, in colorectal cancer and esophageal squamous cell carcinoma, the β-catenin/TCF-signaling pathway (►APC/beta-catenin pathway) has been found to control securin expression. In the process of tumor development, this crucial signaling pathway is deregulated, leading to the accumulation of the oncogenic protein β-catenin, which acts as transcriptional activator in the β-catenin/TCF4 protein complex. The constitutive activity of β-catenin causes at least in part the overexpression of securin and other target genes with the potential to contribute to tumor initiation and progression. Although the molecular mechanisms of securin participation in tumor development are currently poorly understood, further research will reveal whether it is relevant as potential diagnostic or therapeutic target.

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"Seed and Soil" Theory of Metastasis

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Definition

A theory proposed to explain the metastatic preference of ►cancer cells for specific organs is called the "seed and soil" theory, the cancer cells being the "seeds" and the specific organ ►microenvironments being the "soil." Interaction between the "seeds" and the "soil" determines the formation of a secondary tumor.

Characteristics

Historical Development of the "Seed and Soil" Theory

►Metastasis is the spread of a cancer from its primary location to distant sites in the body, forming the secondary tumors. When cancer cells metastasize, they usually do so to preferential organs, depending on the type of cancer. For example, breast cancer cells usually metastasize to the lymph nodes, bones, lungs, liver, and brain; colon cancer cells often metastasize to the lymph nodes and liver.

The propensity of certain organs to harbor metastatic tumors was noticed in the middle of nineteenth century. When Fuchs studied the metastasis pattern of uveal melanoma in 1882, he found that a favorable site for secondary tumor development should be taken into account. Paget, a surgeon, reported the autopsy results of 735 cases of breast cancer and summarized the studies of others, and clearly proposed the "seed-and-soil" theory in 1889, pointing out that metastasis depends on interaction between cancer cells and specific organ microenvironments. The theory has had a great influence on the field of cancer research.

Recent Evidence Shows that the "Seeds" Can Even Prepare the "Soils"

A primary tumor can induce reorganization of the vasculature and lymph channels in the ►sentinel lymph node before metastasis, that is, before the cancer cells arrive. The dramatically remodeled vasculature of the sentinel lymph node can then integrate into the tumor vascular system after metastasis and nurture the fast growing metastatic tumor. The molecular basis for this pre-metastatic remodeling is not yet known.

The preparation of a pre-metastatic "niche" in bone marrow before the arrival of cancer cells has also been reported. The bone marrow-derived hematopoietic progenitor cells that express ►vascular endothelial growth factor receptor 1 are responsible for creating a favorable niche for incoming tumor cells.

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Seer

Definition

Surveillance epidemiology and end results.

►Childhood Cancer

SELDI-TOF MS

Definition

►Surface-enhanced laser desorption/ionization time-of flight mass spectrometry.

Selectins

Definition

A family of cell-adhesion molecules mediating inflammatory cell arrest. E-selectin is found on activated endothelium. P-selectin (CD62-P) is expressed by endothelium and platelets upon stimulation with, e.g., thrombin. L-selectin is expressed on leukocytes. Adhesion molecules that contain a lectin-like domain that recognizes and binds carbohydrates. Relevant examples for adhesion mediated by sialyl-Lewis antigens are E(endothelial)-selectin and P(platelet)-selectin.

►Lewis Antigens
►Tumor-Endothelial Cross-talk

Selection Template

Definition

An oligonucleotide, typically double-stranded DNA, that is used either directly or through a derivative (e.g. RNA transcript) in a combinatorial selection method. Selection templates usually contain a region of selectable randomized sequence flanked by defined sequences used minimally in ►PCR amplification but also containing additional information for use in the selection process (e.g. bacteriophage promoter for RNA transcript production in ►SELEX, IISRE binding sites for IISRE cleavage in REPSA).

►Combinatorial Selection Methods

Selective Estrogen Receptor Modulator

Definition

SERM; Is a designer estrogen that possesses some, but not all, of the actions of prototypical estrogen. Some prevent bone loss and lower serum cholesterol but (unlike estrogen) do not stimulate the endometrial lining of the uterus. Therefore, they are considered more safe.

►Isoflavones
►Estrogenic Hormones

Selective Serotonin Reuptake Inhibitors SSRIs

►Fluoxetine

Selenium

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Definition

Selenium (Se) is an essential trace element having multiple effects in many functions of the normal human organism and inducing disturbances when deficient. Thus, it has an important role in health maintenance, being furthermore implicated, when in low levels, in various chronic pathological conditions such as rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, renal insufficiency, and cancer.

Characteristics

More than 10 million new cancer cases each year are recorded worldwide, with cancer being one of the leading causes of death. Much progress has been made in the quest of the etiology and pathogenesis of cancer in humans, however there are still a lot of issues to be elucidated. Identifying cancer risk factors is critical both in prevention and treatment. Epidemiological and genetic studies suggest that factors such as smoking, bioactive food components and hormones can influence the incidence and mortality of this disease. Among them a lot of attention has been drawn in those that indicate a strong relationship between low Se concentration in the serum and increased risk of various types of cancer in humans.

Trace elements such as Se, zinc, arsenic, cadmium, and nickel, found naturally in the environment, are delivered to humans from a variety of sources including air, drinking water and food. In the human body Se is absorbed by the gastrointestinal tract, skin, and respiratory system. Concentrations of this trace element in the air are low. Thus, diet and drinking water supplies are the primary sources of Se intake. Se enters the food chain through plants, which uptake it from the soil. Notably, there is a wide geographic distribution of Se in the soils varying from high concentrations in the former USSR. and USA. to low concentrations in China, and some other parts of Asia, as well as in many European countries. Se is present in a wide range of foods such as grains, meat, fish, eggs, as well as in some vegetables. Average selenium consumption from foods ranges from 71 to 152 μg daily. The suggested daily Se uptake in adults varies between scientists groups, ranging from 60–120 μg to 200–300 μg daily. Se intake from dietary sources is assessed by 24-h and weekly dietary recalls, diet histories and food frequency questionnaires. The latter is the least preferred method, since potential differences in Se absorption from the intestinal lumen, various food preparations methods and variations depending on geographical Se distribution, impair the results. In humans, high Se concentrations are detected in the thyroid gland, kidneys, genitals and liver, whilst the lowest Se concentrations are detected in pancreas and lymph nodes.

Organic Se is present in foods mainly in the form of selenomethionine, selenocysteine and selenium-methylselenocysteine, whilst inorganic Se either in the form of selenite or selenate is found infrequently and in very low amounts. Both organic and inorganic Se are utilized with similar efficacy in the human body, producing selenoproteins (SPs), although, Se enters at different points in the metabolism processes depending on the chemical form. Twenty-five SPs have been identified until now, out of a possible 50 thought to exist. Glutathione (GSH) reduces the inorganic forms (selenate and selenite) of Se. The originally discovered cytosolic glutathione peroxidase GSH-Px or GPx-1, the phospholipid hydroperoxide GSH-Px, and the secretory GSH-Px represent three isoenzymes of glutathione peroxidase. The latter, ubiquitously expressed, is the first and best characterized SP in mammals. Other selenoproteins such as thioredoxin reductase (TrxR) and selenoprotein P (SeP) also contain molecular Se in their active center and act in a similar fashion. Through all of them Se regulates the cellular antioxidant defence system, DNA damage and protein function. Se also controls cell-mediated immunity and B-cell function. TrxR is considered to be a key enzyme in Se metabolism, reducing Se compounds and controlling the intracellular redox state and SeP appears to protect endothelial cells against damage from free radicals. The varying degree of anti-carcinogenic activity of different forms of selenium, may be attributed to their metabolism in vivo. High Se concentration can lead to either cytotoxic effects or possibly to carcinogenesis, due to DNA strand breaks. Thus, in the first half of the twentieth century Se was considered an undesirable element for higher organisms. In the second half of the twentieth century the role of Se in human nutrition and biology was appreciated, since there was growing evidence that Se has multiple roles in many biological systems. Among them, Se controls cell-mediated immunity and B-cell function. Se role as an antioxidant,

as well as a cancer-preventing factor nowadays is well established by accumulating evidence. A bulk of studies strongly supports the issue that Se supplementation is effective in the reduction of cancer incidence when non-toxic doses of the element are provided with the diet, possessing thus, anticancer properties. Experimental data have shown that the chemopreventive effect of Se is due, at least partly, to its inhibitory effect on cell growth, DNA, RNA, and protein synthesis in transformed cells. Furthermore, changes in stress-related cellular proteins are widely implicated in explaining the protective role of Se. Several reports have described the inhibitory effect of Se on kinase enzyme activity. Cell cycle cyclin-dependent-kinase 2 (cdk2) and/or cell signaling protein kinases and/or some redox regulated proteins with critical transcription factors, have been proposed as targets against which Se exerts its chemopreventive actions. An increase in cyclin B expression as well as phosphorylation of the cdk2 coincidentally with the cell cycle arrest has been demonstrated. In addition, Se exerts an antiproliferative effect by modulating cellular proliferation in G1 phase in both normal and neoplastic cells and possibly impairing the expression of c-fos and c-myc oncogenes.

Furthermore, human defense mechanisms against reactive oxygen species (ROS), which induce oxidative damage are amplified by Se. Cellular oxidative damage is a general mechanism for cell and tissue injury. Selenocystein reduces the levels of hydrogen peroxide and a number of organic hydroperoxides, thereby, acting as antioxidant. Oxidative stress in the target tissue has been suggested to play an important role in carcinogenic process. Thus, Se may act as an antitumoral agent although more studies are needed to investigate the actual role of antioxidants and their possible relationships with trace elements alterations, and Se in particular, in the pathogenesis of various cancer types. It has also been shown that in colorectal cancer cells Se supplementation decreases the COX-2 protein and PGE-2 levels.

Se plasma and tissue concentration is regulated by incompletely understood homeostatic mechanisms. For estimation of the body Se amount levels, whole blood, serum, plasma, erythrocytes, urine, hair, and nails represent biological specimens suitable for sampling. Each of these differs in terms of the exposure period represented. Plasma and serum measures tend to reflect short term exposure, whilst Se levels in erythrocytes represent a long term exposure. Longer term exposure is measured by toenails samples.

Early epidemiological studies ~40 years ago suggested an inverse association between Se levels and risk of cancer. Studies from about 30 countries showed a significant inverse correlation with age-adjusted mortality for colorectal (CRC), prostate, breast, ovary and lung cancer as well as for some hematological malignancies, whilst only a weak association was found for pancreatic and skin cancer. Low Se is associated with risk of lung, colorectum, oesophagus, stomach, liver, breast, prostate, and urinary bladder cancer. Results from many chemoprevention trials strongly suggest that Se supplementation may have some protective effects against the above mentioned cancer types in populations where average dietary Se levels are low.

There are a few data regarding the association between Se and CRC or adenomas. These results depending mainly on small studies are inconclusive and probably new prospective studies with large series of individuals are needed. However, epidemiological studies have reported an inverse association between Se and CRC, cancer stage and survival, whilst some others failed to detect this association reporting null results. A pooled analysis reported by Jacobs et al. indicates that individuals with high blood Se concentration (median value 150 ng/ml) had 34% lower odds ratios (OR 0.66, 95% CI 0.50–0.87, P_{trend} 0.006) for developing a new adenoma throughout the follow up time period, in comparison with those individuals with lower plasma Se levels (median value 113 ng/ml). Thus, new prospective studies regarding Se alone or in combination with other trace elements must be designed in the near future. In breast, contradictory studies have been reported. Although there is some evidence indicating the chemopreventive role of Se in breast cancer, rigorous retrospective and prospective studies are needed to confirm this issue.

The urinary bladder mucosa differs from other tissues, being exposed to Se directly, via the urine, and indirectly, via the blood. An inverse association between serum Se levels and bladder cancer has been reported in the literature. Serum Se levels and glutathione peroxidase activity in patients suffering from transitional cell carcinoma were inversely correlated with tumor grade. Similarly, this strong inverse association has also been detected in prostate cancer. In recent reports, individuals taking Se supplementation exhibited a significant reduction in the risk of prostate cancer. Regarding the other cancer types the usual reverse association between serum Se levels and neoplastic development has been observed, although this phenomenon is not universally accepted, since there are also not confirmatory studies.

By several studies it has been indicated that the process of the underlying tumor development can lead to an uptake of trace elements by neoplastic cells explaining, thus, the increased levels of Se in tumor mass. The same phenomenon is also observed with some other trace elements such as Zn, Fe, Cu in neoplastic tissue. It is not clear whether the increased Se concentration in the cancerous tissues is responsible for the decreased serum Se levels found in these patients

or if the decreased serum Se levels precede the development of cancer.

In conclusion, Se is found naturally in the environment and is uptaken by humans through a variety of sources, including food, drinking water and air. Intake and plasma levels differ depending on geographical distribution and genetic factors. Se chemopreventive action has been demonstrated in some types of human cancers. More clinical trials are needed to investigate the role of Se supplementation in reducing cancer incidence in humans.

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Selenocysteine

Definition

A ►selenium analog of cysteine, where a selenium atom in selenocysteine has taken the place of sulfur in cysteine. Selenocysteine is naturally present in 25 known human proteins, of which thioredoxin receptor (TrxR) isoenzymes constitute three.

►Thioredoxin System

Selenoenzyme

Definition

An enzyme containing selenocysteine as one of the amino acids of the protein.

►Thioredoxin System

SELEX

Definition

Systematic evolution of ligands by exponential enrichment (SELEX) is a combinatorial selection method that identifies oligonucleotide aptamers exhibiting high affinity and binding specificity to a variety of ligands, including proteins and small molecules.

►Combinatorial Selection Methods
►Aptamer Bioconjugates for Cancer Therapy

Self-Renewal

Definition

Is the ability to undergo multiple rounds of cell division while maintaining an undifferentiated state.

►Stem Cell Markers

Self-Sufficiency in Growth Signals

Definition

Capacity of tumoral cells, which refers to the ability that they have to proliferate in the absence of stimulatory growth signals. Normal cells require growth signals in order to actively proliferate. However, tumor cells are characterized by a greatly reduced dependence on exogenous growth stimulation. The explanation is that tumor cells generate many of their own growth signals, inducing a growth signal autonomy.

►Funnel Factors

Sella Turcica

Definition

The bony cavity in the skull that houses the pituitary gland.

►Prolactin

SEMA

►Semaphorin

SEMA Domain

Definition

Is commonly found in ►semaphorins – a large family of secreted and transmembrane proteins. Some semaphorins function as repellent signals during axon guidance.

Semanová II syndrome

►Nijmegen Breakage Syndrome

Semaphorin

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Synonyms

SEMA; Collapsin; Growth guidance cue; Neuropilin ligand; Plexin ligand

Definition

The semaphorins (abbreviated SEMA) constitute a family of genes encoding secreted and membrane associated proteins which share a common domain called the sema domain.

The name semaphorin is derived from the greek words "*sema*" meaning "signal" and "*phor*," which means "to carry." This name was given to semaphorins owing to the function of the first semaphorins in ►axon guidance.

Characteristics

Subclasses

To date, the semaphorin family comprises at least 18 different members in vertebrates and at least three different members in invertebrates. All semaphorins contain an approximately 500 amino acid extracellular sema domain and a class specific carboxy terminus that may encompass additional sequence motifs. At present the known semaphorins are divided amongst eight subclasses on the basis of these structural features and phylogenetic analysis.

Other protein families including ►plexins and the ►receptor tyrosine kinases MET and ►RON contain sema domains, but on the basis of phylogenetic analysis these proteins are only distantly evolutionary related to semaphorins.

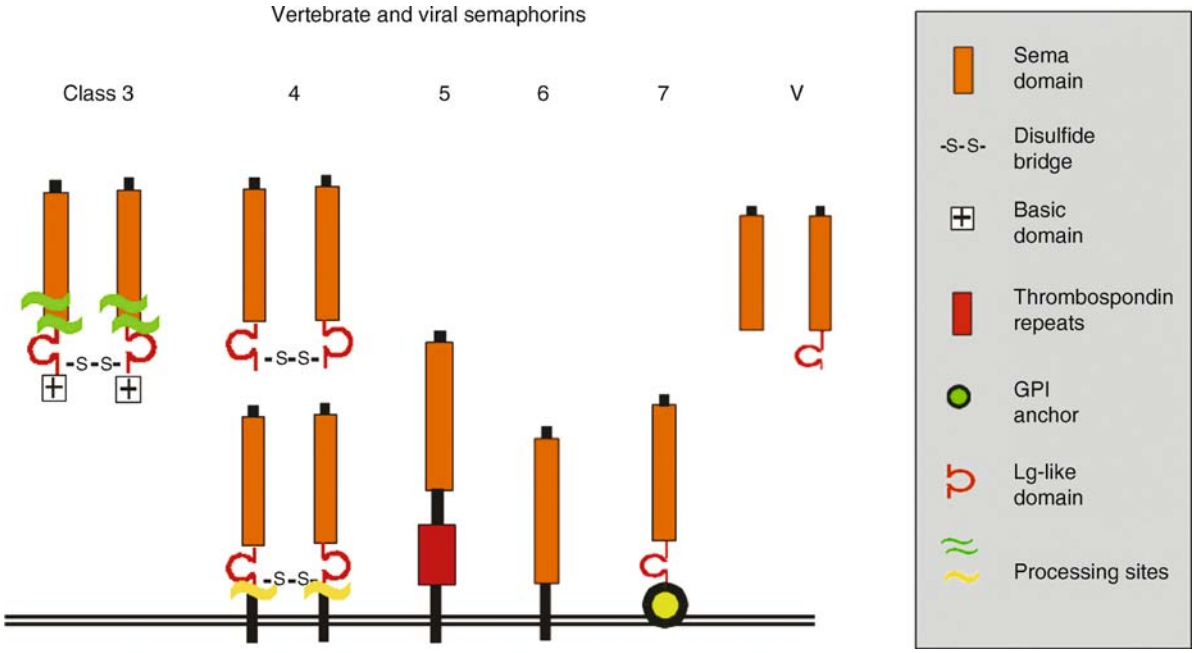
Structure and Processing

With respect to cellular localization semaphorins fall into three categories: secreted (subclass 2, 3, and V), transmembrane (subclass 1, 4, 5, and 6) and ►GPI-linked (subclass 7) (Fig. 1). Class 3 semaphorins require posttranslational modifications to gain activity. During maturation these proteins are proteolytically processed by ►furin or related serine endoproteases, possibly glycosylated (see ►Glycosylation) and they undergo dimerization. Their activity is further regulated by proteolytic cleavage of an internal RXK/RR site which separates the sema domain from the rest of the dimer. This was shown to cause a loss of the repellent activity of these semaphorins in neurobiology. However, such processing may positively add to semaphorin activity during tumor ►progression as recently shown in the case of Sema3E.

In the case of membrane-associated semaphorins, proteolytic processing may cause shedding of the extracellular domains from the cell surface, as shown for the class 4 semaphorin Sema4D.

Brief History of the Semaphorin Family

The first discoveries of semaphorins were made in 1992 and 1993 by Alex Kolodkin and co-workers in Corey Goodman´s lab at the Howard Hughes Medical Institute, Berkeley, University of California. They were attempting to identify molecules involved in the fasciculation of nerve axons in Grasshoppers and this led to the isolation of a transmembrane protein, which they first called Fasciclin IV. This protein turned out to possess a repelling activity which guided nerves in developing insect limp buds. The sequence was unique and used in the cloning of related sequences from other species including human SEMA3A. Meanwhile, in 1993, Yuling Luo and co-workers working in Jonathan Raper´s lab at the University of Pennsylvania isolated a protein from chicken brain extracts responsible for the growth cone collapsing activity observed when such extracts were added to sensory ganglion neurites *in vitro*. This protein was named Collapsin-1 and subsequently realized as the chick counterpart to human SEMA3A. Based on these sequences the



Semaphorin. Figure 1 The structure of vertebrate and viral semaphorin proteins. Semaphorins are secreted, transmembrane or linked to the surface by GPI anchor. They are found as dimers which may undergo proteolytic processing. This processing may cause shedding of the extracellular part of transmembrane semaphorins.

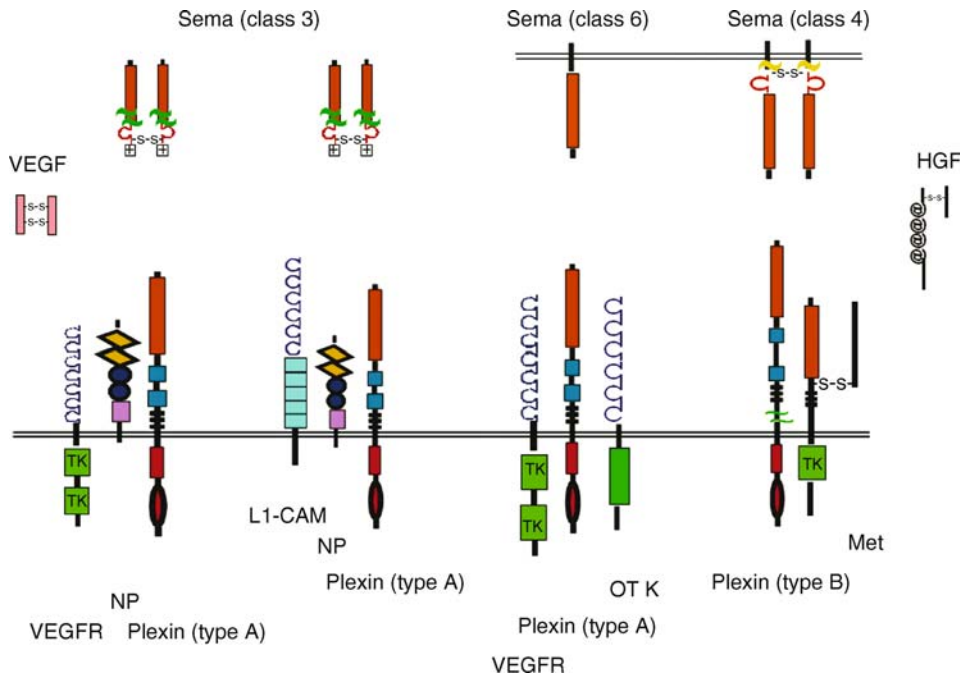
semaphorin/collapsin family was defined. The common family trait was a ~500 amino acid region, named the sema domain, constituting the better part of these proteins and containing a number of unique and highly conserved motifs (see characteristics). In the following years a number of new semaphorin members were isolated from different species. Semaphorins isolated from chick continued to be named collapsins until the nomenclature of semaphorins was changed by initiative of the Semaphorin Nomenclature Committee [1]. Evidence was rapidly mounting to suggest that the semaphorins were acting within the nervous system as ►guidance cues that guided migrating ►axonal growth cones as the complex wiring of the nervous system was established. Semaphorins were typically expressed by neighboring cells and at first considered to act primarily as repelling factors. This notion was changed in 1999 when a semaphorin was for the first time shown to be able to act as an attractive guidance cue. Today, semaphorins are considered to be bidirectional guidance factors, exerting repelling or attractive guidance depending on the composition of receptor complexes on the responding cells. Today, semaphorins are furthermore known to take part in different aspects of biology outside the nervous system including ►angiogenesis, immunobiology, and ►cancer. In cancer biology semaphorins appear to be bifunctional acting either as suppressors (see ►Tumor Suppressor Genes and ►Tumor Suppression) or promoters of tumor progression.

Semaphorins and the Composition of their Receptors

Although many different types of receptors and ►adhesion molecules are today implicated in semaphorin receptor complexes, two distinct types of receptors form the core of most of these complexes: the neuropilins and the plexins (Fig. 2). Members of the membrane-anchored class 1, 4 or 7 semaphorins bind plexins independent of neuropilins. Hence, Plexin-B1 and Plexin-C1 function as receptors for the transmembrane Sema4D and the GPI-linked Sema7A, respectively. On the contrary, the secreted class 3 semaphorins have for long been considered to require a complex of neuropilin and plexin. Hence, the earliest studies addressing this question showed that plexin-A1 combined with NP-1 and NP-2 constituted functional receptor complexes for Sema3A and Sema3F, respectively. But, this concept was challenged in 2004, when Sema3E and plexin-D1 were shown to control vascular development together independently of neuropilins. The reason for this discrepancy is currently unknown.

Neuropilins

The neuropilin family consists of neuropilin-1 (NP-1) and neuropilin-2 (NP-2), the latter existing in different splice variants. Neuropilins are transmembrane proteins that extracellularly possess two CUB (complement-binding) domains, two domains of homology



Semaphorin. Figure 2 Semaphorin receptor complexes. The core of semaphorin receptor complexes is made of plexins or plexins in combination with neuropilins (NP). Plexins bind semaphorins directly. In the case of class 3 semaphorins, it is generally so that a neuropilin is required in order to bind this class of semaphorins. Neuropilins also act as co-receptors for vascular endothelial growth factors (VEGFs). Other proteins may be involved in semaphorin receptor complexes including vascular endothelial growth factor receptors (VEGFRs), the off-track kinase (OTK), the cell adhesion molecule L1-CAM or the receptor tyrosine kinase Met. Hepatocyte growth factor (HGF) is the ligand for Met.

to coagulation factors V and VII, and an oligomerization domain, abbreviated MAM (short for meprin, A5,μ). The CUB domain is involved in the binding to class 3 semaphorins. NP-1 binds Sema3A but not Sema3F; whereas NP-2 binds Sema3F but not Sema3A. The other class 3 semaphorins Sema3C, Sema3D and Sema3E bind both neuropilins. The intracellular domains of NP proteins are very short and devoid of classical signaling motifs. Yet, they are highly conserved and bind possible adapter molecules with a ►PDZ domain.

Neuropilins may facilitate clustering of neuropilin-plexin receptor complexes but neuropilins also serve as bridges to other families of adhesion- and receptor molecules. Notably, neuropilins bind members of the ►vascular-endothelial-growth-factor-receptor (VEGF-R) family. In addition, NP-1 binds to L1-CAM, which is a member of the immunoglobulin (Ig) superfamily of ►cell adhesion molecules (Fig. 2).

Plexins

Nine plexins have been identified and they are grouped into four subclasses (plexin-A, -D). There are four A-type, three B-type, one C-type and one D-type plexin. Like semaphorins, sema domains are a characteristic of the extracellular part of plexins. In addition, plexins have two to three ►Met related sequences (PSI domains), and three IPT domains. Intracellularly plexins have two highly conserved stretches, separated by a variable linker region. The conserved domains include motifs distantly related to ►GTPase-activating proteins (►GAPs). In type-B plexins the carboxy terminus contains PDZ-domain binding motifs.

Plexin Signaling

At least some plexins may be locked in an autoinhibited configuration when not binding semaphorins. As shown for Plexin-A1 this involves the binding of the plexin sema domain to the rest of the extracellular part. It is believed that the plexins upon binding to semaphorins change configuration in a manner that allows phosphorylation of tyrosine residues in the intracellular domain as well as binding to various ►small GTPases and ►GTPase exchange factors (►GEFs).

The phosphorylation of the tyrosine residues in the intracellular part of the plexins is not because of autokinase activity inherent to the plexins but occurs because plexins are coupled to tyrosine kinases. Upon the binding of the semaphorin ligand, the plexins become phosphorylated by the tyrosine kinases with which they

interact. Hence, Plexin-B1 binds the receptor tyrosine kinase Met, and when Plexin-B1 is stimulated by Sema4D, Met causes phosphorylation of Plexin-B1. A similar interaction has been reported between Plexin-B1 and the tyrosine kinase receptor ErbB-2. Other examples of plexins interacting with tyrosine kinases are Plexin-A1 and Plexin-A2, which bind the intracellular tyrosine kinases Fes and Fyn, respectively. Plexin-A1 also interacts with the Off-track receptor, which possesses a kinase domain yet no kinase activity, as well as the VEGF receptor KDR/VEGFR-2. The binding of these two receptors to Plexin-A1 appears to have opposing effects on heart morphogenesis (Fig. 2).

The GAP-related domains in the cytoplasmic part of plexins have been shown to serve as docking sites for small GTPases such as Rac1 and Rnd1 (see ►GTPase and ►Rho family proteins). Furthermore the activation of RhoA is implicated in the axonal collapse mediated by B-type plexins. However, Plexin-B1 does not bind RhoA directly. Instead it binds to leukemia-associated Rho-GEF (LARG) and PDZ-Rho-GEF (PRG) through the PDZ-domain-binding motifs that are specific to B-type plexins. Despite the presence of a segmented GAP domain in plexins, at first plexins were considered not to have any intrinsic GAP activity. However, Plexin-B1 does exert GAP-activity when Sema4D binds to the extracellular domain and Rnd1 at the same time binds to the linker region. Under such circumstances Plexin-B1 exerts a GTPase activation activity on R-Ras (see ►RAS). R-Ras appears to function mainly in the regulation of ►integrins (see ►Integrin Signaling and Cancer).

The small GTPases Rho, Rac, and Rnd are known for their regulatory function with respect to the actin filament assembly and actin-myosin contraction, which constitute the basis for cellular structure and locomotion. Integrins couple this machinery to the extracellular matrix and provide much of the stability during ►migration (see also ►Motility). By recruiting activated forms of small GTPases or affecting integrin function plexins may influence the shape and migratory behavior of cells and axonal growth cones.

Brief History of Semaphorins in Cancer Research

The first discoveries of semaphorins in cancer biology were made in 1996 when SEMA3B and SEMA3F were located at the chromosomal region 3p21.3, which is deleted in small cell lung cancers. Today these semaphorins are thought to act as tumor suppressors through their ability to compete with ►vascular endothelial growth factors for the binding to neuropilin coreceptors. An alternative model predicts that SEMA3F acts by reducing expression of β_1 integrin in cancer cells.

Whereas SEMA3F and SEMA3B are located together on chromosome 3, the other subclass 3 semaphorins, SEMA3A, SEMA3C and SEMA3E are located on chromosome 7. The present studies of SEMA3C and SEMA3E suggest functions of these genes that promote tumor progression.

In 1997 SEMA3C was identified in cis-diaminedichloroplatinum (CDDP) (see ►cisplatin and ►platinum drugs) resistant ovarian TYKnuR cells as a gene capable of conferring chemoresistance to other cells upon ectopic expression. The underlying mechanism is presently unknown. Later SEMA3C has been shown to be upregulated in metastatic human lung adenocarcinoma cells and in malignant glioma cells.

In 1998 Sema3E was identified in murine mammary adenocarcinoma cells (see ►Breast Cancer) and reported to be expressed in a manner correlating with the metastatic potential of such cells. It was later shown to stimulate experimental lung ►metastasis when overexpressed in a nonmetastatic cell line of the same origin. The exact mechanism is currently unknown but seems to deviate from existing models since it requires proteolytic processing of Sema3E. So far human SEMA3E has also been associated with mammary adenocarcinoma, where it seems to be overexpressed in a subset of clinical samples and cell lines as seen in the case of murine Sema3E.

In 2002 Sema4D was shown to stimulate invasive growth *in vitro* through a receptor complex consisting of Plexin-B1 and Met. Later Sema4D has been shown to promote angiogenesis *in vitro* and *in vivo* in a manner that likewise requires a coupling between Plexin-B1 and Met. Experimental evidence for a role of Sema4D in tumor angiogenesis has been reported in the context of head and neck squamous cell carcinoma. Sema4D is also expressed in activated B and T lymphocytes and at a high level in lymphoid and myeloid leukemia cell lines as well as in some T-cell and B-cell non-Hodgkin lymphomas (see ►Hematological Malignancies; Hodgkin Disease and ►Malignant Lymphoma, Hallmarks and Concepts). Whether Sema4D contributes to the progression of these cancers is not known, and if so it might involve a plexin-independent mechanism since the major Sema4D receptor within the immune system appears to be CD72 rather than Plexin-B1.

In 2003, Sema5C was identified in a genetic screen for genes that would suppress a tumor phenotype in *Drosophila* that arises from inactivation of the *lethal giant larvae l(2)gl* gene. This work showed that inactivation of Sema5C blocked tumor growth in such flies and pointed to a mechanism involving a TGF-beta-like signal pathway.

Other class 5 semaphorins (SEMA5A, -5B and -5D) as well as the class 6 semaphorins SEMA6A and SEMA6B have also been associated with different human cancer cells, but the significance of these findings is unknown. Table 1 summarizes the expression and alleged functions of semaphorins in cancer according to published material.

Semaphorin. Table 1 The expression and possible functions of semaphorins in cancer. Whenever the semaphorin has been implicated with human cancer it is listed by its human name

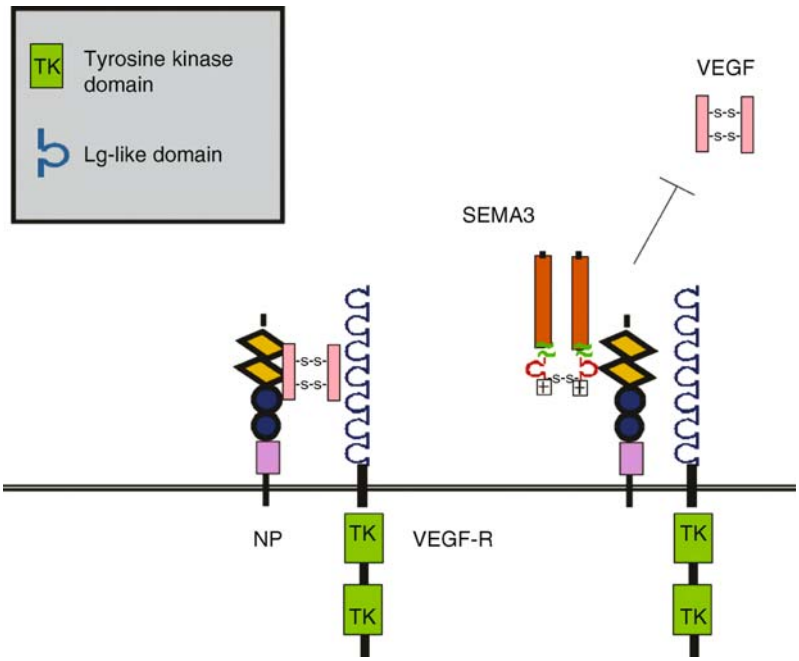
Semaphorin	Type of aberrant expression	Tumor type(s)	Function(s)
SEMA3A	Down-regulated	Mesothelioma	Inhibition of angiogenesis and migration
	Expressed	Breast carcinoma cell lines	
SEMA3B	Maps to 3p21.3 deleted in lung cancer	Lung cancer	Inhibition of angiogenesis
	Down-regulated	Lung, ovarian and breast cancer cell lines	
SEMA3C	Over-expression	Ovarian, lung, glioma cancer cell lines	Promotes cancer cell survival (chemo resistance)
	Differentially expressed (only seen in metastatic cell line)	Lung adenocarcinoma cell lines	
SEMA3E	Differentially expressed (only seen in metastatic cell lines)	Breast carcinoma cell lines	Promotes metastasis
SEMA3F	Maps to 3p21.3 deleted in lung cancer	Lung cancer	Inhibition of angiogenesis
	Down-regulated	Malignant melanoma	Inhibition of adhesion and migration
SEMA4D	Expressed	Head and neck squamous cell carcinoma	Promotes angiogenesis and invasive growth
	Differentially expressed	Lymphoid and leukemia cell lines non-Hodgkin lymphoma	Unknown
SEMA5A	Expressed	Malignant Melanoma cell line	Unknown
	Over-expressed	Uterine leiomyomata	
SEMA5B	Differentially expressed	Renal cell carcinoma	Unknown
Sema5C	Inactivation (experimentally by P element insertion)	Drosophila l(2)gl tumor model	Necessary for tumor growth
SEMA5D	Expressed	Malignant melanoma cell line	Unknown
		Ovarian cancer cells	
SEMA6A	Maps to 5q21-22 deleted in lung cancer	Lung cancer	Unknown
SEMA6B	Down-regulated when cells treated with retinoids	Glioblastoma	Unknown

Different Mechanisms may Account for Semaphorin Functions in Cancer Biology

Model I: Semaphorins may act as antagonists of Vascular Endothelial Growth Factor (VEGF) signaling.

Both class 3 semaphorins and vascular-endothelial growth factors are known ligands of the neuropilin receptors, meanwhile neuropilins exist in complexes with either plexins and VEGF receptors (VEGFR). This creates the basis for a mutually antagonistic relationship between class 3 semaphorins and VEGF ligands (see Fig. 3). Hence, NP-1 is a coreceptor of VEGFR-2 (also known as KDR) mediating the activity of certain VEGF-A isoforms in angiogenesis. However SEMA3A binding to NP-1 blocks this activity in endothelial cells. Likewise, NP-2 is a coreceptor for VEGFR-1 and VEGFR-2, and binding of NP-2 to SEMA3F or SEMA3B may influence signaling through these two receptors. Recently, NP-2 was also shown to act as a coreceptor for VEGFR-3 mediating the activity of VEGF-C and VEGF-D during ►lymphangiogenesis.

Initially, class 3 semaphorins were considered to be tumor-suppressors due to their antagonistic activity on VEGF signaling in endothelial cells (see ►Antiangiogenic and ►Antiangiogenesis). Today, the picture is made complicated by the fact that both endothelial cells and many cancer cells express class 3 semaphorins and VEGF ligands, and both cell types may express any combination of neuropilins, plexins and VEGF-receptors dependent on tumor type and localization. The effect these expressions have on the overall migratory, adhesive and proliferative capabilities of the EC and cancer cells within a given tumor may depend upon the ratio between the different receptors and between ligands. One updated model



Semaphorin. Figure 3 Model for how semaphorins may suppress tumorigenesis by blocking VEGF signaling. Neuropilins (NP) are co-receptors for both class 3 semaphorins and vascular endothelial growth factors (VEGFs). When class 3 semaphorins bind neuropilins they prevent the binding and activation of VEGF-receptors (VEGF-R) thereby blocking the pro-angiogenic signaling of VEGF.

depicts that down-regulated expression of class 3 semaphorins such as SEMA3F and SEMA3B shifts the balance in favor of VEGF ligands and their pro-migratory activity in cancer cells or pro-angiogenic activity in EC.

Model II: Semaphorins may act through complexes of receptor tyrosine kinases and plexins.

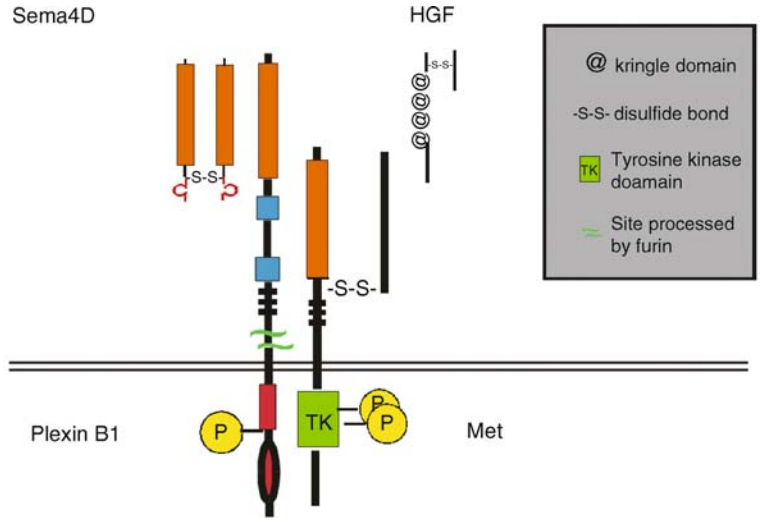
The reason for both tumor-suppressive and tumor promoting activities of semaphorins may originate from the interaction between different plexins and tyrosine kinases. The most prominent example of a potential tumor growth promoting mechanism is the interaction between Plexin-B1 and Met (see Fig. 4). The Met receptor is a known ►oncogene, and both Met and its ligand hepatocyte growth factor (HGF; see ►Scatter Factor) contribute to increased invasiveness and angiogenic activity in tumors. Sema4D and HGF have been shown to act in synergy to cause activation of Plexin-B1 and Met and facilitate invasive growth *in vitro* and angiogenesis *in vivo*. Although it remains to be shown, other semaphorins may contribute to cancer progression through similar interactions between plexins and receptor-tyrosine kinases.

Model III: Semaphorins may act through signaling to integrins.

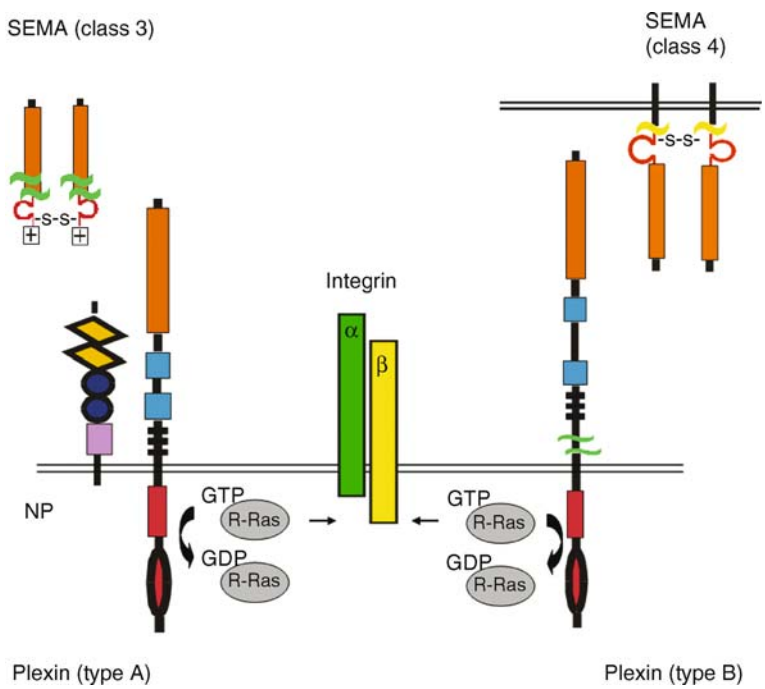
Integrins constitute a family of adhesion molecules of major importance to the migration of cells through the extracellular matrix. At the same time migration is a crucial aspect of the behavior of endothelial cells during angiogenesis as well as the behavior of cancer cells during ►invasion and metastasis. By affecting the function of integrins semaphorins may therefore affect the ability of a tumor to invade or stimulate angiogenesis. Today, compelling evidence suggests that semaphorin signaling as well as plexin activation inhibit integrin-dependent adhesion and migration of different cell types (see Fig. 5). Notably, in 2004 it was shown that SEMA3F expression in melanoma cells induces a poorly vascularized, non-metastatic phenotype, which was partly attributed to a decrease in β_1-integrin mediated migration of the cancer cells. This highlights an alternative way by which class 3 semaphorins may act as tumor suppressors.

Model IV: Semaphorins may act as suppressors of the immune system.

Semaphorins are part of the viral genomes of different ►pox viruses and the *alcelaphine* herpes virus. This has sponsored ideas concerning possible immunosuppressive functions of semaphorins. In accordance, the *Vaccinia* virus encoded semaphorin called A39R have been shown to negatively affect the migration of monocytes and phagocytosis by dendritic cells. Furthermore, the closest ancestor to the viral semaphorins is Sema7A, which seems to play a role endogenous to the immune system although its exact function is unknown.



Semaphorin. Figure 4 Semaphorins may promote tumorigenesis because of the synergistic interaction between Plexins and receptor tyrosine kinases. A prominent example of this is the interaction between Plexin-B1 (the receptor of Sema4D) and Met (the receptor of hepatocyte growth factor (HGF) Met causes phosphorylation of Plexin-B1, when Sema4D binds Plexin-B1.



Semaphorin. Figure 5 Model for how semaphorin signaling may influence the adhesion and migration of tumor cells. When semaphorins do not bind plexins, R-Ras is active (binding GTP), resulting in integrin-mediated binding to the extracellular matrix leading to higher adhesive and migratory behavior of the tumor cells. When semaphorins bind and activate plexin receptors, the GAP (GTPase activating protein) activity of plexins causes the conversion of R-Ras from a GTP-bound state (active) to a GDP-bound state (inactive). This leads to a decrease in integrin-mediated attachment and hence diminished cellular adhesion and migration. The activation of the plexin GAP activity requires the intracellular binding of the small GTPase RND1 to the plexins (not shown).

These findings make it likely that certain semaphorins expressed by cancer cells add to the ways by which the cancer cells evade the immune system.

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Semaphorin Receptors

►Plexins

Seminomatous Germ Cell Tumor

►Testicular Cancer

Sendai Virus

Definition

►Hemagglutinating Virus of Japan.

Senescence

Definition

Is the limited capacity of cells to divide, an irreversible growth arrest state that depends on the age or cell doublings of a cell, a stage in the life cycle of a cell at which it can no longer divide. This state is dependent on the number of cell divisions and is generally brought about through the gradual shortening of the telomeres (repeat sequences at the ends of chromosomes) with each successive population doubling. Senescence is a permanent growth arrest that occurs after cells have exhausted their proliferative capacity. In normal human fibroblast, senescence takes place after approximately 60 population doublings in culture.

►Telomerase
►Senescence and Immortalization
►Aging

Senescence Accelerated

Definition

Accelerated senescence, the process of rapid terminal growth arrest, is accompanied by phenotypic features of cell senescence (enlarged and flattened morphology, increased granularity, expression of specific biochemical and enzymatic markers such as senescence-associated β-galactosidase activity). It can be induced in normal cells by DNA damage or introduction of mutant RAS and is also induced in tumor cells by different anticancer drugs or ionizing radiation.

►Mitotic Catastrophe
►Senescence and Immortalization

Senescence and Immortalization

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Definition

Senescence is the permanent exit of a cell from the cell division cycle, accompanied by morphological and biochemical changes characteristic of ageing.

Immortalization is the ability of cell populations to undergo an unlimited number of cell divisions.

Characteristics

Senescence

Normal mammalian somatic cells can proliferate only a limited number of times in vitro, and the maximum

number is often referred to as the "Hayflick limit." When this limit is reached, the cells undergo various morphological and biochemical changes suggestive of ageing, so the process is referred to as senescence. Senescent cells can remain metabolically active for a long period of time, even though they have permanently exited from the cell cycle. Senescence is thus distinct from cell death (including ►apoptosis and ►autophagy). It is also distinct from terminal differentiation, where cells also exit permanently from the cell cycle and undergo changes that allow them to perform specialized normal functions. Senescent cells have been extensively studied as an in vitro model of ageing. In humans, cellular senescence appears to be a major barrier to the development of cancer.

Immortalization

It is not practicable to test whether cells are truly capable of continuing to divide forever, so cells are usually regarded as being immortalized if they have undergone many cell divisions (typically 100) beyond the Hayflick limit. Many cancers contain immortalized cells and some cancer-derived cell lines have been proliferating in vitro for many decades.

Relevance of Senescence and Immortalization to Cancer

Although the Hayflick number may be quite large (fibroblasts from an adult, for example, may divide up to 40 times before they become senescent), in most situations it is not large enough to permit tumor formation. A tumor containing 2^{40} cells would be big enough to be lethal, but there are two major reasons why 40 cell divisions does not result in a tumor of this size. The first is that cell death occurs at a very substantial rate within tumors, for reasons that include genetic instability (see chapters on ►chromosomal instability and ►microsatellite instability) and difficulties with blood supply (see chapter on ►angiogenesis) that result in cell death. The second is that the genesis of a fully malignant tumor cell requires the accumulation of a number of critically important genetic changes. Most of these changes occur at random and provide a growth advantage to the nascent tumor cell. This process consumes many more cell divisions than a normal cell is able to undergo before it becomes senescent.

Consequently, senescence forms a major barrier to carcinogenesis in humans. A cell containing some of the genetic changes required for carcinogenesis will not usually be able to proliferate sufficiently to form a clinically significant tumor while the senescence barrier is intact. Human cells become immortalized at a very low frequency (so low, that no clear example has yet been found of a normal human cell undergoing immortalization spontaneously in cell culture), so immortalization is a rate-limiting step in human carcinogenesis. In contrast, mouse cells become immortalized spontaneously at a measurable frequency and, correspondingly, the probability of a mouse cell becoming malignant is many orders of magnitude higher than for human cells. The ability to suppress tumor formation is a major selective advantage for a long lived species such as H. sapiens.

A Cell Division Counting Mechanism

The existence of a limit to the number of times a cell can divide implies that there must be a cell division counting mechanism. According to the telomere hypothesis of senescence, the counting mechanism is based on the progressive shortening of the ends of chromosomes (►telomeres) that occurs with cell division. Telomeres form protective caps that prevent the cell recognizing the ends of chromosomes as double strand breaks and repairing them, for example by fusing the ends to each other. They contain repetitive DNA (in all vertebrates the repeat unit is a hexanucleotide, TTAGGG), which ends in a single-stranded G-rich tail. Telomeres are able to fold back on themselves and form a loop structure (referred to as a "t-loop") when the single-stranded telomere invades duplex telomeric DNA and anneals to the complementary strand, thus hiding the free single-stranded telomere end. Telomeric DNA is recognized by specific binding proteins, including TRF1 and TRF2 which bind to double-stranded telomeric DNA and POT1 which binds to single-stranded telomeric DNA. The reasons for telomere shortening include the following. First, DNA replication depends on small RNA primers, which get degraded and replaced by DNA. However, there is no mechanism for replacing the terminal RNA primer required for lagging strand DNA synthesis, which results in the template for the next round of DNA synthesis being shorter. This is known as the "►end replication problem." Second, there appears to be a 5′-3′ exonuclease that shortens the C-rich strand, which creates or increases the length of a single-stranded G-rich telomeric tail.

Regardless of the exact mechanism of telomere shortening, eventually telomeres become so short that they trigger the cell to exit permanently from the cell cycle. In order for a cell to become immortalized, it must somehow prevent telomere shortening. In most cancers this is achieved by the activity of an enzyme, ►telomerase, and in a minority it is achieved by another mechanism referred to as ►alternative lengthening of telomeres (ALT). Every immortalized cell line examined to date has either telomerase or ALT activity.

Telomerase

The telomerase holoenzyme complex is normally expressed in cells of the germ-line. It is also found in some normal somatic cells, especially those that are

required to undergo extensive proliferation, but at levels that are insufficient to prevent telomere shortening. Telomerase synthesizes telomeric DNA to replace the DNA lost during cell division. The essential subunits include an RNA molecule (TElomerase RNA; TER; encoded by a gene designated TERC, which is an abbreviation of TElomerase RNA Component) that acts as the template for synthesis of telomeric DNA, the reverse transcriptase catalytic subunit (TElomerase Reverse Transcriptase; TERT) that carries out the synthesis, and dyskerin (encoded by the DKC1 gene), a protein that binds to TER. Telomerase activity can be detected in some normal human somatic cells, especially cells in highly proliferative tissue compartments such as the bone marrow, skin, mucous membranes and epithelia of the gastrointestinal tract (GIT), but not at sufficient levels to completely prevent telomere shortening. Telomerase has an important role in these tissues, because inherited mutations in any of the genes that encode one of the three telomerase components (TERT, TERC or DKC1), result in a condition called Dyskeratosis Congenita which is characterized by premature failure of proliferative capacity in tissues such as the bone marrow, skin and GIT. In contrast, at least 85% of all cancers contain sufficient levels of telomerase to prevent telomere shortening and the percentage is even higher in most types of carcinomas. The factors controlling hTERT expression are not well understood but it is known that hTERT can be upregulated by ►MYC. If TERT expression is artificially switched on by genetic manipulation in normal cells, it is usually able to induce telomerase enzyme activity, because there is usually expression of the other telomerase subunits. This prevents telomere shortening and in some types of human cells this results in immortalization. Inhibiting telomerase activity in cancer cells may cause cellular senescence or cell death, so telomerase is an attractive target for the development of new anticancer treatments.

Alternative Lengthening of Telomeres (ALT)

Some immortalized cell lines and cancers have no detectable telomerase activity and maintain their telomeres by an alternative mechanism, referred to as Alternative Lengthening of Telomeres (ALT). Overall, about 8–10% of human tumors utilize ALT to prevent telomere shortening. Although the details are not fully understood, ALT is likely to be a recombinational mechanism in which one telomere uses another telomere (or itself via looping back) as a template for synthesis of new telomeric DNA. The ALT mechanism depends on the activity of the ►MRN complex which is known to be involved in ►homologous recombination. Cells that maintain their telomeres by ALT characteristically have very heterogeneous telomere lengths, ranging from undetectably short to extremely long. They also have substantial quantities of extrachromosomal telomeric repeat DNA, that may be either linear or circular, some of which is sequestered within ►PML nuclear bodies. The presence of telomeric DNA and telomere binding proteins within PML bodies is highly characteristic of ALT-positive cells, and may be used to determine whether a tumor utilizes the ALT mechanism. Types of tumors where ALT is common include ►glioblastoma multiforme (the most common primary brain tumor in adults), ►osteosarcomas, and some types of ►soft tissue sarcomas such as ►malignant fibrous histiocytomas and ►liposarcomas.

Tumor Suppressor Genes

Immortalization is facilitated by loss-of-function of the ►p16INK4a or ►RB1 genes, and the ►p53 gene. Loss of the normal function of these genes results in a significant, but finite, increase in cellular proliferative potential. This permits the accumulation of additional genetic changes and increases the probably that activation of a telomere maintenance mechanism will occur. Cells containing an inherited p53 mutation from individuals with ►Li-Fraumeni syndrome are the only type of human cells known to undergo immortalization spontaneously.

Clinical Relevance

Treatments that reverse the immortal phenotype may be a useful form of cancer therapy. An attractive target is telomerase, but inhibitors of telomerase may need to be combined with inhibitors of ALT to prevent the emergence of drug resistance.

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Senescence-associated Chronic Inflammation

►Aging-Associated Inflammation

Senile Involution

►Lobular Involution

Sensorineural

Definition

Associated with the inner ear or nerves involved in hearing.

►Connexins

Sentinel Lymph Nodes

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Synonyms

First-tier node; First-echelon node

Definition

►A sentinel lymph node is a lymph node upon which the primary tumor drains directly.

Characteristics

General anatomy and physiology of the lymphatic system

Lymphatic capillaries are 10–50 μm in diameter, consist of a single endothelial layer with a discontinuous membrane and are supported by collagen filaments. They are filled with lymph fluid originating from the interstitial space due to an osmotic pressure gradient and fluctuating intraluminal pressures. These intraluminal pressures are caused by lymphatic flow that is generated by lymph formation, contractions of the vessel wall and external pressure. Lymph fluid absorbed by lymphatic capillaries drains into larger collecting ►lymphatic vessels. Such lymphatic vessels drain into marginal and medullar sinuses located between germinal centers within a lymph node. These centers contain large numbers of phagocytic cells that accumulate protein colloids. Then, a plexus within the lymph node drains to the efferent lymphatic vessel that joins the artery and vein in the hilum. Direct drainage of the marginal sinus into the efferent vessel also exists (Fig. 1).

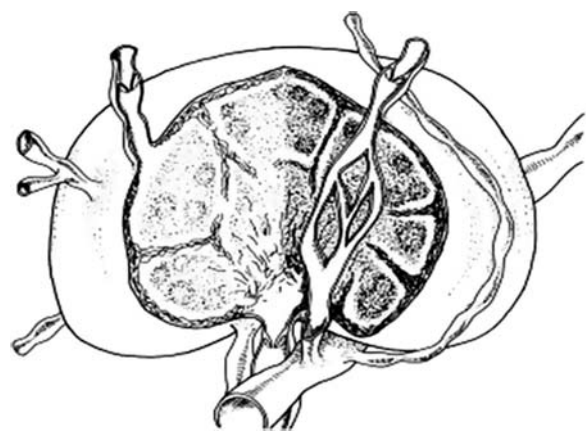
The Sentinel Node

The ►sentinel node is the lymph node upon which the primary tumor drains directly. Lymph fluid moves subsequently to ►second-tier and ►third-tier lymph nodes. Lymph from the primary tumor region does not necessarily travel to the nearest node. Two lymphatic channels originating in the primary tumor can run to two different sentinel lymph nodes (Fig. 2).

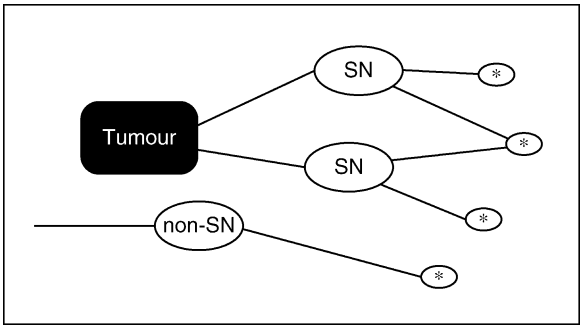
The sentinel node hypothesis implies orderly progression of ►metastases from a primary lesion through the ►lymphatic system. The concept is only relevant in tumors (►cancer) with pre-dominant lymphatic dissemination, such as melanoma and cancer of the breast, penis or colon. If the first node contains a metastasis, there is a chance of tumor spreading downstream. In case of a tumor-negative sentinel node, second-tier and third-tier nodes are generally without metastases.

Lymphatic Mapping

The lymphatic drainage pattern can be visualized by ►lymphoscintigraphy after injection of a ►radio-labeled tracer in or near the site of the tumor. The radio-labeled tracer is cleared from the lymphatic channels and accumulated by the phagocytic cells in the lymph node. ►Lymphoscintigraphic images depict



Sentinel Lymph Nodes. Figure 1 The different relations between lymphatic vessels and lymph nodes. Afferent lymphatic ducts on the left discharge their contents into the marginal sinus. One lymphatic duct runs through the node on the right and another over its surface, bypassing the germinal centers. (Illustration made by Tanis PJ; Reprinted from Tanis et al. (2001) Anatomy and physiology of lymphatic drainage of the breast from the perspective of sentinel node biopsy. J Am Coll Surg 192(3):399–409, with permission from "The American College of Surgeons").



Sentinel Lymph Nodes. Figure 2 A sentinel lymph node (SN) is the lymph node upon which the primary tumor drains directly. Two lymphatic channels originating in the primary tumor can run to two different sentinel lymph nodes. Lymph fluid moves subsequently to second-tier (*) and third-tier nodes. The SN is not always the node nearest to the primary tumor (Non-SN).

the lymph channels and the lymph node or nodes that contain the injected tracer. Dynamic scintigraphy and intraoperative ►blue dye mapping give insight in the lymphatic drainage pattern, which enables the surgeon to find the sentinel node(s).

The earliest sentinel lymph node identification techniques involved the injection of a vital blue dye, usually isosulfan blue. It was a key point in the general acceptance of ►sentinel node biopsy. The blue dye was injected intradermally at the primary tumor site in melanoma patients. An incision was made over the expected lymph node region and the lymphatic channel was visually identified. This channel was dissected and followed to the first draining lymph node.

Subsequent reports described the use of radio-labeled tracers, such as technetium-99m-bound colloids. Colloids with a small particle size can rapidly pass the openings of interendothelial junctions and allow visualization of the lymphatic channels leading directly to the sentinel node. A disadvantage of small sized particles is that some of the tracer moves on to nodes further downstream because phagocytic cells in the first node cannot trap them all. Larger colloid particles enter lymphatic channels more slowly. The tracer almost never moves on to subsequent nodes, but the channels are visualized less often.

Nowadays, the ►lymphatic mapping technique mostly used involves administration of a radio-labeled tracer into or near the primary lesion in combination with blue dye. During surgery, the sentinel node is found with the assistance of both blue dye and a ►gamma-ray detection probe. Preoperative lymphoscintigraphy is added for better specification of the location and number of sentinel nodes. Different methodologies based on these lymphatic mapping techniques are nowadays applied all over the world.

Sentinel Node Biopsy

A sentinel node biopsy is a minimally invasive technique that was initially developed as an alternative to complete lymph node dissection in patients with a melanoma. The majority of patients are spared a more complex surgical procedure with a higher morbidity rate while the same staging information is obtained.

All nodes of a complete node dissection used to be bisected and evaluated by haematoxylin-eosin staining. This way, metastases larger than 2 mm were usually identified. With the selective sentinel node biopsy, the pathologist can focus on the one or few nodes that are most likely to contain metastatic disease. The sentinel nodes are evaluated by both haematoxylin-eosin and immunohistochemistry staining, which occasionally distinguish metastases with a size of one tumor cell.

The combined procedure of lymphatic mapping and sentinel node biopsy provides prognostic information, identifies patients who may benefit from early regional therapy (►locoregional therapy) and, depending on the situation, from adjuvant systemic treatment (►adjuvant therapy). This way optimal survival rates may be realized.

Breast Cancer

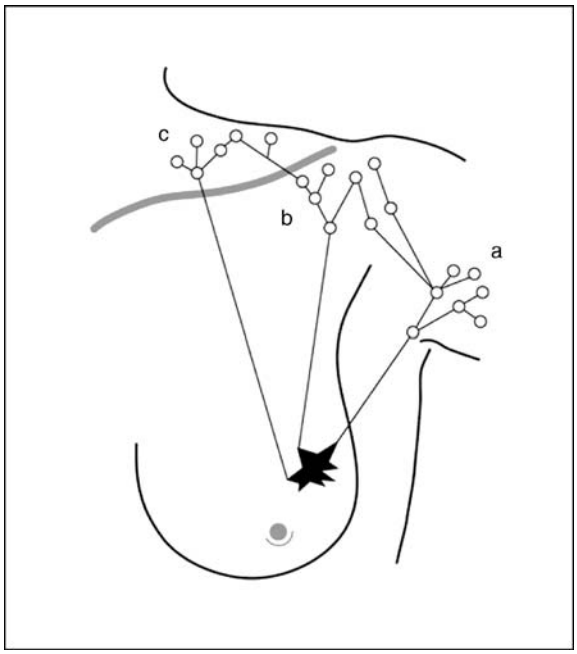
The predominant lymphatic drainage pathway from the breast is towards the axilla. Metastases initially remain localized in the lower axilla and then may travel higher up the chain to the subclavicular and the supraclavicular basins (Figs. 3 and 4).

Axillary lymph node dissection used to be performed in almost every breast cancer patient. This operation has several side effects, such as lymph oedema, pain and decreased mobility of the arm, and often no metastases were found. With the introduction of sentinel node biopsy, axillary lymph node dissection is only indicated if this node is involved. As a result, many patients are spared an unnecessary operation.

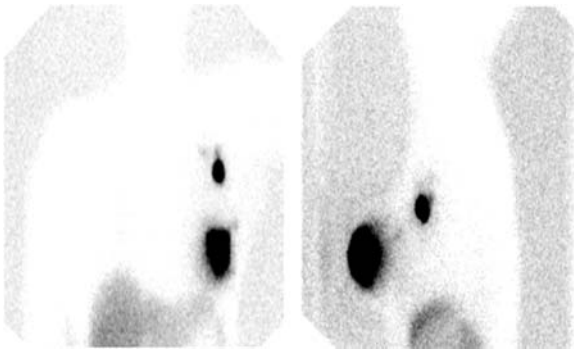
Whether the omission of routine axillary node dissection jeopardizes regional tumor control and survival is still subject of research. Large observational studies revealed excellent results in patients who did not receive axillary node dissection because of a tumor-negative sentinel node. Recurrence rates vary between 0.12% and 0.6% and these numbers do not exceed the known recurrence rates after routine axillary clearance.

Melanoma

There is consensus on the way lymphatic mapping should be carried out in melanoma patients. Preoperative lymphoscintigraphy and intraoperative use of blue dye and a gamma-ray detection probe are standard. The first large studies on sentinel node biopsy in melanoma showed a 95% sensitivity. Recent studies show false-negative rates of around 10%. False-negative means that



Sentinel Lymph Nodes. Figure 3 A tumor (black area) with lymphatic channels to nodes in the axilla (a) and to lymph nodes below (b) and above (c) the clavicula. A metastasis may be found along this lymphatic pathway.



Sentinel Lymph Nodes. Figure 4 Anterior and lateral lymphoscintigrapic images of a woman with left-sided breast cancer. A sentinel node and several second-tier nodes are visualized in the axilla.

the sentinel node is disease-free, while there are metastases in the lymph node basin.

Patients with an involved sentinel node have a 5-year survival rate of around 65% and in patients with a tumor-negative sentinel node this is 90%. A large randomized study showed that early regional node dissection based on a positive sentinel node improves survival in patients with an intermediate-thickness melanoma.

Concluding Remarks

The development of the sentinel node concept is a milestone in the understanding of dissemination of solid malignancies. The introduction of lymphatic mapping in 1989 initiated the widespread use and general acceptance of this approach. Now that the technique has been validated, many patients are spared unnecessary surgery without compromising regional control and the accuracy of staging.

Lymphatic mapping with ►sentinel lymph node biopsy has become a standard component in the management of patients with breast cancer or melanoma. This suggests potential in other tumors that spread primarily through lymphatic channels.

In the future, studies need to focus on more peripheral issues such as the prognostic significance of ►micrometastases and techniques such as molecular assays or markers. These may provide more information to optimize the staging of tumor dissemination and will enable the fine-tuning of therapy.

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Sentinel Node

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Synonyms

Draining lymph node

Definition

Sentinel means a lookout, and the sentinel node is defined as the first ►lymph node or nodes that are located on the direct draining lymphatic route from the area of a primary tumor.

Characteristics

The ►lymphatic system was first described in the seventeenth century by Olof Rudbeck who systematically studied the lymphatic vessels collecting extra cellular fluids from tissues emptying into ►lymph nodes. The lymphatic system is more variable then the blood system and is anatomically less well defined. The drainage from tumors seems to vary considerably, making prediction of the draining lymph node difficult without guidance. Tumor cells become metastatic either by invasive growth through basal membranes or by entering into capillaries. The endothelial cells comprising the lymph capillaries are widely fenestrated (►Fenestration) making easy access to the lymph vessel. There is also an active transport of primarily white blood cells into the lymphatic vessels but tumor cells may also use this mechanism. After entering into the capillaries of the lymphatic vessel, the metastatic cell may then enter into the draining lymph node. Thus, the tumor draining lymph node is the primary location to find lymph node metastases. If tumor cells are present in the sentinel node, the risk of systemic dissemination of the disease is high since about half of the lymphatic fluid entering a lymph node continues directly to the blood circulation via ►lymphovenous shunts.

In 1960 Gould first described a sentinel node draining a cancer of the parotid gland. Cabanas introduced the technique in 1977 for guidance of the surgical procedure for inguinofemoroiliac lymph node dissection of patients with penile carcinoma. This first study demonstrated two important principles regarding sentinel node investigations; (i) the sentinel node is the first site of metastases and may be the only lymph node involved containing metastatic cells (ii) sentinel nodes negative for metastatic cells suggest that no spreading of the disease has occurred and this finding is coupled to increased survival. Studies of prognostic factors in colorectal cancer point out the histological status of the regional lymph nodes as the most important predictor of survival and the presence of regional lymph node metastases implies a 50% reduction in 5-year survival rates. Similar observations have been reported in most solid tumors. However, the quality of the staging process of lymph node investigations are dependent on the total number of locoregional lymph nodes identified and the extent of the following histopathology investigation.

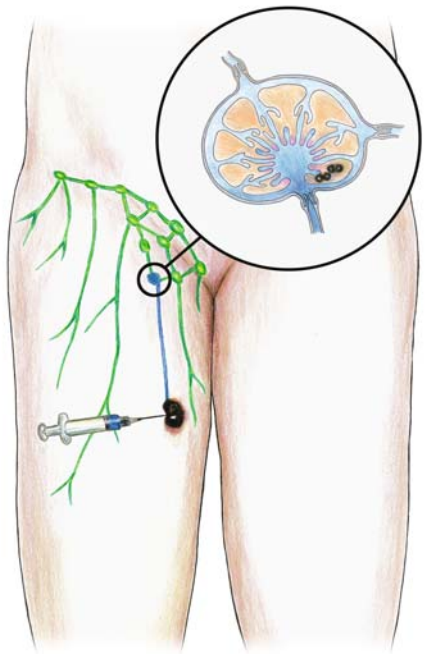
Malignant tumor often induce a peritumoral upregulation of lymphatic vessels and blood vessels by producing ►angiogenic factors such as the vascular endothelial growth factor (►VEGF), making the pathways for lymphatic drainage difficult to predict. Studies have shown that elevated levels of the cytokine cascade of VEGF-C and VEGF-D promote tumoral ►lymphangiogenesis and that inhibition of their joint receptor, VEGFR-3, suppresses the effects. Further, elevated levels of VEGF-C and VEGFR-3 correlate with presence of lymph node metastases and lymphatic invasion in human colorectal cancer. A large study of patients with breast cancer demonstrated an increased presence of lymphatic vessels in metastatic axillary lymph nodes (85%) compared to nonmetastatic lymph nodes (25%) and that intra- and perinodal lymphatic endothelial cell proliferation fractions were higher in metastatically involved lymph nodes. These discoveries underline the association of lymphangiogenesis with lymph node metastases.

Sentinel Node Procedure

The sentinel node is either located before surgery through lymphoscintigraphy or during surgery by the superficial injection of a tracer substance around the tumor (Fig. 1). When the tumor is located in the skin, the injection is usually placed around it in four places subcutaneously or intradermally. In visceral tumors (colorectal and gastric tumors) the injections should be superficial, precisely beneath the serosa whereas in parenchymatous organs (liver) the injections are preferably placed under the capsula into the parenchyma around the lesion. When approaching the tumor from the mucosal side, the injections are made through the mucosa and into the muscular wall (urinary bladder tumors). Within a few minutes the tracer has entered the ►fenestrated lymph capillaries. The tracer is then transported inside lymph vessels and accumulates through phagocytosis by ►dendritic cells in the sentinel node(s). Coal particles, dyes such as patent blue, isosulfan blue, and/or radioactive ^{99}Technetium colloid markers have been used as tracers for intraoperative ocular identification or detection using a hand held γ-counter respectively. A preoperative lymphoscintigraphy can facilitate the location of the regional lymph node basin draining the tumor and may be combined with computerized tomography for precise anatomical information in certain cases. The procedure is dependent on the surgeon's experience with the method and initial procedures are more likely to give rise to false negative sentinel lymph node, i.e., sentinel node does not contain metastatic cells whereas other regional lymph nodes are positive for tumor cells. The false negative rates in large series performed by experienced surgeons are usually as low as 3–5%.

Clinical Relevance

During recent years the importance of correct staging (►Staging of tumors) regarding lymph node metastases including presence of micro metastases has become evident for most types of human solid tumors. However,



Sentinel Node. Figure 1 The sentinel node concept. Identification of the tumor draining (sentinel) node is accomplished by peritumoral injections of Patent blue dye and/or a radioactive tracer ^{99}Technetium. The tracers enter into the lymph capillaries, which follows the lymphatic drainage and accumulation in a lymph node. During surgery the area of drainage is explored after the guidance of a γ-counter and the sentinel node is visualized by dissection. Since the tumor is in contact with the sentinel node, the sentinel node is the preferred site for metastases. When the sentinel node is investigated microscopically, it can be considered diagnostic for the whole lymph node region, guiding prognosis and therapy. In a progressively growing tumor, there is a rapid turnover of cells and disruption of normal tissue architecture, causing release of inflammatory mediators and attraction of macrophages and dendritic cells. Tumor cell debris is phagocytosed by these antigen presenting cells, which after the encounter migrate to the sentinel node, presenting peptides derived from tumor antigens to naïve T lymphocytes. Sentinel node lymphocytes mount proliferative responses and become clonally expanded against tumor antigen in an attempt to fight cancer.

the search for metastases or micro metastases among a large number of lymph nodes in a pathology specimen is time-consuming, labor-intensive, and expensive. Therefore the use of the sentinel node technique has received increasing interest since it entails detailed investigation of (in most cases) only one to three lymph nodes. Thus an increased awareness regarding an extended histopathological investigation of lymph nodes for staging and guidance for therapy has become apparent. The sentinel node concept entails the possibility of multiple drainage pathways from different parts of the primary tumor, thus a patient may have more than one sentinel node. The tumor status of this node reflects the status of the regional lymphatic field and has a strong impact on prognosis. Thus, the sentinel node procedure is increasingly used in malignant melanoma and breast cancer where it has become part of the standard staging (Staging of tumors) procedure and an important factor to consider when deciding about postoperative adjuvant therapy. In fact even insurance companies have taken the status of the sentinel node into account when determining life insurances. In a large randomized study of patients with malignant melanoma, sentinel node biopsy was compared with nodal observation demonstrating that sentinel node biopsy-guided staging provides important prognostic information and furthermore identifies patients that may benefit from immediate lymphadenectomy. In breast cancer, the introduction of sentinel node biopsy has limited the number of axillary lymph node dissections. Thus, the removal of the tumor draining sentinel node permits a smaller surgical axillary procedure and decreases the risk for different side effects including lymphoedema and nerve damage. The 5-year survival rate in patients with negative sentinel node biopsies is not different from women with breast cancer having undergone axillary lymph node dissection without the presence of metastases. Thus, sentinel node biopsy is a safe and accurate metastases screening method for patients with breast cancer. The sentinel node procedure has recently been shown to be applicable in many solid tumors including colorectal cancer, urinary bladder cancer, vulvae cancer, and gastric cancer, and the procedure may be valid for staging the majority of solid tumors.

Immunology of the Sentinel Node

According to the immune surveillance hypothesis, the immune system is continuously sensitized against developing tumors, most of which are eliminated at an early stage. Not only metastatic cells from the tumor enter into the lymphatic vessels but also antigen presenting cells, dendritic cells, which have endocytosed dying tumor cells, or debris from tumor cells containing ►tumor antigens, accumulate in the sentinel node. Since experimental evidence indicates that activation of naïve T cells (►T-cell response) occurs within the highly specialized microenvironment of secondary lymphoid organs, i.e., lymph nodes, the sentinel node may be regarded as the primary site for the immune system to encounter tumor antigens. Thus sentinel node acquired lymphocytes are clonally expanded (►Clonal expansion) T cells recognizing tumor antigens and may therefore serve as a useful source for immunotherapy.

►Adoptive Immunotherapy
►Immunotherapy
►Sentinel Lymph Node

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Sentinel Node Biopsy

Definition

A minimally invasive technique to remove the first node in the draining lymphatic basin. The sentinel node is examined by the pathologist for cancer cells.

►Sentinel Lymph Nodes

Sentinel Vessels

Definition

Dilated episcleral vessels that provide nourishment to an underlying ciliary body tumor.

►Uveal Melanoma

SEP

Definition

Solitary extraosseous or extramedullary plasmacytoma.

►Plasmacytoma

Separin

Definition

A protease, also called separase, that cleaves the cohesin subunit Scc1/Mcd1 to induce sister-chromatid segregation. Its C-terminus is conserved, it.is inhibited by association with securin at the non conserved N-terminus.

Sequence Identification

Definition

Determination of the primary structure of a biomolecule (e.g. amino acids; DNA).

►Oncopeptidomics

Sequestered

Definition

In biochemistry a term, e.g., for a protein, synonymous to covered up, shielded, locked away resulting in biologically not available orphan receptor. The collective term for receptors with unidentified ligands/without known ligands.

►Arachidonic Acid Pathway

Ser/Thr-Phosphorylation

Definition

Protein phosphorylation is a post-translational modification carried out by enzymatic transfer of a phosphate group from a donor-molecule to a polypeptide backbone. The enzymes that catalyze this type of reactions are known as kinases. They transfer phosphate groups either to tyrosine residues (Tyr-kinases) or to serine or threonine residues (Ser/Thr-kinases) according to defined motifs within the polypeptide.

►Cystatins

SERCA

Definition

A pump situated in the membrane of the sarcoplasmic/endoplasmic reticulum that couples ATP hydrolysis to the import of Ca^{2+} from the cytosol to the endoplasmic reticulum lumen.

►Endoplasmic Reticulum Stress

Serex

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Definition

A method for identification and molecular analysis of antigens by recombinant expression cloning; Acronym for *se*rological analysis of antigens by *re*combinant *ex*pression cloning.

Characteristics

For SEREX, a cDNA library is constructed from fresh tumor specimens, cloned into λ phage expression vectors and phages are used to transfect E. coli. Recombinant proteins which are expressed during the lytic infection of the bacteria are transferred onto a nitrocellulose membrane. These membranes are incubated with the diluted serum of the autologous patients and screened for clones reactive with high-titered IgG antibodies. Positive clones are visualized by staining after incubation with an enzyme-conjugated secondary antibody specific for human IgG. Positive clones are subcloned to monoclonality, and the nucleotide sequence of the inserted cDNA is then determined.

The SEREX approach is technically characterized by several features:

- The use of fresh tissue obviates the need for culturing cells in vitro and therefore circumvents in vitro artifacts.
- The analysis is restricted to genes that are expressed by the tissue in vivo.
- The use of high-titered IgG antibodies in the initial screening procedure limits the analysis to such antigens which elicit a strong immune response in the host and imply a cognate T-cell help.
- The serological analysis covers the whole repertoire of proteins expressed by the respective tissue.
- SEREX uses polyspecific sera to scrutinize monoclonal antigens which are highly enriched in lytic plaques. This allows for a direct molecular definition of antigens, since the cDNA sequence of the antigen can be determined instantaneously.
- The specificity of the antigen, i.e. its expression spectrum is determined by the analysis of the mRNA expression pattern by Northern blots and RT PCR.
- If defined types of antigens are to be preselected for, the original SEREX approach can be modified appropriately by using biased cDNA libraries (e.g.normal testis) or modified detection systems (e.g. for IgA, IgM).

To overcome the problem of incorrect folding and the lack of posttranslational modifications, which are both inherent problems of bacterial expression systems, a eukaryotic expression system in yeast, designated as "recombinant antigen expression on yeast surface" (RAYS) has been established. For RAYS, a cDNA-library is cloned for expression as Aga2 fusion proteins on the yeast surface. After incubation with patient's sera, FACS-sorted positive clones are spotted onto 96-well-plates, re-analyzed for specific detection and the nucleotide sequence for the cDNA insert is then determined.

Cellular and Molecular Regulation

High-titered IgG responses imply a cognate T-cell help. Therefore, in an approach of "reverse T-cell immunology" antigens detected by SEREX can be used for the definition of epitopes that are presented in the context of MHC I and MHC II. Preferably, specific T-cell reactivities are looked for in patients with high serum antibody reactivity to the respective antigen, and for many SEREX antigens both CD4 and CD8 stimulating epitopes have been identified.

Clinical Relevance

SEREX allows for an unbiased search and the direct molecular definition of immunogenic proteins based on their reactivity with autologous and allogeneic patient sera. Hence, while SEREX was originally developed for the serological analysis of human ►tumor antigens, it can be used whenever antibody reactivities against tissue antigens are suspected and neither the antibody nor the antigen is known, e.g. for the identification and molecular characterization of ►autoantigens in ►autoimmune diseases. Using the RAYS approach might enable us to identify antigens that have escaped detection to date, because they elicit immune responses against post-translational modified or conformational epitopes not detectable by conventional SEREX. An international effort led by the Ludwig Institute for Cancer Research aims at defining the entire spectrum of antigens expressed by human tumors. All SEREX data are entered into the Cancer Immunome Database,

which is accessible to the public (http://www2.licr.org/CancerImmunomeDB/.)

►Autoantibodies

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Serine Elastase

►Neutrophil Elastase

Serine Protease

Definition

A class of enzymes that cleave peptide bonds in proteins that contain a serine residue in the active site of the enzyme. They play an important role in digestion, blood clotting, and the complement system.

►Protease Activated Receptor Family

Serine Protease Inhibitor

Definition

Large group of proteins that inhibit or antagonize the biosynthesis or actions of serine-type proteases. Serine proteases share a common reaction mechanism based on the formation of an acyl–enzyme intermediate on a specific active serine residue.

►Class II Tumor Suppressor Genes

Serine Protease Inhibitor-like Domain

►RECK Glycoprotein

Serine Proteases (Type II) Spanning the Plasma Membrane

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Synonyms

Matriptase: Epithin, MT-SP1, suppression of tumorigenicity 14

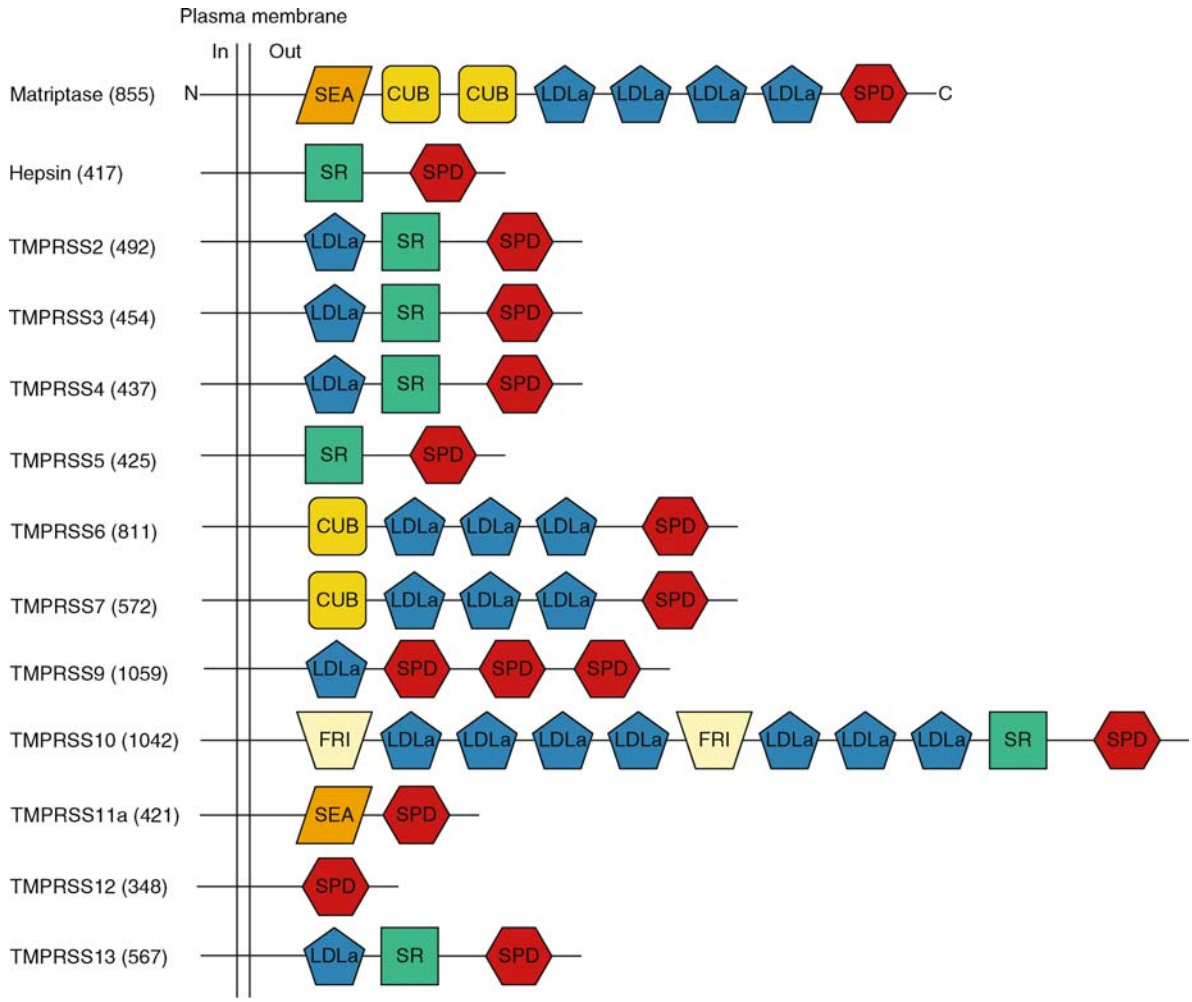
Transmembrane protease serine 1–13: hepsin (TMPRSS 1); Epitheliasin (TMPRSS 2); TMPRSS2 = TMPRSS4; Spinesin (TMPRSS5), matriptase-2 (TMPRSS6), matriptase-3 (TMPRSS7), distal intestinal serine protease (mouse only, TMPRSS8), polyserase (TMPRSS9), Corin (TMPRSS10), TMPRSS11–13 (human genes)

Definition

The family of type II transmembrane serine proteases consists of 14 members identified to date. They share a transmembrane orientation with an intracellular N-terminus and a basic, multidomain structure, which contains several individually folded protein modules in the extracellular domain (Fig. 1). Members of the family possess specific tissue and cell type distributions. For example ►Matriptase and ►Hepsin are epithelial, while ►Corin (TMPRSS10) is expressed in cardiac muscle cells. Matriptase, TMPRSS2 and Hepsin are associated with cancer development or progression.

Characteristics

The serine protease domain, which is located at the C-terminal end of all family members, encodes



Serine Proteases (Type II) Spanning the Plasma Membrane. Figure 1 Domain structures of the known type II transmembrane serine proteases. All are oriented with their amino terminal in the cytoplasm, a single transmembrane spanning domain and their carboxy terminal exposed extracellularlly. They are not drawn to scale but the total amino acid residues for each are indicated in parentheses. TMPRSS11 has known isoforms (a, b, d, e, and f) with similar domain structures and vary by only a few amino acids in length. Domains are abbreviated as; SEA: sea urchin sperm protein, enterokinase and agrin; CUB: complement C1r/C1s, Uegf and BMP-1; LDLa: low density lipoprotein receptor class A; SPD: trypsin like serine protease; SR: scavenger receptor cysteine-rich; FRI: frizzled.

the central function of the extracellular domain. The mechanism of activation, substrate specificity and physiological function of the serine protease domain are best understood for Matriptase and are described below. Hepsin is functionally related to matriptase in its ability to autoactivate, digest epithelial basement membrane proteins and activate hepatocyte growth factor/►scatter factor. Hepsin was discovered in liver and reported as overexpressed in metastatic prostate cancer cells. TMPRSS2 is preferentially expressed in the prostate under the regulation of androgen and the fusion of its promoter with genes from the family of ►ETS transcription factor plays an important role in prostate cancer development and progression (►TMPRSS2/ERG Fusions). TMPRSS2 is, itself, over-expressed in the majority of advanced prostate cancers where it often becomes mis-localized away from the plasma membrane.

Regulation of Activity

The activity of matriptase is regulated through autoactivation and is facilitated by lysophospholipids, steroid hormones and the polyanionic compound, ►suramin. For complete activation, matriptase requires glycosylation and serine protease domains to increase protein stability, an intact ►LDL receptor domain and an initial cleavage at Gly149 in the juxtamembrane

►SEA domain. In addition, the interaction of the LDL receptor domain with the serine protease inhibitor, hepatocyte activator inhibitor-1 (HAI-1) is critical for autoactivation. Active matriptase has a limited range of known substrates. These are ►HGF, urokinase plasminogen activator (►Plasminogen Activating System), ►stromelysin (Matrix Metalloproteinases), ►protease activated receptor (PAR-2) and ►extracellular matrix and cell ►adhesion proteins.

The enzymatic activities of matriptase and hepsin are inhibited by HAI-1, a membrane-associated Kunitz type-1 serine protease inhibitor. HAI-1 shares amino acid sequence homology and a similar domain structure with HAI-2 and consists of two Kunitz domains (N-terminal KD-1 and C-terminal KD-2) intersected by a low-density lipoprotein receptor (LDLR)-like domain. HAI-1 has a remarkable and unique specificity for a serine protease inhibitor and only inhibits the serine proteases matriptase, hepsin, prostasin, hepatocyte growth factor activator (HGFA) and a phosphatidylinositol (GPI)-anchored epithelial serine protease. Through regulation of matriptase, hepsin or HGFA activity, HAI-1 controls the amount of biologically active two-chain HGF on the cell surface and the activation of the Met receptor (►MET).

Biological Responses

Matriptase and hepsin have been shown to facilitate the invasion and metastasis of cancer cells through proteolysis of extracellular matrix, activation of uPA and MMP-3 and increasing in ►angiogenesis. Transgenic mice overexpressing hepsin show a defect in the epithelial basement membrane, characterized by loss and disorganization of collagen-IV and laminin-5 (►Adhesion; ►laminin signaling).

Several proteases and proteolytic cascades mediate the activation of HGF (►Scatter factor). Matriptase and hepsin are central proteases for HGF activation, since pro-HGF is their direct substrate. In addition, matriptase also activates uPA, and thereby magnifies the activity for HGF activation on the cell surface.

Studies in genetic mouse models reveal that matriptase is required for post-natal survival by maintaining the barrier function of the epidermis. Matriptase deficiency phenocopies the loss of its proteolytic target, the serine protease, prostasin/CAP1/Prss8. Matriptase also plays an important role in hair follicle development, thymic homeostasis and keratinocyte differentiation. In humans, matriptase is shed from the cell surface and is found complexed with HAI-1 in human milk.

Expression in Cancer

Matriptase is expressed on cancer cells, that originate from a variety of epithelial origins and has been implicated in cancer development, progression and ►angiogenesis. Matriptase translocates from a perinuclear reservoir to activation foci in cell-cell junctions and later moves to membrane protrusions in response to growth factor stimulation. On the cell surface, the balance between matriptase and hepatocyte activator inhibitor-1 (HAI-1) and HAI-2 regulates matriptase activity. The ratio of matriptase to HAI-1/HAI-2 expression increased with tumor grade and progression in ovarian, cervical, prostate and colon cancer. The resulting increase in active matriptase on the cell surface facilitates invasion and metastasis of cancer cells.

Matriptase and HAI-1 are indirectly regulated by ►androgens. In a ►prostate cancer cell line, treatment with ►testosterone transiently increased matriptase expression and activity and increased shedding of proteolytically cleaved HAI-1. As a result an increase in HAI-1–matriptase complexes occurred in the extracellular space. In normal human prostate glands, HAI-1 protein expression decreased after androgen suppressive therapy, however, in cancerous glands, intracellular protein expression was not affected by androgen.

Clinical Relevance

Pathologic deregulation of matriptase, hepsin or TMPRSS2 can result from increased expression, activation and from an imbalance relative to their cognate inhibitors, e.g. HAI-1. Elevated expression of matriptase and HAI-1 are associated with poor outcome in several human cancers, including node-negative breast, prostate and ovarian cancers. In a transgenic mouse model modest expression of matriptase in epidermis is sufficient to cause ►squamous cell carcinoma, and matriptase expression enhances ►gastric cancer metastasis in ►nude mice. Forced expression of Hepsin in prostate cancer stimulates metastasis while inhibiting local growth. Consistent with matriptase's role in cancer, inhibition of matriptase expression or activity by matriptase-specific small molecular inhibitors, matriptase ►anti-sense, or ►siRNA, suppresses cancer growth in cell culture and ►xenograft models.

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Serine/Threonine Kinase

Definition

Enzyme that adds phosphate group to serine or threonine amino acid residue in substrate protein.

►Herceptin
►Protein Kinase C Family

Serine-Threonine Kinase Receptor-Associated Protein

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Synonyms

hMAWD: Human MAPK activator with WD repeats; Unrip: unr-interacting protein; STRAP

Definition

STRAP is a 39 kDa protein of WD-40 family involved in probable chaperoning function during the formation of multiprotein complexes shown to be active in the ►Transforming growth factor-β (TGF-β) receptor signaling network and U-rich small nuclear ribonucleoprotein (U snRNP) assembly. ►Oncogenic STRAP is up-regulated in human cancers and may be involved in tumor progression.

Characteristics

STRAP was first cloned from mouse embryonic cDNA library while searching for novel proteins that bind the cytoplasmic region of TGF-β type I receptor (TβRI) using yeast two-hybrid screening. Later it was found that STRAP could associate with both type I and type II (TβRII) serine-threonine kinase receptors. Southern blot analyses demonstrate that STRAP is evolutionarily conserved from yeast to mammals. The importance of this conservation was revealed when STRAP knockout mice were generated using the gene-trap mutagenesis technology coupled with ►microarray in the process to identify the probable targets of ►platelet-derived growth factor (PDGF) signaling. *STRAP* was found to be a PDGF-BB-inducible gene. The gene trap insertion results in embryonic lethality between embryonic day (E) 10.5 and E12.5. Homozygous mutant embryos had defects in ►angiogenesis, cardiogenesis, somitogenesis, neural tube closure and embryonic turning. This highlights an indispensable function for STRAP during early development. Later STRAP was also identified in humans as an interacting protein with upstream of N-ras (unr) that is involved in the internal initiation of translation of human rhinovirus RNA and is implicated in cap-independent translation.

STRAP belongs to the family of WD repeat proteins that are known to have four or more repeating units containing a conserved core of approximately 40 amino acids that mostly end with tryptophan-aspartic acid (WD). Most of them are thought to form a circularized β propeller structure. Though the underlying common function is coordinating multiprotein complex assemblies, these proteins are also involved in various cellular processes like signal transduction, transcriptional regulation, programmed cell death, RNA synthesis/processing, chromatin assembly, cell cycle progression and vesicular trafficking. Other common examples of WD repeat proteins are the β subunit of the ►G proteins, TATA box binding protein associated factor II (TAFII), ►apoptotic protease-activating factor 1 (APAF-1), retinoblastoma-binding protein p48 (RbAp-48), receptor activated protein kinase C 1 (RACK1), and phospholipase A2-activating protein (PLAP) or TGF-beta receptor-interacting protein 1 (TRIP-1), which is also known to interact with TβRII.

The human *STRAP* gene is placed in chromosome 12p12.3 near the marker D12S1593. Northern blot analysis, using different tissues in mice, indicated a major transcript of 1.8 kb, though in some tissues a larger transcript was detectable. This may suggest alternative splicing of the STRAP RNA at least in some tissues. It is ubiquitously expressed in all mouse tissues with highest levels in liver and testes and less abundantly in spleen. In humans, STRAP expression has also been shown to be ubiquitous and it forms a 2 kb transcript. Both human and mouse *STRAP* genes contain 10 exons, which finally form a 350 amino acid protein migrating with an apparent mass of 39 kD on SDS-PAGE. Murine STRAP has more than 97% amino acid identity over the entire sequence with its human version. Sequence analysis indicates that STRAP contains six WD40 repeats. STRAP shows a 55% similarity in base pairs and a 19% similarity in amino acid sequence to another known WD40 protein, TRIP-1. Some of these similarities are among the conserved amino acid residues within the WD repeats. STRAP is localized predominantly in the cytoplasm but a good level is also present in the nucleus. It forms homo-oligomers probably through the WD repeats and this may be important for the multi-protein complex assembly. The physical interaction of STRAP with the TGF-β receptor complex raises the possibility that STRAP is a substrate of the receptors. Our findings showed that an increase in the phosphorylation of STRAP

requires the kinase activity of receptors in vivo, but STRAP does not appear to be a direct substrate of the receptors during in vitro kinase assays. The C-terminal 57 amino acids are important for this phosphorylation. Eukaryotic Linear Motif resource (ELM) search indicates the presence of putative phosphorylation sites in the C-terminal region for the casein kinases I and II. Multiple phosphorylation sites also seem to be present inside the different WD repeat domains for different kinases and may serve to modify the function of STRAP.

Apart from TβRI and TβRII, STRAP also binds with ►Smad2, ►Smad3, ►Smad6, ►Smad7, 3-phosphoinositide-dependent protein kinase 1 (PDK1), Ewing's sarcoma protein (EWS), hMAWD binding protein (MAWBP), unr, microtubule associated protein 1B (MAP1B), nuclear export factor (NXF) proteins, Gemin6, Gemin7, and 3 small nuclear ribonucleoproteins (SmB), SmD2, SmD3. Additionally, using ELM motif search, it also shows putative binding sites for other proteins like C-terminal Binding Protein (CtBP), protein phosphatase 1, retinoblastoma protein (pRb), TNF receptor-associated factor 2 (TRAF-2), and glycosaminoglycans and also has binding sites for domains like class II PDZ domain, ►SH2 domain and class IV WW domain. STRAP also shows the presence of multiple potential membrane targeting N-myristoylation sites, at least one of which is outside the WD repeat domains in the C-terminal region. Structural analysis of the WD repeat proteins in general shows that they act as a very rigid platform or scaffold, irrespective of the proteins with which they interact.

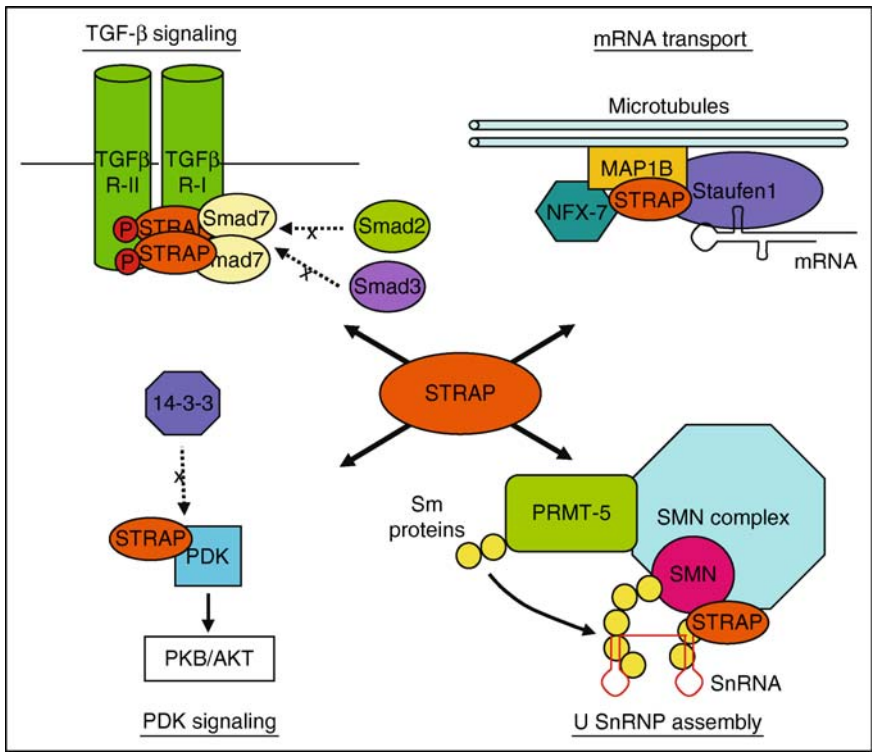
Mechanisms

STRAP binds with both TβRI and TβRII in a ligand independent manner. It synergizes specifically with Smad7, but not with another inhibitor Smad6, in the inhibition of TGF-β signaling. STRAP stabilizes the association between Smad7 and activated receptor complex, thus assisting Smad7 in preventing phosphorylation and activation of Smad2 and Smad3 by the receptor complex. Though Smad6 is also shown to interact with STRAP, this association does not seem to interfere with bone morphogenic protein (BMP) signaling in which Smad6 acts as an inhibitor. STRAP inhibits TGF-β-induced nuclear translocation of Smad2/3 and Smad4 and as a result, activation of TGF-β responsive reporter genes including *plasminogen activator inhibitor 1 (PAI-1)* and ►$p21^{Cip1}$ is abrogated. Downregulation of $p21^{Cip1}$ by STRAP leads to hyperphosphorylation of ►retinoblastoma protein (pRb). In vitro kinase assay demonstrated that overexpression of STRAP can induce extracellular signal-regulated kinase (MEK/ERK) activity in a TGF-β-independent manner. Activation of MEK/ERK pathway by endogenous STRAP was further confirmed by knocking it down using ►small interfering RNA (siRNA). Although STRAP is not a kinase, it may facilitate the activation of MAPK pathway by functioning as a chaperone for upstream kinases. Therefore, STRAP may inhibit activation and nuclear translocation of Smad2 and Smad3 by interacting with receptors and Smad7 and/or by activating MAPK/ERK pathway (Figs. 1 and 2).

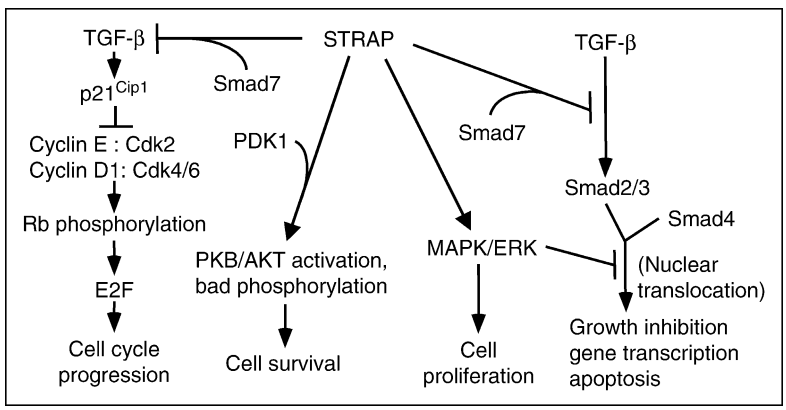
3-phosphoinositide-dependent protein kinase-1 (PDK1) has been shown to phosphorylate and activate many members of the protein kinase A, G, and C subfamily that include protein kinase B (PKB), p70 S6 kinase, protein kinase A and serum/glucocorticoid regulated kinase (SGK). STRAP interacts with the catalytic domain of PDK1 and this interaction is important for the modulation of PDK1 activity. The interaction of STRAP and PDK1 is inhibited by TGF-β and induced by insulin. This induction in binding by insulin is abrogated by wortmannin, a PI3K inhibitor. The mechanism behind PDK1 activation by STRAP is thought to be due to displacement of the 14-3-3 protein from PDK1 complex, which negatively regulates it (Fig. 1). The binding of PDK1 with STRAP also potentiates negative regulation of TGF-β mediated transcription by STRAP. This repression occurs through increased association of Smad7 with both STRAP and TβRI. Cell survival is induced by this interaction between STRAP and PDK1 probably through phosphorylation of Bad and attenuation of ►tumor necrosis factor-alpha (TNF-α) induced ►apoptosis.

STRAP is localized in both cytoplasm and nucleus. It colocalizes and associates with the oncogenic EWS protein in the nucleus through its NH_2 and COOH termini. STRAP inhibits the interaction between EWS and p300, a protein that is a transcriptional co-activator of EWS. This results in downregulation of EWS target genes like *ApoCIII* and *c-fos.* Although TGF-β has no effect on the interaction between STRAP and EWS, TGF-β-dependent transcription is inhibited by EWS.

Unr is an RNA binding protein that plays an important role in the initiation of HRV-IRES dependent translation of the animal picornavirus RNA, like the rhinoviral RNA. STRAP interacts with unr and is thought to be important during the translation stimulation activity. The macromolecular survival motor neuron (SMN) complex that helps in the assembly of spliceosomal Uridine-rich small ribonucleoprotein (U snRNP), contains the SMN protein and six additional proteins, named Gemin2-7, according to their localization (Fig. 1). STRAP seems to be involved in this assembly through its interaction with Gemin6 and Gemin7 as well as SmB, SmD2 and SmD3 components of the SMN complex. Although STRAP is localized in both cytoplasm and nucleus, it is present predominantly in the cytoplasm and may help the nuclear-cytoplasmic distribution of the SMN complex. The presence of STRAP in the SMN complexes was shown to be



Serine-Threonine Kinase Receptor-Associated Protein. Figure 1 STRAP in diverse biological functions through a role in protein complex assemblies in different signaling pathways.



Serine-Threonine Kinase Receptor-Associated Protein. Figure 2 STRAP may be involved in the progression of human cancers by activating multiple oncogenic pathways.

essential for U snRNP assembly. In contrast, STRAP was also shown not to be essential for this assembly by other groups.

STRAP along with EWS was shown to be present in the kinesin driven mRNA transport granules in the dendrites of murine neurons. In eukaryotes, the nuclear export of mRNA is mediated by nuclear export factor 1 (NXF1) receptors. As shown in mouse neuroblastoma N2a cells, NXF proteins bind to brain-specific microtubule associated proteins (MAP) such as MAP1B and also STRAP. Additionally, MAP1B also binds with STRAP. This assembly helps in the nuclear export of mRNA. In an independent setting, NXF-7 binds with MAP1B and STRAP only in the cytoplasm and colocalizes with

Staufen1 containing mRNA transport granules in the neurites of these cells (Fig. 1). As in other cases, STRAP seems to play a role in the multiprotein complex assembly required for both nuclear export of mRNA and the cytoplasmic transport of mRNA containing granules along microtubules.

STRAP is a substrate for SUMO4 (a novel member of the *SUMO* gene family) sumoylation. Although the significance of this is not yet known, SUMO4 sumoylation is predicted to have a role in the regulation of intracellular stress. It will be interesting to determine whether sumoylation of STRAP has any effect on its biological functions.

Clinical Aspects

Although STRAP seems to be involved in mutually independent biological functions, there is increasing clinical and experimental evidence suggesting that STRAP acts as an oncogene. The level of STRAP is found to be altered in different ►cancers. The protein level is elevated in 60% of ►colorectal, 78% of ►lung and 46% of ►breast carcinomas. Several lines of evidence suggest that carcinoma cells frequently lose the tumor suppressor function of TGF-β. Upregulation of TGF-β signaling inhibitors like STRAP and Smad7 and their synergistic function present a novel intracellular mechanism by which a portion of human tumors become refractory to antitumor effects of TGF-β. STRAP also exerts several other biological functions in a TGF-β independent manner that contribute to cell proliferation and inhibition of apoptosis (Fig. 2).

Ectopic expression of STRAP in different cell lines promotes cellular proliferation, induces anchorage-independent growth and increases tumorigenicity during in vitro and in vivo experiments. Downregulation of $p21^{Cip1}$, which results in hyperphosphorylation of pRb as well as activation of MAPK/ERK pathway, may contribute to the tumorigenic effects of STRAP during tumor formation and progression (Fig. 2). As noted earlier, STRAP also has an anti-apoptotic role probably through Bad phosphorylation and inhibition of TNF-α induced apoptosis. STRAP interacts with Ewing Sarcoma protein (EWS), an oncoprotein known to be involved in 80% of Ewing tumors after chromosomal translocations. Normal EWS protein is also upregulated in human cancers, which correlates with the upregulation of STRAP in 71% of colorectal cancers and 54% of lung cancers, suggesting a cooperative role of these two proteins in human cancers. In an attempt to determine whether STRAP is of prognostic value or predictive of ►chemotherapy benefit, *STRAP* gene was found to be amplified in 23% of colorectal tumors and amplification of STRAP in patients without adjuvant chemotherapy was found to exhibit better prognosis. These patients had a worse survival when treated with ►adjuvant therapy when compared with patients without chemotherapy. In contrast, patients carrying tumors with diploidy or deletion of STRAP benefited from the treatment. These results suggest that STRAP is an unfavorable prognostic marker for 5-FU-based adjuvant chemotherapy.

Taken together, STRAP appears to facilitate multiple steps in the process of tumorigenesis and possibly during ►metastasis, and it could be a potentially important drug target for therapeutic intervention in human cancers.

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Serological Analysis of cDNA Expression Libraries

Definition

SEREX; A method to identify cancer-specific antigens based on screening of cDNA expression libraries with serum from cancer patients.

►Cancer-Germline (CG) Antigens

Seropositive

Definition

Indicative of the presence of an antibody to a particular antigen in a patient's blood.

Serotonin

Definition

5-hydroxytryptamine, 5-HT; Is a neurotransmitter synthesized in specific nerve cells (serotonergic) in the central nervous system (CNS) and is believed to play an important role in the regulation of mood, sleep, emesis (vomiting), sexuality and appetite.

►Fluoxetine

Serotypes

Definition

A taxonomic subgroup of a microorganism or virus determined by the antigens expressed.

►Oncolytic Adenovirus

Serpin

Definition

Serine protease inhibitor – a group of structurally related proteins, many of which inhibit proteases.

►Maspin

Sertoli Cell

Definition

Tall columnar cells found in the mammalian testis closely associated with developing spermatocytes and spermatids. Probably provide appropriate microenvironment for sperm differentiation. Its main function is to nurture the developing sperm cells through the stages of spermatogenesis. Because of this, it has also been called the "mother cell." It provides both secretory and structural support.

Sertoli-Leydig Cell Tumor

Definition

Synonym Arrhenoblastoma; Is a rare cancer of the ovaries. The cancer cells produce and release high level of the a male sex hormone testosterone, which may cause the women to develop male physical characteristics, including facial hair and a deep voice. While the tumor can occur at any age, it develops most often in young adults.

►Ovarian Tumors During Childhood and Adolescence

Serum

Definition

The clear liquid that separates from the blood when it is allowed to clot. This fluid retains any ►antibodies that were present in the whole blood.

Serum-Ascites Albumin Concentration Gradient

Definition

Is a parameter of pressure reflecting presence or absence of portal hypertension. It is calculated by subtracting the albumin concentration of the ascitic fluid from the albumin concentration of a serum specimen obtained on the same day. If the gradient is less than 1.1 g/dL, portal hypertension can be ruled out.

►Acites

Serum Biomarkers

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Synonyms

BR 27-29; CYFRA 21-1; Hydroxybutyrate dehydrogenase; Gelatinase B; Breast regressing protein 39 Kd;

brp-39; Chitinase-3-like-1; CHI3L1; Chondrex; Human cartilage glycoprotein-39; HC gp39; 8-kDa heparin-binding glycoprotein; Gp38k; 40 kDa mammary gland protein; MGP-40

Definition

Cancer ►biomarkers are usually proteins detected in the blood, urine or other body fluids that are either produced by the tumor itself or in response to the presence of cancer. Ideally, biomarkers should allow at least one of the following: (i) early cancer detection by screening a healthy or high-risk population, (ii) help confirm the diagnosis of cancer or of a specific type of cancer, (iii) predict prognosis, (iv) monitor treatment response or (v) detect early recurrence.

Characteristics

Most biomarkers are not specific for tumors or organs and their levels may rise in other diseases. The diagnostic value of a tumor marker will depend on the prevalence of a disease in a population group and on its specificity (percentage of normal individuals without disease for whom a negative result is obtained) and sensitivity (percentage of tests which are correctly positive in the presence of a tumor). A cancer biomarker should be measured at a low cost, by a widely available assay with reproducible results in a specimen that is easy to access.

We are interested in developing biomarkers for ►brain tumors. Response to treatment of a ►brain tumor is evaluated by noninvasive imaging. This may be unreliable when assessing novel cytocidal drugs, distinguishing between disease ►progression or radionecrosis and predicting malignant transformation from low to high-grade glioma. ►YKL-40, MMP-9 and ►lactate dehydrogenase (LDH) are potential ►biomarker candidates. Monitoring circulating endothelial and progenitor cells as well as markers of this process including basic fibroblast growth factor and stromal cell-derived factor 1α may help determine treatment effect. As recent methods such as proteomics are utilized, new candidate proteins will be screened as potential biomarkers for a variety of cancers.

Alpha-Fetoprotein (AFP)

►Alpha-fetoprotein (AFP) is a fetal serum protein that shares sequence homology with albumin. It is normally synthesized during gestation by liver, yolk sac, and gastrointestinal tract. Following birth, AFP rapidly clears from the circulation. It may be elevated in patients with cirrhosis, viral hepatitis, drug or alcohol abuse as well as pregnancy, and may be used for screening of fetal spinal cord defects and placental disease.

Serum levels of AFP are elevated in up to 80% of patients with ►hepatocellular carcinoma (HCC). Changes in serum AFP levels reflect the course of disease. In patients with cirrhosis, elevation of serum AFP may be increased for up to 18 months before symptoms of HCC manifest and may be used to assist in the diagnosis. An elevation of AFP greater than 400 ng/mL is predictive for HCC with a specificity >95%. In the setting of cirrhosis and a growing liver mass, many centers use a level greater than 1,000 ng/mL as presumptive evidence of HCC and do not require a biopsy.

In men with nonseminomatous germ cell tumors (NSGCT), AFP is produced by yolk sac (endodermal sinus) tumors and, less often, embryonal carcinomas. In addition, up to 80% of mediastinal NSGCTs are associated with elevated serum AFP, regardless of histologic subtype. Whereas only 10–20% of stage I tumors express elevated levels of AFP, 40–60% of disseminated NSGCT express abnormal levels of AFP. Although these markers may be helpful for the initial diagnosis of a testicular cancer and for prognostication, their main utility is for follow-up of disease status (Table 1).

Beta Subunit of Human Chorionic Gonadotropin (β-hCG)

►Beta subunit of human chorionic gonadotropin (β-hCG) is normally produced by the placenta and human fetal tissue. Elevated serum β-hCG is most commonly associated with pregnancy, gestational trophoblastic disease and germ cell tumors. It can also be found in hypogonadal states and with marijuana use. β-hCG and AFP are elevated in up to 85% of patients with germ cell tumors, but only in about 20% of those with stage I disease. In patients with extragonadal or metastatic disease, highly elevated serum levels of β-hCG or AFP can establish a diagnosis of nonseminomatous germ cell tumor instead of biopsy. Serial measurements of β-hCG every 2–3 months for 1 year following treatment is important as elevation is often the first sign of recurrence, indicating the need to restart treatment. β-hCG is also used to diagnose and monitor response to treatment of gestational trophoblastic disease (Table 1).

Cancer Antigen 15-3 (CA 15-3)

Elevated ►cancer antigen 15-3 (CA 15-3) levels may be found in patients with ►breast cancers, and with much less specificity and sensitivity in patients with ►ovarian, ►lung, or ►prostate cancers. Increased levels may be associated with pregnancy and lactation, benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis.

Because of a lack of sensitivity for early disease detection and lack of specificity, It has not been approved for screening of early breast cancer. However, high preoperative serum levels of CA 15-3 are associated with adverse patient outcome. CA 15-3, like

Serum Biomarkers. Table 1 Tumor markers

Tumor marker	*Primary tumor* and other cancers	Other conditions with elevated levels	Use of tumor marker	
			Diagnosis	Monitoring of treatment response
AFP	*Hepatocellular carcinoma, nonseminomatous germ cell tumor*, gastric, biliary and pancreatic cancer	Cirrhosis, viral hepatitis, pregnancy	Yes (poorly differentiated cancer of unknown origin, patients with cirrhosis and a liver mass)	Yes
β-hCG	*Nonseminomatous germ cell tumors, gestational trophoblastic disease*, gastrointestinal cancer (rare)	Hypogonadal states, marijuana	Yes (poorly differentiated cancer of unknown origin, gestational trophoblastic disease)	Yes (nonseminomatous germ cell tumor and gestational trophoblastic disease)
CA 15-3	*Breast*, ovarian, lung, or prostate cancer	Benign breast disease, benign ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis	No	No (except in selected situations, i.e. follow-up by conventional clinical procedures not possible)
CA 19-9	*Pancreatic and biliary tract*, colon, eosophagal and pancreatic cancer	Pancreatitis, biliary disease, cirrhosis	Yes (selected pancreatic masses)	No
CA 27-29	*Breast*, stomach, ovary, lung, or prostate cancer	Benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis	No	No (except in selected situations, i.e. follow-up by conventional clinical procedures not possible)
CA 125	*Ovarian*, endometrium, fallopian tubes, pancreas, breast, colon and lung cancer	Menstruation, pregnancy, endometriosis, pelvic inflammatory disease, liver disease, pancreatitis, peritonitis and inflammation of the pleura	Yes	Yes
CEA	*Colorectal*, breast, lung, stomach, pancreas, bladder, medullary thyroid, head and neck and liver cancer, lymphoma melanoma	Tobacco, peptic ulcer, inflammatory bowel disease, pancreatitis, hypothyroidism, cirrhosis, biliary obstruction	No	Yes
PSA	Prostate cancer	Prostatitis, benign prostate hypertrophy, prostate trauma, after ejaculation	Yes	Yes
YKL-40	Glioblastoma multiforme, ovarian and endometrial cancer	Inflammatory disease	No	No

►carcinoembryonic antigen (CEA) and ►cancer associated antigen 27-29 (CA 27-29), are most useful for monitoring treatment response in women with breast cancer, especially in advanced disease. Serial determinations of tumor markers after primary treatment can detect recurrent/metastatic disease with lead times of 2–9 months over clinical symptoms. However the clinical value of this lead-time remains to be determined.

The ASCO guidelines do not recommend serial monitoring of CEA, CA 27-29 or CA 15-3 after primary therapy, except in selected situations, where patients cannot be followed by conventional diagnostic techniques.

Cancer Associated Antigen 19-9 (CA 19-9)

►Cancer associated antigen 19-9 (CA 19-9), an intercellular adhesion molecule, is an epitope of sialyated Lewis A blood group antigen. Elevated serum levels of CA 19-9 are found typically in patients with ►pancreatic and biliary tract cancers, and less often in ►gastric, ovarian, ►colorectal, lung, breast and ►uterine cancer. Elevated levels of CA 19-9 are also found in acute cholangitis, cirrhosis or other cholestatic diseases. Five percent of the population is genotypically Lewis-null blood type and will never produce CA 19-9 antigen, even in the presence of tumoral disease. The sensitivity and specificity for pancreatic cancer has been estimated to be 80–90%. These values correlate with tumor size so that CA 19-9 has limited value in identifying patients with small surgically resectable cancers (Table 1).

CA 27-29

Cancer antigen 27-29 (synonym: BR 27-29) is a normal epithelial cell mucin-1 (MUC1) apical surface glycoprotein. Elevated serum levels are highly associated with breast cancer. However they can also be found in cancers of ►colon, ►stomach, ►kidney, ►lung, ►ovary, ►pancreas, ►uterus and ►liver and in a number of noncancerous conditions, including first trimester pregnancy, endometriosis, ovarian cyst, benign kidney, liver and breast disease. Therefore, it has no role in breast cancer screening. Serum CA 27-29 levels are elevated in approximately one third of women with early-stage breast cancer (stage I or II) and in two thirds of women with stages III or IV. There is no agreement regarding the ability of CA 27-29 to detect asymptomatic recurrence after curative treatment. Serial monitoring is currently not recommended by the ASCO guidelines (Table 1).

CA-125

►CA-125 is a glycoprotein that is expressed by celomic epithelium of ovaries, fallopian tubes, endometrium and uterine cervix. Up to 90% of ovarian cancers are celomic epithelial carcinomas and overexpress CA 125. This glycoprotein can be detected in most endometroid, serous and clear cell ovarian carcinomas. Mucinous tumors however express this antigen less frequently. Serum levels of CA 125 may also be elevated in cancers of endometrium, fallopian tubes, pancreas, breast, colon and lung. Non-cancerous conditions with elevated CA 125 levels include menstruation, pregnancy, endometriosis, pelvic inflammatory disease, liver disease, pancreatitis, peritonitis and inflammation of the pleura. CA-125 is an important tumor marker for the diagnosis of epithelial ovarian cancer although it is not perfectly sensitive or specific for ovarian cancer. CA 125 can be used clinically to determine response to treatment and predict relapse and survival (Table 1).

Carcinoembryonic Antigen (CEA)

CEA is a normal mucosal cell oncofetal glycoprotein involved in cell adhesion. It is overexpressed in gastric, ►pulmonary, breast, pancreatic and predominantly ►colorectal adenocarcinomas. It may also be elevated in the serum of heavy smokers and patients with ulcerative colitis, pancreatitis and cirrhosis. Its role as a screening tool remains uncertain because of poor sensitivity and specificity. In patients with established disease, the absolute serum level of CEA correlates with disease burden and has prognostic value. After complete resection, elevated preoperative levels of CEA return to baseline and persistently elevated levels should warrant a search for residual tumor. CEA serum levels should be checked every 3 months for at least 3 years in patients with stage II or III colon cancer if the patient is a candidate for re-resection because elevated levels give a 1.5–6 months lead-time for detection of recurrence detection in comparison to other methods such as imaging. Early detection of asymptomatic recurrence increases the likelihood of a subsequent complete resection (Table 1).

Cytokeratin 19 Fragments

CYFRA 21-1 is a soluble fragment of cytokeratin 19 expressed in normal squamous cells. Elevated serum concentrations are found in tumors of squamous origin, including lung and are associated with short patient survival. The sensitivity of ►cytokeratin 19 fragments (CYFRA 21-1) depends on the histological type, being lowest for small cell lung cancer (about 16–40%). It is not used in early detection or monitoring of disease and has not been incorporated in the ASCO guidelines for patients with lung cancer.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an ubiquitous enzyme that catalyses the conversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. Increased serum levels of LDH have been shown in a number of cancers, including ►melanoma, ►lymphoma, ►germ cell tumors and ►small cell lung cancer. Elevated levels of LDH can also be caused by a number of non-cancerous conditions, including heart failure, hypothyroidism, anemia, as well as lung and liver diseases.

Matrix Metalloproteinase-9 (MMP-9)

►Matrix metalloproteinase-9 (MMP-9) is a member of the zinc-dependent homologous proteinase family that degrades extracellular matrix proteins, such as cell adhesion receptors, chemokines, growth factors and their receptors and protease inhibitors. They are implicated in proliferation of endothelial and hematopoietic stem cells by inducing the release of kit ligand.

MMP-9 expression is elevated in most tumor tissues, including ovarian, thyroid, hepatocellular and gastrointestinal carcinoma. Expression of MMP-9 has been detected in both glioma and its endothelial cells. Increasing levels of MMP-9 as measured by in situ hybridization and immunohistochemistry correlate with higher grade gliomas. In GBM patients, serum levels of MMP-9 are significantly higher in patients with radiologic evidence of disease compared to patients whose disease is inactive. It is a potential future biomarker.

Placental Alkaline Phosphatase (PLAP)

Placental isoenzyme of PLAP is increased in 30% of ovarian cancer (especially serous cystadenocarcinoma), as well as cancers of endometrium, lung, breast and 40% of seminomas (75% in metastatic seminoma). Smokers may also have elevated PLAP levels.

Prostate-Specific Antigen (PSA)

►Prostate-specific antigen (PSA) is a glycoprotein that is expressed by normal prostate tissue and is overexpressed in prostate cancer. It is a sensitive and specific marker for this cancer. Levels of PSA depend upon the age and race of the patient. The predictive value for cancer is 20–30% if serum levels are greater than 4 ng/mL and 50% for values exceeding 10 ng/mL. However 20–30% of patients with prostate cancer have levels within the normal range. The American Urological Association recommends that serial PSA measurements be obtained routinely to detect early recurrence in men who have undergone primary therapy for localized disease. PSA levels should decrease and remain undetectable after radical prostatectomy or at low levels following radiation therapy and cryotherapy. The nadir serum PSA and percent PSA decline at 3 and 6 months predict progression-free survival in men with metastatic prostate cancer treated with androgen deprivation. The degree of PSA decline following second-line treatment of metastatic disease correlates with disease survival (Table 1).

YKL-40

YKL-40 is a member of the mammalian chitinase-like proteins that is highly conserved among different species. It has no enzymatic activity due to amino acid substitutions at the catalytic site. *In vitro* studies suggest that YKL-40 plays a role in proliferation and differentiation of malignant cells, by decreasing apoptosis of cancer cells, stimulating angiogenesis, and inducing proliferation of fibroblasts surrounding the tumor and remodeling of the extracellular matrix. Elevated serum values of YKL-40 have been reported in a variety of cancers, including breast, colon/►rectum, ovary, lung, kidney, glioblastoma, and melanoma. YKL-40 levels may also be increased in other diseases with an inflammatory component.

In a microarray analysis of brain tumors, YKL-40 was found to be highly overexpressed in a subset of patients with glioblastoma multiforme (GBM) in comparison to low-grade gliomas and detected in serum by ELISA. In a prospective study of 143 patients with high-grade gliomas, we found that serum levels of YKL-40 were increased in patients with active disease compared to those with absence of radiographic disease. Elevated levels of YKL-40 also correlated with shorter survival in GBM patients.

In patients with endometrial or ovarian cancer, elevated preoperative levels of YKL-40 may identify a subset of high-risk patients with worse clinical outcome. Studies in a larger sample population are underway to further evaluate its potential role as a biomarker YKL-40.

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Severe Combined Immunodeficiency Disease

Definition

►SCID; ►Severe Combined Immunodeficient Mice represent a model for the human disease.

Severe Combined Immunodeficient Mice

Definition

Scid mice; Spontaneous mutant mice that lack B- and T-cells. As a consequence, scid-mice are severely immunocompromised. Scid-mice do not reject implanted human cells as would normal mice. Hence, these mice allow investigators to study the growth and progression of human tumors in an *in vivo* model.

►Cystatins

Severe Hypoxia

►Anoxia

Sex Cord Stromal Tumors

Definition

SCST; Primary ovarian neoplasms derived from the stromal component of the ovary. During childhood and adolescence, SCSTs most frequently present as juvenile ►granulosa cell tumors or Sertoli-Leydig cell tumors (synonym gynandroblastoma). Less frequent types include sclerosing stromal cell tumor, sex cord stromal tumor with annular tubules, steroid tumors and Brenner tumors.

►Brenner tumor
►Sex cord stromal tumors
►Gynandroblastoma
►Sertoli-Leydig cell tumor
►Ovarian Tumors During Childhood and Adolescence

Sex Hormone

Definition

A chemical substance produced by a sex gland or other organ that has an effect on the sexual features of an organism. Like many other kinds of hormones, sex hormones may also be artificially synthesized.

►Androgens
►Estrogens

Sex Hormone Dependent Cancers

Definition

Prostate cancer is first dependent on androgens (e.g., testosterone) for growth and may become later hormone-independent. Breast cancer is mainly sex hormone-dependent (estrogen and progesterone), but some breast tumors are hormone-independent and are more difficult to treat.

►Gonadotropin-Releasing Hormone

Sezary Syndrome

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Definition

Sezary syndrome (SS), named after the French dermatologist Albert Sezary (1880–1956), is a variant of cutaneous T cell lymphoma (CTCL), a malignancy of mature T-helper cells involving the skin and blood. It is defined by the presence of an ►exfoliative erythroderma, lymphadenopathy, and evidence of neoplastic cells in the skin and blood. "Sezary" cells refer to enlarged mature CD4+ lymphocytes with hyperconvoluted nuclei. Historically, the presence of Sezary cells in the peripheral blood was a defining criteria for SS. However, an increased number of these cells can be found in several benign dermatologic conditions and is no longer the agreed upon standard. The presence of a clonally expanded population of CD4+ cells resulting in an increased CD4/CD8 >10 in the blood is considered a more accurate measure. Immunophenotypic abnormalities which support a diagnosis of SS include decreased/absent expression of T-cell antigens CD2, CD3, CD5, and CD7. The

characteristic histologic features of involved skin seen in other forms of CTCL are often not present in SS and thus may not be a useful tool in its diagnosis. The etiology of SS is unknown.

Characteristics

SS is a rare disease making up less than 5% of all CTCLs. It occurs primarily in adults over the age of 60 with predominance in males.

Clinical features of SS include intractable, debilitating pruritus with an exfoliative erythroderma, edema, ►lichenification, thickening and fissuring of the palms and soles, nail dystrophy, hair loss, and ectropion. Malignant cell infiltrates of the skin can often lead to disfiguring features in the face (known as "leonine faces") and body.

Staging and Treatment

Staging of SS as CTCL stage III or IV depends on the degree of lymph node involvement and evidence of solid organ spread. With SS representing an advanced stage of the disease, the prognosis is usually poor with a median survival of 2–4 years. Immune compromise in these patients leads to increased bacterial infection and often to the demise of the patient.

Since SS involves both the skin and blood, systemic treatment is necessary to control the disease. The most common therapeutic tools used singly or in combination include: ►extracorporeal photochemotherapy (photopheresis), interferon alpha, bexarotene, denileukin diftitox, radiation with total skin electron beam therapy, leukeran, and gemcitabine.

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sFRP

Definition

Secreted frizzled related protein; family of proteins with a cysteine-rich domain (CRD) related to the Fz receptor CRD; in most cases inhibit Wnt signals by binding to Wnt proteins; often downregulated in cancer.

►Wnt Signaling

SH2/SH3 Domains

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Synonyms

Src homology 2; SH2/src homology 3; SH3 domains

Definition

Proteins containing SH2/SH3 domains play critical roles in regulating the formation of intracellular signal transduction complexes. SH2 and SH3 domains recognize amino acid motifs containing phosphotyrosine and polyproline, respectively. Signaling pathways activated by SH2/SH3 domains subsequently lead to cellular responses including differentiation, proliferation and migration.

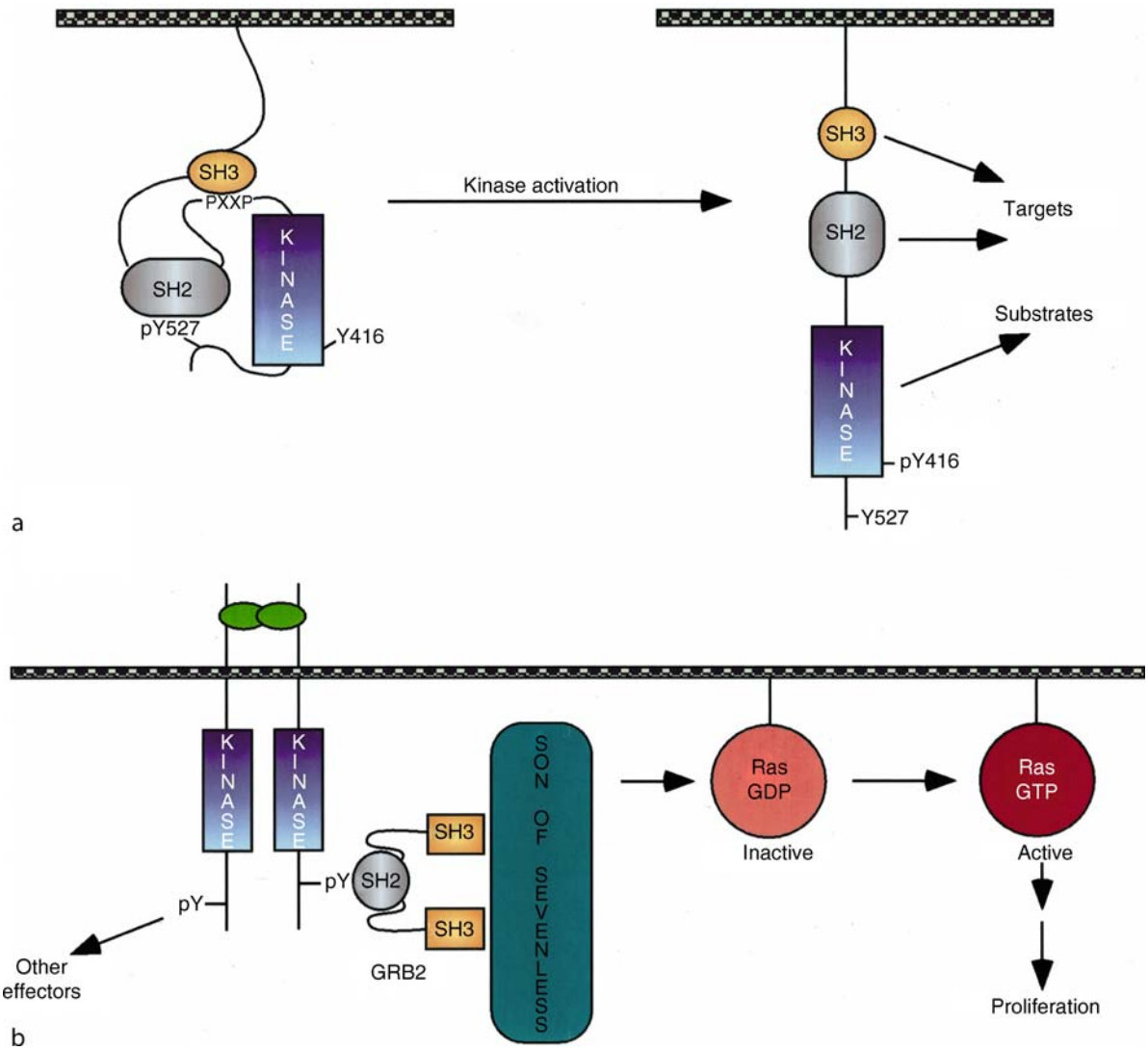
Characteristics

SH2/SH3 domains were originally identified as conserved sequences found in the Src tyrosine kinase and a variety of other proteins. These domains are now known to reside in hundreds of functionally diverse signaling molecules, ranging from tyrosine kinases and phosphatases to phospholipases and transcription factors.

- SH2 domains are composed of ~100 amino acids organized into a modular structure that recognize phosphotyrosine-containing sequences on protein tyrosine kinases and their substrates. The specificity of interaction is generally determined by the 3–4 amino acids C-terminal to the phosphorylated tyrosine position on the target protein. SH2 domain-mediated signaling events lead to cellular responses ranging from DNA synthesis to transcriptional activation.
- SH3 domains are about 50 amino acids in size and bind to target proteins containing the proline-rich consensus sequence PXXP (where P is proline and X is any amino acid). Unlike SH2 domains, SH3 domains generally remain constitutively associated with their cognate ligands, making their function less dependent on tyrosine phosphorylation. Protein interactions mediated by SH3 domains have been implicated in cytoskeletal alterations necessary for changes in cell morphology and motility.

In response to extracellular cues, SH2/SH3 domains act both intramolecularly and intermolecularly to regulate signal transduction. Their functions are well illustrated by two classical signaling cascades exemplified by the Src protein tyrosine kinase pathway and the Ras GTPase pathway.

Src is a membrane bound intracellular tyrosine kinase that contains one SH2 and one SH3 domain (the kinase domain was originally termed the SH1 domain). The post-translational phosphorylation of Src on tyrosine 527 negatively regulates kinase activity. This inhibition is the result of complex intramolecular interactions between phosphotyrosine 527 and the SH2 domain, as well the SH3 domain and a proline-rich central region (see Fig. 1). Concomitant dephosphorylation of tyrosine 527 and phosphorylation of tyrosine 416 relieves this steric block. This allows for the association of the SH2 and SH3 domains with target proteins and enhancement of Src catalytic activity. Thus, Src SH2/SH3 domains both positively and negatively regulate protein tyrosine kinase



SH2/SH3 Domains. Figure 1 Pathways exemplifying SH2/SH3 domain functions. (a) Activation of the Src tyrosine kinase domain serves as an example of both intermolecular and intramolecular functions for SH2/SH3 domains. The Src SH2/SH3 domains serve to inhibit the kinase activity. Dephosphorylation of phosphotyrosine 527 and phosphorylation of tyrosine 416 results in a Src conformational change. The tyrosine kinase is activated and the protein associates with downstream substrates via its SH2 and SH3 domains. (b) The activation of the Ras GTPase by various receptor tyrosine kinases is a classic example of SH2/SH3 domains providing an intermolecular link between critical signal transduction components. Receptor autophosphorylation leads to re-localization of constitutively associated Grb2-Sos, leading to GTP exchange and Ras activation.

activity. Various protein tyrosine kinases utilize a similar mechanism to regulate their function.

The Ras GTPase is a membrane associated growth regulator, and mutated forms are found in many human tumors. When in its GTP-bound state, Ras is considered "on" and activates multiple downstream signaling pathways. In many cases growth factor stimulation of the Ras pathway occurs via the Grb2 SH2/SH3 protein (see Fig. 1). Grb2 is an SH2/SH3 "adaptor" protein, consisting of two SH3 domains flanking a central SH2 domain. The Grb2 SH3 domains constitutively associate with various proteins, including the son-of-sevenless (Sos) GTP exchange factor. Following growth factor stimulation, phosphotyrosine motifs on the activated receptors recruit the Grb2 SH2 domain. This event in part serves to re-localize the Grb2-Sos complex from the cytoplasm to the membrane, placing Sos in proximity to membrane-bound Ras and allowing the exchange of Ras-GDP for -GTP. Based on similar observations made for other SH2/SH3 adaptor proteins, intracellular re-localization may be a common mechanism used to activate a variety of enzymatic pathways.

These examples illustrate important aspects of SH2/SH3 domain function in response to a specific stimulus. It is clear, however, that most signal transduction pathways are interconnected, and that a single extracellular cue can elicit a response involving hundreds of effector proteins. Identifying how various extracellular cues individually and combinatorally affect signaling pathways will be an important challenge for fully understanding SH2/SH3 domain functions.

Clinical Relevance

Many tumor cells possess amplifications and/or mutations in genes encoding components of the tyrosine kinase machinery. Therefore, it follows that proteins regulating cell proliferation have become central targets for drug discovery. The crystallographic structures of many SH2 and SH3 domains in complex with their various ligands has allowed for the rational design of highly specific peptidomimetic molecules. The challenge is to utilize drugs that affect only the pathological action of the specific targeted molecule, while enabling normal signal events to proceed unperturbed. For example, one can envision using SH2 and/or SH3 domain antagonists to inhibit the proliferative capacity of cells transformed due to hyperactivated or aberrantly expressed protein tyrosine kinases.

Importantly, ongoing genomic analyses will undoubtedly identify new SH2/SH3 domain-containing proteins. Characterizing the functions of these newly identified effectors and linking them to the pathogenesis of specific cancers and other disease states will be a worthwhile goal. The use of novel proteomic techniques in combination with classical cell biology will greatly aid this process.

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SH2/src Homology 3

►SH2/SH3 Domains

SH2 Domain

Definition

Src homology (SH) 2 domain; Is comprised of ∼100 amino acid residues capable of binding specifically to a phosphorylated tyrosine residue in a signaling protein. Its binding specificity is determined through interactions between an SH2 sequence and a target sequence of a phosphorylated tyrosine and additional amino acid residues mainly from −1 to + 3 positions surrounding the phosphotyrosine.

►SH2/SH3 Domains

SH3 Domain

Definition

Src-homology domain 3 is a protein domain of about 50 amino acid residues that binds to specific proline-rich sequences; Modular protein interaction domain commonly found in signal transduction proteins. Binds amino acid recognition sequences that include proline-X-X-proline in target proteins. Seen often in adapter proteins that coordinate the function of macromolecular complexes with the actin cytoskeleton.

►SH2/SH3 domains

SHANK

Definition

Refers to large adaptor proteins that can self-associate and bind a variety of different proteins. Best characterized in neurons where they serve to organize receptor proteins at the synapse, shank proteins are also found in epithelia cells where their function is currently unclear.

►Cortactin

Shark Cartilage

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Definition

A dietary supplement derived from cartilage obtained from sharks, commonly hammerhead (*Sphyrna lewini*) and dogfish (*Squalus acanthias*) sharks.

Characteristics

Shark cartilage is a popular dietary supplement that traditionally has been proclaimed to be able to prevent and/or treat established cancer. In the early part of the twenty-first century, it has been one of the ten most common dietary supplements used among cancer patients. It has been suggested that the widespread popularity of this product has partly contributed to an increase in shark fishing, with a resultant sharp, ≥75%, decline in the world shark population in the past 15 years.

Shark cartilage is available as a capsule (taken orally), powder (for oral use when mixed with juice or as an enema), and also as an intravenous preparation. The recommended doses can vary from 500 mg to several grams per day. The adverse effects are predominantly gastrointestinal, such as nausea, abdominal pain, and diarrhea, and even hepatitis. Given its potential anti-angiogenesis properties, it has been advised that pregnant females should not consume the product.

The popularity of shark cartilage soared in the early 1990s after a book (*Sharks Don't Get Cancer*) by William Lane postulated that sharks do not get cancer due to the high proportion of cartilage in their body, suggesting that shark cartilage has chemopreventive properties. The authors predicted that "As events and research continue to unfold, shark cartilage may prove to be the first momentous step towards preventing and conquering cancer." However, subsequent research has not substantiated this prediction. Moreover, sharks do develop cancers, although the rate of cancer incidence among sharks is unclear.

A number of studies have been conducted to evaluate the potential cancer treatment properties of shark cartilage. One of the first reports of a human trial was featured on the TV show "60 Minutes" wherein results from two trials were reported, receiving considerable media coverage. In one trial, researchers from Cuba claimed that 16 weeks of shark cartilage was effective in treating cancer in 3 of the 15 patients that received the treatment. In the other trial, researchers at Simone Protective Cancer Center (NJ) claimed 4 of the 20 cancer patients consuming shark cartilage showed partial or complete response with use of the product. However, these studies had serious methodological flaws (9), the results suggested only a small response rate (20%), and the results were never reported completely in a peer reviewed PubMed indexed journal. In a well designed phase II study, involving 60 patients with advanced cancer, 1 g/kg/day of shark cartilage was reported to be ineffective in reducing the progression of disease, to have no beneficial effect on quality of life, and to have substantial gastrointestinal toxicity in about 25% of the patients. Two other phase II studies reported similar negative results with the use of shark cartilage among cancer patients.

Likewise, no suggestion of efficacy of shark cartilage was seen in a relatively small randomized placebo-controlled, double-blinded clinical trial. Among the 83 patients involved in this trial (42 receiving shark cartilage and 41 receiving placebo), there was no difference in overall survival or quality of life among the two groups. The shark cartilage and its identical smelling, tasting, and appearing placebo were not well tolerated by the study patients, with ~50% of subjects stopping the study medication within a month of initiation. Given the presently available evidence, shark cartilage has generally been considered to be an ineffective agent for cancer and promotion of its use as an anti-cancer agent has been widely criticized.

While shark cartilage supplementation has been found to be ineffective as a chemoprevention or anti-cancer agent in clinical trials, laboratory research has identified a few compounds isolated from shark cartilage that could potentially have anti-angiogenesis properties. In the late 1970s, Langer et al. first isolated a factor from shark cartilage that was found to strongly inhibit the growth of new blood vessels toward tumors and thereby restricted tumor growth. Similar findings have been reported independently by other authors. One

of the standardized extract shark cartilage products obtained from dogfish (AE-941, or Neovastat) has been reported to be a promising agent. In a phase II trial among 80 patients with lung cancer, those receiving higher doses (>2.6 ml/kg/day) of AE-941 were reported to have better survival, compared to patients receiving lower doses (median, 6.1 months vs. 4.6 months; $P = 0.026$). Neovastat is postulated to exert its anti-angiogenesis effects by multiple effects, including inhibition of tissue metalloproteinases, modulation of VEGF (vascular endothelial growth factor), and enhancement of endothelial cell apoptosis. This product was tested in a large phase III lung cancer clinical trial funded by the United States National Cancer Institute. However, the early results of this trial did not support that this agent had any benefit.

Thus, in total, currently available study data do not support the efficacy of shark cartilage as a chemopreventive or anti-cancer agent. Given the lack of established efficacy, its toxicity, and the environmental impact related to harvesting sharks, the use of shark cartilage among patients with cancer should be discouraged.

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Shc

Definition

An adaptor protein that contains both phosphotyrosine-binding and Src homology 2 ►SH2 domain. It is a prominent effector of protein–tyrosine kinase signaling.

►Insulin Receptor

Shedding of Cells

►Exfoliation of Cells

Shimada System

Definition

Is the clinical classification of neuroblastoma.

Short Hairpin RNA

►RNA Interference

SHP-1

Definition

Src homology 2 domain-containing tyrosine phosphatase, non-receptor type 6, PTPN6.

►Erythropoietin
►SH2 Domain

SHP-2

Definition

The Src homology 2 (SH2) domain-containing tyrosine phosphatase which plays a positive role in transducing signals from receptor protein tyrosine kinases down to the MAPKs, Ras, ►PI3K or the Src family kinases.

►Major Vault Protein
►SH2 Domain

SH3P9

►Bin1

shRNA

►RNA Interference

Sialoglycoconjugates

Definition

Are sugar chains terminally modified by an acidic sugar (sialic acid).

►Adhesion

Sialyl Lewis-a/x Determinants

Definition

Are blood group antigens that comprise type 1 (Lewis a) and type 2 (Lewis X) carbohydrates. Lewis a and Lewis x are regarded as tumor-associated markers, and these antigens and their derivatives interact with E-selectin, mediating cancer cell-to-endothelial cell adhesion.

►E-selectin-Mediated Adhesion in Cancer

Sialyltransferases

Definition

Enzymes that catalyze addition of sialic acid (neuraminic acid).

►Lewis Antigens

Sicca Complex

Definition

Refers to dryness of the eyes and mouth; Is a chronic, inflammatory disease that affects the exocrine glands. The primary targets appear to be the lacrimal and salivary gland duct epithelium.

►Sjögren Syndrome

Sicca Syndrome

►Sjögren Syndrome

Signal Sequence

Definition

Synonym signal peptide; Is a *N*-terminal, short (18–26) amino acid sequence that acts like a zip code in nascent polypeptides and determines their subcellular targeting. A signal sequence is a protein region with which a protein can be directed to the appropriate cellular compartment within a cell, they initiate co-translational transfer through the membrane of the endoplasmic reticulum (ER). Proteins are often synthesized in an immature version (pre-protein) that is larger than the mature functional form. This is due to the presence of N-terminal amino acid stretches, referred to as leader sequences. The pre-protein is a transient precursor, since the leader sequence is cleaved off during protein processing. This signal sequence is a short stretch of 15–30 amino acids that mediates the transfer of any attached polypeptide to the endoplasmic reticulum. it provides the means for the ribosomes to attach to the ER membrane (ER regions with associated ribosomes are called “rough ER.” As soon as the first few amino acids of the protein have been synthesized, the nascent protein chain can be co-translationally transferred to the membrane.

The signal hypothesis proposes that the N-terminus of a secreted protein has a signal sequence whose presence marks it for membrane insertion. Once the protein chain is well inserted into the membrane, the signal sequence is cleaved off by a protease within

the membrane and the protein can then enter or even pass through the membrane. This principle does not hold for nuclear proteins, which are synthesized in their mature form.

Signal Transducer and Activator of Transcription

►Signal Transducers and Activators of Transcription in Oncogenesis

Signal-Transducer Proteins

Definition

Proteins function as units in signaling pathways are described as signal-transducers. They have at least two "working parts," one involved in recognition of an input signal (receptor part) and the other in generation of an output signal (generator) recognized by the downstream components. Some signal transducer molecules can respond to additional input signals that modulate their function (modulator) or could, after a short period, terminate generation of the output signal (timer).

►Inositol Lipids
►Signal Transduction

Signal Transducers and Activators of Transcription in Oncogenesis

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Synonyms

STAT; signal transducer and activator of transcription

Definition

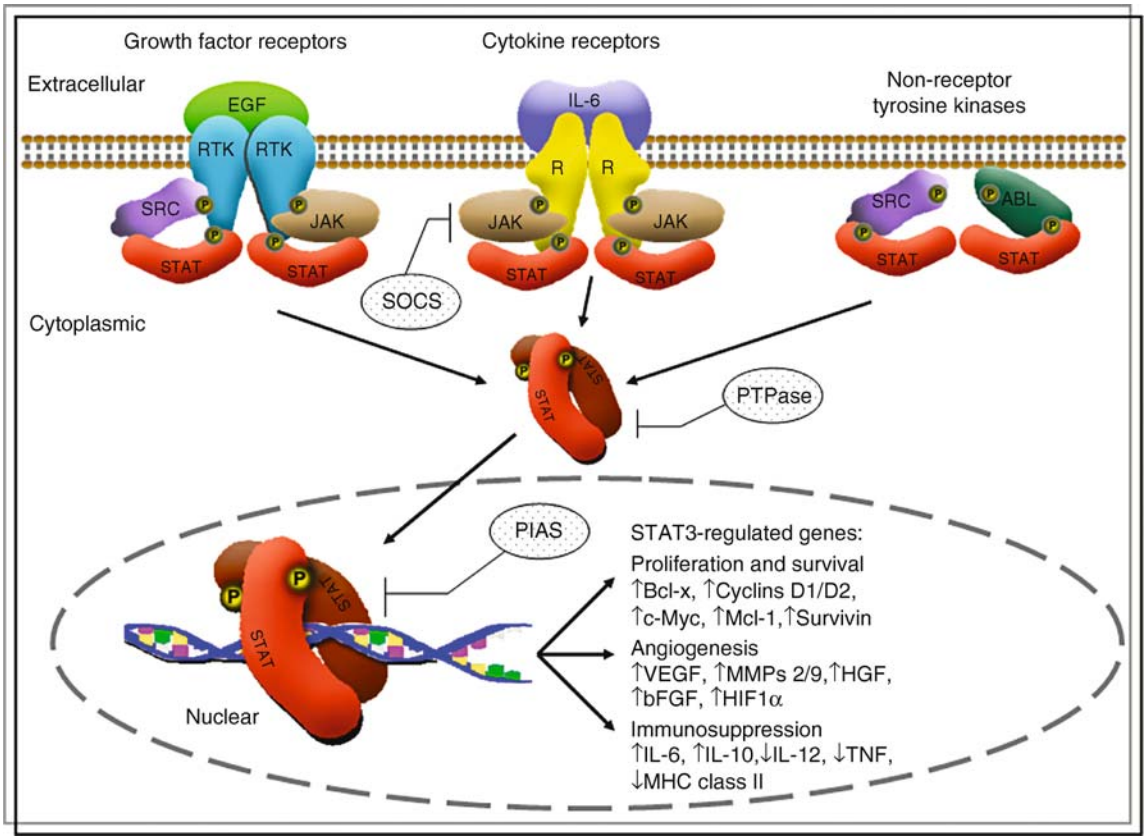
Signal transducer and activator of transcription (STAT) proteins comprise a family of latent ►transcription factors that reside in the cytoplasm and have been shown to control normal ►cytokine and ►growth factor-induced responses. In response to extracellular signals, such as cytokines or growth factors, STATs are activated through ►phosphorylation by ►tyrosine kinases. Subsequently, these activated dimers translocate (►translocation) into the nucleus where they bind to specific DNA sequences and regulate the transcription of cellular genes. Thus, STATs perform dual roles in transmitting receptor-generated signals from the cytoplasm to the nucleus and regulating cellular genes necessary for ►ligand-induced biological responses.

Characteristics

All multicellular organisms possess complex networks of molecular messengers that coordinate vital organ functions. Among the most common mechanisms used by multicellular organisms to ensure appropriate timing and duration of essential biological processes are production and secretion of cytokines and growth factors. Cytokines and growth factors control a wide variety of fundamental biological processes in diverse cell types, including immune responses, cellular differentiation, proliferation and programmed cell death (►Apoptosis; ►Apoptosis Signaling). Although differences exist between the biological processes and cell types regulated by cytokines and growth factors, these ligands possess some overlapping functions and share remarkably similar mechanisms of signal transmission. Cytokines and growth factors generally elicit a biological response by binding to receptor proteins located on the outer cell surface. Binding of these ligands to their specific receptors induces a change in the ability of the receptor to recruit and activate cytoplasmic signaling molecules that participate in signal transmission. Modulation of the activity of these cytoplasmic signaling proteins by the receptors initiates a cascade of biochemical signaling events, which ultimately lead to changes in nuclear gene expression that mediate the biological responses (►Signal Transduction). Cytokine and growth factor-induced processes are normally tightly controlled to ensure proper functioning. Aberrant functioning of these pathways results in deregulated signaling and is associated with development of a variety of pathological conditions including ►cancer.

STATs in Cytokine and Growth Factor Induced Signaling

In response to cytokine or growth factor-induced activation of signaling, cytoplasmic STATs become phosphorylated on specific tyrosine residues by receptors or receptor-associated proteins possessing tyrosine kinase activity (see Fig. 1) (►Receptor Tyrosine Kinases). Tyrosine phosphorylation of STAT proteins



Signal Transducers and Activators of Transcription in Oncogenesis. Figure 1 Constitutive activation of STATs by tyrosine kinases. Growth factors (e.g. EGF) and cytokines (e.g. IL-6) stimulate activation of receptor-intrinsic (RTK) or receptor-associated (JAK, Src) tyrosine kinases. Activated tyrosine kinases phosphorylate receptors to provide docking sites for unphosphorylated STATs. Once recruited, STATs themselves become tyrosine phosphorylated. Oncogenic tyrosine kinases such as SRC and ABL can also phosphorylate STATs independently of receptor engagement. Phosphorylated STAT dimers translocate to the nucleus, bind to specific DNA elements and regulate gene expression. In normal cells, STAT activation is transient and tightly regulated by inhibitory proteins, including PTPase, SOCS and PIAS. In cancer cells, constitutive activation of STATs, in particular Stat3 and Stat5, is associated with changes in the expression of genes that control fundamental cellular processes subverted in oncogenesis. RTK, receptor tyrosine kinase; R, receptor. See text for details. (Buettner et al. (2002) Clin Cancer Res 8(4):945–954 [2].)

allows STAT dimers to rapidly translocate into the nucleus. Once in the nucleus, STAT dimers bind to DNA and control the transcription of specific cellular genes. Under normal circumstances, the biological responses elicited by cytokines and growth factors are transient, and transmission of signaling is terminated through several inhibitory mechanisms, including (i) protein tyrosine phosphatases (►PTPase) that dephosphorylate and inactivate STATs, (ii) ►suppressor of cytokine signaling (►SOCS) family of proteins that prevent STAT activation by tyrosine phosphorylation, or (iii) protein inhibitors of activated STATs (►PIAS), a group of proteins that decrease transcription by binding to and thereby inhibiting STATs. While tyrosine phosphorylation of STAT proteins has been shown to be essential for cytokine-induced signals, activated cytokine receptors generally do not possess kinase activity and are not capable of directly phosphorylating STAT proteins on tyrosine. Cytokines induce activation of STATs indirectly through activation of receptor-associated tyrosine kinases of the Janus kinase (►JAK) family. After cytokine binding, STATs are phosphorylated by the receptor-associated JAKs, allowing signal transmission to proceed. Activation of STAT proteins has also been shown to be required for growth factor-induced cellular responses. However, the majority of growth factors that induce STAT activation bind to receptors that possess intrinsic tyrosine kinase activity, and tyrosine phosphorylation of STATs can be directly mediated by the activated receptor protein, receptor-associated JAK proteins or by additional non-receptor tyrosine kinases such as Src.

Bioactivity

Significantly, constitutive tyrosine phosphorylation and activation of STATs has been detected in cells that have undergone malignant ►transformation in response to expression of a variety of viral oncoproteins, which are often themselves constitutively activated tyrosine kinases (►Cell Transformation; ►Oncogene). Consistent with a role for STATs in oncogenesis, regulation of gene expression by one STAT family member, Stat3, has been shown to be required for induction of malignant transformation by the oncogenic Src tyrosine kinase. Furthermore, expression of a constitutively activated mutant of Stat3 can induce malignant transformation of specific cell types. These results demonstrate that, in addition to signals important to normal cellular functions, activated STATs can also transmit signals critical to oncogenic transformation.

STAT Activation During Progression of Human Cancer

Constitutive tyrosine phosphorylation and activation of STATs, in particular Stat3 and to a lesser extent Stat5, has been shown to occur frequently in a variety of human tumor types including leukemias, lymphomas, multiple myeloma, head and neck cancer, lung cancer, prostate cancer, renal cell carcinoma, colon carcinoma, melanoma and breast cancer. Frequent activation of specific tyrosine kinase signaling pathways, including activation of growth factor receptor tyrosine kinases, has also been detected in many of these tumor types. These findings suggest that constitutive activation of STAT proteins results from the constitutive activation of tyrosine kinases. Moreover, because STAT proteins control the transcription of nuclear genes involved in growth control, constitutive activation of STATs may transmit signals essential for oncogenic signaling and contribute to ►tumor progression. Among the genes that are regulated by STATs are genes involved in controlling cell cycle progression and programmed cell death (including ►Cyclins D1/D2, ►c-Myc, ►Mcl-1, ►BCL-x, and ►Survivin) (►Cyclin D; Myc Oncogene; Mcl Family; Survivin), genes that are involved in immunosuppression (interleukins-6, -10, -12, TNF, and ►MHC class II) (MHC), and genes that also participate in the regulation of angiogenesis (such as ►VEGF, MMPs 2 and 9, HGF, bFGF and HIF1α) (►Vascular Endothelial Growth Factor; Matrix Metalloproteinases; Fibroblast Growth Factors; Hypoxia Inducible Factor-1). Thus, constitutive activation of STATs – in particular Stat3 and Stat5 – in human tumors may contribute to progression of cancer by multiple mechanisms, including proliferation and survival of the tumor cell itself, induction of angiogenesis and suppression of immune cells in the tumor ►microenvironment (immunosuppression).

Targeting STATs for Cancer Therapy

Inhibition of STAT signaling has repeatedly been demonstrated to result in growth inhibition and induction of apoptosis in tumor cells harboring constitutive activation of Stat3 or Stat5. The observed dependence of certain tumors on constitutive STAT activation for survival has wide implications for cancer therapy, offering the potential for preferential tumor cell killing.

Targeting the Tumor Cell

Persistent Stat3 and Stat5 signaling within tumor cells directly participates in the development and progression of human cancers by either preventing apoptosis, inducing cell proliferation, or both. Targeting of tyrosine kinase activity upstream of STAT pathways has drawn special attention because of the recent development of tyrosine kinase-selective inhibitors. However, one potential draw-back of tyrosine kinase inhibitors is that they block multiple downstream signaling pathways in addition to STAT proteins, increasing the likelihood of undesirable toxicity. Since STAT activation is a point of convergence for many different tyrosine kinases, STATs themselves represent promising targets for the development of cancer drugs. One of the attractive features of STAT proteins for cancer therapy is that there are only two molecular targets, Stat3 and Stat5, as opposed to a multitude of tyrosine kinases. Antisense oligonucleotides and small-interfering RNA (►siRNA) that target STATs are just two of the many possibilities to interfere with aberrant STAT signaling (►Antisense DNA Therapy). Alternatively, small-molecule inhibitors are being developed to directly interfere with STAT proteins (►Drug Design). Recent progress has been made in design of short peptides, ►peptidomimetics and other non-peptide compounds that effectively block Stat3 dimerization and DNA-binding activity both *in vitro* and *in vivo*. Importantly, these compounds inhibit cell transformation mediated by activated Stat3 and provide the basis for development of novel drugs for potential cancer therapy.

Targeting the Tumor Microenvironment

Recent evidence suggests that Stat3-positive cancer cells can crosstalk with adjacent "normal" immune cells for the purpose of modifying the tumor microenvironment in favor of the cancer cell. Stat3-positive cancer cells can suppress the production and secretion of pro-inflammatory factors that otherwise would stimulate an anti-tumor immune response (►Inflammation; ►Inflammation in Cancer). At the same time, cancer cells can secrete immune-suppressive factors that act on immune cells. Some of the factors secreted by tumor cells, such as IL-6, IL-10 and VEGF, act on both tumor cells and adjacent immune cells by maintaining Stat3 activation in the tumor cell and simultaneously inducing Stat3 activation in immune cells. It has been shown

for many immune cells in the tumor microenvironment that Stat3 acts as a negative regulator of immune-stimulating molecules. Stat3 activation in immune cells has been found to inhibit the anti-tumor function of natural killer cells (NK), ►dendritic cells (DC), T-cells and ►macrophages, among other cell types (►Natural Killer Cells; Dendritic Cells; ►T Regulatory Cells; Macrophages). In the case of macrophages, for example, it has been demonstrated that ablation of both Stat3 alleles leads to increased levels of several pro-inflammatory cytokines similar to those found in cancer cells in which Stat3 signaling is inhibited. Moreover, simultaneous inhibition of Stat3 signaling in immune cells and tumor cells induces a more pronounced anti-tumor effect, demonstrating the additive nature of blocking Stat3 signaling in both tumor cells and normal cells of the tumor microenvironment. In addition to negatively regulating immune cell function, some of the factors secreted by Stat3-positive cancer cells, such as VEGF, bFGF, HGF and MMPs, are pro-angiogenic and facilitate tumor growth by increasing blood supply (tumor angiogenesis).

Summary

Because STATs play essential roles in regulation of cell proliferation and survival, the frequent activation of these proteins in human tumors suggests that they have essential roles in the malignant progression of human cancers. Furthermore, recent studies indicate that STATs also propagate signals between tumor and normal cells in the tumor microenvironment, thereby promoting tumor immune evasion and tumor angiogenesis Detection of constitutively activated STATs could provide an important marker for activation of oncogenic tyrosine kinase signaling pathways during tumor progression. Currently, characterization of the full complement of STAT-regulated genes that participate in growth regulation and tumorigenesis remains a very active and important area of investigation (►Microarray (cDNA) Technology). These studies have the potential to provide new information critical to understanding how fundamental cellular processes are subverted in tumor cells. Finally, activated STATs in human tumors provide promising targets for the design of novel therapeutics that block STAT functions involved in stimulation of proliferation, prevention of cell death, and induction of immune evasion and tumor angiogenesis. Early results in this area suggest that targeted inactivation of STAT proteins may be an effective approach to halting the growth of various types of human tumor cells. Inhibition of STAT signaling in tumors is predicted to impart therapeutic benefit by inducing growth arrest and apoptosis as well as suppressing tumor angiogenesis and immune evasion. However, targeting STAT proteins for therapeutic intervention in cancer remains to be fully explored.

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Signal Transduction

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Definition

Signal transduction is the process by which information from extracellular stimuli is propagated within a cell to alter cellular function. These signals frequently modulate gene expression, but they may also directly regulate processes such as cellular survival, metabolism, and macromolecular synthesis.

Characteristics

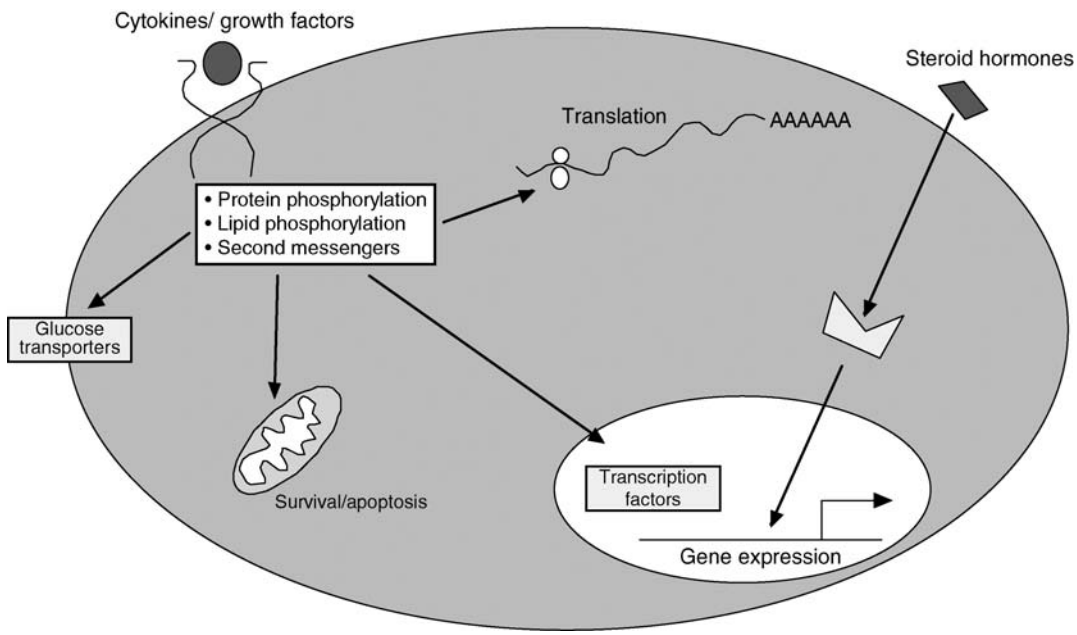
Starting shortly after fertilization, the phenotype of a cell becomes regulated largely by extracellular cues. Soluble factors such as cytokines and hormones, direct contact between cells, and interactions with the extracellular matrix all provide critical information affecting cellular morphology, proliferation, differentiation, and survival. A central question in cellular physiology is how these extracellular processes are converted into signals that can modulate the phenotype of a cell. A number of mechanisms have evolved by which such stimuli arising outside of a cell can regulate the expression of genes in the nucleus and other critical cellular processes. Several processes are used in a variety of ways by mammalian cells to transmit these signals. Each of these processes is also balanced by feedback mechanisms to modulate the cellular response. In essentially every kind of cancer, an abnormality in one or more signal transduction pathways provides a cell with signals for survival, proliferation, or self-renewal in the absence of the normal physiologic cues. Thus, elucidating signal transduction pathways is critical to understanding tumor

pathogenesis and tumor biology. Furthermore, among the most important advances in cancer therapy in recent years has been the introduction of signal transduction inhibitors that specifically target these molecular defects. These agents hold the potential to have much greater efficacy and much less toxicity than conventional cytotoxic agents.

Receptors

The ability to respond to the extracellular environment is mediated by receptors (Fig. 1). Although dozens of receptor molecules have been described in mammalian cells, from a functional perspective they can be grouped into three categories. First are receptors that are coupled to kinases. Activation of these receptors initiates the transfer of the terminal phosphate of ATP (or GTP) to an amino acid residue in a protein. Most receptor-associated protein kinases have specificity for phosphorylating tyrosine residues. This often leads to the subsequent activation of protein kinases that can phosphorylate serine or threonine residues, or lipid kinases. Thus, the interaction of a soluble ligand with an extracellular receptor can lead to the amplification of the signal through kinase cascades that can modulate cellular function through a number of mechanisms. Receptors may contain intrinsic tyrosine kinase activity, as is the case for polypeptide growth factor receptors such as the receptors for insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and others (▶receptor tyrosine kinases). Receptors may lack tyrosine kinase activity of their own, but associate with cytoplasmic tyrosine kinases which become activated by the binding of a ligand. Cytokine receptors, such as receptors for interferons, interleukins, oncostatin M, and leukemia inhibitory factor (LIF) associate with tyrosine kinases of the Janus kinase family whose activation propagates the signal.

Activation of receptor-associated tyrosine kinases leads to two major effects. First, it allows the tyrosine phosphorylation of specific substrates whose activity is regulated by this modification. For example, transcription factors of the ▶signal transducers and activators of transcription (STAT) family are found inactive in the cytoplasm under basal conditions. Once phosphorylated by a tyrosine kinase, STATs form dimers, which allows them to translocate to the nucleus, bind to specific DNA regions, and regulate the transcription of target genes. The second effect of tyrosine kinase activation results from the phosphorylation of the receptor itself and associated proteins. These phosphotyrosine



Signal Transduction. Figure 1 Through signal transduction, extracellular stimuli are converted into biochemical processes that control cellular function. Steroid hormones bind to intracellular receptors which then directly control gene expression. Cytokines, growth factors, and peptide hormones interact with cell surface receptors which then initiate cascades of protein phosphorylation, lipid phosphorylation, and second messenger generation. These mediators then regulate gene expression or directly alter processes such as cellular metabolism, protein translation, and apoptosis.

residues form potential docking sites for proteins that have structural domains, such as the ►Src homology 2 (SH2) domain, that allow them to specifically bind to phosphorylated tyrosine residues. This can trigger a series of interactions with other proteins in which tyrosine phosphorylation of proteins performs a "scaffolding" function, allowing other proteins to associate, which then activates their function.

In addition to the phosphorylation of proteins, kinases that can phosphorylate lipids play a key role in signal transduction. For example, ►phosphatidyl inositol 3-kinase (PI3 kinase), which is activated downstream of many receptors, leads to the phosphorylation of lipids in the cell membrane which can serve as a docking site for proteins with the lipid-binding ►pleckstrin homology domain. The recruitment of proteins such as ►Akt to these sites then leads to their activation.

The second family of receptors do not directly modify other proteins, but rather trigger the production of small molecule mediators that diffuse through a cell and modulate cellular function. Such "►second messengers" include molecules like cyclic AMP (cAMP), cGMP, calcium ions, and lipids released from cellular membranes (such as diacylglycerol). Thus, for example, the binding of glucagon to the glucagon receptor leads to the generation of cAMP by ►adenylyl cyclase associated with the glucagon receptor. cAMP can then activate cAMP-dependent protein kinase, also known as protein kinase A, which phosphorylates a variety of substrates on serine and threonine residues. Among these is the transcription factor ►cAMP response element binding protein (CREB), which becomes activated by this phosphorylation, and mediates the expression of a cohort of target genes.

The third functional group of receptors for extracellular signals are not located on the cell surface, but rather are intracellular. The steroid hormone family of receptors makes use of the fact that the ligands with which it interacts are small lipophilic molecules that are cell permeable. For example, hydrocortisone is secreted by the adrenal glands, circulates in the bloodstream, and then directly enters cells where it binds to the glucocorticoid receptor. The receptor and its associated ligand then binds to specific regulatory sequences in the genome and activates or represses the expression of specific target genes.

Targets of Signaling Pathways

While the modulation of gene expression is a key endpoint of signaling pathways and plays a major role in the modulation of cellular behavior triggered by extracellular stimuli, other important targets of signaling pathways are present in a cell. For example, among the serine, threonine kinases activated downstream of cell surface receptors are kinases that can phosphorylate ribosomal proteins. The ribosomal protein S6 can be phosphorylated by members of the p70S6 kinase family or the ribosomal S6 kinase (RSK) family. Phosphorylation of S6 increases the rate of translation of a subset of mRNAs, which may be important for increasing cellular growth and proliferation. Kinases such as the "mammalian target of rapamycin" (mTOR) can directly phosphorylate and affect the function of proteins involved in both translation initiation and elongation.

Signaling pathways can also directly regulate cellular metabolic function. for example, the serine, threonine kinase ►Akt, which is activated by a variety of receptors, can phosphorylate the glucose transporter Glut4, which promotes its localization to the cell surface, and enhances glucose uptake in ►adipocytes.

For humans and other mammals to develop and function normally, the ability of cells to die through the process of ►apoptosis is a critical step. The development of organs such as the brain and the function of the immune system is dependent on the programmed death of large numbers of cells. While there are several mechanisms by which a cell can die, one central process involves families of proteins that regulate mitochondrial permeability. These proteins may serve to promote survival or apoptosis, and their relative levels and activity are a major determinant of whether or not a cell survives. A number of signaling pathways directly regulate these proteins. For example, the serine, threonine kinase Akt can phosphorylate the pro-apoptotic protein BAD (a member of the ►BCL-2 family of proteins), which blocks its ability to trigger apoptosis, and thereby promotes the survival of a cell.

Signal Transduction in Cancer Pathogenesis

Given the importance of signal transduction in a range of critical cellular processes, it is not surprising that inappropriate activation of signaling pathways can contribute directly to the development of cancer. Signaling pathways may be activated by a variety of mechanisms. Among the most common mutations in human cancer, and the first identified in a human tumor, are activating point mutations of ►Ras family proteins. These mutations lead to the activation of a number of downstream kinases that promote cellular survival and proliferation. Cell surface tyrosine kinases can become activated by overexpression resulting from gene amplification. This mechanism leads to enhanced production of the ►Her2 (ErbB2) protein in ~30% of breast cancers, with the consequent activation of downstream signaling pathways. Receptor tyrosine kinases can also be activated by point mutations, deletions, or internal tandem repeats, all of which can lead to enhanced

tyrosine kinase activity in the absence or presence of ligand. Another common mechanism by which tyrosine kinases are activated in cancer is through ►chromosomal translocations that lead to the formation of a chimeric gene that encodes a tyrosine kinase with enhanced activity. The prototype for this is ►Bcr-Abl, a tyrosine kinase resulting from a translocation between chromosomes 9 and 22 (forming the so-called ►Philadelphia chromosome). The tyrosine kinase c-abl is a relatively weak tyrosine kinase that localizes primarily in the nucleus and is involved in responding to DNA damage. By contrast, Bcr-Abl is a highly active kinase which is found in the cytoplasm and phosphorylates and activates a number of key signaling molecules. Bcr-Abl is found in essentially all patients with chronic myeloid leukemia (CML), and a subset of patients with acute lymphoblastic leukemia (ALL).

Most signal transduction pathways have negative feedback systems that limit their effect. However, signaling pathways can become activated during tumorigenesis by the loss of such a negative feedback component. For example, ►PTEN is a lipid phosphatase that dephosphorylates inositol phosphates, thereby countering the effect of PI3 kinase. The PTEN gene is frequently deleted in cancers which leads to unrestrained activity of the ►PI3 kinase cascade.

Signal Transduction in Cancer Therapy

Current cytotoxic cancer therapies kill cells in a largely indiscriminate manner, and thus have limited efficacy and considerable toxicities. However, increased understanding of how signaling pathways are activated in cancer has presented an opportunity to develop targeted agents which are more effective at inhibiting tumor cells while causing few side effects. Current signal transduction inhibitors generally target activated tyrosine kinases by blocking the ability of ATP to bind to the kinase. Drugs such as imatinib mesylate, which inhibits the abl kinase, have been enormously successful in treating CML, and cause few serious side effects. While much effort is going into the development of kinase inhibitors, there is also an interest in developing inhibitors for signaling proteins without catalytic function. For example, drugs that inhibit the post-translational lipid modifications of Ras might be able to overcome the effects of an activating point mutation in this protein.

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Signal Transduction Cross-Talk

Definition

Is the mechanism by which activated signaling molecules in a primary ►signal transduction pathway can regulate signaling molecules in another primary signal transduction pathway.

►Kit/Stem Cell Factor Receptor in Oncogenesis

Signal Transduction Inhibitor-571

►STI-571

Signaling Cascades

Definition

Cascades involving enzymes that act one on another to relay molecular signals (hormone, growth factor) or physical signals (sensory stimuli) from a cell's exterior to its intracellular response mechanisms.

►Signal Transduction

Signalosome

Definition

Functional definition of a multi-protein complex of various signaling elements, whose association and activities is regulated by scaffold proteins. The

assembly of the signalosome is modulated in a complex spatio-temporal manner and ensures the specificity and speed of intracellular signal ►transduction.

Silencing Mediator of Retinoid and Thyroid Hormone Receptor

Definition

►SMRT co-repressor.

Simian Virus 40

Definition

►SV40; A virus of the genus *Polyomavirus* of the family Papovaviridae, originally isolated from kidney cells of the rhesus monkey. SV40 nucleotide sequences have been identified in certain human cancers, such as ►osteosarcomas and ►mesothelioma.

►SV40

Simulation

Definition

Allows precise radiation dose distribution within the prescribed treatment volume by integrating data from the computed tomography scanner or magnetic resonance imager to the three dimensional treatment planning computer system.

►Radiation Oncology

SIN3A

Definition

Transcriptional repressor protein interacting with ►histone deacetylases.

►Chromosomal Translocation t(8;21)

Single Cell Gel Electrophoresis Assay (SCGE)

►Comet Assay

Single Cell Invasion

Definition

Cell-cell adherence is lost and the cells migrate solitarily. This invasion pattern is usually seen in the presence of ►epithelial-mesenchymal-transition or amoeboid transition (change to a leukocyte-like migration pattern) and requires the expression of β1-integrin.

►Podoplanin

Single Cell Microgel Electrophoresis Assay

►Comet Assay

Single-chain Fv Dimer

►Diabody

Single-Chain Fv Fragment

Definition

Single-chain Fv fragment recombinant antibody molecule composed of the variable light chain domain (VL)

and variable heavy chain domain (VH) of an antibody linked by a short and flexible peptide linker.

►Bispecific Antibodies

Single Dose Toxicity Studies

Definition

Synonyms acute toxicity studies; Are standardized protocols whereby small groups of animals are given a single dose of a chemical usually at several doses to define a dose-response. The top dose is usually expected to be at about the maximum tolerated dose in the particular animal species under study. After euthanasia the animals are subjected to a full autopsy examination and most tissues are examined microscopically for any adverse effects. They are conducted according to Good Laboratory Practice. The so called LD50 (lethal dose 50) study is no longer performed.

►Preclinical Testing

Single Nucleotide Polymorphism

Definition

Refer to SNP; single nucleotide changes (A, T, C or G) in the genome sequence. Such changes can sometimes modify the function of a protein and an understanding of these changes can enhance our understanding and treatment of disease.

►MIC-1

Single Stranded Conformation Analysis

Definition

SSCP; Method applied for mutation screening that is based on the identification of secondary structure differences between mutated und unmutated single strands of a specific amplified region. Detection is performed after ►PCR within non denaturating gels.

►Leukemia Diagnostics

Siomycin A

Definition

Is a thiazole antibiotic compound that was identified from the culture broth of *Actinomycetes, that inhibits FoxM1 activity.*

►Forkhead Box M1

SIOP

Definition

The Societe Internationale Oncologie Pediatrique (International Society of Paediatric Oncology) is an international organization of clinicians involved in the treatment of childhood cancer.

Sipa-1 (Spa-1)

Definition

Signal-induced proliferation associated protein 1; is a GTPase-activating protein specific for Rap1 and is encoded by *Sipa-1* gene. There are several proteins with a homologous structure and function, including human E6TP1 (►human papillomavirus E6-targeted protein 1, also called Spa-L1 in mouse and SPAR in rat), Spa-L2, and Spa-L3.

►Rap1/Sipa-1

Sipple Syndrome

►Multiple Endocrine Neoplasia Type 2

Sir2-like Proteins

►Sirtuins

siRNA

Definition

Short Interfering RNA; Refers to short double-stranded RNA molecules that can be incorporated into ►RISC. They are very similar to ►microRNAs but they are usually synthesised in vitro and not by the cells. If siRNAs are produced in cells (in lower animals or plants) they are produced from long double-stranded RNA and many siRNAs are produced from one such RNA in contrast to microRNAs that are produced from imperfect stem-loop structure precursors and only one from each precursor. SiRNAs are used to knock down the expression of genes using ►RNA interference.

►MicroRNA
►RNA Interference

siRNA

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Definition

The small interfering RNA (siRNA) transcripts are 21–25 bp long, double-stranded transcripts as a defense response to nonendogenous double-stranded RNA (dsRNA), leading to sequence-specific mRNA cleavage. This double-stranded induced cleavage was named ►RNA interference (RNAi).

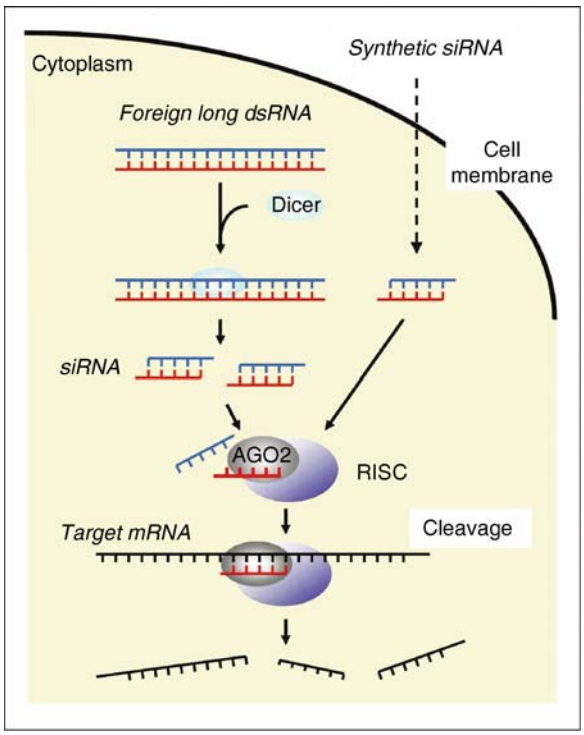
Characteristics

siRNA transcripts are 21–25 bp long, double-stranded transcripts that were found in *Caenorhabditis elegans* in 1998 as a defense response to nonendogenous dsRNA, leading to sequence-specific mRNA cleavage. This double-stranded induced cleavage was named RNAi and was soon to be found in other organisms, for example, *Drosophila melanogaster*, and in vertebrates. The main function of the mechanism is believed to a defense system against introduced viral double-stranded sequences, cutting them to pieces and thereby rendering them unable to infect the cell. The gene regulation part was not discovered until recently. RNAi was also quickly adapted for use in the laboratory as a gene-silencing tool for the silencing of specific genes by introducing the specific double-stranded sequence, and has become a common tool today for the study of gene expression and regulation due to its specificity. siRNA is generated by Dicer cleavage of long dsRNA sequences. After cleavage, the double-stranded siRNA is loaded into the RNA-induced silencing complex (RISC). During the loading process one strand is peeled off, leaving a single-stranded siRNA lodged inside RISC. This strand is then used by RISC as a template to recognize the cleavage target in the target mRNA transcript. A full match induces a cleavage in *Drosophila* RISC complexes, Argonaute2 (AGO2) has been identified as carrying the slicing action, but several other proteins are also involved in the RISC complex (Fig. 1).

Clinical Aspects

There are still unknown details regarding the biogenesis and function of siRNA, especially in mammals. The siRNA technique of using siRNA transcripts to knock down gene expression of specific genes has quickly become a popular method that is used for gene function analysis in molecular biology. The method is still being developed to improve the siRNA vectors, both the specificity and the usability of the technique, but already it exhibits a high specificity. The hope of the future is to be able to use siRNA knock down techniques to treat both genetic disorders, viral infections and, in extension, to be able to use siRNA as a cancer treatment.

The advantage of RNAi technology is that it can be used to target a large number of different genes involving a number of distinct cellular pathways. This is particularly important for a disease as complex as cancer. Most of the RNAi candidate cancer gene targets are involved in pathways that contribute to net tumor growth (either through increased tumor-cell proliferation or reduced tumor-cell death, or both). While mRNAs expressed from mutated cancer ►oncogenes can be directly targeted for RNAi intervention, RNAi can also be used to target and silence gene products that negatively regulate the function of endogenous ►tumor suppressor genes. Other gene products that can be targeted by RNAi include proteins involved in cellular ►senescence, or protein stability and degradation. Although these additional targets are not directly involved in the oncogenesis pathway, they can indirectly



siRNA. Figure 1 Schematic representation of RNA interference (RNAi). siRNA is generated by Dicer cleavage of long double-stranded RNA sequences. After cleavage, the double-stranded siRNA is loaded into the RNA-induced silencing complex (RISC). Synthetic siRNA is directly incorporated in the RISC. During the loading process one strand is peeled off by Argonaute2 (AGO2) leaving a single-stranded siRNA lodged inside RISC. This strand is then used by RISC as a template to recognize the cleavage target in the target mRNA transcript. A full match induces a cleavage.

contribute to net tumor growth, and therefore represent potential candidates for RNAi intervention.

Delivery

RNAi-mediated gene silencing in mammalian cells can be achieved by transfection, for example, using liposomes or electroporation of synthetic siRNA molecules, or by gene transfer using plasmid- or virus-based expression cassettes with RNA polymerase III promoter encoding dsRNA molecules.

The easiest way to induce RNAi is the use of chemically synthesized siRNAs. However, in addition to siRNAs produced by chemical synthesis, siRNAs may be generated by from long dsRNAs in vitro via recombinant Dicer, by in vitro transcription using T7 RNA polymerase, or siRNAs can be isolated from *Drosophila* embryo extracts. Classic transfection of siRNA molecules using different physical methods such as liposome-mediated transfection, electroporation, or single-cell microinjection has been successfully applied. Treatment of mammalian cells with siRNAs typically results in a transient downregulation of the target mRNA after 1–2 days for a duration of 3–5 days. In vivo delivery of chemically synthesized siRNAs to mice was reported by injection into the tail veins. By this approach, a downregulation of 90% of endogenous mRNA transcripts in the majority of liver cells after a single siRNA injection could be achieved. In the liver, the RNAi effect persists for several days.

Although siRNAs are readily taken up into worm and fly cells, most mammalian cells do not efficiently internalize these small molecules. This is even true of cells, such as dendritic cells and ▶macrophages, which are actively sampling their environment. Therefore, the major obstacle for using small RNAs as drugs is to deliver them into the cytoplasm of cells. An exception may be mucosal tissues. In the lung and vagina, siRNA uptake is extremely efficient and occurs even in the absence of transfection reagents. For clinical indications where siRNAs only need to be delivered to a localized region, such as the eye, pulmonary or vaginal mucosa, or superficial tumors, efficient siRNA delivery and silencing can be achieved by mixing siRNAs with cationic lipid transfection reagents used for in vitro transfection and directly injecting the siRNA–lipid complexes into the relevant tissue or instilling it into the body cavity. A similar approach is certain to apply to the skin. Mixing siRNAs with other molecules known to carry nucleic acids into cells, such as certain cationic peptides, might also be used effectively for local delivery. However, some cell types, such as lymphocytes, dendritic cells, and hematopoietic stem cells, are refractory to transfection using cationic lipids. Therefore, even when these targets might be localized, alternate delivery strategies may be needed.

The first demonstrations of the therapeutic potential of siRNAs for silencing disease-related genes delivered siRNAs systemically by rapid high-pressure intravenous injection. This method leads to transient right-sided heart failure, where elevated venous pressures somehow enable siRNAs to get into cells in highly vascularized organs like the liver, pancreas and lungs. Nonetheless, this strategy is too risky for human use. It is, however, possible to deliver siRNAs into an organ, such as the kidney, by rapid retrograde injection via catheter into the draining vein. It may be possible to use hydrodynamic injection into a peripheral vein to treat skeletal muscle by blocking venous outflow using a tourniquet. Elevated venous pressures are generated only in the targeted tissue without inducing potentially fatal heart failure. However, a minimally invasive method for delivering siRNAs requires alternate approaches. A variety of strategies that involve complexing siRNAs to cationic polymers or peptides or incorporating siRNAs into nanoparticles or liposomes have

been proposed. Alternately, siRNAs can be covalently or noncovalently linked to antibody fragments or ligands to cell surface receptors to limit the delivery of the siRNAs to cells that bear the specific receptor. These strategies probably deliver siRNAs via receptor-mediated endocytosis, although the trafficking of siRNAs into and within cells has not been well studied. The directed delivery of siRNAs into specific cells will decrease the amount of siRNAs needed for the efficient silencing of gene expression in the target organ or tissue and will reduce potential toxicity by preventing targeting of unintended cells and tissues. The optimal delivery strategy may differ between different therapeutic indications and will depend on efficiency and duration of delivery and silencing, lack of systemic toxicity, and lack of immunoreactivity, which would interfere with repetitive treatments. The first examples of effective systemic delivery have only recently been described.

Nonspecific Immune Stimulation

While there is a high degree of specificity associated with RNAi, some effects have been observed that are independent of the specific gene targeted for silencing. In general, 21 bp or longer dsRNAs can lead to a sequence-independent ►interferon response. Interferon can also activate the dsRNA-dependent protein kinase (PKR), which phosphorylates and subsequently inactivates the translation factor eIF2, leading to a global inhibition of mRNA translation. The length of the initiating siRNA clearly has some role in triggering the interferon response, with more recent data suggesting that sequences shorter than 19 nucleotides are more likely to escape the interferon antiviral response. It is anticipated that the judicious selection of siRNA sequences together with a greater understanding of their interactions with any given target gene will resolve this issue.

Similarly, evidence exists that siRNAs can activate dendritic cells and other cells of the immune system through a much more specific and restricted class of receptors, the toll-like receptors, which can recognize foreign nucleic acids including dsRNAs and when activated can send a danger signal to trigger a proinflammatory response. These findings raise the possibility that RNAi reagents may trigger unforeseen immune responses, including autoimmune diseases, in vivo.

Off-Target Interference

Nucleic acid-based gene-silencing molecules may also have effects on genes that are not considered targets, the so-called off-target effects, due to similarities in nucleic acid sequences. The degree of the off-target effect is dependent upon the mode of silencing and the stability of the nucleic acid hybrid. If siRNAs are not carefully selected, siRNAs having partial complementarity to an mRNA target can repress translation or subject unintended mRNAs to degradation. A study that compared the gene-expression profiles created by different siRNAs targeted against the same transcript revealed that in extreme cases, as little as seven nucleotide complementarity between the 5′ end of either siRNA strand to an mRNA can cause a reproducible reduction in transcript levels. Interestingly, it has been found from studies in primitive organisms that off-target effects are not observed when complete dsRNAs are introduced instead of synthetic siRNAs. This may be explained by the fact that the siRNAs derived endogenously from the cleavage of dsRNAs are generated and selected by Dicer and the RISC complex, which may have a proofreading mechanism that protects against the generation of siRNA sequences that might result in the silencing of endogenous genes. Therefore, it is possible that mammalian siRNAs generated from dsRNA precursors through the action of Dicer and the RISC complex may be less prone to induce off-target effects than synthetically designed siRNAs. Several algorithms and software are available to select siRNA target sequences with reduced off-target effects, and it will be important to select siRNA targets with relatively sophisticated sequence comparison tools to minimize potential off-target effects.

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SIRS

Definition

►Systemic Inflammatory Response Syndrome.

SIRTs

►Sirtuins

Sirtuins

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Synonyms

SIRTs; Class III histone deacetylases; Sir2-like proteins

Definition

Sirtuins are a family of protein deacetylases (►Histone deacetylases) and ADP ribosyltransferases requiring NAD+ for their enzymatic activity.

Characteristics

Biochemical Features of Sirtuins

Sirtuins are protein deacetylases and ADP ribosyltransferases that target a wide range of cellular proteins in the nucleus, cytoplasm, and mitochondria. Among the substrates are histone proteins, and therefore sirtuins are also referred to as class III ►histone deacetylases. Seven human sirtuins (SIRT1–7, see Table 1) have been identified and share a catalytic domain of 275 amino acids. As opposed to ►histone deacetylases, sirtuins require ►NAD± as a cofactor for their enzymatic activity. Therefore, NAD+/NADH ratio and metabolic status of the cell influences the activity of sirtuins. Nicotinamide is a physiological intracellular inhibitor of sirtuins.

Cellular Functions of Sirtuins

Human sirtuins (SIRTs) are structurally related to the silent information regulator 2 (Sir2) family, which are conserved from bacteria to humans and regulate lifespan in lower organisms. Sirtuins are involved in regulation of the cellular stress response, cell cycle progression, chromosomal stability and aging. SIRT1 knock-out mice show multiple developmental defects and die around birth and is thus required for normal embryonal development.

Mammals have seven sirtuins (Table 1). In addition to the intranuclear SIRT1, there is the cytoplasmic SIRT2, the three mitochondrial sirtuins SIRT3, 4, 5, and also within the nucleus the ►heterochromatin-associated SIRT6 and the nucleolar SIRT7. Based on sequence homology, the sirtuins can be grouped into four classes. SIRT1, 2, 3 comprise class I, SIRT4 is in class II, SIRT5 is in class III, and SIRT6, 7 are class IV sirtuins. Table 1 summarizes the cellular localization and functions of the 7 human sirtuins.

Among the 7 sirtuins, SIRT1 has been most intensively studied, whereas the functions and target proteins of SIRT2–6 are less well understood. SIRT1 regulates the acetylation status of histone proteins and is thereby involved in the control of ►chromatin structure and regulation of transcriptional activity. For example, SIRT1 directly deacetylates lysine 16 of histone H4, which is a mark for transcriptional inactive heterochromatin, whereas acetylated lysine 16 is found in transcriptionally active ►euchromatin. SIRT1 is a component of the polycomb group repressor complex, a multiprotein complex which controls differentiation and developmental programs in embryonic cells. In addition to its function in regulating chromatin structure, SIRT1 regulates the activity of several cellular proteins via modulating their acetylation status. For example, deacetylation of the ►tumor suppressor protein ►p53 by SIRT1 results in downregulation of its transcriptional and ►apoptosis-inducing as well as ►senescence-inducing activity. Other proteins known to be targets of SIRT1 are MyoD (a transcriptional regulator of muscle gene expression and differentiation), FOXO proteins (family of transcription factors that regulate oxidative stress response, cell cycle arrest, DNA repair, and apoptosis),

Sirtuins. Table 1 Cellular localization and functions of sirtuins

	Localization	Function
SIRT1	Nucleus	Deacetylation of histone and non-histone proteins
		Regulation of chromatin structure
		Regulation of activity of p53 and several other transcription factors (see text)
		Involved in cellular stress response, survival, senescence, cellular life span, differentiation
SIRT2	Cytoplasm	Deacetylation of α-tubulin
	Shuttles into nucleus	Mitotic checkpoint regulation
SIRT3	Mitochondria	Acetyl-CoA production
SIRT4	Mitochondria	ADP-ribosylation, suppression of insulin signaling
SIRT5	Mitochondria	not known
SIRT6	Nucleus	Guards genomic instability, target unknown
SIRT7	Nucleolus	Promotes rRNA transcription

Ku70 (a protein involved in nonhomologous repair of DNA double-strand breaks), PPAR-y (a member of the nuclear receptor superfamily that integrates energy control, lipid metabolism, and glucose homeostasis), and NF-kB (transcription factor that regulates immune response, inflammation, cell proliferation, and cell death). As a result of modulating the function of these proteins, SIRT1 is involved in cell survival, muscle differentiation, and fat metabolism and is upregulated during caloric restriction. SIRT1 plays a role in regulation replicative senescence and limit of proliferation of normal cells. SIRT1 ensures that damaged DNA is not propagated and that mutations do not accumulate in cells. In recent years, much attention has been paid to the function of SIRT1 in controlling lifespan and longevity.

Sirtuins and Cancer

SIRT1 limits proliferation capacity and induces senescence in normal, non-transformed cells exposed to chronic stress stimuli and genomic insults. Therefore, sirtuins are thought to protect against the development of cancer. Sirtuins are considered as targets for chemoprevention of age-related cancers by promoting their function in guarding genomic stability and senescence.

However, SIRT1 is also found to be overexpressed in a number of cancers such as acute myeloid leukemia (AML), skin, breast, and colon cancer, and is upregulated in chemotherapy-resistant cancer cells. High levels of SIRT1 protects malignant cells from apoptosis via deacetylation and subsequent reduction of the activity of proteins involved in programmed cell death. Among the cancer-relevant substrates of SIRT1 is the tumor suppressor protein p53. SIRT1 deacetylates p53 resulting in repression of its functional activity. Another substrate of SIRT1 is the critical B-cell lymphoma protein 6 (BCL6). Various tumor cell lines cease growth and undergo apoptosis after SIRT1 ►knockdown. SIRT1 ►overexpression blocks ►oncogene-induced senescence. At the ►epigenetic level, SIRT1 deacetylates ►histone H4 at lysine 16, a modification which is a common hallmark of human cancer. Thus, inhibition of sirtuins appear to be a promising target for certain cancers which are dependent on high levels of sirtuin activity.

Sirtuin Inhibitors and Activators

The biological activity of sirtuins can be modulated by small molecule inhibitors and activators. Nicotinamide, a natural byproduct of the sirtuin deacetylase reaction, is a general inhibitor of all sirtuin family members. Other small molecule inhibitors are sirtinol, M15, and splitomycin. The observation that calorie restriction increases life span through increase of the activity of sirtuins in animal models lead to the screening of compounds with sirtuin stimulating activity. Among the substances identified were the polyphenols quercetin and piceatannol, and resveratrol, a polyphenol found in the skin of red grapes.

Acknowledgments

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Sister Chromatid Exchange

Definition

SCE; Is the mitotic recombination between the two newly replicated sister chromatids, leading to exchange of genetic information.

Sister-Chromatids

Definition

Chromatids of the same chromosome, which are connected by a centromer. Nonsister-chromatids are the chromatides of homologous chromosomes ►Anaphase-promoting complex.

►Securin

Site-Directed Mutagenesis

Definition

Is a mutation created at a defined site of known sequence in a DNA molecule of a wild-type, by a molecular biology technique.

Site-specific Drug Delivery

►Targeted Drug Delivery

Sivelestat

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Definition

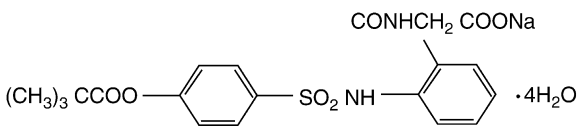
Sivelestate is a low molecular weight synthetic inhibitor of human ►neutrophil elastase (NE) (►Netrophile elastase and cancer), which was produced by Ono Pharmaceutical Company Co., Ltd., in 1991 as ONO-5046, N-[2-{4-(2,2-Dimethylpropionyloxy) phenylsulfonylamino}-benzonyl] aminoacetic acid (MW. 434.47). The structure is illustrated in Fig. 1. This is a potent, specific and intravenously active NE inhibitor and it is widely used to treat patients with lung injury diagnosed as ►SIRS (systemic inflammatory response syndrome), in Japan at a dose of 0.2 mg/kg/h for 14 days.

Characteristics

Mechanisms of Action

Sivelestat inhibits the action of NE which is a member of serine proteases produced by polymorphonuclear neutrophils and monocytes/macrophages. NE degrades a broad spectrum of extracellular matrix (ECM) (►Extracellular remodeling) and cell surface proteins, such as elastin, interstitial collagens, proteoglycans, fibronectin, laminin, and type IV collagens. Under normal physiological conditions, the proteolytic activity of elastase released by recruited neutrophils is strictly regulated by antiproteases, such as alpha-1-protease inhibitor and secretary leukoprotease inhibitor. However, under conditions of physiological disturbance, such as the presence of neoplasms, surgical stress, or inflammation, the balance between elastase and antiprotease is disrupted, and the predominant elastolytic activity causes the destruction of peripheral ECM of the lung.

NE also cleaves pro-►transforming growth factor-alpha (TGF-α) (►Epidermal growth factor receptor ligands) on the surface of human airway epithelial cells via its proteolytic activity and releases mature, soluble TGF-α on human airway epithelial cells. Release of TGF-α activates the ►epidermal growth factor receptor (EGFR) (Epidermal growth factor receptor ligands) signaling cascade (►Transduction of oncogene), resulting in mucinous secretion in the peripheral airways that might cause severe respiratory complications after major surgical stress or severe inflammatory response.



Sivelestat. Figure 1 Structure of sivelestat, ONO-5046, N-[2-{4-(2,2-dimethylpropionyloxy) phenylsulfonylamino}-benzonyl] aminoacetic acid (MW. 434.47).

Clinical Aspects

Clinical Application in Practice

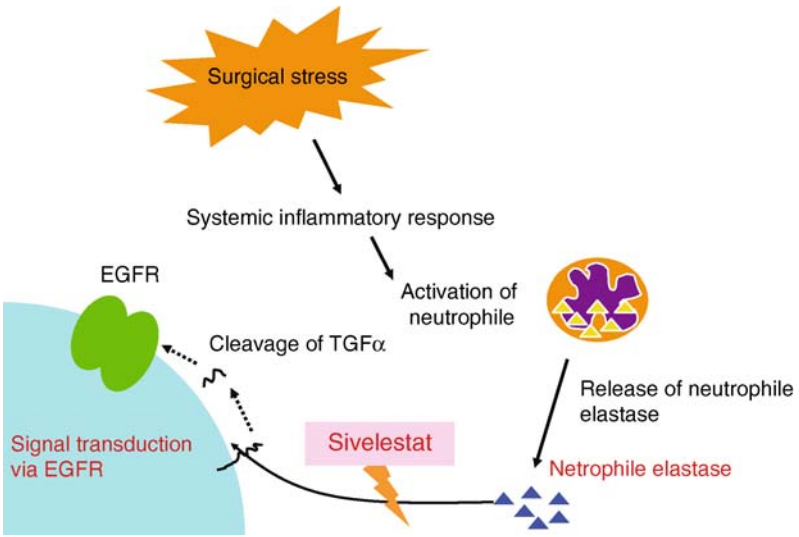
Sivelestat is prescribed clinically for the treatment of various inflammatory diseases in Japan. The anti-inflammatory effects of sivelestat are applied for the alleviation of surgical stress, and can reduce morbidity and organ dysfunction, including ►acute respiratory distress syndrome (ARDS). Considerable amount of NE is released from activated neutrophils which are induced by systemic cytokines including ►TNF-α, ►IL-8 and ►IL-6 produced by ►macrophages in the lung. SIRS is often observed after surgical injury, which influences on the function of leukocytes and endothelial cells leading to the ARDS or ►acute lung injury (ALI) with the destruction of the alveolar construction of the lung.

Application to Cancer Treatment as Molecular Targeting Therapy

Rapid recurrence or regrowth of tumors are often observed after major cancer surgery, or perioperative surgical complications with SIRS that involve the release of NE by neutrophils and also by cancer cells.

On the other hand, cancer cells have multi-►autocrine growth factor and receptor loops including EGFR- TGF-α system for the development of growth and metastatic potential.

The growth and invasion activity of cancer cells are stimulated by NE and the growth stimulation occurs via the cleaved TGF-α from the cell membrane, which activates the EGFR and then activates the subsequent intracellular signal transduction. Moreover, NE stimulates the release of several growth factors including ►PDGF (platelet derived growth factor) and ►VEGF (vascular endotherial growth factor) that are closely



Sivelestat. Figure 2 Action mechanism of sivelestat. NE released from activated neutrophils by SIRS cleaves the pro-TGF-α on the cell surface of the cancer cells, which stimulate the growth of the cell via EGFR. Sivelstat blocks the cleaveage of the TGF-α.

related to the growth and angiogenesis of tumor cells. As mentioned above, sivelestat inhibits the NE-induced release of TGF-α from cell membrane of not only neutrophils but also cancer cells (Fig. 2). Therefore, sivelestat might be a new therapeutic tool for the treatment of anti-inflammatory response and also for molecular targeting therapy (►Molecular therapy) of postoperative cancer patients by inhibiting the rapid cancer cell growth after resection of tumors accompanied with SIRS.

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Sjögren Syndrome

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Synonyms

Sicca syndrome; Sicca complex; Dry mouth syndrome; Dry eyes syndrome; Keratoconjunctivitis sicca; KCS; Xerostomia; Xerophthalmia

Definition

Sjögren syndrome is a chronic, progressive autoimmune disorder that primarily affects the exocrine glands. The classic signs and symptoms include enlargement of the parotid and lacrimal glands, with mucosal dryness manifested by dry mouth (►Xerostomia) and dry eyes (►Xerophthalmia) (►Keratoconjunctivitis sicca). Lymphocytic infiltrates replace functional epithelium, which results in decreased exocrine secretions (exocrinopathy).

Numerous features suggest an immunologic etiology. Patients demonstrate hypergammaglobulinemia and have several types of ►autoantibodies, such as the

S

rheumatoid factor (RF), antinuclear antibodies (ANA), Ro/SS-A and/or La/SS-B, and antibodies to salivary duct epithelium.

Characteristics

Sjögren syndrome is named after the Swedish ophthalmologist Henrik Sjögren. However, the disease was addressed during the nineteenth century in a number of case reports, between the years of 1882 and 1924, describing various combinations of dry mouth, dry eyes, and chronic arthritis. Mikulicz reported in 1892 a man with bilateral parotid and lacrimal gland enlargement associated with massive round cell infiltration. Later on, the link between Sjögren syndrome and ►malignant lymphoma was described in a classic paper in 1964. More recently, a set of preliminary classification criteria was identified by a European Concerted Action in 1993 and they have been widely accepted. The triad of dry mouth (xerostomia), dry eyes (keratoconjunctivitis sicca), and a connective tissue disease, usually ►rheumatoid arthritis (►RA) or ►systemic lupus erythematosus (SLE), is termed secondary Sjögren syndrome. In the absence of a connective tissue disease, the designation primary Sjögren syndrome (or sicca syndrome) is used.

Incidence

Sjögren syndrome is the most common form of autoimmunity disorder and is seen mostly in women (female:male ratio 9:1) in the fourth and fifth decades of life. Its frequency in RA is 20% and therefore Sjögren syndrome is most commonly seen in this context.

Etiology

The etiology of Sjögren syndrome is unknown but may involve genetic and immunological factors.

1. *Genetic Factors*. ►A prominent feature of Sjögren syndrome is its genetic predisposition. The polymorphic ►major histocompatibility complex (MHC) genes are well-documented genetic risk factors for the development of ►autoimmune diseases overall. With regard to Sjögren syndrome, the most relevant MHC complex genes are the class II genes, more specifically the ►HLA-DR and ►DQ alleles. In whites of northern and western European background, including North American whites, Sjögren syndrome is one of several autoimmune diseases associated with the ►haplotypes HLA-B8, DRw52, and DR3. An association with DR2 has been reported in Scandinavians and DR5 in Greeks.
2. *Immunological Factors*. ►Immunological abnormalities include a marked hyperactivity of B lymphocytes with hyperglobulinemia and serum autoantibodies, some organ-specific such as antibodies to salivary duct epithelium, and others nonspecific such as ANA and rheumatoid factor. Impaired cellular immunity with defective suppressor T cell (►Regulatory or suppressor T cells) function has been reported in some patients but not in others. Both ►T lymphocytes and B lymphocytes have been identified in the tissue lesions and local synthesis of ►immunoglobulins has been demonstrated. Since the ►lymphocyte infiltration has been demonstrated to be initially periductular in local lymphocyte cytotoxicity directed against ►antigens on salivary gland duct cells has been postulated.
3. *Environment.* Among the possible etiologic and triggering factors involved in Sjögren syndrome, the discussion about a relationship between viral infections causing development of autoimmune reactions began some decades ago. The putative role of different ►viruses in Sjögren syndrome can be viewed in the light that salivary glands are a site of latent viral infections. Potential viral triggers include a number of viruses. Among these, ►Epstein-Barr virus (EBV) has been widely studied in relation to Sjögren syndrome. A higher prevalence of serum human herpesvirus-6 (HHV-6)-specific antibodies has also been detected in patients with Sjögren syndrome than in normal individuals (36% vs. 10%). Retroviruses are known to infect cells of the ►immune system and cause abnormalities in immune regulation. High serum titers of antihuman T lymphotropic virus type I (HTLV-1) antibodies and a high prevalence of salivary ►immunoglobulin A (IgA) class anti-HTLV-1 antibodies in patients with Sjögren syndrome were reported endemically in Japan. Hepatitis C virus (HCV) infection in some populations has been frequently (14%) detected in patients with primary Sjögren syndrome. Analysis of the association between ►chronic lymphocytic sialadenitis and ►chronic HCV liver disease showed that histologic features of Sjögren syndrome were significantly more common in HCV-infected patients (57%) compared with controls (5%). ►Lymphotropic viruses have the potential to trigger the autoimmune process. Some of the immunoreactive regions within the La/SS-B protein have been found to have sequence similarities with proteins of EBV, HHV-6, and human immunodeficiency virus (►HIV)-1. It seems reasonable that these viruses can promote autoantibody (particularly anti-La/SS-B) production through ►molecular mimicry or exposure of La/SSB homolog sequences on cellular surfaces after translocation of cryptic self-determinants.

Clinical Manifestations

Presentation

The typical case is of a middle-aged woman who developed sicca symptoms insidiously. Less commonly it presents with parotid swelling or with constitutional

symptoms such as malaise and arthralgias. Rarely the presenting feature is cutaneous ►vasculitis, a cranial neuropathy usually involving the trigeminal nerve, a peripheral neuropathy, or the hyperviscosity syndrome. Sicca symptoms tend to develop more rapidly in primary Sjögren syndrome, when episodic parotitis and involvement of other organs is more common.

Involvement of Exocrine Glands

Ocular symptoms due to reduced lacrimal secretion and dessication of the cornea and conjunctiva (keratoconjunctivitis sicca) include a gritty or sandy sensation, reduced tearing, photophobia and the presence of a ropey discharge, particularly at the inner canthus. Recurrent infections are also common. In advanced cases, the cornea may be severely damaged and complications include corneal ulceration and occasionally perforation. Decreased saliva results in dryness of the mouth (xerostomia) that leads to dysphagia, abnormalities of taste, and adherence of food to the buccal surface. Widespread involvement of mucosal glands elsewhere may result in dryness of the nose, posterior pharynx, trachea, bronchial tree, skin, and vagina. Involvement of the respiratory tract leads to an increased frequency of upper and lower respiratory tract infections and otitis media. There is parotid gland enlargement in 50% of cases (80% in primary Sjögren syndrome) sometimes accompanied by pain and fever.

Extraglandular Involvement

Extraglandular lymphoproliferation occurs in 25% of primary Sjögren syndrome, and involves the ►reticuloendothelial system, kidney, muscle, and lungs. Systemic manifestations are varied and include diffuse interstitial pneumonitis, renal tubular abnormalities, peripheral and cranial neuropathy, nonthrombocytopenic purpura, and the development of pseudomalignant and malignant lymphoid proliferation. Malignant lymphoma occurs more often than can be explained by chance alone, and Sjögren syndrome has often been considered to be a link in the spectrum between autoimmune disorders and lymphoproliferative disorders (Malignant lymphoma, Antonino Carbone and concepts).

Diagnosis

The most commonly used tests for the detection of dry eyes are the Schirmer I and the rose bengal (alternatively Lissamine green dye) and subsequent scoring according to van Bijsterveld. Schirmer I is performed using standardized tear tests strips. In the European classification criteria, ►Schirmer I test is positive ►when the wetting is less than 5 mm in 5 min. The scoring according to van Bijsterveld detects destroyed conjunctival epithelium induced by dryness. Saliva production tests are simple screening tests for salivary gland involvement in Sjögren syndrome. Saliva, which is produced by the three major and numerous minor submucosal salivary glands, exhibits great flow variations among healthy individuals and in the same individual under diverse conditions. The test should therefore be standardized; the unstimulated whole saliva collection test is performed for 15 min, and the test is considered positive when 1.5 ml or less whole saliva is collected, being well below the normal mean range. Other tests used to evaluate salivary gland involvement include parotid sialography and salivary gland scintigraphy. The sialography typically shows sialectasis in contrast to the fine arborization seen in normal parotid ductules. In the scintigraphic test, ^{99m}technetium-pertechnate is given intravenously, and in Sjögren syndrome patients, the typical finding is decreased uptake in response stimulation of the parotid and submandibular salivary glands. This test is a sensitive and valid method to measure abnormalities in salivary gland function in the hands of skilled personnel.

SS is Diagnosed on the Basis of Either European Classification

European Classification. (Preliminary criteria for the classification of Sjögren syndrome, Vitali C et al.)

1. Ocular symptoms – Positive response to 1 of 3 questions pertaining to dry eyes
2. Ocular signs – Positive Schirmer test (<5 mm in 5 min) or positive rose bengal staining
3. Oral symptoms – Positive response to 1 of 3 questions pertaining to dry mouth
4. Salivary gland involvement – Objective evidence of salivary gland involvement; salivary scintigraphy; parotid sialography; unstimulated salivary flow less than 1.5 mm/min
5. Serologic or autoantibody test results – Presence of autoantibodies to Ro (SS-A), La (SS-B), or both
6. Categories
   a. Primary SS – Presence of any of 4 of the previous 6 categories
   b. Secondary SS – Presence of potentially associated connective tissue or autoimmune disease with the first 2 categories (ocular symptoms and ocular signs) plus any 2 of the next 3 categories;

Laboratory Abnormalities

Autoantibodies to salivary duct, gastric parietal cells, thyroglobulin, mitochondria, and smooth muscle are frequently present. Rheumatoid factor is found in over 90% and ANA in ~70%. Serum antibodies to two partially characterized cellular antigens Ro (SSA) and La (SSB) are commonly found in primary Sjögren syndrome.

Anti-Ro/SSA and anti-La/SSB Antibodies

The reported frequencies of anti-Ro and anti-La (►Anti-Ro/SSA and anti-La/SSB antibodies) depend on the methods of detection and referral bias of the center performing the study. Overall, anti-Ro precipitins occur in ~60–75% of primary Sjögren syndrome and are also observed in cases of secondary Sjögren syndrome, irrespective of the association with SLE, progressive systemic sclerosis, rheumatoid arthritis, or primary biliary cirrhosis (PBC). Anti-La antibodies were initially reported to occur in up to 40% of patients with primary Sjögren syndrome. Even higher frequencies were reported when anti-La was analyzed by ►ELISA or ►immunoblotting. Further studies have shown that combined detection of anti-La and anti-Ro antibodies has a higher diagnostic specificity for primary Sjögren syndrome than does anti-Ro alone. Although the pathogenetic role of anti-Ro and anti-La in Sjögren syndrome is not established, positive serology is associated with a high frequency of palpable purpura, leucopenia, lymphopenia, and hypergammaglobulinemia, and with more severe glandular disease. Recent studies also have found salivary enrichment of anti-Ro and anti-La in patients with Sjögren syndrome, suggesting local autoantibody production in salivary glands as well as presence of Ro52, Ro60, and La autoantibody-producing cells in salivary gland biopsy samples from patients with Sjögren syndrome (SSA(Ro) SSB(La) in SS, Tsay GJ and concepts).

Anti-CENP-H Antibodies and SS

Anticentromere antibodies (ACA) have been described in patients with different rheumatic disorders including CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactly, telangiectasia), PBC, primary Raynaud's disease (PRD), and rheumatoid arthritis (RA). It is generally accepted that there is a strong correlation between ACA and Raynoud's phenomenon. An increased frequency of ACA and anti-CENP-H antibodies was found in patients with SS. SS can be subdivided serologically into two groups: group one with anti-SSA/Ro and/or anti-SSB/La antibodies, and group two with ACA and/or anti-CENP-H antibodies. In summary, ACA and anti-CENP-H antibodies are associated with SS. ACA or anti-CENP-H antibodies can be used as a serological marker for SS (Anti-CENP-H in patients with Sjögren syndrome, Tsay GJ and concepts).

Pathogenesis

Critical features of pathogenesis include: (i) association with particular class II ►histocompatibility (►HLA-DR/DQ) antigens; (ii) a pattern of particular autoantibodies, including a newly described ►antibody against muscarinic M3 receptor for acetylcholine; (iii) a high proportion of "►high endothelial venules (HEVs)" in the lacrimal and salivary gland biopsies, which express increased levels of ►cell adhesive molecules; (iv) upregulation of major histocompatibility antigens (►H antigens or histocompatibility antigens) and adhesive molecules on epithelial cells in salivary and lacrimal glands, probably in response to local production of interferon gamma (►IFN-γ); (v) expression of cell surface (►Fas, ►Fas ligand) and nuclear molecules (►bcl-2, ►bcl-x) on glandular epithelial cells and on ►lymphocytes infiltrating the glands; (vi) secretion of proinflammatory ►cytokines (such as ►IL-1, ►tumor necrosis factor-α (TNF-α), and ►IFN-γ) by lymphocytes and/or epithelial cells to perpetuate the inflammatory response; (vii) decreased secretion by the residual glandular acini as a result of their decreased neural innervation and defects in their postsignal transduction.

Immunopathology

Humoral Immunity

A large number of autoantibodies have been reported in both primary and secondary Sjögren syndrome, reflecting both B-cell activation and a loss of immune tolerance in the B-cell compartment (►Humoral immunity). Over the past few years, there has been significant progress in defining the fine specificity of these antibodies and characterizing their target ►autoantigens. In some cases, the presence of these antibodies is related to the extent and severity of disease in Sjögren syndrome. The ►B cells make up roughly 20% of the infiltrating cell population in affected glands. The B cells produce ►immunoglobulins (Ig) with autoantibody activity for IgG (rheumatoid factor), Ro/SSA, and La/SSB. A substantial number of the B cells are of ►CD5 + phenotype (►B-1 cells). Production of IgG predominates in Sjögren syndrome, whereas synthesis of IgA is more abundant in normal salivary glands. Nonorgan-specific autoantibodies anti-Ro/SSA and anti-La/SSB are diagnostically the most important and the best-characterized autoantibodies in primary Sjögren syndrome. The majority of anti-Ro-positive sera also react with the denatured from of a 52-kDa protein termed Ro52, which is structurally distinct from Ro60 and probably does not directly associate with the Ro ribonucleoprotein particle. However, the two Ro proteins colocalize to surface membrane blebs on apoptotic cells, where they may become targets of an ►autoimmune response. Human ►monoclonal antibodies reactive with continuous and conformation-dependent ►epitopes on Ro52 have recently been cloned from a patient with primary Sjögren syndrome.

Cellular Immunity

Immunohistologic analysis of lymphoid cell infiltration in exocrine glands in Sjögren syndrome shows a predominance of ►T cells or (T lymphocytes) with fewer B cells and macrophages. ►Adhesion molecules

and activated lymphocyte function-associated ►antigen type I (►LFA-I) (►Leukocyte functional antigen (LFA)) promote homing and occasionally characteristic cell clustering similar to that of follicular structures of lymph nodes. Expression of the mucosal lymphocyte ►integrin $\alpha^E\beta_7$ and its ligand E-cadherin suggest a mucosal origin of a subpopulation of the infiltrating cells. There is an increased expression of ►HLA-DR/DP/DQ molecules on acinar and ductal epithelial cells, presumably due to local production of IFN-γ by activated T cells. The majority of T cells in the lymphocytic infiltrates are CD4 + T-helper cells ►(Helper CD4 T cells) with a ►CD4/►CD8 ratio well over 2. Most of these T cells bear the memory phenotype $CD45RO^+$ and express the ►α/β T cell receptor and LFA-I, and may contribute significantly to B-cell hyperactivity. There is indication of oligoclonal expansion of certain ►TCR Vβ family-expressing lymphocytes. The peripheral blood in Sjögren syndrome has yielded findings similar to those in salivary glands, although a difference in magnitude is occasionally evident.

Immune-Mediated Tissue Destruction

Highly upregulated expression of ►HLA molecules, and the more recently demonstrated B-7 costimulatory molecules, by salivary gland epithelium in Sjögren syndrome is a potentially effective local antigen-presenting mechanism whereby HLA antigens could be involved in exocrine glandular destruction mediated directly or indirectly by $CD4^+$ T cells (►CD4 T cells). Such interaction may lead to further production of cytokines and stimulation of B-cell proliferation and differentiation. Indeed, high levels of ►interleukin (IL)-1B, ►IL-6, and TNF-α, three tissue-destructive cytokines, are produced by epithelial cells. ►IL-10 and IFN-γ are produced mainly by infiltrating T cells, whereas IL-6 and IL-10 also are produced in increased amounts in peripheral blood. A low capacity to produce ►interleukin-2 (IL-2) in Sjögren syndrome might be due to the absence of T cell ►costimulatory signals, resulting in the induction of anergy in the responding T cells population, but other explanations also are possible. Even though the mechanism(S) behind the characteristic glandular destruction of Sjögren syndrome salivary glands remains obscure, immunopathologic findings demonstrate that infiltrating ►cytotoxic T cells (►CTLs) could play a role in this event. On recognition of a proper MHC/antigen complex presented by a ►target cell, CTLs induce cell death through one of two main and independent pathways, the ►perforin-mediated pathway or the ►Fas-mediated pathway. Interestingly, expression of Fas also has been detected among infiltrating mononuclear cells in salivary glands of MRL/lpr mice, a murine model displaying features similar to those of human SLE and Sjögren syndrome.

Expression of Fas, FasL, Bcl-2, and other ►apoptosis-associated genes/proteins has been detected by reverse transcription-polymerase chain reaction (►RT-PCR) and immunohistochemical staining of minor salivary glands in patients with Sjögren syndrome. In particular, ductal and acinar epithelial cells, but to some degree also infiltrating mononuclear cells express abnormal levels of Fas and FasL, especially in cases with heavy mononuclear cell infiltration. Ductal epithelial cells expressing Fas were usually situated inside or close to a dense focus. Most in situ studies have clearly shown a low-grade or even absent apoptosis among infiltrating mononuclear cells. The presence of ►granzyme A (►Granzymes) in Sjögren glands suggests that rather than apoptosis, the ►perforin pathway of CTL-mediated killing may be involved in destruction of salivary glands. Among the salivary gland-infiltrating T cells, some express activation markers such as ►CD25, ►proto-oncogene products, and HLA-DR, but few T cells proliferate as determined by cell cycle studies. It seems difficult also to stimulate the T lymphocytes in Sjögren syndrome with the autoantigens Ro/SSA and La/SSB. These findings suggest that many cells are of memory T cell phenotype; either few of them are autoantigen-specific, or alternatively, many of them are in a state of anergy. In both cases, lack of stimulation of T cells also will hamper the apoptotic signals.

Immunopathogenesis (Summary)

The etiology and pathogenesis of Sjögren syndrome is still a matter of speculation, although several hypotheses prevail. Nevertheless, there is considerable evidence that some as-yet-unknown initiating factor(s) set against the appropriate genetic background may evoke immunologically mediated inflammatory mechanisms, which result in the chronic exocrine gland lesions. T cell-mediated autoimmune responses (►Cell-mediated immunity or cell-mediated immune response) in the glandular tissue and dysregulated apoptosis are currently considered to be of central importance in the pathogenesis. A plethora of autoantibodies has been linked to this autoimmune exocrinopathy, although their role is not always well-defined. Accordingly, B-cell activation is a very consistent immunoregulatory abnormality in Sjögren syndrome.

Treatment and Prognosis

At present, treatment for most patients is essentially symptomatic. The patient should be seen regularly by a rheumatologist as well as by an ophthalmologist and a dentist to prevent and treat the consequences of mucosal dryness, in addition to extraglandular manifestations and other associated complications.

Artificial tears often alleviate the patient's complaints, and are of importance in preventing corneal damage and conjunctivitis. The use of topical steroids is not

recommended because of a high risk of secondary bacterial and viral infections in the eye. Another treatment option for dry eye is "punctal occlusion" by using a variety of "plugs" to occlude the punctual openings at the inner aspects of the eyelids. With this procedure, the instilled artificial tears will remain in the eye for a longer time. The management of dry mouth aims to prevent and treat infections, gum disease, and dental caries. To reduce the risk of caries, it is necessary to keep good oral hygiene and use sugarless sweets and chewing gums to stimulate residual salivary flow. Artificial saliva products and special toothpaste is advocated. Eradication of oral candidiasis usually provides significant improvement of oral symptoms.

Oral ►pilocarpine has recently been shown to be a safe treatment and to provide significant subjective and objective benefits for patients with Sjögren syndrome with symptoms associated with xerostomia. Another potential therapy includes systemic use of interferon-α (IFN-α), which may be of benefit for the symptoms associated with xerostomia.

►Hydroxychloroquine may be useful as an immunomodulating agent reducing immune activation and lymphoproliferation and is sometimes used in patients with Sjögren syndrome. Administration of systemic steroids also has been suggested to improve the signs and symptoms of Sjögren syndrome, but they are mainly used for treatment of severe extraglandular complications such as pulmonary and renal involvement. Serology can be useful in predicting the subsequent outcome and complications in patients with primary Sjögren syndrome. The presence of anti-Ro/SSA antibodies may identify patients with systemic disease, and in anti-Ro/SSA/anti-La/SSB-positive patients, the relative risk of developing non-Hodgkin lymphoma has been reported as high as 49.7 after 10 years' follow-up. The development of extraglandular manifestations seems to be influenced by a number of factors including the MHC HLA B8 and DR3 expression. Spontaneous symptomatic improvement has been described in 12% of patients with primary Sjögren syndrome, especially in elderly patients with some clinical overlap with SLE.

SS and HCV

Patients with SS-HCV and B-cell lymphoma are clinically characterized by a high frequency of parotid enlargement and vasculitis, an immunologic pattern overwhelmingly dominated by the presence of RF and ►mixed type II cryoglobulins, a predominance of ►mucosal-associated lymphoid tissue (MALT) lymphomas, and an elevated frequency of primary extranodal involvement in organs in which HCV replicates (exocrine glands, liver, and stomach). Recently, there has been growing interest in the association of chronic HCV infection with both lymphoproliferative and autoimmune diseases. Several studies have found a higher prevalence of lymphoproliferative disorders in patients with HCV, although others have found no significant association. In addition, the specific tropism of HCV for many extrahepatic cell types has recently suggested a link between HCV and the development of autoimmune diseases, although this extrahepatic replication is not supported by all studies. The sialotropism of HCV may explain the close association with SS and sicca syndrome, whereas its lymphotropism links the virus with the synthesis of cryoglobulins and with lymphoma development. This extrahepatic tropism suggests the probability of the development of both autoimmune and lymphoproliferative processes in some patients with chronic HCV infection. In primary SS, lymphoma seems to be triggered by RF-secreting B cells closely associated with the 17109 and G-6 idiotypes, whereas in patients with HCV, a possible association with an antibody response to the envelope protein E2 of the virus has been postulated. The coexistence of both RF-positive processes (SS and HCV) in the same patient might enhance the possibility of developing diseases related to B-cell proliferation (cryoglobulinemia and low- and high-grade lymphoma). The most frequent type of B-cell lymphoma found in patients with SS-HCV was MALT lymphoma. The predominance of MALT lymphoma in SS-HCV and primary SS but not in HCV suggests an important role for SS in the lymphomagenesis of patients with SS-HCV (►Treatment of SS-HCV, Robert G and concepts). Two therapeutic options should be highlighted as future options. The first is the use of monoclonal agents against B cells (►Rituximab or ►epratuzumab), which have been successfully used to treat not only ►B-cell lymphomas but also cryoglobulinemic vasculitis, with the aim of controlling the marked B-cell hyperreactivity observed in patients with SS-HCV. The second is the use of antiviral agents (►Interferon and ►ribavirin), which aims to eradicate the virus as the main causative agent of this B-cell hyperactivity, and which was successfully used in some of our patients. A combination of anti-B-cell and anti-HCV agents may be a promising option for the successful treatment of B-cell lymphomas in patients with SS-HCV.

SS Cause Lymphoma?

A small percentage of people with Sjögren syndrome develop lymphoma, which involves salivary glands, lymph nodes, the gastrointestinal tract, or the lungs. Persistent enlargement of a major salivary gland should be carefully and regularly observed by your doctor and investigated further if it changes in size in a short period of time. Other symptoms may include the following: (Note that many of these can be symptoms of other problems, including Sjögren syndrome itself. Nevertheless, it is important to see your doctor if you have any of these symptoms so that any problem can be diagnosed and treated as early as possible.)

1. Unexplained fever
2. Night sweats
3. Constant fatigue
4. Unexplained weight loss
5. Itchy skin
6. Reddened patches on the skin

If you are worried that you might develop lymphoma, talk to your doctor to learn more about the disease, the symptoms to watch for, any special medical care you might need, and what you can do to relieve your worry.

SS and Lymphoproliferative Disorders

Sjögren's syndrome is a chronic organ-specific autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands. About half of primary SS patients develop systemic disorders. Primary SS can be divided into three stages according to the extent of organ damage and the course of the disease. In stage I, (~45% of cases), patients have only sicca syndrome and do not experience any systemic involvement, even after 10 years. In stage II, (~50% of cases), patients experience lymphocytic organ damage, which may involve the pulmonary, renal, hepatic, hematologic, and/or dermatologic systems, among the others. Finally, in stage III, (~5% of cases), patients develop malignant ►lymphomas. Lymphomas in salivary glands are through to arise from lymphoepithelial lesions in which there are close interactions among epithelial cells, T cells, and B cells. The B cells in the lesions become activated through the interaction between CD40L and CD40. The progression from polyclonal lymphoproliferation to monoclonal lymphoproliferation, to MALT lymphoma, and finally to high-grade malignant lymphoma is regarded as a multistep process. Antigenic activation of B cells, together with oncogenic events, including ►p53 inactivation and bcl-2 activation, may play important roles in B-cells monoclonal proliferation and malignant transformation. The rheumatoid factor clone is regarded as a candidate B-cells clone that undergoes transformation (Lymphoproliferative disorders in Sjögren syndrome, Yasufumi Masaki and concepts).

Future Directions

The search for susceptibility genes in families with Sjögren syndrome is ongoing with the same approach as in the other chronic autoimmune diseases and with utilization of two major strategies: the position-independent candidate gene approach with mutation screening of suspected disease-related genes and full genome scanning (microsatellite analysis) in humans as well as in animal models to determine susceptibility chromosomal regions, which later will be used in a positional candidate gene strategy. Another challenge in Sjögren syndrome will be to stratify the disease process including genetic and environment triggers. Identification of new genetic markers and better characterization of novel autoantibodies (e.g., those directed against muscarinic receptors in exocrine glands) may lead to the development of better diagnostic and prognostic tests in Sjögren syndrome including its systemic complications. Sjögren syndrome is considered to represent an ideal disease to study the mechanisms underlying autoimmunity because its manifestations are both organ-specific and systemic. The significance of such studies is underlined by the high prevalence of Sjögren syndrome as a common but often neglected systemic autoimmune disease often found in the female and aging population.

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Skeletal Complications (skeletal-related events)

►Bone Loss, Cancer Mediated

Skeletal-Related Events

Definition

Refers to the major complications of tumor bone disease (i.e., pathologic fracture, spinal cord compression, hypercalcemia.)

►Zoledronic Acid

Skeletal Secondary Tumors

►Bone Tropism

Skeletrophin

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Synonyms

Mind bomb homolog 2;Mib-2; Putative NF-kappa-B-activating protein 002N; ZZANK1

Definition

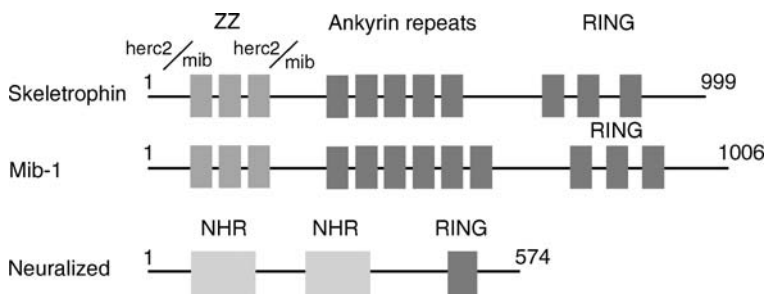
Skeletrophin is one of three ubiquitin ligases, the substrate of which is a cytoplasmic region of Notch ligands and positively regulates ligand-dependent Notch activation. The gene maps to human chromosome position 1p36.32-33, where tumor suppressor factors for neuroendocrine tumors are encoded. Although at least six alternative splicing forms have been found in the public database, the major human product, which is expressed in many tissues, is composed of 999 amino acids and is approximately 110 kDa. Skeletrophin is localized in the cytoplasm and is widely expressed in various adult tissues, with particularly strong expression in skeletal muscle, heart, and brain tissue. There are also alternative splicing forms expressed specifically in the brain, and forms expressed specifically in the developing brain.

Characteristics

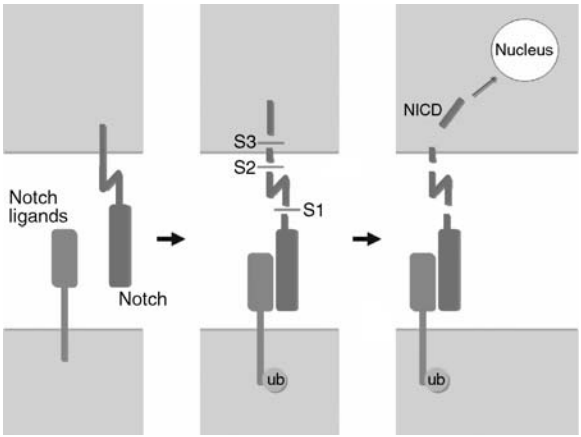
Skeletrophin has two mib/herc2 domains, a ZZ domain, ankyrin repeat domains, and two RING-HC motifs (Fig. 1). A self-ubiquitination assay revealed that a typical RING-HC motif at the C-terminal mediates ubiquitin ligase activity *in vitro*. However, both of the RING-HC motifs are believed to be essential *in vivo*. Both skeletrophin and its paralogue, mib-1 (also known as DIP-1, Fig. 1), also cause mono-ubiquitination of the intracellular domain of Notch ligands. In some tissues, including the brain, both skeletrophin and mib-1 are expressed; however, in other tissues, especially at developing tissues, skeletrophin is expressed only at low levels, with predominant expression of mib-1. Another ubiquitin ligase, termed Neuralized, also causes mono-ubiquitination of Notch ligands.

The other ubiquitin ligases, Neuralized also target intracellular region of Notch ligands. They have two neuralized homology repeat domains (NHRs) and C-terminal RING domain. NHR is believed to be important for ligand binding.

Notch ligands are internalized to the cytoplasm by mono-ubiquitination. In general, mono-ubiquitin-mediated endocytosis removes ligands or receptors from the cell surface membrane, therefore abolishing signal transmission. In contrast, endocytosis of Notch ligands positively mediates ligand-dependent Notch activation, when Notch ligands bind to Notch (Fig. 2). It is thought that endocytosis of Notch ligands may alter the moleular structure of the bound Notch molecules and expose the proteinase cleavage sites, indicated by



Skeletrophin. Figure 1 Schematic representation of molecular structures of skeletrophin and its paralogue mib-1 (mind bomb homologue-1). Skeletrophin and mib-1 have two and three RING-HC motifs, respectively. A typical RING-HC motif at the C-terminus of both skeletrophin and mib-1 mediates ubiquitination *in vitro*. Two unique herc2/mib domains are found at the N-terminus. Distinct molecular functions of herc2/mib are unclear; however, this domain is often found to coexistent with the RING motif. A novel zinc finger domain (ZZ domain), is found in dystrophin and in the first half of skeletrophin and mib-1. The ZZ domain is a putative calmodulin binding site; therefore, it is thought that skeletrophin and mib-1 may be regulated by Ca^{2+} concentration. Ankyrin repeats are a conspicuous feature of ankyrin and act as a binding site for various molecules.



Skeletrophin. Figure 2 Mono-ubiquitination of the intracellular region triggers endocytosis of Notch ligands. Ligand binding to the Notch receptor results in a conformational change, leading to the unmasking of the proteinase cleavage site, indicated by S2 in the figure. Subsequently, the extracellular domain of Notch undergoes proteolytic cleavage, then is further processed at the juxtamembrane proteolytic cleavage site to generate the Notch intracellular domain (NICD). Finally, NICD moves into the nucleus and mediates transcriptional signaling.

S1 in Fig. 2, of the extracellular domain of Notch. This process is linked to the generation of the intracellular Notch domain, which transmits the ligand-dependent Notch activation signal. Mono-ubiquitination-mediated endocytosis is generally followed by lysosomal protein degradation or recycling of surface membrane proteins. Skeletrophin may also be involved in the degradation or recycling of Notch ligands that are not bound to Notch. However, the precise mechanism that determines whether skeletrophin activates or abolishes ligand-dependent Notch activation remains largely unknown.

Clinical Aspects

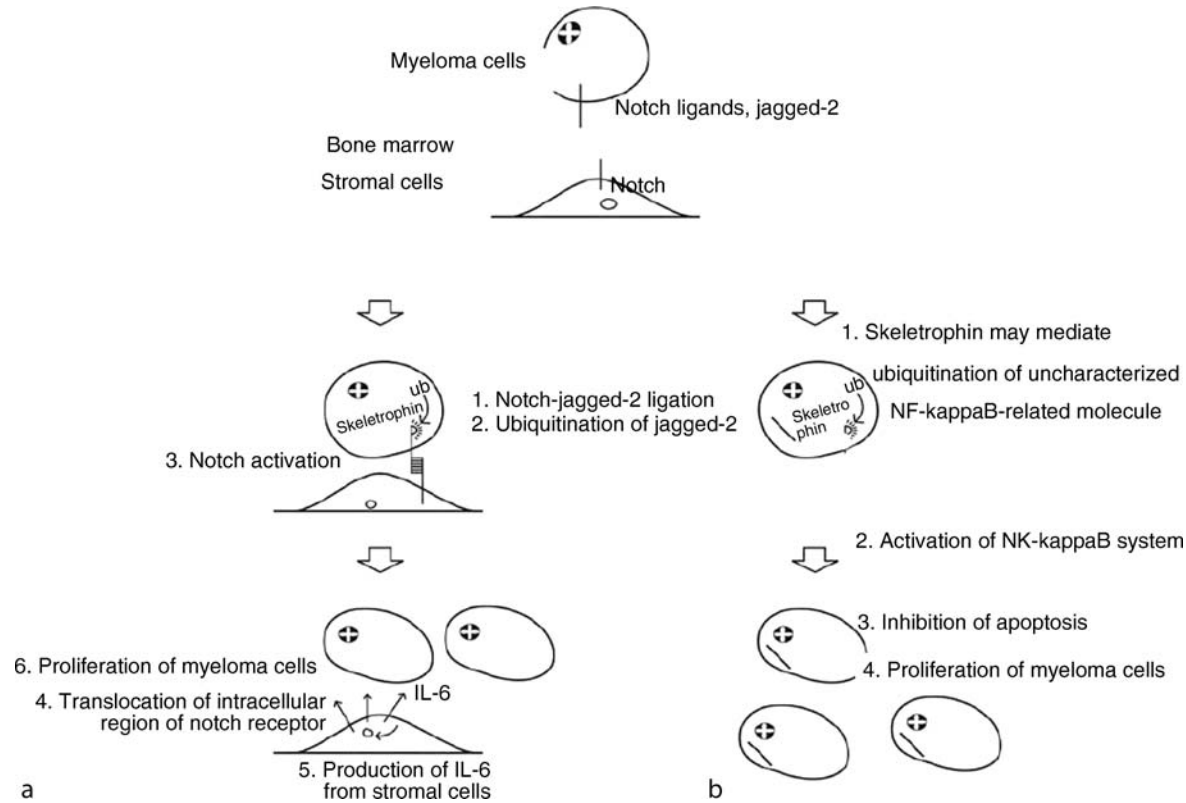
Ligand-dependent Notch activation has a dual antagonistic role in promoting and suppressing tumorigenesis. Skeletrophin also plays a role in both tumor progression and tumor suppression.

Skeletrophin is overexpressed in multiple myeloma, especially from patients whose disease is at an advanced stage and where there are osteolytic bone lesions present. In general, ubiquitin ligase and its substrate, the target molecule, are not constitutively co-expressed in a cell; however, both skeletrophin and its substrate, Jagged-2, a Notch ligand, are constantly and abundantly expressed in many myeloma cells. This aberrant co-expression of skeletrophin and Jagged-2 is caused by epigenetic molecular events in myeloma cells. Both *skeletrophin* and *jagged-2* contain a CpG-rich promoter, harboring CpG islands, which, when hypomethylated, are activated in numerous myeloma cells (Fig. 3). As a result, skeletrophin constitutively acts to cause mono-ubiquitination of the intercellular region of Jagged-2, causing ligand-dependent Notch activation in bone marrow stromal cells, which directly contact with myeloma cells. Finally, activated bone marrow stromal cells secrete cytokines, including IL-6, and create an adequate microenvironment for myeloma progression (Fig. 3a). Jagged-2 is a cell surface membrane protein with a transmembrane domain, whereas in many myeloma cells, Jagged-2 is localized to the cytoplasm, as a result of endocytosis following skeletrophin-mediated-mono-ubiquitination.

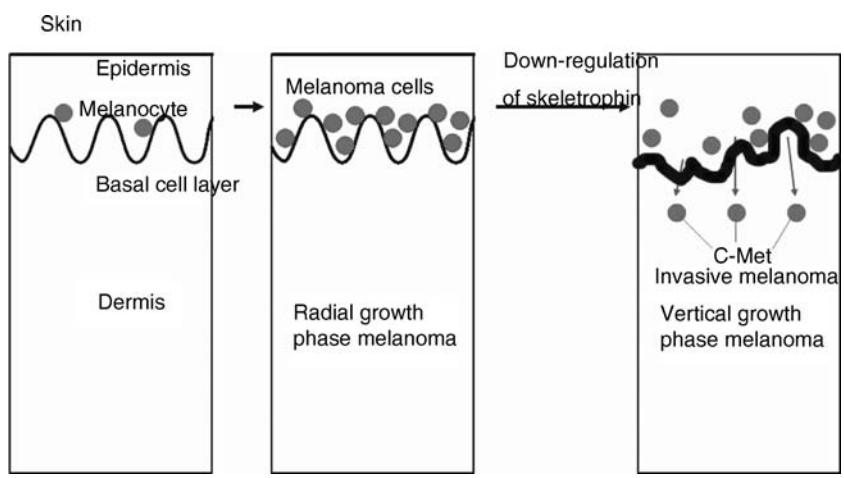
Skeletrophin is also characterized as a putative NF-kappa-B-activating protein, designated 002N. Several independent transcriptional screenings have detected skeletrophin as a powerful NF-kappa-B activator among over 15,000 clones. The anti-apoptotic effect of skeletrophin also contributes to progression of myeloma, especially in patients receiving chemotherapy (Fig. 3b). Notably, the molecular structure of the RING-HC motif in skeletrophin is very similar to that of apoptosis-mediating ubiquitin ligases, such as IAP-1, 2 and XIAP. These findings suggest that skeletrophin has an anti-apoptotic effect and promotes tumor progression by targeting an unknown molecule, other than Notch ligands.

In contrast, skeletrophin suppresses malignant melanoma progression, especially tumor invasion (Fig. 4). Skeletrophin is expressed in normal melanocytes, almost all benign nevi, and in many non-invasive melanoma cells. Skeletrophin is silenced by various epigenetic events, including hypermethylation, in many invasive malignant melanomas at the invasion stage or the vertical growth phase. Skeletrophin down-regulates transcription of the Met oncogene, which encodes the hepatocyte growth factor receptor, and plays a role in determination of the invasive phenotype of many malignant tumors. Restoration of skeletrophin in invasive melanoma has been shown to suppress tumor invasion both *in vitro* and *in vivo*.

To date, three molecular events have been determined to repress skeletrophin expression in melanoma. First, aberrant hypermethylation of the promoter region of *skeletrophin* on a CpG island is found in many invasive melanomas. Second, down-regulation of an activator protein-2 (AP-2), which is known to have a tumor-suppressor role in melanoma, also down-regulates the transcription of skeletrophin. Third, over-expression of Snail, a zinc-finger transcriptional factor, is responsible for silencing of skeletrophin by binding to an E-box in the *skeletrophin* promoter. Notably, Snail is known to be over-expressed in invasive



Skeletrophin. Figure 3 The pathological role of skeletrophin in multiple myeloma. (a) Indirect effect through bone marrow microenvironment. Myeloma cells adhere to stromal cells. Overexpression of skeletrophin and its substrate, Jagged-2, in myeloma cells facilitates ligand-dependent Notch activation in stromal cells. Activated stromal cells secrete various cytokines including IL-6 to promote myeloma. (b) Direct mechanism of skeletrophin in multiple myeloma. Overexpression of skeletrophin activates NF-kappaB signaling and protects myeloma cells against apoptosis.



Skeletrophin. Figure 4 Silencing of skeletrophin in melanoma contributes to tumor invasion. Skeletrophin is expressed in melanocytes, a normal component of melanoma, and in non-invasive melanoma cells at the radial growth phase. In contrast, various epigenetic events down-regulate skeletrophin in invasive melanoma at the vertical growth phase. The silencing of skeletrophin allows melanoma cells to express c-Met. C-met encodes hepatocyte growth factor receptor and contributes to the invasive phenotype of melanoma cells.

melanomas. In addition, as well as being one of the most common karyotypic lesion seen in melanoma, loss of alleles of 1p36 has been detected as a late event, at the invasive and metastatic phase, in melanoma progression. Therefore, loss of heterozygosity may be important for down-regulation of skeletrophin in melanoma.

It has long been speculated that a gene encoding tumor suppressor factor in invasive melanomas is located at human chromosome position 1p36.3. Recent studies indicate that this tumor suppressor factor may be skeletrophin itself. Moreover, it is believed that uncharacterized genes, which encode the suppressor molecule for various neuroendocrine tumors including neuroblastoma, are located at 1p36.3. Since melanoma is a malignant tumor of neural-crest-derived melanocytes, skeletrophin might be a tumor suppressor factor for various neuroendocrine tumors.

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Ski

Definition

Was first identified as a viral oncogene from the avian Sloan-Kettering retrovirus, which transforms chicken embryo fibroblasts. Elevated levels of c-ski have been detected in several human tumor cell lines derived from neuroblastoma, melanoma and prostate cancer. Ski appears to bind to DNA and be part of the ►histone deacetylase complex.

►Smad Proteins in TGFβ Signaling

Skin Cancer

Definition

Refers to cancers involving the skin. About 80% of these skin cancer cases are ►basal cell carcinoma (BCC), 16% ►squamous cell carcinoma (SCC), and 4% ►melanoma.

Skin Carcinogenesis

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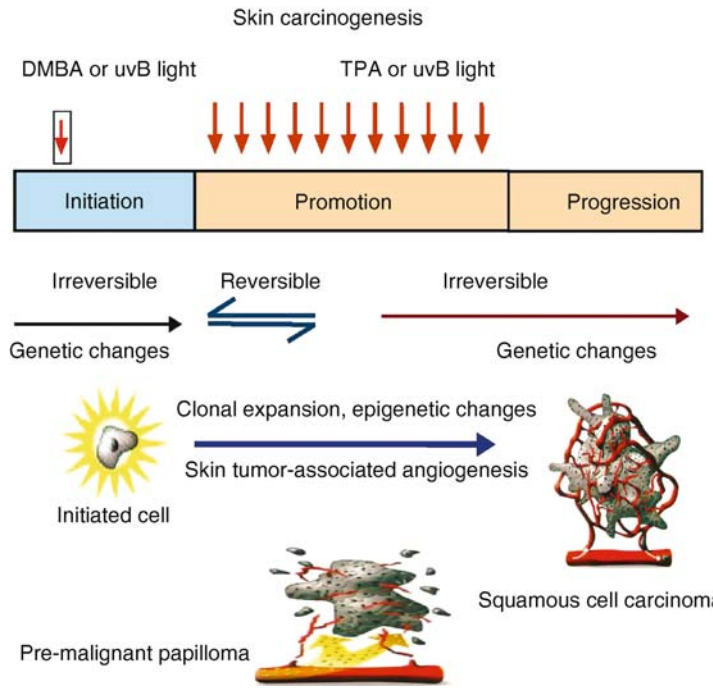
Definition

A multi-step process by which a number of distinct phases occur to form malignant skin tumors. The first step in ►skin carcinogenesis is ►initiation, which is a reversible process during genetic mutations, gene activation or inactivation occur. Examples of initiation are mutations in the v-Ha-*ras* oncogene or inactivation of the p53 tumor suppressor gene. The next phase of carcinogenesis is ►tumor promotion, characterized by a reversible phase of clonal expansion of initiated cells containing mutations/inactivated genes, with a dysregulation of apoptosis of the initiated cells, as well as by accumulation of epigenetic changes such as DNA methylation, inflammation characterized by infiltration of activated leukocytes, production of growth factors, cytokines, reactive intermediates, including oxygen free radicals and nitrogen radicals which stimulate formation of DNA damage, with inhibition of DNA repair enzymes. These alterations become irreversible and first lead to development pre-neoplastic papillomas, which are benign skin lesions. Simultaneous with tumor ►promotion, vascular permeability occurs, which is the first stage of skin ►tumor-associated angiogenesis, which is in itself a multi-step process, during which vasculature develops to provide oxygen and nutrients to expanding pre-neoplastic lesions. With continual accumulation of genetic mutations, the next stage of carcinogenesis, ►tumor progression occurs, which is characterized by the accumulation of genetic mutations leading to conversion of pre-neoplastic skin lesions to malignancy, which are primarily squamous cell carcinomas.

Characteristics

In 1775, Dr. Percival Pott, a British surgeon, made the observation that there was a high incidence of scrotal



Skin Carcinogenesis. Figure 1 Schematic of the multi-step process by which squamous cell carcinomas develop following exposure to either chemicals such as DMBA and TPA or to multiple exposures to sunlight that contains ultraviolet light in the 290–320 nm range, defined as uvB light. Note that this process includes not only proliferation but also includes genetic alterations, epigenetic changes, increased vascular permeability, and tumor-associated angiogenesis.

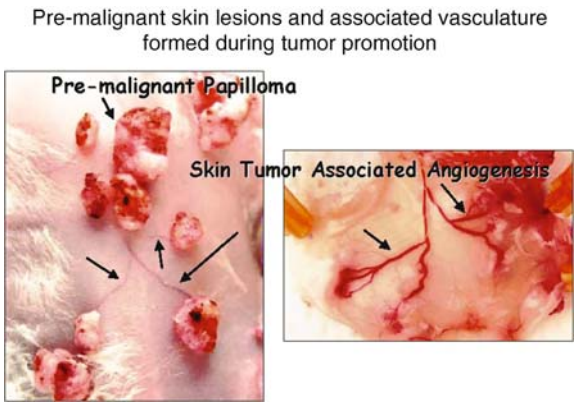
cancer in chimney sweeps compared to that of the general population. This observation is recognized to be the first report of chemical carcinogenesis, suggesting that exposure to environmental agents, such as soot and coal tar, may be directly linked to development of cancer in humans. In addition, this observation also led scientists to evaluate the carcinogenic process using a variety of different models and to identify the specific compounds and/or physical agents that are involved in growth of human tumors.

Thus began the development of rodent models to study what is now understood to be the multi-step process of carcinogenesis. The most widely used models of multi-stage carcinogenesis has been in mouse skin, which have been used over the past 70 years to identify and define distinct and sequential stages of mouse skin carcinogenesis, which is a process that includes multiple steps including tumor initiation, tumor promotion and tumor progression.

The development and use of mouse skin carcinogenesis models have led to the appreciation that the majority of tumors of epithelial origin in humans arise due to a multi-step process, with the majority of experimental models developed using the skin model as an example. There are now multiple models available that have been used to identify the genetic, molecular and cellular basis of rodent lung tumors, gastrointestinal tumors, oral and head and neck tumors, as well as hepatocellular tumors and breast tumors that are based on the commonly held view that human tumors develop as a result of a multi-step process.

There are two primary mouse skin models that have been used to define the genetic, molecular, cellular, and genetic basis of skin carcinogenesis. The first of these models is based on the topical application of a single dose of a chemical carcinogens, primarily polycyclic aromatic hydrocarbons, such as ►7, 12 dimethylbenz [a]anthracene (DMBA), followed by multiple topical exposures to an agent such as the ►tumor promoter, 12-0-tetradecanoylphorbol-13-acetate, (TPA), which is the active ingredient of croton oil, first used in multi-stage skin carcinogenesis studies.

The other model of skin carcinogenesis commonly used is based on induction of skin tumors following multiple exposures to wavelengths of light that are contained in solar radiation, primarily ultraviolet light in the 290–320 nm wavelength range of light, known as ►Ultraviolet Light B (uvB) light. Although humans



Skin Carcinogenesis. Figure 2 Photomicrographs of pre-malignant skin lesions and the dermis underlying the skin lesions which contain vasculature to each pre-malignant skin lesions which provides oxygen and nutrients. These are the classical hallmarks of the process of multi-stage skin carcinogenesis leading to formation of squamous cell carcinomas.

do develop skin tumors from exposure to chemicals, the vast majority of human skin tumors occur due to multiple exposures to solar irradiation, within an increase in skin tumors, due to the depletion of the ozone layer.

Exposure of the skin to a single dose of a carcinogen such as DMBA, a polycyclic aromatic amines derived from fossil fuel by-products followed by multiple exposures to the tumor promoter TPA or to multiple exposures of the skin to uvB light induces both genetic changes and epigenetic alterations, which significantly alters the normal organized pattern of keratinocyte proliferation and differentiation, ultimately resulting in development of skin malignancies.

Genetic Basis of Skin Carcinogenesis

These two mouse models of multi-stage skin carcinogenesis have been used to identify and define distinct and sequential stages of mouse skin carcinogenesis, which include tumor initiation, promotion, and progression. Although exposure of mice to either chemicals or to uvB light results in formation of skin tumors, these different agents induce different genetic mutations.

In the chemical skin carcinogenesis model which uses DMBA as the ►initiator and TPA as the tumor promoter to induce skin tumor formation, the first step is tumor initiation, which is accomplished by topical application of a single sub-carcinogenic dose (25–200 nmol) of DMBA to the dorsal skin of genetically susceptible mice. Exposure of the skin to carcinogen results in formation of mutations in the Harvey-ras (Ha-*ras*) oncogene at codons 12, 13, 59, and 61 of epidermal keratinocytes, which are then considered to be "initiated" cells.

Initiation

Formation of genetic mutations by either a chemical, such as a polycyclic aromatic hydrocarbon, 7,12 dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (BP), derived from incomplete combustion of fossil fuels or as a byproduct of smoking tobacco, respectively, which induce mutations in such areas as codons 12, 13, 59 and 61 of the v-Ha-ras oncogene. Physical entities, such as solar irradiation, particularly ultraviolet light (uv) in the 290–320 nm wavelength range (uvB), induce inactivation of the p53 tumor suppressor gene by formation of mutations in specific codons including codons 151/152, 245, 248, 278, 286 within exons 5–9 of this gene.

Promotion

A process by which chemical or physical agents, such as ►12-0-tetradecanoylphorbol-13-acetate (TPA) or uvB light, stimulate clonal expansion of initiated cells and alter the differentiation pattern of specialized epithelial cells of the skin, epidermal keratinocytes. In addition to these cells undergoing tumor promotion, the stem cells of the skin located in the interfollicular "bulge region" of hair follicles in the dermis also undergo proliferation. Initiated cells undergoing clonal expansion do not undergo the normal process of terminal differentiation, which is a specialized form of programmed cell death, i.e., apoptosis, that normally occurs in epidermal keratinocytes that contain damaged DNA. In contrast to chemicals such as DMBA and BP that act primarily as initiators, uvB light acts both as an initiator as well as a tumor promoter, and is therefore considered to be a "complete carcinogen."

As an initiator of multi-stage skin carcinogenesis, uvB light induces inactivation of the p53 tumor suppressor gene due to formation of mutations primarily by formation of pyrimidine 6-4 pyrimidone photoproducts at codons 151/152. 245, 248, 278, and 286 of the p53 gene, with the frequency of photoproducts very high at codon 286. The importance of the mouse model of ultraviolet light induced multi-stage skin carcinogenesis to human tumorigenesis is based on the fact that ~50% of all human tumors contain defects in the p53 tumor suppressor gene, although the codons that are hot spots for p53 mutations may differ from those induced by uvB light, indicating that there is genetic heterogeneity in human tumors and in mouse models used to study human carcinogenesis. This clearly defines the uvB light-induced model of skin carcinogenesis as having high relevance to the genetic basis of human

skin cancer, as well as to other types of human cancers of epithelial origin.

With the development of transgenic mouse models, the genetic basis of both uvB induced skin carcinogenesis, which is p53 inactivation, and chemical carcinogenesis induced by DMBA/TPA, which is based on v-Ha-*ras* mutations/activation have been verified. Other multi-stage models of carcinogenesis have been developed based on these two mouse models of skin carcinogenesis, such as models for understanding the genetics of cervical cancer induced by human papillomas virus and the genetic basis for colorectal carcinoma using the adenosis polyposis coli gene.

Cellular and Molecular Basis

Ultraviolet light in the 290–320 wavelength range, (uvB) light is a complete carcinogen. This physical agent therefore acts as an initiator and induces mutations in specific codons of the p53 tumor suppressor gene, with clusters of p53 mutated cells formed at very early times after uvB exposure. In addition, uvB light also acts as a tumor promoter to induce clonal expansion of initiated cells, with a dysregulation of apoptosis stimulating this process. A number of different types of DNA damage have been shown to be present in skin exposed to uvB light, including cyclobutane pyrimidine dimers, pyrimidine (6-4) pyrimidone photoproducts, and formation of oxidative DNA adducts such as 8-oxo-deoxyguanine. Epidermal keratinocytes which contain these mutations in the p53 tumor suppressor gene that leads to its inactivation are resistant to apoptosis through the dysregulation of Fas-Fas ligand interactions, thereby allowing keratinocytes with p53 mutations to have a selective advantage in undergoing clonal expansion, first leading to actinic keratosis and subsequently to development of squamous cell carcinomas.

In skin carcinogenesis induced by DMBA/TPA, DMBA serves as the initiator of mutations in the v-Ha-ras oncogene and tumor promotion is accomplished by repeated topical application of a tumor promoter, such as 12-0 tetradecanoylphorbol-13-acetate (TPA), to initiated skin for 20 to 30 weeks. TPA stimulates clonal expansion of initiated *ras*-mutated keratinocytes, ultimately leading to outgrowth of pre-neoplastic skin papillomas. The changes in the skin induced during tumor promotion include epidermal and follicular hyperplasia, altered differentiation patterns of epidermal keratinocytes and interfollicular bulge region "stem cells," with an associated resistance to apoptosis and simultaneous increase in genes associated with cell survival, dermal inflammation characterized by infiltration of activated leukocytes that produce reactive oxygen and nitrogen intermediates, leading to DNA damage and alterations in DNA repair. In addition, other critical factors such as production of growth factors and their receptors, cytokines and chemokines, phosphorylation and subsequent activation of signal transduction molecules, as well as induction of immediate early genes, have been examined for their essential role in multi-stage skin carcinogenesis.

The last phase of mouse skin carcinogenesis is tumor progression, which occurs in a small subset of pre-neoplastic papillomas that acquire additional genetic mutations and subsequently undergo malignant conversion from pre-neoplastic lesions to form malignant squamous cell carcinomas.

Skin tumor-associated angiogenesis is a process that is now recognized to be essential for skin carcinogenesis. The primary growth factors involved in stimulating the vascular permeability and development of new blood vessels surrounding the developing skin lesions are in the family of vascular endothelial growth factor proteins. Further studies which better define other factors involved in skin tumor-associated angiogenesis may hold promise for development of novel strategies to inhibit survival of pre-neoplastic skin papillomas and to block their progression to squamous cell carcinomas.

Relevance to Human Disease

Skin tumors are one of the most prevalent types of tumors diagnosed in humans. Experimental rodent models of multi-stage skin carcinogenesis have been developed to define the genetic, molecular, and cellular basis of this multi-step process by which human skin tumors form. These mouse models have also provided the basis for development of rodent models for other tumors of epithelial origin. The two models of multi-stage skin carcinogenesis described above have allowed identification of the molecular pathways involved in this process and provide models for identification and development of chemotherapeutic agents for treatment of skin cancer patients. In addition, these models of skin carcinogenesis have also been used extensively to evaluate the activity of natural products and their active ingredients for prevention of skin cancer as well as are currently being used to define the role of stem cells in skin tumor carcinogenesis.

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Skin Prick Testing

Definition

A method of testing to determine atopy and the allergens responsible.

►Allergy

Skin-Sparing Mastectomy

Definition

Surgical removal of the breast tissue with preservation of the overlying skin.

►Oncoplastic Surgery

Skinny Needle

►Fine Needle Aspiration Biopsy

Skipper-Schabel Model

►Log-Kill Hypothesis

Skipper-Schabel-Wilcox Model

►Log-Kill Hypothesis

Skp2

Definition

S-phase kinase-associated protein 2; Is part of the ►SCF complex.

►Ubiquitin ligase SCF-Skp2

SL-1

►Stromelysin-1

SLD

Definition

Sphingolipid, the class of lipids based on sphingosine and similar amines.

►Sphingolipid Metabolism

SLE

Definition

►Systemic Lupus Erythematosus.

SLe^x

Definition

Sialyl Lewis x, a naturally occurring glycan of white blood cells and vascular endothelium, necessary for

selectin-mediated ►adhesion, and typically overexpressed on tumor cells.

►Glycobiology

Slit

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Definition

The ►Slit family of secreted proteins has been shown to function in ►axon guidance and neuronal ►migration. There are three Slit proteins in mammals, and while Slit1 expression is mostly restricted to the nervous system, Slit2 and 3 are also expressed in other organs.

Characteristics

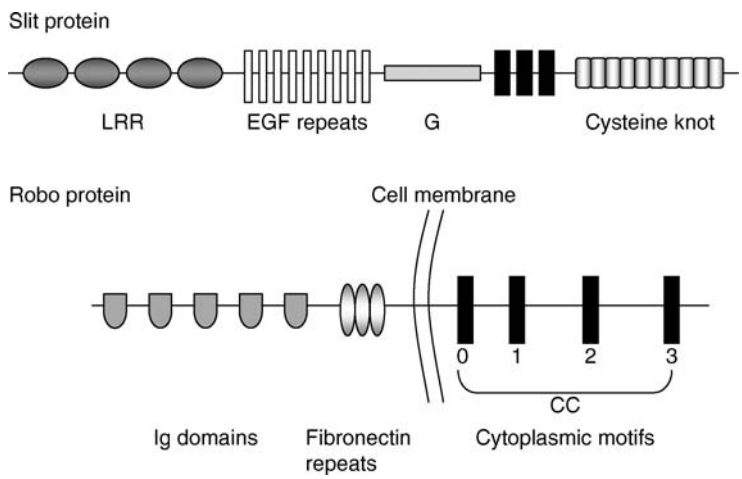
A typical Slit protein contains an N-terminal signal peptide, four leucine-rich repeats (LRRs), seven (in *Drosophila*) or nine (in vertebrates) EGF repeats, a laminin G domain, and a C-terminal cysteine knot (Fig. 1). The receptor for Slit is the transmembrane protein ►Robo (Roundabout), and four *ROBO* genes have been identified. Typically, Robo proteins, including Robo1, consist of five extracellular immunoglobulin (Ig) domains, three fibronectin repeats, and a conserved intracellular region of four cytoplasmic motifs (Fig. 1).

Slit function in the Nervous System

Expression of Robo proteins has traditionally been associated with migrating axons in the developing nervous system. Examples include expression in commissural axon growth cones after ventral midline crossing and expression in olfactory bulb axons on route from the olfactory epithelium to the primary olfactory cortex. In the *Drosophila* spinal cord, Slit functions as a short-range cue to prevent ipsilateral projecting commissural fibers from crossing the midline, and contralateral projecting commissural fibers from re-crossing the midline. A combinatorial code of Robo receptors on medial, intermediate and lateral axons helps control lateral positioning in response to a Slit gradient in the CNS. In addition, Slit proteins act as chemorepellents in axon guidance in the mouse visual system as well as in neuronal migration and axon guidance in the mammalian forebrain both *in vitro* and using knockout mice deficient in Slit1 and/or Slit2 *in vivo*.

Slit-Robo Signaling

Studies examining Slit-Robo signaling can be divided into three basic categories. The first paradigm involves



Slit. Figure 1 Schematic depicting the structure of prototypical Slit and Robo proteins. The mammalian Slit protein contains an N-terminal signal peptide, four leucine-rich repeats (LRRs), nine (in vertebrates) EGF repeats, and a C-terminal cysteine knot. A typical Robo1 receptor consists of five extracellular immunoglobulin (Ig) domains, three fibronectin repeats, and a conserved intracellular region of four cytoplasmic motifs. Robo3 (also known as Rig1) is missing one of the cytoplasmic motifs and Robo4 encodes only two Ig domains, two fibronectin repeats and two cytoplasmic motifs.

the ►RhoGTPases and the regulation of the actin cytoskeleton to generate a turning response.

Evidence has accumulated suggesting an important role for regulation of the actin cytoskeleton machinery in Slit-mediated repulsion. The ►Slit-Robo-GAPS (►srGAPs) facilitate hydrolysis of Cdc42 leading to actin depolymerization. Studies have demonstrated opposing roles for Rac in the regulation of axon repulsion in Drosophila. For example, Slit stimulation leads to recruitment of the SH3-SH2 adaptor protein Dreadlocks (Dock) and the p21-activated serine-threonine kinase (Pak) to the Robo receptor CC2 and CC3 cytoplasmic motifs. Recruitment of this complex increases Rac activity to regulate axon repulsion at the CNS midline. In contrast, Vilse, a conserved family of RhoGAPs has been shown to promote hydrolysis of RacGTP, and less efficiently, Cdc42GTP to mediate Robo repulsion in Drosophila tracheal cells and axons. The contradictory models can be explained in terms of a temporal model of Slit effectors where sequential interaction with the Robo receptors leads to a sustained turning response.

In addition to the involvement of the RhoGTPases, a role for the Abelson kinase (Abl) and its substrate Enabled (Ena) in Slit-mediated repulsion has been elucidated. Abl binds to CC3 and phosphorylates a tyrosine in CC1; whereas, Ena associates with CC2 to regulate the repulsive effect of Slit. Genetic and biochemical evidence suggests that Abl and Ena play opposing roles in Robo mediated repulsion where Abl antagonizes Slit-Robo signaling and Ena promotes the repulsive effect. In addition, Abl has also been linked with a supramolecular complex consisting of Robo and N-cadherin that facilitates inactivation of N-cadherin mediated adhesion in response to Slit. This mechanism uncouples the association of N-cadherin with the actin cytoskeleton and is accompanied by a loss of growth cone traction and axon extension.

Another mechanism associated with Slit-Robo signaling is the "silencing" of the Netrin receptor DCC. Activation of the Robo receptor leads to the silencing of Netrin1's attractive effect through direct binding of Robo's cytoplasmic domain to that of the DCC receptor without a concomitant affect on the stimulation of growth cone extension rate in embryonic Xenopus spinal axons. This hierarchical organization contributes to the finely-tuned controlled mechanisms guiding growth cones to their final targets.

Slit function Outside the Nervous System

Although the function of Slit in axon guidance and neuronal migration is well characterized, other developmental roles have been demonstrated. For example, homozygous knockout mice for the first Ig domain of the Robo1 gene frequently die at birth due to respiratory failure and inadequate lung maturation. Survivors demonstrated severe lung hyperplasia and bronchial abnormalities suggestive of early lung cancer. Human patients with horizontal gaze palsy with progressive scoliosis (HGPPS) were reported to have mutations in Rig1/Robo3, and functional studies have shown defects in commissural hindbrain projections and pontine nuclei. Furthermore, Slit2 and 3 and Robo1 and 2 expression have been detected in non-neuronal cells including pulmonary mesenchyme and airway epithelium, kidney, heart, spleen, thymus and lymph nodes. The temporal and spatial distribution of Slit and Robo mRNAs in fetal and adult tissues suggest that these genes may be associated with functional organization and cell motility during development.

Slit proteins and Cancer

Secreted proteins that guide neuronal and glial cell precursors in the developing central nervous system have also been linked with tumorigenesis. Long-range chemotropic factors including netrins, semaphorins, ephrins and the slit family of proteins are known for their roles in neuronal and glial cell migration. These molecules play an important role in neurodevelopment and it is reasonable to assume that they also influence tumor progression in the nervous system.

Slit-Robo have been shown to be involved in tumor angiogenesis and PI3K signaling has been implicated in this process. Other studies have identified Slit2 as a potential tumor suppressor gene in gliomas, lung, breast and colorectal cancer, as well as in neuroblastoma.

Slit and Medulloblastoma

Slit has also been shown to play an important role in non-neuronal cells, as an inhibitor of leukocyte chemotaxis and promoter of tumor-induced angiogenesis and endothelial cell attraction. ►Invasion of brain tumor cells has made primary malignant brain neoplasms among the most recalcitrant to therapeutic strategies. Slit2 inhibits the invasion of ►medulloblastoma, but not ►glioblastoma (multiforme) cell invasion, in a variety of *in vitro* models. For example, time-lapse videomicroscopy indicated that Slit2 reduced medulloblastoma invasion rate without affecting cell direction or proliferation. Both medulloblastoma and glioma tumors express Robo1 and Slit2, but only medulloblastoma invasion is inhibited by recombinant Slit2 protein. Downregulation of activated Cdc42 may contribute to this differential response.

A role for Rig1/Robo3 in controlling midline crossing of hindbrain precerebellar neurons and axons has been elucidated. Human patients with horizontal gaze palsy with progressive scoliosis (HGPPS) were reported to have mutations in Rig1/Robo3, and functional studies have shown defects in commissural

hindbrain projections and pontine nuclei. Furthermore, early and late stage chick cerebellar rhombic lip fragments are repelled by Slit2. Medulloblastoma cells are thought to arise from external granular layer cerebellar precursors derived from the rhombic lip, thus providing an obvious developmental parallel with Slit2 inhibition of medulloblastoma cell invasion. Slit also inhibits CXCR4-induced motility in breast cancer cells and CXCR4 antagonists have been shown to inhibit medulloblastoma tumor growth *in vivo*.

Slit2 expression has been shown to be downregulated in some gliomas with methylated SLIT2 promoter compared to gliomas and normal brain samples showing no methylation of this promoter. Slit2 and Robo1 are expressed by a variety of glioma and medulloblastoma cell lines and primary tumors. Most CNS neurons in the rat brain express at least one Robo and one Slit during their development, and that levels are maintained from the embryonic to the adult stage. Neurons expressing Robo mRNA could be unresponsive to Slit if molecules or mechanisms regulating Robo expression and function were present.

In light of the evidence demonstrating a role for Slit in leukocyte chemotaxis, angiogenesis, and now, medulloblastoma invasion, it will be necessary to further functionally characterize the intracellular mechanisms mediating non-neuronal Slit effects. The variability in the cell types and models employed will inevitably lead to differences in the intracellular mechanisms responsible for Slit-mediated effects. Selective neurodevelopmental cues such as Slit may provide significant insights into tumor invasion and outline the need for detailed assessments of how to implement this strategy for other tumor types.

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Sloughing of Cells

►Exfoliation of Cells

Slu

►Snail Transcription Factors

Slug

Definition

Snail2; Is a zinc-finger factor that directly represses the transcription of the ►E-cadherin gene by binding to E-boxes (consisting of the sequence 5'-CANNTG) in the proximal E-cadherin promoter.

►Calreticulin

SM

Definition

Sphingomyelin, a phosphosphingolipid, the choline ester of ►ceramide-1-phosphate.

►Sphingolipid Metabolism

SM-5887

►Amrubicin

SMA

Definition

Smooth muscle actin.

SMA- and MAD-related Protein 4

►Deleted in Pancreatic Carcinoma Locus 4

SMAC/Diablo

Definition

The second mitochondria-derived activator of caspase/direct IAP (inhibitor of apoptosis protein) binding protein with low pI. It inhibits the ►inhibitor of apoptosis (IAP) family and promotes caspase activation.

►PUMA

SMAD

Definition

Transcription factor family involved in cell proliferation and differentiation control; regulated by tumor growth factor β (TGFβ).

►Smad proteins in TGFB signaling

SMAD4

►Deleted in Pancreatic Carcinoma Locus 4

Smad Binding Element

Definition

SBE; Was initially defined by a consensus sequence of two inverted repeats of GTCT. More recent data suggest that a single GNCN repeat may also be sufficient for Smad-DNA binding. Due to the low complexity of this consensus sequence it is highly likely this consensus to occur within any promoter sequence suggesting that direct binding of the DPC4-R-Smad complex to DNA is primarily important to stabilize the interaction of the complex with other DNA-binding partners.

SMAD-4/DPC4

►Trefoil Factors

Smad Proteins in TGFβ Signaling

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Definition

The Smad proteins are a family of structurally related molecules which perform a pivotal function in the transforming growth factor-β (►TGF-β) superfamily intracellular cascade. This cytokine superfamily includes TGFβ, activins and ►bone morphogenetic proteins (BMP) and regulate a broad scale of biological responses, including cell fate and ►extracellular matrix production. TGFβ superfamily members signal through heteromeric complexes of transmembrane type I and type II serine/threonine kinase receptors. Upon ligand binding, type II receptor phosphorylates type I receptor, thus activating its kinase. The activated type I receptor then propagates signals to downstream targets such as the Smad proteins. Smads (for Sma and ►Mad proteins from Caenorhabditis elegans and Drosophila, respectively) are currently divided into three classes:

- The receptor-activated Smads (R-Smads) transiently interact and become phosphorylated by specific activated type I receptor. In mammals, Smad1, Smad5 and Smad8 are specifically involved in BMP signalling, and Smad2 and Smad3 are restricted to TGFβ/activin pathway.
- The common-mediator Smad4 proteins (Co-Smad) form heteromeric complexes with either BMP or TGFβ/activin pathway-restricted Smad. These complexes then translocate to the nucleus where they control expression of target genes.
- The inhibitory Smads (I-Smads), namely Smad6 and Smad7, prevent the activation of the R- and Co-Smads through competition with R-Smads for binding to the activated type I receptor. Another mechanism has also been proposed for Smad6; this protein can compete with Smad4 for interacting with receptor-activated Smad1, yielding an apparent inactive Smad1-Smad6 complex (Table 1).

Characteristics

Smad proteins share two highly conserved domains named Mad-Homology domain 1 and 2 (MH1 and MH2) on the N- and C-terminal part of the proteins, respectively. The crystal structure of both domains has been determined. The MH1 and MH2 domains are adjoined by a divergent proline-rich linker region.

Smad proteins do not appear to contain any intrinsic enzymatic activity but rather exert their function through

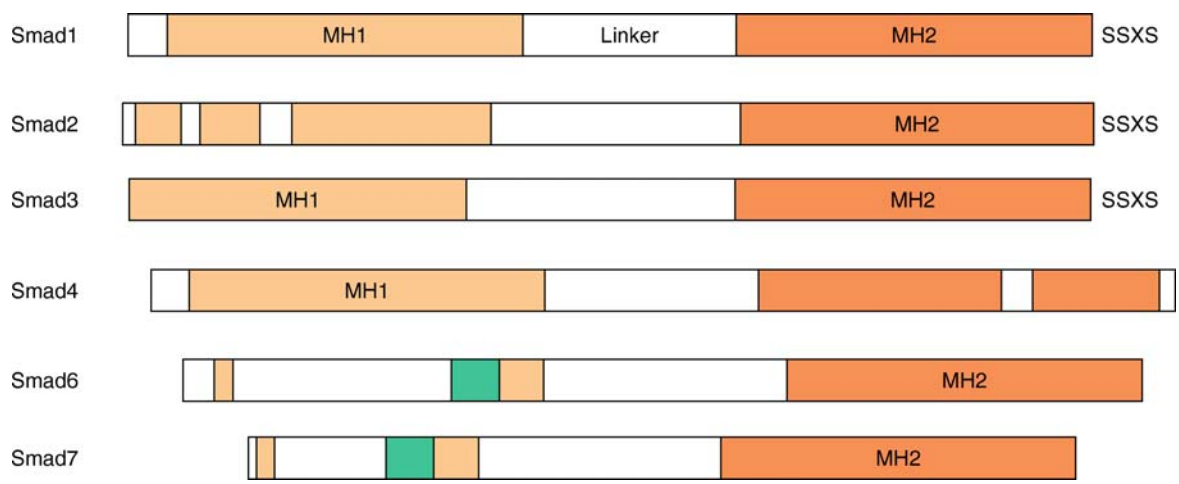
protein-protein or protein-DNA interactions. The MH2 domain mediates the association with other Smads, interaction with activated type I receptors (for R- and I-Smads) and various transcription factors, for example forkhead activin signal transducer (FAST). Furthermore, the MH2 domain enables the interaction of Smad proteins with various transcription co-activators or co-repressors. The MH1 domain also mediates protein-protein interactions with transcription factors, for example ►JUN. Importantly, the MH1 domains of Smad3 and Smad4, but not of Smad2, are able to bind directly to a 5′ GTCT DNA sequence through a β hairpin motif. As Smads bind to DNA with rather low affinity and specificity, these proteins appear to need the interaction with other DNA binding partners to regulate TGFβ target gene expression. In the basal state, MH1 and MH2 domains mutually inhibit each other functions, probably because of a physical interaction. In R-Smads, cytokine-triggered C-terminal serine phosphorylation relieves this auto-inhibition. The non-conserved linker region contains several peptide motifs that participate in Smad activity regulation (Fig. 1).

Cellular and Molecular Regulation

In the absence of cytokine stimulation, R- and Co-Smad monomers are mainly localized in the cytoplasm, whereas I-Smads are predominantly nuclear. Smad anchor for receptor activation (SARA), a protein anchored to membranes, presents the unphosphorylated R-Smads to the TGFβ-activated receptor complexes. This SARA/R-Smad interaction targets the Smad proteins to the plasma membrane and promotes the cytokine intracellular cascade. The type I receptor mediated R-Smad phosphorylation triggers homo- and heteromerization with Smad4 and induces a nuclear accumulation of these proteins. Thus, the R-Smad

Smad Proteins in TGFβ Signaling. Table 1 Smad synonyms

Smad protein	Other names				*Xenopus*	*C. elegans*	*Drosophila*
Smad1	MADR1	Bsp-1	DWF-A	hMAD1	Xmad1		Mad
Smad2	MADR2	JV18-1		hMAD2	Xmad2		Sma 2
Smad3				hMAD3			Sma 3
Smad4		DPC4		hMAD4	Xmad4	Sma 4/DAF-3	Medea
Smad5			DWF-C				
Smad6							Dad
Smad7							
Smad8	MADH6						
Smad10	Smad4β						



Smad Proteins in TGFβ Signaling. Figure 1 Structure of Smad proteins. Pathway-restricted Smads (R-Smads) are phosphorylated by the activated type I receptor on the two most C-terminal serine residues in the SSXS motif. The common-mediator Smad (Co-Smads) contains various small insertions in the MH1 and MH2 domains. The antagonistic Smads (I-Smads) lack most of the conserved MH1 domain. *Boxes in brown* indicate regions that are highly conserved between Smad proteins. *Green boxes* are regions of similarity in I-Smads only.

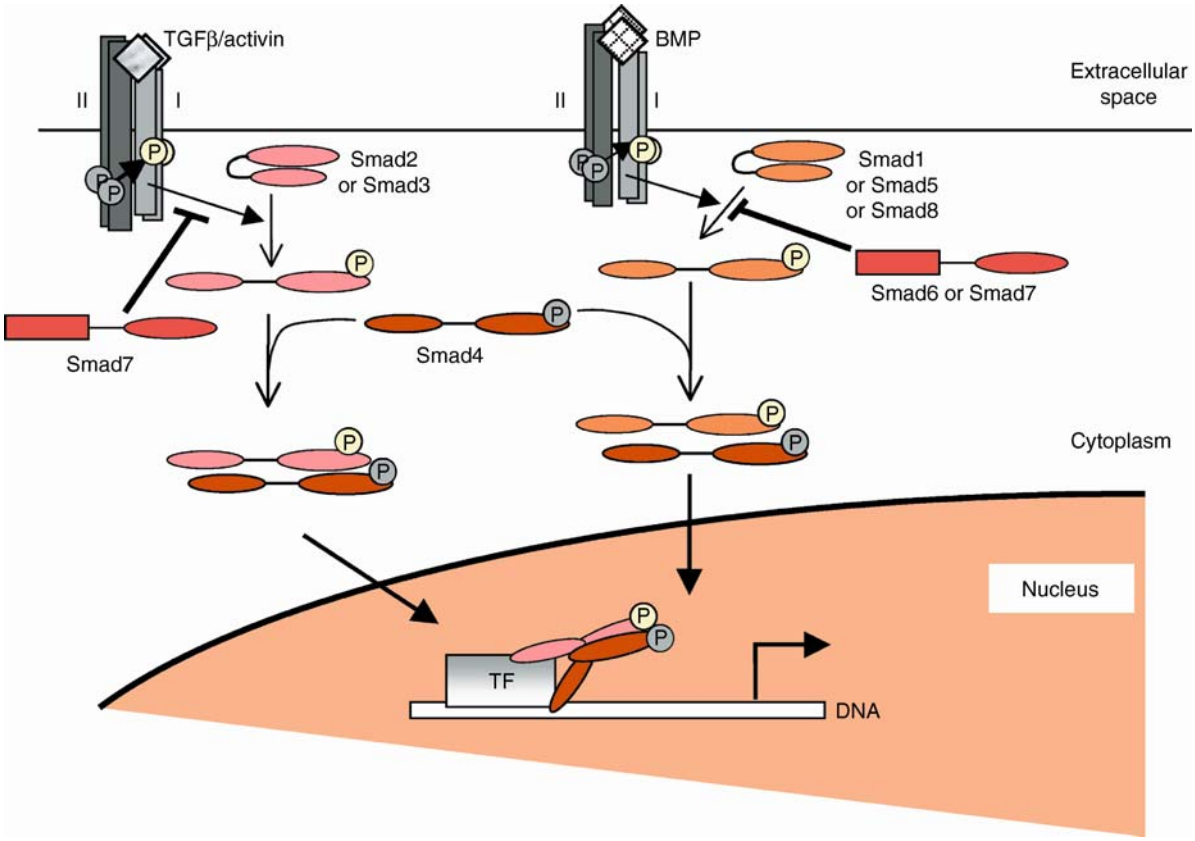
localization prior to or after activation of the pathway is an important feature of TGFβ superfamily signalling. For example, activated ►RAS induces the phosphorylation of R-Smads in their linker region through ►MAP kinase activation, thus preventing Smad translocation to the nucleus. As a result, oncogenic RAS inhibits TGFβ signalling. Interferon γ (IFNγ) also inhibits TGFβ signalling by abrogating R-Smads nuclear translocation. In this case IFNγ promotes the expression of Smad7, an inhibitory Smad that prevents TGFβ-restricted Smad activation.

Smad transcriptional activity is also regulated in the nucleus where Smad interacts with several proteins that promote or repress their activity. For example, the oncogenic ►Evi-1 protein interacts with Smad3 through its MH2 domain and abrogates Smad3 binding to DNA, thus reducing its transcriptional activity. Smads also recruit co-activators like ►CBP/p300 that promote Smad transcriptional activity or co-repressors as the oncoprotein ►Ski. These repressors recruit histone deacetylases to Smad complexes.

R-Smad are activated by phosphorylation, however, no phosphatase has yet been implicated in turning off TGFβ signalling. When R-Smad proteins enter the nucleus, they appear to activate their own degradation through ►ubiquitin-mediated proteolysis (Fig. 2).

Clinical Relevance

The gene encoding Smad4 was originally cloned as a tumor suppressor gene and called deleted in pancreatic cancers 4 (►DPC4). Smad4 appears to have a role in the late stages of a subgroup of colorectal cancers and many pancreatic cancers. In heterozygous mice carrying mutations of Smad4 and ►adenomatous polyposis coli (►APC) genes on the same chromosome, loss of heterozygosity and reduplication of the gene carrying the mutations results in intestinal polyposis



Smad Proteins in TGFβ Signaling. Figure 2 Schematic representation of the TGFβ/Smad pathway. Cytokine binding leads to the formation of a heteromeric receptor complex in which type II receptor phosphorylates and activates type I receptor. Pathway-restricted Smads are then phosphorylated by the type I receptor and form complexes with the common-mediator Smad4. These heteromeric complexes enter the nucleus where they participate, in combination with other transcription factors (TF), in the regulation of target genes. Inhibitory Smads bind to the activated type I receptor thus preventing R-Smads activation.

Smad Proteins in TGFβ Signaling. Table 2 Smad gene characteristics

Smad protein	Human chromosome	Number of exons	Mutation in human cancer
Smad1	4q28-31		
Smad5	5q31	8	
Smad8	13q12-14		
Smad2	18q21.1	11	Colorectal, lung
Smad3	15q21-22	9	
Smad4	18q21.1	11	Pancreas, colorectal, lung, ovary
Smad6	15q21-22		
Smad7	18q21.1		

with more malignant phenotypes than the simple APC heterozygotes (Table 2).

The Smad2 gene is located on the same chromosome region as Smad4 and is also frequently mutated or deleted in colon cancer. The inactivating mutations found in Smad2 and Smad4 mostly affect the MH2 domain in regions important for protein-protein interactions. However, the real role of these proteins in the cancer process has not yet been clearly defined. Mutations in other Smad proteins have so far not been found in human tumors. Nevertheless, one of the mouse Smad3-deficient strains develop metastatic colorectal cancer.

TGFβ is involved in several pathologies, however, the implication of Smad proteins in these disorders remain to be eluciated.

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Small Animal Positron Emission Tomography

►Positron Emission Tomography

Small Bowel Mesentery

Definition

Leaf of connective and fatty tissue connecting the small bowel to the posterior abdominal wall. As well as physically supporting the intestine, it carries the blood, nerve and lymphatic supply.

►Desmoid Tumor

Small Cell Carcinomas

Definition

Are at the highly aggressive pole of the spectrum of ►neuroendocrine tumors.

Small Cell Lung Carcinoma

Definition

A type of ►lung cancer that in which the cells appear small and round under the microscope. Synonym oat cell lung cancer. Small cell lung cancer often grows quickly and spreads to other parts of the body sooner than other types of lung cancer.

►Temsirolimus

Small Cell Neuroendocrine Carcinoma

►Extrapulmonary Small Cell Cancer

Small GTPases

Definition

A group of proteins related to Ras which function in signal transduction. They are active when binding guanosine 5′-triphosphate (GTP), and inactive when

binding guanosine-diphosphate (GDP). Small GTPases have intrinsic ►GTPase activity i.e. the enzymatic ability to hydrolyze GTP to GDP, but this activity is regulated by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs).

►Semaphorin

Small GTP-binding Proteins

►Rho Family Proteins

Small Interfering RNA

Definition

siRNA; Is a molecule consisting of 20–25 double stranded RNA molecules that are used to interfere with the expression of a specific gene.

►RNA Interference

Small Molecule

Definition

Organic compounds of low (typically under ~1,000 g/mol) molecular weight.

►Small Molecule Screens

Small Molecule Drugs

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Definition

A ►small molecule drug in cancer therapy is a molecularly defined chemical entity of low molecular weight, which is applied to a patient with the intention to heal or *palliate* primary ►*proliferative disease*. Or it is directed to prevent secondary consequences of the disease, which are brought about by specific ►*secretory* activity of cancer cells or their general deleterious effect on organ and system functions. A very important aspect is the alleviation of pain.

Characteristics

Differences to Large Molecule Drugs

The molecular weight of a small molecule drug is largely found to be below 1,000 Da, as often it has to pass the cell membrane to reach the target of activity.

Large molecule drugs were often less defined as they were of biological origin e.g. due to ►*posttranslational modifications* and impurities. With the increasing power of analytical and preparative techniques and with the advent of molecularly exactly defined ►*oligonucleotides*, the distinction of exactly defined small molecule drugs from less defined large molecular weight drugs is fading, though antibodies or viruses will remain an independent class of therapeutics. Another distinction between small molecular drugs and those of large molecular weight which once was absolute now becomes relative: the possible permeation of the drug into the cell acting on intracellular targets. Whereas appropriate small molecules might diffuse or be transported by dedicated membrane proteins into the cells, for large molecules biomimetic uptake mechanisms need to be devised like the binding of a drug conjugate to receptors or toxins, followed by an internalization of the receptors toxin, respectively. In other cases viral infections might be feasible. So the natural mechanism is used as a Trojan horse or piggyback.

High Throughput Screening

Many small molecule cancer drugs were derived from natural compounds and used either directly or after some chemical modifications. A second important source came from deeper knowledge of cellular ►metabolism: ►Ligands and coenzymes were modified to obtain antagonists of the corresponding processes. These techniques were expanded with the advent of ►high throughput screening. Mechanistic targets were selected by target identification groups. Binding and functional assays interfering with the relevant mechanism were then adapted to small scale screening encompassing between 96 and 1,536 samples in a plate. Even larger scales are applied experimentally e.g. with various fluorescence read out techniques (e.g. time resolved fluorescence, fluorescence polarization). With appropriate assays up to several million compounds, contained in structurally diverse ►compound libraries, are tested for their interference with specific ►drugable targets (receptors, enzymes) in a few weeks. Also, screens using cultured cells are ongoing in a high throughput mode.

Structure Determination and Modeling

The optimization of the compounds is either at random using the intuition of the medicinal chemist or now increasingly based on the intimate structural knowledge of the respective drug target, which can be obtained from x-ray crystal structure or NMR derived structure determinations. Model calculations are performed fitting chemicals into the drug binding pocket of the target structure and preparing co-crystals of ligands with the target structure to obtain potent compounds. It is a major goal to obtain a high selectivity of a drug for the target chosen, without affecting similar cellular structures necessary for general cell functions. At this point chemical optimization efforts are key.

Physicochemical Properties and Drug Uptake

Small molecule drugs are applied according to their properties by all routes used for general drugs i.e. oral, rectal, s.c., i.m., i.v., nasal or by inhalation to mention the most important ones, and appropriate ►metabolic properties guide its availability for action at the required locus. The small molecule drugs used in cancer therapy need to have the ►physicochemical properties of general small molecule drugs, like appropriate solubility, logP values and pKB, all the metabolic characteristics like stability towards liver and gut metabolism. Physicochemical properties are predicted in model calculations, which in turn are used to predict pharmacokinetic behavior. Optimized molecules will be taken up in the gut and by cells and a useful ►distribution in the body is found. So an appropriate duration of exposure can be secured in adequate concentrations at the locus of desired activity. Depending on the target, a drug may need to pass into the cell by diffusion or an active transport process. Of special importance is their behavior towards elimination from cells by drug transporters, which may become upregulated in the gut preventing its uptake or as a means of cancer cells to acquire ►resistance towards therapy. It may also lead to an accelerated excretion in the gut or kidney. The molecular properties also govern their potential to interact with other drugs influencing their kinetics by inhibition of cytochrome P450 isoenzymes or by induction of metabolizing enzymes (►Drug drug interaction).

Drug Formulations

The drugs need to be ►formulated according to their specific use, e.g. poorly soluble agents like taxanes are formulated for i.v. application as solutions in high amounts of cremophor carriers, which themselves are not neutral to the body, but provoke reactions requiring a ►premedication for a better patient tolerability. Efforts are made to improve formulations avoiding toxic responses due to ►recipients. This is done with for example, taxanes such that the drug substance is dissolved in less awkward carriers or in vitamin E or albumin for i.v. applications permitting higher concentrations with better tolerability and thus efficacy. For others, slow release formulations are prepared to improve their efficacy through a prolongation of drug availability at relevant concentrations. This also might prevent toxicities originating from peak concentrations shortly after application, which are cut off by a slow release formulation.

Side Effects

Selectivity translates into fewer side effects. However, the aim of selectivity will sometimes be compromised by the need to affect several targets by one drug in order to obtain sufficient therapeutic efficacy. Effects which are due to the interaction with one targeted structure in different locations are ►class effects. All compounds interfering with this target will have the same side effects, as this cannot be avoided structurally. Only differential availability needs or periods of blockade may be a way to decrease such effects, if no local application is possible. The tendency to increase hypertension is, for example, a typical class effect of VEGF signal inhibitors, which is independent from the antiangiogenic effect and accompanies it, irrespective of the specific drug used. Avoidable side effects are contained in the specific structure of a molecule and are called compound specific side effects.

Some Examples for the Mechanism of Cancer Drug Action

Small molecule cancer drugs traditionally interfere with ►cell proliferation, one cardinal distinction to normal cells, through damage of DNA or the synthetic process of DNA synthesis. Examples of these drugs are bleomycin and cis-platinum, which result in ►DNA double strand breaks, or fludara, which interferes at various points with ►nucleoside metabolism. Vinca alcaloids or taxanes block tubulin modifications, which are key for nuclear division and cellular transport. With deeper knowledge of cell biology, the cell fate is blocked at very specific sites. So, certain kinases are required for ►signal transductions orchestrating the process of the ►cell cycle culminating in the separation of the chromosomes to form two new nuclei. If this process is disturbed by specific kinase inhibitors, many cancer cells will respond with a standard program resulting in self-abortion, also called ►apoptosis, thus slowing down cancer growth or even reducing its size.

The kinase inhibitors may also interfere with the activity of enzymes which are ►constitutively activated in cancer cells by mutational events e.g. bcr-abl in chronic myelogenous leukemia. Some drugs only hit specific mutants but not others, which may result in resistance, therefore, drugs are investigated for their activity against major mutants found in human disease.

In future it may be possible to discover compounds, which are specific for major mutants of the enzymes found in cancer, resulting in even higher specificity with less adverse effects as normal cells containing the same enzymes are not affected.

Some cancers depend on paracrine or autocrine growth factor signal transduction and small molecules might block protein functions in the signal chain, inhibiting signal flow downstream of that point. This is true e.g. for EGF receptor signal inhibitors at the level of the receptor kinase, e.g. gefitinib, which inhibits the growth stimulus for epithelial cells.

Antagonists of nuclear hormones, which block signaling via or down regulate nuclear receptor proteins, are effective in the therapy of breast and prostate tumor disease.

Another promising branch of small molecule therapeutics attempts to induce a more ▶differentiated and less invasive phenotype of cancer cells. This was originally applied to leukemia cells with the intention of inducing terminal differentiation and is continued with retinoids for AML and other solid tumors. Investigation is now directed towards the inhibition of the epithelial to mesenchymal transition in solid tumors. In recent years small molecule drugs have been developed against stromal components of tumors with little or no effect on tumor cells themselves, like VEGF signal interrupting and antiangiogenic tumor therapeutics.

Interference with chromatin modification like methylation or acetylation recently became an important field of drug research as appropriate test systems are now available.

Systemic Effects of Tumor Growth

Other small molecule drugs block deleterious effects of tumors secreting active products. Hormones or cytokines are discharged from neuroendocrine tumors in excessive manner, which are therapeutically antagonized by specific signal blockers to prevent a flooding of the body with the specific hormone signal. In the case of prolactinomas, treatment with dopamine might be beneficial or in the case of anorexia, a frequent companion of progressive proliferative diseases, megestrol might be helpful. Anticoagulants are advisable in some cases as the incidence of thrombi and emboli is increased in many tumor patients. Standard analgesics are administered if indicated. If they are not effective, as in the case of bone metastases in prostate cancer, antiproliferative therapy is also applied for the purpose of pain relief.

Worldwide, very many new small molecule drugs are intensively investigated with the latest available techniques in newly discovered pathways linked to cancer. They promise a breakthrough for the benefit of cancer patients, prolonging their life for a certain period of time. It is essential that such prolongation of life occurs at a decent quality of life to become a real benefit for the patient and not only one for the health industry.

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Small-Molecule Inhibitors

Definition

Refer to a class of drugs specifically designed to inhibit certain proteins (such as kinases) in the cell that have been implicated in disease.

Small Molecule Screens

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Synonyms

Chemical biology screen; Chemical genetic screen; Compound screen; High throughput screen

Definition

A small molecule screen is a procedure in which small molecules (typically organic compounds with a

S

molecular weight under ~1,000 g/mol) are systematically tested for their ability to activate, perturb, or modify a target or a biological process of interest.

Characteristics

The goal of small molecule screening is to identify compounds that modulate a particular biological process, and thus, can be used as, or developed into, tools for further medical research and/or ►small molecule drugs (►molecular therapy). Typically, an assay is developed into a "►high throughput" screen, meaning that it is optimized for rapidly assaying thousands to hundreds of thousands of compounds in parallel and in an automated fashion. Large amounts of data are collected with the help of robotics, liquid handlers, and processing software. "►Hits," compounds that produce the desired assay result, are hence identified for further study.

Small molecule screening is often the starting point for identifying chemical tools. Developing a chemical probe to inhibit and study a protein, for example, offers several advantages over mutating a gene. A chemical probe can be used for rapid inhibition in different cell lines, organisms, and species, and at varying time points or at different times during the cell cycle or development. Furthermore, protein inhibition may be reversed by removing the compound, and compounds can be developed for versatile specificity (either inhibiting a specific protein or a family of proteins). In classical genetics, however, genes are not easily turned on or off at will, and mutating/deleting a gene that is essential for survival may lead to early embryonic lethality. The application of chemical tools and ideas to biological problems is known as ►chemical biology.

Small molecule screening has now become a very common method for identifying compounds that produce therapeutically desirable biological phenotypes. These compounds can be further modified to optimize their potential as drug candidates. Previously, drugs were discovered either by opportune testing or by identifying the active compounds in traditional medicines (►Chemotherapy of Cancer; Progress and Perspectives).

Types of Screens

Screens are classified into two types; phenotype-orientated or target-based. In ►phenotype-orientated screens, compounds are screened for the ability to induce a particular phenotype. Further work is usually required before the target(s) of the compound is/are known. Little *a priori* knowledge is required about the mechanism by which the desired phenotype is produced. Examples of phenotype-orientated screens include screens for compounds that alter cell division, metabolism, adhesion, viability, or protein localization. Phenotype-orientated screens are a component of ►forward chemical genetics; one may discover novel biological mechanisms involved in producing the desired phenotype by further evaluating the compounds' mechanisms of action.

►Target-based screens are performed to identify compounds that more directly alter a particular target. For example, one may purify a protein and then screen for compounds that perturb the function of this protein. The compound can subsequently be used to perturb the protein's function *in vivo*, and the consequent phenotype can be observed. This methodology is known as ►reverse chemical genetics.

In either phenotype-orientated or target based screens, counter screens or cross screens may be performed to determine if the hits from the first screen, or primary screen, will affect related targets or phenotypes. Multi-targeted compounds are often called "promiscuous" compounds.

"Small" Molecules

Screening may be performed with siRNAs, peptidomimetics, natural compounds, synthetic compounds, or other molecules. The term "►small molecule" typically refers to organic compounds with "small" molecular weights. There is no clear definition for "small," but estimates range from under 2,000 g/mol to under 300 g/mol; most commonly, "small" refers to under 500 g/mol or 1,000 g/mol. Small molecules are commonly used for screening because organic compounds are often able to cross the plasma membrane of cells, and because most marketed medicines have been derived from this class of agents.

Collections of compounds are known as libraries. Pharmaceutical companies have proprietary libraries containing millions of compounds. Only recently have collections of organic molecules numbering in the thousands to hundreds of thousands become commercially available for academic use. Example libraries include the Prestwick Chemical Library (Prestwick Chemicals; 1,120 off patent compounds, 85% marketed drugs), LOPAC^{1280} (Sigma-Aldrich; 1,280 pharmacologically active compounds), Spectrum Collection (MicroSource Discovery Systems; 2,000 biologically active compounds), Maybridge Screening Collection (Maybridge; 56,000 drug-like organic compounds), and EXPRESS-Pick (ChemBridge Corporation; over 435,000 drug-like small molecules).

In the past century, compound libraries were assembled one compound at a time during the synthesis of drug candidate variants or by purifying compounds from natural products (e.g. plants, fungi, bacteria, and other organisms). Recently, progress in ►combinatorial chemistry (the synthesis of a large number of new chemical compounds by combining various sets of compound "building blocks"), automation, and

chemical diversity has allowed for rapid diversity orientated synthesis. Novel small molecule libraries can more readily be created, increasing the rate of production from a few hundred compounds per year per chemist, to millions of compounds per year. At the current time, combinatorial libraries contain simple compounds, meaning that they have one or fewer ►stereocenters (a carbon atom with four distinct functional groups). Natural compounds are typically more complex, and it is thought that compounds with more stereocenters might provide superior target-binding specificity. Proteins, for example, are very stereochemically complex.

Screening Technology and Tools

Small molecules are typically more stable in their dry form, but must be dissolved for screening. Although there is neither a universal solvent nor any solvent proven for long-time compound storage, dimethyl sulfoxide (DMSO), a dipolar aprotic solvent, is commonly used. Dilutions of the DMSO-dissolved compounds may be performed in another solvent, such as in water or phosphate-buffered saline.

Compounds are placed in small plastic rectangular containers called "plates." Each plate contains a grid of individual open divots, called "wells," in a 2:3 rectangular matrix. Screening facilities typically have each compound created by the lab or obtained from a commercial source placed in a different well. The contents of each well and plate are carefully catalogued. Stock plates, or "mother" plates, are not used directly in experiments. Rather, these plates contain concentrated amounts of each compound for storage. "Daughter" plates are copies of the stock plates created by pipetting a small amount of liquid (often microliters) from each well of the stock plate into the corresponding well of an empty plate. The compounds in the daughter plates can be diluted and/or further aliquoted into other plates. The plates used for screening are named assay plates, and their compound contents may be either directly derived from the stock plates, or from other aliquots. Some assay plates may contain mixtures of different compounds in each well to decrease the assay time and cost. In any case, the experimental materials, such as cells or proteins, must be added to the assay plate wells.

In order to decrease the assay cost, length of time, and amount of required materials, a significant amount of effort over recent years has been placed on minimizing the assay volume. In the late 1970s, 96-well plates were used, and a few hundred microliters of assay materials were required in each well. Currently, 384-well plates are in common use, with each well requiring tens of microliters. Some screens are now being performed in 1,536-well, 3,456-well, or even 9,600-well plates, minimizing the assay volume from a few microliters to a few hundred nanoliters.

Accurate, reproducible, and rapid screening of large numbers of compounds necessitates the use of automated robotic systems. These systems are typically built around a core of one or more automated liquid handlers. In addition to a pipetting tool, robotic liquid handlers usually have a gripper tool to manipulate sample containers, assay plates, or pipette tip boxes on a work deck. The handlers are able to transfer liquids, wash tips, and move microplates without human intervention. An example of a liquid handler is the Biomek FX Laboratory Automation Workstation (Beckman Coulter). Other components of automated robotic systems may include incubators, storage containers, liquid dispensers, mixers, plate readers, or other assay equipment (such as automated digital microscopes for high content screens). One or more tracked robotic arms move the microplates from one station to another, and sophisticated software algorithms control almost every aspect of this system. Many screening centers currently have the capacity to screen over 100,000 compounds per day (depending on the assay).

Screening Assays

Numerous assays have been used in small molecule screening. Many have been designed so that hits can be detected by standard plate readers that detect changes in absorbance, fluorescence, or electrochemiluminesence (ECL). Some example screens, among the countless types and variations possible, are described below.

In vitro assays include enzymatic, fluorescent polarization, fluorescence resonance energy transfer, AlphaScreen, scintillation proximity, and biophysical assays. In an enzymatic assay, compounds that inhibit the activity of an enzyme (e.g. from cleaving a fluorogenic substrate) can be screened. In fluorescent polarization assays (FPA), a fluorescent dye is used to label a small peptide (or other molecule), and its speed of rotation is measured. When this peptide is bound to a protein (or other molecule) of equal or greater size, the speed of rotation will decrease significantly. Thus, FPA can be used to identify compounds that inhibit a fluorescently-labeled peptide, for example, from binding a particular protein. In fluorescence resonance energy transfer (FRET), a protein (or other molecule) is first labeled with a particular dye molecule, named a "donor." Then, a protein that interacts with the first protein is labeled with a dye molecule that is excited by the donor, and accordingly named an "acceptor." When the donor molecule is excited, it will in turn excite the acceptor molecule only when the interacting proteins are in close proximity. FRET can thus be used to screen for compounds that inhibit the binding of two proteins (or other molecules). In a FRET variation known as time-resolved FRET (TR-FRET), fluorescent dye signals are read in a time-resolved manner, reducing

assay interference and increasing data quality. AlphaScreen (Perkin Elmer) is similar to FRET in that donors and acceptors are used; however, the interacting proteins (or other molecules) are conjugated to beads. A laser is used to excite a donor bead which produces singlet state oxygen. The singlet state oxygen activates the acceptor bead, which has a chemiluminescer and fluorophores. Scintillation proximity assays also use beads; the donor being radiolabeled, and the acceptor containing a scintillant. Finally, biophysical techniques, such as NMR and X-ray diffraction, have been powerful *in vitro* tools for detecting compounds that bind to a particular protein.

Cell-based assays include bioluminescence resonance energy transfer (BRET), in which one protein is fused to a bioluminescent donor, and another protein, which interacts with the first protein, is fused to an acceptor fluorophore. When the two proteins are in close proximity, energy transfer can be detected; this assay is similar to FRET, but occurs in a cellular context. Cell-based assays are more commonly used for phenotype-orientated screens, such as in identifying compounds that affect cell division, metabolism, adhesion, transporters, or viability.

►High content screens are cell-based assays that utilize a combination of automated digital microscopy and flow cytometry to collect information about spatial or temporal changes in cellular processes. For example, a nuclear protein can be fused to a fluorescent tag, and an automated high content screening apparatus can be used to detect hit compounds that induce a change in protein localization (e.g. to the cytoplasm).

►Virtual high throughput screen or *in silico* screen, are being performed using advances in molecular modeling, combinatorial chemistry, and molecular biology. For example, computers are used to screen virtual libraries for their ability to bind to a model of a protein. Virtual high throughput screening is also used to identify compounds that may or may not potentially possess desirable absorption, distribution, metabolism, excretion, and/or general pharmacokinetic properties.

Screens in whole organisms, such as in bacteria, *Danio rerio* embryos, *Xenopus laevis* embryos, and *Caenorhabditis elegans* have been performed, although screens in larger animals (e.g. mice) are difficult due to the inability to maintain, treat, and observe thousands of these animals in an efficient manner. For screening a single compound on multiple cell lines, the National Cancer Institute has used the hollow fiber assay, in which different human tumor cells are placed within biocompatible hollow fibers. These hollow fibers are implanted subcutaneously or intraperitoneally in mice, and the mice are then treated with the test compound. After treatment, the fibers are removed and the compound's ability to affect different tumor cell lines and to penetrate different physiological compartments can be assessed. This assay, however, is relatively low throughput.

The Screening Epilog

Whether screening for chemical probes or for drug discovery, much research remains to be performed after the initial compound hits are identified. First, the assay is repeated under the same conditions, on the hit compounds to confirm the results. Then, ►dose-response curves may be generated using the same assay, thereby providing ►IC_{50} or ►EC_{50} values. Orthogonal testing may be performed, in which the confirmed hit is tested using a different assay. This assay is often closer to the target physiological condition. Functional assays, or secondary assays, may be performed; for example, compounds identified *via* an *in vitro* screen may need to be tested in a cellular environment, in the presence of membranes or other physical barriers. For phenotype-based screens, the target(s) of the compound(s) must be identified; an often laborious procedure. Various techniques have been developed for this purpose, including covalent labeling of the compound followed by compound-bound protein purification from cellular extracts, and peptide sequencing.

Even after compounds are tested and shown to possess the desired level of target or phenotype specificity, it is very unlikely that the process has revealed a single perfect chemical probe or drug candidate. Likely, several compounds will show some degree of activity. If these "lead" compounds share similar chemical features, one can identify a ►pharmacophore, the set of structural (electronic and steric) features in a molecule that are responsible for the molecule's biological activity. Compound analogs and structure–activity relationships (SAR) will be used to identify or synthesize new compounds that have improved activity, specificity, and ADME (absorption, distribution, metabolism, and excretion) properties.

Conclusions

Small molecule screening is a commonly used method for identifying chemicals probes and drug leads. This type of screening is a key component in the emerging field of chemical biology, with the objectives to identify a small-molecule modulator for each individual function of every macromolecule, and to translate basic research into improved clinical outcome.

►Anti-inflammatory Drugs
►Aromatase Inhibitors
►Apoptosis-Induction for Cancer Therapy
►Imatinib
►Personalized Cancer Medicine
►Vascular Disrupting Agent and Cancer

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Small Round-cell Tumor

►Desmoplastic Small Round Cell Tumor

SMARCB1

Definition

►Tumor Suppressor hSNF5/INI1.

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SMAX1

►Androgen Receptor

Smelting

Definition

Chemical reduction to produce a metal from its ore.

►Lead Exposure

SMN

Definition

Survival of motor neuron; Is a protein associated with spinal muscular atrophy.

Smoking

Definition

Refers to the habit of consuming smoke generated from tobacco in cigarettes, cigars and pipes. Major cause for cancer, emphysema, and heart disease.

►Tobacco Carcinogenesis
►Tobacco-related Cancers

Smoothened

Definition

Abbreviated Smo in mouse; SMO or SMOH in human; The *Smoothened* gene encodes a protein with homology to a G-protein coupled receptor. The smoothened protein forms part of the receptor complex for hedgehog proteins.

►Hedgehog Signaling

SMR (Soluble Mesothelin-related Proteins)

►Mesothelin

SMRT Co-repressor

Definition

The POZ domain of Bcl-6 is associated with the silencing mediator of retinoid and thyroid hormone receptor (SMRT), which was originally isolated as a corepressor of some nuclear receptors without a ligand. SMRT is a component of a larger multiprotein complex including mSin3A and ►histone deacetylase (HDAC). The recruitment of an HDAC-containing complex is a common transcriptional repression mechanism used by transcription factors belonging to various functional classes.

►BCL6 Translocations in B-cell Tumors

SN-38

Definition

The anticancer ►prodrug ►irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is hydrolyzed into its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) by carboxylesterases. Stabilization of the covalent Topo I-DNA complex by SN-38 is a critical step in its anticancer action where by Topoisomerase I-mediated DNA breaks are induced via prevention of DNA religation.

►Pharmacogenomics in Multidrug Resistance
►Topoisomerases
►Irinotecan

Sna

►Snail Transcription Factors

SNAG

Definition

Transactivation domain of Snail proteins, 7–9 amino acids located at the N-terminal region that is conserved between ►snail transcription factor and ►Gli proteins.

►Snail Transcription Factors

Snail Transcription Factors

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Synonyms

Snail1, Snail2 and Snail3 were previously called Snail, Slug and Smuc respectively; Additional synonyms for Snail1: SnR (chick); SNAILH (human); Sna; Additional synonyms for Snail2: hSlug (human), Xslug (Xenopus), Slugh (mouse); Slu; Human symbols for Snail1 and Snail2: SNAI1,SNAI2; SNAIL-like and SNAILP both refer to a human-specific Snail1 retrogene inserted in chromosome 2

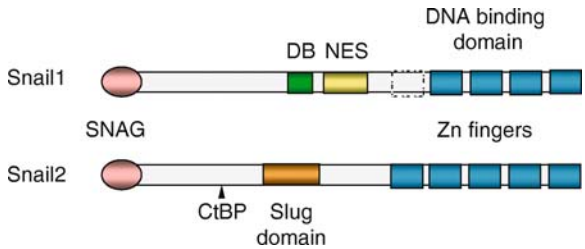
Definition

Snail genes are zinc finger transcriptional repressor of the Snail gene superfamily that participate in developmental and pathological ►epithelial-mesenchymal-transition (EMT) processes. Snail1 was initially characterized as a potent repressor of ►E-cadherin, a major anti-invasive molecule in carcinomas. Snail1 is thus proposed as a potent inductor of tumor invasion, a process that frequently occurs associated to EMT. Besides E-cadherin repression, Snail1-mediated EMT requires the set in motion of a complex genetic program leading to the downregulation of additional epithelial gene markers and to the upregulation of mesenchymal and migratory genes.

Characteristics

Structural Organization

Snail factors share a common organization: a highly conserved carboxy-terminal region, containing from four to six zinc fingers (C_2H_2 type), and a much more divergent amino-terminal region (see Fig. 1). Although three *Snail* genes (Snail1 to Snail3) and a human-specific *Snail1* retrogene (Snail-like) have been described, the vast majority of information available on their function as transcription factors and their involvement in embryonic development and tumor progression is restricted to Snail1 and Snail2. Their zinc-fingers function as the sequence specific DNA-binding domains



Snail Transcription Factors. Figure 1 Schematic representation of the main structural domains of Snail1 and Snail2 proteins. Blue boxes, zinc fingers at the C-terminal region; dotted box indicates the presence of a fifth zing finger in Snail1 protein in some invertebrates; orange box, the specific domain present in Snail2 proteins; DB (green box), destruction box; NES (yellow box), nuclear export signal; SNAG (pink box), N-terminal transactivator domain; CtBP, interacting domain of C-terminal binding protein.

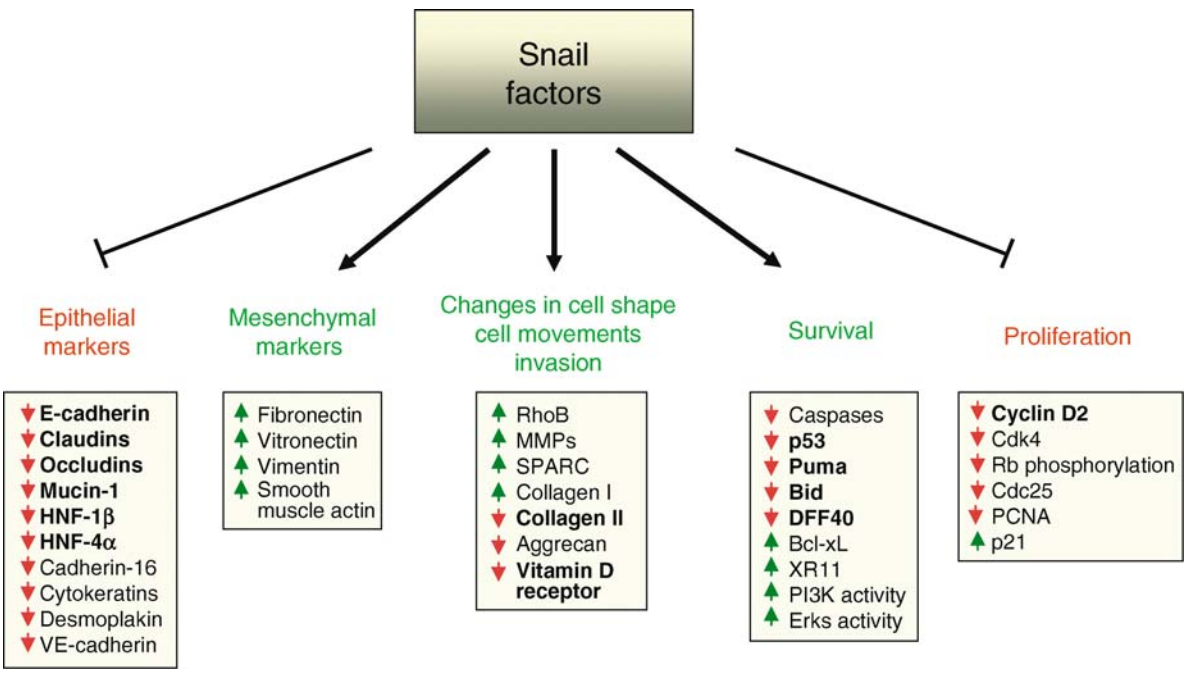
that recognize consensus ►E2-box type elements C/A (CAGGTG). The repressor domain is located at the N-terminal part in the so called ►SNAG domain, conserved between Snail/Gfi proteins. The central region of the Snail proteins is characterized by a Serine-Proline rich region highly divergent between Snail members. While Snail2 contains the so-called Slug domain in the Ser-Pro rich region, whose function remains elusive (Fig. 1b), two different functional domains have been identified in that central region of vertebrate Snail1, a ►nuclear export signal (NES) and ►a destruction box (DB) domain (Fig. 1).

Transcriptional Repression

The transcriptional repression mechanism of Snail1/Snail2 has been established for E-cadherin and many other targets (Fig. 2). In all cases, Snail mediated repression requires interaction with E2-boxes, but with different binding affinities (Snail1 > Snail2). The precise mechanism for Snail1-mediated E-cadherin repression requires the recruitment of specific corepressor complexes containing the mSin3 corepressor and histone deacetylase (HDAC1) and HDAC2. In addition, histone and DNA methylases can be recruited by Snail1 to the repressor complexes to mediate epigenetic silencing of the E-cadherin gene, and potentially of other target genes. The possibility of Snail factors acting as transcriptional activators should be also considered, since Snail2 seems to positively regulate its own promoter during embryonic development in *Xenopus* and at least human Snail2 and Drosophila snail contain a transcriptional activation domain as assayed by transfection.

Regulation of Expression and Functional Activity

Expression of Snail1/Snail2 factors is regulated by a plethora of signals, most of them actively participating in induction of developmental EMT. Among them, growth factors activating ►receptor tyrosine kinases (RTK) and ►MAPK pathways (like fibroblast growth factor (FGF), epidermal growth factor (EGF), ►hepatocyte growth factor/scatter factor (HGF/SF), or oncogenic ►Ras), ►TGF-β/BMP signals and ►Wnt/β-catenin pathways are major inducers of vertebrate Snail1/Snail2 expression. Significantly, crosstalking between distinct signaling pathways are important for Snail1 induction in different systems, particularly those involving TGFβ, Ras/RTKs, ►Notch, and/or Wnt/β-catenin pathways. Additional transcriptional regulatory mechanisms can be exerted through steroid receptors in some particular cellular systems, like in breast mammary carcinoma cells in which ligated ►oestrogen



Snail Transcription Factors. Figure 2 Snail targets. Snail induces a full EMT through the downregulation of epithelial markers, the upregulation of mesenchymal markers and the acquisition of invasive properties. In addition, Snail regulates cell division and survival. Decreased cell division favors invasion versus tumor growth. Altogether, the ability to move, invade and survive confers Snail-expressing cells a selective advantage to delaminate from the primary tumor and to form distant metastasis. Direct targets are shown in bold.

receptors downregulate expression of Snail1 gene through activation of the specific ►MTA3-NuRD correpressor complex. Significantly, Snail factors can exert an autoregulatory control, underlining the necessity of tight regulation of Snail levels.

Besides transcriptional regulation, functional activity of Snail factors can be modulated at posttanscriptional levels. Phosphorylation of Snail1 by GSK3β (►Wnt signaling) in Ser residues located at the central DB and NES domain induces Snail1 nuclear export and cytoplasmic degradation, while potential modification of neighboring Lys residues, phosphorylation by Pak1 and/or interaction with specific ►zinc transporters (like LIV1) positively control Snail1 nuclear localization, protein stability and repressor activity. Much less is known on posttranscriptional regulatory mechanisms of Snail2, except for the induction of proteasome degradation mediated by Ppa2.

Additional Snail Functions

Apart from their implication in EMT, Snail factors can regulate other cellular processes, related to cell proliferation and cell survival with important implications during embryonic development, tumor progression and other pathologies. In some particular cell/tissue contexts, Snail1 expressing cells have a low proliferative potential, exhibiting a partial G1/S cell cycle arrest, a property that may ease migration of cancer cells during tumor progression. For Snail targets related to cell proliferation see Fig. 2.

Snail factors also participate in protection against cell death induced by different external cues, thus acting as survival mediators. Significantly, the pro-survival action of Snail1/Snail2 factors has been demonstrated during embryonic development and in human carcinoma cells under genotoxic stress induced by chemotherapeutic agents and in radioresistance of hematological precursor cells. Snail1/Snail2 negatively regulate p53 (►p53 protein) -dependent (like ►Puma) and p53-independent genes (for additional targets see Fig. 2). Thus, Snail factor expression can contribute to the acquired resistance to ►apoptosis of tumor cells, a characteristic that might be crucial for the metastatic (►metastasis) process. It is very likely that the Snail-mediated pro-survival/resistance function is associated with EMT, as observed in hepatocytes in culture. Nevertheless, the different functions of Snail factors such as induction of EMT, reduced proliferation and survival can be dissociated in some cellular contexts, at least during embryonic development.

Clinical Significance

SNAIL1 expression was originally detected in several carcinoma cells associated to E-cadherin downregulation and invasiveness. Importantly, SNAIL1 expression was also detected at the E-cadherin negative invasive regions of mouse skin carcinomas (►skin carcinogenesis), human breast carcinomas and hepatocarcinomas (►hepatocellular carcinoma), supporting its in vivo implication in induction of tumor invasion. Further studies in different tumor series indicate SNAIL1 expression associated to lymph-node status and/or distant metastasis in breast and ovarian carcinomas (►ovarian cancer), colorectal tumors (►colon cancer) and squamous cell carcinomas, while SNAIL2 has been implicated in ►melanoma metastasis. SNAIL1/2 expression in different tumor series correlates with E-cadherin downregulation and/or induction of several ►matrix metalloproteinases (MMPs). The expression of specific MMPs (i.e., MMP2, MMP9) has been found to be transcriptionally upregulated by Snail1/2 in different cell model systems, further supporting the implication of Snail factors as active invasion inducers through the coordinated regulation of the molecular players of the process. The recent identification of SNAIL1 at the tumor-stroma interface of restricted tumor areas strongly support the implication of SNAIL1 at focally restricted invasion areas where the EMT processes are most likely to occur. Interestingly, SNAIL1 expression has been associated to tumor recurrence in breast carcinoma and to poor prognosis in hepatocarcinomas, while SNAIL2 has been recently associated to poor prognosis or overall patient survival in several tumor types (i.e., colon carcinomas and squamous cell carcinomas). It should be important to analyze in the near future whether the implication of SNAIL1/SNAIL2 in tumor recurrence might be related to the prosurvival function of Snail factors, or perhaps to a potential contribution of Snail factors to cell stemness.

Further studies in large tumor series using highly specific anti-SNAIL1 antibodies, recently developed, (and hopefully anti-SNAIL2 in the near future) are certainly required. This, together with additional functional studies in cellular models and, most importantly, in defined mouse model systems of tumor progression, will contribute to get a complete understanding of the clinical relevance of Snail factors in human tumors.

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SNAILH (Human)

►Snail Transcription Factors

SNCA

Definition

A gene on human chromosome 4 that encodes the α-►synuclein protein. Synonym NACP, PARK1 or PARK4.

SNCB

Definition

A gene on human chromosome 5 that encodes the ►synuclein β protein.

SNCγ

Definition

A gene on human chromosome 10 that encodes the ►synuclein γ protein. Also known as ►BCSG1.

snoRNA

Definition

Small Nucleolar RNAs, are small RNAs (between 60 and 300 nucleotides long) found in the nucleolus. They function as guides for site specific modifications of ribosomal RNA, 2'-O-methylation and pseudouridine formation. More then 300 different ones have been described in humans.

►Cajal Bodies

SNP

Definition

Single nucleotide ►polymorphism: single base variation in the DNA, with a population frequency of >1%.

►Metabolic Polymorphisms and Cancer Susceptibility

SNP Array

Definition

High-density arrays containing thousands of single nucleotide ►polymorphisms (SNPs).

►Modifier Loci

snRNA

Definition

Small nuclear RNA, the RNA components of the snRNPs involved as part of snRNPs in mRNA splicing.

►pre-mRNA Splicing
►Cajal Bodies

snRNP

Definition

Small nuclear ribonucleoprotein; A complex composed of small nuclear RNAs (snRNA) and proteins. snRNPs are important for splice site recognition and catalysis of the splicing reaction.

►pre-mRNA Splicing

SOCS

Definition

The ►suppressors of cytokine signaling (SOCS) family of proteins are key physiological regulators of cytokine responses. Several SOCS proteins have been implicated in the negative regulation of cytokine signaling pathways including STATs.

►Signal Transducers and Activators of Transcription in Oncogenesis
►Suppressors of Cytokine Signaling

SOD

Definition

►Superoxide dismutase.

►Photodynamic Therapy

Sodium Chloride

►Salt Intake

Solar Light Induced Cancer

►Photocarcinogenesis

Solar Ultraviolet Light

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Definition

Solar ultraviolet light is ultraviolet light emitted by the sun.

Characteristics

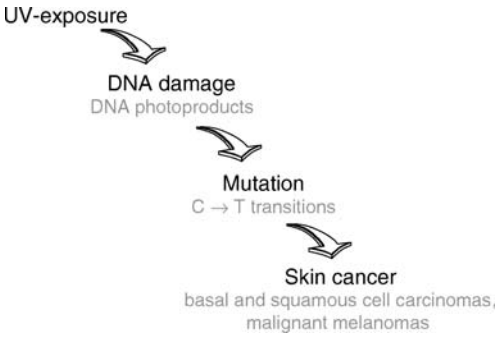
The sun emits ultraviolet radiation as part of the electromagnetic spectrum. It is usually subdivided, rather arbitrarily, into UVA (400–315 nm), UVB (315–290 nm), and UVC (200–290 nm). UVA has been further subdivided into UVA1 (400–340 nm) and UVA2 (340–315 nm). More than 95% of the sun's UV radiation reaching the earth's surface is UVA. Practically all of the UVC, and much of the UVB, is absorbed by the oxygen and ozone in the earth's atmosphere, so that ultraviolet radiation below 290 nm is virtually undetectable at ground level. Nevertheless, the remaining UV radiation can still be absorbed by biological molecules (DNA, proteins, lipids) and elicit photochemical and photobiological responses. A light-absorbing molecule is called a chromophore. Upon absorption of the radiation's energy, this chromophore is elevated to an excited state. Ensuing photochemical reactions may either change the chromophore directly, or, through energy transfer in a so-called photosensitized reaction, indirectly change a molecule other than the chromophore.

Effects of Exposure to Solar Ultraviolet Light on Skin

Exposure of skin to solar ultraviolet light has both short-term and long-term effects. Visible short-term effects are sunburning and/or tanning. Long-term effects are photoaging and induction of skin cancers (►skin carcinogenesis). Skin cancers clearly linked to sunlight exposure are ►basal cell carcinomas and ►squamous cell carcinomas, and ►malignant melanomas. An individual's tendency to develop sunburn and tanning after sun exposure correlates with the individual's susceptibility to long-term effects as well. Therefore, those individuals with higher acute sun sensitivity are generally also more at risk for developing skin cancers after chronic UV exposure.

Within the skin, the depth of penetration of UV light is wavelength-dependent – i.e. the longer the wavelength, the deeper the penetration. While UVA readily reaches the dermis, including its deeper portions, most of the UVB is absorbed in the epidermis, and only a small proportion reaches the upper dermis. UVC, if it reached the earth's surface, would be absorbed or reflected predominantly in the stratum corneum and in upper layers of the epidermis. Both short-term and long-term effects of exposure to UV light are wavelength-dependent. Previously, it was .thought that only UVB is carcinogenic, and only UVA causes photoaging. Today, however, we know that both UVA and UVB cause skin cancer and photoaging. The relative contribution of each, however, continues to be a matter of controversy.

Photocarcinogenesis involves a stepwise accumulation of specific genetic changes in a single cell, with



Solar Ultraviolet Light. Figure 1 Photocarcinogenesis chain of events.

subsequent clonal expansion. Usually, it takes decades until a tumor arises. It is commonly accepted that UV-induced skin cancers develop along a photocarcinogenesis chain of events that involves (Fig. 1):

- DNA damage formation after exposure to solar ultraviolet light
- mutation formation following DNA damage formation
- malignant transformation following mutation formation.

DNA Damage Induced by Solar Ultraviolet Light

Different wavelengths of UV light induce different types of DNA damage. UVC and UVB are capable of exciting the DNA molecule directly and subsequently generating ►DNA photoproducts. DNA photoproducts are dimers, formed by covalently binding two adjacent pyrimidines in the same polynucleotide chain. The two major types of pyrimidine dimers are ►cyclobutane pyrimidine dimers and ►6,4-photoproducts.

►Cyclobutane-pyrimidine dimers (CPDs) are the most common DNA photoproducts formed with solar ultraviolet irradiation of the skin. They are generated upon saturation of the 5,6 double bonds and formation of a four-membered cyclobutyl ring. Cyclobutane-pyrimidine dimers are observed at all possible di-pyrimidine sites, with the thymine–thymine dimer (T–T) being the most common, followed by C–T and T–C dimers. C–C dimers are the least common. The formation of CPDs is not a random phenomenon: it is influenced by the sequence and conformational context of the affected DNA sequence.

The 6,4-photoproduct is a non-cyclobutane dipyrimidine photoproduct, which is formed upon covalent linkage between the C-6 position of one pyrimidine and the C-4 position of the 3′ adjacent pyrimidine. The T–C (6–4) dimer is the most common dimer of this type, but C–C and T–T dimers are also observed after UV irradiation. Upon further irradiation with UV wavelengths between 280 and 360 nm, the normal isomers of 6,4-photoproducts can be converted to their Dewar valence isomers, which are less mutagenic than the normal isomers but may still contribute to solar mutagenesis. A few other rare DNA photoproducts have been described, such as complex purine lesions and pyrimidine hydrates, but their physiological significance in the photobiology of human skin is unknown.

The absorption maximum of DNA is at 260 nm. This makes UVC the most effective wavelength for the induction of DNA photoproducts in naked DNA. However, *in vivo*, due to the absorption of shorter wavelengths in upper layers of the epidermis, 300 nm (UVB) is the most effective wavelength for inducing DNA photoproducts in the basal layer of the epidermis, the anatomical location from which most skin cancers arise. While UVA is much less effective in generating DNA photoproducts, UVA-induced DNA photoproducts may still have a significant contribution to sunlight-induced mutation formation, because of the much higher abundance of UVA in sunlight, as compared to UVB.

UV radiation can also damage DNA indirectly. After absorption of photons by chromophores other than DNA, energy can be transferred either to DNA (type I photosensitized reaction), or to molecular oxygen, with ►reactive oxygen species in turn being able to damage DNA (type II photosensitized reaction).

UV-induced reactive oxygen species include singlet oxygen, and probably other non-radical and radical reactive oxygen species, such as hydrogen peroxide and the superoxide radical. Even the highly reactive hydroxyl radical may be formed by a reaction of hydrogen peroxide with nuclear metals through a Fenton reaction. This oxidative stress not only affects DNA, but also membranes and proteins. The relative contribution of each (oxidative membrane damage, oxidative protein damage, ►oxidative DNA damage) to the different biologic effects of UV irradiation has not been well established. Singlet oxygen and the other reactive oxygen species react predominantly with guanine and generate several DNA changes including the mutagenic and well studied 7,8-dihydro-8-oxyguanine (8-oxoG).

It remains a matter of debate, whether the mutagenic properties of UVA (in particular UVA1: 340–400 nm) are mediated by ►oxidative DNA damage or by the weak ability of UVA to form a few pyrimidine dimers.

Mutations Induced by Solar Ultraviolet Light

Cancer development requires the accumulation of numerous genetic changes in a single cell. Solar ultraviolet light induces mutations in several key genes involved in skin cancer development, e.g. ►*ras* oncogenes, *p53* (►p53 protein, histological and clinical aspects), and *PTCH* tumor suppressor genes. The *p53* tumor suppressor gene encodes a 53 kDa transcription factor that plays a critical role in cellular DNA damage responses.

Mutations in this gene can be found in most cutaneous squamous cell carcinomas and their precursors (actinic keratoses). In addition, chronically sun-exposed skin harbors many keratinocyte clones with *p53* mutations, which are undetectable by light microscopy. This indicates that *p53* mutations are an early event in the pathogenesis of UV-induced cutaneous squamous cell carcinomas.

It is well established that different types of UV-induced DNA damage can lead to the formation of a variety of different mutations, either during attempts by the cell to repair (►repair of DNA) or to replicate these lesions. Most of the *p53* mutations in cutaneous squamous cell carcinomas and its precursors are C → T single and CC → TT tandem transition mutations at dipyrimidine sites. This spectrum of *p53* mutations is very different from *p53* mutations found in malignancies of internal organs; the latter do not have the preponderance of C → T or CC → TT mutations. This, together with the fact that pyrimidine dimers most commonly cause C → T and some CC → TT mutations, provides convincing evidence for a crucial role of pyrimidine dimers in cutaneous photocarcinogenesis. Therefore, C → T, and especially CC → TT mutations have been termed "►UV-signature mutations." They are, in fact, "signature mutations" for DNA photoproducts.

Protection Against Photocarcinogenesis

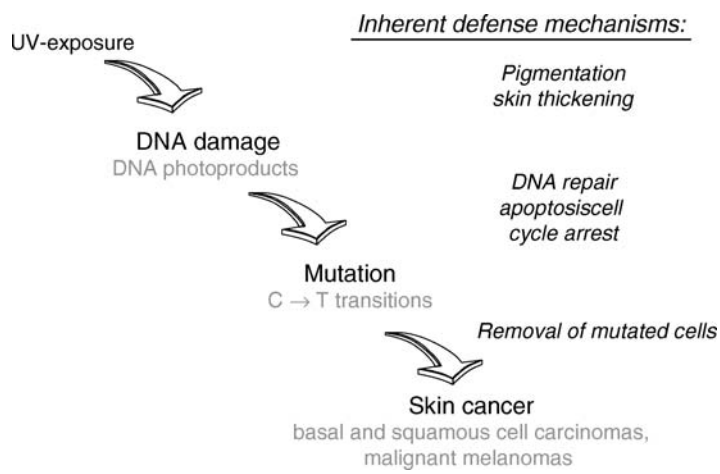
To ensure that most of the damage inflicted by sun exposure will not lead to the formation of skin cancer, UV-exposed cells have several lines of defense against the photocarcinogenesis cascade Fig. 2.

First line of defense – to avoid formation of DNA damage: In order to prevent DNA damage as a consequence of UV exposure, the human epidermis is protected by melanin, the expression of which can be increased in response to ultraviolet light (tanning response) to better protect against subsequent UV exposures. Melanin is a mixture of different polymerized pigments that absorb UV radiation. It is produced by melanocytes and transferred to keratinocytes, where it covers the upper pole of the nucleus in a microparasol to protect the nuclear DNA from the damaging effects of ultraviolet light. Skin can also increase its thickness, which reduces UV exposure of the basal layer, and it contains antioxidative enzymes, which quench reactive oxygen species and reduce the formation of oxidative DNA damage.

Second line of defense – to avoid mutation formation at sites of DNA damage: To counteract potentially mutagenic effects, UV-induced DNA damage requires excision and replacement of damaged nucleotides by DNA repair pathways. No correction procedure is absolutely exact and error-free. If it were, UV-induced skin cancers would not occur in DNA repair-proficient individuals.

DNA photoproducts (pyrimidine dimers) are mutagenic, but they can be repaired by the nucleotide excision repair (NER) pathway. As exemplified by the hereditary disorder ►xeroderma pigmentosum, a defect in this repair pathway increases UV sensitivity and UV mutagenesis in cells, and non-melanoma and melanoma skin cancers *in vivo*. The NER pathway has become well understood, in part through the identification and characterization of the different xeroderma pigmentosum genes.

Individual cancer and skin cancer risks are determined not just by a pronounced NER deficiency, as in xeroderma pigmentosum, but also by more subtle variations in DNA repair efficiency, e.g. as a consequence of polymorphism in DNA repair genes. Likewise, a decline in DNA repair efficiency with age has been linked to the increasing risk of skin cancer with advancing age.



Solar Ultraviolet Light. Figure 2 Inherent cellular defense mechanisms against the photocarcinogenesis chain of events.

Cells from patients with xeroderma pigmentosum variant have an intact NER, yet a phenotype that is indistinguishable from the other xeroderma pigmentosum complementation groups. Cells from these patients do not have a deficit in repairing DNA photoproducts, but a deficiency in what they do with unrepaired DNA photoproducts during replication in the S-phase of the cell cycle. Replicative DNA polymerases usually stall at unrepaired DNA lesions and detach from the DNA strand. For this situation, cells have a number of specialized DNA polymerases that are able to bypass different kinds of DNA damage and extend replication forks through damaged sites. Different polymerases perform this "translesional DNA synthesis" (►translesion DNA polymerases and cancer) with variable fidelity.

Due to a mutation in the pol-η gene, cells from patients with XP variant lack the particular ability of DNA polymerase-η to bypass thymine–thymine dimers with correct insertion of two A residues. This indicates that NER does not always repair all DNA lesions and that the function of a high-fidelity translesional DNA polymerase is crucial for maintaining genomic stability if cells enter S-phase with unrepaired DNA damage. In cells from patients with xeroderma pigmentosum variant, NER can remove most of the thymine–thymine dimers, but, because polymerase-η is missing, any remaining dimers are more likely to be bypassed by polymerases that insert incorrect residues. This causes a UV-►mutator phenotype. If DNA polymerase-η or any other specialized translesional DNA polymerase fails to bypass DNA damage during S-phase, the cell is faced with a stalled replication fork. In these cases, DNA recombination repair, which utilized strand invasion from the sister chromatid, can resolve the stalled replication forks.

Following formation of DNA damage by solar ultraviolet light, cells undergo significant changes. The transcription factor p53 (►p53 protein; biological and clinical aspects), which is both upregulated and activated following exposure to solar ultraviolet light, plays a pivotal role in these DNA damage responses. Genome-protecting effects of p53 in response to UV-exposure are manifold:

- it mediates a cell cycle arrest that allows for more time to repair DNA damage and prevents replication of damaged DNA;
- with overwhelming DNA damage it may induce apoptosis (an affect often seen as sunburn cells, which are apoptotic keratinocytes formed after high dose sun exposure) and thereby prevent survival of damaged cells;
- and it augments DNA repair capacity.

Loss of these protective responses with acquired inactivating p53 mutations results in a UV-mutator phenotype. This explains why p53 plays such a prominent role early in photocarcinogenesis.

Third line of defense – to prevent that cells with mutations expand clonally and form skin cancers: Even after mutations have fixed the inflicted damage for the lifetime of the affected cells, the organism still removes most of these cells, for example, through immune surveillance. However, solar ultraviolet light has several immuno-suppressive effects, which contribute to the carcinogenic properties of solar ultraviolet light. Ultraviolet light is therefore a double-edge sword with regard to photocarcinogenesis: Not only does it generate DNA damage that entails mutation formation and malignant transformation, its immunosuppressive properties and the induction of specific tolerance to UV-induced skin tumors also reduces the ability of the host immune-defense to recognize and remove malignant cells. The latter also impairs the immune-surveillance of cells infected with oncogenic viruses (such as certain HPV types commonly found in squamous cell carcinomas of transplant patients) and may thereby further promote skin cancer formation.

In addition to the intrinsic protective mechanisms against the chain of events leading to the formation of skin cancer, there are extrinsic protective agents and behaviors that can help individuals to reduce their individual skin cancer risk. In order to prevent or reduce UV irradiation of the skin, one can avoid the sun, especially around noontime, stay in the shade, wear protective clothing, and/or wear sunscreens. Sunscreens are topical preparations which attenuate UV radiation before it enters the skin by reflection, absorption, or both. Sunscreens not only protect against the acute skin injury of sunburn, but also against UV-induced immune suppression, photoaging, and skin cancer. The sun protection factors (SPF) of sunscreens, however, which indicate by what factor sunburn is prevented by sunscreen use, do not correlate well with protection factors for other non-erythema endpoints. Therefore, the SPF cannot be regarded as a reliable guide to non-erythema and chronic endpoints.

Topical application of DNA repair enzymes has been shown to increase DNA repair in skin cells and to accelerate removal of DNA photoproducts. In patients with xeroderma pigmentosum, such applications have been reported to reduce the occurrence of actinic keratoses. This indicates that it may be possible to prevent the formation of mutations after the introduction of DNA photoproducts with the use of such "enzymatic sunscreens."

Hereditary Disorders with Increased Photocarcinogenesis

Many disorders with an increased skin cancer risk after exposure to solar ultraviolet light are characterized by a deficiency in the intrinsic protective mechanisms discussed above. For example, men with androgenetic alopecia have a much higher risk of developing

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skin cancers on the scalp than men who have retained their UV-protective hair cover. Lack of protective melanin, as in oculo-cutaneous albinism, increases the amount of DNA damage in the basal layer of the epidermis after UV irradiation and, subsequently, the risk of skin cancer.

The different DNA repair defects of xeroderma pigmentosum and the DNA damage-processing defect of xeroderma pigmentosum variant (see above) generate a UV-mutator phenotype, with an increased chance that DNA damage will result in the formation of a mutation.

Some cases of familial melanoma are caused by a germ-line mutation in the CDKN2A locus, which encodes two gene products, $p^{16INK4a}$ and $p14^{ARF}$. Intact p16 induces a G1 cell cycle arrest by inhibiting ►cyclin-dependent kinases 4 and 6, which in turn inhibit the phosphorylation of the ►retinoblastoma protein. Loss of $p16^{INK4a}$ function, therefore, entails a loss of the G1 checkpoint, leading to abnormal proliferation, unrestricted progression into S-phase, and, importantly, no cell cycle arrest after UV irradiation. $P14^{ARF}$ is an upstream regulator of p53, another important factor mediating UV-induced growth arrest. The disruption of cell cycle control with loss of CDKN2A does not, however, explain why loss of p16/p14 function predisposes mostly to malignant melanoma, and only to a much lesser degree to internal neoplasias, namely pancreatic cancer. A recent finding that loss of $p16^{INK4A}$- or $p19^{ARF}$-function also impairs the ability of affected cells to repair DNA photoproducts, leading to a UV-mutator phenotype, might be the missing link, explaining why alteration in CDKN2a predisposes mainly to UV-induced tumors.

Similarly, p53 protects against UV mutagenesis by inducing a cell cycle arrest after UV-induced damage, by inducing apoptosis in cells with overwhelming UV-induced DNA damage, and by stimulating DNA repair by directly binding to DNA repair enzymes. Consequently, loss of p53 function has been shown to result in a UV-mutator phenotype and reduced repair of UV-induced DNA damage. Li–Fraumeni syndrome is characterized by a germ-line mutation of the *p53* gene, and predisposes to malignancies of various internal organs and cutaneous melanoma.

Patients with the nevoid basal cell carcinoma syndrome, who develop multiple basal cell carcinomas, especially in UV-exposed areas, harbor germline mutations in the *PTCH* gene. PTCH belongs to the ►hedgehog signal transduction pathway that transmits extracellular growth and differentiation signals to the nucleus. While many of the *PTCH* mutations in sporadic basal cell carcinomas are UV signature mutations (C → T and CC → TT), their frequency is lower than in *p53*, and it is unclear whether sporadic *PTCH* mutagenesis is purely UV induced.

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Solitary Bone Plasmacytoma

►Plasmacytoma

Solitary Extramedullary/Extraosseous Plasmacytoma

►Plasmacytoma

Solitary Plasma Cell Myeloma

►Plasmacytoma

Solute Carrier Transporters

Definition

SLC Transporters; Are a large group of membrane transporters that function as secondary-active or passive transporters in the translocation of ions and small molecules, including drugs, across biological

membranes. The group comprises more than 360 members in 46 families.

►Membrane Transporters

Somatic Cells

Definition

Cells other than those of the gamete-forming germ line.

►Adult Stem Cells
►Somatic Tissue

Somatic Cross-Over Point Mapping

Definition

Is a method exploiting the fact that certain genetically unstable cells, e.g. in ►Bloom syndrome, show excessive DNA cross-overs within the BLM gene itself. This leads to restoration of BLM function in cells inheriting two different mutations in BLM.

Somatic Hypermutation

Definition

Somatic hypermutation is a process by which somatic mutations are introduced at a high rate into the variable region parts of immunoglobulin genes. This process is specifically activated in germinal centre B cells. As a result of somatic hypermutation, antibody variants are generated that differ by a few aminoacids from the original antibody. In the germinal centre reaction, B cells expressing antibodies with increased affinity due to favorable mutations can be selected. Somatic hypermutation may be involved in the generation of B cell lymphomas when non-Ig genes are targeted or when chromosomal translocations happen as mistakes of the process.

►Hodgkin and Reed/Sternberg Cell
►Diffuse Large B-Cell Lymphoma

Somatic Recombination of V, D and J Segments

►V(D)J Recombination

Somatic Stem Cells

►Adult Stem Cells

Somatic Tissue

Definition

Most tissues in a multicellular organism. Cells in these tissues do not contribute to the production of gametes and thus mutations in these tissues are not heritable. In humans, many somatic tissues contain cells that are dividing or capable of dividing and thus are capable of renewal, repair, and sometimes regeneration.

►Aging

Somatostatin

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Synonyms

Somatotropin release inhibiting factor; SRIF

Definition

Somatostatin is a bioactive peptide that exists in the two isoforms SST-14 and SST-28, of 14 and 28 amino acids, (SST-14) and SST-28, respectively. It acts as a ►neuropeptide, (neurotransmitter), is produced by neurons and endocrine-like cells and is distributed

throughout the central and peripheral nervous system, endocrine pancreas, gut, thyroid, adrenals, submandibular glands, kidneys, prostate and placenta. Many tumor cells, immune cells and inflammatory cells produce somatostatin.

Characteristics

Somatostatin was initially isolated as an inhibitor of growth hormone (GH) release, but is now best described as a multifunctional peptide, capable of inhibiting secretory processes and cell proliferation. Hypothalamic somatostatin inhibits the release of pituitary growth hormone (GH), thyroid-stimulating hormone (TSH) and corticotropin releasing hormone (CRH). As a neurotransmitter it affects several functions such as autonomous, sensory, locomotive and cognitive. Locally produced somatostatin generally inhibits gut exocrine secretion (e.g. on insulin, glucagon, gastrin), suppresses stomach, small intestine and gall bladder motility, and mediates vasoconstriction, especially of splanchnic vessels. In the adrenals somatostatin inhibits angiotensin II stimulated aldosterone secretion and in the kidneys hypovolemia stimulated renin release. In the immune system it blocks the release of cytokines, including IFN-γ and IL-6, and limits the proliferation of lymphocytes, intestinal mucosar and inflammatory precursor cells. Furthermore, it blocks the action of growth factors such as ►IGF1, ►EGF and ►PDGF. The plasma half life of somatostatin is less than 3 min.

Somatostatin Secretion and Gene Expression

The human somatostatin gene is encoded on chromosome 3q28 in a prehormone form (►preprosomatostatin) with a mRNA of 351bp. The two bioactive forms SST-14 and the N-terminally extended SST-28 are produced by proteolytic cleavage of prosomatostatin. SST-14 is the peptide that is found predominantly. However, up to 30% of immunoreactive SST in the brain is SST-28. Somatostatin secretion is triggered by membrane depolarization, certain ions, nutrients and neurohormones/neuropeptides. Potent stimulators are glucagon, growth hormone releasing hormone (GHRH), neurotensin, corticotropin releasing hormone (CRH), calcitonin gene related peptide (CGRP) and bombesin. Somatostatin gene expression is stimulated by multiple cytokines and growth factors including IGF1 and 2, GH, IL-1, -6,-10, TNF-α, IFN-γ, NMDA receptor ligands and by steroid hormones such as testosterone, glucocorticoids and estradiol. Insulin, leptin, ►TGF-β and some glucocorticoids inhibit somatostatin expression. Transcription of the somatostatin gene is regulated by the intracellular second messengers cAMP, cGMP, NO and Ca2+ and the associated pathways involving ►CREB and ►CBP (cyclic AMP response element binding protein and CREB binding protein).

Somatostatin Receptors (sst)

Somatostatin binds to five currently known G-protein coupled seven transmembrane receptor subtypes (sst1–5) that were initially classified according to their differential binding of somatostatin analogues. The genes for sst1,3,4,5 lack introns, while sst2 contains at least three potential transcriptional start sites, one of which is located in exon 1, 50 kb upstream of the start site in exon 3. All cloned receptor subtypes contain recognition motifs for glycosylation and phosphorylation. Homo- and heterodimerization of the receptors as well as receptor internalization have been described. Upon ligand binding sst induce a multitude of intracellular effects mediated by varying G-proteins coupled to second messengers. All receptors block the formation of cAMP by inhibiting ►adenylyl cyclase and activate tyrosine phosphatases (Table 1).

Molecular Basis of Somatostatins Antiproliferative Effects

Somatostatin limits the proliferation of tumor cells in vitro and in vivo directly and indirectly. Direct regulation is by somatostatin receptors (ssts) that are localized on neoplastic cells; indirect regulation is exerted via ssts on non-neoplastic cells. Mechanistically, this is done by inhibiting the secretion of growth promoting hormones and growth factors (e.g. IGF-1), by promoting vasoconstriction (which leads to a reduced blood flow to tumor tissue), by inhibiting angiogenesis and by influencing the function of immune cells. Theblock of secretion is due to inhibition of the second messengers cAMP and Ca2+ and the inhibition of exocytosis in a G-protein dependent manner.

The direct antiproliferative effects of somatostatin appear to be largely due to the activation of protein phosphatases. Somatostatin induced protein tyrosine phosphatases (PTP) dephosphorylate tyrosine kinases of receptors for growth promoters such as insulin and possibly EGF and IGF-1. Furthermore, somatostatin inactivates MAPK activity via PTP dependent dephosphorylation (sst2), PTP-dependent Raf-1 inactivation (sst3) and inhibitionof cGMP formation (sst5). Activation of PTP is also involved in somatostatin induced apoptosis. While in CHO-K1 cells transfected with each individual sst, sst3 appears to induce apoptosis by activation of TP53, independent of G1 arrest, all other ssts prompt G1 arrest and induction of Rb.

A base pair change in the sst2 gene, found in a lung cancer cell line (COR-L103), lead some authors to the assumption of a tumor suppressor role for somatostatin receptors.

Somatostatin. Table 1 The somatostatin receptor family (sst) (modified after Patel)

	sst1	sst2A	sst3	sst4	sst5
Chromosome	14q13	17q24	22q13.1	20p11.2	16p13.3
mRNA (kb)	4.8	8.5 (?)	5.0	4.0	4.0
Amino acids	391	369	418	388	363
Molecular weight (kD)	53–72	71–95	65–85	45	52–66
Ligand affinity (IC50 nM)					
SST-14	0.1–2.26	0.3–1.3	0.3–1.6	0.3–1.8	0.2–0.9
SST-28	0.1–2.2	0.2–4.1	0.3–6.1	0.3–7.9	0.05–0.4
Octreotide	290–1,140	0.4–2.1	4.4–34.5	>1,000	5.6–32
RC-160	>1,000	5.4	31	45	0.7
Seglitide	>1,000	0.1–1.5	27–36	127-> 1,000	2–23
CH275	3.2–4.3	>1,000	>1,000	4.3–874	>1,000
L-797,591	1.4	1,875	2,240	170	3,600
L-779,976	2,760	0.05	729	310	4,260
L-796,778	1,255	>10,000	24	8,650	1,200
L-803,087	199	4,720	1,280	0.7	3,880
L-817,818	3.3	52	64	82	0.4
Signal transduction					
Adenylyl cyclase	↓	↓	↓	↓	↓
Tyrosine phosphatase	↑	↑	↑	↑	↑
MAP kinase	↑	↓	↑↓	↑	↓
Ca2+ -influx	↓	↓			
Na+/H+ exchange	↑				
Phospholipase C activity		↑			↑↓
Phospholipase A2 activity				↑	
Tumor expression	Gastro- entero-pancreatic tumors	Gastro- entero-pancreatic tumors	Gastro- entero-pancreatic tumors	Meningioma lipoma	Pituitary adenoma
	Medullary thyroid carcinoma	Growth hormone (GH) and thyroid stimulating hormone (TSH) producing pituitary adenomas	Medullary thyroid carcinoma		Not in non-functioning pituitary adenoma
	Ovarian cancer	Breast carcinoma	Ovarian cancer		
	Prostate cancer	Neuroblastoma			
	Pheochromocytoma	Pheochromocytoma			
		Medulloblastoma			
		Meningioma			
		Small cell lung cancer (SCLC)			
		Hodgkin lymphoma			
		Peritumoral vessels			

Characteristics of the five cloned human ssts and information about their distribution in human cancer. Methods that rely on tissue homogenates are unreliable since ssts are found in immune cells and veins surrounding the tumor tissue. Subtype selectivity of ligands is indicated by bold italics for IC50. Subtype expression has only been studied in a limited number of tumor types.

Clinical Relevance

Somatostatin Analogue Therapy

The demonstration of receptor subtype expression in malignancies has paralleled the creation of subtype selective receptor ligands. While the best characterized and oldest analogue, the octapeptide octreotide (plasma half life 2 h), exhibits a preference for sst2 with lower affinities for sst5 and sst3, highly specific nonpeptide

agonists for each of the five subtypes have been developed.

The longest clinical experience exists for the treatment of hormone secreting tumors with octreotide and its microencapsulated longacting release form (LAR). The classic indication for somatostatin analogue therapy is a growth hormone secreting pituitary adenoma in ►acromegaly. Furthermore octreotide and other somatostatin analogues have been used in the treatment of carcinoids, insulinomas, gastrinomas, ►VIPomas, glucagonomas and somatostatinomas, producing symptomatic or subjective responses in 30–75%, and significant reduction in tumor size in 10–15% of patients. In clinical studies octreotide and other analogues have been used as single agents or in combination with conventional cytostatic drugs with varying results in carcinomas of the breast, prostate, pancreas, colorectum, thyroid and lung, inmeningiomas, ►neuroblastomas and Non-Hodgkin-lymphomas.

Somatostatin Receptor Imaging and Radiotherapy

The in vitro detection of somatostatin receptors on a multitude of tumor tissues has led to the development of ►somatostatin receptor scintigraphy (SRS). Apart from neuroendocrine tumors expressing ssts, SRS has successfully been used in the imaging of pheochromocytomas, non small cell lung cancers (NSCLC), meningiomas, ►breast cancer, ►gliomas, ►medulloblastomas, Non-Hodgkin and Hodgkin lymphomas (►Hodgkin disease), granulomatous disease, Sjögren syndrome and rheumatoid arthritis. Binding of radioactive somatostatin analogues has been used in radioreceptor-guided surgery as an asset in the surgery of neuroendocrine gastroenteropancreatic tumors and neuroblastomas with occult metastases.

After ligand binding a fraction of receptors are internalized. This phenomenon has been used for clinical studies of in situ radiotherapy using 111Indium or 90Yttrium labeled somatostatin analogues in terminally ill patients with neuroendocrine tumors.

General Clinical Applications

In carcinoids and neuroblastomas the level of somatostatin or somatostatin receptor expression (e.g. by SRS) has been reported to correlate with tumor differentiation and therapeutic outcome of the disease.

As a therapeutic adjuvant octreotide has been used in the treatment of infectious and secretory diarrhea, L-asparaginase induced pancreatitis, symptomatic treatment of fistulas and in the management of severe pain due to neoplasia.

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Somatostatin Receptor Scintigraphy

Definition

SRS; Is a type of scan used for the diagnosis of neuroendocrine carcinomas. A radioactive form of octreotide (an analog of ►somatostatin) is injected into the patient; this will bind to the tumor cells with somatostatin receptors. A radioactivity-measuring device will detect radioactivity and make a picture showing where the tumor cells are localized.

►Neuroendocrine Carcinoma

Somatostatinoma

Definition

Is a functioning neuroendocrine tumor of the pancreas that produces high amounts of the hormone ►somatostatin that can result in the so-called somatostatinoma syndrome.

►Neuroendocrine Carcinoma

Somatostatinoma Syndrome

Definition

Is a clinical pentad of diabetes mellitus, cholelithiasis, weight loss, diarrhea, and hypochlorhydria/achlorhydria

observed in association with tumors that overproduce the gastrointestinal hormone ►somatostatin.

►Neuroendocrine Carcinoma
►Somastatinoma

Somatotropin Release Inhibiting Factor

►Somatostatin

Sonic

Definition

Gene in hedgehog signaling.

►Hedgehog Signaling

Sonic Hedgehog

Definition

One of three proteins in the mammalian hedgehog family that plays a key role in regulating vertebrate organogenesis, such as in the growth of digits on limbs and organization of the brain.

►Hedgehog signaling

Sorafenib

Definition

An orally available multi-kinase inhibitor that has the chemical structure of *N*-(3-trifluoromethyl-4-chlorophenyl)-*N*Y-(4-(2-methylcarbamoylpyridin-4-yl)oxyphenyl)urea and is capable of simultaneously inhibiting ►VEGFR and ►PDGFRβ tyrosine kinases as well as the RAF serine/threonine kinase. Sorafenib is used to treat patients with ►renal carcinoma.

►Drug Design
►RAF Kinase

SOX18

Definition

A transcription factor in the SOX family, which regulates lymphatic vessel development. Missense mutations or deletions of this gene lead to primary ►lymphedema.

►Lymphangiogenesis

Soy Isoflavonoids

Definition

A group of ►phytoestrogens found in soybean which possess potent antioxidant and antiangiogenic properties. ►Isoflavones interact with animal and human estrogen receptors, causing effects in the body similar to hormone ►estrogen.

►Chemoprotectants

Soy Phytoestrogen

►Genistein

Soy Proteins

Definition

Are a mixture of proteins (α-, β-, and γ-conglycinins, β-amylase, lectin, the Kunitz inhibitor of trypsin, the

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Bowman-Birk inhibitor of chymotrypsin and trypsin, among others) differing in physicochemical and other properties. They are used in human foods in a variety of forms and their consumption is increasing because of reported beneficial effects on nutrition and health. These effects include lowering of plasma cholesterol, prevention of cancer, diabetes, obesity, and protection against bowel and kidney disease.

►Nutraceuticals

Sp

Definition

Specificity protein transcription factor.

►Betulinic Acid

Sp-Like Proteins

Definition

Proteins related to Sp-1 (specific protein-1), the first transcription factor identified. Sp-like proteins contain a C_2H_2-type zinc finger that binds to GC/GT-rich DNA elements. Sp1–Sp4 are four different full length members of the Sp-like protein family, while Sp5-Sp8 seem to be truncated forms of Sp1–Sp4.

►Parathyroid Hormone-Related Protein

SPARC

►Secreted Protein Acidic and Rich in Cysteine

SPB, in Yeast

►Centrosome

SPC1

►Furin

Specific Immunotherapy for Melanoma

►Melanoma Vaccines

Specificity

Definition

The specificity of a test indicates the proportion of patients without the target condition who have a negative result with the test.

►Molecular Pathology

Spectral Karyotyping

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Definition

Spectral karyotyping (SKY) is a multi-fluorochrome ►fluorescence in situ hybridization technique (FISH) in which all the chromosome pairs are simultaneously visualized in different colors in a single hybridization. SKY determines the unique spectral profile of each chromosome generated by specific combinations of different ►fluorochromes. At the present time, SKY can be used to analyze human, mouse and rat chromosomes.

Characteristics

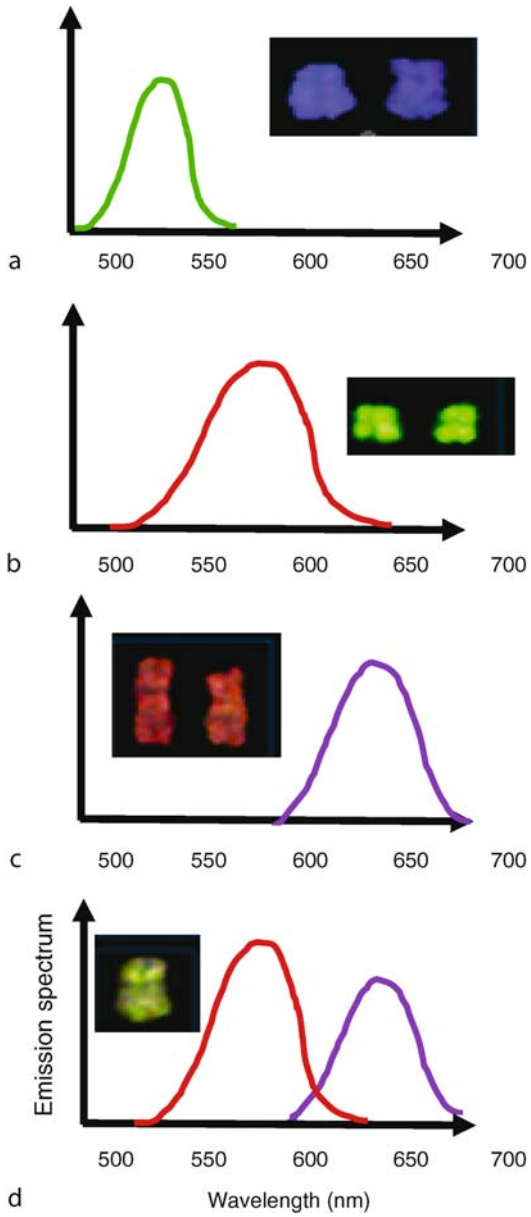
Structural and numerical ►chromosomal alterations (aberrations) are the hallmarks of malignant diseases.

Routine cytogenetic analysis based on ►G-banding techniques provides important information of diagnostic and prognostic relevance both in hematological malignancies and solid tumors. However, detection of chromosomal alterations by this method is complicated by the difficulty in routinely preparing metaphase spreads of sufficient quality and quantity, the clonal heterogeneity of the tumors and the complexity of the many ►chromosomal aberrations. In addition, homogeneously staining regions or double minute chromosomes, results of ►oncogene amplification, are impossible to characterize using G-banding analysis alone. As a result a large number of chromosomal abnormalities are described as so-called marker and derivative chromosomes instead of being precisely defined. Fluorescence in situ hybridization, a highly sensitive and specific tool for the detection of chromosomal aberrations, provides additional information to G-banding analysis clarifying particular problems. However, the ►FISH technique is unable to screen the whole ►karyotype in one experiment and therefore analysis of complex unknown chromosomal alterations requires a large panel of painting probes and several hybridizations.

The fantastic advantage of Spectral Karyotyping (SKY) is the ability to visualize simultaneously all the chromosome pairs in different colors in one hybridization. In this way, karyotype rearrangements are easily detected as a transition from one color to another at the chromosomal breakpoint region.

The SKY method involves several steps:

1. A probe cocktail (Applied Spectral Imaging Ltd, Migdal, Ha'Emek Israel) consisting of fluorescently labeled probes for each chromosome is made by labeling chromosome specific libraries generated by PCR from flow-sorted chromosomes with specific combinations of one or more of the five spectrally distinct fluorochromes (FITC, Rhodamine, Texas Red, Cy5 and Cy5.5).
2. Metaphase preparations are hybridized with this probe cocktail and then stained with 4,6-diamidino-2 phenylindole (DAPI) in antifade medium.
3. The SpectraCube® Imaging system (Applied Spectral Imaging Ltd, Migdal Ha'Emek, Israel) is used to discriminate between the different spectral characteristics of chromosomes. The system measures chromosome-specific emission spectra generated by the combinatorially labeled chromosome-specific painting probes.
4. The spectral signature of the fluorochrome combinations is analyzed using SKYView™ software. It classifies the chromosomes by comparing the acquired spectral characteristics to the combinatorial library containing the fluorochrome combinations for each chromosome. In the classified image the chromosomes appear in a Red-Green-Blue (RGB) display in which FITC is seen as blue, Rhodamine and Texas Red are seen as different shades of green and the infrared dyes not visible to the human eye, Cy5 and Cy5.5, are assigned different shades of red.



Spectral Karyotyping. Figure 1 Schematic presentation of chromosome identification according to the spectral characteristics. Each graph consists of a fluorescence emission spectra and a RGB display of the chromosome. The horizontal axis shows wavelength, the vertical axis shows relative intensity of fluorescence. (a–d) Chromosomes 8, 20, 2 and X, labeled with FITC, Rhodamine, Cy5.5 and a combination of Rhodamine and Cy5.5, respectively.

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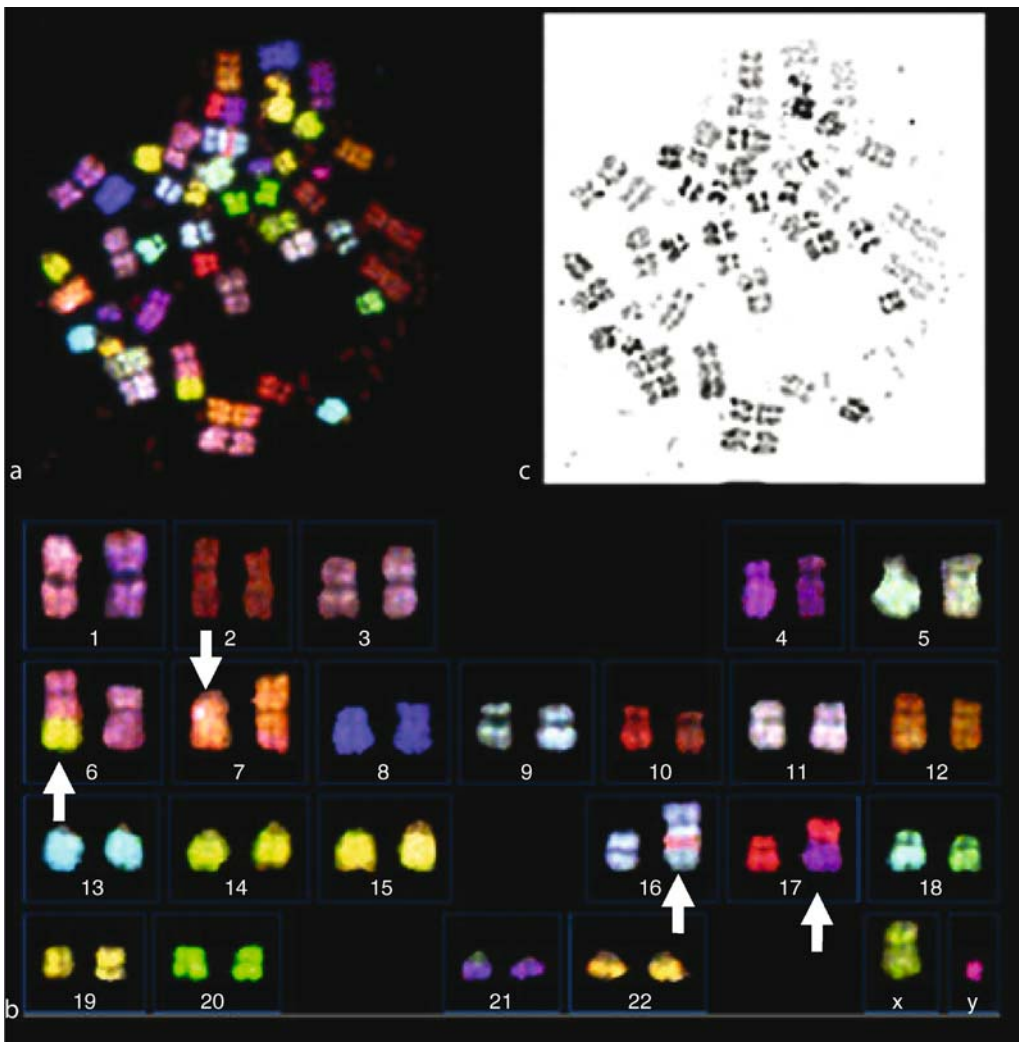
Figure 1 schematically presents the principle of the chromosome classification according to the spectra characteristics. Each graph consist of fluorescence emission spectra of the distinct fluorochromes (or combination of fluorochromes) and a Red-Green-Blue (RGB) display of the appropriate chromosome. Emission maximums for presented fluorochromes are: FITS – 525 nm, Rhodamine – 570 nm, Cy5.5 – 703 nm. Figure 1(a) shows chromosome 8 labeled with FITC, in the RGB display it's seen as blue color; Fig. 1(b) chromosome 20 labeled with Rhodamine, in the RGB display it's seen as green color; Fig. 1(c) chromosome 2 labeled with Cy5.5, in the RGB display it's seen as red color and Fig. 1(d) chromosome 2 labeled with combination of Rhodamine and Cy5.5, in the RGB display it's seen as specific color.

5. The chromosomes are then automatically sorted into a karyotype table according to the nomenclature rules for G-bands. Rearrangements, ▶translocations between different chromosomes and components of marker chromosomes are all easily identified because of a change in color at the point of transition (Fig. 2a and b). Finally, the software assigns a specific classification pseudocolor to each chromosome allowing chromosomal aberrations to be even more easily visualized (Fig. 2b).



Spectral Karyotyping. Figure 2 Spectral karyotyping of metaphase of a neuroblastoma tumor (a) Red-Green-Blue (RGB) display after hybridization with the SKY kit; (b) Karyotype table of spectrally classified chromosomes. Chromosomal aberrations are detected by the combination of two or more colors on the same chromosome (marked by arrows). (c) DAPI-stained separately and inverted to give a G-banding like pattern. (D. R. Betts, Department of Oncology, University Children's Hospital, Zürich, Switzerland; L.Trakhtenbrot, Institute of Hematology, Tel Hashomer, Israel).

6. The DAPI image is captured separately and inverted to give a G-banding like pattern. This image may be used to compliment the SKY analysis with chromosome banding information (Fig. 2c).

Advantages of SKY

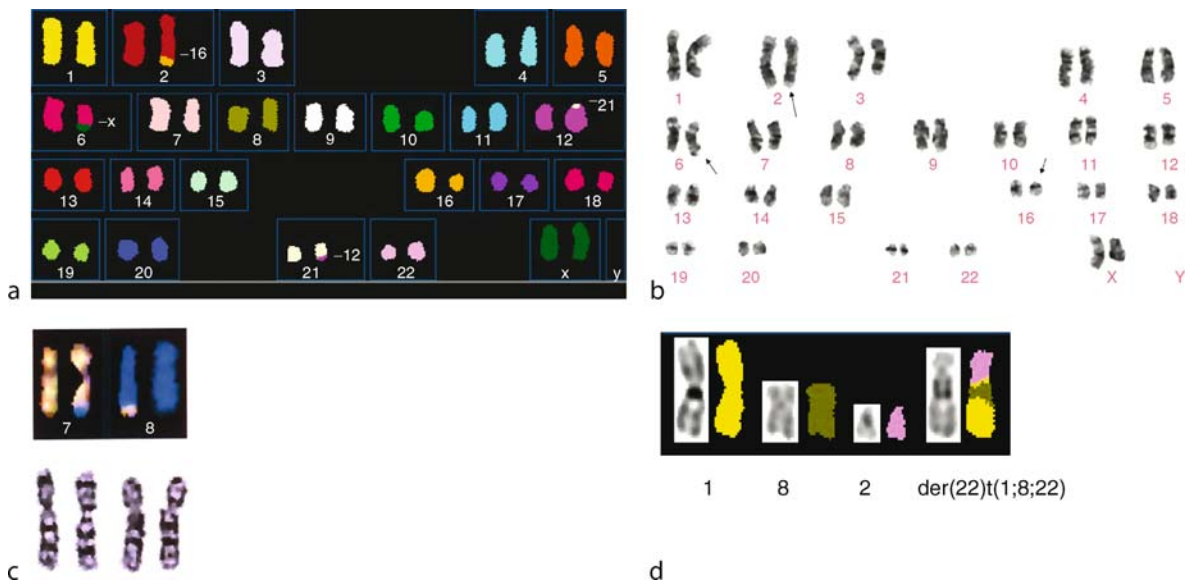
SKY analysis has revealed numerous marker and derivate chromosomes (Fig. 3a, b, d), hidden translocations (Fig. 3a, c), chromosomal ►insertions, homogeneous staining regions and double minutes unidentified or incorrectly identified by G-banding. The combination of SKY with FISH increases the accuracy of karyotype interpretation, permitting precise breakpoint mapping and the detection of small interstitial ►deletions and cryptic translocations. SKY is particularly useful in cancer cytogenetics and provides a much more detailed description of the highly abnormal karyotypes that characterize advanced tumors and cancer cell lines. The precise definition of markers by the SKY technique leads to the determination of an increased number of aberrations per tumor, identification of more chromosomal regions involved in the karyotype evolution and the analysis of more metaphases, especially polyploid. SKY enables the discovery of a larger number of subclones and the revelation of different clonal evolution pathways of karyotype alterations. In general, SKY provides an opportunity to assess the level of tumor instability that is usually associated with advanced or aggressive disease.

The flexibility of SKY permits it to be used with any genome, provided the effective flow sorting of chromosomes can be accomplished. Recently SKY has been developed for murine and rat chromosomes, the most popular species used as model systems for the investigation of tumorgenesis.

Disadvantages

Due to the nature of painting probes, SKY alone can not detect intrachromosomal rearrangements, such as paracentric or pericentric ►inversions, small ►duplications and deletions. The resolution of SKY (1–3 Mb) depends on the level of chromosomal condensation and on the combination of the fluorochromes involved in structural rearrangements. Thus, SKY should be seen as a complement, and not as a replacement, of conventional G-banding analysis.

To overcome these limitations and to elevate the accuracy of cytogenetic analysis a new method named "spectral color banding (SCAN)" was developed on the basis of the conventional SKY analysis. SCAN analyzes a single chromosome, allowing simultaneous visualization of all the chromosome bands in different colors in a single hybridization since each band is labeled with a unique combination of fluorochromes (similar to the SKY technique) with a specific spectral pattern. In this



Spectral Karyotyping. Figure 3 SKY detection of chromosomal aberrations incorrectly identified by G-banding. (a) SKY showing translocations t(12;21), t(2;16) and der(6)t(X;6), displayed in classification colors. (b) G-banding failed to recognized the cryptic translocation t(12;21), and the origin of the additional segments on chromosomes 2 and 6. (c) Translocation t(7;8)(q32;q34) is detected by SKY (RGB display), but not by G-banding. (d) SKY demonstrates a complex translocation involving three chromosomes – 1, 8 and 22. G-banding identifies this rearrangement only as a marker of chromosome 1 origin. (D. R. Betts, Department of Oncology, University Children's Hospital, Zürich, Switzerland; L.Trakhtenbrot, Institute of Hematology, Tel Hashomer, Israel).

way different bands can be distinguished from each other by different colors. SCAN analysis employs a detection system and algorithm for spectral pattern recognition identical to that used in SKY analysis.

The drawback of SCAN is its inability to detect aberrations in the other chromosomes. To overcome this shortcoming, a combination of SKY and SCAN was developed based on a probe cocktail composed of the SCAN banding probes for an individual chromosome and the SKY probe kit without the painting probe for this particular chromosome.

Clinical Significance

Since its introduction in 1996, SKY has been extensively used to elucidate chromosomal aberrations in hematological malignancies as well as solid tumors, including sarcomas, carcinomas and brain tumors. The highly important input of SKY analysis to clinical research of malignant diseases is the identification of novel recurrent aberrations with pathogenic potential and the prediction of a specific phenotype or modified response to treatment or outcome. The results of SKY analyses contribute to diagnosis, therapy decisions, and follow-up studies.

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Spectral Unmixing

Definition

Mathematical algorithm based technique, used in optical image analysis, to distinguish and quantitatively measure the light emission produced by multiple, differently colored fluorescent probes or genetic reporters following their simultaneous detection.

►Bioluminescence Imaging

Spermatocytes

Definition

Spermatocytes are the male germ cells before meiosis I.

►BORIS

Spheroids

Definition

►Multicellular Spheroids.

Sphinganine

Definition

Dihydrosphingosine, the basis of the minor dihydrosphingolipids, generally inactive in cell processes.

►Sphingolipid Metabolism

Sphingolipid Metabolism

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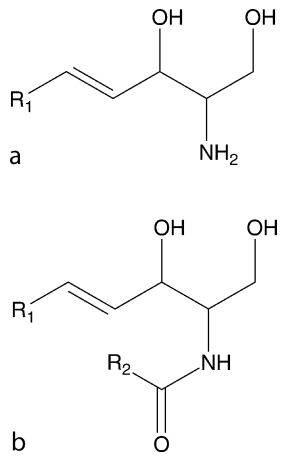
Definition

►Sphingolipids (►SLDs) contain ►sphingosine (►Sph) or a similar moiety. Sphingosine (Fig. 1a) is D-erythro-*trans*-4-octadecene-2-amino-1,3-diol; R_1 is typically a C_{13} alkyl chain. ►Ceramide (Fig. 1b) is a fatty acid amide of sphingosine that plays a pivotal role



Sphingolipid Metabolism. Figure 1 Sketch (a) shows sphinogosine and (b) shows aramide R_1 represents a tridecyl alkyl chain of a saturated or monounsat urated fatty acid

in cell growth and death, in which R_2 symbolizes a saturated or monoenoic fatty acid containing 16–32 or more carbon atoms. Some fatty acids have an OH at carbon-2. Some ceramides contain phytosphingosine instead of Sph; here the double bond is replaced by an OH at C-4.

Hundreds of SLD species occur. The C-1 OH can be phosphorylated to form Sph phosphate or ►Cer phosphate, or esterified by a phosphocholine group to form ►sphingomyelin (►SM), the most common SLD. The OH can also be coupled to a sugar, usually galactose or glucose, to form GalCer or β-glucosylceramide (►GlcCer). GalCer is a major component of human brain; a portion of the lipid has a sulfate group attached to the sugar (►sulfatide). GlcCer, the primary ►glucosphingolipid (►GSL), can add other sugar moieties to the glucose, particularly galactose, N-Ac-galactosamine, and N-►acetylneuraminic acid (a characteristic moiety of the ganglioside series of GSLs).

Characteristics

Sphingolipids control the properties of cell membranes, the rate of cell growth-proliferation-destruction, apoptotic processes, the phosphorylation and dephosphorylation of proteins, the formation of reactive oxygen species (ROS), the hydrolysis of some proteins, the acetylation of nuclear histones, the binding of microbial pathogens and toxins to human cells, ►angiogenesis, telomerase, matrix metalloproteinase, cytosolic and mitochondrial ►glutathione level, and other cancer-relevant factors. Cancer cells appear to synthesize SLDs somewhat faster than normal cells and are more sensitive to SLD manipulation, even in multi-drug resistant cells. This difference in sensitivity bodes well for the design of a drug with a good therapeutic index.

Some SLDs (Cer, ►gangliosides GM3 and GD3) induce ►apoptosis – especially in cancer cells – while other SLDs (Sph phosphate, Cer phosphate, GlcCer, GalGlcCer) promote growth and proliferation and *prevent* apoptosis. Since these two types of lipids can be interconverted by the enzymes that make and hydrolyze them, it is evident that cells must maintain a balance in their concentrations and activities. One or more controlling factors are missing from cancer cells, resulting in unrestrained growth and a high rate of DNA change. Cancer cells contain unusual assortments of SLDs, with dominance of the proliferative lipids. The essence of cancer chemotheraphy appears - *from this viewpoint* – to be the targeting of these lipids, their enzymes, and the factors that control their levels in tumors.

While SLDs occur almost everywhere in cells, there are special aggregates in plasma cell membranes, called ►rafts (GSL- and cholesterol-enriched lipoid/protein ►microdomains). The rafts function as signaling platforms for important cytokines and modification of their compositions can affect many of their functions. Depletion or augmentation of cellular SLDs leads to a remarkable number of important phenomena.

►Poly-Drug Chemotherapy and Multi-Drug Resistance

Because of the unusual sphingolipid Yin/Yang, Janus-faced metabolism, which promotes *and* blocks cell death, cancer therapy calls for the use of a poly-drug cocktail that stimulates the formation of Cer, ganglioside GM3, and GD3, while simultaneously inhibiting synthesis of the proliferative SLDs. The simultaneity is important because manipulation of just one of these lipid clusters leads the cancer cell to induce changes in the other cluster and proliferative factors. This response eventually leads to renewed tumor growth and resistance to the drugs that were used initially. A similar selection occurs when the initially attacked cancer cells mutate to form cells with a more resistant imbalance in SLD composition. Thus, treating tumors with a single, first-line drug can lead to clones that are resistant to the drug and the apparent cure turns out to be illusory. Evidently a cocktail is needed to cover all or most phases of SLD metabolism in an all-out attack, to kill all the cancer clones present in each individual patient. Preliminary trials of the poly-drug approach have indicated that the dose size of each component can be lower than the single-drug approach (i.e., synergistic), so side effects can be expected to be minimized.

Chemotherapy via Ceramide Control

Cer levels can be elevated by the following approaches. Some of the drugs listed are not – or need not be – approved by the FDA.

1. *Stimulate the first step in de novo synthesis of Cer* from serine and palmitic acid, formation of 3-keto ►sphinganine, via pyridoxal phosphate as cofactor. Exogenous palmitate and serine are effective and supplementary pyridoxine may also help. Dietary carnitine, which helps mitochondria destroy the fatty acid, should be avoided. The apoptogenic effect of palmitate is augmented by etomoxir, which inhibits transport of fatty acid by carnitine. Several anticancer drugs (fenretinide, camptothecin, etoposide, paclitaxel, retinoic acid, tetrahydrocannabinol, and valspodar stimulate the de novo synthesis of Cer.

The next steps in Cer synthesis are reduction of the carbonyl oxygen, forming sphinganine, and acylation of the amine group, forming dihydroCer. This somewhat inert analog of Cer is next dehydrogenated to form Cer, producing a double bond between carbons 4 and 5. The crucial segment of Cer is the ►allylic structure, with the C-3 OH and the Δ^4 double bond. Fumonisin, a fungal toxin commonly found in significant concentrations in some foods, inhibits the acylation step and should be avoided via good food handling practices. It also inhibits acylation of free Sph (see below).

2. *Minimize the conversion of Cer to SM* (sphingomyelin), an exchange of the C-1 OH for the phosphocholine moiety of phosphatidylcholine (lecithin) with formation of diacylglycerol. A similar reaction utilizes phosphatidylethanolamine, followed by N-methylation. The enzyme, SM synthase, as well as lecithin, has been found in high concentrations in some tumors, suggesting that they protect themselves against Cer toxicity this way. It is possible to lower the body content of lecithin by restricting the intake of fatty foods. The anticancer phospholipid analog, hexadecylphosphocholine, which inhibits lecithin synthesis, also slows SM synthesis and speeds Cer synthesis. Thus, the various anticancer phospholipid analogs may owe some of their effectiveness to this mechanism. It follows from this also that drugs emulsified with the aid of lecithin should be reformulated. Tamoxifen, which inhibits diversion of Cer by glucosylation, also stimulates phospholipases C and D, thus also lowers tumor lecithin.
3. *Stimulate SM hydrolysis*, which forms Cer + phosphocholine. The major SMases, Mg^{2+}-requiring neutral SMase and an acidic SMase, are normally somewhat inhibited by mitochondrial glutathione (GSH). Thus an agent that lowers GSH levels will greatly elevate Cer level. Radiation, oxidation by ROS, condensation catalysis by glutathionyl S-transferase, inhibition of GSH biosynthesis (buthionine sulfoximine), and Michael reaction of GSH with anticancer drugs that contain allylic ketone moieties (doxorubicin, camptothecin, tetracycline, a keto metabolite of fenretinide, manumycin, actinomycin D, curcumin, quercetin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, illudin, gossypolone, ciprofloxacin, 17AAG (a geldanomycin analog), and others) act this way to produce much of their anticancer activity.

Other agents lower GSH levels in tumors. Cisplatin and dietary thiocyanates (sulforaphane in broccoli) react chemically with GSH. Sulforaphane also induces glutathione S-transferase activity. Cer and many antineoplastic drugs generate ROS in cells, thus raise the sulfur atom to more oxidized states. Even modest oxidation of GSH to form disulfides and mixed disulfides with the cysteine moieties of proteins unmasks the power of SM to form Cer. Many metabolites and nutrients generate ROS in mitochondria in the normal course of oxidative metabolism. Normally the ROS are kept under control by antioxidants. GSH is needed for important enzyme reactions, so its level should not be reduced too far.

The hydrolysis of SM is stimulated by arachidonic acid, a common dietary fatty acid that is converted to prostaglandins. Thus its use would have to be controlled by the use of COX inhibitors. Thalidomide inhibits angiogenesis by stimulating the synthesis of Cer from SM.

4. *Inhibit diversion of Cer to formation of GlcCer* by treatment with D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (►PDMP), ►N-butyldeoxynojirimycin, dietary glucose deprivation, chlorpromazine, tamoxifen, mifepristone, antiandrogens, ketoconazole, irinotecan, doxorubicin, dihydroxy vitamin D3, mitoxanthrone, dexamethasone, arabinofuranosylcytosine, and others. PDMP and its analogs PPMP and PPPP produce Cer in more than one way. GlcCer and its anabolite, GalGlcCer, are growth stimulators and promoters of ►P-gp (MDR1), a major cause of ►multi-drug resistance; thus inhibiting Cer glucosylation is important in several ways. It is possible that food intake reduction, which prolongs life, owes some of its effectiveness to depletion of glucose and UDP-glucose, the co-substrate in GlcCer synthesis.

In addition, GlcCer and GalGlcCer are the precursors of the gangliosides, which prevent the normal action of ►dendritic cells, the major first defense in immunological attack on cancer cells. Tumors shed a significant portion of their rapidly synthesized gangliosides into the extracellular fluid around the cancer cells. The mechanism by which these lipids inhibit dendritic cell development is not clear, possibly by inhibiting Cer glucosylation. The ►ganglioside secretion or shedding also promotes angiogenesis, a vital tool of growing tumors. Thus it seems essential to prevent GSL synthesis so that the patient's immune system can make a final clearing of cancer cells. The accumulated Cer prevents angiogenesis.

A further advantage of inhibiting GlcCer synthesis is that it depletes cell surfaces of the GSLs that act as binding sites for many microbial organisms, including viruses. Alternatively, mimics of the surface GSLs can be used to compete with the cells for binding to infective agents. Thus the complication of ►infection in cancer patients would be minimized.

Patients with ►Gaucher disease, who have a genetic deficiency in the amount of GlcCer hydrolase, are currently being treated by injection of a modified form of the human glucosidase. The enzyme lowers tissue GlcCer levels, forming Cer and glucose, thus should be helpful in cancer patients. A similar effect can be expected by injection of a small peptide that is needed for the glucosidase's activity, ►saposin C.

5. *Inhibit Cer hydrolysis*, which forms Sph + fatty acid, with D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (►D-MAPP), N-oleoyl ethanolamine, and N-octadecylSph. Blocking ►ceramidase seems to be an effective way of elevating Cer and inducing apoptosis. Reacylation of the Sph to regenerate Cer is performed by an acyltransferase produced from the ►longevity assurance gene.
6. *Inhibit the kinase that converts free Sph* to its 1-phosphate ester (►S1P). This ester competes with Cer in controlling a cell's fate, so the *ratio* of the two lipids is critical for rebalancing the SLDs. N,N-dimethyl sphingosine, trimethylSph, and glabridin (an allylic ether in licorice) are suitable inhibitors. DimethylSph is a normal metabolite of sphingosine, but its concentration seems to be too low in tumors.
7. *Chemotherapy with dietary Cer* has shown beneficial effects in intestinal cancer. However it has to be included as part of the total poly-drug approach in order to prevent conversion of the extra Cer to pro-proliferative SLDs. Dietary SM is converted to Cer in the intestine and also has a beneficial effect; it can be considered a pro-drug. Since both lipids and GSLs occur in food, it is likely that they constitute a natural cancer-preventing mixture. SM has been used as part of a liposomal cocktail for dispersing water-insoluble anticancer drugs. A solubilizing chain of poly(ethyleneglycol), attached to Cer by a dicarboxy acid bridge, may solve the problem of administering so insoluble a lipid. It may also be useful for forming a liposomal suspension with SM.

In recent years, analogs of Cer have been synthesized that are more active than Cer in the induction of apoptosis. Adding an additional double bond, conjugated with the ►allylic double bond of Cer, improved the lipid considerably. It is significant that some antineoplastic drugs also have an allylic alcohol group that is part of a chain of conjugated bonds. A Cer analog with a pyridinium group in the fatty acid moiety was found to concentrate in tumors and prevent their growth in vivo. Its effectiveness was greatly enhanced by including gemcitabine, an antineoplastic drug that produces Cer elevation.

Immunological Attack Against Cancer Cells

Some, perhaps many, tumors consist of cells with an unusually high concentration of rare GSLs. Indeed, the ratio of Cer to GSLs decreases as the tumor malignancy increases. Unfortunately, SLDs are not potent inducers of Ab induction (see dendritic cells above) and special techniques had to be developed. Tumors of neuroectodermal origin (e.g.: melanoma and neuroblastoma) are being tested with a mouse/human chimeric ►mAb against ganglioside GD2, a prominent GSL in these cells. Promising results have also been found by active immunization with ►idiotypic peptides that mimic gangliosides. Pathogens containing idiotypes that resemble GSLs sometimes evoke neuropathological autoantibodies, as in Guillain-Barré syndrome.

α-Linked galactose in GalCer (not the usual β-GalCer) is a potent stimulator of NKT cells and a killer of cancer cells. This finding is under active study.

Mechanisms of Sphingolipid Antineoplastic Action

1. Since addition of Cer to whole cells or mitochondria produces ROS and apoptosis, the proposal has been made that the allylic OH of Cer is oxidized to an allylic ketone (keto Cer). Presumably the latter is the therapeutically active form of Cer, since it can undergo adduct formation with GSH, other thiols, and active amines. Antineoplastic drugs that contain an allylic alcohol, ester, or ether moiety may mimic Cer, forming ROS, allylic ketones, and Michael adducts. Cer – and several anticancer drugs – interfere with the mitochondrial electron-transporting oxido/reduction cycle of ►ubiquinone/ubiquinol that normally generates ATP. Ubiquinone is an allylic ketone, thus is likely to oxidize allylic alcohols.
2. Cer can generate pores in mitochondrial membranes, allowing cytochrome *c* and other components to escape into the cytoplasm where they activate the ►caspases and initiate the apoptogenic death sequence. The pores, called barrels, seem to consist of assembled Cer molecules, lined up with their polar (OH) end facing the inside. A certain minimum amount of Cer must be accumulated in the mitochondria to generate one pore. DihydroCer (lacking the double bond) is inert.
3. Cer and Sph activate *kinases* that act on important proteins (e.g.: PKC ζ involved in the control of apoptosis and many other phenomena. Cer also activates ►*phosphatases* (e.g.: PP1 and PP2A) that remove phosphate groups from many proteins (e.g.: ►Akt). These effects are seen with synthetic anticancer drugs too. Generally, sphinganine-based SLDs are inactive in these activations so the

presence of the allylic polar moiety seems to be essential. The mechanism of activation could be ascribed to binding of the active lipid to an allosteric region of the enzymes. However there is also the recently proposed idea that the SLD binds to the active site of the phosphate-transferring region, with the allylic alcohol moiety forming a transitional phosphate ester, functioning as an ►anion-transferring coenzyme for the catalytic processes.

These kinases and phosphatases control the activity of many phosphoproteins that control apoptosis and many other processes. For example, the Cer-activated phosphatase, PP2A, dephosphorylates and thereby inactivates μ- and m-calpains, leading to suppression of the migration and invasion properties of human lung cancer cells.

4. Cer is involved in other anion-transferring reactions of great importance to cancer therapy. It reduces N-acetylation of protein-linked lysine and is involved in ►histone acetylation. It binds to and activates ►cathepsin D, the acid aspartate protease that triggers the apoptotic program by activating Bid. The aspartate COOH in the enzyme's substrate may form a transient ester link with Cer during the peptide cleavage.

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Sphingolipids

Definition

Are a class of lipids derived from the aliphatic amino alcohol sphingosine. The sphingosine backbone is O-linked to a (usually) charged head group such as ethanolamine, serine, or choline. The backbone is also amide-linked to a fatty acid. Sphingolipids are often found in neural tissue, and play an important role in both signal transmission and cell recognition. There are three main types of sphingolipids: ceramides, sphingomyelins, and glycosphingolipids, which differ in the substituents on their head group. Ceramides are the simplest type of sphingolipid. They consist of a fatty acid chain attached through an amide linkage to sphingosine. Sphingomyelins have a phosphorylcholine or phosphoroethanolamine molecule esterified to the 1-hydroxy group of a ceramide. Glycosphingolipids are ceramides with one or more sugar residues and are called gangliosides when they carry three sugars, however one of which must be sialic acid. Phosphorylated forms of sphingosine and ►ceramide, namely sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) are involved in cell survival, angiogenesis and tumorigenesis.

►Lipid Mediators
►Sphingolipid metabolism

Sphingomyelinase

Definition

Sphingomyelin-phosphodiesterase 1 (gene symbol: *Smpd1*) hydrolyses sphingomyelin to ►ceramide. The maximum of the enzyme activity is at an acidic pH.

Sphingomyelins

Definition

Are sphingolipids that possess a phosphorylcholine or phosphoroethanolamine molecule esterified to the 1-hydroxy group of a ►ceramide.

►Lipid Mediators

Sphingosine

Definition

Is an aliphatic amino alcohol that composes sphingolipids.

►Lipid Mediators

Sphingosine-1-Phosphate

Definition

S1P; Is a phosphorylated form of sphingosine and possesses tumorigenic properties.

►Lipid Mediators

Spinal and Bulber Muscular Atrophy (SBMA)

►Androgen Receptor

Spindle Assembly Checkpoint

Definition

SAC; Regulates metaphase-to-anaphase transition of the ►cell cycle in order to ensure that chromosomes do not segregate until each is properly attached by microtubules to bilateral spindle poles during mitosis and meiosis.

►Mitotic Arrest-Deficient Protein 1 (MAD1)
►Checkpoint

Spindle Pole Apparatus

Definition

Develops during the ►cell cycle. Is a highly organized structure that consists of kinetochore microtubules attached to segregating chromatids, and polar microtubules moving apart the spindle poles immediately prior to cell division. Independent changes during G2/M culminate in the formation of the spindle pole apparatus (►G2/M transition). It consists of two opposed poles each arising from one of the two duplicated centrosomes. The bisecting line at which microtubules emanating from each pole of the spindle meet is known as the metaphase plate. Pairs of chromosomes align and attach themselves along the metaphase plate and are then pulled apart from one another by motors that push and pull the separated chromosomes towards opposite poles of the cell. Formation of a spindle pole apparatus requires a change in microtubule dynamics. This is brought about by changes in the phosphorylation states of structural and motor microtubule associated proteins (MAPs); (not to be confused with mitogen activated protein kinase, MAP kinase!). Cyclin-dependent kinase 1 (CDK1) is known to phosphorylate many MAPs.

►G_2/M Transition

Spindle Pole Body

►Centrosome

Spinocerebellar Ataxia Type 1

Definition

SCA1; Is an autosomal dominant progressive neurodegenerative disorder characterized by ataxia, dysarthria, ophthalmoparesis, and variable degrees of amyotrophy and neuropathy. The disease causing mutation is an expansion of a CAG trinucleotide repeat which lies within the coding region of ataxin-1.

►Cajal Bodies

Spinophilin

Definition

synonym neurabin II; Is ubiquitously expressed and interacts with protein phosphatase 1 (PP1), an alternate reading frame (p14ARF, a 14 kDa polypeptide in humans, 19 kDa in mouse), doublecortin and actin, and synergistically suppress osteosarcoma tumor growth with p14ARF.

►Doublecortin

Spirometry

Definition
A physiologic test that measures inhaled and exhaled volumes of air independently and as a function of time.

►Chronic Obstructive Pulmonary Disease and Lung Cancer

Spleen

Definition
A lymphoid organ in the abdominal cavity that is an important center for immune system activities.

Spleen Tyrosine Kinase

►Syk Tyrosine Kinase

Splenic Marginal Zone Lymphoma

Definition
Indolent B-cell neoplasm, composed of small lymphocytes, that is marked by massive splenomegaly and peripheral blood and bone marrow involvement, usually without adenopathy.

►Rituximab
►B-cell Tumors

Splice Variant

Definition
Alternative ►splicing of introns and exons on pre-messenger RNA (mRNA) creates variants of mature mRNA from the same gene, allowing for different forms of the final protein produced.

Spliceosome

Definition
A large multi-protein and snRNA complex that mediates the catalytic steps of the splicing reaction.

►pre-mRNA Splicing

Splicing

Definition
The process by which introns are removed from an RNA transcript.

►Pre-mRNA splicing

Spontaneous Bacterial Peritonitis

Definition
Is characterized by the spontaneous infection of ascitic fluid in the absence of an intraabdominal source of infection involving the translocation from bacteria from the intestinal lumen to the lymph nodes with subsequent bacteremia and infection of ascitic fluid.

►Acites

Sporadic

Definition
Referring to gene mutation, a term frequently used as synonym for non-hereditary.

Sprouty

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Synonyms

Spry

Definition

Sprouty (Spry) proteins are a family of endogenous proteins that negatively regulate the ►ERK/MAP kinase (ERK/MAPK) signaling pathway (Fig. 1) that is activated by ►Receptor Tyrosine Kinases (RTKs).

Characteristics

Sprouty was initially discovered as an inhibitor of ►fibroblast growth factor (FGF) signaling during tracheal development. It was involved in modulating branching in tracheal formation in the fly (Drosophila) and its absence led to excessive "sprouting" of tracheal tubules. As in the fly, mammalian Spry proteins work as feedback inhibitors of RTK signaling during ►branching morphogenesis of the lung, vascular system, kidney tubules and breast ducts.

To date, four mammalian Sprys (Spry1–4) have been identified with sequence similarity to the *Drosophila* protein (Fig. 2). These are expressed in the brain, heart, lung, kidney, limbs and skeletal muscle. The Spry proteins have a highly conserved cysteine-rich C-terminus. The N-terminal half of the Spry proteins are more divergent, however, except for the presence of an invariant tyrosine residue (Y) located in a short, conserved NxYxxxP motif. Many of the inhibitory functions of the Spry proteins are dependent on this residue. Of note, Spry1, 2 and 4 are tyrosine ►phosphorylated/phosphorylation in response to RTK stimulation while the ability of growth factors to induce tyrosine phosphorylation of Spry3 is unknown.

Mechanisms of Action

Various studies conducted in the mammalian system confirm Spry proteins as negative regulators of the ERK/MAPK pathway; they differ in their conclusions as to the specific point in the ERK/MAPK pathway at which Spry acts. Spry has been found to interact with regulators of MAP kinase or mainstream components of MAP kinase (Fig. 1) (a comprehensive description can be found in the review by Mason et al. (2006)).

Regulation of the Activity of Sproutys

As a negative regulator, Spry is subject to tight control through post-translational modifications and through regulation of the protein levels in the cell. The activity of Spry proteins is regulated by post-translational modifications such as serine and tyrosine phosphorylation, palmitoylation and ►ubiquitination.

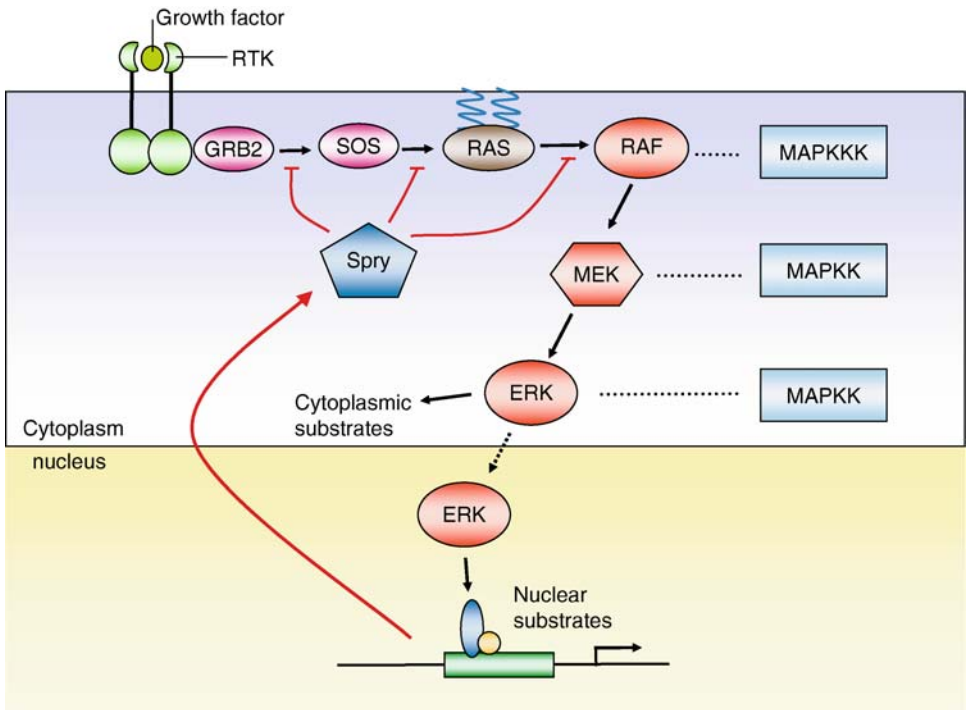
In their N-terminal domain, Spry proteins contain a conserved tyrosine residue (Tyr55 in Spry2) that undergoes phosphorylation in response to growth factor (FGF) and ►epidermal growth factor (EGF) stimulation. This tyrosine residue is necessary for Spry proteins to function as inhibitors of FGF signaling. While tyrosine phosphorylation on the conserved tyrosine on Spry is important for its functional activity, it also promotes the binding of the E3 ubiquitin ligase c-Cbl to Spry2. Tyrosine phosphorylation of Y55 on Spry2, or Y53 on Spry1, creates a consensus binding motif [NxY(S/T)xxP] which serves as a binding site for c-Cbl. The interaction of Spry proteins with c-Cbl promotes the ubiquitination and ►proteasome degradation of Sprys. This pathway has been postulated to serve as a mechanism to control the duration of Spry activity. In addition, several studies have found that serine phosphorylation of Sprys affect the activity of Sprys via influencing the stability and tertiary conformation of Spry proteins.

The activity of Spry proteins is also regulated by controlling the expression of Spry proteins in the cell. Spry proteins are induced by the same RTK signaling pathways they negatively regulate. During vertebrate development, *Spry* genes are often expressed at sites associated with FGF signaling activity. *Sprys* were also observed to be up-regulated upon activation of RTK signaling in *in vitro* cell culture.

Deregulation of Spry Expression in Cancer

The central ERK/MAP kinase pathway is deregulated in many cancers. As Spry proteins are functional inhibitors of this pathway their own regulation is likely highly relevant to the status of some cancers. There is emerging evidence that the expression of the Sprouty family of proteins is deregulated in various cancers including breast, liver, prostate, ►melanoma, ►gastrointestinal stromal tumors (GISTs) and ►lung tumors. *Spry* genes have been shown to be down-regulated in breast, liver and prostate cancers and up-regulated in melanoma, gastrointestinal stromal tumors and Ras-induced lung tumors.

The up-regulation of *Spry* expression in certain tumors (gastrointestinal tumors, melanoma, lung tumors) is often induced by the presence of ►oncogenic lesions (mutation of ►c-Kit, ►Raf kinase, ►Ras) which cause the constitutive activation of the MAP kinase pathways in these tumors. Although numerous oncogenes are also activated in breast, liver and prostate cancers, the same phenomenon of up-regulation of *Spry* genes was not observed. Instead, down-regulation of *Spry* genes was observed. The mechanisms of down-regulation of Spry genes has not been completely elucidated in these



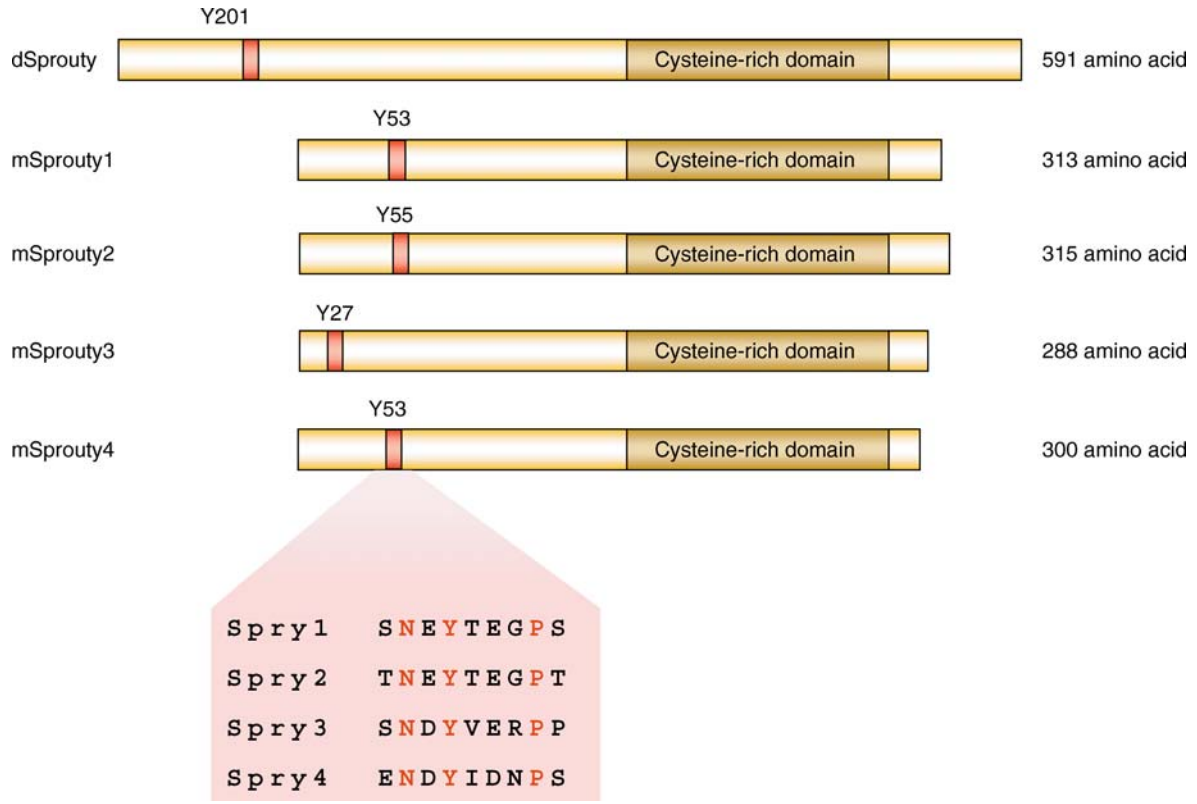
Sprouty. Figure 1 The ERK/MAP kinase pathway. The ERK/MAPK cascade is known for its crucial role in mediating the transduction of signals from Receptor Tyrosine Kinases (RTKs). Following ligand binding, growth factor RTKs such as ►VEGFR and ►FGFR become activated. This induces the binding of adapter proteins such as growth-factor-receptor bound-2 (GrB2) that bind to the activated receptors. In cooperation with Grb2, the guanine-nucleotide exchange factor, son-of-sevenless (SOS) activates ►Ras. This initiates membrane recruitment and activation of ►MAP kinase ►Raf, which leads to the activation of the ►MAP kinase kinase (MEK) and subsequently, ►MAP kinase ERK. ERK phosphorylates several cytoplasmic targets or migrates to the nucleus, where it phosphorylates and activates transcription factors that control the expression of genes that are required for cell growth, differentiation and survival. Various studies conducted in the mammalian system confirm Spry proteins as negative regulators of the ERK/MAPK pathways. Spry proteins are induced by the same RTK signaling pathways they negatively regulate and are observed to be up-regulated upon activation of RTK signaling.

cancers but at least for prostate cancers, some genetic (loss of parts of chromosomes) and ►epigenetic gene silencing by ►methylation mechanisms have been identified to be responsible for silencing of Spry genes.

As the up-regulation of Sprys in cancers is a reflection of an over-active MAP kinase signaling pathway in cancers, Sprys have the potential to be used as ►biomarkers to aid in cancer diagnosis and treatment. Up-regulation of *Spry* genes can be used concurrently with other molecular markers to distinguish between different types of tumors. When gene expression patterns of different soft tissue tumors were analyzed, *Spry* genes were found to be specifically expressed in gastrointestinal stromal tumors (GISTs) but not in synovial sarcomas, neural tumors and leiomyosarcomas. Similarly, *Spry2* expression was found to be up-regulated in melanoma cells with *B-Raf (V599E)* or N-*Ras (Q61R)* mutations but not melanoma cells with wild-type *B-Raf.* Increased MAP kinase activity in these cells was found to contribute to the higher levels of Spry2.

Up-regulation of *Spry* genes could also be potentially used concurrently with other biomarkers to aid ►prognosis or monitor the response of patients to drug treatment. However, depending on the particular type of cancer, up-regulation of *Spry* genes can either be a marker for good or bad clinical prognosis.

Spry4 is shown to be induced by aberrant c-Kit activation in GISTs. Aberrant c-Kit activity derived from activating c-Kit mutations have been shown to be important for development of GISTs. In a study that involved treating GIST cells with an inhibitor of c-Kit, ►Imatinib (Gleevec, STI-571), *Spry4* was identified and confirmed as one of the most significant Imantinib-responsive genes that were consistently down-regulated upon treatment with Imantinib. Imatinib has been shown to be an *in vitro* inhibitor of c-Kit phosphorylation and tumor cell ►proliferation while inducing ►apoptosis in a human GIST cell line. Treatment of GISTs cells with Imantinib resulted in a parallel loss of phosphorylated c-Kit, MAP kinase activation and *Spry4* levels. *Spry4* was found to be a reliable marker



Sprouty. Figure 2 Structure of Sprouty proteins. Four mammalian Sprys (mSpry1–4) have been identified with sequence similarity to the fly (*Drosophila*) protein (dSpry). Spry proteins have highly conserved cysteine-rich C-termini and invariant tyrosine (Y) residues located in a short, conserved motif. Many of the inhibitory functions of Spry proteins are dependent on this residue.

with respect to the clinical response of the patients to Imatinib treatment. In patients responsive to the drug, *Spry4* levels were dramatically decreased. However, in non-responsive patients, *Spry4* levels did not decrease. In patients who initially responded but subsequently relapsed, *Spry4* levels decreased dramatically in the tumor biopsy taken during clinical response but returned to pretreatment levels upon clinical relapse.

Spry2 was also found to be up-regulated in mouse lung tumors which are induced by oncogenic K-Ras. Individual lung tumors were isolated from the mice and examined for Spry expression. The degree of Spry2 up-regulation correlated with the histological grade of the tumor.

While Sprouty is a potential marker of aberrant MAPK signaling in the above-mentioned cancers, in other cancers it is surprisingly a marker for good clinical prognosis. In a study of the gene expression profiles of ►renal carcinoma (clear cell Renal Cell Carcinoma), 51 genes that effectively discriminate between patients with good and poor outcome were isolated. *Spry1* was found to be up-regulated exclusively in the good outcome group.

Ability of Sprys to Inhibit Cancer

Numerous *in vitro* cell-based assays demonstrated that Sprys inhibit cell proliferation, ►migration, and ►invasion as well as ►anchorage-independent cell growth by repressing RTK-induced MAP kinase activation.

Evidence from *in vivo* animal studies indicates that Spry proteins can act to suppress the tumorigenesis process. The first evidence detailing Sprys' ability to interfere with the tumorigenic process was a study where over-expression of Spry2 in an osteosarcoma cell line was found to inhibit tumor growth and metastasis, possibly via the inhibition of MAP kinase activation and cell migration. In a subsequent animal study, Spry2 was shown to inhibit K-Ras-induced lung tumors in mice. In K-Ras-induced lung tumorigenesis, mice develop lung tumors due to the presence of activated K-Ras in their lung tissue. Spry2 was found to be up-regulated in the lung tissue and was found to inhibit the tumor development. Mice with the presence of activated K-Ras in their lung tissue developed greater amount of tumors when the Spry2 expression is abrogated (deletion of Spry2 gene; Spry2 null mice). Analysis of the tumors demonstrated mild increase of

MAP kinase activation in tumors in *K-Ras; Spry* null mice compared to *K-Ras;and Spry2* wild type control mice. This evidence indicates that Spry2 functions to inhibit K-Ras-induced tumor development and that the mechanism may involve antagonism of MAP kinase signaling.

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Spry

►Sprouty

Sputum Cytology

Definition

Microscopic examination of a mucus sample to determine if abnormal cells are present.

►Malignancy-Associated Changes

Squamous Cell Carcinoma

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Synonyms

Epidermoid carcinoma

Definition

Squamous cell carcinoma is a malignant tumor arising from non-glandular lining or covering epithelia.

Characteristics

Squamous cell carcinoma (SCC) is an invasive tumor which may present at numerous body sites, including skin, ocular epithelium, ►oral cavity, alimentary tract, anogenital region, larynx and bronchial epithelium. Thus, the incidence of SCC lesions varies with site. Estimates from the National Cancer Institute indicate that over one million new cases of skin SCC (►Skin carcinogenesis) will be diagnosed in the USA in 2007, representing 16% of all types of skin cancer. Similarly, ~40,000 new cases of head and neck SCC (comprising oral cavity, pharynx, larynx) occur each year. Squamous cell carcinoma is also one variety of ►non-small cell lung cancer, the annual incidence of which is 170,000. Anogenital SCC, comprising lesions of cervix, vulva, vagina, penis and anus, are less frequent. Approximately 23,600 new cases are predicted to occur in the USA each year, of which the majority (12,000) will be ►cervical cancers.

Etiology

As squamous cell carcinomas can occur at several body sites, multiple etiological factors have been implicated in development of these lesions. Skin cancer is largely associated with exposure to ultraviolet light, with the disease affecting those areas of the body exposed to sunlight. Additionally, persons with fair skin susceptible to sunburn are at higher risk. Immunosuppressed individuals, such as transplant recipients, patients suffering from ►epidermodysplasia verruciformis (EV), or those infected with human immunodeficiency virus (HIV) are prone to develop SCC of skin, which is related to infection with ►human papillomavirus (►HPV). For ►lung cancer, the major etiological agents are carcinogens present in ►tobacco smoke (around 90% of male cancer deaths and 80% of those in females are associated with smoking); additional factors include a family history of the disease, or exposure to radon gas. Similarly, head and neck SCC is primarily related to tobacco use, particularly lesions of the oral cavity (►Tongue cancer), oropharynx and larynx. In addition to cigarette smoking, intraoral lesions may also arise in users of chewing tobacco, as well as those who use pan (a combination of areca nut and slaked lime, rolled in a betel leaf to form a quid), with or without tobacco. This practice is popular in southern Asian cultures, and in immigrant populations from this region, resulting in large, exophytic tumors. Further etiological factors for head and neck cancer include alcohol use (also important for ►esophageal SCC) and HPV infection. Anogenital SCCs are primarily caused by infection with "high-risk"

HPV (Human Papilloma Virus) types. Additional etiology includes tobacco usage and HIV infection.

Diagnosis and Clinical Features

Diagnosis of squamous cell carcinoma is based on a combination of clinical examination and histopathological assessment of a biopsy specimen. In some cases, such as ►lung and other internal tumors, clinical examination may be assisted by the use of imaging techniques, such as standard radiography or magnetic resonance imaging. SCCs of skin, oral or genital mucosa may present as exophytic, verrucous (warty) lesions (verrucous carcinoma), or as persistent ulcers that fail to heal. Typically, such ulcers have a raised, rolled border, and larger lesions may show signs of necrosis in the center.

By definition, squamous cell carcinomas arise from squamous epithelium. Histopathologically, therefore, SCCs show signs of ►invasion through the basement membrane into the underlying stromal tissue – the hallmark of carcinomas. Lesions may show varying degrees of differentiation: well-differentiated tumors will be more recognizable as squamous epithelium, forming nests of tumor cells within the stroma and may express an abundance of keratin, forming so-called "keratin pearls." Some tumors can show fewer (or no) signs of squamous differentiation, and may be graded (►Tumor grading) as moderately or poorly differentiated, or anaplastic. Highly aggressive lesions may invade adjacent muscle and bone, and enter into ►lymphatic vessels or blood vessels, thus aiding their spread to secondary sites of growth (►metastasis).

Squamous cell carcinomas may develop through a series of well-defined premalignant stages, during which the epithelium shows increased disruption of normal growth and differentiation and loss of tissue architecture. The epithelium progresses through ever more advanced stages of ►dysplasia, in which some (or all, depending of the degree of dysplasia) of the following ►cellular atypia may be observed:

- Hyperchromatic nuclei
- More frequent mitoses
- Aberrant mitotic figures
- Mitoses in suprabasal cell layers (in stratified epithelia)
- Pleomorphic nuclei, altered nucleus/cytoplasmic ratio
- Loss of cellular polarity
- Dyskeratosis – keratinization deep within the epithelium
- Loss of ►adhesion
- De-differentiation and loss of tissue architecture

Typically, premalignant lesions are described as mildly, moderately, or severely dysplastic depending on the number of cellular atypia observed upon histopathological examination. The most severe cases (but where invasion has not yet occurred) may be referred to as carcinoma-in-situ (CIS). Cervical dysplasias are generally graded as cervical intraepithelial neoplasia (CIN) grade I, II or III, or as CIS, representing increasing severity.

Clinically, potential premalignant lesions may also be noted. ►Actinic keratosis is a well-recognized condition seen on areas of sun-exposed skin which presents as scaly, erythematous patches. Similar lesions on the lip are known as actinic cheilitis. These lesions may range from mild to severe, depending on the degree of cellular atypia present histologically. Full-thickness cellular atypia (CIS) is known as ►Bowen disease. Pre-neoplastic lesions are also recognized at other body sites. In the oral cavity, white patches (►leukoplakia) may be dysplastic, although the majority does not progress to invasive cancer. Red, atrophic areas of ►erythroplakia should be regarded with much more suspicion, as they have a greater propensity for malignant change. A combination of these two lesions – "speckled leukoplakia" or leuko-erythroplakia – are also more likely to undergo malignant transformation. Less frequently, lichen planus (particularly the erosive form) may develop into squamous cell carcinoma. In cervical epithelia, suspicious areas are identified by application of a dilute solution of acetic acid to the area under investigation, producing acetowhite lesions, the histological condition of which is then determined by biopsy. In the development of lung SCC, one of the intermediate histological events is the onset of squamous metaplasia, where the normal respiratory epithelium assumes squamous characteristics. This change may be reversible if exposure to the initiating agent (most commonly tobacco smoke) ceases.

Disease Management

Therapeutic modalities for squamous cell carcinomas include one of (or more commonly a combination of) surgical excision, chemotherapy or radiotherapy. Typically, smaller well-localized lesions are excised with a wide surgical margin of normal tissue. The "normality" of the margin is generally based on clinical and histological assessment. However, this is complicated by the phenomenon known as ►field cancerization. This hypothesis proposes that histologically normal fields or patches of cells are present which have developed from genetically altered stem cells and, thus, are predisposed to undergo malignant ►progression. This underlies the propensity of squamous cell carcinomas to recur following excision or for patients to develop multiple second primary lesions. The likelihood of future tumor occurrence makes preventive strategies appealing. In this regard, the use of ►retinoids has been explored in several clinical trials. However, the success of this is still under debate as,

in some trials, provision of beta-carotene actually increased the incidence of lung cancer. Novel chemotherapeutic drugs that target mutant epidermal growth factor receptors present on the tumor cells are being used for treatment of some ►lung cancers and head and neck SCC. The central role for HPV in cervical ►carcinogenesis has led to the development of a vaccine to prevent viral infection.

Molecular Aspects

A considerable body of research has documented the molecular genetics and biochemical features associated with squamous carcinogenesis. While distinct differences have been noted between tumors arising at specific body sites, there are many common genetic aberrations that are shared amongst SCCs. ►Loss of heterozygosity (LOH), indicative of inactivation of ►tumor suppressor genes, has been reported to occur at multiple chromosomal locations including chromosomes 1, 3p, 4, 5q, 6, 8, 9, 11q, 13q, 14q, 17p and 19q. Two of the most actively studied are the ►*CDKN2A* gene on 9p21 and ►*TP53* on chromosome 17p13 which, respectively, encode p16/►INK4A, an inhibitor of ►cyclin D-dependent kinases, and ►p53, a multi-faceted regulator of cell cycle progression, genomic damage and programmed cell death (apoptosis). Loss of p16/INK4A has been shown to occur through chromosomal loss or deletion, as a result of promoter hypermethylation and, less commonly, through intragenic mutation. The net effect of such a loss is to deregulate the activity of ►cyclin dependent kinases (CDKs) 4 and 6, which are normally active in the G1 phase of the cell cycle. This leads to the hyperphosphorylation of pocket proteins, such as the ►retinoblastoma protein pRB, and more rapid progression through the cell cycle, thus contributing to the enhanced proliferation seen in cancer cells. Loss or mutation of *RB1* is observed less frequently in SCC which may be ascribed, at least in part, to the loss of p16/INK4A, increased expression of cyclin D1 as a result of gene amplification of a locus on chromosome 11p, and the action of the HPV E7 (►Early Genes of Human Papilloma Viruses) oncoprotein.

Loss of expression of a functional ►p53 protein is a common feature of SCC, in some cases through chromosomal loss or deletion. More commonly, however, intragenic mutations result in expression of ►p53 proteins harboring amino acid substitutions that disrupt normal function, or in expression of truncated proteins. The biological consequences of p53 loss can be wide-ranging, given the many functions of this protein, and include failure to activate cell cycle checkpoints in the event of genotoxic stress and failure to execute programmed cell death. C-to-T transition mutations are found commonly in skin cancer, with CC-to-TT changes present at high frequency. These are indicative of ultraviolet irradiation-induced damage, consistent with the known etiology of SCC at this site. Similarly, mutations found in head and neck and lung SCCs are frequently ascribable to the actions of carcinogens present in tobacco smoke, such as the occurrence of G-to-T transversions. The presence of mutation in the *TP53* gene may also be useful to identify clones of genetically altered cells that have arisen during the process of field cancerization.

Deregulation of cell growth by human papillomavirus contributes to the genesis of the majority of cervical cancers. Additionally, HPV has been linked with other anogenital tumors as well as head and neck (primarily pharyngeal), skin and lung cancers. The E6 and E7 (►Early Genes of Human Papilloma Viruses) oncoproteins encoded by "high-risk" HPV types deregulate cell cycle progression by targeting p53- and pRB-dependent pathways. E6 (►Early genes of human papilloma viruses) targets p53 for degradation by the ubiquitin-proteasome pathway (►Ubiquitination). Additionally, E6 (►Early Genes of Human Papilloma Viruses) targets PDZ-motif-containing target proteins such as Dlg, MAGI-1 and MUPP1, each of which has also been shown to suppress cell growth. The E7 (►Early Genes of Human Papilloma Viruses) protein is well-recognized for its ability to bind the retinoblastoma protein and the related p107 and p130 pocket proteins, targeting them for degradation and, hence, also deregulating proliferation. E7 (►Early Genes of Human Papilloma Viruses) also inhibits the function of the transcriptional coactivator FLH2 which may further enhance cellular transformation, while E6 and E7 (►Early Genes of Human Papilloma Viruses) also upregulate expression of the cellular inhibitor of apoptosis protein cIAP2, leading to apoptosis resistance.

Inactivation of the fragile histidine triad gene, ►*FHIT*, located at chromosome 3p14 is another important ►tumor suppressor gene loss that is observed in squamous cell carcinomas. Studies have documented reduced expression in ►oral SCC and in oral premalignant lesions, as well as in esophageal, lung and ►cervical carcinomas. In addition to gene loss, ►epigenetic gene silencing by promoter hypermethylation contributes to reduced transcript levels, and may be attributable to exposure to tobacco smoke. Decreased expression is also reported to be an early event in tumor progression and may be associated with a worse prognosis. Conversely, studies of skin SCCs (non-tobacco etiology) do not provide evidence of altered FHIT expression.

Mutations in the ►*HRAS* gene are reported in only 10–20% of skin tumors, in contrast to initial studies. However, *NRAS* mutations are found with higher prevalence in patients with xeroderma pigmentosum, an inherited condition in which there is a defect in DNA

repair processes. Mutations in *KRAS* are reported in up to 30% of lung cancers. Genes encoding ►Ras proteins are infrequently mutated in head and neck cancers, except for lesions associated with a pan or betel quid chewing habit.

Disruption of growth factor signaling pathways is also common in many squamous cell carcinomas. Overexpression or mutation of the epidermal growth factor receptor (►Epidermal growth factor receptor inhibitors/ligands) is seen with high frequency in head and neck, lung, alimentary and anogenital tumors, and helps to drive proliferation as well as ►motility and ►metastatic spread of tumor cells. Loss of the negative regulatory effects of transforming growth factor beta also contributes to deregulated cell growth, as well as the transition from an epithelial to a mesenchymal (►Epithelial to mesenchymal transition) phenotype that is seen in some advanced cancers. Additionally, the role of ►inflammation is becoming increasingly recognized in squamous cell carcinogenesis, as well as in other tumor types. Notable players in this area include members of the ►chemokine network which can modulate immune function, induce tumor neovascularization (►angiogenesis) and stimulate proliferation and metastasis of tumor cells.

►Epithelial Tumors
►Lung cancer

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SR Proteins

Definition

A family of RNA binding proteins that are characterized by one or more amino-terminal RNA-recognition motifs (RRM), a glycine rich region, and a carboxyl terminal region that is rich in the amino acids arginine and serine which are largely arranged as dipeptides. Individual members of the SR family of proteins are able to complement non-splicing competent cytoplasmic extracts to gain splicing function. SR proteins are important for constitutive ►splicing and have also been found to be important for regulation of alternative splicing. SR proteins facilitate alternative splicing by binding to elements within the pre-mRNA called splicing enhancers and recruiting other components of the ►spliceosome

Src

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Synonyms

c-Src; v-Src; $pp60^{c\text{-}Src}$; $pp60^{v\text{-}Src}$

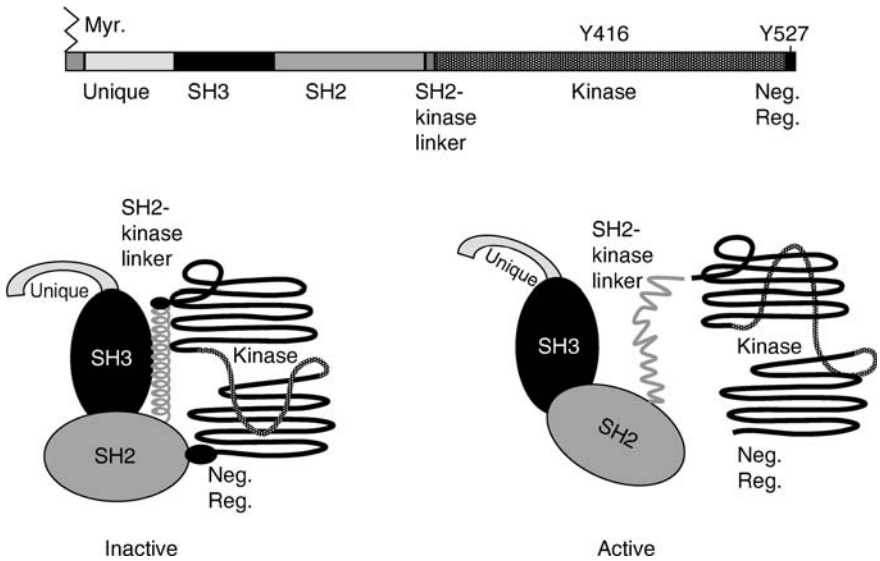
Definition

v-Src (or viral Src) is a 60 kDa protein encoded by the oncogenic retrovirus, Rous sarcoma virus. The protein derives its name from its ability to induce sarcomas in experimental animals and malignantly transform cells in tissue culture. c-Src, or cellular Src, is the normal cellular progenitor of v-Src. c-Src is non- or weakly transforming when overexpressed in tissue culture cells. Both v- and c-Src are cytoplasmic tyrosine kinases that transfer phosphate from ATP to tyrosine residues within specific protein substrates. The resulting phosphotyrosine either conformationally activates the enzymatic activity of the recipient molecule or functions as a docking site for other molecules that transmit growth signals to the nucleus in a chain of events involving multiple phosphorylation and binding reactions. c-Src contains a carboxy-terminal region that maintains the molecule in a mostly inactive state. In v-Src, this 12 amino acid region is deleted, rendering the molecule constitutively active.

Characteristics

Domain Structure

Each molecule of Src contains seven domains that are involved in targeting the protein to cellular membranes (the myristylation domain), in binding other proteins (the Unique, ►SH3 and SH2 domains) and in regulating the catalytic activity (Fig. 1). Src is one of a family of at least nine proteins that have a similar overall structure, including Fyn, Yes, Fgr, Hck, Lck, Blk, Yrk, and Lyn. Some of the family members are present only in certain cell types, such as cells of the hematopoetic



Src. Figure 1 Structure of c-Src. As a linear molecule, c-Src consists of an N-terminal membrane association domain that contains the site of myrisylation, a Unique domain that exhibits the widest sequence divergence among family members, an SH3 domain, an SH2 domain, an SH2/kinase linker, the catalytic domain, and a negative regulatory domain that contains Tyr 527 (531 in human c-Src). When c-Src is activated, Tyr 416 (Tyr 419 in human c-Src) in the kinase domain becomes phosphorylated. How all the domains of c-Src relate to one another in a three-dimensional context to generate the inactive and active states of the enzyme is also shown and explained in the text.

lineage, while others are ubiquitously expressed. c-Src is one of the latter family members.

Subcellular Localization

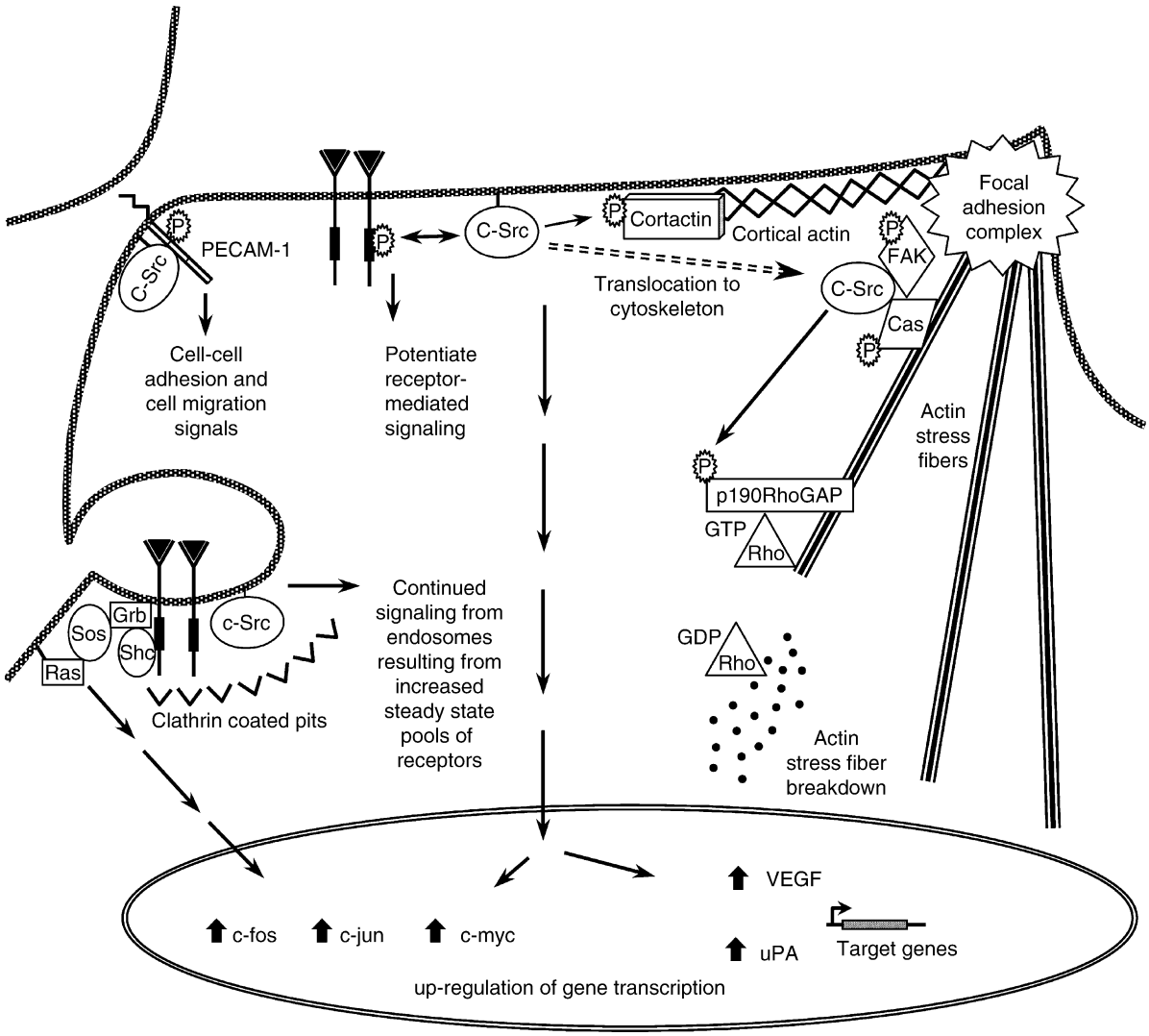
The myristate fatty acid modification on the amino terminus of Src targets it to intracellular membranes, including the plasma membrane and membranes of intracellular organelles, especially those of the endocytic pathway. c-Src has also been found to associate with ►centrosomes in interphase cells.

Interacting Proteins

Src forms complexes with a variety of intracellular signaling molecules via its Unique SH2 and SH3 domains (Fig. 2). These proteins include, but are not limited to, polypeptide growth factor receptors such as the ►epidermal growth factor (EGF) receptor, intercellular ►adhesion molecules such as PECAM and ►E cadherin, gap junction proteins such as connexin 43, and several proteins found in focal adhesions such as focal adhesion kinase (►FAK) and p130CAS. While most of these binding proteins are also substrates of Src, Src can phosphorylate proteins that do not form complexes stable enough to extract from the cell, such as the cortical actin binding protein, ►cortactin, p190RhoGAP (a GTPase activating protein for ►Rho family GTPases) and clathrin, a component of endocytic vesicles.

Cellular and Molecular Aspects

The extreme C-terminal domain of c-Src contains a tyrosine residue (Tyr 527 in chicken c-Src and Tyr 531 in human c-Src) that when phosphorylated binds its own SH2 domain in an intramolecular fashion (Fig. 1). This binding, together with the coupling of the SH3 domain to a pseudo-polyproline sequence in the SH2 kinase linker, renders the protein inactive. c-Src becomes activated when these intramolecular interactions are disrupted by competition with other signaling molecules that contain either phosphotyrosine or polyproline regions that bind the SH2 and SH3 domains, respectively, of c-Src. Such events occur, for example, when the c-Src SH2 domain binds phosphotyrosine 397 of FAK or phosphotyrosines of activated and tyrosine phosphorylated polypeptide growth factor receptors. Full activity is achieved upon dephosphorylation of Tyr 527/531 and autophosphorylation of Tyr 416/419 in the activation loop of the catalytic domain. Antibodies specific for phosphorylated Tyr 416/419 are frequently used to assess the activation state of c-Src in human tumors. c-Src activity has been reported to increase following integrin engagement of extracellular matrix (and subsequent activation of FAK) or upon stimulation of cells with growth factors, such as EGF, PDGF and FGF. v-Src is constitutively activated by deletion of the C-terminal phosphotyrosine 527/531 and mutations within the SH3 domain that reduce interactions with the pseudo-polyproline region.



Src. Figure 2 Examples of c-Src targets and their potential roles in transformation.

S

Clinical Relevance

c-Src is overexpressed or activated in multiple human tumors, particularly in ►glioblastomas and ►carcinomas of the breast, lung, colon, prostate, cervix, stomach and ►ovary. In ►breast cancers, the frequency of tumors overexpressing c-Src approaches 70%. Although analyses of other tumor types are not as extensive as those of breast cancers, existing data suggest the frequency of c-Src overexpression in lung and colon tumors may be similar to that in breast malignancies. Members of the EGF receptor family are also overexpressed in many of the same types of tumors that overexpress c-Src. Recent studies indicate that c-Src and EGFR synergistically promote tumor growth. This enhanced growth is accompanied by an EGF-induced association between c-Src and the EGFR, phosphorylation of EGFR by c-Src on several novel sites, and activation of signaling pathways that are required for EGF-induced mitogenesis and cell survival following genotoxic stress. These findings are providing the impetus for discovering novel therapeutics that disrupt both the physical and functional interactions between c-Src and EGFR family members.

c-Src's involvement in the formation and turnover of focal adhesions and cell-cell contacts also suggests a role for this protein in cell ►migration and ►metastases. Regulation of ►vascular endothelial growth factor (VEGF) expression in response to hypoxia implicates c-Src as a modulator of angiogenesis, further underscoring its potential importance as a target for anti-tumor therapy. To that end, numerous pharmaceutical companies have developed inhibitor compounds

that target c-Src kinase activity, and several of these are being tested for treatment of solid tumors in clinical trials, including AZD0530 (AstraZeneca), BMS-3554825 (Bristol Myers Squibb), and SKI-606 (Wyeth Research).

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SRC-3

►Amplified in Breast Cancer 1

Src8

►Cortactin

Src Family Tyrosine Kinase

Definition

The ►*src* gene is the first discovered viral oncogene carried by the ►retrovirus Rous sarcoma virus and also is the first identified proto-oncogene. The Src family consists of nine family members encoding tyrosine kinases, which are anchored to the inner surface of cytoplasmic membrane with their N-terminal myristylation. Some members such as Src and Fyn are also located within nuclei as well as within mitochondria.

►Membrane-Linked Docking Protein
►Transduction of Oncogenes

Src Homology 2

►SH2/SH3 Domains

Src Homology Domain

Definition

SH; A region of a protein whose tertiary structure allows it to specifically bind to phosphorylated tyrosine residues.

►SH2/SH3 Domains

Src Kinase

Definition

►Src was the first discovered tyrosine kinase. v-Src (viral sarcoma) was the first discovered oncogene, isolated from Rous sarcoma virus and is constitutively activated. Src comprises ►src homology domains, which recruit proteins, expressing these domains to Src.

►Focal Adhesion Kinase

SRC-Homology Domains

Definition

►SH2 Domain.

►SH3 Domain

SRCR Superfamily

Definition

The scavenger receptor cysteine-rich (SRCR) superfamily is a group of glycoproteins comprising cell

surface molecules as well as secreted proteins that are characterized by the presence of at least one highly conserved SRCR-domain.

SREBP

Definition

Sterol regulatory element-binding protein (SREBP): A transcription factor for the transcription of genes that encode the low-density lipoprotein receptors and enzymes in cholesterol synthesis.

►Fatty Acid Synthase

SRF

Definition

Serum response factor (SRF) is a transcription factor that recognizes the CArG box (CC-ATrich-GG) in cellular genes, such as immediate-early-genes. SRF interacts either with an ►Ras-regulated B-box containing ►Ets protein (Elk-1, Sap, Net or FLI-1) or with β-actin/Rho-GTPase (►Rho Family Proteins) -regulated Mal to mediate responses to a variety of extracellular stimuli.

►ETS Transcription Factors

srGAPs

Definition

Family of ►GTPase activating proteins, srGAPs that facilitate hydrolysis of Cdc42 that ultimately leads to actin depolymerization and contributes to the repulsive effect of Slit in neurons.

►Slit

SRIF

►Somatostatin

St. John's Wort

Definition

Synonym hypericum, goatweed. The flowering tops of St. John's Wort are used to treas and tablets with concentrated extracts. Although St. John's wort is not a proven therapy for depression, there is some evidence that St. John's wort is useful for treating mild to moderate depression. It is also used for anxiety and/or sleep disorders. St. John's wort can influence the clearance of specific drugs known to be metabolized by CYP3A (►Cytochrome P450) or excreted via ►ABC drug-transporters.

►Irinotecan

Stage

Definition

Referring to tumors, is an internationally agreed index which objectively measures the extent of tumour growth and progression. Common criteria include tumour size, extent of local spread, and presence of lymph node or visceral metastasis. Each tumour-type has its own staging system, with variations in the common criteria, which correlates with prognosis.

►Transcoelomic Metastasis

Staging of Tumors

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Synonyms

Determination of tumor extent and spread; Tumor staging

Definition

Tumor staging is a clinical procedure aimed at documenting the anatomic extent of a malignant tumor at a specific site, and the extent of its spread locally, regionally, or to distant sites. Upon completion of

the clinical staging procedures, the tumor under consideration is assigned to a particular *stage* (stadium in Latin), i.e. given a semiquantitative designation summarizing the data about the size of the tumor and the extent of its spread.

Characteristics

Purpose

The staging of cancer is used to document the extent of the neoplastic disease in a standardized and consistent manner that will allow clinicians to compare the features of a particular tumor with other similar tumors. The main reasons for staging of tumors have been summarized by the American Joint Committee on Cancer as follows:

- Selection of the most appropriate therapy for each individual patient
- Formulation of prognosis for each cancer patient, i.e., prediction of the natural course of the neoplastic disease and its outcome with or without treatment
- Objective and measurable assessment of the effects of therapy
- Objective performance of clinical cancer studies within a single institution or geographically distant institutions

Clinical staging of tumors is of paramount importance for the proper selection of treatment of each particular cancer patient. Many univariate and multivariate studies have shown that the tumor stage at the time of diagnosis is in most instances the most powerful predictor of outcome of a neoplastic disease and cancer patients' survival with or without treatment. It is also essential for organizing multi-institutional cancer treatment studies and other forms of clinical cancer research.

Methods

Tumor staging is typically based on a multidisciplinary effort including oncologists, surgeons, radiologists, pathologist and even other clinicians. The data may be collected by biopsy, during surgical exploration, during definitive cancer surgery, laparoscopic surgery, and radiologic examination of the patient. The material collected during these procedures is usually submitted for macroscopic and microscopic pathologic examination. Additional studies, such as molecular biologic analysis of the tumor material may be undertaken in research institutions, but it is not routinely included in the staging of most tumors.

The most widely used tumor staging system is called the TNM system. The TNM system is based on analysis of three parameters: the size or extent of the untreated primary tumor (T), the absence or presence of spread to the regional lymph nodes (N), and the presence or absence of distant metastasis (M). For the sake of uniformity and consistency the following standardized definitions are used:

Primary tumor (T)	
TX	Primary tumor cannot be evaluated
T0	No evidence of primary tumor
Tis	Carcinoma in-situ (early cancer that has not invaded into the surrounding stroma)

Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be evaluated
N0	No regional lymph node involvement

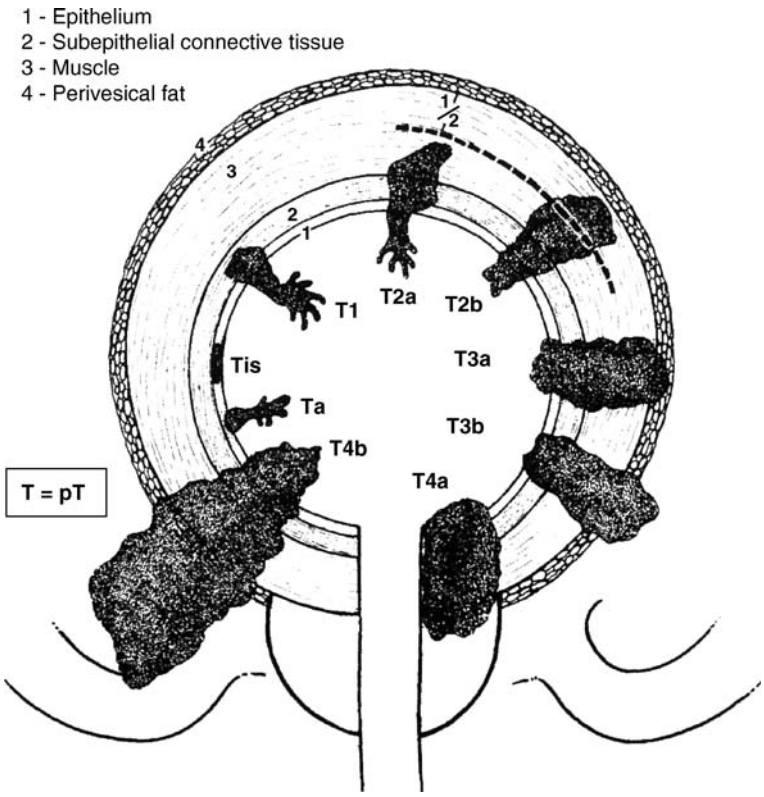
Distant metastasis (M)	
MX	Distant metastasis cannot be evaluated
M0	No distant metastasis (cancer has not spread to other parts of the body)

The staging based on the TNM data typically includes five categories, corresponding to stages 0 and stages I–IV. Each of these stages may be subdivided into subcategories labeled a, b, or c. Stage 0 denotes *carcinoma in situ*. Stage I, II and III tumors are localized to the organ of their origin or have spread regionally. Stage IV tumors have metastasized to distant sites.

The criteria for T, N, and M and for stages 0–IV vary from one anatomic site to another. For example, bladder cancer T3N0M0 is stage III, while colon cancer T3N0M0 is stage II. For specific staging of tumors in various anatomic sites one must refer to specific staging manuals. Figure 1 illustrates the definition of T classifications for bladder cancer.

Perspectives

Staging of tumors has been standardized to a great extent but still many problems remain to be solved. The older staging systems such as the staging of colonic carcinoma according to Dukes are not used any more and are thus only of historical interest. New staging classifications are constantly being proposed and the merits of new approaches are discussed in medical literature. The same holds true for the new studies on the molecular biology techniques or new imaging techniques, which are used in



Staging of Tumors. Figure 1 Tumor staging. Extent of primary bladder cancer (T classification).

experimental protocols in many leading research centers. Overall, one can predict with confidence that the current protocols for staging of tumors will be improved by the introduction of newer technology, but all these modifications will require extensive independent confirmation and validation before they become widely accepted.

Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in-situ: "flat tumor"
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscle
pT2a	– Superficial muscle (inner half)
pT2b	– Deep muscle (outer half)
T3	Tumor invades perivesical tissue
pT3a	– Microscopically
pT3b	– Macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
T4a	– Prostate, uterus, vagina
T4b	– Pelvic wall, abdominal wall

(Reproduced from Greene FL, Page DL, Fleming ID, Fritz AG, Balch

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Standardization

Definition

A consistent set of procedures for designing, administering, and scoring an assessment. The purpose of standardization is to ensure that all individuals are assessed under the same conditions.

►Biomonitoring

STAT

Definition

Signal transducer and activator of transcription, proteins activated in response to interleukins and other cytokines that regulate function of immune system and proliferation, apoptosis, and angiogenesis in various cell lines.

►Suppressors of Cytokine Signaling
►Signal Transducers and Activators of Transcription in Oncogenesis

Stathmin

Definition

Op 18; Is a ubiquitous cytosolic phosphoprotein with various regulatory functions in cell proliferation, differentiation signaling, and activation. In particular, stathmin is involved in the regulation of tubulin dynamics through inhibition of microtubule formation and/or microtubule depolymerization.

►Microtubule-Associated Proteins.
►Oncoprotein 18

Statins

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Definition

Statins are synthetic agents that inhibit HMG-CoA reductase, the rate-limiting enzyme that controls cholesterol biosynthesis. Currently, there are several statins in use for the treatment of hypercholesterolemia in humans. It is well established that these agents induce their therapeutic effects by decreasing low density lipoproteins (LDL). Such effects have been associated with a decrease in the rate of progression of atherosclerotic lesions in patients with coronary artery disease, and it is now well established that the use of statins has changed the natural history of coronary artery disease in humans. Beyond their ability to lower cholesterol, statins have important additional biological effects in vitro and in vivo, including anti-inflammatory and antitumor properties.

Characteristics

There has been substantial experimental evidence establishing that statins inhibit the growth of malignant cells and induce programmed cell death in vitro. Moreover, statins exhibit chemopreventive effects towards certain types of tumors in vivo when administered to humans. The ability of statins to generate such responses in vitro and in vivo is of high interest and may prove to be of clinical value in the future.

Cellular Effects of Statins in Vitro

Statins induce apoptosis of different types of malignant cells in vitro. These include cells of leukemia origin as well as cells originating from a variety of solid tumors, including colon, prostate, breast, thyroid, pancreatic, small and non-small cell lung cancers, as well as malignant melanoma, osteosarcoma, glioma and medulloblastoma. Although the mechanisms by which statins induce apoptosis have not been fully elucidated, there is evidence that distinct cellular events are involved in the induction of statin-dependent pro-apoptotic responses. Blocking protein ►geranylgeranylation correlates with ►lovastatin-induced apoptosis of human leukemia cell lines. In addition, statins activate the pro-apoptotic JNK ►MAP kinase pathway in malignant cells, and the activation of this pathway is essential for statin-dependent programmed cell death. Beyond activation of the JNK pathway, statins inhibit the MEK/ERK signaling cascade, which is associated with increased cell proliferation, and this constitutes another major mechanism contributing to their antineoplastic properties. ►Atorvastatin and ►fluvastatin also induce differentiation of NB4 leukemic cells that are of acute promyelocytic leukemia (APL) origin. Such effects also occur in cell variants that are refractory to the differentiating effects of all-trans-retinoic acid (ATRA). Atorvastatin and fluvastatin exhibit also similar in vitro effects on primary leukemic blasts that have developed resistance to the differentiating effects of ATRA and appear to reverse such ATRA-resistance.

Statins in the Prevention and Treatment of Cancer

There is accumulating evidence from epidemiological studies indicating that statins exhibit chemopreventive effects against certain solid tumor types, including colorectal cancer, lung cancer, prostate cancer, and pancreatic cancer. On the other hand, statins do not seem to exert such chemopreventive effects against

breast cancer. Extensive preclinical evidence has also suggested that statins may have therapeutic effects against certain malignancies. This has led to the recent initiation of clinical trials to examine the clinical activity of statins in certain malignancies. A recent phase I study assessed the combination of high doses of pravastatin with chemotherapy in the treatment of acute myelogenous leukemia patients. Such combination was found to be safe and well tolerated, while a high number of complete responses was seen. Altogether, there is a substantial amount of evidence raising the possibility that statins may eventually find a role in the prevention and/or treatment of certain tumors and hematologic malignancies. Several epidemiological and clinical studies are currently ongoing to address this issue.

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Staurosporine

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Definition

Staurosporine is a natural product originally isolated from the bacterium *Streptomyces staurosporeus* from a soil sample obtained in Japan (Iwate Prefecture) in 1977 during a search for new alkaloids present in actinomycetes and given the name AM-2282. The term alkaloid refers to a naturally occurring amine produced either by a plant, animal or fungus. Actinomycetes are a group of Gram-positive bacteria that had previously been shown to produce the alkaloids pyrindicin, NA-337A and TM-64.

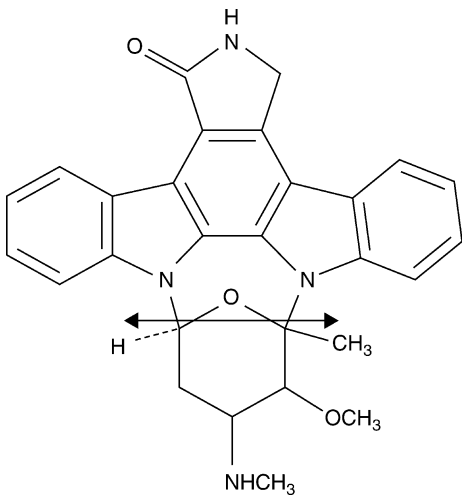
Characteristics

The initial studies on AM-2282 focused on the taxonomy of the producing strain, fermentation, isolation, and physico-chemical and biological properties of this new alkaloid. Besides being identified as a new species, they also discovered that AM-2282 had antimicrobial activity. The term antimicrobial is given to any type of chemical compound that can suppress the growth of, or aid in the death of microorganisms, such as bacteria, yeast and mycoplasma. AM-2282 had antimicrobial activity against fungi and yeast, but no significant effects on bacteria.

The structure of AM-2282, which herein will be referred to as staurosporine, is shown in Fig. 1. Staurosporine was the first of over 50 alkaloids to be isolated with this type of chemical structure, which was elucidated through X-ray analysis. In Fig. 1, the portion of the structure above the arrow, is known as an indole carbazole sub-unit, while the lower part of the structure is a sugar molecule. Although staurosporine was isolated in 1977 and, its X-ray crystal structure was determined in 1978, it was not until 1996 that the first total chemical synthesis was achieved. Part of the challenge to the synthesis of staurosporine was the joining together of the sugar and indole carbazole groups and establishing the sugar stereochemistry. In 1992, the indole carbazole group known as staurosporine aglycon was isolated. Aglycon is the non-sugar compound remaining after replacement of the glycosyl group with a hydrogen atom.

Kinase Inhibition

The anti-microbial activities of staurosporine were initially thought to be via its role as a potent inhibitor



Staurosporine. Figure 1 Structure of staurosporine (AM – 2282).

S

of protein kinase C (PKC). These initial studies revealed that staurosporine was able to inhibit PKC from rat brain. Using other inhibitors such as trifloperazine, chloropromazine, and polymixin B, staurosporine was a more potent inhibitor of PKC than other known inhibitors. Furthermore, PKC inhibitors trifloperazine, chloropromazine, and polymixin B appeared to compete with phospholipids, whereas, the inhibition by staurosporine was not released by increasing the concentration of phosphatidylserine. Studies with cultured cells showed that staurosporine has very potent growth inhibitory activity.

In addition to being an inhibitor of PKC, staurosporine also inhibits several other kinases, such as tyrosine kinases. The in vitro activity of staurosporine was analyzed by investigating the autophosphorylation of $p60^{v\text{-}src}$ in chicken embryo fibroblasts (CEF) that were infected with the rous sarcoma virus (RSV), revealing staurosporine was able to inhibit the autophosphorylation. This was evidence as staurosporine could inhibit tyrosine specific protein kinases similarly to serine and threonine specific kinases such as PKC. Together, staurosporine is a more potent inhibitor of protein-tyrosine kinase than other known inhibitors.

Staurosporine can also inhibit the myosin light chain kinase. Myosin light chain kinase plays a critical role in smooth muscle contraction as well as in the activation of non-muscle cells, by catalyzing the transfer of the γ-phosphate from ATP to the myosin light chain, which is dependent upon Ca^{2+}/calmodulin. The phosphorylation of the light chain is necessary for the activation of actomyosin ATPase, which is a prerequisite for tension development in both smooth and non-muscle cells. Inhibition of the myosin light chain kinase is ATP-dependent.

Other kinases that can be inhibited by staurosporine include the insulin receptor tyrosine kinase activity non-competitive with ATP and the platelet-derived growth factor (PDGF) receptor tyrosine kinase.

Besides having inhibitory activity on these kinases involved in signal transduction pathways, staurosporine was also shown to block cells in different phases of the cell cycle. Staurosporine can block the G1 to S transition at low concentration (1–10 ng/ml) and block cells in late G2 phase at higher concentrations (100–200 ng/ml). There have been several reports showing that the retinoblastoma protein is important in the ability of staurosporine to arrest cells in the G_1 phase of the cell cycle. For example, staurosporine can be used to protect normal cells from the toxic effects of chemotherapy at subnanomolar concentrations, to allow for a reversible G1 arrest through inhibition of the retinoblastoma protein, followed by treatment with camptothecin. Such sequential treatment (with staurosporine first) results in a protection of cells against the toxic affects of chemotherapy. Tumor cells, however, appear unaffected by staurosporine, and are sensitive to low amounts of camptothecin.

The cell cycle associated kinase that is inhibited by staurosporine is Cdc2 (also known as CDK1) which is important in the G2/M transition of the cell cycle. Staurosporine also inhibits the cdk2 and cdk4 kinases which are involved in G1 and S phases.

Collectively, these findings suggest that staurosporine is a non-specific kinase inhibitor because it can inhibit kinases of several different functions with similar efficiencies. This is due to the fact that the serine/theronine kinases conserve the amino acid sequence and 3-D structure of the ATP binding domain. Additionally, staurosporine inhibits the activity of both the insulin receptor tyrosine kinase and of Ca^{+}/calmodulin-dependent protein (CaM) kinase II in a non-competitive manner with ATP. This suggests that staurosporine can interact with other catalytic domains distinct from the ATP binding site.

Staurosporine in Apoptosis

Staurosporine has been a useful tool in analyzing apoptosis because it has been able to induce apoptotic cell death in cell lines that are normally resistant to chemotherapeutic drugs and death-inducing ligands. One of the death-inducing ligands, TRAIL ((TNF)-related apoptosis-inducing ligand), can normally induce apoptosis in two-thirds of melanoma cell lines examined. Staurosporine, however, is able to induce apoptosis in all melanoma cell lines tested, including those resistant to TRAIL.

Although staurosporine was shown to have a strong cytotoxic effect on the growth of several mammalian cell lines, it did not show anti-tumor activity in any in vivo models tested. However, a naturally occurring analog of staurosporine, UCN-01 showed anti-tumor activity in vivo. UCN-01 was also isolated from the same culture broth *Streptomyces* sp. No. 126 that produced staurosporine. UCN-01 differs structurally from staurosporine with a hydoxyl group at the C-7 carbon. Adding this hydroxyl group causes UCN-01 to be specific for PKC inhibition. UCN-01 also shows apoptotic effects in vitro on the cell lines HeLa S3 and MCF-7 similar to what was observed with staurosporine.

Similar to staurosporine, UCN-01 can also inhibit cell proliferation by arresting cells in different phases of the cell cycle. The growth inhibitory affect of UCN-01 is often used to modulate the affects of radiation and chemotherapy. When cells are treated with radiation, which arrests cells in the G2 phase of the cell cycle, in the presence of UCN-01, the cells are no longer able to accumulate in G_2 leading to early mitosis and subsequent apoptosis. UCN-01 also abrogates the S phase of the cell cycle. For example, in p53 mutant cells, treatment with chemotherapeutic drugs such as camptothecin results in S and G2 arrest of

the cells. When cells are subsequently treated with UCN-01, it leads to an abrogation of the camptothecin-mediated arrest resulting in mitotic catastrophe and cell death.

Anti-tumor Activity

Since UCN-01 shows anti-tumor activity in vivo with much less cytotoxicity than staurosporine, UCN-01 was used for further pre-clinical studies. Using a xenograft model system with several tumor cell lines (breast and renal carcinoma, and leukemia cells) and with either intravenous or intraperitoneal injections, UCN-01 was shown to have an anti-tumor effect.

The first case of using UCN-01 to treat patients was in 1996 with a 72 h infusion in the United States (Bethesda, MD) and in Japan as a 3 h infusion. These initial studies showed an unusual pharmacological effect that was not observed in the animal models. The concentrations of UCN-01 in the human plasma were considered abnormally high compared to animal models. Furthermore, the half-life in humans was over 500 h, which was 100 times longer than in the animal models. Eventually it was discovered that in humans, UCN-01 binds strongly to the plasma α_1-acidic glycoprotein resulting in its lack of plasma clearance. Although the initial schedule called for 72-h continuous infusion every 2 weeks, based on the low clearance and prolonged half-life of UCN-01, this was modified to 36-h continuous infusion every 4 weeks. This study has lead to several follow-up studies in which UCN-01 is given as 1–3 h infusions every 4 weeks along with several combinatorial trials. For example, a phase I/II trial of gemcitabine followed by UCN-01 for a 72-h infusion was recently initiated at the National Cancer Institute. Among some of the other agents that are being examined in combination with UCN-01 are cisplatin and 5-fluorouracil. Other ongoing studies with UCN-01 are to use this agent to protect normal cells against the toxic affects of chemotherapy. Since the concentration of UCN-01 required to reversibly arrest normal cells in the G1 phase of the cell cycle is much lower than those required to mediate a toxic affect in tumor cells, this rationale provides a promising alternative to chemotherapy alone. These pre-clinical and ongoing clinical studies provide a novel and potent target that can be used to biologically modify the mechanisms of deregulation of cancer cells and as such provide a novel class of anti-cancer agents.

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Stefins

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Synonyms

Type I cystatins; Cystatins A, B

Definition

►Stefins are members of the ►cystatin superfamily of cysteine proteinase inhibitors localized in the cytosol and thought to protect cytoskeletal proteins from degradation by ►cysteine proteases released from lysosomes.

Characteristics

Type-1 or Family-1 of the cystatin superfamily of cysteine protease inhibitors includes human stefins A and B and their homologues in other species such as ►cystatins α and β in rat, bovine thymus stefin C, porcine thymus stefins D1 and D2, mouse stefins A (1–4) and others (see MEROPS subfamily I25A). The genes for human stefins A and B have been mapped to chromosomes 3q21 and 21q22.3, respectively. The lack of a signal sequence and disulfide bonds makes stefins distinct from other members of the cystatin superfamily. Stefins are single chain proteins consisting of 98–103 amino acid residues, with a molecular mass of 11–12 kDa. Human stefin A is an acidic protein with pI values between 4.5 and 5.0, whereas stefin B is neutral, having pI values in the range of 5.9–6.5. Their tissue and cellular distribution are different, stefin A being localized mainly to epithelial and lymphoid tissue, while stefin B is evenly distributed in various cells and tissues.

Like other members of the cystatin superfamily, stefins are reversible and competitive inhibitors of cysteine proteases. The structural basis of the inhibition

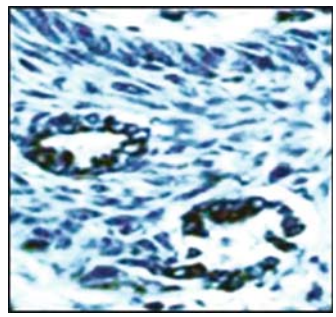
has been elucidated from the X-ray crystal structure of ►papain-stefin B complex and NMR structure of stefin A. Stefins are potent inhibitors of ►cathepsins L and S with Ki values in the picomolar range, whereas cathepsin B inhibition is weaker (Ki 10^{-8} M for stefin A and 10^{-7} M for stefin B).

Physiological and Pathological Roles

Besides protection of cytosolyc and cytoskeleton proteins from degradation by cysteine proteases accidentally released from lysosomes, several other functions have been suggested for stefins. For example, stefin A may be important in the control of normal ►keratynocyte proliferation and differentiation. Also, it has been proposed that it plays a role in ►apoptosis, since apoptotic bodies consistently stain for inhibitor, which also correlates with p53 activation. Stefin A should also protect epithelial and lymphoid tissues from cysteine proteases produced by pathogens invading the body. Increased levels of stefin A were found in inflammatory skin samples, psoriatic epidermis, and in inflamed gingival tissue homogenates from patients with periodontal inflammatory diseases. Decreased levels of stefin B mRNA were detected in patients with ►progressive myoclonus epilepsy (PME) and associated with excessive activity of cathepsin B. Besides protease inactivation, stefin B could bind other proteins in a multiprotein complex that has a specific cerebellar function, which might contribute to the disease in patients with progressive myoclonus epilepsy. Recent genetic studies have also identified mouse stefin A to be involved in a control of ►ovarian follicular growth and maturation.

Stefins in Cancer

Cysteine proteases, the targets for stefin's inhibitory activity, have been implicated in multiple steps of tumor ►progression, including processes of cell transformation and differentiation, ►motility, ►adhesion, ►invasion, ►angiogenesis, and ►metastasis. In most tumor types their levels of expression, protein concentrations and enzymatic activities, in particular of cathepsins B and L, have been demonstrated to be significantly higher than in their control tissue counterparts. The increased levels of cathepsins are usually not accompanied by an equal increase of the cystatins, which was suggested as the main cause of harmful cysteine, cathepsins-dependent proteolytic activity in tumor tissue. However, the balance is selectively altered for type II cystatins, which are in most cases downregulated in tumors, whereas type I cystatins, i.e. stefins A and B, have been found up-regulated in a majority of cases when compared to control tissue counterparts. Higher levels of stefins A and B in tumors have been determined in lung, ►breast, head and neck and ►prostate cancer, as well as in murine lymphosarcomas, ►hepatomas and Lewis lung carcinomas (Fig. 1). These



Stefins. Figure 1 Stefin A immunostaining in lung adenocarcinoma (*Kindly provided by B. Werle*).

higher levels, up to a certain level, may counter-balance the excessive activity of cysteine cathepsins, associated with matrix remodeling, resulting in the progression of the disease. On the other hand, high cytosolic levels of stefins may be relevant for regulation of apoptosis, when initiated via lysosomal cell death pathway inhibiting cathepsin B, which was proposed as a dominant execution protease in the lysosomal apoptotic pathways, induced in a variety of tumor cells by tumor necrosis factor alpha (►TNF-α). In some studies lower levels of stefins in tumors have been reported. For example, stefin A immunoreactivity was lower in lymphomas and in tumors of squamous epithelial cell origin, as well as in prostate and ►brain tumors. Lower mRNA levels of stefin A or B have been reported in breast and ►esophagus tumors as compared to adjacent control tissues.

Although stefins are cytosolic proteins, they have also been detected in body fluids of cancer patients. Stefin A and stefin B have been detected in ascitic fluid from patients with ovarian carcinoma and in bronhoalveolar fluid of lung cancer patients. Increased serum levels of stefin A in patients with ►hepatocellular carcinoma and liver cirrhosis have been correlated with tumor size and with a number of neoplastic lesions. Stefin A, but not stefin B, levels were moderately increased also in patients with ►colorectal or lung cancer.

Increased levels of cysteine protease activity, not being balanced by a corresponding increase of cysteine protease inhibitors, are associated with the progression of malignant disease and patients' poor prognosis. Enhanced expression of stefins is expected to diminish the tumor-associated proteolytic activity and indeed, there is evidence of a suppressive role of stefins in various cancer types. Moreover, higher levels of stefin A and stefin B in tumor tissues have been shown to correlate with a favorable prognosis for cancer patients. A significant prognostic value of stefin A and stefin B was determined in patients with lung and head and neck cancer. In the latter, high stefin A tumor levels were found as a strong factor for prediction of prognosis also in multivariant analysis when correlated with

established clinical parameters. In prostate tumors higher cathepsin B/stefin A ratios were associated with a more aggressive behavior of prostate cancer.

Animal models with excluded expression of particular cystatin did not support a suppressive function for cysteine protease inhibitors in cancer. In stefin B as well as cystatin C ►knock-out mouse, a significantly lower metastatic spread was detected than in wild-type animals. Similarly, higher levels of stefin A and stefin B in body fluids have been associated with a poor prognosis in cancer patients. Alterations in secretion may result in higher extracellular and lower intracellular levels of stefins and, therefore, a reverse correlation with patient survival is to be expected. However, cysteine proteases, and consequently their inhibitors, are involved in various physiological processes, including those which may act in a manner opposing tumor progression, such as apoptosis, activation of T-cell immune response, cell ►migration, seeding, and drug resistance processes. Thus, besides their concentration, cell and tissue localization of stefins, and a number of extrinsic and intrinsic factors, could make a critical switch between harmless and harmful.

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Stem Cell

Definition

Is a founder cell that exists in all multicellular organisms and shows self-renewal ability through mitotic cell division and can differentiate into a wide range of lineage-committed cells. Upon division, each new cell has the potential to either remain as a stem cell or become another type of cell with a more specialized function. There are three kinds of stem cells: embryonic stem cells, germinal stem cells, and adults stem cells that have different developmental potentials: totipotent, pluripotent, multipotent, or unipotent.

►Adult Stem Cells
►Stem Cell Markers
►Stem Cells

Stem Cell Factor

Definition

SCF; Is a glycoprotein that acts as both a positive and negative regulator of hematopoiesis. The cell surface receptor for SCF is KIT, a cancer stem cell marker. SCF is also involved in mast cell development, gametogenesis, and melanogenesis.

►Stem Cell Markers
►Kit/Stem Cell Factor Receptor in Oncogenesis
►Mastocytosis

Stem-Cell Harvest and Purging

Definition

Following stimulation with growth factors, chemotherapy or both, CD34+ stem cells can be mobilized to the blood and harvested via a central vein catheter. In case of malignant bone marrow involvement, tumor cells may be mobilized as well. By in vitro ►purging the stem cells may be selected (e.g., CD34+ cell selection) or the tumor-cells depleted. By in vivo purging of the patient with monoclonal antibodies (e.g., CD20 antibodies in B-cell lymophomas), the amount of tumor cells in the stem cell product may be reduced to undetectable (by ►PCR) levels.

►Mantle Cell Lymphoma

Stem Cell Hypothesis in Cancer

Definition

Tumors arise from cells termed cancer ►stem cells that have properties of ►adult stem cells, particularly the

abilities to self-renew and differentiate into multiple cell types, and that these cells persist in tumors as a distinct population that likely causes disease relapse and metastasis. They are the only cells capable of, by themselves, giving rise to new tumors.

►Pancreatic Cancer Stem Cells

Stem Cell Markers

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Definition

Stem cell markers are molecules used for the identification of unspecialized, undifferentiated cells, and in the case of malignancy, presumptive ►cancer stem cells.

Characteristics

There is increasing evidence that subpopulations of neoplastic cells demonstrate heterogeneity with respect to proliferation, differentiation, and expression of cellular proteins characteristic of ►stem cells. Subpopulations of cells that express stem cell markers that can also be shown to contribute to tumor progression and resistance to chemotherapy are termed cancer stem cells. Cancer stem cells are found in very small subpopulations within tumors, in the range of 0.1-1% of the total cell number. Microscopically, cancer stem cells outwardly appear the same as any other tumor cell. Therefore, in order to identify these rare subpopulations, a number of stem cell markers have been identified and developed as a means of distinguishing stem-like cells from other cells within a cancer population. Unique expression patterns of stem cell markers provide a means for scientists to identify as well as isolate stem-like cells from heterogeneous tumor populations.

Stem cell markers are generally cell surface proteins with the ability to selectively bind to, or activate other signaling molecules. In some cases, stem cell markers are transcription factors that maintain stem cell properties of ►pluripotency and/or ►self-renewal. Stem cell markers are often designated by short-hand terms, based on their cellular function and/or the molecules to which they bind. As yet, there is no unanimously agreed-upon universal stem cell marker. Therefore, multiple markers are used in combination for verification of stem cell identity.

Stem cell markers have been assigned to embryonic, hematopoietic, and neural categories based upon the original location(s) in which they were discovered. These categories work fairly well for embryonic and adult stem cells; distinctions blur in cancer, where, for example, embryonic and/or hematopoietic stem cell markers can be found in tumors of neural origin. Some examples of commonly used stem cell markers with applications in cancer biology include:

ABCG2 (72,300 kDa) (also known as ATP-binding cassette superfamily G member 2, BCRP, BCRP1, BMDP, MXR, MXR1) defines a ►Hoechst-33342-negative phenotype of ►side population (SP) cells. ABCG2 is expressed in cancers of the blood, ►breast, ►prostate, ►lung, germ cell, and retina. ABCG2 may be a potential marker for positive selection of cancer stem cells from a wide variety of tissues.

Bmi-1 (44–46 kDa, murine viral ►oncogene homolog) is a ►polycomb group gene. In addition to its role in development, Bmi is also a tumor-associated antigen, expressed in cancers of the blood, ►brain, lung, oral mucosa and gastrointestinal tract.

CD44 (80–95 kDa) is a ►cluster of differentiation (CD) molecule that functions as a receptor for hyaluronic acid. It is involved in cell/cell and cell/matrix interactions. Overexpression of CD44 has been associated with the development and spread of a range of different types of malignancies. CD44 is found in cancers of the blood, breast, prostate, germ cell and lung.

CD133 (120 kDa), also a cluster of differentiation molecule, was first identified as a hematopoietic stem cell marker. It is a glycosylated protein which recognizes a CD34 + subset of human hematopoietic stem cells. CD133 expression has been demonstrated in human leukemias, prostate cancer, germ cell tumors as well as in ►brain tumors.

CD164 (80–90 kDa), also known as ►sialomucin, is a cluster of differentiation molecule expressed on CD34 + hematopoietic progenitor cells. CD164 has been detected in prostate cancer.

►c-kit (145 kDa, CD117) is the membrane receptor for ►stem cell factor (SCF). It is an oncogene expressed in cancers of the blood, prostate, germ cell, lung and gastrointestinal tract.

Musashi-1, 2 (39 kDa) are RNA binding proteins associated with ►asymmetric cell division (asymmetric cytokinesis) in neural stem cells. Musashi-1 has been detected in cancers of the breast, brain and retina, while Musashi-2 is rearranged in chronic myeloid leukemia.

Nanog (34 kDa) is a human embryonic stem cell marker and transcription factor also found in osteosarcomas, breast cancers, ►retinoblastomas and germ cell tumors.

Nestin (177kDa) is categorized as a neural stem cell marker. It is a class VI intermediate filament protein

primarily expressed in stem cells of the central nervous system, including brain tumors.

Oct-4 (also termed Oct-3 or Oct3/4) is an embryonic stem cell marker. It is a ►POU transcription factor that confers self-renewal and pluripotency to embryonic stem cells. Oct 4 is expressed in both embryonic stem and germ cells, as well as in germ cell neoplasias, ►bone tumors and retinoblastoma.

Sca-1 (18 kDa) is a mouse-specific stem cell marker (stem cell antigen 1, Ly-6A/E), and a member of the ►Ly6 antigen family. Sca-1 is expressed in cancers of the blood, prostate, lung, breast, and retina.

Sox2 (34 kDa) is a transcription factor important in maintaining self-renewal properties of neural progenitor cells. Sox2 is an acronym for "SRY-related HMG-box gene 2." SRY refers to "sex-determining region Y," as the first Sox gene was found to be important in sex determination of developing gametes. HMG refers to ►"high mobility group (HMG group)," which is the DNA binding domain of Sox2. Sox2 has been localized to cancers of the brain, lung and gastrointestinal tract.

A summary of stem cell markers with applications in cancer biology is shown in Table 1. Note that the field is changing rapidly, as new markers and tissue locations are added constantly. Therefore, this is not an exhaustive list, but rather a representative list of the most common stem cell markers to date that are applicable to cancer.

Methods Used to Detect Stem Cell Markers in Cancer Biology

Antibodies directed against stem cell markers can be used to identify and isolate stem cells. Fluorescently-labeled secondary antibodies directed against primary antibodies bound to stem cell proteins identify specific populations of stem-like cells.

Fluorescence-activated cell sorting can isolate rare stem cells from a heterogeneous tumor population. In this technique, a suspension of labeled cells passes by a laser, one at a time, and then through an electric field. The fluorescent cells become negatively charged and can be directed, based on charge, to a separate collecting tube for further analysis. In this way, a very small population of cells expressing stem cell markers can be isolated from a heterogeneous tumor population.

Magnetic beads linked to secondary antibodies also allow for the isolation of rare cell populations based on expression of stem cell markers on the cell surface. Magnetically-beaded cells expressing stem cell markers can be separated based on magnetic attraction while the non-expressing cells are washed away. Once the beaded cells are isolated, the cells of interest can be freed from the magnetic beads for further analysis.

Fluorescent antibodies can also be used to visually assess cells as they exist within the tissue of origin or within mixed cell cultures. Fluorescently-labeled stem-like cells will emit light at specific wavelengths that can be visualized by fluorescence microscopy.

Polymerase chain reaction (PCR) can be used to detect the presence of genes coding for stem cell markers that are expressed in putative stem cells. This method is not as useful for isolating stem cells, but can be used to screen populations for stem cell markers and test isolated cell populations for expression of stem cell genes.

Clinical Applications

The hypothesis that a subpopulation of cancer stem cells drives tumorigenesis and chemo-resistance may

Stem Cell Markers. Table 1 Stem cell markers with applications in cancer biology for each marker, an "X" indicates localization to cancers of various tissues

Marker	Blood	Breast	Brain	Prostate	Retina	Germ Cell	Lung
BCRP/ABCG2	X	X		X	X	X	X
CD133	X		X	X	X	X	X
CD34	X					X	
CD44	X	X		X		X	X
CD164	X			X			
Bmi-1	X		X				X
Sca-1 (mouse)	X			X	X		
Nestin			X				
Musashi-1,2	X	X	X		X		
Sox2			X				X
Oct3/4					X	X	
Nanog		X			X	X	
►c-kit	X			X		X	X

lead to new approaches for cancer prognosis and therapy. Expression patterns of stem cell markers may indicate the differentiated or undifferentiated state of a tumor, and may correlate with a favorable or an unfavorable prognosis in the clinical setting. Once cancer stem cells are identified and characterized using stem cell markers, they can be targeted, using specific stem cell markers for immunologically-based therapies. New stem cell markers will continue to be discovered and are certain to play an important role in the rapidly emerging field of cancer stem cell biology.

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Stem Cell Niche

Definition

The local ►microenvironment that houses the stem cell population in a tissue or organ. Made up of cells, blood vessels and extracellular matrix, ►stem cells adhere to the niche through ►cell adhesion molecules and receive signals e.g. Wnt glycoproteins, that regulate their behavior.

►Stem Cell Plasticity

Stem Cell Plasticity

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Synonyms

Transdifferentiation; Metaplasia

Definition

Stem cell plasticity refers to the ability of some ►stem cells to give rise to cell types, formerly considered outside their normal repertoire of differentiation for the location where they are found. Included under this umbrella title is often the process of "transdifferentiation" – the conversion of one differentiated cell type into another, and ►metaplasia – the conversion of one tissue type into another. From the point of view of this essay, some metaplasias have a clinical significance because they predispose individuals to the development of cancer. Circulating bone marrow-derived cells (BMDCs) that usually generate all blood cell lineages, can switch cell lineage commitment and contribute to the regeneration of several damaged non-hematopoietic tissues, and some carcinomas may even have their origins in BMDCs. The bone marrow origin of some tumor stromal cells and vasculature is now widely acknowledged, but since this pathway was not formerly recognized, it too can be considered a further example of plasticity, which, importantly, has significant biological and therapeutic implications for cancer.

Characteristics

Most adult tissues have *multipotential* stem cells (►Adult stem cells), cells capable of producing a limited range of differentiated cell lineages appropriate to their location, *e.g.* small intestinal stem cells can produce all four indigenous lineages (lysozyme-secreting Paneth cells, mucin-producing goblet cells, absorptive columnar cells and enteroendocrine cells). However, tissue-based stem cells may be more versatile than previously thought, particularly those of bone marrow, and these cells may generate unexpected cell types when engrafted in a damaged non-hematopoietic tissue or organ. This so-called plasticity is being exploited in the field of regenerative medicine where it is hoped to produce new cell therapies for currently intractable diseases such as diabetes and congestive heart failure. Other sources of malleable stem cells include ►umbilical cord blood, ►mesenchymal stem cells (MSCs) from many sources including liposuction waste (fat), skin fibroblasts and spermatogonia.

Stem cell plasticity has been questioned by some investigators who have been unable to reproduce some of the claims: "blood to brain," "brain to blood," "bone marrow to oocytes," "bone marrow to cardiomyocytes." Other instances of plasticity have now been attributed to cell fusion between bone marrow cells (or their macrophage descendants) and cells of the recipient organ. Cell fusion may have implications for tumorigenesis. Such a process could endow differentiated cells with stem cell properties such as infinite self-renewal, while at the same time result in genetic instability with obvious tumorigenic potential.

From the viewpoint of cancer, we should note that while a scattering of engrafted cells of hematopoietic origin (but with a phenotype appropriate to their new location) is often observed in damaged parenchymal organs, these cells appear to have engrafted not as stem cells but either as ►transit amplifying cells or ►terminally differentiated cells, thus their long-term significance for cancer development is highly questionable. If BMDCs did engraft as stem cells in a new location, it is not inconceivable that they could be the founder cells of tumors at these sites. Indeed in a murine model of gastric cancer, BMDCs repopulate the gastric mucosa and over time contribute to metaplasia, dysplasia and cancer in response to chronic infection with *Helicobacterfelis* (►Gastric cancer, ►Helicobacter pylori). BM-derived gastric glands are seen after 20 weeks of chronic infection, leading to a final replacement of 90% of the gastric mucosa with BMDCs after 1 year. Upon progression to epithelial dysplasia and gastric adenocarcinoma, the majority of the dysplastic glands were of BM-origin, most likely from MSCs. Chronic inflammation led to atrophic gastritis, probably ablating the ►stem cell niche of numerous indigenous gastric glands, and the vacant niches were then occupied by BMDCs that subsequently behaved as gastric gland stem cells (Fig. 1) (►Stem cells and cancer).

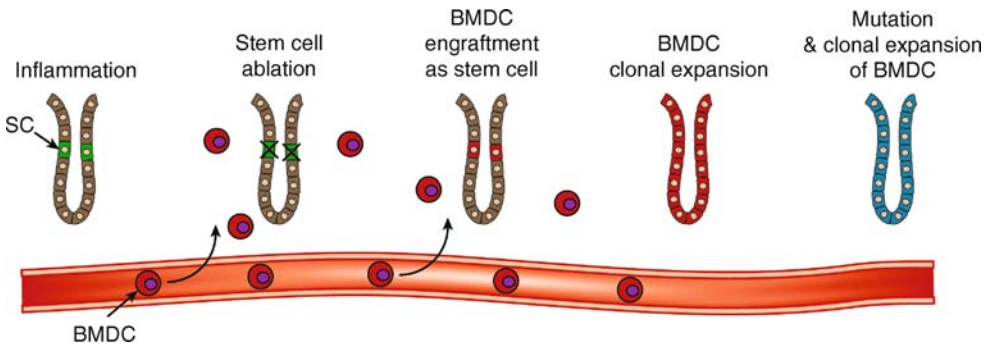
An origin of carcinoma from BMDCs has also been suggested for one case of skin basal cell carcinoma arising in a female recipient of a male kidney transplant. In this case, most of the cytokeratin-positive tumor cells were male, and since BCC rarely if ever metastasizes (so no occult metastasis in the transplanted organ), it is likely that donor BMCs in the graft had migrated to the skin, either fusing with or differentiating into keratinocytes, before undergoing malignant transformation. On a cautionary note, it has been observed that in some murine and human cancers there are occasions when BMDCs incorporate into the tumors, phenotypically mimicking the cancer cells, but of course not actually initiating them.

Metaplasia

Metaplasias are major switches in tissue phenotype, invariably they occur in tissues subjected to chronic trauma, and are likely to represent epigenetic reprogramming of tissue stem cells – *in-situ* stem cell plasticity. Cancers can arise in areas of metaplasia, and we often have a metaplasia – dysplasia – carcinoma sequence. For example, the esophagus is normally lined by squamous epithelia, but due to acid reflux it can become lined by glandular tissue (Barrett's esophagus) and adenocarcinomas usually arise in areas of dysplastic Barrett's esophagus. In the airways of the lung, chronic irritation by tobacco smoke can lead to a switch from columnar to squamous epithelia, and squamous carcinoma of the lung usually arises from patches of squamous epithelia. Metaplasias may well be caused by misexpression of homeotic genes, a good example being intestinal metaplasia in the stomach caused by overexpression of the intestine-specifying *CDX* genes; here too, metaplasia predisposes to gastric cancer.

The Bone Marrow and Tumor Stroma: Clinical Relevance

BMDCs may indirectly influence tumor behavior by contributing to the ►desmoplastic response and to the tumor vasculature, lineages not formerly considered to have arisen from bone marrow (►Desmoplasia, ►angiogenesis). ►Myofibroblasts are a distinguishing feature of pathological fibrosis, normally regarded as having originated by activation of local organ



Stem Cell Plasticity. Figure 1 Stem cell plasticity could provide an alternative pathway for cancer development as seen in experimental gastric carcinogenesis. Continued inflammation and tissue damage leads to eradication of the indigenous stem cell compartment and its replacement by bone marrow derived cells (BMDCs), whose progeny subsequently repopulate the whole gland. Mutation in a BMDC engrafted as a stem cell can then lead to a dysplastic gland and subsequent gastric cancer. Key: indigenous normal epithelial cells are brown, indigenous stem cells are green, BMDCs are red and mutated BMDCs are blue.

fibroblasts, but there is clear evidence that many are generated from bone marrow cells. These BMDCs can contribute to organ fibrosis, including the fibrosis surrounding many cancers – the desmoplastic response. Thus, BMDCs may be useful for the local delivery of anticancer agents such as interferon.

►Endothelial progenitor cells (EPCs) are mobilized endogenously in response to tissue ischaemia or exogenously by cytokine therapy to augment neovascularization. The development of a vascular supply to a tumor is a prerequisite for tumor survival – allowing for the provision of oxygen and nutrients as well as the disposal of waste products. New vessel formation is also required for tumor metastasis. Previously, tumor vasculature was thought to develop exclusively *via* endothelial cell migration and proliferation – *angiogenesis*. However, the creation of new blood vessels by EPCs is known as *vasculogenesis*, and such a process is a significant event in tumorigenesis. This opens the possibility of using bone marrow cells as a vehicle for transporting antiangiogenesis molecules directly to the tumor vascular bed, in effect using BMDCs as Trojan horses.

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Stem Cell Telomeres

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Synonyms

Telomeric repeats of stem cells; Guanine-rich tandem DNA repeats of chromosomal ends

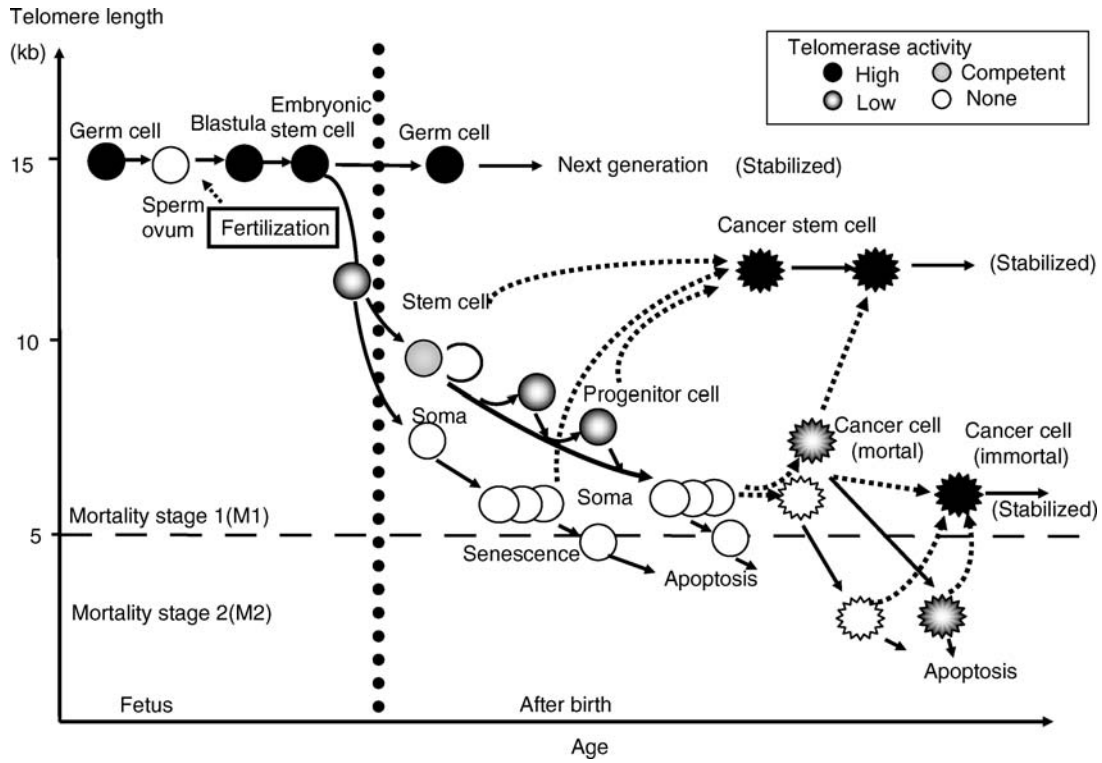
Definition

►Telomeres are guanine-rich tandem DNA repeats that cap the ends of eukaryotic chromosomes. Their primary function is to prevent chromosomal degradation, fusions and instability. During cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes. The slow rate of cell turnover in stem cell compartments means a longer period of ►telomere length stability in stem cells versus somatic cells. Nevertheless, stem cell telomeres do gradually shorten with age.

Characteristics

Human ►telomeres consist of tandem repetitive arrays of the hexameric sequence TTAGGG, with overall telomere sizes ranging from ~15 kb at birth down to <5 kb in ►senescence cells or in some chronic disease states. The end of telomere forms a 3′ overhang of the G-rich strand, which is generate by the postreplicative processing of the C-rich strand, folds back into the duplex telomeric DNA to form a protective t-loop. During the process of cell division, telomeres shorten as a result of the incomplete replication of linear chromosomes, the so-called "►end-replication problem." This progressive telomere shortening is one of the molecular mechanisms underlying aging, since critically short telomeres trigger chromosome senescence and ►apoptosis. A critical length of telomere repeats is required to ensure proper telomere function and avoid activation of the DNA damage pathways that result in replicative senescence or cell death.

The ends of telomeres are protected and regulated by telomere-binding proteins and form a special lariat-like structure called the ►t-loop that prevents degradation by exonucleases or processing as DNA damage signal caused by exposure of DNA ends. A human telomere needs >5 kb of its length to form the t-loop. When the length shortens to <5 kb, cells fall into senescence (►mortality stage 1 (M1)). In immortal cells, telomere length is maintained by ►telomerase, a reverse transcriptase, or another mechanism such as ►alternative lengthening of telomere (ALT). In most human somatic cells, except for stem cells and lymphocytes, telomerase activity is diminished after birth, such that telomere length shortens with each cell division (Fig. 1). Most malignant tumors must have a mechanism for bypassing M1, a cell-cycle checkpoint. The overcome of this checkpoint leads to an extended lifespan but continued telomere losses, eventually producing a crisis or the ►mortality stage 2 (M2) of replicative senescence caused by ►chromosomal instability, unless some mechanisms that escape this stage are activated. To escape M2, the telomeres in immortalized cancer cells must be stabilized by reactivation or up-regulation of telomerase activity.



Stem Cell Telomeres. Figure 1 Telomere dynamics in human stem cells.

Germ cells and ►embryonic stem cells maintain telomere length between each generation with high levels of telomerase activity during rapid proliferation. In the developmental stage, telomerase activity gradually decreases and diminishes in most somatic cells after birth and falls in senescence (M1 stage). Thus, the telomere length of somatic cells is one of the limiting factors of cell division. In ►adult tissue stem cells, the level of telomerase activity is low or undetectable. Activity is up-regulated in committed progenitor cells which have high reproducible activity in each tissue, but it is insufficient to maintain telomere length. On the other hand, most cancer cells bypass M1, which leads to an extended lifespan but continued telomere losses. Eventually, the consequence is crisis or entry into the M2 stage of replicative senescence. ►Cancer stem like cells and cancer cells are immortalized with stabilized telomeres by escaping M2.

Stem cells are capable of karyotypically stable, prolonged self-renewal. They are also characterized by their potential to generate a very large number of committed progenitors and descendants during a small number of self-renewal divisions. Since stem cells have elongated proliferative capacity, they must have a mechanism that maintains telomere length through many cell divisions. In human stem cells, telomere length is maintained for many cell divisions and telomere length remains in longer than somatic cells by telomerase or ALT. The former mechanism is at work in ►hematopoietic stem cells (HSCs) while the latter functions in ►mesenchymal stem cells (MHCs). The mechanism of telomere maintenance in human stem cells is different in each type of stem cell, but telomeres are not completely maintained in human stem cells except for embryonic stem cells. Thus, human ►adult stem cells have an intermediate existence between somatic cells and immortal cells, showing aging-related changes concomitant with telomere shortening. The temporary activation or low expression of telomerase in adult stem cells may partially account fot the resistance to telomere erosion of stem cells in general bu the telomere lengths of these cells were gradually eroded, consequently yielding the ageing of stem cells. The gradual loss of telomeres is interpreted as a regulator for cell life span and is considered a cancer prevention mechanism, because prolonged replications and longevity would subject cells to the accumulation of mutations leading to transformation of stem cells to cancerous cells. If these stem cells are immortalized, they are no longer human stem cells but may become "cancer stem like cells." Recently, the important role of telomeres in human illness has been highlighted by studies of the rare genetic disorder "►dyskeratosis congenita (DKC)." This essay shows the role of

telomeres and telomerase in the function and regulation of the stem cell compartment, and their importance in ►stem cell failure diseases.

In the hematopoietic system, low telomerase is detectable in stem cells with self-renewal potential and their early descendants, but age-dependent telomere loss is observed in both lymphocytes and neutrophils. In bone-marrow transplant patients, the length of telomeres in blood leukocytes was shorter than that in germline cells from the same donor. A subset of stem cells isolated from adult bone marrow showed shorter telomeres than in fetal cord blood, suggesting that a progressive decline in telomere length with age occurs in hematopoietic stem cells. Thus, transplanted HSCs derived from aged donors may reach their proliferative limit during the lifetime of the recipient, resulting in graft failure after HSC transplant. The regulation of telomere length and telomerase activity is a complex and dynamic process that is tightly linked to cell cycle regulation. Therefore, individual stem cell turnover at any given point would be minimal and the up-regulation of telomerase activity in stem cells could be minimal. It is, however, up-regulated in lineage-committed progenitor cells that undergo rapid expansion, such as committed hematopoietic progenitor cells, and activated lymphocytes. In more mature cells, repression of telomerase activity is independent of proliferation. These findings suggest that one important function of telomerase in stem cells is to reduce the rate of telomere loss during cell division, preventing premature critical shortening of telomeres and loss of telomere function. Such a role of telomerase would be critically important in the case of increased proliferative demand, such as infection and regeneration.

In T and B lymphocytes, telomerase activity appears to be up-regulated in response to mitogenically activated proliferation and progressively down-regulated in more mature subsets. Telomerase is also essential for the lifelong maintenance of telomeres in normal memory lymphocytes that have a high rate of turnover in clonal expansion. Loss of the ability to up-regulate telomerase in an antigen-specific highly differentiated memory cell leads to replicative senescence, resulting in a reduced response to reinfection. Low levels of telomerase activity have been found in some non-hematopoietic stem cells, including neuronal, skin keratinocytes, intestinal crypt, mammary epithelial, pancreas, adrenal cortex, and kidney stem cells (Fig. 1). However, some controversial data remain in telomerase activity and TERT (telomerase reverse transcriptase) expression in non-hematopoietic stem cells. The difference of the levels of telomerase activity in the their components of various organs is likely derived from the difference of their cellular turnover among organs. It has been shown that hepatocytes enter senescence due to telomere shortening in the cirrhotic stage of a wide variety of chronic human liver diseases. The replicative senescence in hepatocytes is probably in part the result of continued proliferation during 20–30 years of chronic liver disease. Chronic inflammation, the presence of growth factors, and DNA-damaging agents such as reactive oxygen and nitrogen species may also play a role in this process.

Recently, the critical importance of telomerase activity in some human stem cells has been highlighted by the discovery of the etiology of dyskeratosis congenital (DKC), stem cell failure disease. In this disease, dysfunction of the telomerase RNA template gene causes the absence of telomerase activity and premature telomere shortening, resulting in bone marrow failure, intestinal disorder, or malignancy, at <50 years of age. Positional cloning associated the affected gene in many patients with X-linked DKC, termed *DKC1*, that encodes the dyskerin protein. The association of the telomerase complex with dyskerin suggested that the DKC phenotype may be the result of altered telomerase activity. The subsequent discovery of a 3′ deletion in the gene encoding TERC in a single large family with autosomal dominant DKC confirmed that telomerase deficiency is important to the etiology of DKC, and that telomerase is important for the maintenance of telomeric cellular lifespan and replicative potential in the stem cells of human organs.

In *Terc* knockout mice complete absence of telomerase activity is tolerated without abnormality in the first few generations; however, after the fourth generation, they begin to exhibit abnormalities similar to the phenotype of human DKC. Wild-type mice derived from late generations of *Terc* +/− mice with short telomeres and positive telomerase activity also displayed a similar phonotype of DKC. These findings indicated that short telomeres are the cause of stem cell failure. In addition, the observation that the *Cdkn1a* deletion improves the stem cell function and lifespan of mice with telomere dysfunction indicates that up-regulation of ►p21 (WAF1/CIP1/SDI1) in response to shortened telomeres impairs repopulation capacity of stem cells in age-related diseases and senescence.

Aplastic anemia is generally thought to be the result of HSC damage or loss resulting in the failure of bone marrow stem cells to produce sufficient quantities of all hematopoietic lineages. Telomere length was significantly shorter in the peripheral blood granulocytes and monocytes of patients with aplastic anemia or related disorders. An inverse correlation between age-adjusted telomere length and peripheral blood counts was also observed in aplastic anemia. As in DKC-associated aplastic anemia, a report found mutations in the *TERC* gene of patients with aplastic anemia. Data from congenital disorders, like DKC and aplastic anemia, suggest that disturbed telomere maintenance may play a

role in replicative exhaustion of the stem cell pool *in vivo*, again highlighting that the disturbance of stem cell telomere maintenance is an important etiology of "stem cell failure diseases," which are age-related diseases and premature senility syndromes.

Another mechanism of telomere maintenance in human stem cells is ALT. Mesenchymal stem cells (MSCs), which can be derived from bone marrow, can differentiate into multiple mesoderm-type cell lineages including fibroblasts, adipocytes, osteoblasts, chondrocytes, and endothelial cells, as well as into non-mesoderm-type lineages including neuronal-like cells. In human MSCs (hMSCs), replicative senescence reportedly sets in rather early. Without growth factors, cells cease dividing around 40–50 population doublings, telomere length gradually shortens by 30–120 bp/PD, and telomerase activity is undetectable. However, hMSCs maintain long telomeres without the up-regulation of telomerase activity for more than 100 population doublings in culture with basic FGF. Even using highly sensitive assays, no telomerase activity has been detected in hMSCs so far, and there may be a mechanism of telomere maintenance other than telomerase, such as ALT, in hMSCs. A recent observation of subtelomeric DNA hypomethylation facilitating telomere elongation in mammalian cells suggests that such epigenetic modification of chromatin may occur in hMSCs. However, in hMSCs, overexpression of telomerase indeed resulted in the elongation of telomeres, and *TERT*-transfected cells continued proliferating when untransfected control cells ceased growth. Furthermore, the potential of telomerase-overexpressing cells to form bone *in vivo* was greatly enhanced.

Embryonic stem (ES) cells, primitive stem cells, are likely immortal and are characterized by indefinite self-renewal and the potential to differentiate and contribute to the germ line. To maintain telomere length completely, ES cells display high levels of telomerase activity and hTERT expression, both of which are rapidly down-regulated during differentiation and are much reduced or absent in somatic cells, including stem cells, in self-renewal tissues (Fig. 1). The down-regulation of telomerase activity in differentiating ES cells is reportedly tightly correlated with histone deacetylation and DNA methylation of the *TERT* gene. High telomerase activity or the expression of TERT can, therefore, be regarded as a marker of undifferentiated ES cells.

In cloned animals originating from adult nuclei with shortened telomeres, telomere length in somatic cells has been found to be comparable with that in age-matched normal animals originating from a fertilized egg with long telomeres. This finding indicates that the enucleated oocyte has the ability to reset the telomere length of the nucleus derived from a donor adult somatic cell by the elongation of telomeres. How this mechanism is "reset" remains unknown in oocytes in cloned animals. Solving this mystery would be a technical breakthrough in developing cloned animals.

In summary, because of lifelong cell turnover of stem cells, telomere length in these cells is maintained longer than in somatic cells but gradually shortens due to aging. Insufficiency of telomere maintenance mechanisms in stem cells causes "stem cell failure diseases" such as DKC.

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Stem Cells

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Definition

With the exception of hemopoietic stem cells, morphological criteria do not exist to identify stem cells in most tissues, and therefore, they are generally defined in terms of their properties. *Stemness* is not a single property but a number of properties that a cell has the capability to perform depending upon circumstances. In adult steady state renewing tissues, a stem cell is a relatively undifferentiated cell capable of proliferation and self-maintenance, producing a variety of cell lineages and capable of tissue regeneration following injury. Probably the most important property of stem cells is that of *self-renewal*.

Characteristics

Tissue Renewal and Models of Cellular Hierarchy

Many tissues in the adult undergo self-renewal, and accordingly, establish a life-long population of relatively pliable stem cells – the ▶adult stem cell. The different capacity of tissues to proliferate is the basis for conventionally categorizing tissues in adulthood into three categories:

- Those that are constantly renewing (e.g. bone marrow, intestine)
- Those that proliferate slowly but may renew their population in response to injury (e.g. lung, liver)
- Those that are more static (e.g. nerve, muscle)

Tissues are renewed by cell division and differentiation from a small number of stem cells, which have a high capacity for cell proliferation, but their actual rate of cell division is usually slow in the absence of injury or demand.

Between the stem cell and the mature cell of a particular tissue, a number of different stages of differentiation may be recognized, of which some retain a degree of stemness. Potten and Loeffler classify cells within a system into three types on the basis of their replicative potential as *actual stem cells, potential stem cells*, or *committed cells*. Actual stem cells are defined as undifferentiated cells capable of (i) proliferation; (ii) self-maintenance; (iii) production of large numbers of differentiated progeny; (iv) regeneration of the tissue after injury; and (v) flexibility in the use of their options. The potential stem cells are latent or reserve counterparts of actual stem cells, which may be reactivated to become functioning stem cells. The essential properties of *stemness* may be retained by some proliferating cells located distally in a lineage. This is referred to as the *compartment model of cellular hierarchy* and is illustrated for the intestinal crypt in Fig. 1.

Stem Cell Division

It has been proposed that a specific type of mitosis occurs in the functioning stem cells, which is responsible for the self-maintenance of this cell type. In general, normal stem cell mitosis will proceed by *asymmetrical division* to produce one daughter stem cell and one daughter that continues to divide, mature, and differentiate. However, mathematical modeling suggests that about five percent of the time, a stem cell may undergo *symmetrical division* to produce either two stem cells or two maturing cells. In the former case, a stem cell is lost from its niche by differentiation, displacement or apoptosis to ensure constancy in numbers. Promoting this form of division could be an approach to deplete mutated stem cells (probably the earliest step in carcinogenesis – see below) and may constitute an alternative strategy to inducing cell death to treat early neoplastic lesions.

Stem Cell Markers and Identification Problems

The development of a stem cell specific marker for each tissue of origin is the "holy grail" of stem cell research. Several markers and approaches have been suggested; examples are listed in Table 1, but to-date, (with the exception of hemopoeitic stem cells), there is no ideal stem cell marker for adult stem cells.

In attempting to measure stem cells, one may find oneself in a circular argument. In order to answer the question whether a cell is a stem cell, we have to alter its circumstances, and in doing so inevitably lose the original cell, and one may only see a limited spectrum of responses. This situation has a marked analogy with *Heisenberg's uncertainty principle* in quantum physics – which states that the very act of measuring the properties of a certain body inevitably alters the characteristics of that body.

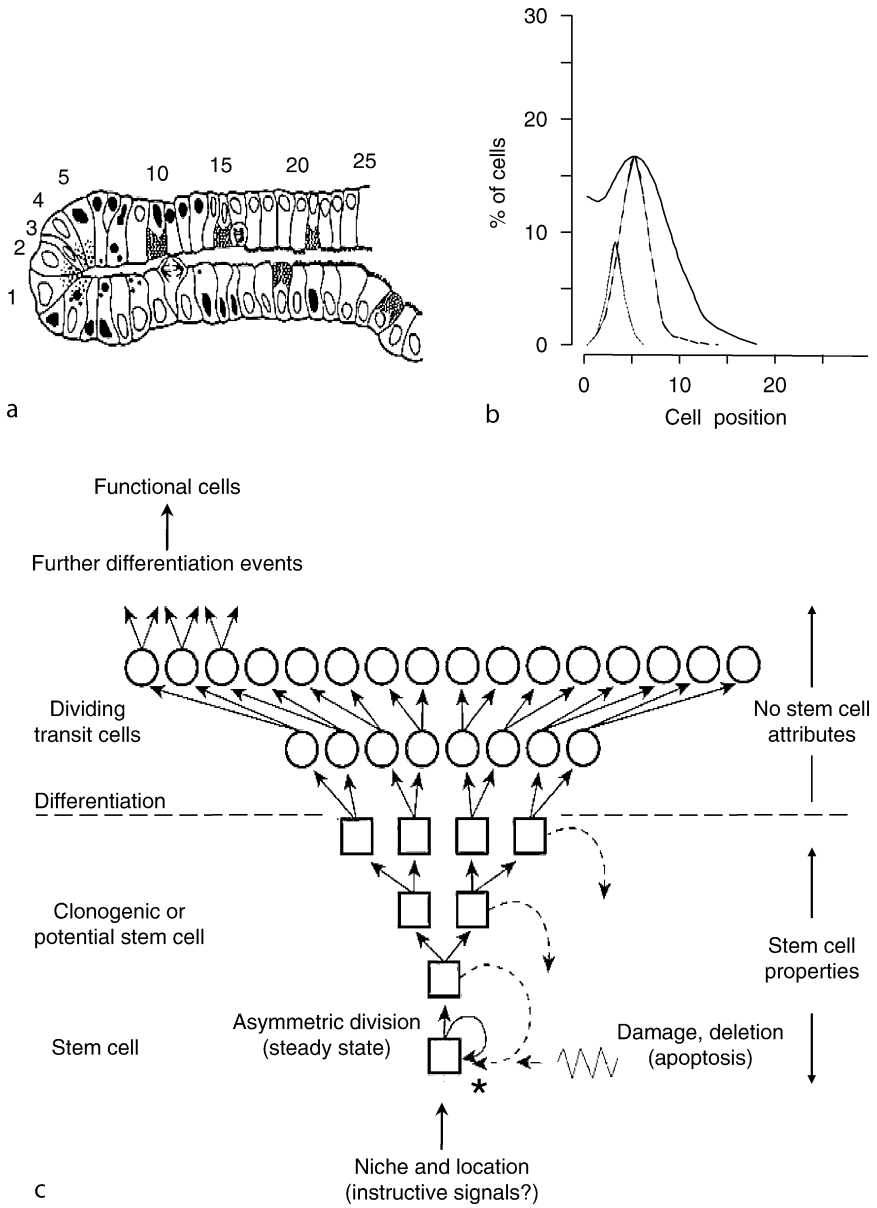
Stem Cell Location and the Niche

Adult stem cells are often localized to specific environments or *niches*. For a number of systems, these niches correspond to specific histologically identifiable locations, but these have not been determined for all tissue. Within each niche, the stem cells are influenced by neighboring cells and extra-cellular matrix. For each tissue, the stem cells in specific niches are *pluripotent* for the cell population of that tissue. Thus, for example, five different cell types (Paneth cell, goblet cell, enteroendocrine cell, M cell, enterocyte) are derived from the intestinal stem cell. Some stem cells give rise to a particular phenotype but there is heterogeneity in the cellular characteristics depending on the microenvironment – for example, blood vessels present in renal carcinoma metastases to the brain display the typical fenestrated morphology of renal vessels and not the continuous morphology of endothelial cells in invading brain vessels. Further studies demonstrated that transplanted bone-marrow cells (in sex-discordant transplant patients) may give rise to new hepatocytes in the livers of the recipients raising the possibility that human stem cells may be reprogrammed to express dormant areas of the genetic code, and thereby regenerate physically distinct phenotypes.

What remains unclear is whether stem cells are intrinsically different from daughter cells or whether they are instructed to be different by their microenvironment. In other words, could any cell behave as a stem cell if given the appropriate niche and signals – a phenomenon referred to as *plasticity*? This has implications for tumorigenesis and clinical situations such as tumor seeding and implantation. It is conceptually possible to manipulate any cell *in vitro* to behave like a stem cell but this is an unlikely scenario *in vivo*. The very early mammalian embryo (less than four cells) – the ultimate stem cells – is strong evidence favoring the argument that stem cells are intrinsically different.

The Concept of Stemness and "Immortal" DNA Strands

A recurrent theme in stem cell biology is whether stem cells are long-lived progenitors with the intrinsic capability to self-perpetuate their pluripotency, or whether "stemness" is not an intrinsic property, but rather a



Stem Cells. Figure 1 (a) Diagram of a longitudinal crypt (large intestine) section showing the cell positions. (b) The apoptosis frequency plot (radiation-induced: *solid line*) can be compared with the theoretical distribution of actual stem cells (*dotted line*), clonogenic or potential stem cells (*dashed line*) based on mathematical modeling. All these are centered round cell position four. The *continuous grey line* represents the distribution of rapidly proliferating cells which are predominantly determined by the committed dividing transit cell. (c) The current model for a three-tiered hierarchical stem cell compartment is also illustrated. There are from 4 to 6 actual stem cells per crypt, but many more cells (potential stem cells) that are capable of stem cell function. When a stem cell undergoes a commitment to differentiation, it often first enters a transient state of rapid proliferation. Upon exhaustion of its proliferative potential, the transiently amplifying cell withdraws from the cell cycle, and executes its terminal differentiation. *Approximately 5% are symmetrical divisions.

non-autonomous feature. The latter model suggests that in every adult tissue with renewal capabilities there must be a niche that determines the stem potential of cells within. Owing to the general lack of specific markers, epithelial stem cells have been traditionally identified by their ability to retain radiolabeled thymidine for long periods of time. More than 25 years ago, John Cairns proposed that stem cells selectively retain old (that is, labeled) replication error-free DNA strands while donating newly synthesized strands to their descendents

Stem Cells. Table 1 Examples of potential stem cell markers and techniques for stem cell isolation

Marker/techniques	Tissue example	Comments
CD34	Bone marrow	A $CD34^+/CD38^-$ cell surface phenotype is found in Acute Myeloid Leukemia stem cells and in normal primitive hematopoietic progenitors
►CD44	Breast	$CD44^+/CD24^-$ cell phenotype isolated from human breast tumors cause breast cancer in SCID mice
CD133 (prominin)	Liver	A transmembrane protein on stem cells from several organ sites
	Bone marrow	
	Neural	
EpCAM (CD326)	Liver	Present on hepatic stem cells but also hepatoblasts and committed progenitors. Overexpressed in many cell lines
NCAM	Liver	Present on hepatic stem cells but not any progenitor cells thereafter
	Neural	
Musashi-1 (Msi-1)	Intestinal crypts	Musashi-1 gene encodes an RNA-bind protein that is required for asymmetric divisions. It may mark intestinal stem cells but also has broader expression in the crypt.
Hes-1	Intestinal crypts	Like Msi-1, Hes1 may be expressed outside the stem cell region of the crypt
SOX2	Neural stem cells	SOX2 is expressed in multipotent neural stem cells at all stages of development
Stem-cell antigen (Sca-1)	Breast	Sca-1 positive cells demonstrate enhanced regenerative potential
^{3}H-thymidine retention	Breast Intestine	Stem cell retain DNA synthesis incorporated label over a long period (see Fig. 2)
Electron microscopy	Breast	Identification of large light cells (ULLC) and small undifferentiated light cells (SLC) justaposed suggest they result from an asymmetric single mitotic event
Hoechst dye side population (SP)	Breast	SP mammary cells are similar to SLC cells and are highly enriched for Sca-1 expression
Laser capture micro-dissection (LCM)	Intestinal crypts	LCM was followed by DNA microarray analyses, which demonstrated increased expression of cell proliferating genes
Gene profiling	Skin	Examples include Wnt inhibitors (sFrp1, Dkk2, Wif1); cell cycle inhibitors (Gas1, Ak1, Inhbb), and TGF-β signaling components

EpCAM, epithelial adhesion molecule; NCAM, neural cell adhesion molecule; Hes-1, a homeobox gene expressed by murine embryonic stem cells; SOX2, a homeobox gene transcription factor derived from murine embryonic stem cells.

which will be lost from the tissue after a short time. Although this has long been controversial, recent work demonstrates asymmetric segregation of chromatids in stem cells of small intestine using specific labels for new and old chromatids (Fig. 2). Retention of old chromatids by stem cells has also been demonstrated using *in vitro* models and in *in vivo* breast stem cell systems. The existence of "immortal" DNA strands has implications for understanding the lifespan and mutagenesis dynamics of stem cells, but it also implies that certain stem cell properties are maintained or inherited autonomously throughout adulthood.

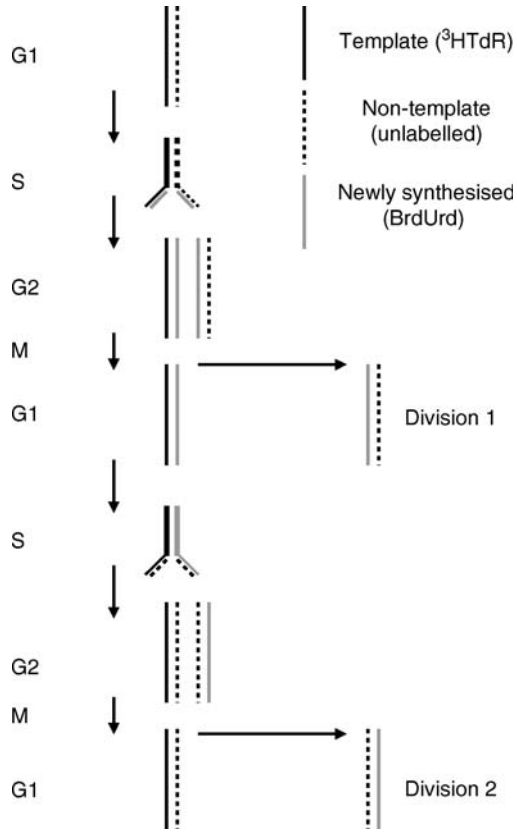
Regulation of Stem Cell Numbers

Until recently, little was understood about the molecular regulation of stem cells. Over the past 5 years, several genes and signaling pathways have been shown to have important regulatory functions for some stem cells. Three key gene systems are ►*Wnt* (Wingless), *Shh* (Sonic ►Hedgehog), and ►*Notch*, and their associated tissue stem cells are listed in Table 2. However, as these genes frequently operate in other cell types, they cannot be called "stemness" genes.

Properties of Stem Cells Which Favor Tumorigenesis

There is a general hypothesis in cancer biology that most cancers arise from a mutated stem cell and are there are a number of characteristics of stem cells which support this hypothesis. These are as follows:

- Capacity for self-renewal and progenitor production via asymmetric cell division



Stem Cells. Figure 2 Diagram showing the segregation of template and newly synthesized DNA strands in one chromosome. The Cairns' hypothesis proposed that all the chromosomes would behave in this way. The template strands are selectively retained by the stem cell daughter of a cell division, whereas the newly synthesized strands are segregated to the daughter cell destined to enter the dividing transit compartment and be shed from the tissue after a few days, thus removing any replication-induced errors. Label introduced into the newly synthesized strands takes two divisions to be removed from the stem cells. Label in the template strand would persist in the stem cell line.

Stem Cells. Table 2 Signaling pathways that regulate stem cells

Signaling pathway	Stem/progenitor cell self renewal	Implicated in tumorigenesis
Wnt	Hematopoietic	Colon carcinoma
	Epidermal	Epidermal tumors
	Intestinal	
Shh	Hematopoietic	Medullablastoma
	Neural	Basal cell carcinoma
	Germ line	
Notch	Hematopoietic	Leukemia
	Neural	Mammary tumors
	Germ line	

Wnt, wingless; Shh, Sonic hedgehog.

- Regulated by a niche – conceivably, disruption of this host control may lead to aberrant expansion of the stem/progenitor cells and cancer initiation
- Long-lived allowing time to accumulate multiple mutations
- Ability to generate multiple lineages via downstream differentiation
- Active telomerase expression
- Activation of anti-apoptotic pathways (high ►BCL-2 and inhibitors of ►apoptosis (IAPs) proteins)
- ►Anoikis resistance allowing survival during dissemination
- Ability to undergo ►migration
- Increased membrane transporter activity, which may in turn be a mechanism of drug resistance
- Distinct signaling pathway patterns including Wnt, Notch, Snail, Twist, and Hedgehog

Evidence that Human Tumors Arise from Stem Cells

Additional evidence that most tumors arise from stem cells comes from the following:

- Many cancers arise in tissues where self-renewal is essential (e.g. skin, gut, bone marrow).
- For the most part, human tumors are monoclonal, suggesting that they arise from a single transformed cell.
- Tissue-specific differentiation – most human tumors contain cell types consistent with an origin from the stem cells of that tissue.
- Changes in stem cell regulation mechanisms occur commonly and early during tumorigenesis.
- Bio-mathematical modeling supports the concept that tumors arise from mutated stem cells.

Cancer Stem Cells

Through increased understanding of the molecular biology of embryonic organ development and self-renewing adult tissues, a clearer idea is emerging that a unique population of stem cells – cancer stem cells (CSC) – may be responsible for the maintenance of tumor growth (Fig. 3), and that these cells are inherently resistant to standard treatments. These concepts are not new, being first promulgated by Cohnheim in 1875. However, recently, a number of investigative teams have been able to successful isolate subpopulations of human cancer cells with dramatically enhanced tumorigenic capacity when transplanted into immuno-deficient mice.

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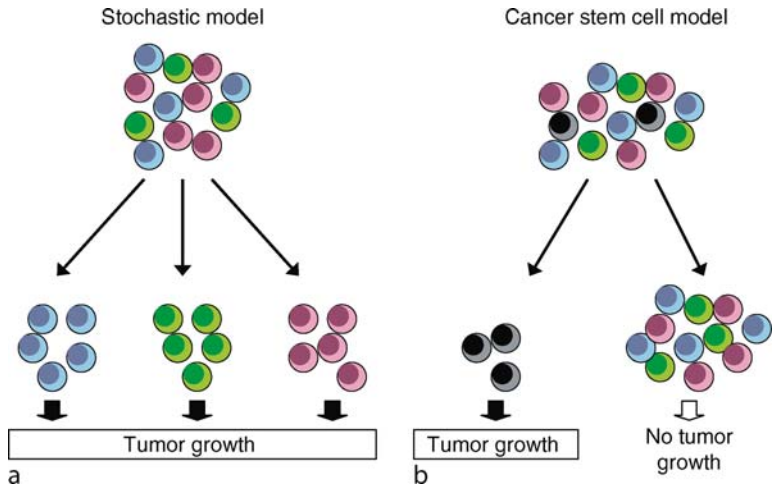
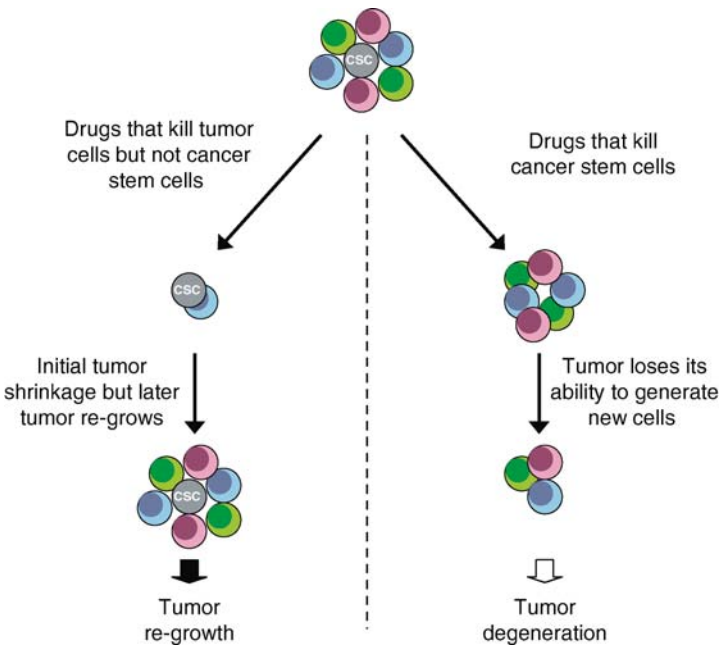


Stem Cells. Figure 3 Cartoon of two common models of tumor growth. In the stochastic model (a), tumor cells are heterogeneous with every cells having a low but equal probability with time of proliferating and forming new tumors. According to this model, the genetic changes leading to cancer development and progression are operative in all cells within the tumor. In the cancer stem cell model (b), tumor cells are heterogeneous but most cells have only limited potential to proliferate and only a small subset – the cancer stem cells (*grey/black shaded* cells) – have the ability to initiate new tumors.



Stem Cells. Figure 4 Conventional therapies (*left-hand side* of panel) may shrink tumors by killing mainly cells with limited proliferation potential. If the putative cancer stem cells (CSC) are less sensitive to these therapies, then they will remain viable after therapy and re-establish the tumor. By contrast, if therapies can be targeted against cancer stem cells (*right-hand side* of panel), then they might more effectively kill the CSC, rendering the tumor unable to maintain themselves or grow.

This has now been successfully demonstrated for ►acute myeloid leukemia, ►breast cancer, ►colon cancer, ►brain tumors and ►pancreatic cancer. However, we do not know with certainty whether or not CSCs are derived from normal stem cells.

As an extension of the CSC model, recent studies have postulated that migrating non-proliferating stem cells, located at the tumor invasion front, exhibit ►epithelial to mesenchymal transition, which may contribute to tumor ►metastases.

Clinical Implications of Cancer Stem Cells

The emergence of a molecular understanding of CSCs will have major implications for how we view conventional anti-cancer treatments, and how we develop novel anti-cancer strategies (Fig. 4). Conventional therapies may shrink tumors by killing mainly cells with limited proliferation potential. If the putative CSCs are less sensitive to these therapies, then they will remain viable after therapy and re-establish the tumor. By contrast, if therapies can be targeted against CSCs, then these might more effectively kill the CSCs, rendering the tumor unable to maintain themselves or grow. Thus, even if cancer stem cell-directed therapies do not shrink tumors initially, they may eventually lead to cure.

A fascinating question, at present, is whether or not stem cells express the molecules that clinicians are currently targeting such as estrogen receptors, ►HER-2, and ►Epidermal Growth Factor Receptor (EGFR). The major signaling pathways so far identified in stem cells are often mutated in cancers but they are not, by and large, the focus of current molecular treatments. Stem cell regulatory pathways (as outlined above) are thus the potential unexploited anti-cancer targets of the future.

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Stem-like Cancer Cells

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Synonyms

Cancer stem-like cells; Cancer stem cells; CSC; Tumor progenitors; Tumor-initiating cells; TIL; Tumor-reinitiating cells; Tumor-repopulating cells

Definition

Cancer stem cells (CSC) are functional rather than having a fixed definition. ►The least stringent definition would be that the prospectively purified CSC population is more tumorigenic than the bulk or the marker-negative tumor cell population(s) in a suitable tumor development assay. Using ►the most stringent definition, a cancer stem cell should be a cell that, at the single-cell level, can reconstitute, in a recipient animal, a tumor that is identical to the parental patient tumor and that can be serially xenotransplanted indefinitely. Therefore, in a strict sense, none of the CSC thus far reported can be truly classified as CSC and should more appropriately be called tumor-reinitiating cells. In reality, it will be very difficult to identify a tumorigenic cell that can fulfill the most stringent definition of a cancer stem cell mentioned above. Firstly, a tumor, especially a solid tumor, is made of numerous cell types. To expect one cell or even a population of cells, when transplanted into a foreign host (i.e., mice) in an exotic environment, to fully reconstitute an original patient tumor in its complete composition is very difficult and essentially impossible to prove. Secondly, when such experiments are actually done, the best one can do is to coinject the putative tumorigenic cell population with stromal components (e.g., fibroblasts, carcinoma-associated fibroblasts or CAFs, urogenital sinus mesenchyme or UGM, etc.) in an extracellular matrix (e.g., Matrigel or collagen) into an "orthotopic" animal site such as brain, mammary fat pad, or prostate lobes. These so-called orthotopic sites are considerably different from their human counterparts and tumor establishment would inevitably require the recruitment of various host (i.e., mouse) cells by the tumor-reinitiating cells. Such "reconstituted" tumors could never be identical to the original patient tumors. Thirdly, during the purification process, the majority of cells are often discarded to obtain marker-positive and -negative populations. These discarded cells would be important in the original tumor composition but they would be very difficult to reconstitute in tumor development assays. Altogether, one can say, at the very best, that the experimental tumor reconstituted from the presumptive CSC histologically "resembles" the (patient) primary tumor.

Therefore, we probably have to seek a middle ground when making the claim to a cancer stem cell. A ►functional definition of CSC is as follows. First, the presumptive CSC (i.e., the cell population enriched in putative CSC) must be prospectively purified from, e.g., cell cultures, xenografts, and/or primary tumors. When purifying candidate populations of CSC from tumors, lineage selection must be performed to remove "irrelevant" cells such as stromal and blood cells that may contain other ►stem cells (SC) including mesenchymal and hematopoietic SC, which might have the ability to undergo transdifferentiation. Second, in vivo

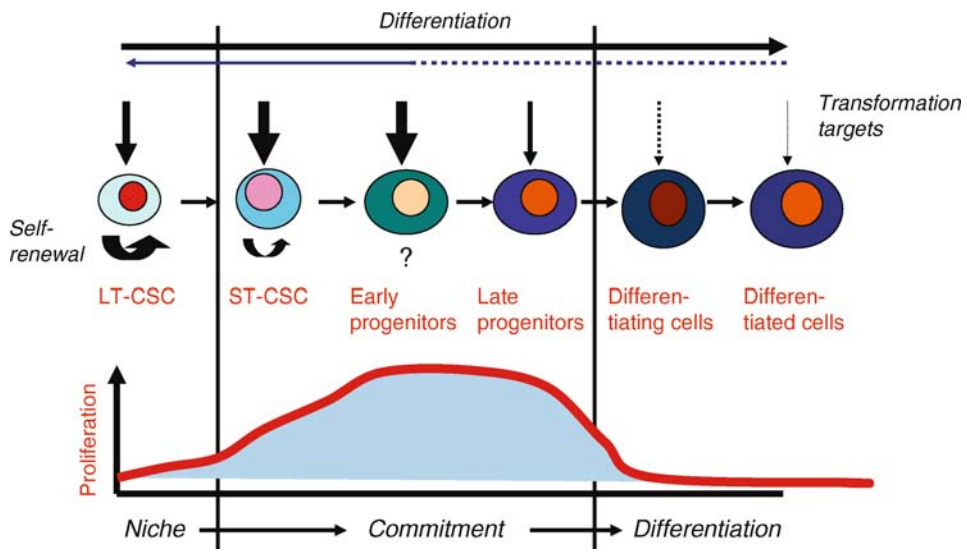
tumorigenicity experiments must be done to show that such cell populations are enriched in tumor-reinitiating cells. When feasible, serial tumor xenotransplantation should be carried out to determine whether the tumors derived from the putative CSC can be transplanted for multiple generations. Histologically, the reconstituted as well as serially xenotransplanted tumors should resemble the original tumor. Third, importantly, the presumptive CSC population, or a subpopulation within, has to be studied to show that they possess certain intrinsic biological properties normally associated with SC elaborated below. Only when these conditions are fulfilled can one confidently claim that the candidate population of tumor cells under investigation is enriched in potential CSC or tumor-reinitiating cells. Importantly, even such tumorigenic populations are likely heterogeneous with true CSC representing perhaps only a very small fraction.

Characteristics

CSC identified in different types of tumors or the same type of tumor but from different patients may manifest very different biological and functional properties. On the other hand, all CSC are expected to share certain common properties such as relative dormancy but with extended proliferative capacity, significant clonogenic potential, preferential expression of stem cell-related genes (e.g., self-renewal genes), the ability to undergo asymmetric cell division, and enhanced in vivo tumorigenic and ►metastatic potentials.

Theoretically, tumor cells in any cancer, depending on the differentiation status, may be organized as a hierarchy that can be roughly classified as long-term CSC (LT-CSC), short-term CSC (ST-CSC), early and late tumor progenitors, and differentiating and differentiated tumor cells (Fig. 1). The LT-CSC should possess indefinite self-renewal and ST-CSC some self-renewal properties. The early tumor progenitors may or may not possess any self-renewal activities. The LT-CSC, localized in putative CSC niche, likely proliferate slowly although these cells possess the highest proliferative potential. By contrast, the cell types that constitute the bulk of the proliferating cell compartment in a tumor are mostly tumor progenitors and ST-CSC (demarcated by two vertical lines, Fig. 1). Differentiating and differentiated tumor cells, which should express tissue-specific differentiation markers, are hypothesized to lack proliferative capacity and self-renewal in vivo. In theory, both CSC (LT- and ST-CSC) and tumor progenitors should have the ability to regenerate tumors in tumor development assays. In principle, however, tumors initiated by CSC should be able to be passaged indefinitely whereas tumors initiated by tumor progenitors can only be propagated for a limited number of times.

Putative CSC are often thought to derive from normal SC, which may or may not be the case. In fact, because normal progenitor cells are the major proliferating cells in a tissue or an organ and they may possess certain self-renewal abilities or they may acquire self-renewal abilities by the transforming events (e.g., silencing of p16, mutations of ►PTEN, etc.), these cells are more likely the targets of initial tumor transformation (Fig. 1). In other words, transformed tissue progenitor cells may



Stem-like Cancer Cells. Figure 1 This figure presents a hypothetical model of tumor cell hierarchy. LT-CSC localized in putative niches make the commitment to develop into ST-CSC and tumor progenitors, which in turn differentiate into "functional" cell types such as PSA-producing prostate cancer cells. In most tumors, tumor progenitors and ST-CSC may constitute the bulk of proliferating cell compartment and their normal counterparts may represent the major transformation targets. See text for more descriptions.

actually be the real CSC. Alternatively, the initial tumor transformation may occur in normal SC but further genetic mutations and/or epigenetic alterations take place in more mature tumor progenitor cells, which thus represent the real CSC that drive tumor formation, ►progression, and recurrence. Both of these concepts have been recently born out in acute and chronic myelogenous leukemia (AML and CML). Finally, although most differentiated cells are thought not to be able to "dedifferentiate" (i.e., going back along the lineage development; Fig. 1), some somatic cells such as hepatocytes and endothelial cells are known to be able to replicate themselves. Therefore, some differentiated cells may also become transformation targets and subsequently become CSC.

Identification

Putative CSC have now been reported in multiple human tumors including AML, CML, multiple myeloma (MM), ►brain tumors (glioblastoma, medulloblastoma, etc), ►breast tumors, ►melanoma, and ►prostate cancer, ►colon cancer, and ►gastric cancer. In principle, CSC can be identified by several experimental strategies.

1. *Marker-Based Analysis*. A variety of adult tissue SC are found to express relatively specific markers, which can be cell surface or intracellular such as nuclear. Interestingly, many reported CSC also seem to express the cell surface markers that identify their normal counterparts. This observation provides a relatively simple enrichment procedure utilizing either flow cytometry-based cell sorting or microbeads-based affinity purification. For nuclear markers, a marker promoter-driving reporter construct such as GFP-tagged retroviral or lentiviral vector system can be developed to track down putative CSC and then purified by flow cytometry. The disadvantages associated with using predetermined marker(s) to identify CSC include that the marker proteins frequently change during cell development in vivo and cell preparation in vitro and that in most cases the functions of "stem-cell" markers in both normal SC and stem-like cancer cells are unclear.
2. *Side Population (SP) Analysis*. Mouse hematopoietic SC are found to preferentially express multidrug resistance (MDR) family proteins such as MDR1 and other membrane transporters such as ABCG2 (also called BCRP for breast cancer resistance protein). This property allows the HSC, in an experimental setting, to pump out the Hoechst 33342 dye. Therefore, on dual-wavelength flow cytometry, the HSC-enriched cell population is identified as a "side" or tail Hoechst^{dim} population at the lower left quadrant of the histogram. By contrast, the major population of cells, devoid of HSC, is displayed as Hoechst^{hi} cells called as non-SP or main population (MP). Recent work reveals that multiple adult tissue SC can also be enriched by the SP protocol and that the SP from several cancer types are also enriched in stem-like cancer cells. The major advantage of this technique when used to identify putative CSC is its simplicity. The potential problems associated with the technique are that chronic accumulation of Hoechst dye in non-SP cells may be cytotoxic (thus invalidating suitable controls) and that SP cells isolated from some normal tissues seem to be enriched in progenitor cells rather than SC.
3. *Sphere-Formation Assays*. Many normal SC such as neural, hematopoietic, and mammary SC, when maintained under special culture conditions, can form three-dimensional spheres, which are like miniorgans that can differentiate into multiple cell types. Putative CSC identified in brain and prostate tumors as well as in melanoma also have the ability to form anchorage-independent spheres. The advantage of using sphere-forming assays to enrich for CSC is its initial independence of specific markers. The disadvantages include the empirical nature of finding culture conditions suitable for sphere formation and the necessity of finding ways later to identify and purify the real CSC from the spheres.
4. *Label-Retaining Properties*. Mammary SC and normal keratinocyte SC in interfollicular epidermis and hair follicle bulges are quiescent and can be identified with a pulse label with the thymidine analog BrdU (bromodeoxyuridine) followed by a long-term "chase" (i.e., removal of the label). Fast-proliferating progenitor cells dilute out the BrdU label after several cell divisions whereas the slow-dividing SC retain BrdU and thus identified as "label-retaining cells" or LRCs by either immunohistochemistry or flow cytometry analysis. Recently, the LRCs purified from the bulge regions in transgenic mice indeed are found to be enriched in SC based on both gene expression profiling and functional assays. Interestingly, the LRCs in human breast tumors coexpress mammary epithelial SC markers and seem to have certain SC properties. Human prostate cancer cell spheres and xenograft tumors also possess slow-cycling LRCs. These tumor LRCs are yet to be prospectively purified out to show that they indeed represent slow-cycling CSC.

Clinical Implications

CSC are thought to have high levels of prosurvival mechanisms such as heightened expression of anti-apoptotic ►Bcl-2 family proteins (including Bcl-2, Bcl-xL, and Mcl-1), ►telomerase, and antioxidant and detoxifying enzymes. In addition, they preferentially express cell surface pumps such as ABCG2 and MDR1. Furthermore, they generally proliferate much slower

than the progenitor cells. Most current anticancer therapeutics targets fast-proliferating tumor progenitor cells. All these together lead to relatively transient therapeutic efficacy as CSC will most likely survive therapeutics due to their relative dormancy, increased survivability, and enhanced capacity to efflux therapeutic agents. CSC, once mobilized to the cell cycle, may regenerate a less differentiated tumor with expanded CSC and tumor progenitor cell compartments and result in drug-resistant tumor progression and ►metastasis. There is emerging evidence that CSC are more resistant to therapeutic regimens.

CSC, due to their different genetic makeups and signaling requirements than in their normal counterparts, might be preferentially targeted. For instance, leukemia SC seem to show some differences in the expression of surface marker repertoire compared to normal hematopoietic SC. Therefore, these different markers may be taken advantage of for the antibody-based therapeutics. CSC with mutant ►p53 and PTEN tumor suppressors may be particularly more sensitive to apoptosis induction induced by restoration of p53 and PTEN expression/functions. Leukemic SC are recently shown to be extremely sensitive to cell death triggered by BH3 mimetics, presumably because of their heavy reliance on Bcl-2 and Mcl-1 for their survival. Finally, normal neural and mesenchymal SC have been employed alone or as vehicles to carry cytotoxic cytokines or gene products to specifically target CSC in certain tumors such as ►glioblastoma multiforme.

Stent

Definition

A synthetic (usually plastic or alloy mesh) tube inserted into a blood vessel, ureter or segment of intestine to prevent or treat blockage.

►Desmoid Tumor

Steradian

Definition

The SI unit (international system of units) for solid angle.

►Bioluminescence Imaging

Stereocenter

Definition

A carbon atom with four distinct functional groups.

►Small Molecule Screens

Stereotactic Radiosurgery

Definition

A radiation therapy procedure that uses special equipment to position the patient and precisely deliver a large radiation dose to a tumor and not to normal tissue. Synonym radiation surgery, radiosurgery, stereotactic external-beam radiation and stereotactic radiation therapy.

►Glioblastoma Multiforme

Sterile-α-Motif

SAM domains are a class of protein modules characterized by a globular structure consisting of five a-helices and can mediate interactions with other proteins, RNA, and lipids.

Steroid Hormone Receptor Coactivators

Definition

Are defined as cellular factors that do not bind DNA directly but are recruited to the promoters by steroid hormone receptors. These promoters enhance agonist-dependent transcriptional activation by facilitating transcription initiation through interaction with components of the basal transcription machinery. Coactivators also enhance transcriptional activity of SHR by other mechanisms such as modulating alternative RNA ►splicing.

►Progestin

Steroid Hormone Receptors (SHR)

Definition

Members of a superfamily of nuclear receptors that function as ligand- or hormone-dependent transcription factors. The term superfamily defines a set of genes derived from a single progenitor gene that has diverged to produce the unique functions of its members.

►Progestin

Steroid Receptor Coactivator-3

►Amplified in Breast Cancer 1

Steroid-Refractory Graft-Versus-Host Disease

Definition

A potentially fatal bodily condition that results when T cells from a tissue or organ transplant, especially a bone marrow transplant, react immunologically against the recipient's antigens, attacking cells and tissues (refractory to steroids).

►Rituximab

Steroid Sulfatase

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Synonyms

Aryl sulfatase C; Steroid sulfohydrolase; Steryl sulfohydrolase; STS

Definition

Steroid sulfatase (STS) is an enzyme which is virtually ubiquitous throughout the human body and its activity is implicated in a range of physiological processes and pathological conditions. Its prime responsibility is for the hydrolysis of alkyl (e.g. dehydroepiandrosterone sulfate (DHEAS)) and aryl steroid sulfates (e.g. estrone sulfate (E1S)) and therefore plays an essential role in regulating the formation of steroids such as DHEA and E1 which can be converted to biologically active steroids (e.g. androstenediol (Adiol) and ►estradiol (E2)).

Characteristics

The activity of STS was first discovered in rat liver microsomes. Now it is known to be active in a plethora of tissue types, including testis, ovary, placenta, skin, lung, brain and bone. Therefore it is thought to be found in small quantities throughout the body. It is a member of a superfamily of 12 different mammalian sulfatases. The gene for the human STS is located on the distal short arm of the X-chromosome. The actions of this enzyme make a large contribution to the in situ estrogen production in hormone-dependent malignant tissues. Little is known about the regulation of STS gene expression or enzyme activity. However, the expression levels of mRNA and enzyme activity are increased in cancerous breast and endometrial tissue compared to normal tissues. During the last decade there has been a significant amount of research carried out to elucidate the role that STS plays in the conversion of steroid sulfates, such as DHEAS and E1S, and the consequent formation of biologically active steroids, such as androstenediol and E2.

The highest incidence of breast cancer occurs in postmenopausal women after the cessation of the production of ovarian estrogens. However, estrogens are still produced by the local conversion of androstenedione to E1, a reaction that is catalyzed by the ►aromatase enzyme. A major proportion of the estrogens that are formed are available for conversion by estrone ►sulfotransferase into E1S which is biologically inactive because it is unable to bind to the ►estrogen receptor (ER). However, steroid conjugates, because they bind to albumin, have a much longer half-life in the plasma (about 9 h) compared to the unconjugated estrogens and are also found in circulating blood at greater concentrations. This acts as a potential reservoir for the formation of biologically active estrogens, e.g. E1, via the actions of STS (see STS figure). The E1 thus formed is further metabolized by 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 to E2 which is the most biologically active natural steroid that can interact with the estrogen receptor (ER) and stimulate tumor cell growth. In postmenopausal women, the production rates of E1 and E2 are ~40 μg/day and 6 μg/day respectively.

There is another route via which STS may influence the growth of some tumors. There is evidence that

DHEAS and its unconjugated metabolite DHEA, stimulate cancer development. These steroids are able to act as precursors for the formation of steroids with estrogenic properties, such as Adiol. Furthermore, various studies have shown that DHEAS, DHEA and Adiol all have the ability to increase proliferation of breast cancer cells in vitro and are capable of stimulating the growth of carcinogen-induced mammary tumors in vivo. The most abundant of these steroids is DHEAS which is secreted by the adrenal cortex. Removal of the sulfate group on DHEAS by STS results in the formation of DHEA which can undergo a further reduction to make Adiol (see STS figure). In postmenopausal women, a significant proportion of Adiol produced comes from DHEAS and DHEA in the peripheral tissues. The importance of Adiol becomes evident when it is known that it can bind to the ER, albeit with a slightly lower affinity than E2. However, as Adiol plasma concentrations are reported to be about 100-fold higher than those of E2 in postmenopausal women, its significance in the development of cancer should not be underestimated.

Regulation of STS

There has been little research carried out as to how the STS enzyme is regulated in situ. It is thought that the cytokines ►interleukin (IL)-6 and tumor necrosis factor (TNF)-α can act synergistically to increase STS activity in some breast tissue and cancer cells. Conversely, IL-1β, an inflammatory cytokine, is capable of reducing STS activity and mRNA levels in human endometrial stromal cells, vascular smooth muscle and human aortas. The presence of these cytokines in breast cyst fluid may explain the differential regulation of STS in breast cancer cell lines by breast cyst fluid. Also, basic ►fibroblast growth factor and ►insulin-like growth factor type I (IGF-I) are known to increase STS activity in some breast cancer cells. This indicates that these growth factors, which are released by malignant breast tissue, can elevate local estrogen levels.

Other steroids are also thought to influence the expression of STS mRNA and subsequent activity. The use of exogenous testosterone treatment in the male mouse causes an induction of STS. The uteri of pregnant guinea pigs have greater levels of STS activity compared to fetal or mature females which implies an estrogenic regulation. The substrate for STS, E1S, is thought to increase STS activity in the liver of ovariectomized rats. Conversely, a reduction in STS mRNA levels was found when breast cancer cells were treated with the ►progestagen, Promegestone. However, exposure to the same cells with a different progestagen stimulates STS activity in these cells. Therefore, it seems likely that STS expression and activity can be regulated by other steroids suggesting various feed-back mechanisms, which are still poorly understood, are active.

STS in Hormone-Dependent Breast Cancer (HDBC)

There is increasing evidence to support the importance of STS in human breast cancers. Breast tumor tissue of postmenopausal women can have as much as ten times the estrogen levels than are seen in the plasma of the same patients. Furthermore, STS activity has been shown to be at least 50 times greater in both pre- and postmenopausal breast tumors compared with normal breast tissue. STS expression is detected in 90% of breast tumors, whereas aromatase expression is only found in 60–70%, and activity of STS in breast tumors is much higher than that of the aromatase complex. This increased STS activity could account for as much as a tenfold greater amount of E1 originating via the STS route than via the aromatase pathway.

Real time RT-PCR experiments have demonstrated that STS mRNA expression in malignant breast tissue is significantly higher than that in normal tissue. Clinical studies have now shown that STS mRNA expression may be a predictor of recurrence in breast cancer patients and that this association and prognosis only applies to ER +ve tumors. Significantly, elevated STS mRNA expression is associated with a poor prognosis in both pre- and postmenopausal women. This indicates that even in premenopausal women intra-tumoral estrogen synthesis could play a major role in the growth of breast tumors. The over-expression of the aromatase enzyme has also been examined as a potential prognostic marker as it is also supposed that its actions are pivotal in regulating tumor estrogen synthesis. However, it was found to have no prognostic value. Therefore, it is possible that the STS pathway may be more important than the aromatase pathway for the production of biologically active estrogens in the tumor. STS mRNA levels have been shown to correlate with tumor size and to be elevated in some tumors exhibiting metastasis compared to non-metastatic cancers.

STS Inhibitors

The importance of STS in the development of various cancers has led to the design and synthesis of a range of STS inhibitors. The main therapeutic focus for these compounds has been targeted on breast cancer in postmenopausal women. These patients are initially treated with standard endocrine therapy, such as antiestrogens, or more recently, aromatase inhibitors. Unfortunately, many breast tumors will fail to respond to these therapies making the use of STS inhibitors a potential option for therapeutic intervention. STS inhibitors come under three main categories:

1. *Alternative substrates*. These compounds (e.g. DHEAS, pNPS, MUS, flavone and isoflavone

sulfates), which contain at least one sulfate group in the structure, are designed to compete with E1S for binding to the active site of STS and, as a consequence, impede the hydrolysis of the natural substrate to E1. However, they are in principle alternative substrates for STS and hence the value of using these agents clinically in the treatment of HDBC is limited.

2. *Reversible inhibitors.* Reversible STS inhibitors are mainly E1 derivatives that are designed to compete with E1S for the enzymes active site but remain metabolically stable by not acting as potential substrates.
3. *Irreversible inhibitors.* The majority of STS inhibitors reported to date belong to this class of inhibitor. EMATE (estrone-3-*O*-sulfamate), the very first highly potent STS inhibitor, inhibits STS in a time- and concentration-dependent fashion. Its sulfamate group (OSO_2NH_2) was originally designed to mimic the sulfate group of E1S. However, despite EMATE being orally active and highly potent, it is not considered as a suitable agent for HDBC therapy because it was found to be strongly estrogenic in rodents. As a result, non-steroidal STS inhibitors were developed, of which STX64 (BN83495, 667COUMATE) has shown excellent efficacy in various in vivo tumor models and also, promising results in a recent Phase I trial in patients with advanced breast cancer. This "proof of concept" provided by STX64 strengthens the role of STS inhibitors in the treatment of HDBC.

There is still a considerable amount of research to be done on establishing the roles of STS in hormone-dependent cancer, mainly in the breast and prostate. The availability of many structurally diverse STS inhibitors may further assist in this work. While STX64 is the first and most advanced development STS inhibitor to date, the considerable therapeutic benefit of STS inhibitors on patients with hormone-dependent malignant tumors should further encourage the development of second-generation inhibitors.

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Steroid Sulfohydrolase

►Steroid Sulfatase

Steroidogenesis

Definition

Refers to the synthesis of steroid hormones such as estradiol, testosterone, and mineralcorticoids.

►Estradiol

Steryl Sulfohydrolase

►Steroid Sulfatase

Stevens-Johnson Syndrome

Definition

►Erythema Multiforme Major.

STI-571

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Synonyms

CGP57148; Gleevec; Glivic; Imatinib; Signal Transduction Inhibiotor-571

Definition

STI-571 is an oral drug marketed by ►Novartis, and is currently used for the treatment of certain types of

►cancer(s), including chronic myeloid ►leukemia (CML) and gastrointestinal stromal tumors (GISTs).

Characteristics

Physicochemical Properties

STI-571 is a small compound belonging to the phenylaminopyrimidine class of compounds (Fig. 1). It is chemically designed as 4-[(4-methyl-1-piperazinyl) methyl]-N-[[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamidine methanesulfonate, with a molecular formula of $C_{29}H_{31}N_7O{\cdot}CH_4SO_3$. Its molecular weight is 589.72.

Pharmacology and Pharmacokinetics

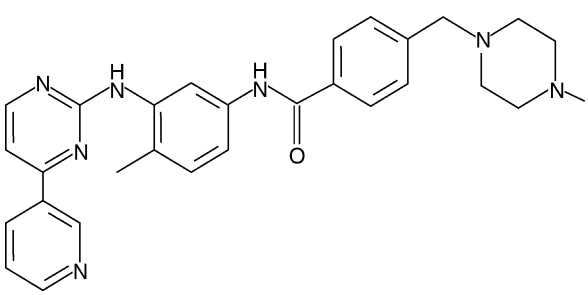
Protein kinases are an important group of ►enzymes that activate a range of substrates through phosphorylation. According to the nature of the phosphorylated –OH group in their substrates, they are classified as protein-serine/threonine kinases, or protein-►tyrosine kinases; in addition, a small group of dual specificity kinases catalyze phosphorylation of both threonine and ►tyrosine. Mutation or dysregulation of these enzymes is a frequent cause of the excess proliferation and/or reduced ►apoptosis of cancer cells. Therefore, protein kinases have become important therapeutic targets.

In humans, at least putative 518 protein kinase genes have been identified, with 385 coding serine/threonine kinases, 90 coding tyrosine kinases and 43 coding tyrosine kinase-like proteins.

STI-571 selectively inhibits certain types of protein-tyrosine kinases, including ►stem cell factor receptor (KIT), ►platelet-derived growth factor receptors (PDGFRs) (PDGFRα and PDGFRβ), ABL and its oncogenic form ►BCR-ABL. These tyrosine kinases catalyze the following reaction by binding adenosine triphosphate (ATP) and transferring phosphate from ATP to tyrosine residues on various substrates:

$$\mathrm{MgATP}^{1-} + \mathrm{Protien} - \mathrm{OH} \rightarrow \mathrm{Protein} - \mathrm{OPO}_3^{2-} + \mathrm{MgADP} + \mathrm{H}^+$$

STI-571 functions by competitively blocking the ATP binding site in these tyrosine kinases, thereby inhibiting their activities. The drug has either no or minimal effects on more than 30 other intensively studied protein kinases.



STI-571. Figure 1 Structure of STI-571.

Data from pharmacokinetic studies in healthy subjects and over 900 patients have shown that STI-571 is well-absorbed after oral administration. Following a single 400 mg dose of STI-571, a peak plasma concentration of 1.9 ± 0.4 μg/ml is reached within 3 h. After multiple dose administrations on a once-daily schedule, the plasma peak concentration reaches a level of 4.4 μM (2.6 ± 0.8 μg/ml). The steady-state plasma trough concentration 24 h after administration of 400 mg daily is over 1 μM. This trough concentration exceeds that required for inhibition of the cellular tyrosine phosphorylation activities of Kit, PDGFs and BCR-ABL in *in-vitro* assays (50% inhibitory concentration of STI-571 ranges from 0.1 to 0.25 μM). When the daily dose is increased to 600–800 mg, a steady-state plasma peak concentration as high as 13 μM can be reached. Mean absolute bioavailability is 98%.

Following oral administration in healthy volunteers, the half-lives of STI-571 and its major active metabolite (the N-desmethyl derivative) are ~18 and 40 h respectively. At clinically relevant concentrations, ~95% of STI-571 binds to plasma proteins (mainly albumin and α_1-acid glycoprotein).

CYP3A4, a ►cytochrome P450, is the major enzyme responsible for metabolism of STI-571. The main circulating active metabolite in humans is the N-demethylated piperazine derivative which has an *in-vitro* potency similar to that of the parent STI-571. Elimination is predominately via bile, mostly as metabolites (75%). After an oral ^{14}C-labeled dose of STI-571, ~81% is eliminated within 7 days, with a faecal to urinary excretion ratio of 5:1. Of the unchanged STI-571, 20% is excreted in urine, and 80% in feces.

Clinical Applications

Chronic Myeloid Leukemia (CML)

CML is a ►clonal hematopoietic malignancy, with an annual incidence of 1–1.5/100,000. The median age of onset is 45–55 years. The disease typically has three phases. Most patients present in chronic phase but, in an average of 3–5 years, the disease progresses to an accelerated phase with increased tumor loads and additional chromosome aberrations. This usually progresses relatively quickly to the blast-crisis phase where the disease comes to resemble acute leukemia. Treatments, including chemotherapy and stem cell transplantation, are usually effective only in the chronic phase of the disease.

More than 90% of cases of CML are caused by the BCR-ABL fusion protein, resulting from a translocation between the bcr gene on chromosome 9 and the abl gene on chromosome 22. This ►cytogenetic abnormality causes the non-receptor tyrosine kinase, ABL, to

become constitutively active, and is necessary and sufficient to induce the malignancy via increased proliferation, reduced apoptosis, and perturbed interactions between CML cells and bone marrow stroma. Therefore, this oncogenic ABL tyrosine kinase has become an ideal therapeutic target in CML.

In 1992, STI-571 was synthesized at Ciba-Geigy Pharmaceuticals (now Novartis) as an effective tyrosine kinase inhibitor. Four years later, the inhibitory effects of STI-571 on *in-vitro* growth of BCR-ABL-positive leukemic cells was confirmed and reported. Phase I clinical trial studies with STI-571 started in June 1998 and phase II trials were initiated in 1999. These trials confirmed that STI-571 is safe and effective in the treatment of chronic-phase CML. In 2001, STI-571 was approved in both the USA and Europe for the treatment of CML.

Large scale, phase-III multi-centre trials have now shown that STI-571 is superior to conventional interferon-α-based therapy in previously untreated chronic-phase CML, with an initial complete hematological response (CHR) rate of 95% versus 55% and a complete cytogenetic response (CCgR) rate of 76% versus 15%. The expected 5-year overall and progression-free survival for STI-571-treated patients has reached ≥90%. Therefore, STI-571 is now used as first-line treatment for CML.

In addition, STI-571 has some activity in the accelerated and blast crisis phases of the disease. Thus, around 40 and 20% of patients in accelerated phase can achieve a CHR and a CCgR respectively. The 3-year progression-free survival has been reported to be as high as 40%. For patients in blast crisis, the rate of response to STI-571 (including CHR, partial hematological response and hematological improvement) is also higher than that in historical controls treated with cytarabine-containing regimens (52% vs. 29%). However, the response of patients with blast crisis is usually temporary, with a median progression-free survival of ≤10 months and a 3-year survival of only 7%.

The standard dose of STI-571 for CML is 400 mg daily. A higher dose, 600–800 mg daily, has also been used, particularly for cases previously treated with conventional chemotherapy. However, whether such increased doses of STI-571 will achieve better responses remains to be determine in prospective studies.

Gastrointestinal Stromal Tumors (GISTs)

Gastrointestinal stromal tumors are a rare type of soft tissue sarcoma, with an annual incidence of 0.68–1.45/100,000 according to epidemiological data from North America and Sweden. More than 90% of GISTs occur in the stomach or small intestine, but they can occur anywhere along the length of the digestive tract. Although the exact causes of GISTs are unknown, most have a constitutively activated ►receptor tyrosine kinase (RTK) pathway. This occurs through mutations in either the KIT (in more than 80% cases) or the PDGFRα (in about 5–7% cases) gene. The abnormally increased activity of either of these two receptor tyrosine kinases is responsible for mediating the signals which stimulate the proliferation and survival of the tumor cells.

The most common treatment for GISTs is surgery to remove the solid tumour. However, GISTs have an extraordinarily high rate of recurrence after surgical resection and are very resistant to radiation and standard chemotherapy. Since STI-571 targets both of these oncogenic tyrosine kinases (as well as ABL), its effects were soon tested in GISTs. The agent was found to have activity, and is now standard therapy for advanced tumors which cannot be removed by surgery alone and for metastatic and recurrent disease.

Since 2000, thousands of patients worldwide with metastatic or recurrent GISTs have been treated with STI-571, and it has been found that a partial response or stable disease is achieved in 80% of cases. Furthermore, survival is prolonged with improvement of quality of life. Seventy percent cases with metastasis now have an expected survival of over 24 months compared to 15 months before the introduction of STI-571. Studies of STI-571 in both the neoadjuvant (STI-571 used both prior to and post surgical resection) and adjuvant (STI-571 used post surgery) settings are now being conducted to evaluate whether the low rates of cure obtained with surgical resection alone can be improved.

A daily dose of 400 mg with or without surgery is the recommended first-line treatment for recurrent or metastatic GIST; a higher dose (600–800 mg) may be considered in patients who progress, develop secondary resistance or present with specific genotypic characteristics. Treatment should be continued until there is progression or adverse effects become intolerable.

Other Diseases

STI-571 is now being used as experimental treatment of other diseases, including BCR-ABL-positive ►acute lymphoblastic leukemia. KIT-positive small-cell lung cancer, and PDGFR-positive ►prostate cancer.

Side Effects

Common side effects include oedema, rash, nausea, diarrhea and fatigue. Anemia, neutropenia and/or thrombocytopenia can develop in some patients, especially those with advanced disease. Almost all of these side effects are dose-dependent and controllable.

Resistance to STI-571

In CML, resistance to STI-571 more frequently develops in advanced disease, although it can be seen in chronic phase, usually after initial response. This resistance can be caused by multiple mechanisms.

Among these mechanisms, mutations in the BCR-ABL kinase domain are the most important. They are detected in more than 90% of cases with resistance to STI-571, and may be acquired during treatment, or may exist in a mutated clone present before therapy. Other mechanisms include BCR-ABL overexpression or amplification, reduced intracellular drug concentrations (e.g. caused by low expression of the drug influx transporter HOCT1 gene and/or high expression of the efflux transporter MDR1 gene), and abnormalities downstream of BCR-ABL, e.g. defects in the pro-apoptotic proteins BAD and Bim.

In GISTs, primary resistance is rare and only seen in <15% of cases. More often, resistance is acquired after more than 1 year of STI-571 therapy. Several groups have shown that such resistance is due to mutations in KIT.

Low Concentrations in the Central Nervous System (CNS)

STI-571 has limited activity against CML in the CNS because of poor penetration of the blood-brain barrier. This results in an 88–100-fold lower concentration of STI-571 in cerebrospinal fluid, compared to that in plasma. Case reports have shown that CNS relapse of CML can occur in patients who have maintained major cytogenetic remission with STI treatment. Although this late CNS disease is still rare, it may become more common over time as more patients are treated successfully with long-term STI-571.

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STI571 STI-571

►Imatinib

Sticker Sarcoma

Definition

Synonym Canine transmissible tumor (CTVT); a contagious veneral tumor found in the domestic dog and potentially in their social canids, such as the gray wolf and the cyote. The tumor cells themselves, rather than another agent such as a virus, constitute the contagious agent of the disease. CTVT is passed through the population by ►allografts, with the tumor cells from one animal directly seeding tumor formation in the next, usually during coitus. The tumor cells carry a particular marker, common to all tumors in the different host animals, which consists in a diagnostic long interspersed nuclear element (►LINE-1) near the ►*MYC* gene. The cells of any canine host do not have this diagnostic LINE-1 insertion. Thus, all tumors represent a single cell lineage that has been propagated over long time. The mechanism for tumor cells to evade the host immune system is not clear.

Stilbenes

Definition

Antioxidant compounds.

►Phytoestrogens

STK5

►Aurora Kinases

STK6

►Aurora Kinases

STK7

►Aurora Kinases

STK12

►Aurora Kinases

STK13

►Aurora Kinases

STK15

►Aurora Kinases

STMY1

►Stromelysin-1

Stomach Cancer

►Gastric Cancer

Stomatitis

Definition

An inflammation of the mucous lining of any of the structures in the mouth.

►Fluorouracil

STR1

►Stromelysin-1

STRAP

►Serine-Threonine Kinase Receptor-Associated Protein

Streptavidin

Definition

A tetrameric protein purified from Streptomyces avidinii that binds very tightly to the vitamin biotin.

Stress

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Definition

There are different ways to investigate the phenomenon of "stress". From the biologist's point of view, stress can be defined as the unspecific biological response of the body to any ►stressor. The biological ►stress response includes the activation of the sympathetic nervous system and the release of several hormones (most prominently adrenaline and cortisol) and is among other reactions associated with an increased heart rate, increased blood pressure, altered immune system response and suppression of the digestive system.

From the psychological point of view, stress is a transactional process which depends on the meaning a person attributes to a stressor. In terms of the transactional stress model which was developed by Lazarus and Folkman, stress results from the subjects' appraisal that a certain stressor or situation is perceived as threatening (as opposed to challenging or harmless or irrelevant) and that the demands of the stressor exceed the individual's resources to deal successfully with the situation. In this model the same situation can lead to severe stress in one person while another person might not even recognize the situation as a potential stressor, depending on a variety of factors, including life experience, personality, coping resources or social environment of the subject.

Generally, there are two main approaches in social research on stress. One approach to define stress is the concept of ►daily hassles. Following this approach, stress is a result of an accumulation of minor stressors in daily life (e.g. misplaced keys or overcrowded public transport). However, the majority of research related to stress and cancer occurrence defines stress as the experience of ►major life events (or synonymical: ►stressful life events).

Major life events are defined as events which cause major changes in the life of the individual and are stressful to almost everybody regardless of individual coping capacity or social environment. In the social sciences, exposure to major life events is often measured by a checklist, the most prominent of these being a checklist which was constructed in the 1960s by Holmes and Rahe as a rank scale of life events. In a reanalysis of this scale in the 1990s, the five most stressful events in the list of 43 events were (in descending order) 'death of a spouse', 'divorce', 'death of close family member', 'marital separation' and 'fired from work'.

Characteristics

The assumption of an association between stress and cancer occurrence is common among cancer patients. Many patients believe that their cancer disease was caused or at least influenced by personal factors such as personality traits, coping strategies, depressive mood or stress. Likewise, many clinicians believe based on their own clinical observations that stress or emotional trauma are associated with cancer risk. Since these beliefs persist among clinicians, researchers, patients and the lay public, the topic has received intensive research attention in the past and many studies have addressed the question if stress is associated with cancer risk.

Possible Pathways of an Association of Stress and Cancer Risk

Different possible pathways for an association between stress and cancer risk have been postulated: The stress reaction of the body includes the release of several hormones and studies on the effects of stress on the immune response have shown that severe or chronic stress can adversely affect the immune system. It has been hypothesized that these changes can directly increase the risk for cancer. A competing theory of a relationship between stress and risk for cancer is based on the assumption that severe stress can influence behavioral factors and thus subsequently increase the risk for cancer. Studies on health behavior have shown that the experience of stress is associated with unhealthy behavior such as smoking, unhealthy diet and lack of physical exercise. These behavioral factors themselves are risk factors for several cancers and an increase in cancer risk in the context of stressful living conditions might be ascribed to an increase of these unhealthy behaviors.

Methodological Problems

Research on the association between stress and cancer risk is methodologically challenging, because stress is difficult to conceptualize and cancer is a multifactorial disease. Any interpretation of research findings must take the methodological basis of the respective study into account, especially with regard to study design, different approaches in the measurement of exposure to stress, outcome measurement (development of a cancer disease) and the consideration of confounding variables.

Many studies on the association of stressful life events and cancer risk which have postulated a link between the two show methodological weaknesses. Some of them are based on small sample sizes and especially many early studies used a retrospective approach and were designed as case-control studies. In a ►case–control study, patients who are diagnosed with cancer are matched with healthy controls and both groups are asked about the experience of stressful events in the past (mostly referring to a period of two, five or ten years before the study). Naturally, a patient who was (maybe even recently) confronted with a cancer diagnosis might recall things differently (and might overreport the exposure to stress retrospectively) than a person who does not have to deal with a potentially life threatening disease. This phenomenon is called ►recall bias and represents a fundamental problem in case-control studies which investigate past events.

The methodologically strongest solution to avoid the problem of recall bias is the ►prospective study design. In prospective studies on stress and cancer risk, only subjects who have not yet been diagnosed with cancer are included in the study. At the beginning of the study, all participants are investigated about their experience of stress and then followed up for a long period of time. The outcome of interest is cancer incidence and the analyses address the question if the subjects who develop cancer during follow up had initially reported more stress (e.g. stressful life events) than the subjects who do not develop cancer.

In addition to study design, the assessment of exposure and outcome plays an important role with regard to the methodological quality of a study. Although it is likely that most people recall especially major events in their life correctly, there is no means to assure that their recollection represents the true exposure. One way to address this problem is the unbiased exposure assessment based on register data. Likewise, the strongest approach to assess the outcome (in this case: the development of a cancer disease) is the

use of register data rather than self-report. Exposure in studies of stressful life events and cancer risk can be ascertained either from register data or from self-reports, which often comprises a checklist.

Another important factor in the examination of an association of stress and cancer risk is the adjustment for other risk factors for cancer. Since it is well established that behavioral factors such as smoking, alcohol consumption, diet, and exercise increase the risk for several cancers, these factors should be taken into account in order not to ascribe a potential finding to stress when it is in fact based on e.g. higher alcohol consumption in the exposed group.

Findings

Positive associations between severe stressors (i.e. stressful life events) and cancer risk have frequently been reported from case-control and ►retrospective studies. Since these studies suffer from the above described methodological problems, this essay focuses on the results of studies which utilized a prospective approach.

Several prospective population-based studies which assessed both exposure and outcome with unbiased data sources such as administrative data from population registers and cancer registers investigated stressful life events such as death of a spouse, divorce, death of a child or serious illness in a child. Most of these studies were conducted in Scandinavia where the availability of complete population registers and cancer registers enable ►cancer epidemiology researchers to link these data and investigate for example thousands of parents whose child died or whose child was diagnosed with a severe illness. Overall, these prospective population-based studies have shown no association between the investigated stressful life events and subsequent cancer risk.

Two studies found a slight increase in the risk for specific cancers though: one study on cancer risk in about 20,000 parents who had lost a child showed that bereaved mothers had a slightly increased risk for all cancers, which was confined to a slightly increased risk for smoking-related cancers. Likewise, a study of the cancer risk of about 20,000 parents of children in whom schizophrenia was diagnosed found that the mothers had an increased risk for ►lung cancer. However, elevated cancer rates were only found for smoking related cancers and since these studies where based solely on administrative data there was no means to adjust for smoking behavior. Therefore these findings seem to support the hypothesis that stress does not directly influences cancer risk, but alters health-related behavior (such as smoking patterns) which in turn might increase cancer risk.

Two other studies utilized a more sophisticated approach by combining administrative data for the unbiased assessment of the outcome with personal information from the participants with regard to potential confounding variables in a prospective study design. Therefore, as opposed to many previous prospective studies, the analyses in these studies could be adjusted for a number of lifestyle factors known to be associated with cancer risk (e.g. smoking patterns and alcohol consumption):

A large prospective study of breast cancer in Finland took exposure to potential confounding factors into account and assessed the outcome on the basis of information in a population register, while experience of stressful life events was assessed from a checklist. It was found that experience of divorce or separation or death of a husband, a close relative or a friend increased the risk for breast cancer. However, a Danish study which used a similar approach (assessment of stress by checklist, assessment of outcome by register data, adjustment for behavioral factors) and investigated all cancer sites did only show that the accumulated experience of stressful life events was associated with an unhealthy lifestyle but was not associated with an increase in cancer incidence.

Conclusion

Although the topic of the presumed association between stress and cancer risk received intensive research attention in the last decades, methodologically advanced studies on the subject are still rare. Due to methodological limitations, many previous studies contribute little to the question of the influence of stressful life events on the development of cancer. Concurrently lay theories on such an influence are widespread among cancer patients and the general public. Only prospective, population-based studies provide reliable information that may support a more evidence-based discussion of these issues.

Based on the repeated results of the prospective studies which have been conducted so far we can conclude that there is no convincing evidence that stress is an independent risk factor for cancer. This conclusion does not only imply that cancer patients have no reason to blame their psychological condition for the occurrence of the disease. The findings might also assist health care professionals working with cancer patients or their relatives since they can reassure patients that there is no scientific proof that stress or the experience of stressful events such as bereavement or divorce causes cancer.

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Stress-activated Protein Kinase

►JNK Subfamily

Stress Response

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Synonyms

Immediate early stress response; Damage response

Definition

Stress response is a process that occurs in response to an altered balance of endogenous homeostasis following altered external and internal stimuli.

Characteristics

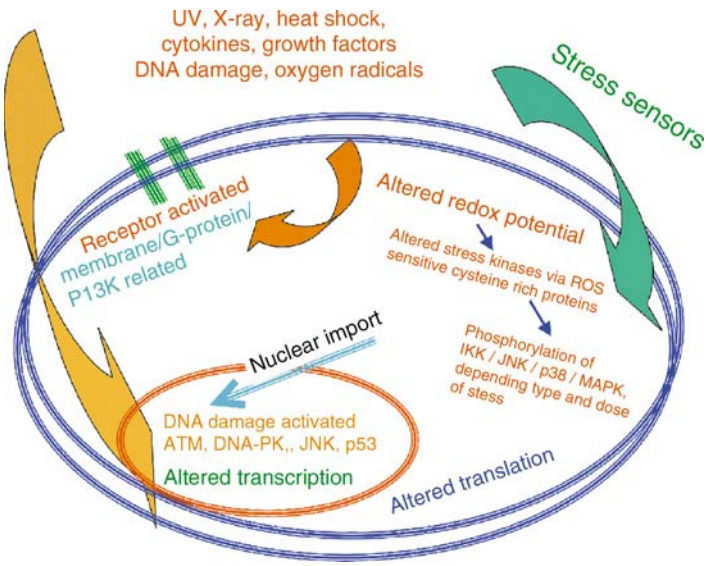
A cellular stress response consists of cellular changes required to accommodate internal or external insult. It can be induced following change in ►ROS which can take place in response to ►DNA damage, ►inflammatory cytokines, ►growth factors and irradiation, as well as osmotic or heat shock. Exposure to anti-cancer drugs or deprivation of survival factors also results in the activation of a stress response. A large set of cellular sensors, which include redox sensitive proteins, cell surface receptors, and proteins that recognize changes in ROS, ►ER stress or DNA damage regulate the activity of stress kinases and their respective substrates. Depending on the type and degree of stress, the combined activation of stress kinases and their corresponding substrates dictate the cellular fate (survival or death) in response to the stress administered (►Apoptosis). The activation of one or more stress activated kinase pathways allows the cell to determine whether DNA repair and growth arrest will prevail over the initiation of programmed cell death or cellular differentiation. The nature of the cellular stress response is therefore dependent on cell type, expression pattern of cell surface receptors and stress kinases in concordance with respective phosphatases and kinase inhibitors.

Cellular Regulation

Recognition of cellular stress can be attributed to one of the following (as well as to any of their combinations):

- Altered organization of cell surface receptors, including changes in the oligomerization of EGFR, PDGFR and IGFR as well as altered localization and conformation of membrane anchored proteins including phosphoinositol 3 kinase (PI3K).
- Change in the redox potential within the cell, primarily due to an altered balance of reactive oxygen radicals (as a result of impaired activity of detoxification and homeostasis maintaining enzymes among which are thioredoxin and glutathione S-transferase π which were shown to inhibit, under non-stressed growth conditions, the activities of ASK1 and JNK, respectively).
- DNA damage which results in the activation of DNA-PK, JNK, and other kinases that sense changes in the presence of DNA lesions (Fig. 1) Each change, or the combination of these changes, has been implicated in the activation of a selective subset of stress kinases and in the subsequent activation of their respective substrates.
- ER stress, which results in activation of ►Unfolded Protein Response (UPR) degradation of misfolded proteins (ERAD) with possible trigger for programmed cell death.
- Change in ubiquitin-mediated signaling that impacts cell cycle progression, activation of protein kinases and DNA damage responses.

Among key stress signaling pathways are the IKK, MAPK, JNK and p38 cascades. Interestingly, the upstream regulatory components that include TRAF2, MEKK1, ASK(1–2), TAK(1–2), MEK(1–4) often share regulation of downstream components of the stress kinase pathways (Fig. 2) The interplay between signaling pathways is controlled through organization via scaffold proteins, which facilitate contact between selective members of a given stress-signaling pathway. Requirements for scaffold proteins were shown for IKK, JNK and MAPK family members. The activation of stress kinases leads to the phosphorylation and/or stabilization of their substrates and to their transcriptional activities. Growing evidence



Stress Response. Figure 1 Outline of major stress sensors. Cell exposure to stress, as exemplified in the list of DNA damage and stress inducing treatments listed, affects one or more of the major cellular stress sensors. Those sensors include cell surface receptors, membrane anchored proteins and cysteine rich proteins which are sensitive to the formation of reactive oxygen radicals, and DNA lesions which are sensed by nuclear-residing protein kinases. The activation of one or multiple sensors depends on the type of stress, its dose and duration. In turn, respective changes in the activities of stress kinases (Fig. 2) will determine cell fate.

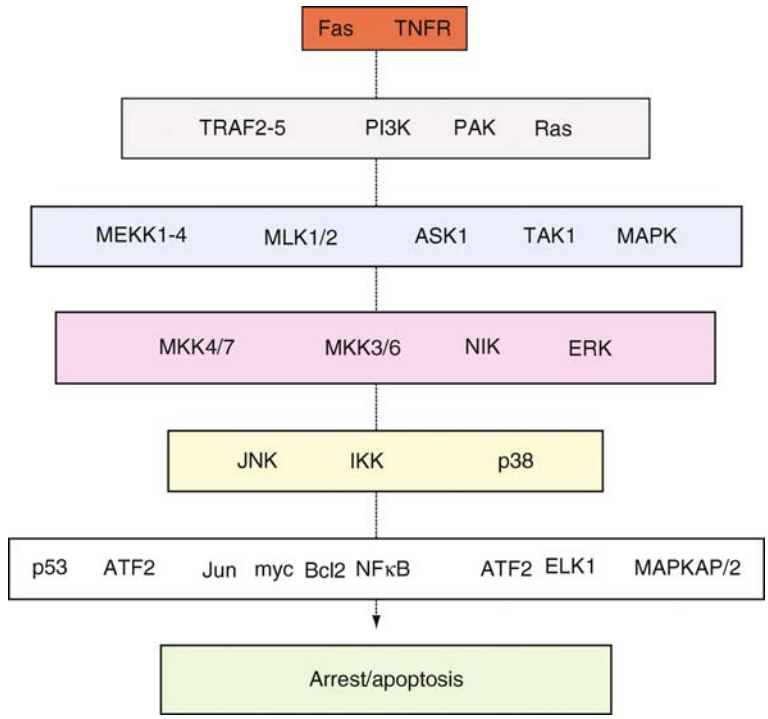
points to re-wiring of signal transduction pathways in human tumors and pathological disorders. Among the four major stress signaling pathways are:

- IKK, a family of kinases implicated in the regulation of IκB phosphorylation and subsequent ►ubiquitination and degradation. Consequently, ►NFκB is free to enter the nuclei to mediate its transcriptional activities. NFκB has been associated with the cellular ability to cope with stress through its positive effect on proteins that antagonize the apoptotic cascade, including IAPs, TNFα, Fas and Bcl2.
- MAPK, which is responsive to Ras and PI3K signaling, has been implicated in the cellular response to ROS and elicits the activation of p42/p44 ERKs.
- JNK and p38 which are activated by the upstream kinases MKK4/7 and MKK3/6, respectively. The latter are tightly regulated by ASK1 and MEKK1 signals. c-Jun/ATF2 are phosphorylated by JNK and p38, which represents divergent signaling cascades that result in transcriptional output that is often shared by the heterodimer ATF2/Jun. Other members of the p38 families are expected to play a role in the phosphorylation of substrates that are not modified by JNK and vice versa, thereby conferring selectivity for the stress response.
- ATM and DNA-PK represents enzymes that recognizes DNA damage and contributes to cellular stress response via phosphorylation of key regulatory proteins including the tumor suppressor protein p53.

Activation of p53 is a good paradigm for stress response as this tumor suppressor stress inducible protein is phosphorylated by multiple stress kinases resulting in the dissociation of proteins that otherwise target its ubiquitination and degradation. Phosphorylation of p53 depends on the nature and degree of damage and stress and is carried out by ATM, DNA-PK, p38, and JNK. Phosphorylation on multiple residues (including 15, 20, 37, 46, 81, 389) depending on the type and dose of damage results in p53 that is able to elicit growth arrest or a signal triggering apoptosis.

Clinical Relevance

The interplay between stress-activated kinases, ligases and other regulatory components appears to play a key role in the type of cellular response to stress. Changes in the expression, activation or in the duration of stress kinase output are expected to result in an altered cellular response to stress. Tumors were found to harbor such changes, which conferred greater resistance to radiation or chemotherapy, with the example of TRAF2/GCK in human melanoma. Mutations in stress kinases are rarely found, although cases reported an MKK4 mutation in certain human tumors. Changes in the activation



Stress Response. Figure 2 Stress kinases. Several stress signaling cascades are based on their affinity to upstream and downstream components. Among the major stress kinases are ERKs (extracellular signal regulated kinases), IKK (inhibitory κ kinase), JNK (Jun kinase) and p38. Each of these signaling cascades is activated at different kinetics and with diverse affinities in response to stress. Thus, various types of stress can activate different subsets of stress signaling cascades. Activation of the upstream stress components results in the phosphorylation of respective downstream targets. For example, TRAF2 (tumor receptor associated factor 2) can elicit the activation of NIK (nuclear factor κ B inducing kinase), MEKK1 (MAP/ERK kinase 1), ASK1 (apoptosis signal-regulating kinase 1), mitogen activated protein kinase (MAPK), which in turn phosphorylates IKK, MKK4/7 (mitogen activated kinase kinase 4/7), p38 or ERKs, respectively. Organization of these signaling cascades via "scaffold" proteins maintains their close contact and the high affinity for related family members. These kinases will in turn phosphorylate their transcription factors substrates. Depending on the combination of activated transcription factors and apoptosis regulatory proteins, the cell can undergo growth arrest, allows proper damage repair or can undergo programmed cell death.

of stress kinases were also reported in heart and neurological disorders.

Our greater understanding of mechanisms underlying the regulation of stress kinases and their substrates is expected to allow the design and use of reagents that would specifically target the selective kinase to alter its activities, which will determine the ability of the cell to cope better (or worse) with the type of stress and DNA damage.

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Stressful Life Event

Definition

Critical event that causes severe stress and requires adjustment to the new situation (e.g. death of a loved one, divorce, job loss).

►Stress

Stressor

Definition
An agent, condition, situation or other stimulus that causes stress to an organism.

►Stress

Striatonigral Medium Spiny Neurons

Definition
MSNs; Projection neurons in the striatum, a major nucleus of the basal ganglia.

►Early B-cell Factors

Stroma

Definition
Meaning mattress or support for parenchymal cells; Is the connective tissue of an organ or a gland. Historically, stroma was recognized as a supportive or scaffolding component. The stroma is believed to "sense" and "react" to paracrine and autocrine physiological or pathological disturbances, thus attempting to maintain the homeostatic balance. In the malignant process, refers to non-malignant cells and connective tissue that surround and support a tumor.

►Desmoplasia
►Stromagenesis

Stromagenesis

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Synonyms
Stromatogenesis; Stromal progression; Tumor-associated stromal progression

Definition
Stromagenesis, from the Greek term *stromatogenesis* (υστρώμα ►stroma = mattress and γένεσις genesis = creation or birth), is used to describe the progressive changes that stroma undergoes during the process of ►epithelial tumorigenesis. Specifically, the term describes tumor-associated changes in fibroblasts and fibroblast-derived ►extracellular matrix, as opposed to the formation of new stromal endothelial blood vessels, identified as ►angiogenesis.

Characteristics
Although the term "►stromagenesis" has only recently entered the scientific literature (2002) describing ►melanoma-fibroblast cross-talk, the process of stromagenesis has been investigated for more than a century. Early publications from several pathologists in the late nineteenth century (e.g. Theodor Langhans in 1879), used the word "stroma" to refer to connective tissue and vessels associated with tumors. They thought of these areas as purely mechanical and nutritional supports with only mild insinuation of their active roles in ►tumorigenesis. The first meaningful hint that the stroma can influence tumor cell behavior was published in 1889, when Stephen Paget presented his "seed and soil" hypothesis. Paget reported that upon autopsy of 735 breast cancer patients, metastases were preferentially distributed to particular organ beds. He proposed that although randomly scattered throughout the vasculature, tumor cells, regarded as "seeds," could only generate viable metastases in specifically permissive territory, or "fertile soil." He further postulated that during cancer progression, tumor cells actively contribute to formation of a pro-metastatic microenvironment. Almost one century after Dr. Paget's theory, in 1975, Dr. Beatrice Mintz, from the Institute for Cancer Research at Fox Chase Cancer Center in Philadelphia, reported that normal host cells could restrict the neoplastic growth of ►teratocarcinoma cells microinjected into blastocysts. The injected blastocysts resulted in ►mosaic yet normal, healthy, and fertile mice. Dr. Mintz's work was the first to show that cancer cells are totipotent (e.g. stem cells) and that a "normal" microenvironment restricts tumorigenic processes, while a tumor-associated stroma promotes tumor development.

Today, it is believed that tumor cells can both influence and be influenced by their stromal microenvironment, and that some stromas are genetically and/or epigenetically more suitable than others to support tumorigenesis and metastases. Some radical theories such as the "tissue organization field theory," propose that stroma is the sole tumorigenic component. This theory describes cancer as a problem of tissue organization comparable to organogenesis, and proliferation as the default state of a cell. Even though the

term "stroma" applies to both connective tissue's endothelial and fibroblastic cells, the term "stromagenesis" is often specifically used to describe the changes that tumor-associated fibroblasts (also known as cancer-associated fibroblasts) and their extracellular matrices undergo during tumorigenesis, while the term angiogenesis is used to describe the endothelial progression manifested as development of new vasculature in the vicinity of the tumor. Finally, the term "tumor-microenvironment" collectively includes stromagenesis, angiogenesis and cells arising from the host inflammatory immune responses to the tumor, which recruits macrophages and lymphoid cells to the tumor milieu.

It is believed that stromagenesis involves a mechanical process whereby the stroma is stiffened due to changes in protein composition, which increases in intrinsic forces proximal to the tumor, inducing the tumor to progress. Stromagenic changes can be observed at the immediate vicinity of a developing tumor, as an integral part of the tumor, as well as at secondary tumor-site (e.g. at the site of a metastasis). Investigators have attempted to describe stromagenesis by recounting its progression in at least three stages: (i) normal or repressive, (ii) primed or inductive and (iii) tumor-associated or activated stroma. The best described stromagenic reaction, known as ►desmoplasia, is regarded as fibrotic or scar-like by pathologists. Oncofetal stroma, less well-described, presents a less organized stroma that is positive for the oncofetal fibronectin isomer and has been associated with, among others, aggressive oral ►squamous cell carcinomas, as well as some breast and colorectal cancers. Since the two described phenotypes share some markers, it is not yet clear whether they are entirely distinct or whether they are dynamically interchangeable, thus emerging as desmoplastic or oncofetal stroma at different times during tumorigenesis. Oncofetal stromal-reactions have been observed relatively far from the primary tumor site (e.g. in the skin of a variety of cancer patients), while desmoplasia has only been reported in direct association to neoplastic lesions. Nevertheless, a clear stroma reaction often represents more than 50% of the total tumor mass such as in the case of pancreatic cancers.

Activated fibroblasts are frequently characterized as myofibroblastic cells expressing ►alpha-SMA. Various tumor-stroma specific markers have been described, e.g. the fibroblast activation protein (►FAP). This protein has been shown to be expressed in the tumor-associated stroma but not in normal stroma or epithelial cells, or in cancerous tissues with the exception of melanoma. Antibodies against FAP were the first used to investigate the possibility of developing an effective chemotherapy by specifically targeting the tumor-associated stroma. Other stroma specific markers include extracellular matrix components such as tenascin-C, hyaluronic acid, and SPARC (also known as osteonectin).

It is now well-recognized that both direct and indirect interactions between cancer cells and their stroma are critical for promoting the growth and invasiveness of tumors. Nevertheless, the mechanisms by which tumor cells promote stromagenesis are not well defined, but some important triggers such as the cytokine TGF-beta have been identified. ►TGF-beta is produced and secreted as a latent molecule by normal epithelial cells, tumors, and stromal fibroblasts. It is activated by extracellular proteases (including metalloproteinases ►MMP-2, MMP-9, ►MT1-►MMP) and receptors (including integrins) on the surface of fibroblasts or early stage tumor cells. TGF-beta suppresses the growth of an emerging population of tumor cells. However, at later stages of tumorigenesis, TGF-beta is indirectly tumor supportive, because during tumor development TGF-beta receptors are often down-regulated on the tumor cell surface causing a loss of TGF-beta responsive growth inhibition in tumor cells. However, because tumor cells continue to produce TGF-beta and stromal fibroblasts maintain high levels of its receptor(s), TGF-beta continues to drive fibroblastic responses (e.g. type I collagen fibrillogenesis), promoting the differentiation of stromal fibroblasts into myofibroblasts (myofibroblasts are also believed to be actively recruited to the tumor site from the circulation). Alpha-SMA also actively promotes stromagenesis and mechanical force is generated by the myofibroblast through the isometric contraction of stress fibers containing alpha-SMA. This force is transmitted by integrin-dependent adhesion-structures, which connect stress fibers with the modified stromal extracellular matrix. It has been shown that TGF-beta induces desmoplastic differentiation of normal fibroblasts into alpha-SMA expressing and collagen type I assembling myofibroblasts *in vitro*. Moreover, it is known that induction of connective tissue growth factor and a specific differential splice form of fibronectin known as ED-A enhance the pro-fibrotic effects of TGF-beta.

The vital interplay between tumor and stroma means that, in principle, it is possible to target signaling pathways that regulate tumor-induced stromagenesis and, thereby, contain tumorigenesis.

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Stromal Cell Response

►Desmoplasia

Stromal Cells

Definition

Supportive cells, usually of a fibroblastic phenotype that provide both structural and nutritional support for the principal cells within a tissue or a tumor.

►Stroma

Stromal-derived Factor 1 Alpha (SDF1α)

Definition

A chemokine recently discovered to play important roles in neovascularization; SDF1α binds CXCR4.

►Antiangiogenesis
►Angiogenesis

Stromal Progression

►Stromagenesis

Stromatogenesis

►Stromagenesis

Stromelysin-1

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Synonyms

Matrix metalloproteinase 3; MMP-3; Proteoglycanase; Transin-1; SL-1; STR1; STMY1

Definition

Stromelysin-1 (EC 3. 4. 24. 17), a secreted proteolytic enzyme whose gene maps to 11q23, is a member of the ►matrix metalloproteinase (►MMP) family. Like the other MMPs, it is characterized by its ability to degrade the extracellular matrix (►ECM) components and its dependence upon Zn^{2+} binding for enzymatic activity. Stromelysin-1 is produced by fibroblastic cells and by normal and transformed epithelial cells in culture and *in vivo*. It plays a pivotal role in the degradation and remodeling of the ECM during a variety of normal and pathological processes. Besides digesting ECM components, stromelysin-1 modulates the activity of biologically active molecules by direct cleavage and release from bound stores, thus influencing many cellular functions.

Characteristics

Enzymatic Properties of Stromelysin-1

Human stromelysin-1 is secreted from cells as an inactive ►zymogen (M*r* latent 57,000 Da) and comprises four distinct structural domains: (i) an N-terminal propeptide that maintains the enzyme in latent form, until it is removed, owing to the interaction of a cysteine residue in this peptide with the zinc ion in the active site, (ii) a catalytic domain containing the active site with the catalytic zinc moiety, (iii) a linker or hinge region, and (iv) a C-terminal hemopexin-like domain that is believed to play a role in the recognition of certain substrates and influence the binding of macromolecular inhibitors. Active enzyme (M*r* active 45,000 Da) is produced by cleavage of the propeptide that can be induced by proteinases like plasmin or *in vitro* by thiol-modifying agents, chaotropic agents and heat, which destabilize and activate the molecule by dissociation of the zinc and the cysteine residue. Once activated, stromelysin-1 is susceptible to inhibition by tissue inhibitors of metalloproteinases (►TIMPs).

Stromelysin-1 has one of the broadest substrate spectra of the MMPs and can degrade most ECM components, but not fibrillar collagens. In addition, stromelysin-1 activates a number of proMMPs, and acts on non-matrix substrates including cell-surface and

matrix-bound growth regulators, releasing them from stores (Table 1).

Regulation of Stromelysin-1

Stromelysin-1 is involved in ECM remodeling during physiological processes such as morphogenesis, growth and wound repair. It follows that a tight and spatiotemporal regulation of its expression is critical for cell and tissue homeostasis. Stromelysin-1 expression is mainly controlled at the transcriptional level. A number of specific DNA elements in the human stromelysin-1 promoter have been shown to be important in the regulation of its transcription. They enable the gene to integrate through binding the transcription factors, which are a large number of stimuli from the different cellular signaling pathways. The specific DNA elements that have been well characterized are: (i) A proximal ►AP-1 site at –70 which binds the transcription factors of the ►Fos and ►Jun families. This site is necessary for the basal level of expression but is not sufficient on its own for activation by numerous inductors as the epithelial growth factor (EGF), the ►platelet-derived growth factor (PDGF), phorbol esters or the proinflammatory cytokine IL-1β. Nevertheless, this site must not be underestimated because it is important for combinatorial regulation by the different members of the Fos and Jun families. (ii) Palindromic head to head ►Ets-binding sites (EBS) at –216/–201, which are not present in the promoter of the other MMPs. These sites bind the oncoproteins of the Ets family, which are transactivators such as Ets-1, Ets-2 or Pea-3 and repressors such as Tel. This EBS palindrome is important for the transcriptional regulation of the stromelysin-1 promoter in response to the oncoproteins ►Ras, Mos and ►Src, phorbol esters which are tumor inducers such as ►PMA and IL-1β. (iii) An upstream regulatory sequence which is composed of an EBS and a NIP (Nuclear Inhibitory Protein) site at –94/–80. It cooperates with the EBS palindrome and the AP-1 site in response to IL-1β and PMA. (iv) A stromelysin IL-1 responsive element (SIRE) at –1614/–1595, which is a distal negative response element to IL-1β responding also to the tumor necrosis factor α (TNFα), another proinflammatory cytokine. This element can present a single nucleotide polymorphism (►SNP) 5A/6A.

Stromelysin-1. Table 1 Stromelysin-1 substrates (Adapted from ref. 1 and completed)

ECM substrates	Bioactive substrates	
Aggrecan	MCP-1 (« monocyte chemoattractant protein-*1* »)	Partial inactivation
Laminin	MCP-2	Agonist > > antagonist
Fibronectin	MCP-3	Agonist > > antagonist
Collagens III, IV, V, IX, X, XI	MCP-4	Agonist > > antagonist
Gelatin	SDF (« stromal cell-derived factor »)	Inactivation
Entactin	Pro-TNFα	Soluble active TNF-α
Perlecan	L-selectin	Shedding
Decorin	Pro-HB-EGF (« heparin binding-EGF »)	Active HB-EGF
Tenascin	Pro-IL-1β	Active IL-1β
Vitronectin	Perlecan	FGF2 release
Fibrin/fibrinogen	Decorin	TGF-β release
LP (« link protein »)	CTGF (« connective tissue growth factor »)	VEGF release
Elastin	SPARC (« secreted protein acid rich in cysteine »)	Angiogenic peptides
	Plasminogen	Angiostatin
	E-cadherin	Soluble ectodomain
	IGFBP-3 (« IGF binding protein-3 »)	Active IGF release
	Pro-MMP-1	Active MMP-1
	Pro-MMP-3	Active MMP-3
	Pro-MMP-7	Active MMP-7
	Pro-MMP-8	Active MMP-8
	Pro-MMP-9	Active MMP-9
	Pro-MMP-13	Active MMP-13
	α1-proteinase inhibitor	Inactivation
	α1-antichymotrypsin	Inactivation
	α2-macroglobulin	Inactivation

The additional adenine in the 6A allele increases the binding of a NF-κB p50/p50 dimer leading to a lower level of stromelysin-1 transcription. This polymorphism has been associated with disease severity in diverse pathologies including ►cancer. (v) A stromelysin-1 PDGF response element (SPRE) at –1584/–1571 which binds the stromelysin-1 PDGF response element binding protein (SPBP). SPBP transactivates the stromelysin-1 promoter in response to PDGF through a κ/ι ►PKC-dependent pathway requiring cooperativity with the AP-1 site. (vi) A nerve growth factor response element (NGFRE) at –241/–229, which binds the interferon response element binding factor-1 (IREBF-1). The transcription of stromelysin-1 was shown to be increased by IREBF-1 in response to the nerve growth factor (NGF).

Besides these well defined responsive elements, variation in the levels of stromelysin-1 transcription by other compounds has been reported: (i) ►oncostatin M, which induces stromelysin-1 in human chondrocytes, (ii) protein synthesis inhibitors such as cycloheximide and anisomycin, which induce an increase in levels of stromelysin-1 messenger RNA in human fibroblasts, (iii) ►transforming growth factor β (TGF-β), which is able to repress the activation of the stromelysin-1 transcription by EGF, Ras and Src in fibroblasts, and (iv) hormones, which are also inducers or repressors of stromelysin-1 transcription. Glucocorticoids are able to inhibit the increase in stromelysin-1 messenger RNA induced by EGF, PMA and IL-1β in fibroblasts. On the one hand, ►retinoic acid, like glucocorticoids in fibroblasts, is able to repress the stromelysin-1 expression and on the other hand to increase its induction by NGF in pheochromocytoma cells. Oestradiol and progesterone inhibit stromelysin-1 expression during the menstrual cycle. Androgens have been reported to decrease the expression of stromelysin-1 induced by PMA in prostate carcinoma cells. Unfortunately, the mechanism whereby all these compounds exert their effects remains unclear. A post-transcriptional regulation of stromelysin-1 was also described. Indeed, activation of p38 alpha mitogen-activated protein kinase (►MAP Kinase) enhances stromelysin-1 expression by messenger RNA stabilization.

All the different responsive elements and effectors listed above point out the complexity and the sensitivity of stromelysin-1 regulation. It reflects more particularly the equilibrium found at every control level between positive and negative signals. This is a strategy required for all the MMPs to obtain a very subtle-tuned expression in response to various stimuli in different cellular types and biological events.

Stromelysin-1 and Cancer

It would be simplistic to consider stromelysin-1 just as a proteolytic enzyme hydrolysing components of the ECM. Stromelysin-1 is able to generate and release numerous active and latent molecular signals or regulate pre-existing molecular signals. In this way, stromelysin-1, like other MMPs, can modulate the cellular environment at different levels and is therefore able to influence the behaviour and the future of the cells. It can be easily understood that misregulation of such a gene is associated with severe erosive and invasive pathologies such as tumor growth and invasiveness.

Stromelysin-1, like numerous other MMPs, is expressed in different tumors and its presence correlates with their aggressiveness. Its expression has been shown *in vivo,* notably in carcinomas: mammary carcinoma, colorectal carcinoma, lung carcinoma, prostate carcinoma, pancreatic carcinoma, oesophageal carcinoma or cutaneous carcinoma. It is noteworthy that stromelysin-1 has been often shown to co-express with the oncoprotein Ets-1, which is considered, among all the other Ets proteins, as an independent marker of poor prognosis in various cancer types. During the earliest stages of tumor development, stromelysin-1 is predominantly localized at the level of the fibroblasts of the ►tumor stroma.

Stromelysin-1 induces, by its proteolytic activity towards numerous compounds, the release of growth factors which act directly on tumor cells and other surrounding cells, including fibroblasts, inflammatory cells and endothelial cells. Stromelysin-1 in degrading perlecan releases FGF2 (or basic ►fibroblast growth factor) which in the setting of cancer is known for its potential to induce ►angiogenesis and might be involved in desmoplasia in tumors, owing to its role in fibrosis. In degrading decorin, another proteoglycan, stromelysin-1 releases TGF-β, a growth factor implied in cell growth and proliferation. Stromelysin-1 is able to cleave extracellular proteins sequestering growth factors such as ►insulin-like growth factor binding protein 3 (IGFBP-3) which binds to insulin-like growth factor II (IGF II). Stromelysin-1 can also release active ►heparin-binding EGF-like growth factor (HB-EGF) from cell surfaces by cleaving it at a site just outside the cell membrane. ►Chemokines are known targets of stromelysin-1 and other MMPs. For example, stromelysin-1 has been shown to transform, by partial proteolysis, the precursor of IL-1β in an active molecule, influencing tumor-infiltrating inflammatory cells. Stromelysin-1 is also involved in the loss of cellular adherence and the phenotypic modification of epithelial cells, which occur during the early stages of carcinomatoid tumor development. Thus, stromelysin-1 cleaves the extracellular domain of the ►adherens junction protein ►E-cadherin. The soluble fragment of E-cadherin which is released prompts cells to disaggregate and promotes tumor-cell ►motility in a paracrine manner, by interfering with the function of other full length E-cadherin

molecules. Cleavage of E-cadherin also triggers the ►epithelial-to-mesenchymal transition (►EMT), promoting cancer cell invasiveness. Acquisition of an invasive phenotype by the tumor often goes with a gain of MMP expression by the tumor itself. Indeed, stromelysin-1 expression is associated with invasive carcinomas. Some ►squamous cell carcinoma (SCC) tumor cells can express stromelysin-1, providing further activity for a tumor-driven proteolytic cascade, in which MMP-13 can be activated by stromelysin-1 expressed by tumor cells. In addition, many compounds secreted by tumor-infiltrating inflammatory cells as well as by tumor or stromal cells are capable of modulating MMP expression. Tumor cells can also secrete factors, such as extracellular matrix metalloproteinase inducer (EMMPRIN), which enhances the expression of several MMPs, including stromelysin-1 by fibroblasts.

Stromelysin-1 has also a role in tumoral neoangiogenesis. Inhibition of the stromelysin-1 activity decreases neovascularization drastically in a murine model of ►colon cancer. In addition, the release of proangiogenic factors by stromelysin-1 is also in agreement with its known role in angiogenesis: (i) release of FGF2; (ii) release of vascular endothelial growth factor (VEGF) isoform $VEGF_{165}$, as a consequence of degradation of its natural inhibitor, the connective tissue growth factor (CTGF); (iii) release of active HB-EGF; (iv) production of an angiogenic polypeptide, owing to the cleavage of a matricellular protein, ►SPARC (secreted protein acid rich in cysteine). Nevertheless, stromelysin-1 can also generate ►anti-angiogenic factors. Indeed, stromelysin-1 generates angiostatin by cleaving plasminogen and might be involved in the generation of ►endostatin, a C-terminal fragment of the basement-membrane collagen type XVIII. It indicates that expression of stromelysin-1 in the tumor periphery might also serve to limit or regulate angiogenesis induced by the tumor. Another role of stromelysin-1 as a negative regulator of tumor expansion might exist. In fact, stromelysin-1 is able to cleave and inactivate ►CXCL12, a ligand for CXC chemokine receptor 4 (CXCR4) on leukocytes. ►Breast cancer cells express CXCR4 and it has been shown that inhibition of the binding of CXCL12 to CXCR4 by blocking antibodies reduces ►metastasis *in vivo*. Therefore, cleavage of CXCL12 by stromelysin-1 might inhibit metastasis.

Stromelysin-1 can act as a natural tumor promoter. It can induce premalignant lesions and favor tumor emergence on its own. These observations come from using transgenic mice overexpressing stromelysin-1 specifically in the mammary glands. Mammary tissue of these transgenic mice presents the characteristics of stroma reaction with collagen accumulation, neovascularization, tenascin-C expression and upregulation of endogeneous stromelysin-1. These changes are hallmarks of cancer ►progression and may even predispose towards neoplastic epithelial transformation. Thus, overexpression of stromelysin-1 gives rise to changes that could potentially promote mammary ►carcinogenesis. This is confirmed in older animals from 6–24 months of age, which develop with an important incidence, spontaneous premalignant lesions and mammary cancers. A recurrent genomic instability has been shown by array comparative genomic hybridization (►ArrayCGH) in these different premalignant and malignant lesions. In addition, stromelysin-1 plays an active role in EMT. Thus, overexpressing this enzyme in normal mammary epithelial cells leads to an important morphological change with a loss of cell-cell adhesions and vimentin synthesis revealing EMT. This was confirmed *in vivo* using transgenic mice overexpressing stromelysin-1. A recent study indicates that stromelysin-1-mediated EMT is due to the expression of Rac1b, an isoform of Rac1 GTPase, which causes an increase in cellular ►reactive oxygen species (►ROS). This increase in the ROS stimulates the expression of the transcription factor ►Snail, promotes EMT and causes oxidative damage to DNA and genomic instability. This may represent a key event in the stromelysin-1-induced phenotypic and genotypic malignant transformation in normally functioning cells.

These results are supported by studies in human cancers where the 5A allele of stromelysin-1 (see above, regulation of stromelysin-1) corresponding to higher levels of stromelysin-1 transcription may represent an unfavourable prognostic feature in breast cancer patients associated with more invasive disease. The 5A allele might also be associated with the increased susceptibility to non small cell lung cancer among smokers and a risk of development and lymphatic metastasis in oesophageal squamous cell carcinoma.

On the contrary, other studies using stromelysin-1 transgenic animals showed a reduction in the number of mice developing mammary tumors following treatment by a chemical carcinogen. An ►apoptosis induction of mammary epithelial cells in transgenic animals overexpressing stromelysin-1 (possibly by degrading laminin) has been reported. In addition, in stromelysin-1-deficient mice, topical applications of carcinogens resulted in skin tumors that grew at a faster initial rate than did carcinogen-elicited tumors in wild type mice. This enhanced tumor development was correlated with a reduction in the tumor inflammatory cell infiltrate. Thus, the presence of stromelysin-1 appears to be a protection by an increased influx of inflammatory cells to the area of carcinogenesis.

All these data point out that the proteolytic activity of stromelysin-1 and other MMPs towards an always growing number of matrix and non-matrix substrates renders it difficult to predict the effect of cleavage in a complex biological context. This is all the more

real in cancer progression which involves a continuous interplay between tumor cells, stroma cells, and inflammatory cells. It becomes clear that the biological consequences of this activity can lead to either stimulation or inhibition of tumoral development. Validation of true substrates *in vivo* and a better understanding of the biological properties of the cleavage products is required to find an efficient therapeutic use for the inhibitors of stromelysin-1 and other MMPs.

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Structural Biology

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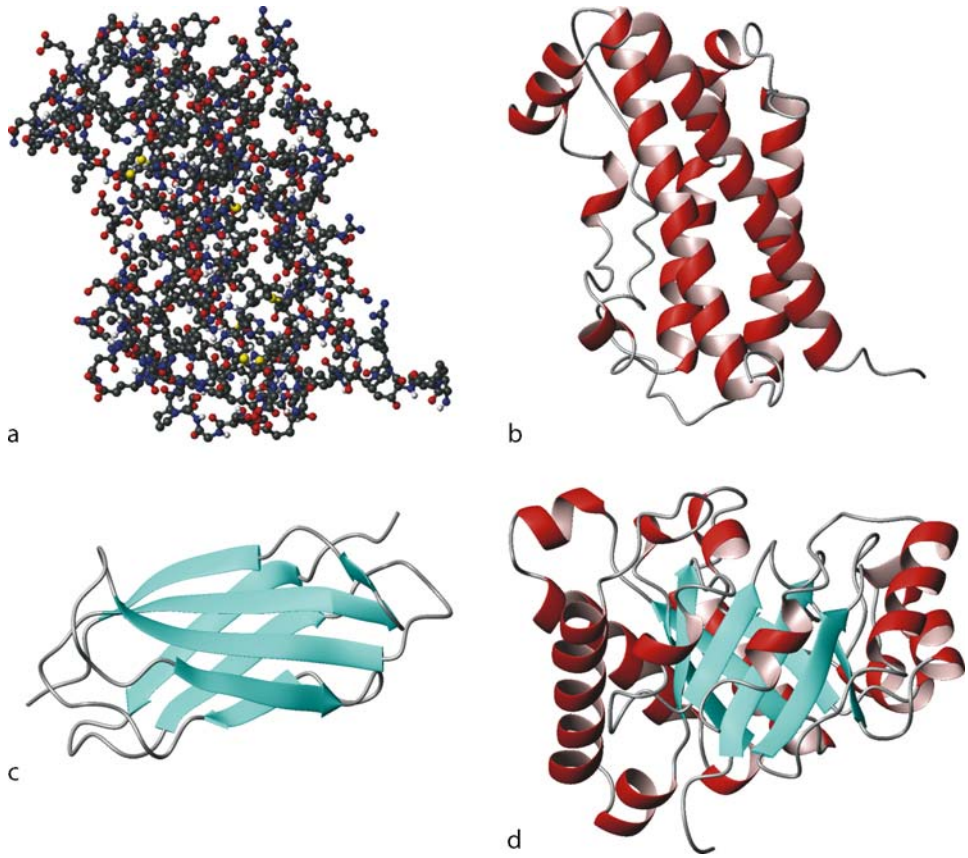
Definition

Structural biology involves biophysical methods that can determine the three-dimensional structure of macromolecules. These structures can lead to an understanding of the molecular basis of cancer and identify the atomic details necessary for drug design, optimization, and development.

Characteristics

The two methods that can elucidate atomic-level structures of biological macromolecules – e.g., proteins, DNA, RNA, and complexes between/among these molecules – are ►X-ray crystallography and ►nuclear magnetic resonance spectroscopy (►NMR). For X-ray crystallography, crystals are necessary for determining structures. In contrast, NMR is used to determine structures of molecules in solution. In crystallography, X-rays are diffracted by the crystal creating the data necessary to determine the structure. NMR experiments are based on the property of nuclear spin, inherent to specific nuclear isotopes such as ^{1}H, ^{13}C and ^{15}N. A majority of NMR-derived structural restraints are derived from magnetic couplings between nearby nuclei. Structures of the same macromolecule determined by each method are essentially identical. However, each technique presents its own advantages and disadvantages and, together, the two techniques are highly complementary. In practical terms, NMR spectroscopy is limited in the size of the protein investigated, with structures <30 kDa being relatively routine and structures up to around 100 kDa anticipated. On the other hand, X-ray crystallography has been successfully utilized to determine the structures of very large macromolecular complexes such as a virus or the large ribosomal subunit. A final important difference involves static versus dynamic views of protein structure. X-ray crystallography excels at providing high resolution detail of the equilibrium, or most stable, state of a macromolecule. NMR structures are normally more dynamic and indicate regions of the structure that have multiple conformations. While this is also possible in crystallography, the end result of an X-ray structure looks more like a static macromolecule. Together, these two views are highly complementary in that knowledge of both the preferred molecular conformation and the possible dynamic excursions around this low energy state are important to understanding molecular function.

The final output of a structure determination project consists of a set of Cartesian coordinates for every atom in the macromolecule, i.e. a listing of their relative positions in the commonly named X, Y and Z geometric axes. Armed with an understanding of the covalent connections between protein atoms, these spatial coordinates allow construction of three-dimensional visualizations of the overall molecular structure. Whereas the "ball and stick" representation (Fig. 1a), localizing each atom (ball) and covalent bond (stick), provides the greatest wealth of detail about the molecular structure, it fails to convey important patterns about the overall structural topology. The simpler "ribbon diagram" (Fig. 1b–d) illustrates only the backbone secondary structural elements, which together assemble into an overall topological pattern. The basic elements of protein ►secondary structure include alpha-helix, beta-sheet, loop and coil regions. Initially produced as a linear chain of amino acid residues, the secondary structure folds to form the ►tertiary structure of the protein. Two or more linear chains or subunits are sometimes necessary to form the final ►quaternary structure. Despite the evolution of tens of thousands of different proteins in the human genome, it appears that far fewer structural topologies are required to carry



Structural Biology. Figure 1 Graphical depictions of protein structures. (a) "Ball and strick" representation of human growth hormone (PDB code 3HHR) compared to (b) a bockbone "ribbon diagram" of the same protein, which more clearly demonstrates its four helical bundle structural topology. Also illustrated are two additional examples of protein structural topologies: (c) the beta-sandwich from human fibronectin (PDB code 1FNF) and (d) the alpha-beta barrel, represented by human triosephospate isomerase (PDB code 1HTI).

out all the necessary functions of human biology. Examples of these fundamental three-dimensional topologies are the four alpha-helical bundle (Fig. 1b), the beta-sandwich (Fig. 1c), and the alpha-beta-barrel (Fig. 1d). Additional examples of experimentally determined protein structures can be found in the ►Protein Data Bank (PDB, http://www.pdb.org), a centralized, public database of nearly all published macromolecular structures. The PDB is a powerful resource for researchers, students and teachers alike, providing graphical tools for visualization of protein structures along with a variety of accompanying genetic, structural and functional data on each submitted molecule.

Macromolecules serve as the cell's machinery and are responsible for a vast majority of the biological activities necessary for life. The atomic level description of macromolecules derived from structural biology provides the framework for defining the fundamental mechanisms by which these molecular machines function. Oftentimes, a newly determined structure of a long-studied macromolecule provides critically needed explanation of previous experimental results. Other times, structural analyses of newly discovered proteins provide important insights into their potential biological functions and serve as a powerful guide for future investigations. The catalytic mechanisms of protein enzymes or nucleic acid ribozymes can be aided by observation of the chemical groups located in the active site of the macromolecule, generally in a series of structures with separately bound reactants, products and intermediates of the reaction. Similarly, the molecular basis of allostery, such as occurs for the binding of multiple ligands to hemoglobin, can be elucidated by comparison of multiple, differently ligated structures. Analysis of transmembrane-spanning and membrane-associated receptor proteins reveals the structural basis of cell-cell communication.

One of the most illustrative case studies of the important role played by structural biology in cancer

research involves the tumor suppressor ►p53 protein. Inactivating mutations in p53 are seen in over 50% of all tumors, highlighting its central function as a cell cycle regulator and tumor suppressor. The p53 polypeptide contains four distinct domains, which vary in their structural stability and their degree of conformational mobility. The most stable region is located in the central portion of the chain and folds into a DNA-binding domain (DBD). A vast majority of inactivating mutations are located in this domain and a combination of X-ray crystallographic structural models of the DBD complexed to nucleic acid along with complementary NMR studies have revealed the precise molecular interactions involved in transcriptional activation. Inactivating mutations directly interfere with DNA-binding, structurally destabilize the DBD, or disrupt critical protein-protein interactions required for cooperative binding of p53 oligomers to DNA. The first 100 residues in p53 are natively unfolded, as demonstrated by NMR and other complementary biophysical techniques. However, portions of this unstructured region are believed to undergo disorder-to-order transitions upon binding various regulators of p53 function. For example, co-crystallization of the p53-binding domain of MDM2, a critical regulator of p53, with its recognition sequence located in the p53 N-terminal domain reveal a helical structure for these residues. Lastly, a large body of structural studies on the "tetramerization domain," located C-terminal to the DBD in p53, have elegantly demonstrated the importance of p53 oligomerization in its function as a transcriptional activator. The tetramerization domain forms a four helical bundle with pairs of beta strands connecting adjacent helices, in what is often referred to as a "dimer of dimers" arrangement. In solution, p53 exists as mixture of dimeric and tetrameric forms in equilibrium, with the tetrameric form preferred for recognition of its DNA consensus sequence. Hence, knowledge of the structural properties of p53 has contributed to a better understanding of its function and has aided the rational design of potential anti-cancer agents, which function by enhancing the tumor suppressing activities of p53.

In the remaining portion of this essay, we will review how the three-dimensional structure of a target involved in cancer, whether a protein or nucleic acid, can be used in a variety of ways to contribute to the development of an anti-cancer drug. We will consider three different methods: (i) the screening of a target by libraries of small molecules using computational techniques, also called *in silico* design, (ii) the use of "molecular fragments" or small molecules in crystallography or NMR, respectively, to design novel compounds, and (iii) optimization of a lead compound or the study of a known therapeutic to meet unmet clinical needs.

If no small molecule inhibitors are initially available, the functional site involved in inducing cancer can directly reveal important properties – such as the volume of the functional site, the electrostatic potential of the macromolecule, atoms that can act as hydrogen bond donors or acceptors, and the overall hydrophobicity of the site – that are important for identifying a drug. Sophisticated software has been developed for ►small molecule screening of millions of compounds into this site and score the best fit of each molecule with respect to all molecules in the library. The molecules predicted to bind most tightly can then be experimentally screened against the target protein. If any of these predicted compounds have the desired chemical or biological effect, they can be used to develop a molecule that goes into clinical trials against a specific tumor or cancer.

An alternative experimental approach is to use either organic solvent molecules (in crystallography) or multiple molecules that bind to adjoining sites (in NMR) to design specific, novel molecules that bind to the active site. The crystallographic approach is known as multiple solvent crystal structures (MSCS) and attempts to find functional groups (such as amides, various alcohols, demethylformamide, acetone, etc.) to create a chemical map of where these functional groups can bind in the active site. The information gained from this approach is used by chemists to design small molecules that incorporate the functional groups in the exact position to create a new therapeutic agent. An analogous approach in solution is known as SAR by NMR. In this case, larger molecules can be used that bind to adjoining regions and can be chemically linked to create a specific compound for the molecular target.

When the structure of an approved drug is studied in complex with its molecular target, it reveals how it binds and what would be necessary to improve potency, if it becomes necessary. A perfect example of this is the ►small molecule drug Gleevec, which is used to specifically target a specific protein that causes chronic myelogenous leukemias. In some patients, mutations occur in the protein that leads to resistance to Gleevec. To overcome this problems, the three-dimensional structure of Gleevec bound to its target, ►bcr-abl, and mutants of this protein have been determined, which has led to both an understanding of how the mutants avoid binding Gleevec and the development of new compounds that are effective against the mutant protein.

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Structural Maintenance of Chromosomes Protein

Definition

SMC; Protein complexes consisting of structural maintenance of chromosomes (SMC) are critical for the accurate segregation of chromosomes during cell proliferation cohesion and condensin are two members of this family. Cohesion is required to hold sister chromatids together during mitosis while condensin is required for proper organization of mitotic chromosomes to allow segregation.

►S-Phase Damage-Sensing Checkpoints

Structural Vascular Stabilization

►Vascular Stabilization

STS

►Steroid Sulfatase

Stuart-Prower Factor

Definition

►Factor X.

Subacute Toxicity Studies

Definition

►Repeat Dose Toxicity Studies

Subarachnoid Space

Definition

Is the space between the brain surface and arachnoid tissue layer filled with cerebral spinal fluid.

►Convection Enhanced Delivery (CED)

Subarachnoidal Spread

►Leptomeningeal Dissemination

Subcellular Compartments

Definition

Parts of the intracellular space divided by various organelles, most of which are enclosed by limiting membranes.

►Modular Transporters

Suberoylanilide Hydroxamic Acid

Definition

SAHA; Is an inhibitor of ►histone deacetylases, undergoing clinical trial.

►Vorinostat

Substrate Channeling

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Synonyms

Enzymic mouth to mouth feeding

Definition

The preferred local utilization within a protein complex of a locally synthesized substrate by shielding the nascent substrate from a molar excess of the same moiety in the surrounding bulk solution. The converse also occurs where that moiety is preferentially drained by an intracomplex "substrate steal" mechanism depriving the complex of essential substrate. The direction of the flux also depends on the accessibility of the relevant precursor pools. Membrane-linked multienzymes in the glycolysis pathway attached to red cell transporter proteins and the fatty acid synthase multienzyme assembly are key examples. Reference [1] and its recent update from the same group (J Biol Chem in press) provide a good example.

The relationship to cancer follows a newly discovered example that utilizes a protein–protein shield that includes one isoform of the first metastatic suppressor ever to be discovered, nucleoside diphosphate kinase (NDPK, nm23, awd) in a complex that can also include ►G proteins. This is described in reference [2] and a comprehensive set of reviews in reference [3]. Briefly, depending on the subcellular site of the complex, the isoform of NDPK and cell type, interacting components (effectors) may include an ion channel in the membrane (e.g., potassium channels). Mitochondrial examples are also known that may maintain *trans*-matrix flux across the inner/outer membranes to/from the cytosol during high demand. Substrate channeling makes it possible to repeatedly create local shielded products and arranges their flux toward or away from a process or organelle without local substrate build-up or deficiency.

Characteristics

Molecular collisions in a liquid such as a cytosol or in a semiliquid lipid membrane typically occur at over one billion per second, which generates a problem for the fidelity of precursor–product relationships in protein–protein-based reactions. These reactions require repeatedly identical products from a pair of substrate reactants say, for example, small ligands at millimolar levels collide in the surrounding water phase that itself exists at a concentration of 55,000 times excess over solute. These solute concentrations are assumed to be even throughout the cell cytoplasm. Substrate channeling challenges that notion.

The Key Features

1. A multiprotein complex contains one or more effector proteins controlling function(s) essential for cell viability (critical nodes of interacting proteins – viability interactomes) that bind one or more interacting regulatory partners involved in sensing some substrate flux. The idea posits a subspace environment (microcytosol) in the interstices of the interface between the protein components forming a shield against the bulk phase 55M water. See latest work from Sariban-Sohraby's group (in press 2007).
2. A relay signal may or may not link to feed the complex of proteins that reflect the prevailing bulk cytosol status. However, small molecules dissolved in water outside the complex do not have unfettered access to the interstices of the multiprotein complex (restricted gate).

The Prototypical Paradigm

Individually, one such protein component (NDPK) of one such complex has been repeatedly linked to the spread of different cancers either by mutation of specific residues that are either (i) known to be permissive for binding/phosphorylation interactions (protein kinase CK2 and NDPK) or (ii) by virtue of altered absolute levels of each component (disordered concentrations of either NDPK or CK2 are linked to cancer). The exact partner protein stoichiometries are unknown but examples exist in cytosol and membrane with different effectors constituting the complex such as ion channels. Thus the proposed sensor complex has the potential to utilize more than one effector protein (ion channel, G protein etc.).

Each effector (output enzyme) shares the property of slotting into some unknown surface of interaction between complexed protein kinases. One kinase is constitutively active when isolated from cells – a necessary prerequisite for a sensor! This cancer-related kinase is CK2 (formerly casein kinase 2); the second is the above dual serine–histidine kinase NDPK (NDPK-A or nm23-H1). In vitro data suggest that AMP activated kinase is also present with NDPK but the function is unknown. AMPK is occasionally misnamed by some authors as AMP-dependent kinase but AMPK was deliberately given the name AMP activated kinase by DG Hardie because of its substantial AMP-independent activity. These three kinases are always active to some degree in a resting cell replete with ATP.

Details

Epithelial Membrane. CK2 also controls the lipid transporter ABCA2 and the efflux pump function of another ABC protein (the MDR protein). Heterotrimeric G proteins receive histidine phosphate on their beta subunits directly from the high energy phosphohistidine on one isoform of NDPK. This idea is similar to control of potassium channel function by direct transfer of phosphohistidine to the target potassium channel, for example, and its removal by a local phosphohistidine phosphatase.

Mechanisms

NDPK not only transphosphorylates nucleoside diphosphates using a high energy phosphohistidine

intermediate (38 kJ/mol, compared with 8 kJ/mol for phosphoserine or phosphotyrosine) but can make this energy available for transfer to a G protein or a K channel. The key authors in the field may be found at www.dundee.ac.uk/mchs/ndpk.

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Subtractive Hybridization

Definition

A technique based on differential hybridization, linker-determined ►PCR and sequencing for determining differences in gene expression under two different conditions or between two tissues.

►Class II Tumor Suppressor Genes

Subtype AML-M7

►Acute Megakaryoblastic Leukemia

Subunit Vaccine

Definition

A vaccine that uses merely one component of an infectious agent, rather than the whole, to stimulate an immune response.

Sugar-Remodeling System

Definition

One of the experimental systems for analyzing the biological functions of oligosaccharides. Cells or animals are manipulated by gene transfection or ►knock-down, leading to changes in their oligosaccharide structures.

►Fucosylation

Sugarbaker

Definition

Peter Sugarbaker, a surgeon from the Washington Cancer Institute, Washington DC, USA, who pioneered intensive loco-regional multi-modality therapy in the management of peritoneal surface malignancies. This is also referred to as ►cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.

SUI1 Domain

Definition

In budding yeast, SUI1 is a translation initiation factor that along with eIF-2 and the initiator tRNA-Met, directs the ribosome to the proper translation start site.

►MCT-1 Oncogene

Suicide Gene

Definition

Genes used in ►cancer gene therapy or ►oncolytic virotherapy encoding proteins that convert non-toxic prodrugs in toxic derivates. A gene when expressed in cells induces cell death. For example, *herpes simplex virus-thymidine kinase* gene. Cells expressing the

thymidine kinase gene phosphorylate a prodrug ganciclovir and phosphorylated ganciclovir kills the cells.

►Oncolytic Virotherapy
►Oncolytic Adenovirus
►Suicide Gene Therapy

Suicide Gene Therapy

Definition

Refers to the two-step process of delivering a gene to tumor cells that is able to transform a non-toxic prodrug into a toxic metabolite and thus killing the cells. Transfer of a gene which, when expressed in cells, activates a normally non-toxic prodrug to a toxic form.

►HSV-TK/Ganciclovir Mediated Toxicity
►Pannexins

Sulcus

Definition

plural: sulci; Is a normal anatomical feature of the brain and represents the groove or fissure of the brain matter seen on gross inspection.

►Convection Enhanced Delivery (CED)

Sulfasalazine

Definition

Sulfasalazine is used to treat bowel ►inflammation, diarrhea (stool frequency), rectal bleeding, and abdominal pain in patients with ulcerative colitis, a condition in which the bowel is inflamed. Sulfasalazine is in a class of medications called anti-inflammatory drugs. It works by reducing inflammation inside the body.

Sulfatases

Definition

Enzymes hydrolyzing sulfuric acid esters. They contain the unusual amino acid formylglycine in their active centre. Formylglycine is post-translationally formed from cysteine (primarily in eukaryots) or serine (mainly in bacteria). Together with sulfotransferases, which form sulfuric acid esters, they regulate local and systemic levels of various hormones and the sulfation/*N*-sulfonation state of glycan structures.

►Sulfotransferases

Sulfate Group

Definition

Is a polar, very water soluble ester of sulfuric acid group that is chemically attached to a drug in a ►phase II metabolism reaction.

►ADMET Screen

Sulfatide

Definition

Galactosylceramide with a sulfate ester on C-3 of the sugar, a strongly acidic sphingolipid.

►Sphingolipid Metabolism

Sulfation

Definition

The process of adding sulfate groups as esters to proteins or other biomolecules. Sulfation is one of the main types of post-translational modifications made

during the protein synthesis process in eukaryotes in addition to ►Glycosylation and ►Phosphorylation.

►Osteopontin

6-Sulfatoxymelatonin

Definition

The major metabolite of melatonin resulting from the hydroxylation of melatonin followed by its sulfation in the liver; it is excreted in the urine.

►Melatonin

Sulfokinases

►Sulfotransferases

Sulforaphane

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Synonyms

4-Methylsulfinylbutyl isothiocyanate; (-)-1-Isothiocyanato-4(R)-(methylsulfinyl) butane

Definition

Sulforaphane belongs to the isothiocyanate family. It is a hydrolysis product of glucoraphanin, a glucosinolate found in broccoli. Its molecular formula is $C_6H_{11}NOS_2$, and its molecular weight is 177.29 Da. Glucoraphanin is also known as 4-methylsufinylbutyl glucosinolate. Sulforaphane is the aglycone breakdown product of glucoraphanin, also known as sulforaphane glucosinolate (SGS). Glucosinolates are beta-thioglucoside-N-hydroxysulfates and are primarily found in cruciferous vegetables (cabbage, broccoli, broccoli sprouts, brussels sprouts, cauliflower, cauliflower sprouts, bok choy, kale, collards, arugula, kohlrabi, mustard, turnip, red radish and watercress). Young broccoli sprouts and young cauliflower sprouts are especially rich in glucoraphanin. The enzyme ►myrosinase present in cruciferous vegetables converts glucoraphanin to sulforaphane upon damage to the plant (such as by chewing).

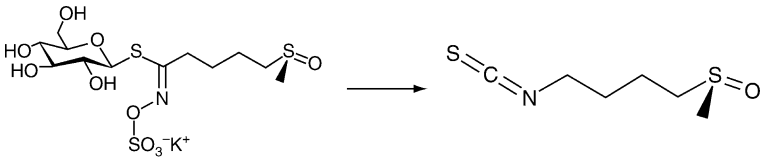
Characteristics

Actions and Pharmacology

Sulforaphane has anticarcinogenic activity in laboratory animals that are exposed to chemical carcinogens. It inhibits mammary gland carcinogenesis in rats induced by ►dimethyl benzoanthracene, colon tumorigenesis in rats induced by ►azoxymethane, and lung carcinogenesis in mice exposed to tobacco carcinogens, ►benzo(a)pyrene and ►4-(methylnitrosamino)-1-butanone (►NNK) (►Carcinogenesis, ►carcinogen metabolism, ►chemical carcinogenesis). It appears to be effective during both initiation and post-initiation (progression) stages. In addition to other cell lines, for example human colon cells, studies have also shown that in prostate cancer cells, sulforaphane can induce ►cell cycle arrest and ►apoptosis, suggesting its potential in preventing prostate cancer. However, so far no tumor bioassays in animals have been reported to test its chemopreventive activity for prostate cancer. It also has anti-bacterial activity as consumption of broccoli sprouts is effective at inhibiting *Helicobacter pylori* growth with sulforaphane being at least one of the active agents.

Mechanisms of Action

Several possible mechanisms have been studied for the chemopreventive activity of sulforaphane, notably inducing phase II detoxification enzymes, such as glutathione S-transferase and quinone reductase [NAD(P)H: (quinone-acceptor) oxidoreductase] (►Detoxification). These enzymes may confer protection against certain carcinogens and other toxic electrophiles, including reactive oxygen species. In addition to phase II enzyme induction, the induction of apoptosis and cell cycle arrest and enhancement of the transcription of tumor suppressor proteins are also likely mechanisms. Numerous studies have shown that SFN activates multiple signaling pathways in various cell lines involving protein kinases. Chief ones among them are the mitogen-activated protein kinase (MAPK) family of serine/threonine kinases, including ERK1/2, JNK1/2 and p38 that play an important role in cell proliferation and apoptosis in response to stimuli and ERK activation mediates PEITC or SFN-induced apoptosis in PC-3 cells. SFN has been shown to induce apoptosis via a p53-dependent or – independent



Glucoraphanin (–)-(R)-sulforaphane

Sulforaphane. Figure 1 Hydrolytical conversion of glucoraphanin to sulforaphane mediated by myrosinase.

pathway. Together, these studies show that ITCs can modulate the kinase pathways and transcription factors, often in a cell-specific manner. As an electrophile, sulforaphane can covalently modify thiols in proteins. The binding to cystein residues in critical proteins has been suggested to underlie its functions as a phase II enzyme inducer and may be one mechanism by which it induces apoptosis. However, detailed molecular mechanisms for the apoptosis induced by sulforaphane remain to be investigated.

Metabolism and Pharmacokinetics

Few studies have been conducted in humans on sulforaphane or its glucosinolate, glucoraphanin. However, broccoli is a rich source of sulforaphane, containing 44–56% of glucoraphanin. It has been used as a substitute of sulforaphane in several human studies to understand how it is metabolized. Like other ITCs, a major route of metabolism of sulforaphane is via the mercapturic acid pathway, involving first conjugation with glutathione (GSH) followed by enzymatic degradation to subsequent cysteinylglycine, cystein and finally N-acetylcysteine. The N-acetylcysteine conjugate is then excreted in urine as a major metabolite of sulforaphane. The bioavailability of sulforaphane from fresh broccoli is approximately three times greater than that from cooked broccoli, in which myrosinase is inactivated. Considering the cancer-chemopreventive potential of ITCs, cooking broccoli may markedly reduce its beneficial effects.

Summary

Results from cell culture and animal studies indicate that sulforaphane can inhibit chemical-induced carcinogenesis (chemical carcinogenesis), however, little direct evidence is available to support its role in protecting against cancers in humans. Epidemiological studies have shown an association of broccoli intake with a reduced risk of colon cancer. Although the active ingredients responsible for the protective effect were not identified, based on results of the animal and cell culture studies, sulforaphane may be involved.

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Sulfotransferases

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Synonyms

Sulfokinases

Definition

Sulfotransferases transfer the sulfo (SO_3) group from a donor substrate to a nucleophilic site of an acceptor substrate (Fig. 1). Whereas 3′-phosphoadenosine-5′-phosphosulfate (PAPS) serves as the sulfo donor for all eukaryotic sulfotransferases studied, the acceptor substrates are highly variable (small endogenous and ►xenobiotic molecules as well as various macromolecules) depending on the individual sulfotransferase enzyme. Sulfotransferases must not be mistaken for ►sulfatases. The latter enzymes hydrolyse sulfo conjugates. Together with sulfotransferases they regulate local and systemic levels of various hormones and the sulfation state of glycan structures.

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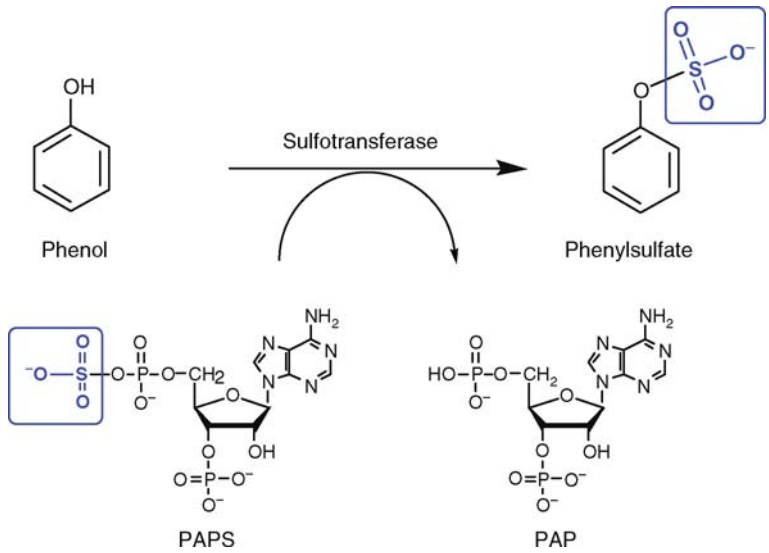


Sulfotransferases. Figure 1 Sulfotransferase reaction illustrated for phenol as the acceptor substrate. In this case the sulfo group is transferred to an oxygen atom, generating a sulfooxy (sulfate) group. Other possible acceptor sites are amino and – rarely – thiol groups, leading to the formation of sulfoamino and sulfothio groups, respectively.

Characteristics

Sulfotransferases can be classified on the basis of the sulfo acceptor: (i) glycan structures of proteoglycans; (ii) carbohydrates of glycolipids; (iii) tyrosine residues of proteins; (vi) small molecules. The amino acid sequences and the gene structures are very different between these four classes.

Proteoglycan carbohydrate sulfotransferases modify sugar and aminosugar residues of glycoproteins. Nearly 30 forms are present in the human. Most forms are localized in membranes of the Golgi apparatus. Some classes of products, such as the glycosaminoglycans (heparan, chondroitin, dermatan and keratan sulfates), are modified in many different positions. This complex and variable modification requires several different sulfotransferases (including combined *N*-deacetylases/*N*-sulfotransferases). *O*-Sulfonated (sulfated) and/or *N*-sulfonated glycoproteins are important components of the cell surface and the extracellular matrix. The negative charges introduced by sulfo transfer affect interactions with other molecules, such as receptors and proteases, and can modulate their function (e.g. as co-receptors or protease inhibitors).

Protein tyrosine sulfotransferases (two forms in the human) modify tyrosine residues of proteins. The tyrosine sulfation motif is identical to the tyrosine phosphorylation motif, and both modifications involve the same increase in mass (80 Da). Nevertheless, there is no competition between these post-translational modifications, as they occur in different cellular compartments. The protein tyrosine sulfotransferases are integral membrane proteins of the Trans Golgi Network. Most tyrosine-sulfated proteins are secretory, others are incorporated into lysosomal and plasma membranes. Protein tyrosine sulfation is irreversible.

A glycolipid sulfotransferase localized in the Golgi apparatus catalyses the formation of various sulfated glycolipids, such as cerebroside sulfate (a major component of myelin) and seminolipid (found in testis).

The fourth class of sulfotransferases modifies small molecules. These enzymes are soluble proteins and are usually localized in the cytoplasm. However, some forms are enriched in cell nuclei in some tissues. Most are present as homodimers with subunit masses of 30.5–41.3 kDa (human forms). Monomeric and heterodimeric proteins may also occur. These sulfotransferases are members of a single superfamily, termed ►SULT. Using the degree of amino acid sequence identity as a guide, the SULT superfamily is further classified into families (SULT1, 2, 4 and 6 in humans), subfamilies and individual forms of subunits (13 in humans). Some SULTs play an important role in the regulation of local and systemic levels of endogenous compounds: SULT1A3/1A4 (identical protein encoded by two different genes) for catecholamines, ►SULT1E1 for estrogens, and SULT2 enzymes for various alcoholic steroids, bile acids and cholesterol (hydroxysteroid and alcohol sulfotransferases are trivial names for the SULT2 enzymes). However, all these forms also metabolize certain xenobiotics with high efficiency. ►SULT1A1, the most abundant SULT form in humans, is characterized by very broad substrate tolerance

towards xenobiotics and a moderate catalytic efficiency for various endogens substrates (estrogens, iodothyronines and catecholamines), for which it may serve as a backup enzyme in the absence of more specific SULT forms. The primary functions of the remaining human SULT forms is less clear, although a number of endogenous and xenobiotic substrates have been identified, except for SULT4A1, a form specifically expressed in brain. Not only no substrate has been found for this form; it also lacks the ability to bind PAPS, the common co-substrate of sulfotransferases. The amino acid sequence of SULT4A1 is conserved to an unusually high extent between different mammalian and other vertebrate species, suggesting an important, hitherto unknown function. Other forms may also have undetected functions in addition to their sulfo transfer activities.

With the striking exception of SULT4A1, the SULT superfamily shows high evolutionary dynamics. While a single SULT1A gene has been detected in all non-primate species investigated, four genes occur in the human. The non-primate enzymes (SULT1A1) metabolize both xenobiotics and catecholamines; they are supported in these functions by SULT1D1. On the contrary, humans have SULT1A enzymes specialized for catecholamine metabolism (encoded by the *SULT1A3* and SULT1A4 genes), and other forms with enhanced xenobiotic-metabolizing activity but strongly decreased affinity for catecholamines (SULT1A1 and SULT1A2); moreover, the SULT1D1 gene is degenerated to a pseudogene in humans. An opposite situation is found for the SULT2A subfamily, which comprises a single form in humans, but at least three different forms in mice and rats. Sexual dimorphism of hepatic expression is another species-dependent characteristic of many SULTs. This dimorphism is dramatic in young adult rats, with Sult1c1 and Sult1e1 exclusively expressed in males and the various Sult2a enzymes exclusively or predominantly expressed in females. Such sexual dimorphisms are absent or minute in humans, much below the interindividual variations within the sexes.

Sulfotransferases and Cancer

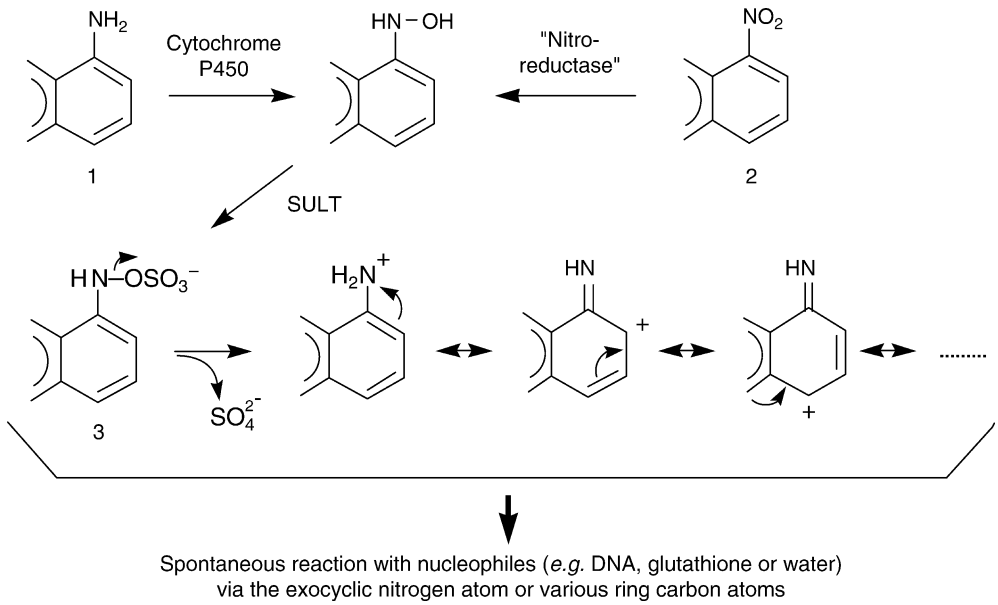
The expression of many sulfotransferases strongly depends on the type and the differentiation state of cells. Changes in levels and patterns of proteogylcan carbohydrate sulfotransferases, glycolipid sulfotransferases and SULTs have often been reported between normal, preneoplastic and neoplastic cells, but without elucidation of the causal relationships. In principle, sulfation-dependent alterations in cell-cell, cell-matrix and protein-protein interactions on the plasma membranes could trigger the process of carcinogenesis. More specific connections with carcinogenesis can be made for SULTs, as they metabolize hormones, carcinogens and anti-cancer drugs.

Estrogen-dependent tissues are major sites of the expression of SULT1E1, which probably is used for tuning the estrogen responsiveness. In the endometrium, SULT1E1 expression strongly varies during the menstrual cycle, roughly in an anti-parallel manner to the expression of the ►estrogen receptor, and is up-regulated in pregnancy. SULT1E1 is present in normal mammary cells, but not in tumor cells. This difference may lead to an increased local level of active estrogen hormones in tumor cells.

Numerous chemical carcinogens require metabolic activation to electrophilically reactive intermediates that can covalently bind to DNA and induce mutations. 2-Acetylaminofluorene was the first carcinogen for which an activation pathway was elucidated. It involved *N*-hydroxylation followed by sulfo conjugation. The *in vitro* findings were corroborated *in vivo*: inhibition of SULT or PAPS deficiency abolished, or drastically decreased, the hepatocarcinogenicity of 2-acetylaminofluorene and *N*-hydroxy-2-acetylaminofluorene in mice and rats. Meanwhile it is evident that SULTs are involved in the formation of reactive, mutagenic and potentially carcinogenic metabolites from many compounds. The reason is in the fact that sulfate is a good leaving group in certain chemical linkages, e.g. when the resulting cation is resonance-stabilized (Fig. 2). Thus, SULT- or *N*-acetyltransferase-mediated esterification usually represents the final activation of various homo- and heterocyclic nitro-, amino- and amidoarenes (Fig. 2). The relative importance of the various SULTs and *N*-acetyltransferases in this activation strongly depends on congener, species and tissue. Other rodent carcinogens that can be activated by SULTs to mutagens include some alkylated polycyclic aromatic hydrocarbons (e.g. 1-methylpyrene), alkenylbenzenes (e.g. safrole), nitroalkanes (e.g. 2-nitropropane), steroidal drugs (e.g. cyproterone acetate), schistosomacidal drugs (e.g. hycanthone) and anti-estrogens (e.g. ►tamoxifen).

SULT-mediated activation is not detected in standard *in vitro* mutagenicity tests, as these systems are SULT-deficient. Addition of an external SULT system is not a reliable remedy for this shortcoming, as sulfo conjugates are charged and may not penetrate into the target cells. cDNA-mediated expression of SULTs within the target cells is a more appropriate alternative. In general, a given promutagen is only activated by a small number of SULT forms, which vary for different promutagens.

In the rat, SULT expression is strongly concentrated to the liver. This is true in particular for those forms that are capable of activating many different promutagens. Indeed, SULT-dependent carcinogens usually induce liver tumors in the rat. The kidney is a potential target for those reactive sulfo conjugates that are sufficiently stable to be transferred via the circulation to this site. Various human SULTs have a much wider tissue distribution than rat SULTs. Thus, it is important to



Sulfotransferases. Figure 2 Metabolic activation of aromatic amines (1) and nitroarenes (2) to reactive sulfuric acid esters (3). In general, these esters are very short-lived in water ($t_{1/2}$ < 1 s). Note that the heterolytic cleavage is not a reversal of the SULT reaction, as the leaving sulfate group contains an additional oxygen atom. "Nitroreductase" is not a specific enzyme in mammalian cells, but a side activity of various enzymes primarily catalyzing other reactions (such as xanthine oxidase, cytochrome P450 reductase and quinone reductase).

consider other candidate target tissues in humans beside the liver and kidney.

Human SULT1A1 is able to activate a particularly wide range of diverse promutagens. However, it also forms stable sulfo conjugates from a much larger number of other xenobiotics and thus may contribute to their detoxification. SULT1A1 shows a common polymorphism, involving a ^{213}Arg/His exchange, which affects enzyme expression and activity. Furthermore, this polymorphism is genetically linked with another functional polymorphism in the neighbouring SULT1A2 gene. The incidence of various tumors significantly differed between SULT1A1 genotypes in epidemiological studies. The high-activity genotype was associated with a reduced risk in some cases and an elevated risk in other cases. Possibly, these findings reflect the dual role of SULT1A1 in detoxification and toxification of xenobiotics.

►Aminoflavone, an aromatic amine, is a new cytostatic drug entering clinical trials. It requires bioactivation by ►cytochromes P450 and SULTs (pathway 1–3 in Fig. 2). The growth-inhibiting activity of aminoflavone in 60 human tumor cell lines was primarily determined by the level of expression of SULT1A1.

The anti-estrogenic activity of tamoxifen, a drug used in the treatment of mammary tumors, is mainly due to its metabolite 4-hydroxytamoxifen, which has much higher affinity for estrogen receptors than the parent drug. 4-Hydroxytamoxifen is a good substrate of SULT1A1. Surprisingly, the high-activity genotype of SULT1A1 was associated with an increased survival time in tamoxifen-treated women with breast cancer; among patients who did not receive tamoxifen, there was no association between survival and SULT1A1 genotype. Thus, it appears that sulfation of 4-hydroxytamoxifen provides a benefit whose mechanism remains to be elucidated.

In conclusion, SULTs are involved in the metabolic activation and inactivation of carcinogens, the regulation of hormones interacting with tumors, and the activation and inactivation of some anti-cancer drugs. Sulfotransferases that modify macromolecules might also play a role in carcinogenesis, as sulfated and *N*-sulfonated macromolecules are major constituents of the plasma membrane and the extracellular matrix, which are important for cell-cell interactions.

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SULT

Definition

Acronym for the gene superfamily encoding soluble sulfotransferases.

►Sulfotransferases

SULT1A1

Definition

Most abundant soluble sulfotransferase in the human organism. It facilitates the excretion of numerous drugs and other ►xenobiotics. It also catalyses the final activation step of various carcinogens and the anticancer drug ►aminoflavone.

►Sulfotransferases

SULT1E1

Definition

Sulfotransferase form with high affinity for estrogens, primarily expressed in estrogen-dependent tissues (for tuning estrogen responsiveness) and in human small intestine (for inactivating dietary estradiol/estrone) and liver. A small number of promutagens are activated by SULT1E1 with much higher efficiency than by other sulfotransferase forms.

►Sulfotransferases

Sumoylation

Definition

Is the reversible, covalent attachment of SUMO, a ubiquitin-like protein (Ubl). Many proteins, such as transcription factors, are regulated by sumoylation. Often sumoylation acts antagonistic against ►ubiquitination, preventing proteins from being degraded. Sumoylation is important for the regulation of DNA damage repair and for the maintenance of genome integrity. There is evidence that sumoylation is involved in cancer metastasis.

Sun Light Induced Cancer

►Photocarcinogenesis

Sunbeds

Definition

The device used for tanning with an artificial source of ►UV radiation may be referred to as a sunbed, a sunlamp, artificial UV, artificial light, a tanning bed, among other terms. Also, a number of terms are used to define a place where indoor tanning may occur: solarium, tanning salon, tanning parlor, tanning booth, indoor tanning salon, indoor tanning facility. In addition, indoor tanning may take place in private, non-commercial premises.

Sunitinib

Definition

Is a small molecule that blocks the ►vascular endothelial growth factor receptor as well as other receptors. It has antiangiogenic and anticancer properties.

►antiangiogenesis

Superantigens

Definition
A class of antigens, including certain bacterial toxins, that unleash a massive and damaging immune response.

Supercooled Phase

Definition
Phase without ice formation, which occurs firstly when tissue is subjected to a constant lowering temperature.

►Cryosurgery in Bone Tumors

Supercooling

Definition
In cryosurgery, pure water with a subzero temperature.

►Cryosurgery in Bone Tumors

Superoxide Dismutase

Definition
SOD; Is an important intracellular antioxidant defense mechanism that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The three mammalian types of enzyme differ with respect to their intracellular localization and the metal atom within their active center (Cu, Zn, or Mn). Overexpression of SOD in cancer cell lines induces an increase in H_2O_2 production and reduces tumor growth.

►Oxidative Stress
►Photodynamic Therapy

Superoxide Radical

Definition
Chemically, an oxygen molecule to which one extra electron has been added, giving it a negative charge. O_2^-

►Nickel Carcinogenesis

Supervised Classification

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Synonyms
Supervised (machine) learning

Definition
Supervised classification is the process in which an artificial system (usually a computer program) is used to generate a predictive model (►Classifier) based on numerical feature descriptions of real-world observations (samples) that are grouped in at least two different categories (classes). The objective of this process is to establish a classifier that predicts with a minimal error the class of new samples that have not been used for construction of the classifier.

Characteristics
Clinical management and basic research of heterogeneous diseases, such as cancer, increasingly covers sophisticated technical systems and laboratory assays that may generate large volumes of high-dimensional data. A prominent example from the field of cancer research is genome-wide expression analysis using ►microarray technology, an approach that may yield tens of thousands of data points (gene-expression levels) for a single biological sample. These comprehensive analyses may reveal important information on the underlying biological phenotype of the sample and are frequently applied to identify novel ►biomarkers for disease outcome or response to therapy. However, processing, analysis and interpretation of this high-dimensional data is not trivial. Deriving

proper conclusions from such complex measurements therefore requires sophisticated computational methods in order to reliably classify the phenotype of each sample. The process in which a computational method is applied to generate or apply a diagnostic or prognostic tool for phenotype classification of a biological sample is called supervised classification.

The techniques applied in supervised classification have their origin in machine learning research and have been developed for the task of learning by examples: Certain real-world observations of interest (samples) can be grouped in different categories (classes). The attributes (features) of the samples can be described, e.g., by numerical quantification through a measurement. The central question addressed by supervised classification approaches is: "Given the features and class labels of an exemplary set of samples (training set), can a pattern of feature values be derived that is indicative of a certain class and is therefore able to accurately predict the class of new samples that were not used for learning (test set) based on their feature description?" Different techniques have been contrived for the task of learning by examples, however, the general framework for the process of supervised classification typically follows a certain standard: A learning machine (a computer program) is confronted with a data set of training samples for which the class affiliation is known and derives a predictive model. This subprocess is known as ►model selection. The predictive value of the derived model is then determined by its ability to generalize from the given set of training samples, i.e., to predict with minimal error the class of new samples that have not been used for classifier generation. This subprocess is also referred to as ►model testing.

Clinical Relevance

In cancer research, techniques for supervised classification have been used particularly for data from molecular and cell biology endeavoring to identify novel biomarkers from ►DNA microarray experiments for gene-expression profiling or comparative genomic hybridization (►ArrayCGH), ►microRNA expression analyses, mass spectrometry or ►proteinchip profiling. The endpoints predicted in such studies usually either cover the classification of disease subtypes, the prediction of patients' outcome or the prediction of response to a certain therapy. With growing experience in dealing with high-dimensional data from these techniques it has become evident that a universally best method for classifier creation does not exist. Therefore, an abundance of strategies and algorithms have been described that may be successfully utilized for supervised classification. Some of the more frequently used learning algorithms are: Linear Discriminant Analysis, Decision Trees, Support Vector Machine (SVM) Learning, Artificial Neural Networks (ANN), and the Nearest Shrunken Centroid Analysis (also known as "Prediction Analysis of Microarrays," PAM).

Caveats and Recommendations

Before tools derived from supervised classification approaches can be used in a clinical setting, it is mandatory that they are both highly accurate and reproducibly applicable. Studies that propose novel predictors by supervised classification should therefore mind the following recommendations:

1. A classifier is not sufficiently characterized by a list of its features. Therefore, a detailed description of the algorithm used and the parameters of the derived predictive model is mandatory. Only a thorough description of the application of supervised classification techniques guarantees reproducible results.
2. Proper evaluation of generated predictive models is mandatory before these models can be introduced into a clinical setting. With high-dimensional data, the number of measured features exceeds that of available samples for classifier construction by far (the feature to sample ratio is high). As a consequence, there is a big threat for "overfitting" classifiers to the unique set of samples from which they have been derived. In this context, overfitting means that although the classifier performs good results on the training set of samples (and therefore seems to "fit" this set quite well) its predictive power might turn out to be poor in an independent data set. To avoid the problem of overfitting, stringent evaluation of a generated classifier should be performed by proper (cross) validation. This implies the use of large sets of samples that have not been used for classifier generation (independent samples).

   In principle, it is advisable to follow the recommendations of Simon and Altman, who suggest classifying studies that promote new diagnostic or prognostic tools into one of three phases. Studies showing an association of a new prognostic factor with real outcome or an improved prognostic performance in comparison to established markers are categorized as phase I or II. Studies investigating the hypothesis that a newly created prognostic tool emanating from phase I and II studies can be independently validated on a large cohort of new patients are of phase III. Only classifiers that successfully pass all three study phases should be considered for utilization in the clinical setting.
3. High classification accuracy of a predictor does not imply biological relevance of its features. A big caveat needs to be put to this fact as even predictive

models with extraordinary accuracy will comprise neither all nor only features that are functionally related to the phenotype of the disease. Deducing biological importance from high discriminative power of features will be misleading in the vast majority of cases. Furthermore, utilizing different algorithms will lead to distinct predictive models, all of which may classify a given set of samples with similar accuracy although they are composed of varying sets of features.

Abiding by these recommendations may save from many of the pitfalls of supervised classification. When applied and evaluated properly, supervised classification approaches will undoubtedly play an important role in the development of improved cancer diagnostics, optimized risk stratification and more individualized, patient-tailored therapy.

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Supervised (Machine) Learning

►Supervised Classification

Supportive Care

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Synonyms

Palliative care

Definition

Supportive care refers to those services, both specialist and generalist, that might be required by people affected by cancer (patients and family members) to meet the many needs associated with cancer and cancer treatment. Thus supportive care is provided across the continuum of the cancer journey, from the point when the possibility of cancer is first raised, through diagnosis and treatment and into palliative care or survival.

Characteristics

Supportive care has been referred to as an 'umbrella' term to describe the many services involved in assisting people affected by cancer and their families to live well and manage the many needs that arise from having cancer and receiving cancer treatment. There is growing evidence that the needs of people with cancer are numerous and that despite increased awareness by health professionals about these needs they frequently remain unmet. Needs relate to many domains but are often classified as informational, physical, psychological and existential. There is also growing recognition that these unmet needs affect both short and long-term outcomes for people with cancer and their families and require more formal approaches to ensure support outcomes are improved. The increasingly organized approach to supportive care in cancer is notable in the formation of the Multinational Association of Supportive Care in Cancer (MASCC) in 1990. MASCC recognizes supportive care to encompass all aspects of care beyond those involved in direct cancer disease management and as involving a range of disciplines and specialists including doctors, nurses, social workers, psychologists, dentists, researchers, educators and others.

Supportive care encompasses many common elements of care provision such as symptom control, rehabilitation, psychological support, self help, information provision, patient education, complementary therapies, spiritual care and palliative care. High quality supportive care assists patients to continue potentially curative cancer treatment, to live well with advanced disease, to manage the return to "normal" as a cancer survivor and to focus on quality living at the end-of-life. Supportive interventions can range from pharmacological interventions such as antiemetics through to psychological interventions such as cognitive behavioral therapy. All health professionals involved in cancer care delivery will be involved in the delivery of supportive care to people affected by cancer and their families and thus such care requires a team approach.

Perhaps the most important related term is palliative care as in many ways the term supportive care seeks to widen the commonly understood elements of palliative care to aspects of cancer care not traditionally understood as requiring palliative care involvement.

Palliative care, as distinct from ▶palliative therapy (as defined elsewhere in this collection) aims to improve the quality of life of people facing life-limiting and terminal illness through provision of pain and symptom relief and attention to the broader psychosocial and existential needs. While many aspects of palliative care are applicable earlier in the course of cancer many people affected by cancer, their families and health providers find the close association between palliative care and end-of-life care a barrier to earlier engagement of these services in meeting non end-of-life support needs. Palliative care is also not recognized as extending beyond cancer treatment into survivorship. Examples of support needs where palliative care services might usefully be included in earlier parts of the illness trajectory include the management of symptoms associated with cancer treatment such as pain from chemotherapy induced mucositis and the psychosocial support of patients and families who face a crisis of meaning following the diagnosis of cancer. However it is more difficult to define other supportive needs as being palliative care, such as pre-chemotherapy education, learning to talk after a laryngectomy or managing issues such as return to work following successful cancer treatment. The rise of modern palliative care has been the catalyst for increased attention to the wider supportive care needs of people with cancer and their families but the term supportive care captures this broader focus of meeting these other support needs.

Supportive care services rely on several key elements for success: assessment of supportive care needs, discussion with patients and families about their priorities for support, referral to appropriate specialist services including the possibility of self-referral and access to appropriate health and support information to enable to patient to understand what is happening to them, to engage in self-care and to participate fully in health related decision making.

Four key principles appear to drive the development of supportive care services internationally in that they are commonly featured in the literature on this topic:

- Supportive care needs require an individualized approach
- Patients and carers are critical members of the supportive care team
- A multidisciplinary and coordinated approach is essential
- The development of an evidence base for interventions to improve supportive care outcomes

Supportive Care Needs Require an Individualized Approach

While supportive care needs are able to be identified, classified and measured amongst groups of patients with similar cancers or receiving similar cancer treatments, to be effective approaches to supporting people with these needs must take account of the person's unique situation. Providing information and support that is tailored to a person's expressed needs improves outcomes. Tailoring of supportive care services to the individual's situation means taking account of their life orientation, values, response to illness, existing support system and goals for care. What is a major problem and cause of distress for one patient or family member might be of only minor concern to another. Thus central to the provision of supportive care services is an ongoing dialogue with the person affected by cancer and their family to ensure appropriate responses to the needs they experience.

Patients and Carers are Key Members of the Supportive Care Team

Importantly supportive care is not the sole domain of qualified health professionals, as is the case in disease related treatment. Indeed patients and their families are considered to be an important component of supportive care delivery. The premise is that supportive care services should allow care to be delivered in home and community settings and that people affected by cancer should be involved in service design and in the development of novel interventions for testing in robust clinical trials.

For many people affected by cancer being at home is made possible through personal and family/friend involvement in supportive care delivery. Supportive care provided by patients and their family/friends can range from technical care such as the management of medications and central venous access devices, through instrumental and daily living care such as assistance with hygiene, mobility and household chores through psychosocial support to cope with the emotional demands of a cancer diagnosis, its treatment and ongoing adverse side effects. Cancer treatment is now largely delivered in the ambulatory setting and thus supportive care interventions increasingly focus on improving the capacity of patients and their significant others to undertake self-management of the many demands of cancer treatment.

A Multidisciplinary and Coordinated Approach

The supportive care needs expressed by people affected by cancer are varied and often complex; this complexity often requiring the input of multiple health professionals. For example, the management of the oral mucosa during chemotherapy might involve a dentist to undertake a pretreatment check and correction of caries, a dental hygienist to remove plaque build-up, a nurse to assist with daily oral hygiene reinforcement and oral assessment to minimize oral complications, enable early detection, and manage oral analgesics, a palliative care physician to manage severe pain and a dietician to assist with maintenance of the patient's nutritional

status. No one discipline will successfully manage these support needs alone.

Supportive care also often requires the involvement of several settings of care such as acute, primary and home care. Coordination of care becomes a key feature of supportive care delivery with a high need for attention to information flow and the avoidance of gaps and/or duplication of care.

In reality many of the disciplines required to be involved in supportive care, such as dieticians, physiotherapists and clinical psychologists, are not well established as specialist disciplines in cancer care in many parts of the world and are also poorly captured in the systems that fund cancer services. This means that the burden of paying for supportive care services, such as those involved in return to work programs or long-term enteral feeding, are part of the large out-of-pocket expenses faced by many people following cancer treatment.

The Development of an Evidence Base to Interventions to Improve Supportive care Outcomes

As the multiple supportive care needs of people affected by cancer have become better recognized so has the need for an evidence base to guide clinicians in how to best address these needs. The increasing focus on supportive care highlights the paucity of evidence to guide interventions to prevent or reduce supportive care needs. In the United Kingdom national guidance has been developed for supportive and palliative care but is largely based on levels of evidence from consensus or descriptive studies with few randomized controlled trials. As a result in many western countries there is an emerging emphasis being placed on the development of research capacity in supportive care. Governments, such as in Canada, the United Kingdom and Australia, recognize that appropriately used *Supportive Care Interventions* can reduce time in hospital, improve quality of life and increase patient satisfaction with health services.

One of the most critical areas of supportive care where there is a growing evidence base is in meeting the psychosocial needs of people affected by cancer, with a specific emphasis on evidence-based communication. Guidelines in this area establish a strong mandate for enhancing the training of health professionals in good communication skills, particularly around the delivery of health information and in eliciting and responding to the patient's concerns. However, even in this area much of the research has focused on women with breast cancer and may miss some of the subtleties of providing for the psychosocial needs of men and individuals with other cancers.

The increasing attention on research is evidenced by the development of specialist journals on supportive care such as Supportive Care in Cancer and The Journal of Supportive and Palliative Care and the creation of a WHO Collaborating Center for Supportive Cancer Care at the MD Anderson Cancer Center in Houston Texas. However, there remain many gaps in the knowledge on how best to prevent, minimize, manage and recover from the many adverse effects of having, receiving treatment for, living with, dying from or surviving cancer.

Conclusion

Supportive care is becoming recognized as a key component of cancer management, standing alongside medical, surgical and radiation treatments to ensure best physical, psychological and social outcomes for people affected by cancer and their families. However there are many barriers to best practice in supportive care including funding, the lack of a strong evidence base in many areas and shortages of specialist services in many disciplines. It is imperative that cancer control programs work to capture the supportive care needs of people affected by cancer and foster research into the best interventions to prevent and minimize these needs is established.

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Suppressive T Cells

►Regulatory T cells

Suppressor of Fused

Definition

Abbreviated Su(Fu) in mouse; SU(FU) in human; A negative regulator of Hedgehog signaling that regulates the nuclear translocation of the Gli transcription factors.

►Hedgehog Signaling

Suppressor of Invasion, Metastasis, and Angiogenesis

►RECK Glycoprotein

Suppressor T Cells

Definition

►Regulatory T cells.

►T regulatory cells
►Treg

Suppressors of Cytokine Signaling

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Synonyms

SOCS

Definition

Suppressors of cytokine signaling (►SOCS) and the cytokine-inducible SH2 protein CIS regulate the responses of various cells to ►interleukins and other cytokines. They are rapidly induced after treatment with a cytokine. SOCS prevent prolonged activation of the signaling pathway of Janus kinase (►JAK)/►signal transducer and activator of transcription (►STAT).

Characteristics

The SOCS/CIS family has eight members: SOCS-1, -2, -3, -4, -5, -6, -7, and CIS. They are composed of an amino-terminal domain of variable length, central ►SH2 domain, and a carboxy-terminal 40-amino-acid module known as SOCS box. Expression and function of SOCS-1, -2, -3 and CIS were characterized in most studies.

SOCS-1 and SOCS-3 downregulate JAK tyrosine kinase activity due to the presence of an inhibitory region in their amino-terminal domain. SOCS-1 could bind directly to JAK kinase through its SH2 domain, whereas SOCS-3 SH2 domain binds the signal-transduction subunit gp130 of a cytokine receptor. The SOCS box is important for interaction with the ubiquitin-transferase system. It suppresses oncogenic activity of the TEL gene fused to JAK2 and STAT phosphorylation in hematopoietic cells. This part of the molecule is involved in the degradation of the nucleotide exchange factor Vav and the papilloma virus oncoprotein E7. SOCS-1 and -3 are major negative regulators of signaling of ►interleukins (IL), in particular ►IL-6, through inhibition of prolonged activation of the JAK/STAT pathway. Mice deficient of SOCS-1 die due to a myeloproliferative disorder caused by uncontrolled ►interferon-gamma and ►tumor necrosis factor-alpha signaling. Deletion of SOCS-3 is associated with polycythemia, a premalignant form of erythroid ►leukemia.

SOCS proteins also interact with either ►insulin-like growth factor or ►insulin receptors thus diminishing the effect of these antiapoptotic molecules. Both regulators of cytokine signaling degrade insulin receptor-substrate-1 and -2 thus inhibiting action of the ►insulin-like growth factors in target tissues.

CIS and SOCS-2 bind to phosphorylated tyrosine residues on activated cytokine receptors. Cytokines which induce ►STAT5, such as ►prolactin, ►erythropoietin, IL-2, -3, and growth hormone signal via upregulation of CIS. Similarly, SOCS-2 acts as a ►negative feedback regulator of growth hormone signaling. SOCS-2-deficient mice present with liver hypertrophy, increase in serum levels of ►insulin-like growth factor-I, and weight.

Role of SOCS-1 in Cancer

SOCS proteins are considered tumor suppressors in most human malignant neoplasms on the basis of expression and functional studies. The expression of SOCS-1 and SOCS-3 is reduced in several tumors in which IL-6 acts as a pathogenetic factor. Mice with haploinsufficiency of the *SOCS-1 gene* develop severe liver fibrosis. In these animals, the development of ►hepatocellular carcinoma was accelerated. Expression of SOCS-1 is decreased in hepatocellular carcinoma because of frequent hypermethylation of CpG islands. It was found in 65% of analyzed liver carcinoma samples. Transfection of hepatocellular carcinoma cells with SOCS-1 cDNA resulted in a reduced growth rate and anchorage-independent growth.

►Epigenetic changes in the *SOCS-1 gene* promoter were also observed in tumors derived from gastrointestinal tract, ►lung cancer, ovary, pancreas, as well as myeloma and chronic lymphoid leukemia. SOCS-1-deficient animals increasingly develop ►colorectal tumors. Decreased SOCS expression because of changes in ►epigenetic mechanisms or increased

degradation leads to continuous activation of JAK2 and STAT thus promoting tumor growth.

JAK activation in cancer could be caused not only by IL-6 and related cytokines but also by ►Src kinase. SOCS could inhibit JAK/STAT activity induced by the cytokines of the IL-6 family but not that induced by ►Src. SOCS-1 is a negative growth regulator of cells transformed by the Kit receptor tyrosine kinase or the v-Abl oncogene. Tumorigenicity and metastatic activity of Tel-JAK2 and Bcl–Abl cells were diminished by expression of SOCS-1. Tumors that overexpress v-Abl may bypass the inhibitory effect of SOCS-1 through phosphorylation, disruption of its interaction with ►elongin, or inhibition of proteasomal (►Proteasome) targeting of JAK.

SOCS-1 expression increased in human ►melanoma in comparison to melanocytes in normal skin and melanocytic nevi. In contrast to several solid neoplasms, SOCS-1 expression correlates with tumor invasion and could be considered a progression marker in melanoma. SOCS-1 mutations were frequently detected in primary mediastinal B-cell and ►Hodgin lymphoma and are associated with increased nuclear phospho-STAT5 accumulation. Thus inadequate action of SOCS-1 in cancer leads to a hyperactivation of signaling through the STAT pathway.

SOCS-3 in Human Cancer

The role of SOCS-3 in human cancer is similar to that of SOCS-1. Its expression is decreased in lung, head and neck, and liver cancer. SOCS-3 promoter hypermethylation was frequently observed in these tumors. Deregulation of the ►STAT3 pathway was reported in cutaneous lymphoma cells in which constitutively active STAT3 and SOCS3 are simultaneously expressed. SOCS-3 levels in lymphoma were reduced by transfection of the dominant-negative STAT3. Under these experimental conditions, the cells became more sensitive to an effect of ►interferon-alpha.

SOCS-1, -2, and -3 and CIS transcripts and immunoreactive proteins are elevated in in situ ductal and invasive ►breast cancer. It seems that SOCS exhibit their action in regulation of STAT3 in tumors in cooperation with other proteins such as ►caveolin. These interactions may be critical for the outcome of STAT3 phosphorylation in breast and ►prostate cancer. Estrogenic induction of SOCS3 in breast cancer cells was blocked by the pure estrogen receptor antagonist ICI 182,780.

IL-6 causes a variety of biological effects in prostate tumors. They may include inhibition of proliferation but also promotion of survival of tumor cells and stimulation of ►angiogenesis. In prostate cancer, the expression of SOCS-3 was investigated in a number of cell lines. SOCS-3 mRNA and protein were found in cells in which there is no expression or phosphorylation of STAT3. In contrast, SOCS-3 was not detectable in LNCaP cells in which treatment with IL-6 induces STAT3 phosphorylation, growth inhibition, and terminal ►neuroendocrine differentiation. Loss of SOCS-3 in that cell line is a consequence of gene promoter hypermethylation. SOCS-3 expression is higher in samples of prostate cancer compared with those obtained from benign tissue. Upregulation of SOCS-3 in prostate and pituitary cancer cell lines was observed after treatment with an AMP derivative. Agents that elevate intracellular ►cAMP cause an inhibition of proliferation and stimulation of ►apoptosis in prostate cancer cells. siRNA approach revealed that SOCS-3 acts as a negative feedback regulator of action of hormones that induce increased cAMP levels.

SOCS-2 in Malignant Diseases

Compared with SOCS-1 and -3, there is a more limited evidence supporting global role for SOCS-2 as a tumor suppressor or promoter. SOCS-2 levels are elevated in BCR–ABL tyrosine kinase-positive in comparison to BCR–ABL-negative chronic myeloid leukemia cell lines. They also increased in patients with chronic myeloid leukemia in blast crisis. SOCS-2 is a component of a BCR–ABL-►negative feedback mechanism. It could inhibit some of its effects in leukemia cells. Leukemia cells, however, develop a resistance to inhibitory effects of SOCS-2. SOCS-2 is induced by estrogen and growth hormone in hepatoma and breast cancer cells.

In short, SOCS proteins are involved in regulation of cellular events in cancer tissue. In most tumors, their expression decreases because of epigenetic changes in respective gene promoters. SOCS reexpression in some of the cancer cell lines leads to retardation of tumor growth. In breast and prostate cancer, SOCS elevation may prevent oncogenic signal transduction through the JAK/STAT signaling pathway.

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Suprachiasmatic Nucleus

Definition

SCN; A region in the hypothalamus of the brainstem that consists of small, bilaterally paired clusters of nerve cells that comprise the central circadian pacemaker or biological clock of the brain.

►Melatonin

Supraparamagnetic Nanoparticle

Definition

A type of nanoparticle composed of iron oxide that is used as a contrast agent for magnetic resonance imaging.

►Nanotechnology

Suramin

Definition

Is a polysulfonated naphthyl urea, inhibiting ►heparanase with an IC_{50} of 48 μM. Suramin inhibited B16 melanoma cell invasion (IC_{50} = 10 μM) through reconstituted basement membrane, but had no effects on melanoma cell growth. Suramin has not been widely used because it has significant toxic effects in humans, including neurotoxicity, renal toxicity, adrenal insufficiency and anticoagulant-mediated blood dyscrasias. In efforts to avoid these side effects, analogs of suramin have been synthesized and are undergoing evaluation. Compounds NF 227, NF 145 and NF 171 are three such analogs, all of which possess heparanase inhibitory activities more potent than that of suramin (the IC_{50} values were 20–30 μM). These compounds effectively inhibited heparanase-mediated ►angiogenesis in an animal model.

►Heparanase Inhibitors

Surface-Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry

Definition

Time-of-SELDI-TOF MS; Is an approach that combines two powerful technologies, retentate chromatography and MS. The core of the SELDI-TOF MS platform is the proteinchip arrays, which have varying chromatographic properties, such as anion exchange, cation exchange, metal affinity, and reverse-phase. The SELDI-TOF MS provides on-chip separation as well as the capability to perform enzymatic reactions directly on the chip. Various complicated biological materials can be uniformly captured, concentrated, and purified on the small chemical surface of the chip. To be able to identify the proteins, the complexity of the sample was reduced by fractionation approaches. A complex mixture of proteins from cells or body fluids can be reduced to sets of proteins with common properties by binding the sample to chips with differing surface chemistries in parallel and in series.

►Proteinchip

Surface Glycoproteins

Definition

Proteins imbedded in the outer membrane of a cell that have polysaccharides attached, particularly on the outer side.

►CD Antigens

Surface Molecules

►CD Antigens

Surface Plasmon Resonance

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Definition

►Surface plasmon resonance (SPR) is one of several optical phenomena known to occur on two-dimensional metal surfaces (typically gold or silver films) when a total internal reflection of incident light occurs at the interface of two different substances, one with a high refraction index and the other with a low refraction index. The SPR ►biosensor, which exploits the SPR phenomenon, is a label-free and surface-sensitive spectroscopic system, which utilizes measured changes in the local refraction index upon adsorption. This sensor may be applicable to disease diagnostics and ►high-throughput screening (HTS) in drug discovery, as well as to studies of biomolecular interaction.

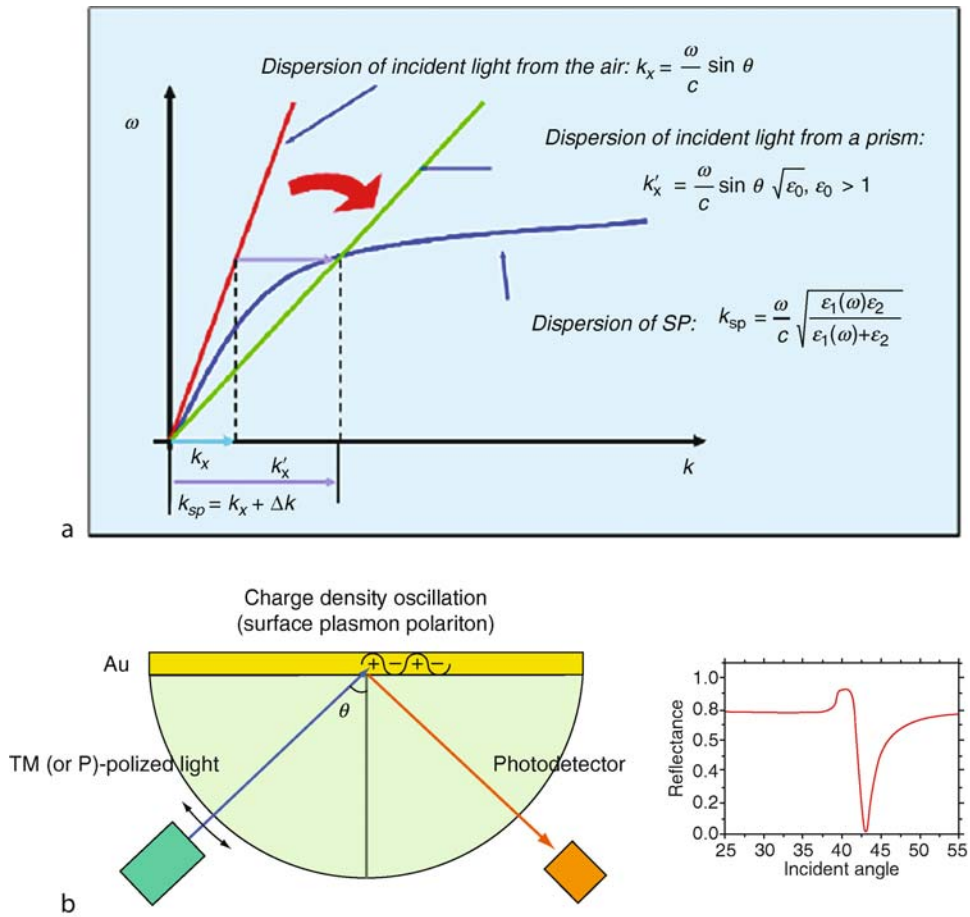
Characteristics

Detection Principle of SPR Biosensor

Surface plasmon polaritons (SPPs), which are also referred to more simply as surface plasmons (SPs), are longitudinal and collective oscillations of electrons occurring on a metal-dielectric interface. The frequency of these longitudinal oscillations are linked to the wave vector (k_{sp}) by a dispersion relation. The incidence of light can induce the excitation of the SPs when the momentum of the SPs matches that of the incident light. In such cases, the well-characterized surface plasmon resonance phenomenon occurs. As SP waves are inherently longitudinal, the waves can be coupled only with the transverse magnetic (TM) mode of electromagnetic waves, the polarized direction of which is parallel to the incident plane. It has been determined that this wave-matching condition can be quite readily disrupted by even miniscule changes in the interface conditions. Hence, in cases in which the light excitation condition is fixed, the SPR technique not only allows for the precise measurement of changes in the refractive index or the thickness of the medium adjacent to the metal film, but also enables the detection of changes in the adsorption layer on the metal surface. As is shown in Fig. 1a, the surface plasmons have a larger wave vector than do light waves of the same energy $\leq\omega$. In order to excite the SPs with photons, the wave vector of the photons must be increased. Thus far, two basic apparatus can be utilized to achieve this: (i) a prism coupler; (ii) a grating coupler. The Kretschmann geometry of the ATR method, using a prism, is currently the approach most often employed in SPR sensors. This is a special case of ATR, in which a thin metal film exists at the ATR interface. As can be observed in Fig. 1b, the light wave is reflected completely at the interface, and excites the SP via the evanescent field. Detection is thus accomplished by recording the changes in the resonance angle or the wavelength.

Properties of the SPR Biosensor

As has been thoroughly documented, the SPR biosensor is a versatile optical spectroscopic system, and represents a promising technology for the real-time, ►label-free analysis of affinity-based measurements in the fields of analytical biochemistry, experimental biology, and medicine. Following the introduction of the SPR biosensor by Jonsson et al. in 1991, the applications of SPR technology have expanded significantly, coming to encompass a wide-ranging field of topics. However, the applications of SPR technology in biomedical science are particularly salient; as many as 5,000 review and research articles concerning SPR have been published over the last 15 years. SPR-based biosensor technologies remain a subject of intensive research, and technical advances in the approach are continually opening new opportunities for the application of the general method. Numerous SPR apparatus are being developed and exploited on the basis of theoretical developments. One such promising approach is the coupling of SPR to mass spectrometry (SPR-MS), an approach that may prove to be extraordinarily useful in the field of functional proteomics. This hybrid SPR-MS system has shown itself to be a rapid and effective method for the identification of interaction partners in complex biological mixtures. The other principal SPR-associated technology involves the application of the technique to imaging systems. With regard to SPR imaging (SPRI), this system detects the change in the reflectivity of incident light, due to the binding of biomolecules to chip surfaces at a fixed angle of incidence, in contrast to SPR systems involving the detection of shifts in the SPR angle or wavelength. SPR imaging using optical array detectors appears to constitute a promising new direction in parallel or multi-channel biosensing, and thus may allow for high-throughput drug screening. Additionally, SPRI technology may also be applicable to the diagnosis of different types of disease including human cancers in the near future. Currently, commercially obtainable SPR instruments are large and expensive, and are therefore inappropriate for applications requiring portability and affordability, such as point-of-care technology (POCT). For this reason, miniaturization efforts that render the development of a portable system feasible have recently been undertaken in parallel with other components of research into more advanced SPR instrumentation.



Surface Plasmon Resonance. Figure 1 (a) The dispersion relation of non-radiative SPs, and (b) the configuration of the ATR method. See text for details.

Biomedical Applications

The most extensively employed application of SPR sensor technology is the monitoring of affinity scale in the study of biomolecular interactions. The many SPR-based affinity analysis applications currently available have become extremely significant in biomedicine and other fields. Biomedical applications of SPR can be categorized into 3 general fields; (i). Biomolecular interaction analysis, (ii). High-throughput screening, and (iii). Proteomics research.

Biomolecular Interaction Analysis (BIA)

The most common application of SPR biosensors is biomolecular interaction analysis (BIA), a critical component of protein function research. SPR technology has been applied to the monitoring of a variety of biological events, including kinetic analyses of ligand–receptor interactions, kinetic analyses of DNA binding to proteins with captured DNA, interaction analyses of enzymes with their substrates, dynamic analyses of antigen-antibody binding, epitope mapping, DNA hybridization, and real-time monitoring of DNA manipulation. In addition, the spectral SPR profile has been shown to be influenced by changes in the optical thickness of the sensor metal film, as well as by changes in the refraction index occurring near the metal surface (within ~200 nm). Because these optical indicators can be affected by structural transitions in proteins, SPR has also been utilized in the characterization of the conformational alterations of immobilized proteins upon binding to small molecules or in a variety of environments.

High-Throughput Screening (HTS)

SPR-based biosensors can be employed not only for the real-time monitoring of the kinetics of ligands with their receptors, but also in the development of pharmaceuticals, as SPR can be employed in drug screening procedures for single molecules in drug discovery studies. The application of SPR technology to high-throughput screening (HTS) is another recent trend in drug screening. SPR systems have been configured into a variety of formats, including array format, multi-channel unit format, and SPR imaging (SPRI)

format, which allow for simultaneous real-time measurement in the range of hundreds to thousands of binding reactions on the surface of a chip. Despite the profound versatility of SPR technology, SPR biosensors are also known to have a significant drawback that makes them inappropriate for high-throughput screening, as this system does not allow for the analysis of many samples in parallel. By way of contrast, SPR imaging technology using optical array detectors not only allows for high-throughput multiplex analysis, but also provides a sensitivity almost identical to that of classical SPR. Therefore, SPR imaging systems are more appropriate for high-throughput label-free detection than any other optical technique. SPR imaging methods allow for the quantitative characterization of biomolecular interactions, including DNA-DNA duplexes and DNA-drug interactions, in an HTS manner. Furthermore, the targeting of single-base mismatches in the alteration of DNA-DNA hybridization properties has been achieved in DNA arrays, using SPRI biosensors. Another technology that uses the SPR imaging system has been applied to the monitoring of real-time interactions of proteins to a DNA-patterned chip surface in a high-throughput manner. For example, this approach has been used for the high-throughput analysis of interactions occurring between the ►p53 protein and multiple DNA sequences. Recently, a novel SPR imaging-based HTS system for anti-cancer drug discovery was developed by Ro et al. in 2006. In the research, in order to determine whether the SPR imaging system was capable of screening for small molecules that inhibit protein-protein interactions, the interaction between the retinoblastoma tumor suppressor ►RB protein and the ►human papillomavirus (HPV) E7 protein was selected for use as a model system. The RB-E7 interaction was challenged by the spotting of the RB protein in the presence of the RB binding peptide (PepC). The SPR imaging results showed that PepC inhibited the RB-E7 interaction in a concentration-dependent manner, thereby indicating that SPR imaging-based HTS technology could potentially provide a versatile tool for the selection of small molecule inhibitors, via the targeting of protein-protein interactions.

Proteomics Research

One powerful method by which the biological function of most proteins can be anticipated is the identification of the interaction partners of "bait" proteins, which results in the discovery of protein biomarkers for disease diagnosis and drug screening. These functional proteomics studies, including "ligand fishing" from complex biological mixtures, can be performed efficiently using SPR biosensors coupled with mass spectrometry (MS). This combined SPR-MS system, when utilized as a tool for ligand fishing, allows for the identification of interaction partners with the desired drug candidate characteristics, biomarkers in a variety of therapeutic areas, epitopes or antibody binding sites on protein antigens, and enzyme inhibitors in extracts constructed from diverse organisms. Conventional SPR-MS approaches can be used to characterize unknown proteins that have been captured on the sensor surface, both via SPR technology and by exact direct mass measurement with ►matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS. As SPR detection is non-destructive and non-labeling, the combination of these two systems is quite relevant to possible approaches to the identification of binding partners directly after interaction analysis, followed by mass spectrometric assays. Recently, a new analytical protocol, in which SPR is coupled to ►electrospray ionization (ESI) MS, has created a new opportunity for the identification and secondary characterization of interaction partners. This system is, potentially, an extremely effective method for the identification of novel binding partners. Therefore, the combined SPR-MS system is expected to become a powerful tool in the area of quantitative analysis of functional proteomics, a field which includes large-scale "ligand fishing" assays.

►Surface Plasmon Resonance

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Surgery

Definition

Removal of the tumor by a surgeon.

Surgical Biopsy

Definition

Synonym open biopsy; Use of surgery to sample or remove tissue.

►Fine Needle Aspiration

Surgical Debulking

Definition

Synonym debulking surgery, also ►cytoreductive surgery, is used to remove just a portion of a cancerous tumor. It is recommended in situations when removing an entire tumor might damage an organ or other parts of the body. It is common for other cancer treatments, such as chemotherapy or radiation, to be used after a debulking procedure.

Surgical Menopause

Definition

Menopause that occurs when a premenopausal woman has both of her ovaries removed.

►Menopausal Symptoms After Breast Cancer Therapy

Surgical Pathology

►Pathology

Surgical Trauma and Cancer Recurrence

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Definition

Surgical trauma occurs after every surgical procedure by using traumatic tools, gauzes, and by rubbing the tissue. This causes an inflammatory response during which loads of cytokines and growth factors are produced. These factors will create an outstanding environment where tumor cells can adhere and flourish into a tumor lesion. Understanding the specific mechanism of tumor cell adhesion can lead to develop specific tools to prevent local and distant recurrence in the future.

Characteristics

The process of cancer metastasis could be compared to an exhausting obstacle race, during which the tumor cell has to pass a series of sequential interrelated steps to become a clinically relevant lesion. In distant metastasis, the tumor cell must succeed in invasion, embolization, survival in the circulation, adhere to a distant capillary endothelium, followed by extravasation and multiplication in another organ. Locoregional tumor recurrence after intra abdominal seeding or spill of tumor cells seems to be less complicated, the tumor cell has to adhere, after which it can flourish and grow out as a lesion.

Although the process of locoregional tumor recurrence gives the impression to be highly comprehensible, peritoneal and local recurrence is a persistent hurdle after curative resection of colon or pancreatic tumors. The resection site is preferable and recurrence to locoregional sites is common. Several theories on local tumor recurrence have been advocated. The most feasible theory is the development of local recurrence after resection of locally advanced disease, which already penetrates the peritoneal surface or adjacent organs. Another source for local recurrence is the presence of cancer cells in the abdominal cavity prior or during surgery. Peritoneal washings, before manipulation of the tumor, are positive in 20–30% of the patients with colorectal cancer. Furthermore, extensive manipulation of the tumor during surgery will cause leakage of tumor cells out of the dissected lesion or out of the transected lymphatic channels or veins. The free floating tumor cells or tumor emboli will precipitate on raw tissue, followed by an inflammatory response during which an outstanding environment for the tumor cells will be created.

Approximately 40% of the patients with colorectal cancer, who underwent surgery for local or locally advanced disease, develop recurrent disease. The intra-abdominal recurrence rate after curative resection for pancreatic cancer is even more deplorable. Methods of prevention and treatment of locoregional metastasis, like neoadjuvant radiotherapy, brachytherapy, adjuvant chemotherapy, photodynamic therapy, (hyperthermic) intraperitoneal chemotherapy and peritonectomy, are developed and implemented in daily surgical practice. The benefit of adjuvant chemotherapy on overall survival for patients with node-positive, locally advanced colon cancer is well established. The adjuvant therapy regimen in pancreatic cancer has not been elucidated yet. The ESPAC-1 randomized controlled trial showed no survival benefit for adjuvant chemoradiotherapy after R0 resection, however, it revealed a potential benefit for adjuvant chemotherapy. However,

a study from Smeenk et al. showed that adjuvant chemoradiotherapy, after irradical resections of pancreatic cancer, gives a significant improvement of local control, nevertheless treatment with chemoradiotherapy does not improve survival.

These (neo)adjuvant treatment strategies are mainly based on diminishing advanced disease. An adequate understanding of the pathophysiology of the tumor cell adherence is needed to clarify the initial step of implantation.

Trauma and Inflammation

Since Virchow's studies in the mid-nineteenth century, the role of inflammation and wounding as an initiator and promoter of tumor development has been implied. Virchow indicated that cancers tended to occur at sites of chronic inflammation.

The investigation aiming to clarify the relationship between inflammation and cancers first led to the determination, whether the reactive oxygen species (ROS) and nitrogen species generated by inflammatory cells, such as leucocytes recruited to the inflammatory foci to kill infectious agents, may cause mutagenic assaults and result in tumor initiation. Nowadays, it has been realized that the development of cancers from inflammation might be a process driven by inflammatory cells as well as a variety of mediators, including cytokines, chemokines, and enzymes, which altogether establish an inflammatory microenvironment.

Inflammation is a process in response to tissue damage caused by microbial pathogen infection, chemical or mechanical induced wounding. At the very early stage of inflammation, neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells present in tissues. As the inflammation progresses, various types of leucocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signaling network involving a great number of growth factors, cytokines, and chemokines.

Surgical trauma induces an acute phase response, during which tissue damage will be controlled, infective organisms will be killed and the repair process will be induced. The acute phase response is initiated by macrophages and monocytes entering the surgical traumatized site, which release proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β). TNF-α and IL-1β stimulate the production and release of other cytokines, like interleukin 6 (IL-6). These cytokines are potential directors of the expression of cell adhesion molecules (CAMs) and regulating in that way the adhesiveness between leucocytes and the endothelium. Parallel to the endothelium, the inflammatory cascade following surgical trauma in the peritoneal cavity, creates an outstanding environment for residual or spilled tumor cells to adhere to mesothelial cells.

Laboratory Investigations

The process of tumor recurrence can be separated into tumor cell adhesion and tumor growth. Initially the tumor cells have to adhere before they nurture and develop into a metastatic lesion. To study the specific pathways of tumor cell adhesion we developed an in vitro model, in which we investigated the interactions of different human colon and pancreatic tumor cell lines on mesothelial monolayers and the endothelium. Our study focuses on the influence of surgical derived inflammatory factors on tumor cell adhesion. IL-1β and TNF-α are crucial enhancing factors of tumor cell adhesion to the mesothelial monolayers and the microvascular endothelium. In addition, IL-1β and TNF-α significantly upregulated the expression of adhesion molecules ICAM-1, VCAM-1, and CD44 on mesothelial cells and endothelial cells.

These cytokines are produced during the acute inflammatory response, which is induced by peritoneal trauma and has to initiate the wound healing process. IL-1β, TNF-α, and IL-6, produced by activated leukocytes, are the major mediators of inflammation and tumorigenesis. Together they generate the production of adhesion molecules, growth factors, nitric oxide, and the activation of the NF-κB pathway. In that way the proinflammatory cytokines will stimulate tumor adhesion, growth, and invasion. Furthermore mesothelial cells have additional active participation in the inflammatory response by producing proinflammatory cytokines and in that way stimulating the expression of adhesion molecules. TNF-α has a dominant function in the abdominal cavity and might modulate the production of ILs from mesothelial cells. Production of IL-1 and IL-8 by mesothelial cells is enhanced after stimulating the cells with TNF-α. In malignant disease, high dose local TNF-α selectively destroys tumor blood vessels and thereby induces apoptosis, but when chronically produced this cytokine may act as an endogenous tumor promoter. It contributes to the modulation of the cell, i.e., CAMs, necessary for tumor spread and growth. The influence of IL-1β on tumor cell metastasis is inevitable as well. In mouse metastasis models, treatment with an IL-1 receptor antagonist significantly decreased tumor development. Additionally, IL-1β-deficient mice are resistant for developing metastases.

During the inflammatory response, PMN are attracted to the site of injury, which is mediated by chemotactic factors and proinflammatory cytokines. PMN are known to aggravate an overwhelming burst of ROS to destroy invading organisms and inducing additional tissue destruction. Furthermore this oxidative burst can be induced by TNF-α, which causes an

upregulation of the FMLP receptors (N-formyl-methionyl-leucyl-phenylalanine) on PMN. In our studies, FMLP-stimulated PMN induce a significant enhancement of tumor cell adhesion. Moreover, the ROS producing system (X/XO) exhibits an even superior enhancement of adhesion of tumor cells and this enhancement was inhibited by antioxidant scavengers.

Clinical Applications

Notwithstanding that surgery remains the treatment of choice in colorectal and pancreatic cancer, local recurrence after curative surgical resection is an incessant drawback. Different pathways of local tumor recurrence have been unraveled, nonetheless in what way can these investigational results be implemented in clinical practice?

Peroperative diminishing surgical trauma, by using less traumatic tools, gauzes, and techniques, seems to be an adequate option. The study done by Bouvy et al. showed in a rat model that laparoscopic surgery is associated with less tumor growth stimulation compared with conventional surgery, due to reduced surgical trauma. Additionally laparoscopic surgery is correlated with less immunological alterations and this may imply less local tumor recurrence as well. Lacy et al. showed that laparoscopic-assisted colectomy was associated with a significantly lower probability of tumor recurrence and a higher probability of overall and cancer-related survival in stage III cancer. Although the recent publication of Law et al. is not a randomized controlled trial, this study put forward a significant survival benefit for patients, who underwent a laparoscopic resection in stage I to III colon cancer. The two randomized controlled trials, in which laparoscopic-assisted colectomy was compared with open surgery, could not reveal this benefit concerning tumor recurrence in the laparoscopic group.

Since the expression pattern of adhesion molecules on tumor cells is tremendously divers, it is not feasible to use single monoclonal antibodies to confront spilled tumor cells. The development of cell adhesion peptides (i.e., RGD peptide) provides promising results by blocking the adherence of tumor cells to the components of the extracellular matrix. The use of RGD peptides has been expanded with preliminary results by using the RGD peptides for delivering drugs to tumor cells that express certain integrin types after which an internalization process takes place of the integrin adhesion complex.

Interfering with the inflammatory process during and after surgery is a reliable option to prevent tumor recurrence, however, wound healing is depending on this process. This interference should be very selective, otherwise the healing process will be disturbed. Interference with the inflammatory response might be accomplished by inhibiting the influx of PMN into the peritoneal cavity with antineutrophil serum (ANS) after curative resections of gastrointestinal tumors. An in vivo study showed a significantly decrease of peritoneal tumor recurrence after intraperitoneal injection of ANS.

Another pathway of interest is the influence of ROS on tumor cell adhesion. Accumulation inhibitors of ROS are superoxide dismutase, catalases, glutathione peroxidases, vitamins C and E, these antioxidant enzymes and nonenzymatic systems are engineered during stress. Since the release of ROS is enormous following surgical trauma, additional exogenous administration of antioxidants might be a therapeutical option.

An antioxidant with potential is melatonin. Melatonin, produced mainly in the pineal gland, possesses a wide spectrum of biologic activities, including its function as a naturally occurring oncostatic neurohormone by inhibiting cell proliferation, inducing apoptosis and reducin metastatic spread. Regarding the scavenger function of melatonin, a synthetic form might be of interest in prevention of tumor recurrence.

Since the discovery of NF-κB in the mid-1980s, this transcription factor has been a subject of intense investigation. The NF-κB transcription factor complex is a pleiotropic activator that participates in the induction of a wide variety of cellular and viral genes. Binding sites for NF-κB are present in the promoter region of many CAMs, cytokines, and growth factors. Antisense inhibition of NF-κB activity causes a block of cellular adhesion to the extracellular matrix, inhibition of in vivo growth of adherent cells, and inhibition of in vivo tumorigenicity in nude mouse models. Recent investigations also showed that ROS is engaged in a unique reciprocal cross talk with NF-κB. The exact mechanism has not been unraveled yet, however, remarkable is that the induction of NF-κB is abrogated by overexpression of ROS scavenging enzymes.

A promising NF-κB inhibitor is pentoxifylline (trental). The inhibition of the transcription of NF-κB might cause a suppression of CAMs and in that way a potential decrease of lung metastasis. In addition, pentoxifylline may reduce the TNF-α induced oxidative burst by reduced binding of FMLP to PMN surface receptors.

Interference with the invasion of tumor cells through the extracellular matrix is another therapeutic option. Matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, play an important role in the growth and invasion of colorectal and pancreatic cancers by degradation of the extracellular matrix. The levels of certain MMPs can be used to estimate the metastatic capacity and recurrence of disease as well as prognosis of patients. However, for effective therapy using MMP inhibitors, highly selective administration may be required. Since MMP-7 is the most critical MMP for colorectal cancer progression, developing

selective inhibitors against this protease and their administration in the early stage of disease may be worth trying.

The inflammatory sequelae enhance tumor cell adhesion the mesothelium in vitro. The pathways involved are orchestrated in a meticulous way by proinflammatory factors produced peroperatively in response to surgical trauma. Interference with the inflammatory sequelae (i.e., cytokines, PMN, ROS, adhesion molecules, NF-κB) produced peroperatively must be well balanced, without disturbing the wound healing process and the systemic immune response. Interference with these pathways may lead to specific tools to conquer the adhesion and growth of spilled tumor cells in vivo.

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Surrogate Endpoint

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Synonyms

Intermediate endpoint; Surrogate endpoint biomarker; Surrogate outcome

Definition

A surrogate endpoint is an outcome observed prior to a health outcome of interest (called the true endpoint) that is used to make conclusions about the effect of intervention on the true endpoint.

Characteristics

Role of Surrogate Endpoints in Cancer Research

The role of surrogate endpoints in cancer research depends on the purposes of the analysis. The main roles are to (i) shorten the length of time needed to evaluate a new treatment for clinical use and (ii) quickly and cheaply evaluate interventions at an early phase of development as a prelude to more rigorous evaluation.

Ideally these roles require the surrogate endpoint to be validated. As with so many terms used in the medical literature, the meaning of the term "validation" can vary among medical scientists. We define validation of a surrogate endpoint as a formal process of data analysis for determining how well the surrogate endpoint can be used to make conclusions about effect of treatment on true endpoint. Some authors use the term surrogate endpoint to mean the early outcome has been validated, based on the literal definition of a surrogate as a substitute; here we use the term surrogate endpoint to refer to an outcome that is considered as a possible substitute for the true endpoint but that still requires validation. If a surrogate endpoint is validated it can, with reasonable confidence, be used to evaluate a new intervention, but subject to very important caveats.

Surrogate endpoints can take a variety of forms related to characteristics of genes, proteins, cells, tissues, or individual health status. Surrogate endpoints have been proposed to shorten the time needed to evaluate a new cancer treatment or preventive intervention. For example three-year disease free survival has been evaluated as a surrogate endpoint for 5-year overall survival in patients in colon adjuvant ▶clinical trials and ▶prostate-specific antigen (PSA) has been proposed as a surrogate endpoint for prostate cancer recurrence but its validation has been questioned. Although adenoma occurrence has been used as a surrogate endpoint for colorectal cancer occurrence in cancer prevention trials, it has not been validated. The validation methods that are discussed below primarily refer to this use of surrogate endpoints to shorten the time to evaluate a new intervention for clinical use.

Surrogate endpoints are also frequently used to evaluate possible treatments to identify those agents that are the best candidates for more rigorous evaluation using a true endpoint. Examples of these surrogate endpoints are markers of cell proliferation and apoptosis. The use of these surrogate endpoints involves much smaller sample sizes than with a true endpoint of cancer incidence which is a major reason (besides shortening the time for evaluation) they are so attractive to researchers. The smaller sample size for the surrogate endpoints arises because the surrogate endpoint is more common than the true endpoint. The more common the endpoint, the smaller the sample size needed to detect a given percentage decrease in probability of endpoint. However the fact that the sample size is much smaller

with the surrogate endpoint than the true endpoint is an indicator that these surrogate endpoints can almost never be validated as currently used in drug development. In this situation, the underlying problem with validation is that a common outcome is not a good substitute for a rare outcome because most subjects with the common outcome do not develop the rare outcome, leading to a large amount of noise. Basically the only way to adjust for the extra noise is with a sufficiently large sample size to reliably predict the effect of treatment on true endpoint. Nevertheless, because selection of candidate ▶chemoprotectants (often termed "chemopreventive agents") is needed, these un-validated surrogate endpoints are used in cancer prevention research. Recently developed schema for assigning levels of evidence may be useful for ranking surrogate endpoints for preliminary evaluation of chemoprotectants.

A third use of surrogate endpoints is for clinical decision-making for individual patients. In this setting, the clinician measures a biomarker on a patient after the start of treatment and uses this marker to predict outcome and inform possible changes in therapy. Validation of surrogate endpoints for this use is not well developed. It is important to realize that most of the literature on validating surrogate endpoints applies to use of surrogate endpoints for evaluating the effect of treatment on true endpoint among a population. Importantly this type of validation does not necessarily imply validation for clinical decision-making.

Methods of Validation

This discussion of validation focuses on the role of surrogate endpoints to shorten the duration of cancer trials used to make a clinical recommendation. At the onset of this discussion, it is important to dispel a common misconception that a high correlation between a surrogate endpoint and a true endpoint implies the surrogate endpoint has been validated. It has been shown mathematically that even perfect correlation between a surrogate and a true endpoint does not guarantee a validated surrogate endpoint. The reason is that the relationship between the surrogate and the true endpoint can differ in different arms of the trial while still being perfectly correlated within each arm. This difference in relationships between arms could lead to incorrect conclusions about the effect of the treatment on the true endpoint when using only information about the effect of treatment on the surrogate endpoint.

When validating a surrogate endpoint it is preferable to use data from randomized trials rather than from observational studies. When data are collected from a randomized trial, each of the two key components, the effect of treatment on surrogate endpoint and the effect of treatment on true endpoint, are not biased from unmeasured baseline covariates. This is not the case when data are collected from an observational study.

There are two major approaches to validation of surrogate endpoints. One approach involves only a single randomized trial with data on a surrogate and a true endpoint. The other approach involves data from multiple randomized trials with surrogate and true endpoints. Once a surrogate is validated it would be applied to a new trial with only a surrogate endpoint.

Many early methods for surrogate endpoint validation were based on data from a single trial and relied on the Prentice criteria. The Prentice criteria, named after the criteria formulated by Ross Prentice in an influential 1989 article on surrogate endpoint validation, were developed to ensure that rejection of the null hypothesis under the surrogate endpoint implies rejection of the null hypothesis under the true endpoint. The main criterion, sometimes called the Prentice criterion, is that the distribution of the true endpoint conditional on the surrogate endpoint does not depend on the intervention. In other words, the Prentice criterion says that, for all treatments under consideration, there is a single pathway from treatment to true endpoint that goes through the surrogate endpoint, so once the surrogate endpoint is known, no other information is needed to determine the distribution of the true endpoint. With data on a surrogate and true endpoint in a single trial one can statistically test if the Prentice Criterion holds. If one statistically rejects the Prentice Criterion, the surrogate endpoint is poor. If one cannot statistically reject the Prentice Criterion, further investigation is needed because lack of rejection of a null hypothesis does not automatically imply that the null hypothesis can be accepted. One approach for validation when the Prentice Criterion is not rejected is to compute the proportion of treatment effect explained by the surrogate endpoint. This computation involves fitting two regression models: (i) a model for the effect of treatment on true endpoint and (ii) a model for the effect of both treatment and surrogate endpoint on true endpoint. The proportion of treatment effect explained equals one minus the ratio of the coefficient for treatment effect in model (ii) to the coefficient for treatment effect in model (i). For a perfect surrogate endpoint, the proportion of treatment effect explained equals one, because the surrogate endpoint captures all the information about the true endpoint. Despite the popularity of the proportion of treatment effect explained, it has many drawbacks including wide confidence intervals. However the major drawback is the difficulty in reaching any consensus as to the proportion needed to validate a surrogate endpoint. Opinions vary, and the "acceptable" threshold may differ from early phases of drug development to definitive testing for drug approval. The acceptable level may also vary among diseases.

Another approach for single-trial validation with a binary surrogate endpoint involves constructing a model for two levels of the surrogate endpoint: (i) the level observed based on the actual assignment of the randomization group and (ii) the level inferred if, contrary to fact, the person were assigned to the other randomization group. While this validation approach has attractive mathematical and conceptual underpinnings, it has not been applied in practice. Also the assumptions required for estimation are very stringent, and may not hold in many situations.

Validation methods based on data from single trials are inherently limited because they do not account for variability over trials in the mechanism by which treatment affects the outcome. This was a major criticism of a single-trial validation of prostate specific antigen as a surrogate endpoint for survival among patients with prostate cancer.

Validation methods based on data from multiple trials of surrogate and true endpoints are becoming more popular. These surrogate endpoint validation methods are sometimes called meta-analytic because, like standard meta-analyses of true endpoints in randomized trials, they combine data over multiple trials although the techniques and purpose differ. A variety of meta-analytic methods for validating surrogate endpoints have been proposed, and there is no consensus as to best approach. In fact some investigators advocate multiple approaches to meta-analytic validation.

Many meta-analytic validation methods are based primarily on two statistics for each trial, the effect of treatment on surrogate endpoint and the effect of treatment on true endpoint, which are often plotted as a set of points. A regression line is then fit to these points to construct a model relating the effect of treatment on the surrogate endpoint to the effect of treatment on the true endpoint. The simplest model is a linear regression based on least squares. More complicated regression models account for additional variability by specifying that the coefficients in the regression vary over trials according to a specified distribution. Different versions of these complex regression models are needed for different types of data (binary, survival, continuous) along with special software. Sometimes there are numerical problems when fitting these models. To apply the results to a new trial, the effect of treatment on surrogate endpoint in the new trial is plugged into the regression model to yield the predicted effect of treatment on true endpoint in the new trial. Alternatively an investigator can compute a surrogate threshold which is the minimum effect of treatment on surrogate endpoint that corresponds to a statistically significant effect of treatment on true endpoint.

Another meta-analytic approach has been recently developed for use with binary surrogate endpoints. This approach has been applied to survival data where the binary endpoint is cancer recurrence at an early time and the true endpoint is probability of overall survival to a later time. The basic idea is that each arm of each trial in the meta-analysis has information relating the surrogate and true endpoints. The same relationships can be applied to the surrogate endpoint in the new trial to obtain an estimate of the predicted effect of treatment on true endpoint in the new trial based on data from each previous trial. These estimates are averaged over predictions from all previous trials to obtain an average estimate of the surrogate-based predicted effect of intervention on true endpoint in a new trial.

Meta-analytic methods are most straightforward when each trial involves two randomized groups, with subjects in one group receiving a control treatment and subjects in another group receiving an experimental treatment that is never used as a control.

Special considerations arise when a trial has more than two randomization groups, when it is not clear which treatment is the control, or when a treatment applies to the control group in one trial and the experimental group in another trial.

There are various approaches for using regression type meta-analytic models to summarize the quality of the surrogate endpoint. One summary measure is an individual-level association, which is the squared correlation between surrogate and true endpoints after adjusting for trial and treatment effects. (Unlike the other validation measures discussed, this measure could be useful for clinical decision making for individual patients.) Another summary measure is a trial-level association, which measures the association between the effect of treatment on surrogate endpoint versus the effect of treatment on true endpoint. Related summary measures based on information theory have also been developed. Sufficiently high values of the summary measure indicate a surrogate endpoint is validated, but more guidance is needed to determine the threshold level.

Another summary measure for the quality of the surrogate endpoint in a meta-analysis is the average prediction error. To compute this measure, one trial is removed from the meta-analysis and treated as a new trial. The other trials are used to predict the effect of treatment on the true endpoint in the "new" trial. The error is the absolute value of the difference between predicted and true effects of intervention on true endpoint in the "new" trial. This procedure is repeated over all trials with a different trial selected as the "new" trial on each iteration. The error is averaged over all trials to obtain the average prediction error. The average prediction error is compared with the average clinically meaningful difference that each trial was designed to detect (which can be inferred based on the sample size of the trial and variability of the true endpoint). As a

rule of thumb, a ratio of average prediction error to clinically meaningful difference of 1/10 indicates a good surrogate endpoint (because errors due to the use of the surrogate are relatively small compared to the difference one hopes to detect), while a ratio of 1 or greater indicates a poor surrogate endpoint. Again this may vary according to how much tolerance one has for making a mistake. This in turn may vary with phase of drug development, lethality or stage of the disease, and toxicity of the intervention.

Caveats in the Use of Validated Surrogate Endpoints

Regardless of the method of validation, there is no guarantee that in a new study the validated surrogate endpoint will in fact yield the correct conclusion about the effect of treatment on a true endpoint in a new trial.

One reason for caution with validated surrogate endpoints is that the treatment under study in a new trial may have different mechanisms for affecting true endpoints than the treatments in previous trials. For example a new treatment to reduce adenomas may have a different impact on the fraction of adenomas that develop into ►colon cancer than would previous treatments. A different mechanism for the effect of treatment on true endpoint could mean that the extrapolation from previous trials, even accounting for extra variability, may be incorrect. To avoid this problem, the intervention in the new trial is often restricted to being in the same "class" as the intervention in the trials used for validation, although this is still no guarantee that the surrogate endpoint will yield the correct conclusions in a new trial. In fact even the same drug could have a different spectrum of mechanistic actions at different doses. For example beta-carotene is likely an antioxidant at low concentrations but a pro-oxidant at high concentrations and acetaminophen is a safe antipyretic drug at low doses, but is a potent liver toxin at higher doses.

A validated surrogate endpoint should also be viewed with caution when the presence of the surrogate endpoint necessitates additional treatments that could affect the true endpoint. For example a surrogate endpoint of cancer recurrence will often lead to secondary treatments designed to alter the true endpoint. If a new type of secondary treatment is adopted in a new trial, results from previous trials using the older secondary treatment will no longer be applicable.

Another caution in using a validated surrogate endpoint is that it applies only to one particular endpoint, usually the primary health benefit. In many trials harmful side effects are a serious consideration. It is possible that in the interval between the surrogate and true endpoints, the intervention can cause serious adverse effects unrelated to the true endpoint. In such circumstances reliance on the biomarker for decision-making can miss a net harm. In essence this is what happened with celecoxib for the prevention of colon cancer. In a clinical trial, celecoxib decreased the risk of incident adenomatous polyps in patients with prior polyps; but an excess of cardiac deaths occurred prior to the average time interval between polyp formation and cancer development. In summary, if a trial is terminated at the time the surrogate endpoint is observed, it may not have continued sufficiently long enough to provide information about harmful side effects.

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Surrogate Endpoint Biomarker

►Surrogate Endpoint

Surrogate Endpoints

►Clinical Cancer Biomarkers

Surrogate Outcome

►Surrogate Outcome

Survival

Is the measure of elapsed time from some well-defined point in time, such as the enrolment into a clinical trial, until an event of interest, such as death, occurs.

►Kaplan–Meier Survival Analysis

Survivin

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Synonyms

BIRC5; baculoviral IAP-repeat containing protein 5; API4; apoptosis inhibitor 4

Definition

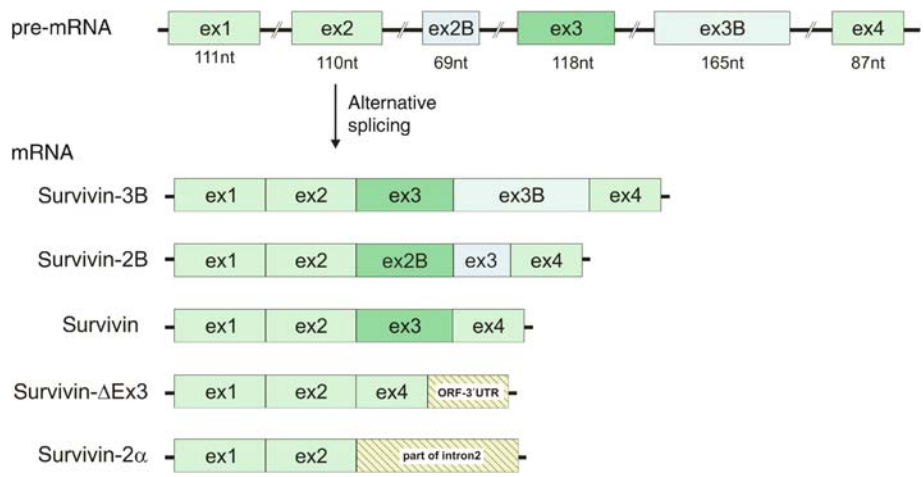
As a structurally unique member of the ►inhibitor of apoptosis protein (IAP) family, survivin is highly expressed in fetal tissues, but not in most adult tissues. Most human cancers return to the fetal pattern of survivin overexpression, thus suggesting a pivotal role of survivin for tumor cell survival.

Characteristics

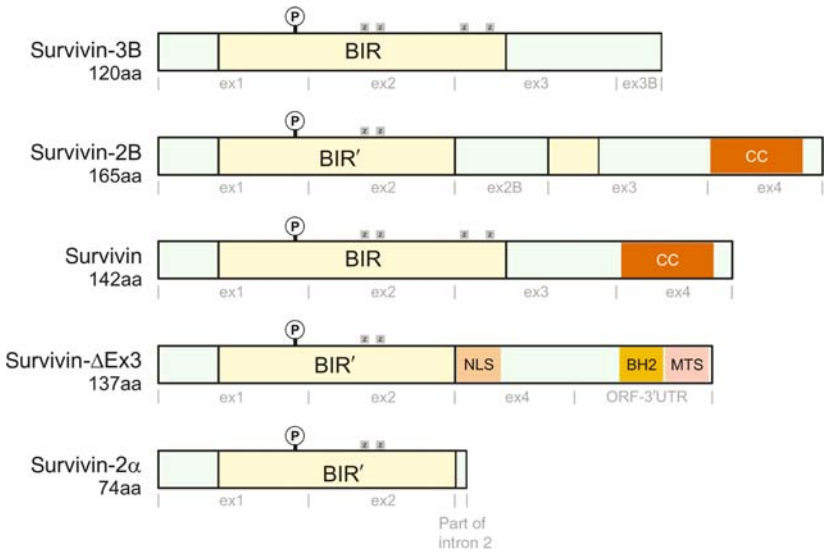
The human survivin gene is located on chromosome 17 (band q25) and encompasses 14.796 base pairs that comprise six exons. The coding strand is preceded by a TATA-less promoter and a GC-rich region corresponding to a ►CpG island. The coding sequence of survivin is largely complementary to the coding strand of the ►effector cell protease receptor-1 (EPR-1) gene. This suggests that survivin and EPR-1 transcripts originate from duplicated genes that were arranged in opposite orientations. The human survivin gene encodes five different splice variants, which may contribute to the fine-tuning of survivin actions (Fig. 1):

- Survivin is the first of five transcripts identified and consists of exon 1 (111 bp), exon 2 (110 bp), exon 3 (118 bp) and exon 4 (87 bp).
- Survivin-2B is characterized by the insertion of an additional exon 2B (69 bp) between exon 2 and 3.
- survivin-ΔEx3 shows a loss of exon 3 as well as a frame shift with extension of the open reading frame into the 3′ untranslated region.
- Survivin-3B contains an additional exon 3B derived from a 165 bp long part of intron 3.
- Survivin-2α is characterized by an addition of 197 nt of intron 2, of which 195 nt are non-coding.

The structure of the corresponding survivin proteins is unique as compared to other IAPs. Thus, most mammalian IAPs contain a carboxy-terminal RING finger domain, a caspase recruitment domain (CARD) and, most importantly, two or three copies of a baculovirus IAP repeat (BIR), a zinc finger domain essential for the inhibition of apoptosis by IAPs. In contrast, survivin proteins exhibit only a single BIR domain, an extended carboxy-terminal α-helical coil that forms a bowtie-shaped homodimer, and lack both the RING finger and the CARD region. The splice variants survivin-2B, survivin-ΔEx3 and survivin-2α exhibit pronounced structural alterations that also affect



Survivin. Figure 1 Structural organization of the alternatively spliced transcripts of the survivin gene. Abbreviations: ex, exon; ORF, open reading frame; UTR, untranslated region; nt, nucleotides.



Survivin. Figure 2 Protein domains of functional divergent survivin splice variants. Abbreviations: P, phosphorylation site at Thr34; z, zinc finger ligand; BIR, baculoviral IAP repeat; BIR′, truncated BIR domain; NLS, nuclear localization signal; BH2, bcl-2 homology domain 2; CC, coiled coil motif; MTS, mitochondrial targeting sequence; aa, amino acids; ex, exon.

their single BIR domain (Fig. 2). The BIR domain in survivin-3B is not impaired by alternative splicing. Survivin is a 16.5 kDa protein (single chain) and can act as a homodimer, while heterodimerisation with its splice variants are also described. To date, research has been mainly focused on the functional properties of the survivin protein, whereas little is known about the four different survivin variants.

Transcription of survivin shows a marked cell cycle-dependent pattern with a pronounced up-regulation in the G2/M phase (►G2M-transition). This cell cycle periodicity has been related to the presence of two Sp1 sites in the proximal promoter region. They might interact with zinc finger transcription factors of the Sp family that are also implicated in the control of other cell cycle-related genes. Suppression of survivin transcription in the G1 phase may be further regulated by a cell cycle homology region (CHR) and by three cell cycle-dependent elements (CDE) of the promoter that are also known from other G2/M-expressed genes.

Functional and Cellular Characteristics

In accordance with other mammalian IAPs, survivin antagonizes a broad range of apoptotic stimuli by inhibiting caspase-3, -7 and -9. Survivin-dependent inhibition of caspase-9 has is restricted on phosphorylation at Thr34. Other mechanisms of action in a cell cycle dependent manner are the association to the ►p53 network, as well as the binding to CDK4, and moreover, the activation of survivin by CDK1-dependent phosphorylation at Thr34 has been demonstrated. Survivin has been reported to interact with several other apoptotic factors, such as XIAP and smac/DIABLO. Moreover, many other proteins are known to also interact with survivin: HBXIP, INCENP, ►Aurora B kinase, tubulin, ►HSP90 *et alii*.

Survivin is abundantly expressed in fetal tissues as revealed by immunohistochemistry in lung alveolar epithelium, proximal tubule epithelium of the kidney, pancreatic islets, endometrial glands, intestinal crypt epithelium, thymic medulla and neurons of the spinal cord. Survivin is predominantly localized in the cytoplasm, whereas survivin-ΔEx3 is found exclusively in the nucleus.

In contrast to other mammalian IAPs, however, survivin expression is not detectable by Northern blot, in situ hybridization and immunohistochemistry in normal adult tissues, with the exception of thymus and placenta. The predominantly restriction of survivin expression to fetal tissues suggests a key role of this IAP protein for the regulation of developmental apoptosis. Of note, several highly proliferating adult cells and tissues could express increased levels of survivin possibly to escape from cell death (e.g. T lymphocytes).

Survivin actions have been located to the microtubules of the mitotic spindle apparatus. Survivin binding to the polymerized microtubules is mediated by a carboxy-terminal ►coiled-coil domain. The increased levels of survivin expression during the G2/M phase of the cell cycle might protect the mitotic apparatus from degradation. Therefore, survivin has been suggested to be an active component of the G2/M

checkpoint control that preserves chromosomal ploidy and genetic stability by induction of apoptosis in aberrant cells. The down-regulation of survivin expression in adult tissues may lower the threshold for apoptosis in replicating cells harboring genetic defects.

Clinical Relevance

Many types of human cancer, such as carcinomas of the lung, stomach, colon, breast, prostate, skin as well as non-Hodgkin lymphomas, neuroblastomas and melanomas, return to the fetal pattern of survivin expression. Survivin reexpression may be an early step of malignant transformation, as evident from its presence in precancerous lesions such as colorectal adenomas and Bowen's disease of the skin. The exact molecular mechanisms involved in the reactivation of the survivin gene in human cancers are currently unknown. Nevertheless, the overexpression of survivin in many histogenetically distinct tumor types indicates a strong selection advantage from survivin-related resistance to apoptosis. This selection advantage may result from the loss of an effective G2/M checkpoint control that permits ►progression of genetically unstable tumor cells through mitosis. Moreover tumor cells may profit from the increased resistance to many different pro-apoptotic stimuli, including – inter alia –hypoxia and death signals from immunocompetent cells.

The clinical implications of survivin-related resistance to apoptosis are profound. First retrospective studies on gastric, colorectal and bladder carcinomas as well as neuroblastomas suggest that survivin may be a prognostic factor, helping to identify patients with an increased risk of rapidly progressive disease.

The presence of survivin in urine may also act as a biological marker for bladder cancer. Different expression patterns of the alternative splice variants of survivin, such as survivin-2B and survivin-ΔEx3, are defined as ►biomarkers on mRNA level for tumor staging and progression in certain tumor entities.

Because survivin also confers increased resistance to certain anticancer drugs, e.g. ►paclitaxel and methotrexate, the level of survivin overexpression may be used as a predictive parameter for anticancer-drug sensitivity. Finally, the disruption of survivin-related antiapoptosis may become an attractive therapeutic target, selectively increasing the susceptibility of cancer cells to apoptosis-based treatment strategies without affecting the viability of non-neoplastic tissues that do not express survivin. Survivin is proposed to play a central role in the progression and resistance to therapy of diverse tumor types. The clinical utility of survivin and its variants as a diagnostic tumor marker can be profoundly improved if the marker is also a therapeutic target. Many studies suggest that an inactivation of survivin prevents tumor progression.

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Susceptibility Loci

►Modifier Loci

Sustained Release

Definition

Drugs released slowly from carriers for a sustained and continuous supply of drugs.

►Drug Delivery Systems for Cancer Treatment

SV40

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Synonyms

Simian virus 40

Definition

A DNA tumor virus (genus Polyomavirus) found to be a contaminant of Salk and Sabin poliovaccines (1955–1961) that propagates naturally in kidney cell lines of

Asian macaque species, specifically the rhesus and African green monkey. SV40 in these species, and related primates, produces no cytopathic effects upon the animals, but the virus injected into hamsters and other rodents causes ependymomas, lymphomas, osteosarcomas, sarcomas and ►mesotheliomas. Subsequent research has shown a possible correlation between SV40 (Fig. 1) and human mesotheliomas.

Characteristics

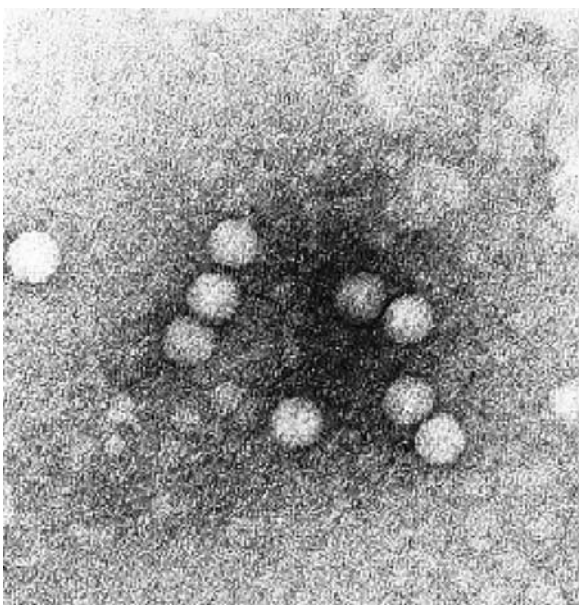
SV40 particles lack a lipid envelope and have a diameter of ~40–50 nm with spherical icosahedral symmetry. The molecular mass of the SV40 virion has been estimated to be 270 kDa. The icosahedral capsid contains three viral proteins (VP1, VP2, VP3), with VP1 being the major protein and VP2/VP3 being minor proteins. Along with the viral proteins, the SV40 virion contains cellular histones (H2A, H2B, H3, H4) that aid in condensing the viral DNA. The SV40 genome comprises of a closed circular dsDNA (5,243 bp) that associates with the various histones to achieve condensation, similar to that of cellular DNA in the form of chromatin. Nucleosomes that number between 24 and 26 on the viral DNA are made from the assembly of the histone-DNA complexes.

The genome of SV40 is numbered in a clockwise direction beginning at the origin (Ori) and continuing around until the site of Ori is reached again, marking the end of the genome. SV40 genome is responsible for coding six genes, synthesizing VP1, VP2, VP3, LP1, and the large (T-ag) and small (t-ag) t-antigens. SV40 large T-ag is comprised of 708 amino acids, while the small t-ag contains 174 residues. The initiation of viral DNA synthesis is mediated by the essential replication protein large T-ag, which in turn is regulated by phosphorylation. The transformation of cells and induction of tumor formation by SV40 is another function of the large T-ag, which causes the inactivation of products made from several tumor suppressor genes, including ►p53, ►pRb, p107, p130/Rb2, p300 and p400. One of the crucial consequences of binding large T-ag to p53 is the inactivation of an essential checkpoint that halts the process of mitosis if DNA damage is present, causing the cell to continue cycling. The small t-ag protein is not an essential mediator in SV40 replication, but it does play a significant role by causing an increase in the production of large T-ag and aiding in inactivating p53.

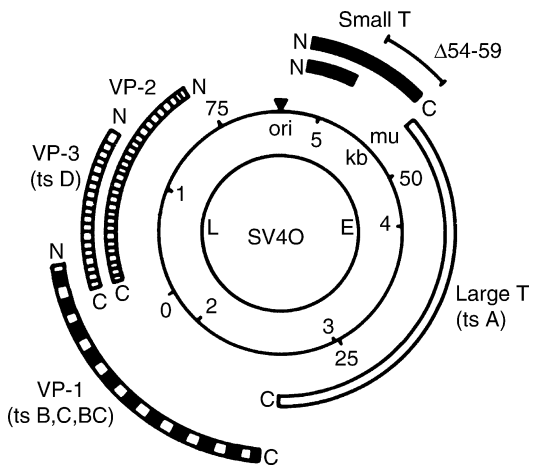
Different regions of the SV40 genome, designated "early" and "late," are expressed at different times during the stages of infection. The early region codes for the large T-ag, the small t-ag, which are the SV40 oncogenes, and for a 17 kDa protein of uncertain biological significance. The late region codes for the capsid proteins VP1, VP2, VP3, and for LP1, a protein involved in the process of SV40 particle assembly late in infection (Fig. 2).

Replication

The early and late regions of the SV40 genome distinctly separate the replication process into two events: Similar to other viruses, SV40 virions come into



SV40. Figure 1 Electron micrograph (250,000×) of SV40 virions (kindly provided by Dr. R. Fresco).



SV40. Figure 2 Genetic map of SV40. *Outer circular* segments represent specific proteins (Tag/tag, VP1, VP2 and VP3). *Inner circle* represent the direction of transcription and region of origin.

S

contact with the outer membrane surface of the host cell and attach to receptors located throughout the outer cell surface. These receptors are thought to represent the major histocompatibility complex class I molecules (MHC class I). Upon being transported into the cell, SV40 virions are moved to the cell nucleus. Once inside the nucleus, the virion capsid disassembles and the viral DNA is released.

At this point in replication, the early region of the SV40 genome is transcribed first, synthesizing the small t-ag/large T-ag proteins. This causes the cells to enter S phase. The SV40 72-bp enhancer elements help positively regulate transcription in the early region of the genome.

It has been identified that there are SV40 strains with either one 72-bp enhancer (archetypal) or two 72-bp enhancers (non-archetypal). Nonarchetypal SV40 replicates more rapidly compared to archetypal SV40. Three G + C-rich domains, also referred to as 21-bp repeat regions, are binding sites for cellular factors. Upon the initiation of S phase, the viral DNA replication and transcription can now begin from the late region, causing the production of necessary structural proteins (VP1, VP2, VP3, LP1). After the necessary proteins and DNA replication is complete, the various viral particles assemble together forming the next generation of SV40 virions. When a high number of virions accumulate in the cells, the cell is lysed and infectious SV40 is released. Infected mesothelial cells, however, can release infectious SV40 without undergoing lysis.

Clinical Relevance

Poliomyelitis was a devastating disease that swept throughout the western world until 1955 when the Salk vaccine began to be used against this crippling disease. Poliovaccines made from monkey kidney cell cultures between 1955 and 1961 and sold until 1963 were found to have been contaminated with numerous supposedly harmless viruses. Knowledge of this contamination was well known, but there was no evidence to suggest any tumorigenic properties of any viruses present in the vaccines. In 1960, Sweet and Hilleman established that an unknown percentage of poliovaccines produced from monkey kidney cell lines was contaminated with SV40. In 1962, Eddy and colleagues produced investigations showing that newborn hamsters injected with rhesus monkey kidney cell cultures developed sarcomas. The resultant sarcomas were attributed to the DNA tumor virus SV40.

It was shown that SV40 was able to successfully replicate, produce infection, and spread throughout humans by oral and respiratory routes. In 1964, when SV40-transformed human cells were injected subcutaneously into volunteer terminally ill patients, those cells were found capable of growth. Vaccines that were produced after 1961 were required by federal law to be tested for SV40, but by that time it has been estimated that ~98 million people, both adults and children, had already been exposed to SV40 through a contaminated poliovaccine. SV40 was able to transform both human and rodent cells in tissue culture. However, epidemiological studies suggested that SV40 was not oncogenic in humans, because the overall incidence of cancer in cohorts injected with contaminated poliovaccines was similar to that of cohorts who had not been exposed to SV40 contaminated poliovaccines.

Subsequent investigations in 1993, showed that when hamsters were injected with SV40 into the pleural space, all of the animals developed mesotheliomas within 3–6 months. Mesotheliomas are tumors that have increased from almost zero to 3,000 cases per year in the USA during the past 50 years. In the USA mesotheliomas are mostly caused by ►asbestos, the finding that SV40 caused mesothelioma in hamsters prompted investigations into the possibility that some mesotheliomas in humans could be attributed to SV40 infection directly or with SV40 acting as a co-carcinogen with asbestos. Mesothelioma samples studied in 1994 showed that 60% of the samples contained SV40 DNA and expressed the SV40 large T (tumor) antigen. The results were confirmed by numerous laboratories using a variety of techniques such as PCR, in situ hybridization, Western blot, immunohistochemistry, Laser dissection/PCR, etc., but the percentage of positive samples varied from 6 to 83%, and a few studies were completely negative. Technical and geographical differences may count for these variances. Significant geographical differences in exposure to SV40 were confirmed by a recent study showing that the poliovaccines used in the former USSR and in the countries under its influence contained infectious SV40 until at least 1978. These findings supported a previous conclusion of the Institute of Medicine of the National Academy of Sciences that the epidemiological data were flawed and therefore it was not possible to accept or reject a causal association between SV40-containing poliovaccines and cancer. In fact, it was not possible to clearly distinguish exposed from non-exposed cohorts. Although the epidemiological data are not available, mechanistic experiments in human mesothelial cells, and animal experiments strongly support a pathogenic role of SV40 in mesothelioma. More recently, SV40 has been shown to be a co-carcinogen in causing mesothelioma in animals and malignant transformation of mesothelial cells in tissue culture. In addition, the data showed that in the presence of SV40 lower amounts of asbestos were sufficient to cause mesothelioma. Co-carcinogenesis was mediated through the activation of the ►extracellular signal-regulated kinases (ERKs) and ►activator protein-1 (AP-1) activity that led to cell proliferation and stromal invasion.

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SVA Repeat

Definition

A hominid-specific composite repetitive DNA element named after its main components, *S*INE-R (Short Interspersed Nuclear Element derived from the Human endogenous retrovirus K10), *V*NTR (►Variable Number Tandem Repeat) and ►*A*lu, elements.

►LINE-1 Elements

SWI/SNF

Definition

Highly conserved multiproteic structures involved in the ATP-dependent chromatin remodeling. Among the core subunits of SWI/SNF, hSNF5/INI1 is responsible for the oncogenesis of rhabdoid tumors.

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily b, member1

►hSNF5/INI1/SMARCB1 Tumor Suppressor Gene

SWISS-PROT

Definition

A highly annotated biological database of protein sequences developed by the Swiss Institute of Bioinformatics and the European Bioinformatics Institute.

►Intrinsically Unstructured Proteins

Syk

►Syk Tyrosine Kinase

Syk Tyrosine Kinase

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Synonyms

Spleen tyrosine kinase; Syk

Definition

Syk ►tyrosine kinase activity was originally recognized in spleen, thymus and lung and was later cloned from spleen. A 72 kDa protein, Syk contains 635 amino acids and the human gene maps to chromosome 9q22. It contains two N-terminal, ►SH2 domains in tandem and a C-terminal tyrosine kinase domain. Interdomain A separates the tandem SH2 domains and interdomain B links the kinase domain to the tandem SH2 domains. An alternatively spliced site in interdomain B deleting 23 amino acids gives rise to a short form of Syk that can be differently expressed according to the tissue. Syk together with the related Zap70 (zeta-activated protein of 70 kDa) constitute a separate tyrosine kinase family.

Characteristics

Distribution and Function

Syk is critical to immune cell signaling where it promotes ►proliferation, survival, ►phagocytosis,

and ►differentiation and is required for lymphocyte development. Syk also influences angiogenesis and lymphangiogenesis. Animals lacking Syk die around birth from a failure of blood and lymphatic vessel separation. Although Syk is now studied as a target for therapeutic control of asthma, allergy and autoimmune disease due its function in immune cells such as mast cells, there are several indications that Syk is potentially a target for cancer therapy. Syk seems to be involved either positively or negatively in tumor formation and ►progression, depending on the cell type. In hematopoietic malignancies, Syk can be constitutively activated or overexpressed. The TEL-Syk gene fusion resulting from a ►chromosomal translocation identified in a myelodysplastic syndrome patient was found to result in constitutively autophosphorylated Syk that promoted growth factor independent growth in a hematopoietic cell line. Syk may also promote cancer growth in B cell lymphomas since inhibitors of Syk suppresses lymphoma growth. Syk overexpression has been reported in anaplastic lymphoma kinase-positive tumors, and in splenic marginal zone and mantle cell lymphomas, however its direct responsibility in lymphoma development is not known. In contrast, loss of Syk has been reported in ►Reed–Sternberg cells of classical Hodgkin disease, and in ►chronic lymphocytic leukemia and ►acute lymphocytic leukemias.

It is now appreciated that Syk, unlike Zap70, is found not only in hematopoietic cells, but also in many other cell types including mammary, gastric, and lung epithelia, and in hepatocytes, melanocytes, neuronal, muscle and endothelial cells and in some fibroblasts. And, Syk appears to behave as a ►tumor suppressor in a growing list of tumors, including ►breast cancer, ►gastric cancer, ►hepatocellular carcinoma, and ►melanoma in which its expression is lost compared with normal cells. In breast, Syk is progressively lost from normal to hyperplastic to ►ductal carcinoma in situ to invasive cancers.

Experimental Evidence for a Tumor/►Metastasis Suppressor Role of Syk

Animal studies using breast or melanoma cells have shown that re-expression by gene transfer of Syk in tumor cells that are Syk negative, blocks primary tumor growth when tumor cells are injected into the mammary fat pad or in the skin of mice, respectively. Conversely, inhibition of Syk function in breast cancer cells that express Syk is accomplished by gene transfer of a kinase-defective, dominant-negative Syk. This results in more efficient tumor initiation and increased tumor growth when cells are injected into mice. An ►experimental metastasis model reveals that re-introduction of Syk potently blocks lung metastasis of breast and melanoma tumors. In keeping with its role in blocking metastasis, in vitro studies correlate Syk loss in breast cancer cells with invasive growth of cells in a three dimensional extracellular matrix culture system. Re-expression of Syk in Syk-negative cells blocks this invasive growth, as well as inhibiting directional cell ►migration due to ►chemotaxis by tumor cells, and ►anchorage-independent proliferation, processes that are associated with the ability of tumor cells to metastasize. Thus, Syk can behave not only as a tumor suppressor, but also as a metastasis suppressor in non-hematopoietic tumors.

Mechanism of Syk Activation and Signaling

Activating receptors on hematopoietic cells contain one or more cytoplasmic ►immunoreceptor tyrosine-based activation motifs (ITAM) that, following activation of the receptor and phosphorylation on tandem tyrosine-containing motifs by Src family kinases, form binding sites for the Syk tyrosine kinase. This occurs via tandem SH2 domains contained in the N-terminus of this molecule. Syk then phosphorylates itself on multiple tyrosine residues, thereby creating binding sites for its substrates and downstream effectors. Amongst these one can distinguish intermediates of the major intracellular signaling pathways (e.g. ►MAP kinase and ►PI3 kinase) and final effectors (e.g. alpha-tubulin and ►cortactin). These signaling cascades finally affect a complex series of cellular responses such as cell proliferation, differentiation, ►adhesion and migration, ►apoptosis, and phagocytosis. Signaling in hematopoietic cells requires immune cell receptors that are lacking in epithelium and other Syk positive cell types raising the question of the mechanism of Syk activation in epithelial cells. However, Syk can also be activated by transmembrane ►integrin receptors in hematopoietic cells. There is some evidence for β1 integrin-mediated activation of Syk in epithelial cells. ►Integrin signaling is involved in cancer and most particularly in the ►invasion and metastasis processes. In epithelial cells, Syk can also be activated when membrane-bound ITAM motifs are introduced into cells either experimentally, or, associated with ►Epstein-Barr virus (EBV) infection. Relevant to EBV infection in ►nasopharyngeal cancer, Syk activation is required for increased cell migration. Oppositely, in breast cancer and melanoma cells, Syk suppresses chemotaxis when re-expressed following gene transfer experiments. Downstream, following Syk activation, a number of pathways can be activated or inhibited depending upon the cell type and microenvironment. Thus, across the spectrum of cancers, Syk function likely depends on the cellular and molecular context, which varies widely between hematopoietic cells and other cell types.

Syk might also influence cell behavior via its differential subcellular localizations in breast epithelial cells. Syk possesses a nuclear targeting sequence that is absent in the alternatively spliced short form, and nuclear localization is associated with suppression of tumor cell invasion and alteration in expression of transcription factors via interaction with SP1 transcription factor in breast cancer cells. Syk also negatively regulates SP1 activation during hypoxic stimulation as occurs during tumor cell growth in areas of low oxygen abundance. An early observation was that re-introduction of Syk into Syk-negative breast cancer cells results in abnormal cell division and cytokinesis. Correspondingly, Syk was found to be present also at the centrosome, the major microtubule organizing center of the cell. This localization is dependent upon its tyrosine kinase activity, and its presence there is tightly regulated during cell cycle progression suggesting a potential to regulate cell proliferation. In other epithelial cells, such as lung, the role of Syk may be more closely aligned with its function in immune cells, since Syk activation is required for production of inflammatory molecules induced by tumor necrosis factor.

Clinical Studies of Syk Expression and Activity

Clinical pathology studies of tissues from breast and gastric cancer patients reveal that the loss of Syk is associated with poor outcome such as reduced overall survival and increased metastasis risk. Syk nuclear localization was significantly associated with improved outcome in gastric cancer. Loss of Syk protein in cancer cells is an independent prognostic marker of poorer overall survival in hepatocarcinoma cells revealed by multivariate statistical analysis but Syk has not been found to be an ►independent prognostic factor in breast or gastric cancers to date.

Loss of Syk is at least partially due to ►epigenetic gene silencing caused by the ►hypermethylation of a ►CpG island in the promoter portion of the SYK gene. This has been documented in breast, transitional cell carcinoma of the bladder, hepatocellular carcinoma, gastric, melanoma, and ovarian cancers; and in T-lineage acute lymphoblastic leukemia. Hypermethylation of the SYK gene is an independent prognostic factor of poorer overall survival in hepatocellular carcinoma. Microarray comparison of genes previously shown to be altered by hypermethylation revealed differential Syk expression in normal versus prostate cancer cells. And, Syk methylation is associated with ►histopathological grade in transitional cell carcinoma of the bladder, loss of Syk being associated with the most invasive tumors. Overall, Syk expression appears to be primarily regulated at the transcriptional level, but presently no transcription factors regulating Syk expression have been identified.

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Sympathetic Ganglia Neurons

Definition

Nerve cells that make-up the sympathetic nervous system.

Symptom Management

Definition

Treatment of the symptoms caused by cancer disease

►Leptomeningeal Dissemination

S

Synaptic Vesicle Recycling

Definition

A specialized class of small vesicles (synaptic vesicles, ~50 nm diameter) in nerve cells store neurotransmitters and releases them upon the arrival of an action potential at the nerve terminal. Neurons can fire in excess of a thousand times per second, which would rapidly lead to depletion of synaptic vesicles. Thus, synaptic vesicles

are efficiently internalized, after their fusion with the plasma membrane by a molecular machinery that is largely overlapping that of ►endocytosis.

Synaptopodin

Definition

A gene expressed in renal podocytes and neurons and interacting with actin.

►Myopodin

Synchronizer

Definition

The regular alternation of light and darkness over 24 h, social time cues, and feeding schedules periodically reset the endogenous bodily rhythms and hence, they are called synchronizers. The light–dark synchronization displays species specificity. Thus, mice or rats that are used for preclinical tests of cancer therapeutics rest during the light span and are active at night, while the reverse is true for humans.

►Circadian Clock Induction

N-Syndecan

Definition

Is a member of the syndecan family of transmembrane heparan sulfate proteoglycans that was cloned initially from neonatal rat Schwann cells and is the principal syndecan expressed during early postnatal development in the central and peripheral nervous systems. Purified N-syndecan binds in vitro with high affinity to several extracellular regulatory ligands, including basic fibroblast growth factor, pleiotrophin and a novel collagen-like protein secreted by Schwann cells that bind to the heparan sulfate chains of N-syndecan. It is suggested that ligand-regulated dimerization of N-syndecan represents a mechanism for regulating downstream signaling activities.

►Pleiotrophin

Syndecans

Definition

Are a diverse group of type I transmembrane heparan sulphate ►proteoglycans with a wide array of ligands and distinct cell signaling capabilities. Four mammalian syndecans have been identified: syndecan-1/CD138, syndecan-2/fibroglycan, syndecan-3/N-syndecan, and syndecan-4/ryudocan. The heparan sulphate glycosaminoglycan chain of syndecans binds a number of extracellular proteins, including growth factors, ►chemokines, extracellular matrix components, cell adhesion molecules, proteases, and protease inhibitors. Although in some cases the family members have overlapping functions, syndecans and their heparan sulphates can bind distinct ligands and produce cellular responses unique to each syndecan. Moreover, the in vivo expression pattern of each syndecan can differ greatly from the others.

►Pleiotrophin

Synergism

Definition

Is opposite to antagonism, i.e., two or more agents create stronger effect than the predicted sum of their individual effects.

►Xenobiotics

Synergistic

Definition

Interaction of two or more agents that results in a combined effect greater than the sum of their individual effects.

►Nilotinib

Syngeneic

Definition

Genetically identical or closely related, so as to allow tissue transplant, immunologically compatible.

Synovial Sarcoma

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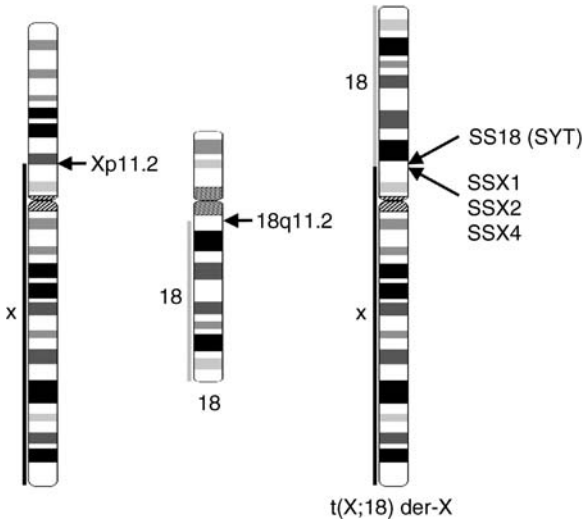
Definition

Human synovial sarcomas are soft tissue tumors that account for up to 10% of all human sarcomas and mainly affect children and young adults. These tumors display relatively high rates of local recurrences and metastases (►metastasis) and are therefore regarded as high grade tumors. Five- and ten-year survival rates of 60–80% and 40–50%, respectively, have been reported in several large retrospective studies. The tumors occur most frequently in the extremities (often associated with the large joints), but may also be encountered in a wide variety of organs. The name synovial sarcomas is misleading since it has become clear that they do not originate from synovial tissue. Instead, synovial sarcomas are thought to be derived from progenitor cells that are capable of differentiating into mesenchymal and/or epithelial structures. A very recent study indicated that these progenitor cells may, in fact, be primary myocytes. Histopathologically, synovial sarcomas can be subdivided in four subtypes: (i) biphasic (with an epithelial and a mesenchymal component), (ii) monophasic (either mesenchymal or epithelial), (iii) calcifying and (iv) poorly differentiated tumors.

Characteristics

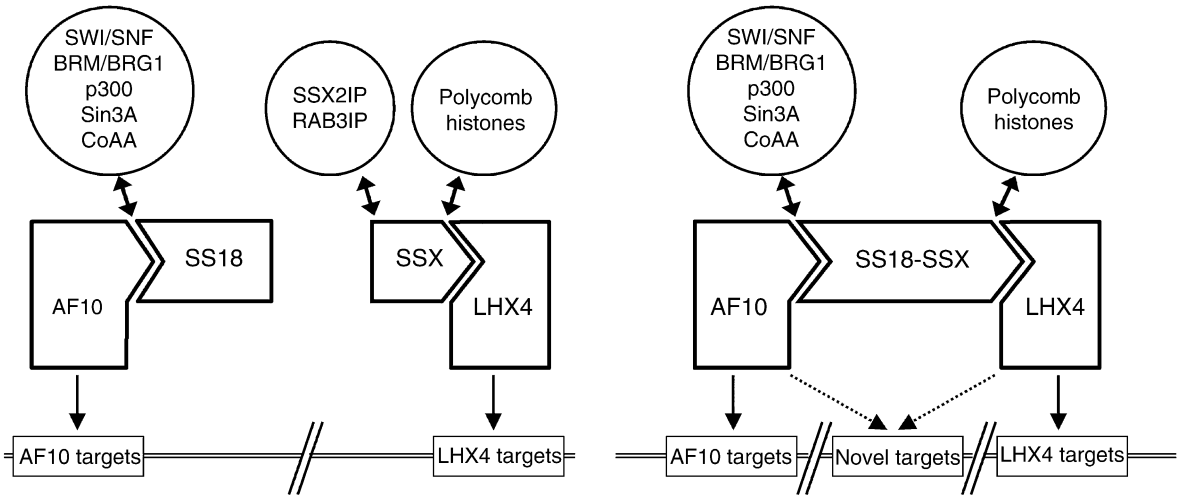
Cytogenetically, the tumors are characterized by a specific chromosomal translocation, t(X;18)(p11;q11) (Fig. 1), which leads to a fusion of the SS18 (previously known as SYT or SSXT) and SSX genes. In the far majority of SS18-SSX fusion proteins identified to date, the C-terminal eight amino acids of the SS18 protein are replaced by the C terminal 78 amino acids of one of the SSX proteins. The SSX genes constitute a family of at least nine (highly related) X-chromosomal genes. Of these, three were found to be involved in the SS18 fusions: SSX1, SSX2 and SSX4. SS18-SSX fusion genes have been detected in almost all synovial sarcomas, but not in any other tumor type examined so far. Clinically, synovial sarcomas display a variable response to common treatment protocols, such as radiation and chemotherapy (►adjuvant Therapy). Over the years, many investigators have reported adverse prognostic factors, such as tumor size (>5 cm), high tumor grade, advanced (metastatic) disease and (higher) patient age. Treatment with chemotherapy (ifosfamide, either alone or in combination with doxorubicin) has been reported to increase the overall survival rates, also



Synovial Sarcoma. Figure 1 The synovial sarcoma specific t(X;18)(p11;q11) chromosomal translocation. The normal chromosomes X and 18 with the respective breakpoints are shown to the *left*. The derivative-X chromosome, with the breakpoint-associated genes (*SS18*, *SSX1*, *SSX2* and *SSX4*) is shown to the *right*.

of patients with high grade tumors and metastases. Despite these findings, there is ample room for further improvement and optimization of the (differential) diagnosis and treatment of human synovial sarcomas. To enable this, detailed information about the molecular mechanisms underlying synovial sarcoma development is of imperative importance.

The tumorigenic nature of the SS18-SSX fusion protein has been established in vitro and in vivo (►oncogene). Further, functional, analysis of the SS18 and SSX genes has revealed that they encode nuclear proteins that exhibit opposite transcriptional regulatory activities. The SS18 protein functions as a transcriptional co-activator which interacts directly with the transcription factor AF10, the co-activator CoAA, and several members of the epigenetic chromatin remodeling (BRM and BRG1) (►chromatin Remodeling in Cancers) and modification machineries (p300 and SIN3A) (►p300/CBP Co-Activators). In contrast, the SSX proteins function as transcriptional co-repressors which interact with the RAB3IP and SSX2IP proteins and the transcription factor LHX4, and are associated with histones and several Polycomb group repressor proteins. The domains involved in these apparently opposite transcription regulatory activities are retained in the SS18-SSX fusion proteins. Therefore, these may function as "activator-repressors" of transcription, which can bind to target DNA through the AF10 and LHX4 transcription factors (Fig. 2). This notion implies that the SS18 and/or SSX protein functions may be impaired in the SS18-SSX fusion protein. Alternatively, the fusion



Synovial Sarcoma. Figure 2 Model depicting the synovial sarcoma associated SS18, SSX and SS18-SSX (fusion) proteins and their respective interactions. In normal cells (*left)* the SSX proteins and their interactors SSX2IP and RAB3IP may associate with the Polycomb repressor complex and histones and in addition, through interaction with the LHX4 protein, bind to cognate DNA sites and affect target gene expression. The SS18 protein can interact with several members of the SWI/SNF chromatin remodeling complex (BRM and BRG1), but also with proteins involved in covalent chromatin modifications (p300 and Sin3A) and the co-activator CoAA. In addition, through interaction with the transcription factor AF10, SS18 may bind to cognate DNA sites and affect target gene expression. In synovial sarcoma cells (*right*) the SS18-SSX fusion proteins have lost the interaction domains for SSX2IP and RAB3IP, but have retained the interaction/association domains for both the SWI/SNF and the Polycomb complexes. Through these interactions, the SS18-SSX fusion proteins may anomalously affect the regulation of these target genes and/or affect the regulation of other (novel) target genes, either through AF10, LHX4, or both.

protein may have gained novel functions. A recent functional analysis revealed that the SS18-SSX fusion protein influences the process of ►epithelial to mesenchymal transition (EMT) which is commonly observed in tumor cells. This example of a SS18-SSX gain-of-function may underlie the above-mentioned histologic differences among synovial sarcomas. A recent study has indicated that novel treatment options for synovial sarcoma patients may include so-called "epigenetic" drugs (►epigenetic Therapy). Synovial sarcoma cell lines were shown to be extremely sensitive to the histone de-acetylase inhibitory drug Romidepsin (also known as FK228 or depsipeptide), both in vitro and in vivo. As yet, the exact mode of action of this broad spectrum "epigenetic" drug on synovial sarcoma growth is unknown. However, it is to be expected that detailed knowledge on the molecular mechanisms underlying synovial sarcoma pathogenesis will be instrumental for obtaining insight into its mode of action and, thus, the development of more targeted therapies.

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Syntenic

Definition

Refers to genes or genetic loci that lie on the same chromosome, i.e. are genetically linked.

►Amplification

Synthetic Cannabinoids

►Cannabinoids

Synthetic Chemoprotectants

Definition

Chemoprotectants that are artificially and chemically synthesized.

►Chemoprotectants

Synuclein

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Definition

Synucleins are small cytosolic proteins of uncertain function, normally expressed at high levels in the vertebrate nervous system. Increased expression of synuclein proteins, especially γ-synuclein, is associated with progression of a variety of tumors.

Characteristics

The precise function of the synuclein proteins is not well understood. All family members (α-synuclein, ►SNCA; β-synuclein, ►SNCB; and ►γ-synuclein, ►SNCG) share a conserved domain related to the lipid-binding domains of the ►exchangeable apolipoproteins, the major lipid transporters in blood. This conserved domain mediates reversible interactions with lipid membranes. α-Synuclein regulates the uptake and incorporation of fatty acids into phospholipids, and mutations in α-synuclein alter the composition of cellular membranes.

Each of the synuclein isoforms possesses a unique tail domain, which may mediate distinct physiological actions. α- and β-synuclein are normally expressed in the ►central nervous system, while γ-synuclein expression is found throughout the central and ►peripheral nervous systems, and at lower levels in some non-neural tissues. Synuclein family genes have so far been identified only in vertebrate species.

Synuclein Expression in Cancer

The first synuclein family member to be associated with cancer was γ-synuclein, which was originally named ►breast cancer-specific gene 1 (►BCSG1), due to its specific expression in infiltrating breast carcinoma as compared to normal breast tissue. The other synuclein proteins are also somewhat associated with certain cancers. For example, 87% of ►ovarian cancers display increased expression of one or more synuclein family members, while 42% express all three. The γ-synuclein isoform is particularly associated with cancer. Overexpression of γ-synuclein is observed in a large percentage of tumors from varied tissues of origin, but not in adjacent non-neoplastic tissues. Expression of γ-synuclein in cancer increases in a stage-specific manner, with moderate expression in stage I tumors, and very high expression in stages III-IV.

Increased expression of γ-synuclein in tumor cells apparently results from deregulation of normal expression, as no γ-synuclein mutations or gene amplifications have been associated with cancer. Tissue specific expression of γ-synuclein is mediated by methylation of ►CpG islands in exon 1, and ►hypomethylation at these sites has been observed in γ-synuclein overexpressing tumor cells. The increased expression of γ-synuclein observed in advanced cancer is mostly likely the consequence of a loss of ►epigenetic gene silencing of γ-synuclein expression during tumor progression, rather than a primary event in tumor initiation.

Misfolding of α-synuclein protein is associated with both familial and sporadic ►Parkinson's disease (PD), and mutations in α-synuclein that alter its sequence or increase its expression cause early-onset PD. PD patients have an increased risk of both ►melanoma and breast cancer, as compared to the general population, but a decreased risk for many other cancers. This may reflect the involvement of common genes in PD and cancer.

Role in Tumor Progression

γ-Synuclein expression in breast cancer cell lines increases cell proliferation. It interacts with the mitotic spindle checkpoint control protein ►BubR1, promoting its degradation by the proteasome and overriding mitotic arrest. Loss of spindle checkpoint control can result in ►aneuploidy, the state of having the wrong number of chromosomes. γ-Synuclein augments proliferative signaling through the ►estrogen receptor pathway, by acting as a molecular chaperone to increase ►estradiol binding to the receptor. γ-Synuclein also alters signaling via the ►MAP kinase pathway, which plays a key role in the regulation of cell proliferation.

γ-Synuclein enhances cell motility and invasiveness, and its overexpression in established tumors may drive malignant tumor progression. γ-Synuclein promotes production of ►matrix metalloproteinases, secreted

proteases that break down the extracellular matrix and allow tumors to invade surrounding tissues and blood vessels, thereby facilitating ►metastasis. γ-Synuclein expression also increases resistance to certain chemotherapeutic agents that trigger ►apoptosis via MAP kinase and/or ►JNK signaling pathways, e.g. ►taxol, vinblastine, and ►paclitaxel.

Overall, γ-synuclein expression in a tumor correlates with a poor clinical prognosis. Its expression in primary tumors is associated with the presence of distant metastases. This protein may prove to be a useful ►biomarker for detecting cancer, ►staging tumor progression, and evaluating metastatic potential.

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Synuclein α

Definition

A member of the ►synuclein protein family. Also known as NACP. Encoded by the gene SNCA.

►Synucleins

Synuclein β

Definition

A member of the ►synuclein protein family. Also known as PNP14. Encoded by the gene SNCB.

►Synucleins

Synuclein γ

Definition

A member of the ►synuclein protein family. Also known as persyn or synoretin. Encoded by the gene SNCG.

►Synucleins

Systemic Antibody-directed Radionuclide Therapy

►Radioimmunotherapy

Systemic Chemotherapy

Definition

Oral or intravenous administration of ►chemotherapy

Systemic Clearance

Definition

A measure of the efficiency with which a drug is removed from the body. It is proportional to the dose and inversely proportional to the Area under the Curve (AUC).

►Lead Optimization

Systemic Inflammatory Response Syndrome

Definition

SIRS; A syndrome proposed by American College of Chest Physicians in 1992. It is defined as a clinical response to a nonspecific insult of either infectious or noninfectious origin. SIRS is defined as two or more of the following variables:

1. Fever of more than 38°C or less than 36°C
2. Heart rate of more than 90 beats per minute
3. Respiratory rate of more than 20 breaths per minute or a PaCO2 level of less than 32 mm Hg
4. Abnormal white blood cell count (>12,000/μl or <4,000/μl or >10% bands)

►Sivelestat

Systemic Lupus Erythematosus

Definition

SLE; Systemic lupus erythematosus is an autoimmune disease in which autoantibodies against DNA, RNA, and proteins associated with nucleic acids from immune complex that damage small blood vessels, especially of the kidney.

Systemic Spread of Cancer

►Metastatic Colonization

Systemic Therapy

►Neoadjuvant Therapy

Systemic Treatment or Therapy

Definition

Therapy theoretically delivered to the entire body, and is distinguished from ►local therapy. ►Chemotherapy is the most common form of systemic therapy. Its purpose can be either curative or palliative.

►Induction Chemotherapy

Systems Biology

Definition

A scientific discipline that seeks to understand the dynamic nature of a life by quantitatively integrating the network of interactive sub-processes (metabolic pathways, gene expression, cell-cell interactions, etc.) occurring within an organismal unit in a manner that can accurately predict the net outcome(s) of perturbing or stimulating any of those sub-processes.

►Drug Design

T

Definition
Testosterone.

►Cyclin G-Associated Kinase

T Cell

Definition
►Thymus-dependent lymphocyte; A leukocyte, synonym as T lymphocyte, which is part of the adaptive immune system; T cell ($CD8^+$) cytolytic action requires presentation of antigens by antigen-presenting cells (APCs) T cells are capable of forming a memory ($CD4^+$).

►Natural Killer Cell Activation

T Cell Receptor Complex

Definition
TCR complex; Consists of the α and β chain of the T cell receptor heterodimer involved in ►MHC/peptide recognition and of the CD3 complex that consists of multiple chains involved in intracellular signal generation.

►Chimeric T Cell Receptors

T_H1 Cells

Definition
Are a subset of CD4 T cells that are characterized by the cytokines they produce. They are mainly involved in activating macrophages, and are sometimes called inflammatory CD4 T cells. Th1 cells enhance cell-mediated immune responses and are essential for controlling intracellular pathogens such as viruses and bacteria.

►Chemokine

T_H2 Cells

Definition
Are a subset of CD4 T cells that are characterized by the cytokines they produce. They are mainly involved in stimulating B cells to produce antibody, and are often called helper CD4 T cells.

►Sjögren Syndrome

T_H3 Cells

Definition
Are unique cells that produce mainly transforming growth factor-β (►TGF-β) in response to antigen; they develop predominantly in the mucosal immune response to antigens that are presented orally.

T Helper

Definition
CD4+ T helper (Th) subsets are characterized by their distinct cytokine production profiles. Th1 cells secrete interleukin-2 (IL-2), IFNγ and TNFβ, which promote cellular immune responses against intracellular pathogens

and viruses. Th2 cells produce IL-4, IL-5, IL-6 IL-10 and IL-13, which promote humoral immunity by aiding in B cell growth and differentiation.

►Interleukin-4

T Helper Lymphocytes

Definition

Subset of ►T lymphocytes that express the CD4 surface marker and are capable of enhancing antibody and cellular immune responses. Helper T cells recognize antigen in the form of peptides complex onto class II major histocompatibility complex molecules and produce lymphokines that regulate immune responses.

►Peptide Vaccines for Cancer

T-Helper 1 Response

Definition

Th1 Response; Type of immune response that primarily induces cell-mediated immunity and the activation of cytotoxic effector T cells. Associated with and induced by the release of a characteristic set of cytokines including interleukin-2, interferon-gamma and interleukin-12. It is generally believed that a strong Th1 immune response is required for effective cell-mediated antitumor immunity.

T-Loop

Definition

Of telomeric DNA is formed in each ►telomere end by invasion of its duplex region by the single-stranded (TTAGGG)-rich 3'-overhang. This structure, which hides the very end of the chromosome, protects chromosome form degradation and loss of vital sequence, block end-end fusion.

►Stem Cell Telomeres

T Lymphocyte

Definition

T cell; type of lymphocyte responsible for cell-mediated immunity; includes cytotoxic cells and helper T-cells.

►Cancer-Germline (CG) Antigens

T-lymphoma Invasion and Metastasis

►Tiam1

T-PLL

Definition

►T-Prolymphocytic Leukemia.

T-Prolymphocytic Leukemia (T-PLL)

Definition

T-Prolymphocytic leukemia (T-PLL) is a disease that represents 20% of prolymphocytic leukemias, occurs at an advanced age of 70–80 years.

►Acute Promyelocytic Leukemia

TAA

Definition

Tumor-associated Antigen.

TACE

Definition
Acronym for tumor necrosis factor-α (TNF-α) converting enzyme.

►ADAM17
►ADAM Molecules

TAK

Definition
Tat-Associated Kinase, also known as positive-acting transcription elongation factor complex (P-TEFb), is a complex of cellular proteins involved in regulation of gene transcription.

►TAT Protein of HIV

Takatsuki Disease

Definition
►POEMS Syndrome.

Tall Cell Variant of Papillary Carcinoma

Definition
Papillary carcinoma of the thyroid characterized by a cell whose height is two- to three times its width and whose cytoplasm is ample and oncocytic. The cell has the characteristic nuclear features of papillary carcinoma.

►Hurthle Cell Adenoma and Carcinoma

TAM

Definition
Acronym for Tumor-associated Macrophages.

►Tumor-Associated Macrophages

Tamoxifen

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Definition
Tamoxifen is a nonsteroidal antiestrogen used for the treatment and prevention of ►estrogen receptor (ER) positive breast cancer. Tamoxifen is the most studied anticancer agent.

Characteristics
Background
Tamoxifen (ICI 46,474) was first described as an effective postcoital contraceptive in rats but the drug induces ovulation in subfertile women. The compound was subsequently reinvented throughout the 1970's as an agent to be targeted to OER positive breast cancers for use as a long-term adjuvant therapy with potential use for the chemoprevention of breast cancer (►Estrogenic hormones and cancer, ►hormones and ►cancer).

Adjuvant Therapy
Studies throughout the 1980's and 1990's demonstrated that long-term adjuvant tamoxifen therapy (5 years) produced dramatic increases in disease-free survival and overall survival. These data were observed in patients who were classified as Stage I and Stage II breast cancers and the compound is effective in both pre and postmenopausal women. Tamoxifen is cheap and effective with availability in generic form in countries throughout the world. Tamoxifen is credited for increasing survivorship and saving the lives of 500,000 women. The appropriate use of tamoxifen as the gold standard for the endocrine treatment of breast cancer throughout the 1990's, is credited in contributing to the decrease death rate from breast cancer observed in the United States and other countries around the world. However, concerns about side effects (see below) and the development of drug resistance has led to the development of the aromatase inhibitors as a substitute

for tamoxifen in the adjuvant treatment of breast cancer in postmenopausal women. Current studies demonstrate that aromatase inhibitors have an improved side effect profile compared to tamoxifen and improved disease-free and overall survival.

Chemoprevention

Laboratory studies first showed that tamoxifen could prevent the development of carcinogen-induced rat mammary tumors. Randomized clinical trials around the world have demonstrated that tamoxifen can reduce the incidence of breast cancer by 50% in high risk pre and postmenopausal women. High risk refers to the mathematical model (Gail Model) that is available on the internet through the National Cancer Institute in the United States that will determine the 5 year and lifetime risk of a woman developing breast cancer. Some of the risk factors used in the Gail Model are age, start of menses, termination of menses, age when the woman had children, if the woman had children, breast biopsies, ductal hyperplasia, and first degree relatives developing breast cancer.

Concerns about the side effects of tamoxifen (see below) have focused the use of tamoxifen in the premenopausal population where the risk/benefit ratio is high. In other words, tamoxifen effectively reduces the incidence of breast cancer but the side effect profile is low. Most importantly, after women take a 5 year course of tamoxifen during their premenopausal years, the beneficial effects of tamoxifen in preventing breast cancer may last for up to another 10 years but the side effects of hot flashes, etc. will disappear. Recent studies of a related compound, Raloxifene that is used for the prevention of osteoporosis, shows that this selective estrogen receptor modulator or (SERM) will prevent breast cancer in women taking raloxifene to prevent osteoporosis. A recent clinical trial in the United States called the Study of Tamoxifen and Raloxifene (STAR) demonstrated in postmenopausal women that tamoxifen and raloxifene were equally effective in preventing an increase in invasive breast cancer but raloxifene had a superior side effect profile.

Mechanism of Action

Tamoxifen is lipophyllic and well absorbed from the gastrointestinal tract. Patients accumulate tamoxifen over the first 4 weeks of treatment when they reach steady-state. The drug has a long biological half-life so that even if treatment is stopped, the drug can be detected in the blood for up to 6 weeks. Tamoxifen is a prodrug that is metabolically activated by the CYP2D6 enzyme system to the compounds 4-hydroxytamoxifen and endoxifen both of which have a high affinity with the OER. It is important to note that CYP2D6 enzyme system can be blocked by certain selective serotonin reuptake inhibitors (SSRIs) and this may impair the antitumor actions of tamoxifen. Patients often take SSRIs to reduce the incidence of hot flashes in patients taking tamoxifen.

Tamoxifen binds to the ligand binding domain of the OER in the breast tumor and causes a conformational change that is distinct from the natural estradiol OER complex. As a result, tamoxifen is unable to cause gene activation and completely mimic estrogen action. However, tamoxifen is not a complete antiestrogen. The tamoxifen estrogen receptor complex retains estrogen-like actions which can switch on and switch off target sites around a patient's body. For example, tamoxifen is an antiestrogen in the breast but has estrogen-like properties in bones and in the uterus. Tamoxifen is classified as a selective estrogen receptor modulator.

Side Effects

Tamoxifen exhibits specific estrogen-like effects and also antiestrogenic effects. Tamoxifen is sufficiently estrogenic to stimulate the uterine endometrium and enhance the growth of OER positive ►endometrial cancers. Tamoxifen causes a fivefold increase in endometrial cancer compared with women not taking tamoxifen. In other words, if a thousand 60 year postmenopausal women were followed for endometrial cancer, one woman per year would develop endometrial cancer. In contrast, if those same women were taking tamoxifen, five women would develop endometrial cancer (low grade, early stage) per year. Additionally, the estrogen-like effects of tamoxifen are reflected in an increase in thromboembolic disorders in postmenopausal women. It is important to note that endometrial cancer and blood clots are not elevated in premenopausal women. There is also a significant rise in the diagnosis of cataracts and cataract operations. The STAR trial demonstrated that raloxifene does not increase the risk of endometrial cancer. There were fewer hysterectomies, fewer cataracts, fewer cataract operations and a lower overall incidence of blood clots.

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Tamponade

Definition

The accumulation of a fluid (blood or serous exudate) in thepericardial sac, leading to compression of the chambers of the heart and leading to increasing heart failure.

►Cardiac Tumors

Tankyrase-2: TNKL, TNKS2, TANK2

►Tankyrases

Tankyrases

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Synonyms

TRF1-interacting, ankyrin-related ADP-ribose polymerases; There are two closely related homologues: Tankyrase-1: TNKS, TNKS1, TANK1; Tankyrase-2: TNKL, TNKS2, TANK2

Definition

Tankyrases are members of the poly(ADP-ribose) polymerase (►Parp) family that regulate telomere length in ►telomerase-positive human cells. There are two related homologues, tankyrase-1 and tankyrase-2. Tankyrase-1 is a protein of 1327 amino acids and 142 kDa. The gene maps to 8p22-p23. Tankyrase-2 is a protein of 1166 amino acids and 127 kDa. The gene maps to 10q23. Tankyrase-1 is relatively abundant in reproductive tissues (i.e. testis and ovary), whereas tankyrase-2 exhibits rather ubiquitous expression.

Characteristics

Structure

Tankyrase-1 has four characteristic domains: HPS, ANK, SAM, and PARP. The N-terminal HPS domain is a homopolymeric run of histidine, proline, and serine residues, the functional significance of which is unknown. The ANK domain is composed of a long stretch of 24 ANK repeats, providing a platform for protein-protein interactions. Distinct from those of ankyrins, tankyrase-1's ANK domain is further divided into five, well-conserved subdomains, ARC (ANK repeat cluster) I-V. Each ARC works as an independent, ligand-binding site. The SAM (sterile alpha motif) domain is another module for protein-protein interaction and contributes to self-multimerization of the protein. The C-terminal PARP domain catalyzes ►poly (ADP-ribosyl)ation of acceptor proteins by using NAD as a substrate. This post-translational modification gives drastic negative charges to the acceptor proteins and often disrupts interactions between the acceptor proteins and their target DNA.

Tankyrase-2 is a closely related homologue of tankyrase-1. Amino acid identities between ANK, SAM, and PARP domains of tankyrase-1 and tankyrase-2 are 83, 74, and 94%, respectively. The most striking feature of tankyrase-2 is the absence of an N-terminal HPS domain. Tankyrase-1 and tankyrase-2 can associate in intact cells via their SAM domains to form a multimer.

Intracellular Distribution

Tankyrase-1 is found at various intracellular loci, including telomeres, mitotic ►centrosomes, Golgi apparatus, and nuclear pore complexes. Telomeric localization of tankyrase-1 is mediated by its interaction with a telomeric repeat-binding factor 1 (TRF1), which directly binds the double-strand telomere DNA, $(TTAGGG)_n$. During mitosis, tankyrase-1 concentrates around the pericentriolar matrices. This accumulation depends on tankyrase-1's interaction with nuclear/mitotic apparatus protein (NuMA), which plays an essential role in organizing microtubules at the spindle poles. In the Golgi apparatus, tankyrase-1 is peripherally associated with the Golgi membranes. In adipocytes and myocytes, it is colocalized with GLUT4 (glucose transporter 4) storage vesicles in the juxtanuclear region of the cells, where it specifically binds to insulin-responsive amino peptidase (IRAP).

The intracellular localization of tankyrase-2 has been characterized less often than tankyrase-1. So far, it has been reported that tankyrase-2 localizes predominantly to a perinuclear region, similar to the properties of tankyrase-1. Upon subcellular fractionation, both tankyrase-1 and tankyrase-2 are predominantly recovered in the low-density microsomal fraction, which contains vesicular ►endosomal compartments.

Binding Partners

To date, various tankyrase-1- and tankyrase-2-binding proteins have been reported with a consensus RXX(P/A)DG motif as a canonical tankyrase-binding site. Such proteins include TRF1, NuMA, IRAP, Grb14 signaling adaptor protein, tankyrase-binding protein of 182 kDa (TAB182), Mcl-1 apoptotic regulator, and Epstein-Barr

virus nuclear antigen-1 (EBNA-1). TRF1, NuMA, IRAP, TAB182, EBNA-1, and tankyrase-1 and tankyrase-2 themselves have been shown to be poly(ADP-ribosyl)ated by tankyrases. PARP inhibitory compounds, such as 3-aminobenzamide, PJ-34, and 4-amino-1,8-naphthalimide, block this poly(ADP-ribosyl)ation.

Functions

Depending on the binding partner and subcellular localization, tankyrase-1 is involved in several distinct biological events, including telomere elongation, cell division control, and insulin-stimulated glucose uptake. Also, the coexistence of multiple ARCs and an oligomer-forming SAM domain is implicated in the master scaffolding function of tankyrase-1 (and tankyrase-2), which could work as an intracellular "molecular lattice."

Telomere Elongation

Classical DNA replication machinery cannot replicate the very ends of linear DNA; ►end replication problem. Accordingly, native capping structures at the ends of chromosomes, telomeres, gradually erode after each round of the cell cycle in somatic cells. Most immortalized cells, including germ cells and 80–90% of cancer cells, maintain their telomere length by activating telomerase. Telomere elongation by telomerase requires its enzyme activity and accessibility to the substrate, 3′-overhang of telomere DNA (also called as the telomeric G-tail). Telomere access to telomerase is repressed *in cis* by the telomeric protein TRF1. Thus, TRF1 directly binds an array of double-strand telomere DNA, $(TTAGGG)_n$, and recruits additional telomere-binding proteins, such as TIN2 (TRF1-interacting nuclear factor 2), TPP1 (originally designated PTOP (POT1- and TIN2-organizing protein), PIP1 (POT1-interacting protein 1) or TINT1 (TIN2-interacting protein 1)), and POT1 (protection of telomeres 1), to the chromosome ends. The resulting TRF1-TIN2-TPP1-POT1 complex diminishes accessibility of telomerase to the telomeres. According to this mechanism, longer telomeres provide more binding sites for TRF1 and therefore become a less reactive substrate for telomerase. Conversely, shorter telomeres provide fewer binding sites for TRF1 and become a more reactive substrate for telomerase. This balance between open and closed states of the telomeres stabilizes the length of telomeric TTAGGG tracts at each chromosome end of telomerase-positive cells.

Tankyrase-1 enhances telomere access to telomerase and contributes to telomere elongation: tankyrase-1 binds to the N-terminal acidic domain of TRF1 via its ANK domain. As described above, the ANK subdomain, ARC, plays a role in association with TRF1. While each of five ARCs can independently recognize TRF1, ARC V, the one closest to the C-terminal, is the most important for telomeric function of tankyrase-1. Interaction between ARC V and TRF1 enables tankyrase-1 to poly(ADP-ribosyl)ate TRF1. This post-translational modification eliminates telomere binding of TRF1, resulting in dissociation of the TRF1-TIN2-TPP1-POT1 complex from telomeres. Telomere-unbound TRF1 is degraded by ubiquitin-dependent proteolysis; ►ubiquitination. These alterations induce a telomere open state and facilitate telomere elongation by telomerase. It is notable that tankyrase-1 enhances telomere access of telomerase but does not increase the enzyme activity of telomerase. Indeed, tankyrase-1 overexpression induces telomere elongation in a telomerase-dependent manner.

Tankyrase-1 can form a ternary complex with TRF1 and TIN2. In this complex, poly(ADP-ribosyl)ation of TRF1 is prevented by TIN2. So far, however, when and how tankyrase-1 is activated or inactivated is not fully understood. Like tankyrase-1, tankyrase-2 also can recognize and poly(ADP-ribosyl)ate TRF1. However, the extent of functional redundancy and specificity between tankyrase-1 and tankyrase-2 are largely unknown.

Cell Division Control

Since ►siRNA-mediated knockdown of tankyrase-1 causes mitotic arrest of multiple cell types, tankyrase-1 is thought to be required for proper cell division. In normal cell division, each pair of sister chromatids is equally divided into two daughter cells. In some tankyrase-1 knockdown cells, however, sister chromatids can separate at the centromeres and arms but not at the telomeres. Accordingly, cell division is interrupted and abnormal chromosome distribution and spindle morphology occur. In other tankyrase-1 knockdown cells, mitotic arrest occurs with intact sister-chromatid cohesion (arrest at the metaphase). Tankyrase-1 is also required for the assembly of bipolar spindles. It recognizes a centrosomal protein NuMA via its ANK domain, and poly(ADP-ribosyl)ates NuMA in intact cells. Consistently, these two proteins are co-localized at the mitotic centrosomes. Meanwhile, tankyrase-1 is phosphorylated during mitosis by glycogen synthase kinase 3 (GSK3). GSK3 inhibitors, such as lithium chloride and indirubin, inhibit this phosphorylation. Currently, the functional significance of NuMA's poly(ADP-ribosyl)ation and tankyrase-1's phosphorylation during mitosis remain unknown.

Insulin-Stimulated Glucose Uptake

The GLUT4 vesicle is an endocytic compartment within adipocytes and myocytes. This storage vesicle contains the glucose transporter protein, GLUT4, and it regulates glucose uptake upon stimulation with insulin; major fractions of the GLUT4 vesicles usually reside in the trans-Golgi reticulum. Insulin stimulation induces exocytic translocation of the vesicles towards the cell surface, where GLUT4 facilitates glucose uptake. In adipocytes, insulin-stimulated translocation of GLUT4

is mediated by IRAP, another GLUT4 vesicle-resident protein.

Tankyrase-1 is implicated in regulation of GLUT4 translocation. As mentioned, tankyrase-1 directly binds IRAP via its ANK domain and can poly(ADP-ribosyl) ate IRAP. Tankyrase-1 knockdown by siRNAs attenuates the insulin-stimulated translocation of GLUT4 vesicles and subsequent glucose uptake. This inhibitory effect of siRNA is reproducible with PJ-34, a PARP inhibitor that is effective against tankyrase-1 (and other PARPs). Tankyrase-1 knockdown does not attenuate the upstream insulin signaling, such as phosphorylation of the ►insulin receptor, IRS-1 (insulin receptor substrate-1), Akt; ►AKT signal transduction pathway in oncogenesis, GSK3, and p42/p44 ERKs (extracellular signal-regulated kinases; ►MAP kinase).

Clinical Aspects

Telomere synthesis by telomerase is the Achilles' heel of unlimited proliferation of most cancer cells. Continuous treatment of cancer cells with telomerase inhibitory drugs (►Small molecule drugs) shortens telomeres and eventually induces cellular ►senescence, ►apoptosis, or both. Thus, telomerase inhibitors have the potential to benefit cancer patients; ►molecular therapy. One concern is that telomere shortening *per se* compromises the effect of telomerase inhibitors since shorter telomeres have fewer TRF1 and therefore allow easier access to residual telomerase activity. This phenomenon results from incomplete shutdown of telomerase activity.

In addition to enzyme activity, accessibility to telomeres could be a rational target for telomerase inhibition. In fact, tankyrase-1 modulates the impact of telomerase inhibitors on human cancer cells. First, tankyrase-1 overexpression, which removes TRF1 from telomeres, confers resistance to telomerase inhibitors. PARP inhibitors, such as 3-aminobenzamide and PJ-34, reverse this drug resistance. Second, even in cells that do not overexpress exogenous tankyrase-1 (but do express endogenous tankyrase-1) these PARP inhibitors enhance telomere shortening by means of telomerase inhibitors, such as MST-312. Accordingly, the cells undergo earlier crisis. Telomerase inhibitor-resistance caused by telomere shortening *per se* is also reversed by 3-aminobenzamide. These observations suggest that tankyrase-1 could be a target for cancer therapy; ►chemotherapy of cancer, progress and perspectives. Pathologically, tankyrase-1 gene expression is elevated in some tumors but not in others.

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TAP

Definition

Is a shuttling mRNA export factor that binds to a GLEBS-like motif on nucleoporin 98 in the nuclear pore complex and functions in both the export of mRNA and import (recycling) of mRNA export factors into the nucleus.

►NUP98-HOXA9 Fusion
►Nucleoporin Family

TARC

Definition

Thymus and activation-regulated chemokine/CCL17 is a Th2 type chemokine expressed by ►dendritic cells and other cell types that binds to CCR4.

►Hodgkin Disease, Clinical Oncology

Tarceva

►Erlotinib

Target Cells

Definition

The functions of effector T cells are always assayed by the changes that they produce in antigen-bearing target

cells. These cells can be B cells, which are activated to produce antibody; macrophage, which are activated to kill bacteria or tumor cells; or labeled cells that are killed by cytotoxic T cells.

Target-Based Screen

Definition

A procedure in which molecules are systematically tested for their ability to activate, perturb, or modify a particular biological molecule.

►Small Molecule Screens

Targeted Deletion

Definition

The process used in generation of a mouse embryonic stem cell (ES cell) with a portion of a specific gene deleted on one allele; embryonic stem cells are transfected with a DNA carrying the deleted gene flanked by regions exactly matching the endogenous allele to be targeted, and in some ES cells the transfected DNA recombines with the homologous endogenous allele, replacing the endogenous allele with the deleted allele.

Targeted Drug Delivery

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Synonyms

Drug targeting; Site-specific drug delivery

Definition

Targeted drug delivery is to site-specifically deliver or activate the therapeutic compounds in tumor. Thus, the targeted drug delivery is expected to enhance drug efficacy by increasing local active drug concentration in tumor, and to decrease side effect by minimizing drug exposure in normal tissues.

Characteristics

Anticancer drugs possess a greater potential of toxicity and much narrower therapeutic index than any other categories of medication. ►Chemotherapy is often dose- and toxicity-limited. A delicate dose regimen is usually required to balance drug efficacy, drug toxicity, and drug resistance; a high dose might cause toxicity while a low dose might induce drug resistance. In addition, anticancer drugs are usually designed to act on the fast proliferating ►cancer cells. However, rapid proliferation is also the feature of some normal cells such as bone marrow, hair follicles, and intestinal epithelium. Although tremendous effort has been explored to improve the protocol of chemotherapy, the success is very limited to enhance drug efficacy and to reduce drug toxicity.

Several targeted drug delivery technologies have been studied to improve chemotherapy by enhancing drug efficacy and reducing drug toxicity, which have achieved certain degree of success.

Intratumoral Drug Administration

The simplest form of targeted drug delivery is local intratumoral drug administration. The therapeutic compounds can be directly injected into tumor tissues to transiently increase local drug concentration with minimal or no exposure to normal tissues. However, this method may not be applicable for many cancers. It is also difficult to maintain effective drug concentration in tumors for a prolonged time, and thus repeated dosing may be required.

Liposomal Drug Delivery

Chemotherapeutic compounds can be encapsulated in liposome. Liposomal drug formulation achieves passive drug targeting in tumor by enhanced permeability and retention (EPR) effect. Tumor tissues have abnormal vasculature. Since these vasculatures are hyperpermeable with no lymphatic drainage, the liposome will be delivered to the tumor tissues by blood circulation and trapped in the tumors. The drug in the liposome will be gradually released to achieve anticancer efficacy. Therefore, liposomal drug formulation will passively enhance drug accumulation in tumor and decrease exposure to susceptible healthy tissues. The successful liposomal formulation of doxorubicin has been used clinically for cancer treatment. Several generations of liposome formulations have been tested. First generation of liposome passively enhances drug accumulation in tumors and decreases the exposure in normal tissues, but it is rapidly cleared in blood (within minutes). Second generation of liposomes (PEGylated long-circulating

liposomes and ►immunoliposome) increase half-life in blood circulation and tumor targeting.

Tumor-Activated Prodrug Therapy (TAP)

The first strategy in TAP therapy is site-specific prodrug activation. Prodrug is an inactive form of a therapeutic compound by chemical modifications. The inactive prodrug can be activated by an overexpressed enzyme in tumor to achieve site-specific activation, while the prodrug remains inactive or less activated in normal tissues to reduce toxicity. The site-specific activation of the prodrug in tumor increases the anticancer efficacy. The clinically used anticancer drug Xeloda (Capecitabine) provides a best example for this strategy. Xeloda is the first oral chemotherapy drug for the treatment of metastatic colorectal cancer. Xeloda is a ►5-fluorouracil (5-FU) prodrug, which is orally administered. Three enzymes (carboxylesterase, cytidine deaminase, and thymidine phosphorylase) activate Xeloda to produce active 5-FU. Xeloda is hydrolyzed by carboxylesterase into 5′-deoxy-5-flurocytidine (5′-DFCR). Subsequently, 5′-DFCR is converted by deaminase to 5′-deoxy-5-flurouridine (5′-DFUR). Finally, 5′-DFUR is converted into 5-FU by thymidine phosphorylase. Due to the high level of thymidine phosphorylase in tumor, more activation of xeloda is observed in tumors than in normal tissues to exhibit better anticancer efficacy than 5-FU.

The second strategy of TAP is to directly link the prodrug to a targeting moiety (antibody or other ligands) for targeted drug delivery. The antibody-prodrug conjugate binds to the antigen on the tumor cells. The drug–antibody–antigen complex is rapidly internalized into the cancer cells to achieve high drug concentration in targeted cells. For example, anti-CD33 antibody can be conjugated with calicheamicin to target acute myeloid leukemia cells due to the high level of CD33 on the cell surface. Antibody trastuzumab-taxane conjugate shows 55–200-fold more potent than Taxol. Daunorubicin and methotrexate have been linked to anti-MM46 antibody or anti-EL4 monoclonal antibody for treatment of human melanoma. Most of those TAP therapies show better efficacy in vitro and targeted drug delivery in animal model. It is still very challenging in many aspects of TAP therapy such as rapid clearance in blood, low potency, inefficient internalization of conjugates, poor penetration, and premature release of the drugs.

Antibody-Directed Enzyme Prodrug Therapy

Antibody-directed enzyme prodrug therapy (ADEPT) is a two step process. In the first step, a drug-activating enzyme is targeted to tumors by a tumor targeting antibody. In the second step, a nontoxic prodrug is administered systemically and converted to the active drug with high local concentration in tumors by the localized antibody-enzyme conjugate. Meanwhile, the prodrug remains inactive (without drug-activating enzyme) in normal tissues and thus decreases its nonspecific toxicity. ADEPT provides many advantages: (i) Amplification effect: each localized antibody-enzyme molecule converts a large number of nontoxic prodrugs to potent active drugs and increases the local active drug concentration in tumor. (ii) Bystander effect: the locally activated drug molecules with high lipophilicity diffuse into the cancer cells regardless of the heterogeneous antigen expression. The bystander effect addresses the issues of poor tumor penetration of the antibody-enzyme conjugate. (iii) The antibody-enzyme conjugate does not need to be internalized into each cancer cell for prodrug activation. The enzymes in ADEPT could be a bacterial enzyme without mammalian homologs to minimize nonspecific prodrug activation in normal tissues, such as carboxypeptidase G2, cytosine deaminase, beta-lactaminase, penicillin G amidase. Other bacterial enzymes with mammalian homologs or mammalian enzymes with low expression in normal tissues can also be used, such as beta-glucuronidase, alkaline phosphatase, and alpha-galactosidase.

Gene-Directed Enzyme Prodrug Therapy

GDEPT is also a two step process. First, a gene that encodes a drug activation enzyme is delivered to tumor cells. The delivered gene will express the drug activation enzyme in the tumor only. In the second step, a nontoxic prodrug is delivered and is converted into active drug in tumor cells by the expressed enzyme. Many enzymes and drugs can be used in this system. For instance, cytosine deaminase for 5-florocytosine prodrug activation, thymidine kinase for ganciclovir, and arabinonucleoside prodrug activation, carboxypeptidase G2 for benzoic acid mustard prodrug activation, carboxypeptidase A for methotrexate-alanine prodrug activation, galactosidase for daunorunbicin-galactose prodrug activation, glucuronidase for epirubicin-glucuronide prodrug activation, alkaline phosphatase for doxorubicin phosphate prodrug activation, cytochrome P-450 for cyclophosphamide and isofamide prodrug activation.

Folate-Targeted Drug Delivery

Many cancer cells overexpress folate receptor on the cell surface. For example, 80% of metastatic breast cancer and 90% of ovarian cancer are folate receptor positive. Folate is absorbed by its carrier, and can also be taken up by cells through folate receptor mediated endocytosis. Folate-drug conjugate can bind to folate receptor to achieve targeted drug delivery. Many compounds can be delivered by folate conjugates such as small molecules of chemotherapeutic agents, protein complexes, radioimaging agents, genes, and antisense oligonucleotides.

Transferrin Targeted Drug Delivery

Tumor cells have been reported to have high level of transferrin receptor. Transferrin-drug conjugates can bind to transferrin receptor and be internalized inside the cancer cell. This strategy has advantages for tumor tissue distribution and prolonged half life. Fro instance, transferrin-doxorubicin has the potential to circumvent cardiotoxicity. Transferrin-diphtheria toxin selectively killed brain tumor cells with high level of transferrin receptor, although this conjugates also show neurological side effects due to low level of transferrin receptor in normal brain endothelial cells.

Albumin-Drug Conjugate for Targeted Delivery

Distinctive characteristics of tumor tissue with lack of lymphatic drainage leads to accumulation of plasma albumin. Thus albumin-drug conjugate can achieve targeted drug delivery. for instance, methotrexate (MTX): albumin (1:1 molar ratio) was accumulated at higher level (14% accumulation) in xenograft tumors compared to MTX alone (0.4% accumulation), and thus showed better efficacy.

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Targeted Drug Design

►Drug Design

Targeted Radioimmunotherapy

►Ionizing Radiation Therapy

Targeted Therapy

Definition

Therapy directed to a specific molecular target present in the cancer cell, which explains the efficacy of the drug. Targeted therapy has been discovered by exploiting the genetic differences between normal and tumor cells. New agents have been specifically developed to target the gene expression and signaling pathways deregulated in the ►cancer cells. For example, the treatment of ►chronic myeloid leukemia has been revolutionized by ►imatinib, a small molecule inhibitor of tyrosine kinases, including ►BCR-ABL, PDGFR, and KIT. Recent studies have also confirmed the effectiveness of ►herceptin in ►breast cancer. Testing for the presence of ►*HER-2/neu* gene mutation to select patients for ►herceptin treatment is rapidly becoming common practice in the health system that can accommodate the cost of treatment.

Targeted Viruses

Definition

Viruses used in oncolytic virotherapy that have been genetically modified to achieve selective replication in tumor cells.

►Oncolytic Virotherapy
►Oncolytic Virus

Targeting, Active

Definition

Active targeting is one of the mechanisms by which drugs or drug delivery vectors can be preferentially delivered to target cells *in vivo*. With this method, vectors that possess tissue-specific molecules, such as antibodies, peptides, and sugar chains, actively recognize target cells by molecular interactions.

►Non-viral Vector for Cancer Therapy
►Drug Delivery System, in Cancer

Tat

Definition

HIV Transactivator protein product of the HIV *tat* gene.

►TAT Protein of HIV

TAT Protein of HIV

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Definition

Tat is a small viral protein that is encoded by the spliced two-exon tat gene in the HIV genome, responsible for transactivation of the HIV genome.

Characteristics

The HIV Tat protein gets its name from its principal activity, Tat stands for Transactivator, which means that it binds to DNA and activates the transcription of DNA into RNA. The Tat protein has an important role in controlling the transcription of the lentivirus HIV genome from its built-in "promoter," known as the long terminal repeat (which refers to its structure) or ►LTR, to make the RNA that forms new HIV virus particles. In addition to this major role, Tat has also been implicated in a wide variety of pathologies encountered in persons infected with HIV. How does this small (about 101 amino acids) Tat protein do this? Tat has a capacity to bind a striking number of different proteins, nucleic acids and even polysaccharides. It is this combination of binding to different partners that has linked Tat to numerous events in AIDS and intrigued many researchers, making Tat one of the most extensively studied HIV proteins.

Transcriptional Regulation: Control of HIV Replication

The HIV LTR acts as a gene promoter. The gene promoter is a portion of DNA that mediates the binding of RNA polymerase (usually through a series of other proteins that bind to the promoter DNA as well) at the beginning of the gene to be transcribed into RNA. This interaction of proteins with DNA controls the transcription of each gene so that it occurs at a certain moment. In the absence of Tat, very little RNA is transcribed from the HIV LTR promoter. When Tat is present, the rate of transcription shoots up several hundred fold, making the transcription of the HIV genome efficient.

After HIV infects a cell, it is reverse transcribed into DNA, which is then integrated into the genome of the cell (similar to ►retroviruses). The viral DNA is then packaged along with the rest of the cellular DNA by winding onto the histone proteins. In this state, the HIV LTR promoter is rather inactive. In fact, in order to be transcribed, the promoter region of any gene must be unwound from these histones. The HIV DNA is bound to the histones in a very specific manner, with one histone group just prior to, and another just after, a site which binds DNA binding proteins of the cell known as SP1 and NFκB. The activity of these proteins may be enough to allow the binding of an RNA polymerase, known as RNA polymerase II or RNAP II, to the HIV LTR.

The binding of RNAP II to the LTR alone is not enough for efficient transcription. In the absence of Tat it does not appear to be able to advance forward to synthesize RNA beyond the first 44 nucleic acid base pairs. The major role for Tat is to unleash this machinery and send it to work. When Tat is present, it binds to a segment of this short initially polymerized RNA, which forms a peculiar loop known as the TAR (TransActivation Responsive) element. The TAR-bound Tat then brings in a series of other proteins that allow the transcription process to proceed.

Tat has been demonstrated to play a key role in the unwinding of HIV DNA from histones, which is one key for allowing RNA transcription. To do this, Tat binds to a group of proteins known collectively as Tat-associated histone acetyltransferase (TAH). The TAH complex can be formed by different cellular proteins such as p300 or is close relative CBP, along with P/CAF and/or TAF250. These TAH proteins have an enzymatic activity that transfers an acetyl group to histones and are known as histone acetyltransferases. The recruitment of the TAH complex to the HIV LTR region by TAR-bound Tat leads to acetylation of the histones that bind to the LTR, causing changes in their conformation that facilitate RNA transcription. In fact, in cells where p300 and P/CAF are limiting, addition of Tat increases transcription only about sevenfold, while addition of both Tat and p300-P/CAF allows increases transcription about 80-fold.

The TAH complex also appears to acetylate the Tat protein itself. This appears to lower the binding of Tat for the TAR, but increases the binding of Tat to another complex of proteins known as the Tat-Associated Kinase (TAK) or positive acting transcription elongation factor complex (P-TEFb). This complex of proteins consists of cyclin-dependent kinase 9 (cdk9) and one of the cyclin T isoforms (T1, T2a, T2b). This protein complex can directly bind Tat. More importantly, the kinase activity of the TAK complex phosphorylates (adds a phosphate group to) the RNA polymerase RNAP

II. This phosphorylation appears to alter the activity of the RNAP II, improving its ability to transcribe the HIV genome. Together, the TAH and TAK complexes brought in by Tat unleash the RNAP II to finish the job it started, giving the 100-fold improvement of transcription, and therefore HIV replication, observed when Tat is added to HIV infected cells.

Tat also transactivates several cellular genes in addition to the HIV LTR. The activation of these genes is also thought to contribute to the pathogenesis of HIV. The genes include the cytokines IL-6, TNF and IL-1, which are known to be increased in AIDS patients and may have detrimental effects on the overall function of the immune system.

AIDS-Associated Pathologies: A Direct Contribution by Tat

One of the most striking properties of Tat is its ability to exit from cells, where it is released into the extracellular environment. Several studies have shown that the HIV-1 Tat protein can exit from cells, including HIV infected cells. As the Tat gene does not encode a signal peptide, the release of HIV-Tat has been suggested to occur via an alternative secretion pathway, like that demonstrated for some cytokines. It may also come from cells dying due to HIV. The Tat that is found extracellularly appears to be intact and active, and substantial levels of Tat protein have been observed found in the serum of many HIV patients. Antibodies can be made against Tat in AIDS patients, indicating that it is released, and interestingly an inverse correlation between anti-Tat antibodies and patient survival has been reported in some studies. These data suggest that extracellular Tat may favor HIV replication, and have spawned tests on the possibility of using Tat as part of an AIDS vaccine.

A wide range of activities have been attributed to the Tat protein released extracellularly. Several studies have demonstrated that the HIV Tat protein, or peptides based on Tat, are capable of entering cells cultured in vitro. The Tat that enters cells is capable of transactivating the HIV LTR. Tat and Tat peptides have even been used to deliver other proteins into cells, and a peptide based on Tat may find use as a signal to move drugs into cells.

In addition to getting into other cells, the Tat that is released also appears to bind to several cell surface proteins, including specific receptors. These activities of Tat have been linked to many of the pathological alterations found in HIV infection. For example, some groups of AIDS patients frequently have ►Kaposi sarcoma, an otherwise rare, benign vascular tumor. Unlike most Kaposi, the Kaposi sarcoma associated with AIDS is very malignant and could be life-threatening for many of these patients. Tat was first linked to Kaposi sarcoma when Kaposi-like lesions were found on mice genetically engineered to express Tat. Soon after, Tat was shown to be a growth factor for Kaposi cells, but the reason for this was not known. Later studies showed that Tat could bind to KDR (VEGFR2), a receptor for the growth factor ►VEGF, on the surfaces of endothelial and Kaposi sarcoma cells. VEGF (vascular endothelial growth factor) is important in the formation of new blood vessels, as is its receptor KDR. The ability of Tat to bind and activate KDR means that Tat could stimulate the formation of vessels found in Kaposi tumors, as well as the growth of cells of the tumor itself. We now know that Kaposi sarcoma is due to an infection with a herpesvirus, HHV8, that occurs when the immune system is unable to control this virus. All Kaposi cells, whether from aggressive AIDS or benign sporadic of iatrogenic (post-transplant), have KDR, however the Tat stimulation of KDR and perhaps other receptors appears to make AIDS Kaposi potentially lethal.

Many Tat proteins have an RGD sequence, three amino acids found in many proteins of the extracellular matrix. Through this RGD sequence Tat can bind to cell surface integrins, proteins that are normally involved in binding to extracellular matrix molecules. Studies have shown that several integrins bind the Tat protein. Tat-integrin binding has been shown to trigger events typical of integrin-extracellular matrix ligand interactions, including activation of p125 Focal Adhesion Kinase. The binding of Tat to these receptors has also been linked to Kaposi sarcoma and other activities of HIV Tat.

The immune suppression seen with AIDS appears to affect cells that are not infected with HIV aside from those harboring the virus. Several studies have shown that there is immune suppression of non-HIV infected cells from AIDS patients, and that the number of immune suppressed cells seems to exceed that of the potentially HIV-infected cells. Proteins released from HIV-infected cells are clearly potential candidates for mediating this immune suppression. Tat has been linked to induction of T-cell anergy (lack of activity), T-cell ►apoptosis (programmed death), but also to a T-cell hyper-activation that appears to prime cells for infection by HIV. These events are probably all closely linked to the same phenomenon. The potential receptor system(s) involved in these activities of Tat include CD26. CD26 is a dipeptidyl peptidase that is known to cleave and alter the activities of chemokines, molecules whose receptors are very important cell surface receptors for HIV and that can regulate HIV infection. Tat has been shown to significantly increase the expression of two key chemokine-HIV receptors, CXCR4 and CCR5, by monocytes and T-lymphocytes, potentially increasing HIV infection.

The Tat protein has been reported to act as a growth factor and protect transfected cell lines from apoptosis. Tat has been consistently found to up-regulate the expression of CD95-►Fas, a protein that signals cells to

die. The increase in apoptosis is typical for partially activated T-cells, as is entry into anergy resulting from an incomplete stimulation of T-cells. Tat may be capable of partial, but incomplete, T-cell activation. HIV does not readily infect resting T-cells, T-cell activation is a key requisite for HIV infection of these cells. A partial T-cell activation may be sufficient for HIV infection yet detrimental to the host immune response, a potential role that Tat may fulfill.

Extracellular HIV Tat has been shown to have wide ranging effects on lymphatic cells such as monocytes, macrophages, dendritic cells and even natural killer cells. Tat has been reported by several groups to be a strong chemoattractant for monocytes. This activity could contribute directly to the recruitment of potentially "infectable" cells toward an HIV-infected cell producing and releasing Tat protein, an activity which may have a direct affect on establishment and spread of HIV infection in the host. Tat has been shown to bind to and activate several chemokine receptors (including CCR3 and CKCR4) and mimic the chemoattractant properties of chemokines. Chronic inflammation is frequently associated with cancer and inflammatory cells can promote tumor growth and tumor angiogenesis, thus Tat may influence the AIDS-associated tumor microenvironment through direct and indirect mechanisms. Tat can even inhibit HIV infection in high doses by binding chemokine receptors, although the physiological relevance of this observation is not yet clear.

The Tat protein appears to inhibit dendritic cell phagocytosis and natural killer cell function, apparently by blockage of certain calcium channels. Finally, Tat has been linked to AIDS associated dementia. Tat has been shown to excite neurons, which is associated with neurotoxicity. The molecular identity of the Tat receptor(s) on neural cells is not yet known. However, the neuroexcitory properties of Tat were blocked by lowering extracellular calcium, suggesting that interference with calcium channel function may be involved.

Conclusion

Tat is known to have a major role in HIV replication through a complex series of interactions with nuclear proteins. In addition, Tat outside the cell appears to be able to stimulate through, or interfere with, several cell surface receptors, sending signals that may be a root cause of many pathologies found in HIV-infected patients.

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TATA Box

Definition

Synonym Goldberg–Hogness box; Is the core promoter sequence (5′-TATAA-3′) of most genes. In eukaryotes, it represents the DNA binding site of the TATA binding protein during the process of gene transcription. Promoters lacking a TATA box were first found in housekeeping genes and have more recently been found in various gene promoters involved in cell-specific transcription.

TATI

Definition

Tumor-associated trypsin inhibitor (TATI) is a low molecular weight protein used as a tumor marker, e.g. ovarian cancer.

►Ovarian Cancer

Tau

Definition

Is a ►microtubule-associated protein (MAP) that is functionally modulated by phosphorylation and that is hyperphosphorylated in several neurodegenerative diseases. Tau proteins interact with ►tubulin to stabilize ►microtubules and promote tubulin assembly into microtubules.

Taurodont

Definition
Unusually large tooth with an unusually large pulp chamber and either multiple roots or a large single root.

►Dental Pulp Neoplasms

Tax

Definition
Is a viral oncoprotein encoded by the ►human T-cell leukemia virus 1 (HTLV-1). HTLV-1 is the causal agent of a human leukemia, adult T-cell leukemia (ATL). During HTLV-1 replication, Tax transcriptionally activates the viral long terminal repeat (LTR). Several major cellular signal transduction pathways including the transcription factors NF-kB, CREB, SRF and AP-1 are induced by Tax. During cell division, Tax binds MAD1 directly to repress its function.

►Mitotic Arrest-Deficient Protein 1 (MAD1)

Tax Binding Protein-181

►Mitotic Arrest-Deficient Protein1

Tax Helper Protein (GLI2)

►GLI Proteins

Taxane

Definition
Are diterpenes synthesized by plants of the genus *Taxus* (yews). A class of naturally-occurring or synthetic chemicals containing a characteristic 15-member ring system, the latter composed of four isoprene units with the molecular formula $C_{20}H_{32}$. Drugs that inhibit microtubule formation resulting in cell cycle arrest and apoptosis, used in cancer chemotherapy. Microtubules are essential to cell division, and taxanes therefore stop this action by freezing the mitosis process.

►Taxotere
►Taxol
►Docetaxel

Taxol

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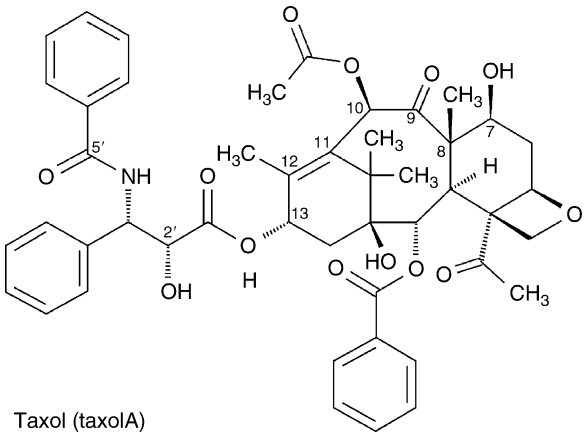
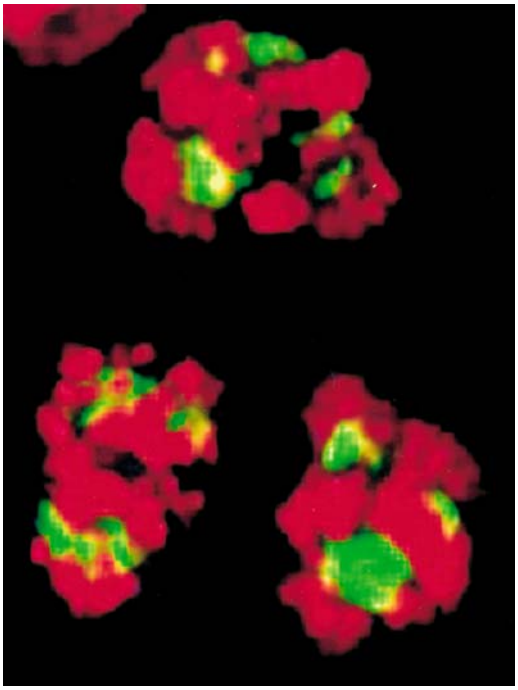
Definition
Taxol (Paclitaxel) was first isolated in 1971 from a crude extract of Taxus brevifolia, a scarce, slow growing yew plant found in the forests of the Pacific Northwest. It is a diterpenoid containing the characteristic taxane ring (Fig. 1). Total chemical synthesis of this compound was achieved in 1994, opening the way for the production of various analogues. Currently, taxol is commercially prepared by hemisynthesis, in which a synthetic side chain is attached to natural products isolated from the needles of Taxus plants. Enzymatic conversion of various taxanes to 10-deacetylbaccatin III (a precursor for taxol hemisynthesis) has been reported.

Characteristics
Mode of Action
Due to its hydrophobic character, taxol readily crosses the ►plasma membrane. Once in the cytoplasm, the drug binds with high affinity to the β-subunit of ►tubulin, modifying and stabilizing ►microtubules. When modified by taxol, these cytoskeletal structures exhibit decreased dynamic instability and increased rigidity. As a result, the function of the microtubule-based machines (e.g., ►mitotic spindle) is compromised and the cells cannot divide properly. Taxol also binds to ►Bcl-2, a protein involved in the process of programmed cell death (►apoptosis).

The cellular effects of taxol vary depending on dose and treatment scheme. In the range of nanomolar concentrations it induces sustained mitotic arrest (Fig. 2), inhibits protein prenylation and triggers apoptosis. At micromolar doses it promotes synthesis and release



Taxol. Figure 1 Chemical structure of taxol.



Taxol. Figure 2 Confocal microscopy image, showing human cervical carcinoma cells in mitosis. Cells were treated with 10 nM taxol for 20 h. Immunostaining was done with anti-tubulin antibodies and counter-staining was done with propidium iodide. Chromosomes are shown in *red*, the mitotic spindle in *green*.

of cytokines, such as ►tumor necrosis factor (TNF) and ►interleukins (IL1 and IL8), increases tyrosine phosphorylation by ►MAP kinases, induces early response genes and stimulates production of ►nitric oxide.

How taxol exerts its cytotoxic action remains elucive. Structure-activity studies differentiate microtubule stabilization from other effects, supporting a direct effect on the genetic and signal transduction machinery. However, other studies suggest that the drug acts by inducing cytoskeletal damage.

Recent observations show that taxol activates Raf-1 kinase and induces phosphorylation of Bcl-2. Phosphorylation of the latter may, in turn, lead to dissociation of Bcl-2/►Bax complexes, unleashing Bax into the cell and thus triggering apoptosis. Although these hypotheses are intuitively attractive, neither of them could fully account for the cell killing action of taxol: programmed cell death is also induced by other microtubule-stabilizing drugs which are free of genomic side-effects (e.g., ►Taxotere), while apoptosis can still occur independently of Raf-1 phosphorylation, or after Bcl-2 is dephosphorylated by cellular phosphatases.

Taxol and other microtubule-acting agents affect dramatically the architecture of the cell nucleus. It is widely known that cells treated with nanomolar amounts of taxanes or ►vinca alkaloids develop lobulated nuclei or multiple micronuclei and missort key cellular constituents. Also, taxol affects the nuclear envelope. Even at nanomolar concentrations, the drug induces focal unraveling of the nuclear lamina and extensive clustering or ectopic localization of nuclear pore complexes (Fig. 3). Cells that possess a defective nuclear envelope and which are treated with taxol remain alive for at least 24 h after the end of the treatment, but are unable to import karyophilic proteins, such as the transcription factor ►NFκB, into the nucleus. It has been proposed that inhibition of NFκB import may render the cells prone to programmed cell death.

Clinical Pharmacology

Antitumor Activity

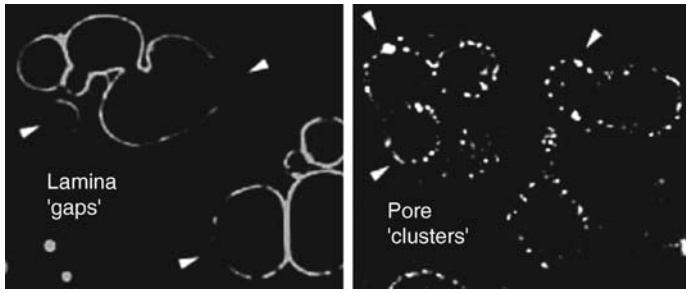
Taxol as a single chemotherapeutic agent has been proven effective against a variety of tumors, including ovarian, breast, head and neck, esophageal, bladder and lung carcinomas. In addition, several schedules of combination therapy have been developed as alternatives for patients with advanced cancer. Pilot studies show that taxol can enhance radiation sensitivity of tumor cells, potentiate tumor response and increase the therapeutic ratio of radiotherapy.

Absorption and Excretion

The drug is administered as a 3-h or 24-h infusion. It undergoes ►cytochrome P450-mediated metabolism to 6-OH derivatives and other products. Less than 10% of a dose is excreted intact from the urine.

Toxicity

The principal toxic effect of taxol is neutropenia. Several other toxic effects, such as myalgias, mucositis,



Taxol. Figure 3 Nuclear lamina and pore complex lesions after treatment of human endometrial carcinoma cells with 10 nM taxol. *Left*: indirect immunofluorescence using anti-lamin B antibodies. *Right*: indirect immunofluorescence using anti-nucleoporin antibodies. The interruption of the nuclear lamina and the formation of large pore clusters is evident in these images.

hypersensitivity reactions, stocking-glove sensory neuropathy and disturbances of the cardiac rhythm, have also been encountered.

Structurally and Functionally Related Compounds

Structurally Related Compounds

- ►Docetaxel (taxotere), produced by semisynthesis (1986) from 10-deacetyl baccatin III, a taxoid precursor. It is the second member of the taxane class to reach clinical use.
- Several chemically synthesized taxoids bearing substitutions or modifications.

Nontaxane, Microtubule-Stabilizing Agents

- Estramustine, a conjugate of estradiol and nor-nitrogen mustard.
- ►Epothilones A and B, two macrolides isolated from myxobacterium, Sorangium cellulosum.
- A family of marine-derived compounds extracted from sponges (discodermolide and laulimalide) or corals (sarcodictyins A - F and eleutherobin).

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Taxotere

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Synonyms

Docetaxel

Definition

Taxotere is a widely used ►cancer chemotherapeutic drug with well-documented clinical anti-tumor activity against a range of human cancers, including ►breast, ►lung, ►prostate and ►ovarian cancers. It is a semi-synthetic molecule belonging to the ►taxane family, and is closely related chemically and pharmacologically to the naturally occurring drug ►Taxol (►paclitaxel). Taxotere binds to the β-►tubulin component of ►microtubules, a key ►cytoskeletal protein in cells, and stabilizes the structure of the microtubule polymer, prevents its disassembly, and suppresses microtubule dynamics. These actions interfere with a number of cellular functions in which the microtubules are involved.

Characteristics

Chemistry

The anti-tumor activity of the taxanes was first observed in preclinical models using a crude extract of the bark of the Pacific yew tree *Taxus brevifolia*, and Taxol was subsequently (1971) identified as the active constituent. Both Taxotere and Taxol are diterpenoid compounds consisting of a 15-member taxane ring system linked to an unusual 4-member oxetan ring. Limited supplies of bark from the *T. brevifolia* tree prompted the search for alternative derivatives and sources of starting material.

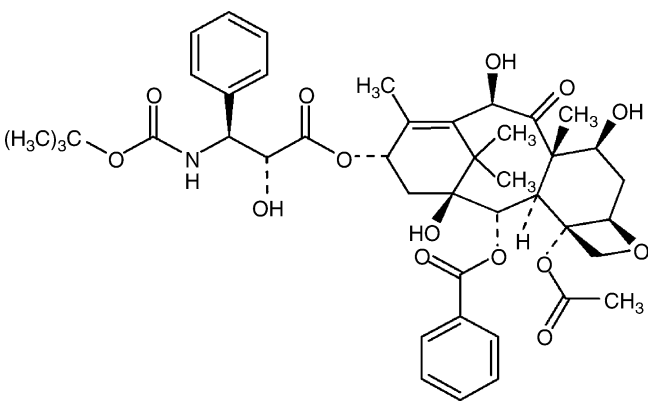
An inactive compound, 10-deacetylbaccatin III, found in the needles (which are more abundant and also are a renewable source) of several yew species, was used as starting material for the synthesis of Taxotere, which was first reported in 1986. Taxotere and Taxol differ in the nature of the substitutions at the C-10 position of the taxane ring and on the ester side chain attached to C-13, and these differences are the basis for the increased water solubility and greater potency of Taxotere, compared to Taxol (Fig. 1).

Mechanisms of Action

Microtubules are a component of the cell's ►cytoskeleton, and form a well-organized network of hollow tubes in which one end is anchored at the ►centrosome (the microtubule-organizing center), and the opposite, free end extends out into the cytoplasm. Microtubules are composed of noncovalent ►heterodimers of α-and β-tubulin, which are arranged head-to-tail to form helical polymers. There is an exchangeable GTP/GDP binding site on β-tubulin which mediates the rapid assembly and disassembly of the microtubules. The free ends of the microtubules undergo frequent periods of slow growth and rapid shortening, a process called dynamic instability, and microtubules also exhibit treadmilling, in which the loss of tubulin at one end is matched by the addition of tubulin at the opposite end. This dynamic behavior is required for the proper functioning of the microtubules. During mitosis, microtubules capture and are responsible for the alignment of the chromosomes and for their subsequent separation to the two daughter cells during anaphase. Properly functioning microtubules are required for the migration of most cell types, and they also play an important role in cell signaling and the intracellular transport of proteins, vesicles, and mitochondria.

Much of the early work on the binding and effect of the taxanes on microtubules was done using Taxol. Taxol preferentially binds to the microtubule polymer, rather than to unassembled tubulin, at a site that is distinct from the GTP/GDP site and which also differs from the sites at which colchicine, vinblastine and podophyllotoxin bind. Studies suggest that in their interactions with purified microtubules, Taxotere and Taxol are qualitatively indistinguishable. Although Taxotere and Taxol have the same apparent binding site on tubulin, Taxotere binds with a 1.9-fold greater effective affinity.

The taxanes promote microtubule assembly, even in the absence of added GTP, by altering the tubulin dissociation constants at both ends of the microtubules. The taxanes reduce the critical concentration of tubulin required for microtubule assembly, with the required concentration 2.1-fold lower in the presence of Taxotere than with Taxol. In addition to promoting both the nucleation and elongation phases of polymerization, they also produce microtubules that are extremely stable and are resistant to depolymerization. The effects on microtubule assembly are maximal at Taxol or Taxotere concentrations that are equimolar to that of tubulin, and evidence indicates that they bind with a stoichiometry of 1 per αβ tubulin dimer. In cells treated with stoichiometric concentrations of taxanes, there is an increase in the microtubule-polymer mass along with the formation of abnormal polymers and a characteristic intracellular "bundling" observed. While these concentrations of the drugs are obtained clinically, it is only for brief periods of time after drug administration. At substoichiometric concentrations, the taxanes effects on microtubule dynamics are more prominent than are their actions on microtubule mass, and the suppression of mitotic spindle dynamics plays a central role in their cytotoxic actions. By interfering with the ►mitotic spindle, the taxanes block cancer cell proliferation by causing cell cycle arrest at the metaphase/ anaphase boundary. The resultant blocking or slowing of mitosis



Taxotere. Figure 1 Chemical structure of Taxotere (docetaxel).

leads to cell death via the induction of the intrinsic mitochondrial pathway of apoptosis.

Taxotere is transported out of cells by the ABC-transporter class of membrane transport proteins, which include P-glycoprotein, MDR1 and MDR2. Overexpression of these pumps and the accompanying multi-drug resistance (MDR) phenotype can lead to profound cellular resistance to the taxanes. Microtubule-related determinants may also confer resistance to the taxanes, and these include the levels of expression of microtubule-associated regulatory proteins, the extent and nature of posttranslational modification of tubulin, and the varying levels of expression of different tubulin isotypes. Interestingly, cancer cells made resistant to Taxol do not necessarily show the same degree of resistance to Taxotere, and this may be due to the observation that Taxotere is retained intracellularly longer than is paclitaxel.

In addition to effects on tumor cell proliferation and apoptosis, Taxotere is a potent inhibitor of angiogenesis, and this is likely due to its direct effects on ►endothelial cells. While it inhibits endothelial cell proliferation at concentrations comparable to those which inhibit cancer cells, it also inhibits endothelial cell motility, invasiveness, and tubule formation *in vitro* at concentrations substantially (10- to 100-fold) lower than required to cause cell cycle arrest or apoptosis. Based on pharmacokinetic analyses of Taxotere, cancer patients are exposed to these anti-angiogenic concentrations of drug for extended periods of time. Taxotere also has anti-angiogenic activity in several *in vivo* preclinical models, and these *in vivo* anti-angiogenic actions were probably not directly due to the anti-proliferative or cytotoxic activities of the drug, as other agents that inhibited endothelial cell proliferation *in vitro* did not affect angiogenesis *in vivo*. The mechanisms for these actions are not fully understood, and may include effects on signaling pathways, including those mediated by cell surface ►integrins and cell surface receptors for the angiogenic factor ►vascular endothelial growth factor (VEGF). The effects of the taxanes on microtubule dynamicity at the low concentrations that inhibit angiogenesis appear to be qualitatively different from those at concentrations that block cell proliferation.

Clinical Use

Taxotere is a highly effective drug with a spectrum of anti-cancer activity that is virtually identical to that of Taxol. Taxotere initially received regulatory approval for use in patients with metastatic or locally advanced ►breast cancer who had failed other drug therapies. In addition to increasing survival when used as second-line therapy in patients with advanced disease, subsequent studies showed it was active in the adjuvant treatment (►adjuvant therapy) of patients with local breast cancer after definitive local treatment. It has been shown to increase survival in unresectable metastatic ►non-small cell lung cancer, and is one of the most effective agents in the treatment of ovarian cancer and in hormone-refractory ►prostate cancer. The wide spectrum of activity that Taxotere was found to have in pre-clinical models has been confirmed in the clinic, and anti-tumor activity has been noted in ►bladder, ►endometrial, ►esophageal, ►gastric, head and neck, and small cell lung ►carcinomas, and in lymphoma and ►melanoma.

Taxotere is often used in combination with other chemotherapeutic drugs, including ►cisplatin, cyclophosphamide, doxorubicin, and prednisone. In addition to being evaluated in a large number of drug combinations, a range of doses and schedules of Taxotere have been studied in clinical trials over the years. The drug is usually administered once a week or once every 3 weeks as short (1 h) intravenous infusions at doses ranging from 60 to 100 mg/m^2. ►Neutropenia is the primary toxicity observed at these doses, with a significantly reduced neutrophil count typically first observed on day 8 after treatment. Complete resolution occurs by days 15–21, and the severity of the neutropenia is related to the dosage of the drug and the extent to which the patients bone marrow function has been compromised by the concomitant or prior use of other myelosuppressive (►myelosuppression) drugs. The neutropenic effect of Taxotere generally is more severe, but shorter in duration, than that of Taxol.

A number of other toxic effects have been associated with Taxotere use. Hypersensitivity reactions (►dyspnea, bronchospasm, and hypotension) can occur acutely with drug administration, and a unique fluid retention syndrome (peripheral edema, pleural and peritoneal fluid) can occur chronically with multiple courses of therapy. In both instances, the frequency and severity of these toxicities are substantially reduced by premedication with a corticosteroid and H_1 and H_2 histamine antagonists. Skin toxicities occur frequently, although these are also attenuated by premedication, with the most common manifestation being an ►erythematous pruritic macropapular rash on the arms, hands and feet. Taxotere causes mild to moderate neurosensory and neuromuscular effects (numbness, ►paresthesia), and although common (up to 40% of patients), these occur less frequently and are less severe than in patients receiving Taxol.

The relative water-insolubility of Taxotere requires it to be formulated for clinical use in the nonionic surfactant polysorbate 80 (Tween 80). Both the hypersensitivity and fluid retention side-effects of the drug have been attributed to the use of this vehicle. A number of different alternatives to the use of Tween 80 have been evaluated in pre-clinical studies, with the objective of developing a less-toxic, better-tolerated formulation, and the

identification of ways to better target Taxotere to malignant tissue. These include polyethylene glycol (PEGylated) liposomal Taxotere, immunotargeted-liposomal Taxotere conjugates, Taxotere-fibrinogen-coated olive oil droplets, Taxotere-encapsulated nanoparticle-aptamer bioconjugates, and submicronic dispersion formulations. Some of these formulations have entered early clinical trials.

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TBI

Definition

Total body irradiation.

►Ionizing Radiation Therapy

T-body

►Chimeric T Cell Receptors

TBP

Definition

TATA-box binding protein; an essential protein binding to the TATA-box found in most eukaryotic promoters of genes transcribed by RNA Polymerase II.

►AAV

TCDD

►Dioxin

2,3,7,8-TCDD

►Dioxin

T-Cell ALL

Definition

Arises from the bone marrow precursors of T cell lymphocytes.

►Childhood Cancer

T-Cell Antigen Receptor

Definition

►T-cell receptor

T-Cell Clone

Definition

Refers to cells derived from a single progenitor T cell.

T-Cell Hybrids

Definition

T-cell hybrids are formed by fusing an antigen-specific, activated T cell with a T-cell lymphoma. The hybrid cells bear the receptor of the specific T-cell parent and grow in culture like the lymphoma.

T-Cell Receptor

Definition

TCR; consists of a disulfide-linked heterodimer of the highly variable α and β chains expressed at the cell membrane as a complex with the invariant CD3 chains. T cells carrying this type of receptor are often called α:β T cells. An alternative receptor made up of variable γ and δ chains is expressed with CD3 on a subset of T cells. Both of these receptors are expressed with a disulfide-linked homodimer of ζ chains, which carries out the intracellular signaling function of the receptor.

T-Cell Response

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Definition

Expansion of antitumor T cells in response to growth of cancer or to a ►cancer vaccine or a cancer immunotherapy.

Characteristics

In living organisms, the growth and division of cells is a tightly controlled and highly regulated process that maintains the integrity of cellular architecture and survival. Cell growth can occur in our body to replace a dead cell, to heal a wound, to maintain normal homeostasis, or to generate immune cells during an immune response. The number of divisions that cells undergo during these processes is highly controlled and the cells stop dividing when they receive signals either through contact inhibition or other means. Deviations from normal regulatory mechanisms may result in uncontrolled cell growth and the development of various types of cancers in our body. Many genes in the human body play pivotal roles in regulating the normal metabolic and growth patterns of cells. Mutations in these genes lead to the production of altered proteins that may result in the uncontrolled cell growth that is observed in cancer.

Role of ►*immunosurveillance* in *antitumor immunity*: Gene mutations occur constantly through the life span of a living organism, resulting in the occasional production of abnormal cells. Most mutant cells with defective metabolism die of a process called apoptosis. The mutant cells that are able to survive with altered proteins will be recognized by the immune system and destroyed by a process termed immunosurveillance. There is strong evidence for the existence of such immunosurveillance against cancer. Cancers develop in high frequencies in people with immunodeficiency diseases associated with immune system defects such as AIDS, or in animals that have an incomplete immune system, suggesting that cancer specific immunity is a major mechanism for elimination of cancers from the body. This immunity is provided by the cytotoxic activity of tumor specific T cells that recognize tumors and destroy them. However, there are many ways that cancer cells circumvent this immunosurveillance. Some cancers secrete immunosuppressive factors such as TGF-β, which attenuates T cell immune response. Down regulation of MHC class I molecules that stimulate the immune system or upregulation of molecules that induce T cell apoptosis are other mechanisms cancers use to inactivate antitumor T cell immune responses.

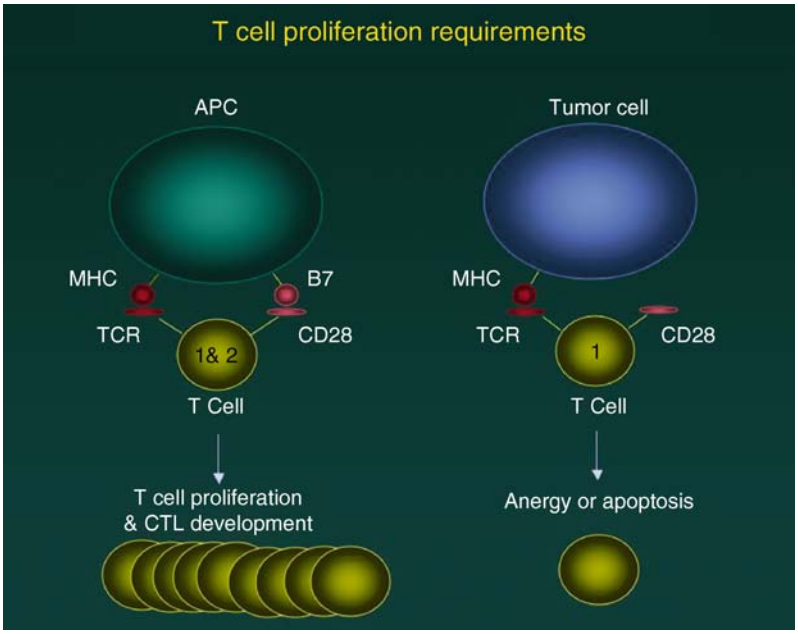
Molecular requirements of T cell responses. T cells are divided into two major subsets. CD4 antigen-expressing T cells are termed "helper T cells" whereas CD8 antigen-expressing T cells are termed "cytotoxic T cells." It has been shown that $CD4^+$ T cells provide help by secreting cytokines for the generation of robust $CD8^+$ T cells that can kill tumor cells. Since T cells play a crucial role in the development of antitumor immunity, most cancer immunotherapy is designed to directly or indirectly activate tumor-specific $CD4^+$ and $CD8^+$ T lymphocytes and induce immunological memory against tumors. Studies have shown that both subsets of T cells play an important role in the antitumor immune response. Recent advances in understanding the molecular and cellular requirements for antigen-specific immune responses have led to a number of promising immunotherapeutic strategies for inducing antitumor T cell responses for the treatment of cancer. Many of these strategies include vaccination with ►dendritic cells (DCs) engineered to express tumor antigens, cytokine transduced tumor cells, peptide vaccines, DNA vaccines, heat shock proteins, hybrid tumor cells, and tumor cells transduced with costimulatory molecules.

The rationale for the vaccination strategies mentioned above is that antigen-specific T cells can be stimulated effectively by providing stimulatory signals arising from either tumors themselves (direct priming) or through ►antigen-presenting cells (APCs) of the host (indirect priming). Normally, T cells are educated in the thymus to react against cells expressing MHC molecules that display peptides derived from foreign or altered proteins. Since tumors express altered proteins, the peptides derived from these altered proteins are associated with MHC molecules and presented to T cells that are specific to the altered protein. For an optimal immune response, antigen specific T cells

require at least two specific signals. One of the signals is provided by engagement of the T cell receptor (TCR) with peptide bearing MHC molecules on the APC. The second signal (costimulatory signal) can be delivered by the interaction of various adhesion molecules on the surface of T cells and the APC, one of which is the interaction of CD28 expressed on T cells and B7-1 (CD80) expressed on APCs such as DCs. The absence of a second signal results in T cell clonal anergy, thus preventing the development of tumor specific T cells (Fig. 1). Tumor cells, which lack costimulatory molecules such as B7-1, are poorly immunogenic since they fail to deliver the costimulatory signal necessary for the generation of an anti-tumor T cell immune response. Therefore, one approach to improve the ►immunogenicity of tumor cells has been to introduce costimulatory adhesion molecules such as B7-1 onto the tumor surface by ►gene transfection. This B7-1 expression results in the induction of T cell mediated antitumor immunity and subsequent tumor rejection in animals. These studies also demonstrated that ►costimulation is required only for the initial stimulation and expansion of tumor specific $CD8^+$ cytotoxic T cells (CTLs) and not required for the killing of tumor cells by CTLs. This basic understanding of the role of T cell immunity in eliminating tumors has given rise to many immunotherapeutic approaches focused on expanding tumor specific T cells in a tumor bearing host using vaccination and other immunotherapy approaches.

Pathways of tumor antigen-specific T cell stimulation in vivo: Tumors transduced with the B7-1 molecule have been suggested to prime T cells directly, whereas DNA vaccines, peptide vaccines, and cytokine transduced tumors may stimulate T cells indirectly through host APC. However, recent studies suggest that tumors transduced with B7-1 molecule use both priming pathways to induce an antitumor immune response. The indirect pathway could occur through host APCs, mainly by DCs taking up the tumor antigens and processing and presenting them to $CD8^+$ T cells through ►cross-priming. B7-1 gene transfected tumor cell vaccines, once thought to work only by activating $CD8^+$ T cells directly, have now been shown to activate T cells indirectly through cross-priming by professional APCs. The enhancement of cross-presentation by tumors expressing B7-1 has also been attributed to the enhanced recognition of these tumors by ►natural killer ►cells. NK cells have been shown to express CD28 and cross-linking of CD28 on NK cells by B7-1 results in the release of factors such as TNF-α and IFN-γ, which subsequently stimulate DCs. Recently it has been shown that activated human NK cells can also directly interact with $CD4^+$ T cells and costimulate TCR-induced proliferation, suggesting a possible cross-talk between $CD4^+$ T cells and NK cells during antigen specific immune response.

Many studies have shown that antigen-specific $CD8^+$ CTLs can be generated without $CD4^+$ T cell help. DCs have



T-Cell Response. Figure 1 T cells require two signals to enpand and become effective cytotoxic T cells. In the absence of second signal of tumor specific T cells and undergo energy or apoptosis. Thus, tumors by not expressing the second signaling. Molecule can prevent generation omtitumor T cell response.

been shown to play a major role in this $CD4^+$T cell independent expansion of $CD8^+$ CTLs. Antigen-specific $CD8^+$ CTLs developed in the absence of $CD4^+$ T cell help are capable of providing protective antitumor immunity in mice. However, recent studies show that $CD4^+$ T cells play a major role in maintaining the CD8 T cell memory developed during antigen exposure. The $CD8^+$ memory T cells developed in the absence of $CD4^+$ T cell help are defective in responding to antigens in a secondary challenge with antigen, suggesting that for induction of an optimal antitumor immunity, both $CD4^+$ T cells and $CD8^+$ T cells are required. These observations demonstrate that interaction of immune cells such as DCs, NK cells and T cells that are activated by direct and indirect pathways cooperate to produce an optimal antitumor T cell immune response during vaccination.

Thus, the introduction of ►immunostimulatory molecules directly into the tumor by gene transfer offers an attractive approach to improve the immunogenicity of tumor cells. Accordingly, the expression of costimulatory molecules such as B7-1 or cytokine molecules by gene transfer results in the induction of tumor immunity capable of inducing wild-type tumor rejection in animals. Apart from these vaccine strategies, it has been shown that adoptive transfer of *ex vivo* expanded and activated ►tumor infiltrating T cells reduced tumor burden in animals and humans. Recent studies have also shown that $CD4^+$ $CD25^+$ regulatory T cells (Tregs) (►T regulatory cells) play an important role in suppressing antitumor immunity in a host. The depletion of Tregs has been shown to increase potency of many antitumor immunotherapeutic methods.

Methods to detect antitumor T cell responses: Studying T cell immune responses during vaccination or other type of immunotherapies is valuable in understanding the nature of the immune response. Moreover, identifying a correlation between characteristics of antitumor T cell response and clinical efficacy of a treatment modality will be useful in predicting clinical outcome of a therapy at an early stage. For example, T cell populations carrying longer telomeres will be the dominant population in mice that are treated with the most potent vaccine since it has been shown that the presence of this T cell population is a predictive indicator of robust antitumor immunity. Similarly, better therapeutic efficacy was observed when a T cell population expressing TCR containing specific Vβ was expanded during a vaccine administration.

Tumor antigen specific $CD8^+$ T cells can be quantified by CTL assays and intracellular IFN-γ staining of cells obtained from blood in humans or the spleen in the case of mice. Limiting dilution assays or CD8 and IFN-γ staining can be used to determine the frequency of activated $CD8^+$ T cells. Increase in IFN-γ has been shown to occur during antigen-specific $CD8^+$ T cell activation. If the frequency of IFN-γ staining cells is too low to be detected by the intracellular cytokine staining method, then the ELISPOT assay to determine IFN-γ secreting cells can be carried out. Alternatively, expansion of tumor antigen specific T cells can be monitored using MHC tetramers. Although measuring antigen specific T cells using MHC tetramers is a very sensitive assay, it indicates the mere physical presence of an antigen specific T cell but does not indicate whether the expanded antitumor T cells are functionally active or not. Therefore, MHC tetramer assays are normally combined with functional assays such as CTL assay or intracellular IFN-γ staining to determine the antitumor efficacy of an immunotherapeutic method.

Lessons from human immunotherapy: Based on the knowledge obtained from *in vitro* experiments and animal models of antitumor T cell responses and vaccines, many clinical trials have been conducted. Both cell-based and vaccine-based therapies have been employed. In cell-based therapies, patients were administered *ex vivo*-expanded tumor infiltrating T cells or DCs loaded with tumor antigens. In vaccine-based therapies, cancer cells or cellular fragments or tumor-associated antigens were modified with adjuvants such as cytokines or other immunostimulatory molecules and administered to induce expansion of tumor antigen-specific T cells. Some of the trials showed moderate success whereas some of them were not as effective as observed in animal models. Interestingly, in many patients, although vaccination produced strong antitumor T cell responses, the regression of the tumor did not occur, suggesting there is a disconnect between the expansion of antitumor specific T cells and efficacy of a cancer vaccine. This could be due to the lack of homing of antitumor T cells to tumor site, or tumors may secrete immnuosuppressive factors that lead to the inactivation of cytotoxic T cells. Also, comparison of the results of T cell response, as measured by MHC tetramer assays and functional assays, showed that not all the tumor antigen specific T cells expanded during a vaccination are functionally active, suggesting aberrations in the development of T cells during vaccination. These results emphasize the need for further studies on the nature and mechanisms of T cell responses during various therapeutic approaches. A careful manipulation of the induction of antitumor T cell immune responses and homing of T cells to tumors will lead to the development of effective therapies to treat various types of cancer.

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T-Cell Zones

Definition

In lymphoid tissues are enriched in T cells and are distinct from the B-cell zones and the stromal elements.

T-cells Recognizing Autoantigens

►Autoimmunity and Prognosis in Cancer

Tcf/LEF

Definition

T-cell factor/ lymphoid enhancer-binding factor; transcription factors comprising Tcf-1 (TCF7), Tcf-3 (TCF7L1), Tcf-4 (TCF7L2) and LEF-1. Repress transcription when bound to ►Groucho and activate transcription when bound to β-catenin.

►Wnt Signaling

TCL1

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Definition

The TCL1 gene is involved in the generation and/or manifestation of mature forms of leukemias, mainly in the T-Prolymphocytic leukemia (T-PLL) and in chronic lymphocytic leukemia (B-CLL).

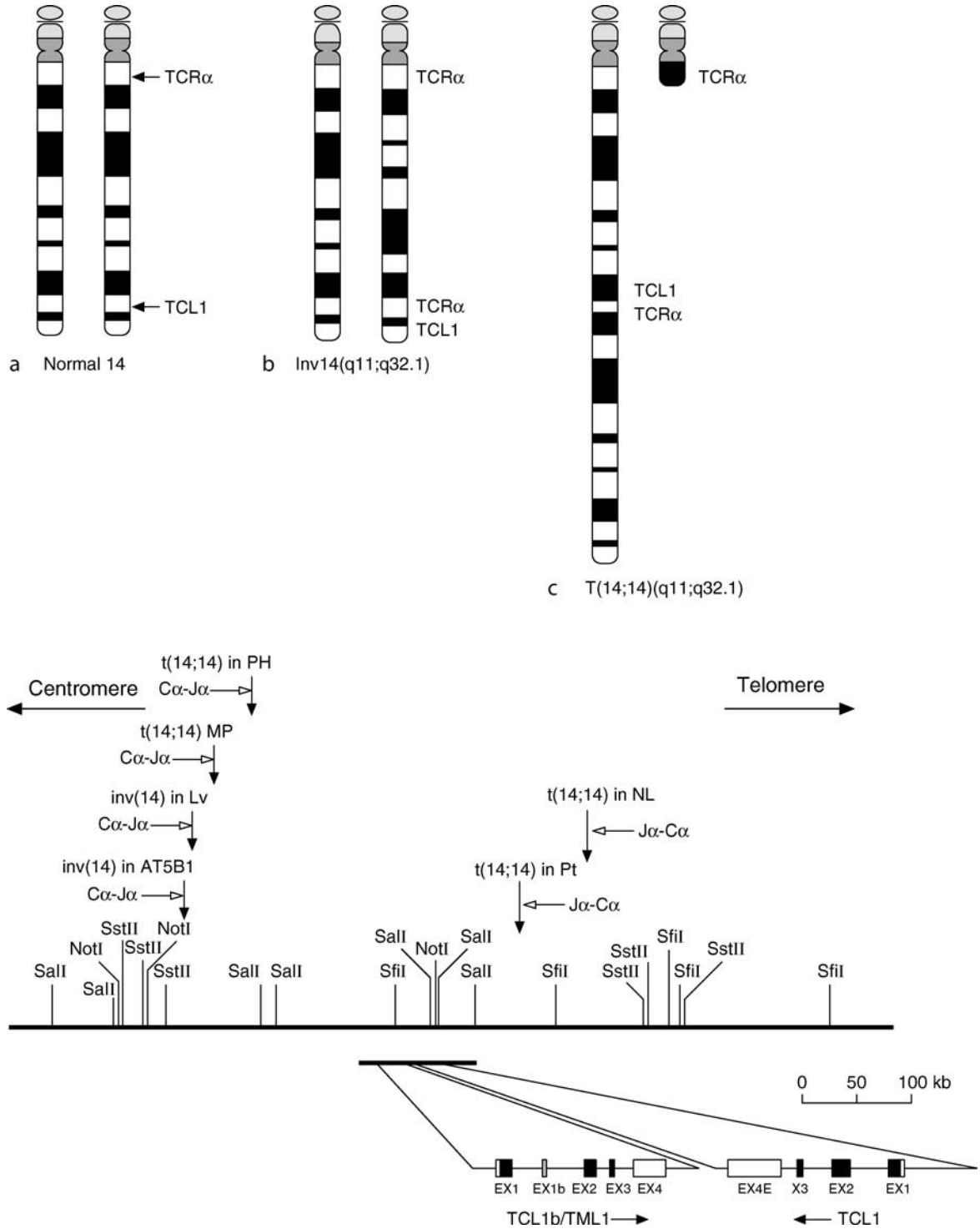
Characteristics

Clinical Characteristics of the T-PLL

T-PLL is a disease that represents 20% of prolymphocytic leukemias. It occurs at an advanced age of 70–80 years, with a slight male predominance. It is, however, quite frequent in patients with the immunodeficiency syndrome ►ataxia telangiectasia (AT) (1–5% of these patients develop it). Clinically it is accompanied by splenomegaly (75%), hepatomegaly (42%), lymphoadenopathy (55%), a high blood count (>200 × 10^9/L), with a very bad survival rate (<8 months). It involves mature T-cells (CD4+ in the 65%; but also CD8+ and CD4+ CD8+); T-cell prolymphocytes usually express CD3, CD5 and CD7. Morphologically the T-prolymphocyte is smaller than its B-counterpart, usually with a single, sometimes irregular, nucleolus. Chromosomal aberrations are quite peculiar and involve either inv 14 (q11;q32); or t(14;14)(q11;q32) and t(X;14)(q28;q11) translocations. Other recurrent changes involve chromosome 8 either as i(8)(q10) or as der(8) t(8;8). Molecular characterization of these breakpoints has been achieved and have brought about the identification of a new class of oncogenes on chromosome 14q32.1 (TCL1) and Xq28 (MTCP1) that are dysregulated (overexpressed) in this disease.

Cytogenetic and Molecular Characteristic of the TCL1 Locus

Intracellular overexpression of the TCL1 gene, is usually the result of a ►chromosome translocation or inversion juxtaposing the human region of chromosome14q32.1 (TCL1) to the region 14q11 of the alpha/delta locus of the T-cell receptor gene (TCR) (Fig. 1, *top*), as a result of a mistake in ►V(D)J recombination. Less frequently the translocation involves the TCL1 gene and the β locus of the TCR, thus giving origin to a t(7;14)(q35;q32.1) translocation. The TCL1 gene was first identified inside an 80 kb area comprised between the group of cloned inversions and translocations. This area has been recently fully characterized and completely sequenced (Fig. 1, *bottom*): other than TCL1, it comprises three other genes: TCL1b (also named TML1), that is highly homologous to TCL1 and lies just 15 kb on the centromeric side of TCL1; TNG1 and TNG2 (also named TCL6), with no homology of TCL1. All of these genes are overexpressed in T-PLL, probably as a consequence of the juxtaposition of the TCR regulatory elements. The chromosomal aberrations described above are present in ~85% of T-PLL, while in the remaining 10 ± 15% it is observed another chromosome translocation between region Xq21 (MTCP1 gene) and region 14q11 (TCR α/δ). The TCL1 and MTCP1 genes share more than 41%



TCL1. Figure 1

of identity and 61% of homology. As mentioned above T-PLLs are rare in the normal population but quite frequent in the patients affected by the autosomic immunodeficiency ataxia-telangiectasia (AT), a syndrome that results from the disruption of the ATM gene (►ATM protein). The disruption of both the alleles of the ATM gene is also present in those cancer cells derived from patients with T-PLL but without AT, thus showing

that alterations in these two genes, i.e. disruption of the ATM loci and overexpression of TCL1 or MTCP1, are necessary for the development of the T-PLL.

Animal Models

Evidence for the oncogenic role of the TCL1 gene is also supported by animal models. Transgenic mice that carried either the TCL1 gene, under the transcriptional control of the $p56_{lck}$ promoter, or the MTCP1 gene under the control of the CD2 regulatory gene, develop a form of chronic leukemia resembling morphologically and phenotypically to the T-PLL. These results indicate that transcriptional activation of the TCL1 and MTCP1 oncogene can cause malignant transformation of T lymphocytes. The leukemogenic effect seems to be dependent on the overexpressed copy number of the transgenes. These mice develop mature T-cell leukemias after a long latency period of around 15 months. The leukemia in these transgenics is almost invariably of the CD8+ phenotype.

More recently, TCL1 has also been shown to play a role in the pathogenesis of B-CLL, the most frequent leukemia encountered in the Western world. In fact, overexpression of the TCL1 gene under the control of a VH promoter and B29 in a transgenic mouse line resulted in B-cell tumors of IgM+ CD5+ cells closely resembling those of B-CLL and mature B-cell lymphoma. These animal models are currently used for in vivo clinical trials of new pharmacological compounds developed to combat mature B-cell leukemias.

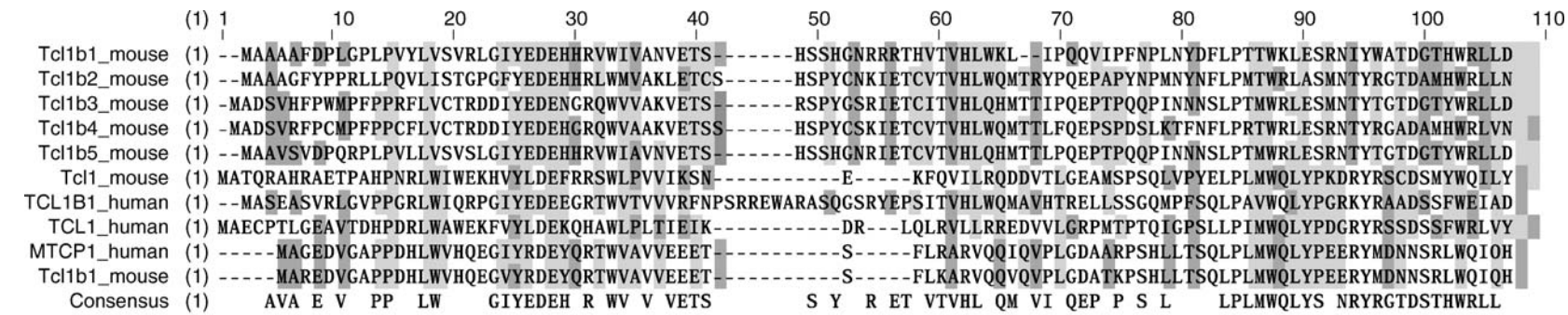
A Tcl1 knockout has been generated and shows impaired early embryonic, and B-cell and T-cell development.

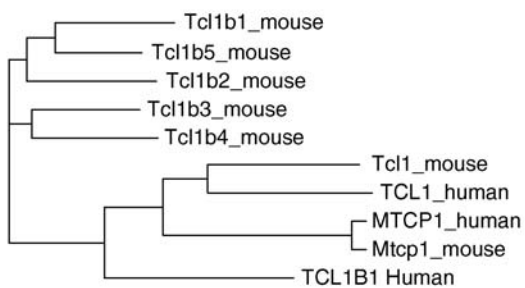
TCL1 Protein

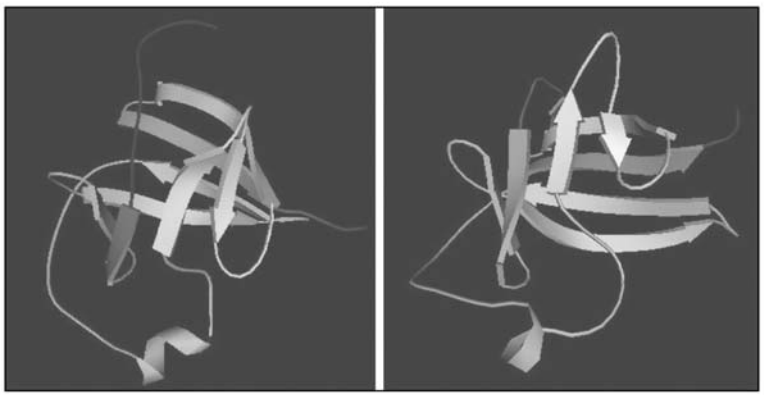
TCL1 codes for a transcript of ~1.3 kb, translated in a protein of 114 aa with a predicted Mr of 14 kD, with homology to no other protein with the exception of the other family members. Ten homologues genes have been identified, seven in the mouse and three in humans (Fig. 2). The 3D structure has been solved by NMR and X-Ray crystallography for both the TCL1 and MTCP1 proteins. These proteins fold in a compact eight-stranded b-barrel structure with a short helix between the fourth and fifth strand, with a unique topology (Fig. 2). The identical residues of the TCL1 and MTCP1 protein are clustered inside the barrel and on the surface at one side of it. Overall the structure resembles those of other proteins involved in the transporting of small molecules (such as lypocalin and calycine): this might lead to the prediction that TCL1 could bind small ligand and function as a transporter. The localization of TCL1 is mainly in the cytoplasm, but a nuclear translocation has also been reported. It is commonly accepted that TCL1 promotes cell proliferation by enhancing the activity of AKT/PKB (►AKT signal Transduction Pathway in Oncogenesis), a serine/threonine kinase having a central role in the signaling pathways that control cell proliferation and survival. Mammalian AKT/PKB is a family consisting of three members, PKBα/AKT1, PKBβ/AKT2 and PKBγ/AKT3, having idiosyncratic roles. TCL1 heterodimerizes with AKT and mediates both AKT transphosphorylation at Ser472/473 residue and AKT transfer to the nucleus. Biochemical approaches have shown that TCL1 binds to the PH domain of AKT enhancing its kinase activity and mediating its translocation to the nucleus. However, there might be other interactors, since both the proteins lack nuclear localization signals. *In vitro* assay demonstrated that this association resulted in a dose-dependent augmentation of AKT activation and suggested that this mechanism might be critical for TCL1-induced neoplasia. Activated AKT increases cell survival/proliferation by phosphorylation of many substrates in distinct signaling pathways. However, *in vitro* analysis concerning TCL1 modulation of AKT targets have shown conflicting results, in fact, some studies have shown that TCL1 overexpression leads to an increased phosphorylation of AKT target substrates FKHR, GSK3-, and BAD, whereas others indicated no effect on the AKT target substrates p70S6 kinase, BAD, IB, and NUR77. However, it is still unclear if these TCL1 functions depend on each other and how they are actually relevant to the promotion of normal/neoplastic cell growth exerted by TCL1.

TCL1 Expression in Normal and Pathological Tissues

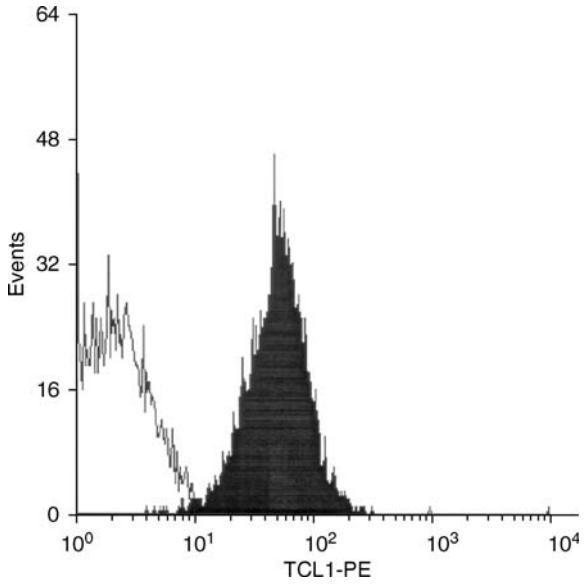
Expression of the TCL1 gene in normal cells is observed mainly in the lymphocytes, but also in ovary and in very early stages of embryogenesis in the mouse. In human T-cells derived from fetal thymus, the gene is confined very early to a CD4-CD8 double negative and no other T-cells seems to express it. FACS analysis of peripheral blood lymphocytes, using a monoclonal antibody, showed that TCL1 is only detectable in malignant T-cells from T-PLL (Fig. 3) while no other T-cell in the blood expresses it. The situation is different in B-cells where TCL1 is absent in the bone marrow in the CD34+ CD19− stem cell rich fraction: weak expression appears in the CD34+ CD19+ subpopulation of pro-B cells, and the expression peaks in the IgM negative pre B-cells with high CD19 and persist in immature IgM+ cells. In the lymph-node TCL1 is expressed by a subset of small lymphocytes of the lymphoid mantles and also by the cells of the germinal centers (centrocytes, centroblasts) of the secondary lymphoid follicles, and by data obtained analyzing a large series of tumors is downregulated in memory and plasma cells. TCL1 was recently found to be highly expressed in the majority of AIDS immunoblastic lymphoma plasmacytoid. The expression of TCL1 was also characterized during preimplantation embryo







TCL1. Figure 2



TCL1. Figure 3

development, namely in the cells from which ES are derived, where it was found to shuttle between blastomere cortical regions and the nucleus by a cell cycle-dependent fashion required for early blastomere proliferation, but not the acquisition of first embryonal differentiation traits. More recently, it has been shown that TCL1 plays a key regulatory role in the maintenance of the proliferation versus differentiation balance embryonic stem (EC) cells. In ES cells, in fact, TCL1 is directly activated by *Oct3/4*, and is among the very few genes that are required for the mitotic self-renewal state, the downregulation of which triggers ES differentiation.

Conclusions

Even if the functional role of the TCL1 and its related genes must be deciphered, this gene plays an important role in the development of the T-PLL, B-CLL, in the B-cell differentiation and in the very early stages of embryo development, and thus its targeting might have therapeutic consequences.

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TCR

Definition

►T-cell Receptor.

TCRα and TCRβ

Definition

TCRα and TCRβ are the two chains of the α:β ►T-cell receptor.

TDA

Definition

Target detection assay (TDA) is a combinatorial selection method that, like ►CASTing, is used to identify consensus protein-binding sites on duplex DNA.

►Combinatorial Selection Methods

TDGF-1

►Cripto-1

Technical Knockout

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Definition

Technical knockout (TKO) is a method for function-based gene isolation in mammalian cells.

Characteristics

Technical knockout application has resulted in the isolation of several novel genes involved in apoptosis and in the genetic dissection of certain processes within this important biological process.

In general, TKO is based on two concepts, both directed at overcoming impediments to the use of genetic screens in cultured cells.

- One concept is the use of nucleic acids as "virtual mutagens" for trapping genes based on their function. This was achieved by transfections with anti-sense cDNA libraries to randomly inactivate gene expression, followed by a strong phenotypic selection.
- The other concept was to develop efficient strategies for introduction and recovery of complex cDNA libraries, to allow multiple rounds of phenotypic selection and rapid screens for identification of functionally relevant genes. To this aim, an expression vector that provides an efficient gene transfer system was developed, with the capability of representing a complex library in a single transfection event.

An ►EBV-based, self replicating episomal vector, was chosen to express a directional anti-sense cDNA library. The unidirectional strategy increased the probability of acquiring "recessive mutations" due to loss of expression. The episomal vector had several advantages over vectors that integrate into the genome. It reduced the background of non relevant phenotypic alterations occurring as a result of random integrations into DNA. In addition, the episomes were easily rescued from the transfected cells by a simple DNA extraction procedure with no need for any other genetic manipulations. This yielded a rapid and convenient way to perform multiple rounds of phenotypic selection. It also solved the issue of plasticity of tissue culture cells, since only the individual cDNA fragments that transduced the phenotypic change in subsequent transfections were scored as real positives. The episomal vector accumulated at multiple copies in the stable transfectants, resulting in high expression levels. In addition, the promoter cassette of the vector had been manipulated to confer much stronger expression levels of anti-sense RNAs during the phenotype selection. Since interferon-γ was the trigger that induced apoptosis, an interferon-responsive enhancer element was introduced into the vector to increase expression during the selection by the killing cytokine. In retrospect, the latter manipulation was found to be critical for the success of the functional cloning, since expression of high anti-sense RNA levels during the phenotypic selection was essential to reduce efficiently the protein levels.

Isolation of Genes in Apoptosis

The application of the TKO approach to apoptosis resulted in the discovery and subsequent characterization of five novel genes that function as positive mediators of this biological process. In addition, an anti-sense cDNA fragment to ►cathepsin D was also isolated, suggesting that this well known lysosomal protease is actively recruited to the death process. It was found that the five novel apoptotic genes (named DAP genes for: Death Associated Proteins) encode proteins that display a diverse spectrum of biochemical activities and different intracellular localizations. The list comprises a novel type of calcium/calmodulin-regulated kinase, which carries ankyrin repeats and the death domain, and is localized to the cytoskeleton (DAP-kinase), a nucleotide-binding protein (DAP-3), a small proline-rich, RGD-containing, protein (DAP-1) found in the cytoplasm, and a novel homologue of the eIF4G translation initiation factor (DAP-5). Extensive studies proved that these diverse activities participate in multiple apoptotic scenarios consistent with the initial design of the strategy which was aimed at targeting genes that belong to the basic machinery of apoptosis.

Preliminary results indicate that some of these rescued genes are functionally interconnected; the others may function independently of each other. The DAP genes were highly conserved in evolution. The C. elegans orthologue of DAP-3 shares 35% identity to the human protein and induces apoptosis in mammalian cells, indicating that the protein is also conserved at the functional level.

Clinical Aspects

The advantage of functional approaches to gene cloning is that they select the relevant rate limiting genes controlling a biological process in a unbiased manner. As a consequence, novel targets and unpredicted mechanisms may emerge, as became evident from the study of DAP proteins. For example, the calcium/calmodulin-dependent DAP-kinase, which was found to be localized to the actin microfilaments, may provide a molecular handle to study the collapse of the microfilament system in ►apoptosis and/or may mediate membrane blebbing. Most importantly, DAP-kinase, was found to possess tumor suppressive activities. This has been analyzed initially in mouse model systems where DAP-kinase display strong anti-metastatic effects and later in other in vitro systems that test suppression of oncogenic transformation. In human carcinoma cell lines and B cell lymphomas, DAP-kinase expression was lost at high frequency. Recent clinical studies have revealed that DAP-kinase expression is lost in non-small lung carcinomas, head and neck carcinomas and B cell malignancies. Thus, screening systems for genes that are rate limiting in apoptosis may target tumor suppressor genes. Another breakthrough step relates to the discovery of DAP-5. The structure/function features of this novel translation regulator resemble the proteolytically cleaved eIF4G initiation

factor, which appears in cells upon infection with some RNA viruses and directs cap-independent translation. The rescue of DAP-5 proved the existence of a strong link between apoptosis and the control of protein synthesis, which seems to be critical in certain apoptotic systems, and focused some of the mechanistic studies towards this direction. Another example refers to the isolation of cathepsin D by the TKO method suggesting that lysosomal proteases are recruited during apoptosis, in addition to the well known caspase family of proteases.

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TEL

Definition

Translocations ►ETS Leukemia Gene; earlier designation for ►ETV6, which was abandoned o avoid confusion with the abbreviation for ►telomere.

►ETV6

TEL-AML1

Definition

In the t(12;21), *AML1* (►acute-myeloid-leukemia-1; synonym Runx-1, CBFA1, and PEBP2A2), a gene that is homologous to the runt gene of *Drosophila* and encodes a DNA-binding transcriptional regulator, fuses with *TEL* (translocation ets-leukemia; synonym ETV6), which encodes a ETS family helix-loop-helix transcription factor. As this ►chromosome translocation is not detected by routine ►cytogenetics, molecular analysis (e.g., ►FISH, ►RT-PCR) is required to identify this favorable chromosomal rearrangement.

►Acute Lymphoblastic Leukemia
►Ets Transcription Factors

Telangiectasia Macularis Eruptive Perstans

Definition

TMEP; A rare form of cutaneous mastocytosis presenting with generalized telangiectatic macules.

►Mastocytosis

Telomerase

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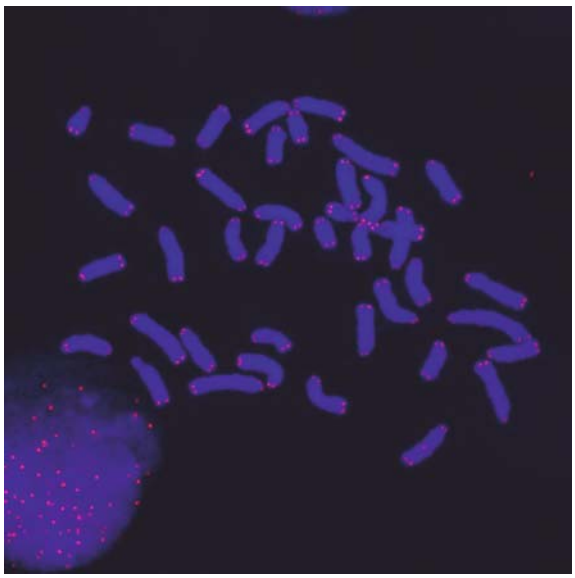
Definition

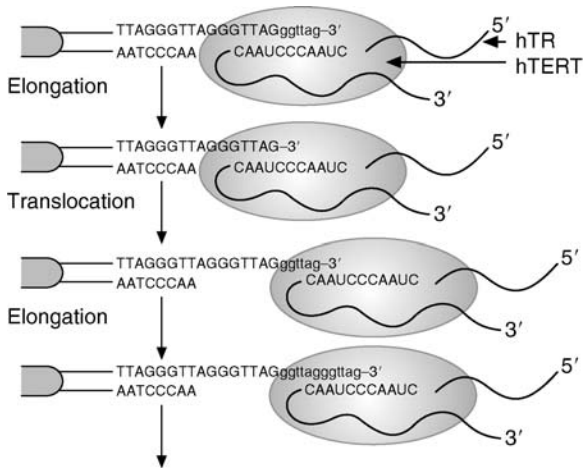
Telomerase (TE-LÓM-ER-ACE) is a ribonucleoprotein enzyme complex (a cellular reverse transcriptase) that maintains chromosome ends and has been referred to as a cellular immortalizing enzyme. Telomerase is composed of both RNA and proteins and uses its internal RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize telomeric DNA (TTAGGG)n directly onto the ends of chromosomes using the catalytic hTERT component. Telomerase is present in most fetal tissues, normal adult male germ cells, inflammatory cells, in proliferative cells of renewal tissues and in most tumor cells. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six base pair repeat. This extension of the 3′ DNA template end in turn permits additional replication of the 5′ end of the lagging strand, thus compensating for the ►end replication problem.

Characteristics

►Telomeres are the repetitive DNA sequences at the end of all linear chromosomes (Fig. 1). In humans there are 46 chromosomes and thus 92 telomere ends that consist of thousands of repeats of the six nucleotide sequence, TTAGGG. The telomere-telomerase hypothesis of aging and cancer is based on the findings that the cells of most human tumors have telomerase activity, while normal human somatic cells do not. Telomere length is maintained by a balance between processes that lengthen telomeres (telomerase) and processes that shorten telomeres (the end replication problem and oxidative damage). Telomerase is a cellular reverse



Telomerase. Figure 1 Telomeres in human chromosomes. Metaphase human chromosomes that have been both stained with DAPI (blue color which stains DNA/chromosomes) and also in situ hybridized with a PNA (peptide nucleic acid) fluorescently labeled telomere probe (red ends of each chromosome).



Telomerase. Figure 2 Telomeric sequences are synthesized by telomerase, a ribonucleoprotein enzyme (composed of both RNA and protein). Telomerase contains RNA-dependent DNA polymerase activity which uses its RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize TTAGGG repeats (elongate) directly onto telomeric ends. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six bp repeat. This extension of the 3′ DNA template end in turn permits additional replication of the 5′ end of the lagging strand, thus compensating for the telomere shortening that occur in its absence.

transcriptase that stabilizes telomere length by adding hexameric (TTAGGG) repeats onto the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomeres that occurs in its absence (Fig. 2). The core catalytic subunit of telomerase, ►hTERT, is expressed in embryonic cells and in adult male germline cells, but is undetectable in normal somatic cells except for proliferative cells of renewal tissues (e.g. hematopoietic stem cells, activated lymphocytes, basal cells of the epidermis, proliferative endometrium and intestinal crypt cells). The hTERT gene maps to chromosome band 5p15.33.

In normal somatic cells, progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and to a limited ability to continue to divide. It has been proposed that telomere shortening may be a molecular clock mechanism that counts the number of times a cell has divided, and when telomeres are short, cellular ►senescence (growth arrest) occurs. It has been proposed, but not proven, that shortened telomeres in mitotic (dividing) cells may be responsible for some of the changes we associate with normal aging.

What Are Telomeres and What Do They Do?

Telomeres are repeated DNA sequences that protect the ends of chromosomes from being treated like a broken piece of DNA needing repair. Without telomeres, the ends of the chromosomes would fuse to each other leading to massive genomic instability. Telomeres are also thought to be the "clock" that regulates how many times an individual cell can divide. Telomeric sequences shorten each time the DNA replicates. When at least some of the telomeres reach a critically short length, the cell stops dividing and ages (senesces) which may cause or contribute to some age-related diseases. In cancer, a special cellular reverse transcriptase, telomerase, is reactivated and maintains the length of telomeres, allowing tumor cells to continue to proliferate.

Why Do Telomeres Shorten?

The mechanisms of DNA replication in linear chromosomes is different for each of the two strands (called leading and lagging strands). The lagging strand is made as series of discrete fragments, each requiring a new RNA primer to initiate synthesis. The DNA between the last RNA priming event and the end of the chromosome cannot be replicated because there is no DNA beyond the end to which the next RNA primer can anneal, thus this gap cannot be filled in (this is referred to as the "end replication problem"). Since one strand cannot copy its end, telomere shortening occurs during progressive cell divisions. The shortened telomeres are inherited by daughter cells and the

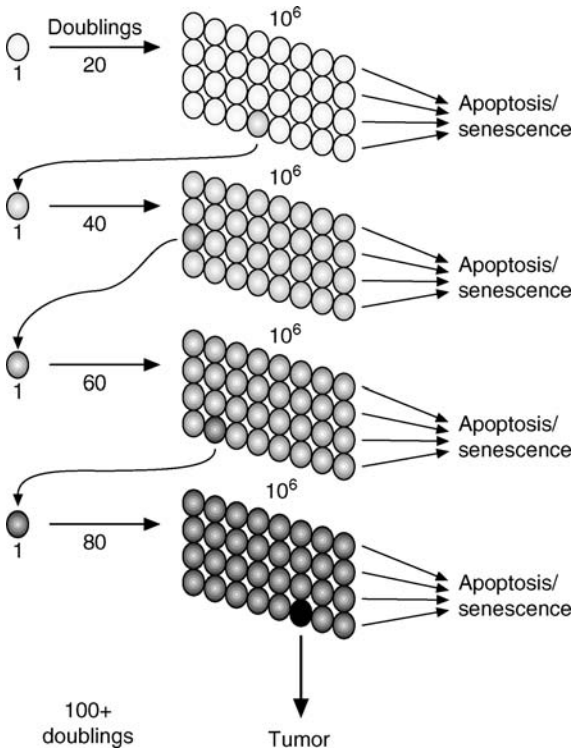
process repeats itself in subsequent divisions. Other factors may lead to telomere loss such as oxidative damage and other end processing events.

What is Cellular Senescence?

In contrast to tumor cells, which can divide forever (are "immortal"), normal human cells have a limited capacity to proliferate (are "mortal"). In general, cells cultured from a fetus divide more times in culture than those from a child, which in turn divide more times than those from an adult. The length of the telomeres decreases both as a function of donor age and with the number of times a cell has divided in culture. There appear to be two mechanisms responsible for the proliferative failure of normal cells. The first, M1 (Mortality stage 1), occurs when there are still at least several thousand base pairs of telomeric sequences left at the end of most of the chromosomes. M1 is induced by a DNA damage signal produced by one or a few of the 92 telomeres that have particularly short telomeres. The M1 mechanism causes growth arrest mediated by the tumor suppressor genes p16, RB1 and p53. If the actions of p53 and p16/pRB are blocked, either by mutation or by binding to viral oncoproteins, then cells can continue to divide and telomeres continue to shorten until the M2 (Mortality stage 2) mechanism is induced. M2 represents the physiological result of critically short telomeres when cells are no longer able to protect the ends of the chromosomes, so that end-degradation and end-to-end fusion occurs and causes genomic instability and cell death. In cultured cells, a focus of immortal cells occasionally arises. In most cases, these cells have reactivated the expression of telomerase, which is able to repair and maintain the telomeres (Fig. 3).

If You Can Stop the Shortening of Telomeres Will This Prevent Cellular Aging?

While there have been many studies indicating that there is a correlation between telomere shortening and proliferative failure of human cells, the evidence that it is causal has only recently been demonstrated. Introduction of the telomerase catalytic protein component into normal human cells without detectable telomerase results in restoration of telomerase activity. Normal human cells stably expressing transfected telomerase demonstrate extension of lifespan, providing more direct evidence that telomere shortening controls cellular aging. The cells with introduced telomerase maintain a normal chromosome complement and continue to grow in a normal manner. Initial concerns that the introduction of telomerase into normal cells may substantially increase the risk of cancer have not proven true. One way to think about this is that special reproductive tissues maintain high levels of telomerase throughout life, and there is no increased incidence of cancers in these special cells compared with other types of cancer. Thus, the major role of telomerase is to maintain telomere stability and keep the cells dividing. These observations provide the first direct evidence for



Telomerase. Figure 3 It has been argued that it may not be necessary to exhaust the replicative potential of normal cells in order to form a massive tumor (e.g. after 50 doublings a single cell could generate a tumor of a size greater than 1,000 kg). However, this theoretical argument assumes that all cells survive, which is highly unlikely to be correct. If the frequency of spontaneous mutations is ~10^{-6}, at least a million cells are needed for a second mutation to occur with reasonable probability. Since these mutations must accumulate in the same cell, a series of clonal expansions must occur as is illustrated in this figure. Since it requires 20 cell doublings to generate approximately one million cells, 20 divisions would accompany each mutation. For example if we assume five mutations are necessary for cancer to arise from a normal cell, more than 100 divisions (doublings) would be required to render a cell malignant. Losses of cells due to apoptosis or inhibition of cell proliferation due to senescence would limit the number of cells in a such a tumor to 10^6–10^8 cells which is less than a 1 g biomass. Thus, with the possible exception of certain stem cells from the bone marrow, skin, and perhaps the intestine, most normal human cells only divide 50–70 times before they growth arrest. Thus cellular senescence could act as a very effective "brake" on the proliferation of cells that had accumulated a few mutations but not all those prerequisite for malignancy.

the hypothesis that telomere length determines the proliferative capacity of human cells.

Can Telomerase be Used as a Product to Extend Cell Lifespan?

The ability to immortalize human cells and retain normal behavior holds promise in several areas of biopharmaceutical research, including drug development, screening and toxicology testing. The development of better cellular models of human disease and production of human products are among the immediate applications of this new advance. This technology has the potential to produce unlimited quantities of normal human cells of virtually any tissue type and may have most immediate translational applications in the area of transplantation medicine. In the future it may be possible to take a person's own cells, manipulate and rejuvenate them without using up their lifespan and then give them back to the patient. In addition, genetic engineering of telomerase-immortalized cells could lead to the development of cell-based therapies for certain genetic disorders such as muscular dystrophy.

Cell and Molecular Regulation

Proteins have been identified that directly interact with telomerase, such as p23/hsp90 (molecular chaperones) and TEP1 (telomerase associated protein 1 with unknown function). In addition, there are likely to be other proteins that help regulate telomerase function that have yet to be identified. The transcriptional regulation of the catalytic subunit of telomerase (hTERT) is clearly complex, but there is evidence that the c-myc gene may be important in some aspects of the transcriptional activation of hTERT. In addition, there is evidence that a gene on chromosome 3 may be involved in the transcriptional repression of hTERT. Since telomerase interacts with the telomeres, there has been a number of proteins identified that directly or indirectly bind to telomeres (TRF1, TRF2, tankyrase, TIN2, hRap1) that are also important in the regulation of telomerase. A single stranded telomere binding protein is called POT1.

There is also regulation of the level of telomerase activity in specific cell types. Telomerase activity is low in the most primitive stem cells of renewal tissues (e.g. crypts of the intestine, bone marrow cells, resting lymphocytes, basal layer of the epidermis), while telomerase activity is increased in the proliferative decendents of these cells. Thus, there are telomerase competent cells that have low activity when quiescent (not dividing) and increased activity when proliferating (dividing). However, these telomerase competent stem cells do not fully maintain telomere length since such cells obtained from older individuals have shorter telomeres than those derived from younger individuals. Thus, in germline (reproductive) cells and tumor cells, telomerase fully maintains telomere length in contrast to stem cells (with regulated telomerase activity) and most somatic cells (with no detectable telomerase activity) in which telomeres progressively shorten with increased age.

Cellular senescence may have evolved, in part, to protect long lived organisms, such as humans, against the early development of cancer. Thus, it has been proposed that upregulation or reexpression of telomerase may be a critical event responsible for continuous tumor cell growth. In contrast to normal cells, tumor cells show no net loss of average telomere length with cell division, suggesting that telomere stability may be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumors may need telomerase to sustain their growth. ►Immortalization of cells may occur through a mutation of a gene in the telomerase repression pathway. Thus, upregulation or reactivation of telomerase activity may be a rate-limiting step required for the continuing proliferation of advanced cancers. There is experimental evidence from hundreds of independent laboratories that telomerase activity is present in ~90% of all human tumors, but not in tissues adjacent to the tumors. Thus, clinical telomerase research is currently focused on the development of methods for the accurate diagnosis of cancer and on novel anti-telomerase cancer therapeutics.

Clinical Relevance

There is mounting evidence that cellular senescence acts as a "cancer brake" as it takes many divisions to accumulate all the changes needed to become a cancer cell. In addition to the accumulation of several mutations in oncogenes and tumor suppressor genes, almost all cancer cells are immortal and, thus, have overcome the normal cellular signals that prevent continued division. Young normal cells can divide many times, but these cells are not cancer cells since they have not accumulated all the other changes needed to make a cell malignant. In most instances a cell becomes senescent before it can become a cancer cell. Therefore, aging and cancer are two ends of the same spectrum. The key issue is to find out how to make our cancer cells mortal and our healthy cells immortal, or at least longer lasting. Inhibition of telomerase in cancer cells may be a viable target for anti-cancer therapeutics, while expression of telomerase in normal cells may have important biopharmaceutical and medical applications. In summary, telomerase is both an important target for cancer and for the treatment of age-related disease.

Could Telomerase be the "Achilles Heel" of Cancer?

We believe that progressive telomere shortening is halted in cancer cells by the presence of the enzyme

telomerase which maintains and stabilizes the telomeres, allowing cells to divide indefinitely. Telomerase activity is detected in almost all human tumors. It is hoped that a therapy can be developed that inhibits telomerase activity and interferes with the growth of many types of cancer. There are several ongoing clinical trials testing these ideas in patients with solid tumors and leukemias.

Will Inhibiting Telomerase Restore the Senescence Program in Cancer Cells and if so Will This Therapy Cure Cancer?

One research strategy is to inhibit the activity of telomerase, forcing immortal cells into a normal pattern of permanent growth arrest (senescence) or death (►apoptosis). Following conventional treatments (surgery, radiotherapy, chemotherapy), anti-telomerase agents would be given to limit the proliferative capacity of the rare surviving tumor cells in the hope that this would prevent cancer recurrence. We believe this treatment would be very selective, in that only cells with an activated telomerase would be affected. As far as we know, that includes only "immortal" tumor cells and germline (reproductive cells) and at lower levels stem cells in renewal tissues.

Will Telomerase Activity be Useful in Cancer Diagnostics?

Telomerase activity is detected in premalignant specimens (in situ lung and breast cancers), while colon and pancreatic cancer have detectable telomerase activity at later (carcinoma) stages. The ability to use almost any clinical specimen and to demonstrate telomerase may allow the detection of cancers at an earlier stage. For example, telomerase activity is detected in lung cells in cancer patients obtained by bronchial alveolar lavage. In addition, fine needle aspirations (breast, liver and prostate cancer), washes (bladder and colon), and sedimented cells from urine (bladder and prostate) provide minimally invasive sources of cells to detect telomerase activity and are likely to have immediate diagnostic utility. Telomerase may also be important in the monitoring of minimal residual disease. In an effort to improve the diagnostic value of telomerase determinations, in situ hybridization methods for the demonstration of telomerase on archival paraffin embedded clinical specimens appears to distinguish cancer from normal cells, correlates well with telomerase activity, and thus may provide added value to telomerase activity assays. In addition, the presence or absence of telomerase may have prognostic value and help risk-stratify patients into those with favorable outcomes (to avoid unnecessary treatments for patients with low or no detectable telomerase) and those with high telomerase activity and with unfavorable outcomes (to help oncologists manage patient treatments more effectively).

Have any Telomerase Therapeutic Agents been Identified and what are the Potential Complications of such Strategies?

Since telomerase is expressed in most advanced cancers, methods for telomerase inhibition using small molecules such as modified oligonucleotides may have utility. There are potential risks in the use of such therapy that must be considered, for example the affects of inhibitors on telomerase-expressing stem cells. However, it is likely that this approach will be less toxic than conventional chemotherapy which affects all proliferating cells, including stem cells. The rate of division of the most primitive stem cells is so much slower than that of most cancer cells that the amount of telomere shortening in the stem cells should be relatively small. Some of the side effects of standard chemotherapy, such as thrombocytopenia, leukopenia, nausea and hair loss due to the death of the cells in rapidly proliferating tissues, may be reduced by the use of telomerase inhibitors, which are predicted to induce cellular senescence or cell death only after a period of growth. This raises what many consider the most important concern with this proposed treatment regimen, the prolonged time potentially required for a telomerase inhibitor to be effective. Since the mode of action of telomerase inhibitors may require telomeric shortening before inhibition of cell growth or induction of apoptosis, there may be a significant delay in efficacy. Thus methods may have to be devised to increase the rate of telomere shortening when telomerase inhibitors are used therapeutically. Telomerase inhibitors will likely be used together with or following conventional therapies, so that once the bulk of the tumor mass is eliminated, anti-telomerase therapy might prevent the large number of cell divisions required for the regrowth of rare resistant cancer cells. They may also be used in early stage cancer to prevent overgrowth of metastatic cells, as well as in high-risk patients with inherited susceptibility to cancer syndromes to prevent the emergence of telomerase-expressing cells (chemoprevention). Finally, there are ongoing telomerase immunotherapy clinical trial. It appears that tumors cells but not normal stem cells express telomerase epitopes on their cell surface. Scientists have identified these epitopes and have produced synthetic peptides and have injected these into patients with advanced cancer. This approach is showing early signs of efficacy and currently Phase III clinical trials for patients with advanced pancreatic cancer are ongoing.

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Telomere

Definition

Refers to repeated DNA sequences ((TTAGG)n at the ends of linear chromosomes that protect the ends of the chromosome from degradation. The segment at the end of each linear eukaryotic chromosome arm, consisting of a peculiar structure (including repeated DNA sequences) required to preserve the sticky ends of chromosomes from randomly joining together. They are involved in genome integrity, chromosome stability, nuclear architecture and chromosome pairing during meiosis. The telomeres of humans consist of as many as 2,000 repeats of the 5′ TTAGGG 3′ sequence.

►Genomic Imbalance
►Senescence and Immortalization
►Telomerase

Telomere Length

Definition

Differs between individuals and species and is under complex genetic control. Mean ►telomere length has emerged as a replicative clock within each population of cells and the tissues and organs. In human normal somatic cells, telomere length ranged between 7–15 kb and shortened 50–150 bp in each cell division.

►Stem Cell Telomeres

Telomeric Repeats of Stem Cells

►Stem Cell Telomeres

TEM7

Definition

Tumor endothelial marker 7; Is a member of a class of transmembrane proteins expressed exclusively in endothelial cells of tumor vasculature. Its function is currently unknown.

►Cortactin

Temodal

►Temozolomide

Temodar

►Temozolomide

Temoget

►Temozolomide

Temozolomide

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Synonyms

Temodar; Temodal; Temoget; Methazolastone; SCH52365; NSC362856; M&B 39831; 3,4-dihydro-3-methyl-4-oxoimidazo [5,1-d]-as-tetrazine-8-carboxamide; 3, 4-Dihydro-3-methyl-4-oxoimidazo(5,1-d)-1,2,3,5-tetrazine-8-carboxamide; 3,4-Dihydro-3-methyl-4-oxoimidazo

(5,1-d)-as-tetrazine-8-carboxamide; 3-Methyl-4-oxo-3,4-dihydroimidazo(5,1-d)(1,2,3,5)tetrazine-8-carboxamide; 3-Methyl-4-oxo-3,4-dihydroimidazo[5,1-d][1,2,3,5] tetraazine-8-carboxamide; 8-Carbamoyl-3-methylimidazo(5,1-d)-1,2,3,5-tetrazin-4(3H)-one; Imidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxamide, 3, 4-dihydro-3-methyl-4-oxo-; 85622-93-1; AIDS-129717; BRN-5547136; CCRG-81045; LS-80558; NCI60_003316

Definition

TMZ is a chemotherapeutic DNA alkylating agent of the imidazotetrazine class.

IUPAC name = 3-methyl-2-oxo-1,3,4,5,8-pentazabicyclo[4.3.0]nona-4,6,8-triene-7-carbo oxamide

CAS number = 85622-93-1

MW = 194.151 g/mol

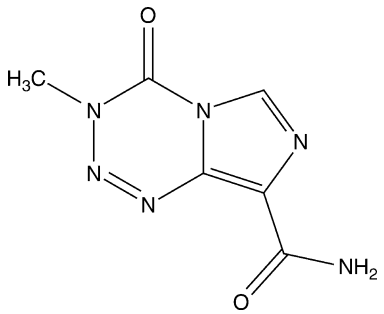
Chemical formula = $C_6H_6N_6O_2$

PubChem Compound information – CID #5394: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD = search&DB = pccompound&term = 5394

Structure – see Fig. 1.

Characteristics

Temozolomide (TMZ) is a chemotherapeutic alkylating agent, a drug used in the treatment of cancer that causes cancer cell death by transferring an alkyl adduct (a methyl group) to bases in DNA. If left un-repaired, the modified or methylated DNA triggers one of many cell death responses to facilitate the destruction of the tumor. TMZ (Fig. 1) is classified as an imidazotetrazine and was developed in the 1990s. The first of the imidazotetrazines to undergo rigorous evaluation was mitozolomide but this compound proved to be too myelosuppressive, possibly because it was found to cross-link strands of DNA in normal cells. TMZ is an N-methyl derivative of mitozolomide and is a monofunctional methylating agent that does not induce DNA cross-links. TMZ is an oral alkylating agent that was recently approved for therapy of brain tumors such as glioblastoma in combination with radiation and is undergoing clinical trials for other cancers including melanoma and leukemia as well as cancers that have metastasized to the brain. TMZ is 100% bio-available, crosses the blood-brain barrier, and produces mild toxicity, with less than 5% of patients experiencing serious myelosuppression. Partial deletion of chromosome 1p/19q in anaplastic and recurrent oligodendroglioma appears to predict for sensitivity to TMZ as it does for other alkylating agents, although the mechanism is unclear. TMZ has also shown some efficacy in non-glial tumors that have metastasized to the brain.

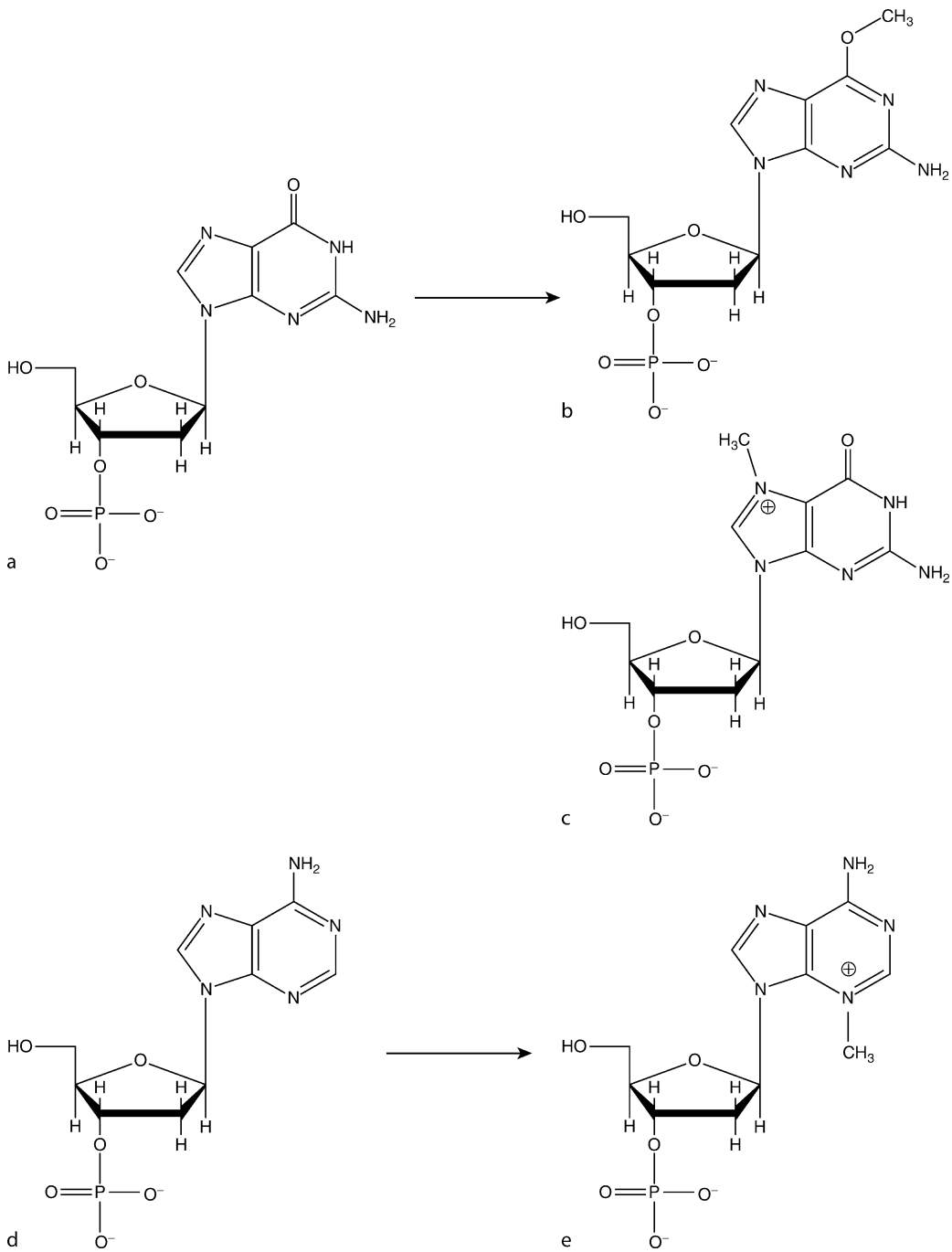


Temozolomide. Figure 1 Diagram of the chemical structure of temozolomide. Only the methyl group (CH_3−) is transferred to DNA. The remainder of the molecule is metabolized in the form of molecular nitrogen (N_2), carbon dioxide (CO_2) and excreted as a molecule of 5-aminoimadozole-4-carboxamide (AIC) in urine.

TMZ is rapidly absorbed after oral administration and undergoes a base-catalyzed addition of water to the C-4 atom and rapid breakdown and decarboxylation to yield MTIC [5-(3-methyl-1-triazeno)imidazole-4-carboxamide] in the peripheral blood. MTIC is the same active metabolite of the alkylating agent dacarbazine (DTIC). DTIC is a pro-drug that requires metabolic activation by enzymes in the liver to generate MTIC. TMZ is also considered a pro-drug although metabolism is spontaneous in that TMZ does not require enzymatic activation to generate MTIC. Once generated, MTIC undergoes acid catalyzed hydrolysis to yield the excreted compound AIC (5-aminoimidazole-4-carboxamide) and the active methyldiazonium ion, which reacts with nucleophilic atoms in DNA: predominantly the N7 and O^6 atom of guanine and the N3 atom of adenine. The major site of methylation is the N7 position of guanine (>70%) followed by the N3 position of adenine (9.2%) and the O^6 atom of guanine (5%). Figure 2 depicts the chemical structure of the naturally occurring bases in DNA (guanine and adenine) and the TMZ-induced alkylated products (O^6-MeG, N7-MeG and N3-MeA).

Temozolomide and MGMT

One of the major cytotoxic DNA lesions or types of DNA damage induced by TMZ treatment is the formation of methyl adducts on the O^6 position of guanine (O^6-MeG; see Fig. 2). However, the cytotoxic guanine lesion O^6-MeG is rapidly repaired by a direct reversal reaction conducted by the DNA repair protein O^6-methylguanine DNA methyltransferase (MGMT or more commonly referred to as AGT). This DNA repair protein is encoded by the O^6-methylguanine DNA methyltransferase gene, *MGMT*, located on chromosome 10 at position 10q26. The MGMT gene encodes a single mRNA (NM_002412) that translates to a single protein of 207 amino acids (NP_002403). MGMT functions to remove O^6-MeG lesions in DNA via a suicide reaction in which the O^6-Me group on guanine is transferred to the cys^{145} residue in MGMT, rendering MGMT inactive. Once alkylated on amino acid residue



Temozolomide. Figure 2 Chemical structures of the guanine and adenine bases in DNA and the temozolomide-induced modification to these bases. The guanine base (a) is modified by temozolomide on the O^6-atom to yield O^6-methylguanine (b) and on the N7-atom to yield N7-methylguanine (c) at a frequency of 5% and >70%, respectively. The adenine base (d) is modified by temozolomide at the N3-position to yield N3-methyladenine (e) at a frequency of 9.2%.

cys^{145}, the MGMT protein is depleted from the cell within a few hours. Depletion of the MGMT protein is facilitated by the ubiquitin-dependent proteosome pathway. The alkylated form of MGMT is targeted for poly-ubiquitylation; a post-translational modification that initiates proteosome-mediated degradation. Interestingly, MGMT activity is observed to be elevated in many tumors (in relation to the surrounding normal

tissue) such as those derived from colon, lung, pancreatic and breast cancer, non-Hodgkin's lymphoma, myeloma and glioma. It is now well established that elevated MGMT expression leads to resistance to clinical alkylating agents due to failure to remove the O^6-MeG lesion.

To improve TMZ efficacy, several strategies have been developed to limit the repair of the O^6-MeG lesion. MGMT activity can be successfully attenuated by the use of free guanine base derivatives, with alkyl groups at the O^6 position, which act as a pseudosubstrate and lead to MGMT depletion. Two of the most promising MGMT specific drugs are O^6-benzylguanine (BG) and O^6-(4-bromothenyl)guanine (Patrin, PaTrin-2, Lomeguatrib). Both of these MGMT-specific small molecule drugs are currently undergoing clinical evaluation to be used in combination with TMZ. In addition, other clinical alkylating agents are found to induce the formation of O^6-MeG adducts to DNA, including the platinum drugs cisplatin and carboplatin as well as the alkylating agent dacarbazine. Combination treatments with TMZ are therefore being evaluated as a novel approach to deplete MGMT protein levels and thereby increase TMZ efficacy. Interestingly, the dosing or scheduling of TMZ administration can also be altered to maximize MGMT depletion. For example, the standard 5-day dosing schedule of TMZ (150–250 mg/m^2/day) leads to complete depletion of MGMT in peripheral blood cells but MGMT levels will recover within 24 h. The observed TMZ-induced depletion of MGMT suggests that variation in the dosing schedules may improve MGMT depletion and clinical response. Compressed scheduling (five doses every 4 h or once every 8 or 12 h) has shown variation in MGMT depletion as well as elevated myelotoxicity. Alternatively, low (75 mg/m^2) extended dosing for 6–7 weeks in cycles of 8 weeks resulted in complete MGMT depletion. Clinical benefit to this or other scheduling paradigms is under intense investigation. The TMZ-mediated induction of the O^6-MeG DNA lesion and the depletion of MGMT has also been used to improve the effectiveness of topoisomerase inhibitors. Topoisomerase inhibitors such as Irinotecan (CPT-11) have been shown to have an enhanced efficacy when delivered in combination with TMZ. The increased level of O^6-MeG in DNA acts as a Topoisomerase I (topo-I) trap and an increase in topo-I/DNA covalent complexes. Recently, pre-treatment with interferon-beta was also shown to down-regulate MGMT expression and to improve the response of xenografts to TMZ in pre-clinical testing.

Conversely, low or undetectable expression of MGMT is generally considered a strong indicator of potential clinical response to TMZ. Loss of MGMT expression in tumors is generally via elevated methylation and hence inactivation of the MGMT promoter. Although MGMT promoter hypermethylation is associated with hypermethylation of many other promoter regions throughout the genome, epigenetic regulation (Epigenetic gene silencing) is not considered a random process in cancer. The development of methylation-specific PCR provides for a highly sensitive and accurate measurement of the methylation status of the MGMT promoter within the tumor tissue. DNA isolated from the tumor can therefore be analyzed for methylation status. Several studies have demonstrated that MGMT methylation status can be used to predict TMZ response. It is possible that MGMT promoter methylation measurements may be used to pre-screen patients for treatment options. However, there are other concerns that define response to TMZ and the cytotoxicity to the O^6-MeG DNA lesion.

Mismatch DNA Repair and Temozolomide Response

The O^6-MeG DNA adduct is not inherently cytotoxic by itself. The adduct is stable and genomic DNA containing O^6-MeG adducts is efficiently replicated during cell division by human replicative DNA polymerases. However, if not repaired by MGMT, either cytosine or thymine may be inserted opposite the O^6-MeG DNA adduct during replication. Insertion of thymine would lead to G to A point mutations in subsequent rounds of cell division and DNA replication, consistent with the elevated level of point mutations in oncogenes and tumor suppressor genes found in tumors with a loss of MGMT expression. The cytotoxicity of the TMZ-induced O^6-MeG adduct stems from the replication-dependent formation of the O^6-MeG:T mispair and the recognition of this mispair by the post-replication mismatch DNA repair pathway (MMR). The MMR pathway is a multi-protein DNA repair and DNA damage signaling pathway that removes errors of DNA replication and functions in meiotic and mitotic recombination as well as in DNA damage signaling and apoptosis following alkylation damage.

Currently, two mechanisms have been proposed for the MMR-dependent cytotoxicity of the TMZ-induced O^6-MeG DNA adduct: a "Futile Cycle of Repair" model and a "Direct DNA Damage Signaling" model. In the "Futile Cycle of Repair" model, the MutSα complex (a heterodimeric complex of the two MMR proteins MSH2 and MSH6) recognizes and binds to the O^6-MeG:T mispair, recruits the MutLα complex (a heterodimeric complex of the two MMR proteins MLH1 and PMS2) to the mispair and initiates repair of the newly synthesized DNA strand. Since this repair process involves removal and resynthesis of the T-containing DNA strand, the O^6-MeG:T mispair is regenerated during each cycle of repair thereby generating the substrate for another cycle. It is proposed that continued rounds of repair may lead to some aborted repair and the formation of double-strand

breaks and cell death. In the "Direct DNA Damage Signaling" model, MutSα binds to the O^6-MeG:T mispair and without repair processing, recruits MutLα and the DNA damage response proteins ATRIP and ATR to initiate the DNA damage checkpoints leading to cell cycle arrest and apoptosis. Critical MMR proteins are therefore required for efficacy of TMZ and hence loss of MMR leads to resistance to TMZ.

By a mechanism similar to that described above for MGMT epigenetic gene silencing, MMR can be compromised by tumor-specific promoter methylation of critical MMR genes. Whereas improved prognosis has been reported in tumors with loss of MGMT expression due to promoter methylation, poor prognosis is observed when MMR capacity is compromised by methylation of the promoter for the essential MMR genes MLH1, MSH2 and MSH6. More recently, loss of expression or inactivating mutations in MSH6 (an essential component of the MutSα complex) have been observed in TMZ resistant glioma tumors and in recurrent tumors following TMZ therapy, implicating an essential role for MHS6 in TMZ response. It is likely that all components of the MutSα complex (MSH2 and MSH6) and the MutLα complex (MLH1 and PMS2) may be essential for TMZ efficacy and response to the TMZ-induced O^6-MeG DNA adduct.

Repair of N7-Methylguanine and N3-Methyladenine DNA Lesions

The N7-methylguanine (N7-MeG) and N3-methyladenine (N3-MeA) DNA lesions (Fig. 2) comprise over 80% of the DNA adducts induced by TMZ. Both of these DNA lesions are repaired by the base excision repair (BER) pathway. BER is the predominant DNA damage repair pathway for the processing of small base lesions derived from alkylation damage. BER is normally defined as DNA repair initiated by a lesion-specific DNA glycosylase and completed by either of two sub-pathways: short-patch BER; a mechanism whereby only one nucleotide is replaced or long-patch BER; a mechanism whereby 2–13 nucleotides are replaced. The majority of repair is currently thought to occur via the short-patch pathway. Repair of N7-MeG and N3-MeA is initiated by the methylpurine DNA glycosylase (Mpg; NM_002434). The paradigm for the short-patch BER pathway initiated by Mpg involves base lesion removal and then AP site hydrolysis by AP endonuclease (Ape1; NM_080649), catalyzing the incision of the damaged strand, leaving a 3′OH and a 5′deoxyribose-phosphate moiety (5′dRP) at the margins. DNA polymerase β (Pol β; NM_002690) hydrolyzes the 5′dRP moiety and fills the single nucleotide gap, preparing the strand for ligation by either DNA Ligase I (LigI; NM_000234) or a complex of DNA Ligase IIIα (LigIIIα; NM_013975) and XRCC1 (NM_006297). Each step throughout BER is coordinated via protein-protein interactions with the XRCC1/DNA Ligase IIIα heterodimer and PARP1 (NM_001618), two important BER scaffold protein complexes. Furthermore, PARP1 has been linked more directly to BER as it interacts both physically and functionally with Pol β and LigIIIα, placing it as a member of the short-patch BER pathway. In addition, PARP1 coordinates with long-patch BER proteins to facilitate the repair of longer stretches of DNA.

BER removes >80% of the DNA lesions induced by TMZ. The advent of TMZ has therefore increased interest in the development of BER specific inhibitors since aborted or blocked BER sensitizes most cancer cells to alkylating agents. Blocking almost any step in the BER pathway will improve TMZ efficacy in cell culture assays, suggesting that BER inhibitors (just as observed with inhibitors of MGMT) may prove useful when administered in combination with TMZ. Few small molecule drugs have been developed that are specific for BER proteins. Lucanthone and CRT0044876 have been reported to inhibit Ape1 and will sensitize cells to TMZ. Methoxyamine is another BER inhibitor; however, this compound does not directly inhibit BER enzymes but instead binds to abasic sites in DNA, making them refractory to further repair and the methoxyamine-bound abasic site is highly cytotoxic. Methoxyamine is currently undergoing clinical trials in concert with TMZ.

The most common BER target for small molecule drugs (inhibitors) is PARP1 although PARP1 is also involved in the repair of double-strand breaks (DSBs) in DNA via a non-homologous end-joining pathway. PARP1 is the founding member of a large family of ADP-ribosyl transferase proteins including six PARPs (poly-ADP-ribose polymerases), eleven MARTs (mono-ADP-ribosyltransferases), seven SIRTs, four PARGs (poly ADP-ribose glycohydrolases) and Pi-MARTs. To date, only PARP isoforms 1, 2 and 3 have been found to respond to DNA damage such as that induced by TMZ. Upon binding to a nick (a single-strand break) or a DSB, PARP is activated to poly-ADP-ribosylate itself and other target proteins and to synthesize poly-ADP-ribose. Inhibiting PARP activation has been shown to significantly improve TMZ-induced tumor cell killing and to improve response to TMZ in pre-clinical xenograft models. Currently, there are at least six PARP inhibitors undergoing clinical trials as anticancer agents, although the specificity of these for PARP1, PARP2, PARP3 or other PARP-family members remains to be determined. Three of these small molecule inhibitors undergoing clinical trials are being evaluated for clinical efficacy in combination with TMZ. These clinical trials (Phase I and II) are for the treatment of solid tumors, metastatic melanoma and glioblastoma multiforme.

Although TMZ is effective in the treatment of glioblastoma and is being evaluated for melanoma and other cancers, efficacy can clearly be improved through combination therapy to inhibit one or more DNA repair pathways. Continued development of MGMT and BER inhibitors is expected to have significant impact on response to TMZ in the near future. Further, analysis of these critical DNA repair pathways in tumors can provide valuable biomarkers to anticipate response.

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Temsirolimus

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Synonyms

CCI-779; Torisel™

Definition

Temsirolimus is a small molecule inhibitor of mammalian target of rapamycin (mTOR) kinase. Aberrant intracellular signaling through mTOR is associated with the cancer cell proliferation. Inhibition of this pathway by temsirolimus result in anti-proliferative effects that may result in improved survival in patients with cancer.

Characteristics

Temsirolimus is a soluble 42-[2,2-bis (hydroxymethyl)]-propionic ester of the macrocyclic lactone rapamcyin (also known as sirolimus). Temsirolimus, sirolimus and other members of this class of agents inhibit the proliferation of normal and malignant cells by inhibiting the activity of mTOR, an intracellular serine-threonine kinase within the Phosphoinositide 3-kinase (PI3K)/Akt signal transduction pathway. Temsirolimus, like sirolimus, reacts with the ubiquitous intracellular FK506-binding protein 12 (FKBP12) to inhibit mTOR function.

Mechanism of Action

Activation of the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway plays a pivotal role in essential cellular functions such as survival, proliferation, migration differentiation and carbohydrate metabolism, and is important in the molecular mechanisms of diseases such as diabetes and chronic inflammation, as well as cancer. Normal cells such as lymphocytes, ►endothelial cells and fibroblasts as well as cancer cells are dependent of this signaling pathway. The observed antitumor and immunosuppressive properties of temsirolimus and other agents within this class are due to their ability to disrupt mTOR function.

Both activating mutations and amplification of oncogenes and loss of tumor suppressor genes occur within the pathway in human neoplasms with remarkable frequency. Activation mutations of growth factor receptors and PI3K, as well as amplification and or overexpression of PI3K and Akt have been reported in different tumor histologies. Similarly, the loss of tumor suppressor proteins that regulate the PI3K-Akt-mTOR pathway such as tuberous sclerosis proteins 1 or 2 (TSC1/2), phosphatase and tensin homologue deleted on chromosome 10 (PTEN), and serine/threonine kinase 11 (STK11 also known as LKB1) has been linked to the pathobiology of a number of tumor predisposition syndromes, including ►tuberous sclerosis syndrome (TSC1/2), ►Peutz-Jeghers syndrome (STK11/LKB1), and ►Cowden syndrome (PTEN). In laboratory models, the resultant aberrant activation of the signaling pathway through oncogene stimulation or tumor suppressor gene loss not only leads to a growth advantage during carcinogenesis but also contributes to tumor angiogenesis, metastasis, and resistance to standard cancer therapy. Of interest and relevance to cancer therapeutics development, aberrant pathway activation may also lead to sensitivity to agents that target mTOR.

Temsirolimus does not directly interact with mTOR kinase and does not inhibit all mTOR functions. mTOR functions are dependent on its forming complexes with other proteins. Two mTOR-containing complexes have been well characterized: a rapamycin-sensitive complex (also called mTOR complex 1, mTORC1), which includes mTOR accessory protein Raptor along with mammalian ortholog of LST8 (mLST8) (regulatory-associated protein of mTOR); and a rapamycin-insensitive complex (also called mTOR complex 2, mTORC2), which composed of mTOR and Rictor (rapamycin-insensitive

companion of mTOR). mTORC1, phosphorylates the well-characterized mTOR effectors S6 kinase 1 (S6K1, also known as p70S6K) and eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1). TORC2 controls the actin cytoskeleton as well as Akt. However, mTOR when bound to Rictor can phosphorylate Akt at its hydrophobic motif leading to Akt activation. This positive feed back loop may result in increased phosphorylation of Akt in the presence of temsirolimus inhibition of the Raptor-mTOR complex.

Activity in Cancer Models

The antiproliferative effects of temsirolimus and other sirolimus derivatives have been evaluated in numerous in vitro and in vivo tumor models. In sensitive cell lines, these agents inhibit tumor and endothelial cell proliferation in picomolar to nanomolar concentrations and may add to the cytotoxicity of other chemotherapeutic agents and radiation. The antiproliferative effects of temsirolimus may be due, at least in part, to its well-characterized inhibitory effects on the activation of S6K1 and 4EBP1. Inhibition of these proteins alters the translation of subsets of mRNAs, particularly those that may be involved in regulating cell cycle progression. In a relatively limited number of tumor models, this class of agents may induce cancer cell death apoptosis or autophagy. The molecular mechanisms leading to apoptosis in cancer cells have not yet been fully deciphered. In addition, mTOR inhibitors can target tumor growth indirectly by inhibiting endothelial cells and ►pericytes proliferation required for tumor angiogenesis.

Activity in Clinical Trials

Results from cancer clinical trials suggest that temsirolimus is well tolerated and appears to have anti-tumor activity. The most common toxicities seen with the drug are mild to moderate skin reactions, ►stomatitis, reductions in blood counts, particularly platelets, and metabolic abnormalities such hyperlipidemia and hyperglycemia. These adverse effects are reversible with interruption of dosing or, for hyperlipidemia and hyperglycemia, with specific treatment. Rarely, pneumonitis has been reported in patients that have received temsirolimus. To date, there has been no evidence of clinically significant immunosuppression with intermittent schedules.

Effective target inhibition has been shown through pharmacodynamic assays assessing inhibition of either S6K1 or 4E-BP1 phosphorylation in surrogate tissue from patients treated with temsirolimus. More limited data from baseline and on treatment tumor tissue specimens supports that temsirolimus inhibited mTOR and downstream targets. However, optimal dose/schedule for intratumoral target inhibition has not well defined due to limitations in numbers of specimens analyzed.

Results from four phase I studies evaluating increasing doses of temsirolimus on different schedules and with oral and intravenous formulations have been reported. The weekly intravenous schedule is the one that has been most extensively evaluated in phase II and III studies.

Phase II studies of single agent temsirolimus evaluating different doses of 25, 75, and/or 250 mg weekly IV have been undertaken in broad range of tumor histologies. The most promising anti-tumor activity has been seen in ►mantle cell lymphoma and ►endometrial carcinoma with objective tumor response rates of 30–40%. Moderate activity has been reported in breast and ►renal cell carcinoma. Minimal single agent activity has been seen in ►small cell lung carcinoma, ►melanoma and ►glioblastoma multiforme. In general, lower doses appear to be as active as higher doses with better tolerability.

A phase III trial of temsirolimus, temsirolimus with interferon versus interferon in poor prognosis patients with renal cell carcinoma has been reported. Of the 626 patients, overall survival of patients treated with temsirolimus was significantly prolonged compared to those treated with interferon (median 10.9 months versus 7.3 months, Harzard Ratio for death 0.73, $p = 0.0069$). The combination of interferon and temsirolimus did not confer greater benefit than interferon alone, possibly due to compromised dose delivery of the agent(s) due to significant toxicity.

The identification of tumor types that respond to mTOR inhibitors remains a major issue for the development of temsirolimus. mTOR is ubiquitously expressed and therefore the sensitivity or resistance of specific tissues to temsirolimus cannot be predicted solely on the basis of whether the target protein can be detected in the tumor tissue. Activation status of the PI3K/AKT/mTOR signaling pathway seems to be the most promising strategy to identifying tumor types potentially sensitive to temsirolimus. As a significant number of patients have cancers that are insensitive to temsirolimus, combinations of the mTOR inhibitor with hormonal therapy (►hormonal treatment), chemotherapy or other targeted therapies, based on the rationale that simultaneous inhibition of multiple signaling pathways are under evaluation.

Temsirolimus, which inhibits the downstream kinase mTOR, has demonstrated that it may be a useful cancer therapeutic as it may confer clinical benefit to patients with acceptable toxicity. The proof of principle that temsirolimus can improve cancer patient survival has been recently obtained from a large randomized trial in advanced poor prognostic renal cell carcinoma. The major clinical development challenges will be efficiently identifying the optimal dose, schedule and combination regimens for patients with susceptible

malignancies and monitoring and managing toxicities to optimize the therapeutic index.

►Rapamycin

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Tenascin-C

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Synonyms

Hexabrachion; Myotendinous antigen; Glial/mesenchymal extracellular matrix protein; GMEM; Cytotactin; J1 220/200; Neuronectin

Definition

Tenascin-C is the founding member of a family of extracellular matrix glycoproteins comprising tenascin-X, -R, and -W in addition to tenascin-C. Its name has been created by Ruth Chiquet-Ehrismann 1986 and represents a combination of the Latin verbs "tenere" (to hold) and "nasci" (to grow, develop, to be born), which provided the roots of the English words "tendon" and "nascent," and reflect the location and developmental expression of the protein observed at that time.

Characteristics

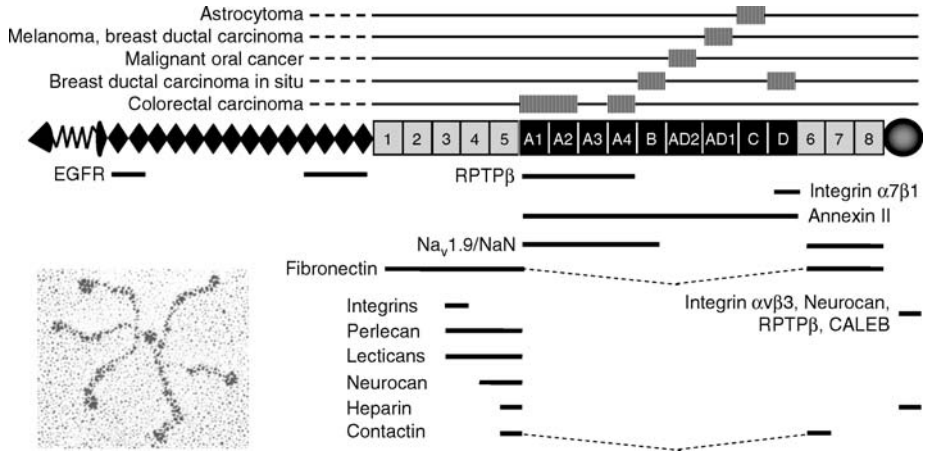
Tenascin-C is part of a ►tumor-specific stroma of most solid cancers, and plays a role in enhancing proliferation, ►angiogenesis, and ►metastasis during tumorigenesis. Moreover, research data support the possibility that tenascin-C contributes to cancer formation via interference with genomic stability, blocking the immunosurveillance and by providing a favorable niche for tumor stem cells (►Cancer stem-like cells, ►stem cells and cancer). Its high expression correlates with bad prognosis for disease-free survival in patients with glioma, lung, and colon cancer.

Molecular Organization

The tenascin-C protein, 2′201 amino acids in length giving rise to a 190–300 kDa monomer, is encoded by 6′603 bp, which are organized into at least 28 exons on chromosome 9q33. Tenascin-C is a modular molecule consisting of an N-terminal region containing a chaperone-like sequence forming coiled coil structures and interchain disulfide bonds that are essential for subunit oligomerization into hexamers. Tenascin-C is comprised of 14.5 epidermal growth factor (EGF)-like repeats, 30–50 amino acids in length, which contain six cysteine residues involved in intrachain disulfide bonds. In tenascin-C, up to 17 ►fibronectin type III domains are present that are ∼90 amino acids in length and that are composed of seven antiparallel β-strands arranged in two sheets. The nature and number of fibronectin type III domains in tenascin-C is generated by alternative splicing that is modulated by the proliferative state of a cell, extracellular pH, and TGFβ1. At least nine different fibronectin type III domains are differentially included or excluded by RNA splicing. This can generate a considerable diversity among different cancers (Fig. 1) and, can cause variable cell responses toward tenascin-C. The C-terminal fibrinogen globular domain resembling the β- and γ-chains of fibrinogen, 210 amino acids in length, forms intrachain disulfide bonds (Fig. 1).

Induction and Processing

Tenascin-C can be induced in a tumor by various pro- and antiinflammatory cytokines and growth factors that are mostly secreted by stromal cells. In addition, hypoxia (►Hypoxia and tumor physiology), ►reactive oxygen species, and mechanical stress, which are also present in tumor tissue, induce tenascin-C expression. In contrast, glucocorticoids suppress tenascin-C expression. Signaling causing activation of transcription factors such as TCF/LEF, NfkB, c-Jun, Ets, SP1, and Prx-1 is involved in tenascin-C gene transcription. Tenascin-C is cleaved by ►matrix metalloproteinases and serine proteases, thus potentially releasing cryptic sites within the fibronectin type III domains of tenascin-C. Cell contact with tenascin-C also induces the expression of matrix metalloproteases, thus presenting a positive feedback loop between induction of matrix metalloproteases by tenascin-C and cleavage of tenascin-C by these enzymes.



Tenascin-C. Figure 1 Domain structure, binding partners and expression of tenascin-C in cancer tissue. The N-terminal oligomerization, EGF-like, fibronectin type III and fibrinogen-like domains are schematically depicted as triangle, rhombomeres, boxes and circles, respectively. The alternatively spliced fibronectin type III domains A1-D are shown in black. An electromicrograph of a tenascin-C hexamer is shown at the left corner. Fibronectin type III domains specifically detected in certain cancers are highlighted above the model. EGFR, epidermal growth factor receptor; CALEB, chicken acidic leucine-rich EGF-like domain containing brain protein; RPTPβ, receptor protein tyrosine phosphatase-β/ζ; Na$_v$1.9/NaN, sodium channel subunit β2. Picture was taken from Orend and Chiquet-Ehrismann (2006) Cancer Lett 244:143–163.

Interaction Partners

Tenascin-C binds to extracellular matrix molecules such as fibronectin, perlecan, aggrecan, versican, and brevican (Fig. 1), thus potentially forming a tumor-specific extracellular matrix network. Cells can interact with tenascin-C via cell surface receptors including integrins (►Integrin signaling in cancer) α2β1, α7β1, α9β1, and αvβ3, syndecan, annexin II, and ►epidermal growth factor receptor (EGFR) among others (Fig. 1).

Cell Rounding and Tumor Cell Proliferation

Tenascin-C has distinct effects on tumor cells, and tumor-associated cells such as carcinoma-associated fibroblasts, tumor-associated macrophages, and endothelial cells within the tumor stroma based on as yet poorly understood cell type-specific responses toward tenascin-C splice variants. Tenascin-C contains adhesive and antiadhesive sequences that coexist in the native molecule. These opposing activities arise as a consequence of tenascin-C binding to extracellular matrix components and to cell surface receptors. One mechanism that induces cell rounding involves tenascin-C inhibition of cell adhesion to fibronectin. This occurs through competitive binding of tenascin-C to fibronectin, thus masking the binding site for integrin α5β1 coreceptor syndecan-4 (►Heparanases). This blocks activation of the small GTPase RhoA and focal adhesion kinase. Activation of oncogenic Wnt (►Wnt signaling), endothelin receptor type A, and ►MAPK signaling induced by tenascin-C and elimination of G0 and G1 cell cycle (►Cell cycle targets for cancer therapy) transition control could contribute to enhanced tumor cell proliferation by tenascin-C.

Metastasis

Tenascin-C is expressed around invasive carcinoma cells that have undergone epithelial-mesenchymal-transition (EMT) (►Epithelial to mesenchymal transition). Tenascin-C supports tumor cell migration and invasion by mechanisms that are little understood. Tenascin-C provides a substratum that supports migration of several cell types including glioma and laryngeal carcinoma cells. A mechanism by which tenascin-C supports colon carcinoma cell invasion involves secretion of tenascin-C by carcinoma-associated fibroblasts, activation of EGFR and, expression of hepatocyte growth factor and activation of its receptor c-Met. This triggered downstream activation and inhibition of the small GTPases Rac and RhoA, respectively in the invading carcinoma cells. In addition to an EMT-associated migration, tenascin-C might also promote other forms of migration in cancer cells.

Angiogenesis

Tenascin-C plays a role during embryonic vascularization and promotes vascular sprouting. It is also expressed during formation of new blood vessels in the adult as, e.g., in granulation tissue of wounds after myocardial infarction, in arthritis, and in neoplastic diseases. In human gliomas, tenascin-C expression correlates with the degree of tumor neovascularization. Tenascin-C may promote angiogenesis by serving as

chemoattractant for endothelial cells, by initiating endothelial cell differentiation, survival, and proliferation, events that involve integrin αvβ3 and vascular endothelial growth factor among not yet identified other molecules.

Phenotype of Tenascin-C Knockout Mice and Cancer

The tenascin-C sequence is highly conserved among species, which suggests that evolutionary forces prevented loss of this gene because of its importance to life. Tenascin-C knockout (►Gene knockout) mice are hyperactive among other neurological defects, which would make them an easy prey. Abnormal behavior in these mice might be due to its role as ligand for neuronal receptors. Despite an otherwise apparently normal phenotype, which is likely due to compensatory mechanisms, tenascin-C knockout mice show difficulties in regeneration upon disturbance of tissue homeostasis such as during healing of wounds in the eye and in the inflamed kidney. Tenascin-C is expressed in stem cell niches (►Adult stem cells) such as those of the bone marrow, brain, and skin. Stem cells are required to maintain and restore tissue homeostasis, in particular upon insults to tissues. This may explain the more severe phenotype in injured tenascin-C knockout mice. The first in vivo evidence for a role of tenascin-C in tumor angiogenesis derives from studies with xenografted melanoma cells into mice lacking tenascin-C expression. In these tenascin-C knockout mice, tumor growth and angiogenesis was strongly reduced in comparison to mice exhibiting tenascin-C expression.

Clinical Aspects

How can we use our knowledge about tenascin-C to combat cancer? Given that expression of tenascin-C correlates with tumorigenesis-enhancing events and with a reduced disease-free survival in patients with some cancers, inhibition of tenascin-C expression at the transcriptional level would be the first choice to block tenascin-C actions in cancer. Unfortunately, this approach is questionable since many factors and conditions that trigger tenascin-C expression are not specific for tenascin-C alone but affect many other genes. Preventing tenascin-C action in a tumor, e.g., by restoration of syndecan-4 function in gliomas offers another approach. However, since tenascin-C has many poorly understood effects at the molecular level on the different cell types within cancer tissue, targeting tenascin-C actions may produce undesirable side effects. The most promising approach today is to target tenascin-C with antitenascin-C-directed antibody fragments that are coupled to cytotoxic reagents in a trojan horse-like strategy, which would trigger destruction of the tumor. Tenascin-C-targeting antibodies are in clinical trials and one needs to await the antitumor response rates in cancer patients.

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Tensin2

Definition

Is a focal adhesion protein of the tensin family that acts as an important link among extracellular matrix, actin cytoskeleton, and signal transduction.

►Deleted in Liver Cancer 1

Tensional Homeostasis

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Definition

A mechano-regulatory network that integrates physical and biochemical cues from the tissue ►microenvironment through mechano—responsive elements such as transmembrane integrins to evoke cytoskeletal re-organization and actomyosin contractility, thereby altering signal transduction and gene expression to modulate cell and tissue phenotype.

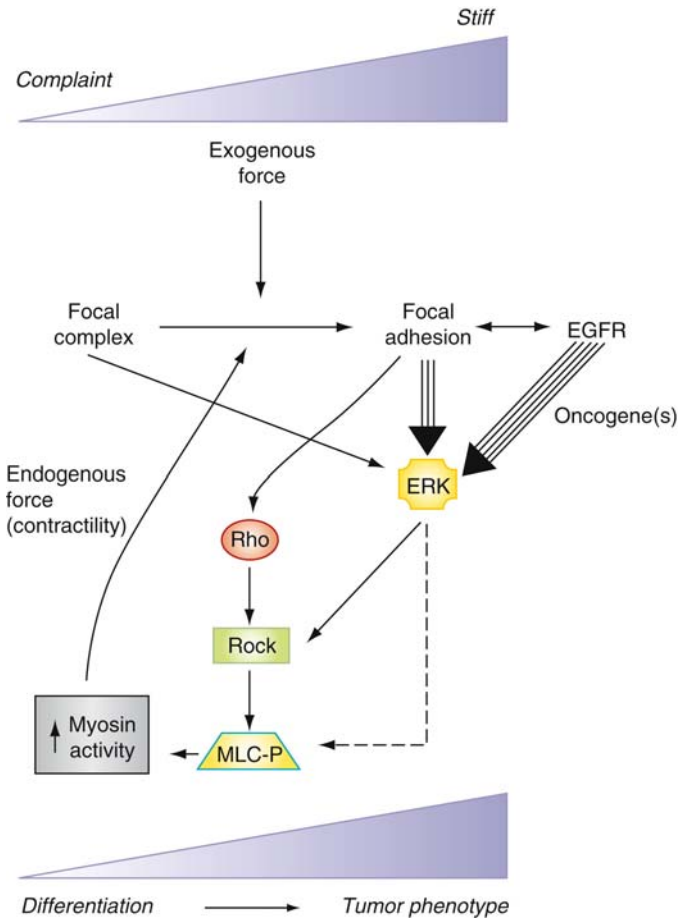
Characteristics

Cells and tissues experience and respond to externally applied mechanical force through mechano-responsive elements that influence signal transduction and result in the generation of reciprocal intracellular force or contractility. The types of ►mechanical stress a cell can experience include compressive or tensile stress which is applied perpendicular to the surface of the cell, and shear stress which is applied parallel to the surface of the cell. For example, osteoblasts and chondrocytes within bone and cartilage are subjected to compressive force induced by walking, lung alveolar cells experience

tensile load resulting from inhalation-induced alveolar sac expansion, and endothelial cells lining the lumens of blood vessels undergo shear force induced by circulating blood flow. Cells integrate external mechanical force on multiple levels. This includes force-dependent changes in the conformation of the plasma membrane lipid bilayers as well as modifications in the orientation and molecular associations of transmembrane proteins. These changes enhance the activity of calcium and potassium ion channels, the extracellular matrix affinity, and cytoskeletal plaque associations of various adhesion molecules including integrins (►Integrin signaling and cancer). Cells also integrate external force cues to generate reciprocal actomyosin-mediated cell contractility and modulate their ►mechanical properties through remodeling of the microtubule, intermediate filament and actin cytoskeletal network. Intracellular mechanical force is transduced to the extracellular microenvironment via functional links between transmembrane receptors that bind to extracellular matrix (ECM) proteins and the intracellular cytoskeletal network and ultimately mediate an equilibrium of extracellular and intracellular forces in the cell. This equilibrium or balance between the extracellular forces and the intracellular forces is called tensional homeostasis. When the extracellular mechanical microenvironment is becomes altered, cells and tissues will coordinately respond by adjusting cell-generated mechanical force or contractility, which in turn elicits changes in cell behavior by modifying the activity and function of signaling pathways and gene expression that determine growth, survival and differentiation. Cells sense and integrate tensional forces by altering the expression and activity of a plethora of putative ►mechanosensors. Nevertheless, integrins are considered key mechanotransducers by virtue of their external associations with the extracellular matrix and their internal links to various adhesion plaque proteins including vinculin, talin and ►focal adhesion kinase (FAK), which in turn mediate interactions with the cytoskeleton and activate various signaling cascades. Extracellular mechanical force can alter the conformation of an integrin from a low ligand-binding affinity state to high ligand-binding affinity state, the conformation of extracellular matrix proteins such as ►fibronectin and collagen I to expose or alter ligand binding sites, and the conformation of vinculin and talin to favor intracellular molecular associations. These mechanically-initiated events promote actin assembly and stabilize adhesion plaque protein assembly and clustering of integrins to convert nascent ►focal complexes into mature ►focal adhesions. Force-dependent integrin activation and focal adhesion maturation increase the magnitude and duration of adhesion signaling including ERK ►MAP kinase and RhoA GTPase (►Rho family proteins). Elevated and sustained activity of ERK and RhoA GTPase drive actomyosin-mediated intracellular contractility by altering the function of Rho kinase (ROCK) and phosphorylated myosin light chain. The elevated intracellular tension in turn promotes focal adhesion maturation, creating a feedback loop of biochemical signaling pathways resulting in an elevated intracellular force generated by actomyosin cytoskeleton and inside out remodeling of extracellular matrix proteins (see Fig. 1).

When the balance between the external and intracellular stress is altered, the cell and tissue will adapt to the new mechanical microenvironment challenge, which can result in positive outcomes such as an increase in bone and muscle density due to exercise, or in negative outcomes such as atherosclerosis mediated by chronically elevated shear force applied by perturbed blood flow and cardiac hypertrophy due to hypertension. Mechanical compression can also regulate gene expression to influence tissue development as has been documented during embryogenesis. Changes in matrix stiffness determine the lineage commitment of mesenchymal stem cells, such that the cells express neurogenic markers when grown in mechanical environment closer to the stiffness of brain (0.1–1 kPa), myogenic markers at an intermediate stiffness (8–17 kPa), and osteogenic markers at a higher stiffness (25–40 kPa). This lineage commitment is regulated by nonmuscle myosin II and is accompanied by an increase in the size of focal adhesions and in the expression of focal adhesion components including talin and phosphoFAK. These results suggest that a cell dynamically probes its mechanical microenvironment through active engagement of integrin adhesion receptors and generation of actomyosin contractility, and that an increase in focal adhesion maturation and intracellular contractility drives downstream signaling events which determine lineage differentiation. Thus, tensional homeostasis is emerging as a critical determinant in cell fate during normal morphogenesis as well as pathophysiological processes.

Solid tumors are characteristically stiffer than normal tissue, which allows detecting tumors by palpation. The elevated stiffness is mediated by increased interstitial tissue pressure and changes in the mechanical properties of malignant cells and the surrounding stroma. The tumor stroma is characterized by an increased deposition and reorganization of matrix proteins including collagen, fibronectin and tenascin, and aberrant ECM cross linking induced by lysyl oxidase, transglutaminase, proteoglycans, and glycation, which contribute to the stiffening of the stroma (►Extracellular matrix remodeling). In addition, transforming ►oncogenes such as ►RAS, ErbB/►HER2 neu and c-Myc (►Myc oncogene) can alter the mechano-responsiveness of cells and cooperate with integrin adhesion signaling



Tensional Homeostasis. Figure 1 Key molecular pathways that mediate tensional homeostasis in cells and tissues. Changes in the mechanical environment of cell, such as an increase in ECM stiffness or elevated extracellular tension, promote integrin clustering to drive the maturation of nascent focal contacts into focal adhesions. The assembly of focal adhesions is associated with increased Rho GTPase activity and elevated and sustained ERK signaling. The combination of enhanced Rho and ERK activity increases actomyosin-mediated intracellular contractility by altering the function of Rho kinase (ROCK) and phosphorylated myosin light chain (MLC-P). Elevated cell-generated force promotes focal adhesion assembly and potentiates growth factor dependent ERK activation in a feed forward vicious cycle. Elevated intra cellular force also alters ECM deposition and organization by orienting and further stiffening ECM. Oncogenes which promote RAS-dependent ERK activation and Rho GTPase activity additionally contribute to cell-generated forces by regulating ROCK and MLCK and myosin II activity.

molecules to enhance cell proliferation, survival and invasion. Indeed, oncogenes such as Ras and ErbB/HER activate Rho and ERK that induce actomyosin contractility and elevate cell-generated forces to further promote the assembly and maturation of integrin adhesions and enhance growth factor receptor cross-talk. This raises the intriguing possibility that in addition to promoting cell growth and survival by directly modifying the activity of various signaling molecules, some transforming oncogenes might promote malignancy by altering the cells tensional homeostasis. Consistently, Paszek et al demonstrated that increasing ECM stiffness from 140 Pa (approximating the compliance of the normal murine breast) to 1,000–5,000 Pa (similar to the stiffness of a malignant murine breast) compromised mammary morphogenesis and induced the malignant phenotype of non-malignant mammary epithelial cells in culture, as demonstrated by an increase in cell growth and survival, and the loss of mammary tissue integrity (i.e. disruption of cell-cell junctions and loss of tissue polarity). Stiffening of ECM also significantly increased recruitment and activation of FAK and actin-binding proteins such as vinculin to β1 integrin adhesion, which was accompanied by an increase of larger, mature focal adhesions, contractility, and enhanced growth-factor dependent ERK activation. More intriguingly, malignantly transformed mammary cells with elevated epidermal growth factor receptor (EGFR) signaling that form colonies of disorganized, invasive and continuously growing cancer cells in

response to a compliant normal matrix also exerted higher cell contractility. Strikingly, inhibiting the activity of Rho GTPase or myosin II or ERK was sufficient to reduce the tumor cells contractility and revert the malignant phenotype of these breast cancer cells toward that of a normal breast acini. Likewise, inhibiting Rho or ERK-dependent myosin activity also normalized the phenotype of non-transformed mammary cells interacting with an abnormally stiffened matrix. Together these findings suggest that breast transformation could arise through the combination of oncogenic mutations that promote cell generated contractility and a progressive stiffening of the ECM which compromises the tensional homeostasis to elevate cell contractility and to increase focal adhesion assembly, which enhance abberant cell growth, survival and invasion.

Clinical studies indicate that mammographic density is strongly and reproducibly associated with an increased risk of breast cancer, independent of other risk factors (►Mammographic breast density and cancer risk). For example breast cancer risk rises to 30% when greater than 50% of the mammography qualifies as dense. Although the high cancer risk linked with breast density could be attributed to decreased detection sensitivity and increased epithelial mass, recent data indicate that elevated collagen and proteoglycan content are also risk factors that contribute to the enhanced transformation frequency associated with this condition. Elevated mammographic density frequently precedes ►ductal carcinoma in situ (DCIS), and DCIS often occurs predominantly in the mammographically dense areas of the breast. Because higher collagen density and elevated proteoglycan-mediated cross linking correlate with an increase in ECM stiffness, these findings are consistent with the prediction that mammographic density could promote carcinogenesis by perturbing the cells tensional homeostasis. If true, an increase in matrix stiffening would herald an altered tissue tensional homeostasis and constitute a tractable predictor of future tissue transformation. Accordingly, an improved understanding of the parameters that promote matrix stiffening and alter tissue tensional homeostasis would assist in the development of improved detection, prognosis and treatment strategies for solid cancers.

To summarize, cells and tissues sense and respond to external force through a process called tensional homeostasis that reciprocally alters the external microenvironment through cell-generated force. Tensional homeostasis is emerging as an important determinant of normal tissue development and adult tissue homeostasis and recent studies indicate that an altered tensional homeostasis likely contributes to the pathogenesis of diseases including cancer and atherosclerosis.

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TEP1

Definition

TGF-β-Regulated and Epithelial Cell-Enriched Phosphatase; Rarely used synonym for PTEN.

►PTEN

Teratocarcinoma

Definition

Is an embryonic or ►germ-cell tumor.

Teratocarcinoma-derived Growth Factor-1

►Cripto-1

Teratogenic

Definition

Refers to substances that cause developmental malformations during gestation.

►Estrogenic Hormones

Teratoma

Definition

Plural Teratomata. Term derived from Greek meaning "monstrous tumor". Is a germ cell tumor derived from pluripotent cells and made up of elements of different types of tissue from one or more of the three germ cell layers. Is a tumor with tissue or organ components resembling normal derivatives of all three germ layers. Rarely, not all three germ layers are identifiable. The tissues of a teratoma, although normal in themselves, may be quite different from surrounding tissues, and may be highly inappropriate, even grotesque (hence the term "monstrous"). Teratomas have been reported to contain hair, teeth, bone and very rarely more complex organs. Usually, however, a teratoma will contain no organs but rather one or more tissues normally found in organs such as the brain, thyroid, liver, or lung.

N- or C-Terminal Processing

Definition

Proteolytic attack of a protein substrate can have different outcomes depending on the protease at work and the protein under attack:

1. The protein can be completely degraded into small fragments and amino acids;
2. The protein can undergo limited proteolysis, also referred to as processing or maturation;
3. If the protein is a protease inhibitor, it may form a complex and neutralize the protease;
4. The protein might be neither susceptible to proteolysis nor interact with the protease.

When limited proteolysis occurs at either end of a protein it is referred to as N- or C-terminal processing.

►Cystatins

Terminally Differentiated Cells

Definition

Cells most distal to the stem cell, being differentiated to perform a specific function, but having permanently lost the ability to divide.

►Stem Cell Plasticity

Tertiary Cancer Prevention

Definition

Prevention of metastatic spread of tumors. A locution used in parallel with ►primary cancer prevention and ►secondary cancer prevention.

►Adjuvant Therapy
►Immunoprevention of Cancer

Tertiary Structure

Definition

The three-dimensional structure of a monomeric protein.

►Structural Biology

Testicular Cancer

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Synonyms

Testicular germ cell tumor; Seminomatous germ cell tumor; Nonseminomatous germ cell tumor

Definition

About 90% of all testicular tumors are malignant germ-cell tumors, and the rest comprise benign tumors deriving from Leydig and Sertoli cells and other interstitial components. Testicular germ-cell tumors originate from totipotent primordial germ cells, which undergo neoplastic transformation as a result of a number of endogenous, exogenous, hormonal and genetic, as well as environmental, events. The neoplastic process results in the development of preinvasive carcinoma in situ (CIS) or TIN representing the common precursor for all testicular germ-cell tumors, except spermatocytic seminoma.

Characteristics

Testicular cancer represents the most common malignant tumor in young men in the age group of 20–40 years. In 1994, ~6,800 new cases of testicular

cancer were diagnosed in the US. There are striking differences in TC incidences around the world, with the highest incidence of 12–14 per 100,000 person-years in Switzerland and Denmark, and the lowest incidence of less than one per 100,000 person-years among African- Americans and the Chinese populations. Cryptorchidism is the best-known risk factor and, according to case–control studies, the relative risk for TC is 2.5–8.8. Familial and genetic factors have been suggested to be involved in the development of TC with a six- to tenfold higher risk in first degree relatives.

Diagnosis

The majority of patients present with a painless scrotal mass and the diagnosis is usually established by physical examination of the tumor-bearing and the contralateral testicle, scrotal ultrasonography and determination of the serum tumor markers alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and lactate dehydrogenase (LDH).

Inguinal exploration and scrotal orchiectomy confirms the diagnosis and is the therapy of choice, revealing accurate information with regard to histopathology and pathological-stage classification. Since benign testicular lesions are recognized with increasing frequency, frozen section analysis should be considered. Contralateral testicular biopsy to diagnosis TIN is recommended in high risk patients (testis volume <12 ml, <30 years) with a risk >34. Only in cases of a second metachronously or synchronously occurring TC an organ-sparing approach might be considered to maintain endogenous testosterone synthesis and to preserve fertility.

Meticulous pathological work-up of the orchiectomy specimen should be performed according to the WHO recommendations, to identify all histological subtypes present and vascular invasion. In clinical stage I non-seminomatous germ-cell tumor (NSGCT), a high percentage of embryonal carcinoma associated with the presence of vascular invasion identifies patients at high risk of lymph-node metastases in the retroperitoneum, thereby enabling a risk-adapted approach.

Following radical orchiectomy, further staging includes computed tomography of the abdomen, the chest and the mediastinum, to detect metastatic lymph-node disease or visceral metastases. The primary landing zones of the right testis include the interaortocaval region and the primary landing zones of the left side include the para-aortic and preaortic lymph nodes. Micrometastases are present in up to 20% and in ~30% of clinical stage I seminomas and non-seminomas, respectively, which will not be detected by classical interpretation of abdominal CT scans owing to the inability of morphological differentiation. Therefore, primary landing zones and the transverse diameter of the largest lymph node might be helpful to identify patients at high risk. Magnetic resonance imaging of the abdomen does not provide additional clinically useful information, and is reserved for patients with contraindications to CT. Positron emission tomography scans are not helpful in the detection of retroperitoneal micrometastases.

Tumor markers are clinically useful for diagnosis, clinical staging, prediction of prognosis (see IGCCCG classification, Table 1) and monitoring the response of therapy. The most commonly used markers are AFP, the ß-subunit of hCG and lactic acid dehydrogenase (LDH); in seminomas, PLAP might be of clinical use. Classical seminomas are always AFP-negative. Following the initiation of therapy, elevated tumor markers should decline according to their half-life (ß-hCG: 24–36 h, AFP: 5–7 days). Any plateau phase or any

Testicular Cancer. Table 1 Classification international germ cell cancer collaborative group (IGCCCG)

	Seminoma	NSGCT
Good prognosis	Any primary localization	Testicular or extragonadal retroperitoneal primary
	No non-pulmonary visceral metastases	No non-pulmonary visceral metastases
Tumor markers	AFP < 1.000 ng/ml	
	ß-hCG < 1.000 ng/ml	
	LDH < 1.5 Norm	
Intermediate prognosis	Any primary localization	Testicular or extragonadal
	Non-pulmonary visceral metastases	No non-pulmonary visceral metastases
Tumor markers	AFP 1.000–10.000 ng/ml	
	ß-hCG 1.000–10.000 ng/ml	
	LDH 1.5–10x Norm	
Poor prognosis		Primary mediastinal GCT
		Non-pulmonary visceral metastases
Tumor markers	AFP > 10.000 ng/ml	
	ß-hCG > 10.000 ng/ml	
	LDH > 10x Norm	

delay in decline is predictive for a poor outcome in terms of response to therapy. The prognostic significance of tumor markers at the time of diagnosis becomes evident for advanced disease only, adhering to the International Germ Cell Consensus Classification Group (IGCCCG) classification.

The Lugano classification represents the most widely used clinical staging system for testicular cancer (Table 2), and describes the extent of metastatic involvement of the lymph nodes and visceral organs. In recent years the IGCCCG has introduced a new staging system for advanced TC defining three prognostic risk groups with regard to therapeutic outcome. Patients are classified to be at good risk (probability of cure 95%), intermediate risk (probability of cure 70%) or poor risk (probability of cure 50%). The IGCCCG classification gives high prognostic evidence and enables an individualized risk-adapted approach in patients with advanced TC.

Therapy

Once TIN is diagnosed, therapeutic intervention is recommended, since 70% of patients will develop invasive germ-cell tumor within the next 7 years. Local radiation therapy with 18 Gy is the therapy of choice in patients with a contralateral invasive germ-cell tumor. In patients with unilateral TIN and a contralateral normal testis, inguinal orchiectomy appears to be the preferred management, since local radiation bears the risk of damaging the healthy testicle.

Non-Seminomatous Germ-Cell Tumors (NSGCT)

Clinical stage I NSGCT represents a troublesome entity concerning recommendations for optimal management since about 30% of patients will exhibit microscopic lymph-node disease. Several treatment options such as primary nerve-sparing retroperitoneal lymphadenectomy, primary chemotherapy, and active surveillance have been developed resulting in the same high cure rates of 98%. The European Germ Cell Cancer Consensus Group recommends an individualized, risk adapted approach based on the results of prospective randomized trials considering the presence or absence of the risk factors vascular invasion (VI) and percentage of embryonal carcinoma (ECA). VI has been identified as the most powerful clinical predictor of lymph-node metastasis with 48% of NSGCTs with VI developing metastases, compared to 14–22% of tumors without VI. A combination of VI and ECA might be even more powerful. Nowadays, nerve-sparing RPLND – if performed – is regarded as the standard approach. Up to 10% of patients will suffer from pulmonary relapse within the first 2 years, and will be cured by platinum-based chemotherapy. Even in low-volume lymph-node disease such as pathological stage IIA, the nerve-sparing RPLND can be performed as bilateral radical surgery without compromising the therapeutic outcome. Primary chemotherapy [two cycles of cisplatin, etoposide and bleomycin (PEB)] or surveillance (absence of VI) result in relapse rates of only 7% and 14%, respectively.

Low-Stage (IIA/B) NSGCT

Low-stage testicular disease comprises clinical stages IIA and IIB associated with a cure rate of ~98%. Patients with low volume disease and abnormal tumor marker levels of AFP, ß-hCG, or LDH are treated with 2–3 cycles PEB chemotherapy, patients with negative markers might be offered nerve-sparing RPLND or surveillance Patients with clinical stage IIB TC will undergo primary chemotherapy depending on the serum tumor marker concentrations with three or four cycles of PEB followed by secondary RPLND in about 30% of cases.

Clinical Stages IIC and III

Inductive chemotherapy represents the therapy of choice, with the number of cycles applied depending on the IGCCCG-based prognostic classification. Patients with "good prognosis" face a long-term survival rate of >90%, and are managed by three cycles of PEB.

Testicular Cancer. Table 2 Clinical Lugano – classification of TC

Stage I	Tumor markers normalized or decline according to their half-life after orchiectomy
	No detectable metastases by imaging studies
	Primary TC confined to the testicle
Stage IIA	Retroperitoneal metastases <2 cm
Stage IIB	Retroperitoneal metastases 2–5 cm
Stage IIC	Retroperitoneal metastases >5 cm
Stage IIIA	Supraclavicular or mediastinal metastases
Stage IIIB	Pulmonary metastases
	Minimal: <5 metastases in each lobe <2 cm
	Advanced: >5 metastases in each lobe or 1 lesion >2 cm
Stage IIIC	Extrapulmonary visceral metastases

T

Patients with "intermediate prognosis" face a survival rate of 70–80% and are managed by four cycles of PEB or cisplatin, etoposide and ifosfamide (PEI). Patients with "poor prognosis" have a survival rate of only about 50%; standard therapy consists of four cycles of PEB or PEI. A major advantage of primary high-dose chemotherapy has not been demonstrated, but this approach is currently being tested in prospective randomized trials.

Seminomatous Germ-Cell Tumors

Clinical Stage I Seminoma

Despite negative CT scans, there is a risk of 12–32% of occult retroperitoneal lymph node metastases depending on the absence or presence negative prognostic markers. The cure rate of clinical stage I seminomatous germ cell cancer is close to 100% and can be achieved by the three different therapeutic options active surveillance, radiation therapy and carboplatin monochemotherapy. Adjuvant retroperitoneal radiation therapy to the paraaortic or paracaval region with 20 Gy or adjuvant chemotherapy with one cycle carboplatin AUC 7 are the standard approach for high risk patients (tumor size > 4 cm, rete testis invasion) and result in a relapse-free long-term survival of 97%. Active surveillance represents the most reasonable approach to patients with good prognostic markers associated with a low recurrence rate of about 12%. Treatment of relapses is more intense with systemic chemotherapy of 3–4 cycles PEB in most cases.

Low-Stage (Clinical Stage IIA/B) Seminoma

Radiation therapy with 30 Gy (IIA) and 36 Gy (IIB), including the ipsilateral iliac and inguinal lymph nodes, is one standard therapeutic approach for low-stage seminomas. Relapse-free survival is as high as 92.5% in clinical stage IIA/B; relapse rates are about 5% in stage IIA and about 11% in stage IIB seminomas. Primary chemotherapy with two cycles of PEB is an alternative to radiation in clinical stage IIB seminoma.

Clinical Stage IIC and III

As pointed out for advanced non-seminomatous germ-cell tumors, therapy should be initiated according to the IGCCCG classification. For patients with good prognosis, three cycles of PEB chemotherapy are the treatment of choice, in patients with intermediate prognosis four cycles of PEB chemotherapy are applied.

Residual Tumor Resection (RTR) Following Chemotherapy for Advanced Testicular Cancer.

RTR represents an integral part of the multimodality treatment of advanced testicular germ cell tumors. The rationale for RTR is to completely resect mature teratoma and vital cancer which will be found in 30–40% and 20% of the patients, respectively. Currently, all residual lesions independent on size should be resected in NSGCT since even small lesions <1 cm in diameter will harbor mature teratoma and vital cancer. The extent of surgery depends on individual risk factors for relapse and quality of life issues. If the histology of the resected retroperitoneal masses is necrosis only, further resection of residual lesions in other organs might be omitted since there is concordance of histology in about 90% of the patients. RTR is a complex surgery associated with the necessity to resect adjacent organs or vascular structures in about 25% of the patients and should, therefore, be performed in experienced tertiary cancer care centers only. According to a recent analysis of prognostic markers patients harbouring a residual mass with >10% vital cancer cells or those with uncomplete resection might benefit from consolidation chemotherapy with two cycles. Postchemotherapy or post-radiotherapy RPLND in seminomas has only to be performed in lesions with a positive PET scan performed about 6 weeks after chemotherapy or radiation therapy in patients with residual lesions >3 cm.

Salvage Chemotherapy, High-Dose Chemotherapy

In seminomas relapsing after first-line radiation therapy a cure rate of >90% can be achieved by cisplatin-based chemotherapy according to the IGCCCG algorithm with regard to advanced seminomas. About 50% of relapsing seminomas following conventional chemotherapy can be salvaged with another combination chemotherapy consisting of PEI– etopside, ifosfamide and platinol (VIP) or – vinblastine, ifosfamide and platinol (VeIP). Currently, a 10% benefit of high-dose chemotherapy with regard to survival has been demonstrated; therefore, it seems advisable that all relapsing patients should be treated in a tertiary referral center.

NSGCT relapsing following conventional chemotherapy, salvage rates are as low as 15–40% using standard salvage protocols such as PEI–VIP or –VeIP. In some institutions the addition of paclitaxel to ifosfamide and cisplatin has been favored due to a high response rate >50%. Conventional-dose cisplatin-based salvage chemotherapy can achieve long-term remission in 15–40% of patients. Early consideration of high-dose chemotherapy seems advisable: trials suggest a benefit for the use of high-dose chemotherapy and autologous bone marrow transfer, with 46% and 50% of the patients being alive and disease-free after a median follow-up of 31 months and 30 months, respectively. Options for third-line chemotherapy are combinations such as paclitaxel and gemcitabine, gemcitabine and oxaliplatin or paclitaxel, gemcitabine and cisplatin, within clinical trials.

Genetics

With regard to predisposing genetic events, the locus Xq27 predisposes for bilateral TC and bilateral cryptorchidism. Other studies have reported the loci 1p36,

4p14–13, 5q21–21, 14q13–q24.3 and 18q21.1–21.3 to be highly associated with TC. Recently, it has been demonstrated that somatic mutations of exons 10, 11 and 17 of KIT occur significantly more often in patients with bilateral TC as compared to patients with unilateral disease. The results indicate that KIT might be involved in the development of familial and a minority of sporadic germ cell tumors and that KIT mutations primarily take place during embryogenesis such that primordial germ cells with KIT mutations are distributed to both testes.

Currently, all molecular markers such as p53, Ki-67, bcl-2, cathepsin D and E-cadherin have not been proven to be clinically useful prognosticators; only reverse transcriptase-polymerase chain reaction for AFP, hCG and germ cell alkaline phosphate (GCAP) mRNA for the detection of circulating tumor cells appears to be an interesting approach, with 60% of clinical stage I testicular cancer patients exhibiting positive signals that turn into negative signals following adjuvant chemotherapy.

Future Directions in TC

Based on the excellent therapeutic outcome, there appear to be only a few developments possible that will have further impact on the survival of testicular cancer patients. However, there might be many options to improve quality of life either due to reduction of acute toxicity or due to the development of treatment regimes associated with a significantly reduced long-term toxicity. It has been that the risk of cardiovascular disease is significantly increased after standard chemotherapy with 3–4 cycles PEB and/or salvage treatment (RR = 2.59). The increased risk is not due to an increase in classical cardiac risk factors but directly dependent on first line therapy. For the future, attempts to minimize treatment should be undertaken especially in patients with good prognosis in whom this type of long-term toxicity might be a greater risk to long-term survival than testicular cancer itself.

Elucidation of those mechanisms involved in the development of intrinsic and extrinsic chemorefractoriness in testicular cancer will be a major issue in the future, to apply effective chemotherapeutic protocols and to save even more lives. There are some promising approaches using modern molecular techniques such as gene expression profiling to explore the role of mismatch repair genes, multidrug resistance genes and potentially unknown genes.

Despite the high cure rates, it will be necessary for testicular cancer to be treated by clinicians and institutions with sufficient experience in diagnosis and management of germ-cell tumors. Specific problems such as extended tumor masses, relapsing tumors or poor prognosis at initial diagnosis must be referred to tertiary centers having the ability of an interdisciplinary approach.

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Testicular Feminization TFM

►Androgen Receptor

Testicular Germ Cell Tumor

►Testicular Cancer

Testicular Tumors

►Germ Cell Tumors

Testosterone

Definition

Testosterone is a steroid hormone from the androgen group. In mammals, testosterone is primarily secreted in

the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone.

►Sex Hormone Dependent Cancers
►Hormonal Carcinogenesis

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

►Dioxin

Tetracopeptide Repeat TPR Domains

Definition
A structural motif that mediates protein: protein interactions. Each TPR motif consists of repeats of a 34-amino acid sequence. It is present in a wide range of proteins; in particular many of the co-chaperones involved in the heat shock protein 70 and 90 folding systems.

►Molecular Chaperones

Tetraploidization

Definition
Process whereby the entire genome is duplicated.

Tetraspanin

►Metastasis Suppressor KAI1/CD82

TF

Definition
Tissue factor.

►Proteinase-Activated Receptors

TFF

►Trefoil Factors

TFIIH

Definition
Is a protein complex that plays a key role in both transcription and DNA repair.

TG2

Definition
An enzyme (EC 2.3.2.13) of the transglutaminase family. Like other ►transglutaminases, it crosslinks proteins between an N of a lysine residue and a glutamine residue in two protein chains, creating a bond (isopeptide) that is highly resistant to proteolysis.

►Transglutaminase-2

TGc

►Transglutaminase-2

TGF

Definition
Transforming Growth Factor.

►Transforming Growth Factor Beta

TGFα

Definition

Transforming growth factor alpha.

►ADAM17

TGF-β

Definition

►Transforming growth factor β; Is a multifunctional dimeric 25 kDa polypeptide growth factor, whose main functions are growth inhibition, immunosuppression and regulation of extracellular matrix formation and turnover. There are three different mammalian gene products. Activation renders the biologically latent TGF-β into its active form, which can bind to the cell surface receptors and initiate signaling. The TGFβ superfamily contains at least forty cytokines currently divided into two classes: the bone morphogenetic proteins (BMP) and the TGFβ/activin. These cytokines regulate cell fate (proliferation, differentiation and apoptosis) and extracellular matrix deposition.

►Smad Proteins in TGFβ Signaling

TGF-β Activation

Definition

An event that renders the biologically latent ►TGF-β into its active form, which can bind to the cell surface receptors and initiate signaling.

TGF-β-Regulated and Epithelial Cell-Enriched Phosphatase

Definition

►TEP1; Alternative, rarely used name for ►PTEN.

TGF-β Superfamily

Definition

Is a group of structurally related multifunctional peptides that control proliferation, morphogenesis, differentiation, migration and a variety of other functions in many cell types. Members of this family include the transforming growth factor βs (►TGF-β), ►bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activins/inhibins, mullerian inhibitory substance (MIS), glial cell derived neurotrophic factors (GDNFs) and macrophage inhibitory cytokine-1 (►MIC-1).

Th1

Definition

CD4+ helper T cells providing cytokines related to cell-mediated immune reactions.

►Allergy

Th2

Definition

CD4+ helper T cells providing cytokines related to humoral immune reactions.

►Allergy

Th1 Immune Response

Definition

Two types of effector CD4 + T helper cell responses can be induced by a professional antigen presenting cell (APC), designated Th1 and Th2, each designed to eliminate different types of pathogens. The Th1 response is characterized by the production of interferon-gamma, which induces B-cells to make opsonizing

(coating) antibodies, and leads to cell mediated anti-tumor immunity.

►DNA Vaccination

Th2 Immune Response

Definition

Is characterized by the release of ►interleukin 4, which results in the activation of B-cells to make neutralizing (killing) antibodies, leading to humoral immunity.

►DNA Vaccination

Thalidomide and its Analogues

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Synonyms

Thalidomide = (ph)thal(ic acid) + (im)id(e) + (i)mide, $C_{13}H_{10}N_2O_4$; Immunomodulatory drugs (IMiDs) = CC-4047, Lenalidomide (CC-5013 or Revlimid)

Definition

First synthesized in 1954 in Germany from the glutamic acid derivative α-phthaloylisoglutamine, thalidomide was used as a sedative and treatment for morning sickness in the 1950s until its teratogenic effects became apparent. Phocomelia is the most well known toxicity of thalidomide, making it absolutely contra-indicated in pregnancy. The IMiDs are immunomodulatory derivatives of thalidomide, rationally designed to be more potent at inhibiting ►cytokine production. The chemical structures of thalidomide and two IMiDs are shown in Fig. 1.

Characteristics

Thalidomide is commonly used in the treatment of moderate to severe erythema nodosum leprosum and less frequently in the treatment of a wide range of non-malignant clinical conditions refractory to standard therapies, such as rheumatoid arthritis, the inflammatory and wasting effects of chronic tuberculosis, Behcet's disease, Crohn's disease, aphthous ulcers, and cachexia associated with HIV infection.

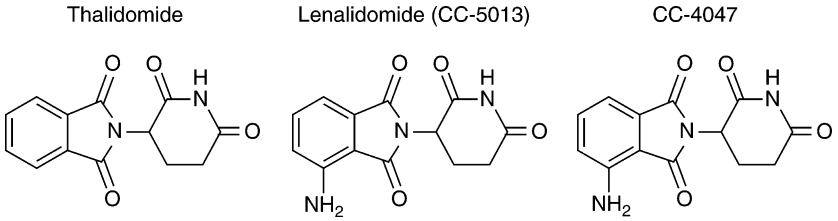
The growth and survival of myeloma cells is critically dependent on the interaction with the bone marrow microenvironment. Thalidomide (Thal) and immunomodulatory drugs (IMiDs) act via mechanisms to disrupt this interaction and inhibit myeloma cell growth and survival. For abbreviations, see main text.

Mechanisms of Action

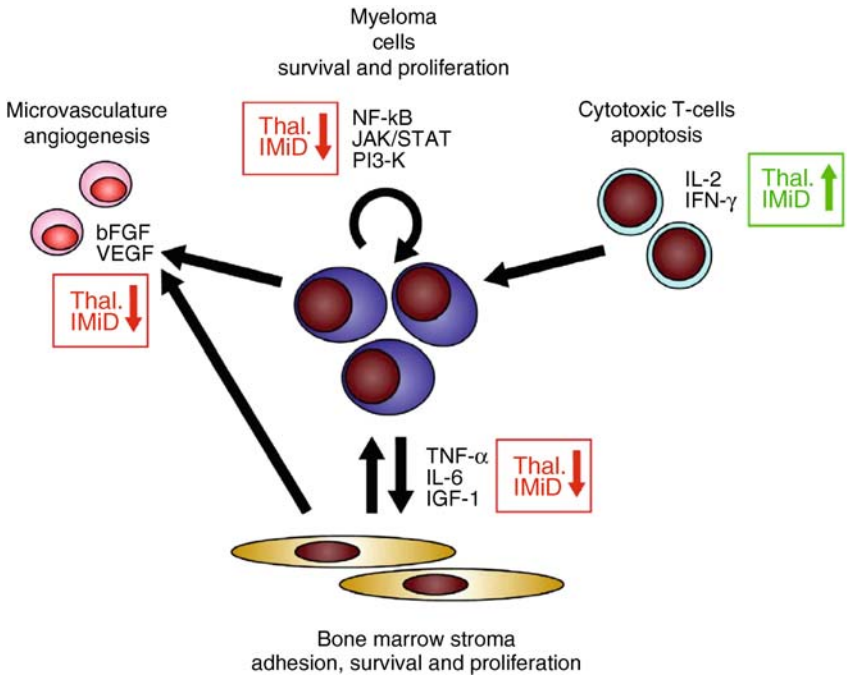
Thalidomide influences the production of and cellular effects of inflammatory cytokines, particularly those involved in ►angiogenesis. It has also been shown to activate cytotoxic T-lymphocytes, a process involving the release of interleukin-2 (IL-2) and ►interferon (IFN)-γ. Thalidomide enhances IL-4 and IL-5 production and down-regulates IL-12 production, effecting a shift in cytokine profile which is generally considered favorable in the treatment of cancer. Thalidomide may also have direct effects on the growth and survival of malignant cells, e.g. it inhibits the activity of ►cyclooxygenase-2 via inhibition of ►nuclear factor κ-B (NF-κB) activity. IMiDs demonstrate a similar range of biological activities to thalidomide, with significantly greater potency in certain activities. For example, CC-4047 exhibits a 20,000-fold higher potency than thalidomide at inhibiting ►tumor necrosis factor (TNF)-α production. Several mechanisms of action are shown in Fig. 2, which lead to alterations in the interplay between ►multiple myeloma (MM) cells and the bone marrow environment.

Multiple Myeloma

Early studies using thalidomide in patients with relapsed multiple myeloma described responses in around half of the patients treated, although typically with a duration of less than 12 months. Initial attempts to achieve high target doses (up to 800 mg/day) resulted in frequent dose limiting toxicity. The reported side effects were dependent on dose and patient age. Somnolence and constipation were common, although manageable in most cases. Peripheral neuropathy occurred in up to a third of patients, particularly with long term treatment, and it was disabling and irreversible in some cases. Particular concerns were raised concerning patients with multiple myeloma who may develop neuropathies for other reasons, thus increasing the severity of the symptom, e.g. concomitant drugs, amyloid deposition or paraproteinaemia. There was also a significant rate of venous thromboembolism (VTE) complicating treatment with thalidomide.



Thalidomide and its Analogues. Figure 1 Mechanism of action of thalidomide and IMiDs in multiple myeloma.



Thalidomide and its Analogues. Figure 2 Chemical structures of thalidomide, lenalidomide and actimid (CC-4047).

More recent clinical studies have suggested that high doses of thalidomide cause unnecessary toxicity since its efficacy in treating multiple myeloma is equivalent at lower doses (100–400 mg/day). A number of groups have investigated the combination (TD) of thalidomide and dexamethasone in patients with relapsed multiple myeloma. Collectively, the results suggest that the TD combination is superior to thalidomide monotherapy for treating relapsed disease and, although there is no published data to directly confirm this advantage, TD combination treatment is widely used in this context. Although an overall survival benefit has not yet been demonstrated, the response rate may be further improved with the addition of cytotoxic agents (Table 1), albeit at the cost of increased toxicity. TD is effective in ~30% of patients resistant to thalidomide when it is used as a single agent.

Based on its activity in relapsed patients, TD has also been tested in patients newly diagnosed with multiple myeloma. The response rate is superior to that obtained using standard infusional chemotherapy with Vincristine, ►Adriamycin and Dexamethasone (VAD) and offers the benefits of an oral regime versus one requiring the insertion of a central venous catheter. These advantages have led to TD being widely adopted as standard first line therapy in younger patients prior to autologous stem cell transplant (ASCT), although whether the improved response rates will translate into an overall survival advantage is not yet known. In patients not suitable for ASCT, two recent studies have demonstrated that the addition of thalidomide to standard chemotherapy (Melphalan and Prednisolone) results in improvements in both response rate and survival (Table 1).

Thalidomide and its Analogues. Table 1 Summary of the activity of thalidomide in the treatment of multiple myeloma

Disease status	Regimen	
Relapsed disease	Thalidomide: Monotherapy	Response rate 25–48% Complete response rare
	Thalidomide + Dexamethasone (TD)	Response rate 41–55% Synergistic in-vitro and in-vivo Effective in relapse post ASCT
	Thalidomide + Steroids + Chemotherapy	Response rate 36–79% Many combinations tested Increased toxicity with intensive regimens
	Thalidomide + Novel agents	Ongoing phase I/II trials Bortezomib, IMiDs, investigational drugs
Newly diagnosed disease (Pre autologous stem cell transplant (ASCT)	Thalidomide + Dexamethasone	Response rate 65–75% CR in 7–15% Survival benefit over standard induction chemotherapy plus ASCT unproven
	Thalidomide + Dexamethasone + Chemotherapy	Response rate up to 90% Many regimens, increased toxicity over TD Survival benefit over standard induction chemotherapy/TD plus ASCT unproven
Newly diagnosed disease (Not candidates for ASCT)	Melphalan + Prednisolone + Thalidomide (MPT)	Response rate 75–80% CR in up to 15% Response rate and event free survival superior to MP but increased non-hematological toxicity
Maintenance post ASCT	Thalidomide + Bisphosphonates	Event free survival and overall survival superior to no maintenance in single study Survival advantage most marked if <90% response to prior treatment Unproven benefit following thalidomide containing induction regimen

Only peer-reviewed published data regarding the efficacy of thalidomide in the treatment of multiple myeloma at various stages of the disease are presented.

Thalidomide has also been investigated in patients with multiple myeloma as maintenance treatment following ASCT. In a single randomized study of patients treated with VAD chemotherapy followed by ASCT, the event free survival was significantly prolonged in patients receiving maintenance compared to those that did not, with a non-significant trend observed towards improved overall survival.

Two IMiDs are currently used in the treatment of multiple myeloma. A phase I study of CC-4047 demonstrated an encouraging response rate of 67% using the drug as a single agent, but the sample size was small. More data are available on the use of lenalidomide. Two phase I studies have demonstrated a maximum tolerated dose of 25–30 mg/day. Subsequent phase II trials have defined the optimal daily dosing schedule and described significant responses in 30–40% of patients, including those previously treated with thalidomide. In 2006, the Food & Drug Administration in the United States of America approved the use of lenalidomide as treatment for relapsed multiple myeloma.

Preclinical data suggesting synergy with steroids has also led to investigation of the efficacy of treatment with a combination of lenalidomide and dexamethasone (Len/Dex). Two large randomized multicenter phase III studies comparing Len/Dex to Dexamethasone in relapsed or refractory disease have produced strikingly similar results. Interim analyses of both studies have demonstrated significantly improved response rates and median time to progression following combination therapy versus dexamethasone alone; both trials were therefore prematurely closed to recruitment. Since activity has also been demonstrated in ►chronic lymphocytic leukemia and ►non-Hodgkin lymphoma, ►clinical trials of lenalidomide treatment in patients with these disorders are ongoing.

►Myelodysplastic Syndromes (MDS)

There has been considerable international interest in the use of lenalidomide in the treatment of MDS. A phase I-II study in 43 patients defined the optimal dose as 10 mg/day and demonstrated responses in almost half

of the patients studied, including a striking response in over 80% of patients with the 5q-syndrome. A subsequent multicenter phase II study of 148 patients, all of whom exhibited the 5q deletion, confirmed the results of the earlier trial. In this trial, 76% of patients responded to lenalidomide treatment, 67% achieved durable transfusion independence (median duration not reached at 2 years) and almost half of all patients attained cytogenetic remission. A second phase II study investigated the use of lenalidomide in 215 patients with low risk MDS without 5q deletions, of whom 44% responded to treatment and 26% became transfusion independent, although the duration of response was less that that reported in the study in patients with 5q deletions. The results of these studies led the Food & Drug Administration in the United States of America to approve the use of lenalidomide as a treatment for anemia in patients with MDS exhibiting a 5q deletion. The optimal duration of treatment is not currently known.

Solid Malignancies

When compared to the use of thalidomide in the treatment of multiple myeloma, the role of thalidomide in the treatment of solid malignancies is less clear, particularly in view of the somnolence, constipation, VTE, peripheral neuropathy and fatigue associated with treatment. Whereas therapeutic doses of 50–400 mg/day orally (2–8 mg/kg/day) are currently used for dermatologic and inflammatory diseases, oncological investigators have generally used 100–800 mg/day either as single agent therapy or in combination with cytotoxic chemotherapy (e.g. carboplatin (►Platinum drugs), docetaxel (►Taxotere) or irinotecan (►Topoisomerase enzymes as drug targets)) or cytokines such as ►interferon-α. Thalidomide at a dose of 400 mg/m^2/day can be safely administered to children with solid tumors in combination with carboplatin.

Thalidomide (100–600 mg/day) has been studied in patients with a variety of solid tumors. In phase II studies, responses have been observed in patients with glioma, metastatic melanoma, pancreatic cancer, Kaposi's sarcoma, malignant melanoma and prostate cancer. Results for patients with renal-cell carcinoma were considered particularly promising, leading to a randomized phase II study in 60 patients with this condition. Patients received either thalidomide or medroxyprogesterone as monotherapy. There was no difference in overall survival between the two groups, leading the investigators to conclude that the risk/benefit ratio did not favor the use of thalidomide as monotherapy in patients with ►renal carcinoma. Similarly, phase II studies of the combination of thalidomide and interferon-α have shown minimal efficacy and unacceptable toxicity in patients with metastatic renal cell carcinoma.

Early data would suggest that the palliative response to thalidomide is separate from the objective radiological response and that it may occur earlier. The most promising palliative results have been reported in Kaposi's sarcoma, malignant melanoma and prostate cancer, especially when thalidomide is combined with chemotherapy. In a study of 40 heavily pre-treated patients with ovarian cancer, thalidomide appeared to be comparable in symptom response and quality of life to single agent intravenous chemotherapy.

Of particular note in the palliative treatment of solid malignancies is the potential for thalidomide to treat ►cancer cachexia. The postulated mechanism of action is compatible with the probable role of TNF, INF-γ, and IL-6 in the aetiology of this debilitating symptom. Randomized, placebo-controlled trials of thalidomide in weight-losing patients with advanced solid malignancies, such as pancreatic cancer, have demonstrated weight gain and improvements in physical functioning.

Future Directions

When one compares IMiDs to thalidomide treatment, in addition to their increased potency in inhibiting the production of cytokines such as TNF-α, one of the most attractive features of the IMiDs is the lack of neurotoxicity caused by drug treatment. Although their safety in long-term administration (i.e. many years) remains to be demonstrated at the phase IV (post-licensing) level of clinical trial development, the toxicity profile of lenalidomide appears compatible with the concept of maintenance treatment for diseases in which its anti-inflammatory properties, anti-angiogenic activity, apoptotic producing activity or inhibition of ►T-cell response would benefit from chronic exposure to the drug. In particular, the ability of IMiDs to inhibit T-regulatory cells (as well as being co-stimulatory) may represent an ideal property for enhancing immunotherapeutic and cancer vaccine approaches to the treatment or prevention of cancer.

Although a large randomized study which was terminated prematurely did not shown any significant impact of lenalidomide treatment on overall survival in patients with stage IV malignant melanoma, several anecdotal responses have been observed in this disease. Patients with malignant melanoma in whom objective responses were demonstrated in a phase II study of lenalidomide treatment, who had also been exposed to melanoma vaccines previously, have led to the suggestion that increased responses may be associated with a reduction in T-cell regulatory activity and co-stimulatory abilities. This highlights the possibility that this class of drugs may work even better in combination with other modalities and may be synergistic with cancer vaccines.

A major target for drug development to inhibit the growth and spread of cancer is angiogenesis. Several ►small molecule drugs which inhibit multiple tyrosine kinase pathways and angiogenesis are currently in

clinical trials. IMiDs inhibit angiogenesis but it is not currently known if they also inhibit ►receptor tyrosine kinase pathways. Indeed, the other immunomodulatory and cytokine modulating properties of IMiDs may in fact be more synergistic in the inhibition of angiogenesis than the inhibition of multiple tyrosine kinases. Since such drugs are likely to be taken for many years by patients with cancer in order to maintain response, lack of toxicity and convenience of oral dosing are important factors.

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Therapeutic Active Targeting

Definition

Systemic delivery of a therapeutic agent to a tumor by linking tumor specific ligands to the therapeutic agent, which facilitates accumulation of the therapeutic agent inside the tumor.

►Nanotechnology

Therapeutic Cloning

Definition

Reprogramming the nucleus of an adult cell through transfer into the cytoplasm of an enucleated oocyte. This is sometimes referred to as "cell-nuclear replacement" or "somatic cell nuclear transfer."

Therapeutic Efficacy

Definition

Beneficial health effect from a treatment.

►Chemoprotectants

Therapeutic Passive Targeting

Definition

Systemic delivery of a therapeutic agent to a tumor which exploits leaky vascular endothelium characteristic of tumors. The therapeutic agent leaks out of circulation through fenestrations in tumor vessels allowing accumulation of the agent in the tumor.

►Nanotechnology

Therapeutic Vaccine Therapy

Definition

Is a form of active ►immunotherapy that treats established diseases by eliciting a systemic, antigen-specific, cellular and humoral immune response. For active immunization against cancer, tumor-associated antigens (TAAs) are presented in an immunogenic formulation to induce a sufficiently powerful acquired immune response. Either the TAAs themselves, or an appropriate mimic such as an anti-idiotypic or mimotopic antibody or peptide, can be used as the immunogenic trigger.

Therapeutic Viruses

►Oncolytic Virus

Thiazolidin

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Synonyms

Thiazolidinone

Characteristics

Thiazolidins are a group of synthetic compounds that can selectively induce ►G2/M arrest (►Cell cycle targets for cancer therapy) and ►apoptosis in cancer cells. They were identified as potential anticancer agents by screening a chemical library for compounds that can kill ►paclitaxel-sensitive and paclitaxel-resistant cancer but not normal cells. The representatives are 5-[(4-methylphenyl)methylene]-2-(phenylamino)-4(5H)-thiazolone and 5-(2,4-dihydroxybenzylidene)-2-(phenylimino)-1,3-thiazolidin. Chemically, the compounds contain three ring structures, a middle oxothiazolidine ring connected with two benzyl rings, one on each side. Several of those compounds can effectively inhibit the growth of human lung, colon, breast, ovary and prostate cancer cell lines. Treating cancer cells with thiazolidin compounds can induce cell cycle arrest in the G2/M phase and apoptosis, activating ►caspase-3, -8, -9, and causing the release of cytochrome *c* from mitochondria. Nevertheless, they had no obvious toxic effects on normal human fibroblasts and mesenchymal stem cells at the 50% inhibitory concentration for susceptible cancer cell lines. The inhibitory effect on cancer cells is independent of the status of p53 and ►P-glycoprotein. They are effective against cancer cells with p53 mutations or cancer cells overexpressing P-glycoprotein. Although the molecular targets and mechanisms of thiazolidin-induced cell cycle arrest and apoptosis are not yet clear, the activation of ►c-Jun-N-terminal kinase (►JNK) (►MAP kinase) by thiazolidin compounds is known to be required for thiazolidin-induced apoptosis in cancer cells. Thiazolidin-induced apoptosis was abrogated when JNK activation was blocked. Moreover, thiazolidins can suppress the expression of ►hypoxia-induced factor 1α (►HIF1α) (►Hypoxia and tumor physiology) and vascular endothelial growth factor in cancer cells. In vivo administration of thiazolidin can suppress the growth of human xenograft tumor in nude mice, indicating that those compounds might be used as anticancer agents.

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Thiazolidinediones

Definition

TZDs; Fatty acid-related, synthetic ligands of peroxisome proliferator-activated receptors that are routinely used in the treatment of diabetes mellitus and can have pro- and anti-cancerogenic effects.

►Peroxisome Proliferator-Activated Receptor

Thiazolidinone

►Thiazolidin

Thin Needle

►Fine Needle Aspiration Biopsy

Thiol

Definition

Functional group consisting of a sulfur and a hydrogen atom (-SH); also referred as sulfhydryl group. An SH-group forms the functional group of the amino acid cysteine. Thiols are important for the function of many enzymes and are crucially important for the formation of protein tertiary and quaternary structures.

►Dimethylfumarate

Thiol-Protease Inhibitors (Cytoplasmic α- and β-TPIs)

►Cystatins

Thioredoxin

Definition

A protein with oxidoreductase activity, having a typical "Trx-fold" and a -CPGC- active site sequence involving a redox active dithiol/disulfide motif. Thioredoxins exist as several isoforms and are found in nearly all cells. Cytosolic Trx1 and mitochondrial Trx2 are the main thioredoxins in human cells.

►Thioredoxin System

Thioredoxin is Identical to ADF Adult T cell Leukemia Derived Factor

►Thioredoxin System

Thioredoxin Reductase

Definition

A flavoprotein with oxidoreductase activity, reducing the disulfide in the active site of thioredoxin to a dithiol. Thioredoxin reductases exist as several isoforms in human cells, with cytosolic TrxR1 and mitochondrial TrxR2 being the two major forms.

►Thioredoxin System

Thioredoxin System

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Synonyms

Protein disulfide reductase; Thioredoxin is identical to ADF adult T cell leukemia derived factor; Trx80 is truncated thioredoxin comprising the 80 or 84 N-terminal amino acids of human cytosolic Trx

Definition

►Thioredoxin (►Trx) is a small ►dithiol-disulfide oxidoreductase existing in all living cells. The thioredoxin system is comprised of Trx, NADPH, and ►thioredoxin reductase (►TrxR) and functions as the cells' major NADPH-dependent protein disulfide reductase. Trx have numerous functions in cell growth such as the electron donor for ribonucleotide reductase, which is essential for DNA synthesis and repair. ►Reduced Trx is alo central in antioxidant defense, regulation of transcription factors such as ►p53, or ►apoptosis inhibiton of ASKI. TrxR is an essential ►flavoprotein with a catalytic ►selenocysteine residue, which is a target for many chemotherapeutic drugs. Both Trx and TrxR are upregulated in most but not all forms of ►cancer.

Characteristics

Trx contains a conserved active site (-Cys-Gly-Pro-Cys), which is present as a disulfide in ►oxidized Trx (Trx-S_2). This disulfide is reduced to a dithiol Trx-(SH_2). Trx-(SH_2) is the cells' major protein disulfide reductase, which is responsible for keeping proteins reduced and maintaining a reducing intracellular environment and controls the cells' redox potential together with GSH and glutaredoxins. As a disulfide-reducing protein, Trx has many targets including ribonucleotide reductase, which makes the deoxyribonucleotide building blocks for DNA, both for DNA replication and repair. In cancer cells ribonucleotide reductase is often strongly upregulated and a target for chemotherapy by, for example, hydroxyurea. Both Trx and TrxR are expressed in mammalian cells as dedicated isoforms for either predominantly cytosolic (Trx-1 and TrxR1) or mitochondrial (Trx-2 and TrxR2) localization. One important function of thioredoxin is to participate in antioxidant response. This is because Trx is the electron donor for the family of thioredoxin peroxidases, which act to remove hydroperoxidases by cysteine residues, which are oxidized to disulfides. Thioredoxin will also reduce and activate proteins, which have been oxidatively inactivated. Thioredoxin is a reducing agent for methionine sulfoxide reductase, which restores Met-SO residues to Met. One function of thioredoxin which is general, is to act as an activator or reducing agent for many transcription factors, which upon DNA-binding in the nucleus require reduced sulfhydryl groups. Examples of such factors are p53, NFκB (►Nuclear factor κB), and ►AP-1. In cells ►oxidative stress often leads to movement of cytosolic Trx1 to the nucleus, where its activity is linked to redox control of transcription factors.

Oxidative stress or activation of many cells leads to secretion of Trx1 by a leaderless pathway, which generates Trx levels of around 25 ng/ml in plasma. In cancer but also in ►inflammation the level of Trx1 in

Thioredoxin System. Table 1 Reactions of importance for cancer involving the thioredoxin system. The thioredoxin system is involved in a number of critical reactions having importance for the progression of cancer. This table shows a list of some of the reactions in which the thioredoxin system is involved. For further discussion or reference to publications describing these reactions, please see the cited review articles

Event	Reaction	Role of the thioredoxin system
Development of ►cancer	Protection from selenium	It is possible that the Trx system is important for the chemopreventive effects of selenium
	Critical role of p53	The transcriptional activity of p53 is regulated by Trx
	Critical role of apoptosis	Several events of apoptosis, including triggering by oxidative stress and signaling through ASK-1, are regulated by Trx
Progression of cancer	Production of DNA precursors	Trx supports ribonucleotide reductase
	Resistance to oxidative stress	The Trx system is an essential part of the cellular antioxidant defense
	Support of growth of leukemia	Extracellular Trx support growth of certain types of leukemic cells
	Histone acetylation	Regulation of histone acetylation by TXNIP links this event to the Trx system
	Telomerase integrity	TrxR1 antisense adversely affects telomerase maintenance
Treatment of cancer	Therapy with anticancer ►alkylating agents	TrxR is a cellular target for many compounds including ►cisplatin, other ►platinum drugs, ►curcumin, and several ►xenobiotics
	Therapy with selenite	Selenite may be cytotoxic toward cancer cells through the action of TrxR

plasma may increase and serve as a diagnostic tool. Extracellular thioredoxin acts as a cocytokine for immune cell growth and was also discovered as adult T-cell leukemia derived factor (ADF) secreted from malignant T-cells resulting from HTLV-I transformation. Plasma also contains a truncated form of Trx called Trx 80, which acts a mitogenic growth factor for monocytes and can induce a Th1 response in the presence of lymphocytes. The levels of Trx80 in cancer have not been investigated.

►TrxR is a ►selenoenzyme, which contains a carboxy terminally located selenocysteine (Sec) containing active site (-Gly-Cys-Sec-Gly) present in all the three isoenzymes of TrxR in a 16-residue elongation to a glutathione reductase-like scaffold structure. Both cytosolic and mitochondrial TrxR are expressed as a large number of isoforms derived from alternative ►pre-mRNA splicing. TrxR has a number of activities, apart from its function to reduce oxidized Trx, such as reducing selenite, dehydroascorbic acid, and members of the thioredoxin fold family such as protein disulfide isomerase (PDI).

Many clinically used anticancer drugs are targeting TrxR and the highly reactive selenol in the active site of the enzyme. Chemotherapeutic drugs targeting TrxR include cisplatin, nitrosoureas, antitumor quinones, melphalan, chlorambucil, and gold compounds.

The special interest in the thioredoxin system in relation to cancer is the potential role of TrxR in the chemopreventive effects of ►selenium compounds in cancer. TrxR is a selenoenzyme, which is dependent on selenium for activity as well as having a role in antioxidant defense and control of p53 and other functions such as the role of thioredoxin in prevention of apoptosis via binding to ►ASK-1, Trx, and TrxR; also has a potential role in the mechanism of selenium in prevention of cancer. However, today the role of TrxR as a target of selenium is not yet proven.

Finally, the protein called TXNIP (also TBP2 or VDUP-1) is critically regulated by binding to Trx in the reduced form and is a key component in the action of, i.e., histidine diacetylase inhibitors on inhibiting cell growth of tumor cells. A general result seems to be that tumor cells are under oxidative stress and the thioredoxin system is thereby upregulated. Normal cells upon treatment with drugs which induce oxidative stress, such as doxorubicin or SAHA, are able to upregulate Trx and TrxR to meet the increased oxidative stress, whereas tumor cells are more sensitive and killed.

The accumulated knowledge from many studies implies that the thioredoxin system is involved in many events of cancer development, treatment, or progression. Some of the critical events in which the thioredoxin system are involved are summarized in Table 1.

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Thiostatins

►Cystatins

Thiotepa

Definition

Is a cytotoxic anti-cancer alkylating agent related chemically and pharmacologically to nitrogen mustard. Its radiomimetic action is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA.

Third-Generation Bisphosphonate

►Minodronate

Third-Generation Nitrogen-containing Bisphosphonate

►Minodronate

Thoracentesis

Definition

The operation of puncturing the chest wall to let out liquids contained in the chest cavity.

►Pleural Effusion

Three-Dimensional Conformal Radiation Therapy

Definition

3D CRT; Is a form of advanced radiation therapy. In 3D CRT, treatment planning is performed using 3D computed tomography (CT), and radiation beams of uniform intensity are delivered to the target through multiple or dynamic ports, conforming the edges of the radiation fields to the shape of the target in order to improve dose localization.

►Proton Beam Therapy

Three-Dimensional Tissue Cultures

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Definition

Three dimensional (3D) tissue cultures are in vitro methods for growing cells in a system that more accurately reproduces the structure, function and ►microenvironment of the tissue of origin.

Characteristics

There is wide recognition that most cells require the complex structure and function of the 3D tissue microenvironment to provide the many signals and interactions for appropriate proliferation, growth, ►apoptosis, ►differentiation and ►invasion necessary to form relevant models of the corresponding in vivo structures. 3D cultures have arisen to overcome the inadequacies of simple (2D) cultures in reliably

representing tissue and organ function in vitro and as a model of intermediate complexity between the cell and the tissue or organ. In cancer research this is reflected in different cell responses to therapeutic agents and other experimental manipulations when cells are in 3D culture rather than (2D) ►monolayer culture which has usually been used for cell propagation and therapeutic testing. For example, promising drugs selected using 2D culture models often fail in 3D cultures, tumor ►xenografts and in human patients. Tumors are a complex tissue consisting of cancer, stromal, immune and endothelial cells, together with ►extracellular matrix which provides support and signaling molecules and binds diffusible growth factors. This unique mechanical and chemical microenvironment has important consequences in cellular function, producing major changes in gene expression and regulation of intracellular signaling as 3D cancer tissue grows. Consequently 3D tumor cultures are used experimentally to better understand tumor biology and provide more realistic testing conditions for therapeutic agents. Below, several of the 3D culture techniques used in cancer research are discussed by increasing complexity, although this scheme is arbitrary since there is often much overlap between categories.

Multicellular Spheroids

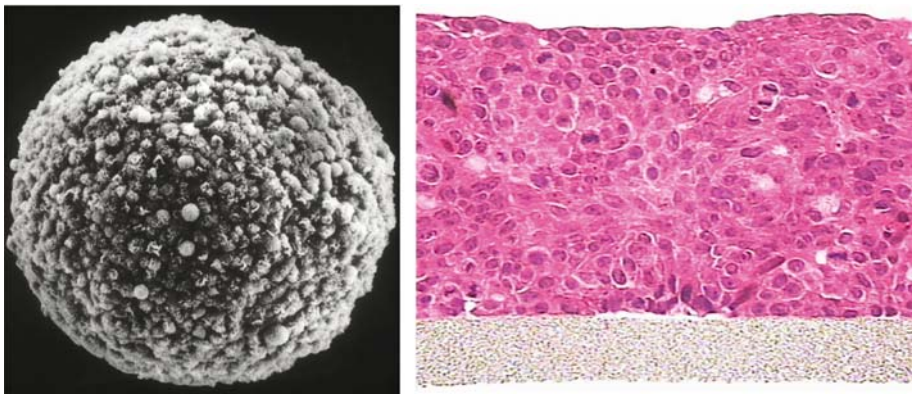
►Multicellular spheroids (MS, spheroids, multicellular tumor spheroids, MTS) are spherical aggregates of cancer cells that model of the extravascular compartment of solid tumors (Fig. 1A). Developed in the early 1970s, they are particularly representative of avascular tumor nodules, micrometastases and densely packed intercapillary regions in large tumors. Spheroids are the most extensively used 3D tissue culture method for cancer cells.

Culture

They are generally grown in mammalian tissue culture medium at 37°C, either as liquid overlay cultures or in suspensions, where attachment to the apparatus is prevent by culturing over agar or in stirred spinner flasks respectively. Spinner cultures allow large scale and economic growth of spheroids with well defined structure and physiology.

Diffusion Limited Growth, ECM and Differentiation

Spheroids generally grow in a Gompertzian fashion, developing many features of the solid tumor microenvironment such as ►hypoxia, ►HIF-1 expression and ►necrosis in the central region when larger than 400–500 μm in diameter. This diffusion limited growth is related to limited penetration of oxygen, nutrients and growth factors, together with depletion of energy reserves in the central region. These gradients, which form over surprising small distances (10–20 cells diameters), limit the thickness of the viable rim of cells to 100–220 μm in most spheroids. Although they will often grow to a diameter of 500–1,000 μm they may be used at any stage for experimentation. During growth the cells secrete extracellular matrix (ECM) components and form cell-cell and cell-ECM interactions necessary for the maintenance of a multicellular structure at cell volume fractions of 45–65%, similar to tumors. One advantage of even simple human 3D cultures such as spheroids is that the ECM, although not completely representative of the in vivo situation, is produced by syngeneic cells rather than by the host cells as may occur in tumor xenografts. Because of the more realistic 3D structure cells in spheroids are more likely to express differentiation markers such as keratins and form cell junctions such as desmosomes and ►tight junctions and often undergo terminal differentiation with formation of pseudoglandular and acinar structures and therefore represent a close



Three-Dimensional Tissue Cultures. Figure 1 Histological structure of spheroids and MCL. *Left*: Scanning electron micrograph of a human cervical squamous cell carcinoma multicellular spheroid ~300 μm in diameter. Haematoxylin and eosin stained HT29 human colon cancer MCL ~150 μm thick.

approximation to the tissue architecture seen in vivo. This contrasts with the homogeneous morphology of reproducing stem cells seen in monolayer culture.

Dissociation, Histology and Other Study Methods

Investigation of the relative effect of the 3D microenvironment is often performed by exposing spheroids to experimental treatments after which they are dissociated into single cell suspensions by exposure to trypsin, and the cellular responses compared to those seen in cells treated after dissociation or grown in monolayer culture. For example, when irradiated under physiological oxygen tensions spheroids demonstrate resistance due to poor killing of the central hypoxic cells, usually demonstrated using the ►clonogenic survival assay (CSA) where dissociated cells are plated in culture dishes and stained colonies are counted ~1–2 weeks later. The so-called radiobiologically hypoxic fraction can be calculated by comparing the sensitivity of cells from spheroids exposed under physiological oxygen concentrations to those exposed under hyperoxic (95%) and anoxic conditions. In some cell lines additional resistance (compared to cell suspensions from monolayer culture) is due to a cell contact effect that lasts at least one cell cycle after dissociation.

Cell Proliferation

A fundamental similarity between spheroids and tumors is that gradients of cell proliferation occur, from rapidly dividing surface cells in the outer 3–5 cell layers (~75 μm) to non-dividing central cells. These proliferation gradients match the gradients of oxygen and nutrients and may be visualized by bromodeoxyuridine assay of histological sections or decreased expression of the proliferation marker ►Ki-67 and increased expression of ►CDK inhibitors such as the quiescence marker ►p27. Consequently, spheroids have a large population of quiescent cells and this explains, in part, the observations of increased resistance to some drugs. Spheroid studies have shown that cells in the central region may exit the cell cycle but remain viable for several days, even under hypoxia, and start reproducing again if conditions change. The lifespan of hypoxic cells was 3–5 days in human spheroids which is similar to (but less variable than) that seen in corresponding xenografts.

pH and Metabolite Gradients

Gradients of metabolites have been demonstrated using quantitative bioluminescence, with increased lactate and lower pH in the central region, due to the marked tumor cell ability to utilize anaerobic glycolysis for energy production and to survive in low pH microenvironments. This is consistent with ATP and glucose concentration gradients not being as steep as O_2 gradient indicating that energy production is partially maintained in central regions. The simple spherical geometry simplifies experimental investigation. This is because the microenvironmental gradients that determine structure and physiology, although present in tumors, are not as highly correlated with distance from the blood vessel as they are with distance from the surface in spheroids.

Cancer Biology

Since function in spheroids may be related in such a direct way to structure they have been extensively used to investigate the biological causes and effects of these microenvironmental gradients. The biology of cell populations from different depth in spheroids may be investigated by histological techniques using fluorescent antibodies and in vivo confocal microscopy and therapeutic response studied using sequential dissociation, centrifugal elutriation, and, especially, fluorescence activated cell sorting (►FACS) where cells are selected by ►flow cytometry based on the differential uptake of fluorescent molecules. Microenvironmental regulation of cell-cell interactions and invasion has been investigated with demonstration of important roles for ►cell adhesion molecules, such as the integrins and cadherins.

Drug Effects and Multicellular Resistance

The variations in tumor cell biology and microenvironment also leads to variability in sensitivity to many drugs in both tumors and 3D cultures. Multicellular resistance in spheroids was found to be partly due to cytokinetics as demonstrated by the difference in sensitivity of freshly dissociated cells to exponential phase monolayer cultured cells. Another key determinant of drug resistance in 3D cultures is survival signals from the microenvironment mediated by adhesion and other molecules. In addition the ►multidrug resistance pump, ►P-glycoprotein, has been demonstrated to be expressed in the centre of large spheroids and gene expression analysis has implicated suppression of DNA mismatch repair in multicellular resistance to alkylating agents.

Drug Transport

With the investigation of drug effects in spheroids it became apparent that poor drug penetration could also cause drug resistance in multicellular structures. Penetration has been studied by microscopy or autoradiography of spheroids after exposure to fluorescent or radiolabeled drugs. This demonstrated poor penetration especially for drugs bound in cells to high concentrations such as anthracyclines and drugs rapidly metabolized such as tirapazamine while others such as 5-fluoruracil and vincristine penetrated well. The reduced cytotoxic response of ►doxorubicin has been correlated with its poor penetration using FACS.

Without this information the interpretation of similar doxorubicin gradients seen in vivo is equivocal. Spheroids have also been extensively used to confirm penetration and binding of nitroimidazole radiosensitizers and hypoxia markers. That tumor penetration is poorest for macromolecules has been convincingly demonstrated in many spheroid studies of antibodies and immunotoxins.

Individualized Therapy

Spheroids, in common with other in vitro methods, have so far found limited use in predicting patient response, mainly because of the difficulties in primary culture of human tumor cells in a timely manner.

Multicellular Layers (MCL, Multilayered Cell Cultures, MCC)

►Multicellular layers (MCL) are flat aggregates of tumor cells grown in culture medium by seeding cancer cells onto a porous support membrane in the commercial culture inserts used for growing cell monolayers. When submerged in culture medium for 3–7 days (depending of the number of cells seeded) they grow to 50–300 μm thick. Histologically they resemble their corresponding multicellular spheroids and tumor xenografts and have similar ECM and matrix components (laminin, collagen types I, IV). They have regions of hypoxia and central necrosis due to oxygen diffusion limitations and differentiated subpopulations of cells. MCL may be studied by similar methods to spheroids and many features in common with spheroids have been demonstrated including radiobiological resistance due to central hypoxia, necrosis and gradients of cell proliferation.

A particular advantage of MCL is the planar geometry allowing drug transport studies by separating two compartments of a diffusion apparatus, introducing drug into one (donor) compartment and measuring its appearance in the other (receiver) compartment, using compound specific analytical techniques such as high performance liquid chromatography (HPLC) and mass spectrometry (LCMS). This allows separation of parent drug from any metabolites or breakdown products which is not usually possibly with observation of fluorescence or radiolabel in histological sections. Consequently drug transport parameters (diffusion coefficients and rates of drug metabolism) may be quantified. Slow penetration has been demonstrated for several DNA binding compounds and limited penetration for tirapazamine, due to rapid drug metabolism, when incubated under anoxic conditions. A pharmacokinetic/pharmacodynamic model combining MCL transport parameters with cellular cytotoxicity is predictive of activity of tirapazamine analogs in tumor xenografts, confirming that the efficiency of penetration is a key determinant of antitumor effect in these hypoxia targeted drugs. The chemical properties which determine transport have been extensively studied demonstrating that low molecular weight, high lipophilicity and low hydrogen bonding capacity are important.

Some cell lines that do not grow well as spheroids will grow as MCL and mixed cultures are easy to establish. MCL formed from mixed cultures of cells able to activate prodrugs with cells than cannot do so have been used to demonstrate the ►bystander effect due to local diffusion of active metabolites.

Multilayered Post-Confluent Cultures (MPCC)

Post confluent cultures in V-bottomed welled plates have been developed in an effort to create a representative of solid tumor multicellular structure that is also simple enough for ►high throughput screening in new drug development. Confluence is reached in 3–5 days after which plateau phase growth and a multilayered structure develops in which there is a range of cell proliferation rates and more heterogeneous and differentiated cell population. These cultures may be used with standard growth inhibition assays using a plate reader in a semi-automated fashion. Multilayers were more resistant to the drugs than monolayers, similar to the situation with spheroids and solid tumors. This model is less amenable to studies of drug transport, but the system is easily controlled to show differences between the drugs effects under altered culture conditions such as hypoxia or in drug combination studies.

Co-Cultures of Tumor and Normal Cells

In the cancer context 3D co-cultures are generally considered growth of cancer cells together with one or more normal cell types which are known to be associated with the cancer in vivo, in an attempt allow development of a more realistic ECM and to add another level of complexity to cell-cell and cell-ECM interactions. Cancer cells have been co-cultured as spheroids together with fibroblasts, macrophages and endothelial cells but have not been as well characterized or used as extensively as cancer cell only spheroids. Co-cultures of human tumor and stromal cells such as fibroblasts or endothelial cells are also possible as MCL, ►Multilayered Post-Confluent Cultures (MPCC) or through the use of microcarrier beads even if these co-cultures will not establish as spheroids.

Histocultures

►Histocultures (Ex vivo Culture or Excipient Culture) are *ca* 1 mm^3 fragments from tumors which are maintained on a collagen matrix for up to 2 weeks. They have the three dimensional multicellular structure and cellular heterogeneity of the original tumor and microenvironmental gradients such as hypoxia. Their advantage is that they contain human stromal cells and ECM in the appropriate proportion and geometrical

arrangement, and retain the true tissue composition and architecture when harvested from patient tumors. They are also thought to contain more cancer cell heterogeneity, such as polyclonal cancer cells, than the above 3D models which are initiated in tissue culture. Histocultures have been used mainly in experimental chemotherapy to investigate the relationship between the extravascular space, cell density and drug penetration and effect. Taxane distribution studies showed that re-arrangement of stromal tissues and interstitial space induced by apoptosis has a marked effect of drug penetration. Hence the delivery of protein-bound drugs may be enhanced, in a schedule dependent manner, by pretreating to induce apoptosis and reduce cell density. Histocultures have also been used in an attempt to individualize chemotherapy for patients in the histoculture response assay.

Organotypic Cultures

In ►organotypic cultures cells are grown in an extracellular matrix which provides the appropriate mechanical as well as chemical microenvironment. They often contain stromal cells as well as cancer cells and may represent the tumor microenvironment better than spheroids due to more typical and extensive ECM. Cells are seeded into medium containing collagen, ►matrigel or synthetic matrix in approximately the concentration found in vivo. The mixture solidifies and is incubated in culture inserts fed by growth medium from above, or from below through a porous membrane of pore size *ca* 8 μm. Stromal cells may be added to the culture or medium to provide additional complexity or diffusible signals. The mechanical properties of the culture may be accurately regulated by mixing matrigel and collagen type I in particular proportions to produce tissue of appropriate stiffness.

These conditions promote growth of a more heterogeneous and differentiated tumor cell population since the ECM and stromal cells are also of human origin. Typical patterns of tumor invasion have been seen in organotypic skin cultures when melanoma cells of different invasive phenotypes were added and in low and high (E-cadherin negative) grade squamous cell carcinomas. Normal mammary tissue will differentiate into acinar structures whereas mammary tumors retain their characteristic non-acinar appearance, and drug induced differentiation typical of that seen in tumors has been observed in ovarian cancer organotypic cultures but not in monolayer culture. Organotypic models of carcinogenesis are under development. At present these cultures are more difficult to use than spheroids for drug studies and this has lead to the use of collagen implanted spheroids as a compromise. Organotypic cultures do not contain elements such as functional vascular or lymphatic systems but cell interactions such as between vascular or lymphatic endothelium and cancer cells have been investigated, mainly in the context of tumor cell migration.

Organ Cultures and Tissue Engineering

Although histocultures may be considered the tumor equivalent of ►organ cultures (often also termed organotypic cultures) the term could also refer to functional 3D cultures which are grown in bioreactors. ►Tissue engineering refers to this process of growing tissues from the start in devices that provide the correct coupling of mechanical (stress, tension), functional (e.g. fluid flow) and chemical (through the cells and ECM components) signals necessary for cells to grow, differentiate and organize into a tissue or organ structure. This is usually achieved by porous artificial scaffolds, hollow fiber matrices or culture inserts modified to allow a constant pressure gradient to provide flow of culture medium. The technology has arisen for the dual purposes of producing human transplantable tissue and organ replacements and developing in vitro models for scientific study and therapeutic testing.

Most success has been obtained with skin, mammary and liver cultures but their use in cancer research is growing. Bioartificial liver tissue has been extensively investigated with the development of cocultures of hepatocytes, stromal cells and endothelium. Using a supporting extracellular matrix such as gelatin or collagen improves cell viability and channel formation induced by the application of a pressure gradient and medium flow, results in architecture similar to hepatic sinusoids which develop similar genotype and metabolism to that in vivo. Models of cancer liver metastasis have been grown in these liver cultures which also have the potential for toxicological drug testing.

Conclusions and Future Directions

Three dimensional culture studies have become a valuable adjunct to investigations in cancer biology and therapy, since these models are of intermediate complexity between tumor cells and xenografts. The use of established systems such as spheroids and histocultures and newly developed systems such as multicellular layers and organotypic cultures is likely to increase further due to recognition that signals from the cell microenvironment are important determinants of phenotype in a quantitative and dynamic fashion. Several experts consider these studies should be mandatory in the development of new cancer therapeutics. The use of multiple realistic in vitro models may also reduce the time and expense involved in preclinical testing. Advances in tissue engineering have brought increased interest in complex 3D tissue culture models for studying the tumor microenvironment. The National Cancer Institute is encouraging these investigations through its “Understanding and defining the role of the

tumor microenvironment" program, key elements of which are training in organotypic culture and promoting better understanding of signaling networks in the stroma and of the complex composition of the tumor ECM (http://plan2006.cancer.gov/biology.shtml).

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Three-Stage Phenomenon

Definition

Vasculogenic mimicry (VM) channels, mosaic blood vessels and endothelium-dependent blood vessels participate in tumor blood supply. A three-stage phenomenon exists among them; that is all three patterns transit in tumor progression. During the early stages of transplanted melanoma, all three patterns are observed in tumor tissues, especially VM. As the tumor grows, more mosaic blood vessels and endothelium-dependent blood vessels are seen, and at the end endothelium-dependent blood vessels become the main blood supply pattern.

►Vasculogenic Mimicry

Thrombin

Definition

Factor IIa; Serine proteinase that activates proteinase-activated receptor 1. It also catalyzes many other coagulation-related reactions, including the conversion of soluble fibrinogen to insoluble fibrin. It can activate proteinase-activated receptors 1, 3 and 4.

►Proteinase-Activated Receptors
►Tumor-Endothelial Cross-talk

Thrombin Receptor

►Proteinase-Activated Receptor-1

Thrombin Receptor-like 1

►Proteinase-Activated Receptor 2

Thrombin Receptor-like 2

►Proteinase-Activated Receptor-3(PAR_3)

Thrombin Receptor-like 3

►Proteinase-Activated Receptor-4

Thrombocyte

Definition

►Platelet

Thrombocytopenia

Definition

A decrease in the number of platelets in the blood that may result in easy bruising and excessive bleeding

from wounds or bleeding in mucous membranes and other tissues.

►Rituximab

Thrombocytopenic Purpura

Definition

A systemic illness characterized by extensive ecchymosis and hemorrhages from mucous membranes, resulting from a reduction in circulating platelets resulting from various causes.

►Thrombocytopenia

Thrombopoietin

Definition

Tpo; A hematopoietic growth and differentiation factor signaling through the Mpl receptor. It promotes megakaryocyte and platelet differentiation. It also stimulates hematopoietic stem cell proliferation via Mpl activation of HOXA9.

►NUP98-HOXA9 Fusion

Thrombosis

Definition

Formation of blood clot.

►Grape Seed Extract

Thrombospondin

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Definition

Thrombospondins (►TSPs, ►THBSs) are a family of multidomain, calcium-binding extracellular glycoproteins which are synthesized, secreted, and incorporated into the extracellular matrix of a variety of normal and transformed cells of both mesenchymal and epithelial origin.

Characteristics

Classification

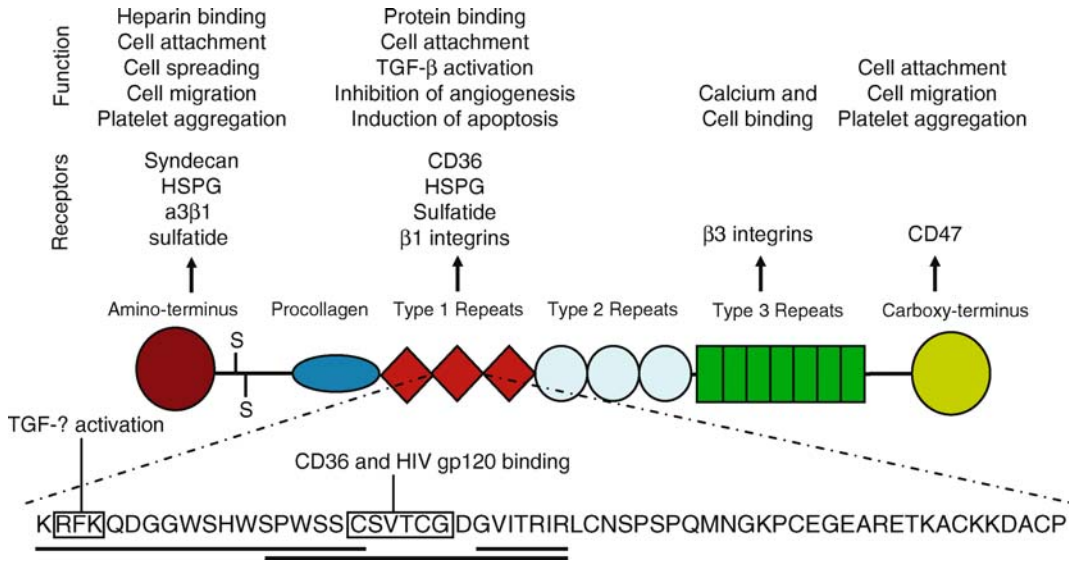
Five distinct forms of thrombospondin have been identified, TSP-1, -2, -3, -4, and -5 (cartilage oligomeric matrix protein, COMP). The TSPs fall into two subgroups, termed A and B, according to their oligomerization status and molecular architecture. Subgroup A includes TSP-1 and TSP-2, which form homotrimers. Subgroup B includes TSP-3, TSP-4 and TSP-5/COMP, which form homopentamers. All five members contain the type 2 repeats, the type 3 repeats and a highly conserved C-terminal domain. The type 2 repeats are similar to the epidermal growth factor repeats, the type 3 repeats comprise a contiguous set of calcium binding sites and the C-terminal domain is involved in cell binding. TSP-1 and -2 also contain three copies of the type 1 repeat (►TSR).

The prototypic member of the family is TSP-1, which has functions in platelet aggregation, inflammatory response and the regulation of ►angiogenesis during wound repair and tumor growth. The structure and functions of TSP-1 are summarized in Fig. 1. The amino sequence of TSR is shown, and the positions of peptides that inhibit angiogenesis are underlined] Many of these properties are shared by TSP-2. TSP-5/COMP (cartilage oligomeric matrix protein) regulates chondrocyte attachment, differentiation and cartilage ►extracellular matrix assembly. TSP-5/COMP has been found highly expressed within the tumor cells of hepatocellular carcinoma, but the pathophysiologic significance is not clear. Little is known about the biology of TSP-3 and TSP-4.

However, the clinical usage of TSP-1 is limited because TSP-1 is a large complicated molecule with numerous receptors and multiple biological functions. However, The anti-angiogenic domain of TSP-1, 3TSR, the three TSP-1 type I repeats, provides a promising alternative for clinical administration.

Previously, recombinant forms of human TSRs have been purified in our lab using a eukaryotic expression system, and showed strong anti-angiogenesis and anti-tumor efficacy in human melanoma and lung cancer xenografts. To date, the effect of TSP-1 and 3TSR on pancreatic cancer progression has not been reported.

Inhibition of angiogenesis by TSP-1 involves the inhibition of endothelial cell ►migration and induction of ►apoptosis. TSP-1 inhibits migration and induces apoptosis in microvessel endothelial cells and consequently inhibits angiogenesis. Besides its anti-angiogenesis efficacy, TSP-1 activates latent



Thrombospondin. Figure 1 Structure and functional domains of TSP-1. TSP-1 is the first naturally occurring anti-angiogenic factor described and a potent tumor inhibitor. The anti-angiogenesis and anti-tumor efficacy of TSP-1 has been well established in animal models.

►TGFβ by interacting with latency-associated peptide, and suppresses tumor cells that respond to TGFβ inhibition

TSP-1 and TSP-2 as inhibitors of tumor angiogenesis.

TSP-1 is the first naturally occurring angiogenic inhibitor to be discovered, and has been shown to play a critical role in inhibiting tumor angiogenesis, resulting in the inhibition of tumor growth and experimental ►metastasis. The suppression of angiogenesis by TSP-1 and -2 involves multiple mechanisms including direct interaction with vascular ►endothelial cell growth factor (VEGF), inhibition of ►matrix metalloproteinase 9 (MMP9) activation, inhibition of endothelial cell migration and induction of endothelial cell apoptosis.

Tumor angiogenesis is regulated by a dynamic balance between angiogenic stimulators and angiogenic inhibitors. The acquisition of an angiogenic phenotype by tumor cells involves the up-regulation of pro-angiogenic factors and the down regulation of endogenous inhibitors. Down-regulation of TSP-1 in tumor cells is a frequent step toward the acquisition of an angiogenic phenotype. In many tumors, down-regulation of TSP-1 accompanies activation of ►oncogenes or inactivation of ►tumor suppressor genes. ►Ras, ►Myc, ►Id1, ►src and ►Jun normally repress TSP-1 expression, and the increased activity of these genes shifts the balance in favor of angiogenesis. Similarly, tumor suppressor genes like ►p53, ►PTEN and ►Smad4 act to increase TSP-1 expression. Loss of their function again leads to a shift in the balance to favor the pro-angiogenic factors. Many gene alterations that lead to decreased TSP-1 expression also lead to increased expression of vascular endothelial growth factor (VEGF). For example, mutations in ras or p53 affect both sides of the angiogenic balance to facilitate the acquisition of a pro-angiogenic phenotype by tumors. In addition, tumor ►hypoxia may also inhibit TSP-1 and induce VEGF expression and thus promote the switch to an angiogenic phenotype.

During tumor progression, the host stromal response increases the level of TSPs. Stromal fibroblasts, endothelial cells and immune cells contribute significant levels of TSP-1 and -2 to the tumor microenvironment, especially at the tumor/stroma border, where TSPs serve as a matrix barrier to halt tumor angiogenesis and prevent tumor cell invasion and expansion. In some cases, tumor growth may be held in check by host TSP-1 and -2. However, genetic changes in tumor cells may eventually enable them to overcome the host defense and induce angiogenesis via further over-expressing angiogenic stimulators.

The roles of TSP-1 and -2 on the progression of spontaneous tumors have been demonstrated in TSP ►knockout mice. In the absence of TSP-1, more rapid tumor progression is observed in ►p53-deficient mice, in APC Min/+ (multiple intestinal neoplasia) mice and in mice that over-express the neu/erbB2 oncogene in mammary tissue. The survival of p53-null and p53-heterozygous mice is reduced in the absence of TSP-1. Genotyping of the tumors in the p53-heterozygotes reveals a higher level of loss-of-heterozygosity in the absence of TSP-1. These data suggest that loss of TSP-1

expression in tumor cells increases their genetic instability and hastens their progress toward life threatening malignancy. Tumor progression of skin cancers is also accelerated in TSP-2-null mice.

The importance of down-regulation of TSPs for tumor progression is further established by the fact that exogenous TSP-1 or -2 inhibits tumor growth. Multiple approaches are designed to systemically or locally increase the levels of TSP-1 or -2, including viral vector mediated or cell-based ►gene therapy, low dose chemotherapeutics and systemic delivery of recombinant proteins or synthetic peptides that include TSR sequences. The TSR domain has been identified as the antiangiogenic domain of thrombospondin, and provides a promising agent for cancer treatment. In general, TSP-based treatment slows tumor growth by inhibiting angiogenesis. Histological analysis revealed a significant decrease in tumor vessel number, vessel size, and ►tumor vessel density. Since the block of ►angiogenesis results in a decrease in tumor blood supply, tumors treated with TSP-1 show increased ►necrosis. The data presented to date indicate that TSP-based reagents are well tolerated and have very little toxicity. A TSR ►mimetic synthetic peptide derived from the GVITRIR sequence, designated ABT-510, is in phase II clinical trials for treatment of cancer. It is clear that therapeutic strategies that are designed to further increase TSP-1 and -2 levels hold great promise for the treatment of cancer. However, the efficacy of these reagents may not be sufficient to be used in single agent therapy, and they may have to be combined with other treatment modalities.

TSP-1 has been shown as an important mediator for some chemotherapeutic agents. A systemic increase in TSP-1 levels reportedly mediates the antiangiogenic and anti-tumor effect of antiangiogenic or metronomic chemotherapy, the frequent administration of low doses of chemotherapeutic drugs. The anti-tumor and anti-angiogenic efficacy of metronomic dosing with cyclophosphamide is associated with increased plasma levels of TSP-1, and is absent in TSP-1-null mice. ►Docetaxel has been reported to up-regulate TSP-1 expression levels in transformed cells. Decreased TSP-1 expression is also associated with tumor cell resistance to taxane-induced apoptosis, and treatment with TSP-1 or TSP-1 mimetic peptide sensitizes tumor cells to taxane cytotoxicity via the interaction of TSP-1 C-terminus and the CD47 receptor.

Mechanism

TSP-1 and -2 act at the interface between the cell surface and extracellular matrix to provide contextual cues that regulate matrix structure and cellular phenotype during tissue remodeling that is associated with inflammation, wound healing and neoplasia. They regulate matrix structure by directly binding to fibrilar components like collagens and fibronectin and by modulating extracellular proteases like ►MMPs and plasmin. The inhibition of angiogenesis by TSP-1 and -2 involves multiple mechanisms including direct interaction with VEGF, antagonizing VEGF-mediated survival signaling, inhibition of MMP9, inhibition of endothelial cell migration, and induction of endothelial cell apoptosis. Since the level of active MMP9 correlates with the amount of VEGF binding to VEGF receptor 2, TSP-1 inhibits VEGF mobilization and signaling by inhibiting active MMP9. In addition, TSP-1 can affect the synthesis of matrix proteins through its ability to activate ►transforming growth factor β1 (TGF-β1) via binding the latency-associated peptide (LAP) of TGF-β1. TSP-1 may also inhibit angiogenesis by decreasing the level of circulating endothelial cell progenitors.

TSP-1 exerts its antiangiogenic effects of on vascular endothelial cells via inhibiting endothelial cell migration and inducing endothelial cell apoptosis. The direct effects on endothelial cell function are reportedly mediated by the interaction of TSR with CD36. Studies with various knockout mice indicate that CD36, the ►Src family kinase, fyn, and c-Jun N-terminal kniase-1 are required for the induction of endothelial cell apoptosis by TSP-1. The CSVTCG sequence in TSR is reportedly the binding site for CD36, however, two other TSR derived peptides, WSHWSPW and GVITRIR, have also been shown to have antiangiogenic function (as shown in Fig. 1). Structural studies indicate that the TSRs fold so that the WSHWSPW and GVITRIR sequences contribute side chains to a single positively charged groove in the surface of TSR that represents the CD36 binding site. Interestingly, TSP-1 or peptides containing TSR sequences can also induce apoptosis of endothelial cells expressing little or no CD36, indicating CD36-independent mechanisms for the induction of endothelial cell apoptosis. The TSRs bind to β1 ►integrins and this interaction can inhibit endothelial cell migration. The interaction between TSRs and the complex of CD36 and β1 integrins results in decreased phosphorylation of VEGF receptor 2. These data suggest that the TSRs may engage a multi-protein complex to inhibit endothelial cell function. In the absence of CD36, β1 integrins may be sufficient to mediate the effects of TSP-1.

Caspase-3, ►Fas ligand and p38MAPK are also involved in the induction of apoptosis by TSP-1. TSP-1 up-regulates Fas ligand expression on endothelial cells, and thus promotes Fas/Fas ligand-mediated apoptosis in endothelial cells. In addition, increased expression of CD47 has also been reported to correlate with increased endothelial cell apoptosis. A peptide that contains the CD47 binding sequence of the C-terminal domain of TSP-1 has been reported to increase endothelial cell

apoptosis and inhibit angiogenesis. In addition to inducing apoptosis, TSP-1 down-regulates survival pathways. TSP-1 inhibits angiogenesis by decreasing expression of ►Bcl-2 and increasing expression of Bax.

Whereas the majority of data indicate that TSP-1 is a potent inhibitor of angiogenesis within the tumor microenvironment, TSP-1 is able to support angiogenesis in some experimental contexts. The pro-angiogenic effect seems to result from the ability of TSP-1 to stimulate recruitment and migration of smooth muscle and inflammatory cells that release pro-angiogenic factors. These divergent responses are probably due, at least in part, to differences in the repertoire of membrane receptors that are expressed by different cell types.

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Thrombotic Cancer Cell Emboli

Definition

Embolization of cancer cell clusters that are protected by a coat formed by activated platelets. This is a particularly efficient way to promote distant ►metastasis and can be induced by ►podoplanin.

Thromboxane

Definition

A group of biomolecules originally derived from prostaglandin precursors in platelets, that stimulate aggregation of platelets and constriction of blood vessels.

Thrombus

Definition

Blood clot, the final product of blood coagulation. A thrombus consists of polymerized fibrin and aggregated platelets.

►Proteinase-Activated Receptors

Thymidine Phosphorylase

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Synonyms

TP; Platelet-derived endothelial cell growth factor; PD-ECGF

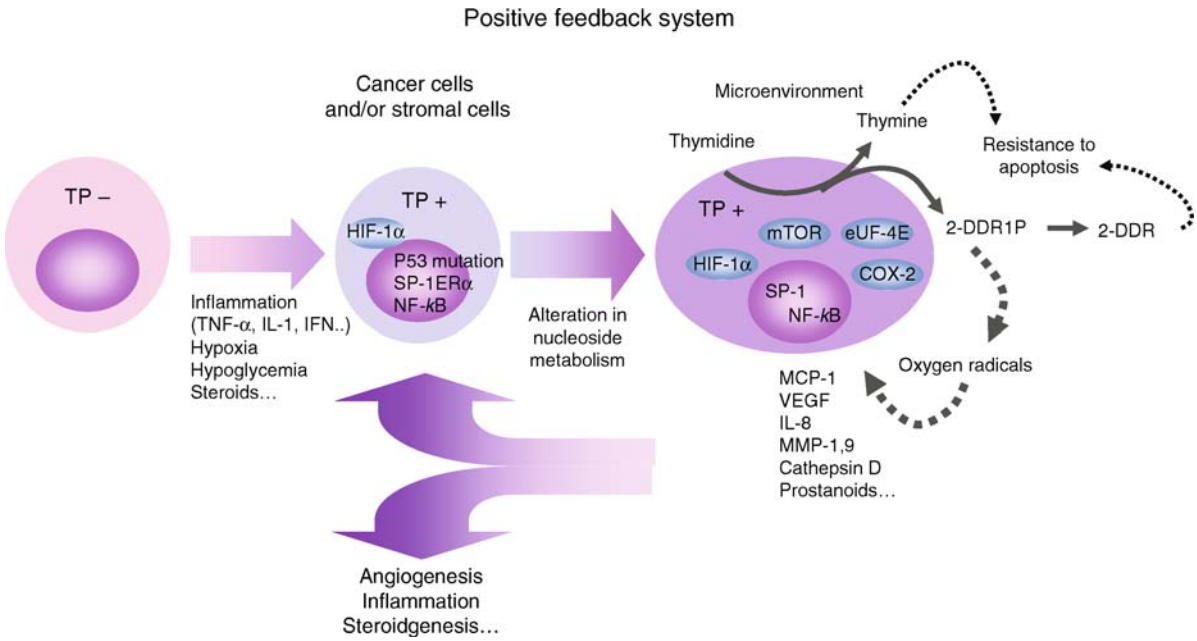
Definition

TP is a nucleoside metabolism enzyme involved in the maintenance of healthy mitochondria and balanced nucleoside triphosphate pool for DNA replication and repair. TP provides multiple cellular biological functions such as stimulation of angiogenesis and antiapoptosis.

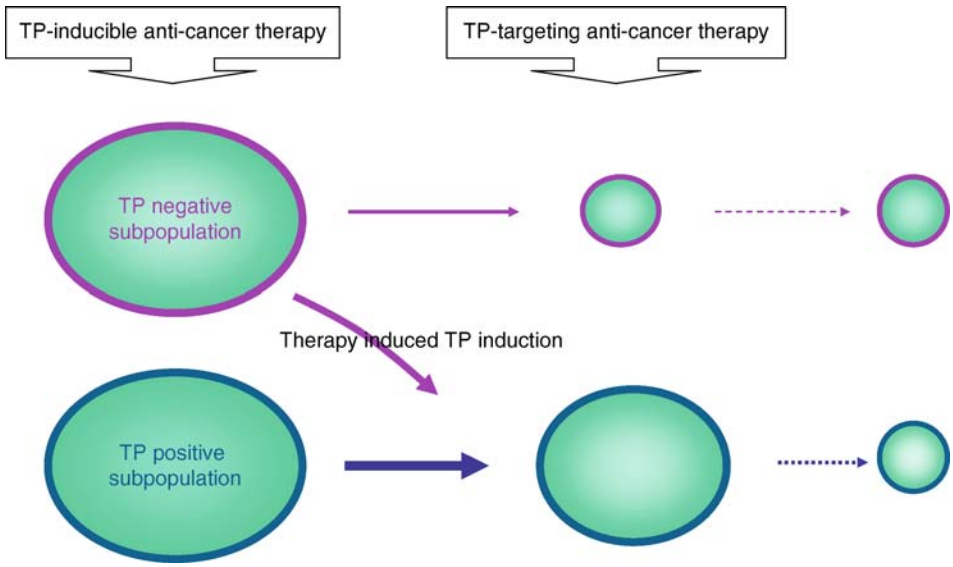
Characteristics

TP was purified in the mid-1970s from both *Escherichia coli* and *Salmonella* as a nucleoside metabolism enzyme; it is a homodimer of 45 kDa subunits. In the middle of the 1980s, a molecule exhibited angiogenic activity was extracted from human platelets and named PD-ECGF. Subsequently, it was discovered that 2-deoxy-D-ribose (2-DDR), a thymidine metabolite, stimulates chemotaxis of the endothelium and angiogenesis, then it was clarified that PD-ECGF had TP activity and these two molecules were the same. Somatic mitochondrial DNA point mutations with TP deficiencies result in mitochondrial neurogastrointestinal encephalomyopathy, with symptoms of gastrointestinal dysmotility, peripheral neuropathy, myopathy, and leukoencephalopathy, indicated that TP plays a role in maintaining mitochondria healthy. TP is induced by chemical and physiological stress such as inflammation and hypoxia. Although the transcriptional control of TP is still unclear, it is known that several cytokines such as tumor necrosis factor ►(TNF)-α, ►interleukin (IL)

►-1, and interferon ►(IFN)-δ are capable of upregulating TP expression in various types of cells including malignant cells. TP is often upregulated in human cancer tissues. Not only tumor cells but also stromal cells such as macrophages and fibroblastic cells possess TP expression. Tumors with overexpression of TP exhibit more angiogenic property as compared with those without TP expression. As to the angiogenic activity of TP, it is reported that TP and 2-DDR stimulated the formation of focal adhesions and tyrosine 397 phosphorylation of focal adhesion kinase (►FAK) and cell surface integrin-$\alpha_5\beta_1$ in human umbilical vein endothelial cells. TP-induced endothelial cell migration was inhibited by antibodies to either integrin-$\alpha_5\beta_1$ or -$\alpha_v\beta_3$, whereas ►vascular endothelial growth factor (VEGF)-induced endothelial cell migration was only blocked by the $\alpha_v\beta_3$ antibody, suggesting that TP and 2-DDR have different roles from VEGF in endothelial cell migration. In addition, a recent report showed that 2-DDR-induced endothelial cell migration and aortic ring formation were blocked completely by rapamycin, indicating that p70/S6 kinase activation is involved in 2-DDR-related endothelial stimulation. In various types of tumors including breast cancer, TP tends to coexpress with other angiogenic factors or cytokines and chemokines such as VEGF, TNF-α, interleukin (IL)-1, and ►macrophage chemoattractant protein (MCP)-1. It is hypothesized that a positive feedback mechanism may exist in tumor microenvironment promoting angiogenesis and chronic inflammation as shown in Fig. 1. In sense, TP-positive cancer stroma correlates with unfavorable prognosis, whereas TP-negative cancer stroma predicts favorable prognosis. In cancer tissues, TP is induced not only by cancer progression but also by anticancer treatments. Various types of anticancer agents such as taxanes, cyclophosphamide (CPA), and oxaliplatin (*l*-OHP) and irradiation are triggers of TP expression. An indirect pathway thorough the induction of inflammatory cytokines may be involved in the induction machinery, however, a direct pathway may also be possible because several chemotherapeutic drugs are known to activate transcriptional factors including ►Sp-1 directly. Therapy-induced TP upregulations are considered to be a prosurvival mechanism of the cells. It is evident that transfection of TP into cancer cells like KB cells renders the cells resistant to apoptosis caused by hypoxia. 2-DDR prevented hypoxia-induced apoptosis and 2-deoxy-*L*-ribose (2-DLR) abrogated the effects. TP also confers resistance to apoptosis induced by chemotherapy such as cisplatin. Therefore, TP functions as angiogenic and antiapoptotic enzyme. TP has been characterized as a therapeutic target in cancer therapy, because 5-FU and its derivatives are, more or less, TP-targeting treatments. In animal experiments using TP-transfected and TP-induced tumor xenografts, 5-FU, 5′-deoxy-5-fluorouridine (5′-DFUR) and capecitabine inhibited tumor growth potently compared with the control. Particularly, treatment with 5′-DFUR or capecitabine is more specific because their conversion to the active compound depends upon TP. Predictive factor research into the role of TP expression in cancer tissues for the clinical efficacy of 5-FU derivatives or 5-FU-containing treatments showed that these treatments were much more effective for TP-positive tumors than for



Thymidine Phosphorylase. Figure 1 A positive feedback system for thymidine phosphorylase upregulation.



Thymidine Phosphorylase. Figure 2 Many chemotherapeutic agents act as an inducer of TP. The addition pf TP-targeting therapy such as 5-FU and its derivatives to TP-inducible therapy would enhance the effect of the TP-inducible therapy by blocking protumor activity of TP-overexpressing cells. In sense, recent clinical trials confirmed that the combination of TP-inducible therapy and TP-targeting therapy achieved additive or synergistic effects on the antitumor effects and/or survival in various types of cancers like breast cancer and colorectal cancer.

TP-negative tumors. Since TP is upregulated by chemotherapy, a unique hypothesis has been raised that TP-targeting chemotherapy might enhance the activity of TP-inducible chemotherapy when it is combined (Fig. 2). Animal experiments data have shown that these combinations of TP-inducible chemotherapy such as taxanes and TP-targeting chemotherapy such as capecitabine often achieve additive or synergistic antitumor effects in various types of human cancer xenografts. Recent clinical trials confirmed that combination treatments between TP-inducible chemotherapy and capecitabine treatment provide favorable combination effects for response rates and time to progression and survival for metastatic cancer patients. For instance, a multicenter, randomized phase III study in metastatic breast cancer patients showed survival advantages by adding capecitabine to standard treatments. Other clinical trials for which the regimen contained TP-inducible chemotherapy, such as paclitaxel, *l*-OHP, *cis*-platinum, CPT-11, CPA, mitomycin C, and irradiation have been examined in the metastatic setting. Furthermore, a variety of combination treatments based upon these concepts are currently being tested for primary diseases as well. As to the possibility for TP inhibition, several agents have been tested preclinically. For example, 2-DLR, a stereoisomer of DDR, inhibited DDR's anti-apoptotic effects and suppressed the growth and metastasis of KB cells overexpressing TP transplanted into nude mice. Oral administration of DLR significantly reduced the number of metastatic nodules in the liver and suppressed angiogenesis and enhanced apoptosis in KB/TP metastatic nodules, which confirmed the importance of TP inhibition and indicated a new direction to control TP-induced protumor functions. In addition to the chemotherapy–chemotherapy combination, the combination of TP-targeting therapy and anti-VEGF therapy has been tested in clinical trials extensively. This strategy looks reasonable because TP and VEGF often coexpress in human tumor tissues. Simultaneous blockade of two different angiogenic pathways would provide some combination effects on tumor growth. The clinical trial results look promising to enhance the antitumor effect and to prolong survival.

Thymidine phosphorylase (TP) is upregulated by various stimuli such as inflammation, hypoxia, hypoglycemia, and steroids. Chemotherapeutic agents and irradiation are also capable of inducing TP. TP upregulation causes the subsequent induction of thymidine metabolites such as 2-DDR-1-phosphate and 2-DDR those are mediators to produce oxygen radicals and to stimulate endothelial chemotaxis, respectively. It is reasonably hypothesized that these reactions may continue chronically in a positive feedback system of the microenvironment.

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Thymocytes

Definition

Are lymphoid cells found in the thymus. These consist mainly of developing ►T cells, although a few thymocytes have achieved functional maturity.

Thymoma

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Definition

An epithelial neoplasm derived from the thymic epithelium.

Characteristics

Thymomas are among the most common epithelial tumors of the thymic gland. The tumor may occur at any age, but it is very unusual for this neoplasm to occur in the pediatric age group. The tumor is most likely to occur in adult individuals of over 40 years of age. The most common anatomic site is in the anterior mediastinum. However, in rare instances, thymomas may also arise in the lung, pleura, and neck. Clinically, patients with thymomas may be completely asymptomatic, and their tumors may become apparent during a routine physical examination. Radiological studies will disclose the presence of an anterior mediastinal mass. On the other hand, thymomas may be associated with other medical conditions such as Myasthenia Gravis. In some series, myasthenia gravis has been associated with thymomas as high as 30–60% of the cases. Although myasthenia gravis is often associated with thymomas, a long and wide spectrum of medical conditions has also been associated with this neoplasm, including neuromuscular, hematologic, immune deficiency, collagen vascular, dermatologic, metabolic, and endocrine processes.

Diagnosis

The two most important parameters in the evaluation of thymomas are whether the tumor is encapsulated or invasive. If the tumor is invasive, pathological staging of the tumor needs to be determined by analyzing the extent of the invasion and/or whether the tumor is limited to the mediastinal region or involves structures such as the pleura, pericardium, lung or other extrathoracic areas. Although, from the histopathological point of view, the diagnosis of thymoma may not represent a challenge, there is very limited information that can be provided in a small biopsy. The same can be said for fine needle aspirate cytology in which one cannot determine the invasion or encapsulation of the tumor. Thus, final diagnosis is best provided after complete surgical resection of the tumor. Histologically, the tumor may not pose a problem in diagnosis, and the vast majority of neoplasms will show similar histopathological features, namely the presence of epithelial cells in a background of lymphocytes, which recapitulates to some extent the normal cellular composition of the thymic gland. The presence of lymphocytes in thymomas may vary, and, in some cases, there is a predominance of lymphocytes, while in other cases the lymphocytes are more inconspicuous. Thick fibrous bands composed of fibroconnective tissue separating the cellular proliferation are usually present giving the appearance of a nodular type of growth pattern. However, the essential component that must be evaluated with more detail is the tissue that is surrounding the tumor. In the great majority of tumors, there is a thick fibroconnective boundary, which is denominated “capsule.” If the tumor is confined within the boundaries of that capsule, then the tumor is diagnosed as encapsulated; in cases in which there is a breach of the capsule by the cellular proliferation, then the tumor is diagnosed as invasive. Although as stated above, the majority of tumors will display the characteristic histology, there is a group of tumors that may show unusual characteristics such as the presence of plasma cells, muscle cells, among others. In this setting, it is very important to be entirely familiar with these neoplasms in order to avoid an equivocal diagnosis. In terms of cytologic atypia and mitotic figures, thymomas do not display marked cellular atypia or increased mitotic activity. In some cases, areas of cystic degeneration, hemorrhage, or necrosis may be present, but it is still the cellular characteristics of the tumor and the presence of capsular integrity that are more significant in predicting outcome.

Thymoma. Table 1 Histological schemes proposed for the classification of thymomas

WHO	Traditional	Muller–Hermelink	Suster and Moran
Type A	Spindle cell	Medullary	Thymoma
Type AB	—	Mixed	Thymoma
Type B1	—	Predominantly cortical	Thymoma
Type B2	Lymphocyte rich	Cortical	Thymoma
Type B2	Lymphoepithelial	Cortical	Thymoma
Type B3	Epithelial rich	Well-differentiated carcinoma	Atypical thymoma

Classification

Over the years thymomas have been the subject of numerous classification systems, which have the goal of predicting their pathologic behavior. Unfortunately, none of the current schemes appears to provide an unequivocal proof regarding the behavior that these neoplasms may follow. Essentially, there is still no cytologic feature that will determine which tumors are more likely to behave aggressively. The most important parameter in the evaluation of the tumors remains the capsular integrity. It has been demonstrated that tumors that are invasive from the outset are more likely to follow an aggressive course, while those tumors that are encapsulated, are more likely to follow an indolent course. Thus, most of the classification schemes that have been presented derived from the need to provide clues as to the clinical course of the tumor. The most common schemes on the classification of thymomas are presented in Table 1.

Treatment and Prognosis

In cases of thymomas, surgical resection is the treatment of choice. However, if the determination is that the tumor is invasive or that the tumor is not completely resected, adjuvant radiation or chemotherapy may be indicated. In cases in which the tumor is large and involves important adjacent thoracic structures, the use of chemotherapy may be necessary prior to surgical resection. The prognosis of patients with thymomas depends largely on the determination of the tumor being invasive or encapsulated. Three main features should be considered in predicting prognosis in patients with thymomas: encapsulation, invasiveness, and resectability of the tumor at the time of diagnosis.

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Thymus

Definition

Is the site of ►T-cell development, is a lymphoepithelial organ in the upper part of the middle of the chest, just behind the breastbone.

►Sjögren Syndrome

Thymus-Dependent Lymphocyte

Definition

►T cell and ►T lymphocyte are short designations for thymus-dependent lymphocyte, the lymphocyte population that fails to develop in the absence of a functioning ►thymus.

Thyroglobulin

Definition

Protein produced by the follicular epithelium of the thyroid gland widely used as a highly specific immunohistochemical marker for follicular cell derived

tumors. Abundantly used as a serum marker to monitor thyroid carcinoma of follicular cell origin.

►Hurthle Cell Adenoma and Carcinoma
►Thyroid Carcinogenesis

Thyroid Carcinogenesis

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Definition

Malignant transformation of thyroid follicle-lining epithelial cells and progression to a clinically manifest thyroid carcinoma. Thyrocytes, embryologically derived from the primitive pharynx, produce thyroid hormones and are thus involved in the regulation of metabolic pathways. Malignant transformation of these cells gives rise to various types of differentiated and undifferentiated thyroid carcinomas. In contrast, medullary carcinomas (5–15% of all thyroid carcinomas) originate from calcitonin-producing neural crest-derived C cells and form a separate entity.

Characteristics

The main groups of non-medullary epithelial thyroid carcinomas include

- Follicular carcinoma (<20% of all thyroid carcinomas), composed of closely packed follicles lined by cuboidal or columnar cells with dark-staining round nuclei and eosinophilic cytoplasm. Capsular and/or vascular invasion, missing in follicular adenoma, is crucial for diagnosis of follicular carcinoma. The tumor spreads via the blood stream, with metastasis preferentially to bone and lung, occasionally to brain and liver.
- Papillary carcinoma (>60% of all thyroid carcinomas), cytologically characterized by typical changes of nuclei with ground glass appearance, overlapping pattern, irregular shape with clefts, grooves and pseudo-inclusions. The main histological variants include
  - Typical papillary variant, classical pattern, showing arborized papillae with fibrovascular core, often containing calcified psammoma bodies
  - Follicular variant with irregularly shaped colloid-containing follicles, composed of cells typical for papillary carcinoma
  - Solid variant exhibiting closely packed cell clusters with the characteristic cytology of papillary carcinoma, separate by a thin fibrovascular stroma
  - Diffuse sclerozing variant containing large areas of fibrosis with small foci of cells typical of papillary carcinoma.

Multicentricity is common to papillary carcinoma. Lymphatic spread and metastasis formation in regional lymph nodes is frequent in all variants of papillary carcinoma.

- Undifferentiated anaplastic carcinoma (5–10%), composed of pleomorphic polygonal, round or spindle cells with mitotic figures, shows rapid growth and invasion and early metastasis to regional lymph nodes and to lungs. This type is more common in older individuals.

General Features

Thyroid carcinomas are the most common endocrine malignant tumors with a variable annual incidence in different parts of the world ranging from 5–100 per million, with females two to four times more often affected than males. The incidence of spontaneous tumors increases with age. Tumors are detected and diagnosed by palpation, ultrasound, thyroid scans and fine needle aspiration biopsy.

- Therapy involves surgery (total or near-total thyroidectomy, often combined with lymph node dissection) and, when distant metastasis of iodine-concentrating carcinomas has to be assumed, radioiodine ablation of disseminated metastatic cells. Metastases that do not take up radioiodine may be treated by surgery, external radiation or chemotherapy.
- Prognosis: Young patients have a more favorable prognosis than older persons, irrespective of the tumor type. Widely invasive follicular or undifferentiated anaplastic carcinomas have an adverse prognosis, particularly in males at older age. Anaplastic carcinomas are most aggressive with a mortality of more than 90%. In contrast, the mortality of patients suffering from papillary carcinomas is low; more than 90% have a 10-year survival. Papillary microcarcinomas may remain clinically undetected and are often found only at autopsy.
- Cases of familial thyroid carcinomas (about 3% of all thyroid carcinomas) indicate that hereditary genetic factors might play a role in thyroid carcinogenesis. However, linkage analyses did not reveal typical susceptibility genes. Instead, genetic heterogeneity in familial nonmedullary thyroid carcinomas is most probable.
- Environmental factors contribute to thyroid carcinogenesis. Iodine deficiency is a risk factor: In iodine deficient regions, particularly in mountain areas, an excess of follicular carcinomas is observed in

goiterous thyroid glands. High TSH production, because of the negative feedback regulation by a decreased thyroid hormone level due to iodine deprivation, may act as a continuous mitotic stimulus for thyrocytes and thus as a cofactor for thyroid carcinogenesis. Iodine supplementation in such areas reduces the risk of follicular carcinoma. However, high thyroid tumor incidence has been observed also in areas of high iodine (Hawaii, Iceland) where nutritional factors might interfere with iodine uptake and/or metabolism. It has been hypothesized that papillary carcinomas may be more frequent in iodine-rich areas. Upward trends of this tumor type, however, are observed in most affluent countries. The reasons are unknown.

Role of Ionizing Radiation

Ionizing radiation is an accepted risk factor for thyroid carcinogenesis. The thyroid gland is one of the most radiation sensitive organs of the body. External therapeutic radiation that had been administered in former years to head, neck or mediastinal regions of children suffering from tinea capitis, enlarged tonsils, adenoids or thymus, hemangioma or Hodgkin disease resulted, after a 10–30 years latency period, in an increased incidence of thyroid carcinomas. It was concluded from large epidemiological studies that the risk for thyroid carcinoma is significantly increased in children before an age of 15 years even after a thyroid dose as low as 0.1 Gy, with a linear dose/risk relation except for very high doses at which the cytocidal effect may be more pronounced than the transforming action. A mean excess relative risk of 7.7 per Gy thyroid dose has been calculated for children. A comparable risk has not been observed for adults after external radiation or after 131I treatment because of benign thyroid diseases or for diagnostic purpose.

Knowledge about correlations between radiation exposure and thyroid carcinogenesis has greatly been increased by studies on survivors of the atomic bomb explosions in Japan, or after the testing of nuclear weapons at Bikini with contamination of the Marshall Island, and, above all, by studies after the Chernobyl reactor accident. After the Chernobyl nuclear power plant explosion in April 1986, about 2 × 1018 Bq of radioiodine isotopes, besides other radioisotopes, were released to the environment. Children who lived in the most severely contaminated regions of Belarus, the Oblast Gomel in particular, received thyroid doses in the range of 0.15–4.7 Gy, in exceptional cases even 10 Gy and more. Thyroid doses in adults were lower by a factor of 3–5. After a latency period of about 4 years, thyroid cancer incidence increased steeply in exposed children, up to a factor of nearly 200 in the regions of largest radioactive fallout. Congruence of areas of radioiodine contamination and of elevated thyroid cancer incidence argues for radioiodine as an etiological factor.

Genetic Aberrations

Molecular studies on structural genetic aberrations did not disclose a characteristic tumor-specific pattern in sporadic thyroid carcinomas: Mutations of codons 12, 13 or 61 of H-, K- or N-►RAS are found in 20–30% of follicular adenomas and carcinomas. Obviously, a constitutive activation of the ras/raf signal transduction cascade as a consequence of these RAS mutations is an important molecular change at an early stage of follicular carcinogenesis. Connections to iodine deficiency or radiation exposure are not apparent. The prevalence of RAS mutations is lower in papillary carcinomas.

Mutations at codon 201 or 227 of the Gsa subunit of the ►GSP gene or ►TSH receptor mutations have been observed in hyperfunctioning thyroid adenomas. These mutations are almost completely missing in follicular or papillary carcinomas. Obviously, the ►TSH receptor-adenylate cyclase-protein kinase A pathway is not primarily affected in epithelial thyroid carcinomas. The prevalence of mutations of the tumor suppressor gene p53 (exons 5–8) is high in anaplastic thyroid carcinomas, but rarely found in other thyroid tumors. p53 mutational inactivation appears closely related to progression from a differentiated to an undifferentiated anaplastic, highly aggressive thyroid carcinoma.

►Loss of heterozygosity on a variety of chromosomes has been reported to be a common feature of follicular neoplasms, particularly carcinomas, suggesting loss of tumor suppressor genes during thyroid carcinogenesis. It is less frequently found in papillary carcinomas.

RET Gene Changes

Rearrangements of the ►receptor tyrosine kinase gene ►RET have been detected in about 17% of sporadic papillary thyroid carcinomas of adults, with very high geographic variation. In addition, another receptor tyrosine kinase gene, NTRK1, is rearranged in rare cases of this tumor type. In contrast to sporadic thyroid carcinomas, radiation-induced tumors of the thyroid show a more homogeneous pattern of molecular aberrations, as evident from analyses of post-Chernobyl papillary thyroid carcinomas: In more than 60%, rearrangements of the proto-oncogene RET were found. Oncogenic RET rearrangements are characterized by loss of the 5′ part of RET including exon 11 and replacement by part of a RET-fused gene. In the rearranged fusion gene, the RET tyrosine kinase domain remains intact at the 3′ end, while the RET transmembrane and extracellular domains, located upstream of the tyrosine kinase domain and essential for the stringent normal regulation of RET activity by

ligand-receptor complex interactions, are missing. They are replaced by 5′ regulatory subunits of the RET-fused genes. This leads to an unphysiological activation of the RET tyrosine kinase in thyrocytes which, under normal conditions, are devoid of this activity.

The preferential RET fusion partners in radiation-induced thyroid carcinomas are

- H4, a gene of yet unknown function, giving rise to H4/RET fusions (PTC1)
- ELE1, a transcriptional activator of the androgen receptor (ELE1/RET fusions, PTC3)

Both types of rearrangement are formed by a balanced reciprocal intrachromosomal paracentric inversion of RET at 10q11.2, ELE1 also at 10q11.2, or H4 at 10q21. Less frequently, RET rearrangements involve interchromosomal translocations, as found with the

- RIα gene, coding for the catalytic domain of cAMP-dependent protein kinase A, at 17q23, leading to RIα/RET fusions (PTC2)

or in the newly detected types of RET rearrangement with the fused genes located on chromosomes other than 10:

- GOLGA5, coding for golgin-84 located in the membrane of Golgi vesicles (GOLGA5/RET fusions, PTC5)
- HTIF1, a transcriptional activator of several nuclear receptors (HTIF1/RET fusions, PTC6)
- RFG7, a homologue of HTIF1 (RFG7/RET fusions, PTC7)
- RFG8 of a yet unknown function (RFG8/RET fusions, PTC8) on 18q21–22
- ELKS with unknown function, on 12p13.3 (ELKS/RET fusion)

It is common to all RET-fused genes detected so far that they are ubiquitously expressed and contain coiled-coil structures. Thus a unique type of fusion protein is formed in thyrocytes combining two characteristic peculiarities: the potential for dimerization and the tyrosine kinase domain. Dimerization of this fusion protein may lead to constitutive, RET-ligand-(GDNF)-independent activation of the RET tyrosine kinase, autophosphorylation and activation of signal transduction pathways not normally active in thyrocytes. The processes involved in the aberrant signal transduction which will finally end up in clonal expansion of the transformed cell(s) are not yet understood. However, the dominant thyroid tumor-inducing effect of the fusion genes has convincingly been demonstrated in H4/RET or ELE1/RET transgenic mice.

The balanced reciprocal translocation does not only cause dysregulation of RET tyrosine kinase by the RET-fused gene, but leads also to a reciprocal transcript with RET at the 5′ end and the RET-fused gene at the 3′ part of the fusion protein. The ensuing disturbance of the physiological function of the RET-fused genes may influence the mode of tumor development. For example, ELE1/RET rearrangements induce preferentially solid variants of papillary thyroid carcinomas, in contrast to H4/RET rearrangements that are related to classical papillary or follicular variants.

The relevance of RET rearrangement as a characteristic radiation effect in human thyroid tissue has recently been experimentally corroborated; radiation leads to the formation of H4/RET rearrangements in normal human thyroid tissue transplanted to ►SCID mice. Epidemiological studies revealed that patients who had undergone radiotherapy earlier in life disclosed more often RET rearrangements in papillary thyroid carcinomas than patients without radiation exposure. On the other side, epidemiological data are yet insufficient to conclude that the presence of RET rearrangement proves radiation as a causative factor in an individual papillary thyroid carcinoma.

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Thyroid Follicle

Definition

A spherical structure filled with colloid, providing storage for the thyroid-hormone precursor ►thyroglobulin, and lined with a single layer of epithelial cells, the thyrocytes, displaying round nuclei with homogeneous chromatin.

►Follicular Thyroid Tumors

Thyroid Hormone Receptor Activator Molecule 1

►Amplified in Breast Cancer 1

Thyroid Hormone Receptors

Definition

THR; Molecules that receive a thyroid hormone and permits it to dock on the nuclear membrane of a cell. Belong to a family of nuclear receptors that function as hormone-activated ►transcription factors and act by modulating the expression of genes. THRs bind DNA in the absence of hormone, usually suppressing the transcription of genes. Hormone binding involves a conformational change in the receptor that lets it to activate transcription.

There are two THR genes THR alpha and THR beta.

Thyroid Lobectomy

Definition

Surgical removal of one half of the thyroid gland.

►Hurthle Cell Adenoma and Carcinoma

Thyroid Receptor Interacting Protein 6

Definition

TRIP6; Is a member of the ►zyxin family of proteins. Synonym ZRP1.

►Lipoma Preferred Partner

Thyroidectomy

Definition

Surgical removal of all the thyroid gland.

►Hurthle Cell Adenoma and Carcinoma
►Thyroid Carcinogenesis

Tiam1

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Synonyms

T-lymphoma invasion and metastasis

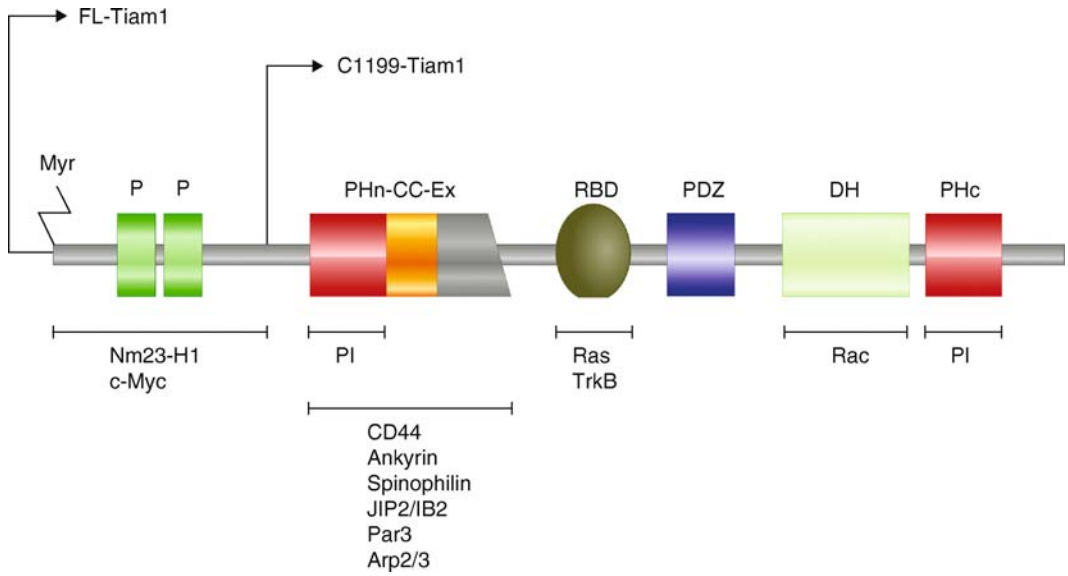
Definition

Tiam1 (T-lymphoma invasion and metastasis) has been identified as a specific activator of the Rho-like GTPase Rac (►Rho family proteins, ►GTPase) and is implicated in the regulation of different cell biological functions, including cell polarity, ►adhesion, ►migration, ►invasion, ►metastasis, and ►carcinogenesis. Tiam1 is a ubiquitously expressed protein of 1,591 amino acids and a predicted molecular mass of 177 kD. The human gene maps to chromosome 21.q22.1. Tiam2 or STEF is a Tiam1-related protein that has been identified in mammals but its expression is mostly restricted to neuronal cells. Still life (SIF) is the *Drosophila* homologue of Tiam1.

Characteristics

Tiam1 is an ubiquitously expressed protein with highest expression levels in brain, testis, and epidermis. It consists of 1,591 amino acids and harbors several distinct domains (Fig. 1). It is myristoylated at its N-terminus, contains two N-terminal PEST domains, an N-terminal pleckstrin homology domain (named PHn), a coiled-coil region with adjacent sequence (named CC-Ex), a Ras-binding domain (RBD), a PSD-95/DlgA/ZO-1 domain (PDZ) and a catalytic Dbl homology (DH)–PH (named PHc) combination. While the PHn–CC-Ex domain of Tiam1 is crucial for membrane localization of the protein, the DH–PHc combination is characteristic for all members of the Dbl-like family of guanine nucleotide exchange factors (GEFs). Accordingly, Tiam1 has been shown to exhibit specific GEF activity toward the Rho-like GTPase Rac in vivo, as reflected by the induction of Rac-mediated membrane ruffling (Fig. 2).

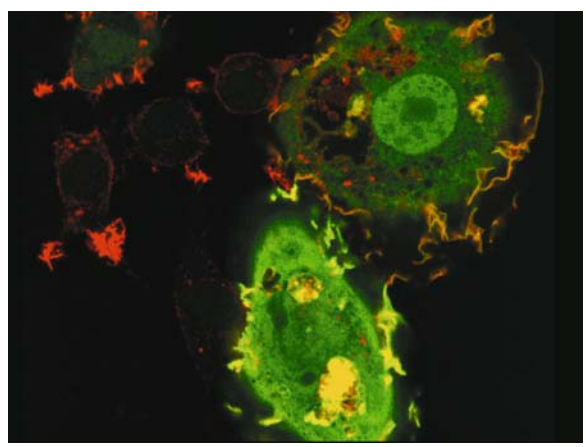
Although the function of Tiam1 as a specific activator of Rac is well-established, the specific regulation of its own activity is far from being fully understood. As was shown for other Dbl-like Rho-GEFs, Tiam1 is activated and translocated to the plasma membrane by N-terminal truncation (as in C1199-Tiam1), but the exact mechanism, underlying the N-terminal autoinhibitory effect of Tiam1 is not yet clear. In addition, Tiam1 may be activated and translocated to the plasma membrane upon stimulation of cells with lysophosphatidic acid (LPA) and ►platelet-derived growth factor (PDGF), and these effects



Tiam1. Figure 1 Domain structures of full-length (FL) Tiam1 and the active (C1199) form of the Tiam1 protein. Myr, myristoylation site; P, PEST sequence; PHn, N-terminal Pleckstrin homology domain; CC, coiled coil region; Ex, extended structure; RBD, Ras-binding domain; PDZ, PSD-95/DlgA/ZO-1 domain; DH, Dbl homology domain; PHc, C-terminal Pleckstrin homology domain. Proteins that have been reported to bind to Tiam1 are indicated below the structure. PI, phospho-inositides.

seem to be mediated by Ca^{2+}/calmodulin kinase II (CaMK-II)-induced threonine phosphorylation of Tiam1. Membrane translocation of Tiam1 is crucial for its capacity to activate Rac-mediated membrane ruffling and activation of c-Jun N-terminal kinase (►JNK subfamily and cancer). Phosphatidyl inositol (PI)3-kinase activity (►PI3K signaling) has often been implicated in the control of Tiam1-driven Rac activation, probably through its effect on membrane translocation of Tiam1. However, a PI3-kinase-independent Tiam1-Rac signaling pathway, stimulated by direct interaction of activated ►Ras with the RBD of Tiam1, has been identified as well. The role of Tiam1 in Ras-induced signaling seems to be complex. On the one hand Tiam1 is activated by active Ras and is required for Ras-induced tumorigenicity. On the other hand Tiam1 reverts Ras-induced ►epithelial-mesenchymal transition and this effect appears to be independent of the RBD of Tiam1. Thus, Tiam1 either potentiates Ras signaling through direct binding or counteracts Ras-induced effects via an independent signaling pathway.

Aside from activated Ras, a number of different cytoplasmic and membrane-associated proteins as well as distinct lipid components of cell membranes, called phospho-inositides, have been reported to bind directly to Tiam1. Of these, the hyaluronic acid receptor isoform ►$CD44_{v3}$ and its cytoskeletal binding partner ankyrin



Tiam1. Figure 2 Characteristic effects of activated Tiam1 (C1199-Tiam1) on cell morphology as determined by ►confocal laser scanning microscopy. BA-HAN-1C ►rhabdomyosarcoma cells of the rat were transiently transfected with C1199-Tiam1 cDNA. C1199-Tiam1 is shown as green fluorescence, F-actin as red fluorescence, and colocalization of both proteins is indicated by yellow fluorescence. While wild type cells (exclusively expressing a red fluorescence) are small and exhibit a round or spindle-shaped phenotype, C1199-Tiam1 transfected cells are much bigger and exhibit a pancake-like morphology as well as characteristic Rac-mediated membrane ruffles (yellow fluorescence).

provide a link between the extracellular matrix and Tiam1. The Par polarity protein Par3 as well as activated ►Rap1 couple Tiam1 to the Par polarity complex and hence to the formation of ►tight junctions and the establishment and maintenance of cell polarity. The Arp2/3 complex links Tiam1 to sites of actin polymerization such as epithelial cell–cell contacts and membrane ruffles. In addition, upon ligand binding the plasma membrane tyrosine kinase receptor TrkB binds and tyrosine-phosphorylates Tiam1, leading to Rac activation and changes in cellular morphology. Other Tiam1 binding partners such as the scaffold proteins JIP/islet brain (JIP/IB2) and spinophilin seem to determine the downstream specificity of Tiam1-induced Rac signaling. Spinophilin enhances Tiam1-induced, Rac-mediated p70 S6 kinase activity in a synergistic manner and at the expense of Tiam1-induced, Rac-mediated activation of p21-activated kinase. In contrast, JIP2/IB2 couples Tiam1-induced Rac signaling to p38 ►mitogen-activated protein kinase activity. Whereas $CD44_{v3}$, ankyrin, Par3, and the Arp2/3 complex bind to the PHn–CC-Ex domain of Tiam1, the metastasis suppressor ►Nm23-H1 and the oncogene c-Myc are suspected to bind to the N-terminus (Fig. 1). However, the biological relevance of the interaction of Nm23-H1 and c-Myc, respectively, with Tiam1 is not yet clear.

Biologically, Tiam1 is involved in the regulation of several cellular functions, including cell polarity, adhesion, migration, invasion, and metastasis. These effects, however, seem to be at least in part cell-type- and cell-substrate-specific. Originally, Tiam1 has been identified as a gene that confers an invasive and metastatic phenotype to otherwise noninvasive murine T-lymphoma cells. In contrast, Tiam1 inhibits migration and invasion of epithelial cells by promoting ►E-cadherin-mediated cell–cell adhesion and by shifting the balance between distinct invasion-promoting ►matrix metalloproteinases (MMP-2 and -9) and invasion-inhibiting ►tissue inhibitors of metalloproteinases (TIMP-1 and -2) toward the TIMPs. However, in other studies, Tiam1 promotes migration and invasion of epithelial cells, as shown for T47D, colon epithelial and Madin Darby canine kidney (MDCK) cells. These seemingly opposing effects of Tiam1 on migration and invasion of epithelial cells were reconciled at least in part by the observations that the effects of Tiam1 and Rac depend on the cell-substrate used, the fact as to whether or not the formation of E-cadherin-mediated cell–cell adhesions is prevented, the relative levels of activity of Rac and Rho, and the cell type studied. For instance, activation of Rac by Tiam1 promoted migration of MDCK cells on a collagen substrate but inhibited migration on a ►fibronectin or laminin (►Laminin signaling) substrate. Moreover, it has been shown that although Rac is required for the formation of E-cadherin-dependent cell–cell adhesions, activation of Rac may also lead to the disruption of these adhesions in a time- and concentration-dependent manner. Finally, Tiam1-induced cell–cell adhesion as well as the inhibitory effect of Tiam1 on cell ►motility was found to require Rac-mediated downregulation of Rho activity, as C1199-Tiam1 downregulated Rho activity and restoration of Rho activity in C1199-Tiam1-transfected cells resulted in a loss of cell–cell adhesion and stimulated cell motility. Nevertheless the maintenance of C1199-Tiam1-induced epithelial-like phenotype in fibroblasts requires at least a basal Rho activity. The mechanism by which Tiam1/Rac antagonizes Rho involves Rac-mediated production of reactive oxygen species that inhibit the low-molecular-weight protein tyrosine phosphatase, resulting in phosphorylation and activation of p190Rho-GAP, a negative regulator of Rho activity.

Aside from these functions in adhesion, migration and invasion, Tiam1 has also been implicated in the development of tumors. For instance, Tiam1 and its effector Rac were shown to be essential for Ras-induced transformation of fibroblasts in vitro. This is underscored by the fact that Tiam1 knock-out mice were proven to be resistant to Ras-induced skin tumors, due to increased ►apoptosis in the basal layer of the epidermis. From this one might speculate that the role of Tiam1 in tumorigenicity is restricted to its function as a mediator of oncogenic Ras signaling. This, however, is not the case, as Tiam1 was also shown to be a Wnt-responsive gene, which is required for the development of intestinal tumors, induced by aberrant ►Wnt signaling. Another mechanism through which Tiam1 may contribute to tumorigenicity is the occurrence of activating Tiam1 mutations. For example, a distinct Tiam1 mutation, located in the PHn domain, has been found in human renal cell carcinomas (►Renal carcinoma) and was shown to be sufficient to transform NIH3T3 fibroblasts in vitro. Recent studies also suggest a role for Tiam1 and Rac in the development and progression of ►prostate cancer, although the underlying mechanisms remain to be determined.

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Tight Junction

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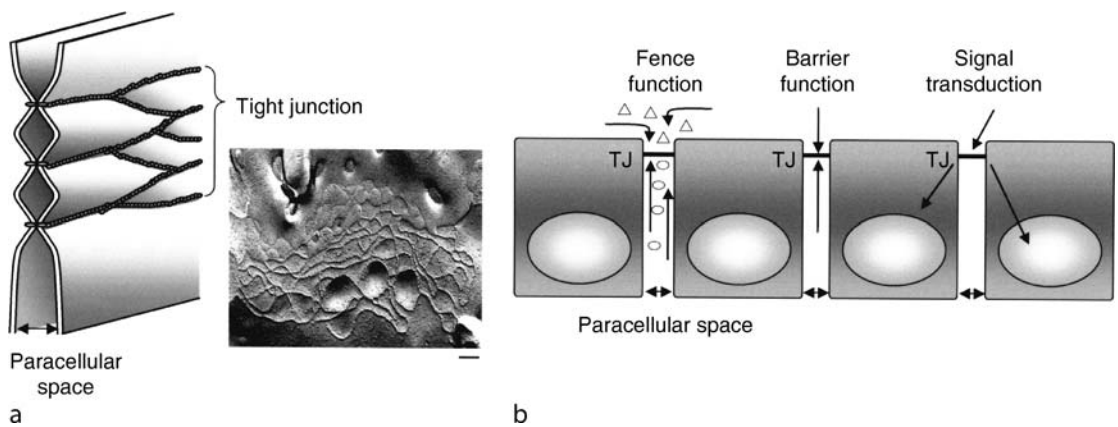
Definition

Tight junctions (TJs) are the apicalmost intercellular structures in epithelial and endothelial cells, playing central roles in the formation of ►cell polarity and functioning as major determinants of paracellular permeability. Cancer cells often exhibit loss of functional TJs, and decreased or impaired TJ formation has been reported for various types of cancer. Disruption of the TJ structure is associated with cancer development, which is causally involved in malignant phenotypes. Endothelial TJs of the host are believed to be the key apparatus to prevent blood-born ►metastasis.

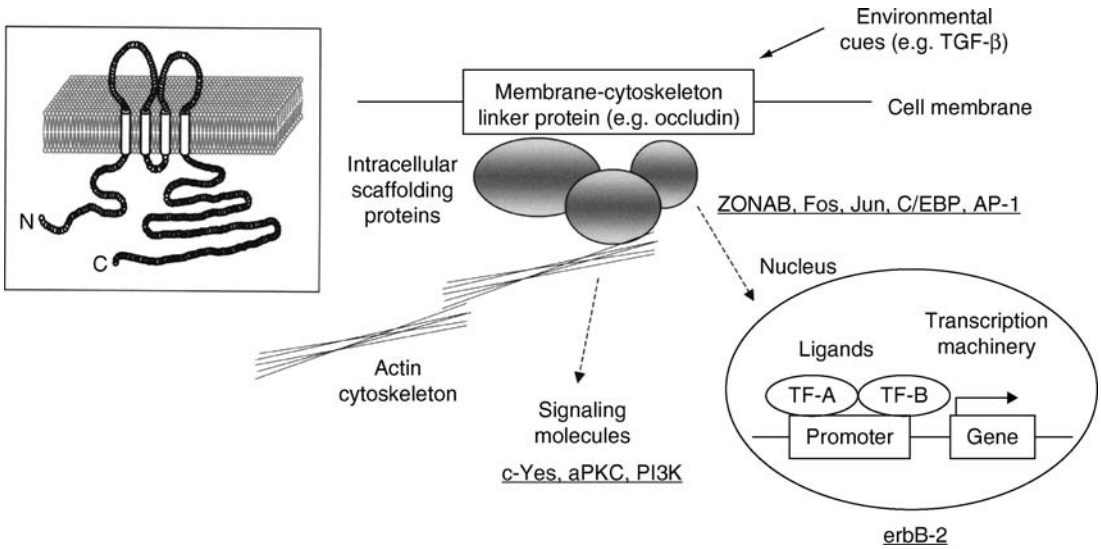
Characteristics

TJs are essential for the tight sealing of cellular sheets, thus functioning as major determinants of paracellular permeability and therefore maintaining tissue homeostasis (Fig. 1). TJs also play a crucial role in the maintenance of cell polarity by forming the fence that prevents lateral diffusion of membrane proteins and lipids, thereby creating a boundary between the apical and the basolateral plasma membrane domains. TJs are involved in the regulation of cellular functions such as proliferation, differentiation, and ►apoptosis, due to the ability of TJ proteins to recruit various types of signaling molecules that have proliferative and differentiative capacities, including ►transcription factors, ►lipid phosphatases, and ►cell cycle regulators. In addition, TJ proteins directly associate with cytoplasmic scaffolding proteins such as zonula occludens (ZO)-1, a submembrane component of TJs, eventually providing a direct linkage to the ►actin cytoskeleton. Another type of apical junctional complex in the epithelial sheet is the ►adherens junction (AJ). The AJ has been extensively studied in cancer, and the expression and/or integrity of several AJ proteins, ►catenins (α, β, and γ), and ►E-cadherin are significantly altered or deregulated in a wide variety of carcinomas. In sharp contrast to the roles of AJ components, the functional impairment of TJ proteins in cancer remains to be clarified.

One of the first identified TJ-associated molecules was occludin, which has been considered to be a requisite integral protein for TJ structure and function (Fig. 2). The long cytoplasmic domain of occludin is rich in serine, threonine, and tyrosine, and accumulating evidence has suggested that occludin is a signaling protein that has functions in receiving or transmitting signals such as atypical protein kinase C (aPKC), Rho proteins, PKC-ξ, c-Yes, and ►phosphoinositide-3-kinase (PI3K). Y-box transcription factor ZO-1-associated nucleic acid binding protein (ZONAB) has been proved to bind the SH-3 domain of ZO-1, and ZONAB and ZO-1 functionally interact in the regulation of erbB-2 promoter activity in cells, indicating that the TJ protein directly participates in the control of gene expression. Another



Tight Junction. Figure 1 Schematic representation of TJ. (a) Left panel: in this structural model of TJ, there are a number of intercrossing TJ strands (depicted as small dots) and three so-called "kissing points" of TJ. Right panel: freeze-fracture replica of a TJ. The TJ consists of an anastomosing network of strands that form irregular interstrand compartments and is comprised of a large number of protein components, including membrane proteins such as occludin and claudins, as well as cytoplasmic scaffolding proteins such as ZO-1. Scale bar, 50 nm. (b) In polarized cells, TJs are positioned at the boundary of the apical and basolateral plasma membrane domains to maintain cell polarity by forming a fence. TJs also seal cells together to generate the primary barrier and prevent diffusion of solutes through the paracellular pathway. In addition, a certain type of TJ protein such as occludin is a signaling molecule that has functions in receiving environmental cues and transmitting signals inside the cells.



Tight Junction. Figure 2 The TJ protein such as occludin is a signal transducer and transmitter for the cell. Inset, schematic presentation of the structure of occludin, with four transmembrane domains and a long cytoplasmic tail. The COOH-terminal region of occludin has many phosphorylated sites, potentially associated with a number of signal transduction pathways. Many of the cytoplasmic TJ components are signaling proteins that have functions in transmitting cellular signals such as c-Yes, aPKC, Rho proteins, PKC-ξ, and ►phosphoinositide-3-kinase (PI3K). Intracellular scaffolding proteins such as ZO-1 and ZO-2 also associate with ZONAB, Fos, Jun, C/EBP, and AP-1, and functionally interact with the regulation of transcription factors (TF-A and TF-B) such as erbB-2 promoter activity in cells, indicating that TJ proteins directly participate in the control of gene expression. In addition, occludin associates with cytoplasmic scaffolding proteins, thereby providing direct linkage to the actin cytoskeleton.

TJ protein, ZO-2, also associates with Fos, Jun, CCAAT/enhancer binding protein (C/EBP), and AP-1 (activator protein-1), suggesting that TJs have a direct functional association with signal transduction pathways. Although transforming growth factor-beta (TGF-β) is known to regulate multiple physiological functions, the second extracellular loop of occludin is a TGF-β receptor, and TGF-β regulates TJ dynamics in vitro via the p38 ►mitogen-activated protein kinase (MAPK) pathway, suggesting that this cytokine plays a crucial role in regulating the opening and closing of the blood–tissue barrier. In addition, occludin regulates TGF-β type I receptor localization for efficient TGF-β-dependent dissolution of TJs during ►epithelial–mesenchymal transitions, which is a cellular transformation crucial for the ►invasion and ►metastasis of many epithelial tumors. Consistent with this, occludin is linked with the apoptotic machinery involving MAPK and Akt signaling pathways. The ►methylator phenotype of occludin provides enhanced tumorigenic, invasive, and metastatic properties of cancer cells via modulation of unique sets of apoptosis-associated genes. It is thus clear that loss of occludin expression favors multiple steps known to be important in tumorigenesis and metastasis, identifying occludin as a likely ►tumor suppressor gene in certain types of cancer.

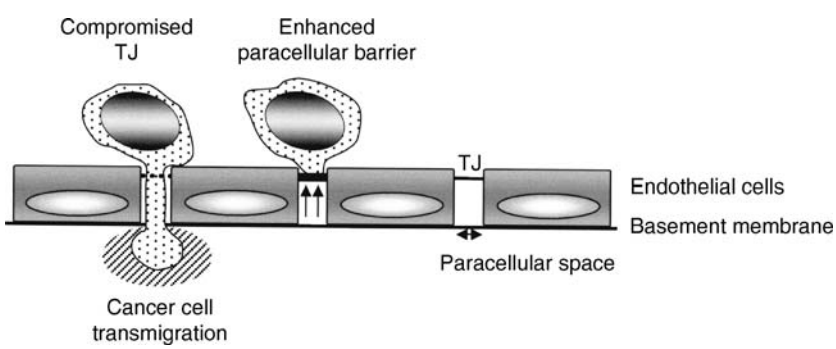
Occludin localizes to the TJ and its overexpression in a number of cell types induces the formation of TJ-like structures, supporting the idea that occludin is a key player in the formation of TJ. However, occludin expression in cells that lack endogenous TJ does not result in generation of typical TJ strands, and occludin-deficient cells and animals have fully developed well-organized TJs structurally. Thus it is now well accepted that the claudin family, which has been shown to contain at least 24 members, is the main constituent of TJs. While many members of the claudin family show a distinct organ-specific distribution pattern within the human body, the role of claudins in cancer has not been clearly defined.

Cancer cells, particularly in those tumors that manifest high metastatic potential, often exhibit loss of functional TJs. In addition, decreased and/or impaired TJ formation has been reported in various types of cancer, and genes having an oncogenic character are known to disrupt TJs. Disruption of the TJ structure is associated with cancer development, which may be causally involved in malignant phenotypes such as local tumor growth, invasion, and metastasis at distant sites. An early study in the field showed that levels of occludin and ZO-1 were often decreased during tumor development and metastasis, and loss of TJ-associated molecules such as occludin was correlated with cancer progression in ►carcinogenesis. Several claudins are regulated by ►oncogenes and tumor-promoting growth factors. In Ras-transformed Madin–Darby canine kidney cells,

claudin-1 was absent from cell–cell contact but was reassembled to the cell membrane after blocking the MAPK pathway, paralleled by TJ formation. Claudin-1 is a possible target of β-catenin/Tcf signaling, which supports a potential role for claudin-1 dysregulation in colorectal carcinogenesis. Transfection of oncogenic *raf-1* into a salivary gland epithelial cell line resulted in downregulation of claudin-1 and loss of TJ function. TGF-β, a potent modulator of invasion and metastasis in cancer cells such as those originating from the pancreas, has been demonstrated to perturb the TJ permeability and assembly of claudin-11 in Sertoli cells. In addition, overexpression of TJ-related proteins such as claudin-1 and -4 in cancer cells induces apoptosis and suppresses the invasive and metastatic potencies of these cells. Histological studies revealed the reduced expression of claudin-1 in breast and colorectal cancers, and down-regulation of claudin-7 in head and neck cancers and metastatic ductal carcinomas of the breast. These reports of decreased TJ protein expression in cancer are consistent with the well-accepted concept that carcinogenesis is accompanied by a disruption of TJs. Phosphorylation of TJ proteins, including claudins, may also affect TJ function in cancer. Typical examples are phosphorylation of claudin-1 by MAPK and PKC, and phosphorylation of claudin-5 by cyclic AMP-dependent protein kinase. Similarly, phosphorylation of claudin-3 and claudin-4 in ovarian cancer has been shown to disrupt TJs.

Aberrant claudin overexpression has been observed in various cancers. Some examples include increased expression of caludin-3 and claudin-4 in pancreatic, uterine, and ovarian cancers, high claudin-4 expression in gastric and pancreatic cancers, and upregulation of claudin-10 in hepatocellular carcinoma and thyroid papillary carcinoma. Besides the overwhelming majority of studies showing the downregulation of claudins in cancer, a number of additional reports have confirmed the paradoxical upregulation of claudins. In addition, certain types of claudins have been shown to modify tumor invasion by the regulation of ►matrix metalloproteinases (MMPs). A number of claudin family members such as claudin-1, -2, -3, and -5 are able to recruit and activate pro-MMP-2 processing mediated by membrane type 1-MMP, which suggests potential involvement in invasion and metastasis in cancer cells. The exact role of claudin overexpression remains to be examined, though the functional importance of these proteins in the development of cancer has been suggested by a number of studies. One finding reported that claudin-4 increased the invasiveness and metastatic potential of pancreatic cancer cells, and claudin-3 and claudin-4, which are highly expressed in ovarian cancer, enhanced ovarian tumorigenesis and metastasis through increased invasion and survival of tumor cells. Future studies will address the detailed molecular mechanisms by which certain types of TJ proteins determine the progressiveness of cancer cells.

Intercellular TJs primarily determine the endothelial barrier, regulating vascular permeability to maintain tightly closed circulating homeostasis. Numerous reports have confirmed the effects of cytokines such as ►vascular endothelial growth factor (VEGF) on endothelial integrity. Although many studies have focused on the strong angiogenic property of VEGF in tumor microenvironments, VEGF is known to have a pathological role in increasing vessel permeability by inducing disruption of TJ dynamics. VEGF overexpression is observed in many tumors, and causally contributes to the extravasation of fluids in the tissue surrounding VEGF-expressing cells (i.e., edema) and in a third space in the body (i.e., ascites and pleural effusion). Vascular endothelial cells also modulate cancer cell extravasation, an important metastatic process of cancer cells, which is believed to be a critical event in a potentially self-limiting step-by-step sequence, including ►migration from the primary lesion, adhesion to the vascular endothelium, extravasation, and growth at a distant site. All-*trans* ►retinoic acid (atRA)



Tight Junction. Figure 3 Enhanced paracellular barrier function prevents cancer cell extravasation. Cancer cells transmigrate through the endothelial monolayer with compromised TJs, which is believed to be an initial step in establishing a metastatic lesion. Therefore, it is feasible that enhancing endothelial integrity by decreasing paracellular permeability of TJs may limit the migration of tumor cells from the vasculature.

has been shown to determine cellular RA bioavailability and is causally an important contributing factor for determining epithelial integrity. In addition, atRA plays an important role in the formation of functional TJs through the involvement of specific RAR/RXR heterodimer-mediated transcription machinery. Based on these observations, it has been demonstrated that enhanced paracellular barrier function in rat lung endothelial cells and mesothelial cells induced by treatment with atRA can significantly suppress the invasion of cancer cells through the epithelial and endothelial monolayer sheet in vitro. It is thus feasible that epithelial and endothelial cells are the defense line of the host against cancer metastasis, controlling cancer cell transmigration by modulating the paracellular permeability of TJs (Fig. 3). This idea is likely to explain the underlying mechanism of endothelial physiology in limiting the migration of tumor cells from vascular structures.

Because of the high specificity of claudin expression patterns in cancer, claudins may represent a useful molecular marker and a prognostic indicator for many different cancers. Importantly, TJ proteins such as occludin and claudins in cancer cells may represent promising targets not only for the tumor detection and diagnosis, but also for therapy that would abrogate the progressiveness of cancer cells. It is clear that the pathology of cancer cells is critical for developing tumors and establishing metastatic lesions; however, epithelial and endothelial TJs will be good candidates for controlling cancer cell transmigration from the compartment defined by intercellular TJs, potentially having promising clinical applications to prevent cancer cell extravasation.

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Tight Junction Protein-1

►Zonula Occludens Protein-1

TIG-IPT

Definition:

Abbreviation for *I*mmunoglobulin-like fold, *P*lexins, *T*ranscription factors, or *T*ranscription factor *I*mmunoglobin) domains. They are found in transcription factors (such as early B-cell factors), cell surface receptors (such as plexins and scatter factor receptors), as well as cyclodextrin glycosyltransferase and similar enzymes. They may be involved in DNA binding and homo- or heterodimerization in some transcription factors, but they functions in other proteins are unknown.

►Early B-cell Factors

TIL

►Stem-like Cancer Cells

Time-of-Flight

Definition

Time-of-flight is a mass analyzer in which ions with a greater velocity and lighter ions flow down to hit the detector first in a field-free region. It is used to measure particle mass-to-charge ratio. An ion of known electrical charge and unknown mass enters a mass spectrometer and is accelerated by an electrical field of known strength. If all ions have the same kinetic energy, the ions with the lower mass will have a higher velocity and reach the detector earlier; whereas the ions with the higher mass will have a lower velocity and hit the detector later.

►Proteinchip
►SELDI-TOF MS

Time Resolved Fluorescence

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

Serene Josiah
Cambridge, MA, USA

Synonyms

High throughput screening; Homogenous assays; Time resolved fluorescence; Fluorescence resonance energy transfer; Homogenous time resolved fluorescence; TR-FRET

Definition

A fluorescence based assay technology used in ►high throughput screening to identify potential ►drug candidates against a variety of diseases; particularly cancer.

Characteristics

Identification of drugs for cancer treatment in today's highly technology driven research environment begins with understanding the biology of cancer and those molecules which can modify cancer cells. Early stages of the drug discovery process focus on the identification of potential drug candidates which are then further refined or excluded based on their ability to meet therapeutic efficacy and safety criteria. Thus, early stage discovery can be generally described in three key areas: (i) identification of ►target protein with causality to the disease (ii) identification of ►assays to measure the effect of putative drug candidates against the target protein or ►disease model and (iii) high throughput screening (HTS) of putative drug candidates with assays. Technology is a key driver in each of these activities. Recent advances in fluorescence based technologies have had a significant impact on the identification of potential drug candidates. Time resolved – ►fluorescence resonance energy transfer (TR-FRET) is a frequently applied technology for the identification and characterization of drug candidates.

Anti-cancer drug discovery is a multidisciplinary field combining knowledge of cancer biology, pharmacology, cell biology, medicinal chemistry and biochemistry. Research has benefited from technology advancements which have incorporated automation into data generation and sophisticated software for data analysis and management. HTS combines a biological assay with automation and specialized software, to rapidly screen through large collections (typically >100 K) of ►small molecule libraries to identify a set of compounds which can be developed into drugs. TR-FRET assay technology allows for the generation of a simple automation friendly assay that is amenable to HTS and can be readily adapted to families of target proteins implicated in disease. Members of the family of protein ►kinases have been tied to cancer and TR-FRET is a valuable technology suited to studying kinases.

The processes by which normal healthy cells are transformed into cancer cells are being elucidated. While there are several mechanisms that drive the transitions from normal to cancer cells, a common feature of all cancer cells is the loss of ability of a given cancer cell to control its growth or ►cell cycle. Regulation of cell growth is a complex process where the cell communicates with its external environment and responds with a series of carefully coordinated internal events. This process is termed ►cell signaling. Cell signaling allows a cell to respond to changes in its external environment and exert its own influence in a localized manner. Understanding and compensating for an aberrant mechanism of cell signaling in cancer cells is an underlying quest in drug discovery and development.

Cell signaling within a cell is mediated by various proteins. Typically, cell signaling proteins are either modifiers (►enzymes) of other proteins (►substrates) or subunits of larger complexes that are formed or disbanded for a particular function. For example, kinases are a family of enzymes that modify their substrates by adding a phosphate group to specific amino acid residues. In turn, the phosphorylated protein may now interact with another protein forming a complex such as a ►transcription factor that is able to enter the nucleus and initiate the ►transcription of specific genes. Thus, the activities of several proteins are coordinated together to obtain a change in the cell such as the production of new proteins. In normal cells, cell signaling is carefully regulated. In cancer cells, events in cell signaling such as the activity of kinases are misregulated leading to inappropriate activation of cellular processes, such as cell growth. A central activity in identifying new therapeutics to cancer is the identification of small molecule compounds that inhibit improper kinase mediated cell signaling.

Kinases are a large family of proteins involved in cell regulation and make up ~2% of the human genome; 518 kinases have been identified. Kinases are intracellular proteins and by modifying their substrates through ►phosphorylation they cause changes in protein localization and/or protein complex formation. Most kinases fall into two categories based on their ability to phosphorylate specific amino acid residues on their substrates; typically tyrosine and serine/threonine. Kinases also typically have high homology in their catalytic domains due to the binding of ►ATP and divalent metal ions (Mg^{2+} or Mn^{2+}) to facilitate transfer of γ-phosphate from ATP to substrate. Substrate to kinase specificity is based on cellular localization or the presence of specialized protein domains which facilitate

protein:protein binding interactions. Kinases may be their own substrates; known as ►autophosphorylation. A complex cascade of protein:protein interactions and enzymatic activity is mediated by kinases and their substrates. Phosphorylation of a protein can result in either activation of signaling (►EGFR kinase) or inhibit further signaling from occurring (►src kinase). Misregulation of kinase signaling as in the case of EGFR results in increased cell growth and ►oncogenesis. Thus, specific kinases and the larger protein family have been targeted as a point of intervention for identifying novel anti-cancer drug candidates.

Kinase activity can be inhibited by small molecules which are able to interfere with ATP binding and thus, prevent phosphorylation of substrate. Anti-cancer drug therapies have been identified which take advantage of a small molecules ability to enter cells, locate and block a target kinase. Examples are: ►Dasatinib, ►Erlotinib, ►Gefitinib, ►Imatinib, ►Lapatinib, ►Nilotinib, ►Sorafenib, ►Sunitinib, and ►Vandetanib. Gifitnib (also called Iressa) targets EGFR kinase which is misregulated due to overexpression in some cancers, including incidences of breast cancer. The inappropriate activity of EGFR kinase in cancer cells leads to the activation of the ►Ras cell signaling pathway. The Ras signaling pathway is comprised of a series of distinct kinases which are involved in either further perpetuating or shutting down the pathway. The inappropriate activation of the Ras pathway leads to changes in the cells which result in increased and misregulated cell growth. A mutation in the ATP binding site of the kinase domain of EGFR renders the cells susceptible to the effect of Iressa. The identification of novel small molecules for additional kinases implicated in cell growth misregulation has key implications for cancer therapies.

Since kinases play a key role in cancer and small molecules have been identified as kinase targeted cancer therapies, HTS has emerged as a useful approach for identifying novel anti-cancer small molecule drug candidates. A key component of a kinase based HTS is a biological assay which can be readily adapted to robotics and measure a test molecule's ability to prevent phosphorylation of substrate by the target kinase. Historically, radioactivity based assays were used to measure kinase activity. The cost of radioactive assays is prohibitive for HTS where large numbers of samples are being processed and tested. Radioactive assays have the additional disadvantage of having multiple steps which increases the challenges of automation. Radioactive assays were preferred to conventional fluorescence based measurements which were relatively insensitive due to their limited assay dynamic range. HTS assays generally have a ►homogenous assay format which is automation friendly. Homogenous assays combine the biological event and assay readout (detection) into a single measurement, minimizing the number of steps needed to perform a given measurement. Radioactive assays are often heterogeneous assay formats, where multiple steps are required to separate the desired biological signal from the total signal generated by the radioactive molecule. TR-FRET technology eliminated these hurdles to HTS by providing (i) a non radioactive assay format (ii) reasonable dynamic range of assay and (iii) homogenous assay format for ease of automation.

TR-FRET is based on ►Time Resolved Fluorescence (TRF) and Fluorescence Resonance Energy Transfer (FRET) technologies. Assay sensitivity can be a significant limitation when using conventional ►fluorophores due to the naturally fluorescent properties of many compounds and proteins. In time-resolved detection technology, there is a time delay between the ►excitation of the fluorophore and the ►emission detection of the fluorophore. When TRF detection is coupled with long lasting fluorophores, sensitivity issues can be overcome for biological assays. FRET technology enabled homogenous assay formats using TRF technology by incorporating two distinct fluorophores into the assay. The two fluorophores are defined as a donor molecule and an acceptor molecule. The excitation of the donor fluorophore causes an emission that in turn is able to excite the acceptor fluorophore and cause a specific measurable emission. The ability of the donor fluorophore to excite the acceptor fluorophore is based on the proximity of the two molecules to each other and is the basis of FRET. FRET enables homogenous assay design based on the proximity of two fluorophores which can be used to measure biologically relevant protein to protein binding interactions. Thus, TR-FRET can be applied to measuring the activity of proteins (such as kinases) and adapted for drug discovery with HTS.

TR-FRET assays for drug discovery are designed to use fluorophores specific for TRF and amenable to FRET. TRF uses specific fluorophores from the family of elements known as the ►lanthanides which have a long emission profile and a large ►Stokes shift. The Stokes shift is defined as the difference between the excitation and emission wavelengths. Lanthanides have Stokes shifts of ~300 nm which is three times greater than conventional fluorophores. The large Stokes shift enables detection of emission signal distinct from excitation energy allowing for greater dynamic range in assay measurements. Fluorescence decay of lanthanides is long-lived (microseconds) relative to conventional fluorophores (nanoseconds). This spectral property allows for measurements removing most background fluorescence noise and further improving the assay sensitivity. The lanthanides are coupled to molecules which are able to capture and transfer light to the lanthanide to generate fluorescence upon excitation. Fluorophores modified for TRF are lanthanide

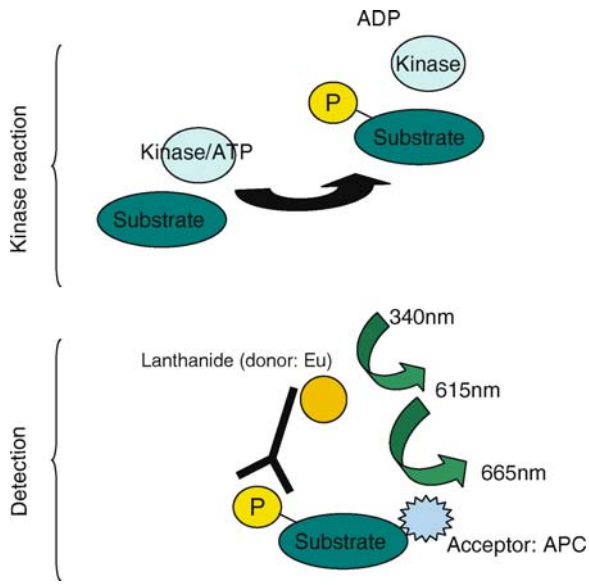
Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery. Table 1 Commonly used TR-FRET pairs for drug discovery

Lanthanide[a]	Stokes shift (nm)	Fluorescence Lifetime (μsec)	Excitation (nm)	Emission (nm)	FRET Acceptor	Excitation (nm)	Emission (nm)
Europium (Eu)	300	730	340	615	APC	615	665
Terbium (Tb)	200	1050	340	495	Fluorescein	495	520
Samarium[b] (Sm)	225	50	340	642			

[a]Variations will occur based on specific fluorophore-chelate or fluorophore-cryprate and acceptor molecules.
[b]Samarium has TRF properties and is not routinely used for TR-FRET.

►chelates or lanthanide ►cryptates. TRF fluorophores have been identified which can serve as donors for a FRET pair. A FRET pair for biological measurements is based on the distance between two fluorophores to activate the acceptor fluorophore. Typically, FRET occurs within 70–90 Å which allows for measuring interactions between proteins. TR-FRET pairs must meet the following criteria to be useful for biological measurements as in HTS; (i) emission spectra of each fluorophore must have a non-overlapping region to allow measurement of each molecules' fluorescence (ii) high energy transfer (high ►quantum yield) to enable sufficient signal detection, and (iii) fluorescence emission must fall outside of spectrum of proteins to avoid high background. Several key properties for fluorophores for TRF and the FRET pairs commonly used in kinase assay design are shown in Table 1.

TR-FRET assay format is applied towards kinase assays for the identification of small molecules which are able to inhibit the enzymatic activity of the target kinase. A sample assay design is presented in Fig. 1 which can be generally applied to kinase family members. Advances in fluorescence technology enabling TR-FRET measurements have led to the availability of a variety of assay reagents which can be applied in kinase assays. Many kinases and their specific or a generic artificial substrate are commercially available. Specific antibodies which detect the presence of a phosphorylated substrate in solution can be coupled with a member of the TR-FRET pair. The second member of the TR-FRET pair can be coupled with the substrate protein. An active kinase will phosphorylate the substrate; which is tagged with the acceptor fluorophore of the TR-FRET pair. The phosphorylated substrate becomes bound to the anti-P-antibody which is tagged with donor fluorophore. This binding event can only occur if the kinase is active on the substrate protein. The binding event causes TR-FRET by bringing the donor and acceptor fluorophores into proximity of each other enabling FRET. The TR-FRET signal measured is only generated by the phosphorylated substrate, minimizing background signal from each individual fluorophore. If a small molecule inhibits the kinase activity, FRET does not occur as there is no phosphorylation event to cause the anti-P-Ab to bind to the substrate. Test molecules can have their own fluorescent properties causing artifactual results in TR-FRET. A ratio of emission/excitation wavelengths is routinely used to minimize fluorescence changes within an assay. The general assay design shown in Fig. 1 is frequently used in HTS.



Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery. Figure 1

The specific conditions of the assay are customized for each kinase and substrate pair and each donor and acceptor TR-FRET detection pair. A commonly used TR-FRET pair is Eu^{3+} as the donor with ►allophycocyanin (APC) as the acceptor molecule (Fig. 1). Excitation of Eu-chelate or Eu-cryptate at 337 nm causes emission at 613 nm which in turn causes excitation of APC which emits at 665 nm. A ratio of 665 nm/613 nm is typically used to monitor specific assay signal.

TR-FRET technology is not limited to small molecule screening or kinases in drug discovery. Macromolecules (antibody base protein therapies, enzyme replacement therapies) are used treat an array of diseases; most frequently by targeting proteins on the surface of cells. Some examples of the use of TR-FRET technology in drug discovery beyond the kinase family are: (i) ►cytokines in the extracellular environment (ii) key intracellular molecules involved in cell signaling such as ►cAMP and (iii) ►protease family of enzymes where misregulation has serious implications in disease. TR-FRET can be applied as long as an appropriate fluorophore pair can be identified such that the proximity of the two fluorophores leads to a sufficient assay signal. In general, TR-FRET has been applied in large scale to kinases as a protein family due to the range of commercially available reagents and the direct link between kinases and their role in cellular regulation. TR-FRET for anti-cancer drug discovery by HTS has the advantage of a homogenous assay format with sufficient sensitivity to identify putative kinase inhibiting small molecules.

The high throughput nature of TR-FRET kinase assays has made it a useful assay format for characterizing the specificity of kinases. Since there is high homology between kinase domains of different kinases and small molecule drugs interfere with the ATP binding site of the kinase domain, small molecules drugs are known to inhibit kinases beyond the original target kinase. In some cases, this activity can be favorable in cancer treatment. Imatinib targets tyrosine kinase ►BCR/ABL and the tyrosine kinase ►c-kit. Another example is Sorafenib which can inhibit both Raf (serine/threonine kinase) and VEGFR(tyrosine kinase), key players in cell signaling. The selectivity profile of small molecule kinase inhibitors is emerging and could provide key information in determining which small molecules to pursue as a drug. Alternatively, selectivity profile information could determine which small molecules to abandon due to their activity as pan-kinase inhibitors or their ability to inactivate a key kinase necessary for cell viability and thus behave as a toxin.

TR-FRET is a key assay technology for identifying and characterizing anti-cancer small molecule drugs; especially against the protein kinase family which has been implicated in cellular misregulation leading to cancer. TR-FRET combines TRF and FRET technologies to provide a sensitive and high throughput assay format for characterizing the activity of small molecules against kinases. TR-FRET is an approach to rapidly screen large collections of putative drug candidates to identify a subset of molecules able to inhibit kinase activity. Application of TR-FRET extends beyond small molecule and kinase drug discovery. TR-FRET is utilized for characterizing macromolecules and a variety of protein families and signaling events. The central role of kinases in cancer and the ease of kinase assay design with TR-FRET has made TR-FRET technology a key component in today's technology rich anti-cancer drug discovery research.

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Time-to-Event Analysis

►Kaplan-Meier Survival Analysis

TIMP

►Tissue inhibitor of metalloproteinases

Tissue Engineering

Definition

Process of growing functional tissues in three dimensions using supporting structures and culture methods that stimulate appropriate growth, morphogenesis and differentiation.

►Three-Dimensional Tissue Cultures

Tissue Factor

Definition

TF; Cell surface receptor which is present in subendothelial tissue, platelets, and leukocytes necessary for the initiation of thrombin formation from its zymogen. Its best known function in blood coagulation. An integral membrane protein which is found on the outside of blood vessel cells and on monocytes during ►inflammation. Upon vessel injury, tissue factor is exposed to the blood stream and binds circulating factor VII. TF can also be produced by tumor cells, triggering their coagulation. Moreover, TF possibly mediates the ►adhesion of cancer cells to exposed subendothelial ►extracellular matrix components at permeabilized vessel sites.

►Proteinase-Activated Receptors
►Coagulopathy
►Protease Activated Receptor Family

Tissue Inhibitors of Metalloproteinases

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Synonyms

TIMPs

Definition

The tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of the ►matrix metalloproteinases (MMPs), proteases that play a central role in the degradation of extracellular matrix components in diseases such as cancer. Four TIMPs (TIMP1–4) which share high sequence homology have been identified. Tumor cells express MMPs that are important for cell ►invasion and metastasis. Because of their implication in tumor progression, TIMPs have been regarded as a potential therapeutic target in the management of cancer.

Characteristics

►Extracellular matrices (ECM) play a central role in the structure and functions of tissues. The ECM consists of a network of fibrous proteins that include collagens, ►laminins, ►fibronectin and proteoglycans that provides a unique structural scaffold for normal tissue function. Alterations in the ECM protein scaffold always develop in pathological conditions such as cancer. In fact, the tumor cells penetrating the subepithelial basement membrane define a malignant tumor. Thus, degradation and remodeling of the ECM are pivotal for tumor growth. These functions are carried out by several enzymatic-cascade systems that control the tumor ►microenvironment and its vascularization. Matrix metalloproteinases, MMPs, are among the major class of enzymes responsible for tumor-associated matrix degradation. This is supported not only by the nature of their association with tumor progression but also by the observations that in animal models MMP inhibitors are effective anti-tumor and anti-metastatic agents. MMPs are a large family of proteases with more than 20 members. Collectively, the MMP family members are capable of degrading essentially all extracellular matrix components. The expression of MMPs by the tumor cells and/or tumor stromal cells determines not only the behavior of malignant cells, but enables them to carry out pivotal functions during the metastatic process such as ►migration and invasion.

It is widely known that the endogenous inhibitors of the MMP family are the tissue inhibitors of metalloproteinases or TIMPs. These proteins regulate the proteolytic activities of the MMPs in the remodeling tissue or tumor microenvironment. TIMPs are a highly conserved, small family of proteins, with sequence homologies present through out evolution including worms, chicken, and *Drosophila*. In contrast to the large number of members in the MMP family, only four *timp* genes are present in the human genome. These genes are located in different chromosomes, *timp-1* on the chromosome X, *timp-2* in chromosome 17, *timp-3* on Chromosome 22, and *timp-4* on chromosome 3. Also, there is an association of the TIMP gene loci with the synapsin gene loci, with the exception of TIMP-2. In both human and mouse, the genes for TIMP-1, 3, and 4 are located in the introns of synapsins I, III and II respectively, but the transcriptional orientation of the two gene families is in opposite direction. TIMPs are small proteins with molecular weight of the core proteins in the range of 21 kDa; TIMP-3 and TIMP-1 are N-glycosylated, while TIMP-2 and TIMP-4 are not. However, they all contain 12 cysteine residues that must correctly pair into six disulfide bonds to give the correct secondary/tertiary structure required for MMP inhibitory activity. The selectivity of TIMPs for inhibiting MMP activity resides in the N-terminal domain. The TIMP family is also unusual in that the N-terminal amino acid is a cysteine residue. X-ray diffraction studies show that it is the amino group of this N-terminal cysteine that actually coordinates with the

Zn atom in the MMP active site, which results in inhibition of the proteolytic activity. Also, TIMP-2 selectively binds to zymogen pro-MMP-2 via the carboxyl terminal domain, and is important for the cell surface activation of pro-MMP-2 by MT-1-MMP. Interestingly, TIMPs -2 and -4 can interact with the catalytic domains in all activated MMPs, but TIMP-1 is a poor inhibitor of MT-1-MMP. TIMP family members have a distinctive pattern of tissue expression. TIMP-2 is unique in that expression of this inhibitor is constitutive in most tissues, whereas TIMP-1, TIMP-3 and TIMP-4 proteins expression is inducible, and TIMP-3 is the only member that is specifically bound to the ECM.

Given that TIMPs regulate MMPs activation, their role in tumor development has been the subject of an intense research. In experimental systems, gene knock-out either in cell lines in vitro or in vivo models reveals that the absence of some TIMPs accelerate tumor growth, as well as tumor-associated ►angiogenesis. In clinical investigations, tumor specimens consistently demonstrate alterations in the balance of MMP/TIMPs. Based on these findings, there has been an interest in these proteins as potential ►biomarkers for prognosis and diagnosis, as well as targets for cancer therapy. In response, synthetic inhibitors of MMPs were developed and tested in clinical trials, but these compounds failed to demonstrate meaningful anti-tumor effects. However, re-examination of TIMP expression in tumor specimens shows no clear pattern when analyzed as an independent variable. For instance, in an increasing number of clinical reports, TIMP-1 has been found to be overexpressed in human tumors and correlated with poor prognosis. These observations lead to the idea that the role of TIMPs in cancer is more complex than one first thought, in that although TIMPs may have MMP inhibition as a main function, these proteins can also regulate steps throughout the malignant process independent of their MMP inhibitory role. Thus, TIMP-1 has been shown as an autocrine growth factor and anti-apoptotic protein even independent of its MMP inhibitory ability. TIMP-3 can directly inhibit tumor growth in vivo by restricting angiogenesis. Recently, TIMP-2 is shown in vitro and in animal models to be an effective anti-tumor and anti-angiogenic factor. These TIMP-2 effects are also direct on cells and independent of its MMP inhibitory activity, mediated by binding to $\alpha_3\beta_1$ integrin.

Despite of their structural similarities, it is clear that TIMPs exhibit a wide spectrum of effects during the process of tumorogenesis. These conflicting TIMP effects in tumor growth are new challenges for the field of MMPs and TIMPs in cancer. Clarifying this will be important for the design of novel anti-cancer therapies. A current approach is being investigated for TIMP-2 and TIMP-3 that involves either over expression of these TIMPs by retroviral-gene transfers into tumor tissues or adenovirus systemic-gene delivery. These are being implemented either as monotherapies or as adjuvants to directly inhibit tumor growth or angiogenesis.

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Tissue Kallikreins

►Kallikreins and Cancer

Tissue Proliferation

►Regeneration

Tissue Regeneration

Definition

Is a regular maintenance cycle in which tissue cells constantly undergo remodeling and restoration.

Tissue Spectroscopy

►Fluorescence Diagnostics

Tissue Stem Cells

►Adult Stem Cells

Tissue-transglutaminase

►Transglutaminase-2

Tissue-Type Plasminogen Activator

tPA; Serine proteinase that converts plasminogen to enzymatically active plasmin, an anticoagulant.

►Proteinase-Activated Receptors
►Plasminogen-Activating System

Tissue Typing

Definition

►Histocompatibility testing.

TJP-1

►Zonula Occludens Protein-1

TKO

Definition

Technical Knockout.

TLR4

Definition

►Toll like Receptor 4 is a membrane protein and is described as a pattern recognition receptor. It co-operates with CD14 in the binding of endotoxins and transmits intra-cellular signals through MyD88 and initiates a cascade that activates nuclear factor κ-B (NFκB). This results in the production of cytokines and other biologically active molecules.

►Kupffer Cells

TMPRSS1

Definition

Hepsin.

TMPRSS10

Definition

Corin.

TMPRSS2/ERG Fusions

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Definition

TMPRSS2/ERG fusions are fusions of the promoter and 5′ portion of the androgen-regulated *TMPRSS2* gene and coding portions of the *ERG* gene that occur in the majority of prostate cancers.

Characteristics

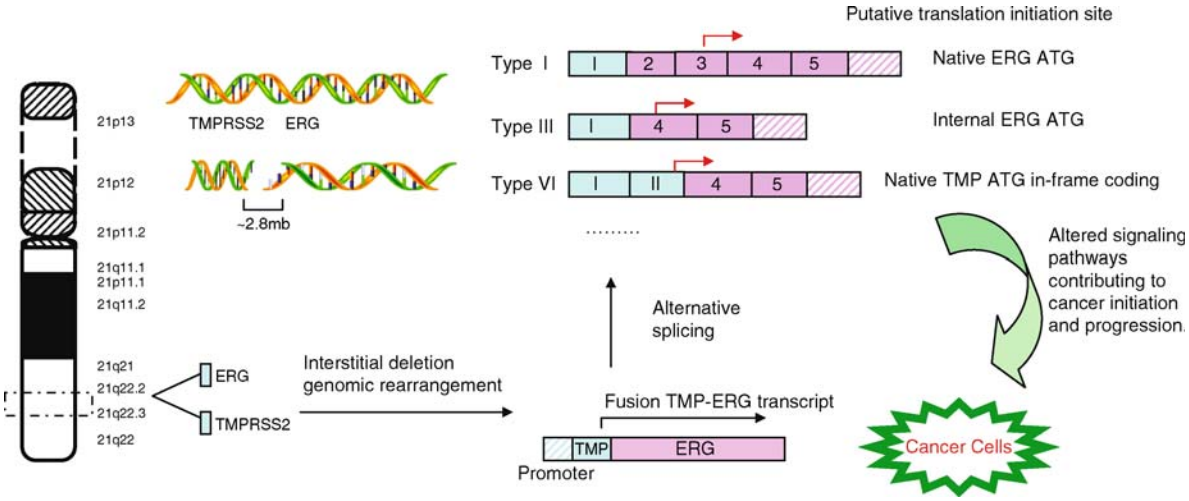
Chromosomal rearrangements resulting in gene fusions with expression of functional proteins are common in leukemias, lymphomas, and sarcomas. The presence of

such ►fusion genes is often linked to specific tumor phenotypes, for example, the ►*BCR–ABL1* fusion encoded by the Philadelphia chromosome in chronic myelogenous leukemia. In contrast, gene fusions with expression of a functional protein have generally been considered to be rare events in common epithelial malignancies such as lung, colon, and breast cancer. This idea has been brought into question by the finding of recurrent fusion of the androgen-regulated *TMPRSS2* gene to the ►ETS transcription factors, particularly the *ERG* gene, in the majority of prostate cancers (►Prostate cancer). Overall, ~50–80% of clinically detected prostate cancers contain *TMPRSS2/ERG* fusion genes, with a much smaller percentage containing fusions with genes for other ETS transcription factors such as *ETV1* and *ETV4*. The existence of a recurrent chromosomal rearrangement in prostate cancer is a paradigm shift in the study of epithelial malignancies and other epithelial tumors may have their own recurrent chromosomal rearrangements that are yet to be identified.

The *TMPRSS2/ERG* gene fusion involves fusion of the promoter region of the *TMPRSS2* gene as well as some 5′ portions of this gene with coding sequence of the *ERG* gene. The *TMPRSS2* gene encodes a transmembrane-bound serine protease that is localized to prostatic epithelium and is overexpressed in neoplastic prostate epithelium. It is well known that normal and neoplastic prostatic epithelium contain the ►androgen receptor and express numerous genes whose expression is regulated by androgens. The *TMPRSS2* promoter contains a 15 bp sequence that is homologous to the consensus androgen response element, which is in accordance with androgen-inducible expression of the gene. Experiments in cells with *TMPRSS2/ERG* fusions indicate that the androgen-responsive promoter elements of *TMPRSS2* mediate the overexpression of ETS family members in prostate cancer. Fusion proteins involving ETS transcription factors are characteristic of ►Ewing sarcoma and occur at lower rates in some leukemias. Based on this finding and the known biology of these transcription factors and fusion proteins, these genes are considered as oncogenes that promote neoplastic progression. Thus, the *TMPRSS2/ERG* gene fusion results in constitutive expression of an oncogenic transcription factor in the neoplastic prostatic epithelium. It is unknown whether decreased *TMPRSS2* expression as a result of loss of one *TMPRSS2* allele due to this rearrangement plays a role in prostate cancer initiation or progression.

The *ERG* gene is located on chromosome 21q22.2 while the *TMPRSS2* gene is ~2.8 Mbp telomeric to *ERG* at 21q22.3. Initial characterization of the mechanism by which the fusion gene arises reveals that about half of the cases there is a large deletion of the genomic DNA between these two genes, while in other cases there appears to be more complex chromosomal translocations. More detailed studies of the mechanism by which the gene fusion arises and the exact regions fused are needed. The fusion gene initiates transcription from the *TMPRSS2* promoter. All reports to date indicate that there is significant heterogeneity in the structure of the mRNA transcripts of the fusion gene, both among different cancers and within a single cancer. Thus some cancers express a single mRNA type, while others express multiple isoforms of the fusion gene. These different isoforms presumably arise via alternative splicing of the initial fusion transcript. In all cases, the fusion mRNA includes the *TMPRSS2* exon 1 and often exon 2 as well. Transcripts with *TMPRSS2* exons 3–5 have also been reported but appear to be uncommon. In some cases, the native ERG translation initiation codon in *ERG* exon 3 is maintained, fused to the untranslated *TMPRSS2* exon 1. The most common transcript contains the TMPRSS2 exon 1 fused to *ERG* exon 4, such that translation would have to arise from an internal ATG codon and give rise to a slightly truncated protein. Of particular interest is an isoform in which *TMPRSS2* exon 2 is fused with *ERG* exon 4. In this case, translation can be initiated from the TMPRSS2 translation initiation codon and results in a true fusion protein containing the first five amino acids of the TMPRSS2 protein fused to a slightly truncated ERG protein. Expression of this isoform is associated with early biochemical recurrence, characterized by detectable serum ►prostate specific antigen (PSA), following radical prostatectomy. Such early recurrence has been shown to be a hallmark of aggressive disease and is associated with cancer progression and death from disease. Thus there is significant heterogeneity in both the structure of *TMPRSS2/ERG* fusion genes and the isoforms of transcripts arising from the fusion gene (Fig. 1).

TMPRSS2/ERG fusion can be detected in high grade prostatic intraepithelial neoplasia, a noninvasive lesion that is generally believed to be the precursor of invasive prostate carcinoma, and thus is the gene fusion may be an early event in prostate cancer initiation. In all series studied to date, 50–80% of clinically detected prostate carcinomas contain the *TMPRSS2/ERG* fusion gene. These findings argue strongly that the fusion gene plays a critical role in prostate carcinogenesis but the exact biological role of the *TMPRSS2/ERG* fusion gene in prostate cancer has not been determined. Analysis of expression microarray databases reveals that ERG expression is associated with increased expression of histone deacetylase 1 (►Histone deacetylases), activation of the ►Wnt signaling pathway and downregulation of apoptotic pathways (►Apoptosis). These observations need to be validated, but indicate that the *TMPRSS2/ERG* fusion gene may have significant biological activities in vivo that promote carcinogenesis. Further studies are needed to characterize the biological



TMPRSS2/ERG Fusions. Figure 1 The TMPRSS2 and ERG genes are located at chromosome 21q22.2–22.3 and undergo interstitial deletions or other genomic rearrangements to yield a fusion gene containing the androgen-regulated TMPRSS2 promoter and 5′ portion of the TMPRSS2 gene fused to coding regions of the ERG gene in 50–80% of clinically detected prostate cancers. The transcript from the fusion gene undergoes alternative splicing to yield various isoforms in prostate cancer cells. The resulting proteins may have a variety of biological activities that promote prostate cancer initiation and progression.

activities of the *TMPRSS2/ERG* fusion gene in human prostate cancer cells.

A number of studies have examined whether the presence of the *TMPRSS2/ERG* fusion gene is associated with more aggressive clinical behavior in prostate cancer. Examination of outcome in patients treated by watchful waiting showed a strong association with death from disease. Retrospective studies have shown an association of the presence of the fusion gene with higher Gleason grade (link Gleason grading), extracapsular penetration, or biochemical recurrence in radical prostatectomy cohorts, although other study did not see these associations. Other factors may also come into play including the isoform(s) of fusion transcript expressed, the total expression level of fusion transcripts and the presence of large interstitial deletions. As described above, certain fusion transcript isoforms are associated with more aggressive disease, possible due to differences in translation initiation efficiency. Increased androgen receptor activity has been associated with prostate cancer initiation and progression and certainly the *TMPRSS2/ERG* fusion gene may be an important androgen receptor target in prostate cancer. Small studies have indicated that when cancers with similar isoforms are compared, that higher transcript expression may be associated with poorer prognosis. Finally, large interstitial deletion of the region between TMPRSS2 and ERG on chromosome 21 may inactivate potential tumor suppressor genes located between these two genes on chromosome 21. Comprehensive studies are needed to understand the impact of the *TMPRSS2/ERG* fusion on prostate cancer progression and clinical outcome.

The presence of a recurrent gene fusion in any malignancy raises the possibility of specific diagnostic tests, such as the use of the Philadelphia chromosome as specific diagnostic test for chronic myelogenous leukemia. A diagnostic test to detect the *TMPRSS2/ERG* fusion transcripts in cancer cells in blood should be specific for prostate cancer and detection of specific isoforms may have prognostic utility to assist in treatment planning. Further studies and development of appropriate technologies are clearly indicated.

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TNF

Definition

►Tumor necrosis factor.

►Neutrophil Elastase

TNF-α

Definition

►Tumor necrosis factor α; Is a potent paracrine and endocrine mediator of inflammatory and immune functions. It regulates growth and differentiation of a wide variety of cell types and acts in combination with other cytokines.

TNF-α in HIV Infection

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Definition

In the 1950s–1960s, it was reported that several patients with malignant tumors had a spontaneous regression of their tumors after bacterial infections. In the 1970s, a bacterially-induced circulating host factor was associated with this anti-tumor activity and was designated "►tumor necrosis factor" (TNF). Subsequently, TNF was isolated, cloned and found to be the leader of the "TNF superfamily" and a pleiotropic cytokine representing a major mediator of the inflammatory and immune responses. The human TNF gene is located on chromosome 6p23–6q12 between class I HLA region for HLA-B gene and the gene encoding the complement factor c; it contains four exons in a total length of 3.6 kb. Human TNF-α is a non-glycosylated protein of 157 amino acids with a molecular weight of 17 kD. A 233 amino acid precursor (26 kD) is synthesized, expressed at cell surface and cleaved by the 85 kD TNF-α converting enzyme (TACE). This TNF precursor is biologically active and considered as the "membrane TNF" form. The 17 kD peptides interact together and form circulating homotrimers. The level of circulating TNF ranges between 10 and 80 pg/mL in non-pathological conditions. TNF is secreted by various cell populations such as cells of the macrophage lineage (monocytes, macrophages, microglial cells), CD4+ and CD8+ T cells, dendritic cells, neutrophils, adipocytes, keratinocytes, astrocytes, neurons and pancreatic cells.

Two distinct membrane receptors for TNF have been identified and cloned:

- A 55 kD receptor (p55) or TNF-R1, newly designated as CD120a and referred as TNF receptor superfamily member 1A (TNF-RSF1A)
- A 75 kD receptor (p75) or TNF-R2, newly designated as CD120b and referred as TNF receptor superfamily member 1B (TNF-RSF1B)

TNF-R1 and -R2 are glycoproteins of 455 and 461 amino acids, respectively. Soluble forms of TNF-R1 (at least two molecular weights 32 and 48 kD) and -R2 (42 kD) are generated by proteolytic cleavage. Cells such as monocytes/macrophages, endothelial cells express both TNF-R1 and -R2. It was first suggested that TNF-α was bound by TNF-R2 and transferred to TNF-R1, which then is activated; in fact, TNF-R1 may be the main receptor for soluble TNF-α, whereas membrane TNF preferentially interacts with TNF-R2.

Characteristics

Physiopathological Role of TNF-α in HIV Infection

Human immunodeficiency virus (HIV) is a ►retrovirus that infects preferentially T CD4+ lymphocytes and ►macrophages, a major source of TNF-α. As a consequence, close relationships exist between HIV and TNF-α; an implication of TNF-α has been reported in HIV replication, neuroAIDS, ►cachexia and in the development of opportunistic infections and tumors.

Relationships Between HIV Replication and TNF-α

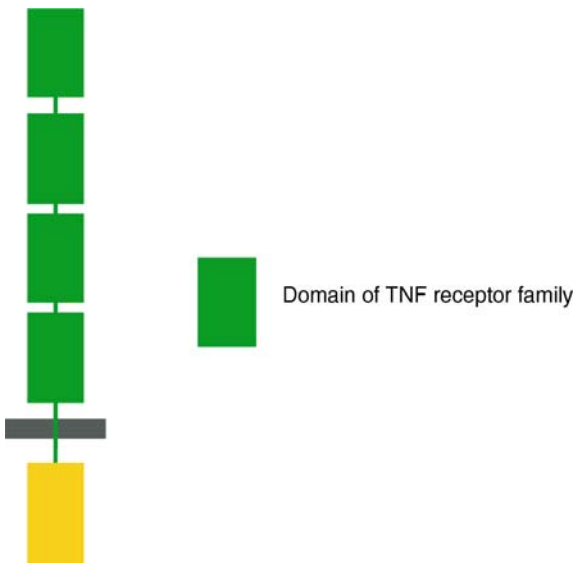
TNF-α is a pro-inflammatory cytokine and thus has been considered as a cytokine that could increase HIV replication. In fact, the effects of TNF-α on HIV replication is dual and opposite:

- Inhibition of HIV entry
- Increase of expression of proviral genome and production of HIV particles

The inhibitory effects on HIV entry is due to the capacity of TNF-α to favor the synthesis of β-chemokines e.g. regulated upon activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α and MIP-1β, the natural ligands that compete with HIV particles to bind their receptor, which is also the co-receptor of HIV. The transcription factor ►NFκB participates, in part, to the deleterious effects of TNF-α on HIV production; this transcription factor is activated by TNF-α, and interacts with domains regulating the

expression of HIV (long terminal repeat or LTR), in which two binding sites has been identified (Fig. 1).

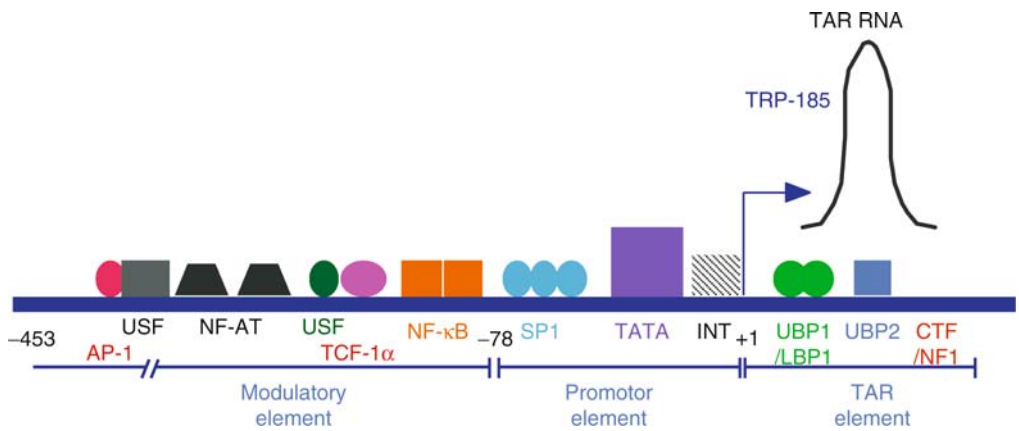
TNF-α is a cytokine that is secreted by cells of the immune system in the area of microbial infections e.g. viral infections. In HIV infection, immune cells are HIV targets, and dysregulations of TNF-α synthesis induced by the HIV infection were explored in macrophages. Several authors demonstrated that the infection of macrophages by HIV is not sufficient to induce the synthesis of TNF-α in the absence of other environmental factors. In contrast, TNF-α synthesis may be increased in the presence of soluble factors or cells; only few contaminating lymphocytes favor, for example, the macrophagic TNF-α synthesis in response of HIV infection.



TNF-α in HIV Infection. Figure 1 Structure of TNF receptor superfamily members. TNF-R1 (p55, CD120a, TNF-RSF1A), TNF-R2 (p75, CD120b, TNF-RSF1Bg).

Role of TNF-α in NeuroAIDS

TNF-α is also a neurotoxic factor that is implicated in the neuronal death in HIV disease. TNF-α can be synthesized in central nervous system (CNS), and the passage of blood brain barrier is not required for its presence in situ. The mechanisms of this neurotoxicity involve the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor and the N-methyl-D-aspartate (NMDA) receptor. TNF-α inhibits the glutamate uptake by astrocytes and favors the synthesis or release of the phospholipid mediator platelet-activating factor (PAF) which enhances excitatory transmission. Moreover, TNF-α also participates in the pathogenesis of neuroAIDS by regulating NFκB activation and HIV replication levels in perivascular macrophages and microglial cells. Indeed, viral factors such as the envelope glycoprotein gp120 and the transactivator protein ►Tat are known to be neurotoxic factors, and Tat can induce TNF-α synthesis. Both TNF receptors are present in the CNS, and on neurons in particular. The role of TNF-R2 in the CNS remains largely unknown. TNF-R1 can promote apoptosis of neurons via the release of silencer of death domains (SODD) permitting the association with intracellular proteins such as TNF receptor-associated death domain (TRADD), FAS-associated death domain (FADD) and caspase-8 (FLICE/CASP8). The activation of acid sphingomyelinase by caspase-8 contributes to the cleavage of sphingomyelin to phosphocholine and ceramide, which activates various enzymes e.g. phosphatases, protein kinases involved in apoptosis (Fig. 2). A new neurodegeneration process has recently emerged; the silencing of survival signal (SOSS). TNF-α, at picogram concentrations, inhibits the survival signaling mediated by insulin-like growth factor 1 (IGF 1) in neurons; TNF-α inhibits tyrosine phosphorylation of insulin receptor substrate 2 (IRS2) and the activation of the survival enzyme, phosphatidylinositol 3′ kinase (►PI3K) and the



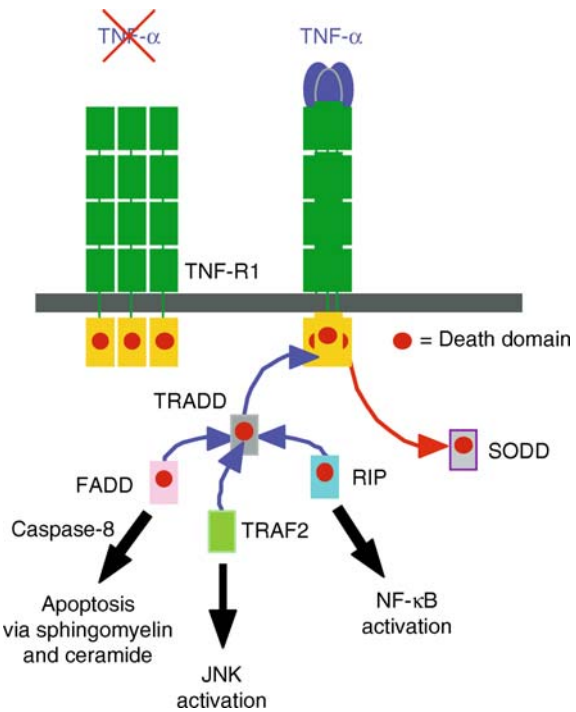
TNF-α in HIV Infection. Figure 2 Different regulatory elements in the HIV LTR domain (LTR: long terminal repeat).

formation of phosphatidylinositol(3,4,5)P3 (►luositoe lipids), known to play a pivotal role in the regulation of cell proliferation and survival.

However, as the effects of TNF-α on HIV replication, the effects of TNF-α in apoptosis may be opposite. TNF-α may be also a neuroprotective factor, for example, in excitotoxic conditions. Members of the family of TNF receptor-associated factors (►TRAF), particularly TRAF2 (Fig. 3), may play a major role in this process; it increases the TNF-α-induced ►JNK and NFκB activation known to favor cell proliferation, and a dominant-negative mutant enhances apoptosis.

Role of TNF-α in Cachexia, Opportunistic Infections and Tumors

TNF-α plays a major role in cachexia in HIV-infected patients but also in the development of opportunistic infections e.g. *Mycobacterium avium*, and tumors e.g. malignant lymphoma and ►Kaposi sarcoma. The immune activation and TNF-α synthesis induced by the Mycobacterium infection amplifies the infection of proximal cells by HIV and their level of viral replication. This viral replication accentuates the TNF-α secretion, and harmful connections between th1e replication of HIV or opportunistic pathogens, and immune activation or inflammation, are created. In Kaposi sarcoma, TNF-α favors the acquisition of the phenotype and functional features of AIDS-related KS spindle cells by endothelial cells. Moreover, TNF-α could be also responsible for deleterious effects of compounds delivered as treatment of opportunistic infections e.g. amphotericin B and cryptococcal meningitis.



TNF-α in HIV Infection. Figure 3 Different signaling pathways mediating biological effects of TNF-α. Tumor necrosis factor-a triggers the release of SODD and the DD domain of TNF-R1, that recruits TRADD via an interaction with the DD domain of this cell factor. Then, TRADD interacts with FADD, TNF-R-associated factor 2 (TRAF2) or kinase receptor-interacting protein (RIP). FADD is involved in the apoptotic signal cascade via caspase-8, TRAF2 in JNK activation, and RIP in NFκB activation.

Clinical Relevance of Molecules Inhibiting TNF-α Synthesis

In order to reduce the TNF-α -dependent HIV replication and the chronic inflammation that is associated with HIV infection, several inhibitors of TNF synthesis and TNF-receptor antagonists were evaluated in vitro or in vivo as therapeutical agent (e.g. RP-55778, pentoxifylline, chimeric humanized monoclonal antibody cA2). These molecules decreased in vitro TNF-α synthesis and HIV-1 replication. In vivo, pentoxifylline and cA2 were tested but no beneficial effects were observed on HIV viral load; only TNF-α synthesis was decreased. These data confirm that an inhibitor of TNF synthesis could not be used as pharmacological agent in monotherapy of HIV disease.

Systemic ►inflammation is decreased by highly active antiretroviral therapy (HAART); therefore, TNF-α and TNF-R2 may constitute reliable predictive markers of efficiency of HAART, or its failure. In tissues, particularly in CNS, HAART is less efficient to decrease HIV replication and chronic inflammation. As a consequence, anti-TNF-α molecules could be theoretically a good pharmacological strategy as adjuvant therapy. Nevertheless, caution is also required because it is important to preserve antimicrobial effects promoted by TNF-α against a variety of infectious agents, and particularly HIV.

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TNF-Related Apoptosis Inducing Ligand

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Synonyms

APO2 ligand; APO2L; TRAIL

Definition

TRAIL is primarily a membrane-bound cytokine that is one of several members of the ►tumor necrosis factor (TNF) gene superfamily. Members of this family induce apoptosis through engagement of cell surface ►death receptors (DRs).

Characteristics

Introduction

►Apoptosis, or programmed cell death, is a genetically conserved physiological event. Apoptosis plays a major role in the elimination of injured or unwanted cells in many physiological and pathological conditions such as normal development, defense against viral infection, dysregulated immune disease and uncontrolled cell growth. Thus, deregulated apoptosis might be related to serious human pathologies including neoplasia, degenerative disorders, and autoimmune diseases. Induction of apoptosis is an important mechanism for ►chemotherapeutic agents or ►radiation to kill cancer cells. Many endogenous cytokines also kill target cells via induction of apoptosis. Among these cytokines, several members of the TNF family have been extensively characterized: TNF-α, Fas ligand (FasL/APO1L/CD95L) and TRAIL.

TRAIL has a unique selectivity for triggering apoptosis in cancer cells, but not normal cells. In agreement with these observations, TRAIL knockout mice were more susceptible to experimental and spontaneous tumorigenesis and ►metastasis. Thus, TRAIL has been considered an important cellular factor both in natural defense mechanisms and as a potential therapeutic agent to treat human cancers.

TRAIL and Its Cell Surface Receptors

TRAIL was first identified in the mid 1990s by a homology search of an expressed sequence tag database with a highly conserved sequence motif characteristic for the TNF family members. The open reading frame for human TRAIL encodes a protein of 281 amino acids long. TRAIL is primarily expressed as a type II transmembrane protein in which the carboxyl terminus of the receptor-binding domain protrudes extracellularly. Similar to TNF-α and FasL, TRAIL can also be cleaved from the cell membrane by ►metalloproteases to yield a soluble and biologically active form. Structural studies have demonstrated that biologically active TRAIL protein forms a homotrimer. A critical cysteine residue that coordinates with a divalent zinc ion stabilizes this homotrimeric structure.

TRAIL has been shown to bind five different cell surface ►TRAIL receptor molecules: TRAIL-R1 (DR4), TRAIL-R2 (DR5/TRICK2/KILLER), TRAIL-R3 (DcR1/TRID/LIT), TRAIL-R4 (DcR2/TRUNDD), and osteoprotegrin (OPG). These receptor molecules, members of the TNF receptor (TNF-R) family, are type I transmembrane polypeptides. TRAIL-R1 and -R2 containing a cytoplasmic ►death domain (DD) that is essential for death signaling are able to transmit apoptosis-inducing activity of TRAIL across the cell membrane. By contrast, the other three receptors lack a functional death domain. Thus, they may act as ►decoy receptors, probably by competing with DR4 or DR5 for TRAIL. Unlike other TRAIL receptors, OPG is a soluble protein, originally identified to regulate osteoclastogenesis. Although OPG has been shown to interact with TRAIL, it has a much weaker affinity for TRAIL than other TRAIL receptors; therefore, it is unclear whether or not OPG can efficiently act as a decoy receptor for TRAIL under physiological conditions. Expression of TRAIL receptors closely parallels that of TRAIL, suggesting that most tissues and cell types are putative targets for TRAIL.

Organization of TRAIL-Induced Apoptotic Signaling

Biologically active trimeric TRAIL protein activates TRAIL receptors via inducing their oligomerization. Similar to other TNF family members, stimulation of the death domain-containing TRAIL receptors DR4 or DR5 recruits the cellular adaptor protein Fas-associated death domain (FADD/MORT1) through interaction of the death domains on each molecule. FADD, interacting with activated DR4 or DR5, then recruits initiator ►caspases (cysteinyl aspartases) such as procaspase-8 and/or procaspase-10, to form a ►death-induced signaling complex (DISC). Procaspase-8 or procaspase-10 molecules undergo autoproteolytic activation induced by proximity of these procaspases leading to the formation of active ►caspase-8 or caspase-10. Although procaspase-10 has been identified as a DISC component, the involvement of caspase-10 in initial death signaling activated by stimulated TRAIL receptors is controversial.

Once activated, caspase-8 initiates proapoptotic-signaling cascades leading to the cleavage of cellular factors. Active caspase-8 transmits its proapoptotic activity to executioner caspases, such as caspase-3 and caspase-7, through two main pathways. One proapoptotic signal pathway, termed the extrinsic or

mitochondria-independent pathway, directly activates executioner caspases. The other, termed the intrinsic or mitochondria-dependent pathway, involves mitochondrial events that activate the executioner caspases. The mitochondria-dependent pathway can be initiated, in part, by Bid (BH3 interacting death domain agonist), a ►Bcl-2 (B-cell lymphoma/leukemia-2) family member or through the uncontrolled production of ►reactive oxygen species (ROS). In the case of the former, caspase-8-cleaved Bid (tBid, truncated Bid) translocates to the mitochondria and induces cytochrome *c* release into the cytoplasm. The cytoplasmic cytochrome *c* binds to ►Apaf-1 (apoptotic protease activating factor-1) and participates in activation of another initiator caspase, caspase-9. This complex is referred to as the ►apoptosome. The activated caspase-9 is then able to activate executioner caspases. Thus, activation of executioner caspases represents the point at which the mitochondria-dependent and -independent proapoptotic signaling pathways, having diverged at caspase-8, meet again.

In most cell types, despite the existence of a mitochondria-independent TRAIL-induced apoptotic signal pathway, the engagement of the mitochondria-dependent pathway is required, as well, for efficient apoptosis. Mitochondrial events also include the release of SMAC (second mitochondria-derived activator of caspases)/DIABLO, apoptosis-inducing factor (AIF) and endonuclease G from the mitochondria. The release of SMAC/DIABLO from the mitochondria appears to be induced by t-Bid and occurs simultaneously with the release of other mitochondrial factors including cytochrome *c*.

Once activated, executioner caspases liberate a DNase termed CAD (caspase-activated DNase) by cleaving an inhibitor of CAD (ICAD/DFF-45). CAD activation leads to DNA fragmentation, a hallmark of apoptosis. Activation of executioner caspases also leads to the cleavage of numerous cytosolic, cytoskeletal and nuclear proteins.

Mechanisms to Attenuate TRAIL-Induced Apoptosis

Regulation of TRAIL-induced apoptosis occurs at multiple points and involves an intricate network of molecules and signals. The expression levels and subcellular localization of the factors in the TRAIL-induced ►apoptosis signaling cascade influence TRAIL signal strength and cellular susceptibility to TRAIL-induced apoptosis. Anti-apoptotic molecules and diverse extracellular cell survival signals influence TRAIL susceptibility of cells by attenuating cellular factors involved in TRAIL signaling.

Expression and mutations of the death domain-containing TRAIL receptors DR4 and DR5 can influence TRAIL susceptibility. ►DNA damaging agents, such as chemotherapeutic agents and ionizing radiation, have been shown to upregulate DR5 expression. These agents, however, also upregulate the TRAIL decoy receptors DcR1 and DcR2 that, consequently, may abrogate the augmented susceptibility due to upregulated DR4 and DR5. Since numerous DNA damaging agents have been shown to sensitize cells to TRAIL, whether or not TRAIL sensitization by such agents requires upregulation of DR5 is unclear.

Early studies suggested that expression levels of DcR1 and DcR2 might be associated with selective induction of apoptosis in tumor cells because the levels of these decoy receptors were higher in normal cells than in tumor cells. Subsequent studies, however, did not show a solid correlation between the expression levels of the decoy receptors and TRAIL susceptibility. This suggests that physiological levels of the decoy receptors might not be sufficient to inhibit TRAIL-induced apoptosis. Nevertheless, these decoy receptors may contribute to TRAIL resistance under certain physiological or pathological conditions, which may regulate the expression levels and subcellular localization of these decoy receptors.

In addition to receptor molecules, many cytosolic factors also modulate TRAIL-induced apoptosis. Caspase-8 plays a critical role in TRAIL death signaling. Accordingly, caspase-8-deficient Jurkat T cells were shown to be resistant to TRAIL-induced apoptosis. In childhood ►neuroblastoma, the gene for caspase-8 was found to be frequently silenced through ►DNA methylation and gene deletion. Furthermore, cell lines derived from these neuroblastoma tissues were resistant to TRAIL-induced apoptosis.

Cellular FLICE ((FADD-like interleukin-1β-converting enzyme)-like inhibitory peptide (c-FLIP)) is structurally related to procaspase-8 but lacks an active site for proteolytic action. This protein inhibits TRAIL-induced apoptosis by competing with procaspase-8 for FADD, preventing formation of a functional DISC. High expression levels of c-FLIP has been observed in many cancer cells that are TRAIL-resistant.

Anti-apoptotic ►Bcl-2 family members such as Bcl-2 and Bcl-X_L have been shown to inhibit apoptosis mediated by various death receptors. Overexpression of these proteins prevents the release of mitochondrial factors by interacting with proapoptotic Bax (Bcl-2-associated X protein) and Bad (Bcl-xL/Bcl-2-associated death promoter) and attenuating their apoptotic function. Overexpression of Bcl-2 and/or Bcl-X_L protects various TRAIL-sensitive cells from TRAIL-induced apoptosis. Bcl-2 and Bcl-X_L are also upregulated by many extracellular cell survival stimuli, including growth factors and ►hypoxia.

The deficiency of Bax results in a significant reduction of apoptosis induced by TRAIL. Treatment of Bax-deficient cells with TRAIL induces the formation of a functional DISC, caspase-8 activation and Bid

cleavage. Mitochondrial events, however, involving the release of mitochondrial factors, such as cytochrome *c* and SMAC/DIABLO, were impaired in Bax-deficient cells. The impaired mitochondrial events prevent post-mitochondrial proapoptotic signaling, including caspase-9 activation. Recently, a line of evidence showed that Bax requires Bak (Bcl-2 antagonist killer 1), another Bcl-2 family member, to act as a gateway for the tBid-induced release of mitochondrial factors. Cells lacking both Bak and Bax, but not cells lacking only one of these proapoptotic components, are completely resistant to tBid-induced cytochrome *c* release and apoptosis. Whether or not Bax-deficient cells express Bak remains to be determined.

Inhibitor of apoptosis protein (IAP) family members, including c-IAP1, c-IAP2, X chromosome-linked IAP (XIAP) and ►survivin, are potent inhibitors of endogenous caspases, which results in blockade of TRAIL-induced apoptosis. Specifically, IAPs exert their inhibitory activity by interacting with caspase-9, -3, and -7, but not caspase-8. SMAC/DIABLO interacts with IAP family members, antagonizing their inhibitory activity and releasing the bound IAPs from caspases. Caspases free of IAPs are more susceptible to proteolytic cleavage and activation. The protein levels of IAPs are regulated by diverse signals and mechanisms, including autoregulation by intrinsic ►ubiquitin protein ligase activity. This intrinsic E3 ligase activity has also been shown to regulate the levels of other proteins such as caspase-3 and SMAC/DIABLO. Downregulation or inactivation of IAPs induces spontaneous TRAIL-induced apoptosis.

Numerous growth factors and cytokines attenuate TRAIL-induced apoptosis. These survival signals regulate many factors involved in proapoptotic and anti-apoptotic signaling. ►Receptor tyrosine kinase (RTK)-mediated activation of the ►phosphatidylinositol-3 kinase (PI-3K)/protein kinase B (PKB/Akt) signaling axis leads to the direct phosphorylation of numerous downstream targets including proapoptotic factors Bad and caspase-9 as well as class O ►Forkhead box-binding family transcription factors (FOXOs). Phosphorylation of Bad and caspase-9 attenuates their proapoptotic activity, ablating the propagation of downstream proapoptotic signaling cascades. Phosphorylation of FOXOs prevents their translocation to the nucleus and results in the transcriptional blockade of genes implicated in promoting apoptosis and cell cycle arrest. A human ►prostate cancer cell line with constitutive activation of Akt is almost completely resistant to TRAIL, indicating that Akt plays a critical role in tumorigenesis and apoptosis.

Augmentation of TRAIL-Induced Apoptosis

Although TRAIL is a potent apoptosis inducer, some cells are resistant to TRAIL. Resistance to conventional therapies such as chemotherapy or radiation poses a major problem in cancer treatment. Subtoxic levels of chemotherapeutic agents facilitate TRAIL-induced apoptosis *in vitro* and *in vitro*, suggesting that this combination of therapy may be superior to TRAIL alone.

Actinomycin D (Act D) sensitizes many TRAIL-resistant cells to TRAIL-induced apoptosis. The combination TRAIL and Act D effectively killed TRAIL-resistant prostate cancer cell lines. In these cells, Act D downregulated XIAP. Act D also promoted TRAIL-induced apoptosis in TRAIL-resistant ►pancreatic cancer cells by decreasing the expression of c-FLIP. Etoposide increased expression of DR4 and DR5 and significantly promoted TRAIL-induced apoptosis in mammary epithelial cells. CPT-11 (Irinotecan/Camptosar®) and taxol were also observed to enhance TRAIL-induced apoptosis in cells from ►colon, ►breast, ►liver, and ►ovarian cancers. Adriamycin treatment also resulted in synergistic cytotoxicity and apoptosis for multiple myeloma that are resistant to TRAIL alone. Although the potential of a TRAIL combination therapy with chemotherapeutic agents is evident, in many cases the molecular basis of the synergistic action of chemotherapeutic agents for TRAIL is poorly understood.

Clinical Potential for TRAIL Therapy

TRAIL is a potent inducer of apoptosis in a wide variety of tumor cell lines *in vitro* and cancer xenograft animal models *in vivo*. Additionally, TRAIL did not show any detectable toxic side effects in safety tests using animals such as mice and chimpanzees.

From a clinical point of view, one of the most important issues in drug development is safety. Numerous studies have demonstrated stringent selectivity of TRAIL to tumor cells but not to normal or non-transformed cells. Recent reports, however, challenge this established apoptotic selectivity of TRAIL to tumors, demonstrating effective induction of apoptosis in cultured normal hepatocytes and neurons. These observations suggest the potential for damage to normal tissues and organs in clinical trials. Reevaluation of these results, however, reveals that the toxicity observed in cultured normal human hepatocytes is associated with the preparation method of the recombinant TRAIL protein. The non-histidine-tagged form of TRAIL versus the histidine-tagged variant is believed to be safer and more appropriate in clinical trials. Recently, an agonistic ►TRAIL receptor antibody specifically targeting DR5 was also shown to be a safe and effective cancer therapy similar to non-histidine-tagged TRAIL.

Perspectives

In the past, most TRAIL research has focused on its proapoptotic activity. Thus, the normal physiological functions of TRAIL remain poorly understood. Recent studies using TRAIL knockout mice and an animal model for the chronic blockade of TRAIL function have

shed light on the role of TRAIL in normal physiology and have demonstrated a key role for TRAIL in immune surveillance of tumor and infected cells. A better understanding of the physiological role of TRAIL will no doubt broaden the possible therapeutic applications of this molecule.

Still, little is known about TRAIL-triggered death signaling. In particular, the modulation of TRAIL death signaling, under the settings of a continuous challenge with extracellular cell survival stimuli, is poorly understood. Many of these stimuli have been shown to attenuate apoptosis. Identification of signaling pathways and cellular factors involved in cell survival will suggest mechanisms whereby potential targets can be specifically blocked, thus enhancing TRAIL activity in therapeutic applications.

Additionally, understanding the mechanisms by which chemotherapeutic agents act and sensitize cells to TRAIL-induced apoptosis will provide various new combination therapies for TRAIL. Although numerous chemotherapeutic agents have been shown to augment TRAIL-induced apoptosis *in vitro* and *in vivo*, toxicity tests of the combinations in cultured normal human cells have not been intensively investigated. Such tests would reduce the concerns raised in clinical trials and provide better combination therapies that have higher efficacy and less toxicity than individual therapies.

TRAIL has great potential to be further developed as a promising new drug for cancers and autoimmune diseases. Even though there is concern for toxic side effects, there remains great interest to determine whether clinical trials will produce similar results for human cancers as in animal models.

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Tobacco

Definition

It is the common name of *Nicotiana* species, particularly of *Nicotiana tabacum*. It can be consumed in many forms although the most common is wrapped in the form of cigarettes. It is consumed worldwide and causes many types of cancers and other diseases due to mainly its content of different carcinogens. The nicotine is the substance causing addiction.

►Tobacco-Related Cancers
►Tobacco Carcinogenesis

Tobacco Addiction

►Nicotine Addiction

Tobacco Carcinogenesis

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Definition

Refers to cancer induction by tobacco products and their constituents in laboratory animals and humans.

Characteristics

Tobacco Products and Human Cancer

Worldwide tobacco use continues to be immense and pervasive. According to estimates by the World Health Organization, there are about 1.3 billion smokers in the world and millions of smokeless tobacco users. Cigarette smoking causes well over one million cancer deaths annually worldwide, and accounts for 26% of all cancer mortality in developed countries. In addition to lung cancer, cigarette smoking causes the following types of cancer: oral cavity, pharynx, larynx, esophagus, pancreas, bladder, nasal cavity, stomach, liver, kidney, ureter, cervix, and myeloid leukemia. ►Secondhand tobacco smoke causes lung cancer in non-smokers, although the risk is less than in smokers. Smokeless tobacco products, such as moist snuff used orally, are accepted causes of oral cavity cancer and are implicated as causes of pancreatic cancer.

Tumor Induction in Laboratory Animals

Experimental studies demonstrate that cigarette smoke as well as its condensate cause cancer in laboratory animals. Inhalation studies have conclusively produced

cancer in the respiratory tracts of hamsters, rats, and mice. Cigarette smoke condensate has been extensively tested on mouse skin, where it consistently induces benign and malignant skin tumors. Smokeless tobacco has been shown to induce oral cavity cancer in rats.

Chemistry of Tobacco Smoke

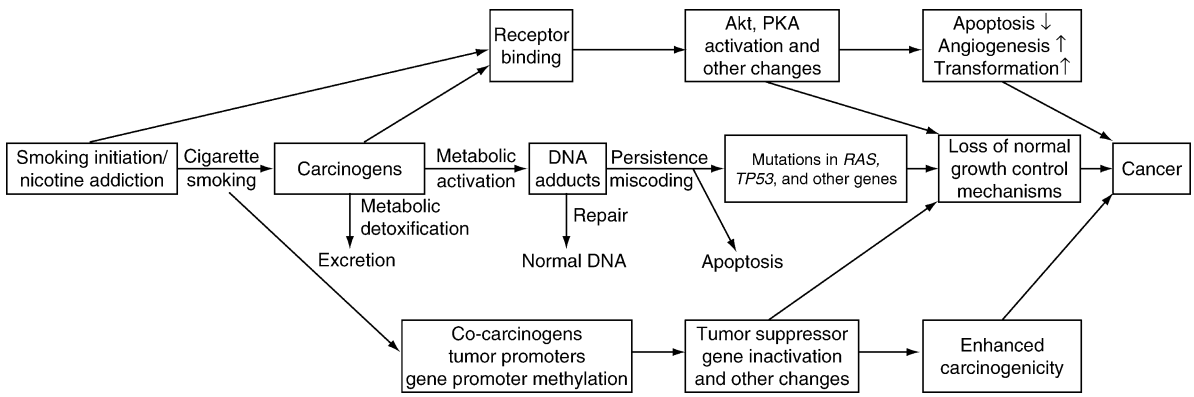
When cigarette tobacco is burned, mainstream and sidestream smoke are generated. Mainstream smoke is the material drawn from the mouth end of a cigarette during puffing. Sidestream smoke is the material released into the air from the burning tip of the cigarette plus the material which diffuses through the paper. Secondhand tobacco smoke is a composite of sidestream smoke and exhaled smoke. Mainstream smoke is an aerosol containing about 1×10^{10} particles per milliliter. About 95% of the smoke is made of gases, mainly nitrogen, oxygen, and carbon dioxide. The particulate phase of mainstream smoke contains more than 4,000 compounds. Many components are present in higher concentrations in sidestream than in mainstream smoke, but a person's exposure to sidestream smoke is far less than to mainstream smoke because of dilution with room air.

There are over 60 ►carcinogens in cigarette smoke that have been evaluated by the International Agency for Research on Cancer, and for which there is sufficient evidence for carcinogenicity in laboratory animals or humans, and 15 are rated as carcinogenic to humans. Carcinogens in cigarette smoke include ►polycyclic aromatic hydrocarbons (PAH) and their nitrogen containing analogues, ►*N*-nitrosamines, ►aromatic amines, heterocyclic aromatic amines, aldehydes, low molecular weight organic compounds such as benzene and 1,3-butadiene, and inorganic compounds. In addition, cigarette smoke contains tumor promoters, co-carcinogens, and toxicants such as acrolein and nitrogen oxides. The most important compounds with respect to human lung cancer appear to be the PAH, typified by benzo[*a*]pyrene (BaP), and the tobacco-specific *N*-nitrosamine ►4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), both considered as human carcinogens. Other compounds that may play a role as causes of lung cancer include 1,3-butadiene, benzene, aldehydes, and metals. Whereas PAH occur in all products of incomplete combustion, ►tobacco-specific *N*-nitrosamines are found only in tobacco products because they are derived from ►nicotine and related compounds. Tobacco-specific *N*-nitrosamines are the most prevalent strong carcinogens in unburned tobacco and are believed to play a significant role in the induction of oral cavity cancer by these products.

Mechanisms of Tumor Induction

Fig. 1 presents a conceptual framework for understanding mechanisms of tobacco carcinogenesis. While this scheme focuses on smokers, similar considerations apply to smokeless tobacco users. The central track of Fig. 1, involving exposure to carcinogens, the formation of covalent bonds between the carcinogens and DNA (►DNA adduct formation), and the resulting permanent mutations in critical genes of somatic cells is the major established pathway of cancer causation by cigarette smoke. While nicotine, the main known addictive agent in cigarette smoke, is not carcinogenic, each puff of each cigarette contains a mixture of carcinogens. Extensive data using specific ►biomarkers, including carcinogen metabolites in urine, DNA adducts, and protein adducts, demonstrate the uptake of these carcinogens by smokers and confirm the expected higher levels of their metabolites in urine of smokers than non-smokers.

Most cigarette smoke carcinogens require a ►metabolic activation process, generally catalyzed by ►cytochrome P450 enzymes (P450s), to convert them to forms that can covalently bind to DNA, forming DNA adducts. P450s 1A1 and 1B1, which are inducible by cigarette smoke via interactions with the aryl hydrocarbon receptor, are particularly important in the metabolic



Tobacco Carcinogenesis. Figure 1 Conceptual framework for understanding tobacco carcinogenesis.

activation of PAH. The inducibility of these P450s may be a critical aspect of cancer susceptibility in smokers. P450s 1A2, 2A13, 2E1, and 3A4 are also important in the activation of cigarette smoke carcinogens. Competing with the activation process is ►metabolic detoxification, which results in excretion of carcinogen metabolites in generally harmless forms, and is catalyzed by a variety of enzymes including ►glutathione-*S*-transferases and UDP-glucuronosyl transferases. The balance between ►carcinogen metabolic activation and detoxification varies among individuals and is likely to affect cancer susceptibility with those having higher activation and lower detoxification capacity being at highest risk. This is supported by considerable evidence from molecular epidemiologic studies of polymorphisms, or variants, in these enzymes.

The metabolic activation of carcinogens results in the formation of DNA adducts which are absolutely central to the carcinogenic process. Starting in the mid 1980s, extensive studies have demonstrated the presence of DNA adducts in human tissues. There is massive evidence, particularly from studies which use relatively non-specific adduct measurement methods, that adduct levels in the lung and other tissues are higher in smokers than in non-smokers, and some epidemiologic data link higher adduct levels with a higher probability of cancer development.

Cellular DNA repair systems remove DNA adducts and return the structure of DNA to normal. These systems include direct base repair by alkyltransferases, excision of DNA damage by base and ►nucleotide excision repair, mismatch repair, and double strand repair. If these repair enzymes are overwhelmed by DNA damage or for other reasons cannot efficiently perform their function, then adducts may persist, leading to higher probability of cancer development. There are polymorphisms in some DNA repair enzymes and the resulting deficient DNA repair can lead to a higher probability of cancer development, according to some molecular epidemiologic studies.

Persistent DNA adducts can cause miscoding during replication when DNA polymerase enzymes process them incorrectly. There is considerable specificity in the relationship between specific DNA adducts caused by cigarette smoke carcinogens and the types of mutations which they cause. G to T and G to A mutations are often found. Mutations have been frequently observed in the *KRAS* ►oncogene in lung cancer and in the *TP53* ►tumor suppressor gene in a variety of cigarette smoke-induced cancers. The cancer causing role of mutations in these genes has been firmly established in animal studies. The *KRAS* and ►*TP53* mutations observed in lung cancer in smokers reflect DNA damage by cigarette smoke carcinogens. In addition, numerous cytogenetic changes have been observed in lung cancer, and chromosome damage throughout the aerodigestive tract is strongly linked with cigarette smoke exposure. ►Apoptosis can remove cells with DNA damage, and serves as a protective counterbalance to these mutational events. The balance between mechanisms leading to apoptosis and those suppressing apoptosis will have a major impact on tumor growth.

While the central track of Fig. 1, proceeding through genetic damage, is clearly established as a major pathway by which cigarette smoke carcinogens cause cancer, epigenetic pathways also contribute, as indicated in the top and bottom tracks of Fig. 1. Nicotine and tobacco-specific nitrosamines have been shown to bind to nicotinic and other cellular receptors leading to activation of Akt (also known as protein kinase B), protein kinase A, and other changes, resulting in decreased apoptosis, increased ►angiogenesis, and increased transformation. Furthermore, the occurrence of co-carcinogens and tumor promoters in cigarette smoke is well-established. These compounds, while not carcinogenic themselves, clearly enhance the carcinogenicity of cigarette smoke carcinogens. This occurs through mechanisms which to date are poorly defined. Another important epigenetic pathway is enzymatic ►methylation of promoter regions of genes, which can result in gene silencing. If this occurs in tumor suppressor genes, the result can be unregulated proliferation.

The chronic exposure of smokers and smokeless tobacco users to the DNA damaging intermediates formed from tobacco carcinogens is consistent with our present understanding of cancer induction as a process which requires multiple genetic changes. Thus, it is completely plausible that the continual barrage of DNA damage produced by tobacco carcinogens causes the multiple genetic changes that are associated with cancer, and lead to a series of well established ►signal transduction mechanisms resulting in uncontrolled cell growth. While each dose of carcinogen from a tobacco product is small, the cumulative damage produced in years of usage is substantial.

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Tobacco Dependence

►Nicotine Addiction

Tobacco-Related Cancers

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Definition

Refers to all those cancers where ►tobacco has an etiologic role either in active or in passive form. Due to the risk for health associated with tobacco consumption and also to its widespread use, tobacco-related cancers have been sometimes studied as a whole.

Characteristics

Tobacco as a Carcinogen

In 2000, 1.47 million deaths from cancer were attributable to smoking, accounting for 32% of all smoking attributable deaths. Tobacco is estimated to cause about 1 of each 5 cancer deaths worldwide, with a higher ►attributable fraction of 29% in high-income countries and 18% in low and middle-income regions. Although the most known tobacco product is cigaret, there are other types of tobacco products, such as pipes, cigars, bidis, or betel quids, among others. The last two ones are especially frequent in India. Scientific evidence has shown that all tobacco forms cause cancer.

Tobacco smoking was declared a human carcinogen in 1986 by the International Agency for Research on Cancer (IARC). Tobacco contains more than 4,000 different chemical substances and about more than 60 have been classified as human carcinogens or probable or possible carcinogens. These substances can be classified mainly in ►polycyclic aromatic hydrocarbons (PAHs) and ►tobacco-specific nitrosamines. The most characteristic substance of the first group is benzo[a]pyrene and of the second is 4-(methylnitrosamino)-1-(3-pyrydil)-1-(butanone), also known as NNK. ►Nicotine, which accounts for 0.5–4% by weight of tobacco leaves, is readily absorbed after tobacco use and is responsible for the addiction. Around the world, there are ~1.1 billions of smokers and a quite high percentage of them will develop a tobacco-related cancer.

When inhaled, tobacco smoke goes through the mouth, pharynx, larynx, main bronchus and then diffuses on the lungs, where its components entry in the circulatory system and are distributed into the body. These components are eliminated mainly on the urine. As can be deducted, all the organism of a smoker is exposed to the harm caused by tobacco, although not all the organs have the same susceptibility for developing a cancer caused by it.

Although it is not exactly known how the tobacco causes cancer on different anatomic locations, one of the most important mechanisms of action is the following: carcinogenic substances contained in tobacco smoke are activated in different cells into more reactive ones. These activated substances can bind to the DNA forming the so-called ►DNA adducts. These DNA-adducts have a covalent union among the activated carcinogenic substance (usually a PAH) and the double-strand of the DNA. If the adduct is not repaired by the replication machinery, it can cause a mutation when the cell replicates that can ultimately lead to cancer. It has also been observed a specific mutation in the ►*p53* gene due to tobacco exposure, especially in ►lung cancer. This mutation appears on the second position on codon 249 and is a guanine to thymine transversion.

Cancers Related with Tobacco

In general, the risk of developing a tobacco-related cancer depends mainly on the duration on smoking and on the number of cigarettes per day. Cumulative exposure increases with time, so the probability of developing a cancer increases with age. The risk of cancer diminishes from the time since quitting and, for some cancers, it can be the same of a never-smoker's risk after some years of abstinence. This is an important message to keep in mind, it is never too late to abandon the tobacco habit and the human health will be benefited with this decision at any age.

Lung Cancer. This is the best known cancer related to tobacco consumption and is the one which causes more deaths. About the 80% of lung cancer deaths are attributed to tobacco in males and the 48% in women worldwide. When comparing a smoker with a nonsmoker, the former has 20-fold more possibilities of developing lung cancer. In the appearance of the disease, the number of cigarettes per day along with smoking duration determines the risk, although the last factor is more important than the first. Some discrepancies exist on the time since quitting for the risk of lung cancer of a former smoker to be the same to the risk of a never smoker. There also exist interactions between tobacco smoking and other risk factors of lung cancer including occupation or radon exposure in the risk of developing the disease.

Oral Cavity and Pharyngeal Cancers. Tobacco is a risk factor for both cancers. Men smokers pose a risk tenfold higher versus never smokers while the average risk in women is fivefold higher. There is also an interaction with alcohol consumption.

Laryngeal Cancer. Tobacco causes laryngeal cancer, there is a strong dose–response relationship between tobacco and the risk of developing this cancer and also an interaction with alcohol intake.

Esophageal Cancer. Tobacco is a risk factor for this cancer; the average risk is about seven to eight times higher than the risk of lifetime nonsmokers. The risk of esophageal cancer due to tobacco increases with heavy alcohol consumption.

Pancreatic Cancer. Tobacco increases the risk of having a pancreatic cancer. For heavy smokers, the risk of this cancer is about three to five times higher than for never-smokers.

Bladder and Kidney Cancers. Smoking increases the risk of bladder, kidney, and renal pelvis cancers. An interesting finding was that the urine of smokers is more mutagenic than the urine of never smokers.

Cervical Cancer. Smoking women have a risk of cervical cancer two to three times higher than never smoking women. Although it is not clear if there is an interaction with human papillomavirus (HPV), it seems to exist a higher risk for smokers that are HPV positive.

Stomach Cancer. Tobacco causes stomach cancer. The risks of smokers versus nonsmokers are around 1.5–2 times higher. Although not confirmed, there seems to be an interaction with Helicobacter Pylori. This infection is harder to be eradicated in smokers compared with nonsmokers.

Acute Myeloid Leukemia. There exists a risk of acute myeloid leukemia for smokers versus nonsmokers and that is 1.5–2 times higher in smokers.

Evidence for Other Cancers

Some cancers have not been related to tobacco. Although the studies performed on them cannot allow us to conclude a causal relationship, we should not disregard a possible association, since there are methodological aspects in the design of such studies that make difficult to assess a possible relationship. In any case, it is true that these cancers would not pose the association (in terms of relative risk) of those cancers with a demonstrated causal association nowadays. For ovarian cancer and endometrial cancer, tobacco does not seem to pose a risk for the former but seems to reduce the risk of the latter. For colorectal cancer, although the published literature seems to indicate a higher risk for smokers, this is not conclusive. For liver cancer, although it seems to be a positive association among smoking and liver cancer, the studies are inconclusive due to the lack of adjustment for other risk factors of this cancer. For prostate, brain, and breast cancers, it seems that tobacco is not associated with the incidence of these cancers.

Passive Smoking

We refer to ►passive smoking exposure when we breath (or are exposed to, i.e., the case of the fetus) tobacco products produced by others. This is also called involuntary smoking. Passive smokers inhale the mixture of the mainstream smoke exhaled by the smoker and also the sidestream smoke produced by the smoldering cigarette. This complex mixture has essentially the same composition that the one to which smokers are exposed and the main difference with smokers is that this mixture is usually diluted on the air, although its concentration depends on many factors. It is called ETS (Environmental tobacco smoke) or more recently ►secondhand smoke. In 1992, the US Environmental Protection Agency (EPA) declared the ETS as a human carcinogen.

In 2006, the US Surgeon General has published an extensive report on the health consequences of involuntary smoking (see references for more information). It states that there is no risk-free level of exposure to secondhand smoke. Some of the results affecting cancer incidence related with this exposure are the following: ETS causes lung cancer, with exposed lifetime nonsmokers having a 20–30% more risk than nonexposed. The evidence is suggestive but not sufficient for nasal sinus cancer. Respecting prenatal and postnatal exposure to ETS the literature suggests that there could be some relationship with ►childhood leukemias, ►lymphomas, and ►brain tumors. These last relationships have to be demonstrated in more rigorous studies.

Conclusions

Tobacco use is nowadays one of the most, if not the most, hazardous agents for human health and also in the cancer appearance. This modifiable exposure causes many different types of cancer (along with cardiovascular and respiratory diseases among others) such as lung, oral cavity, pharynx, larynx, esophagus, bladder, kidney, pancreas, cervix, stomach, and acute myeloid leukemia. The best way for prevention is not to smoke and a clear message has to be given to current smokers that their risk of cancer diminishes immediately from the moment they decide to abandon the habit. A great effort has to be made in order to avoid tobacco use in the new generations. Passive smoking also entails a risk for health, for both adults and for infants. In adults, it causes lung cancer and in infants it could cause childhood cancer (to be further studied), along with other diseases and alterations different than cancer. Exposure to ETS should also be avoided at any concentration in every place.

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Tobacco-Related Lung Cancer

Definition

Cancer that develops in the lungs those who smoke and/or use of smokeless tobacco for long-term period.

►Toxicological Carcinogenesis

Tobacco-Specific *N*-Nitrosamines

Definition

A group of carcinogens structurally related to nicotine which occur only in tobacco products.

►Tobacco Carcinogenesis

Tolbutamide

Definition

Is a potassium channel blocker; may be used in the management of type II diabetes if diet alone is not effective. Tolbutamide stimulates the secretion of insulin by the pancreas. Since the pancreas must synthesize insulin in order for this drug to work, it is not effective in the management of type I diabetes, where the beta-cells of islets of Langerhans are unable to produce insulin, for instance as the result of cell degeneration.

Tolbutamide Intravenous Test

Definition

A test to detect insulin-producing tumors (insulinoma). After a 1-g intravenous dose of tolbutamide, plasma insulin and glucose are measured at intervals up to 3 h; higher insulin responses and lower glucose values characterize patients with such tumors.

Tolerance

Definition

State in which the immune system shows a lack of reactivity toward certain antigens, notably those that are expressed by normal cells and tissues.

Tolfenamic Acid

Definition

A ►non-steroid anti-inflammatory drug (NSAID) that also induces Sp protein degradation.

Toll-Like Receptors

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Definition

Toll-like receptors (TLRs) represent a family of proteins that are involved in the innate sensing of conserved microbial components. They are composed of an extracellular domain containing leucine-rich repeats and a cytoplasmic Toll/Interleukin-1 receptor/Resistance (TIR) domain that is responsible for mediating downstream signaling cascades and subsequent inflammatory response, ultimately leading to the eradication of infectious agents.

Characteristics

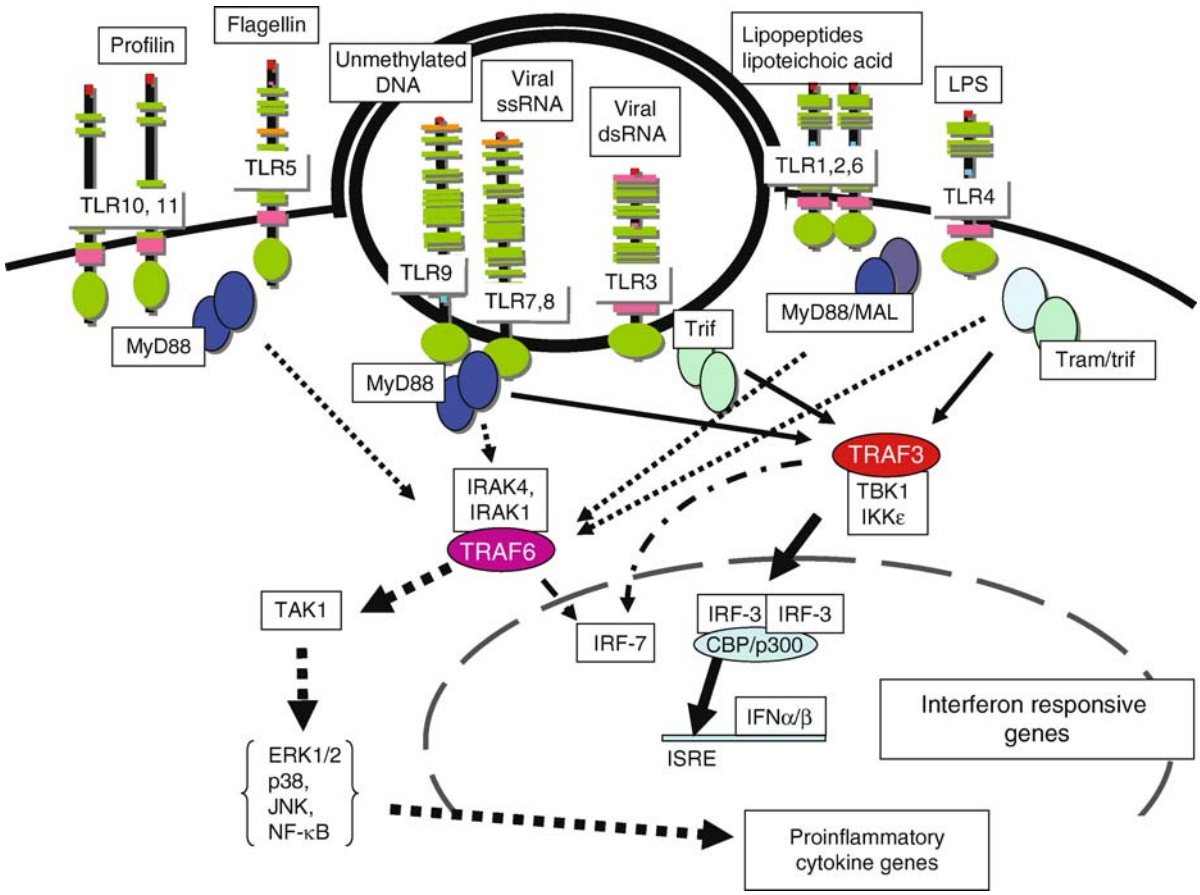
Toll-like receptors were originally described as proteins with homology to the protein Toll, which was first identified in *Drosophila melanogaster*. In flies, Toll served a regulatory function in the embryonic dorsoventral polarity, and was later shown to be an essential component of the *Drosophila* ►innate immune system. Based on the fact that Toll serves an immune function in *Drosophila*, it was proposed that TLRs might "activate ►adaptive immunity" in mammals, but the precise function of TLRs was revealed by forward genetics.

It was long recognized that the inflammatory responses induced by pathogens could be mimicked by specific molecules of microbial origin initially referred to as "endotoxin" and later shown to be lipopolysaccharide (LPS). It was then shown that LPS was capable of augmenting the adaptive immune response to a protein antigen, and in 1975, it became clear that the immuno-►adjuvant effect of LPS depended upon the integrity of a single locus known as *Lps*. It was not until 1998, that positional cloning revealed TLR4 as the membrane spanning component of the LPS receptor and this discovery accelerated our understanding of how the innate recognition of microbes occurs. Further genomic analysis revealed additional TLRs and to date a total of ten human TLRs (1–10) and 12 mouse TLRs (1–9; 11–13) have been described. Each of these recognizes a limited repertoire of broadly conserved molecules of microbial origin. For example, unmethylated DNA (common to prokaryotic genomes and DNA viruses) is recognized by TLR9; gram-positive bacterial lipopeptides are recognized by TLR2, acting in conjunction with TLRs 1 and 6, whereas TLR7 and TLR8 recognize single stranded viral RNAs or guanosine related analogues such as loxoribine and imidazoquinoline. All TLRs have a common cytoplasmic TIR motif that is believed to bind to intracellular adaptor molecules containing TIR motifs as well. Besides TLRs, the TIR motif is shared by the IL-1 receptor and IL-1 receptor-related family of signaling adaptor molecules. To date a total of five TIR domain-containing intracellular proteins have been identified that include MyD88, Mal (also known Tirap), Trif (also known as TICAM-1), Tram (also known as TICAM-2) and Sarm. Although most TLRs activate a common signaling pathway involving the adaptor molecule MyD88, other adaptor molecules involving Tram and/or Trif are able to activate a MyD88-independent pathway specifically downstream of TLR3 and/or TLR4. To date, many of the kinases involved in the TLR signaling pathways have been described and include for instance IRAK-1, IRAK-4 and Transforming Growth factor-β-activated kinase-1 (TAK1) (Fig. 1). Although TLRs are primarily expressed on cells of the innate immune system, including monocytes macrophages and dendritic cells, their expression is not limited to these cells and can also be found in lymphoid or non-hematopoietic cells. Whereas most TLRs are expressed on the surface of the cells, TLRs that recognize nucleotide structures (TLRs 3/7/8/9) are expressed in intracellular compartments such as endosomes and/or endosplasmic reticulum.

TLRs and Cancer Treatment

Although TLRs have limited involvement in the recognition, development or initiation of tumor growth, their immunostimulatory function has been utilized in cancer treatment in a variety of ways. Particularly, TLR7/8 agonists such as ►imiquimod, have long been used as topical immunomodulators, indicated for the treatment of external genital and perianal warts. This drug has also been approved for the treatment of superficial basal cell carcinoma and actinic keratoses, and also has activity against cutaneous metastases of malignant melanoma and vascular tumors. Many of the therapeutic effects of TLR activation can be attributed to the production of proinflammatory cytokines including tumor necrosis factor-alpha (►TNFα), type I ►interferons and interleukin 12. These cytokines have diverse functions that range from direct anti-tumor or pro-apoptotic effects (e.g. TNF), priming of natural killer (NK) cells (e.g. type I IFN) and the maturation of DCs leading to cytolytic T cell activation (e.g. type I IFN and IL-12).

Although many cells of the immune system are involved in tumor surveillance and tumor recognition, the induction of anti-tumor immune responses capable to suppress or eradicate tumors is generally poor. $CD8^+$ cytolytic T cells are the main cells involved in tumor eradication. As the antigens on tumor cells originate from self, most $CD8^+$ T cells have low avidity or are tolerant to these antigens and require a potent



Toll-Like Receptors. Figure 1 Toll-like receptors are key proteins in the innate recognition of microbial components. Upon recognition, TLRs initiate a signaling cascade that involves the TIR domain containing adaptor molecules MyD88, MAL/TIRAP, Trif and/or Tram, ultimately leading to NFκB activation resulting in proinflammatory cytokine production and/or translocation of IRF3/7 leading to type I interferon production (see text for further explanation).

stimulatory environment to become activated and acquire and execute their effector functions. Tumors create a suppressive environment by secretion of anti-inflammatory molecules, prevention of antigen expression and recruiting and inducing regulatory T cells on the site of tumor growth.

The design of experimental therapies is currently geared to implement TLR agonists to act on the above mentioned ►immunosuppressive pathways, thereby enhancing the induction of tumor specific cytolytic T cells. Specifically, vaccines based on the administration of dendritic cells containing tumor antigens or the administration of irradiated autologous or allogeneic tumor cells, have shown improved efficacy when coadministered with TLR-agonists. In both settings, the addition of TLR-agonists is thought to target DC function, leading to proinflammatory cytokine production and the subsequent upregulation of costimulatory molecules. In the event tumor cells were used as a vaccine therapy, only cell associated but not soluble TLR-agonists were shown to improve the adaptive immune response, suggesting targeting of TLRs with antigen in the same DC is crucial for efficacy. In addition, consistent TLR activation also leads to a reversal of T regulatory cell-mediated immune suppression and as such break tolerance towards self antigens. Together, these effects lead to an enhanced tumor-specific $CD8^+$ and $CD4^+$ T cell response ultimately leading to a regression and/or eradication of tumor growth.

►Inflammation

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Tongue Cancer

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Synonyms

Lingual cancer

Definition

Tongue cancers are malignant neoplasms of the tongue affecting oral tongue in the oral cavity or tongue base in the oropharynx.

Characteristics

Epidemiology

The world age standardized incidence is about 1.6 per 100,000 persons. The age standardized incidences of tongue cancer increase with age from about 0.1 per 100,000 persons in age group 15–19 years to about 7.5 per 100,000 persons in age group 65–75 years. The male to female ratio is about 1.5. The common predisposing factors of tongue carcinoma are smoking and drinking. Betel leaf chewing is the main reason for high incidences of tongue carcinoma in a few Asian countries including India and Taiwan.

Pathology and Genetic Aberration

The commonest cancer affecting the tongue is squamous cell carcinoma (►oral cancer) arising from the mucosal epithelial cells. Other pathology including minor salivary gland malignancies and sarcomas are rare. The development of cancer is believed to be a multi-step process with accumulation of many genetic abnormalities. A common genetic abnormality in tongue carcinoma is p53 (►p53 protein, biological and clinical aspects) mutation and its overexpression. The aberrantly underexpressed and overexpressed genes of tongue carcinoma are summarized in Table 1. These aberrantly expressed genes are involved in the control of cell cycle (►cell cycle target for cancer therapy), cell proliferation, ►apoptosis, cell ►adhesion, cell ►motility, and ►angiogenesis.

Clinical Features

The stepwise development of tongue cancer sometimes appears clinically in its premalignant stage as leukoplakia (white discoloration) or erythroplakia (red discoloration). These lesions histologically have various degrees of dysplasia. With further accumulation of genetic abnormalities involved in cancer development, these dysplastic epithelial cells become cancer cells which can invade and metastasize. The risk of malignant change of these dysplastic lesions is in the range of 5–20%. Carcinoma of tongue appears as nonhealing painful ulceration mostly at the lateral border of oral tongue. A typical ulcerative carcinoma of the lateral border of oral tongue with surrounding leukoplakia is shown in Fig. 1.

Subclinical Nodal Metastasis

Tongue carcinoma has high propensity of nodal metastasis even in its early stage. The nodal recurrence rate is 30–50% in untreated clinically N0 neck of T1-2 carcinomas. The commonest site of nodal metastasis is ipsilateral level II, and 95% metastatic nodes are found in the ipsilateral levels I, II, and III. Contralateral nodal metastasis and contralateral nodal recurrence are found only in less than 5% patients.

Most subclinical nodal metastases contain only tiny focal micrometastasis of less than 2 mm diameter within the lymph nodes or on the capsular lymphatic vessels. It has been reported that these cancer cells occupied a median of only 6% of nodal cross-sectional area of a lymph node and only 1% nodes in the neck contained micrometastasis. The small size of the micrometastasis amidst the large number of normal nodes in the neck would cause tremendous difficulty in the clinical detection. Although preoperative radiologic screening including CT, MRI, PET, or ultrasound scans are useful screening methods, they are not sensitive in the detection of most micrometastasis inside the small nonpalpable nodes. The risk of nodal recurrence of observed N0 neck remains high after these radiologic imaging screening.

TNM Staging and Prognostic Factors

The current TNM staging system for carcinoma of oral tongue is shown in Appendix 1. The largest tumor diameter has been used for many years in staging T1-3 oral tongue carcinoma. The largest diameter of oral tongue carcinoma is however either the tumor length or width. Tumor thickness is almost never the largest diameter of oral tongue carcinoma and therefore has no contribution to the current staging system.

It has been shown by many studies that tumor thickness, but not the largest dimension, is a significant independent prognostic factor in predicting subclinical nodal metastasis, local recurrence, and survival of oral

Tongue Cancer. Table 1 Aberrantly expressed genes of tongue carcinoma

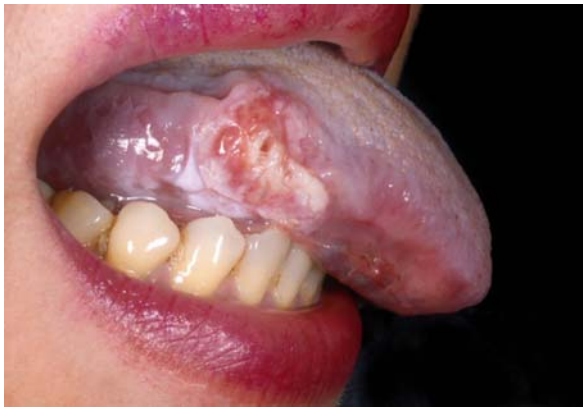
Underexpressed genes	Overexpressed genes
►E-cadherin (83–85%)	Akt (►AKT signal transduction pathway in oncogenesis) (46%)
α-Catenin (94%)	AMFR (42%)
β-Catenin (89%)	►bcl-2 (9–26%)
γ-Catenin (83%)	Cerb-B2 (17–50%)
►Maspin (49%)	C-myc (75%)
P14 (21%)	c-Met (42%)
P15 (47%)	cyclin B1 (37%)
P16 (54–74%)	►cycin D1 (53–68%)
►P21 (40–73%)	EGFR (►epidermal growth factor receptor ligands) (32–34%)
P27 (72%)	EpCam (63%)
►PTEN (29%)	Ets-1 (►ETS transcription factor)(42%)
RARβ (►retinoid receptor crosstalk in cancer) (78%)	►Furin (76%)
	MMP-2 (►matrix metalloproteinases) (49–53%)
	MMP-9 (►matrix metalloproteinases) (78%)
	MT1-MMP (►matrix metalloproteinases) (35%)
	►Osteopontin
	$p34^{cdc2}$ (66%)
	p53 (►p53 protein, biological and clinical aspects) (27–62%)
	PCNA (32%)
	►Podoplanin (60%)
	Rab1A (98%)
	RARα (►retinoid receptor crosstalk in cancer) (65%)
	S100A2 (29%)
	Syndecan-1 (58%)
	TIMP-1 (►matrix metalloproteinases) (65%)
	TIMP-2 (►matrix metalloproteinases) (43%)
	VEGF-A (►vascular endothelial growth factor) (67%)
	VEGF-C (►vascular endothelial growth factor) (49–100%)
	VEGFR-1 (►vascular endothelial growth factor) (89%)
	VEGFR-3 (►vascular endothelial growth factor) (74%)

tongue carcinoma. The thicker the tumor, the higher would be the risk of local recurrence, subclinical nodal metastasis and treatment failure. It is however still unresolved yet on the best cutoff thickness value for staging purpose. The proposed cutoff thickness values for prognosis or staging by various studies vary between 3 and 9 mm. The other important prognostic factor is histologic feature of perineural spread which is a significant risk factor of local recurrence after surgical treatment.

Pretreatment Assessment

Preoperative endoscopy and biopsy should be done to confirm the diagnosis and evaluate the extent of local tumor infiltration. Small neck nodes less than 1 cm along the jugular chain may not be palpable with fingers. Ultrasonography of the neck and ultrasound guided fine needle aspiration for cytology should be done to screen for presence of small nodal metastasis that may not be palpable.

Tumor thickness is an important factor for prognosis evaluation and treatment planning. Preoperative assessment of tumor thickness cannot be done with palpation. Intraoral ultrasonography using 7.5 MHz probe can be used to document the tumor thickness. MRI can also be used to assess the tumor thickness for preoperative evaluation of prognosis. Both T1 and T2 weighted MRI images can show the tumor thickness satisfactorily. MRI images in three-dimensional planes can also help the surgeon in the planning of surgical resection.



Tongue Cancer. Figure 1 Ulcerative carcinoma over the lateral border of tongue with surrounding leukoplakia.

Treatment Options

The treatment of oral tongue carcinoma remains controversial. Brachytherapy alone or surgery alone are each commonly used as primary treatment for early carcinomas by radiation oncologists and surgeons. The curative results of brachytherapy and surgery are similar for early stage carcinoma with over 90% local control. Either surgery or brachytherapy alone are not effective for stage III and IV carcinomas, combined surgery and postoperative chemoradiotherapy are recommended for advanced stage carcinomas.

Other less commonly practiced alternative treatment options include laser surgery or photodynamic therapy for early stage carcinomas. Concurrent intra-arterial regional chemoperfusion of high dose of cisplatin and radiotherapy may be considered as alternative treatment option for advanced T4 stage carcinoma.

Controversy of Elective Neck Dissection Versus Observation of N0 Neck

Since there is high risk of nodal recurrence of observed N0 neck of early tongue carcinoma, elective neck dissection is commonly practiced in many cancer centers worldwide. In retrospective studies of elective selective neck dissection versus observation of N0 neck, the regional recurrence rates could be reduced from 30–50% of observation to 10–15% after for elective selective I,II,III neck dissection. The regional recurrence related mortality rates could be reduced from 20–25% for observation to 4–10% for elective selective I,II,III neck dissection group. From the results of retrospective studies, elective neck dissection can reduce both initial regional recurrence rate and regional recurrence related mortality, and the reduction of node-related mortality contributes to long-term survival benefit.

Appendix I TNM stage of oral tongue carcinoma, AJCC/UICC 2002

Primary tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*
T1	Tumor ≤2 cm in greatest dimension
T2	Tumor >2 cm but ≤ 4 cm in greatest dimension
T3	Tumor >4 cm in greatest dimension
T4	Tumor invades adjacent structures (e.g., through cortical bone, into deep extrinsic muscle of tongue, maxillary sinus, skin; superficial erosion of bone/tooth socket by gingival primary is not sufficient to classify as T4)
Regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in single ipsilateral node, ≤3 cm in greatest dimension
N2a	Metastasis in single ipsilateral node, >3 cm but ≤6 cm
N2b	Metastasis in multiple ipsilateral nodes, all ≤6 cm
N2c	Metastasis in bilateral or contralateral nodes, all ≤6 cm
N3	Metastasis in lymph node >6 cm in greatest dimension
Distant metastasis (M)	
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
Stage	
0	Tis N0 M0
I	T1 N0 M0
II	T2 N0 M0
III	T3 N0 M0, T1-2 N1 M0, T3 N1 M0
IV	T4 N0-1 M0, anyT N2-3 M0, anyT anyN M1

There is however no prospective randomized study comparing the long-term benefit of elective neck dissection compared with observation in the treatment of N0 neck of early stage I and stage II oral tongue carcinoma. There is risk of mortality and morbidity of elective neck dissection. Both elective neck dissection and observation have their proponents in different cancer centers. Instead of performing elective neck dissection for all patients with early tongue carcinoma, patients should be informed of the possible choice of both treatment options of either observation or elective neck dissection of N0 neck. Observation is particularly suitable for patients with thin carcinomas of less than 3–4 mm. The risk of nodal recurrence of patients with thin tumors of 3–4 mm is in the range of

10–15%. Patients choosing observation treatment of N0 neck should be advised to have regular follow-up after primary treatment for early detection of nodal recurrence. Early nodal recurrence can be salvaged with modified or radical neck dissection successfully. Of those patients who cannot be closely followed up, elective neck dissection is a more suitable treatment of choice.

Outcome of Treatment

Local or regional lymph node recurrences account for over 90% of recurrences. Majority of local recurrences cannot be salvaged. Over 90% nodal recurrences of closely observed neck can be successfully salvaged with neck dissection. Of those patients with elective selective neck dissection of cN0 neck, the nodal recurrence rate of pN0 neck is less than 5% and is 30–40% for pN+ neck. Radiotherapy of pN+ neck is therefore advised. The overall 5-year disease free survival rates are in order of 90–100% for stage I, 60–80% for stage II, 30–50% for stage III and less than 20% for stage IV.

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Tonsils and Adenoids

Definition

Prominent oval masses of lymphoid tissues on either side of the throat.

Topoisomerase II

Definition

Is a nuclear isomerase enzyme that alters the topology of DNA. This enzyme transiently cleaves double-stranded DNA, processes an unbroken double-strand DNA and then reanneals the broken strand. It is a major target for antineoplastic agents, such as anthracycline antibiotics, and ►etoposide.

►Adriamycin
►Topoisomerases

Topoisomerase II-DNA Cleavage Complexes

Definition

Transient intermediates in the catalytic cycle of DNA ►topoisomerase II.

►Replication Factories and Foci
►Topoisomerases

Topoisomerase III

Definition

Topoisomerase III is an enzyme that changes the degree of supercoiling of DNA by cutting one strand of DNA and passing an intact strand through the break.

►Topoisomerases
►Poly(ADP-Ribosyl)ation

Topoisomerases

Definition

Enzymes that produce a reversible covalent complex with DNA through which another DNA strand or duplex is passed. Passage of strands or duplexes changes DNA topology introducing or removing supercoils. Type I and III topoisomerases pass single

strands and change DNA supercoiling in units of one. Type II topoisomerases pass duplex DNA and change DNA supercoiling in units of two. ►Topoisomerase II nuclear enzymes induce transient breaks in both strands of the DNA helix simultaneously. The clinically used DNA topoisomerase II inhibitor ►etoposide is known to induce DNA double strand breaks.

►Celastrol
►Decatenation G2 Checkpoint
►Genistein

TOR

Definition

The phosphatidylinositol (PI) kinase homologue TOR ("target of ►rapamycin") is the cellular target of the complex of FK506 binding protein (FKBP) with the immunosuppressant rapamycin. TOR is part of the general mitotic signaling pathway involving a tyrosine kinase, a phosphatidylinositol 3-kinase (PI3K), Akt/PKB kinase, p70S6 kinase (p70S6k) and 4E-BP1 (PHAS-I). TOR is conserved from yeast to mammals and is also known as FRAP, RAFT, RAPT ►mTOR refers to mamalian TOR.

►Autophagy

Torisel

Definition

An inherited condition characterized by benign tissue masses that form on the skin, hair follicles, and gums during infancy, and in the breasts following puberty, and are associated with a higher risk for developing cancers.

►Temsirolimus

Torsade de Pointes

Definition

A peculiar ventricular tachyarrythmia, with characteristic ECG (lengthened QT interval), that can be self limiting or degenerate into ventricular fibrillation and result in death. Drugs that prolong action potential duration (as a result of a reduction in repolarizing current), induce early after depolarizations, which can generate ectopic beats and increase the spatial dispersion of repolarization, which in turn increases the possibility for reentry, have the potential to cause this tachyarrythmia.

►Lead Optimization

Totipotent

Definition

Able to give rise to all cell types. In mammals, only the fertilized egg and early cleavage stage blastomeres are truly totipotent. Cells of the inner cell mass and ►embryonal stem (ES) cells are unable to differentiate into cells of the trophectoderm lineage.

►Adult Stem Cells

Totipotent Stem Cells

Definition

Are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also ►totipotent. These cells can differentiate into embryonic and extraembryonic cell types.

►Adult Stem Cells

Toxic Epidermal Necrolysis

Definition

A life-threatening, exfoliative mucocutaneous disease in which much of the skin becomes intensely red, blisters, and peels off in the manner of a second-degree burn. It is thought to be an idiosyncratic reaction to a drug or other chemical agent.

►Rituximab

Toxicity

Definition

Refers to unwanted biological activity.

►ADMET Screen

Toxicity Testing

►Preclinical Testing

Toxicokinetics

Definition

Refers to study of the fate of administered drug and metabolites in the animals used in toxicity studies. The term usually refers to those studies of drug disposition that form part of toxicity study rather than specialist studies of adsorption, distribution, metabolism and excretion conducted as separate studies.

►Preclinical Testing

Toxicological Carcinogenesis

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Synonyms

Chemical carcinogenesis; Experimental carcinogenesis

Definition

In the broadest possible sense, ►carcinogenesis is a process of generation of benign and malignant neoplasm. Agents such as viruses, radiation, and chemicals are able to induce ►cancer in humans and experimental animals. However, the importance of chemicals as a cause of cancer has long been recognized in basic and clinical studies, and is emphasized by the epidemic of ►tobacco-related lung cancer in the twentieth century. Carcinogenesis may be considered as a form of toxicity in which cells achieve a different steady state from the normal and do not respond normally to homeostatic mechanisms. Carcinogenesis induced by chemicals is called "toxicological (chemical) carcinogenesis." Basic and clinical research in the field of toxicological carcinogenesis has led to many major advances, ranging from the fields of epidemiology and international human studies to laboratory research on mechanisms involved in the complex processes that are associated with the initiation and development of malignant disease (cancer).

Many chemical carcinogens have been identified, and their effects documented in experiments in which animals exposed to the agents at the maximum tolerated dose develop neoplasm. Toxicological carcinogenesis and ►human cancer epidemiology studies have clearly identified specific chemicals that can act as human carcinogens in both occupational and environmental settings. The main groups of relevance to human disease include ►polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, ►alkylating agents, and heterocyclic amines. Cancer resulting from exposure to chemicals in the environment has taken on new importance. Knowledge about the mechanisms and natural history of cancer development from toxicological carcinogenesis as well as epidemiology of human cancer is critical in the control and prevention of human neoplastic disease.

Characteristics

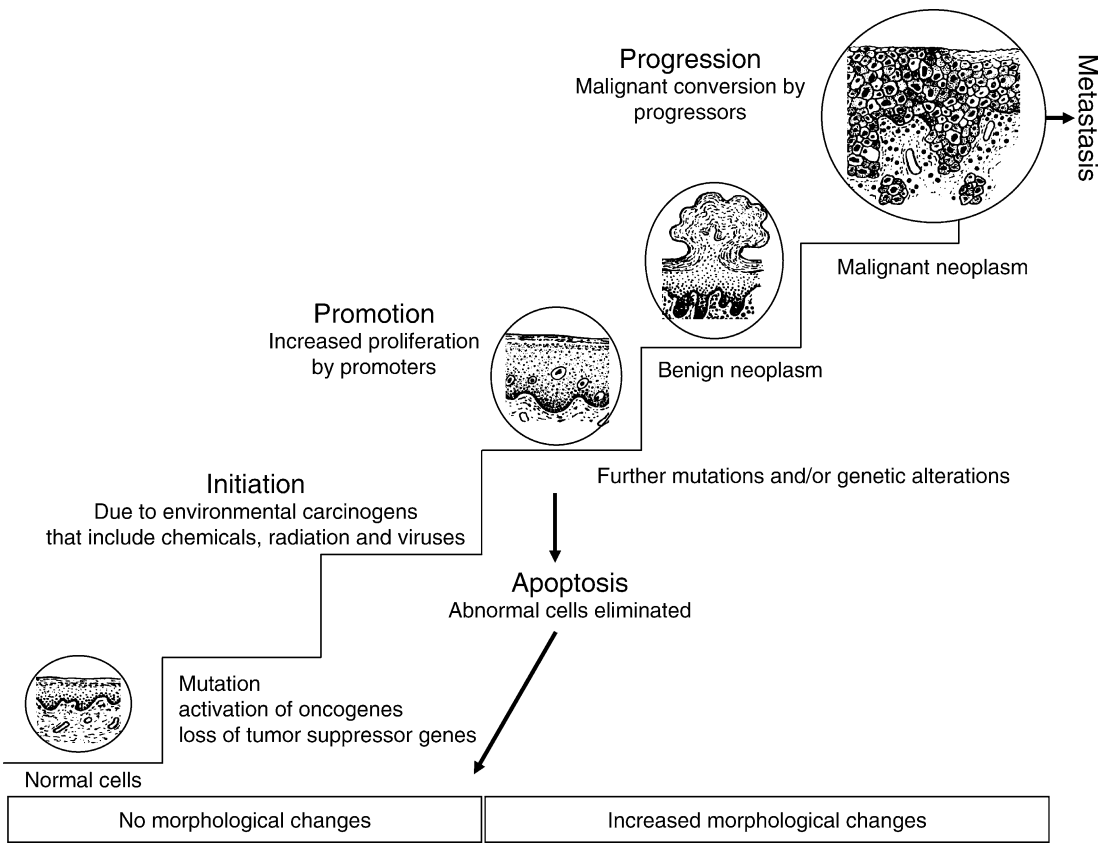
Mutagens are agents that can permanently alter the genetic constitution of a cell. The most widely used screening test, the Ames test, uses the appearance of mutants in a culture of bacteria of the *Salmonella* species. Approximately 90% of known carcinogens are mutagenic in this system. Moreover, most, but not all, mutagens are carcinogenic. This close correlation between carcinogenicity and mutagenicity presumably occurs because both reflect ►DNA damage. The *in vitro* mutagenicity assay is a valuable tool in screening for the carcinogenic potential of chemicals. Cultured human cells are also being increasingly used for assays of mutagenicity.

Chemical carcinogens may cause development of neoplasm either directly or indirectly. They can be grouped into two main classes according to the mechanism by which they stimulate development of neoplasm: (i) ►Genotoxic carcinogen causes direct damage to DNA by forming chemical:►DNA adducts. The abnormal areas of DNA are prone to damage in replication and some adducts are resistant to normal ►DNA repair mechanisms. (ii) ►Non-genotoxic carcinogen is a carcinogen for which there is no evidence of direct interaction with cellular DNA. This type of carcinogen can be divided into two subgroups. ►Mitogenic carcinogen binds to receptors on or in cells and stimulates cell division without causing

direct DNA damage. In experimental skin carcinogenesis such agents have been shown to bind to and activate ►protein kinase C, causing sustained epidermal hyperplasia. ►Cytotoxic carcinogen produces tissue damage and leads to hyperplasia with cycles of tissue regeneration and damage. In some cases it is believed that cytokines generated in response to tissue damage act as mitogenic factors. Chemical carcinogens can be further divided into two groups: (i) ►Direct-acting carcinogen: the agent is capable of directly causing neoplasia. (ii) ►Procarcinogen: the agent requires conversion to an active carcinogen. This conversion takes place through normal metabolic pathways. In procarcinogens the ►cytochrome P450 (CYP) monooxygenase system plays an important role in conversion in many instances. ►Detoxification reactions also occur, the accumulation of carcinogen being determined by a balance between: (i) dose of procarcinogen; rate of detoxification and elimination; and (ii) rate of conversion to the active form.

Three stages have been defined in toxicological carcinogenesis (Fig. 1). Studies of toxicological carcinogenesis among experimental animals have shed light on the individual stages in the progression of normal cells to cancer. From these studies, one can define three stages (►multistep development) of toxicological carcinogenesis:

1. *Initiation* is the first stage and likely represents mutations in a single cell. The nature of the initial changes in cells is still uncertain. In experimental toxicological carcinogenesis in skin, the Harvey ►*ras* gene has been identified as being frequently mutated. This gene is involved in epidermal proliferation and when it becomes abnormal epidermal cells are less responsive to signals that normally cause terminal differentiation. Only relatively few genes have been identified as being mutated in other animal models of toxicological carcinogenesis.
2. *Promotion* follows initiation and is characterized by clonal expansion of the initiated cell. Induction of cell proliferation takes place at this stage. The altered cells do not exhibit autonomous growth, but remain dependent on the continued presence of the promoting stimulus, including an exogenous chemical or physical agent or an endogenous mechanism, such as hormonal stimulation. In this phase of carcinogenesis a promoting agent brings about increased cell proliferation. Promotion is initially reversible if the promoting agent is withdrawn.



Toxicological Carcinogenesis. Figure 1 Toxicological carcinogenesis as a multi-step process.

3. *Progression* is the third stage, in which growth becomes autonomous and is independent of the carcinogen or promoter. At this stage, additional genomic changes presumably endow cells with a relative growth advantage that, in turn, results in their further clonal expansion. Cancer is the end result of the entire sequence and is established when the cells acquire the capacity to invade and metastasize. If there is persistent cell proliferation, initiated cells acquire secondary genetic abnormalities in oncogenes, which first lead to dysregulation and eventually to autonomous cell growth. The ultimate end-point of progression is development of an invasive neoplasm.

The various tests that have been applied to identifying agents with carcinogenic potential may be classified into several general areas on the basis of the time involved in the assay: short, medium, and long. These include short-term tests for mutagenicity (e.g., the Ames test), gene mutation assays *in vivo* (e.g., The LacZ mouse, the LacI mouse, the LacI rat), assay for chromosomal alterations (e.g., ►micronucleus assay, sister chromatid exchange), measurement of primary DNA damage *in vitro* and *in vivo*, and chronic bioassays for carcinogenicity (e.g., chronic 2-year bioassay, medium-term bioassays-Ito model, multi-stage models of neoplastic development, transgenic and knockout mice as models of carcinogenesis).

History

It is widely recognized that exposure to chemicals in the workplace and the environment can contribute to human cancer risk. This was first indicated in 1775 by Dr. Pott, who attributed scrotal skin cancers to prolonged exposure to soot in London chimney sweeps. In 1914, Dr. Boveri first hypothesized that cancer was a genetic disease, prior to the discovery of the genetic material. In 1915, Dr. Yamagiwa and co-workers successfully induced skin cancer in rabbits by painting their ears continuously with benzene solutions of tar. In the 1930s Dr. Kenneway and co-workers demonstrated that pure chemicals isolated from coal tar could also produce tumors in animals. In the 1950s there were parallel discoveries of the structure of the DNA double helix and its establishment as the hereditary material and mutagenic potential of ionizing radiation and certain chemical carcinogens in humans and experimental systems, and extensive investigations into the relationship between chemically induced mutations and human cancer. The 1980s saw the elucidation of the first oncogenes that appeared to be responsible for the initiation of cancer as first predicted by Dr. Boveri. This era also saw the development of the Ames *Salmonella* bacterial mutagenesis assay (the Ames test) and similar genetic toxicology assays. These developments firmly established the basic paradigm for the field of toxicological carcinogenesis: chemicals capable of induction of mutations are presumed to be carcinogens. It was predicted that any chemical or physical agent that can covalently damage DNA could also cause mutations through its DNA-damaging mechanism, and hence can be a carcinogen. The data that followed in the 1990s appeared to strongly support this central assumption, as numerous chemicals that were initially tested for DNA damage or mutations were also carcinogens in experimental animals. Since then, our understanding of the molecular basis of cancer has improved substantially. In addition, investigations into the molecular basis of toxicological carcinogenesis, as well as more extensive human cancer epidemiology studies using modem molecular tools, have greatly expanded our knowledge in this area.

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Toxicological Pathologist

Definition

Is usually a veterinary or medical graduate with experience in the pathological changes that can be induced by chemicals agents including drugs.

►Preclinical Testing

Toxicology

Definition

Study of the nature, effects, and detection of poisons in living organisms. The basic assumption of toxicology is

that there is a relationship among the dose (amount), the concentration at the affected site, and the resulting effects.

►Biomonitoring

TP

►Thymidine Phosphorylase

TP53

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Synonyms

p53

Definition

The TP53 ►tumor suppressor gene is located on chromosome 17p13.1 and encodes an ubiquitous phosphoprotein of molecular mass 51–53,000, essentially expressed in the nucleus. This gene is frequently inactivated by somatic mutation or by loss of alleles in many common human cancers. More than 25,000 such mutations have been described so far. Inherited, heterozygous mutations have been identified in about 400 families with ►Li-Fraumeni Syndrome and Li-Fraumeni-like syndromes (LFS and LFL), characterized by the early occurrence of cancers at multiple organ sites. TP53 belongs to a ►p53 family that also includes TP73 (1p36) and P63 (3p28). In contrast with TP53, these two genes have a restricted, tissue specific and developmental expression pattern and are not frequently mutated in cancer.

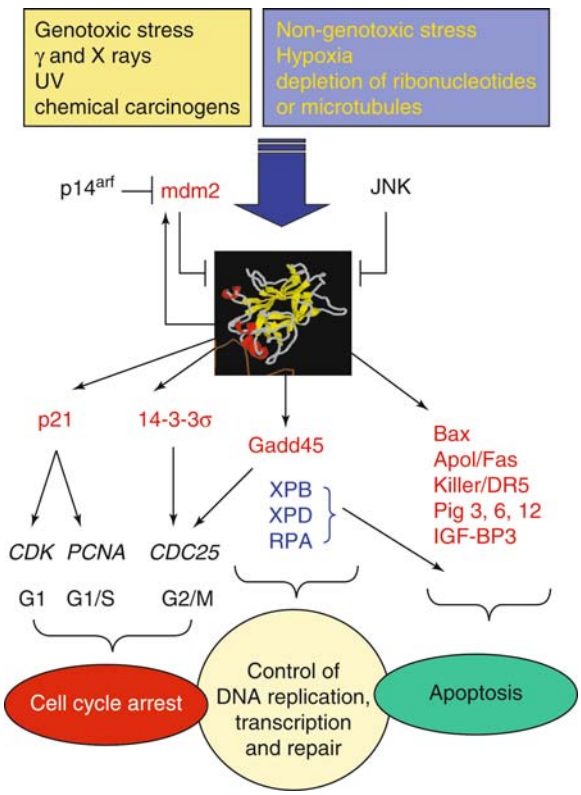
The p53 protein is a latent ►transcription factor that is activated in response to multiple forms of physical and chemical stress to exert diverse, complementary effects in the regulation of cell proliferation, genetic integrity and survival. These effects include:

- Induction of ►apoptosis,
- Control of cell ►division through regulation of ►cell-cycle progression in G1 and G2, ►centrosome duplication and mitosis, and
- Modulation of DNA replication and ►repair of DNA.

The main function of the p53 protein is to act as an "emergency brake" to prevent the proliferation of cells with damaged genetic material, caused by exposure to genotoxic agents (Fig. 1). In a broader context, the protein acts as an integrator of multiple exogenous and intracellular signals to regulate cell proliferation during replicative ►senescence, differentiation and development. Inactivation of TP53 in mice resulted in accelerated development of multiple tumors, while a fraction of p53-deficient embryos displayed a lethal defect in neural tubule closure, resulting in exencephaly.

Characteristics

The TP53 gene spans 20 kb and contains 11 exons, the first one being non-coding. The coding sequence contains five regions showing a high degree of conservation in vertebrates, located in 2, 5, 6, 7 and 8. An ►orthologue has recently been described in

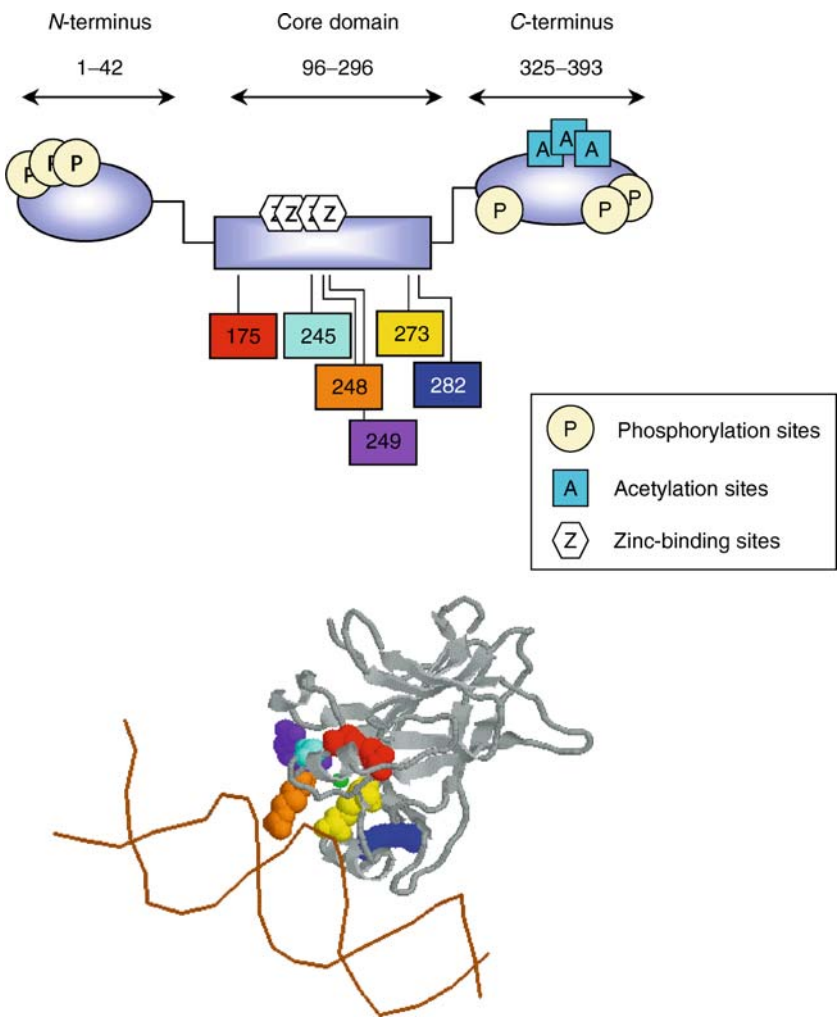


TP53. Figure 1 The p53 pathway. The p53 protein is induced in response to various forms of stress and mediates a set of coordianted, anti-proliferative responses including cell-cycle arrest, control of replication, transcription, repair and apoptosis. Blue: factors that bind to p53 and that are regulated by protein interactions. Red: factors that are regulated by p53 at the transcriptional level.

Drosophila. Several ►gene polymorphisms are identified in the human population, with ►allele frequencies that vary with ethnic origin. However, there is only limited evidence to prove that these polymorphisms play a role in tumor susceptibility.

The TP53 gene does not contain a conventional TATA box, but is under the control of several ubiquitous transcription factors, including ►NFkB, Sp1 and Jun. It is expressed in the form of one major transcript of 2.8 kb and several isoforms generated by alternative splicing or use of an alternative promoter, intron 4.

The protein contains 393 residues and is organized in a hydrophobic, central core (residues 110–296, encoded by exons 5 to 8), flanked by an acidic N-terminus and a basic C-terminus (Fig. 2 *top*). The N-terminus contains two complementary transcriptional activation domains, with a major one at residues 1–42 and a minor one at residues 55–75, specifically involved in the regulation of several pro-apoptotic genes. The central core is made of a scaffold of two β-sheets supporting a set of flexible loops and helices stabilized by the binding of an atom of zinc. These loops and helices make direct contact with DNA sequences containing inverted repeats of the motif RRRC(A/T). The C-terminus contains the main nuclear localization signals and oligomerization domains (residues 325–366). The active form of the protein is a tetramer (in fact, a pair of dimers). The extreme C-terminus has multiple regulatory functions and exerts a negative control on sequence-specific DNA binding activities. Both N- and C-terminal regions contain



TP53. Figure 2 Diagram of the p53 protein structure. *c*: linear structure, showing the three main structural domains. Codon numbers of the main mutation hotspots are shown as coloured boxes. Sites of post-translational modifications are shown as "P" (phosphorylations), "A" (acetylations) and "Z" (zinc binding sites). *bottom*: 3-D structure of the central core of p53 in complex with target DNA. Hotspot residues are shown in the same color code as above.

multiple post-translational modification sites, while only a few have been identified so far in the central core (see Table 1).

Upstream of p53: Signaling of DNA Damage

The p53 protein is constitutively expressed in most cells and tissues as a latent factor. Due to its rapid turnover (5–20 min), the protein does not accumulate unless it is stabilized in response to a variety of intracellular and extra cellular stimuli. Signals that activate p53 include diverse types of ►DNA damage (strand breaks, bulky adducts, oxidation of bases), blockage of RNA elongation, ►hypoxia, depletion of ►microtubules, ribonucleotides or growth factors, modulation of cell ►adhesion and alteration of polyamine metabolism. Most of the current knowledge of p53 protein activation is derived from studies using DNA strand breaks as inducing signals.

The main regulator of p53 protein activity is ►mdm-2, a protein which binds p53 in the N-terminus (residues 17–29); it conceals its transcription activation domain, redirects p53 from the nucleus to the cyclasm and acts as a ►ubiquitin ligase to target p53 for degradation by the ►proteasome. The MDM-2 gene is a transcriptional target of p53, thus defining a regulatory feedback loop in which p53 controls its own stability. The p53/mdm-2 complex is regulated by Arf (Alternative Reading Frame), a 14 kD protein encoded by the p16/CDKN2A gene.

The kinetics, extent and consequences of p53 activation vary according to the nature and intensity of the inducing signals. In response to ionizing radiation, activation of the p53 protein is thought to proceed through several consecutive steps, with first phosphorylation of p53 in the N-terminus by kinases involved in the sensing of DNA damage such as Atm

TP53. Table 1 Factors involved in the activation and post-translational modification of p53

Factor	Biochemical function/activated by p53	Interaction with p53
PARP	ADP-ribose polymerase/DNA strand breaks, nucleotide depletion	ADP-ribose polymers bind to p53
HMG-1	High mobility group 1/?	Binding to N-terminus or to DNA-binding domain
E6AP	E6 accessory protein/ubiquitin-mediated degradation	Binding to p53
Hif-1	Hypoxia-inducible factor/Hypoxia	Binding to p53
14-3-3 s	Cell-cycle regulator/ionizing radiations	Binding, C-terminus (Ser-376)
p300/CBP	Histone acetyl-transferases/co-activators of transcription	Binding, N-terminus acetylation, C-terminus
c-abl	Tyrosine kinase/irradiation, DNA-strand breaks	Binding, proline-rich region
mdm-2	oncogene/negative control of p53	Binding, residues 13–29
NO	Nitric oxide/oxidative stress, inflammation, irradiation	Oxidation of cysteines in DNA-binding domain
Cdc2/Cdk2-Cyclin A/B	Cell-cycle dependent kinases	Phosphorylation of Ser-315; forms complexes with p53
cdk7-cyclin H	Component of TFIIH	Phosphorylation of Ser-33
CKII	Kinase/UV	Phosphorylation of Ser-389; forms complexes with p53
MAPK	Mitogen-activated protein kinase/UV?	Phosphorylation, Thr-73 and 83 (mouse p53)
ATM	Kinase/ionizing radiations	Phosphorylation, Ser-15
DNA-PK	Kinase/UV	Phosphorylation, Ser-15 and Ser-37
Chk-2	Cell-cycle-dependent kinase	Phosphorylation, Ser-20
JNK/p38	Stress-activated kinases/UV	Phosphorylation, Ser-34, mouse p53
PKC	Protein kinase C	Phosphorylation, Ser-378
CKI	Kinase/?	Phosphorylation, several N-terminal serines (including Ser-6 and Ser-9)
p19arf	Cell-cycle inhibitor, alternative product of CDKN2A	Prevents p53-mdm2 interactions
Ref-1	Redox-repair enzyme/oxidative stress, hypoxia	Reduction of cysteine in DNA-binding region binding to C-terminus

(the product of the ►ataxia telangiectasia mutated gene) and Chk-2 (a cell-cycle regulatory kinase). These phosphorylations contribute to the dissociation of the p53/mdm-2 complex and stabilize the protein. Second, p53 binds co-activators with acetyl-transferase activity such as ►p300/CBP co-activators and pCAF. These factors acetylate p53 in the C-terminus. These processes, as well as other coordinated post-translational modifications of the C-terminus, induce conformational changes that turn the protein into an active form with a high affinity for specific DNA binding sites. The third step involves redox regulation of sensitive cysteines within the DNA-binding domain of the protein. This three-step mechanism may account for p53 induction in response to most forms of DNA damage.

Downstream of p53: Cell-Cycle Control, Apoptosis and DNA Repair

Once activated, p53 exerts its effects through two major mechanisms: transcriptional control (activation or repression of specific genes) and complex formation with other proteins. Important downstream effectors of p53 (Table 2) include regulators of ►cell-cycle ►checkpoints (in G1/S, G2 and during mitosis), factors involved in the signaling of apoptosis, and components of the transcription, replication and repair machineries. At the cellular level, activation of p53 most frequently results in either cell cycle arrest (mostly in G1 and/or G2/M) or apoptosis. How a given cell "chooses" between cell cycle arrest and apoptosis in response to specific stimuli may depend upon many factors, such as the nature and intensity of the stress, as well as the cell type. In many tissues, p53 plays a role in drug-induced apoptosis and is thus an important effector in the response of cancer cells to chemo- or radio-therapy. In addition, loss of p53 function results in deficient cell cycle arrest, inefficient ►mitotic spindle checkpoint, aberrant centrosome duplication, premature re-entry into S phase, ►genomic instability and ►aneuploidy.

TP53. Table 2 Some important downstream effectors of p53 functions

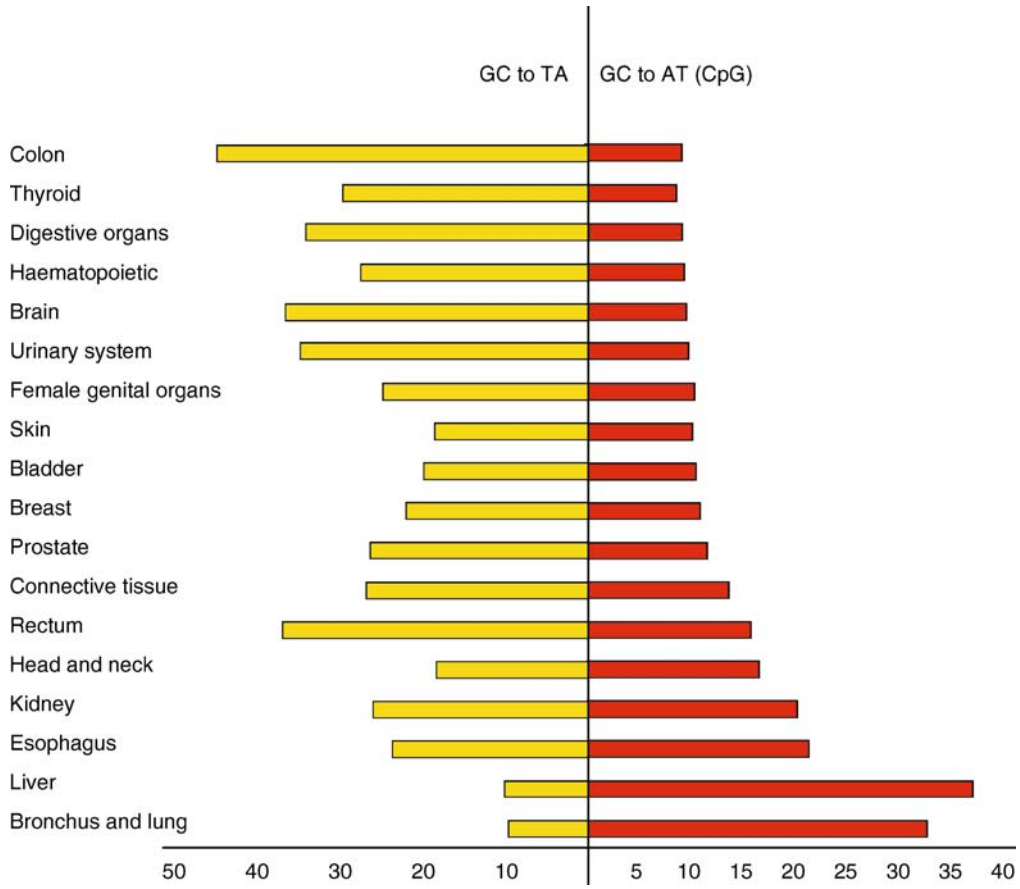
Factor	Activity	Mode of regulation	Function
Apo-1/Fas/CD95	Death signaling receptor	Transcriptional activation?	Apoptosis
Bax-1	Dominant-negative inhibitor of bcl2	Transcriptional activation	Apoptosis
Bcl-2	Repressor of apoptosis	Transcriptional repression	Apoptosis
IGF-BP3	Inhibitor of IGF-I	Transcriptional activation	Apoptosis
Killer/DR5	Death signaling receptor	Transcriptional activation	Apoptosis
P85	Regulatory subunit of PI3 kinase	Transcriptional activation	Apoptosis
Pig-12	Glutathione transferase homologue	Transcriptional activation	Apoptosis
Pig-3	Quinone oxidase homologue	Transcriptional activation	Apoptosis
Pig-6	Proline oxidase homologue	Transcriptional activation	Apoptosis
IGF-I	Growth factor	Transcriptional repression	Apoptosis?
IL-6	Survival factor	Transcriptional repression	Apoptosis?
thrombospondin-1	Inhibitor of angiogenesis	Transcriptional activation	Apoptosis?
Gadd45	Binding to PCNA	Transcriptional activation	Cell-cycle arrest ?
BTG2	Inhibitor of proliferation	Transcriptional activation	Cell-cycle arrest, G1
p21waf-1	Inhibitor of CDK2–4 and 6	Transcriptional activation	cell-cycle arrest, G1 and G2/M
cyclin A	Cell-cycle regulation, S phase	Transcriptional repression	Cell-cycle arrest, G1/S
cyclin G	Cell-cycle regulation	Transcriptional activation	cell-cycle arrest?
GPx	Glutathione peroxidase	Transcriptional repression	Control of oxidative stress
NOS2/iNOS	Inducible Nitric Oxide synthase	Transcriptional repression	Control of oxidative stress
COX2	Inducible cyclooxygenase	Transcriptional repression	Control of oxidative stress?
Pig-1	Galectin-7	Transcriptional activation	Differentiation?
PCNA	Auxiliary subunit of polymerase δ	Transcriptional activation	DNA repair/replication
RPA	Replication protein A	Inhibition by protein binding	DNA repair/replication
ERCC2/ERCC3	Helicases, TFIIH complex	Activation by protein binding	DNA repair/transcription
P53RR2	Ribonucleotide reductase homolog	Transcriptional activation	DNA repair?
TBP	TATA box-binding protein	Inhibition by protein binding	Inhibition of transcription
Mdm-2	Oncogene	Transcriptional activation	Repression of p53
MDR-1	Multi-drug resistance	Transcriptional repression	Resistance to chemotherapy

Clinical Relevance

The TP53 gene is often inactivated by ►missense mutations, in contrast with many other tumor suppressors such as ►APC, ►RB1, ►BRCA1 or p16/►CDKN2A that are inactivated by gene deletion or truncation. The mutations described to date mostly occur in the region of the gene encoding the DNA binding domain. Most of these mutations impair DNA binding by disrupting the structure of the domain or crucial contact points between the protein and target DNA. About 30% of missense mutations affect six "hotspot" codons (175, 245, 248, 249, 273 and 282) (Fig. 2 *bottom*). The other mutations are scattered over 300 different codons. Mutations are very common in the invasive stages of many epithelial tumors. A database of all published mutations is available at the International Agency for Research on Cancer (►IARC TP53 database, http://www-p53.iarc.fr/).

In many cancers, the patterns of mutations show variations, revealing clues about the mechanisms responsible for the formation of the mutations. Specific carcinogen-induced mutations have been identified in ►hepatocellular carcinoma (mutations induced by ►aflatoxins in sub-Saharan Africa and in south-east Asia), in skin tumors (double transitions at adjacent cytosines, a typical signature of mutagenesis by UV in squamous and in basal cell carcinomas) and in lung cancers (G to T transversions associated with exposure to tobacco smoke; ►tobacco carcinogenesis; (Fig. 3).

The usefulness of TP53 mutation detection in molecular pathology is still a matter of debate. As mutation often results in the accumulation of the protein, ►immunohistochemistry (IHC) has often been used as a criterion to detect TP53 abnormalities. However, positive IHC does not always correlate with mutation as several common missense mutants, as well as most



TP53. Figure 3 Prevalence of two common mutation types: G to T transversions and C to T transitions at dipyrimidine (CpG) repeats in tumors of various organs. Tumors with high prevalence of G to T transversions often have a low prevalence of transitions and vice-versa. G to T transitions are a common molecular signature of many environmental carcinogens, such as tobacco smoke components (lung and esophageal cancers) or dietary mycotoxins (liver cancer).

frameshift and nonsense mutants, do not result in protein accumulation. Several well-established methods have been described for the detection of mutations in the TP53 gene, including SSCP (Single Stranded Conformation Polymorphism) analysis, TTGE (Temporal Temperature Gradient Electrophoresis), yeast-based functional assays and, recently, micro-array hybridization assays.

TP53 gene mutations are good markers for the clonality of tumor lesions. In many tissues, mutation correlates with bad prognosis and poor response to therapy, but TP53 mutation has been shown to behave as an independent marker of prognosis only in rare cases such as breast and head and neck cancers. Recent evidence suggests that the nature and position of the mutation may help to predict poor response to treatment. Detection of circulating anti-p53 antibodies as well as of free plasmatic DNA containing mutant TP53 may be of interest in the early detection of cancer lesions.

TP53 is the target of several experimental therapeutic approaches. Gene transfer of wild type TP53 into cancer cells has been tested in several human tumors. However, the effects reported to date are limited and, at best, transient. Another approach is based on the use of cytolytic viruses selectively replicating in TP53-deficient cells (►ONYX vectors). Several pre-clinical studies have investigated the use of small lipophilic coumpounds or peptides to activate TP53 function or to restore the activity of mutant proteins.

►p53
►p53 Family

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TP73L

►p53 Family

tPA

Definition

►Tissue-Type Plasminogen Activator

►Proteinase-Activated Receptors

TPA

Definition

12-O-tetradecanoyl-phorbol-13-acetate. A double ester of phorbol found in croton oil, and commonly known as ►phorbol 12-myristate 13-acetate (PMA). It is a potent tumor promoter often employed in biomedical research to activate the signal transduction enzyme ►protein kinase C. Also can activate JNK and AP-1.

►JNK Subfamily

TR-FRET

►Time-Resolved Fluorescence Resonance Energy Transfer Technology in Drug Discovery

TRA-8

►TRAIL Receptor Antibodies

Trabectedin

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Synonyms

Yondelis; Ecteinascidin 743; ET-743; NSC 684766

Definition

A potent antitumor tetrahydroisoquinoline alkaloid in clinical development originally derived from a marine tunicate and now obtained by a synthetic process developed by PharmaMar starting from microbially produced cyanosafracin B.

Characteristics

Crude aqueous ethanol extracts of the ascidian, or sea squirt, *Ecteinascidia turbinata* were shown to have powerful immunomodulating and antiproliferative properties as early as 1969 but the active principles were not identified until the early nineties. The first six alkaloids that were characterized received the names ecteinascidins 729, 743 trabectedin, (Fig. 1), 745, 759A, 759B, and 770, in accordance with the molecular masses ascribed to these compounds. They revealed a unique chemical structure consisting of a novel pentacyclic skeleton composed of two fused tetrahydroisoquinoline rings (subunits A and B) linked to a 10-member lactone bridge through a benzylic sulfide linkage and attached through a spiro ring to an additional ring system (subunit C) made up of either tetrahydroisoquinoline (as in trabectedin) or tetrahydro β-carboline (as in ET-736). The first two subunits bear a clear structural resemblance to microbially derived safracins and saframycins, and also to sponge-derived renieramycins, all of them less potent anticancer agents than ecteinascidins. On the other hand, a reactive α-carbinolamine or hemiaminal (N-C-OH) group is also present in naphthyridinomycins, quinocarcins, and pyrrolo[1,4]benzodiazepine antibiotics such as ►anthramycin, sibiromycin and tomaymycin. By analogy to these related antibiotics, the potent biological activity of trabectedin and other ecteinascidins was rapidly associated with their ability to form covalent adducts to DNA following *in situ* dehydration of the carbinolamine group to an iminium intermediate that is covalently attached to the amino group of guanine in the minor groove.

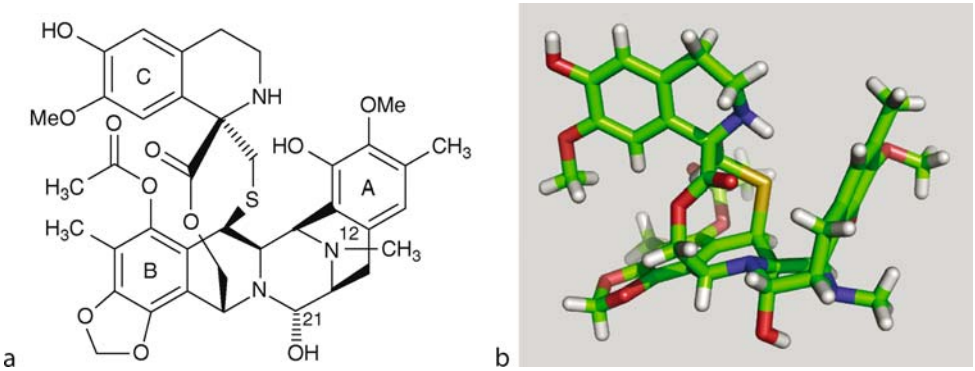
Ecteinascidia turbinata was first harvested from the wild and then successfully grown by the Spanish pharmaceutical company, PharmaMar, in aquaculture facilities in Spain (near Formentera island, in the Mediterranean sea). Subsequently, several synthetic schemes were developed to produce the multigram quantities required for clinical studies worldwide and overcome the limitation of the very low yield (0.0001%) of trabectedin in its natural source.

The first enantioselective total synthesis of trabectedin was achieved in 1996 but industrial manufacturing was made possible through a synthetic route involving the conversion of cyanosafracin B, readily available by fermentation of the bacterium *Pseudomonas fluorescens*, to trabectedin in a very short and straightforward way developed by PharmaMar.

Structural and Biophysical Characterization of Trabectedin-DNA Adducts

Direct evidence that trabectedin alkylates duplex DNA at the exocyclic amino group of guanines was provided by a variety of experiments including gel electrophoresis, DNA footprinting, nuclear magnetic resonance (NMR) spectroscopy, and band shift assays, as well as molecular modeling studies. As a result of this work, it was found that trabectedin was protonated on N12 at physiological pH and a role for hydrogen bonding in sequence recognition and orientation in the DNA minor groove was demonstrated, with TGG, CGG, AGC, GGC, and AGA being established as the preferred DNA triplets for stable adduct formation, and much higher rates of reversibility being measured for site-directed AGT- versus AGC-containing adducts.

The proposed mechanism for activation takes advantage of the increased strength of the hydrogen bond between the proton on N12 and the hydroxyl group on C21 as the trabectedin molecule approaches the minor groove and is desolvated. This proton, which is essential for both sequence recognition and adduct



Trabectedin. Figure 1 Chemical formula and three-dimensional stick representation of the X-ray crystal structure of trabectedin.

stabilization, would then catalyze the dehydration of the carbinolamine yielding the reactive iminium intermediate that undergoes nucleophilic attack at C21 by the exocyclic amino group of the guanine. Since a similar mechanism operates in the activation of pyrrolo[1,4] benzodiazepine antibiotics, it appears that Nature ensures the reactivity of these carbinolamine-containing molecules by the inclusion of an internal catalytic proton adjacent to the leaving hydroxyl group.

As a consequence of trabectedin bonding, the double helical structure is only minimally perturbed except for widening of the minor groove and a net smooth bending towards the major groove due to the introduction of positive roll. This latter feature was novel among minor groove DNA monoalkylating agents as covalent modification of N3 of adenine in AT-rich regions by (+)-CC-1065 and related compounds is accompanied by bending of the DNA into the minor rather than the major groove. Furthermore, if multiple binding sites for trabectedin are properly phased in a relatively short stretch of DNA, the imposed cumulative curvature could bring closer together specified fragments not contiguous in primary sequence, and the drug could be serving a surrogate protein function. On the contrary, binding of three trabectedin molecules in a head-to-tail fashion to three adjacent optimal binding sites would result in no net DNA curvature because the localized bends, brought about by the increase in roll at the sites of covalent attachment, would cancel out over virtually one turn of the helix. In fact, the DNA structure in one such complex (containing the sequence TGGCGGCGG) was shown to be intermediate between the canonical A and B forms of DNA, thereby strongly resembling the conformation that DNA adopts when bound to the consecutive C_2H_2 zinc fingers that are present in transcription factors (►Transcription factor) such as EGR-1 and Sp-1 (which bind to the major groove of GC-rich regulatory sequences in many gene promoters) or that observed in the hybrid double helix of template DNA paired to nascent RNA in the active site of ►RNA polymerase II (RNAPII) elongation complex.

The close contacts and the hydrogen-bonding interaction network that are established between trabectedin and DNA on both sides of the covalent adduct involve both DNA strands and therefore give rise to a significant increment in the stability of the resulting drug-DNA complexes. As a consequence, notable increases in the temperature of thermal denaturation of duplex DNA and substantial blockade of the helicase activities of both simian virus (SV40) large tumor antigen (T-antigen) and bacterial UvrABC and RecBCD enzymes have been reported for DNA oligonucleotides containing trabectedin adducts. This hampering or prevention of strand separation is also expected to result in stalled replication and transcription forks, as observed for a variety of conventional interstrand crosslinkers (e.g. nitrogen mustards, mitomycin or cisplatin). An added advantage in the case of trabectedin would be the minimal distortions inflicted on the normal DNA structure that could help evade some of the recognition and repair mechanisms used for the processing of crosslinks produced in the major groove by these other agents.

Biological Activity

In vitro cytotoxicity studies with trabectedin and other ecteinascidins established subnanomolar potencies against L1210 and P388 mouse leukemia cells, as well as human A549 lung cancer, HT29 colon cancer, MEL-28 melanoma cells and human tumors explanted from patients. Tumor-specific responses and concentration-dependent relationships were observed when a soft agar cloning assay was used to determine the effects of a continuous exposure of trabectedin at different concentrations. These experiments clearly indicated that the duration of exposure to trabectedin was an important factor in human tumors thereby pointing to preferential administration schedules in clinical trials.

In vivo activity was then evaluated in several mouse tumor models and a variety of human tumors xenografted into nude mice, including melanoma, non-small-cell lung carcinoma and ovarian cancer. Long-lasting, complete or partial regressions were observed in both chemo-sensitive and marginally cisplatin-resistant xenografts at the maximum tolerated dose (MTD) but no activity was seen in highly chemo-resistant tumors such as MNB-PTX-1, MEXF 514 and LXFA 629. Importantly, the absence or incomplete cross-resistance with cisplatin and the comparable efficacy against the ovarian carcinoma xenografts justified the clinical assessment of trabectedin in ovarian cancer.

The activity parameters for trabectedin in the panel of 60 human tumor cell lines of the National Cancer Institute (NCI) Anticancer Drug Screen revealed a rather unique profile that encouraged further development as an anticancer agent. The COMPARE algorithm (►COMPARE analysis) established a very high correlation coefficient (0.96) with ►chromomycin A_3, an aureolic acid derivative shown to give rise to a pattern of distinct bands in human metaphase chromosomes, thus suggesting similarities in their apoptotic mechanisms. Despite the fact that these two compounds display very different modes of binding to DNA (i.e. covalent versus noncovalent, carbinolamine activation versus ion-mediated dimerization, etc.), both share a strong binding affinity for some common DNA sites, such as the self-complementary hexanucleotide TGGCCA, to which two trabectedin molecules can bind in a tail-to-tail fashion, each covalently bonded to a different strand. Furthermore, these two natural products are known to exert at least part of their cytotoxicity by interfering with DNA replication and transcription. Thus, at physiologically relevant concentrations (1–100 nM), trabectedin has been

shown to effectively inhibit intracellular DNA synthesis by decreasing replication origin activity and by inducing unusual replication intermediates that may be blocked in fork progression. In addition, trabectedin is able to abrogate the transcriptional activation of a number of genes, including those encoding the multidrug resistance P-glycoprotein (*MDR1*), heat shock protein 70 (*hsp70*), the cell cycle inhibitor p21 ^{Cip1} (*p21*), and collagen α1(I) (*COL1A1*). Nevertheless, global gene expression profiling of trabectedin-treated cancer cells has revealed rather complex patterns of both up- and down-regulation. The reported effects on *MDR1* and additional in vitro data showing enhancement by trabectedin of the cytotoxicity exerted by other chemotherapeutic agents that are substrates for P-gp/MDR1 suggest that combination treatment may be valuable in the clinic.

The extremely low concentrations of trabectedin that are necessary to cause cell cycle arrest and cell death are suggestive of a *trans*-acting mechanism that probably operates through one or more cellular DNA damage response pathways or checkpoints. In this respect, it is notable that cell sensitivity to trabectedin appears to be somehow dependent on a proficient transcription-coupled ►nucleotide excision repair (TC-NER) machinery, and more specifically on the presence of selected components that are implicated in ►xeroderma pigmentosum and Cockayne syndromes. Thus, the initial observation that hamster cells deficient in XPB, ERCC1 or CSB, as well as human XPA and XPC cells, had reduced sensitivity to trabectedin was followed by the report that a human colon carcinoma cell line selected for increased (~20-fold) resistance to trabectedin (following continuous exposure to increasing concentrations of this drug for 1 year) had a truncated and inactive form of the XPG structure-specific endonuclease. Furthermore, drug sensitivity was restored in all cases upon complementation with the respective wild-type protein.

These intriguing effects were recapitulated and expanded using yeast as a simpler eukaryotic model system. It was seen that trabectedin activates the G_2-M and S phase DNA damage checkpoints, in good agreement with the G_2/M block and S phase delay reported in human cells. Likewise, cells deficient in the *XPG* orthologue (*rad13* in *Schizosaccharomyces pombe*) were shown to be much more resistant to trabectedin and underwent much less DNA damage than the corresponding isogenic wild-type strains. However, it became clear that it was not the missing endonuclease activity of this protein that conferred resistance to trabectedin but the lack of part of its DNA-binding domain in the COOH-terminal region. Furthermore, on the basis of a homology model suggesting that the rad13:DNA:trabectedin ternary complex could be stabilized through the direct interaction of subunit C of the drug with a highly conserved arginine residue, an *S. pombe* strain carrying an Arg961→Ala point mutation in rad13 was generated. This mutant displayed normal endonuclease and NER activity but was found to be strongly resistant to the drug.

Haploid yeast mutants with deletions in the *RAD52* epistasis group of genes encoding proteins responsible for ►homologous recombination (HR) and hence most ►double-strand break (DSB) repair in eukaryotic cells (e.g. rad51, rad22 (the fission yeast counterpart of mammalian rad52), and rad54) were found to be extraordinarily sensitive to trabectedin. This result reinforced other indications that the drug is giving rise (directly or indirectly) to DSBs that need to be repaired by homologous recombination, and the fact that the absence of *rad13* partially rescued *rad51Δ* cells supports the view that a rad13-containing complex is somehow involved in the induction or irreparability of lethal DSBs.

These results may have important implications for the optimal use of trabectedin in cancer therapy because patients harboring tumor cells with proficient NER and deficient HR systems would be expected to respond best to the treatment.

Clinical Studies

Trabectedin was selected for clinical development in preference to other related ecteinascidins because of its outstanding potency and greater relative abundance in the tunicate. Among the criteria that were taken into account for bringing it into clinical trials in early 1996 both in Europe and in the United States, we can summarize the following: (i) a novel chemical entity harboring a potential new mode of action, (ii) evidence for a positive therapeutic index, (iii) lack of complete cross-resistance with conventional chemotherapeutic agents, and (iv) feasibility of supply for clinical development. Toxicity to trabectedin so far has been shown to follow a transient-reversible pattern and to be predictable, dose-related, and mostly limited to bone marrow and liver.

Following a favorable opinion adopted by the Committee for Orphan Medicinal Products (COMP) of the European Agency for the Evaluation of Medicinal Products (EMEA), trabectedin was granted by the European Commission orphan medicinal product designation for the treatment of ►soft tissue sarcoma (STS) in April 2001 and for ►ovarian carcinoma (OC) in October 2003. The United States Food and Drug Administration (FDA) awarded Orphan Drug Designation to trabectedin in the indication of STS in October 2004, and in OC in April 2005.

Trabectedin is currently in phase III clinical trials worldwide for ovarian cancer. Extended phase II trials and comparative studies have produced evidence of long lasting responses and tumor control in advanced pretreated sarcomas, breast carcinoma, ovarian carcinoma

and prostate cancer. In July 2007, Yondelis received a positive opinion from the EMEA for the treatment of metastatic or advanced soft tissue sarcoma after failure to anthracyclines and ifosfamide.

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Trabecular Bone

Definition

Type of bone tissue with a low density and strength but very high surface area, that fills the inner cavity of long bones (also known as cancellous, or spongy bone).

►Lead Exposure

Trace Elements

Definition

Refers to microminerals, are mineral nutrients which are typically required to be ingested by humans in amounts of a few milligrams or less per day. This category includes iron, zinc, copper, manganese, selenium, iodine, chromium, molybdenum, and vanadium. Sometimes those mineral nutrients required in amounts of a few micrograms per day are referred to as ultratrace minerals.

►Mineral Nutrients

TRAF-1

Definition

►Tumor necrosis factor-associated factor 1; One of a number of adapter molecules that are involved with tumor necrosis factor receptor superfamily signaling.

►Hodgkin Disease, Clinical Oncology

TRAF2

Definition

Tumor necrosis factor-associated factor 2.

Traffic ATPases

►ABC-Transporters

TRAIL

Definition

►Receptor for TNF-Related Apoptosis-Inducing Ligand
►TNF-Related Apoptosis Inducing Ligand.

TRAIL Receptor Antibodies

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Synonyms

DR4 antibodies; DR5 antibodies; Lexatumumab; Mapatumumab; TRA-8

Definition

►TRAIL induces ►apoptosis preferentially in malignant tissues. Therefore TRAIL is considered to be a potential antineoplastic drug. Agonistic TRAIL receptor antibodies have been developed as alternative pharmacological tool for apoptosis induction via the TRAIL receptors.

Characteristics

After having identified TRAIL as a member of the family of cell death inducing ligands, it became obvious that TRAIL has a strong propensity for transformed or malignant tissues. Therefore TRAIL is considered to be a candidate anticancer drug. TRAIL exerts its apoptosis inducing activity via the two respective agonistic TRAIL receptors DR4 and DR5. TRAIL itself is able to induce cell death in a wide array of cancer cells in vitro or when grown in xenograft settings. The efficacy of TRAIL is increased whenever the ligand is combined with conventional cytostatic agents or ionizing radiation. Depending on the production process, there was concern that TRAIL, like ►CD95-L, could exert considerable hepatotoxicity. Recently released data from phase I trials suggest that TRAIL can be safely administered in patients up to serum concentrations consistent with those demonstrating efficacy in tumor xenograft models.

In parallel to the development of TRAIL as anticancer drug, agonistic antibodies directed against both death inducing TRAIL receptors were developed. Up to now relevant data on three agonistic TRAIL antibodies are available. The signaling pathways triggered by agonistic antibodies have not been reported to differ from the cascades triggered by TRAIL. Treatment of susceptible cells with agonistic antibodies results in the activation of ►caspase-8, caspase-9, and cleavage of ►PARP. As shown for TRAIL, agonistic TRAIL antibodies also induce cleavage of the anti-apoptotic MCL-1 protein. How far key regulatory molecules including ►FADD, ►c-FLIP, and caspase-10 are involved in the regulation of cell death induction via agonistic TRAIL antibodies has not been tested in detail. However, the fact that there is a cross resistance between TRAIL and the agonistic antibodies in such a way that cells being resistant towards TRAIL cannot be killed by either antibody indicates that the signaling pathways are identical or at least highly similar.

The agonistic TRA-8 antibody directed against DR5 has been developed by Sankyo together with researchers from the University of Alabama (Birmingham, USA) and was the first agonistic antibody being described. TRA-8 was generated by immunizing BALB/c mice with a fusion protein containing the extracellular domain of DR5 and the Fc proportion of human IgG1. The antibody does not cross react with murine DR5. The K_d values for TRAIL or TRA-8 binding to DR5 were estimated at 59 and 3 nM, respectively. The high specificity of TRA-8 for DR5 was documented by competition assays showing that TRA-8 efficiently competed with TRAIL for binding to DR5 but not for binding to DR4. In addition, these results indicate that TRA-8 potentially recognizes an epitope within the TRAIL-binding site on DR5. In general, TRA-8 induces apoptosis in tumor cell systems in vitro as well as in murine xenograft model systems (Jurkat and 1321N1 astrocytoma cells). In contrast to the TRAIL preparation used for comparison, the TRA-8 antibody did not induce any signs of hepatopathy in mice.

In subsequent studies, the increased efficacy of multimodal approaches combining either TRA-8 with radiation, chemotherapy or other response modifiers was documented. In this regard it is important to notice that the efficacy of the tested combinations in term of growth delay was shown in ►xenograft models for ►cervical cancer, ►breast cancer and ►pancreal cancer. The overexpression of Bax using an adenoviral vector system increased the efficacy of TRA-8 in a wide array of glioma cells suggesting that bax is involved in the efficacy of the combined treatment. The increased cell death induction translated into an increased growth delay. Besides the TRA-8 antibody, Sankyo also develops an agonistic DR4 antibody 2E12. However, considerably less data are available regarding the pharmacology and efficacy of this antibody.

The second group of antibodies was developed by Cambridge Antibody Technology in conjunction with Human Genome Sciences (Rockville, USA). HGS-ETR1 (Mapatumumab) is directed against DR4 and HGS-ETR2 (Lexatumumab) is directed against DR5. HGSI also develops a third agonistic TRAIL antibody (HGS-TR2, targeting DR5) that was initially developed by Kirin Brewery Ltd. Up to now only data on Mapatumumab and Lexatumumab are available. Mapatumumab is a fully humanized monoclonal antibody and was isolated from 102 different anti-TRAIL receptor mAbs that were generated by phage display technology. The antibody has a high affinity for the DR4 receptor and exerts antitumor activity (EC50 values of ~3.4 nM) in diverse preclinical tumors models including breast, gastrointestinal, lymphoma, ovarian carcinoma, and uterine cancers. Mapatumumab was shown to specifically recognize the TRAIL-R1 protein without any relevant interactions with the TRAIL decoy receptors. The efficacy of mapatumumab is increased by combinations with various cytostatic drugs including carboplatin, cisplatin, camptothecin, topotecan, paclitaxel as well as radiation. Xenograft models for breast, colorectal, nonsmall cell lung cancer as well as uterine cancer revealed a high activity of either the drug alone or in combination with other cytotoxic treatment approaches including radiation.

Like mapatumumab, lexatumumab is a fully humanized IgG1g antibody. The antibody was also generated using a phage display and screening with 383 short chain fragments for DR5 binding properties. In contrast to mapatumumab, no data on specificity, selectivity, and affinity of the antibody are publicly available. Similar to TRA-8, lexatumumab exert pronounced apoptotic reactions in a wide array of malignant cell systems when used alone. Importantly the drug has proven efficacy on tumor growth in xenograft systems from renal cell carcinoma, nonsmall cell lung cancer, breast cancer, and glioma. As already shown for TRA-8, the combination of lexatumumab with various chemotherapeutic agents (camptothecin, cisplatin, carboplatin, paclitaxel, doxorubicin, bortezomib) or radiation increased the efficacy in cell lines and xenografts. The underlying mechanisms of sensitization are still not completely understood. However, it seems likely that the presence of the proapoptotic Bax molecule as well as the upregulation of the respective receptor participate in the increased efficacy of the combined approach.

Clinical Aspects

Mapatumumab (anti-DR5)

Data from several early clinical trials are available and allow a cautious judgment regarding pharmacological and toxicological aspects of mapatumumab. An open label phase Ia/b trial was conducted in 39 patients with various advanced solid tumors.

During the first phase, dose escalation of mapatumumab was performed (0.01, 0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg). The second phase of the trial involved administration of mapatumumab (10 mg/kg) once every 28 days or once every 14 days. The i.v. administration of mapatumumab produced dose-proportional pharmacokinetics up to a dose of 1.0 mg/kg, with a half-life of 15 days for 1.0 mg/kg. The pharmacokinetic data indicate that distribution and clearance follow a two-compartment model, with first order elimination from the central compartment. The best clinical responses reported so far are stable diseases in a proportion of the heavily pretreated patients. No data from ongoing trials combining chemotherapy with TRAIL are available at present.

In addition to various phase I trials, mapatumumab was tested in a multicenter phase II trial in patients with relapsed or refractory non-Hodgkin lymphoma. Patients (n = 40) received either (3 or 10 mg/kg mapatumumab once every 21 days). Partial responses were observed in three patients, and on one patient with relapsed follicular mixed-cell lymphoma demonstrated a more pronounced regression. Two other phase II trials with mapatumumab are ongoing. One trial was conducted in patients with relapsed or refractory colorectal cancer. No data on safety, tolerability, pharmacokinetics, tumor response, time to response, duration of response, and progression-free survival from this trial are available.

The second phase II trial including patients with solids tumor was performed in 32 patients with nonsmall cell lung cancer (median of three previous treatment cycles). These patients received 10 mg/kg mapatumumab every 21 days until disease progression. Mapatumumab was well tolerated with not treatment discontinuations due to drug-related toxicity. In 29% of these patients a stable disease (median duration of 2.3 months) was observed. The most common mapatumumab related adverse events were nausea, fatigue, hypotension, myalgia, pyrexia, peripheral sensory neuropathy, diarrhea, constipation or abdominal pain, rash, hypertension, and thrombocytopenia. A clear maximum-tolerated dose had not been achieved. No antibodies to mapatumumab had been observed. The phase II trial in NSCLC patients revealed that mapatumumab administration was generally safe and well tolerated. In 97% of these patients at least one adverse event reported, however, only 44% of the patients experienced an adverse effect that was considered to be drug related. Again, no immunogenic responses were observed.

Lexatumumab (anti-DR5)

Data from several phase I trials using lexatumumab are available. Results from a US dose escalation trial (0.1, 0.3, 1.0, 3.0, and 10 mg/kg i.v. lexatumumab every 2 weeks) revealed that dose responses were linear up to the 10mg/kg level with a mean half-life of 11 day at the 10-mg/kg dose level. The analysis of a similar trial performed in the UK (37 patients with advanced cancer of different organs sites treated with doses of 0.1, 0.3, 1.0, 3.0, 10, or 20 mg/kg every 3 weeks) revealed linearity over the whole dose range and a distribution model consistent with a two compartment model with first-order elimination from the central compartment (reported pharmacological values: mean parameters for the 1- and 10-mg/kg groups were: C_{max} = 24.0 and 195.9 μg/ml; AUC = 190.8 and 2379 μg.days/ml; half-life = 12.0 and 15.3 days; plasma clearance = 5.7 and 4.8 ml/kg/day; V1 = 44.1 and 50.8 ml/kg and VdSS = 83.7 and 87.1 ml/kg).

Clinical results from both trials have been reported with no major toxicity. Of 31 patients entered in the US trial, 10 experienced disease stabilization and 20 had disease progression. Of 37 patients entered in the UK trail, 11 experienced disease stabilization and 26 had disease progression. Data from the studies indicate that lexatumumab was well tolerated at doses up to 10 mg/kg. The most frequently reported toxicities were: fatigue, nausea, anorexia, constipation, diarrhea, tachycardia, and vomiting. The DLT for Lexatumumab was

defined as 10 mg/kg. No data from phase II trials are available at present.

TRA-8 (CS1008)

Up to now no results from clinical trials have been reported with the humanized version of TRA-8 (CS1008).

Perspectives

TRAIL receptor based treatment strategies are currently entering clinical trials. The feared liver toxicity of TRAIL and agonistic compounds has not been documented in any of clinical trials currently available. No judgment on the definitive clinical anticancer activity of agonistic TRAIL receptor antibodies can be made although the phase I and phase II data revealed some clinical activity.

TRAM-1

►Amplified in Breast Cancer 1

Trans-Golgi Network

Definition

Is part of the Golgi apparatus, which is an ►organelle found in ►eukaryotic cells. The primary function of the Golgi apparatus is to process and package ►macromolecules synthesized by the cell, primarily ►proteins and ►lipids. Trans-Golgi network is the last station of the Golgi apparatus and it directs proteins in the secretory pathways to the appropriate cellular destination.

Transabdominal Metastasis/ Dissemination

►Transcoelomic Metastasis

Trans Activating Protein

Definition

Is a protein that alters gene expression at sites other than those adjacent to where it is being encoded.

►Transcription Factor

Transactivation

Definition

Is the stimulation of gene expression by diffusible mediators, for example proteins that are usually encoded on distant regions of the genome.

►Transcription Factor

Transactivation Domain

Definition

TADs are most frequently found in ►transcription factors. These protein modules interact with the basic ►transcription machinery and functions to actively mediate transcription of downstream target genes.

Transarterial

Definition

Through the artery.

Transarterial Chemoembolization

Definition

An interventional procedure mostly used to treat cancer. Physicians first use a needle to puncture into the artery. Then a thin catheter is threaded into the tumor through

the blood vessel under continuous radiological imaging guidance. Anti-tumoral chemotherapeutic agents and small ►embolization particles are injected into the tumor to induce tumor cell death.

Transcoelomic Metastasis

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Synonyms

Peritoneal metastasis/dissemination; Transabdominal metastasis/dissemination

Definition

Dissemination or spread of malignant tumor throughout the peritoneal (abdominal and pelvic) cavity.

Characteristics

Transcoelomic (meaning "across the peritoneal cavity") ►metastasis refers to the dissemination of malignant tumors throughout the surfaces and organs of the abdominal and pelvic cavity covered by ►peritoneum. Transcoelomic metastasis can occur as a result of ►invasion into the peritoneal cavity by: (i) a primary cancer arising from within the abdominal/pelvic cavity e.g. ►ovarian cancer; (ii) as a manifestation of systemic metastasis following haematogenous or ►lymphatic invasion by a primary cancer e.g. advanced ►breast cancer; or (iii) following intraperitoneal seeding during surgical manipulation e.g. during surgical resection of a colorectal tumor.

The incidence of transcoelomic metastasis is higher with tumors that arise from the peritoneal cavity e.g. ovarian (up to 70% of patients at presentation) and colorectal (up to 28% of patients at presentation). In contrast, extraperitoneal cancers e.g. breast and lung, are associated with a much lower overall incidence of transcoelomic metastasis, although certain histological subtypes e.g. infiltrating lobular breast cancers, have demonstrated a greater predilection for metastases to the gastrointestinal tract, gynecological organs and peritoneum/►retroperitoneum. This suggests that whilst the location of the primary tumor may be a key determinant in the development of transcoelomic metastasis, the tumor phenotype is also an important factor. Hence it appears that a combination of anatomical and tumor-specific factors is involved in the transcoelomic metastatic process.

Transcoelomic metastases contribute considerably to the morbidity associated with carcinomatosis because they have the capacity to affect multiple vital organs within the abdomen. Common examples include bowel obstruction caused by lesions along the gastrointestinal tract, and renal failure caused by obstruction of the ureters. In addition, transcoelomic metastases are frequently associated with the formation of malignant ►ascites, resulting in raised intra-abdominal pressure with consequent abdominal distension and discomfort. This results in early satiety, leading to dietary deficiency, impaired circulation of blood and ►lymphatic vessels, and respiratory compromise secondary to diaphragmatic splinting. Hence, there are potentially significant therapeutic advantages to be gained in understanding the process of transcoelomic metastasis.

Mechanisms of Transcoelomic Metastasis

Models of Metastasis

Two models have been hypothesized for the genetic origins of tumor metastases. The first model, often referred to as the seed-and-soil hypothesis, is that tumors are genetically heterogeneous and metastases arise from clones with a genetically acquired metastatic phenotype, which determines the final site of metastasis. The alternative hypothesis, the stochastic model, is that metastatic cells do not represent a genetically selected clone distinct from the primary tumor, but arise as a stochastic event from tumor cell clones genetically identical to the primary tumor. Recent studies exploring this question using *in vivo* models have suggested a combination of both models of metastasis. Regardless of metastatic model, there are certain observed characteristics that appear to be important for transcoelomic metastastic progression, in which complex cellular adaptations need to occur after cell detachment from the primary tumor mass to ensure survival within the peritoneal cavity.

Cell Detachment

Anchorage-independent growth and the ability to resist ►anoikis is a vital step for the initiation of metastasis. This process appears to involve the increased expression of ►survivin and X-linked inhibitor of ►apoptosis (XIAP), members of the inhibitor of apoptosis protein (IAP) family, which suppress apoptosis by inhibition of ►caspases. Other mediators of anoikis resistance include the family of ►extracellular matrix (ECM) to ►cell-adhesion molecules known as ►integrins. Alterations in levels of integrin-mediated ECM-ligand binding have been found in many different tumor types and can result in decreased cell-adhesion, changes in cell morphology and increased ►migration *in vitro*, and

activation of ECM degrading enzymes including ►matrix-metalloproteinases (MMP).

Peritoneal Fluid and Anatomy

The peritoneal cavity is normally empty except for a thin film of fluid that keeps surfaces moist. Peritoneal fluid arises primarily from plasma transudate and ovarian exudate. Other sources of peritoneal fluid include fallopian tubal fluid, retrograde menstruation and macrophage secretions. The volume of peritoneal fluid is usually 5–20 ml, and varies widely depending on physiological or pathological conditions. Peritoneal fluid contains a variety of free-floating cells, including ►macrophages, ►natural killer (NK) cells, lymphocytes, eosinophils, mesothelial (peritoneal surface epithelial) cells and ►mast cells, which are all involved in immunological surveillance. Intraperitoneal fluid flow is directed by gravity to its most dependent sites and then drawn via the paracolic gutters to the diaphragm by the generation of negative intra abdominal pressure in the upper abdomen during respiration. There is preferential flow along the right paracolic gutter, liver capsule, and diaphragm. Therefore, a natural flow of peritoneal fluid exists within the abdominal cavity, providing a route for the transcoelomic dissemination of detached tumor cells.

As the epithelial surfaces of the female genital tract (i.e. ovaries, fallopian tubes and endometrium) share a common embryological lineage with the peritoneal epithelium, it has been suggested that transcoelomic metastasis from gynecological malignancies, such as fallopian tube and ovarian tumors, are not true metastases but a result of malignant transformation at multiple foci throughout the peritoneum, i.e. peritoneal ►metaplasia. If the metaplasia hypothesis is correct, then one might expect metastatic lesions to be randomly distributed throughout the peritoneum. Alternatively, if the theory of dissemination via peritoneal/ascitic fluid is true, then one might expect that detached tumor cells would, by virtue of gravity, be more frequently implanted in the floor of the pelvis, e.g. the pouch of Douglas (the space between the rectum and back wall of the uterus), followed by the organs in the paracolic gutters, and finally on the diaphragm, i.e. along the normal route of peritoneal fluid circulation. Studies have shown that a high incidence of metastatic implants for all cancers, including ovarian malignancies, within the peritoneal cavity is found on organs where peritoneal fluid resorption occurs (►omentum and omental appendages). In addition, the colon, greater omentum and pouch of Douglas are most often affected, with a reduced incidence of implants seen on the small bowel and its mesentery, which is free to move by peristalsis, compared to the ileocaecal area (the junction between the ileum and cecum), which is fixed to the retroperitoneum. Hence, location and topography with regard to the flow of peritoneal/ ascitic fluid appear to be key determinants in the process of transcoelomic dissemination for all cancers. As such, in the case of gynecological cancers, peritoneal metaplasia alone appears unable to fully account for the peritoneal distribution of carcinomatosis.

Ascites: A Metastatic Milieu

The development of transcoelomic metastasis is often associated with the formation of excess peritoneal fluid known as malignant ascites. It is hypothesized that, in addition to hypoalbuminaemia (low plasma albumin levels) secondary to dietary deficiency, at least three other pathological events can cause ascites: (i) reduced lymphatic drainage from the peritoneal cavity caused by the obstruction of lymphatic vessels by tumor cells; (ii) increased vascular permeability of the peritoneal cavity; and (iii) tumor neo-►angiogenesis. While lymphatic obstruction is a well-recognized cause of ascites, the fact that massive amounts of fluid can accumulate in patients despite relatively little tumor burden suggests the involvement of other non-obstructive factors. These include ►vascular endothelial growth factor (VEGF), a glycoprotein which induces angiogenesis and increased vascular permeability in response to hypoxia. Other immune modulators, vascular permeability factors, and MMPs secreted by both tumor cells and mesothelial cells also contribute significantly to ascites formation and stimulate tumor growth, invasion and angiogenesis.

Immune Evasion

Many immune cells, such as macrophages, are present in peritoneal fluid, and accumulate in so-called "milky spots" within the omentum. These omental macrophages have been found to be cytotoxic against tumor cells *ex vivo*. Consequently, omental macrophages might play an important role in killing tumor cells, thereby preventing development of transcoelomic metastasis and local peritoneal recurrences. Paradoxically, however, *in vivo* studies have shown that cancer cells seeded intraperitoneally specifically infiltrate the milky spots in the early stage of peritoneal metastasis. These studies suggest that omental milky spots are insufficient to prevent tumor progression, and that intraperitoneal metastasis requires tumor cells to possess or acquire mechanisms for evasion of immunological surveillance.

Tumor-infiltrating and malignant ascites-derived lymphocytes, in particular gamma–delta T cells, from patients with metastatic ovarian and colorectal cancer, have also been shown to possess antitumor activity. Hence, it appears that metastatic tumor cells have also developed strategies to evade T cell-mediated cytotoxicity. Fas ligand (FasL) is a transmembrane protein belonging to the tumor necrosis factor superfamily

that can trigger apoptotic cell death following binding to its receptor, Fas. Expression of FasL has been observed in renal, ovarian, colorectal, and head and neck tumors and may be responsible for the immune privilege of tumor cells by inducing apoptosis of antitumor immune effector cells within the tumor microenvironment – the "Fas counterattack". Studies have also shown that tumor progression and metastasis is associated with increased expression of FasL. Other examples of immune evasion include the recruitment of regulatory T (Treg CD4+CD25+) cells to suppress tumor-specific T cell immunity; the presence of high concentrations of soluble forms of the complement pathway inhibitors C1 inhibitor, factor H and FHL-I on isolated metastatic ovarian cancer cells in ascitic fluid; and the phenomenon of spheroid formation observed in ►breast, colorectal and ovarian cancer where tumor cells clump together by upregulating cell-adhesion molecules, thus resulting in increased complement resistance due to insufficient penetration of antibodies and complement into the spheroids.

Tumor Implantation

Although topography appears to be a key determinant in the final site of metastatic implantation within the peritoneum, the actual mechanisms behind tumor implantation remain unclear. However, there is evidence to suggest the involvement of a dynamic regulation of the tumor cell's adhesiveness, and its interaction with the underlying peritoneal mesothelium.

Potential mechanisms for the attachment of tumor cells to the peritoneal mesothelium include binding to ECM proteins like collagen type I and IV, laminin, and fibronectin via tumor cell surface integrins, and to hyaluronan expressed on the surface of human peritoneal mesothelial cells via the ►CD44 tumor cell surface protein, of which there are 10 alternative exon splice variants (v1–v10). Upregulation of certain CD44 variants have been associated with distant metastasis in breast, colorectal and ovarian cancer. Recently, tumor antigen/marker CA125, a glycoprotein overexpressed on the cell surface and secreted by ovarian tumor cells in the majority of ovarian cancer patients, has been shown to bind to mesothelin, a glycosylphosphatidylinositol-linked cell surface molecule expressed by mesothelial cells. Upregulation of the cell adhesion molecule ►E-cadherin may also mediate adhesion of circulating tumor cells to metastatic sites. Adhesion onto the peritoneal surface may be followed by haptotatic migration in which coordinated anti- and pro-migratory signals mediated by ECM proteoglycans confers directionality to tumor cell motility, effectively laying the tracks until a "stop" signal is encountered. Once attached to the peritoneal surface, metastatic cells proliferate and invade into the subjacent epithelium. The MMP family of proteinases and the urokinase-type ►plasminogen activator (uPA) system appear to be major contributors to this process. Human peritoneal epithelial cells and their associated immune and stromal cells, have been shown to release regulatory ►chemokines and cytokines, such as IL-1, ►IL-6 and IL-8, in response to serosal inflammation and injury induced by tumor implantation, which in turn facilitate tumor angiogenesis and ascites formation (via increased secretion of VEGF), and enhanced tumor migration, attachment, proliferation and invasion.

Finally, just as extraperitoneal tumors can metastasize to the peritoneum, intraperitoneal tumors can also metastasize extraperitoneally. Apart from the rich intraperitoneal network of blood and lymphatic vessels which can be invaded by tumors, peritoneal fluid is also continually being returned to the systemic circulation via the subdiaphragmatic lymphatic network and thoracic duct into the left subclavian vein, thus providing a direct "metastatic expressway" for peritoneal metastases to gain access into the lymphatic and circulatory system.

Clinical Aspects

Patients with transcoelomic metastasis often present with signs and symptoms of abdominal pain, abdominal distension secondary to an enlarging tumor or ascites, constipation or diarrhea, shortness of breath, fatigue, loss of appetite, and weight loss. A careful clinical history followed by thorough clinical examination is required to ascertain the likely source of the primary tumor. Investigations should include routine blood tests, including relevant tumor markers, followed by radiological investigations including ultrasound and computer tomographic (CT) scans of the chest, abdomen and pelvis to confirm the likely source of tumor and disease ►stage. In all cases, particularly those in which there is no obvious source of primary tumor (i.e. carcinoma of unknown primary origin), a biopsy of an accessible lesion should be obtained for histopathological and immunohistochemical confirmation and diagnosis.

In the past, clinical situations involving transcoelomic metastasis were treated mainly with ►palliative intent. Increasingly, studies have shown that an aggressive approach to peritoneal surface malignancy involving ►peritoneal debulking (cytoreductive) procedures, combined with optimal perioperative or postoperative systemic or intraperitoneal ►chemotherapy in carefully selected patients can result in long term survival. Clinical assessment parameters that need to be considered include the patient's ►performance status, preoperative abdominal and pelvic CT scans to define the extent and operability of disease, including the presence of extraperitoneal metastases, and tumor histopathology. Key prognostic indicators following surgery include the completeness of ►peritoneal debulking

surgery, the presence of intraperitoneal lymph node and visceral metastases, and tumor type. Of the various scoring systems used to assess the extent of peritoneal carcinomatosis, the most frequently quoted is the peritoneal cancer index (based on the intraoperatively observed distribution and size of intraperitoneal metastasis) and the completeness of cytoreduction score (based on the amount of residual disease following peritoneal debulking surgery), which have been found to correlate well with prognosis in ►colorectal, ►gastric and ►ovarian cancer. A meta-analysis of studies comparing combined peritoneal debulking surgery and perioperative intraperitoneal chemotherapy with systemic chemotherapy alone for the treatment of peritoneal carcinomatosis from ►colorectal carcinoma, has demonstrated improved survival in the combination therapy group. In patients with ovarian cancer and peritoneal metastasis, 2-year survival following radical resection of all macroscopic tumors is 80%, in contrast to less than 22% for the patients with residual lesions larger than 2 cm. Early aggressive treatment of minimal peritoneal surface dissemination appears to confer the most benefit.

In patients with inoperable tumors at presentation, primary systemic or intraperitoneal chemotherapy is recommended, following which reassessment for surgical intervention may be possible if a good treatment response is observed. Palliative measures in the management of malignant ascites include repeated paracentesis (drainage of ascites), which provides relief in up to 90% of patients, and permanent percutaneous drains. The creation of a peritoneovenous shunt (which allows ascitic fluid to drain from the peritoneal cavity into the superior vena cava) prevents the need for repeated paracentesis. Promising experimental approaches in the treatment of transcoelomic metastasis include the use of intraoperative hyperthermic intraperitoneal chemotherapy, anti-►angiogenic agents such as the MMP inhibitors and the VEGF antagonists, as well as ►immunotherapy approaches including antibody targeted T cell therapy and combinations of intraperitoneal immunotherapy and thermochemotherapy.

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Transcript

Definition

Fragment of RNA synthesized from a given DNA template.

Transcription

Definition

Messanger RNA (mRNA); The process by which the genetic code is transferred from DNA to messenger RNA, so that it can be subsequently translated into a protein sequence. Is the first step in gene expression. It involves the faithful synthesis of RNA from the genetic information stored in DNA. It is carried out in the nucleus by DNA-dependent RNA polymerases. A transcriptional activator is a DNA-binding protein that stimulates transcription by interacting with DNA sequence motifs present in the regulatory sequences of the controlled gene. A transcriptional repressor inhibits transcription.

►Orphan Nuclear Receptors and Cancer

Transcription-Coupled Repair

Definition

Is a DNA repair mechanism that operates in tandem with transcription. It directly repairs damaged base on the DNA strand by directly removing or repairing the damaged DNA base. Is the rapid repair of DNA damage in the transcribed strand of expressing genes.

►Homologous Recombination Repair
►DNA-Damage Tolerance

Transcription Factor

Definition

A protein that regulates gene ►transcription by binding to regulatory regions of DNA and/or to other

transcription factors. The presence of these specific DNA sequences confers upon a specific gene the ability to respond in a specific cellular context, to particular stimuli, or at a defined developmental stage. The most fundamental property of all transcription factors is their ability to influence the transcriptional activity of specific genes by acting in either a positive or negative manner. In the context of cancer, there is often a dysregulation in the balance between transcriptional activation and transcriptional repression that alters the expression of critical cancer-promoting or cancer-suppressing genes. Transcription factors are generally divided into two groups: (i) basal transcription factors (BTFs) and (ii) gene-specific transcription factors (GSTFs). The types of GSTFs are named based on the characteristic motifs involved in DNA binding and protein dimerization.

Transcription Factory

Definition

Discrete nuclear compartment where ►transcription takes place.

Transcriptional Complex

Definition

A complex of proteins that directly or indirectly, affects the initiation of ►transcription.

Transcriptional Coregulators

Definition

These are proteins that interact with transcription factors and regulate transcription. They can either be activators of transcription (enhance) or repressors of transcription (inhibit) and may act to modify chromatin structure.

►Orphan Nuclear Receptors and Cancer

Transcriptional Memory

Definition

Mechanism to ensure that specific genes are expressed at specific times so that the genetic information is transmitted to daughter cells to maintain the differentiated cell type.

Transcriptional Regulation

Definition

Regulation of gene expression by ►transcription factors.

Transcriptional Silencing

Definition

Repression of gene ►transcription in a localized region via structural changes in chromatin.

►Histone Deacetylases
►Methylation

Transcriptome

Definition

The full complement of activated genes, mRNAs, or transcripts in a particular tissue at a particular time.

Transdifferentiation

Definition

Transformation of a non-stem cell into a different cell type or the production of cells from a differentiated stem

cell that are not related to its already established differentiation path.

►Adult Stem Cells
►Stem Cell Plasticity

Transduction

Definition

Transfer of genetic material into a cell by means of a virus

►HSV-TK/Ganciclovir Mediated Toxicity
►Transduction of Oncogenes

Transduction of Oncogenes

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Synonyms

Oncogene transduction; Retroviral transduction

Definition

►Retroviruses are RNA-containing viruses that replicate through a DNA intermediate (►provirus) using the enzyme ►reverse transcriptase. During retroviral replication, which requires integration into the host chromosomal DNA for efficient transcription of viral RNA, some retroviruses have acquired specific cellular ►oncogenes, usually with multiple modifications, and often with the loss of trans-acting viral functions. Inclusion of one or more oncogenes in the viral genome then imparts transforming activity on the recombinant virus independent of the site of integration in the cellular genome.

Characteristics

Identification of Cellular Oncogenes

In 1911, Peyton Rous described the isolation of a virus that caused fibrosarcomas in chickens. The Rous sarcoma virus (RSV) subsequently was shown to transform chicken embryos and the surrounding membranes and formed small tumors on the chorioallantoic membrane in proportion to the number of virus particles. Further quantitative assays were developed when RSV and other ►retroviruses were shown to transform or induce morphological and growth behavior changes in cultured cells that mimicked tumor formation in the animal. The ability of RSV to transform cells in culture led to the conclusion that the virus encoded a gene responsible for such changes. Isolation of transformation-defective variants of RSV allowed comparisons with wild-type RSV and the discovery of the viral oncogene, v-*src*. Experiments from the laboratories of Harold Varmus and Michael Bishop revealed that the v-*src* gene is highly related to a specific cellular gene or proto-oncogene, c-*src*, which encodes a protein tyrosine kinase. Unlike the v-*src* gene in RSV, the cellular homologue contained introns, which could be alternatively spliced in various cell types to give different mRNAs. Further characterization showed that the product of the v-*src* gene, v-Src, had substitutions within several functional domains that prevented the normal regulation of the kinase activity during the process of ►signal transduction.

Shortly after the discovery of v-*src*, other transforming viruses were isolated and characterized. Some of these viruses were recovered by treatment of normal cells with halogenated pyrimidines to induce ►endogenous retrovirus expression, by co-culture of primary cells with chemically transformed cells that express non-transforming ►retroviruses, whereas others were isolated by in vivo passage of non-transforming ►retroviruses. In each case, the transforming virus appears to be the result of recombination between a non-transforming ►retrovirus and one or more cellular genes. The ability of ►retroviruses to acquire cellular sequences in their genome and transmit these genes to other cells is known as retroviral ►transduction or, in the case of proto-oncogenes, oncogene ►transduction. Examples of transforming viruses and the acquired proto-oncogenes are listed in Table 1. Deregulation can occur at many steps of ►signal transduction, leading to oncogenesis.

Nevertheless, recombination events leading to the generation of a transforming ►retrovirus appear to be rare in nature. Most of the resulting viruses are defective for replication because the acquisition of cellular proto-oncogenes is accompanied by the deletion of viral structural genes, which are necessary to produce viral particles. Such defective transforming viruses are not transmissible unless they successfully co-infect a cell with a related ►retrovirus that provides the missing gene products in *trans*. Thus, most transforming viruses are more interesting as research tools for the identification and functional characterization of oncogenes and the process of ►transduction than as a major cause of disease in animals and humans.

Transduction of Oncogenes. Table 1 Oncogenes transduced by retroviruses

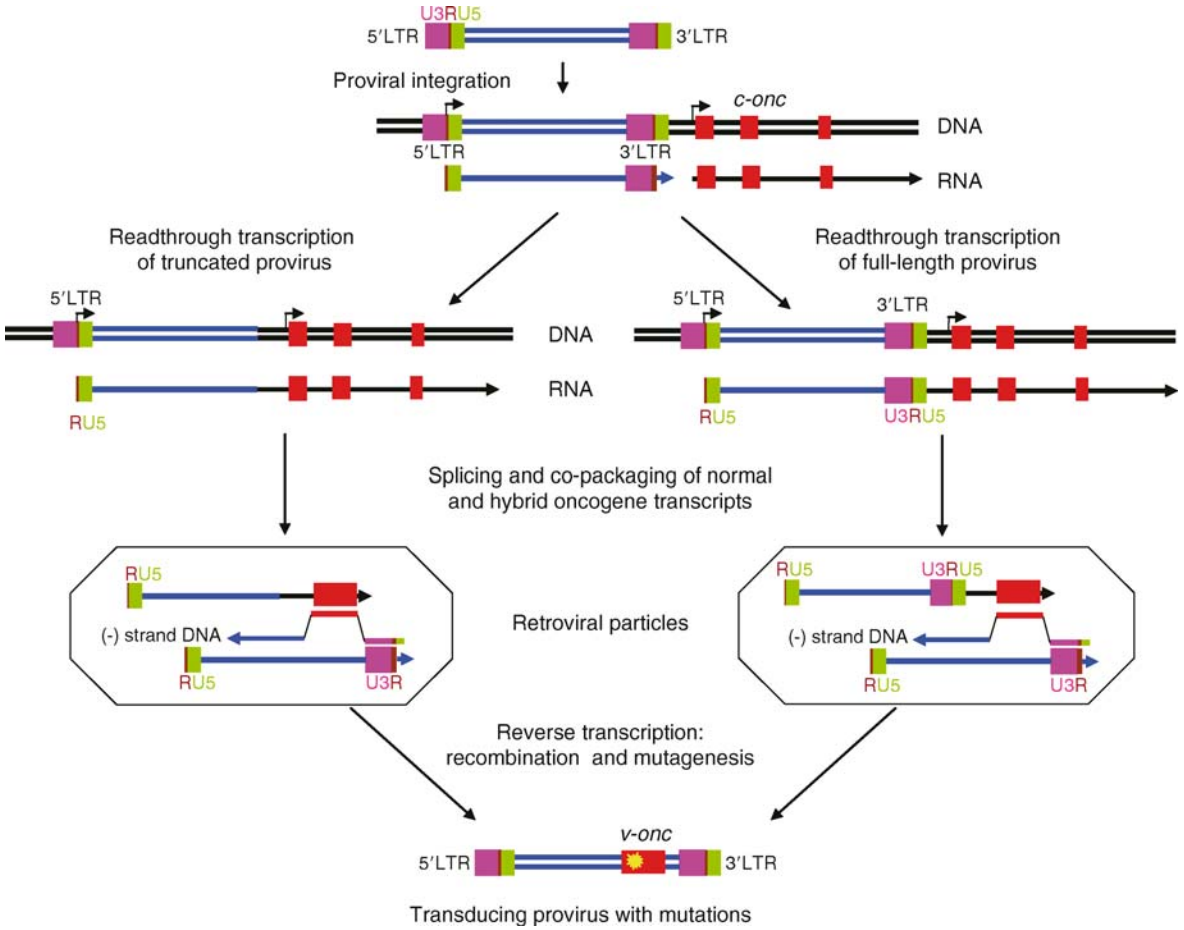
Transforming virus	Acquired proto-oncogene
Abelson murine leukemia virus	*abl*
AKT8	*akt*
Cas NS-1 virus	*cbl*
Avian sarcoma virus CT10	*crk*
Avian erythroblastosis virus ES4	*erbA*
Avian erythroblastosis virus ES4	*erbB*
Avian myeloblastosis virus E26	*ets*
Avian retrovirus RPL30	*eyk*
Snyder-Theilen feline sarcoma virus	*fes*[a]
Gardner-Rasheed feline sarcoma virus	*fgr*
McDonough feline sarcoma virus	*fms*
Finkel-Biskis-Jenkins murine sarcoma virus	*fos*
Fujinami avian sarcoma virus	*fps*[a]
Avian sarcoma virus 17	*jun*
Hardy-Zuckerman-4 feline sarcoma virus	*kit*
Avian retrovirus AS42 (sarcoma)	*maf*
Mill Hill virus 2 (avian myelocytoma virus)	*mil*[b]
Moloney murine sarcoma virus	*mos*
Mouse myeloproliferative leukemia virus	*mpl*
Avian myeloblastosis virus E26	*myb*
Myelocytomatosis virus 29	*myc*
Avian retrovirus ASV31 (sarcoma)	*qin*
3611 murine sarcoma virus	*raf*[b]
Harvey murine sarcoma virus	H-*ras*
Kirsten murine sarcoma virus	K-*ras*
Avian reticuloendotheliosis virus T	*rel*
UR2 avian sarcoma virus	*ros*
S13 avian erythroblastosis virus	*sea*
Simian sarcoma virus	*sis*
SKV770 avian sarcoma virus	*ski*
Rous sarcoma virus	*src*
Y73/Esh avian sarcoma virus	*yes*

[a] *fes* and *fps* are the same oncogene derived from feline and avian genomes, respectively.
[b] *mil* and *raf* are the same oncogene derived from avian and murine genomes, respectively.

Mechanism of Oncogene Transduction

The majority of transforming ►retroviruses are defective for viral replication. Since these viruses are isolated after acquisition of transforming activity, the exact steps required to form these ►retroviruses is unknown, nor is it clear whether every transforming virus has been generated by the same mechanism. However, a general model has emerged for the formation of such viruses (Fig. 1).

First, a non-transforming ►retrovirus integrates upstream of a cellular gene, an event known to occur at a reasonable frequency. Many ►retroviruses integrate preferentially within coding sequences or near sites of active transcription. Non-transforming ►retroviruses often cause tumors by insertion in or near proto-oncogenes, resulting in the activation of transcription or production of abnormal transcripts. These transcripts arise due to enhancer activation of the cellular promoter or activity of the viral promoter on the cellular gene. If the proviral integration results in cellular transformation, cells containing the integration site will be selected for growth. However, some ►retroviruses integrate upstream or downstream and in the opposite orientation relative to the proto-oncogene, and it is believed that the generation of transforming ►retroviruses requires proviral integration upstream and in the same orientation as the oncogene. Such events can result in cancer



Transduction of Oncogenes. Figure 1 Mechanism of retroviral oncogene transduction. (See text for details.)

induction without ►transduction of oncogenes. Thus, many proviral insertion events may lead to cancer, but not formation of transforming ►retroviruses.

Second, transcription initiating in the 5′ LTR generates a transcript that would read through the normal polyadenylation sequences in the 3′ LTR. Recent evidence suggests that increases in retroviral transcriptional readthrough also result in transductive recombination. Potentially, this readthrough transcript could be packaged into virions, although it is generally believed that transcripts longer than 150% of the genome would be accommodated poorly in the virus capsid. Alternatively, a rare deletion of cellular DNA or aberrant splicing could provide a truncated provirus or mutant transcripts that may encompass a much greater portion of the cellular transcripts.

Third, hybrid oncogene transcripts may be packaged along with normal viral transcripts into virions. Retroviruses have a diploid genome that includes cis-acting sequences (usually near the 5′ end of the viral genome) necessary for packaging into the viral capsid (designated the psi sequence). Thus, hybrid transcripts including the psi sequence would be preferentially packaged with normal retroviral sequences to give an RNA heterodimer instead of the normal RNA homodimer. However, normal cellular RNAs can be packaged into retroviral particles at low frequency, and co-packaging would allow copying by reverse transcriptase, which is not template-specific.

Fourth, the hybrid oncogene transcript and the wild-type transcript both will be used as templates for reverse transcriptase, which also is incorporated into viral particles. In some cases, incorporation of the proto-oncogene and expression at high levels from the retroviral promoter appears to be transforming. Nevertheless, most transduced oncogenes have multiple genetic alterations. Reverse transcriptase has several properties that favor the types of genomic changes observed in transforming retroviruses. These properties include template switching, deletion formation, and the introduction of point mutations. If the resulting recombinants retain all of the cis-acting sequences needed for replication, the transforming virus will be capable of propagation in the presence of replication-competent retroviruses. Supporting the importance of readthrough transcripts for this process, transforming retroviral

genomes have been observed that carry poly(A) stretches typical of mRNAs at the junction between the host and 3′ viral sequences. Furthermore, the incorporated oncogenes lack introns.

Lessons from Retroviral Transduction of Oncogenes

Cancer is believed to be a multistep process, where several genetic events contribute to the generation of tumor cells. Some avian retroviruses are known to have transduced two oncogenes, including avian erythroblastosis virus-ES4 and -R (AEV-ES4 and AEV-R) (*erb*B and *erb*A), Mill-Hill-2 (MH2) avian myelocytoma virus (*mil* (aka *raf*) and *myc*), and avian myeloblastosis virus (AMV-E26) (*ets* and *myb*). Some viruses may have transduced more than one cellular proto-oncogene to improve their transforming capacity, a process that may be similar to the acquisition of multiple genetic changes in cancer cells during tumor progression. For example, evidence suggests that *erbA* expression is necessary for the full transforming activity of the *erbB* oncogene in AEV. Also, the MH2 ►retrovirus requires expression of both *mil/raf* and *myc* to transform neuroretinal cells from 7-day-old chicken embryos, an event that *myc*-expressing retroviruses cannot induce. However, AMV-E26 contains two oncogenes, but only one of them has been shown to be necessary for full transforming ability. Deletion of the *ets* oncogene does not diminish the transforming ability of AMV-E26 relative to wild type virus when injected in newborn chickens. Furthermore, many of the transduced oncogenes contain deletions or point mutations that reveal regulatory regions of the encoded gene products, leading to a greater understanding of their normal functions in cellular growth control.

Retroviruses, including simple retroviruses that lack regulatory genes as well as lentiviruses, have become common vectors for therapeutic gene delivery. These viruses have been used to deliver genes for treatment of a variety of illnesses, including cancers and genetic disorders. Lentiviral vectors offer the advantage of being able to infect and replicate in non-dividing cell types. However, simple retroviruses may be advantageous for preferential infection of dividing tumor cells and delivery of the therapeutic gene relative to adjacent normal cells. In both cases, viral structural genes in the vector are replaced with a therapeutic gene of interest. Expression of the transduced genes can be controlled by non-viral promoters, internal ribosome entry sites or splicing. Although these vectors have shown promise as therapeutic agents, the safety of such vectors has been the overriding concern, i.e., preventing the formation of replication competent viruses and avoiding the ill effects of integration. Limitations to replication and the avoidance of multiple insertions within target cells should minimize insertional activation of oncogenes. A recent improvement to the retroviral vector system was removal of the 3′ U3 region, thus creating a self-inactivating (SIN) virus after reverse transcription. Another possible safety concern associated with ►gene therapy is the generation of new transforming viruses, especially in human patients. These events should be extremely rare because of several precautions included during the construction of these vectors, including removal of U3 sequences as well as viral structural genes, thus confining these retroviruses to a single round of replication. Furthermore, the absence of homologous endogenous lentiviruses in humans should reduce recombination events that lead to generation of transforming retroviruses.

In the early part of this century, a major milestone in human gene therapy was achieved. To date, more than 20 children with X-linked severe combined immune deficiency (SCID) or adenosine deaminase (ADA) deficiency SCID have been successfully treated using a Moloney murine leukemia virus-based vector to allow normal expression of the defective gene. Unfortunately, in three of the patients, this vector inserted in close proximity to the *LMO2* proto-oncogene, leading to dysregulation of *LMO2* expression and development of leukemia. Many gene therapy clinical trials are being performed, and current efforts have focused on further improvements to vector safety. Such improvements include prevention of transcriptional readthrough or inclusion of insulator elements to block the formation of hybrid viral-cellular transcripts that may lead to oncogene ►transduction.

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Transfection

Definition

Introduction of DNA into live cells is referred to as:

- Transformation when the cells are bacterial or yeast cells;

- Transfection when the cells are eukaryotic and DNA is offered to the cells as a complex
- Transduction when the cells are eukaryotic and the vector is a non-self-replicating virus
- Infection when the cells are eukaryotic and the vector is a self-replicating virus

Transformation

Definition

Malignant transformation is the collection of events that leads to loss of normal cellular function and acquisition of tumorigenic properties. Transformation of eukaryotic cells describes the failure to observe the normal constraints of growth. It refers to their conversion to a state of unrestrained growth in culture, resembling or identical with the tumorigenic condition. Transformed cells become independent of growth factors usually needed for cell growth. In vitro cell transformation tests are used as a model for predicting in vivo ►carcinogenesis. Hallmarks of transformation in experimental settings include ►anchorage independent growth, cell proliferation without exogenous growth factors, loss of ►contact inhibition and tumor formation in ►xenograft assays.

Transforming Gene

►Oncogene

Transforming Growth Factor Alpha

Definition

TGFα; A potent ►mitogen and ►EGFR ligand. Initially identified as a 50 amino acid polypeptide secreted by transformed cells, it is often overproduced alongside the EGFR in tumors and promotes autonomous proliferation of cancer cells.

►ADAM17

Transforming Growth Factor Beta

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Definition

Transforming growth factors were identified on the basis of their ability to induce soft agar growth and morphological changes in nonmalignant cells. The original observation was of an activity, which was named sarcoma growth factor. Soon afterward the term transforming growth factor, ►TGF, was adopted. Sarcoma growth factor was subsequently found to be composed of an epidermal growth factor like protein, which was named transforming growth factor alpha, ►TGF-α, and of ►TGF-β. TGF-α is a member of the epidermal growth factor family, and is unrelated to TGF-β.

Characteristics

TGF-βs are multifunctional polypeptide growth factors involved in the regulation of cellular growth and differentiation and immune functions. The number of known members of this family is rapidly increasing and stands at more than 40 different growth modulating proteins. Besides TGFβs, these include bone morphogenetic proteins (BMPs), growth and differentiation factors, inhibins and activins. TGF-βs are in many senses unique among growth factors in their potent and widespread actions. Three different mammalian gene products, TGF-βs 1–3, have been molecularly cloned. Almost all types of cells in the body make some form of TGF-β, and nearly all cells have cell surface receptors for it. One of their major effects is inhibition of cell proliferation, a property needed in developmental processes, for instance. TGF-βs have important roles in the control of the pericellular proteolytic balance and in the regulation of the production and structure of the components of the connective tissues and extracellular matrices. TGF-β stimulates the transcription and synthesis of various components of the extracellular matrix like collagens, ►fibronectin, vitronectin, tenascin and proteoglycans. TGF-βs are potent chemotactic factors for many cell types like fibroblasts, eosinophils and various inflammatory cells at very low concentrations. They also suppress matrix degradation by decreasing the expression of proteinases (►Serine proteases (type II) spanning the plasma membrane), such as plasminogen activators (►Plasminogen activating system), numerous metalloproteinases, and by inducing proteinase inhibitors, such as plasminogen activator inhibitor-1 and ►tissue inhibitors of metalloproteinases (TIMPs). In addition, TGF-βs regulate cellular functions by modulating the expression of matrix receptors, the

integrins. For these reasons the activities of TGF-βs must be tightly regulated.

TGF-β Receptors and Signaling Mechanisms

Members of the TGF-β superfamily have diverse functions in cell-cell signaling. TGF-βs play different roles in tissue homeostasis and at various stages of development. The mechanisms of regulation of TGF-β activity are multifaceted and complex. Three different TGF-β isoforms and the types, affinity, and signaling functions of its receptors also add complexity to the regulation of their effects. The effects of TGF-βs and the other family members are mediated from the cell membrane to nucleus through distinct combinations of type I and type II serine/threonine kinase receptors, ►TβRI and ►TβRII and downstream effectors, the ►Smad proteins. The TGF-β receptors form a signaling receptor network with activin like kinase (ALK) receptors. The receptor-regulated Smads become phosphorylated by activated type I receptors, and they form heteromeric complexes with a common partner, Smad4, which gets translocated into the nucleus for gene transcription control. In addition to the signal transducing Smads, inhibitory Smads also play a role in the outcome of the signaling. They down-regulate the activation of receptor-regulated Smads. In contrast to the still growing TGF-β growth factor superfamily, relatively few type I and type II receptors or Smads have been identified. The signaling specificities between different TGF-β superfamily members vary, and a certain family member can elicit a broad spectrum of biological responses.

Latency TGF-β

TGF-βs are produced by the majority of cells in latent complexes unable to associate with TGF-β signaling receptors. Some primary cells and established cell lines secrete active TGF-β. TGF-βs are secreted from cells as latent dimeric complexes containing the mature C-terminal TGF-β and its N-terminal pro-domain, ►LAP, the TGF-β ►latency-associated peptide. The two polypeptide chains of pro-TGF-β associate to form a disulfide bonded dimer. TGF-β is cleaved from its propeptide by furin-like endoproteinase during secretion at RRXR sequence. The LAP propeptide dimer remains associated with the TGF-β dimer by non-covalent interactions. This complex is referred to as small latent TGF-β. TGF-βs are secreted in most cultured cell lines as large latent complexes, consisting of small latent TGF-β covalently bound to one of the three ►latent TGF-β binding proteins (►LTBPs -1, -3 or -4) covalently linked to LAP (Fig. 1). Interestingly, LTBP-2 is unable to form complexes with any of the small latent TGF-βs, suggesting some other functions for this widely expressed molecule. The true LTBPs have a central role in the processing and secretion of TGF-βs, but they evidently have other, for example, structural roles like fibrillins. The expression and secretion of LTBPs and TGF-βs is, in general, coordinately regulated.

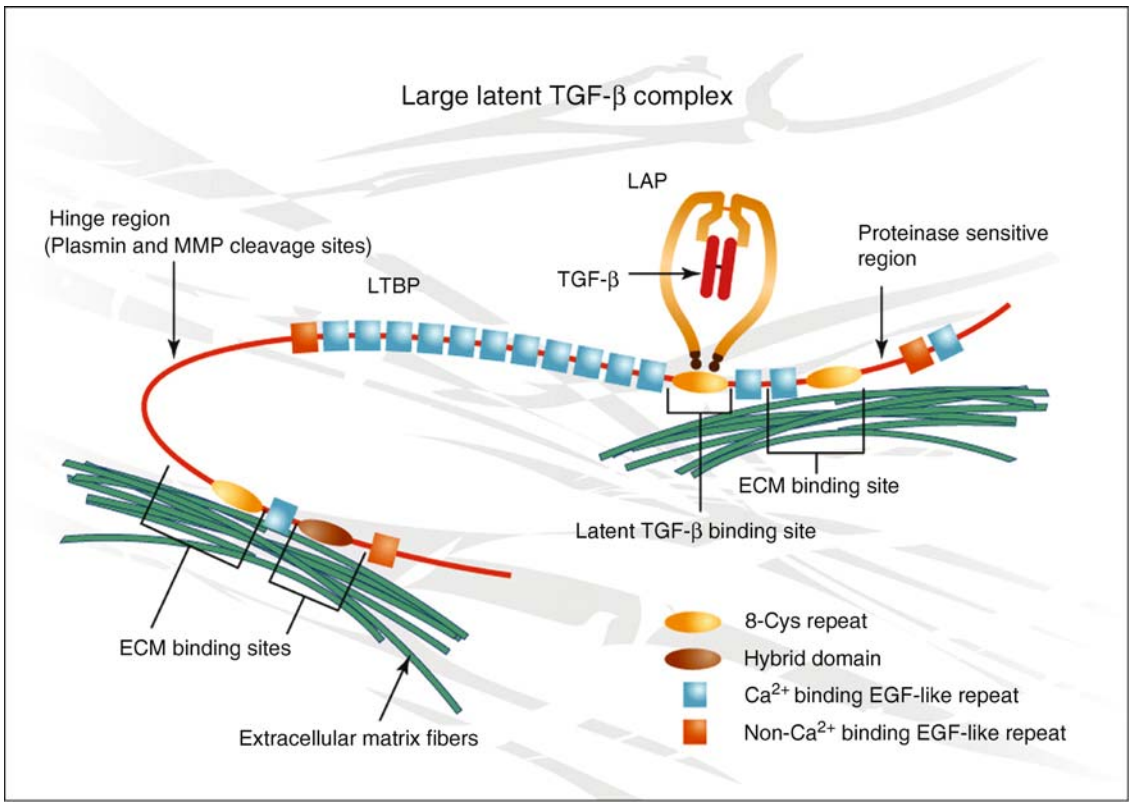
Matrix Association and Release of TGF-β

LTBPs have a central role in the targeting of TGF-β to extracellular matrix structures. LTBPs are produced in excess to TGF-β, and since TGF-β secretion is very inefficient in the absence of LTBP, most secreted cellular TGF-β is in the large latent complexes (Fig. 1). Release of active TGF-β from matrix associated latent complexes appears to require two steps, the release of the large latent complex from ECM by proteolytic truncation, and subsequent activation, which can be achieved by different mechanisms like the integrins. Since TGF-β regulates the cellular production of ECM components as well as the proteolytic balance, the matrix association and activation of TGF-β complexes form a finely tuned control network for the maintenance of the organization of extracellular structures. Cancer cells produce frequently aberrant amounts of both the matrix components and TGF-β. Malignant cells do also frequently fail to deposit TGF-β complexes to the extracellular matrix, probably due to their perturbed deposition of fibronectin-collagen matrix, as well as altered LTBP production.

Latent complexes of TGF-β in the ECM may provide tissues with a readily available storage form of this growth factor. The release and activation of stored growth factors by proteases or migrating cells can generate rapid and highly localized signals like in wound healing or during radiotherapy. Cell movement causes traction of the latent matrix-associated complexes and induces activation. Rapid activation of extracellular signaling mechanisms could be important in the healing of tissues after damage, in the control of cells of the immune system during acute infections and in the initial stages of angiogenesis. It is unclear how soluble growth factors could form gradients in highly cellular tissues. Matrix bound growth factors might generate this kind of an immobilized activity gradient.

LTBPs: Expression and Functions

Relatively few functions have been identified for LTBPs thus far. Structurally they resemble fibrillins, which are components of the extracellular microfibrils. LTBPs have a typical structure consisting of four eight cysteine (8-Cys) repeats and several EGF-like repeats. The association of latent TGF-β with the matrix is mediated by LTBPs (Fig. 1). Not only the N-terminal domains, but also a region of the C-terminus of LTBP are important in this association. The N-terminus contains transglutaminase substrate motifs, and transglutaminase is required for the covalent ECM association. In addition, TGF-β2 and -β3 become associated with LTBPs. It is thus likely



Transforming Growth Factor Beta. Figure 1 Large latent TGF-β complex. The small latent complex contains the C-terminal mature TGF-β and its N-terminal pro-domain, LAP (TGF-β latency-associated peptide). This complex forms a disulphide-bonded complex with the third 8-Cys repeat of LTBP. LTBP associates with the ECM mainly via the 8-Cys domains and some adjoining regions.

that LTBPs mediate and target the binding of all three TGF-β isoforms to various extracellular matrices.

The TGF-β1 binding region in LTBPs is located close to their C-terminus in the third 8-Cys repeat. The association between LTBP-1 and the propeptide part, LAP, is mediated by disulfide bonding. The respective 8-Cys repeats of LTBPs-3 and -4 also bind small latent TGF-βs. Of the numerous known 8-Cys repeats of the LTBPs and fibrillins only three have been found to have the capacity to associate in a covalent manner with the small latent TGF-βs.

Activation of Soluble and Extracellular Matrix Forms of Latent TGF-β

TGF-β can be activated *in vitro* by multiple mechanisms, including proteolysis, enzymatic deglycosylation and extremes of pH. Activation of latent TGF-β involves proteolytic disruption of the non-covalent interaction between the propeptide LAP and TGF-β, which releases biologically active TGF-β capable of binding to its signaling receptors. LAP may also undergo conformational changes in such a manner that TGF-β is released or exposed to its receptors. The existence of different TGF-β isoforms and latent complexes, as well as the number of different LTBPs, suggests that there are variable mechanisms for the activation of TGF-βs.

The electrostatic interaction between LAP and TGF-β can be dissociated *in vitro* by extremes of pH, chaotropic agents, and heat treatment. From the physiological point of view, the acidic environment in the bone (osteoclasts) or during wound healing could induce this kind of ►TGF-β activation.

In vivo analyses of tumor-bearing mice indicated that irradiation (02372) causes rapid activation of TGF-β in the tumors. This effect appears to result from the activation of existing, most probably of matrix-bound latent TGF-β. Irradiation produces reactive oxygen species leading to redox-mediated activation of latent TGF-β complexes. Redox-mediated TGF-β activation may be involved in chronic tissue processes, where oxidative stress is implicated, such as carcinogenesis.

TGF-β can be activated by deglycosylation of LAP. Mechanisms involving proteolysis are, however, more diverse and more likely to operate *in vivo*. Various proteases can degrade LAP and release active TGF-β. Protease inhibitors prevent the activation of TGF-β in cell culture. Cell-cell contacts, targeting of TGF-β, as

well as transglutaminase activity appear to be important in the generation of active TGF-β in vivo.

The processing of pericellular matrix-associated LTBPs and activation of TGF-β are constant events in the ►apoptosis or ►anoikis of endothelial and epithelial cells, pinpointing the importance of pericellular latent complexes as a physiological source of TGF-β.

Thrombospondin-1 (TSP-1), a platelet α-granule and ECM protein, plays a role in the activation of latent TGF-β complexes via a mechanism that does not involve cell surfaces or proteases. Using purified plasma TSP-1 or the recombinant protein it was found that it is able to activate both small and large latent TGF-β complexes. The activation mechanism is not fully understood, but seems to involve the N-terminal end of LAP and the type I repeats of TSP-1, possibly by inducing a change in the conformation of LAP and thus releasing the active TGF-β. TSP-1 interacts with LAP as a part of a biologically active complex, and this may prevent the re-association of the inactive complex of LAP with TGF-β. The expression of TSP is induced during wound healing. TGF-β may thus get focally activated at sites of injury by enhanced TSP synthesis. Accordingly, TSP deficient mice display many phenotypic features, similar to those detected in TGF-β1 deficient mice. The abnormalities in some tissues of the TSP null animals were even reverted by TSP-derived TGF-β activating peptides, further emphasizing the role for TSP in TGF-β activation.

The LAP part of TGF-β contains an RGD-motif, which is recognized by integrins (►Integrin signaling and cancer) $\alpha_v\beta_1$ and $\alpha_v\beta_6$. Integrin $\alpha_v\beta_6$ is also able to activate TGF-β. This activation model is particularly interesting, because $\alpha_v\beta_6$ integrin is expressed solely on epithelial cells, which are very sensitive to TGF-β mediated growth inhibition, and also because the overlap of the phenotypes of TGF-β1 and integrin β_6 chain deficient mice. β_6 integrin deficient mice show increased inflammation and decreased fibrosis, processes that are regulated by TGF-β.

Hormonal effectors (►Tamoxifen) can also affect TGF-β activation. Originally it was found that antiestrogens could induce the production and secretion of active TGF-β in cultured breast cancer cells. Activation of TGF-β has subsequently been observed in a number of cell culture models using estrogens and antiestrogens, retinoids and vitamin D derivatives. Steroid hormone superfamily members are efficient regulators of the expression of TGF-β isoforms, and TGF-βs are likely to act as local mediators of the diverse actions of steroids. Estrogens and antiestrogens regulate TGF-β1 formation in different cells and tissues like in mammary carcinoma cells and in fetal fibroblasts. TGF-β functions, for instance, as an autocrine negative growth regulator in breast carcinoma cells.

TGF-Beta and LTBP Knockout Mice

The importance of the three different TGF-βs is elucidated in the gene knockout studies. Knockout of TGF-β1 results in multifocal inflammatory disease leading to the death of the animal. TGF-β2 knockout is embryonally lethal. The mice develop severe cardiac, lung and craniofacial defects. Inner ear and eye are also affected. TGF-β3 null mice develop cleft palate.

Accordingly, null-mice unable to produce LTBPs develop serious physiological defects. LTBP-3 knockout mice develop multiple defects such as growth retardation, emphysema, bone malformations and abnormalities of thymys and spleen. Their lifespan is, however, normal and they are able to reproduce. Hypomorphic LTBP-4−/− mice develop early emphysema and colorectal tumors, indicative of missing growth inhibitor. The short and long splice forms have different functions. LTBP-1L null mice exhibit a cardiac phenotype, which reveals a crucial role for Ltbp1L and matrix as extracellular regulators of Tgf-beta activity in heart organogenesis.

Perspective

Growth factors of the TGF-β family are important autocrine and paracrine regulators of cell proliferation and differentiation. The regulation levels of their activities include the expression of TGF-β receptors, availability of TGF-βs, their activities and modulation of the cellular response. Most cells secrete TGF-β in a large latent complex, which associates with the extracellular matrix and is unable to bind to the TGF-β signaling receptors. LTBPs have a central role in TGF-β secretion, extracellular matrix deposition and activation. In addition, LTBPs have structural and other functions not directly related to TGF-β signaling. Structural diversity in LTBP proteins is tremendous, and the possible functions of the different forms include, amongst others, the modulation of cell adhesion and the functions of integrins.

Focal activation of latent TGF-β in the matrix by physicochemical means offers a rapid way to induce TGF-β signaling. In addition to plasmin-mediated TGF-β activation novel mechanisms have been found including other proteases, reactive oxygen species, thrombospondin and integrin mediated activation.

The modulation of pericellular proteolytic activity by TGF-β supports a general cascade of events, where proteinases and latent matrix-bound growth factors are components of extracellular signal transduction machinery. This directs tissue construction and remodeling, and probably also regulates the activity of infiltrating immune cells. Disturbances in these control systems could participate in the pathogenesis of a variety of disease states like atherosclerosis, cancer, various fibrotic diseases and chronic inflammation.

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Transforming Retroviruses

Definition

A class of ►retroviruses that is capable to transform host cells. Two classes of transforming retroviruses can be distinguished:

1. Acute transforming retroviruses are characterized by the presence of an oncogene in their genome that has been acquired during host cell passage by retroviral transduction. Elimination of this retroviral oncogene (v-*onc*) by deletion or mutations within the oncogene that affect its function (e.g. a temperature-sensitive mutation), results in the loss of the transforming activity of the virus. This demonstrates that the presence of v-*onc* in the retroviral genome is necessary and sufficient to induce host cell transformation. Examples for this sub-class are the Rous Sarcoma Virus (v-*src*), Abelson leukemia virus (v-*abl*) and Mill Hill 2 virus (v-*myc*; v-*mil*/*raf*-1).
2. Slow transforming retroviruses lack a v-*onc* gene, but often trigger tumorigenesis by inserting their proviral genome into genomic regions that control the expression of proto-oncogenes (insertion mutagenesis). For example, avian B cell lymphomas often contain proviral sequences derived from the Avian leukosis virus in the promotor region of the c-*myc* proto-oncogene. As the retroviral control elements represent strong promotors, the transcription of the c-*myc* gene is largely driven by the provirus and uncoupled from its tight control by the cellular signaling network. This results in Myc overexpression and malignant transformation.

►Retroviral insertional Mutagenesis
►Myc Oncogene
►Transduction of Oncogenes

Transgene

Definition

A foreign gene introduced into a cell by viral or non-viral gene transfer techniques.

Transgenic

Definition

Transgenic animals or plants are created by introducing new DNA sequences into the germ line via addition to the egg. The use of transgenic mice carrying an alien gene in every cell is a widely used experimental system in biomedical science. They are generated by microinjection of DNA (transgene) into fertilized eggs, which are then implanted into pseudopregnant foster mothers. The offspring often carries the transgene in all cells, including germ cells. Thus, the transgene will be transmitted according to Mendelian genetics. Transgenic mice represent a tool to study gene effects in the context of the whole organism.

►Mouse Models

Transgenic Animal

Definition

An animal to which new DNA has been inserted into its genome.

►Mouse Models

Transgenic Mice

Definition

Are mice in which a foreign gene has been inserted in their genome.

►Transgenic

Transglutaminase-2

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Synonyms

Tissue-transglutaminase; tTGase; Transglutaminase type-2; TG2; Cytosolic transglutaminase; TGc; Endothelial transglutaminase; Liver transglutaminase and G_h

Definition

Tissue transglutaminase (►TG2; EC 2.3.2.13) is a ubiquitous and most diverse member of the transglutaminase family of enzymes. TG2 catalyzes calcium-dependent post-translational modification of proteins by inserting highly stable ►isopeptide bonds between polypeptide chains or by conjugating ►polyamines to proteins. In addition, TG2 exhibits ►GTPase activity and can serve as a ►signal-transduction G protein. Less studied functions of TG2 include its protein disulfide isomerase and ►protein kinase activities.

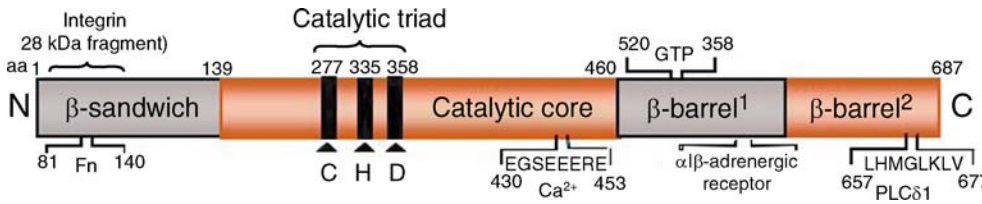
Characteristics

TG2 is a multifunctional protein whose expression in some cell types (e.g., endothelial and smooth muscle cells) is constitutively high. In other cell types, TG2s expression is up-regulated via discrete signaling pathways, such as those induced by certain stress factors, inflammatory stimuli, differentiation agents, and growth factors. Although predominantly a cytosolic protein, TG2 can translocate to the nucleus by "piggy-back riding" other proteins, such as ►importin-alpha-3, or translocate to membranes in association with ►integrins. TG2 can also be secreted outside the cell (by an as-yet unknown mechanism), where it can crosslink extracellular matrix (ECM) proteins and promote ►adhesion of several cell types. An important feature of TG2 is its high binding affinity for ►fibronectin; in cancers, membrane-associated TG2 can promote a stable interaction between cell surface integrins and fibronectin and promote cell growth and survival (Fig. 1).

TG2 and Apoptosis

A role for TG2 in apoptosis was initially suggested by Dr. Laszlo Fesus and his coworkers in 1987 based on the observation that lead-induced hypertrophy of the liver in rats was associated with cellular expression of increased TG2. Since then, many reports have supported the role of TG2 in apoptosis. In general, the expression of TG2 is markedly increased in cells undergoing apoptosis. Forcibly increasing the expression of TG2 in several cell types results in apoptosis or makes them susceptible to death-inducing stimuli. Conversely, reducing TG2 levels by antisense RNA renders the cells more resistant to apoptosis. It is believed that TG2 promotes apoptosis by crosslinking intracellular proteins, preventing their leakage from cells and induction of an inflammatory response. These observations suggest that cells generally do not tolerate the increased expression of TG2 and that TG2 overexpression leads to apoptotic death.

However, some recent reports have provided paradoxical evidence and suggest that TG2 expression and apoptosis do not always go hand in hand. For example, $TG2^{-/-}$ knockout mice (mice lacking all TG2 expression) did not show any genetic alterations that are suggestive of perturbed apoptosis. The possibility that some other proteins compensate for the loss of TG2 in these mice cannot be ruled out. Furthermore, various other studies have provided data suggesting that increased expression of TG2 can prolong cell survival by preventing apoptosis.



Transglutaminase-2. Figure 1 Schematic representation of various functional domains of the TG2 protein. In addition to catalyzing calcium-dependent protein crosslinking function, TG2 can catalyze calcium-independent GTPase, ATPase, protein kinase, and protein disulfide isomerase activities. TG2 can modulate the functions of other proteins by directly interacting or associating with them; examples include ►phospholipase-δ_1, members of the β-integrin family, focal adhesion kinase, fibronectin, osteonectin, ►RhoA, multilineage kinases, and ►retinoblastoma protein. Through these activities, TG2 plays a role in biological processes such as ►apoptosis, wound healing, and cataract formation. Recent work suggests that TG2 can also serve as a signaling molecule and promote cell growth, drug resistance, and metastatic functions in tumor cells.

TG2 in Drug Resistance and Metastasis

Evidence is accumulating that cancer cells that are resistant to chemotherapeutic drugs or that are isolated from metastatic sites express elevated levels of TG2. Also, there is evidence that drug-resistant and metastatic cancer cells share some common pathways. For example, cells from advanced-stage cancers accumulate a large number of genetic alterations that can render them resistant to apoptosis. Resistance to apoptosis can enable cancer cells not only to grow and survive in the stressful environment of distant tissues (i.e., to metastasize) but also to withstand the toxic effects of drugs. Moreover, cell lines selected *in vitro* for resistance against chemotherapeutic drugs are more metastatic *in vivo*, while cancer cells isolated from metastatic sites, in general, exhibit higher resistance to chemotherapeutic drugs. Based on these observations we hypothesized that aberrant expression of TG2 in drug-resistant and metastatic cancer cells may deregulate some intrinsic apoptotic pathways in order to protect cells from apoptosis. Indeed, down-regulation of endogenous TG2 by antisense RNA, TG2-specific ribozyme, or small interfering RNA (►siRNA) could reverse drug resistance in ►lung cancer and ►breast cancer cells. Similarly, inhibition of TG2 by siRNA in breast cancer and malignant ►melanoma cells augmented their response to chemotherapeutic drugs and reduced their invasiveness in laboratory experiments. In pancreatic cancer cells, inhibition of TG2 by siRNA resulted in massive accumulation of lysophagosomes and onset of ►autophagy (type II apoptosis). These properties suggest that TG2 expression in cancer cells contributes to the development of drug resistance and ►metastasis.

Studies to elucidate the mechanisms involved in the development of TG2-mediated drug resistance in cancer cells revealed that TG2 expression augments cell survival signaling by promoting a stable interaction between cell surface integrins and the ECM proteins. Depending on the cell type, 20–30% of total TG2 can exist in complex with β-integrins (e.g., β1 β4 and β5). The association of TG2 with integrins occurs primarily at their extracellular domains and promotes their interaction with ECM ligands such as fibronectin, collagen, and vitronectin. Down-regulation of TG2 in glioblastoma cells resulted in decreased assembly of fibronectin in the ECM and cell death. Importantly, treatment of mice that had orthotopic glioblastomas with the TG2 inhibitor ►KCC009 sensitized the tumors to chemotherapy, induced apoptosis of cancer cells, and prolonged survival of the animals. Further, the interaction between TG2 and integrins is independent of the crosslinking activity of TG2 and results in increased cell adhesion, ►migration, and activation of downstream survival signaling pathways such as ►focal adhesion kinase (FAK). Interestingly, TG2 can also interact directly with focal adhesion kinase and result in its autophosphorylation (pY397) and consequent activation of the downstream ►PI3K and ►Akt signaling.

Activation of the ►nuclear factor-κB (NF-κB), which plays an important role in regulating cell growth, apoptosis, and metastasis, has also been associated with increased TG2 expression in cancer cells. Tumor cells that overexpressed TG2 exhibited increased levels of constitutively active NF-κB. Activation of TG2 led to activation of NF-κB, and conversely, inhibition of TG2 activity inhibited the activation of NF-κB. Similarly, ectopic expression of TG2 caused activation of NF-κB, and inhibition of TG2 expression by siRNA abolished the NF-κB activation and rendered drug-resistant breast cancer cells sensitive to doxorubicin-induced cytotoxicity. Notably, immunohistochemical analysis of pancreatic ductal adenocarcinoma tumor samples further supported a strong correlation between TG2 expression and NF-κB activation. These observations suggest that TG2 induces constitutive activation of NF-κB in tumor cells via a novel pathway. Therefore, TG2 may be an attractive target for inhibiting constitutive NF-κB activation and rendering cancer cells sensitive to anticancer therapies.

Clinical Relevance

Drug resistance and metastasis are major impediments to the successful treatment of cancer. More than 90% of cancer-related deaths can be attributed to the failure of chemotherapy. On the basis of published results that drug-resistant and metastatic tumors and tumor cell lines express high levels of TG2, that TG2 expression promotes cell survival and invasion and that down-regulation of TG2 results in increased sensitivity of cancer cells to chemotherapeutic drugs and to undergo programmed cell death (apoptosis or autophagy), TG2 may offer an attractive target for treating drug-resistant and metastatic tumors.

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Transglutaminase Type-2

►Transglutaminase-2

Transient Amplifying Cells

Definition

Progeny of stem cells that undergo replication, but are not able to self-renew and eventually give only one or more differentiated cell types.

Transin-1

►Stromelysin-1

Transit Amplifying Cells

Definition

Cells that are born from the asymmetric divisions of stem cells, having limited division potential. They become increasingly differentiated with successive divisions, finally giving rise to reproductively sterile, terminally differentiated cells.

►Stem Cell Plasticity

Transition

Definition

Mutations from purine to purine or pyrimidine to pyrimidine.

Transition Metals

Definition

Are metallic elements that have an incomplete inner electron shell. They are characterized by multiple valences.

►Particle-induced cancer

Transitional Carcinoma

►Nasopharyngeal Carcinoma

Transitional Cell Carcinoma

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Synonyms

Urothelial tumor; Transitional cell carcinoma of bladder; Transitional cell carcinoma of renal pelvis; Transitional cell carcinoma of ureter; Urothelial Carcinoma, Clinical Oncology

Definition

Transitional Cell Carcinoma (TCC) arises in the urothelium that covers the lining of the renal calyx, renal pelvis, ureter, bladder and part of the urethra. Although the WHO/ISUP consensus conference has determined that the term *urothelial cancer* is preferable to the term *transitional cell cancer*, the latter remains in widespread use. "Urothelial cancer" may also be confusing because cancers of other histologic types, such as squamous cancers and adenocarcinomas, also arise in the urothelium.

Characteristics

Epidemiology

It was estimated that in 2007, 67,160 new cases of bladder cancer would be diagnosed and 13,750 patients

would die of invasive bladder cancer in the United States. ►Bladder cancer is nearly three times more common in men than in women and more than 90% of bladder cancers are TCCs. The median ages at diagnosis for TCC are 69 years in males and 71 years in females.

Upper urinary tract urothelial tumors involving the renal pelvis or ureter are relatively uncommon, accounting for about 5–7% of all renal tumors and about 5% of all urothelial tumors.

Etiology

One of the genetic changes that must occur for malignant transformation is the induction of oncogene. Oncogenes associated with TCC include those of the ►RAS gene family, including P21 RAS oncogene, and up to 50% of TCCs have been claimed to have RAS mutations. Another important molecular mechanism in the process of carcinogenesis is the inactivation of tumor suppressor genes. These include that of ►P53, the most frequently altered gene in human cancers, the retinoblastoma (RB) gene (►Retinoblastoma Protein, Biological and Clinical Functions), and genes on chromosome 9. Overexpression of normal genes including those for EGF receptor (ERBB1) and ERBB2 (►Epidermal Growth Factor Receptor Ligands) occur in most TCCs.

Cigarette smokers have a fourfold higher incidence of TCC than do people who have never smoked (►tobacco-related cancers). Ex-smokers have a reduced incidence of TCC, but the reduction of this risk down to baseline takes nearly 20 years. ►Nitrosamines, 2-naphthylamine and 4-aminobiphenyl are suggested as being responsible for TCC in cigarette smoke. Women treated with radiation for carcinoma of the uterine cervix or ovary, have a two- to fourfold increased risk of developing bladder cancer. Patients treated with ►cyclophosphamide have up to a ninefold increased risk of developing bladder cancer.

Signs and Symptoms

Microscopic or gross ►hematuria is the most common presenting symptom. Patients with gross hematuria have reported rates of bladder cancer of 13–35%. With microscopic hematuria, the rates decreased to 0.5–10.5%. So, if a patient has unexplained hematuria, either microscopic or gross, cystoscopic examination is usually warranted, especially in individuals older than age 60 or younger people with a smoking history.

The second most common presentation is the constellation of lower urinary tract irritative symptoms such as urinary frequency, urgency, and dysuria. These irritative symptoms usually occur with hematuria. In fact, the risk of TCC may be doubled in patients with irritative voiding symptoms that coexist with hematuria.

Evaluations

In all patients with signs and symptoms suggestive of bladder cancer, ►excretory urography (IVU) is indicated. It is useful in examining the upper urinary tracts for associated urothelial tumors. Large bladder tumors may appear as filling defects in the bladder, but small ones may not be detected. More recently, computed tomography (CT) has replaced IVU in the evaluation of hematuria. After imaging studies, all patients suspected of having bladder cancer should have cystoscopy. Retrograde ►pyelography should be done if the upper tracts are not visualized on IVU or CT.

CT can help to assess the extent of the primary tumor and provides information about the presence of pelvic and para-aortic lymphadenopathy and visceral metastases. But CT fails to detect nodal metastases in up to 40–70% of patients who have them. MRI is not much more helpful than CT. Pelvic lymphadenectomy, which can be done with cystectomy, is the most accurate means of determining regional node involvement. The primary regions of lymphatic drainage of the bladder are the perivesical, hypogastric, obturator, external iliac and presacral nodes. As some patients with limited nodal metastases can be benefited by lymphadenectomy, bilateral node dissection should be done. The usually recommended metastatic evaluation for invasive bladder cancer includes a chest radiograph, abdominal-pelvic CT, bone scan and liver function tests.

A flexible cystoscope is often used for the initial diagnosis and follow-up of patients with bladder tumors. It has much less discomfort than a rigid cystoscope. Bugbee electrode devices can be inserted through a flexible cystoscope to allow destruction of small, non-invasive papillary tumors.

Ta	Papillary, epithelium confined
Tis	Flat carcinoma in situ
T1	Lamina propria invasion
T2a	Superficial muscularis propria invasion
T2b	Deep muscularis propria invasion
T3a	Microscopic extension into perivesical fat
T3b	Macroscopic extension into perivesical fat
T4a	Cancer invading pelvic viscera
T4b	Extension to pelvic sidewalls, abdominal walls, or bony pelvis
N0	No histologic pelvic node metastasis
N1	Single positive node ≤2 cm in diameter, below common iliacs
N2	Single positive node 2–5 cm in greatest diameter or multiple positive nodes
N3	Positive nodes >5 cm in diameter
M0	No distant metastases

Malignant urothelial cells have large nuclei with irregular, coarsely textured chromatin and can be observed on microscopic examination of the urinary sediment. This microscopic cytology is more sensitive in patients with high grade tumors or ►carcinoma in situ (CIS). The specificity and positive predictive value of cytology are quite high.

Staging (1997 AJCC-UICC, TNM Staging)

As tumor stage forms the foundation for determining therapy, accurate staging is critical. The first treatment decision based on tumor stage is the presence or absence of muscle invasion. Because metastases are very rare with a superficial (non-muscle-invasive) tumor, treatment strategy can be grouped into superficial (Ta, T1 and Tis), muscle-invasive, and metastatic tumors. About 70% of bladder tumors are superficial at presentation. Of these, 70% present as stage Ta, 20% as T1, and 10% as CIS.

Treatment of Superficial Bladder Cancer

Local resection of a bladder tumor usually enables complete removal of the tumor and provides diagnostic information about the depth and the grade of the tumor. For this, first the bulk of the tumor and then the deep portion with some underlying bladder muscle should be resected. To detect dysplasia or CIS elsewhere in the bladder, selected site mucosal biopsies from areas adjacent to the tumor, bladder dome, trigone and prostatic urethra have been recommended. If 5-aminolevulinic acid (ALA) is administered into the bladder in conjunction with fluorescent cystoscopy, lesions invisible with normal cystoscopy can be detected.

The most important issue in tumor biology of superficial tumors is recurrence and progression to higher stages. Low-grade Ta tumors recur at a rate of 50–70% and progress in about 5%. High-grade T1 lesions recur in 80% and progress in 50% of patients. The most important risk factor for progression in superficial bladder tumors is grade, not stage. Prognosis also correlates with the presence of CIS, tumor size, multiplicity, lymphovascular invasion and the configuration of the tumor (papillary vs. sessile). Of patients with CIS, 40–83% will develop muscle invasion if untreated.

For T1 tumors, the depth of lamina propria invasion determined by the muscularis mucosa invasion or the extent of invasion below the urothelial surface has been known to be correlated with prognosis. CT and MRI appear to be inaccurate in determining the microscopic muscle infiltration and the minimal extravesical spread, which can also be aggravated by post-tumor recurrence (TUR) changes.

To prevent recurrence and progression of bladder tumors, intravesical immunotherapy using BCG (►Bacillus Calmette-Guerin) has been used. Treatments are generally begun 2–4 weeks after TUR and a 6-week course is usually administered. With BCG, tumor recurrence was reduced by 20–65% and progression was reduced by 23–27%. Intravesical chemotherapy using ►Mitomycin C, ►Doxorubicin, ►Thiotepa, ►Epirubicin and ►Gemcitabine also has been administered.

Treatment of Invasive Bladder Cancer

The standard surgical approaches to muscle-invasive bladder cancer are radical cystoprostatectomy in the male patient and anterior exenteration in the female patient, with bilateral pelvic lymphadenectomy. Anterior exenteration in the female requires removal of the uterus, fallopian tubes, ovaries, bladder, urethra, and a segment of the anterior vaginal wall. A nerve-sparing modification has been proposed in the male patient and results in improved postoperative return of erectile function.

The more prevalent the orthotopic reconstruction becomes, the stricter indications for urethrectomy have been applied. The most significant factor for the anterior urethral recurrence and local/distant failure in the male patient has been identified as prostatic urethral involvement. The estimated 5-year probability of urethral recurrence is 5% without any prostate involvement and 12–18% with prostate involvement. CIS of the bladder neck and trigone was also significantly associated with prostatic urethral involvement. In the female patient, overt cancer at the bladder neck and urethra, diffuse CIS, or positive margin at surgery should be treated by en bloc urethrectomy as a part of the radical cystectomy.

The mortality rate for radical cystectomy is 1–2% and the overall complication rate is about 25%. After urinary tract diversion, bowel obstruction rate is 4–10%. Stricture of anastomosis between ureter and bowel are found in less than 3%. Depending on the type of neobladder, metabolic disorders, vitamin deficiency, and urinary tract infection can occur.

As for the ►neoadjuvant chemotherapy, recent results suggested improvement in overall survival of 5–6% among patients with locally advanced disease (stage T3 to T4a). Some reports suggest that for patients with locally advanced disease and lymph node involvement, ►adjuvant chemotherapy may also provide a survival advantage. Due to the small numbers of patients, these results are as yet insufficient for the routine use of adjuvant therapy.

Treatment of Metastatic Bladder Cancer

These patients are routinely treated with systemic chemotherapy. The most commonly used agents are ►methotrexate, ►vinblastine, ►doxorubicin, and ►cisplatin (MVAC). MVAC chemotherapy produces a complete response in about 20% of patients, although long-term disease-free survival is rare. The combination of cisplatin and a newer agent, ►gemcitabine (GC) has

produced similar survival outcomes with less toxicity compared with MVAC. ►Paclitaxel and ►docetaxel have also been used in clinical trials and demonstrate response rates of 25–83%.

►Urothelial Carcinoma, Clinical Oncology

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Transitional Cell Carcinoma of Bladder

►Transitional Cell Carcinoma

Transitional Cell Carcinoma of Renal Pelvis

►Transitional Cell Carcinoma

Transitional Cell Carcinoma of the Urinary Bladder

►Urothelial Carcinoma

Transitional Cell Carcinoma of Ureter

►Transitional Cell Carcinoma

Transjugular Intrahepatic Portosystemic Shunt

Definition

TIPS; Artificial shunt (expandable metal stent) in the liver from the portal vein (carrying blood from the intestines to the liver) to a hepatic vein (carrying blood from the liver into the inferior vena cava). The procedure is performed via the internal jugular vein under local anesthesia with sedation. TIPS is primarily used in patients with liver cirrhosis with portal hypertension.

►Acites

Translation

Definition

Process by which proteins are synthesized from messenger RNA templates. It has three stages: initiation, elongation and termination.

Translation Initiation Complex

Definition

Protein complex that promotes the proper association of ribosomes with messenger RNA and is necessary for the initiation of ►translation.

Translesion DNA Polymerases

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Definition

Translesion DNA synthesis (TLS) is a highly conserved mechanism for the completion of replication of

T

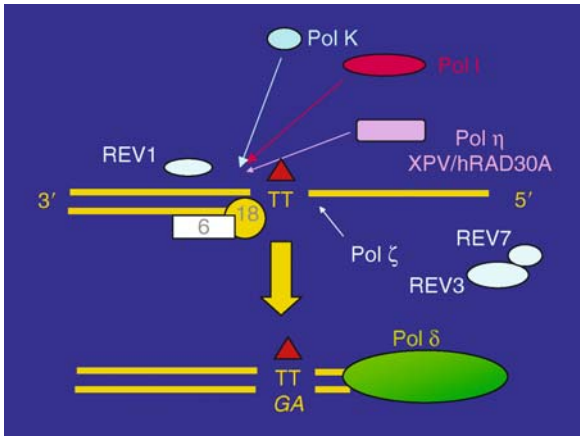
damaged genomes. Analogous pathways exist in bacteria, and homologs with remarkable similarity exist in all eukaryotic cells, including post-mitotic organisms such as *D. melanogaster*. Recent advances in elucidating the molecular mechanisms of carcinogen-induced mutagenesis indicate that replication of DNA templates that contain replication-blocking adducts is accomplished with error-prone DNA polymerases. These polymerases have relaxed base-pairing requirements, and can insert bases across from adducted templates, but with potentially mutagenic consequences.

Characteristics

Most mutations induced by genotoxic carcinogens occur when a DNA template that contains residual (unrepaired) damage is replicated during S-phase of the cell cycle. Presumably, the replication complex is blocked by bulky adducts in the DNA such as those induced by ultraviolet light (UV) or a variety of chemical carcinogens (►Adducts to DNA).

As diagrammed in Fig. 1, recent advances indicate that error-prone translesion synthesis (TLS) is responsible for the majority of base substitutions induced in the DNA. TLS is defined as the incorporation of a nucleotide across from DNA damage followed by extension of the potentially mispaired primer-template. This process is undertaken by at least five accessory DNA polymerases, several of which have been purified and studied in vitro (►DNA damage responses). The properties of these polymerases have been extensively reviewed. Based on structural homology, these polymerases fit into one of two families: the Y-family (REV1, pol η, ι, and κ), or the B-family (pol ζ). The cellular roles of this universe of polymerases are not known. In particular, the extent to which each of these polymerases participates in TLS most likely depends on the structure of a particular adduct and on the sequence context. As shown in Fig. 1, it has been suggested that pol η, ι, and/or κ inserts a base directly across from a lesion, and that pol ζ extends the mispair to form a template-primer that can be extended by pol δ. Although REV1 is a DNA polymerase, its role in mutagenesis is thought to be structural rather than catalytic.

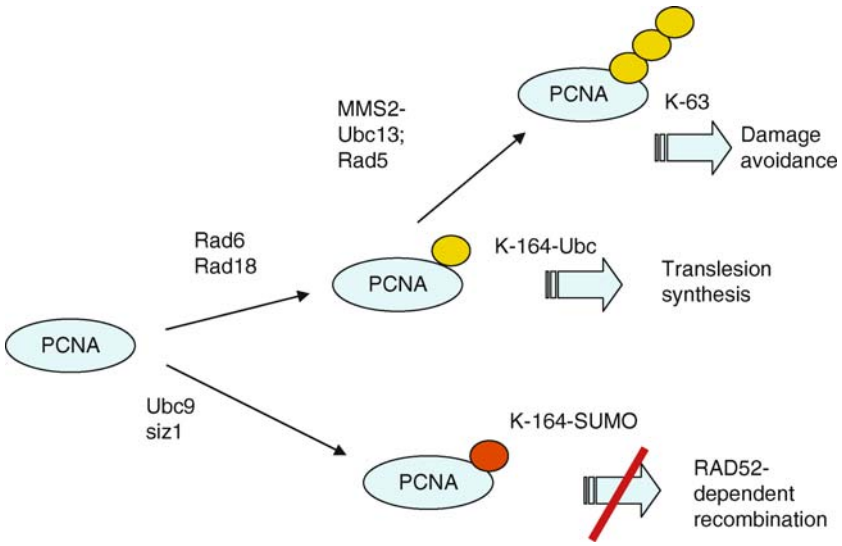
The unrestrained activity of error-prone polymerases would lead to widespread mutagenesis and genomic instability, so there are signaling mechanisms that tightly control polymerase switching events. Although not fully understood, the mechanisms used by cells to accomplish polymerase switching events at blocked primer temini have been studied most intensively in the budding yeast, *Saccharomyces cerevisiae*. In this organism, replication-blocking lesions in the template strand can be bypassed by proteins in the Rad6-dependent DNA damage tolerance pathway. This process



Translesion DNA Polymerases. Figure 1 Model for translesion replication. The replicative polymerase complex stalls at sites of helical distortion induced by DNA damage, such as UV-induced photoproducts. The presumed ubiquitin ligase RAD18 targets a ubiquitin conjugating enzyme, RAD6, to the site of damage. There are two closely related homologs of RAD6 in higher eukaryotic cells, termed RAD6A and RAD6B. One of the targets of ubiquitination appears to be PCNA, which signals accessory polymerases in ways that are not fully understood, although at least one TLS polymerase, pol η, has a higher affinity for monoubiquitinated PCNA. Current thinking is that one of the Y-family polymerases (pol η, pol ι, or pol κ) may insert a base directly across from the lesion, but pol ζ is required to extend the resulting primer such that the pol ***δ*** can continue processive DNA replication. REV1 is required for mutagenesis, but this role is probably separate from its dCMP transferase activity. Recent data indicate that REV1 may tether pol ζ to the other accessory polymerases.

prevents the collapse of stalled replication forks, and replication of the damaged template is completed by TLS with potentially mutagenic consequences, or by damage avoidance mechanisms mediated by recombination that are largely error-free.

As diagrammed in Fig. 2, the ubiquitin conjugating enzyme encoded by *Rad6* and the presumed ubiquitin ligase encoded by *Rad18* are central to this process, since mutants cannot bypass replication-blocking lesions in the template and are sensitive to many DNA damaging agents. Insights into the biochemical function of this complex were gained when the Rad18/Rad6 complex was found to be responsible for the monoubiquitination of PCNA at K164. PCNA modified in this fashion is thought to signal translesion synthesis and further ubiquitination is thought to signal damage avoidance (Fig. 2). Although the molecular details of the signaling pathways downstream of monoubiquitination are unknown, at least one TLS polymerase, pol η, has



Translesion DNA Polymerases. Figure 2 Regulation of lesion bypass in the budding yeast *Saccharomyces cerevisiae* is thought to be signaled by modification of PCNA at lysine 164 (K-164). The presence of a stalled DNA replication fork recruits Rad18, which is a presumed ubiquitin ligase, and Rad6, a ubiquitin conjugase, to the site of the replication-blocking lesion. Monoubiquination at K-164 leads to recruitment of TLS polymerases with potentially mutagenic consequences. Polyubiquitination at lysine 63 (K-63) of ubiquitin by MMS2-Ubc13 leads to damage avoidance. A proposed mechanism for damage avoidance is uncoupling of the replication fork, such that the undamaged strand is replicated for some distance beyond the blocked replication complex. The nascent strand, which has the same sequence as the damaged strand, then acts as a template for replication. The damage is thereby avoided in an error-free manner. A competing reaction is sumoylation at K164 by the SUMO (small ubiquitin modifier)-specific ligase Siz1 and conjugase Ubc9. This reaction is thought to suppress Rad52-dependent recombination and damage-induced genomic instability.

been shown to have enhanced affinity for monoubiquitinated PCNA.

The strategies used by yeast cells to complete the replication of damaged genomes appear to have been conserved in higher eukaryotes, but with additional layers of complexity. For example, higher eukaryotic cells have at least two Y-family polymerases that are not found in yeast and one of these (pol κ) appears to be independent of RAD18. Human RAD18 was cloned and the protein was purified. It is a 56 kDa protein that shares 26% identity and 59% similarity with its yeast counterpart. The protein interacts with the two RAD6 homologs found in higher eukaryotes (RAD6A and RAD6B) with equal affinity and is ubiquitously expressed in all human tissues. Among the conserved regions are a RING finger motif found in the N-terminus that is required for the interaction with RAD6A/B, and a zinc finger that is presumably required for interaction with DNA.

In principle, the accessory DNA polymerases and associated proteins described herein represent potential targets for antimutagenesis strategies. However, deficiency of individual polymerases may result in enhanced carcinogenesis. The most well-studied example of this is the human syndrome xeroderma pigmentosum variant, which is a skin cancer-prone condition that results from an inherited deficiency of DNA polymerase η. This enzyme is posited to be specialized for the error-free bypass of cyclobutane dimers between adjacent thymidine bases. In its absence, recent data indicate that polymerase iota assumes its function and is error-prone when doing so. Unexpectedly, however, when both polymerases are deficient in mouse models, UV-induced skin cancer is accelerated despite reduced UV-induced mutant frequencies in the double knockout. These data support a role for polymerase iota as a tumor suppressor separate from its role in TLS.

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Translesion Synthesis

Definition

Error-prone DNA-repair process that involves a switch to low fidelity DNA-dependent polymerases to bypass an unhooked interstrand crosslink. Since both DNA strands are damaged at the site of such lesion, the antisense strand cannot serve as a template for repair.

►Translesion DNA Polymerases

Translin

Definition

TSN; A Protein encoded by the TSN gene is reported to recognize single-stranded DNA ends of staggered breaks that may occur at recombination hotspots. A role in recombination repair has been suggested, but has still to be confirmed.

►ALU Elements

Translocase

Definition

Is an enzyme that translocates lipids between the two leaflets of the lipid bilayer.

►P-glycoprotein

Translocation

Definition

- Translocation of chromosomes is the illegitimate recombination between nonhomologous chromosomes, a rearrangement in which part of a chromosome is detached by breakage and then becomes attached to another chromosome. The translocation may or may not be reciprocal; in reciprocal balanced translocations, genetic material is exchanged without loss between nonhomologous chromosomes; in unbalanced translocations chromosomal material, often the translocated material from one of the nonhomologous chromosomes is deleted.
- Translocation of proteins is the regulated movement of proteins or other molecules from one cellular compartment or organelle to another.

Translocation ETS Leukemia Gene

►ETV6

Translocation Non-homologous

Definition

Non-homologous translocation; rearrangement of chromosomes that results in the fusion of two chromosomal segments that are not normally attached to one another, often resulting in a microscopically visible alteration of karyotype; unbalanced translocations often involve deletion of genetic material.

Translocation Reciprocal

Definition

Reciprocal translocation; exchange of chromosomal segments between two chromosomes from different chromosome pairs, resulting in the conservation of all participating chromosomal segments; no phenotypic change will occur when the translocation is balanced, which means at the same position of the two homologous chromosomes.

Translocation t(14;18)

Definition

Chromosomal translocation commonly associated with non-Hodgkin B-cell lymphomas. This chromosomal

translocation event places the ►Bcl-2 gene into juxtaposition with powerful enhancer elements associated with the IgH locus, causing a transcriptional deregulation of the Bcl-2 gene and resulting in elevated levels of Bcl-2 mRNA and Bcl-2 protein production.

Transmembrane

Definition

Referring to the domain of a protein that is threaded through a membrane and therefore exists in the hydrophobic environment of a lipid bilayer.

Transmembrane 4 Superfamily Protein

►Metastasis Suppressor KAI1/CD82

Transmembrane Domain

Definition

The portion of a protein that spans the lipid bilayer of the cell membrane.

►Prostate-Specific Membrane Antigen (PSMA)

Transmembrane Protein Type I

Definition

A transmembrane protein that exposes its carboxyl terminus into the cytosol.

►Endoplasmic Reticulum Stress

Transmembrane Protein Type II

Definition

A transmembrane protein that exposes its amino terminus into the cytosol.

►Endoplasmic Reticulum Stress

Transmembrane Signaling

Definition

The plasma membrane of mammalian cells provides a protective barrier and many extracellular agonists are unable to enter the cell. Instead, they recognize and activate specific receptors at the cell surface that in turn stimulate activity of proteins (e.g., lipid-hydrolyzing enzymes) present inside the cell and in this way transmit the signal across the membrane, i.e., from extracellular to intracellular environment.

Transmigration Assay

Definition

Is an *in vitro* assay where cells are placed on one side of a chamber containing a porous membrane (usually coated with an artificial ►extracellular matrix), a hormonal stimulus is applied, and the rate at which cells migrate across the membrane is determined. It measures cell invasiveness, or the ability of metastatic cells to escape from a tumor.

Transphosphorylation

Definition

When two receptor tyrosine kinases are brought into close proximity to each other, the tyrosine kinase domain of one of the receptors phosphorylates the kinase domain on the other receptor. Likewise, the

freshly phosphorylated kinase domain is now more active and can phosphorylate and activate the kinase domain on the remaining receptor.

Transplacental Carcinogen

Definition

A substance that is able to cross the placenta, inducing cancer in the offspring.

►Diethylstilbestrol

Transporter Nomenclature

Definition

Refers to the names used to describe the various biological transporters and pumps used to move a drug through the body.

►ADMET Screen

Transporters

Definition

Are biological protein pumps which move a chemical from one place in the body to another.

►ADMET Screen
►ABC Drug-Transporter

Transposon

Definition

A mobile DNA element that is capable of moving within the genome, either by copying itself or by cutting itself out of its original site and inserting in a new location.

►Retroviral Insertional Mutagenesis

Transpupillary Thermal Therapy

Definition

Near-infrared frequency laser used to concentrate thermal injury on the site of interest (e.g., tumor) leaving the surrounding tissue generally undisturbed.

►Uveal Melanoma

Transurethral Resection

Definition

TUR; Endoscopic operation to remove tumor tissue from the bladder or the prostate via the urethra.

►Urothelial Carcinoma, Clinical Oncology

Transversion

Definition

A point mutation in which a purine base is mutated to a pyrimidine base or vice-versa. Mutations from purine to purine or pyrimidine to pyrimidine are transitions.

►UV Radiation

Trastuzumab

Definition

Synonym ►Herceptin; is a humanized IgG1κ monoclonal antibody specifically binding to HER-2.

Human epidermal growth factor 2 (►HER2/neu), also known as ErbB-2, is a member of the epidermal growth factor receptor (ErbB) family and is notable for its role in the pathogenesis of ►breast cancer and as a target of treatment. It is a cell membrane receptor tyrosine kinase normally involved in the signal transduction pathways leading to cell growth and differentiation.

HER2 is named because it has similar structure to human epidermal growth factor receptor, or HER1. ErbB2 was named for its similarity to ErbB (avian erythroblastosis oncogene B), the oncogene later found to code for EGFR. Gene cloning showed that *neu*, HER2, and ErbB2 were the same.

Trastuzumab is approved for treatment of HER-2 overexpressing metastatic breast cancer in combination with chemotherapy and as single-agent therapy in those patients with metastasic breast cancer who do not respond to chemotherapy.

►Monoclonal Antibody Therapy
►Drug Design
►Herceptin

βTrCP

Definition

Beta-transducin repeats-containing proteins (βTrCP) serve as the substrate recognition subunits for the SCF complexes. βTrCP interact with substrates phosphorylated within the DSGXX(X)S destruction motifs. SCFβ-TrCP mediate ubiquitination and proteasomal degradation of phosphorylated substrates.

►Anoxia

Treatment-refractory Germ Cell Tumors

►Platinum-Refractory Testicular Germ Cell Tumors

Trefoil Factors

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Synonyms

Trefoil peptides; SMAD-4/DPC4; TFF

Definition

Trefoil factors (TFF) belong to a family of heat, acid, and protease-resistant regulatory peptides ubiquitously expressed in brain, blood, and peripheral organs. In inflammatory conditions and generation of cancer lesions, they are induced, lost, or modified by gene silencing and somatic mutations. Thus, TFF overexpression or invalidation is either the consequence or the causal origin of human solid tumors and their ►progression to metastatic situations. While the TFF receptors or recognition systems are not still clearly identified, TFF are involved in mucosal and epithelial cell cytoprotection, wound healing, cancer cell survival and ►invasion, ►angiogenesis, through several oncogenic pathways involved in neoplasia. Finally, TFF are now considered as multifaceted factors with beneficial and pejorative functions on inflammatory and cancer diseases, according to their dual and divergent impacts at early and late stages of these pathological states.

Characteristics

TFF Discovery and Expression

Since the discovery and molecular annotation of the trefoil factor pS2 (TFF1) in human breast cancer, much attention has been devoted on TFF1 and its structurally related protease and acid-resistant factors spasmolytic polypeptide (SP-TFF2) and intestinal trefoil factor (ITF-TFF3). These TFF contain either one (TFF1 and -3) or two (TFF2) trefoil domains delimited by three disulphide bridges. TFF are involved in the stabilization of the mucus layers secreted by mucosal epithelial cells. The three human trefoil genes are located in a cluster region of 55 kb on chromosome 21q22.3. A novel two trefoil domains Bm-TFF2 protein activating platelet aggregation has been recently purified from the frog *Bombina maxima* skin secretions. TFF are widely expressed in brain, the urogenital system (breast, kidney, ►prostate), the lymphoid tissue, the respiratory and the digestive tract (esophagus, ►stomach, intestine, exocrine and endocrine ►pancreas, and liver), in conjunctival goblet cells and pterygium. TFF1 and -2 are predominantly detected in the normal stomach whereas TFF3 is found mainly in the small and large intestine. TFF are regulated via genetic, ►epigenetic, and tissue-specific mechanism including amplification of the chromosomal region 21q22 harboring TFF family genes in ►cholangiocarcinoma, promoter methylation, chromatin modification, histone H3 acetylation, and transcription factors downstream signaling pathways involved in cellular ►stress responses, ►inflammation, and cancer. These pathways include gastrin and bFGF growth factors, the ►interleukin 6 family cytokine receptor gp130/►STAT1–3 and ►SHP-2/►ERK cascades, ►ras, the ►hypoxia-induced HIF-1α transcription factor, allergens in lungs, nuclear ►estrogen receptors and ►GATA-6, ►NF-κB,

peroxisome proliferator-activated receptor gamma (PPAR-γ), hepatocyte nuclear factor 3 (HNF3), the homeodomain transcription factor CDX2, and the activator protein ►AP-1 via the negative control of COBRA1, the cofactor of ►BRCA1 (breast cancer-associated protein 1) involved in ►DNA damage repair.

TFF are secreted by the gastrointestinal mucosa in a mucus, inflammation, and ulcer-associated cell lineage (UACL)-dependent manner. Chronic inflammation and ulceration in the gastrointestinal tract is associated with the development of the reparative UACL from mucosal ►stem cells. UACL was originally described as pyloric metaplasia in the ileum, reflux esophagitis associated with Barrett's esophagus, peptic ulcer in the stomach, and chronic cholecystitis. TFF display multifaceted roles in mucosal repair and during cancer progression.

TFF in Mucosal Repair and Protection

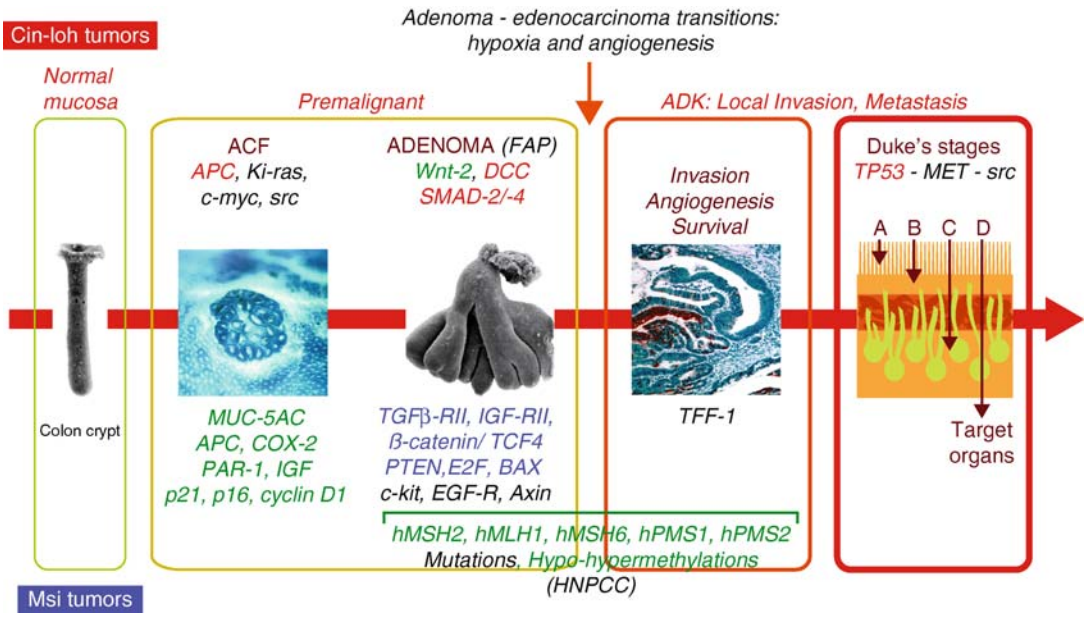
It is now well accepted that TFF are involved in maintenance and repair of the mucosal barrier, wound healing, and cytoprotection during hypoxia and transient inflammatory situations in experimental ulcerative colitis. Local administration of recombinant TFF and ectopic expression of TFF3 in cellular models and transgenic animals supported this general idea of a cytoprotective role for TFF in mucosal repair. Both epithelial and stromal cells contribute to wound healing and mucosal repair. Consistent with a signaling role of TFF in mucosal protection, the tetraspanin family member Vangl1 is involved in the migratory response to TFF3 through Ser/Thr phosphorylation in intestinal epithelial cells. In addition TFF3 improved intestinal crypt stem cells survival following combined radiation and ►chemotherapy in both wild-type and TFF3$^{-/-}$ knockout transgenic mice.

TFF in Chronic Inflammation and Cancer Progression

Although some studies argue for a therapeutic potential of TFF in mucosal injury and wound healing, recent advances in the field support their adverse effects during chronic inflammation and cancer progression. Persistent inflammatory situations initiate several genetic, molecular, and cellular dysfunctions associated with tumor promotion and cancer progression. Notably, self-induction and cross-talk between TFF at their regulatory sequences have been described as a molecular signature of chronic inflammatory situations and neoplasia.

For example, TFF1 exerts divergent functions in the digestive mucosa. In the stomach, gastric TFF1-deficient mice develop antropyloric adenomas and carcinomas, suggesting that *TFF1* is a candidate gastric-specific ►tumor suppressor gene to protect the mucosa against repetitive injury from digestive secretions, ulceration, and chronic inflammation induced by acid, proteases, and pathogens, such as ►*Helicobacter pylori*. Somatic mutations and loss of heterozygosity (LOH) of the *TFF1* gene is observed in human gastric cancers, in association with TFF3 overexpression. In coherence with these observations, ►cyclooxygenase (COX)-2 was strongly induced in pyloric adenomas induced by genetic ablation of the *TFF1* gene in mice. Similarly, COX-derived products are reported to exert beneficial roles in mucosal protection and wound healing, but deleterious functions during chronic inflammation and neoplasia. Conversely, in the normal human colon TFF1 is absent but is induced at high levels in Crohn disease, colitis, and colorectal cancers. It is therefore likely that TFF1 is a cancer progression factor in the human colon, according to its aberrant expression and transforming functions at the adenoma and carcinoma transitions (Fig. 1). Thus, TFF exert opposing functions, one counteracting transient inflammatory situations and the other linked to pejorative functions, in cooperation with other dominant genetic and molecular alterations during the neoplastic progression in the human colon. These include the cancer predisposition pathways controlled by ►Wnt/►APC/β-catenin, ►TGF-β/►SMAD-4, ras, ►src, and deleted in colon cancer (DCC). Validation of this model can be explored in transgenic animals harboring selectively these oncogenic alterations, in cooperation with forced expression of TFF1 in intestinal stem cells, through intestinal promoters that are functional in this cellular compartment, such as the villin promoter (pVIL) and the carcinoembryonic antigen (p►CEA) regulatory regions.

The emergence of colorectal adenocarcinomas (ADK) is a complex multistep process linked to genomic and ►chromosomal instability (CIN) and LOH, ►microsatellite instability (MSI), DNA ►aneuploidy, and generalized deregulation of gene expression and signal transduction pathways. The first mechanism, which accounts for 80% of sporadic cases, is connected with CIN and LOH targeting the tumor suppressors *APC* (5q), ►*TP53* (17p), *DCC* (18q), and TGFβ pathway signaling elements *SMAD-2* and *SMAD-4* (►*SMAD-4*/*DPC4*) at 18q. Sporadic MSI tumors are frequently mucinous, predominantly localized in the right colon, and generally diploid. In MSI patients, alterations in TGFβ-RII, IGF receptors IGF-RII, β-catenin, TCF-4, and E2F transcription factors, as well as loss of the ►*PTEN* tumor suppressor, and the ►apoptosis regulator BAX are frequently reported. Sporadic cancers are also driven by epigenetic mechanisms, hypo and ►hypermethylation of promoter genes encoding cancer markers and/or effectors, such as TFF, MUC-5AC, COX-2, ►protease-activated receptors PAR-1, ►IGF, ►p21/p16/cyclin D1. Dominant activation of protooncogenes by point mutations or constitutive activation by other oncogenic pathways are also frequently observed at early stages (►ACF, polyps: src, ras, ►c-myc) and late stages concomitant with cellular invasion, angiogenesis, and ►metastasis (►c-Kit, ►EGF-R, ►VEGF, the hepatocyte growth factor receptor ►MET, src, and many others).



Trefoil Factors. Figure 1 Genetic and molecular alterations linked to the multistep progression of familial and sporadic human colorectal cancers.

Familial adenomatous polyposis (FAP) is induced by mutations of the *APC* gene, a defect that contributes to CIN and appearance of more than 100 colorectal adenomas. Molecular alterations in other elements of the Wnt pathways are also concerned in sporadic ►colon cancers, including Wnt-2, Axin, β-catenin, and TCF4 transcription factor. Nonpolyposis form of the hereditary colon cancer (HNPCC) is more frequent than FAP, and is caused by germ cell mutations that invalidate the DNA repair systems. DNA ►mismatch repair is deficient in 90% of the HNPCC patients. The mutations concern mostly the hMSH2 and hMLH1 DNA repair enzymes, less frequently hPMS1 and hPMS2.

Such genetic and molecular changes lead to the formation of aberrant crypt foci (ACF), which precede the appearance of ►premalignant adenomas anchored in the colon mucosal wall. The next stage is the evolution of the adenoma toward more aggressive lesions (ADK), and irreversible acquisition of dominant and anarchic functions, chronic inflammation, oxidative ►DNA damage, autocrine and paracrine regulatory loops linked to IGF-R, VEGF-R, EGF-R ligands, induction of thrombin protease-activated receptors PAR-1, trefoil factor pS2 (TFF-1), and the immediate response gene *COX-2*.

Aberrant Expression of TFF as Clinical Markers of the Neoplasia

TFF are involved in the neoplastic progression in human epithelial tumors according to their ability to confer several transforming functions including resistance to apoptosis, induction of cellular scattering, anchorage-independent growth in soft agar, proinvasive and proangiogenic activities in vitro and in vivo. Both TFF1 and TFF3 reduced apoptosis induced by serum privation and loss of cellular adhesion (►Anoikis), a major response linked to cancer cell transformation, survival, and dissemination. Accordingly TFF are connected with several oncogenic and tumor suppressor elements such as ►E-cadherins, EGF-R, the ►RhoA-►ROCK axis, ►PI3-kinase (►PI3K), phospholipase C, COX-2, nitric oxide synthase 2, NF-kB, STAT3, and ►Cdc25. The nuclear phosphatases Cdc25A and B are associated with hypergrowth activity and control of the ►G2/M checkpoint in response to DNA damage and repair.

This is supported by clinical investigations on aberrant expression of TFF in human solid tumors of the prostate (TFF3), premalignant► changes and neuroendocrine differentiation in human prostate cancer (TFF1), human hepatocellular carcinomas (promoter hypomethylation of the *TFF3* gene), hepatolithiasis, and cholangiocarcinomas (TFF1–MUC5AC), primary mucinous carcinomas of the skin (TFF1–TFF3), and ulcerating Barrett esophagus, a precancerous lesion considered as a gastric-type metaplasia. Barrett esophagus is characterized by the specific expression of the gastric-type markers TFF1 and MUC5AC with high levels and strong colocalization in the surface epithelium. In contrast, TFF3, MUC6, and MUC5B were found in the deeper glandular structures. Similarly, gastric metaplasia of the duodenum (GMD) is characterized by replacement of the intestinal epithelium with gastric-type mucus cells, villus

damage and atrophy, and is frequently found in association with inflammation and gastritis induced by *H. p*. GMD expressed TFF1, TFF2, and the colon cancer-associated ►mucin MUC5AC, a marker of ACF now considered as precancerous lesions in the large bowel.

Progressive loss of TFF1 and TFF2 associated with reciprocal induction of TFF3 is likely to be involved in the early stages of gastric ►carcinogenesis. In the normal gastric mucosa, TFF2 is expressed in surface mucus neck cells. Decreased TFF2 expression in chronic atrophic gastritis possibly attributes to the decrease in the number of surface epithelial cells expressing TFF2. Reexpression of TFF2 in gastric epithelial dysplasia implies that TFF2 possibly contributes to the progression of ►gastric carcinoma. Recently, it has been reported that TFF3 induction in gastric tumors correlated with an aggressive phenotype with advanced stages, infiltrative growth pattern and positive lymph nodes. TFF3 is now considered as a marker of poor prognosis in human gastric carcinomas and is associated with aggressive behavior and lethality of colon cancer cells in rats. Ectopic expression of TFF3 promoted the invasive phenotype in Rat-2 fibroblast cells associated with upregulation of β-catenin, MMP-9 matrix ►metalloproteinase, and downregulation of the tumor suppressor gene product ►E-cadherin involved in β-catenin-associated ►adherens junctions. In fact, several reports suggest that TFF participate to morphogenesis and differentiation programs for epithelial cells in breast, gastrointestinal tract, and lungs. Conversely, depletion of TFF3 in the human gastric cancer cell line SNU-1 that expresses TFF3 resulted in decreased ability to form colonies in soft agar and in a marked increase in apoptosis and chemosensitivity to anticancer agents. The situation is probably more complicated since both TFF2 and TFF3 are induced in advanced gastric carcinomas and linked with neoangiogenesis, thus having a negative impact on patient survival, and are independent predictor of disease recurrence. Both TFF1 and TFF2 are strongly expressed in diffuse-type gastric cancers, suggesting that the academic definition of TFF1 as a gastric-specific tumor suppressor gene should be applied in relation with the corresponding status of TFF2/TFF3, and the clinicopathologic context and oncogenic status of human gastric tumors. Several reports indicate that TFF2 is a gastric marker of tumor metastasis frequently upregulated in diffuse gastric cancers in correlation with decreased survival. TFF2-expressing cells are upregulated in the stomach of *Helicobacter*-infected mice and seem to give rise to invasive cancerous lesions. Consequently, both TFF2 and COX-2 are overexpressed in patients with *H.* ►*pylori*-induced chronic fundic gastritis in association with dysplasia. In the established *H.* felis/C57BL/6 mouse model of gastric cancer induced by chronic infection with *Helicobacter* felis, bone marrow-derived mesenchymal progenitor cells, but not hematopoietic stem cells, are recruited to site of gastric mucosa injury and inflammation. This proliferative zone gives rise to spasmolytic expressing metaplasia (SPEM) and differentiation toward an epithelial phenotype, evidenced by positive staining for TFF2 and the epithelial cell cytokeratin KRT1–19 in deep antral and fundic glands. Thus, experimental *Helicobacter* infection can give rise to a new mucosal microenvironment in the infected gastric mucosa following upregulation of the stem cell factor SCF-1 (the ligand of the c-kit tyrosine kinase) and the ►chemokine ►SDF-1 binding the ►G-proteins coupled receptor CXCR4, two key factors involved in the mobilization of bone marrow progenitors and cancer metastasis. It remains to be elucidated whether subpopulations of human gastric cancers may originate from the neoplastic transformation of bone marrow progenitors with gastric mucosal cell gene expression pattern. In addition, SPEM was suppressed by invalidation of the ►TNF-α gene in $Tnf^{-/-}$ K19-C2mE transgenic animals expressing simultaneously COX-1/-2 in gastric mucosa via the cytokeratin 19 gene promoter. Finally, we cannot exclude the possibility that illegitimate and constitutive expression of TFF2 by mesenchymal bone marrow stem cells may also target the gastric progenitor niche for metaplasia and dysplasia. Notably, SPEM is associated with gastric *H.* ►*pylori* infection, aberrant expression of the mucin 6 (*MUC-6*) gene, and progression of human gastric adenocarcinoma. Therefore, the combined loss of the gastric-specific tumor suppressor gene TFF1 with induction of TFF2 and TFF3 provide insights into the complex mechanisms underlying the biological significance and versatility of TFF in gastric cancer progression and neoplasia.

In breast cancer, TFF1 and TFF3 but not TFF2 were identified as informative markers for the detection of ►micrometastases in axillary lymph nodes and blood. Significance analysis of microarrays identified a positive correlation between TFF1 overexpression and breast cancer metastasis to bone in a cohort of 107 patients with primary breast tumors who were all lymph node negative at the time of diagnosis. The involvement of the FGF signaling pathway was also incriminated in preference of tumor cells that relapse to bone. The fact that TFF1 may contribute to tumor relapse to bone is underscored by its abundant presence in breast cancer micrometastases.

In addition, morphogenetic effects have been attributed to TFF2 in human breast cancer cells following the induction of highly complex branched ductular structures typical of migratory, invasive, and survival functions, in a TFF1-dependent manner. TFF3 was also expressed in breast ductal and lobular breast carcinomas in situ, and invasive lobular carcinomas. While TFF1 is a surrogate indicator for the response to antihormonal therapy and favorable outcome in estrogen receptor-α (ERα)-positive and well-differentiated breast cancers, its deregulated expression is now considered to contribute

to the progression of both ER-α-positive and -negative human breast cancers. Of note, plasma levels of TFF were found elevated in patients with advanced prostate cancer.

Conclusions

TFF are now considered as valuable therapeutic tools for the treatment of injured mucosal epithelial cells and protection against mucosal and tissular damages following transient injury and other damages caused by radiation therapy and chemotherapy. Selective loss, induction, and overexpression of TFF observed during inflammatory processes and neoplasia deregulate TFF signaling cross-talks and signals, and compensatory functions linked to TFF. The molecular complexity of TFF is further illustrated by their ability to form covalent disulphide-linked dimers in vitro and in vivo. The possibility that TFF could form heterodimers adds further complexity for their relevance in receptor and signal transduction. Despite increasing interest on TFF in molecular research and clinical applications, TFF are still orphan signaling peptides facing unknown receptors in the classical definition of receptor-mediated signal transduction pathways from the plasma membrane, cytoplasmic and nuclear domains, and vice versa. Recent advances in the field pointed the discovery of new TFF-binding proteins apparently linked to mucosal protection, such as MUC-5AC and the gastrokine-1-like peptide blottin. It is conceivable that TFF are not released via normal secretory pathways in inflammatory situations and neoplasia. The epithelial cell polarity and its normal microenvironment with stromal and vascular cells, immune cells, and extracellular matrix components and their receptors, are lost during cancer progression. In this case, it is tempting to assume that TFF are in the abnormal situation interact with key signal transducers and cancer-associated stromal cell lineages during cancer progression, via illegitimate, pejorative, and persistent mechanisms. In this scenario, TFF were shown to signal through distinct transduction pathways following external addition of the peptides versus ectopic expression. Future attempts and new strategies to identify the stricto sensu TFF receptors and direct transducers are therefore expected in order to exploit the positive facets of TFF for therapeutic purpose and to fight against their deleterious functions in pathological states.

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Trefoil peptides

►Trefoil Factors

Treg

Definition

►T Regulatory cells; are a subset of CD4+ T lymphocytes responsible for the regulation of immune responses. Treg have a defined phenotypic profile (CD4+ $CD25^{high}$Foxp3+) and can suppress functions, (e.g. proliferation or cytokine production) of other (responder) cells. Treg are expanded in the periphery and at tumor sites of patients with cancer. In patients with autoimmune disease, Treg are few or, if present, mediate no suppressor functions. Thus, the quantity and quality of Treg control the immune responsiveness in health and disease.

►Autoimmunity and Prognosis in Cancer
►Regulatory T Cells

Tremolite

Definition

Is an amphibole form of ►asbestos with a basic composition of calcium and magnesium silicates. Upon increased iron content, its color changes from C reamy white to dark green. The iron-containing samples are carcinogenic.

TRF1-interacting, ankyrin-related ADP-ribose polymerases

►Tankyrases

TβRI and TβRII

Definition

Type I and type II serine/threonine kinase receptors of ►TGF-β.

►Transforming Growth Factor Beta

Tribbles Homologue 3

Definition

TRB3; Also synonym TRIB3, NIPK and SKIP3. A pseudokinase that has been shown to regulate several targets potentially involved in controlling tumor growth including Akt.

►Cannabinoids
►Akt Signal Transduction Pathways

Trichilemmoma

Definition

Benign tumor of the hair follicle infundibulum. Multiple trichilemmomas are present on the face in ►Cowden Syndrome.

Trichostatin A

Definition

TSA; Is a potent reversible inhibitor of the family of ►histone deacetylases (HDAC). Was originally reported (1976) as a fungistatic antibiotic obtained from a culture broth of Streptomyces platensis. TSA has some uses as an anti-cancer drug.

Trichothiodystrophy

Definition

PIBIDS, this is an acronym for photosensitivity, ichthyosis, brittle hair and nails, intellectual impairment, decreased fertility and short stature. Disease related to ►nucleotide excision repair. Patients with PIBIDS have mutations in the ►xeroderma pigmentosum (XP) genes XP-B or XP-D.

Trident

►Forkhead Box M1

Trifunctional Antibody

Definition

A hybrid bispecific IgG antibody containing 2 Fab segments with specificity for different antigens and Fc portions that may be derived from different species.

Triglyceride

Definition

A glyceride in which the glycerol is esterified with three fatty acids. It is the main constituent of vegetable oil and animal fats.

►Fatty Acid Synthase

4′,5,7-Trihydroxyisoflavone

►Genistein

3,4´,5-Trihydroxystilbene

►Resveratrol

Trinucleotide Repeat

Definition

Any three nucleotide sequence that is repeated. CGG, CAG, and GAA expansions are associated with human genetic disorders.

►Fragile Sites

Triple Negative Breast Cancer

Definition

Breast cancer that lacks expression of estrogen receptor and progestaron receptor and amplification/overexpression of HER2; a feature of many basal-like breast tumors that is often used as a synonym for basal-like breast cancer.

►Basal-like Breast Cancer

Tripterine

►Celastrol

Trisomy

Definition

Presence of three copies of a particular chromosome instead of the normal two.

Trivalent Chromium (Cr^{+3})

Definition

Chromium ion in the highly reduced state, with a valence of +3. This is thermodynamically the most stable state of chromium ion.

►Chromium Carcinogenesis

Trk Family Tyrosine Kinase Receptors

Definition

Includes Trk (synonym TrkA), TrkB, and TrkC; they are responsible for mediating the tropic effects of the ►NGF family of ►neurotrophins. Nerve growth factor (NGF) specifically recognizes Trk, a receptor identified in all major NGF targets, including sympathetic, trigeminal, and dorsal root ganglia as well as in cholinergic neurons of the basal forebrain and the striatum. ►Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) specifically activate the TrkB tyrosine kinase receptor. TrkB transcripts encoding this receptor are found throughout multiple structures of the central and peripheral nervous system. Trk was originally cloned as an oncogene fused with the tropomyosin gene in the extracellular domain. The rearranged Trk oncogene is often observed in non-neuronal neoplasms such as ►colon cancer and ►papillary thyroid carcinoma, while the signals through the receptors encoded by the proto-oncogene Trks regulate growth, differentiation and apoptosis of the tumors with neuronal origin such as ►neuroblastoma and ►medulloblastoma. The intracellular Trk signaling pathway is also different depending on the Trk family receptors, cell types and the grade of transformation Furthermore, developmentally programmed cell death of neuron, which is largely regulated by ►neurotrophin signaling is at least in part controlled by tumor suppressors ►p53 and ►p73. Thus, Trk and its downstream signaling function in both ontogenesis and oncogenesis.

TRK-A

Definition

Member of the Trk Family Tyrosine Kinase Receptors.

►TRK

Troglitazone

Definition
A synthetic that can suppress cancer expression as a ligand for peroxisome proliferator-activated receptor-γ.

►Fucoxanthin

TROP-1

►EpCAM

Trophoblast

Definition
Extra embryonic layer of epithelium that forms around the mammalian blastocyst and attaches the embryo to the uterus wall.

►Cancer-Germline (CG) Antigens

Tropism

Definition
Generally movement of an organism or cell; for viral vectors: Targeting of specific tissues or cell types within an organism. The predilection of a virus or another pathogen to invade, and replicate in, a particular cell type or tissue or organism.

►Oncolytic Virotherapy

Tropomyosin

Definition
Structural protein involved in muscle contraction.

Trousseau Syndrome

Definition
Armand Trousseau initially described the phenomenon that malignancies are sometimes associated with spontaneous thrombotic events. Unexplained thrombotic episodes may occasionally lead to the diagnosis of cancer

►Tumor-Endothelial Cross-talk

Trp63

►p53 Family

Trp73

►p53 Family

Trx

Definition
►Thioredoxin.

Trypsin

Definition
Serine proteinase expressed not only in the pancreas for food digestion but also by uPA (urokinase-type plasminogen activator).

►Proteinase-Activated Receptor

Tryptase

Definition
A protein secreted by mast cells and related blood and tissue cell types, involved in allergic reaction, and used as a marker for mast cell activation.

►Mastocytosis

TSC

Definition
►Tuberous sclerosis complex.

►Tuberous Sclerosis Complex

TSC1

►Hamartin

TSH

Definition
Thyroid-stimulating hormone; Synonym thyrotropin; Is secreted from cells in the anterior pituitary called thyrotrophs, finds its receptors on epithelial cells in the thyroid gland, and stimulates that gland to synthesize and release thyroid hormones.

►Thyroid Carcinogenesis

TSH

Definition
Thyroid-stimulating Hormone.

TSH Receptor

Definition
The receptor for thyroid stimulating hormone (TSH), which is also called thyrotropin. Encoded by a gene on chromosome 14q, TSHR is largest of all known glycoprotein hormone receptors. It is one of the primary antigens in autoimmune thyroid disease. Autoantibodies to TSHR act as TSH agonists in ►Graves disease and as TSH antagonists in ►Hashimoto thyroiditis.

►Thyroid Carcinogenesis

TSP

Definition
Thrombospondin.

TSR

Definition
►Thrombospondin type 1 repeat.

►Thrombospondin

TTF-1

Definition
Thyroid transcription factor 1 is a marker of thyroid and lower respiratory epithelium often used in immunohistochemistry to detect tumors arising from the thyroid or lung.

►Hurthle Cell Adenoma and Carcinoma

tTGase

►Transglutaminase-2

Tuberin

Definition

Heterodimer partner of ►hamartin, forming the ►Tuberous sclerosis complex (TSC). Hamartin is encoded by the TSC1 gene, tuberin by TSC2. These two proteins function within the same pathway(s) regulating ►cell cycle, cell growth, ►adhesion, and ►vesicle trafficking.

Tuberous Sclerosis Complex

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Synonyms

TSC

Definition

►Tuberous sclerosis complex (TSC): An autosomal dominant disorder caused by a mutation in either the *TSC1* or *TSC2* genes and characterized by the development of hamartomatous growths in multiple organ systems.

Characteristics

Clinical Aspects

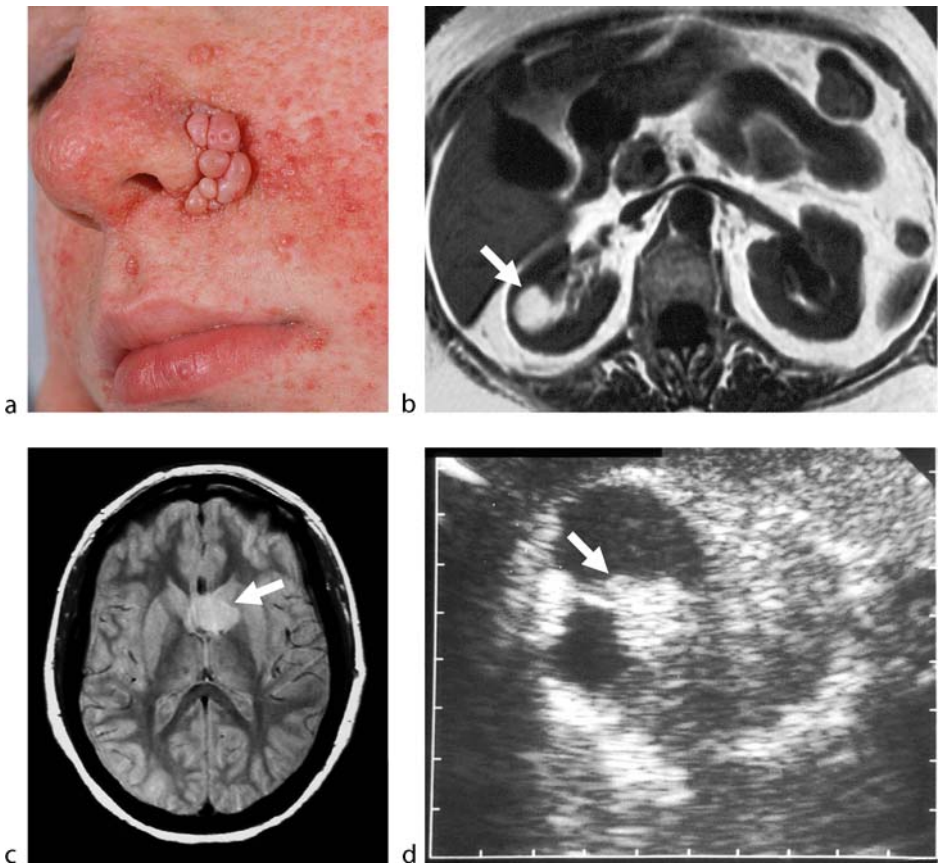
Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that occurs in up to 1 in 6,000 live births, without apparent ethnic clustering. TSC is characterized by the development of unusual tumor like growths, called hamartomas, in a variety of tissues and organs. Subependymal giant cell tumors in the brain occur in about 1 in 10 individuals with TSC, with reported incidence ranging from 6.1 to 18.5%. On serial imaging, these tumors appear to arise from subependymal nodules which are believed to be present in 88–95% of individuals with TSC. Involvement of the brain is associated with some of the most problematic clinical manifestations of TSC, including intellectual handicap, epilepsy, and abnormal behavioral phenotypes, particularly autism and attention deficit disorder with hyperactivity. Other organs commonly and significantly involved in TSC include the skin, kidneys and heart, where associated hamartomatous growths include facial angiofibromas, subungual fibromas, forehead plaques, shagreen patches, renal angiomyolipomas and cysts and cardiac rhabdomyomas (Fig. 1). Renal cell carcinoma (RCC) occurs at an earlier age and is more frequently multifocal in individuals with TSC than in the general population, although it is still uncommon (<5%).

Identification and Characterization of the TSC Genes

Linkage of TSC to chromosome 9q34 was reported in 1987 (locus termed *TSC1*); however, subsequent studies provided strong evidence for locus heterogeneity and led to the identification of a second locus at 16p13.3 (*TSC2*). Among families large enough to permit linkage analysis, approximately half show linkage to 9q34 and half to 16p13, and there is no evidence for a third locus.

Initial definition of the 1.5 Mb *TSC1* candidate region on chromosome 9q34 was achieved by identification of key meiotic recombination events in large *TSC1* families. Two putative recombinants in unaffected individuals then narrowed this region to 900 kb. Large deletions and other rearrangements of the candidate locus were sought in patients with TSC, but no abnormalities were detected. Complete genomic sequencing of the region was initiated and GRAIL2 (Gene Recognition and Assembly Internet Link) and BLAST (Basic Local Alignment Search Tool) were employed to predict putative coding exons and genes. Systematic amplification and mutation screening of exons using TSC patient DNA samples, revealed mobility shifts corresponding to small truncating mutations in the 62nd exon screened. This exon corresponded to a previously identified cDNA clone and various techniques were used to defined the remainder of the open reading frame (ORF). Comparison of cDNA and genomic sequences revealed 23 exons, the first two of which were untranslated. The 8.6 kb full-length transcript was predicted to encode a novel 1,164 amino acid/130 kDa protein termed hamartin.

During 1992–1993 linkage studies identified an ~1.5 Mb region of chromosome 16p as likely to contain the *TSC2* gene. At the same time, a family with both tuberous sclerosis and autosomal dominant polycystic kidney disease was found to segregate a translocation between chromosomes 16p and 22q. The translocation breakpoint on chromosome 16 in this family was shown to disrupt the previously unidentified *PKD1* gene and the *TSC2* gene was predicted to lie telomeric to this breakpoint. The breakpoint was mapped to 150 kb telomeric to 16AC2.5 (the most centromeric flanking marker then identified for *TSC2*). The telomeric limit of the candidate region was greatly reduced by the position of a second breakpoint in a previously reported patient who had a de novo truncation of 16p but no clinical or radiological evidence of TSC. The deletion in this patient effectively excluded ~1.1 Mb of the remaining 1.4 Mb *TSC2* candidate region. A cosmid contig was constructed for



Tuberous Sclerosis Complex. Figure 1 Tuberous sclerosis complex is characterized by the development of hamartomas in a variety of tissues and organs. (a) A facial angiofibroma, (b) an abdominal MRI scan showing a renal angiomyolipoma (arrowed) (c) a cranial MRI scan showing a partly intraventricular subependymal giant cell astrocytoma (arrowed), and, (d) a renal unltrasound scan showing a renal cell carcinoma (within a cyst) (*arrowed*).

the remaining 300 kb candidate region and probes generated from it were used to analyze a panel of TSC patients for rearrangements by pulsed field gel electrophoresis and Southern blotting. Five TSC patients were found to have genomic deletions of between 30 kb and 100 kb, which involved the same 120 kb interval. cDNA clones were isolated corresponding to four genes in the interval, and one was found to be disrupted by all five deletions, making it a strong candidate for *TSC2*. Four smaller intragenic deletions were then identified in TSC patients, including a de novo deletion that was associated with a truncated TSC2 transcript. These findings confirmed the identity of the *TSC2* gene. The 5.5kb *TSC2* transcript was predicted to generate a novel 1,807 amino acid protein product of ~198 kDa termed tuberin.

TSC1 and *TSC2* Act as Tumor Suppressor Genes

Most individuals who have tuberous sclerosis carry a mutant tuberous sclerosis gene in each of their somatic cells. However, it is clear that a huge majority of these cells proliferate, differentiate and function normally, while very occasionally, further localised events result in focal tumorigenesis. In 1971, Knudson proposed that inherited predisposition to tumors might reflect the germ-line mutation of "tumor suppressor genes" and that tumor development might be the result of somatic "second hit" mutations. Investigations of somatic mutations in a variety of tuberous sclerosis hamartomas supports classification of the TSC genes as tumor suppressor genes - several groups have reported evidence for large somatic deletions of the wild-type *TSC1* or *TSC2* allele, manifested as "loss of heterozygosity." The observation of loss of heterozygosity implies clonality of hamartomas, and this has also been confirmed by demonstration of non-random X chromosome inactivation in hamartomas from female patients with tuberous sclerosis.

Lymphangioleiomyomatosis and Pulmonary TSC

Lymphangioleiomyomatosis (LAM) is a disorder seen almost exclusively in females and is characterized by

bronchiolar smooth muscle infiltration and cystic changes in the lung parenchyma. LAM patients often have angiomyolipoma of the kidneys and/or abdominal and hilar lymph nodes. Symptomatic LAM is estimated to occur in ~1 per million of the population without other evidence of TSC, but in up to 5% of females with TSC, implicating a role for the TSC genes in the etiology of LAM. It has recently been shown that somatic mutations of the *TSC2* gene occur in the angiomyolipomas and pulmonary LAM cells of women with sporadic LAM, strongly supporting a direct role of *TSC2* in the pathogenesis of this disease. Interestingly, a mutation in the *TSC2* gene was identified in pulmonary and lymph node LAM cells from a patient with sporadic LAM prior to lung transplantation and the same mutation was subsequently found in the recurrent LAM. These data indicate that histologically benign LAM cells can migrate or metastasize in vivo to the transplanted lung.

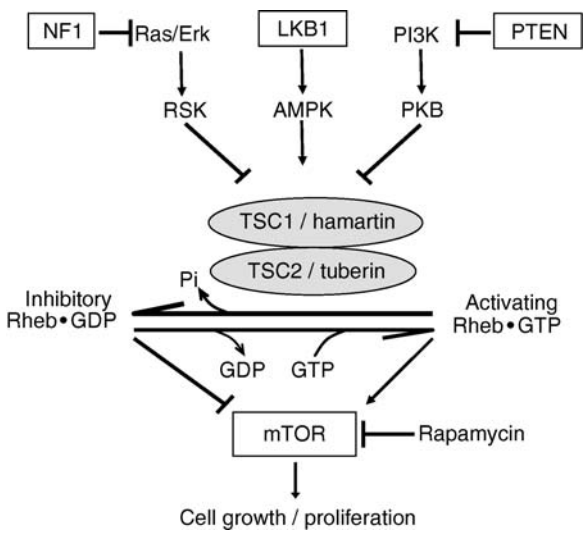
A model in which LAM cells migrate or metastasize to the lung challenges the boundary between benign and malignant diseases. There are other examples of histologically benign diseases in which cells appear to metastasize, including benign metastasizing leiomyoma, and disseminated peritoneal leiomyomatosis. If LAM cells containing *TSC2* gene mutations have the potential to migrate in vivo, it may indicate that the TSC genes have key functional roles related to cellular metastasis.

Role of hamartin and Tuberin in the mTOR Pathway

Genetic screens for growth suppressors in *Drosophila Melanogaster* showed that dTsc1 and dTsc2 play a pivotal role in the conserved insulin-signaling pathway to suppress cellular growth. Hamartin and tuberin function together to repress cell growth signaling through the mammalian target of rapamycin (mTOR). Tuberin possesses a guanine nucleotide triphosphate (GTP)-ase activating protein (GAP) domain which acts on the small G proteins Ras homologue enriched in brain (Rheb) and Rheb like 1 (RhebL1). Mutations in *TSC2* that are associated with tuberous sclerosis often result in the loss of the GAP domain through the deletion of the C-terminus. Furthermore, a clustering of single amino acid mutations within the GAP domain of *TSC2* has also been reported, implying that the GAP domain of tuberin is necessary for normal cell growth control. The normal function of hamartin and tuberin is to suppress the activity of Rheb and RhebL1: when in a complex with hamartin, tuberin reverts Rheb from an active GTP-bound state to an inactive guanine nucleotide diphosphate (GDP)-bound state through loss of the third phosphate on the guanine nucleotide. Active Rheb and RhebL1 have both been shown to associate with mTOR kinase and to specifically promote mTOR-directed phosphorylation of downstream signaling molecules involved in cell growth control that includes eukaryotic initiation factor 4E-Binding Protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1). mTOR also coordinates other critical cellular processes that if deregulated, can lead to cancer. These include cell cycle progression, autophagy (breakdown of cellular proteins), and nutrient uptake that feeds the growth of the cell. As a consequence, elevated mTOR signaling contributes to the pathology in numerous other human diseases associated with cancer.

mTOR and other Hamartoma Syndromes

Germline mutations that impair the normal tumor suppressor function of *PTEN* (phosphatase and tensin homolog), *NF1* (neurofibromin 1), and *LKB1* (also known as *STK11* (serine/threonine kinase 11)) can also lead to inherited hamartoma syndromes. Loss of *PTEN* function causes Cowden's disease, Lhermitte-Duclos disease and Bannayan-Zonana syndrome, while functional loss of either *NF1* or *LKB1* causes Neurofibromatosis "-type1" Peutz-Jeghers syndrome, respectively. Tuberin's ability to function as a RhebGAP is regulated by multiple phosphorylation events that involve cell signaling pathways that are regulated by PTEN, LKB1 and NF1 (Fig. 2); therefore lesions associated with each of these hamartomas syndromes show activation of mTOR and enhanced cell growth signals. PKB and RSK kinase, which lie within the PI3K/PTEN and Raf/NF1 signaling pathways, phosphorylate tuberin on overlapping Serine and Threonine sites. PKB inhibits



Tuberous Sclerosis Complex. Figure 2 Converging signaling pathways that regulate hamartin and tuberin. The NF1, LKB1 and PTEN tumor suppressors negatively regulate mTOR via the PI3K/PKB, AMPK and Ras/Erk/RSK signaling pathways. The hamartin/tuberin heterodimer act as a RhebGAP that converts the active Rheb˙GTP it to an inactive GDP-bound state. Rheb˙GTP activates mTOR, which promotes cellular growth.

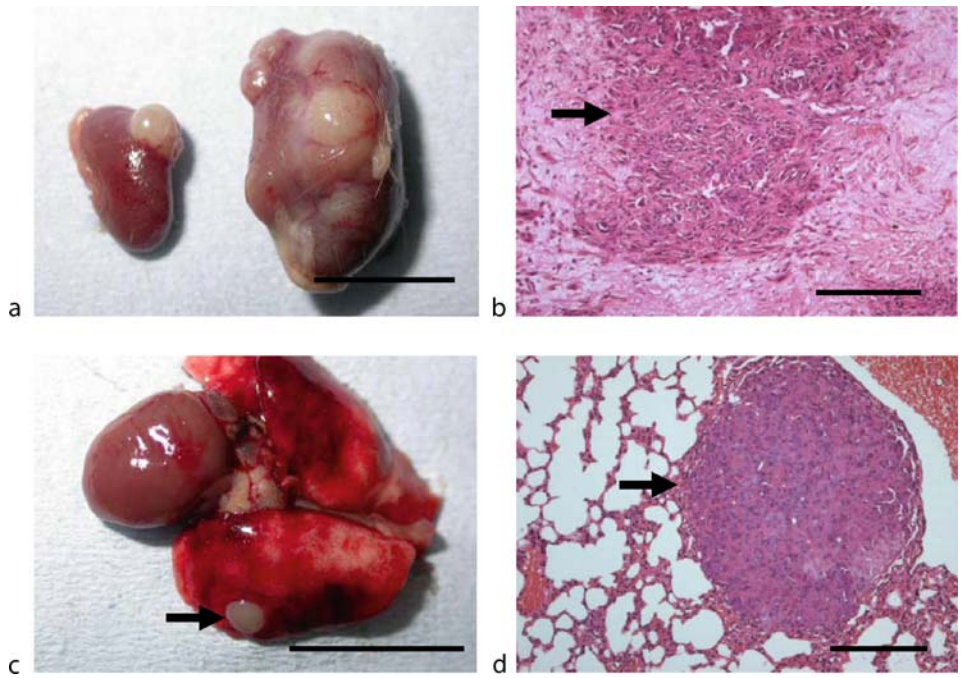
tuberin through the direct phosphorylation of Ser939 and Thr1462 that leads to elevated mTOR activity. Conversely, the LKB1 regulated energy-sensing pathway inhibits cell growth through AMPK-mediated phosphorylation of tuberin, which activates tuberin and represses mTOR. Interestingly, cancer cells deficient in *PTEN* have heightened sensitivity to the anti-proliferative and anti-cancer properties of rapamycin, which potently and specifically inhibits mTOR.

Rodent Models of Tuberous Sclerosis

Genetically engineered mice that carry a single mutant *Tsc1* allele (*Tsc1*$^{+/-}$ mice) develop renal cysts which develop into cystadenomas and RCCs. In one such model, the RCCs had a sarcomatoid morphology consisting of spindle cells with nuclear anaplasia arranged in whorled patterns and occasionally metastasized to the lungs (Fig. 3). Although the occurrence of RCC in humans with TSC is unusual, an association is recognized. The carcinomas are typically discovered at a young age and are thought to evolve from the lining of hyperplastic cysts. The proportion of sarcomatoid features in human TSC-associated renal carcinomas is far greater than in sporadic RCC.

Tsc2$^{+/-}$ mice develop renal lesions by 6 months, which appear to grow progressively throughout the life of the mouse. Histologically, the lesions resemble cystadenomas consisting of a spectrum of lesions including pure cysts, cysts with papillary projections and solid adenomas. Renal carcinoma, characterized by nuclear atypia, massive growth and metastatic disease developed in 5–10% of mice by 18 months, suggesting a very low rate of malignant progression for the cystadenomas (~1 in 1,000), indicating that additional genetic or epigenetic events are required for transformation. Liver hemangiomas, characterized by endothelial and smooth muscle proliferation with large vascular spaces, are seen in about half of *Tsc2*$^{+/-}$ mice by 18 months. Hemangiosarcomas also develop on the tail, paws, or mouth region in about 7% of *Tsc2*$^{+/-}$ mice by 12 months; these lesions do not metastasize but are malignant by cytologic criteria and exhibit bone invasion.

The Eker rat harbors a naturally occurring mutation (insertion of a 6.3 kb intracisternal A particle) in one allele of *Tsc2* and was first described as an autosomal dominant, hereditary model of predisposition to renal adenoma and carcinoma in 1954. Kidney lesions vary in morphology and include pure cysts, cysts with papillary projections and solid adenomas which can be seen as early as 4 months. A small minority of these tumors become malignant, with nuclear atypia and expand to include the entire kidney and metastasize to the lungs, pancreas and liver. Eker rats also develop pituitary



Tuberous Sclerosis Complex. Figure 3 *Tsc1*$^{+/-}$ mice described by Wilson et al. (2005) are predisposed to renal cysts that develop into RCCs that occasionally metastasize to the lungs. (a) Paired kidneys from a Tsc1$^{+/-}$ mouse with RCC ≥5 mm. (b) Microscopic view of a sarcomatoid RCC with elongated sheets of spindle cells. (c) Macroscopic, and (d) microscopic RCC metastases in the lungs. Macroscopic bars are 1 cm and microscopic bars are 200 μm.

adenomas, uterine leiomyomas and leiomyosarcomas, splenic hemangiomas and, at a low frequency, brain hamartomas resembling human TSC subependymal nodules.

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Tuberous Sclerosis Complex 1

►Hamartin

Tuberous Sclerosis Syndrome

Definition

A rare congenital disease in which the essential pathology is the appearance of multiple tumors in the brain and in other organs, such as the heart or kidneys. The name tuberous sclerosis comes from the characteristic tuber or root-like growths in the brain, which calcify with age.

►Tuberous Sclerosis Complex

Tubulin

Definition

A tubulin is a globular protein. There are five kinds of tubulins such as α-, β-, γ-, δ-, and ε-tubulins. Microtubules are assembled from dimers of α- and β-tubulins. γ-tubulin is involved in the nucleation and polar orientation of microtubules. δ- and ε-tubulins are localized at centrioles.

►Doublecortin
►Microtubule Associated Proteins

Tubulin Interacting Proteins

►Microtubule Associated Proteins

Tubulogenesis

Definition

Tube forming process, that gives rise to hyperpermeable tumor capillaries.

►Anoxia

Tumor

Definition

A neoplasm, or "new growth," of cells; a group of cells that has proliferated beyond the constraints of the normal tissue from which it was derived, in an unregulated, uncontrolled fashion, leading to an abnormal mass of cells.

►Chemical Carcinogenesis

Tumor Antigens

Definition

Proteins or carbohydrates that can be recognized by lymphocytes. Malignant cells can be specifically recognized by T lymphocytes or antibodies in cancer patients. This specificity is mediated by the somatically rearranged antigen-receptors in T cells and B cells,

i.e. the T cell receptor alpha and beta chains and the immunoglobulin light and heavy chains. The T cell receptor "sees" peptide fragments derived from any cellular protein of tumor cells in the binding-groove of the major histocompatibility complex on the cell surface. In contrast, the B cell receptor/immunoglobulin is able to bind various three-dimensional macromolecular structures with its variable domain. Common tumor antigens include normal proteins expressed by tumor cells in a lineage-restricted fashion (►Lineage differentiation tumor antigens), proteins expressed by malignant cells and cells in the germ line (►Cancer-testis-antigens) as well as individually mutated or viral antigens.

►Melanoma Vaccines
►Sentinel Node

Tumor-Associated Angiogenesis

Definition

The process of use of blood vessels already existent as well as the development of new blood vessels which provide critical nutrients and oxygen to tumors which are undergoing rapid expansion in mass. This process occurs at a time that is simultaneous with tumor promotion, and is first noted as an increase in vascular permeability. Tumor associated angiogenesis is in itself a multi-step process. There are angiogenic growth factors as well as anti-angiogenic growth factors and when the balance favors the positive signals, tumor-associated angiogenesis begins. This tipping over of pro-angiogenic factors to outweigh anti-angiogenic factor production has been termed "the angiogenic switch."

►Skin Carcinogenesis
►Angiogenesis

Tumor-Associated Antigen

Definition

TAA; Synonym Tumor Antigen. Malignant transformation of the cell, caused by viral infection, mutations, gene ►amplification, chromosomal deletion or translocation, can result in the expression of additional proteins in the malignant cell or in alterations of the expression of normal proteins. These changes are possibly immunogenic and can be recognised by the host's immune system. Over the years, several different antigens with tumour-dependent expression profiles were characterised and can be subdivided in different groups:

1. Tumour-specific shared TAA: encoded by genes that are frequently expressed in a wide variety of tumours, but not in normal tissues, except testis and placenta. Examples are MAGE, BAGE, GAGE and NY-ESO families. This group also includes antigens that arise from mutations in normal proteins such as ►p53, ►Ras, ►β-catenin, or from aberrant fusion proteins (e.g. ►bcr-abl).
2. Patient-specific TAA: arise from patient-specific mutations that occur in DNA, generating altered cellular proteins (e.g. the mutated form of the ►*CASP-8* gene,). These antigens are tumour-specific, hence not found in normal cells. Because they are patient-specific, this type of TAA are difficult to identify and to characterise.
3. Viral antigens: expressed in tumours which arise by infection with oncogenic viruses, for example ►human papillomavirus (HPV) E7 protein in ►cervical cancer.
4. Overexpressed TAA: present at low levels in normal cells, but overexpressed in tumour cells. Examples are PRAME, ►PSA and ►Her2/neu.
5. Differentiation TAA: present in tumour cells and their normal counterparts, e.g. Melan-A, gp100 and tyrosinase in melanoma).

►CD Antigens
►Melanoma Antigens

Tumor Associated Carbohydrate Antigens

Definition

TACA; Structure of carbohydrate nature that is produced by, but not unique for, tumor cells and which can evoke an immune response.

►Glycobiology

Tumor-Associated Fibroblasts

Definition

►Myofibroblasts.

Tumor-Associated Macrophages

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Definition

Tumor-associated ►macrophages (TAM) define a subset of myeloid cells that highly infiltrate solid tumors. Accumulating evidence clearly demonstrates, in various mouse and human malignancies, including colon, breast, lung, and prostate cancer, a strict correlation between increased numbers and/or density of TAM and poor prognosis. Based on this, recruitment and activation of TAM are regarded as pivotal steps of tumor progression, and TAM are putative targets for therapeutic intervention.

Characteristics

Experimental and clinical studies have revealed that chronic ►inflammation predisposes to different forms of cancer, including ►colon cancer, ►prostate cancer, and ►liver cancer, and that usage of ►nonsteroidal antiinflammatory drugs can protect against the emergence of various tumors.

In the late 1970s, it was found that a major leukocyte population present in tumors, the so-called TAM, promote tumor growth. Over the years it has become increasingly clear that TAM are active players in the process of tumor progression and invasion. In several experimental tumor models, the activation of an inflammatory response (most frequently mediated by macrophages) is essential for full neoplastic transformation and progression. This evidence strongly supports the idea that cancers originate at sites of chronic inflammation and suggests that the inflammatory circuits activated at the tumor microenvironment may represent suitable targets of novel anticancer therapies.

Macrophages

Macrophages (Mφ) play an indispensable role in the immune system with decisive functions in both ►innate immunity and ►acquired immunity. In innate immunity, resident Mφ provide immediate defense against foreign pathogens and coordinate leukocyte infiltration. Mφ contribute to the balance between antigen availability and clearance through phagocytosis and subsequent degradation of senescent or apoptotic cells, microbes, and possibly neoplastic cells. Their role is essential for triggering, instructing and terminating the adaptive immune response. Mφ collaborate with T and B cells, through both cell-to-cell interactions and fluid phase-mediated mechanisms, based on the release of cytokines, chemokines, enzymes, ►arachidonic acid metabolites, and reactive radicals. Mφ activation can be either proinflammatory or antiinflammatory, thus contributing to tissue cell destruction or to tissue regeneration and wound healing. These polar phenotypes are not expressed simultaneously, but regulated in such a manner that Mφ display a balanced, harmonious pattern of functions.

Mφ are critical effector cells in the acute innate response, for delayed-type hypersensitivity reactions and T cell-mediated immunity. In 1986 Mosmann et al. described two polarized sets of mouse T helper (Th) cells – Th1 and Th2 – with distinct cytokine secretion patterns. Th1 cells secreted interleukin-2 (IL-2), interferon-γ (IFN-γ), and lymphotoxin (LT, TNF-β). Th2 cells secreted IL-4, IL-5, and IL-6 and promoted B cells proliferation and antibodies secretion. Moreover, additional studies clarified that Th1 and Th2 cells may play opposite roles in pathological conditions, including infections and cancers.

Although excluded from the original "type I–type II" paradigm, Mφ role in the balance of polarized immune responses is being increasingly appreciated. Mφ are able to secrete either IL-12 or IL-10, cross-regulatory cytokines crucial for the elicitation of IFN-γ production and development of Th1 cells and IL-4/IL-13 secretion and Th2 cells proliferation correspondingly. The preferential production of IL-12 and IL-10 sets the basis for the M1/M2 Mφ polarization paradigm, elsewhere defined as the elicitation of functionally distinct Mφ populations, in response to the factors that dominate the inflammatory scene. In analogy with the Th1 and Th2 dicotomi, macrophages can be phenotypically polarized by the microenvironment to mount specific M1 or M2 functional programs. Chronic infections can tightly regulate the immune responses, being able to trigger highly polarized-type I or -type II inflammation and immunity. Classical or M1 macrophage activation in response to microbial products or IFN-γ are characterized by: high capacity to present antigen; high expression of proinflammatory cytokines, such as interleukin-12 (IL-12) and tumor-necrosis factor-α (TNF-α), and consequent activation of a polarized-type I response; and high production of toxic intermediates (►nitric oxide (NO), ►reactive oxygen species). Thus, M1 macrophages are generally considered potent effector cells that kill microorganisms and tumor cells and produce copious amounts of proinflammatory cytokines. In contrast, various signals (e.g., IL-4, IL-13, glucorticoids, IL-10, immunoglobulin complexes/TLR ligands) elicit different M2 forms, able to tune inflammatory responses and adaptive Th2

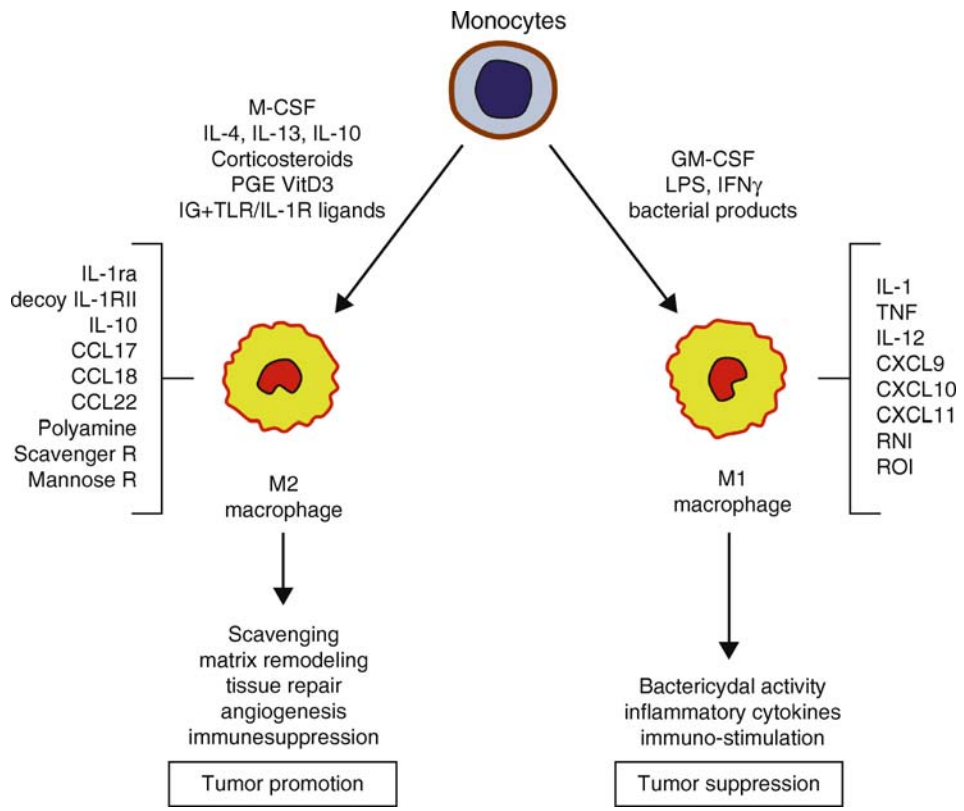
immunity, scavenge debris, promote ►angiogenesis, tissue remodeling and repair. Figure 1 shows selected functions of M1 and M2 polarized macrophages. Microenvironmental signals expressed at the tumor ►microenvironment have the capacity to pilot recruitment, maturation, and differentiation of infiltrating leukocytes and play a central role in the activation of specific transcriptional programs expressed by TAM.

Tumor-Associated Macrophages

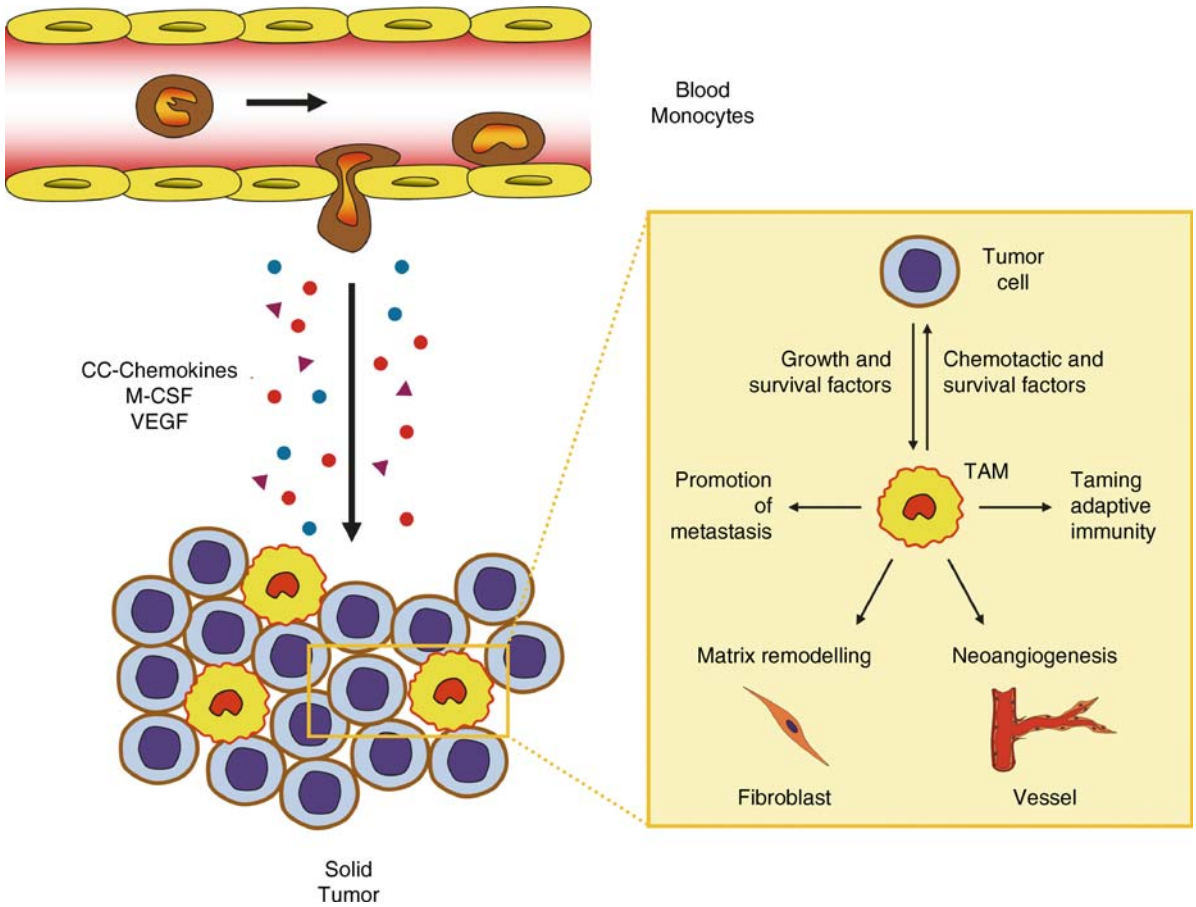
To the extent that they have been investigated, differentiated mature TAM have a phenotype and function similar to type II or M2 macrophages. TAM have been shown to exert a negative effect on antitumor immune responses.

TAM Recruitment

TAM are protumoral cells that are derived from circulating monocytes (Fig. 2) and are recruited to the tumor by a tumor-derived chemotactic factors, originally identified as CC-chemokine ligand 2 (CCL2; also known as MCP-1). Following this observation, other chemokines active on TAM were detected in neoplastic tissues as products of either tumor cells or stromal elements. These molecules have an important role in tumor progression by directly stimulating neoplastic growth, promoting inflammation, and inducing angiogenesis. Evidence supporting a pivotal role for chemokines, in addition to CCL2, in the recruitment of monocytes to neoplastic tissues includes a direct correlation between chemokine production and monocyte infiltration in mouse and human tumors. Molecules other than chemokines can also promote TAM recruitment. In particular, tumor-derived cytokines such as ►vascular-endothelial growth factor (VEGF) and ►macrophage-colony stimulating factor (M-CSF) promote macrophage recruitment, as well as macrophage survival and proliferation, and their expression correlates with tumor growth.



Tumor-Associated Macrophages. Figure 1 Monocytes differentiate into polarized macrophage subsets when exposed to different cytokine milieu. In the presence of GM-CSF, IFN-γ, LPS, and other microbial products, monocytes differentiate into M1 macrophages. In the presence of M-CSF, IL-4, IL-13, IL-10, immunosuppressive agents (Corticosteroids, Vitamin D3, prostaglandins, and immunocomplexes (IG) in combination with IL-1 or TLR ligands), monocytes differentiate into M2 macrophages. M1 and M2 subsets differ in terms of phenotype and functions. M1 cells have high microbicidal activity, immunostimulatory functions, and tumor cytotoxicity. M2 cells have high scavenging ability; promote tissue repair and angiogenesis, immunosuppression and favor tumor progression.



Tumor-Associated Macrophages. Figure 2 Tumor-derived chemotactic factors (CC-chemokines, e.g., CCL2, macrophage-colony stimulating factor (M-CSF) and vascular endothelial growth factor (VEGF)) actively recruit circulating blood monocytes at the tumor site. In the tumor microenvironment monocytes differentiate into tumor-associated macrophages (TAM) that establish a symbiotic relationship with tumor cells. The above tumor-derived factors positively modulate TAM survival. From their own, TAM secrete growth factors that promote tumor cell proliferation and survival; regulate matrix deposition and remodeling, thus favoring metastasis formation, activate neoangiogenesis and promote immunosuppression.

TAM Express Selected M2 Protumoral Functions

The cytokine network expressed at the tumor site plays a central role in the orientation and differentiation of recruited mononuclear phagocytes, thus contributing to direct the local immune system away from antitumor functions. Immunosuppressive cytokines IL-10 and tumor-growth factor-β (TGF-β); are produced by both cancer cells (ovary) and TAM. IL-10 promotes the differentiation of monocytes to mature macrophages and blocks their differentiation to dendritic cells (DC). Thus, a gradient of tumor-derived IL-10 may account for differentiation along the DC versus the macrophage pathway in tumors, resulting in tumor promotion. IL-10 promotes the M2c alternative pathway of macrophage activation and induces TAM to express M2-related functions. Under many aspects, TAM summarize a number of functions expressed by M2 macrophages, involved in tuning inflammatory responses and adaptive immunity, scavenge debris, promote angiogenesis, tissue remodeling and repair. The production of IL-10, TGF-β, and PGE2 by cancer cells and TAM contributes to a general suppression of antitumor activities.

TAM are poor producers of nitric oxide (NO) and, in situ in ►ovarian cancer, only a minority of tumors and, in these, a minority of macrophages localized at the periphery scored positive for ►iNOS. Moreover, in contrast to M1 polarized macrophages, TAM have been shown to be poor producers of ROI, consistent with the hypothesis that these cells represent a skewed M2 population. Moreover, TAM were reported to express low levels of inflammatory cytokines (e.g., IL-12, IL-1β, TNF-α, IL-6). Activation of the transcriptional factor NF-κB is a necessary event promoting transcription of several proinflammatory genes. TAM display defective NF-κB activation in response to M1 polarizing signals lipopolysaccharide (LPS) and TNF-α. Thus, in

terms of cytotoxicity and expression of inflammatory cytokines, TAM resemble the M2 macrophages.

Angiogenesis is an M2-associated function that represents a key event in tumor growth and progression. In several studies in human cancer, TAM accumulation has been associated with angiogenesis and with the production of angiogenic factors such as VEGF and platelet-derived endothelial cell growth factor. Additionally, TAM participate to the proangiogenic process by producing the angiogenic factor thymidine phosphorylase (TP), which promotes endothelial cell migration in vitro and whose levels of expression are associated with tumor neovascularization.

Moreover, TAM accumulate in hypoxic regions of tumors, and ►hypoxia triggers a proangiogenic program in these cells. Therefore, macrophages recruited in situ represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumor cells. On the antiangiogenic side, in a murine model, GM-CSF released from a primary tumor upregulated TAM-derived metalloelastase and angiostatin production, thus suppressing tumor growth of metastases.

Finally, TAM express molecules that affect tumor cell proliferation, angiogenesis, and dissolution of connective tissues. These include epidermal growth factor (EGF), members of the FGF family, TGF-β, VEGF, chemokines. In lung cancer, TAM may favor tumor progression by contributing to stroma formation and angiogenesis through their release of PDGF, in conjunction with TGF-β1 production by cancer cells. Macrophages can produce enzymes and inhibitors that regulate the digestion of the extracellular matrix, such as ►MMPs, plasmin, ►urokinase-type plasminogen activator (uPA), and the uPA receptor. Direct evidence has been presented that MMP-9 derived from hematopoietic cells of host origin contributes to ►skin carcinogenesis. Chemokines have been shown to induce gene expression of various MMPs and, in particular, MMP-9 production, along with the uPA receptor. Evidence suggests that MMP-9 has complex effects beyond matrix degradation including promotion of the angiogenesis switch and release of growth factors.

Modulation of Adaptive Immunity by TAM

It has long been known that TAM have poor antigen presenting capacity and can actually suppress T cell activation and proliferation. The suppressive mediators produced by TAM include ►prostaglandins, IL-10, TGF-β, and indoleamine dioxygenase (IDO) metabolites. Moreover, TAM are unable to produce IL-12, even upon stimulation by IFN-γ and LPS. With this cytokine profile, which is characteristic of M2 macrophages, TAM are unable to trigger Th1-polarized immune responses, but rather induce ►T regulatory cells (Treg). Treg cells possess a characteristic anergic phenotype and strongly suppress the activity of effector T cells and other inflammatory cells, such as monocytes. Suppression of T cell-mediated antitumor activity by Treg cells is associated with increased tumor growth and hence, decreased survival. For instance, in patients with advanced ovarian cancer, an increase in the number of functionally active Treg cells present in the ascites was predictive of reduced survival.

The complex network of chemokines present at the tumor site can play a role also in the induction of the adaptive immunity. Chemokines also regulate the amplification of polarized T cell responses. Some chemokines may enhance specific host immunity against tumors but on the other hand other chemokines may contribute to escape from the immune system, by recruiting Th2 effectors and Treg cells. Figure. 2 summarizes symbiotic relationship between TAM and cancer cells.

Conclusion

Though the presence of TAM has been long considered as evidence for a host response against the growing tumor, it has become increasingly clear that TAM are active players in the process of tumor progression and invasion. Molecular and biological studies have been supported by a large number of clinical studies that found a significant correlation between the high macrophage content of tumors and poor patient prognosis. TAM share many similarities with prototypic polarized M2 mononuclear phagocyte population, in terms of gene expression and functions. In line with known properties of M2 macrophage populations, several lines of evidence suggest that TAM promote tumor progression and ►metastasis by activating circuits that regulate tumor growth, ►adaptive immunity, stroma formation, and ►angiogenesis. This hypothesis is now receiving new supporting evidence indicating that in vivo functional switching of infiltrating M2 macrophages toward an M1 phenotype provides therapeutic benefit in mice bearing tumor ►xenograft. Identification of mechanisms promoting functional diversion of macrophages toward an M2 direction may disclose new valuable therapeutic targets against tumors.

►Endothelins

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Tumor-Associated Stromal Progression

►Stromagenesis

Tumor Cell Invasion

►Invasion

Tumor Cell-Induced Platelet Aggregation

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Synonyms

Tumor-induced platelet aggregation; Pathological tumor cell-platelet interaction; Tumor cell-platelet interaction; Tumor cell-platelet aggregate; Cancer cell-platelet microemboli

Definition

►Tumor cell-induced platelet aggregation (TCIPA) is the ability of cancer cells to generate crucial molecules or surface receptor molecules that mediate platelet aggregation and accelerate the survival advantages of tumor cells in the vasculature, which is important for distant ►metastasis of cancer.

Characteristics

Hematogenous metastasis of cancer cells to critical distant organs is one of the major reasons for death in most cancer patients. After ►invasion into blood vessels, circulating cancer cells may interact with various vascular cells such as leukocytes, platelets and endothelial cells that may affect the survival and metastasis of tumor cells. Although normal platelet functions are important to control vascular hemostasis and thrombosis, cancer cells may express many factors which modulate the platelet activities. Clinically, the interactions between circulating cancer cells and platelets are crucial for tumor metastasis in patients with cancers. Cancer cells may induce the morphological changes and aggregation of platelets and subsequently trap the cancer cells in capillaries and enhance the adhesion of cancer cells to capillary endothelial walls. These events facilitate further tumor cell invasion and metastasis into distant organs. During these processes, the activated platelets release various factors (like serine proteases factor VIIa, factor Xa, ►platelet-derived growth factor (PDGF), ►vascular endothelial growth factor (VEGF), fibrinogen and thrombospondin), which increase vascular permeability and promote the growth, survival, motility and the extravazation of metastatic cancer cells to neighboring organs. Expression of PDGF-receptor in breast cancer tissues has been reported to increase the risk of lymph node metastasis. Similarly, the expression of von Willebrand factor (vWF) in tumor tissues and serum may also affect the metastasis of ►osteosarcoma patients. A number of clinical studies further suggest the increased risk of thrombosis in patients with various cancers, indicating the roles of TCIPA and activation of the coagulation system in the progression of cancer.

Mechanisms

Induction of TCIPA may form platelet-cancer cell aggregates to prevent cancer cells from killing by human cellular and humoral immunities, and from the damage by shear force of blood flow. Platelet activation further facilitates ►angiogenesis, tumor cell adhesion to endothelium and subsequent invasion into distant organs. Various cancer cells may produce differential amounts of critical factors such as ►ADP, ►thromboxane A_2 (TXA_2), thrombin, cathepsins, ►matrix metalloproteinases (MMPs), ►membrane type matrix metalloproteinases (MT-MMP), cancer procoagulant, mucin, tissue factors (TF) and ►Aggrus/Podoplanin etc. These molecules may activate various receptors ($P2Y_{12}$ purinergic receptor, thromboxane receptors, ►protease-activated receptors (PARs), integrin receptors etc.) and downstream signaling cascades to initiate platelet aggregation and coagulation disorders.

TF-bearing cancer cells can stimulate platelet aggregation via generation of thrombin and signaling by ►glycoprotein IIb/IIIa and VEGF release. The localized production of thrombin and fibrin may increase endothelial cell motility as well as platelet activation. Tumor cells have the capacity to convert fibrinogen into fibrin *in vitro* and histologically the deposition of fibrin is popularly noted in the connective tissue surrounding tumor cells, an event being critical for tumor angiogenesis and metastasis. Generation of MMPs by tumor cells may activate platelet integrin receptors followed by induction of platelet aggregation. This event can be inhibited by anti-MMP antibodies and phenanthroline, an MMP inhibitor. MMPs generated by tumor cells and platelets are further shown to degrade the basement membrane and facilitate metastasis.

Cancer cells may also activate platelets to release ADP, which binds further to platelet $P2Y_{12}$ receptors, leading to full platelet aggregation. The ADP-mediated platelet aggregation by tumor cells can be attenuated through degradation of ADP by apyrase (APT102). Moreover, the interaction between platelets and tumor cells can be mediated by cell adhesion proteins, e.g. P-selectin in platelets and P-selectin glycoprotein ligand-1/CD162, heparin sulfate proteoglycan and sialyl-Leqis A/X in cancer cells. This is followed by β3 integrin mediated processes with concomitant activation of thrombospondin and fibrinogen.

Using venous blood from cancer patients for testing, current techniques can detect the presence of circulating tumor cells in the blood stream. Interestingly, podoplanin (Aggrus), a mucin-line glycoprotein, has recently been shown to induce platelet aggregation and affect the invasion/metastasis of cancer. The expression of podoplanin in astrocytic tumors has been linked to the malignancy of astrocytic tumors. This is possibly due to early interactions by surface mucins and other selectin ligands of tumor cells with the platelet p-selectin and L-selectin. Heparin as an antithrombotic agent is able to inhibit metastasis further by blocking the tumor cell mucin P-selectin ligands and thus also the platelet-tumor cell interaction.

Clinical Aspects and Therapy

Higher platelet numbers have been found in many kinds of cancers including lung, gastric, colorectal and breast cancers. Clinically, cancer patients also have higher risk for development of vascular thromboembolism. This can be attributed to abnormalities in platelet functions as well as in the thrombotic and haemostatic systems by tumor cell- released factors. Understanding the mechanisms for TCIPA is helpful for the clinical design of therapeutic agents to control tumor metastasis. A number of clinical studies have found higher levels of thromboxane and lower levels of prostacyclin in plasma and tumor tissues from lung, bone and breast. Moreover reports have also observed the over-expression of ►cyclooxygenase-2 (COX-2) and thromboxane synthase in the tumors of colon, breast, prostate, brain and endocrine. Some tumors may generate excessive amounts of TXA2 to enhance tumor growth and metastasis. An elevation of urinary 11-dehydro-►TXB_2 (a major enzymatic metabolite of TXB2) in patients with colorectal cancers has been noted, suggesting the beneficial use of a low dose ►COX-1 inhibitor to reduce platelet activity in some cancer patients. BM-567, a thromboxane synthase inhibitor and TXA2 receptor antagonist, can inhibit the MG-63 osteosarcoma cell-induced platelet aggregation. Administration of SQ29548, the other TXA2 receptor antagonist, also reduces chondrosarcoma tissue TXB2 levels, vascular permeability and tumor size in experimental animals in vivo. Whether TXA2 receptor antagonists can be effectively used clinically to prevent tumor metastasis should be further addressed. ►Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen may reduce the risk of cancer in colon, esophagus, stomach, prostate and lung as well as the metastasis of prostate cancer. Aspirin as a COX-1 inhibitor and antiplatelet agent is also tested for its anti-metastatic effects. In experimental animals, aspirin may reduce the metastasis of injected ►hepatocellular carcinoma cells to lung. However, a multicenter study shows little effect of aspirin in combination with chemotherapy to improve survival of 303 patients with small cell ►lung cancer. More studies on the dosage, duration and timing of aspirin or other therapeutic administrations are needed.

In experimental animals, heparin administration seems effective in the suppression of tumor metastasis, but not primary tumor growth. This can be due to its inhibition of blood coagulation, tumor cell-platelets and ►tumor-endothelial cross talks. Accordingly, clinical administration of aspirin and heparin to humans may suppress platelet aggregation and therefore promote survival in cancer patients. In addition, heparin, warfarin (a vitamin K antagonist) and antiplatelet agents (prostacyclin and dipyridamole) are able to inhibit the metastasis of ►pancreatic cancer cells and ►melanoma cells to the liver and lung. Intriguingly, recent studies have found that low molecular weight heparin (LMWH) successfully prevents the progression of cancer and improves the survival of patients with advanced ►non-small cell lung cancer, colon, pancreatic, breast and pelvic cancers.

Since tumor cells may also express PARs, glycoprotein IIb/IIIa and integrin αvβ3, inhibition of these receptors and activation and signaling by agents such as Hirudin, PPACK, antithrombin III, receptor neutralizing antibodies are potentially useful methods for inhibition of TCIPA and metastasis by some tumor cells. XV454, a glycoprotein IIb/IIIa antagonist, has

recently been shown to suppress the metastasis of Lewis lung carcinoma cells in experimental mouse metastasis models. A combination of LMWH and XV454 is even more effective in reducing cancer-induced thrombosis in vitro. Currently, some new reagents are being tested for their inhibition of TCIPA. Administration of antiplatelet agents (such as antiplatelet antibody, dansylarginine N-(3-ethyl-1,←5-pentanediyl)amide) before inoculation of tumor cells has been shown to effectively reduce tumor metastasis to lung and bone tissue. Integrilin, a platelet-specific integrin inhibitor, was able to effectively suppress TCIPA, but showed only partial inhibitory effects on physiological platelet functions. An elevated expression of aggrus/podoplanin, a new platelet aggregation inducing factor in tumor tissues has been found and promotes pulmonary metastasis in experimental animals. Administration of anti-podoplanin antibody is also shown to inhibit the neuroglioblastoma cell-induced platelet aggregation. More clinical trials by inhibition of TCIPA using antiplatelet and anticlotting agents are now in progress. These results will be helpful in developing effective clinical strategy and regimens for antimetastatic therapy of cancer in the near future.

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Tumor Cell-platelet Aggregate

►Tumor Cell-Induced Platelet Aggregation

Tumor Cell-Platelet Interaction

►Tumor Cell-Induced Platelet Aggregation

Tumor Endothelial Cell

Definition

Cells that line the blood vessels in a tumor. Tumor endothelial cells can proliferate to form new capillary vessels.

►Angiogenesis

Tumor–Endothelial Communication

►Tumor–Endothelial Cross-talk

Tumor–Endothelial Cross-talk

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Synonyms

Tumor–endothelial communication

Definition

Research in the field of tumor–endothelial communication focuses on specific interactions and pathways that allow circulating tumor cells to interact with the endothelium – the inner lining of the vascular wall. These interactions lead to modifications of the endothelium that eventually facilitate both tumor cell adhesion and ►extravasation. These processes are frequently accompanied by microthrombotic events and tumor–endothelial communication relies in many aspects on mechanisms known from the inflammatory response.

Characteristics

Neoplastic growth and survival of tumor cells in the host environment critically depends on effective mechanisms of tumor–host interactions. This dependency of tumor cell survival in the ►microenvironment is not only limited to the early phase of primary tumor growth, where obviously, e.g., nutritive supplies are needed, but persists throughout the entire life span of

tumor presence that is actually characterized by continuous multiple tumor–host interactions.

A characteristic hallmark of malignant tumor cells is their ability to spread via the host vascular system, settling up colonies distant from their place of origin, a process generally referred to as "►metastasis." There is general agreement that in this process of metastasis via the vascular system – which is the most fearsome aspect of cancer as to the survival prognosis – tumor cells that have managed to leave their site of origin need to apply ►escape mechanisms allowing avoidance of the host immunosurveillance and an eventual extravasation from the blood stream. The transmigration of circulating tumor cells into the host tissue is a highly regulated process, which up-to-date has not been completely understood. Circulating tumor cells extravasating to the host-stroma tissue will have to cross the natural barrier between blood and tissue: the endothelium. This interaction is a complex process that requires molecular mechanisms similar to those known from the proinflammatory and prothrombotic response. This encyclopedic entry will display the mechanisms that have coined the expression of tumor–endothelial communication. On the one hand, it summarizes the findings on tumor cell–endothelial interactions facilitating the transmigration of tumor cells; on the other hand, it will highlight the molecular mechanisms that are currently known for describing how tumor cells modify endothelial functions that eventually may lead to tumor-induced thrombotic events. It goes without saying that the current trend of linking thrombotic events to mechanisms of the inflammatory response also holds true for the understanding of tumor–endothelial communication.

There are multiple evidences that neoplastic cells from a variety of tumor entities actively modify the vascular endothelium. Tumor cells are capable of transforming the endothelial physiological quiescent disposition into a ►prothrombotic, proinflammatory, and proadhesive endothelium. One of the oldest documented observations of this phenomenon is generally accepted to date back to works by Trousseau in the late nineteenth century (1865). These studies showed that tumor cells are associated with thrombi of platelets and neutrophils. In addition, there are multiple clinical reports that a thromboembolic event of so far unknown origin might occasionally be the first hint to an ongoing metastatic tumor disease of a patient. So evidently, these observations reflect tumor–host cross-talk.

Adhesion Molecules in Tumor–Endothelial Cross-talk Mediating Extravasation

The extravasation from the flowing blood stream is a highly regulated process that relies on a specific interaction of ligands on the circulating cell and their receptors on the vascular endothelium. Much of the current knowledge in this field derives from the studies on thrombosis and inflammation, where platelets and leukocytes are specifically directed to bind to the activated endothelium and vascular wall. So-called ►selectins are a family of adhesion molecules that are crucial in this process. There are L-, P-, and E-selectins that all recognize carbohydrate structures. While L-selectin is constantly present on leukocytes, P- and E-selectin become upregulated on activated endothelium. Absence of selectins severely delays or even prevents the adhesion of circulating cells. The lectin domain of selectins recognizes sialylated, fucosylated structures displayed mostly on mucin-type glycoproteins containing a terminal tetrasaccharide sLe^x and sLe^a. Next to platelets and neutrophils, selectin-ligands have been shown to be expressed by several tumor entities and thus support tumor–endothelial communication. In different mouse models of metastasis, it could be shown that P- and E-selectin deficiency reduces the amount of extravasation of tumor cells. It was also reported that heparin, apart from its properties as an anticoagulant, interferes with binding-ligands for selectins and thus prevents metastatic tumor spread. The use of heparin for prevention of tumor metastasis has been shown to be beneficial in many human studies and has become an accepted option for the clinical treatment of cancer patients. In continuation of the initial selectin-mediated interaction of tumor cell and endothelium, ►integrins are recognized to play an important role in cell adhesion. Integrins are heterodimeric transmembrane proteins, that consist of noncovalently bound alpha and beta chains. They play a crucial role in cellular processes such as cell adhesion and migration as well as in the control of cell differentiation, proliferation, and survival. Several binding partners for integrins on and below endothelial cells have been characterized so far, such as VCAM-1, ICAM-1, and extracellular matrix proteins such as fibronectin, vitronectin, and ►laminin.There is also well documented expression of integrins on several tumor entities. During the malignant transformation, the expression of integrins has been reported to alter and leads to different binding avidity. Research on ►integrin-mediated tumor extravasation currently focuses on alphavbeta3 integrin and alpha5beta1 integrin, as their inhibition could decrease metastasis in several studies.

In summary, crossing the endothelial barrier, tumor cells rely on mechanisms that resemble those of cell arrest in the inflammatory and thrombotic response.

Tumor–Endothelial Cross-talk Leading to Prothrombotic Endothelial Activation

Apart from modifying the vascular endothelium towards a proadhesive state, tumor–endothelial cross-talk also

activates the prothrombotic activities of the vascular wall. The clinical observation is that of the so-called ►Trousseau's syndrome, where especially visceral cancers lead to venous thrombotic events. In addition, there are several reports showing that progressive cancer is associated with elevated levels of circulating ►von Willebrand Factor (VWF), a plasma surrogate marker for endothelial activation. The pathogenesis of the prothrombotic state in cancer is complex, and probably multifactorial, however, some studies could elucidate molecular mechanisms critically involved in tumor-induced prothrombotic endothelial activation. A well studied protein involved in endothelial activation is ►tissue factor (TF). Several tumor cells can directly release TF or stimulate circulating leukocytes to increase TF-release, e.g., by the generation of prothrombotic TF-rich microparticles. Once released, TF activates coagulation factor VII, which in turn activates factorX, thus activating prothrombin to ►thrombin. On the endothelium, via activation of the thrombin-receptor (Proteinase-activated receptor 1/PAR-1), thrombin leads to an immediate release of VWF and P-selectin promoting thrombosis and inflammation. A similar mechanism has been described for cancer procoagulant (CP). CP is a single chain cysteine protease that has been identified in a variety of tumors. It can directly activate factorX and thus contribute to the generation of thrombin. Recently, another direct mechanism of endothelial cells by metastatic tumor cells could be demonstrated. It was shown for human ►melanoma and ►colon cancer that metastatic tumor cell lines of these cancers are capable of generating and releasing matrixmetalloproteinase-1 (MMP-1). MMP-1 has been demonstrated to directly activate the thrombin-receptor on endothelial cells followed by VWF and IL-8 release. This mechanism does not rely on the presence of coagulation factors and is therefore proof of immediate tumor–endothelial communication.

Conclusion

The prothrombotic reaction of the endothelium upon contact with malignant circulating cells might be understood as a mechanism for prevention of further dissemination of the tumor, making trapped tumor cells more accessible to the host defense system. Indeed, it could be shown that in the absence of surrounding platelets tumor cells were more prone to ►natural killer cells attacks than when protected in platelet-emboli. However, it can also be speculated that tumor cell arrest in a thrombus might facilitate tumor cell extravasation. As antithrombotic treatment and depletion of circulating platelets has been demonstrated to positively influence tumor metastasis, specific strategies of interference with tumor–endothelial cross-talk might eventually prove beneficial for the prevention of tumor cell metastasis.

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Tumor Glucose Metabolism

►Warburg Effect

Tumor Grading

►Grading of Tumors

Tumor-Induced Platelet Aggregation

►Tumor Cell-Induced Platelet Aggregation

Tumor-Infiltrating Lymphocytes

Definition

TILs; Recognize unique cancer antigens isolated from human tumors and/or inflammatory infiltrate present around solid tumors. ►Adoptive immunotherapy is based on the *ex vivo* selection of tumor-reactive lymphocytes, and their activation for use in an autologous, tumor-bearing host. TILs are activated and expanded *in vitro* in the presence of cytokines (e.g. IL-2) and the patient's own tumor antigens, and then transferred into the same patient followed by

maintenance treatment with cytokines. TILs are capable of lysing autologous cancer cells in a highly-specific fashion restricted by the major histocompatibility complex (►MHC) class I molecules.

►Immunotherapy

Tumor Infiltrating T Cells

Definition

T cells that are found in the tumor tissue.

►T-Cell Response

Tumor-Initiating Cells

►Cancer Stem-Like Cells
►Stem-like Cancer Cells

Tumor Lysis Syndrome

Definition

TLS; Is a very serious and sometimes life-threatening complication of cancer therapy. It can be defined as a constellation of metabolic abnormalities resulting from spontaneous or treatment-related tumor ►necrosis or fulminant ►apoptosis. The metabolic abnormalities observed in patients with tumor lysis syndrome include hyperkalemia, hyperuricemia, and hyperphosphatemia with secondary hypocalcemia. These can lead to acute renal failure (ARF).

►Rituximab

Tumor Markers

Definition

Tumor markers are broadly divided into three groups, which include the oncofetal protein, cancer-related antigen and hormone. They are detected by the analyses of blood, tissue, or body fluid. Their productions result either from the increased or decreased expression of a normal or cancer gene/protein. Theoretically, tumor markers are useful in diagnosis, predicting treatment response, and prognosis, as well as aiding the follow up of patient. However, the value of different tumor markers varies. Some markers may not be tumor specific, whereas some are important tumor-associated genes/proteins that may become the target of cancer therapy.

►Alpha-Fetoprotein – Historical
►Biomarkers
►Clinical Cancer Biomarkers
►Serum Biomarkers

Tumor Matrix

Definition

Synonym tumor stroma, summarizes cells and connective tissue elements that embed the tumor (fibroblasts, endothelial cells, collagen fibers, proteoglycans, etc).

Tumor Metabolism

Definition

The overall biochemical processes that occur in tumors to provide energy for cellular function and growth. Tumor metabolism can proceed through glycolysis and/or oxidative phosphorylation. Factors that determine the pathway and rate through which tumor metabolism proceeds include oxygen content, glucose availability, and pH of the tumor environment.

►Hyperthermia
►Oxygenation of Tumors

Tumor Microenvironment

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Synonyms

Cancer (or tumor) stroma

Definition

The specific conditions existing in the tumor tissue, the nonmalignant cells and the molecules present in proximity to the tumor cells.

Characteristics

It is now widely accepted that "Although abnormalities of cancer genes (►Oncogene; tumor suppressor genes) are essential contributors to cancer, most abnormalities in these genes occur relatively early in the disease process and none of them is known to be associated with the metastatic stage. It is this final stage – the seeding and growth of satellite lesions in other organs – that is ultimately responsible for the great majority of neoplastic deaths." It is the tumor microenvironment that determines and shapes the malignancy phenotype of cancer cells, in other words its metastatic behavior (►Metastasis).

The tumor tissue can be viewed as an ecosystem composed of two compartments being intimately associated with each other. The first compartment constitutes the malignant cells. The second is the tumor microenvironment composed of resident cells such as fibroblasts, endothelial cells (►Tumor-endothelial cross-talk), and other nonmalignant cells; of infiltrating cells such as lymphocytes or macrophages (►Tumor-associated macrophages) and of numerous molecules released by the tumor cells as well as by the non malignant cells. These molecules may be in complex with other molecules, for example, in the extracellular matrix. Other molecules such as growth factors, cytokines, ►chemokines, antibodies, proteases, other types of enzymes, various metabolites, or drugs may be present in soluble form. The microenvironment of many solid tumors may be characterized by hypoxia (►Hypoxia and tumor physiology; ►hypoxia inducible factor-1); low extracellular pH and by low glucose concentration. Cellular products released from necrotic tumor cells are also present.

Although the term tumor microenvironment is used most often with respect to solid tumors, other types of malignancies have also their specific microenvironments. The bone marrow serving as a microenvironment for certain leukemias (►Leukemia diagnostics) and for ►multiple myeloma is a case in point.

Stephen Paget, over 100 years ago, is credited with being the first to postulate the important role played by the microenvironment in metastasis formation. The concept of his "►seed and soil" theory, explaining site specific metastasis in ►breast cancer, has been supported and confirmed. However, numerous studies published in the last three decades demonstrate very clearly that the "soil" functions also, or even primarily, as an active "educational/inductive" venue in which cancer cells are directed, by interacting with microenvironmental factors, into one of several molecular evolution pathways. In other words, by exerting regulatory functions and selective pressures, the tumor microenvironment determines and shapes the malignancy phenotype of cancer cells.

The tumor microenvironment is an interaction arena between microenvironmental components and tumor cells and between different microenvironmental components. This arena is characterized by four major hallmarks: complex regulatory circuits; a yin-yang (double edged sword) interplay; plethora of vicious cycles; abnormality of its "normal," nonmalignant compartment.

Complex Regulatory Circuits. The major function of the tumor microenvironment is a regulatory one. Many genes in the tumor cells and in nontumor cells residing in or infiltrating to the tumor microenvironment are regulated by microenvironmental components.

Several hundred proteins were identified in the microenvironment of breast cancer. An extremely large number of signaling cascades (►Signal transducers and activators of transcription in oncogenesis; signal transduction) would operate in this microenvironment even if only a small portion of these proteins would interact with tumor cells or with nontumor cells. It is safe to predict that a similar number of proteins will be detected in the microenvironment of other types of solid tumors.

These signaling cascades take part in the regulation of genes in tumor and in nontumor cells thereby shaping the phenotype of cancer cells and drive their ►progression.

The regulatory power of the microenvironment can be amplified by the agonistic or antagonistic cross-talk (►Receptor cross-talk) between different signaling cascades. Furthermore, several signaling cascades in cancer cells are aberrant. This may well increase the number of combinatorial signaling pathways, augment their complexity, and decrease the capacity of physiological feedback mechanisms to confront these malignancy-associated processes. Another factor contributing to the complexity of the interactions taking place in the tumor microenvironment is tumor heterogeneity. It is thus to be expected that different tumor variants, expressing different profiles of signaling receptors (►Receptor tyrosine kinases), would respond differentially to microenvironment-derived signals.

The Yin-yang (Double Edged Sword) Interplay in the Tumor Microenvironment. The cross-talk between tumor cells and microenvironmental factors may result in diametrically opposed effects which could either enhance or block tumor formation or progression. There are several examples of such a yin-yang interaction. The activity of transforming growth factor-beta (TGFβ) (►Transforming growth factor) is an example for a microenvironmental molecule manifesting a "love–hate

relationship" with tumor cells. Whereas TGFβ is a potent inhibitor of normal mammary epithelial cells, it enhances tumor cell ►invasion and metastasis of advanced breast cancer cells (►Epithelial tumors). Moreover, cancer cells may secrete TGFβ which augments ►angiogenesis and is capable of suppressing antitumor immune responses of the host. On the other side of the coin, it was recently demonstrated that the progression of pancreatic and of intestinal tumors is enhanced by the inactivation of the TGF signaling cascade.

Another prominent example for yin-yang interplay in the tumor microenvironment is inflammation (►Inflammation in cancer) versus protective tumor immunity. Cells and molecules of the immune system may, under certain circumstances, inhibit tumor growth and under different circumstances promote it.

Vicious Cycles in the Tumor microenvironment. A vicious cycle may be described as an input event that drives and amplifies other events which, in turn, promote tumor progression. Among such activities, the input event may also augment itself (positive feedback). A well studied vicious cycle in the tumor microenvironment is the cross-talk between osteoblasts, osteoclasts and other microenvironmental factors on the one hand and breast, prostate (►Prostate cancer, clinical oncology) and lung (►Lung cancer) tumor cells on the other hand. This cross-talk promotes bone metastasis (►Bone tropism and cancer). Tumor-derived molecules such as cytokines cause either an osteoblastic or an osteolytic response. Such molecules feedback on the tumor and on various cells in the microenvironment causing the release of factors driving tumor progression.

Another example of a vicious cycle in the tumor microenvironment is the cross-talk between chemokines (►Chemoattraction and cancer; chemokine) such as CCL2 and CCL5 secreted from mammary tumors of mice or from breast cancer cells of humans and cytokines such as TNFα secreted from macrophages infiltrating into these tumors. The tumor-derived chemokines attract monocytes to the microenvironment. These monocytes differentiate into macrophages which secrete TNFα. This cytokine up regulates the secretion of CCL2 and CCL5 from the tumor cells. CCL2 and CCL5, in turn, promote the secretion of TNFα from the tumor-associated macrophages. In this vicious cycle, the tumor cells and the macrophages promote each other's ability to express and secrete pro malignancy factors.

Hypoxia (Hypoxia and tumor physiology; hypoxia inducible factor-1) characterizes the microenvironment of solid tumors. Hypoxia-induced changes in the proteome (►Proteomics) may lead to either impairment of tumor growth and spread or, alternatively, to tumor propagation and progression. In the later case, a vicious cycle is created in which tumor cells surviving and propagating under hypoxia will aggravate the state of tumor hypoxia which in turn promotes genomic instability and further progression.

The Nontumor Cells in the Tumor Microenvironment may Express a Different Phenotype than Their Counterparts at Distant Sites. The conditions in the tumor microenvironment, for example, hypoxia, may induce or promote genetic instability and cause mutations and alterations in gene expression profiles of cancer cells (►Genetic disorders associated with cancer predisposition and chromosomal instability). It is not unlikely that such conditions may induce genetic alterations also in nontumor cells present in the microenvironment. The question, if the phenotype and functions of nontumor cells in the tumor microenvironment are similar or different from those of their counterparts in normal microenvironments, is by and large open. Several studies clearly demonstrate that at least some of the nontumor cells in the tumor microenvironment may not represent faithfully the characteristics of their counterparts in other sites of the body. Cancer-associated fibroblasts and endothelial cells are two prominent examples that illustrate the abnormality of tumor-associated nontumor cells.

Cancer-associated fibroblasts have genetic changes both at the DNA level as well as at the expression level. For example, fibroblasts in human carcinomas have tumor suppressor gene mutations. DNA microarrays (►Microarray (cDNA) technology) identified over 100 genes differentially expressed by prostate carcinoma (Prostate cancer, clinical oncology)-derived fibroblasts and by systemically derived ones. These alterations may well manifest themselves by altered functions of such tumor-associated cells. Similar findings were also reported for endothelial cells. Cytogenetic abnormalities have been shown to occur in tumor endothelium and such cells may also express proteins that are not expressed by endothelial cells of the corresponding normal tissue.

The fact that nontumor cells in the tumor microenvironment express a different phenotype than that expressed by the corresponding cells residing in other microenvironments might be exploited in cancer therapy modalities, targeting these differentially expressed molecules.

►Desmoplasia
►Tissue Inhibitors Of Metalloproteinases (Timps)

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Tumor Necrosis Factor

Definition

TNF; Cytokine refers to a class of released by various cell types, including inflammatory cells, responsible for the breakdown of cancer cells, and the propagation of ►inflammation. Antibodies against tumor necrosis factor alpha have been used successfully in combating colitis and cancer associated with colitis.

►Inflammation

Tumor Necrosis Factor-α

Definition

TNF-α; Is a cytokine produced by ►macrophages and ►T cells that has multiple functions in the immune response. It is the defining member of the TNF family of ►cytokines. These cytokines function as cell-associated or secreted proteins that interact with receptors of the tumor necrosis factor receptor (TNFR) family, which in turn communicates with the interior of the cell via components known as TRAFs (tumor necrosis factor receptor-associated factors).

►Sjögren Syndrome

Tumor Necrosis Factor-Alpha Converting Enzyme

►ADAM17

Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

Definition

TRAIL; Is a transmembrane protein that can engage a "death" receptor on cells and induce a process of cell death called ►apoptosis. It is homologous to members of the ►tumor necrosis factor (TNF) family.

►Immunoediting

Tumor Pathology

►Pathology

Tumor Progenitors

►Stem-like Cancer Cells

Tumor Progression

Definition

Tumor progression is a process characterized by progressive accumulation of genetic defects in tumor cells and the outgrowth of tumor cell populations possessing more invasive properties, increased ability to spread to other sites and reduced response to therapeutic intervention. It represents genetic and epigenetic changes of tumor cells resulting in higher malignancy of the tumor. ►progression of tumors; multistep development.

Tumor Promoter

Definition

A non-genotoxic substance that induces tumor formation, after tumor initiation by genotoxic carcinogens.

A well-known tumor promoter is phorbol 12-myristate acetate, (►PMA).

►Protein Kinase C Family

Tumor Promotion

Definition

Characterized by a reversible phase of clonal expansion of initiated cells containing mutations/inactivated genes, with a dysregulation of apoptosis of the initiated cells as well as by accumulation of epigenetic changes such as DNA methylation, inflammation characterized by infiltration of activated leukocytes, production of growth factors, cytokines, reactive intermediates including oxygen free radicals and nitrogen radicals which stimulates formation of DNA damage, with inhibition of DNA repair enzymes. These alterations become irreversible and lead to development preneoplastic papillomas, which are benign skin lesions

►Skin Carcinogenesis
►PMA

Tumor-Reinitiating Cells

►Stem-like Cancer Cells

Tumor-Repopulating Cells

►Stem-like Cancer Cells

Tumor-Specific Stroma

Definition

The ►stroma in a malignant tumor, comprising ►extracellular matrix and embedded cells, constitutes up to 90% of tumor mass and has characteristics not found in normal tissues. In tumor stroma, tumor-associated cells such as carcinoma-associated fibroblasts, inflammatory cell and endothelial cells among others are found that secrete soluble growth factors and cytokines as well as extracellular matrix molecules and extracellular matrix degrading enzymes that promote transformation and malignant progression of tumor cells. Cancer appears to be a product of the tumor-host ►microenvironment, where mutual stimulation of tumor and stromal cells induces tumor formation and, extensive tumor ►angiogenesis and ►metastasis.

Tumor Spread

►Metastasis

Tumor Staging

Definition

►Staging of Tumors.

Tumor Stroma

Definition

Tumor ►microenvironment that is largely a product of the host and is induced as the result of tumor cell-host interactions. Stroma consists of non malignant supporting tissues with ►extracellular matrix and includes connective tissue, blood vessels and inflammatory cells. It is essential for the growth of the tumor and its ability to progress and metastasize.

►Stromagenesis

Tumor Suppression

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Definition

Tumor suppression is the consequence of the functional presence and activity of ►tumor suppressor genes

Tumor Suppression. Table 1 Predisposing Germ Line Mutations in Tumor Suppressor Genes

Associated cancer syndrome	Tumor suppressor gene	Human chromosomal location	Gene function	Cancer type
Familial ►retinoblastoma	*RB1*	13q14	Transcriptional regulator of cell cycle	Retinoblastoma, osteosarcoma
►Wilms tumor	*Wt1*	11p13	Transcriptional regulator	Nephroblastoma
►Li-Fraumeni Syndrome	*p53*	17q11	Transcriptional regulator/growth arrest/apoptosis	Sarcomas, breast/brain tumors
►Von Recklinghausen Disease	*NF1*	17q11	Ras-GAP activity	Neurofibromas, sarcomas, gliomas
►Neurofibromatosis type 2	*NF2*	22q12	ERM protein/cytoskeletal regulator	Schwannomas, meningiomas
►Von-Hippel Lindau Disease	*VHL*	3p25	Regulates proteolysis	Hemangiomas, renal, pheochromocytoma
Familial adenomatous polyposis	*APC*	5q21	Binds/regulates β-catenin activity	Colorectal cancer
Familial ►melanoma	*INK4a*	9p21	$p16^{Ink4a}$ cdki for cyclinD/cdk4/6; $p19^{ARF}$ binds mdm2, stabilizes p53	Melanoma, pancreatic
►Gorlin syndrome	*PTC*	9q22.3	Receptor for sonic hedgehog	Basal cell carcinoma, medulloblastoma
Juvenile polyposis	*DPC4*	18q21.1	Transduces TGF-β signals	Pancreatic, colon, hamartomas
►Cowden syndrome BZS, LDD	*PTEN*	10q23	Dual specificity phosphatase	Glioblastoma, prostate, breast
►Tuberous sclerosis complex	*TSC2*	16	Cell-cycle regulator	Renal, brain tumor
Familial prostate carcinoma	*NKX3.1*	8p21	Homeobox protein	Prostate
►Peutz-Jeghers Syndrome	*LKB1*	19p13	Serine/threonine kinase	Hamartomas, colorectal, breast
Familial gastric cancer	*E-Cadherin*	16q22.1	Cell adhesion regulator	Breast, colon, skin, lung carcinoma
►Ataxia telangiectasia	*ATM*	11q23	P13K-like kinase	Leukemias, lymphomas
►HNPCC	*MSH2*	2p22	Mut S homologue, mismatch repair	Colorectal cancer
HNPCC	*MLH1*	3p21	Mut L homologue, mismatch repair	Colorectal cancer
HNPCC	*PMS1*	2q31	Mismatch repair	Colorectal cancer
HNPCC	*PMS2*	7p22	Mismatch repair	Colorectal cancer
HNPCC	*MSH6*	2p16	Mismatch repair	Colorectal cancer
►Bloom Syndrome	*BLM*	15q26.1	DNA helicase	multiple
►Fanconi anemia				
Complementation Gr A	*FAA*	16q24.3	Involved in DNA cross-link repair	Leukemia
Complementation Gr C	*FAC*	9q22.3	Involved in DNA cross-link repair	Leukemia
►Xeroderma pigmentosum (seven complementation groups)	*XPA*	9q34.1	Binds damaged DNA	Skin
	XPB	2q21	helicase; part of TFIIH	
	XPC	3p	?	
	XPD	19q12.3	Helicase; part of TFIIH	
	XPE	?11	Binds damaged DNA	
	XPF	16p13	Structure-specific endonuclease	
	XPG	13q23-33	Structure-specific endonuclease	
►Nijmegen Breakage Syndrome	*NBS1*	8q21	Involved in DNA doublestrand break repair	Lymphomas
►Familial breast cancer	*BRCA1*	17q21	Transcriptional regulator/DNA repair	Breast/ovarian tumors
Familial breast cancer	*BRCA2*	13q12	Transcriptional regulator/DNA repair	Breast/ovarian tumors

(TSGs). TSGs are recessive genes whose protein products appear to directly or indirectly negatively regulate cell proliferation, promote ►apoptosis and maintain in vivo homeostatic growth and differentiation potential.

Characteristics

Three major classes of genes are involved in cancer causation and progression:

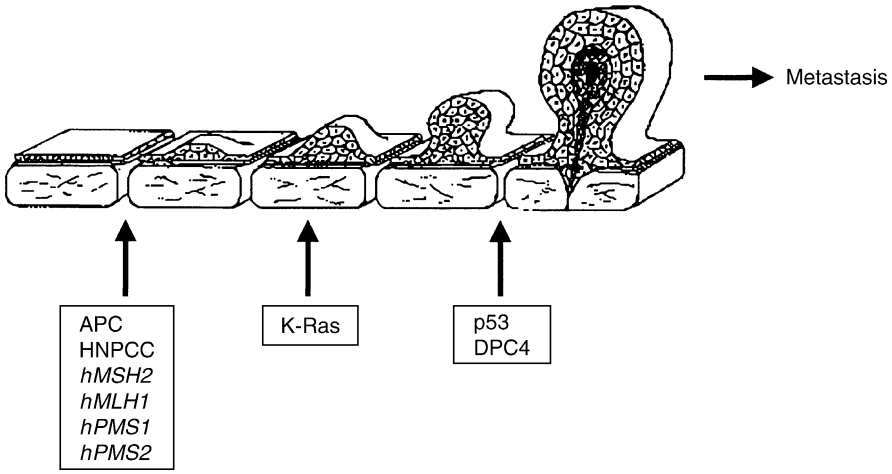
- Dominantly-acting ►oncogenes, whose proteins serve to stimulate cell growth and survival
- Recessive genes involved in ►repair of DNA
- Recessive TSGs

The recessive TSGs and ►DNA repair genes are often included under the rubric of tumor suppressor genes. The importance of TSGs in the genesis of cancer became apparent when individuals predisposed to early onset cancer were found to contain a mutated allele of a certain TSG in their germline. This condition predisposes the individual to earlier onset cancer at a significantly higher probability than individuals who possess a sporadic cancer of the same histologic type. This is best illustrated by the prototypic TSG, ►RB1, that predisposes to childhood ►retinoblastoma. The incidence of sporadic retinoblastoma is ~1:40,000, and is often unilateral, whereas the incidence of heritable retinoblastoma within an affected family is ~40%, and often involves both eyes (bilateral retinoblastoma). The affected proband inherits the mutant allele from one of the parents. Thus, the classic presentation of a heritable recessive TSG is one of dominant autosomal inheritance. In the classic ►two-hit model, the affected individual inherits one mutant RB1 allele and the second allele is eliminated somatically in the retinoblastoma tumor. In the case of sporadic retinoblastoma both alleles are eliminated somatically.

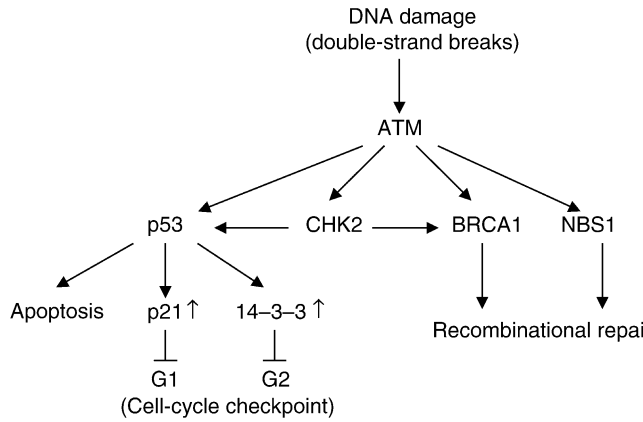
Since the identification of the RB1 TSG, a large number of cancer-predisposing germline mutations in TSGs have been found (Table 1), and they include DNA repair genes. Many, but not all (e.g. ►BRCA1 and ►BRCA2), are commonly found to be mutated in sporadic cancers of the same histologic type as those seen in the relevant familial cancer cases.

Progression to the cancerous condition is a multistep phenomenon. This is best illustrated by the colorectal cancer model (►multistep development; Fig. 1). Tumorigenesis proceeds through a series of cellular alterations, including hyperplasia of the colonic epithelium, benign polyps of increasing size and disordered growth, and ►carcinoma in situ with localized invasion. Distant metastases may occur. Accompanying these cellular alterations are genetic alterations, including activating mutation of the K-ras proto-oncogene and loss-of-function mutations in multiple TSGs. In the case of TSGs, mutations in both alleles must occur for complete loss of function. Loss-of-function of multiple TSGs is a hallmark feature of most, if not all cancers.

TSGs span a broad range of functions (Table 1). These include kinases, phosphatases, cyclin-dependent kinase inhibitors, transcription factors, cell adhesion molecules, proteins involved in specific protein degradation pathways and a variety of DNA repair processes. There is increasing evidence that tumor suppressor proteins and DNA repair proteins interact in functional networks (an example is given in Fig. 2). The ATM kinase phosphorylates and activates the tumor suppressor ►p53 and CDSI/►CHK2 proteins, and the



Tumor Suppression. Figure 1 Multistep progression model for colorectal cancer. Tumorigenesis proceeds through a series of cellular alterations, including hyperplasia, benign polyps of increasing size and disordered growth, and carcinoma in situ with localized invasion. Genetic alterations associated with this progression include inactivation of TSGs, e.g. APC, p53 and DPC4, mutational activation of the K-Ras, oncogene and inactivation of one of the DNA mismatch repair genes.



Tumor Suppression. Figure 2 Networking of tumor suppressor and DNA repair proteins. In response to DNA damage, ATM phosphorylates and activates p53 and the cell cycle kinase CHK2. This kinase also enhances activation of p53 via phosphorylation. Activated p53 upregulates expression of the cyclin dependent kinase inhibitor, p21, and 14–3–3 proteins, resulting in cell-cycle arrest at the G1 and G2 checkpoints, respectively. The activated p53 protein may also induce apoptosis. Transient cell-cycle arrest allows for repair of damaged DNA by preventing the duplication and propagation of damaged DNA. ATM also plays an important role in DNA repair by phosphorylating and activating BRCA1 and NBS1 proteins. See Table 1 for identification of the genes. → = activation; ⊣ = inhibition.

DNA repair proteins, BRCA1 and ►NBS1. These activations lead to cell-cycle checkpoint arrest and repair of DNA damage. Loss-of-function of any one of these factors compromises DNA repair, culminating in genomic instability and increased probability of progression to the cancer phenotype.

Clinical Aspects

Identification of specific germline mutations in TSGs in affected families allows the recognition of those members who are carrying the mutant allele and, therefore, are at a significantly higher risk of getting cancer. Detection of mutant tumor suppressor proteins, e.g. p53, or loss of expression of such proteins may aid in cancer diagnosis and prognosis. Experimental investigations have established that restoration of TSG function in cancer cells that are defective for that function result in suppression of tumor growth or death of the cancer cells. Clinical trials are in progress using ►gene therapy and pharmacologic approaches that aim to apply these procedures to the treatment of human cancers.

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Tumor Suppressor

Definition

A functional gene that reduces the probability that a cell in a multicellular organism will turn cancerous. Defects in these genes increase the probability of cancer formation.

►Tumor Suppressor Genes

Tumor Suppressor Genes

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Synonyms

Recessive oncogenes

Definition

Tumor suppressor genes are genes whose products normally negatively regulate cell growth or cell behavior (Fig. 1).

Characteristics

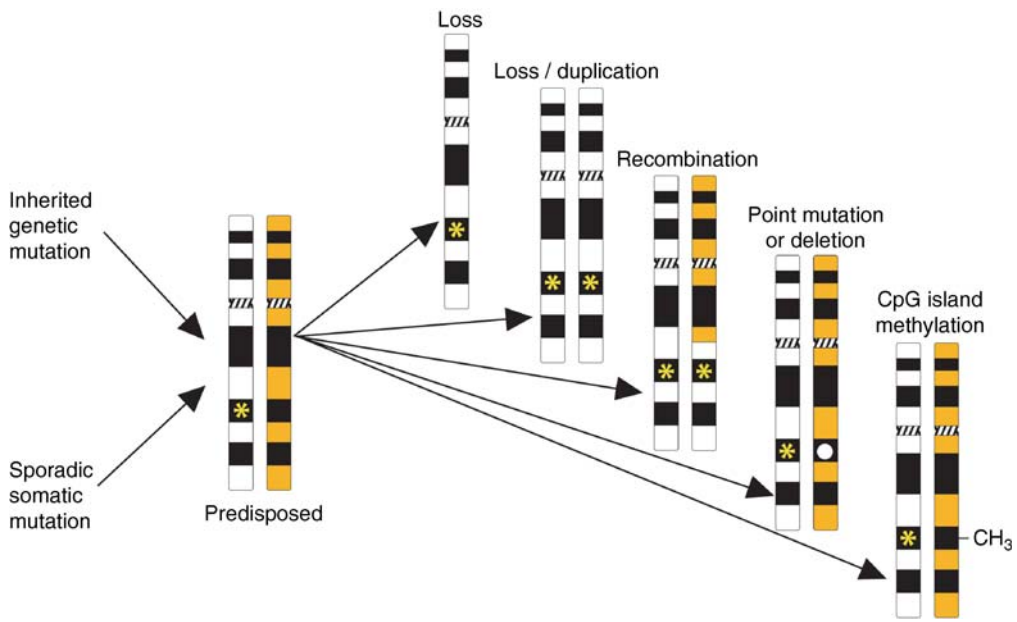
The hallmark of a tumor suppressor gene is that its function is lost during tumor initiation or progression. This typically occurs by one of a set of chromosomal processes called ►loss of heterozygosity but, in some cases, can occur by forming dominant negative forms of the tumor suppressor gene product. Their presence is usually inferred through the cytogenetic or molecular detection of subchromosomal loss. Upon molecular isolation, the genetic inference can be confirmed and dissected by demonstrating a restoration of growth regulation upon ectopic expression of the gene and/or by the formation of tumors or growth abnormalities in animals lacking the functional gene, either naturally occurring mutant strains or those constructed by in vivo homologous recombination "►gene knockout" techniques.

What was the Evidence for Tumor Suppressors?

The primary lines of evidence are genetic: One is that specific kinds of cancer can cluster in families. In most cases, the inheritance pattern is autosomal dominant which means that it is not sex-linked, may be transmitted from either parent and involves the transmission of a gene whose presence is sufficient to cause disease. In addition to familial clustering of the common cancers, two additional clinical observations provide strong epidemiological support for the contention that cancer has a genetic etiology.

- First, some individuals and their families have an ►autosomal dominant transmission of cancer predisposition, not to a single tumor, but to multiple tumors occurring independently at different body sites.
- Second, individuals with a variety of multi-organ developmental defects often also develop specific rare tumors. A statistical argument can thus be made that the combined occurrence of multiple independent tumors or the routine association of developmental defects with tumors which are very rare in the general population is so unlikely as to suggest an etiologic relationship.

The apparent dominant transmission of cancer traits is paradoxical in light of three observations. First, hybrid cells formed from the experimental fusion of highly malignant tumor cells with normal cells are not usually tumorigenic, suggesting that the normal phenotype is dominant in the presence of tumorigenic mutations. Furthermore, the occasional hybrid cell that regains tumorigenicity in these experiments has lost specific chromosomes originally contributed by the normal cell,



Tumor Suppressor Genes. Figure 1 Chromosomal mechanisms for tumor suppressor gene inactivation. Left side, the first mutation (*) can occur in a single somatic cell and result in sporadic disease. Alternatively, it can occur in a germ cell (de novo mutation) or be inherited from an affected parent and result in heritable disease. Right side, the first mutation can become completely inactivated by (from top to bottom) physical deletion or recombination of the wild type chromosome, by a targeted second mutation or deletion of the remaining wild type gene or by methylation of the promoter of the wild type gene leading to loss of expression.

implying that it is not gain of a dominant cancer trait but specific chromosomal loss that is responsible for the tumor phenotype. Second, if a single mutation was sufficient in itself to elicit a tumor, then families segregating for autosomal dominant forms of cancer would be expected to have no normal tissue in the diseased organ. This expectation is in direct contrast to the clinical description of these tumors as focal lesions surrounded by normal, functioning tissue of the same organ. Finally, epidemiological analyses of sporadic and familial forms of several human cancers have indicated that the conversion of a normal cell to a tumor cell requires multiple events.

Retinoblastoma – The First Suppressor

►Retinoblastoma is a relatively rare tumor (1 in 20,000 births) of young children and occurs in both a sporadic and autosomal dominant inherited form. Based entirely on statistical data from epidemiology and clinical observations, several remarkable conclusions were made regarding the nature of events leading to retinoblastoma tumor formation. First, the inherited mutation alone was not sufficient to cause the disease, since there are at least 107 retinoblast cells which are potential targets for retinoblastomas, each carrying the inherited mutation, yet on average, only three independent tumors form per affected individual. This also suggested that at a genetic level, mutations leading to retinoblastoma may be recessive, rather than dominant as suggested by the inheritance pattern. The hereditary tumors were proposed to arise through an initial germline mutation followed by a second mutation in a somatic cell. The rate at which somatic mutations occurred was similar in hereditary and sporadic cases, although sporadic tumors required two somatic mutations, each in the same retinoblast for tumor formation. Entirely consistent with this was the observation that hereditary cases usually occurred at an earlier age, were often bilateral and had multiple tumors, whereas the sporadic cases were invariably unilateral and single tumors. Because of the small possibility that a second somatic mutation may never occur in hereditary cases, ~5% of carriers do not develop any tumor. The nature of the two mutational targets in the genome was unknown at the time of these clinical observations, but cytogenetics and molecular genetics eventually led to the answer as well as to a general approach to other human cancers.

Analysis of the ►chromosome band patterns from hereditary and sporadic retinoblastoma patients revealed a deletion of chromosome 13q14 (chromosome

Tumor Suppressor Genes. Table 1

Gene	Chromosomal location	Function	Cancer sites
►*RB1*	13q14.2	Cell-cycle regulator	Retina, bone, bladder, breast, pancreas
►*p53*	17p13.1	Genome-stability regulator	Brain, breast, leukemia, soft tissue
►*p16*	9p21	Cyclin dependant kinase inhibitor	Brain, melanocyte
p15	9p21	Cyclin dependant kinase inhibitor	Leukemia
p18	1p32	Cyclin dependant kinase inhibitor	Esophagus, lung, bladder, pancreas
►*p21*	6p21	Cyclin dependant kinase inhibitor	Prostate, lung
►*E2F*	20q11	Transcription factor	Erythroleukemia
►*BRCA1*	17q21	Transcription factor (?)	Breast, ovary
►*BRCA2*	13q12–13	Transcription factor (?)	Breast, ovary
►*WT1*	11p13	Transcription factor	Kidney
►*VHL*	3p25–26	Modulator of RNA polymerase	Kidney, central nervous system
►*PTCH*	9q22.3	Transcription repressor	Skin
TGFβR1	9q33–34	►TGF-β receptor	Colon, retina, liver, stomach
TGFβR2	3p21.3	►TGF-β receptor	Colon, retina, liver, stomach
►*DPC4*	18q21.1	TGF-β pathway growth inhibitor	Pancreas, colon, bladder, liver
►*CDH1*	16q22.1	Intercellular adhesion	Breast, ovary, liver, skin, endometrium
►*APC*	5q21	Cell signaling	Colon
►*MCC*	5q21	(?)	Colon
►*NF1*	17q11.2	Cell signaling	Peripheral nervous system, skin
►*NF2*	22q12	cell signaling	Central nervous system
►*MSH2*	2p22	Mismatch repair protein	Colon
►*MLH1*	3p21.3	Mismatch repair protein	Colon
►*DCC*	18q21	Differentiation factor	Colon
►*PTEN*	10q23.3	Protein/lipid phosphatase	Brain, melanocytes, prostate, thyroid, breast

13, q or long arm, band one-four), suggesting that the gene for retinoblastoma (Rb) resided somewhere within this region. DNA from hereditary tumors was then analyzed with cloned DNA probes (termed "DNA markers") that could distinguish the two copies, or ►alleles, of chromosome 13 within each cell. It was found that, in tumors of affected individuals, the region containing the suspected Rb gene on chromosome 13 was present in a mutant only state. This conversion from a heterozygous state to homozygosity for the mutation was termed loss of heterozygosity (LOH), and constituted the second hit required for tumor formation in hereditary cases. Furthermore, LOH on chromosome 13q14 also occurred in sporadic retinoblastoma. These data lent strong support to the idea that retinoblastoma tumor formation occurs by the unmasking of a recessive genetic defect. The discovery that LOH occurs in other hereditary and most sporadic cancers in humans marked the simultaneous emergence of somatic cell cancer genetics and its coupling to the genetics of hereditary cancer. By identifying the region of chromosome 13q14 with the most consistent LOH in tumor DNA, the gene responsible for retinoblastoma was eventually isolated and its functionality assessed. Most importantly, the gene was shown to be mutationally inactivated in retinoblastoma tumors. When a normal copy of the gene was transferred to tumor cells, their growth and tumorigenic behavior was reduced. Thus, the conjoint application of epidemiology, cytogenetics, molecular genetics and molecular biology allows the identification of a gene with tumor suppressing function.

Are There Other Tumor Suppressors and What Cellular Role do They Normally Play?

Since the first suppressor was isolated, many others have been molecularly identified. As might be expected, these represent genes whose products are involved in many different aspects of cell growth and behavior. These include regulators of the ►cell cycle, growth and transcriptional regulators, ►DNA repair enzymes, differentiation factors, elements of cell ►motility and regulators of cellular signaling. Thus, elucidation of the function and nature of tumor suppressors is not only of importance for understanding cancer etiology but also useful for dissecting normal cellular function.

Clinical Relevance

The intimate involvement of tumor suppressor genes in the etiology of most human cancers places them at the center of cancer research. Such knowledge has been exploited for the first prenatal and premorbid predictions of cancer occurrence, for molecular pathology approaches to tumor subtyping, for ►gene therapy approaches toward gene replacement and as targets for agonist/antagonist development in rational drug design. Just as in research, the continued exploitation of tumor suppressor genes for clinical benefit to cancer patients is likely to assume a central role in modern therapies (Table 1).

►CCCTC-Binding Factor (CTCF)
►von Hippel-Lindau Tumor Suppressor Gene

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Tumor-Transforming 1

►Securin

Tumor Typing

►Pathology

Tumor Vessel Density

Definition

The percentage of total vascular area in a given visual field of tumor section.

►Thrombospondin

Tumor Xenografts

Definition

Tumors obtained by inoculation of tumor cells not from the individual in whom the tumor is generated (xeno).

These tumors are widely used as an animal model to investigate the therapeutic efficacy of different anti-tumoral agents.

►Cannabinoids
►Xenograft

Tumoricidal

Definition

Describes an agent or a treatment that may kill cancer cells.

►Chemoattraction

Tumorigenesis

Definition

Abnormal cell proliferation that produces, or is predisposed to produce tumors.

TUNEL

Definition

TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick end labeling (TUNEL), also known as ISEL (in situ end labeling), is a DNA-tailing reaction using TdT. DNA degradation is considered the key biochemical event in ►apoptosis, resulting in cleavage of nuclear DNA into ►nucleosome-sized fragments (approximately 180 base pair unit size). TUNEL can be used to determine apoptosis in individual cells, preferentially labeling apoptotic rather than necrotic cells. The principle is that DNA ends generated during apoptosis are extended by TdT using biotin- or digoxigenin-labeled BrdUrd (bromodeoxyuridine). The incorporated material is detected by fluorescent reagents.

►Mitotic Catastrophe
►Apoptosis

Tunicamycin

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Definition

Tunicamycin is an antibiotic identified and isolated from the fermentation broth of *Streptomyces lysosuperificucs*. Since the antibiotic interferes with the formation of viral and cellular surface coats, it was termed "tunicamycin" after the Latin word "tunica" for coats.

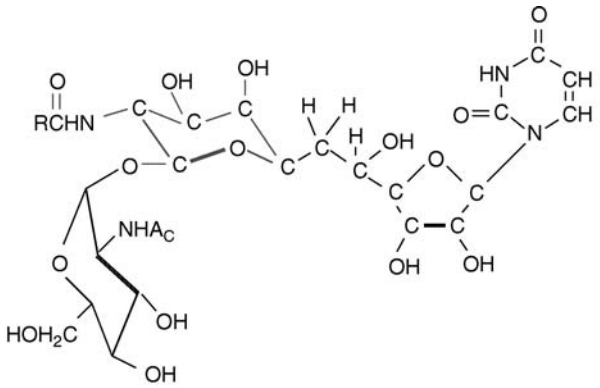
Characteristics

Structure and Function

Tunicamycin is a nucleoside antibiotic composed of uracil, a fatty acid and two glycosidically linked sugars. The sugars are *N*-acetylglucosamine and an unusual 11-carbon aminodeoxydialdose, which has been named tunicamine. Tunicamycin is a white crystalline powder which is soluble in alkaline water, pyridine, and hot methanol, slightly soluble in ethanol and *n*-butanol and insoluble in acetone, ethylacetate, chloroform, benzene and acidic water. High performance liquid chromatography (HPLC) of tunicamycin shows that the antibiotic is separated into ten different components that have molecular weights ranging from 802 to 858. These differences in molecular mass are attributable to differences in the acyl chain length of C_{13}–C_{17} (Fig. 1) Tunicamycin inhibits the first step in the lipid-linked saccharide pathway. Tunicamycin specifically inhibits the formation of dolichyl(pyro)-phosphate *N*-acetylglucosamine (dolichyl-PP-GlcNAc) in the step of the transfer of GlcNAc-1-P from UDP-GlcNAc to dolichyl-P. As a result of inhibition in the lipid-linked saccharide pathway, tunicamycin consequently inhibits the *N*-linked oligosaccharide formation of ►glycoproteins in the ►endoplasmic reticulum (ER) (Fig. 2).

Anti-viral Effect of Tunicamycin

Tunicamycin strongly inhibits the multiplication of enveloped RNA and DNA viruses such as Newcastle disease virus, vesicular stomatitis virus, semlici forest virus, fowl plague virus, Sindbis virus, measles virus, influenza virus, Rous sarcoma virus, Rauscher murine sarcoma virus and herpes simplex virus. Hemagglutinin and neuraminidase are glycoprotein components of the viral envelope. Tunicamycin specifically inhibits the biosynthesis of viral envelope glycoproteins. Tunicamycin possesses cytotoxic activity towards transformed mammalian cells infected with virus such as mouse 3T3 cells transformed by ►SV40 virus,



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II: $(CH_3)_2CH(CH_2)_8CH=CH-$
III: $CH_3(CH_2)_{10}CH=CH-$
IV: $CH_3(CH_2)_{11}CH=CH-$
V: $(CH_3)_2CH(CH_2)_9CH=CH-$
VI : R=$(CH_3)_2CH(CH_2)_{11}-$
VII: $(CH_3)_2CH(CH_2)_{10}CH=CH-$
VIII: $CH_3(CH_2)_{12}CH=CH-$
IX: $CH_3(CH_2)_{13}CH=CH-$
X: $(CH_3)_2CH(CH_2)_{11}CH=CH-$

Tunicamycin. Figure 1 Structure of tunicamycin.

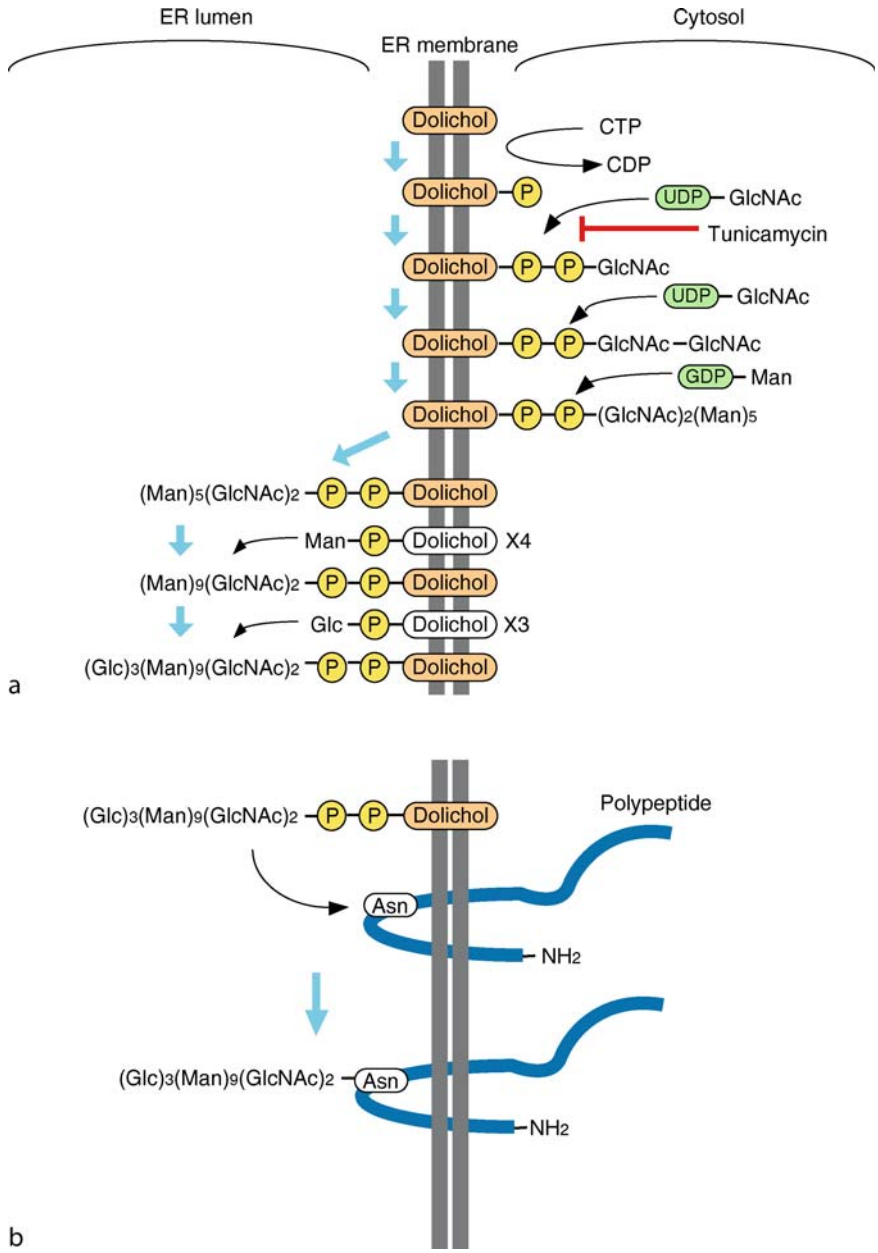
Moloney sarcoma virus or Polyoma virus, and human WI38 fibroblasts transformed by SV40. Parental cell lines are resistant to tunicamycin cytotoxicity prior to neoplasmic transformation.

Apoptosis Induction by Tunicamycin

Tunicamycin induces ►apoptosis in various types of human cancer cells containing ►neuroblastoma, ►breast cancer, ►hepatocellular carcinoma and ►cervical cancer. Recent studies have revealed the mechanism of tunicamycin-induced apoptosis. In eukaryotic cells, ER provides an environment for the synthesis and modification of membrane proteins and secreted proteins. These co- and post-translational modifications including N-linked ►glycosylation are involved in subsequent protein folding and assembly. The accumulation of unfolded proteins causes ►endoplasmic reticulum stress, the term given to an imbalance between the cellular demand for ER function and ER capacity. Cells respond to ER stress by activation of the ►unfolded protein response (UPR). The UPR is mediated through three ER transmembrane receptors, activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1) and pancreatic ER kinase (PKR)-like ER kinase (PERK). Under unstressed conditions, these sensor proteins are maintained in an inactivated state by an association with ER chaperone protein, glucose-regulated protein (GRP78) (also called Bip). The UPR is a cytoprotective response to reduce the accumulation of unfolded proteins and restore normal ER function; however, prolonged ER stress switches from pro-survival to pro-apoptotic signaling. Inhibition of *N*-linked glycosylation by tunicamycin leads to ER stress. Tunicamycin is a representative ER stressor used to elucidate the mechanism of ER stress and UPR followed by apoptosis. The accumulation of unfolded proteins in ER results in the release of GRP78 from IRE1, PERK and ATF6. PERK phosphorylates the eukaryotic translation initiation fctor-2α (eIF-2α and allows the activating transcription factor 4 (ATF4). ATF4 transactivates C/EBP-homologous protein (CHOP). CHOP is an important factor in the induction of apoptosis by tunicamycin. CHOP-deficient mouse embryonic fibroblasts (MEF) provide partial resistance to apoptosis induced by tunicamycin treatment. MEF are not cells derived from cancer tissues but normal cells. MEF is often used to elucidate the mechanism of tunicamycin-induced apoptosis, since MEF leads to apoptosis by tunicamycin treatment and specific gene deficiency is easily provided in MEF. CHOP is a transcription factor and transactivates various genes. Tribbles-related protein 3 (TRB3) is a CHOP-downstream gene induced by CHOP at the transcriptional level. Knockdown of ATF4 and CHOP expression represses tunicamycin-induced TRB3 upregulation. Furthermore, down-regulation of TRB3 by small interfering RNA (►siRNA) partly blocks the apoptosis induced by tunicamycin treatment in human cervical cancer HeLa cells. Tunicamycin treatment also induces a ►p53-up-regulated modulator of apoptosis (►PUMA) and NOXA in a tumor-suppressor p53 gene-dependent manner. Both PUMA-deficient MEF and NOXA-deficient MEF partially reduce tunicamycin-induced apoptosis. These factors are key molecules of tunicamycin-induced apoptosis.

Tunicamycin Increases Effects of Anti-cancer Agents

Many glycoproteins exist on the plasma membrane of tumor cells, including the efflux pump of anti-cancer



Tunicamycin. Figure 2 Oligosaccharide formation and the action site of tunicamycin. (a) Lipid-linked oligosaccharide formation. Oligosaccharide is assembled onto the carrier lipid dolichol in the ER membrane. Tunicamycin inhibits the formation of dolichyl PP-GlcNAc. (b) N-linked oligosaccharide formation. Synthesized oligosaccharide $(Glc)_3(Man)_9(GlcNAc)_2$ is transferred from the lipid dolichol to the asparagine side chain of a nascent polypeptide on the ER membrane. GlcNAc, *N*-acetylglucosamine; Man, mannose; Glc, glucose.

agents. ►P-glycoprotein is a product of the MDR1 gene and protects cells against a broad spectrum of anticancer agents by functioning as an energy-dependent drug efflux pump. Inhibition of *N*-linked glycosylation of P-glycoprotein results in a reduced multidrug-resistance phenotype of cancer cells. Tunicamycin treatment increases the cytotoxicity of doxorubicin, epirubicin, ►cisplatin and vincristin against NIH-3T3 cells into which an exogenesis MDRs gene had been introduced. The human epidermoid carcinoma KB-8-5-11 cell line contains the amplified MDR1 gene. The sensitivity of this cell line to ►doxorubicin, epirubicin, cisplatin and ►vincristin also increases by treatment with tunicamycin. Tunicamycin does not influence the

uptake of these anti-cancer agents but reduces efflux of the agents in cells with a ►multidrug-resistance phenotype. Human ►colon cancer cells with a strong multidrug-resistant phenotype mediated by high constitutive levels of the expression of P-glycoprotein increase daunorubicin accumulation by incubation with tunicamycin. Tunicamycin exposure reduces P-glycoprotein expression on the surface of the cell membrane. Tunicamycin also decreases the 50% inhibitory concentration (IC_{50}) of cisplatin in human pharyngeal carcinoma KB cells and human maxillary squamous-cell carcinoma IMC-3 cells. Combined administration of tunicamycin with cisplatin injected by s.c. around the tumor inhibits tumor growth in C3H/He mice bearing cisplatin-resistant squamous-cell carcinoma *in vivo*, and increases the *in vivo* apoptosis of tumor cells.

Tunicamycin Enhances Death Ligand TRAIL-Induced Apoptosis

TNF-related apoptosis-inducing ligand (►TRAIL) is a type-II membrane protein belonging to the TNF family, which preferentially induces apoptosis in a variety of tumor cells but not in normal cells *in vitro* and *in vivo*. Since some tumor cell lines are resistant to TRAIL, an agent that can overcome resistance to TRAIL has been sought. Tunicamycin sensitizes ►hormone-refractory ►prostate cancer cells to TRAIL-induced apoptosis. The combination of tunicamycin and TRAIL activates ►caspases under the condition in which a single agent hardly activates caspases. Tunicamycin enhances TRAIL activity through the induction of a TRAIL receptor, TRAIL-R2 (also called death receptor 5 (►DR5)). Tunicamycin up-regulates the transcription of TRAIL-R2 through a transcription factor, CHOP.

Normal Cellular Toxicity of Tunicamycin

The hepatotoxicity of tunicamycin has been reported in guinea pigs given a single dose of 400 μg/kg of tunicamycin. A periportal pattern of hepatocellular damage was observed with the death of many hepatocytes up to 72 h post-injection. Swollen hepatocyte cytoplasm protruded into many hepatic blood vessels and detached portions of hepatocytes producing emboli in pulmonary and cerebral capillaries. The toxic effects of tunicamycin have been examined in rats at gestation day 15. At 16 h post-dosing, all pregnant rats had moderate to extensive vaginal bleeding and 1/4 died. The other rats had free blood in the uterus and large decreases in red cell counts, hemoglobin and packed cell volume. Tunicamycin toxicity has been reported in 6- to 8-week-old mice given a single injection of tunicamycin at a dose of 3 μg/g body weight intraperitoneally (i.p.) After 2 days, characteristic renal lesions were detected in tunicamycin-treated mice. Apoptosis was caused in the renal tubular epithelium. Moreover, tunicamycin treatment inhibited mammalian embryogenesis. Although early cleavage was normal, mammalian embryos did not undergo normal compaction and blastocyst formation. Trophoblast cell adhesion may be disrupted since tunicamycin is cytotoxic to these cells. In later development, tunicamycin inhibits kidney tubule formation when present during the embryonic induction of these structures.

Tunicamycin induces apoptosis in a variety of malignant tumor cells and possesses cytotoxic activity to virus-transformed cells compared with parental normal cells. Moreover, tunicamycin enhances the effects of anti-cancer agents; however, tunicamycin also has cytotoxicity against normal cells and causes crucial damage during *in vivo* administration. The mechanism of tunicamycin-induced apoptosis through ER stress has been revealed, but it has not been completely elucidated yet. If tunicamycin is applied to cancer treatment, its usage should be restricted to local administration, but further studies are needed.

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Turban Tumor Syndrome

Definition

►Cylindromatosis.

Turcot Syndrome

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Definition

Turcot syndrome (TS) is a rare inherited neoplastic disease characterized by the association of primary malignant neuroepithelial tumors of the central nervous system and colon cancers and/or multiple colorectal adenomas.

Characteristics

Clinical Criteria

The 130 or so Turcot syndrome (TS) cases described to date include various histopathologic types of ►brain tumors, e.g. glioma, ►medulloblastoma and astrocytoma, associated with a broad spectrum of colorectal findings, from a single adenoma to typical adenomatous polyposis. Usually, polyps are fewer in number than in familial adenomatous polyposis (FAP [►APC gene in familial adenomatous polyposis]) but are larger in size, and multiple adenomas or colorectal cancers occur at an early age and undergo an earlier malignant transformation than in FAP or in hereditary non-polyposis colorectal cancer (►HNPCC).

The clinical definition and the mode of inheritance of Turcot syndrome is controversial; some authors propose that TS is an allelic variant of FAP and support an autosomal dominant inheritance, while others postulate that TS is a disease independent of FAP with an autosomal recessive pattern of inheritance.

Genetics

Dominantly inherited cases have been associated with germline mutations in either the tumor suppressor adenomatous polyposis coli gene (APC), usually mutated in FAP, or in the DNA ►mismatch repair (MMR) genes, which are usually mutated in HNPCC.

Few recessive cases have been reported and only in two cases were the causative mutations found to be within the PMS2 gene, a minor MMR gene that is only rarely involved in HNPCC. In the first recessive case described, a germline nonsense mutation (PMS2-134) that was inherited from the healthy mother was found in one allele. The second recessive case was found to be a compound heterozygote for two frameshift germline mutations within the PMS2 gene; a G deletion (1221delG) in exon 11 and a four-base pair deletion (2361delCTTC) in exon 14, both of which were inherited from the patient's unaffected parents. This was the first evidence of recessive dominance of TS because two germline mutations in PMS2 are not individually pathogenic, but become so when occurring together in a compound heterozygote. Since all carriers of only one PMS2 gene mutation are clinically healthy, a lower penetrance of PMS2 gene mutations may be inferred for colorectal tumor development compared with mutations in other MMR genes, e.g. MLH1 and MSH2. Accordingly, there are no reports of HNPCC patients compound heterozygous for MLH1 or MSH2 mutations, which suggests compound heterozygosity is lethal.

PMS2 Gene

The PMS2 gene, localized to chromosome band 7p22, encompasses 16 kb and consists of 15 exons. It encodes a protein involved in the mismatch repair system that physically interacts with the MLH1 protein through the carboxyl terminus. Both PMS2 mutations (1221delG and 2361delCTTC) found in the compound heterozygous TS patient, caused the loss of the MLH1 interaction domain with PMS2, thus indicating that a disruption of the MLH1-PMS2 heterodimer might block the process of the MMR system.

Microsatellite Instability

►Microsatellite instability, an indicator of the defective DNA mismatch repair system, is characteristic of HNPCC colorectal tumors, of a small fraction of sporadic colon and brain cancers, and of colon carcinomas and adenomas in TS patients. It results from germline mutations in each of the MMR genes, i.e. MSH2, MLH1, PMS1 and PMS2, with somatic mutation inactivating the second allele in the tumor tissues or loss of protein expression in the tumor tissues. Brain and colon tumors in TS patients exhibit severe microsatellite instability at several DNA marker loci, at the repeated regions of the TGFβRII and of MSH3 and MSH6 genes as well as somatic mutations within the APC and p53 genes. High frequencies of the microsatellite instability have also been found in normal colon mucosa from TS patients bearing germline PMS2 mutations, in contrast to HNPCC patients in which microsatellite instability is very rare in normal tissues. Therefore, the high DNA hypermutability in normal tissues of TS patients is associated with a severe biochemical defect in the MMR system and it might trigger the early development of multiple cancers in this syndrome.

Clinical Aspects

Patients with hereditary forms of colon cancer and neurologic symptoms require immediate and thorough investigation because of their significantly increased risk of developing central nervous system (CNS) tumors. Patients diagnosed with a CNS tumor with a family history of colon tumors should undergo screening and

surveillance colonoscopy because the CNS lesion may precede colonic symptoms. The elucidation of the gene defects responsible for TS has therefore important implications in the genetic testing of relatives at risk and in the management of young patients with brain tumors and asymptomatic subjects.

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Turmeric Yellow

►Curcumin

TUTR1

►Securin

Two-Hit Model

Definition

Based on epidemiological and clinical data, it has been inferred that as few as two mutations are sufficient to initiate the development of ►retinoblastoma (Rb). Specifically, it was hypothesized that

- In hereditary Rb one mutation is transmitted via the germline and the second mutation occurs in a somatic cell
- In non-hereditary Rb both mutations arise in somatic cells

In principle this may apply to the inactivation of other tumor suppressor genes.

►Tumor Suppression
►Knudson Hypothesis

Two-Step Carcinogenesis

Definition

The process by which animals are treated first with a low, non-carcinogenic dose of a carcinogen, and then this is followed with a tumor promoter, once per week, leading to a synergistic induction of tumors.

►Chemical Carcinogenesis
►Skin Carcinogenesis
►Tumor Promotor

TXBP181

►Mitotic Arrest-Deficient Protein?1

Type I Cystatins

►Stefins

Type-1 Hypersensitivity

►Allergy

Type I Interferon

►Interferon-a

Type I Programmed Cell Death

►A5-aza-2′ Deoxycytidine

Typical Neurocytoma

►Neurocytoma

Tyrosine

Definition

Is one of the 20 amino acids that are used by cells to synthesize proteins. Although it is among nonessential amino acids that can be made in vivo by animals, tyrosine is synthesized by the hydroxylation of an essential amino acid phenylalanine obtained from dietary sources.

Tyrosine Kinase

Definition

Tyrosine Kinase is a kinase enzyme that modifies other proteins by chemically adding phosphate groups from ATP (phosphorylation) to the tyrosine residues in them. Phosphorylation usually results in a functional change of the target protein by changing enzyme activity, cellular location, or association with other proteins. This process is an important mechanism in signal transduction for regulation of enzyme activity.

►Nutraceuticals
►Tyrosine Kinase Inhibitors
►Receptor Tyrosine Kinases

Tyrosine Kinase Inhibitors

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Synonyms

Protein-tyrosine kinase inhibitors; PTKIs

Definition

Low molecular weight or macromolecular compounds that selectively inhibit the enzymatic activity of one or more protein-tyrosine kinases and which may therefore be useful in the treatment of diseases associated with unregulated kinase activity.

Characteristics

Tyrosine kinase inhibitors are a relatively new class of anticancer drugs which beautifully illustrate the promises and pitfalls of molecularly-targeted drug design. Since the market approval of the first drug in this class, ►imatinib (Gleevec® Imatinib), there has been a steady growth in market approvals of tyrosine kinase inhibitors. To date, there are seven tyrosine kinase inhibitors approved for cancer therapy. In addition to imatinib (Gleevec®), these include dasatinib (Sprycel™), erlotinib (Tarceva®), gefitinib (Iressa®), lapatinib (Tykerb®), sorafenib (Nexavar®), and sunitinib (Sutent®).

The term "tyrosine kinase inhibitor" is usually reserved for low molecular weight compounds that are capable of directly interacting with the catalytic site of the target enzyme and thereby inhibiting catalysis (►Inhibitors, molecular and chemical). Macromolecular drugs, most notably recombinant monoclonal antibodies such as cetuximab (Erbitux®) and trastuzumab (Herceptin®), have also been targeted against tyrosine kinases. Because recombinant monoclonal antibodies employ a different mechanism of drug action and have different pharmacological properties than low molecular weight kinase inhibitors, they will not be further discussed in this essay.

The molecular targets for these drugs are protein-tyrosine kinases, a class of enzymes originally reported as oncoproteins in 1980. It is now recognized that there are at least ninety distinct tyrosine kinases encoded in the human genome. There are two major families of tyrosine kinases. Receptor tyrosine kinases have an intracellular catalytic domain, a transmembrane domain and an extracellular ligand-binding domain; the ►epidermal growth factor receptor tyrosine kinase is a member of this family. Non-receptor tyrosine kinases lack both the extracellular ligand-binding domain and

the transmembrane domain and are hence cytosolic enzymes often non-covalently associated with other receptor systems; Janus kinases, which are typically associated with a variety of cytokine receptors, are members of this family.

Many tyrosine kinases are essential transducers of mitogenic signals from a variety of intercellular stimuli, such as cytokines or growth factors. The normal forms of these enzymes do not appear to be oncogenic when their expression levels and activity levels are properly regulated. It is only when these regulatory mechanisms break down by the introduction of mutations, chromosomal translocations, or due to epigenetic events, that tyrosine kinase activities result in the loss of cellular homeostasis. This loss of homeostasis can be manifested in diseases ranging from relatively benign conditions such as polycythemia vera to serious diseases such as chronic myelogenous leukemia to highly aggressive cancers such as non-small cell lung carcinoma. In such circumstances, cellular physiology appears to become dependent on these tyrosine kinase pathways, a circumstance referred to as "oncogene addiction" (►Pathway Addiction).

While tyrosine kinase inhibitors can sometimes effectively inhibit normal forms of tyrosine kinases under circumstances of oncogene addition, they appear to yield the greatest therapeutic benefit when the target is a mutated form of the enzyme. This may be because tyrosine kinase inhibitors do not equally affect all conformations or activity states of a given tyrosine kinase, but rather appear to be state-selective inhibitors. To illustrate, here are several examples. Imatinib (Gleevec®) preferentially binds and inhibits a form of the ABL kinase in which the activation loop is in a closed configuration, rather than the open configuration that ensues upon phosphorylation of tyrosines within the loop. Gefitinib (Iressa®) shows greatest therapeutic benefit to patients whose epidermal growth factor receptor (►EGFR, ►EGF Receptor, EGFR, ►Epidermal Growth Factor Receptor) kinase contains mutations within the P-loop region of the catalytic domain, but showed little to no benefit to patients with the normal form of the EGFR kinase.

Such examples raise the issue of using suitable diagnostic biomarkers to identify those patients who are most likely to benefit from tyrosine kinase inhibitor therapy. The fact that patients with chronic myelogenous leukemia (►Chronic Myeloid Leukemia) ►could be readily screened for the ►Philadelphia chromosome greatly facilitated the clinical trials which led to the approval of imatinib (Gleevec®). Precise DNA sequencing now identifies those patients who will not likely respond to imatinib (Gleevec®), but who would likely respond to dasatinib (Sprycel™). And it is now evident that gefitinib (Iressa®) is most effective against mutated EGFR kinases which harbor certain mutations in the P-loop of the catalytic domain.

It should be noted that therapeutically useful tyrosine kinase inhibitors are not specific for a single tyrosine kinase, but rather they are selective against a limited number of tyrosine kinases. Such selectivity has generally provided more clinical advantages than if the drugs were truly specific. In the case of imatinib (Gleevec®), which inhibits BCR/ABL, c-kit (►Kit/►Stem Cell Factor Receptor in Oncogenesis), and platelet-derived growth factor receptor (PDGFR) kinases, this leads to a broader range of indications for the treatment of several different diseases related to these tyrosine kinases. In the case of sunitinib (Sutent® ►Anti-Angiogenic Drugs), which inhibits most isoforms of PDGF receptor kinase and vascular epithelial growth factor (VEGF) receptor kinase, this leads to the simultaneous inhibition of tumor proliferation and tumor angiogenesis and greater therapeutic efficacy by inhibiting multiple targets involved in the progression of cancer.

Tyrosine kinase inhibitors are based on only a few common structural motifs, such as phenylaminopyrimidines, anilinoquinazolines, and indolinones. Most of these drugs bind reversibly to the ATP binding site of the tyrosine kinase catalytic domain. An important feature of these drugs is that they are somewhat water soluble, which improves their oral bioavailability. These drugs are formulated as pills. Thus, unlike drugs such as taxol which must be administered through an intravenous infusion in the clinic, tyrosine kinase inhibitors let cancer patients follow a more normal lifestyle because they can be administered in a home setting, rather than in the clinic. Furthermore, these tyrosine kinase inhibitors have a much better safety profile than classical cytotoxic anticancer drugs, which allows them to be taken on a daily basis.

However, a tyrosine kinase inhibitor is not a "magic bullet." Tyrosine kinase inhibitors are generally cytostatic, rather than cytotoxic, drugs and hence drug resistance often emerges after several years of excellent response to therapy. As is the case with other molecularly-targeted anti-cancer drugs, the medical community must learn the most efficacious way to use these new drugs by matching these drugs with the patients most likely to respond to them and to rationally incorporate them into effective combination drug regimes.

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Tyrosine Kinase Receptors

Definition

A vast family of membrane receptors involved in signal transduction of stimuli relevant to proliferation, differentiation and apoptosis. They all contain, in their intracytosolic region, a conserved domain encoding a ►tyrosine kinase activity. Upon ligand engagement receptors undergo autophosphorylation on tyrosine residues. This modification serves the dual purpose of further propagating the signal inside the cell and of activating the process of ►endocytosis, which ultimately leads to signal attenuation. It is controversial whether signaling can also occur in the various intracellular compartments of the endocytic route.

Tyrosine Phosphorylation

Definition

Binding of a phosphate group to the amino acid tyrosine in a protein. Most proteins undergo then conformational changes and are activated. ►Tyrosine kinases catalyze this process and phosphatases again eliminate these phosphate groups.

►Focal Adhesion Kinase (FAK)

Ubiquitin

Definition

Is a small 76 amino acid protein that can be conjugated to other proteins via its C-terminus. The primary function of ►ubiquitination is to target acceptor proteins for degradation by the ►proteasome.

Ubiquitin-directed Protein Degradation

Definition

Plays an important role in regulating a broad spectrum of biological processes, including transcription, signal transduction, cell cycle progression, apoptosis, cell growth and differentiation. Proteins that are targeted for degradation by this mechanism undergo series of ubiquitin-modification steps carried out by the successive activity of E1 (activating), E2 (conjugating), and E3 (ligating) ubiquitin enzymes.

►Ubiquitin Ligase SCF-Skp2
►Ubiquitination

Ubiquitin Ligase

Definition

Synonym E3 ubiquitin ligase; Is a protein which covalently attaches ►ubiquitin to a lysine residue on a target protein. The ubiquitin ligase is typically involved in polyubiquitination: a second ubiquitin is attached to the first, a third is attached to the second, and so forth. Polyubiquitination marks proteins for degradation by the proteasome.

►Microtubule Associated Proteins
►Ubiquitination

Ubiquitin Ligase SCF-Skp2

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Synonyms

S-phase kinase-associated protein 2; Skp2; CDK2/cyclin A-associated protein p45; F-box and leucine-rich repeat protein 1 – Fbxl1; Fbl1

Definition

The SCF-Skp2 ubiquitin ligase belongs to the multi-subunit ►RING-type of E3 ligases involved in ►ubiquitin-directed protein degradation of key cell cycle regulators.

Characteristics

Cell cycle progression is coordinated by the activation of cyclin-dependent kinases (CDKs), the activities of which are controlled by the ubiquitin-directed degradation of key regulators such as ►cyclins and ►CDK inhibitors (CKIs). Two major classes of ►ubiquitin ligases, the SCF (Skp1-Cul1-Fbp) and ►APC/C (anaphase-promoting complex/cyclosome), have a central role in cell-cycle regulation. The SCF complexes are used throughout the ►cell cycle, whereas the APC/C acts primarily during ►mitosis and G1 phase. The SCF complex and the APC/C are structurally similar. Each is constituted of common subunits and a variable substrate-recognition subunit: F-box proteins for the SCF and activators for the APC/C.

Three F-box proteins in the SCF complex – Skp2 (S-phase kinase-associated protein 2), FBW7 (F-box and WD-40 domain protein 7) and β-TRCP (β-transducin repeat-containing protein) – and two activators in the APC/C – CDC20 (cell division cycle 20) and CDH1 (►cadherin 1) – are the most important in cell-cycle regulation. In the SCF ligase, the common subunit Cul1 functions as a molecular scaffold that simultaneously interacts at the amino terminus with the adaptor subunit Skp1 (S-phase-kinase-associated protein-1) and at the carboxyl terminus with a RING-finger protein (Rbx1) and a specific ►ubiquitin-conjugating enzyme (E2). Skp1, in turn, binds to one of many F-box proteins. Each F-box protein appears to be matched with a discrete number of specific substrates through a protein-protein interaction domain. Conventionally, the F-box protein is superscripted after "SCF" to indicate distinct SCF complexes (e.g. SCF^{Skp2}).

Several lines of evidence from both biochemical and genetic studies have shown that the SCF^{Skp2} complex is required for cell cycle progression at multiple stages, including G1/S, S phase progression, and S/G2 transition. As the substrate-recognition subunit in the SCF^{Skp2} complex, Skp2 targets CDK inhibitors such as ►$p27^{Kip1}$, $p21^{Cip1}$, and $p57^{Kip2}$ for degradation. Up to now, it has also been reported that Skp2 targets other key cell cycle regulators such as ►cyclin D1, cyclin E, ►E2F-1, p130, cyclin A, and ►Myc. However, it is $p27^{Kip1}$ that seems to be the primary target of Skp2, given that $Skp2^{-/-}$ ►knockout mice show a marked accumulation of $p27^{Kip1}$, and that prominent cellular phenotypes apparent in $Skp2^{-/-}$ knockout mice, including polyploidy, nuclear enlargement, and ►centrosome amplification, disappear in $Skp2^{-/-}$ $p27^{Kip1}$ double-knockout mice.

Regulation

Cells have different ways to regulate SCF^{Skp2} activity. The Rb tumor suppressor protein has a dual role in controlling the SCF^{Skp2} activity. Skp2 expression is cell cycle regulated, with *Skp2* transcription being activated at the ►G1/S transition by activating E2Fs – *Skp2* is a direct E2F target. In addition, during ►pRb-mediated cell-cycle arrest, pRb associates with Skp2, favors the degradation of Skp2, and promotes the stability of $p27^{Kip1}$. This is at least partly controlled by an interaction of pRb with another ubiquitin ligase, APC/C^{Cdh1}, *via* its activator Cdh1. Beside the degradation of Skp2, APC/C^{Cdh1} also favors the ubiquitin-directed degradation of Cks1, an important cofactor of the SCF^{Skp2} complex. The SCF^{Skp2} complex itself is also subject to post-translational modifications which controls activity of the complex. Skp2 ubiquitylation is inhibited by Cks1. Intriguingly, increased destabilization of Skp2 in response to ►transforming growth factor-β has been linked to loss of expression of Cks1, whose interaction with Skp2 appears to physically protect it from ►ubiquitination. Another post-translational modification that controls the activity of the SCF^{Skp2} complex is ►neddylation on the Cullin subunit. Neddylation describes the linkage of Nedd8 – a small 76-residue protein with sequence similarity to ubiquitin – to a lysine residue on the substrate. Although Nedd8 conjunction is known to dramatically increase ubiquitin transfer by ubiquitin-conjugating enzymes bound to the Rbx1/Cullin complex, the precise mechanism for this stimulation is unknown.

An important step in the process of substrate recognition by the SCF^{Skp2} complex is post-translational modification of the substrate itself. The SCF^{Skp2} complex targets $p27^{Kip1}$ only after phosphorylation on threonine 187 (T187). Active cyclin E/CDK2 complexes are crucial in the phosphorylation process on T187 of cyclin/CDK-bound $p27^{Kip1}$. While free and active cyclin/CDK2 efficiently phosphorylates CDK-bound $p27^{Kip1}$, $p27^{Kip1}$-bound CDK2 is thought to be catalytically inactive. This suggested that degradation by the SCF^{Skp2} complex may require $p27^{Kip1}$-free cyclin E/CDK2 and led to the enigma of how $p27^{Kip1}$ degradation can be initiated in G1 when all CDK2 complexes are bound by $p27^{Kip1}$. $p27^{Kip1}$ phosphorylation on T187 and SCF^{Skp2} dependent $p27^{Kip1}$ degradation at the G1/S transition can be initiated even in the absence of free cyclin/CDK2 complexes. $p27^{Kip1}$ can be phosphorylated on a tyrosine residue at position 88 (Y88) within its CDK-binding domain by tyrosine kinases. Y88-phosphorylated $p27^{Kip1}$ still bind to cyclin/CDK complexes, but the associated kinase retained significant catalytic activity. Furthermore, Y88-phosphorylated $p27^{Kip1}$ is an efficient substrate for phosphorylation on T187 by CDK2 within the trimeric complex (cyclin/CDK2/$p27^{Kip1}$).

Beside its function in ubiquitin-dependent degradation of cell cycle regulators, Skp2 has an unanticipated function as a transcriptional regulator. Skp2 controls mRNA synthesis not only by mechanisms involving ubiquitin-dependent degradation of transcription factors by the proteasome, but also by mechanisms that appear to be independent of the proteasome. Skp2 is a cofactor for transcriptional activation by ►MYC oncoproteins. It has been shown that Skp2 interacts with c-Myc, and thereby mediates its ubiquitylation and degradation. However, Skp2 unexpectedly increases the transcriptional activation activity of Myc, indicating that Skp2 is a transcriptional cofactor. Consistent with this, Myc accumulates, but its transcriptional activity is reduced, in mouse $Skp2^{-/-}$ cells.

Clinical Significance

Skp2 was originally identified as a protein that associates with cyclin A-CDK2 in transformed cells, but not in normal cells. It has become evident that Skp2

expression is negatively correlated with levels of $p27^{Kip1}$ in many cancers, and positively correlated with the grade of malignancy in certain human tumors. It is widely accepted that $p27^{Kip1}$ is a tumor suppressor, not only because of its activity as a CDK inhibitor, but also because of evidence from mouse models and the marked correlation between reduced $p27^{Kip1}$ levels and poor prognosis found in clinical studies of patients with cancer. Indeed, a reduction in the concentration of $p27^{Kip1}$ is common in many types of human malignancies. However, in contrast with other tumor suppressors such as ►p53 or Rb, mutation or deletion of the *$p27^{Kip1}$* gene is an uncommon event in the development of human cancers, indicating that deregulation of $p27^{Kip1}$ expression in human tumors is often due to post-transcriptional mechanisms. Deregulated components of the ►ubiquitination machinery for $p27^{Kip1}$, primarily Skp2, have been described in many cancer types. Overexpression of Skp2 or Cks1 is strongly and independently associated with a loss of tumor differentiation and poor survival. Frequent ►amplification and overexpression of the *Skp2* gene has been observed in lung cancers and in cell lines expressing high-risk ►human papillomavirus. Furthermore, Skp2 has oncogenic potential in transgenic mouse models.

The role of Skp2 in cancer initiation and progression is further supported by findings describing a new tumor suppressor function of pRb. It has been shown that pRb mediates cell cycle arrest also independent of its function to repress E2Fs, but through a mechanism by which pRb directly interacts with Skp2, and inhibits ubiquitylation of $p27^{Kip1}$. Intriguingly, full-penetrance mutations of *Rb* fail to repress both, E2F and Skp2 activities, whereas a naturally occurring partial-penetrance mutant, RbR661W, still inhibits cell cycle progression *via* controlling the SCF^{Skp2} activity despite impaired E2F repression. Together, available evidence strongly supports the notion that Skp2 is a growth promoter and an oncoprotein.

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Ubiquitination

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Definition

Is the covalent modification of a protein by conjugation to ►ubiquitin (Fig. 1). Ubiquitin is a small, 76-residue protein found in all eukaryotes. Conjugates are formed through the ligation of the C-terminus of ubiquitin to the ε-amino groups of protein lysine residues. The major, but not sole, function of ubiquitination is to target proteins for degradation by the ►proteasome. A large number of proteins are substrates for this regulatory pathway. Efficient targeting of proteins for degradation usually involves formation of a multiubiquitin chain on the target protein. Such chains are characterized by specific ubiquitin-ubiquitin linkages.

Characteristics

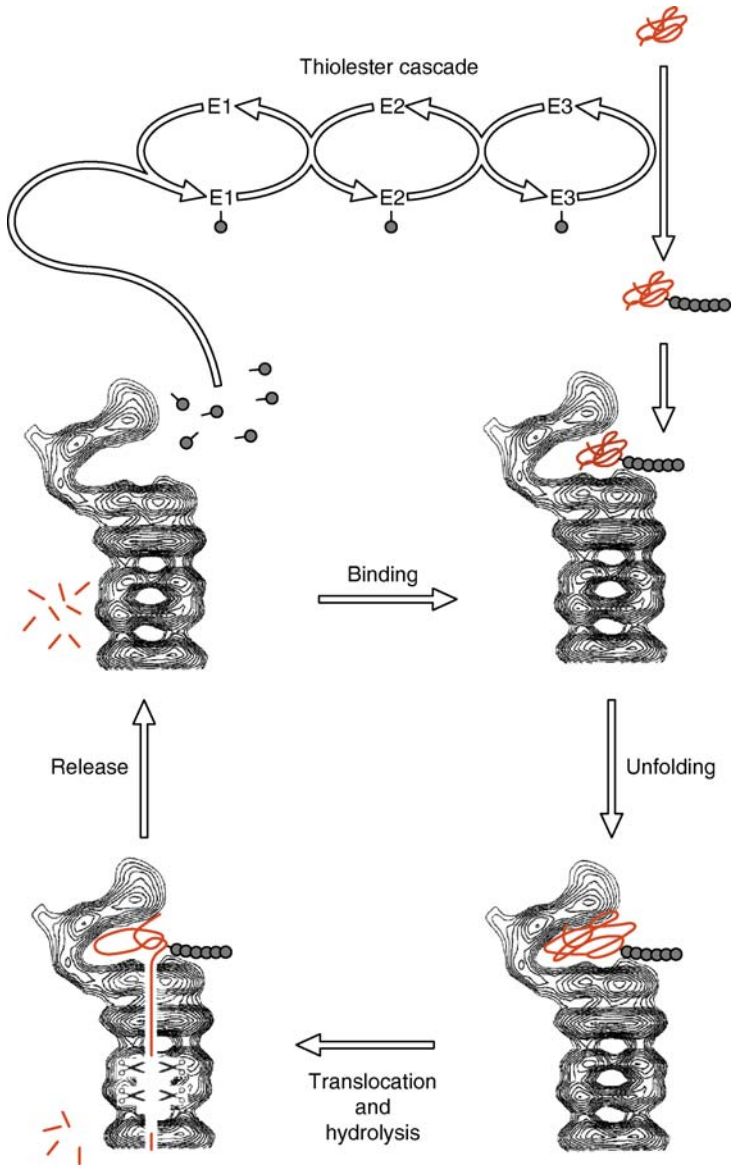
Intracellular proteins are degraded at rates that vary over 100,000-fold. While most proteins are relatively stable, critical regulatory proteins such as oncoproteins and tumor suppressor proteins are often short lived. Examples include ►p53, ►Mdm2, ►receptor tyrosine kinases, β-catenin (►E-cadherin), ►NFκB, ►Fos, ►Jun, cyclins (►cyclin D) and inhibitors of cyclin dependent kinases. The degradation of these proteins is subject to complex controls and is in many cases a dominant aspect of their regulation. For example, the cell cycle is driven principally by temporally controlled variations of cyclin levels, which are enforced by sudden periods of cyclin degradation. Exit from mitosis is marked by the deregulation of one cyclin, while other cell cycle transitions follow upon degradation of other cyclins and cyclin dependent kinase inhibitors.

Protein degradation has several advantages as a regulatory mechanism. As compared to regulated gene expression, it is capable of changing the level of a gene product very rapidly. Down-regulation by repressing gene transcription is very slow when the gene product is stable. As compared to phosphorylation, degradation allows a regulatory switch to function irreversibly. Regulation by degradation is a kind of ratchet mechanism, which in the case of the cell cycle or circadian cycle, for example, can prevent the possibility of reverse movement through the cycle.

Cellular Regulation

Two major systems for protein degradation have been identified in eukaryotic cells:

- The lysosomal pathway
- The ubiquitin-proteasome pathway



Ubiquitination. Figure 1 The proteasome cycle in protein degradation. Ubiquitin is shown as a large dot, and the peptidase sites are shown as scissors. For simplicity, the 26S particle is shown with a single regulatory particle and the 19S particle is shown without the protein mass that occupies the mouth of the wedge. Degradation of the substrate to oligopeptides is accompanied by the regeneration of free ubiquitin. The cycle begins when ubiquitin is activated at its carboxy terminus by the adenylyl transferase E1, then the activated ubiquitin is passed to an E2 enzyme. The E2 may the pass the activated, thiolester-bound ubiquitin to the active-site cysteine of an E3 enzyme (ubiquitin-protein ligase), as shown, or directly to substrate (not shown). For simplicity only one mechanism is shown. The former mechanism is exhibited by the HECT class of ligases and the latter by the RING class, which is the more common. The proteolytic substrate is linked to ubiquitin by an isopeptide bond. Multiple ubiquitin groups are added to the substrate sequentially to form a multiubiquitin chain, which finally targets the substrate for degradation (Figure taken from Ref. 4).

Short lived regulatory proteins, such as those discussed above, are degraded by the ubiquitin-proteasome pathway, with few exceptions. The ubiquitin-proteasome pathway is unusual in that it is a two-step pathway for protein turnover; attachment of the small protein ubiquitin to the substrate protein targets it to be recognized and degraded by the proteasome. Ubiquitin is attached to proteins via its C-terminus, which can form either an isopeptide bond with the ε-amino group of a protein lysine residue, or in rare

cases ubiquitin may be conjugated to the α-amino group at the N-terminus of the target protein. Efficient recognition by the proteasome requires multiple ubiquitin molecules to be ligated to the substrate, usually in the form of a multiubiquitin chain. Once bound to the target protein, ubiquitin itself becomes a preferential target of ubiquitination, and thus ubiquitin chains can be assembled on the substrate.

The ubiquitin-proteasome pathway is highly elaborate. The proteasome itself has over 30 subunits arranged in a remarkable self-compartmentalized structure. The active sites of the proteasome are thus inaccessible to the ubiquitinated substrate proteins unless the substrates are unfolded and translocated through an internal channel in the enzyme into its hydrolytic chamber for degradation. The initial attachment of ubiquitin to the substrate protein involves three classes of enzymes, known as E1, E2 and E3. E1 uses the energy of ATP to form a high energy thiolester linkage with the C-terminus of ubiquitin, thus "activating" ubiquitin for conjugation. E1 then transfers ubiquitin to any of a large number of E2 enzymes, which also bind ubiquitin covalently through a thiolester bond. Ubiquitin is finally donated to the substrate protein, either from the E2 enzyme or from the E3. In either case, the critical substrate recognition component of the machinery is generally the E3 enzyme. Many E3 enzymes are large protein complexes subjected to intricate controls. Substrate recognition by the E3 enzyme can be regulated by phosphorylation of either the substrate or E3 itself.

Ubiquitin conjugates are not always degraded by the proteasome. One alternative fate for such proteins is for the substrate-ubiquitin linkage to be reversed through the activity of any of a large family of ►de-ubiquitinating enzymes. De-ubiquitinating enzymes which probably number 75–100 in humans, can remove ubiquitin from ubiquitin-protein conjugates, thus sparing the protein from proteasomal degradation. These enzymes also break down abundant multi-ubiquitin chains that are not attached to any substrate, and produce mature ubiquitin from the precursor forms in which it is synthesized. A second alternative fate for ubiquitin-protein conjugates is that ubiquitinated cell surface proteins may be targeted for endocytosis and eventual degradation via the lysosome rather than the proteasome.

Still other deubiquitinating enzymes dismantle ubiquitin chains that, because they contain variant sites for ubiquitin-ubiquitin linkage, do not target their substrates for degradation, but rather perform other signaling roles. An example is the tumor-suppressor protein CYLD. Loss of function mutations in this deubiquitinating enzyme lead to multiple physiological defects, but notably inherited cancers of epidermal origin (cylindromatosis). The signal transducers NEMO and TRAF2 are prominent substrates of CYLD. Both NEMO and TRAF2 are positive regulators of the transcription factor NF-κB. Since signaling via NEMO and Traf2 is potentiated by attached ubiquitin chains, loss of CYLD results in enhanced signaling through the NF-κB pathway, which is largely proto-oncogenic.

The physiological importance of the ubiquitin pathway has only recently been recognized, and the field is now expanding rapidly. Cancer is one of the many diseases in which the ubiquitin pathway has been implicated, as might expected from a biochemical pathway that has myriad regulatory proteins as substrates. However, ubiquitination is particularly important for cancer because cancer results from aberrant growth regulation, and many growth regulatory factors are unstable substrates of the ubiquitin pathway. For example, the increased degradation rate of a particular tumor suppressor protein could confer a growth advantage on the affected cell over surrounding non-tumor cells. On the other hand, for oncoproteins whose degradation is perturbed, the opposite effect of stabilization and increased accumulation is generally observed. In each case, the physiological effect is to promote cell transformation. Some of the better established examples of the role of protein turnover in cancer are given below.

Clinical Relevance

p53 and HPV-Associated Cervical Carcinoma

p53 is a tumor suppressor protein, the levels of which rise rapidly after DNA damage. Increased p53 levels result in growth arrest or apoptosis. In normal cells, p53 is degraded through the ubiquitin-proteasome pathway and has a half-life of ∼30 min. p53 accumulation after DNA damage is thought to result principally from its stabilization. p53 is inactivated by mutation or constitutively destabilized in many transformed cells. One example of the latter is that of ►human papillomavirus (HPV)-associated anogenital carcinomas. Strains of HPV that confer high risk for cancer encode an E6 protein that targets p53 for degradation. Consequently, p53 levels are very low in cervical carcinomas associated with HPV. E6 acts in cooperation with the cellular factor known as E6-AP to bind p53. E6-AP is a member of a large class of E3 enzymes (the HECT domain E3′s) that are distinguished by the ability to form thiolester linkages with ubiquitin, in analogy to E2 enzymes. E6 acts as an ancillary factor to modify the specificity of E6-AP. It appears that the normal pathway of p53 turnover does not involve either E6-AP or a cellular homolog of E6. The oncoprotein Mdm-2, previously recognized as a negative regulator of p53, is apparently a ubiquitin ligase for p53. Mdm2 is overexpressed in many human soft tissue tumors, also occasionally in

►neuroblastomas, and elevated Mdm2 levels are associated with poor prognosis of several human cancers.

von Hippel Lindau (VHL) Disease

Like p53, the von Hippel Lindau (►VHL) gene is a ►tumor suppressor gene. In contrast to p53, however, the VHL protein is a component rather than a substrate of the ubiquitin-proteasome pathway. VHL disease affects 1 in 36,000 individuals and is characterized by a range of cancers, including (but not limited to) blood vessel tumors (hemangioblastomas) as well as tumors of the adrenal gland, kidneys, pancreas and lymphatic system. While the complete set of proteins that are stabilized when VHL activity is lost is not yet known, it appears that the major effect of the loss of VHL function is to potentiate angiogenesis and thus allow tumor outgrowth. VHL tumors are richly supplied with blood vessels due to their production of ►vascular endothelial growth factor (VEGF). VEGF is overproduced in these cells as a result of their expressing high levels of transcription factors for the VEGF gene, HIF-1α and HIF-2α. Under normal conditions these proteins are present at low levels as a result of their rapid degradation via the ubiquitin-proteasome pathway. However, under hypoxic conditions their degradation is inhibited, leading to VEGF induction. Even in the presence of normal oxygen levels, however, the HIF proteins are stabilized in VHL tumors. This stabilization is directly due to the loss of VHL in these tumors; the VHL protein binds HIF protein directly and functions as part of an E3 enzyme, the VBC complex, in the ubiquitination of HIF proteins. This complex is closely related to the SCF complex described below.

β-Catenin Turnover in Colorectal Tumors

Aberrant regulation of the Wingless/Wnt signal transduction pathway is a key event in the development of ►colon cancer. In the absence of the Wnt signal, ►glycogen synthase kinase-3β (GSK-3β) is active and phosphorylates β-catenin, causing it to be degraded. Upon Wnt signaling, phosphorylation of β-catenin is inhibited, and β-catenin accumulates and drives proliferation through the activation of downstream transcription factors. The ►APC (adenomatous polyposis coli) tumor suppressor promotes β-catenin degradation by assisting in its phosphorylation by GSK-3β. Among the most common genetic events leading to colorectal cancer are mutations in the APC gene.

The E3 enzyme that recognizes β-catenin and catalyzes its ubiquitination is known as β-TRCP. β-TRCP is an F-box protein and functions in the context of an SCF complex. In general, the key component in the SCF complexes is the F-box protein, which is the primary, if not always exclusive, mediator of substrate recognition. β-TRCP recognizes β-catenin only when it is phosphorylated by GSK-3β. In some tumors, β-catenin is found with substitutions in the serine residues, whose phosphorylation induces ubiquitination. These β-catenin mutants are stabilized as a result of their inability to be recognized by β-TRCP. Substrate recognition by F-box protein is often dependent on substrate phosphorylation.

Degradation of p27, a CDK Inhibitor

Cell-cycle progression is controlled through the activity of cyclin-dependent kinases (CDK enzymes). CDK activity is regulated by two major classes of factors; cyclins and CDK inhibitors. Both cyclins and CDK inhibitors are regulated through the ubiquitin-proteasome pathway (as well as by other mechanisms). Among CDK inhibitors, p27 has been found to be present at significantly reduced levels in common tumors, such as colorectal tumors and in breast cancer. The abundance of p27 is a valuable prognostic marker for epithelial cancers, brain tumors and malignant lymphomas. Reduction of p27 levels has been shown to be due to an increased rate of degradation in epithelial cancers, lymphomas and astrocytic brain tumors. It should be noted, however, that p27 mutations in human cancers are rare. The acceleration of p27 degradation may therefore reflect alterations of the ubiquitination machinery in tumor cells.

The mechanism of p27 ubiquitination is not fully understood, but recent data indicate the S-phase kinase-associated protein 2 (Skp2) and F-box protein functions as the E3 enzyme for p27, in the context of an SCF ubiquitination complex. Recognition of p27 by Skp2 requires phosphorylation of p27 at threonine-187. This phosphorylation event is catalyzed by specific CDK/cyclin complexes such as CDK2/cyclin E, the same kinase complex that p27 inhibits. Given that cyclin E levels fluctuate during the cell cycle, it is not surprising that degradation of p27 is normally under cell cycle control. The lowest rates of p27 phosphorylation and degradation are found during G1. Both p27 regulators, cyclin E and Skp2, are often found overexpressed in tumors.

Cbl and Receptor Tyrosine Kinase Degradation

c-Cbl is encoded by a ►proto-oncogene and negatively regulates signaling via cell surface receptor tyrosine kinases, such as receptors for ►platelet-derived growth factor, epidermal growth factor and colony-stimulating factor-1. Transforming variants of Cbl have been identified in transforming retroviruses and in pre-B cell lymphomas. These oncogenic forms of Cbl contain mutations affecting the RING finger motif of the protein (a zinc-chelating domain).

RING finger motifs have been implicated in the association of various proteins to E2 enzymes. As such,

the presence of a RING finger in a given protein could indicate that it functions as an E3, as an ancillary component of a ubiquitination complex, or even as a substrate for the E2 to which it binds. In the case of Cbl, the RING finger domain has been shown to mediate the association of Cbl to the E2 enzyme known as Ubc4, and to allow Cbl to function as an E3 for receptor tyrosine kinases. The RING finger is essential for ubiquitination and down-regulation of activated receptor proteins. Loss of the RING finger domain is presumably oncogenic because it results in an inactive and dominant negative form of Cbl.

Another domain in Cbl, the SH2 domain, is required for recognition of its substrates. In general, SH2 domains recognize phosphotyrosine residues that are produced upon ligand binding and receptor activation, and thus the involvement of an SH2 domain in receptor ubiquitination may explain the selectivity of down-regulation for active forms of receptor tyrosine kinases.

Fanconi Anemia

As mentioned above, ubiquitin modification can function nonproteolytically in some cases. Often this results from modification of a substrate with a single ubiquitin molecule. Such monoubiquitin modifications are apparently of widespread importance in the regulation of chromatin, in particular DNA repair and transcription. One such modification has been linked to ►Fanconi Anemia (FA), a rare inherited disease with hallmarks including genomic instability, high risk of cancer, and bone marrow failure. Most genes that when mutated can cause FA are involved in the monoubiquitination of chromatin proteins known Fanconi Anemia D2 (FANCD2) and its paralog FANCI. A complex multisubunit ubiquitin ligase is responsible for this ubiquitin event, which is stimulated by DNA damage and promotes proper repair of DNA crosslinks. How ubiquitination of FancD2 and FANCI promotes DNA repair is not yet clear.

Proteasome inhibitors

►Proteasome function is essential in cells, and strong proteasome inibition is unlikely to have any therapeautic utility. However, carefully graded doses of the proteasome inhibitor ►Bortezomib are effective against multiple myeloma in many patients. Bortezomib, also known as Velcade, has been approved by the FDA for this application, as well as for ►Mantle Cell Lymphoma, and is in widespread use in the US and tens of other countries at this time. Bortezomib is effective both in single-agent and combination therapies. Bortezomib inhibits the proteolytic active sites of the proteasome, within the hydrolytic chamber described above. There is considerable debate about why Bortezomib is so effective against certain cancers. Most likely a number of distinct physiological effects of proteasome inhibition underlie its anti-cancer activity.

Summary

Protein turnover pathways that are normally under tight regulation are converted by genetic defects to either constitutively active or inactive states. The protein turnover defects that can lead to cancer are various in nature. The primary defect can lie in:

- E3 enzymes, as in the case of Mdm2, Cbl and VHL
- An E3-associated ancillary factor, as in the case of E6
- A component that regulates phosphorylation, as in the case of APC
- The substrate itself, as for β-catenin

Given the large number of important substrates for the ubiquitin-proteasome pathway, it is likely that more examples of protein turnover defects underlying cancer will be found.

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Ubiquitous

Definition

Something with widespread distribution.

Ubiquitous Immunopoietc Polypeptide

Definition

Former name for ►Ubiquitin.

UCH

Definition

Ubiquitin C-Terminal Hydrolases. Another sub-class of enzymes that belongs to the de-ubiquitinases. UCHs are predominantly responsible for processing ubiquitin precursors that form as either poly-ubiquitin chains or fused to ribosomal precursors.

►HAUSP De-Ubiquitinase

UCN-01 Anticancer Drug

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Synonyms

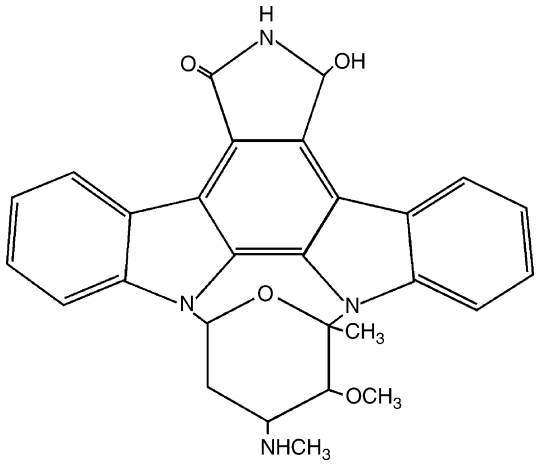
7-hydroxystaurosporine; NSC 638850

Definition

UCN-01 is an indolocarbazole compound originally isolated from *Steptomyces spec*. cultures, similar to ►staurosporine, but with an additional hydroxy group on the lactam ring (Fig. 1). It acts as an ATP competitive inhibitor targeting several kinases including protein kinase C (PKC), ►cyclin dependent kinases and ►Chk1 and shows high preclinical and clinical antitumor activity. UCN-01 can abrogate ►DNA damage induced cell cycle arrest in S and G2 phases of the cell cycle and thereby sensitize tumor cells to DNA damage-induced toxicity. Currently, several phase I/II clinical trials explore UCN-01 as a stand-alone therapy or in combination with various DNA damaging chemotherapeutic drugs.

Characteristics

UCN-01 was originally described as a potent inhibitor of ►protein kinase C (PKC). Its chemical name is: (3R, 8S, 9R, 10R, 12R)-3-hydroxy-9-methoxy-8-methyl-10-(methymamino)-8, 12.epoxy-2,3,9,10,11,12-hexahydro-1H, 8H-2, 7b, 12a-triazadibenzocyclononatrinden-1-one, $C_{28}H_{26}N_4O_4$. Since it acts as an ATP competitive inhibitor, it is not surprising that it is not specific for PKC, but inhibits several cellular protein kinases, although it exhibits a much greater selectivity as staurosporine. Nevertheless, UCN-01 has a high antitumor activity,



UCN-01 Anticancer Drug. Figure 1 The structure of UCN-01. UCN-01 is an indolocarbazole compound and derivative of staurosporine with an additional hydroxy group on the lactam ring.

inhibits growth and induces ►apoptosis in many cancer cells. These effects appear to be independent of PKC inhibition and are rather related to the inhibition of cell cycle kinases including cyclin dependent kinases (CDK), ►PDK1, which positively regulates the AKT/PKB survival pathway (►AKT Signal transduction pathway in oncogenesis) and the checkpoint kinase ►Chk1, which is inhibited by UCN-01 in a low nanomolar range.

Although efficient in several animal models, clinical pharmacology studies have revealed a low bioavailability of UCN-01 in humans, which is due to a high affinity to the α-1 acid glycoprotein in human plasma. Therefore, novel derivatives of UCN-01 with lower retention are currently being explored. However, recent clinical trials have demonstrated that despite the high binding capacity to human plasma proteins a schedule of 34 mg/m^2/d for 3 days is sufficient for antitumor activity.

UCN-01 has completed several phase I clinical trials in the United States and in Japan as a stand-alone therapy and phase II trials are currently underway to investigate the efficacy of UCN-01 in lymphomas (National Cancer Institute).

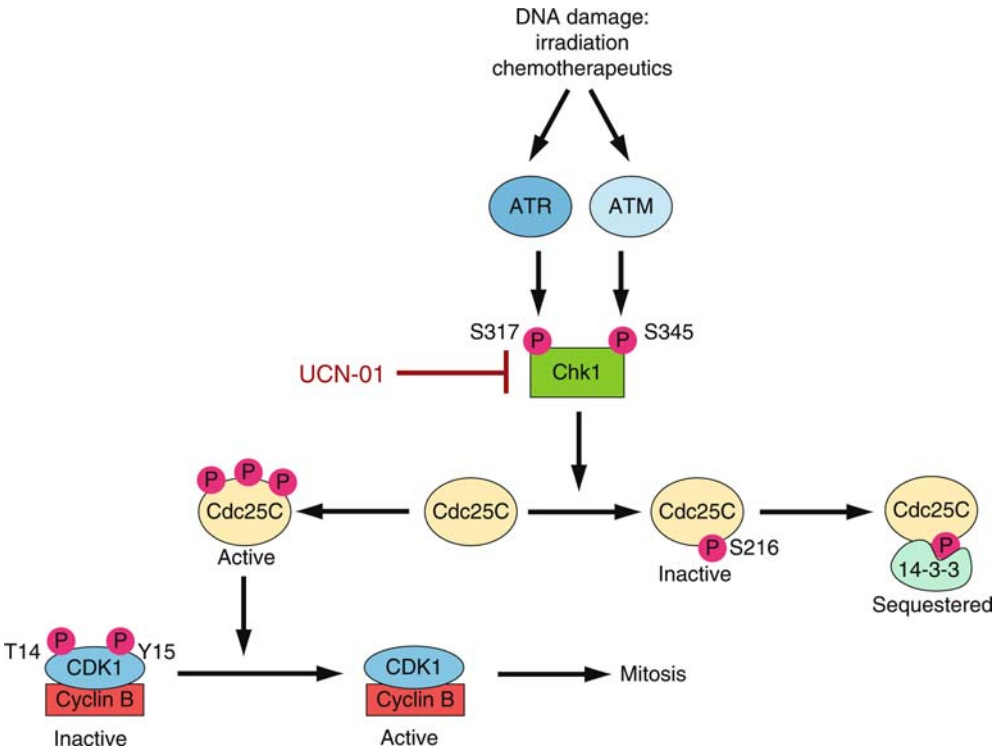
Inhibition of Chk1 Kinase and G2 Checkpoint Abrogation by UCN-01

An important cellular target of UCN-01 is the Chk1 kinase, which has an important function during a normal ►DNA replication. Recent results indicate that abrogation or inhibition of Chk1 results in replication stress followed by DNA damage, which might

contribute to the ability of UCN-01 to induce apoptosis as a stand-alone therapy.

The Chk1 kinase has also an important function in ►DNA damage response in human cells. Upon DNA damage, which is induced for instance by irradiation (►ionizing radiation therapy) or by treatment with ►platinum drugs (►Cisplatin) or topoisomerase inhibitors (e.g. etoposide, topotecan, doxorubicin), cells activate DNA damage checkpoints resulting in the halt of the cell cycle allowing DNA repair to occur. The first step of the DNA damage response is the recruitment of sensor complexes to the sites of DNA damage where the ATM/ATR (►ATM protein; ►ATM-related protein kinase) kinases are activated. These kinases can phosphorylate and activate either the transcription factor p53 (►P53 protein) directly or the Chk2 kinase, which in turn can phosphorylate and activate p53. The transcriptional activation of p53 results in an induction of its target gene *p21* (►p21 (WAF1/CIP1/SDI1), which encodes a CDK inhibitor protein that binds to and inhibits CDK-cyclin complexes in G1. This finally leads to a cell cycle arrest in G1 before cells enter S phase and is thus termed the G1 checkpoint. In addition, the ATM and ATR kinases can also phosphorylate and activate the Chk1 kinase, which is an essential component of the p53 independent G2 checkpoint (Fig. 2). Activated Chk1 kinase phosphorylates and inactivates the dual specificity phosphatase Cdc25C leading to its cytoplasmic sequestration. Cdc25C is the phosphatase responsible for removing two inhibitory phosphates from CDK1, which is required for activation of CDK1 and entry into mitosis. Thus, DNA damage induced inhibition of Cdc25C is mediated by Chk1 and prevents the entry into mitosis constituting the G2 DNA damage checkpoint.

Since many tumor cells lack functional p53 they activate only the G2 checkpoint in response to DNA damage, while p53 proficient cells predominantly arrest in G1, which still allows subsequent DNA repair. This circumstance gives rise to the therapeutic concept



UCN-01 Anticancer Drug. Figure 2 Inactivation of the G2-Checkpoint by UCN-01. In response to DNA damage, the ATM and ATR kinases are activated, which in turn phosphorylate and activate the Chk1 kinase. Chk1 phosphorylates the Cdc25C phosphatase on serine-216 and keeps it in an inactive state. Chk1 mediated phosphorylation of Cdc25C also facilitates the sequestration of Cdc25C into the cytoplasm by promoting complex formation with 14–3–3- proteins. Inactivation of Cdc25C prevents the dephosphorylation and activation of CDK1 at the G2/M transition and thus prevents entry into mitosis. Inhibition of Chk1 by UCN-01 in the presence of DNA damage results in an override of the G2 arrest and cells can enter mitosis in the presence of DNA damage.

of ►G2 checkpoint abrogation by small molecules (►Small molecule drugs), which is expected to target p53 deficient tumor cells selectively. UCN-01 is such a G2 checkpoint abrogator by potently inhibiting Chk1 (IC_{50} = 7–10 nM). Inhibition of Chk1 by UCN-01 in the presence of DNA damage results in an unscheduled activation of Cdc25C and of CDK1-cyclin B kinase and thus leads to entry into mitosis in the presence of DNA damage. The unscheduled entry into mitosis is associated with the induction of cell death, a process also known as "►mitotic catastrophe" or "mitotic cell death." Thereby, UCN-01 greatly enhances the efficacy of DNA damaging drugs selectively in p53 deficient tumor cells. Due to the promising preclinical results that support the concept of G2 checkpoint abrogation, several phase I and II clinical trials for leukemia, lung cancer and advanced solid tumors are now underway to explore the efficacy of UCN-01 in combination with various DNA damaging agents, including platinum compounds and topoisomerase inhibitors (National Cancer Institute, USA).

Mechanisms of UCN-01 Induced Tumor Cell Death

Entry into mitosis in the presence of DNA damage results in the induction of mitotic cell death, which significantly enhances the efficacy of DNA damaging drugs. Recent results have shed light on the mechanisms involved in mitotic cell death. UCN-01 mediated entry into mitosis leads to the activation of the mitotic ►spindle assembly checkpoint, which is responsible for a prolonged mitotic arrest. Subsequent to the activation of the spindle checkpoint, a mitotic form of apoptosis is initiated, which requires the function of the spindle checkpoint. Remarkably, the pro-apoptotic role of the mitotic spindle checkpoint is counteracted by the Aurora-B/►Survivin complex (►Aurora kinase), which possesses a pro-survival function during mitosis. Importantly, pharmacological inhibition of this pro-survival function by inhibitors of Aurora or CDK1 kinases greatly enhances the efficacy of UCN-01 mediated mitotic cell death. Due to this synergistic effect, clinical trials combining UCN-01 and Aurora or CDK1 kinase inhibitors are highly desired.

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UDP

Definition

Uridine diphosphate glucose (uracil-diphosphate glucose, UDP-glucose) is a nucleotide sugar. It is involved in glycosyltransferase reactions in metabolism.

UDP-Glucuronosyl Transferase

Definition

Refers to a family of enzymes that catalyses conjugation of UDP-glucuronic acid with ►xenobiotics containing an electron-rich nucleophilic heteroatom (O, N or S), such as those bearing aliphatic alcohols, phenols, carboxylic acids, aromatic and aliphatic amides, and free sulfhydryl groups.

►Detoxification

UGT

Definition

UDP-glucuronosyltranferase, is a family of drug-metabolizing enzymes. It catalyzes the glucuronidation of lipophilic ►xenobiotics and endobiotics, giving rise to normally less electrophilic, more water soluble, and more disposable products.

►Phase 2 Enzymes

Ulcerative Colitis

Definition

A heterogeneous, idiopathic, chronic, relapsing, inflammatory condition that is immunologically mediated. Disease activity is triggered by environmental factors that transiently break the colonic mucosal barrier, stimulate immune responses or alter the balance between

beneficial and pathogenic enteric bacteria. There is also a genetic component that can be broadly characterized as causing defects in mucosal barrier function, immunoregulation or bacterial clearance. Over time, there is an increased risk of ►colon cancer.

►Inflammation

Ultimate Carcinogen

Definition

Activated, and chemically reactive, form of a carcinogen or procarcinogen that is capable of direct covalent binding to nucleic acid and/or protein macromolecules. The form of a carcinogen that directly interacts with a cell constituent (presumably DNA) to initiate carcinogenesis.

►Carcinogen Macromolecular Adducts
►Toxicological Carcinogenesis

Ultrasound

Definition

Acoustic waves with frequencies greater than 20 kHz, *i.e.*, frequencies higher than sounds audible to humans. Medical ultrasound imaging systems transmit acoustic waves at frequencies greater than 1 MHz to form images of tissue structures that reflect the transmitted waves. In cancer, the most common applications are in diagnostic imaging to create images of internal anatomy, and in ►hyperthermia as a mechanism for heating tissue.

►Ultrasound Micro-Imaging

Ultrasound Biomicroscopy

►Ultrasound Micro-Imaging

Ultrasound Micro-Imaging

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Synonyms

High-frequency ultrasound imaging; Micro-ultrasound imaging; Ultrasound biomicroscopy

Definition

►Ultrasound micro-imaging is imaging of small animals using ultrasound instruments capable of resolving features in a mouse at a level of anatomical detail comparable to clinical ultrasonography of human patients.

Characteristics

Ultrasound micro-imaging systems are an example of technology for ►preclinical imaging of small animals in biomedical research. Ultrasound micro-imaging systems, while similar in design to conventional clinical ultrasound systems, are distinguished by their use of ultrasonic frequencies from 20 to 60 MHz, *i.e.*, frequencies above the 1–15 MHz band typically employed for clinical ultrasonography. The use of high frequencies enables images to be acquired with finer spatial resolution than clinical ultrasound images, but reduces the depth of penetration within the subject because soft tissues absorb ultrasound at a rate approximately proportional to frequency. A typical high-frequency ultrasound system provides spatial resolution on the order of 50–100 μm and penetration depths from 5 to 15 mm. Those penetration depths are suitable for most mouse imaging applications and many rat imaging applications.

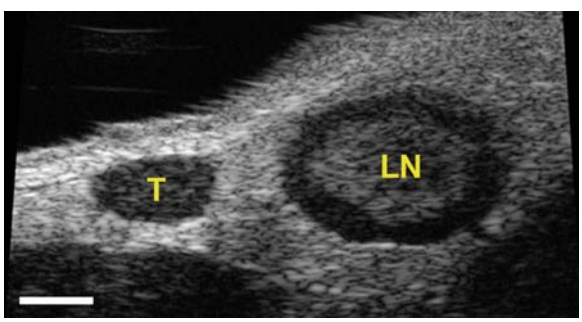
Preclinical imaging is a relatively new technology whose role in cancer research continues to expand due to the importance of ►mouse models in the discovery of biological mechanisms of human cancers and ►preclinical testing of new treatments. Preclinical imaging of small-animal cancer models can be performed using instruments analogous to any of the prominent medical imaging modalities, including x-ray computed tomography (CT), magnetic resonance imaging (MRI), radionuclide imaging, and ultrasound, as well as optical techniques such as ►bioluminescence imaging and optical coherence tomography. Preclinical imaging provides an alternative to research methods that require the use of subcutaneous tumor models with visible lesions or require animals to be sacrificed to obtain tissue specimens for analysis. Imaging facilitates evaluation of tumor progression and responses to

treatment in orthotopic (►Orthotopic model), metastatic, and ►genetically engineered models, where lesions develop in clinically relevant locations in internal organs. Imaging enables measurements to be made in live animals, which is valuable for studying the biological effects of dynamic processes such as blood flow. Imaging also permits data to be acquired from the same animal at multiple time points during an experiment, thereby reducing the number of animals needed for a study, which should accelerate and reduce the costs of developing new anticancer therapies.

Two- and Three-Dimensional Anatomical Imaging

Ultrasound micro-imaging is performed by positioning an ultrasound probe, which can be used while handheld or clamped to a support, against the skin of the mouse. The mouse is anesthetized during imaging so the animal will remain still. The probe transmits ultrasound pulses that reflect from tissue structures and the instrument detects the echoes returning to the probe. Images are displayed in shades of gray such that the brightness of a pixel represents the relative magnitude of the echo received from the corresponding location in the animal. Figure 1 shows an illustrative image of a murine breast cancer tumor and a nearby lymph node within a mammary fat pad. Ultrasound is best suited for imaging soft tissues, so, in addition to mammary fat pad tumors, ultrasound micro-imaging is typically used with subcutaneous or intradermal tumor models or tumors located at sites such as the abdominal organs or prostate.

Ultrasound micro-imaging systems are capable of two-dimensional (2-D) and three-dimensional (3-D) imaging. Two-dimensional imaging can be performed in real time, meaning at least 30 frames are acquired and displayed each second. In cancer studies, real-time imaging is valuable when a tumor is in a location such as the liver that experiences respiratory motion to prevent movement from degrading the images.



Ultrasound Micro-Imaging. Figure 1
Two-dimensional ultrasound micro-image of a murine mammary carcinoma tumor (T) and nearby lymph node (LN). Scale bar denotes 1 mm. (Image acquired by Graham KC and Wirtzfeld LA, University of Western Ontario.)

Ultrasound micro-imaging systems acquire 3-D images by mechanically sweeping the probe in the direction perpendicular to the 2-D imaging plane to acquire a set of parallel 2-D images that are combined to create a 3-D image. Acquisition of a 3-D image requires 20–30 s, but the image volume can be reconstructed as the planes are acquired and displayed immediately. This on-the-fly reconstruction is a significant advantage over other preclinical imaging technologies, which can require tens of minutes for 3-D image reconstruction.

One cancer research application of anatomical imaging is screening animals for tumor development. An investigator can thereby ensure that all mice receiving an experimental treatment actually possess tumors and that the treatment is administered when the tumors are at similar stages. Ultrasound's rapid acquisition and immediate display of images is beneficial because the presence or absence tumors can be determined in a few minutes, thereby permitting many animals to be screened in a reasonable amount of imaging time.

Imaging tumors repeatedly over the course of a study to measure their growth is a second important use of anatomical imaging. Delays in tumor growth and changes in the rate of growth are commonly used preclinical measures of treatment response. Tumor sizes can be determined in a variety of anatomical sites using 2-D or 3-D ultrasound micro-imaging. If 2-D imaging is used, the diameter of the tumor is measured along the long and short axes of the largest cross section through the tumor, and those diameters can be used to estimate tumor volume. Alternatively, the tumor can be outlined in parallel planes through a 3-D image and the total volume enclosed by those boundaries can be determined. Ultrasound is nonionizing and repeated exposure carries no risk to the animal, so it is an attractive technology for treatment studies that require repeated longitudinal measurements of tumor size.

Ultrasound possesses some disadvantages that make the technology inappropriate for some applications. Ultrasound waves traveling through tissue are strongly reflected by bone and by pockets of air, so ultrasound is not effective for imaging bony or air-filled organs. Ultrasound micro-imaging is therefore inappropriate for studies using brain, skeletal, or lung tumor models. Second, the field of view of an ultrasound micro-image is typically 10–15 mm along each side, which is much smaller than the area that can be depicted in an x-ray micro-CT or high-field MRI image. Ultrasound micro-imaging is therefore not an efficient method of searching for ►metastases if lesions are expected to be distributed throughout the animal's body. Third, acquisition of satisfactory images depends on the skill of the ultrasound system operator and the images can be challenging to interpret. For example, the accuracy of

tumor volumes estimated from 2-D images depends on the operator's ability to identify and image the plane containing the largest tumor cross section. Three-dimensional imaging ensures that the entire tumor is measured, but a significant amount of the investigator's time must then be invested in outlining the tumor because automated image analysis algorithms tend to be unreliable for identifying tumor boundaries in ultrasound images.

Doppler Blood Flow Imaging

Preclinical cancer studies often require information about the distribution of blood flow in different regions of a tumor or changes in vascularization as a tumor progresses or responds to treatment with an ►anti-angiogenesis or vascular disrupting agent. ►Doppler ultrasound is an attractive technology for imaging tumor blood flow because Doppler images are produced from echoes received from moving blood cells, which enables blood flow to be visualized without using injected ►contrast agents. Doppler micro-imaging systems provide sufficient spatial resolution to distinguish adjacent vessels separated by 100–200 μm, which is the spatial scale needed to depict tumor microvasculature. However, as in anatomical imaging, this resolution comes at the cost of reduced penetration depth. Furthermore, Doppler systems ignore echoes suggesting flow at velocities slower than a few millimeters per second to prevent the images from being overwhelmed by artifacts arising from tissue motion. Doppler micro-imaging is therefore more effective for imaging vessels 100 μm diameter and larger than for imaging microvessels, which tend to exhibit slower blood velocities.

Two methods for displaying 2-D or 3-D images of tumor blood flow are widely used. In the first method, called color Doppler imaging, pixels at locations where flow is detected are color-coded to represent the mean blood velocity averaged over the resolution volume. Color Doppler images enable discrimination between regions of slow and fast flow and between arterial and venous flow. However, accurate estimation of flow velocities requires knowledge of the angle between the flow direction and the ultrasound beam, which can be difficult to determine in the tortuous vessels frequently associated with tumors.

A second approach to blood flow imaging, termed power Doppler, color-codes images to indicate the total ultrasound power reflected from moving blood cells. Power Doppler images display a quantity proportional to the number of moving blood cells, rather than their velocity, at each point in the image. This approach avoids the angle dependence of the Doppler velocity measurement and reduces the sensitivity of the measurement to noise in the image. The reduced noise sensitivity can be beneficial when the echoes received from the blood are relatively weak, which is often the case when imaging tumor vasculature.

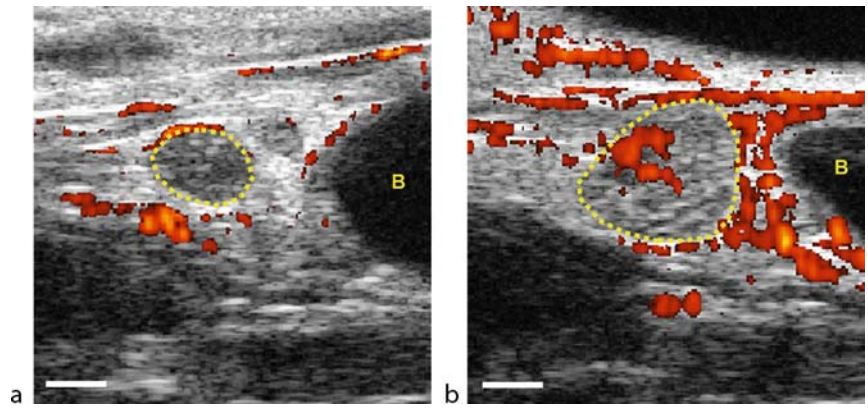
Most cancer research applications require a method of quantifying the vascularity depicted in color or power Doppler images so changes in blood flow can be assessed objectively. Color pixel density is an example of an uncomplicated but useful Doppler vascularity metric. Color pixel density is equal to the fraction of pixels within a region of interest that exhibit detectable flow. The region of interest is usually defined manually by the investigator and may encompass the entire tumor, the core or margin of a tumor only, or a nearby region of normal tissue. A change in color pixel density provides evidence of a change in tumor vascularity. For example, Fig. 2 compares power Doppler images of a genetically engineered mouse prostate cancer model acquired a few days before and during a period of rapid tumor growth. The color pixel density measured over the entire tumor volume increased from 0.7% at the first time point shown (Fig. 2a) to 30.9% at the second time point (Fig. 2b).

Contrast-Enhanced Imaging

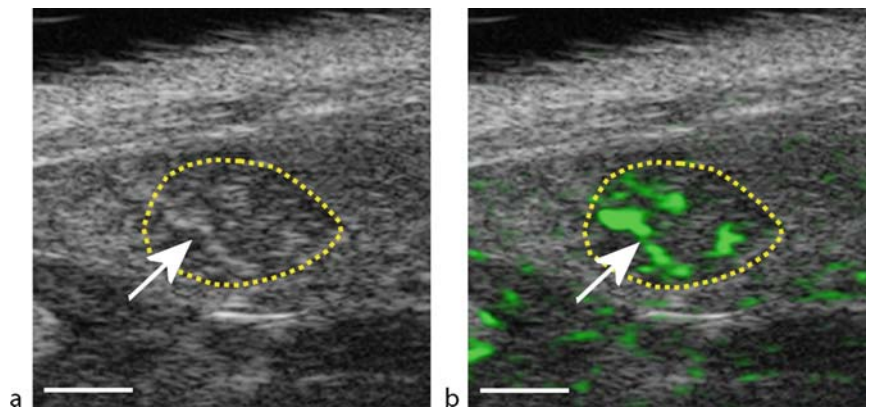
►Microbubble contrast agents provide another means of imaging tumor vascularity using ultrasound. Microbubbles injected intravenously circulate with the blood and increase the magnitude of echoes received from the blood pool. A single microbubble produces echoes several orders of magnitude more powerful than an echo from a single blood cell. Furthermore, scattering of ultrasound from microbubbles includes significant nonlinearity that permits microbubble echoes to be distinguished from tissue echoes using specialized signal processing. These aspects of microbubble physics improve the ability of ultrasound to detect the very slow flow characteristic of tumor vessels. This capability is obtained at the expense of increased experimental complexity, because administration of microbubbles to a mouse involves a tail vein cannulation or similar procedure.

Microbubbles can be used to enhance anatomical or Doppler images, or images can be colored to highlight locations where microbubbles are detected. Figure 3 shows a comparison of the first and third methods of displaying a contrast-enhanced image. Microbubbles appear as bright features in anatomical images, and the path of a microbubble along a vessel can be followed visually when real-time imaging is performed.

Quantification of contrast-enhanced images can be performed to analyze the spatial distribution of microbubbles (and, hence, blood flow) or to analyze the timing of contrast enhancement after injection of a microbubble bolus. The former type of quantification provides a measure of tumor vascularity similar to the Doppler color pixel density. Evaluating the timing of enhancement indicates whether a tumor receives a



Ultrasound Micro-Imaging. Figure 2 Two-dimensional power Doppler micro-images of a transgenic mouse prostate cancer model acquired in a sagittal plane through the bladder (B). Pixels where flow is detected are highlighted with an orange-yellow color scale, with brighter colors indicating regions where higher echo powers were received from moving blood. (a) Image acquired when the tumor (dotted yellow outline) was small and growing slowly. (b) Same tumor imaged 18 days later during a period of rapid growth. Scale bars denote 1 mm. (Figure Adapted from Xuan JW, Bygrave M, Jiang H et al (2007) cancer Res 67:2830–2839.)



Ultrasound Micro-Imaging. Figure 3 Contrast enhanced ultrasound micro-images of an experimental murine liver metastasis (dotted yellow outlines) model. (a) Microbubble contrast agents appear as moderately bright features in a gray-scale image. (b) Same 2-D image with green highlighting indicating regions where microbubbles were detected. A vessel running through the tumor (arrow) is evident in both images. Scale bar denotes 1 mm. (Image acquired by Graham KC, Mackenzie LT, and Wirtzfeld LA, University of Western Ontario.)

majority of its blood supply from the arterial or venous side of the circulation, which is, for example, an important aspect of the physiology of liver tumors. Microbubbles can also be used to perform relative measurements of perfusion by injecting a bolus of microbubbles, waiting a few minutes until the distribution of circulating agents reaches a steady state, temporarily increasing the transmitted power to burst all of the microbubbles within the 2-D image plane, and then reducing the ultrasound power to normal levels and measuring the time required for contrast enhancement to return to steady state. Parameters proportional to the blood volume, volume flow rate, and perfusion (volume flow rate divided by tissue mass) within a region of interest can be estimated from measurements of the change in ultrasound signal intensity as a function of time.

Microbubbles also enable ►molecular imaging using ultrasound. Ultrasound molecular imaging is performed by either modifying the shell composition or conjugating ligands to the shell of the microbubbles to create agents that are targeted to endothelial cell receptors specific to

a biological process. Imaging bound microbubbles provides an *in vivo* assay for the targeted receptor. In a cancer study, the agents are typically targeted to an ►angiogenesis marker such as a ►vascular endothelial growth factor or to a potential site of drug activity. Preclinical studies may be an effective means of developing targeted microbubbles for clinical use, assuming the receptors identified in mouse models are also expressed in humans.

Targeted microbubbles can also be formulated with a therapeutic agent incorporated in the shell or contained in the core of the agent. The therapeutic cargo can be released by bursting microbubbles with high-power ultrasound pulses. This technique is an attractive method of ►targeted drug delivery because ultrasound images can first be acquired to confirm that the tumor has bound the targeted agent, and then high-power pulses can be focused on the tumor only, thus reducing toxicity to healthy tissues by limiting the release of the drug to the immediate vicinity of the tumor.

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Ultratrace Minerals

Definition

►Trace Elements

Ultraviolet Light

Definition

UV light is that portion of sunlight in the 100–400 nm range. Sunlight contains three types of UV light: UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). UVC rays are highly mutagenic but are blocked from reaching the planet's surface by the ozone layer surrounding the earth. Thus, only UVA and UVB rays reach the planet's surface and are responsible for essentially all nonmelanoma skin cancer. However, in areas without an ozone layer, UVC rays may reach the earth's surface and contribute to ►skin carcinogenesis.

►Photochemoprevention
►Solar Ultraviolet Light
►UV Radiation
►Photocascinogenesis

Ultraviolet Radiation

Definition

►UV radiation is electromagnetic radiation with a wavelength between 100 and 400 nm.

►Xeroderma Pigmentosum

Ultraviolet Radiation Induced Cancer

►Photocarcinogenesis

Umbilical Cord Blood

Definition

Cord blood is a rich source of primitive hematopoietic ►stem cells that may be useful for cell therapies for a variety of diseases. Such cells may be as versatile as embryonic stem cells and it is becoming very popular to store newborn cord blood in a cord blood bank. The connective tissue surrounding the cord (Wharton jelly) is a rich source of mesenchymal stem cells.

►Stem Cell Plasticity

Uncertain or Unknown Histogenesis Tumors

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Definition

Tumors of unknown histogenesis are those in which the normal cell counterpart has not as yet been identified. Recent advances in the molecular understanding of cancer have led to classification of these tumors in terms of differentiation, regardless of their location and the presumed cell of origin.

Characteristics

Tumors are classified as epithelial, mesenchymal, lymphoid or neuroectodermal depending on their cell of origin. Most tumors show the histopathological features of their normal cellular counterpart and can be classified accordingly. Currently, pathologists are attempting to classify tumors according to their patterns of gene expression and markers of differentiation. An important related discovery is the so-called cancer stem cell, which has the potential to self-renew and form additional cancer cells of a similar phenotype, and to give rise to phenotypically diverse cancer cells. These cancer cells can arise from mutational transformation of normal stem cells or from more differentiated cells that acquire the properties of cancer stem cells, such as self-renewal potential. This origin could explain the heterogeneity observed in tumors, as well as the diverse phenotypic patterns shown in some tumors.

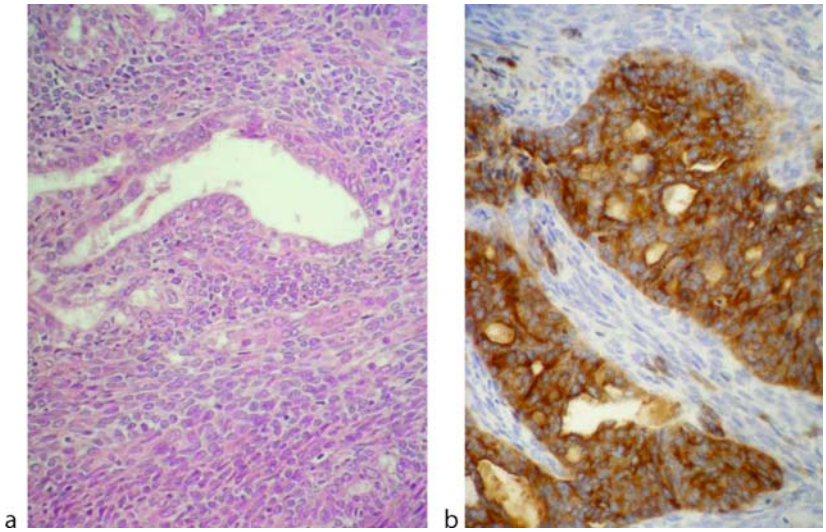
In the majority of carcinomas, lymphomas, glial tumors and sarcomas, tumor cells show histopathological or genetic patterns of differentiation according to their location and normal cellular counterparts. Nevertheless, in approximately twenty types of tumors a normal cellular counterpart has not been identified, even though the tumor may show a clear line of differentiation. Most of these tumors arise in mesenchymal tissues and show mesenchymal, epithelial or even neuroectodermal markers of gene expression. These are known as tumors of uncertain histogenesis.

Many different tumors are included in the group of unknown histogenesis (Table 1). These can have a benign or malignant pattern, and several clinical and pathological characteristics. The following are the most important clinically: ►synovial sarcoma, ►epithelioid sarcoma, ►alveolar soft part sarcoma, ►clear cell sarcoma, ►extraskeletal myxoid chondrosarcoma, ►desmoplastic small round cell tumor, ►extrarenal rhabdoid tumor and ►neoplasms with perivascular epithelioid cell differentiation (PEComas). ►Myxoid tumors constitute a heterogeneous group with peculiar pathological features and clinical settings. ►Ewing tumors are now considered peripheral neuroectodermal tumors with a characteristic chromosomal translocation and varying degree of neuronal differentiation.

In brief, tumors of uncertain histogenesis harbor specific genetic alterations, usually chromosomal translocations, and their molecular signature is essential to establish the diagnosis. It is believed that these

Uncertain or Unknown Histogenesis Tumors. Table 1 Tumors of uncertain differentiation (OMS)

Benign	Intermediate (rarely metastasizing)	Malignant
Myxomas (intramuscular, juxta-articular and deep "aggressive" angiomyxoma)	Angiomatoid fibrous histiocytoma	Synovial sarcoma
Pleomorphic hyalinizing angiectatic tumor	Ossifying fibromyxoid tumor	Epithelioid sarcoma
Ectopic hamartomatous thymoma	Mixed tumor/ myoepithelioma/ parachordoma	Alveolar soft part sarcoma
		Clear cell sarcoma of soft tissue
		Extraskeletal myxoid chondrosarcoma
		PNET/Extraskeletal Ewing tumor
		Desmoplastic small round cell tumor
		Extra-renal rhabdoid tumor
		Malignant mesenchymoma
		Neoplasms with perivascular epithelioid cell differentiation (PEComa)
		Intimal sarcoma



Uncertain or Unknown Histogenesis Tumors. Figure 1 (a) Biphasic synovial sarcoma with glandular and spindle cell component (HE, ×250); (b) Strong immunoreactivity of the glandular component of the tumor for cytokeratins (×250).

tumors arise from mesenchymal stem cells or multipotential cells and that specific genetic alterations can induce the peculiar histopathological characteristics of each tumor. Hence, we now think in terms of lines of differentiation, which are determined by patterns of gene expression. In some neoplasms we can identify a line of differentiation, but we are unable to define a cellular counterpart among normal mesenchymal tissues.

Distinctive Tumors of Uncertain Histogenesis

Synovial Sarcoma

Synovial sarcoma (SS) is a mesenchymal spindle cell tumor that displays variable epithelial differentiation, including glandular formation. ►SS accounts for 5–10% of soft tissue sarcomas, and 90% of cases occur before the age of 50 years. These tumors can arise at any site, although about 80% of cases originate in the deep soft tissues of the extremities, particularly around the knees. The tumors display mesenchymal and epithelial markers and have a specific chromosomal translocation t(X; 18) (p11; q11). Five-year survival is around 40–70% (Fig. 1).

Epithelioid Sarcoma

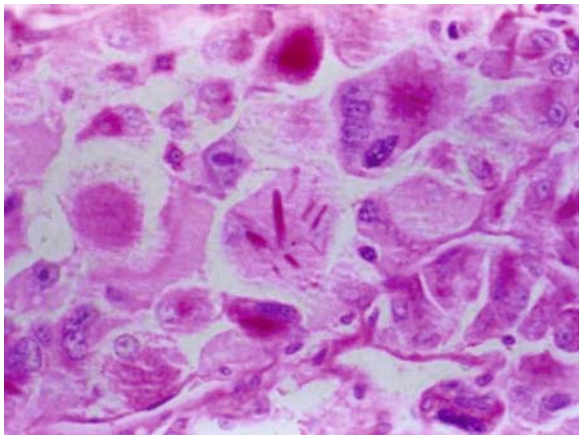
►Epithelioid sarcoma (ES) is a distinctive sarcoma of unknown lineage showing predominantly epithelioid cytomorphology, and affecting mainly adolescents and young adults. ►ES arises more frequently in the distal extremities, particularly the fingers, hands, forearms, knees and lower legs. Histopathologically, ES has a pseudogranulomatous appearance with a nodular growth pattern and mixed proliferation of eosinophilic epithelioid and spindle cells. These tumors are positive for epithelial markers and, in half of the cases, for CD4. The overall recurrence rate is around 80% at 10 years, and 5-year survival rates range from 50 to 80%, with an unpredictable clinical course. Moreover, there is a much more aggressive subtype called proximal-type ►epithelioid sarcoma that arises in the pelvis.

Alveolar Soft Part Sarcoma

►Alveolar soft sarcoma (ASPS) is a rare tumor affecting mainly adolescents and young adults. It is composed of large, uniform, epithelioid cells having abundant eosinophilic, granular cytoplasm arranged in solid nests and/or alveolar structures, separated by thin, sinusoidal vessels. ►ASPS is a rare tumor with a reported frequency of less than 1% of all soft tissue sarcomas. It can occur at any age, but is most common between 15 and 35 years. ASPS usually presents as a slow-growing lesion. Early metastasis is a characteristic feature of this tumor. Histopathologically, the large round or polygonal cells show light atypia and contain rod-shaped crystalline inclusions. Immunohistochemically, no consistent findings or markers have been described although muscle markers have been observed in some cases. In cytogenetics, these tumors harbor genetic alterations such as del (17), and t(x;17) (p11;q25) The 5-year survival rate is around 60% (Fig. 2).

Clear Cell Sarcoma of Soft Tissue

►Clear cell sarcoma (CCS) is a soft tissue sarcoma of young adults with melanocytic differentiation, typically involving tendons and aponeuroses. These rare tumors



Uncertain or Unknown Histogenesis Tumors.
Figure 2 Alveolar soft part sarcoma: PAS stain with diastase digestion demonstrates the presence of crystalline inclusions in the cytoplasm of tumor cells (PAS, ×600).

usually affect young adults, with a peak incidence in the third and fourth decade. The tumor usually presents as a slowly growing mass, being present for several weeks to several years. Histopathologically, the tumor displays a typical uniform, nested to fascicular growth pattern. The cells are epithelioid or spindle with a typical vesicular nuclei and prominent nucleolus. Tumor cells are positive for melanin stains in about 50% cases. The cytogenetic hallmark of this tumor is the presence of a t(12;22) (q13;q12) translocation. The 5-year survival is about 65% and 20-year survival only 10%.

Extraskeletal Myxoid Chondrosarcoma

►Extraskeletal myxoid chondrosarcoma (EMC) is a malignant soft tissue tumor characterized by multinodular architecture, an abundant myxoid matrix, and malignant chondroblast-like cells arranged in cords, clusters, or delicate networks. These tumors account for less than 3% of soft tissue sarcomas and mainly affect adults over 50 years of age. The thigh is the most common location. The tumors form cords or clusters and display an epithelial appearance with abundant eosinophilic cytoplasm, Despite the name, there is no convincing evidence of cartilaginous differentiation. The only marker found in all cases is vimentin. The t(9;22)(q22;q12) translocation is seen in around half of cases. Five-year survival is over 90%, although the tumor has a high potential for local recurrence and metastasis.

Desmoplastic Small Round Cell Tumor

►Desmoplastic small round cell tumor (DSRCT) is composed of small round tumor cells associated with prominent stromal desmoplasia and polyphenotypic differentiation. ►DSRCT primarily affects children and young adults, usually with abdominal serosal involvement. There is a male predominance. Histopathlogically, the tumor cells are small and arranged in nests. Immunohistochemistry can show multi-phenotypic differentiation with positive epithelial, mesenchymal, muscle, and even neuroectodermal markers. The t(11;22) (p13;q12) translocation is a consistent cytogenetic feature. Five-year survival is less than 20%.

Extrarenal Rhabdoid Tumor

►Soft tissue rhabdoid tumor (RT) is a malignant tumor of infants and children, characterized by neoplastic cells with large nuclei, prominent nucleoli, and abundant eccentric cytoplasm with variably prominent eosinophilic cytoplasmic "inclusions." RT can arise in the liver, heart, gastrointestinal system or soft tissues. The rhabdoid cell co-expresses mesenchymal and epithelial markers and, in many cases, neuroectodermal markers. Mutations, deletions or translocations of chromosome 22 involving the SMARCB1 gene are characteristic. Five-year survival is dismal. Since a rhabdoid phenotype can be present in a wide spectrum of tumors, particularly those occurring in adults, the diagnosis of rhabdoid tumor requires exclusion of underlying alternative lines of differentiation.

Neoplasms with Perivascular Epithelioid Cell Differentiation (Pecomas)

►Neoplasms with perivascular epithelioid cell differentiation (PEComas) are mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells. The ►PEComa family of tumors includes angiomyolipoma (AML), clear cell "sugar" tumor of the lung (CCST), lymphangioleiomyomatosis (LAM), clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres (CCMMT), and unusual clear cell tumors of the pancreas, rectum, abdominal serosa, uterus, vulva, thigh, and heart. Histopathologically, these tumors show epithelioid and spindle cells with clear or lightly eosinophilic cytoplasm surrounded by small arcing vessels. They are immunohistochemically positive for melanocytic and muscle markers. A consistent genetic alteration has not as yet been described in the few cases studied. Most PEComas have a relatively benign prognosis but malignant PEComas with aggressive clinical progression have been reported.

Ewing Sarcoma/Primitive Neuroectodermal Tumor (Pnet)

►Ewing sarcoma and ►PNET are defined as round cell sarcomas that show varying degrees of neuroectodermal differentiation. The term Ewing sarcoma has been used for tumors that lack evidence of neuroectodermal differentiation as assessed by light microscopy,

immunohistochemistry, and electron microscopy, whereas the term PNET has been applied to tumors that demonstrate neuroectodermal features. ►Ewing sarcoma/primitive neuroectodermal tumor (PNET) is relatively uncommon, accounting for 6–8% of primary sarcomas. It is the second most common bone and soft tissue sarcoma in children. Most cases arise in patients younger than 20 years. Histopathologically, Ewing tumors are composed of small round cells and are positive for neuroectodermal markers such as CD99. In cytogenetics, this family of tumors is characterized by a distinctive t(11;22)(q24;q12) chromosomal translocation in over 85% of cases. Other translocations are also described and interestingly, the prognosis differs according to the type of translocation (see Ewing tumor chapter).

Capillary Hemangioblastoma

►Capillary hemangioblastoma (CH) is a low-grade (WHO grade I) tumor of uncertain histogenesis, composed of stromal cells and abundant capillaries. ►CH occurs predominantly in the cerebellum, although multiple hemangioblastomas associated with Von-Hippel Lindau (VHL) disease arise at any site in the central nervous system. Approximately 25% of hemangioblastomas are associated with Von Hippel Lindau disease. The tumor cells are vacuolated and are negative for most of the markers studied, including endothelial markers. CH are well-circumscribed tumors; the prognosis depends on the surgical excision and the location.

Angiomatoid Fibrous Histiocytoma

A rare tumor (0.3% of soft tissue tumors), also known as angiomatoid malignant fibrous histiocytoma, which generally affects the deep dermis and subcutis of the extremities in children and young adults, simulating a hematoma. These tumors show multinodular proliferation of epithelioid or spindled eosinophilic cells (myoid cells), pseudoangiomatoid spaces, a thick fibrous capsule, and a pericapsular lymphoplasmacytic infiltrate. Cellular pleomorphism and increased mitotic activity have been identified, particularly in spindled tumors. Fifty percent of cases express desmin and/or EMA. These tumors have an indolent behavior with some local recurrence (10%) and a low metastatic potential (1%), generally to the regional lymph nodes.

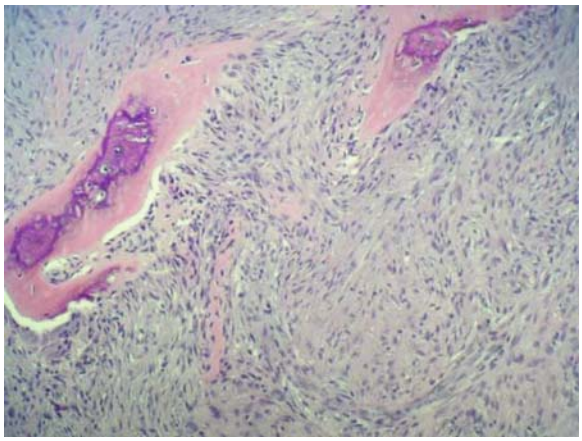
Myxoid Tumors

Within the large variety of predominantly ►myxoid tumors with peculiar clinico-pathological characteristics, we mention some having a generally benign pattern, with a tendency for local recurrence but no metastatic potential. Among these, there is intramuscular myxoma (IMM), which arises mainly in women (40–70 years) and has an excellent prognosis. Some IMM with hypercellular areas are defined as "cellular myxomas." The so-called Juxta-articular Myxoma has histological features similar to cellular myxoma; the majority occur in the vicinity of the large joints. Another myxoid tumor is the Deep "Aggressive" Angiomyxoma, which has a predilection for the pelvic and perirenal regions. And lastly, there is the Ossifying Fibromyxoid Tumor, which arises in the extremities in adults and occasionally acquires a malignant phenotype. It is important to identify myxoid tumors and differentiate them from malignant myxofibrosarcomas, a group of malignant fibrohistiocytic lesions with variable myxoid stroma and cellular pleomorphism that has a much poorer prognosis (Fig. 3).

Other (Benign) Tumors

Some rare, slow growing tumors of uncertain differentiation with tendency to local recurrence, but no metastatic potential, have been described. Pleomorphic hyalinizing angiectatic tumor of the soft parts (PHAT) occurs in adults and arises in the subcutis of the lower extremities. It is characterized by clusters of ectatic thin-walled vessels surrounded by spindled pleomorphic neoplastic stroma with a variable inflammatory component. The tumor cells occasionally express CD34.

Mixed tumor/myoepithelioma/parachordoma form a recently described group of benign tumors that arise in the subcutaneous or subfascial soft tissues of the extremities. Mixed tumors are similar to their salivary gland counterparts, with uniform epithelioid cells in a chondromyxoid stroma. Myoepitheliomas differ from mixed tumors in that they typically lack a definite ductal component. Parachordomas resemble mixed tumors, with the exception that there is prominent cytoplasmic vacuolation. All three tumors express myoepithelial markers: cytokeratin, vimentin and S 100 protein.



Uncertain or Unknown Histogenesis Tumors.
Figure 3 Ossifying fibromyxoid tumor showing fusiform cells arranged in nest and cords set in fibromyxoid stroma and surrounded by incomplete metaplastic lamellar bone (H\E, ×200).

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Uncoupling Protein-1

Definition

UCP-1; A unique mitochondrial protein of brown adipose tissue, diverts energy from ATP synthesis to thermogenesis in the mitochondria by catalyzing a regulated leak of protons across the inner membrane.

►Cachexia

Unfolded Domain

►Intrinsically Unstructured Proteins

Unfolded Protein Response

Definition

UPR; Is an integrated intracellular signaling pathway that transmits information about the protein folding status in the endoplasmic reticulum lumen to the cytoplasm and the nucleus.

►Anoxia

Unipotent Stem Cells

Definition

Cells that can only produce one cell type.

►Adult Stem Cells

Unrip: Unr-interacting Protein

►Serine-Threonine Kinase Receptor-Associated Protein

Unstructured Domain

►Intrinsically Unstructured Proteins

Untranslated Region

5' UTR is the portion of an mRNA from the 5' end to the position of the first codon used in translation. The 3' untranslated region is the portion of an mRNA from the position of the last codon used in translation to the 3' end of the mRNA.

uPA

Definition:

►Urokinase-type Plasminogen Activator – a serine protease.

►Maspin
►Plasminogen Activating Systems
►Proteinase-activated Receptors

p53 Upregulated Modulator of Apoptosis

Definition

►PUMA.

Uranium

Definition

A radioactive chemical element which is important for the nuclear fuel cycle and for atomic weapons. Naturally, two isotopes occur: 99.3% is ^{238}U and 0.7% is ^{235}U.

►Uranium Miners

Uranium Miners

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Definition

In a very strict sense, uranium miners are those involved in uranium production, either in underground or in open pit mining. Further more, the term includes individuals in milling and processing, and in an even broader sense those persons who are employed by the mining company but working in unexposed areas.

The most important carcinogen to which uranium miners are exposed to is the radioactive noble gas radon and its progeny (henceforth called radon). Radon itself is a daughter product of uranium decay. And since uranium is not only part of those geological formations containing this metal in a content rich enough for mining but also in other geological environments, the term "uranium miners" does sometimes not only include miners working in uranium mines, but also miners exposed to elevated levels of radon.

The miners' exposure to radon is given in Working Level Months (WLM). A Working Level (WL) is defined as 1.3×10^5 MeV of potential alpha energy/L air. A Working Level Month equals an exposure to 1 WL for 170 h.

Characteristics

Uranium Mining

Uranium is needed for the nuclear fuel cycle and for the production of nuclear weapons. In the early years of large scale uranium mining it was mainly produced for developing and building nuclear weapons. Large scale uranium mining started in the mid 1940s. Before that, uranium production was low and the metal was used for other purposes.

In 2005, the largest share of uranium from mines came from Canada (28%), followed by Australia (23%). In terms of produced uranium, these countries are followed by Kazakhstan, Russia, Namibia, Niger, and 11 more countries. Overall, 41,595 tons were produced in 2005.

Miners' Cohorts

To date, there are 13 miners' cohorts with sufficient information on each individual miner to conduct epidemiological investigations into health effects, namely cancer. Twelve of these cohorts were jointly analyzed in two slightly different settings consisting of 11 cohorts each, whilst the thirteenth is new and has not been included in any of the joint analyses so far.

A pooled analysis was conducted for 11 cohorts with respect to radon-related lung cancer risk. The included cohorts are, in order of their individual size: Ontario, Canada (uranium); China (tin); Beaverlodge, Canada (uranium); Czech Republic (uranium); New Mexico, USA (uranium); Colorado Plateau, USA (uranium); France (uranium); Newfoundland (fluorspar); Radium Hill, Australia (uranium); Port Radium, Canada (uranium); Sweden (iron). Altogether, this analysis included 60,606 exposed miners with a cohort-depending follow-up from 1943–1991. The mean length of follow-up was 17.7 years. After the publication of the joint analysis, further follow-up was conducted for the French, the Czech, and the Newfoundland cohorts.

Again 11 cohorts were jointly analyzed in terms of radon-related risk for cancers other than lung cancer. In this case, the Radium Hill cohort was replaced by a tin miners' cohort from Cornwall, England. Overall, this pooled study included 64,209 miners with a follow-up from 1941 to 1990 and a mean length of follow-up of 16.9 years.

The largest single cohort is based on uranium miners having worked with the Wismut company in Saxony and Thuringia, Germany. Saxony and Thuringia are the southern part of the former GDR, and mining was stopped after the German unification. The cohort includes 59,001 miners. The follow-up covers the years 1949–1998 with a mean length of 30.5 years.

Exposures

Uranium miners are not only exposed to radon, but also to other agents. Depending on the geological

environment, this can be gamma radiation, long lived radionuclides, ►arsenic, ►asbestos, silica, and others. Nonetheless, the most important agent with respect to cancer induction is ►radon. Among the above mentioned cohorts, the exposure ranges from 0 WLM for unexposed cohort members, e.g., in the German cohort, to over several thousands of WLM as for the Colorado Plateau and the German cohorts. The highest average cumulative exposure of 579 WLM was observed among the Colorado Plateau miners.

The Risk of Lung Cancer

Of major health concern is the induction of ►lung cancer from radon exposure. Results from uranium miners' studies were the first to indicate that radon is the most important risk factor for lung cancer next to smoking. The analysis of the joint 11 cohorts revealed a linear increase of risk with increasing cumulative exposure. The Excess Relative Risk (ERR) per WLM was calculated as being 0.76%/WLM, i.e., the baseline lung cancer risk increases by 0.76%/WLM, but the risk estimates for each single cohort varied by more than one order of magnitude. For a more detailed analysis aimed to study the influence of effect modifiers, two models were used. While the first included time since exposure, attained age, and duration of exposure (the so-called exposure–age–duration model, EAD), the second model included time since exposure, attained age, and concentration (the exposure–age–concentration model, EAC). ERR/WLM decreased constantly with increasing time since exposure and increasing attained age. Furthermore, ERR/WLM was modified by either duration of exposure or concentration, i.e., depending on the model, ERR/WLM increased with increasing duration of exposure or with decreasing radon concentration. The effect of an increasing ERR/WLM with decreasing radon concentration is called the inverse dose-rate effect. For the 11 cohorts, it could be observed only at exposures exceeding 50 WLM. The inverse dose-rate effect might be explained on a microdosimetric basis. During the cell cycle, one or several periods might show a much higher sensitivity to irradiation than the others. Protracted exposure to α-emitters over a longer time subsequently leads to a larger proportion of these sensitive cells being irradiated compared with the same exposure occurred in a shorter time. Another explanation might be that a reduction of the dose-rate may allow for proliferation of cells being initiated by radiation earlier during exposure. A third suggestion is that this effect could be explained as a bystander effect.

The German cohort is as big as all the 11 cohorts put together, but less heterogeneous in various aspects: same societal and geographical background, same way of follow-up, and one system for exposure estimation. When applying those models used for the 11 cohorts to the Wismut miners, the overall estimated ERR per WLM was 0.21% with a 95% confidence interval of 0.18–0.24%. This is somewhat lower than the one observed in the 11 cohorts. For these, no confidence intervals were given. Thus, no clear answer can be given to whether or not there is a significant difference in the risk estimates derived from the two studies. Applying the EAD model to the German cohort revealed no meaningful results, but the EAC model gave results comparable to those from the 11 cohorts to that extended that the inverse dose-rate effect was in the same order of magnitude. Here, it could be observed only at exposures exceeding 100 WLM. The effects of time since exposure and attained age were less pronounced compared to the 11 cohorts. A synopsis of the results is given in Table 1. Though the results seem to be different to some extent, it has been shown that the estimates for the lifetime attributable lung cancer risks are comparable.

Histopathologic Findings

Those studies referring to radiation and cell type have provided conflicting results. Early investigations conducted in the 1960s announced that ►small-cell lung carcinoma (SCLC) was the characteristic cell type of radiation-induced lung carcinoma, whereas later it was suggested that radiation also was associated with other cell types, and that SCLC was simply the one that appeared first. Accordingly, a shift from the predominance of SCLC to squamous cell carcinoma (SqCC) was reported in the Colorado Plateau miners when the follow-up was extended. A short latency for SCLC was also reported for the Ontario uranium miners, amongst which there was a high proportion of SCLC especially 5–14 years after exposure. A study among Chinese tin miners found SqCC to be the predominant type, but SCLC appeared to be more strongly associated with radon exposure, namely among miners who developed lung carcinoma at younger ages. Results from two independent studies among German uranium miners suggest that SCLC and SqCC are more likely related to high radon exposure than adenocarcinomas (AC) and other cell types.

The Risk of Cancers Other than Lung Cancer

The radon-related risk for cancers other than lung cancer was analyzed based on 11 cohorts of radon exposed miners, most of them working in uranium mines. Twenty-eight individual cancer categories were analyzed. Among those, statistically significant increases in mortality were observed for stomach and liver cancer, while statistically significant decrease were observed for ►tongue cancer, mouth cancer, pharynx cancer, and ►colon cancer. For leukemia, an increased mortality was observed within the first 10 years since starting work, but not subsequently. Among the remaining individual categories, mortality

Uranium Miners. Table 1 Exposure–age–concentration model: comparison of BEIR VI parameters with parameters estimated from the WISMUT cohort

	BEIR VI	Wismut cohort
ERR/WLM (%)	0.83	0.25 (0.13–0.36)[a]
Time since exposure (years)		
5–14	1.0	0.66 (0.44–0.89)
15–24	0.78	1.0
25+	0.51	0.50 (0.40–0.60)
Attained age (years)		
<55	1.0	1.0
55–64	0.57	0.80 (0.64–1.0)
65–74	0.29	0.66 (0.50–0.88)
75+	0.09	0.49 (0.30–0.83)
Concentration (WL)		
<0.5	9.09	8.19 (4.36–15.4)
0.5–1.0	4.45	4.27 (2.28–8.0)
1.0–3.0	3.36	2.97 (1.93–4.57)
3.0–5.0	2.91	2.52 (1.66–3.83)
5.0–15.0	1.55	2.08 (1.39–3.13)
15.0+	1.0	1.0

[a]95% confidence interval.

was related to cumulative exposure only for pancreatic cancer and for other and unspecified cancers. For this latter category, the finding again was restricted to the period less than 10 years after start of work. Overall, this analysis gave no evidence for a considerable risk for cancers other than lung cancer from radon. However, later studies from France and the Czech Republic revealed some indication for an increased risk for kidney cancer and leukemias, respectively. The risk for these causes of death can be considered as low, but further analyses based on new and/or extended cohorts are needed. Based on dosimetric assumption, a risk for pharyngeal cancer, cancer of the liver and the bone, and leukemias cannot be ruled out.

Conclusion

In conclusion, there is no doubt about the lung cancer risk deriving from radon exposure among uranium miners and other radon-exposed miners. The risk for other cancers cannot be ruled out, if at all it can be considered as small. Still, it needs further attention, since dosimetric models give hint that doses to some organs might be high enough for cancer induction. Another topic for further research is the analysis of combined effects. Some studies have looked at the interaction of smoking and radon. Results indicate that radon-related lung cancer risk exists both among smokers and nonsmokers. Little is known about combined effects of radon and arsenic or other cocarcinogenic substances.

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Urea Cycle

Definition

Synonym ornithine cycle; Is a metabolic cycle of biochemical reactions occurring in many animals that produces urea from ammonia (NH_3). In mammals, the

urea cycle takes place only in the liver. Eliminating the excess nitrogen in this way prevents it from accumulating in the form of ammonia, which is toxic.

►Arginine-depleting Enzyme Arginine Deiminase (ADI)

Uridine Diphosphate-Glucuronosyltransferase

Definition

Microsomal uridine diphosphate-glucuronosyltransferase (UGT) enzymes catalyze the Phase II glucuronidation. The human UGT superfamily has been classified into the UGT1 and UGT2 families, further classified into three subfamilies (UGT1A, UGT2A, and UGT2B). All nine functional family-1 members of the UGT1A subfamily are encoded by a single gene locus on chromosome 2q37. UGT1A1 is highly expressed in the gastrointestinal tract and the liver and is the primary enzyme responsible for forming bilirubin glucuronides and for the detoxification of the active irinotecan metabolite SN-38.

►Irinotecan

Urine Cytology

Definition

Morphological analysis of cells shed in the urine, nowadays often enhanced by cytogenetic or immunohistochemical techniques

►Urothelial Carcinoma

Urokinase-Type Plasminogen Activator

Definition

UPA; An activator of plasminogen that is distinguished from ►tissue-type plasminogen activator by its ability to regulate cell behavior and tissue remodeling upon binding to its specific cell surface receptor. Serine proteinase which converts plasminogen to enzymatically active plasmin, an anticoagulant. It is overexpressed in a variety of malignant tumors with poor prognosis.

►Proteinase-activated Receptors
►Coagulopathy

Uroplakins

Definition

A group of proteins covering the apical surface of transitional epithelia.

►Urothelial Carcinoma

Urothelial Cancer

Definition

Tumors originating from the urothelium, i.e., from the epithelium lining the urinary tract.

►Urothelial Carcinoma

Urothelial Carcinoma

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Synonyms

Transitional cell carcinoma of the urinary bladder; Bladder cancer

Definitions

Urothelial carcinoma is a cancer arising from the urothelium, a specialized epithelium lining the urinary tract from the renal pelvis into the urethra, retaining

discernable morphological aspects and molecular markers of urothelial cells.

Characteristics

Distribution, Histology, and Clinical Course

Urothelial carcinomas are derived from the urothelium, an epithelium lining the urinary tract from the renal pelvis into the urethra. Most tumors initiate in the urinary bladder, but some are localized in the renal pelvis, the ureter and the urethra. Urothelial carcinoma is the most common histological subtype of ►bladder cancer, often retaining aspects of normal urothelial differentiation including markers like ►cytokeratins 7, 13, and 20, and ►uroplakins. Another histological subtype of bladder cancer, squamous cell carcinoma, is prevalent in selected countries, e.g. Egypt. It typically arises in the context of chronic inflammation, e.g. caused by ►Schistosomas hematobium infection. Limited squamous metaplasia is found in many, especially high-grade urothelial carcinomas. Adenocarcinomas are very rare in the urinary tract. Overall, bladder cancer incidences vary between 4–40/100,000 worldwide, with a two- to fourfold male predominance and a maximum in the over 70 age group.

About 70–80% of urothelial carcinomas exhibit a papillary morphology (Fig. 1a). Non-invasive papillary tumors are designated pTa. They can be removed by resection, but tumor recurrence is observed in 50–70% of the patients. The high recurrence rate and frequent multifocality of non-invasive urothelial tumors suggest a "field effect" in urothelial carcinogenesis. Indeed, multifocal urothelial carcinomas can arise independently, but also through intraluminal or intraepithelial spreading of one initial tumor. Clinical and molecular data suggest that a subset of well differentiated pTa tumors have a particularly low risk of recurrence and progression. These are now classified as a new subentity, PUNLMP (papillary urothelial neoplasia of low malignant potential). Low grade pTa tumors are rarely lethal, but high grade pTa tumors may progress to the invasive stages (Fig. 1b and c) pT1 (invading submocusal tissue), pT2 (invading the muscular layer), pT3 (invading the perivesical adipose tissue layer), or pT4 (invading neighboring organs). Most invasive urothelial cancers, however, develop from a different precursor stage, carcinoma in situ (pCIS), a highly dysplastic flat lesion. With increasing grade and stage of muscle-invasive tumors, metastasis to lymph nodes and distant organs, preferentially liver, lung, bone, and adrenal, becomes more frequent.

Causes

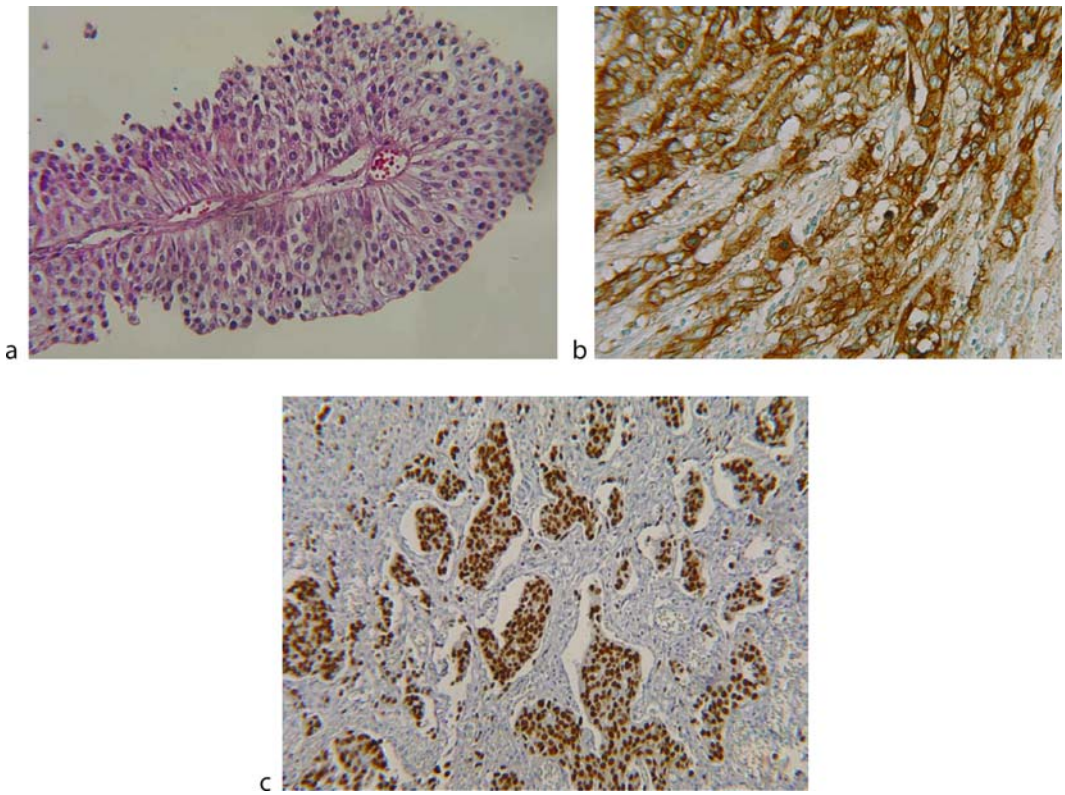
Urothelial carcinoma is caused by chemical carcinogens excreted in the urine, prominently arylamines, but also nitro-compounds, nitrosamines, polyaromates, arsenite, metabolites of drugs like phenacetin and cyclophosphamide, and of endogenous amines eg. from tryptophane. As tobacco smoke is a main source of carcinogens, smokers have an up to sixfold enhanced risk of bladder cancer. Specific exposures occur in certain occupations like, historically, aniline and azo-dye production. Less dramatic risks persist in some industries, e.g. those producing dyes, plastics, and rubber. Tobacco smoke is a source of many urinary carcinogens and together with occupational exposures may be responsible for the overall higher incidence of urothelial carcinomas in industrialized countries. There is little evidence for familial inheritance of urothelial carcinoma, but individual carcinogenesis risk is modulated by polymorphic xenobiotic metabolism enzymes. For instance, lack of GSTM1 (►Glutathione S-transferase M1) increases urothelial cancer risk ~1.5-fold, and more strongly in smokers. Hypomorphic alleles of NAT2 (►N-acetyltransferase 2) underlying the "slow acetylator" phenotype strongly increase the risk of individuals exposed to arylamine carcinogens. HNPCC (►Lynch syndrome) patients also have an increased risk of urothelial cancers, intriguingly only of the upper urinary tract.

Therapy

Nonmetastatic urothelial cancers can be successfully treated by surgery, either by transurethral resection for pTa and most pT1 tumors or by removal of the affected bladder (►Cystectomy), ureter, or kidney. Modern operation techniques have diminished the morbidity and the mortality of radical cystectomy. Continent techniques of urinary diversion allow patients to maintain a good quality of life. Chemotherapy is administered in an ►adjuvant or ►neoadjuvant fashion. Common chemotherapy regimes use MVAC (►methothrexate, ►vinblastine, ►adriamycin, ►cisplatin) or a ►cisplatin/►gemcitabine combination. Both regimes have similar, significant but limited efficacies. To prevent recurrences after transurethral resection nowadays postoperative intravesical chemotherapy is administered routinely. Patients with a high risk of tumor recurrence (due to big, multifocal, or high grade pTa or pT1 tumors or carcinoma in situ (►CIS)) are treated by the more efficacious ►BCG installation immunotherapy. All patients treated for urothelial cancers must be regularly monitored for recurrences. Due to its high recurrence rate and requirement for long-term monitoring and treatment, in many countries urothelial carcinoma is economically the most costly tumor type on a per patient basis.

Diagnosis and Monitoring

Definitive diagnosis of urothelial carcinoma requires endoscopy of the urinary tract. Resected tumors and biopsies from suspect areas are used for tumor staging



Urothelial Carcinoma. Figure 1 Histology of urothelial carcinoma. (a) Typical papillary tumor. (b) Invasive urothelial carcinoma immunostained for EGFR, which is detected at the membranes of the tumor cells growing as sheets and files. (c) Invasive urothelial carcinoma immunostained for TP53, which is detected in the nuclei of the tumor cells growing as clusters. All histological photographs were kindly provided by Dr. Christopher Kramer.

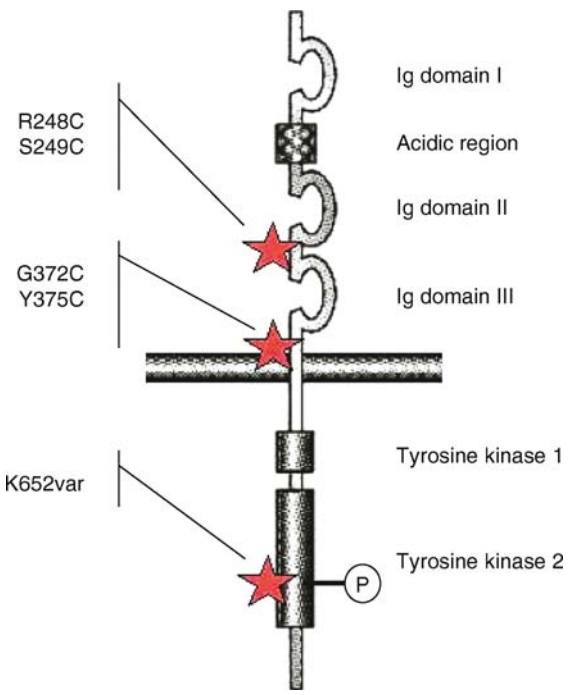
and grading. Visualization of flat lesions, in particular CIS, is improved by porphyrin fluorescence detection following instillation of aminolevulinic acid esters (►Photodynamic diagnostic). Urography is also helpful for diagnosis, especially for tumors in the upper urinary tract, i.e. ureter and renal pelvis. Computed tomography and magnetic resonance tomography are used to determine the extension of more advanced carcinomas and identify metastases. Monitoring for recurrences involves regular endoscopic investigations, as a minimum.

►Urine cytology, a noninvasive technique, is used for the initial diagnosis of urothelial carcinoma, but is more important for detection of recurrences. The technique is highly specific, but not very sensitive, especially for low grade tumors, and depends on observer experience. Therefore, cytogenetical, biochemical, and molecular assays of proteins and cells in the urine are entering clinical practice. Multicolor ►FISH analysis aimed at typical chromosomal changes in urothelial carcinoma is also highly specific, but not very sensitive. ►ELISA-based assays detect proteins released by urothelial carcinomas into the urine. They include the bladder tumor antigen assay (BTA) measuring complement factor H-related protein, ►NMP22 BladderChek measuring a nuclear matrix protein, and ImmunoCyt detecting mucin-like proteins and a ►carcinoembryonic antigen (CEA) isoform. Their specificity is limited by the fact that inflammatory processes common in individuals at risk for bladder cancer also increase urinary levels of these proteins. Novel assays measuring ►Survivin levels, ►telomerase activity, ►DNA methylation alterations, or ►microsatellite allelic loss are under development. These and future molecular diagnostic assays are expected to facilitate the long-term monitoring of patients with urothelial cancers by reducing the number of cystoscopies required, and to improve the classification of this highly diverse tumor type for individualized therapy.

Molecular Genetics

Invasive bladder carcinomas are characterized by pronounced ►chromosomal instability, with typically 7–10 gross aberrations. In contrast, low grade pTa tumors show few chromosomal aberrations and

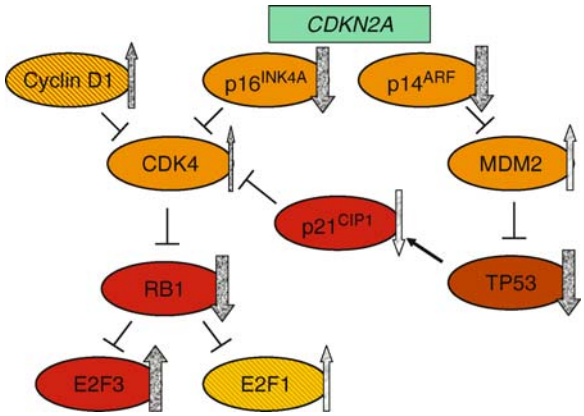
mutations. In such cases, losses affecting either or both arms of chromosome 9 are the only discernable chromosomal abnormality. Additional changes comprise i(5p) and trisomy 7. The 9p losses clearly target the twin gene ►*CDKN2A* which encodes two functionally and biochemically distinct tumor suppressors, $p16^{INK4A}$, and $p14^{ARF}$. Candidate tumor suppressor loci on 9q comprise ►*DBCCR1*, *PTCH1* (a negative regulator of ►hedgehog signaling), *TGFBR2* (a ►TGFβ receptor subunit), and *TSC1* (a component of the ►tuberous sclerosis complex). Many low grade pTa tumors harbor oncogenic missense mutations in a tyrosine receptor kinase, ►FGFR3, which are rare in invasive urothelial cancers and in other cancer types. The most frequent mutations are S249C, Y375C, S248C, and G372C (Fig. 2). They appear to stabilize and prolong the activated state of FGFR3 following binding of fibroblast growth factors or lead to growth-factor independent activation by formation of disulfide bonds. Possibly, they also increase FGFR3 protein levels. The prevalence of FGFR3 mutations decreases from PUNMLPs through pTa towards pT1 tumors and they are rare in muscle-invasive carcinomas. Thus, FGFR3 mutations appear to elicit a comparatively low malignant phenotype. Alternatively to those in FGFR3, oncogenic mutations are found in ►HRAS, but, like *CDKN2A* deletions, these are similarly frequent throughout all tumor stages. Apparently, FGFR3 mutations lead primarily to excessive urothelial cell proliferation, which otherwise resembles that during tissue regeneration after injury. Normal urothelial regeneration is also crucially dependent on autocrine ►EGF-like growth factors, especially HB-EGF and amphiregulin, and their receptors, especially ►EGFR, encoded by the *ERBB1* gene at 7p12. An increased dosage of chromosome 7 is frequent in early stage cancers and amplifications at 7p12 are found in later stages. Overexpression of the EGFR is accordingly common in urothelial carcinoma (Fig. 1b) and may deregulate autocrine stimulation of urothelial cell proliferation. Unlike FGFR3 mutations, EGFR overexpression is often associated with more aggressive cancers.



Urothelial Carcinoma. Figure 2 Common FGFR3 mutations in urothelial carcinoma.

Additional chromosomal changes accumulate in high grade papillary and in CIS tumors indicating the onset of genetic instability and the potential for progression towards invasive stages. In invasive cancers, multiple and variable changes are found. Among the more consistent additional alterations are losses at 3p, 8p, 10q, 11p, 13q, 17p, and 18q, and gains or amplifications at 1q, 6p, 8q, 11q, 17q, and 20q. Missense mutations of the ►p53 tumor suppressor leading to nuclear accumulation of the protein (Fig. 1c) are likewise characteristic of progressive urothelial carcinomas. Strikingly, the groups of tumors with p53 or FGFR3 mutations are almost distinct. In keeping with its precursor property, genetic changes in CIS resemble more those in invasive cancers than those in pTa tumors, although they are not as extensive. DNA methylation alterations also accumulate with tumor progression. In particular, hypermethylation of several genes including *RASSF1A* (an antagonist activated by ►RAS signaling), *SFRP1* (a ►WNT signaling antagonist), ►*APAF1*, and ►*APC* in various combinations becomes more frequent in invasive cancers, although beginning hypermethylation can be detected already in morphologically normal urothelium of cancer patients. Hypomethylation of repeat sequences is also prevalent in urothelial cancers, usually increasing with tumor stage.

All urothelial carcinomas show defects in two regulatory systems, cell cycle regulation and the p53 network, albeit to different extents (Fig. 3). Even low grade pTa tumors display losses of one or both *CDKN2A* alleles which impede both systems. In invasive carcinomas, cell cycle regulation is disturbed by inactivation of both ►RB1 alleles through mutations and allelic loss at 13q14, by amplification of *CCND1* at 11q13 encoding ►Cyclin D1, CDK4 (►Cyclin-dependent kinase 4) amplification, or most frequently complete *CDKN2A* inactivation by homozygous deletion, point mutation, or promoter hypermethylation. In more aggressive cancers, ►CDK inhibitors of the CIP/KIP family are additionally downregulated, usually by epigenetic mechanisms. In case of the imprinted



Urothelial Carcinoma. Figure 3 Common changes in cell cycle regulators in urothelial carcinoma. The direction of the arrows indicate the change in function (down: loss, up: increase), their width the frequency. Red color indicates changes associated with worse histopathological parameters, orange indicates distribution across all stages and grades. TP53 mutating correlate best with grade (indicated by the brown color). Increased expression of E2F1 is found in many cases, but has been postulated to indicate a better prognosis (thus yellow/orange). Overexpression of Cyclin D1 is typical of many low stage tumors (indicated by yellow), but can be caused by gene amplification in advanced cancers (indicated by red). *CDKN2A* encodes both $p16^{INK4A}$ and $p14^{ARF}$.

CDKN1C gene at 11p15 encoding $p57^{KIP2}$ loss of the active (maternal) allele is a further inactivation mechanism. Gains and amplifications at 6p22 lead to overexpression of ►E2F3 and at 8q24 to that of ►MYC, both of which aggravate cell cycle deregulation. Overall, the severity of cell cycle loss of control parallels the aggressivity of urothelial carcinoma. However, since so many different mechanisms contribute, it is difficult to employ this insight for prognostic purposes,. Similarly, defects in TP53 function appear to be associated with tumor progression and are brought about by different mechanisms, most commonly missense mutations in *TP53* and allelic loss of the gene at 17p and $p14^{ARF}$ loss by 9p21 deletions. Rarer cases show overexpression of the TP53 inhibitor ►MDM2. More commonly, mediators of TP53 action such as APAF1 and $p21^{CIP1}$ become downregulated by epigenetic mechanisms. The defects in TP53 function contribute to defective checkpoint function in urothelial carcinoma, which facilitates the accumulation of chromosomal alterations. Moreover, the concomitant loss of proper RB1 and TP53 function prevents hyperproliferation-induced ►senescence. In addition, almost all urothelial carcinomas reexpress active ►telomerase. Detection of hTERT RNA or active enzyme by the TRAP assay is therefore explored for diagnostic purposes.

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Urothelial Carcinoma, Clinical Oncology

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Synonyms

Transitional cell carcinoma

Definition

The ►urothelium covers inner surface of almost the entire urinary tract, extending from the renal pelvis, through the ureter and bladder, to the proximal urethra. Usually it is composed of three to seven layers of cells. The luminal surface of the superficial cells is covered with an asymmetric unit membrane, which functions as the permeability barrier between urine and blood. Nearly 90% of the ►urothelial carcinoma arises in the bladder and renal pelvic and ureteral carcinomas account for only approximately 7% of the total. Worldwide, there are approximately 336,000 new cases of urothelial carcinoma and 132,000 deaths annually.

Characteristics

Morphological and pathological characteristics of urothelial carcinoma, mainly bladder carcinomas (►Bladder cancer), are a combination of ►papillary superficial ►carcinoma and ►nodular invasive carcinoma. Primary and/or associated ►urothelial carcinoma in situ (CIS) are occasionally seen. Papillary carcinoma is a basic pattern and consisting of approximately 70% of urothelial carcinoma; however, during repeated recurrences, 10–15% of them turn to nodular invasive carcinoma.

Another characteristic feature of the urothelial carcinoma is simultaneous or metachronous multifocal tumor development in the urinary tract (Fig. 1). (i) There are patients in which lesions in the renal pelvis, ureter, and bladder are observed simultaneously (Fig. 1a). (ii) When standard nephrectomy is performed for renal pelvic and ureteral carcinomas, approximately one-third of the lower ureter is left intact. In this remaining ureter, urothelial carcinomas develop subsequently in 20–50% of the patients (Fig. 1b). Consequently, at the present time, the state of the art surgery for renal pelvic and/or ureteral carcinomas is ►total nephroureterectomy, including removal of a small portion of the bladder with the ureteral orifice (bladder cuff) in the affected side. (iii) Even if total nephroureterectomy is carried out, however, 15–50% of patients exhibit subsequent carcinomas in the bladder (Fig. 1c). (iv) When superficial papillary urothelial carcinomas of the bladder are resected transurethrally (TUR), the rate for subsequent development of urothelial carcinomas of a similar biological nature in the normal-appearing bladder mucosa is reported to be 50–80% (Fig. 1d). (v) When cystoprostatectomy (i.e., removal of the bladder and prostate) is carried out for male bladder cancer patients, 4–17% incidences of urothelial carcinomas in the remaining urethra have been described (Fig. 1e). In female bladder cancer patients, involvement of the urethra is reported to occur in 1.4–36% of cases. This phenomenon is frequently called recurrences (as stated earlier) in a clinical setting. Two distinct concepts have been proposed to explain this phenomenon; the "►field cancerization" hypothesis indicating urothelial cells are equally exposed to the previous carcinogenic insults, the other hypothesis is "►clonal expansion or implantation" indicating that the multifocal development is caused by the seeding or implantations of originally transformed cells (►Carcinogenesis).

Diagnosis

Patients most frequently complain of gross hematuria. In some of the bladder carcinoma patients, vesical irritability is observed such as micturition pain, frequency, and sense of residual urine.

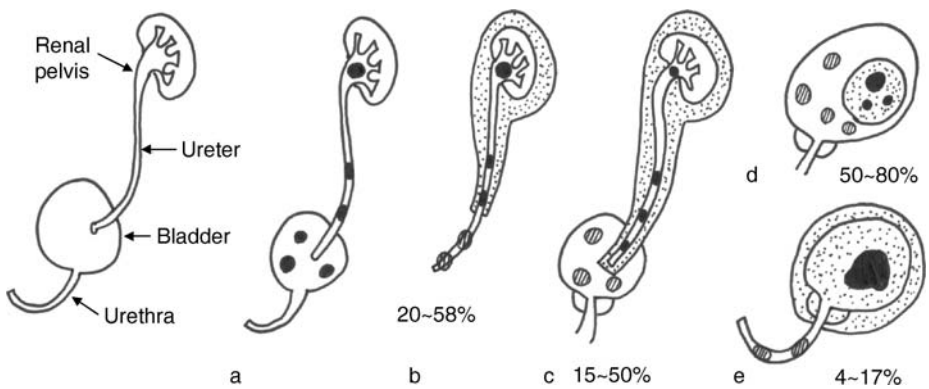
To diagnose urothelial carcinoma, urine cytology is essential, although in many low-grade papillary carcinomas, urine cytology is negative. On the contrary, nodular invasive carcinomas and CIS, usually exhibit positive cytology. For diagnosis of bladder carcinoma, cystoscopy during gross hematuria is particularly important to identify the presence and nature of bladder carcinoma and also to observe the possibility of gross hematuria from either side of the ureteric orifice. For diagnosis of renal pelvic and/or ureteral carcinomas, intravenous urography and enhanced CT are useful.

To evaluate the extent of urothelial carcinomas, chest X-ray, CT from the chest to the pelvic cavity, MRI of the pelvic cavity, bone scintigram, and blood counts/blood chemistry are done.

Biological nature and extent of disease are expressed by grade of tumors (G) obtained by biopsy and TNM staging (tumor, nodes, metastases by UICC) determined by imaging studies. Therapy of urothelial carcinoma is based on these information.

Therapy

In case of bladder carcinoma, treatment of papillary superficial bladder carcinoma is carried out by ►transurethral resection (TUR). Since papillary bladder carcinoma is notorious for its high rate of post-TUR intravesical recurrences, depending upon clinical stage and grade, various options are selected. For cTa, G1–2, just observation by cystoscopy and urinary cytology at 3 months interval at the initial year. For cTa, G3 or cT1, G1–2, one course of intravesical injection of ►Bacillus Calmette–Guerin (BCG) is preferred or just



Urothelial Carcinoma, Clinical Oncology. Figure 1 Clinical findings indicating multifocal tumor development in the urothelium. Hatched lesions are original tumors and oblique line lesions are recurrent tumors. Shaded area indicates the area of surgical resection.

observation. For cT1, G3, if cancer is completely resected, one course of intravesical injection of BCG is preferred. If primary or concomitant CIS is identified, intravesical BCG injection is definitely planned.

If the tumor has the nature of papillonodular or nodular invasive carcinoma indicating G2–3, cT2 or greater, as muscle invasive carcinoma, again, various options are selected such as radical cystectomy or partial cystectomy (if cancer is located at suitable part of the bladder and no associated CIS on multiple mucosal biopsies) or bladder sparing treatment after neoadjuvant ►chemotherapy and maximal TUR. If pathology reveals pT3 or more, positive lymph nodes, then, adjuvant chemotherapy is recommended.

After radical cystectomy, urinary tract is reconstructed by ileal conduit or neobladder. The former procedure needs appliance of pouch to the abdominal stoma to collect urine and the latter procedure ensures the patient to void from the urethra by replacing the bladder by ileal neobladder. In order to use neobladder to replace the bladder, cancerous lesions on the bladder neck and prostatic urethra should be denied by preoperative biopsies or intraoperative frozen section to avoid urethral recurrences of urotherial carcinoma after neobladder construction.

For metastatic urothelial carcinoma or neoadjuvant/adjuvant setting for deeply invasive or nodes positive bladder carcinoma, cisplatin-based combination chemotherapies such as methotrexate, vinblastine, actinomycin D, cisplatin (MVAC), or cisplatin/paclitaxel or gemcitabine/paclitaxel are used (►Cisplatin).

In case of urothelial carcinoma in the renal pelvis and/or ureter, total nephroureterectomy including bladder cuff is carried out because of multiple development of urothelial carcinomas (Fig. 1).

Genetics

Assessment of the risks of bladder carcinoma recurrence and of progression to invasive carcinoma is an important clinical task to plan the treatment and postoperative management of these patients. Genetic information in bladder carcinoma is indispensable for this purpose in addition to clinical grade and stage of bladder carcinoma.

Moreover, these information are essential to elucidate the pathogenesis of urothelial carcinoma.

Mutation in codon 12 of the *HRAS* oncogene was first identified in T24 bladder carcinoma cell line; however, in human bladder carcinoma, the frequency of *HRAS* mutations in codon 12, 13, and 61 was 6% and *HRAS* mutations are relatively infrequent genetic alterations in urothelial carcinoma of the bladder. Overexpression of c-erbB-2 has been observed in 37% of patients with muscle-invasive bladder carcinoma. *FGFR3* which encodes fibroblast growth factor receptor 3 is reported to be mutated as many as 74% of stage pTa superficial bladder carcinomas, 84% of G1, and 55% of G2 tumors which make clear contrast to only 7% of G3 tumors.

Retinoblastoma-associated protein (RB, encoded by ►*RB1*) and ►p53 are key proteins in cell-cycle regulations. *TP53* is located on chromosome 17p 13.1, where frequent lose of heterozygosity (LOH) has been reported in bladder carcinoma. Somatic mutation in *TP53* and its product p53 is regarded as the tumor suppressor being most frequently mutated in cancer. There are many reports on *TP53* mutations in bladder carcinoma; in G3 invasive carcinoma, approximately 50–70% are positive for *TP53* mutations.

RB protein is another key regulator of the cell cycle and ►LOH of the *RB1* locus is reported in 29–33% of bladder carcinomas, often observed in invasive carcinomas. Combination assays of RB and p53 protein are used to improve the accuracy of prognostic predictions.

Loss of chromosome 9 is the common genetic alteration in urothelial carcinomas. Approximately half of these carcinomas, all of the informative loci in both 9p and 9q demonstrate LOH. LOH of chromosome 9 is reported as high as 70% of bladder carcinomas more than pT1, and even 50% of G1 superficial carcinomas.

Promoter hypermethylation could be responsible for inactivation of genes which is called epigenetic alterations, and such inactivation can occur as a result of methylation or in combination with allelic loss and methylation. For example, promoter hypermethylation which encodes epithelial E-cadherin is reported to be associated with tumor recurrence.

Methylation and/or loss of the *CDH1* gene results in lack of E-cadherin, a cell-adhesion molecule. Reduced expression of E-cadherin is commonly observed in high grade G3 bladder carcinomas and CIS.

Loss of chromosome 9p and 9q are frequent early genetic events in the development of urothelial carcinomas, and involvement of chromosome 17p or the *TP53* locus is characteristic of invasive bladder carcinoma.

Molecular analysis including gene chip technology is now becoming a basic procedure in most laboratories; however, hard evidence of its benefit is still limited in the clinical setting. Genome-wide DNA analysis, using gene chips or complementary DNA arrays, are powerful tools for data mining; however, further statistical validation is required before these procedures are applied to the clinics for molecular staging of urothelial carcinoma.

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Urothelial Carcinoma In Situ

Definition

Flat carcinoma arising in the urothelium not elevating into the lumen.

►Urothelial Carcinoma

Urothelial Nodular Invasive Carcinoma

Definition

30% of ►urothelial carcinoma belongs to this type having nodular growth and deeply invading, occasionally metastatic.

Urothelial Papillary Superficial Carcinoma

Definition

Basic pattern of ►urothelial carcinoma having papillary growth but not deeply invading to the wall of organ.

Urothelial Tumor

►Transitional Cell Carcinoma

Urothelium

Definition

Epithelium covering the luminal surface of the renal pelvis, ureter, bladder, and urethra.

►Urothelial Carcinoma

Ursolic Acid

Definition

A triterpenoid from plant species.

►Betulinic Acid

Urticaria Pigmentosa

Definition

UP; Fixed, hyperpigmented, and vascular maculopapular lesions, generally a few centimeters in diameter; a type of ►mastocytosis.

USP

Definition

►Ubiquitin Specific Proteases. One of several subclasses of enzymes that belong to the de-ubiquitinating Enzymes (DUBs) that contain a conserved cysteine-histidine catalytic motif.

►HAUSP De-Ubiquitinase

USP44

Definition

►Ubiquitin-Specific Protease44.

Uterine Artery Embolization

Definition

A procedure that blocks the blood vessels that supply the tumor by injecting small particles into the arteries feeding the uterus.

►Uterine Leiomyoma

Uterine Fibroid

Definition

A common term for ►uterine leiomyoma.

Uterine Leiomyoma

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Synonyms

Uterine fibroids; Fibromyomas; Fibromas; Myofibromas; Myomas

Definition

Uterine leiomyomata are benign tumors comprised of smooth muscle cells of the ►myometrium and of extracellular matrix components, such as collagen, proteoglycans and ►fibronectin. Commonly known as fibroids, uterine leiomyomata are the most prevalent pelvic tumor in women.

Characteristics

Clinical, Epidemiological, and Histological Characteristics. Uterine leiomyomata are symptomatic in ~20–25% of reproductive-aged women. However, the prevalence is likely to be much greater (>75%) as fibroids can be present but asymptomatic. Although uterine leiomyomata rarely (<0.1%), if ever, become malignant (►cell transformation), the symptoms are medically and socially significant and include prolonged and profuse uterine bleeding, pelvic pain, urinary incontinence, constipation, infertility, recurrent miscarriage and premature labor. Clinical diagnosis is typically made by physical examination and can be confirmed by imaging studies such as ultrasound. Small lesions, however, can go undetected.

Although leiomyomata primarily affect women during childbearing years, the only consistently curative therapy is ►hysterectomy. In fact, uterine leiomyomata are the most common indication for hysterectomy in the US, accounting for over 200,000 procedures, or more than one-third of procedures annually. Effective alternative treatment options are limited. For example, ►myomectomy is associated with a high rate of recurrence and medical therapies are not curative and are often ineffective. ►Uterine artery embolization (UAE) has been shown to be effective in reducing symptoms and tumor size and is less invasive than traditional surgical interventions; however, the effect on future fertility is uncertain. ►Magnetic resonance imaging-guided focused ultrasound surgery (MRgFUS), a new non-invasive thermoablative therapy that promises noninvasive, outpatient therapy, is not yet widely available and the long-term effects of the procedure are not known. Currently, non-surgical medical therapies are limited.

Histologically, uterine leiomyomata appear as whorled bundles of smooth muscle cells in well circumscribed masses. Generally, mitoses are rare and appear normal, which is consistent with the benign nature of these tumors. Uterine leiomyomata are classified by their location in the uterus. Tumors within the uterine wall are called intramural fibroids. Tumors located below the outermost uterine layer are termed subserosal fibroids. Alternatively, fibroids that expand toward the innermost uterine layer and distort the uterine cavity are termed submucosal fibroids. Subserosal and intramural locations comprise the majority (95%) of all leiomyomata; submucosal leiomyomata make up the remaining 5%. Tumor location and size correlate with symptoms such as bleeding, pelvic pain and infertility. For example, submucosal fibroids are more often associated with bleeding than are subserosal or intramural tumors.

Despite the major public health impact of uterine leiomyomata, little is known about their etiology. Cellular, molecular, cytogenetic and epidemiological studies have advanced our current understanding of fibroid biology.

Cellular characteristics. Until recently, the steroid hormones estrogen and progesterone were considered the most important regulators of leiomyoma growth. There is abundant evidence that sex steroids promote fibroid growth, including the clinical observations that fibroids grow in the presence of high levels of estrogen or progesterone, such as during the reproductive years or during treatment with synthetic progesterones; fibroids regress in the presence of low levels of estrogen

or progesterone, such as following menopause or during treatment with ►gonadotropin releasing hormone agonists (i.e. GnRHa) or with antiprogesterone agents (i.e. RU-486). Furthermore, fibroids have higher estrogen concentrations, bind more estrogen, have more ►estrogen receptors and progesterone receptors and convert ►estradiol (a more active form of estrogen) to estrone (a less active form of estrogen) more slowly than does normal myometrium. Other hormones, such as growth hormone and prolactin, are also thought to promote fibroid growth, but their roles are less well defined.

Growth factors, which are small proteins that affect cell growth, have been shown to mediate the growth-promoting effects of estrogen and to play an important role in the development of fibroid tumors. Potentially important factors in fibroid growth include ►transforming growth factor-β, basic ►fibroblast growth factor, ►epidermal growth factor, ►insulin-like growth factor and ►platelet-derived growth factor. Overall, estrogen, progesterone and growth factors likely promote tumor growth, but only after the initiation of tumor formation. This initiating event(s) remains largely unknown, although there is evidence to suggest there is a strong genetic component to fibroid development.

Molecular Genetic and Cytogenetic Characteristics. Uterine fibroids are considered to be independent monoclonal lesions as determined by X-inactivation studies using polymorphisms in either the glucose-6-phosphate dehydrogenase (G6PD) isoenzyme or the ►androgen receptor gene on the X chromosome.

Although 60% of fibroids studied cytogenetically have a normal karyotype, approximately 40% of fibroids have nonrandom chromosomal abnormalities. Analyses of these leiomyomata with abnormal karyotypes have revealed consistent cytogenetic rearrangements, which can be divided into several subgroups; ►chromosome translocation between chromosomes 12 and 14, trisomy 12, deletion on the long arm of chromosomes 3 or 7 and the short arm of chromosome 1, rearrangements of the short arm of chromosome 6, and rearrangements of chromosomes 1, 3, 10, 13, and X.

Translocation between chromosomes 12 and 14, t(12;14)(q14-q15;q23-q24), is the most common translocation in fibroids and occurs in approximately 20% of fibroids with karyotypic rearrangements. ►*HMGA2* (previously called ►*HMGIC*) is the critical gene on chromosome 12 involved in these translocations. Rearrangement of *HMGA2* is observed in many other ►mesenchymal tumors including angiomyxomas, breast fibroadenomas, endometrial polyps, hemangiopericytomas, lipomas (►adipose tumors), pulmonary chondroid hamartomas and salivary gland adenomas. However, the consistency of chromosome 14 as a translocation partner of chromosome 12 is notable in fibroids; chromosome 12 translocations in other mesenchymal tumors have a diverse number of partner chromosomes. *RAD51L1* on chromosome 14 has been identified to be involved in some fusion transcripts with *HMGA2*.

Rearrangements of the short arm of chromosome 6 (6p21) are present in fewer than 5% of karyotypically abnormal fibroids and include translocations with chromosomes 1, 2, 4, 10, and 14, as well as inversions and translocations with various other chromosomes. *HMGA1* (previously called *HMGIY*), a gene related to *HMGA2*, is involved in these rearrangements. Chromosome 6 abnormalities are also consistently present in other benign mesenchymal tumors such as lipomas, hamartomas, and endometrial polyps.

The HMGA genes play a role in cell growth. In general, the highest levels of *HMGA* expression are seen in tumor cells and during normal development in embryonic tissue, whereas expression is reduced or absent in adult tissues. Studies of chromosome 12 translocations in fibroids and other benign mesenchymal tumors have revealed various mechanisms of *HMGA2* dysfunction, including creation of fusion transcripts, truncation of *HMGA2*, disruption of *HMGA2* regulatory sequences, and creation of alternative splicing patterns. Interestingly, rearrangements within the coding region of *HMGA2* are frequently observed in lipomas, and rearrangements outside of the coding region are more common in fibroids. This suggests that dysregulation of *HMGA2* expression, not disruption of coding sequence, is a potential mechanism of fibroid growth.

Interstitial deletion of the long arm of chromosome 7, del(7)(q22q32), is present in approximately 20% of karyotypically abnormal fibroids as well as in other benign mesenchymal tumors, such as lipomas and endometrial polyps. Deletion mutations most often result in loss of gene function, and suggest the presence of a ►tumor suppressor gene in this area. Mapping projects have progressively narrowed the critical region, however none of the genes in this area have yet been shown to have a definitive role in the pathogenesis of the del(7q) subgroup of fibroids.

Other less common cytogenetic abnormalities in fibroids include trisomy 12 and rearrangements of chromosomes 1, 3, 10, 13, and X. In cases of trisomy 12, the presence of an extra copy of chromosome 12 may increase the level of *HMGA2* transcript and thus increase the gene product that is involved in fibroid growth.

Rearrangements of chromosome 1 frequently involve ring chromosome formation, as well as translocations with the short arms of chromosomes 2 and 6. Recently, fibroids with a specific deletion on chromosome 1 have been shown to exhibit gene transcription profiles similar to those of leiomyosarcomas, suggesting that subsets of fibroids may have differential potential to

progress to malignancy. In addition, attention has focused recently on rearrangements of chromosome 1 in fibroids since the gene, ►fumarate hydratase, was identified in the region 1q42-q44 and shown to be involved in two syndromes characterized by the development of fibroids. Fumarate hydratase is important in energy metabolism and also appears to act as a tumor suppressor. Cytogenetic analysis of fibroids with 1q42 rearrangements suggests that loss of fumarate hydratase may be important in the development of a subset of fibroids in women without inherited fibroid-related syndromes.

Rearrangements of chromosome 3 include insertions, long and short arm deletions and translocations with chromosome 7. To date, no candidate genes on chromosome 3 have been identified as having a role in fibroid growth or development. Rearrangements of chromosome 10 include loss of one copy of chromosome 10 (monosomy 10) and deletions affecting the long arm (especially band q22). *MORF*, a gene involved in chromatin remodeling, is disrupted in fibroids with translocations involving chromosome 10. Rearrangements of chromosome 13 include deletions and translocations. To date, no candidate genes on chromosome 13 have been identified as having a role in fibroid growth or development. Rearrangements of the X chromosome preferentially involve the region Xp11-p22 and include translocations with chromosomes 5 and 12, deletions, and inversions. Of interest, the region Xp11-p22 contains an *HMGA1*-like sequence, *HMGIYL1*, but aberrant expression of this gene in fibroids remains to be demonstrated.

In fibroids that are cytogenetically normal, genetic alterations that cannot be visualized by karyotype, such as point mutations, may be present. Genes that are differentially expressed in myometrium and fibroid cells include *WNT7A*, a gene involved in patterning in embryogenesis, and *CYP19*, which produces aromatase enzyme involved in estrogen metabolism. DNA polymorphisms that influence leiomyoma risk have been identified. Polymorphism are small sequence variations, often single nucleotide changes. They are commonly interpreted to be without, clinical significance, but, rarely, may result in a change in protein folding or in a change in codon for which there is limited tRNA availability. These changes in term could cause a change in clinical phenotype. The polymorphism of another steroid hormone metabolizing gene, *CYP17*, appears to increase risk of leiomyomata among black African women.

Even among karyotypically abnormal tumors, ~30–40% are mosaic with chromosomally normal cells. These chromosomally mosaic tumors also have been shown to be clonal by X-inactivation analysis as described above, suggesting that cytogenetic abnormalities may be secondary changes in tumor development and that other genetic changes may be primarily responsible for initial tumor growth in genetically susceptible cells.

Heritability and genetic epidemiological characteristics. A variety of epidemiological studies have assessed whether there might be a genetic basis for uterine fibroids, including ethnic predisposition, twin, and familial aggregation studies. For example, women of African origin experience an approximately threefold higher incidence of fibroids than women of other racial and ethnic groups, after adjustment for known risk factors, such as obesity and number of births, as well as socioeconomic status and access to health care. Identical twins have twice the rate for hysterectomy as compared to fraternal twins, which is consistent with the expected rates for a genetically influenced trait. In addition, fibroids are at least twice as common in women who have a first degree relative with fibroids than in women who have no relatives with fibroids. This increases about sixfold in women with early onset of fibroids (<45 years), an expected observation for a genetically influenced trait. Finally, two rare Mendelian disorders, Reed syndrome, characterized by fibroids in association with multiple cutaneous leiomyomata, and hereditary leiomyomatosis and renal cell cancer (HLRCC), a cancer syndrome characterized by fibroids and papillary renal cell carcinoma, are known to be inherited as autosomal dominant traits with reduced penetrance.

Together, these observations indicate that fibroids have a potentially significant heritable component to their development. This genetic predisposition is likely to involve a complex interaction of multiple genes. Future genome-wide studies among sister pairs affected by fibroids may identify susceptibility genes for uterine fibroids.

Overall, cytogenetic analyses, together with molecular clonality and epidemiological studies, suggest that karyotypic abnormalities in fibroids may be important in the pathobiology of these tumors and may represent secondary somatic changes in genetically susceptible cells. The susceptibility gene(s) that are crucial for fibroid pathogenesis remain mostly to be identified. Ultimately, understanding the mechanisms of fibroid tumor development could lead to innovative, less-invasive treatment options for this significant women's health problem.

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Uterine Leiomyoma, Clinical Oncology

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Synonyms

Leiomyomata; Fibromyoma; Myoma; Fibroids

Definition

Uterine leiomyomas are benign neoplasms, composed of smooth muscle cells with variable amounts of fibrous stroma.

Characteristics

Leiomyomas are usually found in the myometrium of the uterine corpus; however, they can also originate in the smooth muscle organs. They are composed of smooth muscle and extracellular matrix (collagen, proteoglycan, fibronectin).

Incidence

Uterine leiomyomas are the most common neoplasms of the female pelvis. They occur in ~20–25% of women of reproductive age, but careful pathologic inspection of the uterus reveals that they are present in more than 80% of women. The neoplasm is more frequently found in the fourth and fifth decades of life and most commonly in patients of African descent. Age-standardized rates by race were 8.9 new cases per 1,000 woman per year for Caucasian women and 30.6 new cases per 1,000 woman per year for black women. Hispanic and Asian women had rates similar to those seen in Caucasian women: 11.0 and 8.0 new cases per 1,000 woman per year, respectively. There is an increased risk of leiomyomas in women with greater ►body mass index (BMI), but risk is decreased in women who smoke, who have given birth or who use oral contraceptive pills. Leiomyomas rarely occur before menarche and typically regress after menopause, indicating ►estrogen as a promoter of growth. Rarely, malignant changes may occur, usually in postmenopausal women. The most common warning sign is rapid enlargement of a ►fibroid with definitive diagnosis usually made at the time of surgery.

Gross Features

Uterine leiomyomas may have solitary nodule or multiple nodules ranging in size from microscopic to huge tumors weighing more than 100 lb. They are firm and well demarcated from the surrounding myometrium. On sectioning, the tumor bulges from the surface and the pseudocapsule, produced by compression of adjacent myometrium, is readily apparent. The surface is smooth and glistening white with a whorled, fasciculated pattern. Variations in this appearance include hemorrhage, hyalinization, hydropic, myxoid or mucinous degeneration, true ►necrosis and calcification. These variations are important because they may also be seen in ►leiomyosarcoma. Leiomyomas are characterized by their location in the uterus:

- Subserous leiomyoma (just under the uterine serosa)
- Interstitial leiomyoma (within the thick myometrium)
- Submucous leiomyoma (just under the uterine mucosa)

Microscopic Features

Histologically, uterine leiomyomas are bundles of interlacing smooth muscle fibers with varying amounts of collagenous fibrous tissue and few blood vessels. The nuclei have a uniform appearance, mitotic figures are infrequent, and there is never nuclear atypia. The variants of uterine leiomyoma listed in the International Society of Gynecological Pathologists' Classification are:

- Cellular leiomyoma
- Epithelioid leiomyoma
- Bizarre leiomyoma
- Lipoleiomyoma

Symptoms

It is estimated that only 10–40% of leiomyomas are symptomatic. Patients may complain of a self-detected mass, abnormal uterine bleeding, pelvic pain or pressure related symptoms.

Bleeding is the most common presenting symptom in uterine leiomyomas. The most frequent presentation includes the development of progressively heavier menstrual flow that lasts longer than the normal duration (menorrhagia). Although menorrhagia can occur in any women with leiomyomas, women with submucous leiomyomas appear to be particularly prone to this complication. Blood loss from this type of menstrual bleeding may be heavy enough to contribute to iron-deficiency anemia.

Another common presenting symptom is pelvic pressure. This is caused by slowly enlarging leiomyomas,

which may attain a massive size. Pressure on the bladder produces urinary frequency, urgency and rarely and the inability to void. Constipation may result from pressure on the rectum. Pressure of the uterine leiomyomas on the ureters may cause hydroureter and, on occasion, hydronephrosis.

Acute onset of pain in previously asymptomatic leiomyomas raises the possibility of necrosis, inflammation or torsion of a pedunculated subserous leiomyoma. Prolapse of submucous leiomyoma may present as intense cramping pain, often accompanied by discharge or bleeding. Pain in the low back or legs may reflect alterations in body posture or pressure on lumbosacral nerve trunks.

Uterine leiomyomas can also be associated with other kinds of reproductive dysfunction, including recurrent miscarriage, infertility, premature labor, fetal malpresentation and other complications of labor.

Diagnosis

The diagnosis of such tumors is usually made by the pelvic examination. A leiomyoma may be suspected based on a bimanual examination that reveals an enlarged, firm, nontender and irregularly shaped uterus. Ultrasonography is the most common method of confirming the diagnosis. A combination of transabdominal and transvaginal ultrasonography should provide information regarding uterine volume, the number of leiomyoma, their location relative to the endometrial stripe, and evaluation of the adnexa. Of other available imaging techniques, magnetic resonance imaging (MRI) may prove to be the most useful but does not improve the findings seen on ultrasonography and has much higher costs.

Treatment

The majority of patients with uterine leiomyomas do not require treatment. If the tumors are stable and slow growing, an annual follow-up including an optimized symptom control is a viable option. Symptomatic patients should usually be offered a trial of conservative management before considering surgery.

►Gonadotropin-releasing hormone (GnRH) agonists are analogs of gonadotropin-releasing hormone. With continuous administration, they induce a hypoestrogenic pseudomenopausal state. Because uterine leiomyomas are estrogen-dependent benign tumors, this causes shrinkage of these tumors and of the myometrial mass. In addition, treatment with a GnRH agonist induces amenorrhea, allowing women with menorrhagia-induced anemia to increase iron stores and hemoglobin concentrations leading to a technically easier surgery with markedly diminished blood loss. But once the treatment is stopped, the leiomyoma tends to regrow to pretreatment size. The major use of GnRH agonists is as a preoperative treatment to facilitate surgical procedures, either myomectomy or hysterectomy. This class of drug cannot be used long term (>6 months) because of the attendant risks of prolonged hypoestrogenism, such as osteoporosis and cardiovascular disease. Combinations of GnRH agonists and low doses of estrogen and progesterone (that is, "add-back" regimens) can minimize the adverse hypoestrogenic effects caused by GnRH agonist treatment.

Androgenic agents (danazol, gestrinone) and progestins (medroxyprogesterone acetate, depomedroxyprogesterone acetate, norethindrone) have also been used to minimize uterine bleeding in women with uterine leiomyomas. However, these kinds of medication do not consistently decrease uterine or leiomyoma volume and their mechanism of action is thought to be the induction of endometrial atrophy. If there is significant endometrial cavity distortion by interstitial or submucous leiomyomas, these agents are often not successful in controlling menorrhagia, because the excessive bleeding is usually related to profound anatomic and vascular distortion.

Hysterectomy is considered to be the definitive treatment for uterine leiomyoma in symptomatic women who have completed childbearing. If the tumors are small, the hysterectomy may be done vaginally, particularly if there is associated pelvic relaxation. The American College of Obstetricians and Gynecologists (ACOG) criteria for hysterectomy for uterine leiomyoma are as follows:

- Presence of 1–3 asymptomatic leiomyomas of palpable size abdominally and if they are a concern to the patient.
- Excessive uterine bleeding:
- Profuse bleeding with flooding or clots or repetitive periods lasting more than 8 days
- Anemia, due to acute or chronic blood loss
- Pelvic discomfort caused by myomas:
- Acute and severe pain
- Chronic lower abdominal or low back pressure
- Bladder pressure with urinary frequency not due to urinary tract infection

When women want to preserve childbearing potential, a ►myomectomy may be performed. Myomectomy has a higher complication rate than hysterectomy. These complications include excess intraoperative blood loss, risk of postoperative hemorrhage, adhesions and bowel obstruction. It is important to inform the patients that there is a 25–50% recurrence of leiomyomas after myomectomy. Therefore, a significant number of women undergoing a myomectomy will require a subsequent hysterectomy. Preoperative criteria for myomectomy published by ACOG are as follows:

- Failure to conceive or recurrent pregnancy loss
- Presence of leiomyomas of sufficient size or specific location to be a probable factor

- No other likely explanation for the failure to conceive or for recurrent pregnancy loss

The use of endoscopic resection will be more widely used for the surgical management of uterine leiomyomas. This technique causes less patient discomfort, less bleeding and has a shorter recovery time. Hysteroscopic resection of submucous leiomyomas can be done using a resectoscope with unipolar cautery loops or with a neodymium:yttrium-aluminium garnet laser (Nd:YAG). The use the Nd:YAG laser has its problems and the operating surgeon must have expertise not only with the hysteroscope but also with the intrauterine laser. Laparoscopic myomectomy and laparoscopically assisted vaginal ►hysterectomy (LAVH) are safe and reliable treatment options, but can be difficult to perform technically and are not available universally.

Uterine artery ►embolization is a new, investigational treatment of leiomyomas. The method involves the catheterization of both uterine arteries using a femoral arterial approach. Preliminary studies in women who had large, symptomatic leiomyomas have shown significant improvement in their symptoms. More trials need to be carried out to confirm the efficacy and safety of this surgical treatment.

Cytogenetic Changes

Evidence from glucose-6-phosphate dehydrogenase isoenzyme analysis and from polymorphism analysis in the androgen receptor demonstrate that uterine leiomyomas are monoclonal and that each tumor within the same uterus arises independently. Although the classic paradigm suggests that the sex steroid hormones (►estrogen and ►progesterone) are the only important modulators of leiomyoma growth and transformation, it is now clear that chromosomal abnormalities play a role in the pathogenesis of these neoplasms. In uterine leiomyomas, non-random chromosomal changes such as translocations, duplications and deletions have been identified in ~50% of tumors studied by cytogenetic analysis. The most frequent abnormalities are translocations between chromosomes 12 and 14, t(12;14)(q14–15;q23–24) and deletions on chromosome 7, del(7)(q22–32). The frequencies of these abnormalities are ~20% and 15%, respectively.

Recently, the ►high-mobility group protein gene HMGIC was identified as the target gene affected by the 12q14–15 aberrations. HMGIC is an architectural transcription factor in the nuclear scaffold, a function critical for the correct assembly of stereo-specific transcriptional complexes. The frequent rearrangement of the HMGIC gene in uterine leiomyomas suggests that this gene is directly involved in the aberrant growth control observed in these tumors. Gene-targeting experiments indicate that HMGIC plays an important role in mammalian growth and development, since inactivation of the murine HMGIC gene results in the pygmy phenotype. In uterine leiomyomas, the mitochondrial aldehyde dehydrogenase (ALDH2) gene in 12q24.1, the recombinational repair gene RAD51B in 14q23–24, and the cytochrome c oxidase subunit VIc (COX6C) gene in 8q22–23 were identified as translocation partners to HMGIC. Deletions of chromosome 7 imply that tumor enlargement in some leiomyomas is probably due to loss of tumor suppressor genes.

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Utrafine Particles

Definition

Are airborne particles formed by combustion which have an aerodynamic diameter of 0.1 μm or less.

►Particle-induced Cancer

UV Radiation

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Definition

UV radiation belongs to the ►non-ionizing radiation part of the electromagnetic spectrum and spans between 100 and 400 nm. Conventionally UV radiation is further divided into three regions: UVA (>315–400 nm), UVB (>280–315 nm) and UVC (>100–280 nm).

Characteristics

Sunlight consists of visible light (400–700 nm), infrared radiation (>700 nm) and UV radiation. The quality (spectrum) and quantity (intensity) of sunlight are modified during its passage through the atmosphere. Solar UV radiation at ground level represents about 5% of the total solar energy; the radiation spectrum is between 290 and 400 nm, and is comprised of approximately 95% UVA and 5% UVB; UVC is completely filtered out by the Earth's atmosphere.

The spectrum of solar UV radiation to which an individual may be exposed varies with latitude, altitude, ground reflectance, season, time of day, weather, stratospheric ozone and other atmospheric components such as air pollution. For most individuals, solar radiation is the major source of exposure to UV radiation. ►Sunbeds and sunlamps used for tanning purposes are the main source of deliberate exposure to artificial UV radiation. Solar radiation is classified as a Group 1 carcinogen by the International Agency for Research on Cancer.

Cancers of the Skin

Skin cancer (see ►skin carcinogenesis) is the most frequent form of cancer worldwide. Skin cancers can be classified into three major histological types: ►malignant cutaneous melanoma and non-melanoma skin cancers, which comprise ►basal-cell carcinoma and ►squamous-cell carcinoma. Basal-cell carcinoma, which represents 70% of all skin cancers, develops from the basal cells of the epidermis, grows locally and never metastasizes. Squamous-cell carcinoma, which represents 20% of all skin cancers, develops from the epithelial cells of the epidermis, has a more aggressive behavior, can metastasize, but is rarely fatal. Cutaneous melanoma develops in the melanocytes within the basal layer of the epidermis; it is the least common form of skin cancer, but is the most serious as it readily metastasizes and is only curable if detected at an early stage.

Epidemiological Evidence of the Carcinogenic Potential of UV Radiation

Host Factors

There is a considerable range of susceptibility of the human skin to UV radiation. Susceptibility to UV radiation is closely related to pigmentary traits, and subjects who have the following characteristics are at increased risk for developing skin cancer (melanoma, squamous-cell carcinoma and basal-cell carcinoma):

- Red hair, followed by blond hair, followed by light brown hair.
- Skin phototype: subjects with a skin phototype I or II (always burn and never tan or always burn before developing a light tan) have a much higher risk for skin cancer than subjects with a skin phototype IV (never burn and always develop a deep tan). Intermediate risk categories are subjects who sometimes burn and always develop a tan (skin phototype III). Subjects with skin phototypes V and VI belong to populations with natural brown or black skin, and are resistant to sunlight.
- Freckles (ephelides) on the face, arms or shoulders. The risk for non-melanoma skin cancer increases with increasing sensitivity to freckling.
- Skin color: pale color, followed by increasing depth of pigmentation.
- Eye color: blue, followed by grey/green eyes, then by brown eyes.
- For melanoma, a large number of common naevi and the presence of a typical naevi.

Subjects with red hair, many freckles, fair skin and who never tan are at particularly high risk for skin cancer.

UV Radiation as an Environmental Risk Factor

The body of evidence from epidemiological studies indicates that there is a causal relationship between exposure to solar radiation and skin cancer.

1. These cancers affect more specifically individuals with fair skin.
2. These cancers occur predominantly on the parts of the body most commonly exposed to the sun such as the face, ears, neck and forearms; however, the distribution of melanomas and basal-cell carcinomas is not as closely related to the distribution of exposure to the sun as that of squamous-cell carcinomas.
3. There is a strong inverse relationship between latitude of residence and the incidence of and mortality from skin cancer.
4. There is a positive relationship between measured or estimated ambient UV radiation and the incidence of and mortality from skin cancer.
5. Migration to a country with high solar irradiation (e.g. Australia) is associated with an increase in risk.
6. There is a dose–response relationship between total cumulative dose and risk for skin cancer, although for melanoma this is not the only determining factor (see below).
7. Actinic keratosis, a sun-induced skin damage, is a precursor lesion of squamous-cell carcinoma.
8. Treatment with PUVA therapy (UVA radiation) increases the risk for melanoma and squamous-cell carcinoma.

However, the type and conditions of exposition (chronic or intermittent exposure, high exposure of short duration, exposure at young age) influence the type of skin cancer induced.

- Squamous-cell carcinoma develops at the sites of the body that receive chronic exposure; in contrast, basal-cell carcinoma and melanoma are associated with intermittent exposure.
- The risk for squamous-cell carcinoma is cumulative; consequently, people who work outdoors tend to have a higher incidence of squamous-cell carcinoma.
- For melanoma, the cumulative dose is not the only determining factor. In particular, the risk is correlated with ambient UV radiation at the place of residence during childhood. In fact, exposure to UVB during childhood is a necessary event for the development of melanoma.
- The risk for melanoma is inversely correlated with intense and continuous exposure to UV.
- Squamous-cell carcinoma is induced by UVB radiation. The wavelengths primarily involved in the development of melanoma and of basal cell carcinoma have not been firmly established.

Intraocular Melanoma

The results of studies on exposure to solar radiation and ocular melanoma would suggest that there is an association, but the data are inconsistent and their interpretation is difficult.

Biological Effects of UV Radiation Relevant to Carcinogenesis

DNA Damage

The biological effects of UV radiation vary enormously with wavelength. In addition, there is an estimated 1,000-fold variability in DNA repair capacity after exposure to UV radiation in humans (►photocarcinogenesis).

UVB is a complete carcinogen that is absorbed by DNA and can damage DNA directly. The DNA damage induced by UVB irradiation typically includes the formation of ►cyclobutane pyrimidine dimers (CPD) and 6–4 photoproducts. If these lesions are not repaired correctly, mutations are likely to occur. These mutations are C ⇒ T and CC ⇒ TT ►transversions, commonly referred to as "UVB fingerprint" or "UVB signature" mutations. UVB can also induce oxidative stress and the formation of singlet oxygen species (O_2^-), and can thus also cause DNA damage indirectly.

UVA is not readily absorbed by DNA and thus has no direct impact on DNA. Instead, UVA induces DNA damage indirectly through the absorption of UVA photons by ►chromophores, with the formation of ►reactive oxygen species (such as singlet oxygen and hydrogen peroxide [H_2O_2]) that can transfer the UVA energy to DNA via mutagenic oxidative intermediates such as 8-hydroxydeoxyguanosine (►8-OHdG). DNA damage by UVA radiation typically consists of T ⇒ G transversions, called "UVA fingerprint" or "UVA signature" lesions. The possibility that indirect DNA damage induced by UVA could play a major role in the development of melanoma is underlined by reports showing that patients who are genetically highly susceptible to oxidative agents develop multiple cutaneous melanomas.

Cellular Damage

Both UVA and UVB lead to altered expression of ►p53 and ►bcl-2 proteins, which may play an important role in regulating UV-induced apoptosis. Irradiation of melanocytes with UVA or UVB leads to the alteration of different intracellular proteins, which suggests that UVA and UVB may initiate the development of melanoma via separate intracellular pathways.

Differential Effects of UVA and UVB

UVA radiation is of longer wavelength and is therefore weaker but more penetrant. In humans, UVA penetrates deeper into the skin than UVB. The basal layer of the epidermis receives 50% of the total UVA and 10% of the total UVB to which the skin is exposed. Since solar radiation at ground level comprises a maximum of 5% UVB, the basal cells receive approximately 100 times more UVA than UVB from exposure to the sun. In contrast, the induction of an erythemal response requires 100–1,000 times more UVA than UVB.

As a result, UVA fingerprint mutations are mostly detected in the basal germinative layer of these lesions, whereas UVB fingerprint mutations are found predominantly more superficially in these lesions. In addition, UVB produces numerous immediate mutations whereas UVA produces fewer immediate mutations and more delayed mutations than UVB.

Consequently, squamous-cell carcinoma is clearly associated with the carcinogenic effects of UVB. In contrast, UVA could play a significant role in the causation of melanoma and basal-cell carcinoma.

Changes in the Immune Response

Both UVA and UVB can affect the immune system, but the two types of radiation seem to act differently. UVB can induce immune suppression at both local and systemic levels whereas UVA does not suppress the systemic immune response. In addition, UVA radiation may affect the local immune response differently from UVB.

Drug-Induced Photosensitivity

A variety of commonly used drugs such as diuretics, antibiotics and non-steroid anti-inflammatory drugs (►NSAIDs) increase cutaneous sensitivity to UV radiation, and are therefore predicted to increase the risk for skin cancer. Most drugs have a phototoxic rather than a photo-allergenic effect. Topical agents that have a

phototoxic effect include plant-derived photosensitizers (psoralens) such as bergamot, which is widely used in perfumed products.

Cancer Preventive Effects of UV Radiation

Several ecological studies have suggested that exposure to UV radiation may reduce the incidence of or the mortality from certain cancers (breast, colon, prostate) and of lymphomas. UV radiation induces the formation of vitamin D from its precursor in the body. Daily exposure of the face, forearms and hands to moderate sunlight for 10–15 min is generally sufficient to maintain vitamin D levels. Any vitamin D deficiency should be alleviated through dietary supplements.

Indoor Tanning and Cancer

The UV spectrum of indoor tanning facilities has varied over the years. The first fluorescent tubes designed for tanning purposes emitted up to 5% of the UV output as UVB. As a result of the growing concern about the carcinogenic potential of UVB, the UV output was then shifted towards UVA. Lamps that produce large quantities of long-wave UVA (>335–400 nm) per unit of time were marketed; these lamps can emit up to 10 times more UVA than that present in sunlight. More recently tanning appliances have been equipped with fluorescent lamps that achieve a balance between total UV, UVB and UVA similar to tropical sun.

The UV output and spectral characteristics of tanning appliances vary considerably as a result of differences in tanning appliance design (e.g. type of fluorescent tubes, materials that compose filters, distance from canopy to the skin), tanning appliance power and tube ageing.

A ►meta-analysis of epidemiological studies showed that ever-use of sunbeds is positively associated with an increased risk for melanoma (relative risk, 1.15; 95% confidence interval, 1.00–1.31), and there is a prominent and consistent increase in risk for melanoma in people who first used sunbeds in their twenties or teen years (relative risk, 1.75; 95% confidence interval, 1.35–2.26). Limited data suggest that the risk for squamous-cell carcinoma is similarly increased after first use as a teenager (relative risk, 2.25; 95% confidence interval, 1.08–4.70). Data also suggest detrimental effects from the use of sunbeds on the skin's immune response and possibly the induction of ocular melanoma.

Radiation emitted by lamps used in tanning appliances (mainly UVA) significantly increase the carcinogenic effect of broad-spectrum UV radiation, which indicates the possibility of a complex interplay between UVA and UVB radiation in human skin. The few studies that have addressed the biological changes in the skin induced by indoor tanning have shown that they are similar to those induced by sunlight.

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UVC Light

Definition

Short-wave ultraviolet light, predominantly induces *cis–syn* cyclobutane pyrimidine dimers and (6–4) pyrimidine–pyrimidone photoproducts between adjacent bipyrimidines.

►Fragile Histidine Triad
►UV Radiation

Uveal Melanoma

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Synonyms

Choroidal melanoma; Ciliary body melanoma; Intraocular melanoma

Definition

Uveal melanoma is an intraocular melanocytic neoplasm that originates from within the ►uveal tract of the eye. The tumor is thought to arise most frequently from melanocytes within a nevus, but may also occur de novo. It is the most common primary intraocular malignancy in adults.

Characteristics

Epidemiology

Worldwide, the incidence of uveal melanoma is highest in northern Europeans. Uveal melanoma has an incidence rate of around 6/1,000,000 in the United States. Similar to cutaneous melanoma, the incidence of uveal melanoma is much higher in whites than nonwhites. There is a slight male preponderance. The average age at diagnosis is 50–60, but any individuals of any age can be affected.

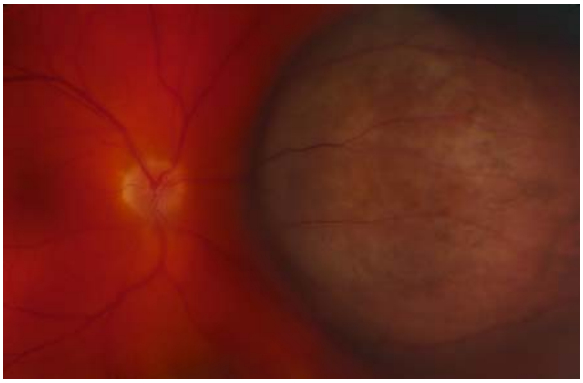
Risk Factors

Certain patient characteristics have been associated with increased risk of uveal melanoma, including light eye color, light skin color, and ability to tan. Individuals with ocular and ►oculodermal melanocytosis (nevus of Ota) are at increased risk of uveal melanoma. Interestingly, although these conditions can occur in any race, the increased rate of uveal melanoma appears to be primarily in whites.

Clinical Diagnosis

Signs and Symptoms

Uveal melanoma can be asymptomatic or associated with a variety of symptoms including blurred vision, visual field defects, distorted vision, flashes and floaters. On clinical examination, uveal melanomas can range in pigmentation from light tan to dark brown, and they are typically dome- or mushroom-shaped (Fig. 1). Features associated with uveal melanoma on fundus examination include orange lipofuscin pigmentation on the tumor surface, exudative retinal detachment, and "collar button" formation where the tumor herniates through the overlying Bruch's membrane, which can cause intraocular hemorrhage. Ciliary body melanomas are located near the crystalline lens and can induce subluxation, cataract formation, and astigmatism (from tilting the lens). Dilated episcleral feeder vessels, so-called "►sentinel vessels," can be associated with an underlying ciliary body melanoma. In larger tumors, neovascular glaucoma and necrosis may occur. Pain may be present if these complications develop. Clinical features associated with increased risk of metastasis include advanced patient age, increased tumor size, and ciliary body involvement.



Uveal Melanoma. Figure 1 Elevated choroidal melanoma adjacent to the optic nerve.

Diagnostic Modalities

Ophthalmoscopy remains the mainstay of diagnosis. Several adjunctive modalities can be used to further characterize the tumor and aid in diagnosis for ambiguous cases. Both ►A-scan and B-scan ultrasonography are critical for the complete evaluation of a suspected uveal melanoma. A-scan provides a single dimensional image characterizing the echogenic interfaces within the tumor. This provides an accurate way to measure the tumor height as well as confirming the typical homogenous characteristics of uveal melanoma. B-scan provides a two-dimensional image that allows for a cross-sectional view of the tumor. Most tumors are dome- or mushroom-shaped, the latter being nearly pathognomonic for uveal melanoma (Fig. 2).

►Fluorescein angiography is also a useful adjunct in to further characterize the tumor exclude other mimicking disorders. Common angiographic findings include early hyperfluorescence, pinpoint leakage and occasionally a double circulation pattern from vessels within the tumor (Fig. 3).

Fine needle aspiration biopsy (FNAB) is required in about 5% of intraocular tumors for the purpose of confirming the diagnosis of a melanocytic tumor. In the setting of an experienced surgeon and cytopathologist, FNAB can yield a diagnosis in over 90% of cases. In addition, FNAB is increasingly being used to assess the metastatic potential of melanomas using molecular tools described below.

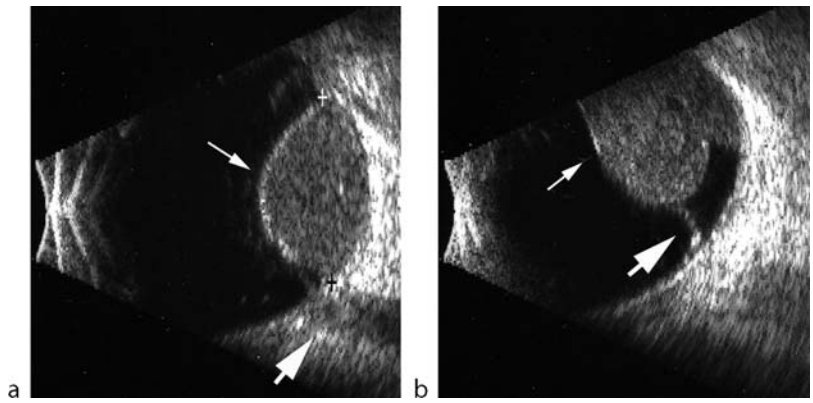
Other imaging modalities such as CT and MRI may provide further information in selected settings, such as when orbital or optic nerve invasion of an intraocular tumor is suspected.

Treatment Options

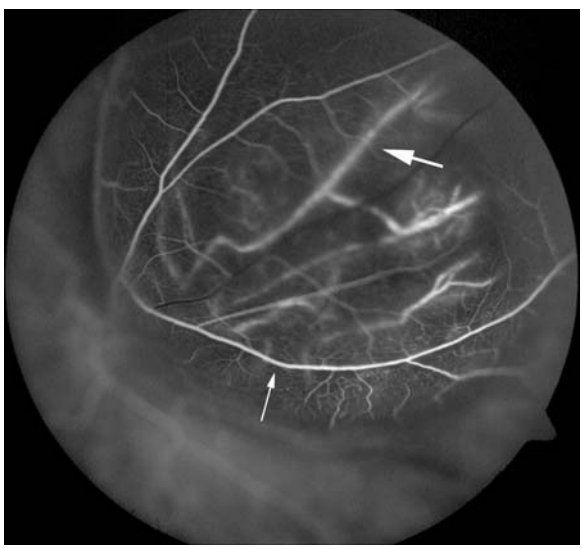
Several treatment options exist for uveal melanoma. The appropriate choice is based on patient age, health and preference, and on tumor characteristics.

Observation may be appropriate for small lesions where the diagnosis is in doubt, and in elderly or infirmed individuals with limited life expectancy. Choroidal melanocytic tumors less than about 3 mm in thickness and less than about 12 mm in diameter are usually considered suspicious nevi until growth is documented, at which point treatment is usually recommended. Serial fundus photography and ultrasonography are used to detect tumor growth.

►Transpupillary thermal therapy (TTT) has been advocated as primary therapy for smaller tumors and as an adjunctive treatment following radiotherapy. In



Uveal Melanoma. Figure 2 Ultrasound (B-Scan) of dome-shaped (a) and mushroom-shaped (b) melanoma. Thin arrows identify the tumor; thick arrows identify (a) the optic nerve and (b) the detached retina.



Uveal Melanoma. Figure 3 Fluorescein angiogram showing double circulation. The thin arrow shows a normal retinal arteriole; the thick arrow shows the intrinsic tumor vessel.

recent years, enthusiasm for primary TTT has waned as longer follow-up has revealed a high rate of local tumor recurrence. Most centers now reserve primary TTT for small tumors in patients who are unable to undergo surgery. The most widespread use of TTT today is as an adjunctive treatment following plaque or charged particle radiotherapy in tumors that are at high risk for recurrence, such as those located adjacent to the optic disc. In this setting, TTT appears to be effective at hastening the regression and reducing local recurrence risk compared to radiotherapy alone. Complications from TTT include retinal vascular occlusion, retinal fibrosis, retinal neovascularization, cystoid macular edema, retinal tears, retinal detachment and vitreous hemorrhage. These complications are much more common with primary TTT than with adjunctive TTT.

►Enucleation was once the mainstay of treatment for uveal melanoma. Today, however, enucleation is usually reserved for tumors that are very large or have demonstrated extensive local invasion (e.g., transscleral or optic nerve invasion). Enucleation may also be preferred in eyes with poor visual potential. At the time of enucleation surgery, a spherical implant is placed in the orbit and attached to the extraocular muscles to imitate the movement of the eye. Subsequently, the patient is fit with a prosthetic eye, which is a shell painted to match the other eye.

Plaque radiotherapy is the most common treatment for uveal melanoma. This treatment approach allows a large dose of radiation (85–100 Gy) to be delivered to the tumor over 4–5 days while minimizing radiation toxicity to the rest of the eye and surrounding structures. Various radioisotopes have been used, including ^{125}Iodine, ^{106}Ruthenium, ^{103}Paladium, but ^{125}Iodine is the most common choice. The medium tumor trial of the Collaborative Ocular Melanoma Study compared ^{125}Iodine plaque radiotherapy to enucleation and found no difference in survival. The plaque is surgically implanted on the scleral surface of the eye overlying the tumor. Treatment outcomes are significantly enhanced when intraoperative ultrasound is used to verify accurate placement of the plaque over the tumor. Local tumor control following plaque radiotherapy is greater than 90% in most centers. Complications include cataract, radiation retinopathy and papillopathy, neovascular glaucoma, vitreous hemorrhage, and rarely scleral necrosis. Incidence and severity of these complications are dose and location dependent.

Focal delivery of high dose radiotherapy can also be achieved with ►proton beam radiotherapy and other forms of charged particle therapy. A surgical procedure is required to place tantalum rings on the scleral surface overlying the tumor to direct the charged particle beam. Local tumor control appears to be slightly better with charged particle therapy than with plaque radiotherapy, especially in centers that do not use intraoperative

ultrasonography to localize their plaques, but complications are also more common with charged particle therapy. Complications include eyelash loss, dry eye, cataract, neovascular glaucoma, and radiation retinopathy and papillopathy. Newer radiosurgery techniques such as Gamma Knife and CyberKnife are being evaluated for their role in uveal melanoma.

Local tumor resection was once a popular alternative treatment that avoided radiotherapy and its attendant complications. However, as more experience has been obtained, it has become clear that local resection has its own set of serious side effects, including a high rate of local tumor recurrence, retinal detachment, vitreous hemorrhage and proliferative vitreoretinopathy. Today, local resection is usually reserved for selected tumors with small basal diameter and anterior location. There are many variations on this surgical technique, but most involve the creation of a partial thickness scleral flap, through which the tumor is resected en bloc. Adjunctive radiotherapy is often used postoperatively.

Pathology

The Callendar classification is the most widely accepted histopathologic grading system for uveal melanoma and is based on the spindle and epithelioid cell types (Fig. 4). Spindle A cells have narrow nuclei with a longitudinal fold. Spindle B cells have larger nuclei with a defined nucleolus. Epithelioid cells are larger and polygonal in shape with large nucleoli. Uveal melanomas are usually described as predominantly spindle, epithelioid, or mixed. Spindle cells portend a better prognosis, and epithelioid cells a worse prognosis. Other pathologic features that have been associated with worse prognosis include transscleral extension, increased number of mitotic figures, increased nucleolar area, and presence of looping extracellular matrix patterns.

Systemic Evaluation and Metastasis

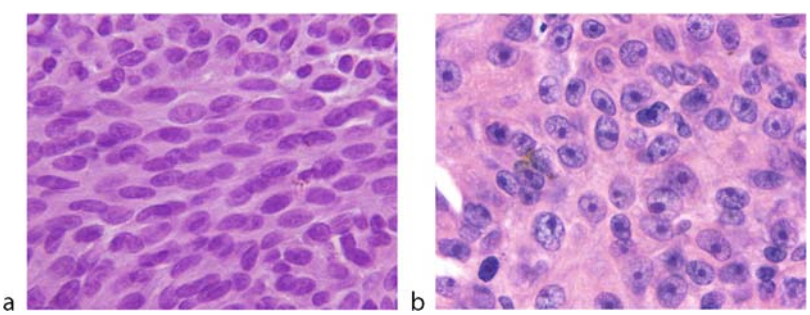
Metastatic uveal melanoma involves the liver in approximately 90% of cases. Other sites include lung, subcutaneous tissues, and bone. Systemic evaluation and monitoring should include liver function tests (especially lactate dehydrogenase, alkaline phosphatase, and gamma-glutamyl transpeptidase), and imaging of the abdomen with CT or MRI. Median survival after clinical diagnosis of metastasis is about 5–7 months. Metastatic disease limited to the liver can occasionally be treated with partial hepatectomy or hepatic arterial chemoembolization. Systemic chemotherapy and immunotherapy have met with limited success.

Prognostic Factors

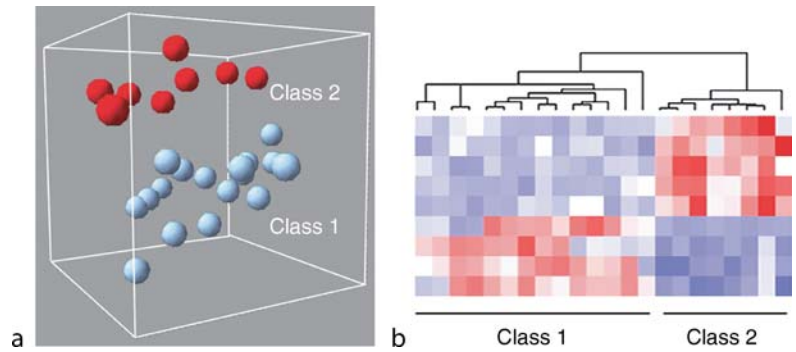
As mentioned in the previous sections, there have been many clinical and pathologic features of uveal melanoma that are statistically associated with metastasis. However, none of these features has demonstrated predictive accuracy sufficient for use in making treatment decisions in individual patients. This has led many researchers to investigate genetic features of the primary tumor that may predict metastasis with greater accuracy. The chromosomal alteration that is most closely linked to metastasis is loss of one copy of chromosome 3 (monosomy 3), which is found in about half of primary uveal melanomas. Various techniques have been employed to assess chromosome 3 status in clinical settings, including karyotype analysis, comparative genomic hybridization, fluorescence in situ hybridization, and loss of heterozygosity for polymorphic markers across the chromosome. More recently, ►microarray gene expression profiling has identified two molecular subgroups of primary uveal melanoma, referred to as class 1 and class 2 (Fig. 5). Virtually all of the metastatic deaths occur in class 2 tumors. The class 2 gene expression signature is closely linked to monosomy 3, but there are some exceptions, and the former outperforms the latter in terms of predictive accuracy. Consequently, the gene expression-based classifier has been refined to require less than ten genes and a small number of tumor cells that can be obtained by FNAB. Clinical studies are now underway to determine the best combination of molecular testing approaches for stratifying patients based on metastatic risk for inclusion in clinical trials and implementation of preemptive, adjuvant systemic therapy.

Systemic Therapy and Future Directions

Recent work on tumor doubling times, disease-free interval and metastasis strongly imply that those uveal



Uveal Melanoma. Figure 4 Uveal melanoma histopathology showing spindle cells (a) and epithelioid cells (b).



Uveal Melanoma. Figure 5 Gene expression profiling is the most accurate predictor of metastasis currently available for uveal melanoma. (a) Unsupervised principal component analysis showing clustering of tumors (spheres) in to class 1 (blue) and class 2 (red), with low and righ risk for metastasis, respectively. (b) Heatmap with supervised analysis with only nine discriminating genes (rows), which classify all tumors correctly (columns). Since a small number of genes can be used, this test is being adapted for use as a clinical test that can be applied to enucleation and biopsy samples.

melanomas that have the capacity to disseminate (e.g., class 2 tumors) have already done so prior to ocular treatment in most cases. This would explain why successful local treatments have not been associated with a demonstrable improvement in survival. Consequently, it may only be possible to improve survival in uveal melanoma by identifying high risk patients and treating them preemptively with systemic therapies that delay or prevent the development of clinical metastasis by maintaining micrometastases in a dormant state. The centerpiece of such a preventative strategy will be the newly emerging molecular prognostic tests described here. This strategy will also require the identification of new therapeutic approaches targeting the micrometastatic cell population.

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Uveal Tract

Definition

Pigmented highly vascular layer of the eye consisting of the choroid, iris, and ciliary body.

►Uveal Melanoma

Uvomorulin

►E-Cadherin

UV-Signature Mutation

Definition

A C → T single or tandem transition mutation that is typically formed at ►DNA photoproducts and commonly observed in UV-induced, but not other cancers.

►Solar Ultraviolet Light
►UV Radiation

V-erb-B2

►HER-2/neu

V-*raf* Murine Sarcoma Viral Oncogene Homolog B1

►B-Raf Signaling

Vaccine Therapy

Definition

Is an active, specific immunotherapy (ASI) mediated by stimulating the host's immune system to generate antibodies and/or immune cells (►cytotoxic T-cells) specifically against the target antigen to destroy a tumors or infectious microorganisms. A therapeutic (treatment) vaccine is given after the onset of disease and is intended to reduce or arrest disease progression. A preventive (prophylactic) vaccine is intended to prevent the initial onset of disease. Tumor vaccines include both native and artificial (mimic) tumor-associated antigens or tumor markers.

►Immunotherapy

Valproic Acid

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Synonyms

2-Propylpentanoic acid; Dipropyl-acetic acid

Definition

Valproic acid is a short chain branched fatty acid. It was first synthesized by B.S. Burton in 1882. During the last 40 years valproic acid has been extensively used for the treatment of epilepsy, bipolar and other neurological disorders. In the human brain, valproic acid affects the function of the neurotransmitter ►gamma aminobutyric acid (GABA) (mainly as a GABA transaminase inhibitor). Valproic acid has ►histone deacetylase inhibitory activity and affects behavior and growth of various cancers and ►cancer cell lines. Valproic acid induces cell differentiation and ►apoptosis of diverse cancer cell lines, increases ►recognition of cancer cells by the immune system, inhibits cell proliferation, decreases ►metastatic and angiogenic potential of cancer cells. The anticancer effect of valproic acid is based on its histone deacetylase inhibiting activity, modulation of ►MAPK signaling and ►beta-catenin pathway. Other mechanisms of action of valproic acid were also proposed. The relative safety of administration of valproic acid and its involvement in multiple pathways are making it a valuable lead in search for more potent and more specific drugs with anti-cancer activities.

Molecular Formula: $(CH_3CH_2CH_2)_2CHCOOH$
Molecular Mass: 144.21

Characteristics

The name valproic acid originates from its descriptive chemical name 2-propylvaleric acid (valeric acid is a synonym for pentanoic acid that was isolated from the flowering plant valerian (*Valeriana officinalis*)). Valproic acid was used as a solvent of neurologically active compounds and its therapeutic potential was discovered by chance. Valproic acid is a relatively safe drug, with only infrequent major side effects that include hepatotoxicity, thrombocytopenia, and prolonged ►coagulation time. In about 5% of pregnant users, valproic acid can cause congenital anomalies such as ►spina bifida.

Valproic acid inhibits proliferation and induces differentiation of neuroectodermal tumor cells.

Valproic acid and its analogs modulate the behavior of various tumor cell types by inducing apoptosis and differentiation, inhibiting proliferation, decreasing

►angiogenetic potency and increasing immunogenicity of cancer cells.

Valproic acid induces apoptosis in two ways: through the effect on ►caspases and ►chromatin fragmentation and trough its effect on membranes linked to phosphatidylserine externalization and release of cytochrome c from mitochondria. Valproic acid induced apoptosis in various animal cell lines including rat hepatoma cell line FaO, many human leukemia cell lines of B-, T-, and myeloid lineage, murine B-lymphoid cell lines, MV4-11 and KOCL-44, prostate carcinoma cell line, human thyroid cancer cells and other cells.

Treatment with valproic acid leads to inhibition of cell proliferation and induction of cell differentiation in different cell lines, mainly of neuroectodermal and leukemic origin. Valproic acid increased differentiation of human ►neuroblastoma cells (SJ-N-KP, AF8), which was documented by neurite extension and up-regulation of ►neuronal markers. The decreased net proliferation rate after valproic acid administration was observed in ►prostate cancer cells – androgen receptor positive (LNCaP and C4-2) and androgen receptor negative (DU145 and PC3). In leukemic cell lines HL-60 and MOLT-4, valproic acid induced cell differentiation was marked by an increase of CD 11b and co-stimulatory/adhesion molecule CD86. ►Hepatocellular carcinomas (HCC) that are resistant to conventional ►chemotherapeutics showed decreased proliferation in response to the treatment with valproic acid. A down regulation of anti- and up regulation of pro-apoptotic factors indicated that modulation of intracellular pro- and anti-apoptotic proteins is a key event in valproic acid induced tumor cell death. The influence of valproic acid in the ►cell cycle was determined to be in the G1 phase.

Valproic Acid is a Potent Inhibitor of Tumor ►*Angiogenesis*. Valproic acid caused inhibition of proliferation, migration and tube formation of endothelial cells and also caused a decrease of endothelial nitric-oxid synthase (eNOS) protein level. The inhibition of angiogenesis in vivo was documented on the chicken chorioallantoic membrane assay and the matrigel plug assay in mice. Valproic acid also caused disturbed vessel formation.

The indirect effect of valproic acid was observed in neuroblastoma cells. A higher production of anti-angiogenic-molecules thrombospondin-1 and ►activin A were detected. Similarly the treatment of colon adenocarcinoma cell line Caco-2 caused significant reduction of ►vascular endothelial growth factor (VEGF) secretion, down-regulation of protein expression and mRNA of VEGF, basic fibroblast growth factor (bFGF) protein level and inhibition of the ►ubiquitin-proteasome proteolytic system activity.

Valproic Acid Increases Immunogenicity of Cancer Cells. Treatment of human ►hepatocellular carcinoma cells with valproic acid mediated recognition of cancer cells by cytotoxic lymphocytes via the immunoreceptor NKG2D. Valproic acid induced transcription of MICA and MICB in hepatocellular carcinoma cells, leading to increased cell surface, soluble and total MIC protein expression. The induction of MIC molecules increased lysis of hepatocellular carcinoma cells by natural killer cells. In primary human hepatocytes, valproic acid treatment did not induce MIC protein expression indicating that valproic acid mediates specific priming of malignant cells for innate immune effector mechanisms.

Valproic Acid was Shown to Modulate Behavior of Numerous Tumors. Valproic acid was repeatedly used in patients with acute myeloid leukemia (AML) and combined with all-trans retinoic acid (ATRA). In some subtypes, the AML is connected with translocations that generate fusion genes including ►retinoic acid receptor alpha (RARα) which functions as an oncoprotein that inhibits differentiation pathways of myeloid lineage. ATRA was shown to partially release the differentiation block by binding to the RARα part of the fusion protein with subsequent increased expression of target genes (likely connected with disruption of binding of transcriptional co-repressors and induced degradation of the fusion protein). Valproic acid markedly increased the efficacy of the treatment by ATRA and resulted in transient disease control in subsets of patients with AML.

The antitumor efficacy of valproic acid was observed in ►medulloblastoma and supratentorial primitive neuroectodermal tumor (sPNET), which are the most common malignant brain tumors in children with poor prognosis. Two medulloblastoma (DAOY and D283-MED) and one sPNET (PFSK) cell lines were treated with valproic acid with resulting potent growth inhibition, cell cycle arrest, apoptosis, senescence, differentiation, suppressed colony-forming efficiency and tumorigenicity in a time- and dose-dependent manner at clinically safe concentrations (0.6 and 1 mmol/l).

Valproic Acid Affects Cell Behavior by Multiple Mechanisms

Valproic Acid Inhibits Histone Deacetylase Activity

The effect of valproic acid on behavior of cancers and cancer cell lines initiated studies directed at its mode of action. Behavior of cells depends on their gene expression that in turn is regulated by the basic transcription machinery, cell and tissue specific ►transcription factors and ►co-factors and by the organization of chromatin. ►Post-translational modification of chromatin, including histone acetylation, methylation, phosphorylation, ubiquitination and other modifications is a critical part of regulation of gene expression. Valproic acid was shown to down-regulate the HDAC activity in ►teratocarcinoma and neuroblastoma cells. Nevertheless, this effect may have been caused by a

direct action of valproic acid on enzymes involved in adding or removing acetyl residues on lysines of nucleosomal histones (mostly histones H3 and H4) or by a modulation of other targets. It was shown that valproic acid affects the in vitro deacetylation assay and acts as an HDAC inhibitor. Inhibition of HDAC activity was analyzed by measuring the acetylation of core histones H3 and H4 in the leukemic K562 and U937 cell lines. Valproic acid and its analogs (2-methyl-2-n-propylpentanoic acid (2M2PP), 4-pentenoic acid (4PA), 2-methyl-pentenoic acid (2M2P), 2-ethylhexanoic acid (2EH), and valpromide (VPM)) were shown to inhibit class I HDAC (HDACs 1–3), and class II HDAC (HDACs 4, 5, and 7). Valproic acid was the strongest inhibitor. The mechanism by which valproic acid affects behavior of cancer cells differs from its antiepileptic effect since the efficacy of antineoplastic and antiepileptic effects differ between particular analogs.

Valproic acid did not inhibit the activity of class II HDAC 6 and 10, in contrast to another HDAC inhibitor, ►trichostatin A (TSA). This implies a more selective effect of valproic acid on HDAC inhibition compared to ►TSA. Although valproic acid affects both class I and class II HDACs, the effect on these two classes differs. Valproic acid has been shown to inhibit the catalytic activity of class I HDACs and induce the proteasomal degradation of class II HDACs in contrast to TSA.

Valproic acid and its analogs induce expression of multiple exogenous reporter genes which are associated with HDAC inhibition, i.e. SV40, p21 and gelsolin.

Studies directed at the genome wide expression pattern showed that valproic acid affects expression of selective groups of genes (by their induction or repression).

Valproic Acid Interferes with MAPK Signaling

Valproic acid increases the DNA binding and transactivation activity of the transcription factor ►AP-1. In vitro studies showed that valproic acid specifically triggers the phosphorylation of ►ERK, the upstream modulator of AP-1, but does not act via the JNK (c-jun N-terminal kinase) and p38 pathways. Valproic acid and its derivatives (named above) activate MAPK. In clear contrast to their effect on inhibition of class I HDACs, the analogs of valproic acid have a stronger effect on MAPK activation than valproic acid itself. This may reflect a possibility that the effect on MAPK is not mediated by HDAC inhibition.

Therefore valproic acid-induced increases in AP-1 binding and function are likely due, at least in part, to activation of ERK followed by phosphorylation and increase in expression of c-Jun. Expression and phosphorylation of c-Jun in ERK pathway is required to direct cellular differentiation in poorly differentiated cells. Nevertheless, it seems likely that the way valproic acid affects cell behavior may to a large extent depend on the particular cell type.

Valproic Acid Affects the Beta-Catenin Pathway

GSK-3beta (►glycogen synthase kinase-3 beta) is a negative regulator of the ►Wnt signaling pathway, which regulates numerous processes, including cellular proliferation, cell migration, cell polarity, organo- and carcinogenesis. GSK-3 beta phosphorylates beta-catenin and this leads to its rapid degradation. Inhibition of GSK-3 beta by Wnt signaling leads to stabilization and accumulation of the beta-catenin protein. Consequently, beta-catenin translocates to the nucleus where it activates transcription of Wnt dependent genes by binding to factors ►Tcf/Lef (T cell factor, Lymphoid-enhancer factor).

Valproic acid has been reported to inhibit GSK-3 beta-mediated phosphorylation of a peptide derived from ►CREB protein in human embryonic kidney 293T and murine Neuro2A ►neuroblastoma cells and increase levels of beta-catenin in human neuroblastoma cells. Nevertheless, it was also proposed that valproic acid activates Wnt-dependent gene expression through the inhibition of histone deacetylase activity. The involvement of histone deacetylase inhibition by valproic acid in beta-catenin dependent regulation is supported by its effect on the expression of E-cadherin. Expression of numerous ►tumor suppressor genes including E-cadherin is linked to hypermethylation of specific regions of DNA and may be partially reverted by ►increased histone acetylation.

Valproic Acid may Influence Additional Pathways

Valproic acid was shown to be involved in other regulatory pathways. In many of them, it is likely that the effect is mediated primarily by the inhibition of histone deacetylase activity but some may be distinct. Valproic acid increases the levels of 5-►lipoxygenase (5-LOX) protein in murine hippocampus. 5-LOX produces ►leukotrienes from ►arachidonic acid and this is likely to be involved in the regulation of ►chromatin remodeling. Since increased levels of 5-LOX are linked to aging and neurodegeneration, it may be expected that the increase of 5-LOX expression may contribute to tumor cell aging and differentiation through chromatin remodeling.

Valproic acid is likely to inhibit invasiveness of cancer cells at concentrations lower than those necessary for its anti-proliferative effect. Increased expression of genes that inhibit invasiveness of cancer cells was observed in response to treatment with relatively low doses of valproic acid (150 μmol/l). These genes included ►signal transducer and activator of transcription 6 (STAT6), Ring1, RYBP and PCDHGC3.

Valproic acid may affect the regulation of gene expression by ►nuclear receptors which is critically

influenced by ►HDACs. Moreover, some members of this superfamily of genes may be affected by valproic acid more directly. PPARδ and PPARγ (but not PPARα) were activated by valproic acid in Chinese hamster ovary cells and F9 teratocarcinoma cells. At least in the case of PPARδ, this effect was not based on the binding and activation of the receptor by valproic acid as an agonistic ligand since it did not induce formation of heterodimers of PPARδ with retinoid X receptor on DNA response elements. Activated expression of PPARγ dependent genes may significantly contribute to the anticancer activity of valproic acid. PPARγ regulates the expression of the tumor suppressor gene PTEN (phosphatase and tensin homologue deleted from chromosome 10) that is often mutated in cancers. ►PTEN, a lipid phosphatase, dephosphorylates phosphatidylinositol (3,4,5)-triphosphate (PIP-3) to phosphatidylinositol (4,5)-diphosphate (PIP-2) and antagonizes the regulation by phosphatidylinositol-3 kinase (PI-3K).

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Vanadium

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Definition

Vanadium, a member of group VB and in the 4th period of the periodic table is a first transition series, d-block, grayish metallic element with an atomic number of 23 and forms different oxidation states of –1, 0, +2, +3, +4 and +5. The oxidation states +3 (vanadic), +4 (vanadyl) and +5 (vanadate) being most common, the oxidation state +4 is most stable in biological systems. Vanadium is an endogenous constituent of most mammalian tissues and is further considered a ►dietary micronutrient. It is an essential element for the proper growth and development of mammals owing to its diverse physiological and biochemical functions.

Characteristics

The uniqueness of vanadium is that it is included in the list of 40 essential micronutrients required in small amounts for normal cell metabolism. Accordingly, it has been incorporated into many multinational pharmaceutical preparations for maintenance of normal health. Although micronutrients never have had pharmacological potencies, they can prevent the minor wear and tear of the essential critical molecules of the cell; vanadium may thus have a role in DNA maintenance reactions and may prevent genomic instability leading to ►cancers. The main source of vanadium intake for the general population is food, such as chicken, fish, grains, cereals, ►liver, spinach, black pepper, parsley, fruits, and vegetables. The total body pool of vanadium in humans is about 100 μg, with the actual daily dietary intake estimated to be 10–60 μg, depending on individual human diet.

Studies have established vanadium as a novel biological regulator and have confirmed the biphasic effect of this trace element in biological systems, that is, essentiality at low concentrations, and toxicity at higher concentrations. Vanadium compounds can influence the behavior of enzymes, mimic growth factor activities, regulate carbohydrate and lipid metabolisms, modulate gene expression and signal transduction pathways, and in particular exhibit anticarcinogenic/antineoplastic activities. The diverse biological action of vanadium results from its capacity to function as an oxyanion, oxycation, or prooxidant.

Vanadium in Cancer Treatment

Vanadium has a potential role in tumor growth inhibition and in prophylaxis against ►carcinogenesis in different experimental cancer models namely ►liver cancer, ►colon cancer, ►breast cancer and others and in various types of malignant cell lines. Vanadium compounds have been found potentially effective against murine ►leukaemia, fluid and solid Ehrlich ►ascites tumor, murine mammary ►adenocarcinoma, HEp-2 human epidermoid carcinoma cells and human ►lung cancer, ►breast cancer, and ►gastric cancer. Synthetic complexes of vanadium with amino acids, peptides, proteins, organometallic and inorganic ligands, and pharmacologically active moieties are an area of current interest in anticancer research. A cytostatic effect is observed with vanadium(III)–L-cysteine complex on chemically-induced ►leiomyosarcoma bearing Wistar rats. [VIII (Hcys)3].2HCl.2.5H2O or compound 1 exhibits significantly greater total antioxidant capacity along with inhibition of neutral endopeptidase activity as potent as thiorphan. Moreover, compound 1 prevents lung

►metastasis, thus proving its role as an antimetastatic agent. These beneficial effects of the above complexes, in combination with their low toxicity, provide evidence for their possible application in the treatment of human malignant diseases. A good candidate for the development of new vanadium derivatives with organic ligands is the flavonoid quercetin because of its own anticarcinogenic effect. The quercitin-vanadyl complex [VO(Quer)(2)EtOH](*n*) (QuerVO) stimulates ►extracellular signal-regulated kinase (ERK) phosphorylation and this seems to be involved as one of the possible mechanisms for the biological effects of the complex. Organometallic vanadocene complexes have been found to be potent antiproliferative and antimitotic agents and block cell division at the G2/M phase of cell cycle in human cancer cells by disrupting bipolar spindle formation. Furthermore, vanadocenes as potent ►apoptosis-inducing cytotoxic agents against human ►testicular cancer cells in vitro as well as in experimental mice model in vivo may therefore have potential utility in the treatment of testicular seminomas in humans. Vanadyl complexes of 1,10-phenanthroline [$VO(Phen)^{2+}$] and related derivatives possess strong antitumor chemopreventive activities against human nasopharyngeal carcinoma, and the observed effects are found to be superior to the chemotherapeutic drug, ►cisplatin. Bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) or Metvan [VO(SO4)(Me2-Phen)2] has been identified as the most promising multitargeted anticancer bisperoxovanadium (bpV) complex with apoptosis-inducing activity in human leukaemia cells, ►multiple myeloma cells and solid tumor cells derived from ►breast cancer, ►glioblastoma, ►ovarian cancer, ►prostate cancer and ►testicular cancer. It is highly effective against cisplatin-resistant ►ovarian cancer and testicular cancer cell lines. Metvan inhibits the constitutive expressions of [►Matrix metalloproteinases (MMPs)] -2 and 9 proteins and its gelatinolytic activity in HL-60 cells, and leukemic cells from patients, and also inhibits the leukemic ►cell adhesion to the extracellular matrix proteins laminin, type IV collagen, vitronectin, and fibronectin and the invasion through Matrigel matrix. Metvan exhibits significant antitumor activity in severe combined immunodeficient mouse xenograft models of human malignant glioblastoma and breast cancer. The broad spectrum anticancer activity of Metvan together with favorable pharmacodynamic features and lack of toxicity of this oxovanadium compound may represent the first vanadium complex and a novel anticancer agent as an alternative to ►platinum-based ►chemotherapy.

Chemopreventive/Anticancer Mechanisms

Several mechanisms for the cancer chemopreventive role of vanadium have been proposed. The biochemical basis of the chemoprotective effect of vanadium may primarily be attributed to the substantial elevation of phase II conjugating enzymes, which may lead to a move and shift of the metabolic profile that may reduce the intracellular concentration of carcinogen-derived reactive intermediates. However, it is not yet clear whether the activity of specific ►CYP isoforms is directly influenced/modulated by vanadium treatment. Ortho- and meta- vanadate have been found to drastically reduce the mutagenicity of metabolically activated carcinogens by modulation of protein phosphorylation through the inhibition of protein phosphotyrosine phosphatases (PTPases). This micronutrient is able to exert in vivo anticlastogenic effect through suppression of ►micronucleus formation, sister-chromatid exchange, and structural, numerical and physiological chromosomal aberrations and may thereby prevent genomic instability. In aqueous solution, vanadium is found predominantly as oxo-anions (e.g., VO_4^{3-}) and as such may exhibit nucleophilic character for the electrophilic agents to attack, thereby preventing DNA ►alkylation damage as per the "carcinogen interception mechanism." although at the moment we cannot say if such processes take place within cells.

Experiments on various cell lines reveal that vanadate exerts its antitumor effects through activation of protein tyrosine kinases (PTKs) and/or inhibition of cellular PTPases leading to an accumulation of phosphotyrosine residues in cellular proteins. Both effects activate signal transduction pathways leading either to apoptosis and/or to activation of tumor suppressor genes. The stimulatory effect of vanadate on the cellular phosphorylation status of cytosolic proteins may be mediated in a dose-dependent manner probably via a ►protein kinase C-dependent mechanism. Vanadyl state is readily converted to vanadate in presence of H_2O_2, and thus vanadate seems to be the species that inhibits specific PTPase, and correspondingly increases the steady states of phosphorylation and thereby activates cytosolic PTKs at relatively low concentrations. Vanadium compounds have been shown to inhibit cell proliferation by limiting the expressions of several potential marker proteins, such as proliferating cell nuclear antigen, Ki-67 nuclear antigen, etc. In vitro antiproliferative activity of a variety of vanadium compounds may be exerted by their ability to interfere with the molecular interactions between GATA binding protein 1 and ►Nuclear Factor kappa B (NF-kappaB) transcription factors and target DNA elements. Vanadium complexes with a +5 oxidation state and their discrete anionic units appear essential for inhibition of tumor cell growth with induction of apoptosis, whereas a +4 oxidation state appears to be important in inhibiting transcription factors/DNA interactions.

Vanadium-induced apoptogenic signals are mediated through upregulation and overexpression of ►p53 tumor suppressor and proapoptotic Bax and by down-regulation of Bcl2. Reactive oxygen species generated

by Fenton-like reactions and/or during the intracellular one-electron reduction of V(V) to V(IV) by, mainly, NADPH participate in the majority of the vanadium-induced intracellular events. ROS/H_2O_2 generated by vanadate triggers ►DNA damage, and also activates ►mitogen-activated protein kinases (MAPKs) signal transduction pathways leading to an increased p53 protein expression and p53 phosphorylation, respectively. This in turn induces p53 transactivation, which subsequently leads to cell apoptosis. p53 is also an important transrepressor of inducible ►nitric oxide-synthase expression and thereby attenuates excessive nitric oxide production in a regulatory negative feedback loop.

Future Direction

Given what is known of vanadium effects on animal physiology, individual responses to vanadium treatment might be influenced by plasma- and cellular- binding proteins and genetic predisposition. Nonetheless, the potential therapeutic advantage of vanadium compounds and the available options for mitigating toxicities suggest that further development should yield safe and effective pharmacological formulations as antineoplastic agent.

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VANGL

Definition

Van Gogh-like proteins 1 and 2; four-transmembrane domain proteins involved in PCP signaling. VANGL2, also known as KITENIN, enhances tumor ►metastasis.

►Wnt Signaling

Variable Number Tandem Repeats

Definition

VNTRs.

►Minisatellite

Variant Allele

Definition

Gene containing one or more single nucleotide ►polymorphisms.

Variant Isoforms

Definition

Existence of more than one protein, generated from the same gene by alternative ►splicing of the nuclear RNA. Unlike some years ago, nowadays it is anticipated that variant protein isoforms are not the exception, but rather the rule.

►CD44

Vascular Disrupting Agents

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Synonyms

VDAs; Vascular targeting agents; Vascular targeted therapies

Definition

Vascular Disrupting Agents (VDAs) disrupt tumor blood flow by specifically targeting vascular abnormalities associated with solid tumor development. The

result is tumor vessel occlusion followed by extensive tumor necrosis.

Characteristics

Tumor growth is limited by the diffusion of oxygen and nutrients from blood vessels into the surrounding tissue. In order to grow beyond a size of ~1 mm^3, tumors are dependent upon their ability to induce ►angiogenesis (the formation of new blood vessels). As the tumor outgrows its blood supply, angiogenic factor (i.e. ►VEGF and ►bFGF) expression is induced, which stimulates the formation of new blood vessels.

The growth rate of tumors is such that angiogenesis must be continuously induced to keep a steady supply of oxygen and nutrients to the tumor tissue, resulting in a state of endothelial proliferation not normally present in the adult body. The vessels produced by this elevated angiogenic state are also abnormal in structure: tortuous vessels that experience transient interruptions in blood flow, and are often leaky with reduced or non-existent ►pericyte.

The abnormality of the tumor vasculature provides a novel target for anticancer therapy, with the idea of interfering with tumor growth through the pharmaceutical disruption of the tumor blood supply (termed ►vascular targeting). Several vascular targeting agents have since been identified and have begun entering clinical trials. The vascular targeting approach can be broadly divided into categories: the antiangiogenics and the Vascular Disrupting Agents (VDAs). The anti-angiogenic agents prevent the formation of new blood vessels thereby slowing tumor growth, while VDAs target and disrupt the existing tumor vasculature, which results in acute destruction of tumor tissue.

The angiogenic process is a multi-stage event including the upregulation of pro-angiogenic factors, increased endothelial cell proliferation, degradation of the vessel basement membrane, and endothelial cell migration and new tube formation. Each of these stages is a potential target for antiangiogenic agents, but agents inhibiting pro-angiogenic factors are perhaps the most common. The most studied angiogenic factor involved in tumor pathophysiology is VEGF, and several agents have been developed which either prevent the binding of VEGF to its receptors or inhibit activation of the receptor. The agent Bevacizumab is an anti-VEGF antibody that prevents VEGF from binding and activating its receptors and has had therapeutic success in clinical trials. The tyrosine kinase ZD6474 has been shown to inhibit angiogenesis by selectively preventing activation of the ►VEGFR-2 receptor and is also undergoing testing in clinical trials.

Vascular Disrupting Agents

The VDAs can be further divided into two basic categories: biological agents and small molecules. Biologic approaches, such as antibodies and fusion proteins, utilize antigens specific to tumor endothelium to target and destroy these cells, while the small molecules rely upon physiological differences to selectively destroy tumor, not normal, endothelial tissue.

The Biologic Approach

The concept of using fusion proteins to target and disrupt tumor vasculature relies upon two necessary components: a ligand that selectively binds tumor endothelium and a conjugated toxin. The result is precise delivery of the toxic agent to only the tumor endothelium and not normal tissues. Two fusion protein VDAs that have produced positive preliminary results have utilized angiogenic proteins to specifically target the tumor endothelium. Testing of a $VEGF_{121}$/rGelonin fusion toxin in nude mice bearing ►PC-3 tumors revealed specific localization of the conjugate to the tumor vasculature and resulted in tumor vessel thrombosis. The fusion complex of antibody against fibronectin conjugated to Tissue Factor was also shown to specifically target the tumor endothelium and treatment of tumor bearing mice with this agent resulted in disruption of tumor blood flow.

Small Molecule VDAs

►Flavonoids and ►tubulin binding agents are two classes of small molecule VDAs that are currently being evaluated. The flavonoids are believed to induce endothelial cell death primarily through the induction of ►inflammatory cytokines such as ►TNF-α. The tubulin binding agents' primary mechanism of action is the disrupting of the ►microtubule network of the tumor endothelium.

Flavonoids Agents

Flavonoids such as flavone acetic acid (FAA) and its fused tricyclic analogue 5,6-dimethylxanthenone-4-acetic acid (DMXAA) have been shown to induce extensive hemorrhagic necrosis in tumors as a result of vascular collapse. Mechanistically, the action of this class of agent is believed to be largely indirect, through the induction of cytokines, particularly TNFα. This view is supported by experimental evidence showing that antibodies to TNFα could inhibit FAA-induced vascular collapse. It is of interest to note that although both FAA and DMXAA selectively damage tumor blood vessels in pre clinical tumor models, only DMXAA induces TNFα in both human and mouse macrophages. Consequently DMXAA is currently considered to be the lead agent of this class of VDAs.

Tubulin Binding Agents

The tubulin binding agents ►colchicine and the ►vinca alkaloids were recognized to have anti-vascular

properties as early as the 1930s. However, the high toxicity of these agents limited their usefulness as vascular targeting therapies. More recently, agents with much more favorable therapeutic indexes, such as Combretastatin-A4-Phosphate (CA4P) and ZD6126, have rekindled interest in the use of this group of VDAs. These agents have shorter half-lifes and bind tubulin in a reversible manner, which reduces their toxicity to normal tissues.

Tubulin binding agents bind tubulin subunits and prevent their polymerization. The result is depolymerization of the microtubules, reorganization of the actin cytoskeleton, and increased cellular permeability, which has been shown to be particularly destructive to dividing endothelial cells. In vivo, VDA treatment induces rapid disruption of blood flow and collapse of the vascular network. Although the precise mechanism of tumor blood flow shut down and vessel disruption is not fully understood, in vitro experiments have demonstrated endothelial cell shape changes and interruption of vascular endothelial-cadherin (►VE-cadherin) signaling as a result of tubulin binding agent treatment. In vivo endothelial cell shape changes could augment vessel occlusion, while disruption of VE-cadherin results in reduced endothelial cell adhesion and could lead to vessel disruption. The tumor cells downstream of the disrupted blood vessels die due to a lack of oxygen and nutrients and the build up of toxic biological byproducts. After VDA treatment, necrosis is induced throughout the central portion of the tumor.

The tubulin binding VDAs should not be confused with other microtubule binding anticancer agents such as the ►taxanes. These agents bind to ►microtubules and prevent depolymerization, contrasting with the VDAs which bind the tubulin subunits to prevent microtubule elongation. Treatment with these agents has not been shown to result in tumor vascular collapse, and, consequently, anticancer therapies like taxanes are designed to directly target and destroy tumor cells, not the tumor vasculature.

VDAs as Adjuvants to Conventional Therapy

A common characteristic observed after VDA treatment is the presence of a so-called "►viable rim" of tumor cell existing at the tumor periphery. This thin layer of viable tumor tissue remains at the tumor periphery, presumably because this tumor tissue is supported by the normal vasculature, which is not affected by VDA treatment. The cells of this region are able to survive regardless of VDA dose or treatment schedule. These tumor cells continue to divide and rapidly repopulate the tumor, limiting the success of VDAs as single agents.

While VDA treatment does not entirely eradicate the tumor, a large proportion of the center of the tumor is killed. In an untreated tumor, the cells of this central region are typically hypoxic, slowly dividing, poorly perfused, and located in a high interstitial fluid pressure and low pH environment, making them more resistant to conventional anticancer therapies. As VDAs destroy this typically resistant region, addition of VDAs to current treatment regimens could improve treatment outcome when used in combination with this therapies.

Several VDA and conventional therapy combinations have been tested in the pre-clinical setting and demonstrated beneficial treatment outcome over either therapy alone. These studies found improved treatment outcome when the VDAs CA4P or ZD6126 were combined with radiation. VDA combination with radiation was also found to effectively treat large tumors successfully, more so than smaller tumors. The combination of ZD6126 with the antiangiogenic agent ZD6474 produced improved treatment outcome in the ►HT29 and ►OW1 tumor models over either treatment alone. A few combinations are currently being tested in various clinical trials.

VDAs such as ZD6126 and CA4P have been shown to produce maximum effective at doses well below the maximum tolerated dose. Neither does treatment with these agents alone result in significant growth delay, presumably due to the rapidly dividing tumor cells at the tumor periphery which survive VDA treatment. Consequently, there have been difficulties evaluating VDA treatment efficacy in the clinical setting. Preclinical evaluations of efficacy centered on the percent induced tumor necrosis, but these measurements are not practical in the clinical setting as they require highly invasive procedures that are not applicable to tumors in all locations. The viable tissue that causes difficulties with the evaluation of treatment efficacy also limits the usefulness of VDAs as single agents.

However, while the effects of these VDAs have been extensively studied for the tumor as whole, little data exists that describes how the characteristics of the tissue that survives VDA treatment change during the phase of VDA-induced damage. Changes induced in this region by VDA treatment have the potential to negatively impact the efficacy of agents used in combination with VDAs. By understanding the VDA effects upon the surviving tumor tissue, insight may be obtained into possible means of overcoming these limitations. For example, decreases in perfusion as a result of VDA treatment that do not result in tumor death may inhibit the delivery of chemotherapeutic agents to the affected region and decrease the ►radiosensitivity of tumors cells in the affected area. A few studies have reported varying combination treatment efficacies with differing treatment schedules. Therefore, a better understanding of the effects of VDA treatment on the surviving tumor tissue could lead to more effective combination treatment strategies.

►Vascular Targeting Agents

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Vascular Endothelial Growth Factor

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Synonyms

VEGF; vascular endothelial growth factor; VPF; vascular permeability factor

Characteristics

VEGF Family

Four VEGF family members have been described in mammals, VEGF-A through VEGF-D. VEGF-E, the fifth member of the family is coded by the Orf virus. An additional relative is the placenta-derived growth factor, PlGF. Whereas the VEGFs are potent growth promoting and vascular permeability enhancing factors, PlGF is incapable of inducing permeability. All members exert their biological functions as homodimers. VEGFs act almost exclusively on endothelial cells. VEGF is expressed by almost all cell types with the exception of endothelial cells which express only marginal amounts of the growth factor. Expression is controlled by a number of different mechanisms. Extracellular signals such as growth factors and cytokines are able to induce transcription of the VEGF gene. Activated oncogenes, such as ras, raf, or src, as well as inactivated tumor suppressor genes, such as ►p53 or ►von-Hippel-Lindau (VHL), contribute to enhanced transcription. The newly identified p53 analogue p73 in its wildtype form can cause repression of VEGF transcription. Hypoxia, a physiological signal in early embryonic development and a pathophysiological signal in many tumors, causes enhanced production of VEGF mRNA and also stabilizes the VEGF mRNA. The pattern of VEGF expression is strictly controlled by some of these factors in a time- and tissue-specific fashion during embryonic development and physiological angiogenesis. In pathological situations VEGF expression proceeds with no specific control, exceeds physiological concentration and occurs at wrong times and locations.

VEGF-Receptor Family

All VEGF family members mediate their signals through a family of distinct high affinity receptors; VEGF-R1 through VEGF-R3. All three VEGF receptors are tyrosine kinases that become stimulated upon ligand binding, with VEGF-R1 tyrosine kinase being much less activated than VEGF-R2 and VEGF-R3. VEGF-R1 is expressed as two distinct forms; the entire transmembrane receptor VEGF-R1 and a soluble variant sVEGF-R1 generated by alternatively spliced mRNA. VEGF-R1 and its soluble variant bind VEGF-A, VEGF-B and PlGF. VEGF-R2 has high affinity for VEGF-A, VEGF-C, VEGF-D and VEGF-E. VEGF-R3 binds VEGF-C and VEGF-D. All three VEGF receptors are exclusively expressed on the surface of endothelial cells. VEGF-R2 is the most important VEGF receptor mediating a proliferative response to endothelial cells. Upon transfection into non-endothelial cells VEGF-R2 becomes autophosphorylated in response to VEGF binding, but is no more mitogenic. This indicates the involvement of cell type-specific signaling mechanisms. The endothelial cell proliferation and survival in response to VEGF requires the association of cell surface adhesive molecules. Activated VEGF-R2 associates with integrins $\alpha v\beta 3$. VE-cadherin colocalizes with VEGF-R2 and upon stimulation by VEGF becomes associated with VEGF-R2, β-catenin and PI-3-kinase. This leads to activation of PKB/Akt and initiation of a survival signal. Disruption of VE-cadherin leads to prevention of VEGF-mediated cell survival. VEGF-receptor expression is under tight control in embryo development and normal physiology. In pathological situations, such as tumor angiogenesis, VEGF-R1 and VEGF-R2 are transcriptionally upregulated in response to VEGF and thus generate an amplification mechanism to enhance this fatal process.

VEGF in Vasculogenesis and Physiological Angiogenesis

VEGF is widely and abundantly expressed in many tissues during fetal development and has been implicated in the process of vasculogenesis, i.e. the de novo formation of the vascular system. Mice deficient for VEGF die at day 8.5–9.0 and show a delayed differentiation and an impairment of both vasculogenesis and angiogenesis, i.e. the sprouting of new capillary vessels from pre-existing vasculature. Similarly, the receptors VEGF-R1 and VEGF-R2 are strongly expressed in the developing embryo. In particular, VEGF-R2 is expressed in the hemangioblasts, the common precursor to both endothelial cells and hematopoetic lineages. VEGF-R1, being non-essential for endothelial development, is required

at a later stage of the organization of the embryonic vasculature. Disruption of the VEGF-R2 gene interferes with endothelial cell development, leading to death of embryos at day 8.5–9.5. Disruption of the VEGF-R1 gene permits endothelial cell differentiation but results in thin walled vessels of larger than normal diameter, and the embryos die at day 9.

In the adult organism, large amounts of VEGF are also found in the female reproductive tissues in association with hormonally regulated angiogenesis that takes place in the ovary and endometrium at specific stages of the menstrual cycle and in pregnancy. Strong expression of VEGF can be detected in several tissues of the adult in the absence of angiogenesis, particularly kidney, lung, adrenal gland and heart. However, some of these tissues express only low levels or no VEGF receptors which might explain the absence of angiogenesis. As VEGF has been shown to be a survival factor for vascular endothelial cells it might also be that this function, requiring only low levels of VEGF receptors, is important for maintaining vascular homeostasis without angiogenesis to occur.

Clinical Relevance

VEGF in Pathophysiological Angiogenesis

VEGF, as well as its receptors VEGF-R1 and VEGF-R2, are strongly overexpressed at both the mRNA and protein levels in almost all malignant tumors. Tumor metastases exhibit overexpression of VEGF similar to that found in the primary tumors from which they arose. Elevated VEGF levels have been found in the blood of tumor patients, correlating in many cases with poor clinical prognosis of the disease. Accordingly, the soluble variant of the VEGF-R1 is also found to be elevated in the blood of tumor-bearing patients indicating the presence of activated tumor endothelium in the diseased areas. Thus, VEGF and the soluble variants of VEGF-R1 could be regarded as surrogates for pathological tumor ►angiogenesis. In addition to the intimate involvement of VEGF and its receptors in tumor angiogenesis, VEGF is also capable of increasing vascular permeability. VEGF-induced leakage of plasma from hyperpermeable microvessels results in fluid accumulation within tumors. This is also favored by the fact that tumors in general lack lymphatic vessels and hence are unable to drain extravasated proteinaceous fluid effectively. This is in particular obvious in ►brain tumors, showing increased intracranial pressure and in tumors metastasizing to body cavities leading to substantial accumulation of fluid.

VEGF and both of its receptors are also overexpressed in a number of pathological entities that involve angiogenesis, but are not associated with neoplasia. These include diabetic and other retinopathies, rheumatoid arthritis and psoriasis. In all of these examples, overexpression of VEGF and its receptors is accompanied by increased microvascular hyperpermeability and pathological angiogenesis.

VEGF/VEGF Receptor System: Therapeutic Opportunities

As VEGF and its receptors are intimately involved in the pathology of many diseases, such as cancer, rheumatoid arthritis, diabetic retinopathies, considerable efforts have been made to interfere therapeutically with this signaling system. Monoclonal antibodies against VEGF and the binding domain of its receptor VEGF-R2 have been generated. Animal experiments show efficacy against tumor vascularization, tumor growth and metastases formation. Both antibodies have been humanized (►humanized antibodies) and are being evaluated in the clinic. Low molecular weight compounds were developed to inhibit the tyrosine kinase activities of VEGF-R1 and VEGF-R2. These compounds show considerable activity against growth of highly vascularized tumors and also inhibit metastasis formation. Some of these inhibitors also show activity against other non-VEGF receptor tyrosine kinases. Clinical evaluation is being carried out to demonstrate whether the additional non-VEGF receptor related activity is beneficial or not. Furthermore, combination of anti-VEGF strategies together with low dose cytotoxic strategies have revealed synergistic effects in animals. This indicates that anti-angiogenic therapy could be useful to enhance the therapeutic potential of conventional chemo-therapeutic drugs.

►Vascular Endothelial Growth Factor

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Vascular Maturation

►Vascular Stabilization

Vascular Normalization

Definition

Is the process whereby ►antiangiogenesis prunes and remodels the abnormal tumor vessels, which become closer to normal tissue vasculature in terms of structure and function.

Vascular Permeability Factor

►Vascular Endothelial Growth Factor

Vascular remodeling

►Vascular Stabilization

Vascular Stabilization

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Synonyms

Structural vascular stabilization; Functional vascular stabilization; Vascular maturation; Vascular remodeling

Definition

Vascular stabilization stands for the basic steps of the morphogenetic processes, finally leading to vascular maturation such as establishment of inter-endothelial contacts, development of a regularly structured basement membrane enveloping both endothelial cells and pericytes, and integration of one layer of peri-endothelial cells (pericytes or smooth muscle cells) into the vascular wall.

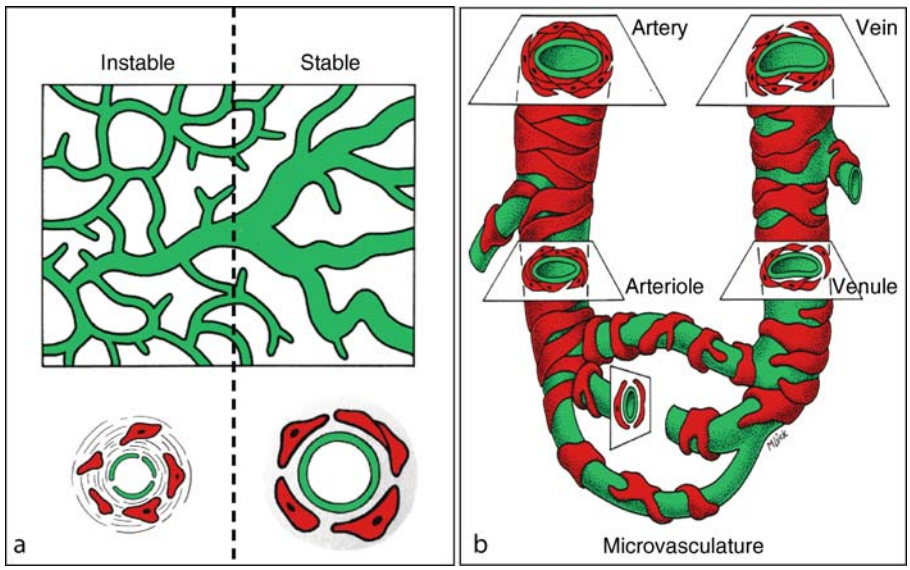
Characteristics

Normal Vascular Hierarchy

The adult vascular system is hierarchically organized in large, middle sized, and micro vessels at both the arterial and venous sides. The lumen of all blood vessels is lined by endothelial cells (EC). EC are of mesodermal/mesenchymal origin and differentiate from hemangioblasts; they are probably the common precursor of endothelial and hematopoietic cells. The first hemangioblasts are visible in the blood islands of chorionallantoic membrane and the peripheral cells of these islands differentiate to endothelial cells, while the rest serve as the source for hematopoiesis. The primitive vascular network developed in the chorionallantoic membrane subsequently connects to the cardiac system and serves as the basis for a functioning blood circulation. However, this system comprising nascent blood vessels will undergo enormous remodeling processes containing several sequential steps which are precisely coordinated timely and spatially. The essential step of the vascular remodeling in the early phase of vascular development is structural stabilization, which involves the development of inter-endothelial contacts and basement membrane and integration of peri-endothelial cells into the vascular wall (Fig. 1). Accompanied by organogenesis and the demand on blood perfusion, further processes of vascular maturation will lead to the development of the vascular wall containing several layers of smooth muscle cells and connective tissue, depending on perfusion pressure and exchange processes between the vascular and interstitial compartments. The factors and mechanisms regulating these processes are also involved in the regulation of vascular permeability, which also depends on organ-specific requirements. The end of these processes is the construction of a vascular hierarchy comprised of large and mid-sized arteries and veins macroscopically visible, as well as arterioles, capillaries and venules making up the micro vascular part of the blood vessels (Fig. 1). As far as we can observe, the vascular stabilization and the final vascular maturation result in a decrease of vascular density as blood vessels which are not included in the organ perfusion by blood undergo regression and successively disappear (Fig. 1).

Vascular Destabilization and Activation of Angiogenesis

The initiation of ►angiogenesis is marked by structural destabilization of the vascular wall, generally accompanied by an abnormal vascular permeability. This is also a common sign of the tumor vascular bed. Looking at the structure of tumor vessels, a structural instability marked by the loosening of pericytes/smooth muscle cells from the endothelial layer, opening of inter-endothelial junctions (►Tight junctions), development of endothelial fenestration and/or transendothelial gaps



Vascular Stabilization. Figure 1 From the primitive vascular plexus to the hierarchy of normal vascular system: the primitive dense vascular plexus is mainly composed of unstable nascent blood vessels of approximately equal state of vascular stabilization (a, *left*). Cross section shows that BM is not regularly structured and pericytes (*red*) are not tightly connected to the endothelial layer (*green*). Vascular stabilization during embryogenesis and fetal development have led to a decrease of vascular density (a, *right*). In cross section (a, *right*) a dense BM (*gray*) is visible, endothelial cells (*green*) build a closed layer and mural cells (*red*) are enclosed by the same BM and are tightly organized around the endothelial layer. These processes serve as the basis for the first step of vascular hierarchy in the adult as demonstrated in b.

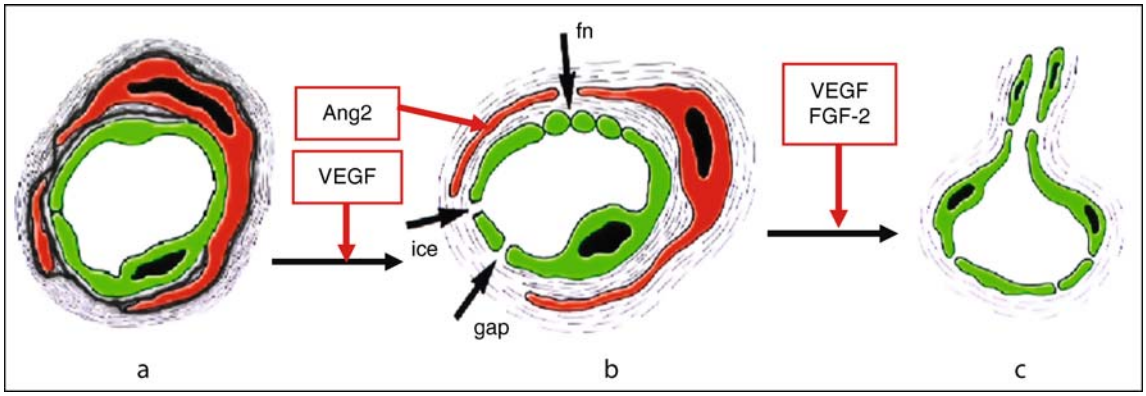
and degradation of the vascular basement membrane are frequently observed alterations of the vascular wall in this initial phase of angiogenesis (Fig. 2). This initial destabilization is probably induced by a switch of the angiogenic balance towards the activation of angiogenic sprouting. The further duration of this process is mediated by pro-angiogenic factors such as VEGF (►Vascular endothelial growth factor), FGF-2 (►Fibroblast growth factors) and Ang2. It would lead to complete destabilization and disintegration of the endothelial layer. The detachment of endothelial cells from the basement membrane results in the migration and subsequent proliferation of these cells. Finally, new capillary sprouts formed by the new endothelial cells are nascent and structurally unstable. The maintenance of pro-angiogenic activation would hold these new vessels in structural instability and in an abnormal leaky state. This phenotype of vessels dominates in the tumor vascular bed. Particularly, the concerted action of VEGF and Ang2 at certain sites of tumor vasculature has been shown to be very effective in keeping the blood vessels unstable and angiogenesis ongoing.

Vascular destabilization accompanied by abnormal vascular leakiness is also frequently observed during inflammation. It is well known that several cell types involved in the inflammatory process such as macrophages or lymphocytes produce high amounts of pro-angiogenic factors including VEGF and bFGF. Also Ang2 which is involved in vascular destabilization has been detected in several inflammatory processes. Thus, inflammation seems to provide high amounts of VEGF and Ang2, and the concerted action of both is the main mediator of vascular destabilization.

Vascular Stabilization

While it has been assumed for several years that new vessels provided by angiogenesis are lined only by endothelial cells and would not be able to enter further steps of vascular maturation, detailed studies in the last decade have revealed without doubt that some of the new vessels developed by physiologic or pathologic angiogenesis as in tumors (►cancer) do exhibit a basement membrane and mural cells such as pericytes and/or smooth muscle cells. As of now, only a few factors have been characterized as being involved in these processes. To give a systematic overview of the vascular stabilization, it makes sense to evaluate the following morphogenetic steps.

1. *Inter-endothelial contacts:* Like cell-cell contacts between epithelial cells, TJ or ►zonula occludens, adherence junctions (AJ) or zonula adherens and ►gap junctions (GJ) are the main cell-cell contact types between endothelial cells, but in contrast to



Vascular Stabilization. Figure 2 Vascular destabilization and initiation of angiogenesis: a normal stabilized capillary with a dense BM (*gray*) enclosing both endothelial cells (*green*) and pericyte (*red*) (a) will be destabilized by the action of pro-angiogenic factors as shown by VEGF and Ang2 leading to endothelial fenestration (fn), opening of inter-endothelial contacts (iec), development of transendothelial gaps (gap), degradation of BM and finally detachment of pericytes from the endothelial layer (b). These morphogenetic events are accompanied by an abnormal vascular leakiness. The further duration of pro-angiogenic action would finally lead to the sprouting of new nascent and unstable blood vessels (c), a process defined as angiogenesis.

epithelial cells, ►desmosomes are absent between endothelial cells. Also, in endothelial cells, TJ are localized at the boundary between the apical and basolateral sides of endothelial cells and thus, they are important for the barrier function. The members of the claudin family, occludin and junctional adhesion molecule-A (JAM-A) as well as their cytoplasmic interaction partners such as the members of the ZO (zonula occludens proteins) are the main players that govern the establishment of endothelial TJs. Also ESAM, the endothelial cell-selective adhesion molecule, is localized at the TJ and regulates the paracellular permeability of the endothelial barrier. VE-cadherin (vascular endothelial cadherin) (►Adherens junctions) is essential for the establishment of adherence zones between endothelial cells. The main intracellular partners of VE-cadherin are β- and γ-catenin. They link α-catenin, which anchors this complex to actin. A further intracellular partner of VE-cadherin is p120, a substrate of src. Of particular impact for vascular stabilization versus destabilization is the interaction of VE-cadherin with endothelial signaling proteins. VE-cadherin, VEGFR-2 (VEGF receptor type 2, KDR) and Src-kinase form a complex which is obviously essential for the maintenance of the endothelial barrier controlling the paracellular transport of molecules and cells through the endothelial layer. In contrast, the interaction of VE-cadherin with VE-PTP (vascular endothelial-specific receptor-protein tyrosin phosphatase) strengthens the AJ between endothelial cells. Gap junctions (GJ) represent intercellular channels mediating exchange of ions and small molecules between neighboring cells by diffusion. The establishment of GJ is governed by ►connexins; ~20 connexins (Cx) have been identified until now. Expression studies demonstrate the presence of Cx37, Cx40 and Cx43 between endothelial cells but the role of connexins in the inter-endothelial communication is not sufficiently understood. Considering the fact that the expression pattern of these connexins in the blood vessels depends on the vessel type and the position in the vascular tree a regulating role of the connexins in differentiation or maturation of blood vessels can be postulated. Although there are several excellent studies regarding the expression and the role of each factor involved in cell-cell contact between endothelial cells, it is not clarified at exactly which stage of vascular morphogenesis these contacts are established. Particularly the determination of temporary and spatial sequences of these processes needs to be evaluated. Based on recent studies it can be postulated that in the first step of vascular morphogenesis, where the nascent vessels are lined only by endothelial cells, the first provisional inter-endothelial contacts are mediated by ►cell adhesion molecules like integrins, VCAM, ICAM and ►CEACAM1. Particularly, CEACAM1 has been shown to be involved in vascular stabilization in a dual way: CEACAM1 overexpression in endothelial cells induces a signaling leading to vascular stabilization, while its down-regulation in epithelial cells as it occurs in several tumors leads to vascular destabilization. In a second step the special cell-cell contacts mentioned above will be established between

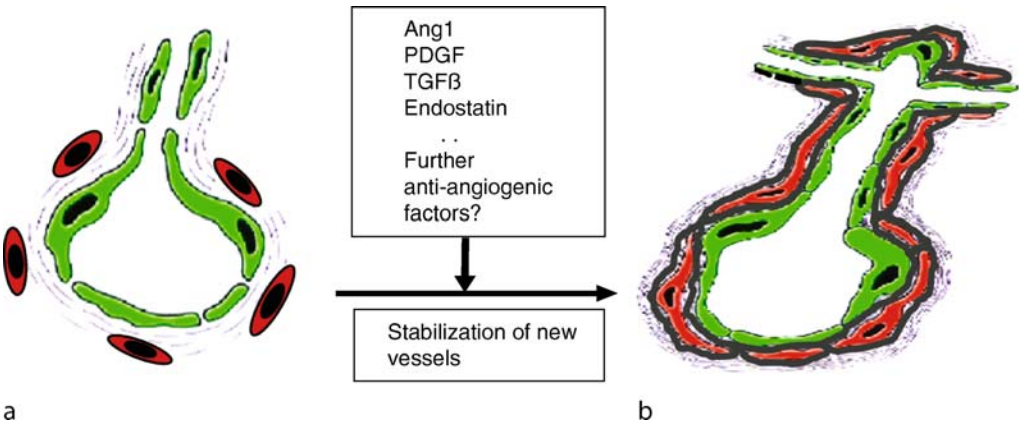
endothelial cells serving the basis for the entering of nascent and still unstable vessels into the stabilization process.

2. *Construction of the vascular* ►*basement membrane (BM):* The BM (or electron microscopically basal lamina) is an extracellular matrix structure of flexible thickness of 40–120 nm underlying all epithelial cell sheets and tubes formed by endothelial cells. In general, the assembly of BM is mainly provided by collagen type IV and laminin, which have the capacity to self-assembly, but several other molecules like perlecan, nidogens, and collagen type XVIII are identified components of BM and necessary for its regular construction. In transmission electron microscopic studies of the normal vasculature, the basal lamina (corresponding structure to basement membrane) shows an amorphous and dense structure. In small blood vessels BM encloses both endothelial cells and pericytes. The dense structure of BM is mostly not present in the basal lamina of tumor blood vessels, indicating a degradation or insufficient construction of the BM. This is one of the essential parameters leading to the loss of endothelial anchoring to the extracellular matrix and to detachment of pericytes from the endothelial layer with subsequent migration and proliferation of endothelial cells. The components of well structured BM signal an inhibitory effect on angiogenesis while the direct contact of endothelial cell to the "provisional" matrix components such as collagen type I, fibronectin and vitronectin accelerates the proliferation and migration of endothelial cells. BM collagens contain cryptic domains with anti-angiogenic activity (►Antiangiogenesis) if they are released from their mother substance as demonstrated by ►endostatin, a fragment of collagen type 18, and tumstatin, a fragment of collagen type IV. Endostatin has been shown to stabilize newly formed endothelial tubes and to strengthen the endothelial barrier by protecting the basement membrane and interendothelial contacts in normal structure. Taken together, these data suggest that a well constructed vascular basement membrane stabilizes the newly formed endothelial tubes and reduces the angiogenic potency. Indeed, in experimental models and in preclinical treatment with different types of angiogenic inhibitors (►Antiangiogenesis) ►stabilization of the vascular wall and the decrease of vascular leakiness are frequently observed phenomena.
3. *Integration of vascular mural/peri-endothelial cells into the vascular wall:* The assembly of pericytes or smooth muscle cells to the vascular wall is an essential step of vascular stabilization. Factors involved in this process are Ang1 and Tie-2-system, TGFβ, PDGF and their receptors. Although the vascular mural cells are of multiple origins, their presence and interaction with endothelial cells are crucial for vascular stabilization. Ang1 is not only involved in the assembly of pericytes into the vascular wall but it also mediates cell-cell and cell-matrix interaction, leading to a significant reduction of vascular leakiness. Ang1- and Tie-2 knock-out mice are lethal because they lack vascular plasticity and remodeling resulting in disorganization of the primitive vascular plexus and apoptosis of endothelial cells. In contrast, Ang1 overexpression in mice blocks the VEGF-induced vascular leakiness and increases the diameter of blood vessels. To reduce the role of pericytes in vascular morphogenesis only to a mechanical supportive function for the endothelial cells would be very simplifying and not appropriate. Via direct signaling facilitated by cell-cell contacts between endothelial cells and pericyte processes through the basement membrane, pericytes influence the maturation and quiescence of endothelial cells. But these actions of pericytes on endothelial cells can only be effective when pericytes are well integrated into the vascular wall enclosed by the same basement membrane as endothelial cells (Fig. 3).

Clinical Implications

In several diseases the vascular dysfunction is either an accompanied clinical problem making therapeutic handling much more difficult such as in diabetic retinopathy and microangiopathy, or it is an essential prerequisite for the full development of diseases such as cardiovascular failure or tumor growth and metastasis. Since cardiovascular diseases rank first and tumor second in the list of fatal diseases world wide, the therapeutic managing of vascular morphogenesis is a big challenge in medicine. The role of vascular stabilization in the process of vascular morphogenesis, plasticity and remodeling is still not sufficiently understood because it was neglected for a long time. In the last few years, the mechanisms of vascular stabilization, particularly the interaction between endothelial and vascular mural cells, have received more attention in both cardiovascular and tumor angiogenesis research. While anti-angiogenesis targets the tumor vasculature to cut blood supply to the tumor tissue, the pro-angiogenic therapeutic strategies deal with the creation of new vessels, which should have the capacity, as far as possible, to achieve a structural stabilization and functional normalization.

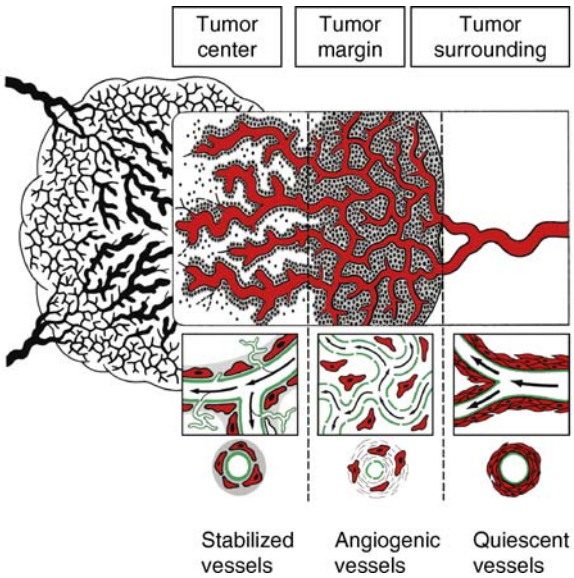
Therapeutic angiogenesis and vascular stabilization: "One is as old as one's arteries" is a frequently cited statement from Virchow and underlines the impact of the arteries in determining the life span, and also the quality of life. Cardiovascular disorders such coronary artery disease or the sclerotic changes of the peripheral



Vascular Stabilization. Figure 3 Vascular stabilization: the wall of a newly formed unstable blood vessel is constructed by endothelial cells (green) with provisional interendothelial contacts, provisional BM without the regular dense organization and mural cells (red) which are present but not regularly integrated into the vessel wall (a). Several endogenous factors as shown by Ang1, TGFβ, PDGF and endostatin, promote the structural stabilization and serve in a concerted interplay with other factors such as VE-cadherin, occludin, claudins, collagen IV, laminin and integrins as the basis for establishment of durable interendothelial contacts, a regular BM and mural cells tightly integrated into the vascular wall, a process named vascular stabilization.

arteries are mostly caused by degenerative alteration of the vascular wall at the arterial part of the vascular system. In all these disorders the formation of new vessels, for example the initiation of the growth of collateral vessels, is desirable, but it is difficult to bring the new vessels in a stable state, fulfilling the specific tissue demands on vascular perfusion and permeability. Although therapeutic angiogenesis in preclinical studies shows promise, no clear success has been achieved in controlled clinical phases II and III studies until now. These results demonstrate how complex the processes are which govern the morphogenesis of blood vessels, but they also show how difficult the way is from experimental models to human trials. Despite these ups and downs, the therapeutic angiogenesis still remains a fascinating perspective, highly promising for the treatment of cardiovascular and ischemic disorders.

Anti-angiogenic tumor therapy and vascular stabilization: The destabilization of blood vessels accompanied by abnormal vascular leakiness is one of the earliest signs of angiogenic activation. This vascular phenotype dominates the tumor vascular bed. Although it was believed for a long time that tumor vessels lack BM and ►pericytes, detailed studies in the last 5–6 years have revealed that in tumor vessels also, endothelial tubes are underlined by a BM and that pericytes are organized around these tubes. But in contrast to normal vasculature, the BM of tumor blood vessels is not well structured, the interendothelial contacts are not strong enough, and the pericytes are not tightly connected to the endothelial tubes. This vascular phenotype has a high plasticity, making it highly susceptible to pro-angiogenic factors such as VEGF and Ang2. While the link between vascular destabilization and angiogenic activation, as well as tumor vascularization, has been known for several years, well studied and understood to a great extent, the impact of vascular stabilization on tumor vascularization, tumor growth and metastasis (cancer) was mostly neglected until a few years ago. Based on emerging data from anti-angiogenic therapy, a "normalization" of tumor vessels has been postulated. According to this hypothesis, the anti-angiogenic therapy would create a "therapeutic window," enabling a more effective use of chemo- or radiation therapy for tumors. This suggests an indirect relation between vascular stabilization and tumor therapy, but what is the role of vascular stabilization per se on tumor angiogenesis and tumor growth and metastasis? The best known factor leading to vascular stabilization is Ang1. Ang1 overexpression blocks the VEGF-induced vascular leakiness by stabilization of blood vessels. In mice overexpressing Ang1, tumor vascularization was seen to be less active than those overexpressing Ang2 or tumor growth was suppressed. But there are also studies suggesting that vascular stabilization, for example by Ang1, results in the acceleration of tumor growth. These controversial findings demonstrate clearly that this aspect of tumor angiogenesis has not been studied sufficiently. Hypothetically, one would expect that vascular stabilization reverses the angiogenic phenotype of blood vessels to a more quiescent phenotype by reducing susceptibility of tumor vessels to pro-angiogenic factors. This in turn would cause a reduction of vascular density and part



Vascular Stabilization. Figure 4 Vascular stabilization and tumor growth: Stabilization of tumor vessels occurs mostly in the tumor center but significantly extends to the marginal zone under anti-angiogenic therapy, e.g. with endostatin. This is accompanied by a dramatic regression of a part of the blood vessels, resulting in a significant reduction of vascular density. This process apparently changes the perfusion of tumor tissue. In the marginal tumor zone with unstable blood vessels, there is a nearly equal perfusion of the whole vascular bed as marked by arrows. In contrast, in the tumor center, the main route of blood flow is served by stabilized vessels while unstable blood vessels were successively cut of function and underwent regression. This may result in further necrosis of tumor tissue. Green: endothelial lining, grey: vascular basal lamina.

starvation of tumor tissue, resulting in tumor necrosis (Fig. 4). This has been observed in several experimental tumor models and experimental tumor treatments with different anti-angiogenic substances. It is imaginable that we principally need a two-step approach of anti-angiogenic tumor therapy: the first step should aim to achieve a disruption of new nascent and unstable blood vessels and a suppression of endothelial proliferation, and the second would deal with the stabilization of tumor vessels switching tumor vasculature from the angiogenic to quiescent phenotype. Both these steps would serve the basis for a therapeutic "window" enabling the combination of angiogenesis inhibitors and chemotherapeutic drugs (►Chemotherapy) and/or radiation (►Radiation oncology) in the final step of tumor therapy. Taken together, emerging data recommend a stronger consideration of mechanisms that govern vascular stabilization in future anti-angiogenic tumor therapy strategies.

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Vascular Targeted Therapies

►Vascular Disrupting Agents
►Vascular Targeting Agents

Vascular Targeting Agents

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Synonyms

VTAs; Vascular targeted therapies; Angiogenesis inhibiting agents; Vascular disrupting agents

Definition

Vascular targeting agents (VTAs) are primarily cancer therapies that are specifically designed to target the vasculature of tumors and as a consequence will inhibit tumor growth and development. They may also be used to treat other pathophysiological conditions in which the tissue vasculature plays a role.

Characteristics

Background

In cancer, the vascular supply to tumors is critical. For most solid tumors to grow beyond a size of a few

millimeters it is necessary for them to develop their own functional blood supply, which they do from the already established normal tissue vasculature by a process called ►angiogenesis. This process begins with the tumor cells secreting various angiogenic growth factors. These factors are upregulated by different environmental changes such as ►hypoxia, loss of ►tumor suppressor function or ►oncogene activation. Of these angiogenic factors, the most potent and specific is ►vascular endothelial growth factor (VEGF), which is not only crucial for endothelial cell proliferation and blood vessel formation, but also induces significant vascular permeability and plays a key role in endothelial cell survival signaling in newly formed vessels. These growth factors react with various receptor kinases on the endothelial cells and then put in motion a series of physical events that include destruction of the basement membrane of the normal endothelial cells, migration of endothelial cells into the extracellular matrix in the form of a sprout, division of endothelial cells away from the sprout tip, the formation of solid strands of endothelial cells in the extracellular matrix, the development of a lumen within the strands, fusion with other sprouts to form loops, and the formation of new sprouts and loops from these primary loops. All this ultimately results in the establishment of a functional vascular supply for the tumor. Once this occurs not only can the primary tumor begin to grow, but the tumor cells have a means of entering the systemic circulation and so move to other areas in the body and then ultimately form ►metastases.

This importance of the tumor vasculature makes it an excellent target for therapy and two major VTA approaches have now evolved. The first is based on controlling the development of the tumor blood vessels by inhibiting the angiogenesis process, while the second involves a disruption of the already established tumor blood vessels. It has been demonstrated that vascular effects are involved in the action of other therapies, including certain types of ►chemotherapy, radiotherapy, and inhibitors of epidermal growth factor receptors or cyclooxygenase-2. But, in these situations the vasculature is far from being their principal target and as such it is technically incorrect to classify them as VTAs.

Mechanisms

Although angiogenesis inhibiting agents (AIAs) and ►vascular disrupting agents (VDAs) both target the tumor vascular supply they are two distinct approaches. AIAs are designed to prevent further development of the tumor neovascular network. The complex process of tumor angiogenesis offers many possible targets for ►antiangiogenesis strategies. These strategies vary from regulation of angiogenic factor expression in tumors to endogenous inhibitors of angiogenesis. Based on their biological activities, these strategies can be categorized into several broad classes. One class of agents specifically targets the angiogenic growth factors, which play the most significant role in neo-vascularization, especially VEGF. VEGF has been targeted by a variety of strategies, including inhibitors of endothelial cell receptor signaling that interfere with associated ►receptor tyrosine kinase activities (e.g., SU5474, SU6668, ZD6474 and PTK787/ZK 222584), as well as monoclonal antibodies directed against pro-angiogenic growth factors (e.g., Bevacizumab/Avastin and DC101). Bevacizumab/Avastin, a recombinant humanized monoclonal antibody to VEGF, is the first anti-angiogenic therapy to have demonstrated a survival advantage when given to patients with cancer. It is currently being investigated in a variety of tumor types. A second class of agents includes those designed to inhibit endothelial cell functions, basement membrane degradation, endothelial cell migration, proliferation, and tube formation. One example is ►Endostatin, a naturally occurring fragment of collagen XVIII, which has been identified as a potent endogenous inhibitor of endothelial cell function. A third class consists of agents that target survival factors of neovascular blood supply, such as integrin antagonists. Integrins are heterodimeric transmembrane proteins that control cell motility, differentiation and proliferation via interactions with extracellular matrix molecules. The αvβ3 integrin is an attractive target for anti-angiogenic therapy because it is almost exclusively present on the cell surface of activated endothelial cells and is considered a survival factor for angiogenic vessels in tumors. Finally, non-specific therapies that impact new vessel development are also being actively considered. ►Thalidomide and its analogues are one such group of agents that inhibits angiogenesis, but the mechanism of action is poorly understood.

VDAs are agents that cause direct damage to the already established tumor endothelium. These include physical treatments like hyperthermia or ►photodynamic therapy, which have been well documented to induce direct tumor cell killing and an indirect effect through the induction of vascular damage. They also include biological response modifiers or cytokines like tumor necrosis factor (TNF) and interleukins; certain established chemotherapeutic drugs such as vinka alkaloids and arsenic trioxide; and various ligand-based approaches that use antibodies, peptides or growth factors that can selectively bind to tumor vessels. But, more commonly VDAs involve the use of small molecule drugs, of which there are two major classes of agents. The first includes flavone acetic acid (FAA) and its derivative DMXAA, which have a complex mechanism of action that is poorly understood, but their main effect on vascular endothelial cells is thought to involve a cascade of direct and indirect effects, the latter

involving the induction of cytokines, especially TNF-α, leading to the induction of hemorrhagic ►necrosis. A second group includes the tubulin-binding agents CA4P, ZD6126, AVE8062, NPI2358, MN-029, and OXi 4503. These tubulin depolymerizing agents are believed to selectively disrupt the cytoskeleton of proliferating endothelial cells, resulting in endothelial cell shape changes and subsequent thrombus formation and vascular collapse. Since they preferentially target dividing endothelial cells this accounts for their tumor specificity. Both types of small molecular drugs have been shown to have potent anti-vascular and anti-tumor efficacy in a wide variety of preclinical models and the lead agents are undergoing clinical evaluation.

Since AIAs and VDAs induce vascular effects by very different mechanisms their anti-tumor activity and optimal application will be very different. Generally, AIAs are given as a chronic administration and essentially slow tumor development. There are examples where tumor growth can be completely inhibited or the treatment of established tumors can result in tumor regression, but these tend to be exceptions rather than the norm. As a result AIAs are probably best suited for early stage or metastatic disease. With VDAs the administration is of a more acute type to induce substantial vascular shut down. Anti-tumor effects should also be possible with lower doses given over a prolonged period, but that would probably increase the risk of normal tissue vessel damage and defeat the potential benefit. Following treatment with VDAs tumor shrinkage has been observed, but this appears to be tumor and drug dependent and although significant it is generally modest and thus tumor growth is only temporarily delayed. There is good evidence that VDAs have a superior effect on bulky disease. Given the key differences between AIAs and VDAs it should be clear from a therapeutic perspective that targeting the tumor vasculature with AIAs and VDAs is complimentary and not redundant. Such approaches are being actively pursued.

Clinical Aspects

It is clear that neither AIAs nor VDAs, whether given alone or even in combination, can induce tumor control. Therefore, their clinical potential as anti-cancer therapies requires that they must be combined with other cancer therapies, and numerous pre-clinical studies have demonstrated that when this is done significant improvements in tumor response are possible. For AIAs, this has been demonstrated when they have been combined with conventional treatments like radiation (►ionizing radiation therapy) and chemotherapy, including ►alkylating agents (e.g., ►cisplatin, carboplatin, melphalan, cyclophosphamide and dacarbazine), nitrosoureas (e.g., BCNU), antimetabolites (e.g., ►gemcitabine, 5-►fluorouracil, and pemetrexed), anthracyclines (e.g., doxorubicin), topoisomerase inhibitors (►topoisomerase enzymes as drug targets) (e.g., ►irinotecan, topotecan and etoposide), taxanes (e.g., ►paclitaxel and ►docetaxel), corticosteroid hormones (e.g., prednisone) and ►bioreductive drugs (e.g., ►mitomycin C). AIAs have to a lesser extent also been combined with hyperthermia and photodynamic therapy. With VDAs the combinations have also included radiation and various chemotherapy agents, such as alkylating agents (e.g., cisplatin, carboplatin, melphalan, cyclophosphamide and chlorambucil), antimetabolites (e.g., 5-fluorouracil), anthracyclines (e.g., doxorubicin), topoisomerase inhibitors (e.g., irinotecan and etoposide), taxanes (e.g., paclitaxel and docetaxel), vinka alkaloids (e.g., vincristine and vinblastine) and bioreductive drugs (e.g., mitomycin C, tirapazamine and AQ4N). Less conventional therapies combined with VDAs include hyperthermia, radioimmunotherapy, and antibody/clostridia directed enzyme prodrug therapy.

One important issue here concerns the pathophysiological effects induced by VTAs. As a result of targeting tumor vasculature, VTAs modify the tumor pathophysiology and these include changes in vascular density, blood perfusion, oxygenation, metabolic activity, intracellular/extracellular pH and interstitial fluid pressure. Many of these pathophysiological changes can have a profound influence on the activity of the other combination therapy, and since the reported changes include both increases and decreases the effects can be in both a negative and positive fashion. For example, increasing tumor oxygenation status will enhance tumor radiation response, while decreasing oxygenation will reduce it. With the AIAs these pathophysiological changes can be highly variable between the different drug types. The same AIA can also produce completely opposite pathophysiological affects in different tumor types, even when administered using similar drug doses and treatment schedules. VDAs are more consistent in the pathophysiological changes induced. Essentially they all decrease tumor blood perfusion and as such will make the microenvironmental conditions, especially oxygenation and pH, worse. These effects suggest that timing and sequence between the different treatments must be considered in any clinical application, especially when combining VTAs with therapies that already have some beneficial effect in patients. Despite these potential limitations, preclinical studies with VTAs in combination with other therapies do show an enhanced tumor response without any significant increased damage in dose limiting normal tissues.

Numerous VTAs are currently under clinical evaluation. With AIAs these include both specific and non-specific inhibitors of angiogenesis, and the phase of testing ranges from Phase I to IV. The most popular agents in clinical testing are the anti-VEGF antibodies

(e.g., Avastin), followed by receptor kinase inhibitors (e.g., Bay 43–9006, SU 11248, PTK 787/ZK 222584), and the non-specific inhibitor thalidomide. Far fewer trials are underway with the VDAs, and those agents that are being investigated are either in Phase I or II. The lead agent in this series is CA4P. Experimental studies strongly support the concept that the application of angiosuppressive and vascular disrupting strategies as adjuvants to standard anticancer therapy can improve treatment outcomes. Lead vascular targeting agents are now under active investigation in such settings in patients. Ultimately, it is possible to envisage future treatment protocols consisting not only of the current mainstays of cancer management, surgery, radiotherapy, and chemotherapy, but will also include a "vascular targeted therapy" consisting of a battery of tumor vessel directed agents.

►Vascular Disrupting Agents

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Vascular Targeting Agents

►Vascular Disrupting Agents

Vasculogenesis

Definition

Is the reorganization of randomly distributed cells into a blood vessel network.

►Vasculogenic Mimicry

Vasculogenic Mimicry

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Definition

The generation of microvascular channels by genetically deregulated, aggressive tumor cells was termed "vasculogenic mimicry" (VM) to emphasize their de novo generation without participation by endothelial cells. VM is thought to represent a vascular channel formation without the involvement of endothelial cells, in contrast to ►angiogenesis. Vasculogenic mimicry refers to a blood supply pathway in tumors that is formed by tumor cells and that is independent of endothelial cell-lined blood vessels. Three factors are thought to govern the formation of functional and patterned microcirculation channels by VM: (i) plasticity of highly malignant tumor cells, (ii) remodeling of the ►extracellular matrix (►ECM), and (iii) connection of the VM channel with host blood vessels to acquire blood supply from the host tissue. Formation of VM in tumors may have substantial impact on clinical outcome of tumor patients. Tumor patients in the presence of VM have a poorer prognosis than those without VM, and ►VM-targeted therapy is a perspective for tumors showing VM. At this point, VM is a new concept originally described for ►melanoma that needs to be studied further in detail.

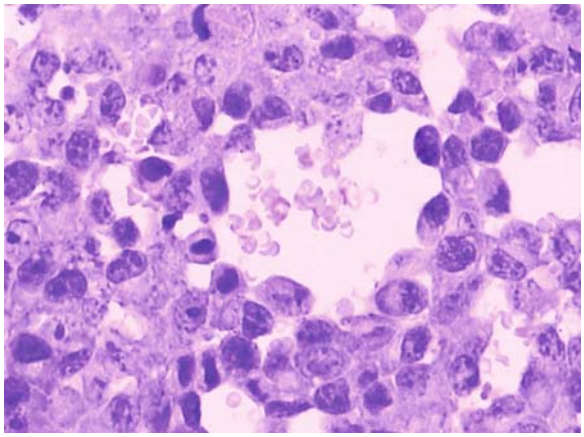
Characteristics

Introduction

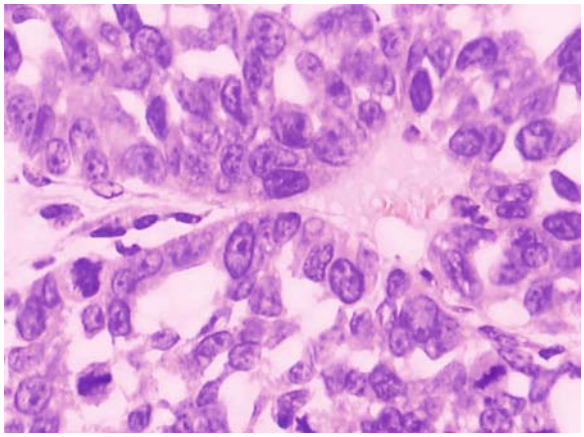
Tumor angiogenesis is a key for tumor growth, ►invasions, and metastasis. Tumor growth is ►angiogenesis-dependent, and angiogenic switch is an essential step for a small and noninvasive tumor to transit into a tumor with invasive and metastatic ability. Blood vessels are assembled by two processes: (i) ►vasculogenesis, the reorganization of randomly distributed cells into a blood vessel network, and (ii) ►angiogenesis, the sprouting of new vessels from preexisting vasculature in response to external chemical stimulation.

Current Status of Studies on VM in Tumors

It is believed that VM consists of tumor cells and PAS-positive ECM on the inner wall of channels. The constituents of PAS-positive ECM are ►laminin, collagens IV and VI, mucopolysaccharide, and heparin sulfate glucoprotein (HSPG). Initially, PAS-positive ECM was considered as an absolutely indispensable element. However, VM channels in the absence of



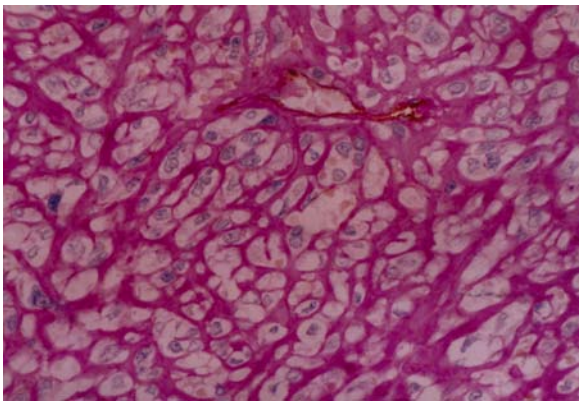
Vasculogenic Mimicry. Figure 1 Vasculogenic mimicry (VM). Melanoma cells form VM channels, and red blood cells (RBC) flow into the channel. Necrosis and inflammatory cells are not observed in tumors undergoing VM.



Vasculogenic Mimicry. Figure 2 The connection of VM channel and endothelium-dependent vessel shows VM is a functional microcirculation.

PAS-positive ECM were observed in a melanoma mouse model.

VM is an independent blood supply pattern in tumors (Figs. 1–3). Compared with endothelium-dependent vessels, it has several characteristics as follows: (i) VM channels are lined by tumor cells but not endothelial cells. (ii) There is red blood cell (RBC) leakage into tumor tissue near to endothelium-dependent vessels, while leaking RBCs are lacking in tumors with VM. (iii) Necrosis and inflammatory cells are not observed in tumors undergoing VM.



Vasculogenic Mimicry. Figure 3 VM consists of tumor cells and PAS-positive ECM on the inner wall of channels. The PAS-positive patterns are lined by tumor cells, and there are red blood cells in the center of the pattern.

Molecular Mechanisms Underlying VM

Compared with less aggressive melanoma cells, highly aggressive melanoma cells express higher levels of ►matrix metalloproteinases (►MMP-1, 2, 9, and 14) and the 5γ2 chain of laminin. This increases expression of MMPs and presence of the laminin receptor on the surface of tumor cells help cells adhere to more laminin. The activated MMPs cleave laminin into several short chains and eventually promote the formation of VM. Phosphoinositide-3-kinase modulates the function of MMP-14 (MT1-MMP), which activates MMP-2 with the help of tissue inhibitor of MMP-2 (TIMP2), and the activated MMP-2 then cleaves 5γ2 chain into γ2′ and γ2x chains. The two chains facilitate the formation of VM. The cleavage fragments of 5γ2 can be secreted by highly malignant melanoma cells directly.

VE-cadherin has been proved to be closely related to the formation of VM channels. Highly aggressive melanoma cells express VE-cadherin but less aggressive ones do not and inhibition of VM formation by downregulating the expression of the VE-cadherin gene.

Dedifferentiation of Tumor Cells is the Key to Formation of VM Channels

Much information on VM has come from studies of highly malignant melanomas. Tumor cells having the ability of VM formation show an embryonic phenotype. A ►cDNA microarray study of 5,000 genes from a patient with poorly and highly aggressive melanoma cells revealed that there was a differential expression in 210 genes, including some genes associated with the phenotypes of endothelial and hematopoietic stem cells.

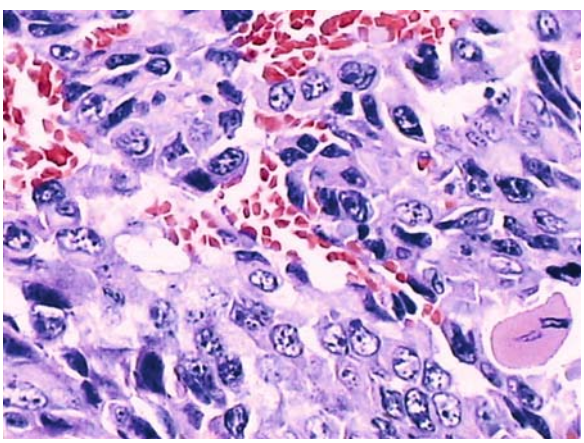
Except for embryonic genotypes, cells of tumors with VM express various angiogenesis-related cytokines. Flt-1 and Tie-2 are expressed by tumor cells of naked

mice bearing human inflammatory breast carcinoma cells. Ovarian ►cancer cells with high aggressivity express the vascular endothelial growth factor (VEGF) and other angiogenesis-related cytokines (e.g., Ang-1 and Ang-2), whereas those with low aggressivity express VEGF only.

The expression of tyrosine kinase (an enzyme that catalyzes the phosphorylation of several signal transduction proteins) is upregulated in highly aggressive melanoma cells than in less aggressive melanoma cells. Aggressive melanoma cells show an increased activity of tyrosine kinase around VM channels. ►Epithelial cell kinase (►EphA2), a tyrosine kinase receptor, is specifically expressed in highly aggressive melanoma cells. Inhibitors of tyrosine kinase activity hinder VM channel formation, and a transient knockout of EphA2 shows reduced VM channel formation.

Linearly Patterned Programmed Cell Necrosis and Three-Stage phenomenon

►Linearly patterned programmed cell necroses (►LPPCN) and ►three-stage phenomenon are thought to play essential roles in the blood supply for melanoma. At the early stage of tumor generation, endothelium-dependent vessels do not sprout into tumor center. Under the pressure of hypoxia, some tumor cells activate ►apoptosis-associated genes, and lacunas left by dissolving LPPCN cells connect with each other and form channel networks (Fig. 4). The channels coming from LPPCN cells face two opposite ends. If they connect with endothelium-dependent vessels, blood will flow into these channels lined by tumor cells and the channels will be a functional microcirculation. In contrast to this, the mass of tumor cells will undergo necrosis if these channels fail to link to endothelium-dependent vessels.



Vasculogenic Mimicry. Figure 4 LPPCN. Cells undergoing LPPCN have spindle-like figure and dark blue nuclei. They connect with each other as lines and some cells enter the wall of VM channels.

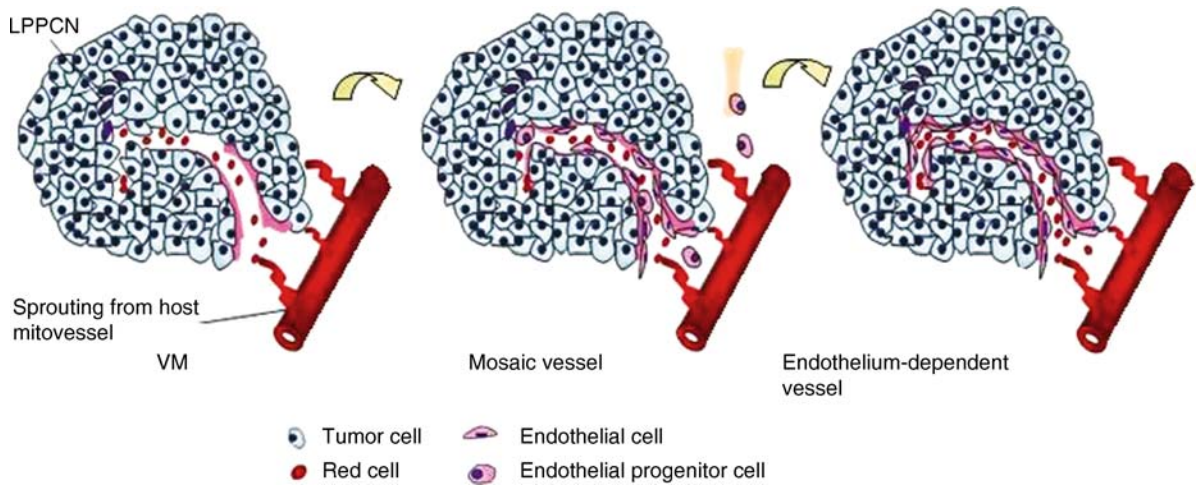
Three microcirculation patterns – VM, ►mosaic vessels, and endothelium-dependent vessels – coexist in melanoma tissue. Angiogenesis requires the recruitment of normal endothelial cells, which may not be efficient and/or sufficient enough for sustaining aggressive tumor growth at the initial stage of rapid growth. Some tumor cells dedifferentiate, connect with other tumor cells or endothelium, and finally line the wall of tube. They are vasculogenic mimicry and mosaic vessels.

Mosaic vessel may be a transition between VM channel and endothelium-dependent vessel. The three-stage phenomenon on tumor blood supply pattern assumes that there is a transformation among VM channels, mosaic vessels and endothelium-dependent blood vessels (Fig. 5). In the stage of rapid tumor growth, endothelium-dependent vessels sprout from normal tissue but are insufficient to support the rapid tumor growth. VM occurs on the base of LPPCN and acts as the major blood supply pattern for tumor growth. As tumor size becomes bigger, endothelial cells from peripheral blood home, proliferate on the wall of VM, and cover some tumor cells forming VM. Mosaic vessels appear and become the major microcirculation pattern in tumors. Finally, endothelium-dependent vessels take over the dominant role in tumor blood supply.

The Effect of Local Tumor Microenvironment on the Formation of VM Channels

Tumor growth and evolution are regulated by a good many factors and tumor cells display distinguished blood supply pattern and biological behavior to adapt different microenvironment. The environmental factors impacting VM channel formation include oxygen pressure, interstitial fluid pressure (IFP) in tumor tissue, pH, focal concentration of cytokines, and ECM.

►Hypoxia is a two-edge sword for tumor generation and development. Hypoxia and ischemia induce tumor cells to necrosis and tumor suppression, whereas hypoxia actives metastasis-related genes to promote tumor invasion. The hypoxic condition enhances the formation of VM channels, which exerts its function though ►HIF-1α and its downstream molecules. In the hypoxic environment, accumulated HIF-1α in tumor cells induces ►MMP-2, MMP-9, and VEGF expression and activation. MMP-2 and MMP-9 proteinases degrade the ECM components and facilitate VM formation, tumor invasion, and metastasis. VEGF secreted by tumor cells results in permeability of blood vessels, resulting in an increase and leaking-out of many serum proteins. Such a milieu provides a temporary matrix for VM channel formation. Under the stimuli of hypoxia, LPPCN-associated genes can be triggered and some tumor cells undergo LPPCN. Interspaces left by



Vasculogenic Mimicry. Figure 5 Three-stage phenomenon on tumor blood supply pattern. During rapid tumor growth, endothelium-dependent vessels sprouting from normal tissue can not satisfy the need for growth. VM occur on the base of LPPCN and acts as the major blood supply pattern for tumor growth. As endothelial cells from host microvessels migrate and endothelial progenitor cells from peripheral blood home into the wall of VM, endothelial cells cover some tumor cells forming VM. Mosaic vessels appear and become the major microcirculation pattern in tumors. Finally, endothelium-dependent vessels get the dominant role in tumor blood supply.

dissolving cells connect with each other as networks to provide the space basement for VM.

Interstitial fluid pressure (IFP) is another important factor affecting tumor microcirculation patterns. Increased IFP is a characteristic of malignant tumors because of its rapid proliferation. It is similar to hypoxia and has double impact on tumor development. High IFP inhibits both endothelial cells of blood vessels and lymphatic vessels to migrate into the tumor center, with the result of tumor hypoxia. Elevated IFP stimulates tumor cells to secret invasion-associated proteins. High IFP is a barrier for endothelium-dependent vessels sprouting into tumor tissue, but hypoxia induced by it is an inducer for VM. Moreover, the expression of MMP-2, MMP-9, ►integrin, ►selectin, and ►kinesin increase significantly in tumor cells growing in the ►microenvironment with high IFP, which promote VM formation to provide sufficient nutrition and oxygen for tumor growth.

Clinical Significance of VM

VM has been observed in several human malignant tumor types, such as highly aggressive uveal ►melanomas, ►breast cancer, ►liver cancer, ►glioma, ►ovarian cancer, ►melanoma, ►prostate cancer, malignant ►astrocytoma, and ►bidirectional differentiated malignant tumors. Tumor cells lining on the inner surface of VM channels are directly exposed to blood flow, may move into the bloodstream and metastasize to other organs.

VM is associated with poor prognosis in patients. Tumors with VM have a higher rate of metastasis compared with tumors without VM, and the patients have a lower 5-year survival rate. Routine ►antiangiogenic drugs, such as angiostatin and ►endostatin, which target endothelial cells, have not achieved a therapeutic effect on tumors that exhibit VM because of the absence of endothelium-dependent vessels (Fig. 6).

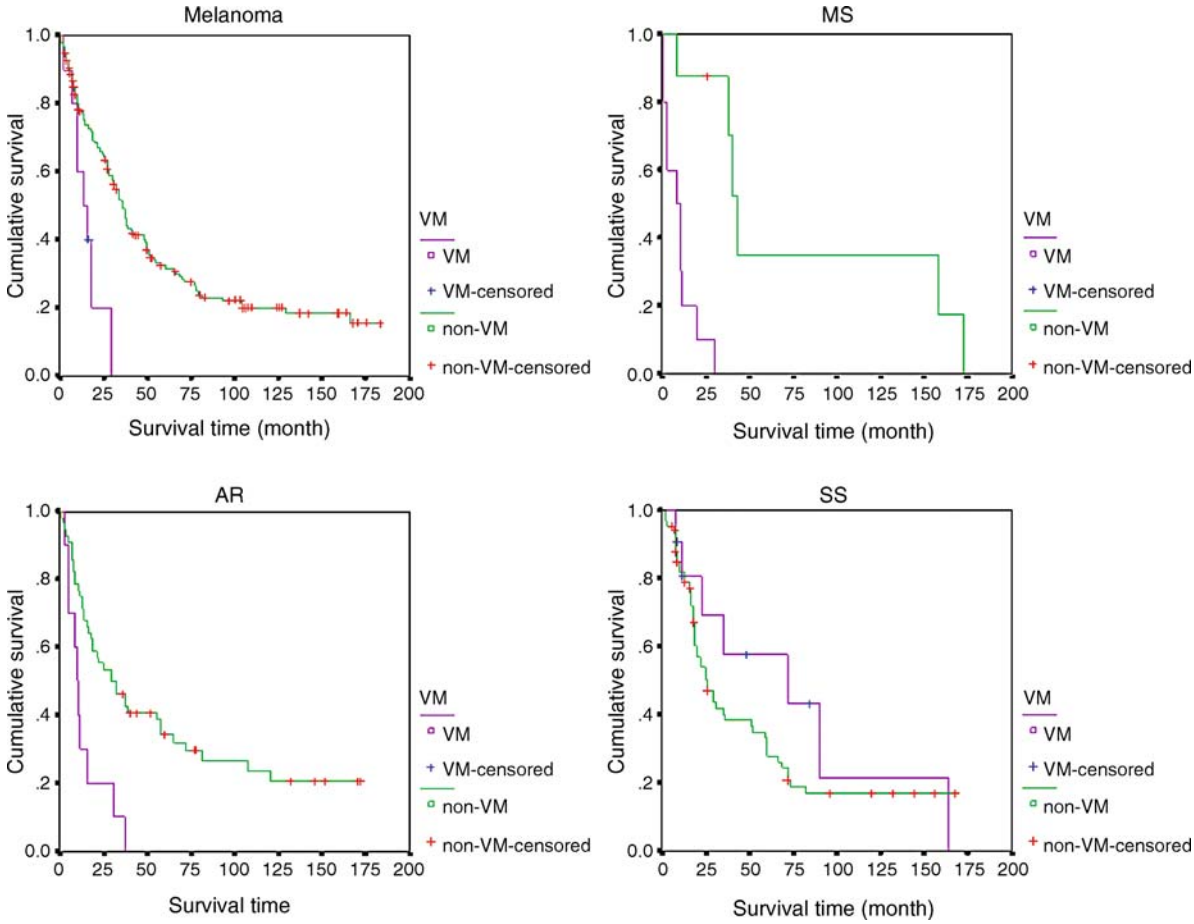
Advances and Challenges

VM and Lymphagenesis in Tumors

VM, a new pattern of blood supply to the tumors, has attracted the attention of many researchers, but many phenomena unique to VM channel formation remain to be elucidated. As a functional tumor microcirculation, VM channels need to be studied with regard to their connection with endothelium-dependent vessels, their relationship with lymphatic tubes, and their dual function as vessels and lymphatic tubes. Uveal melanoma cells have a specific vortex vein but no lymphatic tube. Highly aggressive melanoma expresses lymphatic-vessel endothelial hyaluronan receptor1 (LYVE1) and VEGF-C, a lymphatic tube-related growth factor.

VM-Targeted Therapy

Given the important role of angiogenesis in tumor growth and metastasis, therapies aiming at endothelial cells represent promising antitumor strategies. As VM has a different structure from endothelium-dependent vessels, traditional antiangiogenic agents targeting at endothelial cells, such as anginex, TNP-470, and ►endostatin, have no remarkable effects on malignant tumors with VM.



Vasculogenic Mimicry. Figure 6 Comparison of overall survival time of patients with bidirectional differentiated malignant tumors. A Kaplan–Meier algorithm reveals that the survival time of melanoma, mesothelial sarcomas (MS), alveolar rhabdomyosarcomas (AS), and synovial sarcomas (SS) without VM are both significantly longer than that of patients with VM.

One of the distinguished features of tumors with VM is that cell adhesion molecules, tumor invasion-related proteinases and ECM synthesis and secretion-associated proteins are overexpressed by tumor cells. These molecules represent potential targets for anti-VM strategies of highly aggressive and blood metastatic tumors with VM. Suppressing tyrosine kinase activity, using a knockout ►EphA2 gene, downregulating VE-cadherin, using antibodies against human MMPs and the laminin 5γ2 chain, and using anti-►PI3K therapy are strategies that have been employed to inhibit VM.

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Vasoactive Intestinal Contractor

►Endothelins

Vasoconstrictor

Definition

Any factor that causes the constriction of blood vessels, which increases blood pressure.

►Endothelins

Vasodilation

Definition

Is the regulated widening of blood vessels by a controlled relaxation of the surrounding smooth muscle cells, thus permitting a higher blood flow at a lower pressure.

►Relaxin

Vasostatin

Definition

Refers to the N-terminal domain of ►calreticulin (CRT) (amino acids 1–180), is an endogenous inhibitor of ►angiogenesis and tumor growth. The potency of vasostatin in mice is 4- to 10-fold that of ►endostatin or ►angiostatin.

VCAM-1

Definition

Is a ►cell adhesion molecule that belongs to the immunoglobulin superfamily. It mediates the ►adhesion of lymphocytes to endothelial cells, thereby being involved in various inflammatory diseases including ►angiogenesis and atherosclerosis.

►Minodronate

V(D)J Recombination

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Synonyms

Somatic recombination of V, D and J segments; Immunoglobulin- or T cell receptor-gene rearrangement

Definition

Surface immunoglobulin (expressed on B lymphocytes) and T cell receptors (expressed on T lymphocytes) represent the central molecules for antigen recognition in adaptive immune responses. Variable (V), diversity (D) and joining (J) gene segments, which together encode immunoglobulin or T cell receptor variable chains, are present in every somatic cell. However, in order to acquire coding capacity, V, D and J gene segments need to be assembled in a functional configuration. The mechanism for the assembly of V, D and J gene segments, termed V(D)J recombination, represents a unique capacity of both B and T lymphocytes.

Characteristics

More than 30 years ago, Hozumi and Tonegawa discovered that immunoglobulin genes in B lymphocytes as opposed to any other somatic cell type undergo a complex rearrangement process in order to assemble a functional immunoglobulin variable region. This process, later termed V(D)J recombination, is in fact unique for B and T lymphocytes and critical for the expression of immunoglobulins and T cell receptors, respectively. Immunoglobulin and T cell receptor genes represent gene families in mammalians that are arranged in segments and, hence require somatic recombination of individual variable (V), diversity (D) and joining (J) gene segments to assemble a coding immunoglobulin or T cell receptor gene. V(D)J-recombination defines an early step during B- or T-lymphocyte development within the bone marrow or the thymus, respectively. In humans, three immunoglobulin and four T cell receptor gene loci are known (see Table 1). Each locus carries gene segments that together encode one protein chain. Immunoglobulins or T cell receptors typically represent the assembly of two heterodimers: For instance, immunoglobulins expressed on the cell surface of B lymphocytes comprise of two identical heterodimers of each one immunoglobulin heavy chain and one immunoglobulin κ or λ light chain molecule.

V(D)J Recombination. Table 1 Immunoglobulin and T cell receptor gene loci in the human

Locus	V segments	D segments	J segments	Gene product
IGH, 14q32	123	27	6	Ig heavy chain
IGK, 2p11.2	76	None	5	Ig κ light chain
IGL, 22q11.2	74	None	9	Ig λ light chain
TCRA, 14q11.2	49	None	61	TCRα chain
TCRB, 7q34	64	2	13	TCRβ chain
TCRG, 7p14	14	None	5	TCRγ chain
TCRD, 14q11.2	8	3	4	TCRδ chain

Mechanism

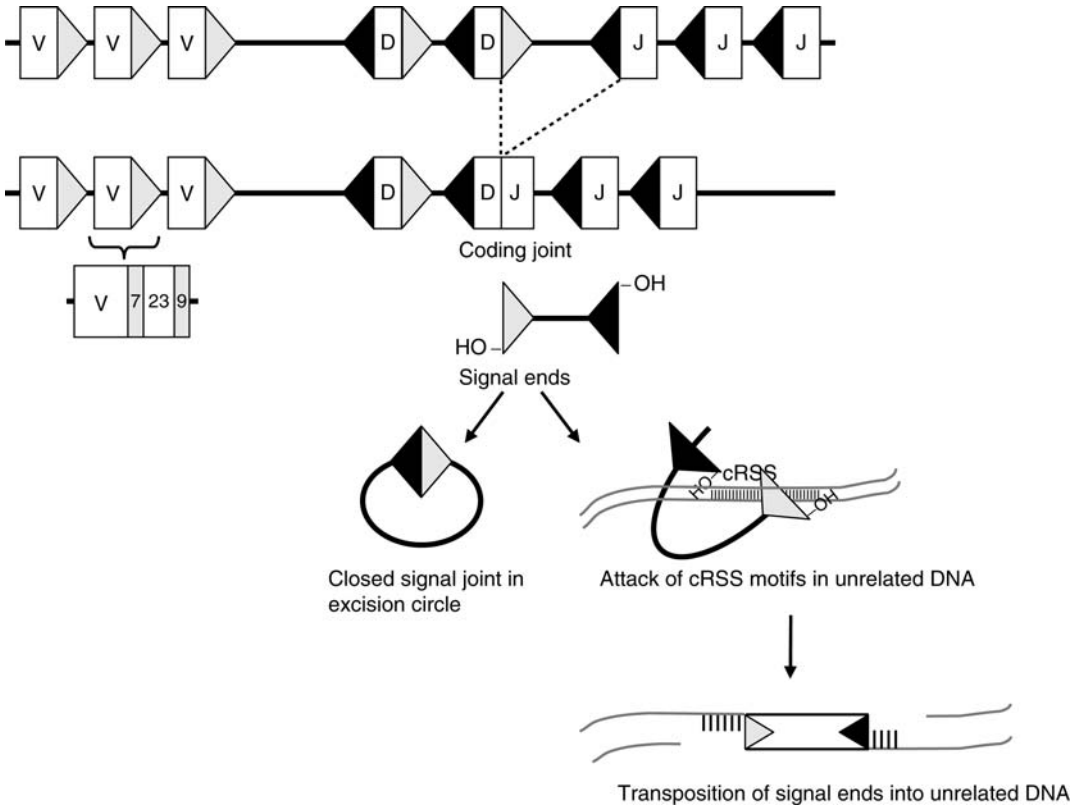
As opposed to meiotic recombination, V(D)J recombination represents a site-specific DNA recombination event in B or T lymphocytes. Site-specific recombination is conferred by recombination signal sequences (RSS) immediately flanking V, D and J gene segments. RSSs are composed of a conserved heptamer and nonamer and a non-conserved spacer of 12 or 23 bp in length. The length of the spacer determines the pairing of RSS-motifs, which only allows synapse formation between RSS motifs of different spacer-length. For the recombination process, RAG1 and RAG2 proteins (encoded by the recombination activating genes 1 and 2) are essential. RAG1 and RAG2 proteins are only expressed in B and T lymphocytes and their expression is tightly controlled during their development. RAG1 and RAG2 form a heterodimeric complex that only recognizes synapses between two RSS motifs of different spacer length, i.e. only 12/23 RSS pairs. The 12/23 rule for RSS pairing ensures that V, D and J segments are recombined in a coordinated manner to prevent mispairing of gene segments.

The RAG1/RAG2 complex then introduces a DNA double-strand break exactly at the border of the heptamer element (Fig. 1). These DNA double strand-breaks are blunt ended and 5′-phosphorylated for signal ends, which facilitates self-ligation and formation of signal joints within so-called excision circles (Fig. 1). In contrast, the broken-ended DNA of gene segments (coding joint; Fig. 1) are protected against immediate re-joining by the formation of a closed hairpin-structure. Of note, even though the targeting by the RAG1/RAG2 complex is very precise, the sequence of coding joints, i.e. the junction between two rearranged gene segments, is extremely variable. This variability of coding joints is owed to further processing of the hairpin structure by the enzymes Artemis and DNA ligase IV. The diversity of coding joints is further increased by the activity of the lymphoid-specific terminal deoxynucleotidyl-transferase (TdT): TdT is able to introduce additional nucleotides, the so-called N-nucleotides, into the junction between two gene segments in a template-independent manner.

Classical RSS motifs containing the conserved heptamer (CACAGTG) and nonamer (ACAAAAACC) sequences and a non-conserved 12 or 23 nucleotide spacer are exclusively found adjacent to immunoglobulin and T cell receptor gene segments. However, RSS-like motifs, so called cryptic RSS sites, have been identified in a number of genes outside the immunoglobulin and TCR loci. Functional assays have shown that these cryptic RSS motifs (cRSS, Fig. 1) can indeed be targeted by the V(D)J recombinase machinery. Given that many genes, among them tumor suppressor and proto-oncogenes, harbor cryptic RSS sites, targeting by the V(D)J recombinase may predispose early B and T lymphocyte precursors to malignant transformation. In this regard, it is important to note that the RAG1 and RAG2 enzymes also possess transposase activity. Thereby, 5′-phosphorylated signal ends carrying a complete RSS motif on each end may attack unrelated DNA, preferentially at cryptic RSS sites (cRSS; Fig. 1). If both RSS motifs of the excised signal ends participate in this attack, this may lead to the integration of the RSS-flanked DNA fragments at positions staggered by 3–5 bp, resulting in target site duplication at the integration site (Fig. 1, bottom). It is obvious that integration of DNA that was excised during V(D)J recombination into unrelated loci carries the risk of oncogenic transformation. Even though classical transposition events are rarely seen in lymphoid malignancies, chromosomal translocations and deletions bearing the hallmarks of illegitimate V(D)J recombination are frequent events during malignant transformation of lymphocytes.

Clinical Aspects

Malignant transformation of human B and T lymphocyte precursors towards ►acute lymphoblastic leukemia (ALL) often coincides with the timing of activity of the V(D)J recombinase machinery. Therefore, it was an obvious hypothesis that aberrant or "illegitimate" V(D)J recombination may be the causative mechanism for many if not all subtypes of ALL. In many cases, ALL cells carry ►chromosomal translocations. The breakpoints of these gene rearrangements, however, exhibit the hallmarks of



V(D)J Recombination. Figure 1 Schematic representation of RSS pairing during D-J segment joining. A simplified model of the *IGH* locus is shown including a group of V, D and J gene segments. Each of these segments are flanked by either a recombination signal sequence (RSS) with a spacer length of 23 bp (V, *light gray*) or 12 bp (J, *dark gray*) or both types of RSS motifs (D). During the RAG1/RAG2-mediated recombination process, two types of break-intermediates are generated: a coding joint (*top*) with a junction between two rearranged gene segments and signal ends (*bottom*) containing a fragment of excised DNA between two RSS motifs. These signal ends are processed by self-ligation to a signal joint within a closed excision circle. These excision circles are very stable in lymphocytes but are not replicated during mitosis. On the other hand, broken ended RSS motifs may attack unrelated DNA, preferentially at cryptic RSS sites (cRSS). Thereby, RAG1 and RAG2 can act as transposases, in that they catalyze the integration of the intervening DNA between two signal ends into the attacked unrelated DNA.

V(D)J recombination in only a few cases (Table 2). For instance, targeting to immunoglobulin (*IGH*, *IGK* or *IGL*) or T cell receptor (*TCRA*, *TCRB*, *TCRG*, *TCRD*) loci would argue for involvement of V(D)J recombination. While *BCL1* and *BCL2* gene rearrangements typically target the *IGH* locus, rearrangements of the *CCND2*, *HOX11*, *LMO2*, *TAL1*, *TAL2* and *TTG1* gene target at least one of the four *TCR* loci (Table 2). In addition, one would expect that breakpoints reflecting illegitimate V(D)J recombination exhibit traces of site-specific targeting in that they precisely flank RSS or RSS-like motifs. Virtually all genes involved in an *IGH*- or *TCR*-specific gene rearrangement show targeting of an RSS or RSS-like motif at least for one of the two translocation partners. In addition, genetic abnormalities were found, in which RSS or RSS-like motifs were targeted even though no immunoglobulin or *TCR* locus was involved. This applies mainly to intragenic/interstitial deletions. Such deletions often occur in T cell lineage ALL cells and deletions of the INK4 family genes *CDKN2A*, *CDKN2B*, *CDKN2D* and within the *HPRT*, *NOTCH1* and *SIL/SCL* loci (Table 2) are examples for such a second type of genetic aberration induced by illegitimate V(D)J recombination. Finally, addition of N-nucleotides (i.e. a junction sequence that cannot be attributed to either of the two fusion partners) by enzymatic activity of TdT represents a unique feature of V(D)J recombination. Indeed, junctional diversity compatible with the introduction of N-nucleotide was found in many cases, in which a gene rearrangement was mediated by V(D)J recombination, but not in all.

Applying these three criteria (involvement of *IGH* or *TCR* loci, site-specific targeting of RSS- or RSS-like motifs and presence of N-nucleotides) to B and T cell lineage ALL, it appears that T cell lineage as opposed to

V(D)J Recombination. Table 2 Frequent genetic aberrations in lymphoid malignancies related to illegitimate V(D)J recombination

Locus	References	Genetic aberration	Target	Malignancy
BCL1	Tsujimoto et al. (1998)	t(11;14)(q13;q32)	*IGH*	Mantle cell lymphoma (B cell)
BCL2	Van Drager et al. (2000)	t(14;18)(q32;q21)	*IGH*	Follicular B cell lymphoma
CCND2	Clappier et al. (2006)	t(7;12)(q34;p13)	TCRB	T cell lineage ALL
	Clappier et al. (2006)	t(12;14)(p13;q11)	TCRA	T cell lineage ALL
CDKN2A	Kitagawa et al. (2002)	9p21 deletion	RSS	B- and T cell lineage ALL
	Cayuela et al. (1997)			T cell lineage ALL
CDKN2B	Kitagawa et al. (2002)	9p21 deletion	RSS	B- and T cell lineage ALL
CDKN2D	Cayuela et al. (1997)	9p21 deletion	RSS	T cell lineage ALL
E2A	*Wiemels et al. (2002)*	*t(1;19)(q23;p13)*	*RSS*	*pre-B cell ALL*
HOX11	Kagan et al. (1989)	t(10;14)(q24;q11)	TCRD	T cell lineage ALL
	Zutter et al. (1990)			
HPRT	Finette et al. (1996)	del Xq26-q27	RSS	Normal lymphocytes
	Chen et al. (1996)			T cell lineage ALL
LMO2	Champagne et al. (1989)	t(11;14)(p13;q11)	*TCRD*	T cell lineage ALL
	Cheng et al. (1990)			
NOTCH1	Tuji et al. (2004)	del 9q34	RSS	T cell lineage ALL
SIL/SCL	Aplan et al. (1990)	del 1p32	RSS	T cell lineage ALL
	Raghavan et al. (2001)			
TAL1	Finger et al. (1989)	t(1;14)(p34;q11)	*TCRD*	T cell lineage ALL
	Chen et al. (1990)			
TAL2	Tycko et al. (1998)	t(7;9)(q34;q32)	*TCRB*	T cell lineage ALL
TCRB/TCRG	Retière et al. (1998)	inv(7)	*TCRB*	Normal T cells
TTG1	Boehm et al. (1988)	t(11;14)(p15;q11)	*TCRD*	T cell lineage ALL
	McGuire et al. (1989)			

B cell lineage ALL frequently arises through genetic aberrations that were caused by aberrant V(D)J recombination. With the exception of the ►*E2A-PBX1* gene rearrangement, none of the classical chromosomal translocations in B cell lineage ALL are compatible with V(D)J recombination: ►*BCR-ABL1*, *MLL-AF4* and ►*TEL-AML1* gene rearrangements are not related to the *IGH* locus, do not show site-specific targeting at RSS-like motifs and do not carry intervening nucleotides within the junction between the two fusion partners. The case of *E2A-PBX1* is complicated because only the breaks of the *E2A* gene are site-specific and localized at RSS-like motifs, while the breaks within the *PBX1* gene are scattered over a large genomic region. Unlike *BCR-ABL1, MLL-AF4* and *TEL-AML1* gene rearrangements, however, the *E2A-PBX1* fusion carries N-nucleotides in almost all instances, which argues for a contribution of the V(D)J recombinase.

►Chromosomal translocations affecting one of the immunoglobulin loci in mature B cell lymphomas may also be owed to somatic hypermutation and/or immunoglobulin class-switch recombination. These two mechanisms are active in mature B cells during affinity maturation within germinal centers of tonsils, lymph nodes and spleen. Indeed, frequent recurrent gene rearrangements in germinal center-derived B cell lymphoma most likely result from somatic hypermutation and/or class-switch recombination and not from V(D)J recombination.

V(D)J recombinase-related genetic aberrations indeed seem to have distinct mechanisms in B cell lineage (pre-B ALL and B cell lymphoma) and T cell lineage (T cell precursor ALL) malignancies. According to a recently proposed model, chromosomal translocations in T cell lineage ALL are more frequent and are mainly caused by illegitimate V(D)J recombination between a TCR locus and a proto-oncogene locus, i.e. both loci were targeted by V(D)J recombination. Conversely, for translocations in B cell lineage malignancy, involvement of the V(D)J recombinase machinery is rare. In these rare cases, in which aberrant V(D)J recombination contributes to B cell lineage malignany, V(D)J recombination typically targets only one of the two translocation partners: In t(1;19)((q23;p13) pre-B ALL, the *E2A* locus is targeted by the V(D)J recombinase but

not its fusion partner *PBX1*. In both ►Mantle cell lymphoma and ►Follicular lymphoma, the *IGH* locus is targeted by aberrant V(D)J recombinase activity but not the *BCL1* and *BCL2* genes on the respective partner chromosome.

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VE-Cadherin

Definition

Vascular endothelial cadherin is a ►adhesion molecule important in maintaining endothelial permeability. Inhibition of VE-cadherin by antibodies increases both permeability and neutrophil transmigration in vivo.

►Vascular Disrupting Agents

Vector

Definition

Genetic information encoding e.g. a therapeutic gene, plus those sequences required for gene expression and for integration into the host genome where applicable. In some situations, the term “vector” denotes the vector sequences in the context of a gene transfer vehicle (viral or non-viral).

►Gene Therapy

VEGF

Definition

►Vascular endothelial growth factor; Is a cytokine made by cells that stimulates new blood vessel formation, by mediating numerous functions of endothelial cells including proliferation, migration, invasion, survival, and permeability. VEGF is also known as vascular permeability factor. VEGF naturally occurs as a glycoprotein and is critical for ►angiogenesis.

►Vascular Endothelial Growth Factor

VEGF-C

Definition

A member of the ►VEGF growth factor family that stimulates lymphangiogenesis and is required for lymphatic vessel development.

►Lymphangiogenesis

VEGFR

Definition

►Vascular endothelial growth factor receptor; ►VEGF.

VEGFR-2

Definition

►Vascular endothelial growth factor receptor 2 gene is transcriptionally regulated during ►angiogenesis.

►Vascular Disrupting Agents

VEGFR-3

Definition
A member of the ►VEGF receptor family that recognizes the VEGF-C and VEGF-D isoform.

►Lymphangiogenesis

Verapamil

Definition
A calcium ion influx inhibitor (calcium entry blocker or calcium ion antagonist). Verapamil is used for reduction of blood pressure and for treatment of cardiac diseases.

►ABC-Transporters
►Fluoxetine

Verner-Morrison Syndrome

Definition
Is a clinical syndrome characterized by a profuse watery diarrhea that results in massive loss of water, potassium, sodium, and bicarbonate, leading to dehydration, electrolyte deficiency, and metabolic acidosis. It is associated with endocrine pancreatic tumors that produce excessive amounts of the vasoactive intestinal polypeptide (VIPomas).

►Neuroendocrine Carcinoma

Vesicle Trafficking

Definition
The process through which membrane-bound molecules are routed to various cellular compartments. Cell surface receptors and their associated signaling complexes are routed to internal endosomal organelles through this process. Signal transduction and vesicle trafficking processes are coordinated and trafficking events can generate discrete cellular signals.

V-Gene

Definition
Variable regions of ►immunoglobulin genes that encode light and heavy chains.

VHL

Definition
Is the gene involved in von Hippel-Lindau disease (renal carcinoma).

►von Hippel-Lindau Tumor Suppressor Gene

Video-assisted Thoracoscopic Surgery

Definition
VATS; Minimaly invasive surgical technique used to diagnose and treat problems in the chest. One or more small incisions are made in the chest and a fiberoptic camera called thoracoscope is inserted through one incision and surgical instruments are inserted through this or other small incisions.

►Pleural Effusion

Villin 2

►ERM Proteins

Vinblastine

Definition
Vinblastine is a ►chemotherapy drug of the group of ►vinca alkaloids that is used as a treatment for some

types of cancer including ►leukemia, ►lymphoma, ►breast cancer and ►lung cancer. It binds ►tubulin, thereby inhibiting the assembly of ►microtubules. It is M phase ►cell cycle specific since microtubules are a component of the ►mitotic spindle and the ►kinetochore.

Vinca Alkaloids

Definition

A class of chemotherapeutic drugs that disrupts ►mitosis by binding to ►tubulin, thus preventing ►microtubules from forming. These are also known as anti-mitotic, antimicrotubule agents, or mitosis inhibitors. Common vinca alkaloids include ►vinblastine, ►vincristine, ►vindesine, and ►vinorelbine.

Vincristine

Definition

Chemotherapeutic agent; ►vinca alkaloid; inhibits ►tubulin polymerization.

Vinorelbine

Definition

Chemotherapeutic agent; ►vinca alkaloid; inhibits ►tubulin polymerization.

VIPoma

Definition

Is a functioning ►islet cell tumor that produces excessive amounts of the vasoactive intestinal polypeptide (VIP) that can result in the ►Verner-Morrison syndrome.

►Neuroendocrine Carcinoma

Viral Oncogene

Definition

A viral gene that contributes to malignancies in vertebrate hosts.

►Gastrointestinal Stromal Tumor

Viral Oncology Epigenetics

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Definition

►Viral oncology epigenetics can represent the ►epigenetic alterations that occur within the host cell genome as a result of viral infection or virally induced ►carcinogenesis. Alternatively, viral oncology ►epigenetics can refer to epigenetic alterations of the viral genome during latent or lytic infection or during carcinogenesis.

Characteristics

One of the emerging concepts in cancer biology is that epigenetic alterations are important in the initiation and early progression of the majority of human cancers. However, differentiation of the early cancer causing epigenetic alterations from later consequences is difficult. Oncogenic viruses are typically very small and with very few genes and yet can induce transformation. Therefore, investigations into the epigenetic alterations that viruses make to the host cell genome may provide an indication of which epigenetic alterations are critical for early carcinogenesis.

Oncogenic viruses include any virus that has been identified as the causative agent for cancer. They can induce cellular alterations that enable transformed cells to evade host responses, including the immune system and apoptosis. In vitro, oncogenic viruses induce cellular transformation creating cells capable of unrestrained proliferation and are often tumorigenic in athymic mice. Oncogenic viruses in humans include DNA viruses, such as ►hepatitis B viruses (HBV), ►human papillomavirus (HPV), polyomaviruses (BKV, JCV, and ►SV40) and the gamma-herpesviruses ►Kaposi sarcoma-associated herpesvirus (KSHV) and ►Epstein-Barr virus (EBV) as well as ►retroviruses, such as ►human T cell lymphotrophic viruses 1 and 2

(►HTLV1/HTLV2), the RNA flavivirus ►hepatitis C virus (HCV).

Epigenetics is a term used to describe the regulation of gene expression and genomic stability by heritable, but potentially reversible, changes in ►DNA methylation and chromatin structure. DNA ►methylation is controlled in the genome by various epigenetic regulators, including the DNA methyltransferases (*DNMT1*, *DNMT3A* and *DNMT3B*), methylated DNA binding proteins (e.g. *MECP2*, *MBD1-MBD4*) and DNA demethylases (e.g. *GADD45A*, *GADD45B*). Chromatin structure is regulated by posttranslational modifications to the tails of the core histones that make up the ►nucleosome including lysine acetylation, lysine and arginine methylation, lysine ubiquitination, serine phosphorylation and proline isomerization. The enzymes that catalyze these modifications (►histone deacetylases, histone acetyltransferases, histone demethylases and histone methyltransferases) interact with other chromatin structure regulators, such as the polycomb group (repression) and trithorax group (activation) genes, ATP-dependent chromatin remodeling complexes and ►microRNAs and the RNAi machinery to coordinate together the regulation of gene expression.

In normal cells transcriptionally active genes typically contain unmethylated promoter ►CpG islands, gene-wide histone hyperacetylation and a number of specific histone modifications, such as H3 lysine 4 (H3K4) methylation, H3K79 methylation, H3 arginine methylation, H3S10 phosphorylation and H2B ubiquitination. Transcriptionally repressed genes often contain methylated promoter CpG islands, histone hypoacetylation and H3K27 and H3K9 methylation. In cancer cells, however, there are marked differences in the epigenetic landscape of the genome. Genome-wide, there is an overall ►hypomethylation associated with repetitive DNA in cancer cells, as well as promoter hypermethylation of specific tumor suppressor genes and hypomethylation of ►oncogenes. In addition to altered DNA methylation, ►chromatin remodeling in cancers is also considered a common epigenetic alteration, including loss of H4K16 acetylation fand H4K20 tri-methylation and gains in H3K4 di- and tri-methylation, H3K79 methylation and H3 and H4 hyperacetylation.

Host Epigenetic Changes Due to Viruses and Virus-Associated Cancers

Differentiating epigenetic causes of cancer from epigenetic consequences is one of the more challenging goals of current research in the epigenetics field. This conundrum can equally be applied to virally induced cancers. It is challenging to differentiate epigenetic changes that are directly due to viral infection, due to the anti-viral response of the host or due to downstream effects of the virally induced transformation. Important early epigenetic changes in cancer may be distinguished from consequences via the identification of specific interactions between epigenetic regulators and the viral proteins effecting those changes and then determining the downstream effects of these interactions.

Kaposi Sarcoma Associated Herpesvirus

►Kaposi sarcoma associated herpesvirus (KSHV) is an oncogenic gamma-herpesvirus identified as the causative agent of the endothelial tumor Kaposi sarcoma (KS) and associated with the lymphoproliferative disorders primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD). Like other gamma-herpesviruses KSHV has both a lytic and latent phase of its life cycle. In the majority of tumor cells the more restricted set of latent genes are often expressed. Recent evidence suggests that KSHV can reprogram the cellular gene expression profiles of both blood and lymphatic vessel endothelial cells, although the mechanisms behind this reprogramming remain unclear.

A direct link between the KSHV protein latency associated nuclear antigen (LANA) and the de novo methyltransferase DNMT3a was examined in a study revealing recruitment of DNMT3a by LANA to the chromatin possibly via its interaction with histones H2A and H2B. This targeted repression of ~80 cellular genes, many of which are typical targets of epigenetic inactivation in numerous cancers. KSHV has been shown to hypermethylate the ►*CDKN2A* gene promoter in the majority of PEL cell lines harboring KSHV and in primary PEL samples. This is not unexpected, given that *CDKN2A* is probably the gene most commonly hypermethylated in cancers. This suggests that KSHV has the ability to invoke DNA methyltransferase activity, via the LANA protein, and may inactivate numerous cellular genes by promoter hypermethylation. In addition, LANA has numerous other roles in epigenetic gene regulation via interactions with a methylated DNA binding protein MECP2, as well as the mSin3 repression complex and the SUV39H1 histone methyltransferase. Numerous studies have also proposed a direct interaction between the KSHV encoded interferon regulatory factors (viral IRF 1, IRF2 and IRF3) and the histone acetyltransferase complex ►p300/CBP. The binding of CBP by the cellular IRF3 is thought to be a necessary interaction for transcriptional upregulation of the antiviral cytokine interferon-β (IFN-β). In effect, the interactions of the viral IRFs with CBP inhibit histone acetyltransferase activity and promote histone hypoacetylation, altered chromatin structure and reduction of cytokine gene expression. Presumably other genes that are activated by p300/CBP would also be down regulated.

Epstein Barr Virus

Epstein Barr virus (EBV) is another gamma-herpesvirus that has been identified as the causative agent of

numerous lymphoproliferative diseases such as ►Burkitt lymphoma (BL), ►Hodgkin Disease (HD) and post-transplant lymphoproliferative disease (PTLD). EBV is also involved in carcinogenesis of epithelial origin tumors, particularly ►nasopharyngeal carcinomas (NPC) and some gastric cancers. The EBV latent membrane protein 1 (►LMP1) increases DNA methyltransferase activity by upegulating the maintenance methyltransferase DNMT1 as well as both de novo methyltransferases, DNMT3b and DNMT3a. In EBV-related epithelial cancers this causes increased promoter methylation and reduction of e-cadherin expression, resulting in increasing cell migration, an important step during carcinogenesis. Other cellular genes that are methylated by EBV are yet to be identified. The EBV latent nuclear antigens EBNA 2 and 3c appear to alter histone acetylation via their interactions with either the p300/CBP complex or with histone deacetylases, respectively.

One of the interesting observations regarding the EBV proteins that coordinate epigenetic regulation (EBNA2, EBNA3c, and LMP1) is that they are all latent genes that are not expressed in most Burkitt lymphoma, EBV-associated gastric cancer or nasopharyngeal carcinomas. This may lead one to conclude that the role of the virus induced host epigenetic alterations may be limited in these cancers. However, in these cancers and in other virally induced cancers it cannot be ruled out that the early cancer precursor cells may have been infected with the virus expressing these proteins that could alter the host epigenome. Epigenetic fingerprints such as histone modifications and DNA methylation are mitotically heritable and therefore even if the progeny cancer cells no longer express these latent genes, the epigenetic history of the cell may remain.

Hepatitis B Virus

The hepatitis B virus (HBV) is a DNA virus from the hepadnavirus family and is the causative agent of viral hepatitis, a chronic inflammation of the liver, which can develop into ►hepatocellular carcinoma. The hepatitis B virus oncogenic protein HBx has been shown to increase the activity of DNMT1, and similar to EBV related cancers, this results in increased DNA methylation of e-cadherin (*CDH1*) and an increased cell migration. Whether this can be attributed directly to increased activity of the maintenance methyltranserase, DNMT1, is not clear as EBV also activates the de novo methyltransferases, DNMT3a and DNMT3b which HBV does not. The tumor suppressor gene *CDKN2A* and the glutathionine-S-transferase (*GSTP1*) genes are both commonly hypermethylated in ►hepatocellular carcinomas associated with HBV, however, there is currently no direct evidence that the virus causes the increased DNA methylation of these genes.

Human Papillomavirus

The human papillomavirus (HPV) family is a large family of DNA viruses of which some subtypes (particularly HPV 16 and 18; ►early genes of human papillomaviruses) are associated with ►cervical cancer and rarer epithelial origin cancers. The two important HPV oncoproteins E6 and E7 have both been implicated in epigenetic alterations during carcinogenesis. HPV E7 protein increases the DNA methyltransferase enzymatic activity by direct interaction with DNMT1 and E6 can bind and inhibit the histone acetyltransferase activity of p300 and CBP similarly to KSHV. This transcriptional repression by HPV is supported by E7 protein interaction with the Nurd ATP-dependent chromatin remodeling complex and ►histone deacetylase 1 which are both involved in transcriptional repression. Numerous cellular epigenetic alterations have been described in ►cervical cancers including hypermethylation of tumor suppressor genes *RB*, *CDKN2A*, *MLH1*, *VHL* and *CDH1*. Whether the E7 mediated increase in DNA methyltransferase activity is responsible for methylation of these genes is yet to be established.

Polyomaviruses (SV40, BK Virus and JC Virus)

Polyomaviruses are very small DNA viruses (~5kb) encoding only six genes. In humans the BK virus is associated with some brain tumors and the JC virus has been associated with gliomas, medulloblastomas and a minority of colorectal carcinomas, however, neither virus has been implicated as direct causative agents in these tumors. The simian virus SV40 is not a causative agent of any human tumors, but has been found in some mesotheliomas. These three polyomaviruses encode the T-antigen oncoprotein which is involved in inducing DNA methylation alterations. The SV40 virus upregulates the de novo methyltransferase DNMT3b which results in increased DNA methylation and increased tumorigenicity in normal human bronchial cells. Abberrant methylation of *RASSF1A*, *HPP1*, *CCND2*, *DCR1*, *TMS1*, *CRBP1*, *HIC1* and *RRAD* have all been detected in SV40 associated malignant mesotheliomas or SV40 infected human mesothelial cells. The BK virus increases transcription of DNMT1 through the pRB/E2F pathway in cells that have pRB inactivated, however, this has not yet been linked to increased methylation of any specific genes. The JC virus T antigen expression is associated with the methylator phenotype in colorectal cancer, however this link with methyltransferase is still rather speculative.

Adenovirus

The ►adenovirus protein E1A can induce transformation in vitro and can induce tumors in animal models but the virus does not in itself cause any human cancers. E1A interacts with the p300/CBP complex and is likely to result in a loss of histone acetylation across the

genome. It has been proposed that this interaction could be one of the key events in E1A induced cellular transformation. Another important role could be its capability of increasing DNMT1 activity, although which cellular genes are hypermethylated as a result of this increased activity is yet to be determined.

Human T Cell Lymphotrophic Viruses

Human T cell lymphotrophic viruses (►HTLV1 and HTLV2) are single stranded RNA retroviruses that are causative agents of adult T-cell leukemias. While there is little data on host DNA methylation alterations directly mediated by this virus, the HTLV1 Tax protein does interact with the ►p300/CBP complex to mediate transcriptional repression.

Epigenetic Alterations in the Virus Due to the Host

The opposite side of viral oncology epigenetics is the epigenetic alterations that occur on the viral genome during the lytic and latent stages of infection and during carcinogenesis. In addition to regulation of their own genome, DNA methylation of viral genes has also been proposed as a mechanism for silencing potentially highly immunogenic proteins that would otherwise elicit an immune response.

Due to spontaneous deamination of methylated cytosines to thymine there is a reduced rate of CpG dinucleotides (►CpG island) in the genomes of organisms that use DNA methylation as a mechanism of gene silencing. This mechanism, known as CpG suppression, is seen in the human genome with an observed CpG dinucleotide rate of ~1% where the statically expected rate would be 1/16 (6%) of the total bp. The genomes of many gamma-herpesviruses, including EBV, show CpG suppression suggesting that their genomes have been methylated similarly to the DNA of the host cells in which they reside. In this way the epigenetic state of the viral genome can be considered due to the host in which they reside. KSHV on the other hand only shows CpG suppression at the lytic switch promoter, ORF50, suggesting that the rest of the viral genome is not extensively methylated. KSHV in fact controls its entry into the lytic phase by employing both demethylation and chromatin remodeling of the lytic switch gene Rta (ORF50) promoter and its latent cycle replication is controlled by hyperacetylation of the replication origin. The EBV C promoter which drives the expression of the latent gene transcripts is often methylated and silenced in most EBV associated tumors. This prevents the expression of highly immunogenic Epstein Barr nuclear antigen (EBNA) proteins that would elicit a cytotoxic T-cell response and provides a very good example of how viruses exploit epigenetic mechanisms to evade the immune system.

Conclusions

It is clear that oncogenic viruses increase the activity of DNA methyltransferases and have the ability to decrease ►histone acetylation via ►p300/CBP. These are both likely to be essential for the inactivation of tumor suppressor genes. That both of these events occur in many non-viral cancers as well suggests that they may be some of the earliest epigenetic alterations in carcinogenesis. The common pathways utilized by viruses to effect these epigenetic changes suggest that perhaps very few changes are required to initiate epigenetic misregulation in cancer. For example the BK virus increases transcription of DNMT1 through the pRB/E2F pathway in cells that have pRB inactivated, however this has not yet been linked to increased methylation of any specific genes. The HPV E7 protein, KSHV LANA, polyomavirus T antigen and adenovirus E1A are all known to inactivate pRB as well as increase DNA methyltransferase activity, suggesting that inactivation of pRB may be a critical step towards epigenetic alterations in carcinogenesis. It is also interesting to note that many of the viral proteins described here, LMP1, E6 and E7, LANA, E1A, large T antigen and Tax, are all often portrayed as the viral "oncoproteins" as they are often either essential components of viral transformation or oncogenic on their own. This is often used as evidence that the functions of these proteins, in this case the epigenetic interactions, are indeed essential for carcinogenesis induced by these viruses.

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Viral Vector-mediated Gene Transfer

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Synonyms

Virus vector; Oncolytic virus; Virus vector-mediated gene transfer

Definition

Refers to the process by which virus vectors are used to deliver functional genes of interest into target cells and tissues, either *in vitro* or *in vivo*.

Characteristics

Viruses have evolved natural mechanisms to efficiently transport their own genetic materials into host cells while also commandeering host cell machinery for replication. Therefore, the use of viruses for the introduction of therapeutic genes and stimulation of the immune system has become an attractive and promising method for the treatment of a variety of diseases, including cancer.

The genomes of a wide array of viruses can be modified and used as a tool for the efficient transfer of exogenous genes into living cells or organisms. In broad terms, two types of virus vectors exist: replication-competent, and replication-defective. Replication- competent vectors are exemplified by live-attenuated strains of many common viruses, including both DNA viruses (such as ►adenovirus, herpes simplex virus type-1 (HSV-1) and vaccinia virus) and RNA viruses (such as recombinant derivatives of vaccine strains of measles virus, poliovirus and vesicular stomatitis virus (VSV)).

Replication-defective vectors have an intrinsically more favorable safety profile than replication-competent vectors, but can be more difficult to engineer and manufacture. Commonly used replication-defective vectors include both small (adeno-associated virus (AAV)) and large (adenovirus, HSV-1) DNA viruses, as well as RNA viruses (including alphaviruses such as Venezuelan equine encephalitis virus (VEE), lentiviruses and other retroviruses). In all cases, the vectors are designed such that one or more genes essential for viral replication or assembly have been deleted or otherwise rendered defective.

Some viral vectors combine aspects of both replication-competent and replication-defective vectors. These include vectors that are fully competent for replication in cells of one type or species, but not in cells of a different type, or from a different host species. Widely used examples include baculovirus vectors, which are able to transduce mammalian cells but which can replicate only in insect cells, and certain poxviruses such as the Modified Vaccinia Ankara (MVA) strain of vaccinia virus, which replicates efficiently in chick embryo fibroblasts, but undergoes abortive infection in human cells. Conditionally replicating virus vectors, such as oncolytic viruses, are another example – and are especially attractive as potential therapeutic agents for cancer treatment, as will be discussed later in this essay.

Finally, there is considerable interest in the development of artificial virus-like vectors, including virus-like particles (VLPs). VLPs offer potential advantages for vaccine delivery or display of vaccine antigens due to the densely repetitive nature of the surface of many VLPs. These properties explain, in part, the tremendous effectiveness of the recently licensed VLP-based vaccines for oncogenic human papillomavirus (HPV) subtypes. Completely synthetic gene transfer vectors have particular appeal because of the potential to mass-produce such agents using conventional chemical technologies, rather than the more complex and cumbersome biological production methods required to manufacture conventional virus vectors. However, the efficiency of gene transfer by completely synthetic vectors currently remains far inferior to that of conventional virus vectors.

Detection of Viral Vector-Mediated Gene Expression and Vector Distribution. In order to analyze the success of viral vector-mediated gene transfer, it is important to be able to monitor both the distribution of the vector and the effectiveness of vector-mediated gene expression. This can be achieved by subcloning a reporter gene into the viral vector backbone. Several reporter genes are commonly used for this purpose including: fluorescent proteins of various colors (Including green fluorescent protein, GFP), *E. coli* β-galactosidase (*LacZ*), and various forms of luciferase *(Luc)*. It is simple to detect and quantitate cells expressing these marker genes *in vitro*, using FACS analysis or fluorescence microscopy. Detection of vector-transduced cells *in vivo* often requires a different approach. Modified luciferase reporters such as the Gaussia luciferase can generate a very bright light emission that can be detected and localized to specific tissues by using highly sensitive light detection methods in combination with 3D tomography.

For in vivo imaging applications, a number of marker genes are compatible with the use of currently available gamma cameras or ►positron emission tomography (PET) instruments. These include the sodium iodide symporter (NIS), which has been used to target radioisotopes of iodine to cancer cells. This allows both *in vivo* imaging of the distribution of the vector, quantitation of gene expression, and also delivery of therapeutic radiation to cancer cells. The ►HSV-1 thymidine kinase (TK) gene has also been a successful tool for imaging when coupled with PET tracers. Furthermore, in the case of cancer therapy, the TK gene can be combined with ►prodrugs, such as ►ganciclovir (GCV), which become activated by TK and exert cytotoxic effects on rapidly dividing cancer cells.

Viral Vectors for Cancer
►*Gene Therapy*. A number of vectors, including AAV, adenovirus, HSV, measles virus and ►retroviruses have been developed to promote the selective elimination of tumor cells. Cancer gene therapy approaches include

immunotherapy and suicide gene therapy. In addition, naturally oncolytic viruses such as reovirus (exemplified by Reolysin) and oncolytic viral vectors that result in the destruction of transduced tumor cells are also being pursued. For reasons of space, we will focus the rest of this review on the latter. Virus platforms that are being used to develop oncolytic vectors include adenovirus, HSV-1, measles virus and vaccinia virus. The field of ►oncolytic virotherapy has made rapid progress in the past decade, resulting in human clinical trials of multiple agents based on all of the above vector platforms. Clinical trials have included phase III studies, and in 2005, the field received a major boost when the Chinese government approved the first oncolytic virus therapy for cancer treatment. This is a significant landmark that likely presages the approval of other oncolytic virus therapies elsewhere in the world.

There are several approaches that can be taken in the use of oncolytic vectors for cancer therapy. One approach simply utilizes the replicating virus itself as therapy. As the virus replicates within the tumor cells, the cells are lysed and destroyed. Considerable efforts are being made to improve tumor-selectivity, and to ensure that the ►virotherapy spares surrounding healthy tissue. This can be accomplished by: (i) placing viral genes under the control of tumor-specific promoters (such as the PSA promoter in ►prostate cancer); (ii) altering the receptor binding properties of the vector in order to target tumors; (iii) utilizing the oncolytic properties of the vector in conjunction with "arming" of the virus with therapeutic genes. The use of "armed" vectors is attractive due to the fact that it draws on multiple mechanisms to achieve the desired effect. For example, engineering oncolytic HSV vectors to deliver a therapeutic gene, such as an antiangiogenic factor, has been shown to enhance the therapeutic efficacy of the vector in small animal model systems. It is also possible to introduce a tumor suppressor gene, such as ►p53, into cancer cells via an oncolytic vector in order to exert control over the cell cycle of the tumor cells while also subjecting the cells to the oncolytic activity of the virus itself.

The success of oncolytic vectors as anti-cancer therapies can be further enhanced by combining the virotherapy with either chemotherapy or radiation treatment. Not only is it possible to use radiation-inducible promoters to direct the expression of essential viral genes, but also it has been shown that radiation and chemotherapy are able to enhance the replication of certain oncolytic vectors. For example, in the case of oncolytic HSV-1 vectors, the induction of DNA repair genes by chemotherapy actually augments viral replication. Also, the use of a reporter gene, which has therapeutic effects when combined with radiation, such as NIS, offers the promise of an effective synergistic approach to tumor treatment.

Overall, the field of cancer gene therapy has made important advances within the past few years, and while oncolytic virotherapy may not offer a simple "magic bullet," it has considerable potential to synergize with, and enhance the effectiveness of, other established therapies such as chemo- and radio-therapy.

Viral Vector Cancer Gene Therapy – Pitfalls. While important advances have been made, concerns remain. First, oncolytic virotherapies may not be sufficiently effective to provide optimal therapeutic benefit as a stand-alone treatment. Therefore, the effectiveness of the vectors needs to be increased. In addition, the issue of pre-existing immunity to common viruses such as HSV-1 and adenovirus serotype 5 may need to be overcome, in order to make most effective use of oncolytic viruses based on these agents. Second, biosafety questions must be addressed. These include the potential for vector replication in normal tissues, such as rapidly dividing stem cells, and the inherent genetic instability of certain viruses. For example, it was recently found that an oncolytic HSV-1 vector being used in human clinical trials contained a previously unrecognized mutation (a truncation in the UL3 open reading frame).

In conclusion, virus vector-mediated gene transfer holds significant promise as a potential therapeutic approach for many human diseases, including cancer. The recent approval of the world's first oncolytic virus for human tumor therapy is indicative of the rapid and exciting progress in the field. Moreover, evolving success in combining oncolytic virotherapies with sophisticated tumor-targeting and gene transfer approaches, as well as conventional chemo- and radio-therapy, provide a compelling reason to anticipate new cancer treatments that more effectively exploit the potential of viral vector systems.

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Virilization

Definition

Refers to the process of normal male sexual development (puberty) regulated by the production of testosterone. In adrenocortical carcinoma, ►virilization can occur in boys (precocious puberty) or girls (pseudo-precocious puberty) as a result of excessive amounts of androgens.

►Childhood Adrenocortical Carcinoma

Virology

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Definition

Virology addresses the molecular nature of viruses, their genetic content, the pathway by which they enter cells and multiply using the molecular machinery of the host cell, and the mechanisms by which they elicit diseases. Tumor virology is a specialized discipline analyzing the association of particular virus types with cancers in animals and humans.

Characteristics

In 1964 the first human tumor virus, the ►Epstein-Barr virus (EBV), was isolated from tumor samples of a patient with African ►Burkitt lymphoma. Subsequently, EBV was linked to the development of other forms of cancer. In the 44 years since the discovery of EBV, other human tumor viruses have been identified (Table 1).

Tumor Virus Epidemiology

The development of cancer is an infrequent consequence of viral infection and often occurs many years after any initial infection. Therefore, tumor viruses often infect individuals without adverse effects. For example, approximately 95% of the World's adult population are infected with EBV. The majority of these individuals carries the virus asymptomatically and will not develop cancer as a consequence of EBV infection. Likewise, tumors associated with the human T lymphotropic virus-1 (HTLV1) arise infrequently in populations where the virus is endemic. Thus, presumably alone tumor viruses are usually insufficient to cause malignancy; virus infection is only one step in the multistep process leading to a cancer. Although virus infection may be linked to a particular cancer type, in order to establish a clear association between the virus and the development of a cancer it is usually necessary to detect the virus within the tumor cells.

Immunosurveillance and Viral Oncogenesis

Virus-associated cancers occur in both immunocompetent and immunodeficient patients. However, the latter group has a particularly high risk for the development of these tumors, suggesting that the immune system can prevent the development of virus-associated cancers. ►Cytotoxic T cells (CTLs) are particularly important in the recognition and elimination of virus-infected tumor cells. CTLs recognize virus-derived peptides that are presented by the infected cell in association with MHC class I. The development of virus-associated cancers in immunocompetent individuals suggests that the virus-infected tumor cell or its progenitor has developed mechanisms to escape immune recognition. In some cases virus-specific CTLs have been used to treat virus-associated cancers (►adoptive immunotherapy). In some cases gene therapy may be used to modify CTL function (►CTL therapy).

Tumor Viruses

There are a number of different viruses that have been associated with the development of cancer. The acutely transforming ►retroviruses cause cancer in animals but to date none have been associated with the development of human tumors. The following sections consider some of the important human tumor viruses.

Herpesviridae

The major oncogenic herpesviruses are the Epstein-Barr virus (EBV) and the Kaposi sarcoma-herpesvirus (KSHV).

Epstein-Barr Virus

EBV (Epstein-Barr virus) is a double stranded DNA virus of the gamma herpesvirus family. EBV infects the majority of the World's adult population and following primary infection the individual remains a lifelong carrier of the virus. In poorly developed countries, primary infection with EBV usually occurs during the first few years of life and is often either asymptomatic or produces only a mild febrile illness. However, in developed populations primary infection is frequently delayed until adolescence or adulthood, in many cases producing the characteristic clinical features of infectious mononucleosis (also known as glandular fever) including sore throat, fever, malaise, lymphadenopathy and mild hepatitis. EBV is often transmitted from one individual to another in saliva and the oropharynx is

Virology. Table 1 Major oncogenic viruses

Virus family	Virus	Natural host	Host in which virus is oncogenic	Tumor	Major oncogenic protein (s)
Adenoviridae	Human Adenoviruses A, B, D	Man	Hamster, rat	Various	E1A, E1B
Polyomaviridae	Polyoma	Mouse	Mouse	Various	Middle T antigen, Large T antigen
	SV40	Monkey	Hamster, rat	Various	Large T antigen
Papillomaviridae	HPV 16,18	Man	Man	Skin cancer, cervical cancer, anal cancer	E6, E7
Herpesviridae	EBV	Man	Man	Burkitt lymphoma, nasopharyngeal carcinoma, Hodgkin lymphoma	LMP1
	KSHV	Man	Man	Kaposi sarcoma, multicentric Castleman disease, primary effusion lymphoma	
Hepadnaviridae	Hepatitis-B virus	Man	Man	Hepatocellular carcinoma	Hepatitis-B x-antigen
Flaviviridae	Hepatitis C virus	Man	Man	Hepatocellular carcinoma	HCV core, NS3, NS4B, NS5A
Retroviridae	HTLV1	Man	Man	Adult T cell leukaemia	Tax

believed to be both the primary site of infection and where virus replication occurs. Virus replication in the oropharynx ensures the production of new virions for transfer in saliva to other susceptible hosts. Because EBV is transmitted in this way infectious mononucleosis is often referred to as the 'kissing disease'.

Soon after primary infection EBV infects B-lymphocytes through an interaction of the viral envelope glycoproteins, gp350/220, with the cellular EBV receptor, CD21. EBV does not usually replicate in B-lymphocytes but instead establishes a latent infection during which no new virions are produced and only a subset of viral genes are expressed. As a consequence of the host immune response, the number of latently infected B-lymphocytes in the peripheral blood falls to approximately 1 in 106 during the months following primary EBV infection. This low number is maintained in healthy carriers by the continual elimination of proliferating EBV-transformed B-lymphocytes by virus-specific CTLs.

The EBV genome usually exists in cells as extrachromosomal pieces of circular DNA, known as episomes. During latency only a limited number of viral genes are expressed; these include six nuclear proteins, referred to as the Epstein-Barr nuclear antigens (EBNAs), two latent membrane proteins (LMPs), two non-translated RNA molecules known as the Epstein-Barr encoded RNAs (EBERs 1 and 2), and transcripts from the BamH1A region of the viral genome (BamH1A rightward transcripts; BARTs). Three major forms of viral latency exist in tumors (Table 2). The restricted forms of latency have evolved partly to prevent expression of the immunodominant EBNA proteins and are controlled by differential ▶methylation of viral promoters. Demethylating agents can induce

Virology. Table 2 Three major forms of viral latency are associated with EBV-positive malignancies

Latency	Viral genes expressed
I	EBERs, BARTs
	EBNA1
II	EBERs, BARTs
	EBNA1,
	LMP1, LMP2A, & LMP2B
III	EBERs, BARTs
	EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C & EBNA Leader Protein (LP)
	LMP1, LMP2A, & LMP2B

During EBV latency only a limited number of viral genes are expressed; these include six nuclear proteins, referred to as the Epstein-Barr nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and EBNA-LP), two proteins found in the cell membrane of infected cells known as the latent membrane proteins (LMPs), two non-translated RNA molecules known as the Epstein-Barr virus encoded RNAs (EBERs) and the BARTs.

expression of these EBNAs and in some cases can also induce lytic cycle; this has been proposed as an alternative treatment for some EBV-associated cancers (►epigenetic therapy).

Many viruses, including EBV, are able to subvert the cellular epigenetic machinery, not only to regulate virus gene expression, but also potentially to influence cell growth by silencing ►tumor suppressor genes).

LMP1 is the major transforming protein of EBV; it is a transmembrane protein which mimics a constitutively activated ►tumor necrosis factor receptor (TNFR). In normal cells binding of the appropriate ligand to these receptors causes intracellular signaling and a number of effects including either proliferation or ►apoptosis, depending upon the nature of the signal and the cell type involved. In contrast, LMP1 requires no ligand for its activation and delivers constitutive intracellular signals leading to cell proliferation and protection from apoptosis, which in turn accounts for the transforming properties of this protein. Signaling pathways activated by LMP1 include ►NF-κB, JNK/AP-1, p38/MAPK, and JAK/STAT (►signal transducers and activators of transcription). Several of these pathways are aberrantly activated in EBV-associated tumors, such as ►Hodgkin lymphoma. LMP1 can decrease ►ATM expression, and may represent one of a number of ways in which EBV can disable DNA repair.

The malignant diseases associated with EBV include Burkitt lymphoma, ►nasopharyngeal carcinoma, Hodgkin lymphoma and a variety of other cancer types.

EBV-Encoded microRNAs

►MicroRNAs (miRNA) are a class of small RNAs that are probably major regulators of gene expression. miRNA are complementary to their cognate mRNA sequences; their interaction in the RNA-induced silencing complex (RISC) results in the cleavage of the target mRNA or in some cases inhibits its translation. The first virus-encoded miRNAs that were discovered were those of EBV, from which five miRNAs were cloned. EBV miRNAs are clustered in two distinct regions; the first is located within the 5′ and 3′ UTR of the BHRF1 transcript and the other within the BART region. The precise functions of these and other viral miRNAs are yet to be defined but are likely to play key roles in the regulation of virus and cellular gene expression, with potential involvement in oncogenesis.

Burkitt Lymphoma

►Burkitt lymphoma (BL) is an aggressive tumor of B-lymphocytes. There are two main types of BL. The endemic (African) type occurs with high frequency (5–20 cases/100,000 children/year) in equatorial Africa and Papua New Guinea and with a distribution that matches that of holo-endemic malaria. Almost all cases of endemic BL are EBV-positive. On the other hand, sporadic BL occurs world-wide with a much lower incidence and only around 15% of cases are EBV-positive. A third form of BL occurs in AIDS patients and approximately one-third of these tumors harbor EBV.

The cells of EBV-infected BL tumors usually display a latency I phenotype – that is they only express one viral protein, EBNA1. More recently variant forms of BL have been described in which expression of some of the other EBNA genes is observed. These are known as Wp using BL since they use a distinct viral promoter (Wp) to drive EBNA expression. Although infected BL tumor cells could process peptides from the EBNA1 protein and present them to specific CTLs, the processing of endogenous EBNA1 through the Class I pathway is inhibited. This, together with the down-regulation of MHC class I and ►adhesion molecules that is a feature of BL cells, contributes to their ability to evade immunodetection.

BL is characterized by reciprocal translocations that result in deregulation of the ►myc gene. In endemic BL, EBV-driven proliferation of B-lymphocytes, together with a general polyclonal stimulation of B cells induced by malaria infection, is thought to increase the chances of one of these specific translocations occurring in the B lymphocytes that will eventually give rise to BL.

Nasopharyngeal Carcinoma

►Nasopharyngeal carcinoma (NPC) is an epithelial tumor of the nasopharynx that is rare in the West but endemic in China, South East Asia, and North Africa. There are three main types of NPC; undifferentiated, non-keratinizing and squamous NPC. EBV is strongly associated with the undifferentiated type (UNPC). UNPC is an aggressive tumor with ►metastasis early to bone, liver, and lung and to the lymph nodes of the neck. The exact contribution of EBV to the pathogenesis of NPC has yet to be established, although recent studies suggest that EBV infection is preceded by genetic changes which include deletions of chromosome regions rich in tumor suppressor genes. Dietary factors, including nitrosamines from preserved fish, as well as EBV, are important risk factors for UNPC.

Hodgkin Lymphoma

►Hodgkin lymphoma (HL) is characterized by relatively low numbers of malignant, so-called ►Hodgkin/Reed–Sternberg cells (HRS cells) surrounded by a mass of ‘reactive’ non-malignant cells. HL is classified into four major subtypes on the basis of the relative proportions and morphology of the HRS cells, the nature of the reactive component, and the degree of fibrosis. In most cases the HRS cell is believed to derive from germinal centre (GC), or post-GC B-lymphocytes.

The frequency of the EBV association in HL is dependent upon a number of factors. EBV is most often associated with the mixed cellularity form and less commonly with the other subtypes. In North America and Europe, fewer (20–40%) tumors are EBV-associated, compared to developing countries where the association approaches 100%. Males also seem to be more at risk than females for EBV-positive HL. In EBV-positive cases, HRS cells express EBNA1, LMP1 and LMP2 (latency II pattern, Table 2). Epitopes from both LMP1 and LMP2 can be processed and presented to CTLs by infected cells. Thus, EBV-infected HRS cells should be recognized by EBV-specific CTLs; however, their survival in immunocompetent patients suggests that they, like BL cells, can escape the immune response. EBV-infected HRS cells express interleukin-10 (IL-10) which can inhibit EBV-specific CTL responses. Regulatory T cells have are also detected in the microenvironment of HL. It is likely that EBV and other persistent virus are able to recruit regulatory T cells to inhibit virus-specific T cell responses.

Lymphoproliferative Disease in Immunosuppressed Patients

In immunosuppressed patients the lack of EBV-specific CTLs can lead to an increase in the numbers of EBV-infected B-lymphocytes. In persistent ►immunosuppression states, such as in post-transplant patients or in AIDS sufferers, EBV-infected B-lymphocytes can proliferate to produce tumor like masses. Later, the acquisition of other genomic changes, such as those that affect ►p53, ►MYC or ►BCL-6 can lead to the formation of a classic ►lymphoma. Therefore, these lymphoproliferative diseases constitute a spectrum of disorders ranging from relatively benign atypical lymphoproliferations which will regress if the immunosuppressive therapy is withdrawn through to highly aggressive lymphomas which do not respond to immune reconstitution.

Other Tumors

EBV is associated with a number of other tumors, including some T cell lymphomas, such as nasal T/natural killer cell lymphomas (►NK cell lymphomas). EBV is also associated with some gastric cancers and with smooth muscle tumors that arise in some immunodeficient patients. Thus, EBV is apparently able to infect and contribute to neoplastic growth in a number of different cell types.

Kaposi Sarcoma Herpesvirus

►Kaposi sarcoma (KS), originally described in 1872, is a malignancy of endothelial cells that usually presents as a brown/purple skin tumor with more aggressive forms involving the lungs, lymph nodes, and gastrointestinal tract. Until the advent of the AIDS epidemic it was a relatively rare disease whose etiology remained obscure. KS occurs frequently in ►HIV-positive individuals, particularly in homosexual/bisexual males. Suspicions that KS might be due to an infectious agent were confirmed when a new human herpesvirus, known as Kaposi Sarcoma herpesvirus or KSHV (also referred to as human herpesvirus-8, HHV8), was discovered in KS tumors from AIDS patients. In fact, viral sequences are present in all types of KS including KS that arises in HIV-negative individuals. Serological assays to detect antibodies to KSHV were developed and showed a higher prevalence of infection in those groups at high risk for the development of KS. KSHV is also associated with primary effusion lymphomas and a rare lymphoproliferative disease known as multicentric Castleman disease.

KSHV is a double stranded DNA virus that is closely related to EBV. Its genome encodes many genes with homology to human genes (Table 3). Some of these are involved in the regulation of both innate and adaptive immune responses; many of them function to inhibit the immune responses to viral infection. For example, several virus-encoded ►interferon (IFN)-regulatory

Virology. Table 3 KSHV encodes many homologues of cellular proteins, some of these viral proteins and their effects upon the infected cell are summarized

Cellular gene	KSHV gene	Effect of virus gene expression
Cyclin gene (cyclin D2)	v-Cyclin D	Phosphorylates Rb and releases cell from cell cycle arrest
IL-6	v-IL-6	Autocrine stimulation of cell growth
IL-8R	v-GPCR	Constitutive activation of phosphatidylinositol pathway leading to cell growth
FLICE inhibitor protein (FLIP)	v-FLIP	Inhibits CD95-mediated apoptosis
Interferon regulatory proteins (IRFs)	v-IRFs 1–4	Inhibits interferon signaling
Bcl-2	v-Bcl-2	Protects infected cell from apoptosis

factors (IRFs) negatively influence anti-viral interferon responses, while others protect cells from apoptosis (e.g. v-►FLIP, v-►Bcl-2).

Polyomaviridae

Polyomaviruses are DNA viruses with small circular genomes encoding only six proteins. They include the mouse polyoma virus, the ►simian virus 40 (SV40), and the human viruses, ►BK virus and ►JC virus. With the exception of the mouse polyoma virus, these viruses do not cause cancer in their natural hosts but do induce tumors in newborn animals, including hamsters and rats. Polyomaviruses do not themselves encode replication proteins and must drive cells into S-phase where host DNA replication proteins can be utilized for virus replication. The large T (tumor) antigen (T-Ag) and the small t antigen (t-Ag) are major effectors of this. T-Ags bind to pRb encoded by the ►RB gene and displace ►E2F thereby promoting cell cycle progression; this is a major mechanism by which T-Ag promote the inappropriate cell proliferation leading to oncogenic transformation. The release of E2F from pRb activates p14ARF which can stabilize p53. However, SV40, JCV and BKV T-Ags can bind to and inactivate p53 and thus prevent inhibition of the cell cycle or apoptosis.

Apart from their well established role in the binding and inactivation of p53 and pRb, T-Ag can influence other pathways leading to oncogenesis. Thus, JCV T-Ag can interact with insulin receptor substrate-1 (IRS-1); this is associated with transformation and might be involved in the development of childhood medulloblastomas. JCV T-Ag can also bind β-catenin, causing it to translocate to the nucleus where it can stimulate the expression of genes such as c-myc and cyclin D1. SV40 t-Ag binds and inhibits protein phosphatase 2A (PP2A), a major serine/threonine specific protein phosphatase; this leads to the activation of several pathways that promote cell proliferation, including the MAPK pathway.

Although the polyomaviruses can transform cells in culture and under certain conditions are oncogenic in laboratory animals, their association with clinical human tumors has yet to be definitely proven (►association of polyomaviruses with human cancers).

Papillomaviridae

►Human papillomaviruses (HPVs) are small DNA viruses that commonly infect epithelial tissues. At present there are over 100 known subtypes of HPV and the majority are responsible for benign lesions of the genital, upper respiratory and digestive tracts. However, some HPV subtypes are associated with malignant diseases of the skin and cervix (►cervical cancers). Cutaneous HPV infection normally results in the appearance of benign warts, however in the rare but lifelong skin disease, ►epidermodysplasia verruciformis (EV), these multiple benign warts can progress to malignant squamous carcinoma when exposed to ►ultraviolet light. The tumor cells often contain HPV 5 or 8. These two HPVs are also associated with the skin carcinoma observed in long-term immunosuppressed renal transplant patients.

Virtually all squamous cancers of the cervix are HPV-positive; HPV16, followed by HPV18 are the commonest subtypes found in this disease. HPV18 is the type most strongly associated with adenocarcinoma of the cervix. HPV is sexually transmitted and the association of HPV with cervical cancer explains many of the risk factors for this disease including early age at first sexual encounter and multiple sexual partners. Although most women will have been infected with HPV at some time, very few will develop invasive cancer.

In cancers, integration of HPV18 is almost universally observed, whereas integration of HPV16 is less common. Integration usually disrupts the E1 or E2 viral genes (►early genes of human papillomaviruses); this results in the loss of negative feedback control of E6 and E7 expression by the viral regulatory E2 protein. Overexpression of E6 and E7 proteins is important in oncogenesis; E6 binds to and inactivates p53 and E7 binds to Rb.

A bivalent HPV16/18, and a quadrivalent HPV 6/11/16/18 vaccine are being evaluated in phase III clinical trials. Preliminary data suggest that these prophylactic HPV virus-like particle vaccines are effective in preventing infections and also in reducing epithelial abnormalities (►human papillomavirus vaccines).

Hepadnaviridae

Hepatitis B Virus

►Hepatitis B virus (HBV) is associated with the development of ►hepatocellular carcinoma (HCC) where integrated HBV DNA can been detected in the majority of tumors. The exact role of HBV in the development of HCC is yet to be established. However, the HBx protein is likely to be important since it can induce a number of cellular changes that contribute to transformation, including the activation of several intracellular signaling pathways, including NF-κB. Other factors in addition to HBV status contribute to the risk of developing HCC; these include smoking, dietary components such as ►aflatoxin, and exposure to other hepatotoxic agents, including ►hepatitis C virus.

Flaviviridae

Hepatitis C Virus

►Hepatitis C virus is a ►blood-borne RNA virus that can cause chronic hepatitis and later ►cirrhosis and hepatocellular carcinoma. HCV is spread by

blood-to-blood contact. Many people with HCV infection have no symptoms and are unaware of the need to seek treatment. An estimated 150–200 million people worldwide are infected with HCV. At least four HCV gene products, namely HCV core, NS3, NS4B and NS5A, have been shown to exhibit transformation potential in tissue culture. Both HCV core and NS5A induce the accumulation of wild-type β-catenin.

Retroviridae

Human T lymphotropic Virus-1

Human T lymphotropic virus-1 (HTLV1) (►human T cell leukaemia virus) infects ►CD4-positive T-lymphocytes and is associated with the development of adult T-cell leukaemia/lymphoma (ATLL). Like other retroviruses, the HTLV1 genome consists of gag, pol, and env genes, which encode important structural and functional proteins, flanked by long terminal redundancies (LTRs). HTLV1 has an additional 3Y region which encodes several proteins implicated in transformation; these include Tax, Rex, p12, p13, p30, and HBZ. ►Tax has been shown to be necessary and sufficient for transformation by HTLV-1. Tax activates both the canonical and non-canonical NF-κB pathways, in turn leading to increased expression of many ►cytokines and their receptors, including ►interleukin-2 and the interleukin-2 receptor, which leads to polyclonal proliferation of HTLV-1-infected cells by ►autocrine and ►paracrine mechanisms. Additional potentially transforming effects are contributed by some of the other HTLV1 genes.

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Virosomes

Definition

Are vesicles mimicking the envelopes of various viruses, such as influenza virus, Sendai virus, and human immunodeficiency virus. These vesicles lack the functional viral nucleocapsid, including the viral genetic material, but can still be used to incorporate low molecular weight drugs, proteins, and nucleic acids. Virosomes can introduce these molecules into target cells by the same mechanism as viral infection because they retain the cell entry and membrane fusion properties of the parent virus.

►Non-viral Vector for Cancer Therapy

Virotherapy

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Synonyms

Oncolytic virotherapy

Definition

►Virotherapy utilizes ►oncolytic viruses, which may occur naturally or more commonly be engineered, such as conditionally replicative ►adenoviruses (►CRAds), to selectively infect tumor cells and replicate within them, thus causing their demise while sparing surrounding normal cells in the host. ►Oncolysis results from the replicative life cycle of the virus, which lyses infected tumor cells and releases viral progeny for propagation of infection and resultant lysis of neighboring cancer cells whereby normal host cells are spared.

Characteristics

Virotherapy represents an exciting and novel interventional strategy for a range of neoplastic disorders. In this strategy a virus is rendered conditionally replicative for tumor cells whereby direct oncolytic target killing is achieved. A variety of viral species have been adapted as virotherapy agents with the majority of human clinical trials exploiting conditionally replicative ►herpes simplex virus (HSV) and adenovirus. Of these, adenovirus has emerged as a promising model oncolytic

vector to target tumor cells. Over 40 different human serotypes of adenoviruses have been identified with types 2 and 5 being the most extensively used in developing oncolytic constructs.

Oncolytic Adenoviruses and Cancer Gene Therapy

The concept of using conditionally replicative adenoviruses (CRAds) for the treatment of cancer, also known as ►oncolytic virus therapeutics, originated in the 1950s. The knowledge that adenoviruses could eliminate cancer cells *in vitro*, as a consequence of their reproducitive cycle leading to cell lysis ("oncolysis"), resulted in clinical studies in which various wild-type adenoviral serotypes were examined for their effect on ►cervical cancer patients. In these studies, no significant toxicity was reported after intratumoral injection (i.p.) or intravenous (i.v.) administration and a moderate tumor response was observed. The most studied CRAd is the one originally generated (*dl*1520) by Arnold Berk and used initially by Frank McCormick as a selective vector, named ONYX-015. This viral vector originally was believed to only replicate in p53-defective cells (present in ~50% of human tumors). It is now recognized that these agents have beneficial properties for cancer ►gene therapy when compared with their nonreplicating counterparts, although this mechanism has subsequently been questioned. The adenovirus-based vectors have maintained their positions as the leading candidates for *in vivo* oncolytic virotherapy because they are safe, produced in high titers, do not integrate into the host chromosome, and have a wide tropism in neoplastic cells. While adenoviral transfection is efficient, the expression of the transferred gene is transient, as the viral genome remains episomal. To maximize CRAd mediated cell killing, one needs to achieve an amplification effect for transduction, via replication of the delivered viral vector post-infection, resulting in lateral spread of the progeny vector and cell killing via viral oncolysis. In addition, viral proteins expressed late in the course of infection are indirectly cytotoxic, including the E3 11.6 kDa adenovirus death protein, E4ORF4.

CRAds in Human Clinical Trials and the Limitations

Significant antitumor activity has been demonstrated using ONYX-015 both *in vitro* and *in vivo* using murine models. The preclinical potential of CRAds led to their rapid translation into human clinical trials, including those targeting recurrent head and neck, pancreatic, colorectal, ovarian, and ►hepatobiliary cancer. The overall conclusion was that adenoviral virotherapy is a safe method when applied via various routes, thus validating the concept *in vivo*. In clinical practice, however, employing CRAd as a single agent has demonstrated limited efficacy. This is due, in part, to relatively inefficient gene transfer to tumor cells. The replicative Ad system of ONYX-015, although demonstrating promise in preclinical studies, yielded no clinical effect in 16 patients with ►ovarian cancer treated intraperitoneally with up to 1×10^{11} plaque-forming units of adenovirus daily for 5 days. Important in this regard, cancer cells have been specifically shown to be profoundly resistant to Ad infection. The results obtained in these studies have been helpful in determining the limitations of the current generation of CRAds. The critical problems that have been encountered involve two main limitations: (i) poor infectivity of cancer cells by adenovirus at the transductional level due to the lack of native adenoviral receptor, the coxsackievirus-adenovirus receptor (CAR), on the surfaces of tumor cells; (ii) poor tumor selectivity of CRAd agents at the transcriptional level due to the lack of tumor specific promoters to selectively drive the viral replication in tumor cells.

CAR-Deficiency on Tumor Cells Results in the Poor Infectivity of Ad Agents

Cancer cells have been specifically shown to be profoundly resistant to Ad infection because of their lack of the primary receptor for viral entry, the coxsackievirus-adenovirus receptor (CAR). As these primary tumor cells often express relatively low levels of the CAR, this results in the poor infectivity of CRAd agents and the difficulty of lateral dispersion of virus in tumor tissue. This has been demonstrated by the fact that low CAR levels strongly reduced viral replication and oncolysis in monolayer cultures and murine models. On this basis, it was proposed that delivery be achieved via a heterologous entry pathway, or CAR-independent pathway to circumvent this key aspect of tumor biology.

Infectivity Enhanced CRAd Agents-Retargeting CRAds to Tumor Cells

Native adenoviral tropism is mediated by two capsid proteins, fiber and penton base. These proteins bind to the primary, high-affinity cellular receptor, CAR, and the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, respectively. Manipulation of these molecular interactions has been carried out to modify adenovirus tropism by routing entry through either a heterologous entry pathway or a CAR-independent pathway. Many approaches have been described to enhance the viral infectivity. These include: (i) Ad capsid modification for circumventing tumor cell CAR deficiency. Specifically, Dmitriev et al. (1998b) reported that construction of modified adenoviral vectors containing the RGD peptide in the HI loop region, which targets integrins $\alpha v\beta 3$

and αvβ5 instead of CAR, increased gene transfer to ovarian cancer cell lines (30–600 fold) and to primary ovarian cancer cells obtained from patients (2–3 fold). Many other approaches have been reported which include targeting Ad to the serotype 3 receptor with a chimeric fiber protein, such as F5/3, targeting Ad to tumor cells with the non-human canine Ad type 2 knob, targeting Ad to a heparin sulfate-containing receptor with a Ad fiber incorporating polylysine (pK7) and targeting Ad to the junction adhesion molecule 1 (JAM1) with an Ad fiber incorporating reovirus sigma 1 fiber. All these fiber modifications enhanced the viral infectivity of Ad vectors but with different levels of success dependent upon the tumor types. (ii) Transductional retargeting using heterologous targeting adapters. In adapter-mediated targeting, the tropism of the virus is modified by an extraneous targeting moiety, the ligand, which associates with an Ad virion either covalently or non-covalently. Zhu et al. described in 2004 that an adenoviral vector was successfully induced to transport itself across polarized epithelial monolayers by use of a fusion protein (sCAR-transferrin), a bi-functional adapter, which bound to the knob of Ad through a sCAR (secretory CAR) domain and to the transferrin receptor on cell surfaces through a transferrin domain. In addition, sCAR-EGF, sCAR-SCF, and sCAR-scFv have all been reported as having successfully targeted EGF receptor-positive cells, c-Kit(+) and CAR(–) hematopoietic cells and ovarian carcinoma cells, respectively. The genetic fusion proteins of single-chain variable fragment (scFv) antibodies directed against the fiber knob and receptor on the surfaces of tumor cells have also been used in virotherapy. In this schema, an anti-fiber-knob Ab has been employed to attach to the cell recognition motif of the fiber knob and, importantly, ablate the native tropism determinants. An Ab, or Fab derivative, was then conjugated to a second moiety, which provided targeting specificity. To that end, receptor ligands such as folate and basic fibroblast growth factor-2 (FGF-2) have been used to successfully target tumor cells. Thus, the strategy of tropism modification either using bispecific conjugates or scFv molecules allowed dramatic augmentation in gene delivery to tumor targets, with a specificity that would predict an improved therapeutic index.

Tumor Specific CRAd Agents – Using a Tumor Specific Promoter to Drive Specific Viral Replication

Although viral replicative specificity is not the main limit of CRAd efficacy, our work and those of others have also sought to address this aspect to improve the overall therapeutic index. The method used to direct CRAd vector specificity is by regulation of viral replication via cellular promoters that are over expressed or reactivated in selected tumor cells, sometimes referred to as "tumor specific" promoters. A number of CRAd agents have been developed, which harbor the essential Ad E1A gene under the control of such promoters. They include the alpha-fetoprotein promoter, Cox-2 promoter, DF3/MUC1 promoter, midkine promoter, PSA/PSMA enhancer, secretory leukocyte protease inhibitor (SLPI) promoter, CXCR4 and survivin promoters, human telomerase reverse transcriptase (hTERT) promoter, and the tyrosinase promoter/enhancer. Most of these agents have demonstrated remarkable preclinical results in eradicating tumors in xenograft mouse models.

A Double Targeted CRAd for Ovarian Cancer

Based on the foregoing considerations, we have developed a CRAd agent which addressed the limits of the current systems. As previously discussed, CAR deficiency in the context of the target tumor would clearly confound this process, thus undermining the overall efficacy of CRAd agents. Previous studies have demonstrated that incorporation of an Arg-Gly-Asp (►RGD)-containing peptide in the HI loop of the fiber knob domain results in the ability of the virus to utilize an alternative receptor during the cell entry process. In the context of ovarian cancer, the RGD modified nonreplicative adenovirus mediates enhanced gene transfer *in vitro* in both established cell lines and freshly cultured ►ovarian cancer cells. It exhibits preferential gene transfer to primary ovarian cancer cells when compared to non-transformed human mesothelial tissue, thus, indicating a degree of specificity for tumor cells. In addition, the RGD modified nonreplicative adenoviral vector demonstrated enhanced infectivity of primary ovarian tumor cells where infectivity was known to be inhibited by preformed neutralizing antibodies against adenovirus found in the ascites fluid in which the tumor cells float. Investigators at the University of Alabama at Birmingham (UAB) and the University of Texas-MD Anderson Cancer Center have constructed a novel infectivity enhanced CRAd, designated Ad5-Δ24RGD, that has a 24 bp deletion in the E1A gene and that incorporates the RGD modification in its fiber. The deletion in the E1A gene is from Ad5bp923 to 946 which corresponds to the amino acid sequence L122TCHEAGF129 of the E1A protein known to be necessary for host cell Rb protein binding. Adenoviruses having this partial deletion cannot induce resting cells to pass the ►G2/M checkpoint and progress to mitosis and lysis. In contrast, most human tumor cells bypass the Rb/p16 pathway, thus allowing for selective replication of adenoviruses with this deletion. Preliminary experiments demonstrated that Ad5-Δ24RGD propagated more efficiently than Ad5-Δ24 in

A549 cells. Specifically, A549 cells transfected with Ad5-Δ24RGD yielded 43 fold times increase in viral progeny (3.75×10^9 pfu/ml) when compared to Ad5-Δ24 infected cells (8.74×10^7 pfu/ml). Cell lysis in Ad5-Δ24RGD infected A549 and LNCap cells was 7 and 3.5 times higher, respectively, compared to that achieved with Ad5-Δ24. Validation of the potential benefits of incorporating infectivity enhancements into a CRAd provided the rational to translate this into clinical trials which has been recently approved by FDA.

Future Directions for Improvements

CRAd human trials were historically limited by lack of useful information regarding the biologic basis of adenovirus efficacy barriers. Absent "surrogate endpoints," imaging could potentially provide this information. We have thus developed an imaging system which addresses this problem, based upon fluorescently labeled adenovirus carrying EGFP (enhanced green fluorescent protein) on the pIX minor surface protein of Ad for vector detection. Furthermore, positron emission tomography (PET) scanning has been used to detect herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) which was fused to this same Ad protein IX (pIX). Other conventional imaging systems for virotherapy have been designed for the detection of transgene expression of reporters such as sodium iodide symporter, somatostatin receptor type 2 (SSTR-2), and luciferase. The combination of a noninvasive imaging modality with a genetic adenoviral labeling system for detection of viral replication and progeny localization has begun to provide a powerful means of real-time monitoring of CRAd function *in vivo*.

►Oncolytic Virus

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Virtual High Throughput Screen

Definition

A procedure in which collections of computerized molecular structures are tested for their ability to activate, perturb, or modify a target or biological process of interest using digital models.

►Small Molecule Screens

Virus-like Particles

Definition

Non-infectious assembly of viral proteins lacking a genome. Biotechnological virus-like particles elicit antiviral immunity without risk of infection and can be used to make vaccines. The term is also applied to some natural non-infectious mycoviruses with an RNA genome.

►Immunoprevention

Virus Therapy of Cancer

►Oncolytic Virotherapy

Virus Vector

Definition

Refers to genetically modified viruses or virus-derived particles that can be used to deliver a (non-viral) gene of interest to some specified target cell or tissue.

►Viral Vector-mediated Gene Transfer

Virus Vector-mediated Gene Transfer

►Viral Vector-mediated Gene Transfer

Viruses

Definition

Viruses are pathogens composed of a nucleic acid genome enclosed in a protein coat. They can replicate only in a living cell, as they do not possess the metabolic machinery for independent life.

►Virology

Vital

Definition

Crucial or extremely important.

Vitamin A

Definition

Is an essential human nutrient. It exists not as a single compound, but in several forms. In foods of animal origin, the major form of vitamin A is ►retinol.

►All-Trans Retinol
►Retinoids

Vitamin C

Definition

A water-soluble vitamin found in fruits and vegetables and used as an ►antioxidant.

►Chemoprotectants

Vitamin D

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Definition

The biologically most active form of vitamin D, 1α,25-dihydroxy-vitamin D_3, and many synthetic analogs exert anti-proliferative actions on different cancer cell types, most typically breast, colon, and prostate. Recent clinical studies indicate a potentially important role for vitamin D in the prevention and treatment of cancer.

Characteristics

1α,25-dihydroxy-vitamin D_3 (1,25D), or ►calcitriol, the biologically most active form of vitamin D, is essential to bone and mineral metabolism. 1,25D deficiency has been traditionally associated with bone diseases characterized by decreased bone mass and reduced mineralization, such as rickets, osteomalacia, and osteoporosis. In addition to its well known actions on bone and calcium homeostasis, this ►secosteroid hormone exerts a number of effects on many different target organs and systems. 1,25D actions include regulation of ►cell cycle, cell proliferation and differentiation in breast, colon, and prostate, among others. 1,25D binds to the nuclear ►vitamin D receptor (VDR), which acts as a ►transcription factor for the regulation of gene expression. In addition, 1,25D stimulates signal transduction cascades implicated in non-genomic responses of target cells such as modulation of the electrical state of the plasma membrane, secretory activities, cell survival, and establishment of the apoptotic phenotype.

Mechanisms

In recent years, natural compounds and synthetic analogs of vitamin D have proved to have significant effects on cell cycle, differentiation, survival and ►programmed cell death. More specifically, 1,25D has been shown to act as a powerful anti-proliferative agent on certain cancer cell types expressing the VDR, including breast, colon and prostate, both *in vitro* and *in vivo*. Typically, cell cycle arrest promoted by physiological doses of 1,25D is linked to cell differentiation, and therefore reduction in the probability of cells to become cancerous.

Human trials on anti-cancer properties of calcitriol and synthetic analogs have increased in recent years, with ►prostate cancer being the most studied one. Calcitriol and numerous analogs have shown potential to become therapeutic agents for the treatment of certain cancer types in the future. However, the molecular mechanisms by which 1,25D inhibits cancer cell proliferation remain only partially understood. To date, more

than 1,000 vitamin D analogs have been synthesized, and many of these analogs have shown to have significant anti-proliferative effects.

The earliest evidences on a relationship between vitamin D and cancer come from epidemiological studies conducted in the 1990s. An inverse relationship was discovered between sunlight exposure –which is lower as latitude increases– and ►prostate cancer cases in the United States over 45 years of data (1950–1994). A main source of vitamin D in our bodies is by means of production of vitamin D_3 or cholecalciferol, a hormone precursor, in the skin by ►UV radiation. The second main source is vitamin D_2 or ergocalciferol in the diet. However, these products are biologically inactive. They require two sequential hydroxylations, first in the liver (to produce 25-hydroxy-vitamin D_3, or 25D), and then in the kidney (to render 1α,25-dihydroxy-vitamin D_3). The major circulating source of the secosteroid hormone is 25D, therefore measurement of circulating concentrations is a good indicator of the overall vitamin D_3 status of the individual. In addition, many tissues express 25-hydroxy vitamin D_3 1α- hydroxylase, the enzyme that transforms 25D into 1,25D, which indicates that local production of the biologically active form may play an important role in ►autocrine and ►paracrine functions. Early epidemiological studies suggested that elevated circulating levels of 25D main protect against cancer, and that ingestion of vitamin D supplements may be helpful in the treatment of tumors.

In addition to case studies, extensive *in vitro* work has demonstrated that high concentrations of 1,25D (10^{-9}–10^{-7} M) inhibit the growth and induce differentiation of tumor cells, including malignant ►melanoma, myeloid leukemia, ►prostate cancer, ►breast cancer, ►glioma cancer, and ►colon cancer. Clinical trials and treatment of cancer patients with high doses of 1,25D or synthetic analogs have been limited due to the fact that, at these high vitamin D_3 doses, there is a significant risk to develop ►hypercalcemia with fatal side effects. This has encouraged the pharmaceutical industry to develop new synthetic analogs with high potency for cell growth inhibition and low hypercalcemic effects (for example, analog EB1089).

The highly conformationally flexible 1,25D molecule offers a broad source of possibilities for the design of chemical compounds with high affinity for the VDR. These are ideally capable of potentiating the genomic regulation of the cell cycle, while inducing only low calcium effects more likely to develop via non-genomic mechanisms. Recently, an alternative binding site for non-genomic analogs has been proposed on the basis of molecular modeling of the crystal structure of the VDR. This new hypothesis for the binding of ligands to the VDR brings an even higher potential for the design of analogs with more specific anti-proliferative actions and reduced hypercalcemic side effects.

Additional aspects that contribute to the tumor suppressive activity of vitamin D compounds include the ability of 1,25D and analogs to reduce ►angiogenesis and ►metastasis. While use of 1,25D and analogs with low calcemic effects for the treatment of cancer offers high promise, combination therapy with other anti-tumorigenic drugs may signify even higher beneficial effects. *In vitro* studies have demonstrated, for example, that 1,25D in combination with the antiestrogen ►tamoxifen has greater efficacy in the inhibition of breast cancer cell lines. Different combinations of vitamin D_3 and ►retinoids, ►cytokines, and a number of cytotoxic compounds including ►adriamycin, ►carboplatin, ►cisplatin have proven to act synergistically on the suppression of cancer cell growth.

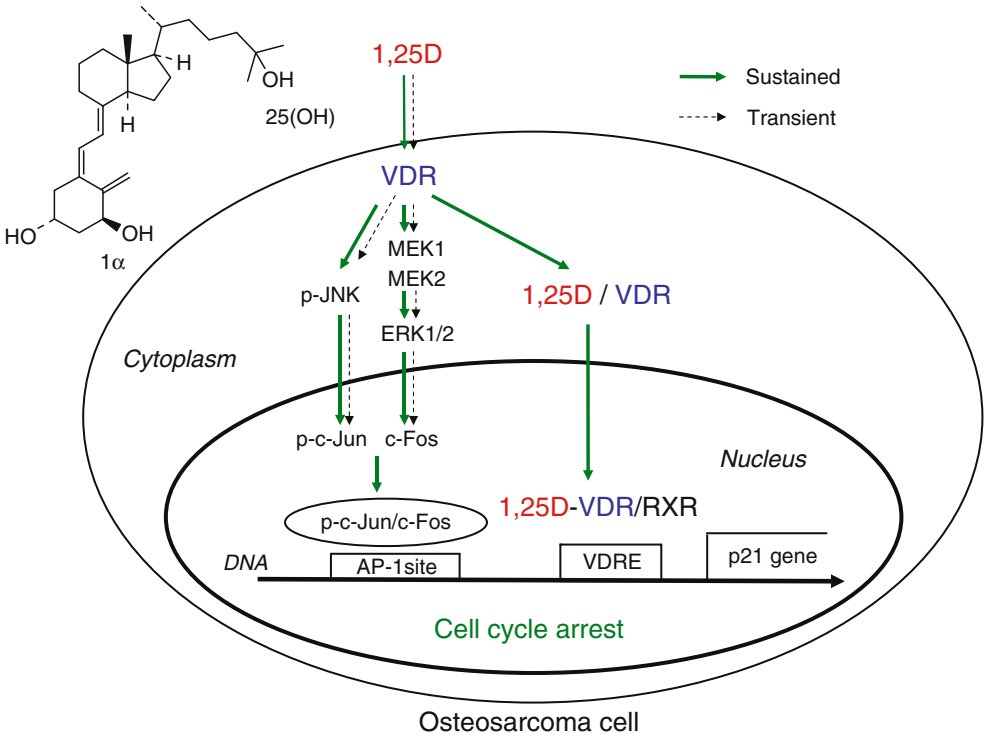
The anti-cancer effects of vitamin D compounds and analogs can be explained on the basis of different aspects of cancer cell biology. More specifically, 1,25D effects on tumor cells include:

1. Regulation of the ►cell cycle
2. Induction of ►apoptosis or programmed cell death
3. Modulation of the expression of ►oncogenes and ►tumor suppressor genes
4. Induction of cell differentiation

Studies performed with 1,25D and analogs on different cancer cell lines *in vitro* have shown that cells grown in the presence of the steroid significantly reduce their growth rate mostly by blockade of the transition from the G_0/G_1 phase (differentiated, non dividing cells) to the S phase (DNA synthesis). In addition to promoting cell cycle arrest and inhibition of cell division, 1,25D and related compounds have proved to induce apoptosis in glioma, breast, leukemia and colon cancer cells. It is not clear, however, how a cell follows its path to either cell cycle progression or apoptosis once it reaches early G_1 phase. Vitamin D_3 compounds appear to act at this point through different molecular mechanisms, depending on whether they induce cell death or stop cells from dividing.

In human ►osteosarcoma, for example, 1,25D treatment reduces cell proliferation *in vitro* by approximately 25% after 3 days. The mechanisms involve sustained activation of ►MAP kinase/►AP-1/p21(waf1) pathways. Upregulation of *p21* gene expression led to control over the cell cycle and subsequent cell cycle arrest (Fig. 1).

The modulation of the expression of certain oncogenes and tumor suppressor genes has been also shown to be affected by 1,25D treatment of cancer cell lines. Among genes that modulate cell growth and apoptosis, the ►*bcl-2*, ►*c-myc*, and retinoblastoma gene products are the most widely studied. The *bcl-2* oncogene product protects against apoptosis, therefore inducing cell survival. In MCF-7 breast cancer cells, 1,25D and analogs KH1060 and EB1089 decreased *bcl-2*



Vitamin D. Figure 1 1,25D reduction of human osteosarcoma cell proliferation occurs via sustained activation of mitogen activated protein kinases ►JNK and MEK1/MEK2 downstream of non-genomic VDR signaling, leading to upregulation of a c-Jun/c-Fos (AP-1) transcription factor complex, which in turn modulates *p21(waf1)* gene expression. Transient refers to 1,25D treatment for 15 min; sustained implicates a treatment with hormone for 3 days. RXR: ►retinoic acid receptor, VDRE: vitamin D responsive element, p: indicates phosphorylation of the protein.

expression, and cells progressed through apoptosis. The expression of the oncogene *c-myc* has also been shown to be reduced by 1,25D treatment of breast cancer cells, and resulted in reduction of cell proliferation. Finally, induction of cell differentiation by vitamin D_3 compounds has been widely described in combination with its anti-proliferative effects. Typically, 1,25D inhibits cell proliferation and induces differentiation in the hematopoietic system, osteoblasts, and keratinocytes.

Currently, the safe upper limits for vitamin D intake as supplements have been set in the US by The Institute of Medicine to be 1,000 IU/day for children under 1 year of age, and 2,000 IU/day for adults. Although rare, vitamin D intoxication may develop if intake of higher doses happens over significantly prolonged periods of time, and lead to hypercalcemia, hyperphosphatemia and hypercalciuria. A recent clinical trial conducted on patients with premalignant colon and rectal tissues showed that a treatment with 1,500 mg of calcium carbonate and 400 IU of vitamin D_3 per day significantly decreased the levels of expression of markers of cell proliferation in polyp tissues, which is an indicator of tumor size reduction. In Europe, fortification of milk and other food products with vitamin D has been prohibited because of an increase in hypercalcemia cases in infants after World War II. However, in consideration that vitamin D deficiency is widely recognized as a cause of impaired bone formation in children, and that there are clear beneficial effects of the hormone in cases of cancer and osteoporosis, many European countries currently fortify margarine and cereals with vitamin D.

Conclusions

In vitro and *in vivo* studies on anti-cancer effects of 1,25D and synthetic analogs have indicated that the steroid has high potential for therapeutic applications in a wide range of cancers. Of special interest is the development of future combination therapies with other anti-tumor drugs with synergistic effects on cell growth. However, it is imperative that the precise molecular mechanisms by which 1,25D modulates cell proliferation and death be investigated. In addition, more clinical studies are needed in order to establish whether 1,25D and analogs can be used as an effective treatment of cancer.

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Vitamin D Receptor

Definition

Member of the family of nuclear receptors transcription factors. The vitamin D receptor is activated upon binding of the active form of vitamin D to its ligand binding site. Once activated, the ligand-bound VDR interacts with a vitamin D responsive element in the DNA to modulate the expression of specific genes in target cells.

►Vitamin D

Vitamin E

Definition

A fat-soluble antioxidant that is known to benefit human health.

►Chemoprotectants

Vitiligo

Definition

Is a chronic skin condition that causes loss of pigment, resulting in irregular depigmented skin patches. The precise pathophysiology of vitiligo is complex and not fully understood. Some evidence suggests that it is caused by a combination of autoimmune, genetic, and environmental factors.

►Melanoma Antigens

Vitrification

Definition

In cryosurgery, pure water that shifts into a solid state.

►Cryosurgery in Bone Tumors

VLP

Definition

►Virus-like particles

VM-targeted Therapy

Definition

Vasculogenic mimicry-targeted therapy; A totally different structure from endothelium-dependent vessels and it reveals several molecules for antitumor therapy. Therapeutic strategies that target endothelial cells have no effect on tumor cells that engage in VM. VM-targeting strategies include suppressing tyrosine kinase activity using a knockout EphA2 gene, downregulating VE-cadherin using antibodies against human ►matrix metalloproteinases (MMPs) and the laminin 5γ2 chain, and using anti-►PI3K therapy.

►Vasculogenic Mimicry

VNTR

Definition

►Variable Number Tandem Repeats

Volume of Distribution

Definition

The theoretical volume required to distribute a drug at a defined concentration, as measured in plasma, throughout the body.

►Lead Optimization

von Hippel-Lindau

►von Hippel-Lindau Tumor Suppressor Gene

Von Hippel-Lindau Disease

Definition

►von Hippel-Lindau Tumor Suppressor Gene

von Hippel-Lindau Tumor Suppressor Gene

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Synonyms

von Hippel-Lindau; VHL; tumor suppressor gene

Definition

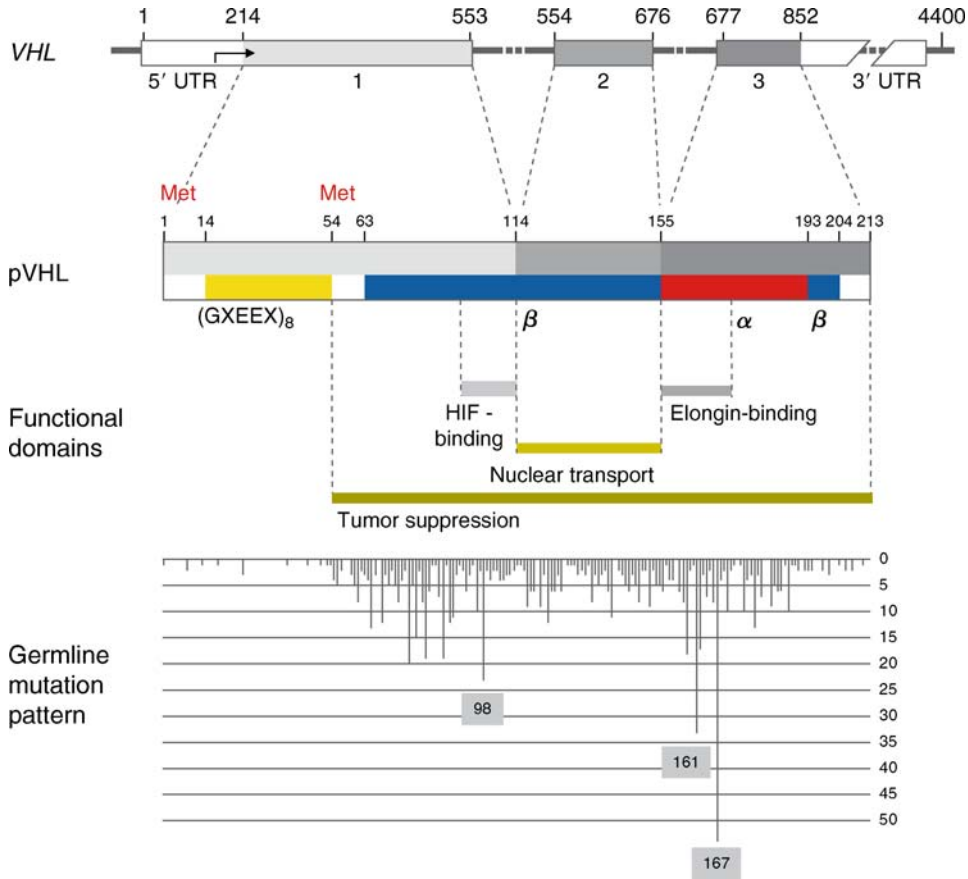
The von Hippel-Lindau ►tumor suppressor gene (VHL) is a cellular gene that is required for normal development, differentiation and cellular stress response (hypoxia, lack of glucose). VHL was discovered in families with the hereditary von Hippel-Lindau (VHL) syndrome by virtue of its two-hit mechanism of inactivation, and identified in 1993 following a positional cloning strategy. The VHL gene may be subject to mutation either in the germline giving rise to VHL disease or in somatic renal epithelial cells giving rise to sporadic renal conventional (clear cell) carcinoma (CCRCC) functioning as a ►gatekeeper. VHL mutation patterns suggested two functional domains within the protein pVHL. Current knowledge indicates similarities to the SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex that targets proteins for degradation. Therefore, pVHL may function as a molecular adaptor in a similar proteolytic pathway. Today's best understood function of pVHL is its role in the VHL/HIF (►Hypoxia inducible factor 1 α) pathway.

Characteristics

Molecular Features

- Gene located at 3p25.3, single copy locus: NCBI GenBank, range: bp 10.158.319–10.168.762, as published in Nature 431(7011):931–945 (2004) (Fig. 1)
- 639 Nucleotides in three exons (originally reported sequence contained 852 nucleotides with 213 untranslated base pairs at the 5′ end)
- Exon 1 is a CpG island (G + C content is 70%, CpG/GpC > 1)
- TATA-less and CCAAT-less promoter
- Two transcription initiation codons (amino acid 1 and amino acid 54)
- Follows a two-hit mechanism of inactivation characteristic for tumor suppressor genes
- Evolutionarily highly conserved
- No homologies known
- The full length protein pVHL contains 213 amino acids
- Known isoforms result from tissue specific and developmentally selective alternative splicing (skipping of exon 2)
- Differentially phosphorylated at serin 68 by casein kinase 2 or glykogen synthase kinase 3
- Expressed in a variety of adult and fetal tissues including those of VHL target organs
- There are two protein binding domains that allow pVHL to function as an adaptor molecule in a proteolytic pathway
- The gene may be subject to mutations at almost any nucleotide of the 470 bp COOH terminal sequence
- Phenotypic variation may result from confounding effects of modifier genes
- No imprinting reported
- Posttranslational negative regulation by E2-EPF UCP (E2-EPF ►ubiquitin carrier protein)

Structure of the von Hippel Lindau gene (*VHL*), and protein, indicating the functional domains. The pattern of germ line mutations published so far, reflects the functional importance of the binding sites to the target as well as to the multimeric protein complex.



von Hippel-Lindau Tumor Suppressor Gene. Figure 1 Structure, Function, and Germ Line Mutations of the VHL gene.

Role in Diseases – Clinical, Molecular and Cellular Characteristics

von Hippel-Lindau (VHL) disease (OMIM 193300) is an ►inherited tumor susceptibility syndrome predisposing gene carriers to a variety of benign and malignant tumors. VHL segregates in affected families as an autosomal dominant inherited trait. Phenotypic expression is highly variable.

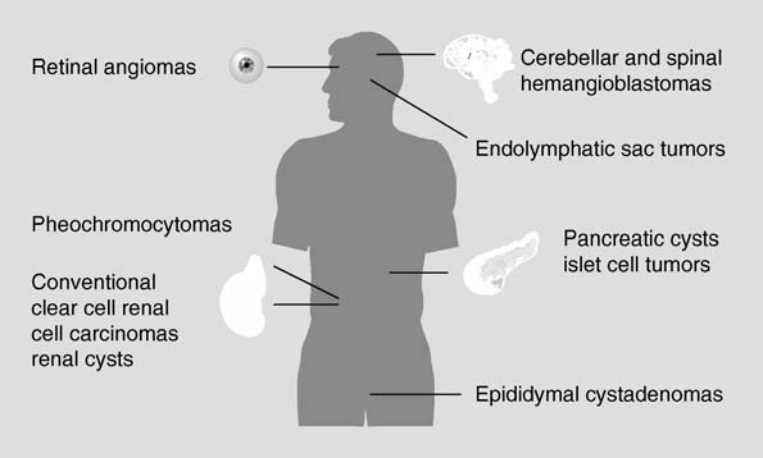
Clinical and Molecular Features

Lesions Associated with von Hippel-Lindau Disease. (Fig. 2) VHL patients may present with a variety of tumors affecting eye, central nervous system, inner ear, adrenal gland, kidney, pancreas, epididymis. Most frequent tumors include retinal angiomas, and ►hemangioblastoma of the cerebellum and of the spinal cord which are usually benign. Other benign lesions include ►pheochromocytoma, renal and pancreatic cysts. Renal clear cell carcinomas are malignant.

- Tumors and cysts are frequently bilateral and/or multiple in origin.
- All ethnic groups are involved, there is no sex bias.
- Birth incidence is estimated 1/39,000 (Germany) to 1/53,000 (East Anglia).
- Prevalence is 1/85,000–1/31,000.
- Incidence of *de novo* mutations is about 5% (up to 20%).
- Mean age at diagnosis is 26 years.
- Penetrance by age of 65 years more than 90%.
- Most severe complications are hemangioblastomas due to unrestricted growth in the confined space of skull or vertebral canal, and CCRCC due to metastasis.
- Major cause of death are hemangioblastomas.

Classical Clinical Definition

- Known family history of retinal or cerebellar ►hemangioblastoma: the presence of a single hemangioblastoma or a visceral manifestation, i.e. diagnosis of a CCRCC in a family member will define this patient as a disease gene carrier.
- Isolated cases, possibly indicating a *de novo* mutation: two or more hemangioblastomas (spinal,



von Hippel-Lindau Tumor Suppressor Gene. Figure 2 Lesions associated with von Hippel-Lindau disease.

cerebellar or retinal) or a single hemangioblastoma in association with a visceral manifestation, i.e. CCRCC) are sufficient to establish the diagnosis.

It is possible in most cases to determine VHL carrier status by mutation testing of DNA derived from nuclear blood cells. This can either be performed to assist clinical diagnosis or to establish carrier status presymptomatically in at risk individuals. Based on the presence or absence of pheochromocytoma, VHL disease is phenotypically subclassified into VHL type 1 (without pheochromocytoma, applies to the majority of all families) and VHL type 2 (with pheochromocytoma, about 7–20% of all families). The VHL type 2 syndrome is further subdivided into type 2A (without CCRCC), type 2B (with CCRCC), and type 2C (pheochromocytomas as the sole manifestation). In VHL type 2C disease, it is especially important to carefully establish the molecular diagnosis in affected patients since pheochromocytoma is also a manifestation of other inherited syndromes such as multiple endocrine neoplasia type 2, neurofibromatosis type 1, and others.

Germline Mutations

Comprehensive germline mutation analysis allows the detection of more than 95% of VHL predisposing mutations in gene carriers (Fig. 1). As of today as many as 1,000 VHL mutations have been described in data bases, of which some 140 have been described as unique mutations. About 50–60% are missense mutations, 20–30% large intragenic deletions, 12–20 microdeletions, or insertions and 7–11% nonsense mutations. Most mutations can be readily identified by sequencing analysis. Large genomic and intragenic deletions may be identified by Southern blotting including quantitative Southern blotting, pulsed field gel electrophoresis (PFGE) or/and fluorescence *in situ* hybridization (►FISH), and more recently by Q-RT-PCR (quantitative real time polymerase chain reaction) and MLPA (multiplex ligase probe amplification). In those rare cases where germline mutations escape detection it is possible that an affected individual may be a mosaic, with some cells carrying the VHL mutation and others that don't. Although it may be difficult to find a VHL mutation in a mosaic individual, offspring are at high risk to develop the disease. Once the mutation is passed on into the next generation, due to affected germ cells, it should be easily identified in the affected offspring who now will carry the germline mutation in all of the body cells.

Most germline mutations cluster within exon 1 and exon 3 suggesting two functional protein domains. In particular, there is a mutational hotspot affecting codon 167, either changing an arginine to tryptophane or to glutamine. The affected amino acid is located within a 35 residue domain necessary for ElonginC binding for the formation of the ternary pVHL/ElonginC/ElonginB complex (VCB). Another frequently identified mutation causes a change of histidine to tryptophane at codon 98. The frequency of this mutation also known as Black Forest mutation is due to a founder effect. The affected amino acid is located within the HIF binding domain. Other commonly described mutations are delPhe76, Asn78Ser, Arg161Stop, and Leu178Pro. A VHL mutation data collection is available at http://www.umd.necker.fr (Beroud C, INSERM).

A unique hematological hereditary disease has been identified as a subtype of VHL germline mutations (Type Chuvash, OMIM 263400). Specific homozygous missense mutations C598T (Arg200Trp), and C571G (His191Asp) present exclusively with clinical features of polycythemia vera with elevated ►erythropoietin level. Interestingly, so far no other typical VHL complications have been seen in any carrier of these homozygous

von Hippel-Lindau Tumor Suppressor Gene. Table 1 Lesions associated with von-Hippel-Linder Disease

Affected Organs	Clinical Symptoms	Frequency
Eyes	Angiomatosis retinae	50–57%
Central nervous system	Cerebellar hemangioblastomas	55–59%
	Spinal hemangioblastomas	13–14%
Adrenal glands	►Pheochromocytomas	7–19%
Kidneys	Renal cysts	76%
	Conventional (clear cell) renal cell carcinomas (CCRCC)	24–52%
Pancreas	Pancreatic cysts	22%
	Serous cystadenomas	Occasionally
	Islet cell tumors usually asymptomatic	Infrequent
Inner ear	Endolymphatic sac tumors	<10%
Epididymis	Cystadenomas	10–15%
Broad ligament	Benign adnexal papillary tumors	Occasionally
Liver	Cysts	Occasionally

mutations. Of note, these mutations have also been observed as compound heterozygote alterations.

Genotype/Phenotype Correlation

There is a difference of frequencies of the types of germline mutations between VHL type 1 and VHL type 2 families. With few exceptions, most VHL type 2-associated germline mutations are of the ►missense type. The predicted consequence of any ►missense mutation is a single amino acid change in an otherwise full length protein. Although these mutations seem to cause minor damage to the VHL protein, they have a tendency to compromise pVHL function in a tissue specific manner, which may explain their frequent association with the formation of pheochromocytoma. The spectrum of VHL type 1-associated germline mutations is much more diverse and includes large deletions, microdeletions, insertions, nonsense, frameshift and missense as well as splice site mutations. Most of these mutations cause severe damage and most likely loss of VHL function due to the destruction of the domain required for VCB complex formation.

A high prevalence of CCRCC was seen in patients with partial germline VHL deletions relative to complete deletions. Deletion mapping revealed that development of CCRCC had an even greater correlation with retention of HSPC300 (C3orf10), located within the 30-kb region of chromosome 3p, immediately telomeric to VHL (52.3 vs. 18.9%, $p < 0.001$).

Sporadic Tumors

There are sporadic equivalents to all VHL tumors. For example, CCRCC is not only a feature of the rare hereditary VHL disease, but more frequently occurs sporadically in the general population. Likewise, hemangioblastomas frequently develop sporadically.

Tumor suppressor genes by definition are subject to a two-hit inactivation mechanism. There are two possibilities to achieve homozygous mutation. For example a germline mutation accompanied by a somatic mutation at the homologous allele will result in a hereditary tumor. Alternatively, two somatic mutations at homologous alleles will result in a sporadic tumor. Thus the same tumor suppressor gene accounts for both hereditary and sporadic tumors. Functional evidence for the tumor suppressor activity of VHL comes from transfection experiments that showed inhibition of tumor proliferation upon reintroduction of wild-type VHL into the $VHL^{-/-}$ tumor cells.

Somatic Mutations

Homozygous somatic VHL mutations were identified in sporadic CCRCC (up to 80%), sporadic cerebellar hemangioblastomas (up to 25%) but only in a few sporadic pheochromocytomas (<10%). Mutation types and locations in sporadic tumors differ from those in the germline. Most mutations in sporadic CCRCC are predicted to severely compromise the VHL protein structure and may therefore cause loss-of-function. About 20% of the cases show epigenetic events, such as abnormal ►methylation of the ►CpG islands (exon 1), which accounts for gene silencing with the predicted consequence of loss of pVHL function. Also, exon 2 is more frequently affected in sporadic tumors than in the germline. In particular, a mutational hotspot was identified in patients with CCRCC, which may reflect possible environmental factors to be involved in tumorigenesis. Altogether, differences in the VHL mutation pattern may reflect the influence of as yet unknown carcinogens, which on their way to become excretable compounds may target the VHL gene in kidney epithelial cells at preferential target sites. One

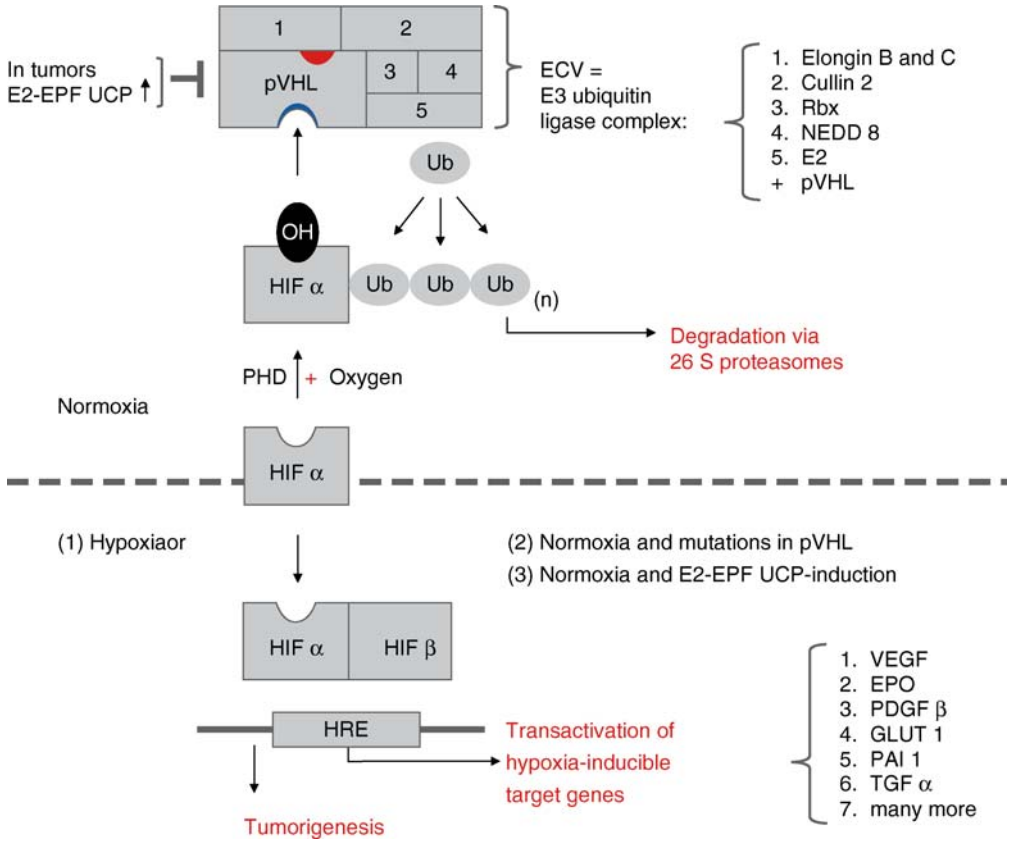
such carcinogen was identified to be trichloroethylene, an important industrial solvent. Somatic VHL mutations and alterations are detectable at all stages of tumor growth, however the presence of somatic VHL alterations may provide a growth advantage to any tumor and therefore indicate a bad prognosis. With the exception of a subgroup of papillary (chromophilic) renal cell carcinomas other pathological entities of renal epithelial tumors, such as chromophobe and the majority of papillary (chromophilic) renal cell carcinomas as well as benign renal oncocytomas, are not associated with VHL mutations.

Somatic VHL mutations have also been found in CCRCCs in members of families with a rare hereditary form of these tumors segregating with a constitutional chromosome 3 rearrangement. These patients had no VHL alterations in their germ line.

The upper part (Fig. 3) illustrates the physiological cellular situation: under normal oxygen conditions (normoxia) HIF α is hydroxylated on conserved prolyl residues (PHD). The pVHL complex polyubiquitinates (Ub) HIF α which is then destroyed by ►proteasomes. The lower part reflects the today known three conditions when HIF α is not degradated and thus will activate a large number of hypoxia-inducible genes: (i) ►hypoxia, or ►normoxia and inactivation of pVHL (ii) via *VHL* mutations, or (iii) via inhibition of pVHL by E2-EPF UCP-induction.

Cellular/Functional Features

Subcellular localization: pVHL localizes mostly to the cytoplasm at steady state but engages in a dynamic transcription-dependent nucleocytoplasmic shuttle. VHL does not carry a classical nuclear export signal. Therefore, shuttling may be facilitated by a sequence that may function as nuclear export domain. VHL-►ElonginC-ElonginB (VCB) complex formation and transcription elongation control: pVHL binds to ElonginC and ElonginB forming the ternary protein complex VCB. Initially, it was proposed that by binding to the Elongins, pVHL may be involved in the control of transcription elongation. Whereas association of Pol II (RNA polymerase II) with the ternary complex ElonginA/ElonginC/►ElonginB allows transcription of downstream genes, the heterotrimer VCB, in which ElonginA is replaced by pVHL, is responsible for the pausing of Pol II at attenuation sites. In normal cells, transcription elongation is controlled by these adverse activities. Mutated forms of



von Hippel-Lindau Tumor Suppressor Gene. Figure 3 VHL/HIF pathway.

V

pVHL are no longer able to associate with ElonginC/ElonginB, consequently resulting in amplified transcription of genes involved in tumorigenesis.

Data of the three-dimensional structure of the VCB complex provided insight into the spatial distribution of amino acids, the α- and β-domain structure of pVHL, and its interactions with ElonginC/ElonginB. Tumorigenic mutations cluster in two surface patches on the molecule; the 35 residue domain responsible for ElonginC binding and a separate remote domain containing the target capture site.

Multimeric complex formation and protein degradation: pVHL forms multimeric complexes with ElonginC and ElonginB, Cul2 and Rbx1. These complexes resemble so-called SCF ubiquitin ligase complexes (Skp1/Cdc53/F-box protein). Thus, by analogy, pVHL is expected to play a role in ►ubiquitination, a biological process that allows a cell to identify proteins in a timely and specific manner in order to destroy them. Cells that lack pVHL are unable to degrade members of the HIF (►hypoxia inducible factor) transcription factor family. Whereas pVHL associated ubiquitination activity depends on ElonginC and Cul2 binding via the α-domain, the β-domain is responsible for target capture, stressing the adaptor function of pVHL between target and the protein degradation machinery. The ability of pVHL to recognize and ubiquitinate HIF can be prevented by mutations in the α-domain. Mutations in the β-domain interfere with the ElonginC/ElonginB binding site.

Role in tumor ►angiogenesis: VHL-related tumors including CCRCC overexpress ►VEGF (Vascular endothelial growth factor), one of the most potent angiogenic factors. This upregulation of VEGF is likely to be responsible for the highly vascular presentation of VHL-related tumors. VEGF expression is under the control of hypoxia inducible transcription factors (HIF). In normal cells, HIF is stable only under hypoxic conditions. pVHL co-immunoprecipitates with the HIF-1 α-subunit, suggesting that pVHL is necessary for the oxygen dependent proteolysis of the HIF-1 α-subunit. Further, cells lacking pVHL are no longer able to degrade members of HIF. Thus, due to the presence of VHL mutations, cells lacking pVHL mimic oxygen deprivation and consequently may unleash HIF. Downstream consequences are the activation of the transcription of a large number of hypoxia responsive genes, such as VEGF, ►EPO (erythropoitein), ►PDGF (platelet derived growth factor), TGFα (transforming growth factor), and GLUT-1 (glucose transporter 1) as well as more than 60 other genes.

Other up-regulated target genes and role in maintenance of extracellular pH: HIF also controls expression of the carbonic anhydrases CA9 and CA12. These enzymes are involved in the reversible reaction of carbon dioxide hydration. They are overexpressed in VHL tumors and in CCRCC due to absence of pVHL, suggesting a role in glycolytic acidification of tumors and a role for pVHL to regulate the maintenance of extracellular pH. CA9 is identified by the antigen binding monoclonal antibody mAbG250, that reacts with CCRCC. This antibody is currently under investigation for an application in the treatment of kidney cancer.

Other capture targets and functions: Other likely target proteins for pVHL are transcription factor Sp1, fibronectin and two isoforms of protein kinase C. Thus, additional proposed functions of pVHL include regulation of the correct assembly of the extracellular matrix and controlled exit from cell cycle.

Animal models: There are VHL homologues in rodents (rat and mouse), Drosophila and worm. Homology to the predicted human pVHL is 50% in Drosophila, 87% in rats and 98% in mice. Homology is highest in the protein binding motifs.

Observations during tracheal development of Drosophila suggests that the Drosophila homologue of VHL (*dVHL*) plays a role in halting of cell migration at the end of vascular tube outgrowth. Considering a common evolutionary origin of the Drosophila tracheal system and the mammalian vasculature, this observation may be helpful to explain the loss-of-function phenotypes in knockout mice and human tumors. In the mouse embryo, loss of VHL activity may lead to uncontrolled cell movement, causing disruption of major vasculature in the placenta. In the adult human with a completely laid out vasculature, lack of VHL activity may be involved in extensive cell migration and vessel branching, leading to overvascularization, as observed in VHL associated tumors.

In homozygously VHL deleted knockout mice, embryos die at day 9–9.5 of gastrulation, due to lack of placental vascularization. Heterozygotes do not show any specific phenotype within the observation period of 2 years. Current scientific efforts focus on the development of a conditional knockout mouse model (deletion of VHL in kidney cells only) to provide a model for the study of kidney tumor initiation and progression and to test gene therapies.

Applications in Diagnosis and Clinical Management

Molecular diagnosis and implications: Patients with VHL disease should be subjected to molecular analysis to determine their family-specific VHL germline mutation. Once a mutation has been identified, all family members need to be tested for VHL mutation carrier status, that is to identify gene carriers and family members not carrying the mutation. Only family members with a VHL germline mutation should then be subjected to clinical diagnostic procedures. This approach significantly reduces the psychological stress of unnecessary clinical screening examinations for the non-carrier. Also, by this approach, costs will be reduced. Affected patients should be tested according

to a standard protocol ((Maher ER (1993) VHL disease. In: Hodgson SV, Maher ER (eds) A practical guide to human genetics. Cambridge Press, pp 157–162; American Cancer Society (1999)). Good clinical practice includes genetic counseling prior to molecular testing and prior to disclosure of tests results. Patient information on all aspects of VHL disease are available online from patient support groups: http://www.vhl.org/ and http://www.hippel-lindau.de/.

Clinical Management. Molecular testing of VHL disease today is used to sustain clinical diagnosis. In many cases, the disease will be discovered presymptomatically. Early detection allows treatment of lesions prior to the onset of symptoms. For retinal hemangioblastomas, adequate laser treatment significantly reduces the risk of blindness. Cerebellar or spinal hemangioblastomas are benign tumors and need to be removed prior to the onset of neurological impairment. Pheochromocytoma should be treated when symptomatic. Renal lesions require specific attention. Cysts may be critical, as their lining epithelium may be the origin of malignoma. Thus, any cysts in VHL patients should be regarded as potentially malignant, especially when they are accompanied by solid and/or fast growing tumors. With respect to the size and location, nephron sparing surgery is the treatment of choice for renal conventional (clear cell) carcinoma. The involvement of a variety of different organ systems requires interdisciplinary cooperation of experts from different medical fields for the appropriate management of VHL disease.

Today an increasing number of drugs are clinically applied in advanced renal cell carcinoma, hemangioblastomas, and age-related macula degeneration that have been developed based upon the detailed understanding of the VHL/HIF/VEGF pathway. This includes administration of small molecule inhibitors of HIF-responsive growth factors, an approach that holds the potential of target-specific treatment that might improve treatment efficacy while minimizing adverse effects.

Remaining Questions

Current knowledge of VHL and the VHL regulatory pathway still leave many questions unanswered. It is still unclear why VHL mutations cause this highly restricted subset of tumor types and whether specific VHL mutations are associated with different site-specific tumor risks. Finally, it will be of interest to know whether VHL in addition to its capacity to inhibit HIF exerts additional tumor suppressor functions.

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von Recklinghausen Disease

Definition

►Neurofibromatosis Type 1
►Neurofibromatosis 1

von Willebrand Factor

Definition

vWF; A large multimeric glycoprotein circulating in the blood stream, produced by ►endothelial cells, ►platelets and subendothelial connective tissue. Since it binds to platelets, collagen which is exposed during tissue injury, and factor VIII, it plays an important role in blood coagulation.

►Proteinase-activated Receptors

Vorinostat

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Synonyms

Suberoylanilide hydroxamic acid; SAHA; MK-0683, SKI390; Zolinza™

Definition

Vorinostat is a small molecule (MW 264) inhibitor of ►histone deacetylase (HDAC) activity (see Fig. 1).

Characteristics

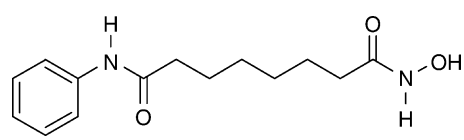
Background

HDACs are enzymes that catalyze the removal of acetyl groups from the lysine residues of proteins, most notably the ►histones contained within ►nucleosomes. Three classes of HDACs have been identified. Class I human HDACs are homologous to the yeast HDAC Rpd3, and include HDAC1, HDAC2, HDAC3 and HDAC8. Class II HDACs are subdivided into Class IIa enzymes (HDAC4, HDAC5, HDAC7 and HDAC9) and Class IIb enzymes (HDAC6 and HDAC10). Class II HDACs are homologous to the yeast HDAC Hda1. The key catalytic residues have been conserved in both the Class I and Class II HDACs. The third class of human HDACs consists of homologues of yeast Sir2. The catalytic site of this third HDAC class is not similar to the catalytic site of the Class I and Class II enzymes. Class III HDACs require nicotinamide-adenine dinucleotide (NAD+) for activity.

Vorinostat inhibits the enzymatic activity of Class I HDACs, (HDAC1, HDAC2, and HDAC3) and the Class II HDAC, HDAC6, at low nanomolar concentrations (IC50 < 86 nM). Vorinostat is approximately tenfold less potent in inhibiting HDAC8, a Class I HDAC, and ~100-fold less potent in inhibiting the Class IIa HDACs, HDAC4, 5 and 7. Vorinostat does not inhibit the activity of the Class III HDAC, SIRT1, at concentrations up to 100 μM. Thus, vorinostat demonstrated potent selective inhibitory activity against specific Class I and Class II HDACs.

Mechanism of Antitumor Action

Vorinostat is active in inducing differentiation, cell growth arrest, or apoptosis in a wide variety of transformed cells in culture. Vorinostat was originally identified as an inducer of differentiation of murine ►erythroleukemia (MEL) cells. Vorinostat has also been found to inhibit proliferation of cultured transformed cell lines derived from lymphoma, leukemia, myelomas, non small cell lung carcinoma, colon carcinoma, central nervous system tumors, melanoma, ovarian carcinoma, renal cell carcinoma, prostate cancer, and breast cancer.



Vorinostat. Figure 1 Vorinostat.

The molecular mechanisms of vorinostat-induced growth arrest, differentiation and ►apoptosis have yet to be fully clarified. The acetylation of nucleosomal histones plays an important role in the transcriptional regulation of gene expression. Hypoacetylation of histones is associated with a condensed ►chromatin structure (►Chromatin Remodeling) and repression of gene transcription. Conversely, acetylated histones are associated with a more open chromatin structure and activation of transcription. Defects in the enzymes regulating histone acetylation have been found in a variety of cancers. These defects can cause an imbalance in histone acetylation that may lead to changes in chromatin structure and transcriptional dysregulation of genes involved in the control of cell cycle progression, differentiation, and apoptosis. A potential mechanism for the anti-tumor action of vorinostat is that vorinostat inhibition of HDAC activity, and subsequent accumulation of acetylated histones, leads to the activation of genes whose expression causes the observed anti-proliferative effects. This model is based on the finding that the expression of a finite number of genes is regulated following exposure to vorinostat. One of the most commonly induced genes is the cell cycle kinase inhibitor $p21^{WAF1/CIP}$.

Vorinostat-induced apoptosis occurs through the intrinsic apoptotic pathway, characterized by the release of mitochondrial membrane proteins such as cytochrome c and the subsequent activation of caspases. Apoptosis mediated by vorinostat can be inhibited by overexpression of ►Bcl-2. Depending on the cell type, caspase activation may or may not be necessary for vorinostat-induced cell death. One study has demonstrated a dependence of ►p53 for vorinostat-induced apoptosis, however, it is generally believed that vorinostat can function in the absence of wild-type p53. Vorinostat has also been cited to induce autophagic cell death. This mode of cell death is characterized by massive degradation of cellular contents by means of intracellular membrane/vesicle reorganization and lysosomal activity.

While most models suggest that histone hyperacetylation leads to transcriptional activation of genes, expression profiling studies have revealed that vorinostat treatment can also lead to repression of gene expression (►Histone Modification in Cancer Biology). Possible mechanisms to explain vorinostat inhibitor-mediated transcriptional repression involve both direct and indirect effects resulting from histone acetylation, or alternately, may involve the hyperacetylation of non-histone proteins. For example, a recent study has demonstrated that vorinostat prevented the transactivation of cytokine-induced signal transducer and activator of transcription (►STAT) 5 target genes. A second study demonstrated that vorinostat

suppressed the induction of c-jun transcription by inhibiting the recruitment of the preinitiation complex to the c-jun promoter resulting in reduced COS-2, cyclin D1 and collagenase-1 transcription.

Vorinostat may affect cancer growth independently from its direct action on tumor cell proliferation and survival by regulating the expression of genes involved in tumor angiogenesis and ►inflammation processes. Vorinostat is a potent anti-angiogenic agent that can alter vascular endothelial growth factor (►VEGF) signaling. The anti-angiogenic activities of vorinostat may arise through its ability to suppress the expression of VEGF receptors 1 and 2, and ►neuropilin. In addition, vorinostat induces expression of the VEGF competitor ►semaphorin III and is likely to suppress angiogenesis by preventing endothelial cells from responding to the angiogenic stimulus generated by VEGF. A separate study found that vorinostat exhibits anti-inflammatory properties in vivo and in vitro and that vorinostat may stimulate the expression of genes that control the synthesis of cytokines and ►nitric oxide or hyperacetylate other targets.

HDAC inhibitors, such as vorinostat, may also affect cell cycle progression by altering the ability of tumor cells to enter and exit mitosis. Histone acetylation is tightly regulated during the cell cycle and may be important for proper deposition of histones during DNA synthesis and chromosome segregation during mitosis. For example, an increase in acetylated histones during S and G2 phase could activate a G2 checkpoint and induce G2 arrest. Loss of this checkpoint, a frequent event in cancer cells, may result in the inability to arrest in G2 phase leading to aberrant mitosis and induction of apoptosis. Likewise, a similar disruption of the mitotic spindle checkpoint may also predispose tumor cells to HDAC inhibitor-induced apoptosis.

While results from mechanistic studies support the hypothesis that the effects of vorinostat on histone acetylation play an important role in its biological activities, the activity of a number of other proteins is regulated, at least in part, by ►histone acetyltransferases (HATs) and HDACs and may be targeted by vorinostat. Several nonhistone proteins, (e.g., ►tubulin, ►Hsp90 and ►p53) are known to be reversibly acetylated on lysine residues and undergo hyperacetylation following exposure to vorinostat. Acetylation of these proteins may also contribute to the antitumor activity of vorinostat.

Studies on the activity of vorinostat in combination with other cancer therapies have demonstrated synergistic or additive activity in a variety of cultured human transformed cell lines. Vorinostat has been tested in combination with other cancer therapies including radiation, kinase inhibitors, cytotoxic agents, and differentiating agents.

Vorinostat has demonstrated anti-tumor activity in a variety of in vivo rodent tumor models whether administered by the intraperitoneal, intravenous, or oral route in both carcinogen induced tumor formation and established tumor models. Administration of vorinostat causes significant tumor growth inhibition in human breast, colon and prostate carcinoma xenografts in mice. Tumor growth inhibition is observed at doses of vorinostat that do not produce toxic side effects.

Clinical Studies

The initial phase I trial (►phase I and II clinical trials) of oral vorinostat was conducted in patients with advanced hematologic malignancies or solid tumors. Seventy-three patients received treatment once or twice daily on a continuous basis or twice daily for three consecutive days per week. The median age was 60 years (range, 20–79) and the majority of patients had received at least three prior systemic therapies. Patients received a total of 416 vorinostat treatment cycles (median, 2; range, 1–37+). The pharmacokinetics were linear from 200 to 600 mg, the mean apparent half-life was 1.5–2 h, and the estimated bioavailability was 43%. Accumulation of acetylated histone H3 was observed in patient peripheral blood mononuclear cells at all dose levels. One patient with transformed diffuse large ►B-cell lymphoma achieved a complete response for 17 months, three patients (diffuse large B-cell lymphoma, laryngeal cancer, ►papillary thyroid carcinoma) had partial responses, and two (metastatic mesothelioma) had unconfirmed partial responses. An additional 16 patients achieved stable disease for 4–37+ months. The major dose-limiting toxicities were anorexia, dehydration, diarrhea, and fatigue. The maximum tolerated dose was 400 mg once daily or 200 mg twice daily for continuous dosing or 300 mg twice daily for three consecutive days per week.

A phase II trial of oral vorinostat in patients with refractory ►cutaneous T-cell lymphoma (CTCL) was conducted. Thirty-three patients were enrolled and received vorinostat 400 mg daily, 300 mg twice daily for 3 days with 4 days rest, or 300 mg twice daily for 14 days with 7 days of rest followed by 200 mg twice daily. The median age was 67 years (range, 26–82), the median number of prior systemic therapies was 5 (range, 1–15), and 85% of patients had Stage IIB or higher CTCL. Eight of 33 patients (24%) achieved a partial response, including 4 of 13 (31%) who received 400 mg daily dosing. The median time to response was 11.9 weeks (range, 3.6–21.9) and the median duration of response was 15.1 weeks (range, 9.4–19.4). The median time to progression was 12.1 weeks overall and 30.2 weeks for responders. One patient who was enrolled on two consecutive dose regimens was treated for 1.4 years. Fourteen of 31 patients (45%), including

73% of those who received 400 mg daily dosing, had symptomatic pruritus relief. The most common adverse experiences were fatigue, thrombocytopenia, diarrhea, nausea, and dysgeusia. Grade 3/4 drug-related adverse experiences included thrombocytopenia and dehydration. Serious adverse experiences and discontinuations due to adverse experiences were least common in patients who received 400 mg daily dosing. These findings supported further investigation of vorinostat 400 mg daily in patients with refractory CTCL.

An international, multicenter, phase IIb trial of oral vorinostat 400 mg daily in patients with persistent, progressive, or recurrent CTCL was conducted. Seventy-four patients were enrolled, including 61 with Stage IIB or higher disease. The median age was 60 years (range, 39–83) and the median number of prior systemic treatments was 3 (range, 1–12). Twenty-two of 74 patients (30%) achieved a partial response, including 18 of 61 (30%) with Stage IIB or higher disease. The median time to response was 55 days (range, 28–171). The median duration of response and time to progression for responders were not reached but estimated to be at least 165 and 256 days, respectively. Response to vorinostat did not appear to be impacted by response to last prior therapy. Eleven patients who did not meet the response criteria had stable disease for at least 24 weeks. Twenty-one of 65 patients (32%) with baseline pruritus scores of at least 3 (scale, 1–10) had pruritus relief. The most common adverse experiences were diarrhea, fatigue, nausea, and anorexia and were mostly mild to moderate in severity. Grade 3/4 adverse experiences included fatigue (5%), pulmonary embolism (5%), thrombocytopenia (5%), and nausea (4%). Fifteen patients received vorinostat for at least 1 year (range, 12.0–34.2+ months). Eighty-five percent of patients did not require dose reduction and 9% discontinued due to a drug-related adverse experience.

Vorinostat (Zolinza™) was approved on October 6, 2006 by the US Food and Drug Administration for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies. Further investigation of vorinostat in phase II and III trials for patients with other hematologic malignancies and solid tumors is ongoing. Clinical activity has thus far been observed in patients with acute myeloid leukemia, multiple myeloma, non-Hodgkin lymphoma, glioblastoma multiforme, and metastatic mesothelioma, as well as breast, laryngeal, nasopharyngeal, papillary thyroid, ovarian, and non-small cell lung cancer.

Activity of Vorinostat in Non-oncology Indications

Vorinostat has also shown activity in preclinical studies in indications beyond cancer, including neurodegenerative disorders such as Huntington disease and as an anti-inflammatory agent. Clinical studies in non-oncology indications will be required to determine whether vorinostat may be used for the treatment of a variety of human diseases in addition to cancer.

►Epigenetic Therapy

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Voxel

Definition

Unit volume of patient tissue, determined by the digital image pixel size and slice thickness.

►Dynamic Contrast-enhanced Magnetic Resonance Imaging

v-Src

►Src

vWF

Definition

►von Willebrand factor

WA

►Withaferin A

WAGR Syndrome

Definition

Acronym for **W**ilms tumor, **A**niridia, **G**enitourinary malformations, and mental **R**etardation; is an unusual complex of congenital developmental abnormalities, such as aniridia, genitourinary (GU) malformations, and mental retardation. Individuals are at high risk (>30%) of having a Wilms tumor. At birth, the association is aniridia, GU malformations, and mental retardation (AGR) syndrome. With the discovery of a ►Wilms tumor in these patients, the association is referred to as WAGR syndrome. These syndromes result from the loss or mutation of the ►Wilms tumor gene from the short arm of chromosome 11.

Waldenstrom Macroglobulinemia

Definition

Is a low grade ►B-cell lymphoma that is characterized by the secretion of IgM. The tumors are also called lymphoplasmacytoid lymphoma in the REAL classification. The absence of CD5 and CD10 expression is useful for its distinction from ►chronic lymphocytic leukemia.

►B-cell Tumors

Walker A Motif

Definition

A consensus sequence of amino acids observed in the nucleotide binding fold among distantly related sequences of ►ABC transporters, ATP synthase, myosin, kinases, and other ATP-requiring enzymes.

Warburg Effect

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Synonyms

Aerobic glycolysis; Tumor glucose metabolism

Definition

Warburg Effect describes the propensity for cancer cells to convert ►glucose to ►lactate even when oxygen is ample. This effect, also known as aerobic ►glycolysis, contrasts with the anaerobic glycolysis that occurs as an adaptive response to low oxygen tension or ►hypoxia. Adaptive responses through the induction of the ►hypoxia-inducible factor 1 (HIF-1) and cell autonomous changes that activate ►AKT, ►HIF-1, ►MYC or ►RAS oncogenes or inactivate the ►tumor suppressor P53 or ►VHL could all contribute to the Warburg effect.

Characteristics

In reports of pioneering studies, which were translated from German by Frank Dickens in 1930 and made accessible to English readers as a monograph entitled "The Metabolism of Tumors," Otto Warburg described the propensity for cancer cells to convert glucose to

lactate even in the presence of oxygen. The experiments described by Warburg were prescient in determining the maximum allowable thickness of normal and cancer tissue sections to be studied without interference from diffusion limitations. The maximum tissue section thickness was calculated from differential equations and determined to be 2.1×10^{-2} cm, which correlates remarkably well with modern experimental microprobe measurements indicating that oxygen becomes limited for cells >150 μm away from ambient oxygen. By studying human and animal cancer tissues, Warburg reached the profound conclusion that tumors prefer to convert glucose to lactate and speculated that "If the respiration of a growing cell is disturbed, as a rule the cell dies. If it does not die, a tumor cell results."

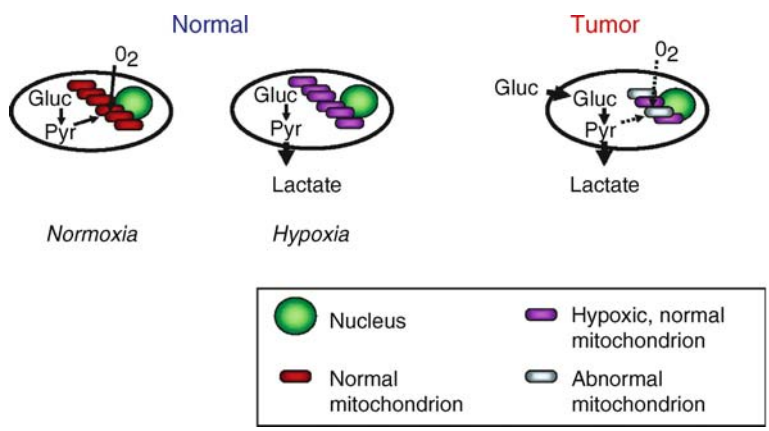
Through the glycolytic pathway, normal tissues convert glucose to pyruvate and generate ATP and NADH. Pyruvate is taken up and then further metabolized by mitochondria through the Krebs or tricarboxylic acid (TCA) cycle. From a single pyruvate molecule, the TCA cycle donates eight high-energy electrons to the mitochondrial transport chain, which creates a proton gradient across the inner mitochondrial membrane, with oxygen as the terminal electron acceptor. Dissipation of the proton gradient produces ATP through ►oxidative phosphorylation. In the absence of oxygen, pyruvate is instead converted to lactate. Cancer cells, in contrast to normal cells, tend to convert glucose directly to lactate even when oxygen is available (Fig. 1).

Mitochondrial DNA Mutations and the Warburg Effect

Warburg's postulation that mitochondrial dysfunction or disturbance contributes aerobic glycolysis is now partially supported by studies of cancer mitochondrial DNA (mtDNA) mutations. Mitochondrial components are encoded by both nuclear and mtDNA, whose mutations can be heritable through maternal transmission, resulting in specific muscle diseases, blindness or deafness. Acquired somatic mtDNA mutations have been discovered in large fractions of human tumors; however, the functional consequences of these mutations are not firmly established. While many mtDNA mutations have unknown effects, some mutations may diminish components of the respiratory chain thereby cause disturbances in respiration. Cell fusion experiments support a functional role of defective mitochondria, which enhance tumorigenicity of prostate cancer cells.

Tumor Suppressors and the Warburg Effect

Since the mitochondrion plays a central and crucial role in programmed cell death or ►apoptosis, it is possible that acute disturbances of respiration could result in cell death as a means to rid the organism of defective cells. However, as proposed by Warburg, if a growing cell with disturbance in respiration does not die presumably as the result of activation of anti-apoptotic pathways, then a tumor cell results. Intriguingly, the tumor suppressor protein ►P53, which plays an intimate role in ►apoptosis, was recently shown to directly activate genes that enhance cellular respiration, such that loss of P53 favors the conversion of glucose to lactate and cell survival. In particular, ►P53 activates Synthesis of Cytochrome c Oxidase 2 (SCO2) which is critical for positively regulating the cytochrome c oxidase (COX) complex, a major site of oxygen consumption by the mitochondrion. Additionally, P53 activates TIGAR, which shares sequence similarity with the bisphosphatase FBPase-2 and diminishes fructose-2,6-bisphosphate



Warburg Effect. Figure 1 The Warburg Effect. In contrast to normal cells that utilizes glucose through mitochondrial oxidative phosphorylation in the presence of oxygen (normoxia) and undergo anaerobic glycolysis in hypoxia, Warburg hypothesized that tumor cells with disturbances in respiration, presumably due to defective mitochondria, have the propensity to convert glucose to lactate even in the presence of oxygen. In tumor cells oxygen and pyruvate are presumably inefficiently utilized (dashed arrows) by the mitochondria. Gluc, glucose; Pyr, pyruvate.

levels, thereby blocks glycolysis. Hence loss of the tumor suppressor P53 would be expected to diminish respiration and unblock glycolysis.

Several ►familial cancer syndromes have been linked to mutations of tumor suppressors that are enzymes in the TCA cycle enzymes or proteins involved in metabolism. Mutations of succinate dehydrogenase are associated with paragangliomas, while mutations of fumarate hydratase are linked with ►renal carcinoma and uterine leiomyomatosis. These mutations result in high levels of succinate, which inhibit prolyl hydroxylase (PHD) function resulting in increased stability of the hypoxia inducible factor HIF-1 protein. PHDs hydroxylate HIF-1 and target it for recognition by the von Hippel-Lindau protein (vHL) and subsequent proteasomal degradation. Hence inactivating mutations of vHL also stabilize HIF, which is important for tumorigenesis through ►transcriptional regulation and stimulation of glycolysis and angiogenesis. These observations indicate that mutations of tumor suppressors may not only decrease mitochondrial function, but may also activate glycolysis directly or through the stabilization of HIF-1.

Mutations of the LKB1 tumor suppressor result in the rare autosomal dominant form of Peutz-Jeghers syndrome that is characterized by early age onset of gastrointestinal polyposis. LKB1 is a kinase that phosphorylates and activates AMP kinase (AMPK) in the presence of AMP when intracellular levels of ATP are low. AMPK, in turn, increases ATP production through stimulation of pathways including fatty acid oxidation. AMPK also activates two other tumor suppressors, TSC1 and TSC2, which are mutated in the familial syndrome ►tuberous sclerosis complex characterized by benign tumors commonly affecting the brain, but also affecting the kidneys, heart, eyes, lungs, and skin. TSC1 and TSC2 form heterodimers that inhibit ►mTOR, the ubiquitous kinase which stimulates cell growth and protein synthesis in response to growth factors. Hence, loss of LKB1, TSC1 or TSC2, activates the mTOR pathway. Hyperactivation of the mTOR pathway through loss of tumor suppressors also appears to increase translation of HIF-1α mRNA and thereby stimulates the Warburg effect.

Oncogenic Activation of the Warburg Effect

In vivo and *in vitro* studies have demonstrated that many tumors are severely hypoxic due to a disordered, inefficient neo-vascular system. Hypoxia results in the stabilization of HIF, which in turn transcriptionally activates genes encoding glucose transporters, glycolytic enzymes, and angiogenic factors. As a result, tumor uptake of glucose is avid and lactate production is robust. It is apparent however, that not all areas of a tumor that are highly glycolytic are necessarily hypoxic, suggesting that cell autonomous oncogenic alterations could also cause the Warburg effect. In one scenario, oncogenes could stabilize HIF itself or alternatively directly inhibit cellular respiration or activate glycolysis.

Since HIF-1 has been found in non-hypoxic areas of tumors, it is likely that HIF-1 could be stabilized by non-hypoxic factors, such as loss of vHL. HIF-1 levels are increased by insulin-like growth factor stimulation, activated v-►Src, ►c-Src or ►Ras oncogenes. Signaling through the EGF receptor, ►HER2, or ►PI3 kinase also promote increased synthesis of HIF-1 protein. Another view contends, however, that the PI3-kinase/AKT and HIF1 are independent pathways. Hence, oncogenic activation of a number of pathways could result in the stabilization of HIF-1 under normoxic conditions. However, oncogenic activation of glycolysis also occur independent of HIF.

Additional insights into the Warburg effect are provided through a novel HIF-1 target gene that actively inhibits mitochondrial function. Pyruvate dehydrogenase kinase 1 (PDK1) is one of four PDK family members and was identified as a direct transcriptional target of HIF-1. PDK1 inactivates the mitochondrial pyruvate dehydrogenase (PDH) complex by phosphorylating the PDH E1α catalytic subunit. Suppression of PDH inhibits the conversion of pyruvate to acetyl-CoA, thereby decreases mitochondrial respiration. As such, it is expected that the non-hypoxic oncogenic stabilization of HIF would increase PDK1 levels, which divert pyruvate from PDH and enhance lactate production. These studies indicate that PDK1 activation could be a key regulatory switch contributing to the Warburg effect.

The ►MYC oncogene is over-expressed in 20–70% of commonly occurring human cancers (www.myccancergene.org). Myc directly binds and transactivates the promoters of several key glycolytic genes (HK2, ENO1, and LDHA), which have highly evolutionarily conserved Myc binding sites, as well as many other glycolytic genes and glucose transporters. The activation of glycolytic genes by Myc could be physiological or pathological under non-hypoxic conditions. It is notable that Myc is also implicated in stimulation mitochondrial biogenesis and hence could normally play a role in driving glucose metabolism toward oxidative phosphorylation in non-transformed cells. In tumor cells with defective or suppressed respiration, however, constitutive activation of Myc is expected to contribute to aerobic glycolysis by directly activating glycolytic genes. Hence, in certain tumors Myc may contribute to a cell autonomous state of converting glucose to lactate even in non-hypoxic conditions.

The ►AKT oncogene, which encodes a protein serine-threonine kinase, also activates a cell autonomous state of aerobic glycolysis in a seeming HIF-independent fashion through mobilization of glucose

transporters to the cell surface, and activation of hexokinase 2 and phosphofructokinase 2. Intriguingly, Akt increases glycolytic conversion of glucose to lactate without an increase in oxidative phosphorylation. Similar to Bcl-X_L, a ►Bcl-2 family member, Akt is anti-apoptotic such that its ability to increase aerobic glycolysis is coupled with suppression of cell death. However, unlike Bcl-X_L, Akt is able to transform cells. Hence, activation of Akt appears to be sufficient for the Warburg effect and tumorigenesis under certain conditions.

The Warburg effect has also intriguingly emerged in a model of ►tumor progression triggered by serial transduction of normal human cells with immortalizing and oncogenic genes. By multidimensional metabolic profiling of primary human fibroblasts transformed step-wise by ►hTERT, ►SV40 large T antigen, small T antigen, and H-Ras, the highly tumorigenic cells were observed to display the Warburg effect with high rates of lactate production and low mitochondrial mass. Cells transformed by the other three genes but lacking H-Ras had high mitochondrial mass and a high oxygen consumption rate with lower lactate production. These observations suggest that a certain stage in tumorigenesis could be associated with increased respiration, but H-Ras appear to be associated with the final conversion of human fibroblasts to a highly tumorigenic phenotype associated with aerobic glycolysis. In fact, Ras is implicated in the regulation of PFKFB3, a 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, which diminishes the intracellular concentration of the activator, fructose-2,6-bisphosphate (F2,6BP). Paradoxically, while increased PFKFB3 appears to be necessary for Ras transformation of fibroblasts, the associated diminished levels of F2,6BP, which allosterically activates the rate-limiting phosphofructokinase 1 does not appear to correlate with cellular transformation. In this regard, the effects of Ras on PFKFB3 contrasts with the activation of TIGAR by P53 discussed above.

In summary, many studies support the activation of glycolysis and lactate production as an adaptive response to hypoxia as well as a result of direct oncogenic activation. Although, distinction between these two possible mechanisms of activation of tumor aerobic glycolysis is necessary for our understanding of tumor metabolism, it is the cell autonomous oncogenic activation of glycolysis that appears to substantiate Warburg's original observations.

Does the Warburg Effect Contribute to Tumorigenicity?

If aerobic glycolysis mediated by the Warburg Effect is much less efficient for ATP production as compared to oxidative phosphorylation, then what are the selective pressures for tumor aerobic glycolysis? Hypoxia is pervasive in tumors in vivo and likely to be a selective pressure on cancer cells. Although independence from oxygen requirement through the Warburg effect could be advantageous to a cancer cell, there must be additional advantages since an adaptive hypoxic response of activated glycolysis should also confer the same advantage to normal cells. Yet, hypoxia diminishes the growth potential of normal cells, except endothelial cells. Several glycolytic enzyme genes, including glucose phosphate isomerase (GPI) and phosphoglucomutase, were found capable of immortalizing primary fibroblasts in an expression cloning study. The associated increases in glycolytic rates are hypothesized to suppress mitochondrial reactive oxygen species production, which has been implicated in cellular senescence and apoptosis. In this regard, ►immortalization by glycolytic enzymes could be a step in tumorigenesis that is poised for additional oncogenic conversions. It should be noted that there are non-glycolytic functions, such as the roles of LDH and GAPDH in transcriptional regulation, of these enzymes that may participate in tumorigenesis.

The multi-functional roles of glycolytic enzymes, which include the anti-apoptotic roles of glucokinase and hexokinase through direct interactions with the mitochondria, could contribute to tumorigenesis. In addition, GPI is also known as autocrine motility factor that was isolated as a factor capable of stimulating motility of cells in an ►autocrine or ►paracrine fashion. The increased motility could contribute to the malignant phenotype of metastasis. How these non-glycolytic functions directly contribute to tumorigenesis remains to be further studied.

Clinical Aspects and Summary

The characteristics of cancer cells including the avid consumption of glucose and robust lactate production were first described by Warburg about 80 years ago. Over the past two decades, the molecular basis for tumorigenesis unfolded in the discovery of oncogenes and tumor suppressor genes that are now linked to altered glucose metabolism. While the analogies of a defective automobile brake to loss of tumor suppressor and a stuck accelerator to oncogene activation are touted as central mechanisms in tumorigenesis, the fuel line or tumor energy metabolism has been an overlooked, yet critical aspect of tumorigenesis. In fact, the avid uptake of glucose by tumors, observed by Warburg decades ago, is now the foundation for the detection and monitoring of human cancers by 2-fluorodeoxyglucose ►positron emission tomography (FDG-PET). Although Warburg suggested that cancer cells underwent aerobic glycolysis as a result of mitochondrial disturbances, more recent studies suggest that both cell autonomous genetic alterations as well as adaptation to hypoxia through the stabilization of HIF-1 contribute to this characteristic tumor metabolic adaptation. This common metabolic perturbation of aerobic glycolysis or the

Warburg effect in cancers appears attractive as a therapeutic target pathway, although the true therapeutic window remains to be established.

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Warts (wts) – Drosophila lats

►Lats in Growth Regulation and Tumorigenesis

WARTS (WTS) – mammalian LATS1

►Lats in Growth Regulation and Tumorigenesis

N-WASp

Definition

Neuronal-►Wiskott-Aldrich Syndrome protein is expressed in most tissues. It the main protein in cells that binds and activates the Arp2/3 complex to initiate actin polymerization and cell movement.

►Cortactin

WD Repeats

Definition

A motif of approximately forty amino acids that commonly ends with a tryptophan and aspartic acid dipeptide. These motifs often occur as tandem repeats. Many of the proteins containing WD repeats are subunits of protein complexes. WD proteins commonly have 4–16 repeating WD units. WD proteins are a large family found in all eukaryotes and are implicated in a variety of functions, such as signal transduction, regulation of transcription, cell cycle control, and apoptosis. The underlying function of WD proteins is the assembly of multimeric protein complexes with the repeating units serving as a rigid scaffold for protein interactions that are mediated by non-WD repeat regions. The specificity of the protein's function is usually determined by its sequence that lies outside of the tandem repeats.

►Molecular Chaperones

Wegener Granulomatosis

Definition

An uncommon disease occurring mainly in the fourth and fifth decades that is characterized by vasculitis of small vessels, granuloma formation in the respiratory tract, and glomerulonephritis, possibly caused by an immune disorder.

►Rituximab

T1- and T2-Weighted Images

Definition

Two types of the images in the magnetic resonance imaging with different length of repetition time (TR) and echo time (TE).

►Hepatic Epithelioid Hemangioendothelioma

T_1-Weighted

Definition

Magnetic resonance imaging that predominantly reflects the longitudinal (spin – lattice) relaxation time of tissues.

►Dynamic Contrast-enhanced Magnetic Resonance Imaging

T_2-Weighted

Definition
Magnetic resonance imaging that predominantly reflects the effective transverse (spin – spin) relaxation time of tissues accounting for local magnetic field in homogeneity.

►Dynamic Contrast-enhanced Magnetic Resonance Imaging

Well-differentiated Neurocytoma

►Neurocytoma

Wermer syndrome

►Multiple Endocrine Neoplasia Type 1

Wharton Jelly

Definition
Connective tissue surrounding the ►umbilical cord.

►Stem Cell Plasticity

Whey Acidic Protein

Definition
WAP; is the principal whey protein found in rodent milk.

►Cripto-1

Whipple Triad

Definition
Is the diagnostic hallmark of ►insulinoma; which consists in symptoms of hypoglycemia (catecholamine release), low blood glucose level (40–50 mg/dL), and relief of symptoms after intravenous administration of glucose. The triad is not entirely diagnostic, because it may be emulated by factitious administration of hypoglycemic agents, by rare soft tissue tumors, or occasionally by reactive hypoglycemia. The clinical syndrome of hyperinsulinism may follow one of two patterns or sometimes a combination of both.

White Blood Cell

Definition
The two most common types are the ►lymphocytes and ►neutrophils.

►Leukocytes

WHO

Definition
World Health Organization.

WHO Grade IV Astrocytoma

►Glioblastoma Multiforme

Wide Excision

Definition
Tumor surgery by which the excision takes place at 2 cm outside the pseudo capsule of the tumor.

►Cryosurgery in Bone Tumors

Wild-type

Definition

Refers to the most prevalent gene variant (encoding a protein, or an active enzyme) in the population. If a variant is predominant in one population, but has a low frequency in another population, the terminology usually refers to the population in which the variant was first described. For this reason, it is advisable to refrain from using this terminology on its own because it may easily create confusion in a number of cases.

Wilms Tumor

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Definition

Wilms tumor (synonym nephroblastoma) is a childhood embryonal cancer of the kidney. It was named after a German physician, Max Wilms, who described the first large collection of cases of this tumor in a paper published in 1899. The term Wilms tumor used to be applied to other types of childhood kidney cancer (clear cell sarcoma of kidney and malignant rhabdoid tumor of kidney) but these are now recognized as distinct entities with different clinical behavior and requiring different treatments.

Characteristics

How Common is Wilms Tumor?

Wilms tumor affects ~1 in 10,000 children before their fifteenth birthday, typically at around age 3–4 years. Ninety percent of cases will have been diagnosed before the age of 7 years. There is a degree of ethnic variation; it is commoner in blacks and relatively rare in Asians. Usually only one kidney is affected, but in 5–8% of cases there are tumors in both kidneys (bilateral disease).

What Causes Wilms Tumor?

Although the majority of cases of Wilms tumor are "sporadic" with no obvious cause, it is known that genetic predisposition to Wilms tumor can occur. One to two percent of affected children inherit a defective gene from a parent. There are at least four such "familial Wilms tumor genes," only one of which has been identified to date. More commonly, in ~5% of all cases, a child has a developmental defect associated with genetic predisposition to Wilms tumor and sometimes other forms of cancer. The first of these to be defined at a molecular level was the association of Wilms tumor with aniridia, a lack of development of the iris in the eye. Such children usually have other defects including abnormal genital development and variable mental retardation, and hence the acronym, WAGR syndrome (for Wilms tumor, Anividia, Genitourinary malformations, and mental retardations). This defect is due to a chromosomal deletion that deletes one copy of the ►Wilms tumor gene, WT1, and the adjacent ►PAX6 gene at 11p13. Loss of one allele of PAX6 is dominant, but development of the tumor requires loss or mutation of the remaining WT1 allele in one or more kidney cells (i.e. tumor development is recessive, hence WT1 belongs to the class of ►tumor suppressor genes). Heritable mutations in WT1 are also responsible for the predisposition to Wilms tumor associated with abnormal kidney development seen in ►Denys-Drash syndrome, where children lose large quantities of protein in their urine (nephrotic syndrome) and often have abnormal genital development. Thus, Wilms tumor provides a fascinating example of how normal development of an organ can be intimately linked to cancer predisposition in that organ. Indeed, Wilms tumor can bear an uncanny resemblance to cell types seen during normal embryonic kidney development, hence the term "embryonal tumor."

There are an increasing number of children recognized with nephrotic syndrome with underlying germline WT1 mutations who do not develop Wilms tumor. Hence, it is possible that inheriting a mutation in WT1 provides only a relatively weak stimulus to developing Wilms tumor (i.e. the gene is of low ►penetrance).

A second category of genetic predisposition to Wilms tumor occurs in the overgrowth syndromes of childhood, of which ►Beckwith-Wiedemann syndrome (BWS) is the best recognized. The genetics of BWS are complex, involving several different genes within the 11p15.5 chromosomal locus and the phenomenon of ►genomic imprinting, whereby expression of a gene depends from which parent it was inherited. The overall tumor risk is ~10%, of which half are Wilms tumors. It appears that children with early nephromegaly (i.e. overgrowth of the kidneys) or asymmetrical overgrowth (hemihypertrophy) are at greatest risk.

Finally, there is some evidence from case control studies that the risk of Wilms tumor may be somewhat increased by certain parental occupations or exposures. However, the relative risk to the child is usually small, of the order of two- to tenfold.

Clinical Characteristics

Wilms tumor is one of the most curable of childhood cancers, even when it has spread beyond the kidney to distant sites. The commonest site of such metastases

is the lung, followed by lymph nodes and liver. Wilms tumor rarely metastasizes to bone, bone marrow or brain. The treatment consists of chemotherapy with one to three different drugs (usually ►vincristine, ►actinomycin D+/– ►adriamycin) together with surgical excision of the affected kidney. Radiotherapy is also used when there is residual or spilt tumor in the abdomen or metastasis to the lungs. With these regimens, ~90% of children with a tumor confined to the kidney (stages I and II) are cured, as are over two-thirds of patients with metastatic disease (stage IV). There is a different philosophical approach to the organization of treatment between different national and international childhood cancer study groups. The National Wilms Tumor Study Group (►NWTSG) of North America favors immediate surgical excision of the affected kidney, followed by chemotherapy with or without radiotherapy, according to the tumor extent found at the time of surgery. The approach of the International Society of Paediatric Oncology (►SIOP) is to use pre-operative chemotherapy to shrink the tumor prior to surgery. This study showed a reduction in the risk of tumor rupture during operation and also reduction in the tumor stage, hence allowing less intensive post-operative treatment. The two groups have comparable cure rates, but the NWTSG approach uses slightly more radiotherapy whereas the SIOP approach uses more anthracyclines (adriamycin). Both these treatments have the potential for long term side effects on growth and fertility and on the heart muscle, respectively. The majority of children with Wilms tumor are cured without the need for either of these agents and are unlikely to suffer any long term sequelae.

Is Wilms Tumor One Disease?

As with most cancers, various "prognostic factors" can be recognized in Wilms tumor. The most obvious adverse factor is increasing tumor ►stage. However, a distinct histological subtype called ►Wilms tumor anaplasia carries a poor prognosis, especially when associated with advanced stage disease. Anaplasia is associated with mutations in the ►p53 gene, which can occur focally as part of clonal evolution of a tumor. Other molecular characteristics that may be associated with worse outcome are allele loss or ►loss of heterozygosity for markers on chromosome 16q, 1p and possibly 22q and 11q. Some of these are being tested prospectively in the current NWTS 5 clinical trial, which aims to use molecular characteristics of a tumor to better define risk groups.

Molecular Characteristics of Wilm Tumor

As soon as the WT1 gene was isolated in 1990 it became clear that it was not mutated in the majority of sporadic Wilms tumors. Among over 600 published cases analyzed for intragenic mutations, WT1 mutation occurs in only ~10%. In some tumors, mutation of both WT1 alleles appears to be sufficient for tumorigenesis, in accordance with ►Knudson hypothesis of two hits. However, in other tumors, either only one WT1 allele is mutated or other genetic events are clearly interacting. The molecular biology of the WT1 protein is fascinating and has led to insights into both tumor and normal development and their inter-relationship. Although it is known that several different genetic loci exist for other Wilms tumor genes, their relative contribution to sporadic Wilms tumor is not yet known. A database of WT1 mutations is maintained (http://www.umd.necker.fr).

Treatment of Relapsed Wilms Tumor and Future Therapeutic Possibilities

Although Wilms tumor is one of the most curable of childhood cancers at initial diagnosis, those cases that relapse carry a much worse prognosis, even with intensive retreatment. Less than a third of relapses are due to the anaplastic variant. Hence, one of the goals of current Wilms tumor clinical studies is to identify factors present at diagnosis that are predictive of outcome. Future treatment intensity could then be stratified according to predicted risk of relapse using molecular characteristics of individual tumors. Another potential avenue is to use knowledge of the biology of the Wilms tumor genes to devise novel therapeutic approaches. In the future this might also lead to preventative strategies for children at increased genetic risk of Wilms tumor.

Heritability and Screening

By clinical criteria it appears that <10% of children with Wilms tumor have a potentially "heritable" form of the disease. Approximately 1–2% have a family history of Wilms tumor, a further 1–2% have the WAGR syndrome, ~3% have either Beckwith Wiedemann syndrome or some degree of asymmetrical overgrowth. However, it now appears that there are several genes that may be responsible for familial Wilms tumor and they may be of relatively low ►penetrance. This means that a proportion of patients with apparently sporadic Wilms tumors may transmit a germline mutation to their offspring predisposing them to Wilms tumor. However, even if the proportion of heritable cases is larger than previously believed, the absolute risk to offspring must be low; only four cases among 362 offspring of 462 survivors of unilateral Wilms tumor have been reported.

Early detection of Wilms tumor has the potential to increase survival and reduce treatment morbidity. Children known to be genetically predisposed to Wilms tumor, such as those with WAGR, Beckwith-Wiedemann or Denys-Drash syndromes, are candidates for screening. This is usually done by regular

abdominal ►ultrasound scanning, although teaching the parents to perform regular palpation of the abdomen is an acceptable alternative. Since Wilms tumors can grow very rapidly, ultrasound screening is recommended at intervals of no >3–4 months. However, there are no definitive clinical trials to determine which screening method or interval is superior for detecting tumors at a low stage (I or II). In the future, if a larger proportion of children are shown to be carriers of one of the familial Wilms tumor genes, then more information should become available on the efficacy of screening. A further benefit of the application of molecular genetics is that children with germline WT1 mutations appear to be at risk of late renal failure and hence require appropriate monitoring.

►Nephroblastoma

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Wilms Tumor Anaplasia

Definition

The term "anaplasia" in ►Wilms tumor is used to describe a histological pattern defined as the presence of all of the following three features:

- Cells with a nuclear diameter at least three times of adjacent nuclei of the same cell type;
- Marked hyperchromatism of these cells, indicative of increased chromosome numbers;
- Abnormal mitotic figure.

►Anaplasia may be focal or diffuse and is associated with mutation of the ►p53 gene. Anaplasia is felt to be a marker of cellular resistance to therapy but not of increased tumor aggressiveness as anaplasia in stage I tumors has no adverse prognostic significance.

Wilms Tumor Suppressor Gene

Definition

WT1 gene; Is located at 11p13 and consists of ten exons spanning approximately 50 kb of genomic DNA. The gene encodes a zinc finger protein that has two alternative splice sites affecting the whole of exon 5 and a three amino acid insertion (KTS) between the third and fourth zinc fingers. The WT1 protein is multifunctional and the various isoforms can act as transcription factors or may be involved in RNA processing or splicing. WT1 is essential for formation of the kidney and gonad in the mouse. There is a genotype-phenotype correlation, with germline missense mutations as seen in ►Denys-Drash syndrome having a more pronounced effect on genitourinary development than complete deletion of one allele, as seen in WAGR syndrome. Germline intronic mutations affecting splicing of the KTS linker also act in a dominant fashion on genitourinary development.

►Wilms Tumor

WIN

►Forkhead Box M1

Wiskott-Aldrich Syndrome

Definition

WAS; is a rare X-linked ►recessive disease with variable expression, but commonly includes immunoglobulin M (IgM) deficiency. WAS always causes persistent ►thrombocytopenia (low platelet counts), and, in its complete form, also causes small ►platelets, atopy, cellular and humoral immunodeficiency, and an increased risk of ►autoimmune disease and ►hematologic malignancies. The exact function of the WAS protein is not fully elucidated, but it seems to function

as a bridge between signaling and movement of the actin filaments in the cytoskeleton. WAS is also sometimes called the eczema-thrombocytopenia-immunodeficiency syndrome.

Withaferin A

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Synonyms

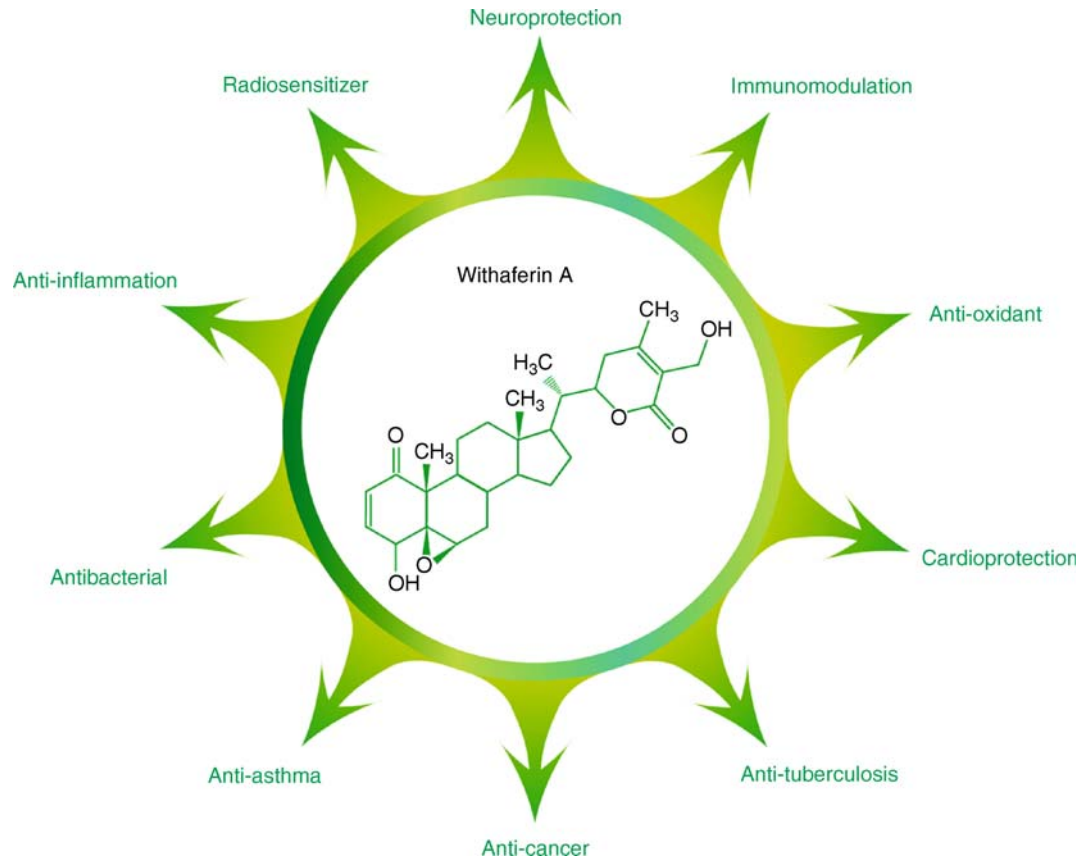
WA

Definition

The natural product Withaferin A (WA) is an important bioactive component of *Withania somnifera* (WS), a medicinal plant of the Solanaceae family that is used in the Indian Ayurvedic medical system, as well as many other indigenous medical practices. A structural analysis of WA indicates that it is a highly oxygenated C-28 ergostane-type steroid with a 22, 26-lactone and a 1-oxo-group (Fig. 1).

Characteristics

WA has been used in several ethnobotanical medical practices (►Natural products) to treat a variety of ailments, including cancer, inflammatory conditions, and cardiac and neurological disorders (Fig. 1). With respect to cancer, seminal *in vivo* and *in vitro* studies have revealed that WA possesses chemopreventive, chemotherapeutic, and radiosensitizing character (►Adjuvant therapy). The chemopreventive properties of WA were first identified in a Swiss albino mice tumor model, and were subsequently confirmed in mice using *Withania spp.* root extracts, as well as several withanolides isolated from these extracts. Similarly, early studies also revealed the chemotherapeutic utility of WA, as it potently inhibited mouse Ehrlich ascites carcinoma *in vivo* and induced G_2/M arrest (►G_2/M Transition) of the cell cycle in Chinese Hamster V79 cells. This WA-mediated G_2/M arrest has been shown to result in significant radiosensitization in cell culture



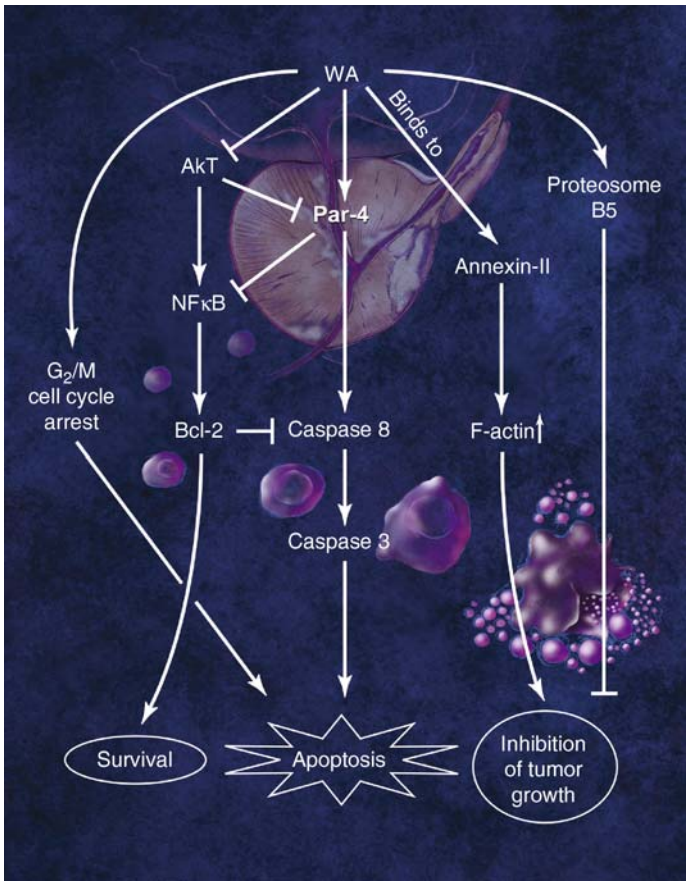
Withaferin A. Figure 1 The diverse spectrum of Withaferin-A activity.

models, as well as in mouse fibrosarcoma and melanoma when administered prior to radiotherapy. Notably, when used in combination with radiotherapy *in vivo*, WA increased tumor cure and disease-free survival. Several reports substantiate these findings and support the activity of WA against various cancer models, including prostate, colon, breast, and lung cancer.

The mechanistic details of WA anti-cancer activity have been extensively studied in prostate cancer (►Prostate cancer, clinical oncology) models *in vitro* and *in vivo*. WA induces ►apoptosis in androgen refractory prostate cancer cells, and causes regression of PC-3 xenografts in mouse models. These studies also reveal that WA alone fails to induce apoptosis in androgen-responsive prostate cancer, but the combination of WA and an anti-androgen induces apoptosis in androgen-responsive cells (►Androgen receptor). Published reports from several labs confirm these selective effects of WA on prostate cancer. Given that androgen-responsive prostate cancer often progresses to an androgen-refractory phenotype, which is resistant to current therapeutic approaches, these findings are significant.

WA targets multiple signaling pathways in prostate cancer cells to induce its apoptotic effects (Fig. 2). In prostate cancer cells, WA up-regulates the gene expression of prostate apoptosis response-4 (Par-4), which induces p53- and phosphatase and tensin homolog (PTEN)-independent cancer-selective apoptosis. The pro-apoptotic effects of WA are dependent on Par-4 expression and action. In normal and malignant cells, Par-4 is phosphorylated and inactivated by Akt (also known as Protein Kinase B), which is an evolutionarily conserved regulator of the cell cycle. However, as shown in prostate, breast, and lung cancer cells, WA inhibits Akt-mediated pro-survival signaling (►BCl-2) and initiates G_2/M cell cycle arrest (unpublished data).

WA also negatively regulates NFκB activity in variety of cancer cell lines. NFκB is a transcription factor, a regulator of key cellular processes, such as apoptosis, immunoresponse, differentiation, and proliferation, and its constitutive activation has been linked to cancer. Under normal conditions, NFκB activity is held in check in the cytoplasm by IκB. Upon phosphorylation of IκB by IκB kinase (IKK), NFκB translocates to the nucleus to effect gene transcription. Importantly, WA inhibits IκB kinase activity, thereby blocking NFκB nuclear translocation, and targets various cysteine residues of multiple kinases and phosphotases.



Withaferin A. Figure 2 Withaferin-A mechanism of action in prostate cancer.

As a consequence, WA alters the phosphorylation status of p38, MEK/ERK, JNK, and IKKβ, thereby inhibiting the pro-survival machinery and triggering apoptosis.

Beyond WA effects on Par-4 and pro-survival signaling pathways (►Apoptosis Signaling), it has been reported that WA "function both as a ►proteasome" and ►angiogenesis inhibitor. The proteasome is a proteinase complex that selectively degrades ubiquitinated proteins, and is considered to be a potential target for cancer chemotherapy. WA targets the proteasome β5 subunit, inhibiting cell survival *in vitro* and tumor growth *in vivo*. Structural modeling analysis indicates WA contains two conjugated ketone bonds that target the hydroxyl group of the N-terminal threonine of the proteasomal chymotrypsin subunit β5. With respect to angiogenesis, a recent study demonstrates that WA binds to annexin II to promote F-actin polymerization and growth inhibition in Prostate cancer cells. This observation complements a study that shows WA inhibits angiogenesis through its ability to negatively regulate NFκB signaling, in addition to ►cyclin D1.

Although preclinical studies reveal that WA impacts multiple signaling cascades associated with cancer cell proliferation and survival, careful analyses are needed to establish the clinical merit of WA in treating human cancers. The precise mechanism by which WA elicits such diverse biological effects remains unclear, thus additional studies are required to identify unknown targets of WA, and clarify the molecular nexus that directs this broad spectrum of WA activity.

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μMEN1

►Multiple Endocrine Neoplasia Type 1

Wnt

Definition

The name comes from the *Drosophila* gene wingless and the proto-oncogene *int-1*. The wnt/β-catenin pathway is highly conserved and regulates gene expression by ►TCF/LEF transcription factors. The pathway is of importance in embryonic development, stem cell differentiation, cell death, and tumor progression.

►Wnt Signaling

Wnt/beta-Catenin Pathway

Definition

In the canonical Wnt/beta-catenin pathway, Wnt ligand binding to its cell surface receptor triggers changes of cytoplasmic effector activities and stabilization of beta-catenin protein. Beta-catenin then accumulates in the nucleus where it interacts with ►high mobility group (HMG) box transcription factors to regulate gene expression.

►APC/β-Catenin Pathway
►Wnt Signaling

Wnt Signaling

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Definition

The name ►Wnt comes from wingless (Wg) and Int-1. Wingless is a *Drosophila* gene important during development and Int-1 is a gene into which the mouse mammary tumor virus (MMTV) *int*egrated to cause tumors. Wg and Int-1 were subsequently found to be related in sequence. Wnt signaling is the result of binding of Wnt proteins to cell surface receptors. Wnt family members exist in all multicellular organisms.

Characteristics

Wnt Proteins

Vertebrate Wnts comprise a family of 19 hydrophobic cysteine-rich secreted glycoproteins of ~350 amino acids in length. Their hydrophobicity stems from lipid modification – cysteine palmitoylation and serine palmitoleoylation – that is essential for signaling activity and secretion. Wnt proteins are quite insoluble and associate with ►HSPGs (heparan sulfate proteoglycans) in the extracellular matrix, so they often signal only over short distances. Long-range Wnt signaling does take place, however, for example, when Wnts form gradients to pattern developing tissues. Long-range Wnt signaling is mediated by ►lipoprotein particles and facilitated by the ►retromer. Wnt signals can elicit a number of cellular responses including proliferation, differentiation and migration (►Migration). The nature of the response is dictated by the responding cell and the specific Wnts and Wnt receptors present.

Mutations of Wnt genes are rare and have not been found in human tumors. However, Wnt gene expression patterns are often altered in tumor cells and inhibition of Wnt activity using antibodies or siRNA (►siRNA) induces apoptosis (►Apoptosis) in some tumor cell types (see Table 1). Both increases and decreases in Wnt5a expression have been observed in cancer, perhaps because Wnt5a plays different roles in different tissues or at different stages during tumor progression.

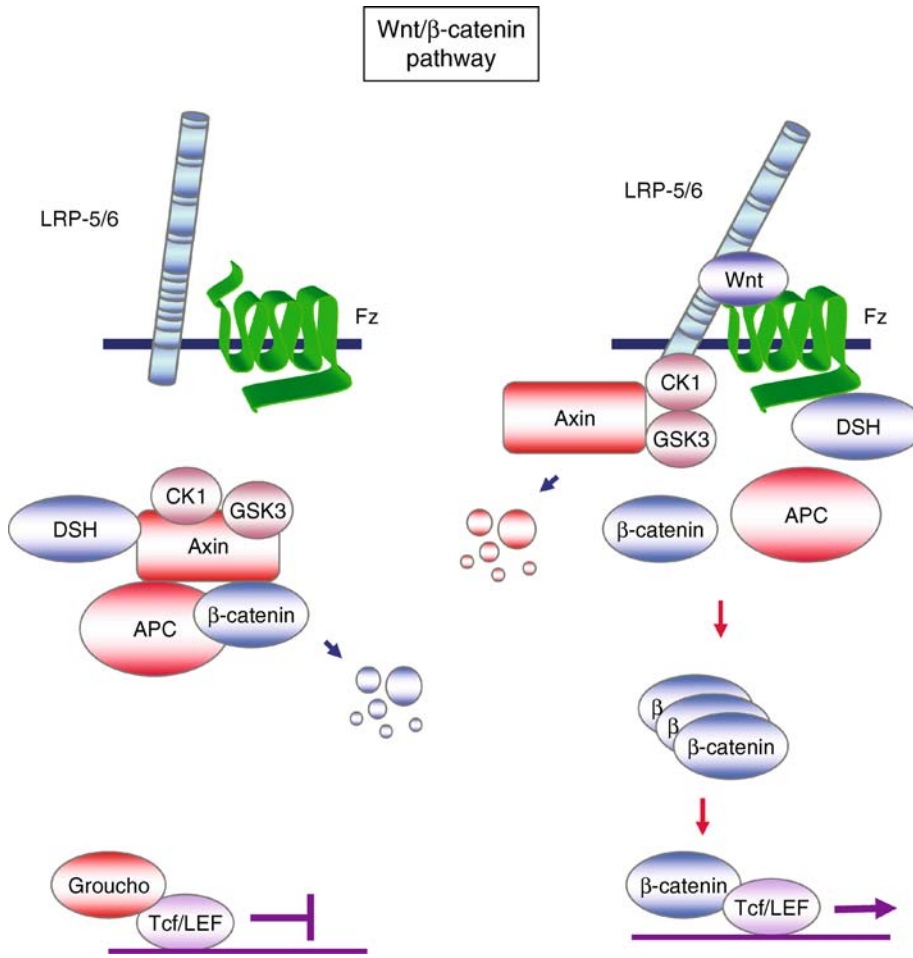
Wnt Signaling Pathways

Wnt proteins normally bind to cell-surface receptors of the frizzled (►FZD) family, which activate intracellular disheveled (►DVL) family proteins. At this stage the Wnt signals bifurcate and can activate distinct pathways. The best understood of these is the Wnt/►β-catenin pathway, also referred to as the canonical pathway (►APC/β-catenin pathway) (Fig. 1). In this pathway, Wnt proteins also bind to ►LRP5/6, which interact with the β-catenin-binding protein ►Axin. In the absence of Wnts, a so-called β-catenin destruction complex controls the level of β-catenin in the cytoplasm. This complex, which includes Axin, glycogen synthase kinase-3(►GSK-3), casein kinase-I (►CKI) and adenomatous coli polyposis protein (APC) (►APC in familial adenomatous polyposis), facilitates β-catenin phosphorylation, ubiquitination (►Ubiquitination), and ultimately degradation in the ►proteasome. Canonical Wnt signals disrupt this complex by eliciting changes in Axin localization and stability, resulting in the stabilization of β-catenin in the cytoplasm. Stabilized β-catenin enters the nucleus and binds to transcription factors, primarily of the ►Tcf/LEF family, thereby regulating gene expression. The Wnt/β-catenin pathway is permanently active in many tumor types, in particular in colon cancer (►Colon cancer), as a result of mutations in APC, β-catenin or Axin. In rare cases, inactivation of Wnt/β-catenin signaling leads to tumor formation, for example, in sebaceous tumors with LEF-1 mutations (Table 2).

Non-canonical Wnt signals do not stabilize β-catenin. Instead, they overlap with other known signaling pathways. The planar cell polarity (►PCP) pathway is a noncanonical pathway that was first defined in *Drosophila*, where it controls the uniform orientation of hairs and bristles on the body. PCP signaling has also been studied extensively in frogs and zebrafish, where it regulates ►convergent extension. In mammals, the best example of PCP is the uniform orientation of the hair cell stereociliary bundles within the cochlea. PCP pathway proteins include FZD, DVL and several proteins not involved in other Wnt signaling pathways; those relevant to cancer include ►VANGL, which is overexpressed in tumors and promotes metastasis (►Metastasis), and PTK7, a catalytically inactive transmembrane tyrosine kinase (►Receptor tyrosine kinase) that is overexpressed in tumor-derived cell lines. The PCP pathway activates the small GTPases Rac and Rho (►Rho family proteins) and the protein kinases c-Jun NH2-terminal kinase (►JNK) and Rho-associated kinase (►ROCK) to induce changes in the cytoskeleton. Whether Wnt ligands activate the PCP pathway is still unclear; there is no strong evidence for this in flies or humans, but mutations

Wnt Signaling. Table 1 Examples of Wnt genes with altered expression in cancer

Wnt	Tumor type	Change in expression	Comments
Wnt1	Several	Increase	Anti-Wnt1 antibody induces apoptosis
Wnt2	Mesothelioma (mesothelioma), melanoma (melanoma)	Increase	Anti-Wnt2 antibody and siRNA apoptosis
Wnt5a	Gastric cancer, melanoma, leukemia	Increase (gastric cancer, melanoma) decrease (leukemia)	Increase probably linked to metastasis
Wnt16	Leukemia, basal cell carcinoma	Increase in expression of alternatively-spliced isoform b	Anti-Wnt16 antibody induces apoptosis



Wnt Signaling. Figure 1 A simplified view of Wnt/β-catenin signaling. In the absence of Wnt (*left*), a protein complex that includes Axin, APC, GSK-3 and CK-Iα promotes the phosphorylation, ubiquitination and subsequent degradation of β-catenin in the proteasome; Tcf/LEF proteins associate with Groucho to repress target gene expression. On the *right*, Wnt binds to FZD and LRP5/6 receptors resulting in DVL and LRP5/6 phosphorylation and Axin recruitment/degradation. This allows accumulation of β-catenin, which then enters the nucleus and associates with Tcf/LEF to activate gene expression. For simplicity several other components of the pathway are not shown.

Wnt Signaling. Table 2 Wnt/β-catenin pathway mutations in cancer

Intracellular component	Common tumor types	Effect of mutations
APC	Colon cancer	Stabilization of β-catenin
β-catenin	Liver cancer	Stabilization of β-catenin
	Ovarian cancer	
	Colon cancer	
Axin	Medulloblastoma	Stabilization of β-catenin
	Liver cancer	
	Colon cancer	
LEF-1	Sebaceous tumors	Reduced Wnt/β-catenin signaling

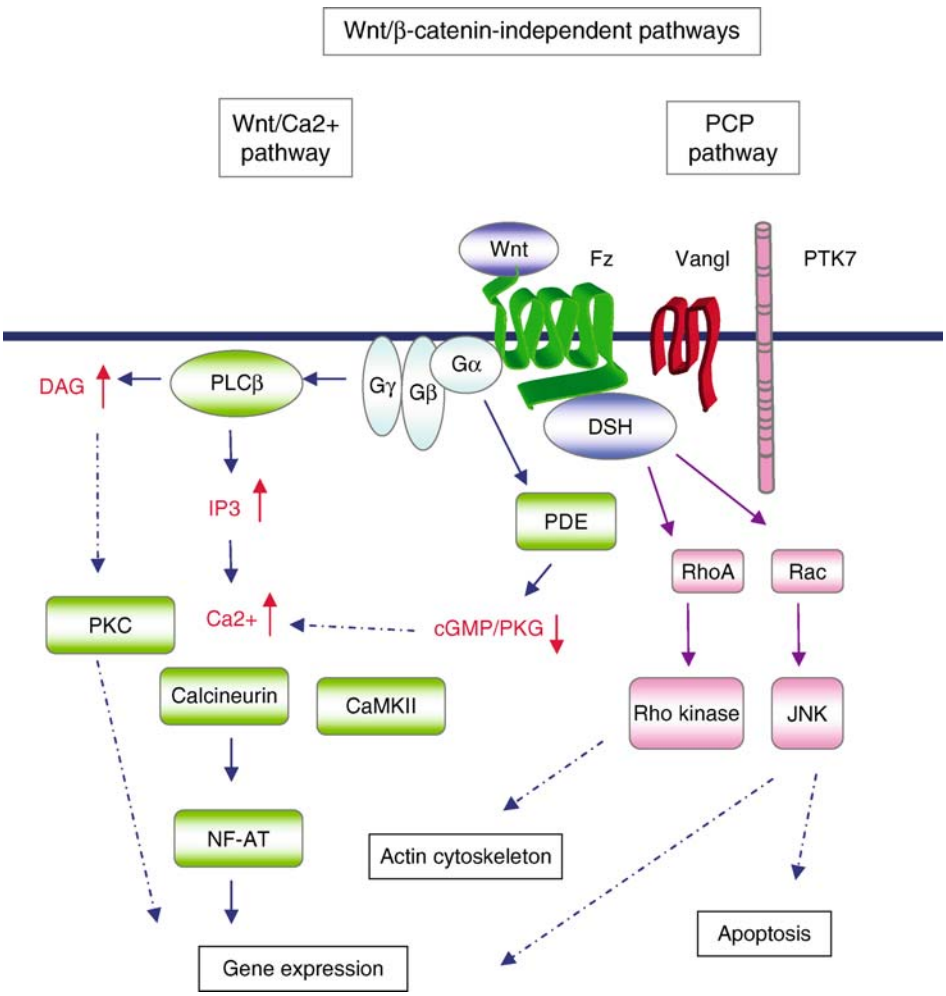
in *Wnt5* and *Wnt11* disrupt convergent extension in zebrafish.

In the Wnt-calcium (Ca2+) pathway, binding of Wnt to FZD leads to activation of ►G proteins that then activate phospholipase C and phosphodiesterase, leading to increased concentrations of free intracellular Ca2+. These events result in activation of ►protein kinase C (PKC) and Ca2+/calmodulin-dependent

protein kinase II (CaMKII) and the Ca2+-sensitive protein phosphatase ►calcineurin. PKC and calcineurin directly and indirectly regulate various transcription factors such as nuclear factor of activated T cells (NF-AT). The Wnt-Ca2+ pathway has been studied extensively in frogs and zebrafish, where it can be activated by Wnt4, Wnt5a and Wnt11 (Fig. 2).

There is increasing evidence for the involvement of heterotrimeric ►G-protein α subunits in canonical and noncanonical signaling pathways. For example, Gαo and Gαt2 participate in Wnt/Ca2+ signaling by activating a phosphodiesterase that lowers cGMP levels, thereby inactivating protein kinase G; Wnt3a signals through Gαq to activate PKCδ; and prostaglandin E2 receptors stimulate proliferation of colon cancer cells through the β-catenin pathway by association of Gαs with ►Axin. The existence of Wnt-Ca2+ and Wnt/G protein signaling pathways in mammalian cells is largely supported by overexpression studies using cell lines and still requires confirmation using genetic approaches.

Just how important non-canonical Wnt signaling is in cancer is still not clear. Wnt5a is probably the most studied non-canonical Wnt and its roles in cancer are complex. For example, Wnt5a expression is reduced in human leukemia and Wnt5a heterozygous mice (►Haploinsufficiency) develop B-cell lymphoma. In contrast, the expression of Wnt5a is increased in gastric cancer (►Gastric cancer), where it correlates with tumor aggressiveness. Wnt5a is likely to have more than one function, controlling cell proliferation and/or differentiation in some tissues and promoting cell migration in others. At the molecular level these differences might



Wnt Signaling. Figure 2 A simplified view of Wnt/β-catenin-independent signaling. In the Wnt-Ca2+ pathway, Wnt binding to FZD activates G proteins, thereby activating a number of downstream signals. The PCP pathway involves activation of FZD and DVL, which together with several additional proteins including VANGL and PTK7, affect Rac/Rho signaling pathways. See text for more details. For simplicity several other components of the pathway are not shown.

Wnt Signaling. Table 3 Examples of ligands and receptors involved in Wnt signaling

Ligand	Receptor	Key events	Examples of changes in cancer
Wnt ligands			
Wnt (several)	FZD	FZD recruits DVL	FZD7 overexpressed in hepatocellular carcinoma (►hepatocellular carcinoma)
Wnt (several)	LRP5/6	LRP5/6 recruits Axin	–
Wnt5a	Ror2	Inhibits Wnt/β-catenin signal	–
Wnt (several)	Ryk	Ryk also binds FZD8 and DVL; activates Wnt/β-catenin signal	Ryk overexpressed in ovarian cancer (►ovarian cancer)
Non-Wnt ligands			
sFRP family	(FZD family)	Inhibit Wnt signals by binding to Wnt	Reduced expression (CpG methylation) (►CpG islands)
DKK family	LRP5/6	Inhibit Wnt/β-catenin signal	Reduced expression (CpG methylation)
WIF1	–	Inhibits Wnt signals by binding to Wnt	Reduced expression (CpG methylation)
Norrin	FZD4	Activates Wnt/β-catenin signal	–
Sclerostin	LRP5/6	Inhibits Wnt/β-catenin signal	–
R-spondin	LRP6, FZD8	Activates Wnt/β-catenin signal	Reduced expression

be explained by the ability of Wnt5a to activate discrete signaling pathways through distinct receptors. For example, in transfected cells Wnt5a inhibits Wnt/β-catenin signaling by binding to the tyrosine kinase Ror2 and activates Wnt/β-catenin signaling by binding to FZD4. There are also examples of canonical Wnts that activate β-catenin-independent signals, such as Wnt3a activation of PKCδ. Moreover, although Wnt1 is best known as an activator of Wnt/β-catenin signaling, anti-Wnt1 antibodies can induce apoptosis in cells that do not express β-catenin, suggesting that Wnt1 noncanonical signals are important for cancer cell survival.

Other Ligands and Receptors that Directly Regulate Wnt Signaling

There are several other proteins that modulate Wnt signaling by binding to Wnts themselves, binding to Wnt receptors or by acting as Wnt receptors (Table 3). Most of the secreted proteins are so-called Wnt antagonists that normally inhibit Wnt signals. The secreted frizzled related protein (►sFRP) family and Wnt inhibitory factor-1 (WIF1) have the potential to inhibit all Wnt signals, since they interact with Wnt proteins themselves. sFRP proteins also bind to FZD receptors and in some cases have been shown to augment rather than inhibit Wnt/β-catenin signaling. The expression of sFRP proteins and WIF1 is downregulated in many tumors as a result of promoter methylation. In contrast to sFRP proteins, dickkopf (►DKK) family members and Sclerostin interact with LRP5/6 and so are thought to inhibit only Wnt/β-catenin signaling. The expression of DKK family members is often reduced in tumors as a result of promoter methylation. However, DKK1 is highly expressed in myeloma (►Multiple myeloma), where it contributes to osteolytic bone disease by inhibiting the differentiation of osteoblasts. Although DKK1 and DKK4 inhibit Wnt/β-catenin signaling, DKK2 can activate Wnt/β-catenin signaling, while DKK3 does not directly affect Wnt signaling at all since it cannot bind to LRP5/6. DKK1 is itself a β-catenin/Tcf target gene that provides a negative-feedback loop for Wnt/β-catenin signaling. Finally, Norrin and R-spondin are secreted proteins that activate Wnt/β-catenin signaling. Examples of proteins that act as Wnt receptors are Ryk, a catalytically inactive receptor tyrosine kinase that binds Wnt proteins using a domain related to WIF1, and Ror2, which binds to Wnt5a.

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Working Level

Definition

WL; Describes the concentration of radon and its progeny in the mining environment. A Working Level (WL) is defined as 1.3×10^5 MeV of potential alpha energy/l air.

►Uranium Miners

Working Level Month

Definition

WLM; equals the exposure to one working level for a working month (170 h). A working level is equivalent to any combination of radon progeny in 1 liter of air that will result in the ultimate emission of 1.3×10^5 MeV of potential alpha particle energy.

►Uranium Miners

Working Level Months (WLM)

Definition

A miner's exposure to radon and its progeny is given in Working Level Months (WLM). A Working Level Month equals an exposure to 1 WL for 170 h or any equal combination of WL and time.

►Uranium Miners

Wound Healing

Definition

Refers to the predictable series of events that takes place after damage to dermal or epidermal tissue.

►CXC Chemokines

Wound Healing Assay

Definition

Is an *in vitro* assay used to study cell ►migration. A wound is created in a cell monolayer and the open gap is inspected microscopically over time as cells migrate and fill the damaged area.

Wox1

►WWOX

Wright-Giemsa Stain

Definition

Synonym wright stain; A specially prepared mixture of methylene blue and eosin in methanol, used to stain cells so that they can be seen microscopically. Most commonly used in hematology for peripheral blood smears and in cytopathology for fine needle aspirations and body fluids.

►Fine Needle Aspiration
►Pathology

WT1

Definition

►Wilms tumor suppressor gene 1, a gene encoding a zinc-finger containing transcription factor that is mutated in a subset of ►Wilms tumor.

WTIP

Definition

WT1-interacting protein. Is a member of the ►zyxin family of proteins.

►Lipoma Preferred Partner
►Wilms Tumor Suppressor Gene

WW Domains

Definition

Named after two highly conserved tryptophan residues within the sequences of small protein modules (~30 amino acids in length) that recognize and bind to proline-rich peptide motifs in interacting proteins.

►WWOX

WWOX

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Synonyms

FOR; Wox1

Definition

Wwox, a 414 amino acid protein of 46 kDa, mapping to a common ►fragile site at chromosome region 16q23.3, exhibits two N-terminal ►WW domains and a central short chain oxidoreductase-like domain. Wwox is a cytoplasmic protein that binds other proteins containing PPxY-containing ligand proteins through the first Wwox WW domain. Through binding of its ligands, Wwox controls transcriptional activation and repression of nuclear genes and thus controls signaling pathways that affect aspects of cell growth and ►apoptosis. Wwox expression is nearly ubiquitous in normal tissues but is lost or decreased in many cancers due to genomic or ►epigenetic modification, and its restoration causes apoptosis of Wwox deficient cancer cells; thus *WWOX* is the second known fragile ►tumor suppressor gene.

Characteristics

The Gene

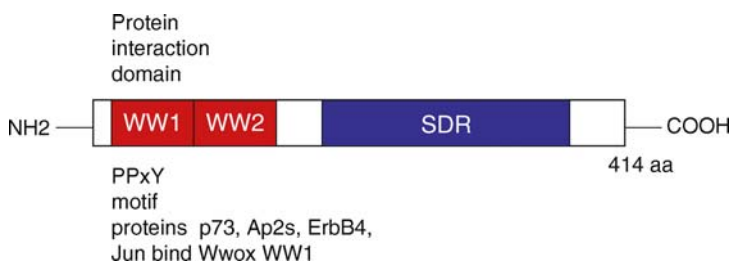
The *WWOX* gene spans a genomic locus of >600 kb and is composed of nine exons encoding an open reading frame of 1,245 bases; the protein sequence includes two WW domains and a short chain dehydrogenase/reductase domain that may be involved in sex-steroid metabolism, considering its sequence homology to 17β-hydroxysterol reductase 3 (Fig. 1).

The *WWOX* gene also spans fragile site ►FRA16D, encompasses a region involved in ►loss of heterozygosity (LOH) in cancers, is associated with ►homozygous deletions in cancer-derived cell lines, with chromosome translocations in ►multiple myelomas, and its promoter region is frequently hypermethylated in cancers (Fig. 2).

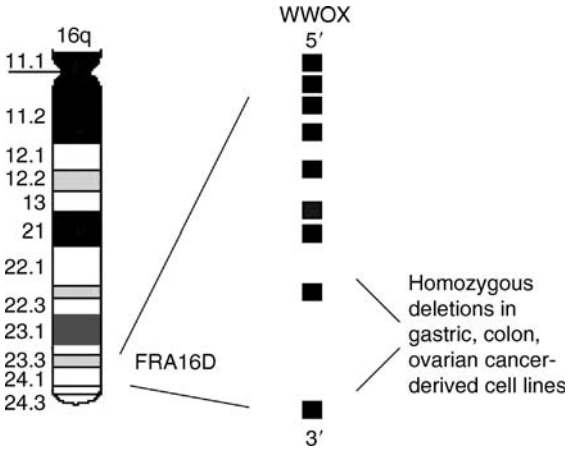
Most cancer cell lines with FRA16D homozygous deletions also exhibit deletions in ►FRA3B and the ►*FHIT* gene, consistent with the finding that common fragile sites or regions are highly susceptible to ►DNA damage and recombination. The mouse ortholog, *Wwox*, at murine chromosome region 8E1, is also fragile and is highly homologous to the human locus. Internally deleted *WWOX* transcripts have been observed in human breast, ovarian and other tumor types, though *bona fide* point mutations have not been observed. The highest level of *WWOX* expression in normal tissues occurs in hormonally regulated tissues. The *WWOX* promoter is hypermethylated in many cancers in association with loss of Wwox expression, and detection of the ►methylation status by ►methylation-specific PCR (MSP) amplification may serve as a marker of cancer development or prevention.

The Protein

Wwox protein binds the proline-rich ligand PPxY and a number of proteins interacting with the first WW domain, WW1, have been identified. WW domains are grouped by binding preference for specific types of proline-rich ligands and the Wwox WW1 domain belongs to group I, that binds PPxY ligands; among



WWOX. Figure 1 Schematic of the Wwox protein showing the WW and short chain dehydrogenase/reductase domains. Proteins with a PPxY motif that interact with Wwox through its first WW domain are listed.



WWOX. Figure 2 Schematic of the WWOX gene. The long arm of chromosome 16 is shown on the *left*. The dark boxes on the *right* represent WWOX exons. Many of the deletions detected in tumor cell lines are within WWOX intron 8.

these ligand-containing proteins are p73, Ap2α, Ap2γ, ErbB4, Jun and the SIMPLE protein; ►p53 has also been reported to bind Wwox or Wox1, the murine ortholog, but other studies have not confirmed this ligand. The Cytogen Corporation has developed, through informatics and binding studies, a proprietary database that lists all potential Wwox ligands among known gene products, and the first three ligands listed above were selected from the database and confirmed as Wwox-interacting proteins by *in vitro* analyses.

The cytoplasmic Wwox protein binds its ligands and prevents the interacting proteins from entering the nucleus, where some have roles in transcriptional activation or repression. There are thus far unconfirmed reports of Wwox subcellular location also in mitochondria and nuclei of some cells.

Biological Role

In normal cells: human tissue samples of more than 30 organs have been analyzed by immunohistochemistry for expression of Wwox; Wwox is expressed in most organs but is expressed at highest levels in secretory epithelial cells such as those of breast, ovary, testes and prostate. Wwox is also expressed in various cells of neural origin.

►Targeted deletion of the mouse *Wwox* gene revealed important roles of Wwox in tumorigenesis and metabolism. Using ►homologous recombination, a mouse lacking exons 2, 3 and 4 of the mouse *Wwox* gene was generated. Progeny from *Wwox* heterozygous (*Wwox*+/–) intercrosses resulted in offspring of all three genotypes with ratios consistent with ►Mendelian distribution. At birth, homozygous Wwox-deficient (*Wwox*–/–) pups were indistinguishable from wild type or heterozygous littermates up to 3 days postpartum; after 3 days, homozygous pups were easily identified by their smaller size. *Wwox*–/– pups continued to grow more slowly than littermates, and all homozygous knockout mice died by 4 weeks after birth. Serum chemistry analysis of *Wwox*–/– mice showed marked hypoproteinemia, hypoalbuminemia, hypoglycemia, hypocalcemia, hypotriglyceridemia and hypocholesterolemia, indicating that *Wwox*–/– pups suffered severe metabolic defects.

Macroscopic and histological examination of the organs confirmed atrophy of many organs in *Wwox*–/– animals without significant microscopic lesions, though *Wwox*–/– mice are born with gonadal abnormalities and display bone growth retardation. Juvenile *Wwox*–/– mice displayed impaired steroidogenesis; levels of steroid biosynthesis enzymes, including Cyp11a1 (cytochrome p450 side-chain cleavage enzyme) and Hsd3b (3-β-hydroxysteroid dehydrogenase), were reduced in mutant testis and ovary compared to wild type and heterozygous testis. Radiography and high-resolution microtomography (μCT) of limb bones showed that *Wwox*–/– mice develop less dense bones with slow growth rates.

Analysis of the *Wwox*-mutant mice demonstrated that Wwox functions as a *bona fide* tumor suppressor. Spontaneous ►osteosarcomas in juvenile *Wwox*–/– and lung papillary carcinoma in adult *Wwox*+/– mice were observed, and *Wwox*+/– mice developed significantly more ethyl nitrosourea (ENU)-induced lung tumors and ►lymphomas in comparison to wild type littermates. These tumors still express Wwox protein, suggesting ►haploinsufficiency of Wwox is cancer-predisposing.

In cancer cells: Esophageal squamous cell carcinoma, ►non-small cell lung cancer and ►breast cancer showed high ►LOH rates, low mutation rates and expression of aberrant transcripts of the *WWOX* gene. Wwox expression is reduced in 63% of invasive breast carcinomas and is correlated with ►estrogen receptor alpha level, and prognostic features; Wwox and Fhit expression is coordinately lost in breast cancers. The *WWOX* gene is also inactivated in breast and lung cancers by regulatory region DNA methylation; promoter methylation was also detected in tissues adjacent to breast cancer, and methylation in *WWOX* exon 1 distinguished breast cancer DNA from DNA of adjacent and normal tissue. Wwox restoration in lung cancer cells *in vitro*, and in ►xenografts, suppressed growth. Wwox-deficient breast cancer cells, by treatment with ►5′-Aza-2′-deoxycytidine to demethylate the *WWOX* promoter, was associated with effective induction of apoptosis *in vitro* and suppressed breast cancer xenograft growth *in vivo*, without affecting the Wwox-sufficient cells or causing persistent changes in global methylation levels.

►Tamoxifen is commonly used for treatment of ►estrogen receptor alpha positive breast cancers, but

de novo or acquired tamoxifen resistance occurs frequently. Wwox protein, which binds and retains Ap2α and γ transcription factors in the cytoplasm, may mediate tamoxifen sensitivity *in vitro*; Wwox loss initiates tamoxifen resistance through release of Ap2 factors to the nucleus where Ap2γ up-regulates ErbB2 (►Her2) expression. Restoration of Wwox in tamoxifen-resistant breast cancer-derived cells restored tamoxifen sensitivity and abrogated ErbB2 expression.

Wwox expression was significantly (p = 0.013) reduced in tamoxifen resistant breast cancers, and a reliable marker of tamoxifen resistance, especially in premenopausal and stage 3 patients. Thus, the Wwox signaling pathway may provide new targets for therapeutic intervention in antiestrogen-resistant breast cancers.

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X Chromosome Inactivation

Definition

A gene dosage compensation mechanism by which one of the two X chromosomes in female cells becomes transcriptionally silenced early in embryonic development through a process understood to be controlled by an inactivation centre located on the X chromosome. The X-inactivation centre determines the X chromosome to be inactivated in each individual cell and produces a noncoding Xist transcript that coats the X chromosome in *cis*, triggering its silencing and further inducing a cascade of chromatin and ►methylation changes completing the ►epigenetic silencing process.

►LINE-1 Elements

Xanthine Oxidase

Definition

Is a ►flavoprotein enzyme catalyzing the oxidation of certain purines; it is normally found in the liver of humans and it is released to blood during liver damage and can cause renal failure.

►Polyphenols

Xanthophylls

Definition

Are ►carotenoids where some of the double bonds have been oxidized, such as lutein and zeaxanthin.

Xenobiotic Biotransformation

►Detoxification

Xenobiotic Metabolism

►Detoxification

Xenobiotic Receptor

►Aryl Hydrocarbon Receptor

Xenobiotic

Definition

Chemicals not normally found in the body (including most carcinogens).

►Carcinogen Metabolism

Xenobiotics

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Synonyms

Foreign substances; Exobiotics

Definition

Xenobiotics are chemicals present in an organism or environment, which did not produce them. Some naturally occurring chemicals (endobiotics) become xenobiotics when present in an environment at excessive concentrations. The word "xeno" is derived from the Greek word "*xenos*," meaning a guest, friend, or foreigner.

Characteristics

Origin of Xenobiotics

Xenobiotics are mostly produced by human activities and excite public awareness due to their ability to interact with the living environment. Some organisms may also form them as a part of their defense system, e.g., mycotoxins, bacterial, and herbal toxins, etc., and xenobiotics become harmful when entering the alimentary chain. Contemporary human exposure to xenobiotics is unavoidable, as xenobiotics are omnipresent. Human exposure to some xenobiotics is voluntary because of their anticipated beneficiary effects on human health (e.g., drugs, antibiotics, dietary supplements as antioxidants, etc.). Daily use of xenobiotics as food ingredients (dyes, stabilizers, emulsifiers, salt compounds, preservatives, etc.), cosmetics and personal care products (makeup, hair dyes, soaps, perfumes), and household products (chlorine bleach, bug sprays, cleaners, etc.) further increases exposure extent. Nevertheless, the positive role of xenobiotics as components of modern technologies enabling further progress of human civilization is inevitable. At present time, it is not possible to divide xenobiotics into straightforward categories as, e.g., good versus bad ones and simply eliminate the latter from living environment.

Metabolism of Xenobiotics

Metabolism or in other words biotransformation leads to conversion of xenobiotics in the organism. After entering the human body, blood vessels transport xenobiotics to the liver as the main ►detoxification site. Hepatocytes (liver cells) contain xenobiotic-metabolizing enzymes (XMEs). The major task of XMEs is to convert xenobiotics soluble in fat into water-soluble products and thus facilitate their elimination from the cell and excretion from the body. XMEs are divided into three groups: ►phase I enzymes (activation), ►phase II enzymes (conjugation), and phase III (transport) enzymes (Table 1). ►Cytochromes P450 (P450s) represent a major player in the field of phase I biotransformation. P450s and other phase I enzymes as NAD(P)H:quinone oxidoreductases (NQOs) usually perform activation reactions aiming at formation of reactive ►intermediates. Such highly electrophilic intermediates efficiently conjugate with ►nucleophiles, e.g., glutathione in reactions catalyzed by phase II enzymes as ►glutathione S-transferases (GSTs). The majority of conjugated xenobiotics undergo transport outside of the cell by ►efflux pumps driven by ►membrane ATP-binding casette transporters (ABC), e.g., ►P-glycoprotein. Main routes of elimination of hydrophilic conjugates are urine, feces, sweat, or breath. Xenobiotics are often highly lipophilic and thus may accumulate in fat tissues and slowly drain into blood circulation long time after exposure event occurred. The typical example may be exposure to organic solvents as styrene or polychlorinated biphenyl ►dioxin. Various physiological factors, e.g., age, gender, nutritional status (starving), or pathological factors, e.g., hypertension, diabetes mellitus, liver cirrhosis, renal failure, etc., significantly affect metabolism. An understanding of xenobiotic metabolism is critical for the pharmaceutical industry because it is responsible for the activity but also for the toxicity of drugs.

Effects of Xenobiotics

Common concern about xenobiotics is due to their toxicity toward living organisms and potential to cause environmental effects on global level. ►Acid rains and production of glasshouse gases increase global warming. Sea pollution or soil contamination by agricultural chemicals facilitates incorporation of heavy metals into highly toxic organic compounds. High concentration of metropolitan transport and local heating enhances concentration of particulate matter containing ►polycyclic aromatic hydrocarbons, ►polychlorinated biphenyls, dioxins, etc. Toxicity classification concerns the grouping of chemicals into general categories according to their most important toxic effect. Such categories can include allergens, neurotoxins, carcinogens, mutagens, teratogens, ►immunotoxins, etc. Xenobiotics act either directly as parent compounds or indirectly as intermediates or products of their metabolism. ►Oxidative stress presents another secondary product of metabolism of xenobiotics. ►Reactive oxygen species may then attack DNA, proteins, or stimulate ►inflammation. In some cases, an inactive conjugated metabolite is reactivated, for example, by enzymatic cleavage and causes tissue-specific toxic effect, e.g., tumor promotion. Certain xenobiotics entering human body deregulate important cellular and organ signaling pathways because they mimic physiological substrates. Xenoestrogens or endocrine disruptors, e.g., phthalates or polychlorinated biphenyls (DDT, dioxin), etc., impair reproductive functions, disturb wildlife, and may cause sterility by decreasing sperm count in males. Another important issue is the widely discussed oncogenic potential of xenoestrogens. Evaluation of environmental exposure to xenobiotics complicated by the fact that combined exposures to several xenobiotics simultaneously occur rather than separate simple exposures. Chemicals acting via the same mechanisms produce ►additive effects. However, interaction between chemicals may result in an

Xenobiotics. Table 1 Major xenobiotic-metabolizing enzymes

	Protein	EC number	Gene	Reactions
Phase I				
Cytochrome P450 monooxygenases	P450	1.14.14.1	CYP	Oxidation, reduction, peroxidation
Flavin-containing monooxygenases	FMO	1.14.13.8	FMO	Oxidation
Alcohol dehydrogenases	ADH	1.1.1.2	ADH	Alcohol oxidation
Aldehyde dehydrogenases	ALDH	1.2.1.5	ALDH	Aldehyde oxidation
Monoamine oxidase	MAO	1.4.3.4	MAO	Oxidative deamination
NADPH-cytochrome P450 reductase	CPR	1.6.2.4	POR	Reduction
Carbonyl reductases	CR	1.1.1.184	CBR	Reduction
Aldo-keto reductases	ALR	1.1.1.21	AKR	Reduction
NAD(P)H-quinone oxidoreductase	NQO	1.6.5.2	NQO	Quinone reduction
Epoxide hydrolases	EPHX	3.3.2.9	EPHX	Epoxide hydrolysis
Carboxylesterases	CE	3.1.1.2	PON	Hydrolysis of ester-containing xenobiotics
Deaminases	CD	3.5.4.1	CDA	Hydrolytic deamination
Phase II				
Glutathione S-transferases	GST	2.5.1.18	GST	Conjugation with glutathione
UDP-Glucuronosyltransferases	UGT	2.4.1.17	UGT	Conjugation with glucuronide
N-Acetyltransferases	NAT	2.3.1.5	NAT	Acetylation
Sulfotransferases	SULT	2.8.2.3	SULT	Conjugation with sulfate
Phase III				
ATP-binding cassette transporter	MDR	3.6.3.44	ABCB	Xenobiotic transport across cell membranes
	MRP/MOAT	3.6.3.44	ABCC	
	BCRP	3.6.3.44	ABCG	
Lung resistance-related protein	LRP/VAULT1	3.6.3.44	MVP	Nucleo-cytoplasmic transport

inhibition (►antagonism) or in ►synergism, a more pronounced effect than would be expected by addition. Estimation of effects of combined mixtures by animal experiments and computer modeling belongs to the most difficult tasks of modern toxicology.

Interactions

Xenobiotics are able to enhance their own metabolism by ►induction of XMEs. Induction of battery of XMEs through ►arylhydrocarbon receptor (AhR) was the first proved example. Expression of XMEs is regulated also through other nuclear receptors, e.g., constitutive androstane receptor (CAR), ►peroxisome proliferator-activated receptor (PPAR), pregnane X receptor (PXR), and others. Some xenobiotics cause inhibition of XMEs by covalent binding to active site or other functionally important part of enzyme or by competition for enzyme with physiological substrates or other xenobiotics. Interactions between xenobiotics and XMEs may cause severe health effects. Induction of P450 2E1 by ethanol consumption enhances metabolism of acetaminophen (paracetamol) by P450 2E1 to hepatotoxic intermediates leading to death of sensitive individuals. Inhibition of P450 3A4 by components of grapefruit juice may either decrease effect of P450 3A4-activated ►prodrugs or/and prolong toxicity of P450 3A4-detoxified drugs. P450 3A4 metabolizes about 50% all currently prescribed drugs, e.g., amiodarone, budesonide, codeine, digitoxin, ►irinotecan, lidocaine, midazolam, simvastatin, ►tamoxifen. Drug–drug interactions may of course have similar consequences as interactions between drugs and nutrition.

Individual Variability

XMEs are highly genetically variable and contain a number of functionally relevant polymorphisms (►metabolic polymorphisms). These inherited DNA variations may influence both level and activity of expressed XMEs. Altered enzyme then lacks critical detoxifying activity or gains activating properties, which may form excess of toxic metabolites and impair important physiological functions. Thus, genetic variability may explain why certain individuals are more susceptible to

toxic effects of xenobiotics than others are. Various populations differ in frequencies of XMEs polymorphisms, e.g., GSTT1-null polymorphism conferring lack of enzyme activity affects 10–20% of Caucasian populations but 30–60% of Oriental ones. Thus, depending on exposure type and extent this polymorphism may be more important for Orientals. Polymorphisms in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) converting ethanol to acetaldehyde cause unpleasant adverse effects in large group of Orientals and are suspected to modify acetaldehyde-induced ►carcinogenesis. Polymorphisms in pharmacologically important P450 2D6 belong to the most studied ones and progress in science and technology enabled genotyping individuals to assess so-called poor metabolizer phenotype (persons with weak metabolizing ability), medium, and rapid phenotype (persons with high metabolizing ability). Such effort pays off both to society on the cost-benefit basis and to individual patient whom physician prescribes the most efficient drug with least adverse effects without blind tests of different medications with potential danger of longer periods of illness or even hospitalization. However, individual variability still greatly complicates assessment of drug–drug interactions and presents a challenge for contemporary pharmacology. Among possible solutions of this puzzle, design of new generation of drugs, which are not subjects to metabolism by polymorphic XMEs will soon play pivotal role.

Xenobiotics and Cancer

Various xenobiotics promote carcinogenesis or cause genotoxicity by interaction with genetic information. The majority of procarcinogens undergo metabolic conversion to become ultimate carcinogens. The extent of carcinogen production is dependent on ratio between activation and detoxification capacity. Thus, understanding the individual variability in XMEs may shed more light on the molecular basis of diseases apparently caused by interaction between genetic background and environment. Experts in molecular epidemiology now evaluate prognostic value of reported associations between genetic variants in genes coding XMEs and various diseases, especially cancer. Although the concept of association between metabolic capacity modified by genetic variation in XMEs and disease caused by metabolically activated procarcinogens seemed reasonable, ►meta-analysis shows that the task will be extremely difficult. Association studies usually use very limited numbers of patients due to problems with recruiting. Moreover, each cancer type presents distinct disease with distinct etiology, so pooling of patients with different diagnoses is questionable. Quite often, the identification of individual chemicals and levels of exposure in studied individuals is very complicated by the long latency period from exposure to onset of cancer (usually decades). Finally, interplay between different groups of low-penetrance genes (such as XMEs) most probably multiplies the effect of associations. The final goal of molecular epidemiology is to predict cancer susceptibility based on individual variability and prevent interacting exposures. In individuals already suffering from cancer, individualized therapy tailored to XMEs overexpressed in tumor cells will be available soon.

Exploitation of Knowledge about Xenobiotics and XMEs

Besides the use in pharmacology and ►cancer epidemiology, the knowledge gained through intensive research on xenobiotics, their origin, and fate in living organisms over last decades suggested promising applications in many commercial areas. Genetically engineered XMEs are evolving tools for selective production of chemicals with valuable properties including drugs whose production by chemical synthesis is impossible or connected with overwhelming costs. Sewage is increasingly contaminated with xenobiotics of all sorts including human waste. Sorting of sewage is becoming extremely expensive and for its bioremediation treatment systems based on immobilized XMEs seem to be the most natural choice. After obtaining solution for some technical aspects broader applications of XMEs and their optimization for commercial needs will soon be performed.

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Xenogeneic

Definition

Derived or obtained from an organism of different species and, therefore, immunologically incompatible.

Xenograft

Definition

The engraftment of cells or tissue originating from one species into the body of a different, often immune-suppressed, host species. In the field of oncology, a commonly used term to describe small rodent tumor cell implantation models. For example, human breast cancer cells injected into nude mice.

►Bioluminescence Imaging
►Mouse Models

Xenograft Models

►Mouse Models

Xeroderma Pigmentosum

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Definition

Xeroderma pigmentosum (XP) is a genetic disease with clinical and cellular hypersensitivity to ►ultraviolet radiation and defective ►repair of DNA. XP patients display a marked increase in the frequency of skin malignancies. XP, thus, serves as a model disease to study the relationship between defects in DNA repair and (skin) cancer. It is a skin cancer prone disorder, due to defective ►nucleotide excision repair (NER).

Characteristics

Xeroderma Pigmentosum (XP) Subgroups

The DNA repair pathway that deals with the removal of DNA damage induced by UV radiation is NER (nucleotide excision repair). In 1972 it was demonstrated by using cell fusion techniques that fibroblasts from one patient were able to compensate the repair defect in fibroblasts from another patient. Heterokaryons (cells with nuclei from different donors in a common cytoplasm) were found to exhibit mutual correction of the defective removal of UV radiation-induced DNA damage. Such cells were said to be in different ►complementation groups. If both nuclei in a heterokaryon have the same genetic defect, then the heterokaryons show defective repair of DNA damage and the patients are in the same complementation group. Such studies have revealed the existence of at least seven complementation groups in XP (XP-A through XP-G). Each complementation group may represent a gene that, if mutated and in homozygous condition, causes XP. In addition, a subgroup exists with the clinical symptoms of XP but with normal NER of UV-induced DNA damage. Patients in this subgroup have a defect in an alternative repair process, viz., in postreplication repair. Such patients are called XP-V (XP variants).

Clinical Aspects

XP is clinically characterized by photosensitivity, pigmentary changes, premature skin ageing and a high incidence of ►skin cancer. In the majority of cases the first symptoms are noticed between 6 months and 3 years after birth. Freckling, sensitivity to sunburn and an increased dryness on sun-exposed skin are usually the earliest manifestations. The first malignant tumors may develop as early as the third or fourth year. ►Basal cell carcinoma, ►squamous cell carcinoma, and ►melanoma, are common and may be multiple. Besides the skin, the eyes and the nervous system may be affected. In the large majority of patients, photophobia and conjunctivitis are early symptoms. Neurological abnormalities occur in ~20% of the cases, which are predominantly patients in the XP-A and XP-D complementations groups. These abnormalities may comprise mental retardation, spacticity, ataxia, dysphasia and areflexia. Two-thirds of XP patients die before 20 years of age from metastases, neurological complications or infections, to which they are also abnormally susceptible. Table 1 summarizes the clinical features of the different XP complementation groups.

Cellular Parameters

The two major types of DNA damage induced by UV radiation are CPD (cyclobutane pyrimidine dimers) and [6–4]PP (6-pyrimidine-4-pyrimidone photoproducts). Both CPD and [6–4]PP are formed between two adjacent pyrimidines (cytosine and/or thymine) on a DNA strand. Both lesions lead to a considerable distortion of the three-dimensional structure of the double helix. CPD and [6–4]PP are removed from the genome by nucleotide excision repair (NER), which comprises two subpathways:

- GGR (global genome repair)
- TCR (►transcription-coupled repair)

The GGR subpathway is responsible for the removal of lesions from the transcriptionally inactive DNA and from the non-transcribed strand of active genes.

Xeroderma Pigmentosum. Table 1 Clinical properties of the separate XP complementation groups

Group	Skin cancer	Neurological abnormalities	Relative frequency
XP-A	+	++	High
XP-B	±	++	Very rare
XP-C	+	–	High
XP-D	+	+	Intermediate
XP-E	±	–	Rare
XP-F	±	–	Rare
XP-G	±	++	Rare
XP-V	+	–	High

In the TCR subpathway, the repair machinery is directed preferentially to the transcribed strands of active genes to avoid unrepaired lesions interfering with transcription. Generally, the transcribed strand is corrected up to 5–10 times as fast as the nontranscribed strand. As different mutations in the same gene can lead to different levels of impairment, patients within the same complementation group can vary quantitatively in their residual GGR and/or TCR capacity. All NER-defective XP complementation groups are more or less defective in both GGR and TCR, with the exception of XP-C and XP-E, which are defective in GGR only.

Molecular Parameters

►NER is the process in which damaged DNA is removed and replaced with new DNA using the intact strand as a template. This complex system involves the concerted action of multiple proteins. The first step in mammalian NER is damage recognition. The XP-C and the XP-E protein play a role in recognition of UV-damaged DNA in nontranscribed DNA. In transcriptionally active DNA, the arrest of transcription at the site of a DNA lesion serves as the damage-recognition signal. Subsequently, the XP-A protein and a protein complex called ►TFIIH are recruited to the lesion, and the double helix is opened around the site of damage. It is assumed that in both transcription and NER the function of TFIIH is the unwinding of the double-stranded DNA helix. Both the XP-B and the XP-D protein are part of the TFIIH complex. After damage recognition and partial unwinding of the double helix the actual removal of DNA damage is identical for the GGR and TCR subpathways. The XP-F and XP-G proteins play a role in cutting the DNA on either site of the damage, thereby releasing a 24- to 32-residue oligonucleotide. Subsequently, the gap is filled in by a DNA polymerase and sealed by a DNA ligase.

XP-V cells have normal removal of UV-induced DNA damage from both transcribed and nontranscribed DNA. Cells can tolerate unrepaired damage in their genome and removal of damage does not have to be complete before DNA replication takes place. As CPD and [6–4]PP are effective blocks to the progression of replicative DNA polymerases, cells have developed specialized DNA polymerases that can bypass DNA damage and extend replication forks through damaged sites. One of these DNA polymerase can bypass CPD at thymine-thymine sites and usually correctly inserts two A residues opposite the lesion. In XP-V cells this specialized polymerase is defective. Consequently, in XP-V cells lesions are bypassed by polymerases that insert incorrect residues, leading to a high level of mutations in XP-V cells.

Animal Models

Experimental studies on the relationship between NER defects and clinical phenotype are difficult to carry out as XP is a rare disease, which consists of at least seven NER-defective subgroups. In addition, experimental studies with UV radiation in XP patients can be considered questionable for obvious ethical reasons. Therefore, mouse models for XP have been developed, which are well suited to study the relationship between deficiencies in NER and susceptibility to skin cancer. The first viable animal models for XP were XP-A-deficient and XP-C-deficient transgenic mice (XP-A and XP-C knockouts). These animals develop normally, are fertile and do not show signs of a NER-disorder. However, after exposure to UV radiation both animal models strongly mimic the phenotype of humans with XP, i.e., they show a severely increased susceptibility to skin cancer. Neurological disorders, common in XP-A patients, were not found in XP-A knockout mice. A direct comparison in skin cancer susceptibility between XP-A and XP-C ►knockout mice has shown that XP-A knockouts are more cancer prone than XP-C knockouts. Hence, skin cancer susceptibility is determined by both GGR and TCR, and defective GGR contributes more prominently to skin cancer development than defective TCR. However, acute UV effects

appear to be related primarily to TCR; the minimal dose required to induce a slight sunburn is strongly reduced in XP-A knockouts but not in XP-C knockouts. Probably blockage of RNA synthesis during transcription, by persistent CPD or [6–4]PP, triggers the influx of pro-inflammatory molecules leading to sunburn.

As parents of XP patients usually are clinically normal, inheritance of XP is considered to be autosomally recessive. Whether carriers of XP genes (heterozygotes) have a subtle increase in skin cancer risk can easily be addressed by XP mouse models. Heterozygous XP-A animals did not show a higher skin cancer susceptibility than wildtypes, whereas heterozygous XP-C animals have been reported to have a higher susceptibility to UV carcinogenesis than their wildtype litter mate controls. The reason for this difference has not yet been elucidated.

Other NER-Related Syndromes

In addition to XP, two other rare genetic diseases have been associated with a defect in NER. The first is CS (Cockayne syndrome), which comprises at least two complementation groups (CS-A and CS-B). CS patients have a defect in TCR and they exhibit a severe clinical phenotype. CS patients show growth failure, progressive neurological degeneration, retinal degeneration, photosensitive skin and deafness, and most CS patients die at an early age. The second NER-related disease is a photosensitive form of ►trichothiodystrophy called PIBIDS. This is an acronym for photosensitivity, ichthyosis, brittle hair and nails, intellectual impairment, decreased fertility and short stature. Patients with PIBIDS have mutations in the XP-B or XP-D gene, which are both components of the ►TFIIH complex. It has been suggested that specific mutations that preserve the transcription function of TFIIH leads to XP, whereas mutations that also modify the transcription function lead to PIPIDS.

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Xerophthalmia

►Sjögren Syndrome

Xerostomia

►Sjögren Syndrome

XIAP

Definition

A member of the IAP (►inhibitors of apoptosis) family of proteins containing one or more characteristic BIR domains. These proteins bind and inhibit ►caspases.

►APAF-1 Signaling

Xiphophorus

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Synonyms

Gordon-Kosswig melanoma system; Platyfish-swordtail melanoma

Definition

Small aquarium fishes of the genus Xiphophorus are known by the common name of platyfish (*X. maculatus*) and swordtail (*X. hellerii*). Introgressive hybridization results in offspring that develop melanoma according to Mendelian principles. This represents the first animal model, described in 1927, systematically employed for studies of genetic factors in cancer and to show induction of melanoma by ultraviolet light (►UV-A).

Characteristics

Genetics of Melanoma Formation

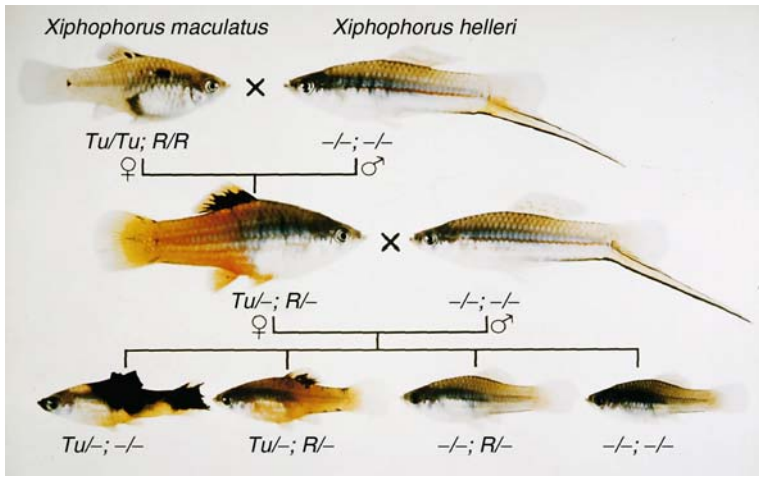
The genetic basis of melanoma formation after introgressive hybridization is explained by the independent

segregation of a pigmentation locus (Sd), which contains a dominantly-acting ►oncogene, designated Tu, and a trans-acting regulatory gene R (also termed Diff, *MelSev* or R_{Diff}) that suppresses the oncogenic activity of *Tu* (Fig. 1). Independent segregation is possible because *Tu* and *R* reside on different chromosomes. Crossing and backcrossing the fish carrying both *Tu* and *R* (platyfish) to the swordtail results in the progressive replacement of platyfish chromosomes bearing the R by swordtail chromosomes lacking *R*. The stepwise elimination of *R* from the hybrid genome allows expression of the *Tu* phenotype, leading to a benign hyperpigmentation in cases where one functional allele of *R* is still present (F1 and 25% of backcross) or to malignant melanoma in cases where *R* is completely absent (25% of backcross).

The *Xmrk* Oncogene

The melanoma oncogene from the *Tu* locus is referred to as *Xmrk* (Xiphophorus melanoma receptor tyrosine kinase, also termed *Xmrk-2*). It encodes a transmembrane ►receptor tyrosine kinase (RTK) that is an oncogenic version of the epidermal growth factor (EGF) receptor. During evolution, *Xmrk* was generated by gene duplication from its corresponding proto-oncogene. The new copy was fused to the 5′ non-coding region of another (anonymous) sequence. This process generated a novel promoter for the oncogenic version (called *Xmrk* to distinguish it from the proto-oncogenic copy *Xegfrb*) of the gene.

As a consequence of this rearrangement, the proto-oncogene and the oncogene are subject to different transcriptional regulation. Specific overexpression of the *Xmrk* oncogene in the pigment cell lineage of hybrid fish is responsible for melanoma formation. An *R* locus-dependent transcriptional control of the oncogene promoter allows high levels of expression exclusively in pigment cells of certain hybrid genotypes but not in non-hybrids. This explains why the dominantly-acting *Xmrk* oncogene is ineffective in the pure bred parental Xiphophorus fish and is a non-hazardous constituent of the genome in natural populations for many generations. This is reminiscent of the situation found for the ►RET and ►MET oncogenes in humans, where dominantly-acting mutations are transmitted through the germ line and elicit ►multiple endocrine neoplasia type 2 (MEN2A, MEN2B) and hereditary papillary renal-cell carcinomas (HPRCC), respectively. Transcriptional control of *Xmrk* by *R* involves the suppression of an Sp1 transcription factor-mediated constitutive promoter activity in non-melanoma cells and a specific hypomethylation of the oncogene promoter, compared to the proto-oncogene promoter. The *Xmrk* oncogene is necessary for melanoma formation since transposon inactivation of the oncogene in one



Xiphophorus. Figure 1 Genetic tumors in Xiphophorus. The classical cross-breeding experiment: a female platyfish (*X. maculatus*), which is homozygous for the X-chromosomal locus *Sd*, encoding the pigment pattern "spotted dorsal" (small black spots in the dorsal fin composed of a specific type of pigment cells – so-called macromelanophores) is mated to a swordtail (*X. hellerii*), which does not have the corresponding locus. The F1 hybrids show enhancement of the *Sd* phenotype. Backcrossing of F1 hybrids to *X. hellerii* results in offspring that segregate. Fifty percent do not inherit the *Sd* locus and are phenotypically like the *X. hellerii* parental strain. The other 50% carry the *Sd* locus and develop melanoma. Here, in approximately half of the fish, the severity of melanoma ranges from very benign (phenotype like the F1 hybrids) to extremely malignant in the others. Highly malignant melanomas become invasive and exophytic, and are fatal to the individual. These melanomas grow progressively, following transplantation into thymus-aplastic "nude" mice. Symbols below the fish describe their genotypes in respect to the *R* and *Tu*-loci.

mutant strain results in loss of melanoma formation. However, overexpression of the receptor is not the only reason for tumor induction. Mutations in the extracellular domain that create covalently linked receptor dimers with constitutive activity and a cell type-specific signal transduction machinery downstream of *Xmrk* are necessary for tumor formation in transgenic fish and for transformation of cells in tissue culture.

Signal transduction by Xmrk affects all cellular events needed for a full transformation. Some, though not all of the pathways induced by the oncogene are also shared by mammalian EGFR. It starts with the parallel activation of the ►Ras/Raf/MAP kinase pathway, the ►PI3 kinase pathway, the ►signal transducer and activator of transcription STAT5, the small src kinase fyn and the ►focal adhesion kinase. These events result in the transcription of numerous target genes belonging to different functional categories (secreted proteins, anti-apoptotic effectors, cell cycle regulatory proteins etc.). Finally, an induction of cellular proliferation, protection from ►apoptosis, ►migration and contact-independent survival takes place. In addition, transcriptional and post-translational regulation of the differentiation and survival factor MITF impairs the final differentiation of the pigment cell. Consequently, *Xmrk* alone is able to elicit all the processes that are necessary for establishing the full malignant neoplastic phenotype of a melanoma cell.

UV-Induced Tumors

Melanoma in certain hybrid crosses can be induced by UV-B and photoreactivation can reverse this melanoma incidence to background levels. When young backcross hybrids were irradiated with wavelengths from 365–436 nm, melanomas could be induced. This led to the appreciation that UV-A, and perhaps the visible light spectrum as well, are important in the etiology of human melanoma. Research on UV-induced melanoma uncovered *CDKN2* a/b as a candidate gene for *R*.

Carcinogen-Induced Tumors

Besides the propensity to develop melanoma on a hereditary basis, certain backcross strains have a susceptibility to develop cancer after exposure to carcinogens. While fish of wild type strains resist the development of tumors after exposure to N-methyl-N-nitrosourea (MNU) or to X-rays, certain hybrids are highly sensitive and respond to treatment by developing a large spectrum of tumors.

Further Information

http://www.xiphophorus.org/

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XME

Definition

Xenobiotics Metabolizing Enzymes; ►Aryl Hydrocarbon Receptor.

X-Ray Crystallography

Definition

A method that uses x-ray diffraction from a crystallized molecule to determine its three-dimensional structure.

►Structural Biology

X-ray Induced Cancer

►Radiation Carcinogenesis

XRF

Definition

X-ray Fluorescence.

►Lead Exposure

YAC

Definition

Acronym of yeast artificial chromosomes. A vector system used to clone large DNA fragments.

►ArrayCGH

Yin and Yang

Definition

Is a philosophical concept developed in ancient China. According to the theory of yin and yang, things and phenomena in the natural world oppose and complement each other. Yin and yang not only means two things opposing each other, but they also means two opposite components in the same thing. The contradiction movement between unity and opposite of yin and yang exists universally within everything and its consequence is the occurrence, development, and changes of all things in the universe. The theory of yin and yang has been widely used in Chinese medicine to refer to various antitheses in anatomy, physiology, pathology, diagnosis, and treatment, such as feminine, interior, cold, and hypofunction being yin while masculine, exterior, heat, and hyperfunction being yang.

►Chinese versus Western Medicine

Yinxing

►Ginkgo Biloba

YKL-40

Definition

Is a member of the mammalian chitinase-like proteins. Elevated serum values of YKL-40 have been reported in a variety of cancers, including breast, colon/rectum, ovary, lung, kidney, glioblastoma, and melanoma. YKL-40 levels may also be increased in other diseases with an inflammatory component.

►Serum Biomarkers

YM-529

►Minodronate

YM-529/ONO-5920

►Minodronate

Yondelis

►Trabectedin

Z´ factor

►Z-Factor

Zeolite

Definition

Are inorganic, porous, lattice-like structures in which the principal bases are aluminum, calcium, and magnesium. Zeolites often are found where volcanic rock has been immersed in water with resulting leaching of internal components.

►Asbestos

Z-Factor

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Synonyms

Z′ factor

Definition

The data quality of an assay can be estimated by the Z′-factor formulae:

$$Z' = 1 - (3\sigma_{c+} + 3\sigma_{c-})/(|\mu_{c+} - \mu_{c-}|),$$

where (σ_{c+}) and (σ_{c-}) is the data standard deviation for the high reference control and low reference control, respectively, and $|\mu_{c+}-\mu_{c-}|$ is the absolute value of the difference of the two control signal means and it defines the *usable* dynamic range (usually the linear range) of the assay.

Characteristics

Z factor (Z′ factor) is a statistical data quality indicator for a bioassay, particularly that used in the field of high throughput screening (HTS). In most HTS programs in drug discovery, each compound from a chemical library is only evaluated in a single test during primary screening. A high degree of accuracy and sensitivity in the assay is therefore critical for identifying active compounds (or "hits"). Due to various unavoidable sources of errors, the measurements from any assay will contain a certain degree of variability. Yet hits need to be identified in the presence of such variations in signal measurement. Reducing the measurement variation will give higher fidelity of the screening data. Therefore, in the design and validation of HTS assays, assessment of the screening data variability, by metrics such as the standard deviation (SD) and coefficient of variation (CV) of the replicate data set, is critical in determining whether an assay can identify hits with high confidence.

The quality of an assay has also been loosely expressed as signal-to-noise ratio (S/N) and/or signal-to-background ratio (S/B). Both ratios reflect the assay signal strength and therefore are useful parameters in assay quality assessment. In HTS, the compound activity is normally expressed as a percentage measured against some selected reference control signals, usually a high reference control and a low reference control. However, both S/N and S/B ratios fail to simultaneously take into account all of the data variability information of the sample and reference controls, as well as the assay dynamic range. To overcome some of the pitfalls of S/N or S/B ratios, Zhang and Oldenburg (1999) proposed a new, simple statistics, the Z factor (Z′ factor) as defined above, for use in evaluation of assay performance and assay signal robustness in HTS.

The Z′-factor contains data variability information of both the high and low reference controls (the same high and low reference controls usually used for normalization of the measured raw data to a percentage activity) as well as the dynamic range information. Therefore, compared to CV, S/B or S/N, the Z′-factor is more

appropriate for evaluating overall assay quality. It is a useful tool for the evaluation, comparison, and validation of any assays in general and has been widely used for quality control (QC) of HTS assay quality.

As originally proposed, the Z-factor refers to the specific Z′-factor when the mean and standard deviation of one of the corresponding reference controls are replaced by those of the testing samples. Therefore, the Z-factor is affected by the screening conditions such as the compound concentration. Z-factor has been used as a screening quality assurance parameter for assay plates owing to its sensitivity toward signal strength and data variability of test samples on the plate as well as the hit rates on the plate.

Generally, a assay with Z′-factor value of close to 1 is an ideal assay for HTS, with Z′ values between 0.5 and 1 are considered good quality, and with values between 0.5 and 0 are considered to have a moderate to poor quality. An assay with $Z' < 0$ can not be used in HTS. These are, however, only rough categorizations of assay quality for HTS and should not be regarded as absolute rules. Also, this metric applies to single point screening only. When multiple measurements (n replicate) of a testing sample are taken, the standard error (SE) should be used instead of the standard deviation and consequently the assay quality will be improved by a factor of square root of n.

There are several caveats and limitations in using the Z′-factor statistics in HTS. First, the Z′-factor should be assessed only when using large data sets, and both the high and low reference control signals should approximate a normal distribution. In some rare cases where either the data distributions deviate severely from a normal distribution or (σ_{c+}) and (σ_{c-}) values are hugely different, e.g., >10-fold, the Z′-factor values can become less effective in indicating the assay data quality. Another limitation is that, due to its dependence on two reference control states of the assay, sometimes the Z′-factor becomes a less defined measure of assay quality for stimulator or agonist screens when there is no known stimulator/agonist reference control available. When there is a strong or natural agonist/stimulator available as reference control, the Z′-factor can be rightfully defined.

Connections to Other Assay Data Quality Indicators

Besides the commonly used Z′-factor, S/B and S/N ratios, there are several other assay performance statistical parameters used in the HTS literature. A "signal window" (SW) ratio had been reported to evaluate the performance of HTS assays. The so called assay variation ratio (AVR) was also used to evaluate assay quality. AVR and Z′-factor are inter-related and inter-changeable to one another, except that the original recommended values for the acceptable and unacceptable assay for screening were somewhat discrepant. Some simulation studies compared the performance of the Z′-factor with both the SW and AVR and recommended the Z′-factor as a preferred assay performance measure. The power analysis is another approach for assay quality indication. However, the power analysis is usually based on one reference state of the assay signal distribution, and may not reflect the quality of the assay over the entire usable signal dynamic range. Compared to the power analysis, the Z′-factor is a much simpler statistical parameter to use. Z′-factor can be used in conjunction with CV, S/B or S/N to give a more comprehensive assessment of the assay variation and performance.

Z′-factor and Lower Limit of Detection of an Assay

The assay sensitivity can be evaluated by the lower limit of detection (LLD) of the assay measurement. The LLD as assay sensitivity limits are evaluated in terms of signal strength versus variability. The S/N ratio is a good indicator for strength of signal. The concentration of an analyte that gives a S/N = 3 is conventionally regarded as the LLD of the assay measurement with regard to that analyte. Similarly, one can use an analyte concentration that gives $Z' = (-1)$ as LLD of the assay.

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Zinc-Finger Proteins

Definition

A zinc finger is a protein domain that can interact with DNA. Folding of the polypeptide chain, into finger-like projections that can intercalate with the DNA helix, is stabilized through interactions with a zinc atom. Many

transcription factors and other regulatory proteins that interact with DNA contain zinc finger domains.

►Mineral Nutrients

Zinc Transporters

Definition

Proteins that coordinate zinc ions and transport them to different intracellular compartments.

►Snail Transcription Factors

ZO-1

Definition

Zonula occludens-1 was the first tight junction protein identified. It serves to link the actin cytoskeleton to proteins that control cell-cell contacts and paracellular permeability in ►tight junctions.

►Cortactin
►Zonula Occludens Protein-1

Zoledronic Acid

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Definition

Zoledronic acid is a stable synthetic derivative of the naturally occurring endogenous pyrophosphate, which acts as a powerful anti-bone degradation drug and exhibits anticancer activities.

Characteristics

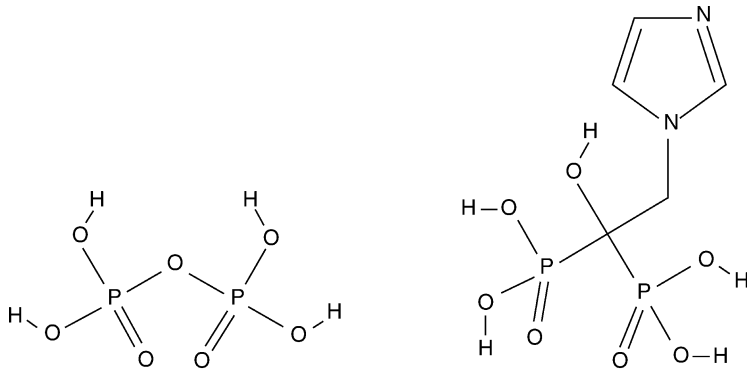
Structural Characteristics

Zoledronic acid belongs to a large drug family named ►bisphosphonate derived from endogenous ►pyrophosphate (Fig. 1). In contrast to pyrophosphate, bisphosphonates have a carbon atom replacing the central oxygen which is associated with two additional substituents. There are three main generations of bisphosphonates: (i) the first generation, including compounds called clodronate and etidronate, possess simple substituents attached to the central carbon; (ii) the second generation such as pamidronate, alendronate, and ibandronate are characterized by aliphatic side chain containing a single nitrogen; (iii) the third generation such as risedronate and zoledronic acid is composed by a heterocycle side chain containing one or two nitrogen atoms. Thus, zoledronic acid is a nitrogen bisphosphonate composed by two nitrogen atoms in an imidazole ring (Fig. 1). Zoledronic acid, like the other bisphosphonates, possesses a high affinity for calcified matrix such as bone tissue via its phosphate groups.

In vitro Activities of Zoledronic Acid and Mechanisms of Action

Due to the high tropism of bisphosphonates for ►hydroxyapatite crystals in bone and the ability of ►osteoclasts to release bone-bound bisphosphonates, osteoclasts represent the main target of zoledronic acid. Thus, after administration, zoledronic acid selectively concentrates on the bone surface at the interface with the active osteoclasts where bone tissue is most exposed. Zoledronic acid is one of the most potent bisphosphonates in terms of anti-bone resorptive activity. While the first generation of bisphosphonates has a relative potency of 1–10, the potency of the second generation reaches 10–10,000, and zoledronic acid has a relative potency more than 10,000. To exert its activities, zoledronic acid must be internalized by the cells. Although the internalization of zoledronic acid is still controversial, two mechanisms have been proposed to explain its entry into the cells: in the first case, the cellular uptake of zoledronic acid would require fluid-phase endocytosis, and in the second case, ►integrins located at the cell membrane would represent the binding site of zoledronic acid which could explain why zoledronic acid is able to inhibit cell adhesion. The main targets of nitrogen-bisphosphonates are the two enzymes involved in the ►mevalonate pathway: farnesyl diphosphate synthase (FPP) and geranylgeranyl diphosphate synthase (GGPP). FPP and GGPP are required for the ►prenylation of small GTPases (i.e., Ras, Rho, and Rac), a biochemical reaction essential for the anchorage of small GTPases to cell membranes and to protein–protein interactions. In fine, their inhibition by zoledronic acid results essentially in the blockade of osteoclast function and also osteoclastogenesis decreasing the proliferation, viability, and recruitment of preosteoclasts.

In addition to their powerful anti-►bone resorption effects, recent in vitro studies evidenced a direct antitumor activity exerted by zoledronic acid on several cancer cells including breast and prostate ►carcinomas, ►osteosarcoma, ►neuroblastoma, ►multiple myeloma.



Zoledronic Acid. Figure 1 Zoledronic acid structure compared with pyrophosphate and basic bisphosphonate structure, characterized by a central carbon substituted by two phosphate groups and two other substituents R1 and R2.

Similar to its effects on osteoclasts, zoledronic acid treatment impairs protein geranyl-geranylation in these cancer cells. The main biological effects of zoledronic acid on malignant tumor cells include the inhibition of cell adhesion (associated with an alteration of cytoskeleton organization), of cell proliferation, and induction of cell death. Indeed, zoledronic acid treatment results in the cell cycle arrest in S and G2/M phases through the control of the intra-S DNA checkpoint and induces cell death independently of ►caspase activation but via the mitochondrial pathway, in particular, the apoptosis-inducing factor and endonuclease G translocation. Furthermore, zoledronic acid exerts its antitumor activity independently of the ►p53 and ►retinoblastoma (Rb) protein status of the cells. This point is of paramount importance, because several mutations or inactivations of the ►antioncogenes *p53* and *Rb* are detected in high percentage of patients suffering from malignant pathologies. Zoledronic acid also exerts synergistic effects in vitro with conventional ►chemotherapy (carboplatin, cisplatin, YDFUR, docetaxel, epirubicin, fluvastatin, gemcitabine, imatinib, paclitaxel, trastuzumab, or vinorelbine, etc) on cancer cells.

Antitumor and Bone Resorption Activity of Zoledronic Acid in Preclinical Models

Although nitrogen-bisphosphonate has been widely used to treat ►osteoporosis and to limit the decrease of bone mineral density, zoledronic acid has been successfully used to reduce the skeletal complications (►osteolysis, hypercalcemia) associated with bone ►metastases. According to the in vitro studies, several animal models demonstrated that zoledronic acid can reduce skeletal-tumor burden in prostate, breast, and renal carcinomas, multiple myeloma, osteosarcoma, chondrosarcoma. For instance, zoledronic acid inhibits the development of ►osteoblastic bone lesions, as well as osteolysis in a murine model of ►prostate cancer. The efficacy of zoledronic acid can be explained by several mechanisms. First, a "vicious cycle" has been described in osteolytic tumors consisting in the activation of osteoclasts by mediators produced by tumor cells, which in turn release osteoclastic factors and/or bone-stocked factors favorable to the proliferation of cancer cells. In this context, zoledronic acid inhibits osteoclastogenesis and bone degradation and then reduces tumor growth. Second, zoledronic acid can affect directly the growth of tumor cells (induction of tumor-cell apoptosis and/or inhibition of tumor-cell proliferation and/or inhibition of tumor-cell spreading and invasion). Indeed, such direct activity can be underlined in nonosseous tumor models such as lung metastases. Thus, zoledronic acid diminishes osteosarcoma-induced lung metastasis in a murine model, thereby prolonging the animal survival. Third, zoledronic acid exerts an antiangiogenic activity and inhibition of tumor cell bone invasiveness by a transient reduction of circulating levels of several growth factors and matrix metalloproteinase. Moreover, it also sensitizes endothelial cells to cytokine-induced, caspase-independent programmed cell death. Fourthly, zoledronic acid exerts a variety of immunomodulatory effects that might contribute to its antitumor activities. It stimulates the proliferation of a specific γδ T-cell subpopulation which exhibits cytotoxicity against numerous tumor cells. Taken together, the published studies demonstrated that zoledronic acid must be considered as a multifunctional molecule exerting both antiresorption and tumor activities.

Therapeutic combinations of zoledronic acid and chemotherapeutic agents have also been assessed in animal models. Thus, in murine models of bone metastases, zoledronic acid combined with UFT (a combination drug of tegafur and uracil) decreases not only bone metastases but also lung metastases and

visceral metastases. In a rat osteosarcoma model, zoledronic acid associated with ifosfamide enhances tumor regression and tissue repair and increases animal survival by inhibiting lung metastases. Similarly, zoledronic acid associated with ►Glivec increases the survival of the leukemia animals. In light of these preclinical data, the main benefit of combined treatment with zoledronic acid and anticancer drugs is to prolong significantly the life of cancer-bearing animals.

Clinical Aspects

On the basis of results obtained from randomized phase III clinical trials, zoledronic acid was approved in the United States for the treatment of patients with documented bone metastasis from solid tumors in conjunction with conventional chemotherapy and patients suffering from multiple myeloma. Zoledronic acid was also approved in Europe for the prevention of ►skeletal-related events (hypercalcemia, osteolysis, bone pain, fractures, etc) in patients suffering from advanced malignancies implicating bone. Thus, zoledronic acid as the other bisphosphonates has became the standard treatment for metastatic disease spread to the bone from prostate, breast, lung, and renal cancers and for multiple myeloma. Zoledronic acid therapy is generally well-tolerated, is safe for long period, and provides durable therapeutic benefits but can be associated with an upmodulation in serum creatinine. In this context, patients must be adequately hydrated, monitoring of renal function being required for all patients receiving zoledronic acid treatment. Caution must also be exercised for patients receiving other potentially nephrotoxic treatment. The current treatment guidelines recommend intravenous administration of 4 mg zoledronic acid diluted in calcium-free solution (approximately 100 ml of saline or 5% dextrose) and infused over 15 min, every 3–4 weeks. This recommended 15 min infusion time is significantly shorter than other i.v. bisphosphonate such as pamidronate (90 mg infusion for 2 h). As the optimal duration of the zoledronic acid treatment is not really documented, the treatment should be continued as far as it is well-tolerated or until significant effects on pathologic bone tissues are observed. Plasmatic concentrations of zoledronic acid quickly increase and reach their maxima at the end of infusion. Thereafter, these concentrations rapidly decrease (<10% of zoledronic acid infused detectable after 4 h, <1% after 24 h) and a very low level of zoledronic acid (<0.1%) can be measured after 20 days, date corresponding to the next administration. In the blood, around 60% of zoledronic acid is associated with albumin, and 60% of the injected molecule binds to the bone calcified matrix before being slowly released in to systemic circulation. Zoledronic acid is not metabolized but is mainly excreted by kidney pathway under its native form. Similarly to the other bisphosphonates, zoledronic acid induces some side effects in approximately 30% of the patients. The adverse reaction includes especially a influenza-like syndrome with fever, fatigue, nausea, vomiting, arthralgia or myalgia, bone pains, and conjunctivitis. Symptoms will generally occur within 24 h and disappear within 48 h. Moreover, these adverse events are less severe with the second injection and do not occur anymore with the third infusion. Alterations of renal function (23% of the patients) have also been reported in the literature. Furthermore, clinical trials using zoledronic acid also pointed out the potential risk (but rare) of osteonecrosis of the jaw in the treated patients, length of exposure appearing to be the most important risk factor for this complication. The effects of zoledronic acid on the reproductive function have been evaluated only in animal models. Thus, low doses (0.01mg/kg) induced dystocias in rat; however, this potential risk is unknown in human. In this context, the main counter indication of zoledronic acid is pregnancy and breast feeding.

Recent advances evaluated the therapeutic interests to combine zoledronic acid with ►taxanes, thalidomide, or with bioactive compounds such as interferon-γ or ►somatostatin in patients with metastatic androgen-refractory ►prostate cancer ►hormone-refractory prostate cancer or with ►renal carcinoma. These combinatory therapies that have synergistic effects offer ►palliative clinical responses to zoledronic acid alone.

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Zollinger-Ellison Syndrome

Definition

Is a rare clinical syndrome characterized by the presence of numerous stomach and duodenal peptic ulcers in unusual locations, associated with an overproduction

of the hormone ►gastrin by a functioning neuroendocrine tumor.

►Neuroendocrine Carcinoma
►Gastrinoma

Zonula Adherens

►Adherens Junctions

Zonula Occludens Protein-1

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Synonyms

Tight junction protein-1; TJP-1; ZO-1

Definition

The zonula occludens (ZO-1) protein-1 is a membrane-associated component of both ►tight junctions (TJ) and ►adherens junctions (AJ) found at the site of cell-cell contact. Functionally, ZO-1 is an intracellular adaptor protein and is known to interact with claudins, occludin, the cadherins, the actin cytoskeleton and downstream signaling pathways and transcription factors (such as ZONAB).

Characteristics

In order for organs to perform their diverse functions, the constituent cells that make up a tissue must be able to arrange themselves in an organized fashion. The complex architecture of a given tissue is regulated by the differential expression of cell-cell adhesion molecules. Epithelial cells adhere to each other through a set of intercellular junctions that consist of adherens junctions (AJ), tight junctions (TJ) and ►desmosomes. These different types of junctions perform discrete adhesion, organizational and transport functions. TJs typically form a "belt" that circles the apical end of the cell to form a paracellular barrier regulating the flow of growth factors, ions, and other macromolecules. The ability of TJs to regulate molecular traffic in this manner is one way of ensuring that the apical/basolateral ends of the cell inhabit different microenvironments and allows for the maintenance of epithelial polarity. The extracellular portion of TJs consist of either claudins (Claudin 1–24), occludin, or the junctional adhesion molecule (JAM) proteins. Of these, the claudins and occludin are four trans-membrane domain proteins that polymerize with their counterparts on adjacent cells to form the physical barrier. JAMs are different from claudins and occludin and instead associate with the TJ strands rather than directly constituting them.

AJs and ►desmosomes do not regulate molecular transport and are primarily involved in cell-cell adhesion. Although AJs are often found in close proximity to tight junctions they are instead cadherin-based, and link directly to the actin cytoskeleton. Unlike AJs, desmosomes are distributed along the cell membrane and link to intermediate filament proteins. The cytoplasmic portions of both AJs and TJs consist of many adaptor scaffolding proteins. These proteins serve many functions such as linking the claudins/occludin and the cadherins (►E-cadherin) to the actin cytoskeleton, as well as serving as molecular docking sites for signaling proteins involved in junctional regulation and cell proliferation. One major adaptor protein is the zonula occludens (ZO-1) protein-1, which has close homology to the rat synaptic protein PSD-95, and the discs large (dlg) drosophila tumor suppressor gene. The ZO-1 protein is part of a larger family that also includes the closely related ZO-2 and ZO-3.

Molecular Biology of ZO-1

ZO-1 was first identified as a ~220 kDa antigen with a monoclonal antibody raised against a cell-cell junction enriched liver extract. The close association of ZO-1 with the claudins and occludin led to the initial identification of ZO-1 as a tight junction protein. Sub sequent work showed that ZO-1 is also expressed in many non-epithelial cells, such as fibroblasts, astrocytes, Schwann cells, glioma (►Glioblastoma multiforme) and sarcomas, where it instead associates with AJs. In cadherin-based adherens junctions, ZO-1 facilitates cell-cell adhesion through its function as a cross-linker between α-catenin and the actin cytoskeleton. ZO-1 exists in at least two alternately spliced isotypes, ZO-1^{+} and ZO-1^{-}, which confer differences in cell-cell junction dynamics. Although most epithelial and endothelial cells express both the + and − forms of ZO-1, some specialized cells, such as the endothelia of the glomerular, the peritubular capillary and the eye's trabecular meshwork express only the ZO-1^{-} form.

Sequence analysis has shown that ZO-1 is a membrane associated guanylate kinase (MAGUK) protein. The molecular structure of ZO-1, with its many protein-protein binding sites facilitates its role as a molecular scaffold. Structurally ZO-1 is composed of three PDZ domains (so named after being identified in *P*SD-95/*D*lg/*Z*O-1 proteins), one SH3 domain (►SH2/SH3 domains) and one guanylate kinase (GUK) domain.

The ►PDZ domains are protein binding motifs that are located almost exclusively at synapses and cell-cell junctions. PDZ domains facilitate the direct interaction of ZO-1 with the C-terminal domain of the claudin/occludin proteins. The SH3 (►SH2/SH3 domains) domain of ZO-1 is thought to be critical for the recruitment of a specialized serine protein kinase called ZO-1 associated kinase (ZAK) that phosphorylates a region immediately C-terminal to the SH3 domain. The guanylate kinase domain of ZO-1 is thought to be catalytically inactive, and is instead more likely to also mediate protein-protein interactions (Figs. 1 and 2).

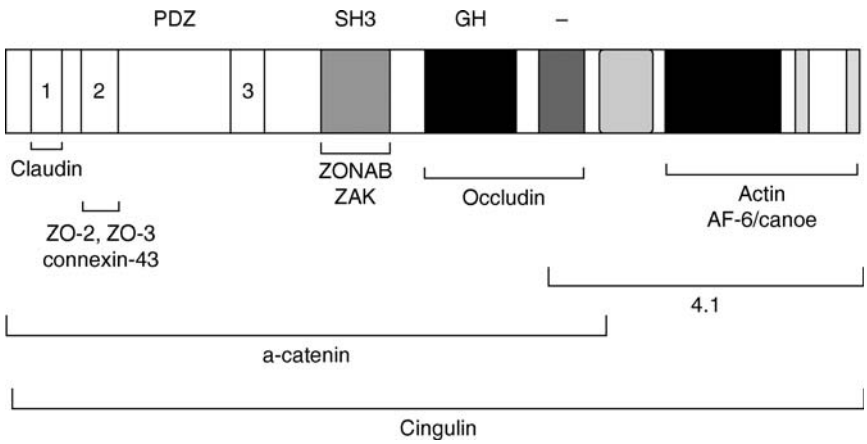
Regulation and Function of ZO-1

The cellular localization of ZO-1 is determined by the level of confluency. In a sub-confluent monolayer, most ZO-1 is localized in the nucleus. As the cells become more confluent, ZO-1 migrates, and becomes associated with the cell-cell contacts of fully confluent cells. Because of this, there is evidence that ZO-1 plays a role in sensing and regulating the extent of cellular confluency by controlling cell proliferation at the level of gene transcription. In accordance with a gene transcription role, ZO-1 associates with its own SH3 motif binding Y-Box transcription factor ZONAB. The ZO-1/ZONAB interaction appears to be critical for controlling cell density in Madin-Darby canine kidney (MDCK) cell monolayers. In these cells, ZO-1 regulates proliferation both at the G_1/S transition checkpoint by sequestering cyclin-dependent kinase (CDK)-4 (►cyclin dependent kinases) and by directly binding to ZONAB to regulate transcriptional initiation. The ZO-1/ZONAB interaction is also regulated by cellular stress. Apg-2 is a novel member of the heat shock protein (HSP)-70 family that directly competes with ZONAB at the SH3 domain of ZO-1 to regulate ZO-1/ZONAB binding. Other studies have shown that ZO-1 can also regulate cell-cell junction formation through interaction with AF-6, which is a target of the small GTPase ►Ras.

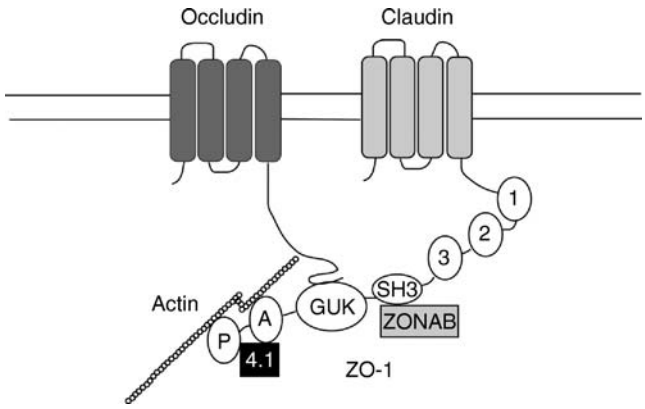
ZO-1 in Cancer

Disruption and alteration of both AJs and TJs are major hallmarks of cancer, and it is likely that altered ZO-1 expression contributes to this phenomenon in an important fashion. Under normal tissue homeostasis, the behavior of an individual cell is tightly controlled by the immediate tissue ►microenvironment. The loss of this local cellular control is associated with epithelial disorganization, invasiveness and hyperproliferation. Many epithelial tumors lack functional TJs, and this is often associated with loss of polarity and de-differentiation. Loss of TJ integrity may be particularly important for allowing the free diffusion of nutrients and other factors responsible for the growth and survival of cancer cells. Another important driving force for behind the enhanced motility and invasiveness of early tumor cells is the loss of AJ function. Loss of E-cadherin, which is often also accompanied by a switch ►N-cadherin expression, is a key early event in tumor formation and is characteristic of the ►epithelial-to-mesenchymal transition (EMT). The critical function of ZO-1 as a scaffold for both claudin/occludin-based TJ and E-cadherin-based AJ is highly suggestive of its role in cancer.

Membrane ZO-1 expression is often reduced or lost in breast, gastric, pancreatic and esophageal ►squamous cell carcinomas (SCC) as well as in SCC of the skin. In breast cancer, high ZO-1/E-cadherin expression is associated with strong cell-cell adhesion and reduced invasiveness. Consequently, loss of ZO-1 expression correlates with a more invasive breast cancer phenotype. Reduced ZO-1 levels are also associated with invasion in other tumor types, such as pancreatic cancer



Zonula Occludens Protein-1. Figure 1 Molecular structure of ZO-1, showing the major protein binding motifs and the major interacting proteins.



Zonula Occludens Protein-1. Figure 2 Structure of ZO-1 based plaque at a tight junction. The ZO-1 is scaffold molecule linking the extracellular tight junction proteins (claudin and occludin) to both gene transcription (ZONAB) and the actin cytoskeleton.

(►Pancreatic cancer, Basic and Clinical Parameters). Altered ZO-1 expression is not the only indicator of aberrant behavior. Mislocalization of ZO-1 from the membrane to the cytoplasm/nucleus is also an important factor in determining the oncogenic behavior of the protein. A loss of membrane-bound ZO-1 and its translocation to the cytoplasm upregulates expression of membrane type-1 matrix metalloproteinase (MT1-MMP) and increases breast cancer cell invasion. A further role for ZO-1 in oncogenic behavior arises from the deregulation of the ZO-1/ZONAB axis. Some epithelial tumor types such as ►hepatocellular carcinoma and pancreatic cancer overexpress the human homolog of ZONAB (Dbpa), which is known to drive the proliferation of epithelial cells through regulation of ►PCNA and ►cyclin D1. Depletion of Dbpa, using an ►shRNA against symplekin, reduced the growth of human adenocarcinoma cells, suggesting that ZONAB could be a potential therapeutic target.

The role of ZO-1 in cancer seems to depend on whether the cancer in question is epithelially-derived or not. As such, some tumor types actually exhibit markedly increased levels of ZO-1 that are not usually found in the non-transformed parent cell. In ►melanoma, which arises from a non-epithelial cell type (the melanocyte), there is an association between high ZO-1 expression and tumor progression. In this instance, ZO-1 associates with N-cadherin leading to increased melanoma cell invasion. In a similar vein, melanomas often exhibit aberrant expression of TJ proteins such as claudin-1. Another tumor type with aberrant ZO-1 expression is ►Merkel Cell carcinoma, although in this instance the precise function of the overexpressed ZO-1 protein is unknown.

At present, knowledge about ZO-1 protein expression in cancer is best used as a diagnostic tool. Loss of ZO-1 expression in epithelial tumors is strongly associated with loss of epithelial organization and increased invasion. In non-epithelial tumors, such as melanoma, the opposite is true and increased ZO-1 expression may contribute to increased tumor invasion.

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ZRP1

Definition

Zyxin-related protein 1 is a member of the ►zyxin family of proteins

► TRIP6
►Lipoma Preferred Partner

Zymogen

Definition

Inactive enzyme precursors that require a biochemical change (such as the hydrolysis of a fragment that masks an active enzyme or configuration change) in order to become active

►Protease Activated Receptor Family

Zyxin

Definition

Is an 82-kDa protein that is localized at cell-substratum and cell-cell adherens junctions. It is the founding member of the zyxin family of proteins which includes seven members: zyxin, TRIP6/ZRP-1, LPP, Ajuba, LIMD1, WTIP and FBLP-1/migfilin/Cal. The name "zyxin" is derived from the New Latin combining form "zyxi," a derivative of the Greek word "zeuxis" meaning "a joining" to indicate that it is found at regions where extracellular ligands are structurally and functionally joined to the cytoskeleton. Under certain circumstances, zyxin accumulates in the nucleus. Zyxin is an ►adaptor protein containing three carboxyterminal copies of the ►LIM domain and a proline-rich pre-LIM region harboring several protein-protein interaction domains. Interaction partners include: CRP1, α-actinin, VASP, Vav, warts/LATS1, E6 protein from ►human papillomavirus type 6, p130Cas, TES, LIM-nebulette, Lasp-1, and myopodin. In humans, the gene encoding the zyxin protein is localized at chromosome bands 7q34-q35.

ZZANK1

►Skeletrophin